# DNA Recombination

## UNIT II

# Introduction

## Homologous recombination

- The exchange of genetic material between two strands of DNA that contain long stretches of similar base sequences.
- Homologous recombination occurs naturally in eukaryotic organisms, bacteria, and certain viruses and is a powerful tool in genetic engineering.
- In eukaryotes, homologous recombination occurs during meiosis, playing a critical role in the repair of double-stranded nicks in DNA and increasing genetic diversity by enabling the shuffling of genetic material during chromosomal crossover.
- In bacteria, homologous recombination is a major mechanism of DNA repair and facilitates the incorporation into DNA of genetic material received via horizontal gene transfer and transformation.
- In viruses, homologous recombination helps shape viral evolution.
- In genetic engineering, homologous recombination is used as a form of gene targeting, in which an engineered mutation is introduced into a specific gene as a means of investigating the gene's function.
- In this approach, foreign DNA with a sequence similar to that of the target gene but flanked by sequences identical to the ones upstream and downstream of the target gene's location is introduced into a cell.

- The cell recognizes the identical flanking sequences as homologues, causing target gene DNA to be swapped with the foreign DNA sequence during replication.
- The exchange inactivates, or “knocks out,” the target gene. In mice, this method is used to target specific alleles in embryonic stem cells, enabling the production of knockout mice.
- Artificial genetic material similar to the target gene is introduced into the nucleus of the embryonic stem cell, which represses the target gene by the process of homologous recombination.
- With the target gene rendered inactive, scientists are able to deduce and investigate its biological functions in the mouse.
- Numerous mouse genes have been knocked out with the help of gene targeting, resulting in the production of hundreds of different mouse models of human disorders, including cancer, diabetes, cardiovascular diseases, and neurological disorders.
- Groundbreaking work on homologous recombination in mouse stem cells was carried out by scientists Mario Capecchi, Sir Martin J. Evans, and Oliver Smithies, who were awarded the 2007 Nobel Prize in Physiology or Medicine for their discoveries.

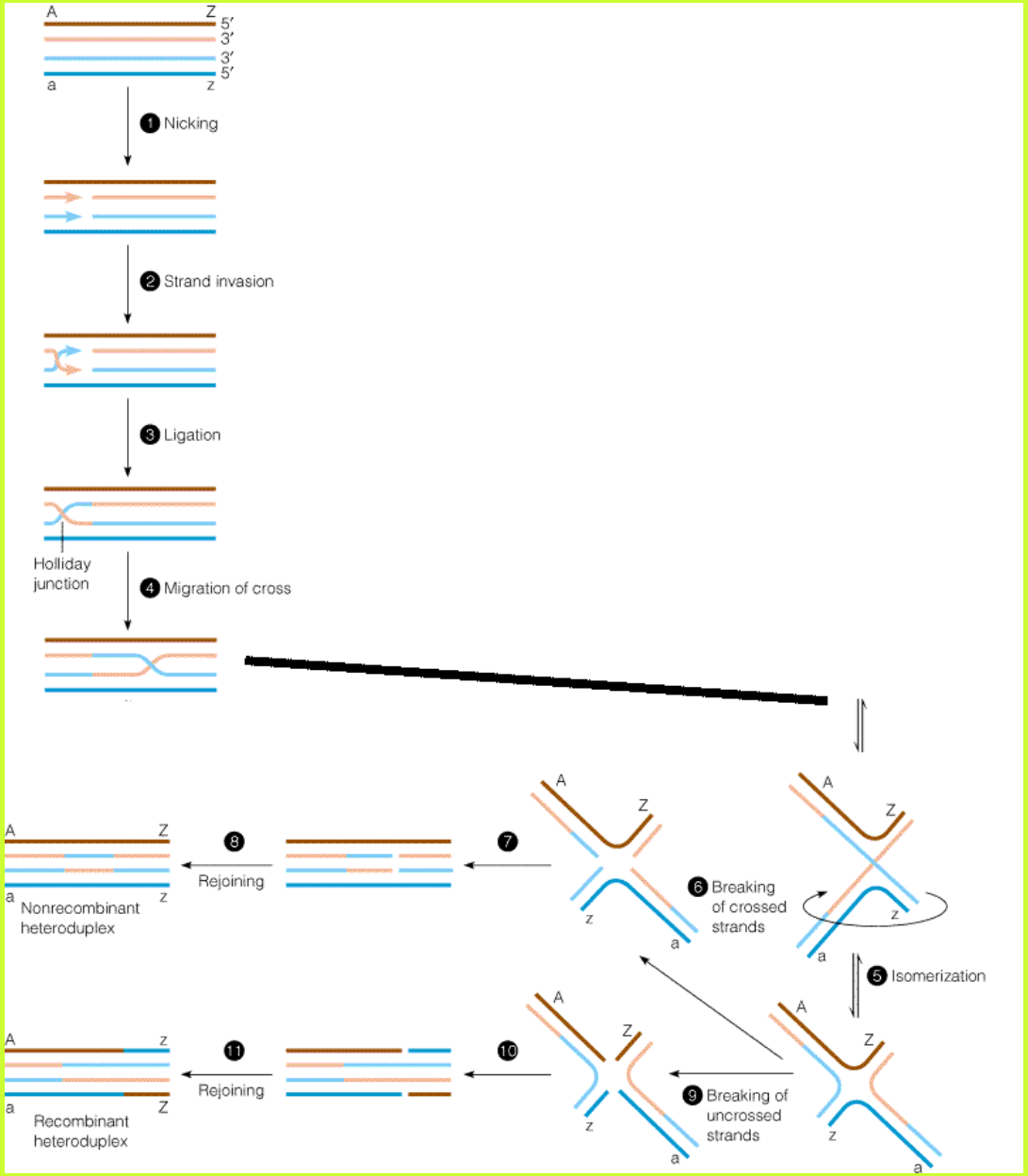
When a DNA double-strand break occurs, nucleotides located at the 5’-end of the cleavage site are removed. During the invasion step that follows, the resulting overhanging 3’-end of the DNA strand “invades” a similar or identical DNA molecule. This results in the generation of one or two cross-shaped structures denoted as the Holliday junctions, which connect both the invaded and the damaged DNA strands. The resolution of the Holliday junction gives rise to homologous recombination, implying or not a crossing over.

A Holliday junction is a branched nucleic acid structure that contains four double-stranded arms joined together. These arms may adopt one of several conformations depending on buffer salt concentrations and the sequence of nucleobases closest to the junction. The structure is named after Robin Holliday, the molecular biologist who proposed its existence in 1964.

In biology, Holliday junctions are a key intermediate in many types of genetic recombination, a biological process that increases genetic diversity by shifting genes between two chromosomes, as well as in double-strand break repair. These junctions usually have a symmetrical sequence and are thus mobile, meaning that the four individual arms may slide through the junction in a specific pattern that largely preserves base pairing.

The Holliday junctions in homologous recombination are between identical or nearly identical sequences, leading to a symmetric arrangement of sequences around the central junction. This allows a branch migration process to occur where the strands move through the junction point. Cleavage, or resolution, of the Holliday junction can occur in two ways. Cleavage of the original set of strands leads to two molecules that may show gene conversion but not chromosomal crossover, while cleavage of the other set of two strands causes the resulting recombinant molecules to show crossover. All products, regardless of cleavage, are heteroduplexes in the region of Holliday junction migration.

# Holliday model




One of the first plausible models to account for the preceding observations was formulated by Robin Holliday. The key features of the **Holliday model** are the formation of **heteroduplex DNA;** the creation of a **cross bridge;** its migration along the two heteroduplex strands, termed **branch migration;** the occurrence of **mismatch repair;** and the subsequent **resolution,** or splicing, of the intermediate structure to yield different types of recombinant molecules. Looking at Figure, the two homologous double helices are aligned, although note that they have been rotated so that the bottom strand of the first helix has the same polarity as the top strand of the second helix. Then a nuclease cleaves the two strands that have the same polarity. The free ends leave their original complementary strands and undergo hydrogen bonding with the complementary strands in the homologous double helix. Ligation produces the structure shown in Figure. This partially heteroduplex double helix is a crucial intermediate in recombination, and has been termed the **Holliday structure.**

# Site-specific recombination

- A second type of recombination, called site-specific recombination, can alter gene order and also add new information to the genome.
- Site-specific recombination moves specialized nucleotide sequences, called *mobile genetic elements,* between nonhomologous sites within a genome. The movement can occur between two different positions in a single chromosome, as well as between two different chromosomes.
- Mobile genetic elements range in size from a few hundred to tens of thousands of nucleotide pairs, and they have been identified in virtually all cells that have been examined.
- Some of these elements are viruses in which site-specific recombination is used to move their genomes into and out of the chromosomes of their host cell. A virus can package its nucleic acid into viral particles that can move from one cell to another through the extracellular environment.
- Many other mobile elements can move only within a single cell (and its descendents), lacking any intrinsic ability to leave the cell in which they reside.

- The relics of site-specific recombination events can constitute a considerable fraction of a genome.
- The abundant repeated DNA sequences found in many vertebrate chromosomes are mostly derived from mobile genetic elements; in fact, these sequences account for more than 45% of the human genome. Over time, the nucleotide sequences of these elements have been altered by random mutation. As a result, only a few of the many copies of these elements in our DNA are still active and capable of movement.
- In addition to moving themselves, all types of mobile genetic elements occasionally move or rearrange neighboring DNA sequences of the host cell genome.
- These movements can cause deletions of adjacent nucleotide sequences, for example, or can carry these sequences to another site. In this way, site-specific recombination, like general recombination, produces many of the genetic variants upon which evolution depends.
- The translocation of mobile genetic elements gives rise to spontaneous mutations in a large range of organisms including humans; in some, such as the fruit fly *Drosophila*, these elements are known to produce most of the mutations observed.
- Over time, site-specific recombination has thereby been responsible for a large fraction of the important evolutionary changes in genomes.

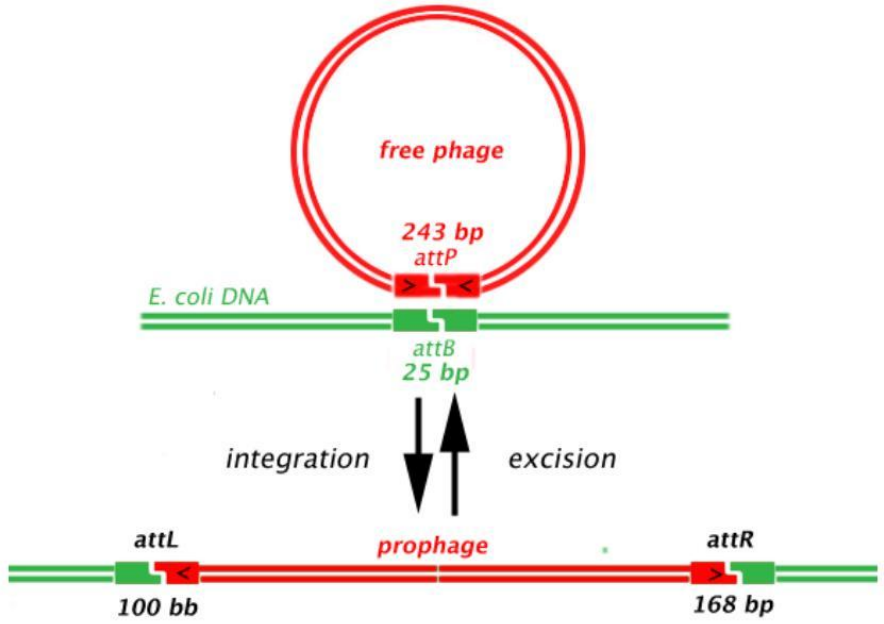
- Unlike general recombination, site-specific recombination is guided by recombination enzymes that recognize short, specific nucleotide sequences present on one or both of the recombining DNA molecules.
- Extensive DNA homology is not required for a recombination event. Each type of mobile element generally encodes the enzyme that mediates its own movement and contains special sites upon which the enzyme acts.
- Many elements also carry other genes. For example, viruses encode coat proteins that enable them to exist outside cells, as well as essential viral enzymes. The spread of mobile elements that carry antibiotic resistance genes is a major factor underlying the widespread dissemination of antibiotic resistance in bacterial populations.

Site-specific recombination can proceed via either of two distinct mechanisms, each of which requires specialized recombination enzymes and specific DNA sites.

1. **Transpositional site-specific recombination** usually involves breakage reactions at the ends of the mobile DNA segments embedded in chromosomes and the attachment of those ends at one of many different nonhomologous target DNA sites. It does not involve the formation of heteroduplex DNA.
2. **Conservative site-specific recombination** involves the production of a very short heteroduplex joint, and it therefore requires a short DNA sequence that is the same on both donor and recipient DNA molecules.

- One example of site-specific recombination is the integration of DNA from bacteriophage λ into the chromosome of *E. coli* (figure). In this reaction, bacteriophage λ DNA, which is a linear molecule in the normal phage, first forms a circle and then is cleaved by the enzyme λ-integrase at a specific site called the phage attachment site.
- A similar site on the bacterial chromosome is cut by integrase to give ends with the identical extension. Because of the complementarity between these two ends, they can be rejoined so that the original circular λ chromosome is inserted into the chromosome of the *E. coli* bacterium.
- Once integrated, the phage can be held in an inactive state until signals are generated that reverse the process, allowing the phage genome to escape and resume its normal life cycle of growth and spread into other bacteria.
- This site-specific recombination process requires only λ-integrase and one host DNA binding protein called the integration host factor.
- A third protein, called excisionase, recognizes the hybrid sites formed on integration and, in conjunction with integrase, catalyzes an excision process whereby the λ chromosome is removed from the bacterial chromosome.

# Site Specific Recombination




**b. Site-specific recombination**

In phage lambda, the integration site is known as **att P**, in E. coli the site is **att B**.
The att sites contain the binding sites for the proteins that mediate lambda recombination.
The integration reaction (att B x att P) is mediated by the proteins
integrase (Int) and
host integration factor (IHF).

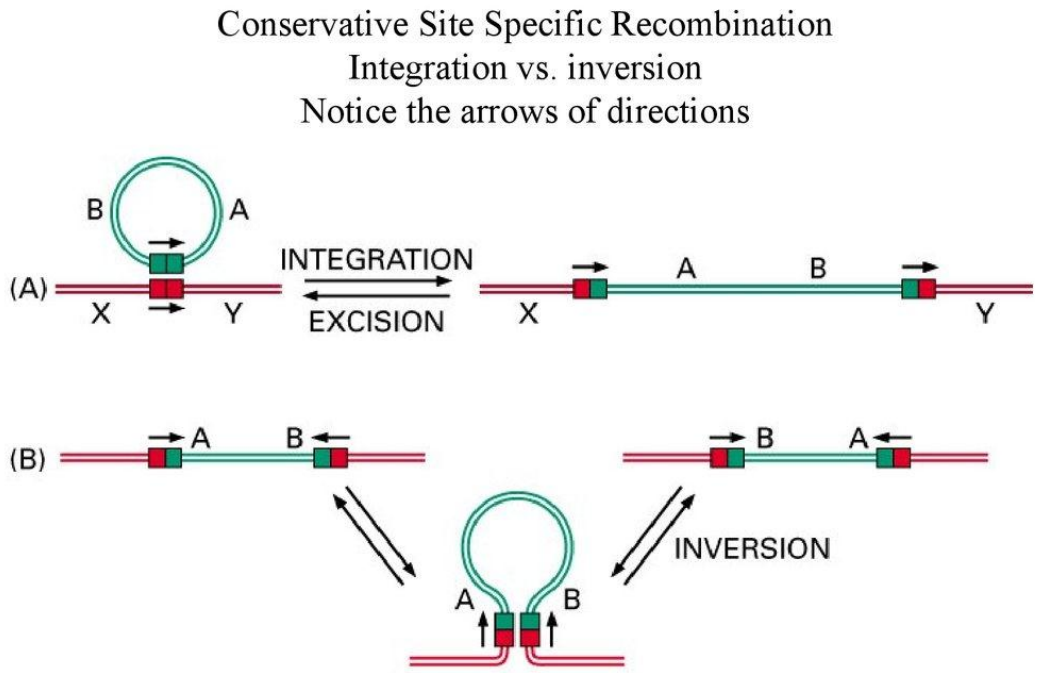



Figure 5–79. Molecular Biology of the Cell, 4th Edition.

# References


- https://www.britannica.com/science/homologous-recombination
- **Molecular Biology of the Cell. 4th edition.**