Replication:
Replication of eucaryotic linear chromosomes
- Nucleosomes as obstacles during replication
- Replication of chromosome ends (telomeres)

DNA repair

Homologous recombination
- in DNA repair
- for exchange of genetic information
- applications

Site specific recombination
- applications

Replication:

- DNA polymerases synthesize 5'->3' and need a primer (free 3' OH, RNA, Primase)
- Replication fork with leading and lagging strand (Okazaki fragments)
- Helicases, ssDNA binding protein, Topoisomerase, sliding clamp
- Prokaryotic origin of replication
- Eukaryotic ARS: Replication is linked to the cell cycle (S-Phase)
- Eukaryotic chromatin: nucleosomes as fundamental packaging unit
  ->nucleosome is histone-octamer (H2A, H2B, H3, H4, synthesized during S-phase unlike most other proteins)

Nucleosome: 2x H2A, H2B, H3, H4 (octamer)
Steric obstacle for the replication fork
Chromatin remodeling proteins destabilize DNA-histone interactions
-> H3-H4 parental tetramers remain DNA associated, randomly segregate to one of the new strands
H2A-H2B dimers dissociate

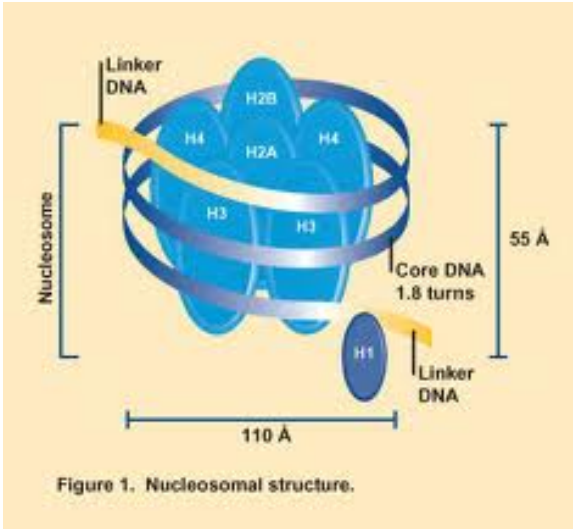


Figure 1. Nucleosomal structure.

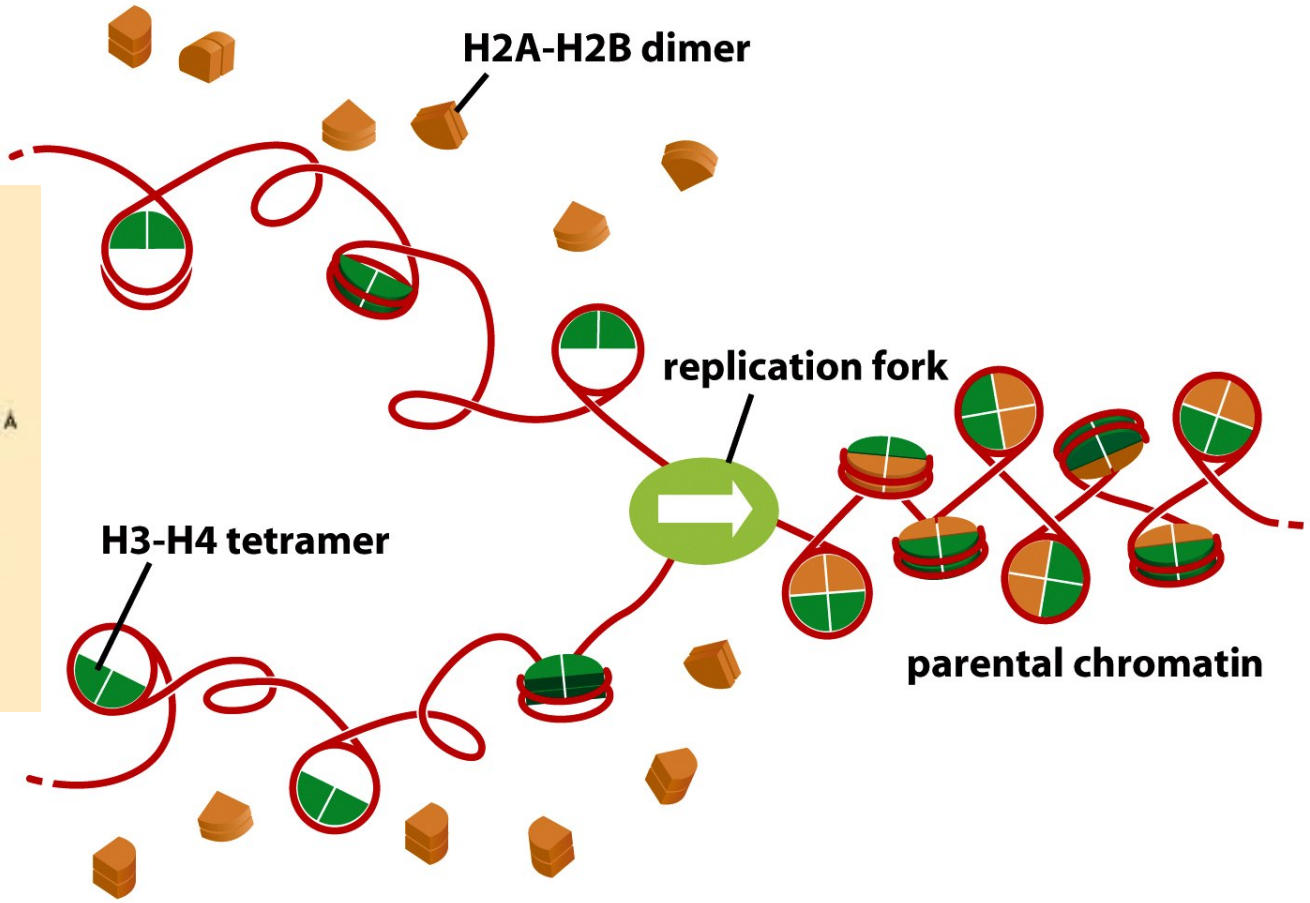


Figure 5-38a *Molecular Biology of the Cell* (© Garland Science 2008)

New H3-H4 tetramers are added
Mix of old/new H2AB dimers are added
NAP/CAF: histone chaperones
(CAF: chromatin assembly factors)

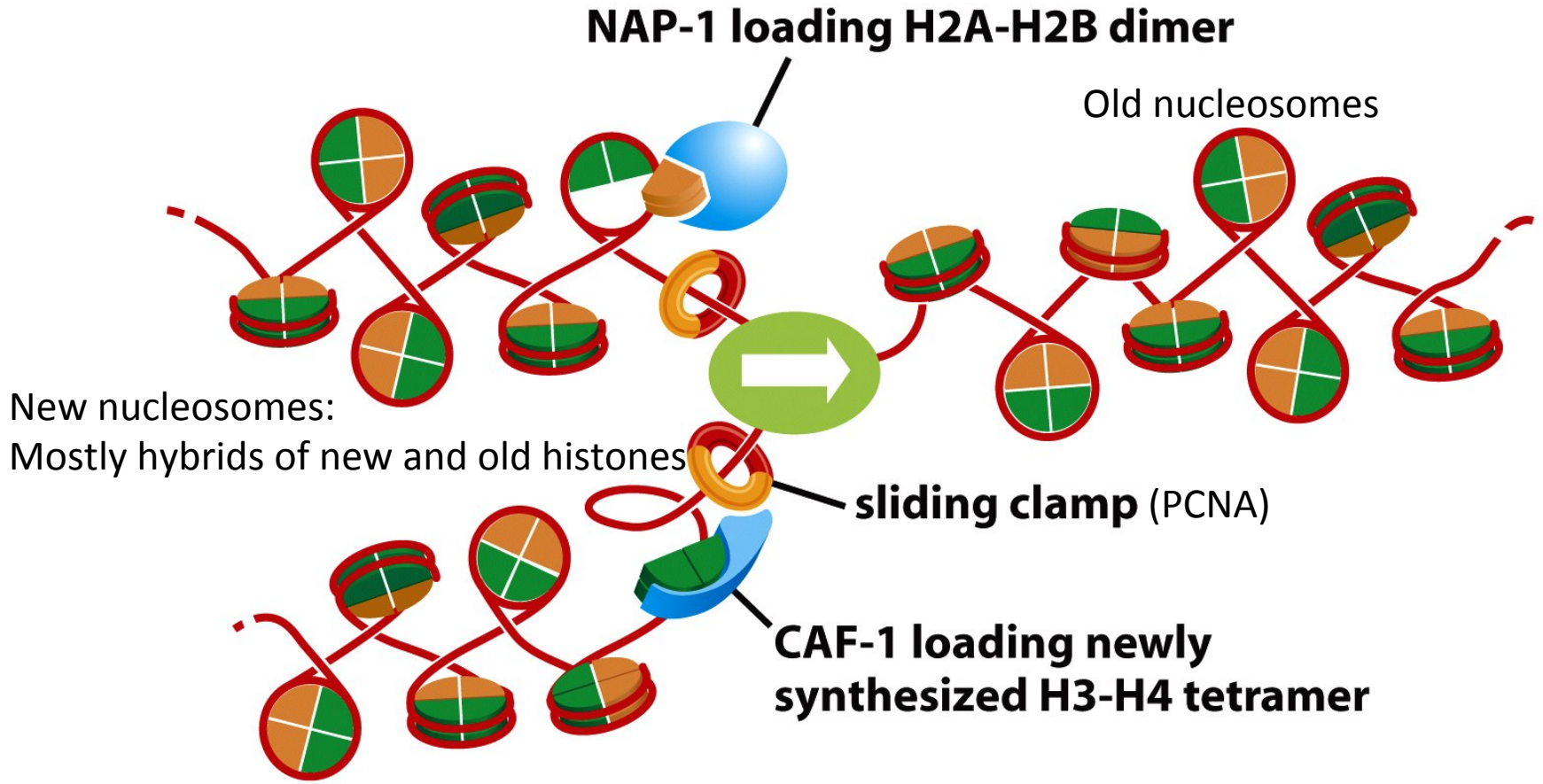


Figure 5-38b *Molecular Biology of the Cell* (© Garland Science 2008)

Copying of histone modification through H3-H4 tetramer distribution to both DNA strands
This mechanism allows segregation of epigenetic modifications

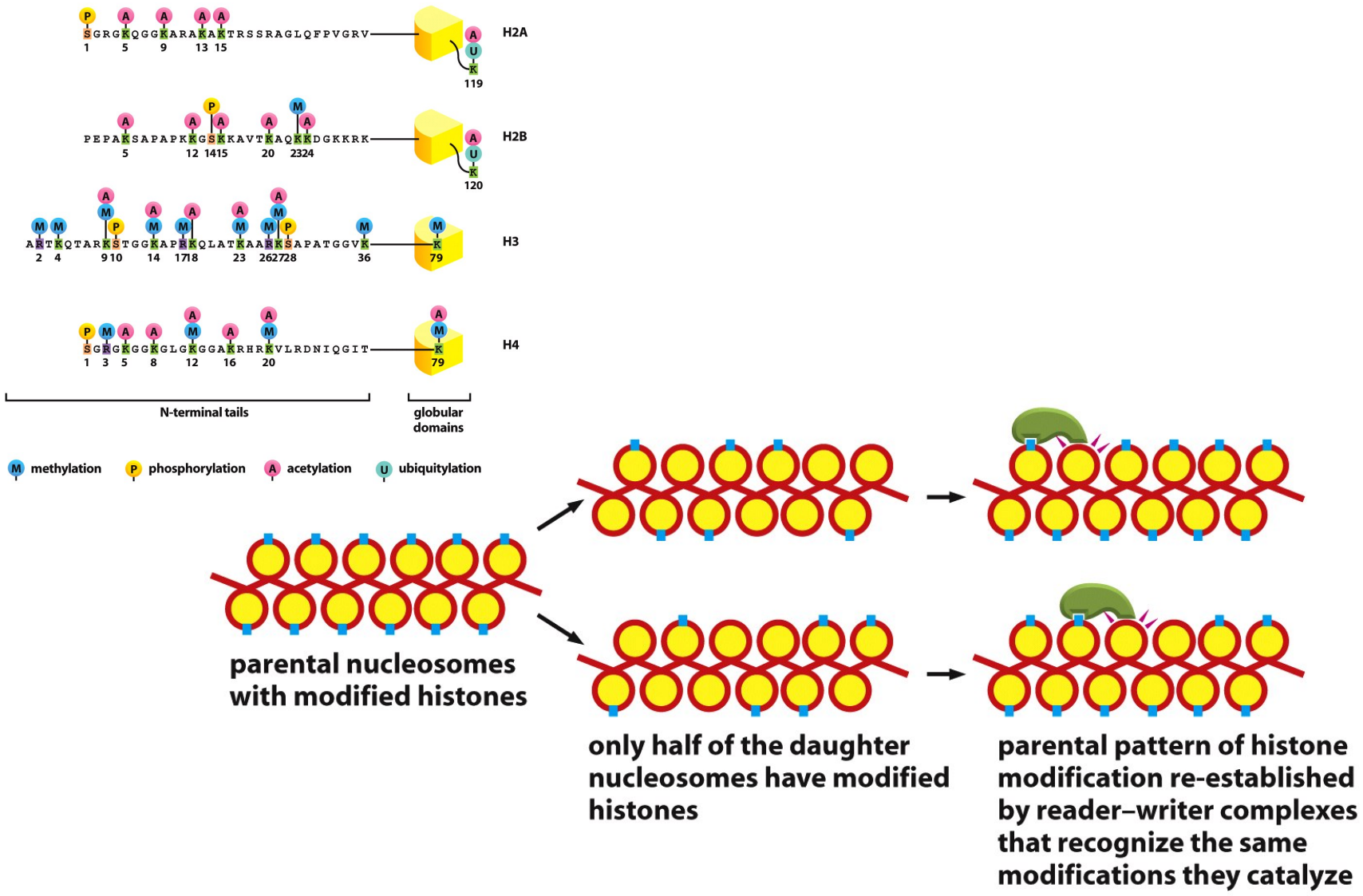
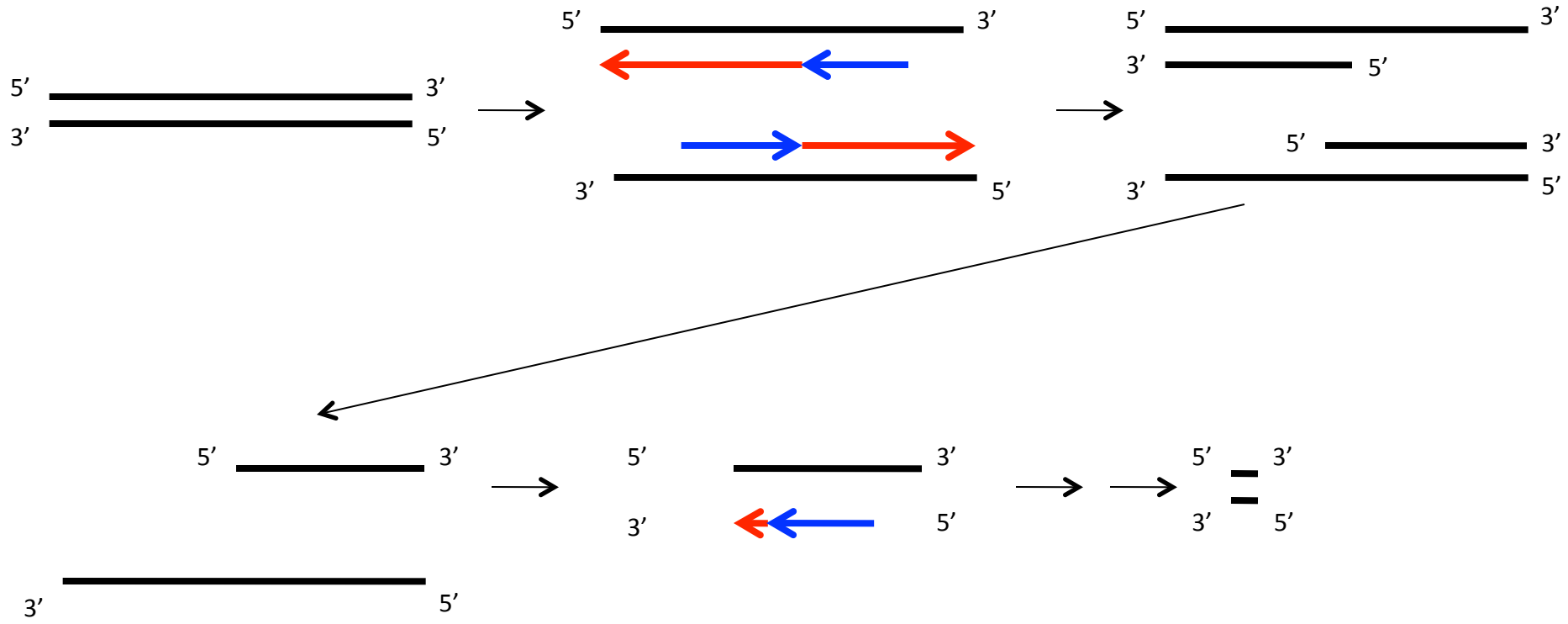
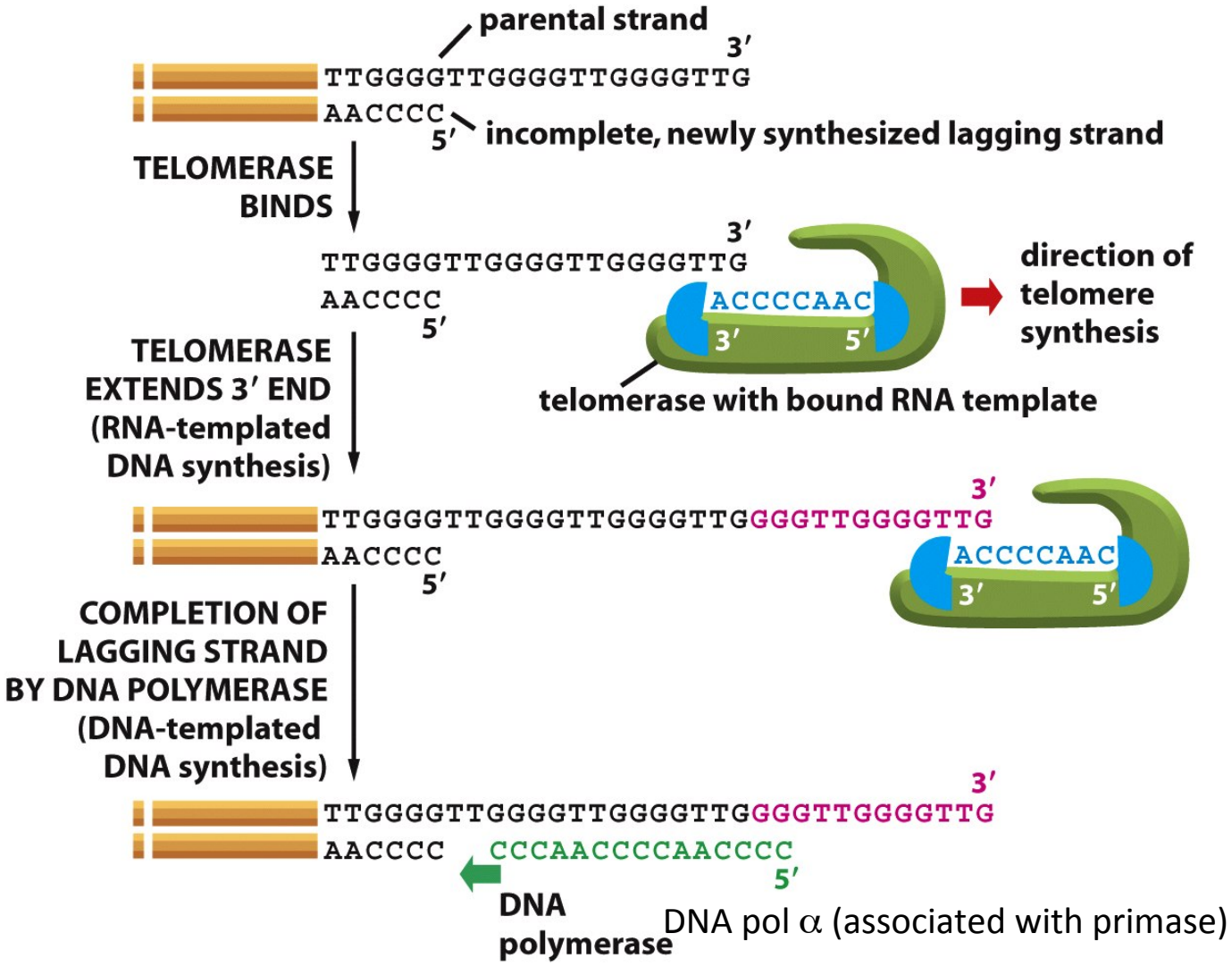


Figure 5-39 *Molecular Biology of the Cell* (© Garland Science 2008)

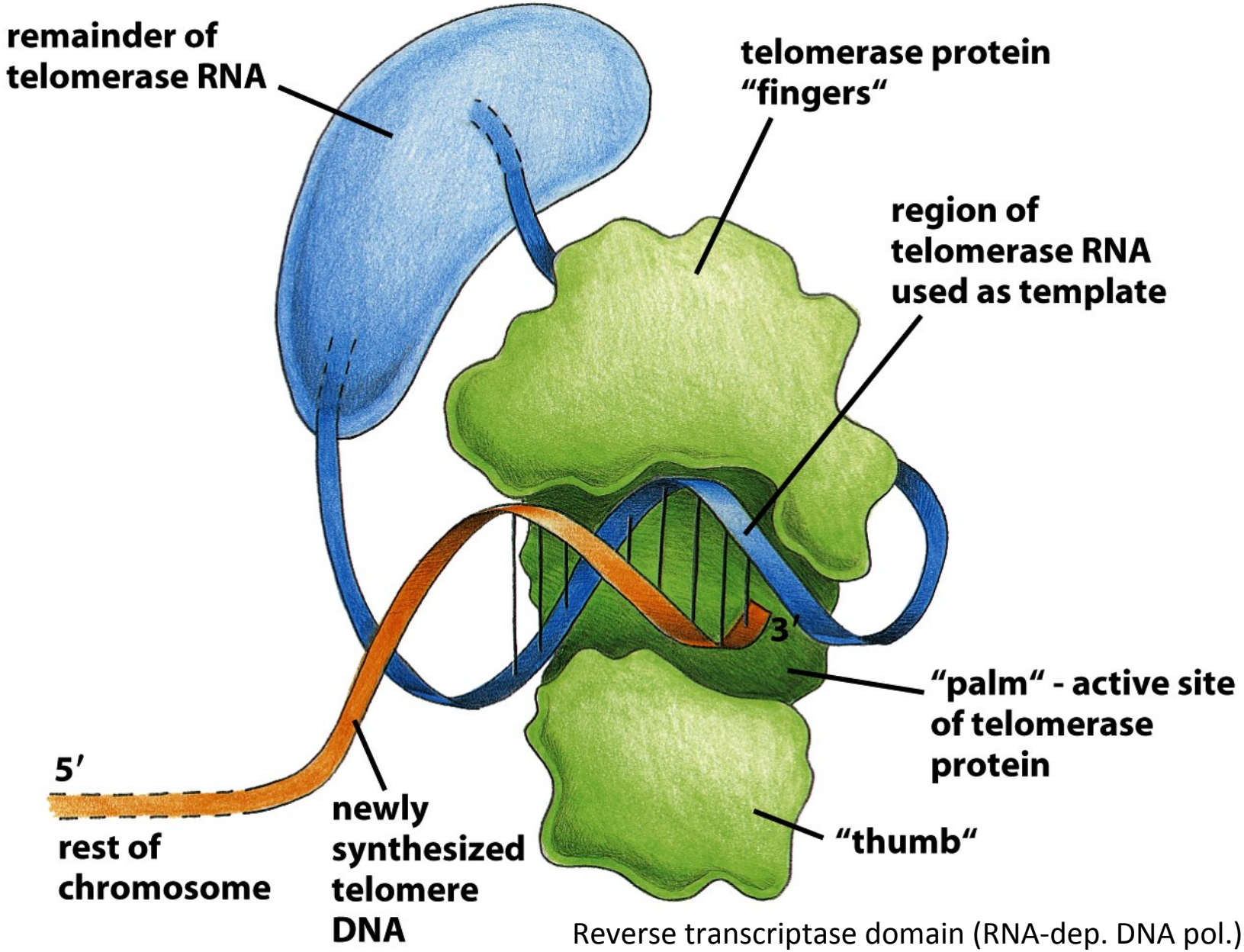
Replication of DNA ends (linear chromosomes)





Telomerase: Nobel prize 2009 (Blackburn, Greider, Szostak)

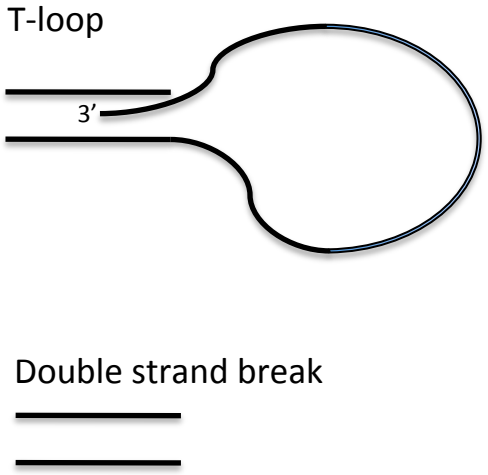
Figure 5-41 *Molecular Biology of the Cell* (© Garland Science 2008)



Reverse transcriptase domain (RNA-dep. DNA pol.)

Figure 5-40 *Molecular Biology of the Cell* (© Garland Science 2008)

T-loop of protruding 3’ end
After lagging strand synthesis, 5’ end is shortened to produce 3’ overhang (to ensure free ss for further elongation by telomerase).
3’ overhang loops back and inserts into the double strand
T-loop provides unique structure for DNA ands that distinguishes them from broken DNA molecules ->protection against nucleases



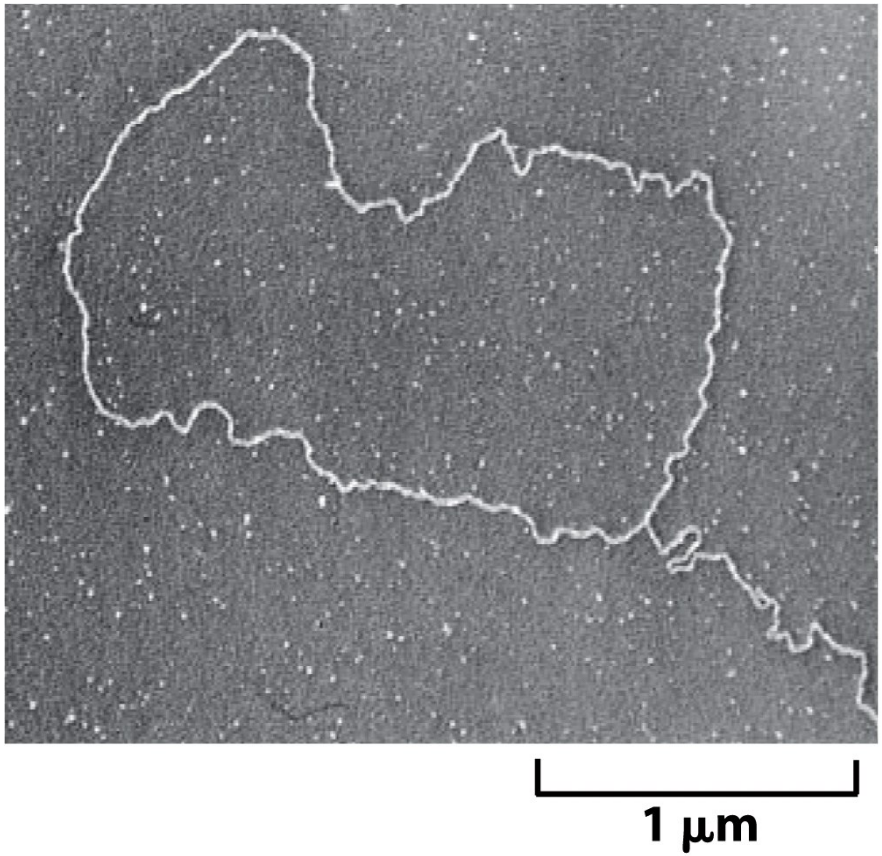
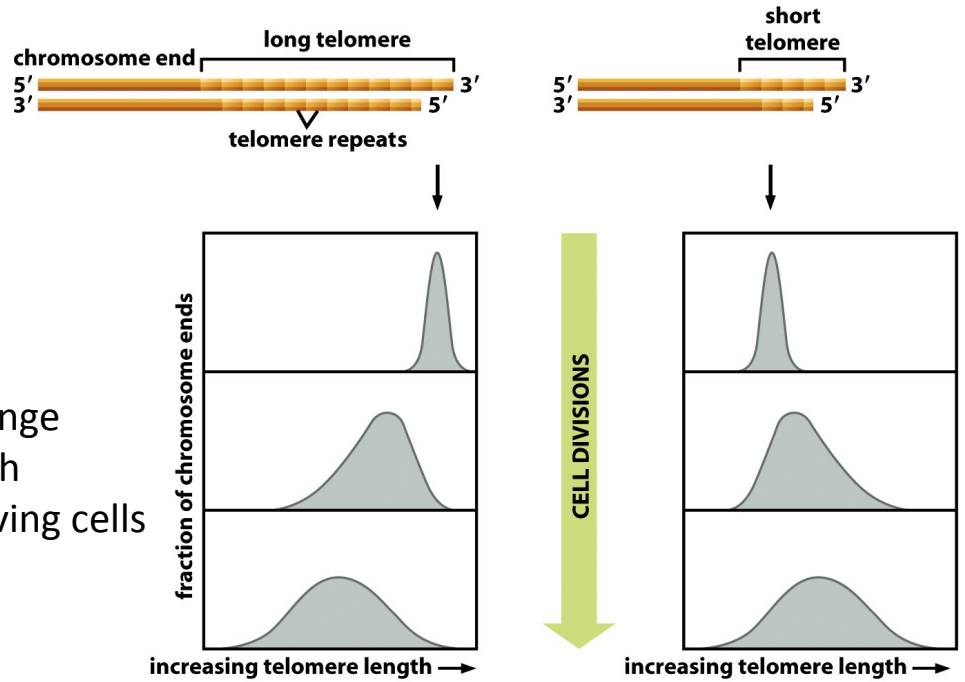


Figure 5-42 *Molecular Biology of the Cell* (© Garland Science 2008)

Experimental change
of telomere length
is readjusted in living cells



Telomere length is important
Stem cells: divide often-> high telomerase activity
Somatic less: limited number of divisions -> low telomerase activity
->Cells cannot proliferate uncontrolled (protection against cancer)

In some cell culture systems increasing telomere length can provide increased potential to divide

Telomerase ko mice develop cancer and age prematurely: cells divide in the absence of telomerase activity which results in disease due to unstable chromosomes

Dolly's telomeres (somatic cells <-> stem cells of germline or adult)

Figure 5-43 *Molecular Biology of the Cell* (© Garland Science 2008)

DNA repair:

Random changes created by environmental influences (heat, radiation, chemicals) and metabolic accidents

Estimate: ≤1/1000 are fixed in genome, the others are eliminated (repaired)

Mutations that affect DNA repair lead to increased mutation rates and cause disease

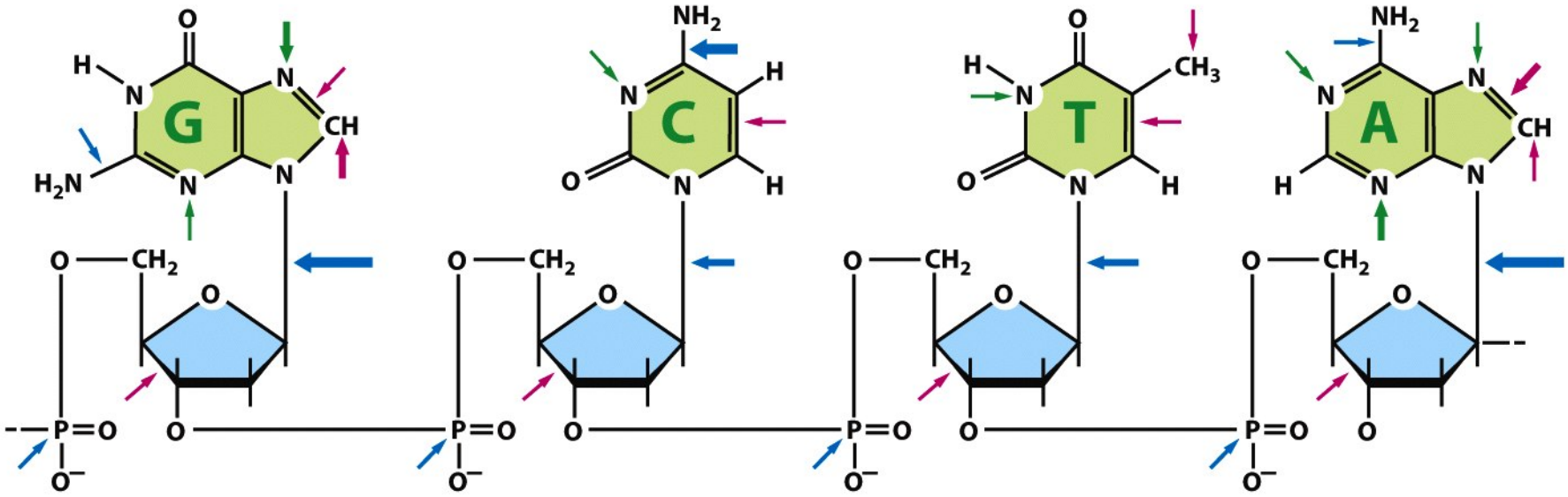
->importance of DNA repair systems

Table 5–2 Some Inherited Syndromes with Defects in DNA Repair

NAME	PHENOTYPE	ENZYME OR PROCESS AFFECTED
MSH2, 3, 6, MLH1, PMS2	colon cancer	mismatch repair
Xeroderma pigmentosum (XP) groups A–G	skin cancer, UV sensitivity, neurological abnormalities	nucleotide excision–repair
XP variant	UV sensitivity, skin cancer	translesion synthesis by DNA polymerase η
Ataxia telangiectasia (AT)	leukemia, lymphoma, γ-ray sensitivity, genome instability	ATM protein, a protein kinase activated by double-strand breaks
BRCA2	breast, ovarian, and prostate cancer	repair by homologous recombination
Werner syndrome	premature aging, cancer at several sites, genome instability	accessory 3′-exonuclease and DNA helicase
Bloom syndrome	cancer at several sites, stunted growth, genome instability	accessory DNA helicase for replication
Fanconi anemia groups A–G	congenital abnormalities, leukemia, genome instability	DNA interstrand cross-link repair
46 BR patient	hypersensitivity to DNA-damaging agents, genome instability	DNA ligase I

Table 5-2 *Molecular Biology of the Cell* (© Garland Science 2008)

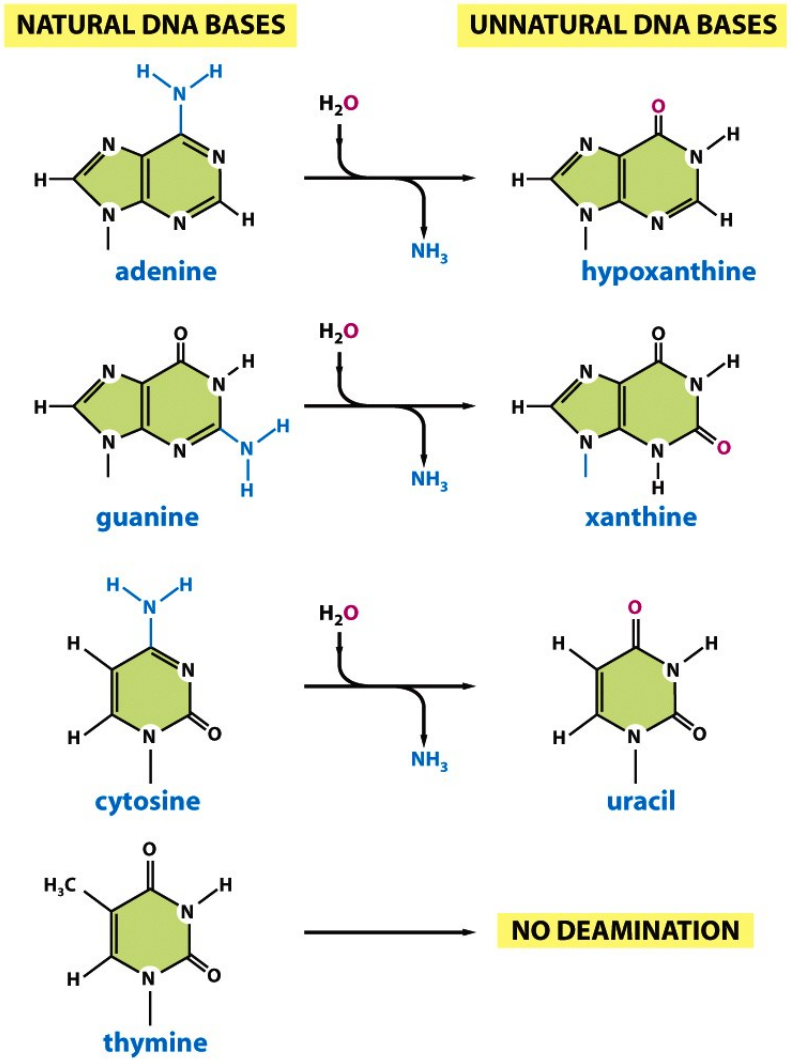
Spontaneous changes in DNA
Depurination: loss of G, A base due to hydrolysis; 5000/cell/day
Deamination of C (->U); 100/cell/day
Oxidation (red arrows)
Uncontrolled methylation (green arrows)

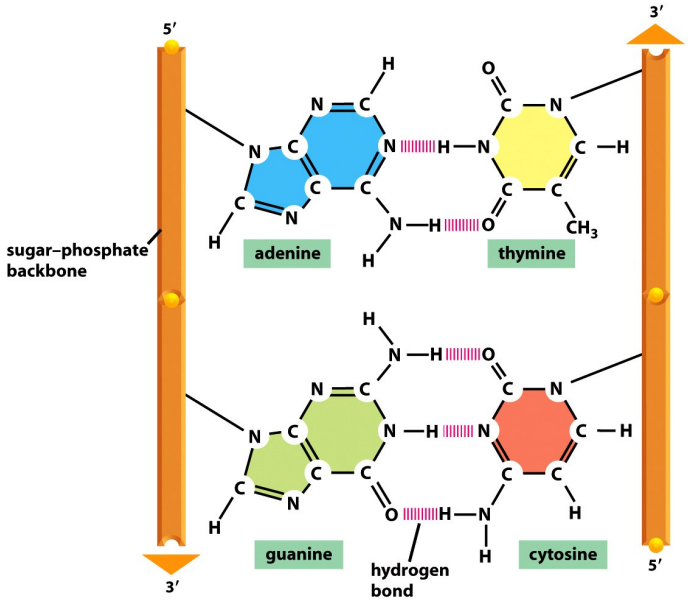


Uncorrected changes may result in mutations (deletions, base-pair substitutions)

Figure 5-44 *Molecular Biology of the Cell* (© Garland Science 2008)

Deamination results in unnatural bases that can be recognized in DNA

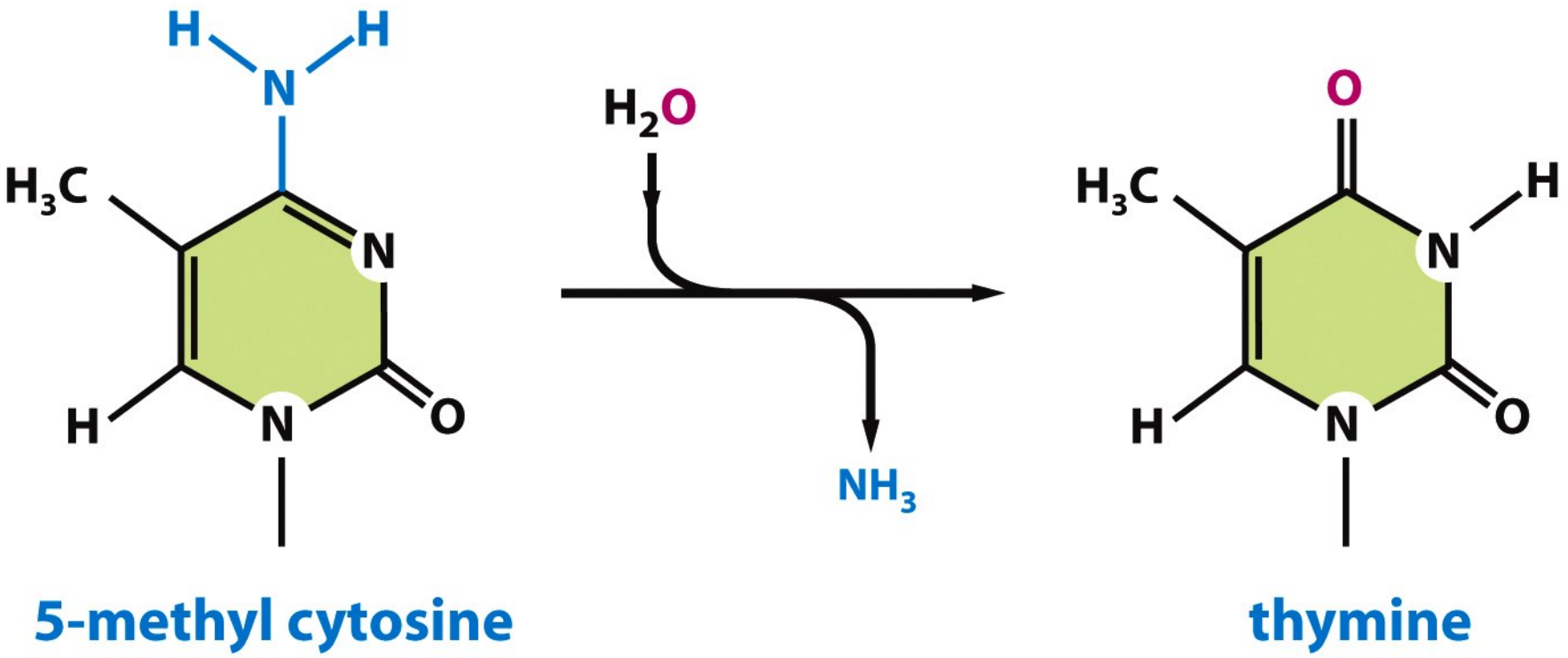




Uracil is not used in DNA
Therefore C->U deamination can be detected

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

Deamination of methylated C results in T, mis-pairing of T-G can be detected by the repair systems
The repair of T-G mismatches is biased to exchanging T for C and thus restoring the correct sequence



Ineffective repair: about 3% of C are methylated, mutations in these nucleotides account for about 30% of single-base mutations in inherited human disease.

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

The most frequent DNA damages/changes

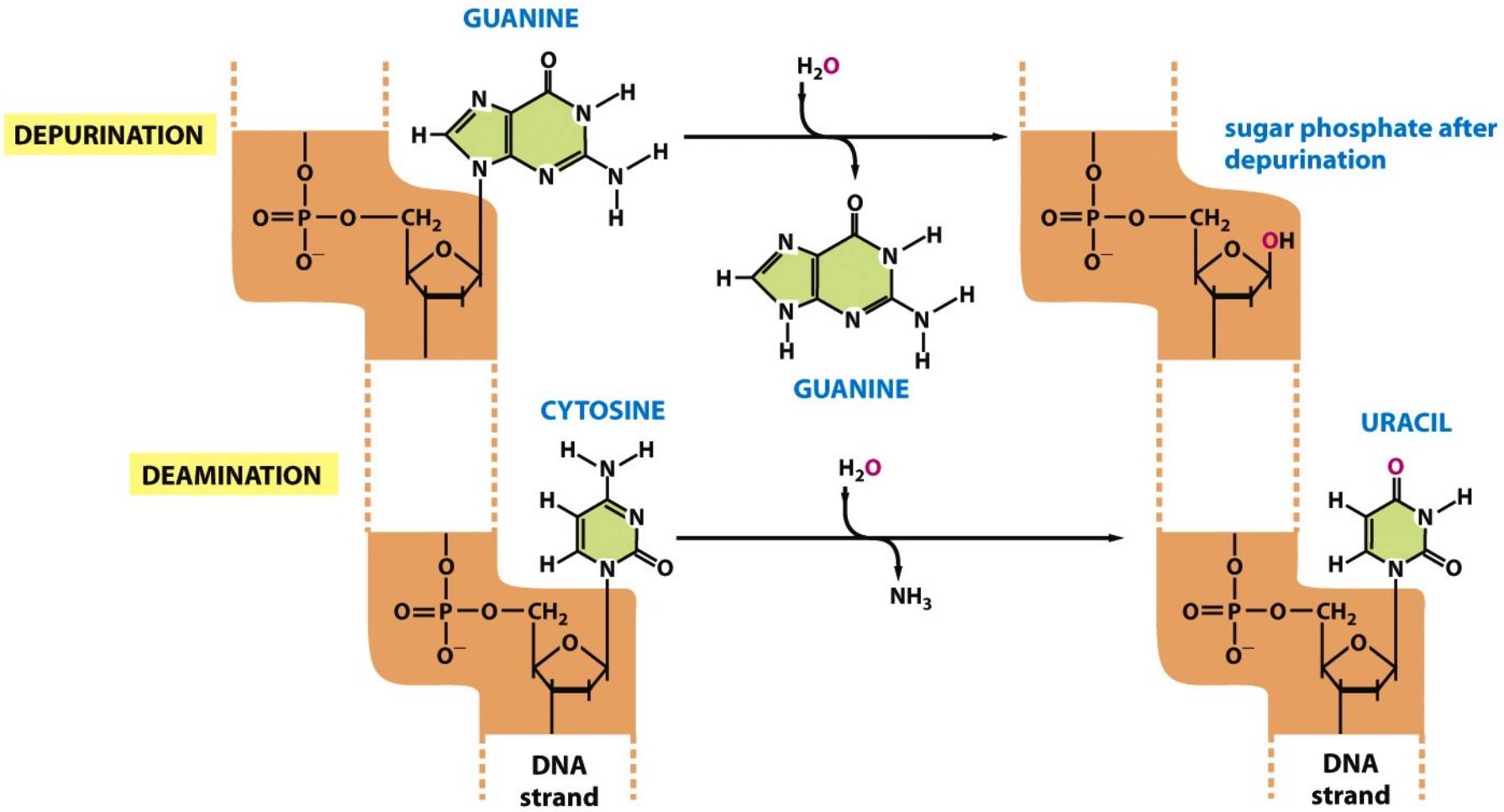
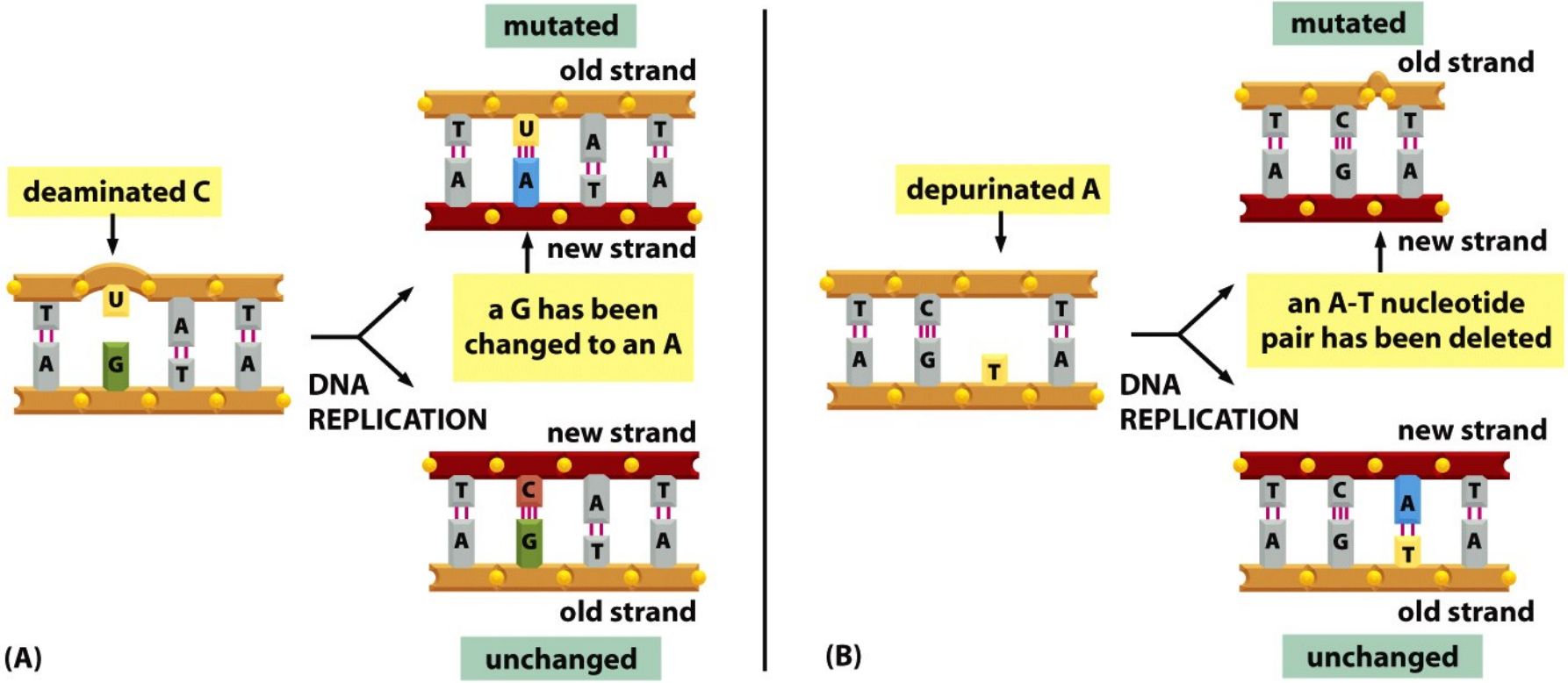


Figure 5-45 *Molecular Biology of the Cell* (© Garland Science 2008)



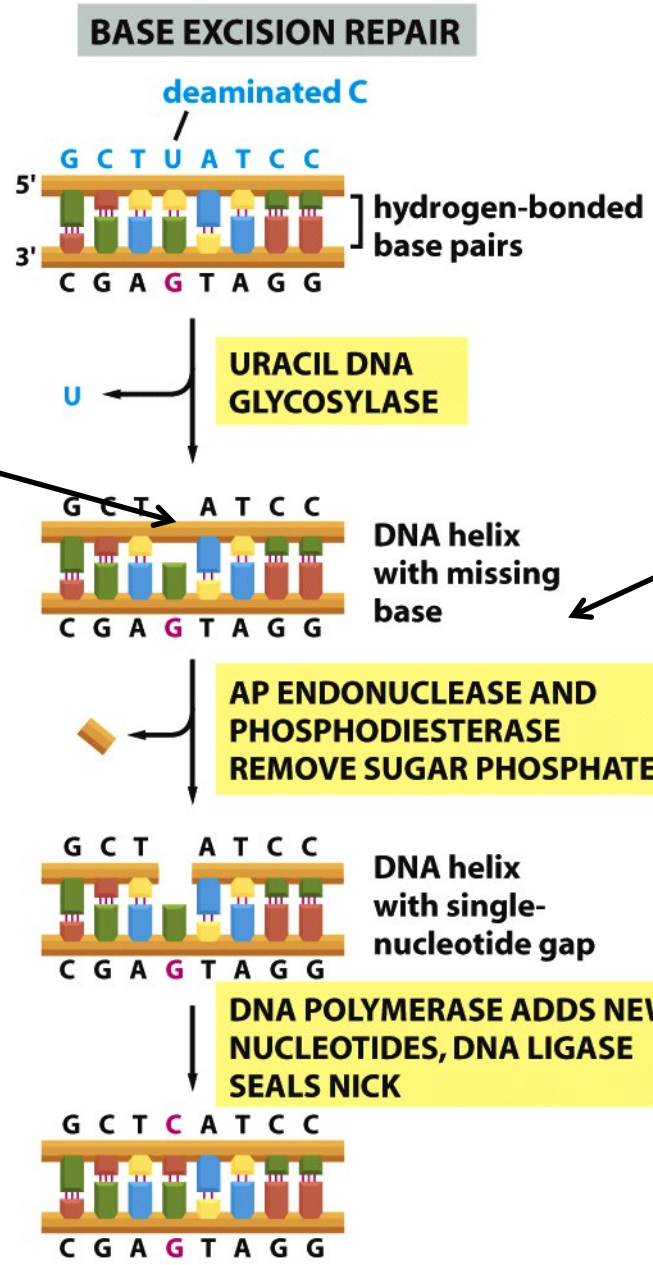
Advantage of DNA double helix for repair:

Double-helical structure of DNA allows repair as long as only one strand is affected

The complementary strand provides the template for the repair.

All genomes are ds, except some small viral genomes (ssDNA or ssRNA)

Figure 5-47 *Molecular Biology of the Cell* (© Garland Science 2008)



Depyrimidated DNA
Missing base

Depurinated DNA
-> missing A or G

AP: apurinic or apyrimidinic
Endonuclease: cleaves within the DNA
(not at the end such as an exonuclease)

DNA pol β

Figure 5-48a *Molecular Biology of the Cell* (© Garland Science 2008)

Glycosylase mediated base-flipping to probe individual bases for alterations
Altered (unnatural) bases are then removed by hydrolysation

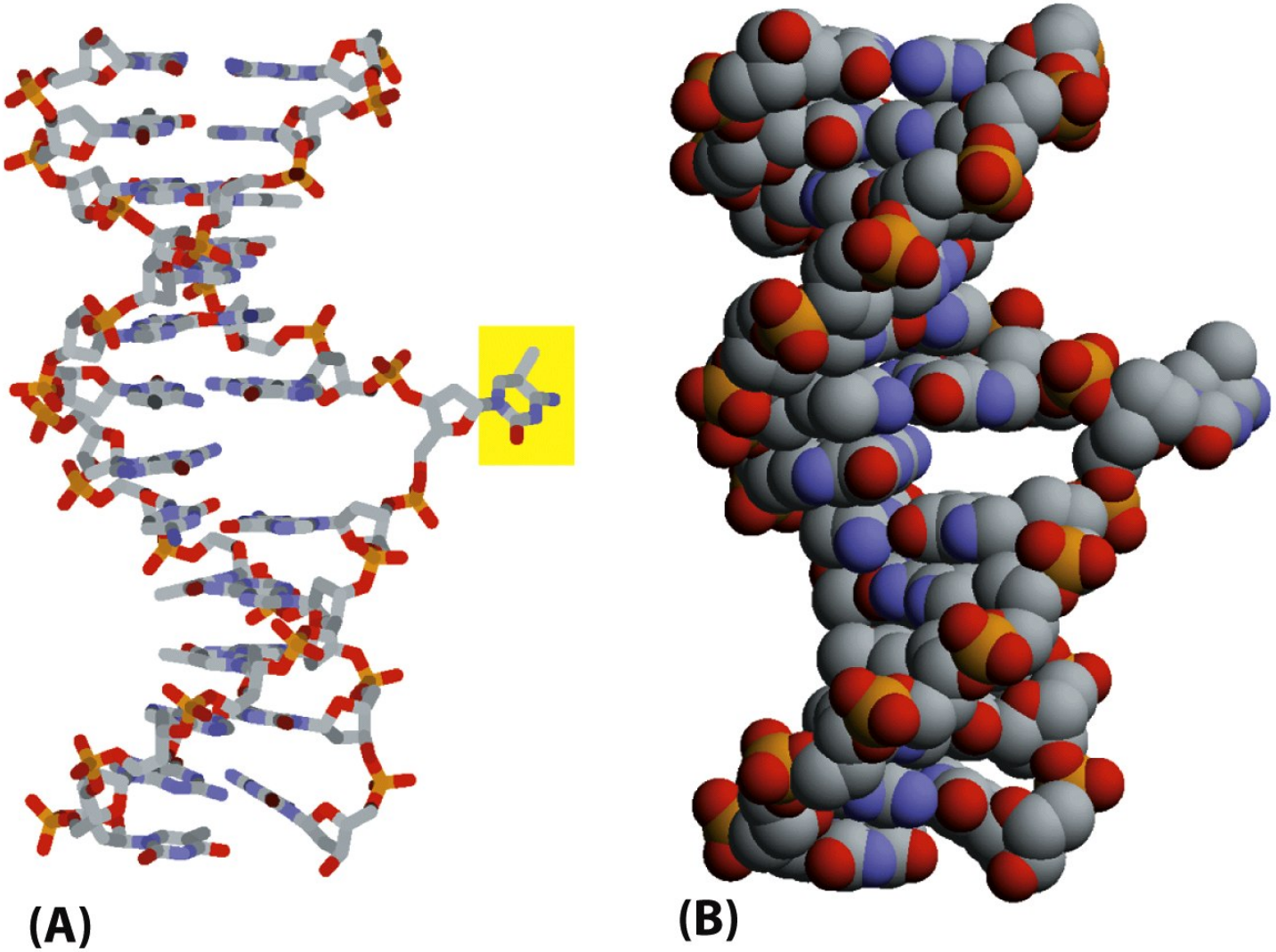


Figure 5-49 *Molecular Biology of the Cell* (© Garland Science 2008)

Thymin dimer caused by UV-irradiation

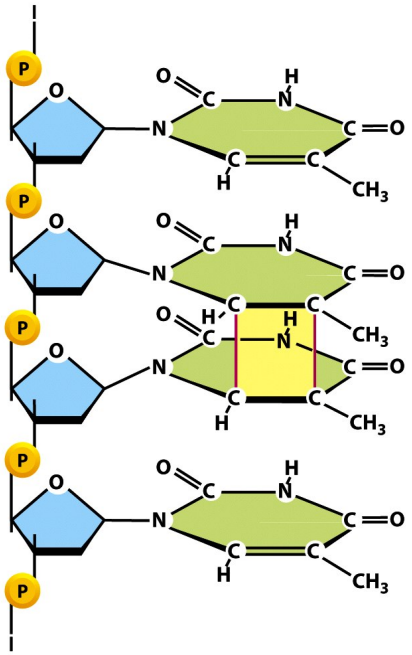


Figure 5-46 *Molecular Biology of the Cell* (© Garland Science 2008)

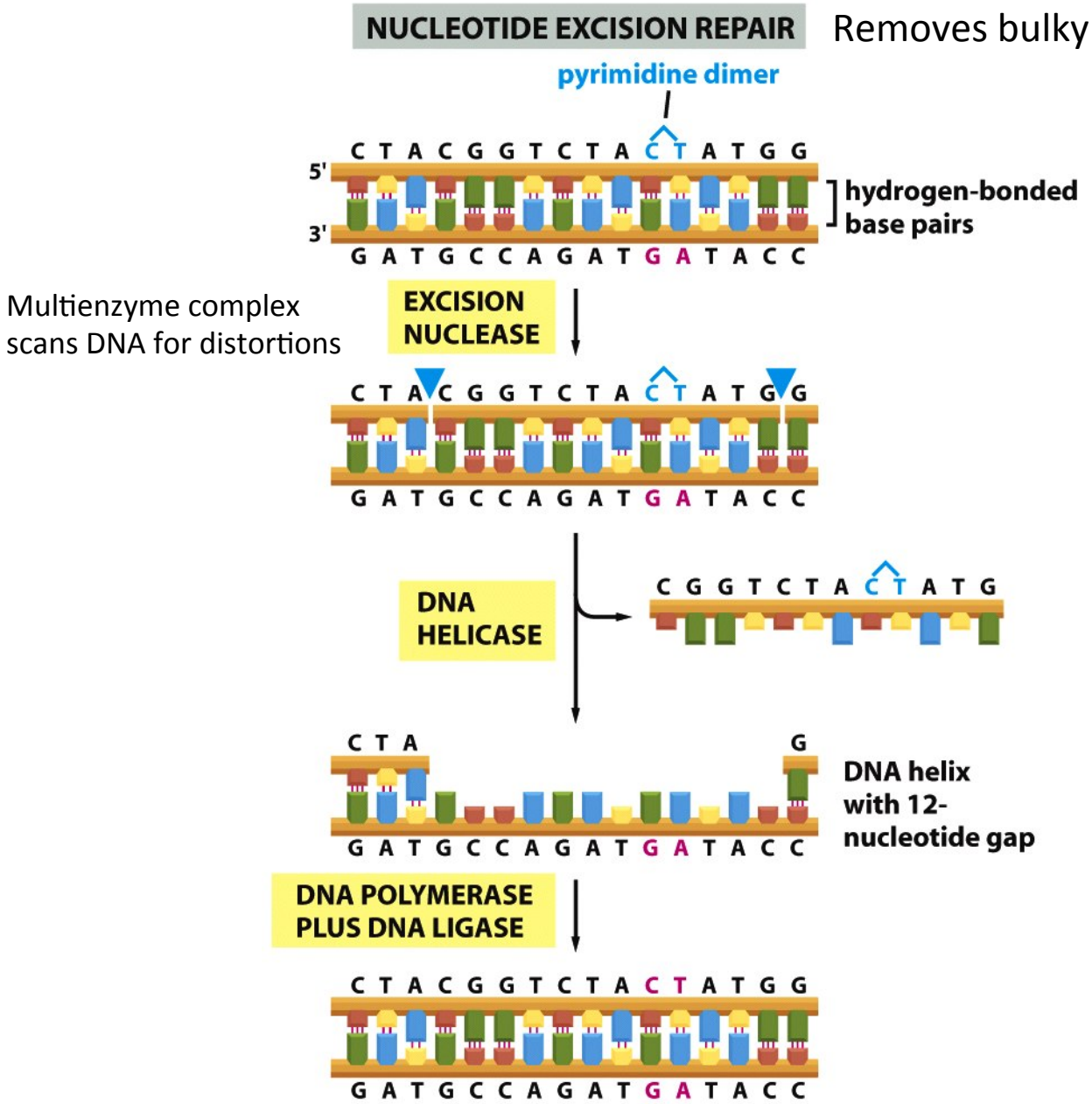


Figure 5-48b *Molecular Biology of the Cell* (© Garland Science 2008)

Error prone repair:

Heavily damaged DNA (stalled DNA replication) may activate 'rough and ready' DNA repair systems (E.coli: SOS repair system), where special DNA polymerases insert nucleotides with less stringency (no 3'->5' exonuclease activity, insertion of random nucleotides) . This results in increased mutation rates.

-> aim is to revert the damaged DNA into a replicable state at all cost.

Controlling the direction of mismatch repair:

In E.coli the parental strand only is methylated. Thus in hemimethylated DNA the unmethylated (i.e. new) strand is corrected.

Nicks rather than methylation are used in eukaryotes to identify the parental strand.

Transcription and DNA repair are coupled:

Transcription-coupled repair (TCR): when RNApol is stalled, specific repair proteins are attracted to the transcription bubble, repair the template strand (without displacing the RNApol) and let transcription resume. The other strand is not repaired by TCR.

DNA repair and progression of the cell cycle are coupled:

DNA damage can block entry from G_1 into S phase, slow S phase and block transition from S into M phase (check points for cell cycle progression). This gives the cell time to repair the DNA and prevent segregation of damages to daughter cells.

Repair of double strand brakes

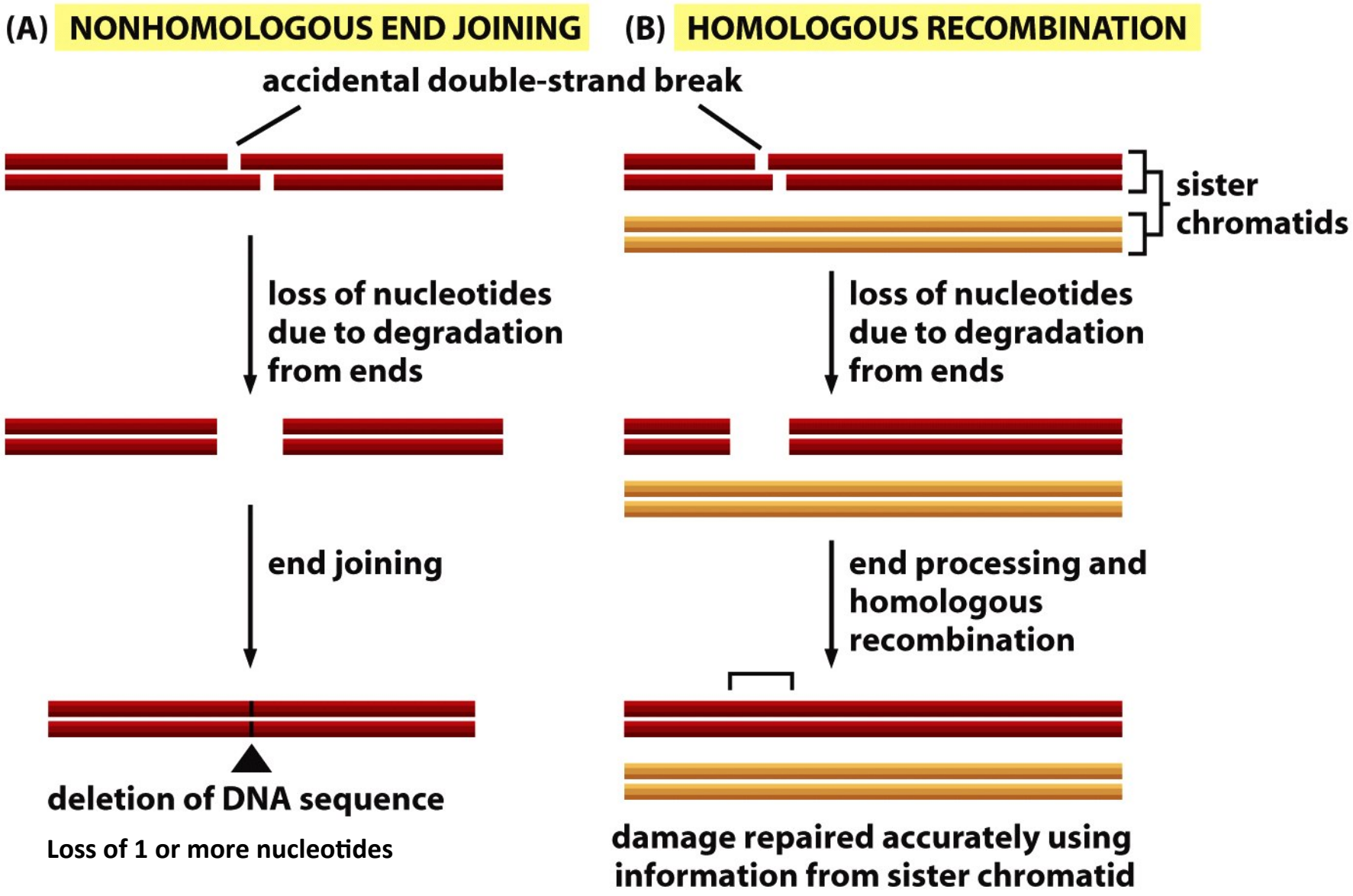
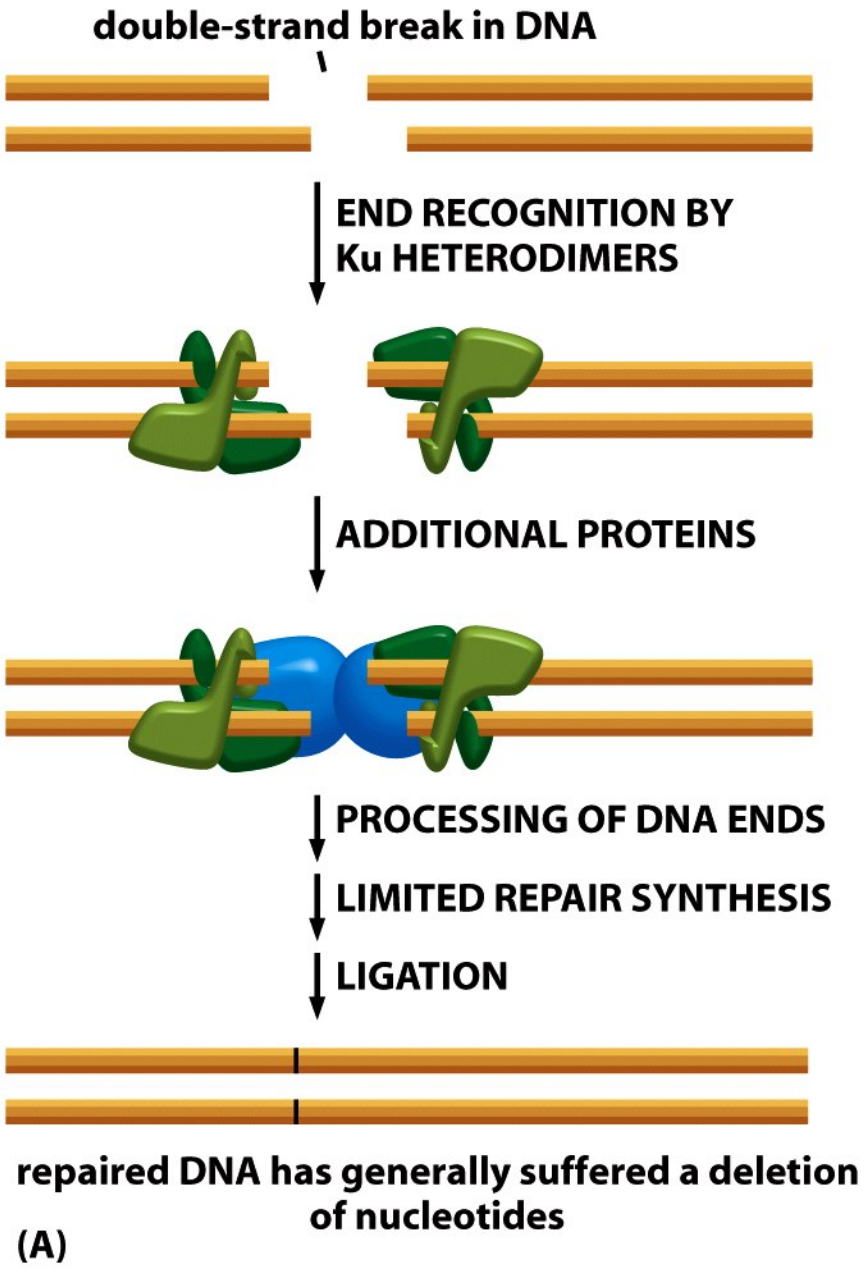
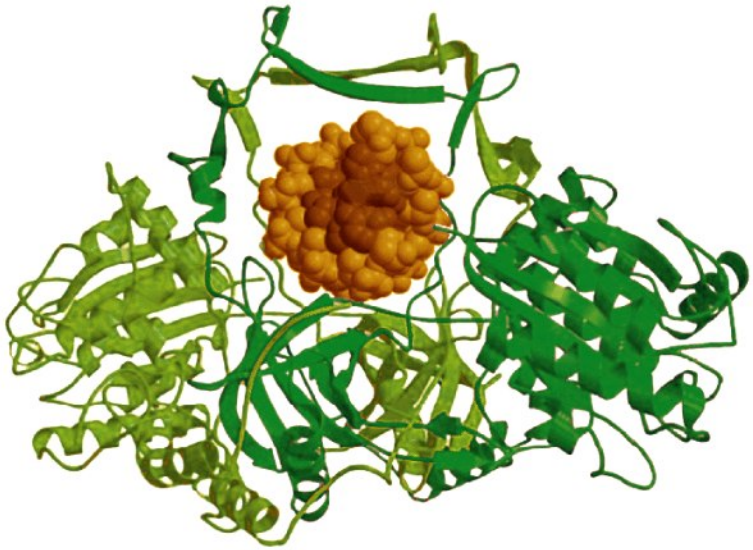


Figure 5-51 *Molecular Biology of the Cell* (© Garland Science 2008)





Common repair for somatic cells
Most of the genome is non-coding
-> Small errors do not matter in most cases

Figure 5-52 *Molecular Biology of the Cell* (© Garland Science 2008)

Homologous recombination:
Exchange between (very) similar DNA molecules

- Repair of ds breaks
- Exchange of genetic information between chromosomes

-> creating new genetic combinations (genotypes)

Evolutionary conserved mechanism: also present in bacteria

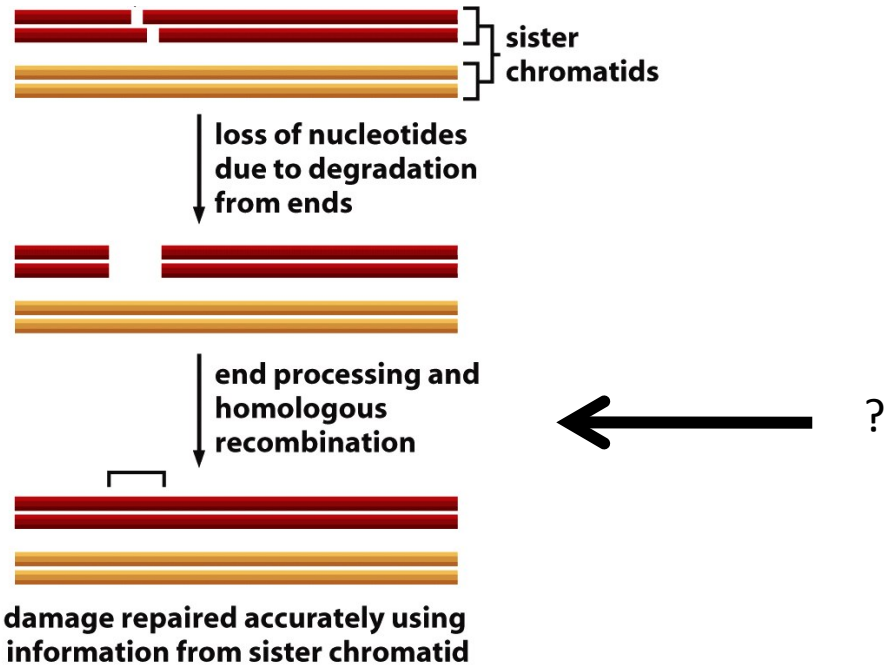
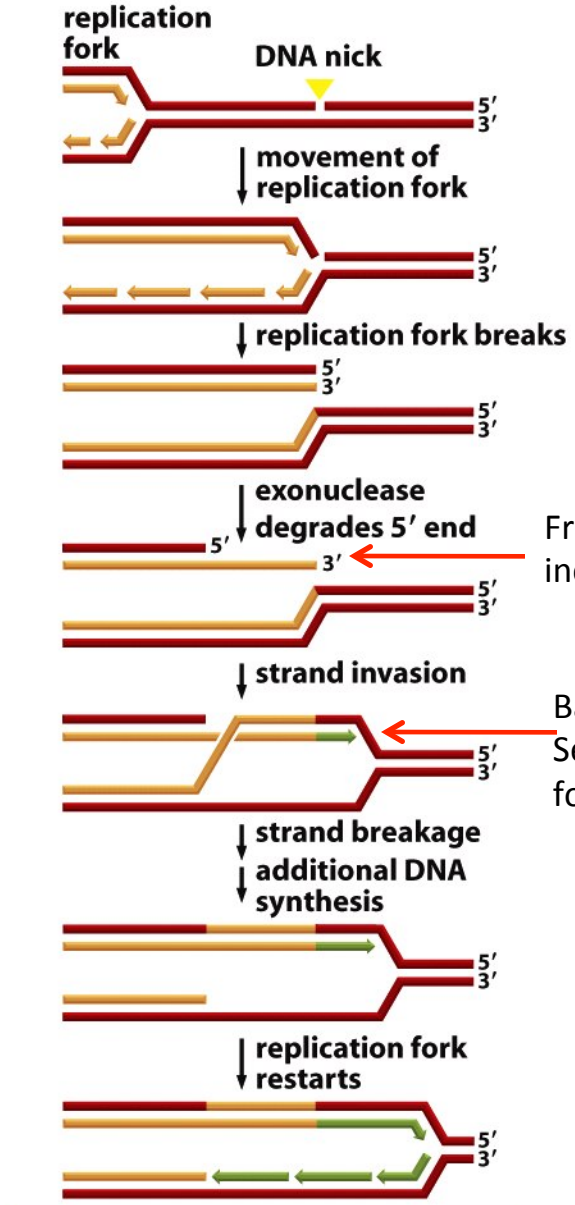


Figure 5-51 *Molecular Biology of the Cell* (© Garland Science 2008)

Repair of a broken replication fork by homologous recombination

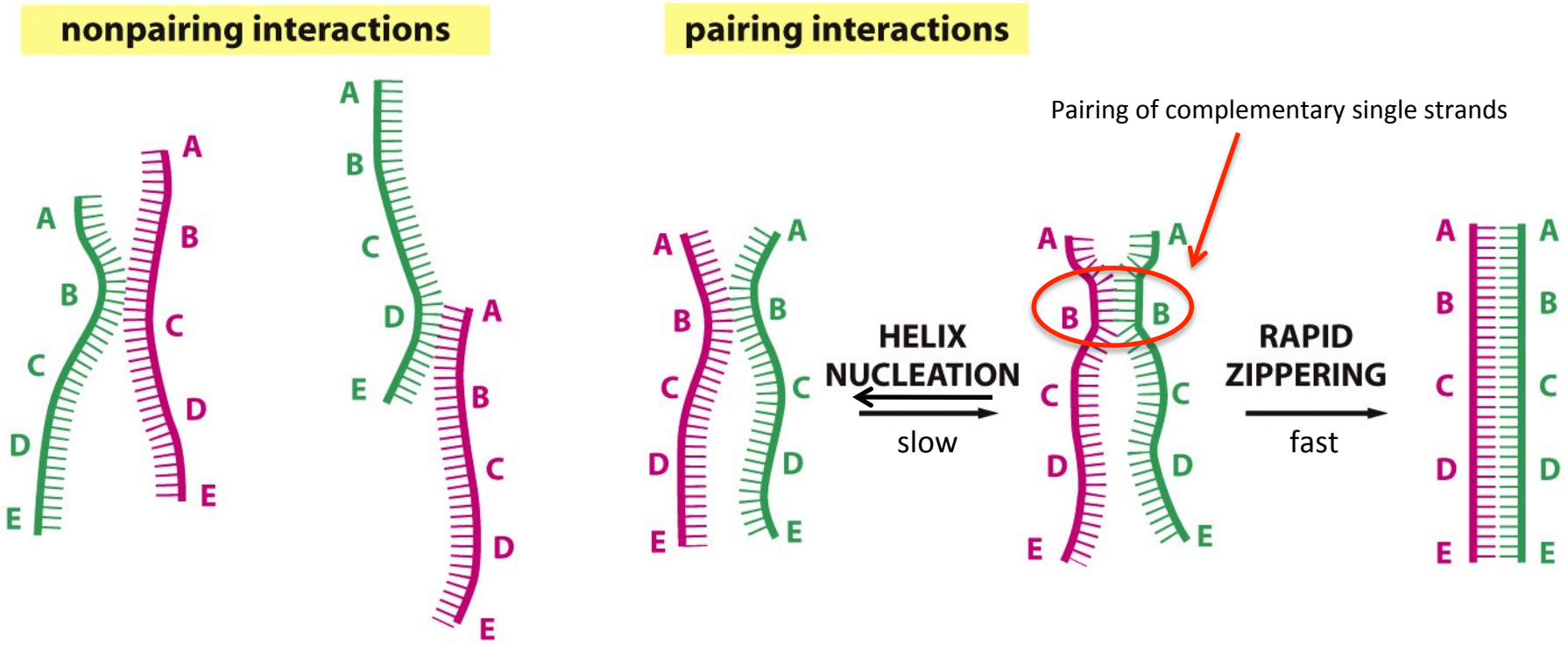


Free 3′ end for the pairing with the incoming DNA ss strand

Base pairing requires similar (homologous) Sequence, invading strand serves as template for DNA synthesis

Figure 5-53 *Molecular Biology of the Cell* (© Garland Science 2008)

Homologous recombination requires base pairing of complementary, similar ssDNA. Base pairing of ssDNA (denatured) in the test tube: **hybridization** (DNA renaturation) of complementary strands. No enzymes are required! Driving force are A-T and G-C base pairing that lead to stable double helix formation.



DNA hybridization in the lab:
Microarrays, hybridization of probes to DNA immobilized on membranes

Figure 5-54 *Molecular Biology of the Cell* (© Garland Science 2008)

In vivo: ssDNA have to be stabilized for base pairing of homologous strands
RecA/Rad51: binds in long cooperative clusters to ssDNA
-> nucleoprotein filament
can at the same time interact with a dsDNA molecule

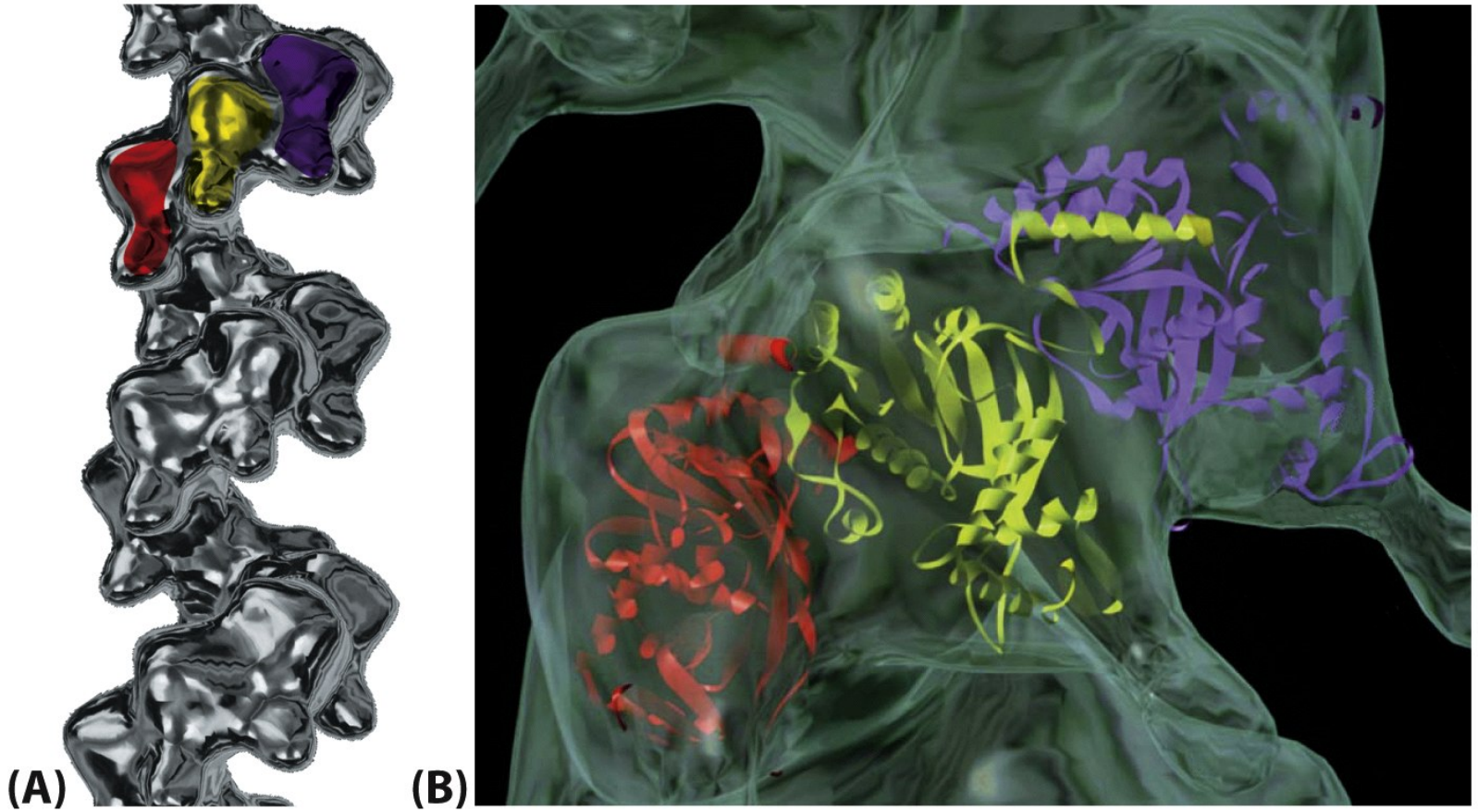
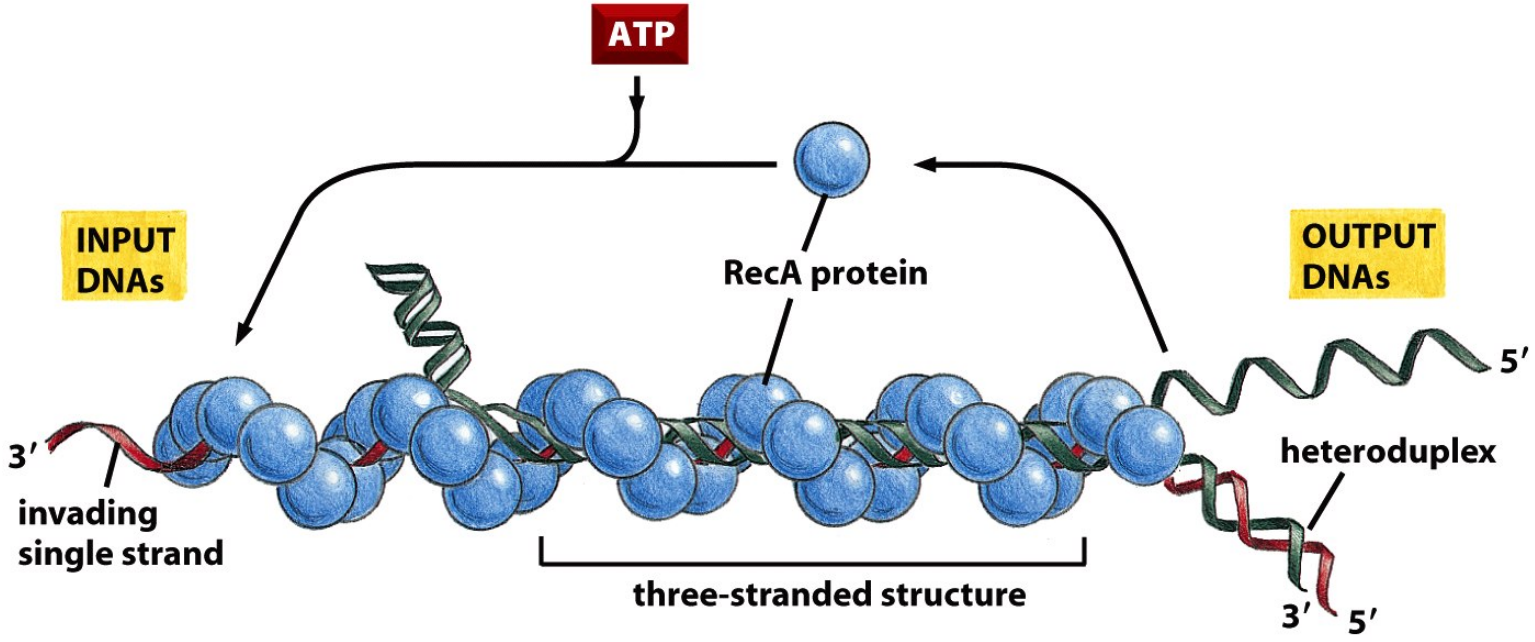


Figure 5-55 *Molecular Biology of the Cell* (© Garland Science 2008)

Multistep DNA synapsis reaction:
RecA intertwines ssDNA with dsDNA molecules (sequence independent)
ssDNA 'searches' for homology (transient base-pairing by 'flipped-out' bases?)
Strand invasion: ssDNA displaces the respective strand of the double helix
-> hetero-douplex formation



Strand invasion

Figure 5-56 *Molecular Biology of the Cell* (© Garland Science 2008)

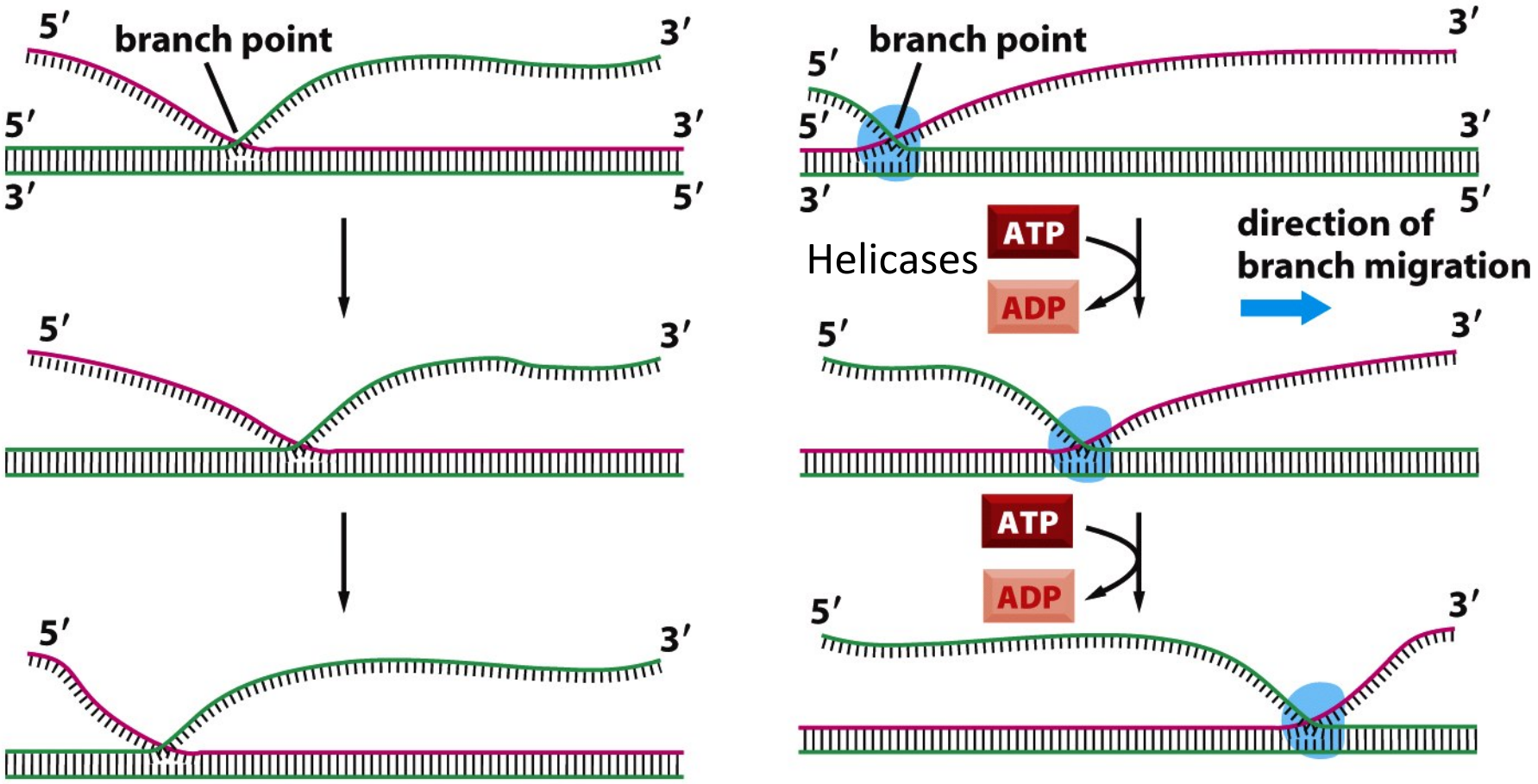
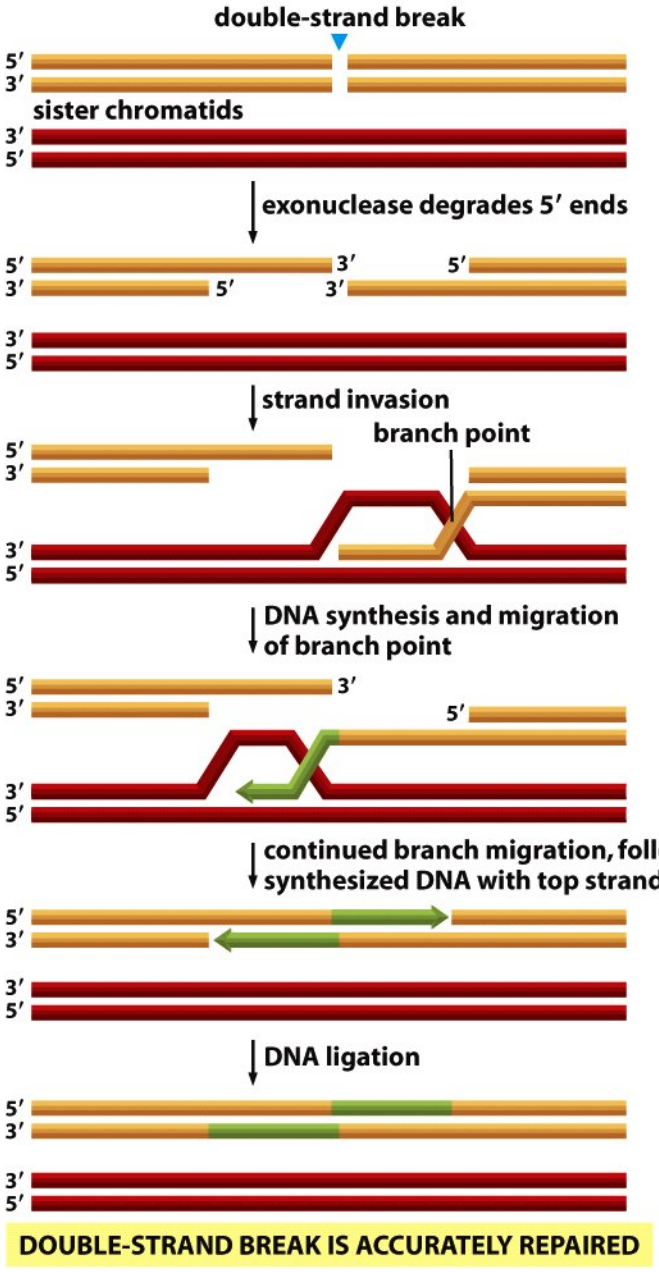


Figure 5-58 *Molecular Biology of the Cell* (© Garland Science 2008)

Repair of double strand brake



-> recombinogenic ssDNA

RecA/Rad51

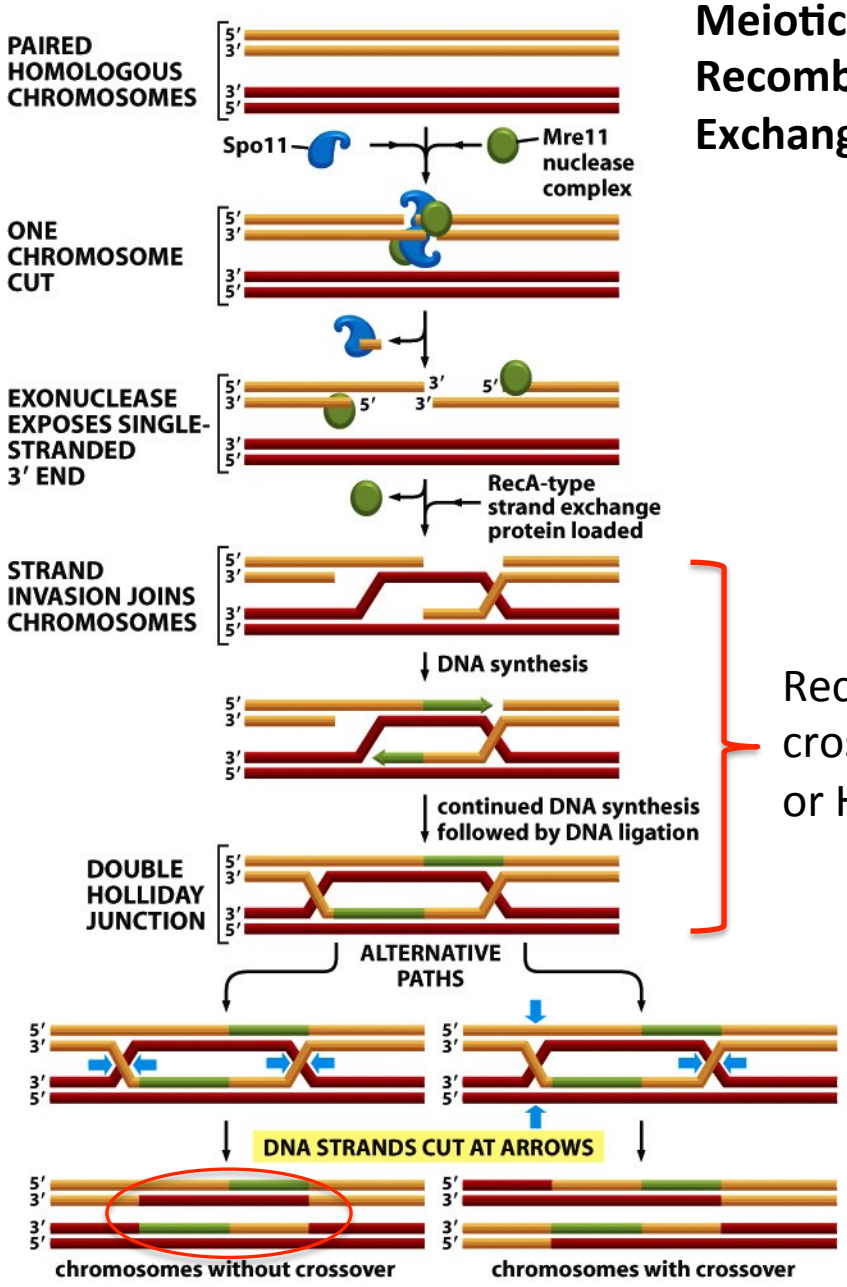
Helicase, DNA polymerase

Gene conversion: template mediated repair using the homologous dsDNA: repaired region corresponds to template
-> loss of heterozygosity: assuming red and yellow were different, after the repair both strands are the same (same as red)

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

Meiotic homologous Recombination: Exchange of genetic information

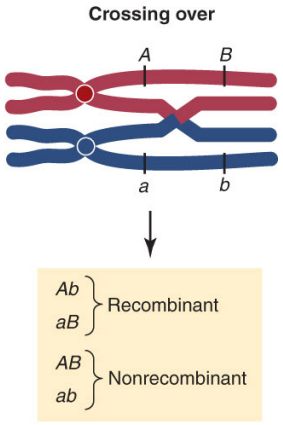
Spo1 induces DSB



Reciprocal strand invasion: cross-strand exchange or Holliday junction

90%

10%



Hetero duplex:
can lead to gene conversion

Figure 5-64 *Molecular Biology of the Cell* (© Garland Science 2008)

RuvA and RuvB: promote branch migration of cross over (Holliday junction)
Endonuclease RuvC cleaves Holliday junction (resolution)

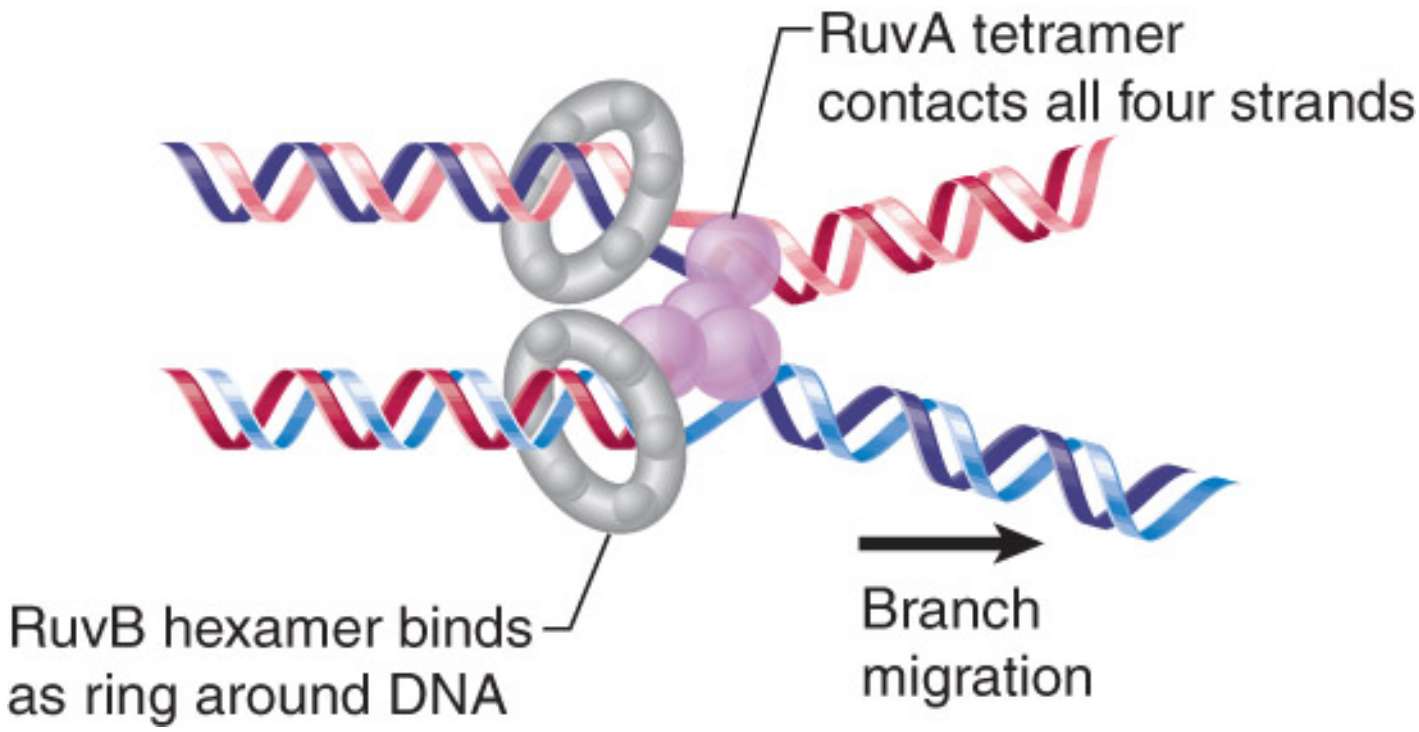
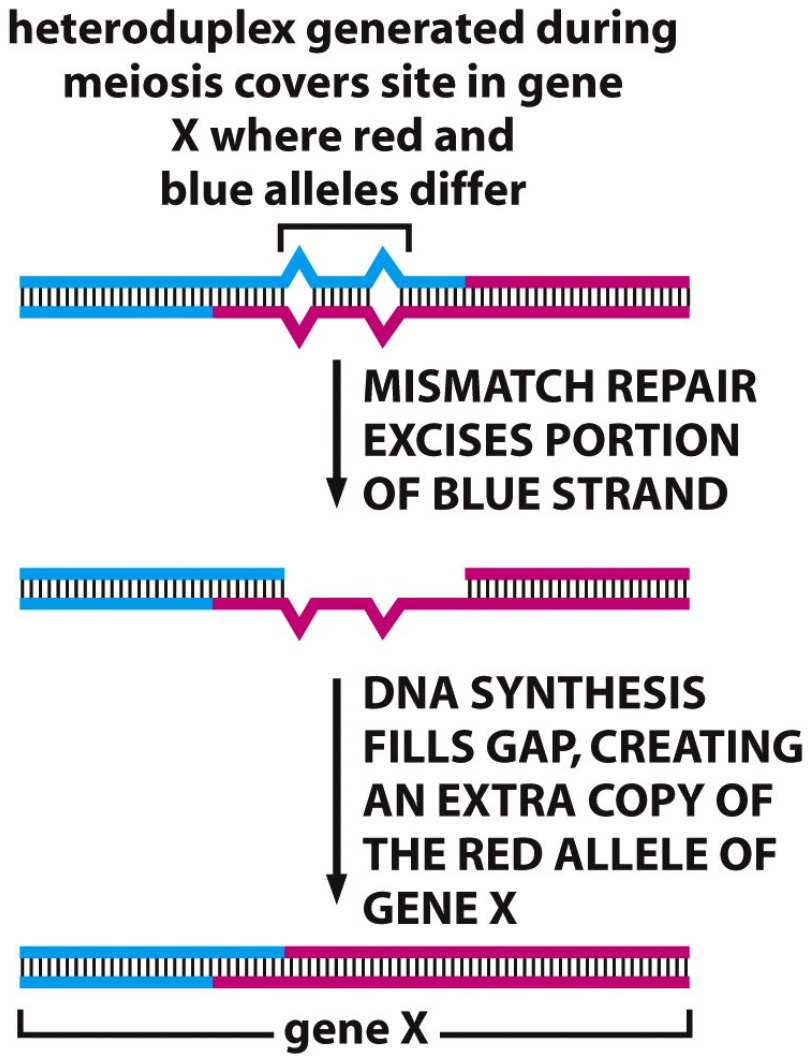


Figure 5-62 *Molecular Biology of the Cell* (© Garland Science 2008)

Heteroduplex repair and gene conversion



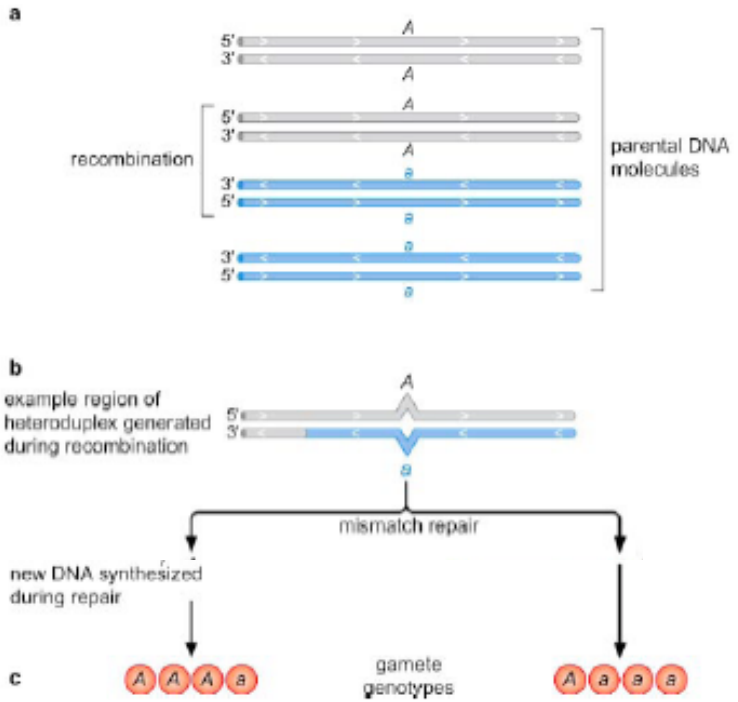


Figure 5-66 *Molecular Biology of the Cell* (© Garland Science 2008)

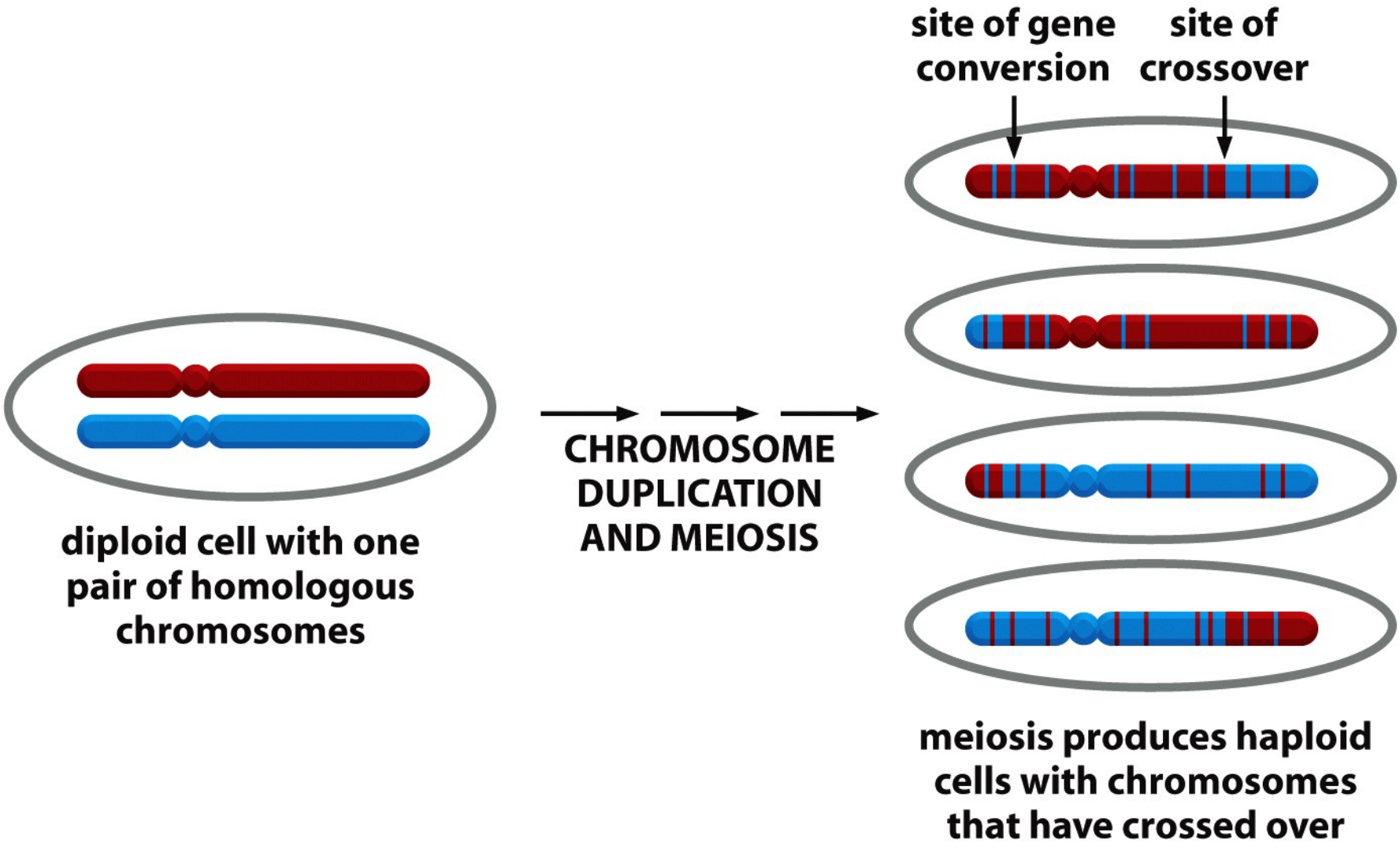
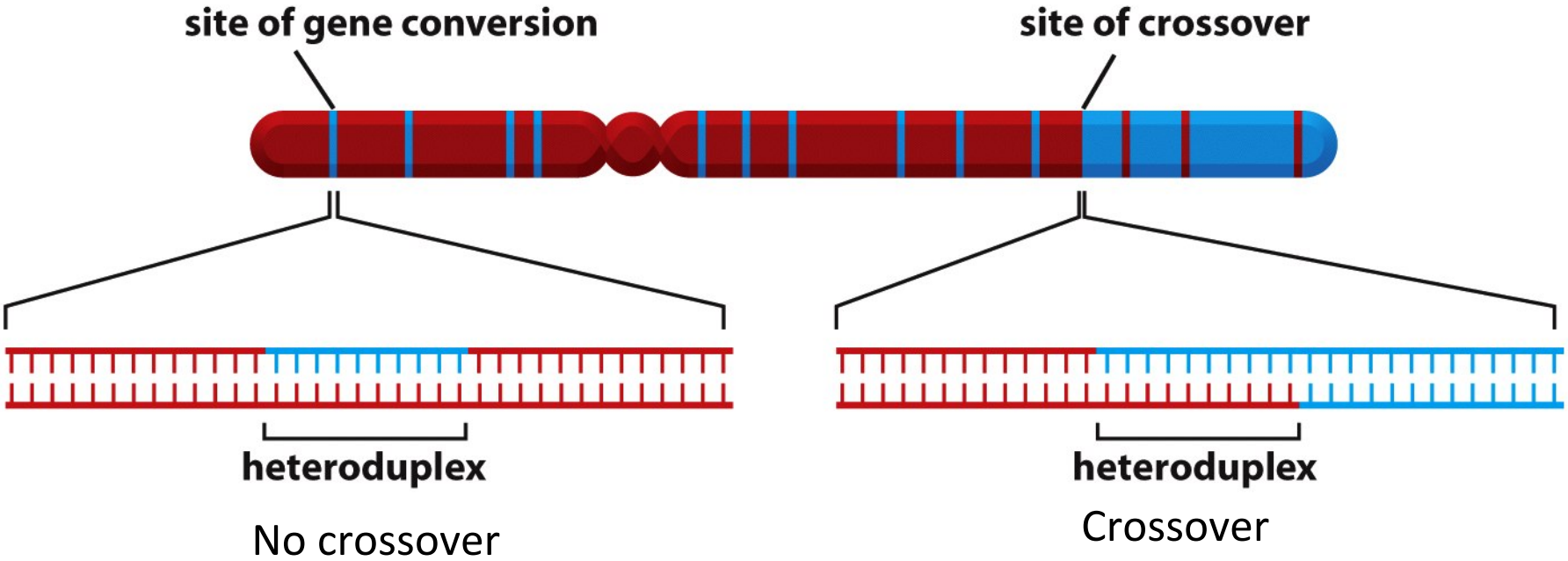


Figure 5-63 *Molecular Biology of the Cell* (© Garland Science 2008)



- Homologous recombination always results in heteroduplex (length is determined by extend of branch migration)
- Resolution of holliday junction determines whether crossover occurs

Figure 5-65 *Molecular Biology of the Cell* (© Garland Science 2008)

Components of the mismatch repair system prevent recombination between poorly matched sequences

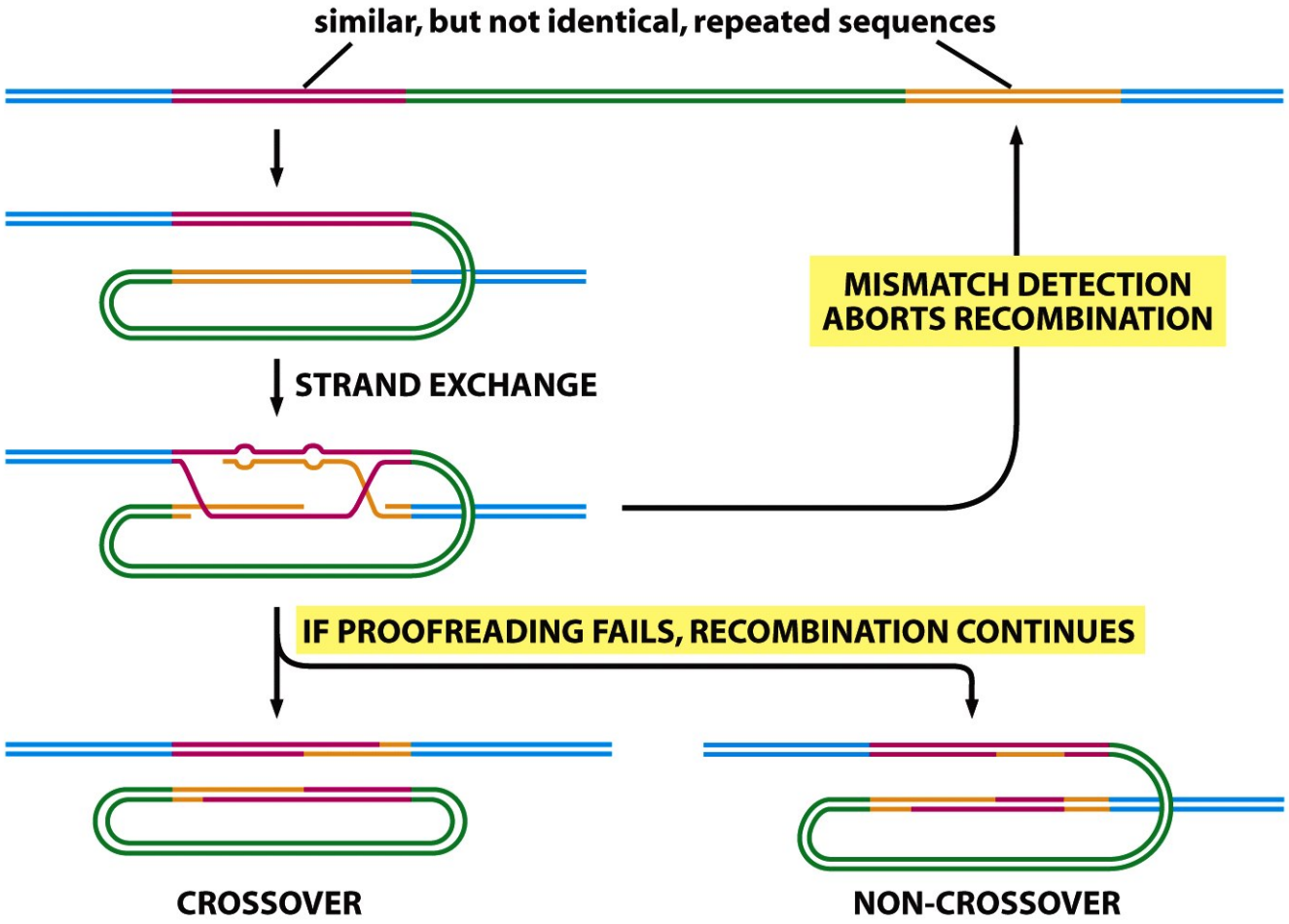
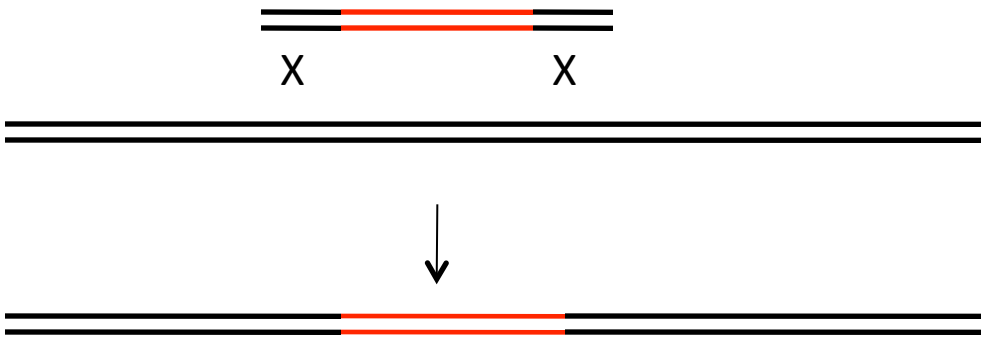


Figure 5-67 *Molecular Biology of the Cell* (© Garland Science 2008)

Homologous recombination as a tool to manipulate genomes



Introduce the DNA into the genome of interest by homologous recombination

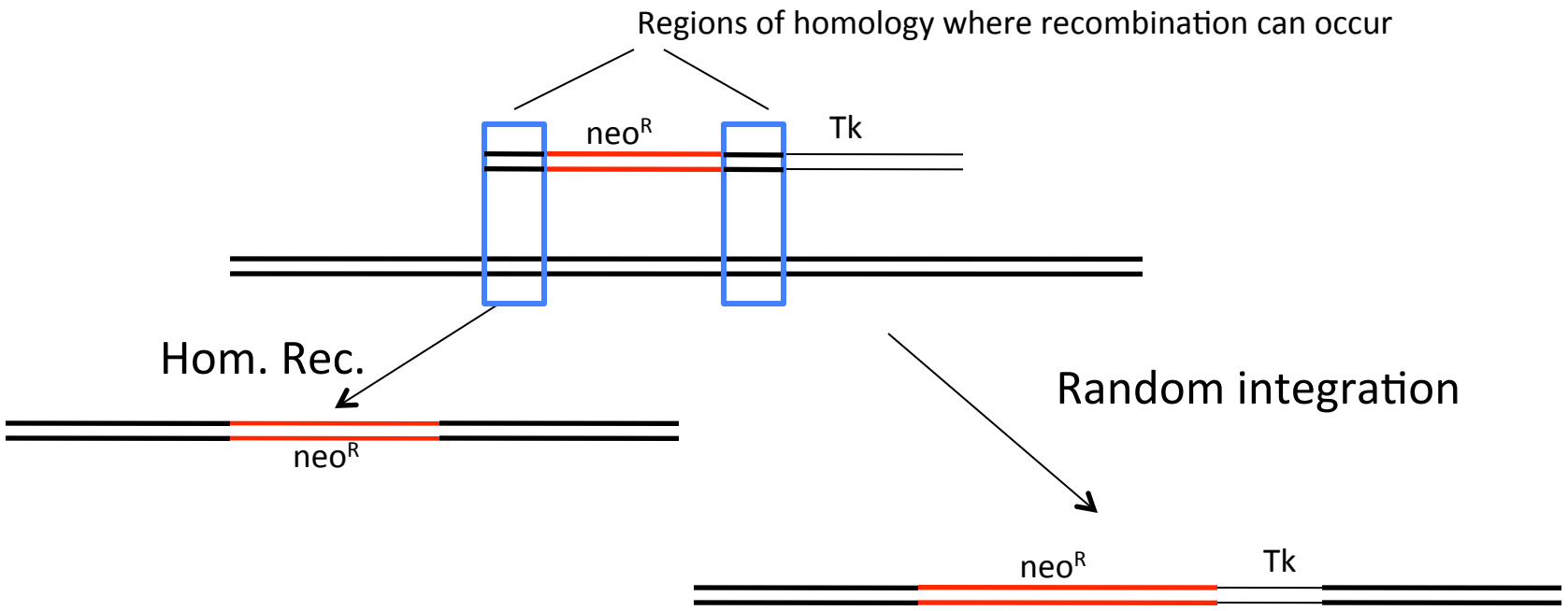
Bring the template DNA into the nucleus (injection, electroporation)

Select for the cell where homologous recombination took place (selectable marker: antibiotics resistance, fluorescence). Rare event, estimate: 0.5×10^6/cell

Make an organism of the cell with the manipulated genome, -> requires totipotent cells

-> embryonic stem cells (ES cells)

Positive/negative selection for a homologous recombination event



neo^R

neo^{R,} Tk$^+$
Tk: viral thymidine kinase, can convert gancyclovir into cell toxic drug (chain termination during replication)

Selection with neomycin and gancyclovir:

cell growth **cell death**

1 : 500'000

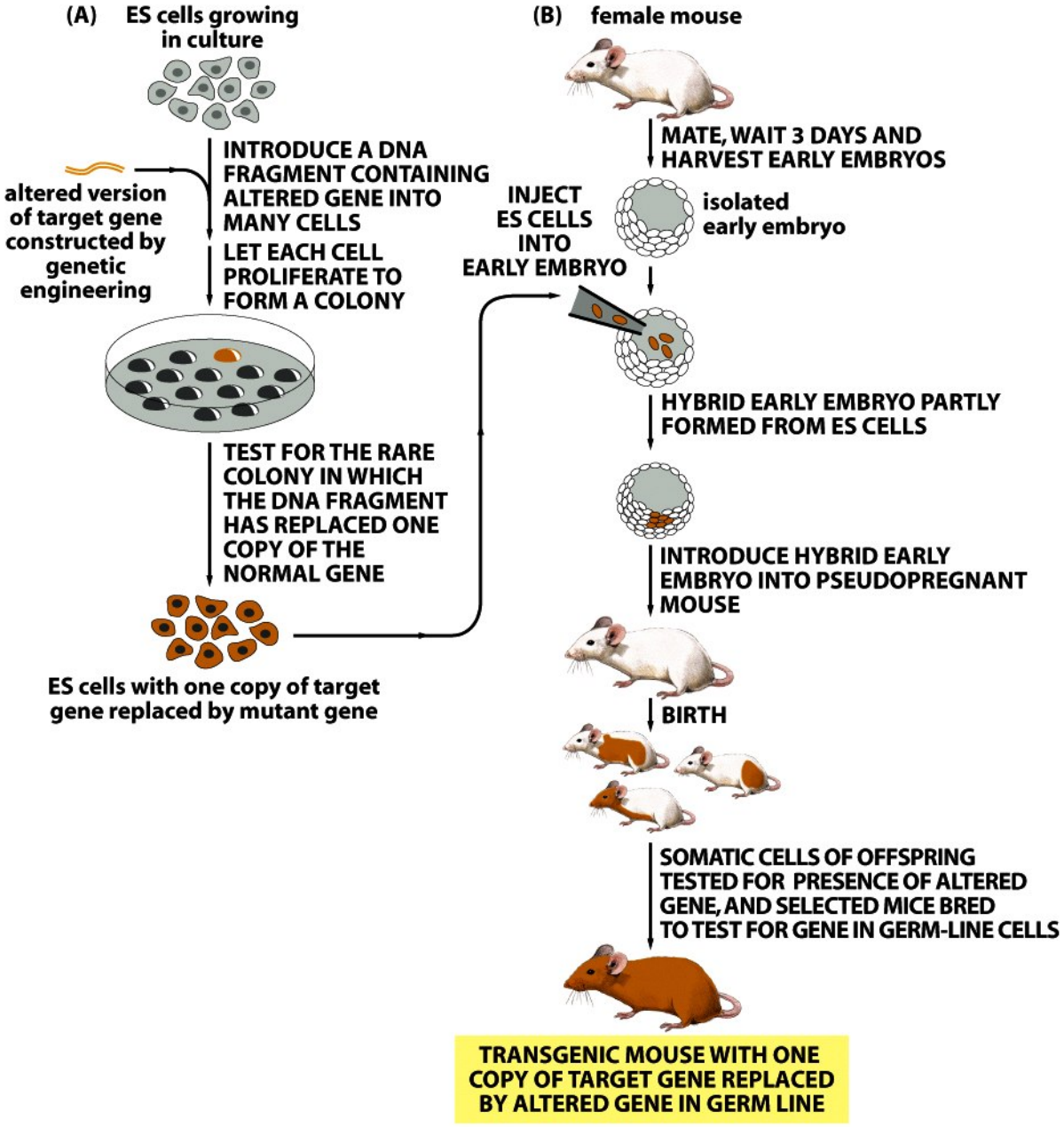


Figure 8-65 *Molecular Biology of the Cell* (© Garland Science 2008)

Conservative site specific recombination

- requires specific sequences (target sites for the recombinases)
- requires specific enzymes (recombinases); resemble topoisomerases I since they form transient covalent bonds with the DNA. Require no energy. Reversal of the integration (excision) restores original sequence (-> conservative).

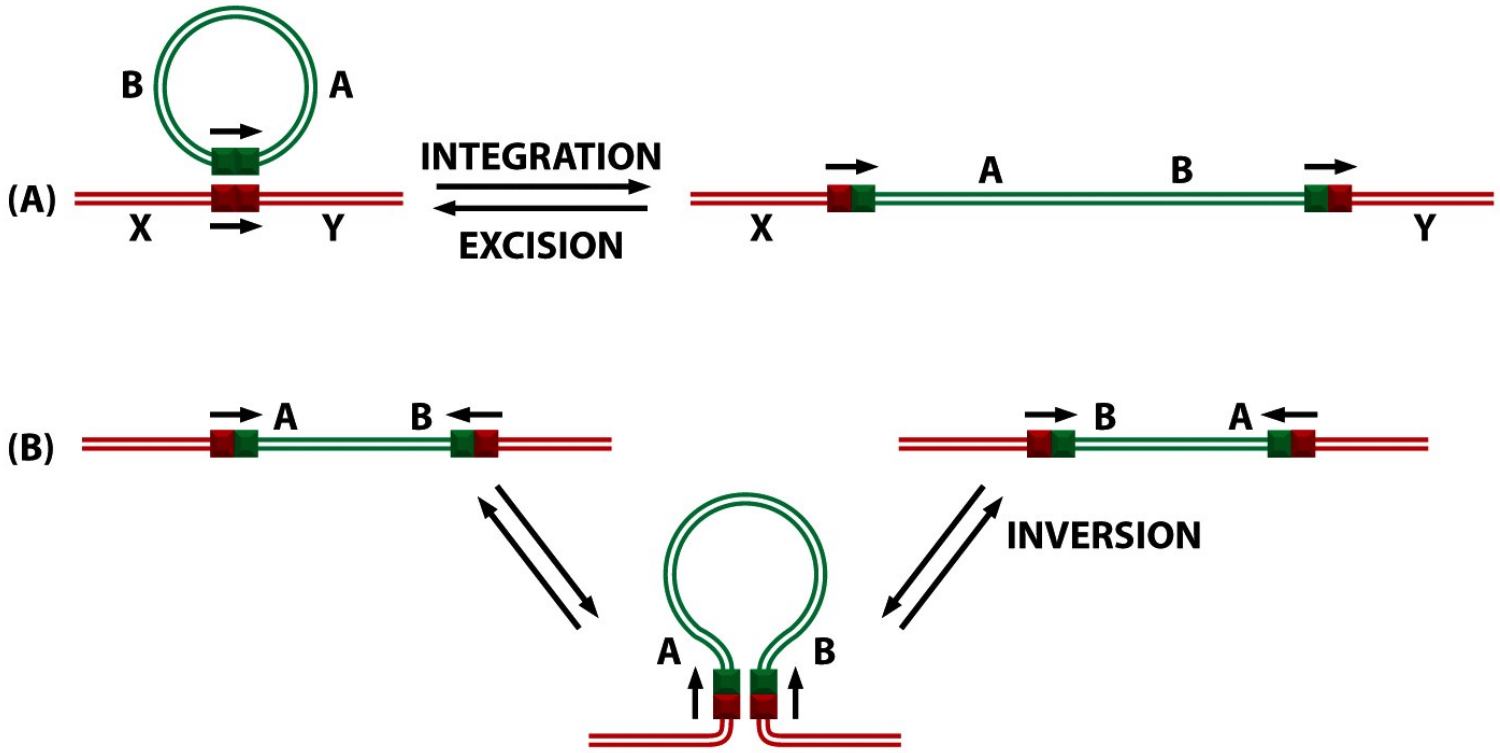
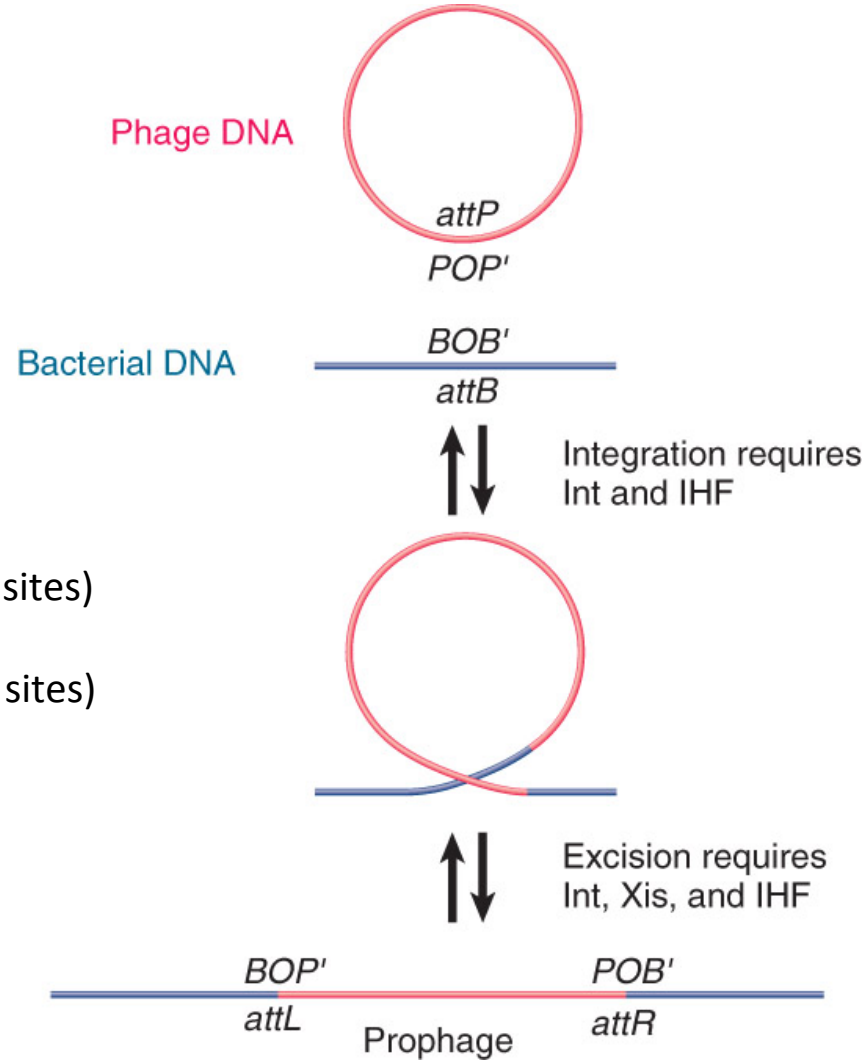


Figure 5-76 *Molecular Biology of the Cell* (© Garland Science 2008)

Phage λ genome integration into the E.coli host genome (lysogenic pathway)

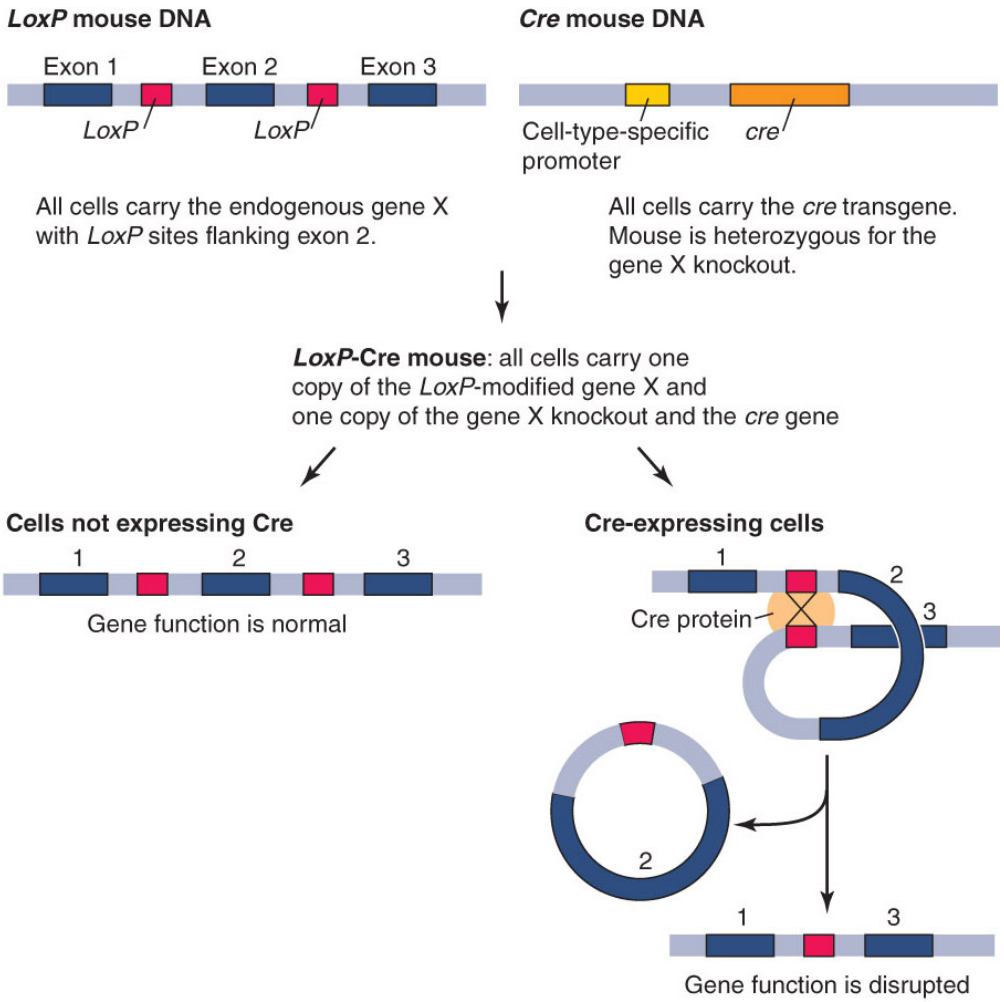
Integration: Int and IHF (attP, attB rec. sites)

Excision: Int, Xis and IHF (attl, attR rec. sites)



Cre/lox system for conditional knockouts in mouse

Phage P1 Cre recombinase recognizes and cleaves lox sites (34bp: 2 x 13 bp inverted repeat with 8bp spacer)



Adapted from H. Lodish, et al. Molecular Cell Biology, Fifth edition. W. H. Freeman & Company, 2003.