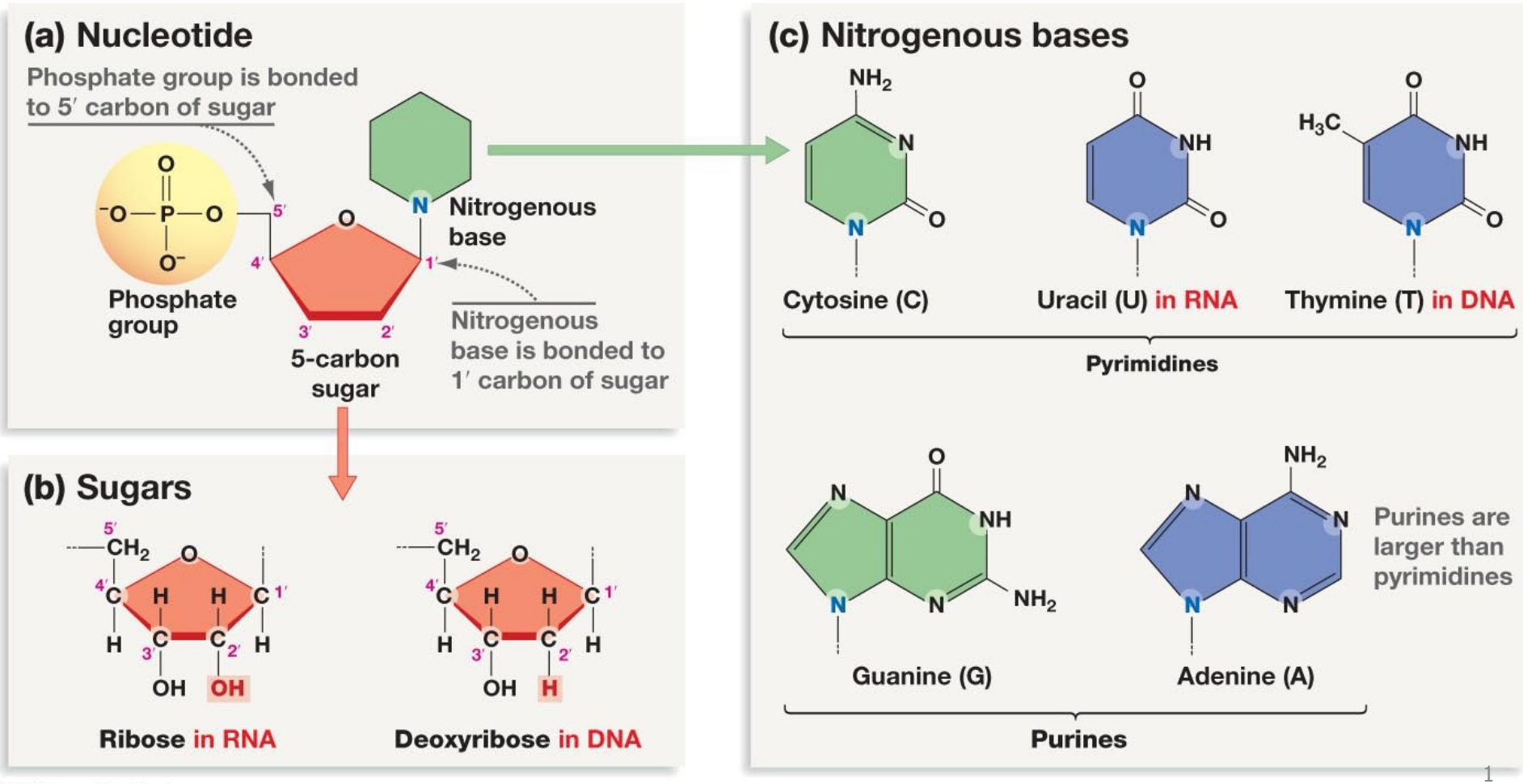
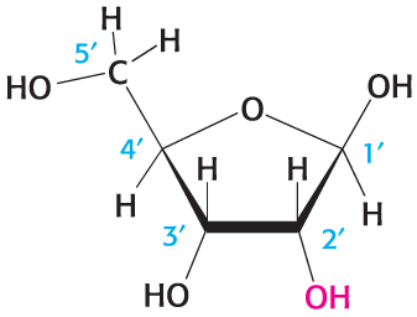
Nucleotides And Nucleosides

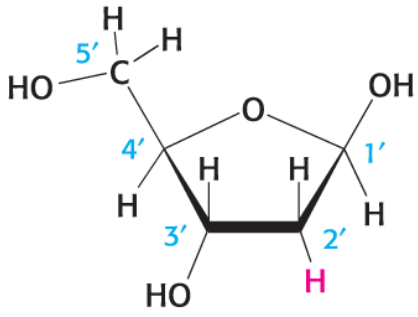


© 2011 Pearson Education, Inc.

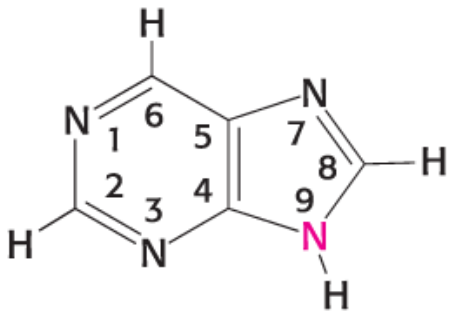
Nucleotides And Nucleosides



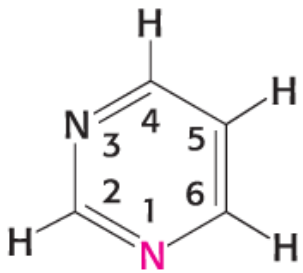
Ribose



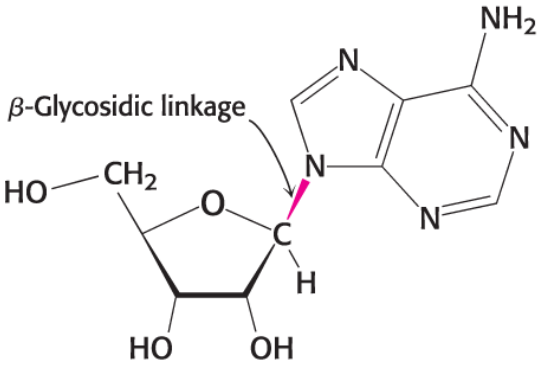
Deoxyribose



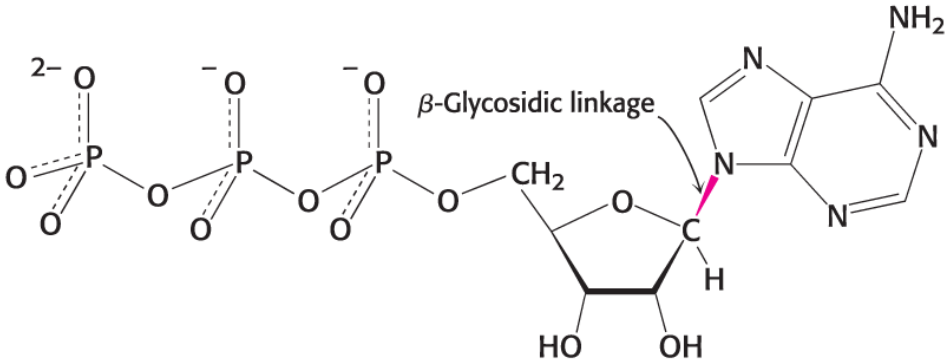
Purine



Pyrimidine

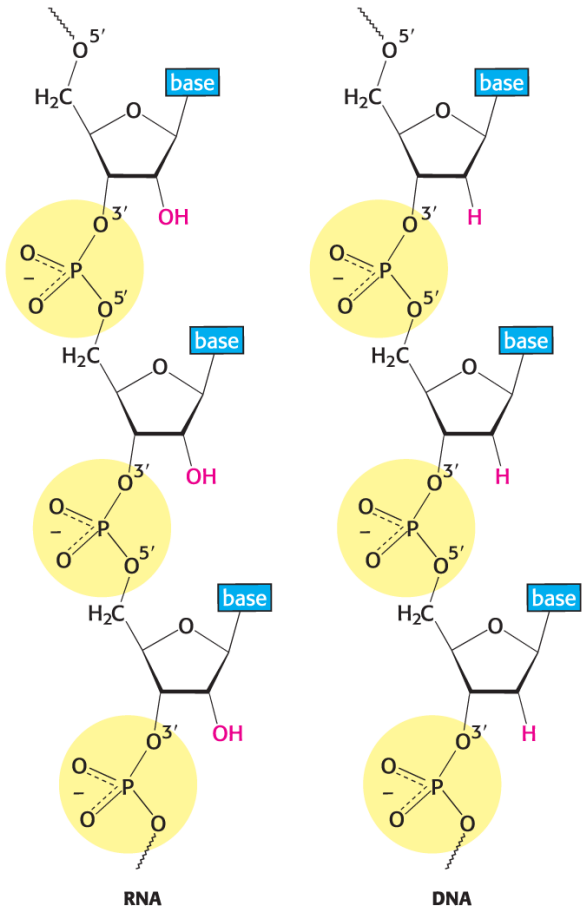


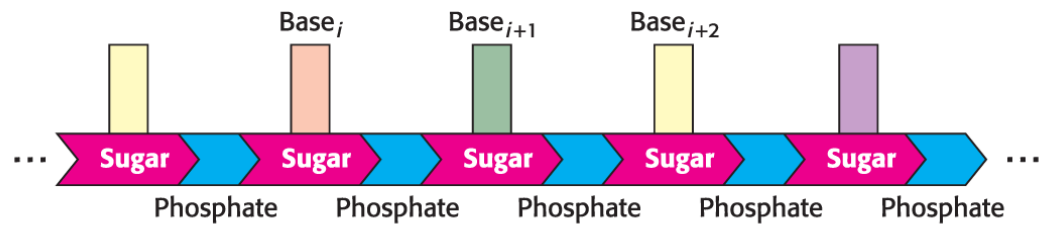
A nucleoside
(Adenosine)

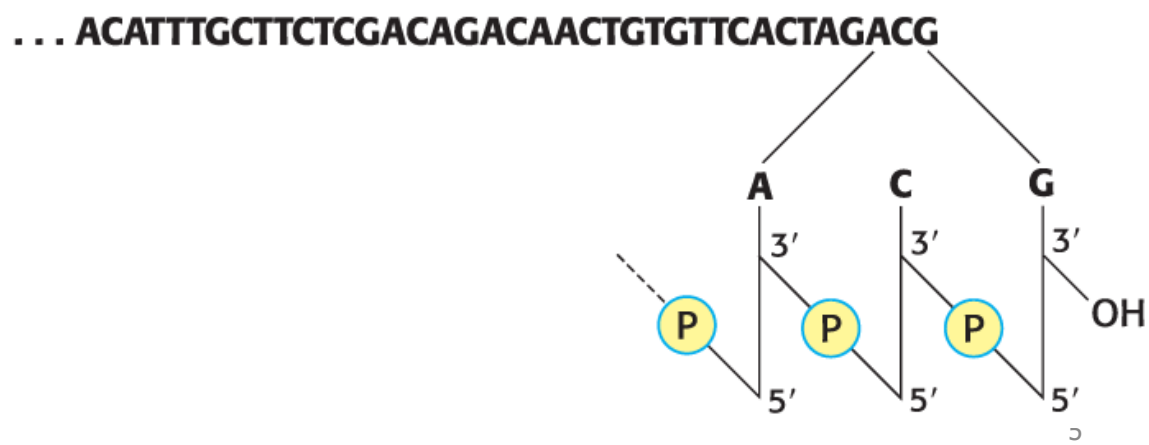


A nucleotide
(Adenosine 5′-triphosphate [5′-ATP])

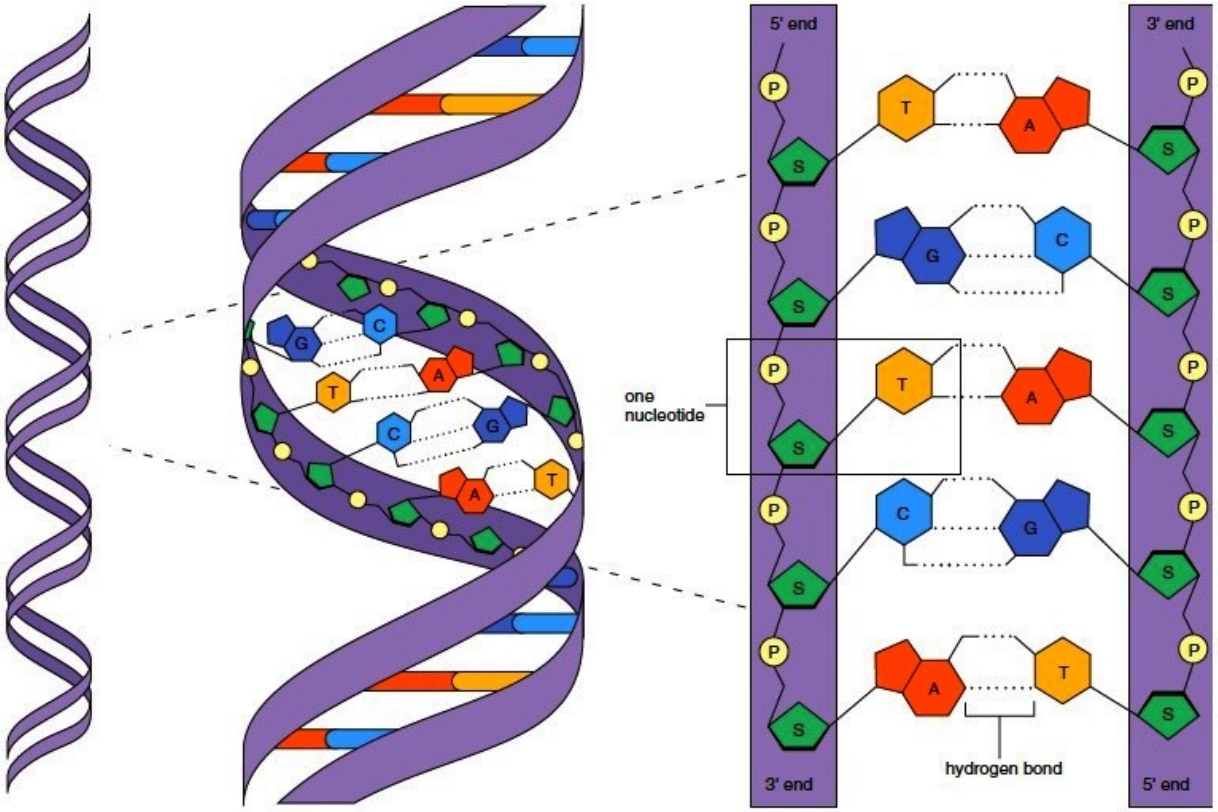
Nucleic Acid Structure:
bases linked to a sugar-phosphate backbone







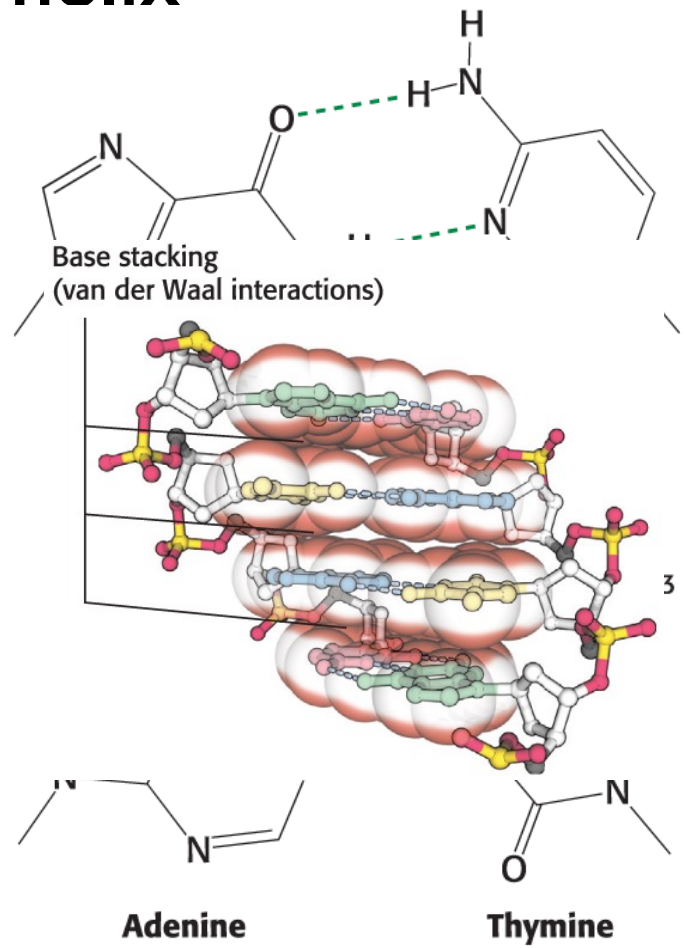
DNA forms a double helix



a. DNA double helix

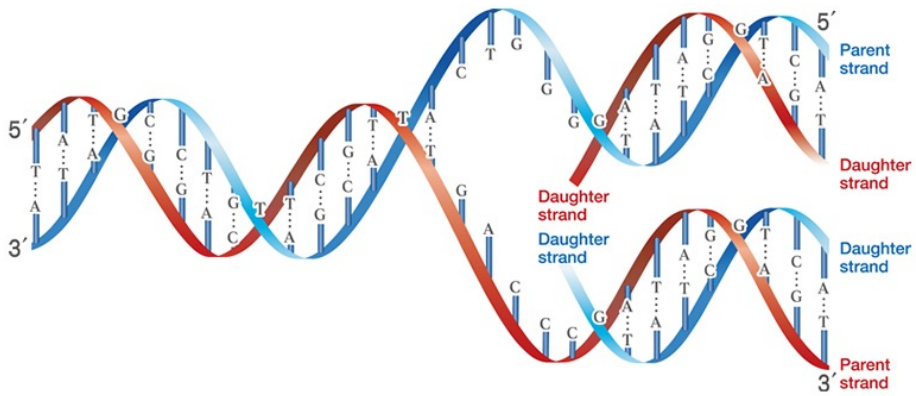
b. Complementary base pairing

c. Ladder configuration

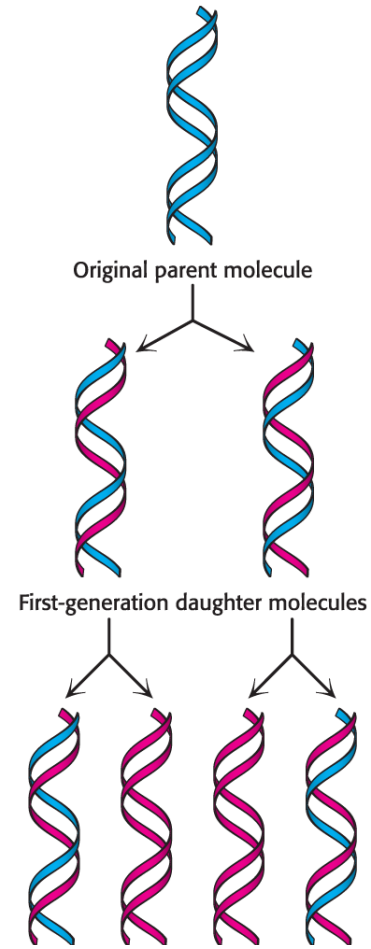


The Double Helix Facilitates the Accurate Transmission of Heredity Information

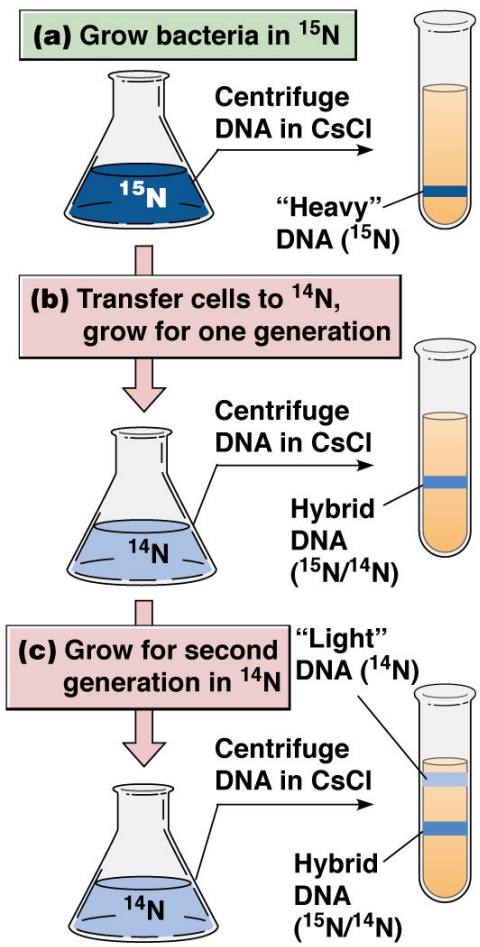
- Because of the base pairing rules, the sequence of one strand determines the sequence of the partner strand.
- The two strands can be separated and complementary sequences synthesized to generate two identical daughter strands.
- Because the two daughter helices have one parent strand and one newly synthesized strand, the replication process is called semiconservative replication.



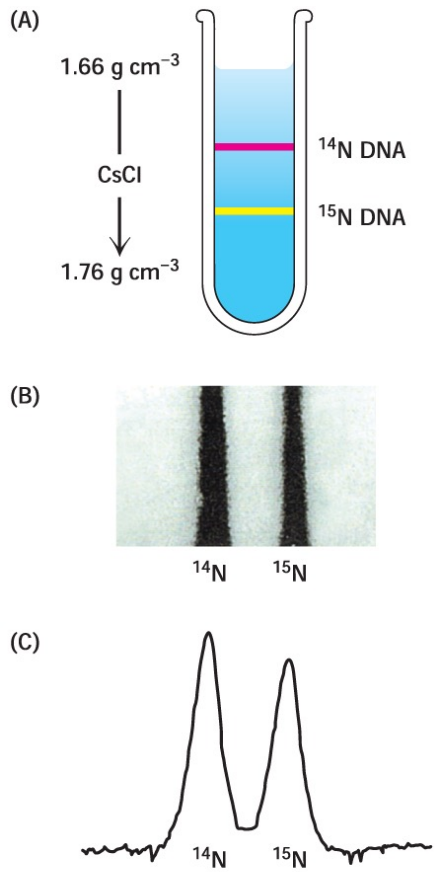
© CSLS/The University of Tokyo

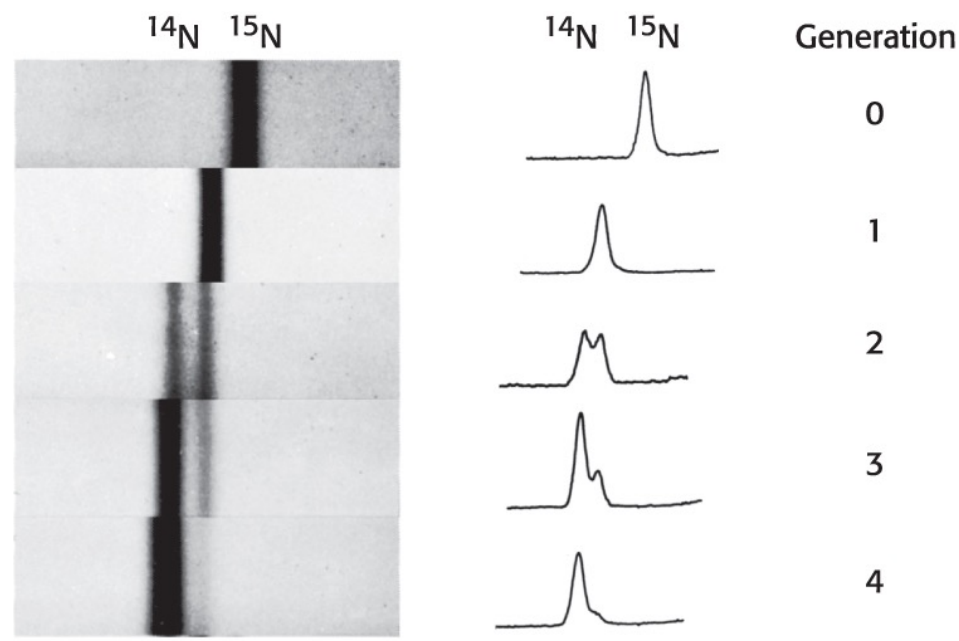


The Double Helix Facilitates the Accurate Transmission of Heredity Information

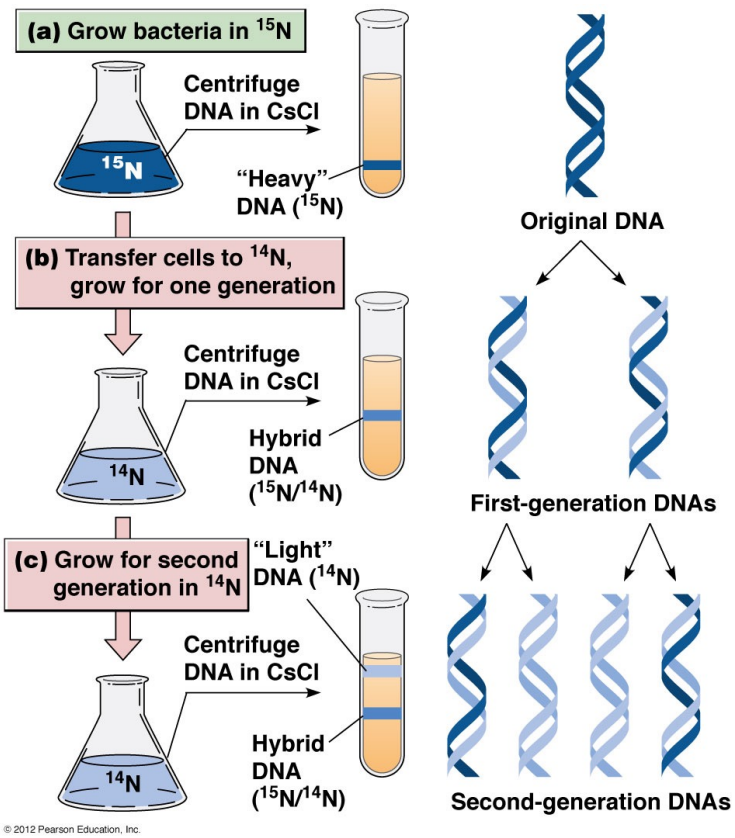


© 2012 Pearson Education, Inc.





The Double Helix Facilitates the Accurate Transmission of Heredity Information

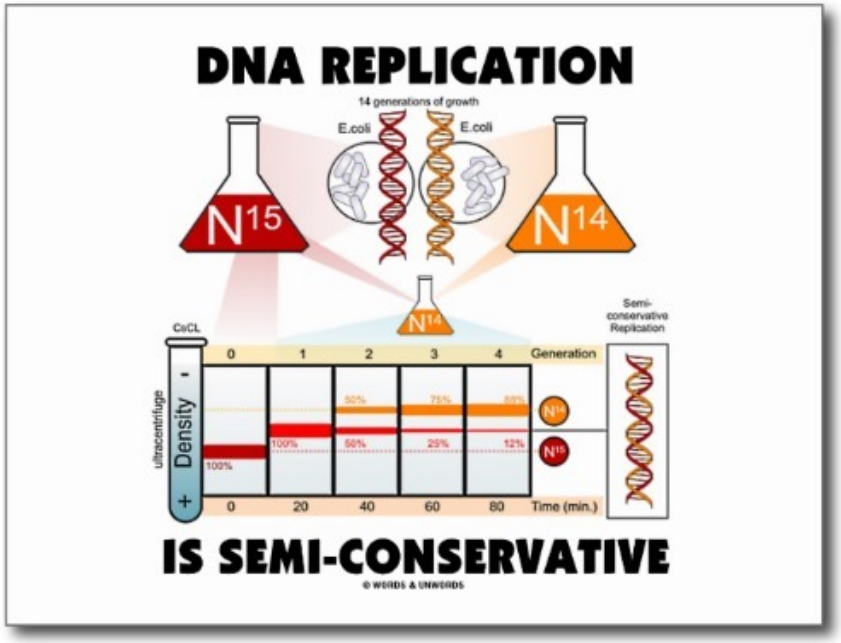


© 2012 Pearson Education, Inc.

- Meselson and Stahl elegantly demonstrated that replication is semiconservative by growing bacteria in growth media supplemented with ^{15}N. The bacteria were then shifted to growth media with ^{14}N as the nitrogen source.
- Density gradient centrifugation established that upon the shift to ^{14}N medium, newly synthesized DNA consisted of DNA with equal parts ^{15}N-DNA and ^{14}N-DNA, a result consistent with semiconservative replication.

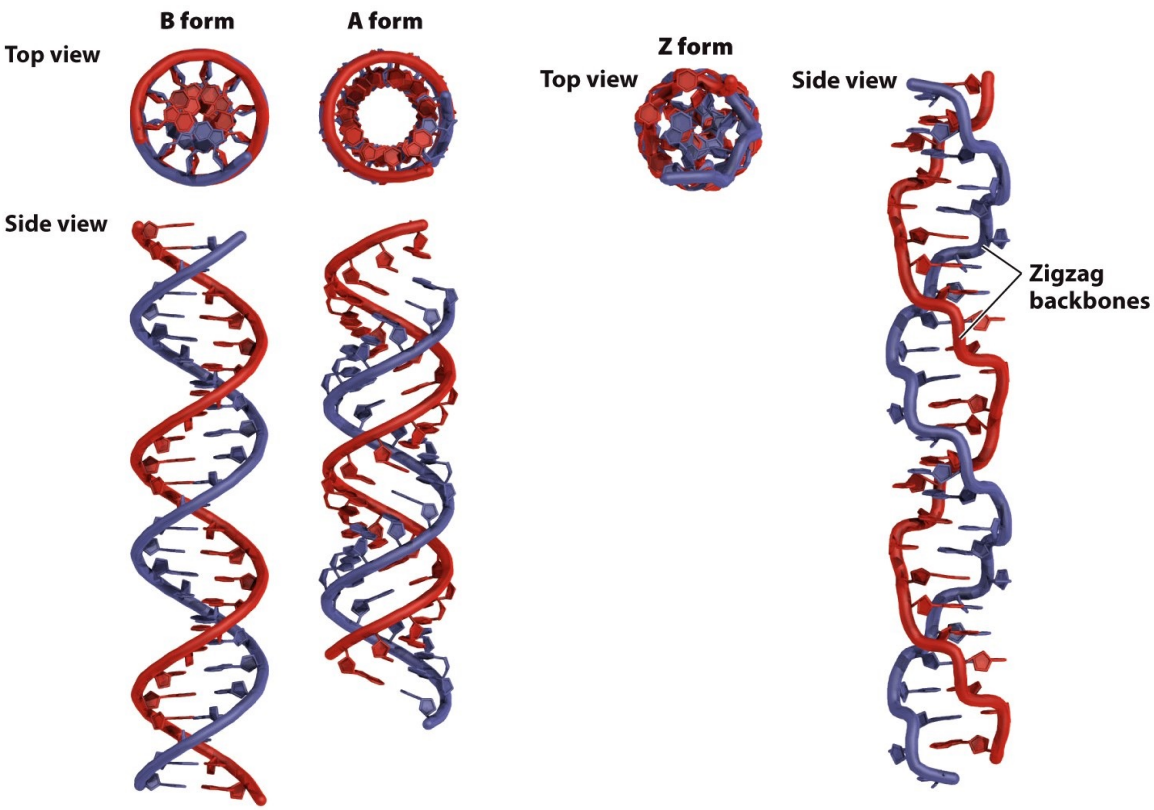
The Strands in the Double Helix can be Reversibly Separated

- During replication or transcription, the two strands of the DNA double helix must be separated.
- In the laboratory, DNA strands can be separated by heating a solution of DNA, a process called denaturation or melting. The temperature at which half of the DNA molecules are denatured is called the melting temperature (T_m).
- Upon cooling, the two strands can bind to one another to reform the double helix, a process called reannealing.



DNA Double Helices Can Adopt Multiple Forms

- In the cell, the most commonly seen form of DNA double helix is called the **B** form or the Watson-Crick helix.
- The double helix can also exist in an **A** form, which is shorter and wider than the B form with the bases at an angle rather than perpendicular to the helix axis.
- The **A** form is seen in RNA double helices and in RNA-DNA hybrid helices, structures observed in transcription and RNA processing.
- The double helix can also form **Z** DNA. **Z DNA** is left-handed and the backbone is zigzagged, accounting for the name "**Z DNA**."



The Major and Minor Grooves

- DNA in the **B** form has a ***major groove*** and a ***minor groove***.
- The presence of the grooves allows access to the hydrogen-bonding capabilities of the exposed bases.
- The hydrogen bonding capability provides a means of sequence-specific interactions between DNA and the molecules it must interact with in the processes of replication and transcription.

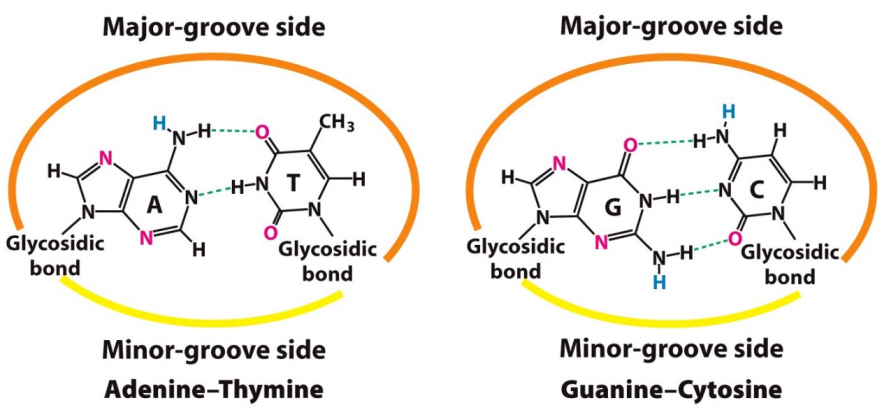


Figure 33.19
Biochemistry: A Short Course, Second Edition
© 2013 W. H. Freeman and Company

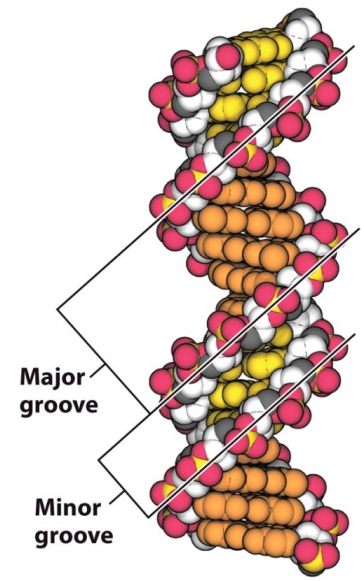
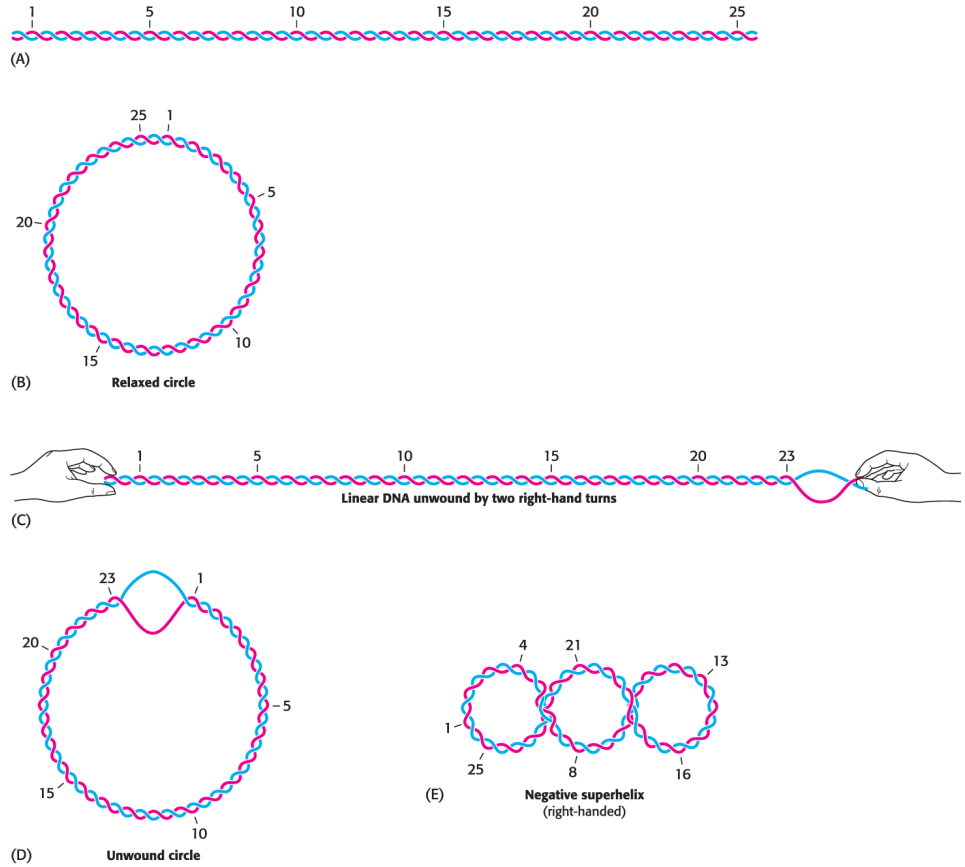


Figure 33.20
Biochemistry: A Short Course, Second Edition
© 2013 W. H. Freeman and Company

Double-Stranded DNA can wrap around itself to form supercoiled structures

- The DNA molecules in human chromosomes are linear.
- The term *circular* refers to the continuity of the DNA chains, not to their geometric forms.



Eukaryotic DNA Is Associated with Specific Proteins

- There are 3.6 meters of DNA in a human cell, packaged into 46 chromosomes.
- Supercoiling accounts for some of the compaction of the DNA, but further compaction occurs by binding certain proteins to the DNA.
- ***Chromatin*** is the entire complement of a cell's DNA and its associated proteins.
- ***Histones*** are highly basic proteins that are components of chromatin.
- Two copies each of histones H2A, H2B, H3, H4 and 200 bp of DNA comprise a ***nucleosome***.
- Nucleosomes are joined by ***linker DNA***, to which histone H1 binds, so that the histone-DNA complex has the appearance of beads on a string.
- Digestion of the linker DNA yields the ***nucleosome core particle***.

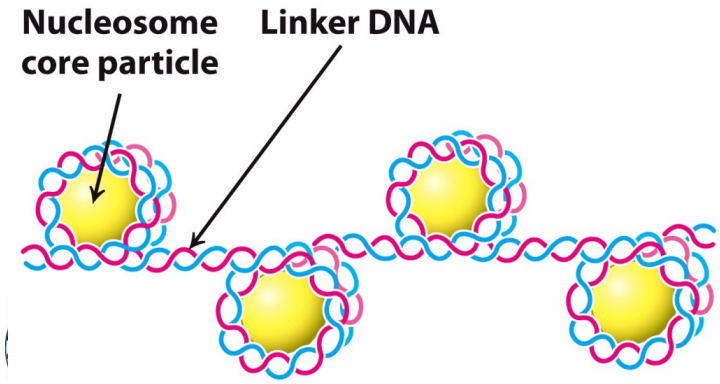
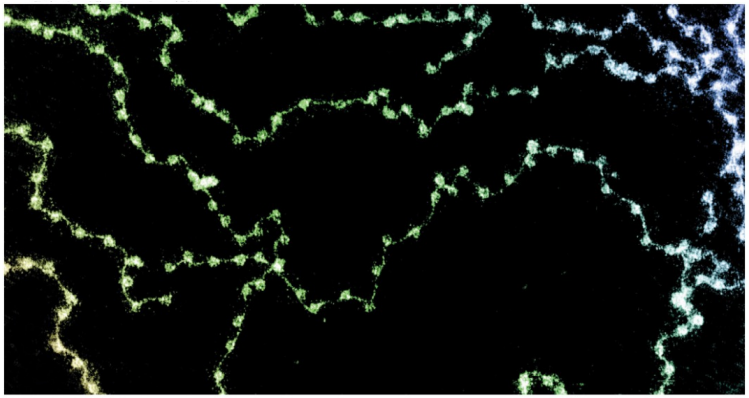


Figure 33.25



Don. W. Fawcett/Science Source.

Eukaryotic DNA Is Wrapped Around Histones to Form Nucleosomes

- The eight histones of the core particle are arranged as an octamer composed of (H3)2(H4)2 tetramer and a pair of (H2A-H2B) dimers.
- DNA wraps around the outside of the octamer.
- Nucleosomes themselves are arranged in a helical array that further compacts the DNA. Further folding generates the ***chromosome***.

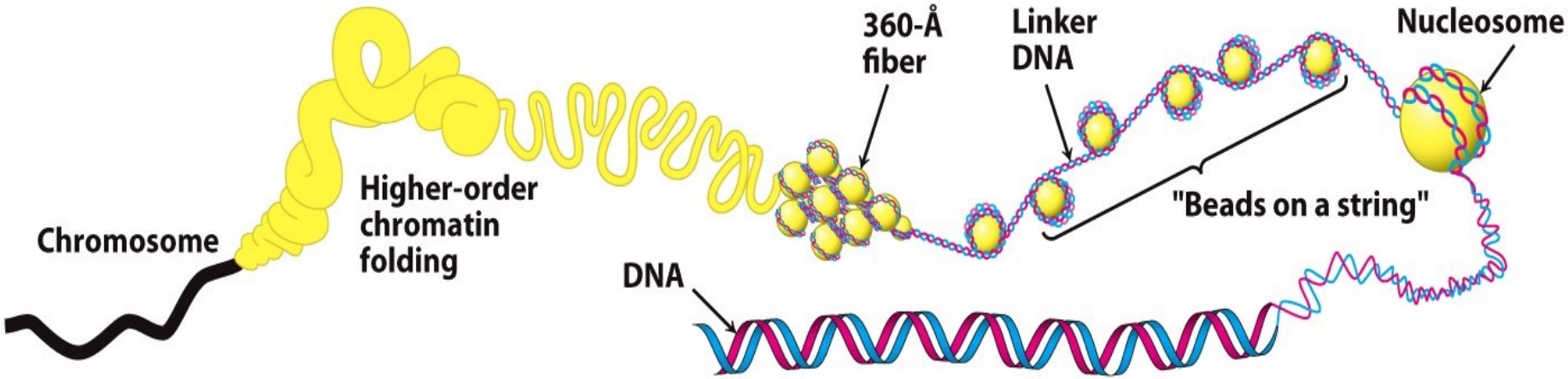


Figure 33.28
Biochemistry: A Short Course, Second Edition
© 2013 W. H. Freeman and Company

Quick Quiz 3

Eukaryotic DNA is tightly bound to a group of small basic proteins called .

A. chromatin

B. histones

C. nucleosomes

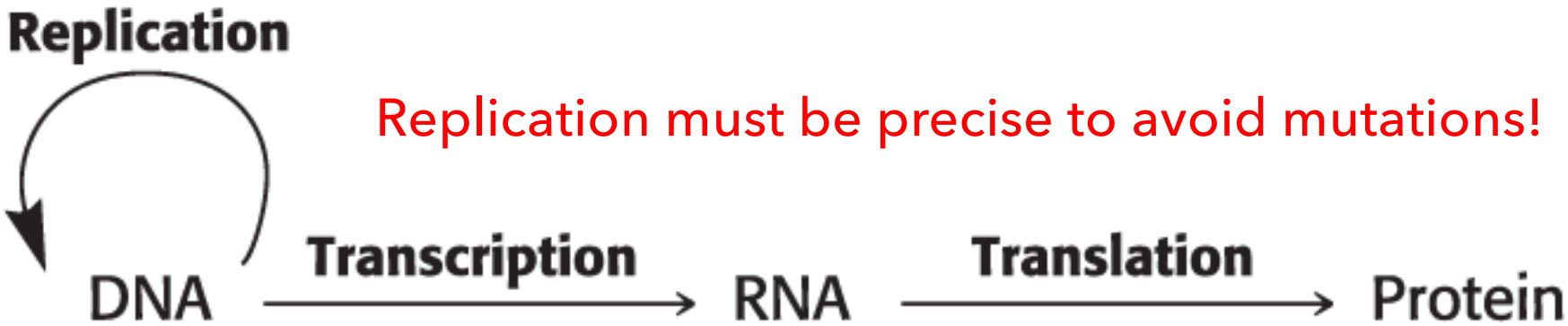
D. transcription factors

E. ubiquitin

Answer:

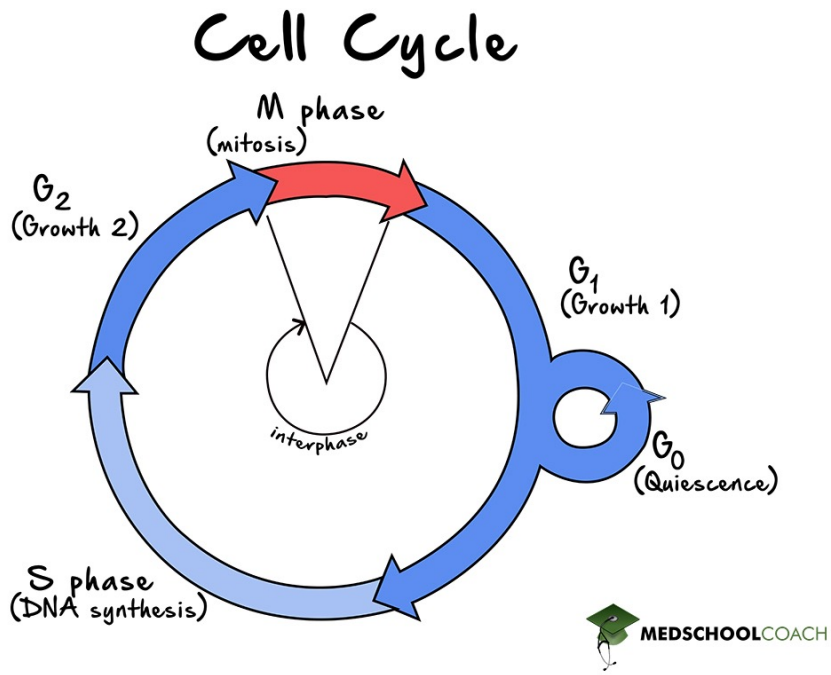
B

DNA Replication

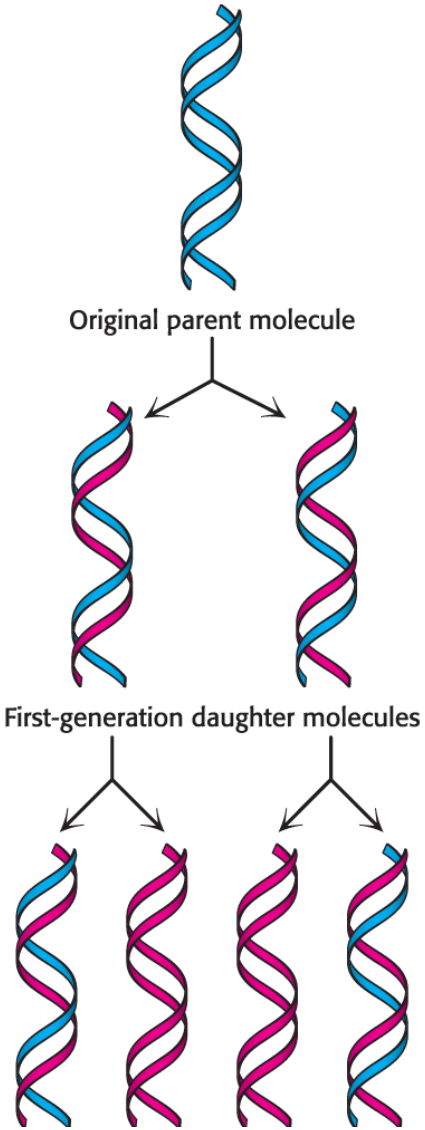


Replication must be precise to avoid mutations!

- **DNA contains the blueprint for the development of the organism.**
- It occurs during S-phase of the cell cycle.
- DNA replication is semi-conservative– each new DNA molecule has one original (parental) strand and one newly synthesized strand.
- The goal is to accurately copy genetic information for cell division.

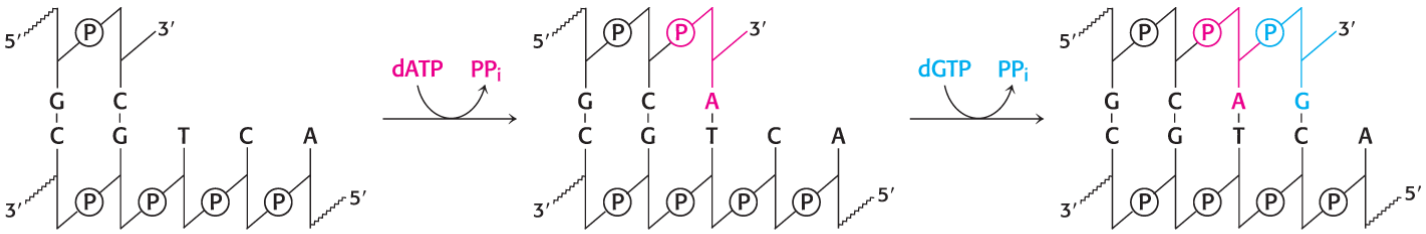


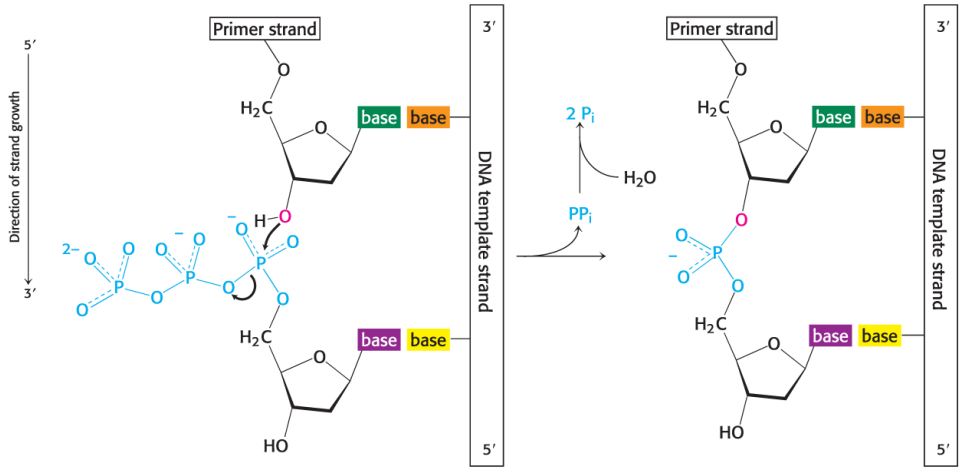
DNA Replication



➢ The polymerization reaction catalyzed by DNA polymerase is:

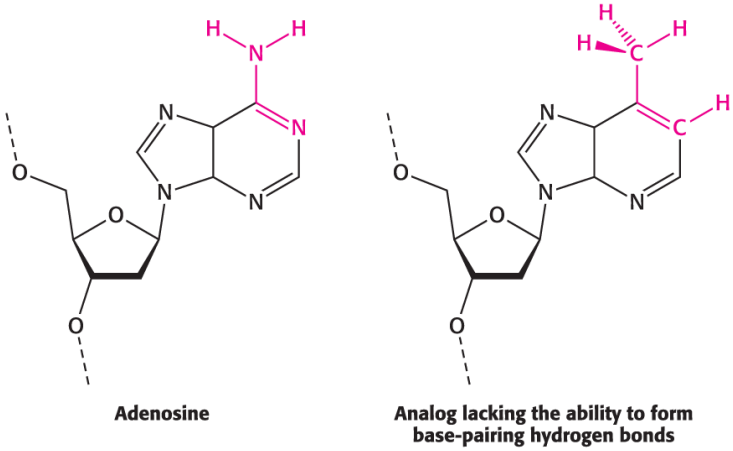
$(DNA)_n + dNTP \rightleftharpoons (DNA)_{n+1} + PPi$

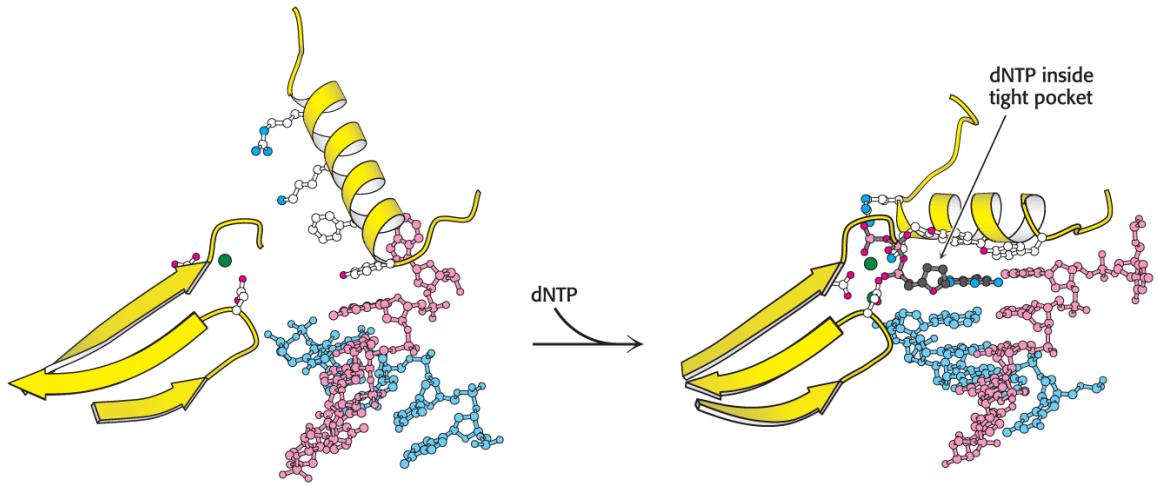




➢ Elongation of the DNA strand proceeds in the 5'-to-3′ direction:

Shape complementarity and selectivity



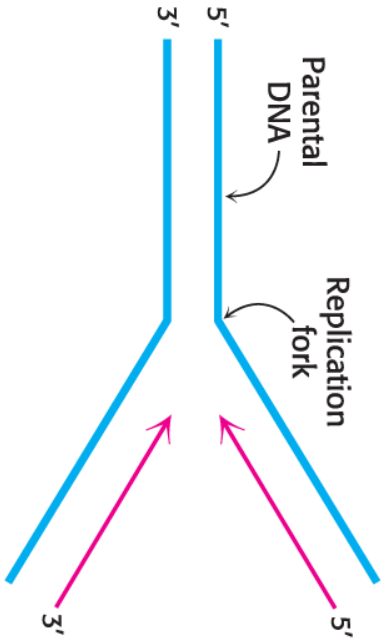


Adenine analogs can still direct the incorporation of thymidine

Binding of a dNTP to DNA polymerase induces a conformational change ➜ generates a tight pocket for base pairing of dNTP and its partner.

Only a properly shaped base pair will readily fit!

DNA Replication



Tymoczko et al., *Biochemistry: A Short Course*, 4e, © 2019 W. H. Freeman and Company

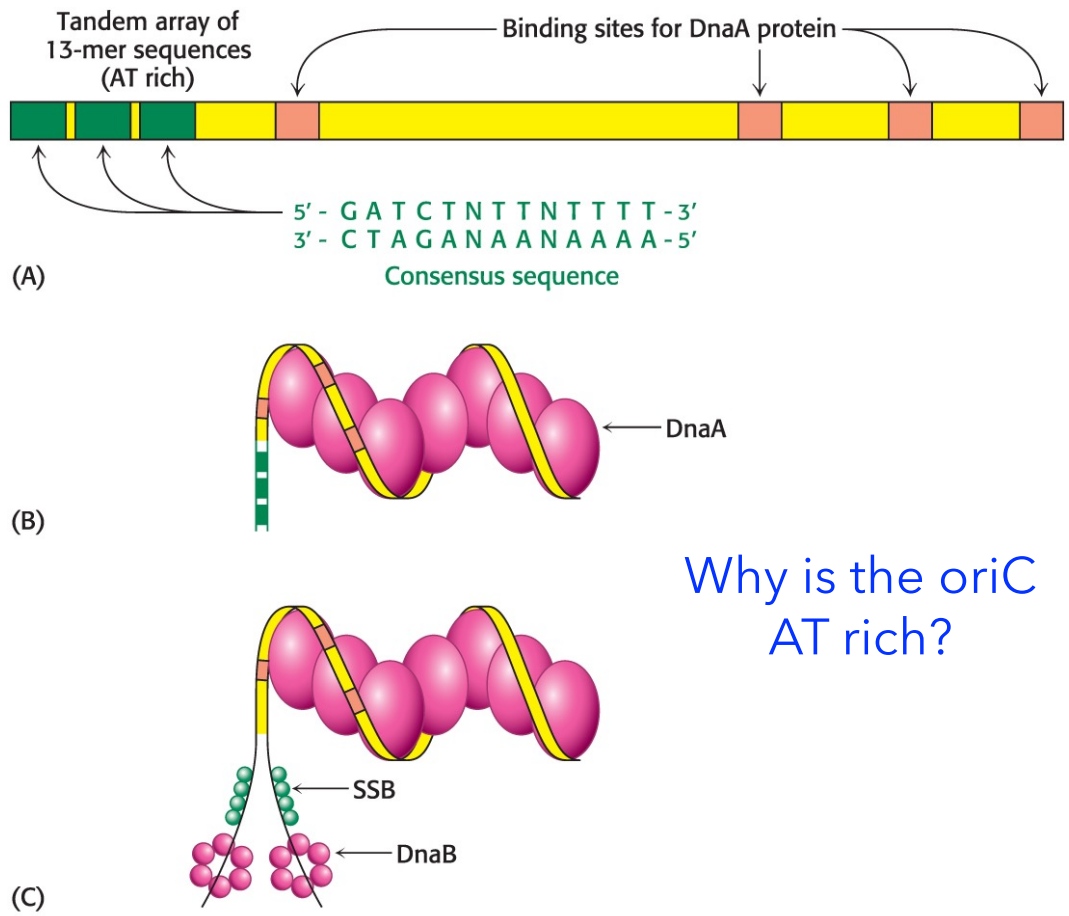
- Negative supercoiling prepares DNA for processes that require strand separation
- Presence of supercoils (overwound) near the "unwound" area makes it difficult for strand separation to continue
- Topoisomerases eliminate supercoils by temporarily cleaving DNA

- Origin of replication (OriC) recognized
- Helicase unwinds DNA
- SSBs stabilize single strands
- Topoisomerase prevents supercoiling

DNA replication in *E. coli* begins at a unique site

- DNA replication is rapid.
- *E. coli* genome, with its 4.6 million base pairs, is replicated in less than 40 min., a rate of 2000 nucleotides per second.

- oriC: origin of replication is the preferred binding sites for the origin-recognition protein DnaA.
- DnaB helicase unwind the duplex and single-strand-binding proteins prevent double helix reformation.
- Pre-priming complex is formed

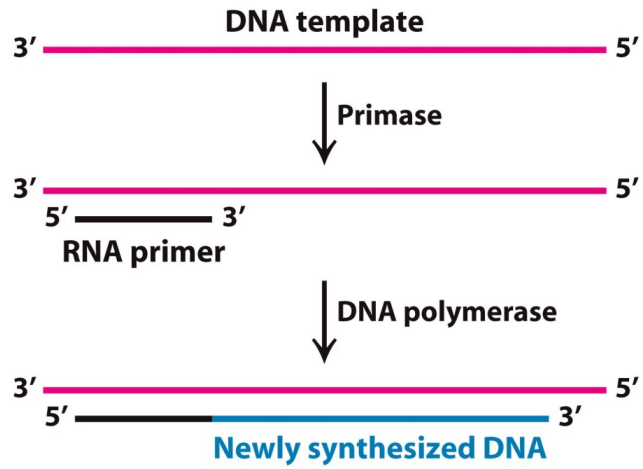


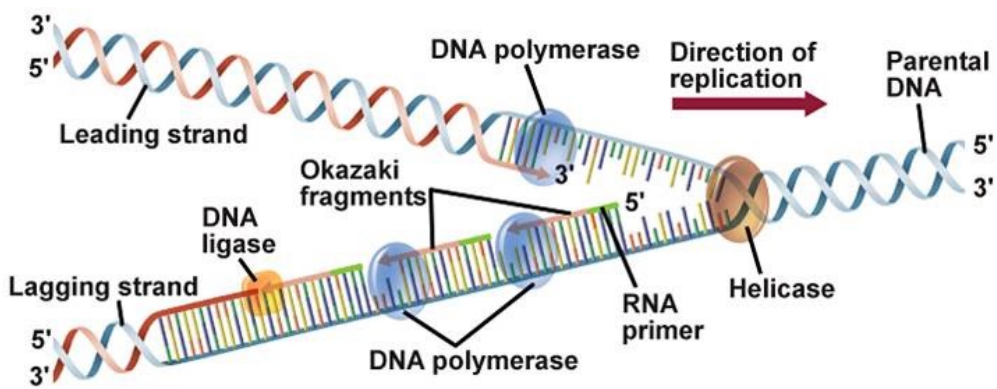
Why is the oriC AT rich?

Tymoczko et al., *Biochemistry: A Short Course*, 4e, © 2019 W. H. Freeman and Company

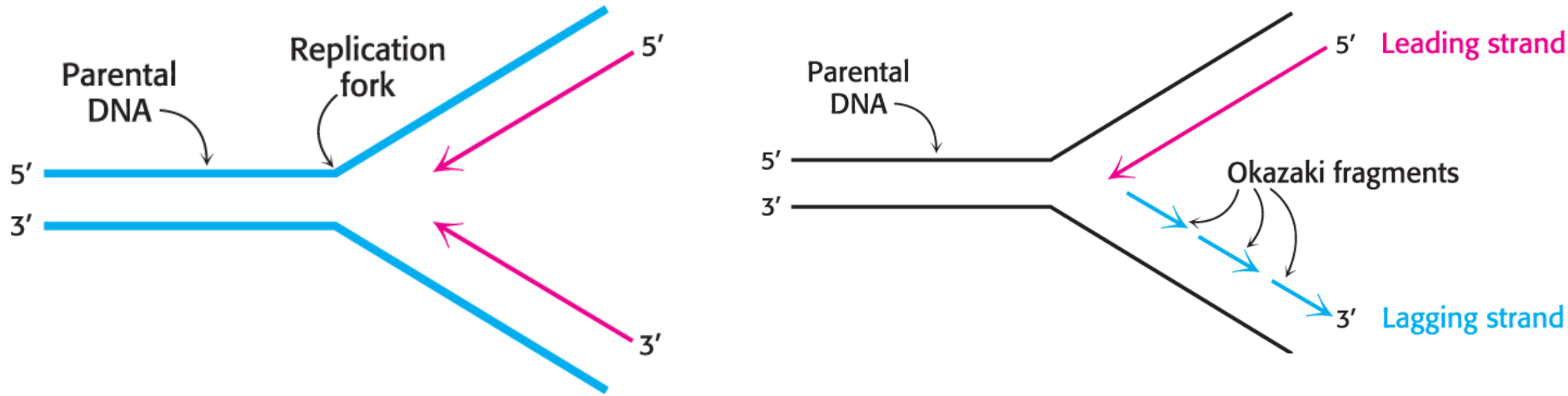
RNA primes the synthesis of DNA

- An RNA primer synthesized by ***primase*** enables DNA synthesis to begin.
- A special type of RNA polymerase called *primase* synthesizes short RNA (≈10 nucleotides) complementary to a DNA strand that then serves as a primer for DNA synthesis.
- The primase is part of a large complex called the ***primosome*** that joins the pre-priming complex to synthesize the required primer.
- DNA polymerase I, employing a third enzymatic activity, a 5′→3′ exonuclease activity, subsequently removes the primers and replaces the RNA with DNA.





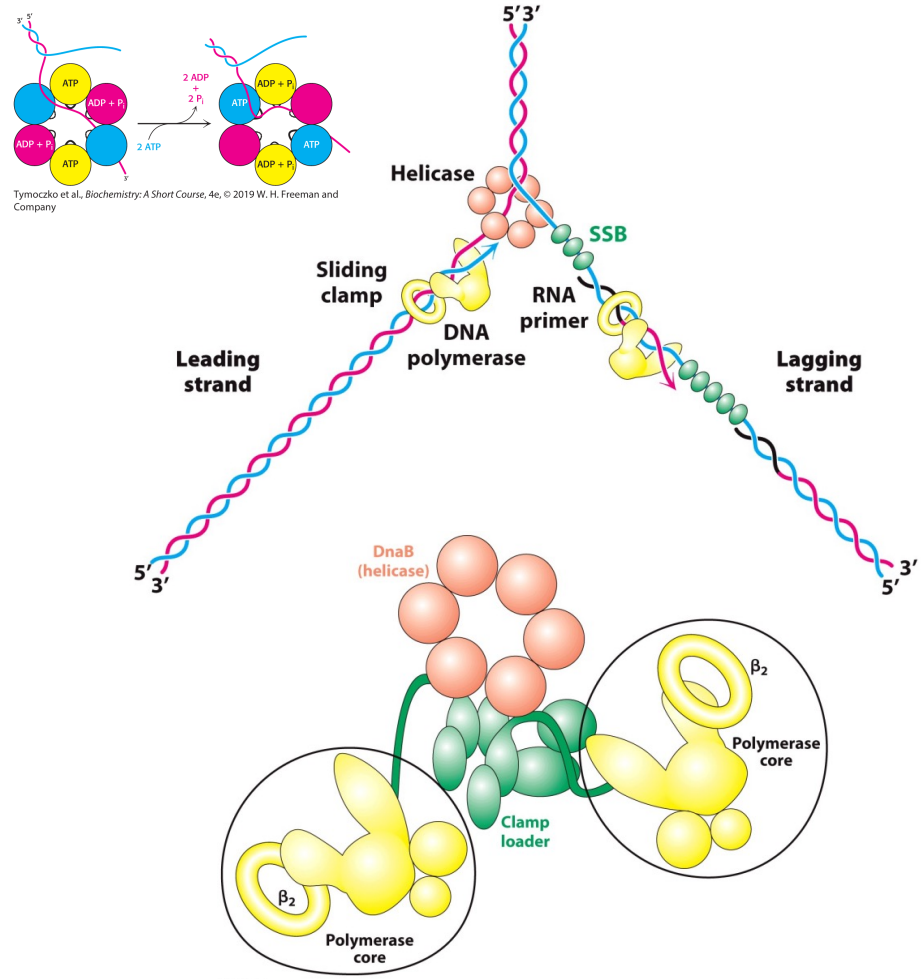
DNA Replication



- DNA polymerases synthesize DNA in the 5′→3′ direction but not in the 3′→5′.
- The strand of DNA that is synthesized continuously is called the leading strand.
- The lagging strand is synthesized discontinuously as Okazaki fragments.
- The lagging strand is looped so that it passes through the polymerase active site in the 3′→5′ direction, allowing synthesis to occur in the 5′→3′ direction.

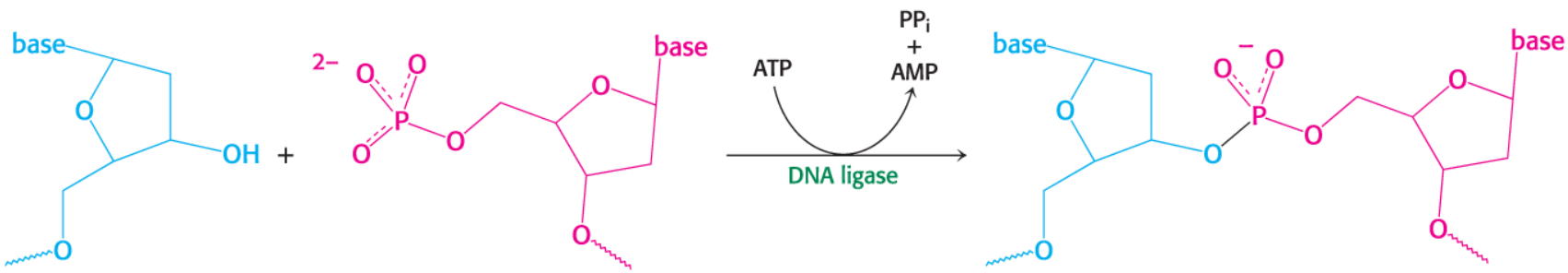
DNA Replication Is Highly Coordinated

- After DNA of ≈1000 nucleotides in length is synthesized, the loop is released and a new loop is formed, a process called the ***trombone model***.
- ***DNA polymerase*** removes the RNA primer and replaces the sequence with DNA.
- ***DNA ligase*** joins the fragments to yield an intact strand.



The DNA Ligase Reaction

- DNA ligase catalyzes the formation of a phosphodiester linkage between 3′-hydroxyl group at the end of one DNA chain and the 5′-phosphate group at the end of other.



Key Enzymes and their Roles

Enzyme	Function
Helicase	Unwinds the DNA helix
SSBs	Prevents reannealing of single strands
Topoisomerase	Relieves supercoiling tension
Primase	Lays down RNA primers
DNA Polymerase III	Synthesizes the new DNA strand
DNA Polymerase I	Replaces RNA primers with DNA
Ligase	Seals Okazaki fragments

DNA Replication

Step 1: INITIATION

- Origin of replication (OriC) recognized
- Helicase unwinds DNA
- SSBs stabilize single strands
- Topoisomerase prevents supercoiling

Step 2: ELONGATION

- Primase lays RNA primers
- DNA Polymerase III extends strands
- Leading strand: continuous synthesis (5' → 3')
- Lagging strand: Okazaki fragments joined by Ligase

Step 3: TERMINATION

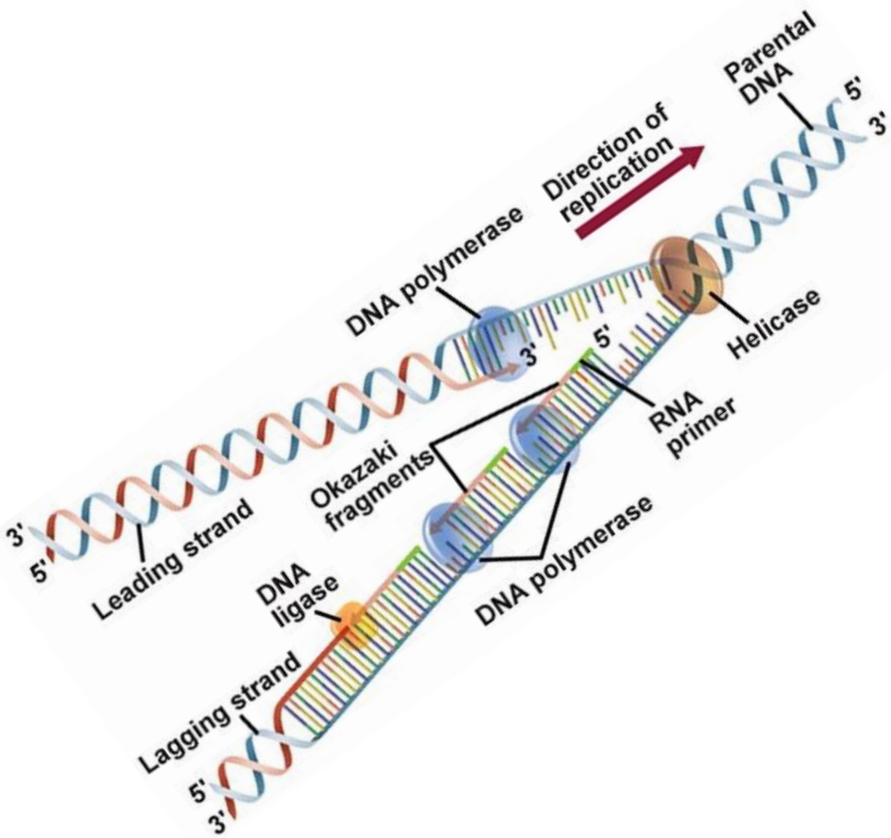
- Replication forks meet and process stops
- DNA Polymerase I replaces primers
- Ligase seals final nicks
- Proofreading ensures accuracy

Step 4: PROOFREADING

DNA Replication

Key characteristics of DNA synthesis are:

1. Four deoxynucleoside triphosphates and Mg^{2+} are required.
2. A template strand is used to direct DNA synthesis.
3. A primer from which the new strand grows must be present.
4. Many DNA polymerases have nuclease activity that allows for the removal of mismatched bases.



Quick Quiz 4

Which of the following is released during the addition of deoxyribonucleotides to the nascent DNA chain?

A. Okazaki fragments

B. pyrophosphate (PPi)

C. AMP

D. ATP

E. dNTP

Quick Quiz 5

DNA polymerases can add nucleotides only to a free hydroxyl group. As a result, a primer made up of _____ is added by the enzyme _____.

A. RNA; DNA polymerase II

B. DNA; DNA polymerase I

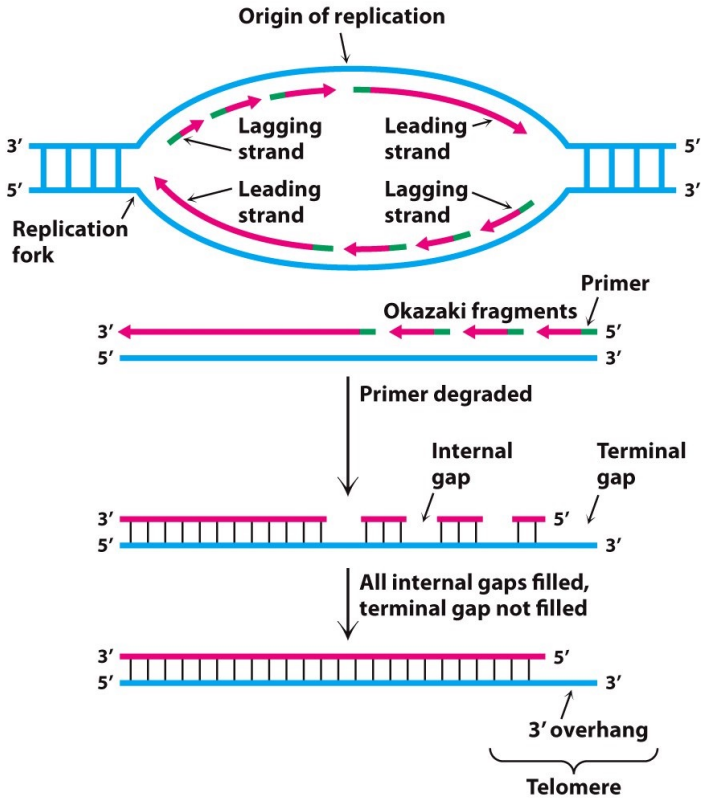
C. DNA; primase

D. Okazaki fragments; primase

E. RNA; primase

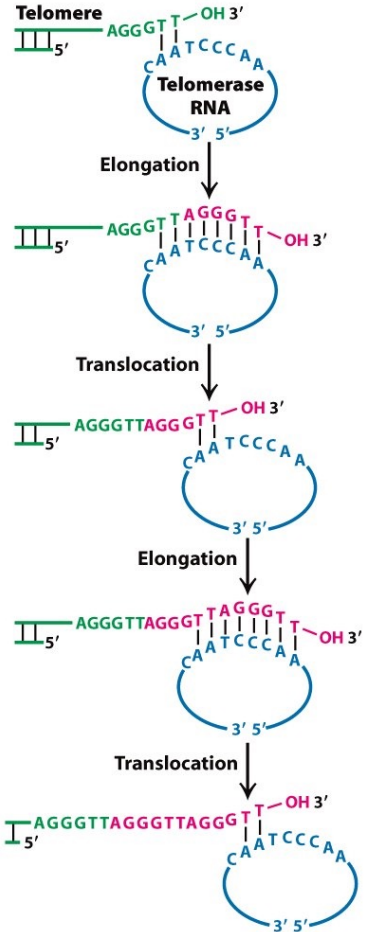
DNA Synthesis Is More Complex in Eukaryotes Than in Bacteria

- Eukaryotes have much more DNA than bacteria. Moreover, the DNA is present as linear molecules in multiple chromosomes.
- Multiple origins of replication are required with each origin of replication representing a replication unit or replicon. Humans have ≈ 30,000 replicons.
- Proteins called licensing factors allow only one replication per replicon per round of DNA synthesis.
- DNA polymerase α, which displays primase activity, initiates DNA replication in eukaryotes, generating a DNA molecule ≈ 20 dNTP in length.
- DNA polymerase δ, a more processive enzyme, extends the chains. The switch from polymerase α to δ is called polymerase switching.



DNA Replication Is Highly Coordinated

- The G-rich leading strand of the telomere can be maintained by the enzyme telomerase.
- Telomerase contains an RNA template that it uses to extend the leading strand.
- In rapidly dividing cells, including cancer cells, telomeres must be maintained by the telomerase to prevent shortening of the lagging, which would lead to cell death.
- High telomerase activity is a characteristic of cancer cells.



Assigned Problems

Chapter	Tymochko, Berg, Stryer, Biochemistry, 2nd Edition,	**Chapter**	Tymochko, Berg, Stryer, Biochemistry, 2nd Edition,
33	1, 3, 4, 5, 7, 10, 12, 16, 17, 19, 20, 21.	34	5, 6, 8, 9, 11, 12, 14, 17.
Chapter	Tymochko, Berg, Stryer, Biochemistry, 3nd Edition, 4th Edition (bottom line)	**Chapter**	Tymochko, Berg, Stryer, Biochemistry, 3nd Edition, 4th Edition (bottom line)
33	1, 3, 4, 5, 7, 11, 13, 17, 18, 20, 21, 22. 1, 3, 4, 5, 7, 11, 13, 17, 18, 20, 21, 22.	34	5, 6, 8, 9, 11, 12, 14, 17. 5, 6, 8, 9, 11, 12, 14, 17.