Lecture 25 (11/16/20)

TODAY

- Reading: Ch25; 990-995, 1005-1012
  Ch26; 1035-1038
  Ch27; 1077-1085, 1092-1096

- Problems: Ch25 (text); 1-3,5-7,10,13-16,12
  Ch25 (study-guide: applying); 1,4
  Ch25 (study-guide: facts); 3,4,6
  Ch26 (text); 1,2,5,6,12
  Ch26 (study-guide: applying); 1
  Ch26 (study-guide: facts); 1,3,5
  Ch27 (text); 6,7,9
  Ch27 (study-guide: applying); 1,3,5

NEXT

- Reading: Ch27; 1088-1091, 1096-1108

- Problems: Ch27 (text); 5,8,10,11,13,16,17
  Ch27 (study-guide: applying); 2,3
  Ch27 (study-guide: facts); 4,6

Nucleic Acids

A. The 4 S's of Nucleotides & NA
B. Structure of the Information
C. Recombinant DNA: Biochemical Basis of Biotechnology
   1. Restriction enzymes, DNA ligase
   2. Vectors and Inserts to make recombinant DNA (rDNA)
   3. Transformation of hosts
   4. Selection of transformants
   5. Expression
   6. Site-directed mutagenesis

D. Replication
   1. Polymerases
   2. Fidelity
      a. Polymerase recognition
      b. Exonuclease
      c. Mis-match repair
      d. Post-replication repair
         i. Direct reversal
         ii. Base excision
         iii. Nucleotide excision
   3. Sequence determination
   4. PCR

E. Transcription
   1. RNA polymerase
   2. fidelity

F. Translation
   1. Genetic code
   2. tRNA

DNA Replication

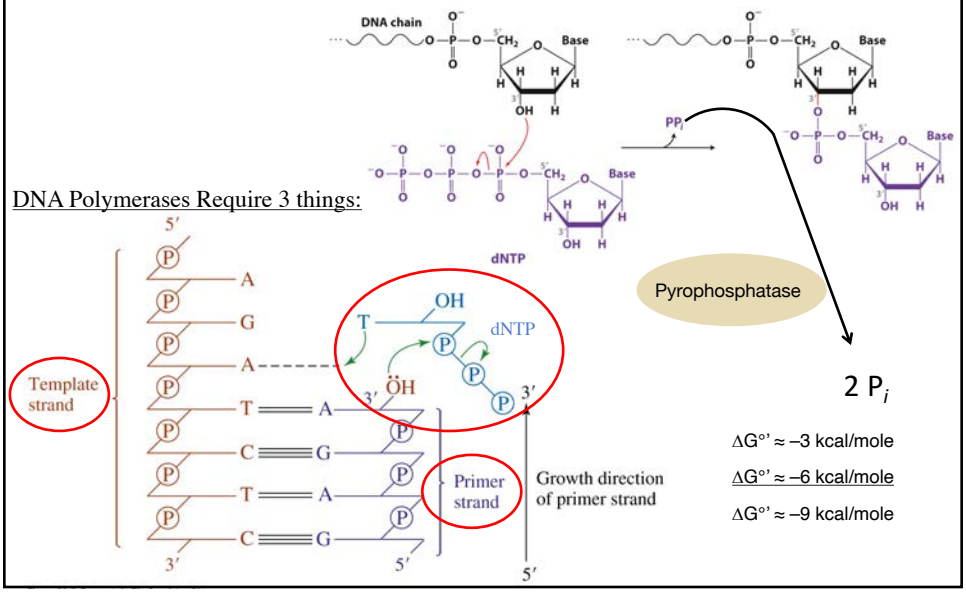
- DNA Replication
  - Polymerases
    - Activities
    - Structure
    - Mechanism
- Replication/polymerase fidelity
  - Exonuclease Activities
  - Mismatch repair
  - Post-replication repair
    - Reversal
    - Base Excision repair
    - Nucleotide Excision repair
- DNA Sequencing
- PCR

DNA Replication

Nucleic acid function: Central Dogma

DNA Replication

Addition at 3' end



DNA Polymerases Require 3 things:

Comparison of Polymerases

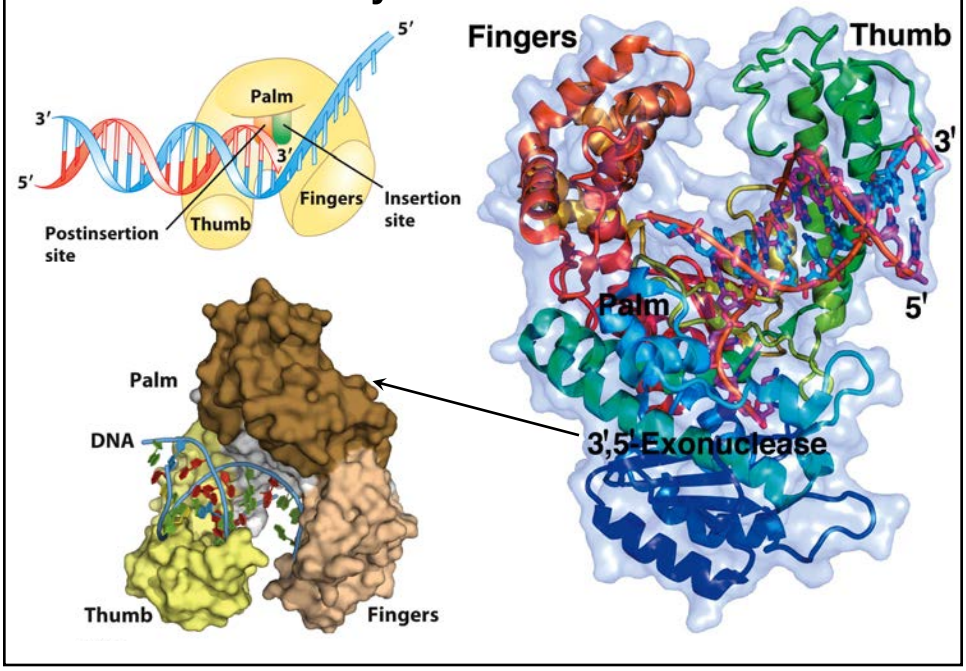
TABLE 25-1 Properties of *E. coli* DNA Polymerases

	Pol I	Pol II	Pol III
Mass (kD)	103	90 (α_4)	130 *
Molecules/cell	400	?	10–20
Turnover number[a]	20	5	1000
Structural gene	*polA*	*polB*	*polC*
Conditionally lethal mutant	+	–	+
Polymerization: 5′ → 3′	+	+	+
? → Exonuclease: 3′ → 5′	+	+	+
? → Exonuclease: 5′ → 3′	+	–	–
Processivity	100	10,000	500,000 [b]

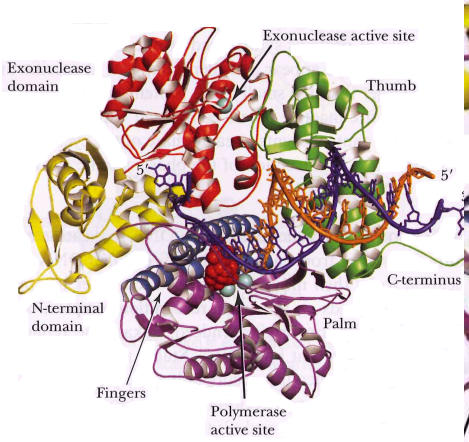
[a]dNTP polymerized sec^{-1} at 37 °C. [b]not including Okasaki fragments

*In a complex with 10 proteins (26 subunits) of >900 kD. Core is trimer of α, θ, ε

DNA Polymerase Structure

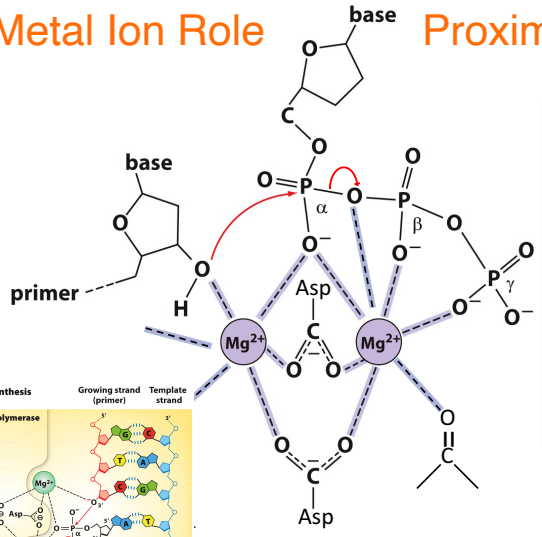


DNA/RNA polymerase mechanism

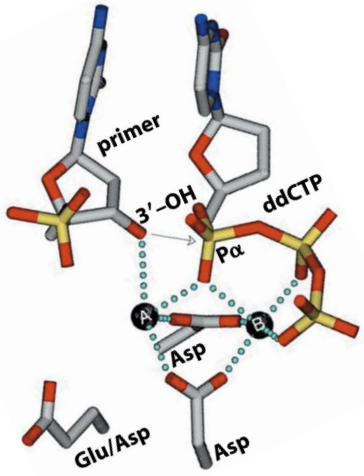


DNA/RNA polymerase mechanism

Metal Ion Role



Proximity Role



This is just the chemistry step, there are other more important enzymatic steps…

DNA Replication

- DNA Replication
  - Polymerases
    - Activities
    - Structure
    - Mechanism
- Replication/polymerase fidelity
  - Exonuclease Activities
  - Mismatch repair
  - Post-replication repair
    - Reversal
    - Base Excision repair
    - Nucleotide Excision repair
- DNA Sequencing
- PCR

Replication/polymerase Fidelity

Geometry of Base Pairing Accounts for High Fidelity

- Errors in replication for *E. coli*: $1/10^9 – 1/10^{10}$ bp
  - $3x10^6$ bp/genome x $1/10^{-10}$ mistakes/bp = 0.0003 mistakes/genome
  - 1 per 1,000–10,000 replications
- DNA polymerase active site excludes base pairs with incorrect geometry
  - At BOTH the insertion site and the post insertion site
  - BUT, DNA polymerases still insert wrong base 1/10,000 times.
  - Repair mechanisms fix these errors.

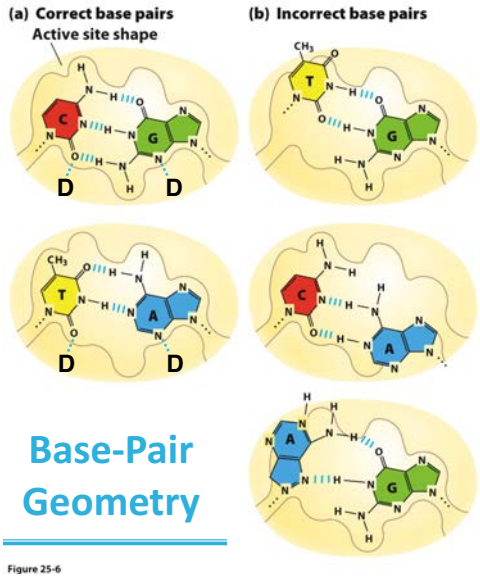
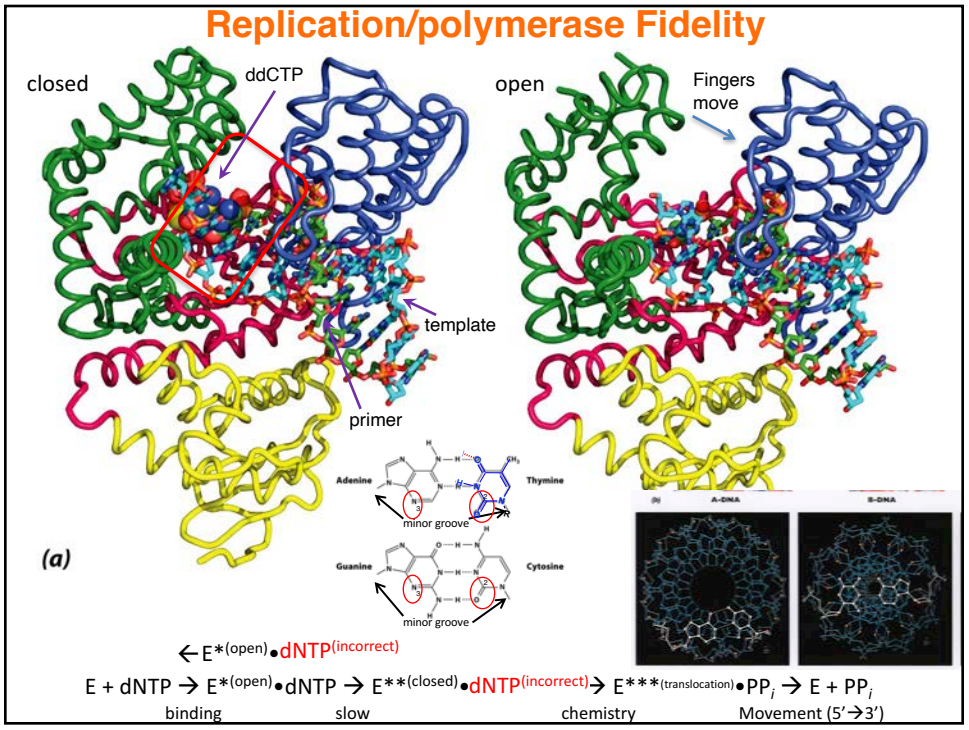
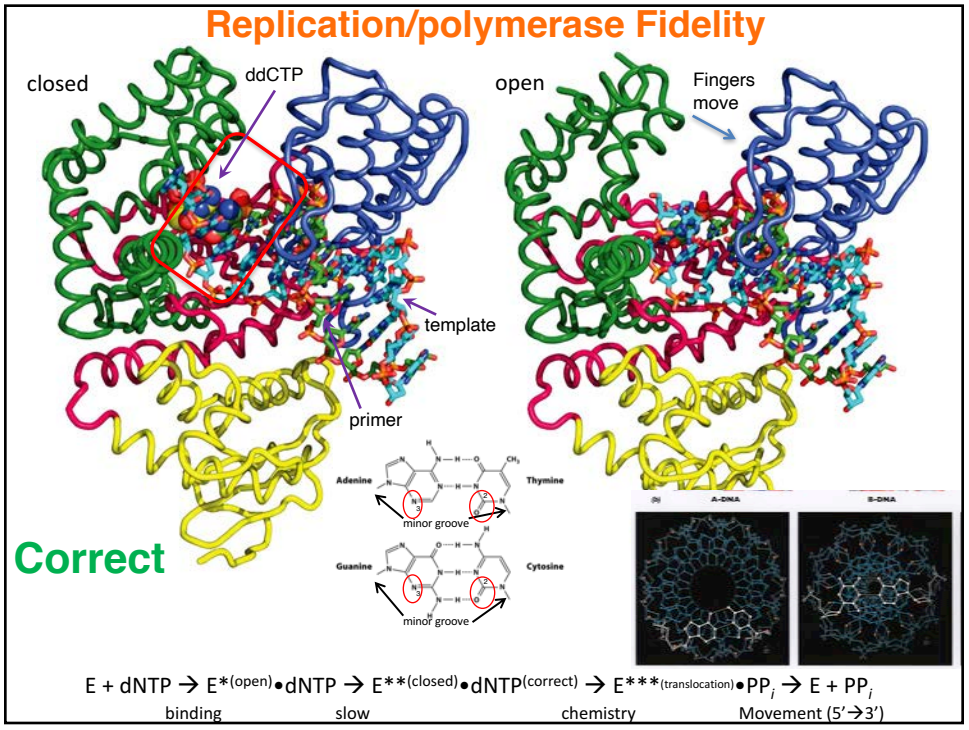
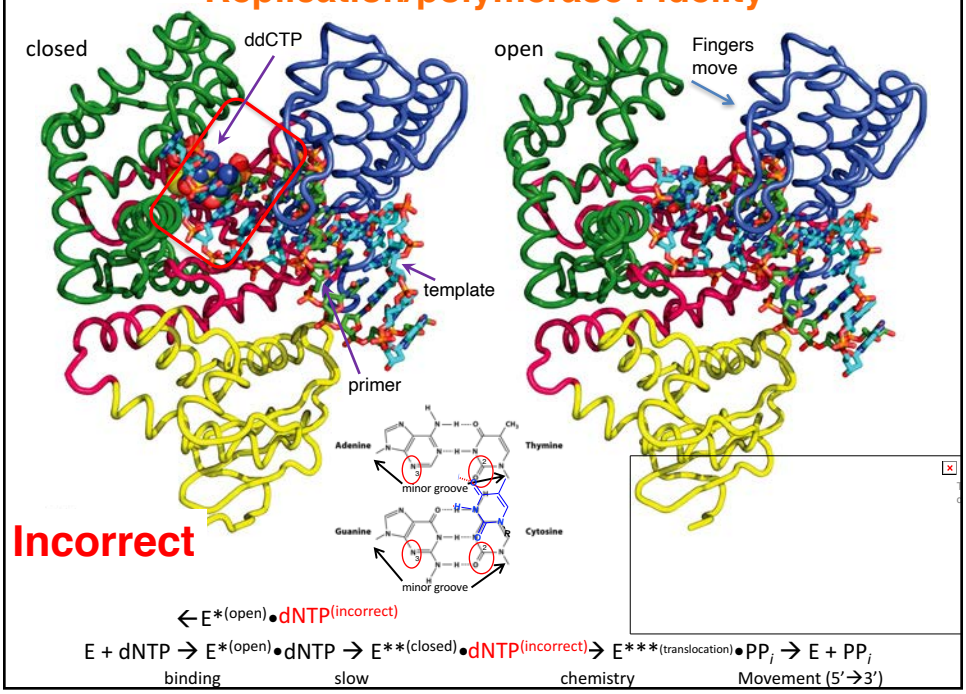


Figure 25-6


Replication/polymerase Fidelity
closed
ddCTP
open
Fingers move
template
primer
Adenine
Thymine
minor groove
Guanine
Cytosine
minor groove
(a)
A-DNA
B-DNA
←E*(open)•dNTP(incorrect)
E + dNTP → E*(open)•dNTP → E**(closed)•dNTP(incorrect) → E***(translocation)•PPi → E + PPi
binding
slow
chemistry
Movement (5'→3')


Replication/polymerase Fidelity
closed
ddCTP
open
Fingers move
template
primer
Adenine
Thymine
minor groove
Guanine
Cytosine
minor groove
Correct
A-DNA
B-DNA
E + dNTP → E*(open)•dNTP → E**(closed)•dNTP(correct) → E***(translocation)•PPi → E + PPi
binding
slow
chemistry
Movement (5'→3')

Replication/polymerase Fidelity



Incorrect

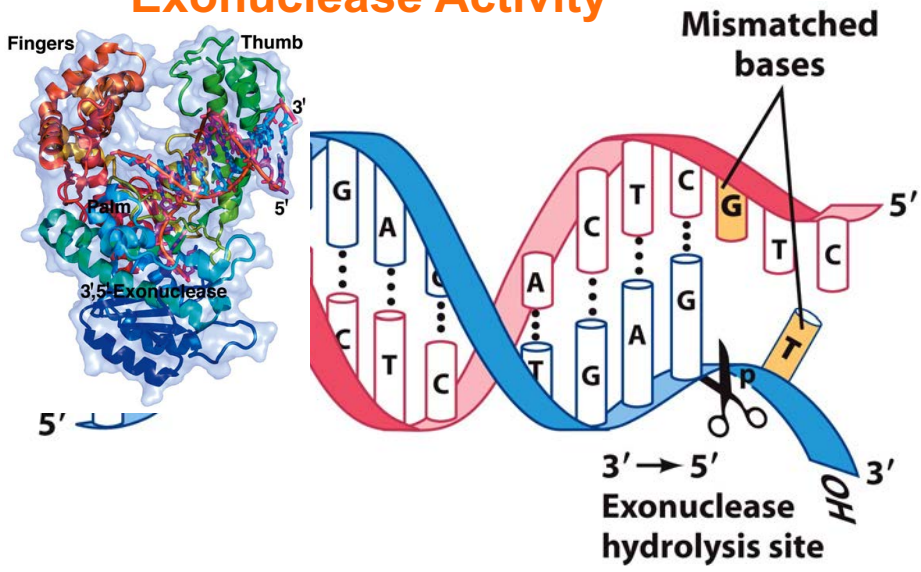
$\leftarrow E^{*(open)} \bullet dNTP^{(incorrect)}$

$E + dNTP \rightarrow E^{*(open)} \bullet dNTP \rightarrow E^{**(closed)} \bullet dNTP^{(incorrect)} \rightarrow E^{***(translocation)} \bullet PP_i \rightarrow E + PP_i$

binding slow chemistry Movement (5′→3′)

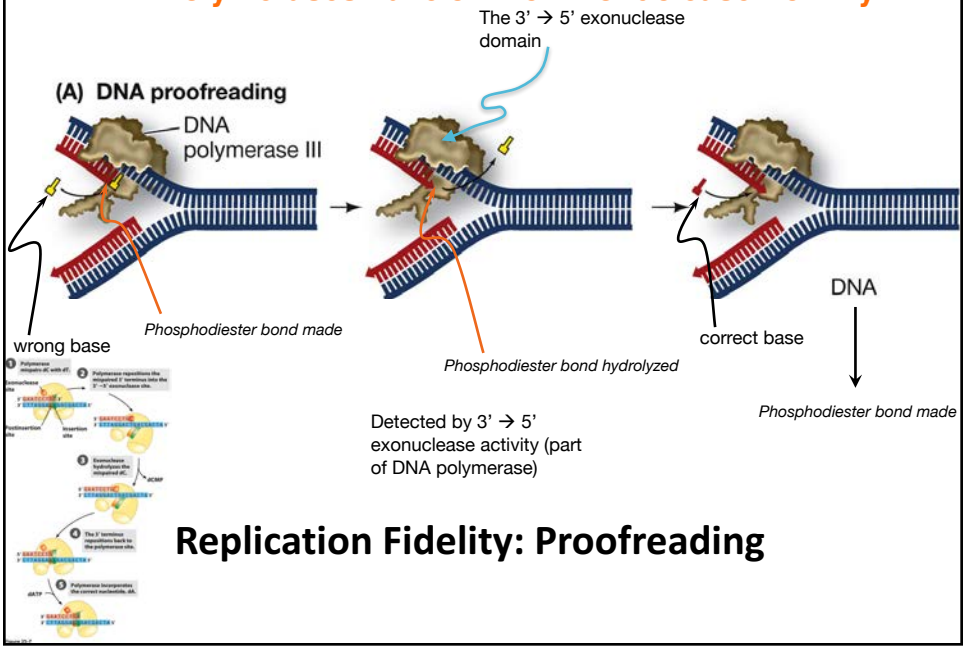
Replication/polymerase Fidelity

DNA Polymerases have 3' → 5' Exonuclease Activity



Replication/polymerase Fidelity

DNA Polymerases have 3' → 5' Exonuclease Activity



Replication Fidelity: Proofreading

Replication/polymerase Fidelity

Comparison of Polymerases

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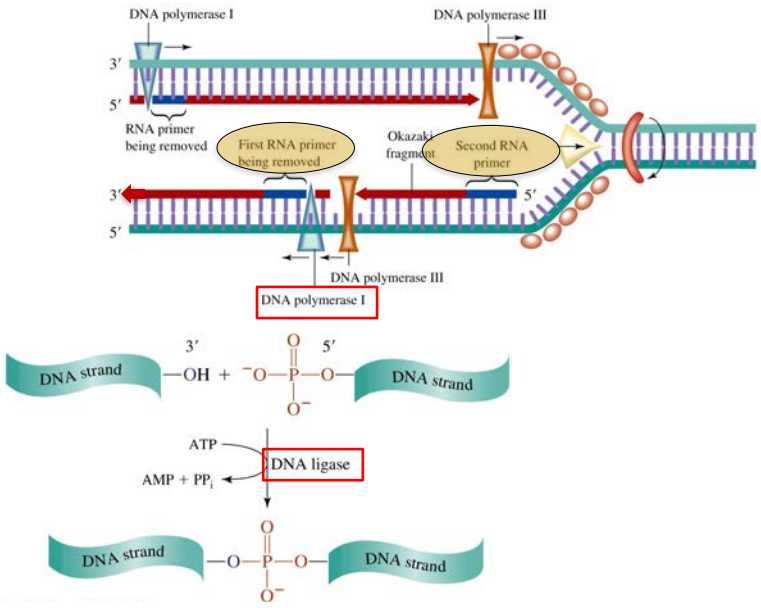
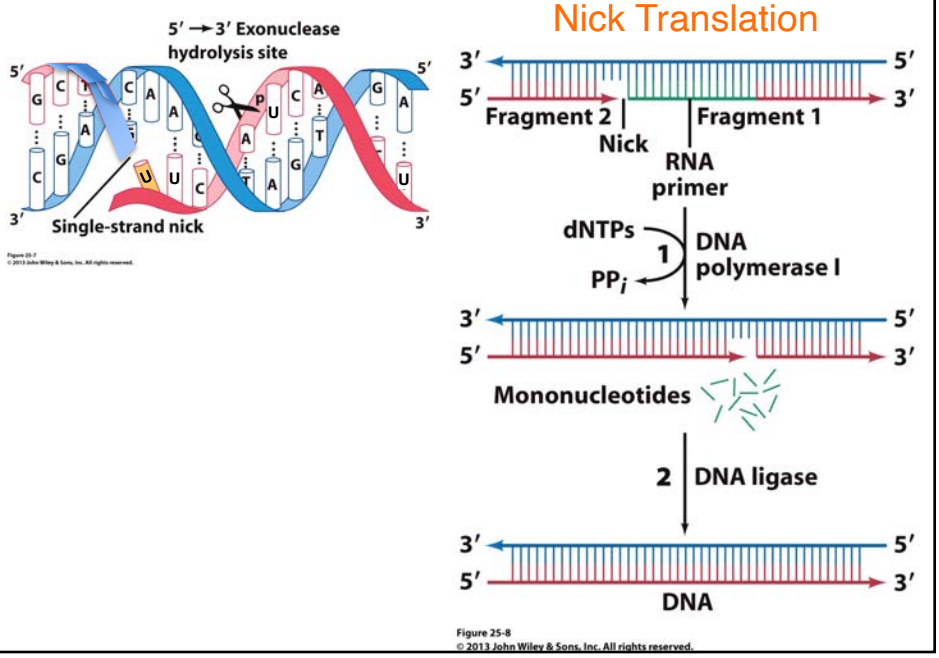
[a]dNTP polymerized sec^{-1} at 37 °C. [b]not including Okasaki fragments

*In a complex with 9-10 proteins (26 subunits) of >1000 kD. Core is trimer of α, θ, ε

DNA Replication Process



Pol I Has 5' → 3' Exonuclease Activity



Replication/polymerase Fidelity

Polymerases make mistakes in synthesis (polymerase 5'→3' activity) = 1/10,000

DNA polymerases have proofreading ability (3'→5' exonuclease activity) = 1/10,000

After replication fork, any mistakes are corrected by **mismatch repair** mechanism. This misses = 1/100

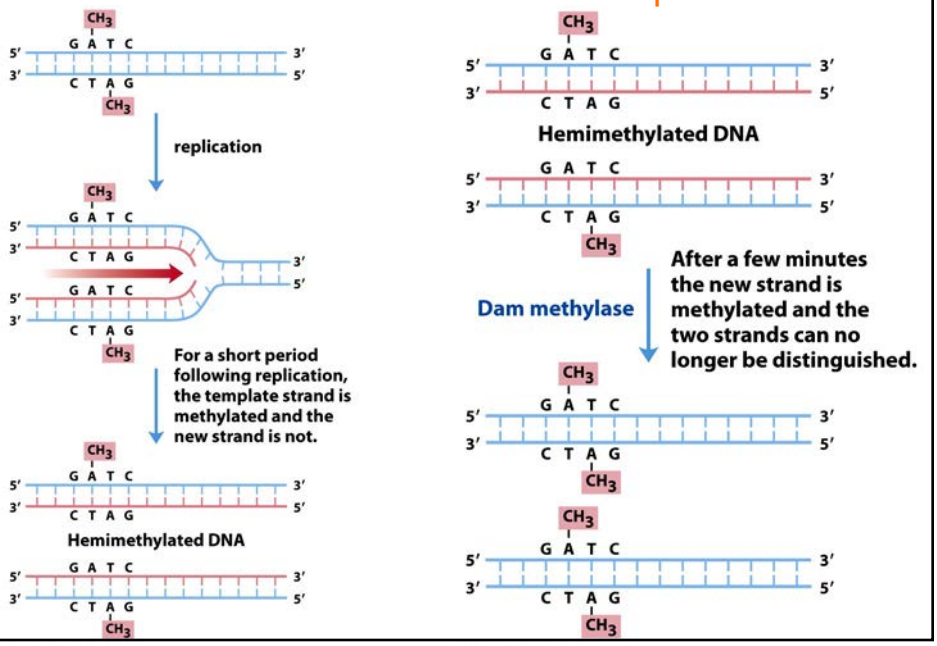
Overall for Replication = $1/10^{10}$

$3x10^9$ bp/haploid genome x $1/10^{10}$ mistakes/bp = 0.3 mistakes/haploid genome

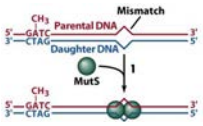
Plus DNA can be damaged in living cells. These damages are repaired by other mechanisms called Post-replication DNA Repair:

- **Direct reversal of modification**
- **Base-excision repair**
- **Nucleotide excision repair**

Replication/polymerase Fidelity
Mechanism of Mismatch Repair



Replication/polymerase Fidelity
Mechanism of Mismatch Repair



In *E. coli* a mismatch is recognized by MutS, which then recruits MutL.

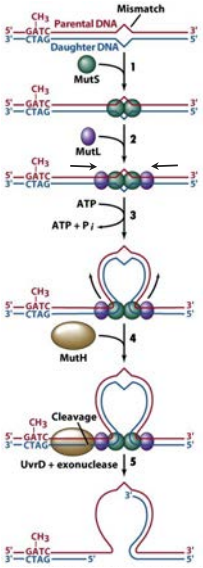
In an ATP dependent manner, the complex translocates along the double stranded DNA to generate a loop.

On encountering a hemi-methylated GATC sequence, it recruits MutH (an endonuclease) and MutH cuts the unmethylated DNA.

A helicase (UvrD) then separate the two strands and the exonuclease completely removes the defected strand.

The gap is then filled by DNA polymerase and DNA ligase.

Replication/polymerase Fidelity
Mechanism of Mismatch Repair



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DNA Repair

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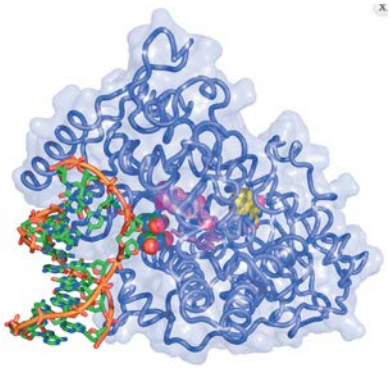
Plus DNA can be damaged in living cells. These damages are repaired by other mechanisms called Post-replication DNA Repair:

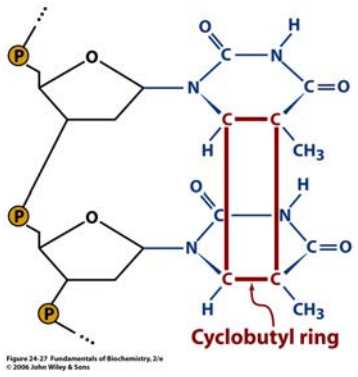
- **Direct reversal of modification (I)**
- **Base-excision repair (II)**
- **Nucleotide excision repair (III)**

DNA Repair

DNA Repair (I): Direct Reversal of Modification



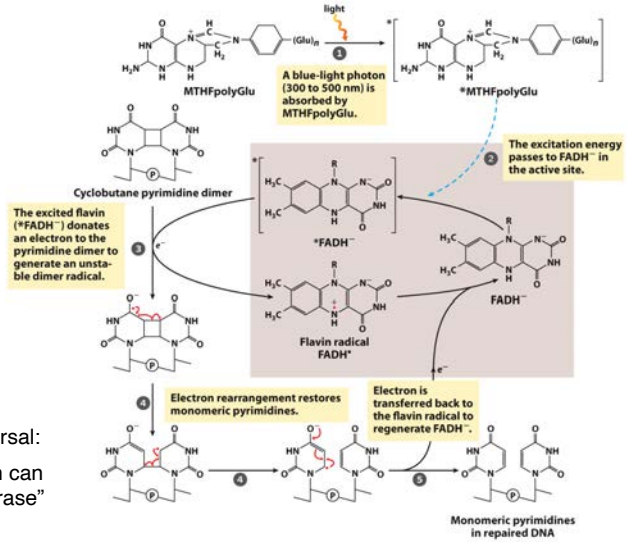
DNA photolyase



UV-crosslinking: pyrimidine dimers

DNA Repair

Repair of Pyrimidine Dimers with Photolyase



Another example of direct reversal:

O^6-alkylguanine modification can be reversed by "alkyltransferase" (*alkB*)

AlkB demethylates 1-methyladenine and 3-methylcytosine.

DNA Repair

Direct Repair of Alkylated Bases by AlkB

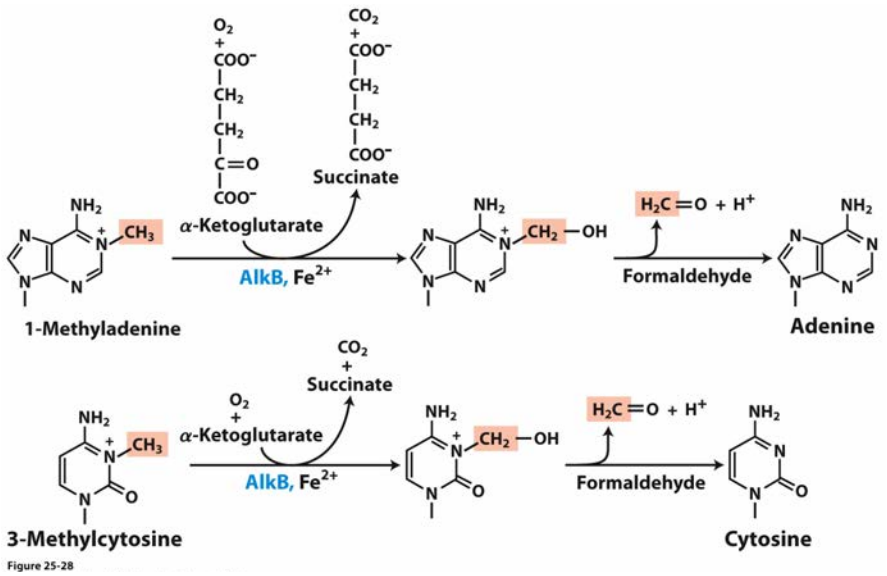
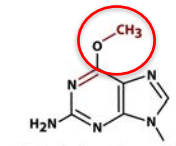


Figure 25-28

RECALL: *Processes which chemically degrade DNA*

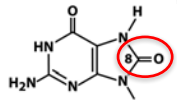
	Process	Events/cell/day
1.	Spontaneous hydrolysis of glycosidic bonds [G ~ A ≈ 20X C,T]	2-10 x 10^3
2.	Deamination of cytosine (and 5-MeC) [C ~ 5-MeC ≈ 50X A ≫ G]	100-500
3.	Oxidation of guanine to 8-hydroxyguanine	100-500
4.	Formation of 3-methyladenine and other bases	600
5.	Photochemical pyrimidine dimer formation [cyclobutane type; 6,4-product; spore photoproduct]	variable

Oxidations/Alkylations:

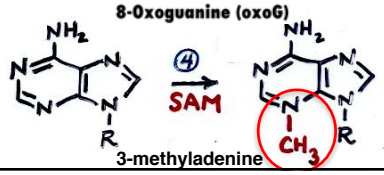


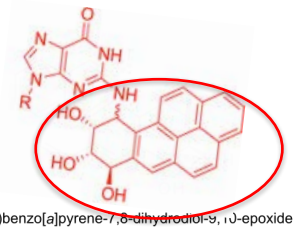
O^6-Methylguanine residue

- Methylation of G at O6 can base-pair with C or T, which causes G → A mutations



8-Oxoguanine (oxoG)

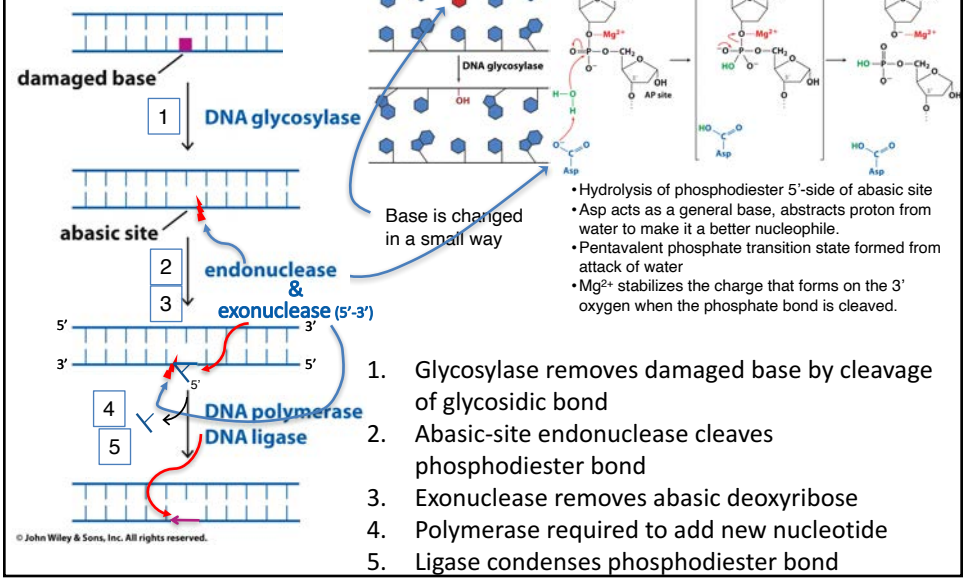




(+)benzo[*a*]pyrene-7,8-dihydrodiol-9,10-epoxide

DNA Repair

DNA Repair (II): Base excision Repair



abasic endo-nuclease mechanism

- Hydrolysis of phosphodiester 5'-side of abasic site
- Asp acts as a general base, abstracts proton from water to make it a better nucleophile.
- Pentavalent phosphate transition state formed from attack of water
- Mg^{2+} stabilizes the charge that forms on the 3' oxygen when the phosphate bond is cleaved.

Base is changed in a small way

1. Glycosylase removes damaged base by cleavage of glycosidic bond
2. Abasic-site endonuclease cleaves phosphodiester bond
3. Exonuclease removes abasic deoxyribose
4. Polymerase required to add new nucleotide
5. Ligase condenses phosphodiester bond

DNA Repair

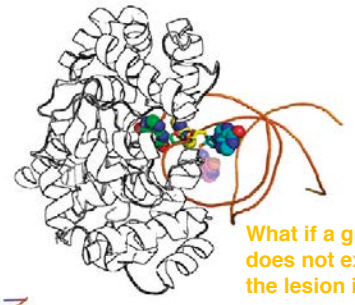
STEP 1

DNA Repair (II): Base excision Repair

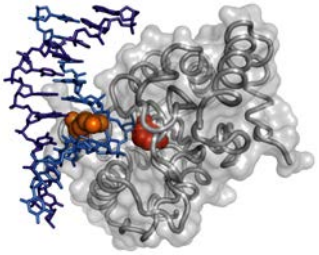
Depends on there being a specific Glycosylase

[Glycosylases exist for 8-Oxo-guaninie (8-hydroxyG), uracil, hypoxanthine, 3-methyladenine]

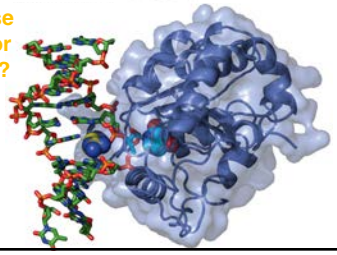
Oxo-guanine glycosylase
Bends DNA helix and extrudes damaged base into enzyme active site



Uracil DNA glycosylase
Flips out base and fills hole with Arg



What if a glycosidase does not exist and/or the lesion is too big?

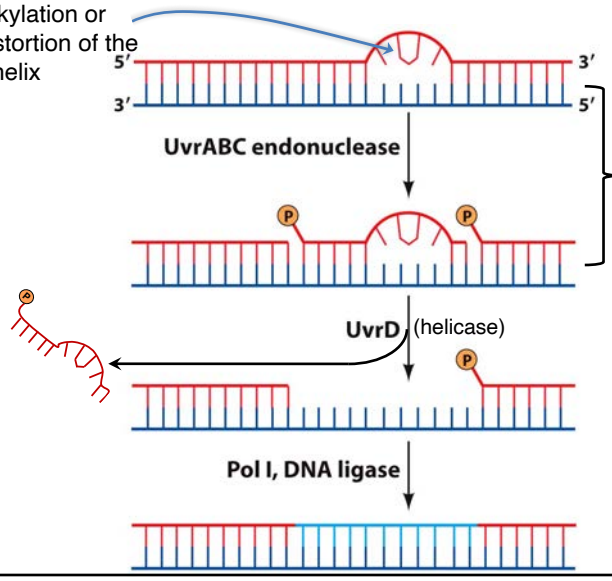


DNA Repair

DNA Repair (III): Nucleotide Excision Repair (NER)

Most common and versatile mechanism of repair

Bulky alkylation or other distortion of the double helix



UvrABC endonuclease

(ATP-driven)

(A_2B_2 tetramer bends DNA at damage)

(UvrC is the endo-nuclease)

DNA Repair

NER in Bacteria and Humans

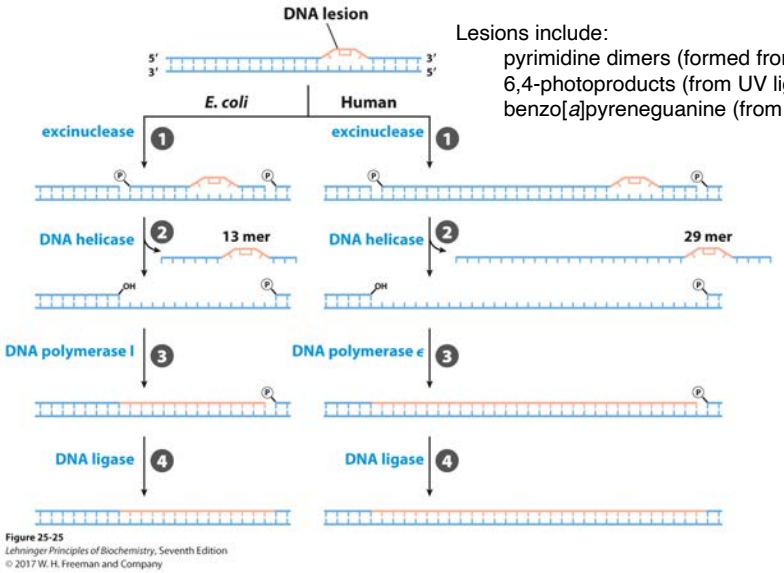


Figure 25-25
Lehninger Principles of Biochemistry, Seventh Edition
© 2017 W. H. Freeman and Company

Lesions include:
- pyrimidine dimers (formed from UV light)
- 6,4-photoproducts (from UV light)
- benzo[*a*]pyreneguanine (from cigarette smoke)

DNA Replication

- DNA Replication
  - Polymerases
    - Activities
    - Structure
    - Mechanism
- Replication/polymerase fidelity
  - Exonuclease Activities
  - Mismatch repair
  - Post-replication repair
    - Reversal
    - Base Excision repair
    - Nucleotide Excision repair
- DNA Sequencing
- PCR

DNA Sequence Determination (Sanger)

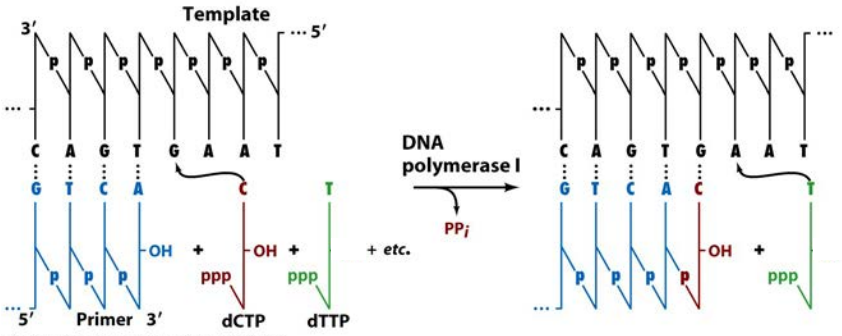


Figure 3-20 Fundamentals of Biochemistry, 2/e
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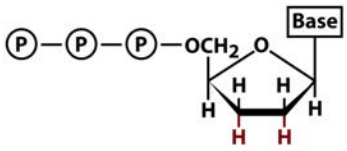

2′,3′-Dideoxynucleoside triphosphate

DNA Sequencing

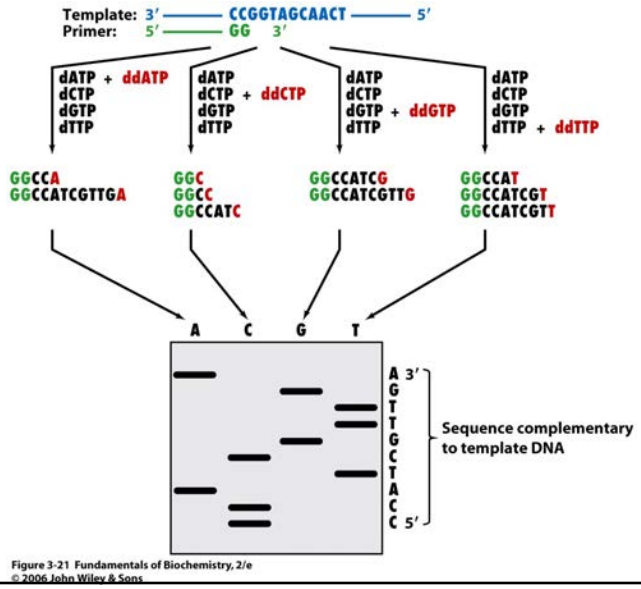

Figure 3-21 Fundamentals of Biochemistry, 2/e
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Visualize the DNA Sequence (I)

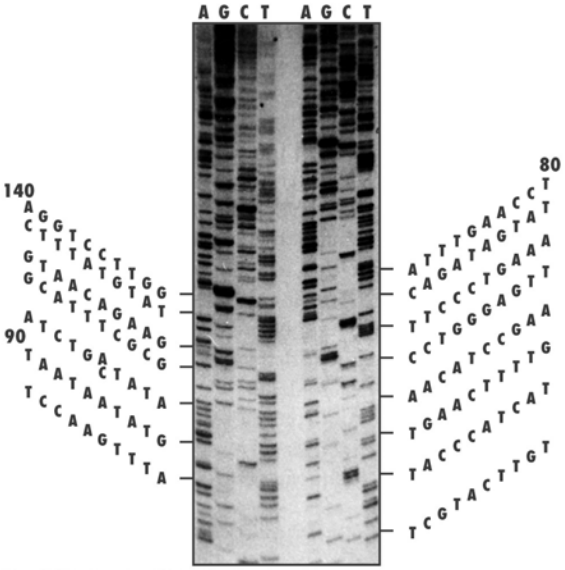
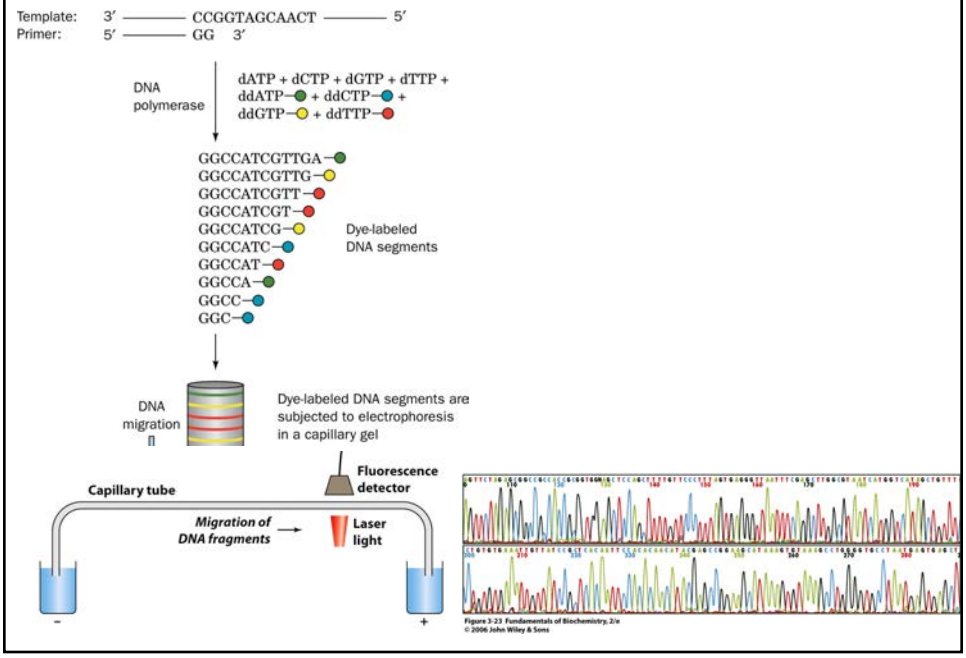


Figure 3-22 Fundamentals of Biochemistry, 2/e

Chain-Terminator Sequencing Method



DNA Replication

- DNA Replication
  - Polymerases
    - Activities
    - Structure
    - Mechanism
- Replication/polymerase fidelity
  - Exonuclease Activities
  - Mismatch repair
  - Post-replication repair
    - Reversal
    - Base Excision repair
    - Nucleotide Excision repair
- DNA Sequencing
- PCR

Molecular Cloning: Polymerase Chain Reaction

Besides using host to MAKE MORE copies of DNA sequences, they can be made by the **polymerase chain reaction** (**PCR**) technique (*in vitro)*.

PCR is a cyclical process:

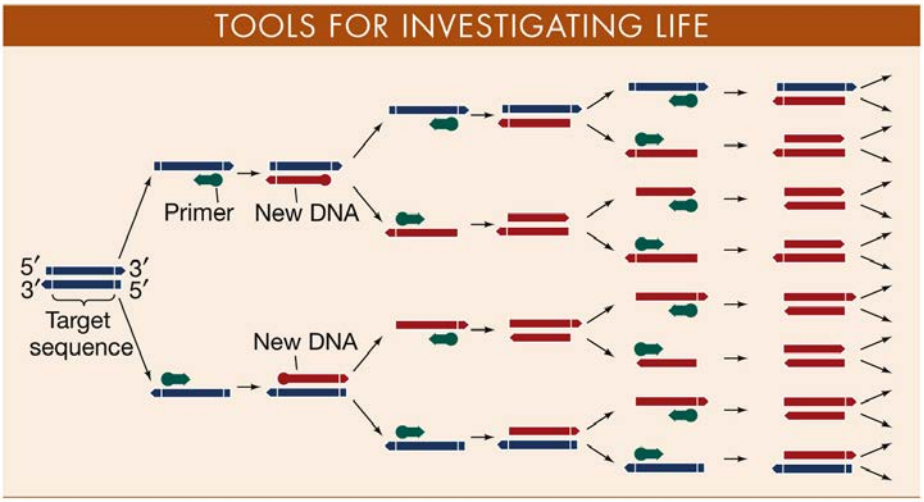
- DNA fragments are **denatured** by heating → single-stranded DNA
- Primers (one complimentary to each strand), plus dNTPs, and DNA polymerase are added → **annealing** and **extension** → new double-stranded DNA
- Repeat

An initial problem with PCR was its temperature requirements.

The key insight was the use of an enzyme that could withstand the heat needed to denature the DNA → DNA polymerase from *Thermus aquaticus,* ***Taq* Polymerase** (insight of Kerri Mullis).

The amplified DNA can then be inserted into plasmids to create recombinant DNA and cloned in host cells. Synthetic DNA can be manipulated to create specific mutations in order to study the consequences of the mutation: Site-directed Mutagenesis.

Process Diagram: Polymerase Chain Reaction (PCR)



Molecular Cloning: Polymerase Chain Reaction