List of available projects 2022 - XXXVIII cycle

PhD program in Genetics and Molecular Biology, Sapienza Università di Roma

PhD Program in Genetics and Molecular Biology (XXXVIII cycle)

Title: Studying the role of long non-coding RNA in neuromuscular physiology and disease

Proponent: Monica Ballarino
Affiliation: Department of Biology and Biotechnology "Charles Darwin", Sapienza University of Rome

Tissue-specific long noncoding RNAs (lncRNA) play essential functions in the regulation of cell growth, differentiation and physiology. Their dysregulation was often associated with neuromuscular diseases, although the knowledge concerning their mechanisms of action is still far from complete. By using a high-throughput transcriptome screening, our group identified several lncRNAs that are expressed in muscle or motor neurons and conserved in human. On these premises, we plan to apply an experimental design where the synergy between CRISPR-Cas9 gene editing and induced pluripotent stem cells technology will allow to study the effect of their ablation on the function of neuromuscular junctions (NMJ), through the generation of 2D (skeletal muscle-motor neuron co-culture) or isogenic 3D (neuromuscular organoids) KO neuro-muscular organoids (NMOs), in which the spinal cord and the skeletal muscle counterparts functionally interact. This study will provide a suitable platform to track the developmental contribution of the human lncRNAs to neuromuscular disorders, particularly those in which the formation of a functional NMJ is early impaired.

References

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Desideri F, Cipriano A, Petrezselyova S, Buonaiuto G, Santini T, Kasparek P, Prochazka J, Janson G, Paiardini A, Calicchio A, Colantoni A, Sedlacek R, Bozzoni I, **Ballarino M.** "Intronic Determinants Coordinate Charme lncRNA Nuclear Activity through the Interaction with MATR3 and PTBP1". Cell Rep. 2020; 33:108548.

Ballarino M, Cipriano A, Tita R, et al. Deficiency in the nuclear long noncoding RNA Charme causes myogenic defects and heart remodeling in mice. EMBOJ. 2018;37:e99697.

PhD Program in Genetics and Molecular Biology (XXXVIII cycle)

Title: Mapping the histone variant H3.3 at human telomeres
Proponent: Stefano Cacchione
Affiliation: Department of Biology and Biotechnology "Charles Darwin", Sapienza University of Rome

Abstract:
In humans, telomeres play a pivotal role in several regulatory pathways that determine the cell fate. At birth, human telomeres are 10–15 kb long, consisting of thousands of TTAGGG repeats organized in a unique and compact chromatin and bound by the six-protein complex, shelterin. The enzyme telomerase maintains telomere length in germinal and embryonic stem cells but is inactive in somatic cells. Consequently, telomeres shorten at each replication cycle till they reach a critical length that triggers a DNA damage response (DDR) pathway leading to permanent cell cycle arrest. To proliferate indefinitely, cancer cells must acquire a telomere maintenance mechanism, in most cases by reactivating telomerase, in 10–15% tumors developing an alternative mechanism named ALT, based on homologous recombination. About 80% of telomeric DNA is organized in nucleosomes characterized by an unusually short spacing and enriched in the histone variant H3.3, deposited in a replication-independent way by the complex ATRX/DAXX. Strikingly, dominant mutations in H3.3 are frequent in ALT pediatric cancers, often in combination with loss of ATRX. Structural and functional features of telomeric chromatin, as well as the role of H3.3 at telomeres are still largely unknown. Telomere heterogeneous length (about 2-10 kbp in humans) coupled with the uniformly repeated sequence renders hard to establish whether the telomere has a regular structural organization along its overall length and how its structure changes when telomeres shorten and uncap. To go deeper in telomeric chromatin organization, new tools and strategies are needed. This project, combining specific chemical cleavage and single molecule sequencing of long telomeric fragments by Oxford Nanopores, aims to obtain a more detailed map of nucleosome organization at human telomeres, particularly of H3.3 nucleosomes. Furthermore, the development of these techniques could allow studying other relevant issues in telomere biology, such as replication fork stalling or DNA damage double-stranded breaks.

References

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- Micheli E, Galati A, Cicconi A, Cacchione S (2017) Telomere maintenance in the dynamic nuclear architecture. Chromatin Regulation and Dynamics (Chapter 13). Anita Göndör, ed., Elsevier publ., pp. 325-352.
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- Galati A, Scatolini L, Micheli L, Bavasso F, Cicconi A, Maccallini P, Chen L, Roake CM, Schoeftner S, Artandi SE, Gatti M, Cacchione S, Raffa GD (2022) The S-adenosylmethionine analog sinefungin inhibits the trimethylguanosine synthase TGS1 to promote telomerase activity and telomere lengthening. FEBS Letters 596: 42-52.

PhD Program in Genetics and Molecular Biology (XXXVIII cycle)

Title: Role of the polyamine-EIF5A-autophagy axis in Myotonic Dystrophy 2 pathogenesis
Proponent: LAURA CIAPPONI
Affiliation: Department Biology and Biotechnologies C. Darwin /Genetics

Abstract of the project: Myotonic Dystrophy 2 (DM2) is a genetic disease primarily affecting skeletal muscle, caused by a mutation of the CNBP/ZNF9 gene. To date the molecular mechanisms underlying DM2 are not understood, and a resolutive therapy is currently missing. In a previous study we have discovered that reduction of the CNBP gene product in the muscle affects locomotor function by causing a decrease of polyamines, small molecules that are critical for muscle cell survival. The working hypothesis is that mutation of the *CNBP* gene in DM2 causes reduced ODC biosynthesis and polyamine depletion in muscle cells, which leads to decreased EIF5A hypusination and reduction of key translational targets involved in autophagy, thus causing locomotor defects. Using newly generated and some already available models, we plan to elucidate how CNBP reduction impairs the autophagic flux and to what extent this mechanism contributes to muscle dysfunction.

References

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2. Antonucci L., D'Amico D, Di Magno L., Coni S., Di Marcotullio L., Cardinali B., Gulino A., Ciapponi L. and Canettieri L. (2013) CNBP regulates wing development in *Drosophila melanogaster* by promoting IRES-dependent translation of dMyc. *Cell Cycle*. 13:434-439. doi: 10.4161/cc.27268.

PhD Program in Genetics and Molecular Biology (XXXVIII cycle)

Title: Genetic population history of Sahelian pastoralists
Proponent: Fulvio Cruciani
Affiliation: Department of Biology and Biotechnology "Charles Darwin"

Abstract of the project:
The Sahel, i.e. the belt separating the Sahara desert from tropical forests further south, is characterized by an intermediate environment mainly composed of savannah and grasslands. The northern part of the Sahelian belt shows a semi-arid climate, where no extensive agriculture is possible, while the more humid southern area allows agricultural practices. However, pastoralist groups can exploit the pastures in the northern Sahel and then move southwards during the driest months. So, the Sahel represents a natural contact area between the pastoralist and agricultural lifestyle, represented by groups speaking languages from three out of four African linguistic families, namely Afro-Asiatic, Nilo-Saharan and Niger-Congo [1]. In this project, we will perform the next generation sequencing of several whole genomes from Sahelian pastoralism groups in order to shed light on their past history, based on preliminary data from the Y chromosome variability [2,3]. The large number of genetic variants that we will be able to identify will be compared with selected whole genome sequences available from the literature and used to reconstruct the population structure and admixture events of the selected groups with neighboring people. Then, the data will be used to analyze the adaptation history of the pastoralist populations of Africa, considering their particular lifestyle and the peculiar environment of the Sahelian belt. To this aim, both the coding and non-coding portion of the genome will be considered in order to identify possible targets of selection, using the most updated bioinformatic tools and approaches [4].

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[3] Della Rocca C, et al. (2020) Ethnic fragmentation and degree of urbanization strongly affect the discrimination power of Y-STR haplotypes in central Sahel. Forensic Sci Int Genet. 49:102374.
[4] Choudhury A, et al. (2020) High-depth African genomes inform human migration and health. Nature. 586:741–748

PhD Program in Genetics and Molecular Biology (XXXVIII cycle)

Title: The epigenetic control of seed germination: how to improve the germination potential of edible Brassicaceae plants

Proponent: Raffaele Dello Ioio **Tutor:** Paola Vittorioso
Affiliation: Department Biology and Biotechnology "Charles Darwin"

Abstract of the project:

Seed germination is the first developmental program in plants, and as such the variability of this trait in crops has a great impact on seedling establishment and crop yield. We are currently chracterising the molecular network underlying this process in new Brassicaceae species suitable for human consumption. Preliminary data revealed that these species show a high germination potential even under adverse environmental conditions. Since epigenetic strictly controls this process in the model plant Arabidopsis thaliana (Bouyer et al., 2011; Molitor et al., 2014; Gu et al., 2019), it is conceivable that it plays a key role in the germination behaviour of these species.

The field of drug discovery has been widely used in animal systems, while in plants it is only recently becoming popular. Although chemical inhibition of epigenetic markers provides an effective approach to study and influence epigenetic modifications, so far it has not been widely exploited. We have previously assessed the efficacy and selectivity of an inhibitor of the human EZH2 subunit of PRC2, and of two p300/CBP inhibitors on Arabidopsis seeds and seedlings (Ruta et al., 2019; Longo et al., in preparation).

Aim of this project is to reveal if and how epigenetic control impacts on the seed germination behaviour of Brassicaceae species characterised by a high germination potential irrespective of environmental conditions. To this end, we will use a chemical genetics approach, unfolding the possibility of identifying a range of molecules that may be useful for both basic research and agronomic applications.

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Bouyer D, Roudier F, Heese M, Andersen ED, Gey D, Nowack MK, Goodrich J, Renou JP, Grini PE, Colot V, Schnittger A. (2011)Polycomb repressive complex 2 controls the embryo-to-seedling phase transition. PLoS Genet. (3):e1002014.

Molitor AM, Bu Z, Yu Y, Shen WH. (2014) Arabidopsis AL PHD-PRC1 complexes promote seed germination through H3K4me3-to-H3K27me3 chromatin state switch in repression of seed developmental genes. PLoS Genet. 10(1):e1004091.

Gu D, Ji R, He C, Peng T, Zhang M, Duan J, Xiong C, Liu X. (2019) Arabidopsis Histone Methyltransferase SUVH5 Is a Positive Regulator of Light-Mediated Seed Germination. Front Plant Sci. 10:841.

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PhD Program in Genetics and Molecular Biology (XXXVIII cycle)

Title: Moonlighting roles of chromatin-remodeling proteins in cell division.
Proponent: Patrizio Dimitri
Affiliation: Department of Biology and Biotechnology "Charles Darwin", Sapienza University of Rome

Abstract:
Chromatin remodeling complexes are specialized multi-protein machines that modulate chromatin organization, with relevant impact on regulation of transcription, DNA replication and DNA repair. We have recently found that subunits of evolutionary related chromatin remodeling complexes perform functions beyond chromatin regulation. In fact, they are massively recruited to the mitotic apparatus and participate to the control of spindle and midbody function during cell cycle progression.
This project aims at gaining mechanistic insights of the roles played by subunits of SRCAP and p400/Tip60 chromatin remodeling complexes in ensuring a faithful cell division in human and Drosophila cells. We will use an interdisciplinary approach that combines cellular and molecular biology, gene editing, targeted protein degradation systems, proteomics and time-lapse analyses.

The project consists of the following experiments:
1. Generation of fusion constructs to study the localization of corresponding tagged proteins to the mitotic apparatus in RPE-1 cells by time-lapse.
2. Generation and expression of the fusion constructs of the protein of interest, to induce the rapid degradation of the corresponding tagged proteins and dissecting in detail their role in the different phases of the cell cycle.
3. Characterize the interaction networks of BAF53a and GAS41 in cell division by Mass spectrometry.

Selected publications related to the project
1) Messina G., Prozzillo M.T., Delle Monache F., Santopietro M.V. and P. Dimitri (2022) Unconventional roles of SRCAP and P400/Tip60 chromatin remodeling complexes in cell division: a new class of moonlighting proteins preventing genetic instability. BMC Biology, In press.
2) Messina G., Prozzillo Y., Atterrato M.T., Delle Monache F., Santopietro M.V. and P. Dimitri (2021) ATPase SRCAP is a new player in cell division, uncovering molecular aspects of Floating-Harbor syndrome. BMC Biology, 19(1):184. doi: 10.1186/s12915-021-01109-x.
3) Prozzillo Y., Cuticone S., Ferreri D. M., Fattorini G., Messina G. and P. Dimitri (2021) In vivo silencing of genes coding for dTip60 chromatin remodeling complex subunits affects polytene chromosome organization and proper development in *Drosophila melanogaster*. International Journal of Molecular Science. 22:4525. doi: 10.3390/ijms22094525.
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9) Messina G., M. T. Atterrato, L. Fanti, E. Giordano and P. Dimitri (2016) Expression of human
Cfdp1 gene in *Drosophila* reveals new insights into the function of the evolutionarily conserved BCNT protein family. Scientific Reports. 6:25511. doi: 10.1038/srep25511.
10) Yokoyama H: Chromatin-Binding Proteins Moonlight as Mitotic Microtubule Regulators. Trends in cell biology 2016, 26(3):161-164.
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PhD Program in Genetics and Molecular Biology (XXXVIII cycle)

Title: Chromosome instability and Neocentromere formation in *D.melanogaster*

Proponent: Laura Fanti
Affiliation: Department of Biology and Biotechnology "Charles Darwin", Sapienza University of Rome

Abstract:
Centromeres are epigenetically determined chromatin structures that specify the assembly site of the kinetochore, the multiprotein machinery that binds microtubules and mediates chromosome segregation during mitosis and meiosis. The centromeric protein A (CENP-A) and its Drosophila orthologue centromere identifier (Cid) are H3 histone variants that replace the canonical H3 histone in centromeric nucleosomes of eukaryotes. CENP-A/Cid is required for recruitment of other centromere and kinetochore proteins and its deficiency disrupts chromosome segregation.
The centromere position can shift in response to altered chromosome structures, but how and why neocentromeres appear in a given chromosome region are intriguing and still open questions. Models of neocentromere formation have been developed during the last few years.
The PhD project aims to study how a neocentromere is formed following a condition of induced stress in *D. melanogaster*.

Selected publications:

Fanti L., B. Perrini, L. Piacentini, M. Berloco, G. E. Marchetti, G. Palumbo and S. Pimpinelli. 2008. The trithorax-Group proteins are involved in heterochromatin formation in Drosophila. Chromosoma. 117: 25-39

Piacentini L, Marchetti M, Bucciarelli E, Casale AM, Cappucci U, Bonifazi P, Renda F and Fanti L. (2019) A role of the Trx-G complex in Cid/CENP-A deposition at *Drosophila melanogaster* centromeres. Chromosoma https://doi.org/10.1007/s00412-019-00711-x

Leo L., Marchetti M., Giunta S. and L. Fanti . 2020. Epigenetics as an evolutionary tool for centromere flexibility. *Genes*, 11, 809. doi:10.3390/genes11070809

PhD Course in Genetics and Molecular Biology (XXXVIII cycle)

Title: Transposable elements: major players in shaping genomic and evolutionary patterns

Proponent: Laura Fanti
Affiliation: Department of Biology and Biotechnology "Charles Darwin", Sapienza University of Rome.

Abstract:
The ability to react to environmental changes is essential for survival and reproduction. Therefore, organisms develop mechanisms to sense external changes and activate appropriate cellular responses. Among the genes involved in the stress response are the heat-shock coding genes. Heatshock proteins protect genomes subject to environmental changes by helping other proteins maintain the right conformation and avoid denaturation. One of the stress proteins, HSP90, affects the silencing pathway mediated by Piwi-interacting RNA, causing the activation of transposable elements and consequently the induction of morphological mutants through an insertional mutagenesis. In addition, flies exposed to heat-shock during the pupal stage frequently show an extensive range of morphological abnormalities, some induced by transposon insertions. Recent results show that other types of biotic and abiotic stressors such as chemicals, biological clock dysfunction and aging cause the activation of transposable elements. For these reasons, transposons are considered tools of evolutionary processes. This project proposes to investigate two important issues: 1) the dysfunction of circadian rhythms as an inducer of the TE-mediated stress response and its relationship with aging; 2) the evolvability of the TE-mediated stress response in different Drosophila populations with a different adaptation to heat and cold.

Selected publications:

Specchia V., L. Piacentini, P. Tritto, L. Fanti, R. D'Alessandro, G. Palumbo, S. Pimpinelli and MP. Bozzetti. 2010. HSP90 prevents phenotypic variation by suppressing the mutagenic activity of transposons. Nature. doi: 10.1038/nature08739. Epub 2010

Bozzetti M.P., Fanti L., Di Tommaso S., Piacentini L., Berloco M., Tritto P., Specchia V. 2012. The "Special" crystal-Stellate System in Drosophila melanogaster Reveals Mechanisms Underlying piRNA Pathway-Mediated Canalization. Genet Res Int. Vol. 2012 doi: 10.1155/2012/324293

Piacentini L**,** Fanti L, Specchia V, Bozzetti MP, Berloco M, Palumbo G and Pimpinelli S. 2014. Transposons, environmental changes, and heritable induced phenotypic variability. Chromosoma 123: 345-54

Fanti L, Piacentini L, Cappucci U, Casale AM, Pimpinelli S. 2017. Canalization by selection of de novo-induced mutations. Genetics 2017 pii: genetics.117.201079. doi: 10.1534/genetics.117.201079

Cappucci U., Noro F., Casale A.M., Fanti L., Berloco M., Alagia A.A., Grassi L., Le Pera L., Piacentini L. and Pimpinelli S. 2019. The Hsp70 chaperone is a major player in stress-induced transposable element activation. Proc Natl Acad Sci U S A. 2019 Sep 3;116(36):17943-17950. doi: 10.1073/pnas.1903936116. Epub 2019

Colonna Romano Nunzia and Laura Fanti. 2022. Transposable elements: major players in shaping genomic and evolutionary patterns. Cells 11, 1048. doi: https://doi.org/10.3390/cells11061048

PhD Program in Genetics and Molecular Biology (XXXVIII cycle)

Title: Investigating the catalytic-independent function of METTL3 in chronic myeloid leukemia
Proponent: Alessandro Fatica
Affiliation: Department of Biology and Biotechnology "Charles Darwin"

Abstract of the project:
Chronic Myeloid Leukemia (CML) is a hematopoietic neoplasm characterized by the clonal expansion of myeloid cells. CML is associated in about 95% of patients with the production of the oncogenic BCL-ABL1 fusion gene. Recently, chemical modification of RNA has emerged as a new mechanism of gene expression regulation. Among many, N^6-methyladenosine (m6A) influences almost every stage of mRNA metabolism and accumulating evidence indicates a strong correlation between aberrant cellular m6A level and leukemia. The METTL3/METTL14 m6A modifying complex has been shown to play critical roles in CML by both catalytic -dependent and -independent functions. In CML, METTL3 mis-localized in the cytoplasm where it binds and stimulates the translation of specific mRNAs. The specific aims of this proposal are:

1. To identify mRNAs translational regulated by cytoplasmic METTL3 in CML
2. Identify cytoplasmic regulators of METTL3 function in the cytoplasmic compartment

References

Ianniello Z, Sorci M , Ceci Ginistrelli L, Iaiza A, Marchioni M, Tito C, Capuano E, Masciarelli S, Ottone T, Attrotto C, Rizzo M, Franceschini L, de Pretis S, Voso M T, Pelizzola M, Fazi F, Fatica A (2021). New insight into the catalytic -dependent and -independent roles of METTL3 in sustaining aberrant translation in chronic myeloid leukemia. *Cell Death & Disease 12*: 870.

Ianniello Z, Paiardini A, Fatica A. (2019). N6-Methyladenosine (m6A): A Promising New Molecular Target in Acute Myeloid Leukemia. Frontiers in Oncology, 9: 251.

PhD Program in Genetics and Molecular Biology (XXXVIII cycle)

Title: Regulation and function of the Aurora-A kinase

Proponent: Giulia Guarguaglini
Affiliation: Institute of Molecular Biology and Pathology, CNR, c/o Department of Biology and Biotechnology-Sapienza University of Rome

Abstract of the project:
The mitotic Aurk-A kinase and its regulator TPX2 are key players in cell division. Aurk-A and TPX2 are frequently co-overexpressed in tumours, and the complex is emerging as a major driver of cancer and chemoresistance. Recently, evidence accumulated about relevant Aurk-A non-mitotic roles in cancer cells, some of which are kinase-independent, but dependent on its nuclear localization.
Our preliminary observations and literature data suggest that TPX2, regulating spindle-associated Aurk-A, is also crucial to define the nuclear pool and functions of Aurk-A. A thorough understanding of nuclear Aurk-A/TPX2 regulation and activity, and their interplay with the well-known mitotic roles, are needed to fully understand the oncogenic functions of the complex and effectively target them for therapeutic purposes.
Aim of this project will be to elucidate the interphase regulation of nuclear Aurk-A in human cells, by exploring the mechanisms controlling interphase nuclear localization and stability of the kinase under physiological conditions and when overexpressed. Cell biology, biochemistry and -omics methodologies will be used, in 2D and 3D cell cultures; cellular imaging approaches, in fixed and live samples, will be largely employed. We expect to understand (i) how TPX2 or newly identified factors regulate the nuclear pool of Aurk-A and (ii) how TPX2 contributes to the nuclear functions of Aurk-A.

References

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PhD Program in Genetics and Molecular Biology (XXXVIII cycle)

Title: Role of Drosophila HP1 in Neural Stem Cell behavior: at the crossroads between neurodegeneration and cancer

Proponent: Lucia Piacentini
Affiliation: Department of Biology and Biotechnology (BBCD)

Abstract of the project:
Only a small number of cells in adult tissues (the stem cells) possess the ability to self-renew at every cell division, while producing differentiating daughter cells to maintain tissue homeostasis for an organism's lifetime. A better understanding of stem cell biology will not only reveal crucial molecular mechanisms that control the formation and maintenance of tissues but will also influence stem cell-based therapies in regenerative medicine and cancer treatments. Much progress has been made in recent years in understanding the molecular mechanisms underlying intrinsic and extrinsic factors controlling stem cell regulation, but the complex network of genetic and epigenetic pathways is only partially understood.
Heterochromatin Protein 1 (HP1) is a dynamic epigenetic determinant mainly involved in heterochromatin formation, epigenetic gene silencing and telomere maintenance. Recently, we found that HP1 controls post-transcriptional regulation of stemness genes thus playing a crucial role in maintaining Germline Stem Cell (GSC) homeostasis in *Drosophila*. Our preliminary results suggest that HP1 might be an important regulator also of Neural Stem Cell (NSC) behavior. The purpose of this research project will be to investigate the molecular mechanism underlying HP1 function in neural stem cell maintenance and differentiation. Since alterations in NCS homeostasis are implicated in a broad spectrum of human diseases, from neurodegeneration to cancer, a further goal of this project will be to study a possible involvement of HP1 in neurodegenerative disorders and cancer using *Drosophila* as an experimental model.

References

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PhD Program in Genetics and Molecular Biology (XXXVIII cycle)

Title: Analysis and modeling of protein structure and protein-ligand interactions

Proponent: Domenico Raimondo
Affiliation: Molecular Medicine Dep., Sapienza University of Rome

Abstract of the project:
Proteins are structurally complex and functionally sophisticated molecules, whose existence is essential to all forms of life with their wide ranging roles in all organisms.
They need to bind to other biomolecules, (inorganic or organic small molecules and peptides) to create specific interactions to achieve corresponding functions. These molecules are called ligands.

In our research group we concentrate our interest to predict (1) if, (2) in which conformation and (3) how strong a biomolecule can bind to a protein, in order to better understand molecular recognition and molecular interactions.

We combine multiple computational methods or experimental methods with computational approaches to address all three questions, trying to model all aspects of the protein-ligand system realistically and efficiently.

Our main objectives include:

- Structural analyses of the protein structures by considering flexibility to understand the deformation induced by ligand interactions.
- Characterization of ligand and target binding-site properties and their corresponding profiles in the aim to predict their interactions, using molecular dynamics simulations and structural bioinformatics methods.
- Study the impact of mutations on the protein structure and its interactions.

Our research topics include medically relevant molecular processes (infectious diseases, cancer, …).
At the moment our structural bioinformatics studies are applied on the protease from coronavirus SARS-COV-2 (MPro), human proteins as CD38, GADD45β and CES1.
We have established collaborations with experimental groups at the Sapienza University and elsewhere to explain the pathogenesis of diseases, which provides insights for drug discovery and design.

References

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PhD Program in Genetics and Molecular Biology (XXXVIII cycle)

Title: The nuclear envelope in physiology and pathology

Proponent: Isabella Saggio

Affiliation: Department of Biology and Biotechnology Charles Darwin

Abstract of the project:
The main aim of the proposed project is to investigate and dissect the connection between nuclear integrity and genome organization. The first goal is to define the ultrastructural organization and dynamics specific macro-complexes at the nuclear rim. Secondly, we want to analyze the impact of these macro-complexes in aging and cancer. Thirdly, we plan to explore the use of the nuclear envelope reshaping drugs to impact on aging and cancer.
We will define the organization of protein macro-complexes at the nuclear envelope by super-resolution, structured illumination and electron microscopy and by confocal live imaging. To get insights into the impact on genome organization, we will analyze the integrity of telomeres by FISH and the dynamics by live imaging. Finally, to define the interrelationship between nuclear envelope integrity and disease, we will perform studies in vitro and in vivo in animal models. In the same systems, we will test the impact of remodeling drugs.
We believe that this study will contribute to the characterization of the molecular mechanisms ensuring nuclear envelope integrity implicated in the control of genome integrity, along with giving insights into their impact in diseases.

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Maccaroni K, La Torre M, Burla R and Saggio I (**2022**). Phase Separation in the Nucleus and at the Nuclear Periphery during Post-Mitotic Nuclear Envelope Reformation. Cells, 11, 1749. IF 6.6

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La Torre M, Burla R, Merigliano C et al. (**2018**) Mice with reduced expression of the telomere-associated protein Ft1 develop p53-sensitive progeroid traits. Aging cell 17(4):e12730. IF: 7.346

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PhD Program in Genetics and Molecular Biology (XXXVIII cycle)

Title: Computational Design of RNA molecules

Proponent: Gian Gaetano Tartaglia
Affiliation: The Department of Biology and Biotechnology (BBCD) and Italian Institute of Technology (IIT)

Abstract of the project:

Using proprietary methods developed in the *RNA System Biology Lab* [1,2], our team is able to generate RNA sequences, or aptamers, that bind with high affinity to specific aggregation-prone proteins [3,4].

We aim to design RNA aptamers to monitor and/or reduce the aggregation [5] or other dysfunctional activities of proteins [6] involved in neuromuscular diseases (NMD; cases of interest are TDP-43, FUS, KMT2D/MLL4, MBNL1, FMR1, FRG1 and CELF2A). The student will implement and improve our *in silico* methods to generate artificial RNAs for the specific protein targets and will exploit available high-throughput experiments to enhance the predictive power. Advanced machine learning methods will be exploited and also developed in the design. The effects of the predicted aptamers will be subsequently tested *in vitro* and *in vivo* in our laboratory. The student will analyze the results of high-throughput experiments (RNA-Seq and Mass spectrometry analyses of the pull-down) conducted in our laboratory or in collaboration with other labs to evaluate the binding specificity of the aptamers. Collaborations with internationally recognized researchers in the field are planned.

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PhD Program in Genetics and Molecular Biology (XXXVIII cycle)

Title: Genomic portrait of Italic Copper Age populations
Proponent: Beniamino Trombetta
Affiliation: Dipartimento di Biologia e Biotecnologie "C. Darwin"

Abstract of the project:
Central Italy has always been a melting pot of different cultures thanks to its strategic geographic position in the Mediterranean sea. Archeological and genetic evidence show that influences in this area from the Near East date back to the Neolithic, with new inputs from the massive Asian Steppe migrations starting from the Bronze Age. In this context, despite the abundance of sites, little is known about the population dynamics during the Copper Age, i.e. the period between the Neolithic and the Bronze Age. Different studies based on the material culture, the isotopic analysis and the genetic components gave contrasting results, showing both populations continuities and discontinuities. In a pilot project, we identified a Copper Age individual from central Italy that showed striking features: while the material culture in its burial place him in the context of the local communities, his genetic components identify it as Anatolian. This observation suggest that he could have been a few-generation descendant of Anatolian immigrants or an immigrant himself. Moreover, its Y chromosome (i.e. the portion of the genome inherited from father to sons) belongs to a nowadays frequent European lineage previously linked to the Steppe ancestry, despite this sample not harboring this component. To shed light on the history and the population dynamics in central Italy during the Copper Age, we want to perform a detailed archaeological, isotopic and genetic analysis of this individual together with more than 30 samples belonging to other copper age necropolises. The data will then be compared to the available data from the literature from other Italian, European and Near Eastern samples. Our results would provide, for the first time, a description of the gene pool of the populations from which the Italic populations of the iron age differed (e.g. Etruscans, Picenes, Umbrians etc.).

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PhD Program in Genetics and Molecular Biology (XXXVIII cycle)

Title: Role of vitamin B6 in cancer using *Drosophila* as a model system
Proponent: Fiammetta Vernì
Affiliation: Department of Biology and Biotechnology "Charles Darwin", Sapienza University of Rome

Abstract
Vitamin B6 or pyridoxal 5'-phosphate (PLP) serves as coenzyme for about 200 metabolic reactions; in addition, it counteracts the formation of reactive oxygen species (ROS) and advanced glycation end products (AGEs). Reduced vitamin B6 availability modulates cancer risk, but underlying mechanisms are not fully elucidated yet (1). We previously demonstrated that low PLP levels induce chromosome aberrations (CABs) in *Drosophila melanogaster* and human cells (2,3). More recently, we found that PLP inhibitors such as 4-deoxypyridoxine (4DP) and ginkgotoxin (GT) promote loss of heterozygosity (LOH) at the *Drosophila* tumor suppressor *warts* (*wts*) locus, thus inducing epithelial *wts* cancers (4). We also collected evidence that PLP deficiency can transform ras^{G12V} benign tumors in more aggressive forms.
Starting from these premises, the purpose of this project is that of shed light on the molecular mechanisms through which PLP deficiency leads to cancer in *Drosophila*. In particular, by focusing on the relationship between vitamin B6, DNA damage and cancer, we will investigate whether PLP impacts on cancer due to its antioxidant role or due to its role as cofactor of enzymes working on the folate cycle, an essential pathway which provides DNA precursors. To this purpose, genetic, cytological, and biochemical approaches will be applied to investigate the role of PLP in some *Drosophila* cancer models such as Ras^{G12V}, Ras^{G12V}/Src, and Ras^{G12V} $dlg^{-/-}$. Finally, will be performed a metabolomic analysis to gain a comprehensive view of the molecular processes involved.

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PhD Program in Genetics and Molecular Biology (XXXVIII cycle)

Title: Targeting RNA-binding proteins to control neurodegenerative and neurodevelopmental diseases
PNRR-CN3, Spoke 3 - Neurodegenerative

Proponents: Bozzoni, Ballarino e Rosa
Affiliation: Department of Biology and Biotechnology

Abstract of the project:
Due to their intrinsic properties, RNA molecules combine the dual function of tethering proteins as well as other nucleic acids. RNA-RNA and RNA-protein interactions allow the nucleation of different membrane-less compartments where the most essential cellular processes occur, such as transcription, processing, translation and intracellular transport. The main goal of our project is to be able to control the structure and function of specific ribonucleoprotein (RNP) complexes by targeting the RNA-RNA and RNA-proteins interactions and to understand how they contribute to gene expression control, to RNP assembly and function, and intracellular trafficking in neurons and muscles. To this aim, we will use integrated experimental and computational approaches. In neuro-degenerative diseases such as ALS, Alzheimer and many others, a causative link has been found between the pathology and the conversion of RNP granules into solid-like aggregates indicating that, by trapping crucial RNAs and proteins, they can lead to the dysfunction of many cellular processes. The ability to control the aggregation of these nuclear and cytoplasmic assemblies, by acting at the level of RNA or RNA-binding protein components, represents highly innovative research. We expect that these studies will strongly increase our understanding of basic molecular processes controlled by RNA molecules and RNA-binding proteins and should also constitute a largely unexplored territory for the development of novel therapeutics and diagnostics.

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Title: Novel therapeutic approaches based on the modulation of m6A RNA modification levels in cancer

Proponent: Alessandro Fatica
Affiliation: Department of Biology and Biotechnology "Charles Darwin"

Abstract of the project:

Accumulating evidence indicates a strong correlation between aberrant RNA modifications and cancer initiation, progression, and drug resistance. Therefore, RNA-modifying enzymes are becoming important anticancer drug targets. One of the most relevant RNA modifications in cancer is the N6-methyladenosine (m^6A). m^6A is the most abundant internal modification in mRNA and it is reversible and dynamic. m^6A is mainly installed by the nuclear complex composed of two methyltransferase-like proteins, METTL3 and METTL14, and can be removed by the ALKBH5 and FTO demethylases. Interestingly, in some types of cancer both writers and erasers can play oncogenic roles. Furthermore, in different tumors mis regulation or new oncogenic activities can be acquired by their altered subcellular localization. Chronic Myeloid leukaemia (CML) is a cancer characterized by the clonal expansion of myeloid cells. CML is associated in about 95% of patients with the production of the oncogenic BCL-ABL1 fusion gene. The use ABL1 tyrosine kinase inhibitors (TKI) made CML a clinically manageable and a cured disease. However, in many cases, treatment with TKIs is not curative BCR-ABL1-independent pathways play important roles in TKI resistance and persistence of leukemic stem cells. We will investigate the oncogenic roles of the METTL3 methyltransferase and the FTO demethylase in CML and the impact of their selective inhibition by small molecules.

We aim at: 1) characterize the molecular function of FTO and METTL3 inhibitors in TKI-sensitive and -resistant CML cells to identify new relevant pathways; 2) test the therapeutic efficacy in preclinical *in vitro* models for CML; and 3) test inhibition of METTL3 and FTO alone or in combination with drugs already use in the clinic to prevent and/or overcome TKI resistance.

PhD Program in Genetics and Molecular Biology (XXXVIII cycle)

Title: Functional identification and effects on crop yield of bioactive compounds obtained from plants grown in marginal areas at low environmental impact.

Proponent: Giovanna Serino
Affiliation: Department of Biology and biotechnology, Sapienza University, Rome, Italy
Abstract of the project:

The aim of this PhD project is to provide experimental evidence supporting the use of natural, bioactive compounds such as (but not limited to) essential oils on crop growth and yield. Selected compounds will be extracted from plants that can be grown by farmers in patches of non-cultivated land ("marginal areas"). These compounds will be tested for their biological activities on different plants belonging to the *Brassicaceae* family: the model plant *Arabidopsis thaliana* and the crop *Brassica sylvestris*. Several growth traits will be evaluated, but we will mainly focus on flower opening, as this is a key biotechnological trait for the *Brassicaceae* family. The molecular and physiological basis of the plant response to these compounds will be also evaluated in detail, using a genetic and whole genome approach. This will lead to the identification of the molecular networks responsible for the effect of a given bioactive compound.
In summary, by combining field and laboratory data, the results obtained within this research project will provide farmers with validated protocols to optimize crop growth and yield under a sustainable farming regimen.

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PhD Program in Genetics and Molecular Biology (XXXVIII cycle)

Title: circular RNAs as vectors for protein expression

Proponent: Irene Bozzoni
Affiliation: Department of Biology and Biotechnology and IIT, Sapienza University

Abstract of the project:
We want to exploit the ability of circular RNA to be translated in order to improve the formulation of RNA active in the production of proteins for vaccination. In the last two years we have seen how technologies based on the use of RNA (linear messenger RNA) are gaining the upper hand in vaccination treatments and how they are proving effective in providing protective antibody responses to viral infections. This research is aimed at improving the formulation of RNAs active in the production of proteins for the production of vaccines through the use of circular RNAs. Circular RNAs (circRNAs) are a new class of RNAs with a characteristic covalently closed circular shape; compared to messenger RNAs currently in use, they offer the advantage of greater intracellular stability, of being able to be efficiently translated into proteins and of not requiring modifications necessary to escape from the innate immune response.

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PhD Program in Genetics and Molecular Biology (XXXVIII cycle)

Title: non coding RNAs in neurodegenerative processes

Proponent: Irene Bozzoni
Affiliation: Department of Biology and Biotechnology and IIT, Sapienza University

Abstract of the project:
A large part of mammalian transcriptomes is composed of RNA molecules that do not encode for proteins (non coding RNAs) and whose role is to control gene expression at many different levels. These molecules, thanks to their intrinsic scaffolding ability, combine the dual function of tethering proteins as well as other nucleic acids. RNA-RNA and RNA-protein interactions allow the nucleation of different membrane-less compartments where the most essential cellular processes occur, such as transcription, processing, translation and intracellular transport. All these assemblies have a vast importance in physiological conditions; however, under specific conditions, as for instance upon stress, they can increase their viscosity leading to solid-like aggregates. Liquid-solid phase transitions can lead to the dysfunction of many cellular processes and their formation has been causatively linked to several neuro-degenerative diseases such as ALS, Alzheimer and many others. The main goal of our research is to study the activity of lncRNAs and circRNAs in normal and ALS pathological conditions in order to understand how they contribute to control RNP assembly, function and intracellular trafficking in motor neurons. We expect that these studies will strongly increase our understanding of basic molecular processes controlled by ncRNAs and should also constitute a largely unexplored territory for the development of novel therapeutics and diagnostics.

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PhD Program in Genetics and Molecular Biology (XXXVIII cycle) industry co-funded

Title: Functionality of genetic and epigenetic changes in the human genome

Proponent: Massimo Reverberi
Affiliation: SARA ENViMOB S.r.l., Rome

Tutor: Simona Giunta
Affiliation: Dpt. of Biology & Biotechnology Charles Darwin, University of Rome "La Sapienza".

Abstract of the project:

The last decade has seen great strides being made in human genomics. We are moving toward comprehensively mapping the genetic and epigenetic status of the human genome. However, understanding changes associated with individual polymorphism, how the genome and epigenome vary across tissues and cell types and especially, the connection with pathological states remains elusive. Using novel biochemical methods including CUT&TAG and third generation sequencing, this Ph.D. project proposes to explore and characterize changes in chromatin features across the human genome under conditions biomimetic of endogenous stress and cytotoxicity. Specifically, the work aims to (1) identify novel polymorphisms and mutational signatures, (2) investigate how they affect the epigenome and chromatin behavior and (3) find causative association with human diseases. To this end, changes will be characterized using the latest assembly and explore their relevance comparing across different experimental conditions, especially those of damage and endogenous cellular stress. The project includes both wet lab component and bioinformatic analyses.

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PhD Program in Genetics and Molecular Biology (XXXVIII cycle) industry co-funded

Title: Early detection of somatic mutations associated with the presence of endometrial and ovarian gynecological tumors during routine Pap smears

Proponent: Francesca Spinella
Affiliation: Eurofins Genoma, Rome

Tutor: Fulvio Cruciani
Affiliation: Dpt. of Biology & Biotechnology Charles Darwin, University of Rome "La Sapienza".

Abstract of the project:
Epithelial ovarian cancer (OC) is the leading cause of gynecologic cancer-related deaths in women, primarily because most patients present with advanced-stage disease. Moreover, in the last 20 years, the incidence rate of another gynecologic cancer, endometrial cancer (EC), has increased by 125% worldwide and despite improved diagnosis and treatment methods, up to 30% of EC patients are primarily diagnosed with stage III or IV EC and have poor outcomes. Cervical cancer, on the other hand, is much less frequent in developed countries thanks to the wide availability and effectiveness of screening with the Pap test (Papanicolau test) patients allowing for early diagnosis and surgical therapy. Unlike cervical cancer, for OC and EC there are no primary (vaccination) or secondary (population screening) prevention tools that allow for an early diagnosis and timely therapeutic intervention. Also, the identification of endometrial and ovarian cancer cells through microscopy examination of the specimen often fails to discriminate such cells from benign conditions or from cervical tumors.
Recently, it has been shown that malignant cells exfoliate from the ovaries and may be detected in Pap specimens, routinely collected through cervical cancer screening. By analyzing the cells shed from the cervix, the health state of other female reproductive organs, such as the ovaries, fallopian tubes and endometrium can be also evaluated. Endocervical DNA or intrauterine DNA can be obtained through vaginal tampons, intrauterine lavage, Pap smear, and Tao Brush to determine the presence of malignant tumor cells in the reproductive tract. Circulating tumor DNA is a type of cell-free DNA that accounts for a small part of circulating cell-free DNA. It is usually released from tumor tissues in vivo and harbors tumor-specific DNA mutations, which can be detected using massive parallel sequencing (NGS).
In this project, we will develop NGS-based tumor DNA screening tests on cervical specimens, with the potential to increase the chances of early detection of endometrial and ovarian cancers in asymptomatic women. The purpose of the new test is to facilitate the diagnosis in non-advanced stages of endometrial, ovarian and cervical cancers, stages in which the cancer is still treatable, combining the cytological screening of the routine Paptest with genetic screening of tumor DNA.
The development of kits that exploit the new NGS technologies fits well into the programmatic research objectives at regional, national and European level.

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PhD Program in Genetics and Molecular Biology (XXXVIII cycle)

Title: A multi-omics approach to unveil the therapeutic resistance in Multiple Myeloma

Proponent: Maurizio Fanciulli
Affiliation: Istituto Fisioterapici Ospitalieri (IFO), Department of Research, Advanced Diagnostics, and Technological Innovation.

Abstract of the project:
Multiple myeloma is a hematological neoplasm arising from the transformation of plasma cells and characterized by an abnormal production of immunoglobulins. Despite important advances in the treatment of MM, relapse of the disease with a more aggressive phenotype is observed in most patients. Therefore, understanding the molecular factors driving therapeutic resistance in MM is critical to implementing new cancer care strategies.
This project aims to understand the role of chromatin plasticity and non-coding variants during the emergence of therapeutic resistance of MM. The research plans to investigate chromatin accessibility shift in MM cells for identifying possible cell states and phenotypical niches resistant to treatment using data from a large patient cohort, in-vitro and in vivo model. This investigation aims to 1) map epigenetic variegation sustaining MM drug resistance 2) define the epigenetic events leading to the arising of therapeutic resistance in a unique primary cell lines model develop in our lab 3) propose a translation of key findings into the clinic.
The project proposes to build a comprehensive understanding of the regulatory events sustaining the evolutive trends which contribute to drug resistance in MM by using the most advanced technologies. Understanding the functional impact of the regulatory asset on the evolutionary trajectory of cancer has fundamental clinical implications. Comprehending the principle ruling the evolutive force leading to cancer drug resistance would provide an advantage in predicting if cancer would metastasize, acquire resistance give a more punctual prognosis. Ultimately, targeting the molecular drivers of the evolutive forces with ad-hoc therapy would give the clinician an advantage in accurately battling cancer.

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PhD Program in Genetics and Molecular Biology (XXXVIII cycle)

Title: Plant transcription factors as key molecular targets for genetic improvement of crop species

Proponent: Giovanna Frugis
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Abstract of the project:

In living organisms, the process of adaptation to the environment involves the accumulation of random mutations in the DNA that can be fixed /selected in the population if they confer an evolutionary advantage. Genetic biodiversity, in natural or domesticated populations, constitutes a source of allelic variants "tested" naturally in the field of evolution, and are therefore an important resource for genetic improvement. More than 60% of adaptive mutations are associated with genes encoding transcription factors (TF), proteins that bind to specific DNA sequences to increase, decrease or modulate the level of gene expression in response to the environment and developmental signals. Transcription represents the most important level of regulation in determining the set of morphological and functional characteristics of an organism (phenotype) starting from the genetic information contained in the DNA (genotype). TF therefore constitute the main target genes on which the processes of adaptation of the genotype to the environment in wild populations and in domesticated species naturally act.

This PhD project will develop innovative molecular technologies based on transcription factors to study the effects of natural genetic variability and use this information to accelerate the breeding of new resilient crop varieties that maintain their productive and typical characteristics in more variable and extreme environmental conditions. The project will address an agronomic trait that is important for the production and adaptation to the environment of plant species: flowering time. Fundamental knowledge of the genetic control of flowering time will be translated from the plant model species *Arabidopsis thaliana* to horticultural species. The following technologies will be developed and acquired during the doctorate program: advanced techniques of molecular biology for DNA cloning, gene expression analysis (qRT-PCR, RNAseq), genome editing and plant genotyping; plant growth and tissue culture; statistics and bioinformatics for data analysis using specific software, including R and RStudio (differential gene expression, cluster analysis, gene co-expression network, systems biology and "omics" analysis for the identification of "hub" genes, identification of DNA binding sites, screening of genomes for natural genetic variation).

The PhD program will be carried out in the framework of the “SMART-BREED” Regione Lazio project.

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PhD Program in Genetics and Molecular Biology (XXXVIII cycle)

Title: Understanding the role of CENP-A in modulating responses to DNA damage and R-loops formation in human cancers

Proponent: Simona Giunta

Affiliation: Department of Biology and Biotechnology "Charles Darwin", Sapienza University of Rome

Abstract of the project: The histone H3 variant CENP-A acts as a locus-specifying seed to assemble kinetochores for mitotic functions and is essential for faithful chromosome segregation. Inability to connect the centromere to the spindle causes aneuploidy, a hallmark of many cancers. Using a novel technique called Cen-CO-FISH, we have identified a critical role for CENP-A in the maintenance of centromeric DNA repeats by repressing homologous recombination resulting in sister chromatid exchange. In addition to chromosome missegregation, chromosome whole arms translocations are prevalent in cancers, but how such rearrangements arise remains unclear. Recently, we identified a role for CENP-A in maintaining the integrity of centromere-associated repetitive sequences by ensuring their effective replication in human cells. In the absence of CENP-A, DNA–RNA hybrids called R-loops are generated due to transcription– replication conflicts. The consequences of R-loops are delayed DNA replication, centromere breakage, recombination, and chromosome translocations similar to those observed in cancer cell. To go deeper into CENP-A role in modulating R-loops, new experimental strategies and tools are needed. This Ph.D. project will harness CRISPR-Cas9 genome editing combined with the latest application of CUT&RUN to obtain a more detailed map of R-loops generation within repetitive DNA in presence and absence of the CENP-A nucleosome. Furthermore, the development of these techniques will enable to study the dynamic formation of these mutagenic structures throughout the cell cycle and to assess their impact on other DNA based transactions such as replication fork stalling or DNA damage repair processes. Finally, third generation sequencing and bioinformatic analysis will be applied to allow the identification of locus-specific mutational signatures.

Selected publications:

Giunta S, Herve S, White R, Wilhelm T, Dumont M, Wong CK, Rancati G, Smogorzewska A, Funabiki H, Fachinetti D. **2021** 'CENP-A preserves genome integrity during alpha-satellite DNA replication by modulating R-loops at human centromeres'. *Proceedings of the National Academy of Science (PNAS)*, 9;118(10):e2015634118

Balzano E, Pelliccia F, **Giunta S**. **2021** 'Genome (in)stability at tandem repeats'. *Seminar in Cell & Developmental Biology*, S1084-6.

Black EM & **Giunta S**.**2018** 'Repetitive Fragile Sites: Centromere Satellite DNA as a Source of Genome Instability'. *Genes*, 9, 615.

Giunta S,. **2018** 'Centromere Chromosome Orientation Fluorescent *In Situ* Hybridization (Cen-CO-FISH) detects Sister Chromatids Exchange at Centromeres in Human Cells'. *Bio-protocol*, 7(8), e2792.

Giunta S & Funabiki H. **2017** 'Integrity of the human centromere DNA repeats is protected by CENP-A CENP-C and CENP-T'. *Proceedings of the National Academy of Science (PNAS)* 114(8), 1928-33

PhD Program in Genetics and Molecular Biology (XXXVIII cycle)

Title: Coordination of inflorescence architecture and stem development

Proponent: Dr. Alice Pajoro
Affiliation: Institute of Molecular Biology and Pathology, National Research Council
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Abstract of the project:
Sufficient food production is threatened by the fast-growing world population and global climate change. To produce sufficient food for future generations in a sustainable manner, it is necessary to develop innovative strategies for plant production. This can only be achieved with an increase in the knowledge of fundamental mechanisms that govern plant development. This project intends to contribute to increase plant yield by studying pathways that control inflorescence development. The architecture of plant inflorescences is an important factor for crop yield because it determines the number of developing flowers, and thereby fruits and seeds. The shape of the inflorescence is established by the activity of the apical meristem. The inflorescence meristem, which is indeterminate, define the size and the complexity of the inflorescence and thereby the numbers of flowers that can develop, whereas the determinate flower meristems form the final stage and stop further growth of the inflorescence. Therefore, the timing of the meristem identity transition from indeterminate to determinate importantly influences crop yield. In this project the regulatory pathways that control these meristem transitions will be studied with a focus on inflorescence meristem longevity. The complexity of an inflorescence and the quality of the fruits and seeds that develop is not only dependent on reproductive meristems activities but also on the inflorescence stem that has an important function in transporting the necessary nutrients. Increasing the numbers of fruits and seeds will need increased allocation of resources to the newly developing organs. Therefore, the project also includes the study of stem development. The project will mainly focus on the model plant Arabidopsis and the master regulator TERMINAL FLOWER 1 (TFL1).

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Balanza et al., (2018) Genetic control of meristem arrest and life span in Arabidopsis by a FRUITFULL-APETALA2 pathway. Nature Communications 9, 565
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PhD Program in Genetics and Molecular Biology (XXXVIII cycle)

Title: Exploring the relationships between transcription-dependent DNA damage and neurodegeneration

Proponent: Grazia Daniela Raffa
Affiliation: Department of Biology and Biotechnology "Charles Darwin", Sapienza University of Rome

Abstract of the project:
Spinal Muscular Atrophy (SMA) is caused by loss of the Survival Motor Neuron (SMN) protein, which mediates the assembly of small nuclear RNAs (snRNAs) into snRNPs. Previous work has suggested that SMN mutations impair the splicing of mRNAs essential for motoneuron survival. It has been also proposed that SMN-dependent defects in transcription termination, causing R-loops formation and DNA damage, may contribute to SMA pathogenesis. We found that the TGS1 hypermethylase is required for snRNA 3' end processing and neuron survival, and that TGS1 overexpression ameliorates the SMN loss-of-function phenotypes. Transcriptome analysis by both Illumina and Nanopore sequencing has shown that loss of human SMN and TGS1 in HeLa cells induces formation of mRNA transcripts that carry alterations in splicing and 3′ readthrough extensions. This project is aimed at investigating whether transcriptional defects induce R-loops and DNA damage accumulation in SMN- and TGS1-deficient cells and to which extent these defects contribute to neuron death, using both human iPSC cells from SMA patients, and Drosophila, to explore the relationships between transcriptional defects and R-loop formation and whether R-loop-dependent DNA damage contributes to neurodegeneration. Another aim of the project is to screen for RNA surveillance factors which affect the rate of R-loop formation and the neuronal death phenotype in both model systems.

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PhD Program in Genetics and Molecular Biology (XXXVIII cycle)

Title: Translating knowledge from Arabidopsis to tomato to improve crop yield

Proponent: Giovanna Sessa
Affiliation: Institute of Molecular Biology and Pathology, National Research Council

Abstract of the project:
As world population increases, global food demand is set to keep enhancing despite a predicted decrease in total arable land. One option to solve this problem is to grow plants at higher densities. When grown in dense stands plants compete for light with their neighbors and employ a suite of developmental adjustments known as the ‘‘Shade Avoidance Response” (SAR). Shade avoidance is an adaptive response that results in phenotypes with a high relative fitness in individual plants growing within dense vegetation (Sessa *et al*. 2018). However, it affects the growth, development, and crop yield. Thus, in order to generate more resilient plants, the design of new strategies aimed at attenuating shade avoidance at defined developmental stages and/or in specific organs is a major challenge for the future. The research activity looks at the generation of Arabidopsis thaliana plants impaired in the activation of specific traits of the shade avoidance response, targeting specific elements of the regulatory network. The strategy devised consists in the manipulation of transcriptional cascade controlled by HDZIPII transcription factors, known as central regulator of individual aspects of SAR (Carabelli *et al*. 2018; Sessa *et al*. 2018). Since the fundamental understanding of the core SAR pathways obtained in Arabidopsis has the potential to be highly translatable to distantly related crop species, the same alteration will be introduced in the tomato orthologous genes by CRISPR-CAS9 system to impair shade avoidance in a crop species.

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