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Società Italiana di Mutagenesi Ambientale

JOINT MEETING AGI-SIMA

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BOOK OF ABSTRACTS

LECTURE: Causes of Cancer and Chance Events in Oncogenesis
(Chair: Margherita Bignami)
Lucio Luzzatto (Scientific Director, Istituto Toscano Tumori and Honorary Professor of Haematology, University of Firenze)

Causes of Cancer and Chance Events in Oncogenesis

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The current model of the pathogenesis of cancer is that a normal cell becomes a cancer cell through a sequence of somatic mutations. Each mutation confers onto the progeny of the cell in which it has occurred a growth advantage, no matter how small. After the first mutation the resulting clone will be just somewhat more abundant than the progeny of surrounding normal cells; after a full set of oncogenic mutations, it will be massive. This mutation-driven selective growth process mimics, in a population of somatic cells, the Darwinian evolution of organisms. Mutations, being random errors in DNA replication, can occur at every round of cell division (estimated frequency of 10^{-7} per gene per cell division): thus, the total number of mutations accumulated by a person will be $M = \mu D$, where μ is the mutation rate and D the total number of cell divisions. Environmental factors (smoking of cigarettes, viruses, bacteria, inflammatory processes, exposure to a number of chemicals, urban air pollution) and hereditary factors (high penetrance and low penetrance genes) are important causes of cancer. A reasonable working hypothesis is that all of these factors are causative of cancer by virtue of an increase in either μ, or D, or both. Thus, since mutations are stochastic events, there is always in the development of cancer an element of chance (it has been referred to as 'bad luck'); however, that chance can be increased, sometimes by orders of magnitude, by known agents. Thus, it is imperative that we mobilize individual and collective forces for the prevention of avoidable cancers.

LECTURE: 150 anni delle leggi di Mendel (Chair: Rodolfo Costa)
Bernardino Fantini (Emeritus Professor of the History of medicine, University of Geneva, Switzerland) e Telmo Pievani (University of Padova)
(Iniziativa aperta a tutta la città di Cortona)

Gregor Mendel e il libro della vita: perché la genetica può dirsi ancora mendeliana a 150 anni dall'enunciazione delle sue leggi

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La metafora del 'libro della vita', sempre più ampiamente utilizzata nella biologia contemporanea, sottintende l'idea che tutte le funzioni e le strutture dei sistemi viventi siano 'descritte' e 'ordinate' sulla base di messaggi testuali e prodotte dall'espressione controllata di tali messaggi in un determinato ambiente. Ciò spiega l'uso vasto e pervasivo nelle scienze biologiche di concetti e modelli tratti dai campi disciplinari della linguistica e della teoria dell'informazione.

La costruzione di questo paradigma esplicativo trova origine nei lavori di Mendel e dal suo uso di simboli letterali (AaBbCc …) per indicare i fattori ereditari, la cui combinazione costruisce il 'messaggio' trasmesso ad ogni generazione. Sviluppata dalla scuola di TH Morgan, l'idea che il genoma siano una sequenza di 'segni' organizzati gerarchicamente in modo lineare trova un'equivalenza solo nel linguaggio, nel quale tutti i fonemi e i relativi segni sono egualmente organizzati in modo lineare. La sequenza come concetto esplicativo resta costante attraverso la genetica classica e la genetica molecolare, sino alle definizioni attuali del genoma come 'libro della vita', riconfermando con forza il valore e l'attualità del lavoro scientifico di Gregor Mendel..

Mendel e Darwin: storia di un incontro postumo

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Il primo ci ha insegnato a leggere i caratteri ereditari nel "libro della vita". Il secondo ci ha insegnato che ogni specie è legata da un rapporto genealogico a tutte le altre nel grande "albero della vita". Mendel e Darwin non si sono incontrati e non hanno potuto spiegarsi l'un l'altro, forse per ragioni non soltanto connesse agli accidenti casuali della storia. Le ricerche sui piselli a Brno risalgono esattamente a 150 anni fa, ma soltanto un secolo fa si è iniziato a comprendere che la genetica mendeliana completa la rivoluzione darwiniana in quanto ne fornisce un elemento decisivo prima mancante: l'origine delle variazioni e il modo in cui le variazioni possono essere trasmesse alle successive generazioni. La fusione di mendelismo e darwinismo ha dato origine a un potente programma di ricerca interdisciplinare, la Sintesi Evoluzionistica Moderna, che oggi è sottoposta a revisioni ed estensioni. Fra queste, le più importanti riguardano proprio la natura interna della variazione che interessava a Mendel: in termini contemporanei, quanto la variazione è sottoposta a selezione e quanto è neutrale; quanto è casuale e quanto vincolata; quanto è genetica e quanto epigenetica. Il futuro della teoria dell'evoluzione dipende ancora oggi, a 150 anni da Mendel, dalla comprensione dei molteplici intrecci fra le sorgenti di variazione e gli influssi ambientali. Il "libro della vita" aperto da Mendel ha ancora molto da raccontare.

Mendel and Darwin: history of a posthumous meeting

Telmo Pievani

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The former taught us to read the hereditary traits in the "book of life". The latter taught us every species is linked to all the others in a genealogical relationship in the branching "tree of life". Mendel and Darwin never met and were unable to exchange their ideas, perhaps for reasons not only related to the accidents of history. The researches on peas in Brno date back exactly 150 years ago, but only a century ago we began to understand that Mendelian genetics completes the Darwinian revolution providing a crucial and at that time still missing element: the origin of variations and how such variations can be transmitted ~~in~~to the following generations. The merging of Mendelism and Darwinism gave rise to a powerful interdisciplinary research programme, the Modern Evolutionary Synthesis, that now is undergoing revisions and extensions. Among these, the most important ones concern the internal nature of variation that hit Mendel's curiosity: in contemporary terms, how much variation is under selection and how much is neutral; how much is random and how much is constrained; how much is genetic and how much epigenetic. The future of evolutionary theory still depends, 150 years after Mendel, on our understanding of the subtle intertwining between sources of variation and environmental influences. The "book of life" opened by Mendel still has a lot to say.

Mini SYMPOSIUM I: Epigenetic changes (Chair: Maria Pia Bozzetti, Univ. of Salento, Lecce)
Epigenetic modifications involved in stem cell pluripotency and lineage commitment.
Salvatore Oliviero (University of Torino)
DNA damage response during cell differentiation. Eugenia Dogliotti (ISS, Roma)

Epigenetic modifications involved in stem cell pluripotency and lineage commitment.

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In mammals, the methylation at 5mC occurs mainly at CpG dinucleotides and is required for fundamental physiological processes, such as embryonic development, X-chromosome inactivation, and genomic imprinting. DNA methylation is mediated by DNA methyltransferases (Dnmt), which include the *de novo* Dnmt3a, Dnmt3b and Dnmt3L enzymes and the maintenance enzyme Dnmt1, which ensures that DNA methylation is maintained at replication. Indeed DNA methylation at position 5 of cytosine (5mC) is believed to be a stable and heritable epigenetic mark required to create and maintain the cell identity. DNA demethylation involves the oxidation of 5mC by the Ten-Eleven Translocation (TET) enzymest o 5-hydroxymethylcytosine (5hmC), 5 formylcytosine (5fC) and 5 carboxylcytosine (5caC), which can be excised by the thymine-DNA glycosidase (TDG). However, the regulation of DNA methylation and its interplay with other epigenetic modifications during embryo development remains elusive.
In embryonic stem cells (ESCs) the promoter of the genes involved in development are maintained inactive by the activity of the Polycomb Repressive Complex 2 (PRC2) in the socalled “bivalent” status by the presence of the contrasting epigenetic marks H3K4me3/H3K27me3. Later, according to the specific pattern of expression in different cell types, the developmental genes either loose the PRC2 repressive mark or become methylated on their promoter. The interplay between Dnmts, Tet1, and PRC2 in ESCs ensures epigenetic plasticity throughout cell differentiation.

DNA damage response during cell differentiation

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DNA damage constantly arises throughout life either by endogenous or exogenous sources. Depending on the time maintenance and function of a specific cell type the risk of accumulating damage may vary. For instance damage to embryonic stem cells that are rapidly proliferating, self-renewing and pluripotent cells if not repaired can lead to mutation amplification and propagation. Therefore, these cells are expected to have stringent control of their genome integrity. Tissue-specific adult stem cells are quiescent cells that when activated accomplish their differentiation program. During maturation the propagation of cells with unrepaired lesions should be avoided but once differentiation is completed the control of genome integrity may become dispensable although the integrity of the genes required for proper function of differentiated cells should be maintained. The information available on the coordination of DNA repair, DNA damage signaling, cell death along the differentiation program will be reviewed and data on an in vitro cell system of skeletal myogenesis will be presented.

Mini SYMPOSIUM II: Cell signaling and proliferation (Chair: Alberto Inga, University of Trento)
ATM kinase modulates HER2 tumorigenicity. Daniela Barilà (Univ of Roma – Tor Vergata)
RTKs signaling: new functional approaches to genetically dissect the tumor landscape. Silvia Giordano (University of Torino)

ATM kinase modulates HER2 tumorigenicity

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Homozygous mutations of the ATM gene cause a rare autosomal genetic disorder, Ataxia Telangiectasia (A-T), characterized by several features among which cerebellar neurodegeneration and increasing predisposition to cancer development. The product of ATM gene is a serine/threonine kinase that acts as a key regulator of the DNA damage response and as a safe-guardian of genomic stability, therefore considered as a tumor suppressor. However, ATM has also been identified as a component of several signalling networks involved in sustaining cell proliferation and tumor growth: ATM is activated downstream Receptor Tyrosine Kinases (RTKs) and in response to hypoxia, and it may sustain tumorigenic signals such as AKT activation, suggesting a dualism for ATM in cancer.
To test this hypothesis we chose as a model system the HER2 Receptor Tyrosine Kinase positive breast cancer. We found that ATM targeting significantly impairs HER2-dependent tumorigenicity in vitro and in vivo. ATM promotes HER2 protein stability and as a consequence it sustains AKT activation downstream HER2. HER2 positive patients (not treated with the anti-HER2 monoclonal antibody trastuzumab) bearing ATM phosphorylation on S1981 (ATM-p) have a shorter Disease Free Survival than those with ATM-p negative tumors, suggesting that the identification of ATM phosphorylation on Ser1981 may have a prognostic and therapeutic significance and revising the canonical role of ATM as pure tumor suppressor.

RTKs signaling: new functional approaches to genetically dissect the tumor landscape.

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The complexity and heterogeneity of tumors are difficult to reproduce in *in vitro* studies, which often cannot adequately elucidate the molecular events involved in tumor initiation and progression. Moreover, histological subtypes of a cancer type that are being treated with similar surgical and therapeutic approaches, are in fact characterized by distinct phenotypes, cell of origin, and underlying key genetic and genomic alterations. Thus, a better understanding of the molecular

characteristic of each tumor, will lead to more personalized treatment approaches that will improve patients' prognosis.

In the last few years there has been a growing interest in the development of patient-derived tumor xenograft (PDX) models for cancer research. These models, in fact, offer several advantages compared to *in vitro* and *in vivo* models derived from conventional stable cell lines. Usually PDX models recapitulate the histologic and genetic characteristics of the tumors from which they derive and do not change across further *in vivo* passages. Several works have shown that PDXs are endowed with predictive clinical value and that they are of incredible importance in the field of precision medicine as they can be used for preclinical trials testing drug efficacy and for biomarker identification.

We aimed at identifying and validating novel targeted therapeutic strategies in gastric cancer, through the generation of a platform of gastric PDXs. This platform will be exploited for: 1) Validation of candidate oncogenes as relevant targets and identification of efficient therapeutic strategies 2) Identification of novel molecular targets; 3) identification of genetic predictors of response/resistance. As a whole, the results of this project should provide a scientific basis for future clinical applications and guide the rational design of molecularly-oriented clinical trials for gastric cancer.

Mini SYMPOSIUM III: Genome instability (Chair: Antonio Antoccia, Univ of RomaTre)
Telomere instability in natural and premature aging. Isabella Saggio (Univ La Sapienza)
Nijmegen breakage syndrome gene, NBS1, and molecular links to factors for genome stability. Alessandra Di Masi (University of RomaTre)

Telomere instability in natural and premature ageing

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The implication of telomeres in ageing was first suggested in the sixties when the Hayflick cell division limit was defined. Animal models have then related organismal ageing to telomeres. Telomerase-deficient mice display progressive tissue atrophy, stem cell exhaustion and multi-organ failure. Telomerase reactivation reverses tissue degeneration in these mice. A DNA damage response is detected in these animals and p53 co-depletion rescues the aged phenotype, suggesting that DNA instability plays a role in the ageing process. Interestingly, in premature ageing syndromes, in laminopathies for example, telomeres are defective and the progeroid phenotype is rescued by telomerase expression and by p53 KO, marking a connection among telomeres, lamins and ageing.

We have characterized a gene named *AKTIP* (Ft1 in mouse) implicated in telomere metabolism. We have shown that AKTIP contributes to the replication of telomeres, interacts with the telomeric proteins TRF1 and TRF2, with the replication factor PCNA and with lamins. AKTIP depletion causes telomere defects and enchains a DNA damage response. In addition, we have observed that Ft1 hypomorphic mice display progeroid characteristics, including bone alterations, absence of subcutaneous fat and impaired development. Taken together the properties of *AKTIP* put this gene at the crossroad of the lamin and telomere fields and suggest that its study could contribute to the definition of the mechanics of the ageing process.

Nijmegen breakage syndrome gene, NBS1, and molecular links to factors for genome stability

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The DNA double-strand break (DSBs) response (DDR) includes damage recognition by the MRE11/RAD50/NBS1 complex, ATM protein activation, histone H2AX phosphorylation at Ser139 (γ-H2AX), MDC1 and 53BP1 recruitment. Many proteins involved in the DDR localize within the promyelocytic leukemia (PML)-nuclear bodies (PML-NBs), which may represent structures where protein complexes are assembled, anchored and/or post-translationally modified. PML-NBs disruption, as a consequence of the PML-RARα fusion gene formation following the t(15;17), causes the acute promyelocytic leukemia (APL) pathogenesis. All-*trans*-retinoic acid (RA) treatment induces PML-RARα degradation, restores PML-NB functions, and causes terminal cell differentiation of APL blasts. However, the precise role of the APL-associated PML-RARα oncoprotein and PML-NBs integrity in the DSBs response and tumor suppression is still lacking. By addressing the consequences of IR-induced DSBs response in primary APL blasts and myeloid cell lines carrying endogenous or ectopically expressed PML-RARα, before and after treatment with RA, we found that the disruption of PML-NBs is associated to delayed DSBs response, as revealed by the impaired kinetic of disappearance of γ-H2AX and 53BP1 foci and activation of ATM and of its substrates H2AX, NBS1 and CHK2. Of note, the disruption of PML-NBs integrity by PML-RARα affects the IR-induced DSB response also in a preleukemic mouse model of APL *in vivo*. Therefore, the oncoprotein-dependent PML-NBs disruption and DDR impairment represent relevant early events in APL tumorigenesis.

LECTURE: Organization and evolution of primate genomes.
(Chair: Guido Barbujani, University of Ferrara)
Mario Ventura (University of Bari)

Organization and evolution of primate genomes

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One of the most fascinating questions in evolutionary biology is how human specific traits, such as high cognitive abilities, erect bipedalism, and hairless skin, are encoded in the genome. Recent advances in genomics and the advent of next generation sequencings technologies have begun to reveal differences between the genomes of the great apes. It has become evident that segmental duplications (SDs) have drastically increased in the primate genomes, and most remarkably in the human genome. SDs trigger mutations such as structural rearrangements (duplications, deletions, inversions and translocations) consequently play a crucial role in both human disease and genome evolution. Several human diseases (genomic disorders) are caused by non-allelic homologous recombination (NAHR) between highly similar SDs, as well as genes contained in segmental duplications have a tremendous potential to cause genetic innovation, probably accounting for the acquisition of human-specific traits. Both increase in gene copy number and formation of new genes by NHAR can explain human evolution. A key example of this effect has been recently reported for SRGAP2 protein and its human-specific paralogs involved in human brain development and evolution.

Mini SYMPOSIUM IV: Mutations: pros and cons (Chair: Antonella Russo, Univ of Padova)
Somatic hypermutations (AID/APOBEC). Silvestro Conticello (Ist Toscano Tumori, Florence)
Germline mutations. Francesca Pacchierotti (ENEA, Roma)

Somatic hypermutations (AID/APOBEC)

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The AID/APOBECs are a class of deaminases that induce editing of cytosines in nucleic acids. These enzymes exert their activity in diverse physiological contexts, but their common ability to mutate nucleic acids represents a double edged sword: AID, a key player in the secondary diversification of the antibody genes, is the trigger of chromosomal translocations in lymphoproliferative diseases. Moreover the mutational signature of the AID/APOBECs has been identified in many cancer genomes. Among the AID/APOBECs, APOBEC1 is the only family member to physiologically target RNA, as the catalytic subunit in the Apolipoprotein B mRNA editing complex. APOBEC1 has been linked to cancer development in mice but its oncogenic mechanisms are not yet well understood. Indeed, we show that expression of APOBEC1 induces a mutator phenotype in vertebrate cells, likely through direct targeting of genomic DNA. Moreover, we find the presence of an AID/APOBEC mutational signature in esophageal adenocarcinomas, a type of tumor where APOBEC1 is highly expressed.

Our findings suggest that the ability of APOBEC1 to trigger genetic alterations represents a major layer in its oncogenic potential. Such APOBEC1-induced mutator phenotype could play a role in the onset of esophageal adenocarcinomas. APOBEC1 could be involved in cancer promotion since the very early stages of carcinogenesis, as it is highly expressed in Barrett's esophagus, a condition often associated to esophageal adenocarcinoma.

Germline mutations

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Until a short time ago, human germline mutation rate estimates relied primarily on the prevalence of monogenic diseases or on phylogenetic distances among primates. More recently, the application of next-generation sequencing to genome analysis in pedigrees has allowed to identify and to enumerate germline point mutations as variants present in children but not their parents. Whereas interspecies comparisons provide insight into long-range processes, the direct measurement of the *de novo* mutation spectrum and rates across generations is crucial for understanding mechanisms of mutation formation. Surprisingly, whole-genome pedigree based estimates are 2 fold lower than the rates obtained by phylogenetic approaches, whereas the various approaches agree in showing that more mutations originate from the fathers than from the mothers, and the incidence of mutations increases with father's age. The next generation sequencing approach has been applied also to estimate mutation rates in multi-generation pedigrees of mice, showing the impact of DNA repair defects, and providing a powerful tool to study the origin and evolution of germline mutations, to generate new mutations for phenotype screening, and to assess the genotoxic effects of environmental factors on mammalian germ cells. Finally, new data in yeast and humans provide solid evidence that meiotic recombination is linked to point mutation formation, a theory originally proposed by Magni and von Borstel in the 60's. Together with new mechanistic insights into drivers of hotspot recombination map, these data led to evolutionary models fitting gene distribution in the genome, gene conversion events at recombination sites, and the large diversity of the recombination map across rodent strains and primate species.

Mini SYMPOSIUM V: Ageing (Chair: Giuseppe Passarino, University of Calabria, Rende)
Ageing genetics and longevity in humans. Alberto Montesanto (Univ of Calabria, Rende)
Tricothiodystrophy as a model of premature aging. Donata Orioli (CNR, Pavia)

Ageing genetics and longevity in humans

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Healthy aging and longevity in humans are modulated by a lucky combination of genetic and non-genetic factors. Twin studies demonstrated that about 25% of the variation in human longevity can be due to genetic factors.

The search for genetic and molecular basis of aging has led to the identification of genes correlated with the maintenance of the cell and of its basic metabolism. Indeed, mutations in genes encoding proteins involved in DNA repair, telomere conservation, heat shock response, and the management of free radicals' levels were found to contribute to longevity or, in case of reduced functionality, to accelerated senescence and the consequent organism aging. These observations led to the idea that healthy aging and longevity may arise from an efficient process of maintenance of cellular and organismal activities.

In parallel, studies showing that calorie restriction may increase lifespan in model organisms, suggested that longevity may be "directly" promoted. The identification of pathways modulated by calorie restriction has shown that the variability of genes associated with nutrient-sensing signaling, may have a key role in healthy aging and lifespan.

Current studies are showing that the interaction between genetic background and environment is essential to determine the individual chance to attain longevity. In this context, epigenetic studies are showing to be important to monitor such interaction and to be very sensitive in revealing individual biological age.

Relevance of transcription deregulations in the clinical hallmarks of trichothiodystrophy

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Trichothiodystrophy (TTD) is a rare hereditary disorder with symptoms affecting several tissues and organs. The most relevant features are hair abnormalities, physical and mental retardation, neurodegeneration, ichthyosis and premature aging. Photosensitivity is present in about 50% of cases and is associated with an altered cellular response to ultraviolet (UV) light caused by a defect in nucleotide excision repair (NER), the DNA repair pathway that removes a wide spectrum of DNA lesions, including UV-induced damage. The genes identified as responsible for the photosensitive form of TTD, namely *XPB, XPD* and *TTDA*, encode distinct subunits of the general transcription factor IIH (TFIIH) that besides acting in transcription is also a key player in NER. Thus, clinical features of TTD may result from DNA repair alterations as well as gene expression deregulations. By whole transcriptome sequencing, we identified TFIIH-dependent transcriptional impairments affecting specific signalling pathways in TTD cells. We found the expression deregulation of genes related to the extracellular matrix (ECM), a complex network of macromolecules that provide structural support to cells and tissues and elicit signalling events controlling cell behaviour and tissue homeostasis. We demonstrated that ECM alterations impair the migration and wound healing features of TTD dermal fibroblasts, which are fully recovered by inhibiting the activity of specific ECM molecules. These findings may explain some of TTD clinical features.

INTEGRATED LECTURES (Chair: Alessandra Pollice, University of Napoli)
Systems biology of host-microbiota interactions. Duccio Cavalieri (Fondazione Edmund Mach, San Michele all'Adige)
Friends with benefits: Microbes, stress and social behaviour. Roman M. Stilling (Cork University, Ireland)

Systems Biology of Host Microbiota Interactions.

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To quote Hippocrates: "All disease begins in the gut". This statement holds especially true for nutrition-related diseases, polarized as undernutrition or overnutrition (malnutrition and/obesity). The impact of gut microbiota here culminates in early infancy, when the immune responsiveness and metabolic phenotype is consolidated. The interaction of food components with the immune system or, indirectly, through their effect on gut microbiota, will influence the immune maturation process and responsiveness. Metagenomics approahces are revolutionizing our vision of the human, we are walking symbiomes and selection acts on the host and the microbes that inhabit his body parts.
We have evolved for millions of years with these microbes, but the changes in our interaction wth these companions changed drastically with the advent of antibiotics, sanitation and diet standardization in the globalized world.
The relative homogeneity of the microbial composition of Europeans and Americans reflects this globalization, rather than a transcendent tendency of humans to select the same bacteria worldwide. Since inflammatory and autoimmune disorders are virtually absent in rural African or Asian communities (Hygiene Hypothesis), understanding the establishment of homeostasis at birth and its robustness in a non globalized-westernized setting is extremely relevant.
We therefore hypoithesize that if dietary standardization affects the mother's microbiome, this change is inheritable by the newborn, thus affecting how his/her immune system interacts with environmental microbiomes. Predisposition to autoimmune diseases might result from early misalignment and potential conflict between maternally transmitted and environmental microbiomes. Understanding the effect exerted by the diversity of human microbiota and mycobiota on immune training will help selecting and preserving cultural food heritage, with resulting impact on health (decreased risk of chronic diseases such as obesity, allergies and inflammatory conditions) and on safety and food production policies.

Friends with Benefits: Microbes, Stress and Social Behaviour

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The genes present in our microbiome, the collective genomes of the microbes that live inside and on the human body, outnumber our own genes by about 400 to 1. Accumulating new evidence points to a role for the microbiome in regulating brain development, function and behavior through a variety of pathways, collectively known as the microbiota-gut-brain axis. Many efforts have focused on delineating a role for this symbiosis in health and disease, ranging from stress-related disorders such as depression, anxiety and irritable bowel syndrome to neurodevelopmental disorders such as autism. In addition, the evolutionary formation of a complex gut microbiota in mammals

may have played an important role in enabling brain development and perhaps sophisticated social interaction. Germ-free (GF) mice, devoid of any microbiota throughout organismal maturation, are a well-established tool to study the effects of the microbiome on host physiology. A growing body of independently replicated findings confirms that GF animals demonstrate altered anxiety-related behaviour and impaired social behaviour. However, the underlying mechanisms of this interaction and the nature of the pathways involved are poorly understood. Our recent findings support the hypothesis that differential gene regulation in specific brain regions of GF mice are involved in regulating to the behavioural phenotypes.

SIMPOSIO: Mitochondria in evolution and disease (Chair: Antonio Torroni, University of Pavia)
Nuclear-gene mutations and OXPHOS disorders. Massimo Zeviani (Director, MRC Mitochondrial Biology Unit, Cambridge, UK)
Mitochondrial Genome Variation: a Female Perspective in Evolution. Alessandro Achilli (University of Perugia)
The role of mitochondrial dysfunction in Cockayne syndrome. Maria Rosaria D'Errico (Istituto Superiore di Sanità, Rome)

IDENTIFICATION AND CHARACTERIZATION OF NEW MITOCHONDRIAL DISEASE GENES

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Mitochondria are the major source of ATP that is synthesized by the respiratory chain through the process of oxidative phosphorylation (OXPHOS), a complex biochemical process carried out through the dual control of physically separated, but functionally interrelated, genomes, nuclear and mitochondrial DNAs. The genetic and biochemical intricacy of mitochondrial bioenergetics explains the extreme heterogeneity of mitochondrial disorders, a group of highly invalidating human conditions, for which no effective treatment is nowadays available. In addition to bioenergetic failure, other mechanisms are probably predominant in the pathogenesis of specific syndromes, such as alterations of cellular redox status, the production of reactive oxygen species, compromised Ca^{2+} homeostasis, mitochondrial protein and organelle quality control, and mitochondrial pathways of apoptosis. By investigating selected families and patients, we have identified several new disease genes, each responsible of distinct defects of the respiratory chain, mtDNA metabolism, or both. Recently published and still unpublished findings will be presented and discussed. Structural analysis and the creation of ad hoc recombinant lines in yeast, flies, and mice have allowed us to dissect out the molecular consequences of the ablation or defects of some of these proteins, and their physical status in normal and disease conditions. These models have also been exploited to implement experimental therapeutic strategies, based on gene and cell replacement, or pharmacological control of mitochondrial biogenesis.

Mitochondrial Genome Variation: a Female Perspective in Evolution

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Mitochondrial DNA (mtDNA) sequences are powerful genetic records of the history of populations and species, and the study of their evolution is profoundly changing our perception on how modern humans and animals evolved and colonized the entire planet. By studying concomitantly the sequence variation in individuals or populations, it is possible to acquire sufficient and reliable information concerning genetic ancestries and migrations, starting from their homelands, all the way to the four corners of the earth.

A remarkable case study in human population genetics is represented by the Native Americans. They belong to one of the few extant human groups whose ancestors entered a vast

uninhabited area over a relatively short interval and then remained isolated from other human contacts for a considerable period of time. The overall picture about the first peopling of the Americas is gradually emerging much clearer and detailed also thanks to the contributions provided by phylogenetic surveys of entire mtDNAs.

The molecular and phylogenetic survey of complete mitogenomes is now also applied to reconstruct the major processes that lead to the domestication and spread of some important mammals (e.g. cattle, horses and goats). Moreover, since after domestication, the diffusion of livestock completely depended on human events and migrations, it is also conceivable that the genetic diversity of modern livestock might provide further details about the history of human movements.

The role of mitochondrial dysfunction in Cockayne Syndrome

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Cockayne syndrome (CS) is a rare hereditary multisystem disease characterized by neurological and development impairment, and premature aging. The molecular bases of this clinical trait are still unknown. CS cells are hypersensitive to oxidative stress and accumulate oxidative DNA damage, but the molecular mechanisms involved remain unresolved. Emerging evidence indicates that the lack of CS proteins is associated with redox unbalance, increased steady-state levels of intracellular ROS, dysfunctional mitochondria and an alteration in metabolic profile. Altered autophagy, that is often affected in neurodegenerative diseases, has also been observed.

We have investigated whether CS proteins play a direct role in mitochondrial maintenance or the mitochondrial dysfunction is a secondary response to the hyperactivation of nuclear DNA damage sensors. We show that CSA localizes to mitochondria and its deficiency leads to accumulation of fragmented mitochondria. The analysis of mitochondrial dynamics showed that in CS-A cells the dynamin-related protein (DRP1) is hyperactivated concomitantly to p53 activation and the mitophagic flux is increased. Efficient rescue of the dysfunctional mitochondrial phenotype and inhibition of mitochondrial apoptosis of CS-A cells was achieved by improving the cell cleaning system via overexpression of Parkin. These findings provide new promising tools for limiting cell loss in CS diseased brain region

POSTER SESSIONS

A - Cell cycle, cell division and chromosome segregation
B - Regulation,differentiation and development
C - Epigenetics
D - Evolution and genome structure
E - Human disease and ageing
F - Neurobiology
G - Genome integrity and DNA damage response
H - Cancer genetics

A1. A role for p14ARF tumour suppressor in human keratinocytes inflammatory response

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p14ARF (mouse p19ARF) is among the most important tumor suppressors in humans. The discovery of a plethora of ARF interactors during the last years and the observation that also viral, genotoxic, hypoxic and oxidative stresses activate an ARF response, suggest that ARF has a wider role to protect the cell and behaves more than a simple tumor suppressor. Our studies have largely contributed over years at defining the mechanisms through which ARF is regulated and in unraveling the signal transduction pathways by which ARF mediates its effect on cell growth (1,2,3). Recent observations indicate that ARF is able to regulate the cell's inflammatory response establishing a role in the regulation of innate immunity (4). This prompted us to analyze the ARF's role in the response to cellular stresses, in particular during the inflammatory response. We choose to analyze the ARF's response to bacterial LPS treatment in human HaCat cells as keratinocytes represent the first barrier to microbial insults in the context of the skin. Interestingly, ARF intracellular levels increase following LPS stimulation both at the RNA and protein levels. We are now defining this effect analysing the potential involvement of epigenetic mechanisms in ARF's activation.

1) Pollice, A. , Sepe, M., Villella, V.R., Tolino, F., Vivo, M., Calabrò, V., La Mantia, G. "TBP-1 (Tat Binding Protein-1 protects the human oncosuppressor p14ARF from proteasomal degradation". Oncogene, Aug 2;26(35):5154-62 (2007)
2) Pollice, A. , Vivo, M., La Mantia, G. "The promiscuity of ARF interactions with the proteasome." FEBS Letters 582, 3257-3262 (2008).
3) Vivo, M., Matarese, M., Sepe, M, Di Martino, R., Festa, L., Calabrò, V., La Mantia, G., Pollice, A. "MDM2-mediated degradation of p14ARF: a novel mechanism to control ARF levels in cancer cells". PLoS One.;10(2):e0117252. Epub Feb 27 (2015)
4) Través PG, Luque A, Hortelano S. "Tumor suppressor ARF: The new player of innate immunity" Oncoimmunology, Sep 1;1(6):946-947 (2012).

A2. YBX-1 protein as a potential player in the timing control of cell proliferation during zebrafish development

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The Y-box binding protein 1 (YBX1 or YB-1) is a member of the family of DNA/RNA -binding proteins with an evolutionarily conserved cold shock domain. YBX-1 plays multiple functions including the regulation of transcription and translation and is involved in the control of cell proliferation, differentiation and stress response. Both oncogenic and anti-oncogenic activities of YBX-1 have been documented. We have identified YBX-1 as a partner of ΔNp63α, a transcription factor involved in the homeostasis of epithelium (1). YBX-1 is highly conserved from fish to man. The high similarity between zebrafish and human YBX-1 makes zebrafish a highly relevant model for studying the physiological role of YBX-1.
Our preliminary experiments indicate that, in zebrafish cells and tissues, YBX-1 protein level, post-translational modifications and subcellular localization are regulated according to the light-dark cycle with a maximum of nuclear localization in the beginning of the light phase and a minimum in the beginning of the dark phase. This nuclear oscillation also persists in constant dark conditions suggesting an involvement of YBX-1 in clock-regulation. Moreover, we have shown that YBX-1 is able to regulate the cyclins also controlled by the circadian clock making this protein a potential link between the core circadian clock mechanism and the cell proliferation control.

Reference: (1) Troiano A. et al. J. of Cell Physiol. 2014, 230(9):2067-74.

A3. Functional interaction of nuclear SAMHD1 and mitochondrial deoxyguanosine kinase in the maintenance of mtDNA

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The dNTP triphosphohydrolase SAMHD1 is a major regulator of dNTP concentrations in human cells. In normal human fibroblasts its expression increases during quiescence, contributing to the small dNTP pool sizes of these cells. In cycling normal human fibroblasts we observed that silencing of SAMHD1 induces expansion and imbalance of dNTP pools with accumulation of cells in G1. On the other hand SAMHD1 mutated fibroblasts derived from AGS patients grew in culture normally although their dNTP pools were larger than those of silenced wt fibroblasts and imbalanced. The main change concerned the concentration of dGTP. The deoxyguanosine produced by dGTP hydrolysis by SAMHD1 may be recycled in mitochondria by deoxyguanosine kinase (dGK). Mutations of dGK cause mtDNA depletion in non cycling cells and hepato-cerebral mtDNA depletion syndrome in humans. We studied if SAMHD1 and dGK interact in the regulation of the dGTP pool during quiescence. When SAMHD1 was silenced by siRNA transfection in quiescent dGK mutated fibroblasts the composition of the mt dNTP pool approached that of the dGK-proficient controls and mtDNA copy number increased, compensating the depletion to various degrees in different mutant fibroblasts. Our results prove the importance of SAMHD1 in the regulation of all dNTP pools and suggest that dGK inside mitochondria has the function of recycling the deoxyguanosine derived from endogenous dGTP degraded by SAMHD1 in the nucleus.

A4. Mitotic stability of a horse satellite-free centromere

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The centromere is the *locus* that controls chromosome segregation during cell division. Most eukaryotic centromeres are characterized by the presence of extended arrays of tandemly repeated DNA, called satellite. Repetitive DNA has been hypothesized to provide a suitable chromatin environment for centromere maintenance and for the cohesion and separation of sister chromatids. However, centromeres completely devoid of repetitive DNA exist. In the horse, we demonstrated that the centromere of chromosome 11 (ECA11) is satellite-free and that the centromeric function is associated to different domains across a region of ~500 kb (Wade *et al* Science 2009 326: 865-7; Piras *et al* PLoS Genet 2010 6: e1000845; Purgato *et al* Chromosoma 2015 124: 277-87).
To our knowledge, no literature data exist on the mitotic stability of satellite-less centromeres. By means of FISH with chromosome specific probes, we compared the mitotic stability of ECA11 with that of ECA13, whose centromere contains the canonical satellite DNA sequences. Two aneuploidy assays, FISH on interphase nuclei and the micronucleus test, were performed in control cells and in cells treated with known aneuploidizing agents (nocodazole and griseofulvin). Our results indicated that the mitotic behaviors of ECA11 and ECA13 are comparable, thus demonstrating that, in the horse, the complete absence of satellite DNA from a centromere does not perturb its proper segregation at mitosis.

A5. Mechanism of SAMHD1 regulation at the G1/S transition of the cell cycle

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Sterile alpha motif and HD-domain containing protein 1 (SAMHD1) is a deoxynucleotide triphosphohydrolase that degrades DNA precursors (dNTPs). SAMHD1 plays important roles in the innate immune response to viral infections and it contributes to the control of balanced dNTP pool sizes in not infected mammalian cells. SAMHD1 concentration oscillates during the cell cycle and shows an opposite pattern of regulation compared to ribonucleotide reductase (the key enzyme of dNTP de novo synthesis) being more abundant in G1/G0 phase and lower in S-phase. The mechanism controlling the quantitative variations of SAMHD1 along the cell cycle is unknown. Considering the cell-cycle related phosphorylation of SAMHD1 on residue T592 and the drop of SAMHD1 concentration during S-phase, we hypothesize that the phosphorylation of SAMHD1 primes the ubiquitination of the enzyme by the SCF^{Skp2} ubiquitin ligase at the G1/ S border, followed by proteasomal degradation of ubiquitinated SAMHD1. We find that SAMHD1 interacts via its C-terminus with cyclin A or E and both cyclins complexed with CdK2 induce SAMHD1 phosphorylation. In cells transiently transfected with either wild type SAMHD1 or with the not phosphorylable mutant T592A SAMHD1 co-immunoprecipitates with Skp2 and its phosphorylation affects the interaction. Preliminary data suggest that the ubiquitin/proteasomal system could be involved in SAMHD1 degradation.

A6. Role of *Drosophila* Cdk7 in mitotic progression

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Cdk7 is the kinase subunit of the Cdk-activating-kinase (CAK) complex, required for Cdk1 phosphorylation and the complete activation of the Cdk1/Cyclin B complex. We are currently characterizing a new EMS-induced mutant allele at the *cdk7* locus. This mutation causes lethality at the larval/pupal boundary, allowing cytological analysis of proliferating tissues for the presence of mitotic defects. Immunostaining of larval brains with antibodies against spindle and/or chromosomal components revealed that *cdk7* mutants exhibit a specific failure in mitotic progression, with most dividing cells arrested in metaphase. In some of these metaphases the spindle elongated, assuming an anaphase-like morphology but the chromosomes remained at the center of the spindle. In the few anaphases present in mutant brains, the spindle and chromosome dynamics were not coordinated and sister chromatids failed to separate properly. Remarkably, in *cdk7* mutant cells Cyclin B was consistently low and did not fluctuate during the different phases of mitosis, while in wild type cells it accumulated in prometaphase and metaphase, started to decreases in anaphase and completely disappeared in telophase. These results led us to hypothesize that the mitotic defects observed in *cdk7* mutants are due to a precocious degradation of Cyclin B occurring in the absence of the Cdk7-mediated phosphorylation of the Cdk1/Cyclin B complex, and highlight a previously undescribed role of this kinase in mitotic progression.

A7. h-prune gene mutated in microcephaly with neurodevelopmental impairment shows microtubule assembly defects during mitosis

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The centrosome forms the main cellular cytoskeleton-organizing center and is the classical site of microtubule nucleation and anchoring processes intimately linked to cell-cycle progression and thus neuromorphogenesis. Mutations in microtubule-regulating genes have been associated with disordered neuronal migration and microcephaly syndromes. Microcephaly is defined as a head circumference of greater than or equal to three standard deviations below the expected age, sex and ethnicity mean. Microcephaly may be developmental resulting from abnormalities of proper development, or degenerative in which cell loss follows a period of normal brain development. Here, we define PRUNE, the *Drosophila* human homologue gene, as a new interactor of b-tubulin and show that it is important for microtubule polymerization, and that it enhances cell migration and cell division. Consistent with this role we show that mutations in PRUNE, discovered in families from Oman and India, result in microcephaly, developmental stagnation, truncal hypotonia and peripheral spasticity. The mutations identified impair the microtubule polymerization abilities of PRUNE, and also diminish it's cell migration and proliferation properties. Together our data establish PRUNE as a molecule fundamental for normal microtubule assembly and function and reinforce the importance of microtubule-related proteins in human cortical development. These results pave the way of the discovery of the human gene, highly represented through evolution, with a new function in cell division during mitosis, thus future experiments will address in details these findings.

B1.Altered Myc/Max ratio induces an eye to wing homeotic transformation by controlling HOX gene expression

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Neuroblastoma is a common tumour of infancy and develops from sympathetic neurons derived from the neural crest. Bad prognosis correlates with amplification of the MYCN oncogene and with its overexpression. MYCN interacts with the MAX protein and activates transcription of target genes but recent studies show that MYCN without MAX can also repress transcription promoting tumour development. Interestingly, about 27% of human pheochromocytomas, another neural crest cells derived tumour, carry mutations in the MAX gene but not MYC gene amplification suggesting tumour development may rely on the ratio between MYC and MAX instead on MYCN level *per se*. To explore this hypothesis, we generated *Drosophila* lines to induce specifically in eye cells uncommitted to differentiation different MYC levels and/or different levels of RNAi against MAX. We observed that MAX downregulation reduces proliferation and organ growth but does not affects retina differentiation and, conversely, MYC overexpression alters eye cells differentiation. Surprisingly, animals expressing both high MYC and low MAX show an eye to wing homeotic transformation and, as animals only with high MYC, show ectopic expression of the wing specific HOX gene *Antennapedia* in the eye primordium. This raises the possibility that in cells uncommitted to differentiation MYC with MAX promotes proliferation and tissue growth but without MAX alters regulation of body segment specific HOX genes to negatively modulate cell differentiation.

B2.Deregulation of a c-myc-miR34a circuitry in tumorspheres from transformed human fibroblasts

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Evidence indicates that a subset of cells endowed with high tumorigenic potential and stemness features (cancer stem cells: CSCs) is responsible for tumor initiation and maintenance in several cancers. In this study, we used a tumor cellular model developed in our laboratory from telomerase immortalized human fibroblasts (named cen3tel) and the tumorsphere assay to possibly isolate and characterize CSCs from *in vitro* transformed cells. Cen3tel cells were able to form spheres (frequency ~ 2-10%), which showed some stemness features, but were not more tumorigenic than adherent cells in *in vivo* assays, suggesting an uncoupling between sphere formation capacity and high tumorigenic potential. Moreover, compared to adherent cells, sphere cells showed a reduced expression of genes involved in tumorigenesis and stemness, as c-*MYC*, *GNL3*, *Notch* and *β-Catenin*, as well as increased levels of the tumorsuppressor miR-34a. We found that deregulation of these genes was reversible and concerted; in fact, one day after sphere cell seeding in adherent culture conditions, all the genes regained the expression levels of adherent cells, suggesting that they are connected in a circuitry and epigenetic mechanisms likely modulate their expression. Here we show that deregulation of these genes is possibly involved in protecting cells from apoptosis during growth in suspension. This mechanism could play a role as a survival mechanism for cancer cell growing after detachment from the extracellular matrix.

B3.The Heterochromatin protein 1 (HP1) is involved in germ line stem cells maintenance

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HP1 (Heterochromatin Protein 1) is a non histone chromosomal protein first discovered in *Drosophila melanogaster* by its association with the heterochromatin and through mutations that suppressed the silencing effect of heterochromatin in position-effect variegation. Numerous studies have shown that such protein is phylogenetically highly conserved and play a role in heterochromatin formation and gene silencing in many organisms. More recently, cytogenetical and molecular studies, performed in Drosophila and in other organisms, have revealed that HP1 associates also with telomeres and multiple euchromatic sites. All these studies collectively have shown that these three different positions are related to three different functions of HP1: heterochromatin formation and gene silencing, telomeric capping and silencing, and positive control of gene expression. Since it has been observed that HP1 is highly abundant in adult ovaries, we have performed studies to test if this abundance could be related to its involvement in *germ-line development*. We will presents the results of our experiments showing that the reduction of HP1 level, with three different driver, is sufficient to compromise both oocyte and follicle development suggesting an important function for HP1 activity during oogenesis and in germ line stem cells maintenance.

B4.The *TCP* genes of the Mediterranean orchid *Orchis italic*

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The TCP proteins are plant-specific transcription factors involved in many different processes, from leaf and flower development to circadian pathways and response to various stimuli. Although their role has been widely investigated in different plant species, in orchids studies on this transcription factor family are almost completely missing.
The analysis of the inflorescence transcriptome and miRNome of *Orchis italica* revealed the presence of 12 *TCP* transcripts expressed in the inflorescence tissues of this wild Mediterranean orchid species. The phylogenetic analysis showed that they belong to different TCP classes (I and II) and groups (PCF, CIN and CYC/TB1) and display a number of conserved motifs when compared to the TCPs of *Arabidopsis* and *Oryza*. In addition, for the first time in an orchid species, we validated the specific cleavage activity of the microRNA miRNA319 on one *TCP* transcript of *O. italica*, demonstrating the evolutionary conserved role of this miRNA in regulating the activity of specific TCP proteins in orchids. The analysis of the expression pattern of the 12 *TCP* transcripts in different inflorescence organs at two developmental stages and in leaf tissue showed that some of them are expressed in all the tissues examined, suggesting their involvement in multiple pathways, while others are expressed only in specific organs, indicating a more specialized function.

B5.Identification of miR-21 as a novel regulator of mesothelin (MSLN) expression

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MicroRNAs (miRNAs) are small non-coding RNA molecules that control mRNAs expression at a post transcriptional level, mainly acting as negative regulators through translation inhibition. The identification of *bona fide* targets represents the most challenging aspect for researchers working on the functional aspect of miRNAs. Recently, we developed a new method (miR-CATCH) based on biotinylated DNA antisense oligonucleotides that facilitates the characterisation of miRNAs::mRNA interactions within the physiological cellular context. Here, the miR-CATCH technique was applied to *mesothelin* (*MSLN*) gene and coupled with next generation sequencing (NGS), to identify miRNAs possibly responsible for the steep increase of MSLN protein levels found in malignant pleural mesothelioma. Biotinylated MSLN oligos were employed to isolate miRNA::MSLN mRNA complexes in Mero-14 and Met-5A cell lines, used as model of high and low MSLN expression, respectively. MiRNAs targeting *MSLN* were then identified by NGS and miR-21 and miR-100 were selected for further validation analyses. Although not differentially expressed in normal versus malignant cells, miR-21 was shown to be able to modulate MSLN expression in miRNA mimic and inhibitor experiments and thus represents a novel regulator of this transcript. This work shows that the miR-CATCH technique, coupled with NGS and *in vitro* validation, represents a reliable method to identify native miRNA::mRNA interactions

B6.JNK-dependent and independent effects triggered by Drosophila pseudouridine synthase gene silencing

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Members of eukaryotic pseudouridine synthases family are highly conserved from Archaea to man. These multifunctional proteins are essential components of the H/ACA snoRNP
complexes involved in ribosome biogenesis, pseudouridylation of target RNAs, as well as multiple additional functions. We used Drosophila melanogaster as animal model to investigate the biological roles of H/ACA snoRNPs and followed the developmental effects triggered by silencing of the *Nop60b*/*minifly* (*mfl*) gene (that encodes the fly pseudouridine synthase) in wing development. Intriguingly, we found that localized *mfl* silencing induces context-dependent effects, being able to trigger ectopic activation of either the JNK stress pathway and the Wg/Wnt mitogenic pathway in different wing areas. Moreover, depletion of MFL protein also causes alteration of apico-basal polarity and disruption of adherens cell junctions. Rescue experiments showed that simultaneous silencing of *mfl* and *jnk* can recover either cell-death and ectopic Wg/Wnt secretion, but not alteration of cell polarity and loss of adherens junctions. We conclude that *mfl* silencing induces JNk-dependent cell death and Wg/Wnt-dependent compensatory proliferation, while defects in cell polarity and adhesion are JNK-independent. Hence, disfunction of multiple essential developmental pathways is likely to account for the variety of different symptoms shown by X-DC patients, the human disease caused by reduced pseudouridine synthase activity.

B7.The heat shock protein 90 (hsp90) in the aphid *Myzus persicae* (Hemiptera; Aphididae)

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Heat-shock proteins 90 (hsp90) are a class of proteins stabilizing a network of "client" proteins that are involved in diverse signal transduction pathways affecting several processes. Recent studies indicated that hsp90 are involved in canalization and genetic assimilation. Indeed, in both flies and plants, mutations in the hsp90-encoding gene induce a wide range of phenotypic abnormalities, which have been interpreted as an increased sensitivity of different developmental pathways to hidden genetic variability. In order to verify the role of hsp90 in aphids, we amplified and sequenced the hsp90-encoding gene in 20 clones of the aphid *Myzus persicae* looking for the presence of mutations. In particular we compared clones with different reproductive modes, propensity to develop winged females and karyotype stability. We identified 9 clones with severe mutations (including frameshift and nonsense mutations) resulting in the production of inactive hsp90. Mutated hsp90 genes were present in aphid clones characterized by the absence of winged females and by the presence of obligate parthenogenesis. No mutations have been observed in aphids with karyotype instability. Literature data reported that hsp90 modulates juvenile hormone (JH) signalling in *Drosophila*. In aphids, JH is involved in the wing development and in the production of sexual morphs suggesting that mutations in the hsp90 genes could disrupt the proper JH signalling during the aphid development.

B8.Analysis of transcriptional factors involved in the maintenance of totipotency among Eukaryotes

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In most animals, the only cells that are truly totipotent are found in early embryos, while adult somatic stem cells are considered to be merely pluripotent. Totipotency in plants is not restricted to early embryonic cells, but it is also present in somatic differentiated cells, whose daughter cells can revert back to a stem cell fate under certain situations. Only recently the notion of stem cell reversal has been recognized for animal somatic stem cells. Specific transcriptional factors have been heralded to have an important role in the maintaining/restoring of totipotency in animals and plants. Several attempts have also been made to compare the molecular mechanisms underlying animals and plants. However, to which extent plants and animals are similar in this context remains an unsolved question. In the present study we provide an analysis of the transcriptional factors that play an important role in the maintaining/restoring of totipotency in plants and animals. Proteins, mainly selected from Uniprot database, were analysed through an automated workflow aimed at describing a phylogenetic relationship between factors involved in the totipotency among eukaryotes. The analysis of the tree suggests a connection between basic principles involved in regeneration in the two kingdoms. This indicates that a better understanding of mechanisms underlying the process of differentiation/de-differentiation in plants could be illuminating for stem-cell biology in other systems as well.

B9.Eukaryotic pseudouridine synthases and stem cell maintenance

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H/ACA snoRNP pseudouridine synthases belong to a highly conserved family whose members play essential functions, including ribosome biogenesis and pseudouridylation of target RNAs. Loss of function mutations of *DKC1,* the pseudouridine synthase human coding gene, cause Dyskeratosis Congenita X-Linked (X-DC), a multisystemic syndrome accompanied by telomerase defects, premature aging and stem cell dysfunction. Drosophila represents a valuable system in which to assess the mechanisms by which pseudouridine synthases regulate stem cell self-renewal. We thus triggered silencing of the *mfl* gene, the Drosophila ortholog of *DKC1*, with the aim to investigate the effects on the formation of larval Adult Midgut Precursor (AMPs) cells, which are an ideal model system for the study of epithelial stem cell lineage. We found that ubiquitous and localized silencing totally disrupts the formation of the imaginal islands, the typical stem niche in which AMPs are organized, and impedes AMPs to proliferate and expand their number. Intriguingly, the AMPs, but not the enterocytes, activate a premature autophagic program. This finding indicates that gene silencing induces a stress response specifically in the stemness compartment and unreveals for the first time a link among depletion of *mfl*-encoded pseudouridine synthase, AMP maintenance and autophagy. We suggest that this effect can be evolutively conserved and possibly accounts for stem cell dysfunction caused by X-DC.

B10.Antimicrobial Peptide-Mediated Immune Response in the Silkworm *Bombyx mori*

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The silkworm *Bombyx mori* has an innate immune system, whose most important effectors are the antimicrobial peptides (AMPs). Silkworm strains can be grouped into 4 geographical types (Japanese, Chinese, European and Tropical) characterised by a different resistance to infections, which is inversely correlated to the silk productivity.
We selected 4 strains (Japanese, Chinese, European and Indian) and we performed oral infections to assess if their different sensitivity was related to a diverse AMP-mediated immune response. We characterised morphologically their midgut *epitelia*, the first defence barrier against oral infection. In addition, we analysed the 21 AMP coding sequences to identify possible strain-specific protein isoforms.
The 5th instar larvae were infected with 2 silkworm pathogens: the G+ *Enterococcus mundtii* or the G- *Serratia marcescens.* The differential pathogen sensitivity of the 4 strains was determined by comparing the survival curves, the rate of the melanization response, the hemolymph antimicrobial activity and the expression induction of 9 representative AMP genes in fat bodies and midgut.
The 4 strains are characterised by a differential resistance to infections. There is a general correlation between the survival profile and the AMP transcriptional activation at systemic level when *B.mori* strains are exposed to *S.marcescens*. On the contrary, the resistance to *E.mundtii* appears to be related to the activation of specific AMP types in the midgut *epithelia*.

B11.dFmr1, a protein with a known role in the nervous system, belongs to the piRNA pathway, active in the *Drosophila melanogaster* gonads, for the silencing of transposons

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Mutations in the fragile X mental retardation protein (FMRP) cause Fragile X syndrome, that represents the most common form of mental retardation in humans. Beyond the mental retardation, Fragile-X patients, exhibit physical abnormalities and defects in the gonadal tissues. The role of the RNA binding protein FMRP has been elucidated in the nervous system. Indeed, it has been suggested that it associates and interacts with components of the Argonaute family, acting as a translational regulator assisted by small RNAs, at synapses.
We have recently demonstrated that dFmr1, the Drosophila homolog of FMRP, participates in the piRNA-mediated silencing of the transposons and the repetitive sequences in the male as well as in the female gonads, affecting the fertility. Hence we suggest that dFmr1 has a role in the piRNA-pathways in the *Drosophila melanogaster* gonads. Our data provide novel perspectives for understanding the phenotypes observed in Fragile X patients and support the view that piRNAs might be at work in the nervous system.

C1.Long Noncoding RNAs in muscle pathophysiology

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Long non-coding RNAs (lncRNAs) have been shown to be involved in different epigenetic mechanisms of gene regulation.
To investigate the function of lncRNAs during muscle pathophysiology we analyzed microarray expression data identifying 173 lncRNAs differentially expressed in different types of muscle fibers. We compared the subcellular localization of the 173 lncRNAs and found that 27 are expressed more in the nucleus, 16 in the cytoplasm and that 8 have a different localization depending on muscle type and/or lncRNA isoform.
We then chose 22 lncRNAs with different subcellular localization and analysed their expression in proliferating and differentiating C2C12 cells using quantitative Real Time PCR. We found 18 lncRNAs differentially expressed during differentiation. We then analysed the expression of these 22 lncRNAs in denervated muscle evidencing their alteration after sciatic nerve cut.
The subcellular localization of 16 lncRNAs was validated through qRT-PCR associated with differential extraction of nuclear and cytoplasmic RNA in C2C12 model and by in situ hybridization.
After mapping the subcellular localization we aim to identify interactions between lncRNAs and proteins or other ncRNAs and to study the effects of lncRNA knockdown/overespression on myoblast differentiation to better understand the role of each lncRNA in muscle development/regeneration.
Study supported by Dr. Cagnin's PRAT (Progetto di Ateneo) from University of Padova.

C2.Unicellular to multicellular: there and back again. A yeast tale

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The model organism *Saccharomyces cerevisiae* can undergo a morphological transition from the typical unicellular morphotype to a filamentous/multicellular state. This process, defined as dimorphism, represents a powerful adaptive mechanism exploited by both pathogenic and not pathogenic fungi (Ryan et al., 2012). Recent findings revealed the involvement of prion-based epigenetic mechanisms in the generation of complex multicellular phenotypes in yeast (Holmes et al., 2013). To contribute to this challenging topic we will take advantage of the natural M28 *Saccharomyces cerevisiae* meiotic derivatives which show 2:2 segregation of the unicellular/multicellular phenotype. Interestingly, these strains are able to epigenetically switch from a filamentous to a unicellular phenotype and vice-versa, with a high reversion rate. The analysis of protein structural changes through the recent mass spectrometry-based LiP-SRM technique (Feng et al., 2014) allowed the identification of several prion-like proteins whose conformation changes during the phenotypic switch, suggesting a multi-faceted regulation of this transition. The integration of transcriptomic, genomic and proteomic data allowed the identification of 10 prion-like proteins as potential regulators of multicellularity in natural yeast. The experimental validation of these candidates is in progress. However, our preliminary results showed that these putative prion proteins are able to drive the multicellular-to-unicellular switch.

C3.A unique mouse model for the study of epi(genetic) defects of two growth-related imprinting disorders: Beckwith-Wiedemann and Silver-Russell syndromes

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Genomic imprinting is an epigenetically regulated process determining allele-specific expression in a parent-of-origin dependent manner. Altered expression of imprinted genes can be associated with at least ten congenital diseases, sharing common clinical features such as growth, metabolic and behavioral disturbances. Epigenetic alterations affecting the *IGF2/H19* imprinting locus at 11p15.5 chromosome region cause two imprinting disorders with opposite growth phenotypes, the overgrowth-associated Beckwith–Wiedemann syndrome (BWS) and the growth restriction-associated Silver–Russell syndrome (SRS). Since the 11p15.5 imprinting cluster is broadly conserved between human and mouse, we decided to study the aetio-pathological mechanisms of these two imprinting disorders by generating a mouse model.
We have produced a mouse line through a knock-in mutation, in which the *Igf2/H19* ICR (H19 DMR) is replaced by the homologous human sequence (ICR1), carrying a mutation found in BWS patients. This mouse line shows increased ICR1 methylation, *Igf2* activation, *H19* repression and overgrowth mimicking BWS, upon maternal transmission of the mutation, and an opposite growth and molecular phenotype resembling SRS upon paternal transmission. A number of tests for in-depth phenotypic characterization of the mouse line upon maternal and paternal transmission of the mutation is in progress with the aim to identify causes of growth alteration and metabolism dysfunction.

C4.Sperm DNA methylation level at interspersed repetitive sequences and environmental exposure

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DNA methylation is pivotal for a wide array of biological processes and is the epigenetic change most studied in relation to environmental exposures. Aberrant DNA methylation has been associated, in somatic cells, with exposure to a variety of environmental contaminants, POPs (persistent organic pollutants) included. DNA methylation can be assessed by a variety of methods but determining its levels after pyrosequencing in retrotransposons, such as Alu, and long interspersed nucleotide elements (LINE-1) has become a popular strategy for the assessment of epigenetic modifications in epidemiological studies. To investigate whether POPs may operate at epigenetic level in the male germ cell line, we applied the same approach to measure DNA methylation level for Alu and LINE-1 repeats, integrated by the analysis of the non-transposonic tandem repeat sequence Satα. We evaluated the mean methylation level of the 3 repetitive sequences in frozen archived semen samples from some 270 fertile men from Greenland, Ukraine, and Poland. We assessed the environmental exposure to PFASs (perfluoroalkyl substances), PCBs (polychlorinated biphenyls), and DDT (dichlorodiphenyltrichloroethane). General linear models were used to analyse associations between POP body burden, measured in serum, and DNA methylation endpoints. No strong, consistent associations were detected between internal POPs concentrations and sperm DNA methylation biomarkers. *Supported by EU FP7 Contract 226217, Project CLEAR*

C5.Methy-sens comet assay and DNMTs transcriptional analysis as a combined approach in epigenotoxicology

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Epigenetic effects of environmental contaminants are currently becoming a major concern, considering their role in influencing development, adult life and etiology of disease. Given the need of simple and adaptive tools to assess these effects we propose a revised version of a comet assay modification designed to detect global methylation changes through enzymatic digestion with two restriction enzymes (HpaII and MspI). We developed a new protocol of a methylation-sensitive (Methy-sens Comet) comet procedure and tested its repeatability on several cell lines. Methy-sens Comet preserves a comet assay trademark features such as single cell analysis allowing the user to identify sub-populations with different sensibility to epigenotoxic stressors. The protocol was used to identify effects of known demethylating or hypermethylating substances as decitabine and nickel chloride. Changes in the transcriptional activity of genes encoding DNMTs, the major actors of the methylome maintenance system, were analyzed by means of real-time RT PCR to identify if the activity of these genes was suitable as an additional bio-marker in the characterization of the epigenotoxic activity of xenobiotics. Our data demonstrated that this combined study design represents a simple and multifunctional approach to implement biomonitoring studies on epigenotoxicological effects of known and unknown xenobiotics.

D1.Maternal history of Southeast Asia and Oceania: a landscape genetics approach

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Southeast Asia and Oceania were home to both one of the earliest and the last great human migration events. The first modern humans to exit Africa arrived in Southeast Asia around 60,000 years BP, subsequently reaching New Guinea and Australia at least 49,000 years BP. This initial wave of colonists was much later followed by a second migration, which eventually completed the colonization of Remote Oceania as late as 1000 years BP. Aside from these two main waves of colonization, several small range movements of populations may have shaped the current genetic diversity of Southeast Asia and Oceania. Mitochondrial DNA (mtDNA) has been extensively used to investigate the history of Oceania/Southeast Asia and, as a result of these studies, we now have the geographical resolution and the sample size required to make inference about past population events. Unfortunately, several past works on mtDNA have been focused on understanding the ancestry of single haplogroups, instead of providing a broad picture of current population diversity. Here we have assembled, to our knowledge, the most comprehensive data set of mtDNA hypervariable region I sequences from Southeast Asia, Near and Remote Oceania. We aim to use this data to provide a better understanding of the landscape of mitochondrial DNA diversity in island Southeast Asian populations and more insight into the migration patterns of humans in this region of the world.

D2.Genome-wide segmental duplication analysis in mammals

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Differences in fixation and copy number polymorphisms have contributed to the phenotypic "plasticity" and species-specific differences between humans and great apes. Segmental Duplications (SDs) have disproportionately affected the African great ape lineages where they appear to have accumulated at an accelerated rate. Widening the view on other mammalian genome assemblies and providing high quality sequence resolution of selected loci in seven mammalian species (macaque, marmoset, dog, cow, elephant, opossum and platypus) we elucidated the mechanisms involved in SDs evolution and SDs distribution. Gene-rich regions embedded in segmental duplications can result as phenotypic traits own of a particular lineage. Two bioinformatics pipelines, the former dependent from the assembly (Whole Genome assembly comparison, WGAC), the latter useful in the retrieval of duplications that are lost by the assembling algorithms (Whole-genome shotgun sequence detection, WSSD) have been used. In order to detect duplications, unique clones identified and validated by FISH, were used to set the threshold for WSSD. SDs calls have been correlated with contig and scaffold statistics in every genome release. Intra and inter SDs ratios have been compared in all considered species and gene content has been analyzed in order to detect specie-specific gene copy number variants

D3.Stress, transposons and genome evolution

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It has been shown that in flies and plants mutations in the stress protein Hsp90 induce a wide spectrum of heritable phenotypic variants. The interpretation was that Hsp90 is a capacitor of morphological evolution and buffers pre-existing genetic variation that is not expressed and accumulates in neutral conditions. This stress-sensitive storage and release of genetic variation by Hsp90 would favour adaptive evolution.
However, our recent study has suggested a different explanation of these results (Specchia et al., 2010). It has been demonstrated that Hsp90 is involved in repression of transcription and mobilization of transposable elements in germ cells by affecting piRNA biogenesis. The reduction of HSP90 causes stress response-like activation and transposition of mobile elements along with a wide range of phenotypic variants due to the transposons insertions to the corresponding genes. In addition a molecular analysis of a phenotypic variant, isolated in Hsp90 mutant strain, has also shown a transposon insertion in the corresponding gene. Intriguingly, it has also found that other mutations that impair piRNA biogenesis as capable to induce phenotypic variation. This further indicates that the expression of morphological variability could be related to the disruption of the piRNA silencing mechanism.
So that, we proposed that, in general, the stress causes the activation of transposons that induce *de novo* gene mutations affecting development pathways.

D4.The first mitochondrial survey on the current population of the Maltese cattle breed testifies a strong and significant founder effect and a maternal influence from Northern Europe

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Local breeds represent an important and often unique pool of endangered sources of genetic variability, particularly when confined to an isolated geographic area. The Maltese breed of cattle is considered to be of ancient origin. Late Pleistocene Oxen skeletal remains and Neolithic representations of primitive cattle have been suggested as proof of a possible local domestication. The objective of the present study is to explore the current mitochondrial DNA (mtDNA) diversity of this breed in order to reconstruct its maternal origins and to identify any residual genetic variants to be preserved.
A mtDNA control-region analysis performed on the entire Maltese cattle population identified only two different mtDNAs (out of a total amount of 19 samples), one encompasses about 90% of the current population and confirms a strong founder effect on the mitochondrial gene pool; the remaining 10% seems to testify for the importation of British cattle, documented in historical records since 1809. The complete mtDNA has defined two novel clades T3c and T3d, both dated to ~9.5 thousand years ago, encompassing the Maltese breed and only a few other breeds of Northern European ancestry.
This new piece of information does not support the hypothesis of a local domestication in Malta since the Maltese cattle mtDNAs are nested within the known domestic founding lineage T3, but confirms a strong maternal influence from Northern Europe rather than from the African coastline.

D5.Genomic technologies uncover inter-varietal structural variation in grapevine

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Grapevine (*Vitis vinifera* L.) is worldwide recognized for its economically valuable role in fruit and wine production; therefore, great interest has been shown in identifying genomic variations and their functional effects on inter-varietal phenotypic differences. Characterization of all genetic variations will be crucial to reach a full understanding of the genetic basis of phenotypic differences.

Our approach combines high-throughput sequencing, array CGH, FISH, and qPCR; and was able to create an inter-varietal atlas of structural variations and single nucleotide variants (SNVs) for the grapevine genome analyzing 4 economically and genetically relevant grapevine varieties. We found 4.8 million SNVs and detected roughly 8% of the grapevine genome affected by genomic variations. Noteworthy, we were able to find genes subjected to CNV as candidate for phenotypic differences between varieties. For example, we mapped 6 different copies of the genes coding for the germacrene D synthase and a flavonol synthase gene in a region, which showed the highest rate of duplication in Italia cultivar being reported as the only aromatic in analysis. Likewise comparison among the different grape genomes showed differences in gene dosages playing critical roles in response to biotic and abiotic stresses.

Overall, our data highlight the significance of these genome-wide studies on CNVs in grapevine genome and identified candidate genes for some of the most complex and desired traits in breeding.

D6.Improvement of the bovine Y-chromosome phylogeny through the identification of novel markers

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Compared to the mtDNA phylogeny, the *Bos taurus* Y-chromosome (BTAY) phylogeny is inadequate both for the number of haplogroups identified and for the level of detail attained. This is mainly due to the lack of Y-specific markers: only six SNPs, derived from the genes *DBY*, *UBE1Y*, *UTY*, *USP9Y* and *ZFY*, are currently available. This set of markers allows the classification of modern cattle breeds into three Y-chromosome haplogroups: Y1, which is prevalent in Northwestern Europe, Y2, which is prevalent in Southern Europe and Anatolian cattle, and Y3, which is zebu-restricted. A limited increase in the BTAY markers coverage has been achieved by combining SNPs with Y-specific microsatellites. Overall, these studies confirmed the outcome of phylogenetic analyses based on mtDNA variation, but netted a modest improvement in the resolution of the BTAY phylogeny by hinting just a likely Y2 sub-haplogroup.

The aim of our study was to mine the sequence of BTAY for additional STS. Thus, we analysed 1.2 millions bp of BTAY sequence proximal to the pseudoautosomal region, and characterized 107 novel STS that are readily usable for population studies. After having tested the STS for polymorphisms, we typed 16 of them showing genetic variation in a sample of 1267 cattle, obtaining an improved and deeply structured Y-chromosome phylogeny that shows a robust separation of the three known haplogroups, Y1, Y2 and Y3, and highlights for the first time a complex pattern of Y2 subhaplogroups.

D7.The youth of an evolutionary novel centromere

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Evolutionary new centromeres (ENC) are centromeres that have repositioned along a chromosome without any marker order variation. The most likely hypothesis is that they were seeded in an anonymous sequence devoid of satellite DNA. Then they evolved toward the normal complexity of mature centromeres with block of satellite DNA and segmental duplications etc... Recently we discovered that a supposed complex structural rearrangement of orangutan chromosome 9 (homolog to human 12) was an ENC. It is present as a polymorphism (~25%) in both Sumatra and Bornean orangutans and is devoid of satellite DNA. This ENC represents a unique opportunity to study an ENC at an early stage of development. We recently identified 7 heterozygous and one homozygous orangutan individual. We investigated the sequence and compaction of the ENC domain with respect to wild type as well as the potential crossover suppression effect upon the chromosomal segment encompassed by the normal and ENC locus. Our striking preliminary results show that the sequence of the ENC domain appears identical to the wild type even after at least 400.000 years of evolution, the minimum time of the separation of Borneo (*Pongo pygmaeus*) and Sumatra (*Pongo abelii*) orangutans. This finding strongly supports the hypothesis that ENCs are initiated by an essentially epigenetic phenomenon.

D8.Geographical distribution of Y-chromosomal STR alleles in Turkey

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We used the AmpFLSTR® Yfiler® kit to generate the genetic profile at 17 Y-STR loci from 124 males sampled in 6 different Turkish locations. We analyzed this dataset in the context of 1489 additional Turkish males from the literature. Overall, the 1613 males represented 9 different regions, spanning from Istanbul (extreme North-West) to the Eastern Black Sea coast and the South Eastern boundary with Syria, plus Cyprus. Seven loci (DYS19, DYS389I and II, DYS390, DYS391, DYS392, DYS393), shared across all studies, were used for the analysis. The Eastern regions displayed slightly lower haplotype diversities (<0.999) and the highest Fst values in pairwise comparisons. The DYS391 and DYS392 loci displayed Fst values above the average. Moreover, we found clear instances of East-West and North-South gradients in the frequency of individual alleles within the Anatolian Peninsula (DYS389II-16, DYS392-11 and DYS393-12). Interestingly, these are also highly predictive of the Y-chromosomal haplogroup J, the clearest paternally inherited marker of eastward migrations across the Northern Mediterranean. Our results lead us to infer a detectable structuring for haplogroup J within Turkey, which may be informative on the source sub-populations which were mostly involved in such migratory movements. Grant sponsor: MIUR-PRIN 2012JA4BTY_003.

D9.Genome-wide exploration of DNA conformational flexibility in Saccharomyces cerevisiae

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We provide the first comprehensive map of DNA conformational flexibility in *Saccharomyces cerevisiae* genome. Flexibility is crucial in DNA supercoiling and DNA/protein binding, regulating DNA transcription, replication or repair. Specific interest in flexibility analysis concerns its relationship with human genome instability. Enrichment in flexible sequences has been detected in unstable regions of human genome defined fragile sites, where genes map carrying frequent deletions and rearrangements in cancer. Flexible sequences have been suggested to be the determinants of fragile gene proneness to breakage, but their actual role remains elusive. Our *in silico* genome-wide analysis shows the conserved presence of highly flexible regions in budding yeast as well as in genomes of other Saccharomyces *sensu stricto* species. Flexibile peaks in *S. cerevisiae* map on 3'UTR of 175 ORFs characterized by decreased half-life transcripts and shared function on cell cycle regulation or stress response. (TA)n repeats shape the central structure of peaks and co-localize with polyadenylation efficiency element (EE) signals. Our findings support the functional importance of flexibility peaks, suggesting that the flexible sequences derive by an evolutionary neofunctionalization of canonical TAYRTA elements leading to a differential 3'-end processing and regulation in genes with peculiar function. They provide a strategy for the characterization flexible sequences inside the human fragile sites.

D10.Identification of conserved chromosomal regions and fragile sites in the holocentric chromosomes of aphids (Hemiptera; Aphididae)

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Literature data suggested a possible link between a reciprocal heterozygous translocation involving autosomes A1 and A3 and the presence of red morphs (in place of green) in the aphid *Myzus persicae*. To verify this relationship, we mapped the genes coding for carotenoids (that are at the basis of the aphid colour) in the genome of the aphids *Acyrthosiphon pisum* and *M. persicae*. This analysis showed the occurrence of a large DNA sequence (more than 240kb) in synteny between the two aphid species. According to the results obtained by *in situ* PCR, genes for the carotenoid synthesis (then referred as carotenoid genes) are located in a subterminal portion of autosome 1 in both species. The study of the map of the carotenoid genes in two *M. persicae* clones with 1 and 3 translocations revealed that these genes were localized in proximity to the chromosomal region involved in the translocation. Therefore, carotenoid genes are new chromosomal markers for the study of chromosomal rearrangements in aphids. In view of the availability of the complete sequence of the *M. persicae* scaffold containing the carotenoid genes and its localization in a chromosomal region recurrently involved in rearrangements, by bioinformatics analyses we identified the presence of fragile sites in *M. persicae* genome. Our results assessed, for the first time that the chromosomal architecture could be at the basis of recurrent karyotype rearrangements in aphids.

D11.Genetic affinities among North-Eastern Mediterranean population samples using the 16 STR loci for human identification.

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Because of their broad variability, autosomal STRs are powerful tools in human identification and forensics. In this work, we report the genetic profiles (16 autosomal STRs) generated with AmpFLSTR® NGM SElect™ on 1493 individuals from 41 sampling locations relevant to reconstruct genetic affinities along the Northern Mediterranean: Central and Southern Italy, Continental Greece, Aegean Islands and Turkey. Additional 99 individuals from the Czech Republic and Palestine were typed as Western and Eastern outgroup samples, respectively. Quality control included repeated typing, blind cross-check with external laboratories and estimation of hidden relatedness. Our results show a low degree of differentiation among samples (overall Fst = 0.0022). They also confirm the common occurrence of drift/founder effects in the same locations, previously detected by us with uniparental markers. Geographically coherent patterns of allele frequency variation could be nevertheless detected at some loci. Some uncommon allelic variants were found to be shared only between Southern Italy (Apulia and Calabria) and Western Greece, suggesting recent genetic exchange between these two areas. Overall, a higher degree of allele sharing was observed between Southern Italy and Continental Greece than Turkey. In conclusion, this STR dataset may help in separating the genetic contributions of different migratory movements along the East-West axis, which impacted Southern Italy over several millennia.
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D12.Mitogenomes from Egyptian cattle breeds: new clues on the origin of haplogroup Q and the early spread of *Bos taurus* from the Near East

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Genetic studies support the scenario that *Bos taurus* domestication occurred in the Near East about 10 thousand years (ky) ago, with the likely exception of a minor secondary event in Italy. However, despite the proven effectiveness of whole mitochondrial genome data in providing valuable information concerning the origin of taurine cattle, until now no population surveys have been carried out at the level of mitogenomes in local breeds from the Near East or surrounding areas. In this study, by using Illumina high-throughput sequencing, we characterized the mitogenomes from two autochthonous taurine breeds, Menofi (N=14) and Domiaty (N=17), from the Nile Delta region. Phylogenetic analyses of the 31 mitogenomes confirmed the prevalence of haplogroup T1, similar to most African cattle breeds, but showed also high frequencies for haplogroups T2, T3 and Q1, and an extremely high haplotype diversity, while Bayesian skyline plots pointed to a main episode of population growth ~12.5 ky ago. Comparisons of Nile Delta mitogenomes with those

from other geographic areas revealed that (i) most Egyptian mtDNAs are probably direct local derivatives from the founder domestic herds which first arrived from the Near East and the extent of gene flow from and towards the Nile Delta region was limited after the initial founding event(s); (ii) haplogroup Q1 was among these founders, thus proving that it underwent domestication in the Near East together with the founders of the T clades.

D13.Maternal and paternal contributions to gene-language correlations in the Old World

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The central goal of the ERC advanced grant project LanGeLin (LANguage-GEne LINeages) is to investigate the relationship between genetic and linguistic diversity, the latter inferred from structural language features, rather than from the vocabulary.

Y chromosome (Y-chr) and mtDNA (mitochondrial DNA) provide complementary information and allow one to investigate the different migrational histories of males and females, and their impact over the global language-gene relationships.

We assembled two datasets including 36 Eurasian populations for which both mtDNA/Y-chr and linguistic data were available.

We calculated and compared phylogenetic trees and Mantel's correlations between genetic, linguistic and geographical distances starting from three matrices: d_{GEN} based on F_{ST} (genetic distances); d_{SYN} based on syntactic features (linguistic distances); and d_{GEO} based on geographical distance between pairs of populations.

Both similarities and differences were evident between patterns of genetic and linguistic variation, casting light on both the genealogical ties between populations, and the mechanisms of language change.

D14.Extensive non-allelic gene conversion among LTR elements in the human genome

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Human endogenous retroviruses (HERVs) arise from retroviral infections of germ-line cells and subsequent integration int
o the host genome. After integration, such inherited proviruses may experience numerous amplifications. These elements are flanked by two long terminal repeats (LTRs) that may play important roles in the regulation of the host gene expression. Recent data suggested that ectopic gene conversion might have had a role in the evolution of LTRs in humans, but the dynamics and the pervasiveness of this process has never been exhaustively explored. To understand the extent to which gene conversion occur and gain new insights into the evolutionary history of LTRs, we undertook an intra-species phylogenetic study of 52 LTRs on 16 different human Y chromosomes. By exploiting the haploid nature of the human Y chromosome and its known phylogeny, we defined several LTR elements as gene-conversion-acceptor-sequences and re-sequenced two of them in a wider sample set. Our comparative re-sequencing analysis revealed the existence of at least two new gene conversion hotspots on the human Y chromosome and suggested complex genetic links among LTRs from different chromosomes. Moreover, we found that these elements are characterized by an extremely high density of polymorphisms showing one of the highest nucleotide diversities ($\pi = 2.2 \pm 0.5 \times 10^{-3}$) in the human genome, as well as a complex patchwork of sequences derived from different LTR elements.

D15.Phylogeographic refinement of human Y chromosome haplogroup E provides new insights into the early dispersal of herders in subSaharan Africa

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Haplogroup E is the most common human Y chromosome clade within Africa and its internal branches have been linked to a wide range of human movements. To increase the level of resolution of haplogroup E, we disclosed the phylogenetic relationships among 729 mutations found in 33 haplogroup DE Y chromosomes sequenced at high coverage (50×) in previous studies and further dissected the E-M35 subclade by genotyping 62 informative markers in about 5000 samples from 118 worldwide populations. The phylogeny of haplogroup E showed novel features compared to the previous topology, including a new basal clade. Within haplogroup E-M35, we resolved basal polytomies and assigned all the E-M35* chromosomes to different new monophyletic clades. Through a Bayesian phylogeographic analysis, we associated each node of the tree to specific geographic areas. By this analysis, we identified a new E-M35 sub-Saharan clade, which originated about 11 kya in the northern part of the Horn of Africa. SNP-based dating, phylogenetic structuring and geographic distribution of this clade are consistent with a multistep dispersal of herders within eastern Africa and its subsequent diffusion to sub-equatorial areas. Our results provide new insights into the evolutionary hypotheses about the spread of pastoralism in Africa and increase the discriminative

power of the E-M35 haplogroup for use in forensic genetics through the identification of new ancestry-informative markers.

E1.*Drosophila melanogaster* as a model to study mitochondrial diseases: functional characterization of *dMpv17*

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Mitochondrial disorders are defined as clinical entities associated with defects of oxidative phosphorylation, which are ultimately genetically determined. During the last decade, an increasing number of nuclear genetic defects have been identified causing mitochondrial DNA (mtDNA) depletion syndromes (MDS), either through the accumulation of deletions (multiple deletions) or through a reduction in mtDNA copy number. Myopathic, encephalomyopathic, and hepatocerebral forms of MDS are known, due to mutations in gene products involved in mtDNA maintenance, either controlling the supply of deoxynucleotides or carrying out the synthesis of mtDNA. In mammals MPV17 is expressed in pancreas, kidney, muscle, liver, lung, placenta, brain and heart. Mutations in MPV17 are a prominent cause of hepatocerebral MDS, accounting for about 50% of the cases. In *D. melanogaster*, *Mpv17* maps on chromosome 2p23-21 and encodes a small hydrophobic mitochondrial inner membrane protein of 176 amino (38% identity with mouse and human; 35% with zebrafish and 28% with yeast SYM1). Studies on SYM1 suggest a role for this protein in controlling the flux of Krebs cycle intermediates across the inner mitochondrial membrane. However, how this functional data are linked to mtDNA maintenance and integrity is still unknown. In order to address these issues we have focused our attention on the functional and molecular characterization of *Mpv17* in *D. melanogaster* by using behavioral, biochemical, molecular and genomic approaches.

E2.TAS2R38 gene and chronic rhinosinusitis: a bitter ending

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Chronic rhinosinusitis (CRS) is a frequent disease with a high social impact and multifactorial pathogenesis. Recently, single nucleotide polymorphisms (SNPs) within the TAS2R38 gene have been implicated as possible contributors to the complex gene-environment interactions in CRS.
The purpose of this study was to confirm the proposed correlation between TAS2R38 genotype, CRS and related comorbidities and to assess whether the presence of a particular allele could be considered a prognostic marker.
53 CRS patients and 39 healthy individuals were genotyped at the TAS2R38 locus. CRS patients were treated by endoscopic sinus surgery and medical therapies and subdivided in “refractory” and “responsive”, depending on the clinical outcome, assessed by internationally accepted scoring systems. Chi-square analyses were used to assess the effect of genotype on CRS and CRS-related comorbidities.
The distribution of the different genotypes at the TAS2R38 locus was not significantly different between refractory CRS patients, responsive CRS patients and controls (χ 2 [10] = 2.75, p = 0.99). Besides, no association was found between the different genotypes at the TAS2R38 locus and CRSrelated comorbidities.
In conclusion, no association was found between TAS2R38 alleles or genotypes and CRS, thus questioning its role in the pathogenesis of CRS. Further studies on larger cohorts are needed to verify these findings and to shed light on the role of bitter taste receptors in CRS.

E3.Analysis of telomere variability in a population sample of Calabria

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Telomeres are specialized structures protecting chromosomes from degradation. The age related decrease of telomere Length (TL) has been associated to replicative senescence and is then considered a biomarker of age related physical decline. In order to better understand the correlation between TL and aging, we have examined TL in 684 subjects aged 64-107 years in a population sample from Calabria.
We did not find any association with the gender. As expected we found an inverse correlation between TL and age ($p<<0.01$). However, when the sample was split into two groups (<90 years: 90-; >90 years:90+), we observed that in the first group the inverse TL/age correlation was maintained, while in the 90+ we observed a direct TL/age correlation. In addition, in the group of 90- no correlation was observed with functional parameters; by contrast, within the 90+ we observed a correlation of TL with cognitive performance, and daily living performance.
Finally, among 90- it was observed to be correlated with lipid parameters and Thyroid function, which are among the main biomarkers of well being in this age group; among 90+ we observed a correlation of TL with the protein pattern of the serum (beta globulin, creatinine), which is, in this age a group, a parameter of metabolism well being.
These results confirm that TL may be a valuable marker of health status in the elderly but that its significance has to evaluated in the context of the specificity each age group.

E4.Genetic and epigenetic studies on DNA methyltrasferases in patients with thymoma-associated Myasthenia Gravis

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Thymoma is a rare neoplasm derived from epithelial cells of the thymus characterized by histologic variability, heterogeneity of malignant behavior and by the presence of autoimmune diseases, particularly Myasthenia Gravis (MG) in 30% of cases. MG is an autoimmune disease mediated, in the most of cases, by antibodies against the nicotinic acetylcholine receptor. Some studies suggest aberrant DNA methylation occurs in thymic epithelial cells and increased methylation significantly correlated with tumor severity. So in order to asses a role of epigenetic processes to the risk and progression of thymoma, we analyzed the methylation levels of DNA methyltrasfereases gene promoters: *DNMT1*, *DNMT3A*, *DNMT3B*, in blood, healthy and tumor tissue of 41 patients with thymoma associated to MG. We observed that in general *DNMT1* and *DNMT3B* gene promoters are not methylated, while *DNMT3A* is completely not methylated in blood but is partially methylated both in healthy tissue and tumor tissue. Interesting correlations among *DNMT3A* promoter methylation in tumor tissue and both histological type of tumor and Osserman classification of MG were found. Analyzing *DNMT3B* promoter polymorphisms (*DNMT3B* -579G>T and *DNMT3B* -149C>T) in a larger population, 216 thymoma patients with MG and healthy controls, we observed that after gender stratification the effect of *DNMT3B* polimorphisms was restricted to males. Present data suggest an involvement of DNMTs to the risk of developing thymoma-associated MG.

E5.Ageing genetics and longevity in humans

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Healthy aging and longevity in humans are modulated by a lucky combination of genetic and non-genetic factors. Twin studies demonstrated that about 25% of the variation in human longevity can be due to genetic factors.
The search for genetic and molecular basis of aging has led to the identification of genes correlated with the maintenance of the cell and of its basic metabolism. Indeed, mutations in genes encoding proteins involved in DNA repair, telomere conservation, heat shock response, and the management of free radicals' levels were found to contribute to longevity or, in case of reduced functionality, to accelerated senescence and the consequent organism aging. These observations led to the idea that healthy aging and longevity may arise from an efficient process of maintenance of cellular and organismal activities.
In parallel, studies showing that caloric restriction may increase lifespan in model organisms, suggested that longevity may be "directly" promoted. The identification of pathways modulated by calorie restriction has shown that the variability of genes associated with nutrient-sensing signaling, may have a key role in healthy aging and lifespan.
Current studies are showing that the interaction between genetic background and environment is essential to determine the individual chance to attain longevity. In this context, epigenetic studies are showing to be important to monitor such interaction and to be very sensitive in revealing individual biological age.

E6.Leukocyte telomere length in the progression of idiopathic pulmonary fibrosis

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Idiopathic pulmonary fibrosis (IPF) is an irreversible, usually lethal, lung disease in which clinical progression can be slow or rapid and the rate of progression strongly influences prognosis. Short leukocyte telomere length (LTL) have been identified in patients with IPF and implicated in disease pathogenesis as an indicator of early biological aging, however its clinical significance is still unknown.
We investigate whether the type of progression, slow or rapid, in IPF could be associated with LTL.
The % predicted FVC change/year was used to classify patients with IPF, followed over time, as slow (<10%pred) or rapid progressors (>10% pred). LTL was measured by real-time PCR in genomic DNA extracted from peripheral blood leukocytes of IPF patients (n=18) and unrelated age-matched healthy controls (n=35). LTL was significantly shorter in IPF patients than in controls (median, range: 1.2, 0.3-3.6 vs 1.8, 0.6-5.0; p=0.005). When IPF patients were stratified on rate of disease progression, LTL was significantly shorter in rapid progressors (1.0, 0.3-1.2) than in controls (1.8, 0.6-5.0;p=0.003) but not in slow progressors (1.3, 0.5-3.6). LTL was negatively correlated with FVC decline/year, expressed as both % predicted (r= -0.65;p=0.01) or absolute value (r= -0.61,p=0.01).
Shorter leukocyte telomere length is associated with a rapid progression of IPF. This finding might improve prognostic information and guide the management of the disease.

E7.Gene-specific methylation in blood DNA of Alzheimer's Disease and Mild Cognitive Impairment individuals

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Folate metabolism plays a crucial role in the regulation of epigenetic processes, particularly DNA methylation. Several evidences suggest that a deregulation of the folate pathway and DNA methylation might be involved in the pathogenesis of Alzheimer's disease (AD).
In this regard, we collected blood DNA samples from 205 late onset AD patients, 74 subjects with Mild Cognitive Impairment (MCI) and 175 healthy controls and we analyzed the methylation status of genes involved in amyloid-beta peptide production (*PSEN1* and *BACE1*), in DNA methylation (*DNMT1*, *DNMT3A* and *DNMT3B*), and in one-carbon metabolism (*MTHFR*), searching for correlation with age and gender, and biomarkers of one-carbon metabolism. Except for *DNMT3A*, our analysis revealed that MCI subjects showed higher methylation levels for all the studied genes, although the only significant ones after multivariate analysis are *DNMT1* and *DNMT3B*. We did not find any difference between AD patients and healthy controls in the mean methylation levels of the genes we have considered. Several of the well-known AD risk factors, including age, folate and homocysteine levels, showed a significant contribution to the methylation levels in particular of *MTHFR* gene.
Collectively those data suggest a link between biomarkers of one carbon metabolism and gene-specific methylation levels in patients and aged-individuals.

E8.Mitochondrial DNA Polymerase Gamma (POLG) deficiency triggers Hypoxia signaling pathway activation in zebrafish

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POLG-related disorders are a group of multi-organ pathologies characterized by the dysfunction of the mitochondrial DNA polymerase gamma, encoded by the nuclear gene POLG and essential for mtDNA replication, repair and stability. Despite many years of intensive research using rodent and cellular models, many aspect of the POLG-related disorders have not been elucidated. In this context, the zebrafish (*Danio rerio*) represents an ideal alternative vertebrate model of human mitochondrial diseases because of its high conservation of physiological processes and genomic structure, low-cost maintenance, transgenic lines availability, embryonic transparency.
Using zebrafish embryos, we have performed transient knock-down of the *polg* gene, obtaining a phenotype characterized by homogeneous developmental delay without obvious malformations, except for a dilated cardiomyopathy and an increased heart bit rate. Moreover, taking advantage of specific signaling pathway reporter lines, we have found that Hypoxia and CREB pathways are up-regulated in zebrafish *polg* morphants, while Wnt signaling appears unmodified. Our preliminary results support the zebrafish as a suitable model to perform Crispr/Cas9-mediated mutagenesis of POLG and other genes involved in Mitochondrial DNA depletion syndromes (MDDS). In conclusion, the evidence of Hypoxia reporter activation under *polg* knock-down conditions, suggests the existence of cross-talk mechanisms sensing mitochondrial dysfunction and changing Hypoxia signaling, as confirmed also by in-vivo analysis we recently performed using mitochondrial-complex inhibitors.

F1.Expression of *BDNF* mRNA in zebrafish treated with *Lactobacillus rhamnosus*

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Several researches on human and animal models have been performed to assess the role of microbiota on brain function, showing how this microcosmic world can influence brain chemistry and behavior. Neurotrophins constitute a family of structurally related proteins required for the development and function of the vertebrate nervous system where they regulate survival, differentiation and synaptic plasticity of specific neuronal populations. Alterations of gut microbiota have been associated with stress and decreased expression of brain neurotrophic factors in the CNS. The *brain-derived neurotrophic factor* (*BDNF*) gene expression in zebrafish has been documented in many tissues and organs. The purpose of this study was to determine whether the probiotic strain *Lactobacillus rhamnosus* IMC 501® influences brain neurochemistry in zebrafish. The *BDNF* mRNA expression was evaluated by using RT-qPCR in zebrafish, with dietary administration for 28 days of *Lb. rhamnosus*. The probiotic treated group showed a statistically significant two-fold increase in *BDNF* expression compared to the control group. This first investigation on zebrafish describes the impact of probiotics on neurochemistry and lays the foundation for advancements in knowledge of the microbiota–gut–brain axis in this popular "striped" animal model.

F2.Pleiotropic Effects of Micro RNA-210 Over Expression in Clock Neurons of *Drosophila melanogaster*

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Micro RNAs (miRNAs) are a novel class of small RNAs which act as modulators of gene expression either by inhibiting the translation or by inducing the degradation of their target mRNAs. Several studies suggest a role for miRNAs as regulators of the circadian clock in mammals and *Drosophila*. Based on computational predictions of target mRNAs of clock (or clock related) genes, we have selected the miR-210 as a putative regulator of the *period* clock gene. We demonstrated that flies over-expressing this miRNA in the canonical clock neurons show an impaired locomotor activity pattern in both light-dark (LD) and constant darkness conditions (DD). Moreover, the projections of the Pigment Dispersing Factor (PDF)-expressing clock neurons in the optic lobe are abnormal showing peculiar "star" shaped body cells. The micro-array analysis performed in the adult fly brain, revealed that this miRNA is affecting indirectly the expression of some circadian genes (ie: *pdf*, *npf*, *cry*, *tim*, *pdp1*) but not the *period* gene, and directly genes like *echinus* and *RhoGAp92B*. The *in-vivo* down regulation of *echinus* indeed can be associated with a severe impairment of the locomotor activity of flies, while the GTPase RhoGAP92B, important for the regulation of the cellular shape of neurons, could be involved in the morphological development of the PDF-expressing neurons.

F3.WDR79/TCAB1 is a conserved SMN modifier

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SMN (Survival Motor Neuron) depletion is the predominant cause of spinal muscular atrophy (SMA). Although SMN is required for RNA metabolism in every cell, it is particularly critical in the motor system. No effective cure has been identified so far for SMA and current therapeutic approaches are aimed primarily at ameliorating SMA through increasing SMN levels. However, SMA genetic models have identified several modifiers that can ameliorate the deficits induced by SMN depletion. Here we investigate WDR79/TCAB1, a gene involved in several aspects of RNA metabolism.

We show that WDR79/TCAB1 depletion induces locomotion defects, similar to those caused by pan-neuronal depletion of SMN in both Drosophila and C. elegans. In both species we also show that WDR79/TCAB1 overexpression ameliorates the SMN-dependent phenotypes. Our data establish that WDR79/TCAB1 plays a conserved role in the SMN pathway and may provide a novel therapeutic target for SMA .

F4.G6pd delta derived neurons as a model to "in vitro" mimic neurons aging

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In neurodegenerative diseases, oxidative stress is a relevant process associated to neuronal cell loss. It has been describe that oxidative stress increases in the brain with age and in the same time cell ability to respond to this stress declines.

In several neurodegenerative diseases before the onset of the disorder, neurons show impaired glucose uptake.

In the cells NADPH is an essential cofactor for the scavenging of oxidative stress. It is mainly generated in the pentose phosphate pathway (PPP), a metabolic pathway that supplies reducing energy to cells oxidizing glucose-6-phosphate and generating pentose sugars. We have generated a mouse G6PD-null embryonic stem cell line (G6pd delta ES cells), and we have shown that these cells are extremely sensitive to oxidative stress. Differentiating G6pd delta ES cells into neurons, we observed that these cells are able to differentiate into all neuronal lineages but GABAergic neurons fail to fasciculate.

We demonstrated that failing in fasciculation of G6pd delta derived neurons is a consequence of their impairment in defence against oxidative stress. Moreover, at molecular level we detected an increased expression of some aging-related molecular markers. These data suggest that G6pd delta derived neurons, unable to respond properly to oxidative stress, could be a useful model to mimic in vitro the condition of aged neurons.

F5.Nuclear localisation of tau protein in Alzheimer disease

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Tau is a multifunctional microtubule-associated protein (MAP) localised in different cellular compartments such as cytoplasm and nucleus. It is typically involved in the cytoskeletal organization and represents a key factor for the regulation of microtubule cytoplasmic dynamics. In the nervous system, tau promotes assembly and stabilization of microtubules, which is required for neuronal morphogenesis and axonal transport. Alteration of the normal tau cytoplasmic protein is directly related to Alzheimer disease. Tau protein was observed in both nuclear and cytoplasmic compartment also in non-neuronal cells such as fibroblasts and lymphocytes. However, the function of the non-cytoplasmic tau remains unclear. TAU gene (MAPT) is expressed in two transcripts of 6kb and 2kb. However while the 6kb mRNA is related to the microtubule associated protein, the 2 Kb mRNA has a non-microtubular function, and is mainly present in the nucleus. Here we present data on the nuclear distribution of tau protein in different cell types, including neuronal cells from normal and Alzheimer disease subjects. Obtained data showed that tau protein endowed by specific phosphorylation patterns is located prevalently in the nucleolus, with a distribution kind related to the functional status of the cells. A possible protective effect on nucleolar DNA or RNA given by binding with phosphorylated tau protein, also in the arising of Alzheimer disease, will be discussed.

F6.CRYPTOCHROME and the circadian control of visual sensitivity in *Drosophila melanogaster*

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In *Drosophila melanogaster* a functional circadian clock in the visual system is important to control visual coding efficiency and optimize vision under different light intensity regimes. We have observed that the diurnal rhythmicity of two different visual behaviours, electroretinogram (ERG) and optomotor turning response, relies on the presence of a functional circadian photoreceptor Cryptochrome (CRY) in the compound eyes.

We have found that CRY is present in the rhabdomeres of all photoreceptor cells, interacts with F-actin during day and night, and may therefore enhance the binding of the phototransduction cascade signaling components to the rhabdomere cytoskeleton in a light-independent way. Moreover the expression of CRY in photoreceptor cells 1-6 of the compound eyes enhances fly sensitivity to daylight.

We have observed that CRY interacts also with Bruchpilot (BRP), a scaffolding protein on the membrane of the presynaptic active zone of the lamina (the first optic neuropil of the fly's optic lobe). Both the formation of the presynaptic T-Bars and the BRP abundance at this structure show circadian variation, resulting in daily rhythms in the morphology and physiology of neurons, important for visual plasticity.

Taken together, these results show that CRY plays multiple circadian roles: in the clock neurons it is involved in the light-resetting of the clock, while in the compound eyes it contributes to the modulation of visual sensitivity.

F7.The CRISPR/Cas9 technology: a tool to generate new circadian mutants in *Drosophila melanogaster*

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The prokaryotic clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) is a powerful method to manipulate the genome of different organisms, including Drosophila. Cas9 is a sequence-specific endonuclease that recognizes and cleaves a target DNA with a specificity that is determined by a guideRNA (gRNA) molecule. Different systems have been developed to express Cas9–gRNA in germ cells of Drosophila. One of such strategies includes two transgenic strains: one expressing Cas9 protein from the germline-specific *nanos* promoter and the other ubiquitously expressing a custom guide RNA (gRNA) that targets a unique site in the genome. The two strains are crossed to form an active Cas9–gRNA complex specifically in germ cells: mutations induced in the founder germline are then transmitted to the next generation (Kondo and Ueda, 2013).
We have applied this strategy to generate new mutants alleles of the circadian gene *timeless* and tested them for some of the main features of the Drosophila clock, like endogenous rhythmicity and temperature compensation. Analysis by PCR and sequencing showed that most of the lines with an interesting circadian behaviour have insertion-deletion (indel) mutations encompassing the target site. These mutations are of different entity, providing us with a number of different alleles of the circadian gene *timeless*. We are now planning to introduce precise modifications using donor templates by homology-directed repair.

G1.Cell cycle-dependent resolution of DNA double-strand breaks repair

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Following exposure to genotoxic agents and DSBs formation, cells activate different repair pathways. These pathways are complementary and operate optimally under different circumstances. It is well known that an important step to determine the choice between one of two major pathways, HR and NHEJ, in non-synchronized cells is the cell cycle phase in which DSB occurs. Conversely, little is known about DNA damage response in quiescent cells. To shed light on the differences between the response to DNA damage in quiescent and proliferating cells, we generated an inducible MCF10A cell line for AsiS1-ER chimera expression. MCF10-AsiSI-ER cell line allows to study the accumulation of repair factors at specific DSBs throughout different cell cycle phases, asynchronously proliferating, G0-arrested, G0-reentry and G1 cells. ChIP-seq experiments show that γ-H2AX mapped with a similar efficiency in both G0 and proliferating cells. However, DSBs occurring in G0 cells are irreparable with a sustained activation of p53-pathway. Conversely, reentry of G0-damaged cells into cell cycle progression shows a delayed clearance of recruited DNA repair factors bound at DSBs. This study shows that cell cycle phases do not interfere with the recognition and recruitment of repair factors at DSBs, but rather profoundly affected resolution of DSBs.

G2.Incorporation of ribonucleotides during base excision repair: interference between ribonucleotide and base excision repair pathways

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The cellular pool of ribonucleotide triphosphates (rNTPs) is higher than that of deoxyribonucleotide triphosphates. To ensure genome stability, DNA polymerases must discriminate against rNTPs and incorporated ribonucleotides must be removed by ribonucleotide excision repair (RER). We investigated DNA polymerase beta (POL beta) capacity to incorporate ribonucleotides into trinucleotide repeated DNA sequences and the efficiency of base excision repair (BER) and RER enzymes (OGG1, MUTYH, and RNase H2) when presented with an incorrect sugar and an oxidized base. POL beta incorporated rAMP and rCMP opposite 7,8-dihydro-8-oxoguanine (8-oxodG) and extended both mispairs. In addition POL ! was able to insert and elongate an oxidized rGMP when paired with dA. We show that RNase H2 always preserves the capacity to remove a single ribonucleotide when paired to an oxidized base or to incise an oxidized ribonucleotide in a DNA duplex. In contrast BER activity is affected by the presence of a ribonucleotide opposite an 8-oxodG. In particular MUTYH activity on 8- oxodG:rA mispairs is fully inhibited, although its binding capacity is retained. This results in the reduction of RNase H2 incision capability of this substrate. Thus complex mispairs formed by an oxidized base and a ribonucleotide can compromise BER and RER in repeated sequences.

G3.Oxidative stress induces persistent telomeric damage and decreases the binding of TRF1 to telomeric sequences in human primary fibroblasts.

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In our previous study we demonstrated that oxidative stress induced telomere shortening/dysfunction in human primary fibroblasts (MRC-5 cells) 48 hrs after H_2O_2 treatment. Our hypothesis was that the persistent telomeric 8-oxoguanine (8-oxoG), the main biomarker of oxidative stress, could be responsible of the telomere shortening observed. In order to confirm the telomeric oxidative damage we tested the presence of telomere dysfunction induced foci (TIF) after treatment. MRC-5 cells were treated with two doses of H_2O_2 (100 μM and 200 μM) for 1 hr. Our preliminary results demonstrated that there were an increase of γ-H2AX foci 48 hrs after treatment and a parallel increase of TIF. About the 50% of this foci colocalized with telomeric protein TRF1 indicating that there were a persistent telomeric damage after treatment. Furthermore, to understand if the telomeric binding protein were involved in telomere shortening/dysfunction observed, ChIP assay for TRF1 and TRF2 were performed. Results demonstrated that oxidative stress decreases the binding of TRF1 with the telomere. TRF2 did not show differences between treated and control samples. This data confirm that oxidative stress induced a persistent telomeric damage that could be responsible of telomere shortening observed 48 hrs after treatment. Therefore the reduction of TRF1 telomere binding protein could be responsible of t-loop instability and consequently of telomere dysfunction

G4.Separase is required for genome stability in human cells

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The removal of cohesin from chromosomes is tightly regulated by a set of cohesin regulators. Bulk of cohesin on chromosome arms is removed by the phosphorylation of the subunit SA operated by CdK1, PLK1 and Aurora B during prophase and prometaphase. At the metaphase-anaphase transition, remaining cohesion is dissolved by the endopeptidase Separase, which cleaves the cohesin subunit RAD21. This cleavage permits to open the cohesin ring causing it to dissociate from chromosomes. Maintenance of ploidy is ensured through the concerted action of many cohesin proteins and in this regard Separase plays a key role. Separase knockout results in embryonic lethality whereas its depletion by RNA interference (iRNA) causes genome instability in mouse embryonic fibroblasts. Loss of Separase or its overexpression have been shown to cause mitotic spindle defects, premature sister chromatid separation and lagging chromosomes. These data have been produced in model organism or in human cancer cells. However, little is known about the role exerted by Separase on genome stability in normal human cells. To address this, we used iRNA to inhibit Separase expression in human primary fibroblasts. We find that depletion of Separase leads to both chromosome aberrations and aneuploidy. In addition, we identified, by co-immunoprecipitation followed by mass spectrometry, MCM proteins as novel molecular partners of Separase, shading light on the importance of separase in genome stability preservation.

G5.ALT mechanism induced by X-rays in human primary fibroblasts

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We have previously established that X-ray irradiation induces an increase in telomere length by 15 days after treatment in HFFF2 human primary fibroblasts. In order to better understand the mechanism underlying the telomere elongation observed, we irradiated HFFF2 with X-rays (4 Gy) and analyzed cells at 3, 4, 5, 8, 10, 13 and 15 days post exposure. By Q-FISH we observed a modulation of telomere length during this time frame. Specifically, we observed a fluctuating trend in telomere length: a telomere shortening at 2-3 days after treatment, a telomere elongation between 5-8 days, again a telomere shortening 9-11 days from treatment and a telomere elongation 13-15 days from treatment. HFFF2 did not activate telomerase after a 4 Gy X-ray irradiation at any of the time points tested. For this reason we focused our attention to the ALT (Alternative Lengthening of Telomeres) mechanism by performing CO-FISH, Immunofluorescence-FISH for PML-telomeres and the C-Circle assay. Our results showed an increase in all ALT-associated hallmarks tested at 4/5, 8 and 13 days after irradiation, which coincided with the bouts of telomere elongation measured by Q-FISH. We conclude that X-ray exposure induced a transient activation of a functional ALT pathway in human primary fibroblasts that caused restoration of telomere length in these cells. The fluctuating trend indicated that after X-ray treatment there were periods in which telomeres shortened and periods in which telomere length recovered.

G6.Induction of autophagy by mutant p53-targeting molecules in cancer cells

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Several studies support the oncogenic gain of function of mutant p53 (mutp53). Mutp53 are frequently present in tumors at higher levels than the wild-type protein (WT) and this accumulation is correlated with higher cell proliferation, cell invasion, tumorigenicity and chemoresistance.
We have previously demonstrated that in cancer cells PRIMA-1, a p53-reactivating small molecule known to exert anti-tumoral effects in various tumor cell lines, stimulated mutant, but not WT, p53 degradation through autophagy. Mutp53 knock down cells were more sensitive than the parental ones, suggesting that the removal of mutp53 through autophagy could promote cell death.
We are exploring the autophagic potential of other mutp53-degradating molecules such as Gambogic Acid (GA) and SAHA to study the correlation between autophagy, mutp53 degradation and cell death. GA is a molecule used in the traditional Chinese medicine that stimulates the ubiquitin/proteasome-mediated degradation of mutp53 and increases the sensitivity of cancer cells to chemotherapeutic agents. SAHA, a FDA-approved histone deacetylase inhibitor, strongly sensitizes cancer cells carrying mutp53 towards chemotherapy, due to its ability to degrade mutp53. Our results show that both GA and SAHA promote mutp53 degradation that is correlated with the activation of autophagy. The impact of autophagy on cell death induced by this molecules is currently being investigated.

G7.Genotoxic effects of *in vitro* MW radiation exposure in human foetal fibroblasts

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In recent years, Microwave (MW) radiation has been increasingly used in a variety of applications: medical, security, telecommunications and military areas. However, few and contradictory data are available on the biological effects of this type of electromagnetic radiation.This study is part of the multidisciplinary and multifrequency GREAM project in which we analysed in addition to MW also the biological effects of Terahertz radiation (De Amicis et al, 2015). The MW irradiation source is a YIG tuned oscillator, delivering 20 mW output power in the frequency range 18-40 GHz. Radiation is launched using a horn antenna and the size of the beam naturally expands by diffraction up the 5 cm diameter of the Petri dish.To evaluate the effects of MW radiation, we performed *in vitro* studies on human foetal fibroblasts. For the assessment of biological damage, several methodologies have been applied for the analysis of different genetic markers (gamma-H2AX, conventional and CREST-micronuclei assays, chromosome non-disjunction analysis and telomere length modulation).

The results indicate no statistically significant increase in phosphorylation of H2AX histone in irradiated samples, confirming results obtained from the analysis of negative MN induction. On the contrary, we observed an increase of centromere positive micronuclei frequency and an increase of non-disjunction events in treated samples. Interestingly, we also demonstrated a telomere length modulation.The preliminary results of this study are reported.

Reference: De Amicis et al., "Biological effects of *in vitro* THz radiation exposure in human foetal fibroblasts" Mut. Res. (2015). In press, available on line at doi: 10.1016/j.mrgentox.2015.06.003

G8.DNA damage and Autophagy

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Agents such as UV, IR, genotoxic chemicals and ROS could affect DNA integrity. Cells counteract their action through the induction of DNA repair systems and occasionally the activation of cell responses such as cell senescence and death. Autophagy is emerging to be linked to DNA damage response although its role is still not well known.

We have analyzed the involvement of autophagy during the treatment of U937 cell line with chemicals that induce different injuries inside cells (cisplatin, menadione, EMS, bortezomib, bleomycin). To reach this goal we have analysed the toxic and genotoxic effect of each compound and of its combination with rapamycin and chloroquine, inducer or inhibitor of autophagy respectively. Activation of the autophagy was assessed by the use of a plasmid coding for the LC3-GFP protein. The cytotoxic effect of the chemicals on U937 cells was measured by MTS. Genotoxicity was evaluated by the Alkaline Comet Assay.

Modulation of the autophagy brings, in almost all cases, to variation in the cyto- and geno-toxicity induced by the compounds. We have observed a dual role of autophagy in response to injuries: the activation of the pathway induces sometimes the reduction of the toxic potential of the compound and sometimes causes an increase of their toxicity. Dissecting the molecular pathways involved in the autophagy activation in response to DNA damage may open innovative strategies able to reduce toxicity of many compounds, including anticancer drugs.

G9.Crosstalk between alterations in ribosome functionality and DNA repair

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Alterations in ribosome biogenesis caused by mutations of genes encoding ribosomal proteins (RPs) can induce a particular kind of cellular stress named "Ribosomal Stress". Some genetics pathologies (ribosomopathies) have been associated to this condition, such as Diamond Blackfan Anemia caused by mutations in *RPS19*. "Ribosomal Stress" condition causes the activation of the tumor suppressor gene *p53*. P53 plays also a relevant role after DNA damage induced in DNA double-strand breaks (DSBs). Ionizing radiations or radiomimetic drugs cause the activation of DNA damage response pathway (DDR) that requires the recruitment of MNR complex and the activation of proteins including p53. The aim of our work is to identify a link between "Ribosomal Stress" condition and the activation of DDR in different cellular systems, focusing on a possible extra ribosomal function of RPS19 during DNA damage. We perform siRNA transfection in normal MRC5 fibroblasts and tumor U251 and HCT116 cell lines. Our preliminary data show changes in activation of DDR in RPS19-depleted cells. Immunofluorescence analysis describe an increase of γ- H2AX and 53BP1 levels after IR treatment in all RPS19-depleted cell lines. According to these data, western blotting analysis describe an increase in p53 and p21CIP1 proteins levels after IR treatment in all RPS19-depleted cell lines. Moreover we perform micronucleus assay in RPS19-depleted cell lines.

G10.Involvement of M1 and M2 subunits of ribonucleotide reductase in Ni(S-Tcitr)$_2$ resistance/sensitivity in human cell lines

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The metal complex [Ni(S-tcitr)$_2$] shows interesting antiproliferative/antimicotic characteristics. It enters the cell and induces G_2M cell cycle arrest, p53 independent-intrinsic-apoptosis by down-regulation of Bcl-2, mitochondrial membrane potential loss and caspase activation. A DNA damaging action was observed through the alkaline Comet Assay not due to DNA oxidation. Furthermore, [Ni(S-tcitr)$_2$] do not induce gene mutation or chromosomal damage, but alters the DNA conformation creating knot-like structures and hairpins.
Analysis of a collection of deletants of *S. cerevisiae* has shown an enrichment in the classes of genes that code for components involved in nucleic acids metabolism such as ribonucleotide reductase (RNR). This enzyme is necessary for the synthesis and repair of DNA; alterations in RNR gene expression have been identified in murine and human tumor cells.
In this study, we aim to evaluate, through RT-PCR, the expression of genes coding for the two subunits (RRM1 and RRM2) of RNR, as potential cellular target of [Ni(S-tcitr)$_2$], on a panel of human tumor cell lines with different sensitivity against this compound. For each cell line, we have chosen two different concentrations: a toxic one, corresponding to the value of IC_{50}, and a subtoxic one, 10 times lower than IC_{50}. Cells were treated for 1-48h according to their sensitivity/resistance against the drug.
Generally, [Ni(S-tcitr)$_2$] induces a remarkable modulation of gene expression of RRM1 and RRM2.

G11.Naphthalene diimides derivative bind telomeric G-quadruplex determining telomere dysfunction and increasing sensitivity to ionizing radiations in glioblastoma cells

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G-quadruplex (G4) interacting agents are a class of ligands able to bind to and stabilize secondary structures located in genomic G-rich regions such as telomeres. Stabilization of telomeric G4 leads to telomere architecture disruption and consequent detrimental effect on cell proliferation making these agents good candidates for chemotherapeutic purposes. In this work, we evaluated the effects of 4 different naphthalene diimides (NDI) derivatives, recently proposed as telomeric G4-ligands, in a glioblastoma multiforme (GBM) cell line (U251MG) as single agents and in combined treatments with X-rays. NDI used in the present works, named C1, C2 C3 and C6, were able to induce cell growth reduction, 53BP1 and telomere-induced dysfunctional foci (TIF) formation. Data obtained indicate a higher effectiveness in the inhibition of cell growth by C1, C2 and C6 when compared to C3, whereas telomere destabilization was observed only in C1 and C2 treated samples. Response to combined treatment of NDIs and X-rays was assessed evaluating long term growth curves, surviving fraction, repair kinetics of DNA damage, and cell cycle analysis. Data indicate a radiosensitizing effect of C1 and C2 whereas no effect was observed in cells exposed to C6. These findings provide evidence of radiosensitizing effects of two out of four NDIs tested through telomere destabilization in a glioblastoma cell line supporting the possibility of a future use of telomere destabilizing agent as radiosensitizers in therapeutic strategies.

G12.Hsp90: a novel sensor of the DNA double-strand break response

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Hsp90 is one of the most abundant and conserved molecular chaperones playing an essential role in eukaryotic cells proteostasis. Hsp90 is involved in cellular homeostasis, transcriptional regulation, chromatin remodeling, and DNA damage response (DDR). Indeed, the efficacy of the DDR pathways is influenced by the nuclear levels of DNA repair proteins, which are regulated by balancing between protein synthesis and degradation as well as by nuclear import and export. The inability to respond properly to DNA damage leads to genetic instability, which in turn may enhance the rate of cancer development. Here, we show that Hsp90 represents an important sensor of ionising radiation (IR)-induced DNA double-strand breaks (DSBs), its levels increasing following irradiation in MRC5, HEK293 and lymphoblastoid cells. In particular, results obtained by mass spectrometry and immunoblot experiments indicate that the DSBs sensing protein NBN directly binds Hsp90 though its *N*-terminal region. In turn, the DNA damage-dependent increase of Hsp90 is strictly associated with an increased interaction with NBN. Moreover, the inhibition of Hsp90 by 17-AAG results in altered levels and nuclear localization of further DSBs sensing protein such as ATM and RAD50, as observed in MRC5, HEK293 and MCF7 cell lines. Overall, our results highlight the role of Hsp90 as a regulator of the DSBs response, thus contributing to the maintenance of the genome integrity.

G13. The replication of *Frataxin* alleles carrying a GAA-repeat expansion is assured by activation of dormant origins

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It is accepted that DNA replication plays a crucial role in affecting trinucleotide repeat (TNR) stability and expansion (although DNA repair and recombination are also implicated). Whether and how these events occur in the endogenous context is however poorly understood.
We used lymphoblastoid cell lines derived from Friedreich's ataxia patients, homozygous for a GAA-repeat expansion within the first intron of the *Frataxin* gene, and control cell lines from healthy relatives. By interphase FISH, we had previously demonstrated the occurrence of delayed replication of the expanded alleles with respect to the normal one. Within a wide genomic segment (850 kb) centred on the *Frataxin* gene we monitored origin usage and replication fork progression by molecular combing. Replication patterns of mutated alleles were remarkably different from the normal one. Indeed, firing was never observed in the normal allele, passively replicated by incoming replication forks, while dormant origins were recruited for the replication of the expanded alleles. This finding indicates that the recruitment of dormant origins is a necessary event to complete replication of the expanded alleles. Furthermore, the proportion of alleles using the expanded GAA-repeat as template for leading strand is higher than in wildtype, suggesting the involvement of an origin switch effect in the presence of the GAA-repeat expansion. Interestingly, frequency and length of forks with unidirectional progression were significantly different between mutated and normal sequences. Our results give new insights in clarifying the effects of a trinucleotide-repeat expansion on DNA replication in the endogenous context, and confirm that in human cells the successful replication of an expanded repeat relies on the plasticity of the process.
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G14.Genetic and epigenetic effects of pristine and coated titanium dioxide nanoparticles in human lung fibroblast cells.

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Several studies have reported the toxic effects of TiO_2 Nanoparticles (NP). However still little is known about epigenetic effects of these NP. We investigated cytoxicity and genotoxicity of uncoated and coated with silica or citrate TiO_2 NP as well as of the benchmark material P25 by using a panel of *in vitro* assays in the human lung epithelial cell line A549. Cell viability was evaluated using colony forming efficiency assay, while cytostasis, apoptotic and necrotic responses as well as chromosomal aberrations, evaluated by micronuclei, nucleoplasmic bridges and nuclear buds induction, were evaluated by means of cytokinesis block micronucleus cytome assay. We also investigated clastogenic and aneuploidogenic origin of micronuclei by performing fluorescence *in situ* hybridization. Further genotoxic analyses were performed by comet assay, with and without the implementation of the restriction enzymes EndoIII and Fpg in order to detect primary and oxidatively damaged DNA. We investigated also the global DNA methylation effect, by means of the LINE-1 methylation analysis. We observed that after 72h of treatment all the NP studied induced a demethylating effect. Our results are in line with the existing literature data and confirm that TiO_2 NP possess cytotoxic and genotoxic effects. Moreover our data demonstrate that TiO_2 NP also possess the capacity to induce impairment of DNA methylation of cells.

G15.Resveratrol and its methoxy derivatives as modulators of DNA damage induced by ionizing radiation

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Various naturally occurring stilbene-like compounds that are related to resveratrol have been reported to possess some of the beneficial effects of the parent molecule and provide even further benefits. Therefore a series of methoxylated analogs of resveratrol were prepared with the aim of increasing the antitumor and proapoptotic activity. In a previous paper we studied two methoxy-derivatives, pterostilbene and trimethoxystilbene, the first formed by the substitution of two hydroxyl groups with two methoxyl groups (trans-3,5-dimethoxy-4'-hydroxystilbene) and the second by the replacement of all three OH groups with methoxyl groups (trans-3,5,4'-trimethoxystilbene), showing their stronger antioxidant activity when compared to resveratrol (RSV). In the present paper we focused on the analysis of the ability of RSV and its two methoxylated derivatives in protecting proliferating non tumoral cells from damage induced by Ionising Radiation (IR). First of all we showed that the methoxy derivatives contrary to their parental compound are unable to affect topoisomerase enzyme and consequently are not clastogenic *per sè*. Secondly both pterostilbene and trimethoxystilbene much efficiently reduce chromosome damage induced by ionizing radiation. Furthermore trimethoxystilbene but not pterostilbene causes a delay in cell proliferation particularly in mitosis progression increasing the number of cells in metaphase at the expense of prophases and ana/telophases.
Lastly we show confocal microscopic analysis of the action of trimethoxystilbene treatment on tubulin polymerization in CHO cells.

H1.The expression of genes up-regulated upon doxorubicin and TNFα combined treatment in MCF7 cells correlates with breast cancer clinical features

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Cellular responses to changes in the microenvironment often involve modulation of large transcriptional networks by sequence-specific master regulators. NFκB and p53 family of transcription factors exert critical functions and in part opposing functions. We addressed the transcriptional cooperation between these transcription factors by expression microarrays. Human breast adenocarcinoma-derived MCF7 cells (Luminal-type) were exposed to single or combinatorial treatments with the chemotherapeutic agent Doxorubicin (Doxo – able to stabilize the p53 protein) and the NFκB inducer Tumor Necrosis Factor alpha (TNFα). 239 up-regulated and 161 repressed genes were synergistically regulated by the Doxo+TNFα double treatment. Transcriptome data were confirmed for 12 of 15 selected Doxo+TNFα synergistic DEGs and for seven the responsiveness was shown to depend on both p53 and NFκB. Gene ontology of Doxo+TNFα up-regulated DEGs showed enrichment for cell migration terms. Indeed, three different types of migration assays showed that the double treatment could increase the motility of MCF7 cells. Moreover, a signature of 29 Doxo+TNFα highly synergistic DEGs exhibited prognostic value for luminal ER positive breast cancer patients, with adverse outcome correlating with their higher relative expression. Finally, the expression of the seven selected genes was analysed from RNA-seq available data obtained in breast cancer cell lines of different origin (Luminal vs Triple Negative) and in breast cancer patients (ER+ vs Triple Negative). Results showed an increased expression of these genes in both Triple Negative-derivative patients and cell lines. We propose that the crosstalk between p53 and NFκB can lead to the activation of specific gene expression programs that may impact on cancer phenotypes and potentially modify the efficacy of cancer therapy.

H2.PDGFRβ, TIMP3 and SULF1: possible role in tumorigenesis of Malignant Pleural Mesothelioma

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Malignant Pleural Mesothelioma (MPM) is a very aggressive tumor that shows poor response to conventional therapy. Thus the identification of biomarkers and therapeutic targets is increasingly necessary. Through a previous research we identified novel genes potentially involved in the malignancy of MPM, among which PDGFRβ, TIMP3, SULF1. The expression of these genes was analyzed in a panel of 4 MPM cell lines (NCI-H28, Mero-14, Mero25, IstMes2) and in one normal mesothelial cell line (Met5A). In order to investigate on their role in the carcinogenesis of MPM, we performed a phenotypic screening, after their downregulation by RNA interference techniques, through the Sulphorhodamine assay, the Colony Formation assay, Wound-Healing assay and the Caspase luminescence assay. PDGFRβ-silencing caused an increase of apoptosis and a decrease of proliferation and clonogenicity in Mero14 and ISTMES2; moreover the migratory ability is decreased in Mero14. TIMP3 depletion caused a lower proliferation in Mero14 and IstMes2 and a reduction of clonogenity ability in Mero14, IstMes2 and Mero25. After SULF1 silencing we observed a decrease of proliferation, clonogenicity and migration and an increment of apoptosis in Mero14. These results confirm the pro-tumorigenic role of PDGFRβ in MPM and suggest an oncosuppressive role of SULF1 through the stimulation of pathway involved in proliferation and clonogenity.

H3.Proline dehydrogenase is overexpressed in lung adenocarcinomas

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Lung cancer is one of the most common tumors worldwide and the leading cause of cancer related mortality, mainly due to a late diagnosis and to the paucity of biomarkers and cures. Lung tumors are highly heterogeneous, comprising Small Cell Lung Carcinomas (SCLC) and Non Small Cell Lung Carcinomas (NSCLC), the most frequent histotypes of which are adenocarcinomas (ADC) and squamocellular carcinomas (SCC), representing 50% and 40% of NSCLCs, respectively. Recent research is devoted at identifying the molecular lesions that may be used for an early diagnosis and to distinguish between different histotypes. Proline dehydrogenase (PRODH) is the enzyme key to proline metabolism and plays a role in induction of apoptosis and autophagy, thus influencing cell fate decisions. Its expression is finely regulated and is altered in several types of cancer. To investigate if PRODH was deregulated in lung tumorigenesis, we analyzed its expression in 62 lung cancer samples by immunohistochemistry. Thirtyfour out of 51 ADC (67%) and 3 out of 11 SCC (27%) were positive for PRODH staining. The increase in protein expression was accompanied in most cases by an increase in transcript levels and did not correlate with p53 or EGFR mutations. Further experiments are ongoing to increase the number of SCC, to investigate if the increased expression is observed also in preneoplastic lesions and if it is associated to upregulation of specific transcription factors.

H4.Association between rs4644 within *LGALS3* and risk of differentiated thyroid cancer: a case-control study

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Differentiated thyroid cancer (DTC) is the most common endocrine malignancy. Here we report a hypothesis-driven study aimed to detect genetic markers of susceptibility to DTC. The selection criteria included genes differentially expressed in DTC found somatically mutated at least in one cancer study, coding SNPs affecting the function of the encoded protein (predicted *in silico*), SNP heterozygosity>0.15 among Caucasians, and the complete conservation of the aminoacid residue in phylogenetically distant organisms. Excluding two SNPs already studied, rs4644 in *LGALS3* gene accomplished all criteria and was tested in a case-control study (1155 DTC and 1222 controls).The association between the SNP and risk to develop DTC was evaluated with the multivariate logistic regression. The results showed a protection role for the carriers of the Histidine-64 (OR=0.66; IC 95% 0.46-0.93) versus the Proline-64 allele. Galectin-3 could affect cancer biology by modulating the expression of target genes. Thus, an *in vitro* assay was performed to verify the functional role of this SNP. TPC1 cells were transfected with the coding sequence of *LGALS3* gene carrying C- or A- variant at the position 191 and the expression of putative target genes was measured by real-time qPCR. The results showed that the C-allele caused the overexpression of *MDM2*. In

conclusion, further studies are needed to clarify the *LGALS3* role in the malignant process of transformation in papillary cancer cells.

H5.Precocious effects of dyskerin silencing in colon cancer cells

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DKC1 is an essential gene causative of the X-linked dyskeratosis congenital disease. The gene encodes a main isoform, called dyskerin, a nucleolar protein involved in cell growth, proliferation, and telomere maintenance. A main characteristic of X-DC is cancer susceptibility, while dyskerin over-expression is observed in many sporadic cancers and considered as a useful prognostic marker. With the aim to better characterize the effects triggered by dyskerin depletion in human cells, we generated a stable and inducible colon carcinoma cellular model able to silence the *DKC1* gene without affecting the expression of a minor dyskerin alternatively spliced isoform that exhibits cytoplasmic localization. In order to define the set of telomere-independent effects triggered by dyskerin depletion, we first focused our analyses on the first days after silencing induction, well before the occurrence of telomere erosion. Intriguingly, we found that dyskerin silencing causes an immediate alteration of cell morphology and perturbs cell-cell and cell-substratum adhesion. These findings are further supported by the ability of dyskerin to interact directly with membrane adhesion molecules. Thus, we suggest that dyskerin might participate in tumorigenesis by regulating trafficking to and from cell membrane.

H6.*HLXB9* gene expression in leukemic cells and its correlation with the compositional properties of rearranged chromosomal regions

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Chromatin organisation in the cell nuclei and, more specifically, the radial positioning of genes within the nucleus has been associated with expression patterns. Disruption of the gene organisation in the nucleus may results in a compromised cellular health. In cancer cells, where chromatin altered re-organisation may occur in the presence of chromosomal rearrangements such as deletions, duplications or translocations, seems to have an important role, as previously demonstrated in leukemic cells. Using cells, carrying interstitial deletions of chromosome 7, from leukaemic patients, we observed a nuclear re-localisation of the *HLXB9* gene (mapping at 7q36, and involved in leukemic progression) correlated to the compositional properties of chromosomal bands neighbouring the breakpoints. Moreover, *HLXB9* transcriptional activation follows the different type of relocation in the nucleus. Here we propose a new model of chromatin reorganization, in leukemic cell nuclei, involving the compositional properties of chromosomal bands included or nearby the deletion breakpoint.

H7.The tumor suppressor p14ARF can promote survival in some cancer cell lines inducing autophagy

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p14ARF tumor suppressor is an important sensor of hyperproliferative stimuli inducing cell cycle arrest or apoptosis through both p53-dependent and independent pathways. In line with its tumor-suppressive role, INK4A-ARF locus is frequently mutated in human cancer. Nevertheless, a significant fraction of human tumors retain persistently high levels of ARF, suggesting that ARF may possess a pro-survival function. Moreover, it was been reported that loss of p19ARF in prostate epithelium does not accelerate but rather partially inhibits the prostate cancer phenotype. We show for the first time that ARF knocked down (KD) HeLa cells present round phenotype, defects in cellular spreading and actin cytoskeleton organization. Although it has been always reported that ARF has prevalent nucleo/nucleolar distribution, we demonstrate that during adhesion and spreading it localizes at focal adhesion where it interacts with Focal Adhesion Kinase (FAK). We report here that in HeLa cells the depletion of p14ARF induces a strong reduction of cell viability and apoptotic cell death. Moreover, ARF KD HeLa cells show reduced autophagy when exposed to nutrient starvation. These data suggests that ARF promotes autophagy in this cancer cell line. Thus we propose that autophagy induced by ARF is necessary to sustain viability in HeLa cells.

H8.RTKs signaling: new functional approaches to genetically dissect the tumor landscape

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The complexity and heterogeneity of tumors are difficult to reproduce in *in vitro* studies, which often cannot adequately elucidate the molecular events involved in tumor initiation and progression. Moreover, histological subtypes of a cancer type that are being treated with similar surgical and therapeutic approaches, are in fact characterized by distinct phenotypes, cell of origin, and underlying key genetic and genomic alterations. Thus, a better understanding of the molecular characteristic of each tumor, will lead to more personalized treatment approaches that will improve patients' prognosis.
In the last few years there has been a growing interest in the development of patient-derived tumor xenograft (PDX) models for cancer research. These models, in fact, offer several advantages compared to *in vitro* and *in vivo* models derived from conventional stable cell lines. Usually PDX models recapitulate the histologic and genetic characteristics of the tumors from which they derive and do not change across further *in vivo* passages. Several works have shown that PDXs are endowed with predictive clinical value and that they are of incredible importance in the field of precision medicine as they can be used for preclinical trials testing drug efficacy and for biomarker identification.
We aimed at identifying and validating novel targeted therapeutic strategies in gastric cancer, through the generation of a platform of gastric PDXs. This platform will be exploited for: 1) Validation of candidate oncogenes as relevant targets and identification of efficient therapeutic strategies 2) Identification of novel molecular targets; 3) identification of genetic predictors of response/resistance. As a whole, the results of this project should provide a scientific basis for future clinical applications and guide the rational design of molecularly-oriented clinical trials for gastric cancer.

H9.Combined pharmacological inhibition of MYCN and LSD1 cooperatively inhibit suppressor genes and reduces Neuroblastoma cell viability

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Neuroblastoma is the most common extra cranial solid tumor of infancy. One of the most important biological markers is MYCN oncogene amplification, which occurs in approximately 25% of cases. Patients whose tumors have MYCN amplification tend to have rapid tumor progression and poor prognosis. Furthermore, histone demethylase LSD1 over-expression is a common finding in undifferentiated Neuroblastoma with an adverse outcome. Recently we demonstrated that MYCN and LSD1 cooperate to repress tumor suppressor genes in Neuroblastoma. Based on that, we investigated the efficacy of pharmacological inhibition of MYCN and LSD1, or both, on Neuroblastoma cell viability. Time- and dose-dependent inhibition of cell grow was observed in Tet21/N and SK-N-BE(2) cells after treatment with TCP and 10058-F4, LSD1 and MYCN inhibitor respectively. We found that combination of TCP and 10058-F4 resulted in a stronger inhibition of viability in more then one Neuroblastoma cell lines. Moreover RNA sequencing of untreated, TCP and LSD1siRNA treated Neuroblastoma cells was employed to search for target genes and pathways regulated by LSD1 in Neuroblastoma. Our studies demonstrate that inhibition of LSD1, in combination with MYCN inhibition, reduces Neuroblastoma cell viability and aim to address their contribution to regulation of common gene targets in a genome wide approach.

H10.Germline defects predisposing to colorectal adenomas and carcinomas

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Mendelian colorectal cancer (CRC) syndromes explain ≈5% of all CRC cases. Most of the 13 high-penetrance genes predisposing to CRC primarily predispose to colorectal polyps. The most common polyposis syndromes are FAP/AFAP, a dominant form caused by germline defects of *APC* tumor suppressor gene, and MAP, a recessive form associated with *MUTYH* base excision repair gene. Recently, a new dominant form of polyposis (PPAP) has been associated with constitutive mutations in the proofreading domains of Polε and Polδ polymerases.
We investigated 100 patients, with/without clear-cut family history of polyposis/CRC, diagnosed with ≥15 adenomatous polyps; 66 showed a milder phenotype (15-100 adenomas) and 34 a more aggressive disease (≥100 adenomas). By different methods, we identified 3 *APC* (4.5%) and 10 *MUTYH* (15.2%) mutation carriers in the first group. Carriers were 17 for *APC* (50%) and 4 for *MUTYH* (11.8%) in the second group, indicating that a more aggressive phenotype is commonly associated with *APC* impairment. In total, by also taking into account family history, mutation carriers were 35% and 68% in the first and second group, respectively. In 36 selected patients tested negative for both *APC* and *MUTYH*, we searched for Polε and Polδ mutations. No pathogenic mutations, nor variants of uncertain significance, could be identified in Polε and Polδ proofreading domains, suggesting that defects in *POLE* and *POLD1* genes are rarely associated with polyposis and CRC predisposition.

H11.Functional dissection of an unforeseen MYCN/p53 complex in the genesis and progression of childhood neuroblastoma

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Neuroblastoma, a neural crest derived tumour, is one of the most common extracranial solid cancer in childhood, and it is often characterized by several genetic abnormalities, among which MYCN gene amplification is the most relevant. As the majority of pediatric tumours, more than 95% neuroblastomas present wt p53, though its functional activity is somehow crippled. Here we show that MYCN interferes with the p53 function by repressing p53-regulated genes. More specifically, through a dual-luciferase assay we could investigate the combined effect of both MYCN and p53 in the regulation of common gene promoters (Killin, E2F8, ABCC1 and ABCC4) and show that MYCN can negatively affect the transcription regulatory ability of p53. Furthermore, Co-IP assays, performed using protein nuclear extracts from Nutlin 3a pre-treated IMR-32 neuroblastoma cells, revealed the existence of a MYCN-p53 nuclear protein complex. Direct interaction between the two proteins was confirmed through in-vitro and ex-vivo binding assays, and mutational analyses revealed that boxes IIIb and IV of the MYCN protein are required to bind the c-terminal moiety of p53. Overall, these results show that MYCN can directly engage with p53, possibly when p53 is released from its inhibitor MDM2, and impinge on the p53 transcriptional regulatory network. Our data provides a first rationale for why p53 in neuroblastoma cannot exert its normal function despite being normal.

H12.Two cases of anaplastic large cell lymphoma with extracopies of ALK gene

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Systemic anaplastic large cell lymphoma (ALCL), is a well defined subtype of non-Hodgkin's lymphoma with characteristic morphologic features, immunophenotype, and molecular genetic alterations which can be divided into two major groups. The first is a spectrum of CD30+ T-cell lymphoproliferative disorders including primary cutaneous ALCL and lymphomatoid papulosis, usually affecting older patients, but characterized by an excellent prognosis. The second is systemic nodal ALCL, which, on the basis of genetic and immunophenotypic features combined with clinical parameters, can be divided into two subgroups: anaplastic lymphoma kinase (ALK)-positive and ALK-negative systemic ALCL. ALK^+ ALCL, is characterized by the presence of *ALK* gene rearrangements and consequent ALK protein expression, while the anaplastic large cell lymphoma ALK negative (ALK^- ALCL) is a provisional entity lacking ALK protein expression but that cannot be distinguished morphologically from ALK^+ ALCL. The most frequent chromosomal aberration characterizing ALK-positive ALCL is t(2;5)(p23;q35)/NPM-ALK (about 75-80% of cases) or variant ALK-involved translocations, while little is known about the genetic changes in ALK-negative ALCL. We investigated the structural and numerical aberrations of the *ALK* gene using interphase fluorescence in situ hybridization (FISH). Here we describe two cases of ALCL negative for the expression of ALK protein, showing extra copies of *ALK* genes, as detected by FISH analysis.

H13. The Ornithine Decarboxylase G317A Polymorphism is Prognostic of Outcome in Primary Neuroblastoma and Differentially Affects Promoter Binding by the MYCN Oncogene

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Polyamines are highly regulated essential cations that are elevated in rapidly proliferating tissues. Ornithine decarboxylase (ODC1), rate-limiting for polyamine synthesis, is an oncogenic MYCN target and an independent prognostic marker in neuroblastoma (NB). We examined the prognostic significance of a single nucleotide polymorphism (SNP) within the ODC1 promoter. Specifically, 839 primary NBs were genotyped for the G317A (rs2302615) promoter SNP by qPCR. In MYCN amplified NB, the GG genotype strongly predicted poorer outcome, whilst in non-amplified patients, the presence of one or two G alleles was associated with worse outcome. The effect of G317A on MYCN/MAX regulation of ODC1 was examined by E-box mutational analyses, EMSA and luciferase reporter assays. Results showed that MYCN/MAX binding to the E-box closest to the G317A SNP was 3-5 fold stronger in the presence of the G-, rather than A-allele. The G-allele/E-box element was also 2-fold more efficient in driving luciferase transcription in MYCN-inducible cells. In support of that, NB BE(2)-C cells (GG) edited to AG through the CRISPR-Cas9 system showed reduced ODC1 expression.

Overall our results demonstrate the functional importance of the ODC1 G317A promoter polymorphism in the regulation of ODC1 expression and provide a mechanistic explanation for how the G317A (rs2302615) promoter SNP can influence neuroblastoma progression.

H14.MYCN coordinately regulates the entire polyamine pathway to maintain high polyamine levels in neuroblastoma

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The MYCN transcriptional target, ODC1, is rate-limiting for polyamine biosynthesis and a therapeutic target in childhood neuroblastoma (NB) and elevated polyamine levels are found in many cancer types, including NB. We examined the prognostic impact of all polyamine pathway genes in a large cohort of 650 primary untreated NB. Gene-expression profiles revealed that high levels of each polyamine biosynthetic gene and low levels of each catabolic gene strongly predicted poor outcome. A direct correlation between MYCN and biosynthetic polyamine gene expression was observed, but an inverse correlation between MYCN and catabolic polyamine gene expression. ChIP and luciferase reporter assays confirmed that MYCN associates with biosynthetic gene promoters by binding to E-box sites in order to activate transcription. In contrast, MYCN binds the promoters of the catabolic genes by interacting with the Sp1 protein in order to repress transcription. Levels of polyamines and their metabolites were measured in a MYCN transgenic mouse model of NB and increased levels observed in tumours of 6 week old mice compared to wild type ganglia. These results provide an unprecedented demonstration of an oncogene coordinately regulating the expression of every gene in a metabolic pathway in order to drive cell proliferation. The findings highlight the critical importance of polyamines in NB and suggest that targeting polyamine pathway genes in addition to ODC1 will be a valuable therapeutic approach.

H15.Specific patterns of somatic mutations in colorectal cancer patients affected by biallelic mutations in the MUTYH base excision repair gene

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Biallelic germ-line mutations in the *MUTYH* DNA glycosylase predispose to colorectal adenomatous polyposis (MUTYH-associated polyposis, MAP). This autosomal recessive syndrome is characterized by a cancer prone phenotype, because of defective repair of 8-hydroxyguanine-containing mispairs. It has been suggested that colorectal carcinogenesis follows a different route when MUTYH is inactive. Seven colorectal cancers (CRCs) from patients affected by MAP were collected and the full complement of individual tumour mutations associated with MUTYH inactivation was identified by exome sequencing. MAP patients were either homozygous for the common G396D/Y179C variants or compound heterozygotes. Data analysis deciphered a mutator phenotype associated with MUTYH inactivation as well as a specific signature of the mutational process, i.e. an excess of G>T mutations due to unrepaired 8-hydroxyguanine. These mutations were located in a sequence context identified by specific 5' and 3' neighbouring bases. G12C mutations in

KRAS codon 12 were present in 100% of cases and were all located at these identified “hot-spot” for G>T transversions (TG*G). Surprisingly the specific signature associated with MUTYH inactivation has also been found in neuroblastoma, suggesting that oxidative damage is a major factor in the onset of this disease. Finally we found that colorectal carcinogenesis in MAP patients is associated with mutations in a characteristic subset of oncogenes and/or tumor suppressors.