Micro 201
Heller Lecture 1/ Class 25 - Recombination and Repair
April 25, 2017

Overview:

In bacteria, genetic recombination between homologous DNA sequences underlies well-known gene transfer processes such as conjugation and generalized bacteriophage-mediated transduction. In these contexts, homologous recombination is required for the stable integration of genetic information from a donor strain into a recipient strain. At the same time, homologous recombination contributes critically to the repair of damaged DNA in all organisms. Furthermore, work from the last decade or two has established an essential role for homologous recombination in the rescue of stalled or broken replication forks, also in all organisms. The background reading (a review chapter by Kuzminov and Stahl and a review article by Persky and Lovett) provides an introduction to homologous recombination and a nice overview of the relationship between recombination and DNA repair. Please make sure that you know what a Holliday junction is and what the double-strand break repair model is before coming to class. The double-strand break repair model, which describes how a DNA double strand break can be repaired via homologous recombination and the formation of a double Holliday junction structure, is nicely presented in the Lovett review (see Fig. 2).

The research paper we will discuss is a foundational study that describes the isolation of a recombination deficient mutant of *E. coli* (*recA*), the first such mutant to be identified. In order to understand this paper, you will need a basic understanding of bacterial conjugation before reading this paper (see the extra reading selection). Specifically, Clark and Margulies identified the *recA* mutant by screening a mutagenized population of F^- cells for those that were unable to acquire chromosomal markers through conjugation with an Hfr donor strain. Pay particular attention to the logic of the screen and the strategy that was used to distinguish recombination deficient mutants from other types of mutants that might be expected to come through the primary screen.

We will begin class by discussing the research paper. Then, we will discuss homologous recombination from a mechanistic perspective, going over the Holliday model and the double-strand break repair model (see above). Finally, if there is enough time, we will begin to discuss the SOS response to DNA damage. For an overview of this topic, I have included a short review by Graham Walker, which he wrote as an introduction to a volume of the Cold Spring Harbor Symposia entitled *Biological Responses to DNA Damage*. This is a particularly nice piece as it gives a feeling for the history of this subject and how progress was made over the years.

Paper for Discussion:

1. Clark AJ and Margulies AD (1965) Isolation and characterization of recombination-deficient mutants of *Escherichia coli* K12. *PNAS* **53:** 451-59.

Required review on SOS:

2. Walker GC (2000) Understanding the complexity of an organism's responses to DNA damage. In *CSH symposia on quantitative biology*, vol. **65**: 1-10.

Background reviews:

2. Kuzminov A and Stahl FW (2005) Overview of homologous recombination and repair machines. *In* The Bacterial Chromosome (chapter 19); NP Higgins *ed*.

3. Persky NS and Lovett ST (2008) Mechanisms of recombination: Lesson from *E. coli*. *Crit Rev Biochem and Mol Biol* **43**: 347-370.