Felix Loosli
ITG/KIT (Karlsruhe)
Developmental neurobiology of vertebrates

Introduction to Genetics

DNA Replication/Repair/Recombination

Molecular biology of the cell, 5th ed. (Alberts et al.)
Genes X (Lewin et al.)

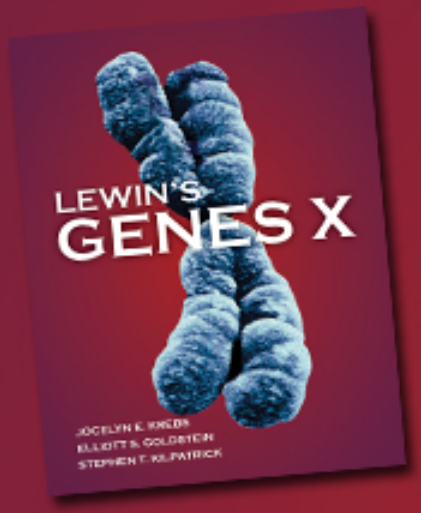
Alberts • Johnson • Lewis • Raff • Roberts • Walter

Molecular Biology of the Cell

Fifth Edition

Chapter 5

DNA Replication, Repair, and Recombination



JOCELYN E. KREBS
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LEWIN'S GENES X

Chapter 11-16

Replication:

- Basic molecular mechanism of DNA replication
  - proteins/activities
  - consequences for DNA replication
- How are chromosomes replicated

Repair:

- Basic molecular mechanism of different types of DNA repair

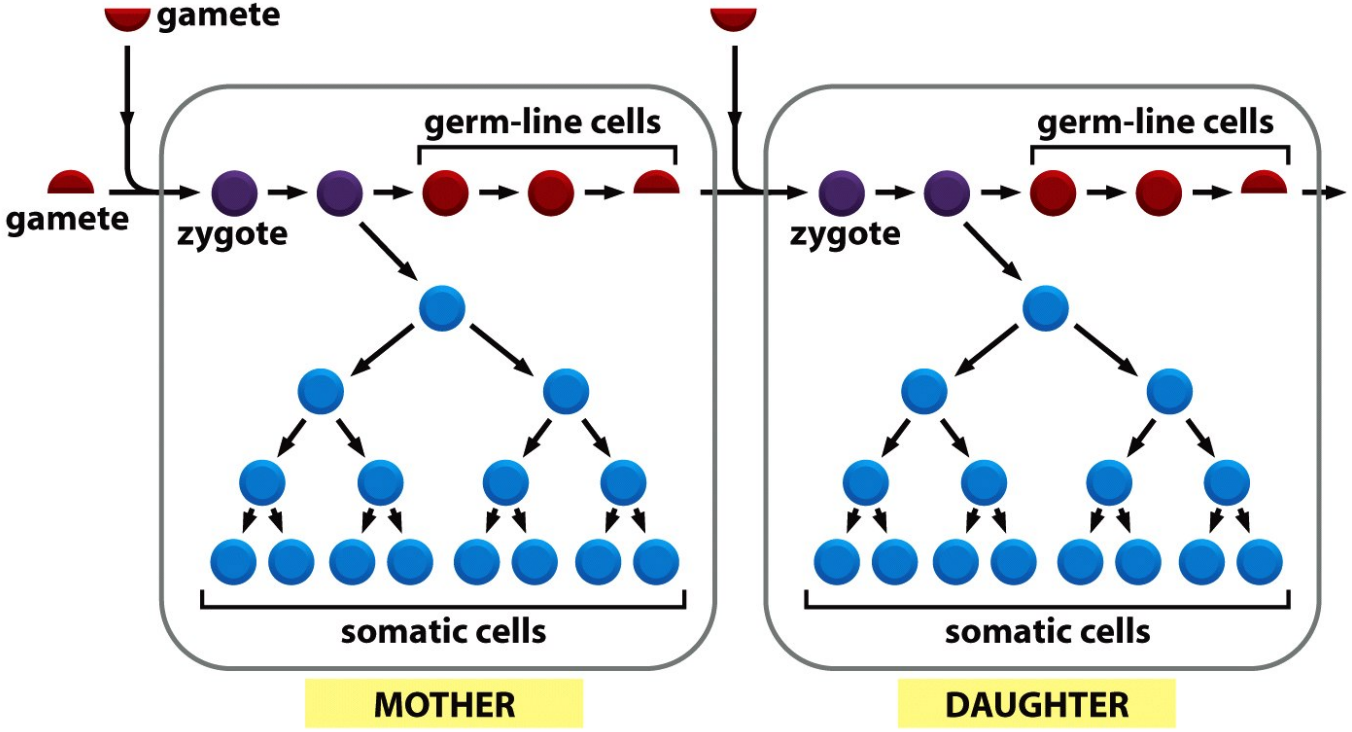
Homologous recombination

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

Site specific recombination

- applications

DNA replication must be very accurate to prevent accumulation of mutations
Somatic cells: important for individual; risk of developmental defects, mal-functions, cancer
Germ cells: to maintain population/species



Procaryote/eucaryote DNA polymerases: $1/10^9$ nucleotides changes per replication

Figure 5-1 *Molecular Biology of the Cell* (© Garland Science 2008)

DNA replication is semi-conservative:
In every replication new single strands are synthesized, old single strands are preserved

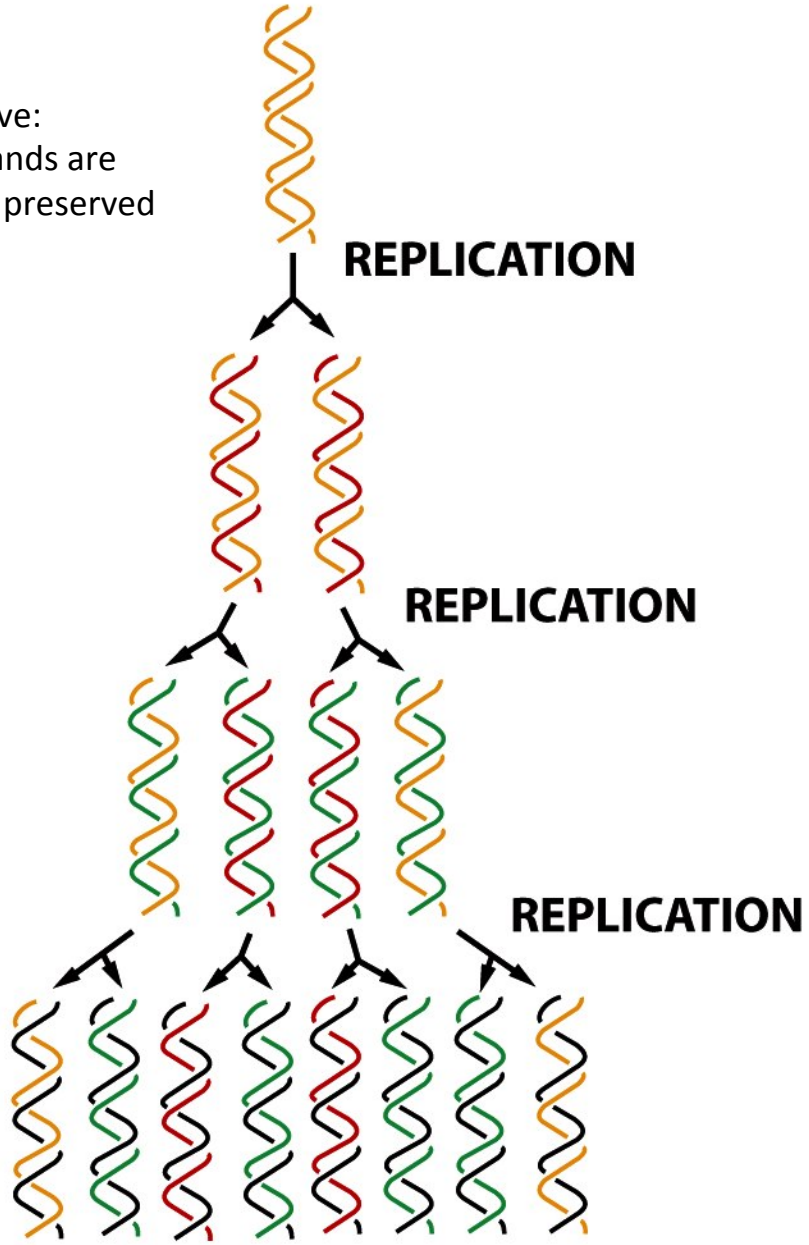
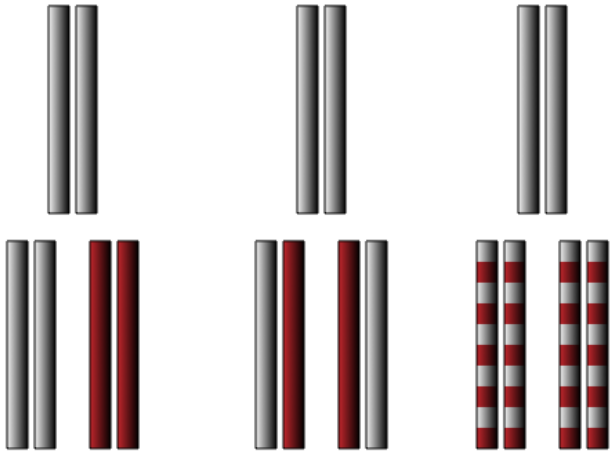
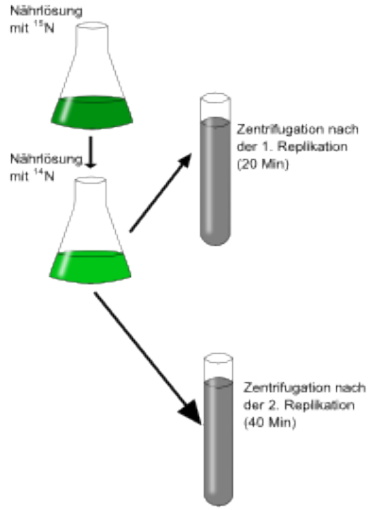


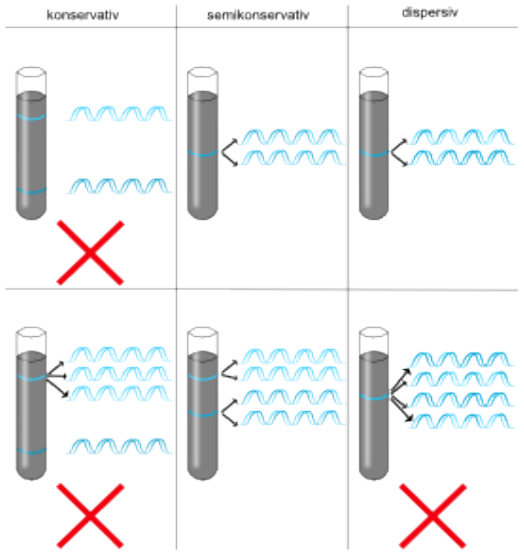
Figure 5-5 *Molecular Biology of the Cell* (© Garland Science 2008)

Experimental proof for semiconservative DNA replication using ^{15}N by Meselson and Stahl

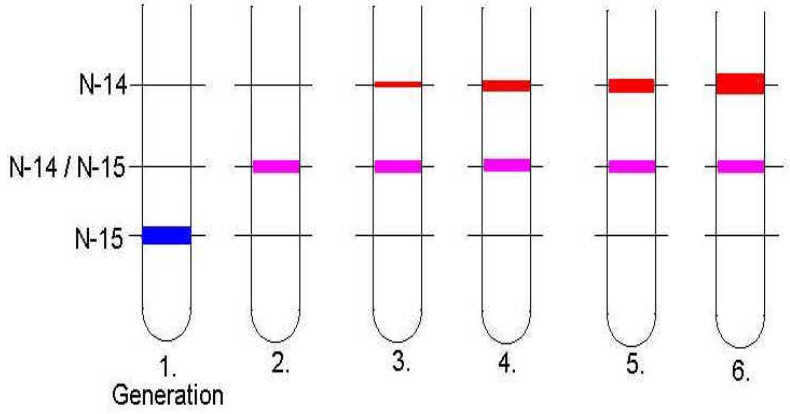


Cons. Semi-cons. dispers





Old DNA: ^{15}N -> heavy
New DNA: ^{14}N -> light



- DNA replication is semi-conservative: each DNA single strand serves as a template for the synthesis of a complementary new strand
- Base-pairing underlies template-based synthesis

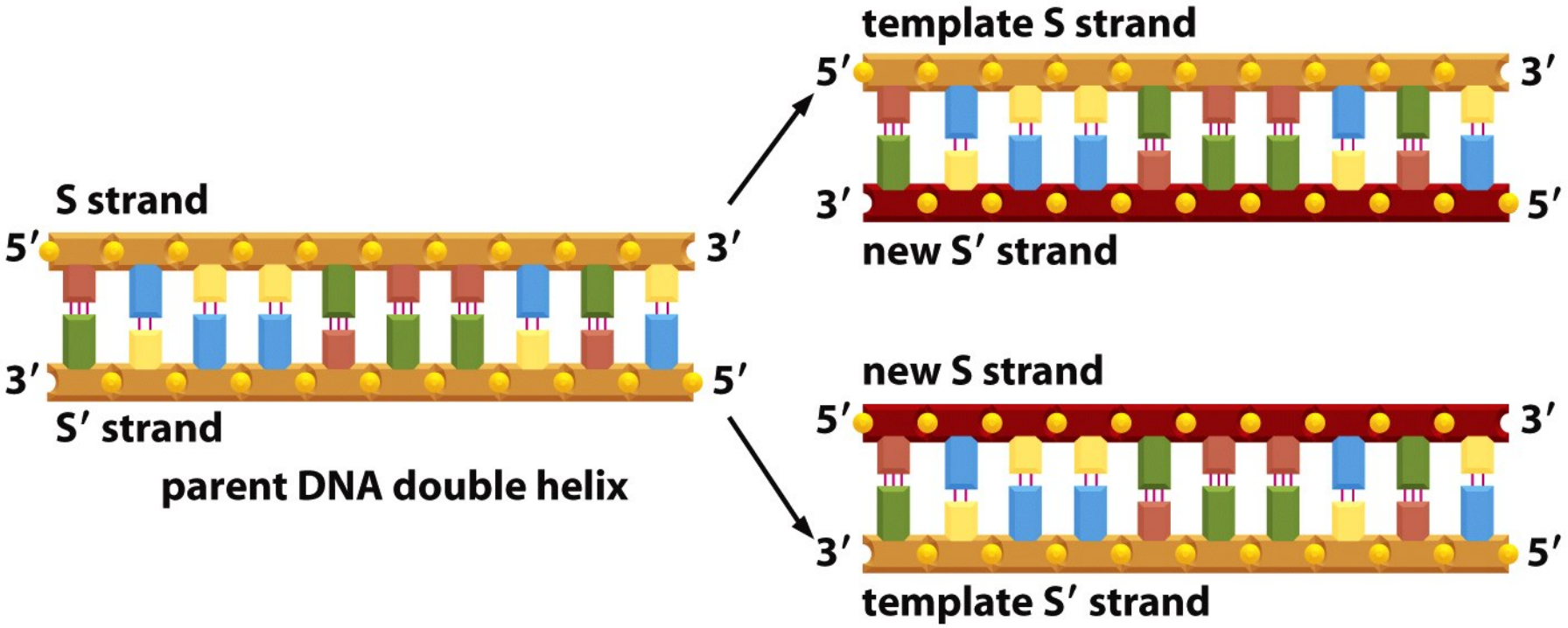
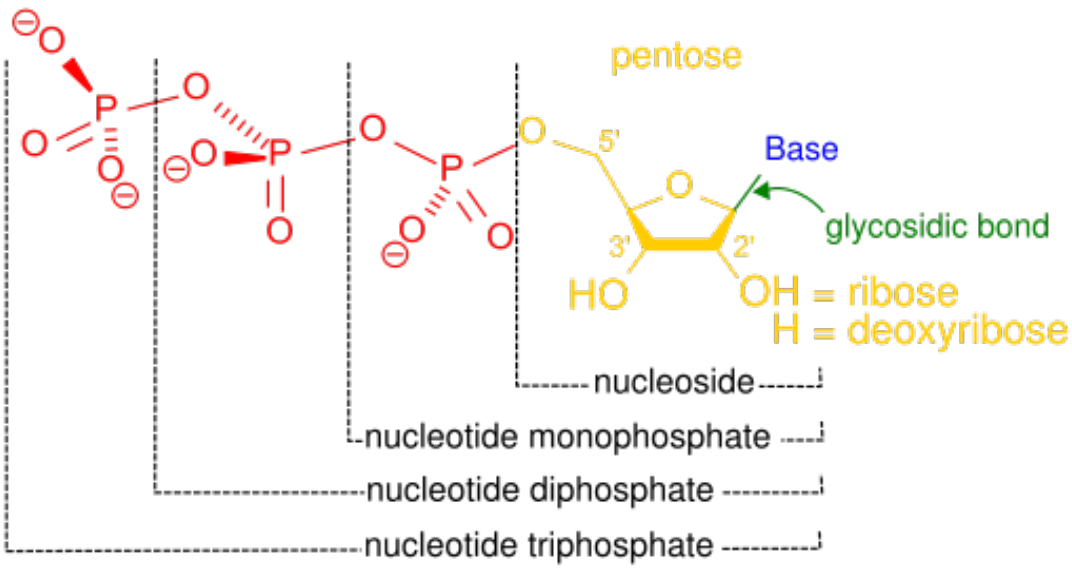
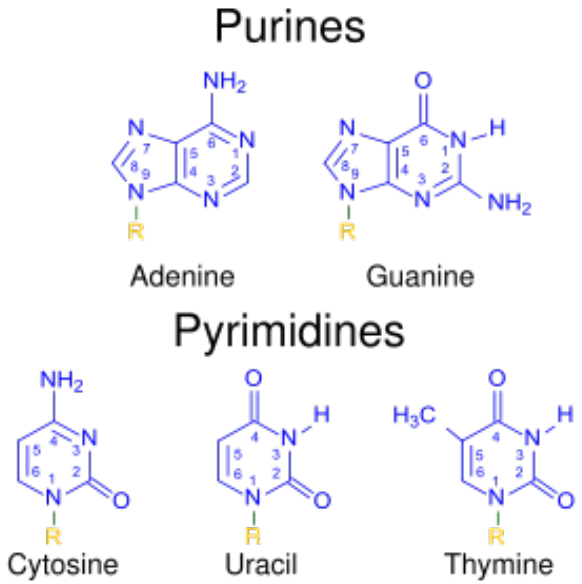
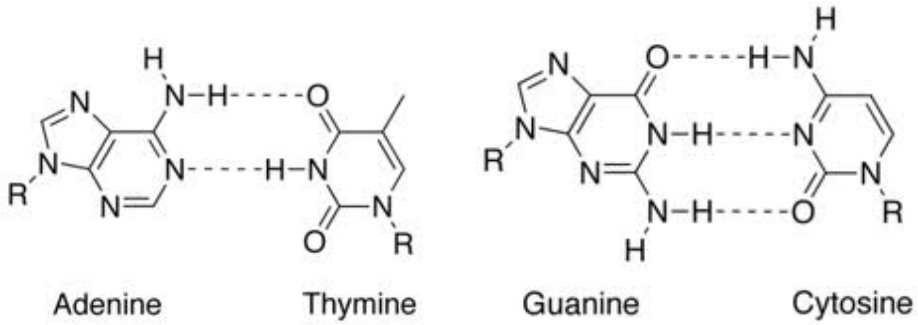


Figure 5-2 *Molecular Biology of the Cell* (© Garland Science 2008)

ACGTU nucleotides

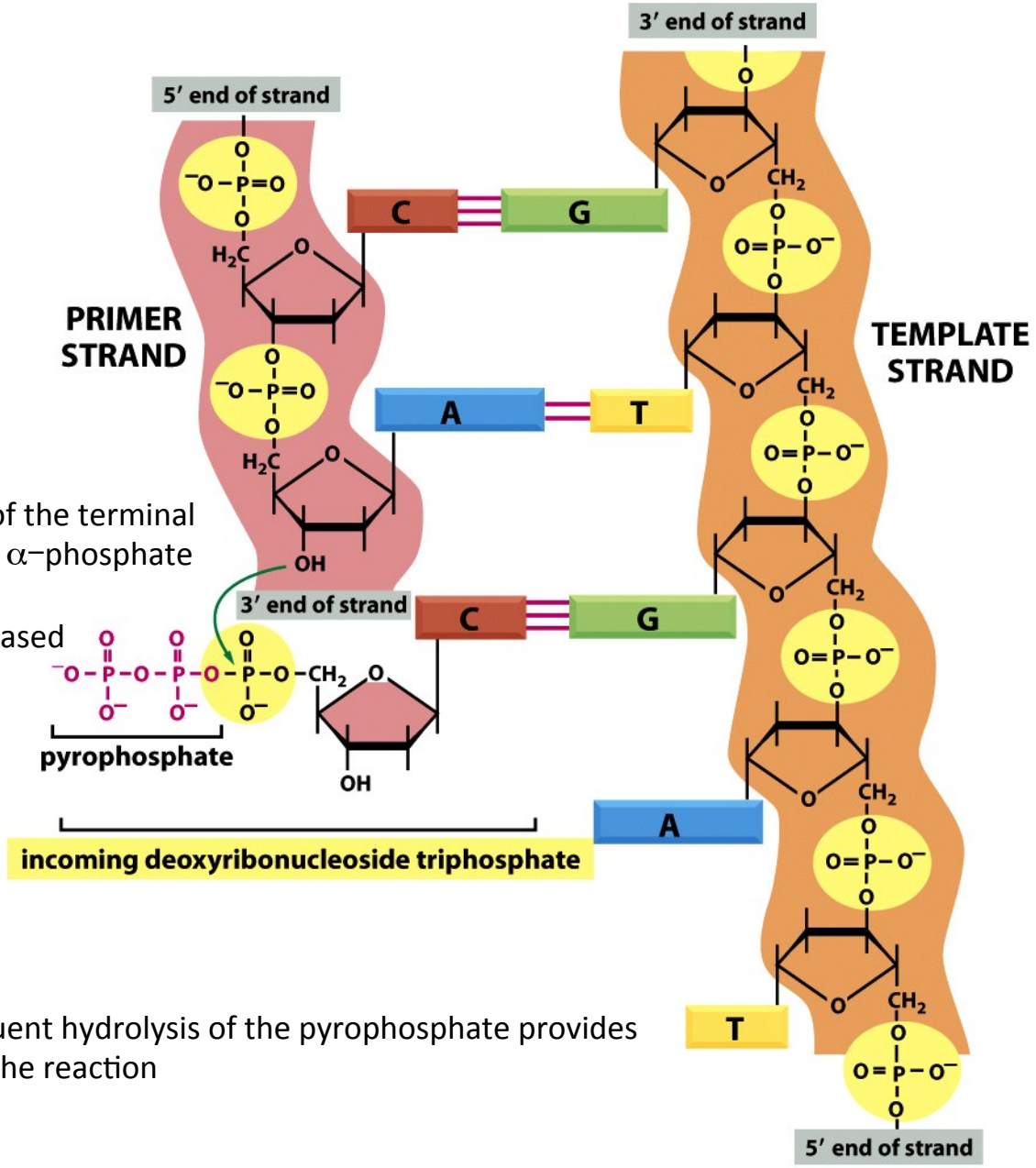






Adenin base -> adenosin nucleosid
Guanine -> guanosin
Cytosine -> cytidin
Thymine -> thymidin
Uracil ->uridin

Nucleophilic attack of the terminal 3′ –OH group on the α–phosphate of the nucleotide; pyrophospate is released



Release and subsequent hydrolysis of the pyrophosphate provides the energy to drive the reaction

Figure 5-3 *Molecular Biology of the Cell* (© Garland Science 2008)

DNA synthesis catalyzed by DNA polymerases

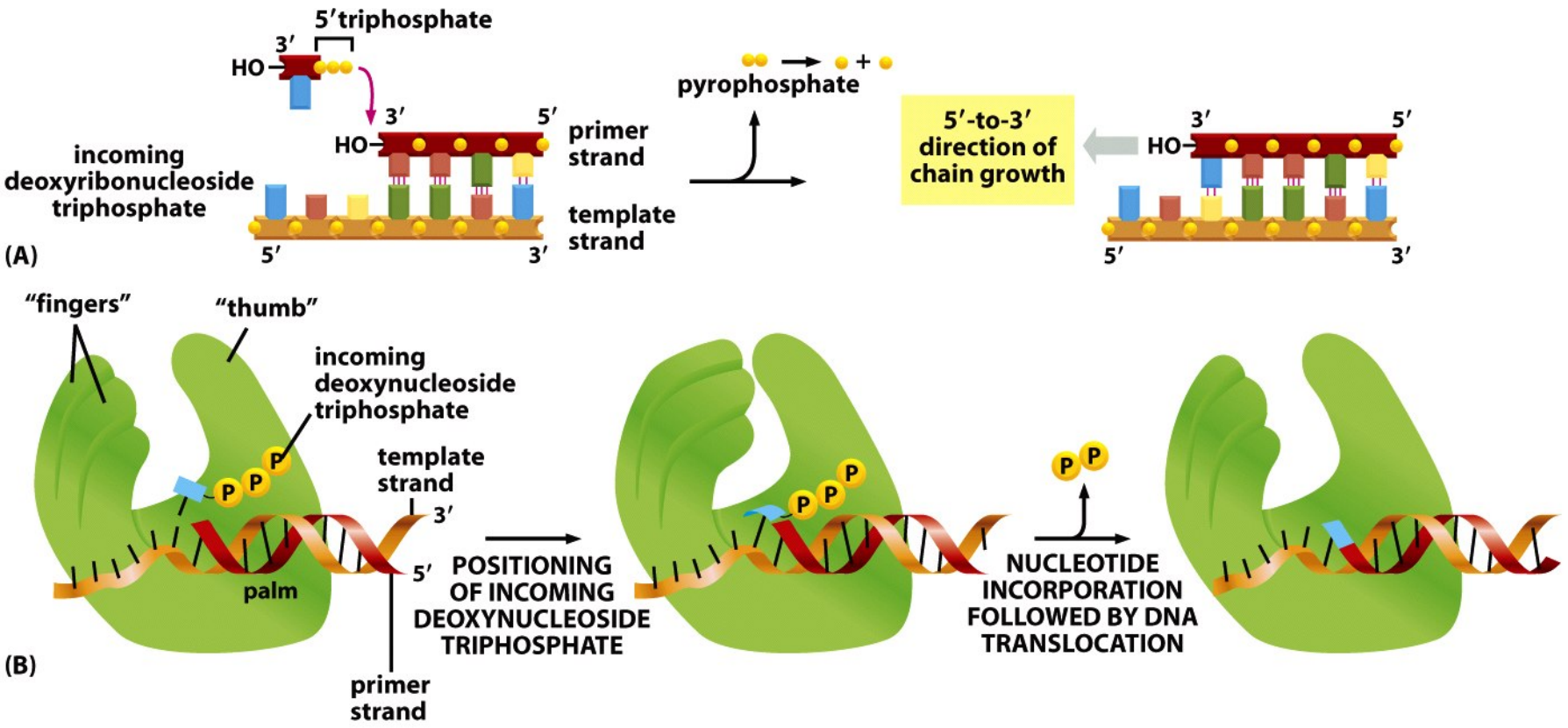
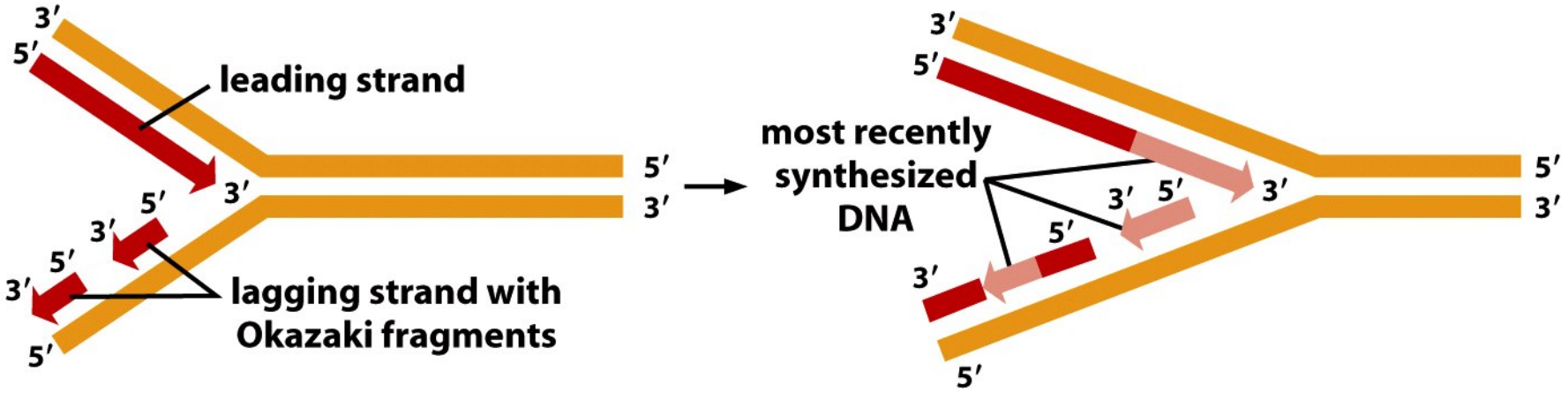


Figure 5-4 *Molecular Biology of the Cell* (© Garland Science 2008)

All known DNA polymerases can synthesis DNA only in 5'-> 3' direction!!
Therefore the replication fork is asymmetric



Leading strand: direction of synthesis and replication fork are the same
Lagging strand: synthesis and replication fork move in opposite directions

Figure 5-7 *Molecular Biology of the Cell* (© Garland Science 2008)

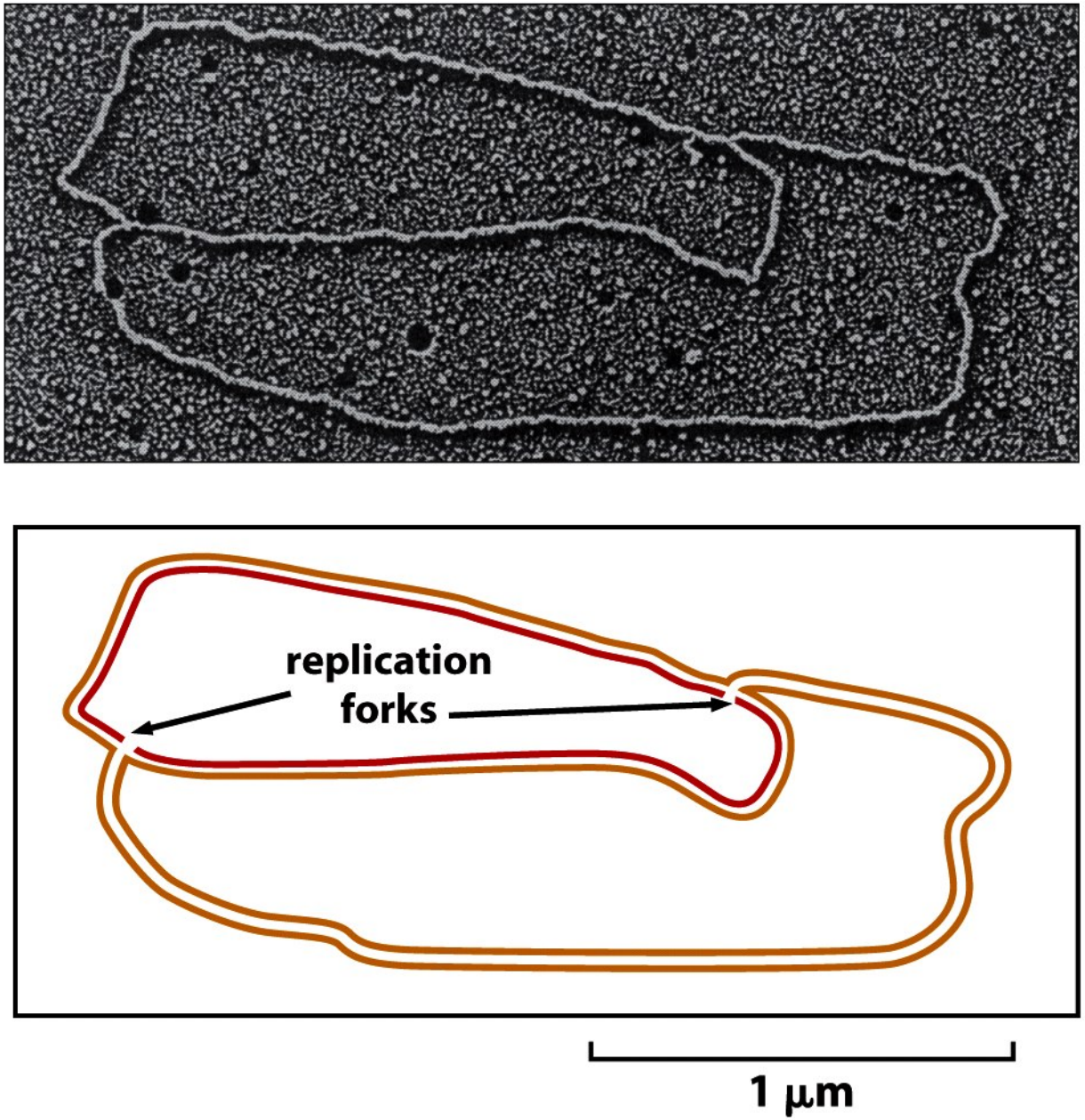
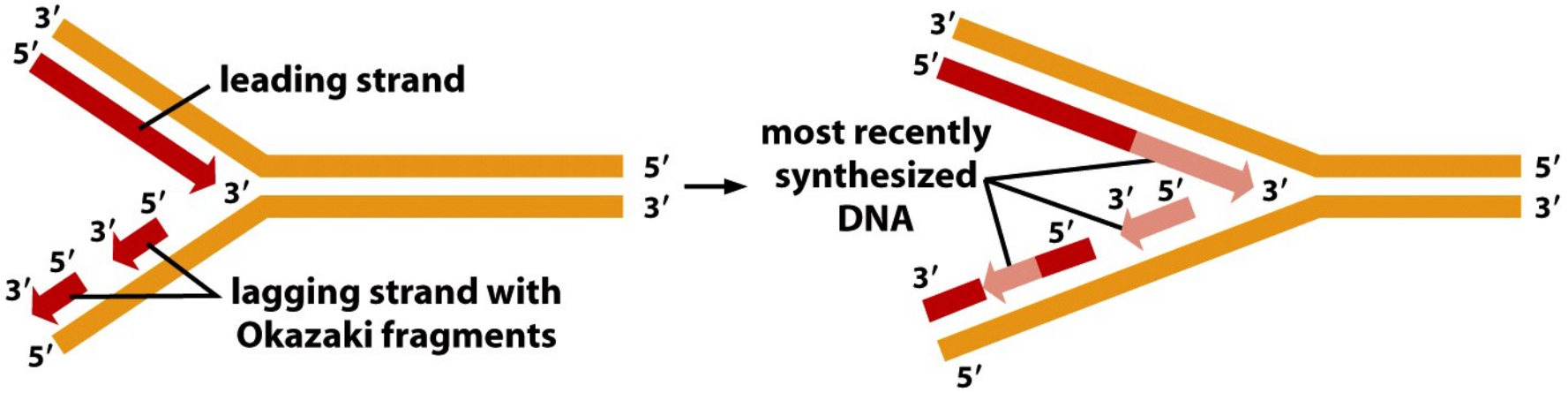


Figure 5-6 *Molecular Biology of the Cell* (© Garland Science 2008)

All known DNA polymerases can synthesis DNA only in 5'-> 3' direction!!
Therefore the replication fork is asymmetric



All known DNA polymerases require a 3' hydroxyl group for the DNA synthesis
-> No de novo synthesis! Only strand elongation!
Therefore a 'primer' that provides the 3'-OH is required!!

Figure 5-7 *Molecular Biology of the Cell* (© Garland Science 2008)

All known DNA polymerases require a 3’ hydroxyl group for the DNA synthesis
Therefore a ‘primer’ (RNA, approx. 10bp) is required!!

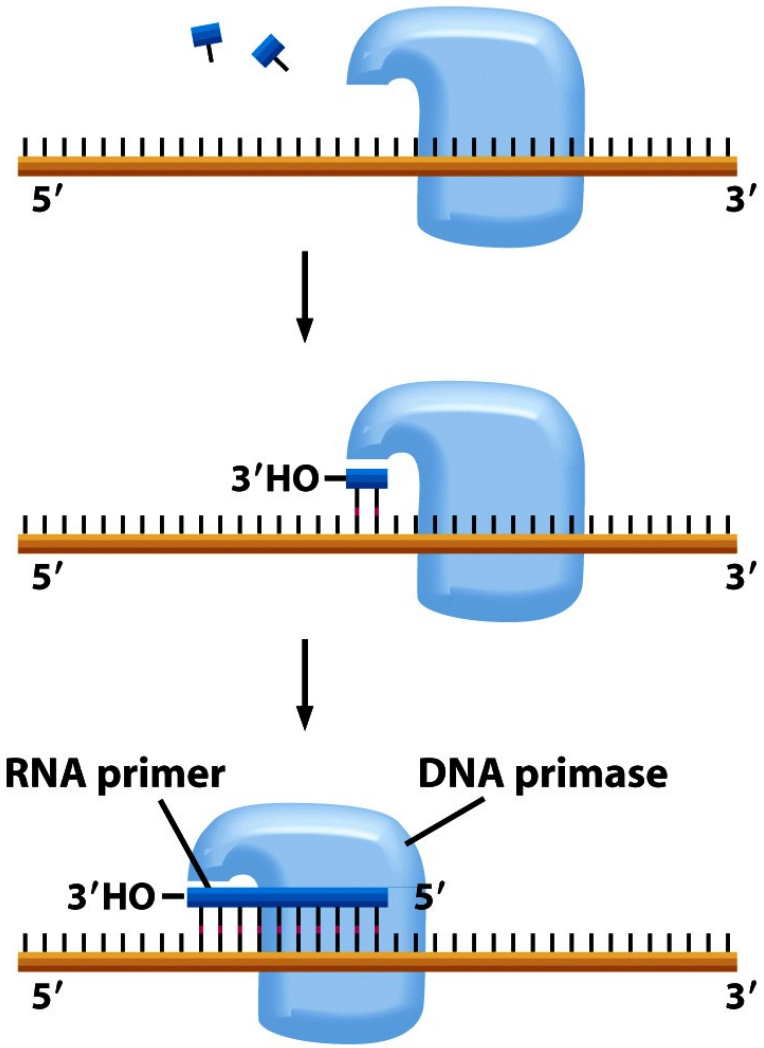
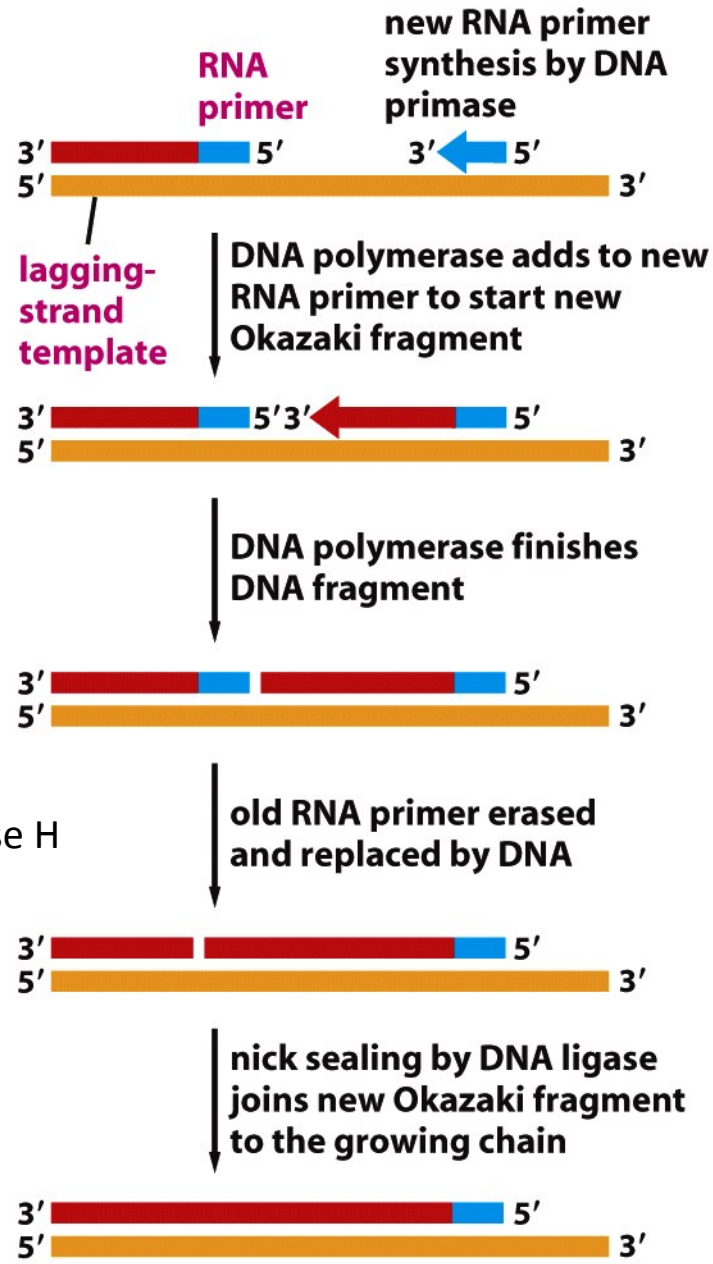


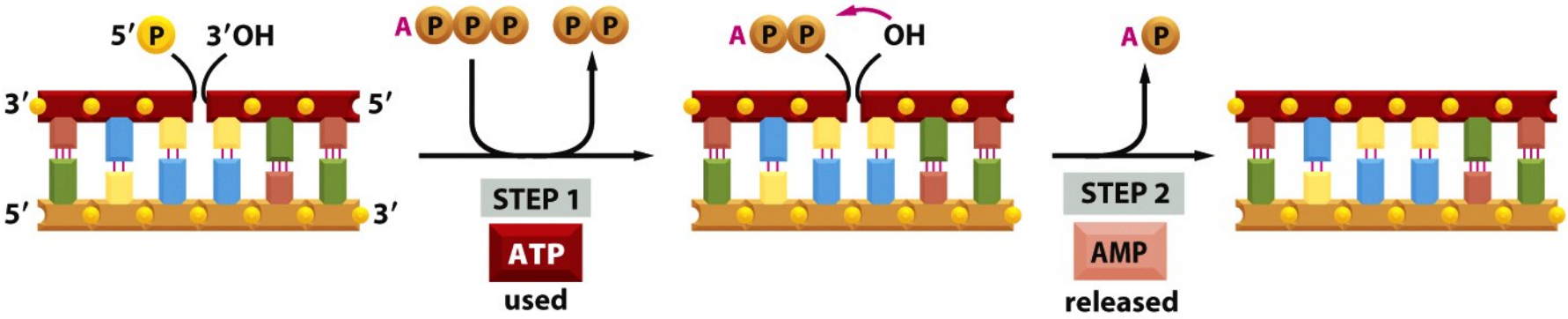
Figure 5-11 *Molecular Biology of the Cell* (© Garland Science 2008)



Low fidelity of primase
->RNA primers are removed
By RNAse H (DNA-RNA hybrids)

Figure 5-12 *Molecular Biology of the Cell* (© Garland Science 2008)

DNA ligase seals the nicks in the lagging strand



Lagging strand:
Primase sets primer, DNApol synthesises fragment, RNAseH removes primer, ligase repairs nick

Figure 5-13 *Molecular Biology of the Cell* (© Garland Science 2008)

High fidelity of DNA replication requires several proofreading mechanisms

1) Correct nucleotide has higher affinity for moving DNA pol, because correct pairing is favorable to incorrect ones (Watson-Crick base-pairing). After binding, correct nucleotide allows DNA pol. to adopt conformational change for covalent incorporation more easily than incorrect ones.

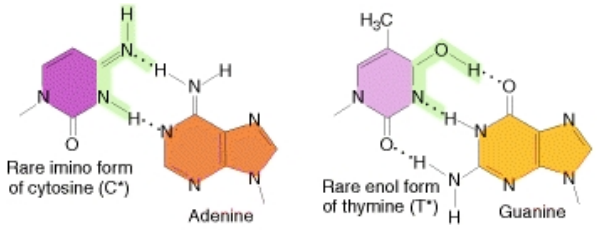
2) Exonucleolytic proofreading (3'->5' proofreading): excision of an incorrect nucleotide at the 3' end that cannot serve as a primer for further synthesis

Table 5–1 The Three Steps That Give Rise to High-Fidelity DNA Synthesis

REPLICATION STEP	ERRORS PER NUCLEOTIDE
5′ → 3′ polymerization	1 in 10^5
3′ → 5′ exonucleolytic proofreading	1 in 10^2
Strand-directed mismatch repair	1 in 10^2
Combined	1 in 10^9

Exonucleolytic proofreading (3'->5' proofreading)

Normal Watson-Crick base-pairing



Rare (instable) tautomeric forms

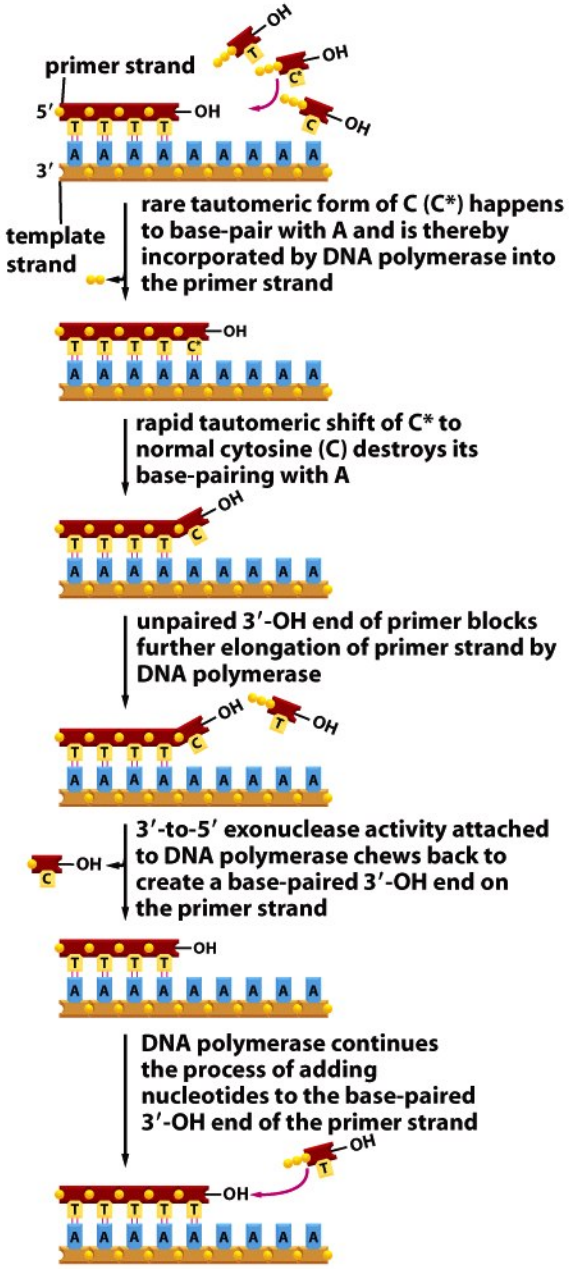


Figure 5-8 *Molecular Biology of the Cell* (© Garland Science 2008)

Separate catalytic sites for synthesis and editing

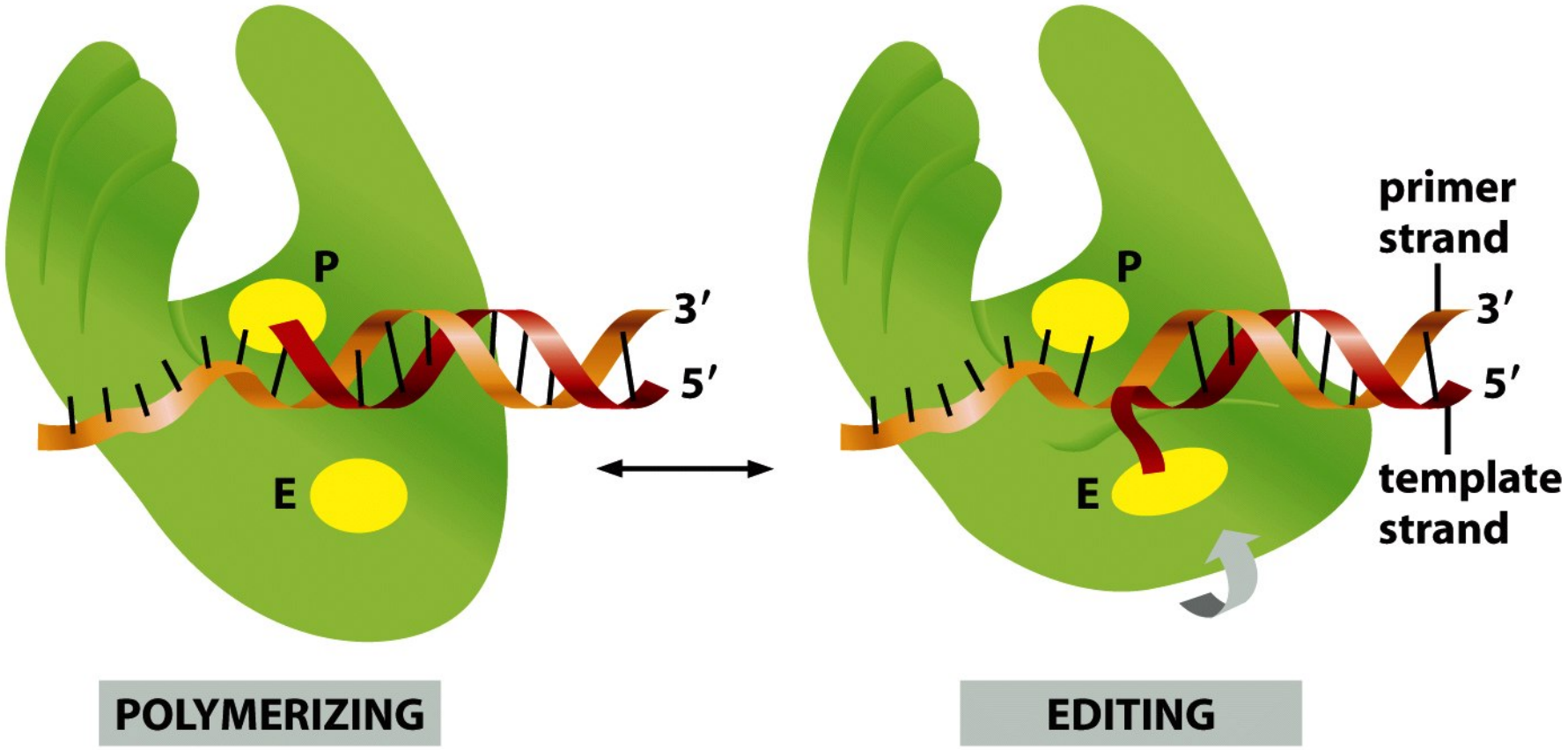
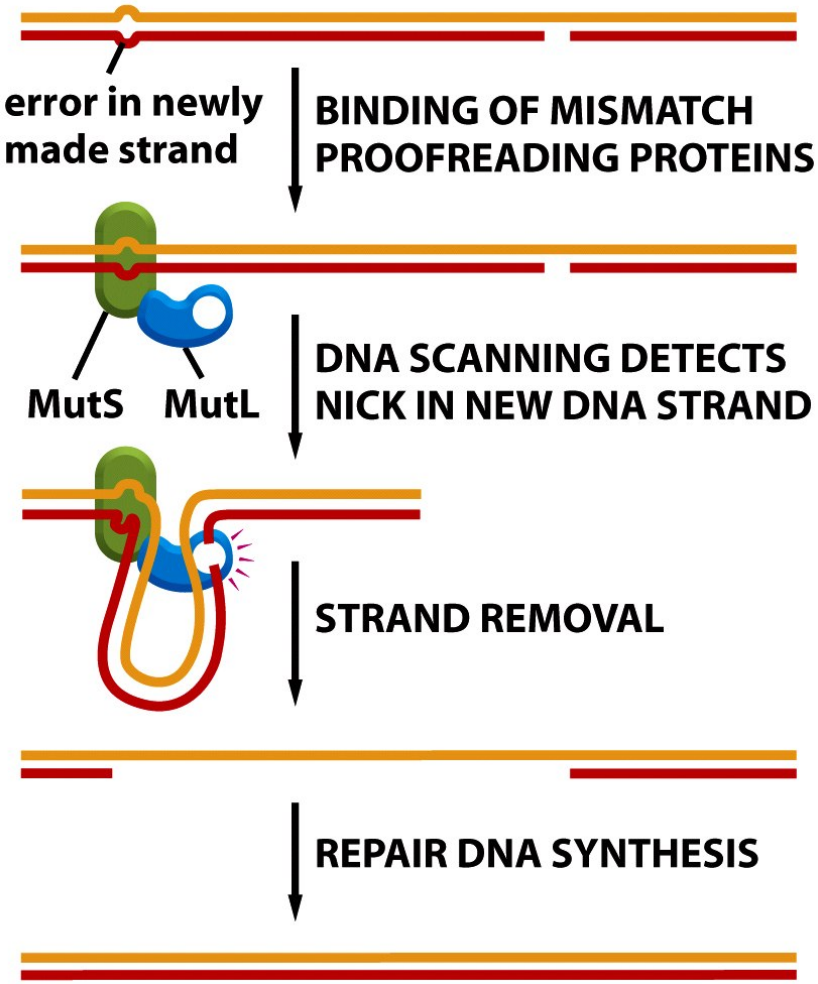


Figure 5-9 *Molecular Biology of the Cell* (© Garland Science 2008)



Heterozygocity in mismatch repair genes
results in increased cancer incidence
(loss of heterozygocity-> loss of repair system)

Strand-directed mismatch repair:
In bacteria, methylation of A in GATC to distinguish new from template strand
In eucaryotes nicks identify new strand

Figure 5-20a *Molecular Biology of the Cell* (© Garland Science 2008)

DNA polymerases:

E.coli:

DNA pol I-III

- DNA pol I/II: DNA repair
- DNA pol III: synthesis of leading and lagging strand holoenzyme consists of seven protein subunits

Eucaryotes:

DNA pol α, β, γ, δ, ε

- DNA pol α: initiation of replication (priming)
- DNA pol β: DNA repair
- DNA pol δ, ε: replication
- DNA pol γ: mitochondrial DNA replication

Accessory proteins that are required for DNA replication:

Structural constraints of the DNA (very stable helical double strand) require proteins that render this structure accessible for the DNA pol. and improve the processivity of the DNA polymerases

1) Unwinding of DNA ds->ss
2) Stabilization of ss
3) Synthesis facilitation

Helicases open the DNA double strand for replication to proceed:
The DNA double helix is very stable
->ATP driven multi-enzyme complexes (helicases) can separate the two strands. Specific helicases move along either of the two possible directions on the single strand.

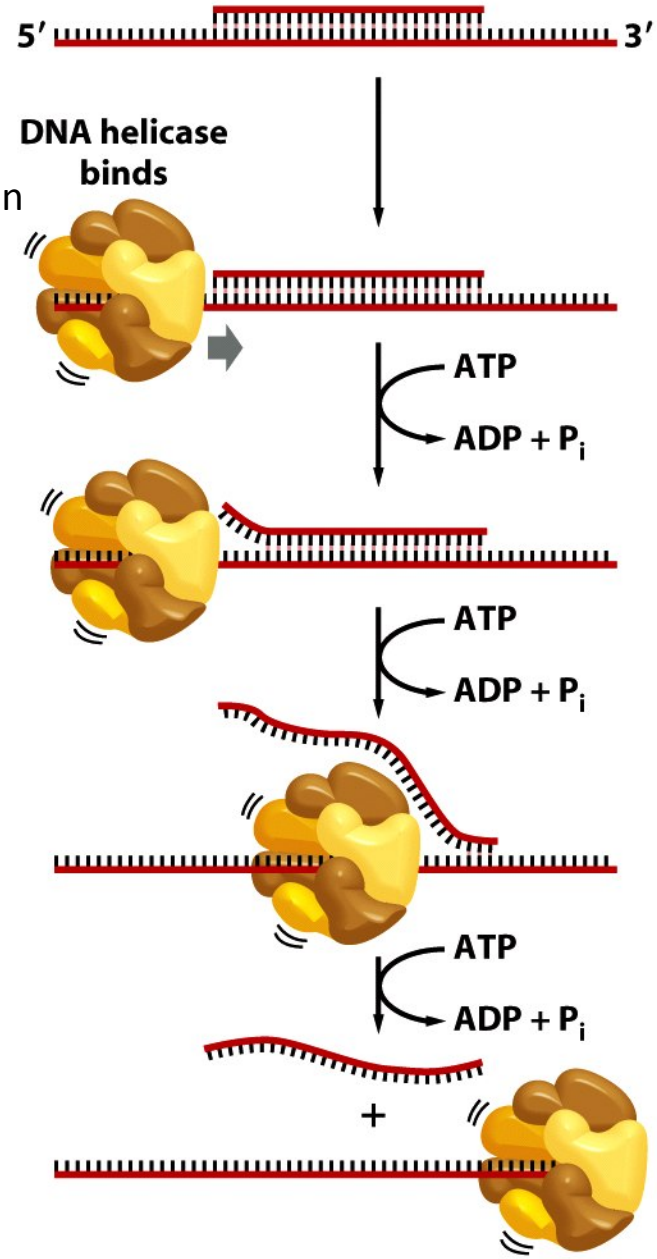


Figure 5-14 *Molecular Biology of the Cell* (© Garland Science 2008)

Single strand binding proteins stabilize unwound single-stranded DNA

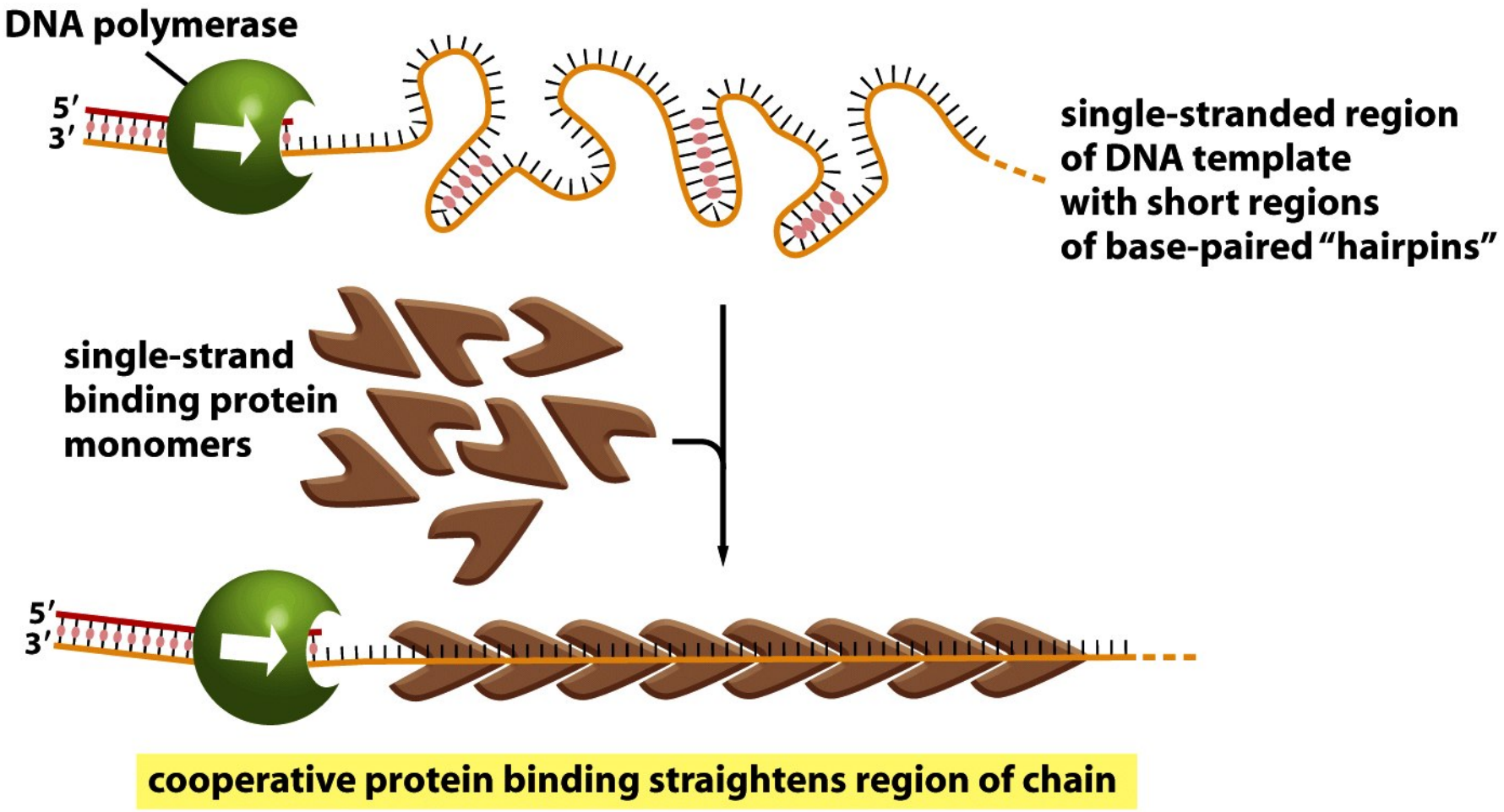
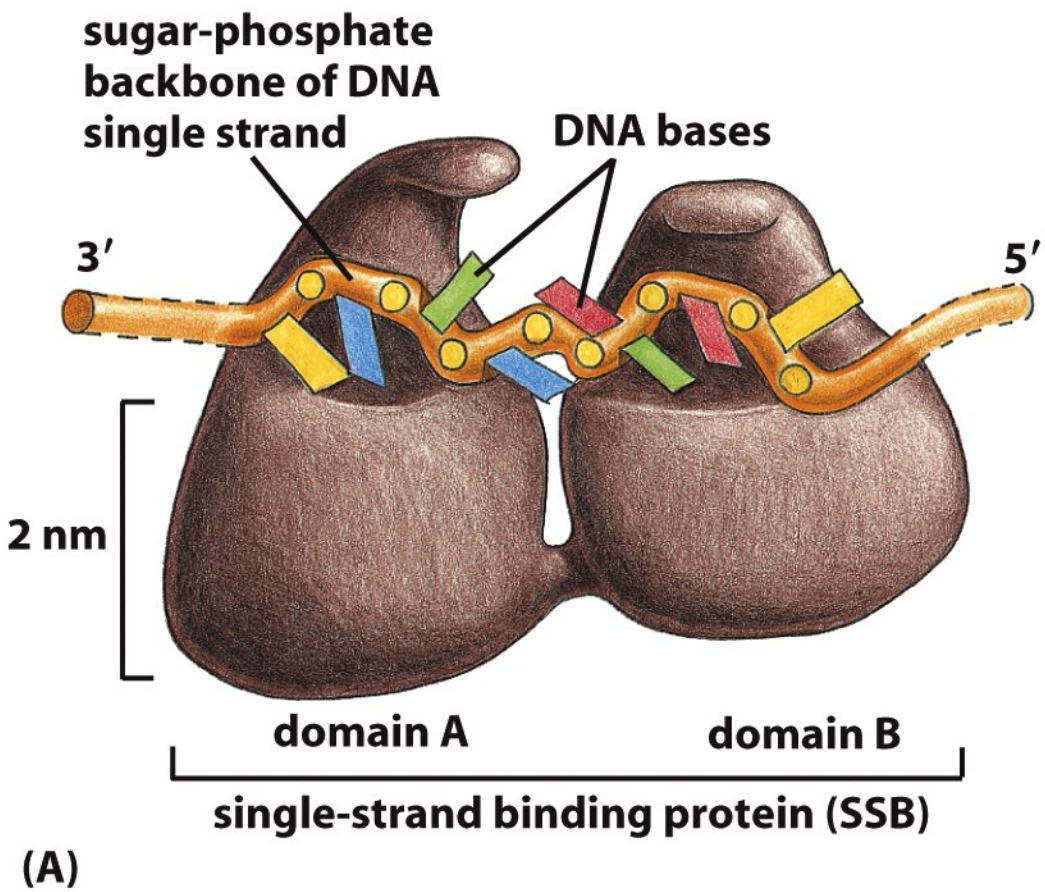


Figure 5-16 *Molecular Biology of the Cell* (© Garland Science 2008)



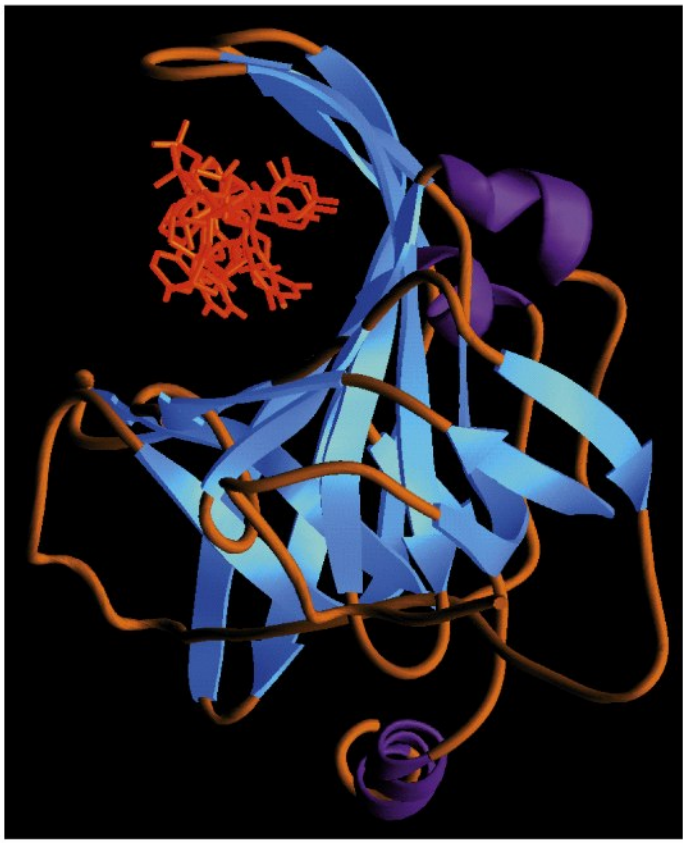
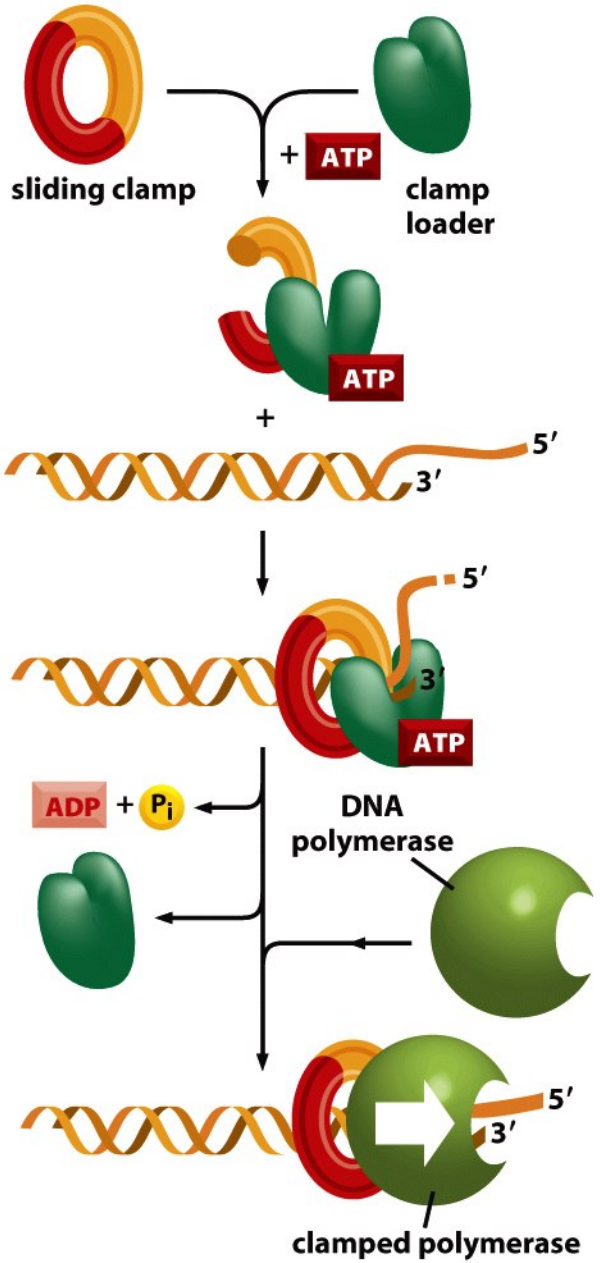


Figure 5-17 *Molecular Biology of the Cell* (© Garland Science 2008)



DNA clamp binds DNA pol III strongly, reduces its dissociation from DNA ->increases processivity

Figure 5-18c *Molecular Biology of the Cell* (© Garland Science 2008)

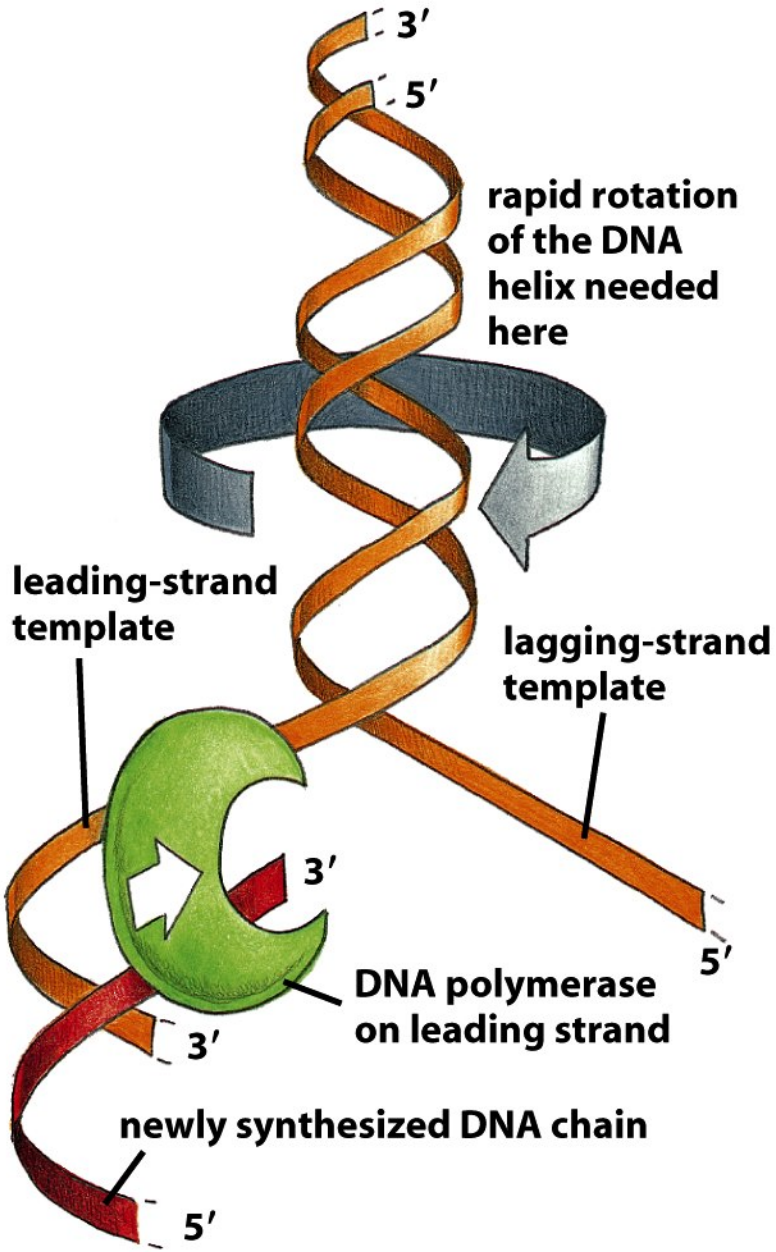


Figure 5-21 *Molecular Biology of the Cell* (© Garland Science 2008)

Topoisomerase I

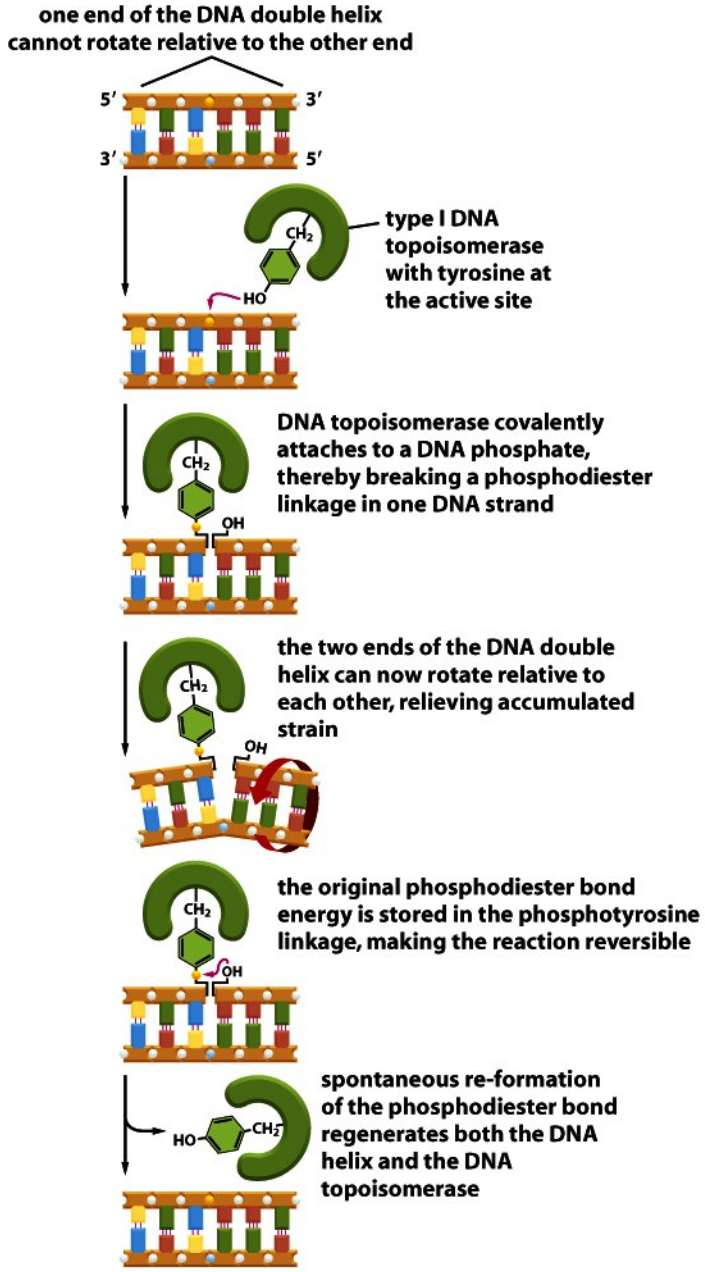
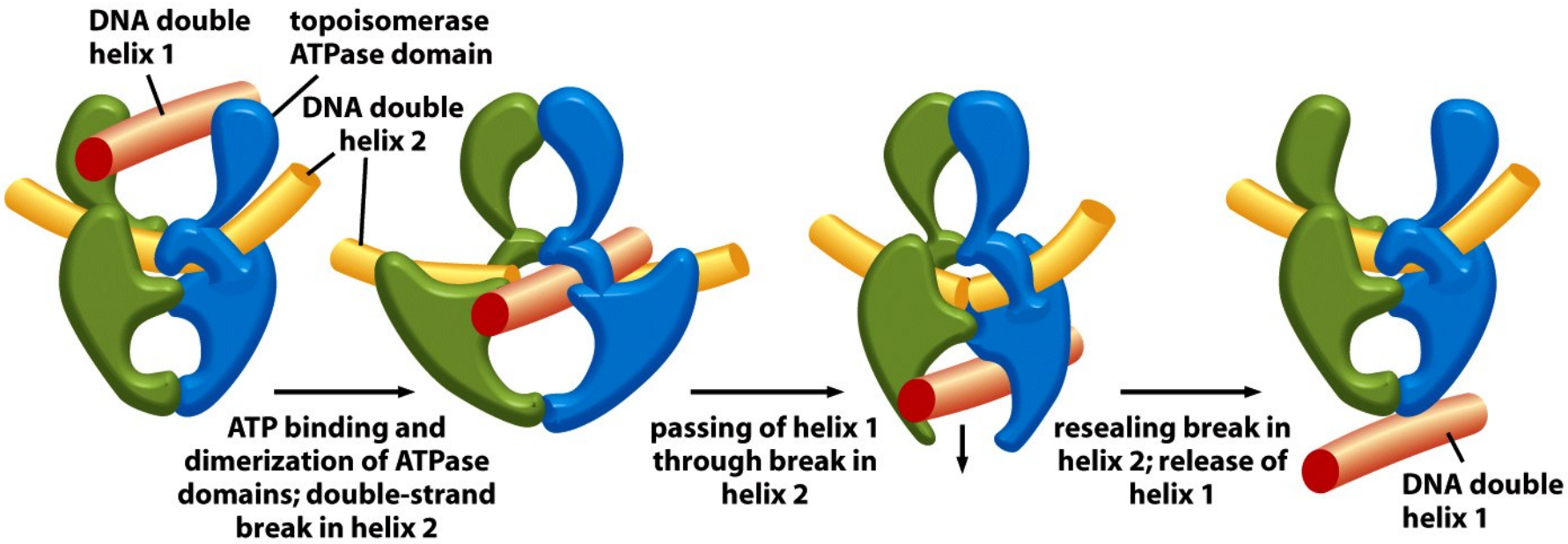


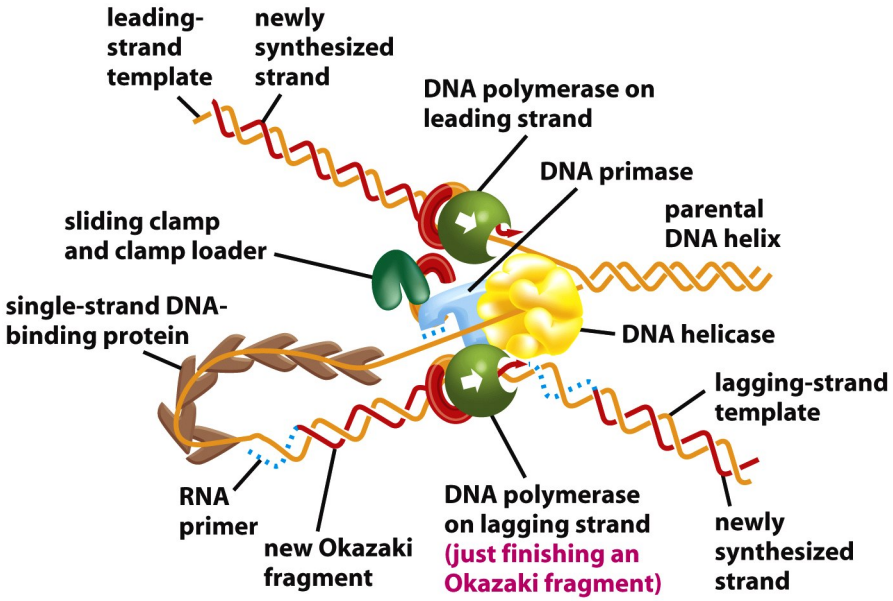
Figure 5-22 *Molecular Biology of the Cell* (© Garland Science 2008)

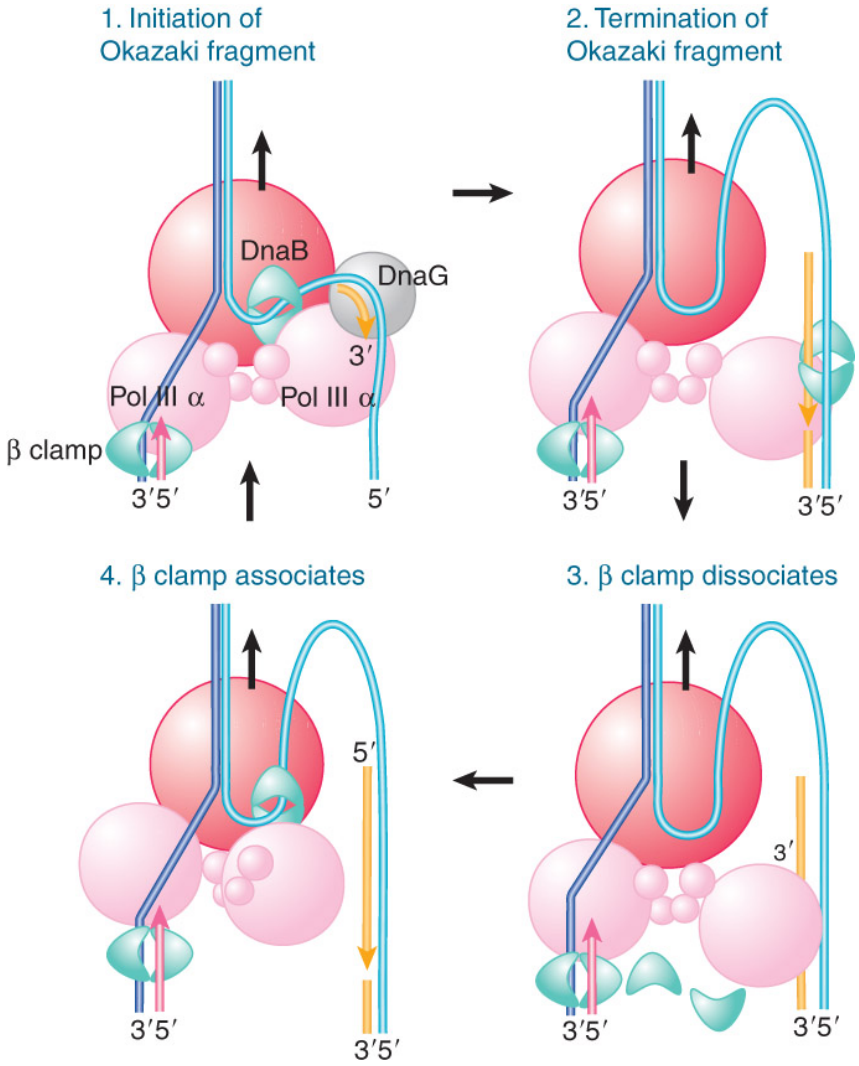
Topoisomerase II



Yeast temperature sensitive Topoisomerase II mutant:
->sister chromosomes remain intertwined after replication

Figure 5-23 *Molecular Biology of the Cell* (© Garland Science 2008)

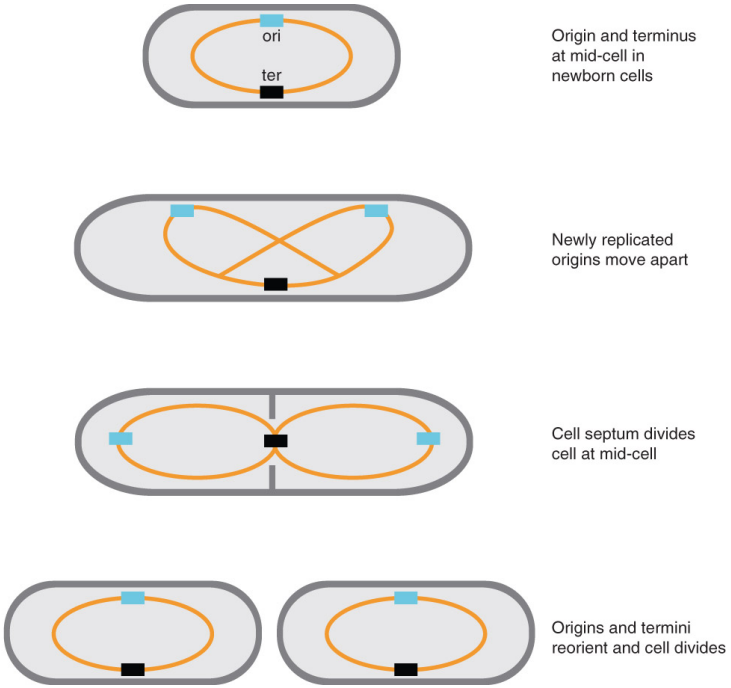


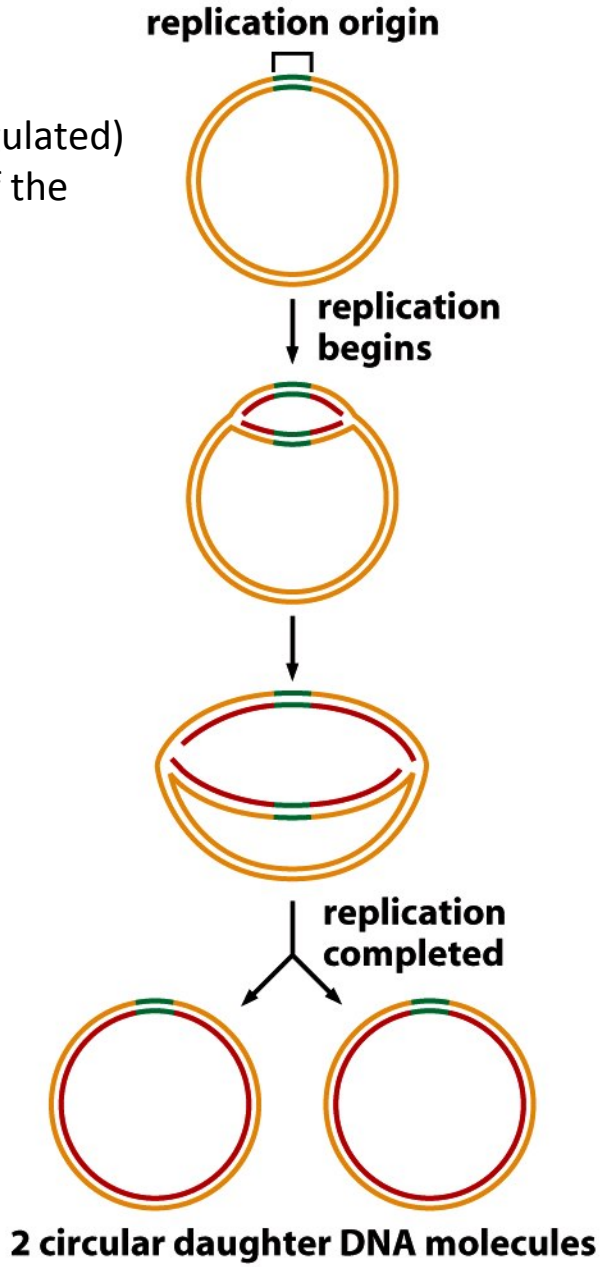


Proteins involved in replication form an orderly large multienzyme complex
fold-back of the lagging strand facilitates loading of clamp-polymerase to ss to initiate synthesis of new okazaki fragment (proteins are kept in place, DNA moves)

Figure 5-19a *Molecular Biology of the Cell* (© Garland Science 2008)

Circular E.coli genome has single origin of replication.
Once initiated replication speed is constant (cannot be regulated)
-> initiation is tightly controlled to ensure that one copy of the genome is made per cell





4.6×10^6bp -> 500-1000bp/second
~40 minutes per genome

Figure 5-26 *Molecular Biology of the Cell* (© Garland Science 2008)

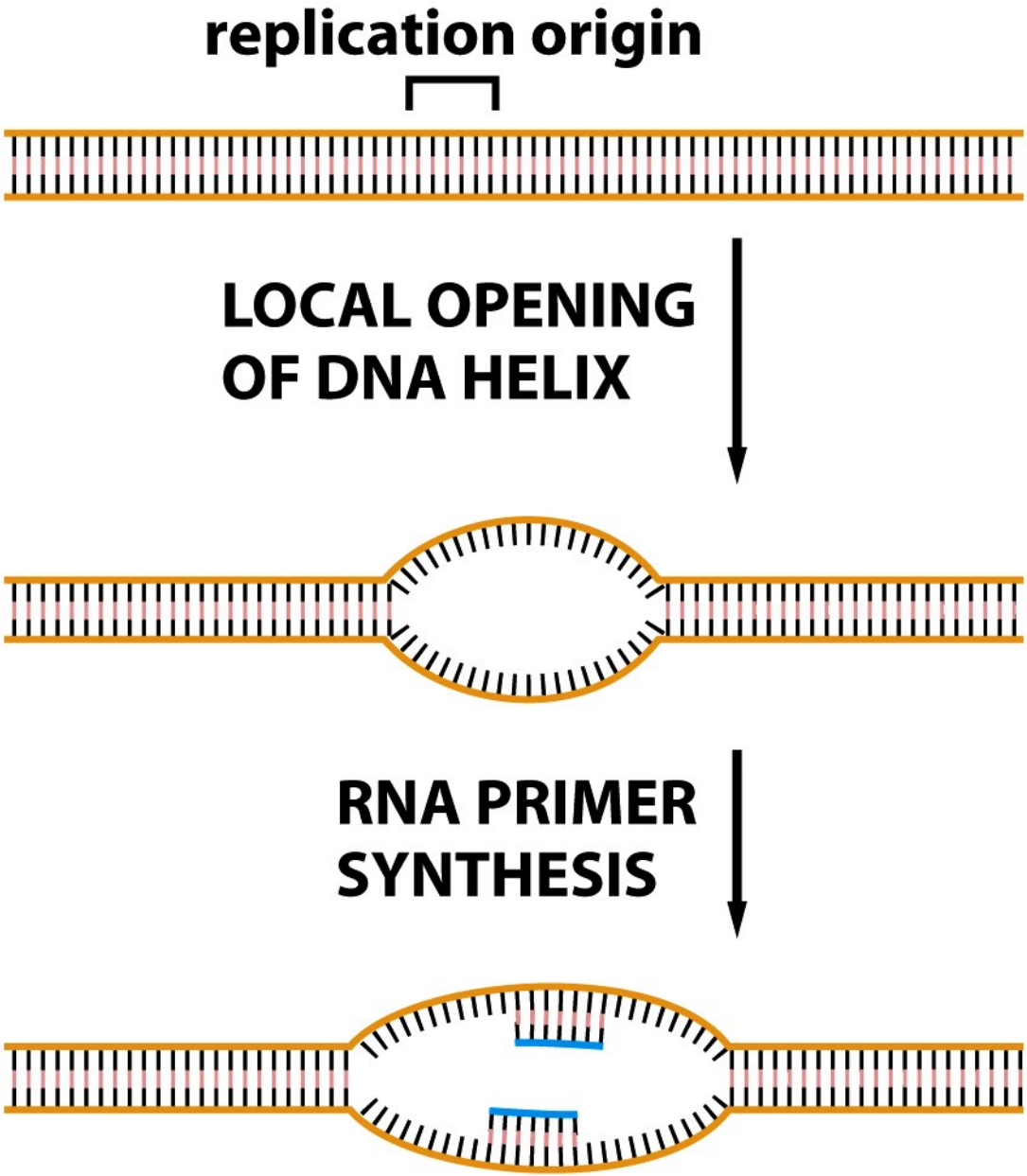


Figure 5-25 (part 1 of 2) *Molecular Biology of the Cell* (© Garland Science 2008)

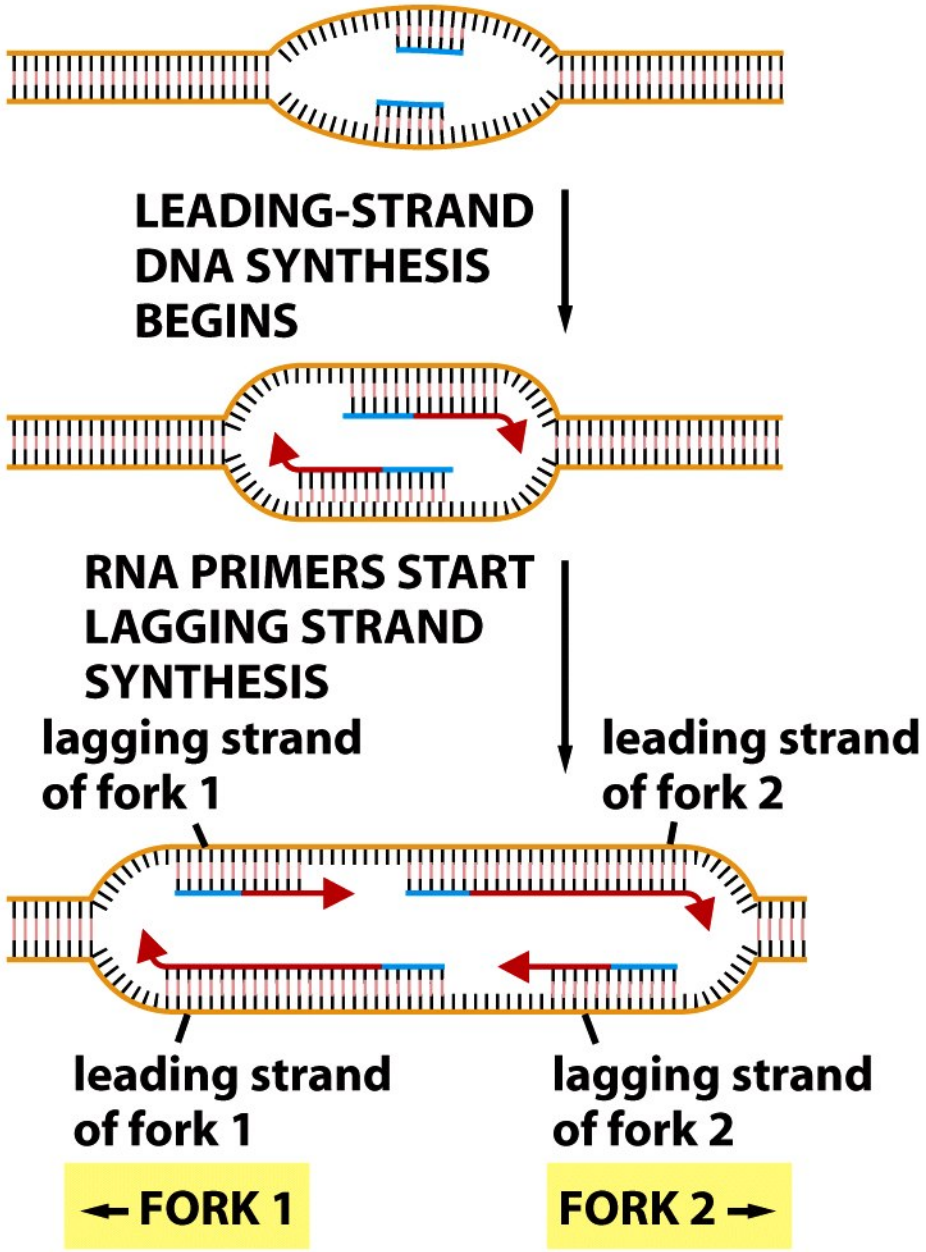


Figure 5-25 (part 2 of 2) *Molecular Biology of the Cell* (© Garland Science 2008)

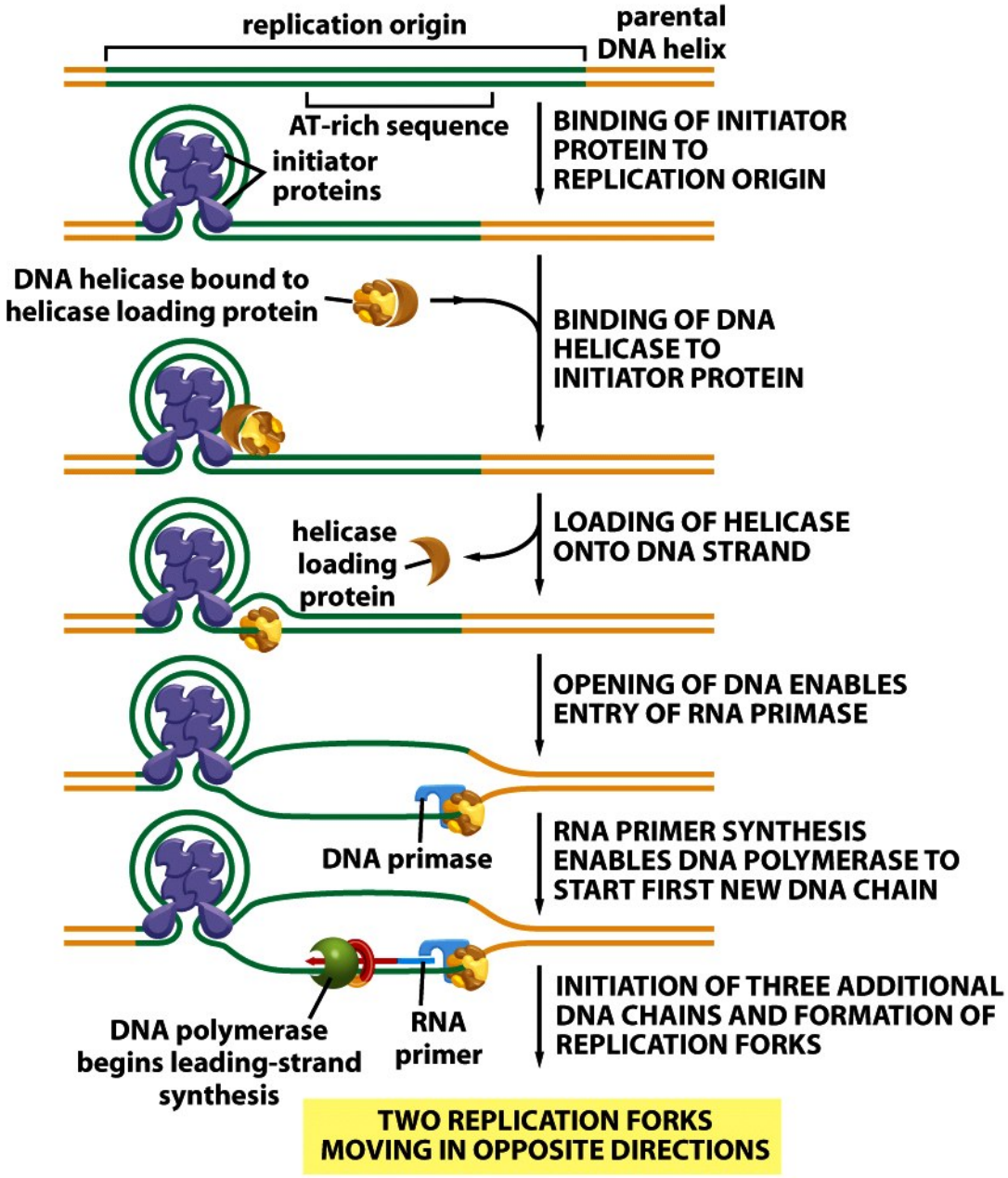


Figure 5-27 *Molecular Biology of the Cell* (© Garland Science 2008)

Origin of replication: ~250bp, contains 11 GATC motifs (which can be methylated)
Refractory period: hemi-methylation of these motifs allows binding of protein that inhibits initiation. After full methylation (~20 minutes later) protein dissociates and re-initiation is possible.

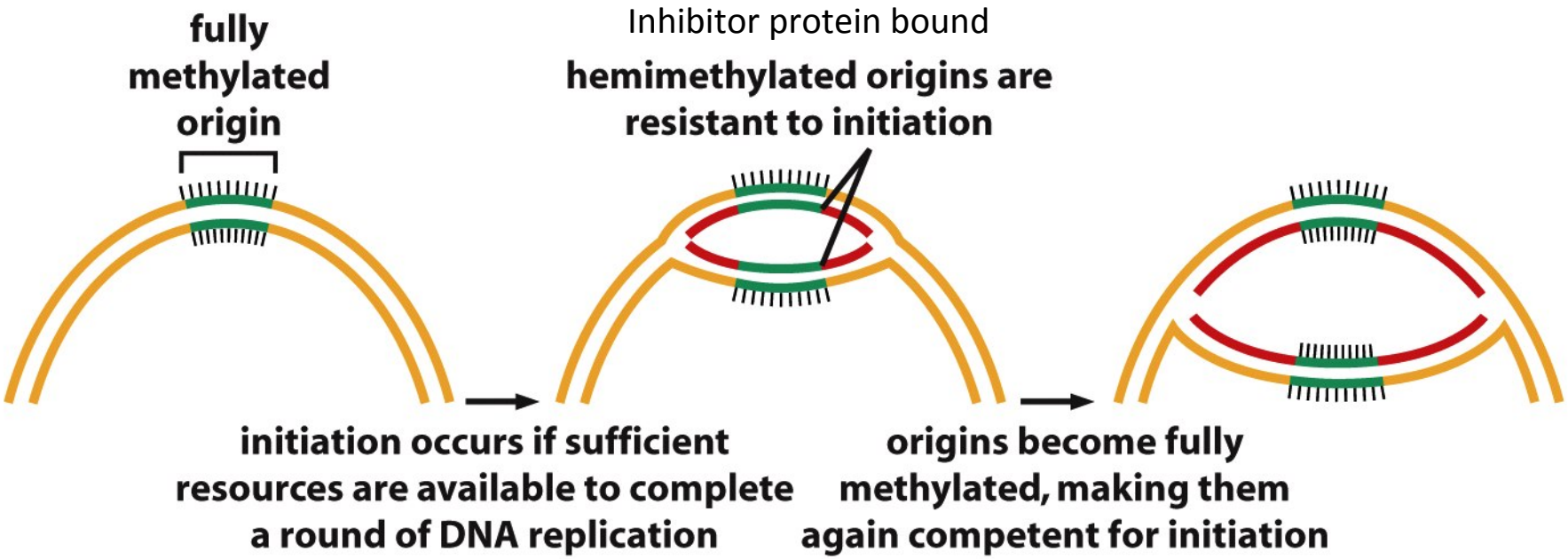
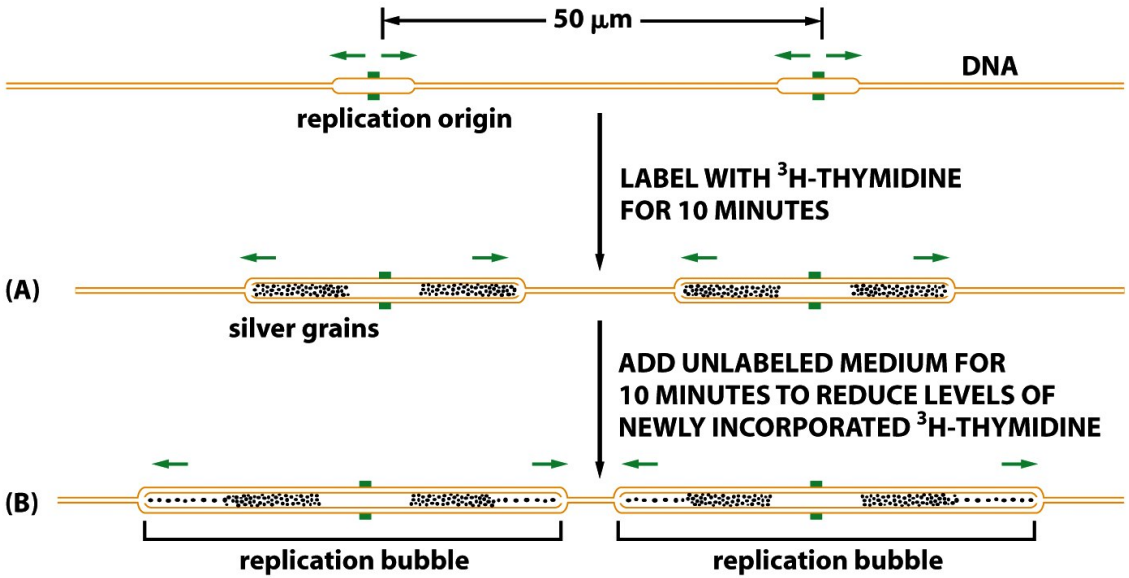


Figure 5-28 *Molecular Biology of the Cell* (© Garland Science 2008)

Autoradiography of eucaryotic replication by exposing ^{3}H-labelled DNA on photo-emulsion coated slides



Speed of synthesis ~50bp/second
Clustered activation of origins (20-80): replication units
Intervals of 30-200kb between origins within one cluster
Replication proceeds as in bacteria (bi-directional replication forks)

Figure 5-29 *Molecular Biology of the Cell* (© Garland Science 2008)

The cell cycle

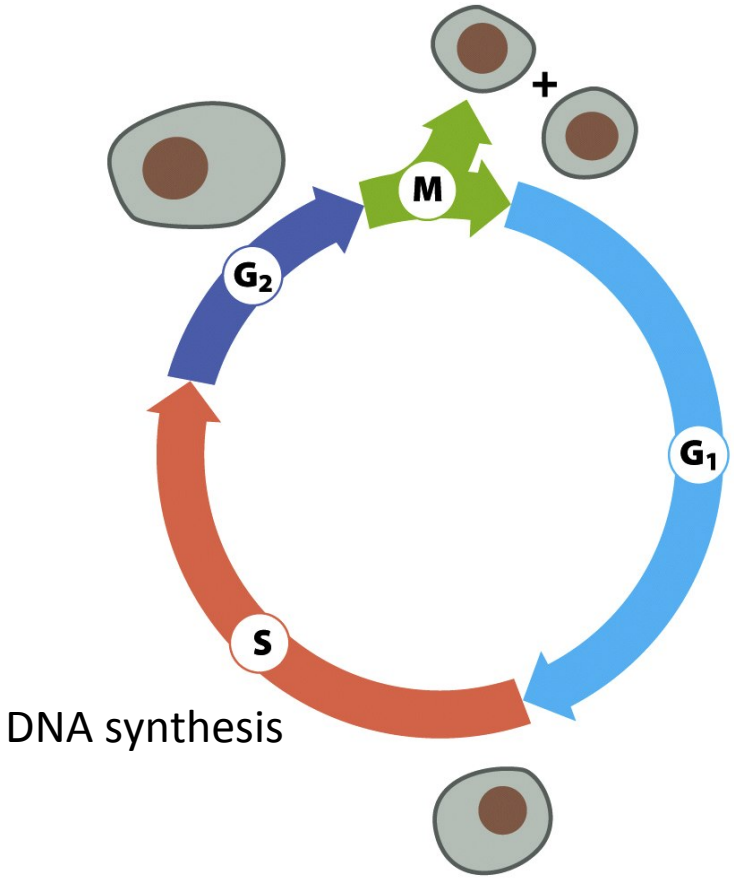
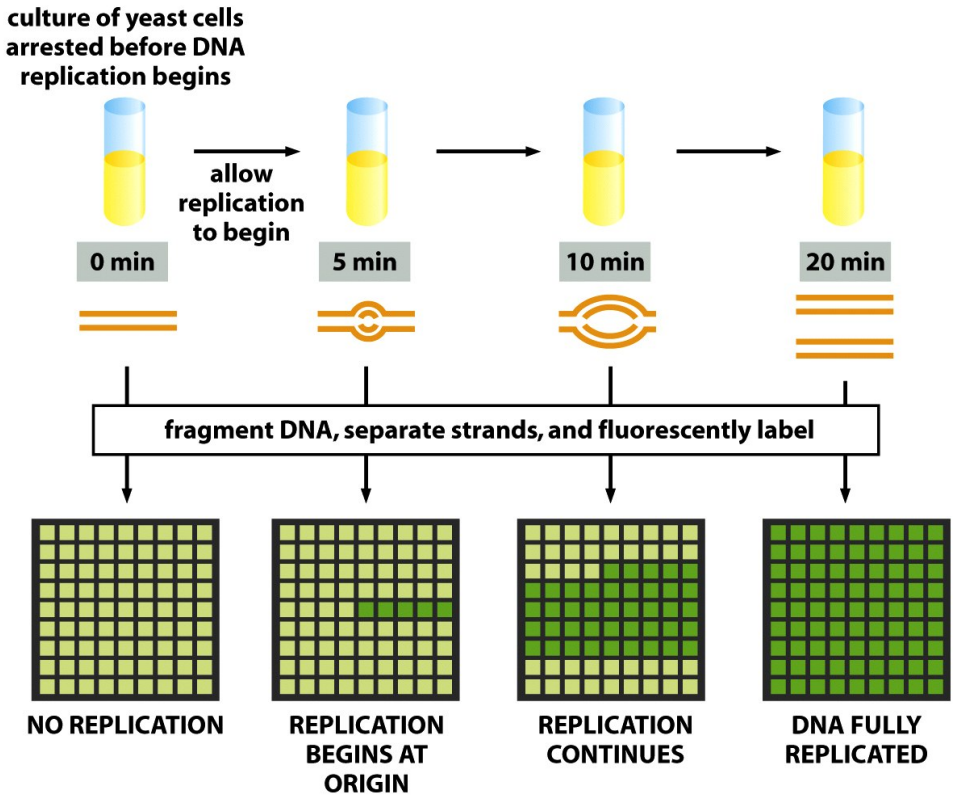


Figure 5-30 *Molecular Biology of the Cell* (© Garland Science 2008)

Micro-array analysis to study global DNA replication



Euchromatin (less condensed) is replicated early in S-phase

Heterochromatin (condensed) late in S-phase

inactivated X-Chr. of females is replicated later than the active one

Figure 5-32 *Molecular Biology of the Cell* (© Garland Science 2008)

The search for autonomously replicating sequences (ARS)

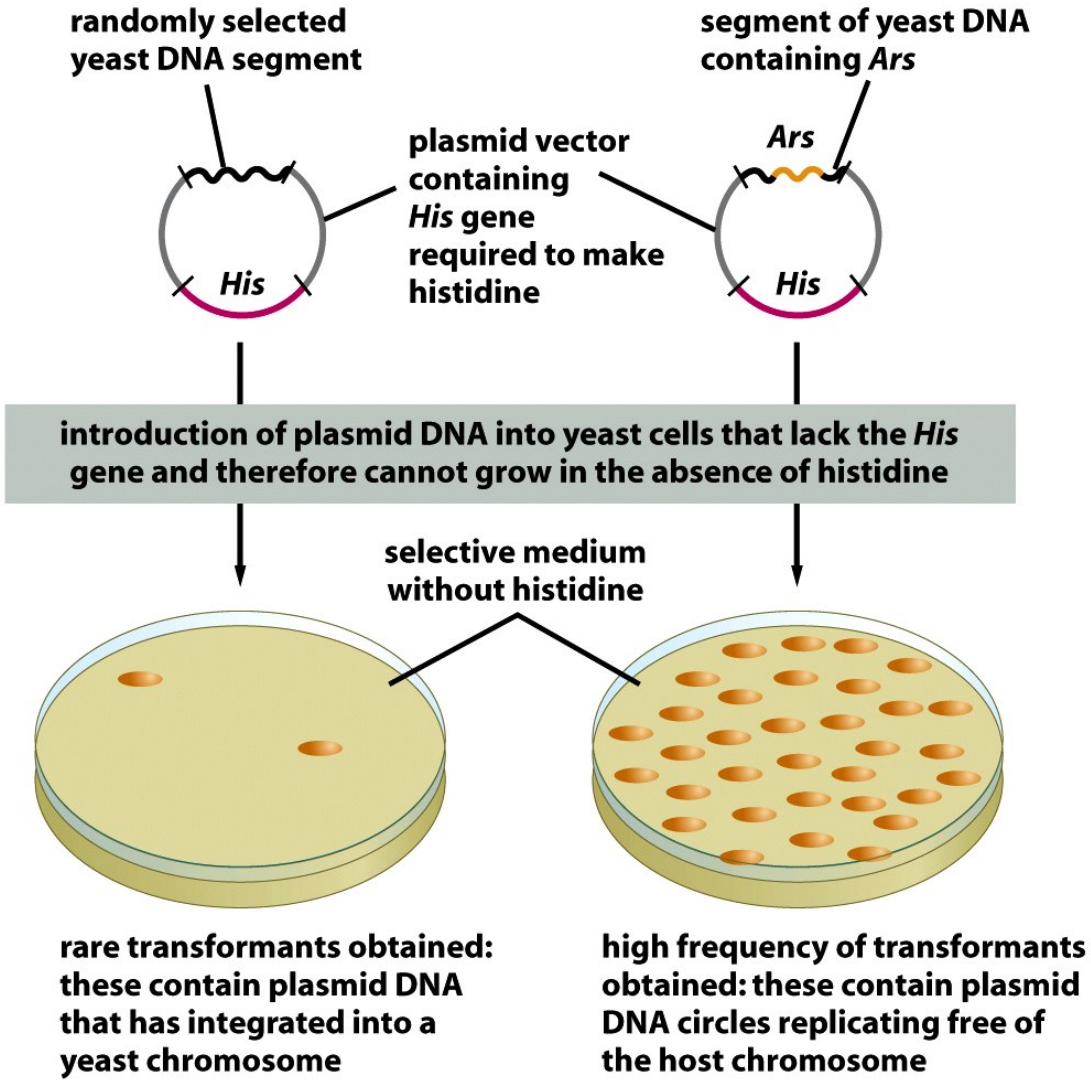


Figure 5-33 *Molecular Biology of the Cell* (© Garland Science 2008)

Positions of ARS on a yeast chromosome:
Not every individual ARS is essential, deletion of a few does not affect replication
ARS are present in excess to ensure efficient chromosomal replication

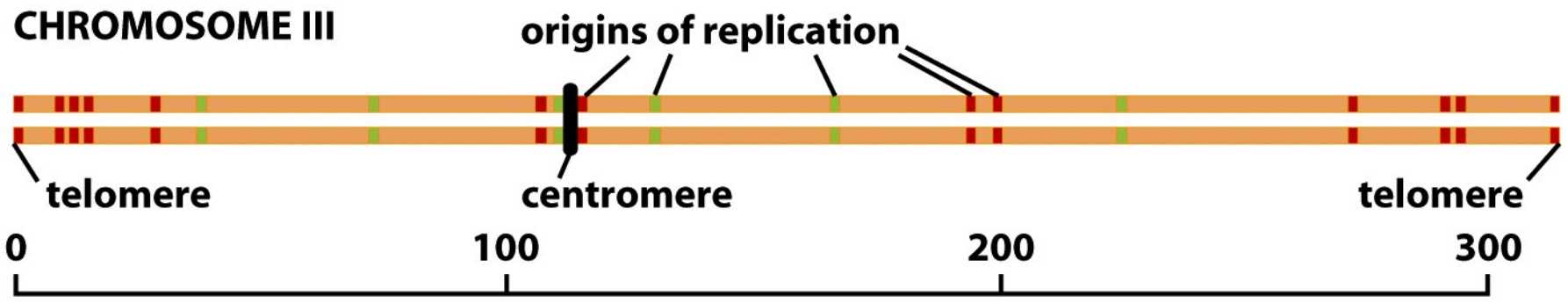
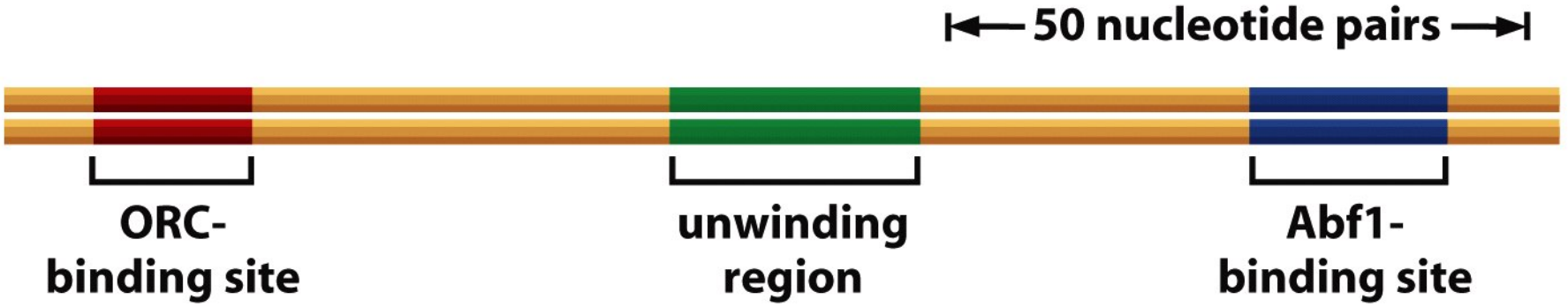


Figure 5-34 *Molecular Biology of the Cell* (© Garland Science 2008)

Yeast ARS



Origin recognition complex

A/T rich (easy to unwind/open)

Auxiliary protein
Facilitates ORC binding

Figure 5-35 *Molecular Biology of the Cell* (© Garland Science 2008)

Replication initiation in eucaryotes: dependence on cell cycle results in sequential activation/inactivation of proteins

Activation of Cdt1

Cdt1,Cdc6: helicase loading proteins

Cyclin-dependent-kinase (Cdk) activates replication and inactivates the pre-RC by phosphorylation

Inhibition of Cdt1 by geminin

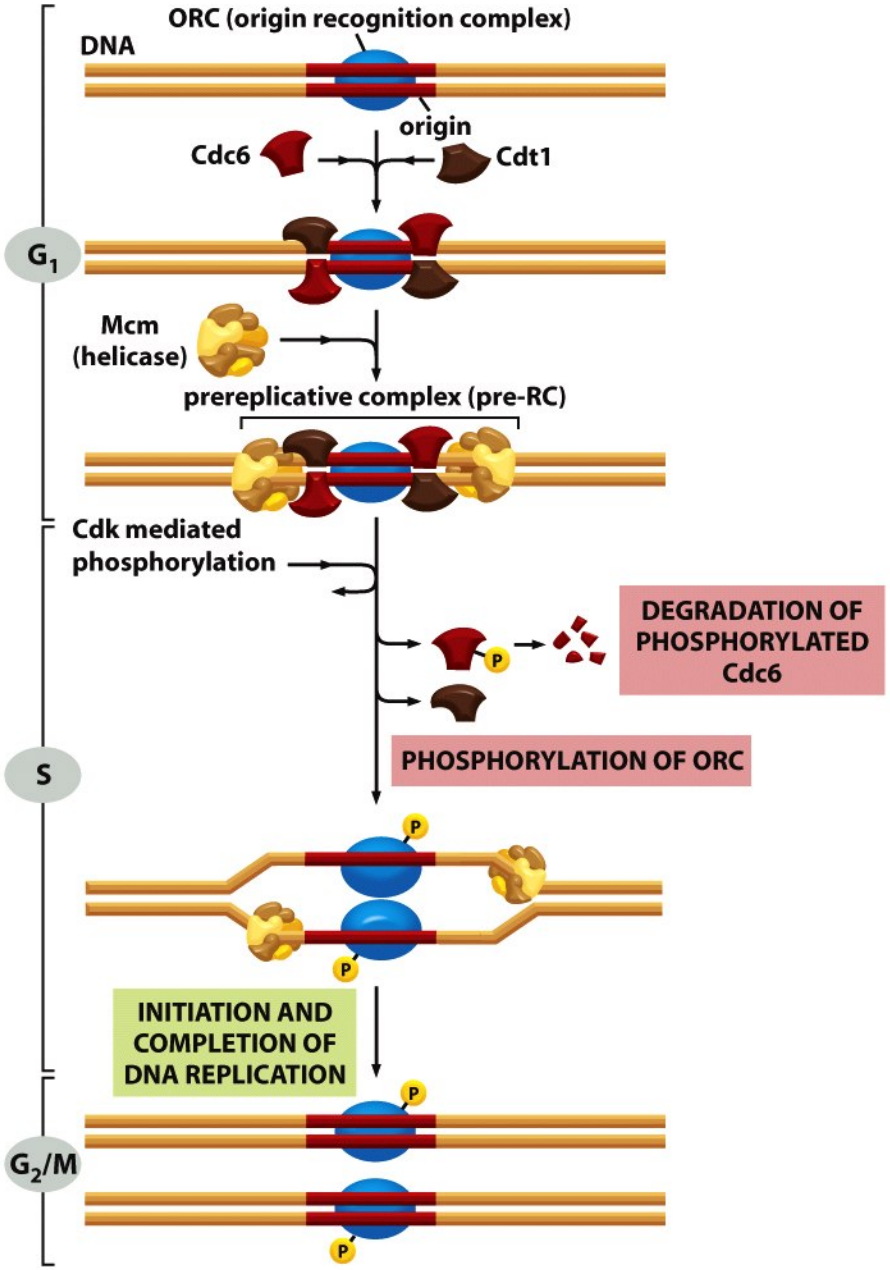
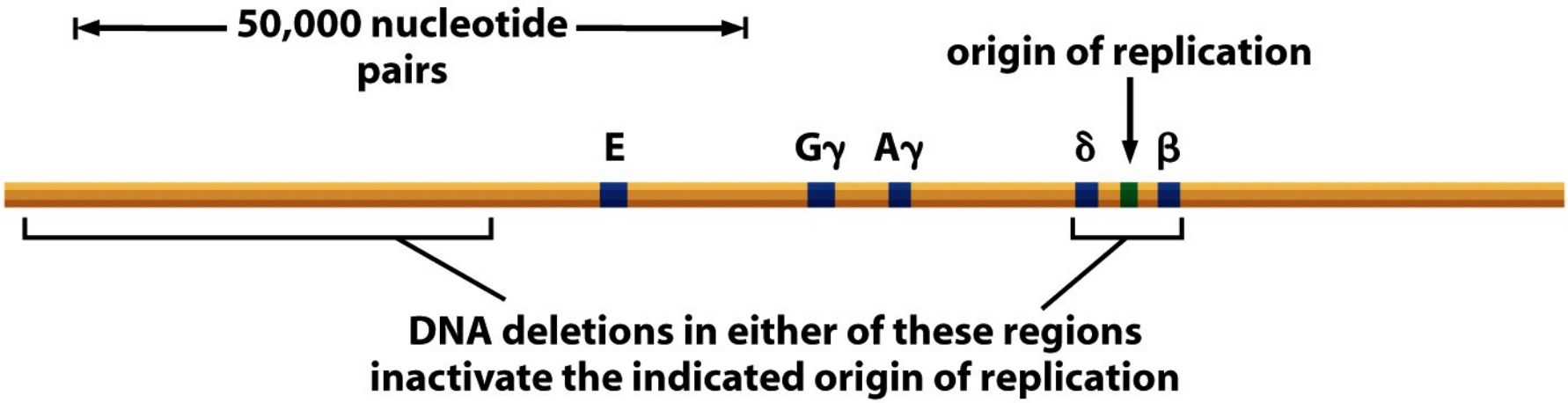


Figure 5-36 *Molecular Biology of the Cell* (© Garland Science 2008)

Human ORC in the β-globin gene cluster



Human ORCs appear to be less well defined.
Possibly chromatin structure has major influence on their function

Figure 5-37 *Molecular Biology of the Cell* (© Garland Science 2008)