MASTER 2 BMC
PARCOURS GENOPATH
ANNEE 2018-19

Titre du sujet de stage :

Repair and nucleolar reorganisation of ribosomal genes.

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Sujet de stage :
Project description:

The DNA is constantly challenged by a plethora of endogenous and exogenous DNA damaging sources. To counteract the deleterious effects of DNA damage, cells are equipped with various DNA repair pathways. Nucleotide Excision Repair is one of the most versatile DNA repair systems, removing helix-distorting DNA adducts in a coordinated multi-step process. Many aspects of the Nucleotide Excision Repair pathways have been revealed and most of the key players identified. Nevertheless, important questions are still unanswered. One of these questions is:

How ribosomal DNA is repaired within the nucleolus?

Ribosome biogenesis is the most energetically costly activity of cells, particularly of high-metabolism cells, such as neurons. RNAP1 transcription, the first and rate-limiting step of ribosome biogenesis, is specifically dedicated to produce the ribosomal RNAs (rRNA) from the ribosomal DNA (rDNA) located in the nucleolus.

Within DNA Repair, uncharted territories still remain to be explored; repair of rDNA is one of these territories. The need of acquiring knowledge on this field is more and more evident in view of the importance of ribosome biogenesis for cells like neurons and myocytes, which require a high amount of protein production and hence a high amount of ribosomes. More specifically, neurological problems in Cockayne Syndrome and Trichothiodystrophy patients can be due to problems in ribosome biogenesis. We have recently demonstrated that the full Transcription Coupled-NER machinery repairs rDNAs.

Interestingly, during repair reaction the complex rDNA/RNAP1 is displaced at the border of the nucleolus, concomitantly with a transcription block. When repair is completed, rDNA/RNAP1 return within the nucleolus and transcription restarts. Importantly, we have demonstrated that this return step is strictly dependent on the full repair of UV-lesions within untranscribed regions proximal to rDNA (manuscript in review). This finding is entirely novel and in contrast with the dogma that solely transcription inhibition can retain RNAP1 at the periphery of the nucleolus.

Currently, our research group is investigating which are the proteins involved in this nucleolar reorganisation: namely, (i) the displacement of the rDNA/RNAP1 at the periphery of the nucleolus after UV irradiation and (ii) its return within the nucleolus after completion of repair.

Recently, we have identified 3 proteins that block the return of the rDNA/RNAP1 complex within the nucleolus even when repair is fully completed. Namely, Centrin-2 CEN-2 (a partner of the repair protein XPC), Nuclear Myosin (NMI) and Nuclear Actin (ACT□) play an essential role in this nucleolar reorganisation (manuscript in preparation).

Our research programme will be focused on:

<u>Defining the molecular mechanism that governs the nucleolar reorganisation during rDNA repair.</u>

In order to achieve this we will:

(i) Define possible interactions between CEN-2, NMI and ACT□ by co-immunoprecipitations on nuclear and nucleolar extracts.

(ii) Investigating the role of Calcium (important for the activity of CEN-2 and NMI) in the nucleolar reorganisation.

(iii) Finding new proteins involved in this nucleolar reorganisation by an si-RNA-based unbiased screening. The read out will be the position of RNAP1-GFP within the nucleolus. The siRNA libraries that will be screened will contain siRNAs against structural proteins, chromatin remodelers, kinases, transcription and DNA Damage Response factors.

The subject of this internship will be within these three objectives.

Technologies utilisées :

Cell culture, transfections, RT-qPCR, Western Blot, RNA Fish, Immunofluorescence, Fluorescence Recovery After Photobleaching, Confocal Real-Time Microscopy, Unscheduled DNA Synthesis, RNA Recovery Synthesis, Western Blot, Chromatin Immunoprecipitation.

Mots clés :

DNA Repair, Transcription, Nucleolus, RNA Pol I

Publications d'intérêt :

Mourgues S., Gautier V., Lagarou A., Bordier C, Mourcet A., Slingerland J., Kaddoum L., Coin F., Vermeulen W., Gonzales de Peredo A., Monsarrat B., Mari PO# and **Giglia-Mari G,#* (2013)** ELL, a novel TFIIH partner, is involved in transcription restart after DNA repair. **Proc Natl Acad Sci U S A**; Oct 29; 110(44): 17927-32.

Nonnekens J, Cabantous S, Slingerland J, Mari PO, and **Giglia-Mari G* (2013).** In vivo interactions of TTDA mutant proteins within TFIIH. **J Cell Sci**. Aug 1;126(Pt 15):3278-83

Nonnekens J, Perez-Fernandez J, Theil AF, Gadal O, Bonnart C#, **Giglia-Mari G**.#* **(2013)**. Mutations in TFIIH causing trichothiodystrophy are responsible for defects in ribosomal RNA production and processing. **Hum Mol Genet** Jul 15;22(14):2881-93.

Kleppa L, Mari PO, Larsen E, Lien GF, Godon C, Theil AF, Nesse GJ, Wiksen H, Vermeulen W, **Giglia-Mari G*,** Klungand, A #* **(2012).** Kinetics of endogenous mouse FEN1 in base excision repair. **Nucleic Acids Res .** 40: 9044-9059. Co-corresponding

Godon C#, Mourgues S#, Nonnekens J, Mourcet A, Coin F, Vermeulen W, **Giglia-Mari G*(2012).** Generation of DNA single-strand displacement by compromised nucleotide excision repair. **EMBO J.** 31**:** 3550-3563.