EuReCa International PhD Program

PhD thesis project

2023 Call for application

Contribution of the roles of RNA species in the regulation of the human NHEJ-factor KU functions during DNA replication

General information

Call	2023
Reference	2023-08-PENNANEACH_LAMBERT
Keyword(s)	DNA replication stress, Genome Stability, Structural Biology, Super-resolution microcopy, Breast cancer.

Director(s) and team

Thesis director(s)	Vincent Pennaneach & Sarah Lambert
Research team	DNA Recombination, Replication and Genome Stability
Research department	UMR3348 - Genome Integrity, RNA and Cancer

Description of the PhD thesis project

Cell proliferation depends on the faithful and accurate duplication of the genome. The integrity of the genome is as its most vulnerable during DNA replication. The DNA replication machinery is continuously threatened by a broad spectrum of unavoidable fork obstacles such as DNA damage causing fork stalling and collapse, a phenomenon known as replication stress (RS) (Zeman, NCB 2014). Failure to safeguard genome stability upon RS is a potent driving force behind the onset and progression of cancer cells. Cells have evolved a range of factors, known as the “DNA Damage Response” (DDR) to protect the genome by repairing DNA lesions and promoting the completion of DNA replication. Understanding the underlying DDR mechanisms is an important aspect of cancer biology, as DDR dysfunction is linked to cancer development and several DDR factors are potent therapeutic targets, especially to target exacerbated RS in cancer cells.

Replication fork processing usually relies on homologous recombination for error-free repair. However, recent works indicates that non-homologous end joining (NHEJ) is unexpectedly active during DNA replication to ensure fork protection, restart and repair (Audoynaud, TIGS 2021). The lab recently discovered a novel RNA-based mechanism to regulate the function of the NHEJ-factor Ku in the processing of stressed forks in fission yeast. This proposal aims at unraveling how RNA species modulate human KU function during RS response.

Objectives

Human Ku is part of the multistep mechanism of fork-degradation (Dhoonmoon Nat Com. 2022). Ku binding to RNA:DNA hybrid at RS site is likely to represent an underappreciated level of regulation yet crucial in fine-tuning NHEJ events at stressed fork. To solve this, this interdisciplinary program integrates state-of the art cell and molecular biology approaches associated to biophysics methods to reveal the fundamental aspects of human Ku functions at stressed forks and how RNA species may modulate these functions. Such understanding may pave new avenues to define novel therapeutics approaches.

We will study:

1 The molecular determinants of Ku binding to nascent DNA in human cells: i) the role of RNA:DNA hybrids and their origin, ii) what type of stressed forks are bound by Ku using distinct RS inducing agents and conditions, iii) the role of DDR kinases Such analysis will be performed in established cell lines and extended to triple negative breast cancers models.

2 The structural determinant of human Ku binds to RNA:DNA hybrids and DNA with the aim to define separation-of-function mutants.

3 The cell response to RS in separation-of-function mutants to elucidate how RNA species modulate its function during RS.

International, interdisciplinary & intersectoral aspects of the project

International

The student will visit the "Single-Molecule Biophotonics research laboratory" (New York, University School of Medicine) to be trained in cutting-edge single molecule fluorescence imaging techniques and computational methods. Super resolution microscopy allows single molecule detection with spatial resolution tenfold improved over conventional confocal microscopy to track Ku at replication fork (Whelan DR, Lee WTC, Marks F, Kong YT, Yin Y, Rothenberg E. Super-resolution visualization of distinct stalled and broken replication fork structures. PLoS Genet. 2020 Dec 28;16(12):e1009256.).

Intersectoral

The student will benefit from mentoring and secondment by people working in private sector will share and pass on his experience to the student, act as accompanying tutor and participate to the yearly PhD committees.

Interdisciplinary

The interdisciplinary of the proposal lies in the complementary approaches used to reach the goals. In addition to receiving training in molecular and cell biology, the student will be exposed to technics and concepts at the frontiers of physics. Training in super resolution microscopy will allow the student acquiring interdisciplinary skills in the physics principles of cell imaging and computational methods for analysis. The collaboration with JB. Charbonnier, who was pioneer to solve crystal and CryoEM structures of human Ku-DNA complexes (Nemoz C, Ropars V, Frit P, Gontier A, Drevet P, Yu J, Guerois R, Pitois A, Comte A, Delteil C, Barboule N, Legrand P, Baconnais S, Yin Y, Tadi S, Barbet-Massin E, Berger I, Le Cam E, Modesti M, Rothenberg

E, Calsou P, Charbonnier JB. XLF and APLF bind Ku80 at two remote sites to ensure DNA repair by non-homologous end joining. Nat Struct Mol Biol. 2018 Oct;25(10):971-980.), the student will acquire skills in state-of-the-art biochemistry (ITC, EMSA) and structural biology. Beyond the technical and specific training, the student will be confronted with diverse and interdisciplinary scientific cultures that will nurture his/her scientific maturity and vision.

Recent publications

1. Zeman MK, Cimprich KA. Causes and consequences of replication stress. Nat Cell Biol. 2014 Jan;16(1):2-9.
2. Audoynaud C, Vagner S, Lambert S. Non-homologous end-joining at challenged replication forks: an RNA connection? Trends Genet. 2021 Nov;37(11):973-985.
3. Charlotte Audoynaud, Anissia Ait Saada, Paloma Fernández Varela, Armelle Gesnik, Virginie Boucherit, Virginie Ropars, Karine Fréon, Jean Baptiste Charbonnier, Sarah Lambert. RNA:DNA Hybrids From Okazaki Fragment are *cis*-Regulators of Ku Function in Safeguarding Replication Fork Integrity. Under revision (https://papers.ssrn.com/sol3/papers.cfm?abstract_id=4017909).
4. The KU-PARP14 axis differentially regulates DNA resection at stalled replication forks by MRE11 and EXO1. Dhoonmoon A, Nicolae CM, Moldovan GL. Nat Commun. 2022 Aug 27;13(1):5063.

Expected profile of the candidate

Applicants should hold a Master degree level in Biology. A solid background in molecular and cellular biology and the mechanisms of maintaining genome stability is strongly recommended. The project highly relies on interdisciplinary aspects for which the applicant should show an ability to integrate into an international research team, to demonstrate a broad scientific curiosity and personal investment.