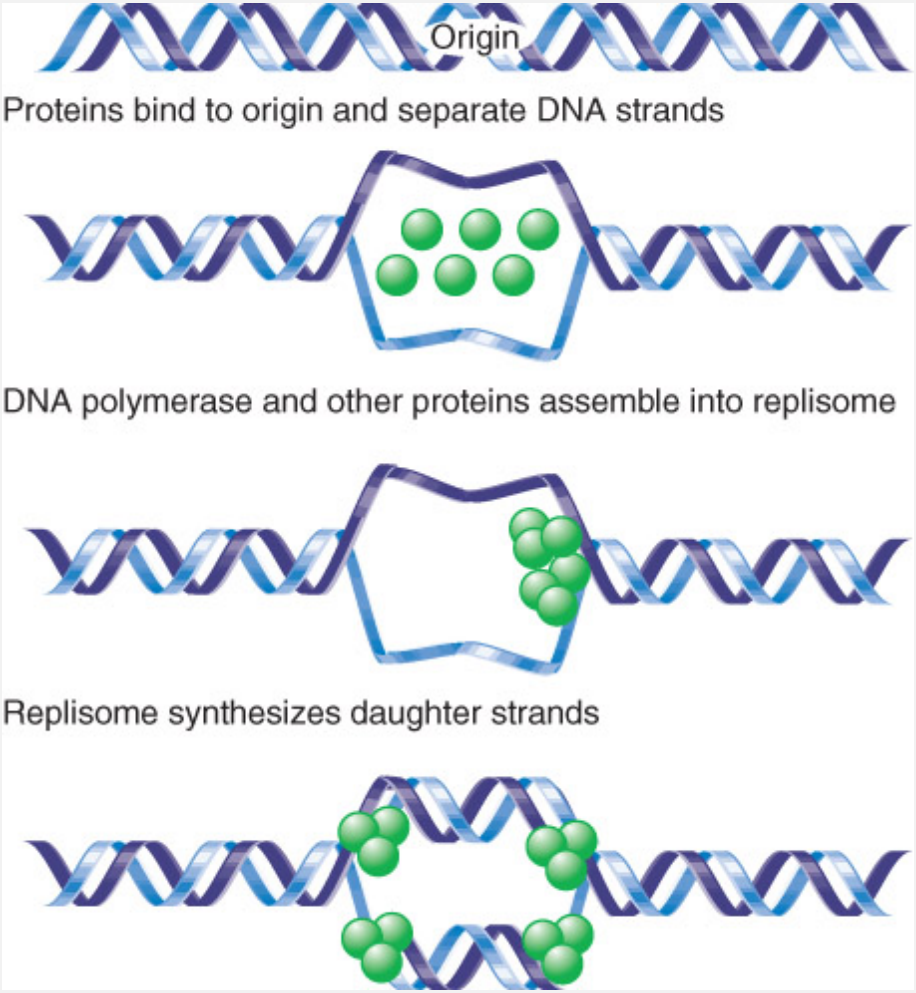
DNA Replication

Introduction

- **topoisomerase –** An enzyme that changes the number of times the two strands in a closed DNA molecule cross each other.
  - It does this by cutting the DNA, passing DNA through the break, and resealing the DNA.
- **replisome –** The multiprotein structure that assembles at the bacterial replication fork to undertake synthesis of DNA.
  - It contains DNA polymerase and other enzymes.

Introduction



Replication initiates when a protein complex binds to the origin and melts the DNA there.

Introduction

- **replicon –** A unit of the genome in which DNA is replicated. Each contains an origin for initiation of replication.
- **origin –** A sequence of DNA at which replication is initiated.
- **terminus –** A segment of DNA at which replication ends.

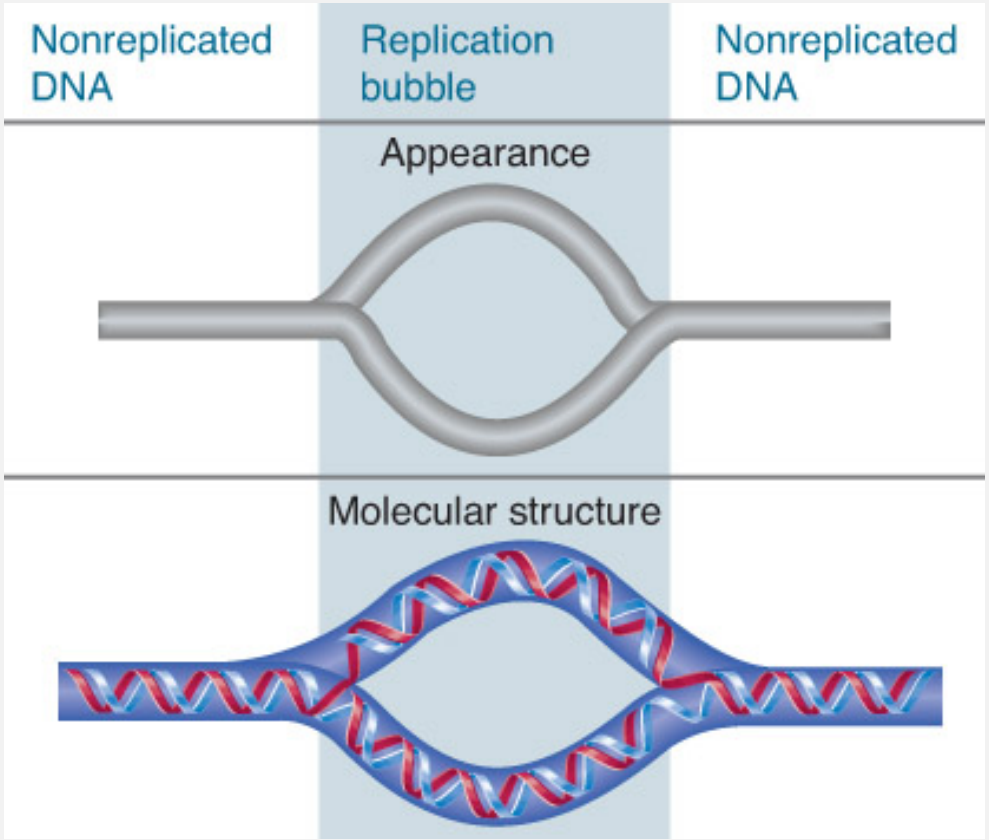
Introduction

- **single-copy replication control –** A control system in which there is only one copy of a replicon per unit bacterium.
  - The bacterial chromosome and some **plasmids** have this type of regulation.
- **multicopy replication control –** Occurs when the control system allows the plasmid to exist in more than one copy per individual bacterial cell.

An Origin Usually Initiates Bidirectional Replication

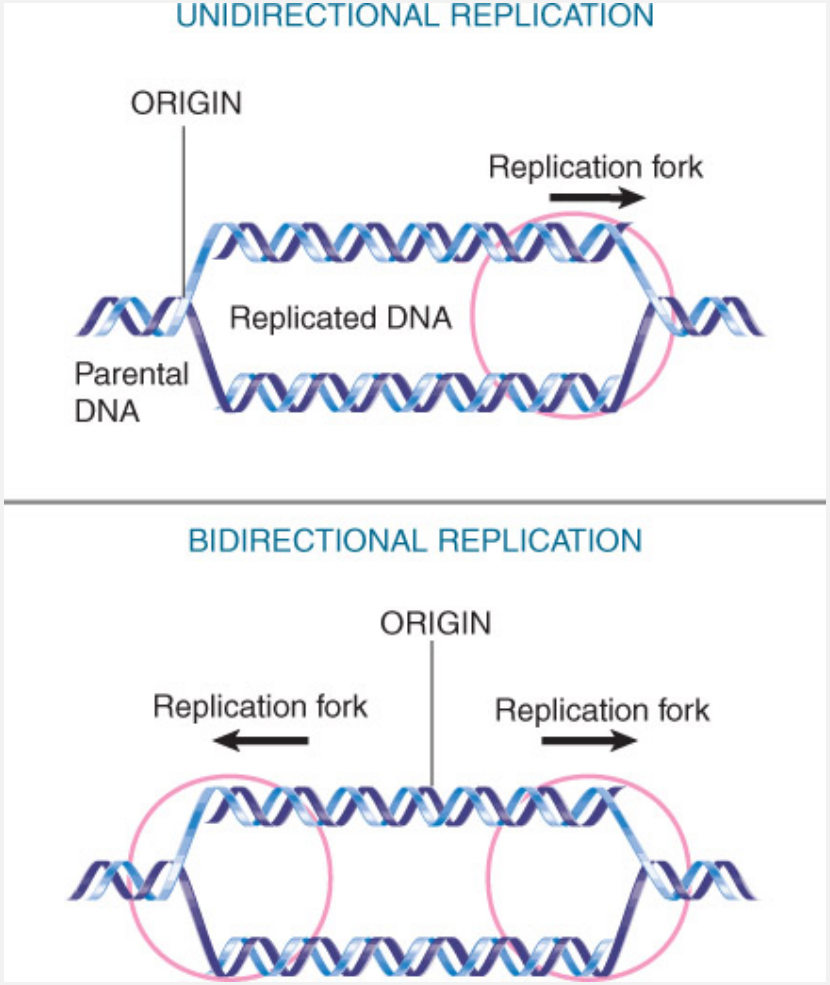
- **semiconservative replication –** Replication accomplished by separation of the strands of a parental duplex, with each strand then acting as a template for synthesis of a complementary strand.
- A replicated region appears as a **bubble** within nonreplicated DNA.
- A **replication fork** is initiated at the origin and then moves sequentially along DNA.

An Origin Usually Initiates Bidirectional Replication



Replicated DNA is seen as a replication bubble flanked by nonreplicated DNA.

An Origin Usually Initiates Bidirectional Replication

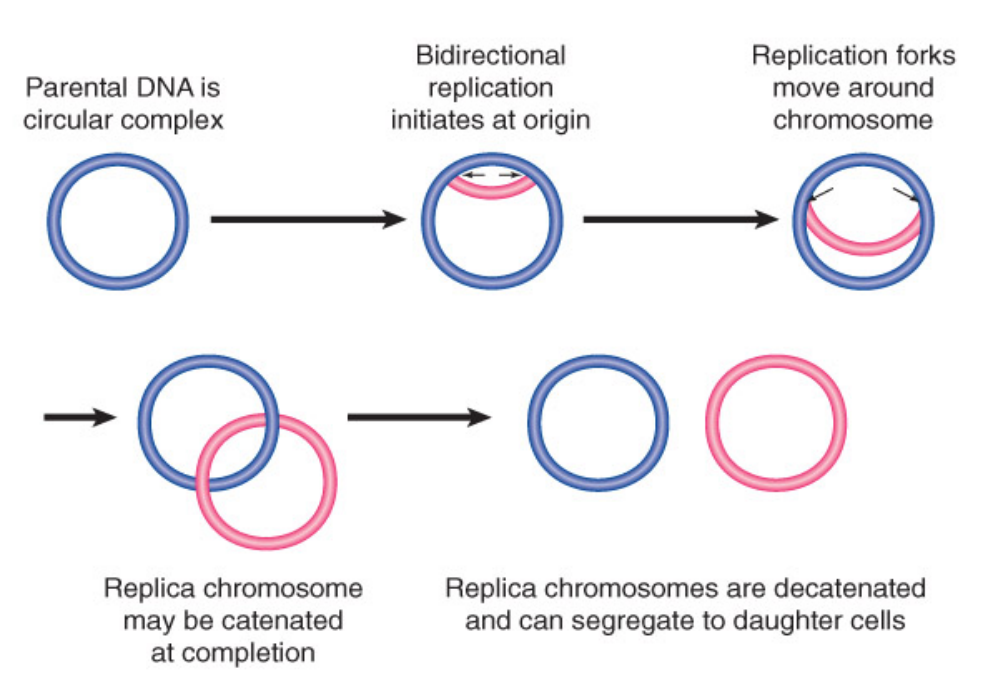


- Replication is **unidirectional** when a single replication fork is created at an origin.
- Replication is **bidirectional** when an origin creates two replication forks that move in opposite directions.

Replicons can be unidirectional or bidirectional, depending on whether one or two replication forks are formed at the origin.

The Bacterial Genome Is (Usually) a Single Circular Replicon

- Bacterial replicons are usually circles that replicate bidirectionally from a single origin.
- The origin of *Escherichia coli*, *oriC,* is 245 base pairs (bp) in length.



Bidirectional replication of a circular bacterial chromosome is initiated at a single origin.

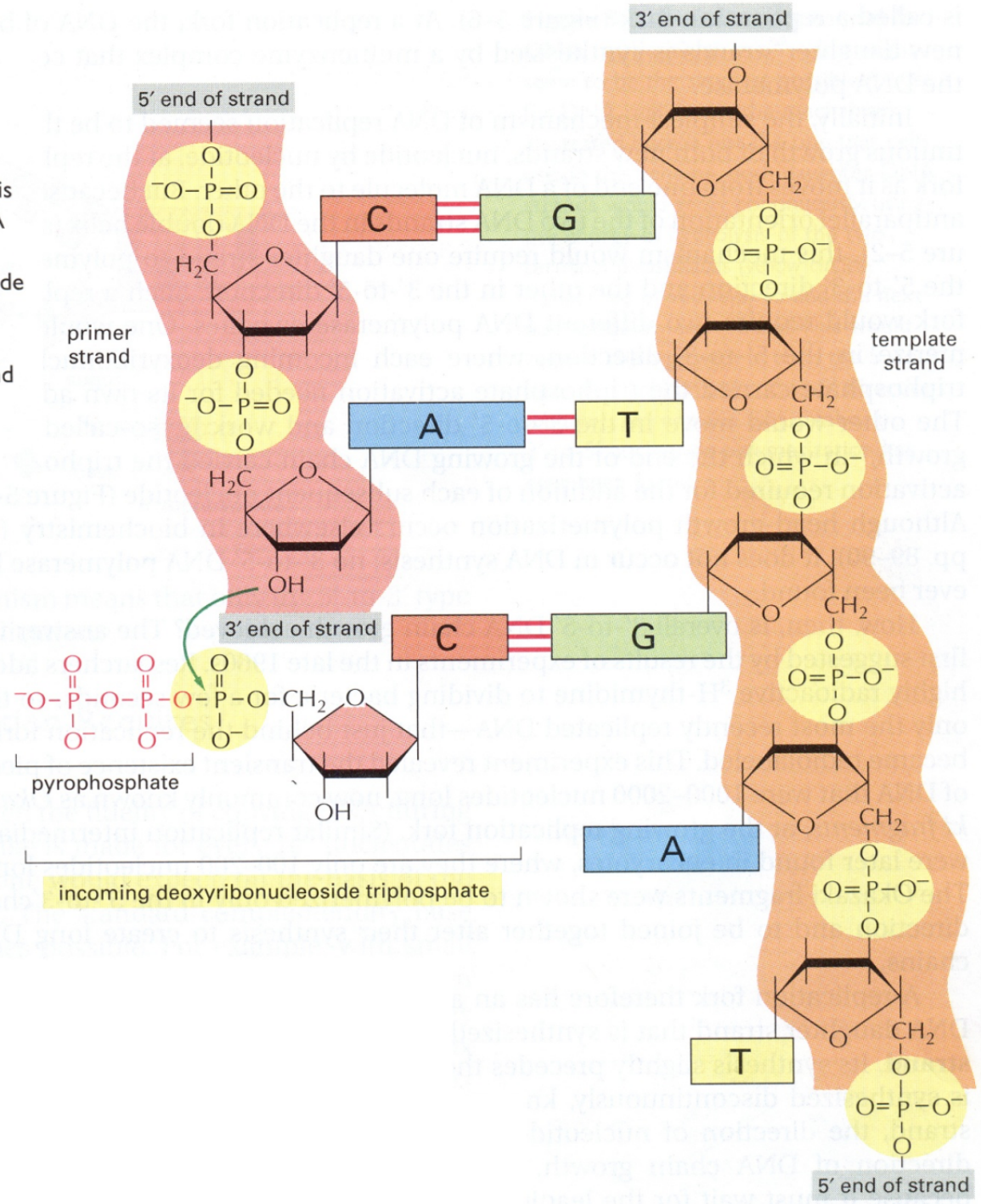
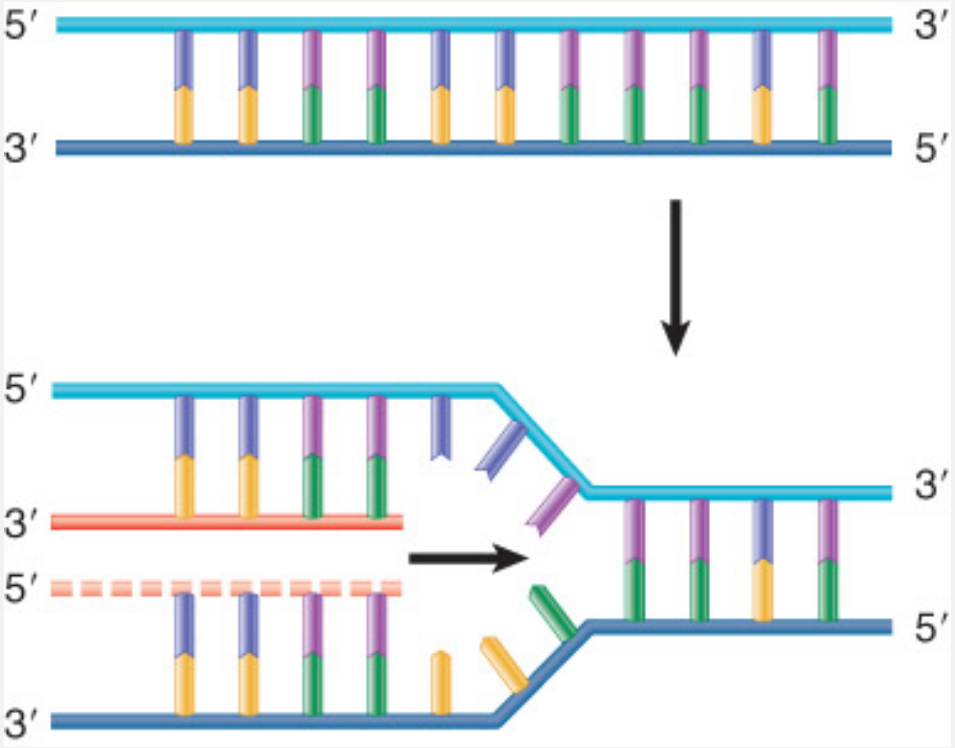


Figure 5–3 The chemistry of DNA synthesis. The addition of a deoxyribonucleotide to the 3′ end of a polynucleotide chain (the *primer strand)* is the fundamental reaction by which DNA is synthesized. As shown, base-pairing between an incoming deoxyribonucleoside triphosphate and an existing strand of DNA (the *template strand)* guides the formation of the new strand of DNA and causes it to have a complementary nucleotide sequence.

DNA Polymerases Are the Enzymes That Make DNA

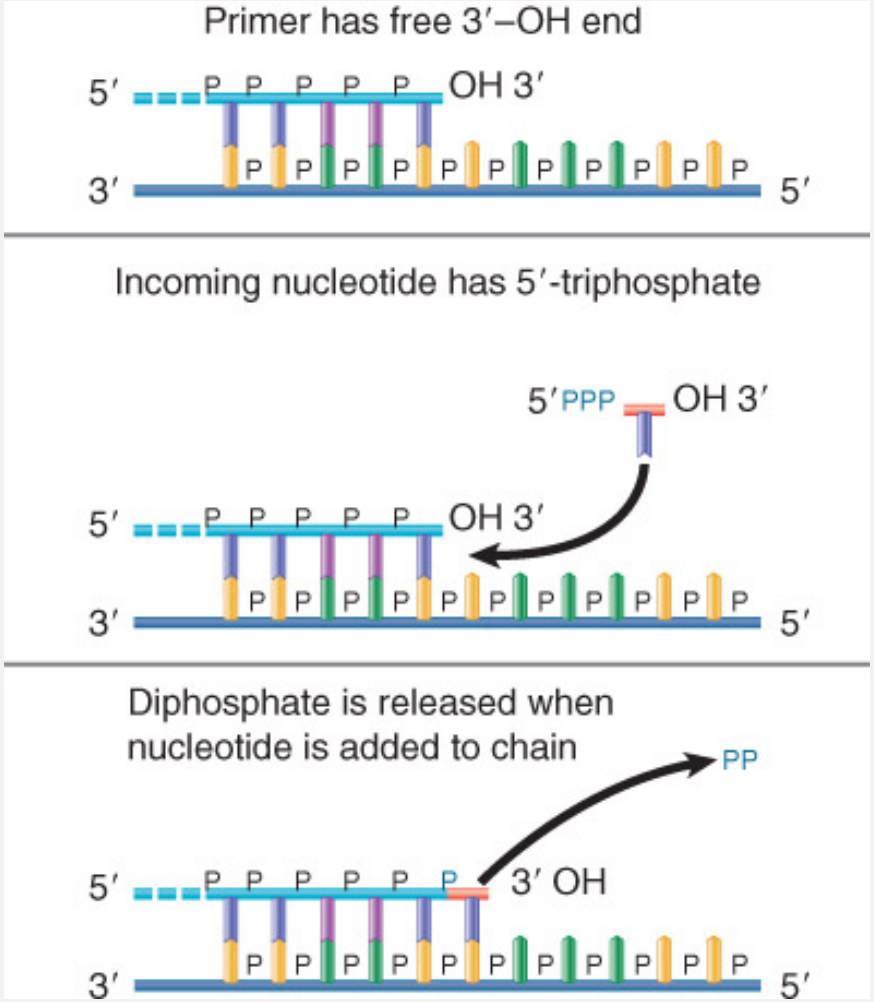
- DNA is synthesized in both **semiconservative replication** and **DNA repair** reactions.



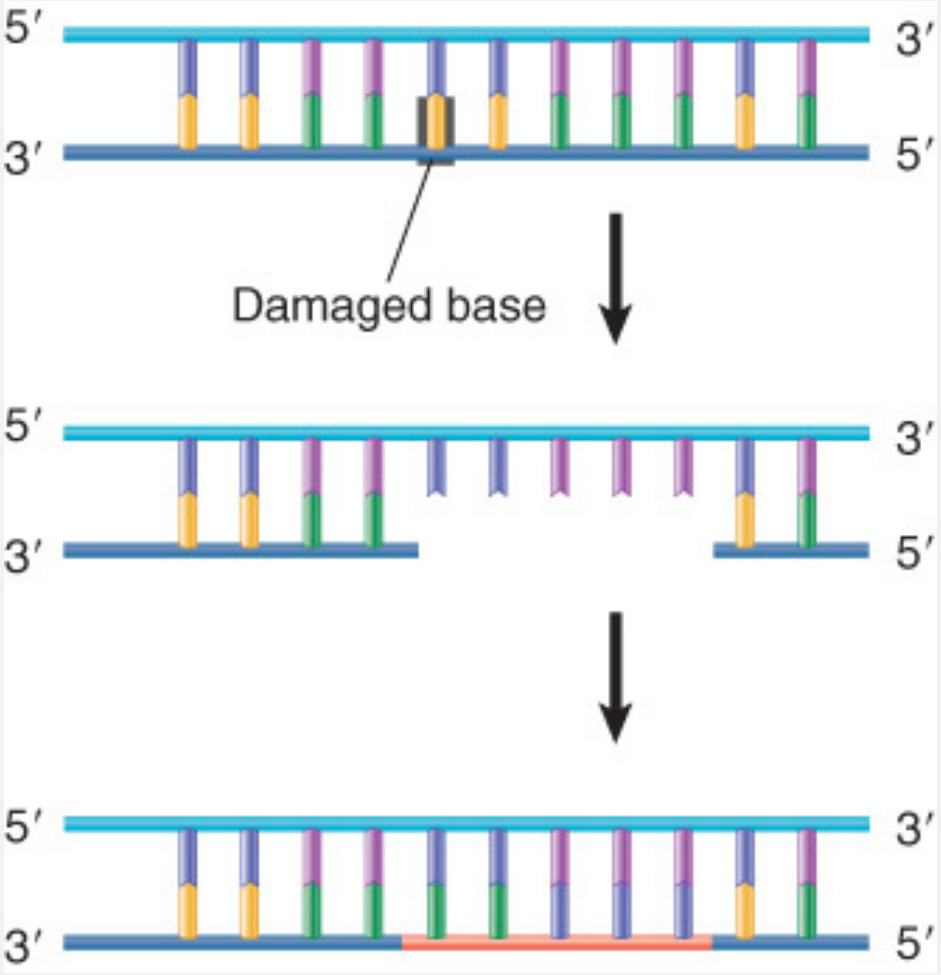
Semiconservative replication synthesizes two new strands of DNA.

DNA Polymerases Are the Enzymes That Make DNA

DNA is synthesized by adding nucleotides to the 3' –OH end of the growing chain.



DNA Polymerases Are the Enzymes That Make DNA



Repair synthesis replaces a short stretch of one strand of DNA containing a damaged base.

DNA Polymerases Are the Enzymes That Make DNA

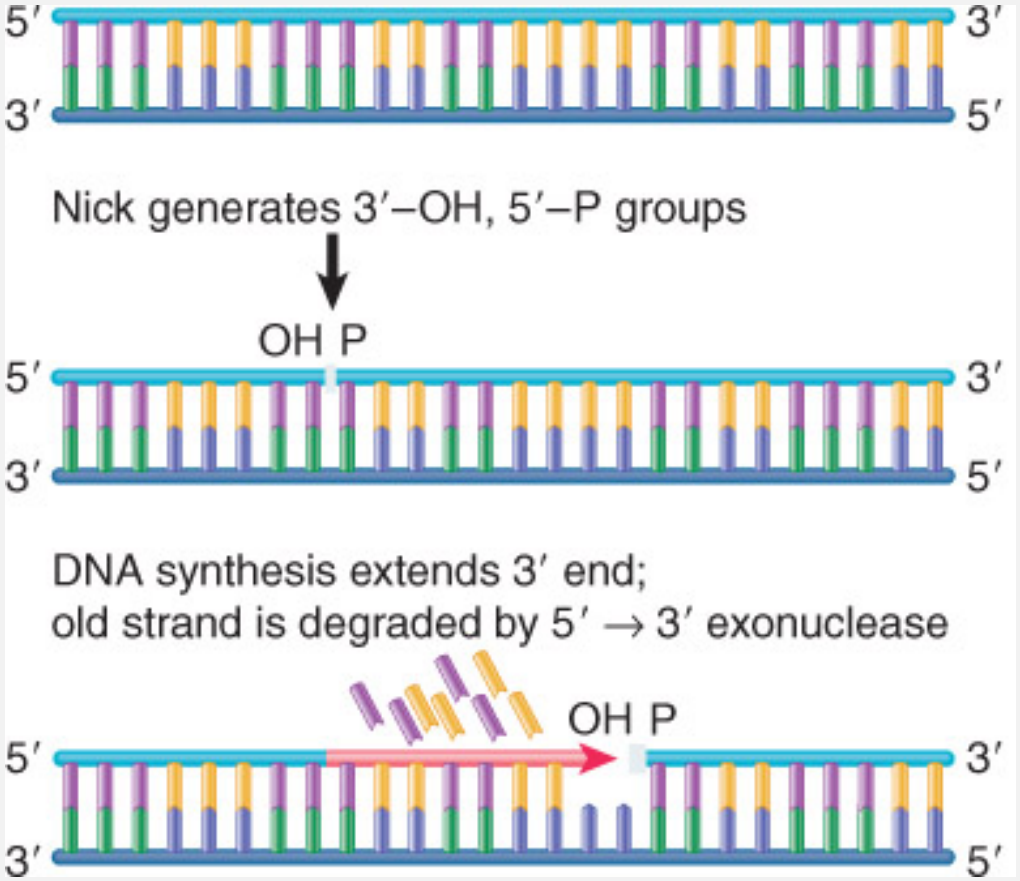
- A bacterium or eukaryotic cell has several different **DNA polymerase** enzymes.
- One bacterial DNA polymerase (a **DNA replicase**) undertakes semiconservative replication; the others are involved in repair reactions.

TABLE 11.1 Only one DNA polymerase is the replication enzyme. The others participate in repairing damaged DNA, restarting stalled replication forks, or bypassing damage in DNA.

Enzyme	Gene	Function
I	*polA*	Major repair enzyme
II	*polB*	Replication restart
III	*polC*	Replicase
IV	*dinB*	Translesion replication
V	*umuD'$_2$C*	Translesion replication

Only one DNA polymerase is the replication enzyme.

DNA Polymerases Have Various Nuclease Activities

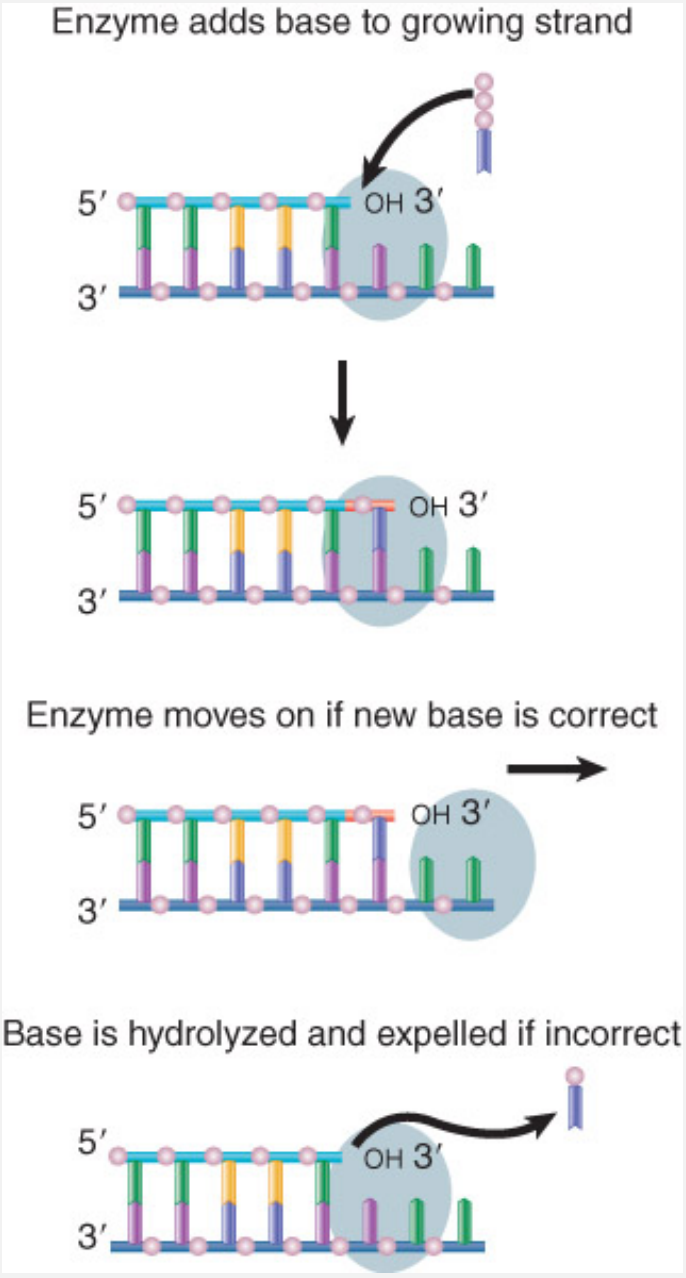


- DNA polymerase I has a unique 5′–3′ exonuclease activity that can be combined with DNA synthesis to perform **nick translation**.

Nick translation replaces part of a preexisting strand of duplex DNA with newly synthesized material.

DNA Polymerases Control the Fidelity of Replication

- High-fidelity DNA polymerases involved in replication have a precisely constrained active site that favors binding of Watson–Crick base pairs.
- **processivity –** The ability of an enzyme to perform multiple catalytic cycles with a single template instead of dissociating after each cycle.



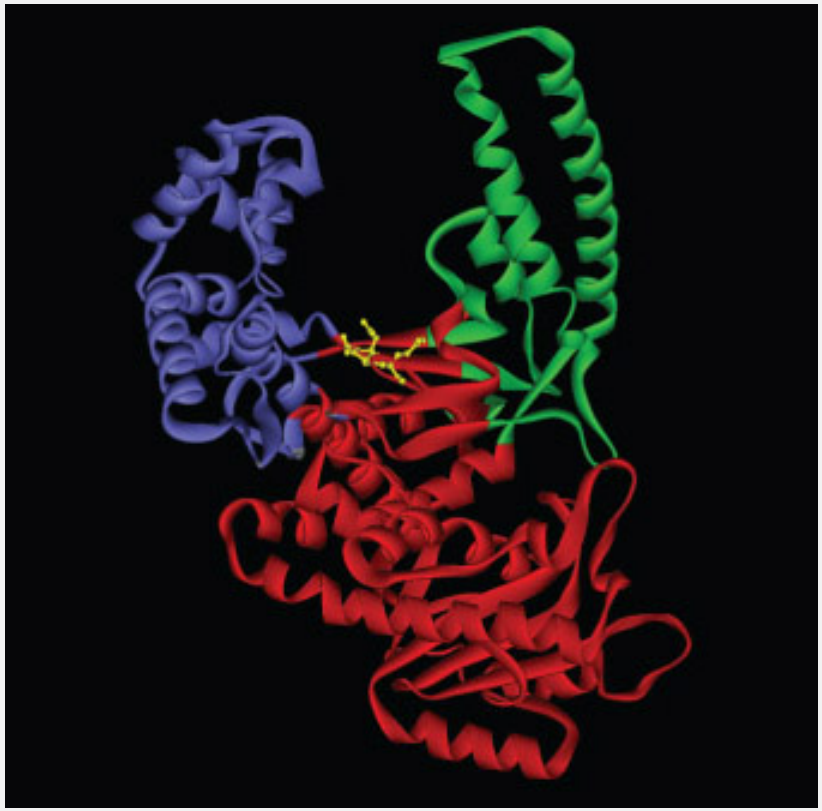
DNA Polymerases Control the Fidelity of Replication

- DNA polymerases often have a 3′–5′ exonuclease activity that is used to excise incorrectly paired bases.
- The fidelity of replication is improved by **proofreading** by a factor of about 100.

DNA polymerases scrutinize the base pair at the end of the growing chain and excise the nucleotide added in the case of a misfit.

DNA Polymerases Have a Common Structure

- Many DNA polymerases have a large cleft composed of three domains that resemble a hand.
- DNA lies across the “palm” in a groove created by the “fingers” and “thumb.”



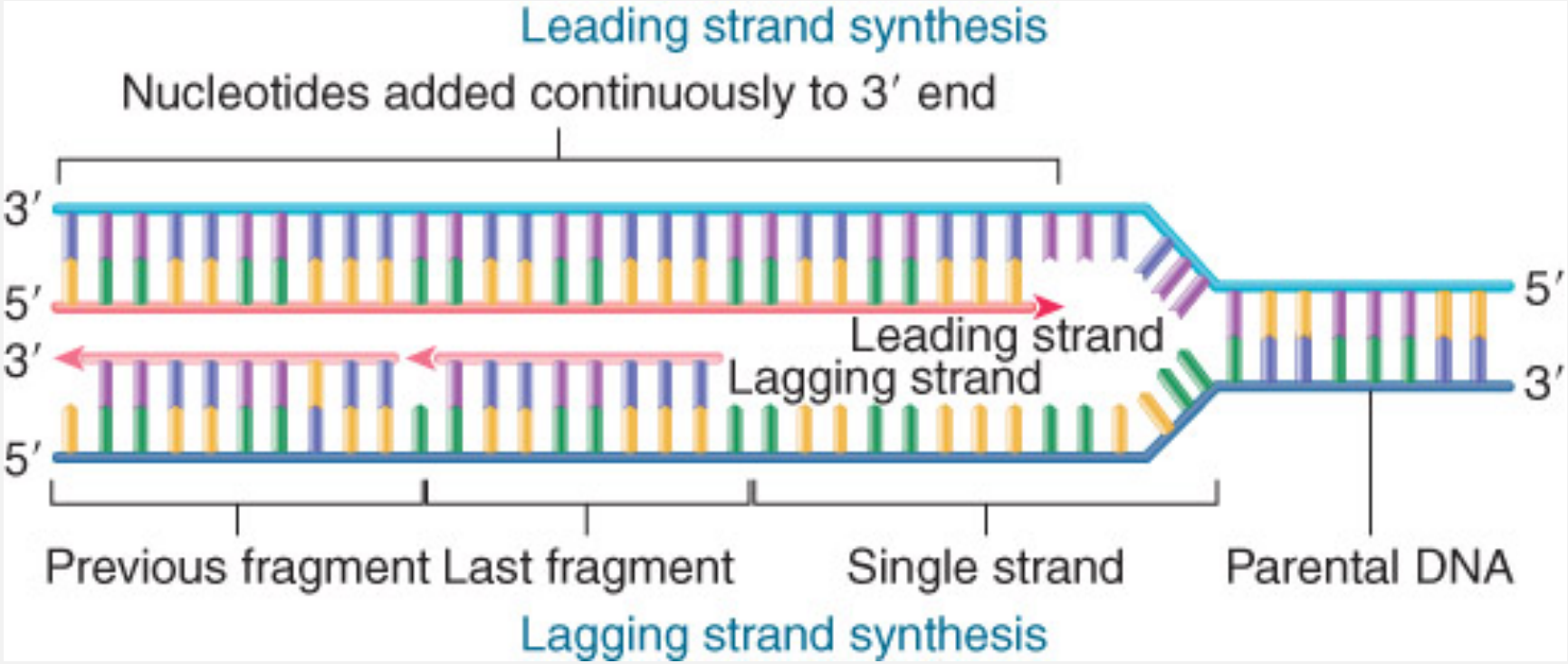
The structure of the Klenow fragment from *E. coli* DNA polymerase I.

Structure from Protein Data Bank 1KFD. L. S. Beese, J. M. Friedman, and T. A. Steitz, *Biochemistry* 32 (1993): 14095–14101.

The Two New DNA Strands Have Different Modes of Synthesis

- The DNA polymerase advances continuously when it synthesizes the **leading strand** (5′–3′), but synthesizes the **lagging strand** by making short fragments (**Okazaki fragments**) that are subsequently joined together.
- **semidiscontinuous replication –** The mode of replication in which one new strand is synthesized continuously while the other is synthesized discontinuously.

The Two New DNA Strands Have Different Modes of Synthesis



The leading strand is synthesized continuously, whereas the lagging strand is synthesized discontinuously.

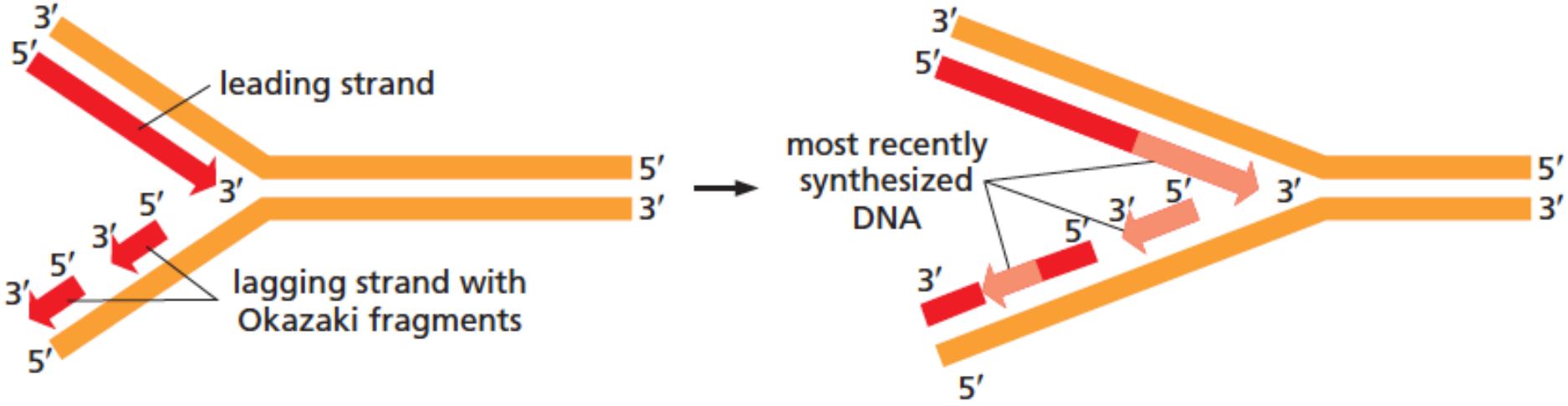
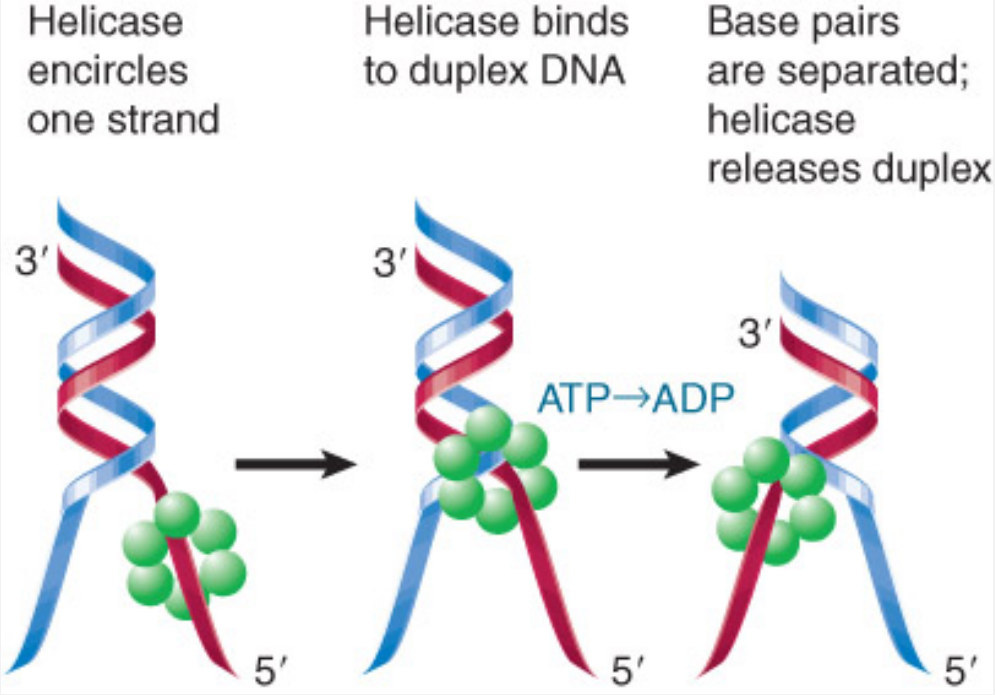


Figure 5–7 The structure of a DNA replication fork. Left, replication fork with newly synthesized DNA in red and arrows indicating the 5′-to-3′ direction of DNA synthesis. Because both daughter DNA strands are polymerized in the 5′-to-3′ direction, the DNA synthesized on the lagging strand must be made initially as a series of short DNA molecules, called *Okazaki fragments*, named after the scientist who discovered them. Right, the same fork a short time later. On the lagging strand, the Okazaki fragments are synthesized sequentially, with those nearest the fork being the most recently made.

Replication Requires a Helicase and a Single-Stranded Binding Protein

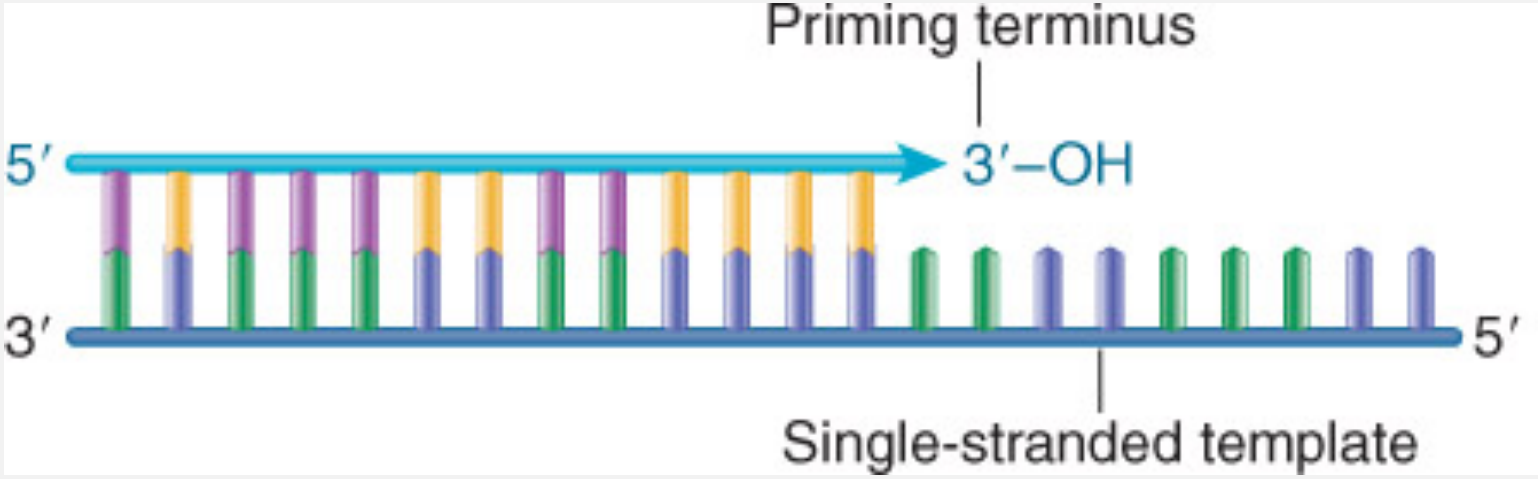
- Replication requires a helicase to separate the strands of DNA using energy provided by hydrolysis of ATP.
- A single-stranded DNA binding protein is required to maintain the separated strands.



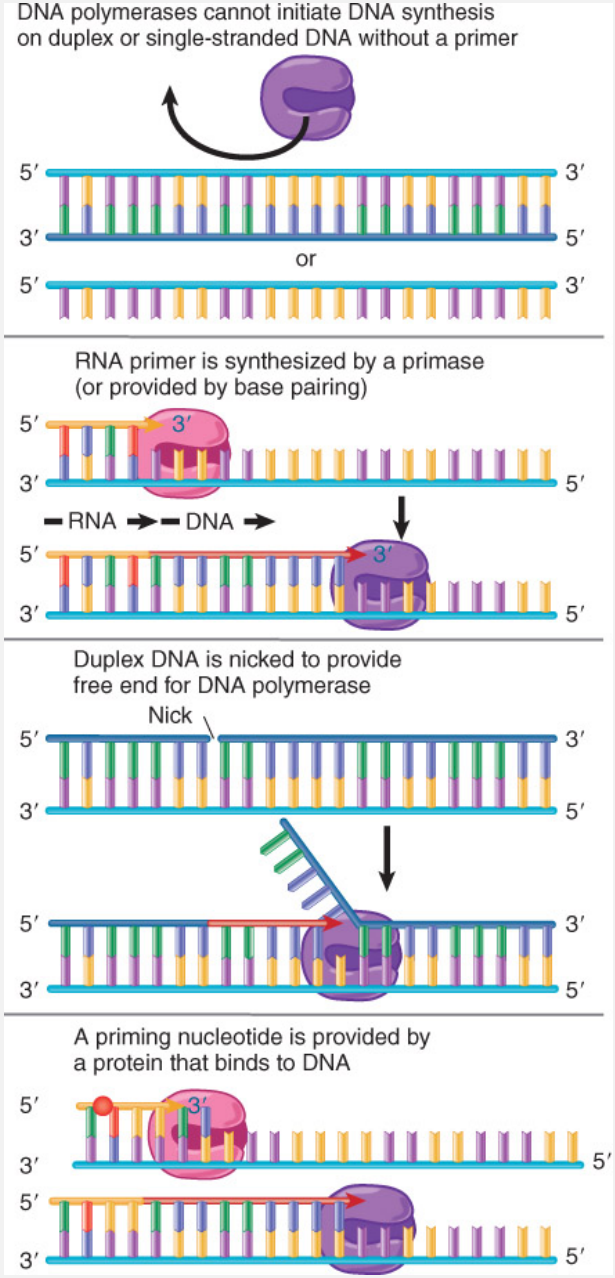
A hexameric helicase moves along one strand of DNA.

Priming Is Required to Start DNA Synthesis

- All DNA polymerases require a 3′–OH priming end to initiate DNA synthesis.



A DNA polymerase requires a 3' –OH end to initiate replication.



Priming Is Required to Start DNA Synthesis

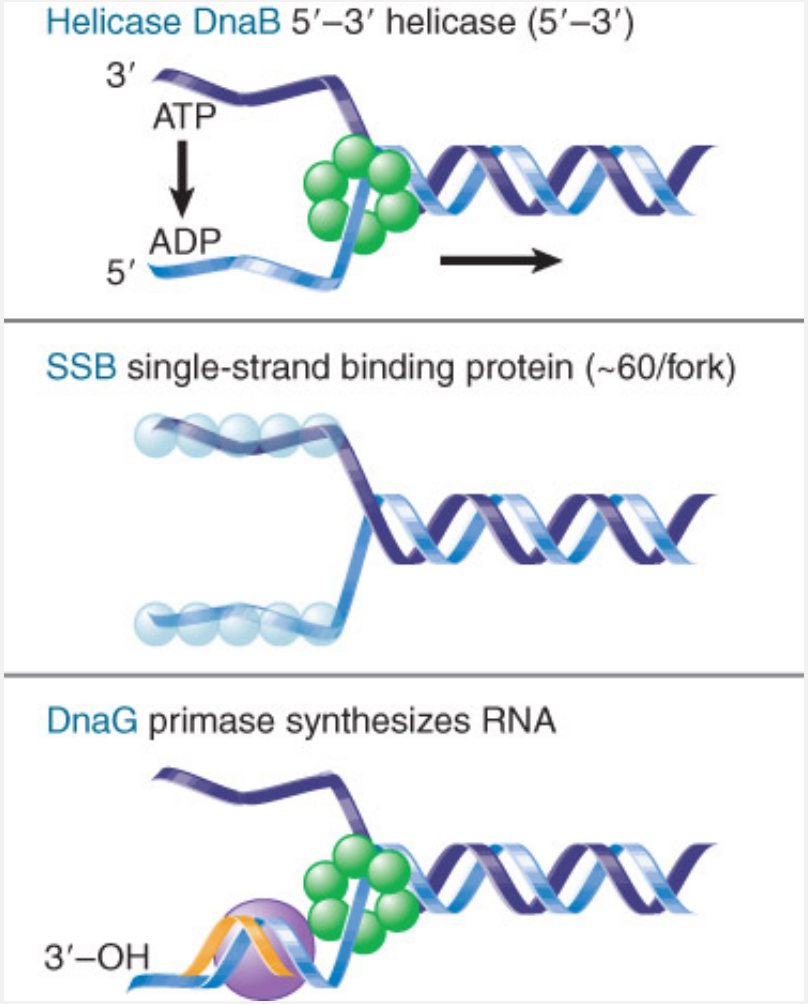
- The priming end can be provided by an RNA **primer**, a nick in DNA, or a priming protein.

There are several methods for providing the free 3' –OH end that DNA polymerases require to initiate DNA synthesis.

Priming Is Required to Start DNA Synthesis

- For DNA replication, a special RNA polymerase called a primase synthesizes an RNA chain that provides the priming end.

Priming Is Required to Start DNA Synthesis

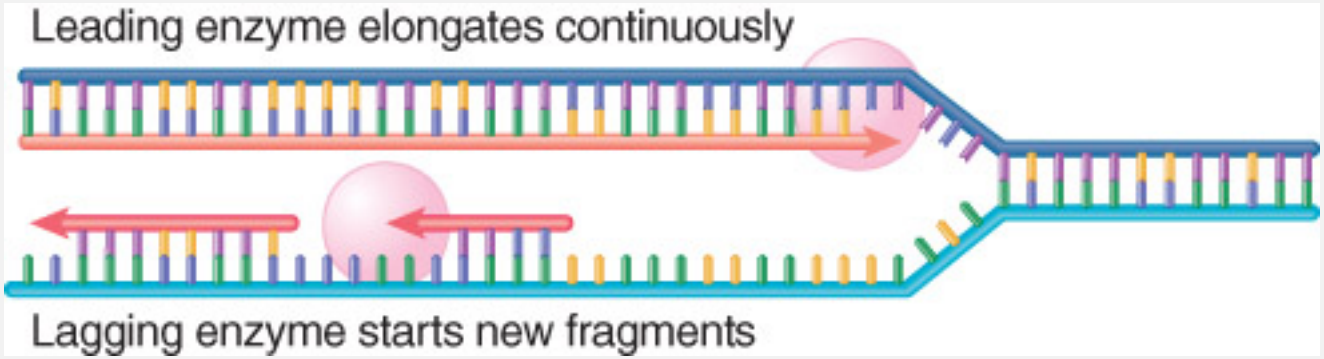


- Priming of replication on double-stranded DNA always requires a replicase, SSB, and primase.
- DnaB is the helicase that unwinds DNA for replication in *E. coli*.

Initiation requires several enzymatic activities, including helicases, single-strand binding proteins, and synthesis of the primer.

Coordinating Synthesis of the Lagging and Leading Strands

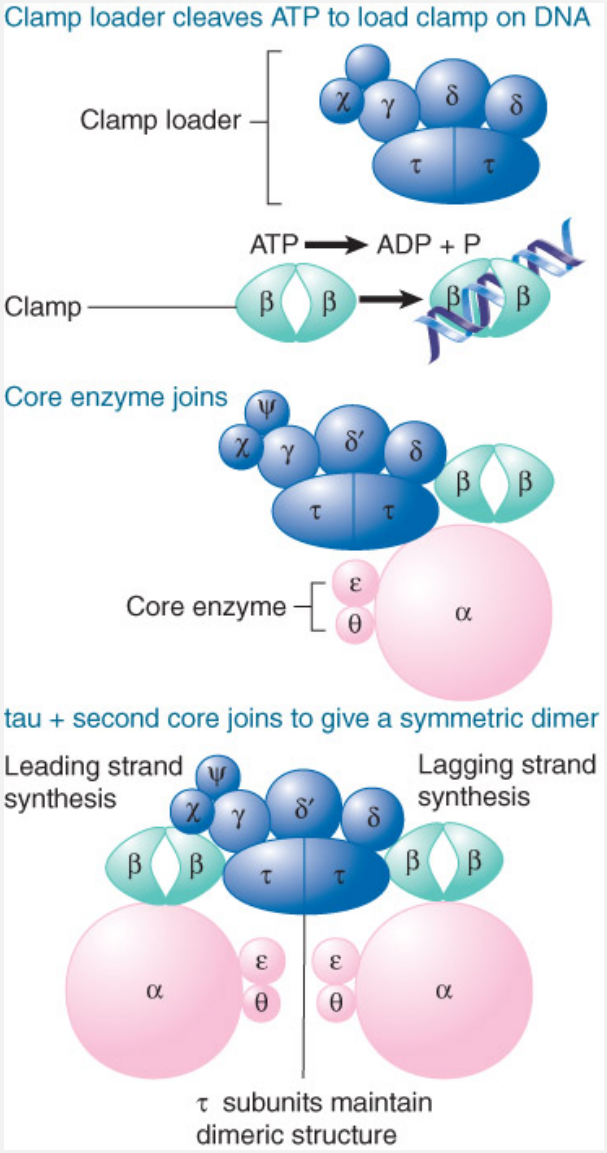
- Different enzyme units are required to synthesize the leading and lagging strands.
- In *E. coli*, both these units contain the same catalytic subunit (DnaE).
- In other organisms, different catalytic subunits might be required for each strand.



A replication complex contains separate catalytic units for synthesizing the leading and lagging strands.

DNA Polymerase Holoenzyme Consists of Subcomplexes

- The *E. coli* DNA polymerase III catalytic core contains three subunits, including a catalytic subunit and a proofreading subunit.
- The DNA Pol III holoenzyme has at least two catalytic cores, a processivity clamp, and a dimerization clamp-loader complex.



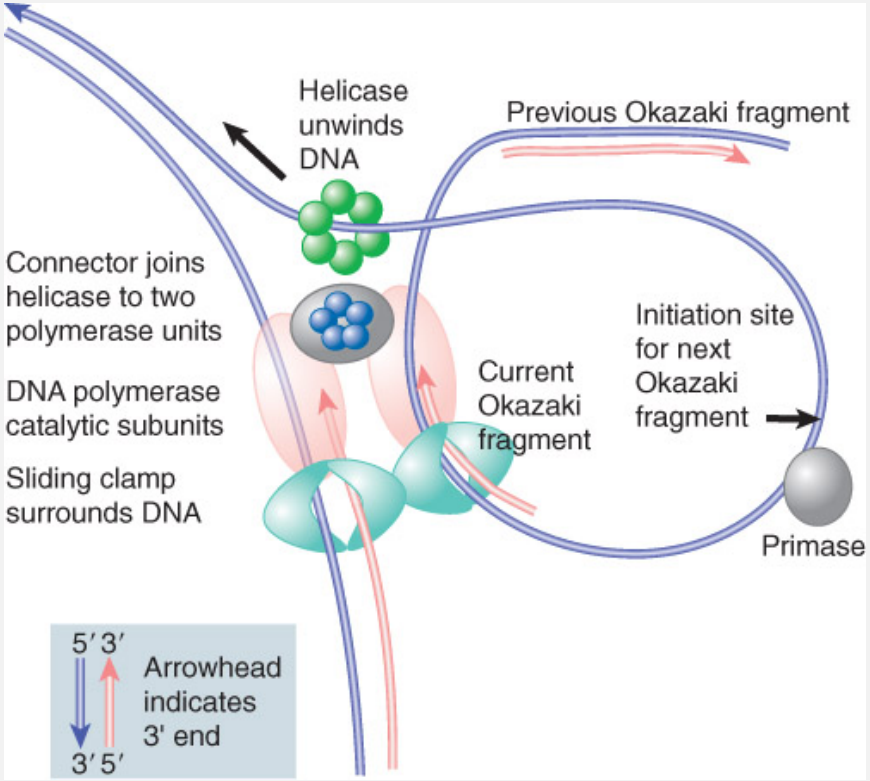
DNA Polymerase Holoenzyme Consists of Subcomplexes

- A **clamp loader** places the processivity subunits on DNA, where they form a circular **clamp** around the nucleic acid.
- At least one catalytic core is associated with each template strand.
- The *E. coli* replisome is composed of the holoenzyme complex and the additional enzymes required for chromosome replication.

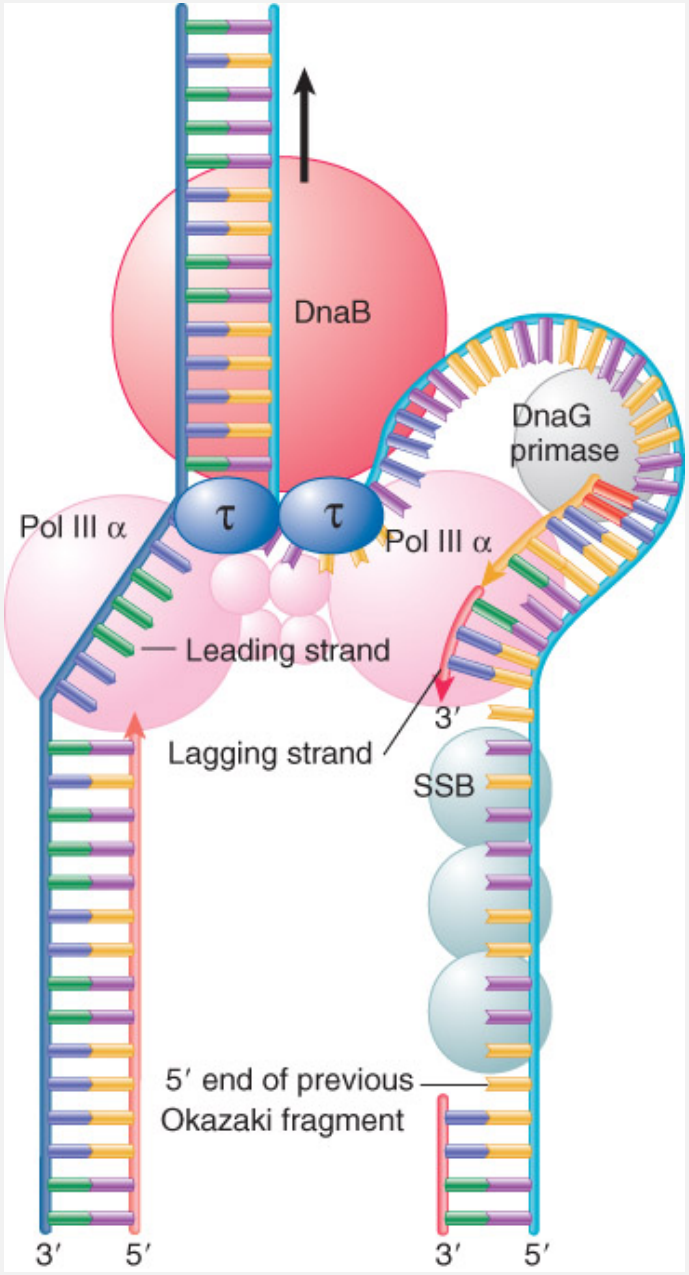
DNA polymerase III holoenzyme assembles in stages, generating an enzyme complex that synthesizes the DNA of both new strands.

The Clamp Controls Association of Core Enzyme with DNA

- The core on the leading strand is processive because its clamp keeps it on the DNA.
- The clamp associated with the core on the lagging strand dissociates at the end of each Okazaki fragment and reassembles for the next fragment.



The helicase creating the replication fork is connected to two DNA polymerase catalytic subunits.

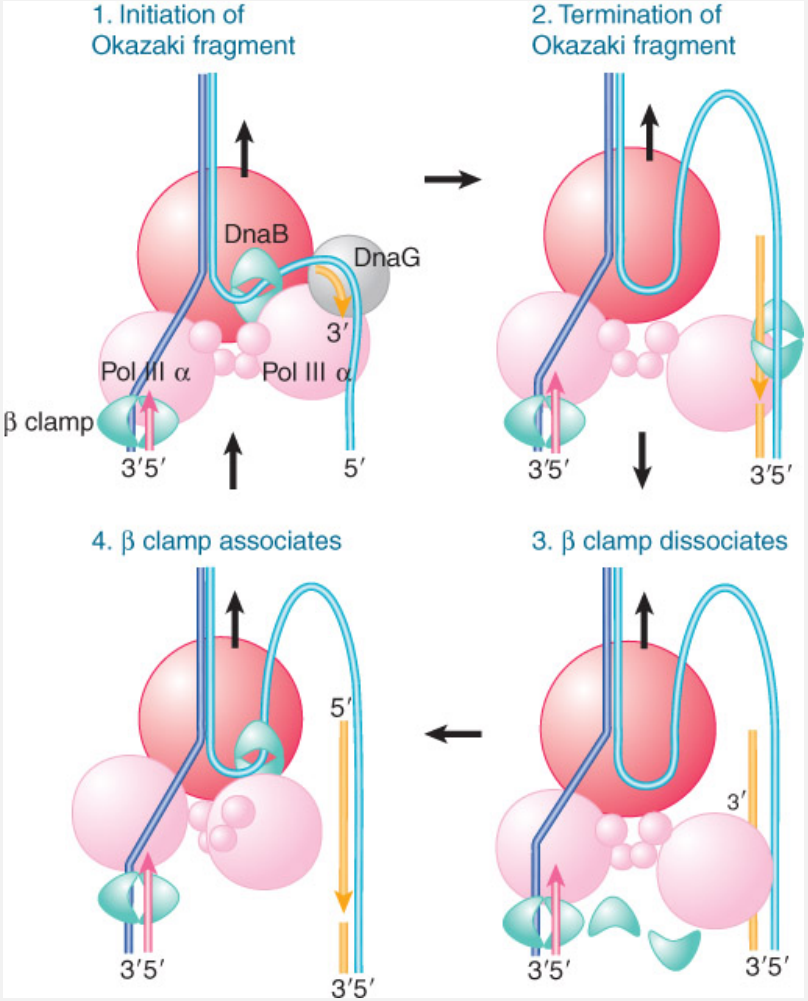


The Clamp Controls Association of Core Enzyme with DNA

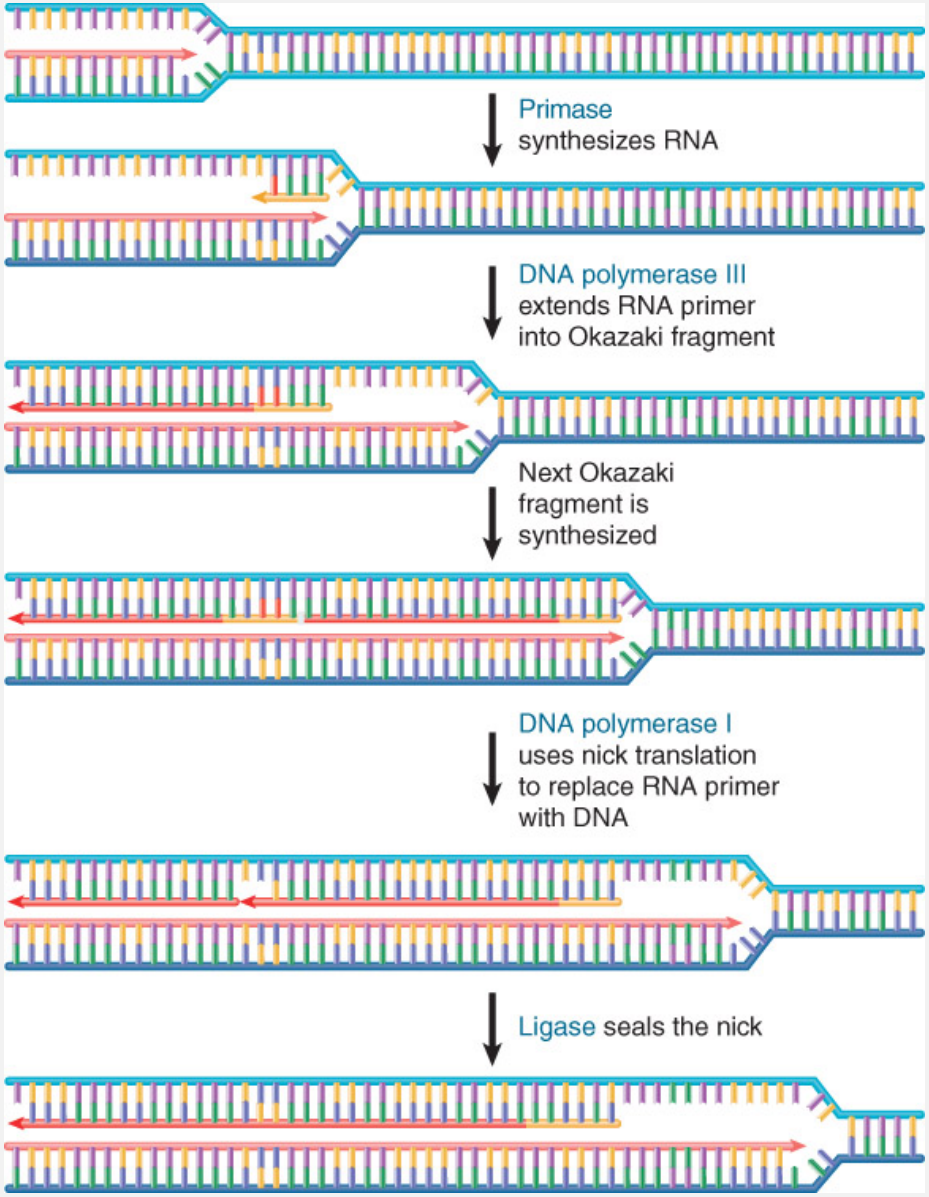
- The helicase DnaB is responsible for interacting with the primase DnaG to initiate each Okazaki fragment.

Each catalytic core of Pol III synthesizes a daughter strand. DnaB is responsible for forward movement at the replication fork.

The Clamp Controls Association of Core Enzyme with DNA



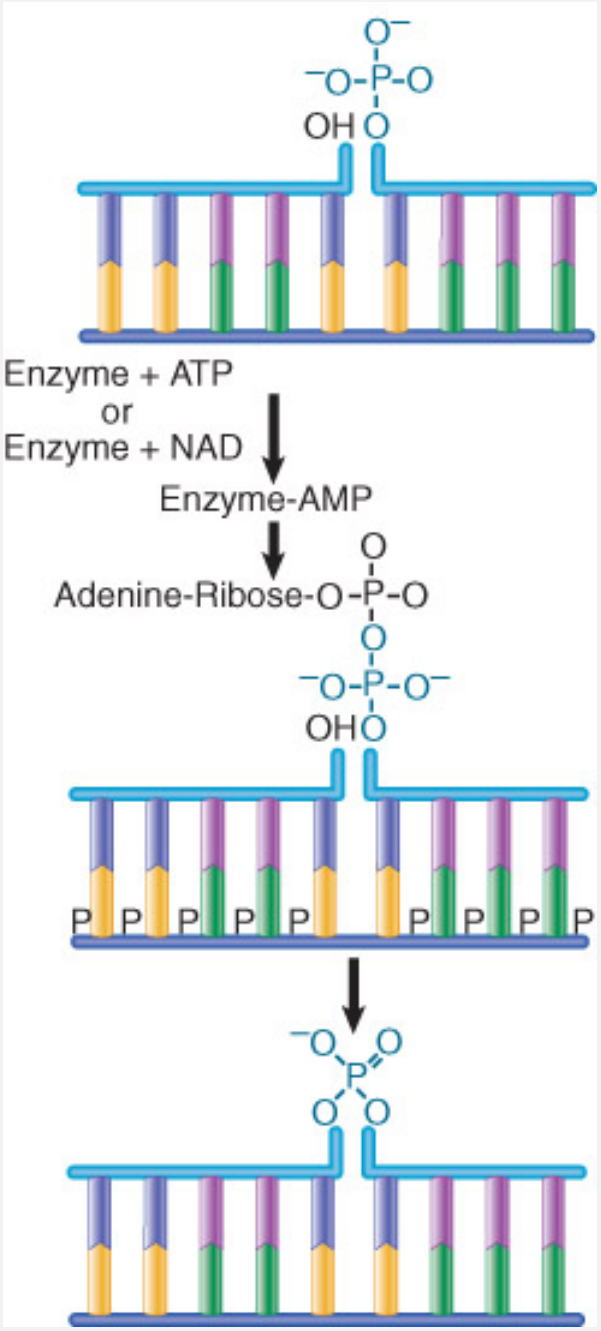
Core polymerase and the clamp dissociate at completion of Okazaki fragment synthesis and reassociate at the beginning.



Okazaki Fragments Are Linked by Ligase

- Each Okazaki fragment begins with a primer and stops before the next fragment.
- DNA polymerase I removes the primer and replaces it with DNA.

Synthesis of Okazaki fragments requires priming, extension, removal of RNA primer, gap filling, and nick ligation.



Okazaki Fragments Are Linked by Ligase

- **DNA ligase** makes the bond that connects the 3′ end of one Okazaki fragment to the 5′ beginning of the next fragment.

DNA ligase seals nicks between adjacent nucleotides by employing an enzyme-AMP intermediate.

Separate Eukaryotic DNA Polymerases Undertake Initiation and Elongation

- A replication fork has one complex of DNA polymerase α/primase, one complex of DNA polymerase δ, and one complex of DNA polymerase ε.
- The DNA polymerase α/primase complex initiates the synthesis of both DNA strands.
- DNA polymerase ε elongates the leading strand, and a second DNA polymerase δ elongates the lagging strand.

Separate Eukaryotic DNA Polymerases Undertake Initiation and Elongation

TABLE 11.3 Similar functions are required at all replication forks.

Function	*E. coli*	Eukaryote
Helicase Loading helicase/primase Single-strand maintenance Priming	DnaB DnaC SSB DnaG	MCM complex Cdc6 RPA Polα/primase
Sliding clamp Clamp loading (ATPase)	β γδ complex	PCNA RFC
Catalysis Holoenzyme dimerization	Pol III core T	Polδ + Pol ε ?
RNA removal Ligation	Pol I Ligase	FEN1 Ligase 1

Similar functions are required at all replication forks.

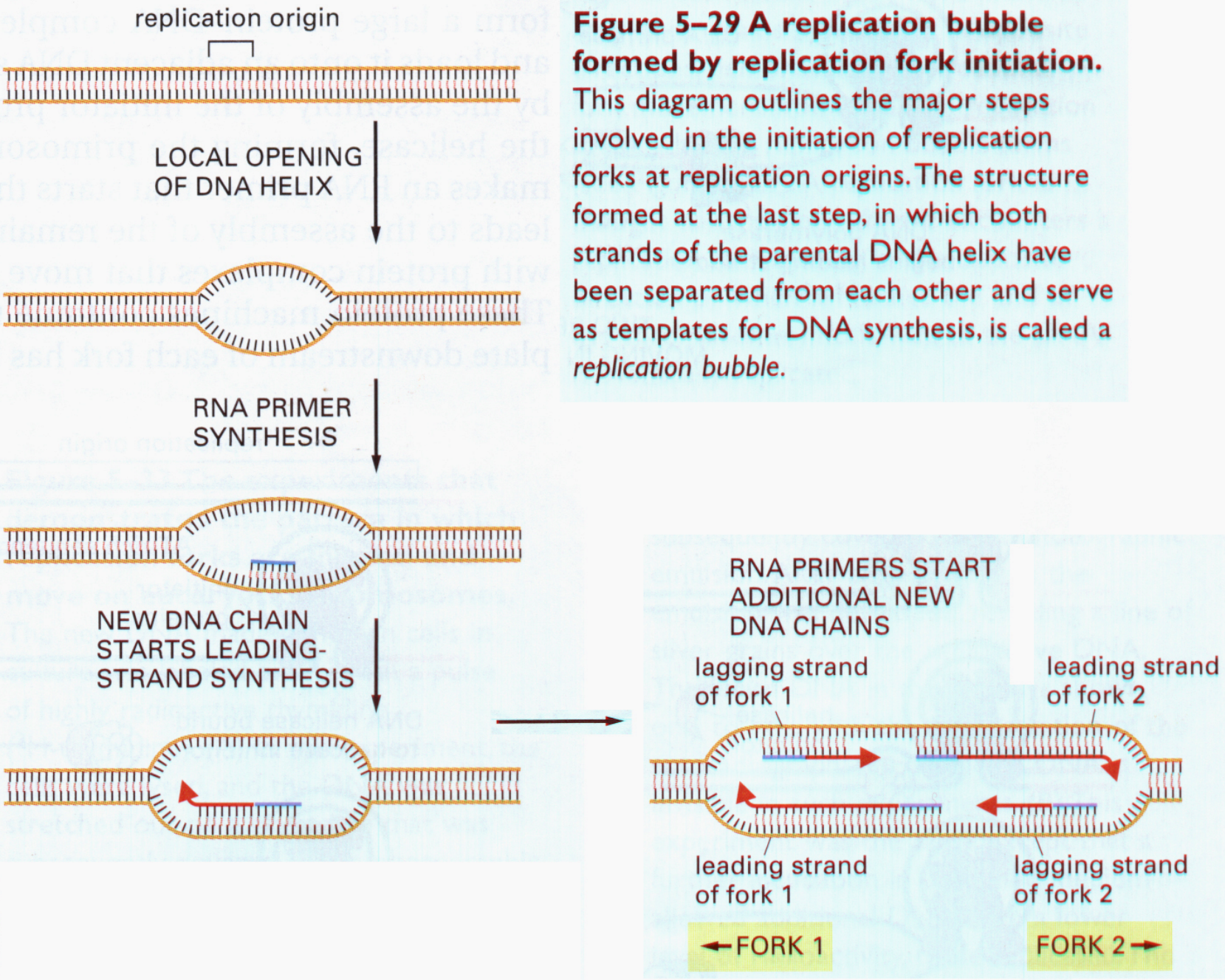
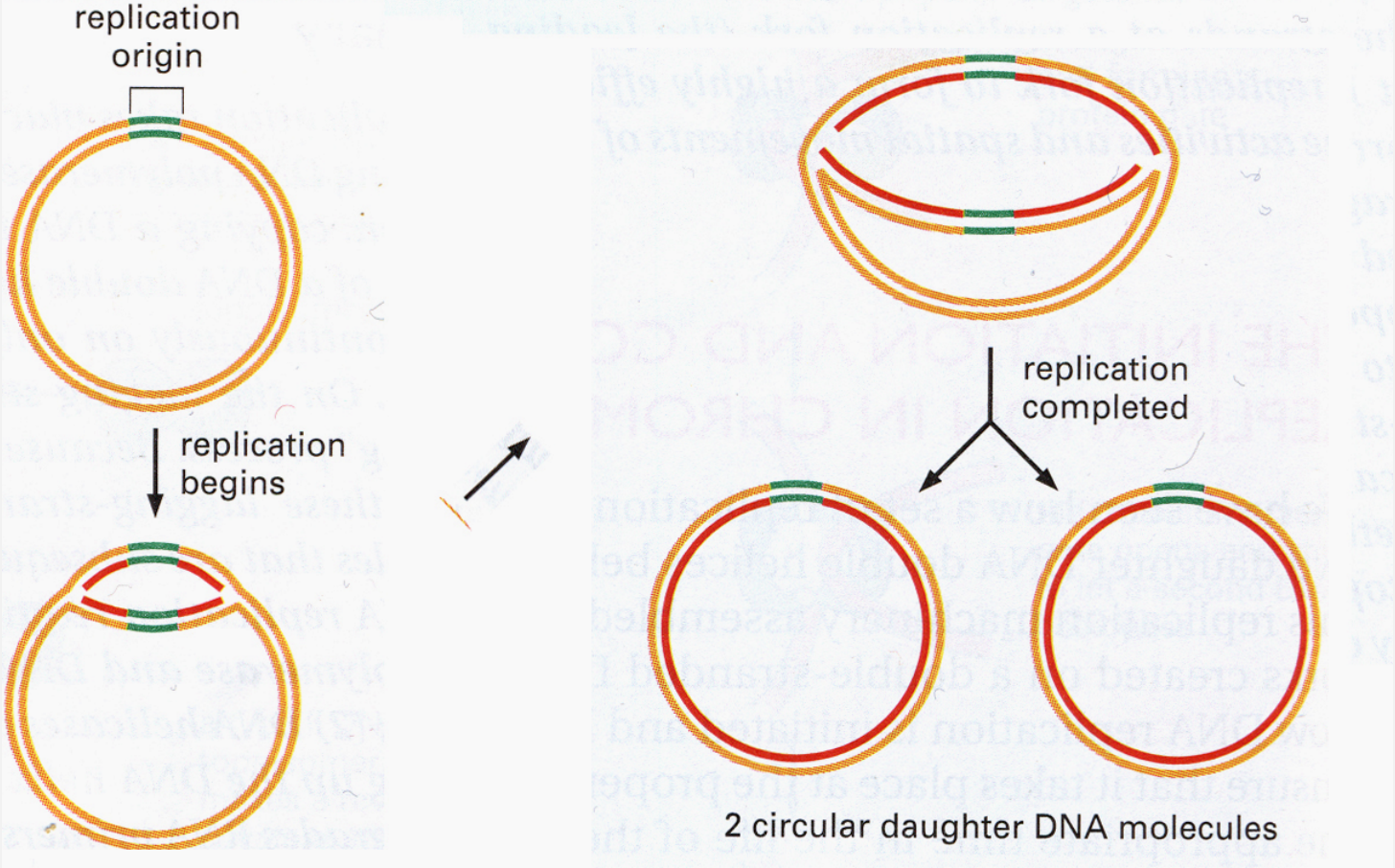


Figure 5–29 A replication bubble formed by replication fork initiation. This diagram outlines the major steps involved in the initiation of replication forks at replication origins. The structure formed at the last step, in which both strands of the parental DNA helix have been separated from each other and serve as templates for DNA synthesis, is called a *replication bubble*.

Figure 5–30 DNA replication of a bacterial genome. It takes *E. coli* about 40 minutes to duplicate its genome of 4.6×10^6 nucleotide pairs. For simplicity, no Okazaki fragments are shown on the lagging strand. What happens as the two replication forks approach each other and collide at the end of the replication cycle is not well understood, although the primosome is disassembled as part of the process.



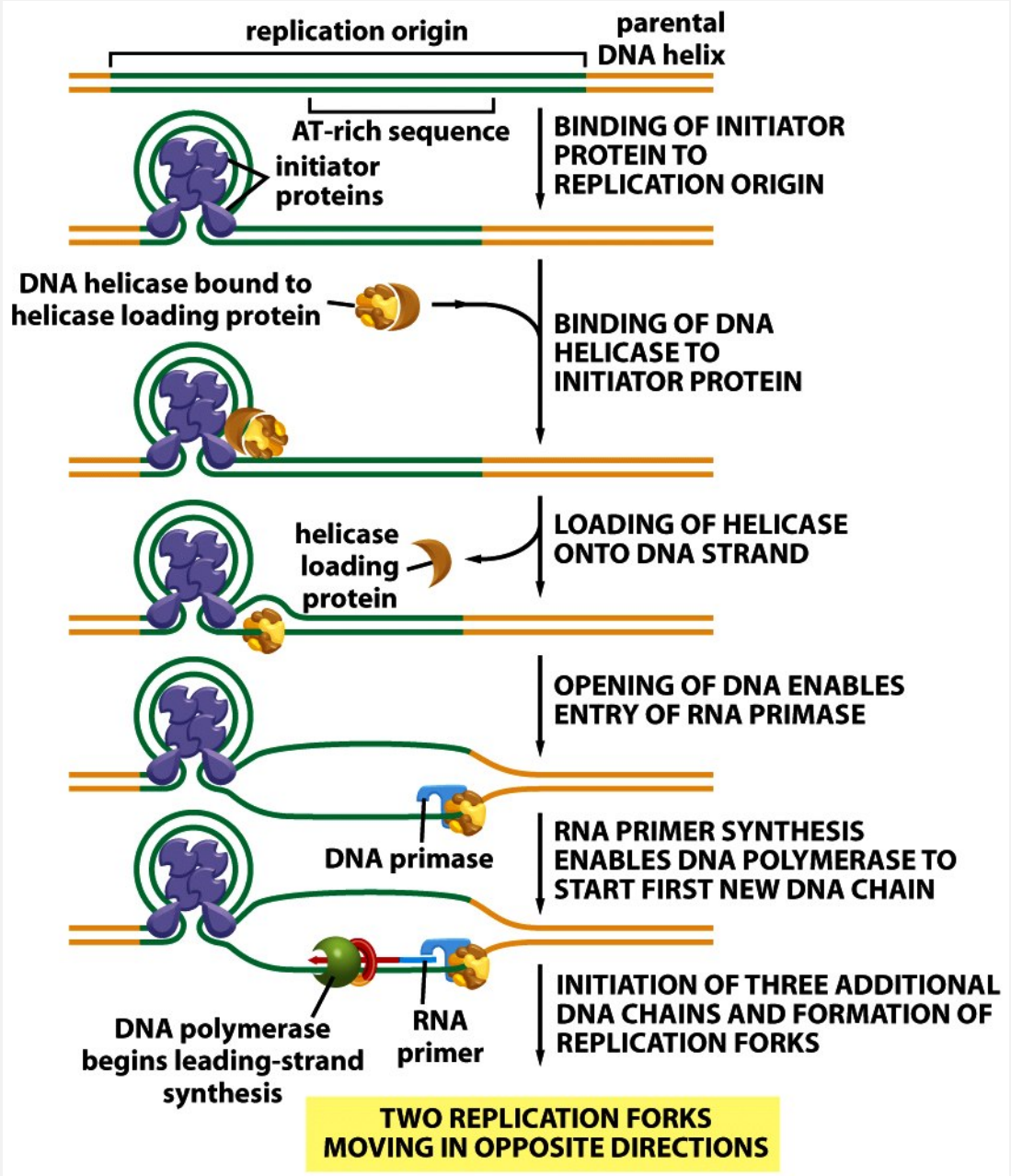
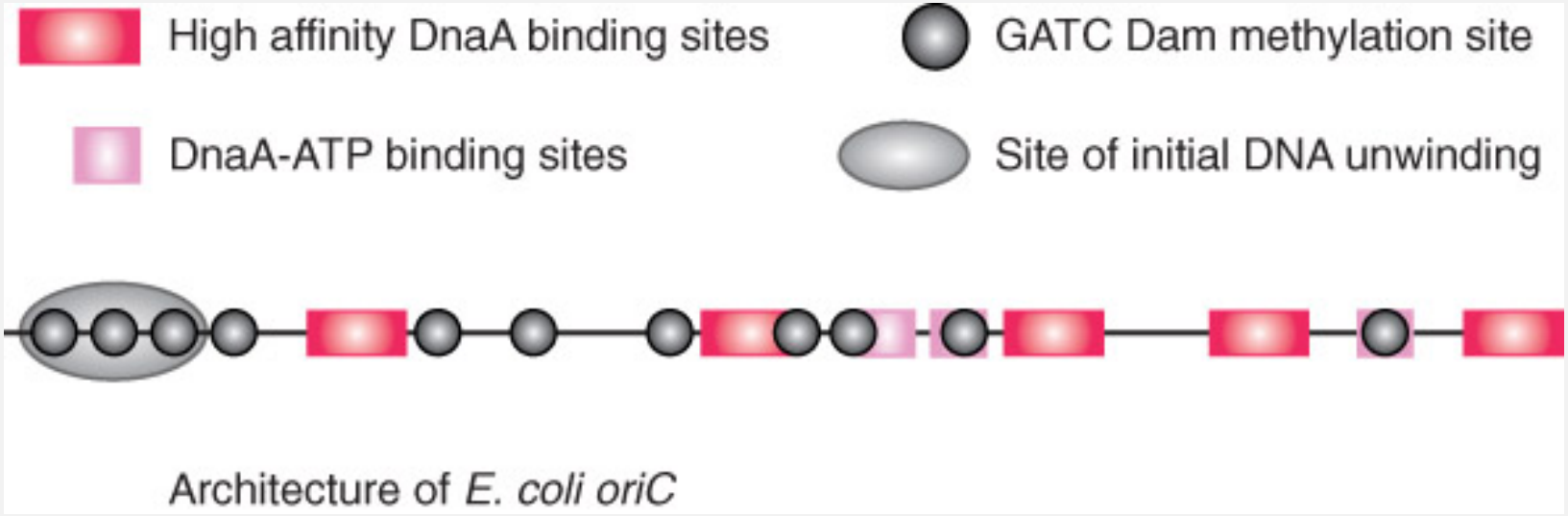


Figure 5-27 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Methylation of the Bacterial Origin Regulates Initiation

- *oriC* contains binding sites for DnaA: *dnaA* boxes.
- *oriC* also contains 11 GATC/CTAG repeats that are methylated on adenine on both strands.



The *E. coli* origin of replication, *oriC,* contains multiple binding sites for the DnaA initiator protein.

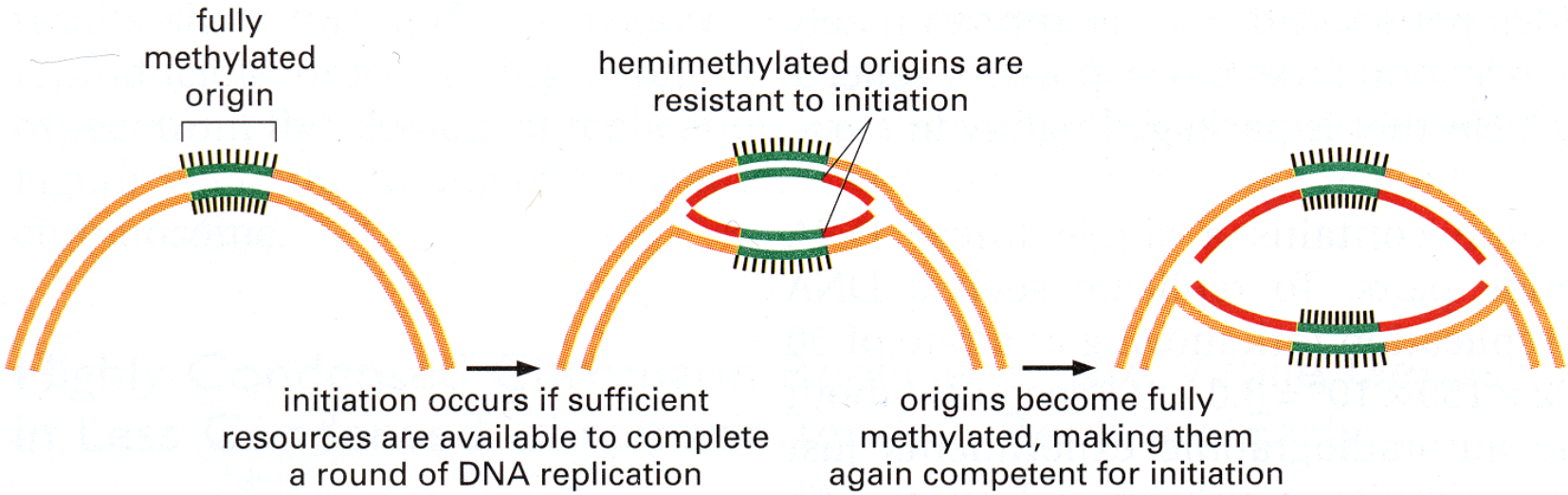
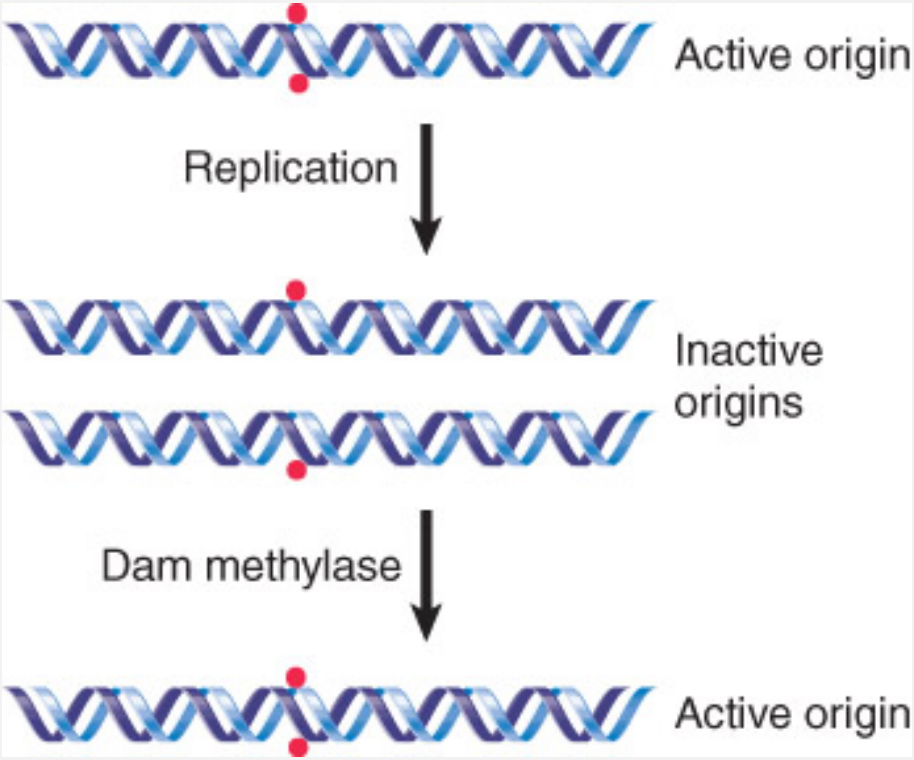


Figure 5–32 Methylation of the *E. coli* replication origin creates a refractory period for DNA initiation. DNA methylation occurs at GATC sequences, 11 of which are found in the origin of replication (spanning about 250 nucleotide pairs). About 10 minutes after replication is initiated, the hemimethylated origins become fully methylated by a DNA methylase enzyme.

As discussed earlier, the lag in methylation after the replication of GATC sequences is also used by the *E. coli* mismatch proofreading system to distinguish the newly synthesized DNA strand from the parental DNA strand; in that case, the relevant GATC sequences are scattered throughout the chromosome. A single enzyme, the *dam* methylase, is responsible for methylating *E. coli* GATC sequences.

Methylation of the Bacterial Origin Regulates Initiation

- Replication generates **hemimethylated DNA**, which cannot initiate replication.
- There is a 13-minute delay before the GATC/CTAG repeats are remethylated.



Only fully methylated origins can initiate replication.

Archaeal Chromosomes Can Contain Multiple Replicons

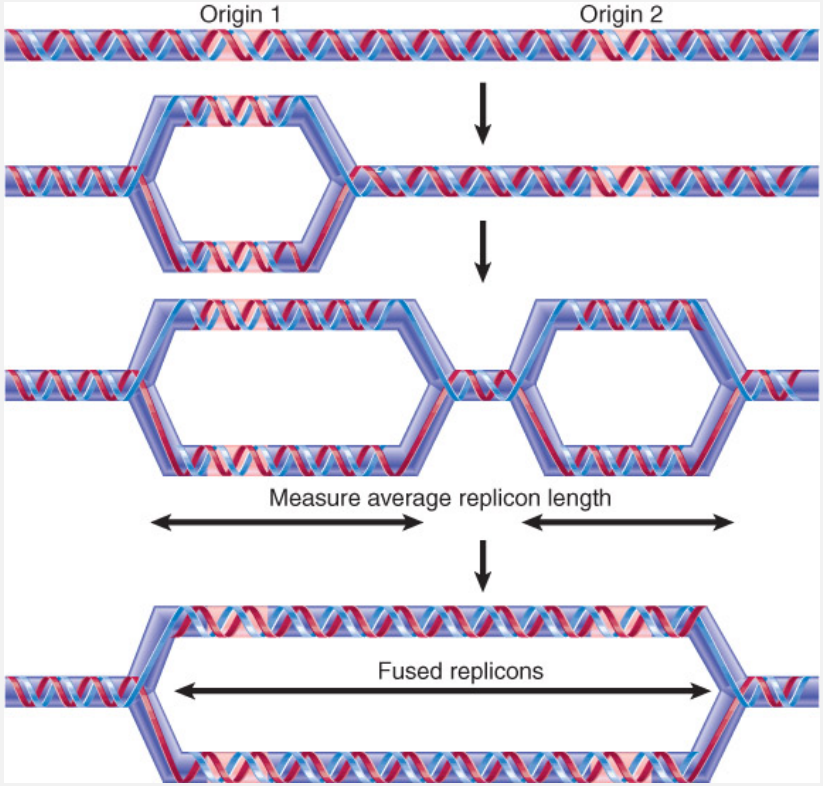
- Some archaea have multiple replication origins.
- These origins are bound by homologs of eukaryotic replication initiation factors.

Each Eukaryotic Chromosome Contains Many Replicons

- A chromosome is divided into many replicons.
- The progression into **S phase** is tightly controlled.
- **checkpoint –** A biochemical control mechanism that prevents the cell from progressing from one stage to the next unless specific goals and requirements have been met.

Each Eukaryotic Chromosome Contains Many Replicons

- Eukaryotic replicons are 40 to 100 kilobases (kb) in length.
- Individual replicons are activated at characteristic times during S phase.
- Regional activation patterns suggest that replicons near one another are activated at the same time.



A eukaryotic chromosome contains multiple origins of replication that ultimately merge during replication.

Replication Origins Can Be Isolated in Yeast

- **A domain –** The conserved 11-bp sequence of A-T base pairs in the yeast ARS (autonomously replicating sequence) element that comprises the replication origin.
- Related origin recognition complexes are found in multicellular eukaryotes.

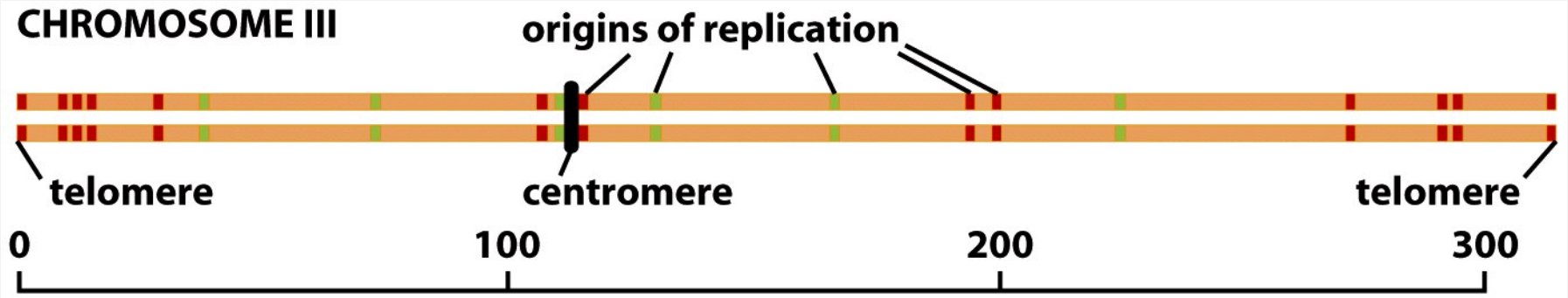


Figure 5-34 Molecular Biology of the Cell 5/e (© Garland Science 2008)

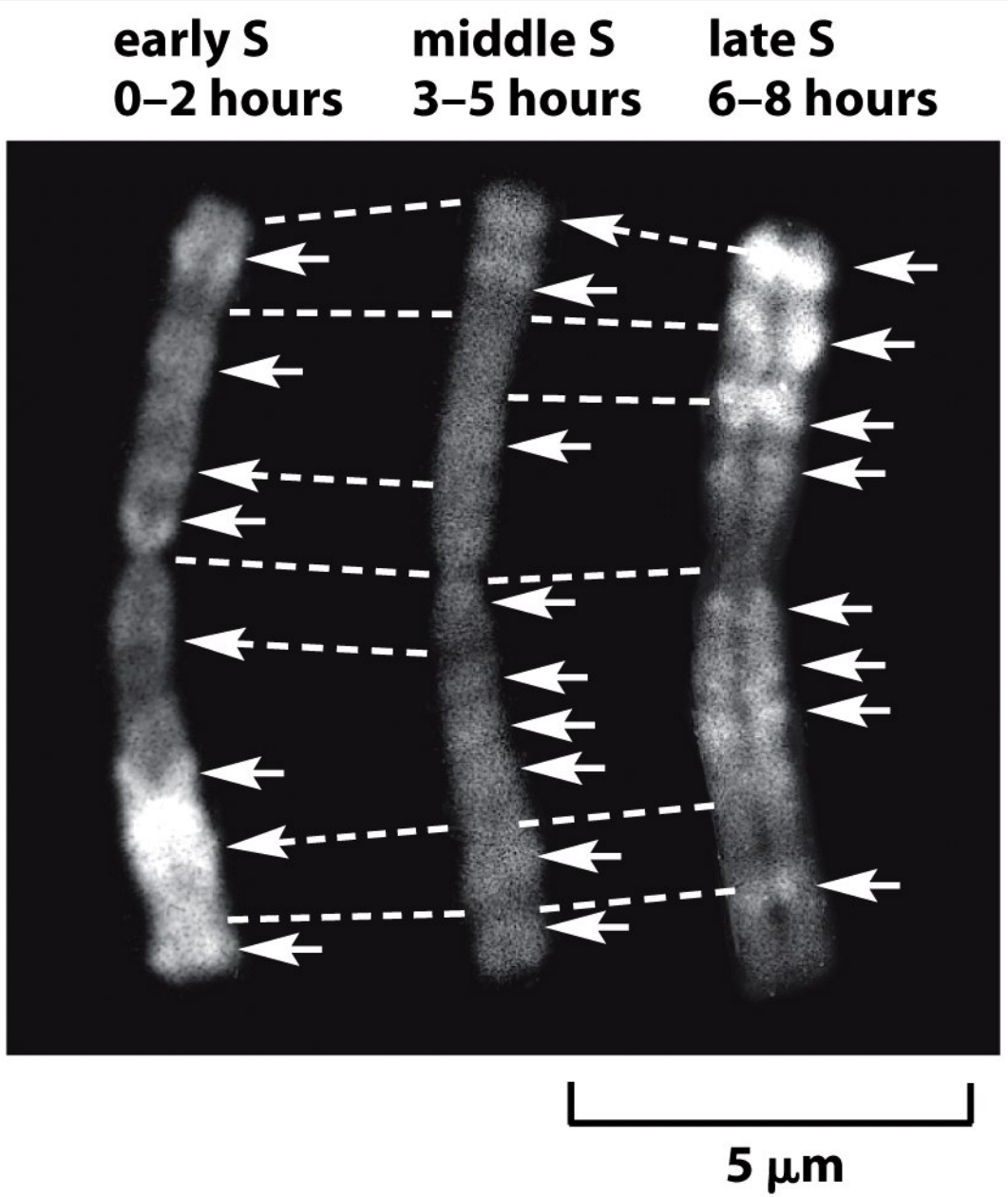
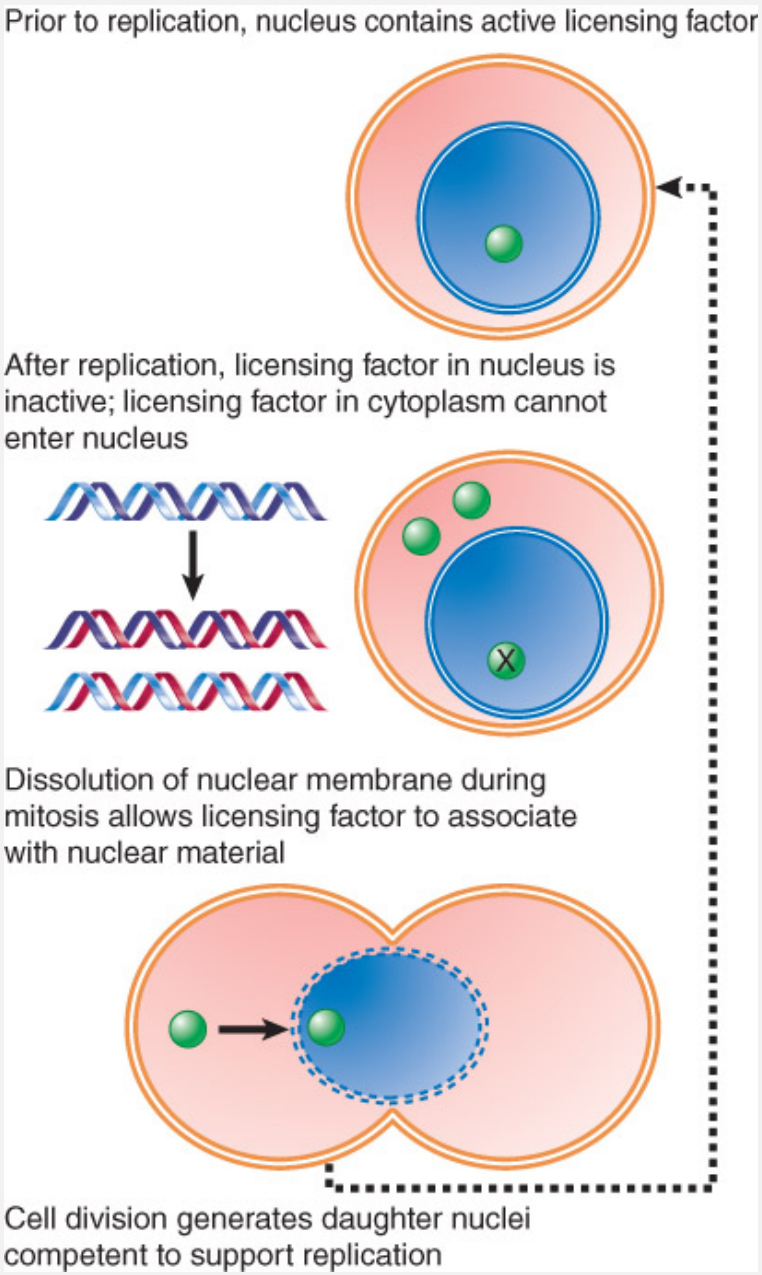


Figure 5-31 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Licensing Factor Controls Eukaryotic Rereplication

- **Licensing factor** is necessary for initiation of replication at each origin.
- Licensing factor is present in the nucleus prior to replication, but is removed, inactivated, or destroyed by replication.



Licensing Factor Controls Eukaryotic Rereplication

- Initiation of another replication cycle becomes possible only after licensing factor reenters the nucleus after mitosis.

Licensing factor in the nucleus is inactivated after replication.

Licensing Factor Binds to ORC

- ORC is a protein complex that is associated with yeast origins throughout the cell cycle.
- Cdc6 protein is an unstable protein that is synthesized only in G1.
- Cdc6 binds to ORC and allows MCM proteins to bind.
- Cdt1 facilitates MCM loading on origins.

Licensing Factor Binds to ORC

- When replication is initiated, Cdc6 and Cdt1 are displaced. The degradation of Cdc6 prevents reinitiation.
- **prereplication complex –** A protein-DNA complex at the origin in *S. cerevisiae* that is required for DNA replication. The complex contains the ORC complex, Cdc6, and the MCM proteins.
- **postreplication complex –** A protein-DNA complex in *S. cerevisiae* that consists of the ORC complex bound to the origin.

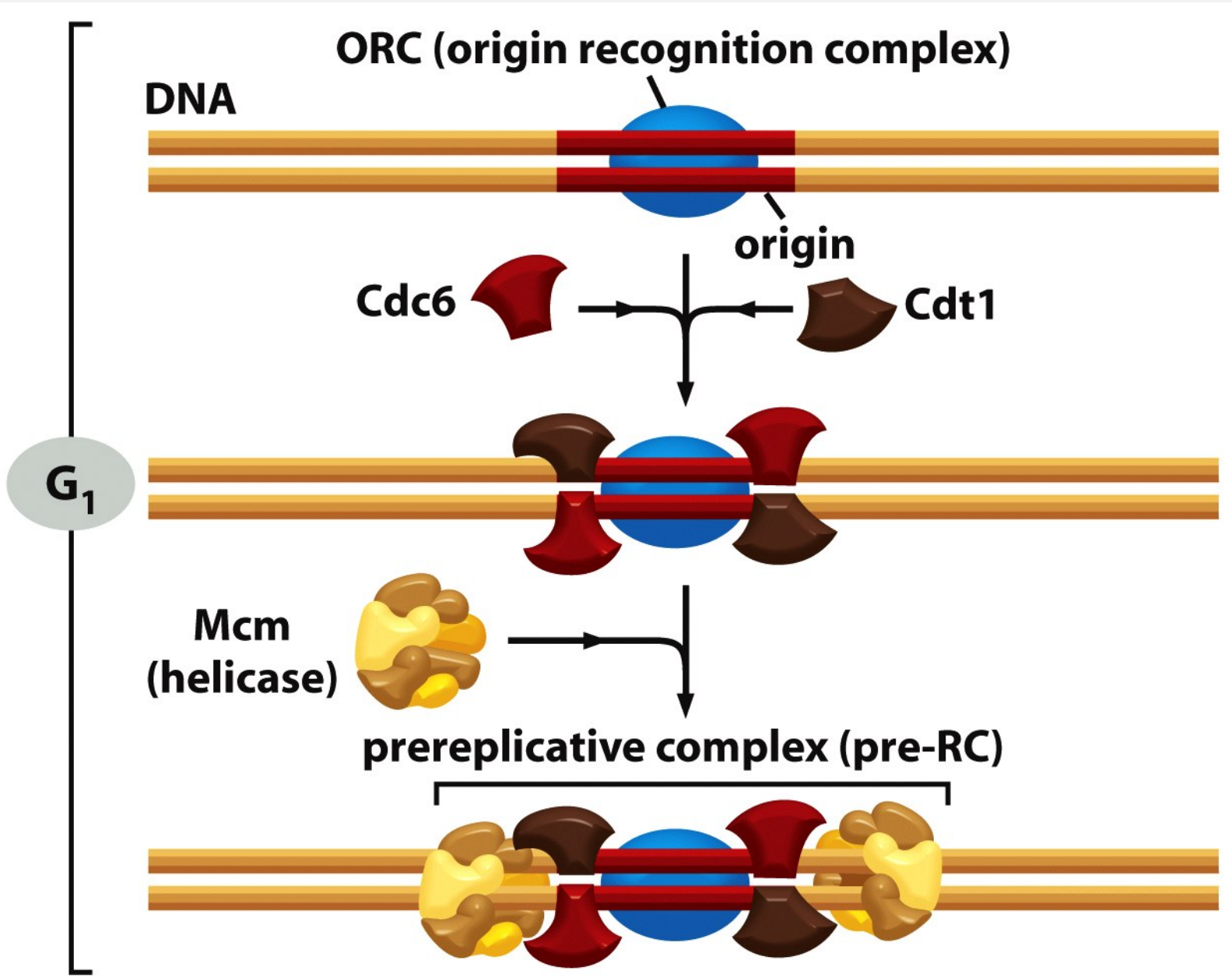


Figure 5-36 part 1 of 3 Molecular Biology of the Cell 5/e (© Garland Science 2008)

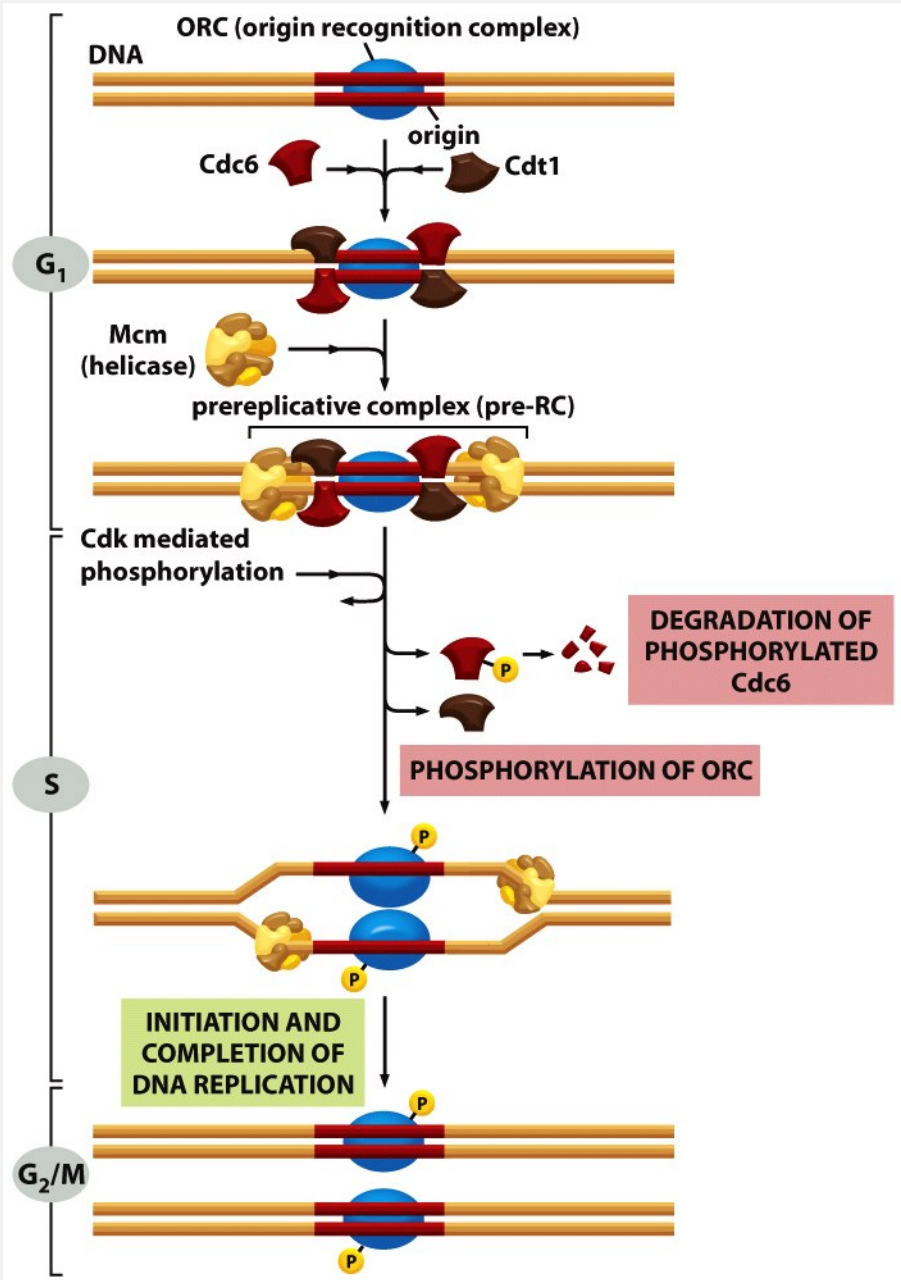
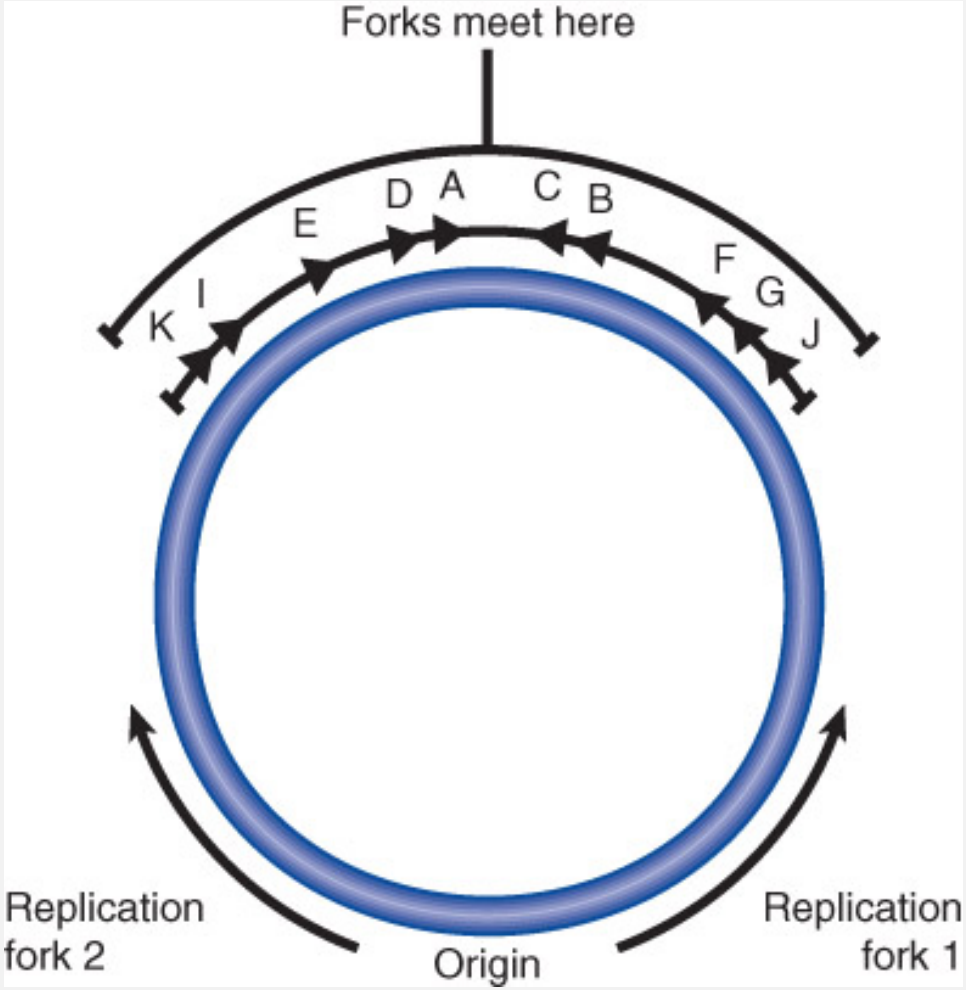


Figure 5-36 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Termination of Replication



- The two replication forks usually meet halfway around the circle, but there are *ter* sites that cause termination if the replication forks go too far.

Replication termini in *E. coli* are located in a region between two sets of *ter* sites.

Telomeres Are Synthesized by a Ribonucleoprotein Enzyme

- **Telomerase** uses the 3′–OH of the G+T telomeric strand and its own RNA template to iteratively add tandem repeats (5′-TTAGGG-3′ in human) to the 3′ end at each chromosomal terminus.
- Telomerase uses a reverse transcriptase to extend the very ends of the chromosomes and solve the so-called end replication problem.

Telomeres Are Synthesized by a Ribonucleoprotein Enzyme

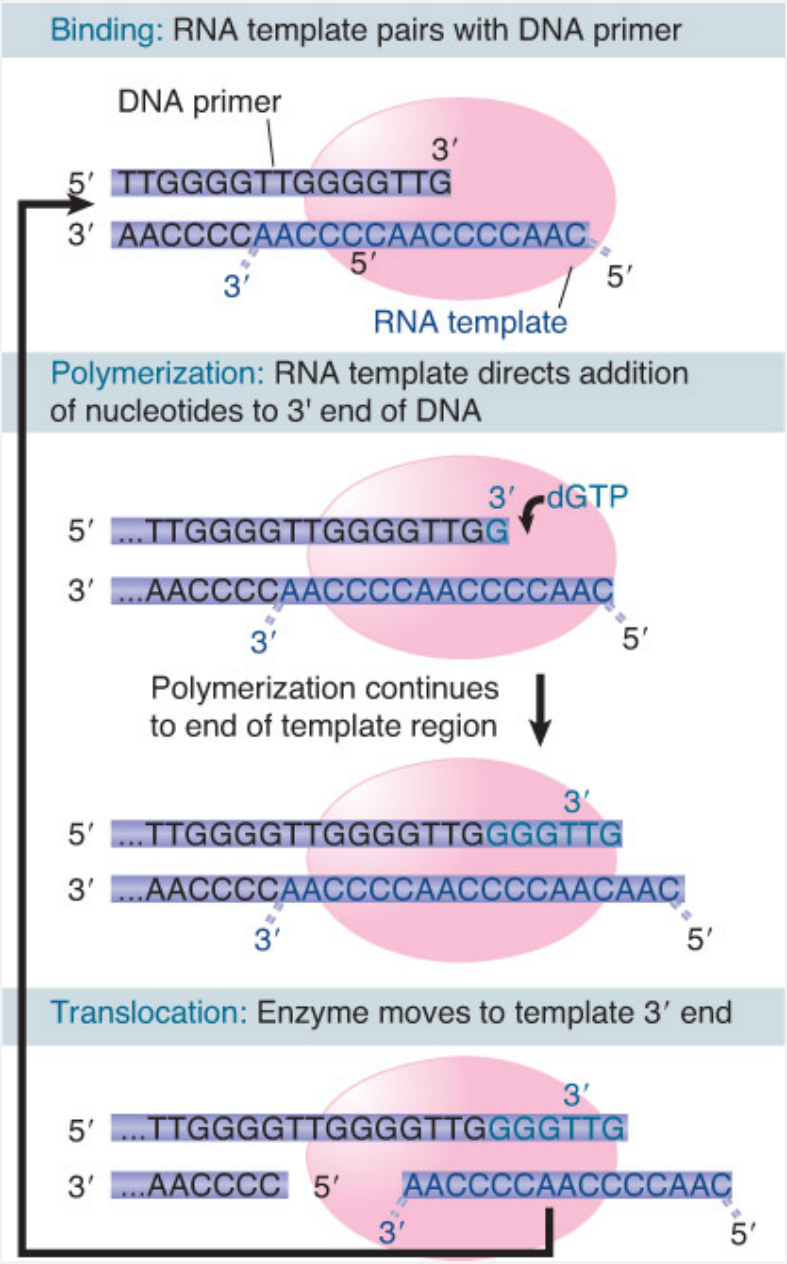
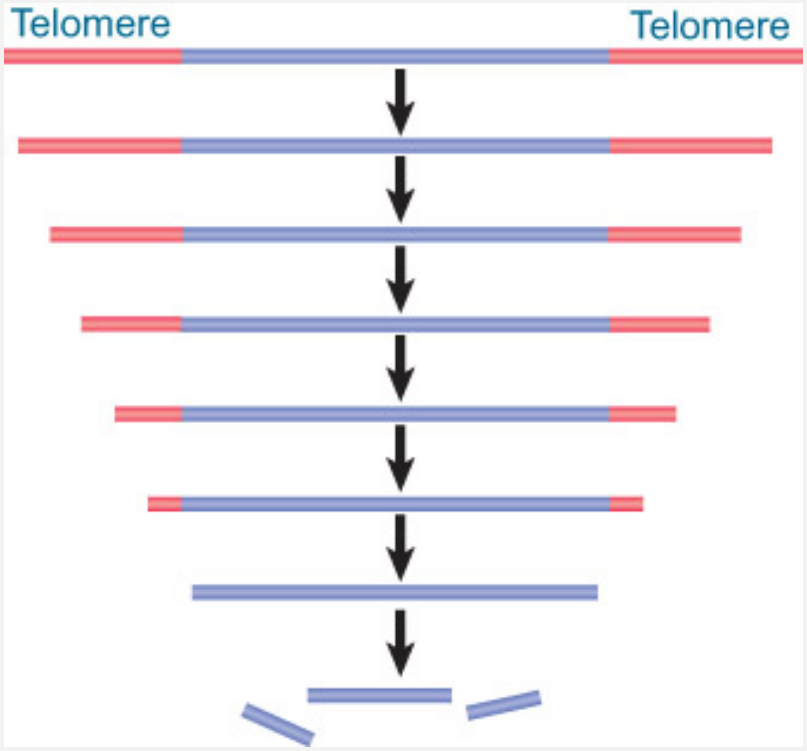


Figure 7.33: Telomerase positions itself by base pairing between the RNA template and the protruding single-stranded DNA primer.

Telomeres Are Essential for Survival

- Telomerase is expressed in actively dividing cells and is not expressed in quiescent cells.
- Loss of telomeres results in senescence.
- Escape from senescence can occur if telomerase is reactivated, or via unequal homologous recombination to restore telomeres.



Mutation in telomerase causes telomeres to shorten in each cell division.