MCB: Molecular Biology

# Recombination

Recombination plays a major role in the generation of variety in eukaryotes. Each generation, the combination of alleles is shuffled anew. In this module we will focus on recombination and its effects at a practical level: When and where does recombination occur? What are its effects on the genome? We will look briefly at recombination at a mechanistic level in *E. coli*, where we understand much of the molecular mechanism involved. It is believed that the mechanism is very similar in eukaryotes although we have not identified all of the players. Finally, we will look at two other recombination mechanisms that have clinical relevance, site-specific recombination and transposition. These mechanisms form part of the life-cycle of several viruses, are involved in gene regulation, and also figure in attempts to develop gene-therapy approaches.

Reading:

**MBoC(6th) Ch5**: *HOMOLOGOUS RECOMBINATION, TRANSPOSITION AND CONSERVATIVE SITE-SPECIFIC RECOMBINATION*. **Ch24**: Ig genes are assembled from separate gene segments during B cell development.

Need to know and understand:

DNA sequence homology

Types of recombination

**homologous recombination**

**site-specific recombination**

**transposition**

Homologous recombination

importance during meiosis in eukaryotes, **crossover**. Importance in all cells as a DNA repair mechanism

found also in prokaryotes

importance of DNA break (nick or double-stranded) for initiation of recombination

formation and resolution of **Holliday Junction**

strand exchange

**isomerization of Holliday Junction** - recombinant and non-recombinant resolution

heteroduplex DNA - **branch migration - Gene Conversion**

In *E. coli* following enzymes are involved:

RecBCD protein complex - cutting, unwinding of DNA and 5'->3' exonuclease activity until Chi site.

Rec A protein polymerization on ssDNA. Involvement in strand exchange.

RuvA, RuvB proteins bind to Holliday junction and mediate branch migration

RuvC is a nuclease which resolves the Holliday junction into two separate DNAs

In Eukaryotes SpoII makes double-stranded DNA cuts, RAD51 and Dmc1 are like RecA.

Site specific recombination

Involves sequence-specific DNA binding by specialized recombination machinery. Site-specific recombination can be used to regulate gene expression: example is *Salmonella* flagellar genes H1 and H2.

Immunoglobulin loci: V(D)J joining. RSS - recombination signal sequences. RAG1 and RAG2 recognition of RSS, only found in lymphocytes. Cut DNA is resolved by V(D)J recombinase activity, much of which is probably part of the general repair machinery and is found in every cell.

Transposition

**Different types**
**DNA -> DNA (direct)**
**DNA -> DNA + DNA (replicative)**
**DNA -> RNA -> DNA (retrotransposition)**

**Transposons** - Jumping genes. Able to move about the genome, inserting themselves at many places almost at random. This makes them mutagens! The transposon contains the genes which regulate its own jumping. e.g. P-elements in *Drosophila*. **In prokaryotes they often carry antibiotic resistance genes**. Examples are Tn5 in *E. coli*. There are no currently active DNA transposons in humans, but there is a fossil record of them in the genome.

**Retrotransposons** - Encode a reverse transcriptase and integrase activity (L1 in humans). These are active players in mutation and evolution of the human genome.

Recombination in Medicine. Recombination is met both on the patient and pathogen sides. Homologous recombination during meiosis is responsible for generating much of human genetic diversity. In the future it will have a key role in gene therapy. It is also the foundation that permits positional cloning of disease-associated genes. In contrast, normally functioning homologous recombination can lead to gene conversion and loss of heterozygosity (LOH) - a process associated with tumor progression. Inborn errors in BRCA1 and BRCA2, components of the homologous recombination machinery lead to increased rates of some cancers, notably breast cancer and prostate cancer. Mutations in RAG1 or RAG2 prevent Ig site-specific recombination and the generation of antibody diversity. Such people have an essentially non-functional adaptive immune system, making them profoundly susceptible to infection (the bubble boy). On the other side, the pathogen Salmonella uses site-specific recombination to change its surface appearance, thus evading recognition by the adaptive immune system. Retrotransposons are one of the major mutagens acting on the human genome - and the damage cannot be fixed by any repair pathway! They are therefore one of the most active agents changing the content of the human genome. On the pathogen side, DNA transposons often carry antibiotic resistance genes and their ‘jumping’ frequently disperses antibiotic resistance genes among different bacterial strains.

Focus illustrations: Source: Molecular Biology of the Cell: Sixth Edition (2015) Alberts et al., Garland Science, NY

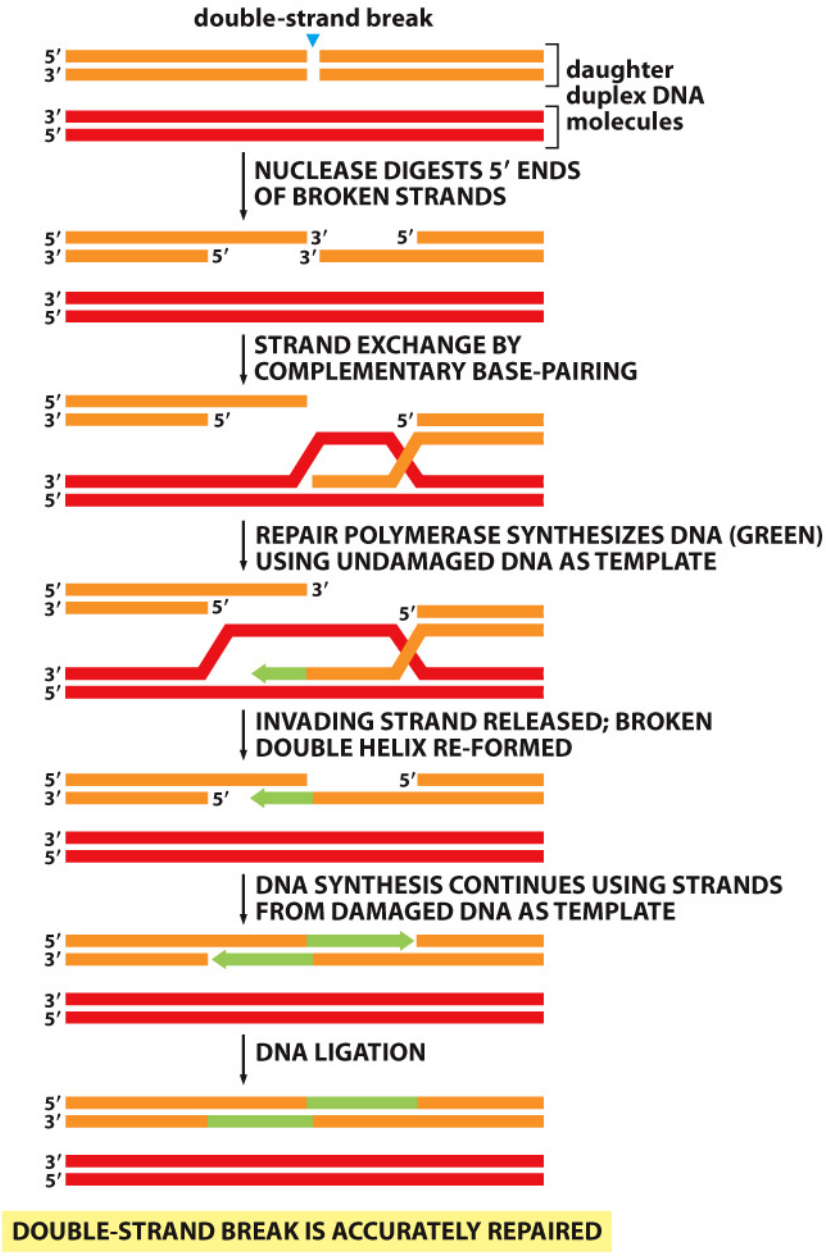


Figure 5-48 Molecular Biology of the Cell 6e (© Garland Science 2015)

Figure 5–48 Mechanism of double- strand break repair by homologous recombination. This is the preferred method for repairing DNA double-strand breaks that arise shortly after the DNA has been replicated, while the daughter DNA molecules are still held close together. In general, homologous recombination can be regarded as a flexible series of reactions, with the exact pathway differing from one case to the next. For example, the length of the repair "patch" can vary considerably depending on the extent of 5′ processing and new DNA synthesis, indicated in green.

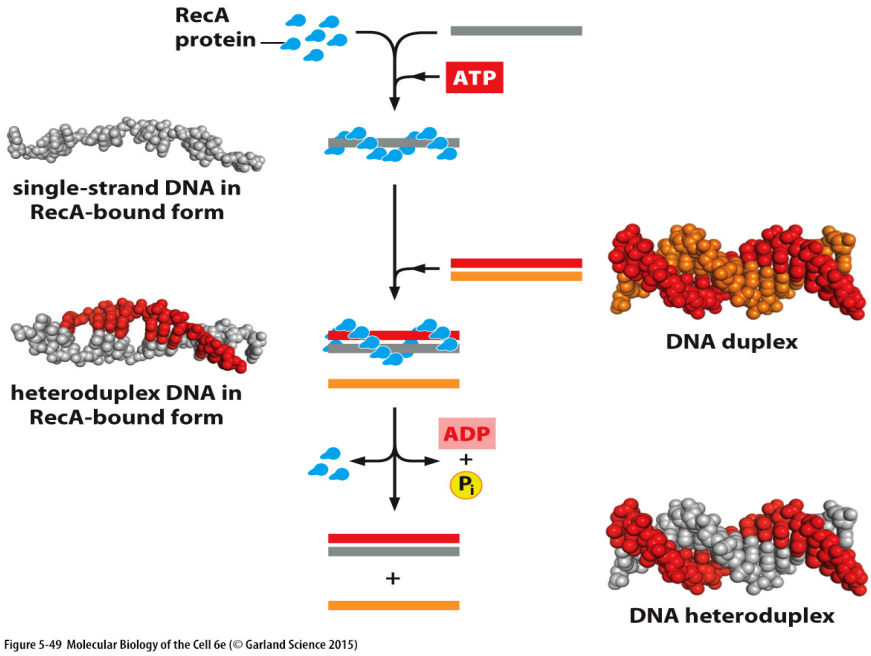


Figure 5-49 Molecular Biology of the Cell 6e (© Garland Science 2015)

Figure 5–49 Strand invasion catalyzed by the RecA protein. Our understanding of this reaction is based in part on structures determined by x-ray diffraction studies of RecA bound to single- and double-strand DNA. These DNA structures (shown without the RecA protein) are on the left side of the diagram. Starting at the top, ATP-bound RecA associates with single-strand DNA, holding it in an elongated form where groups of three bases are separated from each other by a stretched and twisted backbone. In the next step, the RecA-bound single strand then binds to duplex DNA, destabilizing it and allowing the single strand to sample its sequence through base-pairing, three bases at a time. If no match is found, the RecA-bound single strand of DNA rapidly dissociates and begins a new search. If an extensive match is found, the structure is disassembled through ATP hydrolysis, resulting in the dissociation of RecA and the exchange of one single strand of DNA for another, thereby forming a heteroduplex. (PDB code: 3CMX.)

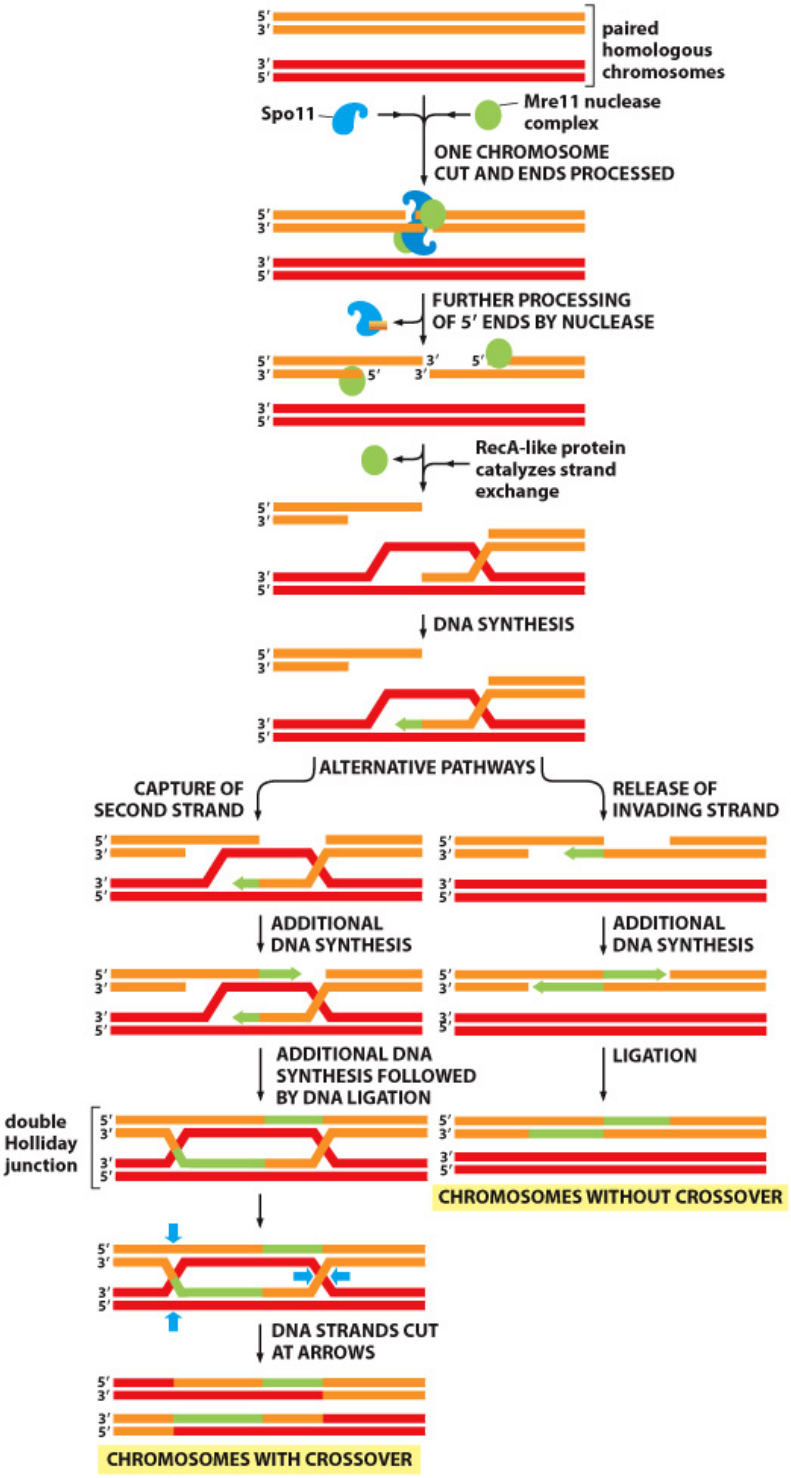


Figure 5-54 Molecular Biology of the Cell 6e (© Garland Science 2015)

Figure 5–54 Homologous recombination during meiosis can generate chromosome crossovers. Once the meiosis-specific protein Spo11 and the Mre11 complex break the duplex DNA and process the ends, homologous recombination can proceed along alternative pathways. One (right side of figure) closely resembles the double-strand break repair reaction shown in Figure 5–48 and results in chromosomes that have been "repaired" but have not crossed over. The other (left side with strand breaks as shown by the blue arrows) proceeds through a double Holliday junction and produces two chromosomes that have crossed over. During meiosis, homologous recombination takes place between maternal and paternal chromosome homologs when they are held tightly together (see Figure 17–54).

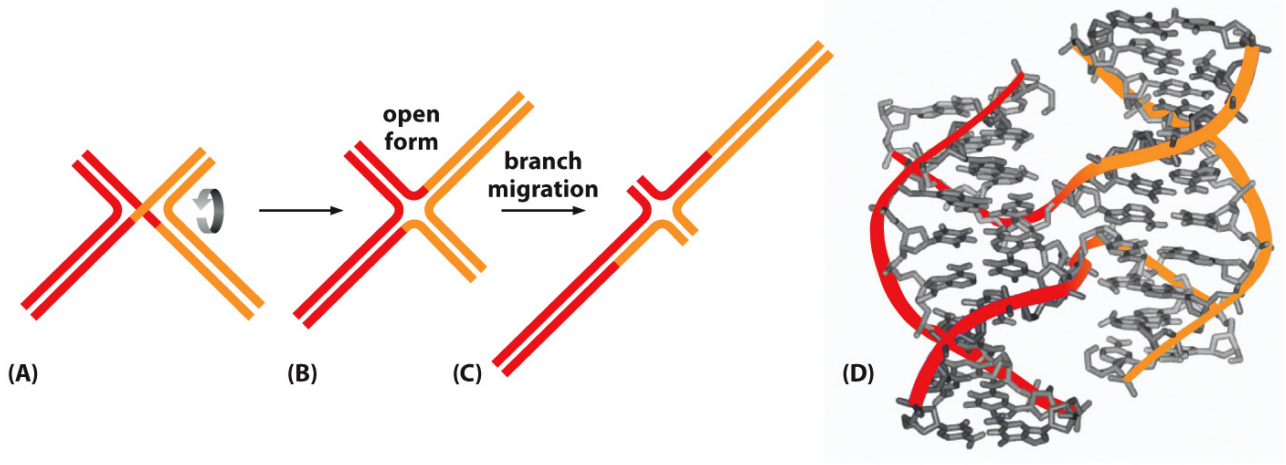


Figure 5-55 Molecular Biology of the Cell 6e (© Garland Science 2015)

Figure 5–55 A Holliday junction. The initially formed structure (A) is usually drawn with two strands crossing, as in Figure 5–54. An isomerization of the Holliday junction (B) produces an open, symmetrical structure that is bound by specialized proteins. (C) These proteins "move" the Holliday junctions by a coordinated set of branch- migration reactions (see Figure 5–57 and Movie 5.8). (D) Structure of the Holliday junction in the open form depicted in (B). The Holliday junction is named for the scientist who first proposed its formation. (PDB code: 1DCW.)

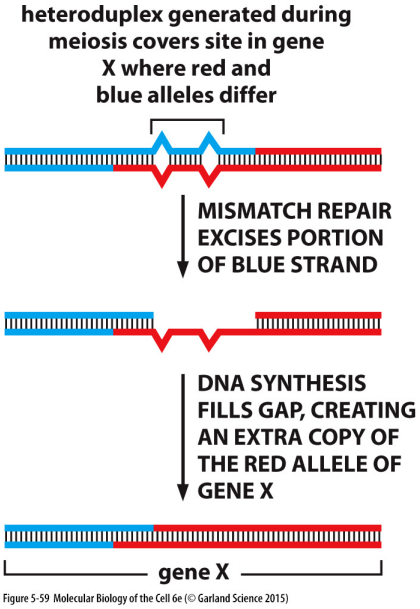


Figure 5-59 Molecular Biology of the Cell 6e (© Garland Science 2015)

Figure 5–59 Gene conversion caused by mismatch correction. In this process, heteroduplex DNA is formed at the sites of homologous recombination between maternal and paternal chromosomes. If the maternal and paternal DNA sequences are slightly different, the heteroduplex region will include some mismatched base pairs, which may then be corrected by the DNA mismatch repair machinery (see Figure 5–19). Such repair can "erase" nucleotide sequences on either the paternal or the maternal strand. The consequence of this mismatch repair is gene conversion, detected as a deviation from the segregation of equal copies of maternal and paternal alleles that normally occurs in meiosis.

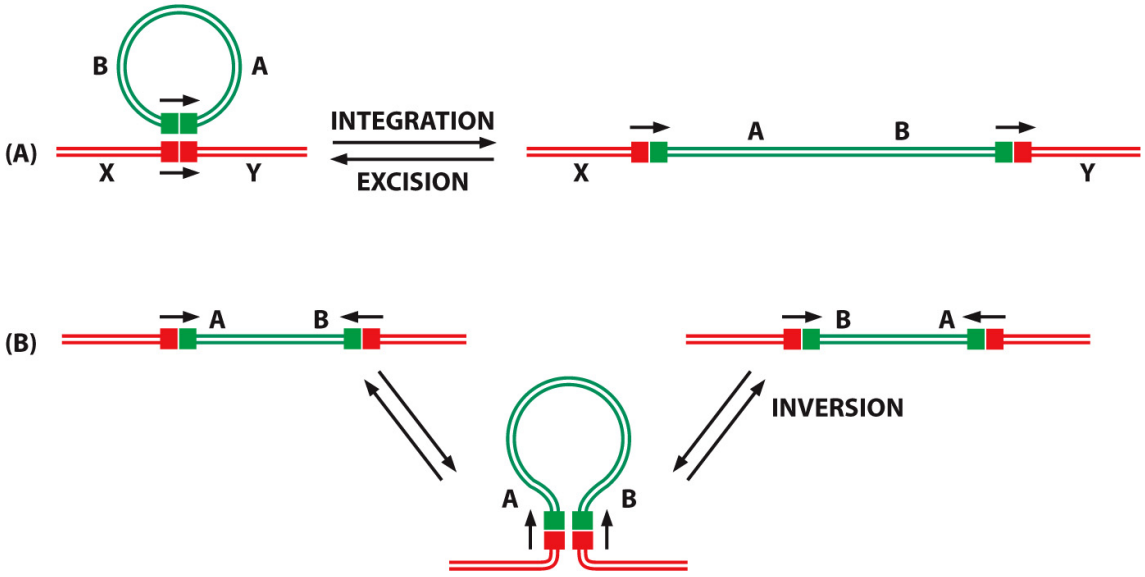


Figure 5-64 Molecular Biology of the Cell 6e (© Garland Science 2015)

Figure 5–64 Two types of DNA rearrangement produced by conservative site-specific recombination. The only difference between the reactions in (A) and (B) is the relative orientation of the two short DNA sites (indicated by arrows) at which a site-specific recombination event occurs. (A) Through an integration reaction, a circular DNA molecule can become incorporated into a second DNA molecule; by the reverse reaction (excision), it can exit to re-form the original DNA circle. Many bacterial viruses move in and out of their host chromosomes in this way. (B) Conservative site-specific recombination can also invert a specific segment of DNA in a chromosome. A well-studied example of DNA inversion through site-specific recombination occurs in the bacterium Salmonella typhimurium, an organism that is a major cause of food poisoning in humans; as described in the following section, the inversion of a DNA segment changes the type of flagellum that is produced by the bacterium.

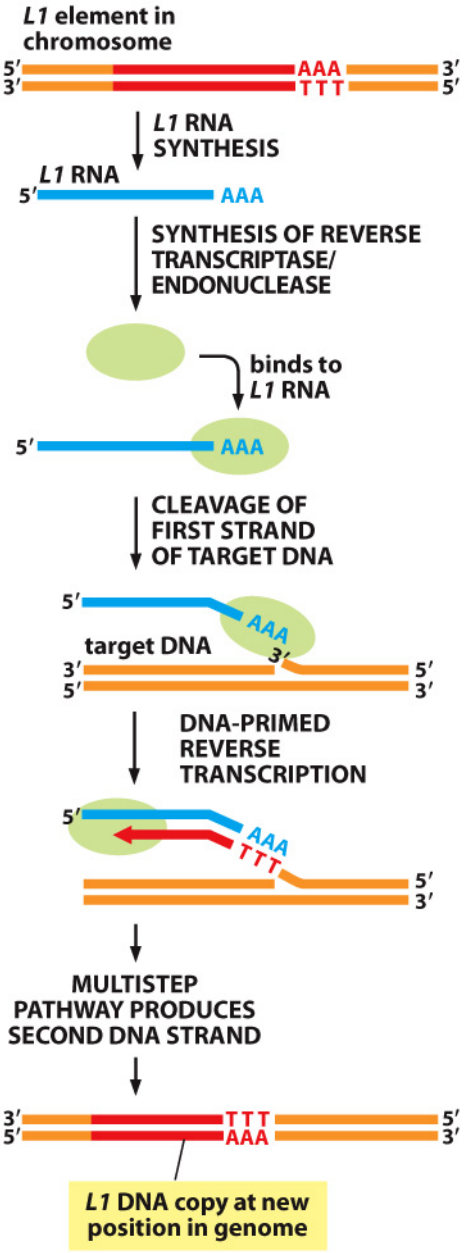


Figure 5-63 Molecular Biology of the Cell 6e (© Garland Science 2015)

Figure 5–63 Transposition by a nonretroviral retrotransposon. Transposition of the L1 element (red) begins when an endonuclease attached to the L1 reverse transcriptase (green) and the L1 RNA (blue) nick the target DNA at the point at which insertion will occur. This cleavage releases a 3′-OH DNA end in the target DNA, which is then used as a primer for the reverse transcription step shown. This generates a single-strand DNA copy of the element that is directly linked to the target DNA. In subsequent reactions, further processing of the single-strand DNA copy results in the generation of a new double-strand DNA copy of the L1 element that is inserted at the site of the initial nick.