FISV

Federazione Italiana
Scienze della Vita

SAPIENZA
UNIVERSITÀ DI ROMA

AAI ABCD AGI SIBBM SIBE SIBV SIC SICA SIF SIGA SIMA SIMGBM SIP SIPaV

12th FISV CONGRESS

Program
and
Abstracts

Rome, Italy
September 24-27, 2012

www.fisv.org

www.fisv2012.azuleon.org

FISV - Federazione Italiana Scienze della Vita

Program and Abstracts of the 12th FISV CONGRESS

University of Rome "Sapienza", Rome, Italy

September 24 - 27, 2012

Disclaimer

This abstract book has been produced using author-supplied copy through the website *fisv2012.azuleon.org*. Editing has been restricted to some corrections of spelling and style.

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WELCOME LETTER

The Italian Federation of Life Sciences (FISV) is pleased to have its National Congress, after a break of 3 years, at La Sapienza, University of Rome. The choice of a such prestigious and easily accessible venue has contributed to attract a great number of attendees, especially young scientists. We are glad of this success and hope that the trend will continue for the years to come.

FISV is constituted by fourteen Scientific Societies dealing with different areas of biological and biomedical research, from environment to plant physiology, from molecular and cellular biology to pathology. The Congress, by tradition, deals with internationally emerging issues, offering original contributions from some of the best Italian and International laboratories. Specific topics have been chosen among the emerging subjects at the International level. During the four days of the meeting there will be 4 keynote lectures, 3 plenary symposia and 4 parallel symposia organized by the Societies belonging to the Federation. Minisimposia will cover almost the entire field of biology and will highlight some of the highest quality contributions chosen from the submitted abstracts.

Finally, two evening events on hot topics with a potential social impact have been organized where the press has been specially invited.

We thank the Societies of the Federation, the Organizing Committee and the Organizing Secretariat which have greatly contributed to the success of this event. We also thank all the attendees who have enthusiastically responded.

With best wishes,
Il Presidente della FISV
Prof. Felice Cervone

MEMBER SOCIETIES

FISV - Federazione Italiana Scienze della Vita

Italian Federation of Life Sciences

AAI	**Associazione Antropologia Italiana**
ABCD	**Associazione di Biologia Cellulare e del differenziamento**
AGI	**Associazione Genetica Italiana**
SIBBM	**Società Italiana di Biofisica e Biologia Molecolare**
SIBE	**Società Italiana di Biologia Evoluzionistica**
SIBV	**Società Italiana di Fisiologia Vegetale**
SIC	**Società Italiana di Cancerologia**
SICA	**Società Italiana di Chimica Agraria**
SIF	**Società Italiana di Farmacologia**
SIGA	**Società Italiana di Genetica Agraria**
SIMA	**Società Italiana di Mutagenesi Ambientale**
SIMGBM	**Società Italiana di Microbiologia Generale**
SIP	**Società Italiana di Patologia**
SIPaV	**Società Italiana di Patologia Vegetale**

COMMITTEES - SECRETARIAT

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Felice Cervone (*FISV President*, Rome)
Rodolfo Negri (*FISV Secretary*, Rome)

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Alberto Chiarugi (Florence)
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Giovanni Destro Bisol (Rome)
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Valerio Orlando (Rome)
Francesca Pacchierotti (Rome)
Sergio Pimpinelli (Rome)
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SESSION ORGANISERS

Plenary Lecture

Francesca Pacchierotti
Sergio Pimpinelli
Giovanna Riccardi

Plenary Symposia

Duccio Cavalieri
Bianca Colonna
Antonio Musarò
Valerio Orlando
Sergio Pimpinelli

Parallel Symposia

Margherita Bignami
Bianca Colonna
Giulia De Lorenzo
Franco Faoro
Francesca Pacchierotti
Sergio Pimpinelli

Mini Symposia

Stefano Alemà
Giorgio Bertorelle
Michele Bianchi
Margherita Bignami
Claudia Bolognesi
Francesco Cecconi
Alberto Chiarugi
Marco Crescenzi
Fabrizio d'Adda di Fagagna
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SAPIENZA
UNIVERSITÀ DI ROMA

University Map

LEGEND

① Rettorato (Aula Magna)
② Fisiologia Generale (Aula A)
③ Edificio di Botanica (Aula Giacomini)
④ Genetica (Aula Montalenti)
⑤ Edificio Nuovo di Chimica (Aula II)
⑥ Matematica (Aula III)
⑦ Edificio Vecchio di Fisica (Aula Amaldi)

PROGRAM OVERVIEW

Monday, September 24

11:00	Registration & Accreditation ①	CNR Symposium "Graziella Persico"
13:00		
14:30	**Opening & Welcome** ①	
15:00	**Plenary Lecture** ① Denis Duboule *"Epigenetics; a new type of Genetics or more of the same?"*	
16:00	**Plenary Symposium** ① *"Stress, transposons and evolution"*	
18:00	**Plenary Lecture** ① Gabriella Campadelli Fiume *"Rethinking herpes simplex virus: the way to oncolytic agents"*	
19:00	**Wine & Cheese**	
20:30	**Round table** ④ *"Determinismo genetico, epigenesi e libero arbitrio"*	

Tuesday, September 25

9:00	**Plenary Lecture** ① Filippo Rosselli *"Inside the role of the FANC pathway: how ensure DNA replication and chromosomes integrity across cell cycle"*
10:00	Coffee Break
10:30	**Plenary Symposium** ① *"Synthetic Biology discovering new words and new worlds"*
12:30	**Poster viewing** ① (topics 1-12) & **Lunch**
14:30	**Mini Symposia** Session I **Topics** 1 - 2 ④ 3 - 4 ⑤ 5 - 6 ⑦ 7 - 8 ② 9 - 10 ③ 11 - 12 ⑥
17:00	**Societies' time** **AGI** ⑦ **SIBV** ③ **SIGA/SICA** ② **SIMA** ④ **SIMGBM** ⑥
20:00	**Congress Dinner** Limonaia Restaurant

Wednesday, September 26

9:00	**Plenary Symposium** ① *"Non-coding RNA in cell function and diseases"*
11:00	Coffee Break
11:30	**Parallel Symposia** *1 - Parassitism and symbiosis:* ⑦ *2 - Molecular mechanisms of DNA Damage Response* ⑥
13:30	**Poster viewing** ① (topics 13 - 24) & **Lunch**
15:30	**Mini Symposia** Session II **Topics** 13 - 14 ⑦ 15 - 16 ④ 17 - 18 ③ 19 - 20 ⑤ 21 - 22 ② 23 - 24 ⑥
18:00	
20:00	**Evening event** *"Giovani e Meritocrazia"*

Thursday, September 27

9:00	**Plenary Lecture** ① Pier Paolo Di Fiore *"Connecting the machineries of cell fate determination and tumor suppression in breast stem cells"*
10:00	Coffe Break
10:30	**Parallel Symposia** *3 - Innate immunity* ⑦ *4 - Plant and microbe secondary metabolites: role in biotic and abiotic stress responses and evolution* ⑥
12:30	**Congress closure & Zanichelli Prize award** ①
13:00	**Armenise - Harvard Symposium** ①
16:30	**Meeting of the EMBO Italian Members**

PROGRAM

Monday, September 24

11:00 - 14:00 **Registration & Accreditation** (Anti-Aula Magna)

11:00 - 13:00 **CNR Symposium "Graziella Persico"**

14:30 - 15:00 **Opening & Welcome** (Aula Magna)

Felice Cervone *(FISV President – Sapienza, University of Rome)*
Rodolfo Negri *(FISV Secretary – Sapienza, University of Rome)*
Filiberto Cimino *(President of the Italian Society of Biochemistry and Molecular – University of Naples)*

15:00 - 16:00 **Plenary Lecture** (Aula Magna)

Denis Duboule *(Lausanne, Switzerland)*
"Epigenetics; a new type of Genetics or more of the same?"

16:00 - 18:00 **Plenary Symposium** (Aula Magna)

"Stress, transposons and evolution"

Chairs:
Patrizio Dimitri *(Sapienza, University of Rome)*, Angelo Viotti *(CNR, Milan)*

Speakers:

Vincent Colot *(Paris)*

Donal O'Carroll *(Rome)*
"Establishment and maintenance of transposon silencing in the male germline"

Valerio Orlando *(Rome)*
"Epigenetic regulation of L1 elements mobilization in health and disease"

Sergio Pimpinelli *(Rome)*
"Transposons and the evolution of evolvability"

18:00 - 18:45 **Plenary Lecture** (Aula Magna)

Gabriella Campadelli Fiume *(Bologna)*
"Rethinking herpes simplex virus: the way to oncolytic agents"

19:00 - 20:00 **Wine & Cheese** (Terrace)

20:00 **Round table** (Genetica, Aula Montalenti)

"Determinismo genetico, epigenesi e libero arbitrio"

Speakers:

Denis Duboule *(Lausanne, Switzerland)*

Bernardino Fantini *(Geneve, Switzerland)*

Piergiorgio Donatelli *(Rome)*

Ignazio Marino *(Servizio Sanitario Nazionale)*

Sergio Pimpinelli *(Rome)*

Tuesday, September 25

9:00 - 10:00 **Plenary Lecture** (Aula Magna)

Filippo Rosselli *(Villejuif, France)*
"Inside the role of the FANC pathway: how ensure DNA replication and chromosomes integrity across cell cycle

10:00 - 10:30 **Coffee Break**

10:30 - 12:30 **Plenary Symposium** (Aula Magna)

"Synthetic Biology discovering new words and new world"

Chairs:
Livia Leoni *(Roma TRE University)*, Duccio Cavalieri *(University of Florence)*

Speakers:

Victor De Lorenzo *(Madrid, Spain)*
"Designing soil bacteria for environmental biocatalysis: then and now"

Ahmad Khalil *(Boston, USA)*
"Programmable Biology: Synthetic Approaches to Transcriptional Regulation and Cellular Microenvironments"

Paola Paci *(Rome)*
"Stochastic modeling of expression kinetics identifies messenger half-lives and reveals sequential waves of co-ordinated transcription and decay"

12:30 - 14:30 **Poster viewing [topics 1-12] & Lunch**

Mini Symposia Session I

14:30 - 15:45 **1. Cell cycle** (Genetica, Aula Montalenti)

Chairs:
Patrizia Lavia *(CNR, Rome)*, Maurizio Gatti *(Sapienza, University of Rome)*

Valeria de Turris *(Rome)*
Proximity ligation assay (PLA): a new tool to visualize regulatory pathways at their site of action

Federico Lazzaro *(Milan)*
RNase H and post-replication repair protect cells from ribonucleotides incorporated in DNA

Federica Lo Sardo *(Rome)*
PcG mediated higher order chromatin structures modulate replication programmes at the Drosophila BX

Maria Elena Pisanu *(Rome)*
Changes in lipid metabolism detected by 1H MRS in HER2-overexpressing ovarian cancer cells exposed to conventional and innovative anticancer treatments

Patrizia Vernole *(Rome)*
DNA damaging agents can induce cell cycle arrest in different phases of mitosis

15:45 - 17:00 **2. Cellular stress, apoptosis and autophagy** (Genetica, Aula Montalenti)

Chairs:
Francesco Cecconi *(University of Rome, Tor Vergata)*, Marco Crescenzi *(Istituto Superiore di Sanità, Rome)*

Adriana La Volpe *(Naples)*
Transgene or dsRNA mediated silencing induces DSBs and apoptosis in the Caenorhabditis elegans germ line

Chiara Murgia *(Rome)*
Intracellular zinc is required for intestinal cell survival signals trigged by inflammatory cytokines

Rodolfo Negri *(Rome)*
The transcriptional response of mammalian cancer cells to irradiation is dominated by a cell cycle signature which is strongly attenuated in non-cancer cells and tissues

Elia Ranzato *(Alessandria)*
New combined therapy for malignant pleural mesothelioma: a preclinical study

Laura Trapani *(Rome)*
Early autophagy inhibition is required for the completion of Activation-Induced Cell Death (AICD) in T cells

14:30 - 15:45 **3. Genomics, proteomics and system biology** (Edificio Nuovo di Chimica, Aula II)

Chairs:
Ferdinando Di Cunto *(University of Turin)*, Graziano Pesole *(University of Bari)*

Daniele Avitabile *(Rome)*
Oxidized Peroxiredoxin 2 levels increase in the nucleus of temperature-entrained human keratinocytes

Mariolina Gullì *(Parma)*
Yeast toxicogenomics: a system biology approach to study the response to 5-fluorouracil and nystati

Lucio Nitsch *(Naples)*
Genes responsible for mitochondrial dysfunction in Down syndrom

Elena Perrin *(Florence)*
Analysis of the RND superfamily in the Burkholderia genus: evolution and putative physiological role

Valeria Zazzu *(Berlin, Germany)*
IT Future of Medicine: integration of -omics data into personalised medicine

15:45 - 17:00 **4. Chromosome biology and dynamics** (Edificio Nuovo di Chimica, Aula II)

Chairs:
Francesca Degrassi *(CNR, Rome)*, Antonella Russo *(University of Padua)*

Michela Bonomo *(Civitavecchia)*
TRF2 regulates nucleosome density and spacing at human telomeres

Mariateresa Carcuro *(Rome)*
AKTIP, a conserved E2 variant enzyme that interacts with lamins and protects mammalian telomeres from replicative damage

Valentina Monti *(Reggio Emilia)*
The dark matter of the evolution: genetic variability at work in clonal lineages of aphids

Gianluca Sigismondo *(Milan)*
Proteomic mapping of the the euchromatic modificome and interactome at enhancers and promoters by combining ChIP and MS analysis

14:30 - 15:45 **5. DNA Replication, Repair and Recombination** (Edificio Vecchio di Fisica, Aula Amaldi)

Chairs:
Marco Muzi Falconi *(University of Milan)*, Margherita Bignami *(Istituto Superiore di Sanità, Rome)*

Marco Barchi *(Rome)*
Reduced proficiency in homologous recombination underlies the high sensitivity of embryonal carcinoma testicular germ cell tumors to cisplatin and poly (ADP-ribose) polymerase inhibition

Alessandra di Masi *(Rome)*
The DNA damage sensor protein NBN: role of BRCT domains in the DNA damage response

Ivana Murfuni *(Rome)*
Regulation of MUS81 pathway by cooperation with RAD52 and post-translational modifications

Sabrina Pinato *(Novara)*
Ubiquitination and genome stability: the role of RNF168's ubiquitin binding domains in the regulation of the DNA damage response

Valeria Simonelli *(Rome)*
Coordination of base excision repair and mismatch repair processing of chemotherapy-induced DNA damage

15:45 - 17:00 **6. Developement, differentiation and aging** (Edificio Vecchio di Fisica, Aula Amaldi)

Chairs:
Stefano Alemà *(CNR, Rome)*, Fabrizio d'Adda di Fagagna *(IFOM - IEO Campus, Milan)*

Maria Ina Arnone *(Naples)*
A gene regulatory network that controls the formation of a functional gut in the sea urchin embryo

Valeria Bevilacqua *(Rome)*
Characterisation of a novel long non coding RNA involved in in vitro neuronal differentiation

Costanza Maria Cristiani *(Rende)*
Single-nucleotide polymorphisms inside microRNA target sites influence aging and longevity

Ubaldo Gioia *(Rome)*
Two microRNAs, miR-23 and miR-125, control the cell fate determinant Musashi1 during astrocyte differentiation

Pompeo Macioce *(Rome)*
The impact of hypoxia in the regulation of β-dystrobrevin (DTNB) and miRNA-143 in retinoic acid (RA)-induced neuronal differentiation of NT-2 cells

14:30 - 15:45 **7. Environmental microbiology and biotechnology** (Fisiologia Generale, Aula A)

Chairs:
Roberto De Philippis *(University of Florence)*, Michele Bianchi *(Sapienza, University of Rome)*

Alessandra Adessi *(Florence)*
The use of vegetable wastes for photobiological H2 production

Elisa Bastianelli *(Rome)*
Deesterified homogalacturonan content as a biochemical trait to select plant varieties useful for bioenergy production

Isabella Gandolfi *(Milan)*
Characterization of anammox populations and microbial communities during autotrophic nitrogen removal in different reactors

Carmine Landi *(Fisciano)*
Production of Lipase A from Bacillus subtilis using different strains of the yeast Saccharomyces cerevisiae as host: a preliminary approach to the feasibility of the bioprocess

Giordano Rampioni *(Rome)*
Development of a quorum sensing-based communication system between natural and synthetic cells

15:45 - 17:00 **8. Immunology** (Fisiologia Generale, Aula A)

Chairs:
Rosa Sorrentino *(Sapienza, University of Rome)*, Rossella Paolini *(Sapienza, University of Rome)*

Giorgio Camilli *(Rome)*
Modulation of HLA-E expression during monocyte differentiation and activation

Luigi Lembo Fazio *(Rome)*
Naip-5 inflammasome governs cell death responses and IL-18 secretion in Shigella infected bone marrow-derived dendritic cells

Gaëlle Noël *(Rome)*
Impact of Shigella flexneri muropeptide shedding modifications in antigen-presenting cell

Barbara Pompili *(Rome)*
ROS contribute to Pseudomonas aeruginosa killing by CF macrophages

Linda Quatrini *(Rome)*
Down-regulation of the NKG2D receptor is differentially controlled by MICA and ULBP2 ligands

14:30 - 15:45 **9. Epigenetics and epigenetic therapies** (Edificio di Botanica, Aula Giacomini)

Chairs:
Alberto Chiarugi *(University of Florence)*, Andrea Mattevi *(University of Pavia)*

Fabio Ciccarone *(Rome)*
Erasure of DNA methylation in mouse primordial germ cells: a role for PARylati

Bruna De Felice *(Caserta)*
Small non-coding RNA signature in Multiple Sclerosis patients after treatment with Interferon-ß

Denise Drongitis *(Naples)*
Epigenetic modifications at retrotransposable sequences: further evidences on their correlation with the whole genome epigenetic changes

Cecilia Mannironi *(Rome)*
A role for Jhd2 de-methylase in transcription regulation in *S. cerevisiae*

Laura Tudisco *(Naples)*
Involvement of HIF-1α in hypoxia-induced Placental Growth Factor expression

15:45 - 17:00 **10. Human genetic and genomic diversity** (Edificio di Botanica, Aula Giacomini)

Chairs:
Rosaria Scozzari *(Sapienza, University of Rome)*, Olga Rickards *(University of Rome, Tor Vergata)*

Vincenza Battaglia *(Pavia)*
The peopling of South America: the last major human dispersal

Giovanni Destro Bisol *(Rome)*
Mine, yours, ours? Sharing data on human genetic variation

Gisella Figlioli *(Pisa)*
Medullary thyroid carcinoma (MTC) and RET proto-oncogene: mutation spectrum in the familial cases and a meta-analysis of studies on the sporadic form

Gabriele Scorrano *(Rome)*
Neolithic revolution: cultural or genetic change in central-south Italy? A mosaic scenario

Francesca Tassi *(Ferrara)*
On the origins of the Etruscan people

14:30 - 15:45 **11. Genetic of microorganism** (Matematica, Aula III)

Chairs:
Anna Maria Puglia *(University of Palermo)*, Ezio Ricca *(University of Naples)*

Simone Battaglia *(Pavia)*
Characterizing the MmpL3 protein, a novel target for new antitubercular agents

Sara Carloni *(Milan)*
Evaluation of the infection-relevant role of small RNA-based regulatory systems in the opportunistic pathogen Pseudomonas aeruginosa

Alessandra Polissi *(Milan)*
New insights into the Lpt machinery for lipopolysaccharide transport to the cell surface: functional dissection of LptC protein

Teresa Rinaldi *(Rome)*
The ERMES complex is essential for mitochondrial inheritance and lipid biogenesis in S. cerevisiae

Ruggero Rusmini *(Milan)*
Universally conserved protein gcp is essential for Pseudomonas aeruginosa viability

15:45 - 17:00 **12. Evolution** (Matematica, Aula III)

Chairs:
Giorgio Bertorelle *(University of Ferrara)*, Renato Fani *(University of Florence)*

Andrea Benazzo *(Ferrara)*
Temporal patterns of divergence and hybridization in three Antarctic fish species

Anna De Gaetano *(Pavia)*
A detailed phylogeny of cattle mtDNA haplogroup T1: old ideas and new perspective

Mariko Forconi *(Ancona)*
Landscape of active transposable elements in Latimeria menadoensi

Francesco Spinelli *(Rome)*
Nucleotide diversity of polygalacturonase-inhibiting protein (PGIP) genes in natural populations of Phaseolus vulgaris

Mario Ventura *(Bari)*
Gorilla genome structural variation reveals evolutionary parallelisms with chimpanzee

17:00 - 20:00 ## Societies' time

AGI (Edificio Vecchio di Fisica, Aula Amaldi)

17:00 - Premi Dottorato AGI/Zanichelli 2011 e 2012 e presentazione dei lavori premiati
18:00 - **Pablo Amati** "Un ricordo di Jack von Borstel"
18:15 - Assemblea dei Soci AGI

SIBV (Edificio di Botanica, Aula Giacomini)

Premio "Assunta Baccarini Melandri"
Assemblea ordinaria dei Soci SIBV
Elezione del Presidente

SIGA/SICA (Fisiologia Generale, Aula A)

Riunione congiunta SIGA-SICA

SIMA (Genetica, Aula Montalenti)

Assemblea dei Soci SIMA

SIMGBM (Matematica, Aula III)

Assemblea dei Soci SIMGBM

20:00 ## Congress dinner

at **Limonaia Restaurant** (Via L. Spallanzani 1, Rome)

Wednesday, September 26

9:00 - 11:00 **Plenary Symposium** (Aula Magna)

"Non-coding RNA in cell function and diseases"

Chairs:

Antonio Musarò *(Sapienza, University of Rome)*, Giuseppe Macino *(Sapienza, University of Rome)*

Speakers:

Irene Bozzoni *(Rome)*
"Non-coding RNA: new functions and perspectives"

Davide Corona *(Palermo)*
"An RNA memory mechanism to inherit epigenetic marks"

Davide Gabellini *(Milan)*
"Chromatin-associated ncRNAs as epigenetic regulators in muscular dystrophy"

Ugo Ala *(Turin)*
"The ceRNA world: a new way of looking at the role of different RNA molecules"

Ernesto Picardi *(Bari)*
"De novo detection of A-to-I RNA editing in human brain and spinal cord by RNA-Seq technology"

11:00 - 11:30 **Coffee Break**

Parallel Symposia

11:30 - 13:30 **1. Parasitism and symbiosis** (Edificio Vecchio di Fisica, Aula Amaldi)

Chairs:

Sergio Pimpinelli *(Sapienza, University of Rome)*, Bianca Colonna *(Sapienza, University of Rome)*

Speakers:

Eugene Rosenberg *(Tel Aviv, Israel)*
"The Hologenome Concept: Role of Microorganisms in the Adaptation and Evolution of Animals and Plants"

Carlotta De Filippo *(Trento)*
"The impact of diet in shaping human gut microbiota: what we can learn from Africa"

Daniele Daffonchio *(Milan)*
"Microbial symbionts: a resource for the management of insect-related problems"

Paola Bonfante *(Turin)*
"An ancient plant-fungal symbiosis: origin and evolution of arbuscular mycorrhizas"

11:30 - 13:30 **2. Molecular mechanisms of DNA Damage Response** (Matematica, Aula III)

Chairs:
Francesca Pacchierotti *(ENEA, Rome)*, Margherita Bignami *(ISS, Rome)*

Speakers:

Gisela Taucher-Scholz *(Darmstadt, Germany)*
"Spatiotemporal dynamics of DNA damage sites in the context of chromatin"

Simona Giunta *(New York, USA)*
"Repeat the repeats: Stability of the centromere in cancer and aging"

Marco Foiani *(Milan)*
"ATR –mediated mechanosensing of topological tension"

Fabrizio d'Adda di Fagagna *(Milan)*
"Molecular mechanisms of cellular senescence"

13:30 - 15:30 **Poster viewing [topics 13-24] & Lunch**

Mini Symposia Session II

15:30 - 16:45 **13. Neurobiology** (Edificio Vecchio di Fisica, Aula Amaldi)

Chairs:
Alberto Chiarugi *(University of Florence)*, Andrea Mele *(Sapienza, University of Rome)*

Elena Ambrosini *(Rome)*
A new cellular model to disclose Megalencephalic Leukoencephalopathy with subcortical Cysts (MLC) pathological mechanisms

Maria Egle De Stefano *(Rome)*
Responsiveness to NGF is reduced in sympathetic neurons of mdx mice, affecting axon outgrowth and regeneration both *in vivo* and *in vitro*

Christina Orru *(Hamilton, USA)*
PcG mediated higher order chromatin structures modulate replication programmes at the Drosophila BX

Federica Sandrelli *(Padua)*
2mit, an intronic gene of timeless2, is involved in memory formation of *Drosophila melanogaster*

Ada Maria Tata *(Rome)*
M2 muscarinic receptor activation contributes to modulate Schwann cell migration and differentiation in myelinating phenotype

16:45-18:00 **14. Cell communication, signal transduction and membrane trafficking** (Edificio Vecchio di Fisica, Aula Amaldi)

Chairs:
Bianca Zani *(University of L'Aquila)*, Claudia Verderio *(CNR, Milan)*, Letizia Lanzetti *(IRCC, Candiolo)*

Letizia Astrologo *(Rome)*
High-throughput analysis of downstream effects of activating Gsα mutations in skeletal progenitor/stem cells

Martina Gabrielli *(Milan)*
Microvesicles released from microglia stimulate excitatory synaptic activity via enhanced sphingolipid metabolism

Davide Gnocchi *(Rome)*
Effects of 3,5-Diiodothyronine (3,5-T2) on lipid accumulation and insulin signaling in a rat model of Non Alcoholic Fatty Liver Disease (NAFLD)

Chiara Paparella *(Rome)*
The LysM receptor-like kinase AtLYK3 negatively regulates defense responses in Arabidopsis thaliana

Simonetta Santini *(Rome)*
ATM kinase modulates ITCH E3 ubiquitin ligase activity

15:30-16:45 **15. Oncogenes and tumour suppressors** (Genetica, Aula Montalenti)

Chairs:
Silvia Soddu *(Regina Elena Cancer Institute, Rome)*, Giuseppe Palumbo *(University of Naples)*

Laura Antonucci *(Rome)*
Hedgehog signaling controls IRES-dependent translation through a CNBP/Sufu complex

Eros Di Giorgio *(Udine)*
Oncogenic properties of the MEF2-HDAC4 axis

Giulia Fianco *(Rome)*
Role of src dependent phosphorylation on tyr380 of caspase 8 in tumorigenesis

Beatrice Salvatori *(Rome)*
The microRNA-26a target E2F7 sustains cell proliferation and inhibits monocytic differentiation of acute myeloid leukemia cells through control of p21CIP1/WAF1 expression

Davide Valente *(Rome)*
HIPK2 in the control of genome stability: a new mechanism in tumorigenesis

16:45-18:00 **16. Stem cells, IPS, cancer stem cells** (Genetica, Aula Montalenti)

Chairs:
Alessandro Rosa *(Sapienza, University of Rome)*, Lucia Di Marcotullio *(Sapienza, University of Rome)*

Cesare Gargioli *(Rome)*
Autologous progenitor cells in a hydrogel form a supernumerary and functional skeletal muscle *in vivo*

Alessandro Rosa *(Rome)*
Generation of patient-specific iPS cells to provide an in vitro model system of Amyotrophic Lateral Sclerosis (ALS)

Tommaso Russo *(Naples)*
miR-125a regulates mouse ESC differentiation

Antonio Simeone *(Naples)*
Otx2 is an intrinsic determinant of the Embryonic Stem Cell state and is required for differentiation to a stable Epiblast Stem Cell condition

Daniela Tosoni *(Milan)*
The Numb/p53 pathway controls mode of division and tumorigenic potential of normal and tumor mammary stem cells

15:30-16:45 **17. Host-pathogen interaction** (Edificio di Botanica, Aula Giacomini)

Chairs:
Antonio Mastino *(University of Rome, Tor Vergata)*, Paolo Visca *(Roma TRE University)*

Valeria Ciancarella *(Rome)*
Modulation of humoral innate immune system during the pathogenesis of *Shigella flexneri*

Matteo Gravino *(Viterbo)*
Shared and distinctive features in plant response to damage- and pathogen-associated molecular patterns

Francesco Imperi *(Rome)*
An old drug suppresses *Pseudomonas aeruginosa* pathogenicity by inhibiting pyoverdine-regulated virulence gene expression

Lorenza Tulli *(Siena)*
Identification of a novel surface exposed *Clostridium difficile* protein potentially involved in the colonization of intestinal mucosa

Luca Zinzula *(Cagliari)*
Biochemical characterization of recombinant Ebola virus VP35 homo-oligomeric profile and in silico 3D modeling of its N-terminal coiled-coil oligomerization domain

16:45-18:00 **18. Plant development and diseases** (Edificio di Botanica, Aula Giacomini)

Chairs:
Franco Faoro *(University of Milan)*, Ida Ruberti *(CNR, Rome)*

Abdellah Ahou *(Rome)*
An *Arabidopsis* polyamine oxidase undergoing proteasomal regulation

Alessandra Boccaccini *(Rome)*
DAG1 and GAI shared functions in light-mediated seed germination

Simone Ferrari *(Rome)*
Role of pectin composition in plant growth

Nora Gigli Bisceglia *(Rome)*
An *Arabidopsis* MAPKKK gene family involved in plant developmen

Fiorella Lo Schiavo *(Padua)*
Programmed cell death induced by high levels of cytokinin in *Arabidopsis* cultured cells is mediated by the cytokinin receptor CRE1/AHK

Paolo Trost *(Bologna)*
AIR12, a b-type cytochrome of the plasma membrane of *Arabidopsis thaliana* is a negative regulator of resistance against *Botrytis cinerea*

15:30-16:45 **19. Plant metabolism and environmental stress** (Edificio Nuovo di Chimica, Aula II)

Chairs:
Giuseppe Forlani *(University of Ferrara)*, Marcello Iriti *(University of Milan)*

Sofia Caretto *(Lecce)*
Effects of cyclic and linear oligosaccharides on artemisinin metabolism in *Artemisia annua* L. cell cultures

Vittoria Locato *(Rome)*
Ophiobolin A effect on cell cycle is mediated by alteration in GSH fluxes among cell compartments

Erica Mica *(Pisa)*
Identification of microRNAs controlling leaf cell development during drought stress in *Brachypodium distachyon*

Chiara Pagliarani *(Grugliasco)*
Effects of stress on miRNA abundance in grapevine

Daniela Trono *(Foggia)*
Possible role of a mitochondrial phospholipase A2 activity in durum wheat (*Triticum Durum* Desf.) response to hyperosmotic stress mediated by activation of the dissipative systems

16:45-18:00 **20. Plant nutrition** (Edificio Nuovo di Chimica, Aula II)

Chairs:
Graziano Zocchi *(University of Milan)*, Gianattilio Sacchi *(University of Milan)*

Simona Carfagna *(Naples)*
O-acetyl-L-serine(thiol)lyase activity reveals the sulphur status in algal cell

Roberto De Michele *(Palermo)*
Watching a protein at work, or how an ammonium transporter was made sensor

Sergio Esposito *(Naples)*
Redox regulation and dependence of oligomeric state of *Populus trichocarpa* plastidic P2-glucose-6P dehydrogenase (*Pt*P2-G6PDH) by pH

Laura Zanin *(Udine)*
Cloning and heterologous expression of the urea transporter ZmDUR3 in *Zea mays*

Liliana Tato *(Milan)*
Integrated responses of Parietaria judaica to iron deficiency conditions

15:30-16:45 **21. Protein synthesis, degradation and homeostasis** (Fisiologia Generale, Aula A)

Chairs:
Giovanna Serino *(Sapienza, University of Rome)*, Fabrizio Loreni *(University of Rome, Tor Vergata)*

Davide Esposito *(Naples)*
Human rpL3 induces mitochondrial apoptosis in Calu-6 cells through activation of p21 expression

Davide Mainieri *(Milan)*
Mecchanism of insoluble protein body formation by the maize storage protein γ-zein

Marilena Mancino *(Milan)*
Cap dependent translation contributes to viability and resistance of myeloma cells to bortezomib

Emanuela Pedrazzini *(Milan)*
The enigmatic, putative potassium channel AtKCO3 of *Arabidopsis* tonoplast

Lisa Ulbrich *(Rome)*
Mutations in Neuroligin 3 and activation of an ER stress response

16:45-18:00 **22. Environmental and molecular mutagenesis** (Fisiologia Generale, Aula A)

Chairs:
Claudia Bolognesi *(IST, Genoa)*, Eugenia Dogliotti *(Istituto Superiore di Sanità, Rome)*

Pasqualina Colasuonno *(Bari)*
Induced mutations for the Lycopene cyclase ε genes by TILLING in durum wheat

Elisa Coluzzi *(Rome)*
Role of telomeres on chromosome instability induced by oxidative stress in human primary fibroblast

Sebastiano Di Bucchianico *(Pisa)*
Size-independent cytotoxicity and size-dependent genotoxicity of AuNP in murine alveolar macrophages, evaluated by two different dose-metrics

Francesca Mussi *(Parma)*
Characterization of the sensitivity of HT29 cell line to different chemoprotective phytochemicals

Roberto Petrillo *(Rome)*
Preliminary results on mutagenic effects of exposure to 900 MHz Radiofrequency radiation: enhancement of SAR increases the MN induction in exposed root cells of *Vicia faba*

15:30-16:45 **23. Regulation of transcription** (Matematica, Aula III)

Chairs:
Giorgio Dieci *(University of Parma)*, Vincenzo Scarlato *(University of Bologna)*

Maria Cristina Bosio *(Parma)*
Transcriptional regulatory proteins binding to the promoters of ribosome biogenesis genes in *Saccharomyces cerevisiae*

Luca Fagnocchi *(Siena)*
The NadR regulon: adhesins and diverse meningococcal functions are regulated in response to physiologically relevant signals

Valeria Lucci *(Naples)*
AMOTL2 interaction with TAZ causes the inhibition of surfactant proteins expression in lung cells

Jessica Marinello *(Bologna)*
Antisense non coding RNAs induced by topoisomerase I inhibition at CpG island promoters of human cells

Chiara Salvi *(Rome)*
Role of the CSN5 subunit of Cop9 signalosome in transcription modulation of genes involved in zinc and lipid metabolism in *S. cerevisiae*

16:45-18:00 **24. RNA biology** (Matematica, Aula III)

Chairs:
Alessandro Fatica *(Sapienza, University of Rome)*, Claudio Gualerzi *(University of Camerino)*

Enrico Baruffini *(Parma)*
Mutations of the mitochondrial-tRNA modifier *MTO1* cause hypertrophic cardiomyopathy and lactic acidosis

Stefano Dini Modigliani *(Rome)*
FUS/TLS can affect selected microRNA levels

Luca Giordano *(Lecce)*
Role of the Drosophila Fragile X gene, *dFMR1*, in the piRNA-mediated silencing of repetitive sequences

Alessandra Rogato *(Naples)*
Identification and characterization of diatom regulatory small non-coding RNAs by integrative computational and experimental analyses

Paola Zuccotti *(Milan)*
Study of trans-acting factors involved in the post-transcriptional regulation of CDK5R1

20:00 **Evening event**

"Giovani e Meritocrazia"

Thursday, September 27

9:00 - 10:00 **Plenary Lecture** (Aula Magna)

Pier Paolo Di Fiore *(Milan)*
"Connecting the machineries of cell fate determination and tumor suppression in breast stem cells"

10:00 - 10:30 **Coffee Break**

Parallel Symposia

10:30 - 12:30 **3. Innate immunity** (Edificio Vecchio di Fisica, Aula Amaldi)

Chairs:
Maria Lina Bernardini *(Sapienza, University of Rome)*, Angela Santoni *(Sapienza, University of Rome)*

Speakers:

Mathias Chamaillard *(Lille, France)*
"Revisiting paradigms on innate immune response to commensals"

Paul Schulze-Lefert *(Köln, Germany)*
"From taxonomic structure to functions of the bacterial Arabidopsis root microbiota"

Giulia De Lorenzo *(Rome)*
"Plant sensing and reacting to cell wall damage"

Bruno Lemaitre *(Lausanne, Switzerland)*
"The Drosophila gut: a new paradigm for epithelial immune response"

10:30 - 12:30 **4. Plant and microbe secondary metabolites: role in biotic and abiotic stress responses and evolution** (Matematica, Aula III)

Chairs:
Franco Faoro *(University of Milano)*, Annamaria Ranieri *(University of Pisa)*

Speakers:

Monika Schreiner *(Großbeeren/Erfurt, Germany)*
"Improving the formation of dietary secondary plant metabolites – especially glucosinolates – by elicitor applications"

Vincenzo Lattanzio *(Foggia)*
"Plant phenolics: some physiological and ecological aspects"

Pawel Bednarek (*Poznan, Poland)*
"Tryptophan-derived metabolites in the immunity of Brassicaceae species"

Francesco Vinale *(Naples)*
"Secondary metabolites involved in plant – beneficial microbe interactions"

Marcello Iriti *(Milan)*
"Melatonin, a newly focused metabolite in plant neurobiology and innate immunity"

12:30 **Congress closure & Zanichelli Prize award** (Aula Magna)

13:00 Armenise-Harvard Symposium (Aula Magna)

Chairs:

Valerio Orlando *(Dulbecco Telethon Institute (DTI) at IRCSS Fondazione Santa Lucia, Rome)*

Speakers:

Rosa Bernardi *(Milan)*
"The role of hypoxic response in leukemogenesis, a view from acute promyelocytic leukemia"

Rosangela Sozzani *(Pavia)*
"Transcription factor-mediated signaling pathway regulates stem cell fate in the Arabidopsis root"

Sabrina Sabatini *(Rome)*
"Spatial coordination between stem cell activity and cell differentiation in the root meristem"

Tiziana Bonaldi *(Milan)*
"Global quantitative proteomics reveals that miR17-92 dampens MYC- gene expression activity in established B-cell lymphomas"

Rosella Visintin *(Milan)*
"The Cdc14 phosphatase and Cdc5 kinase ensure anaphase onset"

16:30 Meeting of the Italian EMBO Members

(Edificio di Botanica, 1° floor, Aula Marini Bettolo)

PLENARY LECTURE

PL.1
Epigenetics; a new type of Genetics or more of the same?

Denis Duboule
University of Geneva, Geneva, Switzerland
Federal Institute of Technology (EPFL), Lausanne, Switzerland

Ever since the proposal of epigenetic landscapes was put forward by Conrad Waddington, the word 'epigenetic' has been used with different meanings, in different contexts. Clearly, the original use of this term was to overcome a lack of understanding of the question associated with what we could refer to as the problem of 'genomic equivalence' and cellular differentiation, including the idea of irreversibility, as suggested by the decreasing altitude of the landscape.
Nowadays, such landscapes can be reversed by reprogramming cells, which involves modifications in several 'epigenetic' marks, a term that refers to either the addition or the subtraction of molecules at the surface of either DNA or histones. While the use of the same term to qualify various phenomenon is not in itself a problem, it is important to clarify what we are talking about whenever wordings like 'epigenetic inheritance' or 'epigenetic regulation' are used.
Waddington clearly believed in a genetic basis for his epigenetic landscapes, and even the role of external forces to build the landscapes can now be interpreted *via* signaling pathways or localized signals, which progressively constrain cell fates. The fact that chromatin or DNA modifications may be parts of these mechanisms does not mean that they have no genetic origins.
Over the past few years, these 'epigenetic modifications' have been potentially associated with the (fixed) inheritance of characters, which would then not be solely based on the DNA sequence. The view that the environment can impact upon our transmitted characters without modifying our genetic program is traditionally well accepted, outside the scientific community, yet a clear demonstration of its validity is still lacking.

PL.2
Rethinking herpes simplex virus: the way to oncolytic agents

Gabriella Campadelli-Fiume, L. Menotti, C. de Giovanni, P. Nanni, P. L. Lollini
Department of Experimental Pathology, Section on Microbiology and Virology, Alma Mater Studiorum - University of Bologna, Italy

Oncolytic viruses infect, replicate in and kill cancer cells. They must be modified relative to their wt counterparts, so as to achieve cancer-specificity and spare the cells usually targeted by the wt-virus. Herpes simplex virus (HSV) has emerged as a most promising candidate because a number of properties, including moderate pathogenicity in humans; it is amenable to attenuation and tropism retargeting; the ample genome provides space for heterologous genes; specific antiviral therapy is available in a worst case scenario. In the first and second generation oncolytic-HSVs (oHSVs), currently in clinical trials, safety was achieved at the expenses of attenuation, and tumor specificity rested in low PKR activity of the tumor tissues. Our laboratories have developed novel, more potent and highly tumor-specific o-HSVs. The strategy is genetic engineering of HSV and modification of the viral tropism through retargeting to cancer-specific receptors. We have identified two site in the receptor-binding glycoprotein gD where we can engineer ligands to the targeted cancer-specific HER-2 receptor, overexpressed in mammary and ovary tumors. In preclinical studies the HER-2-retargeted HSV exerts antitumor efficacy in mice xenografted with human cancers. The challenging issues to be discussed centre on efficacy following systemic routes of administration; efficacy against the highly malignant glioblastoma; retargeting to additional cancer receptors.

PL.3
Inside the role of the FANC pathway: how ensure DNA replication and chromosomes integrity across cell cycle

Filippo Rosselli
UMR 8200 CNRS, Laboratoire «Stabilité Génétique et Oncogenèse», Institut de Cancérologie Gustave Roussy, 114 rue Edouard Vaillant, 94805 Villejuif, France

Fanconi anemia (FA) is a rare hereditary chromosome instability (CIN) syndrome characterized by progressive bone marrow failure and cancer predisposition. Cells derived from FA patients are hypersensitive to DNA-crosslinking agents and exhibit chromosome abnormalities, including chromatid-type gaps and breaks, chromatid interchanges, radial figures, endoreduplication and chromosome gain or loss. The proteins encoded by the FA genes constitute the so-called FANC/BRCA pathway, involved in the control of both DNA replication and DNA damage response. Eight of the 15 FANC proteins so far identified form a large nuclear complex, called the FANC core complex. The only established function of the FANC core is to monoubiquitylate FANCD2 and FANCI in S-phase and in response to replication stress. Monoubiquitylated FANCD2 and FANCI relocalize to the chromatin, forming nuclear foci that co-localize with proteins involved in DNA damage tolerance and repair. The other FANC pathway components, BRCA2/FANCD1, BRIP1/FANCJ, PALB2/FANCN, RAD51C/FANCO, SLX4/FANCP work downstream FANCD2/FANCI in homologous recombination.
We will present and discuss some of our recent results on how the FANC pathway participates in preventing CIN and aneuploidy acting both during S phase and mitosis in both unperturbed cell cycle and in response to genotoxic insults.
First, during the S phase, the FANC pathway collaborates with several DNA damage response proteins, including ATR, BRCA1, MRE11, CHK1, BLM, PCNA as well as one or more TLS polymerases, to the maintenance and re-start of stalled replication forks. This action seems to be particularly important for fragile sites stability.
Secondly, during mitosis, it participates along with the structure-specific endonucleases ERCC1 and MUS81 and in tight collaboration with the BLM helicase in the resolution of DNA bridges that hamper proper chromosome segregation at ana-telophase.
Thus, the FANC pathway appears to be necessary to optimize the achievement of DNA replication and to ensure the transmission of fully repaired (or repairable) chromosomes to daughter cells.

PL.4
Connecting the machineries of cell fate determination and tumor suppression in breast stem cells

Pier Paolo Di Fiore
University of Milan and IFOM-IEO Campus, Milan Italy

Numb is a cell fate determinant that by asymmetrically partitioning at mitosis controls binary cell fate decisions. In human breast and lung cancers, there is frequent loss of Numb expression, due to its exaggerated ubiquitination and ensuing degradation. This causes alterations in two major downstream pathways. On the one hand, lack of Numb allows for unchecked signaling activity of the Notch receptor. On the other, lack of Numb causes attenuation of the p53 signaling pathway. Tumors cells displaying loss-of-Numb expression are addicted to this event and to its molecular consequences. Our recent results point to the mammary stem cell (MSC) compartment as the cellular "target" of Numb misregulation in breast tumors. We have developed a technology to cultivate and purify mammary stem cells. In normal MSC, Numb is asymmetrically partitioned at mitosis. This in turn dictates the replicative fate, in that the Numb(+) cell remains quiescent (and retains MSC capabilities), whereas the Numb(-) cell acquires a progenitor fate and undergoes rapid symmetric divisions. The control of Numb over MSC fate is executed

through the ability of Numb of silencing Notch signaling and maintaining high levels of p53 in the MSC. This latter event is due to Numb-mediated inhibition of the ubiquitinating function of the E3 ligase Mdm2 over p53. Lack of Numb in tumor MSC causes a switch form the asymmetric to the symmetric mode of division, thus forcing both daughter cells to assume the same replicative fate. Our understanding of how Numb is mechanistically involved in all these aspects, with particular emphasis to the Numb-Md2-p53 circuitry, will be discussed.

PLENARY SYMPOSIUM

PS1 - Stress, transposons and evolution

PS1.1

Vincent Colot
Institut de Biologie de l'Ecole Normale Supérieure, Paris
[No Abstract Received]

PS1.2
Establishment and maintenance of transposon silencing in the male germline

Donal O'Carroll
European Molecular Biology Laboratory, Mouse Biology Unit, Monterotondo, Roma

Piwi proteins and piRNAs have conserved functions in transposon silencing. The murine Piwi proteins Mili and Miwi2 direct epigenetic LINE1 (L1) and intracisternal A particle (IAP) transposon silencing during genome reprogramming in the embryonic male germline. Piwi proteins are proposed to be piRNA-guided endonucleases that initiate secondary piRNA biogenesis. To investigate the role of Piwi-catalyzed endonucleolytic activity, we engineered point mutations in the mouse that substitute the second D to an A in the catalytic triad (DDH) of Mili and Miwi2, generating the *Mili*^{DAH} and *Miwi2*^{DAH} alleles, respectively. Analysis of Mili-bound piRNAs from homozygous *Mili*^{DAH} fetal gonadocytes revealed the failure of transposon piRNA amplification resulting in the stark reduction of piRNA bound within Miwi2 ribonuclear particles (RNPs). We find that Mili-mediated piRNA amplification is selectively required for L1 but not IAP silencing. The defective piRNA pathway in *Mili*^{DAH} mice results in spermatogenic failure and sterility. Surprisingly, homozygous *Miwi2*^{DAH} mice are fertile, transposon silencing is established normally and no defects in secondary piRNA biogenesis are observed. We conclude that cycles of intra-Mili secondary piRNA biogenesis fuel piRNA amplification that is absolutely required for L1 silencing. Further adventures in the exploration of the piRNA pathway will also be presented.

PS1.3
Epigenetic regulation of L1 elements mobilization in health and disease

B. Bodega, F. Della Valle[1], V. Saccone[2,3], S. Consalvi[2,3], J. Martone[4], V. Cazzella[4], C. Mozzetta[2], M. Mora[5], P. Carninci[6], I. Bozzoni[4], P. L. Puri[2,3], **Valerio Orlando**[1]
[1]Dulbecco Telethon Institute (DTI) at IRCSS Fondazione Santa Lucia, Epigenetics and Genome reprogramming, Rome, Italy, [2]Dulbecco Telethon Institute (DTI), IRCCS Fondazione Santa Lucia, Rome, Italy, [3]Sanford-Burnham Institute for Medical Research, La Jolla, USA, [4]Department of Biology and Biotechnology "Charles Darwin", Sapienza University of Rome, Italy, [5]Division of Neuromuscular Diseases and Neuroimmunology, Istituto Nazionale Neurologico 'C. Besta', Milano, Italy, [6]RIKEN Yokohama Institute, Omics Science Center, Yokohama, Kanagawa, Japan

Epigenetic regulation and impact of repetitive elements in human genome function and disease is largely unknown. Recent reports indicate that LINE-1 (L1) occurs in brain cells and defects in their mobilization and epigenome regulation are associated with neurological disorder. Whether somatic L1 retrotransposition regulation could contribute to other differentiation programs and impact non-neurological, diseases is currently unexplored.
Duchenne muscular dystrophy (DMD) is a genetically well defined disease being associated with mutations in the dystrophin gene. Increasing evidence indicates that disruption of the dystrophin-associated protein complex (DAPC) at the sarcolemma affects not only the structure of muscle fibers[1], but impact global genome expression (coding and non coding transcripts) through deregulation of the nNOS-HDAC2 pathway. Here we show that during skeletal myogenesis LINE-1 (L1) transcription and copy number variation (CNV) are dynamically regulated while in DMD cells HDAC2 is aberrantly recruited at L1 promoter and their mobilization is impaired, suggesting a role for L1 activity in muscle cell differentiation. Indeed, we found that inhibition of L1 mobilization by reverse transcriptase impaired the differentiation ability of normal human primary muscle cells. Notably, functional restoration of dystrophin-nNOS-HDAC2 signaling and fiber functionality by HDAC inhibitors or dystrophin re-expression by exon-skipping could restore normal L1 expression levels and CNV either in the *md*x mice and in DMD primary muscle cells. We propose that repetitive elements could be potential players in myogenesis, and their deregulation a key epigenetic trait in muscular dystrophy manifestation.

PS1.4
Transposons and the evolution of evolvability

Sergio Pimpinelli
Sapienza, Università di Roma

After Darwin's book on the origin of species by natural selection, the theory epoused by his predecessor Lamarck was never completely abandoned. To explain, some of the apparent Lamarckian-like phenomena in a Darwinian sense, Waddington elaborated the "canalization and assimilation" concepts (Waddington, Nature, 1959). He hypothesised the existence of a cryptic genetic variation that is maintained hidden due to the robustness of the developmental process that he indicated as "canalization". If an environmental stress is strong enough to overcome this robustness, cryptic genetic variants can be expressed and become heritable by an "assimilation" process.
In past years, it has been observed that in fliesand plants, when the activity of Hsp90 is reduced, a wide spectrum of phenotypic variants is induced. The interpretation was that Hsp90 is a "capacitor" of morphological evolution and buffers a cryptic genetic variation that accumulates in neutral conditions.
However, other studies have suggested that Hsp90 could acts as a "mutator" through impairment of RNAi silencing. The reduction of Hsp90 causes stress response-like activation and transposition of mobile elements causing phenotypic variants.
This observation, suggests a mechanism for rapid evolutionary changes: the environmental changes play a direct active role on evolution of genomes by the induction of genetic variability, by means of transposons, and thus allowing for the possibility of selection of more adapted genotypes along with their more adaptive stress-response, i.e. transposons may also make evolvability evolvable.

PS2 - Synthetic Biology discovering new words and new world

PS2.1
Designing soil bacteria for environmental biocatalysis: then and now

Víctor de Lorenzo
Centro Nacional de Biotecnología, CSIC Madrid 28049, vdlorenzo@cnb.csic.es

A widespread tenet in the late 80s and early 90s was that genetically engineered microorganisms (GEMs) could be designed in the Laboratory to remediate every imaginable environmental problem. This paradigm has however failed. Systemic analyses of soil bacteria have revealed that most pollutants are sensed not only as nutrients-to-be but, predominantly, as stressors. This often makes the bulk of the gene expression machinery to be spent in enduring stress instead of metabolizing the target compounds. The bottleneck for effective biodegradation is not the lack of an enzyme or a regulator, but the stress that bacteria undergo during exposure to and metabolism of the target chemical. This shifts the genetic engineering emphasis from discrete enzymatic bottlenecks to refactoring large portions of the metabolic and stress-response networks of the host cells. Specially, reinforcement of the physiological blocks that produce reductive power (in particular NADPH) is necessary to cause a positive balance of biodegradation *vs.* stress. In this context, the current onset of Synthetic Biology provides new avenues to entertain a generation of GEMs 2.0 for non-contained environmental applications.

PS2.2
Programmable Biology: Synthetic Approaches to Transcriptional Regulation and Cellular Microenvironments

Ahmad S. Khalil
Biomedical Engineering Department, Boston University

Synthetic biology is redefining the discipline of biology, helping us to understand how organisms behave and develop through the forward engineering of molecular circuitry with well-understood genetic components. In this talk, I will highlight advances in synthetic biology that have helped us to uncover biological design principles and to bring about practical applications in biotechnology and industry. I will discuss new tools being developed in my lab that are addressing recent challenges in the field and enabling new levels of cellular programmability. I will describe a synthetic framework for designing and assembling transcriptional components and circuits, which allows us to systematically program complex transcriptional functions in eukaryotes. I will also briefly describe a programmable microfluidics system that enables the automated and multiplexed manipulation of cells and small liquid volumes for more sophisticated control of cellular microenvironments and systems-level experimentation.

PS2.3
Stochastic modeling of expression kinetics identifies messenger half-lives and reveals sequential waves of co-ordinated transcription and decay

F. Cacace[1], **Paola Paci**[1,2], V. Cusimano[1], A. Germani[3], L. Farina[4]
[1]Università Campus Biomedico, via Alvaro del Portillo 21, 00128 Rome, Italy, [2]CNR-Institute of Systems Analysis and Computer Science (IASI), BioMathLab, viale Manzoni 30, 00185 Rome, [3]Dipartimento di Ingegneria Elettrica e della Informazione, Universit_a de L'Aquila [4]Dipartimento di Informatica e Sistemistica, Sapienza Universit_a di Roma

The transcriptome in a cell is finely regulated by a large number of molecular mechanisms able to control the balance between mRNA production and degradation. Recent experimental findings have evidenced that fine and specific regulation of degradation is needed for proper orchestration of a cell global response to different environmental conditions. We developed a computational technique based on stochastic modeling, to infer condition-specific individual mRNA half-lives directly from gene expression time-courses. Predictions from our method were validated by experimentally measured mRNA decay rates during the intraerythrocytic developmental cycle of Plasmodium falciparum. We then applied our methodology on publicly available data for the reproductive and metabolic cycle of budding yeast. Strikingly, our analysis revealed, in all cases, the presence of periodic changes in decay rates of sequentially induced genes and co-ordination strategies between transcription and degradation, thus suggesting a general principle for the proper coordination of transcription and degradation machinery in response to internal and/or external stimuli.

PS3 - Non-coding RNA in cell function and diseases

PS3.1
Non-coding RNA: new functions and perspectives

Irene Bozzoni
Dept. of Biology and Biotechnology, Sapienza, University of Rome

One of the greatest surprises of high throughput transcriptome analysis of the last years has been the discovery that the mammalian genome is pervasively transcribed into many different complex families of RNA. It is now becoming largely accepted that the non-coding portion of the genome rather than its coding counterpart is likely to account for the greater complexity of higher eukaryotes. In addition to a large number of alternative transcriptional start sites, termination and splicing patterns, a complex collection of new antisense, intronic and intergenic transcripts was found. Small non-coding RNA have been extensively studied and shown to be key components of regulation and control in many eukaryotes including animals and plants. While they were initially described as negative switches acting with transcription factors to control of gene expression, these RNAs are now seen as modulators or fine tuners of posttranscriptional regulation that are often components of negative or positive feedback loops.
Although many studies have helped unveiling the function of many small non-coding RNAs, very little is known about the long non-coding (lncRNA) counterpart of the transcriptome.
Examples of regulatory circuitries regulated by both small and long non-coding RNAs will be presented and perspectives on their new functions in the control of gene expression will be discussed.

PS3.2
An RNA Memory Mechanism to Inherit Epigenetic Marks

M. C. Onorati, W. Arancio, **Davide F.V. Corona**
Dipartimento STEMBIO – Sezione Biologia Cellulare, Dulbecco Telethon Institute c/o Università degli Studi di Palermo, Palermo – ITALY

A central question in epigenetics is to understand how, terminally differentiated daughter cells can inherit complex patterns of chromatin modifications from their mother cell. Even if several mechanisms have been hypothized to explain the establishment and maintenance of cell identity, it is still unclear how during mitosis covalent and ATP-dependent chromatin modifications are transmitted after DNA replication. Indeed, a simple way for daughter cells to restore the transcriptional profile of mother cells is to directly 'sense' the transcriptome of their mother cells. In order to unveil the molecular nature of somatic cell epigenetic memory, we used classic Position Effect Variegation assays to check if non functional alleles of the *white* gene could modify the eye color variegation caused by an heterochromatin inversion of the *white* gene called *white-mottled 4* (w^{m4h}). Our data show that several *white* alleles suppress the variegation of the w^{m4h} line. Unexpectedly, the presence of *white* alleles causes an increase in the *white* gene transcript as well as an opening in the chromatin structure at the w^{m4h} locus. Remarkably, this effect is inheritable, a phenomenon highly reminiscent of RNA mediated paramutation.
The changes in the levels of expression of the w^{mh4} gene, induced *in trans* by several *white* alleles, indicate that the presence of a non functional gene that does not produce a coding transcript but potentially only ncRNA, could influence *in trans* the expression of a functional copy of the same gene silenced by heterochromatin. Our data indirectly indicates that cells can 'sense' the presence of non coding RNA's inherited from their mother cells and can use them to epigenetically reset their transcriptional program after DNA replication.

PS3.3
Chromatin-associated ncRNAs as epigenetic regulators in muscular dystrophy

Davide Gabellini
Dulbecco Telethon Institute and Division of Regenerative Medicine, San Raffaele Scientific Institute, Milano, Italy

Repetitive sequences account for more than 50% of the human genome but are largely ignored. Recent results indicate that a significant portion of the epigenetic modifications is present in repeats and that these regions are transcribed producing ncRNAs, frequently in a tissue-specific fashion.
Facioscapulohumeral muscular dystrophy (FSHD) is an autosomal dominant disease associated to reduction in the copy number of the D4Z4 repeat mapping to 4q35. We have recently found that the Polycomb group of epigenetic repressors targets D4Z4 in healthy subjects and that D4Z4 deletion is associated with reduced Polycomb silencing in FSHD patients. We have identified *DBE-T*, a chromatin-associated non-coding RNA produced by the FSHD locus selectively in FSHD patients. *DBE-T* coordinates chromatin conformation and de-repression of 4q35 genes leading to FSHD.
Our work provides insights into the biological function of repetitive sequences in regulating gene expression and shows how mutations of such elements can influence the progression of a human genetic disease.

PS3.4
The ceRNA world: a new way of looking at the role of different RNA molecules

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The discovery of many different types of RNA molecules, from the most known protein-coding to the broad non-coding class of RNAs had led to an astonishing view of the transcriptome complexity: this concept was further deepened by introducing the possibility of cross-regulation among different RNA molecules acting as competitive endogenous RNAs (ceRNAs). Messanger RNAs, transcribed pseudo-genes and long non-coding RNAs are targets of miRNAs and can display the ability to talk to each-other by sequestering shared miRNAs. Besides, ceRNAs are organized in vast and complex regulatory networks (ceRNETs) that cooperate with all the other layers of regulation to orchestrate the living cell behavior. These ceRNETs are characterized by a great number of microRNAs and ceRNAs involved. Each ceRNA displays different patterns of microRNA Response Elements (MREs) allowing different efficiency in cross-regulation, depending on the level of available molecules. In this respect an important challenge is to develop tools to accurately predict putative ceRNAs and to investigate their role in tumorigenesis and cancer progression.

PS3.5
De novo detection of A-to-I RNA editing in human brain and spinal cord by RNA-Seq technology

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RNA editing is a molecular phenomenon in which primary transcripts are modified at specific positions. In human it cooperates with alternative splicing in increasing both proteomic and transcriptomic complexity and modulating gene expression. Many events have been identified up to now by next generation sequencing technologies employing massive transcriptome and genome sequencing. However, genome and transcriptome reads from single human individuals are not always available. In contrast, a lot of RNA-Seq data are housed in public databases and provide a source of yet unexplored RNA editing sites.

Here we present a simple computational strategy to identify de novo RNA editing sites in RNA-Seq data. Our strategy is based on a double mapping procedure and the Fisher's exact test to detect statistically significant base conversions.

When applied to the SRA study SRA012427 from human brain we found 19 highly significant A-to-I conversions in known human coding regions. Interestingly, 11 of such changes have been already described in literature and Sanger sequencing experimentally confirmed 6 of them. We also used our strategy in a RNA-Seq experiment from human spinal cord confirming 12 out of 15 RNA editing candidates by whole exome sequencing carried out on the same individual and tissue.

PARALLEL SYMPOSIA

S1 - Parassitism and symbiosis

S1.1
The Hologenome Concept: Role of Microorganisms in the Adaptation and Evolution of Animals and Plants

Eugene Rosenberg
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Animals and plants evolved from prokaryotes and have remained in close association with them. The hologenome theory of evolution posits that a unit of selection in evolution is the holobiont (host plus symbionts). The hologenome is defined as the genetic information of the host and its microbiota, which function in consortium. Genetic variation of the holobiont, the raw material for evolution, can arise from changes in either the host or the symbiotic microbiota genomes. Changes in the hologenome can occur by three processes that are specific to holobionts: microbial amplification, acquisition of novel strains from the environment, and horizontal gene transfer. Recent data from culture independent studies provides considerable support for the hologenome theory: (i) all animals and plants contain abundant and diverse microbiota, (ii) the symbiotic microbiota affects the fitness of their host, and (iii) symbiotic microorganisms are transmitted from parent to offspring. As acquired characteristics (microbes) are heritable, consideration of the holobiont as a unit of selection in evolution leads to neo-Lamarckian principles within a Darwinian framework.

S1.2
The impact of diet in shaping human gut microbiota: what we can learn from Africa

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NGS technologies are opening new frontiers in investigating human metagenome variation. We present results on how differences in diet and environment shaped gut microbial ecology in Europeans and Africans. We characterized the fecal microbiota of African children from Burkina Faso living in a rural village with a diet predominantly vegetarian, versus those living in an urban area, that maintains the consumption of cereals and legumes but introduces protein rich food and European children living in an urban area with a typical western diet (Italy). The first key finding is that diet is the dominant factor in shaping gut microbiota. Burkina children from the rural village (BFR) are differentiated but closely related to the urban (BFU). All the Burkina gut microbiota profiles (BFR & BFU) cluster separately from EU. The second key finding is that BFR metagenome was significantly enriched in distinctive bacterial genera (*Xylanibacter*, *Prevotella*, *Treponema*) that might help to extract energy from the plant polysaccharides (abundant in the BFR children's fiber rich diet) while protecting them from inflammatory gut diseases. At the same time BFR also had decreased numbers of well-known pathogens compared with BFU and EU. The third key finding is that short chain fatty acids levels (SCFAs) are statistically much higher in Burkina children respect the European ones, and associated to the presence of sequences encoding for the enzymes needed to digest these fibers. Interestingly SCFAs decrease in BFU respect to BFR probably due to depletion of SCFAs producing species, such as *Xylanibacter*. The observed different bacterial compositions are likely to have profound influences on the immune system, possibly explaining the absence of inflammatory bowel diseases, and allergies in African children and adults.

S1.3
Microbial symbionts: a resource for the management of insect-related problems

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Insects affect human life in multiple ways, negatively by parasitizing humans, animals and plants and by transmitting diseases, and positively by acting for instance as pollinators or pest parasitoids. The body-associated microbiome of insects, like those of humans and vertebrates, is a complex community of species adapted to the host. Recent observations in higher animals and humans evidenced that the relative species disproportion of the native gut microbiome, a phenomenon known as intestinal dysbiosis, is linked to several diseases. Dysbiosis have been also evidenced in insects like in the sterilized males of the Mediterranean fruit fly Ceratitis capitata that decrease mating competitiveness due to an unbalanced microbiome. Dysbiosis manipulation represents a novel approach for controlling undesired traits linked to insects in a strategy named 'Symbiotic Control'. Here, it is discussed the importance of symbionts for the biology of insects and for insect control applications. Novel strategies of gut microbiome management based on dysbiosis manipulation are presented for improving insect health of pollinators, or for counteracting pathogen transmission by insect vectors.

S1.4
An ancient plant-fungal symbiosis: origin and evolution of arbuscular mycorrhizas

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The arbuscular mycorrhizal (AM) symbiosis involves most of land plants and a number of soil fungi belonging to the ancient Phylum Glomeromycota. This mutualistic association dates back to more than 450Mya and was likely established before the root origin. In the today symbiosis, the fungus improves the mineral nutrition of the plant with the uptake of several nutrients (e.g. phosphate and nitrogen) from the soil, while the plant supplies its heterotrophic partner with sugars. The symbiosis is the result of a complex exchange of molecular information which commences in the rhizosphere before the partners become in physical contact, and continues during all the steps of the colonization process, when the fungus is accommodated inside the root cells
Molecular and genetic tools, coupled to high-throughput sequencing and advanced microscopy, have led to the genome sequencing, transcriptome analysis of some symbionts and detection of many underlying cell mechanisms. In the presentation some of these novel findings will be reviewed focussing on those (i.e. signalling pathways between plants and fungi, transcription factor-like determinants, systemic effects on fruit development) which illustrate how the AM symbiosis is at the basis of plant metabolism.

S2 - Molecular mechanisms of DNA Damage Response

S2.1
Spatiotemporal dynamics of DNA damage sites in the context of chromatin

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Efficient DNA repair mechanisms have evolved since faithful repair of DNA damage is essential for maintaining genome integrity. Ionizing radiation-induced DNA double-strand breaks (DSBs) are one of the most dangerous lesions with severe consequences for cell survival and chromatin stability. The local induction of DNA damage and biological imaging techniques have recently contributed to the dynamic visualization of the cellular DNA damage response to DSBs. Thus, the use of heavy ion irradiation to produce localized DSBs combined with live cell imaging of repair factors have revealed the spatiotemporal behaviour of DNA damage response proteins in real time at the level of single cells.
A mechanism to prevent potential chromosomal translocations arising from distant DNA ends in mammalian cells is the positional stability of DSB, i.e. the absence of long-range roaming of damage sites in the nucleus. On the other hand, in highly repetitive heterochromatic DNA compartments, a small-scale movement leading to the relocation of heterochromatic DSBs to euchromatic regions, where repair can proceed, may prevent potentially deleterious recombination events. This DSB motion could be related to the local DNA decondensation at the sites of ion-induced damage, as measured after targeted irradiation of heterochromatin in murine cells. Therefore, in addition to repair proteins, the participation of factors involved in the decondensation or remodelling of chromatin, as well as in the regulation of chromatin binding proteins have emerged to be important in the DNA damage response.

S2.2
Repeat the repeats: Stability of the centromere in cancer and aging

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Maintenance of genome integrity is essential for preserving cellular homeostasis, healthy longevity and viability. Genome stability must be maintained by accurate distribution of the genome during mitosis. The centromere, a spatially and epigenetically defined region of chromosomes, plays a pivotal role in the assembly of the kinetochore structure and capturing of microtubules, ultimately enabling faithful cell division. Each human centromere is composed of highly organized arrays of repetitive alpha satellite DNA. Extensive sequence homology of the repeats indicates that homologous recombination (HR) plays an active role in maintaining the repeat structure, but also implies that this process must be tightly regulated to avoid loss of centromeric DNA, which could result in kinetochore malfunctions and cause genome instability. To test this, we have utilized the CO-FISH technique to monitor centromere recombination in proliferating human cells. Our data indicate that sister chromatid exchanges and rearrangements at centromeres are frequently observed in cancer cells. We are currently investigating the underlying molecular mechanisms that maintain centromere integrity and examining its disruption during aging and tumorigenesis. Since the level of CENP-A, the centromere-specific histone H3 variant, is altered in cancer cells and during cellular senescence, we are also testing the effect of reduction or over-expression of CENP-A and other centromeric proteins on the stability of the centromere. The potential mechanisms by which the centromere integrity is compromised during senescence and cancer development will be discussed.

S2.3
ATR -mediated mechanosensing of topological tension

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Gene gating couples mRNA transcription with export through the nuclear pores, thus establishing physical links between transcribed chromatin and the nuclear envelope. Gated genes establish topological barriers that prevent replication fork progression. The Mec1/ATR checkpoint counteracts gene gating during S phase to resolve the topological barriers and allow fork progression across transcribed genes. Checkpoint mutants fail to release transcribed chromatin from the nuclear periphery, accumulate topological stress at incoming forks leading to fork reversal and unscheduled recombination events.
We studied the mechanisms sensing the collision between forks and gated genes and we provide evidence that ATR is able to sense the mechanical stress resulting from topological tension.

S2.4
Molecular mechanisms of cellular senescence

Fabrizio d'Adda di Fagagna
IFOM-IEO Campus, Milano

Early tumorigenesis is associated with the engagement of the DNA-damage checkpoint response (DDR). Cell proliferation and transformation induced by oncogene activation are restrained by cellular senescence. We have previously shown that expression of an activated oncogene in cultured normal human cells results in a permanent cell-cycle arrest caused by the activation of a robust DDR. Experimental inactivation of DDR abrogates senescence and promotes cell transformation. Oncogene-induced senescence is also associated with a global heterochromatinization of nuclear DNA. Our most recent results on the interplay between DDR and heterochromatin formation, the differential repair of the human genome, the regulation of DDR in stem cells and our search for novel pathways regulating genome stability will be discussed.

S3 - Innate immunity

S3.1
Revisiting paradigms on innate immune reponse to commensals

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The gut microbiome is composed of innumerable albeit highly diversified microorganisms that interact with epithelial lining throughout the entire life. Importantly, not all commensals have the same potential to instigate disease, referred herein as pathobionts, or alternatively to maintain homeostatic immunity also known as symbionts.
Dysbiosis has been associated with common human intestinal disorders with missing heritability, such as Crohn's disease (CD). Today millions of Europeans suffer from Crohn's disease (CD), which is a polygenic inflammatory bowel disease with far from optimal therapeutic management, as judged by the enhanced risk of immune adverse events from serious infections to colorectal cancer.
Recent studies, including ours, unveiled key negative regulators of intestinal tumorigenesis that license the microbiota against transmissible inflammation and tumorigenesis in the colon. During chronic inflammation, the innate immune system may facilitate colon tumorigenesis in genetically predisposed individuals in response to dysbiosis. In these individuals, therapeutic approaches that reroute the innate immune response to commensals might help correct dysbiosis before it leads to development of advanced neoplasia.

S3.2
From taxonomic structure to functions of the bacterial Arabidopsis root microbiota

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The plant root defines the interface between a multicellular eukaryote and soil, one of the richest microbial ecosystems on earth. We have developed and applied bacterial 16S rRNA gene pyrosequencing to characterize and compare soil and root-inhabiting bacterial communities of *Arabidopsis thalinana*. We show that roots of *Arabidopsis thaliana*, grown in different natural soils under controlled environmental conditions, are preferentially colonized by Proteobacteria, Bacteroidetesand Actinobacteria, and each bacterial phylum is represented by a dominating class or family. Soil type defines the composition of root-inhabiting bacterial communities and host genotype determines their ribotype profiles to a limited extent. The identification of soil type-specific members within the root-inhabiting assemblies supports our conclusion that these represent soil-derived root endophytes. Surprisingly, plant cell wall features of other tested plant species appear to provide a sufficient cue for the assembly of ~40% of the Arabidopsis bacterial root-inhabiting microbiota, with a bias for Betaproteobacteria. Thus, this root sub-community may not be Arabidopsis-specific but saprophytic bacteria that would naturally be found on any plant root or plant debris in the tested soils (Bulgarelli *et al*., in press). In contrast, colonization of Arabidopsis roots by members of the Actinobacteria depends on additional cues from metabolically active host cells. We have begun to systematically purify Arabidopsis bacterial endophytes on the basis of the culture-independent 16S rRNA gene survey. I will present experiments aimed to explore presumed root microbiota functions for plant growth using defined bacterial communities under laboratory conditions.

S3.3
Plant sensing and reacting to cell wall damage

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Like animals, plants have evolved a so-called innate immune system, in which germ line encoded receptors for non-self molecules play a key role, leading to the activation of the immune responses primarily at the site of infection and eventually in the whole plant. Also self molecules, released upon tissue injury caused by pathogens or wounding and indicated as damage-associated molecular patterns (DAMPs), are recognized as danger signals by the plant innate immune system. Oligogalacturonides (OGs), derived from homogalacturonan, the major pectic component of the plant extracellular matrix, are a well-known class of plant DAMPs. Recent insights into the OG signaling cascade will be discussed.

S3.4
The *Drosophila* gut: a new paradigm for epithelial immune response

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The gut combines and integrates very different physiological functions required for maintaining the equilibrium of the whole organism. In addition to its role in digestion, it is the main entry route for pathogens, and a reservoir for resident bacteria that must be tolerated. Finally, the intestinal epithelium undergoes a constant renewal required to maintain the integrity of this barrier. However, little is known about how these functions are regulated and coordinated, or what mechanisms are required to ensure gut homeostasis upon exposure to external challenges such as bacterial infection. Using an integrated approach, we are studying the mechanisms that make the gut an efficient and interactive barrier despite its constant interactions with microbes. We also focus our attention on the regulatory mechanisms that restore gut normal function upon challenge with bacteria. Our projects utilize integrated approaches to dissect not only the gut immune response, but also gut homeostasis and physiology in the presence of microbiota, as well as strategies used by entomopathogens to circumvent these defenses. We believe that the fundamental knowledge generated on *Drosophila* gut immunity will serve as a paradigm of epithelial immune reactivity and have broader impacts on our comprehension of animal defense mechanisms and gut homeostasis.

S4 - Plant and microbe secondary metabolites: role in biotic and abiotic stress responses and evolution

S4.1
Improving the formation of dietary secondary plant metabolites - especially glucosinolates - by elicitor applications

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Inverse associations between fruit and vegetable intake and chronic diseases, such as different types of cancer and cardiovascular disease, have been demonstrated in numerous epidemiological studies. Secondary plant metabolites have been indicated to be responsible for this observed protective effect.
Recently, a number of genotypic and ecophysiological studies demonstrated the potential to affect concentration and composition of various secondary plant metabolites in many vegetables species. Moreover, targeted chemical and/or physical elicitor applications can trigger distinct changes in the plant's secondary metabolism combined with the establishment of adapted plant-based production systems.
This presentation is focused on species of the plant order Brassicales which are especially characterized by a certain group of secondary plant metabolites - the glucosinolates. However, only for certain individual glucosinolates the evidence was found that their hydrolysis products induce health-promoting effects mainly due to their anti-carcinogenic properties. Thus, based on screening studies of Brassicales species and cultivars specific elicitor treatments are used to modify the biosynthesis and degradation process of these desired glucosinolates associated with corresponding gene expression studies.

S4.2
Plant phenolics: some physiological and ecological aspects

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'Plant phenolics' and 'polyphenols' are secondary natural metabolites arising biogenetically from either the shikimate/phenylpropanoid pathway, which directly provides phenylpropanoids, or 'polyketide' acetate/malonate pathway, which can produce simple phenols, or both, thus producing monomeric and polymeric phenols and polyphenols, which fulfil a very broad range of physiological roles in plants. Higher plants synthesize several thousand known different phenolic compounds. Plant phenolics are considered to have a key role as defence compounds when physiological and ecological constraints can lead to increased production of free radicals and other oxidative species in plants. Both biotic and abiotic stresses stimulate carbon fluxes from the primary to the secondary metabolic pathways, thus inducing a shift of the available resources in favor of the synthesis of secondary products. An interesting link between primary and secondary metabolism couples the accumulation of the stress metabolite proline with the energy transfer towards phenylpropanoid biosynthesis via the oxidative pentose phosphate pathway. The alternating oxidation of NADPH by proline synthesis and reduction of $NADP^+$ by the two oxidative steps of the oxidative pentose phosphate pathway lead to a simultaneous accumulation of phenolic compounds.

S4.3
Tryptophan-derived metabolites in the immunity of Brassicaceae species.

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One of the evolutionary conserved responses of flowering plants to pathogen attack involves biosynthesis and secretion of secondary metabolites. Model plant *Arabidopsis thaliana* accumulates upon infection tryptophan derived indole-type metabolites, including indole-3-carboxylic acids (I3CAs) and phytoalexin camalexin. Our recent study revealed in this plant species a pathogen triggered pathway for metabolism and secretion of tryptophan-derived indole glucosinolates (IG), which is critical for pre-invasive defence against a number of fungal and oomycete pathogens. Currently we investigate the conservation and diversification of the pathogen-inducible tryptophan-derived metabolism in close and distant *A. thaliana* relatives by metabolic profiling. We substantiate the observed species-specific metabolic patterns by the presence or absence of candidate ortholog genes encoding enzymes involved in tryptophan metabolism in accessible genomes of *A. thaliana* relatives. Our metabolic survey reveals a surprising conservation of the pathogen-triggered IG metabolic and secretory pathway between the tested plant species, suggesting an ancient and important function of this metabolic branch in Brassicaceae pre-invasive defence responses. In contrast, I3CA and camalexin biosyntheses appear to be clade-specific innovations within the conserved framework of pathogen-inducible tryptophan metabolism and represent relatively recent manifestations of the plant-pathogen arms race.

S4.4
Secondary metabolites involved in plant - beneficial microbe interactions

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The term "secondary metabolite" includes a heterogeneous group of chemically different natural compounds normally of low molecular weight and active in a variety of biological processes. Agriculture-relevant microbes typically produce these molecules during processes of competition with other micro- and macro-organisms, symbiosis, parasitism or pathogenesis. Many secondary metabolites have antibiotic properties, which enable the microbe to inhibit and/or kill other microorganisms (i.e. those competing for a nutritional niche). In fact, some of these compounds have been recently associated to the biological control of plant pathogens and pests, and they may represent, along with the producing microbes, potential alternative to chemical pesticides. The production of different secondary metabolites has been proposed, in some cases, to be related to the activity of various beneficial microbes used world-wide for crop protection and fertilization. Metabolomic analysis of the interactions between plants, fungal phytopathogens and beneficial fungi has aided in the identification of several bioactive fungal secondary metabolites that positively affect plant metabolism. Some of these compounds have direct activity against phytopathogens, but also increase disease resistance by triggering the plant defence system, and/or enhance root, stem, shoot and leaf vegetative growth, which may produce significant yield increases that are detectable in the field.

S4.5
Melatonin, a newly focused metabolite in plant neurobiology and innate immunity

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Melatonin, an indolic derivative of tryptophan, was long thought to be a vertebrate neurohormone, until its recent discovery in a multitude of non-vertebrate taxa. It was found in bacteria, unicellular eukaryotes, macroalgae, fungi and plants, and, now, it can be considered ubiquitous in living organisms. Among angiosperms, melatonin was detected in both mono- and dicotyledons, particularly in some medicinal and food plants, with a great inter- and intraspecific variability, also depending on the phenological stage, agro-meteorological conditions and stress

factors. Its (patho)physiological role(s) *in planta* have not been completely elucidated yet. The indoleamine seems to be involved in radical organogenesis, growth, reproduction, flowering and seed germination. It also exerts anti-senescence effects, regulates the cell oxidative homeostasis and its synthesis increases in response to priming with elicitor of the plant innate immunity. Both endogenous melatonin levels and treatments with this molecule have been correlated with enhanced tolerance to heavy metals, UV, atmospheric pollutant and salt stress. In general, the powerful antioxidant activity of melatonin seems to be responsible for the tolerance to those stresses involving the massive production of reactive oxygen species. Finally, the occurrence of melatonin in plant foods adds a new element in the field of nutritional sciences, contributing to explain the healthy potential of relevant Mediterranean traditional foods.

ARMENISE - HARVARD SYMPOSIUM

AH.1
The role of hypoxic response in leukemogenesis, a view from acute promyelocytic leukemia

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Hypoxia-inducible transcription factors (HIFs) are the main regulators of cellular and systemic adaptation to hypoxia, and are often up-regulated in solid tumors because of intra-tumoral hypoxia and activation of specific oncogenic pathways. In tumors, HIF activation triggers a complex series of adaptive responses ranging from induction of anaerobic metabolism, neo-angiogenesis, cell migration and maintenance of cancer stem cells. For this reason, pharmaceutical agents with HIF-inhibitory functions are beginning to be tested in phase I/II clinical trials for the treatment of solid tumors.
More recently, HIF factors are beginning to be implicated also in hematological malignancies. We investigated the role of HIF-1α in acute promyelocytic leukemia (APL), which is epitomized by the chromosomal translocation t(15;17) and the resulting oncogenic fusion protein PML-RARα. We found that PML-RARα functionally cooperates with the transcription factor HIF-1α, and sustains a number of HIF-mediated pro-leukemogenic functions. The implications of HIF inhibition for APL treatment will be discussed.

AH.2
Transcription factor-mediated signaling pathway regulates stem cell fate in the Arabidopsis root

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In multicellular organisms normal development requires appropriate patterning and specification of diverse cell types. The expression of specific sets of genes determines the fate of each particular cell type. In the Arabidopsis root, the transcription factors, SHORTROOT (SHR) and SCARECROW (SCR), regulate the formative divisions of the immediate progeny of the ground tissue stem cells, known as cortex/endodermis initials (CEI). These cell divisions are asymmetric as the daughter cells adopt distinct developmental fates, one giving rise to the cortex, the other to the endodermis. To understand the specific context and dynamics of the SHR/SCR pathway in regulating these asymmetric divisions, we expressed an inducible version of either SHR or SCR in its respective mutant background, which also contained a ground-tissue specific GFP marker. Using fluorescence activated cell sorting in combination with microarray analysis, we examined the transcriptional effects of SHR and SCR induction specifically in the ground tissue over time. This allowed us to identify novel downstream genes of SHR and SCR in a dynamic and tissue-specific manner. In addition, we are looking at the spatio-temporal inter- and intracellular dynamics of SHR protein movement and activity using fluorescence correlation techniques. Using *in vivo* approaches, we are defining the rate of SHR movement and characterizing the dynamics of SHR/SCR interaction in different cell-types. These complementary approaches are providing novel insight into how SHR and SCR control key root developmental features, such as stem cell niche specification and radial patterning, and are integral to our ongoing efforts to model the dynamics of the SHR/SCR-mediated signaling pathway.

AH.3
Spatial coordination between stem cell activity and cell differentiation in the root meristem

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Understanding the molecular mechanisms through which plant meristems are maintained is a central question in developmental biology. In the root of Arabidopsis thaliana, stem cells in the apical region of the meristem self-renew and produce daughter cells that differentiate in the distal meristem transition zone. To ensure root growth, the rate of cell differentiation must equal the rate of generation of new cells. Cell differentiation takes place in the transition zone that is localized in the distal part of the root meristem, but must be synchronized and balanced with division of the stem cells that are localized in the apical part of the meristem. We have previously shown that maintenance of the Arabidopsis root meristem size - and consequently root growth - is controlled by the interaction between two hormones at the meristem transition zone: cytokinins, which promote cell differentiation, and auxin, which promotes cell division, but it is still unknown how the cytokinin/auxin interaction maintains a balance between cell differentiation at the transition zone and cell division in the stem cell niche. Here we show that SCARECROW (SCR) maintains stem cell activity repressing cytokinin-mediated differentiation input in the stem cell niche through down-regulation of the cytokinin-responsive transcriptional regulator ARR1 thus controlling root meristem size.

AH.4
Global quantitative proteomics reveals that miR17-92 dampens MYC- gene expression activity in established B-cell lymphomas

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Aberrant microRNA expression frequently correlates with cancer pathogenesis.
Until now, the general consensus in literature has been that miR17-92 (a cluster encoding six mature miRNAs) functions as oncogene, promoting tumorigenesis when aberrantly expressed. However, emerging evidence suggests that - under certain circumstances and depending on the set of mRNA targets expressed- the same cluster may display the opposite effect, acting as a tumor suppressor. While the enforced expression of miR17-19b synergizes with MYC and accelerates tumor progression in a mouse B-cell lymphoma model, the function of the same cluster has not been investigated yet in full-blown lymphomas.
We have employed SILAC-based quantitative proteomics to profile global changes in protein levels upon the enforced expression of miR17-19b in mature mouse B-lymphoma cells, whose phenotypic analysis unexpectedly revealed reduced proliferation and increased apoptosis.
We have obtained a high confidence quantitative proteome of more than 4500 proteins. A comparison with the corresponding transcriptome indicates a predominant response at the protein level, with significant down-regulation of miR17/20- and miR19a/b- targets. Statistical analysis selects 238 proteins significantly down regulated in our experimental conditions. Intriguingly, miR17-92 appears to affect at various level the MYC-dependent response: in fact, 40% of the identified targets, as well

as assigned GO functional categories, are transcriptionally regulated by MYC. Furthermore within the class of novel targets we observe a significant enrichment of MYC-miR17/20 Feed Forward regulatory Loops (FFLs).
Altogether our results support the emerging model of a "double-life" for miR17-19b, providing new large-scale data substantiating it at the molecular level: in particular, the mature miR-17 and miR-20 seem to display a specific modulatory action on the MYC-mediated response, thus acting as a tumor-suppressor in the model system investigated.

AH.5
The Cdc14 phosphatase and Cdc5 kinase ensure anaphase onset

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To ensure a correct transmission of the genome during cell division, replicated chromosomes (sister chromatids) must first be separated and then segregated between the daughter cells. Sister chromatid segregation occurs in anaphase and it is triggered by the dissolution of cohesin ring complexes that hold the sister chromatids together. Indeed, cleavage of cohesin by separase is sufficient to reduce the forces that oppose the pulling force of the spindle MTs, thereby promoting sister chromatid segregation. While cohesins are clearly essential to hold sister chromatids together, accumulating evidence has suggested that cohesin-independent forces, such us DNA catenation or condensation provide additional forces that resist mitotic spindle activity. Recently, we found that the simultaneous removal of Cdc5 kinase and Cdc14 phosphatase activities results in cells that arrest with undivided nuclei and short bipolar spindles regardless of having degraded securin and having cleaved cohesin. As artificial removal of cohesin by induced ectopic cleavage also did not suppress the nuclear division defect, our results indicate that in addition to cohesion cleavage, anaphase needs to be induced by a mechanism that is redundantly controlled by the Cdc5 kinase and the Cdc14 phosphatase.

POSTER AND ORAL PRESENTATIONS

1 - Cell cycle

P1.1
Novel mitotic roles of Aurora-A and implications for anti-cancer strategies

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Faithful chromosome segregation in mitosis is orchestrated by the bipolar spindle. The Aurora-A kinase is a key regulator of spindle assembly and function, frequently overexpressed in tumors; the microtubule (MT)-binding protein TPX2 regulates Aurora-A activity and localisation. We described that TPX2 also controls Aurora-A protein stability: then, TPX2 abundance could strongly influence Aurora-A function, with implications for tumorigenesis. We have found that Aurora-A and TPX2 are frequently co-overexpressed in cancer and propose that the Aurora-A/TPX2 complex acts as an oncogenic holoenzyme. Aurora-A is regarded as a potential target in anti-cancer therapies. We reported a novel function of Aurora-A in control of spindle pole integrity with potential effects on the fidelity of chromosome segregation: this raises concern on the therapeutic use of Aurora-A inhibitors. Our preliminary data from single-cell imaging analyses indicate that the Aurora-A inhibitor MLN8237, currently in clinical trials, induces multiple spindle defects and aneuploidy in human cells. High-throughput imaging is enabling us to identify conditions that may influence the outcome of Aurora-A targeted therapies.

P1.2
PACAP and VIP in human ovarian cancer cell lines

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Epithelial ovarian cancer develops from malignant transformation of OSE. The etiology of epithelial ovarian cancer is poorly understood. Some types of cancer cells produce PACAP and VIP which stimulate cell proliferation; instead their receptor antagonists decrease tumor mitogenesis. Concurrent administration of receptor antagonists and chemotherapeutic agents enhances the response. GCs produce PACAP in response to LH surge at the time of ovulation, but its role in ovarian cancer is not known. In this work, we have characterized the effects of these peptides on cell growth and survival in human ovarian cancer cell lines (Skov3, Tov21G). We have demonstrated that both peptides and their receptors are differentially expressed in these cells. Analysis of cell survival demonstrated that these peptides do not affect cell viability. CDDP decreases cell survival and induces an accumulation in S phase in both cell lines. Combined treatments, PACAP/VIP, plus CDDP cause a reduction of cell death. In conclusion we have demonstrated that PACAP and VIP do not play a relevant role in ovarian cell growth and survival, but preliminary analysis shows that probably are involved in cell invasion.

P1.3
Mir-24 regulates cell proliferation by targeting p27

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MicroRNAs may regulate several developmental and physiological processes and may be important in tumorigenesis. Cell cycle's regulatory mechanisms are mainly composed of cyclins, cyclin-dependent kinases, and CDK inhibitors (CDKIs).Alteration of these mechanisms results in uncontrolled cell proliferation, which is a distinctive feature of human cancers. We aimed at investigating the role of miRNAs in the regulation of main CDKIs: p27 and p16. Here, we proved for the first time that p27 is direct target of miR-24.Ectopic miR-24 expression into HEKa reduces p27 protein levels and promotes cell proliferation. It was been demonstrated that also p16 is a target of miR-24 and that ectopic expression of miR-24 suppresses p16 expression in cervical carcinoma cells. We demonstrate that miR-24 also acts as down modulator of p16 expression in HEKa cells.In cancer,p27 is inactivated through several mechanisms and have both prognostic and therapeutic implications. We confirm that downregulation of p27 and p16 miR-24-mediated also occurs in several carcinoma cell lines. Our data suggest that miR-24 is involved in CDKIs regulation and indicate that upregulation of miR-24 play a role in carcinogenesis

P1.4
Importin β regulates kinetochore / microtubule interactions in human mitotic cells

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Importin β, the main vector in interphase nuclear import, also has roles in mitotic spindle assembly. Here we show that importin β is a global regulator of mitotic transitions in human cells. By deletion mapping and time-lapse assays we find that importin β overexpression impairs chromosome congression and microtubule (MT)/kinetochore (KT) interactions via a nucleoporin-binding domain. In proteomic studies we identify importin β as part of a complex on the spindle MTs that includes RANBP2, a nucleoporin with SUMO ligase activity, and the SUMOylated form of the RANGTP-hydrolysing factor, RANGAP1. SUMO-RANGAP1 is normally recruited to KTs after MT attachment, but importin β counteracts this recruitment. In summary, importin β modulates a critical switch at the KT/MT interface: in early mitosis, when RANGTP-dependent MT nucleation is predominant at KTs, it prevents premature RANGAP1 delivery therein; when chromosomes align, it modulates RANGAP1 delivery (hence, RANGTP hydrolysis) at KTs, with concomitant activation of MT/KT attachment monitoring mechanisms. Thus, importin β 'adapts' the topological organization of key factors to ongoing changes during mitotic progression.

P1.5
Molecular imaging of NF-Y transcriptional activity maps proliferation sites in live animals

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In vivo imaging involving the use of genetically engineered animals is an innovative powerful tool for the non-invasive assessment of the molecular and cellular events that are often targets of therapy. Based on the knowledge that the activity of the Nuclear Factor-Y (NF-Y) transcription factor is restricted "in vitro" to proliferating cells, we have generated a transgenic reporter mouse, called MITO-Luc, in which a NF-Y dependent promoter controls luciferase expression. In these mice bioluminescence imaging of NF-Y activity visualizes areas of physiological cell proliferation and during regeneration in response

to injury. Using this tool, we highlight for the first time an unknown function of NF-Y activity in liver regeneration. The MITO-Luc reporter mice should facilitate investigations on the involvement of genes in cell proliferation as well as provide a useful model for studying aberrant proliferation in disease pathogenesis. It should be also useful in the development of new anti/proproliferative drugs, their efficacy assessment and side effects on non-target tissues.

P1.6
Molecular bases of the postmitotic state: investigating reactivated DNA synthesis in terminally differentiated cells

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Terminally differentiated myotubes (Mt) have permanently lost the ability to proliferate and the ultimate reason for such impairment is still unknown. DNA synthesis can be forced in Mt by a variety of means, but reactivated Mt never complete DNA duplication and sustain heavy DNA damage, which triggers apoptosis or mitotic catastrophe. The inability to complete DNA replication could be explained by two non mutually-exclusive causes: deregulated cell cycle machinery and unpermissive chromatin structure. We induced Mt and myoblast (Mb) nuclei to synthesize DNA in Xenopus egg extracts (XEE). Preliminary results showed that Mt nuclei are far less permissive to DNA replication than the Mb ones. Since XEE can complement any functional defect, Mt limited replication is most likely due to their chromatin structure. To determine which DNA regions are under-replicated (UR) in Mt, newly synthesized DNA extracted from Mb and Mt reactivated by suppression of cell cycle inhibitors is being hybridized to genomic chips. Altogether, these results should shed light on the mechanistic bases of the postmitotic state of terminally differentiated cells.

O1.1
Proximity ligation assay (PLA): a new tool to visualize regulatory pathways at their site of action.

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Proximity ligation assay (PLA) is a new technology that combines the capabilities of immunofluorescence and co-immunoprecipitation assays, giving high specific and sensitive information on the subcellular localization of proteins while interacting with their partners. Localising the signals specifically generated by protein-protein interactions inside the cell adds significant insight to the understanding of regulatory processes.
Here we have used PLA to investigate the mitotic roles of RAN GTPase effectors, i.e. Importin beta, RANBP2 and exportin-1/CRM1. To validate our approach, we first verified the interactions of these proteins in the well-known interphase nucleocytoplasmic transport network. Interphase Hela cells were probed for key interactions (RAN-RANBP2, RANBP2-RANGAP1, RANBP2-Importin beta and RAN-CRM1): PLA enabled us to visualize for the first time all of these interactions at discrete sites of the nuclear envelope, which were previously only inferred indirectly from single co-immunoprecipitation or co-localization. We then tested mitotic cells. Our results depict specific patterns of interaction along the spindle microtubules and at the microtubule/kinetochore surface. The newly identified patterns reveal the topological organization and architectural features of regulatory signals operated by RAN and its effectors at the mitotic apparatus.

O1.2
RNase H and post-replication repair protect cells from ribonucleotides incorporated in DNA

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During replication, the chemical identity and integrity of the genome is challenged by the incorporation of ribonucleotides (rNTPs), which are more prone than deoxyribonucleotides (dNTPs) to potentially toxic and mutagenic strand cleavage. During normal replication, DNA polymerase epsilon incorporates ribonucleotides with high frequency. We show that in budding yeast four DNA transactions contribute to surviving the presence of rNMPs in DNA. Both RNase H1 and RNase H2 have roles in removing rNMPs from DNA, and failure to do so causes replication stress and has toxic consequences, when DNA polymerases encounter rNMPs in the template strand at the next round of replication. We provide genetic and biochemical evidence that cells lacking RNase H survive replication stress through two post-replication (PRR) pathways, MMS2-dependent template switching and Pol ζ-dependent bypass of rNMPs in the DNA. Finally, cells lacking RNase H have a constitutively activated PRR and accumulate ubiquitylated PCNA. Our findings describe a new function for PRR in overcoming the obstacles represented by ribonucleosides misincorporated during DNA replication.

O1.3
PcG mediated higher order chromatin structures modulate replication programmes at the Drosophila BX-C

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Polycomb group of proteins (PcG) are conserved transcriptional regulators that maintain repressed transcriptional states, acting directly on chromatin structure. During S-phase, in addition to DNA, PcG epigenetic signatures need to be duplicated to preserve cell identity, requiring a tight coordination between PcG complexes and replication programs. However, to date the mutual regulation between replication and PcG functions is still unknown. To address this issue we performed genome-wide bioinformatic analyses in Drosophila and functional experiments on the well characterized BX-C locus. Our data that transcription per se is not the sole determinant of cellular replication timing, whereas higher order structures can dictate the timing of replication. In line with this observation, analysis on two Drosophila cell lines presenting different BX-C conformations revealed a cell type specific replication program that mirrors BX-C higher order structures. Our work suggests that PcG complexes synergistically contribute to the definition and the maintenance of genomic structural domains where genes that are co-regulated replicate at the same time.

O1.4
Changes in lipid metabolism detected by 1H MRS in HER2-overexpressing ovarian cancer cells exposed to conventional and innovative anticancer treatments

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Magnetic resonance spectroscopy (MRS) studies showed increased levels of phosphocholine (PCho) and activation of choline kinase and PC-specific phospholipase C (PLC) in epithelial ovarian cancer (EOC) vs. nontumoral counterparts (Iorio et al Cancer Res 2010). We focused attention on the highly tumorigenic in vivo passaged SKOV3.ip cells, which showed higher PLC activity, increased PCho and HER2 contents compared with the parental EOC cells. PLC inhibition by D609 induced cell cycle and growth arrest, cell swelling and down-modulation of HER2 expression. These effects were associated with significant decreases in PLC expression and PCho level. Similar biochemical and biological effects were also induced in SKOV3.ip by cisplatin (CDDP). Both agents induced increases in MRS-visible mobile lipids. In vivo diffusion-weighted MRI analyses on CDDP-treated SKOV3.ip xenografts showed that changes in the apparent diffusion coefficient (ADC) of water molecules was an early marker of response to CDDP. These results suggest the interest of using integrated MR and cell biology approaches to evaluate the metabolic effects of conventional and new cytostatic agents in ovarian cancer models.

O1.5
DNA damaging agents can induce cell cycle arrest in different phases of mitosis

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Mitotic checkpoints have been associated to DNA damage response. In this study we analyzed the progression through mitosis of mismatch repair deficient and proficient colon cancer cell lines, after treatment with the DNA polymerase inhibitor aphidicolin, radiomimetic bleomycin and topoisomerase I inhibitor SN38. Cells were treated with the drugs at concentrations and times suitable to detect DNA and chromosome damage without abrogating mitosis. After fixation, cells were incubated with anti-α, -β or -γ tubulin antibodies and a secondary fluorescent antibody. The percentage of cells in the various phases of mitosis, abnormal mitoses (multipolar, with lagging chromosomes) and mitotic indexes were evaluated. Aphidicolin and bleomycin caused cell arrest mainly at the checkpoint between metaphase and anaphase, whereas SN38 induced cell accumulation at the end of mitosis, when tubulins are present only in the midbodies, just before the definitive cytodieresis. Since aurora kinase B is essential in the control of cytodieresis, we plan to analyze its possible intervention in cell response to the drugs under study. Moreover, we will evaluate telomere damage induced by topoisomerase I poisons that may cause cell arrest at the end of mitosis.

2 - Cellular stress, apoptosis and autophagy

P2.1
Does Resveratrol affect radiation-induced chromosome damage? A multiparametric study on human lymphocytes exposed in vitro

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A huge amount of experimental data demonstrated that the natural compound Resveratrol (RESV) exerts various beneficial effects on human health. Controversial data are present in literature about the ability of RESV to affect DNA molecule integrity. Since controlled exposure to ionising radiation (IR) is one of the most effective treatments of cancer patients we focused our attention on a possible radioprotective activity of RESV in normal tissues. We investigated the ability of RESV to modulate the chromosome damage induced by IR using the Cytokinesis-Block Micronucleus (CBMN) assay and the Chromosomal Aberration (CA) test on human peripheral blood lymphocytes. While RESV by itself does not induce any DNA damage, we surprisingly found a controversial effect in combined treatment: a reduction of IR-induced MN and an increase of IR-induced CA. These results suggested a deeper analysis of the influence of RESV both on DNA repair efficiency and on cell viability. The modulation by RESV of DNA repair has been analysed through Comet assay and γ-H2AX foci induction. The influence of RESV on cell viability has been assessed through apoptosis and cell growth analysis.

P2.2
Influence of prolonged nutrient deprivation on virulence properties in *Aeromonas hydrophila*

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Aeromonas hydrophila encompasses a diversity of strains with varying pathogenic potential. Although some *A. hydrophila* isolates show identical patterns of virulence-associated genes, their virulence may be different. Furthermore, studies report the capacity of *A. hydrophila* to support stressful conditions with modifications of virulence properties.
In the present study we evaluated the level of virulence variation under prolonged nutrient deprivation in two strains of *A. hydrophila* (respectively LS and 87) from surface water and fish, showing the same genotype but different cell toxicity. In particular we investigated the adhesiveness, cytotoxicity and the expression profiling of haemolysin gene (aerA).
The results showed that, after a 35 d period, both strains entered into the VBNC state. In addition there was a decrease of the tested virulence factors, tough *A. hydrophila* 87, which was initially less virulent, lost its adhesive ability at day 14 and the expression of the haemolysin gene at day 21. In conclusion nutrient deprivation stress may impair virulence properties in *A. hydrophila* therefore having possible implications in the pathogenesis of the infections.

P2.3
Antioxidant effect of L-Dopa and carbidopa combined treatments on human peripheral blood mononuclear cells

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L-Dopa (LD) is the 'gold standard' drug for the treatment of Parkinson's Disease (PD). Literature studies have reported both the generation of free radicals by LD treatment and evidence of neuroprotective effects on dopaminergic cells. Moreover, since peripheral metabolic pathways significantly deplete the amount of LD reaching the brain it is usually co-administered with carbidopa (CD), a decarboxylase inhibitor, so increasing LD bioavailability. In previous studies on neuroblastoma cells we tested LD effects, either alone or in combination with CD, showing a reduction of DNA damage and oxidative stress exogenously induced by hydrogen peroxide. Since PD patients are characterized by a systemic oxidative stress leading to peripheral damage we tested LD and CD effects on peripheral blood mononuclear cells (PBMCs). PBMCs are easily accessible ex vivo dopaminergic model for exploring the biological effects of LD therapy. Our data confirm the role of CD in increasing the protective effects of LD in the presence of exogenous oxidative stress. These findings provide a preliminary indication that LD+CD combined therapy may thwart the pathogenetic role of systemic oxidative stress in PD.

P2.4
Cytotoxic/genotoxic effects of M2 agonist arecaidine on human glioblastoma cell lines

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Muscarinic receptors have been found in several tumors including glioblastoma. Previously we have demonstrated that M2 muscarinic receptor agonist arecaidine inhibited cell proliferation in a time and dose-dependent manner in two glioma cell lines (U251 and U87). FACS analysis have also confirmed that arecaidine caused cell cycle arrest, with cell accumulation in G1 for U87 and in G2/M for U251 cells and induced apoptosis in both cell lines. Here we report the cytotoxic effects produced by arecaidine in glioma cell lines, causing ROS production, double strand DNA break and SIRT1 and SIRT2 modulation. Particularly in U251 cells arecaidine causes also the formation of multipolar mitotic spindles and misalignment of chromosomes, with an increase of metaphases number and the complete absence of anaphases, probably due to pro-metaphase/methaphase checkpoint activation. SAC genes expression will be evaluate to confirm this hypothesis. The data obtained demonstrate that the cytotoxic effect- arecaidine induced on glioma cell lines may contribute to the severe apoptotic cell death. These results open new interesting therapeutic perspectives for this molecule in glioblastoma therapy.

P2.5
DNA damage response by single-strand breaks in terminally differentiated muscle cells and the control of muscle integrity

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Mouse post-mitotic muscle cells accumulate single strand breaks (SSB) after oxidative and alkylation DNA damage but they are resistant to their killing effects. We demonstrate that, upon SSB induction, H2AX-phosphorylation occurs in myotubes and is largely ATM-dependent. However, DNA damage signaling cascade downstream of ATM is defective as shown by lack of p53 increase and phosphorylation. The stabilization of p53 by nutlin-3 was ineffective in activating the cell death pathway indicating that the resistance to SSB inducers is due

to defective p53 downstream signaling. Conversely, doxorubicin and menadione were able to activate p53 and to kill myotubes. We show that cell killing is p53-dependent although a restriction of p53 activated genes was is observed. To gain insights into the cell death pathways active in post-mitotic muscle cells experiments were carried out to characterise autophagy as a cellular stress response. Autophagy was activated during muscle differentiation and was exacerbated in the absence of p53. Whether autophagy is implicated as a pro-survival mechanism in the resistance of post-mitotic cells to different genotoxic insults is currently under investigation.

P2.6
Chemotherapy and cardiotoxicity: doxorubicin vs imatinib mesylate

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Cardiotoxicity is a side effect of several chemotherapies. Anthracyclines, 5-fluorouracil and taxanes are known to induce cardiotoxicity, but some forms of cardiotoxicity have been described for most antitumour agents, included the new generation anti-cancer molecules belonging to the family of target therapies. In this contest, our attention was focalized on the tyrosine kinase inhibitor (TKI) Imatinib Mesylate (IM). The toxicity induced by IM on human cardiac mesenchimal stem cells (C-MSCs) was compared with the effect induced by the well-known cardiotoxic chemotherapic Doxorubicin. Furthermore, we have analyzed the effect of these drugs on their target cell lines (K562 and MCF7). The tested drugs show induction of cyto- and geno- toxicity on the target cell lines and on C-MSCs. To understand the mechanisms of toxicity induced by IM and Doxorubicin, we analyzed the expression of proteins involved in different cellular pathways like apoptosis, autophagy and maintenance of DNA integrity. The two drugs showed a different behavior: Doxorubicin induce high genotoxicity and apoptotic cell death; IM, instead, seems to induce pro-survival pathways like autophagy.

P2.7
Excessive fatty acid intake may modulate p53 activity and its control of cell cycle and energy metabolism

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Background and Aims: Nonalcoholic fatty liver disease (NAFLD) ranges from steatosis to nonalcoholic steatohepatitis (NASH), which may lead at least to hepatocarcinoma (HCC). Mitochondria are main players in lipid and energy metabolisms, thus also in hepatic lipid storage. P53 protein is a metabolic modulator but its role in NAFLD is unknown. Thus, we aimed to investigate the role of p53 in NAFLD. **Methods:** Huh 7.5.1 HCC cells were treated with a solution of oleic and palmitic acids (molar ratio 2:1, 0,5mM) for 14h and 24h. AdipoRed and Alamar blue assays were performed to evaluate lipid content and proliferation respectively. By Western blot we have investigated p53, p21 and SCO-2. Mitochondrial complex IV activity was evaluated. **Results:** Treatment enhanced intracellular lipid content. At 14h we found an increase in p53 activity, p21 and SCO-2, but at 24h all proteins were decreased. Treatment produced an increased proliferation mainly at 24h, while the complex IV activity decreased progressively. **Conclusions:** Our data suggest a p53-mediated metabolic cell response, which fails after prolonged fatty acid exposure, affecting both energy metabolism and cell cycle control.

P2.8
Synergistic cytotoxicity of copper(II) complexes in combination with cisplatin: an application of artificial neural networks and experimental design

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Polychemotherapy is used to enhance overall inhibitory potentials and reduce selection of drug resistance to single agents. Multidrug protocols are generally based on cocktails of different drugs known to have synergistic type of interactions one with another. In this work we propose a novel approach to search for optimal synergistic drug combinations by using Artificial Neural Networks (ANNs) and the Experimental Design (ED). We applied the experimental approach to the study of the effects of the cytotoxic agent CDDP (cis-Platin) in dual drug combinations with novel antiproliferative Cu(II) complexes containing two 1,10-ortho-phenanthroline units, which were recently prepared and characterized by us (Pivetta et al., J Inorg Biochem 2011 and 2012). We designed and tested ≈ 40 different mixtures of Cu(II) complexes and CDDP against the T-leukaemia CCRF-CEM cell line. Neural network was used to model experiments and predict cytotoxicity values of drug combinations on the whole working space, including validating points. Results will show that ED-ANN is a new, efficient and fast method to search for synergy of two or more drug associations.

P2.9
Ascorbate/epigallocatechin-3-gallate/gemcitabine combined treatment kills mesothelioma via apoptosis pathway

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Malignant mesothelioma (MMe) is an asbestos-related, lethal cancer arising from mesothelial cells of the pleura, with poor prognosis and limited effective therapies.
Ascorbate has been reported to be useful in the treatment and prevention of cancer, and we have found that MMe cells are more susceptible to ascorbate than normal mesothelium. We observed a synergistic cytotoxicity of ascorbate in combination with gemcitabine and the green tea polyphenol epigallocatechin-3-gallate (EGCG).
The induction of apoptosis is a main requisite for chemotherapeutic agents, and we investigated whether the induction of apoptosis was mechanistically linked to the synergistic action of ascorbate/EGCG/gemcitabine combination.
Confocal calcium imaging and caspase 3 assay revealed higher increases of these parameters after exposure to the triple combination with respect to any compound used singularly. Thereafter, we conducted a survey of 96 genes involved in programmed cell death, observing a clear apoptosis pattern with maximum upregulation for Death-Associated Protein Kinase 2 (DAPK2). In conclusion, we have demonstrated that the combination of ascorbate/EGCG/gemcitabine synergistically inhibits MMe growth through the induction of noninflammatory, DAPK2-dependent apoptosis. These data indicate our combined treatment as a possible candidate for a novel, innovative clinical approach to mesothelioma.

P2.10
Role of Bim and p53 mitochondrial-translocation in apoptosis induced by Combretastatin A-4 in human non-small lung cancer cells

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The BH3-only protein Bim and p53 protein are well known apoptosis promoting proteins, but their reciprocal interactions in apoptosis induced by microtubule-disrupting agents, such as Combretastatin A-4 (CA-4) have been poorly investigated. With this purpose, Non Small Lung Cancer Cells (NSLCC) were treated with the microtubule damaging agent CA-4, and various endpoints were investigated. Upon treatment, transient knockdown of Bim in H460 and treatment in H1299 which do not express the p53 protein, drastically reduced apoptosis, indicating that these proteins are involved in cell death induced by microtubule disorganization. In H460 cells, the protein levels of Bim and p53 were strongly up-regulated in a time-dependent manner; they were released from microtubules and translocated to mitochondria, where they interacted each other and with other modulators of apoptosis, leading to Cyt C release, alterations in mitochondrial membrane polarization, cells cycle arrest at the G2/M-phase and cell death. However, the extent of cell death was not reduced after combined treatment of CA-4 with phitirin-μ, an inhibitor of p53 mitochondrial-translocation. Overall, these data support a model of CA-4-induced apoptosis in NSLCC, for which the expression of Bim and p53 protein is essential, but the mitochondrial function of p53, linked to p53-transcription independent apoptosis is negligible

P2.11
Involvement of ERp57/PDI A3 in the response to oxidative stress induced by Amyloid beta peptide

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Alzheimer's disease is a neurodegenerative disorder whose pathological hallmark is the accumulation of Amyloid-beta (Aβ) peptides in the form of senile plaques. Aβ peptides can cause neurodegeneration and disrupt cognitive function by several mechanisms, including oxidative stress. ERp57 is a protein disulfide isomerase involved in the cellular stress response and could efficiently help to reduce the level of oxidized proteins preventing the accumulation and aggregation of misfolded proteins. Aβ peptides are present in the CSF of normal individuals as a complex with ERp57, suggesting it may be a carrier protein which prevent aggregation of Aβ. Our aim is to gain insights into the molecular mechanisms involved in oxidative stress induced neurodegeneration, and unveil whether ERp57 may exert a neuroprotective effect from Aβ toxicity. Thus, human neuroblastoma cell line SH-SY5Y was stably transfected to overexpress ERp57 and treated with the $A\beta_{25-35}$ fragment. Variations in cell functions, redox state, morphology, and protein composition were monitored, focusing the attention on ERp57 expression levels, chemical modifications and cellular distribution in wild type and transfected cells

P2.12
LC3 deregulation and autophagy suppression during hepatosteatosis development

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Currently, hepatosteatosis is considered one of most common liver diseases worldwide. As multifactorial disease, hepatosteatosis development is regulated by several molecular mechanisms. Autophagy suppression has been recently described as potential hallmarks of intra-hepatic fat accumulation and its progression. Here, we studied the regulation of autophagic process during hepatosteatosis development. To this aim we used liver samples from a rat model of hepatosteatosis and children affected by this disease, and HepG2 cells.
We found a reduction in the expression levels of LC3 mRNA and protein, an increase of the levels of p62 (polyubiquitin-binding protein), and an accumulation of ubiquitinated proteins in hepatosteatotic rat livers. Similar results were observed in liver tissues from children with hepatosteatosis. HepG2 treatment with free fatty acids induces in vitro hepatosteatosis altering the autophagic process. In this phenomenon glutathione intracellular levels and PTEN/Akt/mTOR signal transduction pathway seem to play relevant roles.
In conclusion, our findings demonstrate the presence of a deregulation of autophagic process during hepatosteatosis development and suggest potential molecular networks involved in this effect. Further analysis is required to understand if these results may provide novel potential targets to design safe and efficient therapeutic approaches against hepatosteatosis.

P2.13
On the possibility that the effects of copper and nickel on nuclear basic proteins and on DNA oxidative damage depend on copper-arginine and nickel-lysine interactions

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In living cells, DNA is complexed with histones or sperm nuclear basic proteins (SNBPs) to form chromatin. Although histones and SNBPs protect DNA from a variety of potentially dangerous reactive species, such as hydroxyl radicals (•OH), chromatin packaging doesn't protect DNA from metal ion-dependent free radical damage. We report the effects induced by Cu^{2+} and Ni^{2+} ions on DNA and various lysine or arginine rich nuclear proteins: salmin, *Mytilus galloprovincialis* protamine-like II and III, calf thymus H3 histone and poly-L-lysine and poly-L-arginine. Protamine-like II-III and salmin, which contain arginine, as a consequence of conformational changes, acquire proteinase k digestion resistance in the presence of copper and not in the presence of nickel. Copper, probably interacting with arginines, promotes hydrogen peroxide DNA damage when it is complexed with arginine rich proteins while nickel compacts and protects DNA only when it is complexed with lysine rich proteins. This observation corroborates with the findings that nickel, for its preference to specific lysine residues in the H4 N-terminal, influences carcinogenesis acting as potent inhibitor of histone H4 acetylation.

P2.14
The new dyskerin isoform 3 over-expression promotes DNA damage repair inducing p21 (CDKN1A)

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X-linked DC, a multisystemic syndrome -characterized by telomere shortening, stem cell loss and altered DNA damage response- is caused by mutations in the h-DKC1 gene, which encodes at least two mRNAs, called isoforms 1, which encodes dyskerin, a nucleolar protein, and isoform 3, which encodes a cytoplasmic protein with undefined function. To test if Isoform 3 could have a role in cellular stress response, we subjected to DNA damage stably overexpressing (3XF-Iso3) or empty vector transfected (3XF-M) HeLa derived cells. Comet assay performed after X-ray exposure and 0, 2 and 4 hours recovery showed a reduced length of 3XF-Iso3 comets and a reduced amount of DNA in tail respect to 3XF-M comets, similar results were obtained treating cells with hydrogen peroxide. Western blot analysis revealed for 3XF-Iso3 cells constitutive expression of p21 (CDKN1A), which confers, at

least in some conditions, resistance to DNA damaging agents. The p21 expression is probably induced by the change in Notch2 intra cellular domain modification highlighted by western blot. In conclusion the new DKC1 isoform 3 improves the DNA damage response, probably by the Notch2-iduced p21expression.

P2.15
Regulation of functionally related lysosomal genes by miRNA

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Lysosomes are single membrane-bound cytoplasmic organelles present in almost all eukaryotic cells. Specific enzyme deficiencies account for more than 50 lysosomal storage disorders, characterized by intracellular deposition and protein aggregation, events also found in neurodegenerative diseases. Recently it was found that most lysosomal genes exhibit coordinated transcriptional regulation by TFEB. Gene expression at the post-transcriptional levels can be regulated by miRNAs, that play relevant roles in diverse biological processes in which lysosomal system is implicated. A database on human lysosomal genes and their regulation by miRNAs was realized: lists of lysosomal genes were collected by public resources and binding predictions from five softwares (TargetScanS, picTar 4-way and 5-way, PITA, miRanda) integrated. Results showed that there is no particular miRNA coordinating lysosomal system but functionally related genes are regulated by the same miRNAs, such as adaptor proteins which are responsible for vesicular trafficking. Experimental validation of these targets is ongoing.
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P2.16
Relevance of arginine in DNA oxidative stress induced by copper- H1 histone complexes

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Copper chloride on some proteins, including histones and prion protein determines formation of complexes resistant to proteinase K (PK) digestion. We analyze the effect of copper on H1 histones. We used as somatic histone that of calf thymus while as sperm H1 histone that of annelid worm *Chaetopterus variopedatus* (*Ch.v.*). The two histones differ for their K/R ratio that is 2 for sperm H1 histone and 15 for the somatic one. The results show that, in the presence of copper chloride, both histones acquire PK resistance but the sperm histone shows higher resistance. These results indicate the formation of copper-H1 histone complexes PK resistant. The deguanidinated derivative of *Ch.v.* H1 histone in which 80% of arginine are modified in ornitine, results more susceptible to the action of PK respect to native molecule indicating the relevance of arginine in this effect. In addition *Ch.v*. H1 histone, in the presence of copper chloride, shows higher DNA binding affinity respect to the histone in absence of copper in contrast to the somatic one. Finally, only *Ch.v.* H1 histone in the presence of copper chloride and hydrogen peroxide, is able to produce DNA oxidative damage.

O2.1
Transgene or dsRNA mediated silencing induces DSBs and apoptosis in the *Caenorhabditis elegans* germ line

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Transgene mediate cosuppression and RNA interference are trans-generational silencing mechanisms acting both at a post-transcriptional and epigenetic level in *C. elegans* germ line. We found that both these procedures, commonly used to silence genes of interests and that share several common factors, induce germ-line DNA damage and apoptosis. RAD-51 foci, a marker for ongoing DNA repair, increase during these mechanisms indicating induction of DNA double-strand breaks. Furthermore apoptosis is dependent upon genes involved in the DNA damage-response checkpoint.
RNAi or co-suppression have been postulated as defense mechanisms against genomic intruders. We speculate that this novel mechanism may trigger the elimination of germ cells that have undergone virus infection or transposon activation. These observations shed new light on the cross-talk between different pathways devoted to the protection of genome stability in germ cells.

O2.2
Intracellular zinc is required for intestinal cell survival signals trigged by inflammatory cytokines

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Zinc is a functional component of structural proteins and enzymes, recent experimental evidence has highlighted an additional function for free or loosely bound zinc ions as an intracellular signaling factor. We investigated the contribution of intracellular zinc in maintaining intestinal epithelium integrity when exposed to the inflammatory cytokine TNFα. We showed that marginal zinc deficiency affected TNFα signalling, shifting intestinal cell fate from survival to death, this is reverted by zinc itself and other micronutrients such vitamin E. TNFα promotes a zinc dependent survival pathway that includes the regulation, at different levels of gene expression, of transcription factors and signaling proteins. TNFα signal results in cIAP2 mRNA increase through the activation of NFkB, we showed that NFkB nuclear translocation is impaired by zinc depletion and cIAP transcrption inhibited. XIAP, a potent inhibitor of apoptosis protein is quickly degraded in marginally deficient intestinal cells while its transcription is not affected. These results provide a possible molecular explanation for the clinical observation that zinc supplements ameliorate Inflammatory Bowel Disease symptoms and open to the possibility of introducing nutritional intervention to implement standard therapeutic approaches.

O2.3
The transcriptional response of mammalian cancer cells to irradiation is dominated by a cell cycle signature which is strongly attenuated in non-cancer cells and tissues

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Our goal was to identify genes showing a general transcriptional response to irradiation in mammalian cells and to analyze their response in function of dose, time and quality of irradiation and of cell type.
Materials and methods: We used a modified MIAME (Minimal Information About Microarray Experiments) protocol to import microarray data from 204 different irradiation conditions in the Radiation Genes database and performed cut-off-based selections and hierarchical gene clustering.
Results: We identified a set of genes which respond to a wide range of irradiation conditions in different cell types and tissues. Functional analysis of the negatively modulated genes revealed a dominant signature of mitotic cell cycle regulation which appears both dose and time-dependent. This signature is prominent in cancer cells and highly proliferating tissues but it is strongly attenuated in non cancer cells.
Conclusions: The transcriptional response of mammalian cancer cells to irradiation is dominated by a mitotic cell cycle signature both dose and time-dependent. This core response, which is present in cancer cells and highly proliferating tissues such as skin, blood and lymph node, is weaker or absent in non-cancer cells and in liver and spleen. CDKN1A (cyclin-dependent kinase inhibitor 1A) appears as the most generally induced mammalian gene and its response (mostly dose- and time-independent) seems to go beyond the typical DNA damage response.

O2.4
New combined therapy for malignant pleural mesothelioma: a preclinical study

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Malignant mesothelioma (MMe) is an almost incurable tumor, showing resistance to chemo- and radio-therapy, with a median survival of 8 to 18 months, lacking a standard therapeutic approach. Among alternative remedies to cancer, a growing interest has been directed to the preventive action of active nutrients.
We had previously shown that ascorbate is more cytotoxic to MMe cells than to mesothelial cells, due to a redox mechanism. Moreover, ascorbate is synergistically cytotoxic to MMe cells with gemcitabine and epigallocatechin-3-gallate (EGCG). So, we tested this combination in vivo, using an ip xenograft model of MMe in immunodeficient mice. Treatments by ip injections resulted in higher survival of mice, as shown by Kaplan-Meyer curves. Necropsy data revealed marked reduction of tumor growth and complete inhibition of diaphragm metastatization.
Taken toghter, data have shown that the combined therapy increases mouse survival, limits tumor growth and invasiveness, reduces the activation of cell growth signaling pathways and increases apoptosis rates in tumor growing regions. These results strongly indicate the use of this mixture as a potential therapy for MMe clinical treatments.

O2.5
Early autophagy inhibition is required for the completion of Activation-Induced Cell Death (AICD) in T cells

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Autophagy is the major intracellular degradation system by which cytoplasmic materials are delivered to and degraded in the lysosome and a dynamic recycling system that produces new building blocks and energy for cellular renovation and homeostasis. In the immune system, autophagy is mostly related to pro-survival processes in the physiology of the cells. Activation-induced cell death (AICD) is an important physiological mechanism of programmed cell death necessary to select the development of a functional repertoire of T cells and to maintain homeostasis and peripheral tolerance in the immune system. We recently discovered that during AICD, the T cell fate depends on a cross-talk between AICD and autophagy. Indeed, genetic ablation of the autophagic machinery increased AICD, revealing a protective role for autophagy on the onset of T cell death, whereas pharmacological induction of autophagy protected cells from cell death. Thus, the early block of autophagy after AICD induction is necessary for the effective achievement of the cell death. We decided to investigate the putative signaling pathway responsible for the inhibition of autophagy upon AICD induction in order to better elucidate the crucial interplay of these two mechanisms for T lymphocyte destiny.

3 - Genomics, proteomics and system biology

P3.1
Grapevine small RNAs and their involvement in genotype x environment interaction

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Understanding the molecular mechanisms involved in the interaction between genetic composition and the environment is crucial for modern viticulture, given that wine quality depends on the combination of *terroir* and cultivar. We approached the problem by focusing on miRNAs. First we set up a reference by producing a comprehensive miRNA expression atlas of grapevine sequencing 52 libraries derived from 7 tissues at different developmental stages with 2 biological replicates. Both conserved and lineage-specific miRNAs have been characterized, greatly improving knowledge on grapevine miRNAs in different tissues. To investigate the specific role of miRNAs in berry maturation and plant plasticity, we analyzed miRNAs in developing and maturing berries of the two cultivars *Cabernet Sauvignon* and *Sangiovese* grown in parallel in 3 different environments. A total of 48 smallRNA libraries were produced and sequenced. All the data were analyzed using a custom made bioinformatics pipeline, coupled with statistical tests that identified differentially expressed miRNAs. Expression data will be integrated with results of target genes identification experiments to describe miRNA functional role.

P3.2
Ferns share mitochondrial rps3 introns with seed plants

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Plant mitochondrial (mt) genome evolution has seen a frequent coming and going of group II introns before the establishment of the angiosperm lineages. The evolutionary history of introns and the relative contribution of intron loss and acquisition in shaping land plant mt genes and genomes remains on the whole poorly understood.
The mt gene for the ribosomal protein S3 (rps3) has caught our attention because of the multiple changes in intron content that its locus has experienced during plant evolution.
The rps3, generally, harbors one group II intron, namely rps3i74, at the same insertion site from algae up to the angiosperms analyzed so far, except in Beta and Marchantia. Surprisingly, in the mitochondria of some gymnosperm groups the rps3 reading frame harbors a downstream second group II intron, the rps3i249.
Here, we report the remarkable genomic organization and expression of the rps3 encoded by the mt genome of the previously undocumented fern Adiantum capillus veneris L.
Furthermore, a broader survey of additional ferns was carried out to identify the point of acquisition of the rps3i249 and the evolutionary depth of the rps3 intron flow in neglected land plants.

P3.3
*Cv*PL: a new Protamine-like of the marine worm *Chaetopterus variopedatus*. Characterization and sperm chromatin organization model

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The marine worm *Chaetopterus variopedatus* (*Ch.v.*) is the only invertebrate described to date with sperm chromatin organized only by two Sperm Nuclear Basic Proteins (SNBPs): a sperm H1 histone (*Cv*H1) and a molecule defined as a protamine in 1983. We characterize this protein and named *Cv*PL. *Cv*PL contents 41.7 mol% of arginine and 14.4 mol% of lysine. This protein shows similar features to protamine as the molecular weight of 8370.5 Da, the K/R ratio of 0.34, and the CD spectra; while *Cv*PL N-terminal sequence shows high similarity with the arginine-rich C-terminal domain of chordate PLs SNBPs. As some PL-type, *Cv*PL contains a globular domains and shows their electrophoretic mobility in a high resolution acetic-acid urea polyacrylamide gel. Further this protein forms stable oligomers and we have already shown, by specific amino acid side chain modifications, that oligomeric structure depends on anion-mediated lysine-arginine interactions. Finally *Cv*PL binds DNA in a similar fashion as vertebrate PLs and *Cv*H1, but in a different way from the lysine-rich somatic H1 histones. Based on all of this, is proposed a *Ch.v.* sperm chromatin organization model.

P3.4
The proteolytic activity of lactoferrin

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Lactoferrin (Lf) is a glycoprotein, expressed and secreted from glandular epithelial cells and from mature neutrophiles of mammals. Lf is an important component of the aspecific host defence or natural immunity. Lf displays proteolytic activity towards two putative colonization factors of Haemophilus influenzae. The proteolytic activity of Lf was studied by using synthetic fluorogenic substrates. Human Lf proteolytic activity is similar to that of serine-proteases. Such activity is also present in both bovine Lf and hen's ovotransferrin, suggesting that Lf proteolytic function is evolutionary conserved and that it could be of great importance for Lf protective role. However, the physiological substrates and the mechanisms of actions involved in the exploitation of this protective activity are not known. Bioinformatic studies identified several conserved serine residues that could be responsible of the proteolytic activity of Lf. In particular, one putative active site has been found on the basis of structural similarities with Tricorn protease, a protease involved in the cytosolic degradation of viral proteins, suggesting a role of Lf in the degradation of viral proteins.

P3.5
Next generation sequencing approaches to characterize natural populations of *Brachypodium distachyon*

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The present work aims at the application of a Genotyping-by-Sequencing (GBS) approach to the genetic characterization of Turkish populations of *Brachypodium distachyon* (Bd), a model species for monocots, with the aim of identifying the connections between allelic and ecologic variants. GBS produces thousands of SNP markers on a reduced representation of the genomes of a number of samples in a single NGS reaction. GBS is based on the use of methyl-sensitive restriction enzymes which not only reduce genome complexit, but select for fragments which are likely to contain actively coding regions. We collected 96 Bd samples grouped in 8 populations growing at regular intervals along an environmental gradient spanning from the West maritime regions of Hellespont to the inner deserted regions around Lake Tuz. Genotypes have been multiplexed accordingly to GBS protocol and run in two 46-plex lanes on an Illumina NGS platform. The raw data obtained have been referred to Bd complete genome sequence and filtered through different bioinformatic procedures to obtain a solid SNP map from which derive quantitative measures of population genetics and environmental relatedness of genomic traits.

P3.6
Drug repositioning for orphan diseases through Conserved Anticoexpressed Gene Clusters (CAGCs)

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Orphan disease (OD) are commonly defined as rare disorders with a prevalence of less than than 1:2000. Despite the low frequency of the single disorders, OD represent an extremely important medical and social challenge. Drug repositioning, i.e. finding new indications for approved drugs, represents to date one of the most cost- and time-effective strategies to identify a therapy for at least a subset of OD. Recently, different approaches based on high throughput data and computational strategies for drug repositioning have been developed. The main limitation of the current approaches is that they mostly require gene expression profiles directly relevant to the condition under study, such as those obtained from patient cells and/or from suitable experimental models. In this work we have developed a new computational strategy, uniquely based on the search for conserved anti-correlation between known drug targets and human disease genes, performed on public microarray databases. On this basis, we propose new potential candidate drug targets and drugs for rare human diseases for which no specific gene expression data are available.

P3.7
Large scale analysis of the tomato membrane proteome during fruit ripening

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As a step towards understanding the role of membrane proteins in tomato fruit ripening, a subcellular proteomic study was undertaken to provide a qualitative global analysis of total microsomes obtained from tomato mature green (MG) and red ripe (RR) fruit. Microsomes were fractionated using self-generating iodixanol gradient and a shotgun LC-MS/MS approach was used to identify tryptic peptide fragments of the collected fractions. About 2000 proteins from both MG and RR microsomes were identified, many of which are unique and developmental stage-specific. Gene Ontology term analysis, used to categorize the proteomes with respect to subcellular localization, molecular functions and biological processes, showed that proteins related to response to biotic and abiotic stress, protein metabolism, cell developmental processes and transport were the most abundant. Moreover, several cell wall proteins that are known to be localized in the Golgi or other compartments of the secretory pathway were identified. The relevance of some proteins belonging to the classes of signalling and response to stress, cellular trafficking and cell wall synthesis/modification is discussed.

P3.8
Having a look at *Microbispora* sp. ATCC-PTA-5024, a lantibiotic producer, "from the cradle to the grave" at the proteome level

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The actinomycete *Microbispora* sp. ATCC-PTA-5024 produces the lantibiotic NAI-107 which is active against vancomycin-resistant pathogens. *M*. sp. ATCC-PTA-5024 is poorly characterized and high throughput studies may give some insights to improve NAI-107 production.
A differential proteome analysis, based on 2D Fluorescence Difference Gel Electrophoresis (2D-DIGE) and mass spectrometry (MS) procedures, was carried out to unravel changes in global protein expression during producing and not-producing growth stages. This analysis revealed differential regulation for putative pleiotropic regulators, stress response factors and proteins involved in lantibiotic resistance, cell-wall biosynthesis, central carbon metabolism, amino acid and protein synthesis. Due to their expression profile and their putative molecular and cellular function, the identified proteins could play a key role in regulation of processes associated to bacterial growth and lantibiotic production.
These data, shedding light on *Microbispora* life style, could be used as a background to improve fermentation strategies or to design synthetic biology approaches aimed to increase lantibiotic yield production.

P3.9
The 14-3-3 networking in the protozoan parasite *Giardia duodenalis*

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The 14-3-3s, are a family of eukaryotic pSer/pThr binding proteins that play a pivotal role in multiple cellular processes. The flagellated protozoan parasite Giardia duodenalis is the agent of a diarrheal disease, giardiasis, and is also a valuable eukaryotic model due to its simple life cycle and cellular and genomic organization. Giardia encodes a single 14-3-3 isoform, with extensive post-translationally modifications, that is related to parasite encystation process. To characterize the g14-3-3 interactome, a transgenic Giardia line expressing a FLAG-tagged g14-3-3 under its own promoter has been generated. Affinity chromatography coupled with MS/MS analysis have been used to purify and identify FLAG-g14-3-3-associated proteins from trophozoites and encysting parasites. A total of 314 putative g14-3-3 binding partners were identified. Some interactions occured uniquely in one stage, while others were shared. Furthermore, the interaction of g14-3-3 with the giardial homolog of a cell-division-cycle protein kinase was characterized, leading to the identification of a multiprotein complex containing g14-3-3, the protein kinase and a a novel regulatory subunit of the kinase.

P3.10
Genomic organization, expression analysis and evolution of the TR loci in *Tursiops truncatus*

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The dolphin (*Tursiops truncatus*) is a mammal that, during the evolution, has adapted fully to the aquatic environment. Despite of the popularity and even of the iconic status of the dolphin, our knowledge about its immune system and evolution is very limited. Our studies focus on the genomic T cell receptor TRG, TRB and TRA/D loci organization and expression analysis of the peripheral repertoire. Sequences retrieved from the first dolphin genome assembly allowed us to determine the genomic structure of all the TR loci. TRG locus is organized in a single V-J-C cassette spanning ~ 90Kb and contains two variable, three joining and a single constant genes. The TRG repertoire was analyzed using rapid amplification of cDNA ends (RACE) and RT-PCR. The two TRGV and the three TRGJ genes were expressed in every possible rearrangement in blood and in skin, although a bias toward V1-J2 and V2-J3 was found. In particular the bias toward V2-J3 was noted in both the productively and nonproductively rearranged repertoires. The TRB locus is distributed over 5 scaffolds (~210 Kb). It contains 24 TRBV (11 predicted to be functional) genes, assigned to 18 subgroups, two clusters of 6 and 7 TRBJ genes and its organization is broadly similar to that of humans and mice. Preliminary analysis on rough sequence data reveal that dolphin TRA/D locus extends for at least 6 scaffolds and exhibits

a peculiar genomic structure with a gene content distinct from that of humans and artiodactyls.

P3.11
Genotyping and biochemical characterization of strains of *Pleurotus eryngii*

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Fungal genus *Pleurotus* includes species of economic importance such as *Pleurotus eryngii*, which consists of a complex of taxa, whose characterization have not yet been well studied. The aim of this work was to provide new tools for certification and traceability by molecular and biochemical markers useful to discriminate the varieties of *P. eryngii* species. A group of 93 isolates of *P. eryngii* belonging to both var. *eryngii* and var. *ferulae* were analyzed through sequence analysis of two housekeeping genes (*tef*, elongation factor alpha1 and *rpb2*, RNA polymerase II second largest subunit) and through enzymatic pattern (native-PAGE) of proteic markers such as catalase, peroxidase and superoxide dismutase. The polymorphism of enzymatic markers utilized, not unequivocally distinguished varieties *eryngii* and *ferulae*. Conversely, sequencing analysis of the two genes revealed the presence of some Single Nucleotide Polymorphisms (SNPs) able to distinguish the two varieties, useful to set up molecular identification assays.

P3.12
The hidden value of human whole exome sequencing

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Whole-exome sequencing (WES) is a powerful and cost-effective technology to explore variation in protein coding genes and >30,000 WES datasets are available in public repositories.
We developed a novel computational methodology to assembly mitochondrial genomes from off-target WES data. To assess the effectiveness of mitochondrial DNA (mtDNA) reconstruction with different exome enrichment systems, we analysed Illumina paired-end WES reads generated from peripheral blood of a human individual (SRA study SRP007499). We assembled over 98% of the entire mtDNA from all exome platforms (TruSeq. Illumina; SureSelect, Agilent and SeqCap EZ-Exome, NimbleGen).
Extending the study to several other WES data generated by diverse enrichment protocols, we show that exome data contain a sufficiently large number of off-target mitochondrial sequences for complete or near-complete mtDNA assembly, with efficiency depending on the exome enrichment protocol and tissue of origin. Therefore this methodology could provide valuable insights into pathological effects of mutations and structural variations of mtDNA in diverse cytotypes representing a large variety of physiological and disease conditions.

P3.13
Piroxicam and cisplatin combined treatment enhances apoptosis in mesothelioma cells *via* p21 nuclear shifting

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Drug combination has been showed to be efficacious in cancer treatment, acting on cell cycle-through pro-apoptotic proteins or specific miRNA– and enhancing tumor sensitivity to drugs.
We have analyzed the molecular mechanisms associated to the piroxicam/cisplatin treatment in mesothelioma cells by microarray analysis. We observed an increasing of p21 that plays a critical role in apoptosis induction. p21 is a cyclin kinase inhibitor responsible of the growth arrest, after DNA damage. p21 can be pro-apoptotic inducing cell death in response to chemotherapeutic agents. We found a specific p21 increase in the nucleus at protein level. This indicates that translocation of p21 in the nucleus might be associated to its specific role.
To better investigate the molecular mechanisms we analyzed the protein profiles by using a proteomic approach and identified differentially expressed proteins. Their corresponding spots on gel are under investigation by mass spectrometry.
These results in combination with the use of specific bio-informatic tools (MNI) might help to identify potential targets, useful for the design of efficacious mesothelioma therapies.

P3.14
New insights for the study of protein aggregation by the Protamine-like of the annelid worm *Chaetopterus variopedatus*

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Protein aggregation processes have been subject of several studies because of their relevance in many fields of the scientific and technological research, from medicine (Alzheimer's, mad cow disease, cancer development) to alimentary sciences. Also ionic interactions play a role in protein aggregation and the basic protein *Chaetopterus variopedatus* Protamine-like (*Cv*PL), is an ideal model for study of protein aggregation and for the effects induced by metals. *Cv*PL is precipitated from its solution by addition of SDS and has an oligomeric structure depending on anion-mediated lysine arginine interactions. We analyze the effect of different salts and metals on *Cv*PL solubility in SDS. The results show that only copper is able to make the protein soluble in SDS. Furthermore, *Cv*PL in the presence of copper chloride ($CuCl_2$) has a mobility in SDS-PAGE, which corresponds to molecular weight lower than the real, that is 8300 Da. In addition, *Cv*PL acquires also Proteinase K digestion resistance in the presence of $CuCl_2$, that induces *Cv*PL conformational changes as confirmed by circular dichroism and fluorescence spectroscopy.

P3.15
Genome wide analysis and insertion polymorphism of the Equine Repetitive Element ERE1

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Taking advantage of the recent publication of the horse genome sequence, we carried out a genome wide analysis of the perissodactyl-specific SINE family of ERE elements (Equine Repetitive Element). We evaluated the frequency of insertion polymorphism of the youngest ERE subfamily (ERE1) both by bioinformatic analysis and by *locus* specific PCR. We demonstrated a strong correlation between sequence conservation and percentage of polymorphic elements: 30 % of the elements with more than 98% identity to the *consensus* are polymorphic. About 46% of the ERE1 *loci* are inside introns; since intronic sequences cover about 26% of the mammalian genomes, we can argue that ERE1 elements are preferentially located in gene rich regions. We identified several ERE1 *loci* located less than 1 kb from the 5' end of genes, thus suggesting that they might have a role in modulating gene expression. It

must be underlined that 3 of these 5' proximal *loci* are polymorphic in the horse population; thus, they could give rise to horse specific variants in gene expression.

P3.16
Mrs metabolic characterization of idh1-mutated non tumoral cells

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In lower grade human gliomas, mutations in the isocitrate dehydrogenase (IDH) family are relatively early and common events. The most common IDH mutation is the aminoacid substitution of arginine 132 with an histidine in the cytosolic IDH isoform (IDH1) . This "gain of function" mutation imparts the ability to convert α-ketoglutarate in 2-hydroxyglutarate (2-HG). Recent reports correlate this mutation with extensive DNA hypermethylation and more favorable outcome in gliomas patients. Here, we investigated the magnetic resonance spectroscopic (MRS) metabolic alterations in human embryonic kidney (293T) cells with overexpressing mutated IDH1 $^{(R132H)}$. MRS analysis was performed on cells transfected with the wild type and mutated $^{(R132H)}$ IDH1 cDNA at 24 and 48 hours.
We detected 2-HG only in IDH1^{R132H} cells at 24 and 48h after transfection with concentrations respectively of 11.04±0.52 and 13.00±4.43 nmol/10^6 cells, and a reduction of 35% (p=0.004) of glutamate at 48h post IDH1 ^{R132H} transfection. Our results show that MRS could provide the possibility to monitor the metabolic alterations associated with IDH1 mutant tumors and allow to better characterize IDH1 tumor subtype.

P3.17
Identification, synthesis and antibacterial activity of a novel antimicrobial peptide from tomato

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Defensins (also known as gamma thionins) are a class of cysteine-rich proteins which exerts broad-spectrum antimicrobial activities. In this work we identified in the tomato genome 17 putative plant defensins by bioinformatics analysis. All proteins, with the exception of 2, had a mature peptide that includes eight conserved cysteines involved in disulfide bonds essential for structural folding. In addition, all the identified sequences contain a highly conserved γ-motif that consists of two antiparallel β sheets connected through a loop. We synthesized the γ-motif of one the identified tomato defensin which is composed of 16 amino acids and characterized by a total net charge of +5. The antimicrobial activity of the synthesized peptide has been tested against Gram-positive and Gram-negative bacteria. Preliminary data suggest that the peptide has a bactericidal activity against Gram-negative and a bacteriostatic activity against Gram-positive bacteria. The peptide did not display haemolytic activity and tested onTHP-1 cell line showed anti-inflammatory activity and no cytotoxic effects.

P3.18
3D conformational analysis of the immunoglobulin 3'regulatory region

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The human Immunoglobulin (Ig) heavy chain cluster encompasses a duplication of four constant genes together with the 3' Regulatory Region (3'RR) harbouring three enhancers. A long internal palindromic region embeds the central enhancer hs1.2 that is surrounded by the enhancers hs3 and hs4. This palindrome keeps only short portions of sequence similarity among the different species, but is observed in all the mammal genomes that we analysed, suggesting a conservation of the function. The consensus on the genomic sequence for the formation of DNA protein complexes induces 3D structures required for activation of the different genetic territories. New studies on conformational and nuclear localisation of chromatin evidenced the physical contact of the enhancers with the 5' Ig promoter by means of 3C and 4C analyses with ChIP. The polymorphic region of hs1.2 has a predicted tetraplex structure potentially formed by a quartet of guanine with intra or intermolecular DNA looping. We analysed with NMR the tetraplex formation in vitro on DNA oligos corresponding to the predicted sequence. Specific spectra indicating the presence of tetraplex structures are observed. We also studied the feasibility of SNPs frequency analyses for the 3'RRs by means of the 1k Genomes Project database, ascertaining the limits of this tool when duplicated region are under study.

P3.19
On the possibility that *Mytilus galloprovincialis* sperm chromatin has a nucleosomal organization

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The nuclei of the sperm of the marine bivalve mollusks contain a complex protein mixture in which PL-type coexist with a complete set of somatic histones. The informations concerning the organization of sperm chromatin mediated by P-likes are very few. In *Mytilus galloprovincialis*, the nuclear protein composition consists of about 20% histones and three P-likes: PLII, PLIII and PLIV that represent about 80% sperm-specific protein component. The three proteins differ in lysine and arginine content and for their K/R ratio that affect their DNA binding mode and self-association ability. Despite analyses by micrococcal nuclease digestion show the lack of a nucleosomal organization, we present an hypothetical nucleosomal chromatin structure model in which core histones form nucleosomes maintained by PL-II together with PL-IV to form an histone H1-like. PL-III instead probably interacts with DNA-linker that is longer than that of somatic cells. This model is supported by the fact that PL-II and PL-IV have the same DNA binding mode of somatic H1 histone while PL-III has the DNA binding mode of sperm H1 histone. In addition PL-II and PL-III have different self-association ability.

P3.20
Variant detection in genomes of somatic mutants and their characterization

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Somatic mutations that accidentally happen in buds can be vegetatively propagated in many plants and are important for the breeding of some fruit crops. In this work we want to explore the molecular bases of

grapevine somatic mutants selected for their distinguished phenotype. In particular, we aim to identify differences in the clonal genomes within a plant variety and eventually, to discover the mechanisms that generate somaclonal variability. *Vitis vinifera* is an ideal model because: there are many clones with accumulated somatic mutations and a high quality reference genome sequence is available.
Due to the low frequency of the events to be detected, the molecular tools used that include deep sequencing (whole genome scanning approach through Next Generation Sequencing), algorithms for specific variant detection, and a bioinformatic pipeline for automated analysis must be fine tuned to avoid the detection of false positives. We will be focusing on three aspects of variation by looking at the genomic DNA level, at the gene expression level and finally at the the epigenome level always using Next Generation Sequencing methods.

O3.1
Oxidized Peroxiredoxin 2 levels increase in the nucleus of temperature-entrained human keratinocytes.

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A metabolic circadian clock based on the cyclic oxidation of six antioxidant enzymes, the peroxiredoxins (PRDX1-6), has been recently described. In this study, we addressed the existence of such clock in the human HaCaT keratinocytes. The cell clock was entrained by two temperature cycles (12h 37°C, 12h 33°C). Cell fractions were isolated before (T0) and after clock (T8) synchronization. Proteomic analysis revealed PRDX2 in the nuclear fraction of synchronized cells. WB and confocal immunofluorescence on independent experiments confirmed PRDX2 nuclear localization and its increased levels at T8. The oxidized form PRDX $SO_{2/3}$ displayed a similar trend. No changes were observed for PRDX1 and PRDX6. Finally, WB in non reducing conditions demonstrated that PRDX2 was present as an active dimer and increased in the nuclear fraction upon clock synchronization. Our work demonstrates for the first time the presence of a metabolic clock in human keratinocytes. The PRDX2 nuclear localization suggests a possible interplay with the transcriptional clock, opening new perspectives for the study of that patho-physiological processes which are known to be circadianly regulated in the skin.

O3.3
Yeast toxicogenomics: a system biology approach to study the response to 5-fluorouracil and nystatin

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Saccharomyces cerevisiae is used as an eukaryotic model for toxicogenomics studies. We took advantage of two main features that this system offers: i) the availability of heterozygous/homozygous diploid and haploid gene deletion mutants collections with a full barcode gene identification system; and ii) the existence of complete genomic, transcriptomic and proteomic datasets. The coupling of the classical top-down approach (using the mutants), the bottom up approach (starting from proteomic or transcriptomic targets) with a system biology approach, has been used to provide a comprehensive knowledge of the molecular and cellular effects of chemicals in biological systems. For two different types of pharmaceutical drugs, 5-fluorouracil, a widely used anti-tumoral agents with strong cytotoxic effects, and nystatin, an antimycotic agent, which also has toxic effects on eukaryotic cells, we have studied mutants targeting, RNA effects and physiological effects. The results obtained have been analyzed with a system biology approach.

O3.4
Genes responsible for mitochondrial dysfunction in Down syndrome

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The upregulation of chromosome 21 (Hsa21) genes in trisomic human fetuses causes the downregulation of genes coding for mitochondrial proteins. We are interested in demonstrating if and how this contributes to the mitochondrial dysfunction observed in cells from Down syndrome subjects.
To this aim we investigated the expression of Hsa21 and mitochondrial genes, and we characterized the mitochondrial defect, in trisomic human fetal fibroblasts (HFF). Gene expression analysis confirmed the upregulation of Hsa21 genes and the dysregulation of mitochondrial genes in trisomic HFF. We focused on the Hsa21 genes, RIP140, DYRK1A and RCAN1, and on the mitochondrial genes, PGC-1α, NRF1 and IMMT. From our studies we propose that overexpression of the repressor protein RIP140 causes the dowregulation of PGC-1α, a key gene in mitochondrial biogenesis and function. The upregulation of DYRK1A and RCAN1 might further reduce PGC-1α expression via the CaN/NFATc pathway. The reduction in PGC-1α might increase ROS directly and might increase mitochondrial Ca2+ levels via PPARγ. PGC-1α might also affect mtDNA copy number, by decreasing NRF1, and cristae pattern and integrity, by decreasing IMMT.

O3.5
Analysis of the RND superfamily in the *Burkholderia* genus: evolution and putative physiological role

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The genus *Burkholderia* includes a variety of species, with opportunistic human pathogenic strains, whose increasing resistance to antibiotics has become a public health problem. A major role in multi-drug resistance could be played by multidrug efflux pumps belonging to the RND superfamily, an ubiquitous group of proteins that appears to be mainly involved in antibiotic resistance in Gram- bacteria.
We performed a deep analysis of the distribution of representatives of all the 8 families belonging to this superfamily in 26 *Burkholderia* completely sequenced genomes, to evaluate their possible use as a target for novel antimicrobial drugs. A total of 417 putative RND proteins were identified. Through different *in silico* analyses, most of these sequences were characterized and a core of proteins conserved in all *Burkholderia* genomes was identified.
The whole body of data obtained on the presence and distribution of RND proteins in *Burkholderia* genus shed some light on both the physiological role(s) played by these proteins and their evolution in this genus; besides, the core of proteins identified might serve as a basis for future experimental tests aimed at checking their possible use as novel targets in antimicrobial therapy against *Burkholderia* species.

O3.6
IT Future of Medicine: integration of -omics data into personalised medicine

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The IT Future of Medicine (ITFoM) initiative will produce computational models of individuals to enable the prediction of their future health risks,

progression of diseases and selection and efficacy of treatments while minimizing side effects. To be able to move our health care system to treat patients as individuals rather than as members of larger, divergent groups, the ITFoM initiative, with more than 120 academic and industrial partners from 31 countries, proposes to develop a new, data rich computational model based on integrated molecular, physiological, and anatomical data of every person ("Virtual patient"). As one of six Future and Emerging Technologies (FET) Flagship Pilot Projects funded by the European Commission, ITFoM will foster the development in functional genomics and computer technologies to enable the generation of "Virtual patient" models to make them available for clinical application. The establishment of such "Virtual patient" models is now possible due to the enormous progress in analytical techniques, particularly in the '-omics' technology areas and in imaging, as well as in sensor technologies.
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4 - Chromosome biology and dynamics

P4.1
Functional analysis of mammalian centromeric domains by DNA fiber immuno-FISH

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The horse represents a unique model system for the study of centromere function. While the majority of centromeres contain satellite DNA, the centromere of chromosome 11 is completely devoid of repetitive DNA (Wade et al., 2009, Science 326: 865-867; Piras et al., 2010, PLoS Genet. 6: e1000845.). Here we present data on the architectural organization of horse centromeric DNA and on DNA/protein interactions at the single centromeric domain level. To this purpose, we set up *ad hoc* high-resolution molecular cytogenetic procedures. Our results indicate that the two satellites are arranged in alternate arrays intercalated by sequences still to be identified. We demonstrate that the centromeric function involves only a portion of the extended satellite array at each centromere. Finally, we give evidence that the single copy centromere of horse chromosome 11 has an about 400 bp long "centromerizable" domain which, in some individuals, can be organised in two distinct subdomains, only one of which is functionally active in each homologous chromosome.

P4.2
The Drosophila telomeric protein Verrocchio binds single-stranded DNA

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Telomeres are nucleoprotein complexes that protect the ends of eukaryotic chromosomes. In most organisms, telomeres consist of short G-rich repeats, which are added to chromosome ends by telomerase, and end in a 3' single-stranded overhang. In humans, telomeres are protected by six specific telomeric proteins forming the shelterin complex. On the contrary, Drosophila telomeres are maintained by transposition of three specialized retroelements, and do not terminate with GC-rich repeats. Most of the Drosophila telomere-related proteins so far identified have clear human counterparts, with the exception of HOAP, HipHop, Modigliani (Moi) and Verrocchio (Ver). HOAP, Moi and Ver form a telomere-capping complex, called terminin, having the same properties of human shelterin: a specific telomeric localization and a telomere-limited function. Our study focused on Ver, an OB-fold containing protein, that binds ssDNA in a non-specific manner. We examined its capability to bind different ssDNA sequences, also in competition with dsDNA, and the loss of this capability in a dysfunctional mutant. We further investigated the influence of other terminin proteins on Ver binding.

P4.3
Relationships between telomeric protein dysfunction and aneuploidy in aged women

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Aneuploidy has severe consequences on human health at both somatic and germ cell levels. Among factors affecting the fidelity of chromosome segregation, aging has a prominent role, although it is not completely clear which are the age-related mechanisms possibly implicated. A study has been undertaken to evaluate whether a decrease in the function of telomeric proteins could be associated with chromosome missegregation in aged individuals. Preliminary results on the expression of 84 genes central to telomere replication and maintenance, evaluated in young (<35 years) and aged women (>55 years), showed significant differences between the two groups in the expression of genes of the shelterin complex (TINF2, TPP1, OBFC1) and of genes regulating the telomerase activity (DKC1, PAX8, TERT). Interestingly, it has recently been demonstrated a pivotal role of TINF2, DKC1 and TERT in telomere replication, recombination and cohesion, which are key processes controlling accurate chromosome segregation. Work is in progress to evaluate whether these pathways are perturbed in elderly women.

P4.4
Cohesin mutations impair the recriutment of RNA polymerase II at the promoter

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Cohesin ensures correct chromosome segregation by holding sister chromatids together from the S phase until their separation in anaphase. Mutations in cohesin and regulatory cohesin genes, NIPBL, SMC1A and SMC3 are associated to Cornelia de Lange syndrome (CdLS), a rare human development disorder. Since CdLS cell lines show no clear defects in sister chromatid cohesion, the molecular basis underlying CdLS remains elusive. Interestingly, data showing that cohesin is also involved in gene expression regulation has accumulated over recent years. To define the role that cohesin plays in the correct assembly of the transcription complex, we analyzed the recruitment of RNA polymerase II (pol II) at the promoter regions of expressed genes, in SMC1A-mutated cell lines. We found that the recruitment of RNA pol II is strongly reduced in all cell lines, irrespective of SMC1A mutations. This work emphasizes the role of cohesin in transcription regulation and provides new clues to the CdLS pathogenesis.
This work was supported by a grant from Tuscany Region to AM

P4.5
$p14^{ARF}$ re-expression induces apoptosis in aneuploid HCT116 cells

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Weakening the Spindle Assembly Checkpoint by reduced expression of its components such as MAD2, BubR1 and MPS1 induces chromosome instability and aneuploidy both hallmarks of cancer cells. $p14^{ARF}$ that is found frequently altered in human cancers, is overexpressed in response to oncogenic stimuli to stabilize p53 halting cell progression. Previously, we determined that lack or reduced expression of $p14^{ARF}$ is involved in the maintenance of aneuploid cells suggesting that it could be part of a pathway controlling proliferation of aneuploidy cells. To investigate further this aspect of $p14^{ARF}$ function it was ectopically expressed in HCT116 cells, a stable near diploid cell line, after MAD2 depletion used as a trigger for aneuploidy. We observed reduction of aneuploid cell numbers as well as decrease of spindle alterations and mitotic abnormalities in these cells in comparison to MAD2 post-transcriptionally silenced cells. In addition $p14^{ARF}$ ectopic expression in MAD2 depleted HCT116 cells induced apoptosis accompanied by increase of p53 and $p21^{WAF1}$ protein levels. Altogether these results suggest that $p14^{ARF}$ could prevent proliferation of aneuploid tumor cells.

O4.1
TRF2 regulates nucleosome density and spacing at human telomeres

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Mammalian telomeres stabilize chromosome ends as a result of their assembly into a peculiar form of chromatin comprising a complex of non-histone proteins: shelterin. TRF2, one of the shelterin components, binds to double-stranded telomeric DNA and is required to fold the telomeric chromatin into a protective cap. Although telomeric chromatin contains nucleosomes, their relationship with shelterin and their role at telomeres are still elusive. In this study, we found that nucleosome density at telomeres is inversely dependent on TRF2 dosage; this remodeling effect coincides with late or post-replicative events. In addition, TRF2 overexpression alters nucleosome spacing increasing internucleosomal distance. In agreement with in vivo data, we found that addition of TRF2 to in vitro assembled nucleosomal arrays caused an increase of spacing between telomeric nucleosomes. We conclude that TRF2 negatively regulates nucleosome density at telomeres in a cell cycle-dependent mechanism altering the inter-nucleosomal distance. These findings raise the intriguing possibility that the control of nucleosome density by TRF2 contributes to telomere protection.

O4.2
AKTIP, a conserved E2 variant enzyme that interacts with lamins and protects mammalian telomeres from replicative damage

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Telomere maintenance is a process needed to protect the genome against instability and telomere dysfunction linked to tumorigenesis and premature aging. Driven by results assigning a telomeric role to its Drosophila homologue peo, we studied the human gene, AKTIP (Ft1 in mouse). AKTIP KD triggers proliferation impairment and senescence. Ft1 KD p53-/-MEFs show telomeric aberrations, including multiple telomeric signals, indicative of telomere replication impairment. AKTIP mechanistic role appears, indeed, to be linked to replication: AKTIP interacts with DNA and with replisome components (RPA and PCNA), and AKTIP KD cells display an intra-S block. A seducing aspect of AKTIP comes from its localization, a typical punctate signal at the nuclear rim. This pattern is consistent with the interaction of AKTIP with nuclear lamins, which we assessed by GST-pull down, and also with that with components of the replication forks (PCNA), which situate at the periphery of the nucleus in the final part of S-phase.
Our data suggest that AKTIP could become a new important player of the mechanistic scenarios of human diseases linked to "telomeraging" including cancer and laminopathies.

O4.3
The dark matter of the evolution: genetic variability at work in clonal lineages of aphids

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Aphids reproduce primarily by apomictic parthenogenesis, so their offspring should represent a genetically identical lineage. Conversely, literature data indicate the presence of genetic variability (karyotype variations affecting host choice and pesticide resistance) within clonal lineages. FISH experiments carried out on Myzus persicae strains collected on tobacco plants revealed variant karyotypes due to recurrent fragmentations, not only between different individuals within the same asexual lineage, but also within each embryo. Chromosome mosaicism is a peculiar feature of malignant cells, whereas it is a very rare phenomenon in whole organisms. The clastogenic effect of nicotine could have a pivotal role in M persicae chromosome fragmentations. The holocentric nature of aphid chromosomes together with the high telomerase expression and the obligate apomictic parthenogenesis could be at the basis of the stabilization of the observed chromosome instability. The idea that aphid populations are genetically stable on time and space is therefore erroneous but aphid populations seem to be the sum of specimens that can have different karyotypes and could consequently give different responses to the selective environmental forces.

O4.4
Proteomic mapping of the the euchromatic modificome and interactome at enhancers and promoters by combining ChIP and MS analysis

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Histone post-translational modifications (hPTMs) play important roles in modulating chromatin structure, influencing DNA transcription, replication and repair. The"histone code hypothesis" proposes the existence of an hPTMs language that code for specific chromatin functional states. hPTMs are typically studied using either antibodies or mass spectrometry(MS) allowing a global view on bulk chromatin. Recently a few studies tried to carry out proteomics analysis of hPTMs and readers at specific chromatin loci; our team has implemented the Proteomic Mapping of Immuno-purified Chromatin(ProMIC) approach, where antibodies are used to enrich specific chromatin portions and MS-based quantitative proteomics is employed to characterize hPTMs/variants and protein interactors co-enriched in those regions. Here we describe the application of ProMIC to characterize the modificome and interactome of transcription start sites(TSSs) and enhancers, marked by H3K4me3 and K4me1 respectively. We identified hPTMs and complexes showing an euchromatic localization (like H3K79men, H3K18/K23ac/ac2 and FACT complex) and other features overrepresented at enhancers like H3K36me2, SET complex; interestingly in these loci we identified WD40 or PWWP domains containing proteins that could be direct binders. In perspective we plan to use the PromiC approach in RAW264 cells, resting and LPS-stimulated, in order to analyze dynamic changes of hPTMs and proteins in euchromatin upon stimulus.

5 - DNA Replication, Repair and Recombination

P5.1
BRC/Fanconi pathway in the interstrand crosslinking sensitivity and in the development of the model system *Caenorhabditis elegans*

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The BRC/Fanconi pathway comprises a large network of genes whose defects are associated with cancer-prone syndromes, sensitivity to mutagens and developmental defects. The pathway is extremely complex and branches to intersect with cell cycle control and apoptosis, making it difficult to understand essential interactions and redundancy. Genes in the pathway are evolutionary conserved, allowing dissection and mechanistic studies in model systems, such as the nematode *C. elegans*.
We have recently demonstrated that the BRCA1 gene is essential for DNA repair in *C. elegans* (Adamo et al., 2008), and inactivation of the Non-Homologous End Joining (NHEJ) pathway suppresses genomic instability due to defects of the BRC/Fanconi pathway in *C. elegans* and human cells (Adamo et al., 2010).
We intend to make use of *C. elegans* genetic and biochemical screenings in order to identify new genes interacting with the BRC/Fanconi pathway and to clarify the mechanisms of cross-talking between this pathway and the NHEJ.
In particular, the effect of mutations in genes of this pathway on developmental steps will be analysed.

P5.2
Genotoxic effects of *Tdp1α* (Tyrosyl-DNA Phosphodiesterase) depletion in *Medicago truncatula*: impairment of DNA repair pathways and enhancement of cell death fate

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An intron-spliced hairpin RNA approach was used for the silencing of the *MtTdp1α* gene, encoding the α isoform of tyrosyl-DNA phosphodiesterase 1 in *Medicago truncatula* Gaertn. Tdp1, involved in the repair of DNA topoisomerase I-mediated DNA damage, has been poorly investigated in plants (Macovei *et al*., 2010, *Planta* 232: 303-407). The reduction in mitoses frequency and the increase in percentage of PCD events positively correlated with the extent of *MtTdp1α* down-regulation while double strand breaks were the predominant lesions. Differently from animals, the plant cells lacking *MtTdp1α* do not rely on alternative repair pathways for the removal of the topo I-mediated DNA damage. The nucleolus of *MtTdp1α*-depleted cells showed a collapsed structure flanked by chromatin aggregates, highlighting the requirement for the *MtTdp1α* gene in maintaining the nucleolar function. An overall picture of the role played by this gene in the DNA repair response of plant cells is presented, uncovering the possibility that a nucleolar checkpoint might be activated.

P5.3
Role of Werner syndrome protein in the maintenance of common fragile site stability

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Common fragile sites (CFS) are genomic regions unusually prone to breakage following DNA replication stress and are often found rearranged in cancer cells. We have recently unveiled a key role of the WRN helicase activity in protecting cells against CFS expression but the molecular events that lead from replication perturbation to DNA breakage at these sites are incompletely understood. We investigated how WRN affects activation of the ATR-dependent checkpoint following replication perturbation specifically induced at CFS and if WRN and its key interactor WRNIP1 collaborate to CFS stability.
Our data demonstrate that loss of WRN leads to impaired phosphorylation of ATR downstream targets, such as CHK1 and γH2AX. Also, fractionation experiments show an impaired RPA accumulation in chromatin prepared from WRN-deficient cells.
Furthermore, WRN associates with WRNIP1 after CFS-perturbing treatment and down-regulation of WRNIP1 induces CFS expression. Moreover, loss of WRN results in increased chromatin accumulation of WRNIP1, suggesting a more general regulatory role of WRN.
Further experiments are ongoing to provide mechanistic insights into the pathway ensuring CFS stability.

P5.4
Evolutionary conserved mechanisms of genome stability, repair and protection

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The genome of all organisms is threatened by endogenous and environmental factors. Malfunctioning of DNA repair and recombination systems may cause genome instability, which has broad effects on aging, cancer predisposition, fertility and development. Dissection of the cascades of events triggered by DNA damage and the underlying networks of interacting proteins is a major challenge of post-genomic biology. As the protein factors involved in these processes have been conserved over billion years of evolution, great progress in the field of DNA repair has been driven by the use of model systems.
We are investigating mechanisms of genome stability, repair and protection in hyperthermophilic Archaea, microorganisms living at temperatures above 80°C, which makes the maintenance of an intact and functional genome a great challenge. Using a combination of in vivo and in vitro biochemical approaches, we have investigated the activity, function, regulation, physical and functional interaction of hyper-thermostable proteins able to protect and repair damaged DNA or modify its structure. Our results might help understanding the function and relationships of these essential proteins.

P5.5
RNA-Seq analysis discloses early senescence and nucleolar disfunction triggered by *TDP1α* depletion in *Medicago truncatula*

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The stabilized topo I/DNA covalent complexes, which occurs in presence of damages DNA sites, can be removed by tyrosyl-DNA phosphodiesterase I (Tdp1) to prevent deleterious lesion such double strand breaks. The Tdp1 function is poorly investigated in plants. RNA-Seq analysis was carried out in *MtTdp1α*-depleted *Medicago truncatula* Gaertn. plants, obtained with ihpRNAi approach. Different levels of transcriptional modulation (up- and down-regulation, alternative splicing, activation of alternative promoter) in genes involved in DNA damage sensing, DNA repair and chromatin remodeling were highlighted, suggesting for novel roles of the *MtTdp1α* gene in maintaining genome integrity. Up-regulation of senescence-associated

genes and telomere shortening were observed while there was evidence of impaired ribosome biogenesis. This finding is consistent with the key role of topo I within the nucleolus and suggests the requirement for the *MtTdp1α* gene to maintain the nucleolar function. Finally, alteration of cuticle observed in the *MtTdp1α*-depleted lines might reflect the cross talk existing between defense and senescence or existence of a general stress response activated in these plants.

P5.6
Increased levels of p21CDKN1A do not inhibit the recruitment of NER factors at DNA damage sites

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P21CDK1NA is a cyclin-dependent kinase inhibitor playing multiple roles also in the DNA damage response. Therapeutic trials have been developed to contrast tumor cell proliferation, by exploiting the p21 ability to arrest the cell cycle; in particular, proteasome inhibitors increase p21 protein levels, impairing tumor cell growth. However, this approach is may be potentially dangerous because high p21 levels inhibit the apoptotic response and allow DNA repair, rendering tumor cells resistant to chemotherapy. We have investigated whether the accumulation of p21 levels, induced by the inhibitor of proteasome MG132, may affect nucleotide excision repair (NER) and apoptosis. The results have shown that MG132 induced persistent increased levels of XPC, PCNA and p21 proteins at local DNA damage sites, together with accumulation of XPG, DNA polymerase δ and CAF-1, suggesting that the presence of p21 protein did not block the recruitment of NER factors interacting with PCNA. Immunoprecipitation experiments have shown that DNA pol δ interacts with an ubiquitinated form of p21. These results indicate that p21 regulates steps of NER before degradation.

P5.7
HIV-1 Integrase and ribonuclease H inhibition by pyrrolyl diketohexenoic acids derivatives

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HIV-1 integrase (IN) and Ribonuclease H (RNase) H belong to a polynucleotidyl trasferases class and share structural similarities among which the coordination of two Mg2+ ions in their active sites required to catalyze their reactions. Several compounds initially developed as HIV-1 IN inhibitors have been also screened against HIV-1 reverse transcriptase associated RNase H function. Among them, diketo acid (DKA) derivatives are able to chelate divalent metal ions present in the active site of IN and are capable to inhibit also the HIV-1 RNase H function. The simultaneous inhibition of both HIV-1 IN and RNase H activities is an innovative approach to develop dual inhibitors.
We have reported that DKA derivative RDS 1643 inhibit the HIV-1 RNase H activity with IC50 value of 13 μM and it slightly inhibited the HIV-1 IN reaction (IC50 value of 92-98 μM). Moreover, in cell-based assays it was able to block the replication of wild type HIV-1, showing an EC50 value of 13 μM and a CC50 value of 63 μM. We have synthesized and tested a series of RDS1643 derivatives against both HIV-1 IN and RNase H RT-associated functions and the results shown that a series compounds are dual inhibitors with an IC50 value between 0.042 and 10 μM.

P5.8
Transgenic *M. truncatula* lines overexpressing the DNA repair *Tdp2* gene: molecular characterization

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The 5'-Tyrosyl-DNA Phosphodiesterase enzyme (Tdp2) is able to resolve the covalent complexes between DNA Topoisomerase II (topo II) and the oxidized nucleotide 8-oxo-dG (Zeng et al. 2011, J. Biol. Chem. 1:403-9). This novel DNA repair function has been described only recently in animal cells while information on the role played in planta is still lacking. We report on the isolation and molecular characterization of *MtTdp*2 gene in the model plant *Medicago truncatula*. Bioinformatic analyses revealed the presence of a putative chloroplast transit peptide and different Tdp2 isoforms were also identified in the plant kingdom. The *MtTdp2* gene is up-regulated in response to photo-oxidative stress and during photomorphogenesis, while no significant up-regulation was observed in response to the topo II inhibitor etoposide. In order to elucidate if *Tdp2* overexpression might lead to increased stress tolerance, transgenic lines overexpressing the *MtTdp2* gene have been produced and analyzed.

P5.9
Identification of a novel ubiquitin mark at the N-terminal tail of histone H2As involved in the response of DNA damage

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Histone ubiquitination has recently gained enormous attention due to the involvement in important cellular processes count in genome stability and transcriptional regulation. Detection of DNA double-strand breaks on chromatin are under control of regulatory ubiquitination events governed by the RNF8 and RNF168 ubiquitin-ligases that, in concert with the E2 conjugating enzyme Ubc13, modify chromatin by ubiquitinating histones H2A and H2A.X in the proximity of damage. The only known ubiquitination site on histone H2As is represented by a conserved Lys residue at the C terminus of the proteins (K119). Here, we describe for the first time a novel ubiquitination site at the N terminus of histone H2As, formed by two Lys residues at positions 13 and 15 (K13/K15), and required for RNF8/RNF168- and etoposide-dependent ubiquitination of histones H2As.
This unprecedented result indicates that DNA lesions induce a qualitatively different ubiquitin signal on chromatin, by means of the ubiquitin ligases RNF8 and RNF168. This discovery opens new perspectives in the field of chromatin modifications, suggesting that additional epigenetic marks can be appended to histones under particular cellular conditions to transmit specific signals.

P5.10
Mutator phenotype associated with inactivation of the MUTYH DNA glycosylase in human patients affected by MUTYH-associated polyposis

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The MUTYH DNA glycosylase counteracts the mutagenic effects of 8-oxo-7,8-dihydroguanine (8-oxodG) by removing adenine opposite the oxidized purine. In humans biallelic germ-line mutations in MUTYH cause the autosomal recessive MUTYH-associated adenomatous

polyposis (MAP). We identified a considerable variability in the spontaneous mutator phenotype associated with inactivation of the MUTYH gene in lymphoblastoid cell lines (LCL) derived from MAP patients harbouring different mutations. To investigate to which extent specific MUTYH mutations and/or the level of oxidative stress affected the mutator phenotype, mutation frequency was measured at the PIG-A gene in LCLs derived from two sisters affected by MAP and harbouring the same homozygous mutation (Y179C, one of the most common ones). Both spontaneous mutagenesis and oxidant-induced mutation frequencies were evaluated together with the level of DNA damage as provided by measurements of 8-oxodG in DNA. The results support the pathogenic role of this MUTYH mutation and identify the mutator phenotype as a possible relevant factor for a better clinical assessment of MUTYH variant pathogenesis.

P5.11
Investigating the cross-talk between the caretaker gene WRN and the S-phase checkpoint pathways

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An accurate response to stressed replication is crucial for maintaining genome stability and requires both ATR and ATM functions.
We recently demonstrated that the WRN RecQ helicase is directly phosphorylated by ATR and ATM at multiple S/TQ sites, both in vitro and in vivo, in response to replication fork arrest. However, whether a regulated WRN phosphorylation by ATR and/or ATM is required for a correct checkpoint response is unknown. To this aim, we generated multiple WRN phosphorylation mutants and evaluated CHK1 phosphorylation as well as replication fork recovery and DNA damage after treatment with HU or CPT.
Our data suggest that after 2mM HU or 5μM CPT (high-dose) expression of an unphosphorylable WRN allele (WRN6A) increases CHK1 phosphorylation as compared with the wild-type cells. In contrast, expression of the WRN6D phosphomimetic mutant results in less than wild-type levels of CHK1 phosphorylation. Interestingly, at 50 nM CPT (low-dose), CHK1 phosphorylation is impaired by WRN6A but enhanced by WRN6D. Experiments are ongoing to investigate the functional relevance of the observed defective CHK1 activation and to unveil a phosphorylation-dependent WRN-CHK1 interaction.

P5.12
The role of the nucleotide excision repair protein XPA in the control of oxidative stress

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In a previous study, using a panel of nucleotide excision repair (NER) defective mouse embryo fibroblasts (MEF), we demonstrated that base excision repair (OGG1) and NER factors (XPC, XPA and CSB) all contribute to the repair of 8-oxoguanine (8-OH-Gua) with OGG1 as the main determinant of 8-OH-Gua DNA levels. The involvement of XPA was also confirmed in primary fibroblasts from three XP-A patients that, upon treatment with the oxidizing agent KBrO3, showed accumulation of 8-OH-Gua and a slight hypersensitivity as compared to normal fibroblasts (Parlanti et al., submitted) . Here, we show that XP-A fibroblasts are also hypersensitive to ionizing radiation. To gain insights into the molecular mechanisms underlying the hypersensitivity of these cells to oxidizing agents, the redox status of XP-A primary fibroblasts was analysed. ROS levels were measured by oxidation of the spin probe CPH to CP* by EPR. The steady-state ROS levels were significantly higher in primary fibroblasts from XP-A donors as compared to normal. This alteration was associated with a perturbation of the oxidative metabolism in all XPA-defective fibroblasts as analysed by ^{1}H-NMR. Experiments are in progress to understand the role of XP-A in the control of both redox balance and energy metabolism.

P5.13
The dual role of Cockayne syndrome proteins in the response to DNA damage

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The neurological defects in Cockayne syndrome (CS) may be due to loss of mitochondrial function upon oxidative stress, whereas the impaired transcription-coupled repair could account for the skin photosensitivity (Cleaver, Nature Genetics, 2012).
In this study we addressed the question of whether different functions of CS proteins are involved in the susceptibility to exogenous stressors. CS cells are hypersensitive to the mitochondria specific inhibitor menadione (MND) and to methyl methane sulphonate (MMS). Normal human primary fibroblasts exposed to MND showed higher 8-oxoguanine levels in mitochondrial (mt) than nuclear (n) DNA. In the case of CS fibroblasts no induction over background was recorded in both mt and nDNA. The increased sensitivity of CS cells to MND is not related to increased mtDNA damage but is likely due to bioenergetics unbalance.
Following MMS exposure, in CS cells a slight accumulation of DNA breaks as well as increased phosphorylation of H2AX were observed, mostly in S-phase cells. All together these data show that the role of CS proteins in the response to different types of stressors reflect their dual function at nuclear and mitochondrial level.

P5.14
The human LamB2 replicator prevents gene silencing by a novel insulator element

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Gene silencing (GS) is an epigenetic event involved in gene regulation during development. By altering the expression of genes, such as oncosuppressors, GS may participate in cancer formation and progression. GS is also a major drawback for the production of recombinant proteins of biomedical relevance and gene therapy. Origins of DNA replication (ORIs) may prevent GS of nearby transgenes. In metazoans, ORI density is correlated with genomic landscapes with a strong association between ORIs and transcriptional regulatory elements. Thus, replication and transcription appear to be linked to chromatin conformation by yet largely unknown mechanisms.
In this work, we have been investigating the genetic and/or epigenetic mechanisms involved in gene silencing prevention by the LamB2 replicator that triggers DNA synthesis upon integration of a 1.2kb LamB2-ori DNA fragment into nearly all genomic sites. We identified two DNA elements, one required for triggering DNA synthesis (LB2-core) and the other (LB2-IE) for preventing gene silencing. The latter is a novel insulator linked to transcriptional regulatory elements of the nearby transgenes likely by chromatin loop formation.

P5.15
Tab2 controls transcriptional response to estrogen receptor of genes related to cell proliferation and genome stability in drug-resistant breast cancer cells

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Tab2 mediates response to different transduction pathways by dismissing NCoR corepressor from gene regulatory regions, thus substantially modifying the activity of certain transcription factors. We have demonstrated that Tab2 suppression in tamoxifen-resistant MCF7-TAMR breast cancer cells restores the antiproliferative response to tamoxifen, thus implying constitutive activation of Tab2 in resistance. Microarray analysis showed that Tab2 regulates genes with functions in cell cycle, cell assembly and organization and DNA replication, recombination and repair. Furthermore, the highest-score interaction network identified was centered around BRCA1. Tab2 is recruited to estrogen-responsive genes through interaction with the N-terminus of ERα and, in fact, an ERα mimic peptide led to recovery of the antiproliferative effect of tamoxifen in MCF7-TAMR cells. We also mapped the Tab2 domain responsible of ERα interaction very close to several sites of regulatory phosphorylation. We are currently investigating whether phosphorylation regulates ERα/Tab2 interaction and if Tab2 has a more general role in the control of cell proliferation and genome stability.

P5.16
The replication profile of different human cell types, as evaluated by molecular combing at whole genome level and at single loci

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Features of DNA replication are still poorly characterised in mammalian cells. The replication profiles of peripheral blood lymphocytes (PBL), immortalised lymphoblastoid cells, and primary normal fibroblasts were compared by molecular combing. Fork rates ranged 1.75-1.95 kb/min in PBL/lymphoblastoid cells, and were slightly more variable among fibroblast subcultures. Interorigin distances correlated positively with the average fork rates. Unidirectional forks were observed in a small but reproducible percentage (5-10%) in PBL, but in fibroblasts they represented about 1/3 of the total observations. In all samples, 5-10% of the replication forks showed uncoordinated rates between the two arms, or appeared arrested/paused. The replication profiles of early/late replication loci, including common fragile sites FRA6E and FRA3B, were analysed. Slower fork rates (about 1 kb/min) and higher proportions (20-40%) of unidirectional forks characterised these regions with respect to the whole genome. No specific parameters were found at the fragile sites with respect to non fragile loci. These results may contribute to clarify the features of the replication programme of human cells.

P5.17
DDB2 interacts with the Nucleotide Excision Repair proteins at DNA damaged sites

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DDB2 (48kDa) was identified as a cofactor of the heterodimeric complex UV-DDB, which has affinity for the major types of DNA lesions induced by UV irradiation, such as 6-4 PPs and CPDs. These lesions lead to distortion of DNA, increasing cells susceptibility to cancer. Mammalian cells utilize Nucleotide Excision Repair (NER) system to remove bulky DNA structural changes and, in this process, DDB2 plays an important role in the recognition step of UV-damages. DDB2 mutations were found in Xeroderma pigmentosum syndrome, complementation group E (XP-E).
In this study, we analyse the DDB2 possible interaction with NER proteins at damage sites. To this end, HeLa cells were transfected with pcDNA3.1-DDB2 construct and irradiated with UV-C (30 or 100J/m2). DDB2 cellular localization was investigated by western blot and immunofluorescence analyses; the direct interaction between DDB2 and NER proteins were studied by immunoprecipitation experiments. The results showed that DDB2 co-localizes with NER proteins at DNA-damage sites at 5, 10, 30 min post-UV irradiation. In addition, DDB2 recruited on DNA interacts and physical associates with Cullin 4A, XPC, XPG, p21 and PCNA proteins.

P5.18
Development of a series of 3-hydroxyquinolein-2(1H)-ones as selective inhibitors of HIV-1 reverse transcriptase associated RNase H activity

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The current HIV-1 therapy, the Highly Active Antiretroviral Treatment (HAART), consists of a cocktail of drugs which includes reverse transcriptase inhibitors, protease inhibitors and/or a fusion inhibitor. This regimen has many limitations as cost, patient's adherence, drug toxicity and development of multidrug resistance. New strategies aimed at inhibiting virus replication are still necessary. In this regard, we consider the reverse transcriptase (RT) associated ribonuclease H (RNase H) function an attractive target. A few classes of RNase H inhibitors have been identified in the recent years and some of this interact with the Mg2+¬·¬·¬ cofactors within the catalytic core of the enzyme. In the present work we have developed and tested on the HIV-1 RT associated RNase H function a series 3-hydroxyquinolin-2(1H)ones, and showed that the most active compounds are ester and amides substituted with IC50 value between 16 and 22 μM.

P5.19
hMTH1 expression protects mitochondria from Huntington's disease-like impairment

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Huntington disease (HD) is a neurodegenerative disease caused by expansion of CAG repeats in the huntingtin (Htt) gene. The expression of hMTH1, the human hydrolase that degrades oxidized purine nucleoside triphosphates, grants protection in a chemical HD mouse model in which HD-like features are induced by the mitochondrial toxin 3-nitropropionic acid (3-NP). To further examine the relationship between oxidized dNTPs and HD-like neurodegeneration, we studied the effects of hMTH1 expression in a genetic cellular model for HD, such as striatal cells expressing mutant htt (HdhQ111). hMTH1 expression protected these cells from 3-NP and H2O2-induced killing, by counteracting the mutant htt-dependent increased vulnerability and accumulation of nuclear and mitochondrial DNA 8-hydroxyguanine levels. hMTH1 expression reverted the decreased mitochondrial membrane potential characteristic of HdhQ111 cells and delayed the increase in mitochondrial reactive oxygen species associated with 3-NP treatment. Further indications of hMTH1-mediated mitochondrial protection are the partial reversion of 3-NP-induced alterations in mitochondrial morphology and the modulation of DRP1 and MFN1 proteins, which control fusion/fission rates of mitochondria. Finally, in line with the in vitro findings, upon 3-NP in vivo treatment, 8-hydroxyguanine levels in mitochondrial DNA from heart, muscle and brain are significantly lower in transgenic hMTH1-expressing mice than in wild-type animals.

P5.20
Functional studies of CK2-mediated phosphorylation of WRN

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The Werner syndrome protein, WRN, is a RecQ DNA helicase involved in the non recombinogenic pathway of stalled replication fork recovery. WRN is supposed to be finely regulated by different post-translational modifications and its sequence contains several putative phosphorylation sites of unknown functional role. Our results show that CK2, an ubiquitous Ser/Thr kinase, phosphorylates, in vitro and in vivo, the N-terminal region of WRN containing the binding sites for RPA. We found that the association of WRN with RPA in vitro is greatly enhanced by CK2-mediated phosphorylation. We identified phosphorylation sites by MS/MS and generated unphosphorylable (WRN6A) and phosphomimetic (WRN6D) forms of WRN. Using chromatin fractionation analysis and Western blotting, we found that CK2 regulates WRN association with chromatin upon replication arrest. Moreover, expression of WRN6A or WRN6D affected RPA phosphorylation and chromatin localisation, as well as association with chromatin of MRE11. Other experiments are on the way to determine how CK2-regulated WRN-RPA association may affect WRN function and replication fork recovery and how CK2 may modulate WRN protein-protein interaction.

O5.1
Reduced proficiency in homologous recombination underlies the high sensitivity of embryonal carcinoma testicular germ cell tumors to cisplatin and poly (ADP-ribose) polymerase inhibition

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Testicular Germ Cell Tumors (TGCT) and patients-derived cell lines, are characterized by extreme sensitivity to interstrand cross-link (ICL) inducing agents, such as cisplatin. However, the molecular defects at the base of such response have remained elusive. Here we examined the proficiency of five embryonal carcinoma (EC) TGCTs cell lines to repair cisplatin-induced ICLs. We found that, as measured by γH2AX staining (a surrogate marker of double strand breaks formation), EC cell lines were either incapable or had a reduced ability of repair ICL-induced damage. The defect correlated with a reduction of Homologous Recombination Repair (HR), as monitored by Rad51 foci formation and direct evaluation by a GFP-reporter substrate. In line with this observation, EC cell lines were sensitive to the treatment with the inhibitor of poly(ADP-ribose)polymerase (PARPi) AZD2281. The magnitude of their sensitivity correlated with HR-repair deficiency and PARP1 protein expression levels and activity. In addition, we found that AZD2281 enhanced TGCT response to cisplatin, reducing the ability of the cells to overcome the damage, promoting cell death. Overall, our results suggest that AZD2281 might be beneficial in the clinical treatment of TGCTs, improving the treatment of patients otherwise resistant to the standard therapy.

O5.2
The DNA damage sensor protein NBN: role of BRCT domains in the DNA damage response

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The BRCA1 Carboxy-Terminal (BRCT) tandem domains are present in several proteins involved in the response to the DNA double-strand breaks (DSBs) sensing and signaling. These domains are pivotal in phosphorylation-dependent protein-protein recognition, and epidemiological data indicate a relationship between mutations within BRCT domains and cancer susceptibility. The NBN protein, which has a tandem BRCT domain, forms a trimer with MRE11 and RAD50 that is involved in nearly every aspect of DNA damage response (DDR). To date, the exact role of NBN BRCT domains in the DDR is still matter of debate, and controversial data are available in the literature regarding protein-protein interaction and timing of BRCT-containing proteins localization on the DSB. To study the role of the BRCT domains of NBN in the DDR, natural and artificial NBN mutations, all perturbing the relative geometry of the tandem, have been studied by a combined biochemical and cell-biological approach. In particular, DNA damage sensing, protein-protein interaction, and activation of DNA damage pathways have been addressed in order to assess how the tandem BRCT domains influence the overall cellular metabolism, with possible consequences on cancer development. Results obtained indicate that the BRCT domains of NBN are involved in the proper activation of the DDR, altering the localization of repair proteins at the DSB, as well as DNA damage signaling.

O5.3
Regulation of MUS81 pathway by cooperation with RAD52 and post-translational modifications

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In mammalian cells, MUS81, a structure-specific endonuclease, generates DNA double strand breaks (DSBs) to restart perturbed forks and allow cell viability following replication stress. If other recombination factors cooperate with MUS81 and how the function of MUS81 is regulated in human cells is unclear. Using CHK1 inhibition as a model of replication stress, we found that RAD52 cooperates with MUS81 at perturbed forks, probably generating the MUS81 substrate. However, upon CHK1 inhibition, concomitant abrogation of RAD52 and MUS81 results in extremely reduced recovery from replication stress, suggestive of multiple recovery pathways. We show that MUS81 and RAD52 are differently phosphorylated after replication perturbation or stress. MUS81 is targeted by the checkpoint kinase CHK2 and phosphorylation requires priming modification by CK2. Using in vitro approaches and MS/MS, we identified the MUS81 phosphorylation sites and similar studies are ongoing for RAD52.
These results suggest that in human cells there are multiple mechanisms of recovery from replication stress and that they are intertwined and finely regulated.

O5.4
Ubiquitination and genome stability: the role of RNF168's ubiquitin binding domains in the regulation of the DNA damage response

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Ubiquitination is a post-translational modification that regulates many cellular functions, including the DNA damage response (DDR) and DNA repair. It is a multi-step process involving the attachment of the ubiquitin moiety (Ub) to lysine residues on substrate proteins. Ub mediated network requires protein modules known as Ub binding domains (UBDs) to translate and amplify the Ub signal into specific cellular functions. Both the enzymatic activities and the docking capability of the ubiquitinating system represent an important level of regulation of DDR. We have previously identified and characterized a new RING finger protein endowed with Ub ligase activity, namely RNF168. Upon DNA damage, RNF168 modifies chromatin by ubiquitinating histones H2A and H2A.X in the proximity of damage. We found that RNF168 contains three UBDs namely MIU1, MIU2 and the most recently identified

UMI. We demonstrated that UBD's integrity is largely required for the localization and function of RNF168. In fact, we found that the simultaneous inactivation of them abolishes RNF168's Ub ligase activity in vivo and impairs the recruitment of the crucial downstream mediators 53BP1 at DNA damage sites. Finally, we will present data showing the presence in RNF168 of an additional Ub binding region with peculiar features. It represents a novel family of UBDs and might have an important role in the regulation of RNF168's ligase activity.

O5.5
Coordination of base excision repair and mismatch repair processing of chemotherapy-induced DNA damage

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Base excision repair (BER) is a fundamental mechanism to remove endogenous and induced DNA damage. This role is often coordinated with mismatch repair (MMR) that overlaps with BER in the processing of several lesions.
Overexpression of BER proteins, such as DNA polymerase beta (POLB), has been frequently described in association with human tumors.
By transcriptional profiling of sporadic human gastric cancers we found that the downregulation of a MMR gene MLH1 and the overexpression of POLB accounted for the discrimination between tumors with and without MMR deficiency. To gain mechanistic insights into this phenomenon, gastric cancer cells with stable inactivation of MLH1 and overexpression of POLB, in combination or alone, were constructed and treated with model damaging agents. We identified a protective role of POLB from the lethal effects of O^6-methyguanine in a pathway likely independent from MMR. POLB overexpression was also associated with increased resistance to hydrogen peroxide, irrespective of MLH1 status. The sensitivity to chemotherapeutic drugs such as methotrexate and temozolomide is currently under investigation.
Moreover, our data show that lithocolic acid, a specific POLB inhibitor, is able to sensitize POLB overexpressing cells to both alkylating and oxidizing agents leaving open the question about the potential impact of POLB inhibitors on the therapeutic index.

6 - Development, differentiation and aging

P6.1
Toxicological effects of carbon nanoparticles during the development of Paracentrotus lividus embryos

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The term nanopollution is referring to all waste generated during the fabrication of nanomaterials. Ecotoxicological impacts of nanoparticles and the potential for bioaccumulation in plants and microorganisms are very important and still under research. We chose the Paracentrotus lividus as model organism and analyzed the expression of ectodermal GRN in embryos developed in presence of Carbon Nanoparticles (C-NPs). We found that during the early stages of development, the gene expression and the related morphological changes are closely related to the amount of C-NPs. During later stages, the development stops in cultures supplemented with small and medium quantities of nanoparticles and continues accelerated in the medium added with high concentrations of C-NPs. Only in this case, after 48h, it is observed the formation of the larva pluteus. This larva compared to the control is modified: it retains its triangular shape but loses the asymmetry of the arms. In particular, it has the ability to accumulate nanostructures within the bag digestive system; this material is the product of unexpected biomineralization process, activated as self-defense against the outer material.

P6.2
EGR-1 modulates neurite outgrowth and matrix metalloproteinase expression via muscarinic receptor activation

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Mouse neuroblastoma cells provide an interesting model to analyze cellular events involved in neuronal differentiation. The forced expression in these cells of choline acetyltransferase (ChAT) gene leads to a more differentiated state through the transcription factor EGR-1. We analyzed whether EGR-1 over-expression in neuroblastoma cells and ChAT-positive clones may modulate the expression of matrix metalloproteinases (MMPs), important enzymes involved in brain development. We evaluated the role of EGR-1, considering also the interference due to ZnEGR negative dominant. Our results demonstrate that muscarinic stimulation modulates EGR-1 protein expression and EGR-1 positive cells show an increase in fiber outgrowth. The level of MMP-2 was significantly increased in the conditioned medium of EGR-1 clones, while MMP-9 was not present. Conversely, the transfection with ZnEGR causes a strong reduction in MMP-2 levels. Finally, fiber outgrowth was reduced in the presence of MMP inhibitor.
Our results indicate that the progression along the neural differentiation program is at least in part due to the expression of EGR-1, and that gelatinases play a crucial role in neurite extension.

P6.3
RSPO1/ β-catenin/ DKK1 distribution pattern during testis embryonic development

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R-spondins (RSPOs) are a family of 4 secreted proteins that promote β-catenin stabilization. Recently RSPO1 has been demonstrated as a candidate for ovary determination, but it is present also during testicular embryonic development even if in the literature there are few and contradictory data about this subject.
This study clarifies the distribution pattern of RSPO1, its antagonist Dickkopf-1 (DKK1) and β-catenin during testis development from 11.5 to 18.5 dpc. To this end whole mount immunofluorescences of these proteins were analyzed by confocal microscopy.
RSPO1 appears detectable on the coelomic surface of the testis since 11.5 dpc. Testicular cords express RSPO1 since 13.5 dpc to 18.5 dpc.
DKK1 is expressed since 11.5 dpc and shortly after becomes restricted to testicular cords. Coelomic epithelium never expresses DKK1.
β-catenin appears as a cortical cytoplasmic signal on the coelomic epithelium as well as in the testicular cords during all the stages analyzed.
These observations demonstrate that RSPO1/β-catenin/DKK1 machinery is present also in testis differentiation and suggest that the balance between these factors may has a role in the proper control of gonad organogenesis.

P6.4
LPS-induced TNF-α factor (LITAF) mediates hepatic stellate cell-dependent production of inflammatory molecules during nonalcoholic fatty liver disease (NAFLD)

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LPS-induced TNF-α factor (LITAF) has been recently found increased in nonalcoholic fatty liver disease (NAFLD) models. NAFLD is one of most prevalent chronic liver diseases ranging from simple steatosis, with or without necro-inflammation, to fibrosis. Hepatic stellate cells (HCSs) differentiation into myofibroblast phenotype is crucial for NAFLD-dependent inflammation and fibrosis. Here, we studied the potential role of LPS-induced LITAF in these phenomena in in vivo and in vitro models.
By immunohistochemistry, we found LITAF expression increased in NAFLD children liver tissues correlating with histological traits of hepatic inflammation and fibrosis. On the other hand, LX2 (HCSs) treatment with LPS (100 or 500 ng/ml) upregulated LITAF nuclear expression levels even though no significant differences were found in its total lysate protein and mRNA levels. In fact, confocal microscopy confirms a near/intra-nuclear accumulation of LITAF in LPS-treated cells. Moreover, LPS treatment reduced LX2 cell proliferation rate, while increased the IL-6, IL-1β and TNF-α expression. The same inflammatory molecules increased in the plasma of the already examined NAFLD patients.
Concluding, LITAF might be a downstream factor activated by LPS signaling cascade in HSCs, leading to a pro-inflammatory profile typical of NAFLD.

P6.5
Role of EGR-1 in mouse SVZ-derived aNSCs proliferation and differentiation and its interplay with the NSCs proliferative factor EGF

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In adult mammals, NSCs persists only in specialized niches at the level of selected CNS regions, such as the subventricular zone (SVZ). While the presence of NSCs in the adult brain is well established, signalling pathways that regulate cells proliferation and differentiation remain

poorly understood. The early growth response gene 1, (EGR1) is an important transcription factor widely studied in the adult mammalian brain, acting as a convergent point between a variety of extracellular stimuli and activation of target genes. In our study we have explored how EGR1 regulates adult NSCs derived from mouse SVZ and in particular the molecular interplay between EGR1 and the proliferative factor EGF. We have demonstrated that EGR1 expression decrease after EGF deprivation and that EGR1 over-expression, rescue cell proliferation decrease observed after EGF removal, suggesting a cross talk between EGR1 and EGF pathway. To better understand this mechanism we are investigating EGF cascade pathway to individuate possible EGR1direct or indirect target both by gene expression and ChIP.

P6.6
A critical requirement for cyclin D3 in adult muscle stem cell function

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In previous studies we have shown that cyclin D3 is highly induced during myoblast cell differentiation in vitro through mechanisms controlled by MyoD and pRb, two pivotal regulators of skeletal myogenesis. To assess the role of cyclin D3 in the control of muscle progenitor cell function, we used RNAi technology to knockdown cyclin D3 protein levels *in vitro* and cyclin D3 knockout approach *in vivo*.
The inhibition of endogenous cyclin D3 expression in C2 myoblasts resulted in reduced proliferation, premature expression of differentiation markers and impaired myotube formation, indicating that cyclin D3 critically controls the balance between myoblast proliferation and differentiation.
Cyclin D3-null muscles collected from 2-mo-old mice showed fewer quiescent stem cells (satellite cells) and a decrease in myofiber size when compared with wild-type controls. The analysis of primary myoblast cultures established from cyclin D3-null muscle and the analysis of satellite cells activated *in vivo* following muscle injury revealed cyclin D3 is critically required for proliferative expansion of myogenic precursor cells and for generation of quiescent satellite cells.

P6.7
Molecular evolution and expression studies in *Danio rerio* elicit a possible molecular mechanism to explore function gain of Insulin-like 3 in Vertebrate evolution

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Insulin-like 3 (INSL3), is a member of the insulin-IGF-relaxin peptide superfamily, that is synthesized as pre-prohormones mainly in somatic cells of the gonads, in testicular Leydig cells and in the ovarian follicular theca cells. It is encoded by a relatively small gene composed of two exons in vertebrate species. Here we present RT-PCR evidence that at least two *insl3* transcripts are present in adult zebrafish tissues and developing embryos: one of them retains intron as confirmed by cloning, sequencing and our whole-mount in situ hybridization results. Bioinformatics analyses performed on all known *insl3* genes reveal that its structure is conserved among fish but intron length is different. Interestingly, giving support to our hypothesis that more than one transcript could be maturate from the *insl3* pre-mRNA, we found, inside *insl3* intron, a conserved open reading frame, coding for a peculiar peptide domain. Analyses performed on proximal promoter instead reveal molecular evidence about the possible functional role gain of *insl3* in mammalian testis development.

P6.8
Characterization of relaxin system in the vertebrate developing brain

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The relaxin (Rln) peptide family exerts diverse biological effects from reproduction to regulating central nervous processes through different G protein-coupled receptors (RXFP1-4). Our previous studies in vertebrate embryogenesis, using zebrafish as a model system, showed that Rlns could be involved in nervous system functioning in early development. Two Rln family members are expressed in restricted cell clusters in the developing brain and are thought to function as a neurotransmitters. We are now extending the characterization of Rln/Rxfp system by neuroanatomical investigation of Rxfp receptors gene expression. We found that two homologues of mammalian Rxfp3 were broadly expressed in the developing brain. These data are in line with the hypothesis that Rxfp3 is the cognate receptor of relaxins also in non mammalian vertebrate. A detailed neuroanatomical analysis provided evidences for the expression of such Rxfp3 homologues in many brain areas involved in different neural processes such as sensory input processing and stress response. Our findings provide the basis to analyze the involvement of relaxin system in the early life stage of a vertebrate model organism.

P6.9
p63, a new molecular target of the teratogenic drug thalidomide

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The transcription factor p63 plays a key role in limb, epithelial and cranio-facial development and p63 mutations have been associated to many human congenital syndromes. In some cases, the clinical features of p63 patients were similar to those of babies born from mothers exposed to thalidomide. This observation prompted us to investigate whether p63 could be a molecular target of thalidomide.
Our data indicate that ΔNp63α and ΔNp63β proteins, but not ΔNp63γ and p53, are degraded through the proteasome upon thalidomide exposure in several human cell lines expressing either the endogenous or the transfected p63 proteins. By mutational analysis, we identified p63 residues serine 383 and threonine 397 as the amino acids required for thalidomide-induced p63 degradation. Moreover, thalidomide action on p63 requires GSK3 kinase and Fbw7 Ub ligase activity, since GSK3 inhibition and silencing of endogenous Fbw7 blocked the effect of thalidomide on p63.
Finally, thalidomide modulates p63 protein levels, with consequent alterations of p63 target genes expression, also in developing zebrafish embryos in vivo.
The results shed new light on the molecular mechanisms of thalidomide.

P6.10
Male subfertility and sperm epigenome alterations in mice lacking the selenoprotein nGPx4

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We have recently demonstrated that the nuclear form of Glutathione Peroxidase 4 (nGPx4) is required for proper sperm chromatin assembly. In this study we have investigated the impact of nGPx4 on male fertility and sperm epigenome. Matings of nGPx4 KO male mice to wild-type females yielded both litter sizes and percentage pregnant plugged females significantly smaller than those obtained by the WT male mice. In vitro fertilization assays revealed 70% reduction in the percentage of embryos at pronuclear stage when metaphase II oocytes were

inseminated by nGPx4 KO capacitated sperm compared to WT ones. The reduced fecundity of nGPx4 KO males was due to a defect of sperm ability to penetrate zona pellucida, being both the sperm binding and the sperm-oolemma fusion similar between the two genotypes. These results demonstrate the subfertility of nGPx4 KO male mice. We also analysed sperm hyperacetylated histone H4 levels by western blotting and immunofluorescence staining. Interestingly, higher amounts of the modified histone 4 appeared in nGPx4 KO sperm compared to WT sperm. These data link the modifications of histones retained in sperm to paternal chromatin remodeling at fertilization

P6.11
Role of keratinocyte growth factor receptor (KGFR/FGFR2b) expression and signalling in the impairment of the epidermal cell differentiation induced by HPV16 E5 protein

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The KGFR/FGFR2b plays a key role in regulating keratinocyte early differentiation and the HPV 16E5 oncogenic protein is known to perturb this process. To investigate if 16E5 might interfere with the epithelial differentiation by altering the expression and signalling of KGFR, we took advantage of our newly developed in vitro model of synchronous receptor modulation and forced cell differentiation through treatment with Thapsigargin, an inhibitor of Ca-ATPase pump family (Belleudi et al., 2011). Quantitative RT-PCR showed that 16E5 down-regulated both KGFR and the K1 marker in a dose-dependent manner and that miR-125b was involved in the receptor modulation. Molecular, biochemical and immunofluorescence approaches demonstrated that KGFR expression was able to counteract the decrease of K1 and increase of p63 induced by 16E5. Receptor activation and signaling were required for these contrasting effects, while KGFR depletion enhanced the 16E5 action. Our results indicate that the 16E5-mediated KGFR down-modulation is a crucial step in the HPV-induced impairment of epidermal cell differentiation.

P6.12
Expression patterns of Pl-c-jun during Paracentrotus lividus sea urchin development

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The AP-1 transcription factor is involved in many cellular events such as cell cycle, cell proliferation, differentiation. It consists of dimers composed of different proteins, c-jun, junB, c-fos, Fra-1 and Fra-2. We isolated the complete cDNA encoding the Pl-c-jun gene from P.lividus embryos and described the phylogenetic relationships with homologs of different phyla. We found that Pl-c-jun mRNA levels, measured by QPCR, were high in unfertilized egg, and decreased during development if compared to egg's mRNA. The spatial expression of Pl-c-jun mRNA, detected by WMISH in embryos at different developmental stages, showed ubiquitous transcripts in the cleavage stages. Then, Pl-c-jun mRNA was restricted in a few cells at the vegetative region of blastula and in some skeletogenic cells, the Primary Mesenchyme Cells (PMC), in gastrula and pluteus embryos. The localization of c-jun protein was studied by immunofluorescence experiments on whole mount embryos using commercial antibodies. The presence of total c-jun protein in the PMCs was confirmed, showing a cytoplasmic localization, while its phosphorylated form was restricted to the nucleus of the same cells. Preliminary Western Blot of total c-jun protein, in subcellular fractionation experiments, showed nuclear and cytoplasmic localization too. The specific localization of Pl-c-jun in PMC suggests a role as a regulatory element of skeletogenic genes.

O6.1
A gene regulatory network that controls the formation of a functional gut in the sea urchin embryo

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The relative simplicity of the sea urchin tripartite larval gut, the easy of regulatory and functional analyses, and the recently developed genomics resources, afford a unique opportunity for analysis of the gene regulatory network (GRN) involved in the creation of a functional gut. Sea urchins are deuterostomes, and as such they share a common heritage with vertebrates that is not shared by other invertebrate models. The aims of this project are to elucidate the GRN involved in patterning and regionalization of the endoderm, to assess the degree of conservation of this network amongst the deuterostome lineage, and to gain insight into the origin and evolution of the Parahox genes leading to the chordate lineage. We demonstrated that two of the three sea urchin ParaHox genes, SpLox and SpCdx, are expressed in a spatial and temporally collinear fashion within the developing digestive tube and play a key role in partitioning of the mid- and hind-gut. They are involved in mutual regulation: the posterior SpCdx gene is not expressed in the absence of the anterior SpLox gene and the expression domain of SpLox is not restricted posteriorly when t SpCdx is silenced. A comparison with vertebrates showed a striking conservation of topology of gene expression and signaling events between sea urchin and mouse, thus suggesting the existence of an ancient "kernel" of genes involved in gut patterning processes among deuterostomes.

O6.2
Characterisation of a novel long non coding RNA involved in *in vitro* neuronal differentiation

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Long non coding RNAs (lncRNAs) are one of the most abundant class of ncRNAs transcribed in a developmentally regulated and cell-specific manner. Despite their abundance, only a small fraction of them has been associated with biological functions or diseases. Interestingly, it has been shown that a half of all lncRNAs is expressed in the nervous system. Starting from our studies aimed at unveiling microRNAs (miRNAs) involved in neuronal differentiation, we identified a novel lncRNA in which miR-125b-1 is embedded (linc125b-1). As the miRNA, linc125b-1 is upregulated during in vitro differentiation of human neuroblastoma and medulloblastoma (MB) cells and in human derived induced pluripotent stem (iPS) cells committed towards the neuronal fate, suggesting these two ncRNAs are co-regulated during differentiation. Moreover, we found linc125b-1 downregulated in MB primary tumours, suggesting its role in controlling neuronal differentiation and tumour growth.
Notably, linc125b-1 is mainly cytoplasmic and displays putative binding sites for miRNAs known to counteract cellular differentiation. Functional studies are in progress to verify its potential role as competing endogenous RNA.

O6.3
Single-nucleotide polymorphisms inside microRNA target sites influence aging and longevity

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MicroRNAs (miRNA) are short noncoding RNAs that modulate post-transcriptional gene regulation by binding to complementary sequences, primarily in the 3′UTR. It was reported that SNPs in 3'UTR regions

targeted by miRNAs may alter the strength of miRNA binding, resulting in a deregulation of the relevant genes. Since gene deregulation can affect the rate of aging, we hypothesized that SNPs within miRNA target binding sites may play a role in human lifespan. To test this hypothesis, the 3'UTRs of 231 genes involved in pathways correlated with aging were analyzed to identify miRNA-binding sites by *in silico* analysis. The analysis identified 63 SNPs falling in the miRNA binding, 8 of which significantly altered the binding site, according to the bioinformatic analysis. We investigated these 8 SNPs in two age groups (66-85 and >85 years). The rs45592833-SIRT2 and rs2855262-SOD3, whose alleles could modulate gene expression by differential interaction with miR-1275 and miR-545, respectively, were differently distributed in the two age groups, suggesting they could be associated with longevity. The project will continue by testing *in vitro* the actual effect of the SNPs on miRNAs binding.

O6.4
Two microRNAs, miR-23 and miR-125, control the cell fate determinant Musashi1 during astrocyte differentiation

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During nervous system development neural progenitor cells (NPCs) first generate neuronal cell types and then glial cells. So far, the molecular pathways that instruct NPC to turn off neurogenesis and turn on gliogenesis remain elusive. microRNAs (miRNAs) are good candidates for directing such temporarily regulated switch but, so far, only miRNA-dependent regulatory circuitries controlling neuronal and oligodendrocytic differentiation have been elucidated. It is, therefore, crucial to identify the miRNAs and the regulatory circuitries important for astrocyte specification and terminal differentiation. We produced an atlas of miRNAs modulated during the differentiation of astrocytes generated from mouse NPCs and, among the most upregulated miRNAs, we focused on miR-23 and miR-125. We demonstrated that these miRNAs mediate the decrease of Msi1, an RNA binding protein with a critical function in self renewal capability of NPCs. Remarkably, the transduction of miR-23 and miR-125 in NPCs triggers the expression of the astrocyte differentiation marker GFAP, and inhibits cell proliferation, suggesting a role for these miRNAs in the commitment of NPCs towards the astroglial lineage.

O6.5
The impact of hypoxia in the regulation of β-dystrobrevin (DTNB) and miRNA-143 in retinoic acid (RA)-induced neuronal differentiation of NT-2 cells

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Hypoxia is not only a pathological event but also a physiological condition. During hypoxia cells activate a number of adaptive responses including gene regulation by **H**ypoxia-**I**nducible **F**actors, heterodimers consisting of an O_2-labile α subunit (HIF-1α, 2α or 3α) and a stable β subunit. Hypoxia allows HIF-α to escape from normoxia-mediated degradation, translocate into the nucleus and bind **H**ypoxia-**R**esponse **E**lements in the promoter of target genes and miRNAs. We have identified a HRE in the promoter of β-dystrobrevin (DTNB), a member of the Dystrophin Protein Complex and a putative target of miR143 that has recently been suggested to be involved in neuronal differentiation. As mild hypoxia favors neural differentiation, we used RA-treated NT2 cells cultured in mild hypoxia (5% O_2) as compared to normoxia (20% O_2) to study DTNB and miR143 expression during neuronal differentiation. In our model mild hypoxia delays neuronal differentiation and both DTNB and miR143 expression without interfering with the miR143 targeting of DTNB previously observed in normoxia. Moreover, mild hypoxia is sufficient to activate HIF-1α that in turn may regulate a putative hypoxia target gene such as DTNB.

7 - Environmental microbiology and biotechnology

P7.1
Influence of different compounds on a production of two bacteriocins by *Enteroccus casseliflavus* 416 K1 and *Lactococcus lactis* ATCC 11454

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Lactic acid bacteria (LAB) are known for the production of antimicrobial compounds, as bacteriocins, that can inhibit pathogens and spoilage microorganisms. The interest for LAB is increased because they can be used as biopreservation to extend the shelf life of many foods. Bacteriocins are polypeptides with bactericidal activity, generally against bacteria closely related. The employ of bacteriocins as food biopreservatives or as therapeutic agents, require a large-scale production of compounds with high activity. Our study was focused on understanding how two different bacteria: *Enterococcus casseliflavus* 416 k1 and *Lactococcus lactis* ATCC 11454, can increase the production of enterocin 416 K1 and nisin A respectively. Both bacteria were cultured in media added with different compounds at different concentrations, to assess which substances could amplify or even inhibit the production of the two bacteriocins. The compounds, used at 3 different concentrations, were vitamins, proteins, salts, tween 80 and SDS. The bacteriocins produced under the different conditions tested, were studied to evaluate their spectrum of action against several indicators as well as their antimicrobial titer. Our preliminary results indicate in many case an increase of bacteriocin production with a wider spectrum of action and a higher antimicrobial titer related to the compounds added to the culture media, but in particular with the addition of vitamins at low concentration.

P7.2
Abundance, diversity and relations with environmental factors of the airborne bacterial communities in urban areas of Northern Italy

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The presence of microorganisms in the atmosphere is well-known, however the abundance, the diversity of airborne bacteria and the factors influencing their diversity are still scarcely analyzed. In this work we used quantitative PCR and Illumina technology to provide a detailed characterization of microbial communities associated to the atmospheric Particulate Matter. Air samples were collected across seasons. Seasonal variability in the composition and abundance of microbial communities was found. In particular, in the total suspended particles collected in Milan, a significant abundance of Chloroplasts was detected, especially in warmer seasons, due to the presence of plant debris and pollens, while Actinobacteridae was the most abundant taxon in cold days. Therefore, the potential sources of airborne bacteria were investigated. Soil and plants were the sources, which most likely affected the airborne bacterial communities. This study demonstrated the potential of the Illumina technology and quantitative PCR to investigate the microbiological component of the atmosphere, an environment largely neglected by previous studies.

P7.3
Soil bacterial community response to land use types and seasonal changes

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We aimed at determining the effects of land use intensification along with seasonal variations on soil bacterial community. Five Mediterranean agricultural soils characterized by different land-use and management were examined in spring and autumn: cork-oak forest, hayland-pasture rotation, semi-natural grassland, and ploughed and grass covered vineyard. All the soils resulted to be sandy-loam, with sub-acid pH reaction and very low organic matter content, with the highest values in soils with the lowest human impact. A predominance of fast-growing bacteria was found in all samples. Molecular identification and phylogenetic analysis revealed that cultivable bacteria were basically composed of *Proteobacteria*, *Actinobacteria*, *Bacteroidetes*, and *Firmicutes*, with a different seasonal distribution. Cluster analysis of culture-dependent DGGE patterns revealed that soil bacterial communities clearly differed depending on season; within each season, subgroups including bacterial communities associated to different land uses were observed, in agreement with culture-independent T-RFLP results. In conclusion, differences in soil microbial biodiversity can be essential ascribed to seasonal changes rather than land-use.
Funded by MIUR, Project SOILSINK

P7.4
Accumulation of silver by an Aspergillus fumigatus strain isolated from an industrial cyanide waste

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The study focuses on the interaction of an Aspergillus fumigatus strain isolated from a jewelry industrial waste rich in metal cyanide complexes with silver. The fungus was able to grow at concentrations up to 647.208 µg/ml. of silver added to the substrate as AgNO3. Transmission Electron Microscopy revealed cytoplasmic accumulation of silver nanoparticles within 24 hours by the fungus kept in a AgNO3 1 mM aqueous solution, followed by metal translocation and deposition on the cell wall surface after 72 hours. UV-visible spectrum of the solution did not reveal the presence of silver nanoparticles.The results were confirmed by atomic absorption spectrometry which revealed an accumulation of silver in the fungal biomass simultaneously with its disappearance in the aqueous solution. The absorption process was pH more than temperature dependent; the maximum silver accumulation occurred at pH 8.5. In conclusion the Aspergillus fumigatus strain under study did not show extracellular synthesis of silver nanoparticles as conversely reported in literature, making us to suppose that different mechanisms may be involved in silver sequestration even among different strains of a same species.

P7.5
A new bio-engineering treatment for optimization and enhancement of polluted marine sediment

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Petroleum hydrocarbons (e.g. PAHs) are major pollutants of marine sediments can produce adverse effects on environment and on human health. The problem of polluted marine sediments can be approached whit

different in situ (e.g. dredging, capping, solidification/stabilization…) or ex situ treatments (e.g. sediment washing, solvent extraction, chemical oxidation…). However, new trends of environmental ecology, would like all of these techniques were focused on the recovery of impacted ecosystems, rather than simply reducing the massive volume of oil released into the sea (clean up), or the simple removal of the matrix (sediment) contaminated. In this contest, different study shown as better result was obtained by application of bioremediation strategies. In the present study we have developed the creation of a particularly system for optimize the biodegradation process. This system, being designed as a confinement system, can operate directly in the environment but with the advantages of a controlled system. Inside, in fact, all process of biodegradation can occur without any influence on the surrounding.

P7.6
Evaluation of microbial diversity in rizospheric soils of poplars and maples by culture and culture-independent methods

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This work is in the frame of the LIFE+ DEMETRA project, which main purpose is the creation of a Quick Monitoring Index to assess the potential risk generated by transgenic crops in well determined ecosystems. Our research activity aims to define microbial diversity and its seasonal variations in rizospheric soils of trees and herbaceous plants considered as possible targets of unintended interactions with transgenic plants.
Here we report data from rizospheric soil samples of 11 maples and 15 poplars (11 wild and 4 cultivated) collected on a seasonal base (summer 2011- spring 2012) in different study areas of the Regional Parco Migliarino-San Rossore-Massacciuccoli. Both bacteria and fungi populations, have been analysed by culture and culture-independent (T-RFLP) approaches.
Seasonal fluctuations of microbial abundance were evaluated by viable counts on agar plates; correlations among CFU and environmental parameters were evaluated.
Ecological diversity indices (abundance and evenness) were applied to TRFs in T-RFLP profiles.
UPGMA clustering analysis and PCA analysis were used to highlight relationship among T-RFLP fingerprinting of different microbial communities.

P7.7
Cellobiose dehydrogenases of *Chaetomium globosum*: new players in cellulose degradation

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Cellobiose dehydrogenase (CDH) is an extracellular fungal flavo-cytochrome recently exploited in a variety of biocompatible biosensors and biofuel cells [1,2]. CDH oxidises cellodextrins, lactose and, depending on its origin, monosaccharides (e.g. glucose), using a variety of electron acceptors. Putative CDH genes were identified in various fungi by genome sequencing. In ascomycetes a differentiation of CDHs into two classes (IIA, IIB) was proposed, where class IIA contains a C-terminal carbohydrate-binding module missing in class IIB. Moreover, the two classes seem to differ with respect to their catalytic properties, even if little is currently known about their actual role [3]. Genes for both types of CDH are present in several ascomycetes, including *Chaetomium globosum*. The aim of our work was to elucidate the role of *C. globosum* CDHs, correlating gene expression and protein secretion to availability of cellulosic substrates. Furthermore, we are currently investigating their possible use in biotechnological applications.
[1] Kim et al 2012 Biotechnol Bioproc E 17: 55-59
[2] Wang et al 2012 Biosens Bioelectron 31: 219-225
[3] Harreither et al 2011 Appl Environ Microb 77: 1804-1815

P7.8
Lichens: a weapon against fungi and bacteria

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In studies of bioactive natural products many researchers focused on lichens which have been used for medical purposes since ancient times and have been shown to contain substances with remarkable biological activity, as usnic acid. Our work was developed into two parts. In the first, we investigated both the antimicrobial activity of (+)-usnic acid (Sigma-Aldrich) and, as medical application, the antibacterial action of urinary catheters containing this compound. In the second ones, we tested the acetone and cyclohexane extracts of a lichen collected from trees in "Parco della Chiusa" (BO, Italy). The antimicrobial assay of the (+)-usnic acid and of the two extracts was carried out by agar well diffusion method against both bacteria and fungi. Lichen extracts were tested after 1, 7, 16 and 32 days since the extraction procedure. The antimicrobial activity of urinary catheters was assayed after 1, 6, 24 and 48 h against the most common agents of urinary tract infections evaluating residual viable count of indicator suspensions which were put in contact with catheters. The results showed a strong antimicrobial activity of (+)-usnic acid such as lichen extracts and catheters too. Moreover, the antimicrobial activity of extracts was lost after 7 days for most indicator strains, but a good inhibition was maintained against B.subtilis up to 16th days and against C.albicans and A.hydrophila up to 32th days.

P7.9
Exploring the food microbiota: a metagenomic approach to antibiotic resistance

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The food microbiota in traditional fermented products consists of a complex community of environmental origin, which can transiently colonize and interact with the host gut microbiota. Selection and spreading of antibiotic resistance (AbR) genes in foodborne bacteria has gained increasing interest, especially in light of the potential transferability to opportunistic pathogens. Our laboratory has reported the isolation and characterization of AbR genes from foodborne bacteria, associated with mobile elements. To further clarify such linkage, which can lead to horizontal transmission of AbR genes, we have analized the food microbiome through a metagenomic approach. We have constructed a fosmid library with total DNA extracted from a traditional fermented fresh cheese. This library is composed of 20.000 clones, representative of the entire cheese microbiome, including under-represented species. Screening with functional and PCR approaches allowed identification of low frequency AbR genes which escaped detection with a culture-dependent approach. The library is also being used to identify genes involved in metabolism of food bioactives with proven health promoting effects

P7.10
Selection of microorganisms for the mobilization of cellulolytic agricultural biomass for energy production

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The production of biofuels as renewable energy source could significantly reduce the negative impact of fossil fuels. However,

this activity has resulted in increasing of land cultivation specifically with carbohydrate-rich crops for energy production, and resulting shortage of crop production for food. The use of residual agricultural biomass as a substrate for biofuels or biogas production could improve the sustainability of the bio-energy production but still requires the improvement of the technologies for the mobilization of the sugar-polymers cellulose and hemicelluloses that are the main components of the plant biomass. Several microorganisms are able to produce the cellulose-degrading systems (endoglucanases, exoglucanases and β-glucosidases) and purified enzymes are available. A great a diversity is still present in the environment for such traits and needs to be explored. The aim of this work is to illustrate the selection and the characterization of novel bacterial isolates which are able to de-polymerize cellulose. We have performed an enrichment culture from rotten palm tissue and isolated mesophilic strains able to grow on carboxy-methylcellulose and cellobiose as sole carbon sources under aerobic conditions. The phylogenetic affiliation was obtained by sequencing of the 16S rRNA gene and has allowed the identification of Serratia sp., Providencia sp., Enterobacter sp., Lysinibacillus sp., Pseudomonas sp., Raoultella sp.

P7.11
Volatile organic compounds (VOCs) from Antarctic sponges-associated bacteria inhibiting Cystic Fibrosis pathogens

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A panel of 132 bacterial strains isolated from three Antarctic sponges (*H. verrucosa*, *A. joubini* and *L. nobilis*) and affiliated to different genera (e.g., *Pseudoalteromonas*, *Arthrobacter* and *Psychrobacter*) were tested for their ability to produce natural drugs that could be exploited in the control of infections in Cystic Fibrosis (CF) patients. A panel of 70 opportunistic CF pathogens belonging to the *Burkholderia cepacia* complex (Bcc) and resistant to a plethora of antibiotics was used. Data obtained revealed that Antarctic bacteria were able to inhibit the growth of Bcc strains. Cross-streak experiments performed with the most active Antarctic bacteria also revealed that the antimicrobial compounds are very likely VOCs, a finding that was further confirmed by the SPME-GC-MS, technique which revealed the production of 128 different compounds, some of which synthesized via unknown and unusual pathways. These data highlight the potentiality of Antarctic bacteria as novel sources of antibacterial substances to face Bcc infections in CF patients.
Work supported by Ente Cassa di Risparmio di Firenze (Grant # 2008.1103) and Italian Cystic Fibrosis Research Foundation (Grant #12/2011).

P7.12
Archaeal populations dynamics during anaerobic digestion of ultrasound pre-treated activated sludge

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Anaerobic digestion is an efficient technology to stabilize sludge by means of mass and pathogen reduction,recovering energy in the form of biogas. Mechanical pretreatment as ultrasounds aims to convert waste activated sludge (WAS),in which the particulate organics are recalcitrant to anaerobic bacterial hydrolysis,into a soluble form. In the anaerobic digestion process, different bacterial trophic groups cooperate sequentially in order to achieve degradation of a variety of polymeric and monomeric substrates, which are transformed into methane by methanogenic Archaea. Microbial population dynamics were evaluated by Fluorescence In Situ Hybridization, during mesophilic anaerobic digestion of either raw or sonicated WAS, at different food/inoculum (F/I) ratio. Degradation of particulate matter and biogas production were also investigated. Archaea increased over digestion time irrespective of F/I and of sonication treatment.Two predominant acetotrophic archaeal populations belonging to Methanosaetaceae and Methanosarcina spp. were retrieved. This study suggests a strict association of Methanosarcina dominance to an efficient biogas production during mesophilic anaerobic digestion.

P7.13
Phenotype Microarray characterization of biocide-resistant *Staphylococcus aureus* strains

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Resistance to antiseptics based on quaternary ammonium compounds (QACs) is widespread among clinical human bacterial isolates. Evidence suggests that extensive use of biocides may impose selective pressure contributing to the emergence of decreased antiseptic susceptibility and cross-resistance between widely used biocides and antibiotics. In *Staphylococcus aureus*, resistance to QACs is often associated to the presence of plasmid encoded efflux pumps known as QAC transporters.
To study the responses associated to different QAC pumps and to identify new potential efflux targets, we analyzed by the Phenotype Microarray high-throughput technology the metabolic activity of 10 QAC^+ strains of *S. aureus* in presence of 240 different toxic compounds, each one at 4 concentrations, in comparison with 5 QAC^- strains.
Beside the identification of known efflux targets, PM approach highlighted new potential targets for QAC pumps, that must be confirmed through MIC/MBC determination and efflux assays. The identification of new chemicals inducing lower tolerance correlating with the presence of QAC determinants opens new perspectives for the development of more effective antimicrobial treatments.

P7.14
Microbial population dynamics and analysis of reductive dehalogenase genes expression in a PCE-dechlorinating mixed culture throughout the establishment of pseudo steady state operating conditions

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In this study a perchloroethylene (PCE) to ethene dechlorinating culture was analyzed throughout the operation until the establishment of pseudo steady state operating conditions. The dynamics of microorganisms involved in the biodegradation process were analyzed by combining in situ hybridization techniques and nucleic acids – based techniques. Cell detectability by Fluorescence In situ Hybridization (FISH) allowed to discriminate the actively dechlorinating bacteria whereas dechlorinators marginally involved in the reductive dechlorination process were visualized only by Catalyzed Reporter Deposition FISH (CARD-FISH). The observed shift in terms of FISH detectability of dechlorinating bacteria able only to partially dechlorinate PCE (Desulfitobacterium spp., Dehalobacter spp., Geobacter spp., Sulfurospirillum spp.) to those able to completely dechlorinate PCE to ethene (Dehalococcoides spp.) efficiently fitted with the dechlorination kinetics. Dehalococcoides spp. was furthermore analyzed by qPCR and RT-qPCR to quantify both 16S rRNA phylogenetic biomarker and reductive dehalogenase genes (tceA, bvcA, vcrA) involved in the biodegradative process. Under steady state operating conditions, the culture was able to completely reduce PCE to ethene (RD= 5.1 meq/L/d) and Dehalococcoides spp. cells represented up to 40% of the total bacteria with 16S rRNA transcripts > 109 mL-1.

P7.15
BODIPY compounds act as photosensitizers for microbial inactivation

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The photodynamic antimicrobial chemotherapy (PACT) exploits the use of a light-activated photosensitizer which transfers the adsorbed energy to oxygen or to any molecule present in the close proximity causing a lethal effect in bacterial cells, thus being a promising technology for clinical and environmental applications.
BODIPYs are versatile dyes never tested before in photodynamic application against prokaryotes. Two novel BODIPYs were synthesized and administered to two bacterial model strains, the Gram positive Staphylococcus xylosus and the Gram negative Escherichia coli. The two photosensitizers differ only in the moiety linked on pyridine nitrogen atom as PS 3 and PS 4 bear a methyl and a benzyl group, respectively. Despite the small structural difference, the methylated PS (3) and the benzylated PS (4) remarkably differ as regard the MIC and the MBC for both microorganisms, PS 3 being much more efficient. In-depth examinations of the antibacterial activity performed using the more efficient compound 3, showed that the photoinactivation was dependent on PS concentration, light dose and cellular density. BODIPY 3 proved to be very effective against S. xylosus and even against E. coli under very "mild" conditions, i.e. very short time of incubation in the dark, limited light dose and low PS concentration, making this molecule a very promising photosensitizer.

P7.16
Development of new synthetic media for recombinant protein production in Antarctic bacterium P. haloplanktis TAC125

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Pseudoalteromonas haloplanktis TAC125 was the first Antarctic Gram-negative bacterium which genome was annotated (Medigue et al 2005). It is characterized by high growth rates at low temperatures combined with the ability to reach high cell densities. The previously described features make the use of PhTAC125, as alternative expression host for the production of soluble and biologically active proteins at low temperatures (Parrilli et al 2008). The optimisation of cultivation strategies are essential factors to obtain high protein production. In a recent paper, we described the use of a defined medium, containing branched amino acids (L, I, V) as carbon sources. The use of LIV medium resulted in a significant increase in either reporter enzyme production or biomass yield with respect to the previously optimized conditions (Giuliani et al 2011). However, high cost and very poor solubility in water of branched amino acids makes unprofitable the use of this medium in large scale processes. Therefore, different new synthetic media have been optimised for recombinant protein production in PhTAC125, based on gluconate and less expensive amino acids, such as L-glutamate and L-aspartate.

P7.17
Exploring methanotrophic activity in geothermal soils from Pantelleria island (Italy)

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Methane is released to the atmosphere by a wide number of natural (geological and biological) and anthropogenic sources, and is the second most important greenhouse gas after CO_2. Microbial oxidation in soils by methanotrophic bacteria contributes to the removal of CH_4 from the atmosphere, and methanotrophic activity was recently detected in volcanic/geothermal areas, where degassing of endogenous gases occurs. Our aim is to describe the methanotrophs at the main exhalative area of Le Favare site at Pantelleria Island, where high CH_4 consumption (up to 950 ng/g/ per h) was measured.
Soil bacterial diversity was analysed by TTGE of amplified 16S rRNA genes and the diversity of proteobacterial methanotrophs was investigated by creating a clone library of the amplified methane mono-oxygenase encoding genes, *pmmoA*. Enrichment cultures, on a mineral medium in a CH_4-enriched atmosphere, led to the isolation of different strains that were identified as *Methylocistis* spp. Understanding the ecology of methanotrophy in geothermal sites will increase our knowledge of the role of such soils in methane emissions.

P7.18
Identification of bacterial siderophores-producers along seawater column in hydrocarbons-enrichments

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The aim of this study is to identify bacterial siderophore-producers in seawater at different depths, namely: surface, max chlorophyll, min oxygen and max depth. Iron is a limiting nutrient for microorganisms in marine environment because of its low availability in aerobic and neutral pH conditions. Bacteria synthesizes siderophores, low-molecular-weight and high-affinity chelators for the iron uptake. During the oceanographic cruise "Bonifacio_2011" four stations were sampled to study the induction of siderophores-producers in different substrates, namely naphthalene, oil and tetradecane and siderophore-producers were selected on CAS medium. As a result , were isolated microorganisms able to produce siderophores mainly in oil and naphthalene enrichments. The isolated strains, after phylogenetic analysis of 16SrDNA, shared 99% homology with *Halomonas sp., Marinobacter sp., Alteromonas sp., Pseudomonas sp., Vibrio sp., Spongibacter sp., Alcanivorax sp.* Moreover, the higher percentage of siderophores-producers was at maximum chlorophyll depth, confirming the syntrophic relationship between algae and siderophore-producers. Interesting, the most part of selected strains belong to hydrocarbons-degrading bacteria suggesting their role as helper during natural attenuation processes.

P7.19
Looking for cold adapted lipase in Pseudoalteromonas genus: a bioinformatic approach

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Esterases and lipases are the most applied biocatalysts in industrial applications. The reasons of this success are related to the wide diversity in the substrates recognised, combined to the exquisite chemoselectivity, regioselectivity and stereoselectivity frequently displayed by this enzymatic class.
Over the last years, growing interest has been devoted on cold-adapted lipolytic enzymes, due to their applicability, amongst the other, in the production of thermal labile secondary chemical compounds, in "domestic" cold-washing, or in "bioremediation" applications carried

out at ambient temperature (Babu et al., 2007; Hausmann et al.,2010).
The recent availability of ten genome drafts from Antarctic marine bacteria belonging to the Pseudoalteromonas genus allowed us to embark in a data mining approach aimed at identifying gene encoding cold-adapted lipases.
Hausmann S, Jaeger KE (2010) Lipolytic enzymes from bacteria. In: Timmis KN(ed)
Handbook of hydrocarbon and lipid microbiology. Springer, Berlin, pp. 1099-1126
Babu J., Pramod W. R., George T, and Nitisha S. Standard (2007). Review Cold-active microbial Lipases: a versatile tool for industrial applications Biotechnology and Molecular Biology Review Vol. 2 (2), pp. 039-048.

P7.20
Comparison of different clean-up (bioremediation) assays for treatment of bilge water

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Ship operations produce wastes and these compartments also capture fuel and engine oil that may leak within the boat. The substances collected in the blowest part of the hull, called the "bilge area", must be managed properly to avoid environmental pollution; in effect, illegal discharge of bilge oil into the sea accounts for about 10% of the total oils that enters the sea and represent an serious threat to our marine ecosystem. Bioremediation is recognized as one of several effective techniques to clean up outlined bilge water in the environment.
The primary objective of this work is analyze the potentiality of different biostimulation techniques for recovery of bilge water. For this aim four different microcosm systems (only bilge water, BW; bilge water whit inorganic nutrient, BW+IN; bilge water whit dispersant BW+D and bilge water emulsifier agent BW+E) were carried out. In order to monitor the changes occurring in the structure and composition of natural microbial communities measures of bacterial density (DAPI count, CFU, MPN), microbial activity (BOD) and structure (16S rRNA clone libraries) were carried out. Measure of oil degradation were also carried out.

P7.21
Characterization of bacterial communities in tourist ports in the Mediterranean Sea Basin

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Port areas pose major concerns due to the presence of toxic pollutants and to their harmful effects on the marine ecosystems and human health. Hydrocarbon contamination, associated with the heavy boat traffic and related facilities, is one of the major environmental problems. Moreover, tourist ports are subject to seasonal massive impact. This work is part of a multidisciplinary characterization of the tourist ports of Cagliari IT, El Kantaoui TN, Heraklion GR, carried out within the MAPMED project aimed to improve the environmental sustainability of tourist coastal areas in the Mediterranean Sea Basin with regard to monitoring and reduction of marine hydrocarbon pollution. Two sampling campaigns were carried out during winter and late spring, before the touristic season, and a third one is planned after the touristic season. In each port, samples of seawater, superficial and anoxic sediments were collected at different stations. A combination of culture-dependent (MPN of different metabolic groups) and -independent approaches (T-RFLP of 16S rRNA gene and other genes) has been employed in order to define the seasonal and spatial variations in the *Bacteria* and *Archaea* communities.

P7.22
A collection of immobilized and stabilized nucleoside phosphorylases for the synthesis of nucleoside analogues

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Nucleoside phosphorylases (NPs; E.C. 2.4.2) catalyze the reversible cleavage of the glycosidic bond of (deoxy) ribonucleosides in the presence of inorganic orthophosphate to generate the nucleobase and α-*D*-(deoxy)ribose-1-phosphate. If a second nucleobase is added to the reaction medium the formation of a new nucleoside can result.
We have recently reported on the production and characterization of a purine nucleoside phosphorylase from *A. hydrophila* (*Ah*PNPII) [1]. This PNP has been used in the synthesis of a few 6-substituted-purine-9-ribosides.
We have now isolated, cloned and expressed four new NPs from *C. koseri*, *C. perfringens* and *S. pyogenes* (*Ck*PNPI, *Ck*PNPII, *Cp*UP and *Sp*UP). Their substrate specificity has been investigated and compared to that of *Ah*PNPII and other enzymes previously reported. *Cp*UP, *Ah*PNPII and *Ck*PNPI were immobilized and used in the synthesis of antiviral araA and ddI.
[1] Ubiali D. et al. Adv. Synth. Catal. 354, 96, 2012

P7.23
A three-dimensional hepatocyte-alginate culture system as a tool for in vitro pharmacological tests

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Hepatocyte cultures are widely used as a model to investigate physio-pathological liver processes and offer a wide range of biotechnological applications in biomedical and pharmaceutical areas. The use of 3D matrices has produced improvements in culture techniques in maintaining the differentiated phenotype and specific cell functions. In this study, we developed a 3D-construct made of gas-foaming templating alginate scaffolds and human C3A hepatocytes. In this system, we assayed cell viability, morphology, growth, capability to perform specific liver functions, as well as the induction of P450 enzymes following drug addition. An interesting practical application of this device concerned the study of the metabolism of anabolic androgenic steroids of anti-doping interest. Results obtained by means of GC-MS demonstrate that hepatocytes cultured on 3D alginate scaffolds allow a better identification of metabolites of phase I and II, using testosterone as a model drug, in comparison to conventional cultures. This innovative system seems to be promising for the future in the discovery of new drug metabolites and in the identification of low concentrations of bioactive molecules.

P7.24
Microflora DNA (mf DNA): new perspectives for the study of biodiversity and microbial ecology

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Microbial communities are organized in complex ecosystems and can strongly affect the surrounding. The acquisitions of the genome projects, the knowledge of microbiomes, the advances in molecular techniques and next generation sequencing has opened new perspectives for research and the study of biodiversity. The definition of a database of environmental microorganisms (eg. GenEnv) on the basis of their genome has allowed the development of new molecular approaches to the study of the degree of biological diversity in various ecological

niches. The extraction and analysis of microflora DNA (mfDNA) is an new way of interpreting the diversity and dynamics of microbial communities in an specific environment. Specific applications in forensics, security, environmental quality, biotechnology are presented. In particular the identification of a microbial signature, as the result of the detection of DNA from physiological/environmental species, allows scientists to collect information on the equilibrium and the biodiversity of ecosystems.

P7.25
Long-term effects of ocean warming on the prokaryotic community: evidence from the vibrios

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Vibrios are a major source of human and animal diseases and yet in most countries both human and non-human illnesses associated with these bacteria are increasing. The cause of this increase is not known, but since vibrios are strongly thermodependant there is good reason to believe that global warming may have contributed.
To investigate this possibility we examined historical samples from the Continuous Plankton Recorder (CPR) archive which is one of the longest and most geographically extensive collection of marine biological samples in the world using advanced molecular analysis and pyrosequencing.
We show that the genus Vibrio, including the human pathogen V. cholerae, has increased in prevalence in the last half a century in the coastal waters of the southern North Sea and that this increase is correlated significantly, during the same period, with warming sea surface temperature. These findings provide support for the view that global warming may have a strong impact on the composition of marine bacterial communities with important implications for human and animal health into the future.

O7.1
The use of vegetable wastes for photobiological H_2 production

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Research on photobiological H_2 production is focusing on the utilization of low cost substrates as sources of reduced carbon, as a possible route to valorize wastes as sources of clean energy. NH_4^+, often present in this kind of substrates, is an inhibitor of nitrogenase-mediated H_2 production in purple non sulfur bacteria.
A 2-stage process using vegetable residues was investigated. In the 1st stage (acidogenic) the raw residues were dark-fermented by the autochthonous microflora. In the 2nd stage (H_2 photoevolution) the liquid fraction of the fermented residues was utilized by a *Rhodopseudomonas palustris* NH_4^+-insensitive mutant strain for H_2 production.
The aim was to assess the effectiveness of the mutation by using the strain on a waste derived medium.
The mutant strain was able to produce H_2 in this medium without need of dilution. Nitrogenase assays showed a reduction in efficiency probably due to the limited irradiation but not to the presence of NH_4^+.
In conclusion, the 2-stage process seems promising for coupling H_2 production with the treatment of vegetable waste-derived substrates.
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O7.2
Deesterified homogalacturonan content as a biochemical trait to select plant varieties useful for bioenergy production

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Plant biomass is an attractive renewable source for the production of biofuels. The major bottleneck of the lignocellulosic biomass conversion is the recalcitrance of cell wall polysaccharides to hydrolysis (saccharification) due to their complex structure. Cell wall is mainly composed of a cellulose-hemicellulose network embedded in a cohesive pectin matrix. Intermolecular bonds of pectin mediated by acidic homogalacturonan (HGA) influence the accessibility of cellulose to hydrolysis. We have demonstrated that the reduction of HGA regions by the constitutive or inducible expression of a fungal polygalacturonase (PG) or pectin methylesterase inhibitors (PMEIs) in Arabidopsis, wheat and tobacco improves enzymatic saccharification. Here we demonstrate that a low level of acidic HGA can be used as marker to identify Arabidopsis and wheat genotypes with improved saccharification efficiency. We also show that the tissue from tobacco plants expressing a fungal PG more efficiently accumulate intermediates during the anaerobic biomethane production.

O7.3
Characterization of anammox populations and microbial communities during autotrophic nitrogen removal in different reactors

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The anammox process is an alternative to conventional nitrification-denitrification systems for nitrogen removal from wastewater, since aeration and external carbon sources are not needed. However, research on the best engineering solutions to be adopted is still ongoing. In this work, anammox populations and microbial communities were characterized during the nitrogen removal process from the liquid fraction of a piggery manure digestate in two different laboratory reactors, a Sequencing Batch Reactor (SBR) and a Membrane Biological Reactor (MBR). They were initially fed with a mineral medium, later gradually replaced with the wastewater. The presence and variations of anammox, AOB and NOB populations in the reactors were monitored with FISH analyses, while the abundance of total and anammox microorganisms and AOB was determined by qPCR. The structure of the microbial community in each reactor was studied with DGGE, targeting the V3-V5 hypervariable regions of the 16S rRNA gene. Despite the high copy number and activity showed by anammox bacteria during the experiment, the potential ability to completely remove nitrogen was reduced at the highest percentages of wastewater.

O7.4
Production of Lipase A from Bacillus subtilis using different strains of the yeast Saccharomyces cerevisiae as host: a preliminary approach to the feasibility of the bioprocess

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Lipases are enzymes which catalyze the hydrolysis of long chain triglycerides. Lipases have a wide industrial application in the oleochemistry, detergent formulation, organic synthesis and nutrition.
In this work, the Lipase A from *Bacillus subtilis* expressed in three different strains of the yeast *S. cerevisiae* (the industrial diploid strain, Y306, and two haploid laboratory strains, CEN.PK 113-5D and BY4741) as a fusion protein with the cell-wall protein Pir4, was produced in aerated fed-batch reactor.
In all the transformed yeast strains, the lypolytic enzyme resulted to be confined onto the biomass, presumably strongly and stably linked to the cell-wall. For this reason, the heterologous enzyme was considered as an enzyme immobilized on their own producing cells. The performance in fed-batch reactor of the three strains was evaluated in terms of both cell density and activity of the enzyme achieved during the fermentation runs. These latter were set up at a 0.16 h^{-1} μ value. On the basis of experimental results obtained, a study of bioprocess feasibility on industrial scale was made together with an economic evaluation of costs and profits of a single fermentation run.

O7.5
Development of a quorum sensing-based communication system between natural and synthetic cells

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Bio/chemical-Information and Communication Technology (bio/chem-ICT) aims at extending classical ICT, based on the transmission of electrical or electromagnetic signals, to bio/chemical molecules. In this context, we are exploring the possibility to develop a communication system between natural and synthetic cells, a scenario that would pave the way for new rational drug-delivery approaches. As a model system we choose *Pseudomonas aeruginosa*, a human pathogen that coordinates group behaviour via a well-characterized quorum sensing (QS) system, and semi-synthetic minimal cells (SSMCs). SSMCs are liposome-based micro-compartments containing the minimal components required for protein expression from a DNA template.
Preliminary analyses showed that well-defined populations of liposomes can be obtained in both rich and minimal bacterial growth media, and that these liposomes are stable in a *P. aeruginosa* growing culture. Moreover, liposomes loaded with synthetic *P. aeruginosa* QS signal molecules are able to activate the QS response in *P. aeruginosa* mutants impaired in signal molecules production. The generation of SSMCs synthesizing QS signal molecules is now in progress.

8 - Immunology

P8.1
Effects of oxygen-loaded dextrane nanobubbles on regulation of gelatinases in haemozoin-fed human monocytes

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Matrix metalloproteinases (MMP) can activate cytokines or destroy tight junctions, thus playing a crucial role in main features of human cerebral malaria (CM): inflammation, blood brain barrier (BBB) damage, and hypoxia. Here, we studied the effects of oxygen-loaded nanobubbles (OLN) on hypoxia-dependent regulation of gelatinases (MMP-2 and MMP-9) in haemozoin(HZ)-fed human monocytes. Cells secreted basal levels of MMP-9 but not of MMP-2. Hypoxia reduced basal MMP-9 release without affecting expression. HZ and its lipoperoxidation derivative 15-HETE enhanced MMP-9 expression and release, either in normoxia or hypoxia. OLN, constituted by dextran shell and oxygen-storing decafluoropentane core, with sizes of about 500 nm and a negative surface charge, showed good capacity of O2 delivery, not accompanied by O3 generation after UV rays (346 nm) sterilization, and not displaying toxic effects on cells. OLN abrogated both hypoxia-dependent reduction or HZ-dependent increase of MMP-9 secretion. These data suggest that OLN may prevent MMP-dependent inflammation and BBB damage in CM, thus potentially being a promising candidate for adjuvant therapy in complicated malaria.

P8.2
Capsaicin induces surface exposure of immunogenic cell death molecular determinants like carleticulin, HSP90 and HSP70 in human cancer cell lines

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Some anticancer chemotherapeutics (anthracyclines, oxaliplatin) elicit immunogenic apoptosis, meaning that dying cancer cells are engulfed by dendritic cells and tumor antigens efficiently presented to CD8+T lymphocytes, which control residual tumor cells. Immunogenic apoptosis is characterized by early-apoptotic surface exposure of specific intracellular damage-associated molecular patterns like calreticulin (CRT), HSP90 and HSP70. We investigated the ability of capsaicin (CPS; trans-8-methyl-N-vanillyl-6-noneanamide) to induce CRT, HSP90 and HSP70 exposure in the human SD48 and T24 bladder cancer cell lines. Cells were treated with 150-250 μM CPS for different time periods, and, by immunoblot and immunofluorescence, we showed that CPS induced early-apoptotic cell surface exposure of CRT, HSP90 and HSP70. Moreover, HSP90 and HSP70 were also released in the supernatant during apoptosis. The investigation of the exposure/release of other immunogenic molecular determinants is underway. We provide the first evidence that CPS induces early-apoptotic CRT, HSP90 and HSP70 exposure, suggesting that CPS might represent a novel potential anticancer immunogenic chemotherapeutic agent.

O8.1
Modulation of HLA-E expression during monocyte differentiation and activation

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HLA-E are non-classical MHC class I molecules that, through the binding of CD94/NKG2A receptor, protect cells from the killing by Natural Killers (NK). Differently from the classical HLA class I, HLA-E is poorly polymorphic determining an unique change at amino acid 107 which is, however, functionally relevant. The expression and function of HLA-E require peptides derived from the leader sequences of HLA class I molecules. However, they can also bind self or pathogen-derived peptides, whose effect on the NK response is controversial. To partially address this, we compared the expression of the HLA-E molecules in monocytes and in macrophages. The results show how the HLA-E is strongly up-regulated in macrophages as well as in a differentiated monocytic cell line. Other molecules involved in antigen presentation such as ERAP1 and ERp57, but not HLA class I molecules, are also upregulated. These findings strengthen the hypothesis that HLA-E exploits the same machinery as the classical HLA-class I molecules to present self proteins other than HLA-class I. Work is in progress to evaluate the functional effects of this overexpression on the activity of NK and antigen-specific T cells.

O8.2
Naip-5 inflammasome governs cell death responses and IL-18 secretion in *Shigella*-infected bone marrow-derived dendritic cells

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Detection of microbes relies on pattern recognition receptors (PRRs) that recognize bacterial molecules, so-called PAMPs. PRRs also respond to host–derived danger signals termed DAMPs, which are released in response to tissue injury, stress or necrotic cell death. A subset of PRRs, belonging to the intracellular NOD-like receptor (NLR) family induces the assembly of a multi-protein signaling platform, called inflammasome. Several NLR proteins, including NLRP3, NLRC4, AIM2 are known to promote the inflammasomes assembly. Inflammasomes activation results in caspase-1 processing, secretion of pro-inflammatory cytokines and pyroptosis.
Here, we have investigated on the molecular mechanisms, governing the balance between survival and cell death in BMDCs infected with *Shigella*. Our results show that BMDCs respond to *Shigella* infection by triggering two cell death pathways: i) a rapid and massive apoptosis via caspase-3 activation; ii) caspase-1–mediated pyroptosis and the ensuing IL-1β and IL-18 release. We also characterize the inflammasome platforms demonstrating that MyD88–dependent signaling pathway plays a pivotal role in IL-1β release, whereas is dispensable for IL-18. On the contrary, infected caspase-1$^{-/-}$ BMDCs are strongly impaired in both IL-1β and IL-18 release. Moreover, we identified Naip5 as the main NLR-protein, able to induce caspase activation and IL-18 release, allowing BMDCs cell death.

O8.3
Impact of *Shigella flexneri* muropeptide shedding modifications in antigen-presenting cells

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Bacterial pathogens have evolved several strategies to avoid immune system recognition such as peptidoglycan (PGN) modification. PGN is a dynamic structure undergoing to continuous remodeling during the bacterial growth that leads to muramylpeptide shedding. Muropeptides are recognized by the innate immune system through the intracellular PRRs NOD1 and NOD2. As the innate immune system is committed to prepare the development of the adaptive immunity, changes in the first steps of pathogen recognition could dramatically affect its development. With this aim, we have chosen *Shigella flexneri* as model system, mutated in genes involved in PGN recycling to investigate whether and to what extent modifications in PGN muramylpeptide organization and shedding could impact the immune system. We have focused our attention on two cellular systems linking the two branch of the immunity: macrophages and dendritic cells.
Our results demonstrated that PGN fragments composition influences host responses both in macrophages and in dendritic cells. In fact, following contact with these *Shigella* mutants, cytokine/chemokine pattern and antimicrobial peptides activation and release by cells are largely affected, as well as macrophages cell death; an important feature in *Shigella* pathogenesis. Moreover, we showed that PGN mutants – derived commitment of dendritic cells orchestrate adaptive immunity, by manipulating T cell polarization toward Th1, Th2 and Th17 phenotypes.

O8.4
ROS contribute to *Pseudomonas aeruginosa* killing by CF macrophages

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Professional phagocytes destroy pathogens in part through ROS, generated directly or indirectly by the NOX2 NADPH oxidase. The importance of ROS in pathogen elimination is highlighted by individuals with mutations that cause partial or total inactivation of the oxidase which are characterized by severe recurrent infections that can result in death. In a previous study, it was demonstrated that *P. aeruginosa* (Pa) induced ROS release, which was abolished in *Cftr*-deficient murine macrophages and that this impairment was associated with defective bactericidal activity. In order to determine whether this microbicidal mechanism is also impaired in human CF macrophages we measured ROS generation following Pa infection in CF as well as in non-CF cells. Subsequently, we compared the bactericidal activity of non-CF and CF cells treated with DPI, an inhibitor of the NOX2 NADPH oxidase.
The resulting data revealed similar ROS generation by non-CF and CF cells following Pa infection. Accordingly, we showed that DPI caused a significant increase in the survival of intracellular bacteria in non-CF as well as in CF macrophages. Similar results were obtained in macrophages differentiated *in vitro* or isolated from lung.

O8.5
Down-regulation of the NKG2D receptor is differentially controlled by MICA and ULBP2 ligands

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The activating NKG2D receptor on human NK cells mediates "induced self recognition" in that its ligands (NKG2DLs) are up-regulated by stressed or diseased cells. Evidence collected in the past years demonstrated that chronic exposure to NKG2DLs induces receptor down-modulation.
The aim of this study was to evaluate whether different NKG2DLs, namely MICA and ULBP2, are equivalent in their capacities to down-modulate the surface receptor expression on human NK cells.
We analyzed the rate and kinetics of NKG2D down-modulation in primary cultured NK cells and in the NKL NK cell line upon transient stimulation with stably over-expressing MICA or ULBP2 target cells. NKG2D internalization and lysosomal degradation was more rapid and efficient in MICA-experienced cells that also showed a higher tyrosine phosphorylation of the ubiquitin ligase c-Cbl.
All together these results demonstrate that NKG2D down-regulation is influenced by the nature of its ligand and suggest a different contribution of the ubiquitin pathway in the control of NKG2D internalization and degradation in MICA- versus ULBP2-experienced cells.

9 - Epigenetics and epigenetic therapies

P9.1
Evidence of an epigenetic regulation mechanism in primary endothelial cells from gestational diabetes umbilical cords

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Gestational diabetes (GD) is a common disorder present in human. It is associated with increased levels of glucose in the blood that can be dangerous for neonatal growth. The genetic component is believed not to be a component of the disease as no significant association has been reported. New lines of evidence suggest an epigenetic component for GD. Moreover, exposure of cells to high glucose (HG) conditions is believed to induce epigenetic changes. In order to study epigenetic changes by HG in vivo we compared gene expression and promoter methylation in endothelial primary cell lines from umbilical cords of GD patients (n=3) and controls (n=3). In order to assess the effect of hyperglycemia in vivo we firstly analyzed gene effects at the level of the transcriptome using chips. We found induction of several genes. Many genes were confirmed by means of qPCR. Quantitative PCR of methylation-sensitive or resistant restriction enzyme digested genomic DNA from GD-HUVEC using primers amplifying CpG rich island shows differential DNA methylation in TGFB2 and IGFB3. Data obtained at the same time brings new information on DG, i.e. the hypothesis of an epigenetic mechanism regulating TGFB2 and IGFBP3 expression by HG and supports the use of GD-HUVEC cells as a model for the study of the molecular mechanisms involved in the pathogenesis of the disease.

P9.2
A bioinformatics analysis of Lamin A regulatory network: a perspective on epigenetic involvement in Hutchinson-Gilford progeria syndrome

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Hutchinson-Gilford progeria syndrome (HGPS) is a rare human genetic disease that leads to premature ageing and ageing-associated phenotype. HGPS is caused by mutation in the lamin A (LMNA) gene that leads, in affected young individuals, to the generation of progerin, a splicing mutant of lamin A. Classical and Competitive Endogenous RNAs bioinformatics analysis of the LMNA gene network of interactions is presented. Lamin A seems to be involved in epigenetic regulation of transcription, chromatin remodelling, DNA repair, with key roles in stemness regulation, normal ageing and telomere functions. The study suggests particular relevance of chromatin remodellers and histones covalent modifiers in the LMNA network. Specifically, HTATIP histone acetylase seems to be of particular relevance in the network.

P9.3
YETI and CFDP1: two related proteins required for chromatin organization

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The BCNT proteins are characterized by an evolutionarily conserved 80 amino acid region located at the end of the C-terminal portion (BCNT-C domain). Despite a widespread distribution of this protein family proteins in animals and plants, its function remained largely unknown. We have used *Drosophila melanogaster* as a model system to perform in-depth functional studies of the fly BCNT protein, called YETI. Using a combination of P-element mutagenesis, inverse PCR, RNAi knock down and transgene rescue methods, we found that i) the *Yeti* gene correspond to *l(2)41Aa*, an essential Drosophila gene located in pericentromeric heterochromatin and ii) the YETI protein is required for the proper chromosome organization and H2Av loading on chromatin. In addition, overexpression of the human hortologous gene, *Cfdp1*, in wild-type flies and its RNAi-mediated knock-down in HeLa cells strongly suggest that the CFDP1 protein, like YETI, is involved in chromatin regulation, which is extremely interesting in the light of a possible involvement of CFDP1 in human developmental syndromes.

P9.4
SAGA complex regulates centromeric histone variant and centromere

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The centromere is a chromosome region defined by a variant nucleosome needed to prevent chromosome loss and aneuploidy. Loss of HAT Gcn5 causes mitotic defects, here we show that deletion of SAGA components Gcn5 and Ubp8 leads to an aberrant association of histone variant Cse4 at the centromere in yeast. Also, we demonstrate that Gcn5 does not act directly on Cse4 but rather cooperates with the ubiquitin protease Ubp8. Ubiquitin-mediated proteolysis reportedly regulates the localization of Cse4 but the regulatory mechanism was not fully identified. Here we show that Cse4 lysines are required for the association of Cse4 at centromere and are direct targets for Ubp8. Loss of either Gcn5 or Ubp8 affects the Cse4 stability at the protein level and alters its turnover. Collectively, our results demonstrate that SAGA complex is involved in the deubiquitination of Cse4 at lysines and that Gcn5 supports this activity.

P9.5
Methylation of the human mitochondrial 12S rRNA gene is correlated with aging

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Controversial evidences have been reported about epigenetic modifications of mitochondrial DNA. This study was aimed at investigating the presence of methylated cytosines in two CpG sites located within human mitochondrial 12S and 16S rRNA genes, respectively. Methylation of the first gene was analyzed by TaqMan Real-time PCR on bisulfite-treated DNA; in the second case, methylation was analyzed by using a restriction enzyme cutting the bisulfited DNA only if methylated. Both procedures were applied to blood DNAs collected from 301 individuals (63-107 years old). Methylated cytosines were found within the sole 12S gene. In particular, in most samples the presence of both methylated and unmethylated molecules was detected. By a linear regression model, we found that in 90-107, but not in 63-89 year-old subjects, methylation percentage significantly decreased with age in males (β=-0.03; p=0.006).
Overall, our data represent the first evidence about the presence of methylated cytosines within the human mitochondrial 12S rRNA and, more importantly, the susceptibility to extreme longevity of this epigenetic modification.

P9.6
Candida parapsilosis DNA contains modified bases as shown by amplified fragment length polymorphism (AFLP) analysis and by HPLC and mass spectrometry

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Candida parapsilosis is an opportunistic pathogenic fungus, characterized by very little genetic variability. Sequence analysis of its genome suggests that it is almost completely homozygous. AFLP analysis of 400 clinical isolates confirmed its low genetic variability. Only 6 additional bands were found using the EcoRI/MseI enzyme combination. Notably, the band intensities varied considerably between isolates. To explore the possibility that this variation was caused by base modification(s) at the restriction sites, other enzyme combinations (EcoRI/HapII, EcoRI/MspII, EcoRI/MboI, EcoRI/Sau3A, EcorI/CviQI and EcoRI/Csp6I), differing for their sensibility for base modifications, were used. Indeed, additional bands not present in in silico AFLP analysis were observed. Sequence analysis of selected AFLP bands, confirmed their C. parapsilosis origin and showed that most of them contained a modified site. Using HPLC followed by mass spectrometry, no detectable levels of the 5-mC or 5-hmC modifications were observed in the genomic DNA, while the quantification of the 6-mA marker demonstrated appreciable levels of the modified base (0.031%).

P9.7
The ZFP57 imprinting factor and epigenetic control: further clues stemming from expression profiling, ChIP seq and in vitro-induced differentiation

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Epigenetic marks are key to embryogenesis and are linked to the regulation of pluripotency, cell fate and cellular identity. Extensive epigenetic reprogramming occurs at early embryos stages and exceptions are the parent of origin-specific signatures at Imprinting Control Regions (ICRs) driving regulation of imprinted genes. ZFP57 was shown to be required for the maintenance of DNA methylation at ICRs in embryos and of both DNA methylation and histone H3 modification in embrional stem cells (ES). ZFP57 associates with KAP1, a recruiter of chromatin modifiers including the H3 methyltransferase SETDB1 and HP1, thus promoting H3 K9-trimethylation and chromoproteins assembly at targeted sites. ChIP seq profiling in ES cells of endogenous ZFP7 and associated factors shows assembly at hundreds regions in addition to imprinted loci and we will report on ongoing studies that integrate expression profiling, genome-wide occupancy, the status of selected targets and *in vitro* cell differentiation that suggest a wider role for ZFP57 in shaping the pluripotent epigenome and provide further clues for its involvement in development and cell fate.

P9.8
The WD-repeats chromatin remodeling component *nfc102* regulates maize development through distinct epigenetic-related mechanisms

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The maize *nfc102* gene encodes a WD-repeat protein and displays homology with Arabidopsis *FVE*: a component of the autonomous flowering pathway that regulates epigenome stability.
We observed that maize *nfc102* down-regulation mutants exhibited various developmental defects, suggesting a pleiotropic *nfc102* effect. In particular, *nfc102* mutation alters the transcription of key regulators of maize flowering, such as *Indeterminate 1* and the florigen *ZCN8*, and partially releases epigenetic silencing of transposable elements. We reported that *nfc102* regulates transcription by mediating histone modification changes. However, this occurs by means of different mechanisms, depending by distinct targets. Indeed, *nfc102* can either directly target histone modifiers (e.g. histone deacetylases) toward its target or regulate RNA processing by modulating formation of the RNA antisense strand. Altogether, these observations support the hypothesis that nfc102 is part of different chromatin remodeling complexes, involved in distinct pathways and acting through different mechanisms, where it may act as a scaffold to facilitate and stabilize protein interactions between others complex components.

P9.9
Evidence of methylation within the control region of human mitochondrial DNA

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5-methylcytosine occurs in most of prokaryote and eukaryote DNA. However, the presence of this modified base within human mitochondrial DNA (mtDNA) is currently debated. In this study methylation status of mtDNA control region (D-Loop) was analyzed by bisulfite sequencing and methylated DNA immunoprecipitation in peripheral blood DNAs collected from 30 human subjects and in DNA samples from cultured cells obtained from different tissues. We demonstrated the presence of methylated cytosines within human D-Loop. In particular, both 5-methylcytosines and 5-hydroxymethylcytosines were detected. Moreover, an observed variability among blood and cells from other tissues suggests that tissue specificity of mtDNA methylation mechanisms could occur. Immunoblotting analyses revealed the presence of DNMT1 and DNMT3b within mitochondria, thus suggesting that these enzymes may be responsible of the maintenance of 5mC and h5mC, respectively, within D-loop region. On the whole, we prove as epigenetic mechanisms also occur in human mtDNA, thus suggesting that epigenetic modifications may affect, through yet unknown mechanisms, replication and transcription of this genome.

P9.10
Role of cis-acting elements and trans-acting factors in genomic imprinting defects

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Gamete-of-origin dependent gene expression is controlled by allele-specific epigenetic modifications of Imprinting Control Regions (ICRs). Either hypo- or hyper- DNA methylation abnormalities at the ICRs result in altered gene expression and disease. DNA methylation changes are accompanied by changes in histone modifications and long-range protein interactions of the ICRs. ICR epigenetic alterations may result from either mutations acting in cis or affecting factors acting in trans. Among the factors interacting with the non-methylated allele, we showed how the binding of CTCF with multiple adjacent target sites maintains the DNA methylation-free status at the IGF2/H19 ICR. Concerning the factors interacting with the methylated allele, we demonstrated that the binding of the ZFP57/KAP1 complex is required to maintain DNA methylation and histone H3K9 trimethylation at multiple ICRs. We observed that ZFP57 specifically interacts with a methylated target motif that is enriched at the ICRs in both human and mouse. Sequence determinants for ZFP57 binding and DNA methylation maintenance at imprinted loci are being investigated by DNA-protein interaction studies and targeted mutagenesis.

O9.1
Erasure of DNA methylation in mouse primordial germ cells: a role for PARylation

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Poly(ADP-ribosyl)ation (PARylation) drives epigenetic events regulating chromatin structure and transcription. In particular, Parp1 is directly able to influence DNA methylation patterns controlling the activity of Dnmt1. Here, we investigate the involvement of PARylation during germline epigenetic reprogramming in mouse embryo. Notably, we showed that Parp1 activity is stimulated in primordial germ cells (PGCs), the embryonic precursors of gametes, prior to and at the beginning of DNA demethylation. We demonstrated that PARP inhibition impairs both genome-wide and locus-specific DNA methylation erasure in PGCs. Besides participating in the base excision repair (BER)-mediated DNA demethylation, we showed that PARP activity regulates the expression of genes involved in active DNA demethylation as Tet hydroxylases. In conclusion, our results indicate a more general involvement of PARylation during the epigenetic reprogramming of germline. Moreover, the transcriptional control of Tet genes by PARylation opens new perspectives on the mechanisms that may underlie events of genome hypomethylation typical of several pathologies as tumors and neurological disorders.

O9.2
Small non-coding RNA signature in Multiple Sclerosis patients after treatment with Interferon-ß

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Non-coding small RNA molecules play pivotal roles in cellular and developmental processes by regulating gene expression at the post-transcriptional level.
In human diseases, the roles of the non-coding small RNAs in specific degradation or translational suppression of the targeted mRNAs suggest a potential therapeutic approach of post-transcriptional gene silencing that targets the underlying disease etiology.
The involvement of non-coding small RNAs in the pathogenesis of diverse diseases such as cancer, cardiac failure, and neurodegenerative diseases such as Alzheimer's , Parkinson's disease and Multiple Sclerosis has been demonstrated. Particularly, it is becoming increasingly evident that non-coding small RNA species are associated with the Multiple Sclerosis (MS) development and the responsiveness to IFNß.
We analyzed the composition of the entire small transcriptome in IFNß treated Relapsing-Remitting MS patients and, beside the altered expression of several miRNAs, our analyses revealed differential expression of small nucleolar RNAs (snoRNAs) and misc-RNAs. Hsa-mir- miR-26a-5p expression was significantly higher in IFN-b treated RRMS patients at 3 months treatment, slightly declining at 6 months treatments. The results might provide insights into the mechanisms of action of IFN-b treatment in MS and provide fundamentals for the development of new therapeutic tools.

O9.3
Epigenetic modifications at retrotransposable sequences: further evidences on their correlation with the whole genome epigenetic changes

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Epigenetic modifications have a pivotal role in normal brain development and functioning and in the etiology of different neurological diseases. Previously, we showed that tolerance to ischemic brain episode, induced by preconditioning with Cortical Spreading Depression (CSD) phenomenon, might act through epigenetic control of gene expression. The analysis of dimethyl-H3K4 and dimethyl-H3K9 at specific loci led to hypothesize that such modifications might act through at least two effects. Firstly, they could represent a molecular memory of early transcription process for neuroprotective genes; secondly, they could contribute to an epigenetic silencing of retrotransposable sequences. These conclusions have been validated by the results on the epigenetic pattern of DNA methylation; in particular, retrotransposable sequences were hypermethylated in CSD-preconditioned brain hemispheres. The data on histone tail modification and DNA methylation corroborate the idea that epigenetic status of repetitive sequences are correlated with the whole genome epigenetic status and that they could represent potential biomarkers in specific normal and pathological conditions.

O9.4
A role for Jhd2 de-methylase in transcription regulation in *S. cerevisiae*

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Histone tails are subjected to several modifications which form a combinatory code which is read and interpreted by a plethora of regulatory protein complexes. Among the various modifications, Lysine (K) methylation is particularly interesting, due to its widespread roles in transcriptional regulation, DNA repair and epigenetic inheritance. In S.cerevisiae, Set1 performs K4 di- and tri-methylation which are removed by the histone de-methylase Jhd2
The protein Not4 has recently been shown to have an important role in regulating histone H3 methylation at highly transcribed genes by targeting Jhd2 to proteolysis. We found that Not4 preferentially binds to ribosomal protein (RP) genes and increases H3K4 tri-methylation supporting the model in which H3K4 tri-methylation is controlled by Jhd2 proteolysis.
We also found evidence of a regulation of Jhd2 expression in function of cell cycle and of growth rate. JHD2 mRNA and protein abundance increase after diauxic shift and immediately after release from the stationary phase. We analyzed the effects of Jhd2 expression on H3K4 methylation and gene expression. We focused in particular on a group of genes which are strongly modulated at the diauxic shift that we found to dramatically increase their H3K4 tri-methylation upon induction by ChIP on chip experiments. These results suggest a regulatory role of Jhd2 in DS-specific genes transcription.

O9.5
Involvement of HIF-1α in hypoxia-induced Placental Growth Factor expression

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Placental Growth Factor (PlGF) is a member of Vascular Endothelial Growth Factor (VEGF) family, mainly involved in pathological angiogenesis. Several data indicate a positive modulation of PlGF expression by hypoxia, but the direct involvement of hypoxia-inducible factor-1α (HIF-1α) has not been demonstrated, therefore the molecular mechanism governing this effect is still unclear.
We demonstrated that PlGF expression is effectively up-regulated

by hypoxia in human and mouse endothelial cells. So, we decided to investigate whether the hypoxia-induced PlGF expression could be mediated at epigenetic level. Thus, we analyzed the acetylation status of histones H3 and H4 by ChIP assay: the acetylated isoforms of histones were enriched in the PlGF intron-2, where HREs, never described before, are located. We detected the binding of HIF-1α to this region by ChIP assay. Moreover, PlGF expression was inhibited when we knocked down the expression of HIF-1.
In conclusion, these results suggest that both human and murine PlGF intron-2 have a chromatin structure that undergoes a change under hypoxia condition in endothelial cells and that HIF-1α is directly involved in PlGF hypoxic regulation.

10 - Human genetic and genomic diversity

P10.1
"Human biodiversity in Italy: micro-evolutionary patterns", a national collaborative project (PRIN 2009-2011)

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Due to their low genetic complexity and relative environmental and socio-cultural uniformity, geographical and/or cultural isolates provide an unique opportunity to reconstruct processes shaping human biodiversity. Groups with remarkable historic and linguistic peculiarities, often living in a mountainous setting, are widely dispersed in the Italian territory from the Alps to the main islands. Following and developing previous work by the same research network, the project initiative "Human biodiversity in Italy: micro-evolutionary patterns" is aimed at achieving an extensive genetic characterization of these populations and studying the relations between patterns of genetic diversity and cultural, historical and demographic factors. To this purpose, research units from the Universities of Bologna, Cagliari, Pisa and Roma Sapienza are collaborating in a multidisciplinary framework. The innovative aspects of this project are represented by: i) an accurate sampling strategy based on the history and the demographic structure of the populations; ii) a complete genotyping of uniparental and autosomal markers; iii) a participative involvement of the interested communities.

P10.2
Cultural homogeneity, ethnic boundaries and genetic diversity among Alpine linguistic islands

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German-speaking "linguistic islands" settled in the eastern Italian Alps represent an ideal case study for anthropological investigations of the impact of geographical factors and cultural diversity on the genetic structure of human populations. We analyzed mtDNA variation in four of these groups, Lessinia, Sappada, Sauris and Timau, that share many cultural aspects, but their components do not self-identify as belonging to the same community. We observed a reduced intrapopulation genetic diversity, together with a high differentiation from Italian and European populations, particulary for the latter three, which also lack signs of demographic expansion. As a further test of these signatures of genetic isolation, through a Bayesian approach, we inferred a reduced incoming gene flow in Sappada, Sauris and Timau from both a neighboring open Italian-speaking and a wider European population. Thereafter, we carried out coalescent simulations to weight the effect of differences in effective size and of an "ethnicity hypothesis" on the level of genetic differentiation observed between these historically related isolates.

P10.3
Phylogeographic analysis of human Y chromosome diversity in eastern Africa

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Encompassing an area characterized by enormous geographic variety, as well as ethnic, linguistic and cultural diversity, eastern Africa has seen remarkable levels of human migration and interaction over a very long period of time. Despite its importance for the evolutionary history of our species, this region has nonetheless seen less evolutionary genetic research than other regions in the African continent. In a study of 750 males from 25 eastern African populations, we have analyzed 107 Y-specific biallelic polymorphic markers, many of which here described for the first time. We observed 44 different Y chromosome haplogroups, some of which - haplogroups A-M13/V3, J-M267/V44, E-M215 and E-M329 - showed peculiar and interesting geographic distributions in the region. Phylogeographic analysis of the data showed that the gene pool of eastern Africa has been shaped by different processes associated with the physical geography of the area, social structure of some populations, demic diffusions and important cultural innovations.

P10.4
Allelic frequencies of enhancer hs1.2 from Africa to Eurasia

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The human Immunoglobulin heavy chain 3' Regulatory Regions (3'RR) span over 30kb at the 3' of both constant alfa genes encompassing three enhancers hs3, hs1.2, hs4. The central enhancer hs1.2 is polymorphic with four alleles that seem to control the Ig seric levels in children, even though the mechanism is not fully understood yet. The alleles differ for the number of a 40bp satellite repeated from 1 to 4 times and include the consensus for variable number of transcription
factors probably conditioning the 3D conformation of the entire Regulatory Region. The *2 allele absent or at very low frequency in African populations is present in Europe with a frequency of 40-50% and is associated with higher risk of immune diseases. The genetic distances of the European and Asiatic populations from Africans are confirmed by the Native American and Afro-American who conserved the frequencies

of the Asians or African groups. The increase of frequency of the *2 allele in non African populations could suggest a higher necessity of immuno protection of these populations in childhood correlated with environmental changes since we demonstrated that the allele is associated to higher levels of seric Ig in Italian children.

P10.5
Peopling of South America: new data from Southern Cone mtDNA haplogroups

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Studies of Native American mtDNA variation entered the final phase of the phylogenetic refinement: the molecular dissection of the founding haplogroups into clades of younger age and more restricted geographic distributions. For the Southern Cone of South America, this approach has recently allowed the identification of two autochthonous clades whose age estimates indicate that Paleo-Indians might have reached this region from Beringia in less than 2000 years. In this study, by sequencing 46 mitogenomes from Chile and Argentina, we identified two novel clades, termed B2l and C1b13. Our finding supports the scenario that the mutational motifs characterizing these sub-clades arose and expanded into the Southern Cone region. However, the age estimate for B2l appears to be younger than those previously reported for other local clades. The difference could reflect the different evolutionary origins of the distinct South American-specific sub-haplogroups, with some being already present at the very front of the expansion wave along the Pacific coast prior to its arrival in what is now Chile and others originating later *in situ*, when the tribalization process had already begun.

P10.6
Testing for a Southern route of early human dispersal from Africa

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Although paleontological and genetic data strongly suggest that anatomically modern humans originated in Africa and dispersed from there some 60,000 years ago, there is still disagreement on the details of the dispersal process. Based on analyses of skull shapes, Lahr and Foley (1994) proposed that some populations originated from an earlier (100,000 years ago) expansion of modern humans through the horn of Africa (southern route). To date, analyses of both mitochondrial DNA and modern genomic variation are consistent with a complex "Out of Africa" scenario, involving more than a single dispersal event; nevertheless all these results are still far from settling the issue. In this study we analyze genome-wide SNPs variation of 28 worldwide populations; we combine different approaches, including (a) the analysis of the population structure, (b) a comparison of genetic distances between populations with the expectations of different dispersal models, and (c) a linkage disequilibrium (LD)-based approach to trace the population's changes in Ne through time and to estimate the respective divergence time from Africa. This way it would be possible to explicitly test whether the southern route of early human dispersal from Africa can explain, better than any alternative model, the observed worldwide genomic variation, allowing us to significantly improve our understanding of the evolution of human populations.

P10.7
Y-chromosome diversity in modern Bulgarians: new clues about their ancestral origin

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To better define the structure and origin of the Bulgarian paternal gene pool, we have examined the Y-chromosome variation in 808 Bulgarian males. The analysis was performed by high-resolution genotyping of biallelic markers and by analyzing the STR variation within the most informative haplogroups (hgs). We found that the Y-chromosome gene pool in modern Bulgarians is primarily represented by Western Eurasian hgs whereas hgs common in Altaic and Central Asian Turkic-speaking populations occur at a frequency of only 1.47%. Principal Component analyses group Bulgarians with European populations, apart from Altaic and Central Asian Turkic- speaking groups. Within the country, the genetic variation is structured in Western, Central and Eastern Bulgaria, indicating that the Balkan Mountains have been permeable to human movements. The lineage analysis provided, in addition, notable results about hgs R-L23*, E-V13 and J-M241. Although the genetic legacy of Bulgaria remains somewhat inchoate, the results of this high resolution study provide also new clues on the Proto-Bulgarians who arrived in the present Bulgaria in historical times.

P10.8
Screening of MPM patients for activating somatic mutations within PDGFR-beta

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Platelet-derived growth factor receptor beta (PDGFR-β) plays a role in malignant pleural mesothelioma (MPM). It is a target for the tyrosine-kinase inhibitor imatinib. It was observed that a subset of MPM patients don't respond to the treatment. The causes of the resistance are ascertained in other types of tumours. It was shown that activating mutations are found within exons 12 and 18 of its gene. In the gastro-intestinal stromal tumours the "gatekeeper mutations" within exon 14 make cells insensitive to imatinib. To ascertain the frequency of these mutations in MPM, we performed a mutation screening of the exons 12 and 18 of PDGFR-β on 100 surgically MPMs. We did not observe any mutation. PDGFR-β was found over-expressed at mRNA level in tissues: we hypothesized that it is involved in MPM because of its increased expression (that could be functionally equivalent to a constitutive activation), rather than the somatic mutations. To reveal whether PDGFR-β plays a role in the secondary resistance to imatinib, we induced a long-term resistance to imatinib, in the MPM cell line MERO-14. Once resistant clones are obtained, they will be screened for mutations in exons 12, 14, and 18.

P10.9
Genetic characterization of northeastern Italian population isolates in the context of broader European genetic diversity

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As population isolates have a great potential in detecting disease-causing genetic variants, we aimed to genetically characterize a region from northeastern Italy, hypothesized to harbor several isolates communities. 1,310 samples, collected from 6 isolated villages, were genotyped at more than 266,000 SNPs. Our original data was jointly analyzed with genome-wide datasets of European populations. We analyzed the internal genetic structure of the isolates using unsupervised structure-like analysis, uncovering that some of the village populations are dominated by multiple ancestry components. We split each isolate into two groups: the group in which ancestral component is unique into the European contest, and the other in which is the same of other European populations. Our analysis shows a great level of intra-population variability and a non-homogenous structure in some of our isolates, which could lead erroneous inferences if not taken into account. The methodology presented here for the analysis of genetic structures of isolated populations can provide a useful tool for genetic epidemiology and Genome Wide Association studies that involve such populations.

P10.10
Investigations about ancient DNA require both genomics and HPLC analysis to guarantee reliable data

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Ancient DNA recovered from archaeological remains makes it possible to go back in time and study the etiopathology of genetic diseases. Several conditions, typical of death, destroy nucleic acids leading to DNA fragmentation of ancient samples, all events that can negatively affect genotyping tests usually based on PCR methods [a]. Some studies refer aspartic acid and alanine racemization [b], evaluated by RP-HPLC chromatography [c], indicative of aDNA preservation and contamination. This work was undertaken to prove integrity and authenticity of ancient samples analyzed first by HPLC and then by genomic HLA typing including PCR-SSP, PCR-SSO (respectively "forward" and "reverse" hybridization with sequence-specific probes) and SBT (Sequence Based Typing). Six human rests were studied: the Cardinal Carlo de Medici skeleton (XVI-XVII century A.C.) and five mummies, from Lazio and Abruzzo, (XIX A.C.). We demonstrated that the determination of amino acid racemization extent is crucial for supporting the reliability of molecular anthropology studies being effective in identifying aDNA samples suitable of amplification.

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O10.1
The peopling of South America: the last major human dispersal

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To investigate the colonization of South America, one of the topics that are still a matter of debate in the human dispersal, we have analyzed haplogroup Q (Hg Q), which is virtually the only clade of the Y-chromosome phylogeny observed in modern-day Amerindians of Central and South America. The analysis of ~400 samples belonging to Y-chromosome Hg Q identified in a survey of more than 2,000 Native Americans from all over the double continent from the Sorenson database (www.smgf.org), resulted in the identification of two distinct Y-chromosome founding lineages, named Q-L54 and Q-M3. While Q-L54 is mainly observed in Central America, Q-M3, the most diffused Native American sub-lineage, displays two branches: one with a spread all over Central and South America, and the other limited to the Andean region. On the whole, the observed diffusion of the main Hg Q lineages, together with the diversification of Q-M3 into clades of younger age and more restricted geographic distribution, are indicative of two different episodes of early settlement of South America with subsequent Mesoamerican and Andean expansions.

O10.2
Mine, yours, ours? sharing data on human genetic variation

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To ascertain whether the sharing of primary datasets is common-practice in studies of human genetic variation, we analyzed 508 Pubmed indexed papers from 2008 to 2011. A substantial portion of datasets (21.9%) was found to have been withheld, while neither strong editorial policies nor high impact factor proved to be effective in increasing the sharing rate beyond the current figure of 80.5%. We could observe a substantially lower sharing in medical than evolutionary and forensic genetics, more evident for whole mtDNA sequences (15.0% vs 99.6%). The low rate of positive responses to e-mail requests (28.6%) suggests that sharing should be regarded as a prerequisite for final paper acceptance, while making authors deposit their results in open online databases which provide data quality control seems to provide the best-practice standard. Finally, we estimated that 29.8% to 32.9% of total resources are used to generate withheld datasets, implying that an important portion of research funding does not produce shared knowledge. By making the scientific community and the public aware of this important aspect, we may help popularize a more effective culture of data sharing.

O10.3
Medullary thyroid carcinoma (MTC) and *RET* proto-oncogene: mutation spectrum in the familial cases and a meta-analysis of studies on the sporadic form

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Medullary thyroid carcinoma (MTC) accounts for 5-10% of all thyroid cancers and it can occur as a sporadic (sMTC) or as a familial (fMTC) form. *RET* proto-oncogene germline mutations are crucial for the onset and the progression of fMTC and the occurrence of single nucleotide polymorphisms (SNPs) could predispose to the sporadic form.
We carefully reviewed PubMed database in order to describe the mutation spectrum and to perform a meta-analysis of the available case-control association studies for sMTC. *RET* germline mutations mostly involve

codons 609, 611, 618, 620 (exon 10) and 634 (exon 11), encoding for the extracellular cysteine-rich domain, and codons 768 (exon 13) and 804 (exon 14) of the intracellular tyrosine kinase domain. The meta-analysis demonstrated a weak association of sMTC susceptibility with S836S and a more convincing association with IVS1-126G>T. We also performed *in silico* predictions and both of the genetic variations showed to have functional effects. To conclude, further studies are warranted to confirm these results and functional analysis are needed to better understand the consequence of such *RET* variants and to improve our knowledge on the disease.

O10.4
Neolithic revolution: cultural or genetic change in central-south Italy? A mosaic scenario

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The aims of the present study is to assess the degree of genetic and cultural changes associated with the introduction of farming in south and central Italy. For this purpose a biomolecular approach based on ancient DNA (aDNA) was applied to the Early Neolithic specimens from different sites: 8 human remains recovered in Mora Cavorso cave (RM) (dated 5,472 – 5,314 BC), 2 human samples from Ripatetta (FG) (dated 5,860 – 5,600 BC) and 2 human remains from Palata (BT) (dated 5,630 – 5,460 BC). The aDNA results together to those obtain from other European Neolithic, Palaeolithic and extant Italian and European populations were analyzed with different statistical softwares to understand the genetic changes if any during these periods. It was highlighted that the demographic transition was not uniform across Europe, but it was rather like a mosaic of population replacement, admixture and adoption of farming practices by indigenous populations. Thus, the transition to farming in the outlying regions of Europe such as Italy was probably a more complex and lengthy process, involving also the acculturation of local hunter–gatherer groups.

O10.5
On the origins of the Etruscan people

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The Etruscan civilization is documented in a region roughly correspondent to current Tuscany from the 8th to the 1st century BC. After the 1st century the Etruscan's language and culture disappeared from the records. The debate on the Etruscans' origins starts when Herodotus described them as a group of immigrants from Lydia, whereas Dyonisus of Halicarnassus regarded them as an indigenous population. To date, claims about the Etruscans' origins come from genetic similarities between the contemporary mtDNAs of Turks and modern Tuscans; this finding was interpreted as evidence of a common descent of these populations from Etruscan ancestors. In this study we took advantage of ancient DNA sequences from classic Etruria to explicitly test models of Etruscan origins by ABC methods. Using contemporary sequences from Tuscan populations for which data strongly suggest an Etruscan ancestry and from Western Anatolia, we found that the observed genetic similarities cannot be attributed to an immigration wave from the Near East leading to the onset of the Etruscan culture in Italy. Rather, our results confirm that these links exist, but date back to possibly to the spread of farmers during the Neolithic period. The genetic evidence is consistent with an Italian population locally developing the Etruscan culture.

11 - Genetic of microorganisms

P11.1
Analysis of genes involved in biofilm formation in *Stenotrophomonas maltophilia*

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S. maltophilia is an emerging opportunistic pathogen which is currently isolated from the airways of cystic fibrosis (CF) patients. For what it concerns its virulence *S. maltophilia* produces a wide range of extracellular enzymes potentially involved in the colonization of respiratory tissues. Moreover *S. maltophilia* is able to adhere to cultured epithelial respiratory cells, as well as to produce biofilm on a variety of abiotic surfaces favouring its adhesion to the host tissues. In this work 54 *S. maltophilia* strains have been analyzed for their ability to form biofilm on abiotic surface. Strains included 41 clinical strains isolated from CF patients, 11 environmental strains, the reference strain K279a (genome sequenced) and the type strain LMG958. The amount of biofilm produced is highly variable and the strains have been classified as strong, moderate or weak producer. In order to investigate the genes involved in biofilm formation pathway K279a mutants for fimbriae and extracellular enzymes (lipases, proteases, etc.) have been constructed and then complemented with their wild-type genes. Mutants and complemented strains have been assayed for their ability to product biofilm.

P11.2
Two different approaches to fight *Burkholderia cenocepacia* infections

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Burkholderia cenocepacia J2315 is a Gram-negative opportunistic pathogen able to cause serious infections in cystic fibrosis patients. The eradication of this microorganism is very difficult due to its resistance to a wide range of antibiotics. Thus, the discovery of new compounds able to inhibit *B. cenocepacia* growth and virulence is extremely important. In this work the mechanism of resistance to a new promising antimicrobial compound was investigated. It was found to rely on a mutation in the repressor of *BCAL2820-22* (RND-4), encoding a Resistance–Nodulation–Division (RND) efflux pump. These data confirmed that RND-4 has an important role in the *B. cenocepacia* multidrug resistance and might allow the improvement of the antimicrobial molecule. Furthermore, the quorum sensing mechanisms (CepIR, CciIR, BDSF-based) of this pathogen were examined to delve into the virulence. The phenotype of *B. cenocepacia* mutants deleted in *BCAM1870* (CepI), *BCAM0239a* (CciI) or *BCAM0581* (BDSF synthase) was analyzed. Data obtained prove that QS has a crucial role in the pathogenesis of *B. cenocepacia*, since biofilm maturation, drug resistance and virulence were affected in the inactivated mutants.

P11.3
Regulation of metabolic changes in response to environmental oxygen in the yeast Kluyveromyces lactis

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Oxygen sensing and signalling are important mechanisms of physiological adaptation to changes of environmental conditions in all aerobic organisms. Traits, either common, either specific can be found in the hypoxic response of bacteria, yeasts, plants and mammals. In humans, the hypoxic response is critical for many causes of mortality, including cardiac ischemia, chronic pulmonary diseases, infarct and cancer, where conditions of oxygen shortage are generated. This work integrates the study of the hypoxic response of the yeast Saccharomyces cerevisiae, worldwide used as eukaryotic model organism, with Kluyveromyces lactis. The use of the latter yeast in this field of research is justified by the fact that respiration and fermentation are not predominantly governed by glucose repression, as occurs in S. cerevisiae, but fairly well sensitive to oxygen availability. As a consequence, the K. lactis mechanism of metabolic regulation by oxygen suggests the possibility to use the hypoxic response and/or adaptation of this yeast as a model for tissues or biomasses suffering non optimal oxygenation. Induction of the metabolic shift from respiration to fermentation and the activation of lipid metabolism will be described and the function of mediators of oxygen signalling and hypoxic transcription factors so far identified will be reported.

P11.4
Identification and transcriptional analysis of exopolysaccharides biosinthesis gene clusters in *Lactobacillus plantarum* WCFS1

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Exopolysaccharides (EPS) from lactic acid bacteria contribute to specific rheology and texture of fermented milk products and finds applications also in non-dairy foods and in therapeutics. The aim of this work is to study EPS biosiyntetic genes in *Lactobacillus plantarum*, a probiotic and food associated lactobacillus, also encountered in the human intestinal tract. By analysis of functional categories in the *L. plantarum* WCFS1 genome, we found six gene clusters, hereby named *eps1-6*, whose deduced amino acid sequences are annoted as putative proteins involved in exopolysaccharides biosynthesis. Based on homology to proteins encoded by polysaccharide biosynthetic gene clusters in other lactic acid bacteria, putative functions in polysaccharide export, polymerisation, chain length determination, and subunit biosynthesis could be assigned to several gene products encoded within the above mentioned clusters. Reverse transcription-PCR analysis revealed that the *eps* loci are organized in six operons and two monocistronic genes. Preliminary transcriptional data suggests that expression of *eps* gene clusters are under the control of the global transcription regulator CcpA.

P11.5
Decaprenyl-phospho-β-D-ribofuranose-2'-oxidoreductase (DprE1): state of the art

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Tuberculosis (TB) is responsible for an estimated 2 million deaths annually. The emergence and spread of *Mycobacterium tuberculosis* multidrug-resistant strains has prompted the discovery for new drugs and the characterization of their resistance mechanisms.
The cellular target of some new antituberculars, including the Benzothiazinones (BTZs) and the Dinitrobenzamides derivatives (DNBs), is the DprE1 enzyme. DprE1 works in concert with DprE2 to catalyze the epimerization of decaprenylphosphoryl-D-ribose (DPR) to decaprenylphosphoryl-D-arabinose (DPA), a precursor for arabinan synthesis, without which a complete mycobacterial cell wall cannot be produced.
Both DNBs and BTZs share the same mechanism of inhibition, which consists in the enzyme-dependent activation of the nitro group that reacts with an active site cysteine residue forming an irreversible covalent adduct. This has been demonstrated by the biochemical and structural characterization of DprE1 and is fully consistent with the observation

that DNB- and BTZ-resistant mycobacterial strains arose from mutations at the DprE1 cysteine residue or from transformation of the original nitro group into a less active amino derivative.

P11.6
Isolation and characterization of *M. tuberculosis* and *M. bovis* BCG mutants resistant to new antitubercular pyrrole derivatives

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Tuberculosis (TB), caused by *M. tuberculosis* (Mtb), is one of the most serious infectious diseases worldwide. The current treatment for TB lasts for an exhausting 6 months and many drugs are bactericidal only against growing bacterial populations. Moreover, multidrug-resistant (MDR) strains that also exhibit resistance to the second-line drugs (extensively drug resistant strains, XDR) are now on the rise. Finally, latent infection presents one of the major obstacles in gaining control over TB. Thus, new drugs that inhibit new targets are urgently required to (i) shorten the duration of effective treatment; (ii) improve the treatment of MDR/XDR-TB; (iii) provide more effective treatment of latent TB infection.
We have demonstrated that BM212, a pyrrole derivative with a potent activity against MDR isolates of Mtb and intracellular bacilli, targets the membrane protein MmpL3, a transporter for cell wall mycolic acids. In this study, we report the isolation and characterization of *M. tuberculosis* and *M. bovis* BCG mutants resistant to three BM212 new more effective derivatives.

P11.7
Carotenoid production and its role in stress response, sporulation and biofilm formation in pigmented Bacilli

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Spore-forming Bacilli are Gram-positive bacteria commonly found in a variety of natural habitats, including the gastro-intestinal tract of animals. Isolates of various Bacillus species are pigmented and have been shown to produce C30 carotenoids, likely involved in the cell stress response (1, 2). We report a physiological characterization of carotenoid biosynthesis in two pigmented Bacilli isolated from marine samples: strain SF214, belonging to the B. pumilus species and producing an orange carotenoid, and strain SF241, belonging to the B. firmus species and producing a pink-red carotenoid. In both strains carotenoid production occurred during stationary growth phase. Only in SF214 production of the pigment was controlled by the temperature and the presence/absence of light and was shown to be a bistable phenomenon. A NTG-mediated mutagenesis allowed the isolation of mutants of both strains with strongly altered pigmentation. Analysis of the mutants indicated that carotenoid production occurs following a developmental pathway alternative to that leading to spore formation.
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P11.8
VirF expression in *Shigella flexneri*: two proteins to the price of one gene

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In *Shigella flexneri* the complex virulence gene cascade is triggered by a major activator, the VirF protein. In this view, it is not surprising that this master regulator is subject to a "layered" regulation strategy. The basic layer is represented by the transcriptional regulation operated in response to environmental stimuli by nucleoid proteins, such as H-NS, FIS and IHF, and by two component systems such as CpxA-CpxR and EnvZ-OmpR. The existence of an additional regulatory layer has been evidenced in this work. We have found that the expression of the *virF* gene consists in a differential translation event giving rise to two forms of VirF. The 30 kDa form is responsible of the activation of the virulence system, while the 21 kDa form exerts a negative feedback control on *virF* expression itself. Our model is further complicated by the involvement of a sRNA, OmrA, in inhibiting, under high osmolality conditions, the production of the 21 kDa form from the *virF* transcript. The OmrA-mediated inhibition might mitigate the negative feedback effect of VirF 21 kDa on *virF* transcription, promoting a more efficient synthesis of VirF 30 kDa and thus a more effective expression of the virulence system within the host.

P11.9
Comparative analysis of the *nadA* and *nadB* antivirulence loci in *Shigella* spp and Enteroinvasive *Escherichia coli* (EIEC)

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Shigella have been derived from *E. coli* by convergent evolution involving both gain and loss of genes and appears to have diverged from commensal *E.coli* more than EIEC, a group of *E.coli* which shares with *Shigella* the same pathogenicity process. Surprisingly, unlike *E. coli*, *Shigella* strains require nicotinic acid for growth on minimal medium, due to alterations in the *nadA* and *nadB* genes, which encode the enzyme complex that convert L-aspartate to quinolinate (QUIN), a precursor of NAD. Recently as been reported that QUIN is a specific inhibitor of several virulence phenotype of *Shigella*. Since EIEC strains are regarded as evolutionary intermediates from *E.coli* to *Shigella*, in this study we analysed if the auxotrophic requirement for nicotinic acid may be selected in EIEC such as in *Shigella* during the evolution toward a pathogenic life-style. For this purpose several EIEC strains, belonging to different serotypes and isolated in different geographical areas, were examined for nicotinic acid requirement. Unlike *Shigella* spp, the majority of EIEC strains examined were prototroph and nicotinic acid auxotrophy of few EIEC strains was due to changes affecting just the *nadB* gene.

P11.10
The *dprE1* gene: a new target to develop diagnostic kits for screening and phylogenetic analysis of pathogenic Mycobacteria

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More than 50 species of the genus *Mycobacterium* are recognized as human pathogens. *Mycobacterium tuberculosis* is the etiologic agent of tuberculosis (TB) which is still a major global health concern, mainly due to the emergence of resistant strains. Other than the species that cause TB [*M. tuberculosis* complex (MTBC)], Non Tuberculous Mycobacteria (NTM) can also cause clinical problems.
In order to better check the antibiotic resistance and to identify Mycobacteria we are developing two new ready-to-use screening and drug resistance molecular assays for TB diagnosis, storable at room temperature. The assay is based on the analysis of *dprE1* gene of *M. tuberculosis* and the 19 orthologous genes belonging to MTBC and NTM. This gene is essential for *M. tuberculosis* growth and encodes for the DprE1 enzyme, involved in arabinogalactan synthesis and target of Benzothiazinones (BTZ) and Dinitrobenzamides (DNB).
Since the *dprE1* gene is highly conserved in Mycobacteria, we are evaluating the correlation of a phylogenetic tree based on *dprE1* gene sequence to define the phylogenetic link of the several mycobacterial

pathogens and BTZs or DNBs sensitive/resistant species.

P11.11
cotH over-expression by-passes the CotE requirement for assembly of outer coat components of Bacillus subtilis

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Bacillus subtilis spores are surrounded by the coat, a multilayered structure composed of proteins and glycoproteins. Proper formation of the coat is essential for spore resistance to toxic chemicals, lytic enzymes as well as for an efficient spore germination. Coat formation is controlled at the transcriptional level by the action of two sigma factors of the RNA polymerase and at least three transcriptional regulators and at a post-translational level by the action of several morphogenetic proteins.
We focused on CotE and CotH, two major morphogenetic factors responsible for the assembly of 24 and 9 outer coat components, respectively. CotE-controlled proteins include CotH, therefore originating a cascade pathway controlling some of the most abundant spore surface proteins.
The analysis of a collection of deletion mutation in the cotE gene allowed us to identify the last 20 C-terminal amino acid residues of CotE as responsible of the interaction with CotH. In addition, we report that over-expression of cotH eliminates the CotE-requirement for the assembly of several outer coat components and by-passes the germination and resistance phenotypes typical of mutant strains lacking cotE.

P11.12
The timing and extent of quorum sensing response affect *Pseudomonas aeruginosa* chronic lung infection in mice

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The $3OC_{12}$-HSL quorum sensing signal molecule plays a key role in *Pseudomonas aeruginosa* infections, mutants impaired in $3OC_{12}$-HSL synthesis displaying a reduced pathogenic potential in mice. However, no data are available on the effect of a deregulated 3OC12-HSL production on *P. aeruginosa* infection. In this bacterium, the QscR, QteE and QslA factors inhibit premature (pre-quorum) $3OC_{12}$-HSL production, while the RsaL regulator limits the post-quorum accumulation of $3OC_{12}$-HSL.
A *P. aeruginosa rsaL* mutant produces 10-fold more $3OC_{12}$-HSL than the wild type. In this study we demonstrate that this mutant is strongly impaired in causing chronic lung infection in mice, while it retains wild type ability to establish a systemic infection. Preliminary data show that also the *qscR* mutant has a similar behaviour. This suggests that factors controlling the timing and the extent of $3OC_{12}$-HSL production are required for the establishment of chronic infection and could constitute novel drug-targets. The impact of QteE and QslA on *P. aeruginosa* pathogenetic potential is currently under investigation.
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P11.13
Screening of gene-specific libraries of *Neisseria meningitidis* fHbp displayed on bacteriophage λ capsid

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Factor H binding protein (fHbp) of *N.meningitidis* is a down-regulatory molecule in the complement cascade, and binding of this molecule to the bacterial surface contributes to the ability of the organism to avoid complement-mediated killing. Bacteriophage λ has emerged as an effective vehicle for the surface display of complex repertoires from natural sources, alternative to the more common filamentous phage systems. We have screened 3 libraries, displaying V.1, V.2 and V.3 fHbp fragments from *N. meningitidis*, with 5 anti-fHbp mAbs from mice immunized with V.2 or V.3 rfHbp proteins to identify fragments of fHbp involved in the binding of fH. Specific phage clones were isolated and sequenced. Multiple sequence alignment allowed us to identify specific amino acid residues corresponding to the mAb reactivity patterns.

P11.14
Mutation in *etfA* causes pleiotropic effects in *Bacillus subtilis*

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Microbial calcium carbonate precipitation is a major biogeochemical process with implications in various fields such as conservation of monumental stones. Even though it is very diffuse among bacteria, it remains poor understood. By a mutagenesis approach in *Bacillus subtilis*, we identified the *etfA* gene as essential for calcite precipitation. Its putative product is the α subunit of the αβ heterodimeric electron transfer flavoproteins (ETFs), involved in electron transfer during fatty acid metabolism. We demonstrated that the mutation in *etfA* causes an excess of H^+ extrusion, impairing precipitation on solid medium in mutant FBC5. Here we report results obtained from comparison of physiological traits of *B. subtilis* 168 and FBC5 during growth on precipitation medium, to evaluate if the *etfA* mutation affects physiological responses other than precipitation. Membrane fatty acid profiles revealed changes in FBC5 phospholipids membrane composition with accumulation of relatively long fatty acids (C17:0). Comparison of motility revealed atypical formation of flagella by FBC5. Sporulation is under investigation.

P11.15
Effect of efflux pumps inhibition on *Pseudomonas aeruginosa* physiology and virulence

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RND efflux pumps confer multi-drug-resistance and are also involved in the secretion of virulence factors and quorum sensing (QS) signal molecules in bacterial pathogens. RND efflux pumps inhibitors (EPIs) are considered promising drugs for potentiating antibiotic activity. However, little is known about the effect of EPIs on bacterial physiology and virulence.
Here, the effect of a known EPI compound, PAβN, on *Pseudomonas aeruginosa* physiology has been investigated. PAβN affects the transcription of about 109 genes, mostly involved in phosphate, iron and nitrate metabolism. Many of the PAβN up-regulated genes are QS-controlled, including genes for the synthesis of the virulence factor pyocyanin. Accordingly, PAβN causes accumulation of the $3OC_{12}$-HSL QS signal molecule and pyocyanin in *P. aeruginosa* culture supernatants. On the other hand, PAβN inhibits other *P. aeruginosa* phenotypes directly related to virulence, *i.e.* swimming, twitching and swarming motility.
In conclusion, EPIs may have unpredictable effect on bacterial pathogenesis, calling for more detailed studies prior to their use in therapy.
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P11.16
Identification of domains of *Lactobacillus plantarum* enolase involved in adhesion to collagen

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Collagen (Cn) is the main component of connective tissue and is the most abundant protein in mammals. Several pathogen bacteria express surface proteins that interact with this extracellular matrix component. By means of Far Western blot assay, we identified some putative collagen-binding proteins in the cell surface fraction of *Lactobacillus plantarum* LM3; one of these is the EnoA1 alfa-enolase (48 kDa), coded by the *enoA1* gene. Indeed, a strain carrying a null mutation in *enoA1* (LM3-CC1) adhered less efficiently than the wild type strain to Cn-coated wells. By in silico analysis we were not able to find Cn-binding domains described in other bacterial adhesins. To characterize the interaction with collagen, GST-tagged EnoA1 was expressed in *Escherichia coli* BL21 and purified to homogeneity. A preliminary analysis allowed the identification of an internal 15,7 kDa protein fragment, retaining the Cn-binding activity. To identify and characterize the Cn-binding domains of the *L. plantarum* LM3 enolase, expression and purification of shorter EnoA1 fragments, are currently in progress.

P11.17
Function, evolution and maturation of the CotG protein of *Bacillus subtilis.*

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Bacterial endospores are quiescent cell forms, extremely stable and resistant to enzymatic and chemical insults. Spore resistance is in part due to the coat, the multilayered proteinaceus structure that surrounds the mature spore. CotG is a highly abundant coat protein of *B. subtilis* that has a structural and regulatory role on the assembly of other coat components. The central region of CotG has a complex modular organization with 12 single and tandem repeats. CotG-homologues have been identified only in species closely related to *B. subtilis*.
We report now that many *Bacillus* species contain a gene with 5' and 3' regions homologous to those of *cotG* of *B. subtilis* but with a non-homologous central part. In all cases the central part has a modular structure. Conservation of such features suggests a relevant, albeit still obscure role of CotG in spore coat formation. To clarify this role we constructed *cotG* mutants that lack either the entire protein or only its modular part and a complex regulatory pattern has been highlighted. Experiments are also in progress to evaluate the potential role of two putative kinases and a glycosyltransferase of *B. subtilis* on CotG maturation.

P11.18
KlMID1 a relevant key player between ER homeostasis and mitochondrial dysfunction in *Kluyveromyces lactis*

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The interplay between calcium metabolism and glycosylation in yeast is largely unknown. In order to clarify this relationship, the effect of the mutation in *KlOCH1* gene, coding for the Golgi α-1,6-mannosyltransferase, on the calcium homeostasis was studied in the Kluyveromyces lactis yeast. In particular, the role of *KlMID1* gene, encoding one of the components of the plasma membrane calcium channel (Cch1-Mid1), was investigated. Almost complete suppression of the phenotypes occurring in the mutant strain, ranging from oxidative stress to cell wall alteration, was observed by increased dosage of *KlMID1*. In addition, this N-glycosylation mutant showed increased calcium accumulation and decreased transcription of *KlMID1* and *KlCCH1*. Moreover, the calcium alterations included the increased expressional profile of the *KlPMC1* gene, coding for the vacuolar calcium ion pump. Furthermore, perturbation of endoplasmic reticulum homeostasis was observed in the *Kloch1-1* cells. Similarly, down modulation of calcium signalling genes as well as altered mitochondria functionality were induced in the wild type cells after treatment with dithiothreitol (DTT). However, no mitochondrial alteration occurred in the treated cells when *KlMID1* was overexpressed.Our results suggest that ER stress taking place in *Kloch1-1* cells appears to be the primary cause for *KlMID1* down-modulation and its resulting effects on the expression of calcium homeostasis genes.

P11.19
Immunomodulatory effects of *Lactobacillus plantarum* LM3

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Modulation of the immune system is one of the mechanisms underlying the beneficial effects of probiotic bacteria on human health. The aim of this study was to investigate the immunomodulatory effect of *Lactobacillus plantarum* LM3 and of the isogenic mutant strain LM3-CC1 (*Δeno*A1) on intestinal epithelial cells Caco-2. We had previously characterized the adhesive properties of the enolase EnoA1 with respect to fibronectin, collagen, and plasminogen. We found that in Caco-2 cells exposed to *L. plantarum* LM3, expression of β-defensin 2 (HBD-2) was induced; a 2 times lower level HBD-2 expression was found in the LM3-CC1 strain. In addiction, we analysed the pro- and anti-inflammatory responses of Caco-2 cells stimulated by both *L. plantarum* strains, evaluating the expression of IL-6, IL-10, and TGF-β. We found different immunomodulatory effects of the two strains, with a lower level of expression of pro- and anti-inflammatory molecules in Caco-2 cells exposed to the mutant strain. Analysis of the protective effect of *L. plantarum* LM3 in murine model will be needed to finally assess the probiotic characteristics of this strain.

O11.1
Characterizing the MmpL3 protein, a novel target for new antitubercular agents

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Tuberculosis (TB) is getting seriously problematic since the emergence of HIV and the appearance of multidrug-resistant (MDR). Therefore, new drugs of different classes against diverse targets are urgently needed. BM212, a pyrrole derivative, shows a potent activity against Mycobacterium tuberculosis (*Mtb*), non-tuberculosis mycobacteria, MDR clinical isolates and intracellular bacilli. We have identified the MmpL3 protein as the cellular target of BM212 in *M. bovis* BCG, *M. smegmatis* and *Mtb*. It has been demonstrated that MmpL3 is involved in the transport across the plasma membrane of a precursor of mycolic acids, essential components of mycobacterial cell wall. Besides BM212, other three different antitubercular molecules target MmpL3, indicating that this protein represents a new potential druggable target for the treatment of TB. To investigate the mechanism of BM212 action and to validate MmpL3 as a new target, two goals should be achieved: protein production/purification and gene inactivation. In this work, we report the preliminary experiments of both MmpL3 expression in heterologous host and *mmpL3* gene disruption.

O11.2
Evaluation of the infection-relevant role of small RNA-based regulatory systems in the opportunistic pathogen *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa is an important opportunistic pathogen in immune-compromised and cystic fibrosis patients. We have performed a comparative small RNA (sRNA) profiling via deep-sequencing of *P. aeruginosa* PAO1 and PA14 strains (1) which share the same host range but differ in pathogenicity. We now aim at identifying infection-relevant sRNA-based regulatory systems. To this end, we are focusing on a short list of 8 validated sRNAs which showed, at preliminary screenings, potential virulence hallmarks such as: specific expression in PA14 or PAO1; responsiveness to infection-relevant host stimuli (e.g. oxygen availability, temperature shift) or invasion signals (e.g. quorum sensing). Our approach is to generate sRNA deletion mutants and test them for i) canonical virulence phenotype(s); ii) ability to invade human respiratory epithelial cells and stimulate an immune response; iii) airways infection in both acute and chronic murine models. The above tests are accompanied by *in silico*, genetic and transcriptomics-based screenings for the genome-wide search of sRNAs target genes.

1. Ferrara S. et al. (2012). PLoS One 7: e36553.

O11.3
New insights into the Lpt machinery for lipopolysaccharide transport to the cell surface: functional dissection of LptC protein

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Lipopolysaccharide (LPS) is a complex surface glycolipid of Gram-negative bacteria relevant for maintaining the integrity of the outer membrane (OM). LPS belongs to the class of conserved microbial molecules (PAMPs) recognized by the innate immune system. The structure and biosynthetic pathway of LPS are known since long time but only recently details on its assembly pathway have emerged.
The Lpt protein machinery for LPS transport to the OM, recently characterized in Escherichia coli, is composed of seven essential proteins (LptABCDEFG). Both biochemical and genetic data indicate that the Lpt proteins form a transenvelope complex and operate as a single device. LptC, an inner membrane (IM) bitopic protein able to bind LPS in vitro, forms a complex with the ATP-binding cassette transporter, LptBFG Indeed LptC binds the periplasmic protein LptA and this interaction is crucial for LPS export to the OM. Bioinformatic analyses revealed three conserved motifs of LptC in several γ-proteobacteria. Interestingly, the most conserved motif 1 seems is highly divergent in the Pseudomonas aeruginosa Pa-LptC homologue and Pa-LptC is not able to complement an E. coli lptC conditional mutant. To understand the rationale for the lack of cross-complementation we constructed E. coli-P. aeruginosa chimerae of LptC. Complementation assays and biochemical analyses are ongoing to dissect the functional role of LptC motifs.

O11.4
The ERMES complex is essential for mitochondrial inheritance and lipid biogenesis in *S. cerevisiae*

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Mitochondria are essential organelles, they cannot be synthesized ex novo and a correct inheritance from mother to daughter cell is essential for viability, due to the essential function performed by mitochondria in lipid and Fe-S protein biogenesis. Recent studies identified the ERMES complex as essential for phospholipid homeostasis, mitochondrial DNA maintenance, mitochondrial motility and inheritance, ER-mitochondria Ca^{++} exchange and mitochondrial protein import. It has recently proposed that a link exists between cell cycle and mitochondrial movement into the bud, in a strain deleted for one ERMES gene. We have investigated the mitochondrial movement defect in yeast strains lacking the ERMES genes and we have demonstrated that this complex is essential for mitochondrial movement into the bud in absence of the MMR1 gene, whose product is involved in mitochondrial bud tip retention. Not only the absence of mitochondria into the bud causes bud lethality, but we observed a multibud phenotype in mother cells with an aberrant septin ring deposition. We will also present data concerning the involvement of ERMES complex in lipid biogenesis.

O11.5
Universally conserved protein gcp is essential for *Pseudomonas aeruginosa* viability

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The significant increase in the occurrence of *Pseudomonas aeruginosa* (multi)-drug resistant strains urgently calls for the discovery of novel essential genes or pathways, not yet targeted by antibiotics. For this purpose we set up a genome-wide identification of "essential-for-growth" genes in *P. aeruginosa* via construction and screening of shotgun antisense libraries. Among several inserts impairing PAO1 growth, we focused on one that targets *locus* PA0580 encoding for the 341 aa protein gcp, belonging to the YgjD/Kae1 family on the top-10 list of universally conserved proteins. It has been recently shown that this family is involved in the biosynthesis of N6-threonylcarbamoyladenosine (t6A), a universal modification found at position 37 of tRNAs decoding ANN codons. YeaZ, an essential YgjD paralog conserved uniquely in bacteria, interacts with and is required for YgjD function *in vivo*. We validated the gcp essential role in *P. aeruginosa* both by insertional and conditional mutagenesis. In addition, we purified gcp through affinity chromatography. This approach will allow us to assess biochemical activity and identify potential functional interactors.

12 - Evolution

P12.1
A tale of two species: genetic structure and gene flow patterns in an *Ulmus minor / U. pumila* complex

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Ulmus minor is a deciduous forest tree suffering from progressive and severe demographic reduction owing to a Dutch elm disease (DED) epidemic. Healthy and mature stands of *U. minor* are nowadays rare in Europe. *Ulmus pumila* is an exotic species resistant to DED and widely naturalized in the ecological niche of *U. minor*. A small relict pure population of *U. minor* and its neighborhoods (a 5 Km radius area with both *minor* and *pumila* individuals) were sampled. Overall, 439 trees and 388 seeds (harvested in two successive years) were genotyped at six nSSR loci in order to investigate gene flow dynamics by paternity analysis. STRUCTURE and sPCA clustering analyses revealed that the genetic structure is probably the result of admixture of four gene pools that are not related to a particular species. In contrast with the neighborhood area the inner relict *U. minor* nucleus exhibits a marked spatial structure in which trees with prevalence of one of genetic pools tends to spatially cluster. Gene flow resulted as high as ~70% (consistent between years and species). Although *pumila* individuals represent only 5% of sampled trees, they pollinate ~25% of locally sired *minor* seeds.

P12.2
Analysis of *ynfB-speG* locus in *Shigella* and enteroinvasive *E.coli*

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Shigella and enteroinvasive *E.coli* (EIEC) are the causative agent of bacillary dysentery. The genomes of *Shigella* and *E. coli*, its commensal ancenstor, are colinear and highly homologous. The genetic differences are the result of an evolutionary process that led to the acquisition of virulence genes and loss of genes that are deleterious to the fitness of the bacterium and the invasive process. We have focused our attention on a particular pathoadaptive mutation leading to the silencing of the *speG* gene, which encodes spermidine acetyltransferase, an enzyme catalyzing the conversion of spermidine into the physiological inert acetylspermidine, since recent evidence stresses the involvement of polyamines in bacterial pathogenesis. The polyamines are polycationic molecules involved in numerous cellular processes. We show that in *Shigella speG* gene is inactive and that the consequent accumulation of spermidine strongly favors the survival of the pathogen under oxidative stress conditions, as well as within the macrophages it invades during infection. *speG* gene is located within the *ynfB-speG* locus. The *ynfB* gene encodes for a protein whose function is not yet known. The aim of this work is to characterize the *ynfB-speG* locus in EIEC strains and to understand the function of YnfB protein to ascertain its hypothetical involvement in the polyamines metabolic pathway.

P12.3
The evolution of African great ape subtelomeric heterochromatin and the fusion of human chromosome 2

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Chimpanzee and gorilla chromosomes differ from human chromosomes by the presence of large blocks of subterminal heterochromatin thought to be composed primarily of arrays of tandem satellite sequence. We explore their sequence and organization and show a complex organization composed of sets of segmental duplications, which have hyperexpanded in concert with the formation of subterminal satellites. The high intra- and interspecies copy number polymorphism of these regions can be accurately estimated by assaying read-depth of next-generation sequencing datasets. Phylogenetic and comparative genomic analyses suggest that the structures have arisen independently in the two lineages with the exception of a few seed sequences present in the common ancestor of humans and African apes. In our model an ancestral human chimpanzee pericentric inversion and the ancestral chromosome 2 fusion both predisposed and protected the chimpanzee and human genomes respectively to the formation of subtelomeric heterochromatin. Our findings highlight the complex interplay between duplicated sequences and chromosomal rearrangements that alter the cytogenetic landscape in a short period of evolutionary time.

P12.4
Molecular characterization of the ancestral centromere of chromosome 2

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Human chromosome 2 is the product of a head-to-head fusion of two acrocentric ancestral chromosomes, IIp and IIq, which remained separated in chimpanzee and gorilla. The dicentric chromosome originated from the fusion reached stability by inactivating one centromere corresponding to the IIq, through the loss of alphoid DNA, via poorly understood mechanisms. Unlike the fusion point, the ancestral centromere mapping at 2q21.1-2q21.2 has been poorly investigated.
Here we performed comparative in silico and molecular analyses in chimpanzee, gorilla, orangutan and macaque genomes in order to shed light on the genomic organization of the 2.1 Mb region encompassing the ancestral centromere. This approach allowed us to track precisely the evolutionary history of the ancient centromere and the corresponding pericentromeric region, whose assembly is still complicated by segmental duplications. In this study we provide the patterns of segmentally duplicated regions among chromosomes for each analyzed species, highlighting species-specific deletions and duplications.

P12.5
Zootoca vivipara as a model for testing evolutionary transition from oviparity to viviparity

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Squamate reptiles offer a unique model system for testing hypotheses about the evolutionary transition from oviparity to viviparity in vertebrates. The lizard *Zootoca vivipara* is one of the few species with different reproductive modalities in different groups; in particular, *Z.v.carniolica* is an egg-laying lizard and *Z.v.vivipara* is a live-bearing one, and both live in the Eastern Italian Alps, sometimes in sintopy. This scenario provides an interesting natural experiment for studying the evolutionary shift in reproductive mode. Some populations have been analysed using classical genetic markers (mitochondrial and nuclear DNA sequences and autosomal microsatellites). The mtDNA results

indicated a marked genetic divergence between the two subspecies (around 5% at the cytochrome B). The existence of hybrid individuals, supported by the nuclear markers, increased the interest on this topic. RADtag sequencing, a next-generation sequencing technique that allows to simultaneously discover and analyze hundreds of thousands of SNPs, was then applied to *Zootoca vivipara* subspecies to identify mutations correlated with the reproductive modality and with related adaptive traits.

P12.6
In lycophytes the minor Photosystem II antenna Lhcb6 undergoes light-dependent phosphorylation

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Vascular plants include two divergent lineages, lycophytes and euphyllophytes (ferns and seed plants). We compared the response of photosynthesis to increasing irradiance in two ferns and two lycophytes, with special emphasis on *Selaginella martensii*. Lycophytes were characterized by a light-dependent increase in the "state transition"-quenching component of non-photochemical quenching (*NPQ*). This component was found to depend on protein phosphorylation. Interestingly, besides D2, CP43 and Lhcb1-2, *S. martensii* also showed phosphorylation of Lhcb6, which forms the minor antenna CP24 of PSII. Bidimensional separation of thylakoid proteins by means of large pore-Blue Native/SDS PAGE allowed us to detect phosphorylated Lhcb6 in association with PSI and the free trimers of LHCII. The light-dependent phosphorylation of Lhcb6 seems to be unique to lycophytes, in fact it has never been observed in other land plants. Our results suggest that, in lycophytes, phosphorylation of Lhcb6 could be linked to the regulation of the energy distribution between PSI and PSII.

P12.7
False signals of bottlenecks in genetic data caused by variance in reproductive success

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Demographic bottlenecks, severe reductions in population size, are important events in the evolutionary history of species and populations as they can reduce genetic variation. Establishing whether a bottleneck has occurred is important in ecological, evolutionary and conservation biology investigations. There are several statistical methods to identify a bottleneck using genetic variation data, but the rate of false positives (a signal of a bottleneck when one has not occurred) has received little attention. We use population genetic simulations to test whether species with high reproductive success (some individuals producing no or few offspring while others produce many offspring) might show false positives, e.g., signals of genetic bottlenecks in a constant size population. We find that moderate and high variance in reproductive success produces 30-50% rates of false positives for several tests and types of genetic markers. We show that using a stricter significance threshold ($0.005 \geq \alpha$) can decrease error rates. These tests should be used with caution in organisms with high variance in reproductive success, such as marine organisms, plants, and amphibians.

P12.8
Molecular and phylogenetic analyses of Italian sheep breeds based on mitochondrial DNA sequences

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Since domestication, sheep could be found in a wide geographic range of distribution due to their extreme adaptability. In recent decades, new livestock technologies drastically changed sheep management, emphasizing the breeding of a small number of selected breeds. In order to preserve autochthonous genotypes of the Italian peninsula, we focused our study on some local breeds (Appenninica, Gentile di Puglia, Sopravissana, Merinizzata Italiana, Sarda, Comisana) aiming to characterize the molecular variation of their mitochondrial genomes. We first sequenced a large segment of the mtDNA control region, including the entire hypervariable segment between nps 15452 and 16263, in more than 300 samples (at least 30 individuals for each breed). Other two European breeds (Spanish Merino and Lacaune) were also included for comparison. The network built on the obtained haplotypes was extremely star-like, with virtually no monophyletic clades detectable and showing only few haplotypes pooled together. The shared mtDNAs, as well as the most divergent ones, were selected for complete sequencing in order to fully clarify the genetic relationships and phylogenetic histories of these breeds.

P12.9
Isotope investigation of Early Neolithic farmers from central and south-eastern Italy

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The arrival of agricultural and breeding practices at around the X millennium BC had prominent repercussion on culture, economy, demography and settlement patterns of Old World and still represents a fascinating and challenging subject matter amongst scholars.
In this work, the question of Neolithic transition was dealt with in dietary terms with the aim to investigate the subsistence strategies at Neolithic times and the economic implications, i.e. the extent of the shift from hunting and foraging to farming, through combined bone collagen stable isotope analyses of carbon, nitrogen and sulphur. The latter were performed on Early Neolithic populations from the poorly investigated and interesting areas of central and south-eastern Italy. The results seem to highlight a significant dietary variability amongst farmers letting suppose a gradual change from food collection to food production. In order to give indication of possible migration events, a further analysis of oxygen stable isotopes was carried out on tooth enamel of samples retrieved from a central Italy site. The results showed that they are unlikely to have undertaken massive movements during their lifetime.

P12.10
Genetic traits of *ATPsyn-c* of *D.melanogaster*: an intriguing model of polycistronic units?

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ATPsyn-c is the only gene of the *D. melanogaster* genome encoding subunit c of the mitochondrial ATP synthase proton channel.
The Drosophila mature *ATPsyn-c* polypeptide shares a high level of amino acid identity with all its Metazoan counterparts.
Extensive mutagenesis analysis shows that the gene is essential in many biological processes *D. melanogaster,* since severe morphological and functional defects are associated with mutations of the ATPsyn-c transcriptional unit.
We also identified conserved sequences encoding the *RNAseP:RNA*, which is involved in tRNA maturation, in the third intron of the gene. This position is strictly conserved in 19 Drosophila species. Intriguingly, a search for ATPsyn-c orthologs in 40 sequenced Arthropoda genomes, indicates that the orthologous intron does not contain the RNAseP:RNA gene, which is instead found, using a BLASTN strategy, within an intron of other unrelated genes, different in each taxonomic families but with the same 5' to 3' orientation of the host gene.
Our data indicate that ATPsyn-c is a bicistronic locus in Drosophila, possessing the genetic information necessary to encode both the ATP synthase subunit c and the RNaseP:RNA ribozyme.

P12.11
A comparative analysis of Bari-like transposons

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Bari-like elements are members of widespread Tc1 superfamily of transposons that has colonized most species of the Drosophila genus.
All identified elements are characterized by an elevated level of structural homogeneity in the genome they habit, suggesting an initial incoming of their evolutive history. Bari-1 and Bari-3 isoated respectively in D.melanogaster and D.mojavensis, share 65% identity at the transposase level while they are much more divergent in the architecture of their terminal inverted repeats.
To get more insight into the biology of Bari-like elements, we are undertaking a detailed study of the strucutural components in these transposons (transposase and TIRs). Preliminary study indicate that both trasnsposases are able to enter into the nucleus of S2R+ and HepG2 cells whithout performing a significative recognition of their target DNA suggesting a specific control in the transposition process mediated by defensive mechanisms of invaded hosts. Preliminar expression studies support the hypothesis that post-transcrcriptional events regulate the transposition of Bari elements.

O12.1
Temporal patterns of divergence and hybridization in three Antarctic fish species

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Divergence and hybridization are important processes during the origin and evolution of a species, and their relative role often depends on specific climatic events. We investigated the demographic histories of the species within the genus Chionodraco, a group of cold-adapted Antarctic fish. It has been suggested that periodic expansion of Antarctic ice sheets have promoted allopatric speciation. On the other hand, warmer ice melting phases could have promoted inter-specific hybridization events. In this study we analysed the genetic structure and variation in 108 individuals typed at 8 microsatellite loci. Different evolutionary models assuming different "pulses" of hybridization were also compared using the Approximated Bayesian Computation approach. Interspecific introgression events were supported by the mixed genetic make-up of some individuals, and the most statistically supported model implied genetic exchanges occurring only during the Holocene and Eemian interglacial periods. Overall, this study suggests that cycles of divergence and hybridization, associated to glacial and interglacial phases, respectively, affected the evolutionary history of Antarctic species.

O12.2
A detailed phylogeny of cattle mtDNA haplogroup T1: old ideas and new perspectives

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The possibility of an independent and/or secondary domestication events of *Bos taurus* occurred after domestication in the Fertile Crescent is still debated. In particular, haplogroup T1 - fixed in African cattle but present also in Middle Eastern, Anatolian and Southern European breeds - has been the subject of several investigations pointing to an independent African domestication event and to a genetic contribution of African cattle to Iberian and Creole cattle. We have identified 281 T1 subjects through the analysis of more than 2000 mtDNA control regions representing breeds from Europe, Africa and America and obtained 54 new T1 mitochondrial complete genomes. This allowed us to redesign the T1 phylogenetic tree which is now composed of 6 distinct T1 sub-haplogroups (T1a-T1f). Our data support the overall scenario of a Near Eastern origin of the T1 sub-haplogroups from as much as eight founding T1 haplotypes, but also raised the possibility that one sub-haplogroup (T1d) arose in North Africa. The previously identified ''African-derived American'' haplotype turned out to be a sub-clade of T1c and we found it for the first time in Egypt.

O12.3
Landscape of active transposable elements in Latimeria menadoensis

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Coelacanths occupy a key position in the evolution, representing one of the closest extant relatives of the terrestrial vertebrates. Moreover some studies suggest that *Latimeria* might be considered a 'living fossil' not only from a morphological point of view but also from a molecular point of view.
In order to shed light on the effective stasis in the genome evolution in this organism, we explored the activity of transposable elements by transcriptome analysis.
The assembly of transcripts from liver and testis samples of an adult specimen of *L. menadoensis* revealed that about the 10% of contigs (representing about 2% of total paired-end reads) contains a TE. Elements belonging to Class I and II were identified. Among them CR1 LINEs, tRNA-SINEs, Gypsy LTR-retrotransposons, and Harbinger DNA-transposons are the most expressed. Moreover the comparison of expressions in liver and testis showed the occurrence of repeated sequences with marked differential expression.
The presence of active transposable elements challenges the 'frozen genome' hypothesis in the coelacanths.

O12.4
Nucleotide diversity of polygalacturonase-inhibiting protein (PGIP) genes in natural populations of *Phaseolus vulgaris*

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PGIPs are plant extracellular Leucine-Rich Repeat (LRR) proteins that inhibit polygalacturonases (PG) produced by phytopathogenic fungi during infection and protect cell wall integrity. The PG-PGIP interaction is considered a model system to understand the structural basis of the interactions between LRR proteins and their pathogen-derived ligands. We present a study on the molecular evolution of the four genes that form the PGIP gene family of Phaseolus vulgaris. Nucleotide diversity was analyzed in a sample of wild accessions that is representative of the geographical distribution of the species. We have used bioinformatics tools such as DnaSP to analyze DNA polymorphic sites, and the statistical package PAML (Phylogenetic Analysis by Maximum Likelihood) to test whether a significant departure from neutral evolution can be inferred for the four PGIP genes. These analyses show different evolution pattern of the four paralogous PGIP genes and suggest a different selective pressure on the different family members.

O12.5
Gorilla genome structural variation reveals evolutionary parallelisms with chimpanzee

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Structural variation has played an important role in the evolutionary restructuring of human and great ape genomes. We generated approximately 10-fold genomic sequence coverage from a western lowland gorilla and integrated these data into a physical and cytogenetic framework to develop a comprehensive view of structural variation. We discovered and validated over 7,665 structural changes within the gorilla lineage including sequence resolution of inversions, deletions, duplications and mobile element insertions. A comparison with human and other ape genomes shows that the gorilla genome has been subjected to the highest rate of segmental duplication. We show that both the gorilla and chimpanzee genomes have experienced independent yet parallel patterns of structural mutation that have not occurred in humans, including the formation of subtelomeric heterochromatic caps, the hyperexpansion of segmental duplications, and bursts of retroviral integrations. Our analysis suggests that the chimpanzee and gorilla genomes are structurally more derived than either orangutan or human genomes.

13 - Neurobiology

P13.1
Functional characterization of human astrocytoma cells overexpressing new Inwardly-Rectifying K+ Channel Kir4.1 mutations associated with epilepsy and autism spectrum disorder

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Two new missense mutations (p.R18Q and p.V84M) in KCNJ10 gene encoding the potassium channel Kir4.1 have been recently detected in children with epilepsy, Autism Spectrum Disorder (ASD) and intellectual disability. When expressed in a heterologous system R18Q and V84M mutations affected current amplitudes by increasing surface expression and single-channel conductance, respectively. With the aim of understanding the molecular mechanism generating pathological defects and since astrocytes through the abundant expression of Kir4.1 channel play a major role in the regulation of $[K^+]_o$ homeostasis essential for normal neuronal activity and synaptic functions, we have generated and characterized astrocytoma cell lines overexpressing Kir4.1 wild type or carrying the new pathological mutations. Immunofluorescences and biochemical analysis of Kir4.1 molecular relationships indicated that mutations differently affect channel intracellular localization and functional interactors opening the way to clarify the pathogenetic mechanism of autism-epilepsy phenotype and possibly identify new targets for novel therapeutic approaches.

P13.2
Opposite roles of TGF-ß1-responsive TSC22D proteins in regulating cerebellar granule neurons differentiation and commitment to apoptosis

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The TSC22D protein family includes widely expressed members controlling multiple biological processes. We demonstrated that TSC22D4 is involved in cerebellar granule neuron (CGN) differentiation and commitment to apoptosis. These opposite functions rely on the existence of multiple TSC22D4 forms (42, 55, 67 and 72 kDa) differing in modification, subcellular localization and function. While the TSC22D4-72 is bound to chromatin, the TSC22D4-67 is associated with Apoptosis Inducing Factor (AIF) in mitochondria. When CGNs are committed to apoptosis the TSC22D4-67 is rapidly transferred from mitochondria to nuclear matrix with a kinetics similar to that of AIF transfer to chromatin. Besides TSC22D4, CGNs also express TSC22D1-1 and TSC22D1-2 splice variants. We have recently observed that TSC22D4-42 interacts with TSC22D1-2 in undifferentiated but not differentiated CGNs.
Present data suggest that TSC22D4-42 – TSC22D1-2 complex is important in regulating CGN balance between proliferation and differentiation, whereas TSC22D4-67- AIF complex is relevant in the sensoring and early transduction of death signals at the onset of CGN apoptosis.

P13.3
Region-specific function and regulation of Retinoic Acid signalling in neural progenitors

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Retinoic acid (RA) is a vitamin A derivative that plays a crucial role in the specification of the posterior nervous system during early stages of vertebrate development, while, at later stages, it is also required for forebrain development. This suggests that epigenetic regulatory mechanisms locally modulate the response of neural progenitor cells (NPCs) to RA, in order to achieve appropriate, position-dependent effects and however very little is known, about this mechanism. We studied the response of NPCs derived from E13.5 mouse cortex (Ctx), lateral ganglionic eminence (LGE) and spinal cord (SC) to RA. Ctx, LGE and SC NPCs all express the RA receptors RARa, RARb and RARg and respond to exogenous RA upregulating the RA target genes RARb and Dhrs3. Thus, in these cells, the RA signalling pathway is functional and elicits a transcriptional respons. However, RA actives specific transcriptional programs in Ctx, LGE and SC NPCs. In particular, RA treatments upregulated HoxB4, HoxB6, HoxB8 and HoxB9 in SC NPCs, Dlx2 and Six3 in LGE NPCs, while none of these genes is upregulated in Ctx NPCs. We are investigating the molecular mechanisms modulating the response of Ctx, LGE and SC NPCs, generating NPCs lines where individual RARs are abrogated, and performing ChIP assays with RA-treated NPCs using anti-RAR antibodies.

P13.4
Dysbindin expression in retinal Müller glial cells

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Dysbindin, the product of the DTNBP1 gene, was identified by yeast two hybrid assay as a binding partner of dystrobrevin, a cytosolic component of the dystrophin protein complex. Although its functional role has not yet been completely elucidated, the finding that dysbindin assembles into the biogenesis of lysosome related organelles complex 1 (BLOC-1) suggests that it participates in intracellular trafficking and biogenesis of organelles and vesicles. Dysbindin is ubiquitous and in brain is expressed primarily in neurons. Variations at the dysbindin gene have been associated with increased risk for schizophrenia. As anomalies in retinal function have been reported in patients suffering from neuropsychiatric disorders, we investigated the expression of dysbindin in the retina. Our results show that differentially regulated dysbindin isoforms are expressed in rat retina during postnatal maturation. Interestingly, dysbindin is mainly localized in Müller cells and astrocytes. The identification of dysbindin in glial cells may open new perspectives for a better understanding of the functional involvement of this protein in visual alterations associated to neuropsychiatric disorders.

P13.5
Dynamin I (DynI), a GTPase involved in endocytosis of synaptic vesicles, as target of peroxynitrite (PN)

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Functional properties of proteins involved in the synaptic vesicle (SV) exo-endocytosis are affected by oxidative stress. In rat brain synaptosomes treated with peroxynitrite (PN), a powerful oxidant able to induce both Tyr phosphorylation (pTyr) and Tyr nitration (NO_2Tyr) in proteins target, we showed that two synaptic proteins DynaminI (DynI) and Synaptophysin (SYP) were modified. We found that PN down-regulated the pTyr of the GTPase DynI in a dose-dependent manner while increased DynI NO_2Tyr. MS/MS analysis identified one nitration site in DynI at Tyr_{354}. The pTyr of DynI by *c-src* induces its self-assembly and increases its GTPase activity, here we showed both these functions of DynI were inhibited by PN. In addition, DynI forms a complex with SYP regulated by pTyr of SYP. Interestingly, in synaptosomes treated with PN, formation of SYP/DynI complex was impaired while SYP

associated higher amounts of VAMP2. Our results suggest that PN promotes the association of SYP to VAMP2, thus blocking the binding of DynI with SYP. In conclusion, site-specific Tyr modifications modulate the association of SV proteins and regulate DynI via control of self-assembly.

P13.6
BDNF genetic variants and the risk of alzheimer's disease without depressive component: an association study in the italian population

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BDNF (Brain Derived Neurotrophic Factor) appears to protect neurons from harmful stimuli and changes in its levels may contribute to the pathogenesis of neurological disorders. Our study focused on the best-known polymorphism in this gene, BDNF 196 G/A (rs6265), and on two other BDNF SNPs: BDNF 270 C/T (rs2030324) and the BDNF 11757 G/C (rs16917205) in 200 Alzheimer patients and 400 healthy controls by PCR-RFLP and RealTime PCR. A statistically significant increase of 11757 GG genotype frequency in AD vs healthy subjects (OR=1.4670;p=0.0331) was observed; whereas the CG genotype demonstrates a statistically significant decrease of frequency in AD patients vs controls (OR=1.1104;p=0.0194). We also focalized our attention on haplotype reconstruction. A statistically significant decrease of the TAC haplotype frequency in AD patients vs healthy controls group (OR=0.4542;p=0.005) and a statistically significant increase of the CAC haplotype frequency in patients vs control (OR=1.9031;p=0.019) was demonstrated. These results strengthen the importance of this molecule in neurological diseases, but also point to some differences with results already found in AD-depressed patients.

P13.7
Inhibition of CXCR4 regulates microglial reactivity and tumor microenvironment markers in a glioma model

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The C-X-C chemokine receptor-4 (CXCR4) is expressed in many different tumors. In gliomas, CXCR4 activation by the agonist CXCL12 can sustain proliferation, angiogenesis, migration, as well as the recruitment of the surrounding microglia/macrophages. The aberrant communication between glioma cells and tumor microenvironment represents one of the major factors regulating brain tumor diffusion. As the CXCL12/CXCR4 axis is involved in several aspects of tumor progression it could be an important target for new therapeutic strategies. In this study we investigated whether a new CXCR4 receptor antagonist, the peptide Phe-7, could affect the proliferation and survival of a human glioma cell line (U87MG) and modulate the tumor microenvironment in vivo. We observed a Phe-7-mediated decrease in the amount of living/proliferating cells after 72h of incubation. Immunohistochemistry analyses on brain sections demonstrated that the Phe-7 treatment induces: i) inhibition of microglial/macrophages cells recruitment at the tumor edge; ii) modulation of the microglial/macrophages reactivity inside the tumor; iii) reduction of angiogenic markers in the tumor core. These data suggest that this new CXCR4 antagonist, the peptide Phe-7, could represent a powerful tool to modulate glioma-microglia interactions and local inflammatory responses, as well as to hamper with intratumoral vascularization, thus interfering with tumor development and progression.

P13.8
Immediate early genes expression in the cerebellar cortex correlates with LTP and LTD induction

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Immediate early genes are thought to contribute to long-term synaptic plasticity (LTP and LTD) in the hippocampus. We employed VSD imaging in rat cerebellar slices in order to map LTP/LTD spatial distribution in the cerebellum granular layer at 15' and 120' following a Theta Burst Stimulus (TBS) delivered to the mossy fibers. Slices were then fixed and processed for immunohistochemistry and in situ hybridization in order to identify c-Fos and P-CREB expression patterns at the protein and mRNA level. The induction of long-term plasticity increased the average level of P-CREB both at 15' and 120' after TBS, while c-Fos was unaltered at 15' and significantly increased at 120'. By spatially correlating long-term synaptic plasticity with the corresponding variation of P-CREB and c-Fos, we observed that regions showing LTP well correlated with positive variations of P-CREB and c-Fos. Conversely, areas showing LTD correlated exclu-sively with negative variations of P-CREB. The analysis performed in the presence of 50 μM APV showed that, in correspondence with the block of LTP and LTD induction, c-Fos and P-CREB levels were unchanged, confirming their involvement in cerebellar plasticity.

P13.9
The transcriptional factor REST is required to prevent aNSC mouse SVZ-derived neural stem cells differentiation in vitro

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The RE1 silencing transcription factor (REST) is a transcriptional regulator involved in neural differentiation. REST levels declines during embryonic stem cells conversion into the neural lineage and foetal neural stem cells (NSC) differentiation into neurons.We explored REST function in NSC lines derived from the adult SVZ (aNSCs). We found that REST is expressed in aNSCs and represses the expression of several well-known target genes. REST knockdown promoted aNSCs differentiation despite the presence of growth factors (GF). Interestingly REST silencing did not modify the neurogenic and gliogenic potential of aNSC induced to differentiate by GF withdrawal. These data suggest that REST knock down is sufficient to induce NSC differentiation but it does not affect their lineage-specific differentiation program. Consistently, knockdown of REST in primary neurospheres impaired their growth and clonogenic potential.

We performed wide-genome analysis and identified a number of genes that are bound and/or regulated by REST, including miRNAs. Among them we focused on miR-124, a well-known direct REST target gene. We are studying the effect of miR-124 over-expression on NSC maintenance.

O13.1
A new cellular model to disclose Megalencephalic Leukoencephalopathy with subcortical Cysts (MLC) pathological mechanisms

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Megalencephalic leukoencephalopathy with subcortical cysts (MLC), is a rare congenital and incurable leukodystrophy characterized by macrocephaly, subcortical fluid cysts and myelin vacuolation. Mutations in the MLC1 protein, a membrane protein highly expressed in brain astrocytes and whose function is still unknown, are responsible for the disease. Since no animal model for MLC is available and human biopsies are extremely rare, we generated human astrocytoma cells stably overexpressing MLC1 wild-type or carrying pathological mutations, as a cellular model where investigating MLC1 function and disease-associated defects. Using astrocytoma cells we demonstrated that MLC1 is localized in the plasmamembrane and endolysosomal organelle and takes part to the regulation of astrocytic response to osmotic changes by its functional interaction with the Na,K-ATPase pump and the calcium channel TRPV4. Pathological mutations alter MLC1 localization, molecular interactions and intracellular calcium influx leading to dysregulation of astrocyte-mediated osmoregulatory processes. These data shed light on MLC pathogenesis and MLC1 function paving the way for the development of specific therapies.

O13.2
Responsiveness to NGF is reduced in sympathetic neurons of *mdx* mice, affecting axon outgrowth and regeneration both *in vivo* and *in vitro*

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Dystrophin (Dp427) is a cytoskeletal protein defective in muscle and brain of Duchenne muscular dystrophy patients and *mdx* mice. To unravel the question whether Dp427 played a role in early axon growth dynamics, we axotomized adrenergic neurons of the superior cervical ganglion (SCG) of wild-type (WT) and *mdx* mice to reactivate development-related mechanisms of axon elongation. Levels of tyrosine hydroxylase, examined at different post-operative times in iris and submandibular gland (SG), two of the SCG targets, decreased. However, while those in *mdx* mouse iris never recovered, re-innervation of SG, enriched in nerve growth factor (NGF), was similar between the two genotypes. Reduction in number and length of regenerated axons was also observed 1d after *mdx* mouse neuron axotomy *in vitro*. Moreover, neurite elongation of SCG neurons grown with 5, 10, 50 or 100 ng/ml NGF was always significantly lower in *mdx* mouse cultures respect to WT, along with levels of proteins downstream to NGF signaling. Our data suggest that lack of Dp427, which mediates cytoskeleton-extracellular matrix linkage, interferes with NGF-associated signal transduction, affecting axon growth and regeneration.

O13.3
Ultrasensitive detection of prion seeding activity by RT-QuIC and eQuIC assays for early diagnosis of prion diseases

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Management of prion diseases requires rapid assays for pre-symptomatic diagnosis. We have used the real-time quaking induced conversion assay (RT-QuIC) to quantify hamster PrP^{res} in brain, nasal lavages and cerebrospinal fluid (CSF), detect deer chronic wasting disease and sheep scrapie PrP^{Sc} in brains (Wilham et al., 2010) and human sporadic Creutzfeldt-Jakob Disease (sCJD) PrP^{res} in brains (Peden et al, 2012). Two studies have shown the utility of RT-QuIC in diagnosing sCJD using CSF (Atarashi et al., 2011; McGuire et al., 2012) and we have used the RT-QuIC in a time course study using CSF (Orru` et al., 2012). Our preliminary studies show that RT-QuIC identifies scrapie positive sheep using CSF. We generated the "enhanced QuIC" (eQuIC; Orru` et al., 2011) that detects attograms of human variant Creutzfeldt-Jakob disease PrP^{res} spiked into plasma and discriminates between plasma and serum samples from scrapie-infected and uninfected hamsters. eQuIC detects ~1fg of human PrP^{sCJD} and 10ag of sheep PrP^{Sc} spiked into plasma and a variety of sheep and mouse-adapted scrapie strains in plasma. Presently, we are validating these techniques for the early diagnosis of prion diseases.

O13.4
2mit, an intronic gene of *timeless2*, is involved in memory formation of *Drosophila melanogaster*

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"Nested gene" defines any gene enclosed in another larger external gene. In *D. melanogaster* ~85% of nested genes are protein-coding genes. In 2010, we characterized the D. melanogaster *timeless2* (*tim2*), showing its involvement in chromosomal stability and circadian clock light synchronization. A protein-coding gene, named *2mit*, maps on the *tim2* 11th intron. Using insertional mutations, tissue-specific overexpression and down-regulation methodologies, we showed that *2mit* is involved in Drosophila memory formation, via its expression in brain mushroom bodies. *tim2* and *2mit* exhibit no evidence of functional relationship, but show a negative correlation in expression levels in specific regions of adult brain. *2mit* orthologs have been identified in 20 Drosophila species and in other insects. The chromosomal organization of *tim2* locus, with *2mit* embedded within a *tim2* intron, is conserved among the 20 Drosophila species, indicating that this structural organization was present before the radiation of Drosophila genus occurred 50-60 million years ago. Our data suggest the existence of some evolutionary constrains which contributed to maintain tim2-2mit host-nested genes association.

O13.5
M2 muscarinic receptor activation contributes to modulate Schwann cell migration and differentiation in myelinating phenotype

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ACh receptors are expressed in glial cells, suggesting new roles for ACh in glial cell development and physiology. Rat Schwann cells express all muscarinic receptor subtypes. M2 receptor is the most abundant and its activation causes a reversible arrest of cell cycle in G1 phase with possible consequence on Schwann cell differentiation. Recently we demonstrated that the M2 agonist arecaidine causes an increased

expression of myelin proteins P0, PMP22 and MBP, induces an up-regulation of transcription factors krox 20 and sox 10, involved in the myelinating phase and a down-regulation of the genes involved in the maintenance of undifferentiated state such as c-jun and Notch-1. Moreover it causes a down regulation of Neuregulin-1 isoforms and a decrease of erbB2 receptor, Notch-1 and jagged-1 proteins. Electron microscopy and morphometric analysis of sciatic nerves of KO M2/M4 mice show an increase in myelin thickness and in degenerating axon number. Furthermore wound healing experiments indicate that M2 receptor activation promotes Schwann cells migration. These data suggest that ACh may contribute to drive Schwann cells differentiation in myelinating phenotype.

14 - Cell communication, signal transduction, and membrane trafficking

P14.1
Selective targeting by PEG-masked ferritin-based multifunctional nanoparticles

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Nanoparticle-based systems are promising for the development of imaging and therapeutic agents. Targeted delivery of nanoparticles requires an accurate system design. We developed nanoparticle constructs based on the heavy chain of the human protein ferritin (HFt), a highly symmetrical assembly of 24 subunits enclosing a hollow cavity. HFt-based nanoparticles were produced using both genetic engineering and chemical modifications to impart functionalities such as: the α-melanocyte-stimulating hormone peptide, as a melanoma-targeting moiety, or a recently-developed peptide, named P12, as a α5β1 integrin-targeting agent; polyethylene glycol molecules, as stabilizing moieties; rhodamine fluorophores and magnetic resonance imaging agents, for detection. The produced constructs were characterized by a number of physicochemical techniques, and assayed for selective melanoma or endothelial cell-targeting in vitro and in vivo. HFt-based nanoparticle constructs were specifically taken up by the targeted cells in vitro. Moreover, experiments in melanoma-bearing mice indicated that these constructs had a good tumor-targeting and vessel-targeting profile in vivo.

P14.2
The Gac/Rsm regulatory network controls siderophore production in *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa is a versatile bacterial pathogen endowed with a wide range of survival strategies. To face iron limitation, *P. aeruginosa* secretes two siderophores, pyoverdine and pyochelin, whose ability to deliver iron to the cell is crucial for biofilm formation and pathogenicity. In this study, we have described a link between iron uptake and the Gac/Rsm system, a well-known signal transducing pathway of P. aeruginosa which controls the switch from planktonic to biofilm lifestyle. We observed that production of both pyoverdine and pyochelin by *P. aeruginosa* is dependent on the state of activation of the Gac/Rsm pathway, which was found to control siderophore regulatory and biosynthetic genes at the transcriptional level, likely independently from the master regulator of iron metabolism Fur. Preliminary assays revealed that the Gac/Rsm system regulates siderophore production through modulation of the intracellular levels of the second messenger c-di-GMP, suggesting that two major global regulatory networks of *P. aeruginosa* (c-di-GMP signaling and the Gac/Rsm pathway) are coordinately involved in the regulation of siderophore-mediated iron uptake.

P14.3
The dark site of LOV

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Light transduction has been mostly studied in the model system *N. crassa*. To gain insight on photoreceptor systems operating in subterranean ascomycetes, we started the analysis of *T. melanosporum* genome. It has been found, by prediction tools and expression analyses, that *T. m.* genome contains photoreceptor-like sequences similar to the white collar (WC) complex, opsin related protein, phytochrome, and velvet-like transducing components. To go deeper into the analysis of these putative photoreceptors a *N. crassa wc-1 ko* strain was transformed with a recombinant gene in which the LOV domain of wc-1 was replaced by LOV domain of tuber. The albino phenotype of the *null* mutant was rescued in the transformants which were able to accumulate carotenoids comparable to the wild type. Moreover, in the recombinant strain the analyses of transcription level, by RT-PCR, of light induced genes confirmed the full complementation of the *null* mutation by the recombinant construct. The information on truffle light-sensing system would be of special interest not only for the evolutionary history of the Perizales but also may open new perspectives for optogenetic applications.

P14.4
Sub-cellular localization and dynamics of an Arabidopsis mitogen-activated protein kinase kinase kinase (MAPKKK) family involved in elicitor-triggered signalling

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Alpha 1-4-linked oligogalacturonides (OGs) derived from plant cell wall pectin are damage-associated molecular patterns (DAMPs) that are recognized by plant receptors as signals of an altered-self, leading to the activation of the plant immune response. So far, elements involved in OG-triggered signalling are mostly unknown. In both animal and plants, Mitogen-Activated Protein Kinase (MAPK) cascades represent a highly conserved signal transduction mechanism. Three types of kinases form the core module of a MAPK cascade: MAP kinase kinase kinases (MAPKKKs), MAP kinase kinases (MAPKKs) and MAP kinases (MAPKs). We have analysed the subcellular localization and dynamics of a family of MAPKKKs by live cell imaging coupled with transient expression experiments. In transgenic plants constitutively expressing a fluorescent form of each MAPKKK, we observe that, upon elicitation with OGs or elf18, these proteins translocate from mitochondria to plastids and to the nucleus; the same organelles appear to be sites of reactive oxygen species (ROS) production in response to the elicitors. These results point to an important role of these MAPKKKs in ROS generation and signaling and in immunity.

P14.5
Phosphoproteomic analysis of Arabidopsis membranes reveals novel elements involved in response to oligogalacturonides

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Oligogalacturonides (OGs) are plant endogenous elicitors that

accumulate during fungal infection and can act as danger signals to activate the plant immune response. We used 2-D DIGE proteomic analysis coupled with phospho-specific ProQ Diamond staining for a quantitative measure of both protein abundance variation and phosphorylation state changes in total microsomes of OG-treated Arabidopsis seedlings. Two proteins PcaP1 and DET3, which undergo phosphorylation changes after 10 min upon OG treatment, were further investigated. PcaP1 is a plasma membrane-associated protein that binds Ca2+ and phosphatidylinositol phosphates, major components of intracellular signaling. DE-ETIOLATED3 (DET3) encodes the subunit C of the vacuolar H+–ATPase. PCaP1 is present as a multispot protein in 2D gels. Null pcap1and det3 mutant seedlings are compromised in OG-induced phosphorylation of the mitogen-activated kinases (MAPKs) MPK3 and MPK6, and in several early responses to both OGs and a bacterial pathogen-associated molecular pattern (PAMP), flg22, indicating that PcaP1 and DET3 are required for full activation of the defense responses triggered by biotic elicitors.

P14.6
A proteomics study of cAMP response in Arabidopsis thaliana

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The second messenger 3'-5'-cyclic adenosine monophosphate (cAMP) and adenyl cyclases (ACs), enzymes that catalyse the formation of cAMP from ATP, are increasingly recognized as important signaling molecules in plant growth, development and plant responses to the environment.. Key functional evidence for a role of cAMP came from whole-cell patch-clamp current recordings in *Vicia faba* mesophyll protoplasts that showed cAMP specific and concentration-dependent increases of outward K^+-currents {Li et al., Plant Physiol., 106: 957-961, 1994). Here we use proteomics to identify cAMP-dependent protein signatures and identify a number of proteins with a role in light-dependent responses. In addition, we make use of on-line data mining tools and the large amount of publicly available Arabidopsis transcriptomics data to infer protein function at the systems level. Based on our proteomics results and supported by a transcriptional analysis at the systems level, we propose, that much like in cyanobacteria and algae, cAMP has a role in light signaling and the regulation of photosynthesis in higher plants.

P14.7
Specific and unique relationship between Notch3 and Jagged1 in T cell leukemia

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The initiation of Notch signaling occurs through a series of proteolytic events upon the binding of Notch receptors to specific cell-bounds ligands (Jagged1 and 2 and Delta1, 3 and 4), expressed in neighboring cells. Although deregulated expression of Jagged1 has been documented in several human tumors, the mechanisms by which their misexpression contributes to leukemogenesis have not been elucidated.
We show here that specific and unique relationships exist between Notch3 and Jagged1 in the animal model of T cell Acute Lymphoblastic Leukemia (T-ALL), represented by the Notch3-IC transgenic mice. Our observations reveal that in N3-232T cells, an immortalized cell line established from N3-IC transgenic thymocytes, exist a bidirectional function of Jagged1 ligand, that is able not only to trigger Notch signaling in neighboring cells, but also to signal through soluble cytoplasmic domain, that is able to move into the nucleous and synergize with Notch3-IC transcriptional complex to activate Notch3 target genes and to sustain the Notch3-IC-dependent leukemia. These data suggest that Notch3 and Jagged1 autocrine/paracrine loop is required for development and progression of T-ALL.

P14.8
Leaf saporin isoforms targeting dependency on signals in non processed proteins

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Ribosome-inactivating proteins (RIPs), a large group of plant enzymes present in a great variety of species inhibit protein synthesis. While these proteins have been studied in the biomedical field as immunotoxins, in plants their function is far from being resolved although a role in pathogen resistance/response was suggested. Saporins are a well studied single chain RIPs, identified in several organs of Saponaria with several isoforms characterized and isolated from both intra- and extracellular fractions. In order to characterize the signals necessary for the localization of these toxic proteins several ammino-terminal GFP fusions were constructed. Several transient heterologue systems (Arabidopsis roots, Arabidopsis protoplasts and onion epidermal layers). Data presented suggests that the carboxylic terminal extension (propeptide) is relevant for correct targeting of the studied isoforms.

P14.9
Role of cAMP during the growth of tobacco BY-2cells

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Although the existence of cyclic AMP in higher plants has been, widely, debated, nowadays its role as second messenger has been, definitively, demonstrated. Indeed, cAMP is involved in several physiological processes such as cell cycle regulation, seed germination and defense responses. However, little is still known on its signal transduction. To investigate the role of cAMP in plant signaling pathways, tobacco BY-2 cells have been transformed with the cAMP-sponge (cAS), a non invasive tool able to selectively reduce cAMP concentration. The cAS is composed of two high-affinity cAMP binding domains of the regulatory subunits IBeta of human PKA that specifically bind cAMP and not cGMP. The cAS under the control of the strong constitutive promoter CaMV 35S and in frame with the reporter gene mCherry was transferred in BY-2 cells via A. tumefaciens-mediated transformation. After the assessment of transgene integration and of its expression, the growth parameters of transformed BY-2 cells were characterized. The obtained results show that low levels of cAMP negatively affect growth of TBY-2 cells during the exponential phase, principally slowing, cell cycle progression.

O14.1
High-throughput analysis of downstream effects of activating Gsα mutations in skeletal progenitor/ stem cells

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Fibrous dysplasia (FD) of bone is a genetic disease caused by mutations of the α subunit of the stimulatory G protein, Gs (Gsα). The mutation

results in enhanced cAMP production, and in a subversion of the structure of bone/bone marrow, mediated by the effects on skeletal stem cells (SSC). To investigate the downstream effects of the mutation in stem cells, we transduced phenotype-purified SSC with a LV vector encoding one of the two mutations causing the disease in humans (R201C). De novo transduction of normal SSC (as opposed to isolation of SSC from bone lesions) and the use of the parent untransduced cell strain as control, allowed to approach a high-throughput analysis of transcriptome changes downstream of mutated Gsα, with a statistical power which would not be permitted by natural variability of clinical material and rarity of the disease. This revealed the up- or down regulation of multiple genes specifically involved in excess bone formation, bone resorption, angiogenesis, hematopoietic control, and adipogenesis. Among these, individual genes were identified which could directly explain the emergence of specific histopathological changes.

O14.2
Microvesicles released from microglia stimulate excitatory synaptic activity via enhanced sphingolipid metabolism

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We have recently shown that microglial cells shed microvesicles (MVs) upon activation. To investigate whether microglia-derived MVs affect neurotransmission, we analysed spontaneus excitatory activity (mEPSC) in hippocampal neurons exposed to MVs and found a dose-dependent increase in mEPSC frequency without amplitude changes. Paired-pulse recordings showed that MVs mainly act at the presynaptic site by increasing release probability. Glutamate exocitosis was promoted by enhanced sphingolipid metabolism in neurons. Indeed, MVs stimulated ceramide and sphingosine production, while the enhanced mEPSC frequency MVs-induced was prevented by inhibiting sphingosine synthesis. Interestingly, this pathway controls only the excitatory neurotransmission. In fact, analysis of spontaneous GABAergic activity after MVs treatment showed a decrease in inhibitory miniature postsynaptic current (mIPSC) frequency, that still persisted after blocking the sphingolipid cascade. These data identify microglia-derived MVs as a new mechanism by which microglia influence synaptic activity and highlight the involvement of neuronal sphingosine in the excitatory modulation pathway.

O14.3
Effects of 3,5-Diiodothyronine (3,5-T_2) on lipid accumulation and insulin signaling in a rat model of Non Alcoholic Fatty Liver Disease (NAFLD)

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Non-Alcoholic Fatty Liver Disease (NAFLD) is an emerging pathology, becoming very common in industrialized countries, due to an excess of fats and/or fructose in the diet, together with poor physical activity. Aim of the work is to define an in vitro model of NAFLD to test pathogenic mechanisms and potentially therapeutic molecules. We used adult primary rat hepatocytes after a 24 h load with oleic acid and/or fructose and examined the effects on lipid accumulation and cell viability, and on the activity of insulin signaling pathway. Moreover, we tested the effect of 3,5-Diiodothyronine (3,5-T_2) in preventing and reverting lipid accumulation, and in the re-activation of insulin signaling pathway. Results show that oleic acid stimulates fat accumulation and induces insulin resistance, upregulating the expression of two proteins involved in insulin signaling pathway (p85 regulatory subunit of PI3K and PTEN), and blocking the phosphorylation of Akt at Ser473 and hence its activation, differently from fructose. We also demonstrated that, in our system, 3,5-T_2, in the range of physiological concentrations, is able to prevent and revert lipid accumulation, and to restore Akt activation.

O14.4
The LysM receptor-like kinase AtLYK3 negatively regulates defense responses in Arabidopsis thaliana

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Recognition of pathogens by plant cells is mediated by transmembrane receptor-like kinases (RLKs). We have investigated the role in innate immunity of a family of five Arabidopsis thaliana genes encoding RLKs characterized by the presence of a LysM domain in the extracellular portion (LYK proteins). Using reverse genetics, we have found that one of these genes, AtLYK3, negatively regulates expression of defense genes and resistance to pathogens. The expression of AtLYK3 is strongly repressed by elicitors and fungal infection, while it is induced by the hormone abscisic acid (ABA), previously shown to have a negative role in resistance against some pathogens. Plants lacking a functional AtLYK3 show reduced sensitivity to this hormone, suggesting that AtLYK3 is important for the cross talk between signalling pathways activated by ABA and pathogen.

O14.5
ATM kinase modulates ITCH E3 ubiquitin ligase activity

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Ataxia Telangiectasia Mutated (ATM) kinase is a Ser/Thr kinase that plays a central role in DNA damage response. More recently different proteomics approaches identified ATM as an important modulator of the ubiquitin-proteasome system. Besides ATM controles the activity of several E3 ubiquitin ligases.
We have previously shown that ATM kinase expression and activity may modulate death receptor induced apoptosis, acting on FLIP-L protein stability, a central modulator of death receptor signaling. This findings point to ATM as a novel interplay between the DNA damage response and death receptor induced apoptosis. It has been shown that ITCH E3 ubiquitin ligase is a main regulator of FLIP-L protein stability. This observation along with the identification of ATM as a main modulator of the activity of several E3 ubiquitin ligases in the DNA damage response, leads to the hypothesis that ATM may modulate FLIP-L stability by the direct modulation of ITCH enzymatic activity.
Here we will present data supporting this hypothesis and we will provide in vitro and in vivo genetic evidence for the functional link between ATM and ITCH activities.

15 - Oncogenes and tumour suppressors

P15.1
TRIM8 suppresses cell proliferation by antagonizing ΔNp63α oncogenic activity

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p53 oncosuppressor protein and its relative p63 are central hubs in controlling cell proliferation. We have recently demonstrated that TRIM8, a member of the TRIM family, is a new p53 direct target gene, which, through its RING domain, stabilizes p53 and promotes the degradation of MDM2 leading to cell cycle arrest and reduction of cell proliferation.
Given the fine and very complex interplay between the TA and ΔN isoforms of the p53 family members, we investigated the effect of TRIM8 on the oncogenic ΔNp63α, the main p63 isoform involved in cancer development.
We found that TRIM8 overexpression induces degradation of both endogenous and transfected ΔNp63α in a dose dependent manner, while TRIM8 silencing results in a pronounced accumulation of endogenous ΔNp63α protein levels paralleled by an increase in cell proliferation.
Consistently, the ΔNp63α transactivation on specific p63 target genes, e.g.ADA and CCND3, decreases upon TRIM8 overexpression.
Altogether, our results reveal a previously unknown regulatory pathway controlling p53 and p63 stability and suggest TRIM8 as novel therapeutic target to simultaneously enhance p53 oncosuppression and impair ΔNp63α oncogenic activities.

P15.2
Expression of a dominant negative mutant of the kinetochore protein Hec1 suppresses tumour cell growth in cancer cells and animals

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Mitotic proteins have become attractive targets for the development of molecular cancer therapeutics, as tumor cells are characterized by high mitotic activity. Highly Expressed in Cancer protein 1 (Hec1) is a constituent of the Ndc80 kinetochore complex, which mediates kinetochore-microtubule attachment at mitosis.
Inducible expression of Hec1 N-terminally fused to EGFP acts as a dominant negative mutant promoting chromosome segregation within multipolar spindles (Mattiuzzo et al., PloOne 2011). Compelling evidence highlighted a double role of aneuploidy so that low chromosome instability (CIN) promotes tumorigenesis, whereas high CIN leads to cell death. In this work we explored the idea of Hec1 as a molecular target to produce massive chromosome malsegregation and cell death in cancer cells, as a gene therapy strategy based on an essential mitotic gene. To this aim we expressed EGFP-N-terminally tagged Hec1 in tumor cell cultures and showed that its expression kills those cells more readily that siRNA mediated Hec1 depletion. To validate expression of the modified protein as a therapeutic tool, we demonstrated that EGFP-Hec1 expression inhibits tumor growth in a mouse xenograft model by disrupting mitosis.

P15.3
Mutual c-MYC-dyskerin regulatory loop in human breast cancer cells

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X-linked DC is a multisystemic syndrome caused by mutations in the DKC1 gene and characterized by bone marrow failure, premature aging, telomere shortening and susceptibility to cancer. DKC1 encodes a multifunctional protein, named dyskerin, mainly involved in telomere stability and rRNA maturation and pseudouridylation. To shed more light into the multiple functions played by dyskerin, we silenced DKC1 in MCF7 cells and checked the expression of key regulatory genes involved in proliferation. We observed that, while the amount of c-myc transcript was invaried, in cMYC protein level was significantly reduced in the silenced cells. Accordingly, the levels of three c-MYC induced targets (Cad, Ncl, and Tert) were found significantly reduced. Since DKC1 is a transcriptional target of cMYC, these data indicated the existence of a mutual c-MYC-dyskerin regulatory loop. As c-MYC can be translated from an internal ribosome entry site (IRES) present in its 5' UTR, and dyskerin is known to be required for efficient IRES-dependent translation, we suggest that this effect can account for most of the proliferation defects observed in the high turnover tissues of X-DC patients.

P15.4
The ΔNp63α protein interacts with Y-box binding protein 1 and promotes its ubiquitination

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The p63 protein is a member of p53 transcription factor family. The TP63 gene encodes isoforms that contain (TA) or lack (ΔN) a transactivation domain. The p63 protein plays an essential role for the development of skin and other epidermal derivatives. The ΔNp63α isoform is mainly expressed in the basal layer of the squamous epithelia and is restricted to progenitor cells with proliferative potential. We demonstrate that ΔNp63α can physically and functionally interact with the Y-box-binding protein 1 (YB-1).
YB-1 belongs to the cold-shock domain protein superfamily and performs a wide variety of cellular functions, including transcriptional and translational regulation of proteins involved in the proliferation, survival and cellular differentiation. Inasmuch, as the level of YB-1 drastically increases in tumor cells, this protein is considered to be one of the most indicative markers of malignant tumors. The YB-1 C-terminal region contains a number of distinct sites that can be targeted for post-translational modifications such as phosphorylation, acetylation and ubiquitination. We have observed that expression of ΔNp63α, but not ΔNp63γ, causes accumulation of YB-1 high molecular weight forms in the nuclear compartment and we provide evidence that these are ubiquitinated YB-1 forms. The functional role of YB-1 ubiquitination by DNp63a is currently under investigation.

P15.5
p14ARF, TBP-1 (Tat Binding Protein 1) and Mdm2 crosstalk in the regulation of cell proliferation

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We recently demonstrated that TBP-1, previously described as a regulator of p14ARF, is involved in the control of cell proliferation also independently from p14ARF. In particular, overexpression of TBP-1

diminishes cell proliferation while its stable knock-down increases cell proliferation, migration and resistance to apoptosis. These effects involve the activation of the Akt/PKB signalling, indicating TBP-1 as an upstream regulator of Akt activation. Furthermore, TBP-1, itself, is a downstream target of Akt/PKB suggesting the existence of a negative feedback loop. Interestingly, MDM2, one of the main direct targets of Akt activation, plays a major role in this regulation, likely placing TBP-1 downstream of the Akt-MDM2 axis. The specific mechanism for MDM2-dependent depression of TBP-1 levels remains to be understood. However, it has to be noted that MDM2 has multifaceted roles in protein degradation beside its well-described role as E3-ubiquitin ligase. On the other hand, it has very recently been reported that MDM2 interacts with components of the 19S proteasome in a ubiquitylation independent manner claiming a wider view of its mechanism of action. In this scenario, we surprisingly found that p14ARF intracellular levels can also be modulated by Mdm2 overexpression, with a yet undefined mechanism that doesn't involve the ubiquitin ligase activity of Mdm2. We are currently investigating on the potential cross talks among Mdm2 action on TBP-1 and p14ARF.

P15.6
Plant secondary metabolites can modulate p53 transactivation potential, as revealed by a miniaturized luciferase assay

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The master regulatory network of p53 is composed of a large number of genes that are direct targets of p53-mediated transactivation. Essential for the transcriptional regulation by p53 is the target response element sequence. Many factors can influence p53 activity including protein levels, levels of cofactors and stress-dependent post-translational changes affecting p53 protein interactions. The aim of this study was to test several plant secondary metabolites alone or in combination with anticancer drugs for their potential to modulate p53 transactivation potential. These experiments were conducted using a previously developed luciferase assay that exploits variable expression of p53 in Saccharomyces cerevisiae and then confirmed in human cells. We tested a panel of saponins, Astraverrucins I, III, VI, and Cycloaralaoside D from Astragalus verrucosus. Our results indicated that these compounds seem to have an inhibitory effect on p53-dependent transactivation. When tested in combination with Doxorubicin or Mitomycin C, saponins led to an increased inhibitory effect on p53 transactivation in yeast, and counteracted p53-dependent activation of reporter genes also in MCF7 cell line.

P15.7
Anti-human ErbB3 mAbs and their combination with the BRAF inhibitor (PLX4032) strongly inhibit human melanoma cells growth

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Melanoma is one of the most aggressive forms of cancer and new therapeutic approaches are highly needed. Approximately 50% of melanomas bear mutations of the BRAF oncogene and this has led to the clinical development and recent approval of the BRAF inhibitor PLX4032 for the therapy of melanoma. Emerging evidences have pointed to the ErbB3 receptor as a key node in the activation of proliferation/survival pathways of melanoma cells. We previously demonstrated that anti-human ErbB3 monoclonal antibodies A3 and A4 inhibit melanoma cells growth and migration and strongly affect receptor internalization, recycling and degradation. Here we tested the combination of our mAbs among themselves and with PLX4032. Proliferation assays demonstrated that A4 mAb strongly synergize with PLX4032 in reducing melanoma cells growth. Moreover, biochemical and immunofluorescence approaches demonstrated that A3/A4 combination induces a surprisingly fast receptor internalization and durable degradation.. These findings pave the way to further studies aimed at identifying the molecular mechanisms responsible for mAb-induced ErbB3 intracellular trafficking and degradation and at exploring this new antibody-small molecule combination for the therapy of melanoma.

P15.8
CLU isoforms expression in human thyroid tumour

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Clusterin (CLU) is an ubiquitous multifunctional factor synthesized in functionally divergent isoforms: sCLU and nCLU play a crucial role probably by keeping a balance between cell proliferation and cell death; a third isoform, denoted as 11036, was predicted by ASPicDB and experimentally observed in colon cancer, but its functional role remains unclear.
By means of an in vivo model we studied CLU expression in normal and diseased thyroid tissues. Immunohistochemical analyses showed that CLU is up-regulated in papillary carcinoma in comparison to the follicular adenoma. We investigated also by RT-qPCR the differential expression of the three CLU isoforms. In the papillary carcinoma, a specific increase of the sCLU isoform was demonstrated in comparison to a decrease of the nCLU and 11036 isoform.
Our results suggest the existence of a differential CLU gene expression during the progression from normal to malignant cell and a specific alteration of the sCLU:nCLU ratio towards sCLU. Finally, we provided the first circumstantial evidence for the potential use of CLU isoforms as effective biomarkers for thyroid cancer.

P15.9
MicroRNA regulation by mutant p53 oncoprotein

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Deregulated miRNA expression has been documented in diverse cancers, but the mechanisms through which this occurs remain unclear. Tumor suppressor p53, mutated in approximately 50% of human cancers, can acquire GOF activities favouring tumor induction, maintenance, spreading. wtp53 interaction with Drosha complex facilitates the processing of pri-miRNAs to pre-miRNAs, while an overexpressed mutp53 disrupted p68/Drosha interaction, suggesting that mutp53 might be responsible for miRNA downregulation present in cancer.
To identify new mechanisms underlying mutp53 dependent dysregulation of microRNA in cancer, we performed a genome wide analysis of miRNA expression in colorectal adenocarcinoma SW480 before and after mutp53 depletion. Our first results reveal that mutp53 depletion is associated with up-regulation of 34 mature miRNAs and down-regulation of only 3. Validation of genome wide miRNA expression profile for mature miRNAs and pri-miRNAs shows that mutp53 plays a role both at transcriptional and posttranscriptional level.
These results suggest a role for mutp53 in the down-regulation of miRNA expression in cancer.

P15.10
HIPK2 splicing variants: a differential role in tumorigenesis?

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HIPK2 is an evolutionary conserved kinase involved in the regulation of various biological function including development, proliferation and apoptosis. HIPK2 activity is regulated by several post-translational modification such as phosphorylation, ubiquitylation and sumoylation, as well as by the interaction with specific cofactors like axin, HMGA1 and PML. In contrast, relatively little is known about HIPK2 post-transcriptional regulation; thus, we focused our attention on alternative splicing. *In silico* analysis showed that at least three different mRNAs are transcribed from the *HIPK2* gene. Besides the full length mRNA, there are two shorter isoforms. One encodes a protein lacking part of the homeodomain-interacting domain, and the other one a protein with its C-terminal region truncated near the site of caspase cleavage and therefore potentially more active and non-ubiquitylatable. RT-PCR experiments confirmed the presence of the three isoforms in different cell types. Moreover, preliminary results obtained by WB suggest that in non-tumor cells the longest isoform is mainly cytoplasmic while a shorter one is nuclear, whereas this specific distribution is lost in tumor cells.

P15.11
Itch-dependent nondegradative ubiquitination of Sufu controls Hedgehog pathway

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Hedgehog (Hh) pathway triggers an intracellular signal cascade leading to activation of the Gli family transcription factors. Gli proteins display both a positive transcriptional function but can also be converted into a cleaved form provided of transcriptional repressor activity. This process is finely tuned by SuFu that, in absence of signaling, interacts and protects Gli3 from degradation and promotes its conversion into a repressor form (Gli3R). We show here that this key event is regulated by Itch, an HECT E3 ubiquitin ligase involved on major biological processes. Itch binds SuFu and promotes its ubiquitination on specific lysines. Of relevance, we find that SuFu polyubiquitination occurs through K63 ubiquitin linkages without affecting its stability, characterizing a regulatory rather than a degradative pathway. In this regard, we observe that this process increases the binding of Sufu to Gli3, promoting its conversion into Gli3R and keeping the Hh pathway off. Indeed, activation of Hh signaling antagonizes the Itch-triggered ubiquitination of Sufu. Our findings suggest that this post-synthetic modification plays an important role in the negative control of Hh signaling.

P15.12
Human RNASET2 as a candidate stress-response gene involved in non-cell autonomous tumor suppression

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The process of cancer development is usually characterized by significant alterations in both the intracellular and extracellular environments. Indeed, the ability to promptly recognize such aberrant changes and to orchestrate a proper response to them provides a powerful defense mechanism against cancer in metazoans. This is particularly evident when the role of several tumor suppressor genes is considered, since a high proportion of them displays biological functions clearly related to stress response. We have recently reported an impressive non-cell autonomous tumor suppressive role for the human RNASET2 gene. In the context of in vivo murine xenograft models, this gene apparently acts by evoking a strong innate immune response against human ovarian cancer cells. This observation raised the question of whether RNASET2 might work as a stress response gene whose role is to detect a pre-cancerous state and to send a proper warning message to cells of the innate immune system. We investigated this issue by means of several in vitro cell models and we provide here preliminary results which are clearly compatible with a role for human RNASET2 as a wide-range stress response gene.

P15.13
PCAF ubiquitin ligase activity inhibits Hedhehog signaling in response to genotoxic stress via p53

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The Hedgehog (Hh) signaling regulates development and cell proliferation. Gli1 transcription factor is the final effector of the pathway and its deregulation leads to tumors formation, including medulloblastoma. Proteolytic processes are the major mechanism by which this pathway is turned off. Here, we identify the role of PCAF to limit Hh signaling in response to DNA damage. We show that the intrinsic E3-ubiquitin ligase activity of PCAF controls the stability of Gli1 by promoting its ubiquitination and proteasome-dependent degradation. We observed that genotoxic stress suppresses Hh/Gli signaling and that a p53-activated PCAF is required for this event. Indeed, DNA damage agents failed to reduce Gli1 levels both in a p53-deficient background and after depletion of PCAF. Mechanistically, DNA damage enhances p53 levels and function, such an increase of p53 transcriptionally upregulates PCAF that in turn degrades Gli1. Our data highlight a p53/PCAF/Gli1 circuitry centered on PCAF that, in a p53-dependent manner, limits Hh activation and DNA damage response abrogation, thus reducing genomic instability e restoring sensitivity to radiotherapy and chemotherapy.

P15.14
Study of p53 localization at the centrosomes: comparison between human and mouse cells

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Centrosomes play critical role in formation of bipolar mitotic spindles. In human mononucleated lymphoid cells, the p53 oncosuppressor moves towards the centrosomes in mitosis, by a microtubule-mediated transport. ATM, an activator of p53 in response to DNA damage, is also required for p53 centrosomal localization and transient phosphorylation of p53 at Ser15 is a critical step. Indeed, when ATM is not functional (i.e., in Ataxia Telangiectasia - AT), p53 fails to localize at centrosomes in mitosis. To investigate the molecular mechanisms of p53 localization at the centrosomes and the contribution of the ATM/p53-dependent centrosomal defect in tumorigenesis, we thought to get clue from the *Atm* KO mouse model that shows many similarities with the human, AT phenotype. To this aim, we first characterized the p53 centrosomal behavior in mouse cells. We found that p53 localizes at the centrosomes in interphase through a microtubule-independent mechanism. Furthermore, in mouse cells p53 is phosphorylated at Ser18 but this phosphorylation take place also upon inactivation of Atm. These results suggest that p53 centrosomal localization might play different function in human and mice.

P15.15
Mimicking p14ARF phosphorylation influences its ability to restrain cell proliferation

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The INK4a/ARF locus on the short arm of chromosome 9 is one of the most frequently altered loci in human cancer. It is generally accepted that ARF is involved in oncogenic checkpoint pathways by sensitizing

incipient cancer cells to undergo growth arrest or apoptosis through both p53-dependent and independent pathways. While ARF activation at the transcriptional level has been the focus of intensive studies, only recently have mechanisms governing ARF turnover been identified. Here, we show for the first time that p14ARF is a PKC target. Prediction analysis showed many potential phosphorylation sites in PKC consensus sequences within ARF protein, and, among them, the threonine at position 8 was the most conserved. Substitution of this threonine influences both ARF stability and localization. Furthermore a phosphomimetic ARF mutant reduces the ability to arrest cell growth although it still retains the ability to bind MDM2 and stabilize p53. Thus we propose that phosphorylation of ARF in both immortalized and tumor cell lines can be a mechanism to escape ARF surveillance following proliferative and oncogenic stress.

P15.16
microRNA-214 controls melanoma progression via the coordination of a novel pathway involving TFAP2 and ALCAM

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Malignant melanoma is fatal in its metastatic stage. It is therefore essential to unravel the molecular mechanisms that govern disease progression to metastasis. MicroRNAs (miRNAs) are endogenous non-coding RNAs proven to control tumor progression. Using a melanoma progression model, we identified a novel pathway controlled by miR-214 that coordinates metastatic capability. Pathway components include protein-coding genes, such as TFAP2C, homologue of a well-established melanoma tumour suppressor, the adhesion receptor ITGA3 and multiple surface molecules such as ALCAM, CD9, ADAM9 as well as specific small non-coding RNAs. Modulation of miR-214 significantly alters tumor cell migration, invasion and survival to anoikis, as well as extravasation from blood vessels and metastasis formation in mice. Considering that miR-214 is highly expressed in invasive human melanomas, our data suggest a critical role for this miRNA in disease progression and the establishment of distant metastases. A detailed analysis of the molecular mechanisms used by miR-214 to control metastatic dissemination will be presented.

P15.17
The p63 protein as a regulator of Y-box binding protein 1 subcellular distribution and functions

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Y-box-binding protein 1 (YB-1) belongs to the cold-shock domain protein superfamily, one of the most evolutionary conserved nucleic acid-binding protein currently known. YB-1 performs a wide variety of cellular functions, including transcriptional and translational regulation, DNA repair, drug resistance and stress responses. Inasmuch, as the level of YB-1 drastically increases in tumor cells, this protein is considered to be one of the most indicative markers of malignant tumors. Here, we present evidence that ΔNp63α, the predominant p63 protein isoform in squamous epithelia and YB-1 can physically interact.ΔNp63α promotes YB-1 nuclear accumulation thereby reducing the amount of YB-1 protein bound to transcripts encoding Snail1, a potent inducer of epithelial-to-mesenchymal transition (EMT). Moreover,ΔNp63α cooperates with YB-1 in the transcriptional activation of PI3KCA, the gene encoding the catalytic subunit of PI3 kinase.ΔNp63α expression reduces tumor cell motility and Snail 1 protein level while increasing E-cadherin. This study identify YB-1 and ΔNp63α as novel interacting partners and provide evidence for the involvement of p63 in controlling cell motility.

P15.18
TRIM8 up-regulation restores p53 tumor suppressor activity in renal cell carcinoma

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In some tumors, despite a wild-type p53 gene, the p53 pathway is inactivated by alterations in its regulators or unknown mechanisms, leading to resistance to cytotoxic therapies.
Recently, we demonstrated that TRIM8 is a new p53 direct target gene. TRIM8 stabilizes p53 impairing its association with MDM2 and inducing cell cycle arrest and reduction of cell proliferation. Moreover, we found that TRIM8 silencing impaired p53 stabilization and MDM2 degradation after U.V. irradiation.
Since clear cell renal cell carcinoma (ccRCC) is an aggressive drug-resistant cancer showing rare p53 mutations, we wondered what was TRIM8 expression level in this cancer.
We found that TRIM8 is down-regulated ccRCC. Therefore, we evaluated the effects of restoring TRIM8 expression in an RCC cell line. We found that Nutlin-3 and Cisplatin treatments resulted more effective in terms of reduction of cell proliferation and p53 pathway activation when TRIM8 is over-expressed.
Therefore, we suggest TRIM8 as a new target for therapeutic intervention in cancers (e.g., ccRCC) where p53 is wild-type and its pathway is defective.

O15.1
Hedgehog signaling controls IRES-dependent translation through a CNBP/Sufu complex

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The Hedgehog morphogen is critical for development, stem/progenitor cell fate and tumorigenesis. The response to Hedgehog ligands is regulated by an interplay between the Gli transcription factors and Suppressor of Fused (Sufu), which finely tunes the transcriptional output. Here we show that Hedgehog regulates IRES-dependent translation independently of

Gli, through a complex formed by Sufu and the RNA-binding protein CNBP. Following Hedgehog activation, CNBP is recruited to 5'UTRs of selected target mRNAs and, in complex with Sufu, promotes their IRES-dependent translation. Sufu binds and protects CNBP from degradation and increases its binding affinity to target mRNAs. Consistent with the developmental and tumorigenic role of Hedgehog, CNBP is upregulated in cerebellar stem cells, medulloblastomas and tumor stem cells, where it mediates selfrenewal and cancer growth. Furthermore, this mechanism is also conserved in Drosophila, where CNBP directs wing development. Collectively we reveal a novel mechanism involved in morphogen-dependent physiologic and pathologic processes.

O15.2
Oncogenic properties of the MEF2-HDAC4 axis

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The role of class IIa HDACs in cancer development is unclear and debated. Here we document for the first time the oncogenic potential of HDAC4 in the NIH-3T3 lineage. Nuclear retention of HDAC4 induces morphological alterations of murine fibroblasts, which acquire a spindle-like shape and lose focal adhesions. Moreover fibroblasts overexpressing HDAC4 display a proliferative advantage and the capability to grow in soft agar and form tumors when injected in nude mice. By microarray experiments we identify 49 genes repressed by HDAC4. Several MEF2 targets belong to this signature and an ER-inducible MEF2 reverts the transformation induced by HDAC4. GSEA analysis unveils that genes repressed by the PI3K/Akt/mTOR pathway are also under the influence of HDAC4. We experimentally prove that the PI3K/Akt/mTOR pathway control the activity of MEF2 transcription factors. In conclusion, our data demonstrate the oncogenic properties coming from MEF2 repression in fibroblasts and that both HDAC4 and PI3K negatively modulate MEF2 through different but additive mechanisms.

O15.3
Role of src dependent phosphorylation on tyr380 of caspase 8 in tumorigenesis.

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Caspase8 (C-8) is required for apoptotic response triggered by death receptors. Resistance to apoptosis contributes significantly to carcinogenesis. However C-8 is rarely deleted or silenced in some tumors implying a potential pro-tumorigenic role and pointing to the presence of alternative mechanisms that may trigger the down-regulation of C-8 activity. We identified a novel pathway by which non receptor tyrosine kinase Src phosphorylates C-8 on Tyr380 impairing its activation therefore protecting cells from apoptosis. Moreover, we and others, demonstrated that C-8 expression and its phosphorylation have a role in cell adhesion and migration suggesting the hypothesis of a pro-tumorigenic function of C-8. To further elucidated this issue we developed a colorectal carcinoma cell line based system that costitutively express active Src, inducible for the expression of either C-8 wt or its unphosphorylable mutant, C-8-Y380F. Here we will present evidence for a role of C-8 expression and of its phosphorylation in the resistance to cell death triggered by loss of cell adhesion (anoikis) and in the anchorage independent growth as mechanism to promote tumor progression.

O15.4
The microRNA-26a target E2F7 sustains cell proliferation and inhibits monocytic differentiation of acute myeloid leukemia cells through control of p21$^{CIP1/WAF1}$ expression

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Acute Myeloid Leukemia (AML) is a heterogeneous disorder caused by malignant transformation of bone marrow-derived cells, which results in enhanced proliferation as well as aberrant differentiation arrest. Small non-coding RNAs together with transcription factors affect fundamental molecular pathways that control survival and proliferation of blood cells and are aberrantly expressed in almost every type of leukemia. Vitamin D3 arrests proliferation of AML cells and induces their differentiation into mature monocytes. This mechanism is mediated by the upregulation of the cyclin-dependent kinase inhibitor p21. We demonstrated that miR-26a contributed to the increased expression of p21 by inhibiting the transcriptional repressor E2F7. Upon silencing of E2F7, we observed a marked induction of monocyte/macrophage differentiation and arrest of proliferation. We demonstrated that E2F7 directly binds p21 promoter repressing its expression in proliferating AML. Finally, we showed that the expression of E2F7 is upregulated in primary blasts from AML patients, while miR-26 is expressed at low levels, compared to healthy monocytes. These findings indicate that the newly identified miR-26a target E2F7, might have an important role in monocytic differentiation and leukemogenesis.

O15.5
HIPK2 in the control of genome stability: a new mechanism in tumorigenesis

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Failure in cytokinesis provokes genetically unstable states, such as tetraploidization and multinucleation, that are among the hallmarks of cancer.
We have recently demonstrated that HIPK2, an oncosuppressor involved in cell proliferation and apoptosis controls cytokinesis and prevents tetraploidization. This HIPK2 unexpected function highlights the possibility that its oncosupressor activity is not only linked to its pro-apoptotic function, but also to cytokinesis control.
We are analyzing the roles played by HIPK2 in the induction of chromosomal instability (CIN) and in tumorigenesis, by studying predisposition to CIN and cancer in vitro and in vivo using murine models.The transforming interactions between endogenous HIPK2 and transfected oncogenes were analyzed by using Hipk2-/-, +/- and +/+ mouse embryonic fibroblasts (MEF), stably expressing both activated ras and E1A oncogenes (r/E1a).
Interestingly, we found that Hipk2 -/- r/E1a MEF show an higher clonogenicity and predisposition to CIN than Hipk2 +/+ r/E1a MEF. Further analysis on the in vivo tumorigenicity of these MEF will be also presented.

16 - Stem cells, IPS, cancer stem cells

P16.1
TrkB and neuroptrophins in primary lung cancer cultures

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Lung cancer represents the leading cause of cancer-related mortality worldwide, that tumors contain a small population of cancer stem cells that are responsible for tumor maintenance and spreading.Neurotrophins are growth factor molecules that regulate the biology of embryonic stem cells and cancer cells.We investigate the role of the NTs and their receptors using as study system primary cell cultures, derived from malignant pleural effusions of patients with adenocarcinoma of the lung. The primary cell cultures, was able to growth both as differentiated cells in adherence and as non-adherent floating spheroids.We assessed the expression of these receptors resulting in higher TrkB expression in spheroid cultures.The effect of K252a, a inhibitor of the activity of the Trk family, induced apoptosis to a greater extent in spheroids compared to adherent cells.Interestingly, treatment with NTs reverted the inhibitory effect of k252a in maintenance and formation of spheroids.We observed that TrkB is involved in AKT pathway activation in our primary cell cultures, thus suggesting that TrkB have a role in tumor progression and strongly supporting target for the therapy of lung cancer.

P16.2
Isolation of murine undifferentiated spermatogonia subpopulations: stem cell potential and gene expression profiling

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Spermatogenesis is a cellular process that depends on the self-renewal and differentiation of a poorly represented population of spermatogonial stem cells (SSC), called spermatogonial type As. The knowledge of the biology of SSC is fundamental for the treatment of male infertility and for tissue regeneration. Based on the expression of GFRA1, the co-receptor GDNF secreted by Sertoli cells is essential for the proliferation of the SSC, in my laboratory has been recently highlighted a phenotypic and functional heterogeneity of spermatogonial stem cells. In order to characterize the SSC GFRA1 positive, was generated a transgenic mouse expressing the fusion protein GFRA1-EGFP. Using this animal model has been verified the ability to isolate subsets of undifferentiated spermatogonial expressing the fusion protein at different levels of intensity. The innovative aspect is to analyze the different patterns of gene expression and the migration potential of the subpopulations isolated from the transgenic mice, for the purposes of a better understanding of the process of regeneration.

P16.3
The Drosophila minifly (mfl) gene is essential for the formation of intestinal stem cells

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The Drosophila mfl gene belongs to a highly conserved family whose members play essential functions, including ribosome biogenesis and pseudouridylation of target RNAs. Mutations in its human ortholog, the h-DKC1 gene, have been associated with the dyskeratosis congenita X-Linked, a multisystemic syndrome accompanied by telomerase defects, premature aging and stem cell dysfunction. Since Drosophila lacks telomerase, we investigated by in vivo RNAi the effect of mfl silencing on the formation of larval intestinal stem cells (called AMPs), which are emerging as an ideal model system for the study of epithelial stem cells. Intriguingly, both ubiquitous and localised gene silencing strongly affected the formation of the larval imaginal islands, the typical structures in which the AMPs are organized, and only very rare and dispersed cells expressing the esg stemness marker were occasionally detected. Moreover, AMP asymmetric division is affected. Hence, mfl is essential for AMP formation, and its expression is specifically required within imaginal islands. DKC1 roles on stem cell formation/maintenance thus appear to be evolutively conserved, and are likely to be telomerase-independent.

P16.4
Malignant pleural effusion as model to assess sensitivity to chemodrugs

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With the aim to find a model to predict drug sensitivity we set-up an in vitro model based on malignant pleural effusions (MPE) from lung cancer. We have optimized isolation procedures and culture conditions to expand in vitro primary cultures from Malignant Pleural Effusions (MPEs) of patients affected by lung adenocarcinomas. By this method we have been able to establish primary cultures from malignant pleural effusions both in adherent and/or in spheroid conditions in almost all cases analyzed. After low pasage banking, cells are immediately characterized for sensitivity to drugs used in standard chemotherapy regimen and analyzed for the expression of cancer stem cells markers and other markers relevant for this type of cancer. In vitro data have shown large variability among primary cultures probably reflecting the interpatient variability. In addition isolated tumor cells were able to give rise to tumors when xenografted in immunodeficient mice. We are currently collecting data from in vitro proliferation assay and looking for a correlation to patient response to validate this as a system potentially useful to predict drug efficacy using patient-derived primary cultures.

P16.5
Membrane complement regulatory proteins and prostate cancer

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Prostate cancer is the most frequently diagnosed cancer in men. Recent reports about cancer stem cells have prompted questions regarding the involvement of normal stem/progenitor cells in prostate tumor biology, their potential contribution to the tumor itself and whether they are the cause of tumor initiation and its progression and even metastatic diffusion.
Our attention has been focused on the study of prostate cancer stem cells and their potential ability to escape complement attack by expressing at very high levels membrane complement regulatory proteins (mCRPs): CD46, CD55, CD59. The expression of mCRPs is proposed to protect cancer cells from complement-depend cytotoxicity, especially when accessing the blood circulation during growth and metastasis. In addition we have studied the role of pseudogenes as competing endogenous RNA (ceRNA) in the regulation of mCRPs expression.
Four prostate cancer cell lines have been studied by flow cytometry and all were found positive for mCRPs molecules but with different intensity.

Prediction of the presence of a pseudogene that may act as regulatory element for mCRPs expression is also presented.

O16.1
Autologous progenitor cells in a hydrogel form a supernumerary and functional skeletal muscle in vivo

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Extensive loss of skeletal muscle tissue results in incurable mutilations and severe loss of function. In vitro generated artificial muscles undergo necrosis when transplanted in vivo before host angiogenesis may provide the amount of O2 required for muscle fibre survival. Skeletal muscle tissue engineering has met with limited success, due to the complex tissue architecture and the presence of a dense microvascular network without which muscle fibers do not survive once implanted in vivo. Here we report a novel strategy exploiting the good survival and differentiation of mouse mesoangioblasts in a recently discovered biomaterial, PEG-Fibrinogen, and their ability, once engineered to express Placenta derived Growth Factor and embedded in this material, to attract host vessels and nerves while myotubes begin to form. Mesoangioblasts, embedded into PEG-Fibrinogen hydrogel, generate an additional muscle on the surface of the Tibialis Anterior. This strategy opens the possibility of in vivo autologous muscle creation for a large number of pathological conditions.

O16.2
Generation of patient-specific iPS cells to provide an in vitro model system of Amyotrophic Lateral Sclerosis (ALS)

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Amyotrophic Lateral Sclerosis (ALS) is a severe neurodegenerative disorder due to the loss of motor neurons. A subset of familial ALS cases is linked to mutations in the FUS/TLS and TARDP genes, encoding for putative regulators of miRNA biogenesis. In the nervous system, deregulation of miRNAs correlates with the initiation and progression of several neurological disorders. Our general aim is to study the possible involvement of miRNAs in ALS pathogenesis.
The molecular analysis of ALS pathogenesis is hampered by the lack of suitable cellular model systems. Human somatic cells can be now reprogrammed to embryonic-like induced pluripotent stem cells (iPSCs), which can be then differentiated in vitro in all adult tissues. As iPSCs can be also generated from somatic cells derived from patients, they provide a unique opportunity for disease modeling in vitro. Our approach consists in the generation of iPSCs from fibroblasts of ALS patients and their differentiation into motor neurons. We have established a collection of ALS-iPSCs covering several FUS/TLS and TARDP mutations, which can be exploited for the analysis of possible alteration of RNA metabolism in ALS motor neurons.

O16.3
miR-125a regulates mouse ESC differentiation

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Mechanisms governing embryonic stem cell (ESC) differentiation are still not completely understood. BMP4 plays an important role in maintaining ESCs in the undifferentiated state and in the regulation of lineage commitment of epiblast stem cells (epiSCs). We recently identified a transmembrane protein, named Dies1, whose suppression by RNAi interferes with mouse ESC differentiation, by downregulating the BMP4 signalling. We asked whether modulation of Dies1 levels could be a physiologic mechanism to regulate ESC pluripotency and/or differentiation. We found that miR-125a targets Dies1 mRNA and regulates its translation in ESCs. The overexpression of miR-125a impairs differentiation and this effect is specifically mediated by Dies1 downregulation and accompanied by a decrease of BMP4 signalling. We also found that BMP4 activates the transcription of miR-125a gene. Therefore, a feedback loop exists which sets ESC sensitivity to BMP4. The analysis of this regulatory mechanism revealed that miR-125a overexpression and the consequent inhibition of the BMP4 signalling arrest the cell differentiation in the epiSC stage. This phenomenon was mimicked by the exposure of the cells to a BMP4 inhibitor. Experimental evidence indicates that the downregulation of BMP4 signaling sustains the epiSC-like phenotype due to the concomitant activation of the Nodal/Activin pathway.

O16.4
Otx2 is an intrinsic determinant of the Embryonic Stem Cell state and is required for differentiation to a stable Epiblast Stem Cell condition

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Mouse embryonic stem cells (ESCs) represent the naïve ground state of pre-implantation epiblast and epiblast stem cells (EpiSCs) the primed state of post-implantation epiblast. Relevant studies revealed that the ESC state is maintained by a dynamic mechanism characterized by cell-to-cell spontaneous and reversible differences in sensitivity to self-renewal and susceptibility to differentiation. This metastable condition ensures indefinite self-renewal and, contemporary, predisposes ESCs for differentiation to EpiSCs. Here we show that Otx2, a transcription factor essential for brain development, plays a crucial role in ESCs and EpiSCs. We found that Otx2 is required to maintain the ESC metastable state by antagonizing ground state pluripotency and promoting commitment to differentiation. In addition, Otx2 is required for ESC transition into EpiSCs and, subsequently, to stabilize the EpiSC state through suppression of neural fate in cooperation with BMP4 and Fgf2. We propose that Otx2 is a novel intrinsic determinant controlling the functional integrity of ESCs and EpiSCs.

O16.5
The Numb/p53 pathway controls mode of division and tumorigenic potential of normal and tumor mammary stem cells

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We characterize the role of Numb in the homeostasis of the normal mammary gland, where Numb is known to be asymmetrically distributed at mitosis of normal mammary SC (NMSC), and to exert a key role as a potent tumor suppressor. We provide evidence that, upon NMSC division, Numb is asymmetrically partitioned in the progeny that retains the SC identity. In compromising Numb function, we uncover profound defects in the behavior of NMSC, which shift from an asymmetric to a symmetric mode of self-renewal division and show a higher proliferative rate, with an ensuing increase in the SC number. Mechanistically, we implicate the Numb-p53 axis as a master regulator of ACD in either

normal and tumor MSC. We also unmask a link between loss of Numb, expansion of the SC compartment, aberrant mammary morphogenesis and increased frequency of mammary tumors. These tumors are characterized by the presence of cancer SC (CSC) with unlimited self-renewal potential. Pharmacological restoration of p53 by treatment with the Mdm2 inhibitor Nutlin, or lentivirally enforced Numb expression dramatically affect tumor growth by decreasing the frequency of CSC symmetric divisions, and therefore ultimately inhibiting their unlimited replicative potential.

17 - Host-pathogen interaction

P17.1
Structural resolution of the complexes formed by the polygalacturonase inhibiting protein 2 from Phaseolus vulgaris and three different fungal polygalacturonases by Small Angle X-Ray Scattering (SAXS)

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Polygalacturonases (PGs) are among the first enzymes produced by phytopathogenic fungi during infections and degrade homogalacturonan, a component of pectin that acts as a cohesive element for the entire cell wall structure. To accommodate pathogenesis in a variety of conditions and on various hosts, many PG isoenzymes are produced by pathogens. Against these PGs, plants have evolved many polygalacturonase inhibiting proteins (PGIPs) that inhibit PGs and slow down the fungal infection by favouring the accumulation of oligogalacturonides, endogenous inducers of the plant defences. Although the crystallographic structure of several PGs and of PGIP2 from Phaseolus vulgaris (PvPGIP2) are already available, the structure of the PG-PGIP complex still remains unresolved. We report here a comparative analysis of the low resolution structures of the complexes formed by PvPGIP2 and the PGs produced by three different phytopathogenic fungi. This study highlights residues involved in the interaction of different PG-PGIP combinations, and paves the way to the design of artificial PGIPs with improved inhibition capabilities.

P17.2
A functional genomics approach to establish the complement of carbohydrate transporters in Streptococcus pneumonia

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The aerotolerant anaerobe Streptococcus pneumoniae is part of the normal nasopharyngeal microbiota of humans and one of the most important invasive pathogens. A genomic survey allowed establishing the occurrence of 21 PTS systems, 7 carbohydrate uptake ABC transporters, 1 sodium:solute symporter and a permease. Despite high genomic variability, combined phenotypic and genomic analysis of 20 sequenced strains did assign the substrate specificity only to two uptake systems. Systematic analysis of mutants for most carbohydrate transporters enabled us to assign a phenotype and substrate specificity to twenty-three transport systems. For five putative transporters for galactose, pentoses, ribonucleosides and sulphated glycans activity was inferred, but not experimentally confirmed and only one transport system remains with an unknown substrate and lack of any functional annotation. Using a metabolic approach, 80% of the thirty-two fermentable carbon substrates were assigned to the corresponding transporter. The present work permits to draw a functional map of the complete arsenal of carbohydrate utilisation proteins of pneumococci, allows re-annotation of genomic data and might serve as a reference for related species. These data provide tools for specific investigation of the roles of the different carbon substrates on pneumococcal physiology in the host.

P17.3
Identification and functional characterization of genes involved in the pathogenesis of *Clostridium difficile*

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Clostridium difficile, a Gram-positive, spore forming, anaerobic bacterium is the leading cause of infectious diarrhoea in hospitals worldwide. Enteric infections caused by *C. difficile* have been associated with antibiotic treatment and disruption of the normal gastrointestinal flora.
The cell surface of *C. difficile* is poorly characterized. The most widely studied components are the proteins that constitute the S-layer, a paracrystalline array surrounding the cell. Few other putative cell wall proteins (CWPs) have been reported, however their functions remain unclear. Characterization of the bacterial surface can be done using a 'surfome' analysis, which involves surface proteolysis of intact cells and identification of released peptides by mass spectrometry. The secreted proteins (secretome) can also be identified, by performing similar analysis on bacterial culture supernatants. Trying both approaches, we were able to identify putative surface and secreted proteins. Among these, some proteins of unknown function were selected for further characterization, in an attempt to understand their role in *C. difficile* pathogenesis.

P17.4
Epitope mapping of a cross-reactive monoclonal antibody to meningococcal factor H-binding protein

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Meningococcal factor H-binding protein (fHbp) is a surface-exposed lipoprotein that binds human factor H (fH), enabling down-regulation of complement activation on the bacterial surface. Binding of fH leads to bacterial evasion of host-mediated immunity. The protein has been divided into 3 variant groups or 2 sub-families. A panel of anti- fHbp mAbs has been produced from mice immunized with the 3 variants of fHbp and their epitopes were previously mapped, except for the mAb designated JAR36, a murine IgG mAb isolated from a mouse immunized with variant 3. We now report epitope mapping of JAR36, this mAb cross-reacts with all fHbp sequences in V.2 and V.3 groups, binds to the bacterial surface and elicits complement-mediated bactericidal activity in combinations with other anti-fHbp mAbs. We screened bacteriophage-displayed random peptide libraries to identify amino acid residues contributing to the JAR36 epitope. Mapping predictions were validated by constructing, through site-specific mutagenesis, corresponding rfHbps single-point variants, and analyzing their reactivity with the mAb.

P17.5
The xylanase inhibitor Taxi-III is involved in wheat resistance against Fusarium graminearum

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Cereals contain xylanase inhibitor proteins (XIs) which inhibit

microbial xylanases. These inhibitors are considered part of the defence mechanisms that plants use to counteract microbial pathogens. Nevertheless, in planta evidences for this role have not been reported yet. Therefore, we produced a number of wheat transgenic plants over-expressing constitutively TAXI-III. Results show that TAXI-III endows the transgenic wheats with new inhibition capacity. These plants showed a significant reduction of disease symptoms caused by Fusarium graminearum but do not show any significant reduction of leaf spots caused by Bipolaris sorokiniana. Possible differences on the efficacy of TAXI-III to inhibit specific xylanases produced by these pathogens during host infection or the presence of additional factors conditioning host infection at floral and leaf tissues, respectively, can be responsible for this different outcome. In conclusion, our results provide for the first time a clear evidence in planta that XIs are involved in plant defence and show the possibility to manipulate TAXI-III accumulation to improve wheat resistance against F. graminearum.

P17.6
A *Fusarium graminearum* endo-xylanase expressed during wheat infection is a necrotizing factor

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Fusarium graminearum is the causal agent of Fusarium head blight of wheat. During the infection process, this fungus secretes a large number of hydrolytic enzymes, some of them able to degrade xylans, main constituents of monocot cell walls. In particular, endo-ß-1,4-xylanases hydrolyze the inner ß-1,4 glycosidic bond and could be important pathogenic weapons. However, xylanases could also exert effects independent from their enzymatic activity, as the case of *Botrytis cinerea* xylanase Xyn11A, which is also a necrotizing factor in tobacco and tomato leaves. *F. graminearum* gene FG03624 encodes a xylanase whose deduced amino acidic sequence has a 55% identity with Xyn11A and is one of the two most expressed endo-xylanases in early stages of wheat spikelets infection. We therefore cloned this gene for heterologous expression in Pichia pastoris and characterized the purified xylanase by studying its enzymatic activity in vitro and by infiltrating wheat tissues to test its necrotizing activity. Results obtained demonstrate that this endo-xylanase is able to induce cell death and production of hydrogen peroxide in the treated tissues, even when its enzymatic activity is impaired.

P17.7
Searching inhibitor peptides of HIV-1 Nef virulence factor

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Nef is a HIV-1 27-kd regulatory phosphoprotein that plays a pivotal role in maintaining high viral load and in progression to AIDS.
After internalization by primary human non infected monocyte-derived macrophages (MDM) Nef mediates the activation of Nuclear Factor kappa B, specific MAPKs and Interferon Regulatory Factor 3, thus the synthesis and release of several cytokines and chemokines that act in autocrine and paracrine manner. Nef works as a molecular adaptor, interacting with and influencing the activity of more than 30 intracellular partners. Modelling analysis performed on TRAF2/4-1BB indicate that the Nef acidic cluster A60QEEEE65 is a putative binding motif for specific TRAF adaptor family members (i.e., TRAF2 and TRAF6) and gene silencing experiments confirm TRAFs involvement in Nef signalling activity.
We are investigating on the Nef/TRAF interactions in order to find a Nef binding inhibitor. We are using the region of Nef protein encompassing the acidic cluster (N-Term Nef) as target sequence to select peptides from a phage display library of random peptides. The selected peptide will be tested on uninfected MDMs to verify its ability to inhibit Nef-induced signalling.

P17.8
Characterization of fungal PGs and studies on their interaction with the plant inhibitor *Pv*PGIP2

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Pectins are a family of highly heterogeneous and branched polysaccharides rich in D-galacturonic acids that are present in plant primary cell walls. They are the major components of the middle lamella, a thin layer of adhesive extracellular material found between adjacent young plant cells. Phytopathogenic fungi degrade pectins by producing polygalacturonases (PG) and other pectic enzymes either to facilitate the invasion of the plant tissue and to release nutrients to be used as carbon source. PGIPs (polygalacturonase-inhibiting proteins) are plant cell wall proteins that specifically inhibit the activity of PGs. The interaction between PGs and PGIPs limits the destructive potential of PGs and favors the accumulation of oligogalacturonides capable of activating plant defense responses. Here we report on the genomic organization and the biochemical characterization of *endo*PG1 from *Sclerotium rolfsii* expressed in *P. pastoris* as well as its interaction with *Pv*PGIP2. We also report on the biochemical characterization of PGs from *Phytophthora nicotianae* and their interaction with *Pv*PGIP2.

P17.9
Metabolic changes in Arabidopsis thaliana treated with Trichoderma secondary metabolites

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Trichoderma fungi are presently marketed as biopesticides, biofertilizers and soil amendments, due to their ability to protect plants by containing pathogen populations, as well as increase plant growth and development under different soil conditions. Some Trichoderma spp. are well known producers of secondary metabolites, compounds potentially related to survival functions of the producing organism, such as competition against other organisms, symbiosis, metal transport, growth differentiation.
In this study, we have examined the metabolic changes in Arabidopsis thaliana treated with three Trichoderma metabolites that have been found to be involved in the induction of disease resistance and growth promotion, by using LC-ESI-QToF MS. The effects of treatments with 6-pentyl-α-pyrone (6PP), harzianic acid (HA) and hydrophobin 1 (HYTRA1) (conc. 10-7 M) on A. thaliana growth were observed. Applications of these compounds increased plant root-length by 48%, 51% and 40%, respectively compared to the untreated control (water). The Trichoderma metabolite treatments produced significant modifications in the plant metabolome by acting on specific pathways involved in plant defence (i.e phytoalexin production). Moreover, rapid changes in the production of major hormones were observed. The results obtained allow a better understanding of the role of some metabolites in the important beneficial interactions of Trichoderma with the plant.

P17.10
Comparative dynamics of plasma membrane potential depolarization and gene expression induced by *Spodoptera littoralis*, *Myzus persicae*, and *Pseudomonas syringae* in *Arabidopsis thaliana*

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Biotic stress invokes plant responses involving different defense mechanisms.However, we do not know whether different biotic stresses share a common response or which signaling pathways are involved in responses to different biotic stresses.We investigated the common and specific responses of *A.thaliana* to 3 biotic stress agents: *Spodoptera littoralis*, *Myzus persicae*, *Pseudomonas syringae*. The plasma membrane potential (V_m) depolarization was induced by insect attack: the response was much more rapid to *S.littoralis* than to *M.persicae*. The later V_m response was related to almost 10-fold more genes, which were differentially regulated by *M.persicae* than by *S.littoralis*. Genes induced by both insects showed opposite regulation, indicating the existence of differences in plant responses to these herbivores.The latest V_m depolarization was found for *P.syringae*, however the number of genes differentially regulated by *P.syringae* was closer to those regulated by *S.littoralis* than by *M.persicae*. The pattern of gene response regulated by both *P.syringae* and *S.littoralis* was also similar, indicating that a common mechanism was involved in the response to these two different biotic stresses

P17.11
Lipopolysaccaride adaptation to host as an immune evasion strategy of *Shigella flexneri*

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The recognition of microbes by innate immune system relies on PRRs/PAMPs interaction. PAMPs include bacterial structures, such as lipopolysaccharide (LPS), peptidoglycan.Bacteria are able to modify their PAMPs in order to modulate the immune response. LPS is an essential component of Gram-negative membrane and consists in lipid A domain and a polysaccharide region. The lipid A represents the immunostimulatory principle of LPS; changes in acylation pattern of this region affect the immunostimulatory properties of LPS, modulating the host innate immune response.On these basis we investigated whether *S. flexneri* could be able to modify the LPS structure during the intracellular residence in epithelial cells.For this purpose, LPS recovered from intracellular bacteria was extracted and analyzed by MALDI-TOFF. The Lipid A of intracellular bacteria is characterized by tri-, tetra- and penta- acylated lipid A, compared to hexa-acylated lipid A of *in vitro* grown bacteria. Then, we compared the biological activity of these two LPS in several cellular model systems, such as HEK293-TLR4 and BMDM. According to its structure, the LPS of intracellular Shigella induce a significantly lower NF-κB activation and cytokine/kemokine release than the LPS *in vitro* grown bacteria.In conclusion, *S. flexneri* can modify their structures for evading PRR recognition, as a strategy to reduce the immune system activation.

P17.12
Biochemical and molecular modifications in sheep brains during natural scrapie

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The success in the control of prion disorders depends on both availability of methods for the early detection of prions in biological fluids and identification of factors that favour prion formation in the brain. We recently reported that scrapie-infected mouse brains have increased content of free cholesterol and of the cholesteryl arachidonate fraction of cholesterol ester pool (Vascellari et al, Lipids Health Dis 2011). To ascertain presence and relevance of above modifications in natural scrapie, we investigated lipid variations in brains of uninfected and scrapie-infected sheep from a farm hit by natural scrapie. Of 18 sheep, 7 were healthy and prion-negative (controls); 3 were healthy and prion-positive (asymptomatic scrapie); and 8 were prion-positive with clinical scrapie (symptomatic scrapie). We used biochemical and molecular methods to identify and measure variations in cerebral cholesterols, lipids, and cholesterol-related proteins and genes. Data were integrated and submitted to multivariate statistical analysis to give a holistic picture of the molecular profile of infected vs. uninfected and symptomatic vs. asymptomatic sheep brains. Preliminary results are discussed.

P17.13
Exogenous HIV-1 Nef treatment induces HMGB1 release in human monocyte and primary macrophages

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The viral protein Nef is a virulence factor that plays multiple roles during hu immunodeficiency virus (HIV) replication. HIV-1 Nef treatment of hu monocyte-derived macrophages from healthy donors starts intracellular signaling triggering production of pro-inflammatory chemo-cytokines that induce tyrosine phosphorylation of signal transducer and activator of transcription (STAT)-1, -2 and -3 in autocrine and paracrine manner (Mangino G. et al. JVI 2007 and PLoS One 2011). High-mobility group box 1 protein (HMGB1), previously considered to be only a nuclear factor, was also identified as a damage-associated molecular-patterns (DAMP). Pro-inflammatory stimuli or molecules that mimic infection increase cell surface expression of HMGB1 or its secretion into extracellular environment (Ciucci A. et al, PLoS One 2011). HMGB1 mediates the response to infection, injury and inflammation and interacts with RAGE and some Toll-like receptors leading to production of pro-inflammatory cytokines, dendritic cells maturation and Th-1-cell responses (Naglova H, Bucova M. 2012). Here we report that HIV-1 Nef treatment induces HMGB1 release in THP-1 cell line and in human primary macrophages.

P17.14
Canine adenovirus (CAV-2) vectors induce an innate immune response and a modulation of cell cycle genes in human dopaminergic neurons

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CAV-2 vectors circumvent the ubiquitous anti-human Adenovirus (HAd) memory immune response, are capable of long term neuron-specific expression, do not induce the maturation of dendritic cells and have been proposed for the treatment of neurodegenerative diseases. In the prospect of clinical applications, we wanted to define the toxico-genomic profile of helper-dependent (HD) CAV-2 in human neurons. We transduced cultures of differentiated midbrain-derived human neuronal progenitor cells with HD CAV-2 and, for comparison, with lentiviral vectors (LV) and HD human (H) Ad. We evaluated gene modulation by Affymetrix gene chip, at 2h and 5 days post transduction. Our analyses of the chip containing 47,000 transcripts showed that, at comparable transduction levels, HD CAV-2 exhibited a specific modulation profile, distinct from that of both HD HAd and LV. HD CAV-2 altered genes belonging to the cell cycle, DNA recombination and repair pathways, as well as genes involved in the immune response and inflammation. Single gene and pathway modulation data emerged from our analysis constitute useful information for toxicity prediction, vector evolution and virus-host

interaction studies.

P17.15
Mitogen-activated protein kinase signalling in plant immunity

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Alpha 1-4-linked oligogalacturonides (OGs) derived from plant cell wall pectin function as damage-associated molecular patterns (DAMPs) and, like pathogen-associated molecular patterns (PAMPs), activate the plant immune response and regulate developmental responses, likely due to their ability to antagonize auxin. So far, little is known about the intracellular signal transduction elements involved in OG signalling. In both animal and plants, Mitogen-Activated Protein Kinase (MAPK) phosphorylation cascades represent a highly conserved signal transduction mechanism. A MAPK cascade consists of a core module of three kinases that act in sequence: a MAP kinase kinase kinase (MAPKKK) that activates, via phosphorylation, a MAP kinase kinase (MAPKK), which in turn activates a MAP kinase (MAPK). We are elucidating the role of a MAPKKK gene family composed by three members in the signal transduction cascade activated by OGs and PAMPs. Analysis of single and double knock out mutants, as well as of a conditional triple mutant, obtained by expressing an inducible artificial microRNA, reveals defective responses of these mutants to both types of elicitors and to pathogens.

P17.16
Evaluation of the human peptide hepcidin 20 as a potential candidate for the topical treatment of *Candida glabrata* vaginal infection

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Vaginal infections due to *Candida glabrata* are difficult to eradicate due to the intrinsically low susceptibility to azoles of this species. In this study, the in vitro fungicidal activity of the human cationic peptide hepcidin 20 (Hep20) was evaluated in: (i) sodium phosphate buffer (SPB), (ii) a vaginal fluid simulant (VFS) and (iii) human vaginal fluid (VF) against fluconazole resistant *C. glabrata* isolates. In SPB, all isolates were susceptible to Hep20 at neutral pH, with a higher fungicidal effect observed under acidic conditions (pH 5.0). The results obtained following a 4-fold dilution of VFS indicated that the peptide maintains its fungicidal activity in the presence of a divalent cationic chelating agent. Interestingly, a synergistic effect of Hep20 in combination with fluconazole was also observed. The Hep20 concentration used in the synergistic experiments were not toxic when examined in erythrocytes, peripheral blood mononuclear cells or an epithelial cell line. The fungicidal activity of the peptide was confirmed in human VF. Overall, these data suggest that this peptide could be a promising candidate for the topical treatment of *C. glabrata* vaginal infections.

O17.1
Modulation of humoral innate immune system during the pathogenesis of *Shigella flexneri*

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The innate immune system consists of cellular and humoral arms. The innate immunity is responsible for early detection and destruction of invading microbes, through the activity of a limited set pattern-recognition receptors (PRRs). To initiate immune responses, PRRs recognize pathogen-associated molecular patterns (PAMPs) and induce several intracellular signaling pathways, leading to inflammatory responses. Components of humoral innate immunity include members of the complement cascade and soluble PRRs, such as pentraxins. They play a key role as effectors and modulators of innate resistance in animals and humans. On these bases, we investigated the role of pentraxin3 (PTX3) during the cellular invasion of *Shigella flexneri*, exploiting mouse monocyte-derived dendritic cells (BMDCs) as a model. We found a high PTX3 release in BMDCs infected with the non invasive *Shigella* variants with respect to the wild type strain. In addition, the PTX3 release was reduced of 50% in BMDCs *myd88*$^{-/-}$ cells infected with both invasive and non invasive strains. Moreover, in BMDCs *trif*$^{-/-}$ and *irf3*$^{-/-}$ the production of PTX3 was drastically reduced. Data obtained through parallel experiments on BMDM confirmed those obtained in BMDCs. These findings suggest that PTX3 secretion involved TLRs, and identify the Myd88-indipendent pathway as the major and important signaling pathway during *Shigella flexneri* infection.

O17.2
Shared and distinctive features in plant response to damage- and pathogen-associated molecular patterns

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During pathogen infection or after injury, homogalacturonan (HGA), a major component of pectin in the plant cell wall, is broken down into oligogalacturonide (OGs) fragments that act as damage-associated molecular patterns (DAMPs) and activate the plant immune response. Analysis of transcript profiles and mitogen-activated protein kinase (MAPK) activity revealed an extensive overlap between early responses triggered by OGs and those induced by bacterial pathogen-associated molecular patterns (PAMPs). Both OGs and PAMPs regulate also developmental responses, likely due to their ability to antagonize auxin. So far, several elements involved in early PAMP signalling have been identified, such as BAK1 and CPKs, but their involvement in OG signalling is still unknown. We have therefore investigated whether transduction components are shared by the PAMP- and OG-activated pathways. Our results suggest that DAMP and PAMP signals act through distinct elements leading to similar defence responses. Notably, we have identified elements that are required for elicitor-triggered activation of defence responses but not for elicitor/auxin antagonism.

O17.3
An old drug suppresses *Pseudomonas aeruginosa* pathogenicity by inhibiting pyoverdine-regulated virulence gene expression

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Pseudomonas aeruginosa is responsible for a broad range of infections in humans, including chronic lung infection in cystic fibrosis patients. *P. aeruginosa* infections are difficult to eradicate by conventional antibiotic therapy, calling for the development of innovative therapeutic strategies. An appealing approach for identifying novel antibacterial drugs is to search for inhibitors of virulence. Anti-virulence drugs have the advantage of reducing the severity of the infection without creating the strong selective pressure imposed by conventional antibiotics.

Here, we explored the regulatory circuit dependent on the siderophore pyoverdine as a target for anti-virulence compounds. We developed a high-throughput screening system to screen a commercial library of more than 1,000 FDA-approved drugs, according to the "selective optimization of side activities" approach, which relies on the search for novel side activities in old drugs. This led to identify an old drug that strongly inhibits pyoverdine-regulated virulence phenotypes without affecting growth. This molecule displayed a wide range of activity against clinical strains and efficiently reduced *P. aeruginosa* pathogenicity in a mouse model of lung infection.

O17.4
Identification of a novel surface exposed Clostridium difficile protein potentially involved in the colonization of intestinal mucosa

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Clostridium difficile, a Gram-positive spore forming anaerobic bacterium, is a frequent cause of hospital-associated infections. Its pathogenicity is mediated by toxin A and B and extensive studies have defined their modality of action and their interferences in cellular pathways of the host.
Whereas it is assumed that colonization is a prerequisite to toxin pathogenicity, little is known yet about the role of nontoxin proteins in C. difficile virulence.
The availability of the whole C. difficile genome allows the identification of putative surface proteins that could mediate the colonization of the intestine. Searching for sequence homologies to known virulence factors, we have selected a protein exposed on the bacterial surface with a potential ability to adhere to extracellular matrix components and to epithelial cells.
Preliminary studies aimed to characterize the selected protein have been carried-out by threee approaches: ability of the recombinant protein to bind to human cells, tissue sections and extracellular matrix components; expression on the surface of Lactococcus lactis to evaluate its capacity to confer adhesiveness to intestinal cells and contribution of selected protein to bacterial adherence to human intestinal cells.

O17.5
Biochemical characterization of recombinant Ebola virus VP35 homo-oligomeric profile and *in silico* 3D modeling of its N-terminal coiled-coil oligomerization domain

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VP35 is a multifunctional protein involved in several steps of the Ebola virus (EBOV) life cycle. As a component of the viral RNA polymerase, VP35 is essential for EBOV replication and it also participates in nucleocapsid formation. Furthermore, VP35 is a key determinant of virulence. In fact, it contributes to EBOV escape from the host innate immune response by suppressing RNA silencing and by blocking RIG-I like receptors pathways that lead to type I interferon (IFN) production. VP35 homo-oligomerization has been reported to be critical for its replicative and IFN-antagonistic functions and it has been proposed to take place via a predicted coiled-coil domain located within the structurally unsolved N-terminal region of the protein. Here we report the homo-oligomerization profile of a recombinant VP35 (rVP35) assessed by size-exclusion chromatography, native polyacrylamide gel electrophoresis and analytical ultracentrifugation. On the basis of our results and in agreement with previous observations, we also performed an *in silico* 3D modeling of the N-terminal coiled-coil domain responsible for VP35 homo-oligomerization. Our structural proposal advances the understanding of how EBOV VP35 may associate to form homo-oligomers, a process that is crucial for both viral RNA synthesis and pathogenicity.

18 - Plant development and diseases

P18.1
Effects of ripening stages on anthocyanins and antioxidant activity in Olea europea olives cv Cellina di Nardò

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The olive cultivar 'Cellina di Nardò' is widespread throughout the Salento region, particularly in the Lecce province, but also in Taranto and Brindisi territories, for a total of 60,000 hectares.
This work reports the phenol content and the antioxidant activity of olive fruits from Cellina di Nardò during ripening. Table olives are harvested after a complete maturation and are naturally black cause anthocyanin accumulation. Different ripening stages were analysed. Total Phenols (TP) content was determined by Folin-Ciocalteau method and HPLC/DAD/MS was used for the anthocyanin characterization. Antioxidant activity (AA) was determined by DPPH, ORAC and O2 superoxide method. Data show that during ripening, TP increase from immature olives to well ripened olives reaching values two-times higher; anthocyanins were present only in mature olives up to 5.3 g/kg dry pulp. AA was determined among four ripening stages but it was particularly high in the totally black olive fruit according to TP and anthocyanin amounts.

P18.2
Antioxidative response in italian rice cultivars showing differential tolerance to salt stress

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Soil salinity, one of the main parameters affecting crop yield, is expected to increase steadily due to climate change and wrong agronomic practices. Rice, staple food for over half the world's population, is the most sensitive crop to salt stress conditions. When the external concentration overwhelms the homeostatic capability of the cell, ion accumulation in the cytosol occurs, leading to the production of reactive oxygen species (ROS). At low level ROS have a crucial role as signaling molecules, but at higher concentration they can in turn seriously damage the cell. To avoid this, the activation of suitable antioxidant systems is mandatory. In the frame of the RISINNOVA project, funded by the AGER Consortium, we previously screened 17 rice commercial varieties for their susceptibility to salt stress. Here we report on the levels of selected antioxidant enzymes (superoxide dismutase, catalase, ascorbate peroxidase and glutathione reductase) and free proline in salt-treated seedlings of 5 rice cultivars with a contrasting capability to cope with hyperosmotic stress. Results could provide the basis for a marker-assisted selection for salt tolerance through increased ROS scavenging.

P18.3
Expression analysis and biochemical studies of defense-related genes in the pathosystem *Fusarium verticilliodes*-maize

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Fusarium ear rot leads to mycotoxin contamination of kernels and loss of yield. We tested in six maize genotypes with contrasting levels of resistance to *Fusarium verticillioides*, genes involved in defense response. At 15 days after anthesis, on caryopses harvested 72 hours after infection, the expression of PR1, PR5, PRm3, PRm6, peroxidase, catalase 2, ascorbate peroxidase and superoxide dismutase 2, was evaluated. The level of induction was stronger in susceptible lines, followed by intermediate lines, while resistant lines responded with lower fold changes values. The trend of expression of the genes matched with the absolute quantification of the fungus *beta-tubulin*, indicating a higher copy number of the gene in susceptible genotypes, than resistant ones. Parallel, the activity of enzymes catalase, ascorbate peroxidase, generic peroxidases, superoxide dismutase, was determined to verify their effectiveness and involvement level in *Fusarium verticillioides*-maize pathosystem. In addition, the redox state of ascorbate and protein thiols as well the hydrogen peroxide and malondyaldheide contents were determined to evaluate the oxidation level in maize caryopses after infection.

P18.4
Homogalacturonan affects Arabidopsis resistance to pathogens

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Microbial pathogens need to breach the epidermal cell walls of the host to infect plants. The impact of the chemical composition of plant cell wall polysaccharides on the outcome of the plant–pathogen interaction is poorly understood. In particular, little is known about the role of the most abundant component of pectin, i.e. homogalacturonan (HGA), which participates in determining cell wall rigidity and is the first polysaccharide that is degraded by microbes during infection. Modification of HGA in Arabidopsis thaliana by expression of a fungal polygalacturonase (PG) or by mutations of a gene involved in HGA biosynthesis (*QUA2/TSD2*) influences cuticle permeability and results in an increased resistance to the fungal pathogen *Botrytis cinerea*. This resistance is abolished by treatments with the hormone abscisic acid (ABA), which also restores a cuticle permeability comparable to that of wild type plants. On the other hand, resistance to the fungus *Alternaria brassicicola*, which is positively influenced by ABA, is compromised in HGA-modified plants. A mechanism by which ABA and pectin interact to influence responses to biotic stress is proposed.

P18.5
An interaction network of the receptor WAK1 regulates OG responses in Arabidopsis

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Conserved pathogen/microbe-associated molecular patterns (PAMPs/MAMPs) and endogenous molecular patterns, which are present only when the tissue is infected or damaged (damage-associated molecular patterns or DAMPs), can act as danger signals and activate the plant immune response. These molecules are recognized by surface pattern recognition receptors (PRRs). Oligogalacturonides (OGs), released from the plant cell wall, are well-known DAMPs that have long been considered as local signals in the wound response. Recently, through a chimeric receptor approach, we have demonstrated that the Arabidopsis Wall-Associated Kinase 1 (WAK1) is a receptor of OGs. WAK1 has been described to form a complex with an apoplastic glycine-rich protein (GRP-3) and a cytoplasmic kinase-associated protein phosphatase (KAPP). Arabidopsis plants overexpressing WAK1 as well as grp-3 and kapp null insertional mutants have been used to characterize the role of the three proteins in the perception/transduction of the OG signal, in the wound response and in resistance to pathogens.

P18.6
Pectin methylesterases affect plant resistance to pathogens

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Pectin is synthesized in a highly methyl esterified form and is de-esterified in muro by pectin methyl esterases (PMEs). The degree and pattern of methyl esterification affect the cell wall structure and properties with consequences on their resistance to pathogens. We show that PME is required for the initial plant tissue colonization by fungal and bacterial necrotrophs, making pectin more susceptible to the action of the hydrolytic enzymes of the pathogens. We have reduced the susceptibility of plants to pathogens by increasing the methyl esterification of pectin through the overexpression of PME inhibitors (PMEI). A natural Arabidopsis ecotype, showing a higher pectin esterification and lower homogalacturonan content than the reference ecotype Col-0 is more resistant to necrotrophic fungal and bacterial pathogens. PME is also required for viral cell-to-cell and long-distance movement of plant virus. Our results support the notion that PME activity affects the mechanical properties of cell wall and plant resistance against pathogens.

P18.7
A KDEL-cysteine protease from *Lilium longiflorum* tepals localises to the vacuole during floral senescence

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Proteolysis is a defining feature of senescent tissues and is linked to the disassembly of cellular contents and nutrient remobilisation. During senescence both compartmentalisation and regulated activation of degradative enzymes is crucial for ensuring that premature cellular destruction does not occur. KDEL-tailed cysteine proteases have been identified in senescent tissues of several species; they are retained in the ER by their C-terminal KDEL sequence, and transported to the cytosol in ER-derived vescicles called ricinosomes. Cytosol acidification following vacuole rupture results in ricinosome rupture and activation of the KDEL proteases from an inactive pre-protein. Here we show that a *Lilium longiflorum* KDEL protease is transcriptionally up-regulated and post-translationally processed in senescent petals. In young tissues the protein is retained in the ER, while during floral senescence it localises to the vacuole. This is in contrast with a previous report showing the presence of a KDEL protease in *Hemerocallis* petal ricinosomes. Our data therefore suggest an alternative translocation route and activation mechanism for KDEL proteases during petal senescence.

P18.8
Endosperm is the main regulator of nucellus programmed cell death in *Sechium edule* through emission of ethylene

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The nucellus is a maternal tissue that feeds the developing embryo and the secondary endosperm. During seed development the cells of the nucellus suffer a degenerative process early after fertilization as the cellular endosperm expands and accumulates reserves. Nucellar cell degeneration has been characterized as a form of developmentally programmed cell death (PCD).
By using biochemical and pharmacological approach, we analyzed the role of the endosperm in the regulation of nucellus PCD. Endosperm produces high amount of ethylene, nitric oxide and indoleacetic acid. We examined the role of these small and diffusible signalling molecules in the regulation of nucellus PCD and we tried to elucidate how they can cooperate and regulate each other into the endosperm. We showed that ethylene acts a positive regulator of nucellus PCD and its synthesis can be in part induced by nitric oxide. High levels of IAA were detected both in the endosperm and in dying nucellus but this hormone is not directly involved in the execution of PCD.

P18.9
Unraveling the regulatory pathway of lysine-specific histone demethylases in plants

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In *Arabidopsis thaliana* four lysine-specific histone demethylases were recently identified (AtLSD1, AtLSD2, AtLSD3 and AtLSD4). These proteins participate in epigenetic regulation of gene expression in association with multi-protein complexes and are involved in important developmental processes, such as flower transition and root elongation.
To identify AtLSDs molecular partners, *Arabidopsis* plants were transformed with a *35S::AtLSD1-FLAG-HA* construct and recombinant AtLSD1 together with associated proteins were immunoprecipitated. Mass spectrometry-based analysis of these proteins leads to interesting insights. Phenotypical analysis of loss-of-function *atlsd* mutants and *35S::AtLSDs* transgenic plants under both physiological and stress conditions is in progress. These mutant plants are also analyzed for the expression of genes associated to plant developmental programs and defense resposes to determine the AtLSDs specific targets. To obtain information on the tissue- and organ-specific expression pattern of the *AtLSDs*, *AtLSD::GFP-GUS* were obtained. Using this multi-disciplinary approach it has been possible to highlight important differences among the various AtLSDs.

P18.10
Protein engineering and improvement of plant resistance to pathogens: exploitation of a polygalacturonase inhibitor

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Polygalacturonases (PGs) are produced by fungi during infections and are an important microbial pathogenicity factors. Against these PGs, plants have evolved apoplastic polygalacturonase-inhibiting proteins (PGIPs) that slow down the fungal infection and favour the accumulation of oligogalacturonides, endogenous inducers of the plant defences. The PGIP2 from Phaseolus vulgaris has been engineered (engPGIP2) to improve its defensive potential. Transgenic Arabidopsis plants have been generated that express the engPGIP2 under the control of a promoter inducible by exogenous chemical treatment. Upon induction, the engineered inhibitor was able to activate a broad range of defence responses, ranging from production of reactive oxygen species to callose deposition, and to confer enhanced resistance to pathogens.

P18.11
Expression profile of APETALA2, mir172 and AGAMOUS in the floral tissue of the orchid Orchis italic

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In Arabidopsis, AP2 and AG are A- and C-class genes that drive the formation of perianth and reproductive organs, respectively. AP2 is a negative regulator of AG and its function is regulated by miR172

through the RNA cleavage. We isolated the homologs of AG and AP2 in the orchid Orchis italica, named OitaAG and OitaAP2, respectively. We performed Real Time RT-PCR experiments to check the OitaAG and OitaAP2 expression within different floral tissues collected before and after anthesis. In addition, we evaluated the expression levels of miR172 and verified the cleavage of the OitaAP2 mRNA at the mir172 target site. In both stages, OitaAP2 is expressed within the perianth organs (tepals and lip) whereas OitaAG is expressed in column (fused male and female reproductive tissues). Mir172 expression pattern is opposite to that of OitaAP2, however in the late column it is not present, indicating that before anthesis OitaAP2 is expressed in all the floral tissues, where its function is regulated by the presence/absence of mir172, whereas after anthesis, OitaAP2 and mir172 are no more expressed. In both stages, the absence of OitaAP2 within column permits the expression of OitaAG.

P18.12
Dynamic changes in Arabidopsis transcriptome during shade avoidance response

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The success of competitive interactions between plants determines the chance of survival of individuals. Daylight contains roughly equal proportions of red (R) and far-red (FR) light, but within vegetation, that ratio is lowered as a result of the R absorption by photosynthetic pigments. This light quality change is perceived as a signal of the proximity of neighbours provoking a set of morphological changes to perceive maximum sunlight. The adaptive reaction driven by low R/FR signal is the shade avoidance response (SAR).
Several key regulators involved in the SAR have been identified. However, very little is known about the cascade of events triggered by low R/FR that give rise to activation of the response and lead to adaptation to an unfavourable light environment. Therefore, SAR was examined by genome wide expression profiling in wild type and genetically altered plants exposed to low R/FR light for different times. To identify gene networks, both computational and experimental approaches are being pursued. Together, these analyses uncovered novel aspects of SAR and generated testable hypotheses on gene regulatory networks underlying plant responses to light quality changes.

P18.13
Grapevine leaf senescence: morphological changes of GFP-labeled mitochondria associated with cell ageing

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In this report, spontaneous and cytokinin-induced senescence occurring in leaf of grapevine plants was described by analysing mitochondrial morphology and dynamics. The senescence process was also analysed in grapevine cultured cells and it was observed that both proliferating cultured cells and mature leaf are characterised by mitochondria organized in dynamic networks. When senescence takes place, mitochondria progressively enlarge, increasing their volume and reducing their number and motility. Transformation of *V. vinifera* embryogenic cell culture allowed the production of plants stably expressing GFP targeted to mitochondria. Different phases of leaf senescence were characterised by analysing photosynthetic parameters, molecular markers and mitochondrial morphology. The results allowed the association of distinctive mitochondrial features to distinct physiological stages. Senescence was induced on plant cuttings treated with high level of cytokinin, and physiological and molecular changes compared to natural senescence. In cytokinin-induced leaf senescence, the process takes place in a quicker way but similar changes in mitochondria morphology and dynamics were observed.

O18.1
An *Arabidopsis* polyamine oxidase undergoing proteasomal regulation

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Polyamine oxidases (PAOs) are FAD-dependent enzymes involved in polyamine catabolism. In *Arabidopsis*, five PAO genes (*AtPAO1-5*) have been identified with important differences among them in subcellular localization, substrate specificity and expression pattern, which suggest distinct physiological roles. In the present work, *AtPAO5*, the only so far uncharacterized AtPAO which is specifically expressed in the vascular system, was partially purified from *35S::AtPAO5-6His Arabidopsis* transgenic plants and biochemically characterized. Data evidenced interesting differences in substrate specificity between AtPAO5 and the other AtPAOs. Furthermore, subcellular localization studies for AtPAO5 through confocal analysis of *35S::GFP-AtPAO5* and *35S::AtPAO5-GFP* transgenic plants demonstrated cytosolic distribution of this enzyme with formation of aggregates. Treatment with the proteasomal inhibitor MG132 increased the number of aggregates, indicating AtPAO5 association with the proteasomal complex. A positive regulation of *AtPAO5* expression by polyamines was also shown. These data give new insights into the complex regulatory network controlling polyamine metabolism.

O18.2
DAG1 and GAI shared functions in light-mediated seed germination

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Seed germination is controlled by environmental and endogenous factors. Germination of Arabidopsis seeds is mediated mainly by the photoreceptor phyB. A key role is also played by the hormones ABA and GA, which play an antagonistic role, as ABA inhibits this process, whereas GA triggers seed germination. PIL5 - a bHLH protein- is the master repressor of phyB-mediated seed germination. It induces expression of RGA and GAI, encoding two DELLA proteins, negative regulators of GA-mediated processes. DAG1, a Dof TF, acts dowstream of PIL5 and negatively regulates GA biosynthesis by directly repressing the AtGA3ox1 gene. We are currently investigating the relationship between RGA, GAI and DAG1. Our results suggest that RGA and GAI have different roles with respect to DAG1. In fact, expression of DAG1 and GAI, but not of RGA, are mutually regulated in the seed. Moreover, GAI, similarly to DAG1, seems to partecipate in the repression of AtGA3ox1. Interestingly, genetic data indicate that DAG1 and GAI could have a function during embryogenesis, as dag1gai-t6 double mutant is embryo-lethal. We conclude that DAG1 and GAI may cooperate in both seed germination and embryo development.

O18.3
Role of pectin composition in plant growth

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Cell wall strongly influences plant growth. To investigate the role of cell wall pectin, and in particular of homogalacturonan (HGA), in this process, we have analyzed in Arabidopsis the ectopic expression of a fungal polygalacturonase (PG), which degrades de-esterified HGA, and mutants for endogenous genes that affect HGA composition and structure such as *QUASIMODO2*, encoding a putative HGA methyltransferase, two putative PG-encoding genes and *AtPME3*, encoding an ubiquitously

expressed pectin methylesterase. We have also analyzed Arabidopsis plants overexpressing PME inhibitors and have increased degree of pectin methylesterification. The data obtained indicate that HGA levels and/or esterification have a major impact on cell expansion and tissue growth rate. Collectively, our results support the hypothesis that pectin structure is an important determinant of plant growth. We have also identified an extracellular peroxidase overexpressed in plants with altered pectin; studies conducted on loss- and gain-of-function lines indicate that this protein negatively regulates growth.

O18.4
An Arabidopsis MAPKKK gene family involved in plant development

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The mitogen-activated protein kinase (MAPK) phosphorylation cascade is a highly conserved signal transduction mechanism that plays a key role in regulating processes such as plant immunity, growth and development. The MAPK cascade consists of a core module of three kinases that act sequentially: a MAP kinase kinase kinase (MAPKKK) that activates, via phosphorylation, a MAP kinase kinase (MAPKK) that in turn activates a MAP kinase (MAPK); this phosphorylates specific target proteins. In Arabidopsis, 60, 10 and 20 genes encode MAPKKKs, MAPKKs and MAPKs, respectively. Our work aims at elucidating the role of an Arabidopsis MAPKKKs gene family, consisting of three members and so far known to be involved both in cytoskeletal organization and cell division. Because homozygous triple knock out mutants are not obtainable, likely because of lethality, we have generated a conditional triple mutant that expresses an inducible artificial miRNA able to silence one member of this family in a double knock-out background. We describe the severe developmental defects displayed by the triple mutant and the results of our investigation on the underlying mechanisms.

O18.5
Programmed cell death induced by high levels of cytokinin in Arabidopsis cultured cells is mediated by the cytokinin receptor CRE1/AHK4

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High levels of cytokinin(CKs) induce programmed cell death (PCD) both in animals and in plant cells. High levels of the CK benzylaminopurine (BA) induce PCD in cultured cells of Arabidopsis thaliana by accelerating a senescence process characterized by DNA laddering and expression of a specific senescence marker. The question has been addressed whether AHK members of the small family of Arabidopsis CK receptors are required for BA-induced PCD. In this respect, suspension cell cultures were produced from selected receptor mutants. Cell growth and proliferation of all receptor mutant and wild-type cell cultures were similar, showing that the CK receptors are not required for these processes in cultured cells. The analysis of CK metabolite instead revealed differences between wild-type and receptor mutant lines, and indicated that all AHK receptors are redundantly involved in the regulation of the steady-state levels of isopentenyladenine- and trans-zeatin-type CKs. To study the role of CK receptors in the BA-induced PCD pathway, cultured cells were analysed for their behaviour in the presence of high levels of BA. The results show that CRE1/AHK4 is required for this kind of PCD

O18.6
AIR12, a *b*-type cytochrome of the plasma membrane of *Arabidopsis thaliana* is a negative regulator of resistance against *Botrytis cinerea*

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The extracellular matrix of plants is a dynamic compartment where environmental cues are perceived and signals are generated to orchestrate adaptive responses. AIR12 is an ascorbate-reducible cytochrome b, GPI-anchored to the plasma membrane, able to promote, in vitro, either ascorbate regeneration or hydroxyl radical formation. In Arabidopsis plants, activity of the AIR12 promoter is observed mainly in (i) sites of auxin accumulation (e.g. stipules, hydatodes) and/or (ii) sites of controlled cell separation processes (e.g. micropilar endosperm during germination, epidermal cells encircling by the emerging lateral root, floral organs abscission zones after shedding) and (iii) sites of lignin deposition (e.g. vascular tissue). AIR12 expression is also stimulated by Botrytis cinerea infection. AIR12 is encoded by a single gene in Arabidopsis and although AIR12 knock-out mutants showed neither evident developmental defects nor altered auxin responsiveness, they showed a strong resistance to Botrytis cinerea. A model is proposed to integrate AIR12 redox activities with the positive correlation observed between AIR12 and physiological cell separation/lignin deposition processes (where AIR12 would play as an antioxidant) and the negative correlation between AIR12 and resistance to Botrytis cinerea (where AIR12 would play a pro-oxidant role).

19 - Plant metabolism and environmental stress

P19.1
Mixotrophic growth of *Neochloris oleoabundans* (syn. *Ettlia oleoabundans*) in the presence of glucose: biotechnological implications

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The Chlorophyta *Neochloris oleoabundans* is a mixotrophic organism (Giovanardi *et al*., 2012 – *Protoplasma*, DOI 10.1007/s00709-012-0390-x), which can accumulate lipids, especially under N starvation, so it is often proposed for green energy production. In other microalgae, mixotrophy due to glucose is referred to promote both growth and synthesis of molecules of interest (lipids). In this work, growth and lipid production ability of the alga in a brackish medium containing 0, 2.5 and 5.0 g/L of glucose were compared. Algal growth was similar in both mixotrophic media reaching the stationary phase after 9 d of cultivation with a cell density of 4 to 5.5 times higher than that of controls. This was parallel to the PSII maximum quantum yield, as the F_V/F_M ratio was higher than in the autotrophic algae until 9 d of growth and then decreased. All mixotrophic algae were filled with lipids starting from the stationary phase of growth. Consumption of glucose was complete only in algae cultivated in the 2.5 g/L medium. Data suggest that *N. oleoabundans* can be very efficiently cultivated with 2.5 g/L of glucose to obtain both biomass and lipids for biotechnological purposes.

P19.2
Acclimation of Chlamydomonas reinhardtii To Different Growth Irradiances

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We report on the changes the photosynthetic apparatus of Chlamydomonas reinhardtii undergoes upon acclimation to different light intensity. When grown in high light, cells had a faster growth rate and higher biomass production with respect to low and control light conditions. However,cells acclimated to low light intensity are indeed able to produce more biomass per photon available as compared to high light acclimated cells, which, indeed, dissipate as heat a large part of light absorbed, reducing their photosynthetic efficiency. This dissipative state is strictly dependent on the accumulation of LhcSR3, a protein related to Light Harvesting Complexes (Lhc), responsible for non-photochemical quenching in microalgae. Other changes induced in the composition of the photosynthetic apparatus upon high light acclimation, consist into an increase of carotenoids content on a chlorophyll basis, particularly zeaxanthin, and a major down-regulation of light absorption capacity by decreasing the chlorophyll content per cell. Surprisingly, the antenna size of both Photosystem I and II are not modulated by acclimation.

P19.3
Durum wheat CMO expression analysis under salt and light stresses

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Durum wheat seedlings were submitted to salt stress under two different light regimes; 350 µE, which is usually the intensity used in growth chambers for experimental studies, and 900 µE, which is close to the real light intensity experienced by plants when grown in their natural environment. The most surprising result was the strong inhibition of leaf glycine betaine (GB) accumulation by high light, under both control and salt stress conditions. To investigate the cause of this inhibition, we determined the transcript levels of leaf choline monooxygenase (CMO), the key enzyme of GB synthesis in higher plants. Two different partial TdCMO cDNA isoforms were isolated: the 867bp isoform potentially encoded the active enzyme, while the 977bp isoform contained frame-shift mutations with 3 stop codons. The RT-PCR study revealed that the active form of TdCMO cDNA was predominant under low light condition and overexpressed under salt stress treatment, in agreement with the overaccumulation of GB in response to salt stress. Conversely, the defective form of the TdCMO gene was predominant under high light, the active form being almost totally absent, even under salt stress (only around 10% of control light). Such a result could explain the very low levels of GB encountered under high light conditions.

P19.4
The good-nature of fructans: prebiotic and antioxidant effects on human health

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Wheat has been recently suggested as an interesting fructan source. Fructans are the most widely used prebiotics and they have quickly gained a great importance as beneficial food ingredients. Here, the metabolism of fructans and other molecules of nutritional value have been studied during durum wheat kernel maturation. Changes in fructan content, activities of the enzymes involved in their metabolism, antioxidant metabolites and related enzymes as well as phenolic compounds and antioxidant total capacity were analyzed. The results showed that fructans were accumulated in the first 20 days after anthesis when the enzymes involved in fructan biosynthesis had also the highest activities. Immature kernels were also richer in antioxidant metabolites and enzymes than mature ones as well as in hydrophilic antioxidant capacity. Taken together these results increase the interest of immature wheat flour in functional food field. Moreover the identification of the maturation stages in which kernels have the highest nutritional value in terms of fructan and antioxidant levels can have interesting implications for the production of novel wheat-based foods with increased healthy value.

P19.5
Ex situ and *in situ* experiments of phytoremediation by use of *Pteris vittata* and *Populus* hybrids grown on pyrite ashes

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Iron-bearing sulphide minerals are largely responsible for the generation of mine drainage and hence of the release of heavy metals and arsenic into the environment. Roasted arsenopyrite in dumped ash resulted from the production of sulfuric acid has raised significant health and environmental concern. Current technologies applied for the remediation of arsenic-contaminated sites are expensive and environmentally disruptive. The phytoremediation has gained interest due to its cost-effectiveness and environmental soundness. The present study refers to the experimental tests carried out either ex-situ in pots and in-situ in dumping site during the years 2010 and 2011.
The plant species used are: 1. poplar, hybrid Orion (Populus deltoides x Populus nigra), to reduce heavy metal concentrations; 2. the arsenic-hyper-accumulating Chinese brake fern (Pteris vittata) for the metalloid-As. Both experiments in pots and in plots were set in order to assess the survival of the plants, arsenic accumulation, physiological and biomass parameters, as well as enzymatic stress responses evaluated through monitoring of catalase, and –SH groups activities in leaves and roots. The study of the parameters above listed, together with improvements in

the site using agronomic techniques (such as additions to the site of plant compost and pot soil) allowed the construction of a pilot-plant with an area of 4.500 m^2.

P19.6
Evolutionary conserved stress-responsive CCCH zinc finger proteins are involved in abiotic stress and seed germination in arabidopsis and durum wheat

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Plant CCCH zinc finger proteins with nucleic acid binding activity are involved in important biological processes. Previous studies indicated that the expression of 2H8, a gene coding for a CCCH zinc finger protein isolated in durum wheat is responsive to cold and dehydration stresses. A functional conservation between a sub-group of stress-related Arabidopsis CCCH genes and 2H8 as well as two durum wheat homologous genes has been suggested. Among the Arabidopsis genes, AtTZF3 is the putative ortholog of the 2H8 gene. To gain information on role of AtTZF3 in stress responses, a functional analysis using AtTZF3 under- and over-expression mutants is underway.
A deep phenotypic evaluation of the germination process under abiotic stress conditions revealed that the knocked-down mutants are more tolerant to stress than the wild type suggesting that AtTZF3 is a negative regulator of seed germination. Similarly, expression analysis under stress conditions suggests the involvement of 2H8 in the regulation of seed germination in durum wheat.

P19.7
An increase in antioxidants helps tobacco BY-2 cells to overcome moderate heat stress

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High temperatures can negatively affect plant growth and productivity. It is known that heat stress induces significant changes in normal physiological processes and generates reactive oxygen species (ROS). In order to limit the oxidative damage, occurring under stress, plants have developed detoxification systems, able to scavenge the highly toxic ROS. In order to clarify the relationship between cell growth, redox homeostasis and activation of defence mechanisms, the effect of moderate heat stress (exposure to 35°C) has been studied in tobacco BY-2 cells. The data indicates that the block of the cell cycle is an initial defence strategy. A strong increase in the expression of HSPs and an enhancement of antioxidant enzymes also occurs. However, these defence mechanisms seems to be not sufficient to cope with a persistent heat stress. Five-seven days after the start of heat treatment, the activity of antioxidant enzymes declines. The parallel increase in ROS determines oxidative damages and cell death. Interestingly, the pre-treatment of BY-2 cells with antioxidants correlates with a better growth capability, due to the recovery of cell divisions and a decrease in cell death.

P19.8
Post translational regulatory mechanisms for plant δ[1]-pyrroline-5-carboxylate reductase

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Besides its role in counteracting hyperosmotic stress conditions, the amino acid proline exerts several beneficial effects, and it is accumulated inside the cell of plants and microorganisms also in response to many other types of stress. Proline can be produced from either glutamate or arginine, with the two pathways sharing the last reaction, catalysed by a δ[1]-pyrroline-5-carboxylate reductase (P5Cr). As it occurs at the converging point of two routes, P5Cr may be subjected to a fine modulation, even if not controlling the rate-limiting step. Its expression was indeed found to be regulated at both the transcriptional and the translational level, whereas no post translational regulatory mechanism has been described to date. We previously showed that the activity of *Arabidopsis thaliana* P5Cr is modulated by the ratio and the redox status of pyridine nucleotide cofactors. Here we report the chromatographic separation of two enzyme forms showing different properties. Since Arabidopsis possesses only a single gene coding for a P5Cr, and their ratio was found to vary as a function of redox conditions, these two forms might have a role in regulating stress-driven proline accumulation.

P19.9
Phenolic metabolism in durum wheat genotypes producing anthocyanin pigmented seed

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During recent years increasing evidence supporting the ability of the so-called functional foods to promote well-being and reduce the risk of certain major diseases prompted a strong interest for the development of strategies to increase the level of health-promoting nutrients in fruits and vegetables. Among these beneficial substances are the anthocyanins, naturally occurring phenols representing an important source of hydrophilic dietary flavonoids with high antioxidant activity. Purple wheat grains are a promising source of anthocyanins, but to ensure protective effects higher levels of these bioactive compounds would be required. In the frame of the ALISAL project, funded by the Italian MiPAAF, we aim at the characterization of the biochemical basis of proanthocyanidins and anthocyanins accumulation in the pericarp and in the aleurone layer of pigmented durum wheat varieties. Here we report on specific activity levels of key enzymes controlling regulatory reactions in anthocyanins synthesis (namely DAHP synthase, phenylalanine-ammonia lyase, calchone synthase, anthocyanidin synthase and anthocyanidin reductase) in total seed extracts of pigmented and non-pigmented genotypes.

P19.10
New vectors for delivering polyphenols: the case of curcumins

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Natural antioxidants with polyphenol structure have limited bioavailability and low solubility in aqueous environments. Therefore, delivery agents are required to exploit polyphenols anti-oxidant properties in vivo with small administration doses. Liposomes are the best candidates for this purpose, due to their biocompatibility and easy preparation. However, the loading capacity of conventional liposomes toward polyphenols is not satisfactory, since the rigid and bulky skeleton resulting from one or more benzene rings is not easily accommodated within the hydrocarbon chains of the liposome bilayers.
Here we devised a top-down approach to obtain novel liposomes from the membranes the cyanobacterium Cyanothece sp.CCY0110 for improving the uptake of curcumin, a polyphenol used in the diet or as natural chemotherapics. The curcumin chemical structure is based on two aromatic rings connected by a poly-unsaturated spacer of eight carbon atoms.
Light and neutron scattering methods showed that the structures obtained from the complex membrane of cyanothece (algosomes) are suitable matrices for the loading of curcumins.

P19.11
An Arabidopsis copper amine oxidase is involved in jasmonate-induced root xylem differentiation

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The polyamines (PAs) spermine, spermidine and putrescine (Put) are oxidized by amine oxidases (AOs) to amino aldehydes, releasing an amine moiety and hydrogen peroxide (H_2O_2). It has been reported that PA-derived H_2O_2 inhibits root growth and promotes xylem differentiation in maize roots, inducing wall stiffening and signaling developmental PCD. Here, we show that the apoplastic copper containing AO (At4g14940) is involved in the methyl jasmonate (MeJA)-induced xylem differentiation in Arabidopsis roots, exploiting a loss-of-function mutant for the *At4g14940* (*AtCuAO**) gene. Conversely, *AtCuAO** is not involved in Abscisic acid (ABA) or Benzyl adenine (BA) signalling pathways affecting root development or vascular tissue differentiation. Consistently, *AtCuAO** expression is induced by MeJA, but not by ABA, BA or α-Naphthalene acetic acid treatment. MeJA induces H_2O_2 production at the site of the first differentiated xylem cells and negatively affects Put level in WT roots, while being ineffective in mutant roots. Further studies are in progress in *AtCuAO** over-expressing plants. Our data suggest that AtCuAO*-produced H_2O_2 behaves as mediator in the JA-induced root xylem differentiation.

P19.12
Subcellular localization and hormone-regulated expression of durum wheat lipoxygenases

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Lipoxygenases (LOX) are non-heme iron-containing dioxygenases that catalyse oxygenation of polyunsaturated fatty acids and lipids leading to the synthesis of oxylipins. Oxylipins have been implicated in a wide range of pivotal physiological functions, such as signal transduction, biotic and abiotic stress response, development and senescence. In higher plants LOX play important roles in fruit development and ripening, and their activity can generate compounds influencing fruit flavour and quality. However, during foodstuff processing LOX can also cause a significant loss of dietary carotenoids. In the frame of the ALISAL project, funded by the Italian MiPAAF, we previously resolved two LOX isoforms in extracts from wheat (*T. turgidum* ssp. *durum* cv Ofanto) cultured cells. Here we report on their functional properties, with special emphasis on carotenoid bleaching activity. One form was purified to electrophoretic homogeneity, and its localization was investigated by subcellular fractionation. The expression of the two LOX at both the mRNA and the protein level was measured in response to hormonal treatments. Results suggest that both may be involved in defence against pathogens.

P19.13
Increased photoprotection in an engineered strain of *Chlamydomonas reinhardtii* with a phytoene synthase exogenous gene

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Microalgae have been recently studied as *green cell factories* for the production of highly valuable molecules, including carotenoids.
In our Lab the nuclear transformation of *Chlamydomonas reinhardtii* cw-less strain cc-3491 with an expression vector containing the cDNA of *At*PSY allowed to obtain one transformant with a yellowish phenotype, B3. It was observed that B3 accumulated zeaxanthin, which conferred to cells photoprotection. To further investigate this behavior, cells were grown at 30 and 250 $\mu mol_{photons}\ m^{-2}\ s^{-1}$ (LL and HL cultures). In B3 LL cultures a slightly increase of the total pigment content was observed with respect to control. Moreover, energy partitioning analyses showed a major proportion of constitutive dissipation yield caused by the accumulation of zeaxathin. B3 cells grown under HL, appeared instead photoacclimated after 4 d of growth. Finally, the comparison between B3 LL after 4 d of growth with a HL photoacclimated control showed similar profiles in the energy partitioning and HPLC. Concluding, transformation did not induce a relevant accumulation of carotenoids, but a change in the methabolic pathway confering a higher photoprotection in transformed cells

P19.14
Gold nanoparticles synthesis using water extracts from different plants

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The exceptional properties of Au nanoparticles are used for many purposes, including opto-electronic devices, catalysis, DNA labeling, drug delivery, cell imaging and biosensors. Extended applications and new uses require synthetic techniques based on "green chemistry", which are both cost-effective and environment friendly. In this context, the reducing properties of plant extracts has attracted increasing attention. Here we used water extracts from both crop (*Cucurbita pepo* L.) and wild (*Silene paradoxa* L.) plants, hydroponically pretreated or not with different metals.
Mild operating conditions (40°C and submillimolar concentrations of metal) allowed to carry out the Au nanoparticle synthesis with different efficiency, according to the plant organ, the species and the treatment considered.
Shape and size of these nanoparticles were investigated by Transmission Electron Microscopy and, when possible, directly in solution by Dynamic Light Scattering. Possible mechanisms are discussed to explain the observed differences.

P19.15
Freeze tolerance of Zoysia matrella (L.) Merrill as affected by late-season nitrogen application, and changes in carbohydrates during cold acclimation

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This study was conducted with the aim of assessing the development of freeze tolerance (LT_{50}) in zoysiagrass under the effect of two different levels (15 and 30 g m^{-2}) of nitrogen applications, and evaluating the dynamics of soluble carbohydrates composition during cold acclimation and overwintering. A combined approach with natural acclimation in the field, followed by monthly controlled exposure to sub-freezing temperatures, was employed to evaluate LT_{50}. Fall color of zoysiagrass was improved by nitrogen applications, the latter extending the green period by more than one month. In October, the higher nitrogen treatment caused less cold hardiness (LT_{50} = -10 °C) compared with the other treatments, but was beneficial to freeze tolerance in March. The controls reached cold hardiness in November, 1 month earlier than the N treatments, in agreement with the color retention data. Controls also showed a more linear freeze tolerance during the experimental period. Variations in total soluble sugars occurred during the acclimation and de-acclimation process. Alteration of metabolism was more evident in the 30-g N treatment, in particular as regards the storage sink.

P19.16
Glucosinolates and Mirosynase activity in Brassica rapa L. susp. sylvestris grown under salt stress

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Glucosinolates are sulphur metabolites found in Brassicaceae that in the last years have acquired high importance for their anticancer properties. Biotic and abiotic stress can highly influence the accumulation of glucosinolates, changing also the nutraceutical value of plant tissues.
In this work the effect of salt stress on glucosinolate and myrosinase synthesis has been studied in ecotypes of Brassica rapa L. cv. sylvestris largely used to prepare many traditional recipes in South Italy. Plants were grown in controlled condition hydroponically. At 40 d from germination half of them were kept in a Hoagland medium supplemented with NaCl (75 mM) and grown up to the formation of the flower sprouts. Glucosinolate, isothiocyanate and myrosinase activities were determined in plant tissues. The results evidenced a significant increase of glucosinolate content and myrosinase activity in the stressed with respect to the control plants. The highest increase occurred in the flower sprouts. The other data were analyzed and discussed.
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P19.17
Variegation in *Erythronium dens-canis* L.(Liliaceae) leaves: a structural-functional study

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Erythronium dens-canis is a small early-flowering lily of wood undergrowth, whose two leaves have a remarkable pattern of red-brown and silver-green patches. The red-brown colour is due to a subepidermal cell layer with vacuolar anthocyanins, whereas silver-green spots are produced by air spaces between epidermis and underlying chlorenchyma. The two kinds of areas do not differ in amounts of photosynthetic pigments, chloroplast organization, photosynthetic parameters (Fv/Fm, NPQ, ETR), or xanthophyll cycle components. Interestingly, in leaves of plants with developing fruits the red-brown parches gradually vanish giving way to an intense green hint, without accompanying changes in the structural-functional features of photosynthetic tissues. This suggests that leaf anthocyanins have no sunscreening role in *Erythronium*. Since fertilization in this early-flowering species may be limited by scarcity of pollinators, we conclude that the initial pattern of leaf pigmentation might be part of an attraction strategy useful for the plant reproductive success. When this role ceases, the resources for anthocyanin synthesis can be allocated elsewhere by plants developing fruits and seeds.

P19.18
Leaf hydraulic resistance is not affected by the root-specific aquaporin *VvPIP2;4N* overexpression

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After overexpressing the grape aquaporin gene *VvPIP2;4N* in *Vitis vinifera* L. 'Brachetto' we analysed i) the expression of *VvPIP2;4N* and five other aquaporins, ii) whole-plant, root, and leaf ecophysiological parameters, and iii) leaf abscisic acid content. The availability of six different transgenic grapevine lines with different *VvPIP2;4N* transcript levels allowed us to study the correlation between expression of this aquaporin and water transport processes at the whole-plant level.
Expression of transgenic *VvPIP2;4N* inhibited neither the expression of the endogenous gene nor that of other PIP aquaporins in both root and leaf. The expression level of *VvPIP2;4N* (endogenous + transgene) was inversely correlated to root hydraulic resistance. Partitioning of plant hydraulic resistance at the organ level showed that leaf resistance was not affected, despite the fact that the transgene was expressed in both roots and shoots. Upon water stress, the overexpression of *VvPIP2;4N* induced a surge in leaf abscisic acid content, and a decrease in stomatal conductance and leaf gas exchange.
Our results show that aquaporin-mediated modifications of root, but not leaf, hydraulics play a substantial role in the regulation of water flow in irrigated grapevine plants, while they have a minor role upon drought, probably because other signals, such as ABA, take over control of water flow.

P19.19
Effects of amino acid analogues on plant metabolism: β-pyrazol-yl-L-alanine

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Dozens of non-protein amino acids have been discovered in plants. The routes leading to their synthesis, their metabolic fate and acceptable human intakes were extensively investigated, yet for most of them little is known concerning the possible physiological role. β-pyrazol-yl-L-alanine was identified in seeds of many cucurbits, mainly in cucumber, where it represents the major free amino acid and accounts for up to 1‰ of total dry weight. Since its presence did not act as antifeedant against aphids, it was early assumed as an unusual nitrogen storage compound. The discovery that it represents a side product of cysteine synthase led some authors to hypothesize on the lack of a function for its synthesis. However, we previously showed that at millimolar concentrations it is able to inhibit other plant growth at both the undifferentiated cell and seedling level. Here we report its effects on rice cultured cells. The mechanism of pyrazolalanine uptake, its charging on tRNAs, free amino acid pools in treated cells, and its ability to inhibit selected enzymes in amino acid metabolism were investigated. Results are discussed in view of understanding the mode of its phytotoxic action.

P19.20
The link between photosynthetic efficiency and productivity in algae cultures for biodiesel production

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Algae are a potential feedstock for biodiesel production but strong research efforts are still needed to make their exploitation competitive. In particular algae light use efficiency must be optimal in all conditions in order to achieve a sufficient productivity. Here the influence of light intensity on Nannochloropsis salina growth and lipids productivity was analyzed using a flat-bed photobioreactor designed to minimize cells self-shading. Results show that Nannochloropsis cells are able to use efficiently even very intense light if illumination is alternated with dark periods. Otherwise, algae experience radiation damages and photosynthetic productivity is strongly reduced. Presented results suggest that in an algae photobioreactor mixing optimization is seminal for algae productivity.
Another major parameter for algae performances is nitrogen, whose depletion induces lipids accumulation but also causes a growth inhibition. It is seminal to find a method to induce lipids biosynthesis without affecting photosynthetic efficiency and to this aim the effect of nitrogen deprivation on Nannochloropsis photosynthetic apparatus was characterized at the molecular level, showing that this species can cope with nitrogen deprivation through a re-organization of entire photosynthetic apparatus which allows maintaining a good efficiency for

the residual proteins.

P19.21
Mitochondrial ferritin is truly a functional iron-storage protein in cucumber roots

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Intracellular Fe trafficking must satisfy both chloroplastic and mitochondrial demands for Fe without allowing its accumulation, in dangerous, redox-active forms, in the plant organelles. The Fe storage protein ferritin is involved in such intracellular control of Fe trafficking and homeostasis in both organelles. The characterization of Arabidopsis atfer4 heterotrophic cells as well as atfer4 plants, both knock-out for the ferritin isoform targeted also to mitochondria, unveiled a putative signalling role of ferritin, beside its known role as Fe store [1,2]. These results prompted us to further investigate the functional role of mitochondrial ferritin in plants.
We could show the presence of the monomer ferritin in mitochondria of cucumber roots, which is strictly dependent on the Fe supply in growth medium; we could also detect, in cucumber mitochondria, the 24-mer ferritin complex, which binds Fe(III) and whose presence is also strictly dependent on the Fe supply. Such results indicate that mitochondrial ferritin is a functional iron-storage protein in cucumber roots.
The functional interaction, in mitochondria, between ferritin and the mitochondrial iron chaperone protein frataxin is currently under investigation in our laboratories
[1] Tarantino et al (2010) J Plant Physiol. 167: 1598–1605.
[2] Tarantino et al (2010) J Plant Physiol 167: 453-460.

P19.22
NH_4^+ toxicity at high concentration is due to K^+ deficiency

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NH_4^+ is often the preferred source of N, due to the lower energy required for its assimilation. However, different algal species show very different ability to cope with high [NH_4^+].
Since NH_4^+ has been shown to cross cellular K^+ channels when no specific NH_4^+ uptake system is available, we hypothesize that the sensitivity of some algae (but not others) to high [NH_4^+] is associated with the absence of high capacity NH_4^+ channels on the plasmalemma and thus on the possibility that NH_4^+ penetrates into the cell at the expenses of K^+.
In this work, the marine diatoms *Phaeodactylum tricornutum* and *Cylindroteca closterium* were grown in the presence of 0.01, 1 and 10 mM either NO_3^- or NH_4^+. They proved to be very different in their responses to the N regimes. In particular, *C. closterium* growth was severely hampered by high [NH_4^+] but stimulated when the [K^+] was higher in the medium, *P. tricornutum* growth rate was stimulated by the increase in [NH_4^+] and not affected by [K^+]. We conclude that in *P. tricornutum* NH_4^+ and K^+ transports are distinct and independent, whereas in *C. closterium* NH_4^+ enters the cells via K^+ channels determining K^+ deficiency at high NH_4^+ concentration.

P19.23
Biochemical and structural characterization of higher plant photosystem II supercomplexes

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Photosystem II (PSII) in higher plants is organized into large supercomplexes, composed by an intrinsic core associated with variable amounts of membrane-bound peripheral antenna complexes (LHCII). Usually 2-4 copies of trimeric LHCII are strongly (S) and moderately (M) bound to the dimeric core (C_2) via monomeric antenna proteins (CP29, CP26 and CP24), thus forming the C_2S_2 or $C_2S_2M_2$ supercomplexes. Homogeneous preparations of C_2S_2 and $C_2S_2M_2$ particles, isolated from pea thylakoids, were characterized by biochemical methods and single particle electron microscopy. Their different size was assessed by BN-PAGE and the polypeptide composition analysed by 2D SDS-PAGE and mass spectrometry. These biochemical analyses revealed: 1) for both particles, an overall integrity of the reaction centre core also in the low molecular mass subunits; 2) the presence of the minor antenna protein CP24 and the extrinsic polypeptides PsbP and PsbQ only in the $C_2S_2M_2$ particle. The final 2D projection maps obtained for the C_2S_2 and $C_2S_2M_2$ supercomplexes elucidated the different organization of their peripheral antenna system in terms of composition, location and orientation of the antenna proteins.

P19.24
Drought tolerance in ornamental plants used for green design in urban areas

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The choice of plants with low impact of maintenance is an important purpose for green design in the urban Mediterranean areas, since the climate is characterized by hot, dry summers, and mild, wet winters. The plants growing in this region are frequently subjected to drought stress during summer, so the drought tolerance is required for a sustainable management of ornamental plants. The aromatic plants represent good candidates, due to their rusticity and for the production of volatile organic compounds (VOCs). This research represents a part of the INTERREG-ALCOTRA Project "AROMA" dealing with the investigation of aromatic plants for environmental and productive activities. Two different species (*Salvia dolomitica* Codd. and *Salvia sinaloensis* Fern.) have been cloned and treated in controlled environmental conditions with three irrigation regimes (100%, 50% and 0% of container capacity - CC), in order to characterize their drought resistance at morphological and physiological levels. The obtained results indicated that the plants are tolerant to mild drought stress (50% CC), but exhibited a different behavior under severe drought condition (0% CC). Different responses between species are observed, particularly on water potential, growth index, and on primary and secondary metabolites composition (pigments and VOCs).

P19.25
Relationship between sulfate assimilation pathways and phytoplankton evolutionary trajectories

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Sulfur assimilation has been studied in depth in few higher plants, but little is known for algae. Secular changes of [SO_4^{2-}] are among the most conspicuous variations in oceans chemical history and probably impacted phytoplankton radiation in the Mesozoic, when algae with red chloroplasts became prominent after a long period of green algae and cyanobacteria dominance (the so called Sulfate Facilitation Hypothesis, SFH). We hypothesize that the SFH is associated to functional differences in SO_4^{2-} assimilation enzymes.
To test this hypothesis we cultured the prasinophyte *Tetraselmis suecica*,

the diatom *Thalassiosira pseudonana*, the dinoflagellate *Amphidinium klebsii* and the cyanobacterium *Synechococcus* sp. in the presence of either 5 or 30 mmol SO_4^{2-} L^{-1}, approximately equivalent to pre- and post-Mesozoic concentrations. Then we studied the influence of [SO_4^{2-}] on the activity, kinetics and expression of the enzymes that concur to its assimilation.
Our results indicate that some functional differences exist in the SO_4^{2-} assimilation pathways of algae, also with respect to higher plants. These differences may have played a role in the SFH.

P19.26
Polyphenol profiling and concentration in peach fruits subjected to UV-B irradiation in post-harvest

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UV-B radiation (280-320 nm) is known to stimulate plant secondary metabolism, thus possibly enhancing the concentration of some healthy beneficial molecules in fruits and vegetables.
To test whether UV-B radiation could be an effective tool to improve the nutraceutical quality of peach, fruits of three cv (Babygold 7, Suncrest and Big Top), harvested at commercial maturity, were treated with 1.68 W/m^2 UV-B for up to 36 h or maintained under UV-B lamps screened with UV-B blocking plastic film.
The concentration of polyphenolic classes was determined by spectrophotometric assays, followed by HPLC ESI-MS quantification of the main compounds.
The effects of UV-B treatment was found to depend on tissue, skin being more susceptible than flesh, as well as on cv. Suncrest and Big Top, even if with different trends, generally reacted to the treatment by increasing phenylpropanoid concentration, while Babygold 7 was less influenced by UV-B light and underwent a decrease in polyphenols after 24 h of irradiation.
In conclusion, UV-B radiation could represent a powerful tool to modulate phenylpropanoid accumulation, provided that the treatment is targeted to the specific cultivar.

P19.27
Characterization of a glutathione S-transferase in *Triticum durum*

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Glutathione-S-transferases (GSTs) are ubiquitous, multifunctional family of enzymes, that catalyse the conjugation of glutathione to a wide variety of electrophilic, lipophilic substrates in bacteria, fungi, animals and plants. Roles for GSTs in endogenous metabolism are less well defined, with the enzymes linked to a diverse range of functions, including signaling, counteracting oxidative stress, and detoxifying and transporting secondary metabolites. A cDNA (AF109714) coding the TaGSTZ1 glutathione S-transferase was identified in Triticum durum (cv Cappelli) and was cloned in pET24a vector for the in vivo expression. Moreover, the expression of the TaGSTZ1 has been analyzed by qRT-PCR under drought and heat stress and following H202 and glyphosate treatment. The results highlighted that the TaGSTZ1 expression is slightly sensitive to drought and heat stress. The glyphosate treatment trigger a very high increase of the TaGSTZ1 transcript, suggesting a key role in cellular detoxification. These data confirm the versatility of GST proteins and their activities in different stresses. Particularly TaGSTZ1 is slightly up-regulated by drought and heat stress.

P19.28
The gene family of (phospho)glucan-water dikinase and their role in starch metabolism: a phenotyping study

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Part of the photosynthetic energy is accumulated in plants as both primary and secondary starch. Beside secondary starch, typically accumulated in storage organs, primary starch is deposited in leaves, following a diurnal rhythm. The first event of the starch degradation pathway is the phosphorylation of few glucose monomers, catalyzed by enzymes known as (phospho)glucan-water dikinases, (phospho)GWD. The aim of the present work is to study the relative contribution to starch degradation of the three (phospho)GWD coded by the genome of *A. thaliana*. To this aim, two T-DNA insertion mutants for each of the genes of interest were obtained from NASC (Nottingham, UK) and homozygous lines were selected. Phenotypic parameters, such as leaf starch content, growth rate, flowering time, flower and silique number, seed number, weight and size, seeds content and dry weight, were determined. Despite the several differences observed between wild type and mutant plants, X-ray powder diffraction patterns of primary starch granules of mutants and WT plants were identical, suggesting that none of these enzymes are involved in modifying the fine architecture of starch granules.

P19.29
Molecular cloning, expression, and characterization of a secretory phospholipase A_2 from durum wheat (*Triticum durum* Desf.) leaves

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Phospholipases A_2 (PLA_2s) hydrolyse membrane phospholipids to release lysophospholipids and free fatty acids, both precursors for signalling molecules active in a wide range of physiological and pathological processes. A cDNA encoding a putative secretory PLA_2 ($sPLA_2$), designated as *TdsPLA$_2$III*, was isolated from durum wheat leaves. The *TdsPLA$_2$III* mRNA was mainly expressed in root, but was also present in culm, glume and seed, and to a lesser extent, in leaf and awn; moreover, its was found to be over-expressed under drought stress. The encoded protein was 162 amino acid long and was predicted to contain a signal peptide of 33 residues, 12 Cys residues, a Ca^{2+} binding loop, and a catalytic domain typical of $sPLA_2$s. The recombinant TdsPLA$_2$III showed preference for phosphatidylcholine (PC) and phosphatidylethanolamine and required Ca^{2+} at millimolar concentrations to reach its maximal activity. When PC was used as substrate the reaction rate showed a hyperbolic dependence on substrate concentration (Km = 280±13 µM, Vmax = 0.043±0.008 units/µg of protein), an optimum at pH 9.0 and inhibition by palmitoyl trifluoromethyl ketone and dithiothreitol, two specific PLA_2 inhibitors.

P19.30
Physiological and morphological responses of a unicellular alga to lead or cadmium stress

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We studied the metabolic responses to the toxicity of lead (Pb) and cadmium (Cd) in the green-fresh-water-microalga *Chlorella sorokiniana* 211-8K (Chlorophyceae). Both the pollutants tested alter the alga cell ultrastructure and its physiological characteristics. The toxic effects of the two heavy metals resulted time-dependent to the exposure. After 24 h of Pb or Cd treatment photosynthesis was strongly inhibited while respiration was enhanced. In the algal cells, Pb or Cd exposure, induced a reduction of the total chlorophylls and a decrease of the soluble protein levels, radically compromising the growth specially in cultures under cadmium treatment. We reported data on ultrastructural alteration induced by the two heavy metals. Most importantly, the O-acetyl-L-

serine(thiol)lyase enzyme activity was significantly increased after only 2 h of Cd exposure indicating the existence of a link between the metal contamination and cysteine synthesis. Then, *Chlorella sorokiniana* cells seem to better tolerate high concentrations of Pb while appear to be more sensitive to Cd. These results provide additional information to a better understanding heavy metal effects in microalgae.

P19.31
Glutathionylation and nitrosylation of cytosolic glyceraldehyde-3-phosphate dehydrogenase from Arabidopsis are controlled by redoxins and reduced glutathione

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In Arabidopsis thaliana, cytosolic GAPDH is involved in the glycolytic pathway and is represented by two differentially expressed isoforms (GapC1, GapC2) that are 98% identical in amino acid sequence. Here we show that GapC1 catalyzes a NAD(H)-specific reaction and its enzymatic activity is strictly dependent on Cys149. Catalytic Cys149 is the only solvent-exposed cysteine of the protein and its thiol is relatively acidic (pK_a 5.7). This property makes GapC1 sensitive to oxidation by NO-donors and H_2O_2, which inhibit enzyme activity by converting the thiolate of Cys149 ($-S^-$) to nitrosothiols and irreversible oxidized forms ($-SO_2^-$, $-SO_3^-$) via a labile sulphenate intermediate ($-SO^-$), respectively. Reduced glutathione (GSH) prevents this irreversible process by reacting with Cys149 sulphenates to give rise to a mixed disulphide (Cys149-SSG), as demonstrated by both MS and biotinylated GSH. Glutathionylated GapC1 can be fully reactivated either by cytosolic GRXs or, less efficiently, by cytosolic TRXs. By contrast, nitrosylated GapC was not reactivated by either GRXs or TRXs and full reactivation was only observed in the presence of GSH. Potential relevance of these findings is discussed in the light of the multiple functions of GAPDH in eukaryotic cells (e.g. glycolysis, apoptosis) that appear to be influenced by the redox state of the catalytic Cys149.

P19.32
Sugars and free amino acids profiling in fruits of sweet cherry landraces of Campania Region (Italy)

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In the last years, the reduction of agricultural biodiversity due to the large scale adoption of few improved varieties that displace the landraces historically selected and cultivated by farmers, significantly increased the vulnerability of existing agro-ecosystems. Agro-biodiversity, being the basis of our agricultural food chain, is an essential resource to meet our food security and is the foundation of sustainable agriculture development and livelihood security, mainly in the globalized world. Its conservation and promotion needs characterization of such genetic resources. This aim can be improved using not only morphological, agronomic and genetic traits, but also metabolomic ones that are markers not of potential development of a given cultivar but of its actual phenotypic expression in the given environment and time important to protect traditional local recipes. Such analyses can allow to trace the products also along the food chain when the morphological traits cannot be used.
Here are reported the metabolic profiles of sugars and free aminoacids occurring in harvested fruits of 27 sweet cherry landraces of Campania germoplasm cultivated in the fields of "CRAA – Azienda Agricola Sperimentale Regionale 'Improsta', and compared with some cultivars cultivated in the same fields. The patterns of sugars and aminoacids allow to discriminate among the landraces and to group them according the percentage of similarity.
Financial support was obtained by "Regione Campania, Italy, PSR 2007/13, Measure 214, Action f2, project Agrigenet"

O19.1
Effects of cyclic and linear oligosaccharides on artemisinin metabolism in *Artemisia annua* L. cell cultures

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Artemisinin (AN) is a sesquiterpene antimalarial compound produced, though at low levels (0.1–1% dry weight), by the herbal plant *Artemisia annua* L. We established *A. annua* cell cultures able to produce intracellular and, more interestingly, extracellular AN. In a previous work, ß-cyclodextrins (ß-CDs), known to increase the water solubility of lipophilic compounds, resulted to significantly enhance AN production in *A. annua* cell cultures. The aim of this work was to better investigate the effects of ß-CDs on AN metabolism. For this purpose, intracellular and extracellular levels of the AN intermediates dihydroartemisinic acid (DHAA) and artemisinic acid (AA) were evaluated after various treatments, using cyclic (ß-CDs) or linear oligosaccharides such as maltodextrins and oligogalacturonides (OGA). OGA were obtained by hydrolysis of polygalacturonic acid with pectoliase from *Aspergillus japonicus*. Fifty mM ß-CDs, 10 mM maltodextrins and different concentrations (20, 100 and 500µg/mL) of various OGA fractions were tested. The results showed that ß-CDs were able to significantly increase both AN and DHAA levels while the 4-5 OGA fraction only induced a significant increase of DHAA.

O19.2
Ophiobolin A effect on cell cycle is mediated by alteration in GSH fluxes among cell compartments

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Ophiobolins are micotoxins produced by Bipolaris and Aspergillus genera, which attack several plants of agronomic interest. Their mode of action is still obscure.
This study reports the effects of ophiobolin A on tobacco cv Bright Yellow-2 (BY-2) cells. Ophiobolin A-induced responses depended on the applied dose. At 10 µM, the toxin induced a programmed cell death (PCD) that appeared not to be mediated by an early overproduction of reactive oxygen species. Whereas, at lower concentrations ophiobolin A did not affect cell viability, but arrested cell cycle in a reversible manner. In particular, 2-5 µM ophiobolin A induced cell block in S/G2 phase. Concomitantly, it freezed the activity of the poly ADP-ribose polymerases (PARPs), nuclear enzymes involved in DNA metabolic transitions, which normally increases during the exponential growth phase in plant cells. Perturbations of GSH fluxes across different sub-cellular compartments were also observed. Indeed, GSH accumulated in the tobacco nuclei during the growth phase at the highest proliferation rate and then it spread across the whole cell. In ophiobolin A-blocked cells, GSH appeared sequestered into the nuclei.

O19.3
Identification of microRNAs controlling leaf cell development during drought stress in *Brachypodium distachyon*

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Brachypodium distachyon (Bd), a model species for grasses, shows a high level of drought tolerance. Aiming at shedding light on the complex network regulating drought response, we focused on the role of miRNAs in fine tuning gene expression in developing leaves.
The reference accession Bd21 was subjected to a non-lethal drought stress, resulting in a strong leaf size reduction caused by reduction of cell size rather than cell number, as revealed by kinematic analyses. To understand the role of miRNAs during stress response in proliferating and expanding cell, we adopted NGS technology to characterize 4 smallRNA libraries obtained from 2 developing leaf areas in stress and control conditions. In-house developed bioinformatics pipeline identified 275 conserved and lineage-specific miRNAs, 213 of which were identified for the first time in *Bd*. Statistical test detected differentially expressed miRNAs with a higher proportion of miRNAs involved in developmental programming, while only a few miRNAs are regulated under drought stress. Putative miRNAs targets were identified, and we are investigating their possible involvement in leaf development both in normal and stressed conditions.

O19.4
Effects of stress on miRNA abundance in grapevine

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In this study, we investigated the population of microRNAs present in leaf and root tissues of Cabernet Sauvignon (CS) and M4 rootstock grapevine genotypes under water and salt stress. Physiological parameters were daily measured on stressed and irrigated plants, in order to set the exact day of sampling (gs < 0.05 mmol H2O m^{-2} s^{-1} and Ψleaf ~ -1.4 MPa). Low Molecular Weight RNA (LMW RNA) was extracted and used to prepare cDNA libraries, which are in process of being sequenced by means of SOLiD platform and which were used for q-PCR analysis.
The expression of several conserved miRNAs was analyzed by qRT-PCR using TaqMan probes. In several cases, expression showed differences between leaves and roots. Some miRNAs (such as e.g. miR159) were overexpressed in both leaves and roots of stressed CS and M4 plants, while the expression of others (miR393) was exclusively activated in leaves. A few miRNAs were differentially expressed between CS and rootstock genotype.
Further analyses are underway on grafted plants, in order to gain evidence on possible miRNA transport between scion and rootstock.

O19.5
Possible role of a mitochondrial phospholipase A_2 activity in durum wheat (*Triticum Durum* Desf.) response to hyperosmotic stress mediated by activation of the dissipative systems

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Phospholipase A_2 (PLA_2) hydrolyses the 2-acyl ester bond of 1,2-diacyl-*sn*-3-phosphoglycerides to give free fatty acids (FFAs) and 1-acyl-2-lysophospholipids, precursors of second messengers involved in plant response to internal and external stimuli. We have previously reported that a PLA_2 activity exists in mitochondria from different plant sources, the characteristics of which resemble those of other known plant PLA_2s: optimum at basic pH values, Ca^{2+}-dependence and sensitivity to specific PLA_2 inhibitors. Here we report evidences that, in mitochondria from durum wheat seedlings exposed to salt (0.21 M NaCl) and osmotic (0.42 M mannitol) stress, the PLA_2 activity increases, respectively, to about 50 and 95%. Consistently, under hyperosmotic stress conditions, we also observe an increase in the mitochondrial FFAs content. This is of particular interest since our results show that, in durum wheat mitochondria, FFAs activate the plant uncoupling protein and the plant mitochondrial potassium channel. These dissipative systems are able to decrease the mitochondrial membrane potential and dampen the generation of ROS, that is known to increase under stress conditions.

20 - Plant nutrition

P20.1
Iron acquisition by barley plants from natural Fe-complexes

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Soluble Fe in soil is represented by a mixture of complexes between Fe and organic ligands such as organic acids and phytosiderophores (PS). In this work we studied the mechanisms of Fe acquisition in barley plants supplied with ^{59}Fe-PS or ^{59}Fe-citrate (1 µM Fe, pH 7.5). Fe-sources has been supplied at the beginning (max release of PS) and at the end (basal release) of light cycle. Results show that Fe-deficient plants accumulate higher amounts of Fe from both sources, compared to the Fe-sufficient ones. The uptake rate changed during the light cycle, especially in Fe-deficient plants, reaching the highest values in the morning, and was dependent on the Fe-sources, being generally higher when Fe was supplied as Fe-PS. The pH influence on Fe uptake was evaluated in the range 5.5 - 8.0; pH increase caused a reduction in the capability of plants to take up Fe. Measurements of Fe-PS stability by LC-ICP/MS at different pHs showed that the formation of the complex is highly dependent on pH. These results highlight the importance of proton gradient not only for the release of PS and Fe-PS uptake, but also for the formation of the complex.
MIUR PRIN and FIRB, Unibz-TN5046 & Rhizotyr TN5218

P20.2
Molecular characterization of the Lotus japonicus NRT1(PTR) and NRT2 families

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Nitrate is an essential element for plant growth, both as a primary nutrient in the nitrogen assimilation pathway and as an important signal for plant development. Low and high affinity transport systems are involved in the nitrate uptake from the soil and its distribution between different plant tissues. We identified putative members of both systems in the model legume L. japonicus. We investigated the transcripts abundance in root tissues of nine and four genes encoding putative low-affinity (NRT1) and high-affinity (NRT2) nitrate transporters, respectively. The genes were sub-classified as inducible, repressible and constitutive on the basis of their responses to provision of nitrate, auxin or cytokinin. Furthermore, members specifically and significantly regulated in root and nodule tissues during the symbiotic interaction with Mesorhizobium loti have been identified. The interpretation of the global regulative networks obtained, allowed to postulate roles for nitrate transporters as possible actors in the cross talks between different signaling pathways triggered by biotic and abiotic factors. A biochemical and functional characterization is in progress.

P20.3
Ionomic maps of *Solanum pennellii x S. lycopersicum* introgression lines variation as response to toxic elements

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A central theme of ionomics is the study of variations in the ionome in response to physiological stimuli, environmental conditions and genetic modifications. Our gol was to characterise the ionome modification induced by mineral elements on introgression lines (ILs) population in wich each line is identical across their whole genome to cv. M82 except for a single introgressed region of the wild species *S. pennellii*. Until now the contribution of the genome of *S. pennellii* in the tomato cultivated variety for ions accumulation has not yet been studied. ICP-MS analysis were performed on apical tips of ILs grown on no-lethal concentration of As, Cd, Cr, Cu, Ni, Pb and Zn. Macro, micro, trace and toxic elements concentration in each IL and in parental cultivated cv. M82 were determined. Ionome variations of ILs were evaluated as the differences between each IL and cv. M82. Data were elaborate by T-test analysis. Results showed that traits correlated to ion homeostasys were significantly modified in response to the treatment and to a specific single introgression. The ionomic maps drawn represent the first obtained on these important genotypes.

P20.4
Brassica rapa plants saved inflorescences under sulphur deficiency

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The strong decrease in S atmospheric emissions and the use of low-S fertilizers caused a widespread S deficiency. Sulphur nutrition strongly influences productivity and nutritional value of crop plants. In particular in Brassica vegetables sulphur is essential for biosynthesis of glucosinolates, that are S-containing secondary metabolites of high nutraceutical value. In this work the effect of sulphur nutrition on metabolite profiles of. Brassica rapa L. subsp. sylvestris ecotypes was investigated. Nitrogen and sulphur metabolite profiles were determined in different organs. The results evidenced that growth was strongly reduced in low S plants. The non sulphur free aminoacids concentration increased. The S-metabolites cysteine, glutathione and glucosinolates that were at lower level in the leaves, were kept high in the flower sprouts, suggesting that plants in any case try to save the reproductive organs.
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P20.5
Root retention activity and accumulation of Cd in the shoot of two barley cultivars

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Manel and Lemsi are two barley (Hordeum vulgare L.) cultivars characterized by relatively high and low Cd accumulations in shoot and grain. With the aim of deciphering the reason of the different behaviours we compared, under low and environmentally realistic Cd concentrations, the activities of components of the root 'firewall system' limiting the translocation of Cd towards the shoot. Kinetic analyses with 109Cd as a tracer showed that the Vmax of the Cd influx in the root was higher for Lemsi than Manel. Nevertheless, the Cd concentration in the roots did not differ between the two cultivars as a consequence of a more efficient loading of the metal into the xylem of Lemsi. The higher Cd translocation observed in Lemsi is related to a lower synthesis of phytochelatins and, moreover, to a lower level of transcript of the P1B-ATPase HvHMA3 actively accumulating the metal into the vacuole. As a consequence, in the root of Lemsi a higher amount of free soluble Cd available for the loading into the xylem exists. The lower HvHMA3

mRNA level in Lemsi might be due to its higher capacity to take up Cd and Zn from the external medium.

P20.6
LaMATE2: a transporter involved in transmembrane vehiculation of genistein in white lupin plants

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White lupin has developed a complex strategy to survive in soils with low availability of nutrients. This behavior involves the modification of root architecture with the formation of cluster roots and the release of root exudates, mainly organic acids and flavonoids. These compounds can mobilize sparingly available nutrients in soils, such as iron and phosphorus, via complexation, ligand exchange, and in the case of flavonoids, reduction/complexation. They can also influence the biological characteristics of the rhizosphere, affecting the activity of the microorganisms.
In this work we have physiologically characterized the exudation of genistein from proteoid roots. The gene involved in this process has been isolated via cDNA-AFLP and it has been named *LaMATE2* which belongs to the MATE transporter family. The expression of this gene was reduced in *knock down* mutant plants, which also showed decreased genistein exudation. Expression in yeast cells allowed the characterization of transport activity. The results show that *LaMATE2* is a genistein transporter, involved in the exudation processes of flavonoids.
MIUR FIRB "Futuro in Ricerca", Unibz TN5046, ProvBZ Rhizotyr TN5218

P20.7
Involvement of two MATE genes in the release of root exudates in apple plants grown under nutrient deficiency

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South Tyrol is one of the most important apple cultivation area in Europe covering 70% of apple production in Italy and 15% in Europe. However, to date, there are still problems in the cultivation which limit the productivity and compromise the quality. One of the major problems encountered is the nutrient availability. Several species of plants are able to overcome these nutritional deficiencies, releasing low molecular weight compounds called "root exudates", as organic acids and flavonoids. The objective of this work was to study the involvement of two MATE (Multidrug And Toxic compound Extrusion) proteins in the release of these substances. We analyzed the root exudates released by apple (*Malus x domestica* Borkh.) rootstocks M9, the most commonly used in the commercial production, grown under different availabilities of iron and phosphorus. After that, we investigated two MATE genes of apple, homologous to a MATE gene of the model plant white lupin (*Lupinus albus* L.), known for its involvement in the release of flavonoids. The levels of gene expression were evaluated in the root tissues via Realtime RT-PCR.
Grants: *Unibz TN5031, Prov. BZ Rhizotyr TN5218 & MIUR-Firb*

O20.1
O-acetyl-L-serine(thiol)lyase activity reveals the sulphur status in algal cell

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Plant cell absorbs sulphate and assimilates it in L-cysteine, which represents the first S-amino acid coming from sulphur assimilation. Unicellular algae, like higher plants, are able to adapt their metabolism to nutrient deficient conditions such as sulphate-deficiency. We analyzed and compared the effects of sulphate-deprivation in the mesophilic-green-alga Chlorella sorokiniana (318/23 ACUF) and in the thermo-acidophilic (extremophile) unicellular red alga Galdieria phlegrea (291/355 ACUF), collected at Pisciarelli (Campi Flegrei, Napoli, Italy). The removal of sulphate from the culture medium caused in Chlorella sorokiniana a time-dependent increase in *O*-acetyl-L-serine(thiol)lyase (OASTL) activity and a reduction in the soluble protein content; the increase of the enzyme OASTL was correlated to Cys level in the cell. Galdieria phlegrea culture medium was modified to obtain S-starved cells to evaluate the effects of S deprivation on growth, protein contents and OASTL activity. The experiments were also conducted in Galdieria cells grown heterotrophically thanks to their ability to use organic compounds to live.

O20.2
Watching a protein at work, or how an ammonium transporter was made sensor

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Genetically encoded sensors are potent tools for the study of a plethora of different compounds. Currently, however, a sensor for ammonium, one of the main nitrogen sources for microorganisms and plants and an important ion in humans, is missing. In most organisms, including plants, ammonium uptake is mediated by ammonium transporters (AMTs). AMTs are assembled in trimers, each monomer consisting of 11 transmembrane helices. As indicated by crystal structure and genetic evidence, helix 5 oscillates during transport of ammonium, and this movement is permitted by the long cytosolic loop 5. By taking advantage of this conformational change, we created the first transporter-derived sensor, fusing the Arabidopsis AtAMT1;3 with a modified circularly permuted green FP (mcpGFP). Different variants of the sensor were obtained by changing the composition of the linkers. The sensors are dose-dependent in their response, specific for ammonium and therefore have the potential to be used as genetically encoded sensors in a variety of organisms. Furthermore, the response of the sensors is strictly linked to their transport activity, as shown by complementation assay in yeast and mutant analysis. This is the first time that the activity of a transporter can be measured by means of fluorescence.

O20.3
Redox regulation and dependence of oligomeric state of *Populus trichocarpa* plastidic P2-glucose-6P dehydrogenase (*Pt*P2-G6PDH) by pH

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In plants, G6PDH (EC 1.1.1.49) is present in the plastids as P1 and P2 isoforms. A key role for the plastidic P2-G6PDH has been suggested in

redox regulation, demonstrating a link between enzyme and reductants poise.
The overexpressed P2-G6PDH from *Populus trichocarpa* was described, but its sensitivity to reductants and oligomeric form should be still defined.
Size exclusion chromatography and light scattering measurements show that the enzyme elute as a dimer at pH 7 while as a tetramer with a higher catalytic activity at pH 9. As expected, both oligomers were observed at pH 8.
The CD spectrum suggests a secondary structure with 42% α-helix and 30% β-sheets. During thermal denaturation, a slight structural disruption was observed, resulting in a loss of activity.
PtP2-G6PDH is inhibited by DTT in vitro, and this redox regulation is attributable in vivo to thioredoxins (Trxs). It is known that chloroplastic P1-G6PDH is similarly inactivated by Trx *f* and Trx *m*. In contrast, PtP2-G6PDH is strongly inhibited by Trx *m*, and a lesser extent by Trx *f* and *z*. Thus, P1-and P2-G6PDHs are regulated by different Trxs, suggesting distinct roles for these enzymes in higher plants.

O20.4
Integrated responses of Parietaria judaica to iron deficiency conditions

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Iron is an essential element for plants, as it is a component of many proteins that play a central role in vital functions such as photosynthetic and respiratory chain. Although highly abundant in most soils, iron bioavailability is low at physiological pH. In alkaline soils, especially in calcareous ones, iron solubility decreases dramatically; in fact iron chlorosis is a common symptom exhibited by crop species grown in these kinds of soils. In dicots and non Poacea monocots iron acquisition is mediated by a reduction-based mechanism localized at the root cell membrane, oriented to create the conditions to reduce Fe^{3+} to $Fe^{2+}$and transport it inside the cell. These specific responses are flanked by other non specific responses as the biosynthesis of low molecular weight organic acids and polyphenols. The active root exudation of reductant and chelating substances to increase iron availability is a well documented strategy developed by plants. *Parietaria judaica* is a weed well adapted to live in extremely calcareous conditions. We studied the different responses displayed by *P. judaica*. to deal with low iron bioavailability at morphological, physiological and metabolic level. In particular, as *P. judaica* has a high content in phenolics, we studied their role in iron acquisition as well as the secondary metabolism rearrangement occurring to sustain their massive synthesis.

O20.5
Cloning and heterologous expression of the urea transporter ZmDUR3 in Zea mays

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In the last decades agricultural N-fertilization has led to a tremendous urea input into biosphere, accounting for over 50% of the world N-fertilizer consumption. Therefore urea, nitrate and ammonium are the three forms of N generally present in cultivated soils. Despite the great agricultural importance of urea for higher plants, the molecular and physiological bases of its transport have been investigated only in Arabidopsis and rice. In order to characterize a high-affinity urea transporter in maize, we isolated the *ZmDUR3* ORF which is highly homologous (84% identity on nucleotide level) to the rice urea transporter *OsDUR3*. *ZmDUR3* encodes an integral membrane protein with 731 amino acid residues. By complementation assays, heterologous expression of *ZmDUR3* could restore growth of a yeast *dur3* mutant on urea medium.
The identification and characterization of urea transporters in higher plants is important not only for understanding the urea-related plant N-nutrition processes, but also for exploring potential strategies to improve urea-based N-fertilizer use efficiency in agricultural crop production.
Work supported by NORGEF project (Friuli Venezia Giulia, LR/26) and MIUR-PRIN 2009

21 - Protein synthesis, degradation and homeostasis

P21.1
Role of Pim1 kinase in the translational control in prostate cancer cells

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Deregolation of one or more steps involved in the protein synthesis control is a mechanism able to promote cancer transformation because an abnormal protein synthesis can contribute to an aberrant cell growth. In the prostate cancer, a common alteration is the constitutive activation of the PI3K/AKT pathway leading to the mTORC1 complex stimulation. mTOR shares some substrates, as 4E-BP1 and AKT, involved in the protein synthesis, with the kinase Pim1, an oncoprotein with a role in the cell growth and cell proliferation. Pim1 is highly expressed in many toumours and is able to interact with the ribosomes through association with the ribosomal protein S19 (RPS19). To address the role of Pim1 in the translational control in prostate cells were used PC3 cells overexpressing Pim1 (with lentivirus infection) to investigate if its overexpression can cause variations in proliferation, cell cycle, polysomal distribution of specific translationally regulated mRNA (TOP mRNA) and in general protein synthesis. Finally, variations in the phosphorilation of proteins involved in the translational control, as AKT, eEF2, S6K, AMPK, were analysed and their changes were examinated after Pim1 overexpression, PP242 (mTOR inhibitor) treatment and Pim1 depletion. The results suggest that Pim1 is involved in translation, acting on proteins as S6K, eEF2 e AKT in an mTOR independent manner.

P21.2
The ubiquitin/26S proteasome pathway in the integration of developmental signals at the plant shoot apical meristem

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Ubiquitin-mediated degradation of regulatory proteins controls key events in plant development and response to the environment. Within the framework of a Scientific and Technological Cooperation between Italy and China (MAE-MIUR) we are investigating the involvement of ubiquitin-mediated proteolysis in the integration of developmental and environmental signals exerted by Knotted1-like homeobox (KNOX) and NAM/ATAF/CUC (NAC) domain transcription factors at the plant shoot apex. KNOXs and NACs play a key role in establishing the fine equilibrium between cell differentiation and stem cell maintenance in the shoot apical meristem (SAM), a population of undifferentiated cells at the tip of the shoot axis that establishes early during plant embryogenesis and gives rise to all shoot organs throughout the plant's life.
We produced transgenic Arabidopsis lines with constitutive or inducible alteration of the activity of a generic E2 UPS component and analyzed the effect on SAM function. *In silico* gene expression analysis and protein-protein interaction studies identified E2 and E3 enzymes putatively involved in the developmental pathways controlled by KNOX and NAC transcription factors.

P21.3
Regulation of plasma retinol-binding protein (RBP4) secretion by vitamin A

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Retinol (vitamin A alcohol) deficiency causes the inhibition of RBP4 secretion from hepatocyte and its accumulation in the endoplasmic reticulum (ER); retinol repletion promptly results in resumption of RBP4 secretion. We have characterized immortalized murine hepatocytes (3A cells), that respond to stress stimuli and represent an innovative tool for in vitro studies on liver function and a useful model to investigate the regulation of RBP4 synthesis and secretion. Similarly to what happens in vivo, in this cell line RBP4 gene transcription is not affected by retinol availability, but this ligand is essential for a regular secretion of the protein from the cell. Using 3A cells, we are studying the mechanism and factors that regulate apoRBP accumulation in the ER during vitamin A deficiency. In particular, we provide evidence of the interaction between RBP4 and ERp44, an ER resident protein involved in thiol-mediated retention mechanism that regulates the secretion of proteins with disulfide bonds, such as adiponectin. These data suggest that ERp44 is involved in the oxidative folding process and may be implicated in the apoRBP4 retention.

P21.4
Analysis of the signaling pathways activated in response to ribosomal stress

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Several studies have shown that any perturbation in the synthesis of ribosomes causes the activation of the so called "ribosomal stress response" leading to block of cell proliferation and/or apoptosis through the activation of p53. To address the molecular mechanisms involved, we used the erythroleukemia cell line K562C doxycycline-inducible for the expression of siRNA specific for ribosomal protein (RP)S19 mRNA. Downregulation of RPS19 causes a reduction of cell proliferation and a decrease in general protein synthesis. Further analysis showed that the inhibition occurs at the level of translation elongation and coherently we found an increase in the phosphorylation of eukaryotic elongation factor 2 (eEF2). eEF2 phosphorylation is mediated by a specific kinase eEF2K, that can be regulated by the mTORC1 downstream target S6 kinase (S6K), or by AMP-activated protein kinase (AMPK). Preliminary data indicate that mTORC1 activity is not altered whereas there is an increase of AMPK phosphorylation in K562C RPS19 depleted cells. We are now further addressing this issue to identify other components of the pathway connecting ribosomal stress with translational elongation inhibition.

P21.5
Development of monoclonal antibodies to block the polymerisation of Z alpha1 antitrypsin

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Alpha1 antitrypsin (AAT) is produced in the hepatocytes and secreted to the bloodstream, reaching the lungs where it inhibits neutrophil elastase. The Z variant of AAT (Glu342Lys) is a common mutant variant that causes polymerisation and retention of AAT within the endoplasmic reticulum, predisposing to liver disease. The lack of circulating AAT leads to early onset emphysema, due to i) an imbalance between AAT and neutrophil elastase that results in tissue destruction, and ii) the inflammatory effect of polymers themselves. Here we aimed to generate monoclonal antibodies (mAb) able to block the polymerisation of Z AAT whilst preserving its inhibitory activity. To this end, mice were immunized with purified monomeric active human Z AAT and mAb were produced following standard procedures. We screened nearly 300 hybridoma clones for their ability to recognize the antigen, and approximately 50 positive ones were further tested for their ability to

block the polymerization of Z AAT in vitro. We obtained one positive result, the 4B12 mAb, which is able to block polymerisation at equimolar concentrations while allowing 36% of the normal inhibitory activity of Z AAT.

P21.6
Investigating the toxicity of neuroserpin polymers in neural progenitor cells

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Point mutations in neuroserpin (NS), a serine protease inhibitor secreted by neurons, are responsible for the dementia familial encephalopathy with neuroserpin inclusion bodies (FENIB). Mutant NS undergoes polymerisation within the endoplasmic reticulum, causing NS retention, loss of activity and cytotoxicity, leading to neuronal death. Current models for FENIB recapitulate the cellular handling of these mutant forms, but fail to explain cell toxicity.
We have developed a new cell model to study the mechanisms involved in the toxicity of NS polymers by means of mouse neural progenitor cells stably transfected with the highly competent pTP6 vector expressing wild type NS (WTNS), or the S52R and G392E mutant variants that are responsible for moderate and severe forms of FENIB, respectively. Our SDS PAGE and western blot results showed the expression of NS in cell lysates from every cell line and the presence of WTNS in the cell medium. Moreover, NS polymers were detected in cell lysates and culture media of S52R and G392E NS expressing cells by non-denaturing PAGE and western blot analysis. These results validate our cell model and set the basis for our studies of NS polymer toxicity.

P21.7
Design and accomplishment of synthetic ion channels

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Kcv is a unique K+ channel formed by the pore module only, without any obvious regulatory domains (1). Kcv is therefore an interesting building block in the construction of synthetic channels according to the "lego-logic" that consists in adding regulatory modules to the Kcv pore unit. Building on our prior in-depth knowledge of Kcv structure-function, we have started a line of research that aims at building synthetic Kcv channels with new properties. So far we have engineered: a voltage-gated Kcv, by adding the voltage sensor unit of CiVSP, a phospahatase of C. intestinalis (2), to the N-terminus of Kcv (3); a calcium-regulated Kcv, by adding a calmodulin moiety to the N- and a M13 peptide to the C-terminus of Kcv; more recently, we have obtained a blue-light regulated Kcv by adding to its N-terminus the light sensor module (LOV2) of the plant photoreceptor phototropin. All constructs form functional channels in Xenopus oocytes. Each channel maintains the K+ selectivity of the Kcv pore but shows unique gating properties specifically determined by the added modules. Even though in some cases we still have to optimize the control on gating exerted by the exogenous module, we consider these results a proof of principle that the modular building approach is correct and that Kcv is indeed a suitable choice. The achievement of a light-regulated K+ channel will be particularly relevant for the new emerging field of optogenetics that uses light-gated channels to trigger or to inhibit action-potentials in neurons (4). (1) Plugge B, Gazzarrini S, Nelson M, Cerana R, Van Etten JL, Derst C, DiFrancesco D, Moroni A, Thiel G. A potassium channel protein encoded by chlorella virus PBCV-1. Science.2000 287(5458):1641-4. (2) Murata Y, Iwasaki H, Sasaki M, Inaba K, Okamura Y. Phosphoinositide phosphatase activity coupled to an intrinsic voltage sensor. Nature. 2005 435(7046):1239-43. (3) Arrigoni C, Cosentino C, Schroeder I, Hansen UP, Van Etten JL, Thiel G, and Moroni A. A synthetic voltage-gated potassium channel engineered from evolutionary unrelated protein modules. Submitted. (4) Fenno L, Yizhar O, Deisseroth K. The development and application of optogenetics. Annu Rev Neurosci. 2011;34:389-412.

P21.8
Role of eIF6 phosphorylation and its activity in tumor growth

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The translational machinery is controlled by Eucariotic Initiation Factor 6 (eIF6) that is able to limit 60s availability and so to prevent 80S subunit formation.
Several studies showed that PKCβII can phosphorylate eIF6 on the phosphosite Serine 235, and that mutation of Ser235 results in reduced tumorigenesis in vitro and in impaired tumor growth, in vivo. Furthermore the overexpression of eIF6 and PKCβII has been evidenced in most tumor cells. These data suggest that eIF6 phosphorylation is involved in tumor progression, raising the question on which oncogenes act on it.
Enzastaurin is a PKC beta blocker recently introduced in lymphomas trials. Since eIF6 is phosphorylated by RACK1/PKC, we tested the action of Enzastaurin in a malignant pleural mesothelioma model. Preliminary data indicate that Enzastaurin does not significantly affect cells growth, but reduces eIF6 phosphorylation in dose-time dependent manner. The effect of Enzastaurin in tumor growth in vivo is being analysed and next studies aim to elucidate the role of eIF6 phosphorylation event in tumor growth.

P21.9
The Arabidopsis COP9 SIGNALOSOME interacting F-BOX KELCH 1 protein forms an SCF ubiquitin ligase and regulates hypocotyl elongation

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The regulation of protein turnover by the ubiquitin proteasome system (UPS) is a key posttranslational mechanism underlying diverse cellular processes. One of the key components of the ubiquitin proteasome system, the COP9 signalosome (CSN), regulates E3 ubiquitin ligases belonging to the cullin-ring family. The CSN is conserved from yeast to animals and has been shown to regulate many aspects of plant development.
We have isolated a new plant-specific protein co-purifying with the COP9 signalosome (CSN) in cauliflower, which we denominated CFK (COP9 INTERACTING F-BOX KELCH). We show that CFK1 is and F-box protein and a component of a functional ubiquitin ligase complex. We further show that CFK1 stability is regulated by the CSN and by proteasome-dependent proteolysis, while light induces accumulation of the CFK1 transcript in the hypocotyl. Analysis of loss of function and gain of function lines indicates that CFK1 promotes hypocotyl length by inducing cell expansion, downstream of gibberellin. We propose a model in which light, CSN and the proteasome might control cell expansion by controlling CFK1 levels.

P21.10
ΔNp63α acetylation is impaired in natural p63 mutants

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The p63 transcription factor, homolog to p53 plays a crucial role in

epidermal and limb development. Dominant mutations in p63 gene give rise to several human congenital syndromes like Split-Hand/Footh Malformations (SHFM) caused by mutations of lysine K193 to glutamic acid (E). p53 transcriptional activation is dependent on lysine acetylation driven by p300 acetyl-transferase; a new lysine acetylated by p300 was identified in p53 (K164) is conserved in p63 and it corresponds to K193. We have evidences that p300 acetylates, *in vitro*, a p63 peptide centered on K193. p300 interacts with p63 in human cell lines and p300 overexpression increases p63 half-life while p300 silencing reduces p63 stability; interestingly, p63K193E mutant is insensible to p300. In addition, p300 enhances p63 transactivation potential on the p63 target genes promoter; interestingly, the K193E mutation seems to be a promoter specific mutation, since it has reduced transactivation potential on development related promoters but not on cell-cycle regulated promoters.
Our results suggest that p300 acetylates p63 on K193 lysine, mutated in SHFM-IV patients.

O21.1
Human rpL3 induces mitochondrial apoptosis in Calu-6 cells through activation of p21 expression

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It has recently reported that some ribosomal proteins regulate, through extraribosomal functions, the cell cycle and apoptosis in response to nucleolar stress. Defects of ribosome assembly promote the binding of some ribosomal proteins to MDM2, activating p53 and p21 to induce cell cycle arrest or apoptosis, depending on the cellular context. Several proteins involved in ribosome assembly, such as Nucleophosmin (NPM), have been proposed as positive regulators of p53-independent p21 expression. We have previously reported that NPM binds to rpL3 in the context of rpL3 expression autoregulatory circuit (Russo et al., NAR, 7576-85, 2011). We wondered whether this interaction could occur also in the regulation of p21 expression. To verify this hypothesis, we first analyzed changes in p21 protein levels in p53-null Calu-6 cells upon rpL3 overexpression. We observed that rpL3 enforced expression resulted in an increase of p21 protein levels. Interestingly, we detected that the rpL3-mediated p21 upregulation activates mitochondrial apoptosis in Calu-6 cells, abrogated by p21 silencing. These data indicate that mitochondrial apoptosis activated by rpL3 overexpression may be p21-dependent.

O21.2
Mechanism of insoluble protein body formation by the maize storage protein γ-zein

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Maize seed storage proteins (zeins) accumulate as insoluble heteropolymers (protein bodies) in the endoplasmic reticulum (ER). γ-zein is a major component, assembles into protein bodies also when synthesized in the absence of its partners and is composed of a C-terminal domain with four intrachain disulfide bonds, preceded by an N-terminal part that contains seven additional Cys residues and a Pro-rich repeated region. By transient expression in tobacco protoplasts, we have analyzed the relationships between assembly, solubility and intracellular traffic of γ-zein in which either the first two, six or all Cys residues of the N-terminal part have been mutated to Ser. Our results indicate that γ-zein can assemble as soluble dimers, most probably through hydrophobic interactions between the repeated domains. In the absence of further assembly, these dimers are largely secreted and in part sorted to the vacuole The formation of interchain disulfide bonds, to an extent that depends on the availability of Cys residues in the N-terminal half, allows further assembly into insoluble polymers and inhibits traffic from the ER. Supported by the 2006 Accordo Quadro CNR-Regione Lombardia.

O21.3
Cap dependent translation contributes to viability and resistance of myeloma cells to bortezomib

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Multiple myeloma (MM) is a blood malignancy. Proteasome inhibitors like Bortezomib have doubled life expectancy, but MM patients develop resistance to therapy. Bortezomib acts through the induction of the Unfolded Protein Response (UPR), i.e. an accumulation of misfolded proteins that can cause a lethal stress response. By this theory, increasing the proteasome load by the stimulation of translation may worsen the UPR. We evaluated the crosstalk between translation and Bortezomib toxicity in multiple myeloma cells. Bortezomib toxicity did not correlate with induction of the UPR but caused a late reduction in global translation. The reduction of translation was accompanied by dephosphorylation of the mTORc1 target 4E-BP1. Infection of myeloma cells with mutant forms of 4E-BP, constitutively dephosphorylated, worsened Bortezomib induced cell death. Since mTORc1 inhibitors cause pharmacological inhibition of 4E-BP phosphorylation, we tested whether they could act synergistically with Bortezomib. We found that rapamycin and PP242 induce the arrest of myeloma cells. We provide a rationale for treating patients with mTOR inhibitors, independently from their response to Bortezomib.

O21.4
The enigmatic, putative potassium channel AtKCO3 of Arabidopsis tonoplast

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AtKCO3 is the only putative voltage-independent K^+ channel subunit of Arabidopsis thaliana with a single permeation pore domain. We show that overexpressed AtKCO3 or AtKCO3::GFP proteins are efficiently sorted to the tonoplast, but vacuoles isolated from these transgenic plants do not have significant alterations in current density. Consistently, analysis of KCO3 knockout plants did not reveal marked growth alterations. Because the permeation pore of K^+ channels is formed by four copies of the pore domain, we have investigated whether KCO3 assembles into tetramers. Upon velocity gradient centrifugation, both AtKCO3 and AtKCO3::GFP were detected as homodimers, an assembly state that therefore would not allow for activity. We conclude that if AtKCO3 functions as a K^+ channel, active tetramers are either held by particularly weak interactions, different from those that allow dimer formation, or are assembled only under unknown, specific physiological conditions. Supported by EU Marie Curie RTN 'Vacuolar Transport Equipment for Growth Regulation in Plants' (MRTN-CT-2006–035833) and by the 2008 PRIN Program.

O21.5
Mutations in Neuroligin 3 and activation of an ER stress response

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Mutations in the genes encoding for synaptic adhesion molecules have been linked to autism spectrum disorders. Neuroligins (NLGNs) are post-synaptic cell-adhesion proteins interacting with presynaptic

partners, the Neurexins, in the specification of synapse identity. The most characterized autism-linked mutation in the NLGN3 gene is an Arg451 to Cys substitution which causes ER retention and decreased surface expression of NLGN3 both in cellular and mice models. The KI mice expressing the NLGN3 R451C mutation show a gain of function compared to the NLGN3 KO mice. Our unpublished data suggest that the R451C mutation activates an ER stress response in over-expression studies indicating a gain of function caused by the mutant protein at the cellular level. We have originated inducible PC-12 Tet-on cell lines expressing either NLGN3 WT or mutant proteins show UPR activation through the ATF6 pathway and offer the opportunity to study possible alteration of functional properties caused by the mutation in relation to ER stress.

22 - Environmental and molecular mutagenesis

P22.1
Automated scoring of lymphocyte micronuclei: an approach to validate the Metafer slide scanning platform

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The micronucleus (MN) assay is one of the best validated techniques in genetic toxicology and in human biomonitoring. Two main technical problems hamper the widespread use of the assay: a) the reproducibility of the results which is still operator dependent and b) the visual scoring of thousands binucleated cells. The automation of scoring seems to be the most promising strategy to overcome these limitations. The automated system used in our study is the Metafer 4. Our first approach to validate the system was carried out through the evaluation of the dose-effect calibration curve for MN frequency in peripheral lymphocyte from healthy subjects in vitro treated at six dose levels of 137 Cs gamma rays in the range 0-2 Gy. We applied the standardized procedure for sample processing and slide preparation and we used fluorescent dye DAPI. A number of classifiers and parameters for the automated search were tested. The comparison between visual and automated scoring showed lower MN frequency levels in automated analysis than in visual counting. The system failed to identify MNi if they are close or attached to the main nuclei or if there are more than one MN in the same BN cell. The analysis of the results allowed to modify critical steps in the experimental protocol and in system set up improving the detection of BN cells and MN. A good correlation (R= 0,94) between visual and automated scoring was observed over the dose range of ionizing radiation 0-4 Gy.

P22.2
In vitro testing for cytotoxicity and genotoxicity of seeds and essential oil of *Foeniculum vulgare* Mill

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Fennel (*Foeniculum vulgare*) infusions are used in traditional medicine both in Asia and Europe, in particular for the prevention of infantile colic and flatulence. The pharmacological activity of Fennel teas is due to several essential oil components such as estragole, an alkylbenzene that has recently drew attention to the scientific community. In fact, it has been reported to possess genotoxic activity in laboratory animals. However animal studies were conducted using high estragole concentrations. In addition, it has been shown that an agent administered in its isolated form may have different effects than applied in natural multicomponent mixtures.
In this approach, we have evaluated the potential cyto- and genotoxicity of Fennel seeds lyophilised extract, very fine Fennel seeds powder and Fennel seeds essential oil; estragole was also tested as reference compound. *In vitro* testing was performed on human hepatic HepG2 cell line. Cytotoxicity was assessed by MTT test, DNA damage was evaluated by both comet assay and micronucleus test. Neither the 3 considered herbal preparations nor the reference compound (estragole) caused cytotoxic or genotoxic effects in human HepG2 cells.

P22.3
Aneuploidogenic effects in peripheral blood lymphocytes exposed *in vitro* to differently sized-AuNP

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Owing to the possible translocation of NP following exposure, it is important to assess biological effects of gold nanoparticles in the major route of distribution in the whole organism. The cytotoxic and genotoxic effects were thus evaluated in primary cultures of human peripheral blood lymphocytes exposed *in vitro* to spherical AuNP citrate-buffered (5, 15, and 40 nm) in a range of mass concentration from 0.1 to 10^3 µg/ml. Cell viability in whole blood was determined by MTT assay and Trypan Blue dye exclusion. The proliferative activity, cytotoxicity, apoptotic markers and genotoxicity were assessed by the cytokinesis-block micronucleus cytome assay. Fluorescence *in situ* hybridization with human pancentromeric probes was applied to distinguish between clastogenic and aneuploidogenic effects. Comet assay was applied for detecting DNA strand breaks and oxidative DNA damage. Citotoxicity assays revealed a statistically significant dose-dependent decrease of viable cells. AuNP genotoxicity showed a dose-dependent and size-dependent trend. Moreover, FISH analysis data revealed a clear cut increase of centromere-positive micronuclei in a size-independent way. *Supported by the Research Project "NanoReTox" funded under 7th EU FWP (Ref. No. 214478)*

P22.4
Cyto- and genotoxic effects in murine alveolar macrophages after exposure to differently sized and shaped CuO nanoparticles

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Reports emerging from literature studies are identifying a strong influence of geometry and surface properties of nanomaterials on their ability of causing cell damage. For this purpose, we investigated the relationships between size and shape of engineered copper oxide nanoparticles and their cytotoxic and genotoxic effects on murine macrophagic RAW264.7 cultures. The cytotoxic responses have been examined through cell viability analysis (MTT assay) along with the cytostatic and genotoxic effects (Cytome assay with cytokinesis block, Comet assay and Comet assay modified by enzymes for the detection of oxidative DNA damage) in RAW264.7 cultures after exposure to three forms of CuO nanoparticles: CuO Rods, CuO Spheres and CuO Spindles. Our results show that the CuO Spheres exerted greater cytotoxic action than other nanomaterials tested while an increase of genotoxic responses was found after CuO Rods exposure. In our experimental system the size and shape seem to interfere with the cytotoxic and genotoxic effects in a differential way. *Supported by the Research Project "NanoReTox" funded under 7th EU FWP (Ref. No. 214478)*

P22.5
Dolichopoda crickets as potential bioindicator of genotoxicity in radon-contaminated confined environments

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Radon represents the major source of natural radioactivity in confined environments. Despite experimental evidence of a direct association between residential exposure and human lung cancer, contradictory results are present relating indoor exposure and genotoxic effects. Our aim

is to evaluate the genotoxic potential of different radon concentrations, from 221 up to 26,000 Bq m-3, in wild cave-cricket (Dolichopoda) populations sampled from six caves in central Italy. Specimens were sampled from each cave and tested for radon-induced DNA-damage by Comet assay in haemolymph and brain cells. Individuals collected from the least radioactive cave, housed for 60 days before analysis, were used as controls. Statistically significant increases of DNA damage were found in both cell types of individuals from all caves. The extremely low control values also indicated a good responsiveness of these organisms to environmental variations. Results indicate cave crickets as reliable tool for detection of genotoxic potential of air-polluted confined environments and can be proposed as bio-indicator system for indoor exposure to air radioactive-pollution.

P22.6
Biological effects in human neuroblastoma cells following *in vitro* co-exposure to extremely low frequency electromagnetic fields and aluminum

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Epidemiological studies have implicated both aluminum (Al) and extremely low frequency (50 Hz) electromagnetic fields (ELF-EMF) exposure in the onset of neurodegenerative diseases (ND). Many experimental evidences have pointed out the ability of Al to induce cellular oxidative stress, a key feature of ND. Whereas, the possible role of ELF-EMF in the pathogenesis of these diseases is still unclear, even though recent studies have reported the capacity of this physical agent to interact with chemicals thus enhancing or inhibiting their effects on biological systems.
Considering that, the aim of this study was to investigate in the neuroblastoma cell line SKNBE(2) whether 1 h co-exposure to Al (4 to 40 μM $AlCl_3$) and ELF-EMF (50 Hz; 10 μT to 1 mT) could exert biological effects. Genotoxicity was assessed by the comet assay, whereas induction of cytoprotective heat shock proteins 70kDa (Hsp70) was determined by Western blotting analysis. Results showed that ELF-EMF and Al did not cause any significant increase in either DNA damage extent and Hsp70 expression in SKNBE(2) cells. To elucidate the impact of co-exposure to ELF-EMF and Al, further studies are planned with different cell lines.

P22.7
Radiation-induced DNA damage and RNA changes in mouse sperm

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Ionizing radiation induces cytotoxic and genotoxic effects in testis cells, however its impact on the male gamete epigenome is still unknown. New findings suggest that sperm RNAs are delivered to the fertilized oocyte, and have a role in the development of early embryo. With the aim of assessing DNA damage and RNA/microRNA changes in spermatozoa deriving from irradiated testicular cells, mice were irradiated with 1 Gy X-rays, and sacrificed 2 or 6 weeks after irradiation to assess DNA and RNA alterations induced by exposure of spermatogonial and post meiotic testicular cells.
DNA/chromatin integrity was assessed by comet and Sperm Chromatin Structure assays. Results demonstrate non-targeted DNA breaks in spermatozoa deriving from surviving irradiated spermatogonia. In total RNA extracted from spermatozoa, the presence of specific microRNAs (miR-34b/c) and transcripts associated to fertility (Clusterin, AKAP4, WNT5A, HSBP1, FOXG1B, CLGN) and to motility (CRISP2, LDHC) was demonstrated, and their concentration was assessed in comparison to testis and liver relative abundance. Preliminary data suggest that X rays modulate the sperm levels of some of the analyzed RNA molecules.

P22.8
Mitochondrial DNA dysfunction in relation to PAH exposure

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Polycyclic aromatic hydrocarbons (PAHs) are established lung carcinogens, that cause nucleus and mitochondrial (Mt) DNA damage. Nucleus and Mt DNA damage are also associated to lung cancer risk. Aim of this study was to explore Mt DNA alterations in relation to PAH exposure.
We examined PAH exposure (urinary 1-pyrenol) and the consequent nucleus DNA alterations (BPDE-DNA adduct, micronuclei, telomere shortening, and p53 methylation) in relation to MtDNA copy number (mtDNAcn) in the PBLs of coke-oven workers (n=46) chronically exposed to PAH and matched controls (n=44). MtDNAcn was measured by a RTPCR assay and is expressed as the ratio between MtDNAcn (MT) to copy number of a single copy gene (S) (MT/S).
MtDNAcn (MT/S) geometric means (GM) unadjusted and adjusted by age were higher in heavily exposed (1-pyrenol >3 μmol/mol creatinine) coke-oven workers [GM 1.06 and 1.07 MT/S] compared to controls [GM 0.89 and 0.89 MT/S] ($p<0.05$). In the whole study population MtDNAcn (ln MT/S) was positively related to PAH exposure ($p=0.0324$) and BPDE-DNA adduct ($p=0.0552$).
Our results indicate that MtDNAcn is related to PAH exposure and could be a central event in PAH carcinogenesis.

P22.9
Pro-genotoxic activity of hyperglycemia

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Diabetes and metabolic syndrome are metabolic disorders characterized by elevated level of glucose in the blood. Hyperglycemia is known to perturb the balance between oxidative stress and antioxidant defense mechanisms in the cells, thereby altering the response of biological system towards various toxic xenobiotics. The aim of this *in vitro* approach was to test hyperglycemia for pro-genotoxicity in human liver cells (HepG2 cell line). Increased cell susceptibility to genotoxic xenobiotics was tested by challenging cell cultures with 4-nitroquinoline-1-oxide (4NQO) as model genotoxic compound. The extent of primary and oxidative DNA damage has been evaluated by the alkaline single-cell microgel-electrophoresis (comet) assay. The results showed a clear correlation between increasing concentrations of glucose in the medium and increasing extent of DNA damage caused by the test (fixed) concentration of 4NQO. Ongoing experiments are underway to evaluate the role of hyperglycemic conditions in altered expression patterns of genes related to inflammation and proliferation processes, apoptosis and antioxidant defense. Gene expression will be evaluated by real-time PCR array.

P22.10
An evaluation of Titanium Dioxide genotoxicity in human lymphocytes

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Titanium Dioxide (TiO2) is a widespread nanomaterial despite little is known about its genotoxicity. In this study, an evaluation of genotoxicity of anatase TiO2 nanoparticle (NP) and fine (F) forms is carried out in isolated human lymphocytes using micronucleus and comet assays and 8-OxoG detection. TiO2 forms, characterized by SEM, displayed an average size above 200nm, for both forms. Interaction of particles with lymphocytes was evaluated by the side scatter (SS) parameter in flow cytometry. Preliminary results indicate the presence of a cell subpopulation (about 17% of all lymphocytes) with a dose dependent increase in SS respect to control after treatment with both forms. For micronucleus assay, lymphocytes were treated for 28 and 48h with 50-200ug/ml. No micronucleus induction with TiO2 NP and a slight increase with the F was shown. No decrease in cell proliferation was observed. For comet assay, cells were treated for 24h with 50-400 ug/ml of TiO2 NP and F. A significant increase in both tail moment and length values was observed after treatment with TiO2 NP whereas preliminary results with TiO2 F suggest a slight but not significant increase of both parameters with dose.

O22.1
Induced mutations for the Lycopene cyclase ε genes by TILLING in durum wheat

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During the last two decades, DNA sequencing has led to availability of gene sequences in model plants encouraging the development of alternative strategies to create novel alleles in specific genotypes of crop species. One of these new emerging technologies is TILLING (Targeting Induced Local Lesions In Genomes) that combines random chemical mutagenesis with high throughput discovery of the induced mutations in target genes. In the present study we developed a new durum wheat TILLING population by treating seeds (cultivar Aureo) with 0.60% ethyl methanesulfonate. The first screening of the M2 mutagenized population concerned the Lycopene cyclase ε genes (LYC-ε) involved in the carotenoid biosynthesis. Non functional alleles of LYC-ε gene is expected to block or reduce the metabolic flux into lutein, thus leading to accumulation of β-carotene. The genomic DNA isolated from 1152 M2 plants, pooled fourfold and organized into 96-well format, were analyzed in the first screening of the TILLING population. Five and three mutants were found for LYC-ε-A genome and for LYC-ε-B genome, respectively, and molecularly characterized.

O22.2
Role of telomeres on chromosome instability induced by oxidative stress in human primary fibroblast

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Telomeres are nucleoprotein structure located at the end of linear chromosomes and their primary role is to maintain chromosome stability. Due to their high content of guanines and their low efficiency in DNA damage repair, telomeres were demonstrated highly sensitive to damage by oxidative stress. This work has the aim to evaluate the role of telomeres on chromosome instability induced by oxidative stress. We used human primary fibroblast treated with H2O2. We evaluate the effect of hydrogen peroxide on cells cycle, and the single strand breaks induced. The results on telomere length showed a significant telomere shortening after 48 hour of treatment. To evaluate the chromosome instability we have analyzed a special biomarkers of instability closely related with a dysfunctional telomere, such are micronuclei, nuclear buds and nucleoplasmic bridges. Our results showed an increase of NBUDs and NPBs related with telomere dysfunction. Taken together our data showed that telomere shortening could account for chromosome instability and that identifying a connection between the two can contribute to understanding the mechanism of chromosome aberration formation.

O22.3
Size-independent cytotoxicity and size-dependent genotoxicity of AuNP in murine alveolar macrophages, evaluated by two different dose-metrics

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There is an increased interest in nanoparticles mechanisms of bioreactivity due to the lack of an in-depth evaluation of the physical-chemical properties responsible for their toxic effects. In this study we evaluated the cyto- and genotoxicity of gold nanoparticles of different sizes (5, 15, and 40 nm) in murine Raw264.7 cells as model of alveolar macrophages. To accurately measure the possible dose- and/or size-dependent toxicity of the AuNP, we performed a definite assessment of their bioreactivity, using two different dose-metrics. In the first approach, we exposed our model system at several mass concentrations of AuNP while in the second approach we used absolute numbers of AuNP. We observed AuNP-induced toxicity, namely a reduction of cell viability, micronuclei induction, presence of DNA fragmentation and oxidation, and cytostatic responses, differentiated in apoptotic and necrotic. Interestingly, we found that for both approaches, the cytoxic effects in the exposed macrophages were directly related to the concentration of the AuNP in a size-independent fashion while the genotoxic effects may be related to the size and the AuNP-15nm the most effective. *Supported by the Research Project "NanoReTox" funded under 7th EU FWP (Ref. No. 214478)*

O22.4
Characterization of the sensitivity of HT29 cell line to different chemoprotective phytochemicals

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Phytochemicals are plant-based chemicals that mediate their positive health benefits affecting specific molecular targets such as genes, or stabilizing conjugates affecting metabolic pathways. Their mechanisms of action are not fully understood but they are probably dependent on the type of cell and tissue involved.
Aim of our study was to characterize the specific response of HT29 (human adenocarcinoma cell line) to the antiproliferative and antioxidant effect of different phytochemicals such as ferulic acid, β-carotene, 2-Hydroxycinnamic acid, lutein, p-coumaric acid, ascorbic acid and 20-hydroxyecdysone. The antiproliferative activity was evaluated by MTS assay that determine the number of viable cells in proliferation after treatment with increasing concentration of phytochemicals. Antioxidant activities were assessed through alkaline Comet Assay on 24h pre-treated HT29 cell line as reduction of DNA migration induced by H_2O_2. Preliminary data suggests that these molecules induce an increase of

endocellular defenses. A complex relationship between the proliferation inhibition and the antioxidant activity emerged.

O22.5
Preliminary results on mutagenic effects of exposure to 900 MHz Radiofrequency radiation: enhancement of SAR increases the MN induction in exposed root cells of *Vicia faba*

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The massive diffusion of Radiofrequency emitting devices such as mobile phones, has created growing concern about possible adverse health effects. Several experimental data are now available in the literature relating SAR (Specific Absorption Rate) values of RF radiation to induction of a wide spectrum of adverse effects at cellular and tissue level. Yet, contradictory results have been found on cancer induction and genotoxic-mutagenic effects obtained on different mammalian cell systems. Studies in plant cells (*Tradescantia* and *Allium cepa*) exposed to RF showed the induction of mitotic disturbance and of chromosomal aberrations. Our aim is to evaluate the mutagenic effect, through the micronucleus (MN) test, of 900 MHz RF irradiation at different SAR values in *Vicia faba* root cells. Plants were exposed for 72h to RF in a TEM (Transverse Electro Magnetic) cell at the SAR values of 1 and 2 W/Kg. An outstanding increase of micronucleus frequencies was found with increasing SAR value. This result poses a warning about possible human health effects of radiofrequency exposure.

23 - Regulation of transcription

P23.1
Characterization of the incoherent feedforward loop governing quorum sensing in *Pseudomonas aeruginosa*

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Quorum sensing (QS) is a communication system that controls virulence-related phenotypes in the human pathogen *Pseudomonas aeruginosa*. The QS receptor LasR responds to the QS signal molecule $3OC_{12}$-HSL and promotes signal production by increasing the transcription of the $3OC_{12}$-HSL synthase gene, *lasI*. LasR also activates the expression of other genes, including the *lasI* transcriptional repressor RsaL, generating a regulatory network motif known as incoherent feedforward loop (IFFL). This network motif confers peculiar properties to the QS circuit of *P. aeruginosa*, including robustness with respect to $3OC_{12}$-HSL fluctuations.
Here we provide evidence that the IFFL generated by LasR and RsaL provides robustness with respect to LasR fluctuations to a sub-group of QS-controlled phenotypes like the production of the virulence factor pyocyanin. Conversely, LasR-controlled virulence factors that are not regulated by RsaL (*e.g.* proteases) are affected by variations in LasR concentration. This is particularly relevant considering the number of environmental and metabolic stimuli known to modulate LasR levels.
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P23.2
Transcriptional regulation of miR-200 family in colon cancer

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A widespread deregulation of miRNAs is commonly observed in human cancers and promotes cellular transformation and tumorigenesis. Thus, miRNAs may be potential targets for cancer therapy; still, the mechanisms through which miRNAs are regulated in cancer remain unclear. Through a computational analysis on 60 kb around the TSS of 117 promoters of miRNA, whose expression is deregulated in colon cancer, we identified in 65 of them the conserved consensus motif (CCAAT) for the transcriptional factor NF-Y. Among them the 2 promoters of miR-200 family members contain multiple CCAAT in a region of 3 kb around the TSS. These miRNAs play an essential role in tumour suppression by inhibiting EMT transition. Using ChIP experiments performed in human colon adenocarcinoma cells, we show that NF-Y directly binds miR-200 promoters; this binding correlates with the appearance of open chromatin marks. Loss of NF-Y binding, by over-expressing a dominant negative mutant form of NF-Y, leads to the down-regulation of both pri-miRNAs and mature miRNAs, demonstrating that NF-Y activates miR-200 family members. Finally, through a computational analysis, we identified NF-Y as putative target of miR-200.

P23.3
Wnt4 expression in thyroid cells is modulated by the transcription factor Pax8

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The transcription factor Pax8 is expressed during thyroid development and is involved in the morphogenesis of the thyroid gland and maintenance of the differentiated phenotype. The silencing of Pax8 expression in FRTL-5 thyroid cells and the analysis of the gene expression profile identified Wnt4 among the downregulated genes. Wnt4 has been implicated in several developmental processes including regulation of cell fate and patterning during embryogenesis.
We analyzed the molecular mechanisms by which Pax8 could regulate Wnt4 expression in thyroid cells. Analysis of the 5'-flanking region of the Wnt4 gene identified putative Pax8 binding sites confirmed by EMSA and ChIP. Moreover, transfection of FRTL-5 cells with progressive deletions of the 5'-UTR of Wnt4 showed that all constructs possess a thyroid-specific activity due to the transcription factor Pax8. Overall, our results show that Pax8 is involved in the transcriptional regulation of the Wnt4 gene. Interestingly, we also revealed that the expression of Wnt4 in FRTL-5 cells is TSH dependent.
Taken together, our data indicate that in thyroid cells Pax8 participates to Wnt4 gene expression directly binding to its 5'-flanking region suggesting that Wnt4 is a new target of this master regulatory gene and we hypothesize that in the reduced expression of Wnt4 correlates with the alteration of the epithelial phenotype.

P23.4
Polymorphysm rs6314 and alternative splicing of the 5HT2a gene may contribute to altered gene expression and to psychiatric disease

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Serotonin receptor 2a (5HT2a) signaling is important for modulation of cortico-striatal pathways and prefrontal activity during cognition. A SNP in the 5HT2a gene (rs6314, C>T) implies a missense substitution at amino-acid 452 of the receptor (his>tyr) and has been associated with differential 5HT2a signaling and with physiological as well as behavioral effects. Our bioinformatic analysis predicted that rs6314 alters splicing signals and 5HT2a expression. rs6314 variation destroys the binding site for SRp40, a protein that binds exonic splicing enhancer and promotes the efficient and/or accurate splicing of pre-mRNA. Moreover, the rs6314 T allele was associated with lower mRNA and protein expression in vivo and in HeLA cells. NCBI database reports two HTR2A transcript variants (the smaller lacking exon 2) encoding different isoforms. Using specific oligonucleotides, we demonstate that the alternative transcripts reported in NCBI are always present. qRT-PCR quantification of the two isoforms in post-mortem cingulate cortex revealed greater amount of the smaller isoform in patients with schizophrenia and with bipolar disorder relative to healthy individuals.

P23.5
Zyxin is a novel target for beta-amyloid peptide: characterization of its role in Alzheimer pathogenesis

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Zyxin is an adaptor protein recently identified as a novel regulator of the homeodomain interacting protein kinase 2 (HIPK2)-p53 signaling in response to DNA damage. We reported an altered conformational state of p53 in tissues from patients with Alzheimer's Disease (AD), due to a deregulation of HIPK2 activity, thus leading to an impaired and dysfunctional response to stressors.
Here we examined the molecular mechanisms underlying the deregulation of HIPK2 activity in two cellular models, HEK-293 cells overexpressing the amyloid precursor protein and fibroblasts from AD patients, starting from recent findings showing that zyxin expression is important to maintain HIPK2 protein stability. We demonstrated that both beta-amyloid 1-40 and 1-42 induce zyxin degradation, thus

affecting the transcriptional repressor activity of HIPK2 onto its target promoter, metallothionein 2A, in turn responsible for the induction of an altered conformational state of p53.
We demonstrated for the first time in AD the existence of a new target of Aβ activities, zyxin. These results may help to better understand the pathogenesis of AD, through the fine dissection of events related to Aβ activities.

P23.6
Triptolide induces CDK7-mediated block and degradation of RNA Polymerase II in human cells

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Triptolide, a natural product and strong transcriptional inhibitor, binds to XPB subunit of the general transcription factor TFIIH. It was known that it can induce degradation of the largest subunit Rpb1 of RNA polymerase II, however details of the molecular mechanisms remain to be clarified. In this study, we show that the cellular RNAPII decrease is subsequent to a block of the enzyme at promoters. Triptolide leads to Rpb1 hyperphosphorylation at Ser5 of the carboxy-terminal domain and Rpb1 ubiquitination. Kinase inhibitors and especially Cdk7 downregulation rescued the cellular and chromatin-bound Rpb1 decrease induced by triptolide. Our data show that triptolide triggers a Cdk7-mediated proteasomal Rpb1 degradation mechanism. Our data also show that triptolide induces Rpb1 degradation in different human cancer cell lines, and this drug activity is proportional to drug cytotoxic potency. Thus, we propose a possible mode of action of triptolide: its binding to XPB can cause a block of RNA polymerase at promoters by activating Cdk7, a TFIIH subunit. Cdk7 can then phosphorylate Rpb1 Ser5, inducing the subsequent Rpb1 ubiquitination and degradation.

P23.7
Role of WT1-ZNF224 interaction in the expression of apoptosis-regulating genes in leukemic cell lines

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The KRAB-zinc finger protein ZNF224 was originally identified as the transcriptional repressor of the human aldolase A gene[1]. Although the function of KRAB-ZFPs is largely unknown, they appear to play important roles in cell proliferation, apoptosis and cancer.
Previously, we demonstrated that ZNF224 is a novel WT1 transcriptional cofactor[2]. Wilms tumor protein 1, is a zinc finger transcriptional factor whose overexpression is associated with a poor response to therapy in leukemia.
In this work we have analyzed the role of WT1-ZNF224 interaction in the expression of apoptosis- regulating genes. By chromatin immunoprecipitation assays we demonstrated that WT1 recruits ZNF224 to the promoter of its target genes. A combination of over-expression and knockdown analyses revealed that ZNF224 acts as a co-activator of WT1 in the regulation of pro-apoptotic genes and suppresses WT1-mediated trans-activation of anti-apoptotic genes. Moreover, we observed that ZNF224 itself is modulated by cytosine arabinoside and Imatinib in chronic myelogenous leukemia K562 cell line, drugs widely used in the treatment of myeloid leukemia. Our findings suggest that the increased expression ZNF224 may represent an important event in the drug-induced apoptosis in leukemia cells.
[1]Medugno et al. Gene 2005;359:35-43
[2]Florio et al. HMG 2010;19:3544-56

P23.8
Altered DNA damage response in Down syndrome cells

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Down syndrome (DS) patients develop an Alzheimer-like neuropathology (AD) characterized by beta-amyloid (Aβ) deposition in the brain and neurodegeneration. However, the mechanisms of Aβ toxicity have not been completely elucidated. Aβ may induce alteration in p53 conformation thereby inactivating the apoptotic pathway, but it may also induce directly DNA damage. In this work we examined the relationship between Aβ overload, impairment of p53 activity and presence of endogenous DNA damage in DS fibroblasts. The results show that in DS fibroblasts there is an increase in unfolded p53, which could be dependent on deregulation of homeodomain-interacting protein kinase 2 (HIPK2). HIPK2 protein and activity are deregulated in AD fibroblasts however they are not altered in fibroblasts from DS patients. HIPK2 protein stability is maintained by zyxin, and we observed that zyxin basal expression is increased in DS fibroblasts, while it results downregulated in AD fibroblasts. DNA damage response is altered in DS fibroblasts as shown by deregulated expression of the DNA repair protein XRCC1, and of checkpoint protein phospho-Chk2.

P23.9
The major trascriptional activator of *KlPDC1*: Rag3

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Kluyveromyces lactis is a respiratory yeast in which fermentation is facultative, a style of living more similar to the majority of eukaryotic organisms than the fermentative yeast Saccharomyces cerevisiae. Moreover, in K. lactis there isn't redundancy of genes such as in S. cerevisiae. In this project we studied the expression of the gene (KlPDC1) encoding the principal enzyme involved in the fermentative pathway of pyruvate metabolism: Pyruvate decarboxylase. KlPDC1 expression relies on positive and negative control mechanisms, such as glucose induction, ethanol repression and a feedback regulation mechanism known as autoregulation. We identified Rag3 as the major transcriptional activator of KlPDC1 in the autoregulation mechanism. We've found that RAG3 expression depends on growth conditions and it's phosphorylated by hypoxia, suggesting a putative role in oxygen response. RAG3 is also involved in Thiamine biosynthesis, whereas Thiamine is an important cofactor of numerous enzymatic processes, including pyruvate decarboxylation. We also studied the interaction between Rag3 and Pdc1 and the localization of both proteins in function of their activities.

P23.11
NAFLD and CVD: a possible role of the liver as a warning of cardiovascular risk

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Background and aim: Non-alcoholic fatty liver disease (NAFLD), is considered the hepatic manifestation of the metabolic syndrome which is one of the main cardiovascular disease (CVD) risks. Mitochondrial damages and oxidative stress are also involved both in NAFLD progression and in CVD. Aim of the study is to evaluate the possible correlation between liver damage and CVD in a mouse model of liver steatosis and inflammation. **Material and methods:** C57BL/6J mice were fed with a chow (LFD) or a High Fat Diet (HFD). Livers and

hearts from mice fed for 3, 6 and 12 months were collected. RT² Profiler PCR Array System was performed to study a panel of genes involved in lipid metabolism, CVD risk, inflammation and cell cycle. **Results:** Preliminary data highlighted a different timing of metabolic changes and oxidative response between the two organs. In the liver of HFD mice, in fact, genes involved in lipogenesis and in glycolysis were induced earlier (3 months) than in the heart (6 months). Genes involved in CVD, inflammation and cell cycle had similar behaviour. **Conclusions:** Our work suggests that the evaluation of hepatic lipid metabolism alteration could be useful to predict CVDs.

O23.1
Transcriptional regulatory proteins binding to the promoters of ribosome biogenesis genes in *Saccharomyces cerevisiae*

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Ribosome biogenesis in yeast is known to require the coordinate expression of several hundred genes, including rRNA and ribosomal protein genes, coding for structural components of the ribosome, snoRNA and the so-called ribi genes, required for rRNA maturation and the assembly of functional ribosomes. Transcription of this large set of genes needs to be tightly co-regulated in order to finely orchestrate the most energy consuming process of the cell, with high expression levels in rapidly dividing cells and prompt repression under stress conditions.
Three proteins, Stb3, Dot6 and Tod6, have been identified as key regulators of ribi gene expression in budding yeast. They bind respectively to RRPE and PAC, two sequence motifs enriched in ribi gene promoters, and act as repressors by recruiting Rpd3 histone deacetylases and turning off transcription [1]. But are these the only elements responsible for regulation of the whole ribi regulon? We addressed this question by a detailed phylogenetic footprinting analysis of ribi gene promoters in Saccharomycetes group. We found a significant enrichment of other sequence motifs in addition to known RRPE and PAC, most of them being recognition sites for the General Regulatory Factors Abf1 and Reb1. What is their role? Do they serve to recruit individual factors or play a more direct role? Are they important for co-regulation or do they act as gene-specific factors?
[1] A.Huber et al., The EMBO Journal 30 (2011), 3052 - 3064.

O23.2
The NadR regulon: adhesins and diverse meningococcal functions are regulated in response to physiologically relevant signals

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The NadR regulator was shown to repress *nadA* (neisserial adhesin A) and play a role in its phase variable expression. In this study we elucidated the NadR regulon and the responses of these genes to physiologically relevant signals.
Microarray analysis revealed over 30 genes deregulated in the NadR mutant and we defined the NadR regulon through *in vitro* DNA-binding assays. All NadR-regulated genes investigated were found to respond to 4HPA, a small molecule secreted in saliva, which was previously shown to induce *nadA*. Two types of 4HPA-responsive regulation, corresponding to two types of promoter architectures, were observed: while NadA and the majority of NadR targets (type I) are induced, only the MafA adhesins (type II) are corepressed in response to 4HPA. This alternate NadR-mediated regulation was confirmed after incubation in saliva. We demonstrated that type I promoters exhibit two upstream NadR binding sites, while type II exhibit one downstream extended NadR binding site.
We conclude that, through a sophisticated dual mechanism, NadR coordinates a wide transcriptional response to signals present in saliva enabling the meningococcus to adapt to the relevant host niche.

O23.3
AMOTL2 interaction with TAZ causes the inhibition of surfactant proteins expression in lung cells

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TAZ (Transcriptional co-Activator with PDZ-binding motif), also called WWTR1, is a 14-3-3-binding molecule and functions as a transcriptional co-activator by binding to the PPXY motif present in several transcription factors. The Hippo pathway regulates TAZ activity through a phosphorylation-mediated mechanism that causes its cytoplasmic sequestration or degradation. Here we describe a Hippo pathway-independent mechanism that modulates the activity of TAZ through the interaction with Angiomotin-like 2 (AMOTL2). We show that AMOTL2 robustly co-immunoprecipitates with TAZ, and their interaction is dependent on the WW domain of TAZ and the PPXY motif in the N-terminus of AMOTL2. Furthermore, we demonstrate that AMOTL2 colocalizes with TAZ in the cytoplasm in H441 human lung cells and regulates TAZ cytoplasm-to-nucleus translocation through direct protein-protein interaction. Since the expression of the Surfactant protein C in respiratory epithelial cells is dependent on the cooperation between the transcription factor TTF-1 and TAZ, we used a luciferase assay to verify whether AMOTL2 could inhibit the transcriptional cooperation of the two factors. Taken together, our results suggest an inhibitory role of AMOTL2 on TAZ ability to co-activate transcription and reveal a novel mechanism to modulate the activity of TAZ through physical interaction with AMOTL2.

O23.4
Antisense non coding RNAs induced by topoisomerase I inhibition at CpG island promoters of human cells

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Non-coding RNAs (ncRNAs) can modulate molecular mechanisms in physiological and pathological states. Camptothecin (CPT) specifically inhibits Topoisomerase I (Top1) by stabilizing a DNA-drug-enzyme complex wherein a DNA strand is cut and covalently linked to the enzyme (Top1cc). Here we use directional RNA-Seq to evaluate transcriptome of CPT-treated vs untreated HCT116 cells, demonstrating that formation of Top1ccs determine stimulation of new transcripts, in particular antisense RNAs at CpG island promoters of annotated genes. A stable reduction of cellular Top1 content significantly reduces the described effect. Data were validated by qrt-PCR in HCT116, but also in U2OS and PC3 cells. As the antisense transcription correlates with promoter activity, we mapped Top1 demonstrating its abundance close to TSS of transcribed genes. We also determined the rapid dynamics of Top1 and RNA polymerase II binding to chromatin and the involvement of CDK7 and CDK9 in the mechanism. We showed that Top1ccs can block RNA polymerase II initially, and, after inhibition of further polymerase recruitment, can trigger in-cis the activation of antisense transcription due to alterations of DNA topology.

O23.5
Role of the CSN5 subunit of Cop9 signalosome in transcription modulation of genes involved in zinc and lipid metabolism in *S. cerevisiae*

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The ubiquitin/proteasome system (UPS) has been shown to be necessary for tight regulation of gene expression with important implications for cellular homeostasis. Recent evidence shows that part of this regulatory action is at transcriptional level. A key component of the UPS is the COP9 signalosome (CSN), a protein complex conserved in all eukaryotes which regulates the activity of cullin-based E3 ubiquitin ligases.
The CSN removes the ubiquitin-like modifier Nedd8 from cullin-based E3s, an activity that resides in the JAMM/MPN+ domain of Csn5. In *S. cerevisiae*, the CSN is smaller than others eukaryotes and its subunits are not required for viability.
We performed a transcriptomic analysis of a S.cerevisiae strain deleted in CSN5 compared with its isogenic wild type strain. Data analysis showed that 183 genes were significantly modulated at least two fold in ΔCSN5 as compared with the wt strain. In particular, we found genes belong to lipid metabolism and zinc homeostasis that are repressed. In order to support a real involvement of the CSN in these functions we performed real time RT-PCR on 11 genes to test their modulation in different CSN-deletion mutants. We also tested CSN deletion strains for phenotypic features related to defects in zinc uptake and ergosterol biosynthesis. We are now trying to define the mechanism of this regulatory activity by proposing both an individual promoter-focused analysis and a genomic-wide proteomic approach.

24 - RNA biology

P24.1
Exon 45 skipping through U1-snRNA antisense molecules recovers the Dys-nNOS pathway and muscle differentiation in human DMD myoblasts

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Duchenne Muscular Dystrophy (DMD) is a rare disorder due to mutations in the dystrophin gene: the absence of dystrophin, besides impairing contractile capacity of the muscle fiber, delocalizes and downregulates nitric oxide synthase (nNOS); this alters S-nitrosylation of HDAC2, its chromatin association and the expression of specific genes dependent on the Dys-nNOS-HDAC2, such as miR-1 and miR-29. Moreover, among several therapeutic approaches, exon skipping has been demonstrated to be a successful strategy which convert severe Duchenne forms into milder Becker ones.Recently we have shown the selection of effective antisense constructs able to rescue dystrophin synthesis from a Δ44 deletion, through skipping of exon 45; moreover we demonstrated nNOS re-localization, corrected timing of myogenic markers expression and recovery of specific miRNAs. Comparing different Becker deletions, we demonstrated that those lacking nNOS localization displayed molecular features closer to Duchenne than to wild type myoblasts. These data suggest that the ability to rescue nNOS localization is a crucial feature that can differentially affect the outcome of exon skipping on different DMD mutations.

P24.2
A cross-platform comparison of Affymetrix and Agilent microarrays reveals considerable differences in the identification of differentially expessed miRNAs in lung tissue of c-Raf transgenic mice

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In order to identify disease associated regulations of miRNAs in precursor lesions of lung cancer RNA extracts from c-Raf transgenic mice and from healthy lung of wild-type (WT) mice were hybridized to the Agilent and Affymetrix microarray platform. Thus less than 60% of miRNAs were commonly identified amongst both microarray platforms. With the Agilent microarray 8 miRNAs were identified as significantly de-regulated, of which three were selectively de-regulated in male transgenic mice. When the same samples were analyzed with the Affymetrix platform only two miRNAs were found as significantly de-regulated of which miR-127 was common between both analyses. Quantitative qRT-PCR of miR-21, miR-146b, miR-127, miR-433, miR-96, miR-183, miR-184 and miR-322 was performed. The data agreed with findings obtained by the microarray platforms. Bioinformatic analyses predicted a total of 152 mouse genes as targets of regulated miRNAs of which 4 and 11 genes were significantly regulated in laser micro-dissected lung dysplasia and lung adenocarcinomas of c-Raf transgenic mice. For many of the predicted target genes their repressed protein expression was also reported for human lung cancer.

P24.3
Antisense RNA-induced exon-skipping for the gene therapy of frontotemporal dementia and Parkinsonism associated with chromosome 17 (FTDP-17)

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The cause of about half the cases of FTDP-17 neurodegeneration are mutations affecting the alternative splicing of exon 10 (E10), in turn causing intraneuronal accumulation of tau protein. We explored the feasibility of an antisense (as-) RNA-based gene therapy to correct tau splicing in FTDP-17.
We first tested whether it was possible to modulate E10 splicing by the use of Antisense Oligonucleotides (AONs) masking specific splicing-regulating sequences. RT-PCR and Western blot analyses showed that AONs are able to alter the splicing behaviour of tau E10 in the rat endogenous transcript (PC-12, rat pheochromocytoma cell lines), with variable efficiencies depending on the concentration of the AONs and on the targeted sequence. Based on these results, we embedded the as-RNA sequences in chimeric U snRNA vectors, whose promoters lead to long-term as-RNA expression. We tested these chimeric antisense snRNAs for their ability in modulating the splicing behaviour of tau in endogenous rat mRNA. To evaluate the effects of AONs/Chimeric Antisense-snRNA on the human tau pre-mRNA, we constructed a luciferase minigene reporter system, we carried out co-transfection into HeLa cells and evaluated the induction of E10 skipping by luciferase expression assay and RT-PCR.
This project was supported by Telethon Italia grant GGP08244.

P24.4
Involvement of TAR DNA binding Protein-43 (TDP-43) in microRNA biogenesis during neuroblastoma cell differentiation

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In the last few years increasing interest has been devoted to TDP-43, a multifunctional protein which is a major signature for neurological proteinopathies. Mutations within the gene encoding TDP-43 have been linked to neurodegenerative diseases, such as amyotrophic lateral sclerosis (ALS) and frontotemporal lobar dementia (FTLD). In these diseases, TDP-43 is mislocalised from its predominant nuclear localization to the cytoplasm, where it forms ubiquitin-positive inclusions.
Recently, a role in microRNA (miRNA) biogenesis has also been highlighted: as a component of both Drosha and Dicer complexes, TDP-43 contributes to the production of a subset of miRNAs.
Since miRNAs are increasingly implicated in neuronal differentiation control, we investigated the nuclear contribution of TDP-43 in miRNA biogenesis during differentiation of human neuroblastoma-derived cells. Knock down experiments of the single components of the Microprocessor complex revealed a crossregulation between Drosha and TDP-43 and highlighted a crucial role of TDP-43 in miRNA production.

P24.5
ADAR2 editing activity inhibits glioblastoma growth through the modulation of the CDC14B/Skp2/p21/p27 axis

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ADAR2-mediated A-to-I RNA editing, an essential post-transcriptional modification event in brain, is impaired in GBMs and astrocytoma cell lines. However, the role played by ADAR2 editing in astrocytomas remains to be defined.
Here, we demonstrate that ADAR2 editing rescue in astrocytomas prevents tumor growth in vivo and modulates an important cell-cycle pathway involving the Skp2/p21/p27 proteins, often altered in glioblastoma. We demonstrate that ADAR2 deaminase activity is essential to inhibit tumor growth. Indeed, we identify the phosphatase CDC14B, which acts upstream of the Skp2/p21/p27 pathway, as a novel and critical ADAR2-target gene involved in glioblastoma growth. These findings demonstrate that post-transcriptional A-to-I RNA editing might be crucial for glioblastoma pathogenesis and so we suggest that ADAR2 editing enzyme may be a novel candidate tumor-suppressor gene.

P24.6
microRNA-mediated regulation of PAX8 expression in Epithelial Ovarian Cancer (EOC)

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Epithelial Ovarian Cancer (EOC) is a morphologically and biologically heterogeneous disease. The most favored hypothesis is that EOC arises from the surface epithelium of the ovary (OSE). It has been shown that PAX8, which is normally expressed in non-ciliated secretory cells of healthy fallopian tube mucosal linings but not in the adjacent ciliated epithelia, nor in OSE cells, is over-expressed in EOC. Recent publications hint at a role for miRNAs in EOC. miRNAs are ~21 nt regulatory RNAs that control development and differentiation acting as post-transcriptional negative regulators of the expression of key target gene. miRNAs have an altered expression in several human cancers and may act as oncogenes or tumor suppressors. We analyzed PAX8 mRNA and protein levels and used an Agilent microarray platform to evaluate miRNAs expression profiles in different cell lines considered EOC models. By using the PITA algorithm we found that 3'UTR region of PAX8 mRNA is predicted to be a target for some of these miRNAs and by Real Time PCR analysis we confirmed their basal level of expression. At present, we're performing transfection experiments to demonstrate a direct binding of selected miRNAs, cloned in a plasmid allowing for their over-expression upon transfection in mammalian cell lines, to PAX8 3'UTR either on the endogenous PAX8 mRNA or on a co-transfected luciferase reporter.

P24.7
EMFs-mediated modulation of miRNA-30a and Beclin1 expression

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Constant exposure to electromagnetic fields (EMFs) seems to increase the risk of several human diseases, including neurodegenerative disorders such as Alzheimer's disease (AD). However, the literature data remain still controversial. Of interest, the effects of EMFs on the biological systems seem to depend on the EMFs "dose" and wavelength, and can shift from cytoprotection to cytotoxicity. At molecular level, EMFs are able to finely modulate gene expression by acting on transcriptional and post-transcriptional processes. Within this context, post-transcriptional mechanisms are key determinants of gene expression modulation, since they allow a rapid adaptation of protein levels to changing environmental conditions influencing, in different contexts, the cell fate. These mechanisms include a fascinating class of small non-coding RNA molecules called microRNAs. We focused on the effects of EMFs on miRNA-30a and its putative negatively-regulated target *beclin-1* and we report here preliminary data. Beclin-1 is a key autophagy-promoting gene that plays a critical role in the regulation of cell death and survival of various cell types, and its expression is down-regulated in AD.

P24.8
The DNA damage response to ionizing radiation is modulated by miR-27a

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Perturbations during the DNA-Damage Response (DDR) pathway can originate from alteration in the functionality of the microRNA-mediated gene regulation, being microRNAs (miRNAs) small noncoding RNA that act as post-transcriptional regulators of gene expression. By integrating the transcriptome and microRNome, we provided evidence that modeled microgravity can affects the DNA-damage response to IR in human peripheral blood lymphocytes (Girardi et al., 2012). By functional assays using luciferase reporter constructs we have shown that miR-27a targets ATM, the gene coding for the main kinase of DDR pathway. Since miR-27a is classified as an oncomir, being overexpressed in several tumors, we investigated the miR-27a-ATM interaction by validating miR-27a as a direct regulator of ATM through site-direct mutagenesis of the reporter vector containing the 3'UTR of ATM gene. We also analyzed the in vitro effects of ionizing radiation in cells overexpressing miR-27a, by assessing cell survival, DNA repair and cell cycle progression. Our results show that overexpression of miR-27a diminishes cellular radiosensitivity, suggesting a role of this miRNA during DDR.

P24.9
Yeast as a model to identify suppressors of respiratory defects due to human equivalent pathogenetic base substitutions in yeast mt tRNA genes

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Human mitochondrial (mt) diseases due to mutations in mt tRNAs genes are heterogeneous disorders that cause muscle and neurodegenerative dysfunction. The absence of effective treatment is partly due to the scarcity of suitable models and to the difficulty in human mt DNA manipulation. This problem can be overcome in yeast, in which biolistic mt transformation is possible.
We identified nuclearly encoded factors such as the yeast mt protein synthesis elongation factor (EF-Tu) and cognate mt aa-tRNA synthetases (aaRS) that rescued the respiratory defects due to mt tRNA mutations. Recently, the nuclear suppressors identified in yeast have also been used to alleviate the defective mt phenotypes in human mutated cultured cells. We also found that the orthologous human aaRS are active in yeast mt tRNA mutants. We hypothesised that the suppressor molecule could correct and protect the altered structure of mutated tRNA in a chaperone-like manner, and that this RNA stabilizing capability could be restricted to a conserved domain of the aaRS. We demonstrate that the C-terminal domain of yeast and human mt LeuRS, maintains the full suppressing activity in mt tRNALeu, Val and Ile yeast mutants.

P24.10
Role of long non coding RNAs in the control of muscle differentiation

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Advances in deep sequencing technologies have demonstrated that genomes of mammals, as well as other organisms, produce thousands of long transcripts that have no significant protein-coding capacity and thus are referred to as long non-coding RNAs (lncRNAs). A detailed analysis of two murine genomic microRNA (miRNA) loci, miR-206/133b and miR-31, led us to identify two lncRNAs, linc-MD1 and linc-31, and to defined their expression profile and function during muscle differentiation. Notably, while linc-31 is expressed in proliferating conditions, linc-MD1 is activated in early phases of myoblast differentiation. We demonstrate that linc-31 and linc-MD1 are localised in the cytoplasm where, acting as "sponges" for specific miRNAs, they regulate the expression of key factors involved in the myogenic program. We also identified an alternative linc-31 transcript, originating from an internal promoter and giving rise to a chromatin-bound RNA species. Our data indicate a relevant role of these lncRNAs in the complex network of regulatory interactions governing the correct timing of the muscle differentiation program.

P24.11
SpliceAid-F: a database of human splicing factors and their RNA binding sites

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A comprehensive knowledge of all the factors involved in splicing, both proteins and RNAs, and of their interaction network, is crucial for a better understanding of the splicing process and functions. A large part of relevant information is buried in the literature or collected in various databases. By hand-curated literature and databases screenings we collected experimentally assessed data about 71 RNA-binding splicing regulatory proteins into a database called "SpliceAid-F". For each splicing factor we annotated its functional domains, protein and chemical interactors, RNA interactions as experimentally validated binding and not-binding sites, including information about the relevant genes where these sites lie, their genomic coordinates, the splicing effects, experimental procedures, as well as the corresponding bibliographic references. The information stored in SpliceAID-F is also cross-linked to ASPicDB.
In total, SpliceAid-F contains 4227 interactions, 2590 RNA binding sites and 896 not-binding sites. The latter have never been collected before but can be of great interest, for example as negative examples to train machine learning algorithms. Our data may help to explain an observed splicing pattern as well as the effect of mutations in functional regulatory elements.

P24.12
MiR-200c can mediate either poor or good outcome in ovarian cancer depending on HuR localization in the cell

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The members of the miR-200 family have been reported to act as both oncogenes and tumor suppressors in independent studies. In a panel of ovarian adenocarcinoma cell lines, we observed a direct correlation between miR-200c expression and chemoresistance. Surprisingly, in A2780 cells miR-200c targeted class III β-tubulin (TUBB3), a factor associated with drug-resistance, while a positive correlation was observed between miR-200c and TUBB3 expression in most of the analyzed cell lines. We found that the miR-200c can increase the association of the RNA-binding protein HuR with TUBB3 mRNA, and HuR binding enhanced TUBB3 mRNA translation. The analysis on 220 ovarian tumors showed that overexpression of miR-200c correlated with poor or good outcome depending on the cellular localization of HuR. When HuR was confined in the nucleus, miR-200c suppressed TUBB3 expression and resulted in a good prognosis, whereas when HuR occurred in cytoplasm, the same miRNA seemed to enhance TUBB3 expression and produce a poor outcome. These findings contribute to a better knowledge of the interplay among RNA-binding proteins and miRNAs and suggests a potential usefulness in ovarian cancer prognosis.

O24.1
Mutations of the mitochondrial-tRNA modifier *MTO1* cause hypertrophic cardiomyopathy and lactic acidosis

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Dysfunction of mitochondrial respiration is a recognized cause of hypertrophic cardiomyopathy. To gain insight into the genetic origin of this condition, we used next-generation exome sequencing to identify mutations in *MTO1*, which encodes mitochondrial translation optimization 1. Two affected siblings carried a maternal p.R620Lfs*8 frameshift and a paternal p.A428T missense mutation. A third unrelated individual was homozygous for the latter change. In both humans and yeast, *MTO1* catalyze the 5-carboxymethylaminomethylation of the wobble uridine base in three mitochondrial tRNAs. Accordingly, mutant muscle and fibroblasts showed variably combined reduction in mtDNA-dependent respiratory chain activities. Reduced respiration in mutant cells was corrected by expressing a wild-type *MTO1* cDNA. Defective respiration of a yeast *mto1Δ* strain failed to be corrected by an Mto1^{P622X} variant, equivalent to human MTO1^{R620Lfs*8}, whereas incomplete correction was achieved by an Mto1^{A431T} variant (human MTO1^{A428T}). The respiratory yeast phenotype was dramatically worsened in the presence of a paromomycin-resistant (P^R) mitochondrial rRNA mutation, which mimics the human 12S rRNA sequence.

O24.2
FUS/TLS can affect selected microRNA levels

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MicroRNAs (miRNAs), are small non-coding RNAs, derived from long primary transcripts by stepwise processes that occur in both nuclear (Drosha cleavage) and cytoplasmic (Dicer cleavage) compartments. The mature miRNA molecules can bind to messenger RNAs regulating their levels or translation. MiRNAs are plentiful in the nervous system where they play a pivotal role in differentiation, synaptogenesis and plasticity. Mutations in the gene encoding for the FUS protein, described as a putative Drosha interactor, are linked to familial forms of Amyotrophic Lateral Sclerosis (ALS).
Data obtained by highthroughput analysis using human neuroblastoma cell line, SK-N-BE, interfered for FUS mRNA and differenziated *in vitro* by retinoic acid treatment, indicates that FUS downregulation alters the expression profile of several miRNAs. We demonstrate by RNA binding assay that these miRNA are able to bind FUS protein *in vitro* and we also provide evidence, by chromatin immunoprecipitation analysis, that FUS localizes on miRNA transcription sites. Altogether these data suggest that FUS may regulate miRNAs biogenesis co-transcriptionally and that there may be a miRNA involvement in ALS pathogenesis.

O24.3
Role of the Drosophila Fragile X gene, *dFMR1*, in the piRNA-mediated silencing of repetitive sequences

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Fragile X syndrome, one of the most common forms of inherited human mental retardation, is caused by the functional loss of the fragile X mental retardation protein, FMRP (1). FMRP is widely expressed in brain and testis where major symptoms are manifested (2).
Drosophila melanogaster contains a single *dFMR1* gene, coding for FMRP protein with a high structural and functional homology with human FMRP proteins. FMRP is an RNA-binding protein and it is a component of the miRNA pathway involved in translational silencing in both Drosophila and mammals (3). In these organisms, FMRP has also been shown to be a component of the RNA-induced silencing complex (RISC) (4).
FMRP function remains understudied outside the neural and synaptic development. Such studies are critical for elucidating the complexities of FMRP biology. In this study we investigated the role of FMRP in the germinal tissues of Drosophila. We demonstrated an "exceptional" role for FMRP in the piRNA pathway that controls the silencing of repetitive sequences and acts as a guardian of the genomic integrity.
References
1 Verheij et al., 1993
2 Devys et al., 1993
3 Jin et al., 2004
4 Caudy et al., 2002

O24.4
Identification and characterization of diatom regulatory small non-coding RNAs by integrative computational and experimental analyses

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Diatoms are a major but poorly understood phytoplankton group. The complete genome sequencing of the centric diatom *Thalassiosira pseudonana* (Armbrust et al., 2004) and the pennate diatom *Phaeodactylum tricornutum* (Bowler et al., 2008) has revealed that these organisms have particular genetic makeup and metabolic pathways. Recently, it has been shown that it is possible to inhibit gene expression in *P. tricornutum* using RNA interference (RNAi) (De Riso et al., 2009). The data opened the way for reverse genetics and also supports the presence of functional gene silencing pathways generating small RNAs. In the last decade, 20-30nt RNA molecules generated by double-stranded RNA (dsRNA) precursor have been found to act as novel regulators of gene expression. Aiming to investigate small RNAs-based regulatory mechanisms in diatoms a library of small RNAs has been prepared from *P. tricornutum* cells grown under different light conditions and sequenced with the Solexa technology, yielding more than 5,000,000 reads with a perfect match to the genome. However, none of them has been predicted as a possible miRNA by the known computational methods. Interestingly for 2 of them Northern blot analyses revealed differentially expressed products of around 21nt and bigger precursors matching in intergenic region. Our data, combining computational and molecular analysis, provides the first experimental evidence that small RNAs may play a regulatory role in diatoms.

O24.5
Study of trans-acting factors involved in the post-transcriptional regulation of CDK5R1

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CDK5R1 is involved in CNS functioning and neurodegenerative disorders. Both the CDK5R1 3'-UTR remarkable size and its conservation are indicative of an important function in post-transcriptional regulation. We showed that CDK5R1 3'-UTR decreases transcript stability and translational efficiency. We identified by luciferase assays a 138 bp region as the most destabilizing portion of the 3'-UTR. To assess if this region interacts with RBPs and to delimit the binding site, we performed UV cross-linking and site directed mutagenesis assays, allowing us to identify a poly-U stretch as a binding site of RBPs. We also demonstrated that nELAVs bind the poly-U motif by UV CL/IP assays. However, over-expression and silencing of nELAVs showed a stabilizing activity on CDK5R1 transcript. To search for destabilizing factors binding this region, we carried out pull-down experiments followed by mass-spectrometry analysis, allowing to detect an interaction with the splicing factor hnRNPA2/B1. The validation of hnRNPA2/B1 binding to the CDK5R1 destabilizing region and the silencing/over-expression studies will shed light on its possible role in post-transcriptional regulation of CDK5R1.

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