GENETIC RECOMBINATION

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Genetic recombination:

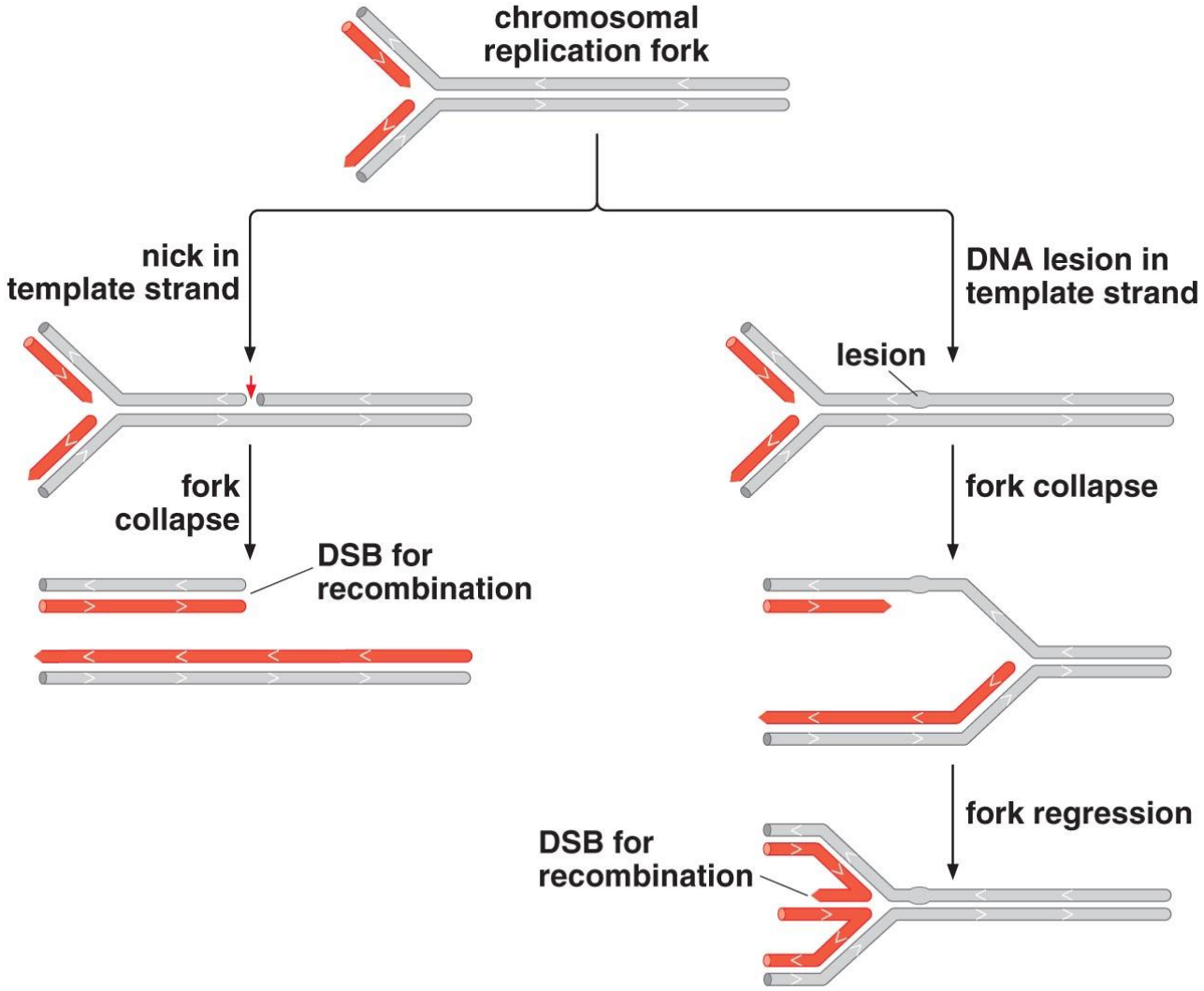
1.Homologous Recombination

2. Site-Specific Recombination

3. DNA Transposition

Homologous Recombination at the Molecular Level

DNA BREAKS ARE COMMON AND INITIATE RECOMBINATION



Recombination repair DNA breaks by retrieving sequence information from undamaged DNA

Double-Strand Breaks are Efficiently Repaired

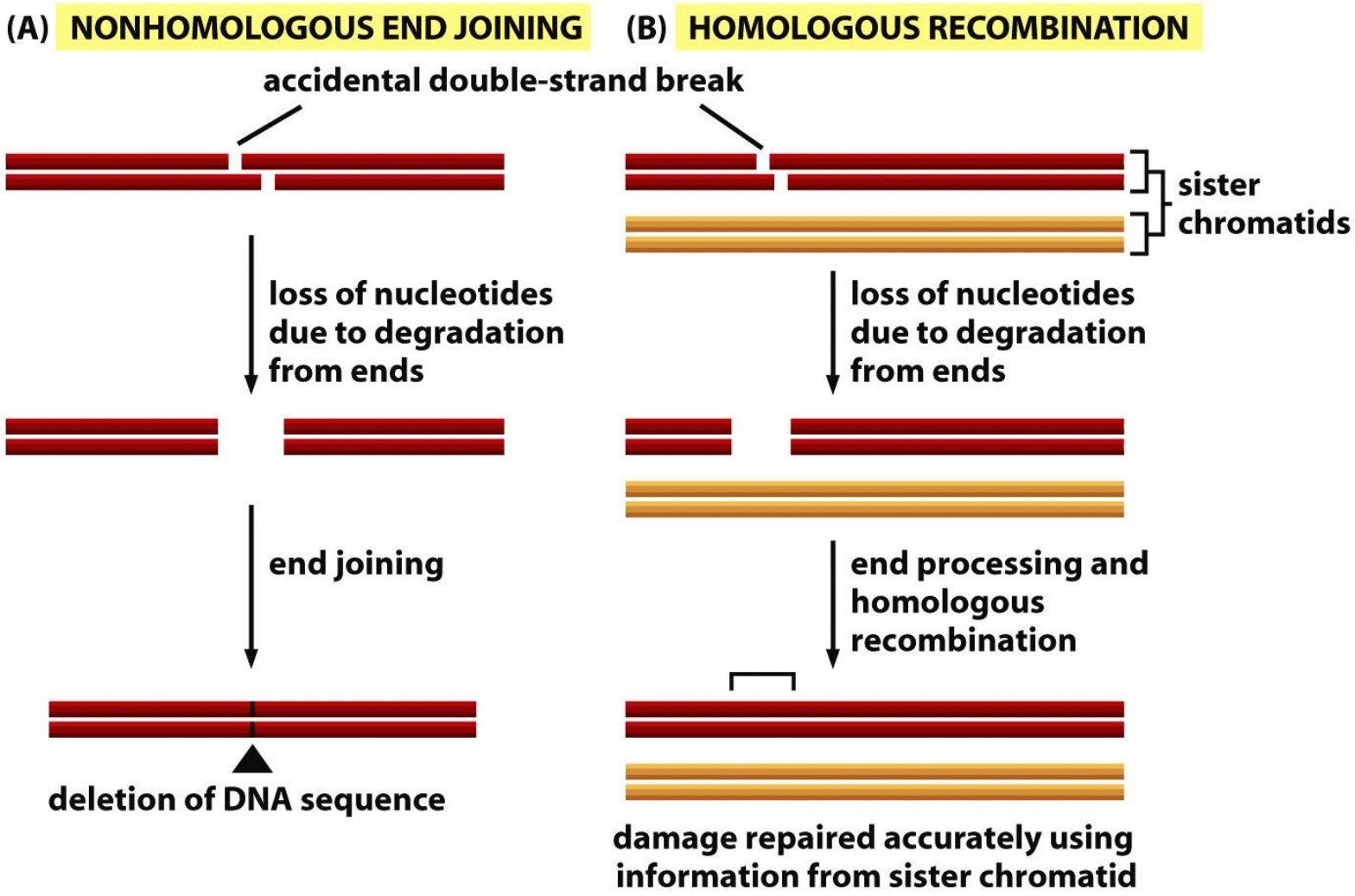
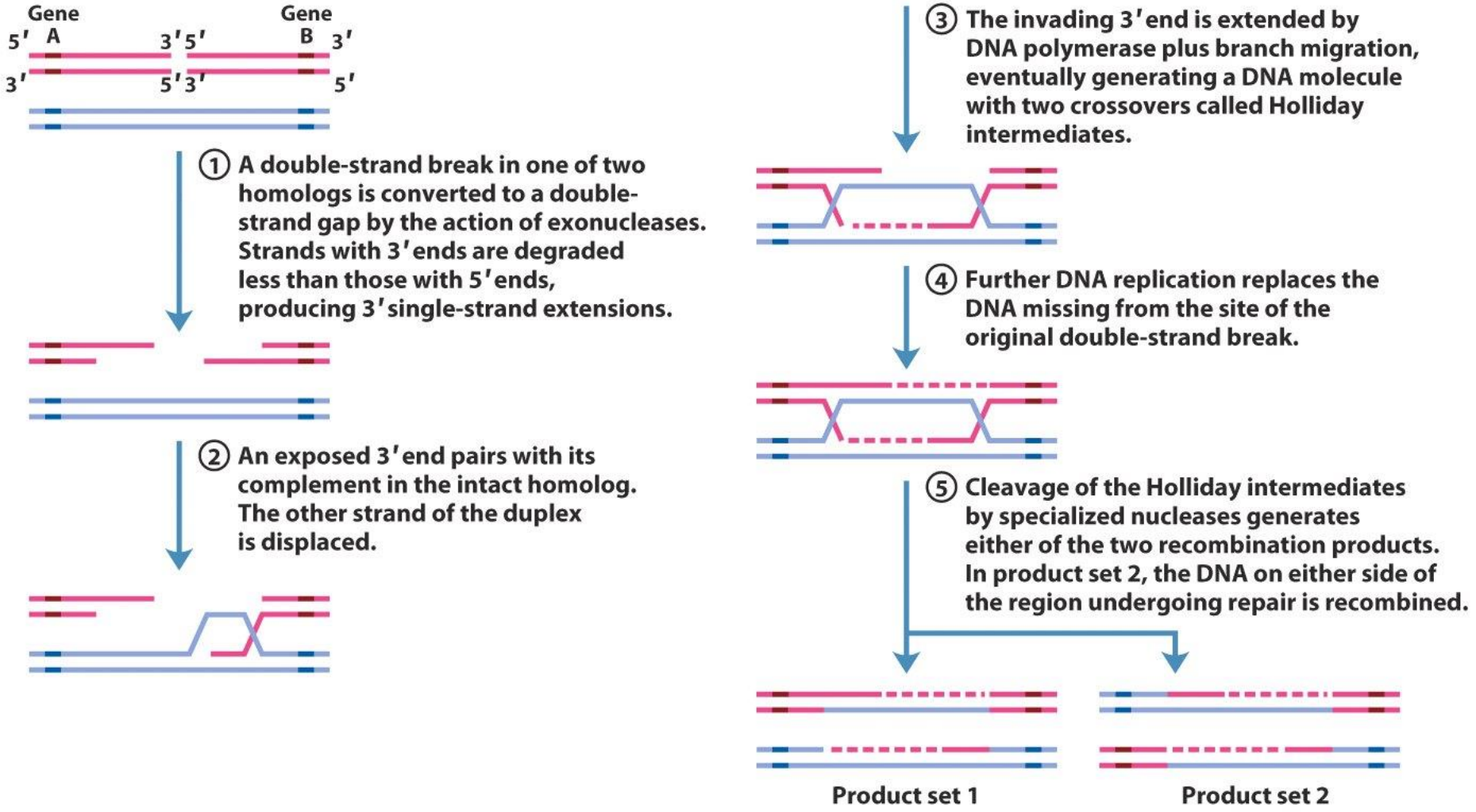
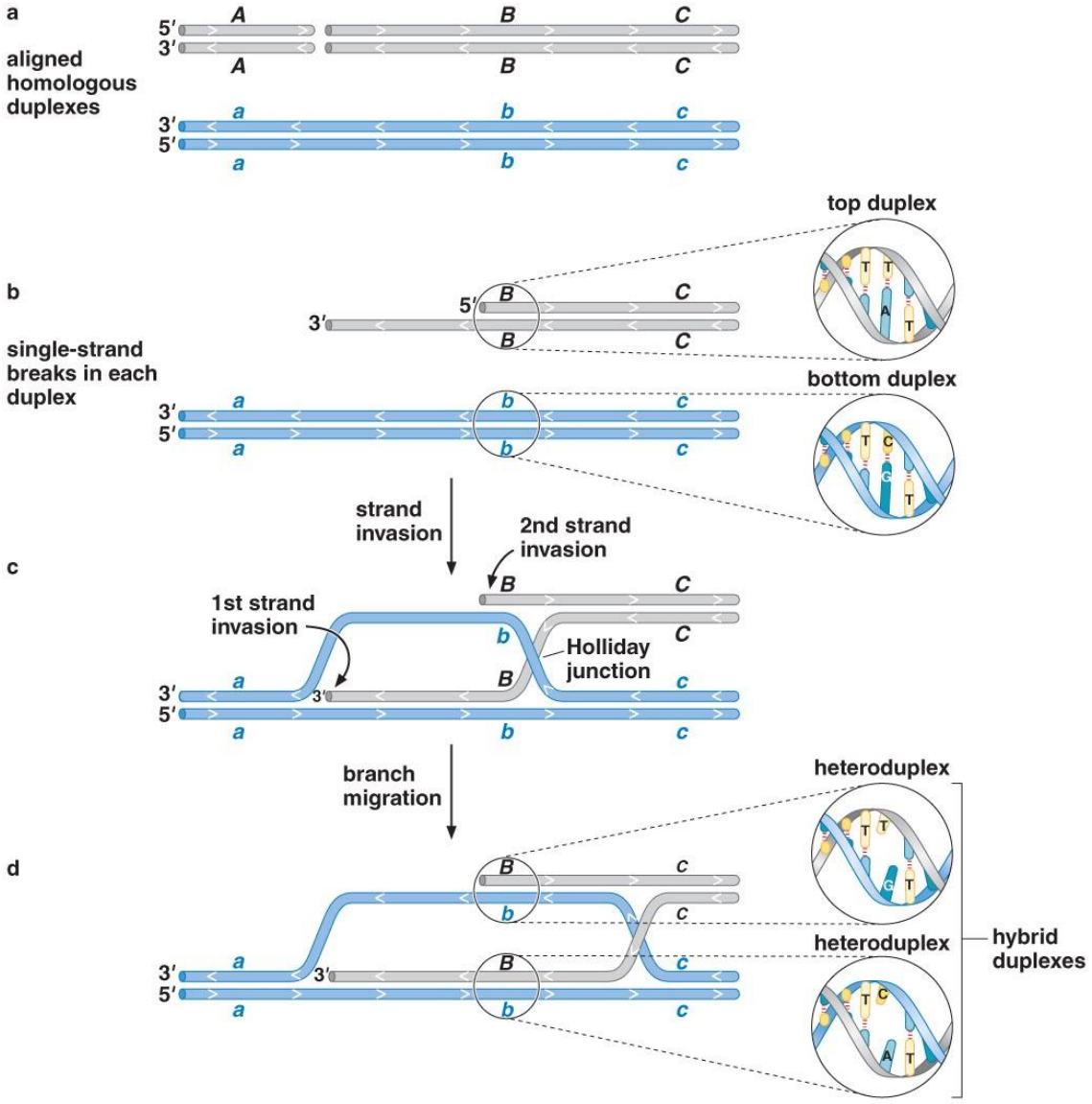


Figure 5-51 Molecular Biology of the Cell 5/e (© Garland Science 2008)

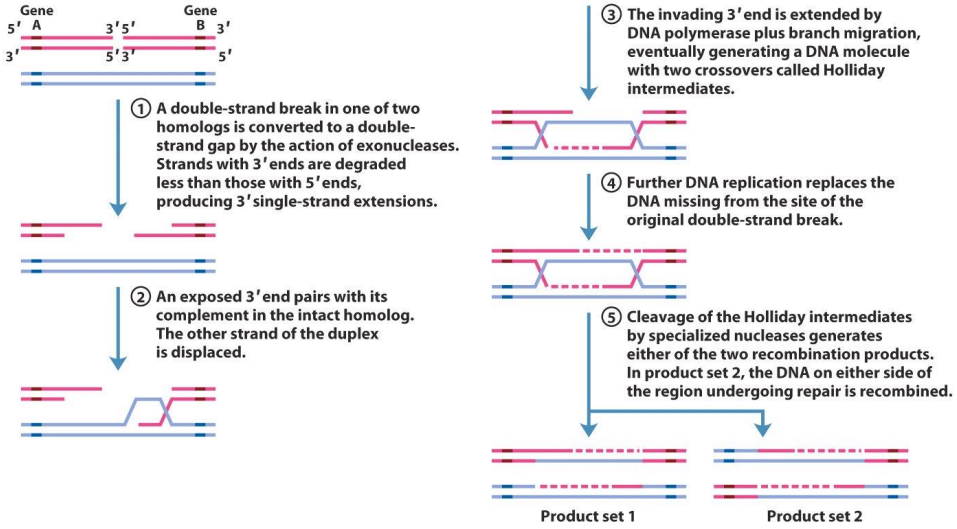
HOMOLOGOUS RECOMBINATION MODELS

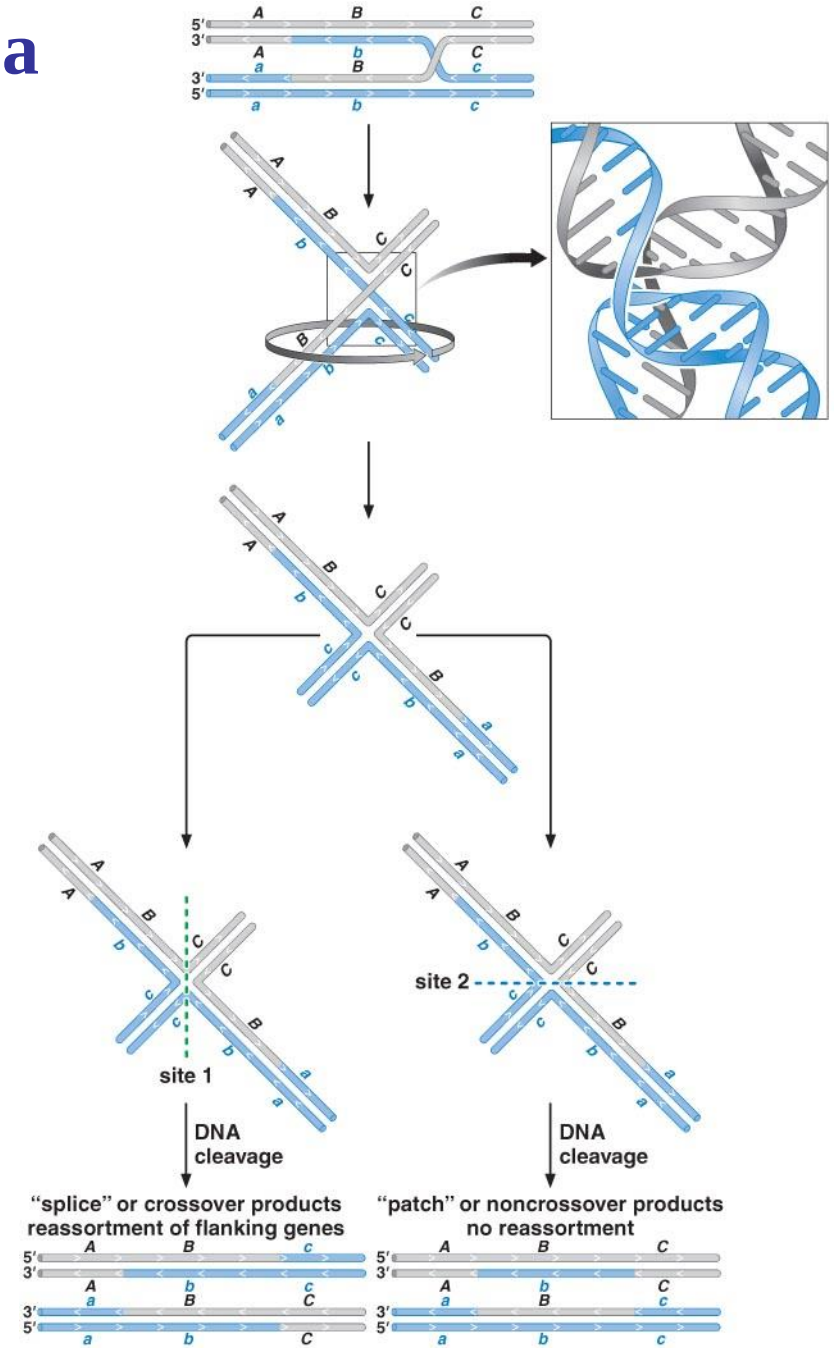


Strand invasion (strand exchange) is a key step in homologous recombination

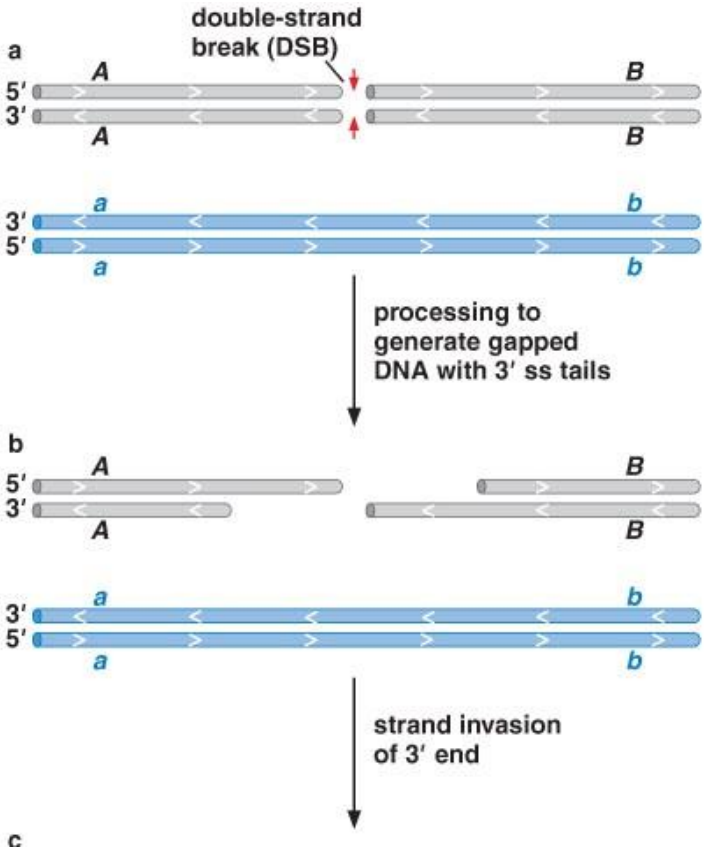


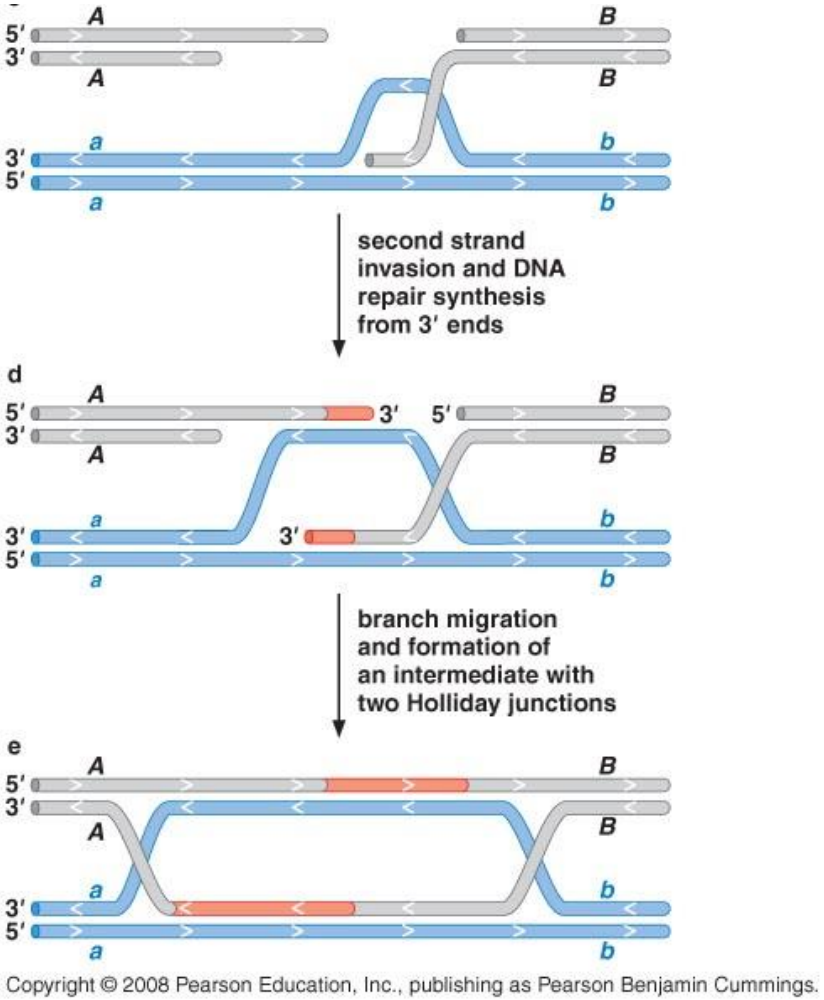
Resolving Holliday junctions is a key step (final step) to finishing genetic exchange





The double-strand break-repair model describes many recombination events



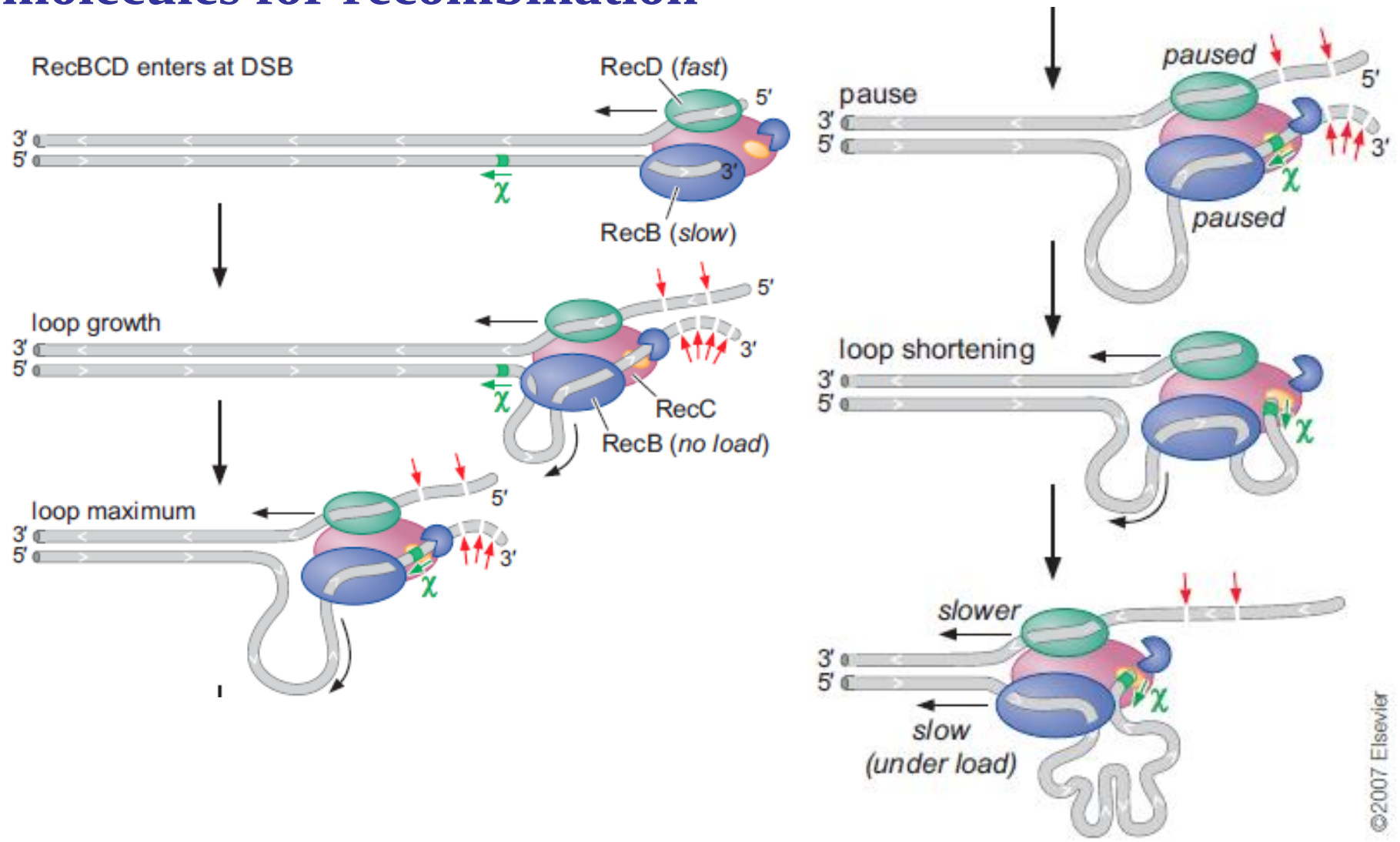


HOMOLOGOUS RECOMBINATION PROTEIN MACHINARIES

TABLE 10-1 Prokaryotic and Eukaryotic Factors That Catalyze Recombination Steps

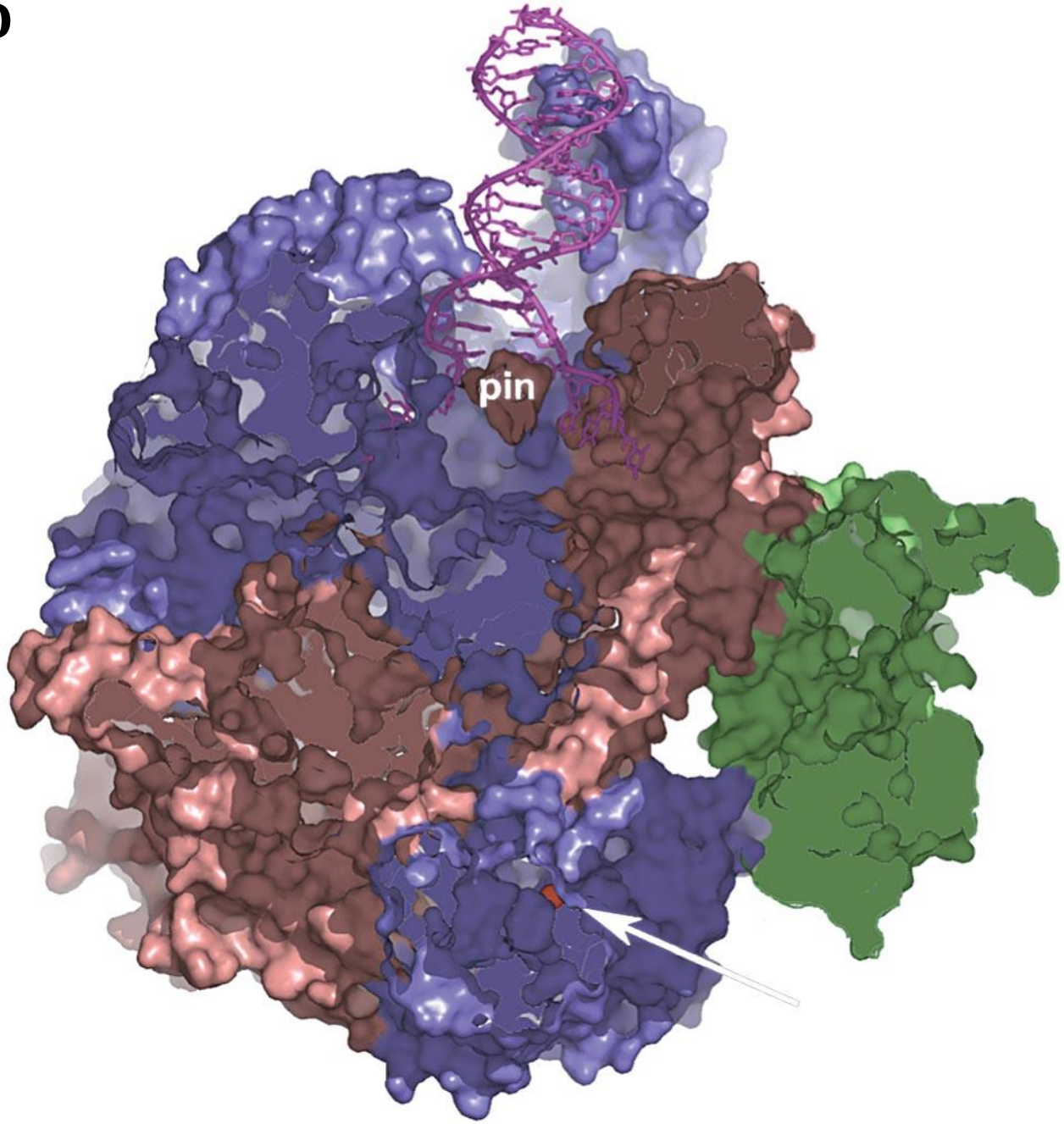
Recombination Step	*E. coli* Protein Catalyst	Eukaryotic Protein Catalyst
Pairing homologous DNAs and strand invasion	RecA protein	Rad51 Dcm1 (in meiosis)
Introduction of DSB	None	Spo11 (in meiosis) HO (for mating-type switching)
Processing DNA breaks to generate single strands for invasion	RecBCD helicase/nuclease	MRX protein (also called Rad50/58/60 nuclease)
Assembly of strand-exchange proteins	RecBCD and RecFOR	Rad52 and Rad59
Holliday junction recognition and branch migration	RuvAB complex	Unknown
Resolution of Holliday junctions	RuvC	Perhaps Rad51c-XRCC3 complex and others

The RecBCD helicase/nuclease processes broken DNA molecules for recombination

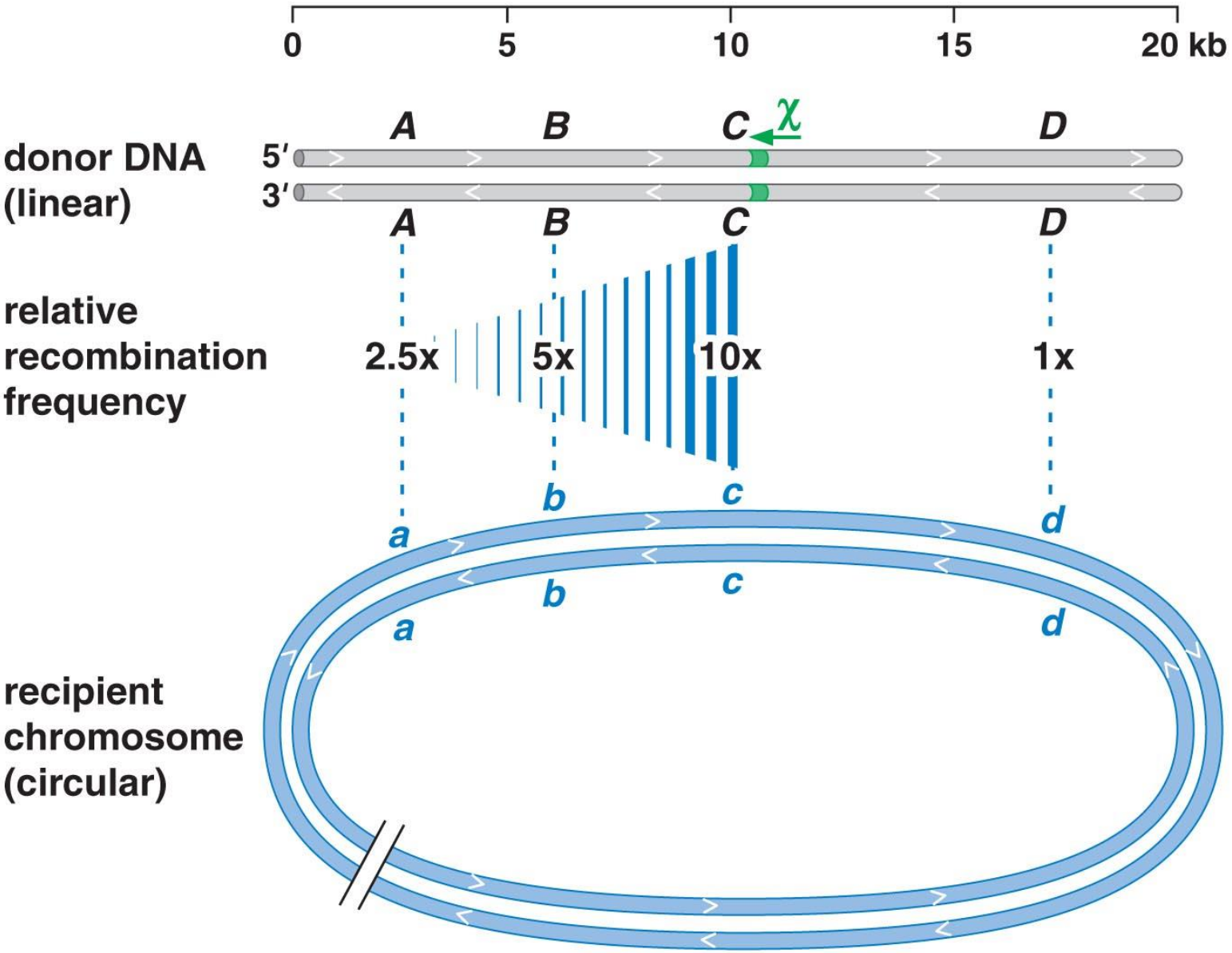


χ-site 5'-GCTGGTGG-3'.

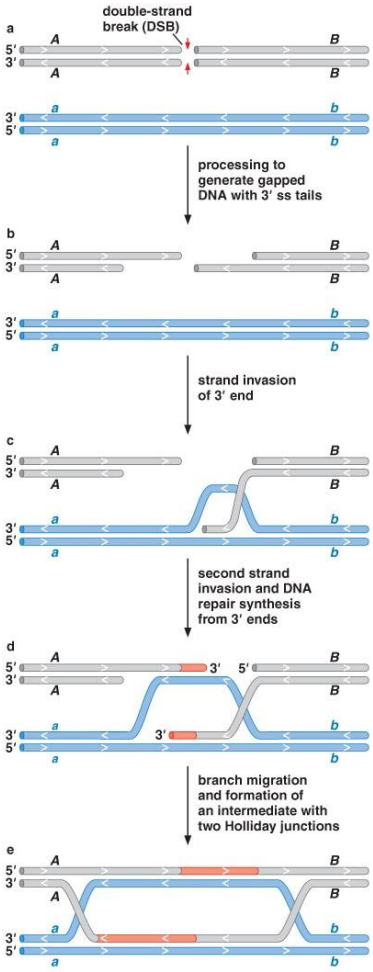
Structure of RecBCD

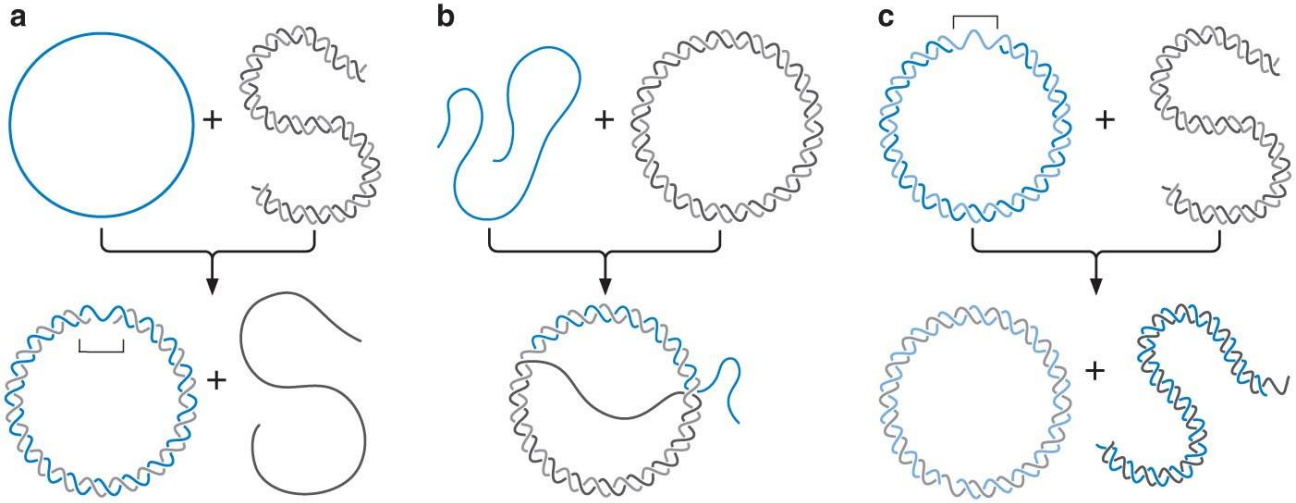


Chi sites control RecBCD (GCTGGTGG)



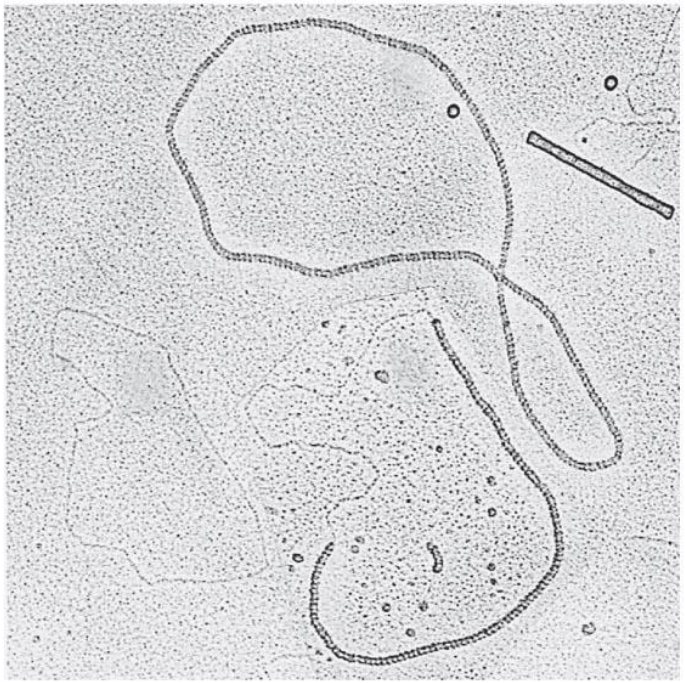
RecA protein assembles on single-stranded DNA and promotes strand invasion





Three views of the RecA filament

a



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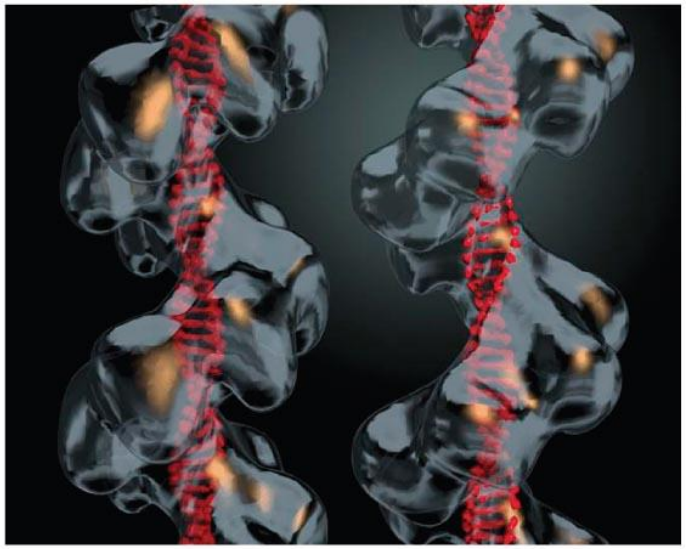
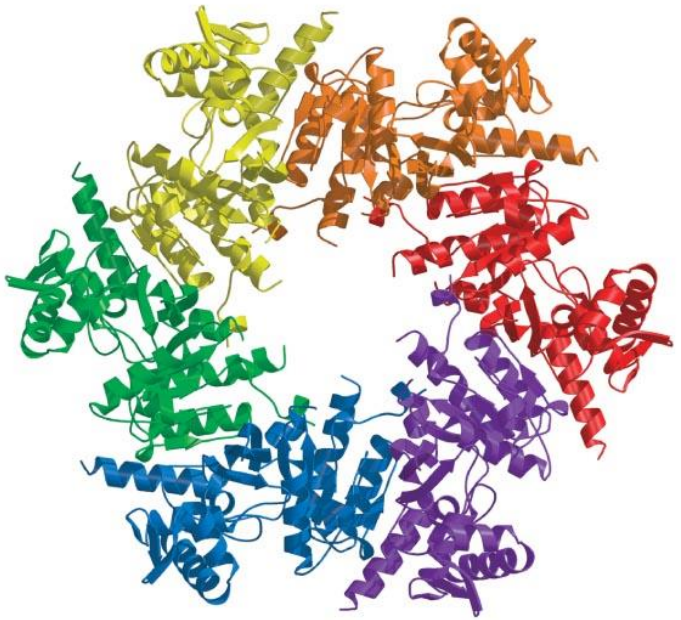
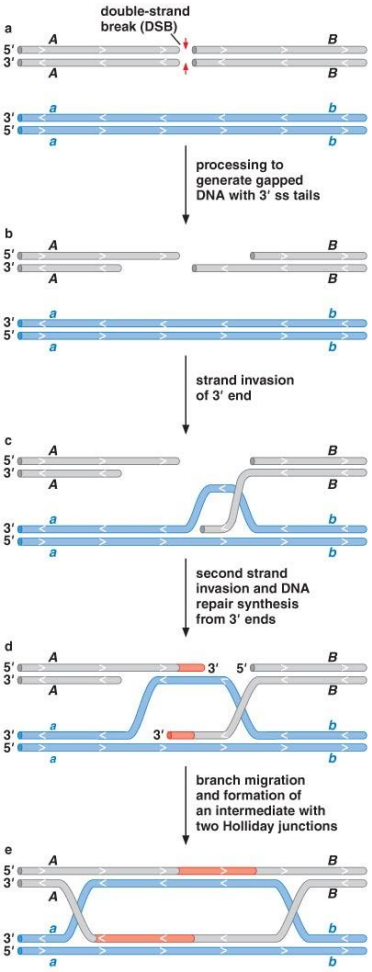
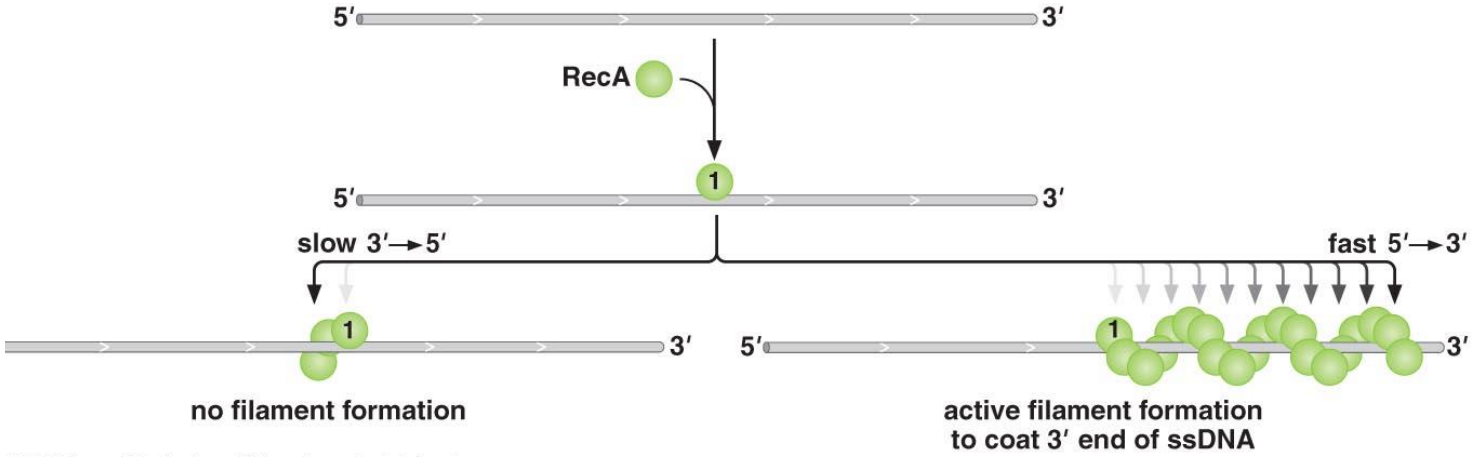


c

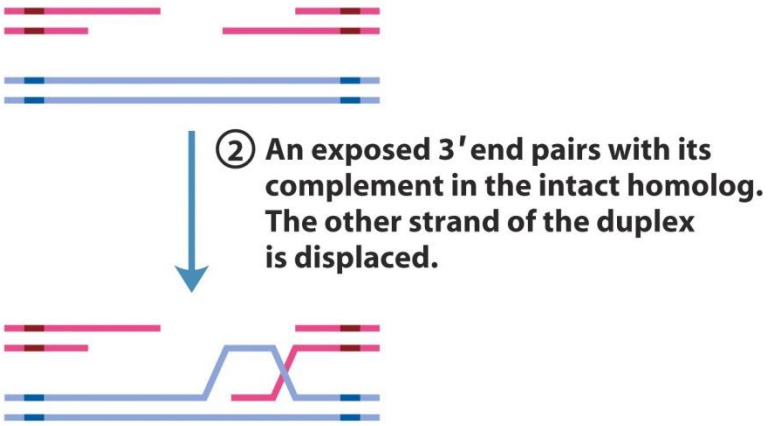


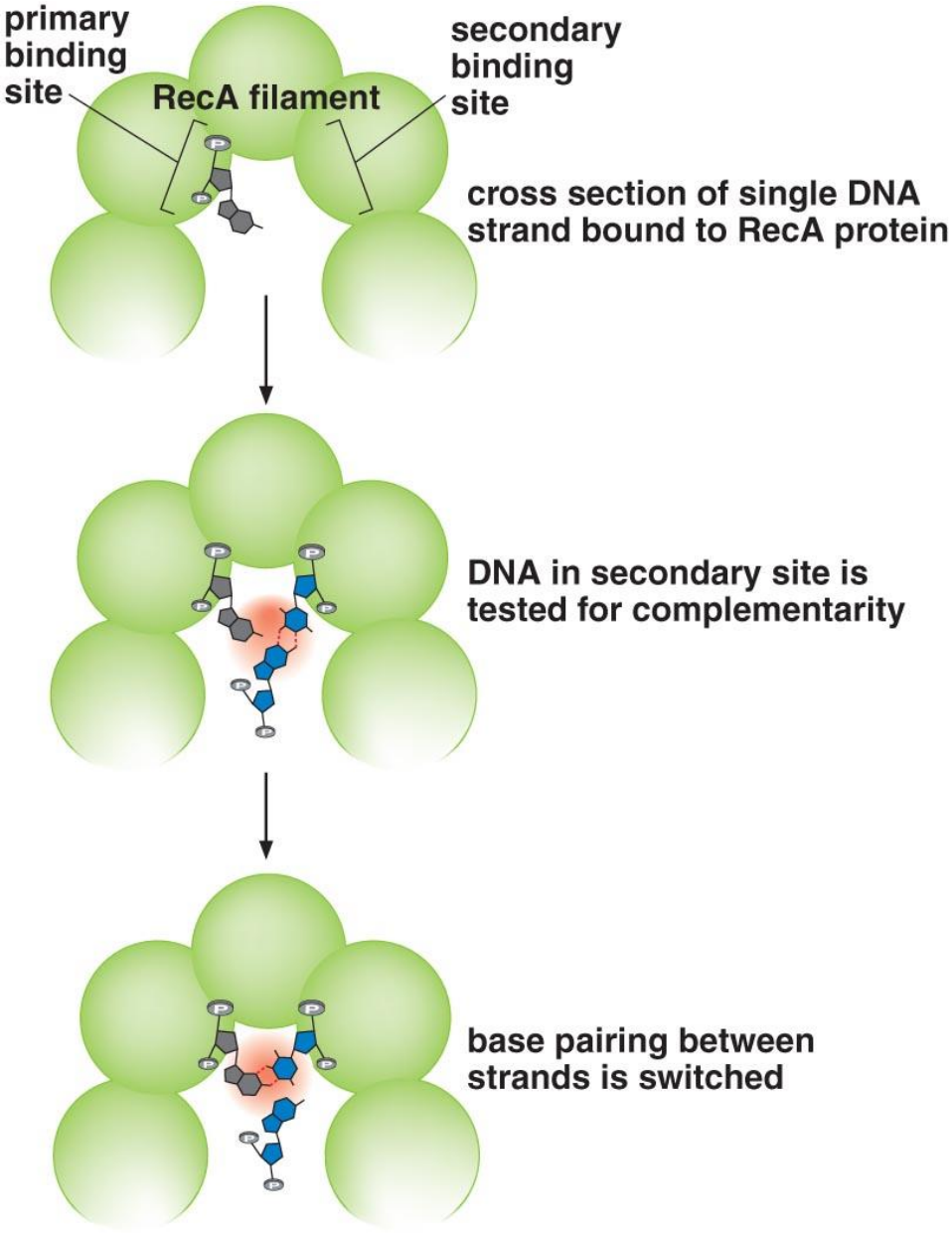
Polarity of RecA assembly





Newly based-paired partners are established within RecA filament

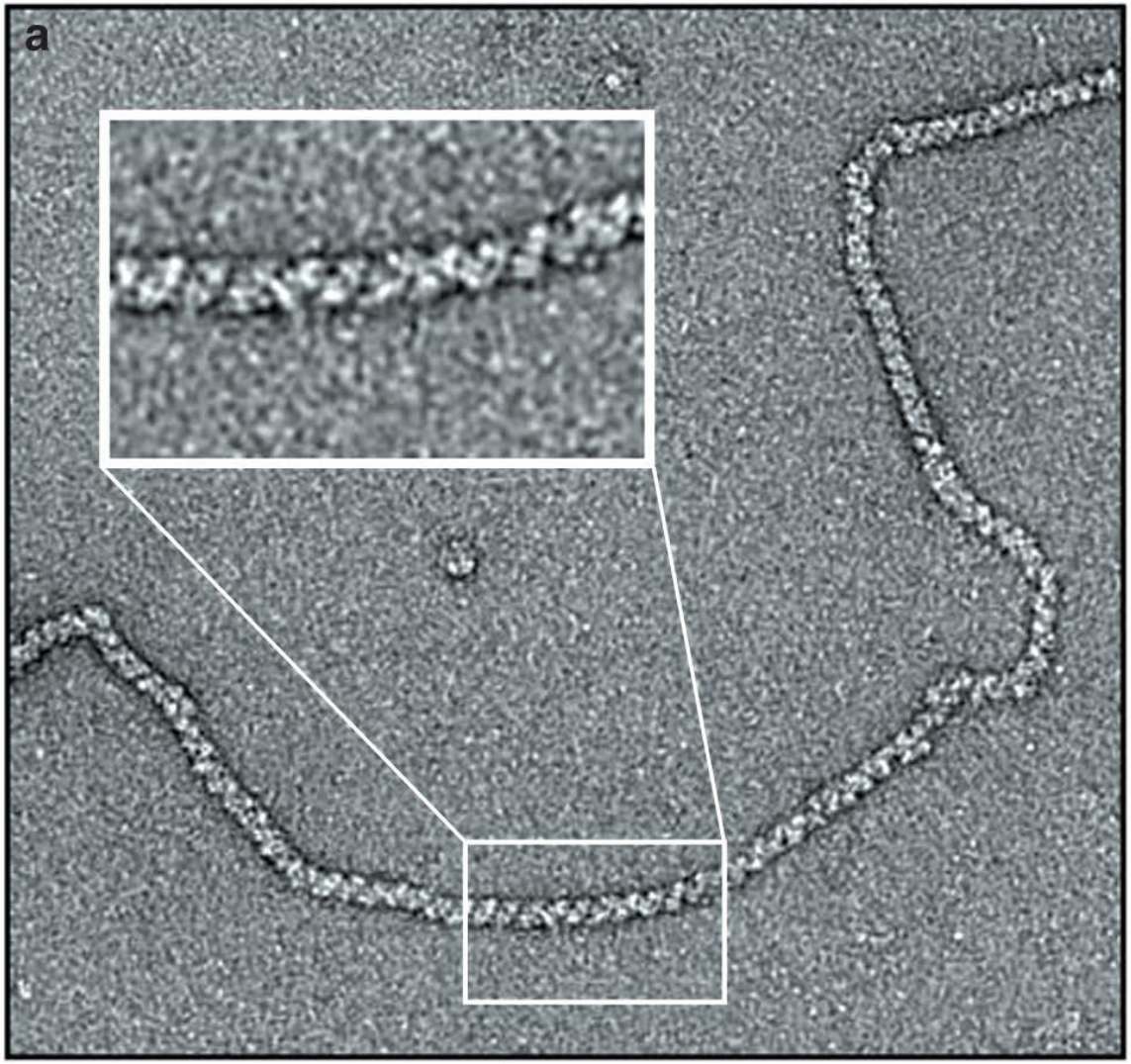


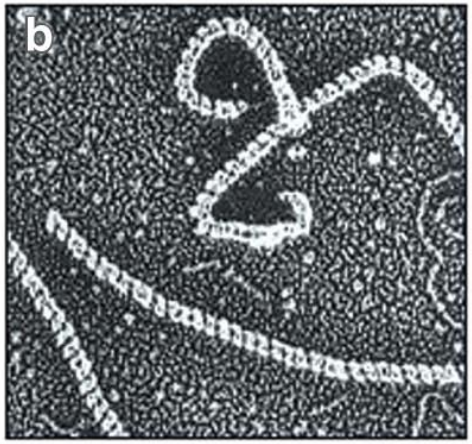


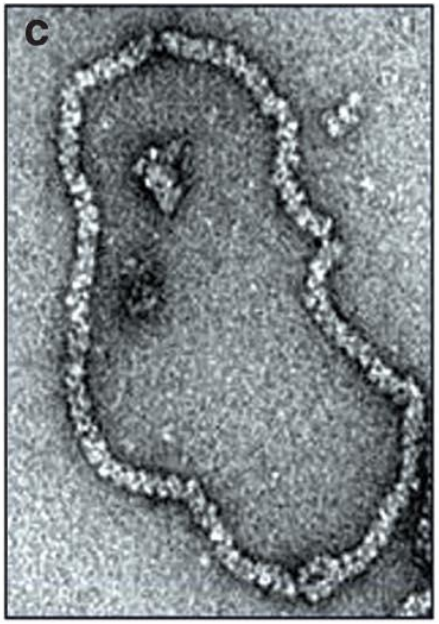
RecA homologs are present in all organism

Human Rad51

RecA







Rad A of Archaea

Branch migration can either enlarge heteroduplex regions or release newly synthesized DNA as a single strand

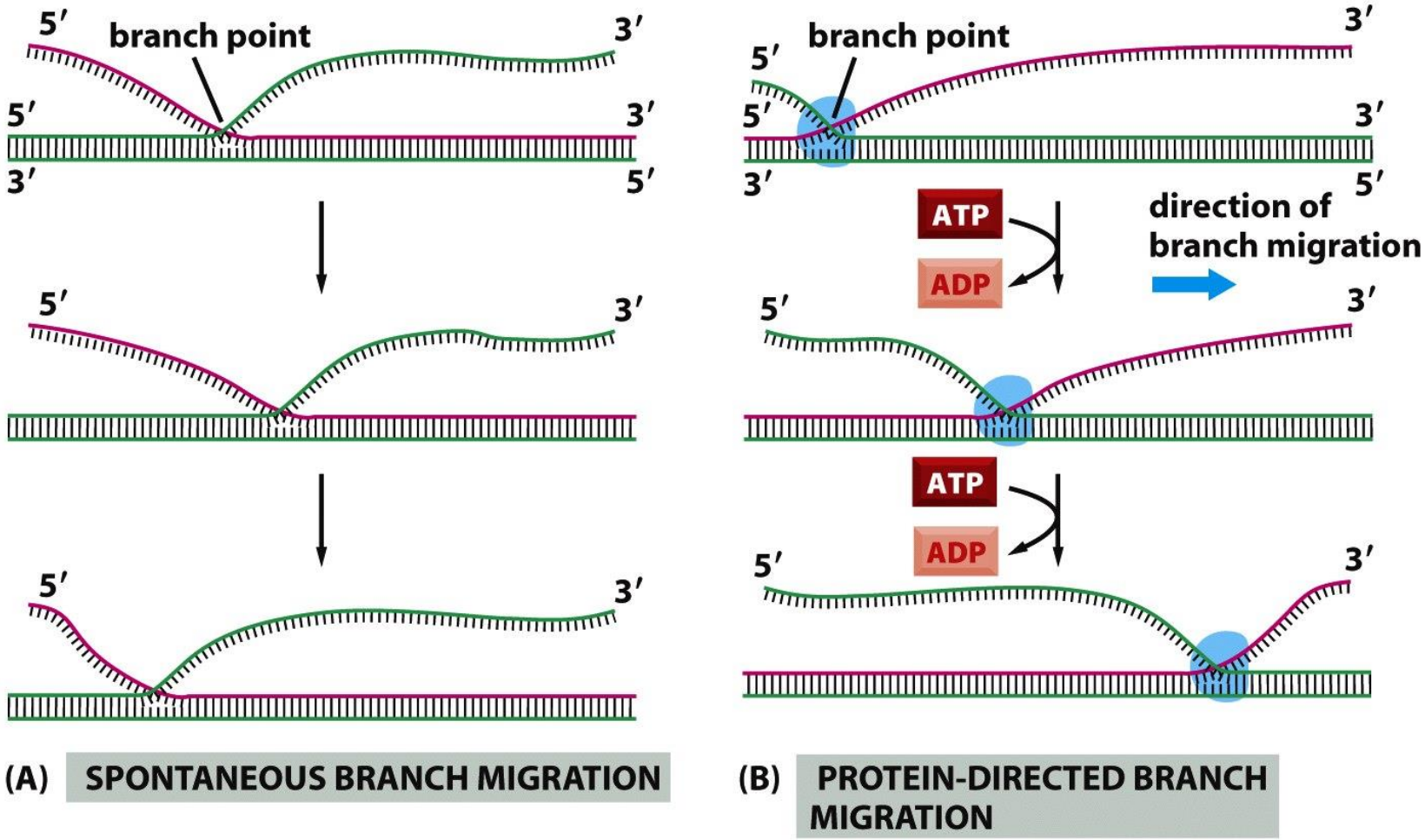
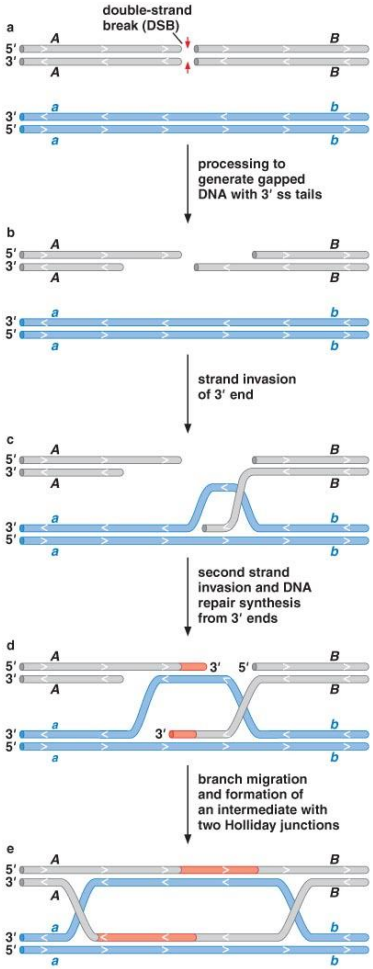
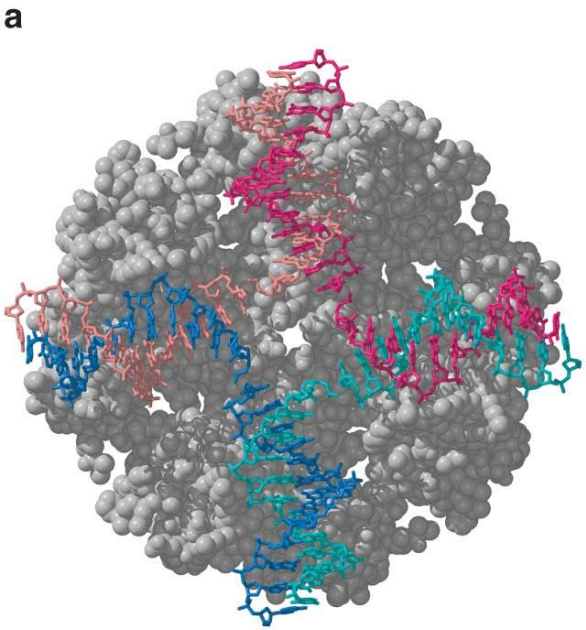
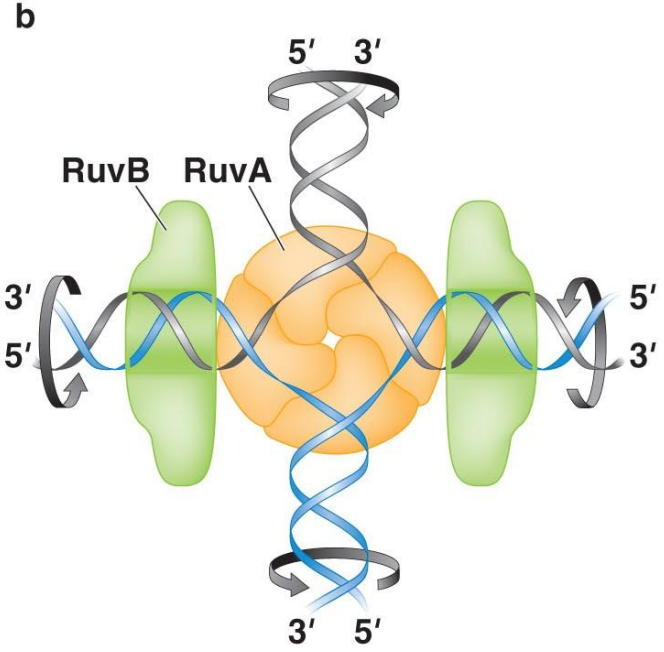


Figure 5-58 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Structure of RuvA and model of RuvAB bound to Holliday junction







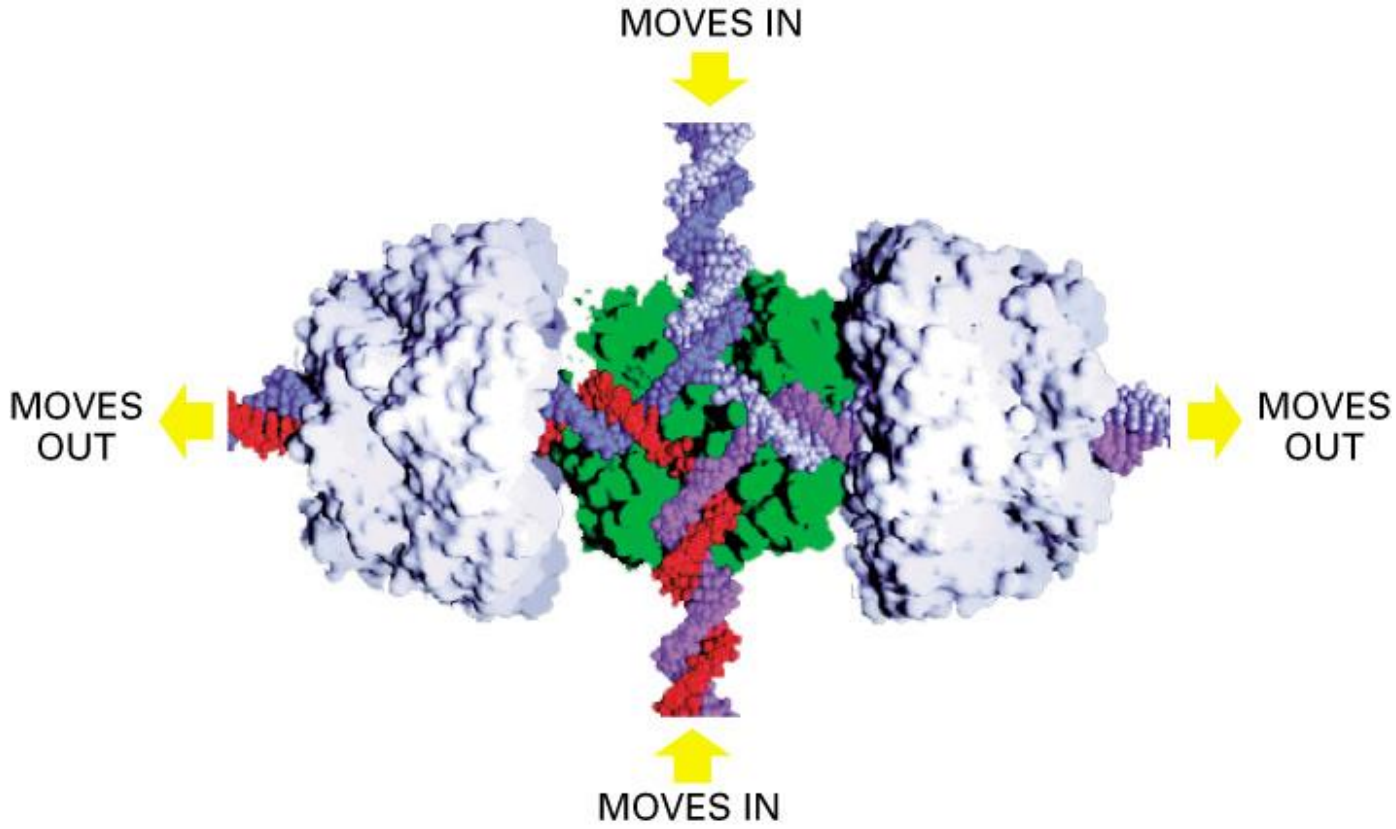


Figure 5–63. Molecular Biology of the Cell, 4th Edition.

Enzyme-catalyzed double branch migration at a Holliday junction.

In *E. coli*, a tetramer of the RuvA protein *(green)* and two hexamers of the RuvB protein *(pale gray)* bind to the open form of the junction. The RuvB protein uses the energy of ATP hydrolysis to move the crossover point rapidly along the paired DNA helices, extending the heteroduplex region as shown. There is evidence that similar proteins perform this function in vertebrate cells. (Image courtesy of P. Artymiuk; modified from S.C. West, *Cell* 94:699–701, 1998.)

RuvC cleaves specific strands at the Holliday junction to finish recombination

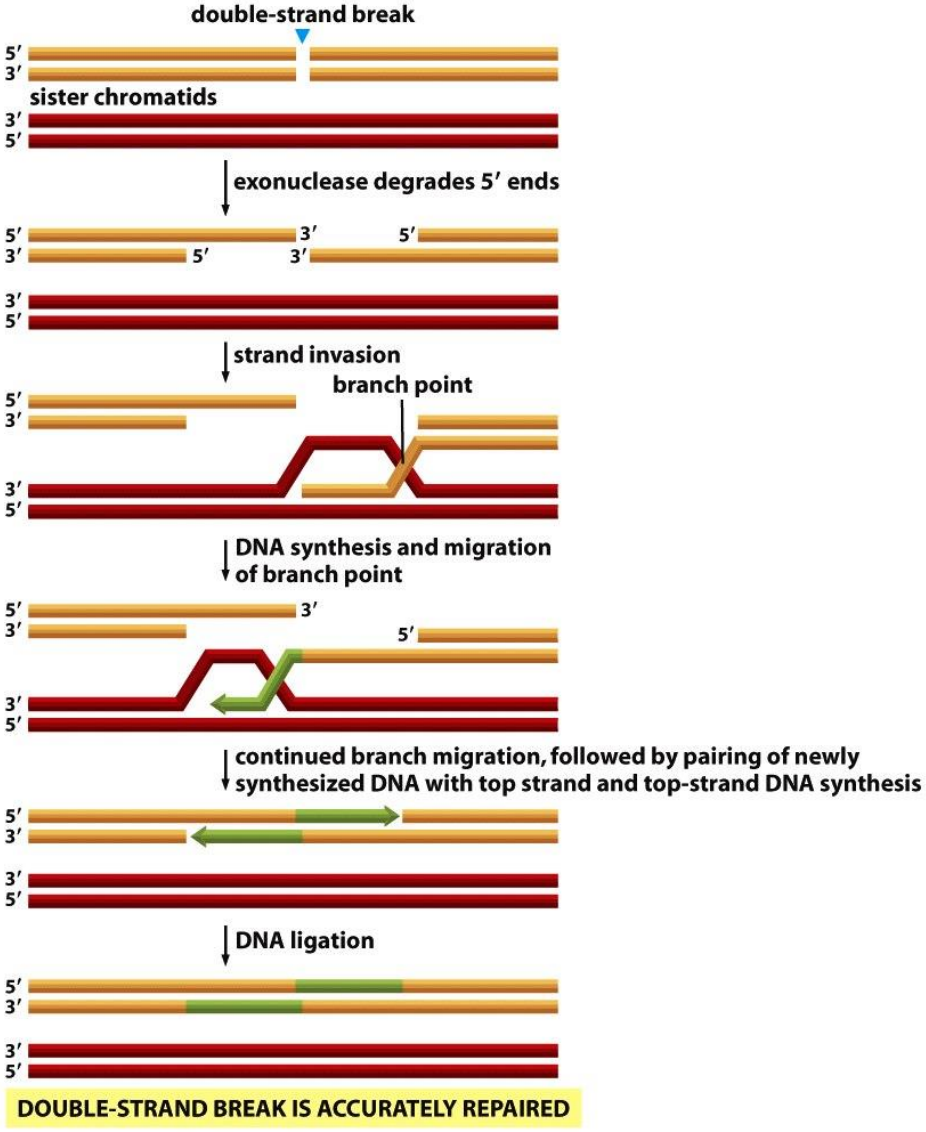
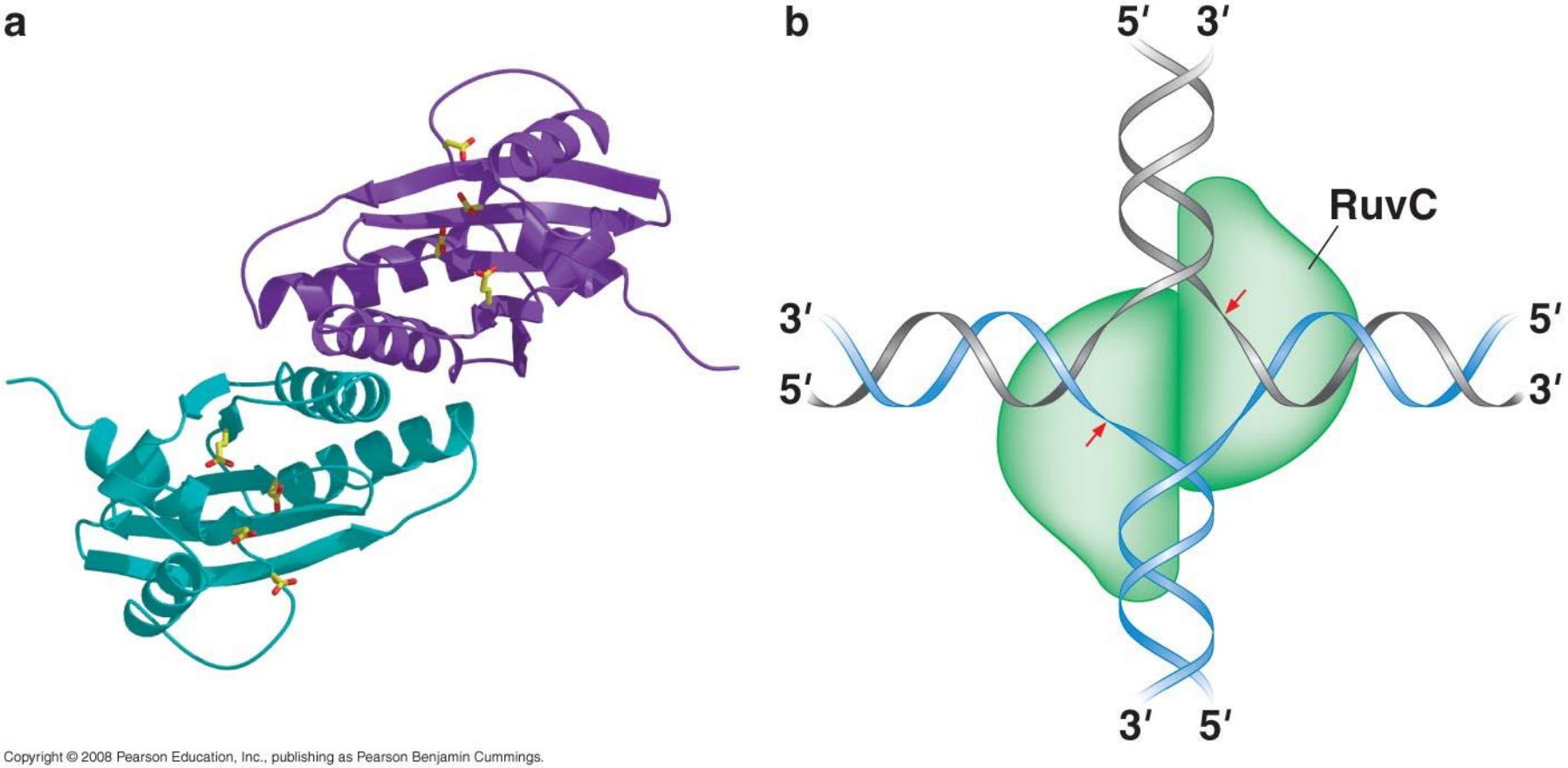
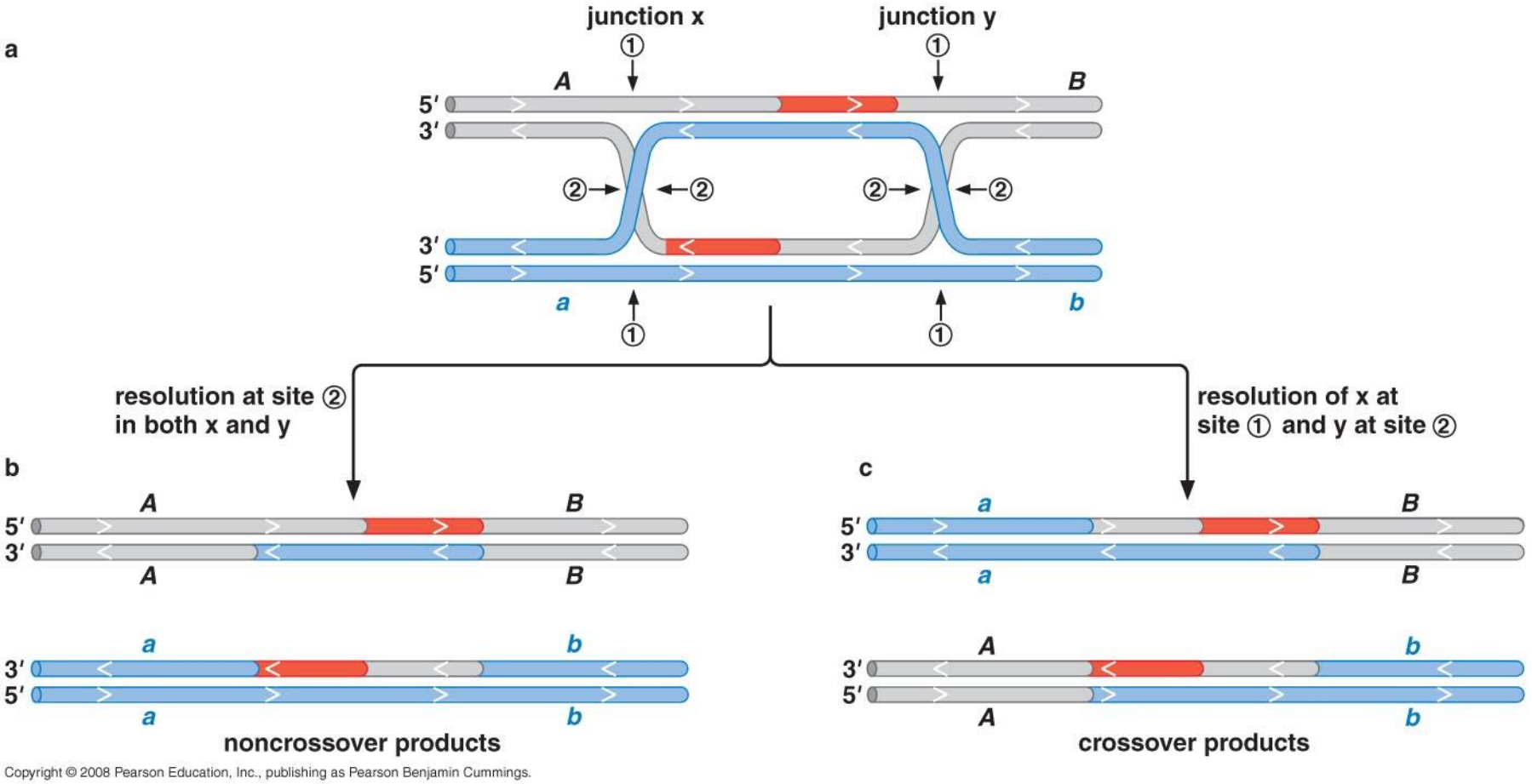


Figure 5-59 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Structure of RuvC and model of RuvC dimer bound to Holliday junction



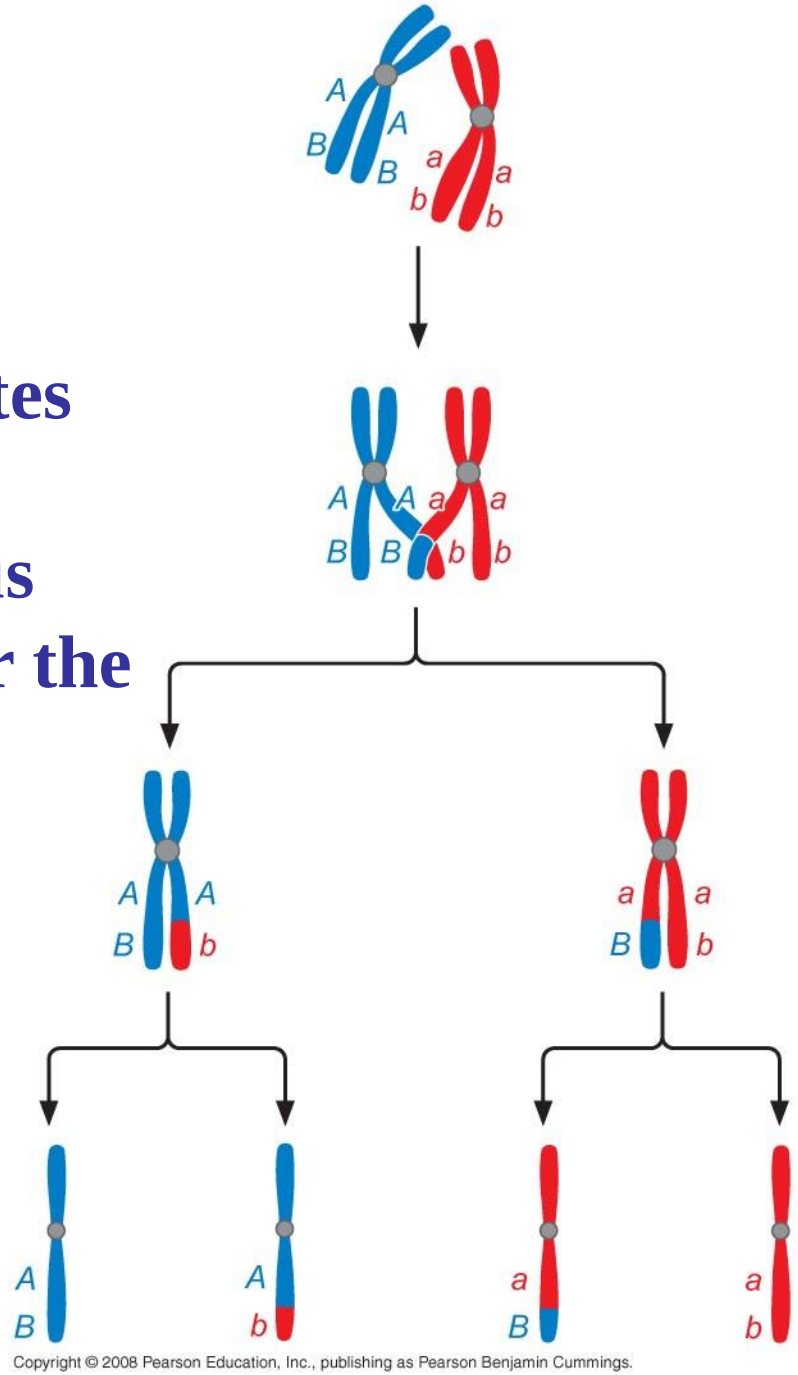
How to resolve a recombination intermediate with two Holliday junctions



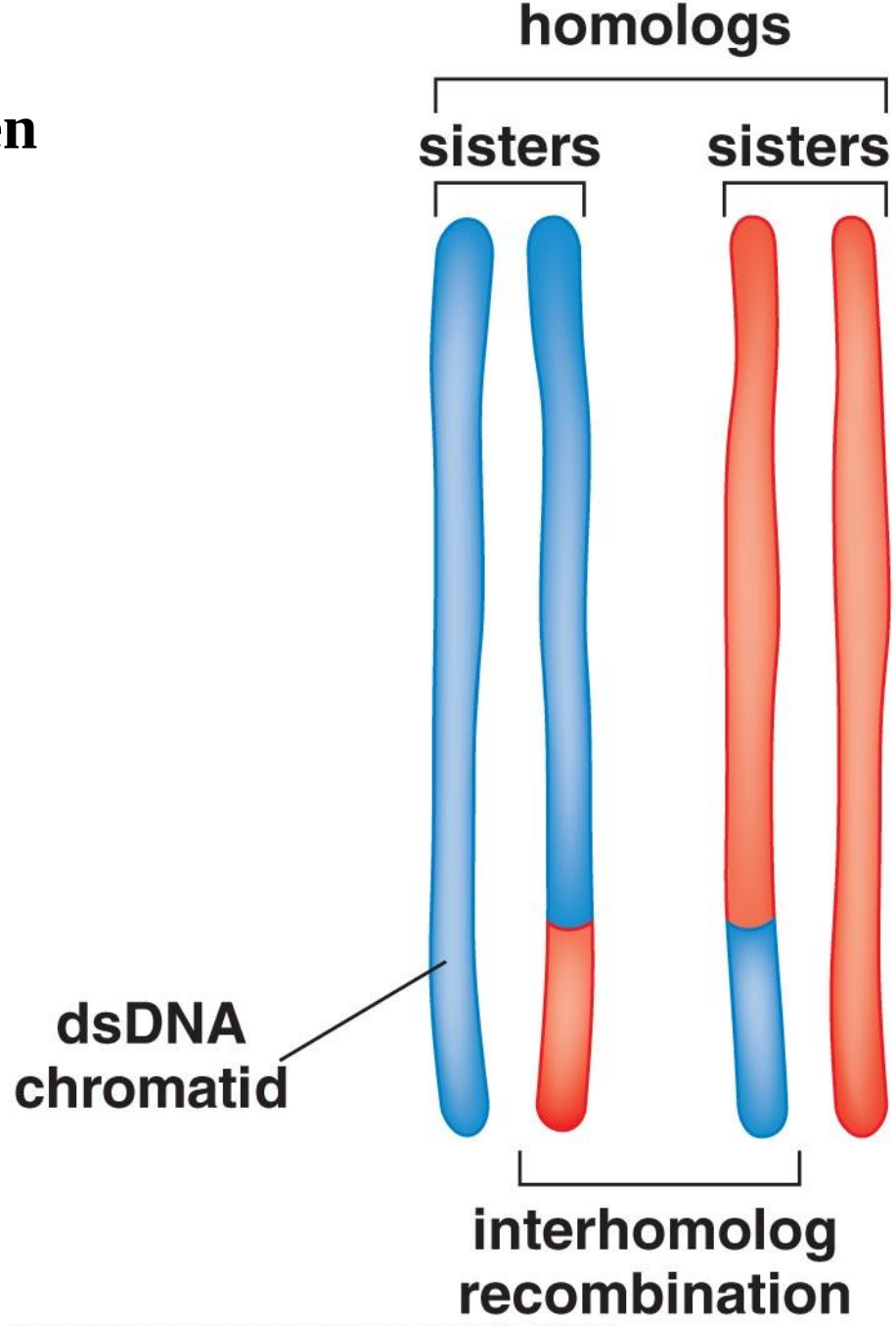
HOMOLOGOUS RECOMBINATION IN EUKARYOTES

Homologous recombination has additional functions in eukaryotes

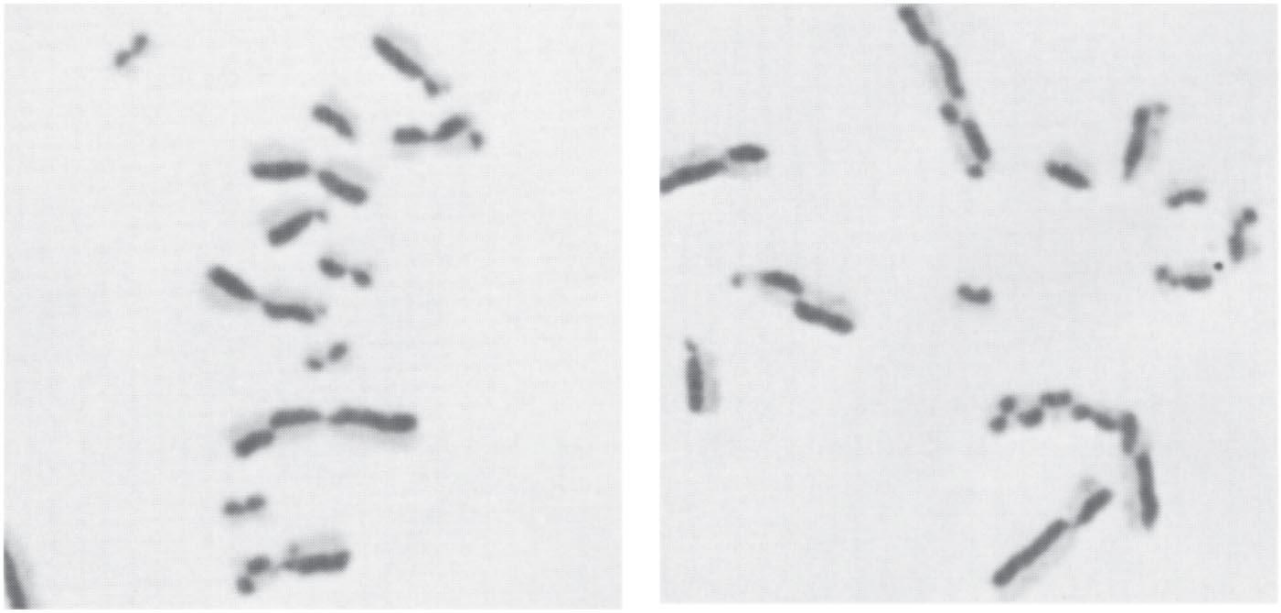
It is required to pair homologous chromosomes in preparation for the first nuclear division and for segregation during meiosis

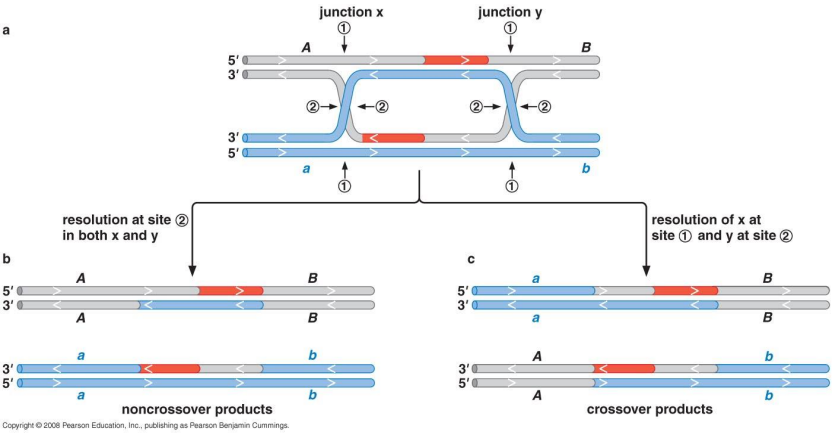


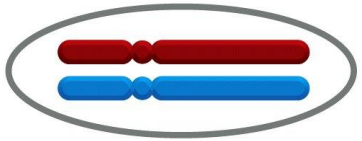
Meiotic recombination between homologous chromatids



Meiotic recombination also frequently gives rise to crossing over between genes on the two homologous parental chromosomes

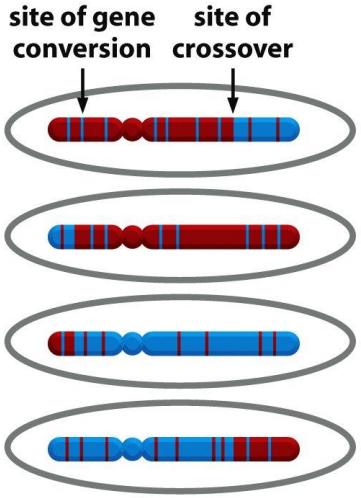






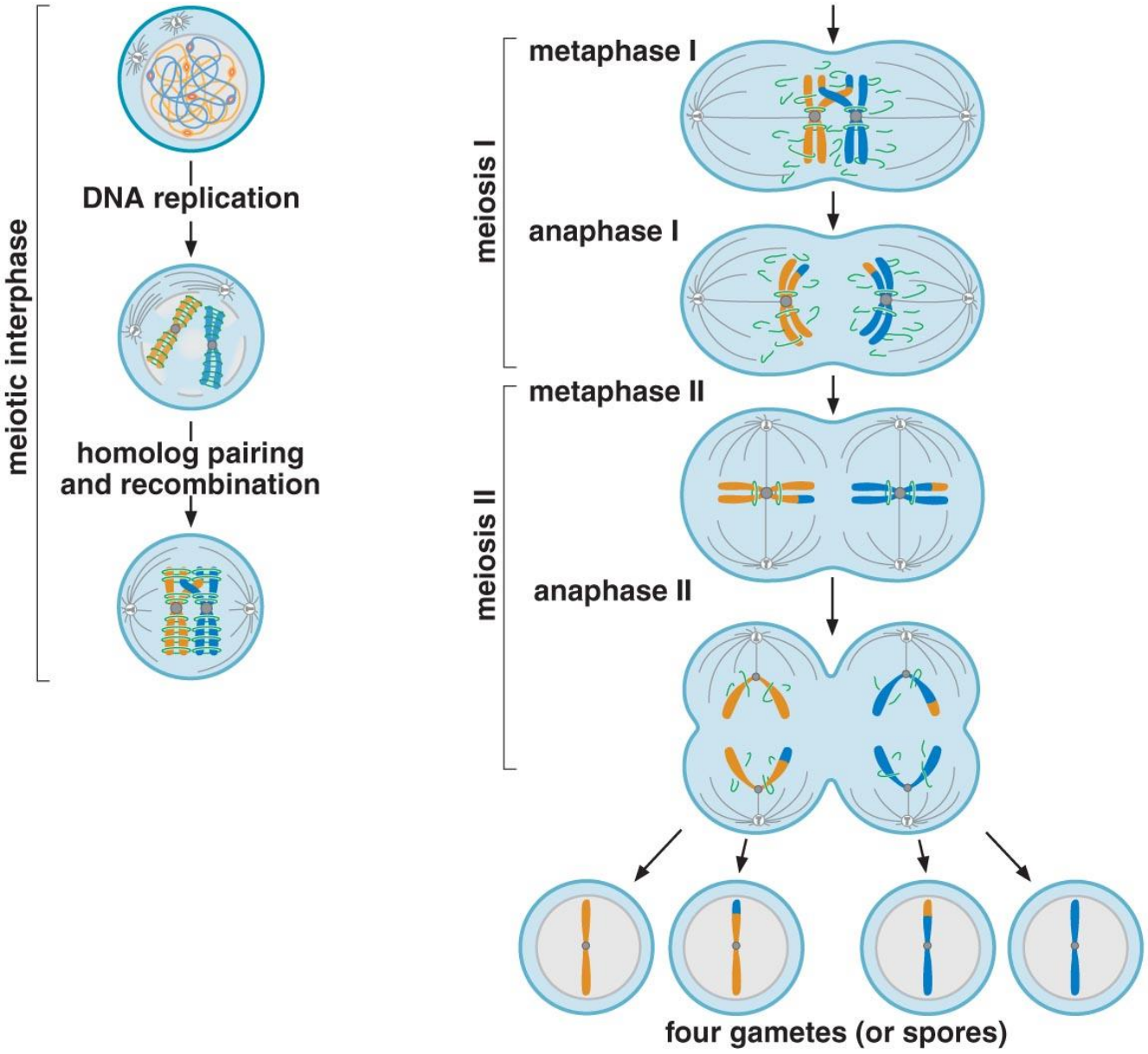
diploid cell with one pair of homologous chromosomes

CHROMOSOME DUPLICATION AND MEIOSIS

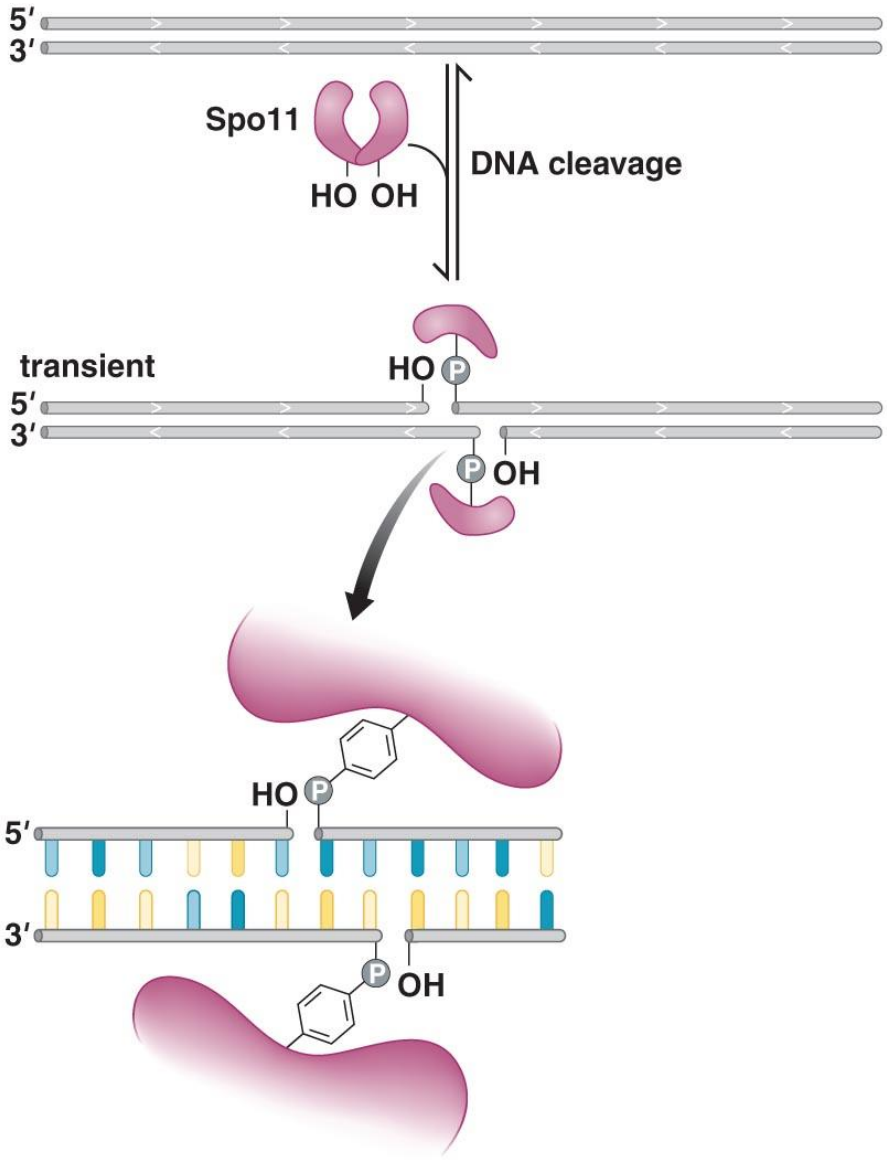


meiosis produces haploid cells with chromosomes that have crossed over

Figure 5-63 Molecular Biology of the Cell 5/e (© Garland Science 2008)

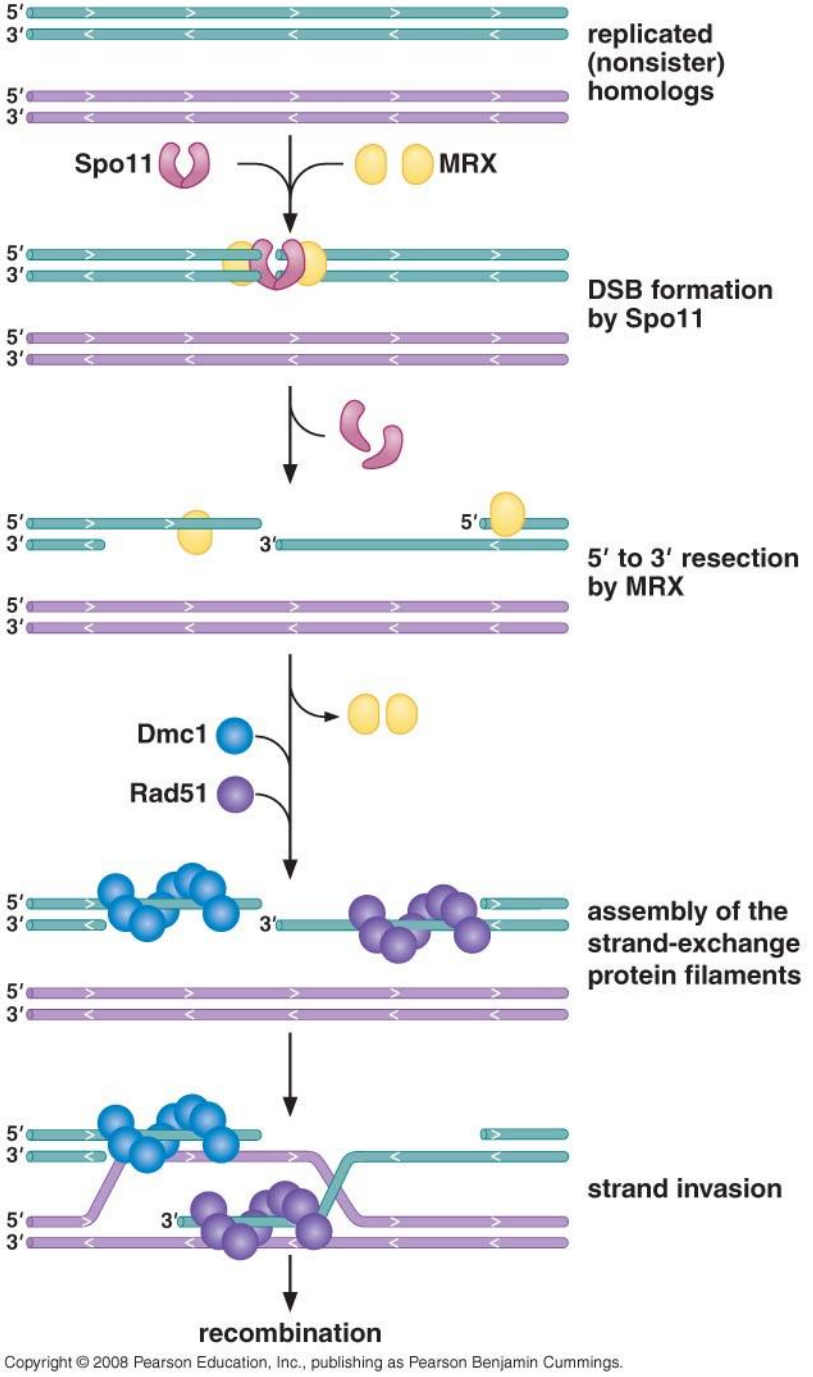


Programmed generation of double-stranded DNA breaks occurs during meiosis

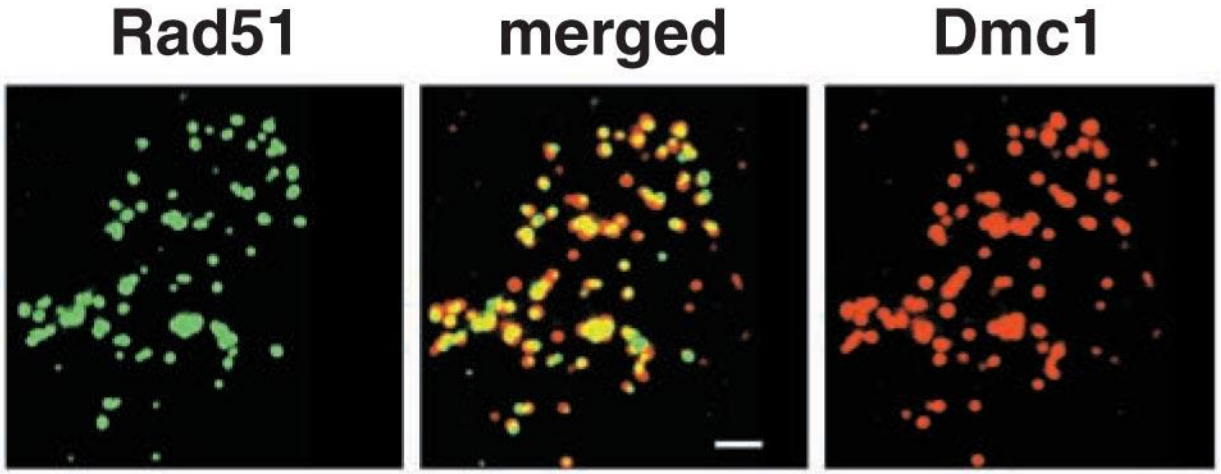


MRX protein processes the cleaved DNA ends for assembly of the RecA-like strand exchange proteins (DMC1, Rad51)

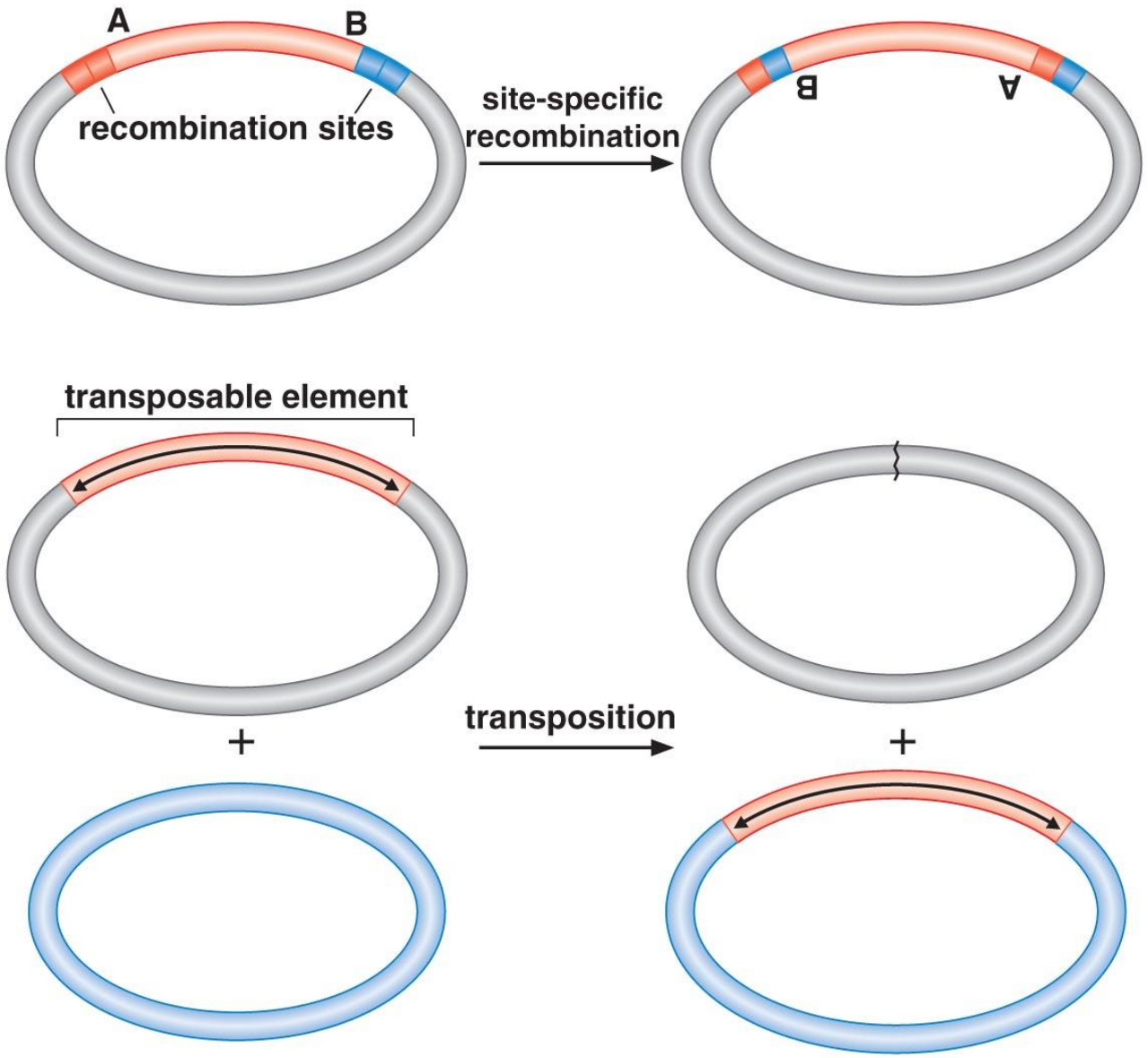
DMC1 specifically functions in meiotic recombination



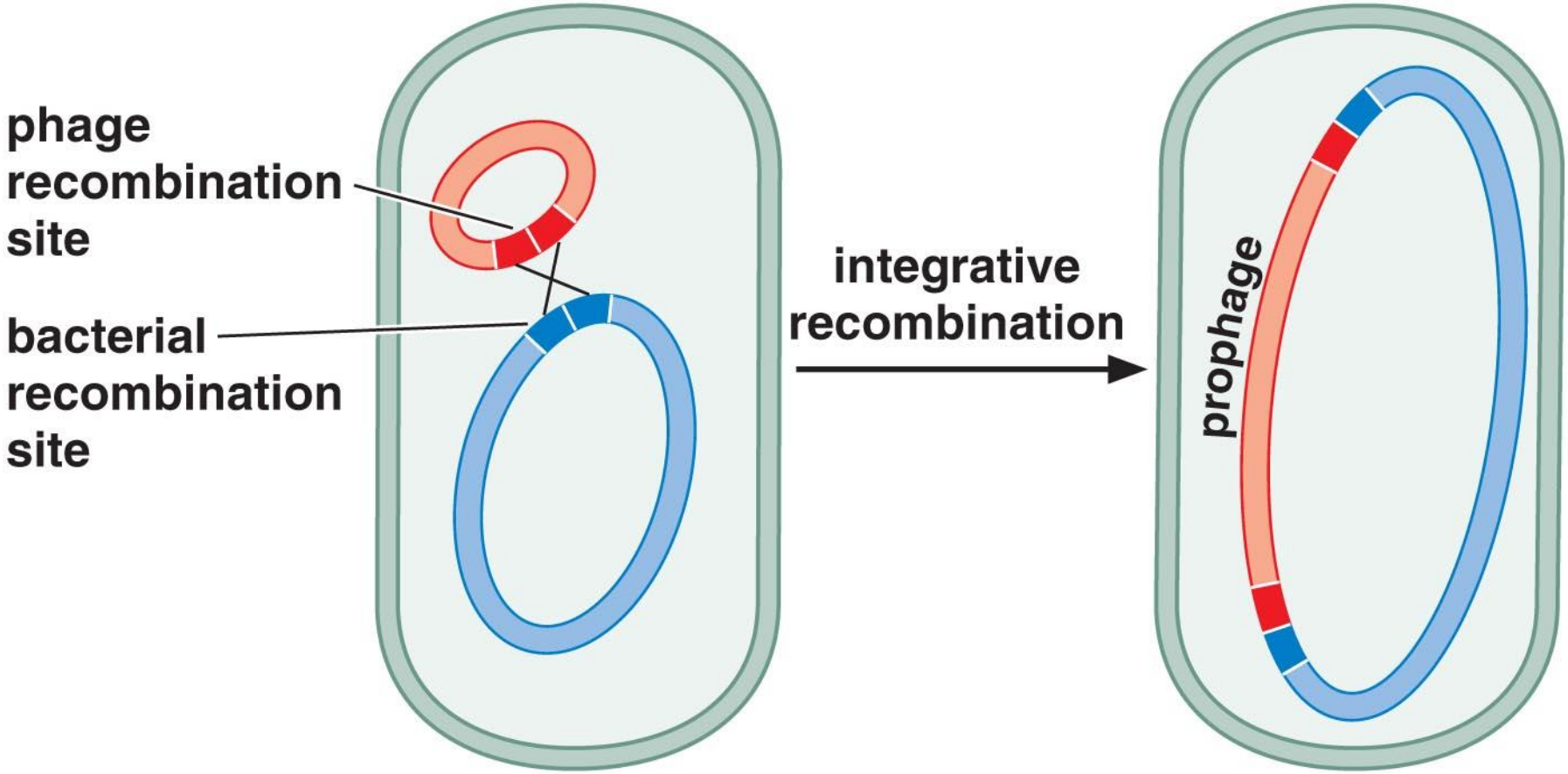
Many proteins function together to promote meiotic recombination (Rad51, DMC1, Rad51 paralogs, Rad52, Rad54)



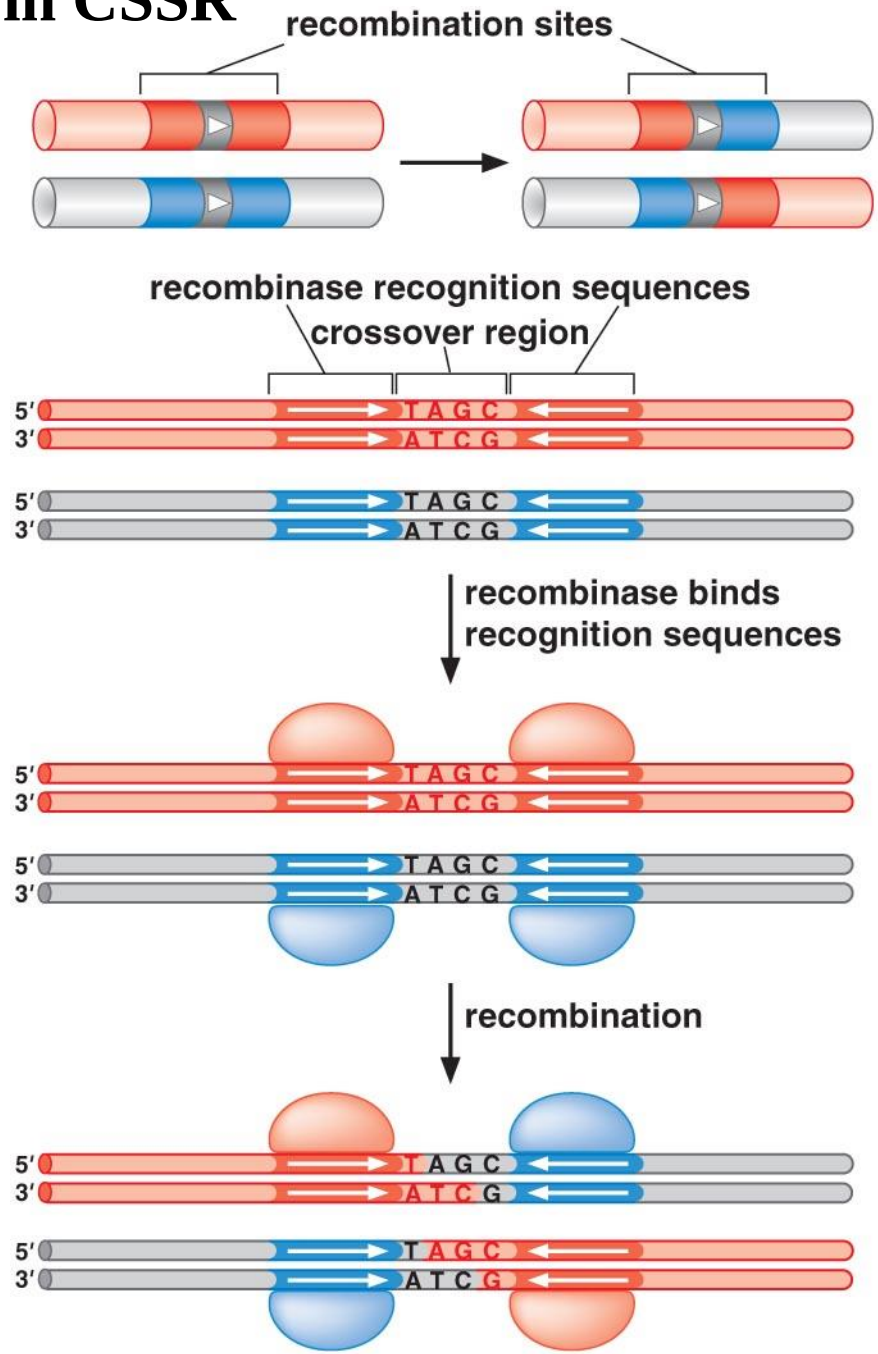
Site-Specific recombination & Transposition



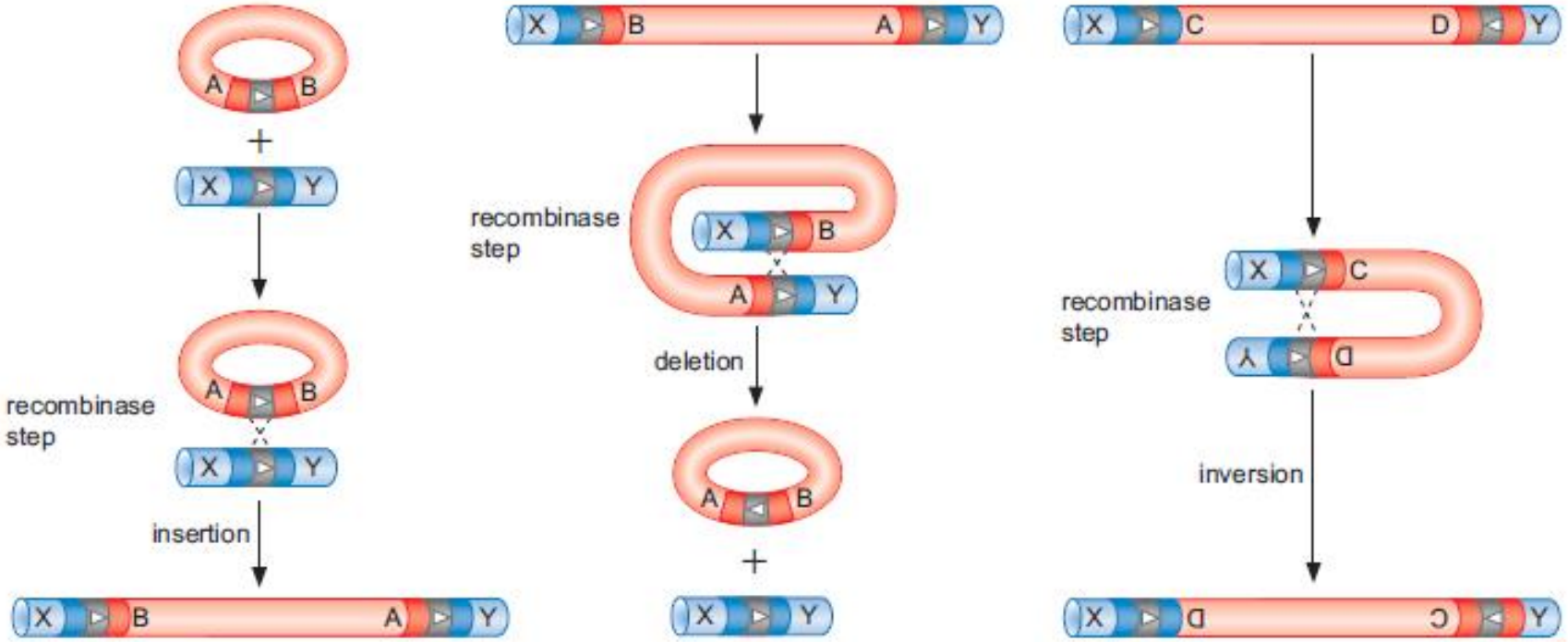
Conservative site-specific recombination



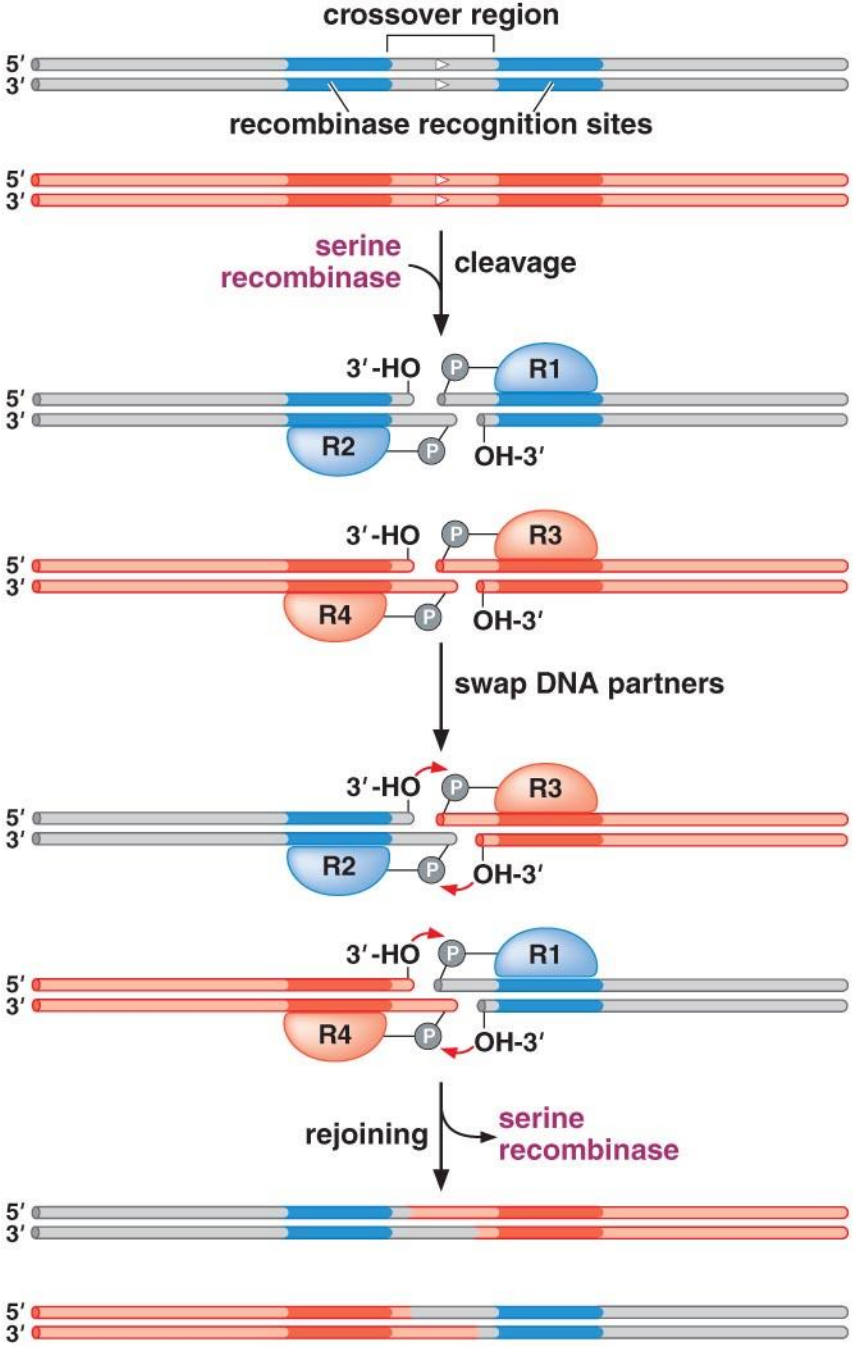
Structures involved in CSSR

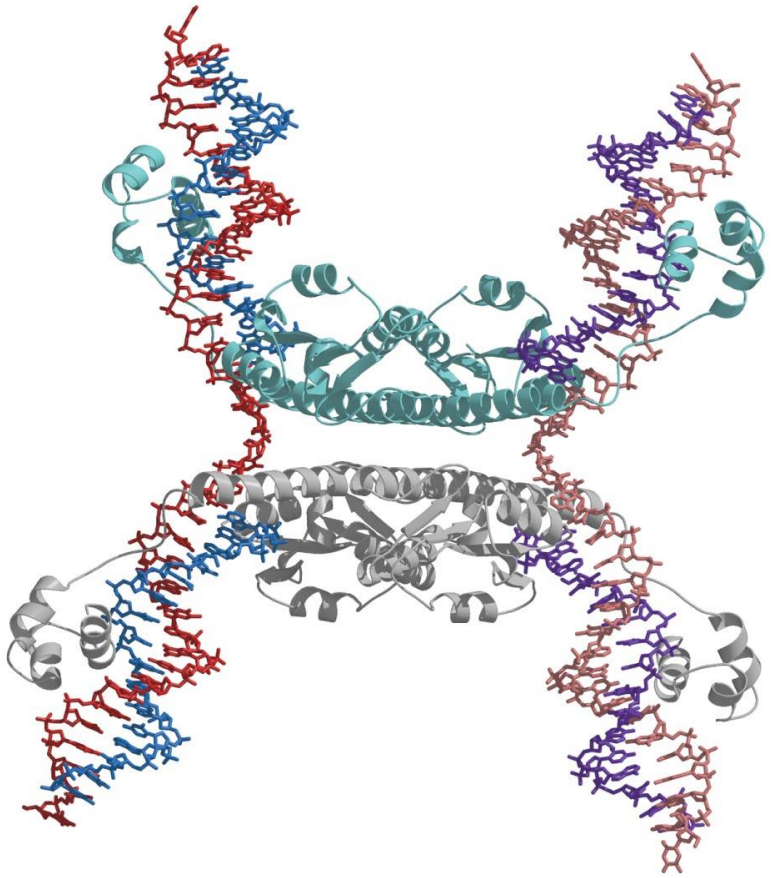


Three types of CSSR

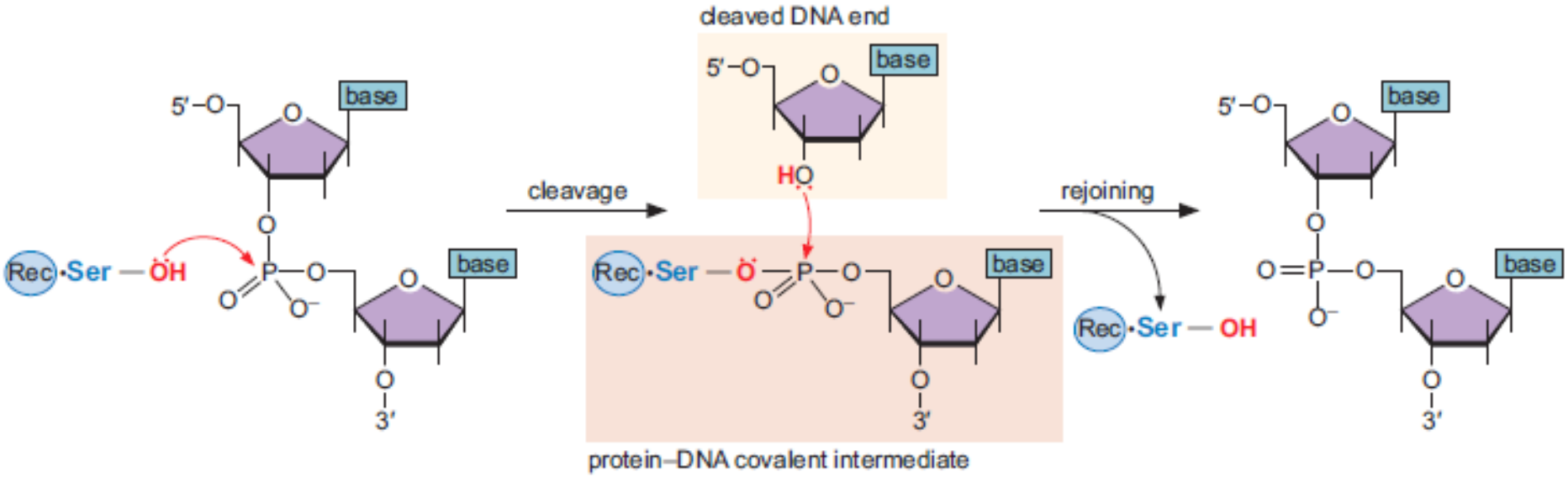


Recombination by a serine recombinase

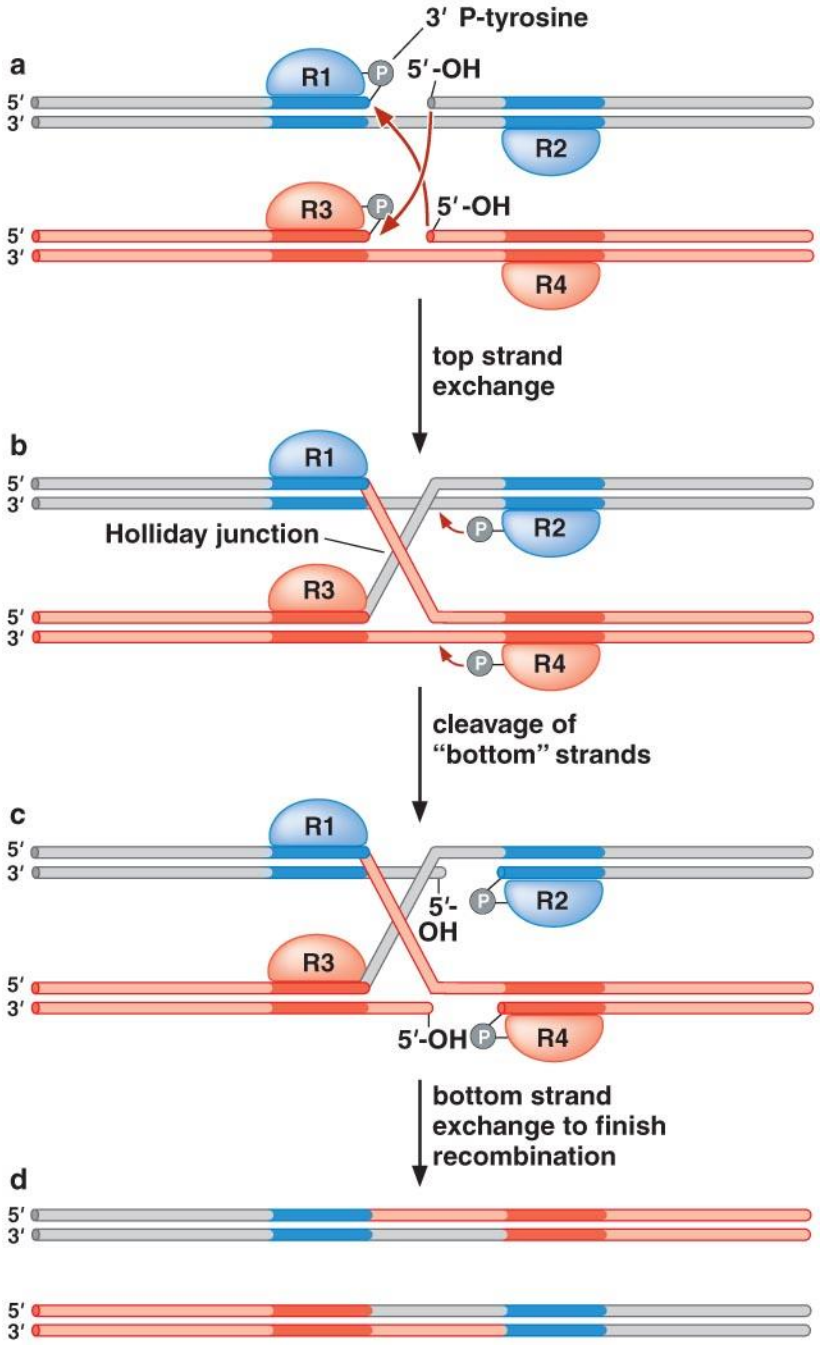




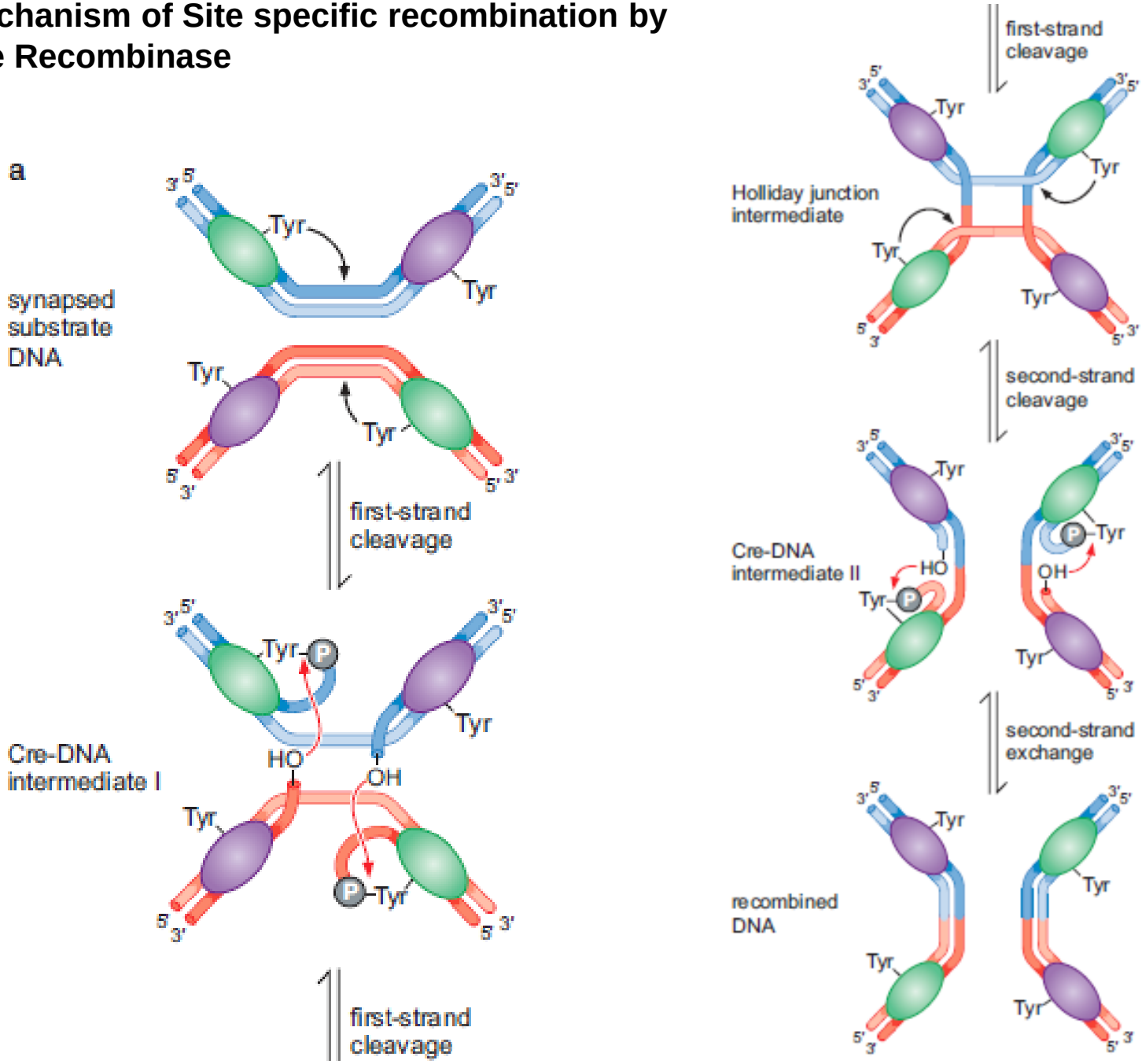
Covalent-intermediate mechanism used by the serine and tyrosine recombinases.



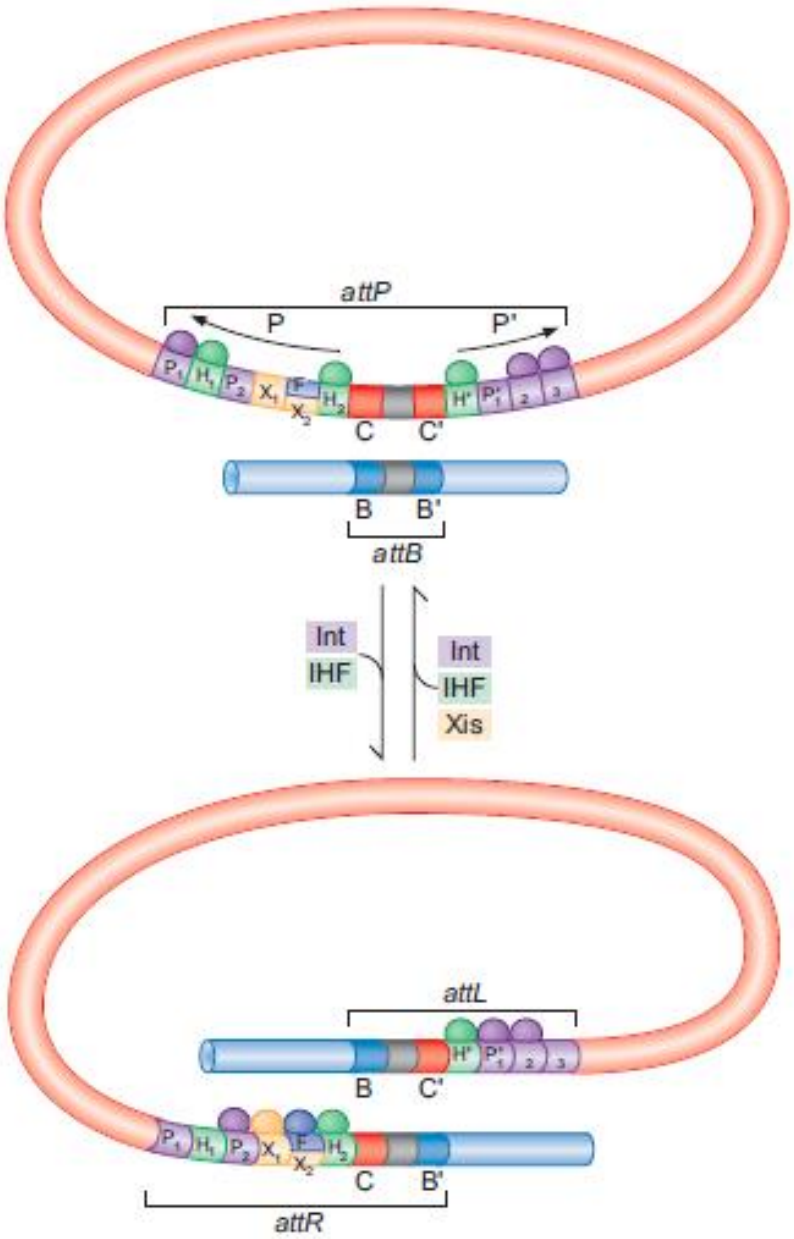
Recombination by a tyrosine recombinase

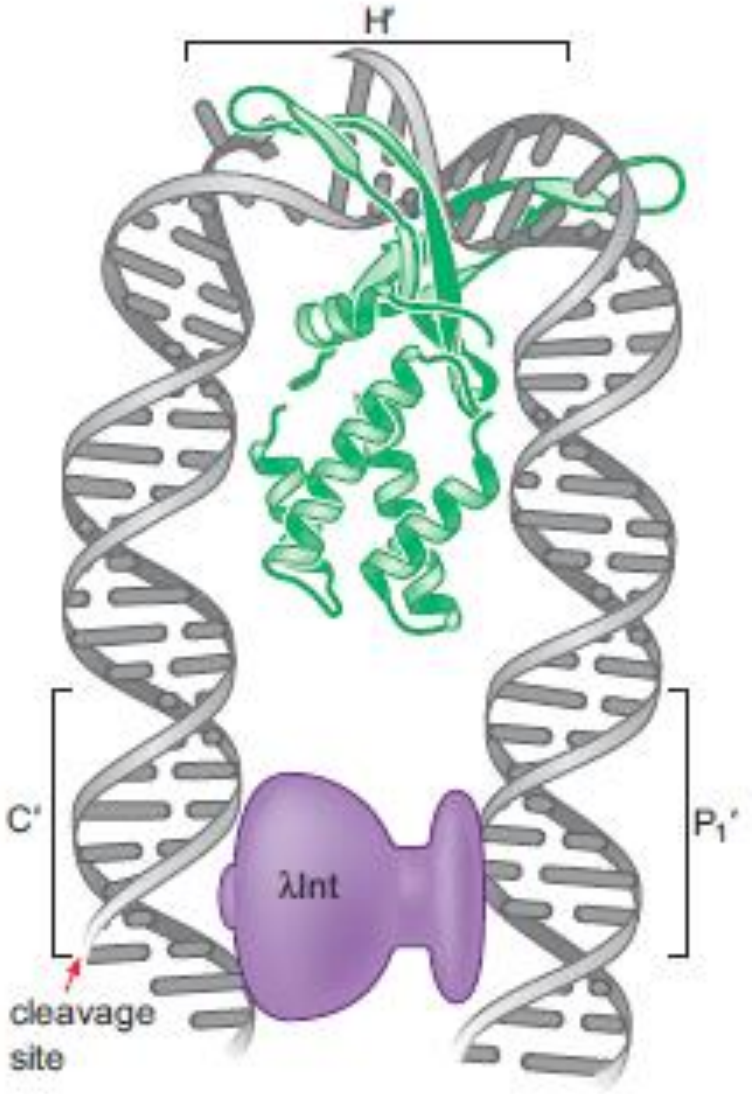


Mechanism of Site specific recombination by Cre Recombinase



Recombination sites involved in λ integration and excision showing the important sequence elements.





Model for IHF bending DNA to bring DNA-binding sites together.

Conservative site-specific recombination was discovered in bacteriophage lambda

The insertion of a circular bacteriophage lambda DNA chromosome into the bacterial chromosome.

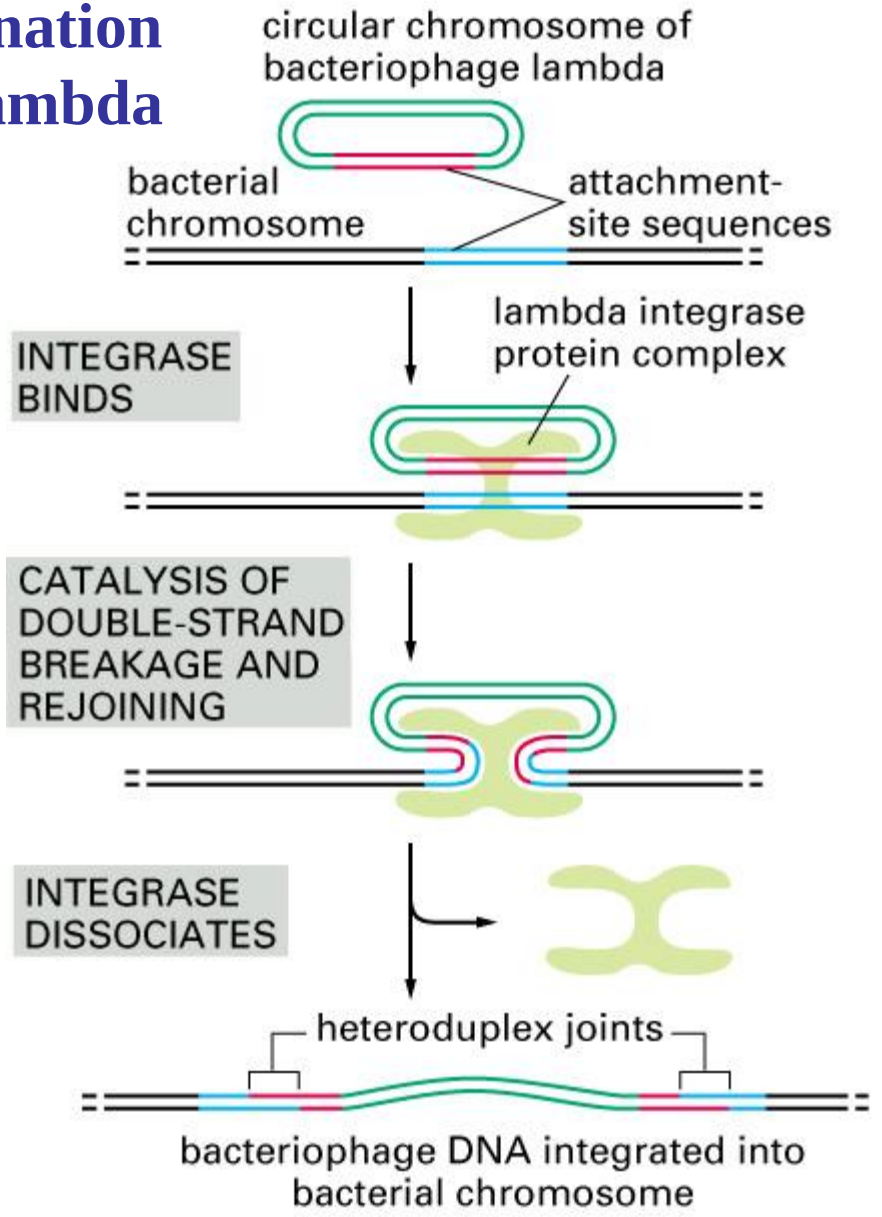


Figure 5–80. Molecular Biology of the Cell, 4th Edition.

The life cycle of bacteriophage lambda.

The double-stranded DNA lambda genome contains 50,000 nucleotide pairs and encodes 50–60 different proteins. When the lambda DNA enters the cell, the ends join to form a circular DNA molecule. This bacteriophage can multiply in *E. coli* by a lytic pathway, which destroys the cell, or it can enter a latent prophage state. Damage to a cell carrying a lambda prophage induces the prophage to exit from the host chromosome and shift to lytic growth *(green arrows)*. Both the entrance of the lambda DNA to, and its exit from, the bacterial chromosome are accomplished by a conservative site-specific recombination event, catalyzed by the lambda integrase enzyme (see Figure 5–80).

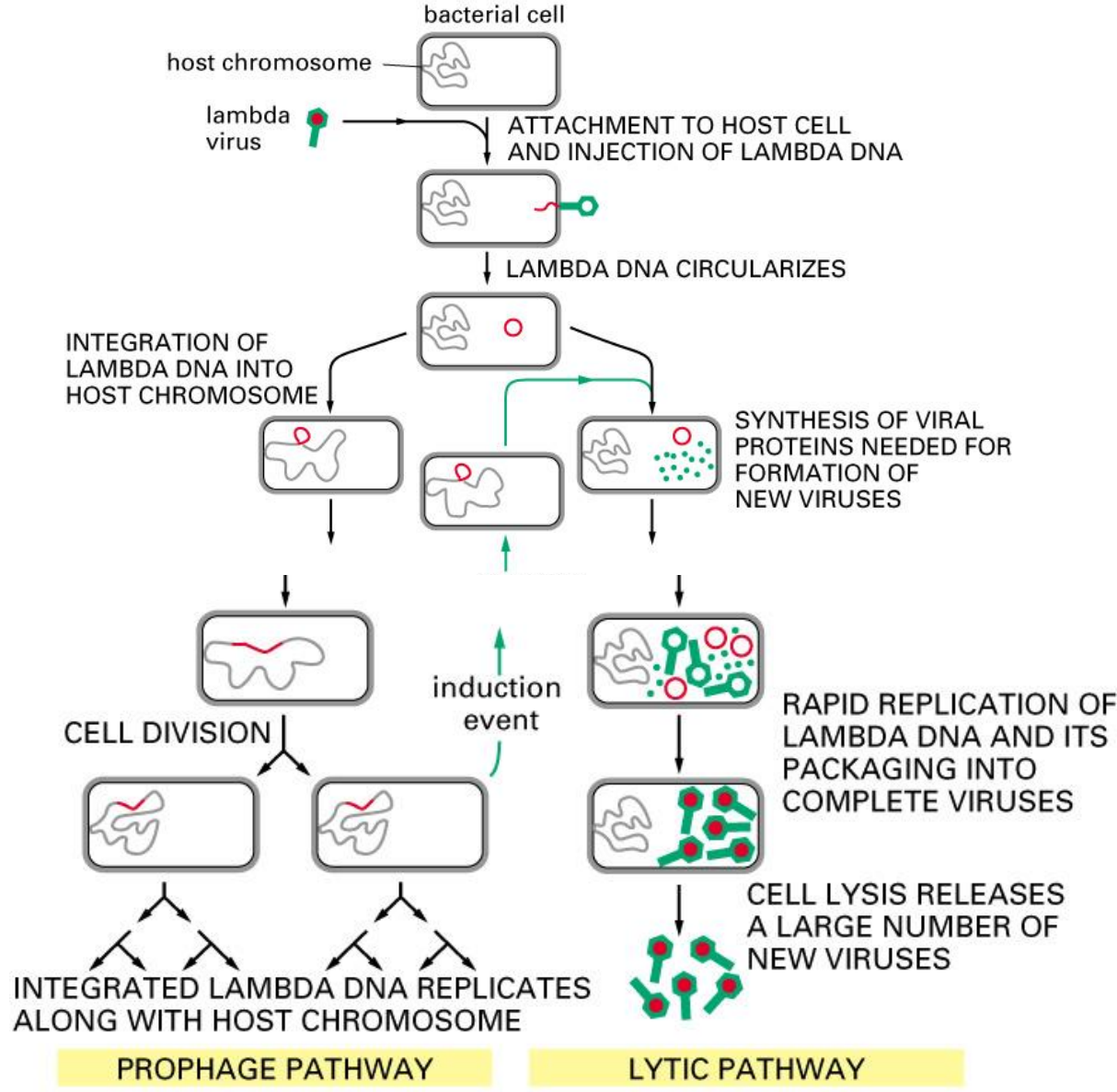
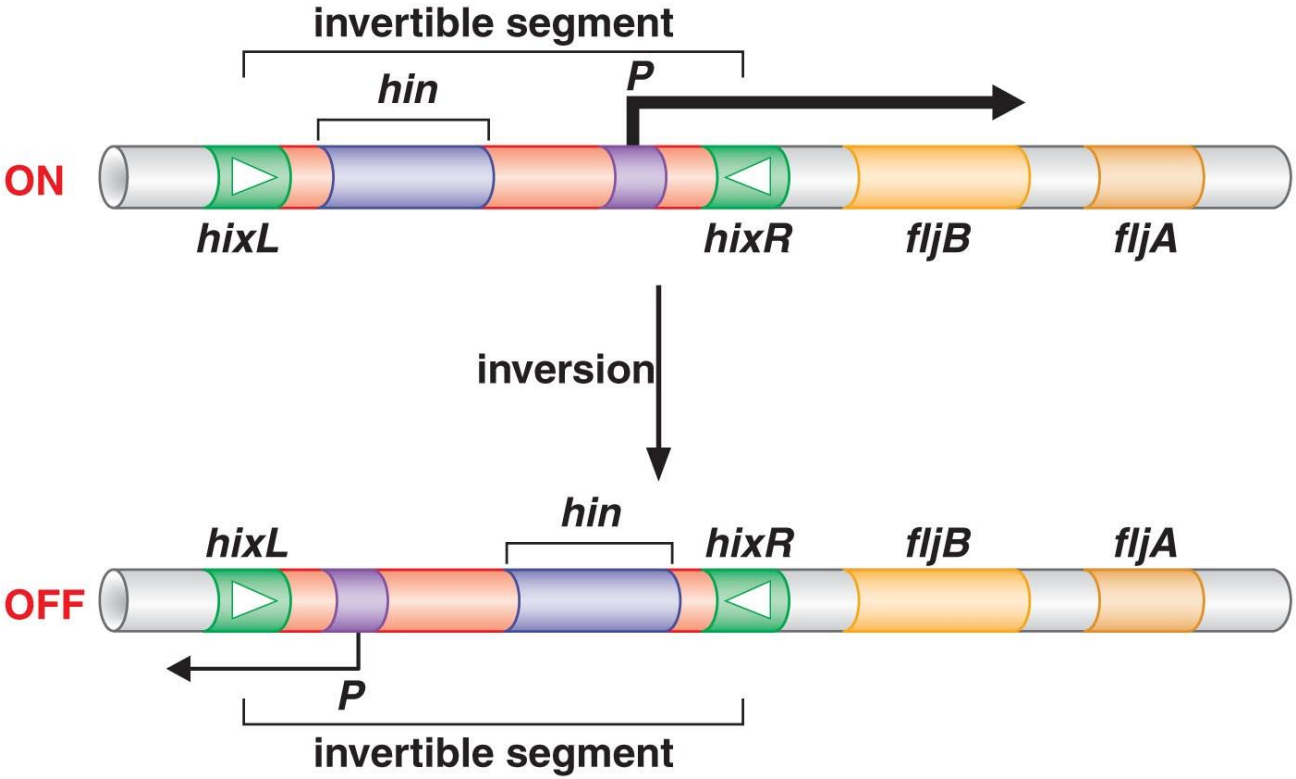


Figure 5–81 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

DNA inversion by the Hin recombinase of Salmonella



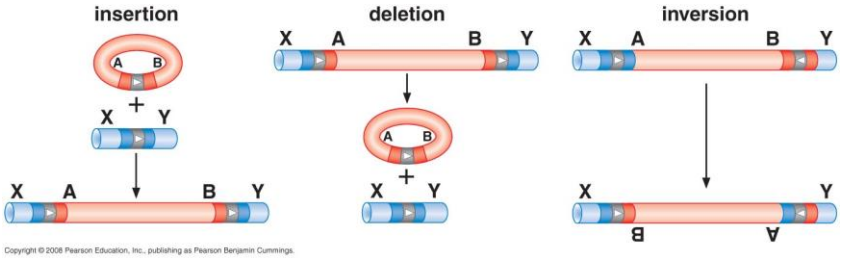
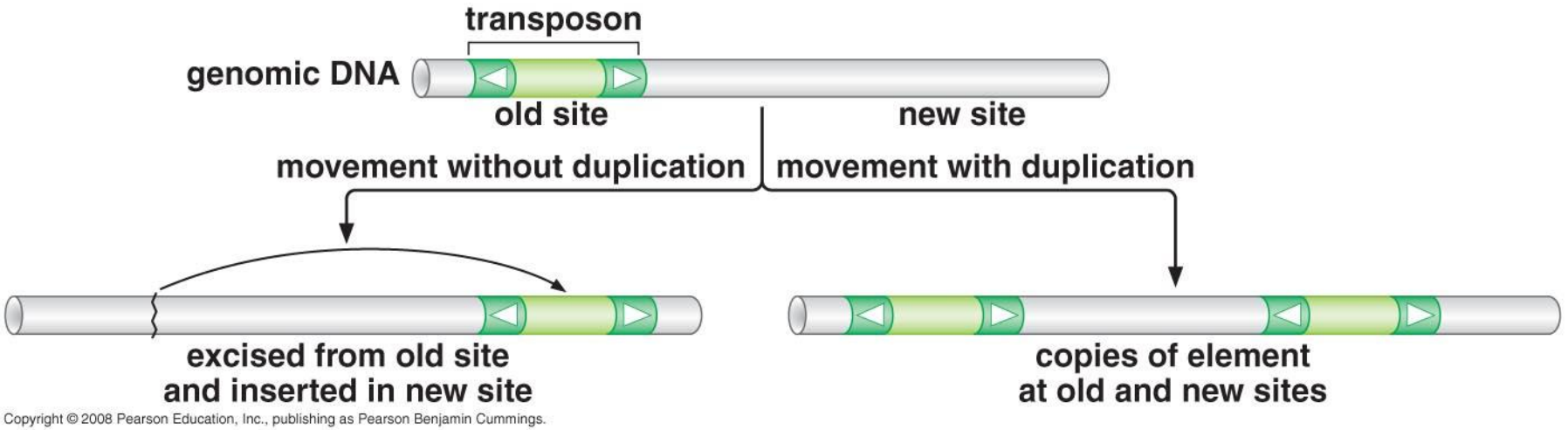


TABLE 12-1 Recombinases by Family and by Function

Recombinase	Function
Serine family	
Salmonella Hin invertase	Inverts a chromosomal region to flip a gene promoter by recognizing *hix* sites. Allows expression of two distinct surface antigens.
Transposon Tn*3* and γδ resolvases	Promotes a DNA deletion reaction to resolve the DNA fusion event that results from replicative transposition. Recombination sites are called *res* sites.
Tyrosine family	
Bacteriophage λ integrase	Promotes DNA integration and excision of the λ genome into, and out of, a specific sequence on the *E. coli* chromosome. Recombination sites are called *att* sites.
Phage P1 Cre	Promotes circularization of the phage DNA during infection by recognizing sites (called *lox* sites) on the phage DNA.
Escherichia coli XerC and XerD	Promotes several DNA deletion reactions that convert dimeric circular DNA molecules into monomers. Recognizes both plasmid-borne sites (*cer*) and chromosomal sites (*dif*).
Yeast FLP	Inverts a region of the yeast 2 μ plasmid to allow for a DNA amplification reaction called rolling circle replication. Recombination sites are called *frt* sites.

Transposition

Some genetic elements move to new chromosomal locations by transposition



Genetic organization of the three classes of transposable elements.

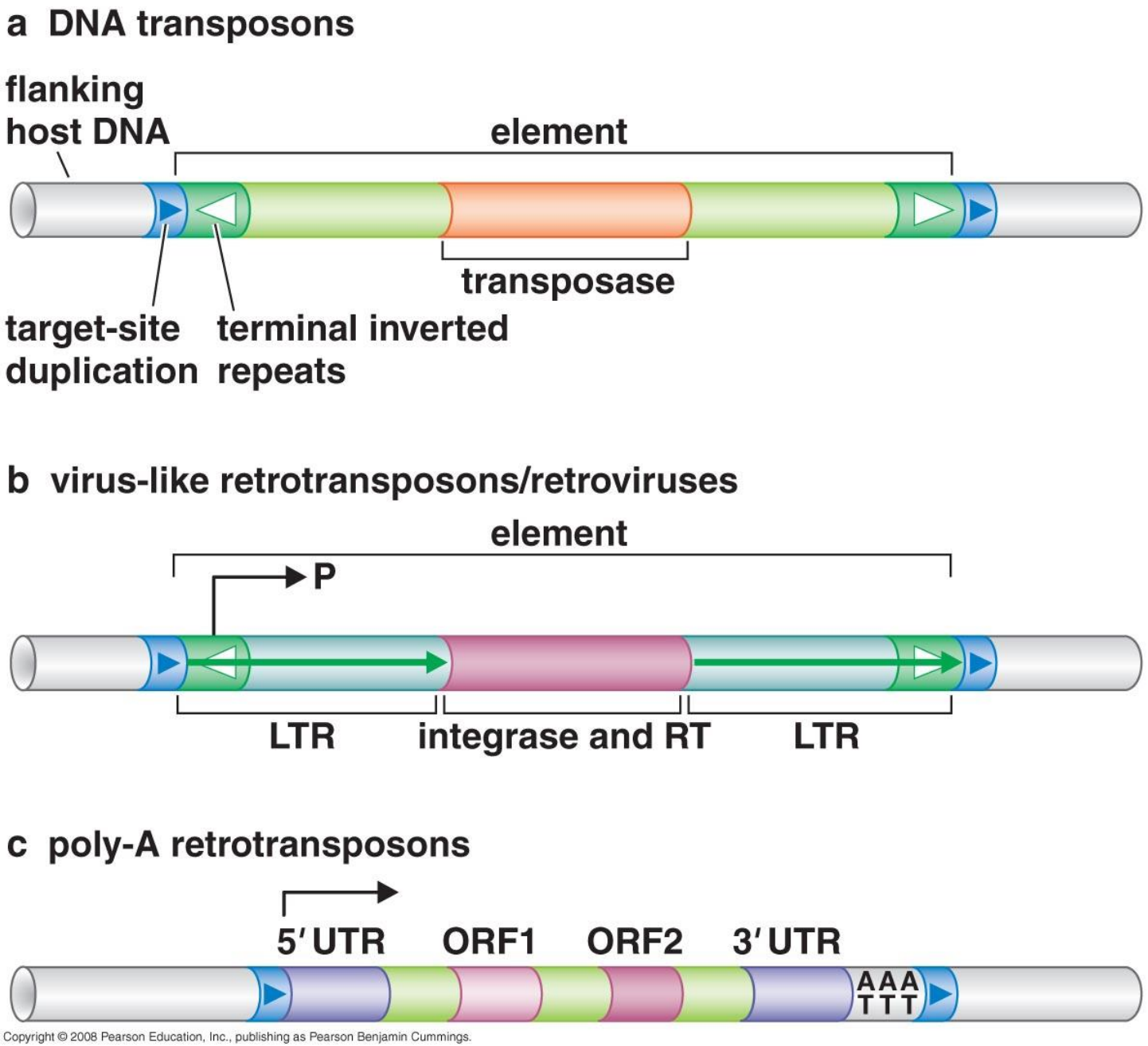
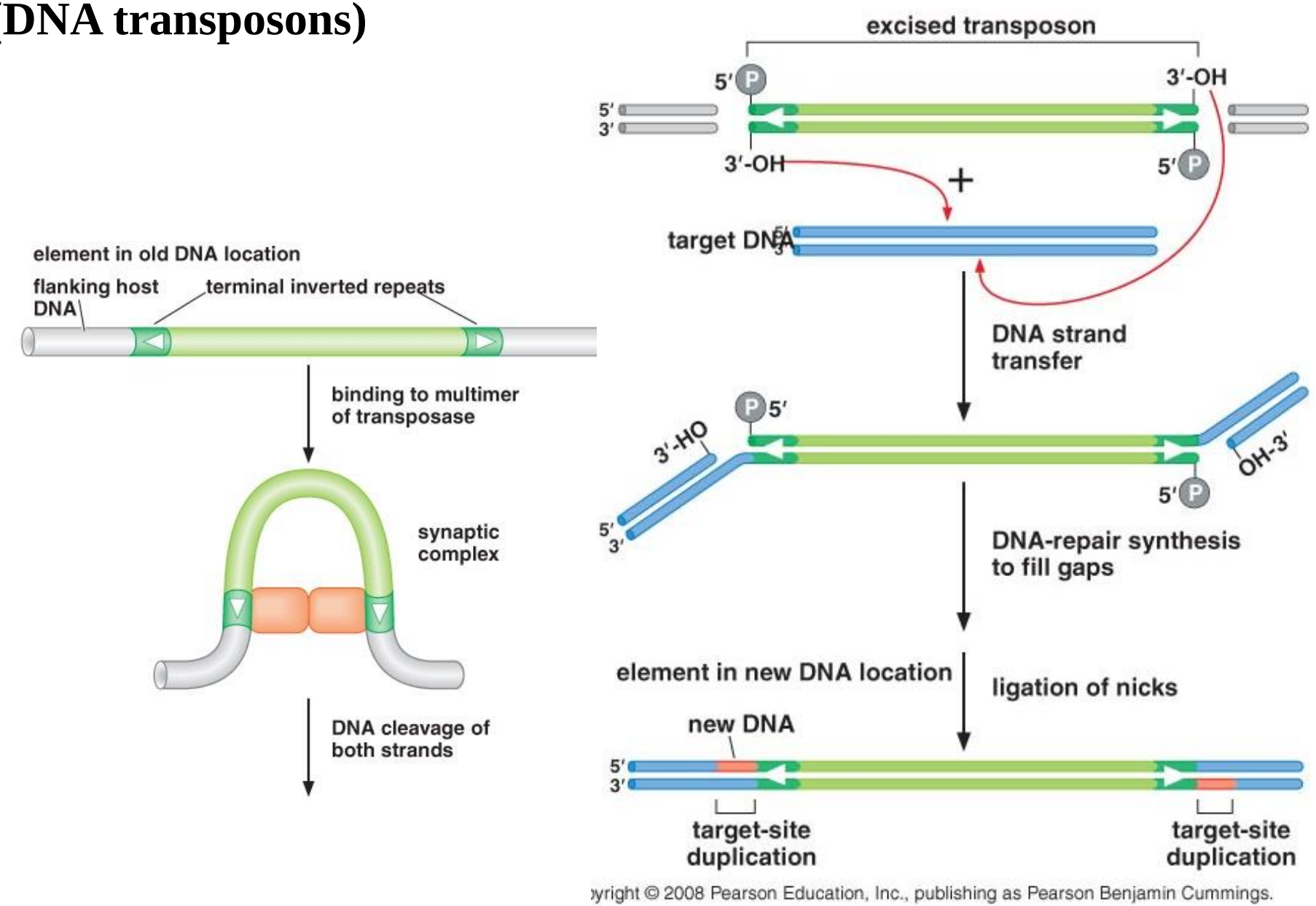


Table 5–3 Three Major Classes of Transposable Elements

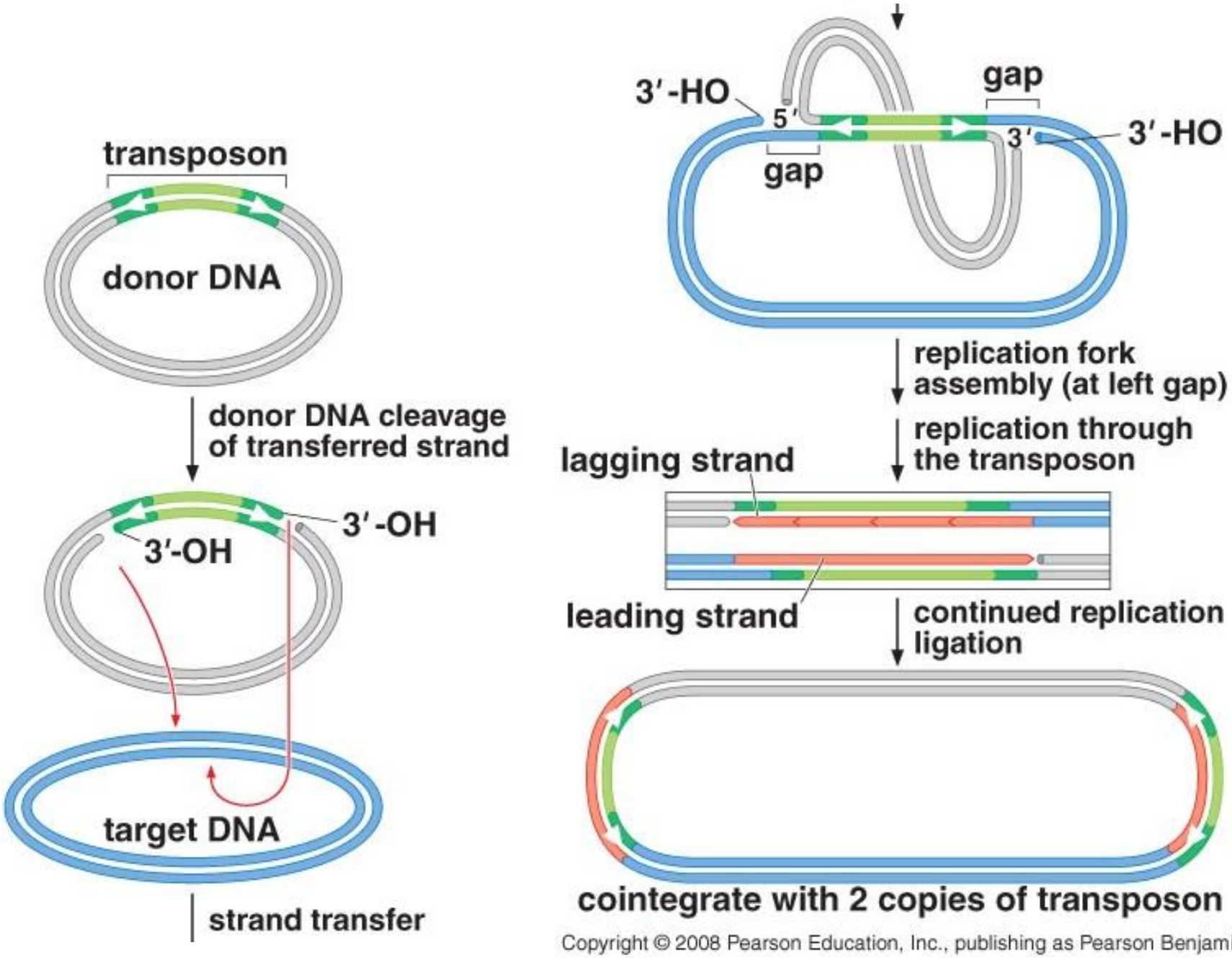
CLASS DESCRIPTION AND STRUCTURE	SPECIALIZED ENZYMES REQUIRED FOR MOVEMENT	MODE OF MOVEMENT	EXAMPLES
DNA-only transposons			
 short inverted repeats at each end	transposase	moves as DNA, either by cut-and-paste or replicative pathways	P element (*Drosophila*) Ac-Ds (maize) Tn3 and Tn10 (*E. coli*) Tam3 (snapdragon)
Retroviral-like retrotransposons			
 directly repeated long terminal repeats (LTRs) at each end	reverse transcriptase and integrase	moves via an RNA intermediate produced by a promoter in the LTR	Copia (*Drosophila*) Ty1 (yeast) THE1 (human) Bs1 (maize)
Nonretroviral retrotransposons			
 Poly A at 3′ end of RNA transcript; 5′ end is often truncated	reverse transcriptase and endonuclease	moves via an RNA intermediate that is often produced from a neighboring promoter	F element (*Drosophila*) L1 (human) Cin4 (maize)

These elements range in length from 1000 to about 12,000 nucleotide pairs. Each family contains many members, only a few of which are listed here. In addition to transposable elements, some viruses can move in and out of host cell chromosomes by transpositional mechanisms. These viruses are related to the first two classes of transposons.

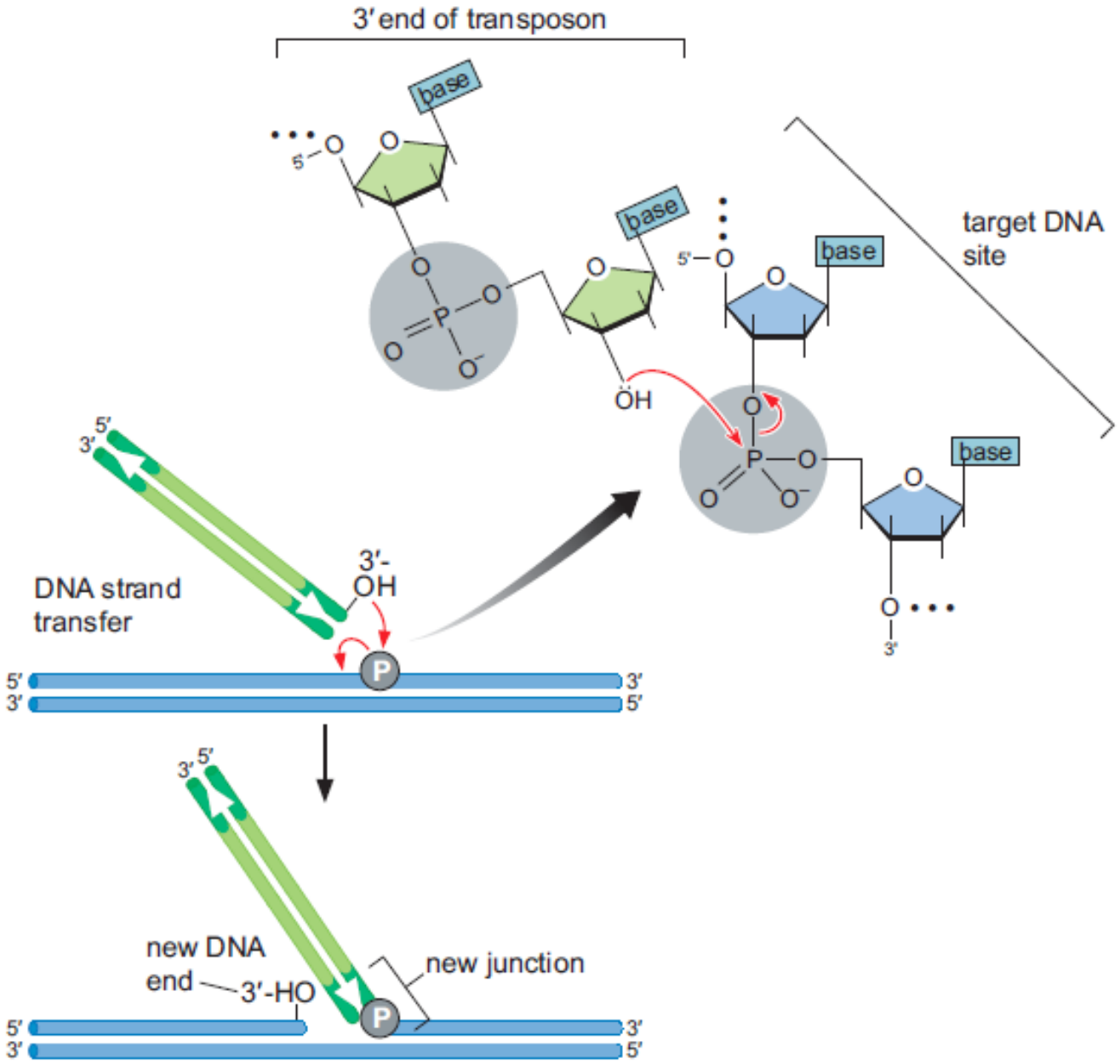
The cut-and-paste mechanism of transposition (DNA transposons)



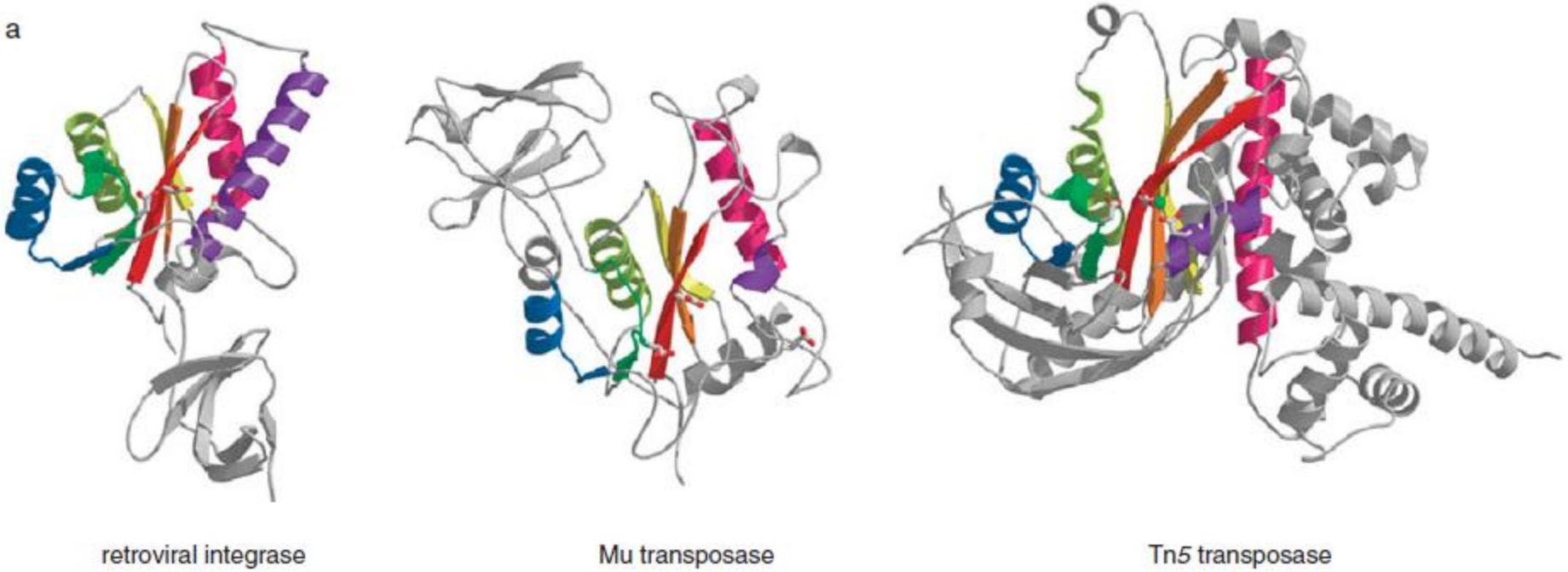
Replicative transposition (DNA transposons)



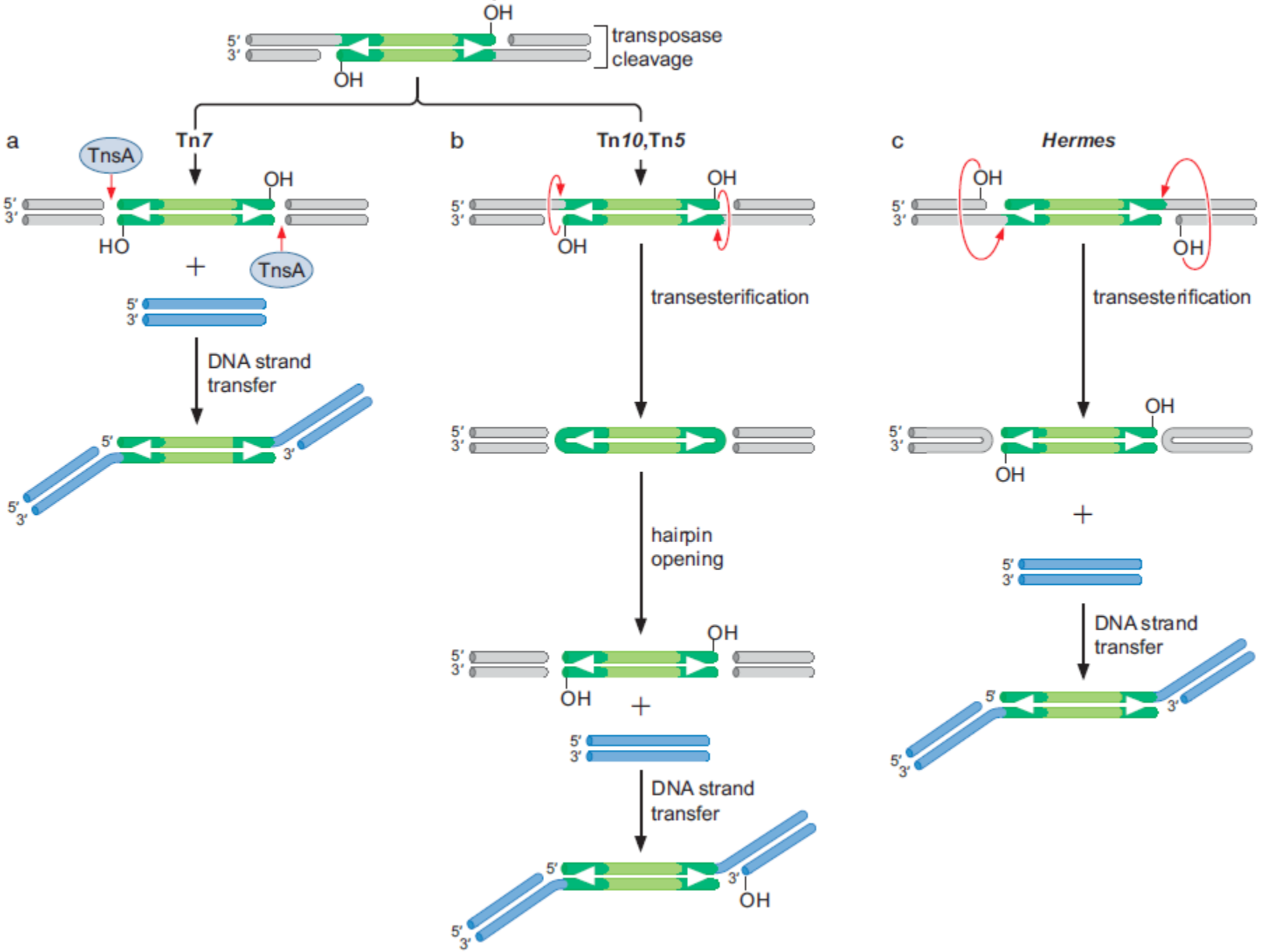
Chemical step of DNA strand transfer



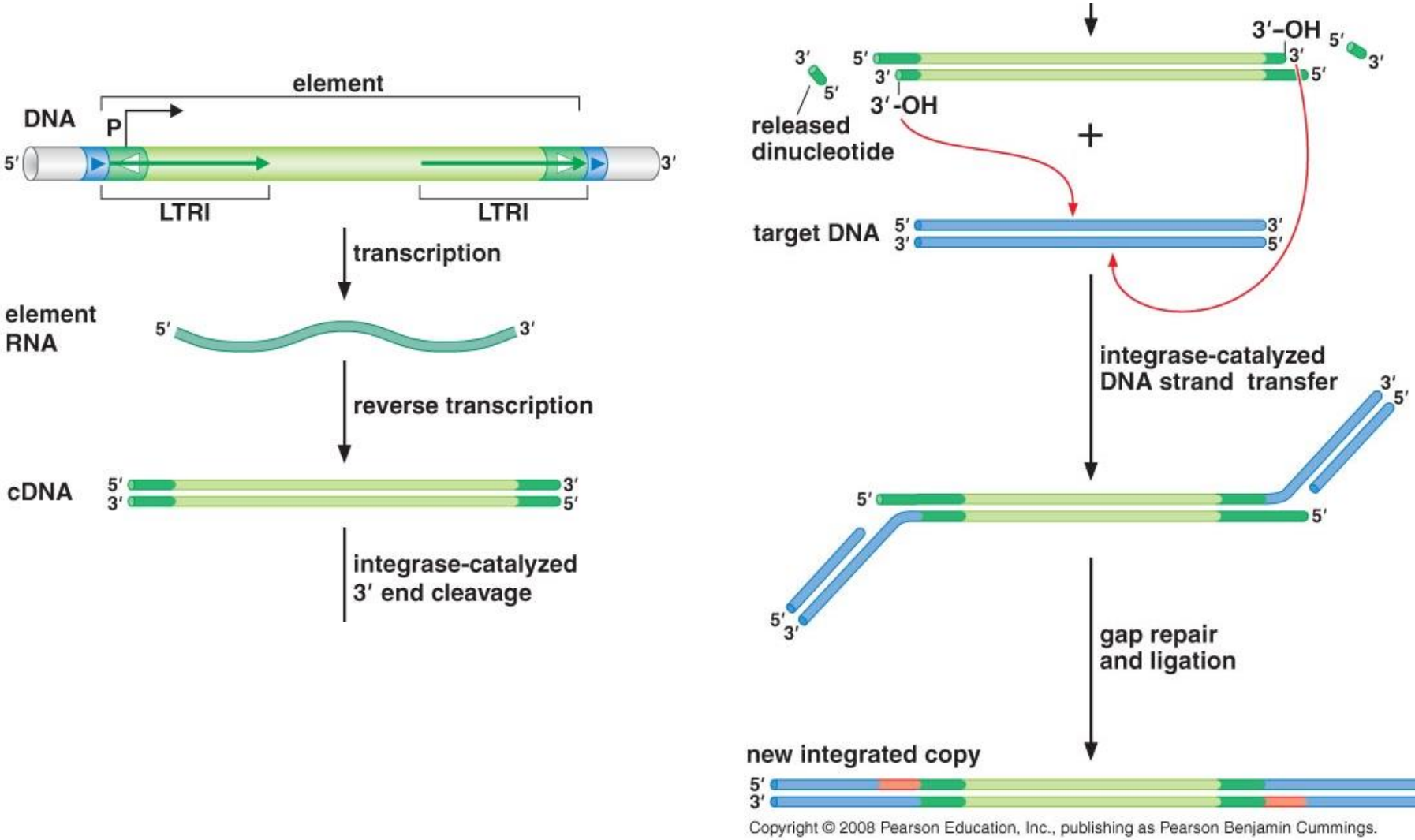
Similarities of catalytic domains of transposases and integrases.



Three mechanisms for cleaving the nontransferred strand.



Virus-like retrotransposons



Some virus use a transposition mechanism to move themselves into host cell chromosomes

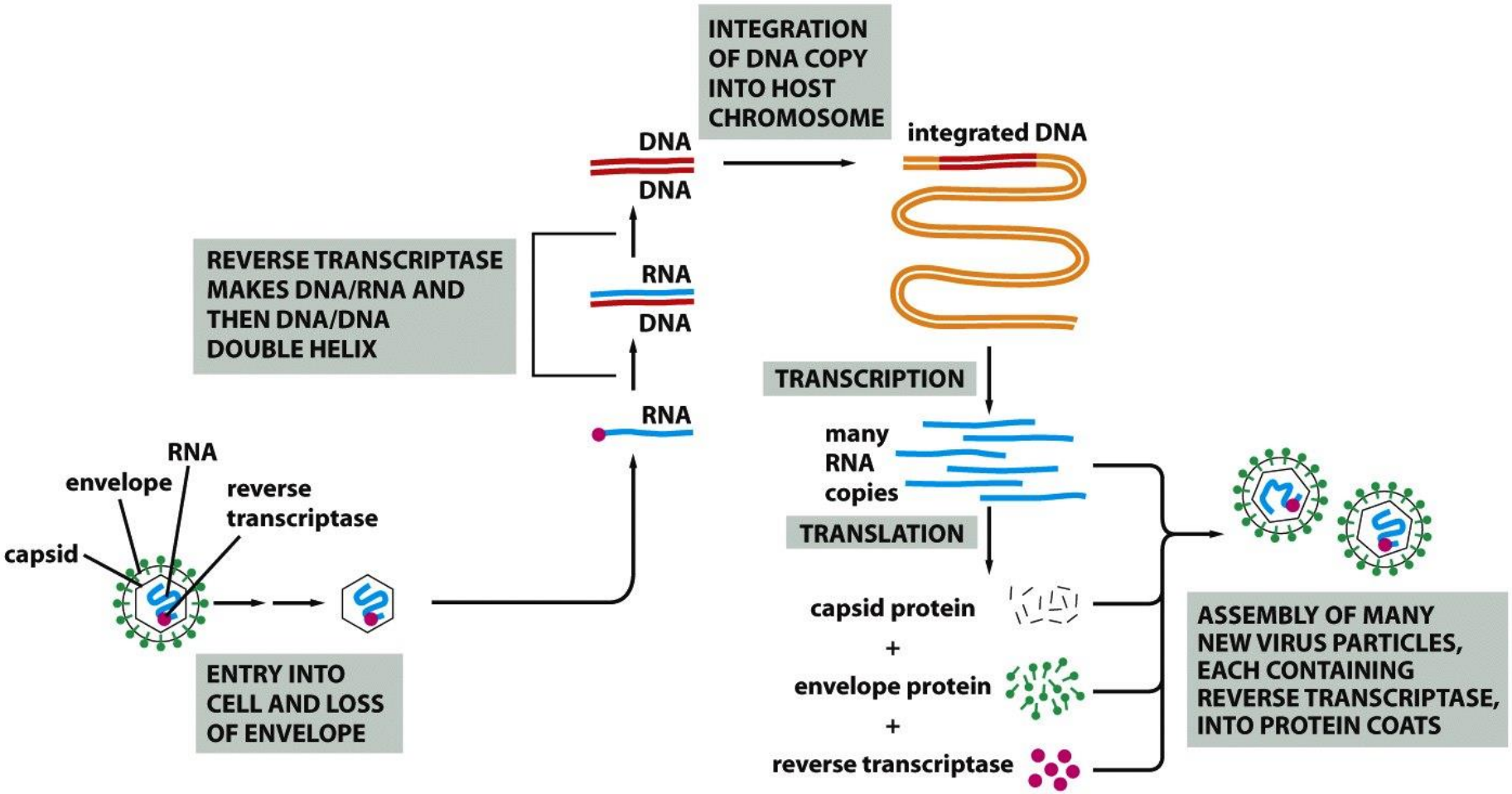
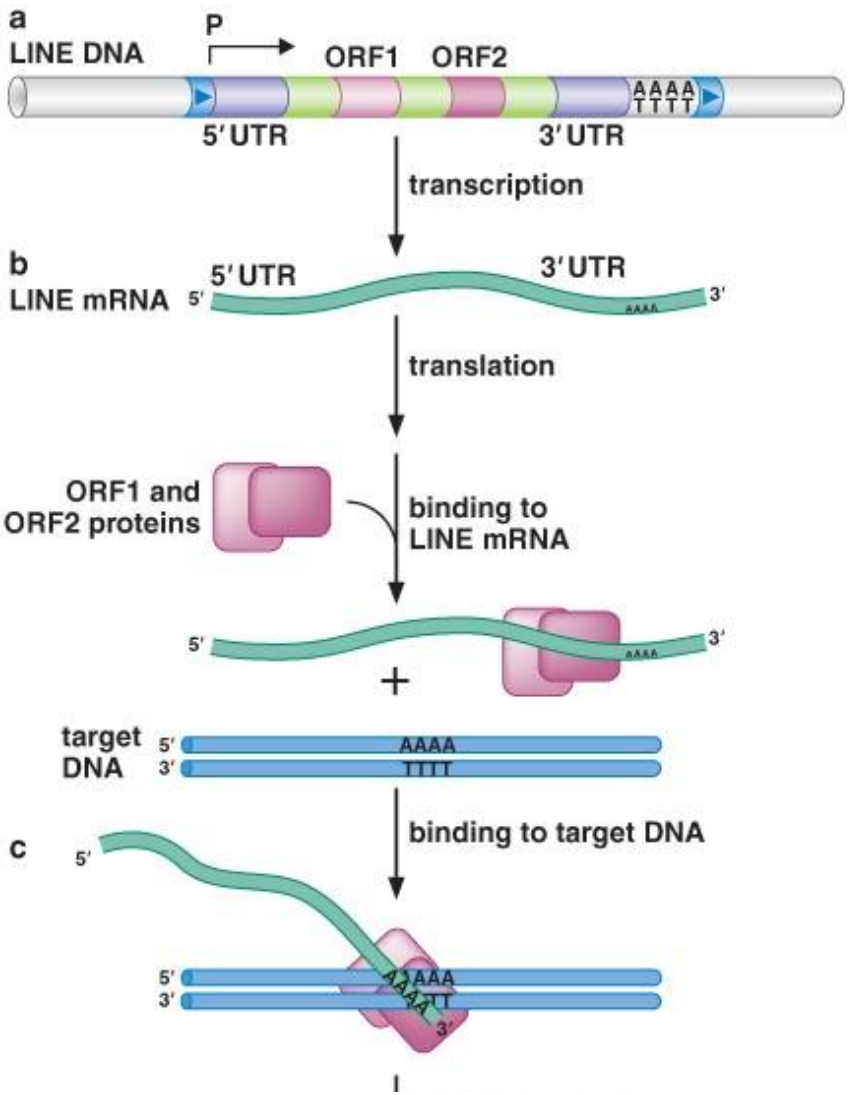
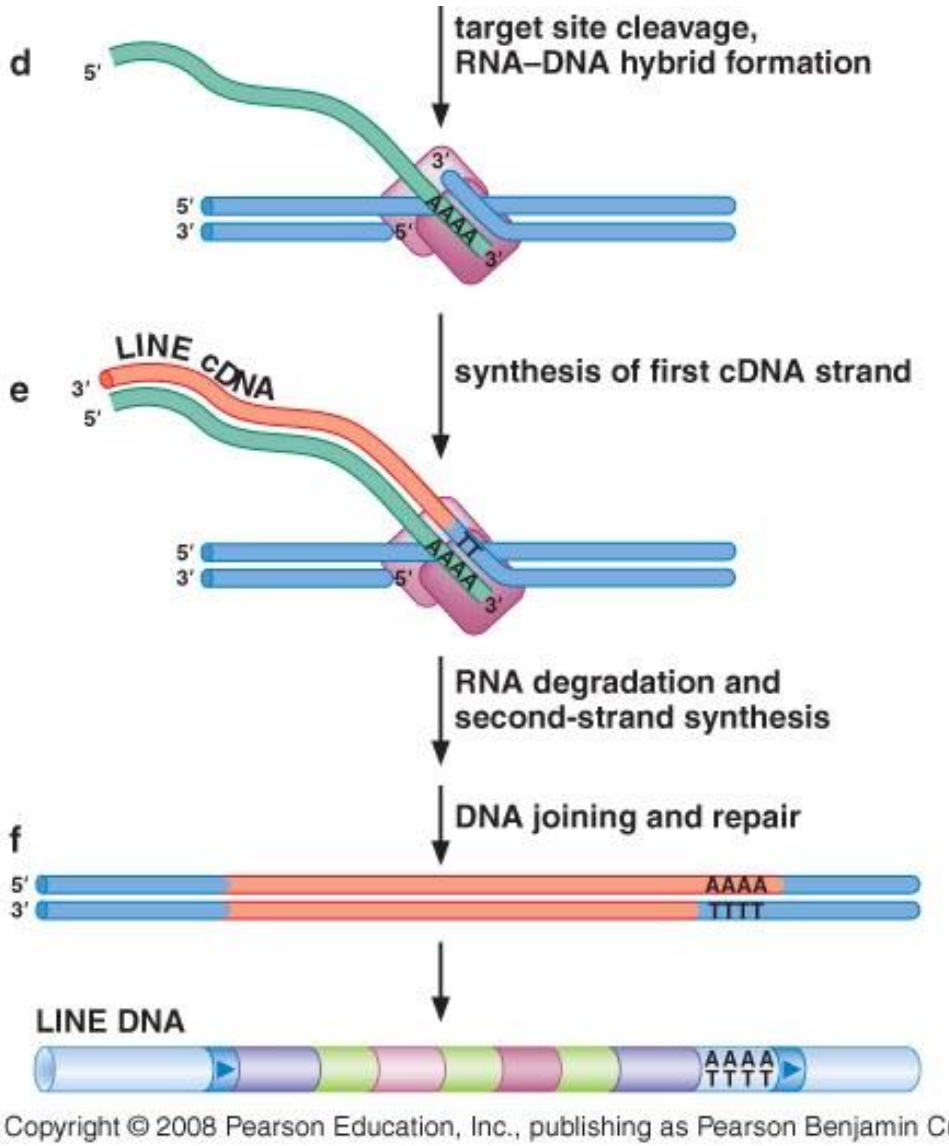


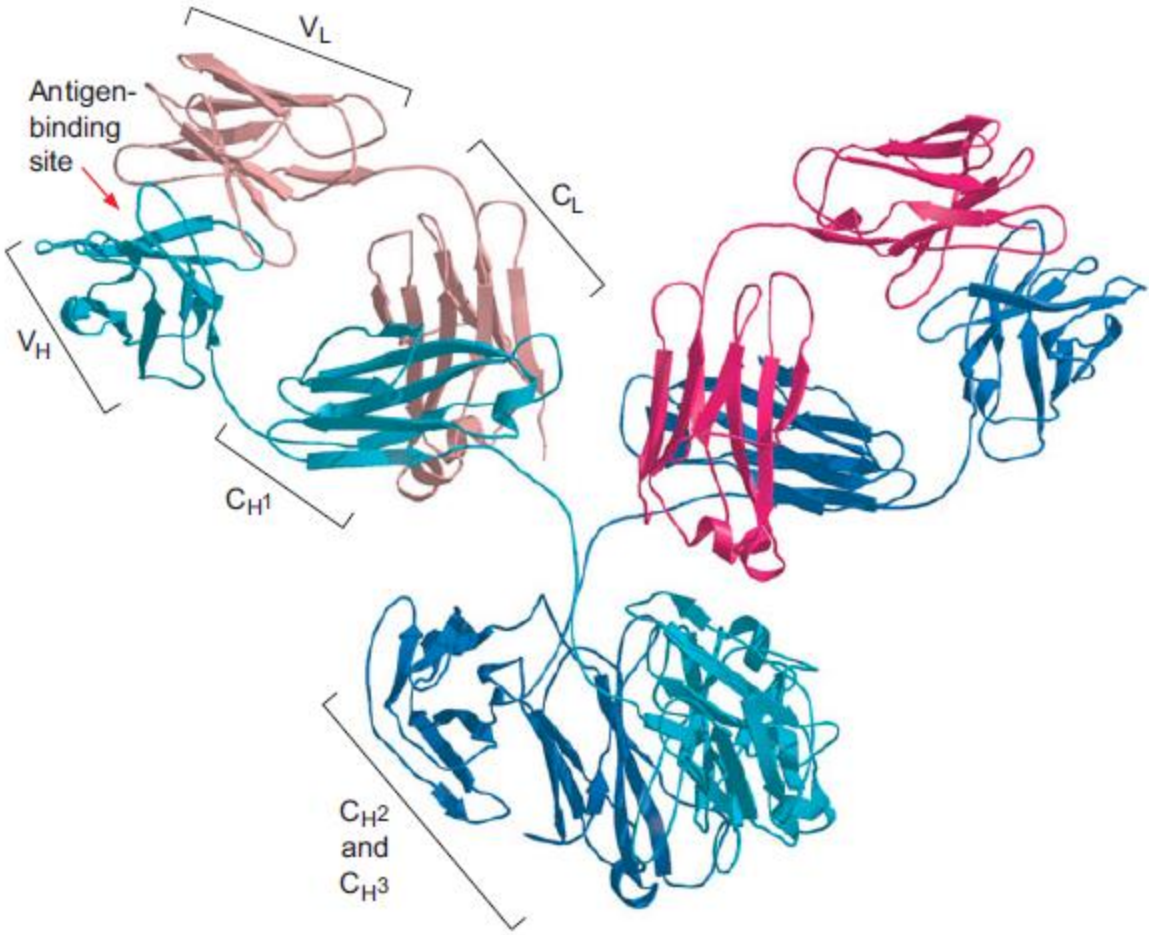
Figure 5-71 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Poly-A retrotransposon

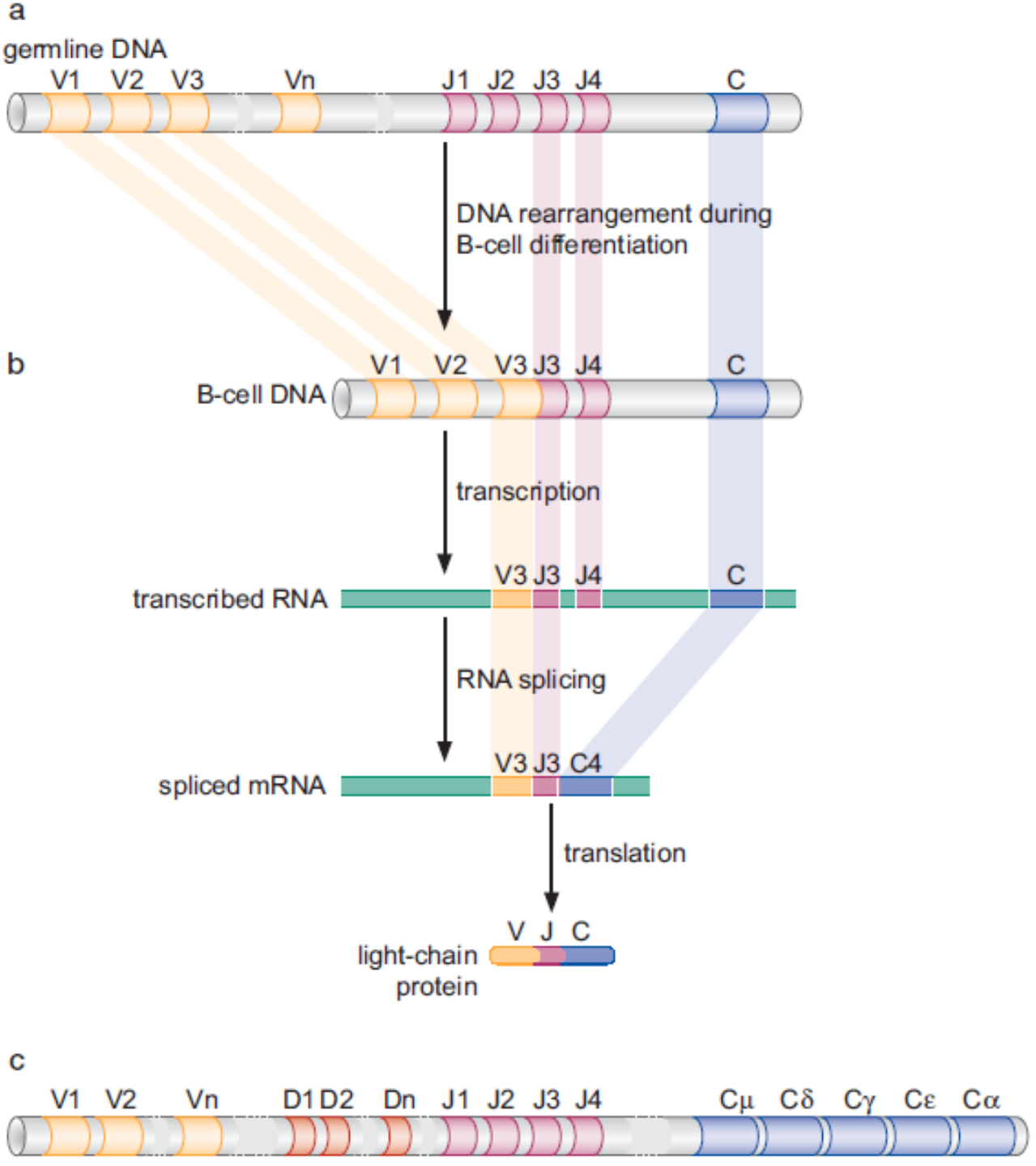




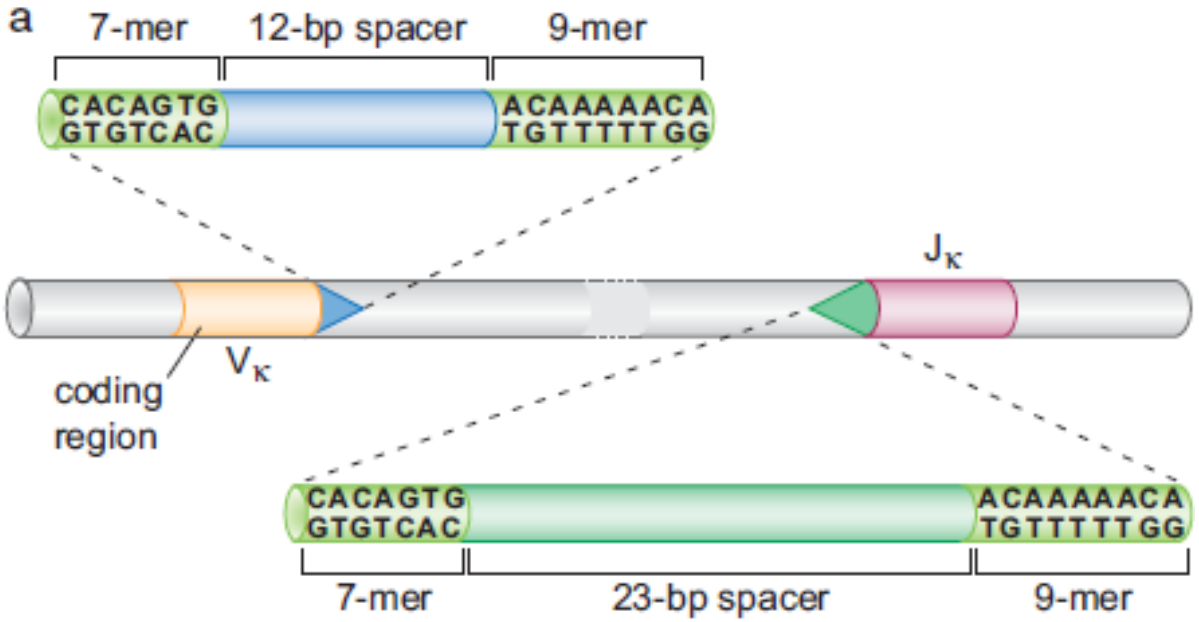
V(D)J Recombination

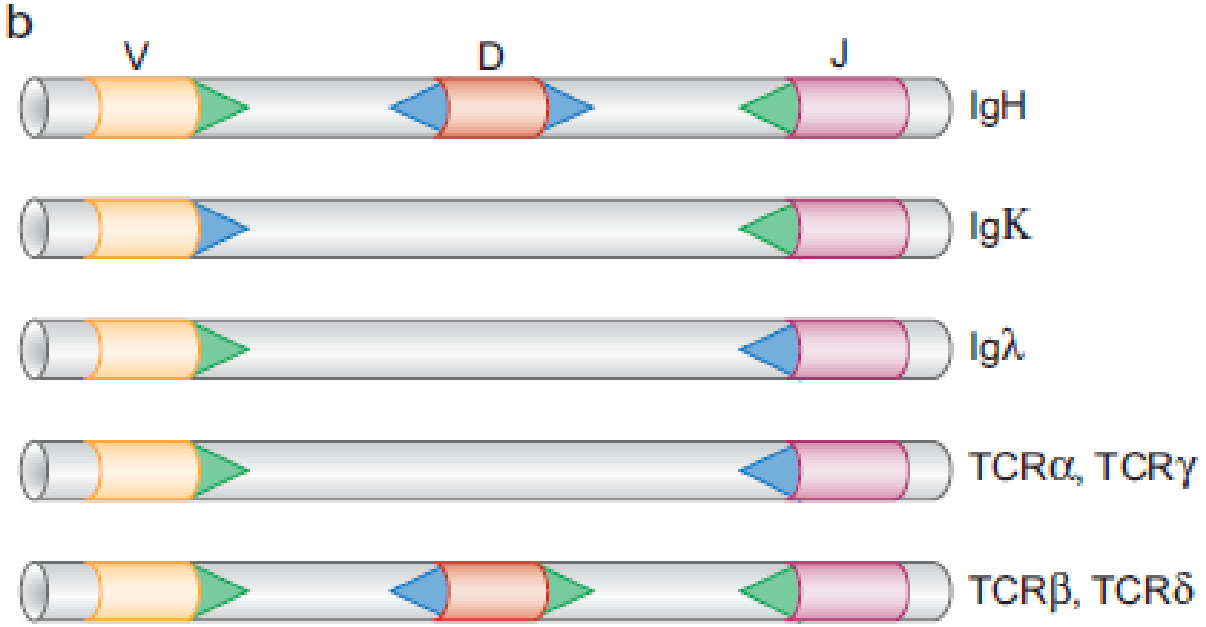


Overview of the process of V(D)J recombination.



Recombination signal sequences recognized in V(D)J recombination.





The V(D)J recombination pathway: cleavages occur by a mechanism similar to transposon excision.

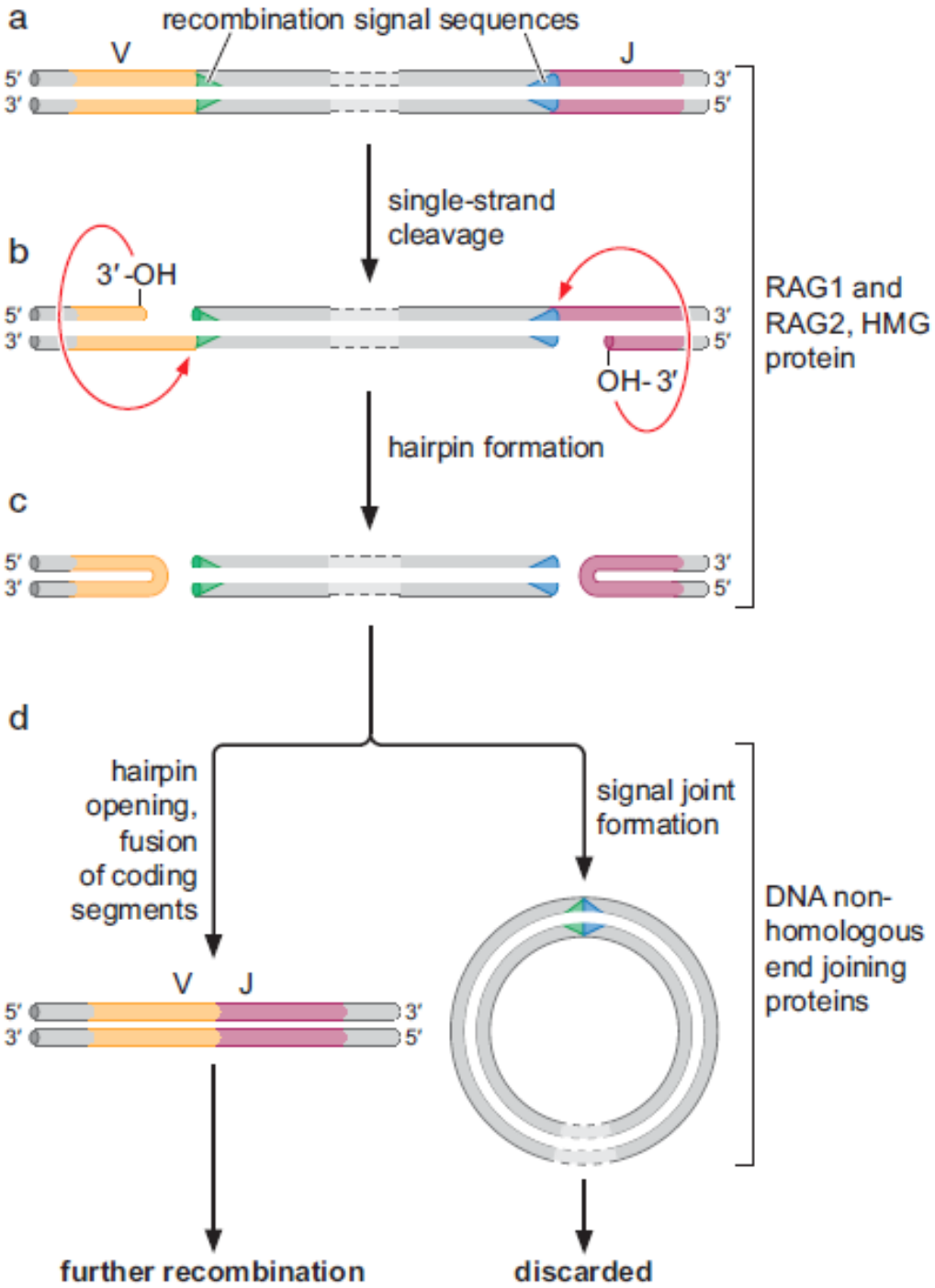


TABLE 11-2 Major Types of Transposable Elements

Type	Structural Features	Mechanism of Movement	Examples
DNA-mediated transposition			
Bacterial replicative transposons	Terminal inverted repeats that flank antibiotic-resistance and transposase genes	Copying of element DNA accompanying each round of insertion into a new target site	Tn*3*, γδ, phage Mu
Bacterial cut-and-paste transposons	Terminal inverted repeats that flank antibiotic-resistance and transposase genes	Excision of DNA from old target site and insertion into new site	Tn*5*, Tn*10*, Tn*7*, IS*911*, Tn*917*
Eukaryotic transposons	Inverted repeats that flank coding region with introns	Excision of DNA from old target site and insertion into new site	P elements (*Drosophila*), *hAT* family elements, Tc*1*/*Mariner* elements
RNA-mediated transposition			
Virus-like retrotransposons	~250- to 600-bp direct terminal repeats (LTRs) flanking genes for reverse transcriptase, integrase, and retrovirus-like Gag protein	Transcription into RNA from promoter in left LTR by RNA polymerase II followed by reverse transcription and insertion at target site	Ty elements (yeast), *Copia* elements (*Drosophila*)
Poly-A retrotransposons	3′ A-T-rich sequence and 5′ UTR flank genes encoding an RNA-binding protein and reverse transcriptase	Transcription into RNA from internal promoter; target-primed reverse transcription initiated by endonuclease cleavage	F and G elements (*Drosophila*), LINE and SINE elements (mammals), *Alu* sequences (humans)

Thank You