BI 8 LECTURE 13

REPAIR, ENGINEERING, SUBVERSION, AND EVOLUTION: MANY USES OF DNA RECOMBINATION

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Reading for this week: Alberts et al. Ch. 5

Unfinished business: a unique problem at the end of linear chromosomes

how do you prime replication of the last few bases of the lagging strand?

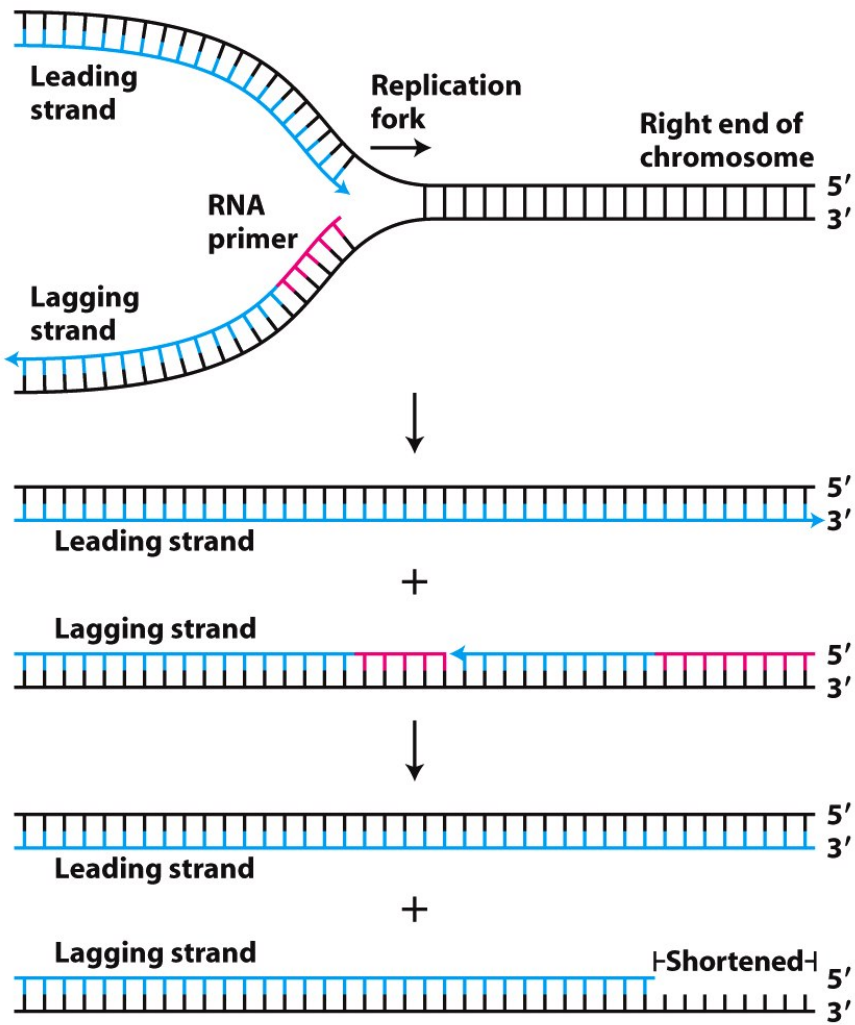


Figure 6-48
Molecular Cell Biology, Sixth Edition

A special simple-sequence repeat element at ends of chromosomes and a special ribonucleoprotein enzyme, TELOMERASE, make it possible to solve this problem

Ends of chromosomes consist of tandem repeats of simple sequences:

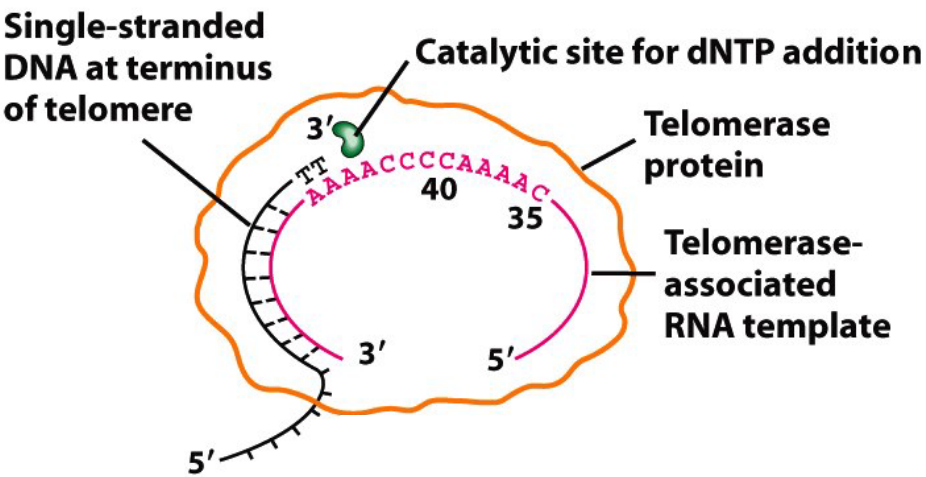
$(TTAGGG)_n$

(n=800-2500 in human germ cells, declining in differentiated cells with age)

$((TG)_{1-3}(TG)_{2-3})_n$

(n= 50-75 in brewer's yeast Saccharomyces cerevisiae)

Telomerase uses these repeats as templates to restore ends and may even extend the number of repeats at certain cell cycles



Telomerase is a "reverse transcriptase": a DNA polymerase that uses RNA template…
and in this case the RNA template is part of the telomerase enzyme

Using repeat sequences, telomerase makes RNA templates for elongation of terminal lagging-strand template beyond original end

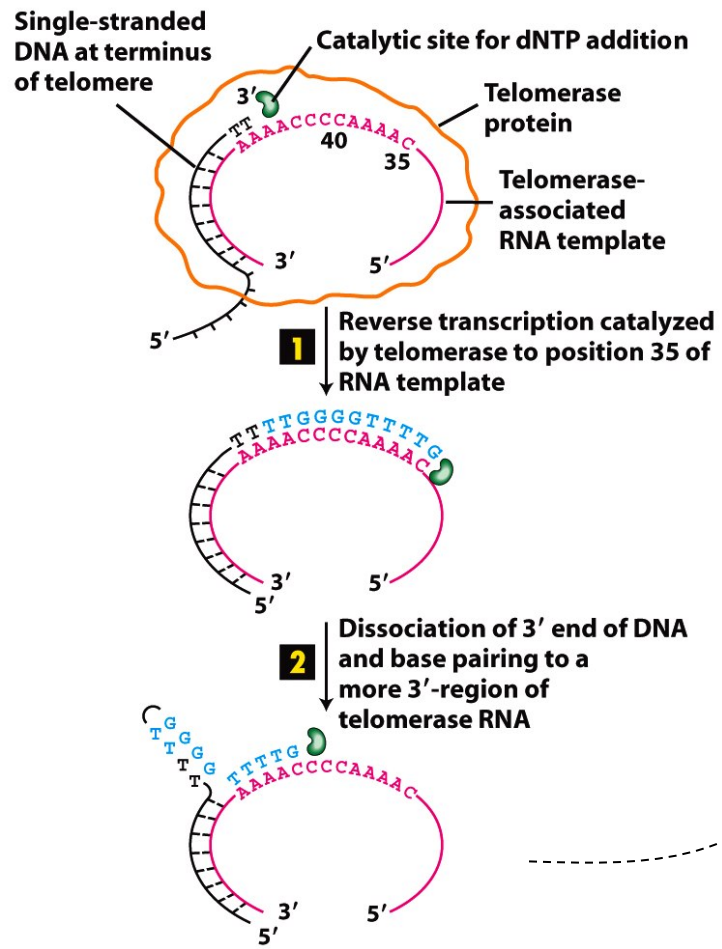


Figure 6-49 part 1
Molecular Cell Biology, Sixth Edition

Plenty of DNA now for primase, DNA pol to work on!

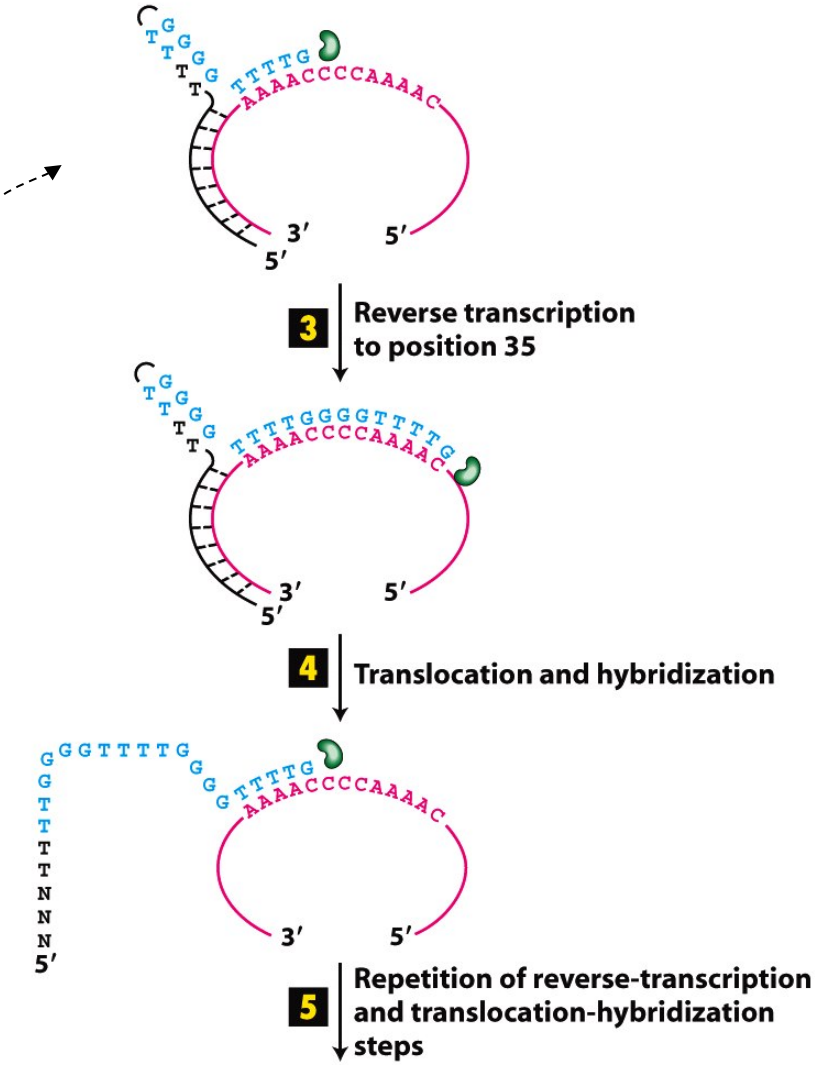
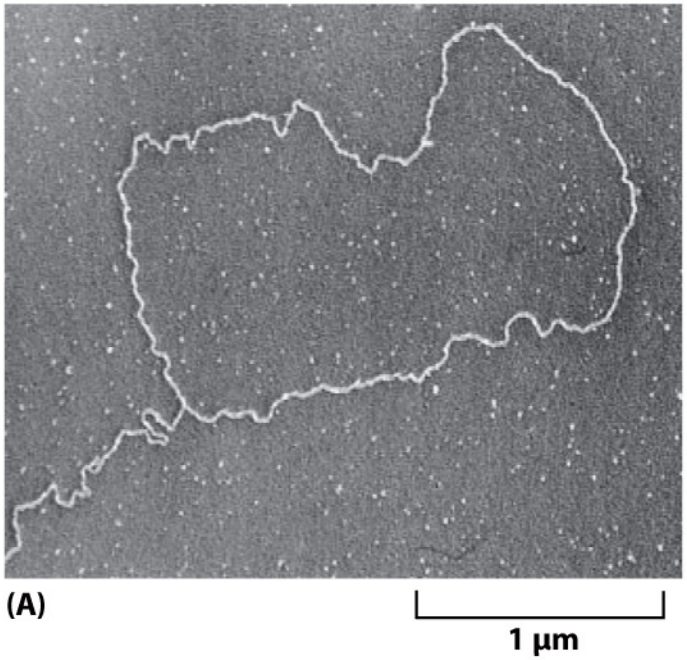


Figure 6-49 part 2
Molecular Cell Biology, Sixth Edition

After extending the telomeres, there's still a ragged end … but it is then "bundled" into a safe nuclease-resistant loop structure

(Telomerase-mediated end extension might have started way back here)



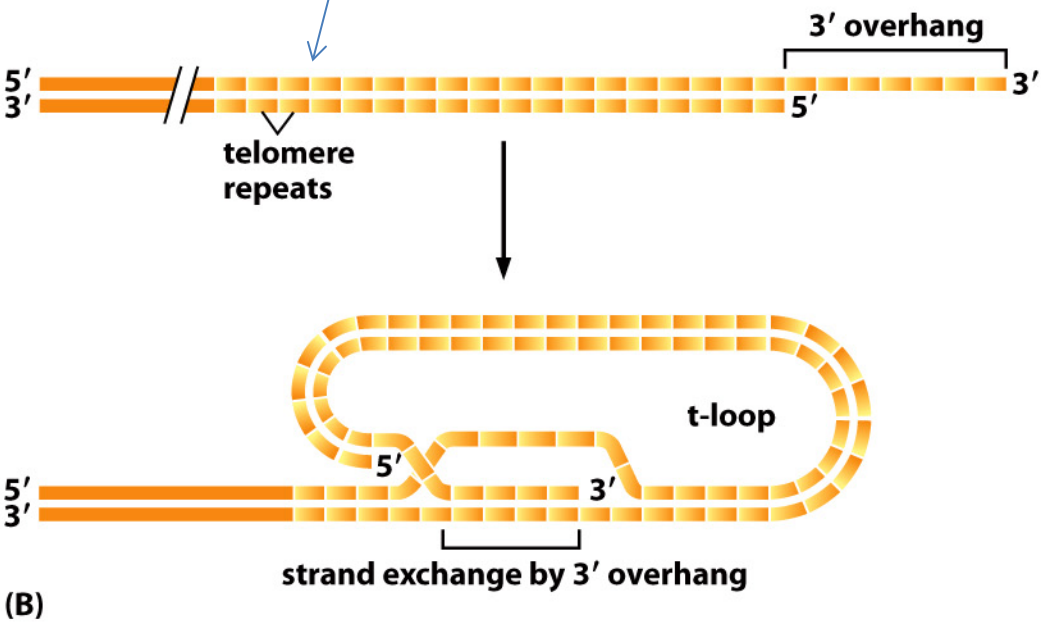


Figure 5-35 Molecular Biology of the Cell 6e (© Garland Science 2015)

Telomerase is expressed specifically by cells that need to self-renew without senescence

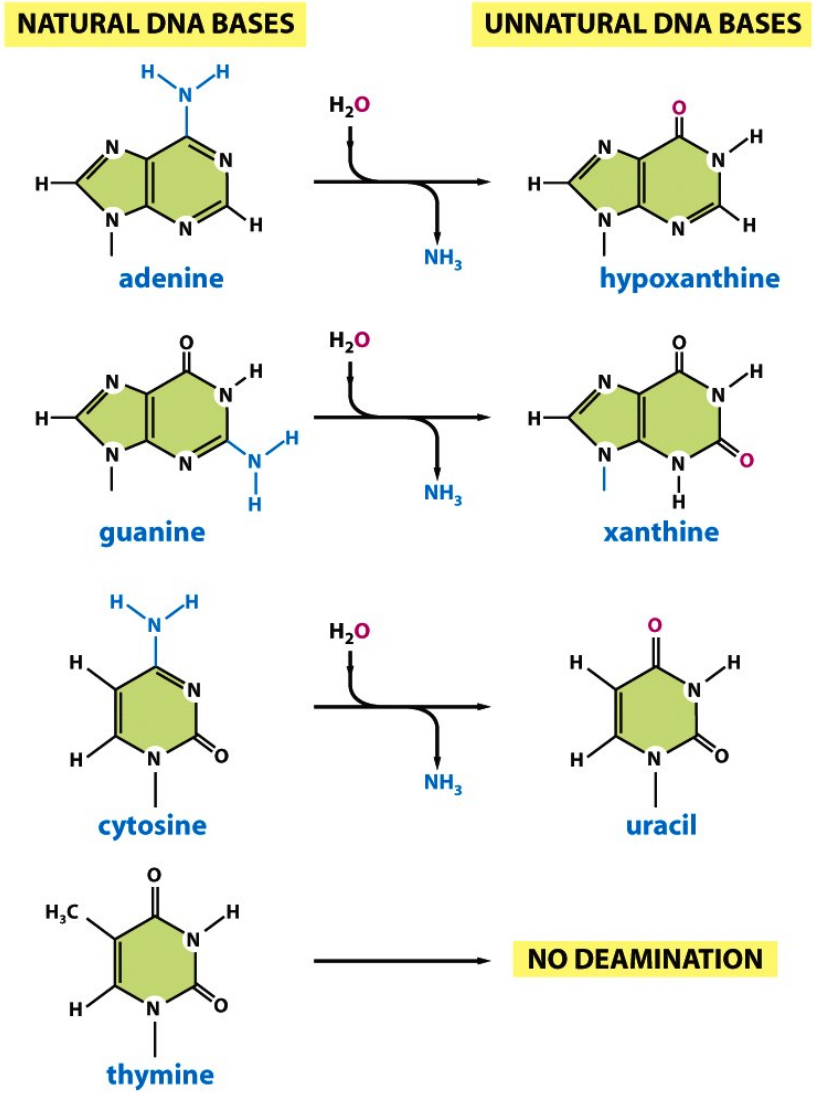
- Most cells in our bodies lose telomere repeats slowly with successive rounds of cell division: internal aging clocks
- Stem cells and germ cells are principal sites of telomerase activity in humans
- Some immune cells specialized for retaining “memory” over many years are induced to express telomerase during cell division
- Normally, lack of telomerase protects against cancer… reactivation of expression in tumor cells is a key event in oncogenic transformation

Reasons why DNA needs repair

- DNA breaks (e.g. due to radiation or chemicals)
- Mistakes during replication that escape proofreading
- Single-base damage or pyrimidine dimer formation (UV radiation or chemical damage)

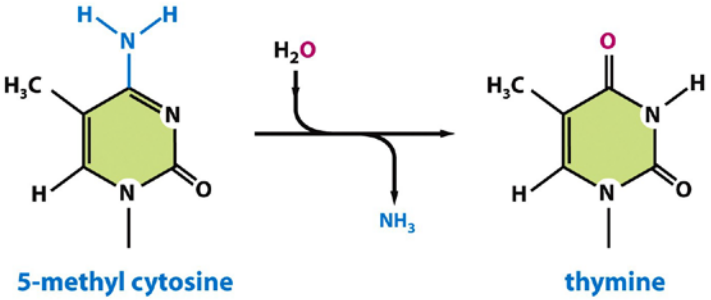
All can result in mutations if not corrected

The problem of damage: even correct bases in DNA can become dysfunctional due to chemical damage



Often by hydrolytic deamination

Note: when methylated C is deaminated, there is nothing to show that the base formed is “unnatural”!



High frequency mutation!

Figure 5-50ab *Molecular Biology of the Cell* (© Garland Science 2008)

Specialized repair systems excise defective base or region: then DNA is filled in by polymerase, sealed by ligase

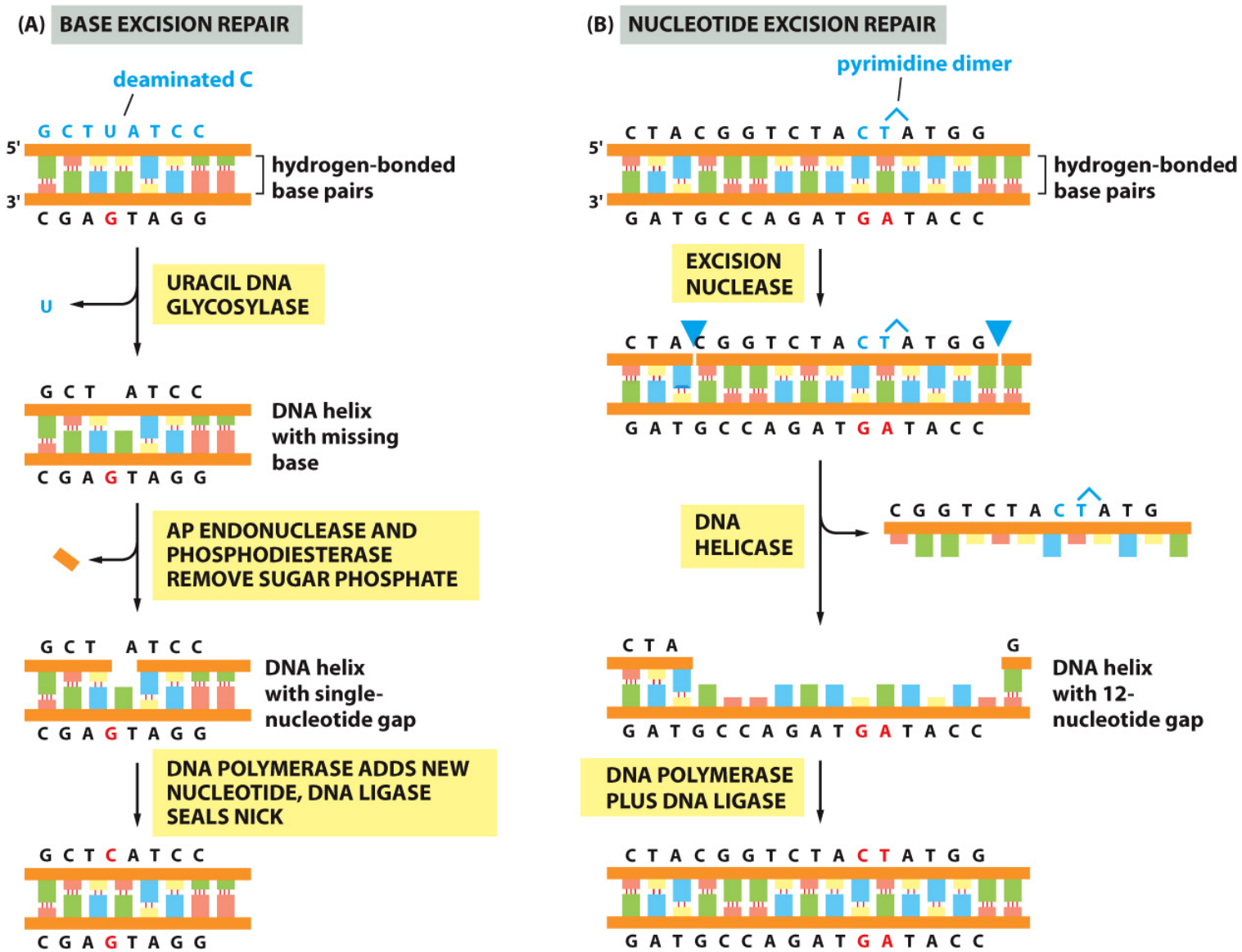


Figure 5-41 Molecular Biology of the Cell 6e (© Garland Science 2015)

The secret sculptor of genomes: recombination

- Crucial for large-scale lesion repair in DNA
- Creates much evolutionary change in genome size and gene order
- Starts with a DNA break, and “search” of a broken denatured DNA strand for something to anneal to as template for extension/repair
- Different types of possibilities based on how long a hybrid the “searcher” needs to find before being able to be ligated

Two major forms of recombination: different problems to solve, different enzymatic solutions

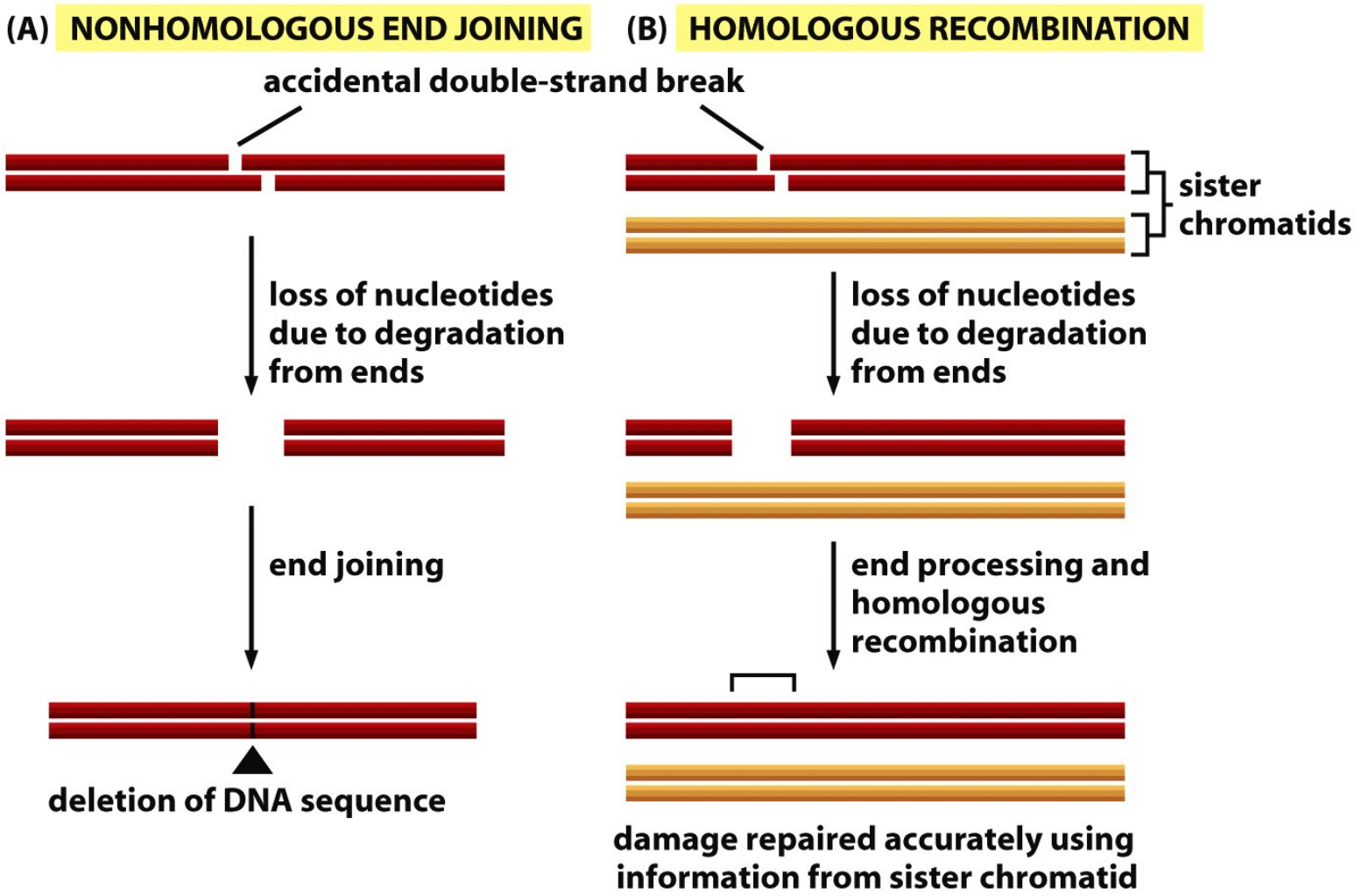


Figure 5-51 *Molecular Biology of the Cell* (© Garland Science 2008)

Key features of homologous recombination

- Requires that broken DNA can find another highly similar, undamaged copy to use as template for repair
- Substantial length of ***sequence identity*** provides the site for the recombination
- Broken DNA is first processed to generate protruding 3’ single strand at break: “invader” and primer for repair synthesis
- Conservative mode of recombination: same homologous sequence that was used to target recombination ends up copied faithfully in ***both*** product DNA molecules

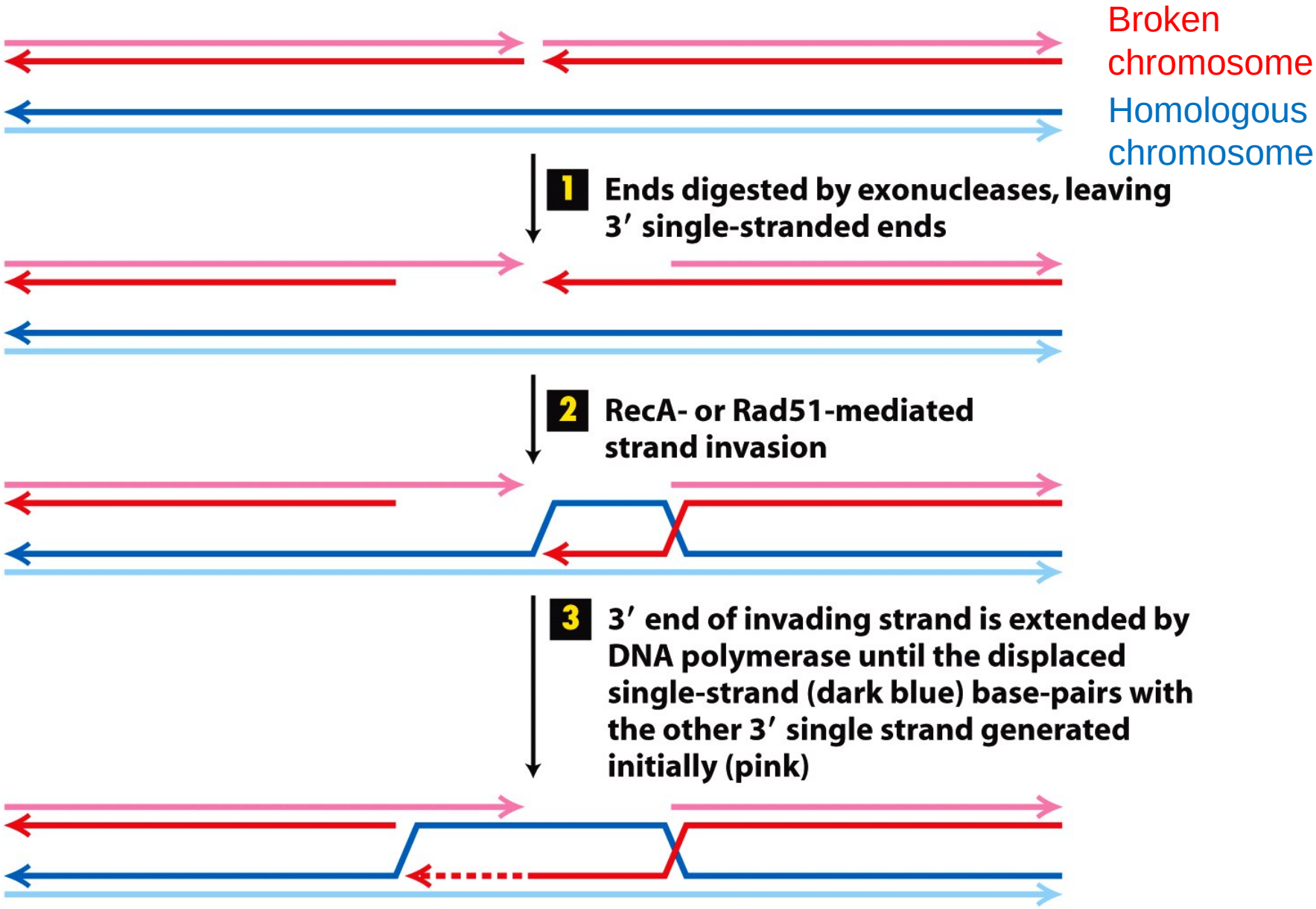


Figure 4-42 part 1
Molecular Cell Biology, Sixth Edition

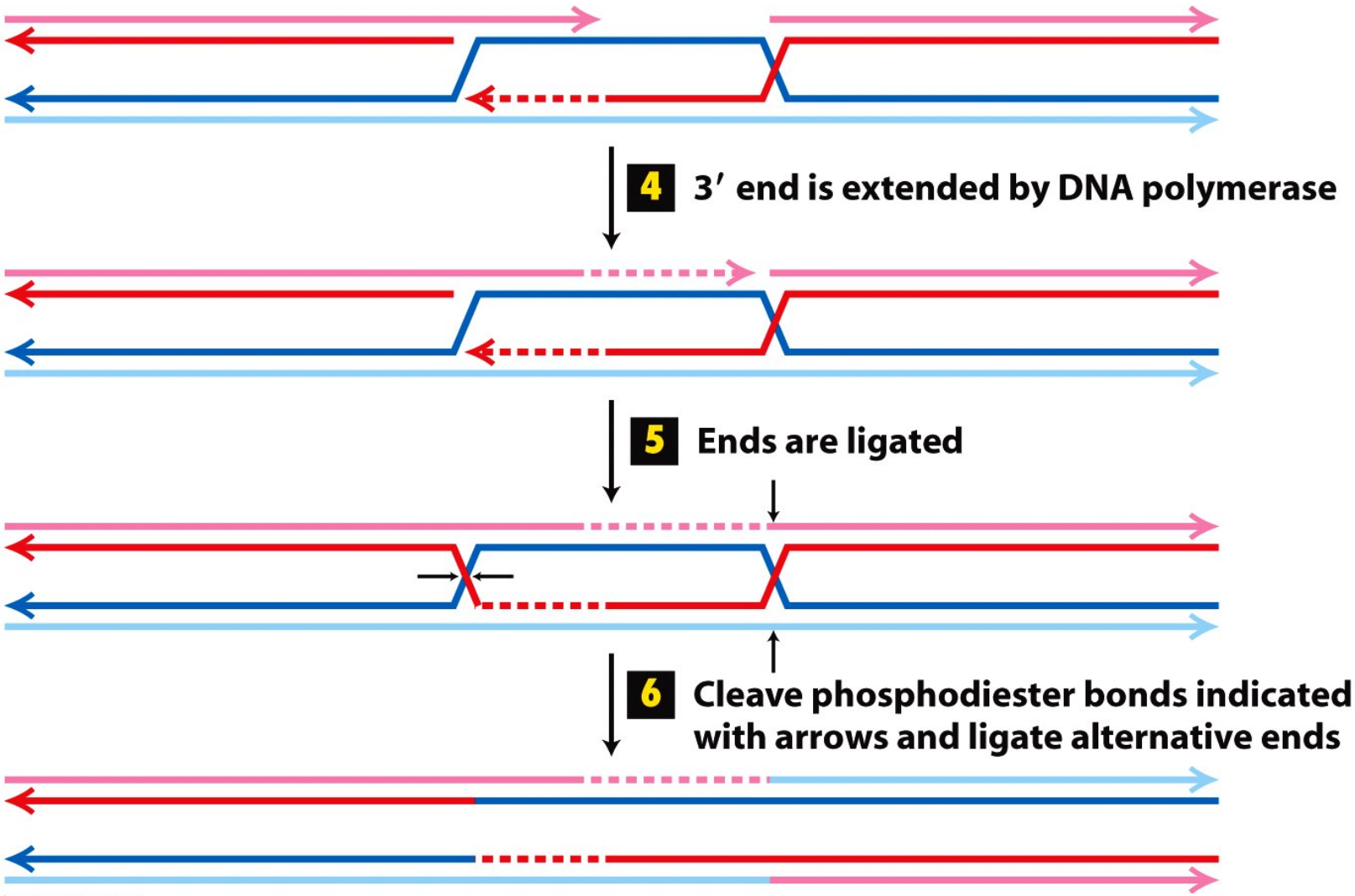


Figure 4-42 part 2
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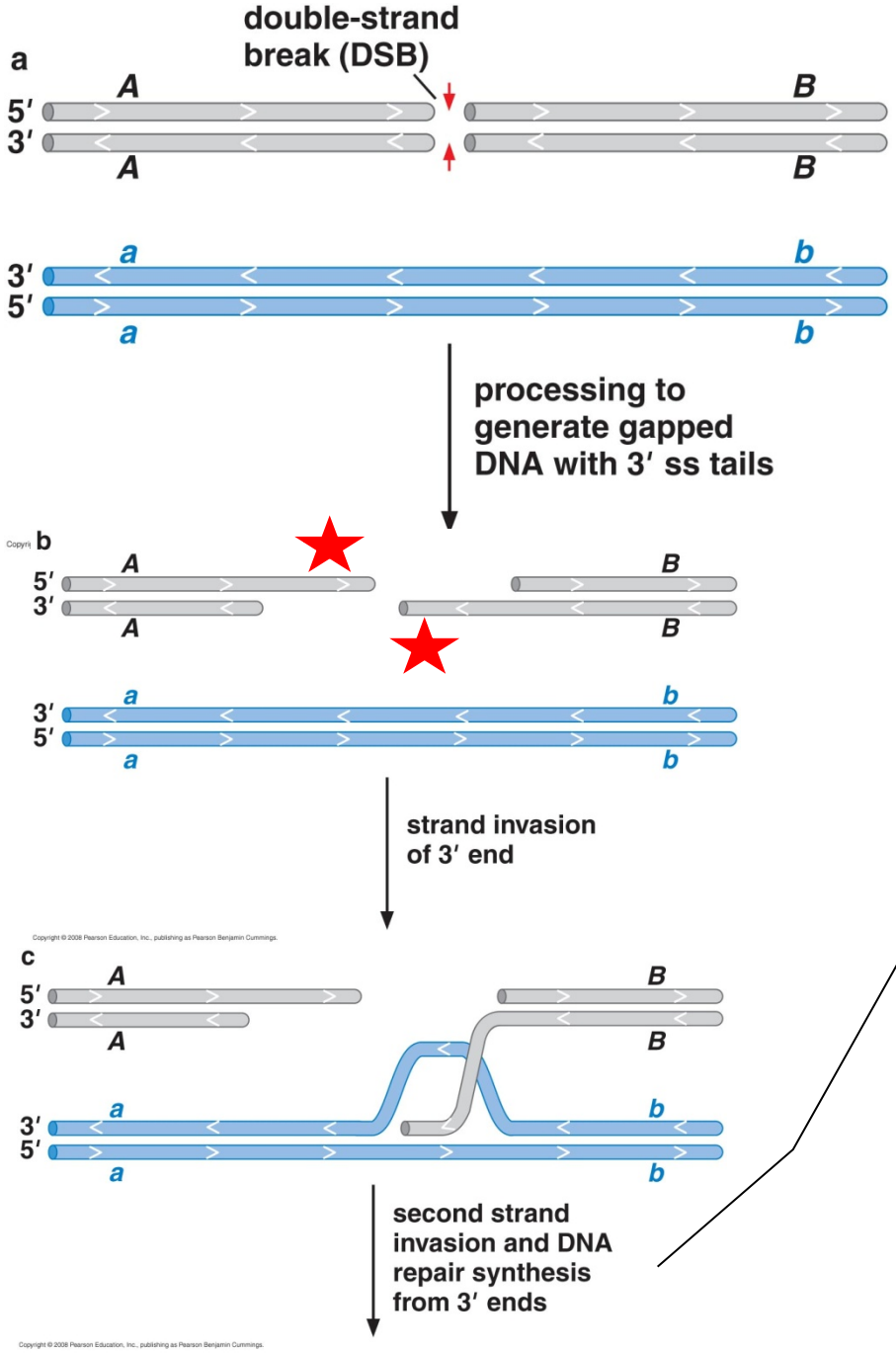
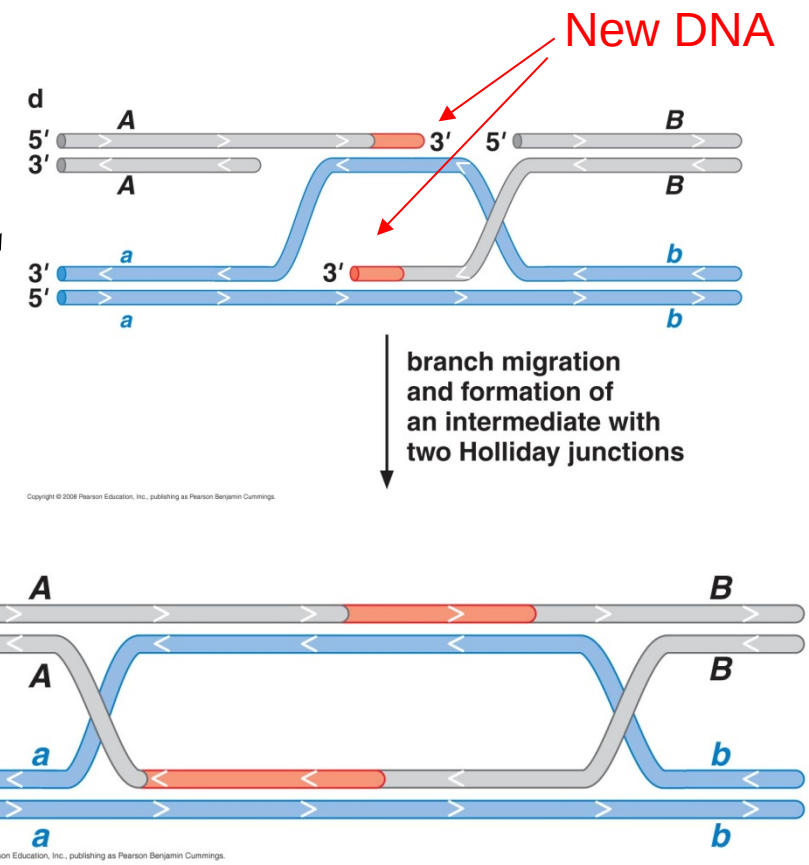
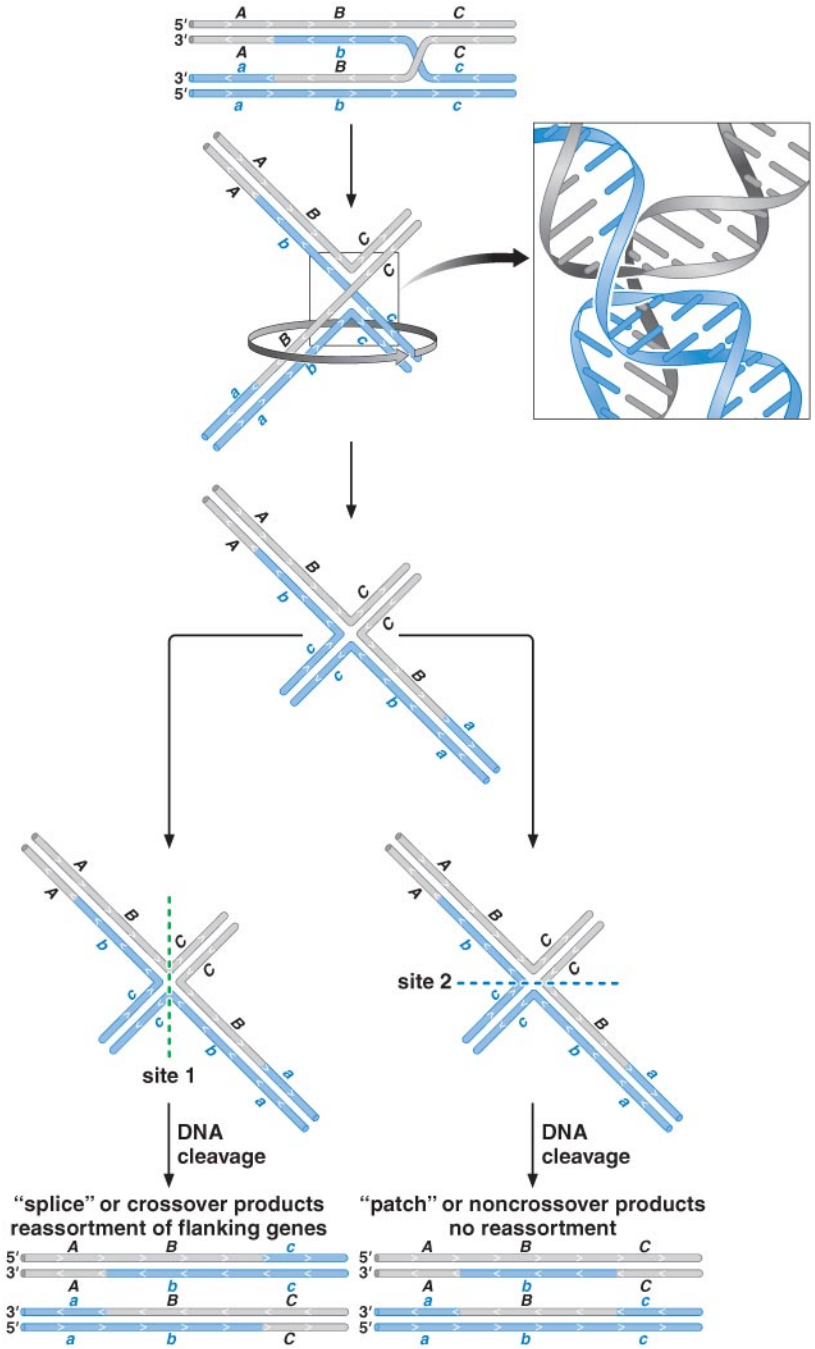
A broken chromosome can be repaired by homologous recombination with related sequences on another chromosome





No privileged strands in Holliday junction: can resolve by cleaving "outer" pair as well as "inner" (strand invaded) pair



Homologous recombination is especially valuable to rescue replication from a nick

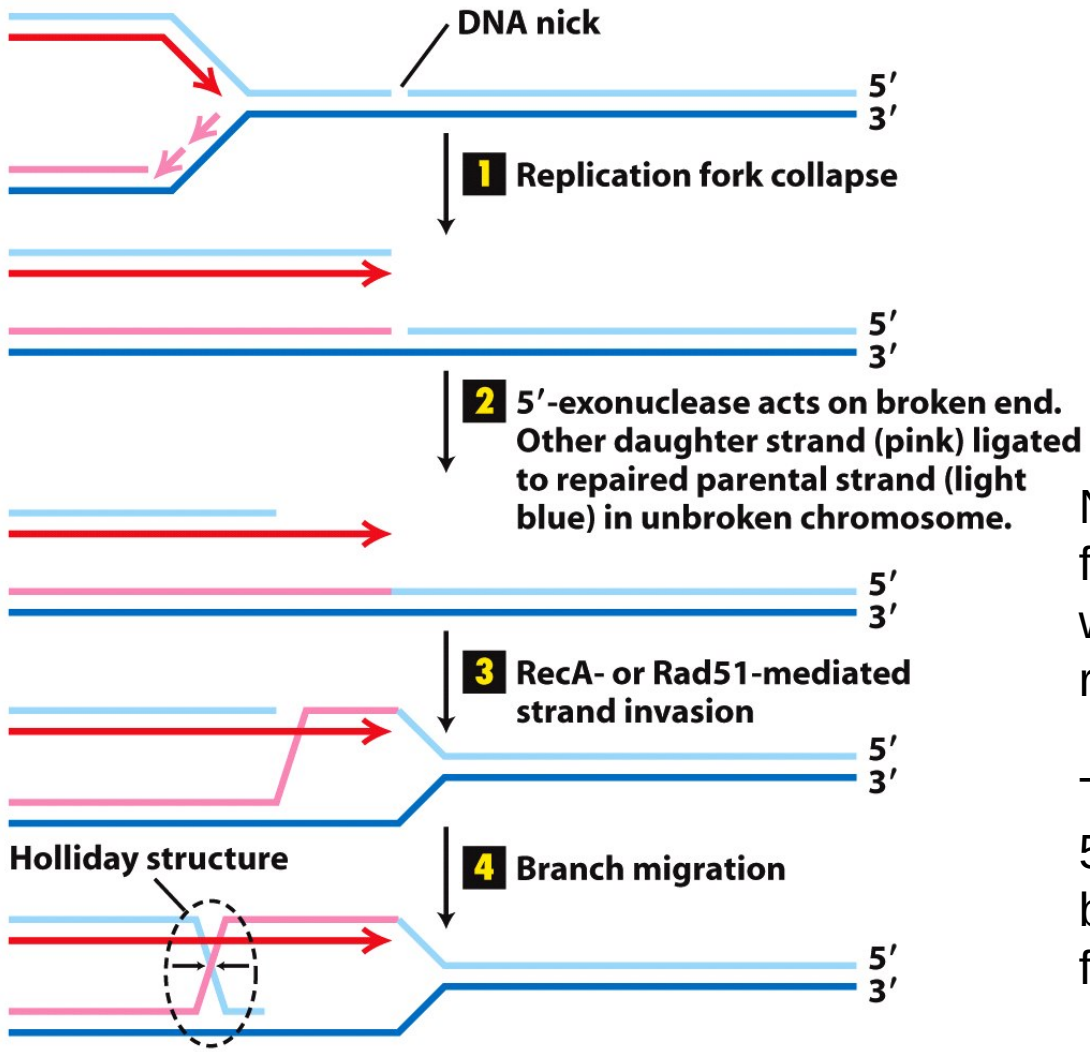


Figure 4-41 part 1
Molecular Cell Biology, Sixth Edition

Need to generate a free ss 3'OH end… which can now invade repaired part of duplex

This gives "orphaned" 5' end behind the break a new chance for a partner

Crossed-over complex now has primers and templates enough for completing all strands: cut, extend, and ligate

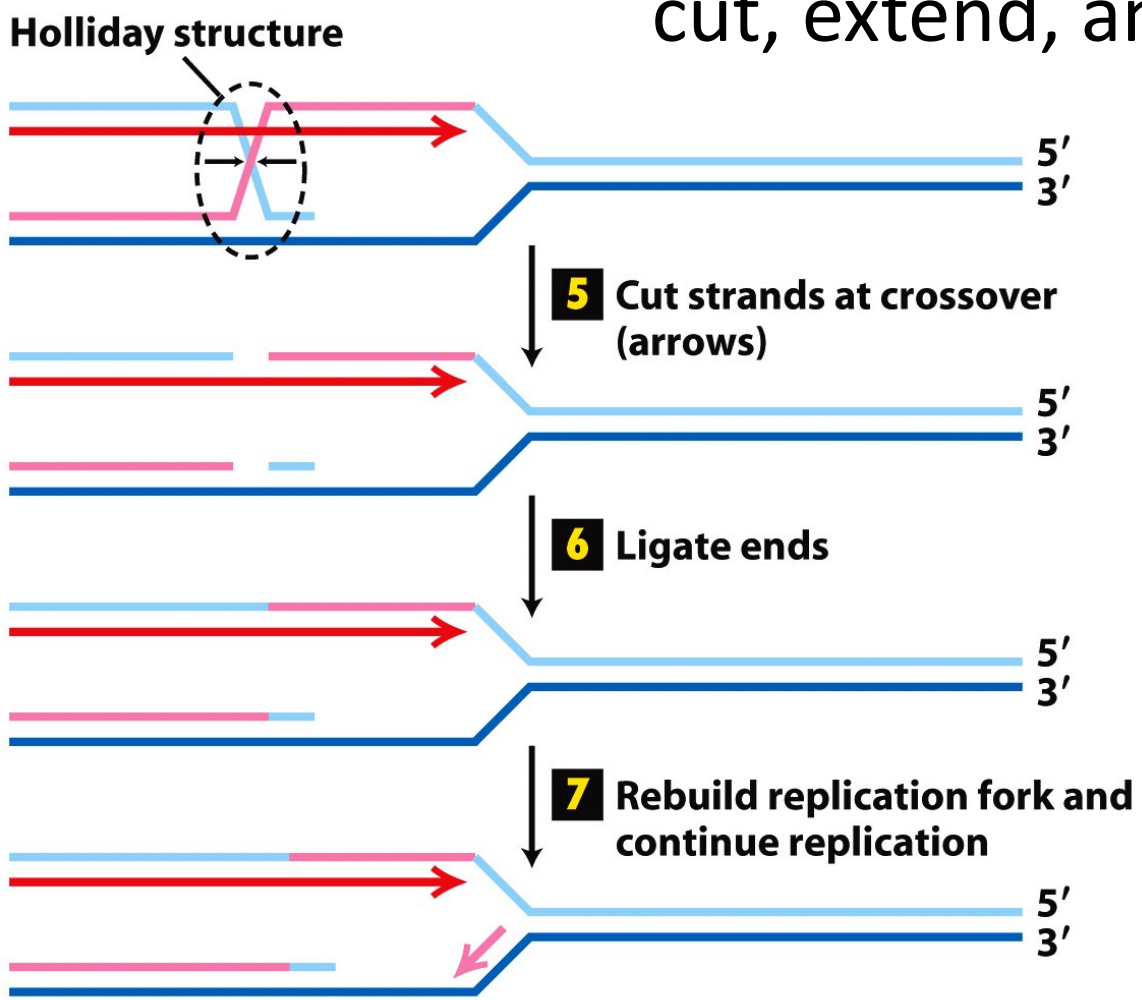


Figure 4-41 part 2
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Recombination between homologous chromosomes, initiated by a DNA ds break, is programmed into meiosis

(to form germ cells)

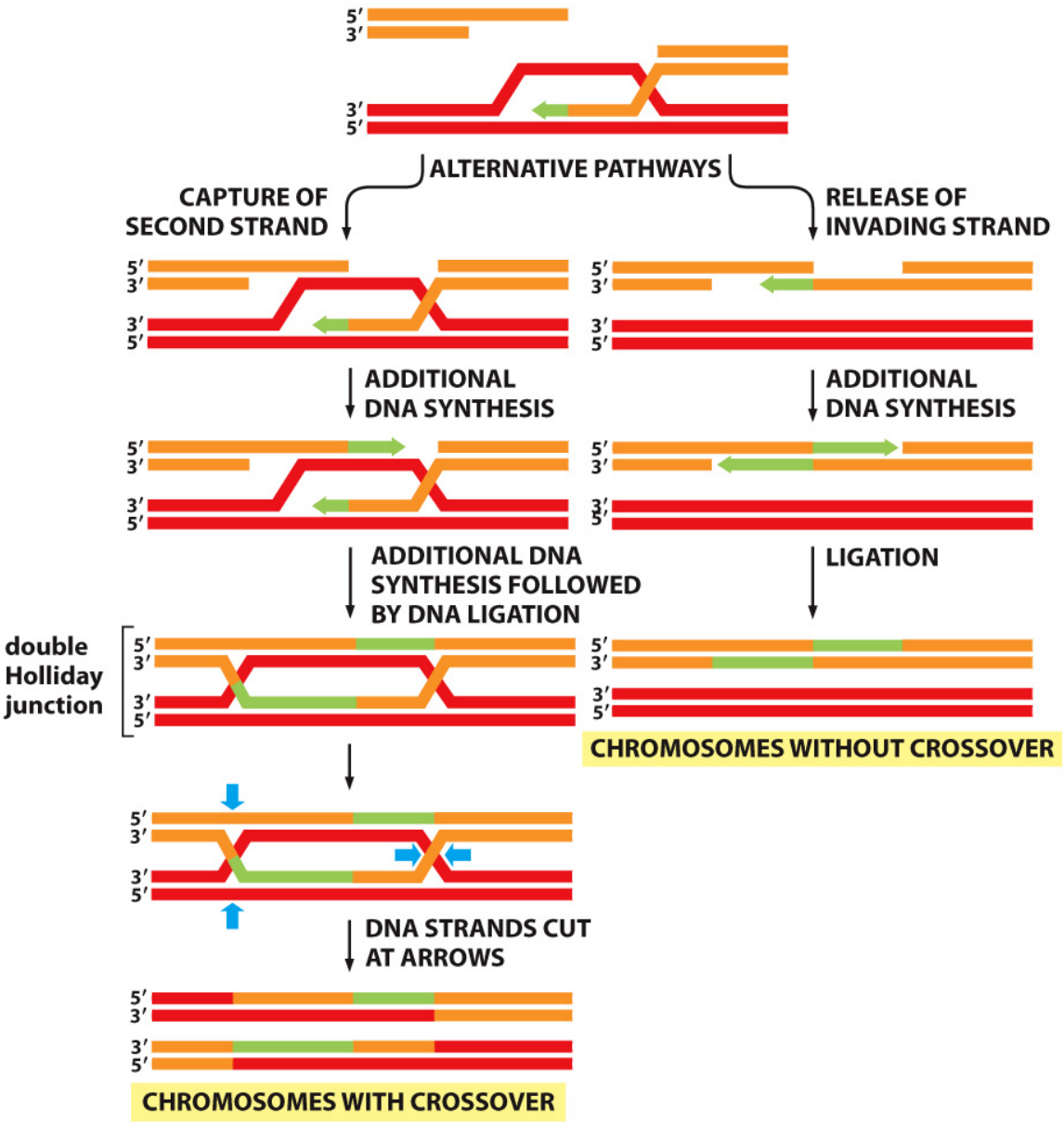

Figure 5-54 (part 2 of 2) Molecular Biology of the Cell 6e (© Garland Science 2015)

Nonhomologous end joining: when the homologous DNA is not easy to find

often imprecise, but a lot better than a broken chromosome

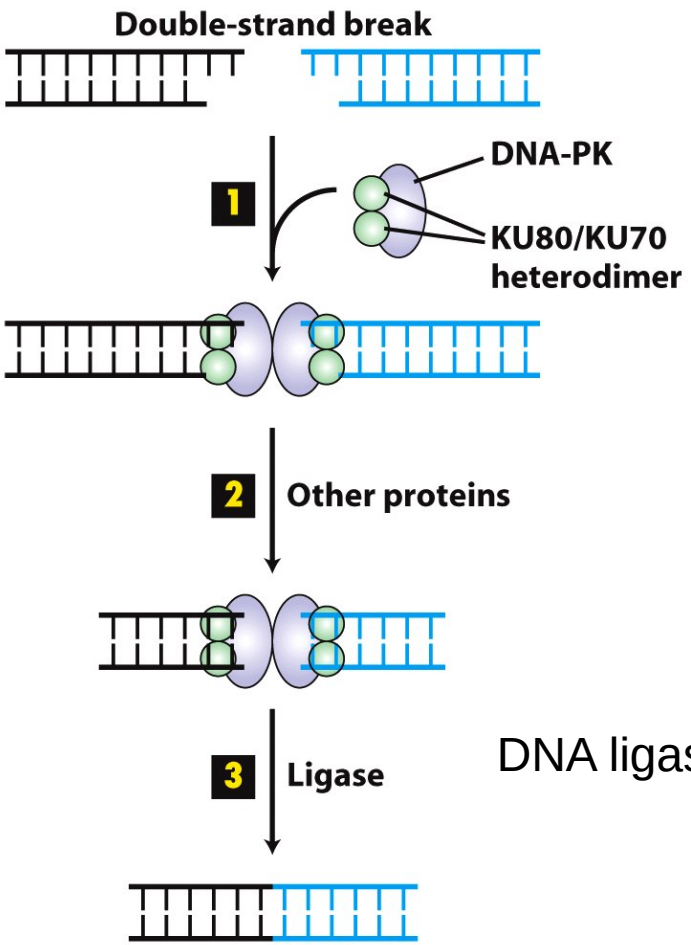

Figure 4-40
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Key: protect broken ends so that they cannot do harm!

But missing bases are not added back

DNA ligase IV, Xrcc4, Artemis, etc

Recombination as a way of life: meiosis and transposable elements

- Formation of gametes – sperm and eggs – involves a step of cell division where recombination between maternal and paternal alleles is specifically enhanced
  - Resorting of mother's/father's alleles among resulting chromosomes offers more chances for new combinations
  - Preserves completely normal chromosome gene order, DNA length for both resulting types of chromosome

- "Selfish DNA" – transposable elements – recombine to exist
  - Passengers in host chromosomal DNA
  - Addition or subtraction of sequence to host chromosomes
  - Often imprecise in insertion, excision → genetic instability
  - Many copies inserted can damage chromosomes (increased recombination among homologous patches on nonhomologous chromosomes)

Typical DNA insertion element in its integrated location

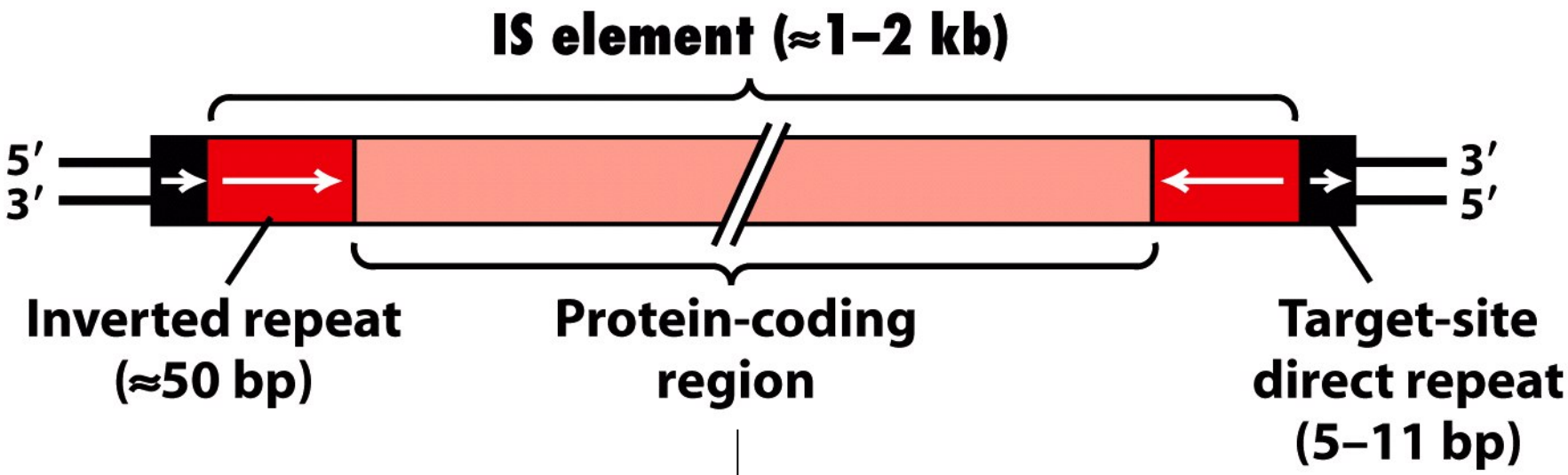


Figure 6-9
Molecular Cell Biology, Sixth Edition

Encodes specific transposase enzyme that promotes recombination & integration:
recognizes inverted repeat sequences of insertion element and ***cuts*** IS element, target DNA

Staggering success of “selfishness”:

Our genomes are full of transposable elements that have greatly increased in copy number … just since mice and humans diverged

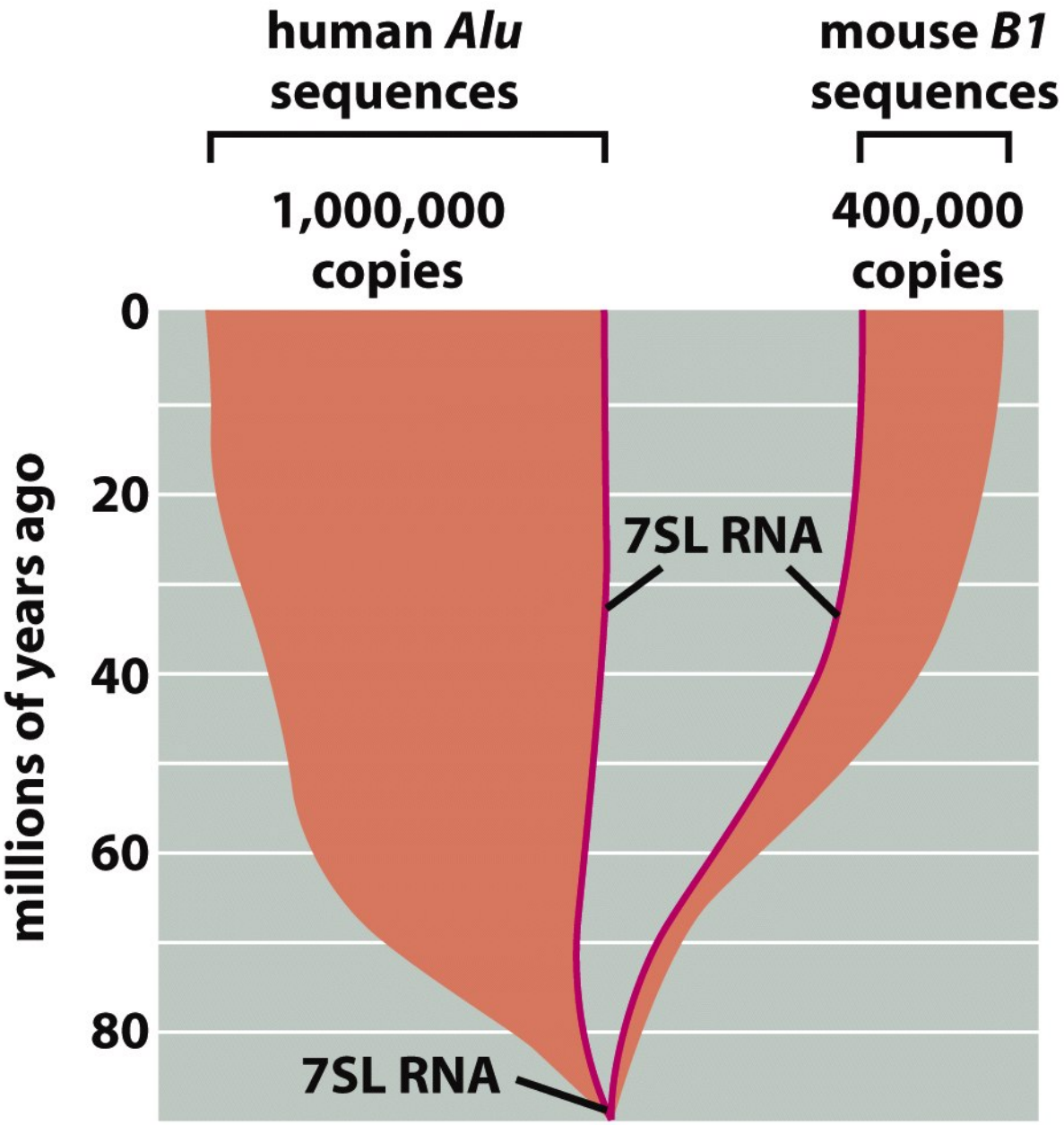


Figure 5-75 *Molecular Biology of the Cell* (© Garland Science 2008)

Randomly inserting transposable elements use blunt-end DNA ligation without homology for inserting into new DNA sites

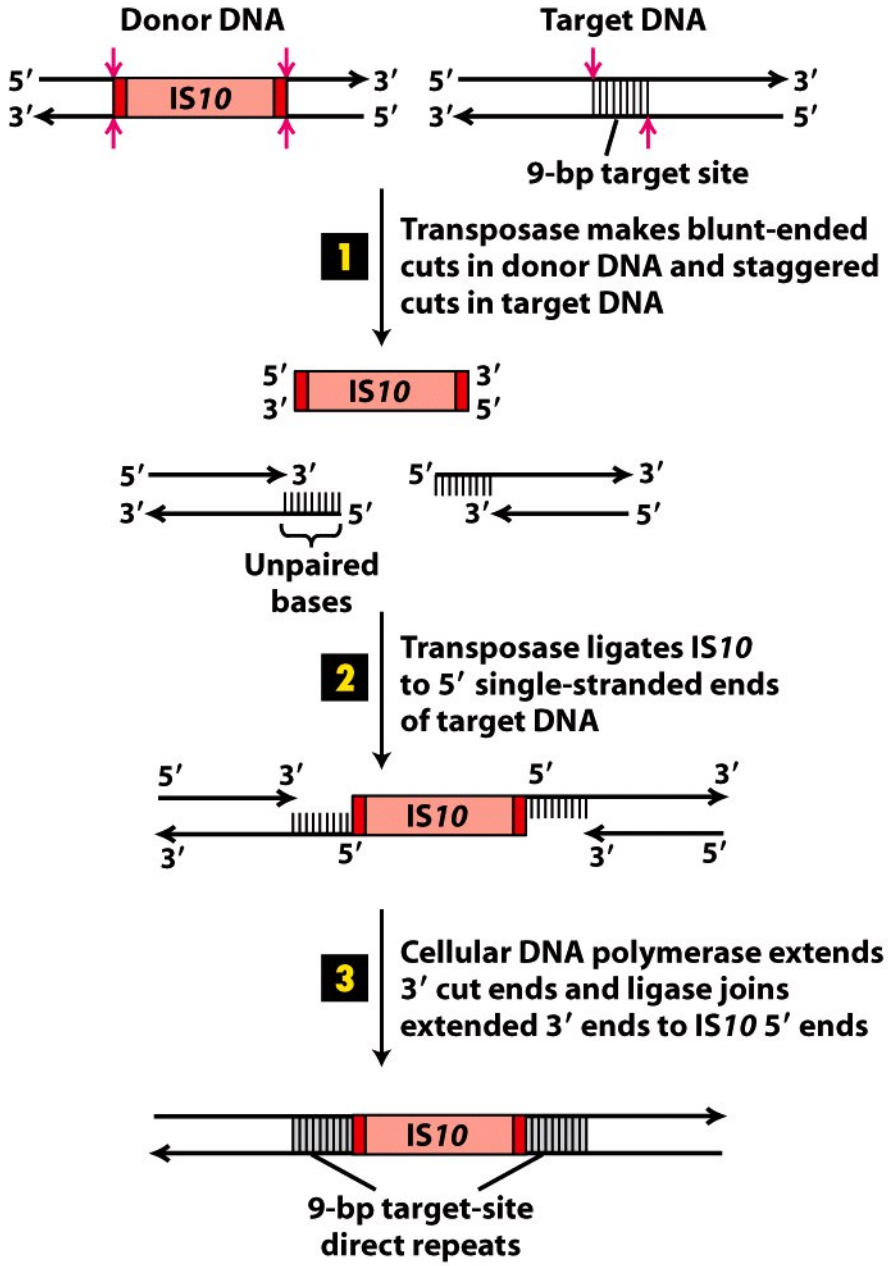
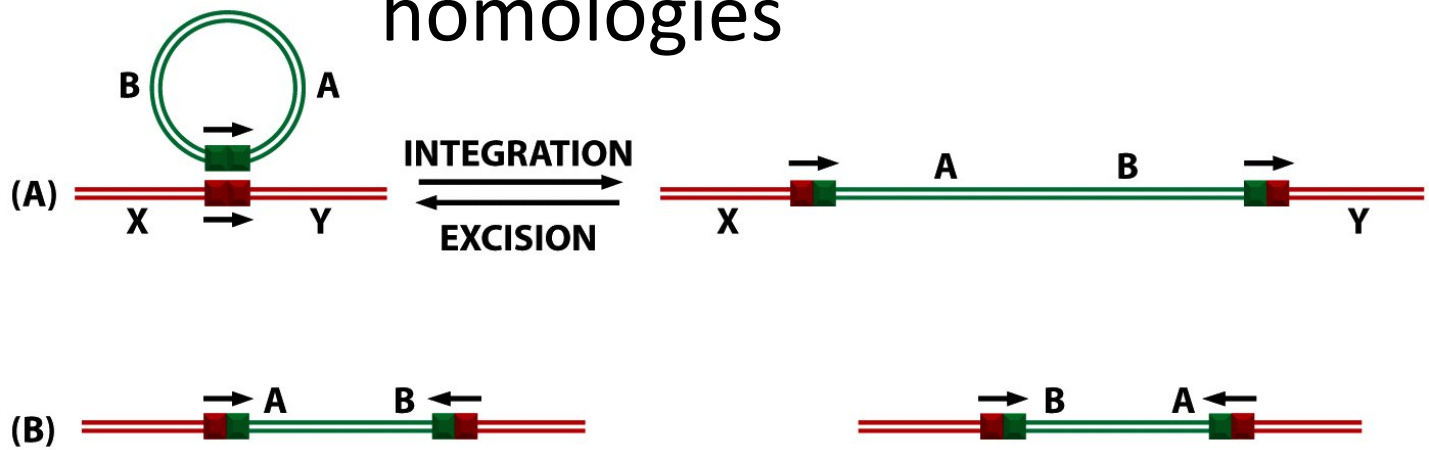


Figure 6-10
Molecular Cell Biology, Sixth Edition

Note: don't allow target DNA sites to have 3' overhangs so that the target DNA cannot "invade" the transposon's sequence…

Some very specific DNA transposons have site-specific modes of integration and excision: basically homologous recombination with short homologies



These are elements that code for ***highly sequence-specific transposases***

Well known bacterial systems: Cre/lox, Flp/Frt, and λ phage integration where specific host target sequences are recognized

Here, precise insertion and excision; may undergo reciprocal recombination to "invert"

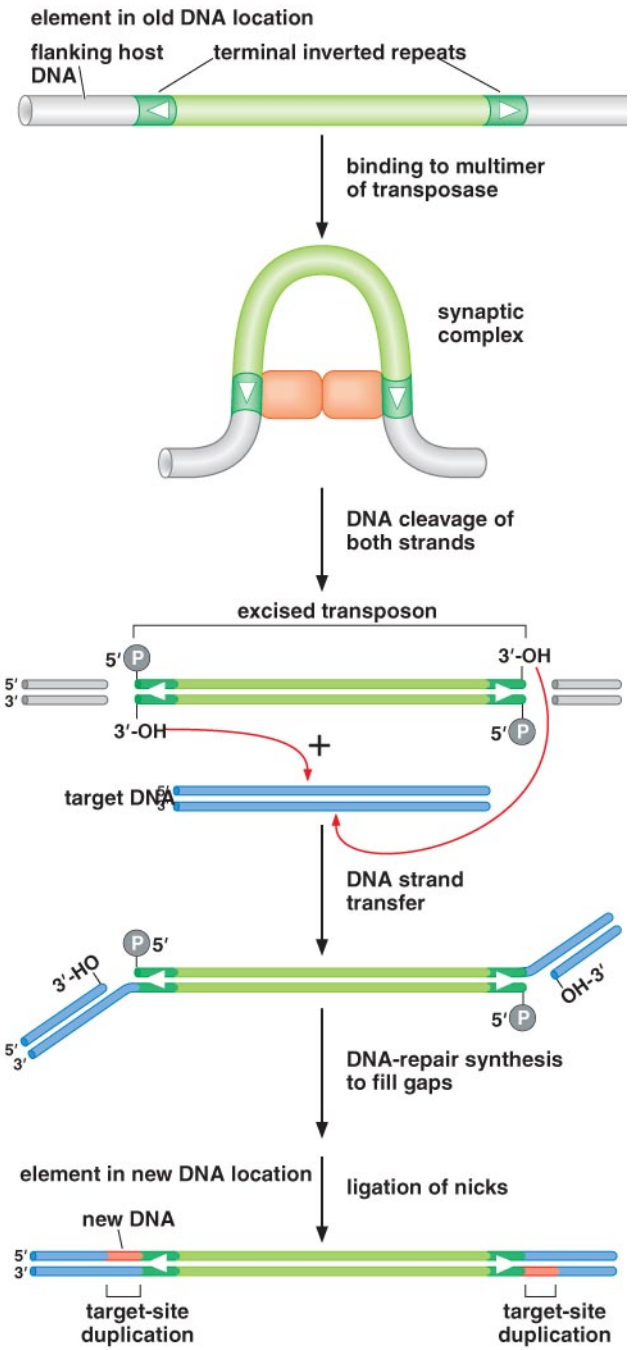
Figure 5-76 *Molecular Biology of the Cell* (© Garland Science 2008)

Site-directed recombination as a tool for genetic engineering

- Highly specific ***recombinases*** such as Cre, Flp, and λ integrase can be cloned into vectors under control of known promoter/enhancers
- Highly specific recombinational ***target recognition sites*** (e.g. LoxP, Frt, λatt's; 30 bp) are very rare in genomes normally
- Target recognition sites can be inserted in pairs around a gene of interest without disrupting function (e.g. "floxing" with Cre target LoxP sites)
- Can make double transgenic with floxed target gene plus specifically regulated Cre recombinase vector… then target gene works normally except in tissue or condition in which Cre expression is induced… and then target gene is ***deleted***
- Can set up any site in any gene as a ***specific target*** for Cre (or Flp, or λ integrase)-mediated ***insertion*** of any DNA of interest, if it is flanked with correct recombination recognition sites

“Cut and paste”: DNA transposons need to cut themselves out of old location to insert in new one

Only way to increase copy number is for already-replicated copy behind replication fork to hop into another site ahead of replication fork



A recombinosome complex keeps ends together during transposition

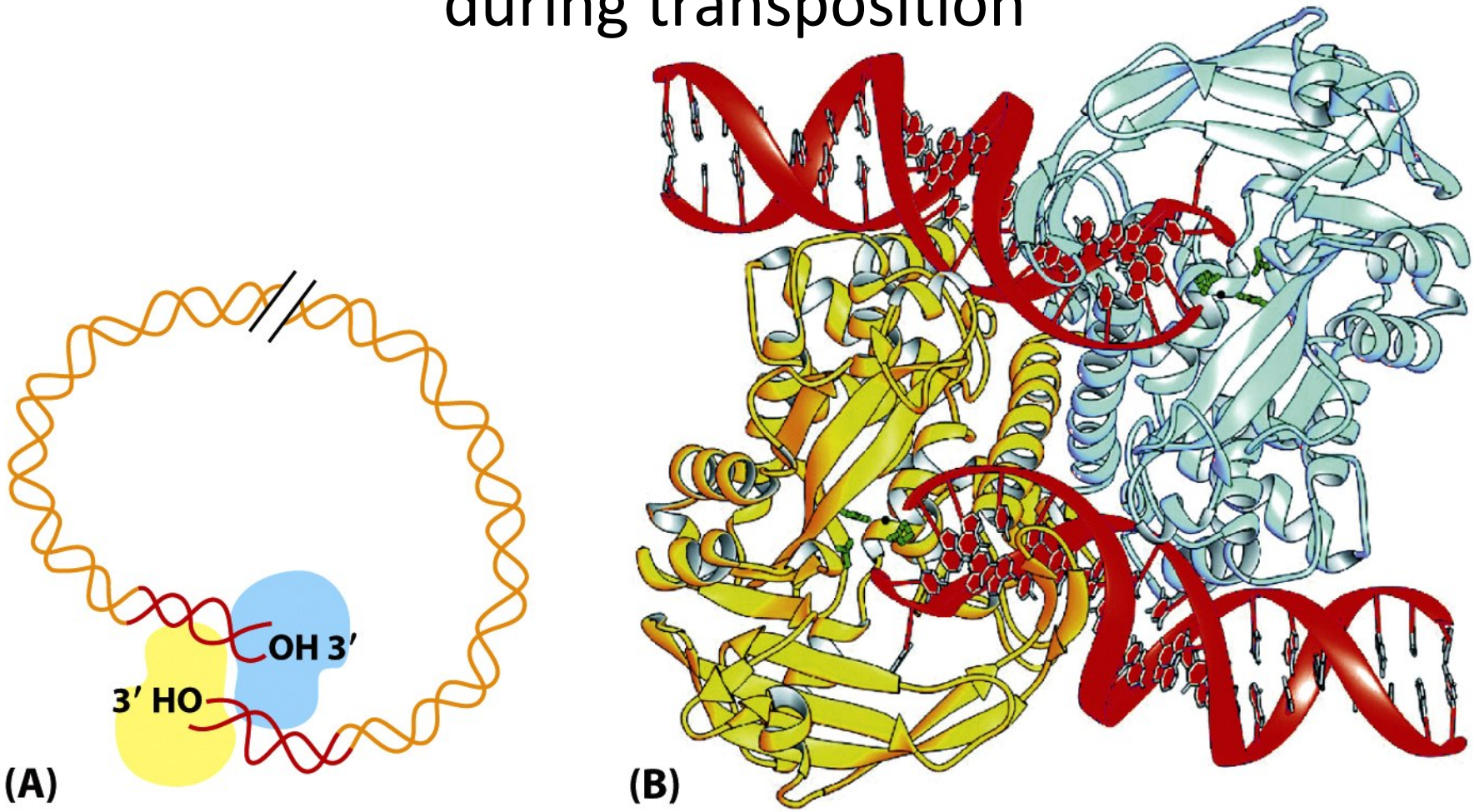


Figure 5-70 *Molecular Biology of the Cell* (© Garland Science 2008)

Viruses that integrate in host genomes use a "transposon" strategy

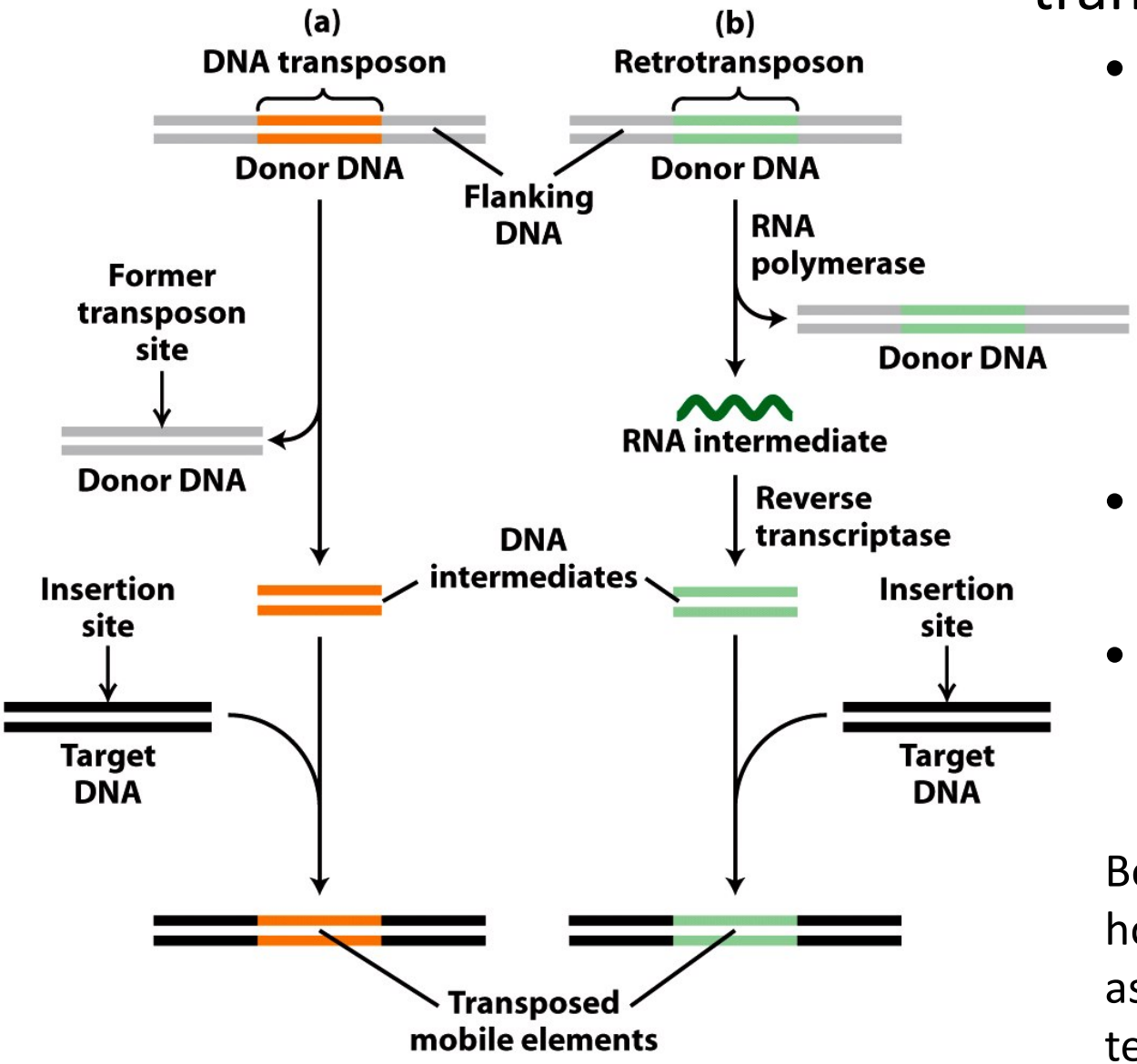
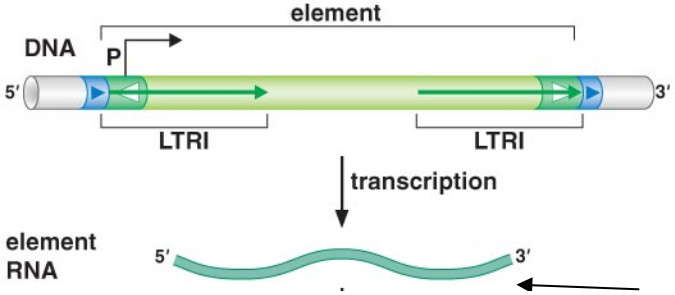


Figure 6-8
Molecular Cell Biology, Sixth Edition

- Viral genome is an element with highly defined, specific sequences at each end recognized by special recombinase
- Two biochemical pathways
- Both invade host DNA by attack on ***random*** sequence

Both make staggered cuts in host DNA a few bp apart to assist insertion: after repair, telltale micro-duplications

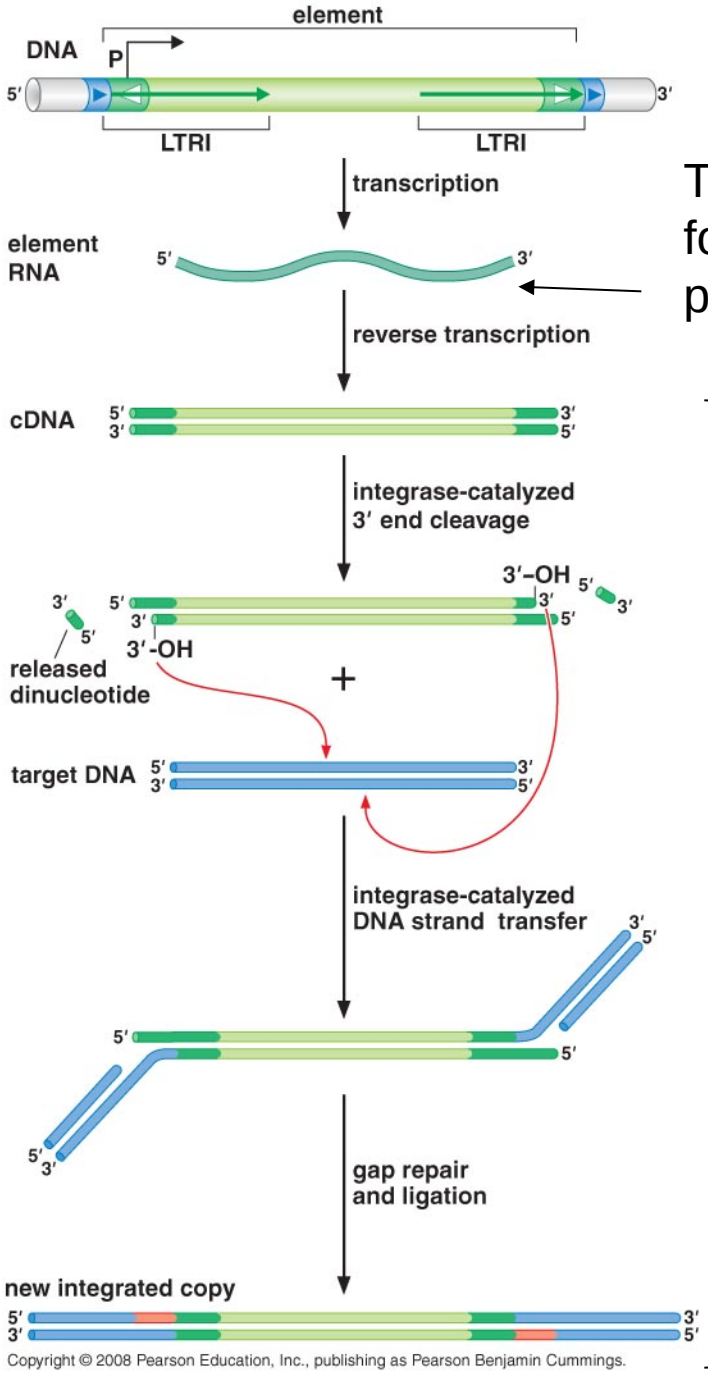
Retroviral integration & retrotransposon integration: use RNA polymerase and reverse transcriptase to make the insertion copy – no need to excise, ever



This RNA is the form that is packaged in virus of infectious retroviruses… with its own encoded proteins coating it

Virus particle itself contains reverse transcriptase, encoded by viral RNA. This enables first RNA-DNA hybrid, then DNA duplex to be made from same viral RNA when it re-enters a cell

Retroviral integration & retrotransposon integration: use RNA polymerase and reverse transcriptase to make the insertion copy – no need to excise, ever



This RNA is the form that is packaged…

in nucleus of the Infected cell

The irony of the arms race: Regulated, but transposable element-like recombination is ***central*** to immune cell development

- Genes that code for T and B cell receptors are not assembled in germline genome

- Clusters of gene elements need to be assembled via DNA recombination in order to create transcription units
  - ***No*** splice donor/acceptor sites
  - Promoters and enhancers can be > 1 Mb apart in germline genome

- Specific recombinases need to rearrange DNA for immune cells to express receptors, and to survive
  - Variable sequences at recombination joints contribute greatly to immune recognition diversity