ZOO 203 – MOLECULAR BIOLOGY
UNIT 1: DNA REPLICATION
(PART –I)

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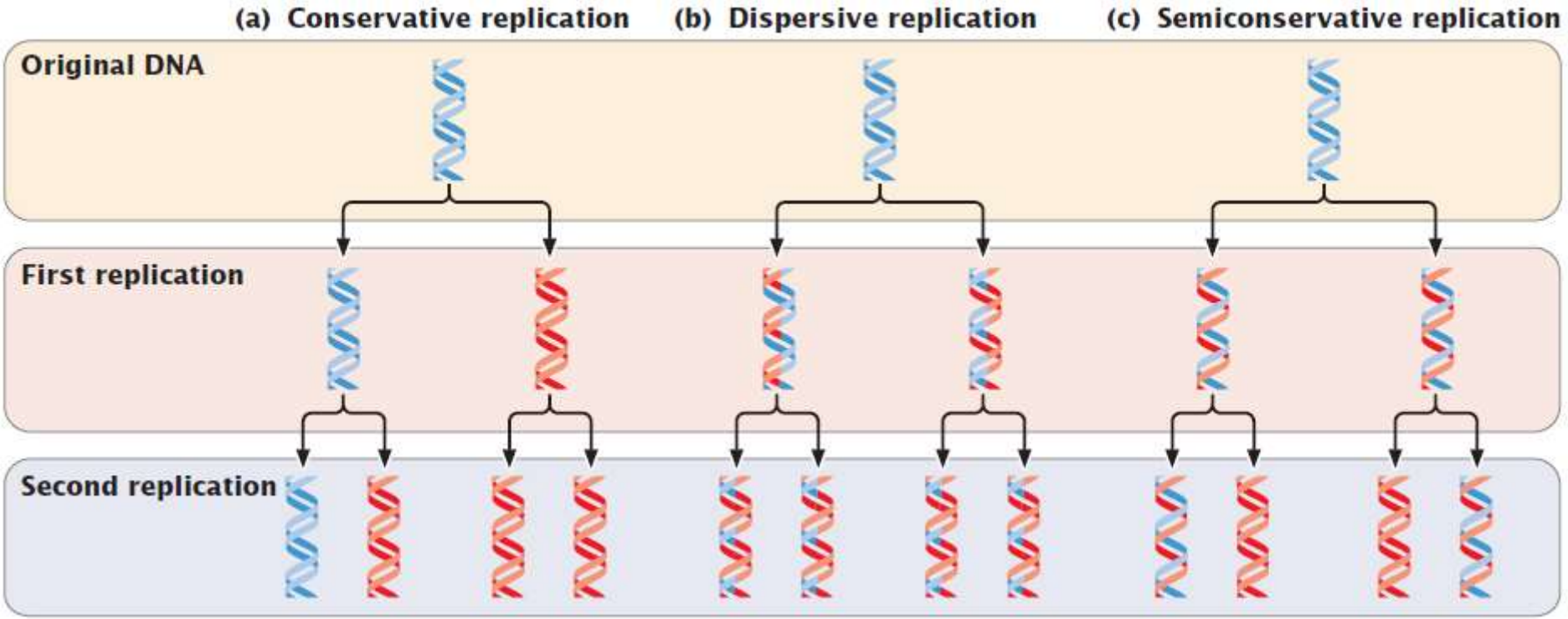
Semiconservative replication

- In **conservative** replication (**Figure a**), the entire double-stranded DNA molecule serves as a template for a whole new molecule of DNA, and the original DNA molecule is ***fully*** conserved during replication.

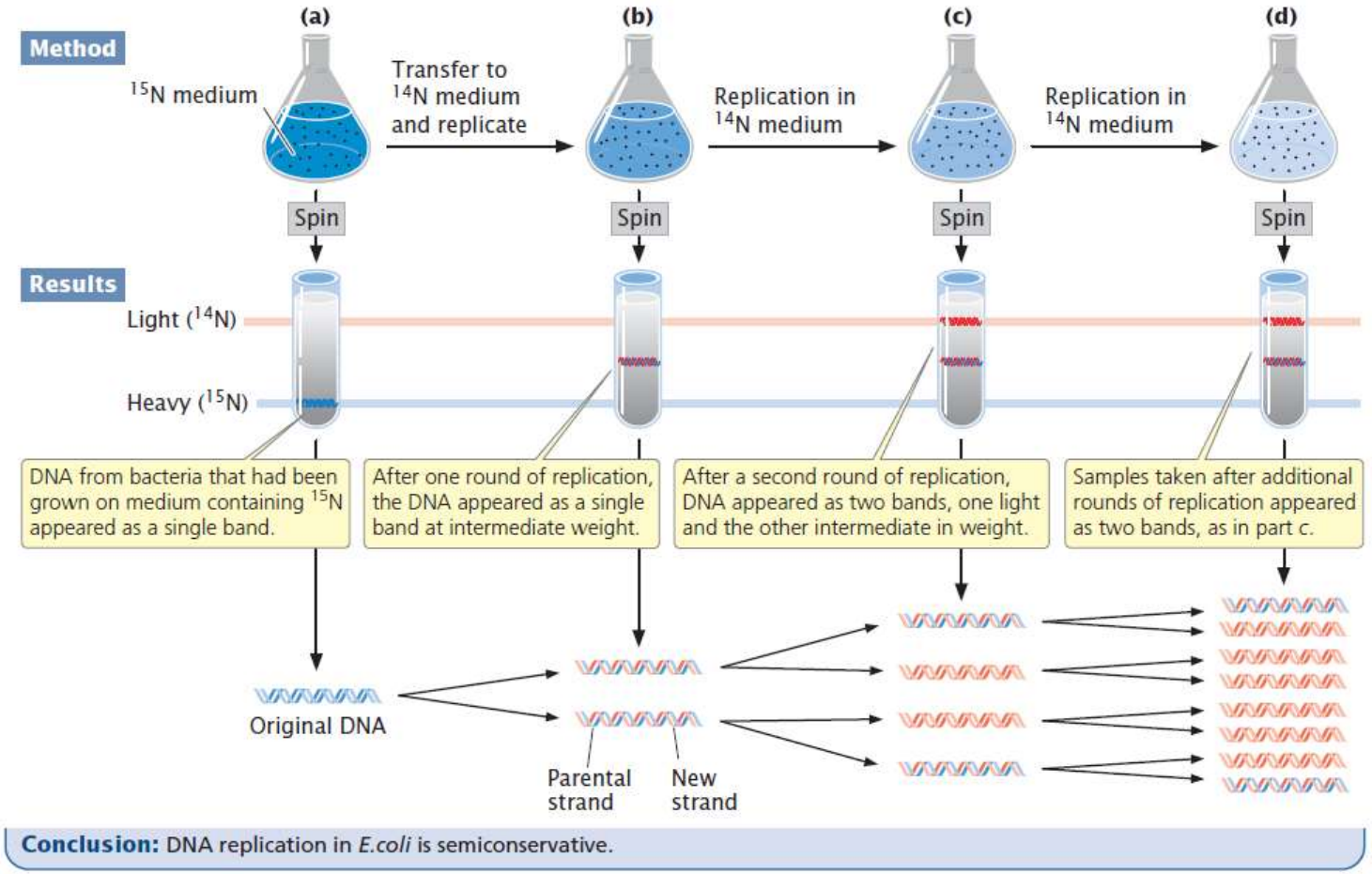
- In **dispersive** replication (**Figure b**), both nucleotide strands break down (**disperse**) into fragments, which serve as templates for the synthesis of new DNA fragments, and then somehow reassemble into two complete DNA molecules. In this model, each resulting DNA molecule is interspersed with fragments of old and new DNA; none of the original molecule is conserved.

- **Semiconservative** replication (**Figure c**) is intermediate between these two models; the two nucleotide strands unwind and each serves as a template for a new DNA molecule

**Three proposed models of replication are conservative replication, dispersive replication,
and semiconservative replication**



Meselson and Stahl demonstrated that DNA replication is semiconservative



Modes of REPLICATION

- **Theta replication**
- **Rolling-circle replication**
- **Linear eukaryotic replication**

- Individual units of replication are called **replicons**, each of which contains a **replication origin .**

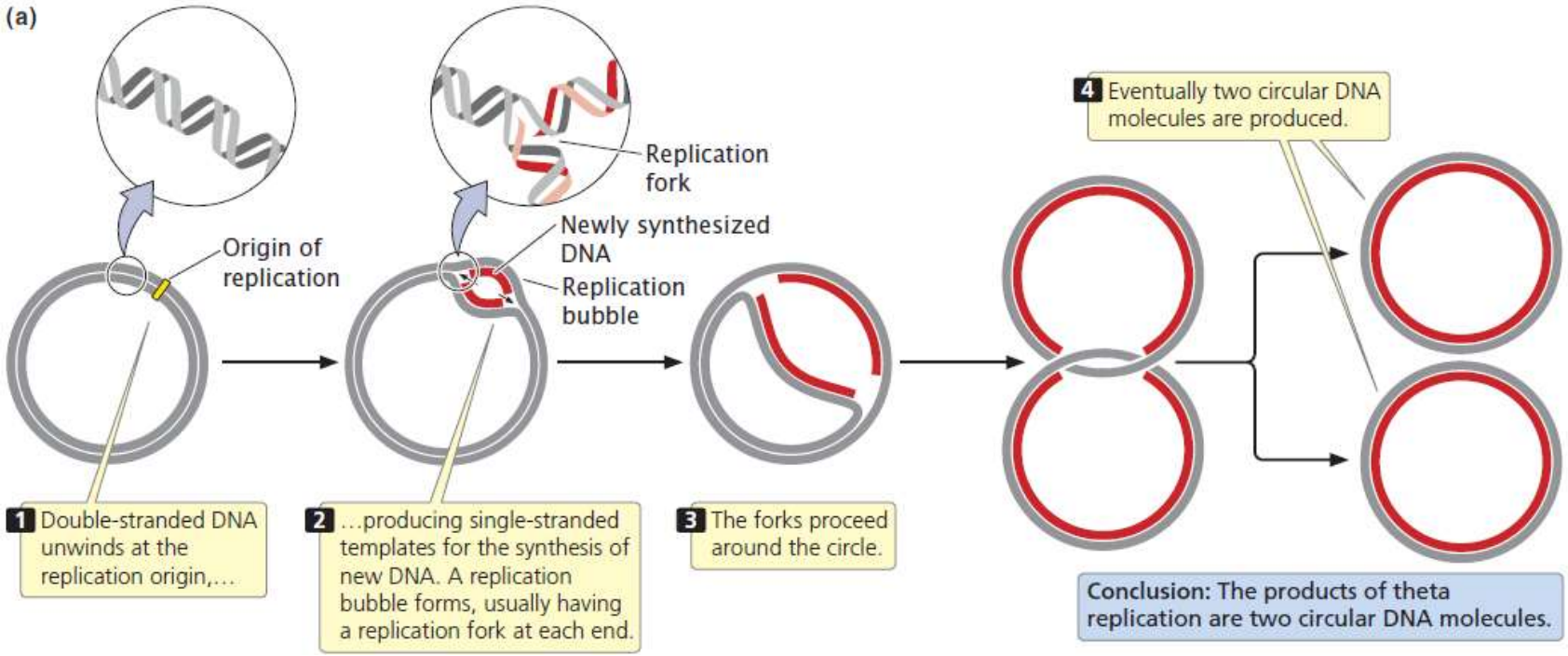
- Replication starts at the origin and continues until the entire replicon has been replicated. Bacterial chromosomes have a single replication origin, whereas eukaryotic chromosomes contain many.

- The unwinding of the double helix generates a loop, termed a **replication bubble**

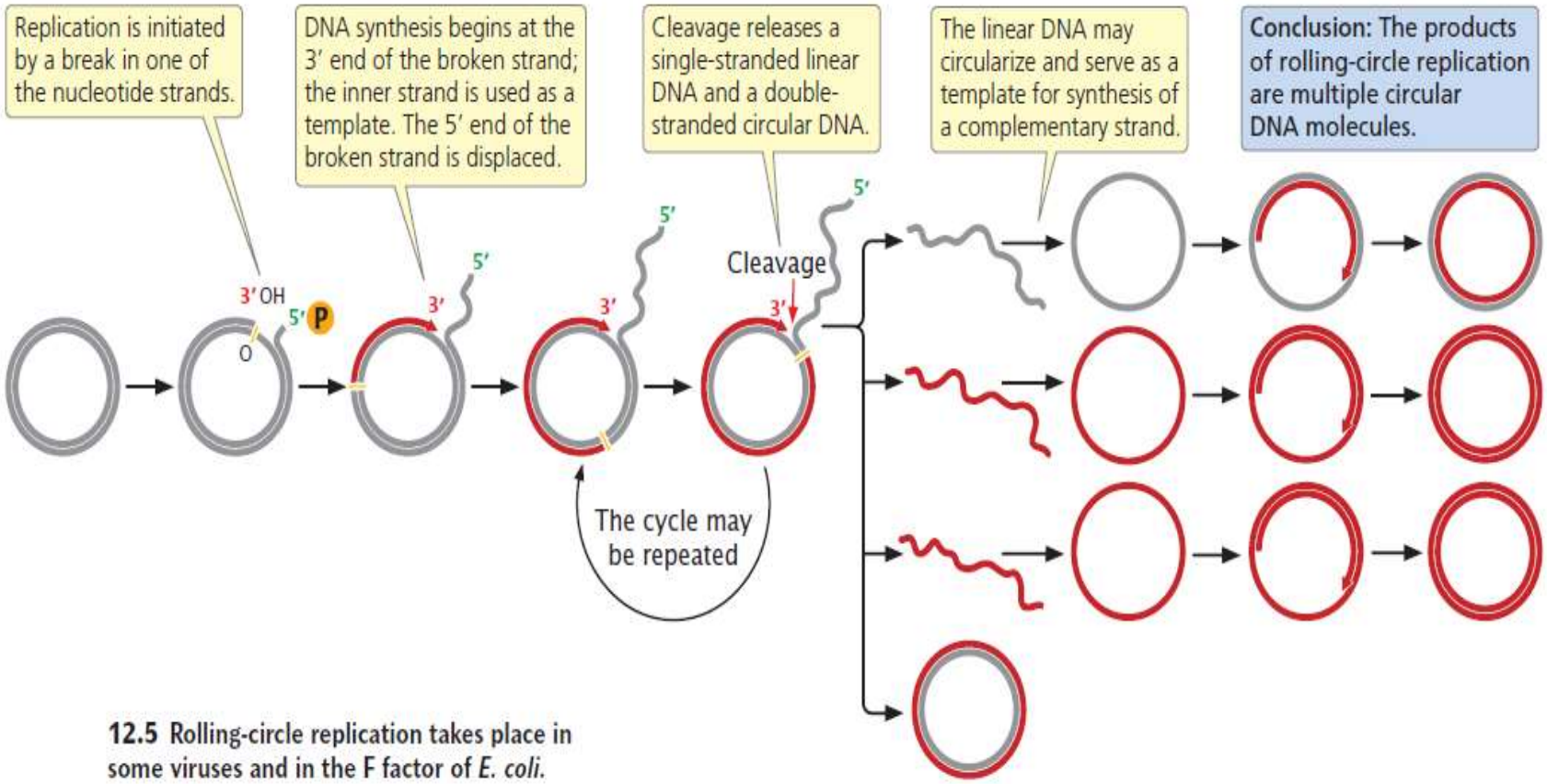
- The point of unwinding, where the two single nucleotide strands separate from the double-stranded DNA helix, is called a **replication fork**

- If there are two replication forks, one at each end of the replication bubble, the forks proceed outward in both directions in a process called **bidirectional replication**

Theta replication is a type of replication common in *E. coli and* other organisms possessing circular DNA.



ROLLING CIRCLE REPLICATION



12.5 Rolling-circle replication takes place in some viruses and in the F factor of *E. coli*.

Linear DNA replication takes place in eukaryotic chromosomes

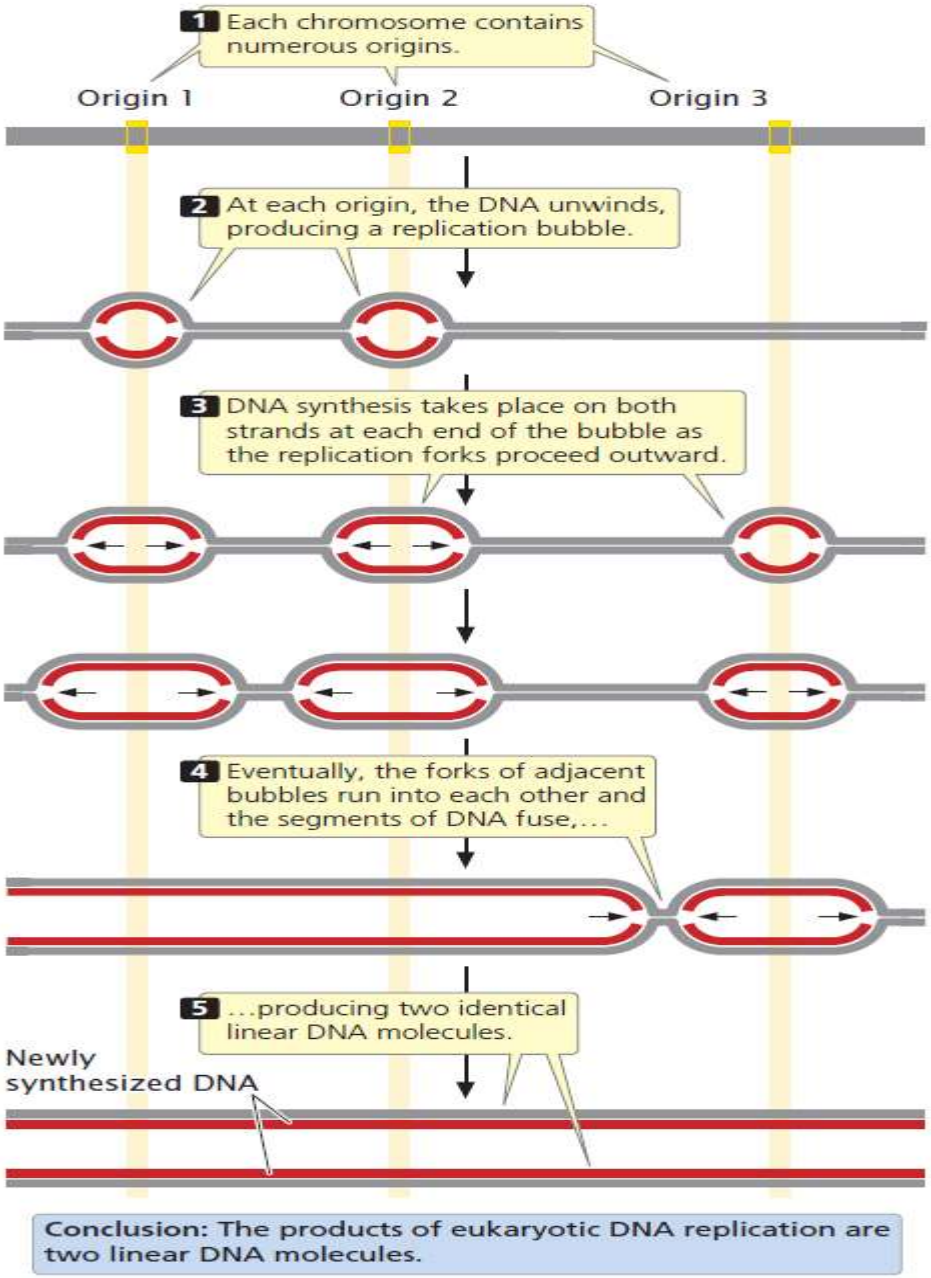


Table 12.2 Characteristics of theta, rolling-circle, and linear eukaryotic replication

Replication Model	DNA Template	Breakage of Nucleotide Strand	Number of Replicons	Unidirectional or Bidirectional	Products
Theta	Circular	No	1	Unidirectional or bidirectional	Two circular molecules
Rolling circle	Circular	Yes	1	Unidirectional	One circular molecule and one linear molecule that may circularize
Linear eukaryotic	Linear	No	Many	Bidirectional	Two linear molecules

Requirements of Replication

- Although the process of replication includes many components, they can be combined into three major groups:
- 1. a template consisting of single-stranded DNA,
- 2. raw materials (substrates) to be assembled into a new nucleotide strand, and
- 3. enzymes and other proteins that “read” the template and assemble the substrates into a DNA molecule.

The chemistry of DNA synthesis

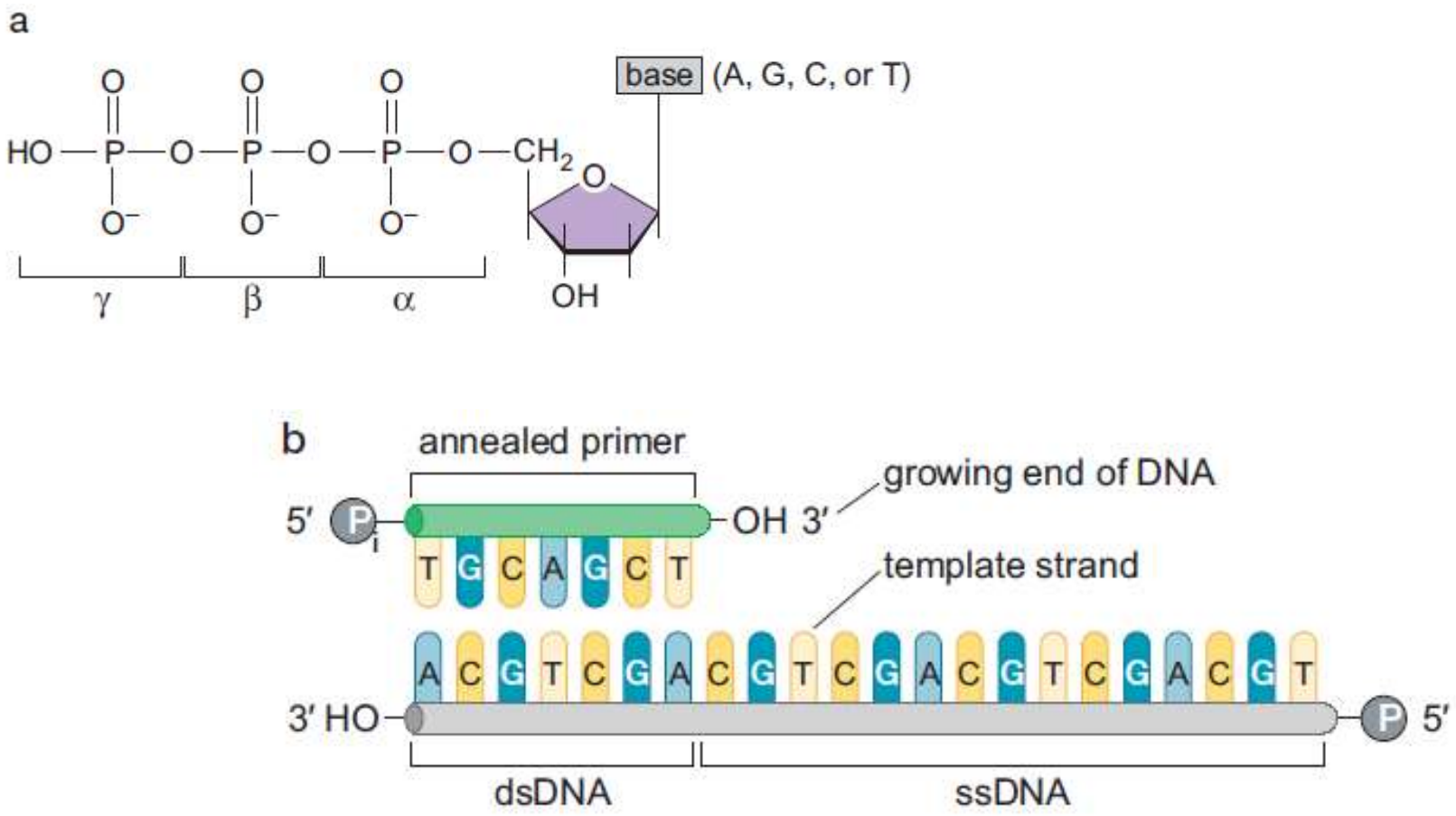
The chemistry of DNA synthesis

- DNA Synthesis Requires Deoxynucleoside Triphosphates and a Primer:Template Junction
- Nucleoside triphosphates have three phosphoryl groups that are attached via the 5’-hydroxyl of the 2’-deoxyribose. The phosphoryl group proximal to the deoxyribose is called the **α-phosphate**, whereas the middle and distal groups are called the **β-phosphate** and the **γ-phosphate**, respectively
- The second essential substrate for DNA synthesis is a particular arrangement of single-stranded DNA (ssDNA) and double-stranded DNA (dsDNA) called a **PRIMER : TEMPLATE JUNCTION**
- **It has two key components:**

  1) The template provides the ssDNA that directs the addition of each complementary deoxynucleotide.

  2)The primer is complementary to, but shorter than, the template. The primer must have an exposed 3’-OH adjacent to the single-strand region of the template which will be extended by nucleotide addition

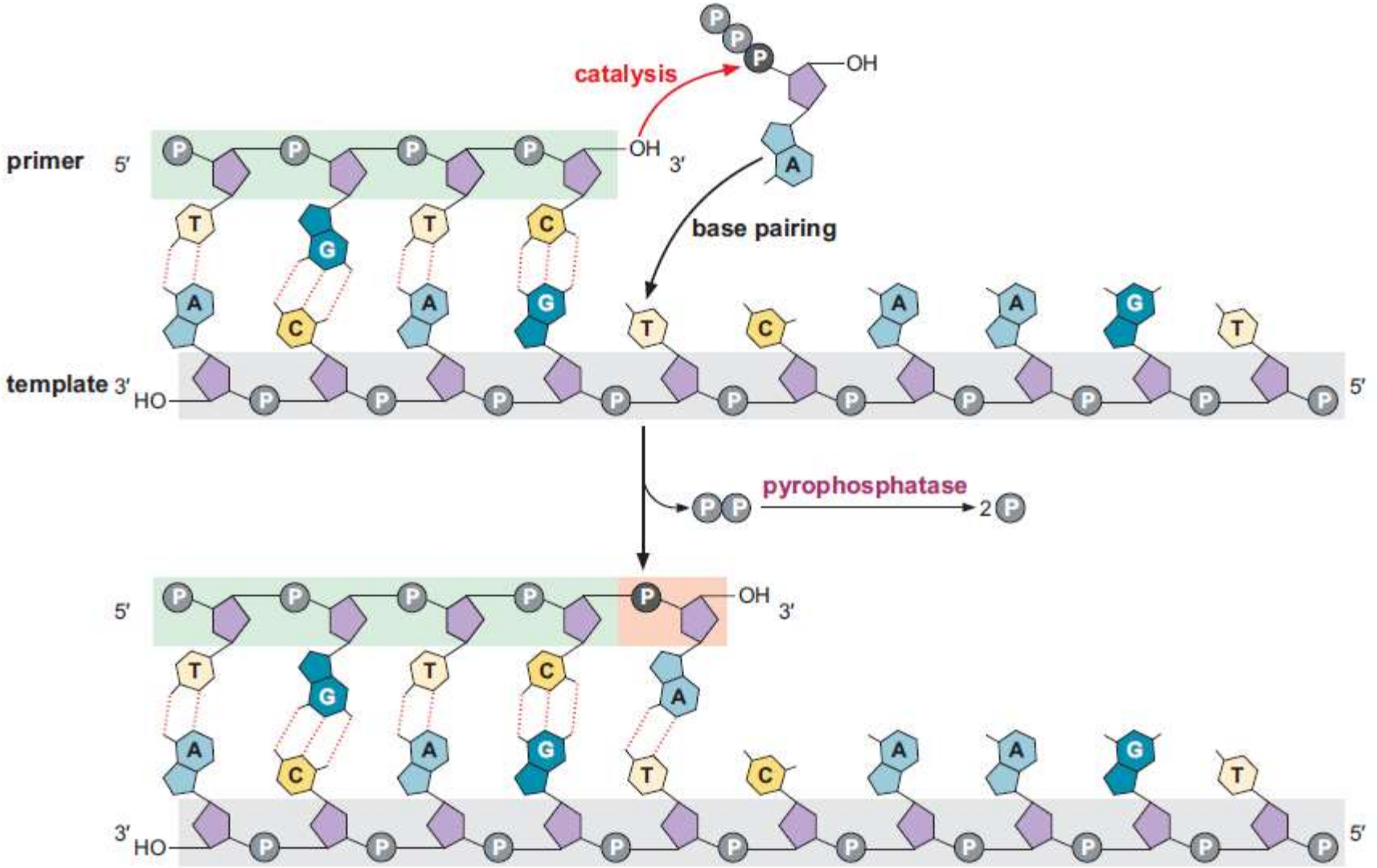
Substrates required for DNA synthesis



DNA Is Synthesized by Extending the 3’ End of the Primer

- The phosphodiester bond is formed in an S_N2 reaction in which the hydroxyl group at the 3’ end of the primer strand attacks the **α -phosphoryl** group of the incoming nucleoside triphosphate.
- The leaving group for the reaction is **pyrophosphate**, which is composed of the **β-phosphate** and **γ-phosphate** of the nucleotide substrate

Diagram of the mechanism of DNA synthesis



Hydrolysis of Pyrophosphate Is the Driving Force for DNA Synthesis

- The addition of a nucleotide to a growing polynucleotide chain of length 'n' is indicated by the following reaction:

$$\text{XTP} + (\text{XMP})_n \rightarrow (\text{XMP})_{n+1} + \text{P} \sim \text{P}.$$

- But the free energy for this reaction is rather small ($\Delta G = -3.5$ kcal/mol). Additional free energy is provided by the rapid hydrolysis of the pyrophosphate into two phosphate groups by an enzyme known as **pyrophosphatase**

$$\text{P} \sim \text{P} \rightarrow 2\text{P}_i.$$

- The net result of nucleotide addition and pyrophosphate hydrolysis is the breaking of two high-energy phosphate bonds. Therefore, DNA synthesis is a coupled process, with an overall reaction of

$$\text{XTP} + (\text{XMP})_n \rightarrow (\text{XMP})_{n+1} + 2\text{P}_i.$$

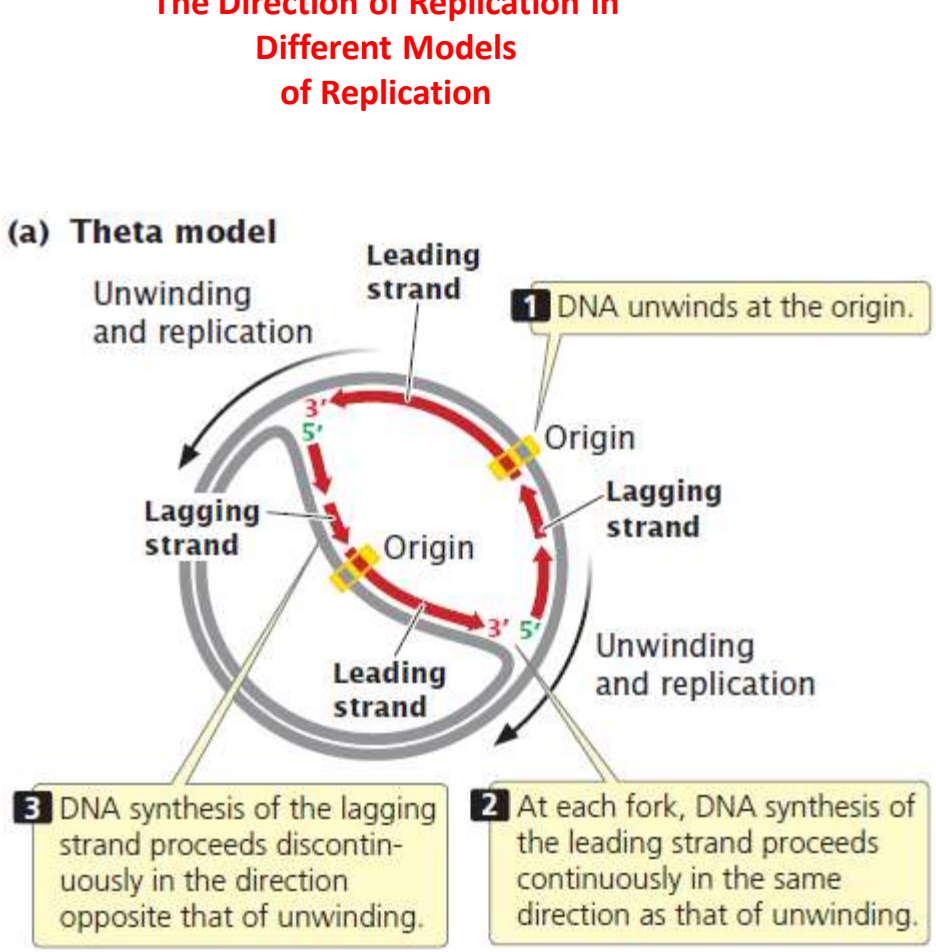
Direction of Replication

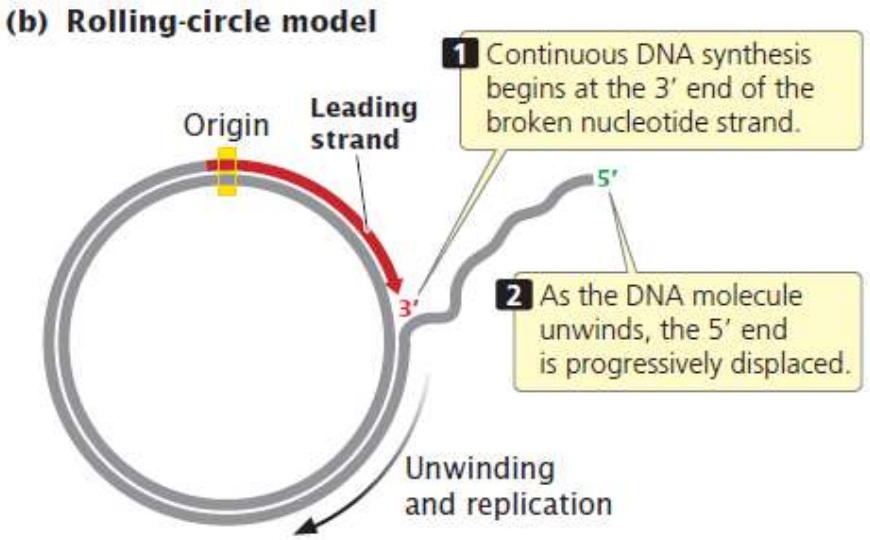
- **Continuous and discontinuous replication**
- This new strand, which undergoes **continuous replication,** is called the **leading strand**
- The newly made strand that undergoes **discontinuous replication** is called the **lagging strand**

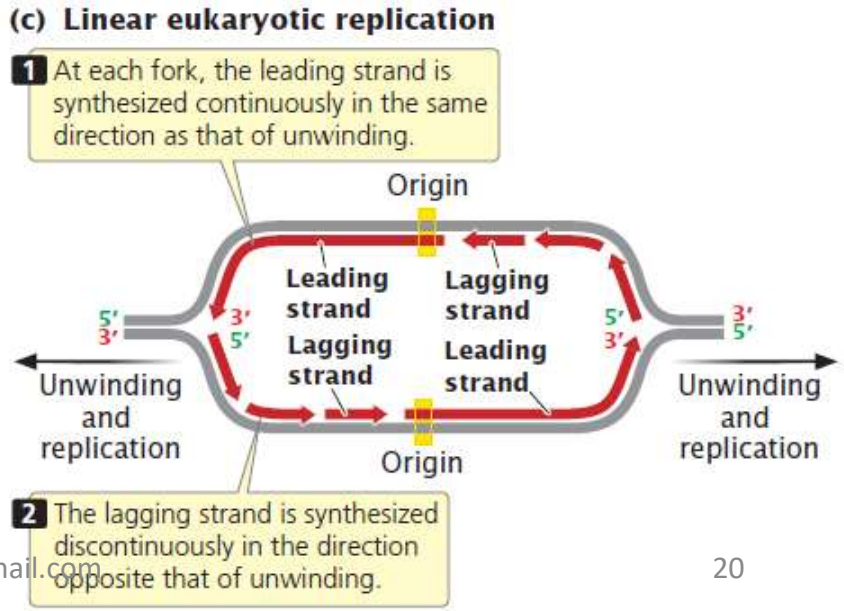
Okazaki fragments

- The short lengths of DNA produced by discontinuous replication of the lagging strand are called **Okazaki fragments,** after **Reiji Okazaki** and **Tuneko Okazaki** who discovered them.
- In bacterial cells, each Okazaki fragment ranges in length from about 1000 to 2000 nucleotides.
- In eukaryotic cells, they are about 100 to 200 nucleotides long.
- Okazaki fragments on the lagging strand are linked together to create a continuous new DNA molecule

The Direction of Replication in Different Models of Replication





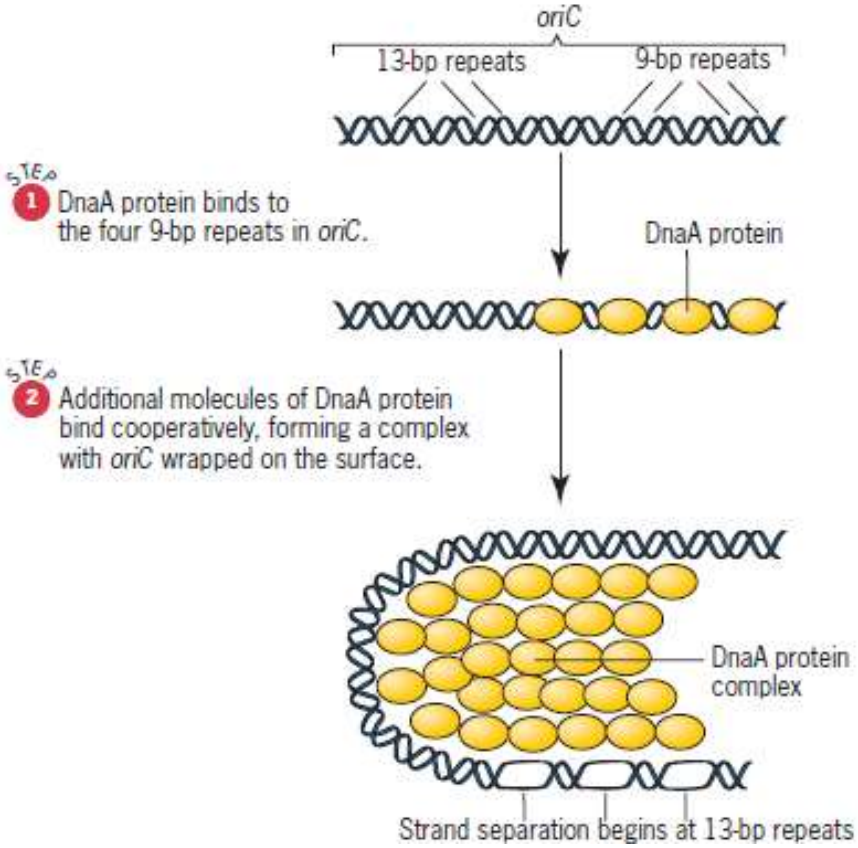


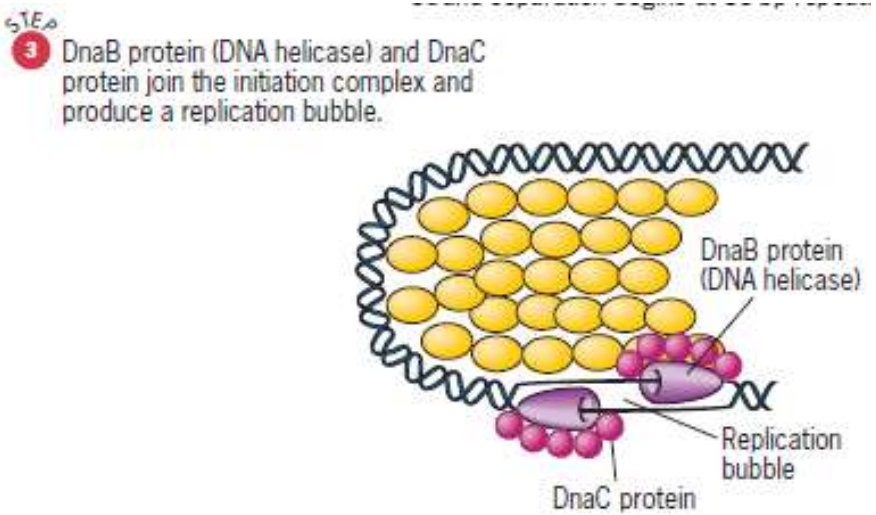
Initiation:

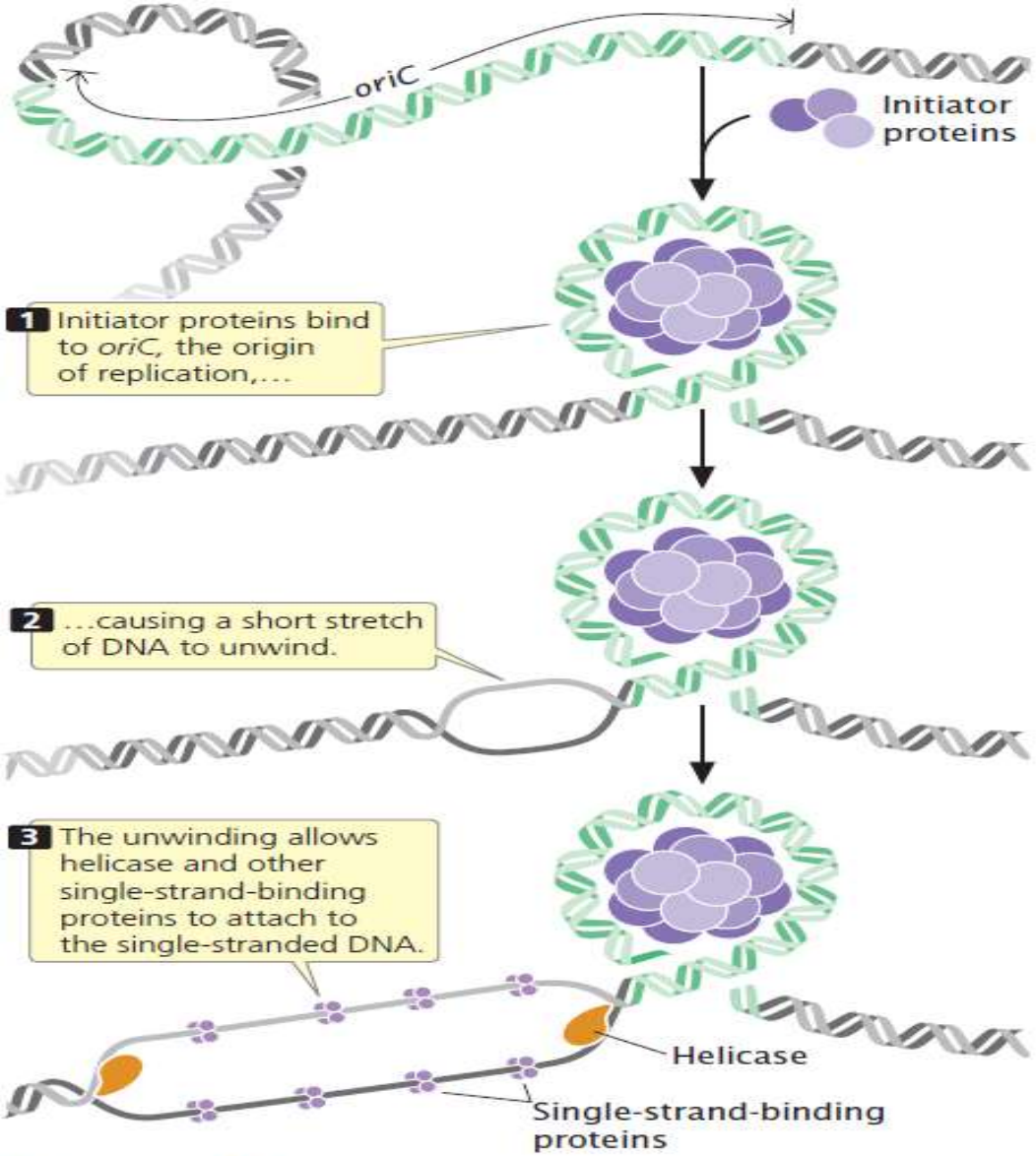
- The replication of the *E. coli* chromosome begins at oriC, the unique sequence (245 bp) at which replication is initiated, with the formation of a localized region of strand separation called the **replication bubble**.
- This replication bubble is formed by the interaction of **prepriming proteins** with oriC .
- The first step in prepriming appears to be the binding of **four molecules of the dnaA gene product—DnaA protein—to the four 9-base-pair (bp) repeats in oriC.**
- Next, DnaA proteins bind cooperatively to form a core of 20 to 40 polypeptides with oriC DNA wound on the surface of the protein complex.
- **Strand separation** begins within the **three tandem 13-bp repeats in oriC** and spreads until the replication bubble is created.

- A complex of **DnaB protein** (the hexameric DNA helicase) and **DnaC** protein (six molecules) joins the initiation complex and contributes to the formation of two bidirectional replication forks.
- The **DnaT** protein also is present in the prepriming protein complex, but its function is unknown. Other proteins associated with the initiation complex at oriC are **DnaJ** protein, **DnaK** protein, **PriA** protein, **PriB** protein, **PriC** protein, DNA-binding protein **HU**, DNA **gyrase**, and single-strand DNA binding (**SSB**) protein.
- In some cases, however, their functional involvement in the prepriming process has not been established; in other cases, they are known to be involved, but their roles are unknown. The DnaA protein appears to be largely responsible for the localized strand separation at oriC during the initiation process.

Prepriming of DNA replication at oriC in the *E. coli* chromosome







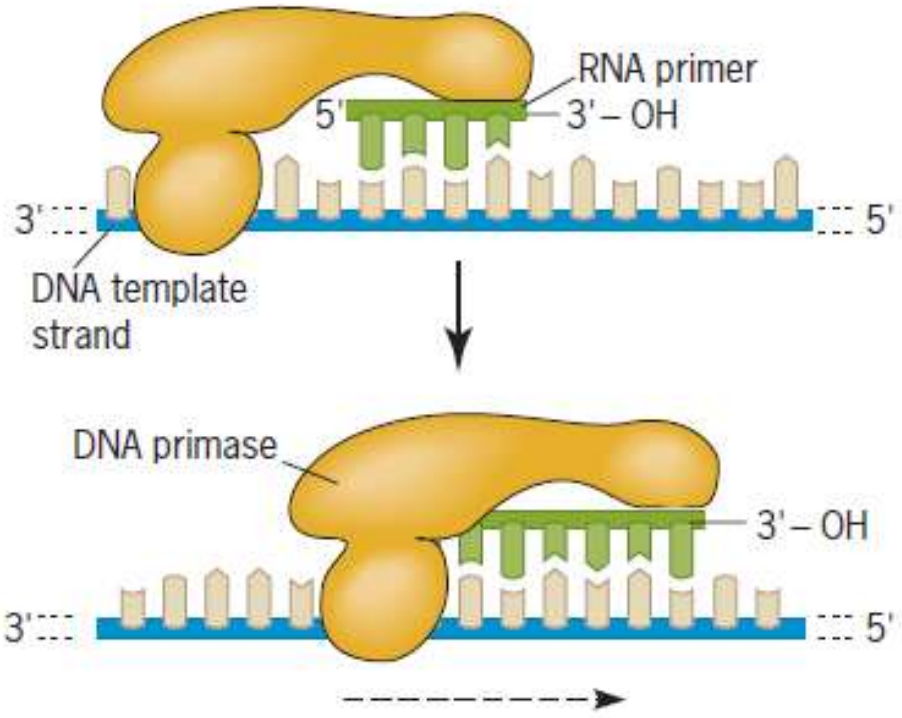
12.11 ***E. coli*** **DNA replication begins when initiator proteins bind to** ***oriC*****, the origin of replication.**

INITIATION OF DNA CHAINS WITH RNA PRIMERS

- DNA chain is initiated by a short **RNA primer synthesized** by **DNA primase** (The E. coli DNA primase is the product of the **dnaG** gene**)**.
- In prokaryotes, these RNA primers are 10 to 60 nucleotides long, whereas in eukaryotes they are shorter, only about 10 nucleotides long, which provides a 3′-OH group to which DNA polymerase can attach DNA nucleotides.
- As because primase is an RNA polymerase, it does not require a 3′-OH group to which nucleotides can be added.
- Primases prefer to initiate RNA synthesis using an ssdna template containing a particular trimer (**GTA** in the case of E.coli primase).
- Analysis of the E. Coli genome sequence shows that the **GTA** target sequence for E. coli primase is **overrepresented in the portions of the genome** that will be the **template for lagging strand** DNA synthesis

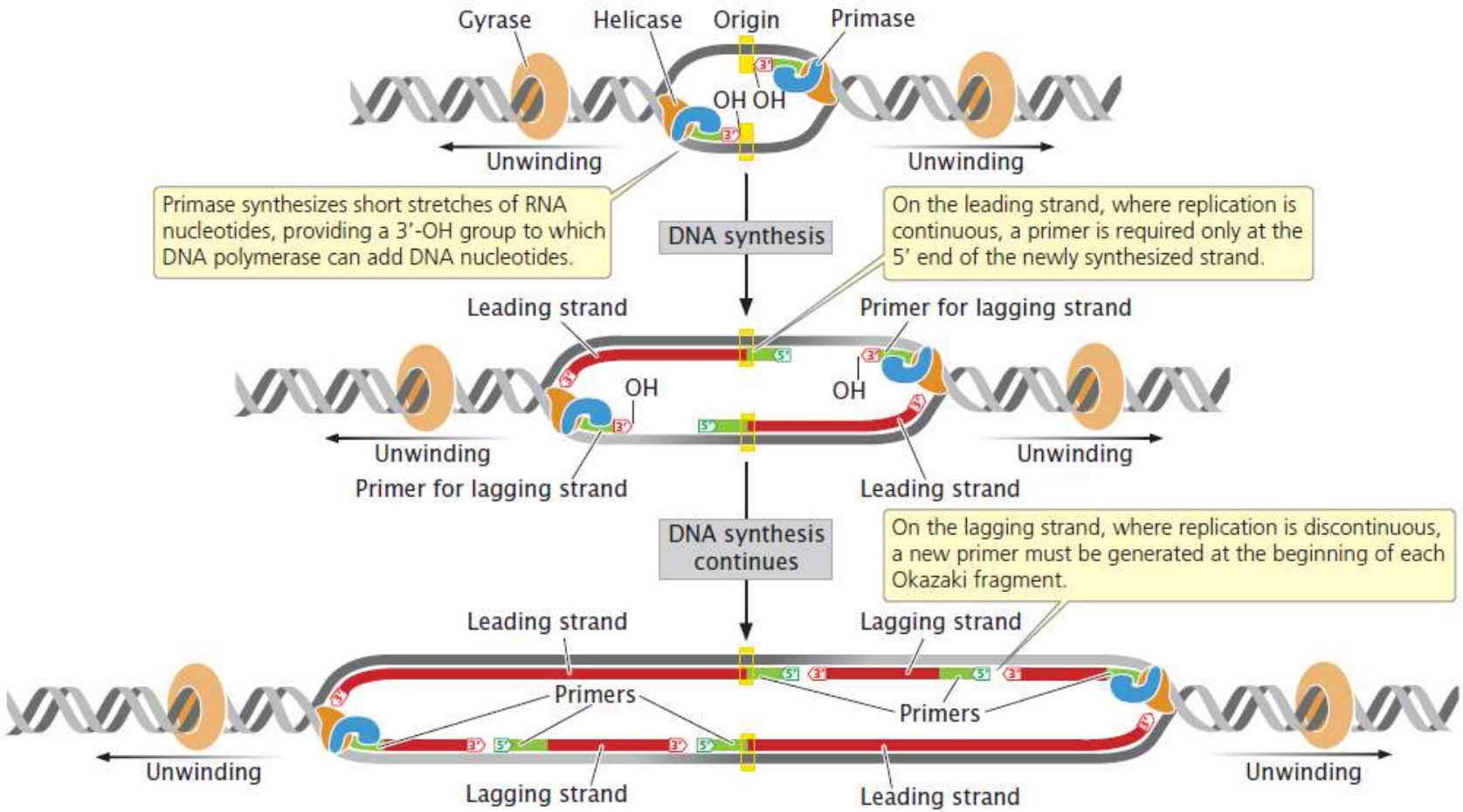
- All DNA molecules initially have short RNA primers embedded within them; these primers are later removed and replaced by DNA nucleotides.

- Primase forms a complex with helicase at the replication fork and moves along the template of the lagging strand. The single primer on the leading strand is probably synthesized by the primase–helicase complex on the template of the lagging strand of the other replication fork, at the opposite end of the replication bubble



The initiation of DNA strands with RNA primers

Primase synthesizes short stretches of RNA nucleotides, providing a 3’-OH group to which DNA polymerase can add DNA nucleotides.



DNA replication requires an unwinding mechanism

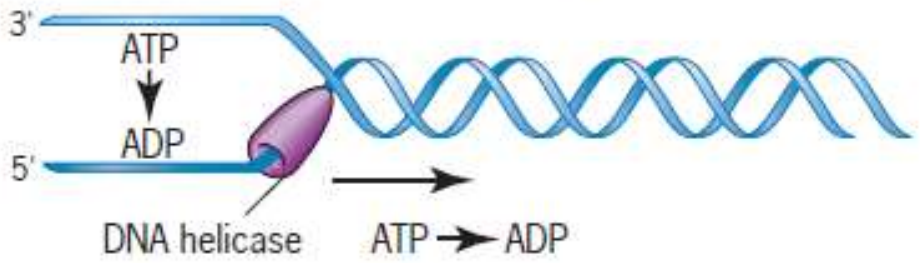
- Each gyre, or turn, is about 10 nucleotide pairs long, so a DNA molecule must be rotated 360^0 once for each 10 replicated base pairs.
- In *E. coli, DNA replicates at* a rate of about 30,000 nucleotides per minute. Thus, a replicating DNA molecule must spin at 3000 revolutions per minute to facilitate the unwinding of the parental DNA strands.
- The unwinding process involves enzymes called **DNA helicases.**

DNA helicase

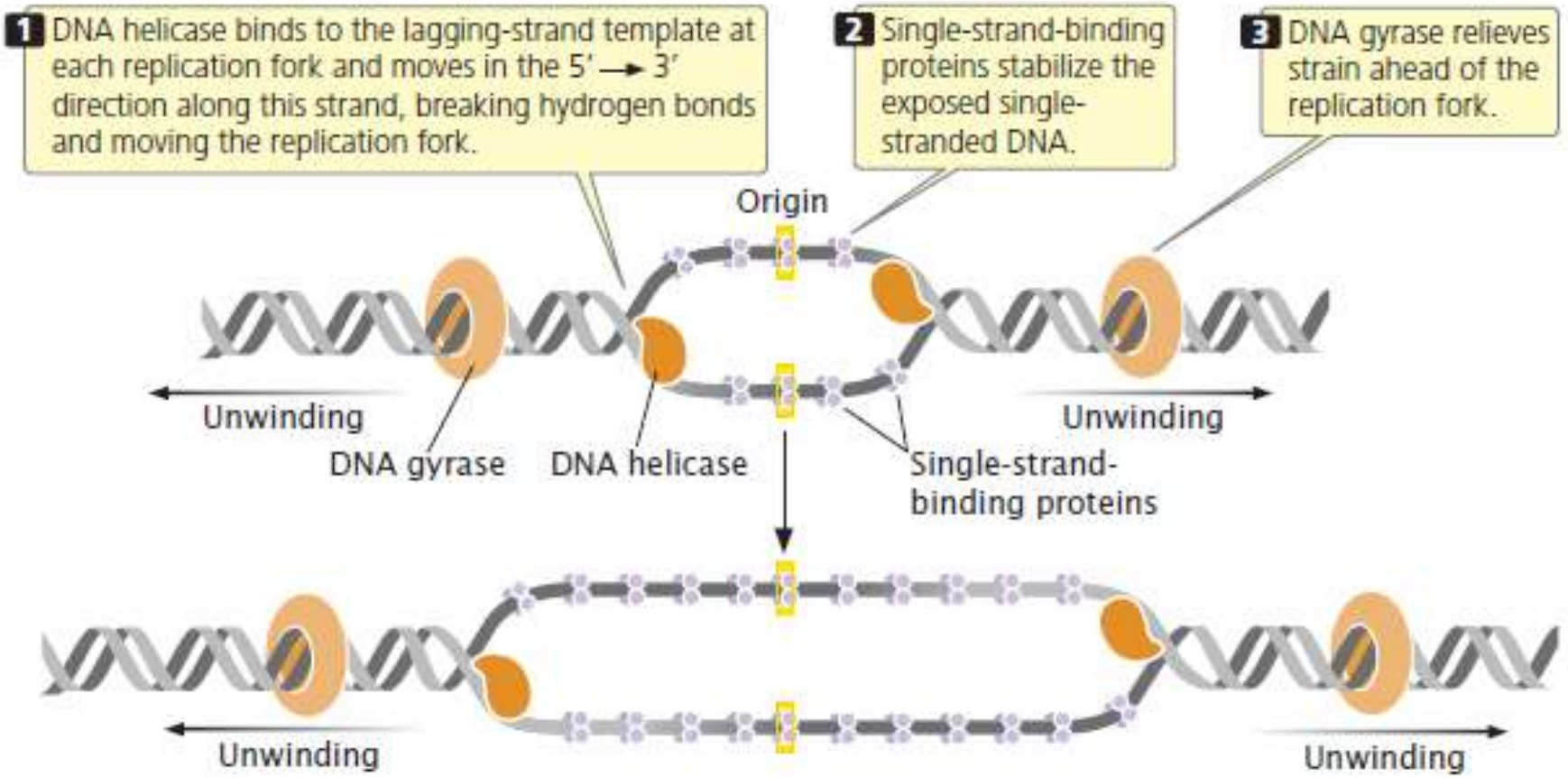
- DNA helicase **breaks** the **hydrogen bonds** that exist between the bases of the two nucleotide strands of a DNA molecule.
- Helicase cannot **initiate the unwinding** of double-stranded DNA; the initiator protein first separates DNA strands at the origin, providing a short stretch of single-stranded DNA to which a helicase binds.
- Helicase binds to the lagging-strand template at each replication fork and moves in the 5′→3′ direction along this strand, thus also moving the replication fork

- DNA helicase in *E. coli* is the product of the *dnaB* gene.
- DNA helicases unwind DNA molecules using energy derived from ATP

DNA helicase catalyzes the unwinding of the parental double helix.



DNA helicase unwinds DNA by binding to the lagging strand template at each replication fork and moving in the 5′→3′ direction

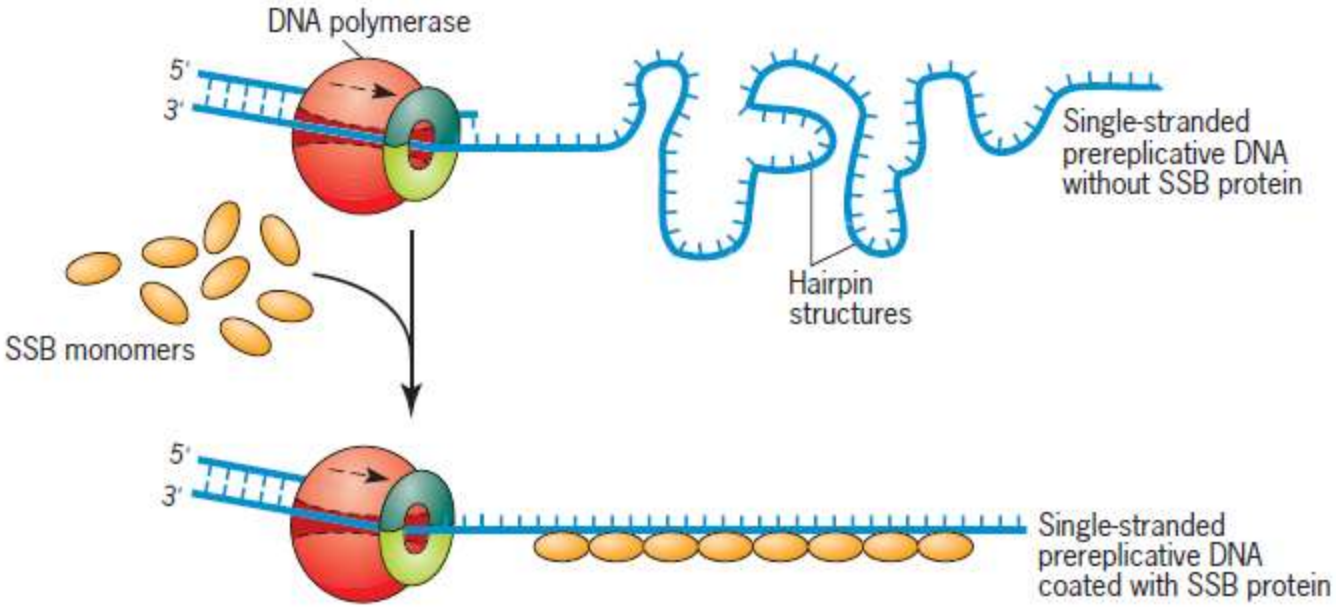


SSBs

- After DNA has been unwound by helicase, single-strand-binding proteins (SSBs) attach tightly to the exposed single-stranded DNA.
- These proteins protect the single-stranded nucleotide chains and prevent the formation of secondary structures such as hairpins that interfere with replication.
- Single-strand-binding proteins form tetramers (groups of four); each tetramer covers from 35 to 65 nucleotides.

- The binding of SSB protein to single-stranded DNA is cooperative; i.e the binding of the first SSB monomer stimulates the binding of additional monomers at contiguous sites on the DNA chain.
- Because of the cooperativity of SSB protein binding, an entire single-stranded region of DNA is rapidly coated with SSB protein.
- Without the SSB protein coating, the complementary strands could renature or form intrastrand hairpin structures by hydrogen bonding between short segments of complementary or partially complementary nucleotide sequences. Such hairpin structures are known to impede the activity of DNA polymerases.
- In *E. coli,* the SSB protein is encoded by the ssb gene.

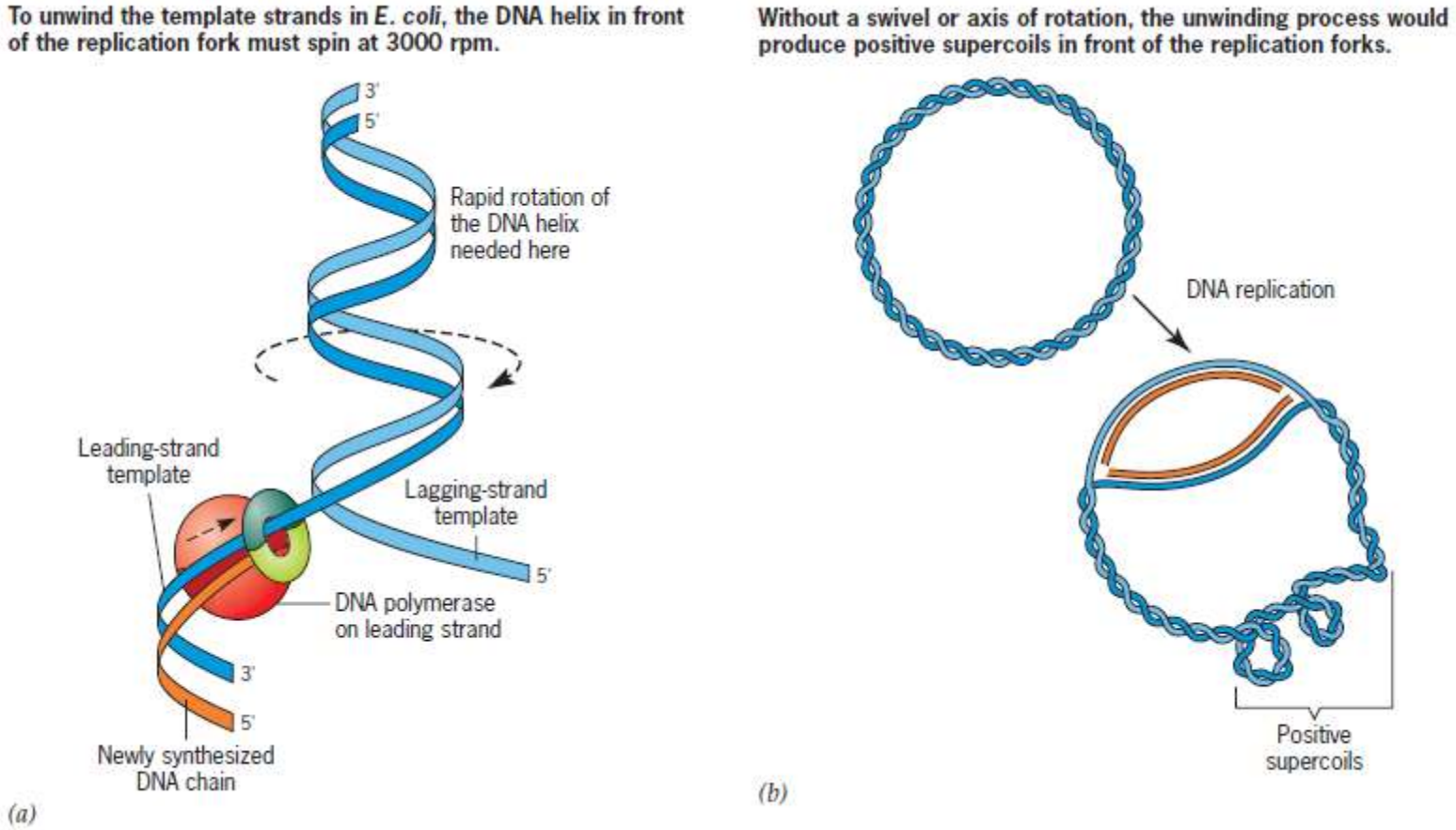
Single-strand DNA-binding (SSB) protein keeps the unwound strands in an extended form for replication.



what provides the **swivel or axis of rotation** that prevents the DNA from becoming tangled (**positively supercoiled**) ahead of the replication fork?

- The circular molecule of *E. coli* DNA spins at 3000 revolutions per minute to allow the unwinding of the parental strands during replication.
- Still **positive supercoil** is not produced ahead of the replication fork

A swivel or axis of rotation is required during the replication of circular molecules of DNA



TOPOISOMERASE

- The required axes of rotation during the replication of circular DNA molecules are provided by enzymes called **DNA topoisomerases.**
- **The** Topoisomerases control the **supercoiling** of DNA by catalyzing transient breaks in DNA molecules but use covalent linkages to themselves to hold on to the cleaved molecules.

Types :

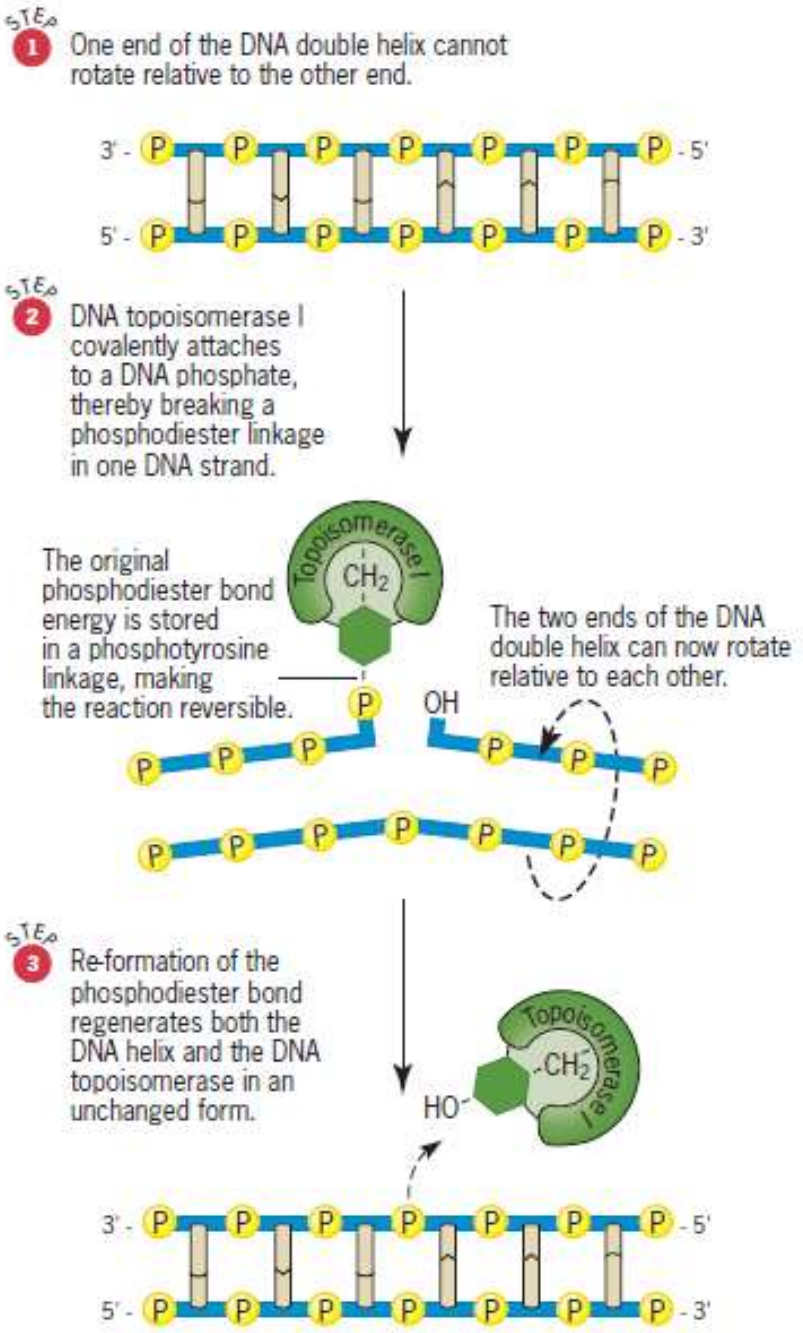
(1) **DNA topoisomerase I** enzymes produce temporary single-strand breaks or nicks in DNA. It **remove supercoils** from DNA one at a time

(1) **DNA topoisomerase II** enzymes produce transient double strand breaks in DNA. It **remove** and **introduce supercoils** two at a time

Topoisomerase I

- The transient single-strand break produced by the this enzyme provides an axis of rotation that allows the segments of DNA on opposite sides of the break to spin independently, with the phosphodiester bond in the intact strand serving as a swivel.
- Topoisomerase I enzymes are energy-efficient. They conserve the energy of the cleaved phosphodiester linkages by storing it in covalent linkages between themselves and the phosphate groups at the cleavage sites; they then reuse this energy to reseal the breaks.

DNA topoisomerase I produces transient single-strand breaks in DNA that act as axes of rotation or swivels during DNA replication



Type II topoisomerase / DNA gyrase in *E. coli*

- DNA topoisomerase II enzymes induce transient double-strand breaks and **add negative supercoils** or **remove positive supercoils** two at a time by an energy (ATP)-requiring mechanism.
- They carry out this process by cutting both strands of DNA, holding on to the ends at the cleavage site via covalent bonds, passing the intact double helix through the cut, and resealing the break.
- In addition to relaxing supercoiled DNA and introducing negative supercoils into DNA, topoisomerase II enzymes can separate interlocking circular molecules of DNA.

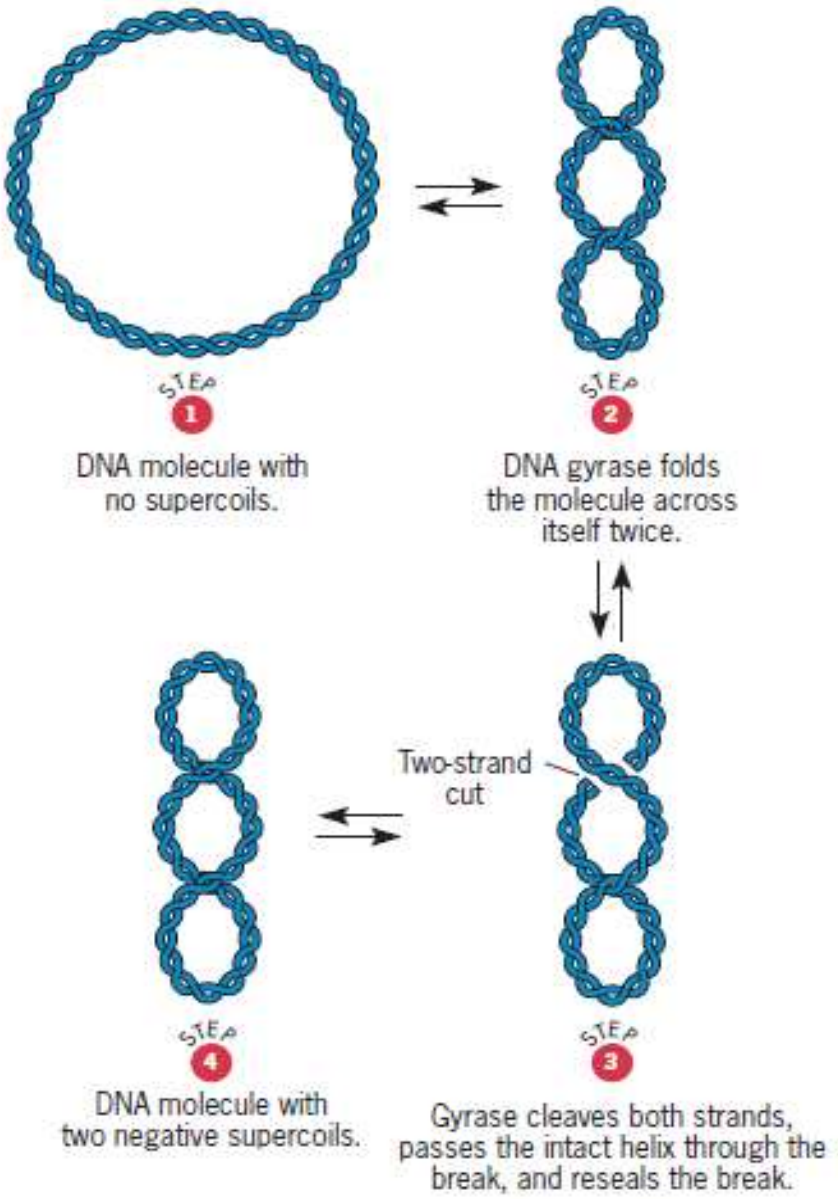
DNA gyrase in *E. coli*

- DNA gyrase is a tetramer with two α subunits encoded by the gyrA gene (originally nalA, for nalidixic acid) and two β subunits specified by the gyrB gene (formerly cou, for coumermycin).
- **Nalidixic acid** and **coumermycin** are antibiotics that block DNA replication in *E. coli* by binding to the α and β subunits respectively, of DNA gyrase there by inhibiting the activity of DNA gyrase.
- Thus, DNA gyrase activity is required for DNA replication to occur in E. coli.

Why DNA gyrase activity is required ?

- Chromosomal DNA is negatively supercoiled in *E. coli.*
- The negative supercoils in bacterial chromosomes are introduced by DNA gyrase, with energy supplied by ATP.
- This activity of DNA gyrase provides another solution to the unwinding problem. Instead of creating positive supercoils ahead of the replication fork by unwinding the complementary strands of relaxed DNA, replication may produce relaxed DNA ahead of the fork by unwinding negatively supercoiled DNA.
- Because superhelical tension is reduced during unwinding—that is, strand separation is energetically favored—the negative supercoiling behind the fork may drive the unwinding process. If so, this mechanism nicely explains why DNA gyrase activity is required for DNA replication in bacteria. Alternatively, gyrase may simply remove positive supercoils that form ahead of the replication fork.

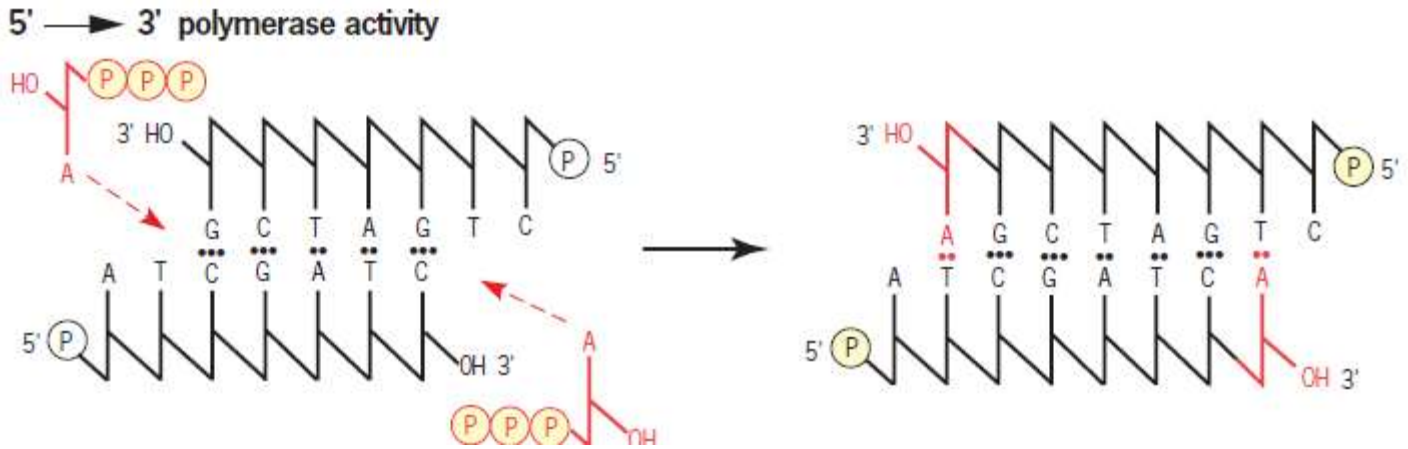
Mechanism of action of DNA gyrase, an *E. coli* DNA topoisomerase II required for DNA replication.

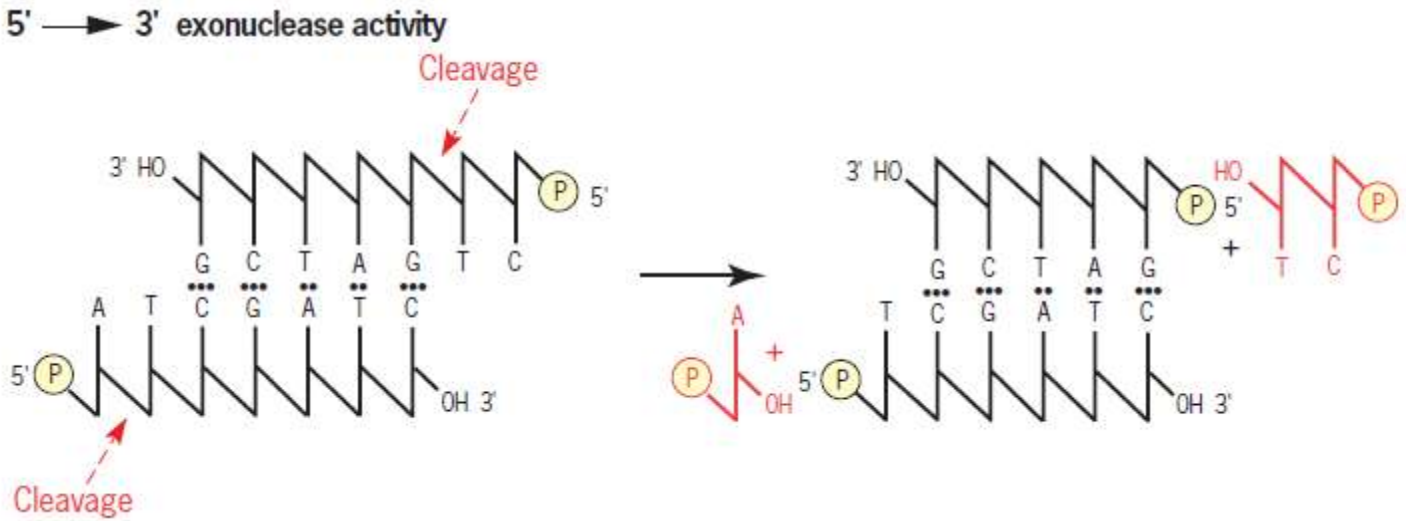


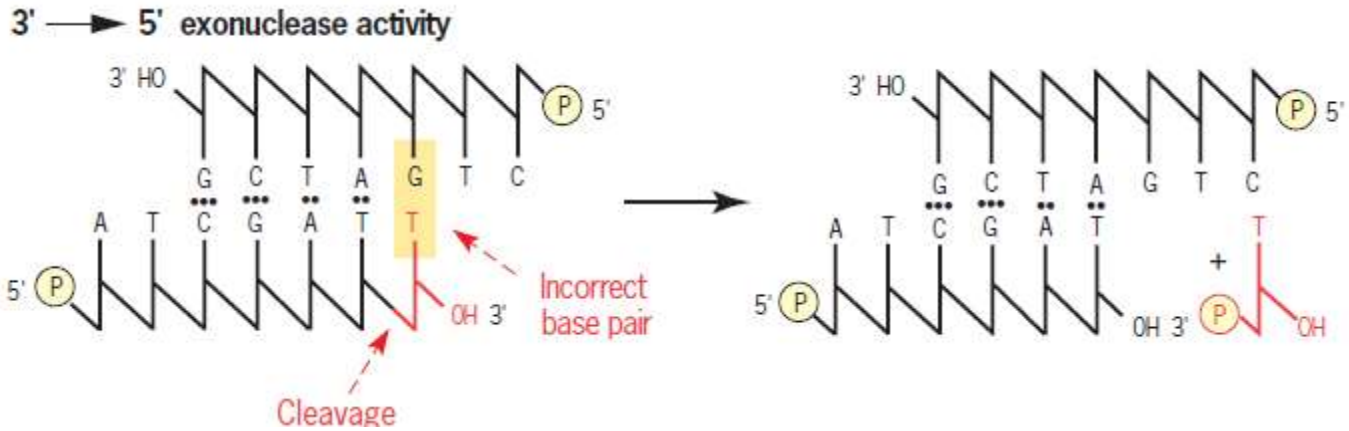
DNA synthesis by DNA polymerases

- **DNA polymerase III -**This enzyme has two enzymatic activities (see Table 12.3). Its 5′→3′ polymerase activity allows it to add new nucleotides in the 5′→3′ direction.
- Its 3′→5′ exonuclease activity allows it to remove nucleotides in the 3′→5′ direction, enabling it to correct errors.

- DNA polymerase III has high processivity, which means that it is capable of adding many nucleotides to the growing DNA strand without releasing the template: it normally holds on to the template and continues synthesizing DNA until the template has been completely replicated.
- The high processivity of DNA polymerase III is ensured by one of the polypeptides that constitutes the enzyme. This polypeptide, termed the β subunit, serves as a clamp for the polymerase enzyme: it encircles the DNA and keeps the DNA polymerase attached to the template strand during replication. DNA polymerase III adds DNA nucleotides to the primer, synthesizing the DNA of both the leading and the lagging strands.

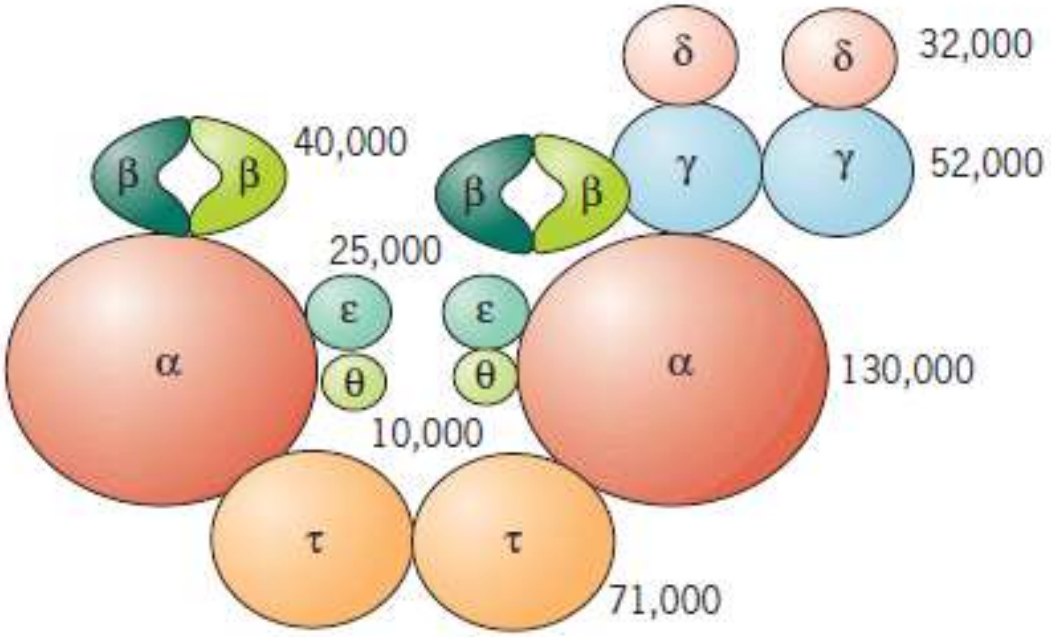

5' → 3' polymerase activity
HO
P P P
A
3' HO
P 5'
G C T A G T C
A T C G A T C
5' P
OH 3'
A
P P P
OH
3' HO
P 5'
A G C T A G T C
A T C G A T C A
5' P
OH 3'


5' → 3' exonuclease activity
Cleavage
3' HO
P
5'
G C T A G T C
A T C G A T C
5'
P
OH 3'
Cleavage
A
+
P
OH
3' HO
P
5'
G C T A G
T C G A T C
5'
P
OH 3'
HO
5'
+
T C
P


3' → 5' exonuclease activity
3' HO
P
5'
G C T A G T C
A T C G A T T
5'
P
OH 3'
Incorrect base pair
Cleavage
3' HO
P
5'
G C T A G T C
A T C G A T
5'
P
OH 3'
+
T
P
OH

DNA polymerase III, the "replicase" in *E. coli*

- a multimeric enzyme with a molecular mass of about 900,000 daltons in its complete or **holoenzyme** form**.**
- The minimal core that has catalytic activity *in vitro* contains three subunits: α (the dnaE gene product), ε (the dnaQ product), and θ (the holE product). Addition of the τ subunit (the dnaX product) results in dimerization of the catalytic core and increased activity.
- The catalytic core synthesizes short DNA strands because of its tendency to fall off the DNA template.
- The β subunit (the dnaN gene product) of DNA polymerase III forms a dimeric clamp that keeps the polymerase from falling off the template DNA in order to synthesize the long DNA molecules
- Proofreading function of DNA polymerase III of E. Coli, is carried out by the ε subunit



Structure of the *E. coli DNA* polymerase III holoenzyme

DNA POL I

- The first *E. coli polymerase to be discovered,* ***DNA polymerase*** **I,** also has **5′→3′ polymerase** and **3′→5′ exonuclease** activities (see Table 12.3), permitting the enzyme to synthesize DNA and to correct errors.
- Unlike DNA polymerase III, however, DNA polymerase I also possesses 5′→3′ exonuclease activity, which is used to remove the primers laid down by primase and to replace them with DNA nucleotides by synthesizing in a 5′→3′ direction.
- DNA polymerase I has lower processivity than DNA polymerase III. The removal and replacement of primers appear to constitute the main function of DNA polymerase I.
- After DNA polymerase III has initiated synthesis at the primer and moved downstream, DNA polymerase I removes the RNA nucleotides of the primer, replacing them with DNA nucleotides. DNA polymerases II, IV, and V function in DNA repair.

The best-studied polymerases are those of *E. coli, which has at least five different DNA polymerases*

Table 12.3 Characteristics of DNA Polymerases in *E. coli*

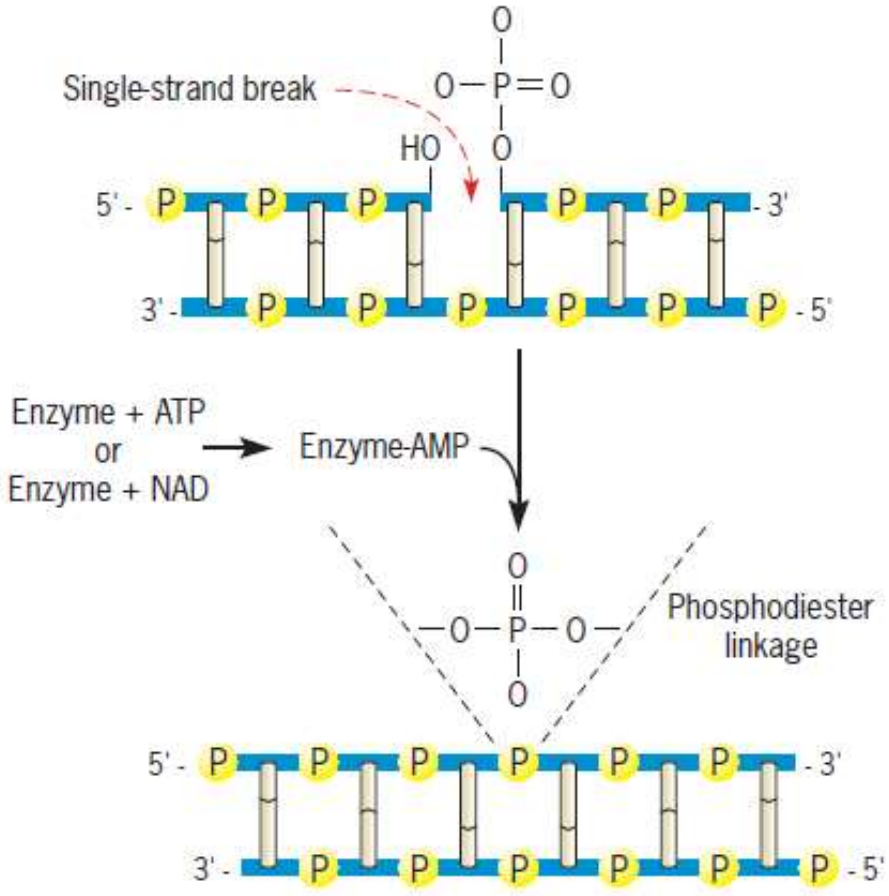
DNA Polymerase	5′⟶3′ Polymerization	3′⟶5′ Exonuclease	5′⟶3′ Exonuclease	Function
I	Yes	Yes	Yes	Removes and replaces primers
II	Yes	Yes	No	DNA repair; restarts replication after damaged DNA halts synthesis
III	Yes	Yes	No	Elongates DNA
IV	Yes	No	No	DNA repair
V	Yes	No	No	DNA repair; translation DNA synthesis

Despite their differences, all of *E. coli's* DNA polymerases do

1. **synthesize any sequence specified by the template strand**
2. **synthesize in the 5′→3′ direction** by adding nucleotides to a 3′-OH group;
3. **use dNTPs to synthesize new DNA**
4. **require a primer to initiate synthesis**
5. **catalyze the formation of a phosphodiester bond** by joining the 5′-phosphate group of the incoming nucleotide to the 3′-OH group of the preceding nucleotide on the growing strand, cleaving off two phosphates in the process
6. **produce newly synthesized strands** that are complementary and antiparallel to the template strand
7. **are associated with a number of other proteins**

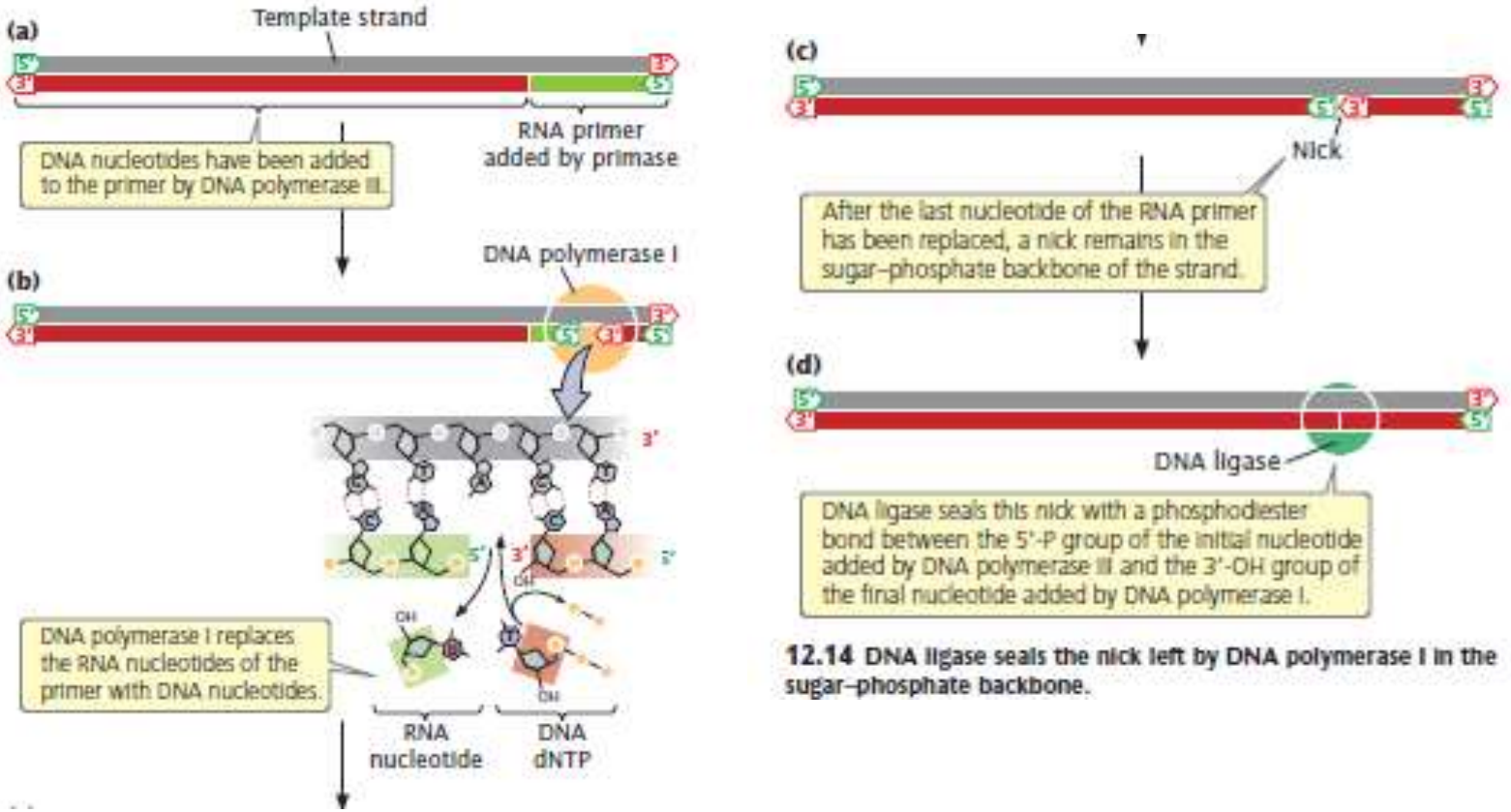
DNA ligase

- DNA ligase catalyzes the covalent closure of nicks (missing phosphodiester linkages; no missing bases) in DNA molecules by using energy from nicotinamide adenine dinucleotide (NAD) or adenosine triphosphate (ATP).
- The *E. coli* DNA ligase uses NAD as a cofactor, but some DNA ligases use ATP.
- AMP of the ligase-AMP intermediate forms a phosphoester linkage with the 5’ -phosphate at the nick, and then a nucleophilic attack by the 3’-OH at the nick on the DNA-proximal phosphorus atom produces a phosphodiester linkage between the adjacent nucleotides at the site of the nick.



DNA ligase catalyzes the covalent closure of nicks in DNA

DNA ligase



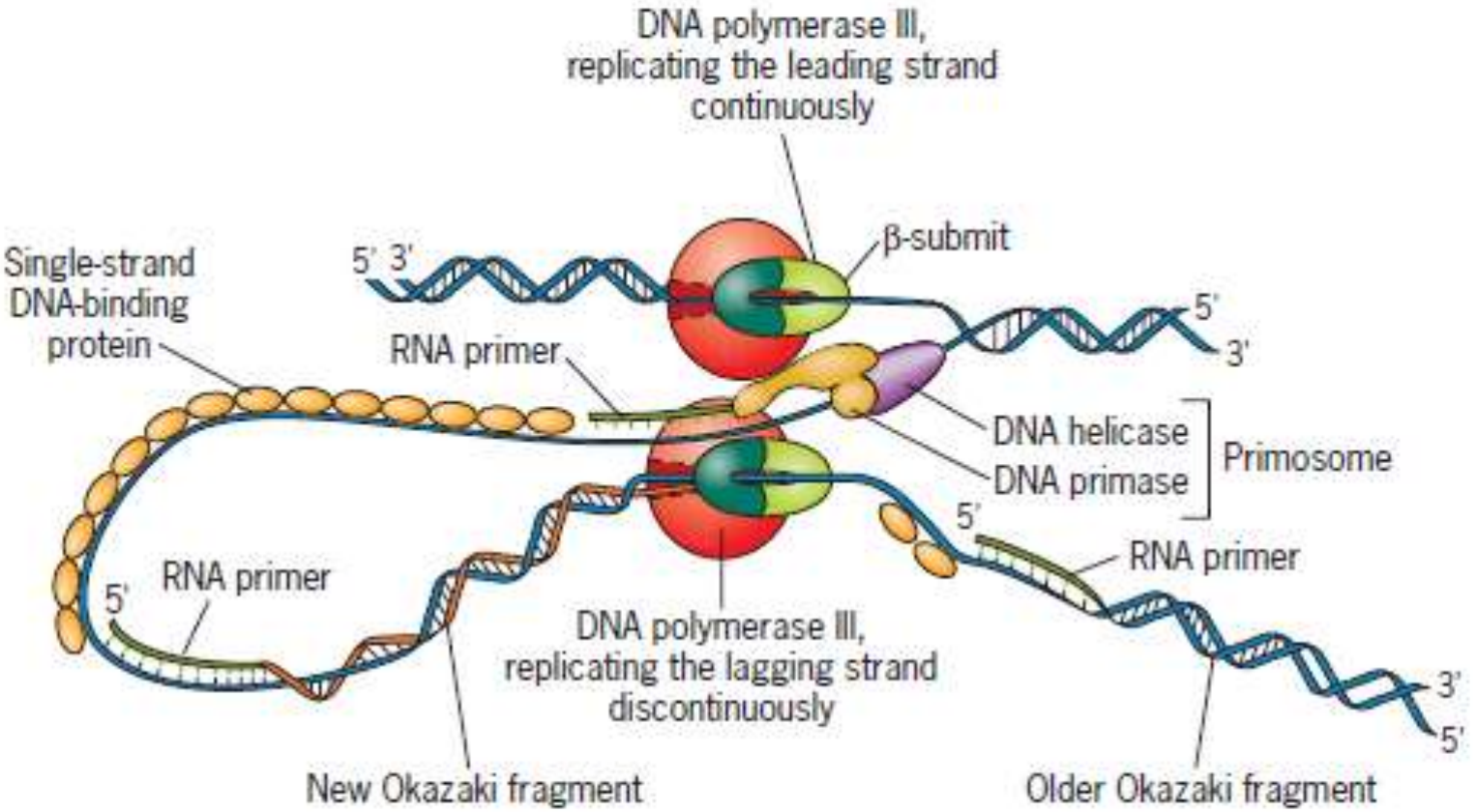
12.14 DNA ligase seals the nick left by DNA polymerase I in the sugar–phosphate backbone.

THE PRIMOSOME

- The initiation of Okazaki fragments on the lagging strand is carried out by the **primosome,** a protein complex containing DNA primase and DNA helicase. The primosome moves along a DNA molecule, powered by the energy of ATP.
- As it proceeds, DNA helicase unwinds the parental double helix, and DNA primase synthesizes the RNA primers needed to initiate successive Okazaki fragments.
- The RNA primers are covalently extended with the addition of deoxyribonucleotides by DNA polymerase III.
- DNA topoisomerases provide transient breaks in the DNA that serve as swivels for DNA unwinding and keep the DNA untangled. Single-strand DNA binding protein coats the unwound prereplicative DNA and keeps it in an extended state for DNA polymerase III.
- The RNA primers are replaced with DNA by DNA polymerase I, and the single-strand nicks left by polymerase I are sealed by DNA ligase.

THE REPLISOME

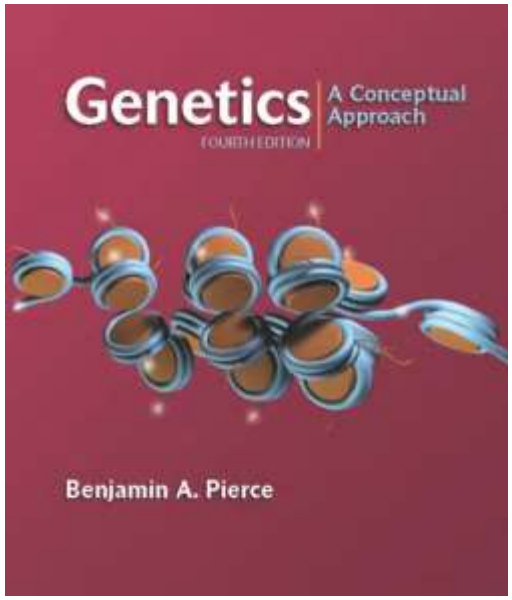
- The replication fork moves along a parental double helix, two DNA strands (the leading strand and the lagging strand) are replicated in the highly coordinated series of reactions.
- The complete replication apparatus moving along the DNA molecule at a replication fork is called the **replisome.**
- The replisome contains the DNA polymerase III holoenzyme; one catalytic core replicates the leading strand, the second catalytic core replicates the lagging strand, and the primosome unwinds the parental DNA molecule and synthesizes the RNA primers needed for the discontinuous synthesis of the lagging strand.
- In order for the two catalytic cores of the polymerase III holoenzyme to synthesize both the nascent leading and lagging strands, the lagging strand is thought to form a loop from the primosome to the second catalytic core of DNA polymerase III


DNA polymerase III,
replicating the leading strand
continuously
β-submit
5' 3'
Single-strand
DNA-binding
protein
RNA primer
5'
3'
DNA helicase
DNA primase
Primosome
5'
RNA primer
5'
RNA primer
DNA polymerase III,
replicating the lagging strand
discontinuously
3'
5'
New Okazaki fragment
Older Okazaki fragment

Termination

- In *E. coli*, the termination of replication occurs at variable sites within regions called terA and terB, which block the movement of replication forks advancing in the counterclockwise and clockwise directions, respectively.
- DNA topoisomerases or special recombination enzymes then facilitate the separation of the nascent DNA molecules.
- The DNA is condensed into the nucleoid, or folded genome, of E. *coli*, in part through the negative supercoiling introduced by DNA gyrase.

REFERENCE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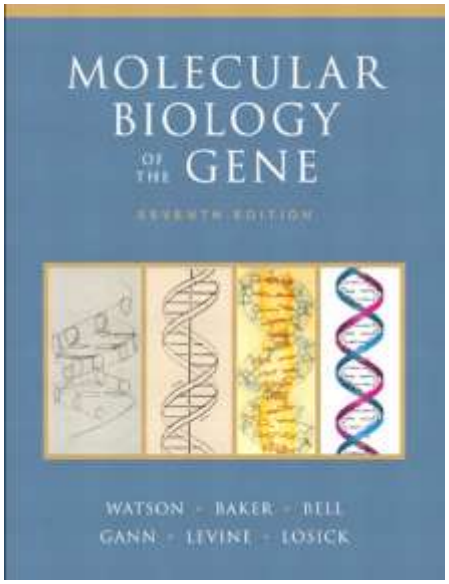
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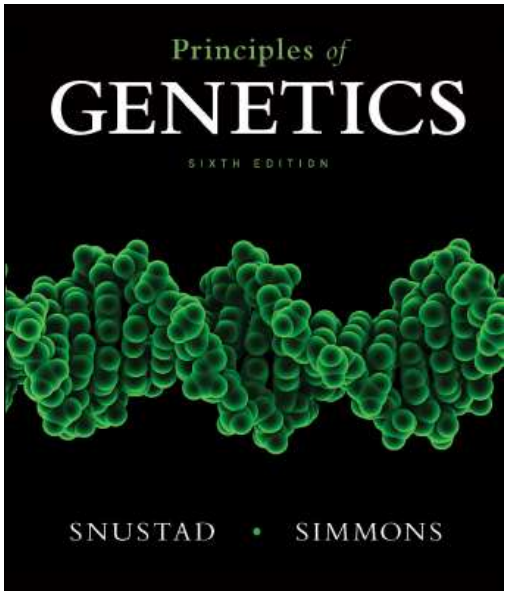
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