Lecture Outline: Feb. 11, 2009

1. Overview of Recombination
2. Requirements of Recombination
3. Mechanism of Recombination and Holiday structure formation.
4. Enzymes involved and Chi-sites

Reading assignment for Regulation of gene expression (Feb. 13, 2009):
   pp71-85; 518-528; 547-556

Holliday junction animation link:
   http://www.sinauer.com/cooper/4e/animations0602.html

Overview: the obvious stuff?

Recombination = “good” No recombination = “bad”

Recombination probably allows species to adapt/evolve more quickly in response to changing environmental conditions
Recombination allows genetic information previously associated with one DNA molecule to become associated with another; also allows the order of genetic information on particular molecule to change.
Recombination also critically important in restarting replication in response to DNA damage.
Requirements for Recombination

1. Identical or very similar DNA sequences in the cross over region.

2. Complementary base pairing between double dsDNA molecules. The point where two dsDNA are held together by complementary base pairing between their strands is called the synapse.

3. Recombination enzymes—the machinery of the recombination complex. The functions of these enzymes include identifying and processing complementary regions suitable for recombination.

4. Heteroduplex formation involving all four strands of DNA from the two DNA molecules in a synapse. This process occurs in all organisms capable of undergoing some kind of genetic exchange.

Requirements for Recombination

1) Identical or very similar DNA sequences in the cross over region and complementary base pairing between double stranded DNA molecules.

- Homologies as short as 23 bases work (but more is better -- longer homology = more frequent cross-overs)

- Breaks occur in both DNA sequences engaged in the recombination

- Rejoining of DNA molecules after recombination done: if enzymes don’t finish the job, the cell dies
Holliday model of recombination (1964)

Evidence for the Holliday model

- Dressler & Potter isolated plasmids from cells to scan for intermediates
- Found figure 8 features on EM grids
- ca. 1-3% produced structure similar to that above after digestion with restriction enzyme
- Formation dependent on RecA
Holliday model needs ssDNA

How is ssDNA generated?

Option #1:
Single strand invasion model

Option #2:
Double strand break model
Holliday model needs ssDNA

Appears that both potential mechanisms are viable options.

What are the protein players in recombination and how do they act?

Requirement 3: Recombination proteins

<table>
<thead>
<tr>
<th>Gene</th>
<th>Mutant phenotype</th>
<th>Enzymatic activity</th>
<th>Probable role in recombination</th>
</tr>
</thead>
<tbody>
<tr>
<td>recA</td>
<td>Recombination deficient</td>
<td>Enhanced pairing of homologous DNAs</td>
<td>Synapse formation</td>
</tr>
<tr>
<td>recBC</td>
<td>Reduced recombination</td>
<td>Exonuclease, ATPase, helicase, χ-specific endonuclease</td>
<td>Initiates recombination by separating strands, degrading DNA up to a χ site</td>
</tr>
<tr>
<td>recD</td>
<td>Rec− χ independent</td>
<td>Stimulates exonuclease</td>
<td>Degrading 3’ ends</td>
</tr>
<tr>
<td>recF</td>
<td>Reduced plasmid recombination</td>
<td>Binds ATP and single-stranded DNA</td>
<td>Substitutes for RecBCD at gaps</td>
</tr>
<tr>
<td>recG</td>
<td>Reduced recombination in RecBC</td>
<td>Single-stranded exonuclease</td>
<td>Substitutes for RecBCD at gaps</td>
</tr>
<tr>
<td>recN</td>
<td>Reduced recombination in RecBC</td>
<td>ATP binding</td>
<td>Substitutes for RecBCD at gaps</td>
</tr>
<tr>
<td>recO</td>
<td>Reduced recombination in RecBC</td>
<td>DNA binding and renaturation</td>
<td>Substitutes for RecBCD at gaps</td>
</tr>
<tr>
<td>recQ</td>
<td>Reduced recombination in RecBC</td>
<td>DNA helicase</td>
<td>Substitutes for RecBCD at gaps</td>
</tr>
<tr>
<td>recR</td>
<td>Reduced recombination in RecBC</td>
<td>Binds double-stranded DNA</td>
<td>Substitutes for RecBCD at gaps</td>
</tr>
<tr>
<td>recG</td>
<td>Reduced Rec in RuvA−B−C−</td>
<td>Branch-specific helicase</td>
<td>Migration of Holliday junctions</td>
</tr>
<tr>
<td>nruA</td>
<td>Reduced recombination in RecG−</td>
<td>Binds to Holliday junctions</td>
<td>Migration of Holliday junctions</td>
</tr>
<tr>
<td>nruB</td>
<td>Reduced recombination in RecG−</td>
<td>Holliday junction-specific helicase</td>
<td>Migration of Holliday junctions</td>
</tr>
<tr>
<td>nruC</td>
<td>Reduced recombination in RecG−</td>
<td>Holliday junction-specific nuclease</td>
<td>Resolution of Holliday junctions</td>
</tr>
<tr>
<td>priA, priB, priC, dnaT</td>
<td>Reduced recombination</td>
<td>Helicase?</td>
<td>Reload replication forks</td>
</tr>
</tbody>
</table>
How do you find *rec* genes?

- Clear that recombination occurs within minutes of DNA coming into cell (via conjugation/transduction, so proteins must assist)
- First *rec* mutations identified using Hfr crosses

**RecBCD/RecA**

Single stranded region formed by RecBCD loads with RecA protein and promotes strand invasion. The D-loop is the displaced strand.
RecA binding to DNA

RecA coats ssDNA and searches for its complementary sequence within the cell. RecA-DNA complex may involve a triple-stranded structure, as shown.

RecBCD and Chi sites

Chi sites identified in screen of special λ replication mutants; general importance later became apparent.

Chi sites impact activity of RecBCD
Cutting Holliday Junctions

RuvABC proteins are required to cleave DNA in these structures.

RecFOR

RecFOR cannot process DNA ends; works as ssDNA gaps.