Recombination from a Biochemical Perspective

Enzymology

Over 25 different different enzymes are involved in homologous recombination in enteric bacteria. The following list includes some of the important players.

- RecA - The RecA protein promotes pairing between a single-stranded DNA molecule and a homologous sequence in another DNA molecule, a process called synapsis. (There are no other proteins in enteric bacteria that can catalyze this reaction, so recA mutants are "completely" defective for recombination.) RecA requires a minimal length of DNA sequence homology between the invading donor strand and the double stranded recipient DNA -- called a Minimal Essential Pairing Sequence (MEPS).

- RecBCD pathway - The RecBCD complex initiates recombination between two homologous dsDNA molecules when one of the molecules has a free, double-stranded end. The RecBCD complex binds to the free end and rapidly degrades the DNA. When RecBCD encounters a short DNA sequence called a chi site, the RecD subunit is altered such that the double-stranded exonuclease is inactivated -- the RecBC complex is converted to a helicase with 5' to 3' single-stranded nuclease activity that unwinds the donor DNA and degrades one strand of the DNA. In addition, the RecB subunit helps "load" RecA protein onto the 3' end of the resulting ssDNA. The ssDNA-RecA complex can invade a homologous dsDNA molecule. (Other enzymes in enteric bacteria that can catalyze similar functions, so recBC mutants are not completely defective for recombination.)

- RecFOR pathway - The RecFOR pathway was discovered by looking for suppressors of recBC mutants (called sbcBCD). RecF binds DNA and has a weak ATPase activity that is stimulated by RecR. RecO promotes the exchange of single-stranded DNA binding protein (SSB) with RecA protein. The RecFOR pathway also includes two additional proteins, RecJ and RecQ. RecJ is a single-stranded exonuclease that catalyzes the removal of deoxy-nucleotide monophosphates from DNA in the 5' to 3' direction. RecQ is a helicase.

- RuvAB - These enzymes catalyze branch migration, resulting in pairing of two homologous strands of DNA. RuvAB has DNA helicase activity that causes branch migration from a crossover in the direction that will extend the DNA heteroduplex.

- RuvC - These enzymes are Holliday junction specific endonucleases that cut two strands of DNA at a "cross-over" (Holliday junction). The resulting DNA strands can be rejoined by DNA ligase, completing the recombination reaction. RuvC interacts with RuvAB to form a RuvABC complex that facilitates the branch migration and recombination reaction.

These enzymes function together to result in recombination via the molecular mechanism shown on the link Recombination from a Molecular Perspective. .

Some important points to remember about the biochemistry of homologous recombination are:

1. Homologous recombination requires RecA protein.
2. Homologous recombination requires substantial DNA sequence homology between the donor and recipient DNA.
3. Recombination results from the cutting of two DNA strands between phosphodiester bonds, and the covalent rejoining of the DNA strands.
4. Recombination is a complex process involving many different enzymes, and many redundant functions.

These biochemical points explain some very important practical aspects of genetic recombination:

1. Recombination between any two sequences is a relatively low frequency event.
2. Because the cutting-rejoining of phosphodiester bonds can occur between any adjacent nucleotides within a homologous pair of DNA molecules, the co-inheritance of two genetic markers is inversely proportional to the distance between the markers.

REFERENCES:

- Amundsen S, Smith G. 2003. Interchangeable parts of the Escherichia coli recombination machinery. Cell 112: 741-744.
- Cox M. 2003. The bacterial RecA protein as a motor protein. Annu Rev Microbiol. 57: 551-577.
- Kowalczykowski S. 2000. Initiation of genetic recombination and recombination-dependent replication. Trends Biochem Sci. 25: 156-165.
- McGlynn P, Lloyd RG. 2002. Genome stability and the processing of damaged replication forks by RecG. Trends Genet. 18: 413-419.

Return to Microbial Genetics supplement.

Please send comments, suggestions, or questions to smaloy@sciences.sdsu.edu

Last modified September 25, 2004