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Choreography of HR

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Part 1: Finding the "perfect match": Increased chromosome mobility facilitates homology search during recombination

Our genome is constantly challenged by damaging agents that can induce double strand breaks in DNA. Improper repair of those breaks leads to genomic instability and cancer predisposition. The first part of this project was performed in the Rodney Rothstein laboratory, in the Department of Genetics & Development at Columbia university, New York USA. The Rothstein laboratory is studying one of the important pathways to repair such DNA breaks, homologous recombination, which uses the undamaged homologous DNA sequence to repair the broken chromosome. The mechanism that brings together two homologous sequences is a key component of the homologous recombination process.

In a recent article published in Nature Cell Biology, we showed that the mobility of chromosomes is dramatically increased in the presence of double strand breaks in the genome (Miné-Hattab *et al*). We showed that increased chromosome mobility facilitates the pairing between homologues and is dependent on early recombination proteins. To explore chromosome mobility in the context of DNA repair *in vivo*, we fluorescently tagged two homologous DNA loci in diploid baker's yeast cells and investigated their dynamics before and after induction of a double-strand break. In the absence of damage, the two homologous loci occupy separate regions of the nucleus and explore approximately 3% of the nuclear volume. Following double-strand break induction, homologous loci colocalize 10 times more often. To facilitate pairing, the mobility of both the cut and uncut chromosomes dramatically increases allowing them to explore a larger nuclear volume. Miné-Hattab and Rothstein propose the "Increased Chromosome Mobility" model in which the induction of a DSB dramatically affects not only the motion of the broken locus but also unbroken loci. As such, the volume inside the nucleus in which homologous loci move is increased, greatly expanding the region that the chromosomes can explore to find homologous sequences after DNA damage. Since increased DNA mobility can potentially lead to unwanted chromosomal rearrangements, investigating the mechanism of how

chromosomes move in the context of DNA repair is an important first step in understanding the role of DNA dynamics in maintaining genome integrity.

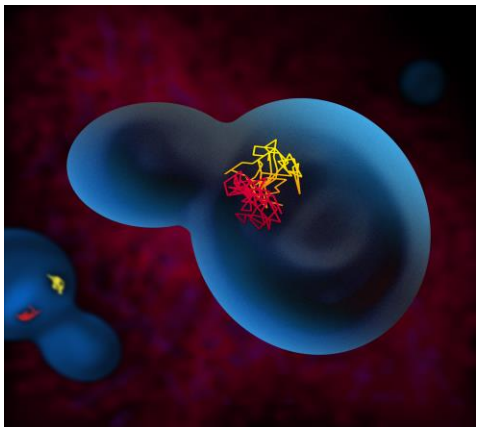

Figure legend: Two budding yeast cells are represented. On the right, the red and yellow lines trace the path of the two chromosomes during the search for homology that was stimulated by a double-strand break in one of the two homologous chromosomes. On the left, another cell in the abcence of double strand break.

Part 2: High-resolution microscopy reveals the multi-time scale nature of DNA motion

The second part of the project was performed in Xavier Darzacq laboratory, a pioneer laboratory in high resolution microscopy at the Ecole Normale Supérieure de Paris, France. Collaborating with Vincent Recamier, a mathematician and Ignacio Izeddin a biophysicist in the Darzacq laboratory, we were able to image DNA mobility up to 1000 times faster to explore the detailed nature of DNA motion in the context of DNA repair. Our results reveal that DNA motion is sub-diffusive and composed of distinct anomalous regimes depending on the time scale DNA motion is observed. The superposition of these anomalous regimes optimizes DNA nuclear exploration because it combines the precision of a fast and local motion visible at short time scale to large scale motions visible at larger time scales. Interestingly, the observation of several anomalous regimes depending on the time scale is in agreement with the “reptation model” developed by De Gennes to describe the mobility of entangled polymers. After DNA damage, DNA motion is also composed of several anomalous regimes but at equivalent time scales, DNA motion is more diffusive allowing a more efficient target search. Overall, using high resolution microscopy allowed us to reveal the multi-time scale nature of DNA motion in the presence and in the absence of double strand break. This work is in progress for a publication.