Résumé du thème de recherche de l'équipe Dynamique des Chromosomes et Recombinaison
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Notre équipe étudie les mécanismes de la recombinaison méiotique, qui assure la bonne ségrégation des chromosomes lors de la méiose et donc la formation de gamètes viables ayant le bon nombre de chromosomes. Nous étudions en particulier les protéines spécifiques à la méiose qui assurent la formation des crossing over, et les facteurs qui déterminent la distribution des évènements de recombinaison le long des chromosomes méiotiques.
Nous utilisons des approches génétiques, génomiques et de biologie moléculaire, et réalisons nos études chez la levure *S. cerevisiae* et chez la souris, les mécanismes de la recombinaison méiotique étant très conservés.

Titre du projet de stage M2 : Etude fonctionnelle du complexe MutSγ dans la réparation des cassures double-brin de l'ADN au cours de la méiose

Projet de stage :
During meiosis, crossover formation between homologous chromosomes is required to promote accurate chromosome segregation and the production of gametes with a balanced chromosome content. Errors in meiotic crossover formation are a major source of aneuploidies, leading to sterility and chromosomal diseases such as trisomy 21. A striking feature of meiosis is that numerous DNA double-strand breaks are simultaneously generated per cell (~170 in *S. cerevisiae* and ~250 in mouse) while only a limited fraction of them is processed to **give rise to crossovers**. 2 to 3 crossover events occur on each pair of chromosomes. One major question is how the different actors successively license DNA breaks, activate dedicated recombination machineries and control the proper processing of crossovers. Two interconnected and conserved molecular systems are required for the formation of crossovers during meiotic prophase I. The first is a set of eight proteins, called the ZMM (an acronym for Zip1-4, Msh4-5, Mer3, Spo16) group, that work collectively to stabilize dHJs intermediates. The second system, the MutLγ (Mlh1-Mlh3)-Exo1 complex, is essential in resolving recombination intermediates to transform dHJs into crossovers. **At the crossroad between the ZMM and MutLγ-Exo1 complexes, lies the conserved MutSγ (Msh4-Msh5) DNA clamp,** which presumably plays a pivotal role in coordinating the processing of dHJs into crossovers. The lab has recently identified an interaction between MutSγ and MutLγ in meiotic cells (Sanchez et al 2020) and in 2-hybrid assays (unpublished). The objective of the project will be to uncover the molecular events allowing MutLγ and ZMM to cooperate with **Msh4-Msh5** and promote crossovers.
For this, the lab has generated in collaboration with computational biologists a structural model of the MutSγ-MutLγ complex based on the analogy with the mismatch repair complexes MutS-MutL conserved from prokaryotes to eukaryotes. From this model, key residues located at the surface of Msh4-Msh5 and of Mlh1-Mlh3 will be tested for their involvement in the interactions. Candidate residues will be mutated *in vivo* and their effect on the different steps of recombination will be assessed.

Techniques mises en œuvre par le stagiaire :
Using budding yeast as a model system, the project will combine a large variety of molecular and genetic assays (2 hybrid, coimmunoprecipitation, chromatin immunoprecipitation, phenotypical studies, indirect immunofluorescence).

Principales publications au cours des 5 dernières années :

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. doi: 10.1073/pnas.2022704118.

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. doi: 10.1093/nar/gkab232

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. doi: 10.1073/pnas.2013012117.

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. doi: 10.1038/s41586-020-2592-2.

De Muyt, A., Pyatnitskaya, A., Andréani, J., Ranjha, L., Ramus, C., Laureau, R., Fernandez-Vega, A., Holoch, D., Govin, J., Margueron, R., Couté, Y., Cejka, P., Guérois, R. and **Borde, V.** (2018) A meiotic XPF-ERCC1-like complex recognizes joint molecule recombination intermediates to promote crossover formation. **Genes and Development** *32*, 1-14.

Adam, C., Guérois, R., Citarella, A., Verardi, L., Adolphe, F., Béneut, C., Sommermeyer, S., Ramus, C., Govin, J., Couté, Y. and **Borde, V.** (2018) The PHD finger protein Spp1 has distinct functions in the Set1 and the meiotic DSB formation complexes. **PloS Genetics** 14(2):e1007223.

Duroc, Y., Kumar, R., Ranjha, L., Adam, C., Guérois, R., Md Muntaz, K., Marsolier-Kergoat, M.-C., Dingli, F., Laureau, R., Loew, D., Llorente, B., Charbonnier, J.-B., Cejka, P. and **Borde, V.** (2017) Concerted action of the MutLβ heterodimer and Mer3 helicase regulates the global extent of meiotic gene conversion. **eLife** 6, e21900.