Molecular Determinants of Homologous Recombination-Associated DNA-synthesis

Cells have to constantly face different types of DNA lesions from diverse sources, which must be repaired in order to avoid mutagenesis and disease appearance. DNA double-strand breaks (DSBs), the most dangerous DNA lesions for genome integrity, can arise accidentally, upon exposure to genotoxic agents or failures during DNA replication, or be part of programmed developmental processes, such as meiosis. Faithful repair of DSBs typically occurs through the homologous recombination pathway, which requires a DNA synthesis step to reconstitute the DNA degraded during DSB resection (Recombination-Associated DNA Synthesis or RADS). How the extent of DNA synthesis is regulated and what are the consequences of its deregulation is poorly characterized. In particular, we do not know which factors are recruited once a DSB end has invaded the intact DNA template to initiate DNA synthesis and which exact DNA polymerases, among which some might be mutagenic, are involved. The project aims to answer these questions by two different approaches: a candidate and an unbiased approach, using budding yeast as convenient model system and the experimental context of meiosis, during which hundreds of programmed DBSs are triggered in the genome. If successful, the unbiased approach will be transferred to human cells undergoing localized DSB formation and repair.

We will identify proteins associated to ongoing DNA synthesis at meiotic DSBs by mass spectrometry. For this, budding yeast is ideal, since homologous recombination processes are highly conserved with those of human cells, and we have the unique ability, thanks to genetic tricks, to obtain cells that form and repair DSBs in a highly synchronous way, at a time well separated temporally from that of bulk DNA replication.

The PhD project will be organized in three tasks, the first two ones being initiated in parallel:

1. Candidate approach to identify recombination-associated DNA synthesis proteins
The main DNA polymerase acting at DSB sites is DNA pol delta. Using a functional TAP-tagged Pol3 subunit, we will perform a purification of the protein complex associated to DNA pol delta at the time of DSB repair recombination from cells undergoing highly synchronous meiosis. We will also trigger a “mitotic” DSB repair pathway from those cells by using well described conditions, to compare the “meiotic” versus the “mitotic” proteome associated to pol delta. The interacting candidates will be identified by quantitative label-free mass spectrometry, at the proteomics platform of I. Curie, and candidates validated by classical co-immunoprecipitation, ChIP-seq approaches and mutant analyses.

2. Unbiased approach to identify recombination-associated DNA synthesis proteins (iPOND-like)
For this complementary approach, the host team has recently obtained experimental conditions to isolate newly-synthesized DNA at sites of DSB repair, thanks to the incorporation of a modified analog of thymidine (similar to EdU) to which a functional group can be attached by “click” chemistry. Using this tool, the team has successfully mapped genome-wide the sites of recombination-associated DNA synthesis (unpublished). The PhD project will use this approach to purify proteins associated to these recombination-associated DNA synthesis sites, using an approach derived from iPOND (isolation of proteins associated with nascent DNA[1]). As in the part 1, the proteins will be identified by quantitative mass spectrometry from various experimental conditions (meiotic versus mitotic, mutant analyses) and validated by complementary and functional tests.

3. Identification of recombination-associated DNA synthesis proteins in human cells undergoing DSB repair
Once the procedure will have been validated in yeast, we will use the iPOND-like approach to identify proteins associate to sites of recombination, using a well-described experimental system where human cells can undergo synchronous genome-wide formation and repair of DSBs. The candidates obtained will be compared to those identified in budding yeast.

This PhD project will shed new light on a poorly explored aspect of homologous recombination, with consequences for fertility and the maintenance of genome stability, unregulated in many pathologies such as cancer.


Faisabilité du projet de thèse (1/2 page maximum, en anglais)
Explicit la faisabilité du projet en termes d'expertise de l'équipe d'accueil, des collaborations potentielles qui pourront être mises en place pour certains aspects du projet, de la disponibilité des appareils nécessaires au bon déroulement du projet...

The Chromosome Dynamics and Recombination team has all relevant expertise for the proposed project, and the student will benefit from the in-house platform for proteomic experiments. The team recently published several articles on the control of recombination-associated DNA synthesis during recombination (Duroc et al, 2017; Vernek et al, 2021) and on the identification of novel protein complexes important for meiotic recombination (De Muyt et al, 2018; Sanchez et al, 2020). The team also has long standing collaborations with computational biologists specialized in predicting protein-protein interactions sites, biochemists and structural biologists that will be important for exploiting the proteomic interaction results.

The project contains aspects that rely on already available preliminary data (the efficient pull-down of newly synthesized DNA at recombination sites), and the combining of the candidate approach and the more exploratory unbiased approaches should open new avenues on the regulation and mechanism of DSB repair associated DNA synthesis.

Thèses actuellement en cours dans l’équipe
Tous les encadrements doivent être indiqués (y compris les co-directions avec un autre HDR pour des doctorants d’une autre ED, et les encadrements dans le cadre de programmes doctoraux tels qu’IPV, FDV...)

<table>
<thead>
<tr>
<th>Nom et Prénom du doctorant</th>
<th>Directeur(s) de thèse</th>
<th>Année de 1ère inscription</th>
<th>ED</th>
<th>Financement</th>
</tr>
</thead>
<tbody>
<tr>
<td>PYATNISKAYA, Alexandra</td>
<td>Valérie BORDE (HDR)/ co-encadrant : Arnaud DE MUYT</td>
<td>2017</td>
<td>CDV</td>
<td>PSL</td>
</tr>
<tr>
<td>MONY Sreelekshmi</td>
<td>Valérie BORDE (HDR)</td>
<td>2019</td>
<td>CDV</td>
<td>Equipe (FRM+ANR)</td>
</tr>
</tbody>
</table>
Trois publications récentes du directeur de thèse (du co-directeur ou du co-encadrant s’il y a lieu). Mettre en gras le nom du directeur de thèse.


Docteurs encadrés par le directeur de thèse ayant soutenu entre la date de dépôt de ce dossier et il y a 5 ans et publications relatives à leur sujet de thèse. Mettre en gras le nom du directeur de thèse et celui du docteur.