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 <u>synapse</u>.
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

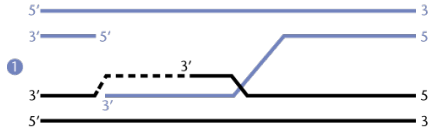# Holliday model of recombination (1964)

Figure 10.1
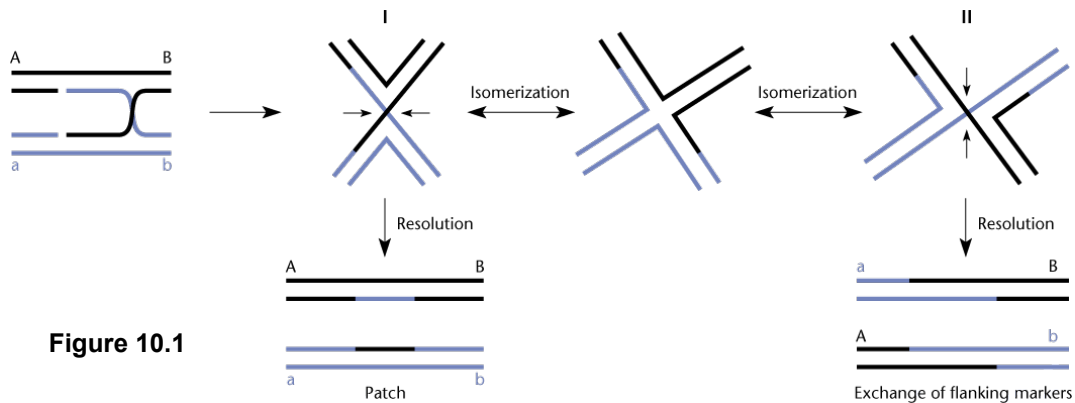
Evidence for the Holliday model
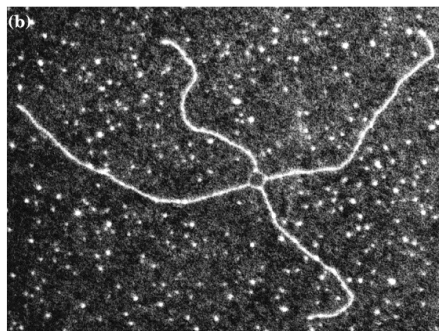
- Dressler & Potter isolated plasmids from cells to scan for intermediates
- Found figure 8 features on EM grids
- *ca.* 1-3% produced structure similar to that above after digestion with restriction enzyme
- Formation dependent on RecA

Holliday model needs ssDNA

How is ssDNA generated?
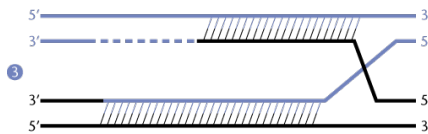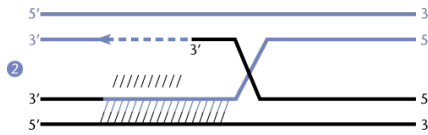
Option #1:
Single strand invasion model

Figure 10.3

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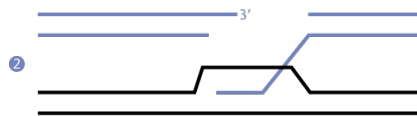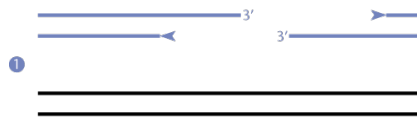
Option #2:
Double strand break model

Figure 10.4

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

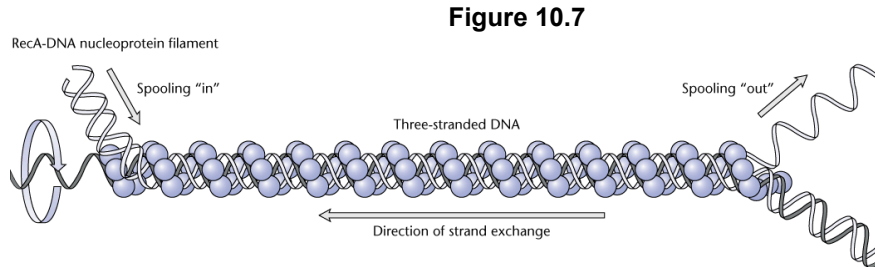| TABLE 10.1	Some genes encoding recombination functions in *E. coli*		
Gene	Mutant phenotype	Enzymatic activity	Probable role in recombination
recA	Recombination deficient	Enhanced pairing of homologous DNAs	Synapse formation
recBC	Reduced recombination	Exonuclease, ATPase, helicase, χ-specific endonuclease	Initiates recombination by separating strands, degrading DNA up to a χ site
recD	Rec⁺ χ independent	Stimulates exonuclease	Degrading 3′ ends
recF	Reduced plasmid recombination	Binds ATP and single-stranded DNA	Substitutes for RecBCD at gaps
recJ	Reduced recombination in RecBC⁻	Single-stranded exonuclease	Substitutes for RecBCD at gaps
recN	Reduced recombination in RecBC⁻	ATP binding	Substitutes for RecBCD at gaps
recO	Reduced recombination in RecBC⁻	DNA binding and renaturation	Substitutes for RecBCD at gaps
recQ	Reduced recombination in RecBC⁻	DNA helicase	Substitutes for RecBCD at gaps
recR	Reduced recombination in RecBC⁻	Binds double-stranded DNA	Substitutes for RecBCD at gaps
recG	Reduced Rec in RuvA⁻B⁻C⁻	Branch-specific helicase	Migration of Holliday junctions
ruvA	Reduced recombination in RecG⁻	Binds to Holliday junctions	Migration of Holliday junctions
ruvB	Reduced recombination in RecG⁻	Holliday junction-specific helicase	Migration of Holliday junctions
ruvC	Reduced recombination in RecG⁻	Holliday junction-specific nuclease	Resolution of Holliday junctions
priA, priB, priC, dnaT	Reduced recombination	Helicase?	Reload replication forks

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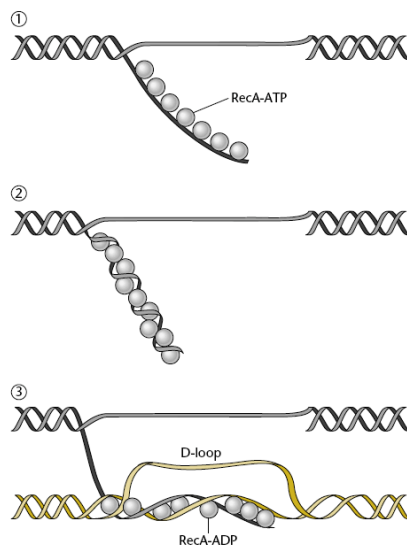
Single stranded region formed by RecBCD loads with RecA protein and promotes strand invasion. The D-loop is the displaced strand.

RecA binding to DNA

Figure 10.7

RecA-DNA nucleoprotein filament

Spooling "in"

Spooling "out"

Three-stranded DNA

Direction of strand exchange

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.
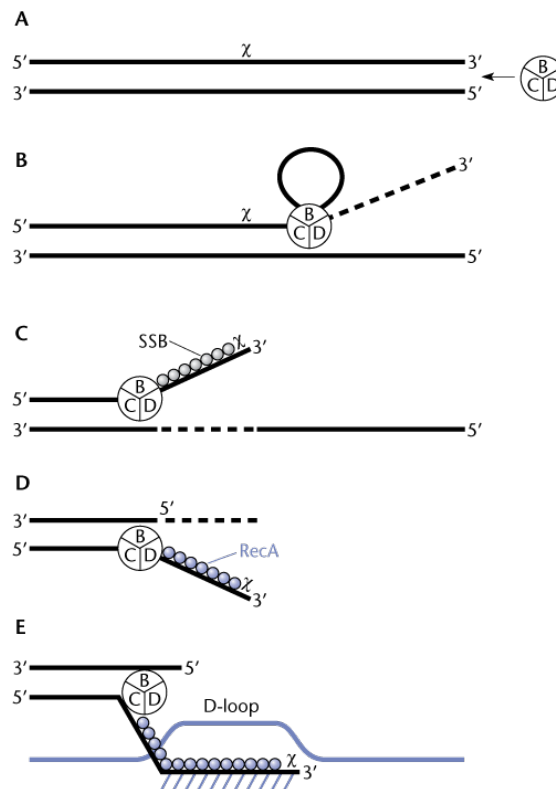
Chi sites impact activity of RecBCD

A

5′ —————— χ —————— 3′ ← B C D

3′ —————————————— 5′

B

5′ ———————— χ — B C D ⟍ 3′

3′ —————————————— 5′

C

SSB χ 3′

B C D

5′ ——— B C D

3′ ——— - - - - - - - - ——— 5′

D

3′ ——— 5′ - - - - -

5′ ——— B C D ⟍ RecA χ 3′

E

3′ ——— 5′

5′ ——— B C D

D-loop

χ 3′

Figure 10.5

Cutting Holliday Junctions

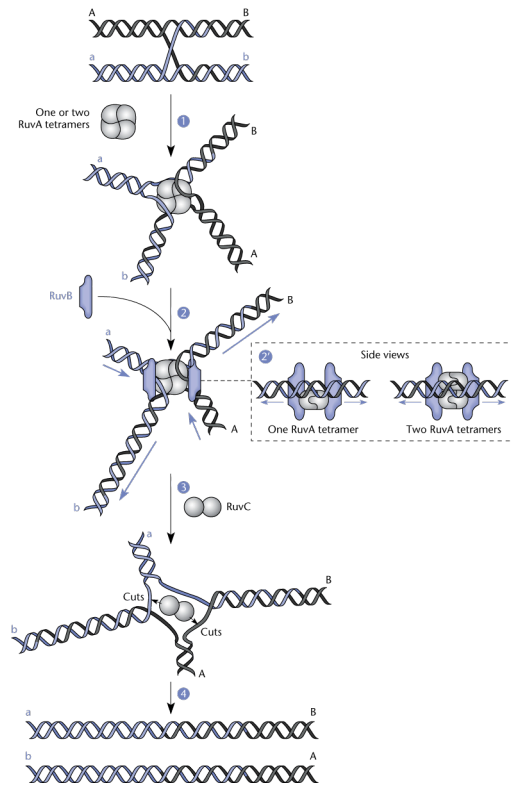RuvABC proteins are required to cleave DNA in these structures.

Figure 10.8
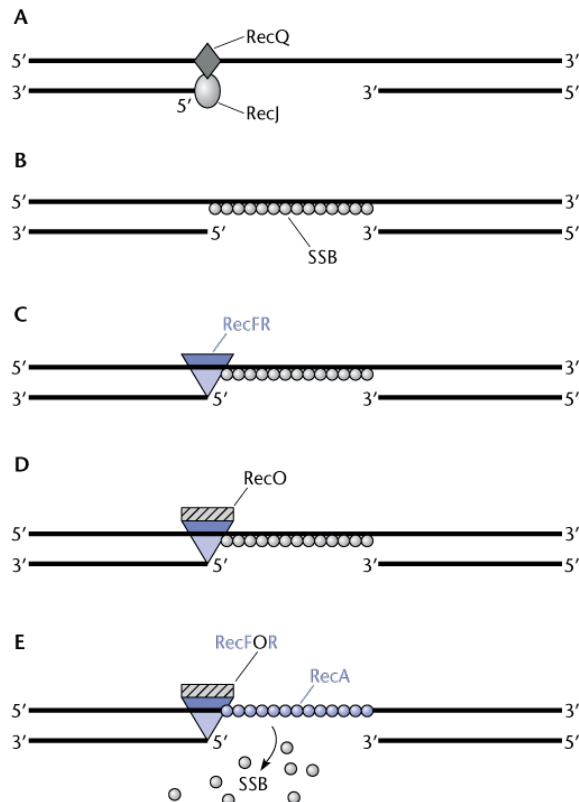
RecFOR

RecFOR cannot process DNA ends; works as ssDNA gaps.

Figure 10.6