RESEARCH PROJECTS SUMMARY

PhD Course in Molecular and Cellular Biology

(XXXVI CYCLE – A.Y. 2020-2021)

Project leader: Alessandro Aliverti (alessandro.aliverti@unimi.it)

Location: Dipartimento di Bioscienze, Università degli studi Milano

From protein structure to novel therapeutics against rare genetic diseases

This project aims to develop an efficient and innovative pipeline to select novel drugs against rare orphan diseases, exploiting a structure-based drug discovery (SBDD) approach. Patients suffering from rare maladies (<5 patients/10000) deserve the same quality of treatment as conventional patients do. However, pharmaceutical companies do not invest in the development of drugs against these diseases and academic research rarely receives the resources for traditional drug discovery projects. SBDD represents a cost-effective alternative to conventional high-throughput screening, as it allows an inexpensive pre-screening (i.e. virtual docking to potential targets) of chemicals. This computational approach will significantly reduce the number of molecules to be tested *in vitro* and *in vivo*, minimizing the experimental burden. Moreover, libraries of molecules already approved by FDA/EMA will be included in the screening, aiming at the repositioning of drugs that could be immediately available for clinical application. In particular, two different hereditary diseases will be object of this study: i) cone dystrophies (CoD) caused by mutations in the guanylate cyclase (GCAP) protein; ii) mitochondriopathies due to allelic variants of the apoptosis inducing factor (AIF);
The proposing labs have already extensively studied the aforementioned etiological proteins and the molecular bases underling the related diseases are relatively well understood. Moreover, crystal structure of wild type and mutated proteins, as well as robust protocols for their functional characterization are available. The project is highly multidisciplinary and offers training opportunities ranging from computational biology to preclinical studies. The student will be primarily responsible for the activities here briefly summarized: i) GCAP (Vocke *et al.*, 2017) and AIF (Sorrentino *et al.*, 2017) structures will be used as targets to screen *in silico* novel ligands of the proteins. Several libraries of small molecules are available, but primarily a 9,000 compounds library of already approved drugs will be used. ii) Proteins will be expressed in heterologous systems and purified to homogeneity. iii) Best binders according to the computational analysis, will be tested experimentally *in vitro* on the purified proteins with the appropriate assay (shift in denaturation profile, enzyme inhibition, alteration of oligomerization/aggregation state, protein-protein interaction, etc.) and their binding affinity measured. iv) The effect of the small molecule on the physiological and pathological function of the target will be thoroughly characterized to dissect the mechanism of action, experimentally and *via* simulations. v) Finally, the crystallographic structure of the target-ligand complexes will be obtained to set the bases for an optimization of the leads.

This project develops in the framework of a collaboration between the Enzymology Laboratory (Alessandro Aliverti), Dept. of Bioscience and the CNR-IBF X-ray crystallography and structure-based drug design group (http://users.unimi.it/biostru/ibf-cnr-lab.html) (Mario Milani, mario.milani@unimi.it).

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Vocke F, Weisschuh N, Marino V, Malfatti S, Jacobson SG, Reiff CM, Dell'Orco D, Koch KW. Dysfunction of cGMP signalling in photoreceptors by a macular dystrophy-related mutation in the calcium sensor GCAP1. Hum Mol Genet. 2017 Jan 1;26(1):133-144.

Project leader: [Andrea Francesco Barbuti-andrea.barbuti@unimi.it]
Location: [Department of Bioscience]

Study of the functional and molecular mechanisms of familial cardiac pathologies using human and murine pluripotent stem cells

The overall goal of the project is to understand the pathological mechanisms underlying different heart diseases using innovative approaches based on human patient-derived induced pluripotent stem cells (iPSC) or mouse embryonic stem cells (mESC). The iPSC models offer the unique opportunity to produce patient-specific cardiomyocytes for characterizing their functional properties and unravelling possible alterations of membrane excitability and contraction. The mESC are instead useful to specifically study sinus-node disease, and developmental disease that affect heart. The PhD student will be involved in the exploitation of these models to study possible alterations of membrane excitability and contraction using molecular and electrophysiological analyses. In particular, the student will acquire skills in the patch clamp technique, multi electrode array (MEA) recordings, stem cells maintenance and differentiation and basic molecular biology techniques (qRT-PCR, WB, ICC)..

Pathologies studied in Barbuti's lab with the above approach are:

1. - Familial form of Atrial Fibrillation (AF). AF is the most common arrhythmia worldwide, however, molecular mechanisms triggering AF are still unclear (Nattel et al. 2020). We exploit the iPSC model derived from patients with mono- and multigenic forms of AF in order to dissect the mechanisms of AF onset (Benzoni et al 2020).

2. Caveolinopathies, a family of genetic disorders arising from mutations of the *CAV3* gene (encoding for caveolin-3) that lead to some rare forms of muscular dystrophies, myopathies and arrhythmias during childhood. The onset of cardiac abnormalities, associated with myopathic CAV3 mutations, is still poorly understood because of the paucity of accessible diseased cardiac specimens and the lack of control samples of human origin. We generate iPSC-CM from patients in order to characterize their functional properties, elucidating the players involved in the possible alterations of membrane excitability and contraction (Meraviglia et al 2018).

3- IDDCA is an autosomal recessive ultra-rare syndrome characterized by global developmental delay evolving into intellectual disability, cardiac abnormalities (most commonly sick sinus syndrome with bradycardia), muscle hypotonia, ocular abnormalities, epileptic encephalopathy, and gastric reflux (Lodder et al. 2018). These disturbances are due to mutations in *GNB5.* How alterations in GNB5 lead to pathogenic mechanisms is still unclear. We generated iPSC-cardiomyocytes derived from patients and a KO-line in order to study the initiation of cardiac abnormalities (Malerba et al 2019). In parallel,

we also applied the mES model GNB5-KO in order to study specifically the developmental and functional alterations of sinus node cells.

Project leader: Aureliano Bombarely (aureliano.bombarely@unimi.it)

Location: Department of Bioscience/Dipartamento di Bioscienze

Transposon Landscape Variation and its Influence on Phenotype Diversity in Plants

Transposable elements, also known as transposons, are fragments of mobile DNA capable of move in a genome. Transposons can be classified in two general types depending of their mechanism of transposition: Class I (or retrotransposons), with a copy-and-paste mechanism and class II (also known as DNA transposons), with a mechanism of cut-and-paste. Plant genomes are rich in transposable elements. There are some well known cases in which a transposon is responsible for the production of a specific phenotype associated to an agronomical trait, for example the plant architecture in the modern maize is associated to a transposon insertion upstream the TB1 gene. Our laboratory has developed several tools for the analysis of the transposon landscape such as DeepTE and TE-marker which we have successfully applied to study some plant models such as rice (*Oryza sativa*).

We hypothesize that the ***transposons are major drivers in the production of new phenotypes during the plant domestication***. The aims of this project are:

- **Aim 1**: Characterize the transposon landscape evolution in Solanaceae species such as tomato (*Solanum lycopersicum*), potato (*S. tuberosum*) and eggplant (*S. melongena*).
- **Aim 2**: Analyze the selection of transposons among wild and domesticated populations.
- **Aim 3**: Perform association analysis between agronomical traits and transposons and infer the number of phenotypes influenced by transposons.

Project leader: M Cristina Bonza (cristina.bonza@unimi.it)

Location: Department of Biosciences

Characterisation of MIZ1-ECA1 interaction to shed light on the mechanisms underlying long-distance Ca^{2+} signalling in plants

Adaptation to environmental hazards and optimisation of resource acquisition requires an efficient long-distance communication within and between organs to communicate and exchange information.

Calcium (Ca^{2+}) is a crucial second messenger in plants where it couples the perception of endogenous and environmental signals to proper physiological responses. Ca^{2+} signals are transmitted by stimulus-specific cytosolic Ca^{2+} elevations commonly referred as "Ca^{2+} signature". These signatures, composed of various physical elements as amplitude, duration, frequency of oscillations and spatial localization, are believed to encode information from primary stimuli and, therefore, to contribute to the stimulus-specificity of the biological response. Although some components of the Ca^{2+} signalling network have been functionally characterised, the mechanisms by which they are regulated to shape complex Ca^{2+} signatures are only beginning to be elucidated. Recent considerations of activities of Ca^{2+} transport systems using mathematical modelling suggested that physiological relevant variations in the activity of Ca^{2+}-extruding systems as Ca^{2+}-ATPases and Ca^{2+}/H^{+} exchangers are sufficient to determine the shape of Ca^{2+} transients in response to environmental stimuli, emphasizing the crucial role that these Ca^{2+} efflux systems play in plant adaptive responses.

We recently found that MIZ1, an ER membrane-associated protein is indispensable for long-distance Ca^{2+} signalling involved in hydrotropic response in Arabidopsis. MIZ1 directly binds to ECA1, an isoform of ER-localised IIA Ca^{2+} pump and acts as a negative regulator of it in response to hydrostimulation, thus causing an elevation of cytosolic Ca^{2+} levels that promotes root bending towards zones with a higher water potential. MIZ1 is the first regulator of plants IIA Ca^{2+}-ATPases to be identified so far. The discovery that MIZ1, a protein unique to land plants interacts with ECA1, is intriguing and merits further investigation. A fundamental question is the understanding of how MIZ1 interacts and regulates ECA1 and how this mechanism can be exploited to improve the plants water foraging.

The PhD student will start investigating, by different biochemical approaches, the MIZ1-ECA1 interaction in detail. The possibility that besides ECA1, MIZ1 might regulate other ECA isoforms will be also assessed. Moreover, the PhD student will produce a MIZ1 recombinant protein suitable for crystallization and structural determination by X-ray diffraction to understand the molecular bases of its biological function. In addition, the results obtained from the biochemical characterisation will be used to understand the physiological context of MIZ1-ECAs interaction, its effect on Ca^{2+} homeostasis and its relevance in plant responses to environmental cues using transgenic plants and *in vivo* molecular imaging analysis.

The project will be carried out in strict collaboration with Prof Hillel Fromm (University of Tel Aviv), Prof Alex Costa and Prof Stefano Ricagno (Department of Biosciences, University of Milano).

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Project leader: Matteo Brilli, matteo.brilli@unimi.it

Location: The working place will be at the Department of Biosciences, but there will be frequent meeting with colleagues involved in the project working at the Policlinico San Matteo in Pavia and the University of Pavia.

Integrating pathogen genomes and health big data for challenging health-associated infections

At its core, the work of clinical microbiologists consists in the retrieving of a few bytes of information (species identification; metabolic capacities; staining and antigenic properties; antibiotic resistance profiles, etc.) from pathogenic agents. The development of next generation sequencing technologies (NGS), and the possibility to determine the entire genome for bacterial pathogens, fungi and protozoans is introducing a breakthrough in the amount of information generated by clinical microbiology laboratories: from bytes to gigabytes of information. In parallel, the development of novel informatics tools, designed for the management and analysis of the so-called Big Data, offers the possibility to search for patterns in databases collecting genomic and microbiological information on the pathogens, as well as epidemiological data and information on the clinical parameters of the patients. Nosocomial infections and antibiotic resistance are major challenges for clinical microbiologists, and efforts for controlling their diffusion are strongly needed. In this project, the candidate will learn the secrets of bacterial genome sequencing, assembly and annotation and then she/he will apply advanced statistical techniques (Random Forests, Support Vector Machines, Adaptive Boosting) to (i) characterize and better understand how genomics characteristics affect pathogen spreading within and outside hospitals and (ii) use additional orthogonal sources of information about patient status for developing precision-medicine approaches tailored to patients with specific characteristics.

At the end of the PhD, the candidate will have a strong background in (i) bacterial genomics (assembly, annotation, positive selection measures, classification techniques…) and (ii) machine learning and artificial intelligence techniques for big data analysis (RF, SVM, AdaBoost..), together with (iii) knowledge of the major pathogens and problems in clinical microbiology thanks to strong interactions with hospitals.

Project leader: Annalisa Bucchi (annalisa.bucchi@unimi.it)

Location: Department of Biosciences

Study of the molecular mechanisms underlying the genesis and control of the cardiac rhythm in physiological and pathological conditions: FGF21 and sinoatrial node activity

The purpose of this study is to investigate whether the Fibroblast Growth Factors 21 (FGF21) modulates the activity of Sino-Atrial Node (SAN), which is a group of cells that confers automaticity to the heart.

FGF21 is mainly produced in the liver; once released in the bloodstream it reaches its target organs where it contributes to the regulation of whole-body energy consumption, and particularly to the metabolism of glucose and lipids. In addition, FGF21 is also produced by other organs (for example the heart) in response to metabolic stress; the increase in circulating levels of FGF21 in several cardiovascular diseases (including atherosclerosis, coronary heart disease, myocardial ischemia, cardiac hypertrophy, and diabetic cardiomyopathy) has been associated with cardio-protective effects. Although SAN cells are not traditionally considered as targets of FGF21 several findings, among which preliminary data of my laboratory indicating the presence of the FGF21 receptor complex in SAN cells, suggest that FGF21 can modulate the activity of these cells.

Patch-clamp experiments will be performed to evaluate the acute and chronic effects of FGF21 on the electrical properties of murine SAN cells. Immunostaining, quantitative RT-PCR, and western-blot experiments will be performed to gain insights on the molecular pathways activated by FGF21.

These results will provide novel insights on the mechanisms implicated in the regulation of pacemaker cells function by FGF21 and will help to understand the pathological consequences of the alteration of the pathways stimulated by FGF21.

Project leader: Lucia Colombo, lucia.colombo@unimi.it

Location: Dipartimento di Bioscienze

Genetic network controlling reproductive development in plants

The female germ cells are formed inside the female gametophyte by complex developmental programs named sporogenesis and gametogenesis. The process starts with the differentiation of a single cell inside the ovule that undergoes meiosis to give origin to four megaspores. One of these megaspores undergoes three mitotic division to give origin to the female gametophyte.
The project will focus on the genetic and molecular control of female germ line initiation and progression. The differentiation of a single germ line initial that undergoes meiosis is of pivotal importance and it is under a complex post transcriptional, translational and post translational control. We have previously identified a complex required for the initial step of germ line progression involved in translational control mediated by miRNA (TRmR). The Ph.D student will identify new components of this network and functionally characterize them. Furthermore, he/she will isolate and sequence miRNAs associated to polysomes in wt and mutant backgrounds to identify targets regulated by TRmR and required for female germ line initiation. To achieve the objectives, the Ph.D student will have to learn and use molecular and genetics techniques such as ChIP, CRISPR-Cas9 genome editing technology, polysome isolation and advanced microscopy techniques.

Project leader: Carlo Camilloni (carlo.camilloni@unimi.it)

Location: Department of Biosciences

Computational design of enhanced class I MHC antigens

In physiological as well as in pathological conditions, the status of nucleated cells is mirrored by a set of processed peptides that are presented to the immune system by the Class I Major Histocompatibility Complex (MHC-I). The interface that results from the combination of the peptide and MHC-I (pMHC) surface is the key for interaction with the T-Cell Receptor (TCR) and is a prerequisite for CD8+ cytotoxic T-lymphocyte (CTL) activation, control and/or clearance of viral infections and tumor cells.

The goal of this project is to develop general rules for the design of altered antigens by characterizing *in silico* the pMHC-TCR recognition mechanism. The underlying hypothesis is that pMHC-TCR recognition is the result of the balance of two processes: the peptide preorganization in solution and the pMHC preorganization, where the former contributes to the pMHC stability and the latter to pMHC-TCR affinity. Both effects are related to the entropy of the system and consequently can be better appreciated by the use of molecular simulations. We propose to compare the conformational dynamics of multiple pMHC-TCR systems for which a crystal structure is already available. In particular we will compare the dynamics of the peptide in solution, with the pMHC dynamics and pMHC-TCR dynamics. From these simulations we aim to 1) verify to which extent the two levels of preorganization hold for different systems; 2) test *in silico* the effect of proline substitutions in non-anchoring positions as well as the role of D- amino acids substitutions on protruding as well as on not-protruding (including anchor) residues to enhance the preorganization of the peptide and pMHC systems; 3) identify the most common interaction patterns between pMHC and TCR to eventually derive a comprehensive set of rules for the design of altered peptides without previous knowledge of the pMHC-TCR bound structure. Eventually, the combination of enhanced sampling molecular dynamics with machine learning techniques will provide a generalized scoring function to evaluate pMHC-TCR interactions.

Project leader: Chiara Rolando chiara.rolando@unimi.it and Graziella Cappelletti graziella.cappelletti@unimi.it

Location: Department of Biosciences, Via Celoria 26, 20133 Milan

Regulation of microtubules and RNA metabolism interaction in the neuron-astrocyte crosstalk during Parkinson's Disease

Parkinson's Disease (PD) is the second most widespread neurodegenerative disease with several pathological features including dopaminergic (DA) neuron loss in the substantia nigra pars compacta [1]. The presence of **α-synuclein aggregation is the key neuropathological hallmark** and there is

emerging evidence that **microtubule alterations can contribute to α-synuclein accumulation** in PD [2].

Post-transcriptional regulation affects several aspects of RNA metabolism, including regulation of RNA stability and translation [3]. Interestingly, α-synuclein interacts with RNA binding proteins (RBPs), the effectors of post-transcriptional regulation. During stress and disease, RPBs can sequester RNAs and form **Stress Granules** (SGs) [4]. However, the contribution of SG formation to PD is still unclear.

We hypothesize that alterations in **microtubules and SG interactions** can contribute to pathogenesis in PD. We will test this hypothesis and focus on **DA neurons** and glial cells, including **astrocytes that are affected and actively contribute to the disease** [5]. The project will have three aims: i) unravel how microtubules and its modifications can contribute to α-synuclein aggregation in DA neurons and astrocytes; ii) identify the role of SGs in astrocyte and DA neuron response in PD; iii) manipulate microtubules and SG interaction in astrocytes to rescue neuropathological alterations in PD. To test our hypothesis, we will perform ***in vivo* and *in vitro* experiments on models of PD**. We will combine state-of-the-art molecular biology tools including **Cross-linking and Immunoprecipitation** (CLIP) and **multiplexed *In Situ* RNA detection** with neuron-astrocyte cultures and **confocal and high content imaging**. We will validate interesting targets on ***in vivo* PD models** and **PD patient brain samples**.

This project will open and define a novel and as yet unexplored field of research. We will show that manipulating microtubules and SG crosstalk will modify pathophysiological hallmarks of PD.

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Project leader: Elena Cattaneo, elena.cattaneo@unimi.it

Location: Department of Biosciences, Laboratory of Stem Cell Biology and Pharmacology of Neurodegenerative diseases at National Institute of Molecular Genetics, Via Francesco Sforza 35, Milano

Stem Cell-based approaches for understanding Huntington's Disease

Huntington's disease (HD) is an autosomal-dominant, progressive neurodegenerative disorder that usually onsets in midlife and caused by a CAG expansion in the Huntingtin (HTT) gene. The disease is characterized by motor, cognitive, and psychiatric symptoms. These symptoms and the progression of HD can be linked to its neuropathology, which is characterized by the loss of specific neuronal populations in many brain regions. Several studies have shown that medium spiny neurons (MSN) are more severely affected and that HTT plays a role in their normal physiology and therefore also in the pathology.

Our aim in this project is to develop strategies to differentiate human pluripotent stem cells into human MSNs for studies related to Huntingtin biology and pathology and for experimental transplantation studies in HD models. One strategy will exploit the single cell RNAseq data from our recent study of the development of the human fetal brain to implement the differentiation of our cells in vitro. Moreover, we plan to use genome editing tools to modify the expression of HTT and/or of the CAG in the gene to modulate and/or knock out HTT in different cell types for functional studies. Quality of the neurons obtained at the end of the differentiation protocol will be verified by a convergence of features such as expression of neuronal markers as well as by neurochemical, transcriptional and bioelectrical analyses. It is expected that the candidate will learn how to expand and differentiate human pluripotent stem cell lines. He/she will then study the impact of gene modifications on the differentiation of the cells in vitro and in vivo, and will characterize the cellular progeny from a transcriptional and biochemical profile. He/she will use the cells to study Huntingtin biology and/or for transplantation studies. The candidate may also develop or exploit bioinformatics skills for data analysis.

The candidate will have the opportunity to learn from exposure to a wide range of technologies and research activities.

Project leader: Alex Costa (alex.costa@unimi.it)

Location: Department of Biosciences

Harnessing the new emerging imaging technologies to uncover the role of Ca2+ signaling in plant nutrient homeostasis

Increasing crop yields by using eco-friendly and sustainable practices is of high priority to tackle problems regarding food security and malnutrition worldwide. To reach these goals, the scientific community aims to decipher the local and systemic nutrient sensing and signaling mechanisms of plants. In this context, several lines of evidence about the involvement of Ca^{2+} as the signal of an

impaired nutrient availability have been reported. Ca^{2+} signaling is a complex and tightly regulated process which requires specific protein toolkits to perceive external stimuli and to induce the specific responses in the plant needed to survive.

The progress obtained during recent decades about new emerging technologies, based on the use of genetically encoded Ca^{2+} sensors and advanced microscopy (wide-field, confocal and Light Sheet) (Candeo et al., 2017; Costa et al., 2018) offers the chance to perform high resolution *in planta* analyses of Ca^{2+} dynamics at cellular and subcellular resolution. As highlighted in Wilkins et al. (2016) Ca^{2+}-mediated signaling appears to play a central role in the regulation of nutrient deficiency stress.

The PhD student will start from the generation of new *Arabidopsis thaliana* transgenic lines expressing genetically encoded Ca^{2+} sensors (e.g. Cameleon, GCaMPs and R-Geco1) (Costa and Kudla, 2015) under the control of ubiquitous or tissues-specific promoters (e.g. phloem and xylem parenchymal cells). These transgenic lines will be used in imaging-based experiments to identify the cell types primarily involved in the sensing of Fe nutrient stress (iron deficiency and excess). Moreover, the effects of Fe stress on Ca^{2+}-based long-distance signaling (Choi et al., 2016) will be also evaluated. To discover the players involved in the Ca^{2+}-based nutrient sensing a reverse genetic approach will be pursued by assaying selected mutants (knock out for genes involved in the Ca^{2+} transport mechanisms expressed in the identified tissues) through traditional phenotypical, transcriptional and imaging analyses.

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Project leader: Luca DEL GIACCO – luca.delgiacco@unimi.it

Location: Department of Biosciences, via Celoria 26 – 20133 – Milan, Italy

A multi-tiered approach to unravel the molecular bases of SOD1-mediated ALS Pathogenesis

RATIONALE_To improve the knowledge on the molecular bases behind the onset/progression of Amyotrophic Lateral Sclerosis (ALS). OBJECTIVES_To identify genes/pathways involved in SOD1-mediated pathogenesis of ALS representing new potential disease markers.

DESIGN AND METHODS_Transgenic zebrafish expressing the ALS-linked G93R mutant form of SOD1 Tg (sod1:G93R) will be employed. We recently revealed profound abnormalities in the properties

of these fish spinal neurons starting from very early embryonic stages, supporting the notion that ALS may have a prolonged presymptomatic period, even dating back to embryonic life. For identification purposes, the transgenic fish have been designed to express the DsRed fluorescent protein in response to heat shock. Strikingly, even when maintained at physiological temperatures, the larvae express the DsRed in several interneurons of brain and spinal cord, indicating stress conditions in these cells due to the expression of the Sod1 transgene.

AIMS_We will:

1. Sort the spinal cord and brain DsRed-positive neurons from SOD1 Tg(sod1:G93R) and control larvae, then measure the changes in gene expression by means of RNAseq.
2. Functionally characterize the singled-out genes (Aim 1) through gain- and loss-of-function analyses in zebrafish larvae.
3. Translate the obtained data to mouse, analyzing the expression of the genes previously characterized in fish larvae also in brain and spinal cord extracts from mice carrying the SOD1 G93A mutation.

Project leader: Diletta Dolfini (diletta.dolfini@unimi.it)

Location: Dipartimento di Bioscienze

Bioinformatic Identification of key transcription factors in epithelial tumors

Synergy between Transcription Factors regulates gene expression. TFs are pivotal in the control of cell growth: alteration of their structure or expression leads to tumorigenesis. NF-Y is a "pioneer" TF binding the CCAAT box (1), a crucial element of promoters. It is a trimer made of histone fold domain dimer NF-YB/NF-YC and the sequence-specific regulatory NF-YA. NF-YA exists in two major isoforms, “short” and “long”. Independent evidence suggest that NF-Y plays a relevant role in cancer progression. (i) Profilings of tumor vs normal cells show enrichment of CCAAT in cancer "signatures" genes (2). (ii) A genome-wide connection to a plethora of oncogenic and growth controlling TFs. (iii) Crucial genes in metabolic pathways altered in cancer cells are controlled by NF-Y (3). (iv) Yet another isoform -NF-YAx- is overexpressed -and plays an important role- in glioblastomas (4). NF-YAs correlates with a poor prognosis in ovarian cancer (5). In the last years, we define NF-YA isoforms role in epithelial cancers -breast (6), lung (7,8), head and neck: we found that NF-YAs levels correlate to a proliferative signature, while NF-YAl is associated to mesenchymal cellular identity.

Tumors rely on CCAAT-binding to activate a significant number of "cancer" genes. An upregulation of NF-YA could corroborate the malignant transformation of cells and a switch between long and short isoforms could push an invasive behavior concurring to formation of metastases. The critical questions are: could NF-YAs subvert normal gene expression and mediate transformation? Could NF-YA isoforms switch be a marker of transformation and aggressiveness of tumor cells?

We aim at obtaining a fine molecular knowledge of NF-YA isoform switching during transformation of cells in breast and sarcoma cancers. We will perform in-depth analysis of available breast and sarcoma gene expression data to characterize the switching of NF-YA and in general of TFs isoforms in these tumors. This project will clarify the role of NF-YA and TF isoforms in defining epithelial or mesenchymal cancer signatures.

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Project leader: JUAN IGNACIO EZQUER GARIN (juan.ezquer@unimi.it)

Location: Dipartimento di Bioscienze

Deciphering new molecular regulators towards next generation hybrids in Agriculture

In many plant species, crosses of parents to partners of a lower ploidy leads to non-viable seeds that are considered to arise due to defects in the seed endosperm. This interesting phenomenon in plant biology is called the “triploid block”. Understanding the molecular effectors and the signalling pathways controlling this phenomenon is the main objective of this PhD project. Thus will pave the ground for designing genetic strategies to overcome interploidy hybridization barriers, holding immense potential for plant breeding. While the majority of triploid block phenomena seem to relate to paternal genome excess progeny, maternal genome excess progeny defects have also been observed. To date a small number of suppressors of the triploid block in Arabidopsis thaliana have been identified. In our laboratory we have identified several epigenetic regulators, transcription factors and natural accessions of Arabidopsis thaliana, which act as candidate suppressors of the triploid block. In order to validate the role of them, we propose to study the role of these candidate genes (or genetic backgrounds in the case of natural accessions) controlling triploid block and related post-reproductive

phenomena in Arabidopsis thaliana. Information will be used in other crops available in the lab (Rice, Solanaceae and Brassicaceae) to design new-generation hybrids of interest.

In this project, the PhD candidate will use a wide range of different techniques and modern methodologies available in the department: from genetic and biochemical analysis, cell biology techniques, advanced confocal imaging, studying protein reporters as well as hormone trafficking and oxidative stress monitoring, together with the integration of NGS data analysis. The student will be involved also in international collaborations with other labs. The thesis hereby proposed will introduce the student to advanced concepts in plant biology with a focus on understanding at the molecular, physiological, and subcellular levels, how seeds develop. This holistic view will form the PhD candidate onto how selective molecular breeding aimed at the generation of new hybrids in agriculture.

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Defining the molecular and cellular mechanisms by which tissue macrophages promote angiogenesis in neovascular diseases

The formation of blood vasculature (angiogenesis) is a physiological process during development

and we previously unveiled that tissue-resident macrophages interact with nascent blood vessel sprouts to promote vessel fusion (anastomosis) during mouse and zebrafish development (Fantin et al., *Blood* 2010, PMID: 20404134). However, angiogenesis can be stimulated following injury and be detrimental during certain diseases, such as solid tumors and neovascular eye diseases. Instead, the formation of new vessel circuits can compensate for the lack of blood flow following ischemia. Macrophages have been implicated in these neovascular processes, still the molecular mechanisms involved in macrophage-promoted angiogenesis remain mostly unclear (e.g. Liyanage et al., *Arter Thromb Vasc Biol* 2016, PMID: 26603154).

Our preliminary data show that tissue macrophages in the developing mouse brain express the insulin like growth factor *Igf1* and are required for IGF1 signaling. Notably, we found *Igf1* mRNA to be upregulated during oxygen-induced retinopathy (OIR), a model of neovascular eye disease in which macrophages are essential for the formation of neovascular lesions, and following hindlimb ischemia (HLI), in which macrophages can promote flow recovery. IGF1 signaling has previously been suggested to promote angiogenesis; however, it is unclear whether macrophages modulate angiogenesis by secreting IGF1 to promote blood vessel anastomosis.

One aim of this project will be to elucidate the role of macrophage-derived IGF1 during blood vessel anastomosis using microfluidic devices for 3D cultures complemented by genetic manipulation in zebrafish and mouse development, as models of stereotyped angiogenesis, as well as more complex disease models, such as OIR and HLI. The other aim will be to combine flow cytometry and transcriptomic profiling with functional studies to identify the full pro-angiogenic gene expression signature of tissue macrophages and define the relevant signaling pathways that are activated by macrophages in ECs during angiogenesis in health and disease.

Recapitulating, the overall goal of this project is to reveal novel molecular and cellular mechanisms by which macrophages promote angiogenesis in neovascular diseases and will provide for the first time a systematic investigation of macrophage function in both development and disease settings from a vascular perspective. The completion of this proposal will deliver innovative knowledge that will help exploit macrophage biology to devise efficient strategies to therapeutically modulate angiogenesis in different human diseases.

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Molecular control of plant reproductive development in response to day length

The transition from a vegetative to a reproductive stage in rice occurs upon measurement of day length and consequent expression of two essential florigens belonging to the phosphatidyl-ethanolamine-binding protein family. Rice florigens are encoded by *HEADING DATE 3a* (*Hd3a*) and *RICE FLOWERING LOCUS T1* (*RFT1*), which are transcribed and translated in the leaf vasculature and then reach the shoot apical meristem through the phloematic stream. At the apical meristem, they bind to a 14-3-3 protein (Gf14c) and to OsFD1, a bZIP transcription factor, to form the Florigen Activation Complex (FAC) and activate inflorescence formation. During this stage, transcription of a third florigenic gene closely related to *Hd3a* and *RFT1*, *FLOWERING LOCUS T LIKE 1* (*FT-L1*), is also activated. Mutations in *FT-L1* alter both flowering time and inflorescence development, suggesting that a triple florigen system is necessary for correct inflorescence development.

In this project the student will explore how the upstream regulators of *FT-L1* are responsible for its activity in the reproductive meristem, and how patterns of accumulation of florigenic proteins are modulated by day length at the shoot apical meristem. The experimental plan will require molecular biology, genetics, and biochemistry approaches.

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Identification and characterisation of new factors involved in maintaining genome stability

The maintenance of genome integrity is essential for the survival of the cell and the faithful transmission of genetic material. Due to the numerous lesions that can damage DNA and compromise its stability, all living organisms have evolved complex mechanisms of surveillance and damage repair.
Mutations in these control systems are extremely harmful to cells because they are a source of genomic instability which, in turn, can lead to cell death and, in the most complex eukaryotes, is the cause of hereditary genetic diseases and precedes and guides the process of carcinogenesis.
Knowledge of the molecular mechanisms that allow to respond to genetic insults and that underpin the maintenance of genome integrity is fundamental for understanding cell survival strategies, for identifying the causes of genetic diseases and tumors and for developing new therapies that are more effective and have less cytotoxic effects.
With this research project we aim to use yeast as a model organism to identify, through a genetic and molecular biology approach, new genes responsible for genome stability and then extend the knowledge acquired to more complex organisms.

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Stress-response signal integration by Transcription Factors interactions

Transcriptional regulation is at the heart of all biological processes and is governed by transcription factors (TFs) which bind to discrete genomic regions. Many protooncogenes and tumor suppressors are TFs and their dysregulation leads to uncontrolled cell growth and cancer.
One mechanism often deregulated in malignant cells is their adaptation to the adverse conditions of nutrient and oxygen limitations in the tumor mass microenvironment, which result in Endoplasmic Reticulum (ER) stress, and upregulation of the Unfolded Protein Response [1]. The UPR comprises diverse effector pathways, which include transcriptional activation mediated by TFs of the bZIP family. Detailed analyses of different ER-stress promoters have established that the critical precisely spaced CCAAT-N9-CACG ERSEs (ER Stress Elements) are bound by the ubiquitous TF NF-Y and the bZIP TF ATF6, recognising the CCAAT and CACG motifs for synergic, stress dependent, transcriptional activation [2,3]. The CCAAT box is a common DNA element also found in promoters of growth controlling genes, bound by the trimeric TF NF-Y, a pivotal transcriptional regulator known to synergyse with and to facilitate TF

binding to DNA [4,5]. Recent genome-wide analyses allowed to map the in-vivo locations of numerous TFs pointing to direct interactions of NF-Y with a discrete set of TFs, including oncogenic bZIPs [6]. We have also shown that NF-Y can play critical roles in cancer progression, by promoting metabolic rewiring, ER-stress resistance, and upregulation of ER-stress response genes [7]. Such information, together with the knowledge of the crystal structure of NF-Y bound to DNA [9], is the foundation of the proposed project.

In this project, we will investigate on fundamental conserved aspects of the UPR which rely on established interactions of central TFs of the bZIP family with the NF-Y heterotrimer, by elucidating the molecular mechanisms of their cooperativity in transcriptional regulation, and their relevance in cellular transformation. We will employ biochemical, molecular genetics and structural biology approaches to pursue the following specific aims: in vitro biochemical analyses of purified proteins to evaluate TFs cooperativity in DNA binding; isolation of TFs ternary complexes with DNA for structural studies, including Cryo-EM analyses, to visualise protein domains involved in TF interactions; in vivo studies of promoter occupancy following TFs inactivation, by ChIP and by ChIP-Seq, to understand possible hierarchy in DNA binding; in vivo functional studies by transient expression with gene promoter-reporter assays to validate and define the molecular bases of cooperativity of the TF partnerships in stress signal responses.

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Location: Department of Biosciences

Pseudomonas aeruginosa determinants for chronic inflammation in cystic fibrosis.

Cystic Fibrosis (CF) is a genetic disease caused by mutations in the CF transmembrane conductance regulator (CFTR). Although the disease affects various organs, the main cause of death in CF patients is chronic lung infections, mostly caused by the Pseudomonas aeruginosa infections. Over

the course of many years of chronic infection in the CF patient, *P. aeruginosa* can undergo multiple adaptive mutations in the CF lung (i.e., increased biofilm production), which in turn makes this bacterium tolerant to the host immune system, and resistant to antimicrobial therapies. An almost paradoxical aspect of CF is that, although *P. aeruginosa* adaptive mutations almost invariably lead to the loss of virulence traits, lung tissue degeneration still progresses, likely due to overly stimulation of the immune system. Thus, while in the majority of bacterial infections activation of the adaptive immune response plays a protective role, in CF the persistent activation of effector T cells can cause exacerbated inflammation and consequent tissue damage triggering an exaggerated inflammatory response ultimately leading to lung failure. In particular, chronic infection of CF lung by *P. aeruginosa* results in triggering differentiation and proliferation of Th1/17 cells that are characterized by co-production of pathogenic cytokines as INF-γ (implicated in epithelial disruption) and IL-17 (sustain additional neutrophil recruitment), that in turn promote chronic inflammation and tissue damage. Based on data recently published in the literature, in which transcriptomic experiments were performed on CF-adapted *P. aeruginosa* isolates taken directly from the sputum of CF patients, we have a clearer picture of what changes in gene expression take place during long term P. aeruginosa adaptation to the host. We will target upregulated genes, selecting them based on their predicted function, and starting with genes involved either in cell surface structure or in gene regulation. We will create isogenic mutants in the selected genes and will test these mutants for their ability to induce differentiation and proliferation of Th1/17 cells. These results will represent a turning point in the understanding of the molecular mechanisms of the disease, as they will, for the first time, define specific P. aeruginosa determinants for the induction of the Th1/17 cell response, which might represent potential targets for the development of new therapeutic strategies.

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Location: Dipartimento di Bioscienze

Functional Analysis of Genes Controlling Inflorescence Architecture in Arabidopsis and Rice

Plants play a crucial role in our society, they are the producers of oxygen, food, feed, medicine, biomass and they decorate our houses and cities. Sustainable production increase is one of the most challenging issues for agriculture since resources like land, water and energy become more and more limited. In this project we focus on inflorescence development in Arabidopsis and rice with the aim to produce more and higher quality seeds. Arabidopsis is used as a model plant and rice is important as a crop but it functions also as a model for cereal research.

The rice inflorescence or panicle has a main axis on which primary branches develop. From the primary branches the secondary branches arise, from which the spikelets are born. The number of branches varies between rice varieties and by that the number of seeds that develop on a panicle. Panicle branching is therefore an important character for rice crop improvement.

Very little is known about the genetic control of panicle branching. Recently, Yoshida et al. (2013) identified a regulator of rice panicle branching called TAWAWA1, however the molecular mechanism is still far from understood. We have in the frame of a French-Italian EVOREPRICE project isolated by laser micro-dissection microscopy isolated the apical, primary and secondary branch meristems of rice inflorescences. This material has been used for RNA extraction and next generation sequencing analysis. The transcriptomes have been analyzed and key genes putatively involved in regulating branching have been identified (Harrop et al., 2016). Similar experiments have been done in Arabidopsis (Mantegazza et al., 2014) and comparisons have been made with the rice datasets. Candidate genes will be subjected to functional analysis using molecular and genetic tools. For making mutants we will use CRISPR-Cas9 genome editing technology. The project is mainly focusing on transcription factors and we are interested in the regulatory pathways that they control. Furthermore, we want to use molecular approaches to

identify their target genes and to obtain a deeper understanding of the molecular and structural aspects of these transcription factors.

In conclusion this project focuses on the molecular genetic characterization of regulatory pathways controlling inflorescence architecture. This information will contribute to the breeding of crops for the sustainable agriculture of the future.

Project leader: Prof. Roberto Mantovani, mantor@unimi.it

Location: Dipartimento di Bioscienze

Role of NF-YA isoforms in breast cancer

Gene expression is governed by the binding of transcription factors to important regulatory elements in promoters and enhancers. The gene expression program of tumor cells is typically altered, and knowledge of these changes has great importance to support the clinical data; a first understanding is appreciated on deregulated genes; a second, deeper level is obtained considering isoforms expression, as it is also acquirable now by NGS. Analysing a huge amount of TCGA mRNA-seq data, we identified (i) a global overexpression of NF-YA, one of the subunits of the trimeric transcription factor NF-Y, in epithelial cancers; (ii) a switch in the two alternatively spliced isoforms of NF-YA in aggressive breast cancers (BRCA). The binding site of NF-Y -the CCAAT box- is typically enriched in promoters of genes overexpressed in tumors, particularly epithelial ones. The two isoforms differ in 28 aminoacids in the transcriptional activation domain, coded by exon 3. In general, an upregulation of NF-YA "short" could push malignant transformation of cells and a switch from "short" to "long" an invasive behavior with formation of metastases. We generated BRCA cell in which exon 3 is ablated by genome editing, and preliminary data concur with this hypothesis. The key question is now: what are the molecular mechanisms? The aim of the Project is focused on the role of NF-YA isoforms in supporting cell transformation and cell migration in selected tumors; in particular, we will dissect the involvement of NF-YA isoforms in determining the acquisition of aggressive migratory aspects in epithelial tumors (BRCA). To do so, we will use genetic approaches, by employing further genome editing approaches, and, subsequently *in vitro* and *in vivo* experiments to characterized edited breast cancer cells.

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Project leader: Michele Mazzanti (michele.mazzanti@unimi.it)

Location: Department of Biosciences

Study of the antiproliferative effect of metformin combined to transcranial stimulation in glioblastoma cellular and animal models

In the last decades, our laboratory focused its attention on tumour physiology, particularly on glioblastoma. Due to its severity, glioblastoma remains incurable and new therapeutic strategies are urgently needed. We recently demonstrated the unique localization of transmembrane CLIC1 protein (tmCLIC1) in glioblastoma stem cells (GSCs), a small subset of the tumour mass considered responsible for tumour resistance and relapse. tmCLIC1 is functionally expressed in the plasma membrane as an ion channel, supporting GSCs proliferation [1, 2]. On the contrary, in healthy cells CLIC1 is mostly cytosolic, suggesting tmCLIC1 as a promising pharmacological target for glioblastoma.
Our previous studies identified tmCLIC1 as a target for the antidiabetic drug metformin [3]. Metformin works as an open channel inhibitor, binding tmCLIC1 inside the pore. However, metformin impairs GSCs proliferation in a millimolar range, a concentration unattainable in the brain upon metformin oral administration.
The goal of this project is to decrease metformin's working concentration, enhancing its action on tmCLIC1. tmCLIC1 is a voltage dependent channel, increasing its open probability upon depolarization. Our working hypothesis envision the possibility that repetitive membrane potential oscillations are able to increase tmCLIC1 open channel time and thus the available metformin binding sites. This approach is supported by strong *in vitro* preliminary data on human GSCs and *in vivo* in glioblastoma mouse models, in which glioblastoma cells were repetitively depolarized through optogenetics tools.
Our translational strategy to glioblastoma patients will be to mimic optogenetic-induced depolarization. Therefore, we propose electromagnetic stimulation in combination with metformin administration. The strategy known *in vivo* as transcranial stimulation should enhance metformin's action causing an impairment of tumour growth, representing a potential adjuvant approach to face glioblastoma.
The graduate student will be involved in the investigation of metformin's antiproliferative effect enhancement by inducing membrane potential oscillations. The research will imply the production of CLIC1 KO cancer stem cells through the utilization of the Crispr-CAS9 technique, cell biology, molecular and electrophysiological experimental procedures.

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Development of genetic and pharmacological approaches to cure Muscular Dystrophies

Muscular Dystrophies (MDs) are severe genetic disorders mainly due to mutations in structural proteins, causing contraction-induced damages (1,2). Previous attempts to treat these diseases raised from the idea that accelerating muscle growth and regeneration would exert beneficial effects. We recently demonstrated that slowing down the degeneration-regeneration cycles and switching muscle fibers towards a slow-twitching phenotype by silencing the transcription factor Nfix leads to a morphological and functional amelioration of the dystrophic phenotype (3-5). The idea to target Nfix in dystrophic muscle was indeed developed, demonstrating that silencing *Nfix* in both *Sgca* null and *mdx-4cv* dystrophic mice morphologically and functionally protects from the degenerative process by promoting a more oxidative musculature and by slowing down muscle regeneration.We therefore provided a new proof of principle for an innovative therapeutic approach based on the idea that slowing down the degeneration-regeneration cycles, instead of increasing regeneration, delays the progression of the pathology. These data are supportive of a new role for Nfix in the progression of MD and suggest Nfix as a novel target to treat this severe disease. Interestingly, we recently identified the upstream signaling responsible for the expression of Nfix (6).
On the basis of the identification of the molecular pathways regulating Nfix expression, we are now developing a pharmacological approach to inhibit Nfix in MDs. Moreover, we set a reporter system to perform a High throughput screening (HTS) of compound libraries to rapidly test thousands to millions of compounds that might inhibit Nfix activity.

The main goal of this three-years PhD project is to evaluate the efficacy of the two FDA-approved drugs and/or new molecules identified by the drug screening in inhibiting Nfix expression and in rescuing the pathological signs in two dystrophic animal models, the *Sgca*-null and *mdx*-4cv. Successful outcome of this project will provide a novel and affordable therapy for MDs.

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Molecular characterization of early infantile epileptic encephalopathy (EIEE) related HCN1 mutations: advancing therapeutics and treatment

Early infantile epileptic encephalopathy (EIEE) is a severe brain disorder with poor prognosis and no adequate therapies characterized by recurrent intractable seizures in newborn and infants. EIEE-causing mutations have been found in the gene coding for Hyperpolarization-activated Cyclic Nucleotide-gated channel 1 (HCN1). HCN1 is a voltage-gated channel widely expressed in the central nervous system (CNS) where it stabilizes the membrane potential of neurons by dampening both inhibitory and excitatory stimuli. *In vitro* studies on EIEE- related HCN1 mutations show that both up- and down-regulation of HCN1 current can be associated with epilepsy, indicating a complex role of HCN1 channels in the development of the disease. The central hypothesis is that HCN1-related EIEE patients who are resistant to conventional antiepileptic drugs may be better treated with drugs that specifically target HCN channels. To this end, we will use our expertise in HCN channel biophysics, structural biology and Molecular Dynamics (MD) simulation together with an array of peptide tools, drugs and noncommercial molecules to mend or attenuate mutation-induced defects in HCN1 channels. Initial results obtained in heterologous systems will be further validated in human iPSC-derived neurons from patients and in knock in mice with HCN1 mutations. The goal is to provide clinicians with robust indications on how to treat individual patients.

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The response to DNA damage in non-proliferating cells: mechanisms and pathologies

Genome stability is continuously challenged by several sources of endogenous and exogenous damage, also at the level of non-proliferating cells. To face this insults and preserve integrity several defense mechanism have evolved, collectively known as DNA damage response (DDR)(1).

According to their location inside the human body, different sources of DNA lesions (e.g. UV light, chemotherapeutic, smoke, pollution) can jeopardize the stability of our genome.

Non-cycling, post-mitotic and terminally differentiated cells like Keratinocytes, Myocytes, Osteocytes, Adipocytes and Neurons compose several tissues, of the human body. Failure to preserve functionality of these non-cycling cells can lead to systematic insults to the affected tissues, resulting in severe pathologies (e.g. neurodegeneration and many types of tissue specific tumors).

For these reasons proper repair of DNA insults in non-cycling cells is as important as in cycling

cells and, from a certain points of view, even more relevant.

The study of genomic integrity maintenance in terminally differentiated cells is therefore of paramount importance, and although several studies have been carried out in the recent years, the underlying molecular mechanisms are still largely unclear.

What happens and which genes are involved in these cells when they experience DNA lesions, is still quite unclear.

We will investigate how the different lesions are resolved and which genes are involved in the different mammalian tissues.

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Structural architecture of the "pioneer" transcription factor NF-Y

and its companions on DNA

Transcription factors (TF) recognize DNA motifs and recruit protein complexes that enable transcription initiation. The binding of most TFs is restricted to regions of the genome that are not packaged into chromatin. Some TFs, the so-called "pioneer" TFs, can however bind to chromatin *via* contacts to its fundamental unit, the nucleosome, and initiate transcription in silent chromatin regions [1]. Pioneer TFs actively help initiate the assembly of regulatory factors on DNA by locally opening chromatin, re-positioning nucleosomes, enabling intrinsic cooperative binding among other DNA-binding factors, and/or recruiting other chromatin modifiers and coregulators. In all cases they are used extensively in developmental gene induction, cell differentiation and reprogramming, and some evolved to promote timely physiologic responses to hormones and are significant in human cancers. Therefore, it has been proposed that inhibitors that block their accompanying pioneer factors may provide novel therapeutic agents [2].

In this context, the present Ph.D. project focuses on **Nuclear Factor Y (NF-Y), a "pioneer" histone-like TF that binds and activates the CCAAT-box [3], its interaction with other relevant TFs, and its inhibition.** In promoters, NF-Y binds the CCAAT-box, typically at -60/-100 bp from the transcriptional start site, cooperating with neighboring TFs, including growth-controlling and oncogenic ones, consistent with the enrichment of CCAAT motifs in promoters of genes overexpressed in cancer. In particular, genome-wide analysis detected a precise stereo alignment between CCAAT and other TF binding sites supporting the concept that the **combinatorial interplay between the pioneer TF NF-Y and other TFs** is at the heart of the regulation of gene expression. Recently, a combination X-ray crystallography [4], Small Angle X-ray Scattering (SAXS) and single-particle Cryo-EM was demonstrated to be successful for the **visualization of NF-Y in complex its target CCAAT-DNA and with other TFs** (MAX and USF1) members of the b-HLH TF family on a promoter oligonucleotide containing the CCAAT-box and the E-box. These data shed first light on the molecular mechanism of DNA-cooperativity binding of the TFs and on the need of a specific and conserved distance in the genome between CCAAT-box and E-box to allow the TF-complex formation (paper in preparation).

In the present Ph.D. project, we propose the following lines of research, aiming (1) to dissect, at a molecular and structural level, the interplay between NF-Y and bZIP (basic leucine-zipper) TFs relevant in cancer and other pathologies, and (2) to identify small molecules able to regulate the function of the NF-Y-based TF machinery.

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Envelope biogenesis in Gram-negative bacteria as molecular target for the development of next generation antibacterial drugs

Gram-negative bacteria are defined by the presence of an outer membrane (OM), which prevents the entry of toxic molecules within the cell, providing resistance to many antibiotics.

The OM is an asymmetric bilayer consisting of a lipopolysaccharide (LPS) outer leaflet and a phospholipid inner leaflet decorated with integral membrane proteins and lipoproteins. The pathways by which the components of the OM are transported and assembled at the OM are partly understood but several aspects remain to be elucidated. LPS is transported from the site of synthesis, at the cytoplasmic side of the inner membrane (IM), to the OM, through the periplasmic space between them, by two essential ABC (ATP-binding cassette) transporters conserved among Gram-negative bacteria: MsbA and Lpt. MsbA catalyses the flipping of LPS across the IM, whereas Lpt allows the transport of LPS across the periplasm to the cell surface.

This project focuses on the Lpt transporter in the Gram-negative model organism *Escherichia coli*. The Lpt transporter is a trans-envelope complex composed by seven essential proteins in *E. coli* which connects IM and OM. The Lpt proteins are grouped into two subcomplexes: LptBFG constitutes the IM ABC transporter, which provides the energy for the extraction of LPS from the IM. LptBFG transporter is physically associated to the bitopic IM protein LptC. At the OM, the integral membrane protein LptD, plugged by the lipoprotein LptE, is responsible for LPS assembly on the cell surface. Lpt inner and outer membrane components are connected in the periplasm by the soluble LptA protein to achieve trans-envelope trafficking of LPS.

Protein-protein interactions among the Lpt components have been studied by our and other groups and have been shown to be crucial to build a functional complex. However, the molecular mechanisms by which the ABC transporter LptBFG couples the energy of the ATP hydrolysis to LPS transport are not completely elucidated, as well as the role of LptC in Lpt transenvelope complex assembly/function.

This project aims at defining the role of LptC by analysing mutants, both arisen by selection as well as generated *ad hoc*, defective in the function of this protein and at gaining mechanistic insights into LptBFG(C)-catalysed LPS transport. Moreover, in collaboration with Prof. Jean Pierre Simorre Institut Biologie Structurale (Grenoble, France) we aim at gaining structural information on wild type

and mutated LptBFG(C) complexes. The information obtained by this work will be used to implement a bacterial two-hybrid system to screen libraries of chemical compounds against the assembly/function of the Lpt transporter.

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RESEARCH PROJECT SUMMARY

Title: Insights into the role of DNA break processing factors in maintaining genome stability to target cancer cells

The ability of cells to detect and properly repair double stranded DNA breaks (DSBs) is essential for maintaining genome stability and preventing cancer development. Indeed, DSBs are the most cytotoxic forms of DNA damage, because inaccurate DSB repair leads to mutations and/or gross chromosomal rearrangements. A critical step in regulating DSB repair is the processing of its DNA ends. Several factors are involved in this mechanism, which are conserved in all eukaryotes. Mutations in most of these factors lead to genome instability, cancer and severe human inherited diseases. Initially, the project will start in model organism *S. cerevisiae* and it will be focused on the characterization of critical factors and mechanisms involved in the processing and repair of Cas9-induced DSB. Indeed, it is an open debate in the literature whether the Cas9-induced DSBs are processed through the classical DSB repair pathways and factors or they are processed by different specialized mechanisms. Importantly, to edit a desired locus in the genome with a donor template (*e.g.* gene sequence substitution or single-codon modifications), resection at Cas9-induced DSB is compulsory. Therefore, all the regulations and cellular aspects (such as cell cycle phase and chromatin context at the target gene), that influence this crucial step of DSB repair, have to be taken into account to foresee the success of the gene editing protocol. In particular, an open question is how Cas9-DSBs are resected, also considering the relative prolonged binding of the Cas9 protein on the substrate once DNA is cleaved *in vitro*. Recently, we

developed a PCR-based method to precisely quantify Cas9-induced DSB in yeast and human genome, which will be of great utility in this project.

In a second part, the project will be extended to human cell lines with the aim to verify the possible conservation of the factors and mechanisms previously obtained in yeast. The Cas9-based technology will be used to create DSBs in defined sites in the genome of different human patient derived cell lines, carrying mutation in critical DSB repair genes. The processing and repair of Cas9-induced DSB will be analysed with standard and innovative PCR-based techniques. The obtained results will be important to define how cells deal with DNA damage and to understand the molecular basis of cancer development. Moreover, the project may clarify certain aspects of the Cas9-based technology and may suggest strategies to improve it.

Impact on cancer

A better understanding of DSB processing and repair in human cells may be informative to design powerful strategies to sensitize cancer cells to chemotherapy. The identification of novel targets that can be modulated by clinically-relevant chemical compounds interfering with DSB resection will be relevant to decrease: i) the dose of DSB inducing agents; ii) the cytotoxicity towards healthy cells; iii) the risk of secondary tumors induced by the therapy and cancer relapse.

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BarPLUS: improving photosynthesis in barley to increase the production of biomass that can be converted into biofuel

Barley is a major crop worldwide, with Europe producing the greatest share (~60 MT/yr). Beside grains barley plants produce an almost equivalent amount of straw that in the past was considered as a secondary product of minimal value. Indeed, most of the genetic progress to increase yield was obtained through a change in biomass partitioning from straw to grains and the current plant architecture has been mainly driven by the necessity of increasing the harvest index. Nevertheless, the increasing demand for renewable materials makes straw, and especially barley straw characterized by the largest content of carbohydrates among cereals, a valuable product for its potential conversion into biofuels and other products. Indeed, barley crop residues are desirable feedstocks because of their low cost, immediate availability, no competition with food, and relatively concentrated location in the major grain growing regions. Given this perspective, we believe that the current barley photosynthesis performance should be revised to maximise the farmer income (grain value plus straw value).

To this aim, BarPLUS will identify genes, alleles and lines needed to increase barley plant biomass, without penalty on grain yield, in the agro-climatic and management scenarios of Italy. This goal will be achieved through improved efficiency of the photosynthetic process and field trial evaluation. We will deploy mutagenized lines and diverse accessions carrying natural allelic variants in candidate genes

(CGs) for components of the light absorption and photo-protection mechanisms of the photosynthetic apparatus to evaluate their usefulness to increase barley biomass production.

Taking advantage of the unique resources of mutants and exome-resequencing data available for barley, BarPLUS will deliver knowledge and tools to develop a new barley variety, which will provide farmers with 5-to-10 % more biomass per hectare without compromising grain yield.

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[Structural bases of amyloid aggregation in Light Chain Amyloidosis]

Systemic immunoglobulin light chain (AL) amyloidosis is a severe protein conformational disease caused by misfolding and deposition of patients-specific monoclonal light chains (LCs). The sequence variability is such that virtually each patient bears a different LC sequence responsible for the disease. Current therapies are of limited help in the presence of advanced organ involvement and do not interfere with LC toxicity, and a significant proportion of patients die within few months after diagnosis, mainly due to cardiac involvement.

We have started decoding the molecular determinants of LC toxicity by biophysically and structurally characterizing 8 patients-derived amyloidogenic and 5 control LCs (Oberti *et al* 2017). We could observe that thermodynamic stability and protein dynamics seem to play relevant roles while changes in structure and hydrophobicity were not relevant. By point mutation, the biophysical properties of pathogenic LC were modified and this resulted into a non-toxic protein variant indicating the fundamental role of kinetic stability and protein dynamics in determining the pathogenicity of LC sequences (Maritan *et al* JMB 2020). Moreover, we have recently elucidated the first Cryo-EM structure of amyloid fibrils extracted from the heart of an AL patient (Swuec *et al* 2019) and the role of proteolysis in the formation of amyloid fibrils is also being investigated (Lavatelli *et al* 2020, under review).

The present project aims to further clarify the relationship between toxicity and protein biophysics using multidisciplinary approaches, which have been already devised in the lab (Le Marchand *et al* 2018). The detailed study of protein structure and flexibility will be performed by X-ray crystallography, spectroscopic techniques, small angle X-ray scattering and site-directed mutagenesis. Such data will be verified on animal models and on human cardiac fibroblasts.

Importantly, given the high sequence variability between pathogenic LCs, the structures of other amyloid deposits from different patients and from several tissues from the same patient will be characterized by Cryo-EM in order to elucidate the structural properties of the aggregated LCs. This structural effort is of paramount relevance towards the design of ligands specifically acting against amyloid aggregates.

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Insight into the Walker-Warburg Syndrome: structure-function of the membrane protein POMT1/2 complex

Walker-Warburg syndrome (WWS) is a severe developmental disorder affecting muscles, brain, and eyes. Most patients do not survive beyond the age of three years. No specific treatment is available[1]. Mutations in the genes encoding for membrane proteins O-mannosyltransferases (POMT) 1 and 2 are associated with WWS (OMIM 236670; 613150). POMT1 and 2 co-assemble to catalyse the initial step of the biosynthesis of O-mannosyl glycans, wherein a mannosyl residue is transferred to the acceptor protein[2]. Because of the co-association, mutations in either of the two genes can equally lead to the development of WWS. A well-known target of POMT1/2 activity is α-Dystroglycan (αDG), a glycoprotein that acts as a linker between the extracellular matrix and intracellular cytoskeleton. αDG expression is widespread, including skeletal muscle and nervous system. Hypoglycosylation of αDG causes typical WWS phenotypes: muscular dystrophy frequently associated with eyes and brain malformation and intellectual disability[3]. Mutations in POMT1 and 2 identified in patients with WWS were found to abolish POMT activity and thus αDG glycosylation[4]. How these mutations cause the loss of POMT activity, however, remains unclear. The development of pharmacological treatments for patients with WWS is urgent and requires: 1) a detailed description of the molecular mechanism of POMT1/2 action; 2) an understanding of the specific effects of the mutations (inhibition of binding to the sugar substrate and/or to αDG).

In order to achieve this objective, the project proposes two complementary and synergistic aims:

- to describe, at atomic level, the POMT1/2 complex by obtaining its 3D structure in the absence and in the presence of the substrate dolichyl phosphate mannose (Dol-P-Man) and the principal target αDG protein. This goal will be achieved by employing the innovative technique of single particle cryo-electron microscopy (cryo-EM);

- to investigate whether mutations associated with WWS affect POMT1/2 interaction with Dol-P-Man substrate and/or with the target protein αDG. This goal will be pursued by employing a protein-ligand binding assay specifically designed to study membrane proteins like POMT1/2.

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Mechanisms of anthocyanin cardioprotection

Dietary flavonoids have received considerable attention since epidemiological studies have suggested that regular consumption of flavonoid-rich foods or beverages is associated with a decreased risk of cardiovascular mortality [1–3], attributed primarily to their antioxidant properties and by modulating cell signaling and metabolic pathways. Among the different classes of flavonoids, anthocyanins (ACNs) are the most recognized, visible members, that contribute to the cardioprotection. In these last years, recent studies have suggested that dietary flavonoids, and more specifically regular ACN consumption, induce a state of myocardial resistance evidenced by a reduced infarct size following regional ischemia and reperfusion [4] that is related, at least in part, to an improvement in the antioxidant defenses of the heart (i.e. cardiac glutathione). Moreover, there are increasing evidences that seem to confirm that many biological effects of ACNs are related not only to their antioxidant properties, but also to their ability to modulate mammalian cell signaling pathways. For instance, an ACN-rich diet modulate the metabolism of (n-3) PUFA and to induce a marked increase in plasma EPA and DHA, fatty acids known to be protective against heart disease complication [5,6]. Our studies on mice treated with the chemotherapeutic agent Doxorubicin (Doxo) have indicated that mice fed ACN-rich diet from purple corn have a better cumulative survival compared to yellow diet upon treatment and do not show the histopathological cardiac alterations (*e.g.* fragmented mitochondria and sarcolemma) associated to Doxo treatment, indicating that dietary ACNs have a strong cardioprotective effect against Doxo [7]. More recently, we have demonstrated that dietary ACNs from purple corn are effective in reducing inflammation associated to obesity and trigeminal pain [8,9].

Aim of this project is to highlight the molecular mechanism of anthocyanin-mediated cardio-protection against Doxo toxicity, focusing on Doxo-induced oxidative stress, inflammation and autophagy dysregulation. To this aim, the project will be divided into the following tasks: (i) to investigate the ability of ACNs to reduce Doxo-induced oxidative stress and inflammation through

activation of the NRF2-mediated antioxidant response and inhibition of the MAPK/NF-kB signaling pathways; (ii) to investigate the Doxo-mediated autophagy dysregulation through the LC3B signaling pathway. This project will therefore shed new light on Doxo-mediated cardiotoxicity, while proposing a counteracting therapy.

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Development and implementation of cloud-oriented bioinformatic tools and workflows

Both the variety and the quantity of data that we can extract from biological systems have been subject to extraordinary growth in recent times due to the advent of Next-Generation Sequencing and other high-throughput technologies. This unprecedented capacity demands a parallel continuous development of the bioinformatic tools that researchers use to organize, manage, share, and analyse the available data to gather meaningful information from them. A technology that is driving the development of new tools for data-intensive bioinformatics is cloud computing, that is the delivery of computing services -servers, storage, databases, networking, software, analytics, intelligence, and the Internet ("the cloud") to offer faster innovation, flexible resources, economies of scale, improved reproducibility and other advantages. This technology, and the strong concurrent emphasis on the expansion of public e-infrastructures devoted to scientific purposes, can significantly improve the availability and capabilities of bioinformatics tools and workflows. Cloud computing effectively removes several barriers that can hamper data-intensive research, as the necessity of acquiring and maintaining local hardware and making sophisticated tools for bioinformatics are readily available in other contexts, e.g., in clinical settings.

Recently we have been involved in the development of Laniakea [1], a software platform based on the INDIGO-DataCloud middleware [2]. This platform can be deployed over any scientific or commercial cloud infrastructure to provide a Galaxy on-demand service to its users. Galaxy is a popular software platform for accessible, reproducible and collaborative analysis of bioinformatics data [3]. The end-

user interacts with Laniakea through a front-end that allows a general setup of the Galaxy instance, then Laniakea takes charge of the deployment of the virtual hardware and the software components. At the end of the process, the user gains access to a private, production-grade, yet fully customizable Galaxy virtual instance. Laniakea supports the deployment of plain or cluster backed Galaxy instances, shared reference data volumes, encrypted data volumes, and the rapid development of novel Galaxy flavours, which is Galaxy configurations tailored for specific tasks.

This Ph.D. project aims to build on this result, expanding Laniakea to make it grow into a complete and integrated virtual research environment. This goal will be pursued from multiple angles:

integration of more bioinformatic tools for our virtual Galaxy instances, support to different workflow engines (e.g., the Common Workflow Language), better connection with data sources, integration of containerization technologies (e.g., Docker, Singularity), provision over the cloud of additional data analysis platforms and tools.

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