Sessione Poster:
1.Ciclo cellulare e cancerogenesi (Alessandra Pollice, Guglielmina Nadia Ranzani)

1.1. Buccal micronuclei assay in a human population from Sicily (Italy) exposed to petrochemical industry pollutants
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1.2. *TPO* genetic variants and risk of differentiated thyroid carcinoma (DTC).
Monica Cipollini[1], Sonia Garritano[1], Alessandra Bonotti[2], Gisella Figlioli[1], Cristina Romei[3], Alfonso Cristaudo[2], Rossella Elisei[3], Stefano Landi[1], Federica Gemignani[1]
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1.3. MicroRNA-dependent regulation of mesothelin gene.
Chiara De Santi[1], Ombretta Melaiu[1], Alessandra Bonotti[2], Rudy Foddis[2], Alfonso Cristaudo[2], Luciano Mutti[3], Sonia Garritano[4], Monica Cipollini[1], Marco Lucchi[5], Elisa Barone[1], Federica Gemignani[1], Stefano Landi[1] cdesanti@biologia.unipi.it,

1.3. p63 as a new player involved in the cancer cell response to chemotherapy
Daniela Di Girolamo[1], Daniela Montariello[1], Viola Calabrò[1] and Piera Quesada[1].
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1.5.ΔNp63α and YB-1 functional interaction regulates proliferation and survival of normal and transformed keratinocytes
Orsola Di Martino[1], Annaelena Troiano[1], Irene Schiano Lomoriello[1], Daniela Di Girolamo[1], Girolama La Mantia[1] and Viola Calabrò[1].
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1.6. Plant secondary metabolites can modulate p53 transactivation potential, as revealed by a yeast miniaturized luciferase assay.
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1.7. Search for novel common variants influencing differentiated thyroid cancer
Gisella Figlioli[1§], Aleksandra Köhler[2§,] Bowang Chen[2], Stefano Landi[1], Rossella Elisei[3], Cristina Romei[3], Monica Cipollini[1], Alfonso Cristaudo[3], Franco Bambi[4], Per Hoffmann[5,6,7], Stefan Herms[5,6,7], Michał Kalemba[8], Dorota Kula[8], Shelley Harris[9], Peter Broderick[9], Richard Houlston[9], Susana Pastor[10,11], Ricard Marcos[10,11], Antonia Velázquez[10,11], Barbara Jarząb[8], Kari Hemminki[2,12], Asta Försti[2, 12*], Federica Gemignani[1*] gfiglioli@biologia.unipi.it

1.8. The *fragile centrioles (fract)* gene is required for the maintenance of centriole integrity during *Drosophila* male meiosis
Ramona Lattao, Maurizio Gatti, and Silvia Bonaccorsi
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1.9. Functional relationships among p14ARF, YB-1 and ΔNp63α

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1.10. Role of mesothelin and calretinin in malignant pleural mesothelioma

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1.11. Heterogeneous molecular mechanisms underlie Hereditary Diffuse Gastric Cancer syndrome

Valeria Molinaro[1], Valeria Pensotti[2], Monica Marabelli[1], Irene Feroce[3], Monica Barile[3], Simonetta Pozzi[3], Lucia Fini[4], Luigi Laghi[4], Davide Serrano[3], Loris Bernard[2], Bernardo Bonanni[3] and Guglielmina Nadia Ranzani[1]. valeria.molinaro01@universitadipavia.it, valeria.molinaro01@ateneopv.it

1.12. A role for splicing factors in microtubule-kinetochore interactions

Claudia Pellacani[1], Elisabetta Bucciarelli[2], Daniel Hayward[3], Fioranna Renda[1], Antonella Palena[2], James G. Wakefield [3], Maurizio Gatti[2] and Maria Patrizia Somma[2]
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1.13. *Int6*, a new gene involved in the regulation of microtubule dynamics in *Drosophila S2* cells

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1.14. Identification of pathways involved in aneuploidy onset and its tolerance using a DNA microarray approach

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1.15. Microfluidic devices for the analysis of tumor cell mechanical properties and invasivity

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1.16. A novel role of the p14arf tumor suppressor in cellular adhesion

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1.1. Buccal micronuclei assay in a human population from Sicily (Italy) exposed to petrochemical industry pollutants

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People living near petroleum refinery are potentially exposed to environmental pollutants due to wide range of petroleum-derived hydrocarbons and chemical substances used in the manufacturing of petroleum derivatives. The aim of this study has been to evaluate the genotoxic effects on the human populations living and/or working in areas exposed to hydrocarbon pollutions produced by petroleum refineries located in the Southern and East of Sicily, using micronucleus (MN) assay on buccal exfoliated cells.

The sample, workers and non-workers in the petroleum refinery industries, plus a control group composed by people living away from these areas, were selected to analyze the frequency of micronucleated cells (MN) and other nuclear anomalies (ONA: pyknosis, karyolysis and karyorrhexis) of buccal epithelial cells. Additionally, information on lifestyle factors from questionnaires filled out by each subject was obtained and studies about correlation were made.

Our sample showed MN and ONA frequencies significantly higher ($P<0.05$) respect to the control subjects. Moreover, MN and ONA frequencies in petroleum refinery workers are not significantly different respect to the people living in petroleum refinery adjacent areas. Our findings indicate that people working in the petroleum refineries, as well as people living near these factories are under risk of cytogenetic damage, possibly due to the high level of environmental pollutants present in the studied areas.

1.2. *TPO* genetic variants and risk of differentiated thyroid carcinoma (DTC).

Monica Cipollini[1], Sonia Garritano[1], Alessandra Bonotti[2], Gisella Figlioli[1], Cristina Romei[3], Alfonso Cristaudo[2], Rossella Elisei[3], Stefano Landi[1], Federica Gemignani[1]

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The thyroperoxidase (*TPO*) has a key role in the iodine metabolism, being essential for the thyroid function. Mutations in the *TPO* gene are common in congenital hypothyroidism, and there are also signs of the implication of *TPO* in thyroid cancer. We performed a case-control association study of single nucleotide polymorphisms (SNPs) in *TPO* (i.e. rs2048722, rs732609 rs1042589), and differentiated thyroid carcinoma (DTC) in 1190 cases and 1290 controls. Multivariate logistic regression analyses were performed separately for each SNP. From the three studied polymorphisms significant associations were detected between DTC and rs2048722 (OR=0.79, 95% CI=0.63-1.00, P=0.045) and rs732609 (OR=0.72, 95% CI=0.55-0.94, P=0.016). The corresponding associations for the subgroup of the papillary thyroid carcinoma were similar to those for all DTC. No association was detected for the third *TPO* polymorphism. Interestingly, rs732609 encodes for Threonine a to Proline missense change in position 725 within *TPO*, that resides near the complement control protein (CCP)-like gene module (aa 741-795), but the functional significance of this change is unknown. Since the proline residue is conserved in most of the vertebrates, it could be hypothesized that the change affects the conformation of the protein, conferring a reduced flexibility to the carbamidic bond (given its cyclic structure). Thus, present results point to *TPO* as a gene involved in the risk of DTC, and could be of relevance for future studies to understand the role of *TPO* in thyroid tumorigenesis.

1.3. MicroRNA-dependent regulation of mesothelin gene.

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Malignant pleural mesothelioma (MPM) is a rare and aggressive tumor characterized, as ovarian and pancreatic cancer, by an overexpression of mesothelin protein encoded by MSLN gene. MSLN is probably involved in cell adhesion processes and several studies suggested a role in cancer signaling pathways. Furthermore, SMRP (soluble mesothelin related peptide) has been proposed as a diagnostic marker for MPM. One of the possible mechanisms of MSLN overexpression in cancer can be the downregulation of microRNAs targeting MSLN mRNA. To test this hypothesis, an accurate literature search was carried out collecting all studies performed using microRNA microarray in MPM, ovarian, and pancreatic cancer, focusing on down-regulated miRNAs. Only few miRNAs were down-regulated in all the three types of cancer. According to algorithm prediction, one of them showed to target MSLN mRNA in its coding region. Then, experiments are ongoing on the MSLN-overexpressing cell lines Mero14 with microRNA mimics. Quantitative real time pcr and western blot analysis aimed to measure MSLN mRNA and protein levels are performed at 48 and 72 hours after transfection. Preliminary results show that some miRNAs could cause a down-regulation of MSLN mRNA at 48hours after transfection, while MSLN protein is almost silenced at 72hours after transfection. Further investigations are ongoing to understand MSLN regulation and its role in cancer development.

1.4. p63 as a new player involved in the cancer cell response to chemotherapy

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Poly (ADP-ribose)polymerase 1 (PARP-1) is involved in cellular processes such as DNA repair and apoptosis. Therefore, PARP-1 inhibitor (PJ34) is considered a potential treatment for p53 proficient breast and ovarian carcinoma cells, when combined with TOP I poisons.
p63 is a member of the p53 family highly expressed in carcinoma cells of epithelial origin. In particular, TAp63 proteins mimic p53 transcriptional and proapoptotic functions whereas the ΔNp63α protein has been shown to repress p53-target genes acting as an oncogene.
We have analyzed the sensitivity of MCF7 ($p53^{wt}$), MDA-MB231 ($p53^{mut}$) breast carcinoma and SCC022 ($p53^{null}$) squamous carcinoma cells to PJ34 and TOP I (CPT, TPT) inhibitors combined treatment. We show that MCF-7 cells exhibit apoptotic cell death while MDA-MB231 and SCC022 cells are resistant to these agents. In MCF7 cells PJ34 reverts TPT-dependent PARP-1 auto-modification and triggers caspase-dependent PARP-1 proteolysis. Furthermore, TPT as a single agent stimulates p53 expression while in combination with PJ34 also induces TAp63 proteins.
In SCC022 cells we observed that degradation of endogenous ΔNp63α by TPT+PJ34 treatment is not sufficient to induce apoptosis thereby indicating that p53 and/or TAp63 is/are required to induce apoptosis. Our data suggest that p63 is a new player in the apoptosis pathway triggered by TOP I and PARP-1 inhibitors.

1.5. ΔNp63α and YB-1 functional interaction regulates proliferation and survival of normal and transformed keratinocytes

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The p63 protein is a member of the p53 gene family [1].The TP*63* gene encodes isoforms that contain (TA) or lack (ΔN) a transactivation domain. ΔNp63α, a critical pro-proliferative factor and a marker of epidermal stemness, is the most commonly expressed p63 protein and is essential for morphogenesis of organs/tissues developing by epithelial-mesenchimal interactions such as the epidermis, teeth, hair and glands [2]. We have recently shown that ΔNp63α is a molecular partner of the Y-box binding protein 1 (YB-1). YB-1, a marker of malignant tumor, is a nucleic acid binding protein with pleiotropic functions, such as alternative splicing, regulation of transcription and translation. Although YB-1 is predominantly cytoplasmic, it is highly expressed in the nuclear compartment of proliferating keratinocytes and squamous carcinoma cells. Here we show that ΔNp63α induces the nuclear accumulation of post-translationally modified forms of YB-1, sumoylation and ubiquitination are both involved in this phenomenon. During keratinocyte differentiation, YB-1 silencing restrains cell proliferation. In proliferating HaCaT and squamous carcinoma cells, YB-1 knockdown causes dramatic cell death and detachment. Our observations suggest that ΔNp63α and YB-1 functional interaction is critically associated with the proliferation and survival of normal and transformed keratinocytes.

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1.6. Plant secondary metabolites can modulate p53 transactivation potential, as revealed by a yeast miniaturized luciferase assay.

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The p53 master regulatory network is composed of a large number of genes that are direct targets of p53-mediated transactivation. Essential for the transcriptional regulation driven by p53 is the target response element sequence. Many factors, including protein levels, availability of cofactors and stress-dependent post-translational changes affecting p53 protein interactions, can influence the p53 activity. The p53 activity is ubiquitously lost in cancers either by mutation in the *TP53* gene, or by inactivation of its protein, thereby indicating its relevance as a therapeutic target in cancer. One important strategy to develop effective chemo-preventive or therapeutic anticancer agents is to study into chemical compounds derived from natural sources. The aim of this study was to test several plant secondary metabolites and fractionated plant extracts alone or in combination with anticancer drugs for their potential to modulate p53 transactivation potential. These experiments were conducted using a simplified luciferase assay that exploits variable expression of p53 in *Saccharomyces cerevisiae* and then confirmed in human cells. We tested a panel of fractions of saponins and saponins alone from *Astragalus verrucosus*. Our results indicated that these compounds exerted an inhibitory effect on p53 transactivation in yeast. When tested in combination with Doxorubicin or Mitomycin C, several fractions showed a significant recovery of the reduction of p53 activity induced by drugs.

1.7. Search for novel common variants influencing differentiated thyroid cancer

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Thyroid cancer is a common endocrine malignancy with a rapidly increasing global incidence in the recent decades. Differentiated thyroid cancer (DTC), arising from follicular cells, includes the most common histological subtypes, papillary and follicular thyroid cancer, representing 80% and 15% of all thyroid cancers, respectively. Genome-wide association studies (GWASs) have identified robust associations with polymorphisms at 9q22.33 (*FOXE1*) and 14q13.2 (*NKX2-1*) and the disease. However, most of the inherited genetic risk factors of DTC remain to be discovered.

To search for new DTC risk variants we performed a GWAS in the high incidence Italian population and followed up the most significant associations in the lower incidence populations from Poland, UK and Spain. After excluding previously identified loci, the strongest association was observed for *DIRC3* at 2q35 (P=6.4×10-10). Additionally promising associations were attained for *IMMP2L* at 7q31 (P=4.1×10-6 and P=5.7×10-6), *RARRES1* at 3q25.32 (P=4.6× 10-5) and *SNAPC4*/*CARD9* at 9q34.3 (P=3.5×10-5).

Our findings provide insights into the genetic and biological basis of inherited genetic susceptibility to DTC. To further improve our knowledge on the disease, new loci, selected on the basis of association signals in our GWAS, will be analysed.

1.8. The *fragile centrioles (fract)* gene is required for the maintenance of centriole integrity during *Drosophila* male meiosis

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We isolated a mutation in a *Drosophila* gene we name *fragile centrioles (fract)*; this mutation disrupts male meiosis and causes male sterility. Premeiotic spermatocytes of *fract* mutants display 2 normal centrioles at each cell pole. However, the cell poles of meiotic ana-telophases I of *fract* mutants often exhibit defective centrioles. As a result, 60% of secondary spermatocytes assemble bipolar monastral spindles that are unable to mediate proper chromosome segregation. *fract* encodes a 322 aa protein expressed only in testes. An antibody raised against Fract decorates the distal ends of male meiotic centrioles. A centriole stability assay indicated that the centrioles of *fract* mutants are more sensitive to colchicine- or cold-induced depolymerization than their wild type counterparts. We also asked which factors are responsible for centriole depolymerization in *fract* mutants not exposed to colchicine or cold. We constructed flies homozygous for *fract* and heterozygous for mutations in either *Dhc64* or *Klp61F*, which encode the dynein heavy chain and the (Eg5-homologue) Kinesin-like protein at 61F, respectively. In these flies the centrioles were substantially more stable than in *fract* mutant flies. Thus, lowering MT-dependent pulling and pushing forces on centrosomes by dynein or kinesin depletion rescues the centriole phenotype of *fract* mutants, indicating that *fract* is essential for stress resistance of male meiotic centrioles.

1.9. Functional relationships among p14ARF, YB-1 and ΔNp63α

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The p14ARF tumour suppressor is one of the most important oncogenic sensor in mammalian cells, able to respond also to a variety of different stresses and activating both p53-dependent and independent pathways through interaction with a multitude of cellular partners. Interestingly, ARF has been shown to cause sumoylation of most of its partners but the function of ARF-induced sumoylation is partly unknown. Recently, we have demonstrated that p14ARF can downregulate ΔNp63α (the pro-proliferative isoform of the p63 family members) intracellular levels in human keratinocytes and in tumor-derived cell lines *in vitro* through sumoylation-induced degradation. Interestingly, we have observed a gradual increase of p14ARF expression levels, opposite to that of the pro-proliferative ΔNp63α during *in vitro* keratinocytes differentiation suggesting a causal relationship between the two. Recently, we showed that ΔNp63α modulates the activity of Y box-binding protein (YB-1), one of the most indicative markers of tumour progression that is also involved in epithelial-mesenchyme transition and in brain development. We preliminary observed that during keratinocytes differentiation, YB-1 protein levels decrease, but this effect is abolished when p14ARF expression is silenced, suggesting a role for ARF in regulating YB-1 intracellular levels. Whether this effect is direct or involves ΔNp63α is under investigation.

1.10. Role of mesothelin and calretinin in malignant pleural mesothelioma

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Genes involved in malignant pleural mesothelioma (MPM) have been poorly characterized and previous studies have yielded conflicting results. Recently, we employed an approach based on the analysis of published transcriptomes and identified a set of deregulated genes. Thus, in the present study an experimental validation of 27 genes (*CALB2*, *MSLN*, *TMEM176A*, *RAN*, *NME2*, *CFB*, *PCNA*, *ALDOA*, *PLK2*, *BUB1B*, *SSBP1*, *COL6A1*, *EEF2*, *RAD21*, *CRIP1*, *FGF9*, *VCAN*, *MCM2*, *PGM1*, *LGALS3BP*, *CDK2AP1*, *RCN2*, *PTGS2*, *FGF2*, *ANXA4*, *AURKA*, *BIRC5*) was undertaken with the aim to establish which were most dysregulated. A comparison of fresh MPM specimens versus controls established that mesothelin and calretinin (*MSLN* and *CALB2*) were candidates involved in tumorigenesis. Transient *MSLN*-silencing caused a decrease in proliferation rates, reduced invasive capacity, and sphere formation in MSLN-overexpressing Mero-14 cells. Moreover, *MSLN*-siRNA combined with cisplatin triggered a marked increase in apoptosis and decrease in proliferation, suggesting a sensitizing effect. While silencing of *CALB2* did not modify the growth of *CALB2*-overexpressing IstMes2 cells, *CALB2* depletion caused a significant change in morphology of spheres in matrigel assays, forming grape-like complexes. These data suggest that *MSLN* and *CALB2* play an active role in MPM progression.

1.11. Heterogeneous molecular mechanisms underlie Hereditary Diffuse Gastric Cancer syndrome

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The inactivation of *CDH1* gene (E-cadherin) is a well-established defect in gastric cancers (GC) of both diffuse and intestinal histotypes. Germline defects of *CDH1* have been associated with Hereditary Diffuse Gastric Cancer (HDGC), an autosomal dominant syndrome (1-3% of GC cases) highly predisposing to diffuse-type GC and to lobular breast cancer.
We searched for *CDH1* germline lesions in 32 HDGC probands selected according to international consensus criteria. We performed a series of complementary approaches on both DNA and RNA including: DNA sequencing, *in silico* analysis, MLPA, SNuPE, RT-PCR, Realtime RT-PCR and bisulfite-sequencing. All these techniques allowed us to identify different types of *CDH1* molecular defects in 19% of probands.
Loss/aberrant expression of E-cadherin has recently been associated with alterations in miR-200 family members targeting the 3'-UTR of ZEB1/2 and SUZ12, and in miR-101 targeting the 3'-UTR of EZH2 and PTGS2 genes. We searched for mutations of these miRNAs and corresponding targets sequencing the DNA of the probands without *CDH1* disease-causing defects. No germline mutations were identified, suggesting that other mechanisms might be involved in E-cadherin loss/downregulation.
To find new genes possibly associated with HDGC we are currently applying next generation sequencing. Results are expected to increase mutation detection rate, improving genetic counseling and management of the families at risk.

1.12. A role for splicing factors in microtubule-kinetochore interactions

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An RNAi-based screen recently performed in our laboratory identified several proteins required for chromosome segregation. Surprisingly, these proteins included several splicing factors (SFs). To understand the mitotic role of SFs we focused on the *CG6876* and *CG10754* genes, which encode the *Drosophila* homologs of the human PRPF31 and SF3A2 SFs, respectively. *Drosophila* S2 cells lacking these proteins exhibit a mitotic phenotype comparable to that caused by depletion of Ndc80, a protein required for stable kinetochore-microtubule attachment. RNAi against either *CG6876/DPrpF31* or *CG10754/D-Sf3A2* inhibited proper kinetochore fiber formation and disrupted chromosome segregation. Consistent with these findings, we found that the D-PrpF31 and D-Sf3A2 physically interact with Ndc80 and facilitate its localization at the kinetochore. Injection of antibodies against D-PrpF31 or D-Sf3A2 in *Drosophila* embryos cause a strong metaphase arrest phenotype within a few minutes. These results exclude that the mitotic phenotype caused by depletion of either D-PrpF31 or D-Sf3A2 is due to problems in splicing of mitotic RNAs, and instead point to a direct role of these SFs at kinetochores. This mitotic role of SFs appears to be evolutionarily conserved as RNAi of human *SF3A2* affected Hec1/Ndc80 localization at the kinetochores and chromosome segregation just like D-Sf3A2 depletion. Our findings suggest that D-PrpF31 and D-Sf3A2 are components of a conserved molecular machinery that mediates proper kinetochore-microtubule interactions in higher eukaryotes.

1.13. *Int6*, a new gene involved in the regulation of microtubule dynamics in *Drosophila S2* cells

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In a recent RNAi-based screen we identified several new genes required for proper spindle assembly, including *int6* that encodes a component of the translation initiation complex that also interacts with both the 26S proteasome and the COP9 signalosome. Several published studies indicate that *Int6* is a proto-oncogene implicated in various types of cancer, but its precise biological activity has not been defined. We found that RNAi-mediated depletion of Int6 in *Drosophila* S2 cells results in a metaphase arrest phenotype, with short spindles, elongated/distorted centromere/kinetochore regions and abnormally shaped centrosomes. Double RNAi experiments to simultaneously deplete both Int6 and a protein required for either spindle formation or metaphase-to-anaphase transition further suggested that Int6 is involved in the regulation of MT dynamics. Time-lapse imaging of Int6-depleted cells expressing tubulin-GFP indicated that they form a normal mitotic spindle but then remain arrested in metaphase. During metaphase arrest, the spindle length progressively decreases leading to a short and compact structure. FRAP analysis further suggested that Int6 activity is specifically required for MT plus-end dynamics at the spindle equator. Collectively, our results lead us to propose that Int6 controls the levels and/or the activities of MT-depolymerising motors by regulating protein degradation complexes such as the proteasome and the COP9 signalosome.

1.14. Identification of pathways involved in aneuploidy onset and its tolerance using a DNA microarray approach

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Genomic instability is a hallmark of the majority of human tumors explaining the heterogeneity shown by tumor cells. This phenomenon is often associated with chromosomal instability (CIN) and aneuploidy, a condition in which tumor cells lose or gain chromosomes. Previously, we showed that posttranscriptional silencing by RNAi of pRb[1], DNMT1[2] and MAD2 is associated with aneuploidy in cultured human cells reinforcing the idea that there are several roads leading to aneuploidy. In the attempt to understand if a common molecular signature exists underlying aneuploidy and its tolerance in tumor cells, we induced aneuploidy in human fibroblasts (IMR90) by depleting Rb, MAD2 and DNMT1 genes and analyzed their transcriptome by Microarray (using a p-value of 0.05 and a fold change greater than 2). 1722 differentially expressed genes in the three sample analyzed against control were identified, of which 282 differentially expressed simultaneously in at least two out of three samples analyzed. These 282 genes were analyzed using freeware bioinformatics software (DAVID, GOrilla) that showed the presence of genes involved in many functional group inherent to the inflammatory response, cell proliferation and apoptosis. A detailed analysis of these results (using other system biology tools like Pathway Studio 9, Reactome, STRING) will be shown to get clues about the pathways involved in the generation and tolerance of aneuploidy.

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1.15._Microfluidic devices for the analysis of tumor cell mechanical properties and invasivity

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Tumor cell invasive and migration capacities are at the basis of metastasis formation, the main cause of cancer death. Cytoskeleton modifications, leading to cells' ability to change shape, are crucial events in cell movement and are frequently altered in cancer cells. Cells' propensity to undergo shape changes is strictly linked to their mechanical properties, such as their ability to respond to external forces; therefore, parameters like cellular deformation and relaxation in response to exogenous forces could give information on cell's invasive capacities and their metastatic potential, as well as on the possible effects of chemotherapeutic drugs on cell motility.

In order to study the relationship between cellular mechanical properties and invasivity, we are setting up devices based on the use of an optical stretcher (OS). OS relies on a double beam trap obtained through two counterpropagating laser beams; increasing the laser power, the stress produced generates a quantifiable elongation of the cell along the beam axis, giving a measure of cellular plasticity. OS allows the analysis of single cells, determining deformability at the entire cell level. We will provide results showing that in tumor cell lines cellular deformability increases with metastatic potential. Moreover, results will be presented on the effect on cellular deformability of paclitaxel, which do not affect cellular proliferation, but able to reduce cellular migration and increase α-tubulin acetylation.

1.16. A novel role of the p14arf tumor suppressor in cellular adhesion

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The INK4a/ARF locus on the short arm of chromosome 9 is one of the most frequently altered loci in human cancer. It is generally accepted that ARF is involved in oncogenic checkpoint pathways by sensitizing incipient cancer cells to undergo growth arrest or apoptosis through both p53-dependent and independent pathways. While intensive studies have been focused on ARF activation at the transcriptional level, only recently mechanisms governing ARF turnover have been identified. We have shown for the first time that p14ARF is a PKC target and that phosphorylated ARF protein is localized in the cytoplasm both in Hela and HaCaT.

In the attempt to clarify the role of cytoplasmic localized ARF, we accumulated evidences of human p14ARF involvement in cellular adhesion. Cell spreading and tyrosine phosphorylation levels of the focal adhesion kinase (FAK) are reduced following ARF depletion in Hela and HaCaT cells. Moreover, we found a molecular interaction between ARF and the Focal Adhesion Kinase (FAK) upon fibronectin stimulation. Thus, the p14ARF tumor suppressor could partecipate in adhesion pathway by positively regulating cell interactions with the extracellular matrix.

Sessione Poster:
2. Genetica di popolazioni (Mauro Mandrioli, Guido Barbujani)

2.1. Darwinian (positive) selection in vitamin D and folate pathway genes in humans.
Elena Arciero[1,2*], Simone Andrea Biagini[2*], Yuan Chen[1], Donata Luiselli[2], Luca Pagani[1,2], Chris Tyler-Smith[1], Qasim Ayub[1]
simoneandreabiagini@gmail.com

2.2. StreamingTrim 1.0: a Java software for dynamic trimming of 16SrRNA sequence data from metagenetic studies
Giovanni Bacci[1,2], Marco Bazzicalupo[1], Anna Benedetti[2], Alessio Mengoni[1*]
alessio.mengoni@unifi.it

2.3. Genomic tools for fishery and conservation of European hake and common sole
Alessia Cariani1, FishPopTrace Consortium2 and Fausto Tinti1
"alessia.cariani" <alessia.cariani@unibo.it>

2.4.Analysis of Clock Genes Variation in worldwide populations
Irene Dall'Ara[1], Silvia Ghirotto[1], Selene Ingusci[1], Guido Barbujani[1] and Cristiano Bertolucci[1].
Irene Dall'ara <dllrni@unife.it>

2.5.Molecular identification of Diptera, used in forensic investigations, by means of variable lenght of intronic sequences
Emanuela De Domenico, Debora Lombardo, Concetta Federico, Claudia Leotta, Alessandro Marletta, Francesco Lombardo, Salvatore Saccone
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2.6. Population structure and adaptive variation to climate in natural populations of Norway spruce (*Picea abies* Karst)
Erica A. Di Pierro[1], Elena Mosca[1], Duccio Rocchini[1], Giorgio Binelli[2], David B. Neale[13] and Nicola La Porta[1]

2.7. Low pass DNA sequencing of 1,200 Sardinians reconstructs European Y chromosome phylogeny
Paolo Francalacci[1], Laura Morelli[1], Andrea Angius[2,3], Riccardo Berutti[3,4], Daria Sanna[1], Antonella Useli[1], Magdalena Zoledziewska[2,4], Michael B. Whalen[2], Chris Jones[3], Gonçalo R. Abecasis[5], David Schlessinger[6], Serena Sanna[2], Carlo Sidore[2,4,5], Francesco Cucca[2,4]
"Paolo Francalacci" <pfrancalacci@uniss.it>

2.8. Cannabinoids abuse: evaluation of genetic vulnerability
Serena Galati[1], Maria Carla Gerra[1], Matteo Manfredini[1] and Claudia Donnini[1]
"Claudia Donnini" <claudia.donnini@unipr.it>

2.9. Early human dispersal from Africa: A model-based test of two hypotheses
Silvia Ghirotto[1], Massimo Mezzavilla[2], **Francesca Tassi[1]**, Sibelle Torres Vilaça[1], Lisa De Santi[1], and Guido Barbujani[1]
Francesca Tassi <tssfnc@unife.it>

2.10. *Across language families: exploration in genomic and linguistic variation in Europe*
Ghirotto S[1], Tassi F[1], Benazzo A[1], Guardiano C[2], Barbujani G[1], Longobardi G[3]
Silvia Ghirotto <ghrslv@unife.it>

2.11. Mitochondrial haplogroup H in the heart of Central Asia: a far echo of the West

Hovirag Lancioni[1], Ugo A. Perego[2], Anna Olivieri[3], J. Edgar Gomez-Palmieri[2], Maria Pala[4], Irene Cardinali[1], Francesca Gandini[3], Norman Angerhofer[2], Dashtseveg Tumen[5], Erdene Myagmar[5], Damdin Bayarlkhagva[5], Munkhjargal Bayarlkhagva[5], Aigerim Dyikanbaeva[6], Scott R. Woodward[7], Natalie M. Myres[7], Antonio Torroni[3], Alessandro Achilli[1] lancioni@katamail.com

2.12. A next generation sequencing analysis of the human Y chromosome provides new clues about ancient genetic events in Africa

Andrea Massaia,[1] Beniamino Trombetta,[1] Giovanna Bellusci,[2] Natalie M. Myres,[3] Andrea Novelletto,[2] Rosaria Scozzari,[1] Fulvio Cruciani[1] Andrea Massaia <and.massaia@gmail.com>

2.13. The Phylogenetic Relationships of Barn Swallow (*Hirundo rustica*) as Inferred by Mitogenomes.

Anna Olivieri[1], Francesca Gandini[1], Hovirag Lancioni[2], Alessandro Achilli[2], Ornella Semino[1], Antonio Torroni[1]. anna.olivieri@unipv.it

2.14. AGREEMENT BETWEEN EVIDENCES OF HEPATITIS FROM HISTORICAL DOCUMENTS AND GENETIC SUSCEPTIBILITY TO PRIMARY BILIARY CIRROSIS ABOUT SOME BONE RELICS FROM PESCINA, L'AQUILA, ITALY, XI CENTURY AD

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2.15. GENOME-CULTURE INTERACTIONS IN THE EVOLUTION OF HUMAN TASTE

Davide Risso[1], Sara De Fanti[2], Andrea Quagliariello[2], Cristina Giuliani[2], Marco Sazzini[2], Sergio Tofanelli[1], Gabriella Morini[3], Davide Pettener[1] and Donata Luiselli[1].
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2.16. Evidence for extensive non-allelic gene conversion between sex chromosomes in humans

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2.1. Darwinian (positive) selection in vitamin D and folate pathway genes in humans.

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Abstract:
Vitamin D and folate are essential vitamins that play important roles in human physiological development and are activated and degraded by sunlight, respectively. We aimed to detect signals of positive selection in gene sets involved in metabolism, regulation and action of these two vitamins using an existing algorithm developed by our group to universally test for evolutionary adaption in any chosen gene set. We made use of the low coverage re-sequencing data from worldwide populations generated by the 1000 Genomes Project and compared frequency spectrum based summary statistics between vitamin D and folate pathway gene sets and matched control genes. Genes interacting with Vitamin D receptor (VDR) were selected in all populations. Within this group genes that directly interact with VDR and retinoid –X-receptor (RXR) were selected in Africans and Europeans, respectively. Genes involved in folate uptake were selected in Europeans only and those associated with methylation were selected in both Africans and Europeans. Some genes (*ARID1A* and *BAZ1B*) were selected in all populations while others such as *FGR*, *NCOA1*, *RXRA* and *NROB2* were selected in non-Africans. The selected genes identified by this method are shared between the folate and vitamin D pathways, enriched in the nucleus and significantly associated with chromosome organization (p-value 1.609 X 10^{-24}) and cellular protein modification process (p-value 1.529 X 10^{-14}). This study provides evidence for convergent evolution operating these pathways and highlights the power of using such an approach to understand how modern humans have genetically adapted to environmental changes during their recent evolutionary history.

2.2. StreamingTrim 1.0: a Java software for dynamic trimming of 16SrRNA sequence data from metagenetic studies

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Molecular microbial ecology is massively using next generation sequencing technologies to describe taxonomic composition and infer the functionality of microbial communities, by using metagenetic applications based on PCR amplicon library sequencing. One of the problems related to the utilization of amplicon libraries data from next generation sequencing technologies is to analyze the quality of every reads present in a sequence file and to be able to trim the low quality segment without lose too much information for the following taxonomic analyses. Here, we present StreamingTrim, a DNA reads trimming software, written in Java, capable of analyzing the quality of a DNA sequence file and to search for low quality zones in a very conservative way, by using a dynamic trimming algorithm. This software has been developed aiming to provide a tool which allow to trim amplicon library data, retaining as much as taxonomic information as possible.
The software is provided with a Graphical User Interface (GUI) for a user-friendly use. From the computational point of view, StreamingTrim reads and analyzes sequences one by one from the input file, without keeping anything in memory, allowing the computation to be run on a normal desktop PC or laptop. Trimmed sequences are saved in an output file and a statistics summary, containing the mean and the standard deviation of length and quality of the whole sequence file, is also displayed.
Compiled software, manual and example data sets are available under the BSD-2-Clauses License at GitHub repository at https://github.com/GiBacci/StreamingTrim/.

2.3. Genomic tools for fishery and conservation of European hake and common sole

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Next-generation sequencing (NGS) technologies offer the opportunity to move from population genetics to population genomics, even in non-model species. In the present study NGS platforms were used to discover Single Nucleotide Polymorphisms (SNPs) in the muscle transcriptome of common sole (*Solea solea*) and European hake (*Merluccius merluccius*), two of the most important demersal fisheries in Europe. Transcriptome sequencing and *de novo* assembly into unique contigs, *in silico* SNP detection, and validation by high-throughput genotyping yielded hundreds of polymorphic SNP loci for both species.

Population samples from several Atlantic and Mediterranean locations were screened at the developed EST-derived loci, in order to find candidate genes for environmental adaptation at different spatial scales and to integrate information on neutral and adaptive evolutionary patterns. With the putative neutral markers we confirmed the major genetic breaks already described for the target species, while analyzing candidate outliers SNPs higher resolution of population differentiation both between and within basins was obtained.

Our results suggest local adaptation as a potential driver of population structure in marine fish species. Although caution should be taken when drawing indirect inferences about adaptive processes in the wild, precautionary management strategies should contemplate the possible presence of locally adapted populations.

2.4. Analysis of Clock Genes Variation in worldwide populations

Irene Dall'Ara[1], Silvia Ghirotto[1], Selene Ingusci[1], Guido Barbujani[1] and Cristiano Bertolucci[1].
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Life on Earth is marked by many biological rhythms, evolved as consequence of environmental periodic changes, such as day-light cycles or the fluctuation of seasons. The circadian rhythm, a period of ~24h, gives rise to oscillations in behaviour and physiological functions, in order to anticipate upcoming daily change and to maximally benefit from the limited natural resources. Comparisons of genotypic and phenotypic information highlight links between clock genes' polymorphisms and several human diseases. In this project, we analyse the global diversity of clock genes and their associations with sleep disorders. To do this we used whole genome sequences from the 1000Genomes data set (1092 individuals from 14 worldwide populations). In the initial phase, we considered 18 SNPs known to be linked with human diseases, describing their levels and patterns of population diversity, and investigating the association between allele frequencies and factors such as latitude and photoperiod. Correlations between genetic and geographical distances were insignificant, contrary to what observed at most neutral loci, and thus suggesting some effects of selective pressures upon the populations considered. However, the global population structure did not seem to reflect differences in photoperiod, whereas differences were observed in the distribution of SNPs at different loci.

2.5. Molecular identification of Diptera, used in forensic investigations, by means of variable lenght of intronic sequences

Emanuela De Domenico, Debora Lombardo, Concetta Federico, Claudia Leotta, Alessandro Marletta, Francesco Lombardo, Salvatore Saccone

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The identification of Diptera at the larval stage is important in forensic investigations as it helps in establishing the correct PMI (Post Mortem Interval). In the present work we describe a procedure that allows the molecular identification of three species of Diptera, useful in the forensic investigations, more quickly and easily respect to the Barcoding system. To achieve this goal we taken into consideration a number of gene sequences, one of which has allowed to obtain informations to distinguish the three considered species of scavenger Diptera: *Lucilia sericata* (Meigen,1826), *Calliphora vicina* Robinseau-Desvoid, 1830 and *Sarcophaga carnaria* (Linnaeus, 1758).

The system developed involves a PCR amplification of a DNA segment within the wingless gene. The amplified segment presents a conserved sequence in the 3' and 5' ends, in order to use the same primers in different species, and a variable length among the three species, due to the amplified sequence that contains an intron of wingless whose size is variable. In this way it is possible to assign larvae of the three Diptera species simply analysing the band size on an electrophoretic gel. This procedure could help, in the forensic investigations, to obtain easily and rapidly the estimation of PMI of a body colonized by these larvae.

2.6. Population structure and adaptive variation to climate in natural populations of Norway spruce (*Picea abies* Karst)

Erica A. Di Pierro[1], Elena Mosca[1], Duccio Rocchini[1], Giorgio Binelli[2], David B. Neale[13] and Nicola La Porta[1]

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Forest trees dominate many Alpine landscapes and are currently exposed to changing climate. Norway spruce is one of the most important conifer species of the Italian Alps due to its economic and ecological value. This study seeks to determine and quantify patterns of genetic diversity for natural populations of this species toward understanding adaptive responses to changing climate. A wide array of potential candidate genes was tested for correlation with climatic parameters characterizing sampled populations. Across the Italian species range, 24 natural stands were sampled; trees were genotyped for 384 selected Single Nucleotide Polymorphisms (SNPs) from 285 genes. To avoid false-positive association between genotype and climate, population structure was investigated. Weak differentiation among populations was revealed by all methods used (pairwise F_{ST} comparisons, STRUCTURE, DAPC). Just one population was clearly divergent from the unique major cluster identified. Selection scan revealed five F_{ST} outliers, and an environmental association analysis detected seven SNPs associated to one or more climatic variables. Precipitation, more than temperature, was often associated with genotype. These findings provide relevant information for understanding and quantifying climate change effects on this species and its ability to genetically adapt.

2.7. Low pass DNA sequencing of 1,200 Sardinians reconstructs European Y chromosome phylogeny

Paolo Francalacci[1], Laura Morelli[1], Andrea Angius[2,3], Riccardo Berutti[3,4], Daria Sanna[1], Antonella Useli[1], Magdalena Zoledziewska[2,4], Michael B. Whalen[2], Chris Jones[3], Gonçalo R. Abecasis[5], David Schlessinger[6], Serena Sanna[2], Carlo Sidore[2,4,5], Francesco Cucca[2,4]

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Human genetic variation is comprised of DNA sequence differences between individuals; its characterization can be used to clarify the origins and genetic relationships of contemporary populations. Genetic variation within the male specific portion of the human Y chromosome (MSY) is particularly well suited for this goal, because its lack of recombination simplifies reconstruction of the sequence of molecular events accumulated over evolutionary time. Here we report a sequence based phylogenetic tree of the MSY at an unprecedented level of resolution. We did whole genome low pass sequencing of Sardinian 1,204 males, an ancient founder population. We then applied a hierarchical approach to filter the Single Nucleotide Polymorphisms (SNPs) that we discovered on the aligned sequences of MSY, assigned the validated SNPs to specific haplotype clusters, thereby increasing the number of phylogenetically informative MSY polymorphisms to 11,763 SNPs. Using these markers, we could detect all main haplogroups present in Europe, define many new clusters of lineages within each of them and infer estimates for the time elapsed since the most recent common ancestor (TMRCA) for all of them. We calculate a putative age for coalescence of ~200,000 years ago, consistent with previous mtDNA based estimates. The remarkable increase in the number of available polymorphisms thus provides an enhanced framework for future studies of human evolution.

2.8. Cannabinoids abuse: evaluation of genetic vulnerability

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The vulnerability to develop drug addiction is influenced by a combination of genetic and environmental factors. The study was aimed to identify, in the population of patients addicted to cannabis, the most prevalent among the drugs in adolescents and young adults, possible genetic factors that may constitute biological correlates of personality traits at risk for addictive disorders.
The polymorphisms of the genes chosen for the study are those involved, according to the literature, in determining the tone of the endogenous cannabinoid and dopamine which presents the complex pathway of endocannabinoids interactions: CNR1: (ATT)n, 1359G>A, rs2023239, rs806380; FAAH: C385A; ANKK1: Taq1 A; DRD2: C957T; DRD4: 7R; COMT Val108/158Met;DAT1/SLC6A3: 9R.
Although preliminary and until now obtained in a small sample of subjects (50 controls and 30 cannabinoid dependent subjects), the results obtained allow us to detect, in the population of drug users, a statistically significant difference, compared to the population of abstinent for the A1 allele of the gene SNP ANKK1 TaqIA that is significantly increased in the population of drug addicts. The TaqIA is located in exon 8 of the gene ANKK1 on which it depends, for reasons not yet clarified, the expression of DRD2, encoding the dopamine receptor. Regarding the functional polymorphism, the C957T SNP in exon 7 of the DRD2 gene, is prevalent in its allelic form T in the population of cannabis-dependent. Other alleles in other genes are prevalent in the target population of cannabis abusers while not showing significant differences. The enlargement of the analysis to a greater number of abstinent drug addicts will allow us to better define the prognostic implications of risk polymorphisms test.

2.9. Early human dispersal from Africa: A model-based test of two hypotheses

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It is unclear whether early modern humans left Africa through a major migration process, dispersing simultaneously over Asia and Europe, or in two main waves, first through the Arab peninsula into Southern Asia, and later through a Southern route crossing Palestine into Western Asia and Europe. We collected genomic data on twenty populations and developed explicit geographic models of the demographic expansions, finding good correlations between geographic and genetic distances, but only insignificant differences between models involving single or multiple dispersals. Because such an uncertainty probably reflects the interplay between population splits and contacts, we used a newly developed method to incorporate both phenomena in the population tree. We thus found evidence for several admixture events, mostly involving Central Asian populations. Once presumably admixed populations were excluded from the analysis, we estimated clearly different divergence times from Africa for Asians and Europeans. A good correlation emerged between linkage disequilibrium estimates and geographic distances from Africa, but only when geographic distances were calculated according to a Multiple Dispersal model. Both findings support an earlier peopling of part of Asia through a Southern route.

2.10. *Across language families: exploration in genomic and linguistic variation in Europe*

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The notion that patterns of linguistic and biological variation may cast light on each other and on population histories dates back to Darwin's times, but turning this intuition into a proper research project has met with serious methodological difficulties. New approaches using measures of linguistic diversity based on syntactic (as opposed to lexical) features have the potential to bypass these difficulties. In this study, we first validated the method, showing that the well-established set of relationships among European languages can actually be reconstructed with good statistical confidence from syntactic comparisons. We then compared the linguistic structure of Europe with its genetic structure, inferred from the analysis of >229,000 SNPs in 15 populations, 12 of them speaking Indo-European languages. We found a highly significant degree of correlation between measures of syntactic and genomic diversity and, contrary to what observed in studies based on smaller datasets, we found that geographic distances are poorer predictors of genomic differences than linguistic distances. In addition, a good correlation was found between syntactic and lexical distances in the subset of the data (among Indo-European speakers) where the comparison was possible. ADMIXTURE analysis identified a genetic clustering of populations in agreement with their linguistic affiliation. We conclude that by focusing on structural linguistic features larger-scale comparisons are now feasible, which may cast light on processes of both biological and cultural change.

2.11. Mitochondrial haplogroup H in the heart of Central Asia: a far echo of the West

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Although Inner Asia is one of the most sparsely populated regions in the world, it played a pivotal role in shaping the history that greatly added to the cultural, ethnic, and genetic diversity observed throughout present Eurasia. Perhaps, the two most historically significant phenomena witnessed in this part of the world were the ambitious expansion strategy employed by Mongolia's most prominent personality, Genghis Khan and the complex network known as the Silk Road.

Through a strategically and diversified collection effort, we collected more than 3,000 biological samples (accompanied by informed consents and genealogical data) from the countries of Kyrgyzstan, Kazakhstan, and Mongolia. Most of these samples were sequenced for the three hypervariable segments of mitochondrial DNA (mtDNA) and subdivided among more than 30 haplogroups and sub-haplogroups. As expected, most haplogroups were typical of modern East Asian populations. However, many different Western Eurasian clades were also identified, with a particular high incidence of H, the most common haplogroup in Europe, thus suggesting a direct link between the heart of Central Asia and Western Eurasia. To discriminate between ancient migrations and more recent historical events, we are now analyzing the complete mitogenome of a subset of these samples, particularly focusing on those H mtDNAs that appear to be the most informative ones when considering their control-region haplotypes.

2.12. A next generation sequencing analysis of the human Y chromosome provides new clues about ancient genetic events in Africa

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The male-specific portion of the human Y chromosome (MSY) has been intensively studied for phylogeographic purposes. However, the deepest lineages of its phylogeny remained largely unexplored, resulting in a biased distribution of known markers over the phylogenetic tree. We characterized by high-coverage next generation sequencing a set of deep rooting lineages, framed in a larger collection of worldwide Y chromosomes; we identified 2,386 SNPs, 80% of which novel. Evidence for some degree of purifying selection emerged in the form of an excess of private missense variants. The resulting MSY tree recapitulated the previously known topology but showed drastically different relative branch lengths, with remarkably older node ages. Our dating results, together with phylogeograpic data, hint a central-western african origin for the MSY variation, and fit recent archaeological evidence about an early exit of *Homo sapiens* out of the African continent. Our experimental design produced an unbiased resource of new MSY markers and novel insights on a period of human evolution previously considered poorly accessible with paternally-inherited markers.

2.13. The Phylogenetic Relationships of Barn Swallow (*Hirundo rustica*) as Inferred by Mitogenomes.

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Despite available data from bird species are significantly increasing, a comprehensive picture of their phylogenetic relationships is not yet available. This is partially due to the fact that taxonomic classifications can be misled by morphological/behavioural convergence, but especially by the lack of adequate, if any, molecular data from many species.

The barn swallow (*Hirundo rustica*) has a worldwide distribution, in part allowed by the switch from natural nest sites to human structures. Some features such as its fast and highly efficient horizontal flight, long-distance migration, and adaptation to aerial feeding classify this species as particularly interesting for analyses of oxidative phosphorylation genes.

Here we provide for the first time the complete mitochondrial genome of the Western Eurasian subspecies *H. rustica rustica*, by adding four new mitogenomes (sampled in North and Central Italy) to the phylogenetic tree of the Passerines.

This pilot project aims to i) fill a gap in the bird mitochondrial DNA tree by adding the first complete mitogenomes of the European barn swallow; ii) clarify the phylogenetic relationships within the *H. rustica* subspecies complex, within the Passerines, and between Passerines and other similar but non-closely related birds (such as hummingbird and swift - belonging to the order of Apodiformes); iii) search for signals of convergent evolution in the mitochondrial DNA of bird species.

2.14. AGREEMENT BETWEEN EVIDENCES OF HEPATITIS FROM HISTORICAL DOCUMENTS AND GENETIC SUSCEPTIBILITY TO PRIMARY BILIARY CIRROSIS ABOUT SOME BONE RELICS FROM PESCINA, L'AQUILA, ITALY, XI CENTURY AD

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The inner areas of L'Aquila (Abruzzo, Italy), such as Pescina old town, have often been theatre of numerous combats in the past and many ethnic groups have come and gone. Migratory events may result in the disappearance of several genes in the local people as well as contribute to introduction of new foreign alleles, among which some responsible for autoimmune diseases HLA-linked (ADHLs). The history of ADHLs diseases (*e.g.,* their appearance or spreading during the ages) has both an anthropological and biomedical interest. We focused our studies on Saint Berardo from Pescina (1079-1130 AD), Cardinal and Bishop of the Marsi (L'Aquila) known for its many miracles and for having fought against simony [1]. Archival documents refer the Saint was caught by pains attacks in flank and viscera, with continuous remission and exacerbation of symptoms, until he died. The clinicians of that time diagnosed him with hepatitis. [2]. Thus, we performed immunogenetic assays on DNA from bone relics of the Saint, after testing his DNA authenticity by means of peculiar RP-HPLC analyses [3]. Our data revealed that Saint Berardo carried HLA-DR8 gene predisposing to primary biliary cirrhosis (PBC) [4], without excluding he could have suffered from the more severe PBC-AIH (AutoImmune Hepatitis) overlap syndrome. Apart from obvious cultural, religious and medical aspects, this work demonstrated the DRB1*08 allele was already present among this local people in the 11th century AD.

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2.15. GENOME-CULTURE INTERACTIONS IN THE EVOLUTION OF HUMAN TASTE

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Several of genes involved in the expression of human phenotypes are supposed to have undergone positive selection in response to changes of cultural practices. In the present study, we especially focus on the biological/cultural co-evolution of taste perception, in the attempt to estimate stochastic and causative patterns of correlation between patterns of human genetic variation, taste-related cultural/phenotypic traits and sensitivity to bitter, sweet and umami. For this purpose, a preliminary investigation of genetic variability on 14 taste-related genes was carried out on around 100 individuals sampled from different Italian regions and for which information about preferences of bitter, umami and sweet foods were collected through a questionnaire on liking of 18 common foods. Observed liking/consumption ratings were used as quantitative variables for statistical analyses. In addition, a perception test constituted by 3 different sections (Taste thresholds, Liking test, Perception) was submitted to each participant, in order to quantify specific levels of taste perception by adopting a modified version of the LMS (Labeled Magnitude Scale). The investigated taste-related genes and SNPs were selected from literature and by surveying genetic variants contained in different databases (dbSNP, 1000Genomes), resulting in 57 informative variants used to design a Sequenom® custom genotyping array dedicated to the genetics of taste.

2.16. Evidence for extensive non-allelic gene conversion between sex chromosomes in humans

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Human sex chromosomes co-evolved from a pair of autosomes through the suppression of recombination over progressively larger regions. It has long been accepted that meiotic recombination between human sex chromosomes is limited to short telomeric portions, known as pseudoautosomal regions, which mark the boundaries of a male specific region (MSY). However, in the last years, the idea that the human sex chromosomes did not have an independent evolutionary history has begun to emerge with the discovery of some MSY regions interested by X-to-Y gene conversion. In this study we explored how pervasive XY gene conversion has been during the evolution of human sex chromosomes. Using human-chimpanzee interspecies X-Y sequence comparisons we identified 19 regions showing signatures of historical gene conversion. Further, to explore the dynamics of XY non-allelic recombination in recent human evolution, we resequenced these gene conversion hotspots in 68 widely divergent Y haplogroups, and found that at least five of them are still active in humans. Our results show evidence of extensive XY non-allelic recombination between gametologous regions of sex chromosomes suggesting that the sequence landscape of MSY could be modulated by the transfer of genetic information from the X chromosome, and providing an additional explanation for the ability of the Y chromosome to retard degradation during evolution.

Sessione Poster:
3.Replicazione, riparazione e ricombinazione del DNA (Paolo Plevani, Margherita Bignami)

3.1. The oxidized dntps pool as a relevant factor in trinucleotide repeat expansion
Piera Cilli[1], Ilenia Ventura[1], Anna Minoprio[1], Alberto Martire[2], Patrizia Popoli[2], Filomena Mazzei[1] and Margherita Bignami[1] Bignami Margherita <margherita.bignami@iss.it>

3.2. DNA Topoisomerase I contributes to epigenetic regulation of Pol II transcriptional silencing at rDNA.
Anna D'Alfonso[1], Francesca Di Felice[1] and Giorgio Camilloni[1,2]. dalfonsoanna@yahoo.it

3.3. Transgene expression in meiotic *C elegans* germ-line
Maria Rosaria Dello Stritto [1], Adele Adamo, [1] Adriana La Volpe[1]
Maria Rosaria Dello Stritto <rosaria.dellostritto@igb.cnr.it>

3.4. Effects of *fcd-2* mutations during early embryonic development in *Caenorhabditis elegans*.
Marcello Germoglio[1], Adele Adamo[1], Adriana La Volpe[1]. <m.germo87@gmail.com>

3.5. Mutator phenotype in cells derived from patients affected by MUTYH-associated polyposis: the role of specific *MUTYH* mutations
Francesca Grasso[1], Elisa Giacomini[2], Massimo Sanchez[3], Paolo Degan[4], Viviana Gismondi[5], Liliana Varesco[5], Caterina Cerasaro[1], Filomena Mazzei[1], Alessandra Viel[2] and Margherita Bignami[1] Francesca Grasso <f.grasso@ymail.com>

3.6. Germ-line apoptosis in response to DNA damages requires evolutionary conserved pro-crossover factors during *C. elegans* oogenesis.
Adriana La Volpe[1], Adele Adamo[1], Pamela Santonicola[1], Enrique Martinez-Perez[2], Nicola Silva[1,2,]
Adriana La Volpe <adriana.lavolpe@igb.cnr.it>

3.7. THE PROTEIN PHOSPHATASE 2A (PP2A) IS REQUIRED FOR THE MAINTENANCE OF *DROSOPHILA* CHROMOSOME INTEGRITY
Chiara Merigliano[1], Antonio Marzio[2], Roberto Piergentili[2], Maurizio Gatti[2], and Fiammetta Vernì[1]
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3.8. Sugar and chromosome stability: Clastogenic effects of sugars in vitamin B6-deficient cells
Chiara Merigliano, Antonio Marzio, Maurizio Gatti and Fiammetta Vernì
Chiara Merigliano <chiara.merigliano@gmail.com>

3.9. Cross-talk between Fanconi Anemia pathway and Non Homologous End Joining in *Caenorhabditis elegans*
Pamela Santonicola [1], Adriana La Volpe[1], Adele Adamo [1]
Pamela Santonicola <pamela.santonicola@igb.cnr.it>

3.10. REPLICATION PROFILE OF THE *FXN* LOCUS IN NORMAL HUMAN CELLS AND IN CELLS CARRYING THE ALLELE WITH GAA/TCC-REPEAT EXPANSION

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3.1. The oxidized dntps pool as a relevant factor in trinucleotide repeat expansion

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Several neurodegenerative diseases derive from trinucleotide repeat (TNR) expansion. It has been proposed that this occurs during long-patch base excision repair (BER) of the oxidized DNA base 8-hydroxyguanine (8-oxoG). Studies in Huntington's disease indicate that TNR expansion might also depend on lack of coordination between DNA polymerase β (Polβ)-dependent repair synthesis and FEN1 cleavage. This might generate loops or hairpins, which are difficult to remove and might be integrated into the genome. To investigate the molecular basis of TNR expansion, we chose an experimental strategy based on *in vitro* BER reactions on 100bp duplex oligonucleotides containing 20 CAG/CTG repeats and a single 8-oxoG located in a triplet and purified enzymes and/or mammalian cell extracts. We hypothesized that TNR expansion might be favoured not only by direct DNA oxidation, but also by incorporation of oxidized triphosphates during repair synthesis. *In vitro* repair assays using a purified polβ and dNTPs supplemented with 8-oxodGTP confirmed the role of BER in modulating TNR expansion. In addition, during Polβ-dependent repair synthesis, 8-oxodGTP was incorporated opposite either C or A. The OGG1 and MUTYH DNA glycosylases were then able to repair respectively the newly-formed 8-oxoG:C and 8-oxoG:A mispairs. We propose that parallel processing of these mismatches initiates closely-spaced repair events on opposite strands and establish a 'toxic oxidation cycle' that favours TNR instability.

3.2. DNA Topoisomerase I contributes to epigenetic regulation of Pol II transcriptional silencing at rDNA.

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In *Saccharomyces cerevisiae,* ribosomal DNA (rDNA) lies on chromosome XII and is organized in 100-200 repeated units. All the *cis* elements responsible for replication, recombination and Pol I, Pol II and Pol III transcription are located in the intergenic region (IGS), which space the units. All these different molecular events occurring at this locus should be finely controlled because strictly connected.
We have demonstrated that in *S. cerevisiae* the epigenetic state of H4K16 at IGS of rDNA is crucial for most of these functions. H4K16 is the main target of Sir2p HDAC, which is responsible of rDNA regulation.
sir2Δ yeast cells show loss of transcriptional silencing with ncRNA hyperproduction from c-PRO and e-PRO cryptic promoters occurring on IGS, alteration of recombination with Extrachromosomal rDNA Circles (ERCs) hyperproduction, general increase of H4 histone acetylation.
The null mutation of *TOP1* gene also causes the same phenotypes acting at rDNA: ERCs accumulation, loss of transcriptional silencing and hyperacetylation of histone residues have been all reported for *top1Δ* cells. Top1p is a DNA topoisomerase, and it is not clear why altered phenotypes shown in *top1Δ* mutants are so similar to those observed in *sir2Δ* mutants.
In our work we investigated how the *top1Δ* mutation can alter phenotypes at rDNA, focusing to the transcriptional silencing of ncRNA and to H4K16 acetylation, and we will display the contribution of Top1p to epigenetic regulation of rDNA.

3.3. Transgene expression in meiotic *C elegans* germ-line

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The introduction by injection of dsDNA carrying a transgene in *C. elegans* leads to the formation of multicopy arrays. If the transgene is driven by a somatic promoter it will be expressed in the corresponding tissue, while if it is driven by a germ-line specific promoter it leads to abrogation of gene expression from the multicopy array and from the endogenous corresponding sequence, this phenomenon is known as transgene mediated cosuppression. The phenomenon of cosuppression therefore is a hindrance for the analysis of meiotic genes expression or for the study of specific mutations introduced in a meiotic transgene. For example, we injected the transgene *pPD96_04* with two reporter coding sequences, GFP and LacZ, under the promoter of the meiotic RecA-like recombinase, *rad-51*, a gene necessary for homologous DNA recombination/repair in soma and in meiosis. Nematodes carrying the transgene array do not express the reporter genes in any tissue. However, after γ-irradiation the reporter gene become expressed in the soma, but it is still absent in thegerm-line, likely because of cosuppression. We decided inject our transgene in a strain carrying the *rde-2* mutation. The *rde-2* gene is indeed required for the functional silencing by cosuppression. In this way we hope to be able to find a simple way to study expression of meiotic genes from a transgene array not only in somatic tissues but also in the germ-line.

3.4. Effects of *fcd-2* mutations during early embryonic development in *Caenorhabditis elegans.*

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We have demonstrated that mutations in the *C.elegans fcd-2* gene induces a significant increase of developmental defects compared to the wild type population. FancD2 (FCD-2 human homologue) plays a central role in FA pathway, repairing double-strand breaks (DBSs) using homologous recombination (HR) and preventing the illegitimate and careless repair by non-homologous end joining (NEHJ). Mutations in this gene causes Fanconi anemia (FA), an autosomal recessive genetic disease characterized by genome instability, cancer predisposition, infertility, occurrences of developmental defects, and cell lines hypersensitivity to inter cross-linking (ICL) agents.
To further investigate the causes of the developmental defects observed in nematodes, we analyzed the early embryogenesis of *C. elegans* at stages at which timing of cell division is crucial for proper development. The treatment with the ICL agent cisplatin (CDDP) leads to a significant delay in cell division, unlike what occurs after treatment with alkylating agents or with γ-irradiation. *fcd-2* mutants treated with CDDP show longer delay in cell division compared to the wild type nematodes treated in the same way. This delay decreases when the DNA ligase LIG-4 is depleted in *fcd-2* mutants. LIG-4 is a key factor of NEHJ pathway joining together DNA ends of DSBs. This results suggest that NEHJ is inappropriately activated in *fcd-2* early embryos, causing cell division delay likely associated with developmental defects.

3.5. Mutator phenotype in cells derived from patients affected by MUTYH-associated polyposis: the role of specific *MUTYH* mutations

Francesca Grasso[1], Elisa Giacomini[2], Massimo Sanchez[3], Paolo Degan[4], Viviana Gismondi[5], Liliana Varesco[5], Caterina Cerasaro[1], Filomena Mazzei[1], Alessandra Viel[2] and Margherita Bignami[1]

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Oxidative stress causes different kinds of DNA damage, including double and single strand breaks and base modifications; 8-oxo-7,8-dihydroguanine (8-oxodG) is the most extensively studied type of oxidative damage because it is potentially mutagenic. DNA 8-oxodG codes ambiguously during replication and directs incorporation of C or A with almost equal efficiency, leading to G:C→T:A transversions. The MUTYH DNA glycosylase counteracts the mutagenic effects of 8-oxodG by removing A opposite to the oxidized purine. Biallelic germ-line mutations in *MUTYH* cause the autosomal recessive MUTYH-associated adenomatous polyposis (MAP).
We previously identified a large variability in the spontaneous mutator phenotype associated with inactivation of the *MUTYH* gene in lymphoblastoid cell lines (LCLs) derived from MAP patients harbouring different mutations. To investigate whether this variability depends on specific *MUTYH* mutations or the genetic background of the patients, we characterized LCLs derived from MAP patients expressing the same variant (Y179C or R245H). LCLs derived from homozygous as well as from heterozygous carriers were analyzed for spontaneous and oxidant-induced mutation frequencies at the *PIG-A* gene and for DNA 8-oxodG levels. Homozygous inactivation of MUTYH by either Y179C or R245H mutations resulted in increased spontaneously or oxidant-induced mutations frequencies and DNA 8-oxodG levels, while this is not always true for heterozygous carriers.

3.6. Germ-line apoptosis in response to DNA damages requires evolutionary conserved pro-crossover factors during *C. elegans* oogenesis.

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Crossover events are essential for correct chromosome segregation at meiosis I. DNA double strand breaks (DSBs) are physiologically induced by the type II topoisomerase-like DNA transesterase SPO-11 to start the recombination process and promote the formation of inter-homologue crossovers. Some conserved proteins such as the heterodimeric partners MSH4 and MSH5 are required at a late step of the process to promote and stabilize crossovers, and their absence results in the accumulation of recombination intermediates. Timely repair of DSBs is an essential part of the meiotic program, since accumulation of unprocessed DSBs during the pachytene stage of meiotic prophase triggers a DNA damage checkpoint response that induces apoptosis of damaged cells. We show that the evolutionary conserved pro-crossover factors MSH-4, MSH-5, and ZHP-3 are also required in the *C. elegans* germ line for the apoptosis of germ nuclei in response to DNA damages derived by defective meiotic recombination/repair, ionizing radiation, and CDDP treatment. These pro-crossover factors seem to act in parallel or downstream of the DNA damage checkpoint activation.
Further studies will be required to identify the specific partners and steps of the apoptotic response at which MSH-4/5 and ZHP-3 are required. Elucidating the pro-apoptotic roles of MSH-4/-5 and ZHP-3 will increase our understanding of how these repair proteins contribute to preserve genome integrity during gametogenesis.

3.7. THE PROTEIN PHOSPHATASE 2A (PP2A) IS REQUIRED FOR THE MAINTENANCE OF *DROSOPHILA* CHROMOSOME INTEGRITY

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A key event in DNA damage response (DDR) is phosphorylation of the histone variant H2AX (H2Av in *Drosophila*) at the sites of DNA breakage to a form □-H2AX, which recruits several additional DNA repair factors. These factors form discrete nuclear foci that dissolve when DNA repair is completed. Completion of DNA repair requires phosphatase-mediated dephosphorylation of □-H2AX. We isolated a new lethal mutation, *tws*^{430}, in the *Drosophila twins (tws)* gene, that encodes the B regulative subunit of the Ser/Thr phosphatase 2A (PP2A). This mutation causes frequent (54%) chromosome aberrations (CABs) in larval neuroblasts. In addition, *tws*^{430} mutations affect the regression of IR-induced repair foci; in *tws*^{430} mutant brains the □-H2Av foci persist much longer than in controls, suggesting that PP2A is required for □-H2Av dephosphorylation. Double mutant analysis showed that mutations in *tefu* (ATM) are epistatic over mutations in *tws* (PP2A); in contrast *mei-41* (ATR) *tws* double mutants showed a higher frequency of CABs than either single mutant. These results suggest that *Drosophila* PP2A mediates dephosphorylation of ATM substrates, whose persistent phosphorylation interferes with the DNA repair processes. Partial RNAi-mediated inactivation of the B55 subunit of the human PP2A causes CABs in HeLa cells. These results highlight the functional conservation of PP2A from flies to humans, and suggest the proper behavior of repair foci is essential for maintenance of chromosome integrity.

3.8. Sugar and chromosome stability: Clastogenic effects of sugars in vitamin B6-deficient cells

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Pyridoxal 5'-phosphate (PLP), the active form of vitamin B6, has been implicated in preventing human pathologies, such as diabetes and cancer. However, the mechanisms underlying the beneficial effects of PLP are still unclear. Using Drosophila as a model system, we show that PLP deficiency caused either by mutations in the pyridoxal kinase-coding gene (dPdxk) or by vitamin B6 antagonists results in chromosome aberrations (CABs). The CAB frequency in PLP-depleted cells was strongly enhanced by sucrose, glucose or fructose treatments, and dPdxk mutant cells consistently displayed a higher glucose contents than their wild type counterparts, due to an acquired insulin resistance. Together, our results indicate that an elevated intracellular level of glucose has a dramatic clastogenic effect if combined with PLP deficiency. This effect is at least partially due to an elevated level of Advanced Glycation End-products (AGE) formation. Treatment of dPdxk mutant cells with alpha lipoic acid (ALA) lowered both AGE formation and CAB frequency, suggesting an AGE-CAB cause-effect relationship. The clastogenic effect of glucose in PLP-depleted cells is evolutionarily conserved. RNAi-mediated silencing of PDXK in human cells or treatments with PLP inhibitors resulted in chromosome breakage, which was potentiated by glucose and reduced by ALA. These results suggest that patients with concomitant hyperglicemia and vitamin B6 deficiency (e.g., due to treatment with PLP antagonists) may suffer chromosome damage. This might impact on cancer risk, as CABs are a well-known tumorigenic factor

3.9. Cross-talk between Fanconi Anemia pathway and Non Homologous End Joining in *Caenorhabditis elegans*

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The Fanconi Anemia (FA) is a cancer-prone syndrome causing anemia, occurrence of developmental defects and cell sensitivity to interstrand crosslinking agents. Genes in the FA pathway are evolutionary conserved, allowing dissection and mechanistic studies in model systems, such as the nematode *C.elegans*. The FA pathway is involved in the choice between the high fidelity repair pathway, Homologous Repair (HR), and the error prone Non-Homologous End Joining (NHEJ). The *C.elegans fcd-2* mutant shows hyper-sensibility to cisplatin (CDDP), occasional developmental defects as well as increase in the apoptotic levels, and in the number of nuclear foci of the RecA-like recombinase RAD-51 in the germline. The DNA repair defects shown in the *fcd-2* mutants are suppressed by eliminating the NHEJ. In fact, when the latest step of NHEJ pathway is blocked, by depleting the DNA ligase *lig-4*, there is phenotype rescue.
Our goal is to better understand the interactions between those pathways.
We have identified a suppressor of *fcd-2* ICL sensitivity we named *clt-1* for Cross Linking Tolerant by screening on CDDP. The double mutant *fcd-2*;*clt-1* resembles the wild type also in the number of RAD-51 nuclear foci in untreated worms. We are now further characterizing the *clt-1* mutant in order to understand its role and function.

3.10. REPLICATION PROFILE OF THE *FXN* LOCUS IN NORMAL HUMAN CELLS AND IN CELLS CARRYING THE ALLELE WITH GAA/TCC-REPEAT EXPANSION

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Friedreich ataxia (FRDA), the most common inherited ataxia, is transmitted as an autosomal recessive trait and 98% of affected individuals are homozygous for an expanded GAA/TTC trinucleotide repeat in the first intron of the *FXN* gene (9q13) encoding the mitochondrial protein frataxin. This mutation causes the transcriptional inhibition of the *FXN* gene. Among several factors contributing to dynamic instability of trinucleotide repeats, DNA replication is a key process. By molecular cytogenetic approaches we are currently evaluating the replication profile of the *FXN* locus, in normal cells, and in cell lines derived from FRDA patients and their heterozygous relatives. By interphase FISH we evaluated the proportions of non-replicated (single fluorescent spot) and replicated (double fluorescent spot) alleles, both in asynchronous proliferating cells and in 4 (early-to-late) S-phase fractions obtained after FACS sorting; in parallel, for the same nuclei the BrdU staining pattern was recorded. The results indicate that replication of the *FXN* domain may start during a wide temporal window corresponding to mid-late S-phase patterns. In particular, nuclei with both replicated alleles start to be numerically consistent from the second S-phase fraction. With respect to the normal sequence, a shift in replication timing of the expanded allele is suggested, but further observations are necessary to confirm the trend. In parallel, by molecular combing, we attempted to evaluate the position of activated origins, and the fork rates within the *FXN* locus; the global replication dynamics of normal and mutated cells has been also considered. Our preliminary data suggest the lack of activated origins within the *FXN* gene; therefore both the normal and the mutated allele are passively replicated by forks firing in the flanking regions and running at speed in the normal range for human cells. The analysis will be completed by the evaluation of possible altered replication patterns linked to the presence of the expanded repeat.

Sessione Poster
4.Espressione del DNA e genomica funzionale (Momi Lanfranchi, Annamaria Puglia)

4.1.The role of long non-coding RNA in pathophysiological conditions of skeletal muscle.
Stefano Cagnin[1], Enrico Alessio[1], Francesco Chemello[1], Chiara Romualdi[1], Gerolamo Lanfranchi[1]
stefano cagnin <stefano.cagnin@unipd.it>

4.2. miRNA and gene regulatory pathways of stage I epithelial ovarian cancer: reconstructing cancer circuits.
Enrica Calura[1], Gabriele Sales[1], Paolo Martini[1], Robert Fruscio[2], Lara Paracchini[3], Eliana Bignotti[4], Antonella Ravaggi[4], Romina Baldo[2], Sonia Magni[2], Mariacristina Di Marino[3], Laura Zanotti[4], Dionyssios Katsaros[5], Germana Tognon[6], Enrico Sartori[6], Sergio Pecorelli[4,6], Maurizio D'Incalci[3], Sergio Marchini[3] , Chiara Romualdi[1]
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4.3. Transactivation specificity is conserved among p53 family proteins and depends on a response element sequence code.
Yari Ciribilli.*[1], Paola Monti*[2], Alessandra Bisio[1], Thien H. Nguyen[3], Abdul S. Ethayathulla[3], Ana Ramos[3], Giorgia Foggetti[2], Paola Menichini[2], Daniel Menendez[4], Michael A. Resnick [4], Hector Viadiu^[3], Gilberto Fronza^[2] and Alberto Inga^[1] Alberto Inga <inga@science.unitn.it>

4.4. A deep sequencing approach to uncover the inflorescence miRNome of the orchid *Orchis italica*
Sofia De Paolo[1], Maria Sica[1], Valeria D'Argenio[2,3], Piergiuseppe Cantiello[2], Francesco Salvatore[2,3], Luciano Gaudio[1], and Serena Aceto[1]. Sofia De Paolo <sofiadepaolo@gmail.com>

4.5. The Antarctic krill *Euphausia superba* shows diurnal cycles of transcription under natural conditions
Cristiano De Pittà[1], Alberto Biscontin[1], Alessandro Albiero[2,3], Gabriele Sales[1], Caterina Millino[2], Gabriella M. Mazzotta[1], Cristiano Bertolucci[4], Rodolfo Costa[1] cristiano.depitta@unipd.it

4.6. Role of intestinal microbiota in diet-induced inflammation
Blanda Di Luccia[1], Raffaella Crescenzo[1], Luisa Cigliano[1], Loredana Baccigalupi[1], Salvatore Cozzolino[1], Ezio Ricca[1], Susanna Iossa[1] Blanda <blanda.diluccia@unina.it>

4.7. Physical stresses interfere with the piRNA-mediated silencing of repetitive sequences and transposons in *Drosophila melanogaster*
Antonella Friscini[1],Valeria Specchia[1],Vincenzo Nassisi[2], Luciano Velardi[2], Domenico Delle Side[2], Ettore De Giorgio[1], Sergio Pimpinelli[3], Maria Pia Bozzetti[1] <maria.bozzetti@unisalento.it>

4.8. Functional and evolutionary diversification in bacterial replicons: the case of *Sinorhizobium meliloti*
Marco Galardini[1], Francesco Pini[1,2], Marco Bazzicalupo[1], Emanuele G. Biondi[2], Alessio Mengoni[1,*]
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4.9. The RNA helicase BELLE interacts with CRYPTOCHROME and participates to the circadian machinery in *Drosophila melanogaster* .
Gabriella M. Mazzotta[1], Laura Caccin[1], Elena Carbognin[1], Moyra Mason[1], Maria Pia Bozzetti[2], Rodolfo Costa[1]
Gabriella Mazzotta <gabriella.mazzotta@unipd.it>

4.10. *trpX*: a small orf involved in *S. coelicolor* tryptophan metabolism
E. Palazzotto[1,2], G. Gallo[2], A. Vassallo[2], P. Fontana[2], A. Sutera[2], A. Giardina[2], A. Fabbretti[1], G. Renzone[3], A. Scaloni[3] and A. M. Puglia[2]. emiliapalazzotto@gmail.com

4.11. Regulation of the expression of the metabolic enzyme Proline Dehydrogenase in cancer cells in response to stress.
Silvia Palombella[1], Ivan Raimondi[1], Raffaella Cinquetti[1], Elisa Taiana[2], Marzia Gariboldi[2], Michela Bistoletti[1], Elena Monti[2] and Paola Campomenosi[1,3] palombella.silvia@gmail.com>

4.1. The role of long non-coding RNA in pathophysiological conditions of skeletal muscle.

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BACKGROUND Epigenetic modifications are involved in the maintenance of lineage commitment and terminal differentiation but, also, in the dynamic nature of cells. In this context skeletal myogenesis, which entails the progression through two antagonistic processes (proliferation and differentiation), is only a recent object of investigation.
RESULTS We identified several differentially expressed genes between different types of muscle fibers. Many of these genes codify for proteins involved in chromatin modification. Fiber type switching is associated with ageing and skeletal muscle pathologies and our findings suggest a link between ageing muscle processes and skeletal muscle pathologies and chromatin remodelling. In addition, genome wide approaches (sequencing and qRT-PCR) were used to quantify and validate the expression of non-coding RNAs in single myofiber. 156 miRNAs and 74 lncRNAs were found as fiber-specific. 57 lncRNAs were localized in the fiber nuclei where they may be involved in chromatin conformation modification. In support of this hypothesis we recognized that one nuclear lncRNA interacts with a specific histone. Moreover this lincRNA is differentially expressed during atrophy induced by denervation.
CONCLUSIONS We evidenced how specific muscle cells can modulate their expression of coding and non-coding RNAs to respond to changed conditions. In particular a specific lncRNA is involved in the chromatin modification through its interaction with histone proteins.

4.2. miRNA and gene regulatory pathways of stage I epithelial ovarian cancer: reconstructing cancer circuits.

Enrica Calura[1], Gabriele Sales[1], Paolo Martini[1], Robert Fruscio[2], Lara Paracchini[3], Eliana Bignotti[4], Antonella Ravaggi[4], Romina Baldo[2], Sonia Magni[2], Mariacristina Di Marino[3], Laura Zanotti[4], Dionyssios Katsaros[5], Germana Tognon[6], Enrico Sartori[6], Sergio Pecorelli[4,6], Maurizio D'Incalci[3], Sergio Marchini[3], Chiara Romualdi[1]
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Gene expression has been widely studied to explain cancer molecular deregulations. The production of cancer gene expression data is nothing less than astounding. However, with the benefit of hindsight we can assert that, since we completely ignored the non-coding transcriptome, we spent at least 10 years to study cancer mechanisms having only half of data in our hands. miRNAs, being the post-transcriptional regulators of genes, deserve special attention. Since their discovery, miRNAs have been thoroughly studied and hundreds of target genes have been found. Despite all these efforts the pathways, which formally describe biological circuits, still lack microRNAs. This penalizes data analyses and result interpretation.

Currently, miRNA and gene circuits are obtained through binding prediction combined to expression correlation analyses. These analyses are useful, but totally miss the biological context of cell signals. To overcome this limitation we developed a new system, based on the new Graphite web-tool (Sales *et al.*, 2012, 2013) able to integrate miRNAs in pathways and identify circuits of functionally related genes and miRNAs showing coordinated expression changes.

In collaboration with the Mario Negri Institute, we used our method to successfully guide the study of the cellular circuits in the early stage of Epithelial Ovarian Cancer (EOC). Gene and miRNA expression of 257 snap-frozen stage I EOC biopsies have been profiled and used for the analyses of circuits. The results offered by our innovative method demonstrate that histotypes and grades of EOC have discriminant regulatory circuits driving the differentiation of the tumour environment (Calura *et al.*, 2013).

4.3. Transactivation specificity is conserved among p53 family proteins and depends on a response element sequence code.

Yari Ciribilli.*[1], Paola Monti*[2], Alessandra Bisio[1], Thien H. Nguyen[3], Abdul S. Ethayathulla[3], Ana Ramos[3], Giorgia Foggetti[2], Paola Menichini[2], Daniel Menendez[4], Michael A. Resnick [4], Hector Viadiu^[3], Gilberto Fronza^[2] and Alberto Inga^[1]

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Structural and biochemical studies have demonstrated that p73, p63 and p53 recognize DNA with identical amino acids and similar binding affinity. Here, measuring transactivation activity for a large number of response elements (REs) in yeast and human cell lines, we show that p53 family proteins also have overlapping transactivation profiles. We identified mutations at conserved amino acids of loops L1 and L3 in the DNA-binding domain that tune the transactivation potential nearly equally in p73, p63 and p53. For example, the mutant S139F in p73 has higher transactivation potential towards selected REs, enhanced DNA-binding cooperativity *in vitro,* and a flexible loop L1 as seen in the crystal structure of the protein-DNA complex. By studying, how variations in the RE sequence affect transactivation specificity, we discovered a RE-transactivation code that predicts enhanced transactivation; this correlation is stronger for promoters of genes associated with apoptosis. We propose the selection during evolution of an RE sequence code within target promoters, that coupled to stress-induced post-translational modifications, can affect intrinsic conformational flexibility of p53 proteins. The combination of these factors can alter DNA binding affinity and cooperative binding so as to modulate *in vivo* selectivity, favoring the activation of apoptotic target genes.

4.4. A deep sequencing approach to uncover the inflorescence miRNome of the orchid *Orchis italica*

Sofia De Paolo[1], Maria Sica[1], Valeria D'Argenio[2,3], Piergiuseppe Cantiello[2], Francesco Salvatore[2,3], Luciano Gaudio[1], and Serena Aceto[1].

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Plant microRNAs (miRNAs) are short, non-coding RNAs involved in the regulation of different pathways such as signal transduction, response to stress, flower development, etc. Although miRNAs have been widely studied in many model plant species, they remain largely unknown in non-model species such as Orchidaceae, one of the largest plant families with floral structures highly specialized and diversified. In order to characterize the floral miRNome of the Mediterranean orchid *Orchis italica*, we constructed and sequenced a small-RNA library of inflorescence tissue using the Illumina MiSeq platform. The 4,718,127 total reads were processed to remove adaptors, low-complexity and invalid sequences, tRNA/rRNA contamination, reads shorter than 18 and longer than 35 nt. The resulting 1,064,237 reads were collapsed, obtaining 37,818 distinct reads, with the highest length distribution at 24 nt. BLAST analysis against the known plant stem-loop and mature miRNAs currently deposited in miRBase 19 gave positive matches for 178 sequences of *O. italica* corresponding to 23 miRNA families. Using the miRDeep-P software, we searched for putative novel miRNAs using as reference the transcriptome of the orchid *Phalaenopsis aphrodite*. We selected eight conserved and two putative novel miRNAs to evaluate the expression in tepals, lip, column and ovary. The expression profile and the putative target prediction revealed conserved patterns, compared with those of the model species.

4.5. The Antarctic krill *Euphausia superba* shows diurnal cycles of transcription under natural conditions

Cristiano De Pittà[1], Alberto Biscontin[1], Alessandro Albiero[2,3], Gabriele Sales[1], Caterina Millino[2], Gabriella M. Mazzotta[1], Cristiano Bertolucci[4], Rodolfo Costa[1]

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Polar environments are characterized by extreme seasonal changes in day length, light intensity and spectrum, the extent of sea ice during the winter, and food availability. A key species of the Southern Ocean ecosystem, the Antarctic krill has evolved rhythmic physiological and behavioral mechanisms to adapt to daily and seasonal changes. The genome sequence of the Antarctic krill is not yet available. A normalized cDNA library was produced and pyrosequenced in the attempt to identify large numbers of transcripts. All available *E. superba* sequences were then assembled to create the most complete existing oligonucleotide microarray platform with a total of 32,217 probes. Gene expression signatures of specimens collected in the Ross Sea at five different time points over a 24-hour cycle were defined, and 1,308 genes differentially expressed were identified. Of the corresponding transcripts, 609 showed a significant sinusoidal expression pattern; about 40% of these exibithed a 24-hour periodicity while the other 60% was characterized by a shorter (12-hour) rhythm. We assigned the differentially expressed genes to functional categories and noticed that those concerning translation, proteolysis, energy and metabolic process, redox regulation, visual transduction and stress response, which are most likely related to daily environmental changes, were significantly enriched. Our work represents the first characterization of the krill diurnal transcriptome under natural conditions.

4.6. Role of intestinal microbiota in diet-induced inflammation

Blanda Di Luccia[1], Raffaella Crescenzo[1], Luisa Cigliano[1], Loredana Baccigalupi[1], Salvatore Cozzolino[1], Ezio Ricca[1], Susanna Iossa[1]

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Emerging evidence suggests that the gut microbiota plays a role in the development of a chronic low-grade inflammatory state in the host that contributes to the development of chronic metabolic diseases and of obesity. The metabolic damages induced by a western diet in animal models can be partially releaved by an antibiotic treatment, suggesting that the composition of the intestinal microflora is influenced by a western diet and that the alteration of the microflora is a potentially important factor involved in the metabolic damages associated to obesity.
To address this issue we used 5 groups of rats fed, for a period of 8 weeks, with normal or high fructose diet (groups 1 and 2, respectively) and with normal or high fructose diet plus a mixture of antibiotics (groups 3 and 4, respectively). The fifth group of rats was treated identically to group 2 but received a daily inoculum of intestinal bacteria from rats of group 1. After the treatments the animals were sacrified and analyzed. Metabolic analysis confirmed that: i) a high-fructose diet induced obesity and an inflammation state; ii) antibiotic treatment alleviated only the latter of such effects; iii) the administration of intestinal bacteria from rats fed with a standard diet also alleviated the inflammation effects caused by the high-fructose diet. A metagenomic analysis of the microbiota of the five groups of animals is currently in progress.

4.7. Physical stresses interfere with the piRNA-mediated silencing of repetitive sequences and transposons in *Drosophila melanogaster*

Antonella Friscini[1],Valeria Specchia[1],Vincenzo Nassisi[2], Luciano Velardi[2], Domenico Delle Side[2], Ettore De Giorgio[1], Sergio Pimpinelli[3], Maria Pia Bozzetti[1]
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In the wild, individuals, populations and species are faced with changes of the environmental conditions. They undergo physiological adaptations to challenge these changes, but many types of stresses can interfere with basic biological processes leading to severe consequences on the fertility and on the viability of organisms. It has long been known that stress conditions induce the activation of transposable elements causing genomic instability. Here we report the effects of physical stresses like constant and pulsed 900 MHz electromagnetic waves (radio waves) and heavy metals, on the activation of Stellate sequences and of transposable elements in the germline of males and females in *Drosophila melanogaster.* Such stresses interfere with the piRNA-mediated regulation of the repetitive sequences and of the TE, resulting in the loss of genome stability

4.8. Functional and evolutionary diversification in bacterial replicons: the case of *Sinorhizobium meliloti*

Marco Galardini[1], Francesco Pini[1,2], Marco Bazzicalupo[1], Emanuele G. Biondi[2], Alessio Mengoni[1,*]

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Many bacterial species, such as the alphaproteobacterium *Sinorhizobium meliloti,* are characterized by open pangenomes and contain multipartite genomes consisting of a chromosome and other large-sized replicons, such as chromids, megaplasmids and plasmids.
The evolutionary forces in both functional and structural aspects that shape the pangenome of species with multipartite genomes are still poorly understood. Therefore, we sequenced the genomes of ten new *S. meliloti* strains, analyzed with four publicly-available additional genomic sequences.
Results indicated that the three main replicons present in these strains (a chromosome, a chromid and a megaplasmid) partly show replicon-specific behaviors related to strain differentiation. In particular, the pSymB chromid was shown to be a hotspot for positively-selected genes and, unexpectedly, genes resident in the pSymB chromid were also found to be more widespread in distant taxa than those located in the other replicons. Moreover, through the exploitation of a DNA proximity network, a series of conserved "DNA backbones" were found to shape the evolution of the genome structure, with the rest of the genome experiencing rearrangements.
The presented data allow depicting a scenario where the pSymB chromid has a distinctive role in intra-species differentiation and in evolution through positive selection, while the pSymA megaplasmid mostly contributes to structural fluidity and to the emergence of new functions, indicating a specific evolutionary role for each replicon in the pangenome evolution.

4.9. The RNA helicase BELLE interacts with CRYPTOCHROME and participates to the circadian machinery in *Drosophila melanogaster*

Gabriella M. Mazzotta[1], Laura Caccin[1], Elena Carbognin[1], Moyra Mason[1], Maria Pia Bozzetti[2], Rodolfo Costa[1]

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In *Drosophila melanogaster* CRYPTOCHROME (dCRY) is a blue light photoreceptor involved in the photic input pathway to the circadian clock. Recently we have shown that dCRY also modulates the fly vision in a circadian fashion, as it is involved in the control of the diurnal cycling of photoreceptor sensitivity and motion vision. Moreover, albeit controversial, it has been suggested that in peripheral clocks dCRY could act as a transcriptional repressor in a light independent manner.
In a search for putative partners of dCRY we identified BELLE, an ATP-dependent RNA helicase, known to be involved in splicing, translation and RNAi. *belle* mRNA levels oscillate in *Drosophila* heads either in LD cycles or in constant darkness (DD) while the protein in expressed at constant levels throughout the day. Nevertheless its localization within the fly brain varies during the 24 hours. Flies mutant for this gene show an altered rhythmicity in their locomotor activity profile, accompanied by an altered PER expression in PDF^+ cells. Moreover, *belle* mutants show altered expression and mobility of transposable elements (TE) in the gonads, further supporting the involvement of this RNA helicase in the fly silencing machinery.
We suggest that BELLE has a role in the circadian machinery of *Drosophila*, where it could act in the post-transcriptional modulation of some circadian components.

4.10. *trpX*: a small orf involved in *S. coelicolor* tryptophan metabolism

E. Palazzotto[1,2], G. Gallo[2], A. Vassallo[2], P. Fontana[2], A. Sutera[2], A. Giardina[2], A. Fabbretti[1], G. Renzone[3], A. Scaloni[3] and A. M. Puglia[2].

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Streptomyces coelicolor is a model actinomycete used for studying microbial differentiation. *S. coelicolor* amino acid biosynthesis is growth-stage dependent and not subjected to end-product repression. Tryptophan biosynthetic genes are organized in a split-operon despite other bacteria such as *E. coli* and *B. subtilis*. *S. coelicolor trpCXBA* operon carries a small orf named *trpX* that encodes a 7 KDa protein (TrpX) with unclear function. A *trpX* mutant strain, created in our laboratory, shows a very slow growth kinetics in minimal medium compared to that of wt. The addition of tryptophan (Trp) in the medium or complementation with a *trpX* wt allele restores growth in minimal medium. Thus, TrpX is involved in tryptophan biosynthesis.
TrpX was overexpressed in *E. coli* and purified by IMAC in order to understand its functions. A pull-down assay using crude extract of *S. coelicolor* was performed and putative interacting proteins were identified by mass spectrometry analysis.
Moreover, differential proteomic analyses, based on 2D-differential gel electrophoresis and mass spectrometry procedures, were applied to identify TrpX-dependent metabolic pathways during the *trpX* mutant growth.
The results suggest that TrpX plays a modulating role in Trp metabolism and controls the expression of regulatory proteins and enzymes involved in aerial hyphae, spores and secondary metabolite production, such as antibiotics.

4.11.Regulation of the expression of the metabolic enzyme Proline Dehydrogenase in cancer cells in response to stress.

Silvia Palombella[1], Ivan Raimondi[1], Raffaella Cinquetti[1], Elisa Taiana[2], Marzia Gariboldi[2], Michela Bistoletti[1], Elena Monti[2] and Paola Campomenosi[1,3]

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Changes in the expression/activity of aminoacid-metabolizing enzymes are a frequent occurrence in tumors. **Proline dehydrogenase** (PRODH) is the key enzyme in proline metabolism, catalyzing the first step in the pathway, whose ultimate products are glutamate and α-ketoglutarate (α-KG). Mutations in the *PRODH* gene, mapping to chromosome 22q11, have been reported to give rise to the mendelian trait hyperprolinemia and possibly to schizophrenia; a role in tumor suppression is also emerging.

The aim of our research is to characterize the regulation of the *PRODH* gene and its role as a tumor suppressor. We recently showed its regulation by p53 and p73, and characterized the response elements responsible for transactivation. Here we present experimental evidence indicating that in glioma cells PRODH downregulates the expression of the inducible α subunit of hypoxia-inducible factor (HIF)-1, the master regulator of cellular adaptation to hypoxic stress, that frequently occurs during tumor growth. In this setting, we found that PRODH reduces the levels of HIF-1α and VEGF, one of its targets, by increasing the availability of α-KG (which is involved in O_2-dependent HIF-1α degradation). Conversely, we found that hypoxic or chemical stabilization of HIF-1α causes a reduction in PRODH transcript and protein levels, suggesting a mutual crosstalk between the two systems. Investigations aimed at elucidating the mechanisms involved in this downregulation are under way.

Sessione Poster

5. Biologia cromosomica ed epigenetica (Sergio Pimpinelli, Maria Pia Bozzetti)

5.1. Myc transactivation and Myc-induced DNA damage: two sides of the same coin

Stefano Amente[a], Giacomo Di Palo[a], Susanna Ambrosio[a], Maria Cristina Sorrentino[a], Cinzia Raimondo[a], Giuliana Napolitano[a], Mario Faretta[b], Gaetano Ivan Dellino[b], Pier Giuseppe Pelicci[b], Luigi Lania[a] and Barbara Majello[a]
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5.2. *Analysis of the physical organization and of the CENP binding ability of horse centromeric satellite DNA families*

Elisa Belloni, Francesca Piras, Alice Mazzagatti, Claudia Badiale, Benedetta Meinardi, Andres Castro, Grazia Savini, Federico Cerutti, Mirella Bensi, Solomon Nergadze, Elena Raimondi and Elena Giulotto
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5.3. Global DNA hypomethylation following 5-aza-2'-deoxycytidine treatment induces aneuploidy in HCT-116 tumor cells

Giuseppe Costa[1,2], Salvatore La Marca[1], Sergio Spatafora[1], Lorena Veneziano and Aldo Di Leonardo[1]
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5.4. Histone amount, genome stability, gene expression and aging in NHP6 mutants of *S. cerevisiae*

Francesca Di Felice[1], Andrea Lukacs[1], Diletta Durano[1], Anna D'Alfonso[1], Davide Gaglio[1], Elisa Cesarini[1], Federico Zambelli[2], Giulio Pavesi[2] and Giorgio Camilloni[1]
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5.5. Chromosomal mapping reveals a dynamic organization of the histone genes in aphids (Hemiptera: Aphididae)

Mauro Mandrioli[1], Gian Carlo Manicardi[2]
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5.6. Cytological maps of heterochromatin of sequenced *Drosophila* species

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5.7. The analysis of *pendolino* reveal unexpected differences between euchromatic and heterochromatic *Drosophila* telomeres

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5.8. The epigenetic role of Bucentaur *(*BCNT) protein family

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5.9. Hsp90 and its interacting partners in the piRNAs pathway

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5.10. Environmental stress, transposons and evolution

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5.11.AKTIP, an E2 variant enzyme that interacts with lamin and protects mammalian telomeres from replicative damage

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5.12. BIT chromosome translocation induces *PDR*-dependent anticancer drug resistance in yeast.

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5.1. *Myc transactivation and Myc-induced DNA damage: two sides of the same coin*

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The proto-oncogene c-Myc (Myc) is an immediate-early serum response gene essential for embryonic development, cellular proliferation and survival. While low Myc levels are necessary and sufficient for cellular viability and proliferation, pathological activation of this proto-oncogene by over-expression and gain of function mutations is observed in a large number on tumors. Activation of Myc oncogene is both associated with the induction and suppression of DNA damage response (DDR) signaling, which acts as a barrier to tumor progression. Moreover, it has been proposed the oncogene-induced DNA damage (OID) model for cancer development and progression to help explain many features of cancer, including genomic instability and the suppression of DDR. Myc represents a paradigm of OID. We proposed a model that involves DNA oxidation mediated by Myc recruitment of the histone H3 demethylase LSD1, as a necessary and early event in initiation of transcription of Myc targets. Based on our working model we suggest that Myc possesses the intrinsic capability to induce DNA oxidation at its target sites, and in condition of overepressed Myc levels, may at least in part, contribute to Myc-induced DDR signaling. We will present data of the DDR associated with oncogenic activation of Myc, with special focus on Myc induced DNA damage directly associated to Myc-induced transcription.

5.2. *Analysis of the physical organization and of the CENP binding ability of horse centromeric satellite DNA families*

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Centromeres are the sites of kinetochore assembly and spindle fibre attachment and consist of protein-DNA complexes in which the DNA component is characterized by the presence of extended arrays of highly variable and rapidly evolving tandem repeats. We isolated, from the horse genome, three centromeric satellite DNA families (37cen, 2PI and 137sat). Here we report on the physical organization of these DNA sequences as studied by three colour FISH on mechanically stretched metaphase chromosomes and high resolution three colour FISH on "combed" chromatin fibres. The overall organization of the different classes of centromeric horse satellite DNA appears to be a mosaic where the three DNA families display a strictly intermingled association of sequence blocks widely variable in size, the 37cen satellite being the most abundant and the most widely distributed. Such an organizational pattern might be common in genomes, such as the horse one, characterized by a high rate of interchromosomal exchange. Further, the interaction among centromeric satellites and centromeric antigens has been investigated by ChIP-seq using a CREST serum and immuno-FISH, with anti CENP-A and anti CENP-B antibodies, on extended chromatin fibres. The results suggest that, in the horse genome, 37cen plays a central role in centromere function, the other satellite DNA sequences being accessory DNA elements, presumably contributing to the organization of pericentromeric chromatin, by interacting with 37cen.

5.3. Global DNA hypomethylation following 5-aza-2'-deoxycytidine treatment induces aneuploidy in HCT-116 tumor cells

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Aneuploidy, the alteration of the normal number of chromosomes, is found in most of the human solid tumors and correlated with to defects in the process of chromosome segregation (*1*). It was also suggested that the alteration of the 5-methylcytosine (5-mC) pattern in the chromosome pericentromeric region, generated to aneuploid cells (*2, 3*). To investigate the relationship between hypomethylation and whole chromosome aneuploidy, we treated HCT-116 cells, a near diploid line, with the demethylating agent 5-aza-2'-deoxycytidine (DAC). The treatment with DAC for 24, 48 and 72 hours produced a progressive reduction of DNA methylation as shown by decrease of 5-mC signal. DNA hypomethylation resulted in a strong change of 5-mC distribution pattern in metaphase chromosomes that was associated with several chromosome abnormalities, such as: highly hypocondensed chromosomes, "rail-road track" chromosomes and endoreduplication. Live imaging experiments of HCT-116 cells expressing H2B-GFP and DAC treated, showed misaligned and lagging chromosomes, micronuclei, and elongation of metaphase-anaphase transition. All together these results provide further strong evidence of the correlation between DNA hypomethylation and aneuploidy in human somatic cells.

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5.4. Histone amount, genome stability, gene expression and aging in NHP6 mutants of *S. cerevisiae*

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HMGB-box proteins are found in most of the eukaryotes and are involved in genome stability, gene expression and, particularly in mammals, in the inflammatory response. In *S. cerevisiae*, Nhp6 A and B (homologue to mammalian HMGB1) encode highly homologous proteins involved in modulation of chromatin structure binding DNA in a non-sequence-specific manner. Despite the high evolutionary divergence, several HMGB1 mutants phenotypes are shown also in yeast. Both yeast and mammal cells lacking Nhp6 proteins or HMGB1 respectively, exhibit chromosomal instability and hypersensitivity to DNA damaging agents. Nhp6 mutation shortens life span and has been associated with high levels of Extrachromosomal rDNA Circles.
Nucleosomes restrict DNA accessibility both to damage and to transcription and their number in cells was considered fixed. Recently aging yeast and mammalian cells were shown to contain fewer nucleosomes. We demonstrated that mammalian cells lacking HMGB1p contain a reduced amount of histones and consequently reduced nucleosomes, as well as yeast *nhp6* mutants, possibly because HMGB1 facilitates nucleosome assembly. In yeast *nhp6* cells, the loss of nucleosome particles affects nucleosomal occupancy, being it non-uniform along the yeast genome. Thus, different nucleosomal sites compete for available histones. Variation in nucleosome number, by affecting nucleosomal occupancy both genomewide and gene-specifically, constitutes a novel layer of epigenetic regulation.

5.5. Chromosomal mapping reveals a dynamic organization of the histone genes in aphids (Hemiptera: Aphididae)

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Despite their involvement in different processes, histone genes have been analysed in few insects. In order to improve the knowledge about this important gene family, genes coding for histones have been analysed in the aphid *Acyrthosiphon pisum* showing that at the amino acid level, aphid histones are highly conserved. In particular, data from *A. pisum* confirm that H1 is the most variable of the five histones, whereas histones H3 and H4 are highly conserved with the H3 almost identical from insects to vertebrates. *A. pisum* histone genes are organized in a quintet with the H1 gene followed by H2A and H2B genes that are adjacent and transcribed in same directions, in the opposite strand in respect to the H1 gene. At the 3' end of the histone cluster, genes H3 and H4 constitute an oppositely transcribed pair. The span of the aphid histone genes (more than 7 kb) is greater than the average length of the histone cluster till now reported in insects (about 5 kb). Furthermore, spacers that separate the aphid histone genes vary in length. The histone genes have been mapped in *A. pisum* and successively in the aphids *Myzus persicae* and *Rhopalosiphum padi* showing that they are present in a single large cluster located in an interstitial position of autosomes 1, differently from what reported in the Russian wheat aphid *Diuraphis noxia,* where histone genes have been localized in a telomere of the two X chromosomes suggesting a dynamic organization of this multigene family in aphids.

5.6. Cytological maps of heterochromatin of sequenced *Drosophila* species

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The genome of 12 Drosophila species have been recently sequenced by whole-genome shotgun sequencing. These species span from closely related pairs to distantly related species of the *Drosophila* and *Sophophora* subgenera.
In *Drosophila melanogaster*, a cytogenetic map of contigs along heterochromatin has been already elaborated by "Fluorescence *in situ* hybridization" (FISH) on mitotic chromosomes and SuUR polytene chromosomes.
To localize the contigs along the heterochromatin of the other sequenced species, we performed a cytological analysis of heterochromatin by banding tecniques. By this approach, we elaborated cytological maps as we have previously done in Drosophila melanogaster.

5.7. The analysis of *pendolino* reveal unexpected differences between euchromatic and heterochromatic *Drosophila* telomeres

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Drosophila telomeres are sequence-independent structures that are maintained by transposition to chromosome ends of specialized retroelements rather than telomerase activity. Fly telomeres are capped by the terminin complex that localizes and function exclusively at telomeres and by a number of non-terminin proteins that do not serve telomere-specific functions. *pendolino (peo),* encodes a non-terminin protein homologous to the E2 variant ubiquitin-conjugating enzymes. Null *peo* mutants exhibit ~ 5 telomeric fusions (TFs) per cell. We have recently identified a viable hypomorphic allele of *peo* (peo^h) that causes ~ 1 TF/cell; 99% of the TFs observed in this allele involve the heterochromatic telomeres (the Y, XR and 4th chromosome telomeres), a TF pattern never observed in the telomere capping mutants so far characterized, where all telomeres fuse with comparable frequencies. This suggests that heterochromatic telomeres are preferentially affected by Peo reduction and that this effect is masked in null mutants in which most telomeres are fused. The preferential fusion of the heterochromatic telomeres in *peo* mutants is likely to reflect a defect in late DNA replication, as *peo* mutants are defective in PCNA recruitment. We also found that peo^h and *Su(var)3-9* double mutants exhibit a strong increase in the TF frequency compared to peo^h single mutants, implicating for the first time the Su(var)3-9 histone H3-K9 methyltransferase in *Drosophila* telomere maintenance.

5.8. The epigenetic role of Bucentaur (BCNT) protein family

Giovanni Messina[1,2], Elisabetta Damia[1,2], Laura Fanti[1], Francesca Romana Mariotti[1,2], Emanuele Celauro[1,2], Maria Carmela Accardo[1,2], Matthias Walther[3] , Fiammetta Vernì[1], Maria Teresa Atterrato[1,2], Daria Picchioni[1,2], Roberta Moschetti[4], Ruggiero Caizzi[4], Ana Losada[5], Lucia Piacentini[1], Giovanni Cenci[1,6] Ennio Giordano[7], and Patrizio Dimitri[1,2]

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Drosophila YETI and human craniofacial development protein 1 (CFDP1) belong to the evolutionarily conserved family of BCNT proteins, whose function remains largely unknown. To investigate the biological role of these proteins, we have performed an in-depth functional analysis by combining cytogenetic, molecular and biochemical approaches. We report that the loss of YETI function result in lethality before pupation and dramatic defects in higher order chromosome structure. These defects are associated with reduced amounts of histone variant H2Av, nucleosomal histones, epigenetics marks such as HP1 and decreased levels of gene expression. We also observed that YETI-GFP fusion protein physically interacts with histone H2Av, Domino and HP1. Based on these findings, we propose that YETI is a new subunits of *Drosophila* DOM/Tip-60 chromatin remodeling complex and plays a role in the epigenetic regulation of gene expression by promoting H2Av exchange and HP1 recruitment at chromatin remodeling sites. Similarly, we found that CFDP1 is an essential nuclear proteins whose depletion leads to severe defects in higher-order chromatin structure and plays a role in chromatin organization via interactions with members of human SRCAP chromatin remodeling complex and with HP1. The results of this work provide evidence in favour of functional conservation of YETI and CFDP1 proteins, in that they are crucial epigenetic regulators essential for chromatin organization and remodeling in *Drosophila* and human cells.

5.9. Hsp90 and its interacting partners in the piRNAs pathway

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Hsp90 is a molecular chaperone stabilizing many key regulatory proteins. Recently it was shown that the functional alteration of Hsp90 causes activation of transposons in Drosophila germ cells due to alterations in the piRNA pathway. This disfunction results in the induction of a series of phenotypic variants. Therefore Hsp90 works as suppressor of variability that can be generated by the movement of transposons.
Preliminary experiments show that the "heat shock" treatment activates the movement of transposons in Drosophila thus suggesting that stress may trigger a functional shift of Hsp90.
To address this point, we performed experiments whose results strongly suggest that a functional shift of Hsp90 induced by stress could be related to the involvement of Hsp90 in complexes that are different in normal and stress conditions. In other words, Hsp90 functions in piRNA pathway in absence of stress, but under stress conditions, its role changes by its interaction with different factors.
A further result obtained in this work is the involvement of GW182 in piRNA pathway. GW182 interact with Hsp90 and localizes in nuage where piRNA biogenesis occurs. In addition, Hsp90 disfunction causes GW182 and Vasa delocalization from nuage. Therefore we can speculate that, in piRNA pathway, the functions of these three proteins are interconnected.

5.10 Environmental stress, transposons and 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.

5.11. AKTIP, an E2 variant enzyme that interacts with lamin and protects mammalian telomeres from replicative damage

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Growing evidence indicates that mammalian G-rich telomeric DNA is a difficult substrate for the DNA replication machinery. Previous work has shown that the shelterin telomere protection complex recruits BLM, RTLE1 and topoisomerase to facilitate unwinding of the secondary structures formed by the TTAGGG repeats. Shelterin also recruits the CST complex that promotes restart of stalled replication forks. We report here that AKTIP/Ft1, an ubiquitin E2 variant enzyme, is required for mammalian telomere replication. AKTIP/Ft1 was identified on the basis of its homology with the *Drosophila* telomere-capping protein Pendolino. AKTIP is enriched at the nuclear periphery and interacts with A/C and B1 lamins. In addition, AKTIP interacts with the TRF1 and TRF2 shelterin components and with the DNA replication factors PCNA and RPA70. Loss of AKTIP/Ft1 impairs DNA synthesis and results in fragile telomeres and sister telomere associations. Our results suggest that AKTIP/Ft1 functions in lamin-associated replication factories to counteract replication fork stalling. We propose that when the replication fork is challenged by telomeric DNA, AKTIP is recruited at telomeres to allow fork progression, possibly through PCNA ubiquitinylation.

5.12. BIT chromosome translocation induces *PDR*-dependent anticancer drug resistance in yeast.

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Until recently, it was not clear whether chromosome translocations were the cause or the consequence of certain neoplastic transformations. We have previously developed a system named BIT (Bridge-induced chromosome translocation) to reproduce this major genomic rearrangement in the model eukaryote *Saccharomyces cerevisiae*. Besides affecting DNA replication and repair, cytokinesis, cell cycle, karyogamy, and producing chromosomal instability, this event can lead to an increased resistance to anticancer chemicals like Doxorubicin and to actin de-polymerizing molecules like Latrunculin A via an endocytic actin network deregulation triggered by over-expression of the *PRK1* serine/threonine protein kinase gene. The same effect can be further enhanced by the overexpression of *PDR1* and *PDR3* transcriptional regulators of pleiotropic drug resistance factors. The coupling of Latrunculin A and fungizone enhances the permeabilization to Doxorubicin, killing translocants more efficiently than wild type cells. Thus, the BIT system helps to elucidate the acquired drug resistance in budding yeast, and provides new approaches based on genome stabilization as alternative methods to classical chemotherapeutic treatments.

Sessione Poster

6. **Sviluppo, differenziamento e invecchiamento** (Laura Fanti, Ileana Ferrero)

6.1. Functional effect of a SNP of TAS2R16 region associated to human longevity

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6.2. Methylation of the human promoter ribosomal RNA genes is correlated with aging

Dina Bellizzi, Patrizia D'Aquila, Teresa Scafone, Alberto Montesanto, Giuseppe Passarino
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6.3. The Heterochromatin protein 1 (HP1) is involved in germline stem cells maintenance

Assunta Maria Casale, Sergio Pimpinelli, Laura Fanti and Lucia Piacentini
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6.4. Genetic variability of telomerase associated proteins has a complex correlation with longevity

Costanza M. Cristiani[1], Daniele Campa[2], Giuseppina Rose[1], Cosmeri Rizzato[2], Francesco De Rango[1], Maura Carrai[3], Federica Tallaro[1], Paolina Crocco[1], Alberto Montesanto[1], Federico Canzian[2], Giuseppe Passarino[1], and Roberto Barale[3] Costanza Maria Cristiani <costanzamariacristiani@yahoo.it>

6.5. Multiple roles of the Tgs1 tri-methylguanosine synthetase in *Drosophila melanogaster*

Maria Laura Di Giorgio[1], Paolo Maccallini[1], Azzurra Codino[1], Federica Lecciso[1] and Grazia Daniela Raffa[1]. maria laura di giorgio <marialauradg@gmail.com>

6.6. RAE1 localization pattern correlates with the functional role in Drosophila melanogaster spermatogenesis

Fabiana Fabbretti[1], Silvia Volpi[1], Silvia Bongiorni[2], Barbara T. Wakimoto[3], Giorgio Prantera[1].
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6.7. Notch signaling requires the Abnormal wing discs (Awd) function during *Drosophila* development

Marilena Ignesti[1], Marilena Barraco[1,4], Gouthami Nallamothu[2], Julie A. Woolworth[3], Serena Duchi[1,5], Giuseppe Gargiulo[1], Valeria Cavaliere[1], Tien Hsu[2] Marilena Ignesti <marilena.ignesti@unibo.it>

6.8.Mitochondria and lifespan extension in *Saccharomyces cerevisiae:* the longevity mutations *sch9*Δ and *rei1*Δ contribute to mitochondrial DNA stability

Jade Quartararo[1], Ileana Ferrero[1]. jade.quartararo@unipr.it

6.9. Effect of tumour suppressor mutations and hypoxia exposure on Abnormal wing discs (Awd) expression during *Drosophila* larval development

Patrizia Romani[1], Antonio Strillacci[2], Tien Hsu[3], Enzo Spisni[2], Giuseppe Gargiulo[1] and Valeria Cavaliere[1]
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6.10. An in vivo study of signaling pathways involved in zebrafish thyroid formation.

Natascia Tiso[1], Federica Marelli[2], Patrizia Porazzi[2], Giulia Melato[1], Olivier Ek[1], Giulia Marchetto[1], Andrea Vettori[1], Nicola Facchinello[1], Marco Schiavone[1], Alessandro Casari[1], Matteo Astone[1], Francesca Benato[1], Elisa Colletti[1], Martina Milanetto[1], Enrico Moro[1], Francesco Argenton[1] and Luca Persani[2] *natascia.tiso@unipd.it*

6.11. The impact of ANK proteins on host-parasitoid interactions

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6.1. Functional effect of a SNP of TAS2R16 region associated to human longevity

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We have recently shown that a single nucleotide polymorphism (SNP), rs 978739 (A/A), which maps 212 b.p. upstream the coding region of the TAS2R16 gene, is associated with an extension of longevity in a population of elderly. We have cloned the promoter region of the gene TAS2R16, including rs 978739, (A/A) in the vector pGL3, upstream the luciferase gene. Two cell lines, SAS and HCT116, were co-transfected either with the pGL3 plasmid bearing alternatively the two allelic variants (A/A and G/G), and with the PRL-TK vector carrying the gene of renilla. In both cell lines it was observed an increased basal expression of the luciferase gene when the variant A/A was present, compared to the variant G/G. SAS transfected cells were also treated with salicin which is an effective ligand of the receptor encoded by the gene TAS2R16. Treatments with salicin 0.2 mM for 10 hours induced a greater expression of luciferase, suggesting that the salicin is able to induce the expression of its own receptor. In conclusion we have shown a functional role of this SNP, in the region upstream of the gene coding for the TAS2R16 receptor, that is associated with a greater extension of human longevity.

6.2. Methylation of the human promoter ribosomal RNA genes is correlated with aging

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The transcription of the ribosomal RNA (rRNA) genes is a control point highly regulated in ribosome biogenesis. Several evidence demonstrated as rDNA expression is subject to epigenetic regulation. *In vitro* data demonstrated that in cultured mouse cells, rDNA transcription is abrogated by methylation. Moreover, the senescence of fibroblasts is accompanied by a significant increase in cytosine methylation within rDNA genes. Significant hypomethylation of the rDNA promoter was also observed in human hepatocellular carcinoma samples with respect to matching normal tissues, with consistent high level of rRNA synthesis. No data are yet available regarding a possible epigenetic regulation of rRNA genes along the lifetime.

This study was aimed at investigating the aging related variability of methylation within the promoter region of the rRNA genes by Sequenom EpiTYPER assay in samples collected from 65-101 years old human subjects. We found that methylation levels are significantly higher in very old subjects (90+) with respect to the 65-89 years old subjects.

To our knowledge, this represents the first evidence about the correlation of the methylation of the rRNA promoter region with aging. As the functional decline of elderly people has been strongly associated to the progressive age-decline of the total protein synthesis rate, our observations suggest that the epigenetic regulation of rRNA genes may represents a key point in this decline and, in turn, in the aging process.

6.3. The Heterochromatin protein 1 (HP1) is involved in germline stem cells maintenance

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HP1 (Heterochromatin Protein 1) is a nonhistone 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 and testis, we have performed studies to test if this abundance could be related to its involvement in germ stem cell (GSC) regulation.
We will presents the results of our experiments showing that HP1 is involved in GSC maintainance and transposon silencing.

6.4. Genetic variability of telomerase associated proteins has a complex correlation with longevity

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Telomere length (TL) has been observed to be highly correlated with longevity. To verify whether the variability of genes correlated to telomere maintenance affects longevity and was associated with telomere length, we studied in a population from Southern Italy (age range 20 - 108 years) thirty-two polymorphisms in nine telomerase associated genes of which twelve in the genes coding for the core enzyme (*TERT* and *TERC*) and the remaining in genes coding for components of the telomerase complex (*TERF1*, *TERF2*, *TERF2IP, TNKS*, *TNKS2*, *TEP1* and *KARS)*.

We did not observe any statistically significant association between SNPs and TL (after correcting for multiple testing). Thus, the effect of variability of these genes either is negligible with respect to the environmental factors, or such an effect is negligible with respect to the variability of the original TL. On the other hand, we found that the variability of genes encoding for TERF1 and TNKS2 shows a significant association with human longevity. This suggests that the maintenance of these chromosomal structures is critically important for preventing, or delaying, senescence and aging.

On the whole, our results suggest that the variability of the genes coding for proteins involved in protecting the integrity of telomere structures, rather than the variability of those directly involved in telomere elongation, is correlated with longevity.

6.5. Multiple roles of the Tgs1 tri-methylguanosine synthetase in *Drosophila melanogaster*

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Drosophila Tgs1 (dTgs1, also known as Drosophila-Tat-like), is the *Drosophila* homologue of the conserved TGS1 hypermethylase.
TGS1 adds a trimethylguanosine cap (TMG cap) to several noncoding RNAs including the snRNAs, the telomerase RNA subunit, and viral RNAs, favouring their compartmentalization in their district of function.
dTgs1 is encoded by a bicistronic locus which also encodes Moi, one component of terminin, the *Drosophila* telomere capping complex. We have found that, besides being cotranscribed on the same mRNA, Moi and dTgs1 physically interact. Although dTgs1 is not required for telomere protection, we have found that it regulates the transcript levels of the *Drosophila* telomere-specific retrotransposons HeT-A and TART.
In vertebrates, TGS1 is enriched at the Cajal body (CB) and its catalytic activity is related to the Survival of Motor Neuron (SMN) complex, whose function is essential to prevent development of Spinal Muscular Atrophy (SMA) in humans. We have found that dTgs1 interacts with Smn, and appears to function in the same Smn pathways controlling proper locomotory activity in flies.
Collectively, our data suggest that dTgs1 controls multiple aspects of RNA metabolism, which are crucial for both telomere homeostasis and the control of locomotory activity.
We are currently investigating the role of dTgs1 in telomeres homeostasis, Cajal body stability and its functional relationships with the SMN complex.

6.6. RAE1 localization pattern correlates with the functional role in Drosophila melanogaster spermatogenesis

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RAE1 is a evolutionarily conserved nucleoporin belonging to the WD40 repeat protein family showing a wide variety of functions. Studies in different organisms suggest two major functions of RAE1 in nuclear membrane traffiking and mitotic cell cycle regulation. *rae1* is involved in poly(A)$^+$ mRNA export in yeast (Brown et al., 1995; Murphy et al., 1996) and in humans where hsRae1 is associated with the nucleoporin Nup98 (Pritchard et al.,1999). In mammals, the interaction of RAE1 with BUB1 (Whang et al., 2001) and NUMA (Wong et al., 2006) suggests a role in cell cycle regulation, moreover in Drosophila culture cells the depletion of *rae1* leads to an arrest in G1 phase (Sitterlin 2004). Very recently, we provided the first evidence for a role of RAE1 in Drosophila meiosis and spermatogenesis (Volpi et al., 2013). Here, we show a confocal microscopy analysis of RAE1 localization pattern during wildtype spermatogenesis in *Drosophila melanogaster*. We used a GAL4/UAS system which allows to express a GFP-tagged RAE1 under a costitutive *tubulinGal4* driver. We followed the RAE1 distribution through the whole spermatogenesis, from mitotic stages to mature sperms and found a GFP-RAE1 pattern fully in agreement with its functional role as revealed by the $rae1^{Z5584}$ mutant phenotype. The observed pattern of RAE1 protein can be considered comparable to the wild type because the RAE1-GFP construct is able to rescue the sterility phenotype of a *rae1* omozygous mutant males.

6.7. Notch signaling requires the Abnormal wing discs (Awd) function during *Drosophila* development

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The *Drosophila awd* gene encodes for the homolog of human metastasis suppressor gene *Nm23.* Many different functions have been assigned to Awd, including a role as an endocytic mediator. Endocytosis is a critical event for the correct regulation of different signaling pathways, including Notch signaling. We focused our efforts to investigate *awd* role during Notch receptor-mediated endocytosis. We used the awd^{J2A4} amorphic allele to induce targeted loss of function of *awd* gene using different genetic approaches.

We found that loss of *awd* gene function causes faulty Notch signaling in different *Drosophila* tissues. awd^{J2A4} mutant clones show no expression neither of Notch activation target genes nor of transcriptional reporters. Notch appears to be trapped into enlarged vesicles, unable to signal. Proper regulation of Notch entry into the appropriate endosomal compartments is critical for signaling. Vesicles could be identified by the presence of specific markers, which allow us to characterize different kind of endosomes in the endocytic route. We therefore analyzed different markers of the endocytic pathway in *awd* mutant cells and we found out that Notch accumulates into early endosomes.

Our results indicate that *awd* is required for Notch intracellular trafficking, which is essential for the correct activation of ligand-dependent Notch signaling.

6.8. Mitochondria and lifespan extension in *Saccharomyces cerevisiae:* the longevity mutations *sch9*Δ and *rei1*Δ contribute to mitochondrial DNA stability

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Mutations that reduce the activity of the nutritional signaling pathways TOR/SCH9 and RAS/PKA induce an increase in chronological lifespan (CLS) extension in different model organisms, redirecting cells towards a respiratory metabolism and promoting stress resistance.

To assess the relationship between mitochondrial function and ageing we investigated whether the increased CLS of well-known longevity mutants correlated with an increase in mitochondrial DNA stability. Among the longevity mutations analyzed only two, *sch9Δ* and *rei1Δ*, accumulate deletions on mtDNA at lower rate than the parental strain, suggesting that deletions on mtDNA may have a primary role in ageing and that maintenance of mtDNA is only one of the actors involved in the regulation of the ageing process.

Furthermore s*ch9Δ* and *rei1Δ* longevity mutants show high rate of respiratory activity accompanied by no significant difference in mtDNA amount. Different mechanisms through which the two longevity mutants promote lifespan extension and mtDNA stability have been identified. Deletion of *SCH9* leads to an increase of ROS production early during growth to promote an adaptive signal that stimulates lifespan extension and reduces oxidative damage in stationary cells, activating a stress response program mediated by Sod2p overexpression. Otherwise the reduced rearrangements on mtDNA and the increased respiratory activity in *rei1Δ* longevity mutant appear to rely on a direct stabilization of mitochondrial DNA through overexpression of nucleoid components.

6.9. Effect of tumour suppressor mutations and hypoxia exposure on Abnormal wing discs (Awd) expression during *Drosophila* larval development

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In *Drosophila*, the *abnormal wing discs* (*awd*) gene is required for the normal development of wing, leg and eye-antenna discs. The entire amino acid sequence of Awd is 78% identical to the isoforms of the mammalian metastatic tumour suppressor Nm23-H1/H2. The *awd* lethal phenotype can be rescued by exogenously expressed Nm23-H2.
The proposal of our study is to elucidate Awd/Nm23 function in tumour tissues.
Since Nm23 protein has been found in the serum of tumour cell lines, we extract cell-free hemolymph from third instar larvae and interestingly, we detected the presence of extracellular Awd protein.
We found that in third instar hyperplastic tumour larvae Awd expression is decreased. Interestingly in metastatic tumour larvae Awd expression and secretion is increased.
The data so far obtained indicate that the use of *Drosophila* model could shed light on the mechanism, which correlates the increased level of extracellular Nm23 and metastatic potential in human carcinomas.

6.10. An in vivo study of signaling pathways involved in zebrafish thyroid formation.

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The molecular pathways contributing to thyroid development and function appear to be highly conserved in vertebrates; similar to mammals, the zebrafish thyroid gland is composed of endoderm-derived follicles, filled with colloid and producing thyroid hormones. However, the precise role of different pathways in the several morphogenetic steps, occurring during thyroid development, and their activity in the surrounding tissues remain to be clarified.
In our study we aim to characterize and systematically dissect the role of key signaling pathways, throughout the main steps of zebrafish thyroid formation.
To this purpose, we are taking advantage of a series of zebrafish signaling pathway reporter lines, maintained or specifically generated by our group. In particular, we are focusing our attention on the following signaling cascades: Bmp, Shh, Fgf, Wnt, Notch, cAMP, Hypoxia, Stat3, Hippo, Glucocorticoid. These transgenic lines, expressing green (GFP) or red (mCherry) fluorescent proteins in response to specific signaling activation, are currently in *vivo* monitored from the early steps of thyroid primordium induction and budding until the final stages of migration, follicular polarization and hormone production. Relevant pathways, activated nearby or within the thyroid region, will be subjected to further validation using pathway-specific agonists/antagonists or mutant/morphant conditions, in order to precisely dissect the role of a given signal on thyroid formation and function.

6.11. The impact of ANK proteins on host-parasitoid interactions

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Parasitic wasps during oviposition disrupt the host immune reaction and endocrine balance in order to create a suitable environment for the development of their progeny. This is mediated by female reproductive secretions, injected along with the egg, which include venom, ovarian secretions and, in certain parasitoids of moth larvae, symbiotic viruses of the family Polydnaviridae (PDV). Analyses of PDV genomes revealed a conserved gene family encoding proteins characterized by ankyrin repeats. These proteins (ANKs) are similar to insect and mammalian IκB, but the lack of regulatory domains for signal-mediated degradation and turnover confers them an immunosuppressive activity.

To gain insights on the role played by the ANK proteins we used *Drosophila* as a model system. We analyzed the function of the *Tn*BVANK1 protein, coded by the PDV associated with the wasp *Toxoneuron nigriceps* (*Tn*BV), which parasitizes the larvae of the tobacco budworm, *Heliothis virescens*. The expression of *TnBVank1* gene in the *Drosophila* prothoracic gland, which synthesizes the ecdysone, deeply affects the physiology of this gland by altering its endocytic pathway. This results in pupation failure, which is similarly observed in *H. virescens* larvae parasitized by *T. nigriceps*. Interestingly, we found that another member of the *Tn*BVANK protein family, *Tn*BVANK3, also causes a developmental arrest of *Drosophila* larvae. The functions of this protein are currently being characterized.