EuReCa International PhD Program

PhD thesis project

2023 Call for application

Optical Mapping on single chromosomes of DSB repair events by recombination in normal and cancer cells

General information

Call	2023
Reference	2023-02-BORDE
Keyword(s)	Genome stability – DNA repair – Optical mapping – single molecule analyses – yeast genetics – cancer cell lines – computational analyses.

Director(s) and team

Thesis director(s)	Valérie Borde
Research team	Chromosome Dynamics and Recombination
Research department	UMR3244 - Dynamics of genetic information: fundamental bases and cancer (DIG-CANCER)

Description of the PhD thesis project

DNA double-strand breaks (DSBs) must be repaired to avoid mutagenesis and disease appearance. Several DSB repair pathways can be used, more or less mutagenic, depending on the chromosomal location and/or the status of the cell (normal vs mutant or pathological). Many studies of DSB repair have used low resolution approaches, such as microscopy, or more resolutive population-based studies (ChIPseq). These preclude finely studying DSBs that occur at non “hotspot” places, their stochasticity, and the way they are repaired depending on their precise genomic location.

Finally, the step of DNA synthesis that occurs during DSB repair is poorly characterized. The PhD student will study, at high resolution and on single molecules, the events of DSB repair, and determine their regulation, in yeast and human normal or breast cancer cells. For this, optical mapping will be combined with the mapping of repair-associated DNA synthesis along stretched long chromosomal molecules (Bionano), using an approach that we have developed.

The project will involve molecular biology and imaging approaches, and collaboration with computational biologists of the department who developed analysis pipelines for the optical mapping combined with S phase replication study. The student will be exposed to a multidisciplinary environment. He/she will get basic knowledge of the mechanisms of DSB repair, in normal and pathological cells, and have privileged contacts with industry, through the Bionano company.

Objectives
Optical mapping has been recently coupled to mapping of DNA synthesis, by Optical Replication Mapping (ORM). We have improved ORM in our lab, and here we will use it to map DSB repair events by Optical Recombination Mapping (OReM) in yeast and in human cells.

There are three main objectives:
1) Apply OReM to budding yeast undergoing DSBs at defined positions during programmed meiotic recombination. This will allow determine the stochasticity of meiotic recombination initiation and the spacing between DSB events, poorly understood. As a proof of principle, the student will also determine, using our optimized ORM, the S phase replication profile during meiosis and mitotic cycle. Results will be compared to published population-based studies.
2) Apply OReM to human cells. First, we will use a system where DSB are induced at well-known places. OReM will be performed on G2 synchronized cells, and in different mutants (different steps of homologous recombination, non-homologous end joining, alternative end-joining) and cell cycle stages (G1). Then OReM will be applied to random DSB induced by irradiation or genotoxic agents, in normal or established breast cancer cell lines affected in different steps of homologous recombination.
3) Optimized ORM in human cells. This will demonstrate the applicability of optimized ORM to a variety of biological questions. Results will be compared to the recent ORM study that used a less efficient labeling approach.

International, interdisciplinary & intersectoral aspects of the project

International
During the PhD project, the student will collaborate with a lab in the UK who has obtained mutants proposed to alter the distribution of the DSBs that initiate meiotic recombination. As part of his training, the student will spend some time in that lab, to introduce the mutations on his/her system to perform OReM.

Intersectoral
The project will be made in tight contact and collaboration with the BioNano company. The student will benefit from the technical support and will make a visit to their headquarters in Europe. The project will generate approaches that will very likely be of great interest to the community and potentially to industrial applications. We intend to patent our technique and/or develop a partnership with the Bionano company for broader applications once the results from this PhD will have been obtained, especially those establishing the replication patterns in yeast and human cells as proof of principle.

Interdisciplinary
This PhD will combine experimental and computational approaches to answer important biological questions of the stochasticity and mechanism of DSB repair events, that are a threat to genome integrity. The project will benefit from our active collaboration with a team in our department, for the computational analyses of the Bionano signals and with whom the team has already obtained meiotic optical recombination mapping data from budding yeast. The project will also include a translational aspect with the Curie hospital, since we plan to analyze sets of breast cancer cell lines deficient for distinct steps of homologous recombination.

Recent publications

1. Pyatnitskaya, A., Andreani, J., Guérois, R., De Muyt, A.* and **Borde, V.*** (2022) The Zip4 protein directly couples meiotic crossover formation to synaptonemal complex assembly. **Genes and Development** *36*, 53-69.
2. Dai, J.[†], Sanchez, A.[†], Adam, C., Ranjha, L., Reginato, G., Chervy, P., Tellier-Lebegue, C., Andreani, J., Guérois, R., Ropars, V., Le Du, M.H., Maloisel, L., Martini, E., Legrand, P., Thureau, A., Cejka, P., **Borde, V.***, Charbonnier, J.-B.* (2021) Molecular basis of the dual role of the Mlh1-Mlh3 endonuclease in MMR and crossover formation in meiosis. **Proc Natl Acad Sci U S A.** *118*, e2022704118.
3. Vernekar, D.V., Reginato, G., Adam, C., Ranjha, L., Dingli, F., Marsolier, M.C., Loew, D., Guérois, R., Llorente, B., Cejka, P. and **Borde, V.** (2021) The Pif1 helicase is actively inhibited during meiotic recombination which restrains gene conversion tract length. **Nucleic Acids Research** *49*, 4522-4533.
4. Cannavo, E., Sanchez, A., Anand, R., Ranjha, L., Hugener, J., Adam, C., Acharya, A., Weyland, N., Aran-Guiu, X., Charbonnier, J.-B., Hoffmann, E.R., **Borde, V.**, Matos, J. and Cejka, P. (2020) Regulation of the human MLH1-MLH3 endonuclease in meiosis. **Nature** *586*, 618-622.
5. Sanchez, A., Adam, C., Rauh, F., Duroc, Y., Ranjha, L., Lombard, B., Mu, X., Wintrebert, M., Loew, D., Guarné, A., Gnan, S., Chen C.-L., Keeney, S., Cejka, P., Guérois, R., Klein, F., Charbonnier, J.-B. and **Borde, V.** (2020) Exo1 recruits Cdc5 polo kinase to MutLgamma to ensure efficient meiotic crossover formation. **Proc Natl Acad Sci U S A.** *117*, 30577-30588.

Expected profile of the candidate

Applicants should have:

- A strong interest for genome stability and DNA repair mechanisms
- A good experience in molecular biology and genetics, either with budding yeast or human cell lines. Knowledge in programming and genomic analyses would be ideal but is not mandatory.
- A strong self-motivation and commitment to his/her project