DNA Recombination Mechanisms

Recombination

- Present in prokaryotic and eukaryotic cells
- Only poorly understood

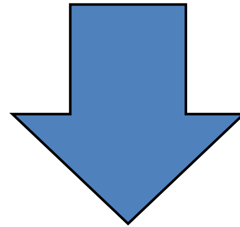
Why do chromosomes undergo recombination?

1. include roles in specialized DNA repair systems,
2. specialized activities in DNA replication,
3. regulation of expression of certain genes,
4. facilitation of proper chromosome segregation during eukaryotic cell division,
5. maintenance of genetic diversity,
6. and implementation of programmed genetic rearrangements during embryonic development.

Recombination

ABCDEFGHIJKLMNOPQRSTUVWXYZ

abcdefghijklmnopqrstuvwxyz



ABCDEFGhijklmnoPQRSTUVWXYZ

abcdefgHIJKLMNOpqrstuvwxyz

Mitotic and meiotic recombination

- Recombination can occur both during mitosis and meiosis
- Only meiotic recombination serves the important role of reassorting genes
- Mitotic recombination may be important for repair of mutations in one of a pair of sister chromatids

Recombination mechanisms

- Best studied in yeast, bacteria and phage
- Recombination is mediated by the breakage and joining of DNA strands

Classes of Recombination

Genetic recombination events fall into at least three general classes.

1. Homologous genetic recombination
(also called general recombination) involves genetic exchanges between any two DNA molecules (or segments of the same molecule) that share an extended region of nearly identical sequence. The actual sequence of bases is irrelevant, as long as it is similar in the two DNAs.

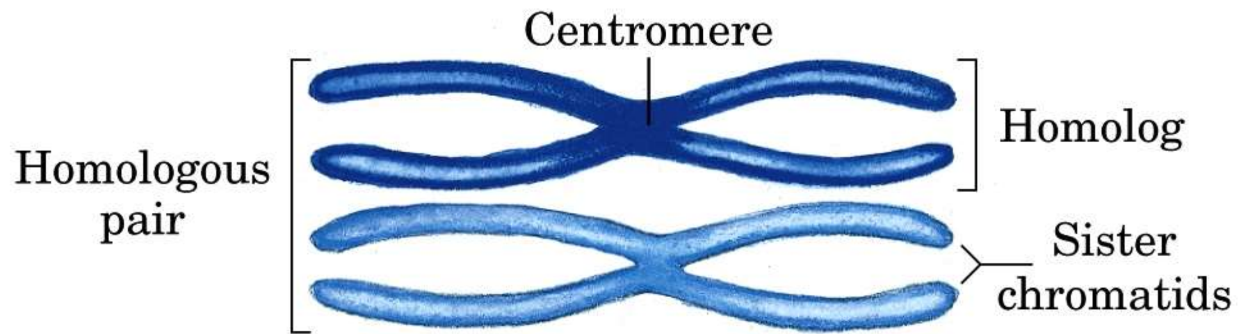
2. In **site-specific recombination, the exchanges occur** only at a *particular DNA sequence.*

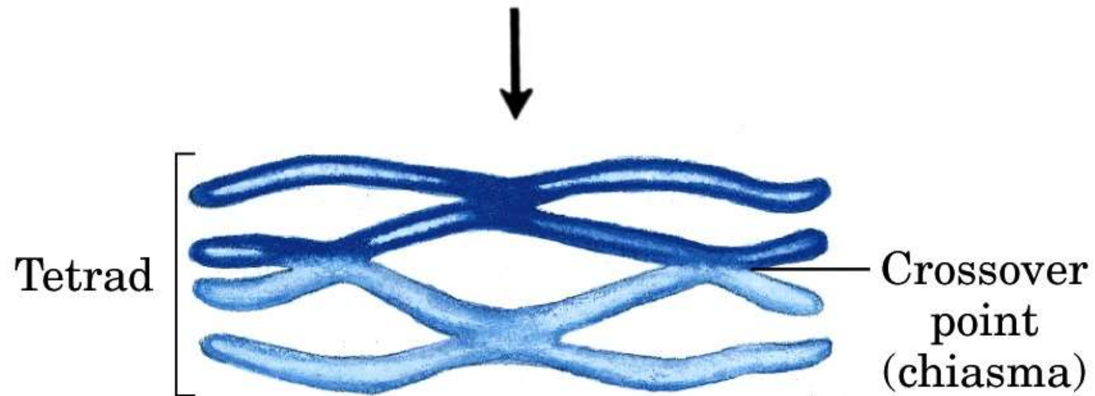
3. DNA transposition is distinct from both other classes in that it usually involves a short segment of DNA with the remarkable capacity to move from one location in a chromosome to another. These "jumping genes" were first observed in maize in the 1940s by Barbara McClintock. There is in addition a wide range of unusual genetic rearrangements for which no mechanism or purpose has yet been proposed

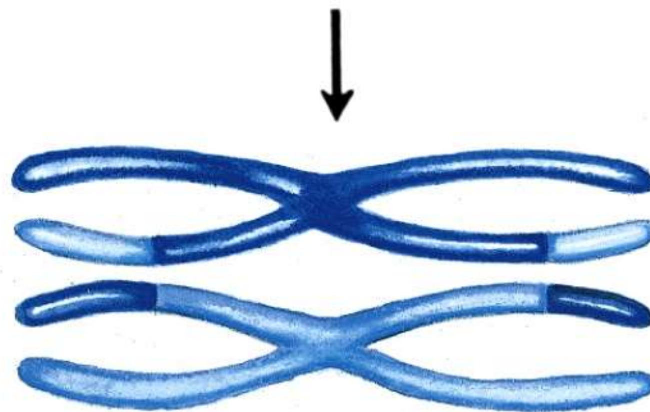
1. Homologous Recombination

Homologous recombination thus serves at least three identifiable functions:

(1) it contributes to the repair of several types of DNA damage;
(2) it provides, in eukaryotic cells, a transient physical link between chromatids that promotes the orderly segregation of chromosomes at the first meiotic cell division; and
(3) it enhances genetic diversity in a population.







(a)

Figure: The homologous chromosomes of a grasshopper are shown during prophase I of meiosis. Many points of joining (chiasmata) are evident between the two homologous pairs of chromatids. These chiasmata are the physical manifestation of prior homologous recombination (crossing over) events.

- *DNA Recombination Is Directed by Specific Enzymes*

- Genetic recombination involves:
  - endonuclease nicking
  - strand displacement
  - ligation
  - branch migration
  - duplex separation to generate the characteristic **Holliday structure** (chi form)

The Holliday model

- Two homologous duplexes are aligned
- Strand exchange leads to an intermediate with crossed strands
- This branch can move: Branch migration
- The branch is resolved by cleavage and sealing

Recombination during Meiosis Is Initiated with Double-Strand Breaks

A likely pathway for homologous recombination during meiosis has four key features.

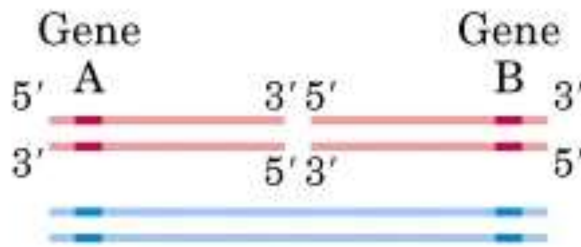
1. First, homologous chromosomes are aligned.
2. Second, a double-strand break in a DNA molecule is enlarged by an exonuclease, leaving a single strand extension with a free 3-hydroxyl group at the broken end (step 1).
3. Third, the exposed 3 ends invade the intact duplex DNA, and this is followed by **branch migration (Fig. 25–32) and/or replication to** create a pair of crossover structures, called Holliday junctions (Fig. 25–31a, steps 2 to 4).
4. Fourth, cleavage of the two crossovers creates two complete recombinant products (step 5).

In this **double-strand break repair model for recombination,** the 3 ends are used to initiate the genetic exchange. Once paired with the complementary strand on the intact homolog, a region of hybrid DNA is created that contains complementary strands from two different parent DNAs (the product of step 2 in Fig. 25–31a).

Each of the 3 ends can then act as a primer for DNA replication. The structures thus formed, **Holliday intermediates** (Fig. 25–31b), are a feature of homologous genetic recombination pathways in all organisms.

FIGURE 25–31

Double strand break model

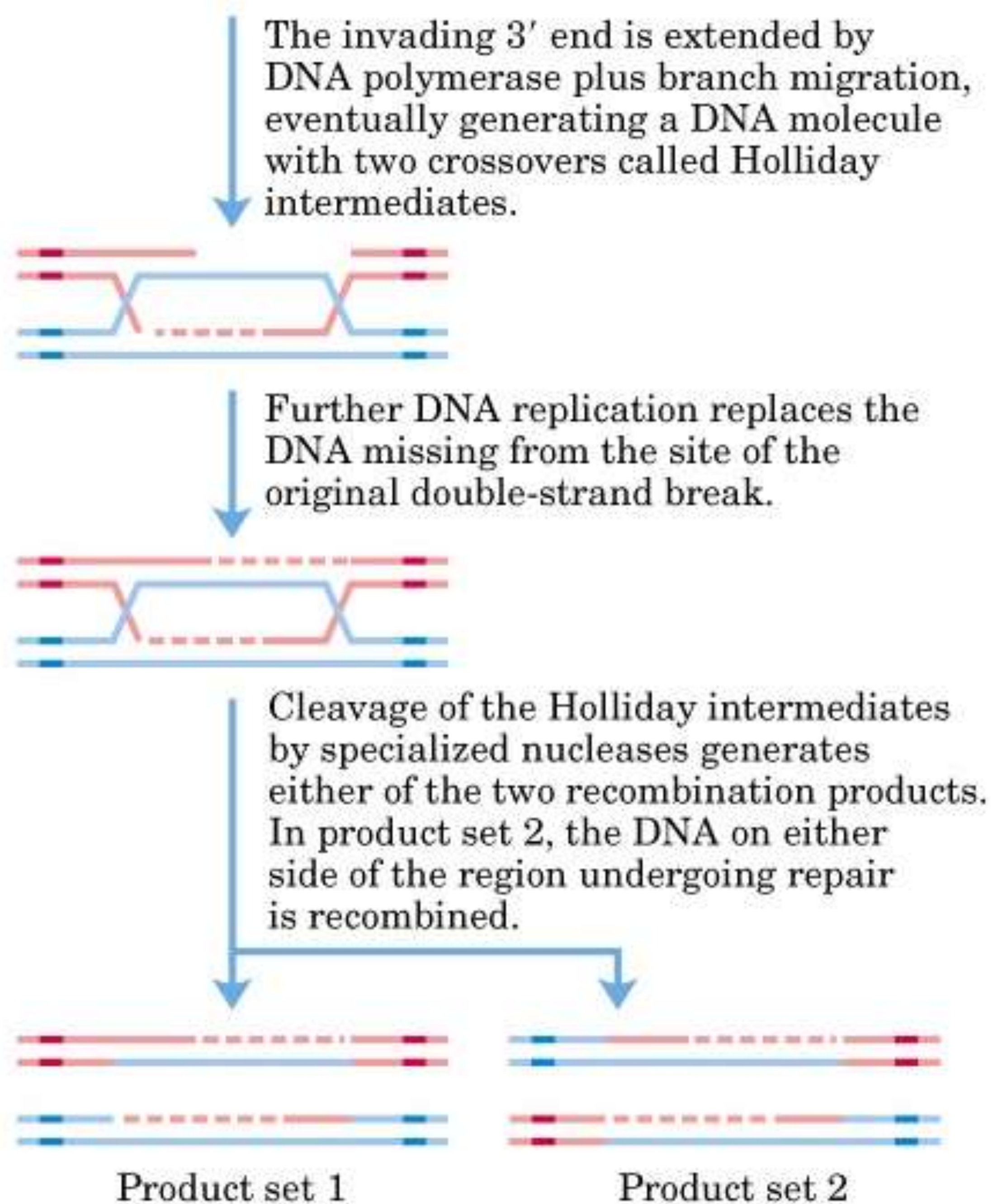


A double-strand break in one of two homologs is converted to a double-strand gap by the action of exonucleases. Strands with 3′ ends are degraded less than those with 5′ ends, producing 3′ single-strand extensions.

An exposed 3′ end pairs with its complement in the intact homolog. The other strand of the duplex is displaced.

The invading 3′ end is extended by DNA polymerase plus branch migration, eventually generating a DNA molecule with two crossovers called Holliday intermediates.

Double strand break model

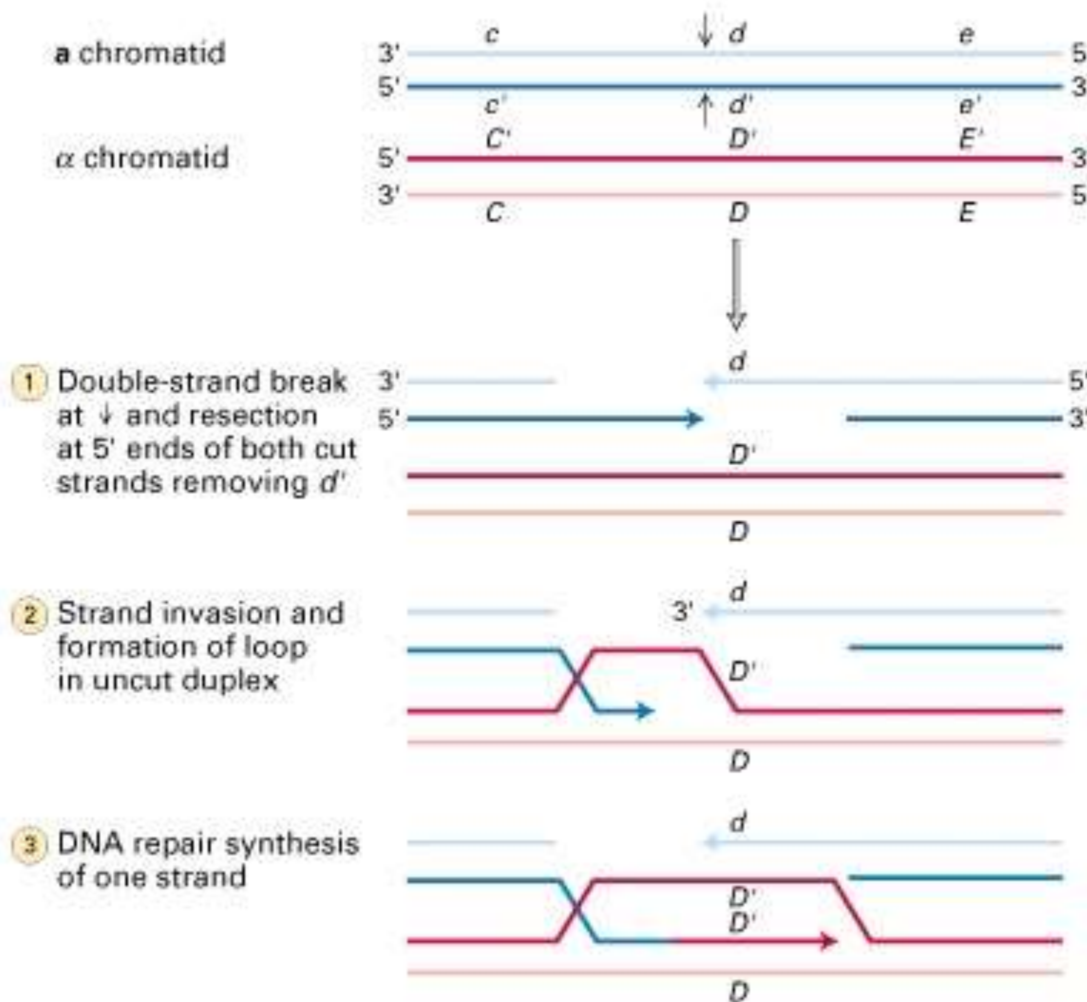


(a)

FIGURE 25–31

A Holliday intermediate formed between two bacterial plasmids in vivo, as seen with the electron microscope. The intermediates are named for Robin Holliday, who first proposed their existence in 1964.

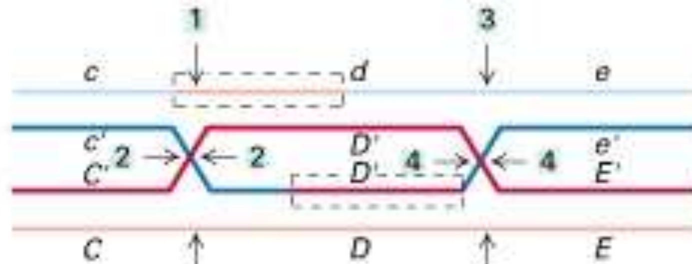
Double-strand breaks in DNA initiate recombination (part I)



Double-strand breaks in DNA initiate recombination (part II)

4a DNA repair synthesis of other strand

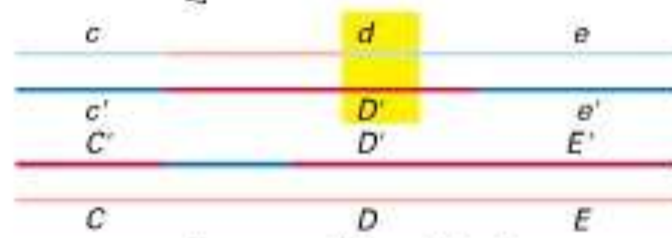
4b Ligation and formation of Holliday structures



5a Cleavage at 2 and 3

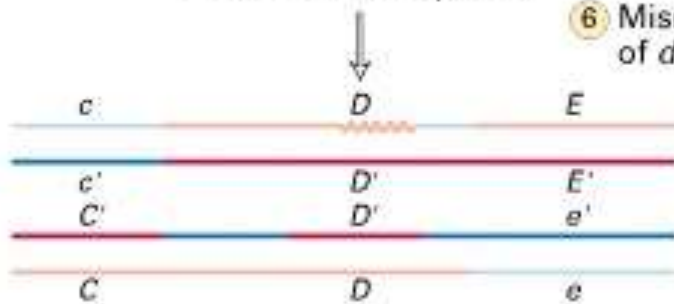
5b Cleavage at 2 and 4

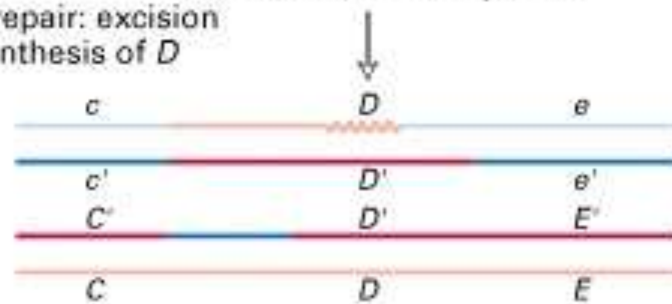
Recombinant duplexes



Nonrecombinant duplexes

6 Mismatch repair: excision of *d* and synthesis of *D*





The cross-strand Holliday structure is an intermediate in recombination

The crossover point can move in either direction, often thousands of nucleotides, in a process known as **branch migration (Fig. 30-67*e, f*)** in which the four strands exchange base-pairing partners. A Holliday junction can be resolved into two duplex DNAs in two equally probable ways (Fig. 30-67*g–l):* **1. The cleavage of the strands that did not cross over** (right branch of Fig. 30-67*j–l) exchanges the ends of the* original duplexes to form, after nick sealing, the traditional recombinant DNA (Fig. 1-27*b).*
2. The cleavage of the strands that crossed over (left branch of Fig. 30-67*j–l) exchanges a pair of homologous* single-stranded segments.

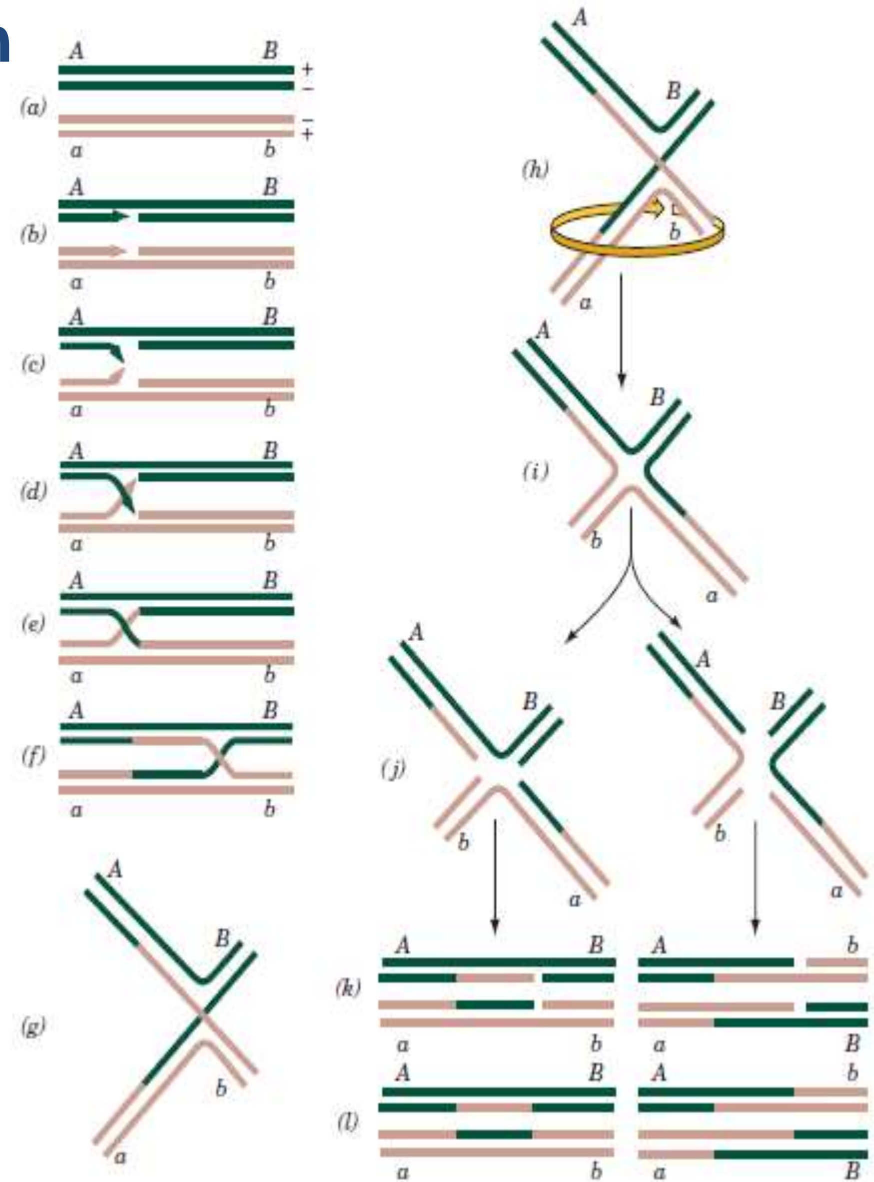
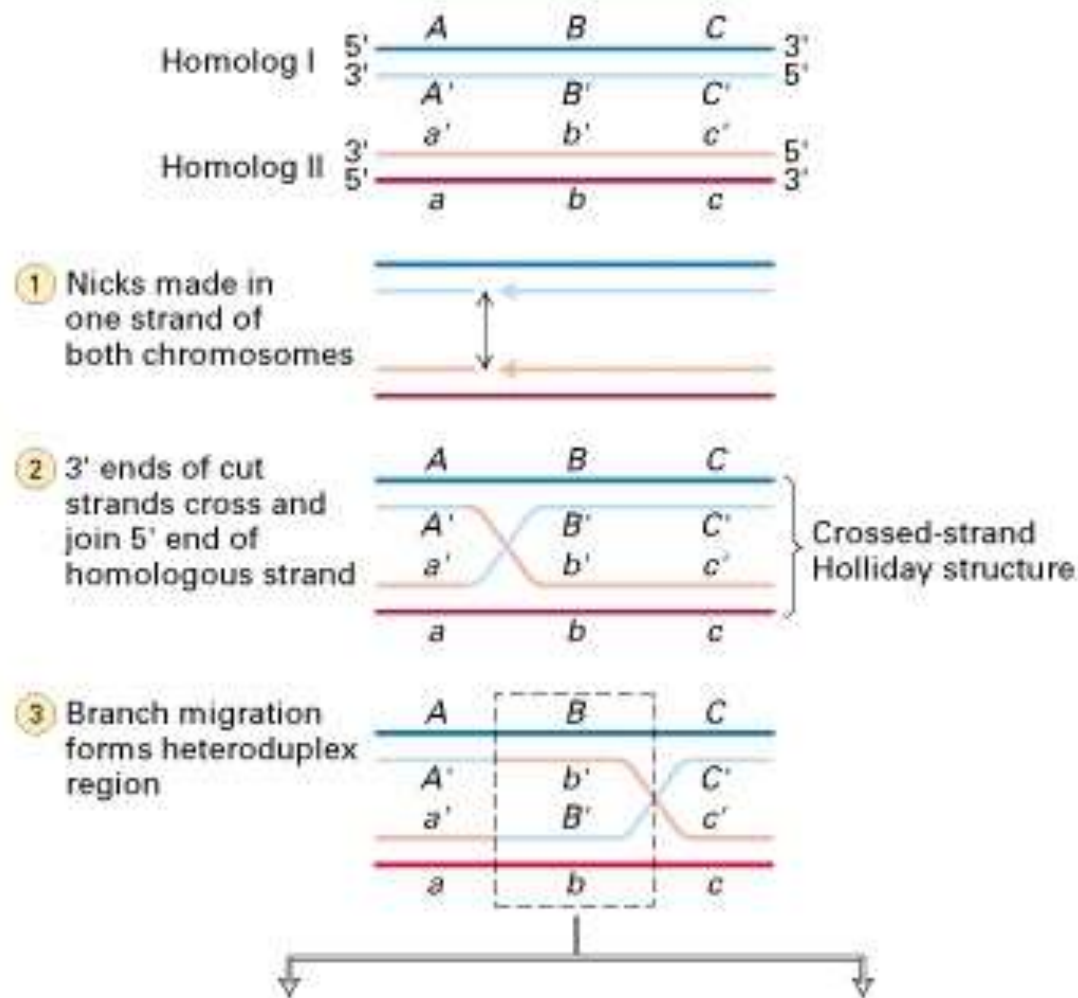
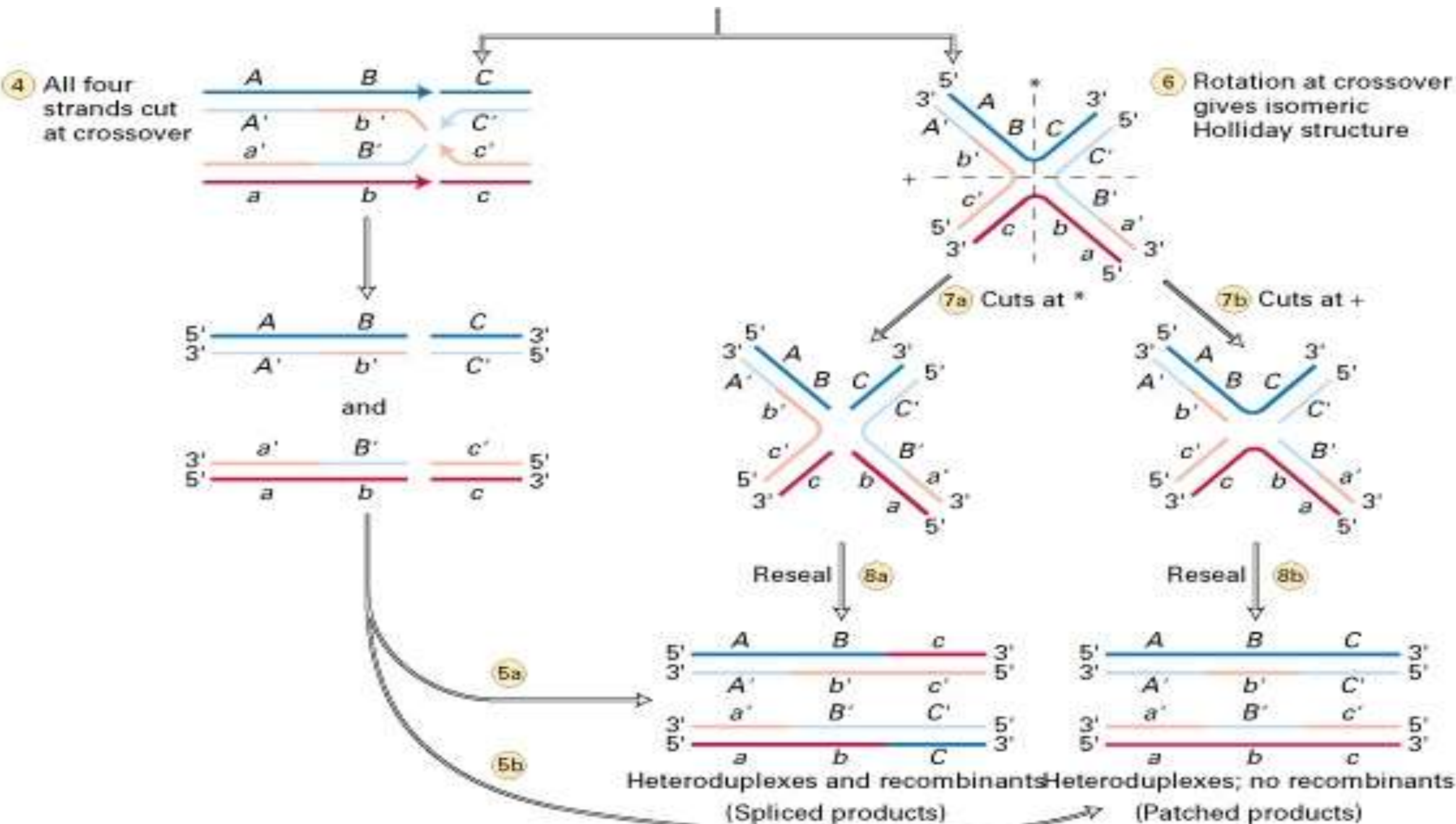


Figure 30-67 **The Holliday model of homologous recombination between homologous DNA duplexes.**
See the Animated Figures

The cross-strand Holliday structure is an intermediate in recombination (part I)



The cross-strand Holliday structure is an intermediate in recombination (part II)

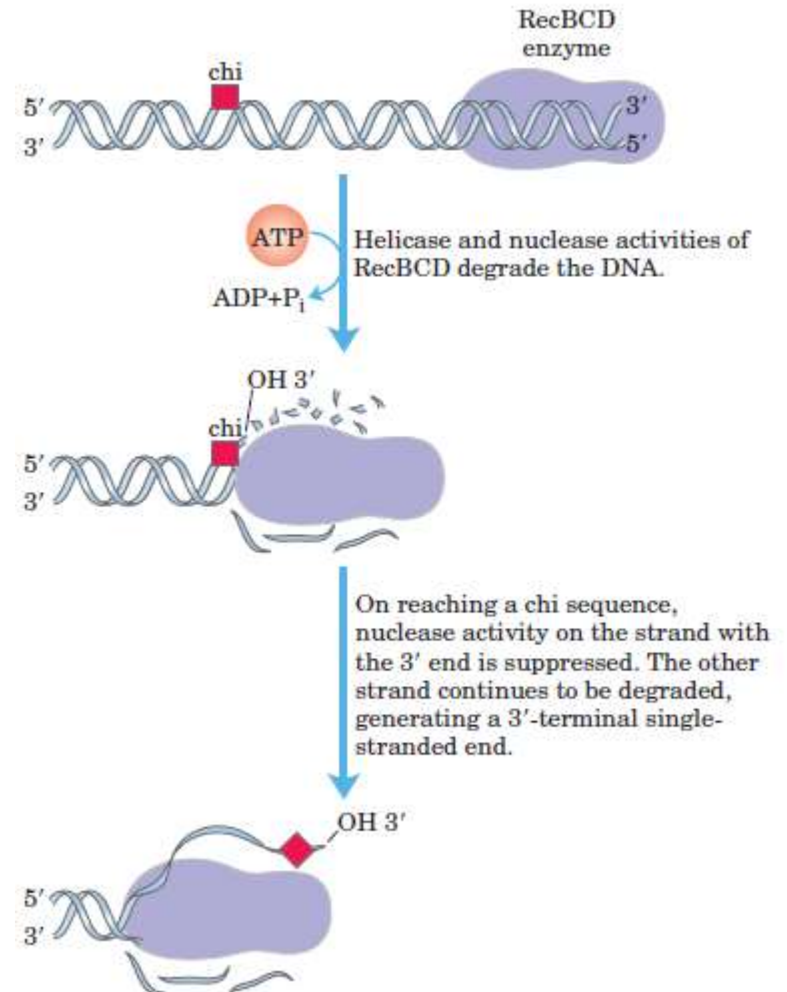


Recombination Requires a Host of Enzymes and Other Proteins

The RecBCD enzyme binds to linear DNA at a free (broken) end and moves inward along the double helix, unwinding and degrading the DNA in a reaction coupled to ATP hydrolysis (Fig. 25–33). The activity of the enzyme is altered when it interacts with a sequence referred to as chi, (5)GCTGGTGG. From that point, degradation of the strand with a 3 terminus is greatly reduced, but degradation of the 5-terminal strand is increased. This process creates a single-stranded DNA with a 3 end, which is used during subsequent steps in recombination (Fig. 25–31).

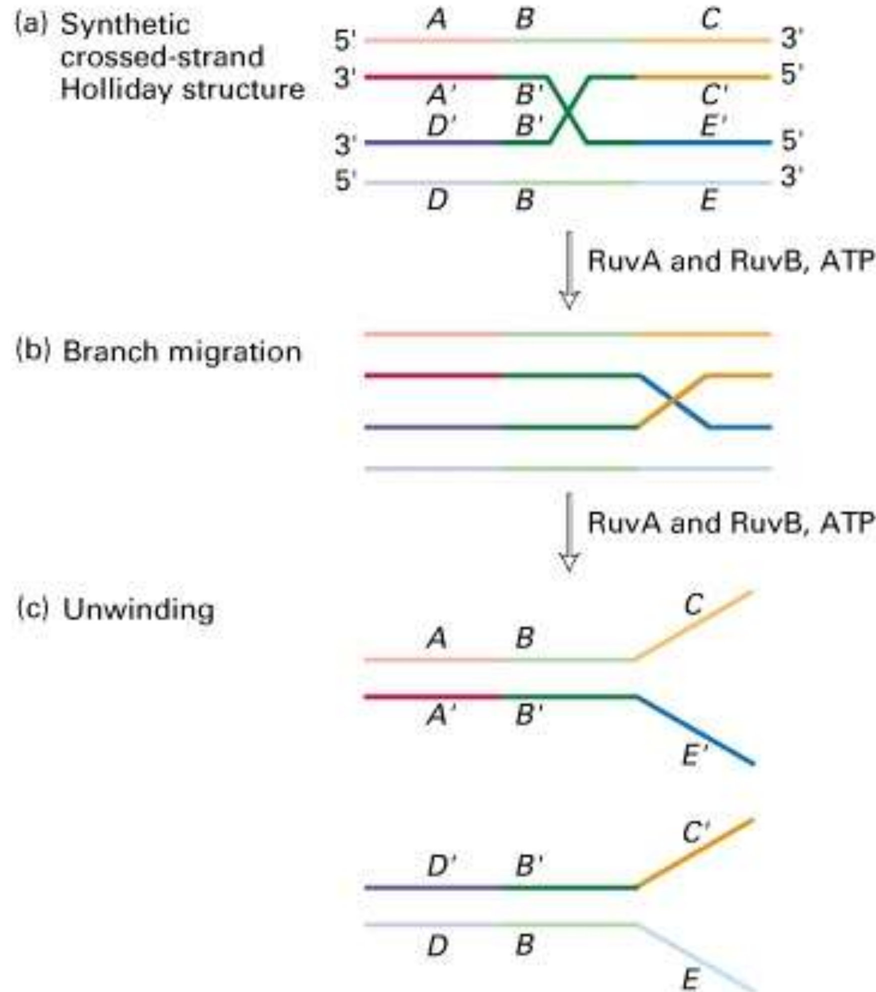
Initiation of recombination by the RecBCD enzyme

FIGURE 25–33 Helicase and nuclease activities of the RecBCD enzyme.
Entering at a double-stranded end, RecBCD unwinds and degrades the DNA until it encounters a chi sequence. The interaction with chi alters the activity of RecBCD so that it generates a singlestranded DNA with a 3 end, suitable for subsequent steps in recombination. Movement of the enzyme requires ATP hydrolysis. This enzyme is believed to help initiate homologous genetic recombination in *E. coli. It is also involved in the repair of double-strand breaks at* collapsed replication forks.



chi, (5)GCTGGTGG.

Branch migration and resolution of Holliday structures depends on Ruv proteins



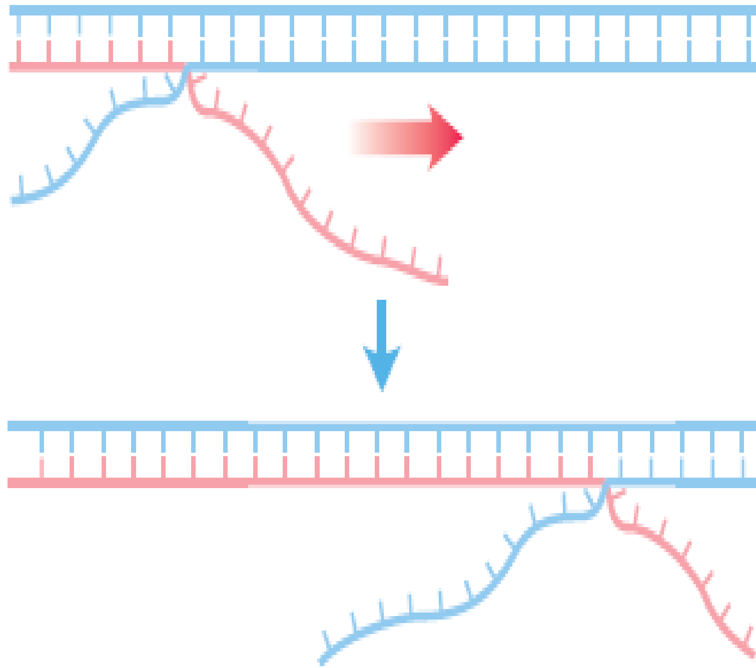
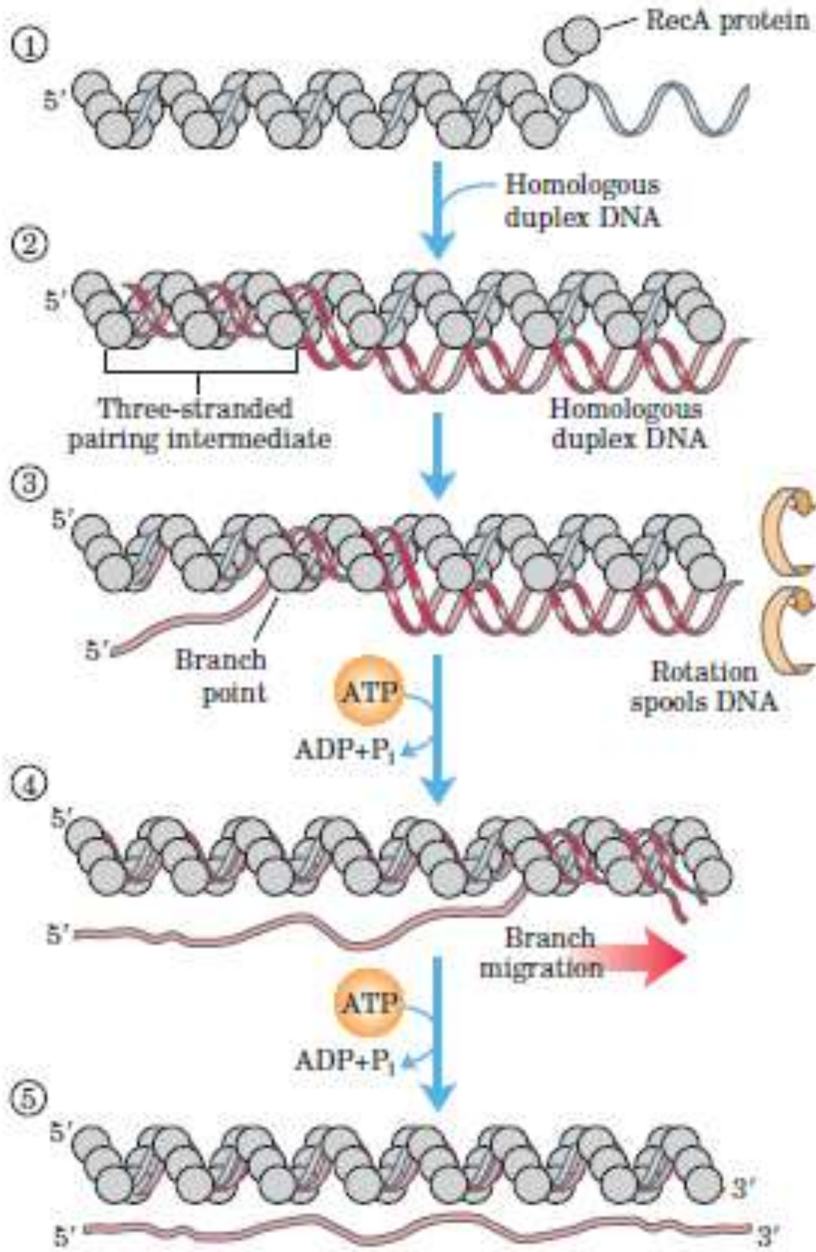


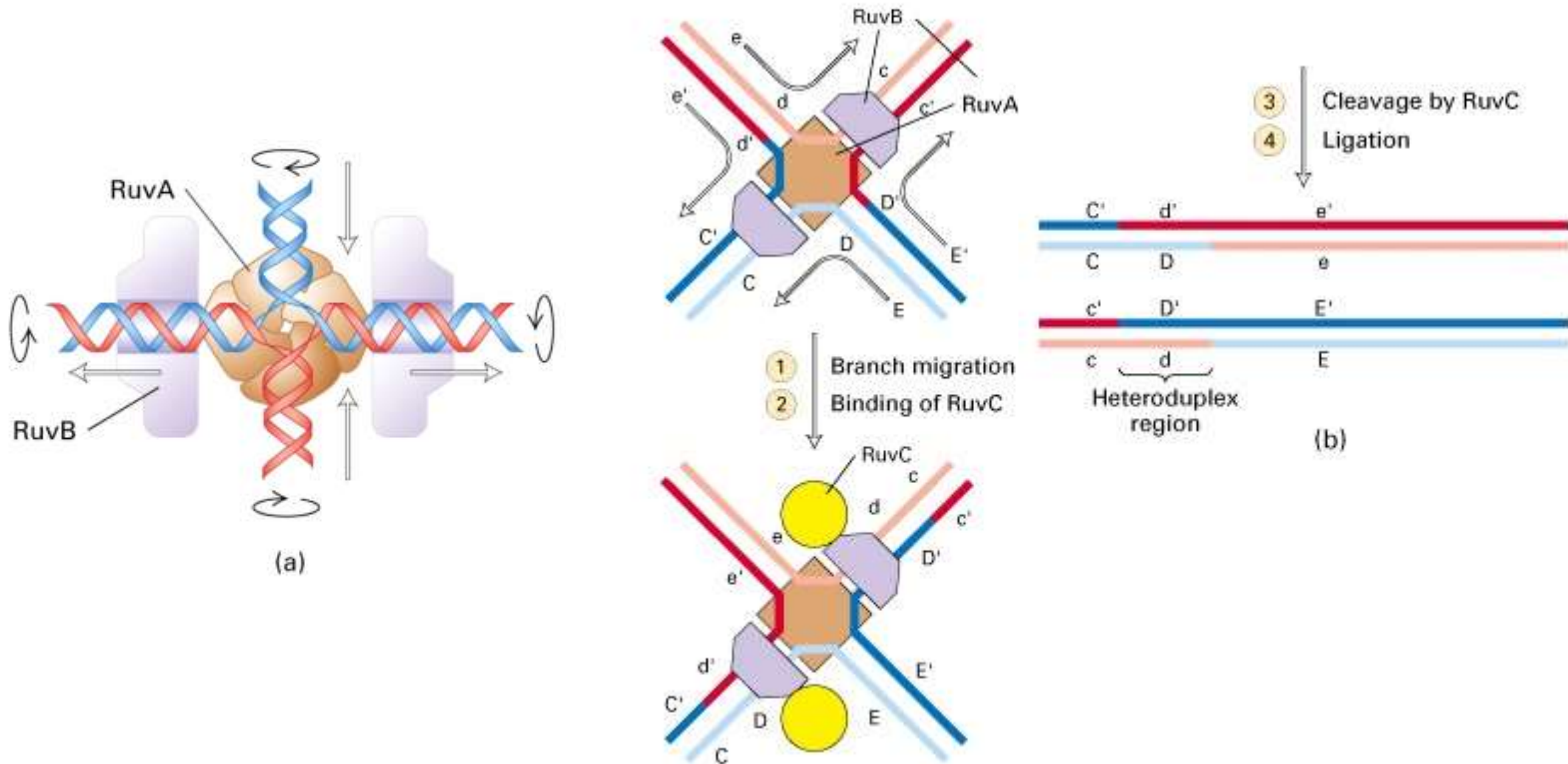
FIGURE 25–32 Branch migration. When a template strand pairs with two different complementary strands, a branch is formed at the point where the three complementary strands meet. The branch "migrates" when base pairing to one of the two complementary strands is broken and replaced with base pairing to the other complementary strand.
In the absence of an enzyme to direct it, this process can move the branch spontaneously in either direction.
Spontaneous branch migration is blocked wherever one of the otherwise complementary strands has a sequence nonidentical to the other strand.

- As the duplex DNA is incorporated within the RecA filament and aligned with the bound single-stranded DNA over regions of hundreds of base pairs, one strand of the duplex switches pairing partners (Fig. 25–36, step 2). Because DNA is a helical structure, continued strand exchange requires an ordered rotation of the two aligned DNAs. This brings about a spooling action (steps 3 and 4) that shifts the branch point along the helix. ATP is hydrolyzed by RecA protein during this reaction. Once a Holliday intermediate has formed, a host of enzymes—topoisomerases, the RuvAB branch migration protein, a resolvase, other nucleases, DNA polymerase I or III, and DNA ligase—are required to complete recombination.
- The RuvC protein (*Mr 20,000) of E. coli* cleaves Holliday intermediates to generate full-length, unbranched chromosome products.

FIGURE 25–36 Model for DNA strand exchange mediated by RecA protein. A three-strand reaction is shown. The balls representing RecA protein are undersized relative to the thickness of DNA to clarify the fate of the DNA strands. 1 RecA protein forms a filament on the single- stranded DNA. 2 A homologous duplex incorporates into this complex. 3 As spooling shifts the three-stranded region from left to right, one of the strands in the duplex is transferred to the single strand originally bound in the filament. The other strand of the duplex is displaced, and a new duplex forms within the filament. As rotation continues (4 and 5), the displaced strand separates entirely. In this model, hydrolysis of ATP by RecA protein rotates the two DNA molecules relative to each other and thus directs the strand exchange from left to right as shown.



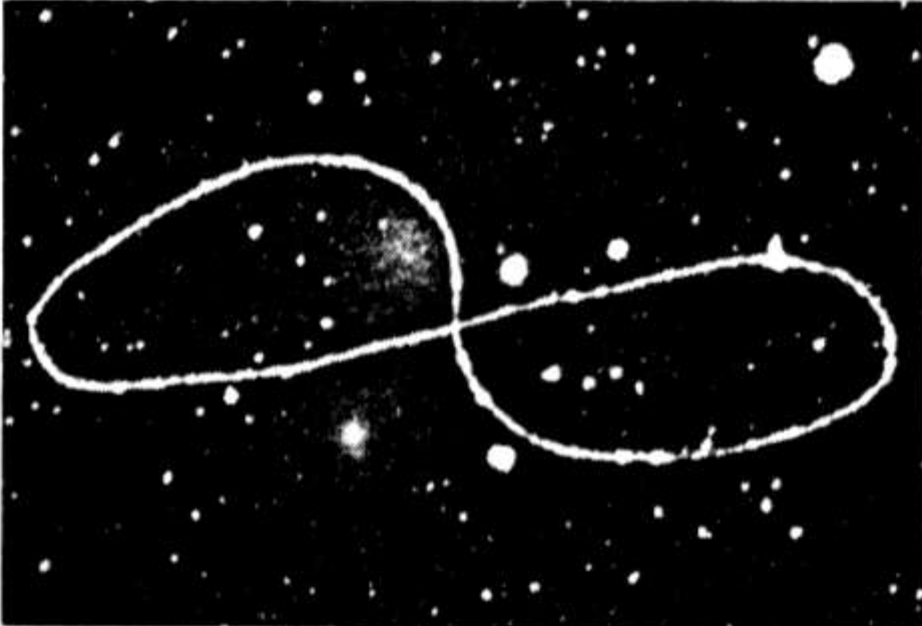
Action of *E. coli* proteins in branch migration and resolution of Holliday structures



Chi structures

- When plasmids recombine figure eight structure is formed
- If the recombined plasmids are cut with a restriction enzyme a χ (chi) is formed

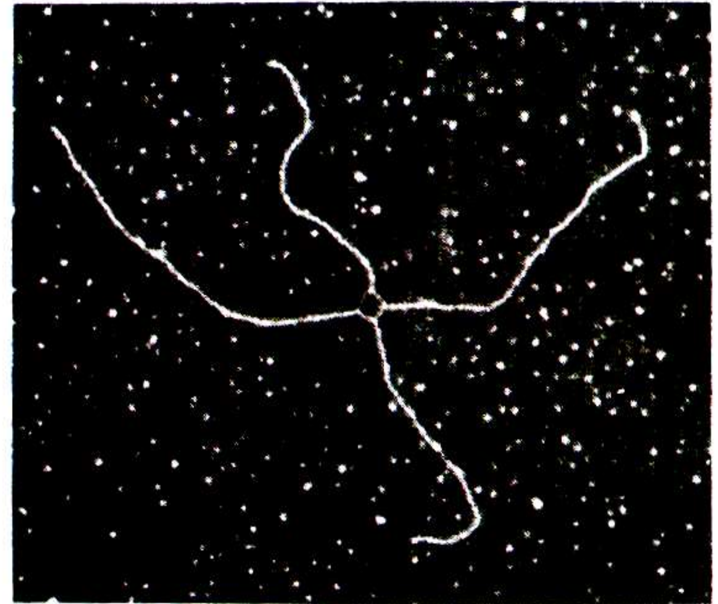
Generation of a chi intermediate

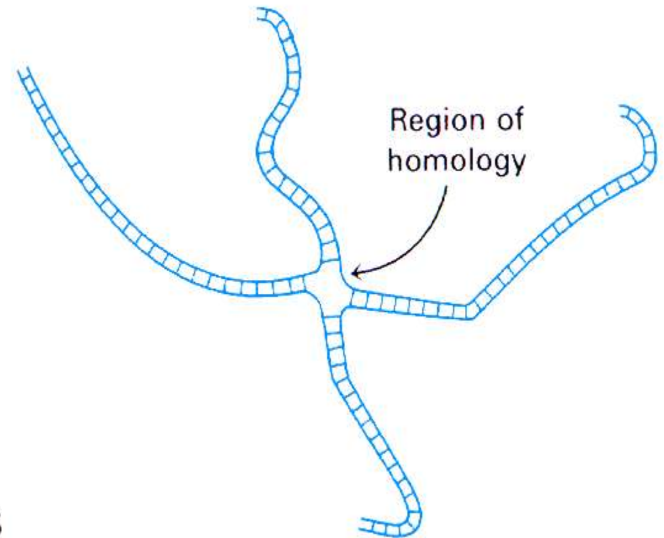


A

B

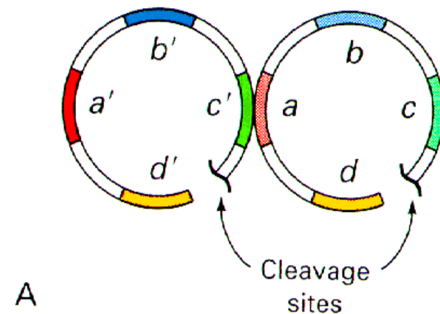
Electron micrograph of the chi form

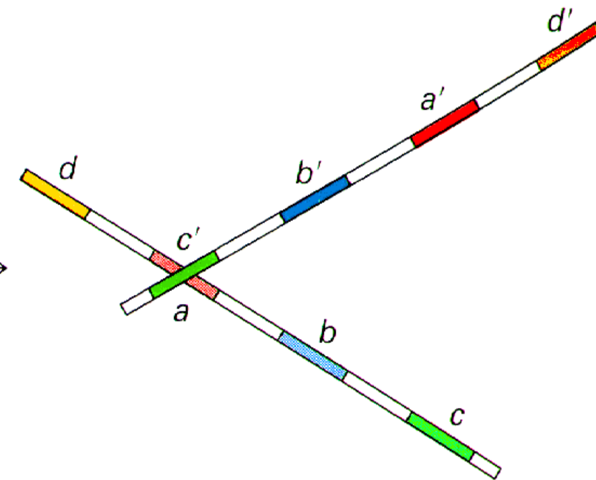


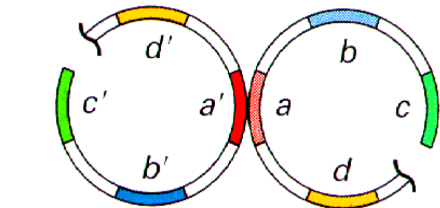


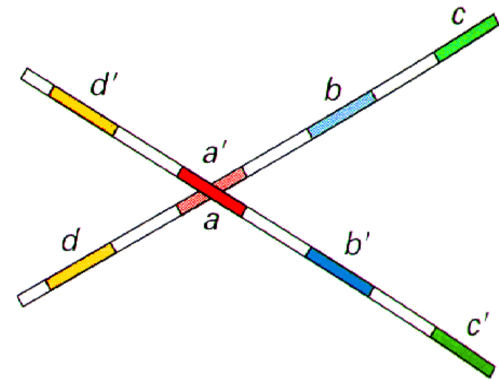
What does the Chi structure prove?

- The fact that each pair of arms is the same length shows that the circles are joined at homologous sites









B

Recombination between homologous DNA sites

- Recombination provides a means by which a genome can change to generate new combinations of genes
- Homologous recombination allows for the exchange of blocks of genes between homologous chromosomes and thereby is a mechanism for generating genetic diversity
- Recombination occurs randomly between two homologous sequences and the frequency of recombination between two sites is proportional to the distance between the sites

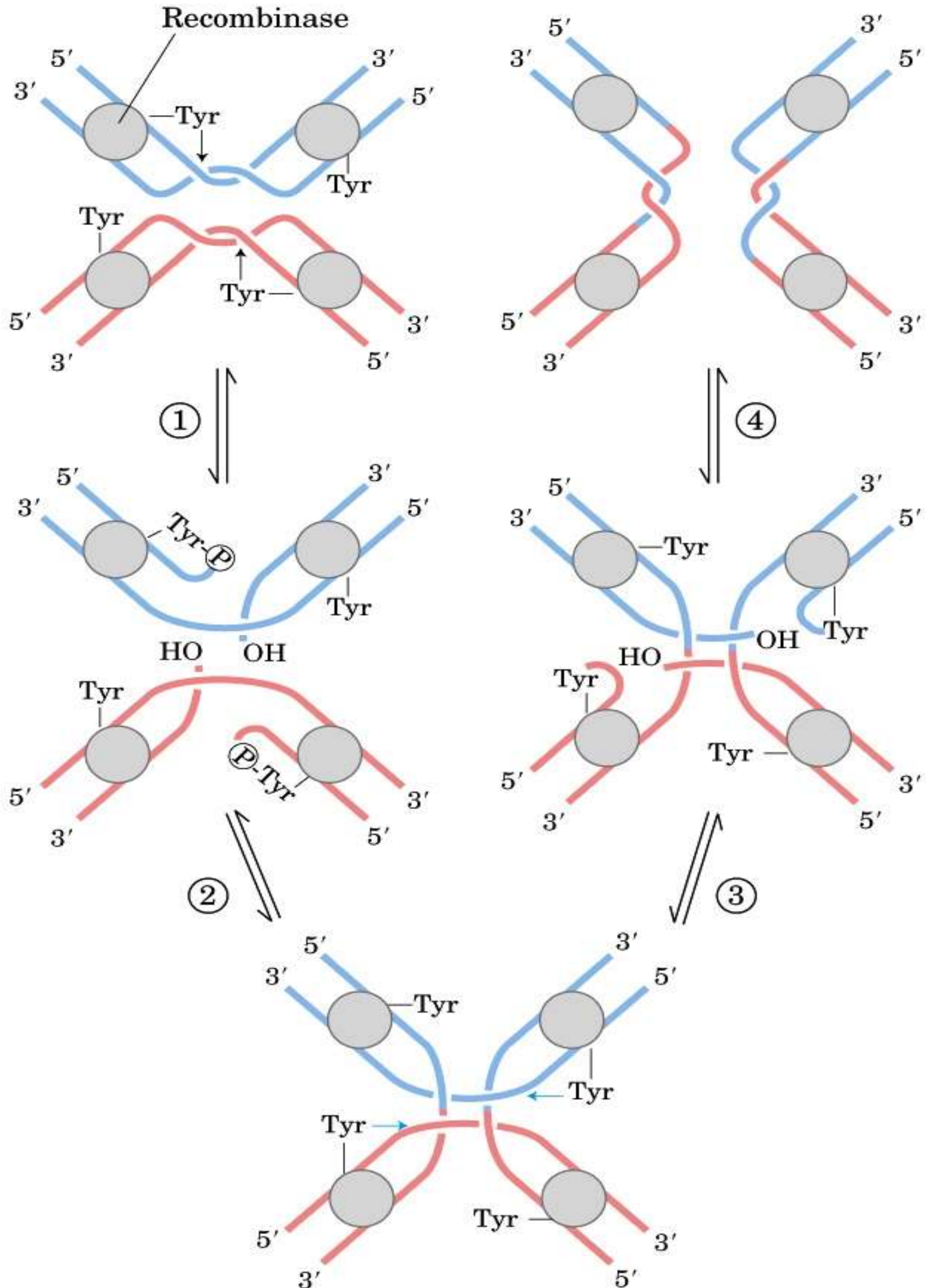
2. Site specific recombination

- Viruses and transposable elements often integrate their genomes into the host chromosome

- Site specific recombination is used by both eukaryotes and prokaryotes to regulate gene expression and to increase the organisms genetic range

Site specific recombination

FIGURE 25–38 A site-specific recombination reaction. (a) The reaction is carried out within a tetramer of identical subunits. Recombinase subunits bind to a specific sequence, often called simply the recombination site. 1 One strand in each DNA is cleaved at particular points within the sequence. The nucleophile is the OH group of an active-site Tyr residue, and the product is a covalent phosphotyrosine link between protein and DNA. 2 The cleaved strands join to new partners, producing a Holliday intermediate. Steps 3 and 4 complete the reaction by a process similar to the first two steps. The original sequence of the recombination site is regenerated after recombining the DNA flanking the site. These steps occur within a complex of multiple recombinase subunits that sometimes includes other proteins.



Each site-specific recombination system consists of an enzyme called a recombinase and a short (20 to 200 bp), unique DNA sequence where the recombinase acts (the recombination site). A separate recombinase recognizes and binds to each of two recombination sites on two different DNA molecules or within the same DNA. One DNA strand in each site is cleaved at a specific point within the site, and the recombinase becomes covalently linked to the DNA at the cleavage site through a phosphotyrosine (or phosphoserine) bond (step 1). The transient protein-DNA linkage preserves the phosphodiester bond that is lost in cleaving the DNA, so high-energy cofactors such as ATP are unnecessary in subsequent steps. The cleaved DNA strands are rejoined to new partners to form a Holliday intermediate, with new phosphodiester bonds created at the expense of the protein-DNA linkage (step 2). To complete the reaction, the process must be repeated at a second point within each of the two recombination sites (steps 3 and 4). In some systems, both strands of each recombination site are cut concurrently and rejoined to new partners without the Holliday intermediate. The exchange is always reciprocal and precise, regenerating the recombination sites when the reaction is complete. We can view a recombinase as a site-specific endonuclease and ligase in one package.

The sequences of the recombination sites recognized by site-specific recombinases are partially asymmetric (nonpalindromic), and the two recombining sites align in the same orientation during the recombinase reaction. The outcome depends on the location and orientation of the recombination sites (Fig. 25–39). If the two sites are on the same DNA molecule, the reaction either inverts or deletes the intervening DNA, determined by whether the recombination sites have the opposite or the same orientation, respectively. If the sites are on different DNAs, the recombination is intermolecular; if one or both DNAs are circular, the result is an insertion. Some recombinase systems are highly specific for one of these reaction types and act only on sites with particular orientations.

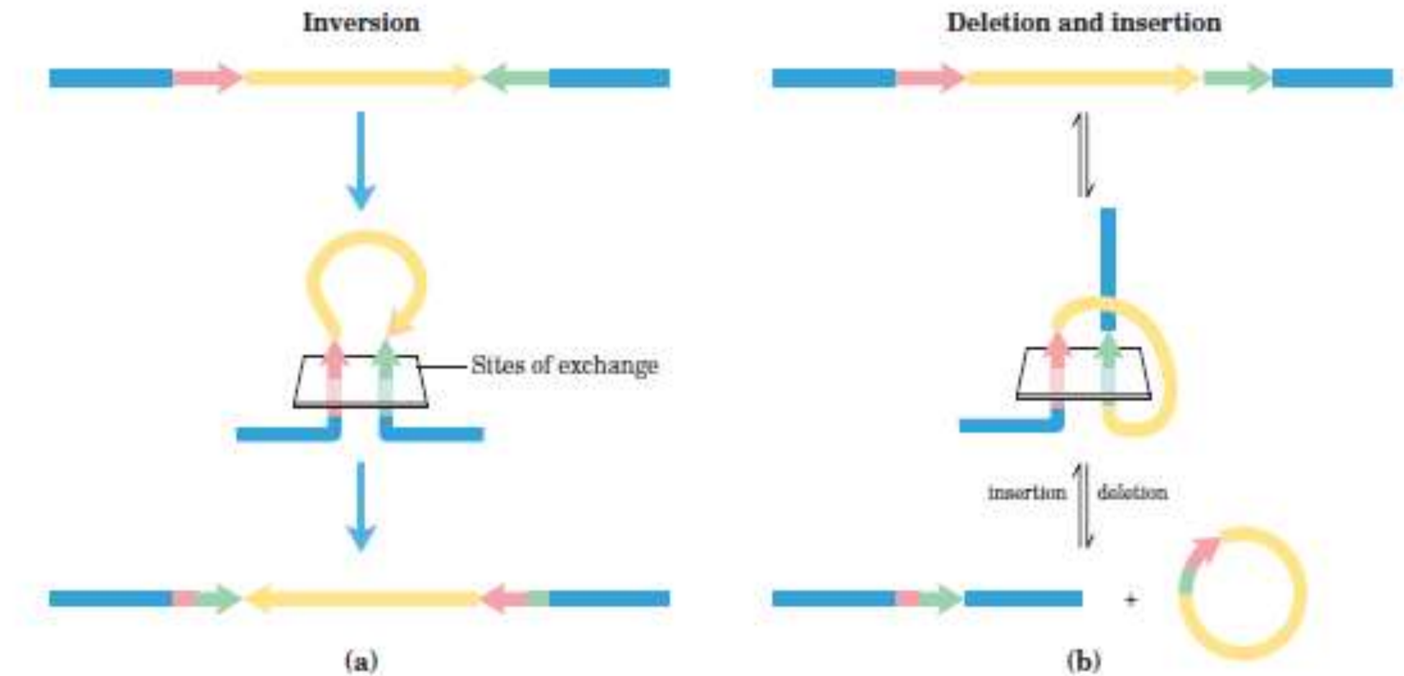


FIGURE 25–39 Effects of site-specific recombination. The outcome of site-specific recombination depends on the location and orientation of the recombination sites (red and green) in a double-stranded DNA molecule. Orientation here (shown by arrowheads) refers to the order of nucleotides in the recombination site, not the 5n3 direction. **(a) Recombination sites with opposite orientation in the same DNA** molecule. The result is an inversion. **(b) Recombination sites with the** same orientation, either on one DNA molecule, producing a deletion, or on two DNA molecules, producing an insertion.

When phage DNA enters an *E. coli cell, a complex series of* regulatory events commits the DNA to one of two fates. The *DNA either replicates and produces more bacteriophages* (destroying the host cell) or integrates into the host chromosome, replicating passively along with the chromosome for many cell generations. Integration is accomplished by a phage-encoded recombinase (*integrase)* that acts at recombination sites on the phage and bacterial DNAs—at attachment sites attP and attB,

respectively (Fig. 25–40).

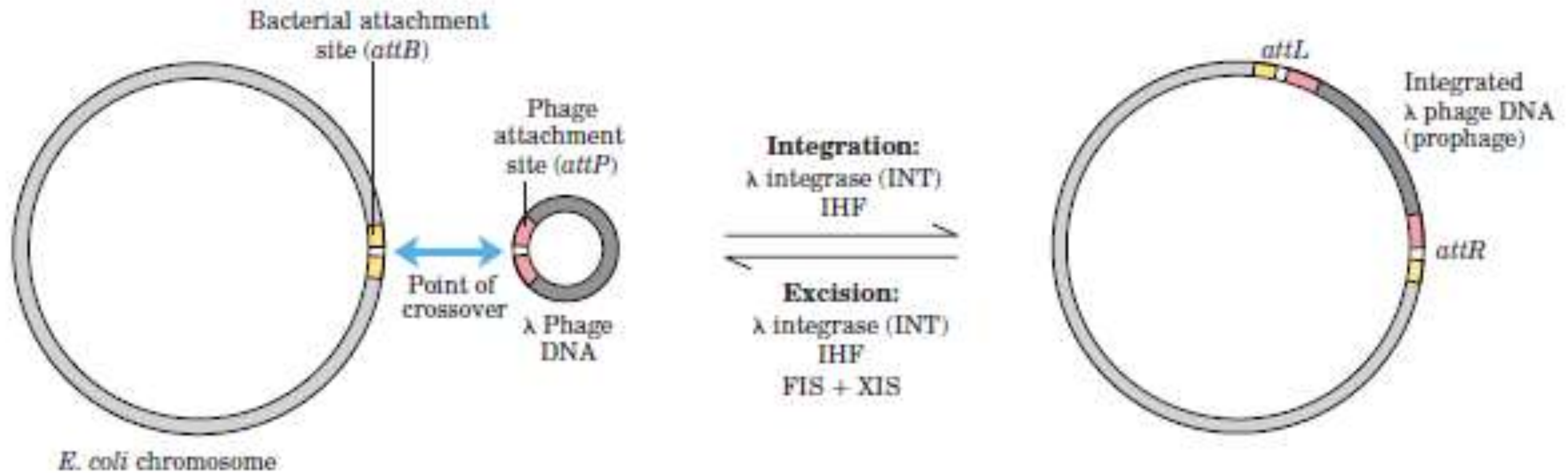


FIGURE 25–40 Integration and excision of bacteriophage *DNA at* the chromosomal target site. The attachment site on the ***phage DNA*** (attP) shares only 15 bp of complete homology with the bacterial site (attB) in the region of the crossover. The reaction generates two new attachment sites (attR and attL) flanking the integrated phage DNA.

The recombinase is the *integrase (or INT protein). Integration and* excision use different attachment sites and different auxiliary proteins. Excision uses the proteins XIS, encoded by the bacteriophage, and FIS, encoded by the bacterium. Both reactions require the protein IHF (*integration host factor), encoded by the bacterium.*

DNA Transposition

recombination that allows the movement of transposable elements, or **transposons. These segments** of DNA, found in virtually all cells, move, or "jump," from one place on a chromosome (the donor site) to another on the same or a different chromosome (the target site). DNA sequence homology is not usually required for this movement, called **transposition;** the new location is determined more or less randomly. Insertion of a transposon in an essential gene could kill the cell, so transposition is tightly regulated and usually very infrequent. Transposons are perhaps the simplest of molecular parasites, adapted to replicate passively within the chromosomes of host cells. In some cases they carry genes that are useful to the host cell, and thus exist in a kind of symbiosis with the host

Classes of Transposons

Bacteria have two classes of transposons.

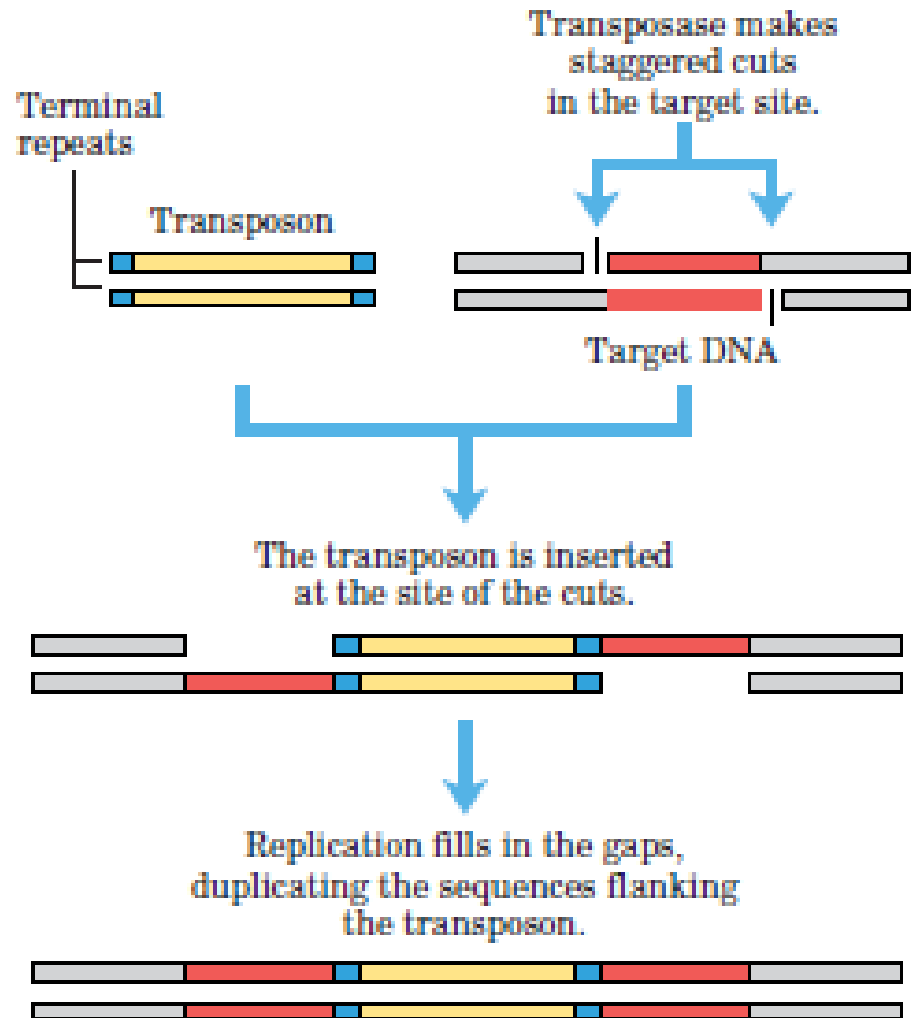
1. **simple transposons**

Insertion sequences contain only the sequences required for transposition and the genes for proteins (transposases) that promote the process.

2. **Complex transposons** contain one or more genes in addition to those needed for transposition. These extra genes might, for example, confer resistance to antibiotics and thus enhance the survival chances of the host cell. The spread of antibiotic-resistance elements among disease-causing bacterial populations that is rendering some antibiotics ineffectual is mediated in part by transposition.

Bacterial transposons vary in structure, but most have short repeated sequences at each end that serve as binding sites for the transposase. When transposition occurs, a short sequence at the target site (5 to 10 bp) is duplicated to form an additional short repeated sequence that flanks each end of the inserted transposon (Fig. 25–42). These duplicated segments result from the cutting mechanism used to insert a transposon into the DNA at a new location.

FIGURE 25–42 Duplication of the DNA sequence at a target site when a transposon is inserted. The duplicated sequences are shown in red. These sequences are generally only a few base pairs long, so their size (compared with that of a typical transposon) is greatly exaggerated in this drawing.



There are two general pathways for transposition in bacteria.

In **direct or simple transposition** (Fig. 25–43, left), cuts on each side of the transposon excise it, and the transposon moves to a new location. This leaves a double-strand break in the donor DNA that must be repaired. At the target site, a staggered cut is made (as in Fig. 25–42), the transposon is inserted into the break, and DNA replication fills in the gaps to duplicate the target site sequence.

In **replicative transposition** (Fig. 25–43, right), the entire transposon is replicated, leaving a copy behind at the donor location. A **cointegrate** is an intermediate in this process, consisting of the donor region covalently linked to DNA at the target site.

Two complete copies of the transposon are present in the cointegrate, both having the same relative orientation in the DNA. In some well-characterized transposons, the cointegrate intermediate is converted to products by site-specific recombination, in which specialized recombinases promote the required deletion reaction.

FIGURE 25–43 Two general pathways for transposition: direct (simple) and replicative. 1 The DNA is first cleaved on each side of the transposon, at the sites indicated by arrows. 2 The liberated 3- hydroxyl groups at the ends of the transposon act as nucleophiles in a direct attack on phosphodiester bonds in the target DNA. The target phosphodiester bonds are staggered (not directly across from each other) in the two DNA strands. 3 The transposon is now linked to the target DNA. In direct transposition, replication fills in gaps at each end. In replicative transposition, the entire transposon is replicated to create a cointegrate intermediate. 4 The cointegrate is often resolved later, with the aid of a separate site-specific recombination system.

The cleaved host DNA left behind after direct transposition is either repaired by DNA end-joining or degraded (not shown). The latter outcome can be lethal to an organism.