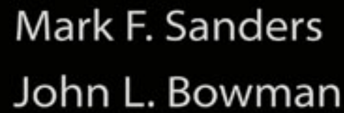

GENETIC
AN INTEGRATED APPROACH
ANALYSIS

SECOND EDITION

7

DNA Structure and Replication

Lectures by Dr. Tara Stoulig
Southeastern Louisiana University

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Models of Replication

- Watson and Crick1953: *" it has not escaped our notice that the specific base-pairing suggests a possible copy mechanism"*

- A consequence of **complementary base pairing** is that nucleotides on one strand of the duplex can be used to identify nucleotides on the other strand

- After the DNA structure was identified, **three competing models of DNA replication** emerged

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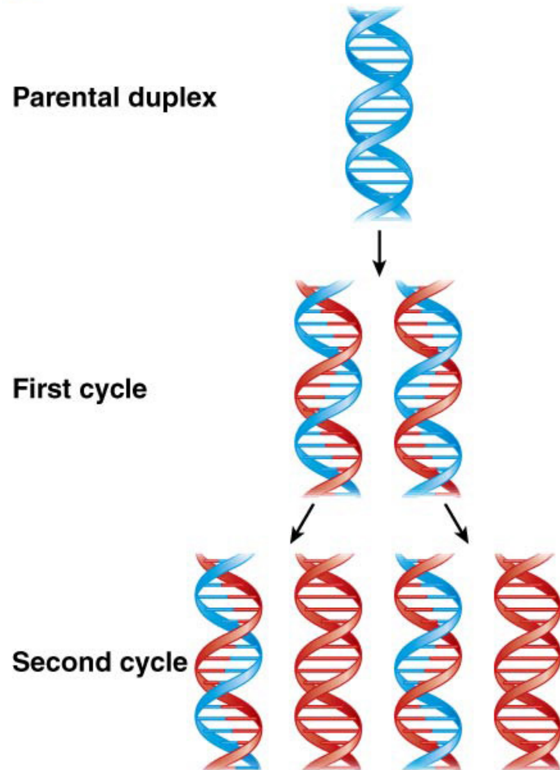
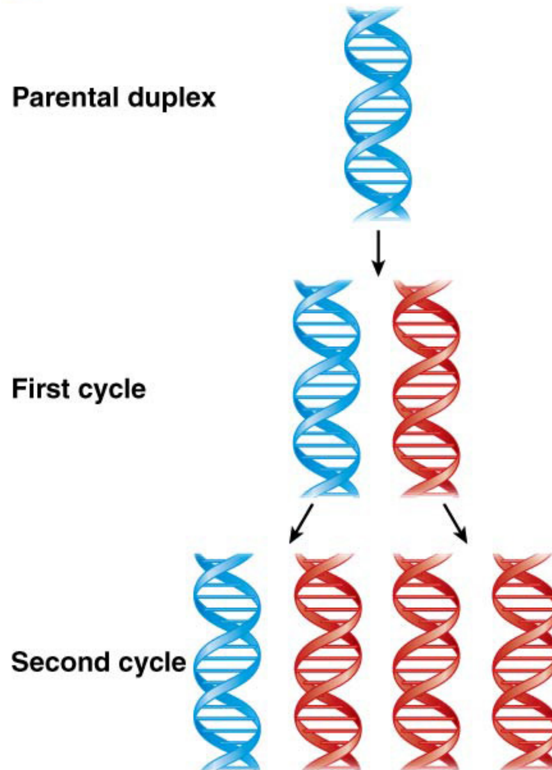
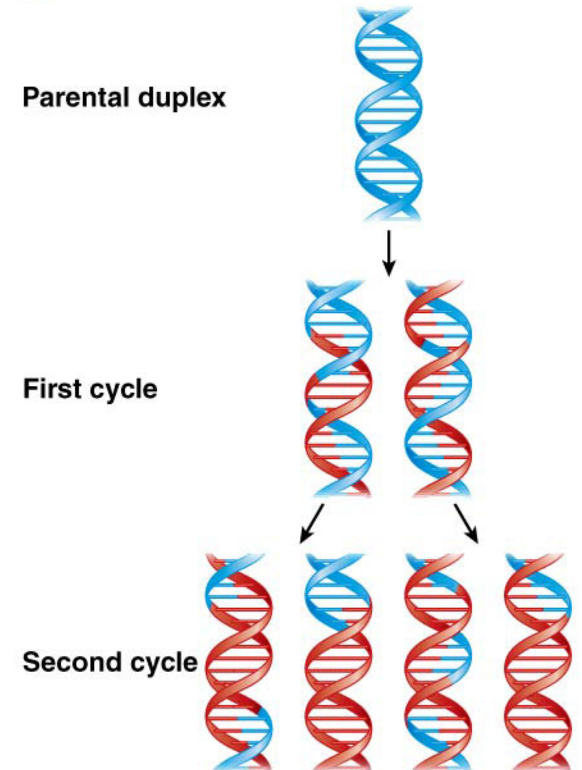
Figure 7.8 Three proposed mechanisms of DNA replication. Meselson and Stahl will test these hypothesis.







Semiconservative DNA replication: each daughter duplex contains one parental and one daughter strand

Conservative DNA replication: one daughter duplex contains both parental strands and the other contains both daughter strands

Dispersive DNA replication: each daughter duplex contains interspersed parental and daughter segments

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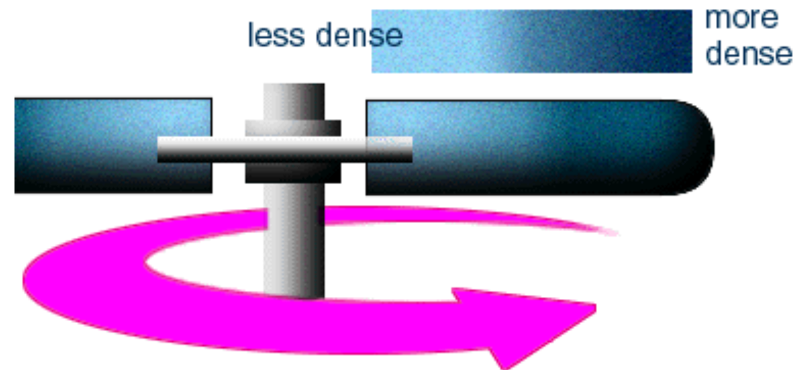
The Meselson-Stahl Experiment

- **In 1958, Meselson and Stahl used cesium chloride (CsCl) centrifugation to test the models of DNA replication**

- method to separate molecules with different molecular weights



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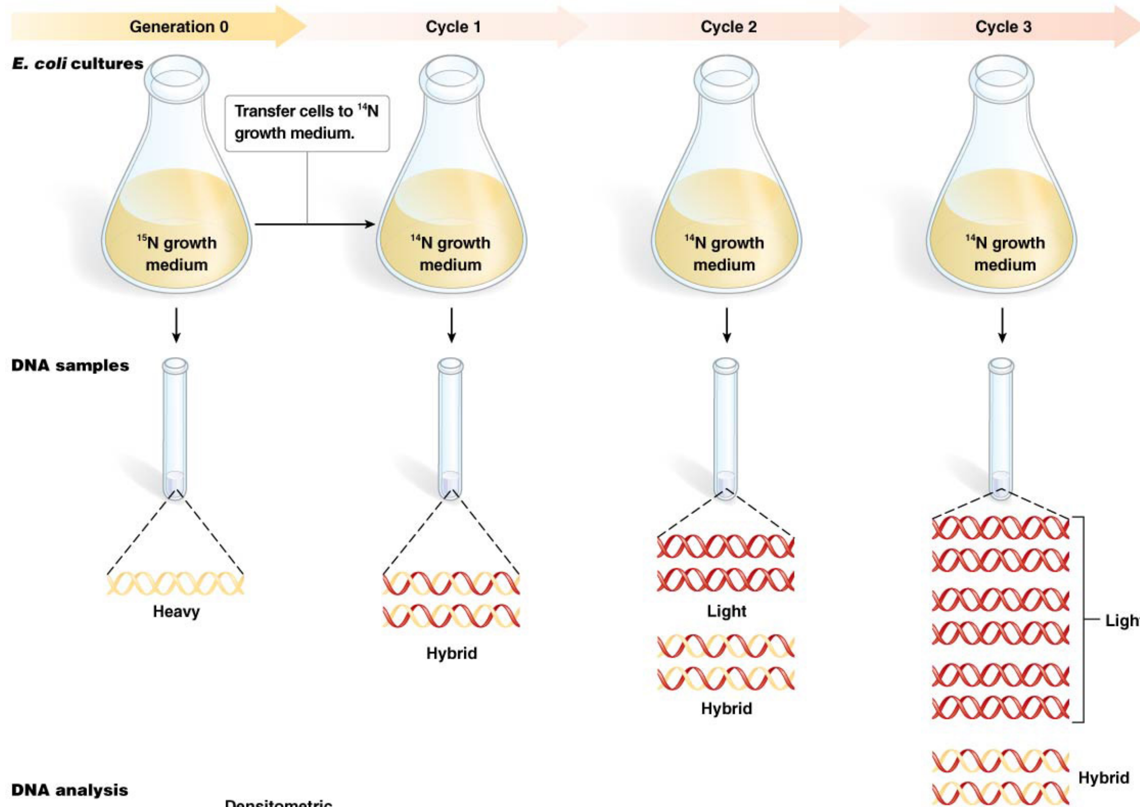
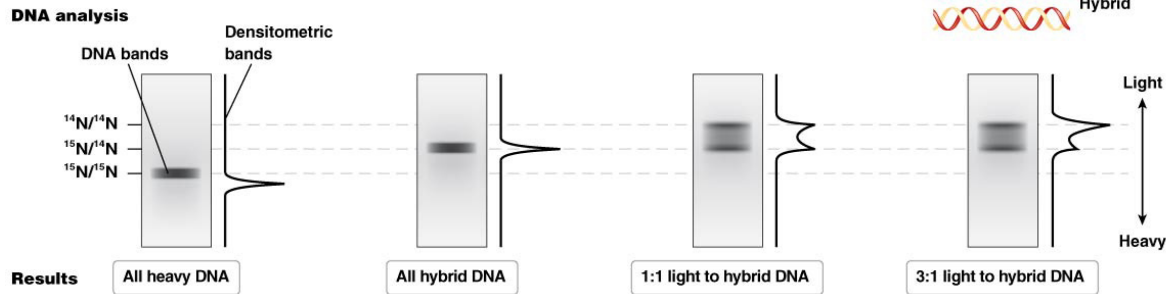
E. coli grow in a medium containing heavy nitrogen (^{15}N)

bacteria are transferred to ^{14}N medium



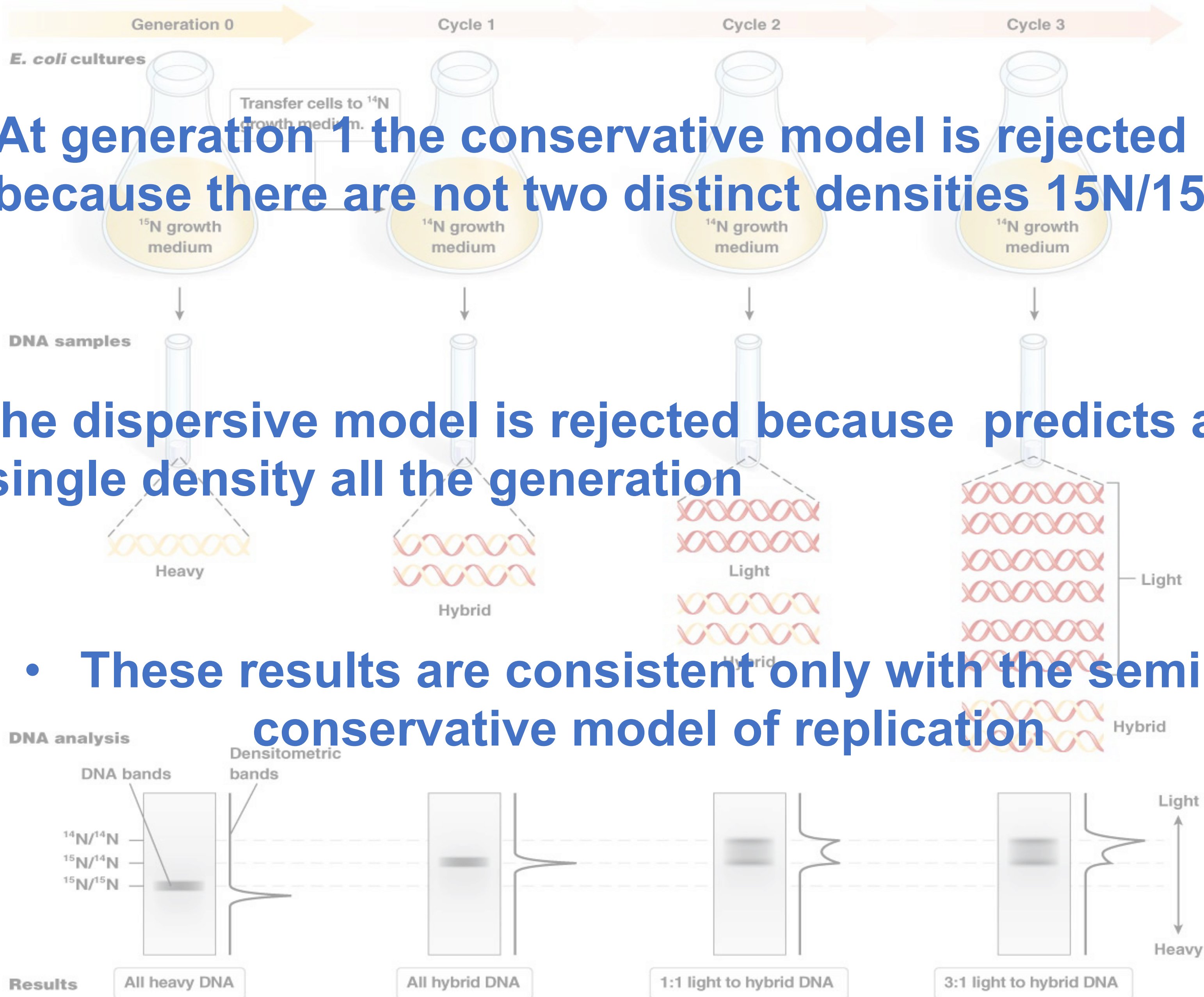
After one round of replication, the DNA of an aliquot of cells was isolated and centrifuged to determine its density



all DNA molecules had the densities $^{14}N/^{15}N$

half the molecules had densities of $^{14}N/^{15}N$ and the other half $^{14}N/^{14}N$

conclusion



- At generation 1 the conservative model is rejected because there are not two distinct densities 15N/15N
- the dispersive model is rejected because predicts a single density all the generation
- These results are consistent only with the semi-conservative model of replication

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One Origin and bi-directionality: replication in Bacterial DNA

- **John Cairns** reported the first evidence of bacterial origins of replication 1963

DNA replication is most often **bidirectional**, proceeding in both directions **from a single o**rigin of replication **in bacterial** chromosomes

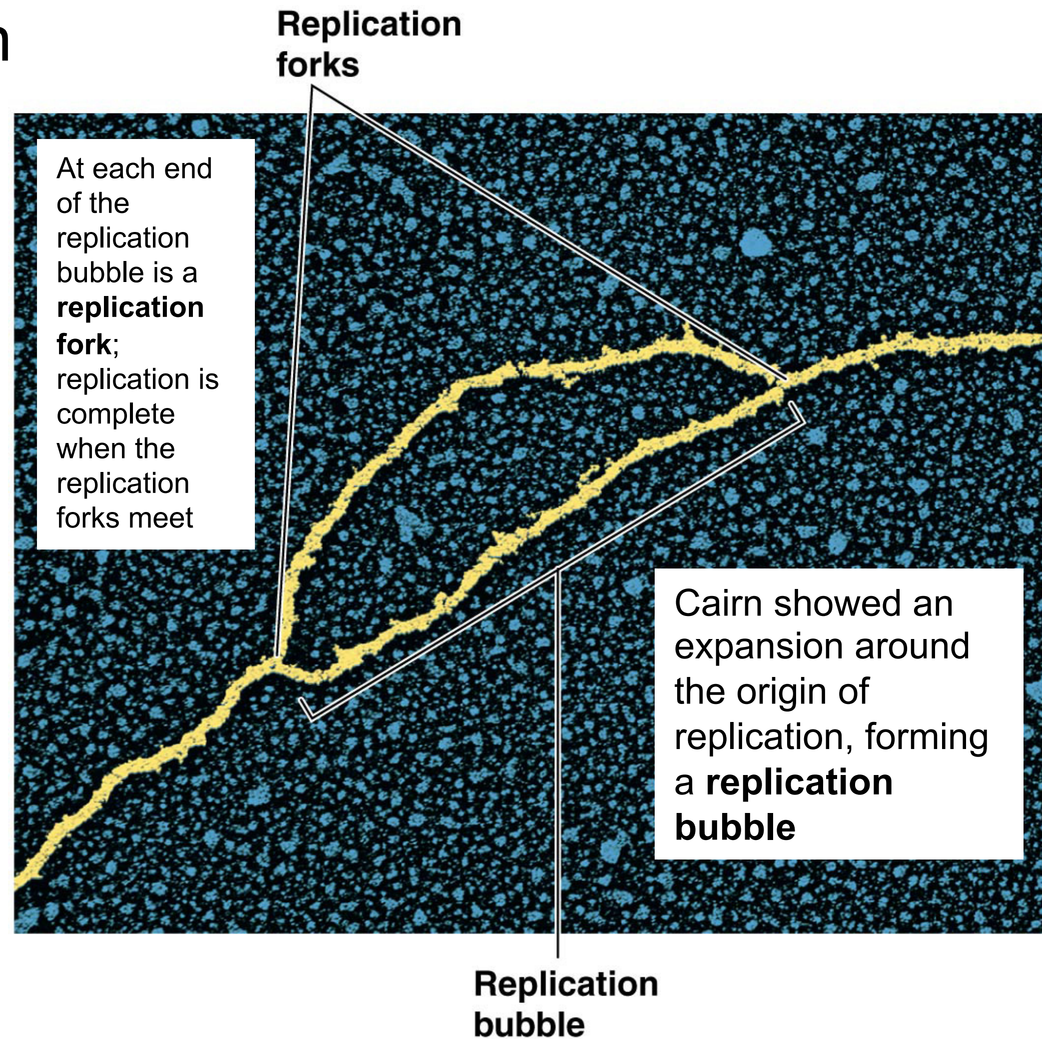

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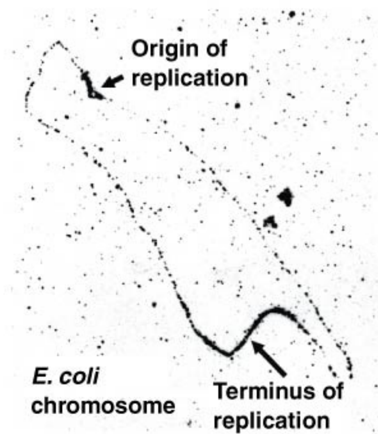
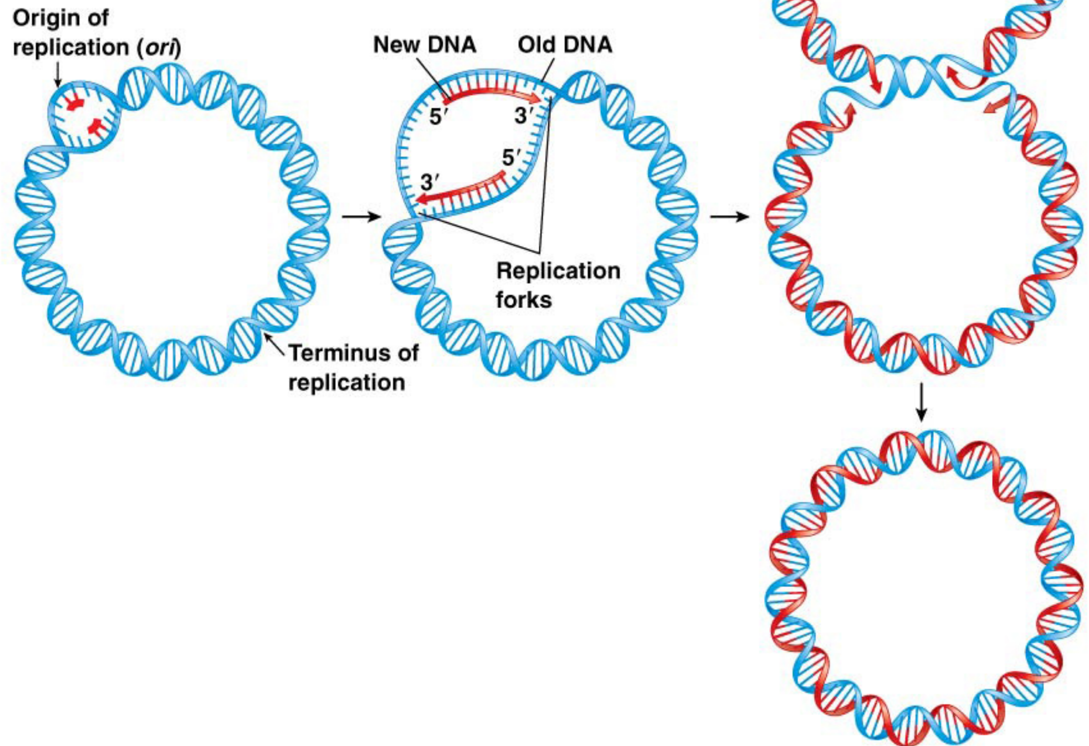
Figure 7.12 Bidirectional DNA replication: SCHEME





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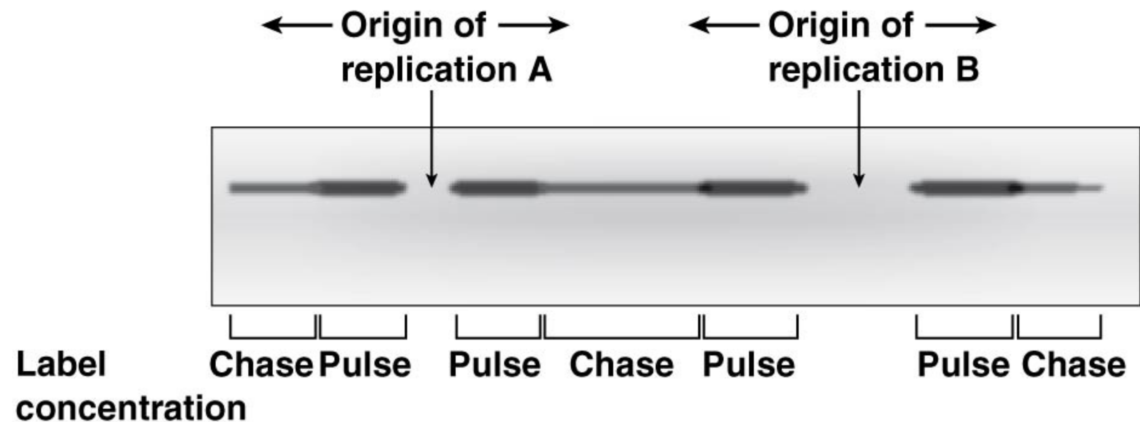
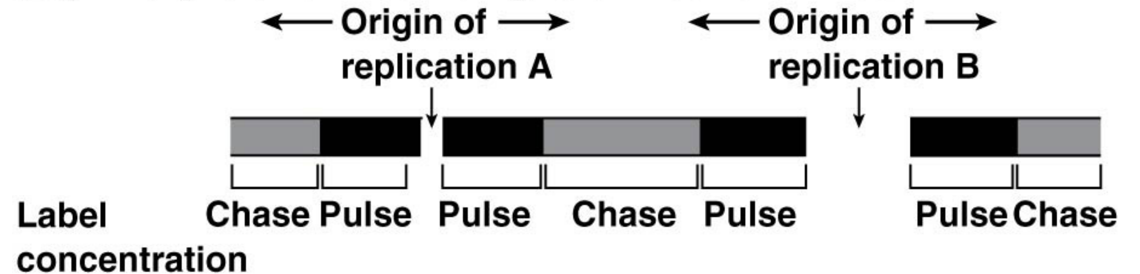
Eukaryotic chromosomes have multiple origins of replication

Cells are exposed to high level of radioactive compound to incorporate in DNA synthesis (pulse) and following the pulse the compound labeled is removed (Chase)

The alternance between radioactive and not is examined by radiography and the pattern of alternating pulse and chase (dark and light) on on both sides of origin is compatible with bidirectionality





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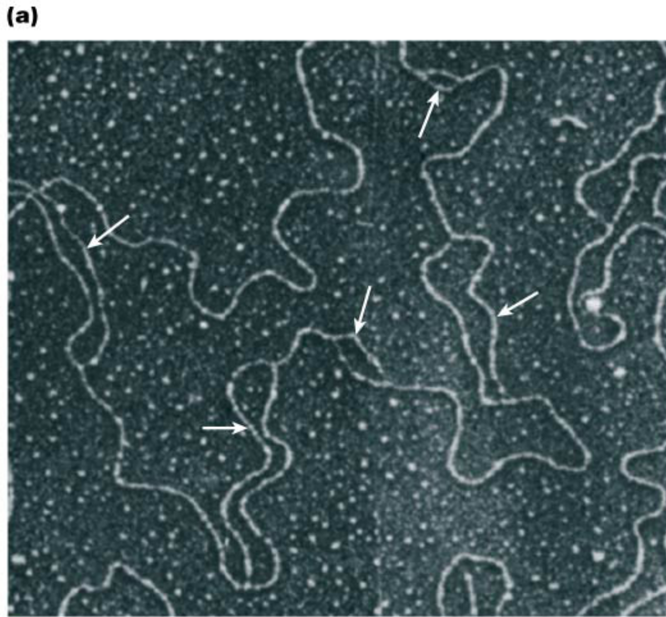
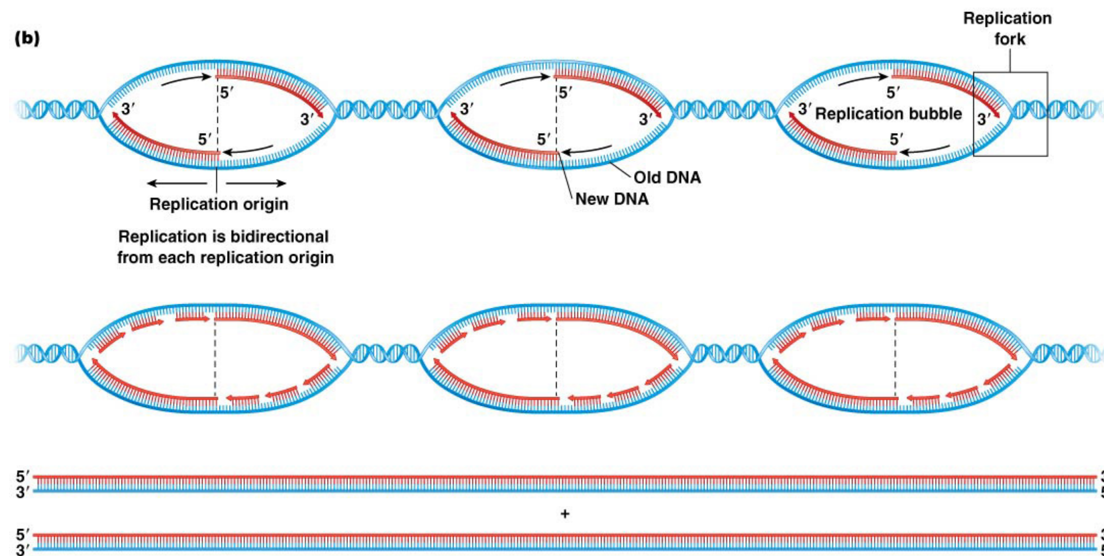
Multiple Replication Origins in Eukaryotes

- Autoradiograph analysis shows multiple origins of replication (thousands: homo 10.000) on eukaryotic chromosomes





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7.4 DNA Replication

- Replication is **best studied in bacteria**
- ***Replisomes**:* large complex aggregations of proteins and enzymes, assemble at each replication fork

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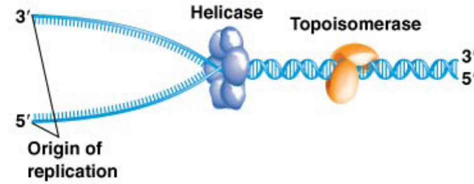
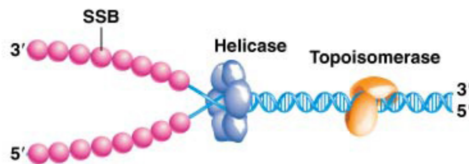
D N A R E P L I C A T I O N

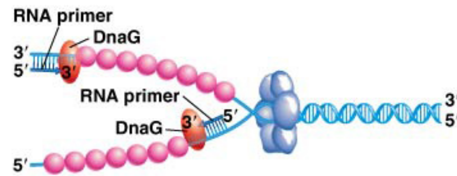
1. Helicase breaks hydrogen bonds. Topoisomerase relaxes super-coiling.



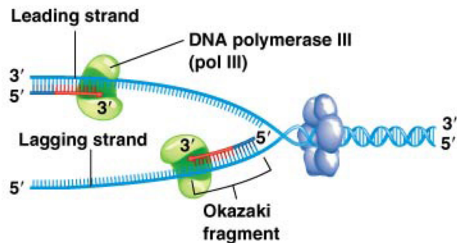
2. Single-stranded binding (SSB) protein prevents reannealing.



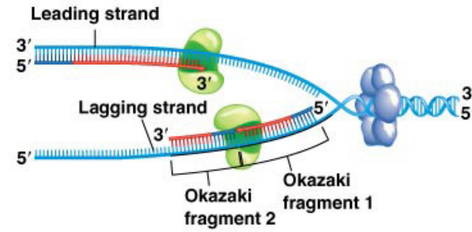
3. DnaG synthesizes RNA primers.



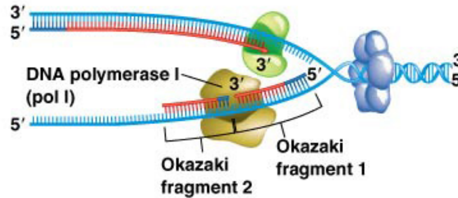
4. DNA polymerase III synthesizes daughter strand.



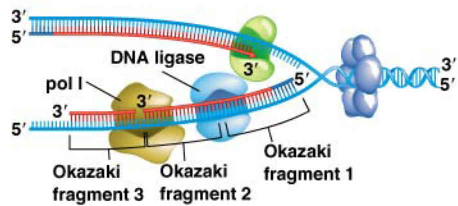
5. DNA polymerase III elongates the leading strand continuously and the lagging strand discontinuously.



6. DNA polymerase I removes and replaces nucleotides of the RNA primer.



7. DNA ligase joins Okazaki fragments.



Protein	DNA topoisomerase	Helicase (DnaB)	SSB	Primase	DNA pol III	DNA pol I	DNA ligase
Icon							
Role	Relaxes supercoiling	Unwinds the double helix	Prevents reannealing of separated strands	Synthesizes RNA primers	Synthesizes DNA	Removes and replaces RNA primer with DNA	Joins DNA segments

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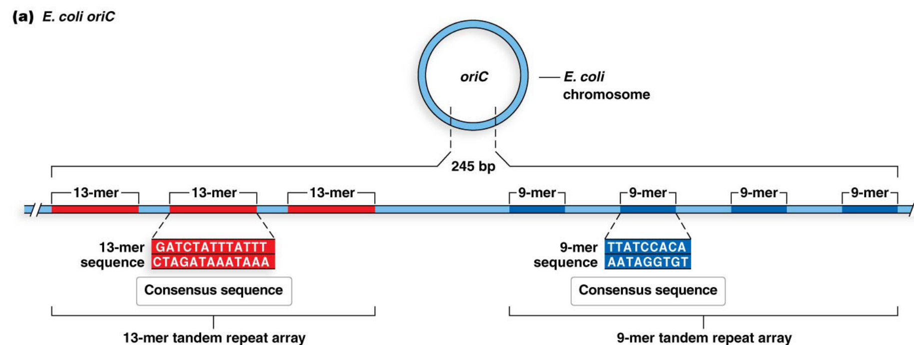
DNA Sequences at Replication Origins

- **Best characterized ORIGIN-OF-REPLICATION: oriC in** *E.coli* (245bp A-T rich)
- Comparison within and between bacterial species leads to identification of **consensus sequences**, characterized by the nucleotides found *most often* at each position of DNA in the conserved region
- **Replication origins** Def conserved nucleotide sequence that acts as the BINDING SITE for proteins that initiate the replication



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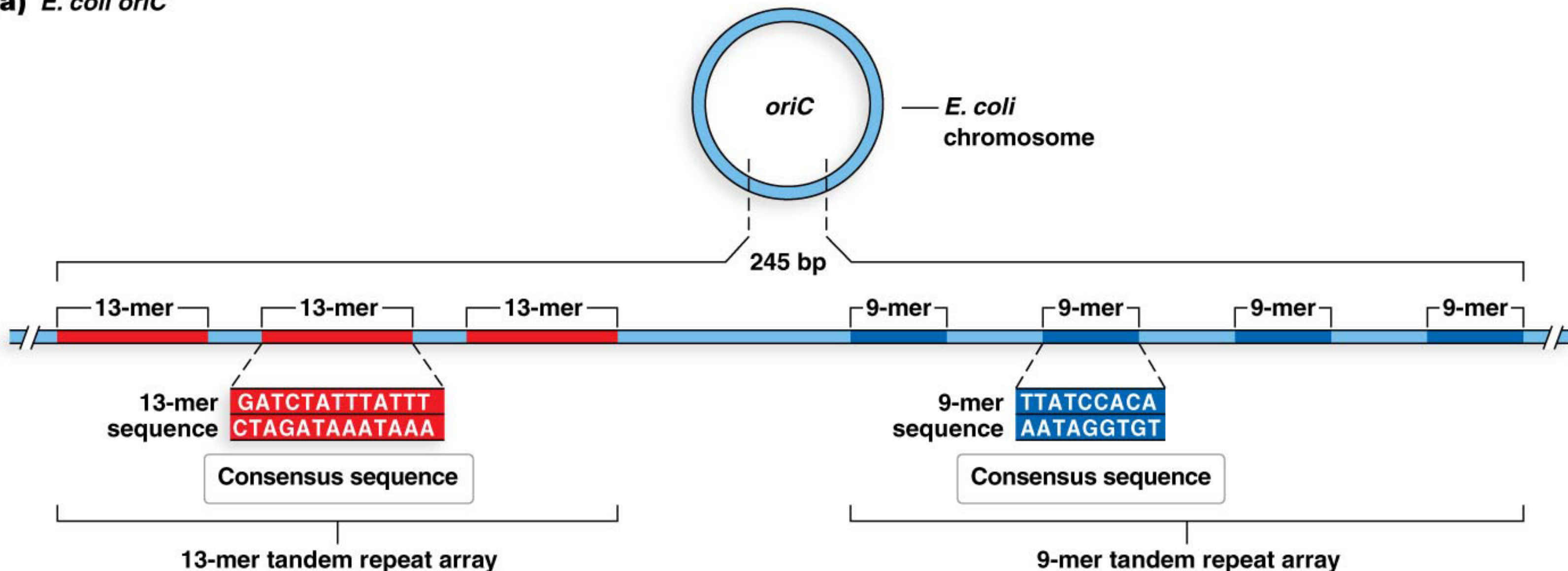
Bacterial Replication Origins

- Replication origins of bacterial different species have similar (conserved) but not identical sequences
- *ori*C is subdivided by **3 sequences made of 13bp** (13-mer) **and 4 of 9bp** (9-mer). These sequences of *oriC* are **conserved**—they play an essential role in replication– (sequence itself + spacing for proteins assemble)



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Eukaryote Replication Origins

- The multiple origins of replication are called **autonomously replicating sequences (ARS)**
- *Saccharomyces cerevisiae* (yeast) has the **most fully characterized origin-of-replication** sequences: ARS1

- Replication origins of other eukaryotes are less well characterized

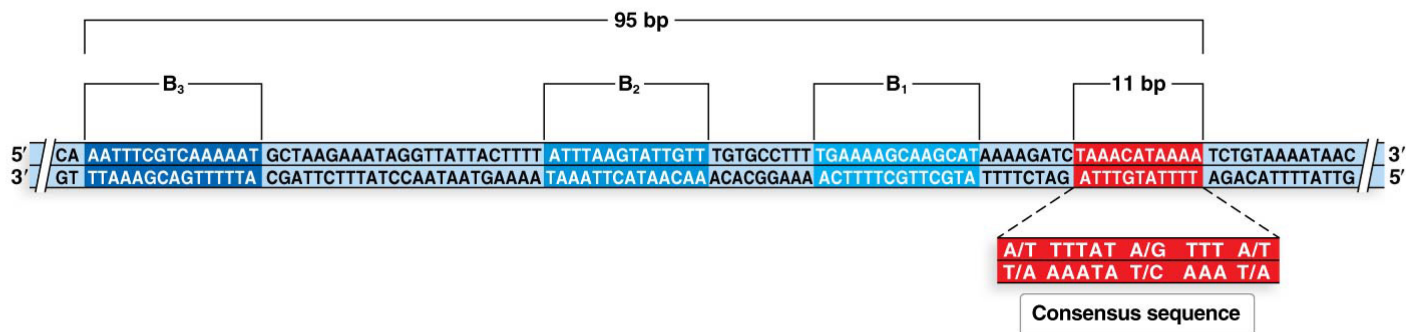

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Figure 7.15a Origin of replication sequences in *E. coli* and yeast.

(a) *E. coli oriC*

oriC
E. coli chromosome
245 bp
13-mer
13-mer
13-mer
9-mer
9-mer
9-mer
9-mer
13-mer sequence
GATCTATTTATTT
CTAGATAAATAAA
Consensus sequence
9-mer sequence
TTATCCACA
AATAGGTGT
Consensus sequence
13-mer tandem repeat array
9-mer tandem repeat array

(b) *S. cerevisiae* autonomous replicating sequence 1 (ARS1)

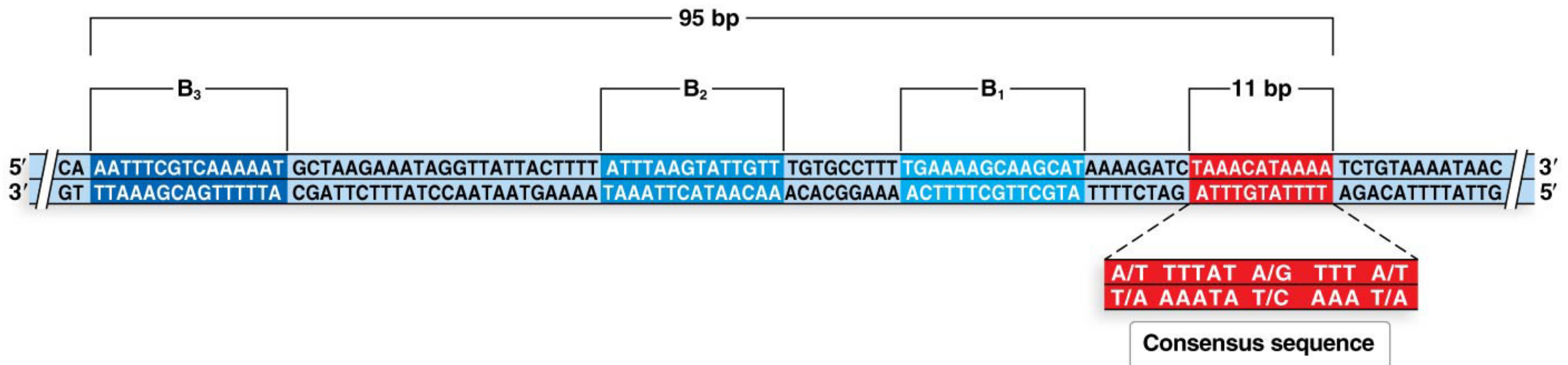

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Figure 7.16 Replication initiation at *oriC*, requiring DnaA, DnaB, and DnaC proteins.

- DnaA binds first, bends the DNA (at 9-mers seq.), and breaks hydrogen bonds in the A-T rich sequences (=13-mers seq.)
- DnaB is a **helicase** that uses ATP energy to break hydrogen bonds of complementary bases to separate the strands and unwind the helix
- DnaB is carried to the DNA helix by DnaC

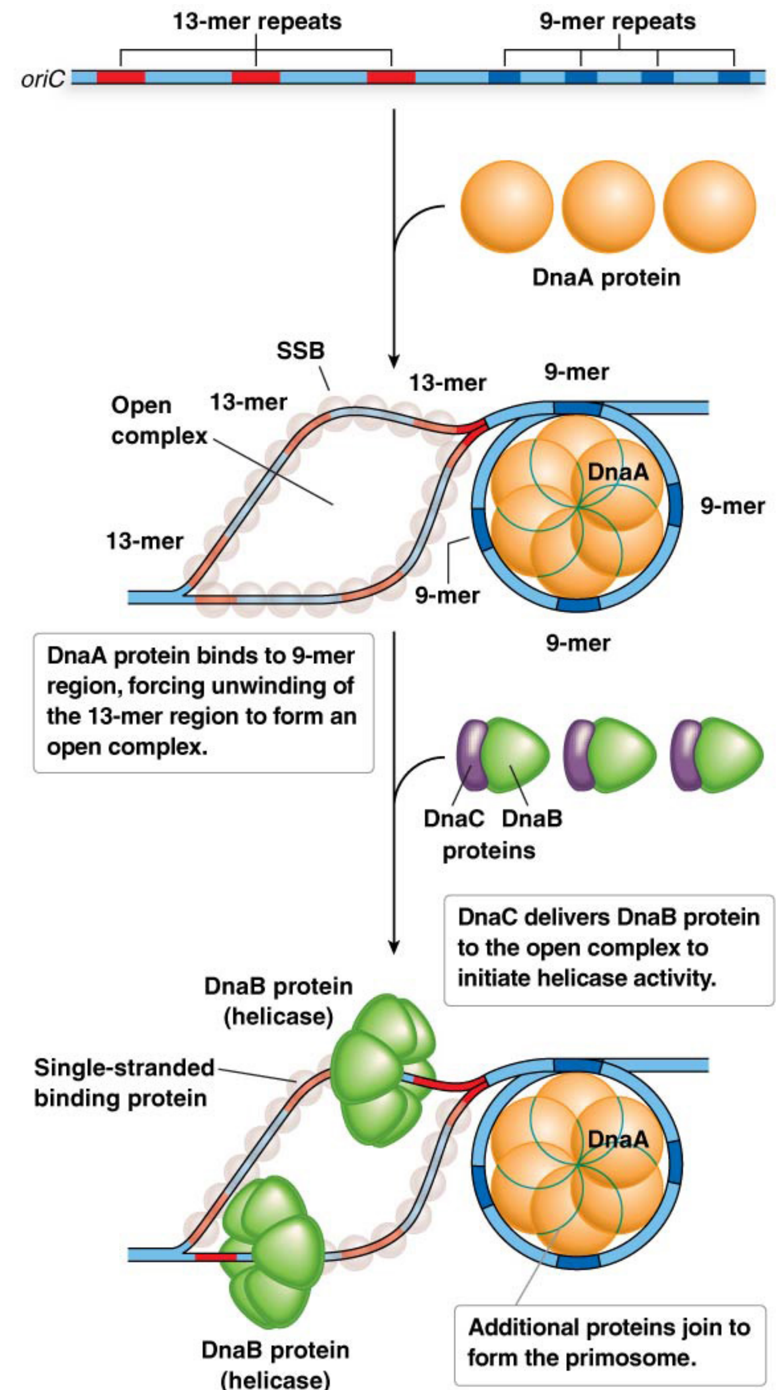

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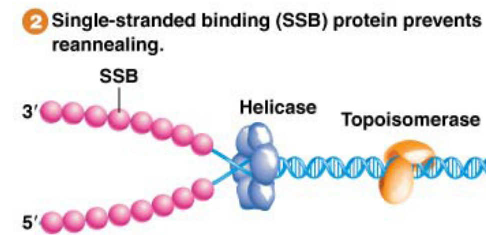
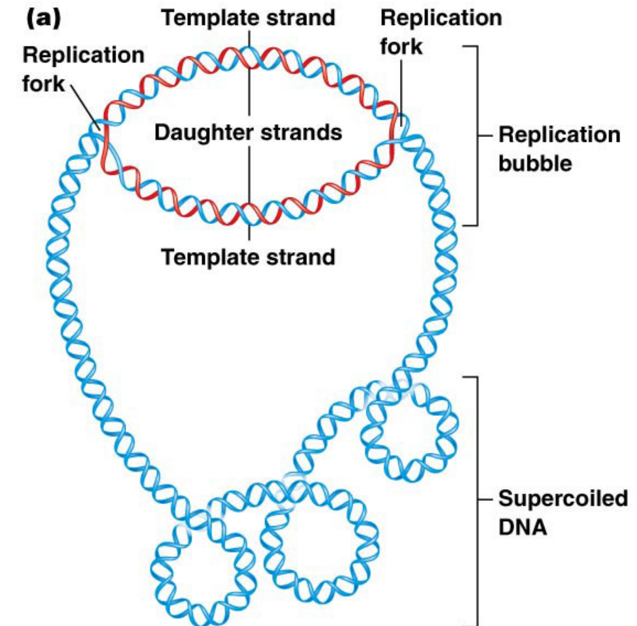
Figure 7.17 DNA supercoiling in bacteria (a) and its cutting and release by topoisomerase (b).

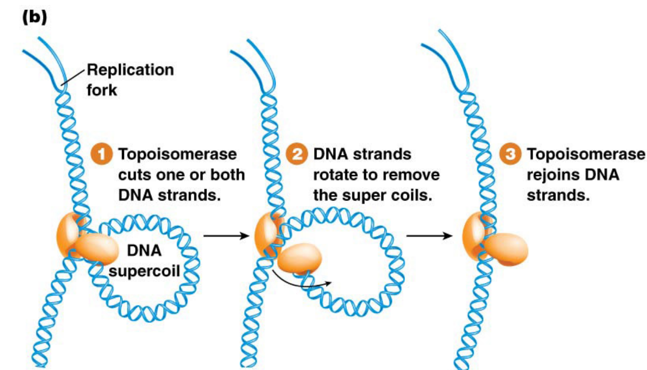
- The unwound DNA strands are more stable **reannealing: it is prevented by** single-stranded binding protein (SSB)



- Unwinding of circular chromosomes will create torsional stress, potentially leading to **supercoiled DNA ____ Fig.a)**



- Enzymes called **topoisomerases catalyze controlled cleavage** and rejoining of DNA that prevents overwinding_______ Fig.b)



- In eukaryotes: linear DNA rotates!

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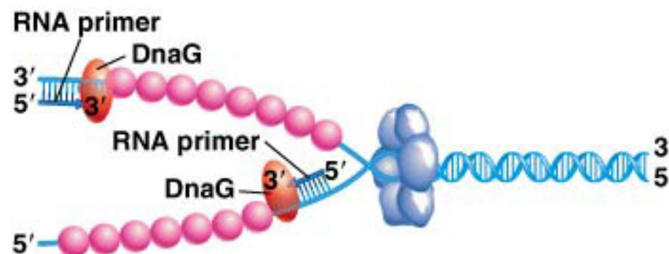
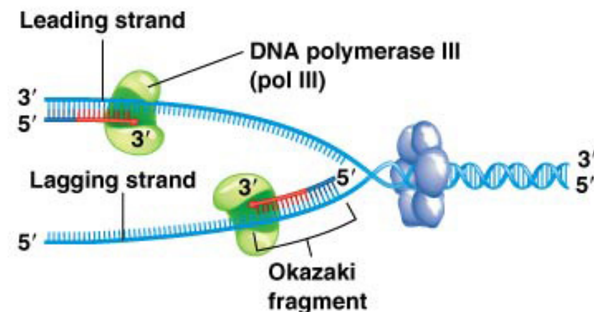
RNA Primers Are Needed for DNA Replication

- **<u>To start the elongation process:</u>** DNA polymerase III elongates DNA strands by adding nucleotides to the 3′ end of a pre-existing strand: the overall direction is 5’➔3’
- **DNA polymerase III cannot initiate DNA strand synthesis on its own… needs a 3’OH-free**
- **<u>IT IS NECESSARY A PRIMER!!</u>** : RNA polymerase (called **primase or DNAG)** synthesizes RNA primers
- Primase (RNA-pol/DNAG) joins DnaA, DnaB, and DnaC at *oriC* (it is used RNA-pol enzyme because no primer is necessary)





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- DO YOU THINK THAT THESE PRIMERS MADE WITH RNA CAN STAY in the DNA FOREVER???

- OBVIOUSLY NO… WE WILL SEE HOW A SPECIALIZED ENZYME CALLED **DNA polymeraseI will remove** the RNA_primers (~12bp and 3'-OH-free)

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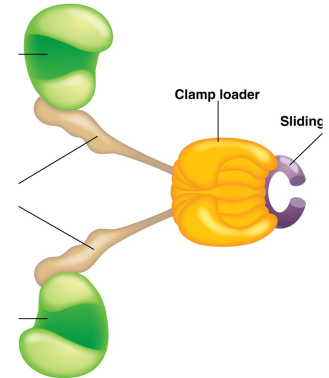
Continuous and Discontinuous Strand Replication

- The parental strands are the template and daughter DNA strands are synthesized by the DNA **polymerase III (pol III) holoenzyme** (*E. coli*)



- ***Holoenzyme:*** refers to a **multiprotein complex** in which a **core enzyme** is associated with the **additional components**

- **Replisome:** many of the enzymes participating in the DNA replication are part of this large complex. The **replisome contains two copies of pol III** and it is **located at the replication fork**

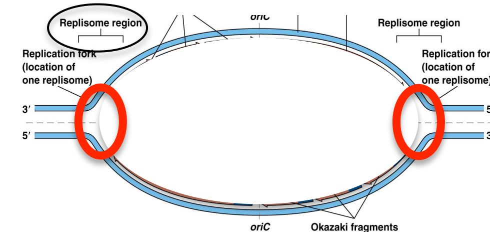


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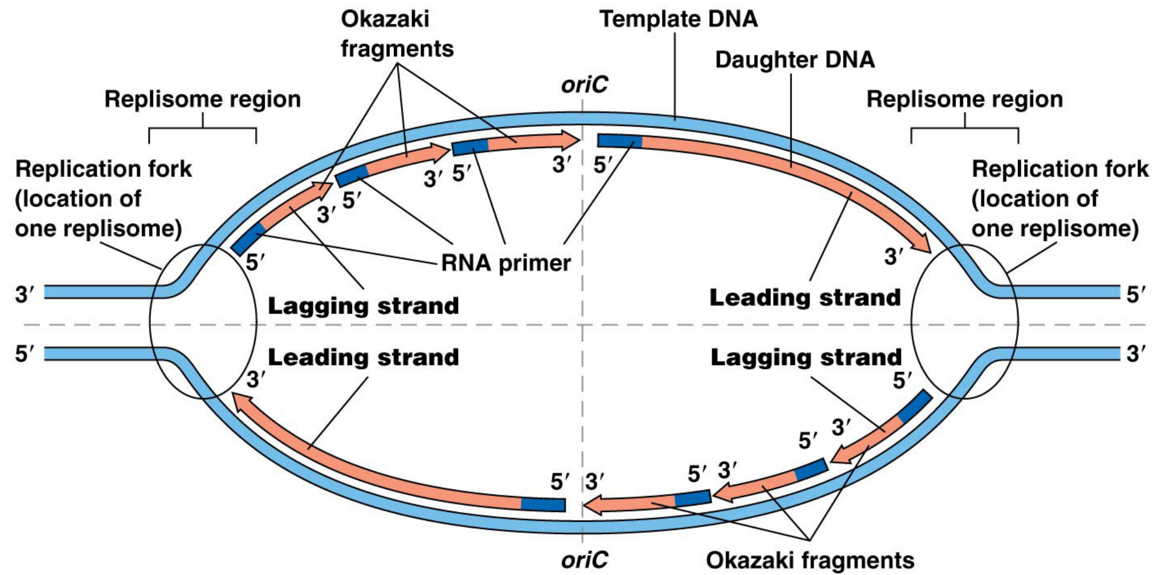
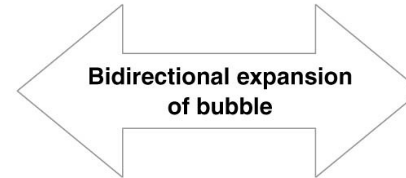
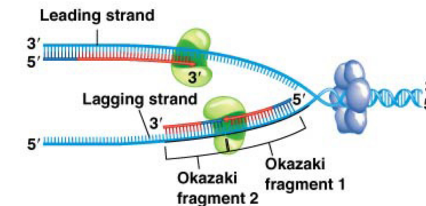
Figure 7.18 The replication bubble: Leading and Lagging Strand Synthesis

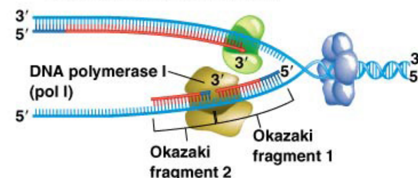
- **Leading strand:** one copy of **pol III synthesizes** one daughter strand **continuously** in the same direction as fork progression



- **Lagging strand:** the other copy of **pol III** elongates the daughter strand **discontinuously**, in the opposing direction to fork progression, via short segments (**Okazaki fragments**)







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DO YOU REMEMBER THE RNA-PRIMERS????

IT'S TIME TO REMOVE THEM

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Figure 7.19 Removal and replacement of RNA primer nucleotides and ligation of Okazaki fragments in *E. coli*.

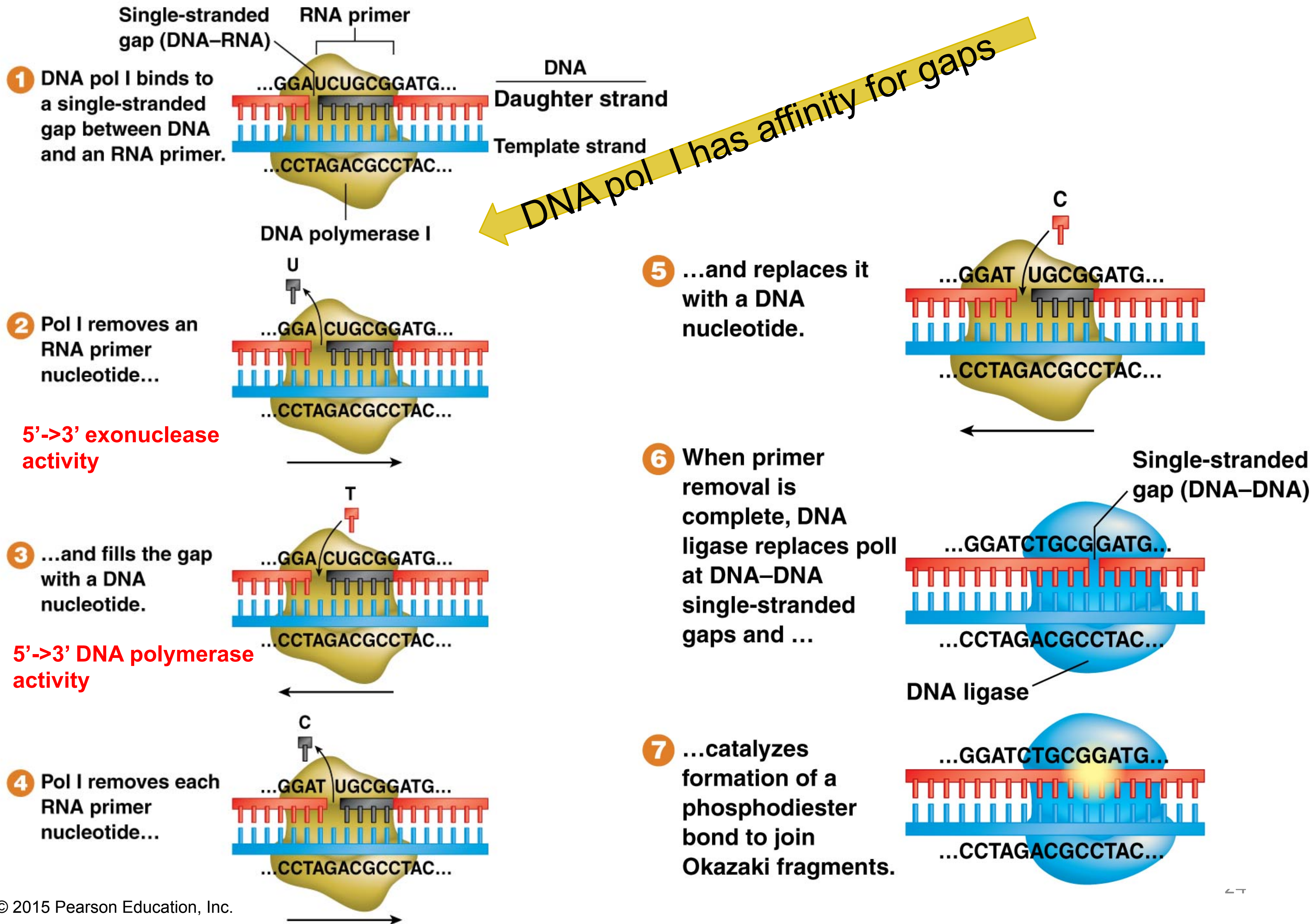

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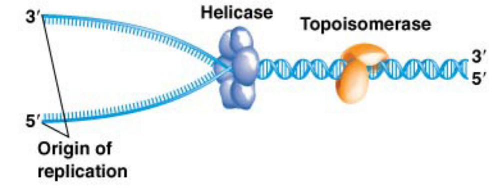
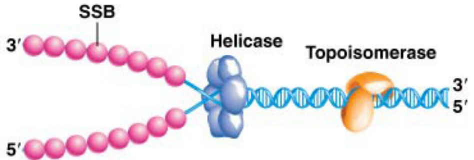
Figure 7.14 DNA Replication.

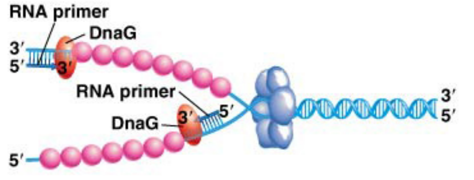
1 Helicase breaks hydrogen bonds. Topoisomerase relaxes super-coiling.



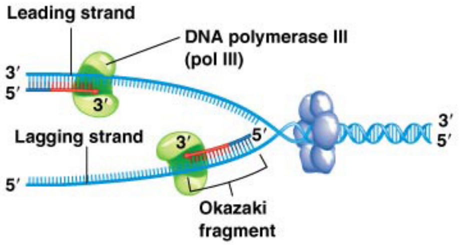
2 Single-stranded binding (SSB) protein prevents reannealing.



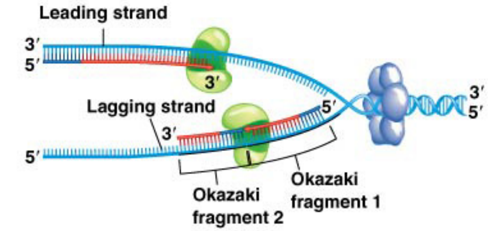
3 DnaG synthesizes RNA primers.



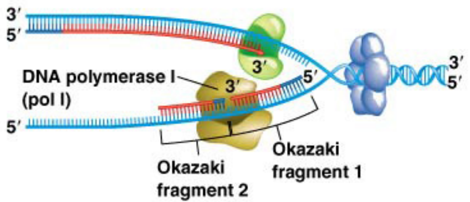
4 DNA polymerase III synthesizes daughter strand.



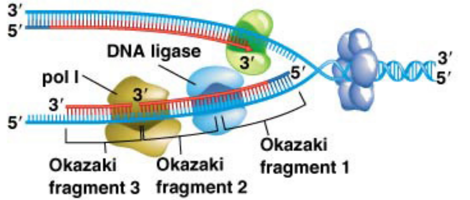
5 DNA polymerase III elongates the leading strand continuously and the lagging strand discontinuously.



6 DNA polymerase I removes and replaces nucleotides of the RNA primer.



7 DNA ligase joins Okazaki fragments.



Protein	DNA topoisomerase	Helicase (DnaB)	SSB	Primase	DNA pol III	DNA pol I	DNA ligase
Icon							
Role	Relaxes supercoiling	Unwinds the double helix	Prevents reannealing of separated strands	Synthesizes RNA primers	Synthesizes DNA	Removes and replaces RNA primer with DNA	Joins DNA segments

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Simultaneous Synthesis of Leading and Lagging Strands

- The DNA pol III holoenzyme contains 11 protein subunits, with the two pol III core polymerases each tethered to a different copy of the tau (τ) protein
- The tau proteins are joined to a protein complex called the **clamp loader**; two additional proteins form the **sliding clamp**

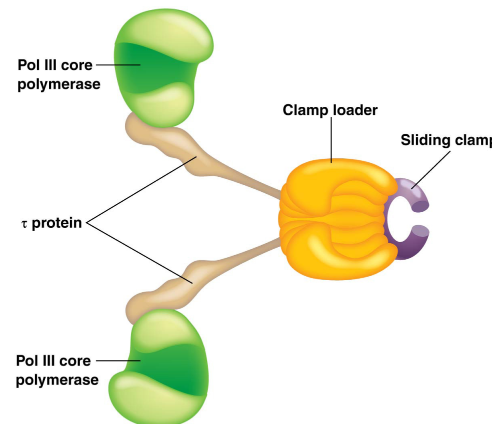

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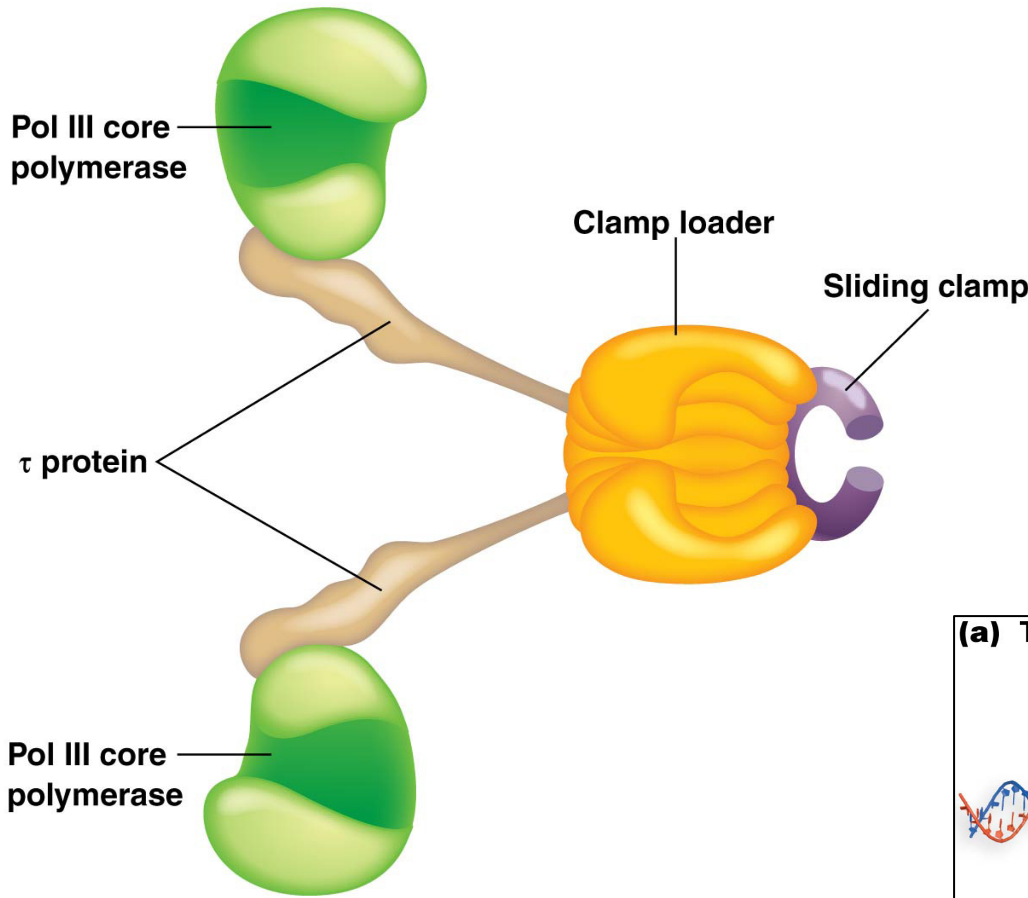
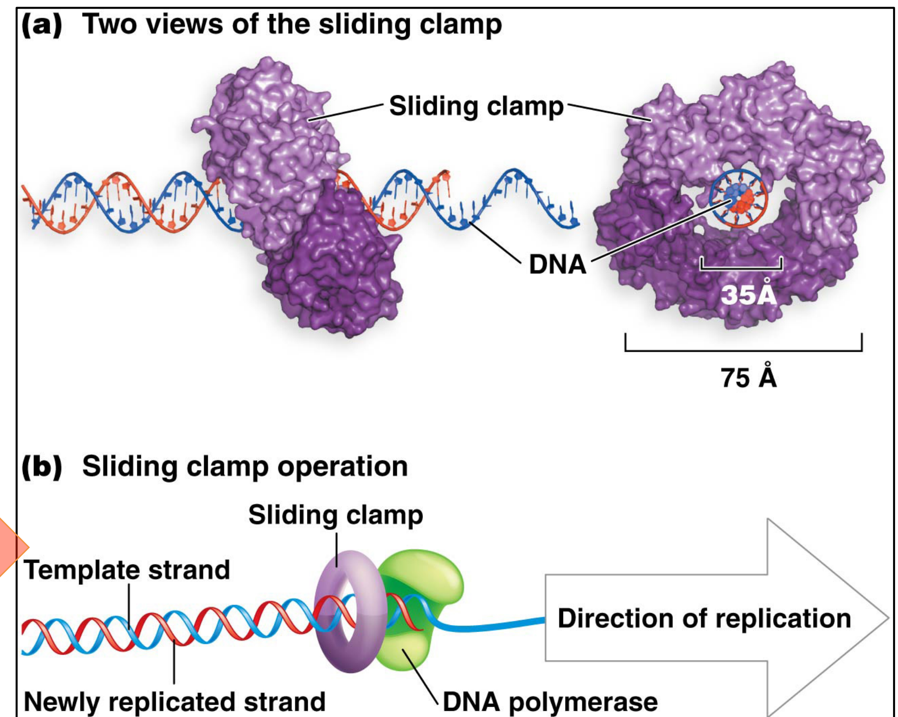
Figure 7.20 DNA polymerase III holoenzyme: the processivity issue and the sliding clamp



- The **DNA pol III holoenzyme** contains the **two pol III core**, each joined to the **clamp loader** by **two** copies of the **tau** (τ) (TOTAL= protein11 protein subunits) and two additional proteins form the **sliding clamp**



The high processivity secret is an association between sliding clamp and core-polymerase III

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The Trombone Model of DNA Replication:
A. Kornberg 1960s one enzyme-two strands

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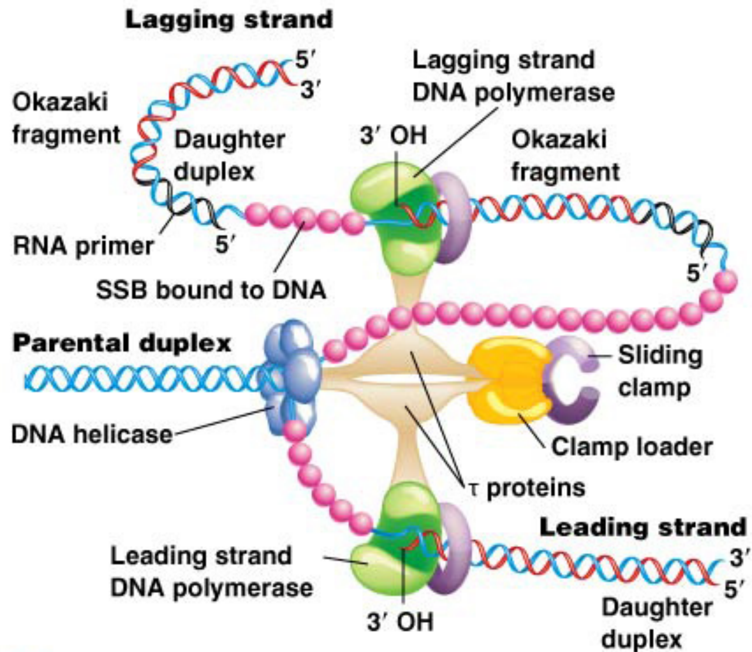
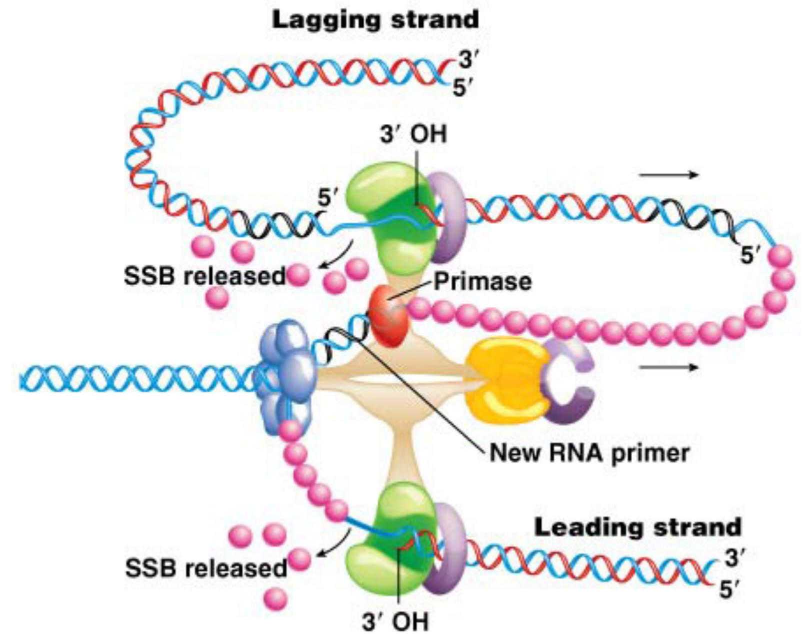
Figure 7.22 The Trombone Model of DNA Replication:
A. Kornberg 1960s one enzyme-two strands



1 DNA helicase denatures the parental duplex, and SSB coats leading strand and lagging strand templates. The leading strand DNA pol III–sliding clamp complex synthesizes the leading strand continuously. The lagging strand pol III–sliding clamp complex synthesizes an Okazaki fragment.



2 Primase binds the lagging strand template and synthesizes a new RNA primer. SSB is released ahead of leading strand and lagging strand synthesis, and ahead of RNA primer synthesis.

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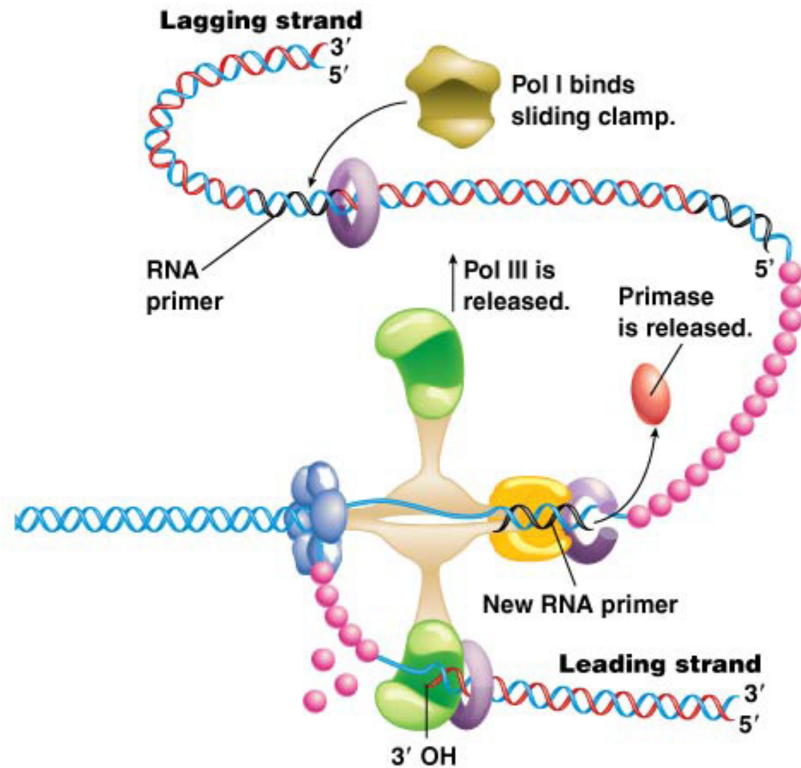
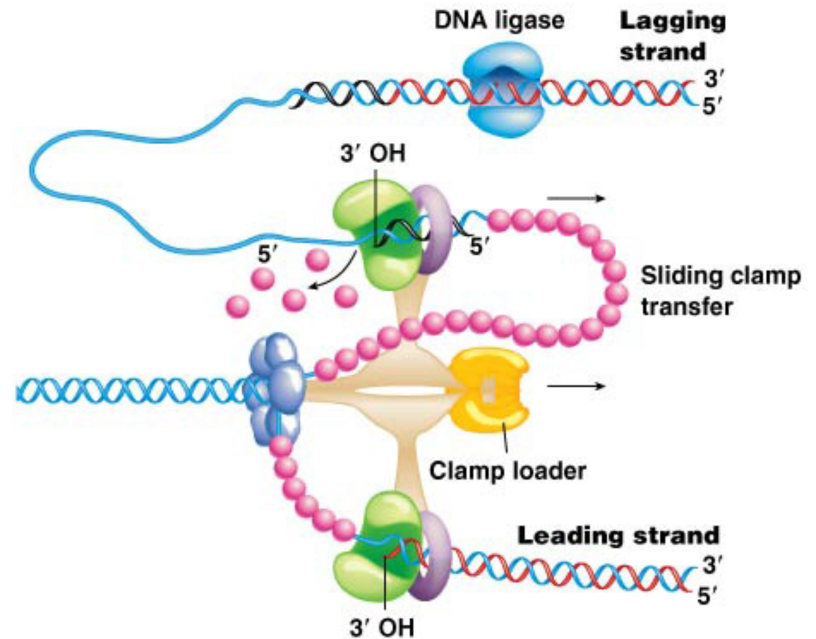


3 Lagging strand DNA pol III completes synthesis of an Okazaki fragment and is released by the sliding clamp. A DNA pol I replaces pol III to begin removal of the RNA primer and replacement of RNA nucleotides by DNA nucleotides.



4 DNA ligase joins Okazaki fragments. The clamp loader places a new sliding clamp near the 3′ end of the RNA primer on the newly primed lagging strand. Lagging strand DNA pol III binds the sliding clamp and initiates synthesis of a new Okazaki fragment.

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Eukaryotic DNA Polymerases

- Eukaryotes have many more DNA polymerases than bacteria
- DNA polymerase α carries out synthesis of RNA primers
- Polymerase δ and polymerase ε carry out lagging and leading strand synthesis, respectively
- Each interacts with **proliferating cell nuclear antigen** (**PCNA**), which functions as the sliding clamp

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Table 7.1 DNA Replication Proteins and Enzymes

Table 7.1 DNA Replication Proteins and Enzymes

Domain			
Bacteria	**Eukarya**	**Archaea**	**Role in Replication**
DnaA	Orc1–6	Orc1/Cdc6	Replication-origin recognition
DnaB, DnaC	Cdc6/Cdt1	Orc1/Cdc6	Helicase activity
	Mcm2–7	Mcm	
	GINS	GINS	Helicase recruitment
DnaG	Primase/pol α	Primase	Primer synthesis
DNA Pol III	DNA pol δ	Pol B	DNA synthesis
	DNA pol ε	Pol D	
DNA pol I	RPA	RPA	DNA synthesis
RnaseH	FEN1/DNA2	FEN1/DNA2	Primer removal
β (sliding) clamp	PCNA	PCNA	DNA polymerase progression
Tau protein	RFC	RFC	Replication fork progression

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Table 7.2 Properties of Selected Bacterial, Eukaryotic, and Archaeal DNA Polymerases

Table 7.2 Properties of Selected Bacterial, Eukaryotic, and Archaeal DNA Polymerases

Polymerase	Functions
Bacterial polymerases	
DnaG	RNA primer synthesis
I	RNA primer removal, proofreading, mutation repair
III	DNA replication, proofreading
Eukaryotic polymerases	
Primase/α	Primer synthesis and lagging strand synthesis
δ	Lagging strand synthesis, proofreading, DNA mutation repair
ε	Leading strand synthesis, proofreading, DNA mutation repair
Archaea polymerases	
Primase	Primer synthesis
PolB	DNA synthesis
PolD	DNA synthesis

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DNA Proofreading (= correction)

- DNA replication is very accurate, mainly because DNA polymerases undertake **DNA proofreading**, to correct occasional errors

- Errors in replication occur $1/10^9$ (billion) nucleotides in *E. coli*

- Proofreading ability of DNA polymerase enzymes is due to a **3′-to-5′ exonuclease activity**

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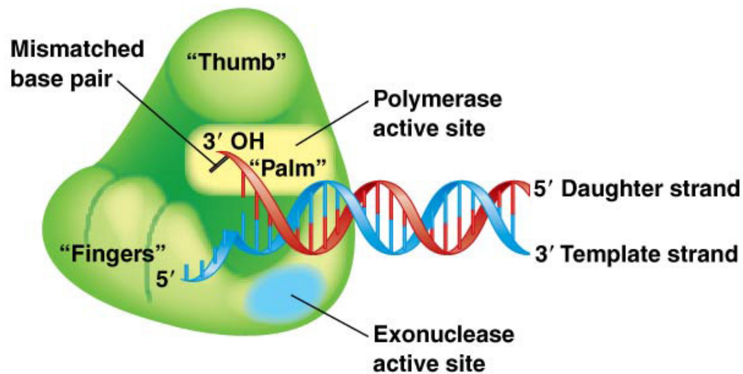
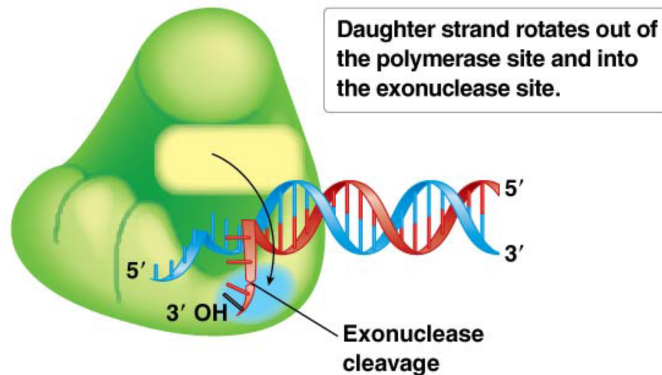
Figure 7.23 DNA polymerase proofreading activity: the example of pol I and pol III

(a) DNA polymerase error



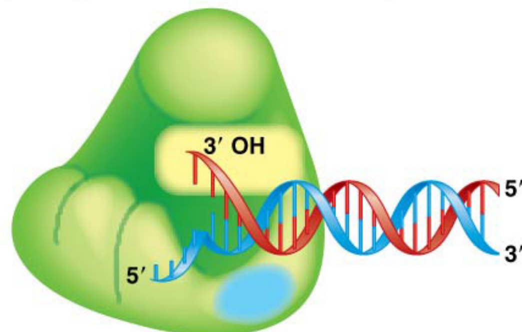
Mismatches give not proper hydrogen bond length

(b) Exonuclease removal of mismatched base pair



The displacement causes a rotation of the new strand into the exonuclease site

(c) Daughter strand resumes DNA synthesis



Several nucleotides are removed (also the erroneous one) and the strand rotate back to the polymerase site

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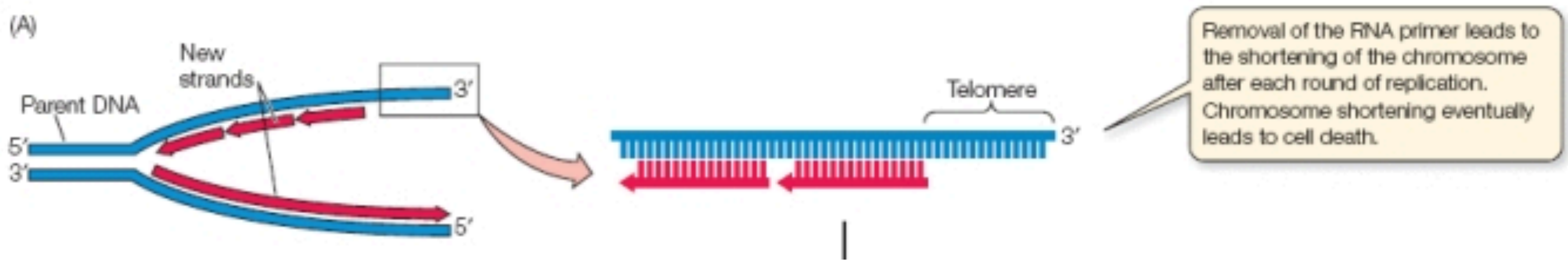
Finishing Replication

- **The leading strand of linear chromosomes can be replicated to the end**

- The lagging strand requirement for a primer means that **lagging strands cannot be completely replicated: the primer of RNA at the very end of the chromosome will be removed and never replaced by DNA**



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- This problem is resolved by repetitive sequences at the ends of chromosomes, called **telomeres, THE ENZYME CATALYZING THIS REACTION IS TELOMERASE (ribonucleoprotein)**

- These repeats ensure that incomplete chromosome replication does not affect vital genes

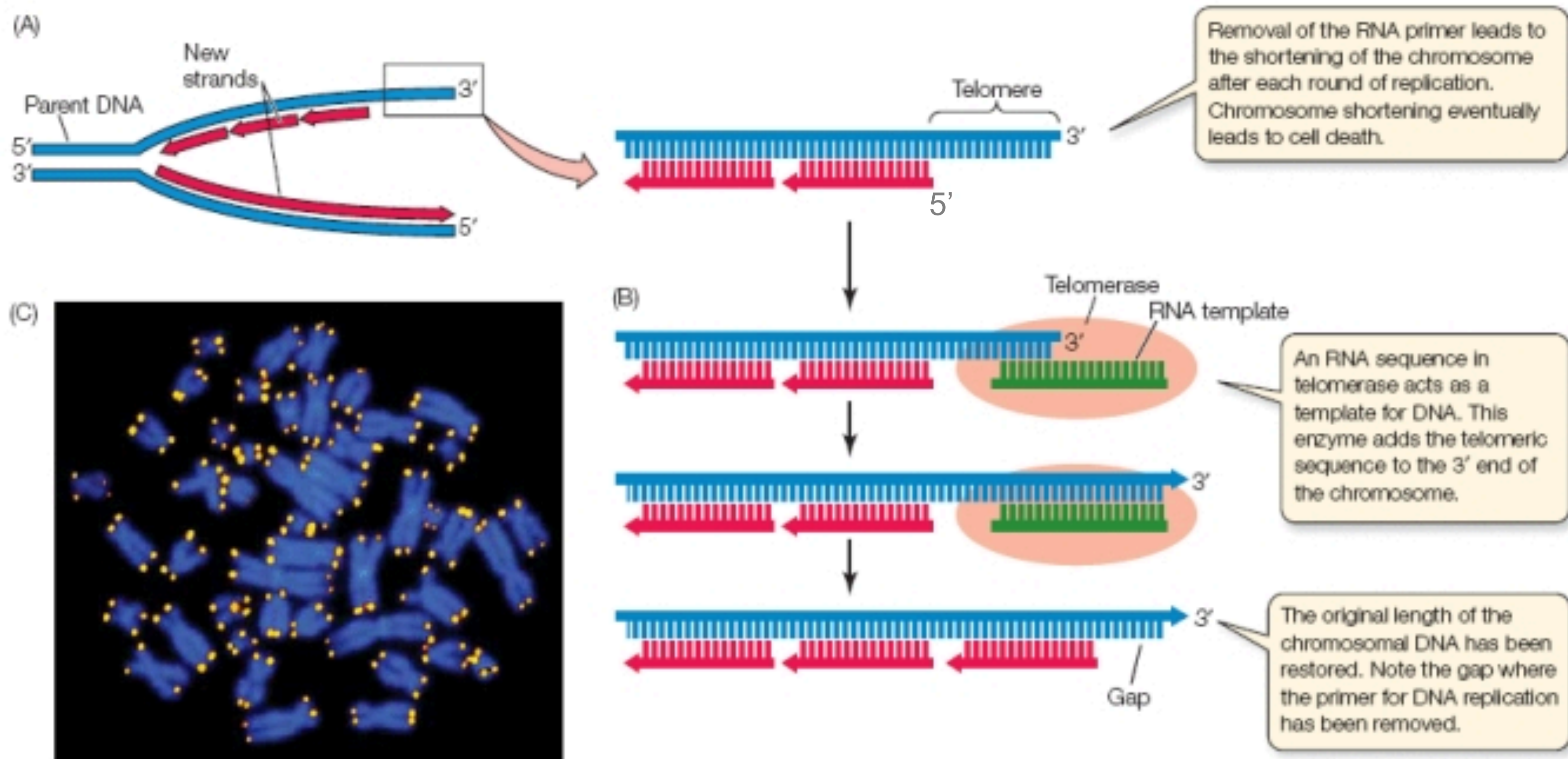

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Figure 7.24 Loss of DNA at telomeres.

- Telomeres are synthesized by the ribonucleoprotein **telomerase**

- **The RNA in telomerase** is complementary to the telomere repeat sequence and **acts as a template for addition of DNA**

- Telomere sequences in most organisms are quite similar (in homo telomere sequence is 5'-TTAGGG-3' and the telomeric RNA molecule is 3'-AAUCCC-5')

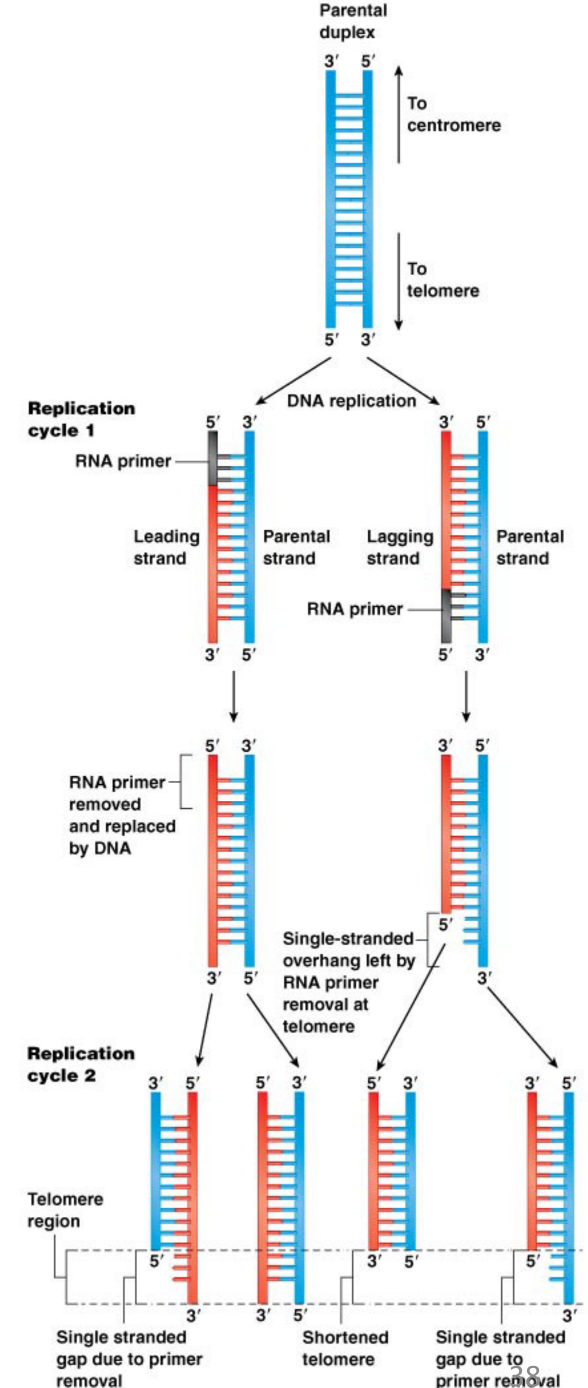

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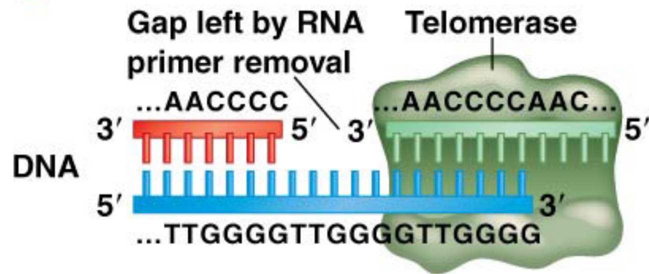
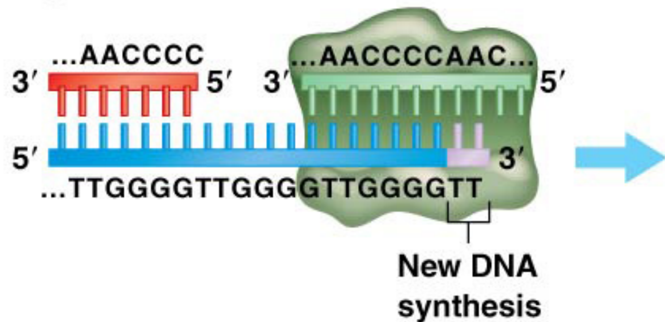
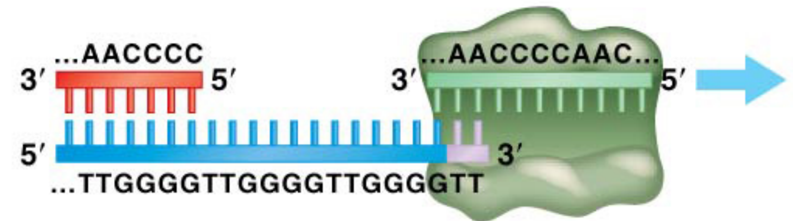
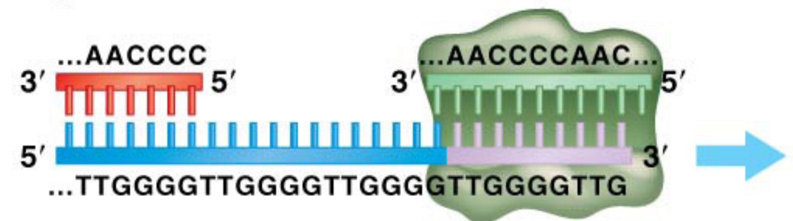
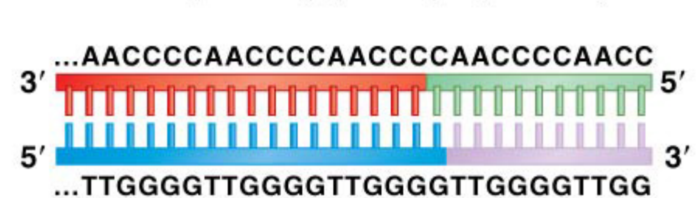
Figure 7.25 Telomerase synthesis of repeating telomeric sequence.











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Importance of Telomerase Activity

- Mice that are homozygous for loss-of-function mutations of the ***TERT*** (***telomerase* reverse *transcriptase*; encodes telomerase**) **gene** give rise to developmental defects
- The defects are detected in the fourth and fifth generations, due to loss of telomere length with each generation
- By the fourth and fifth generations, shortening of chromosomes is critical and apoptosis is induced

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Telomeres, Aging, and Cancer

- **Telomere length is important** for chromosome stability, cell longevity, and reproductive success
- Telomerase is **active in** germ-line cells and some stem cells in eukaryotes
- Differentiated somatic cells and cells in culture have virtually no telomerase activity; such cells have limited life spans. Telomerase is **normally turned off in somatic cells**
- **Reactivation** of telomerase can lead to aging cells that continue to proliferate, a feature of many types of **cancer**
- *TERT* reactivation is one of the most common **mutations** in cancers of all types

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MOLECULAR ASSAYS

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7.5 Molecular Genetic Analytical Methods Make Use of DNA Replication Processes

- Molecular biologists have used their understanding of DNA replication to develop new methods of molecular analysis

- Two widely used methods include:
  - *polymerase chain reaction* (*PCR*)
  - *dideoxynucleotide DNA sequencing*

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The Polymerase Chain Reaction

- The **polymerase chain reaction** (**PCR**) is an automated version of **DNA replication** that produces millions of copies of a short target DNA segment
- PCR requires:
  - A double-stranded DNA template containing the target sequence to be amplified
  - A supply of the four DNA nucleotides
  - A heat-stable DNA polymerase (*Taq*, is isolated from *Thermus aquaticus*)
  - Two different single-stranded DNA primers (starting point for *Taq* polymerase to add nucleotides)
  - A buffer solution

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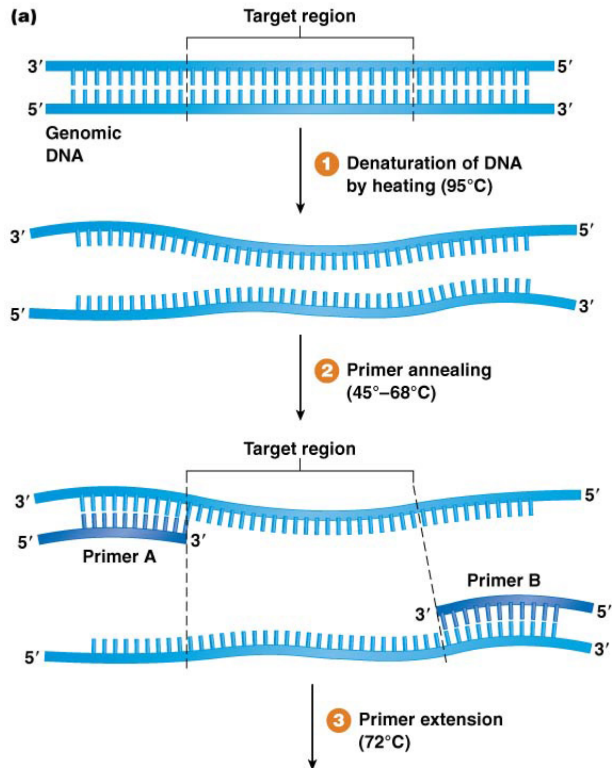
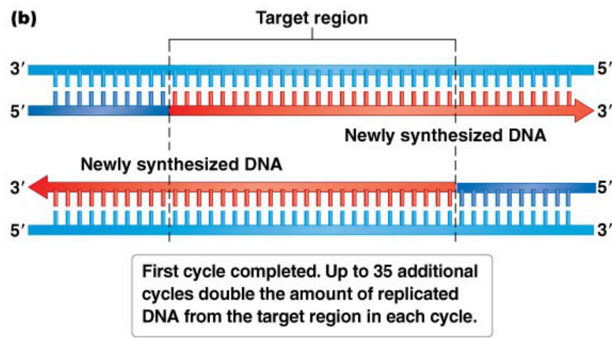
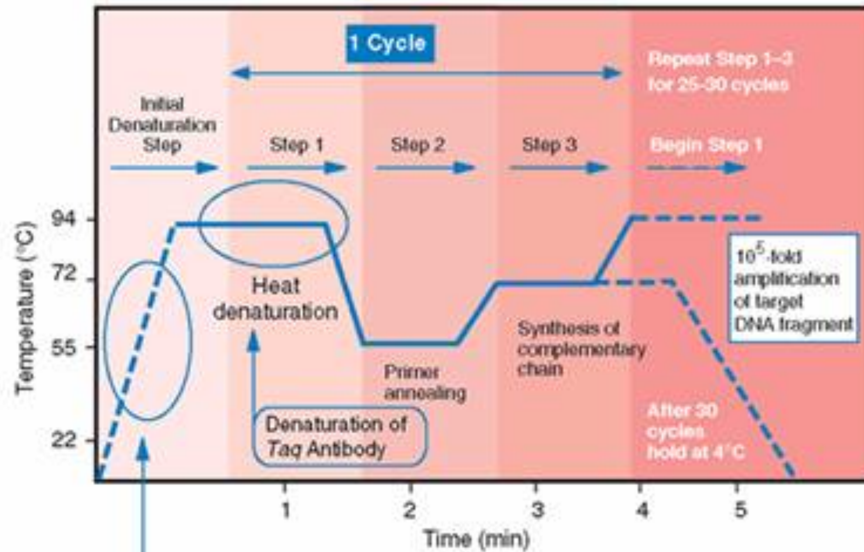
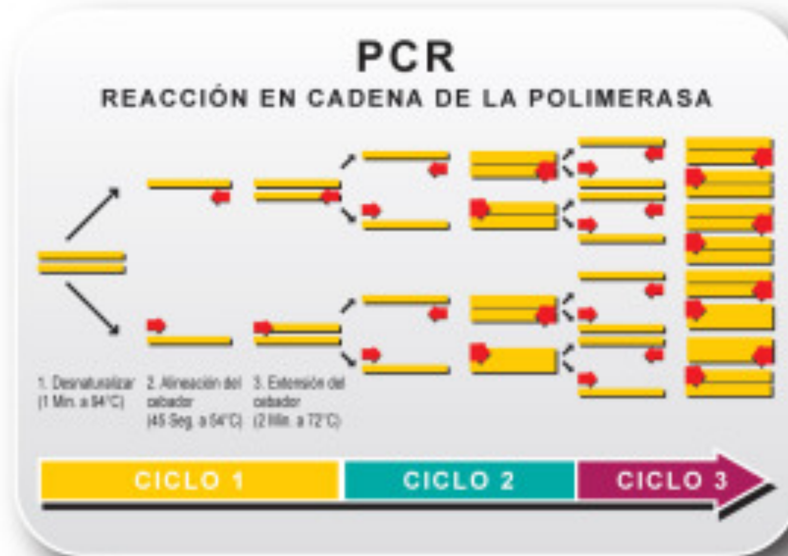
Figure 7.26 Polymerase chain reaction (PCR).









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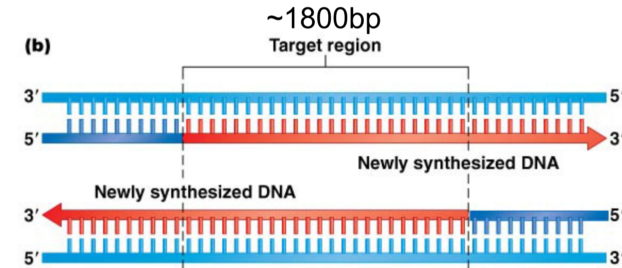
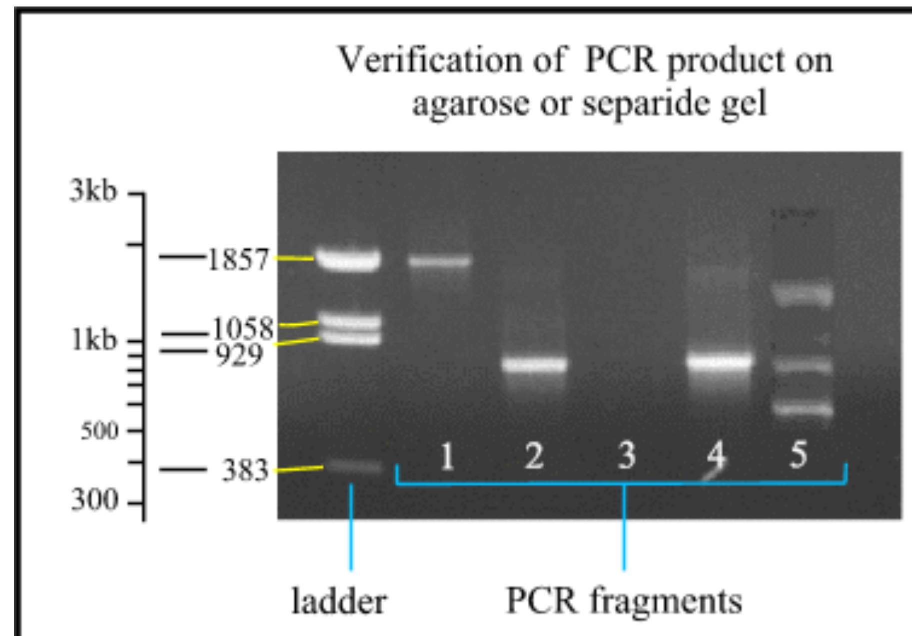
Separation of PCR Products

- **Amplified DNA fragments are separated** from the rest of the reaction mixture **by gel electrophoresis**
- PCR product sizes are measured in base pairs (bp)





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Dideoxynucleotide DNA Sequencing

- Method to know the precise sequence of bases

- The most famous DNA-sequencing protocol is by Sanger in 1977 (di-deoxy nucleotide method)
- Sanger Sequencing requires:
  - DNA polymerase
  - (dNTPs)
  - a small amount of one **dideoxynucleotide** (**ddNTP**), which lacks a 3′-OH group

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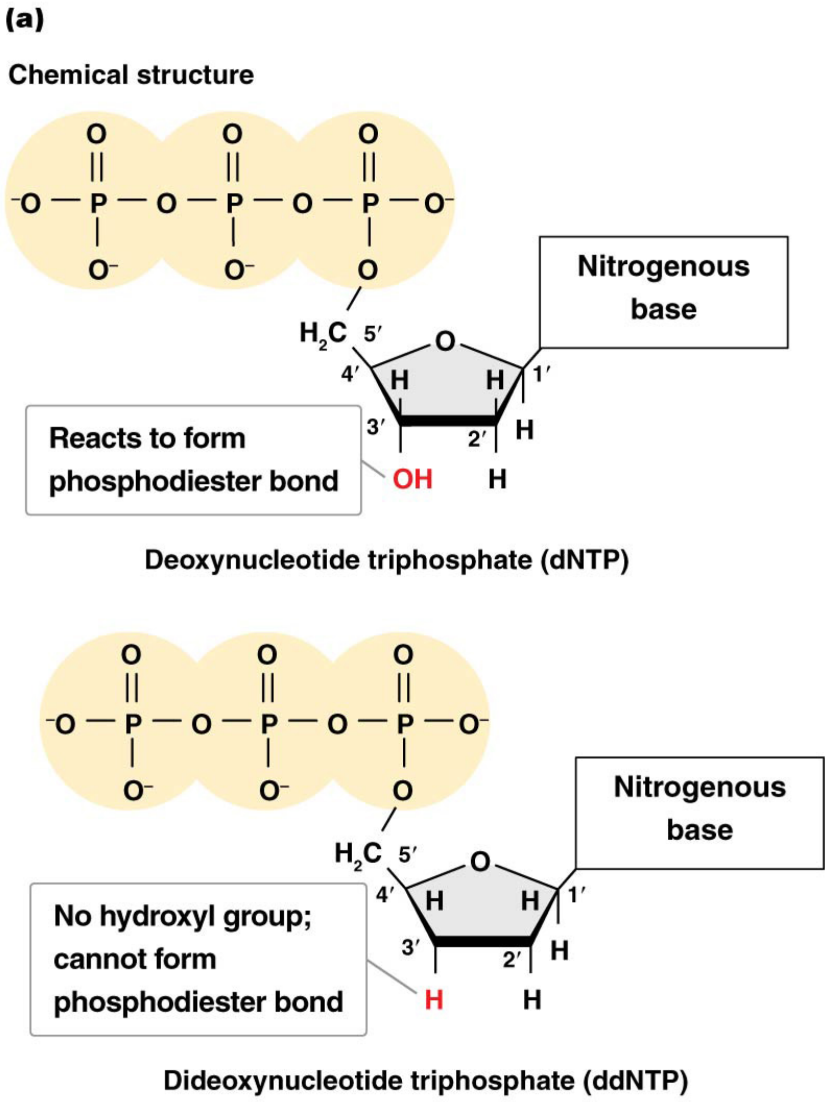
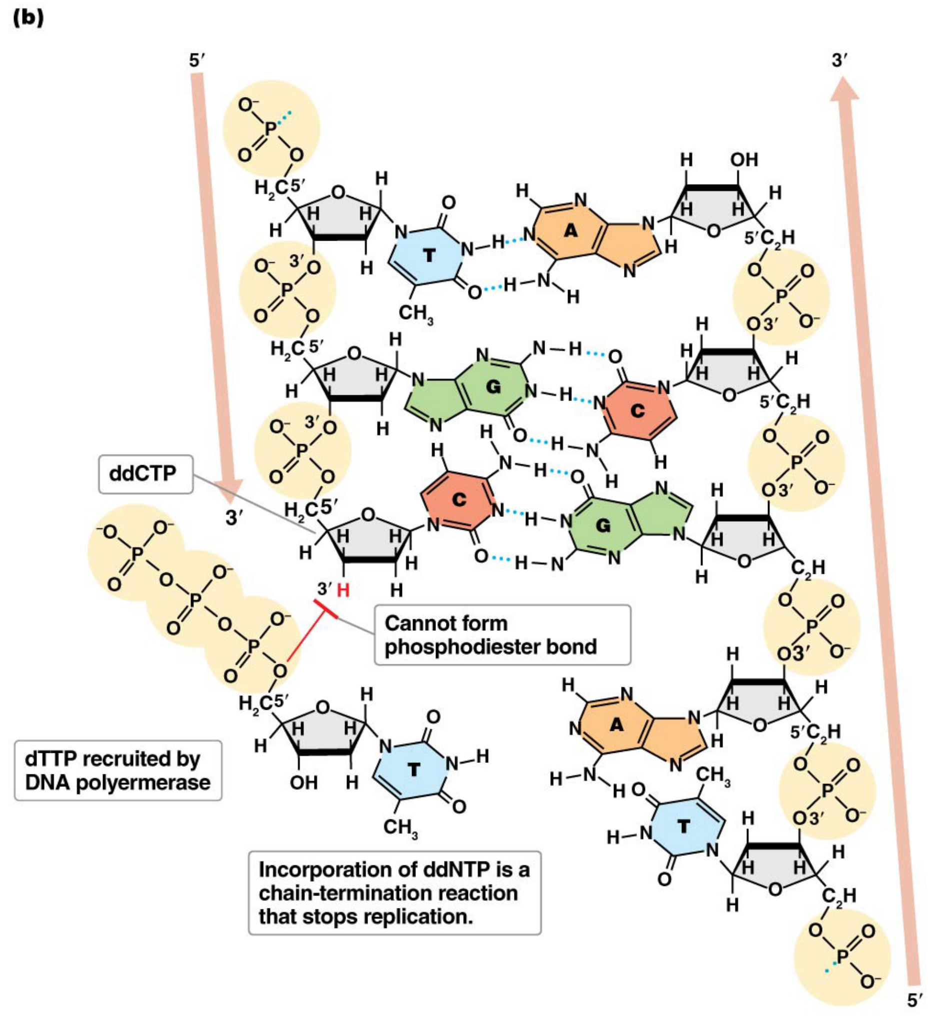
Figure 7.28a Nucleotides used in DNA sequencing reactions.





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Strand to be sequenced

Strand to be sequenced

Strand to be sequenced

Strand to be sequenced

ddG ddA ddT ddC

G A T C

Length of fragment

30
29
28
27
26
25
24
23
22
21

Termination by dideoxy-

GACGCTGCGA
GACGCTGCG
GACGCTGC
GACGCTG
GACGCT
GACGC
GACG
GAC
GA
G

3′

5′

This sequence is complement to DNA template strand

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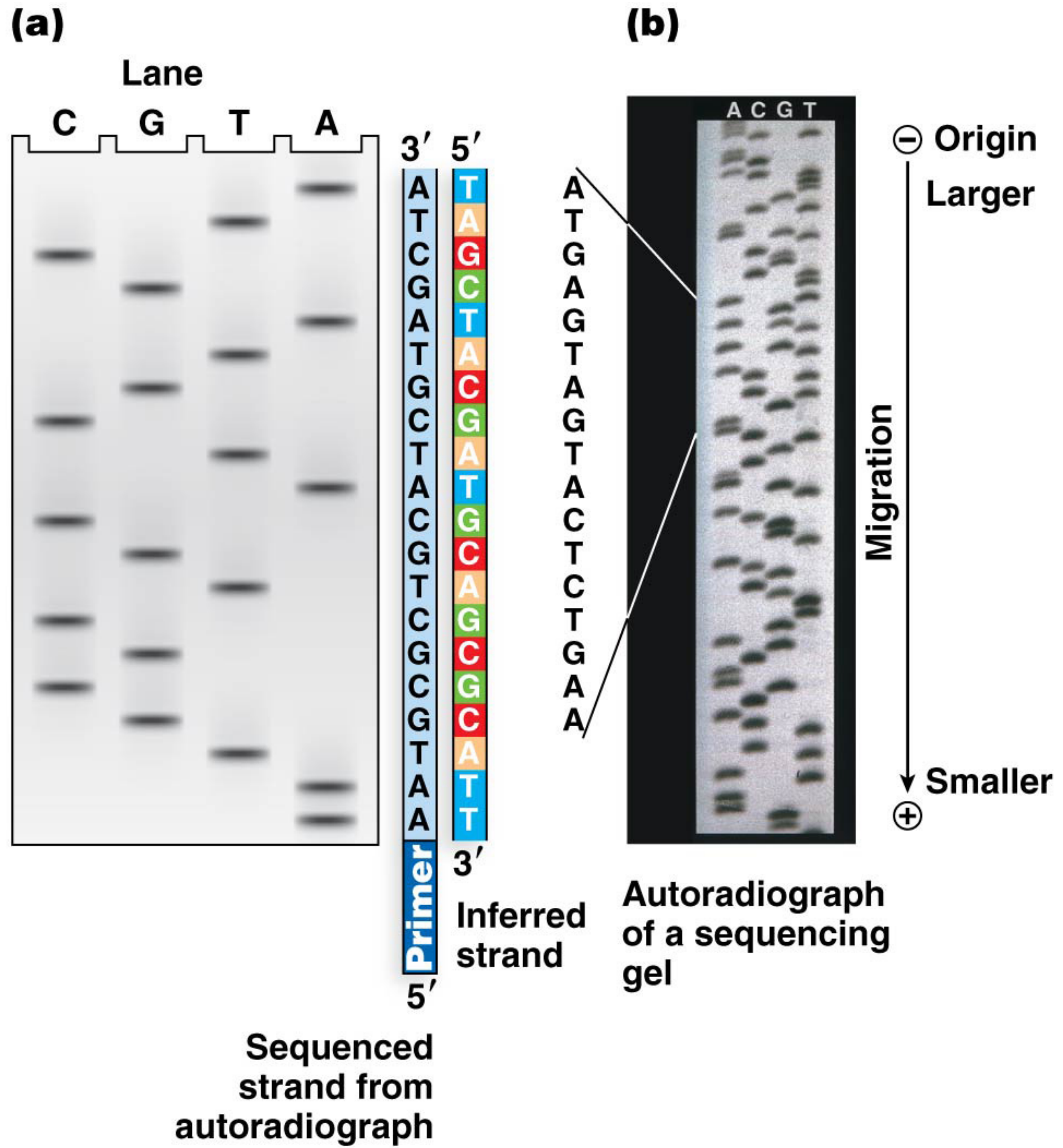
Figure 7.30 Interpretation of a DNA sequencing gel.



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