ÉCOLE NORMALE SUPÉRIEURE DE LYON

Master 2 internship offer (6 months – January-June 2021)

Development of a quantitative model of the homology search process during the repair of a DNA break

Internship supervisor and Host laboratory:

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Keywords:

Spatial genome organization, Modeling, Stochastic simulations, DNA repair

Project description:

Homologous recombination (HR) is a conserved DNA break repair pathway that uniquely uses an intact DNA molecule as a template. The search for this homologous template is carried out by a specialized and conserved nucleoprotein filament (NPF) assembled on the DNA flanking the break. How this molecular platform achieves accurate and efficient identification of a single homologous donor in the vastness of the genome and nucleus remains a main modern biology conundrum. The goal of our lab is to define this mechanism, and the candidate will participate in this endeavor by developing a computer simulation of the process.

Homology search has two clearly identified dimensions: at the molecular scale it requires a machinery capable of efficiently sampling dsDNA and recognizing homology, and at the cytological scale it necessitates the ability for the DSB to explore the nucleus for opportunities to encounter a homologous match. Reconciling these two dimensions is a main technical challenge. Thanks to novel assays to track DNA joint molecules formed early on in the pathway (Piazza et al. Cell 2017; Piazza et al. Mol. Cell 2019) and Hi-C to determine the spatial organization of chromatin genome-wide (Piazza et al, in preparation), we acquire averaged time series data of both the molecular and cytological transformations occurring over the course of DNA repair in yeast. These assays already showed that (i) chromatin broadly reorganizes in response to a DNA break, (ii) this organization inhibits inter-chromosomal homology sampling and donor identification, and (iii) that DNA joint molecules formed upon encounter of significant homology exist in a dynamic equilibrium. Together with the biophysical knowledge of the process of homology sampling, this information will be instrumental in the development of a computational framework of the homology search process. We expect this framework to allow us to gain quantitative insights into the role of multiple nucleoprotein factors involved in the search at the molecular and cytological scales.

Methodology:

The goal of the internship is to develop this computational model for analyzing biological results by easily modifying search parameters. A basic stochastic model has been developed in collaboration with the laboratory of Javier Arsuaga (UC Davis)(**Fig. 1**). Since homology sampling by the NPF binds at 8 nt microhomology, we defined their positions genome-wide for a given NPF sequence, on average 370 per position in the NPF. The simulation thus has a probability of association and dissociation with any possible microhomology, one being present at a long homologous donor and the others dispersed genome-wide. Association to a microhomology, by virtue of physically tethering the NPF to a given dsDNA molecule, increases the probability of engaging a nearby homology, which plays in favor of

the long homology, provided that the dissociation constant is sufficiently high. This model enables us to differentiate from an effect of homology length and association/dissociation from micro-homologies. However, it does not ponder the probability of association to any given microhomology by their contact probability given by the tridimensional organization of the genome, not does it incorporate a threshold determining the transition from microhomologous to homologous pairing and DNA strand invasion (the next step in HR), and the subsequent disruption activities of DNA joint molecules mediated by extrinsic HR factors. These implementations will be made by the student by expanding on the initial code. Thanks to a permanent back-and-forth with experimentalists in the lab, the parameters of the search model will be best fitted. The model will provide a long-term foundation for the laboratory to explore the role of various factors in homology search and predicts consequences of their defects on genome stability.

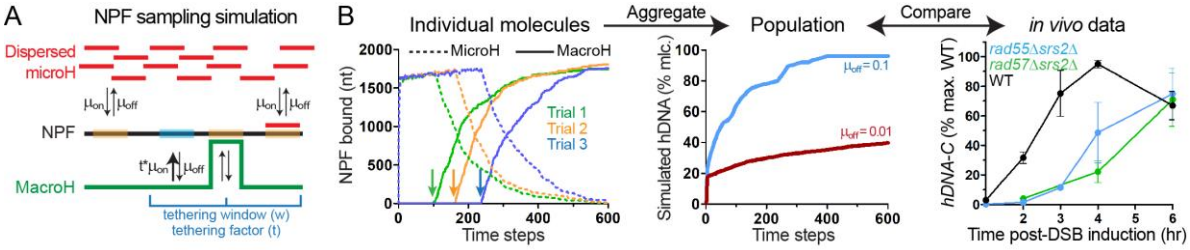

Figure 1: *(A) Rationale and current parameters of the homology search simulation. (B) Individual simulations (left), their aggregation with two different microhomology (microH) dissociation constants (middle) and how these population data can be compared with real biological data.*

Mission:

- Analysis of Hi-C contact data.
- Development of a computer simulation of the homology search process usable by non-bioinformaticians.

Candidate profile:

- Expertise in UNIX environment.
- Advanced knowledge in bash, Python and/or R languages.
- Experience with high-throughput sequencing data.
- Notions in basic molecular genetics (DNA replication, repair, chromosome organization,…)
- Good organization skills, autonomy, and presentation skills.

Lab publications related to the research project:

Piazza, A*, Bordelet H, Thierry A, Savocco J, Girard F, Koszul R* "*Cohesin overlays multiple constraints to homology search during recombinational DNA repair*" **Cell** (submitted/ soon in **BioRxiv**)

Piazza A, Heyer WD "Moving forward one step back at a time: Reversibility during homologous recombination" **Current Genetics** doi: 10.1007/s00294-019-00995-7

Piazza, A, Shah, SS, Wright, WD, Gore, SK, Koszul, R, and Heyer, W (2019). "*Dynamic processing of displacement loops during recombinational DNA repair*" **Molecular Cell**, **2019** 73(6):1255–1266.

Piazza A, Heyer WD "*Homologous recombination and the formation of complex genomic rearrangements*" **Trends in Cell Biology**, **2018** Nov 26; (18) 30186-7.

Piazza, A, Wright, WD, and Heyer, W. "Multi-invasions are recombination byproducts that induce chromosomal rearrangements" **Cell**, **2017** 170(4):760–773.

Full texts can be found at www.piazzalab.org