UNIVERSITÀ DI TRENTO
Dipartimento di Biologia Cellulare, Computazionale e Integrata - CIBIO
Corso di Dottorato in Scienze Biomolecolari

Corso di dottorato in Scienze Biomolecolari
PhD in Biomolecular Sciences
Ciclo 39 / Cycle 39
A.Y. 2023-2024

Reserved scholarship F

Progetti finanziati nell'ambito dei Dipartimenti di Eccellenza 2023-2027.
Curriculum Bio-Industry
Il vincitore sceglierà il progetto di ricerca dall'elenco sottostante.

MUR-funded grants - Departments of Excellence 2023-2027.
Bio-Industry Curriculum
The winner will choose from the list available below the research project.

Principal Investigator	Project title
1 – Marta Biagioli & Toma Tebaldi	*Parsing the role of telomere biology and TERRA non-coding RNA in brain pathologies by high-throughput and single cell approaches*
2 – Erik Dassi & Alberto Inga	*Interplay between the FASTK and DHX30 RNA-binding proteins in pancreatic cancer: mitochondrial metabolism meets translation control and resistance to apoptosis to reveal new therapeutic targets*

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Project 1

Decrypting metastatic potential of primary liver tumors

Laboratory:
NeuroEpigenetics Laboratory (https://www.cibio.unitn.it/184/neuroepigenetics-laboratory)
Laboratory of RNA and Disease Data Science (https://www.cibio.unitn.it/1349/laboratory-of-rna-and-disease-data-science)

Principal Investigator: Marta Biagioli & Toma Tebaldi
This project will be in collaboration with Prof.ssa Maria Caterina Mione and Prof. Emilio Cusanelli

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Synthetic description of the activity and expected research outcome

Located at the chromosomal ends of each eukaryotic organism, telomeres possess a crucial role in protecting genomic DNA from replicative erosion and DNA damage. Characterized by heterochromatic regions, the maintenance of such structures is fundamental for genome integrity and cell viability. Therefore, telomeres need to be strictly regulated by telomerase and shelterin complexes, and telomeric repeat-containing RNA (TERRA), a class of long non- coding RNA transcribed from those regions. With no surprise telomere dysfunction is linked to a wide range of pathologies, including bone marrow failure, premature aging disorders and cancer. In recent years, a connection between telomere shortening and neurologic disorders has been reported in association studies, and little is known about the mechanisms and molecular determinants that drive telomere shortening and pathogenesis in these diseases.

The general aim of this study is to dissect the role of telomere maintenance in brain disorders, focusing on neurodevelopmental conditions (such as Autism Spectrum Disorders, ASD) and pediatric brain tumors. In fact, while the question of how mutations in the same genes can drive both cancer and neurodevelopmental disorders remains puzzling, understanding the underlying mechanisms has important clinical implications.

With this project, we aim to investigate whether altered telomeres' regulation is implicated in the 'promiscuity' of gene mutations (CHD1/8/2, PTEN and others) - associated with oncogenic potential in some cases, andcorrelated with neurodevelopmental syndromes in others.

We aim to address this question by blending molecular biology techniques - aimed at characterizing telomere length, telomerase activity and chromatin landscape at telomeric regions - with computational approaches - to develop specific pipelines to detect and quantify TERRA repetitive, non-coding RNAs in various datasets, publicly available and generated in house from our disease models.

1. Characterization of telomere length and telomerase activity in genetic in vitro and in vivo models of neurodevelopmental disorders and brain tumors.

We aim to generate and characterize in vitro (human induced pluripotent stem cells (iPSCs)) model systems, carefully replicating the genetic mutations correlating with ASD and brain cancers. We then will study the impact of these mutations on transcript, protein levels, evaluate the genomic integrity (by karyotyping) and pluripotency (by in vitro differentiation assays). Human iPSCs will be then differentiated to neuronal progenitors (hNPCs) or directly to terminal differentiated neurons (hTDNs). We will characterize TL on DNA samples via telomere restriction fragment analyses and southern blot. Genomic instability will be evaluated by assessing activation of DNA damage response via immunofluorescence using anti-γH2AX and 53BP1 antibodies followed by DNA-FISH using a telomeric repeat-specific probe. Finally, by using TRAPeze RT Telomerase Detection Kit, a highly sensitive in vitro fluorometric detection and real time quantification kit, analysis of telomerase activity will be conducted. The same studies have already been conducted in an in vivo model of brain cancer (Lab Mione) and the model will be used as a paradigm for the study of telomere maintenance in pediatric brain cancer.

2. Characterization of telomeres' chromatin landscape.

Because genetic promiscuous mutations might directly (by precisely impacting the functionality of chromatin regulators) or indirectly (by altering TERRA non-coding RNAs and/or other mediators) affect chromatin organization and compaction at telomeric regions, we aim to capitalize on the previously generated and characterized in vitro and in vivo model systems, to test whether the status of the major heterochromatic histones modifications (H3K9me3, H4K20me3) and transcription factors/insulators (CTCF) might be altered at telomeres by chromatin immunoprecipitation quantitative dot blot (ChIP-dot blot) analysis.

3. Characterization and quantification of TERRA by sequencing.

A general question addressed by the project is how much of the subtelomeric sequence is expressed in each TERRA molecule, and what is their role. The project aims at gaining an understanding of TERRA expression by high-throughput approaches, in particular next-generation sequencing. In this framework, one aim is to optimize a protocol/pipeline to detect TERRA transcripts from short-read and long-read sequencing libraries, based on alignment to subtelomeric regions and the presence of telomeric repeats. This will allow not only the characterization, but also the quantification of TERRA expression in different conditions, as well as the analysis of TERRA splicing and polyadenylation.

Methods for the high-throughput analysis of TERRA expression:

a) nanopore direct long-read RNA sequencing of TERRA enriched RNA libraries (telomeric repeat probes, polyA probes)

b) TELL-seq: short read sequencing of TERRA enriched RNA libraries (telomeric repeat probes, polyA probes, probes defined by subtelomeric regions)

c) Long-read sequencing by PACBIO

4. Detection or prediction of TERRA expression in single-cell RNA-seq datasets.

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Detection of TERRA expression in single cell datasets will allow us to explore their expression in specific cell subpopulations and their potential association with disease-affected cells. Due to the low expression levels of TERRA RNAs, the detection in single-cell libraries is more challenging. The first analysis will be performed on datasets from single-cell platforms that maximize sequencing depth within each cell, such as Smart-seq. A complementary approach would be the single-cell inference of TERRA expression, by looking at the expression of RNAs and proteins promoting TERRA expression (for example the RALY RNA binding protein, or the activation of ALT mechanism for telomere elongation). The aim of this analysis would be the definition of a TERRA expression score, based on the activity of gene modules associated with TERRA expression. The optimization and benchmarking of these scores will be obtained by employing publicly available single cell datasets (brain tumors/neurodevelopmental syndromes datasets) and later validated with newly generated data.

Candidate's profile (skills and competencies)
The ideal candidate has previous experience in molecular and cellular biology and wants to learn computational biology approaches.

Project 2

Interplay between the FASTK and DHX30 RNA-binding proteins in pancreatic cancer: mitochondrial metabolism meets translation control and resistance to apoptosis to reveal new therapeutic targets

Laboratory:
Laboratory of RNA Regulatory Networks (https://www.cibio.unitn.it/1096/rna-regulatory-networks)
Laboratory of Transcriptional Networks (https://www.cibio.unitn.it/85/laboratory-of-transcriptional-networks)

Principal Investigator: Erik Dassi & Alberto Inga

Synthetic description of the activity and expected research outcome
Pancreatic ductal adenocarcinoma (PDAC) is a frequent cancer with a dismal prognosis and a 5-year survival rate of less than 10%. It carries a complex signature of aberrations impacting core cellular pathways, with KRAS mutated in most tumors. Despite considerable efforts, we struggle to improve survival to PDAC, calling for innovative treatment strategies. Addiction of tumor cells on oxidative phosphorylation (OXPHOS) is an emerging concept, already observed in glioma and few other tumor types. Also in PDAC, survival to ablation of KRAS signaling was shown to depend on OXPHOS. This addiction could thus represent a weakness to be exploited towards PDAC treatment.

The Fas Activated Serine/Threonine Kinase (FASTK) RNA-binding proteins (RBPs) are known players in mitochondrial RNA metabolism. These six evolutionarily conserved proteins (FASTK, FASTKD1-5) are endowed with partially overlapping regulatory mechanisms and also act in the cytoplasm. FASTK has been suggested to have an anti-apoptotic role, while the effect of the other members of the family on mitochondrial activity in PDAC has not been investigated yet.

DHX30 was recently shown by us to participate in a translation control process that negatively buffers p53-dependent apoptosis. We also showed that DHX30 exerts functions both in the cytoplasm and in mitochondria due to two isoforms produced by alternative promoters, that collectively coordinate cell energetic balance with both global and specific mRNA translation control. Both FASTKD2 and DHX30 were previously shown to be required for mito-ribosome biogenesis. Furthermore, data from the Dassi lab revealed that FASTK protein families are dysregulated in more than a quarter of PDACs, and results from depletion experiments in the MIA PaCa-2 cell model led to the proposal that FASTK and FASTKD4 (TBRG4) have opposite functions on cell fitness, with the latter promoting cell apoptosis. Interestingly, FASTKD4 silencing led to upregulation of DHX30.

Based on the data and tools available, the Dassi and Inga lab are joining forces to propose a PhD project to deeply investigate the interplay between FASTK, FASTKD4, and DHX30 in pancreatic cancer, in particular focusing on the mechanisms by which FASTKs can regulate isoform-specific DHX30 expression and the consequences of this interplay for mitochondrial function and cell fitness, including global translation, activation of integrated stress response, and apoptosis proneness. As recently shown by Moody et al, and consistent with our results of isoform-specific depletion experiments of cytoplasmic and mitochondrial DHX30, experiments with single cell resolution will be included to unravel the impact of FASTK-DHX30 functions and interactions on cell metabolism and fitness.
Experiments will also include omics-based characterization of global cell responses by translatome, transcriptome and RNA-protein interactome analyses. These datasets will be integrated by network-based omic analysis tools and machine learning approaches. The results will eventually be augmented with post-transcriptional regulatory annotations to trace the FASTK-DHX30 regulatory network.

Candidate's profile (skills and competencies)
The Tutors have an established track record of collaboration and will provide a multidisciplinary training experience in molecular and computational biology. An ideal candidate profile is a master student with a strong background in quantitative biology that is willing to be involved both in functional assays, sample preparations for omics analysis and data crunching.