DNA RECOMBINATION

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Reference texts: Berg etal, Chapter 28
Weaver, 5th ed, Chapter 22, 23

Recombination
Definitions of recombination

Types of recombination

- Homologous
- Site-Specific
- Transposition

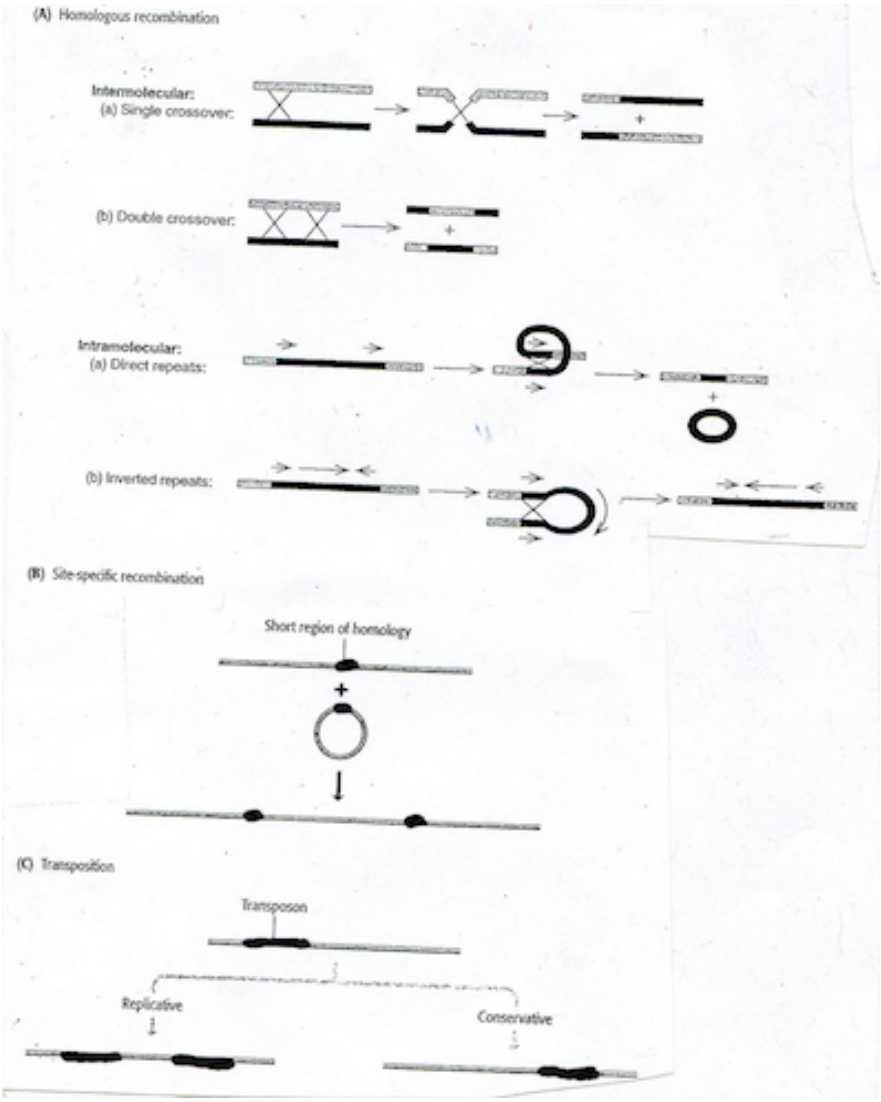
What is it good for?

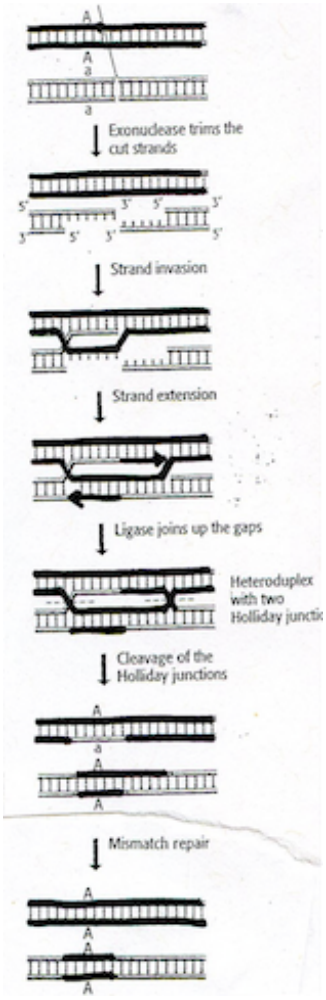
28.5 DNA Recombination Plays Important Roles in Replication, Repair, and Other Processes

Most processes associated with DNA replication function to copy genetic message as faithfully as possible. However, several biochem processes require the recombination of genetic material between two D molecules. In genetic recombination, two daughter molecules are for by the exchange of genetic material between two parent molec (Figure 28.41). Recombination is essential in the following processes.

1. When replication stalls, recombination processes can reset the repl tion machinery so that replication can continue.
2. Some double-stranded breaks in DNA are repaired by recombinatic
3. In meiosis, the limited exchange of genetic material between pa chromosomes provides a simple mechanism for generating genetic diver in a population.
4. As we shall see in Chapter 34, recombination plays a crucial role in gen erating molecular diversity for antibodies and some other molecules in immune system.
5. Some viruses employ recombination pathways to integrate their gen material into the DNA of a host cell.
6. Recombination is used to manipulate genes in, for example, the gen tion of "gene knockout" mice (Section 5.4).

Recombination is most efficient between DNA sequences that are simil sequence. In homologous recombination, parent DNA duplexes align regions of sequence similarity, and new DNA molecules are formed by breakage and joining of homologous segments.





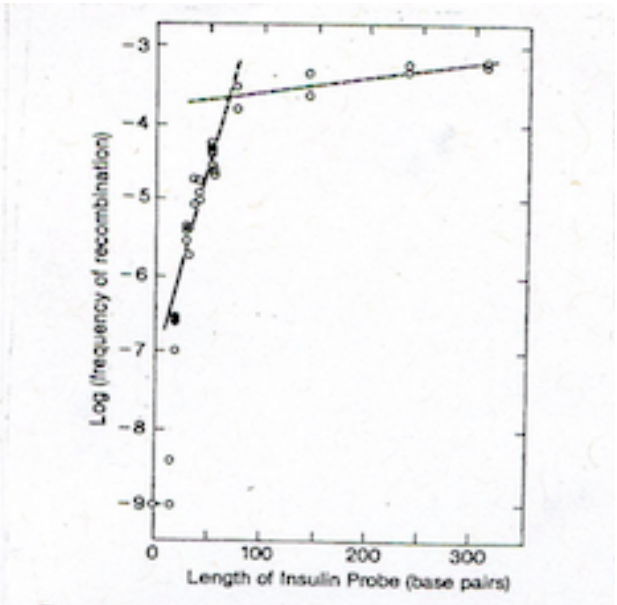


FIG. 2. Frequency of recombination as a function of length of homologous DNA. The frequencies of recombination between insulin DNA fragments in the miniplasmid and the insulin gene in λhI1 have been plotted against the length of the insulin DNA in the probe. Each experimental point represents a single determination of recombination frequency from separate recombination experiments.

Proc Natl. Acad Sci 82, 4768, 1985

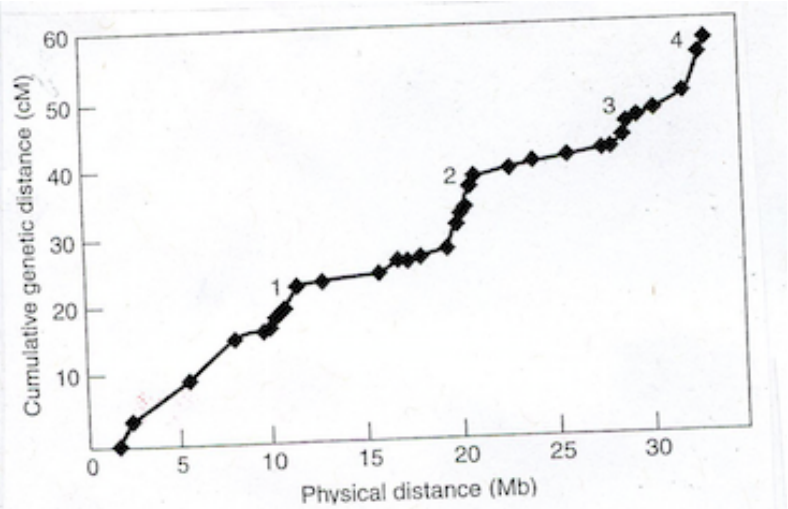


Figure 24.12 Genetic distance plotted against physical distance in chromosome 22q. The cumulative genetic distance between markers (in cM) is graphed versus the physical distance between the same markers (in Mb). The numbers denote four areas of relatively high rates of recombination (as reflected in the steeply rising curves). Weaver

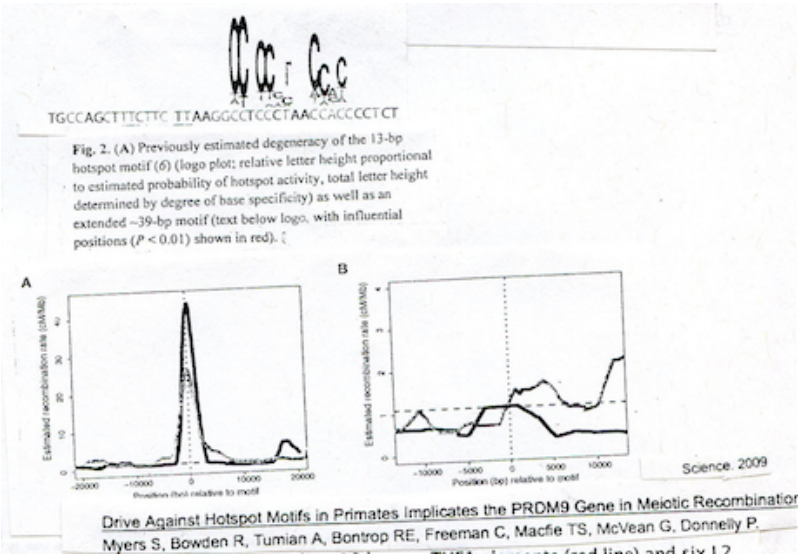


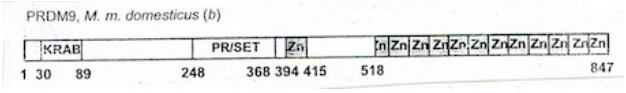
Fig. 2. (A) Previously estimated degeneracy of the 13-bp hotspot motif (6) (logo plot; relative letter height proportional to estimated probability of hotspot activity, total letter height determined by degree of base specificity) as well as an extended ~39-bp motif (text below logo, with influential positions ($P < 0.01$) shown in red).

Science. 2009

Drive Against Hotspot Motifs in Primates Implicates the PRDM9 Gene in Meiotic Recombination

Myers S, Bowden R, Tumian A, Bontrop RE, Freeman C, Macfie TS, McVean G, Donnelly P.

Estimated HapMap Phase II recombination rate across the 40 kb surrounding 16 human THE1 elements (red line) and six L2 elements (blue line) orthologous to the 22 regions analyzed in chimpanzee, and each containing a conserved exact match to the 13-bp core motif. Rates are smoothed using a 2-kb sliding window slid in 50-bp increments, averaged across elements. Horizontal dashed line indicates the human average recombination rate of 1.1 cM/Mb. Vertical dotted line indicates the center of the repeat. (B) Average estimated recombination rate for the western chimpanzee data around the 16 THE1 elements (red line) and six L2 elements (blue line) containing the 13-bp core motif. Other details are the same as (A).



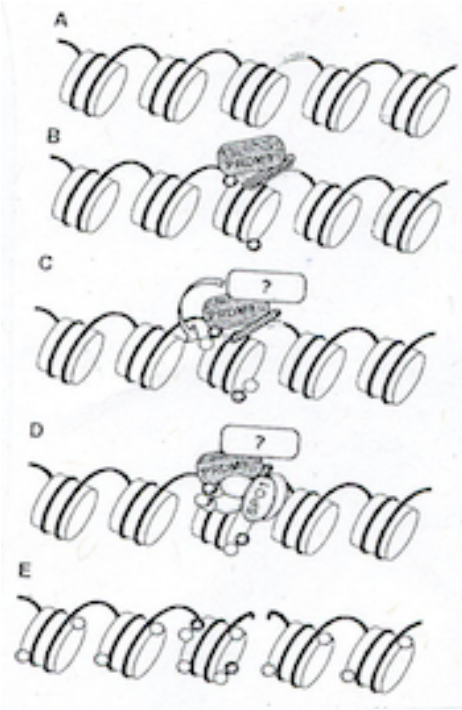


Figure 5. Model of hotspot specification by PRDM9. (A) The DNA and several nucleosomes are represented. A DNA sequence motif recognized by PRDM9 is represented in green. (B) PRDM9 binds to its target DNA motif through the zinc finger array and catalyzes H3K4me3 (orange). (C) A protein partner of PRDM9 may catalyze another post-translational histone modification (grey), allowing for the formation of a hotspot-specific signature. (D) PRDM9, a partner, or other component of the chromatin may recruit the recombination initiation complex containing SPO11 or may create a favorable chromatin environment allowing access of SPO11 to the DNA. (E) A DSB is formed by SPO11 and triggers the phosphorylation of histone H2Ax (yellow) in the surrounding nucleosomes. The DSB is then repaired by homologous recombination and lead to a CO or to gene conversion without CO.
doi:10.1371/journal.pbio.1001176.g005

PLoS Biol. 2011, Grey C. et al

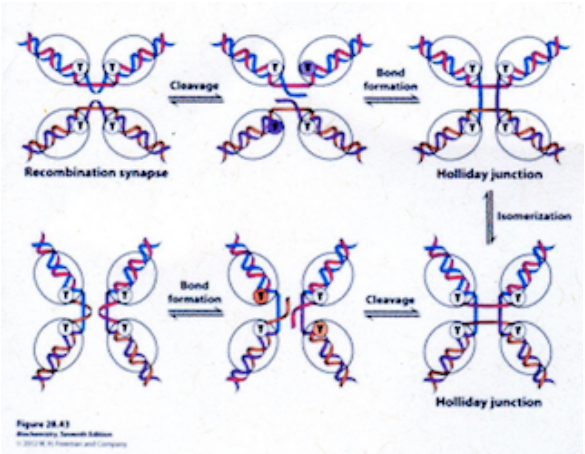


Figure 28.43 Recombination mechanism.
Recombination begins as two DNA molecules come together to form a recombination synapse. One strand from each duplex is cleaved by the recombinase enzyme; the 3′ end of each of the cleaved strands is linked to a tyrosine (Y) residue on the recombinase enzyme. New phosphodiester bonds are formed when a 5′ end of the other cleaved strand in the complex attacks these tyrosine– DNA adducts. After isomerization, these steps are repeated to form the recombined products.

Site-specific recombination to facilitate plasmid partitioning

Austin, S et al. (1981). A novel role for site-specific recombination in maintenance of bacterial replicons. Cell 25, 729-736.

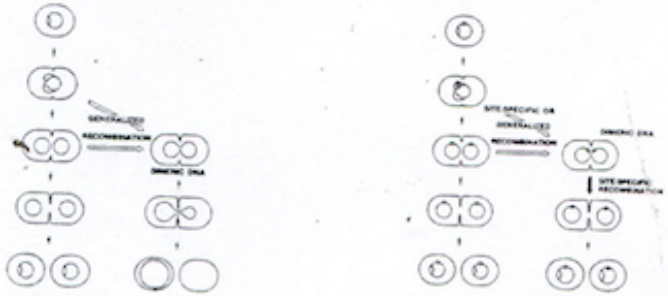
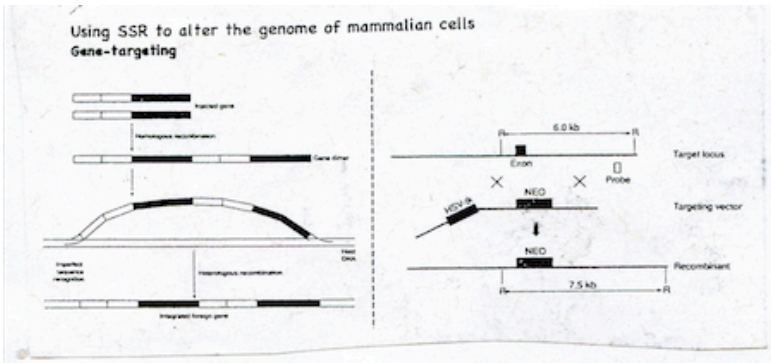
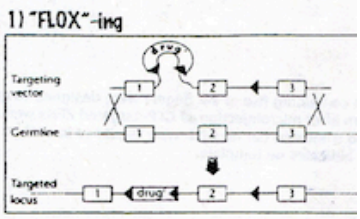
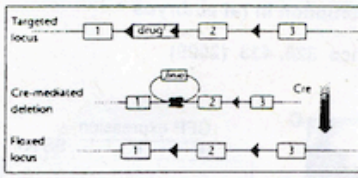


Figure 1 – Hypothetical Effect of Generalized Recombination on the Maintenance of a Unit-Copy Replicon in Dividing Cells
The normal pathway for replicon partition (left pathway) is blocked by dimer formation. When the cell divides, a cured cell results (right pathway).

Figure 2 – Hypothetical Effect of a Highly Active Site-Specific Recombination Site on the Partition of a Unit-Copy Replicon in Recombination-Proficient Dividing Cells
During the course of normal replication and partition (left pathway), any dimers formed by generalized or site-specific recombination are highly unstable because of the high activity of the site-specific recombination. Resolution into monomers rapidly provides substrates for proper partition (right pathway).

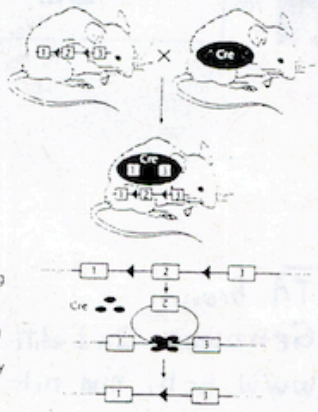






The steps involved in creating a knockout mouse

Step 1: A normal gene is disabled by inserting neo+ gene
Step 2: The disabled gene is transferred to embryonic mouse stem cells
Step 3: The disabled copy recombines with the normal gene on the mouse chromosome
Step 4: The cells are grown on a medium that contains the antibiotic G418. Only the cells with the neo+ gene will survive.
Step 5: Cells containing a neo+ gene are injected into early mouse embryos, which are implanted into a pseudopregnant mouse
Step 6: Variegated mice are then interbred and produce some progeny that are homozygous for the knockout gene



"Conditional" mutagenesis in mice using the Cre-loxP system. Left Top: Introduction of loxP sites into a chromosomal gene by homologous recombination in embryonic stem (ES) cells. Left Middle: Removal of the resistance marker from the targeted locus by Cre-mediated recombination in ES cells to create a modified locus containing only the two loxP sites required for conditional mutagenesis. Bottom: Cell-type specific gene inactivation *in vivo* by Cre-mediated recombination of the floxed locus. Animals carrying the floxed locus are mated with transgenic or "Cre knock-in" mice expressing Cre in the target tissue. Gene inactivation by Cre-mediated exon deletion occurs only in the target tissue.