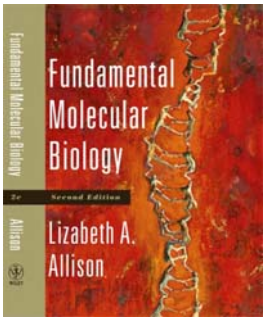
Terry Brown

Genomes
Third Edition

Chapter 17:
Recombination



Fundamental Molecular Biology
Second Edition

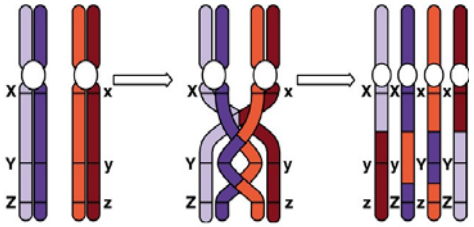
Lisabeth A. Allison

Chapter 7
DNA Repair Pathways

Recombination

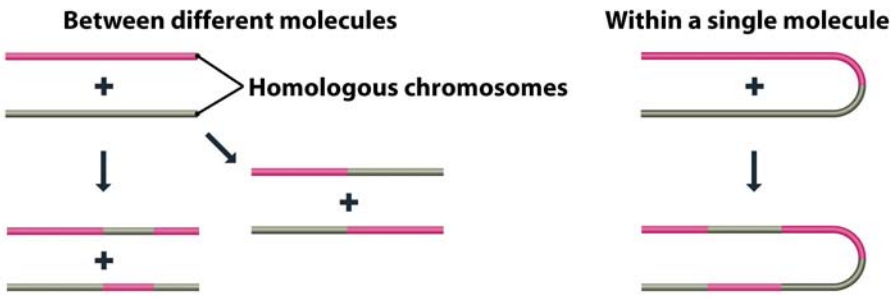
- Outcome of crossing-over between pairs of homologous chromosomes during meiosis.
- Crossing-over results in daughter chromosomes that have different combinations of alleles compared with their parental chromosomes.

Crossing over during meiosis



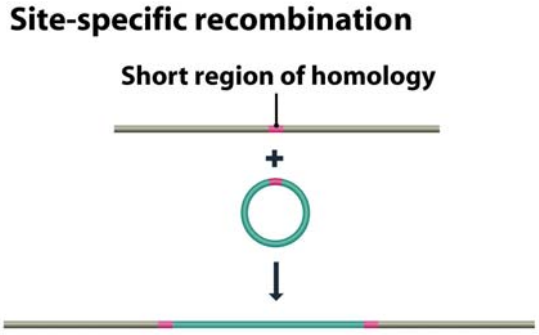
- A variety of processes that involve the breakage and reunion of polynucleotides.
  1) Homologous recombination
  2) Site-specific recombination
  3) Transposition.

Homologous recombination



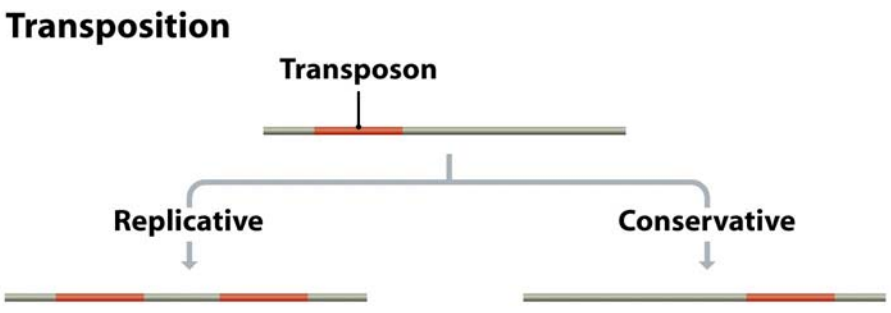
- Homologous recombination is exchange of DNA segments between two similar or identical molecules of DNA.
- It happens between different molecules or within a single molecule.
- It is responsible for crossing-over but its primary function is in DNA repair.

Figure 17.1a *Genomes 3* (© Garland Science 2007)



- Site-specific recombination occurs between DNA segments possessing only a limited sequence homology, possibly just a few base pairs.
- The insertion of phage genome into bacterial genome is mediated by site-specific recombination.

Figure 17.1b *Genomes 3* (© Garland Science 2007)

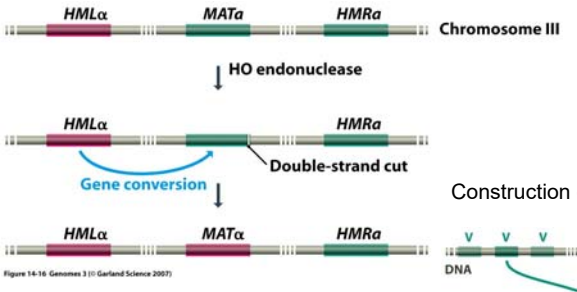


- Transposition is the transfer of a segment of DNA from one position in the genome to another position.

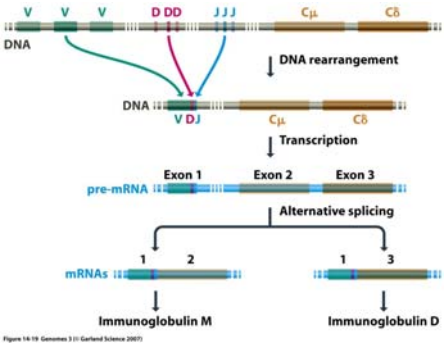
Figure 17.1c *Genomes 3* (© Garland Science 2007)

Other recombination events

Mating type switching in yeast



Construction of immunoglobulin genes



17.1 Homologous recombination

17.1.1 Models for homologous recombination

1. The Holliday and Meselson-Radding models for homologous recombination

- Robin Holliday and Matthew Meselson
- The key feature of Holliday model is formation of a heteroduplex resulting from the exchange of polynucleotide segments between the two homologous molecules, called chi form.

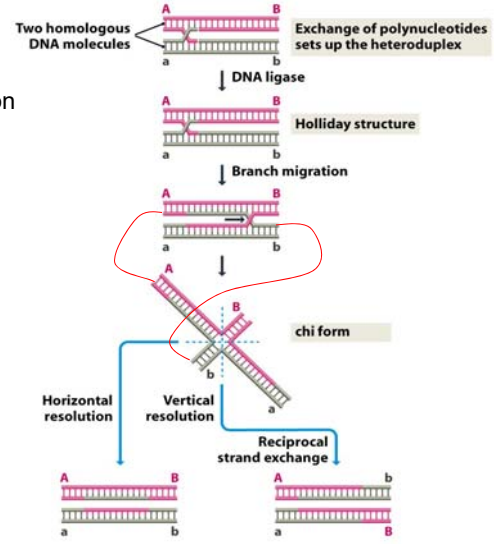
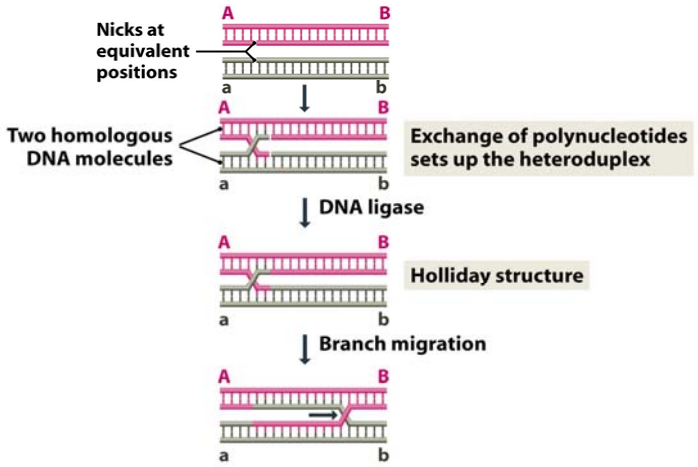
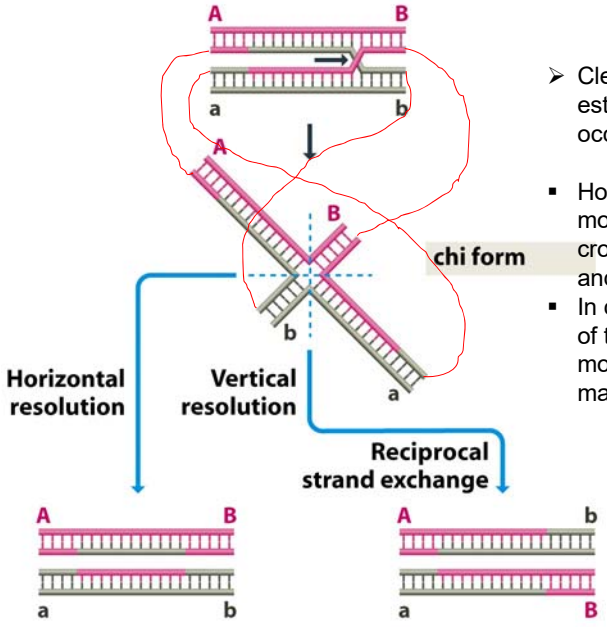


Figure 17.2 *Genomes 3* (© Garland Science 2007)



- According to Holliday model, single-strand breaks occur at the same position on one strand of each parental DNA.
- Free ends of each broken strand then migrate across to the other DNA helix, where the invading strands are joined to the free ends they encounter.
- The resulting crossover junction is called a Holliday junction.
- It displaces the original complementary strand ahead of it, causing the Holliday junction to migrate (branch migration).

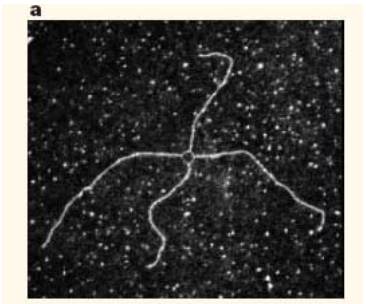
Figure 17.2 part 1 of 2 *Genomes 3* (© Garland Science 2007)



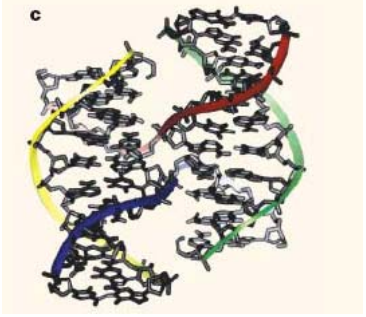
- Cleavage and rejoining to re-establish two separate DNAs occurs in two ways.
  - Horizontal resolution leads to two molecules that do not show crossover of markers in genes A and B.
  - In case of vertical resolution, both of the resulting recombinant molecules show crossover of markers in genes A and B.

Figure 17.2 part 2 of 2 *Genomes 3* (© Garland Science 2007)

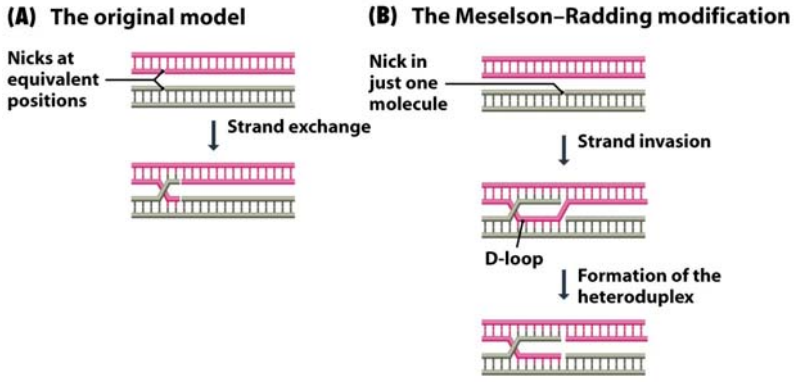
Crossing-over



electron microscopic image of chi form



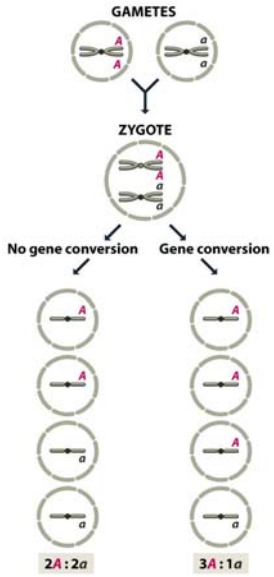
atomic model of chi form



- The original model needs nicks at precisely the same position on each molecule for strand exchange.
- The model was criticized because no mechanism could be proposed to make these nicks.
- According to Meselson and Radding model, the free end generated by a single-strand nick invades the unbroken helix at the homologous position and displaces one of its strands, forming a D-loop.
- Subsequent cleavage of the displaced strand at the junction between its single-stranded and base-paired regions produces the heteroduplex like in the original model.

Figure 17.3 *Genomes 3* (© Garland Science 2007)

2. The double-strand break model for homologous recombination



- Gene conversion is a process that results in the four haploid products of meiosis displaying an unusual segregation pattern.
- Holliday's original and Meselson's modified model cannot explain gene conversion.
- Without gene conversion, two gametes with allele 'A' and allele 'a' form a zygote, and produce four haploid spores 2A;2a alleles.
- If gene conversion occurs, the ratio will be changed like 3A;1a.

Figure 17.4 *Genomes 3* (© Garland Science 2007)

Double-strand break model

1) 100-1,000 times during meiosis
2) homologous recombination is involved in DNA repair (DSB repairing)

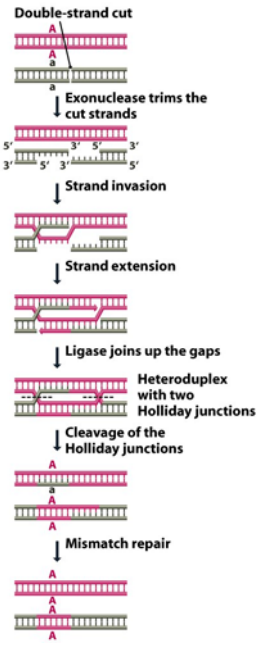
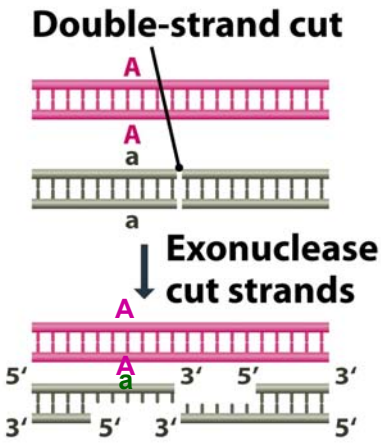


Figure 17.5 *Genomes 3* (© Garland Science 2007)



- Homologous recombination initiates with a double-strand cut.
- Then, exonucelase trims the cut strands so each end has 3'-overhang.

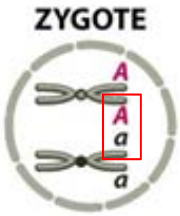
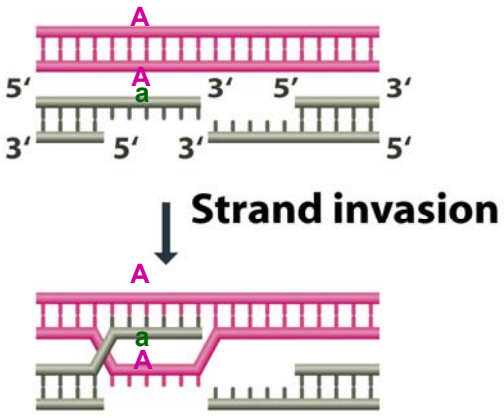
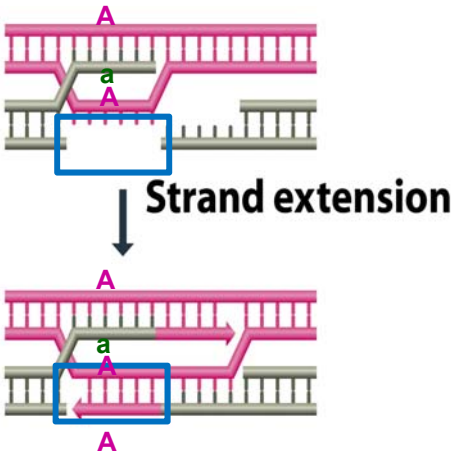


Figure 17.5 part 1 of 6 *Genomes 3* (© Garland Science 2007)



- One of 3'-overhangs invades and form D-loop like Meselson-Radding model.

Figure 17.5 part 2 of 6 *Genomes 3* (© Garland Science 2007)

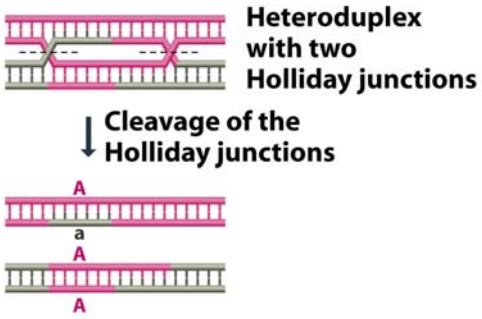


- The 3'-overhangs are extended.

Figure 17.5 part 3 of 6 *Genomes 3* (© Garland Science 2007)

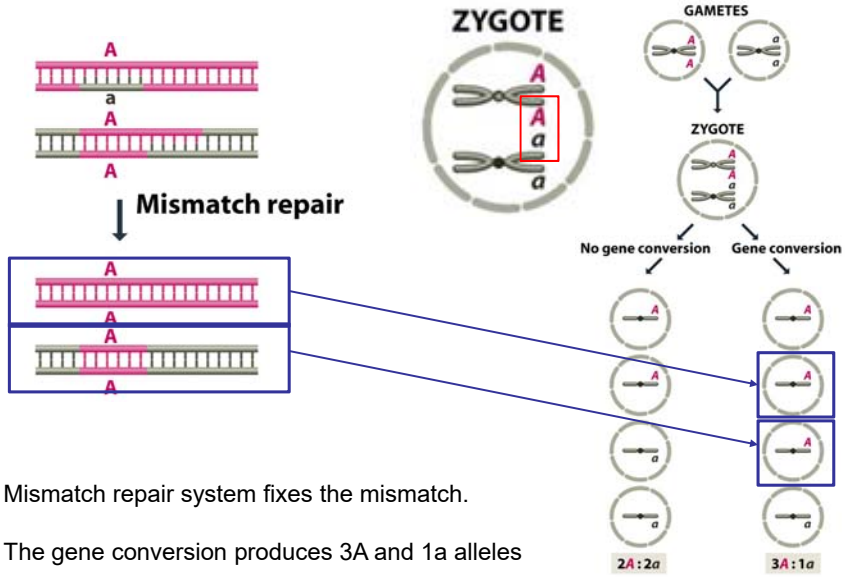
- After ligation, the resulting heteroduplex has a pair of holliday junctions.

Figure 17.5 part 4 of 6 *Genomes 3* (© Garland Science 2007)



- The structure can be resolved in a number of ways, some of them resulting in the mismatched allele.

Figure 17.5 part 5 of 6 *Genomes 3* (© Garland Science 2007)



- Mismatch repair system fixes the mismatch.
- The gene conversion produces 3A and 1a alleles

Figure 17.5 part 6 of 6 *Genomes 3* (© Garland Science 2007)

17.1.2 The biochemistry of homologous recombination

1. The RecBCD pathway of Escherichia coli

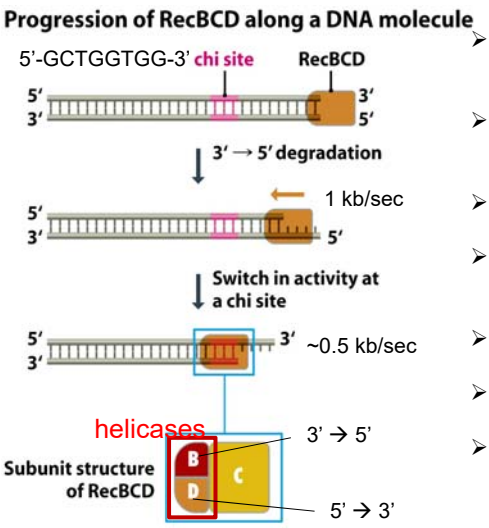
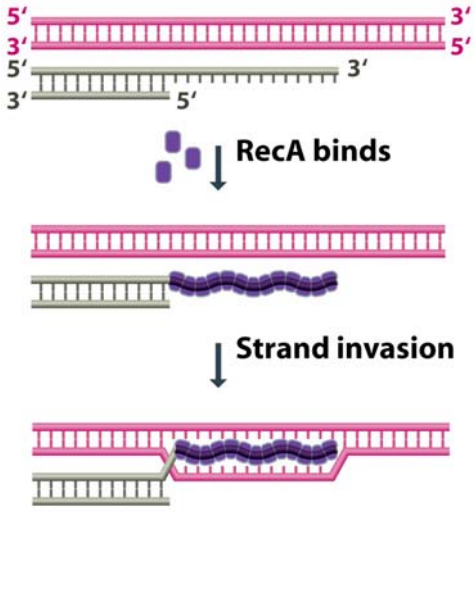


Figure 17.6 *Genomes 3* (© Garland Science 2007)

- RecBCD enzymes mediate E. coli homologous recombination.
- To initatie the recombination, RecBCD complex attaches to the free ends of a chromosome at a double-strand break.
- RecD has a 5' to 3' helicase activity and RecB has a 3' to 5' helicase activity and 3' to 5' exonuclease activity.
- The enzyme complex moves and degrades the strand.
- The enzyme complex progresses along the DNA at a rate of ~ 1kb/sec until it reaches the chi site.
- The chi site occurs on average once every 6 kb.
- At the chi site the RecBCD complex undergoes conformation changes.
- The conformation changes slow down the migration of the RecBCD and abolish the 3' to 5' exonuclease activity of RecB, and make a single endonucleolytic cut in the other strand.



- The result is a double-strand molecule with a 3'-overhang.
- RecA proteins form a protein-coated DNA filament.
- The filament invade the intact double helix and set up the D-loop.

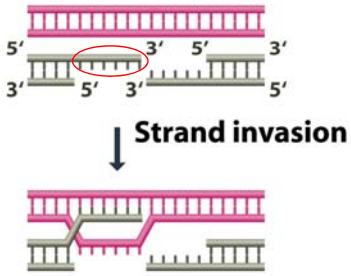
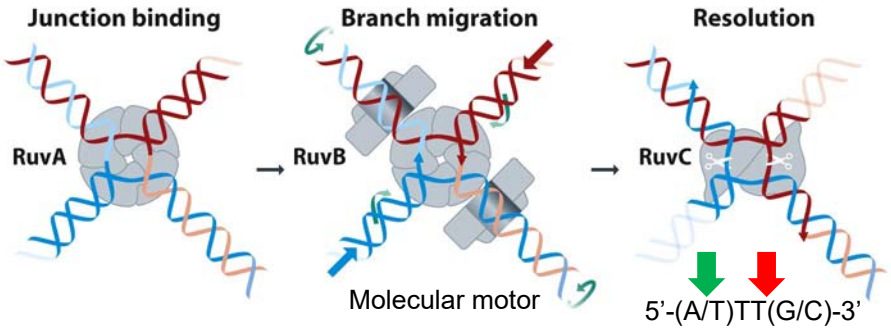


Figure 17.7 *Genomes 3* (© Garland Science 2007)

Branch migration



- Branch migration is catalyzed by four copies of RuvA proteins bound to the Holliday junction with an RuvB ring on either side.
- The complex acts as a molecular motor, so that the branch point moves.
- Branch migration stops preferentially at the sequence 5'-(A/T)TT(G/C)-3'.
- After dessociation of RuvAB comlex, two RuvC proteins bind to the junction and resolve the structure.

Figure 17.8 *Genomes 3* (© Garland Science 2007)

2. Other homologous recombination pathways in E. coli

- E. coli has at least two alternative homologous recombination pathways, RecE and RecF although their recombination efficiency is low.

RecF pathway

1) RecT, RecA, RecF, RecQ, RecJ, RecO, RecR, RuvA, and UvrD
2) RecJ: exonuclease (remove 5'-end)
3) RecQ: helicase
4) RecO: promote annealing of complementary single-stranded DNA ends and strand exchange in RecA-mediated homologous recombination
5) RecR: interact with RecO, enhances RecO's ability to stimulate displacement of SSB protein from ssDNA by RecA
6) UvrD: helicase

RecE pathway

1)RecA, RecJ, RecO, RecR, RecQ, RecF

3. Model for mammalian homologous recombination to repair DSBs

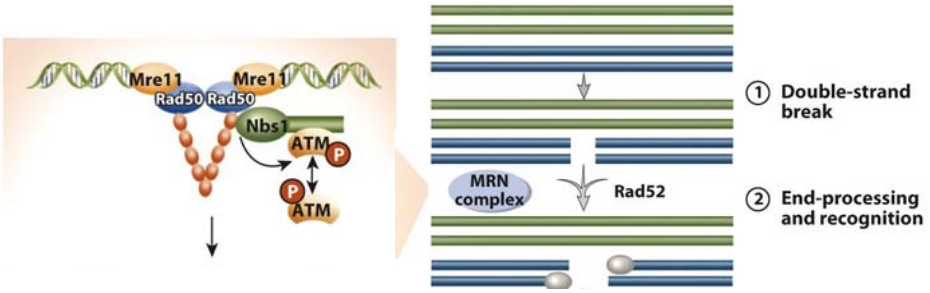


Figure 7.13
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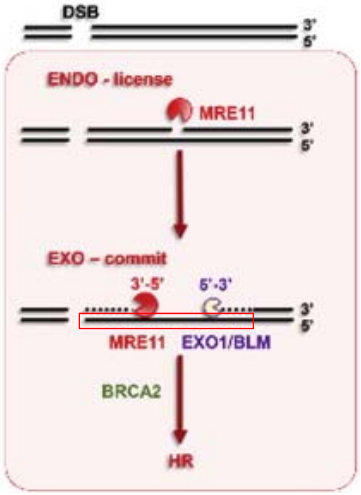
1) Double-strand break (DSB) is induced by ionizing radiation.

2) End-processing and recognition:

a. The MRN (Mre11-Rad50-Nbs1) complex is recruited to the DSB and initiates repair.
b. The 3′→ 5′ exonuclease activity of Mre11 generates single-strand DNA tails.
c. The MRN complex forms a bridge between free DNA ends via the coiled coil arms of the Rad50 dimers.
d. The ssDNA tails are then recognized by Rad52.



Molecular Cell Volume 53, Issue 1, p7–18

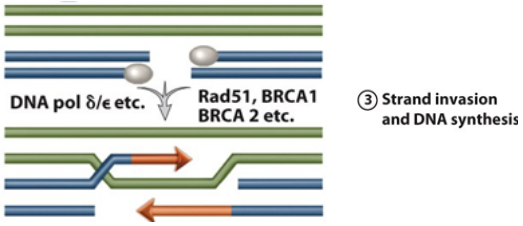


Figure 7.13
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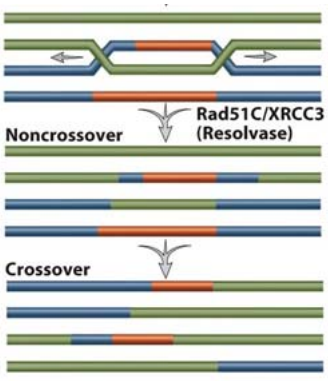
3) Strand invasion and DNA synthesis:
a. Rad51 initiates strand invasion of the 3′ tails with homologous intact sequences.
b. Rad54, Rad55, Rad57, BRACA1, and BRACA2 are involved in homologous recombination.
- BRACA2: interacts with RAD 51
c. Strand exchange generates a hybrid molecule.
d. Missing sequence information at the DSB is restored by DNA synthesis.



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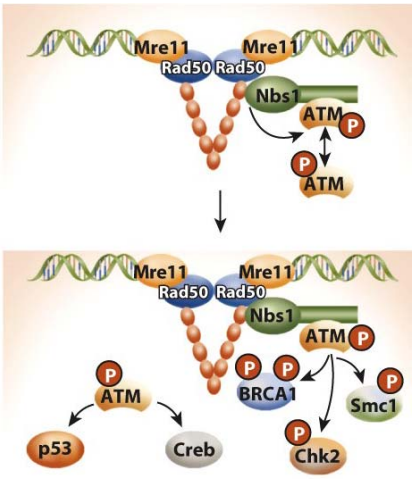
4) Branch migration
a. Rad54 is a motor protein that is important for branch migration.
b. Rad51C/XRCC3 complex is required for Holliday junction processing.

5) Holliday junction resolution and ligation



- ATM (ataxia telangiectasia mutated) and DSBs

1) ATM is a serine-threonine kinase, which is a key signal transducer.
2) MRN complexes form a bridge between free DNA ends via Rad50 dimers.
3) Inactive ATM dimers are recruited to the DSBs.
4) Conformation change of NbsI may activates ATM.
5) Activated ATM monomers either phosphorylate proteins (BRCA1, Smc1, Chk2) involved in DNA repair or phosphorylate proteins (p53, Creb) involved in cell cycle control.
6) Ataxia telangiectasia

- Extreme sensitivity to radiation
- Increased susceptibility to developing cancer
- Immunodeficiency
- Premature aging
- Neurodegenerative disorder

17.1.3 Homologous recombination and DNA repair

1) Single-strand gap repair

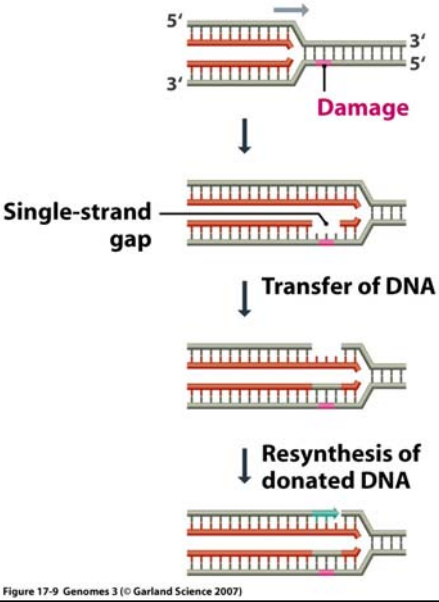


Figure 17-9 Genomes 3 (© Garland Science 2007)

- The primary function of homologous recombination is in postreplicative DNA repair.
- The DNA polymerase encounters heavily damaged region like cyclobutyl dimes, the polymerase jumps ahead and restarts replication.
- The gap is repair by E. coli RecF pathway.

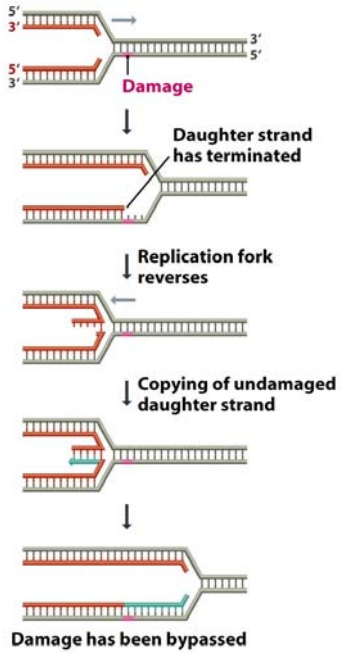


Figure 17.10 *Genomes 3* (© Garland Science 2007)

2) Reversal of the replication fork

- If the damaged region cannot be bypassed, replication terminates.
- One possible mechanism to overcome the mutation is that the replication fork stalls and reverses a short distance and the damaged region is replicated with undamaged strand as a template.
- Then the replication fork moves forward again.

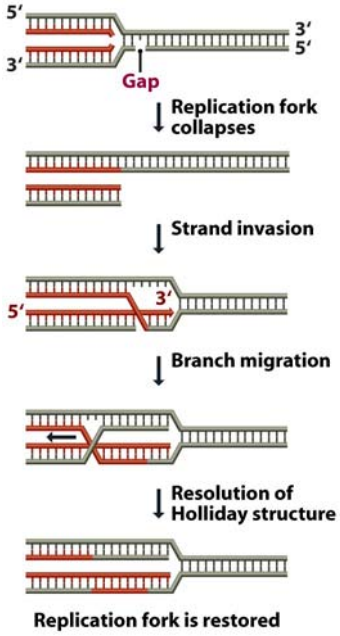


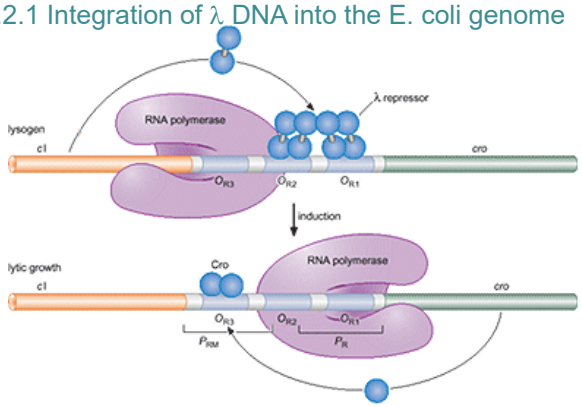
Figure 17.11 *Genomes 3* (© Garland Science 2007)

3) Recovery of a collapsed replication

- A gap in a parent strand causes replication collapse.
- In this case, the broken end of a daughter strand invades parent strands and forms D-loop.
- The replication fork is restored by branch migration and resolution of Holliday structure.

17.2 Site-Specific Recombination

17.2.1 Integration of λ DNA into the E. coli genome

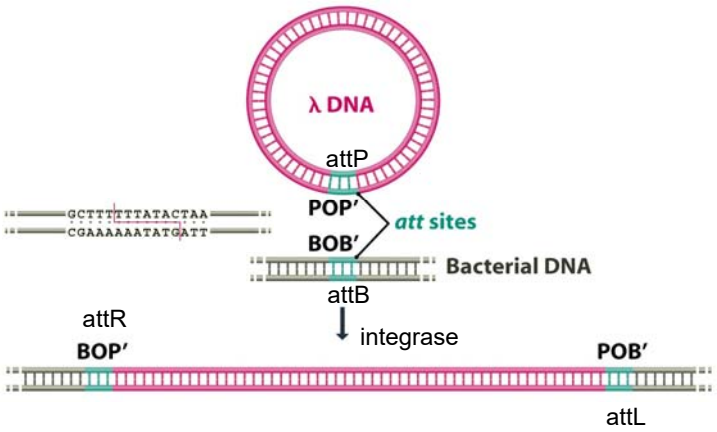


Lysogenic pathway
→ prophage

Lytic pathway

- After injecting its DNA into an E.coil cell, bacteriophage λ can follow either of two infection pathways.
- In the lytic pathway, new phages are rapidly generated in host E.coli and released within about 45 mins after infection.
- In the lysogenic pathway, lambda genome is integrated into bacterial genome and stays resident within the host's genome without apparent harm to the host.

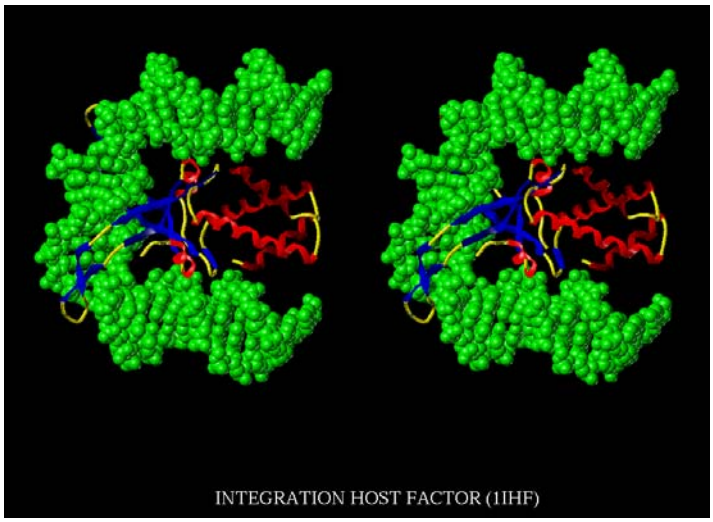
Figure 17.12 *Genomes 3* (© Garland Science 2007)



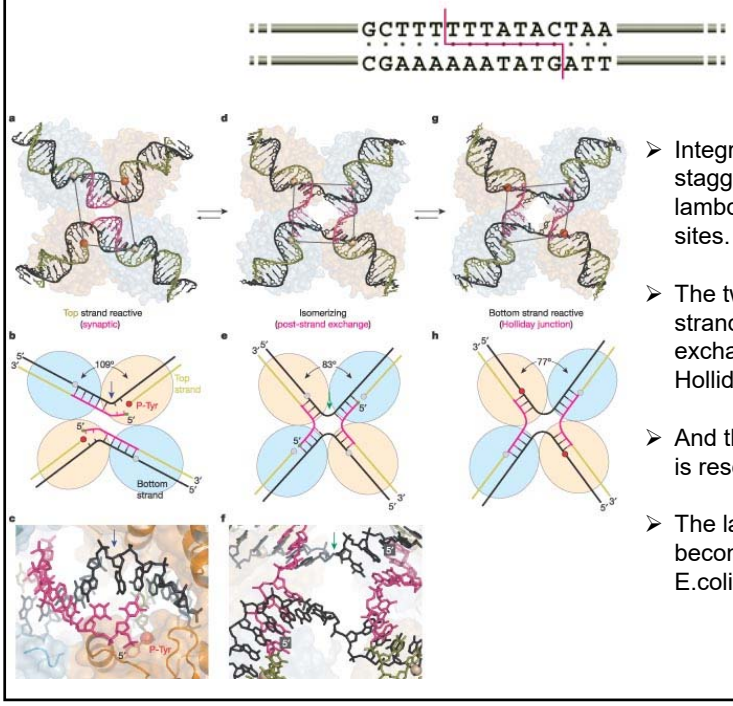
BOB': 23 bp
POP': > 250 bp

- The lambda genome integrates into the E.coli chromosome by site-specific recombination between attP on the phage genome and attB on the bacterial genome.
- The staggered cut is generated in each att site during integration and excision of the lambda phage genome.
- The recombination event is mediated by a type I topoisomerase, called integrase.

Figure 17.13 *Genomes 3* (© Garland Science 2007)



- Integrase and a second protein integration host factor (IHF) coat the phage attachment site.

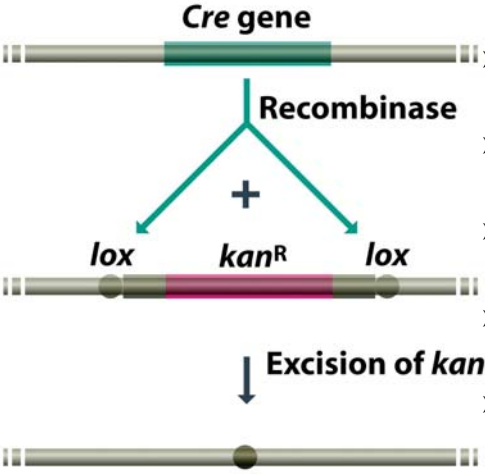


- Integrase makes a staggered cut at both lambda and E.coli att sites.
- The two short and single-strand overhangs are exchanged and produce a Holliday junction.
- And the Holliday junction is resolved.
- The lambda genome becomes inserted into the E.coli genome.

17.2.2 Site-specific recombination is an aid in genetic engineering

Bacteriophage P1

13bp	8bp	13bp
ATAACTTCGTATA -	GCATACAT	-TATACGAAGTTAT



- The Cre-lox system is a bacteriophage P1 site-specific recombination system.
- Cre recombinase recognizes 34 bp target sequence loxB and loxP.
- This system is used for the generation of genetically modified crops.
- To select genetically modified crops, we need a selection marker.
- There is a debate about the possible harmful effects of the selection markers.

Figure 17.14 *Genomes 3* (© Garland Science 2007)

Cre-loxP targeting

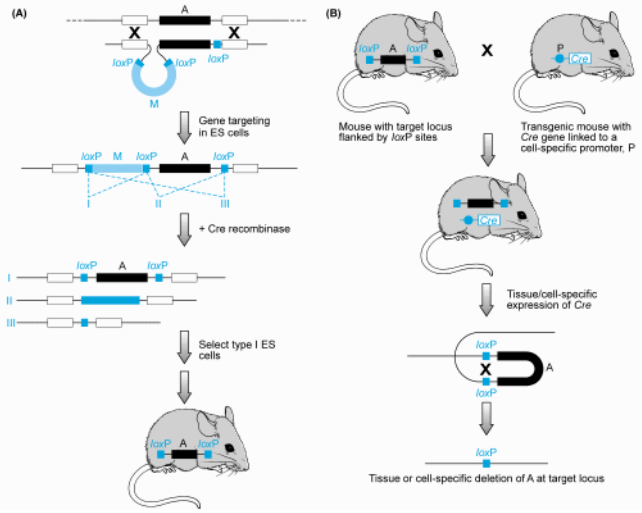
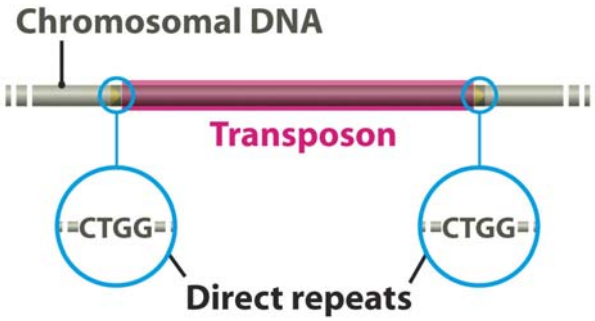


Fig. 21.8

17.3 Transposition



- Transposition is not a type of recombination.
- It often utilizes recombination.
- The end result is the transfer of a segment of DNA from one position in the genome to another position.
- A characteristic feature of transposition is a pair of short direct repeats flanking the transferred segment.
- Barbara McClintock discovered mobile genetic elements in maize (1983 Novel prize)

Figure 17.15 *Genomes 3* (© Garland Science 2007)

- Abundant in the genomes of bacteria, plants, and animals.

1) Mammals: nearly half the genome
2) Some higher plants: ~90%

- Transposition (movement) may disrupt genetic function and result in phenotypic variation.
- In vertebrates and higher plants, only a low percentage of spontaneous mutations are caused by transposable elements.
- The primary function of eukaryotic DNA methylation may be defense of the genome from transposition of transposable elements.

Table 12.3 Classes of transposable elements.

Class	Transposition intermediate	Examples
Class I		
LTR retrotransposons	RNA	Yeast:
		Ty elements
		Human:
		Human endogenous retroviruses (HERV)
		Mouse:
		Intracisternal A particles (IAPs)
Non-LTR retrotransposons	RNA	Human:
LINES (autonomous)		*L1* elements
SINES (nonautonomous)		*Alu* elements
Class II		
DNA transposons	DNA	Bacteria:
		Insertion sequence
		Bacteriophage Mu
		Transposons (e.g., Tn7)
		Drosophila:
		P elements
		Maize:
		Ac and *Ds* elements
		Invertebrates and vertebrates:
		Tc1/mariner superfamily

Table 12.3

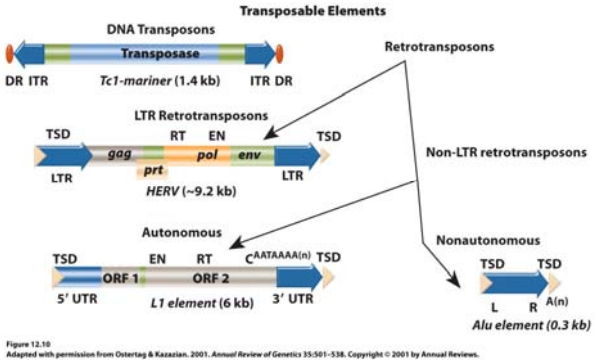


Figure 12.10

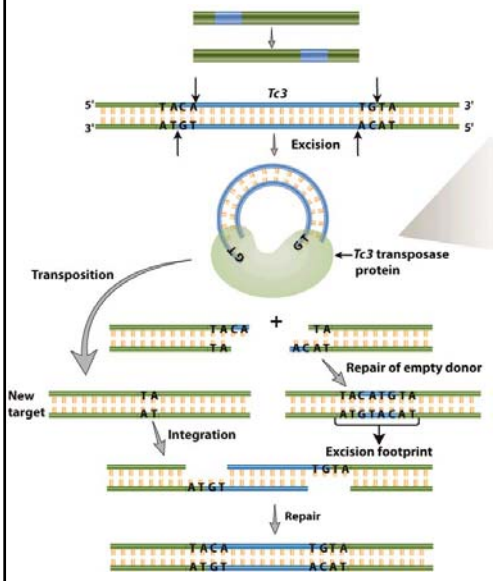
- Two main classes of transposable elements

1) DNA transposons: DNA intermediate during transposition.
- Inverted terminal repeats (ITRs), transposase, short direct repeats (DRs)

2) Retrotransposons: RNA intermediate during transposition.

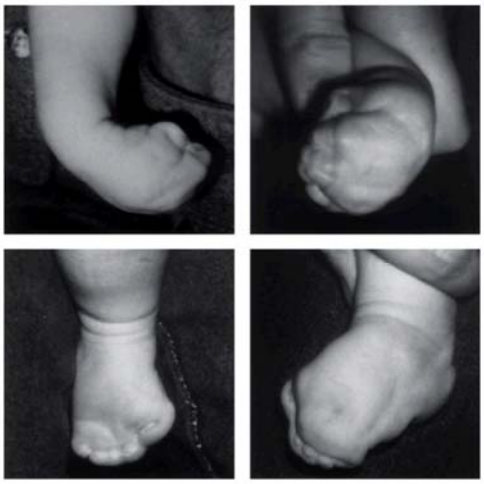
a. LTR retrotransposons: long terminal repeats (LTRs), RT and endonuclease domains (EN), group-specific antigen (gag), protease (prt), polymerase (pol), envelope (env).
b. Autonomous: 5'UTR with promoter, RT, EN, ORF1, ORF2, 3' UTR.
c. Nonautonomous: L and R sequences, poly (A).

17.3.1 DNA transposons move by a “cut-and paste” mechanism



- DNA transposons consist of a transposase gene flanked by inverted terminal repeats that bind transposase and mediate transposition.
- The transposase enzyme has a catalytic domain and a DNA-binding domain.
- The transposase catalyzes hydrolysis between the transposon and franking DNA.
- The free 3’-ends attack at the new target DNA.
- The empty donor is filled in by DNA polymerase and rejoined.

- Transposable elements provide material for DNA mispairing and unequal crossing-over.
- They are potential causal agents of human disease through insertion mutagenesis.



Disease Box 12.4 Figure 1e
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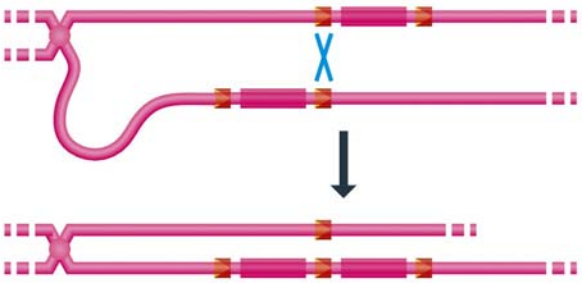
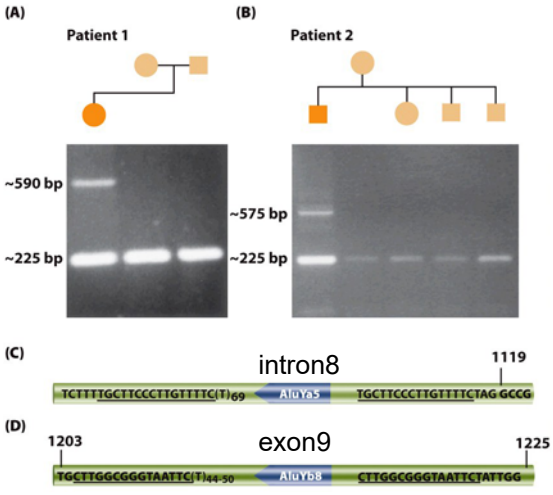
- Apert syndrome

1) Craniofacial abnormalities and syndactyly (condition wherein two or more digits are fused together) of the hands and feet.
2) Usually results from a missense mutation in exon 7 of the fibroblast growth factor receptor II gene.
3) In two cases, insertion of an Alu element in or near exon 9 causes the disease.

Unequal sister chromatid exchange





17.3.2 Retrotransposons move by a “copy-and paste” mechanism

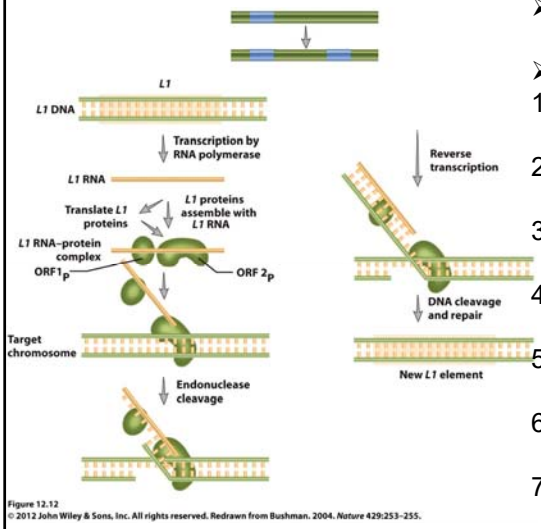


Figure 12.12

- Remnant of ancestral retroviral infections.
- L1 retrotransposon

1) L1 element is transcribed into L1 RNA.
2) L1 RNA is translated into ORF1p and ORF2p.
3) The L1 RNA-protein complex binds to target DNA.
4) Endonuclease makes a nick in the DNA.
5) The RT synthesizes a DNA copy of L1 RNA.
6) The RNA is degraded and ds DNA is produced.
7) The ds DNA is integrated into the target site by DNA cleavage and repair processes.