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Science in the News - Between sisters: Watching replication-associated recombinational DNA repair

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Homologous Recombination Visualization

DNA replication is a very intricate process that that incorporates different mechanisms to prevent mispairings throughout the DNA strand. However, DNA replication is still open to the formation of double-strand breaks (DSB), or "DNA lesions that disrupt the molecular continuity due to the severing of the linear DNA molecule into two fragments and the linearization of a circular molecule" (Schipler, Iliakis, 2013). In turn, these breaks in DNA can "lead to cancer through the promotion of genetic changes due to the increased chances of mispairing" (Lovett, 2018), meaning these breaks increases DNA's greater chances of its bases pairing with the wrong base, causing mutations within the cell that could cause cancer. However, it is possible to repair DSBs through "homologous recombination with the sister chromosome that is present in close proximity to the damaged site immediately after replication" (Lovett, 2018). Homologous recombination is a sort of crossover process in which a Holliday junction is formed between the two chromosomes which is initiated by a recombination protein, such as RAD51, and then DNA is synthesized, and ligation occurs, which is when the two ends of the DNA covalently link together. Afterwards, the junction is resolved through another mechanism.

Even though this process seems to be very straightforward, the process had never been observed because of "the difficulty in inducing sister chromosomes recombination at specific sites in vivo" (Lovett, 2018). Although observations of DSB repair have been made through site-specific exonucleases which cleaves both of the sister chromosomes (instead of one) in a way that is not restrained by the replication fork (where most spontaneous breaks occur), a new pathway, such that only one sister chromosome was cleaved, had to be devised in order to visualize this specific DSB repair. This genetic pathway was first introduced by Vincent Amarh,

Martin White, and David Leach (later denoted as Amarh et al. like in the article found) during their studies of Escherichia coli (E.coli).

Amarh et al. tested this by "placing 246-bp inverted repeats in the lacZ gene on the E. coli chromosome" and "each cleavage site was surrounded by fluorescently tagged repressor proteins to allow both the intact locus and the ends of the break to be monitored in live cells" (Lovett, 2018). The repeats are acting as the portions of DNA that needs to be repaired and the repressor proteins are helping visualization of the process. Replication of the DNA then occurs. There is then a hairpin formation on the lagging strand. SbcCD, a group of enzymes, then cleaves the "DNA carrying inverted repeats that form hairpin DNA secondary structures at a high efficiency" (Lovett, 2018), causing the replication fork to move and leaves a break within one chromosome while leaving the other intact. Afterwards, DNA is resectioned by RecBCD helicase/nuclease, and bound to RecA which helps with strand exchange. As stated earlier, the next steps of the process are the formation of the Holliday junction recombination intermediate and the resolution of said intermediate. (Note: there are other factors that help with this process, such as destabilizing factors like UvrD and RecX that come into play, etc. But these were the general steps taken in the experiment.)

Amarh et al. discovered that SbcCD is "formed only during replication and only on the sister chromosome formed by lagging-strand replication in E.coli" (Lovett, 2018), meaning that only one of the sister chromosomes is broken, instead of two like in exonucleases, during DNA replication. In addition, it was found that "the other (chromosome) remains intact and will act as a source of DNA sequence homology to direct recombinational repair" (Lovett, 2018). Another portion of the study found that "there was no detectable loss of viability: all cell apparently survive this event, despite a high efficiency of cleavage" (Lovett, 2018).

I chose this article because during lecture, we were shown a picture about the chromosome crossover process that usually occurs during meiosis. I vaguely remember learning about chromosome crossover during high school I believe. There wasn't too much detail about the process though. It was more of a "just know it does this" type of situation without much of an explanation. I admit I was not too curious about the process back then, but now, it's interesting trying to learn about other subjects. (I'm a chemistry major so I don't have too much knowledge about biology, except for the vague topics that I remember learning throughout middle school and high school.) So it was interesting to learn about this topic during lecture and learning more about the details through this article.

Works Cited

1. Amarh, V; White, Martin A.; Leach, David R.F. Dynamics of RecA-mediated repair of replication-dependent DNA breaks *J Cell Biol* Jul 2018, 217 (7) 2299-2307; DOI: 10.1083/jcb.201803020
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3. Schipler A, Iliakis G. DNA double-strand-break complexity levels and their possible contributions to the probability for error-prone processing and repair pathway choice. *Nucleic Acids Res*. 2013;41(16):7589-605. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3763544/pdf/gkt556.pdf