Proposed project for a Masters 2 traineeship at the Center for Cancer Research of Marseille

Name of the team: **DNA Damage and genome instability**
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Title of the project: **Mutagenic or error-free bypass of DNA damages *in vivo*.**

Keywords: DNA damage, mutagenesis, DNA replication, homologous recombination

Project summary:

During his Master 2 internship in our team, the student will use bacteria and yeast cells to studies the replication of a damaged DNA, and determine the genes and mechanisms involved in mutagenesis.

It has long been recognized that mutations in DNA are at the origin of genome instability, leading to several diseases among which cancer holds an important place. While mutations in the genome can arise spontaneously, they very often are the result of damages in the DNA. Indeed, the genome of all living organisms is constantly injured by endogenous and exogenous agents (UV light, cigarette smoke…) that modify the chemical integrity of DNA. **Our overall goal is to understand how the cells deal with this damaged DNA.** When damages are not repaired, cells possess two major strategies to tolerate residual lesions: i) translesion synthesis (TLS) where specialized DNA polymerases insert a few nucleotides opposite the lesion, with the possibility of introducing a mutation (Pagès & Fuchs 2002); ii) damage avoidance (DA) where the cells use homologous recombination to recover the genetic information from the sister chromatid, insuring survival in an error-free manner (Kuzminov 1999).

When dealing with accidental DNA lesions, mutagenic DNA damage tolerance mechanisms can lead to unwanted mutations, the initiating cause of cancer. On the other hand, when DNA damaging agents are used as therapeutics during chemotherapies, error-free tolerance mechanisms can lead to resistance to treatments. It is therefore essential to understand these mechanisms that encompass both TLS and DA.

In order to explore DNA damage tolerance, our team has developed an assay to monitor both error-prone (TLS) and error-free (DA) pathways simultaneously, by following the fate of a single replication-blocking lesion that we introduce in the genome of a living cell (Pagès & Fuchs 2018; Maslowska et al. 2019). Using this assay, the student will explore the genetics of lesion bypass and the structure of the replication fork that encounters a DNA lesion. More specifically, after inactivating or modifying specific genes, the studied lesion is inserted in the genome the cell (bacteria or yeast), and the bypass of the lesion is monitored by a colorimetric assay. We are routinely using a wide variety of molecular biology techniques (cloning, PCR, qPCR, Chromatin Immunoprecipitation, Next Generation Sequencing, etc…).

References:

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Pagès, V. & Fuchs, R.P., 2018. Inserting Site-Specific DNA Lesions into Whole Genomes. *Methods in molecular biology (Clifton, NJ)*, 1672(Chapter 9), pp.107–118.

Pagès, V. & Fuchs, R.P.P., 2002. How DNA lesions are turned into mutations within cells? *Oncogene*, 21(58), pp.8957–8966.