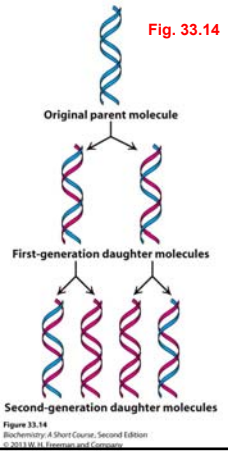
BCMB 3100 - Chapters 34 & 35
DNA Replication and Repair

- Semi-conservative DNA replication
- DNA polymerase
- DNA replication
- Replication fork; Okazaki fragments
- Sanger method for DNA sequencing
- DNA repair
- PCR

- Meselson & Stahl, 1958
- Semiconservative DNA replication
- Each strand of DNA acts as a template for synthesis of a new strand
- Daughter DNA contains one parental and one newly synthesized strand

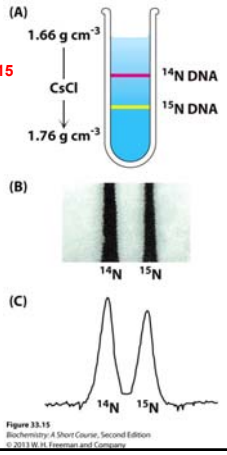
Fig. 33.14



Meselson and Stahl demonstrated DNA replication is semiconservative

- Bacteria grown on media supplemented with ^{15}N.
- Bacteria then shifted to growth media with ^{14}N as nitrogen source.
- Density gradient centrifugation showed, upon shift to ^{14}N medium, newly synthesized DNA consists of DNA with equal parts ^{15}N-DNA and ^{14}N-DNA,
- Demonstrated semiconservative replication.

Fig. 33.15

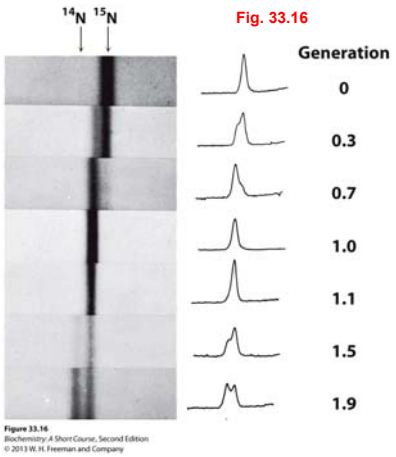


Density gradient centrifugation of DNA from *E. coli* grown first grown on "^{15}N-media" then shifted to "^{14}N-media.

Results show that after one generation all DNA was hybrid with equal parts ^{14}N and ^{15}N.

Demonstrates semiconservative replication.

Fig. 33.16



Enzymatic Synthesis of DNA

Arthur Kornberg (1955-58) discovered an enzyme that synthesized DNA

Experimental Strategy

1) dNTPs as precursors of DNA

2) sensitive assay to detect newly synthesized DNA; radioactive dNTPs & acid precipitation of DNA

3) When animal cell extracts proved unsuccesful they turned to *E. coli*

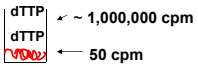
E. coli divides fast (every 20 minutes) and large quantities of cells can be isolated

Results of Kornberg experiments (1955-58)

E. coli extract + ^{14}C-labeled dTTP (1,000,000 cpm)

incubate ↓

acid precipitate



First evidence for DNA polymerase!

50 / 1,000,000 cpm → 0.005% of radioactivity incorporated into DNA

Enzyme purification → DNA Polymerase I

Took approximately 10 years to purify and characterize

100 kg (~220 lbs) E. coli → 500 mg DNA Polymerase I

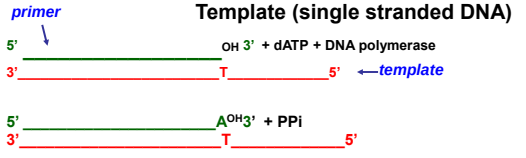
DNA Polymerase I

Molecular Weight: 103 kd; monomer

Activity

$(DNA)_n$ residues + dNTP → $(DNA)_{n+1}$ + PPi → 2 Pi

Requirements: dATP, dTTP, dGTP, dCTP
Mg++
Primer with free 3'-OH
Template (single stranded DNA)



DNA Polymerase is template-directed

** one active site (for polymerase activity) can accommodate all four dNTPs; the correct dNTP is determined by the corresponding base on the template stand.

DNA Polymerase I is moderately processive (~20 residues)

*Polymerization is in the 5' → 3' direction

E. coli DNA Polymerase I has three different active sites on a single polypeptide chain!!

Activities of DNA Polymerase I

1) 5' → 3' polymerase
2) 3' → 5' exonuclease (proof-reading)
3) 5' → 3' exonuclease (editing)

Proof-reading: 3' → 5' Exonuclease Activity

3'-5' Exonuclease activity

5' …pTpApGpCpC-OH

3' …pApTpCpGpApTpCpGpApT…5'

A-C mismatch

5' …pTpApGpCpT-OH

3' …pApTpCpGpApTpCpGpApT…5'

Correct base can then be inserted

*DNA polymerase I examines the result of each polymerization before proceeding to the next.

Editing: 5' → 3' Exonuclease Activity of DNA Polymerase I

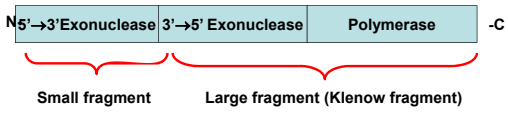
5' …pApCpTpApGpCpC-3'
3' …pTpGpApTpCpGpG-5'

Editing

5' pTpApGpCpC-3'
3' …pTpGpApTpCpGpG-5'

DNA Polymerase I is a tri-functional, single polypeptide protein. It's three activites reside in the N-terminal, middle, and C-terminal region of the polypeptide, respectively (shown below).

The 5'→3' Exonuclease activity can be removed from the enzyme by proteolytically cleaving off the N-terminal region of the protein. The remaining protein, with the remaining two enzyme activities is called the **Klenow fragment.**



Structure of the Klenov fragment of DNA Polymerase I

Binding of dNTP to active site causes conformation shift, yielding tight pocket when correct base is in position
(next slide)

Fig. 34.3

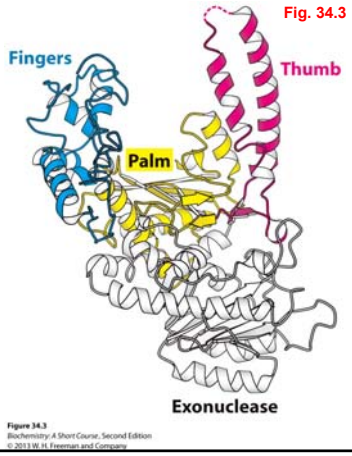


Figure 34.3
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Conformation change in DNA polymerase binding site when correct dNTP binds

Fig. 34.5

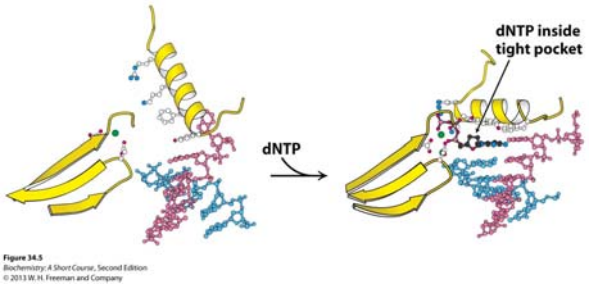

Figure 34.5
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***In vivo* DNA Polymerization**

Delucia & Cairns, 1969

discovery of DNA polymerase II & III

***pol A1 mutant had very low levels of DNA Pol I activity (~1%)**

1) Normal multiplication rate

2) similar bacteriophage replication as wild type

3) more easily killed by UV light than parental strain

4) Conclusion: DNA Pol I is involved in DNA repair!

DNA Polymerse III is the replication enzyme in *E. coli

Activities: 5' → 3' polymerase

3' → 5' exonuclease

Requirements same as for DNA Pol I

>20 protein + DNA Pol III + DNA Pol I required for DNA replication in *E.coli*

Table 34.1 *E. coli* DNA polymerases

Enzyme	Function	Additional enzyme activities	Type of DNA damage
Polymerase I	Primer removal and DNA repair	5′ → 3′ exonuclease	
		3′ → 5′ exonuclease	
Polymerase II	Repair	3′ → 5′ exonuclease	Attachment of bulky hydrocarbons to bases
Polymerase III	Replication	3′ → 5′ exonuclease	
Polymerase IV	Repair		Attachment of bulky hydrocarbons to bases
Polymerase V	Repair		Attachment of bulky hydrocarbons to bases
			Sites missing bases
			Covalently joined bases

Note: Polymerases II, IV, and V can replicate through regions of damaged DNA (Chapter 35). They are called translesion polymerases or error-prone polymerases.

Table 34.1
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DNA Replication and Repair

- Semi-conservative DNA replication
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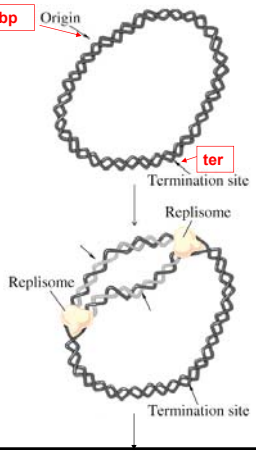
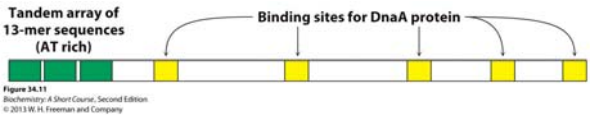
- Sanger method for DNA sequencing
- DNA repair
- PCR

- E. coli has a circular chromosome (4.6 million base pairs)
- Bidirectional DNA replication in *E. coli*
- New strands of DNA are synthesized at the two replication forks where replisomes are located
- Replication rate: ~1000 nucleotides/sec



E. coli Ori C *(245 bp)* Fig. 34.11



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Cartoon of possible structure of DnaA binding Ori C and DnaB helicase unwinding duplex

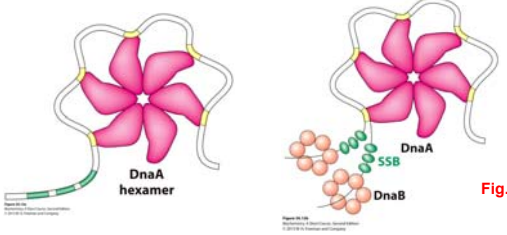
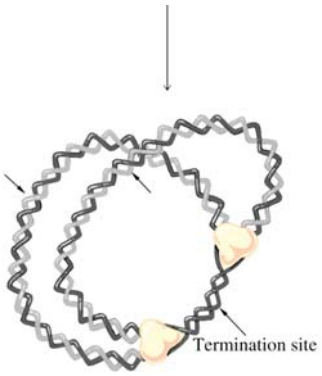


Fig. 34.12

1) Replication starts at OriC (dnaA gene product binds)
2) Replication proceeds simultaneously in opposite directions → 2 replication forks per replicon
3) The replication forks meet at ter (tus: terminator utilization substance binds)



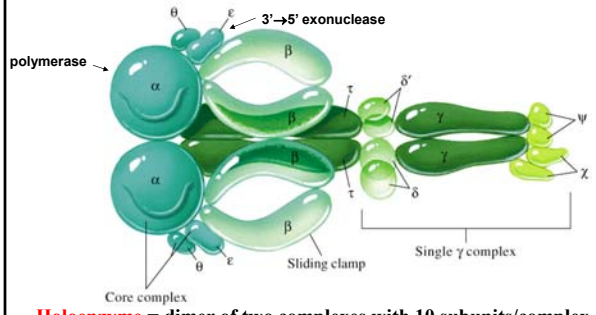
Eukaryotic replication

- Eukaryotic chromosomes are large linear, double-stranded DNA molecules
- Replication is <u>bidirectional</u>
- Multiple sites of initiation of DNA synthesis (versus one site in *E. coli*)

DNA Polymerases in *E.coli*

- *E. coli* contains three DNA polymerases
- _____ - repairs DNA and participates in DNA synthesis by removing & replacing RNA primer
- _____ - role in DNA repair
- _____ - the major DNA replication enzyme, responsible for chain elongation

E. coli DNA Polymerase III: subunit composition



Holoenzyme = dimer of two complexes with 10 subunits/complex
Each complex synthesizes one daughter strand

Sliding clamp of DNA Polymerase III

Fig. 34.15

(β subunits)

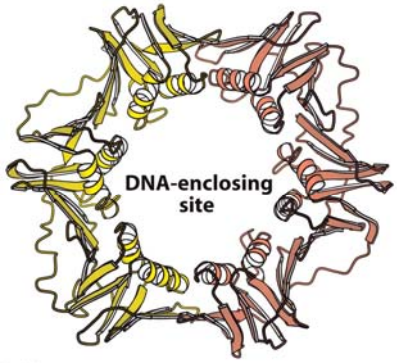


Figure 34.16
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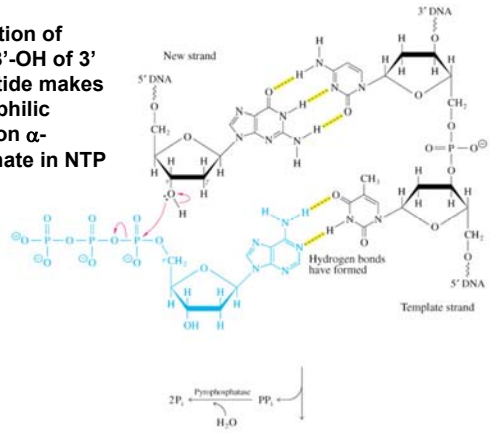
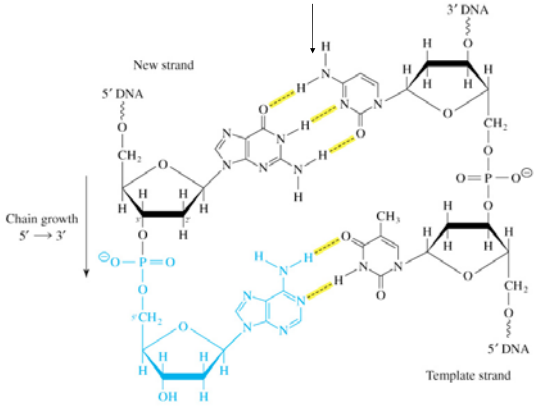
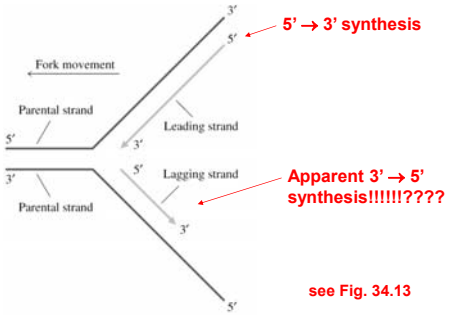
Elongation of DNA. 3'-OH of 3' nucleotide makes nucleophilic attack on α-phosphate in NTP





Events at a replication fork



5' → 3' synthesis

Apparent 3' → 5' synthesis!!!!!????

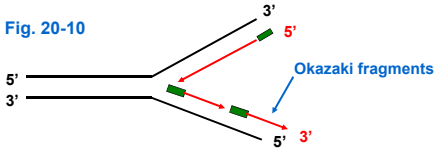
see Fig. 34.13

Is there a 3'→ 5' DNA polymerase that can account for the apparent 3'→ 5' synthesis? No!!

There is discontinuous DNA synthesis

Reiji Okazaki (1968) showed that a significant amount of newly synthesized DNA exists as small (~1000 nucleotides) fragments called _____

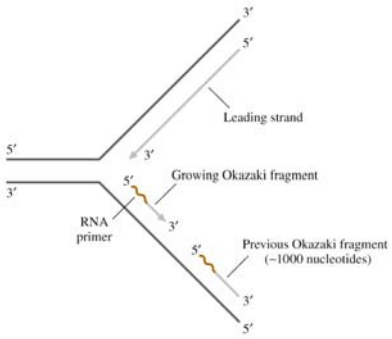
See Fig. 20-10



DNA ligase joins _____ together

see Fig. 34.14

Diagram of lagging-strand synthesis



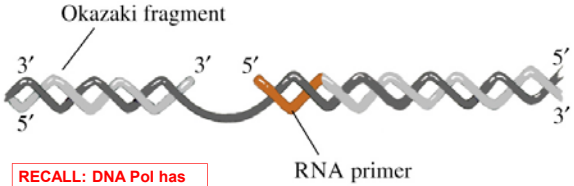
DNA synthesis occurs at the replisome: a complex that includes DNA Pol III, the primosome (helicase + primase) + SSB proteins

RNA primer synthesized by primase

RNA primer synthesized by primase; DNA-dependent RNA polymerase, (product of dnaG gene in *E.coli*) synthesis short RNA primers of ~ 10 nucleotides

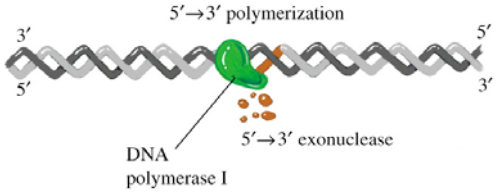
Joining of Okazaki fragments by DNA Pol I and DNA ligase

(a) Completion of Okazaki fragment synthesis leaves a nick between the Okazaki fragment and the preceding RNA primer on the lagging strand.

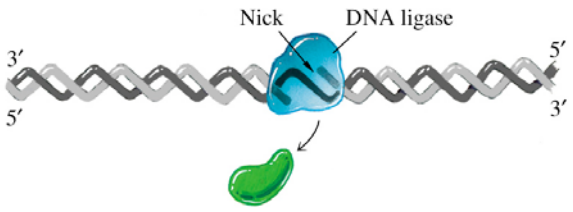


RECALL: DNA Pol has processivity of ~ 20

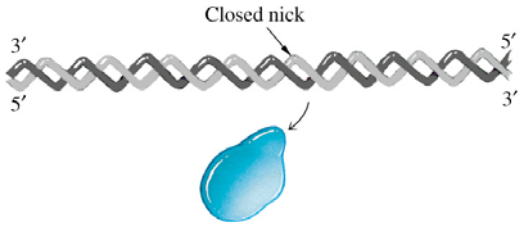
(b) DNA polymerase I extends the Okazaki fragment while its 5′→3′ exonuclease activity ,removes the RNA primer. This process, called nick translation, results in movement of the nick along the lagging strand.



(c) DNA polymerase I dissociates after extending the Okazaki fragment 10–12 nucleotides DNA ligase binds to the nick.



(d) DNA ligase catalyzes formation of a phosphodiester linkage, which seals the nick, creating a continuous lagging strand. The enzyme then dissociates from the DNA.



DNA Replication in *E coli*

1) DNA **supercoil is relieved ahead of & behind replication fork by topoisomerase**
   *cleavage of one (Type I) or two (Type II) strands of DNA
   *Passage of DNA segment through break
   * resealing of break

2) **Replication fork** is site of **simultaneous unwinding** (by **helicase** in replisome) and **DNA synthesis** (**DNA polymerase III**+ single stranded binding proteins, SSB)

3) **Primase synthesizes down RNA** primer

DNA Replication in *E coli* (cont.)

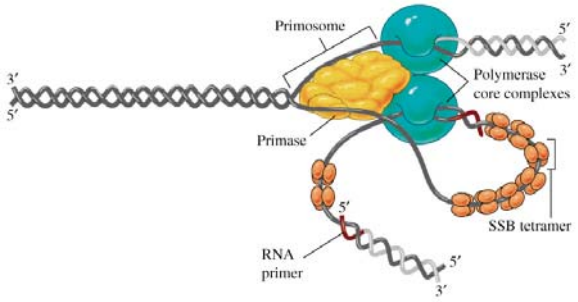
4) **DNA Pol III synthesizes** new **DNA** in 5'→3' direction using parental strand as template

5) **DNA Pol I removes RNA primer** (5'→3' exonuclease) and **fills in gap** (5'→3' polymerase)

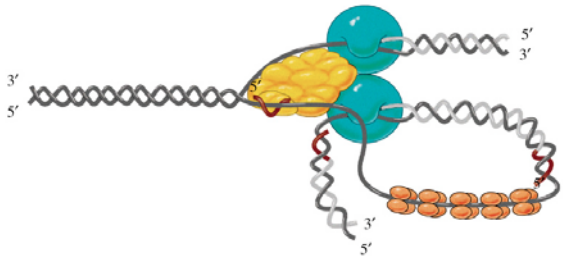
6) **DNA ligase joins** ends of **daughter strands** (i.e. closes nick)

Replisome DNA synthesis

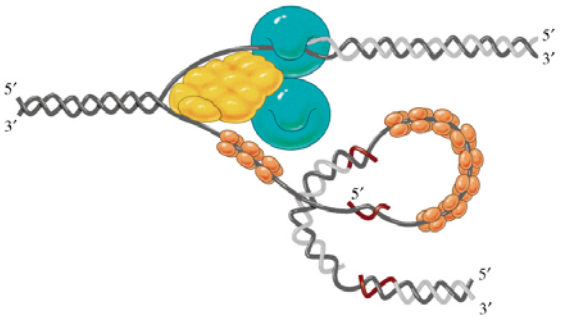
(a) The lagging-strand template loops back through the replisome so that the leading and lagging strands are synthesized in the same direction. SSB binds to single-stranded DNA.



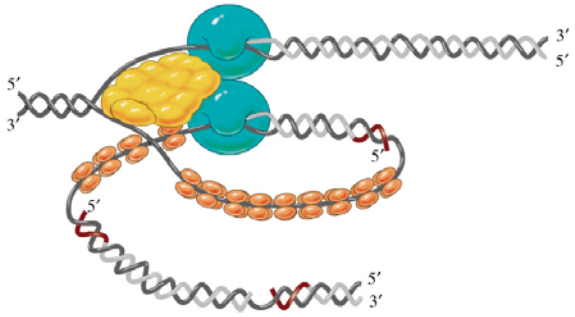
(b) As helicase unwinds the DNA template, primase synthesizes an RNA primer. The lagging-strand polymerase completes an Okazaki fragment.



(c) When the lagging-strand polymerase encounters the preceding Okazaki fragment, it releases the lagging strand.



(d) The lagging-strand polymerase binds to a newly synthesized primer and begins synthesizing another Okazaki fragment.



DNA replication in eukaryotes is similar to that in prokaryotes

Differences

1) **Chromosomes are linear with multiple origins of replication**
2) **Replication fork moves more slowly → Okazaki fragments of 100-200 nucleotides; primer = 10 nucleotides**
3) **Eukaryotes have at least 4 DNA polymerases: α, δ, ε (DNA replication); β (DNA repair); γ DNA replication in mitochondria**

Telomeres and DNA synthesis

Fig. 33.22/33.21

- The ends of linear chromosomes are sensitive to degradation by DNAse. Also synthesis of lagging strand would be slightly incomplete with each division cycle. 5'→3' polymerase activity makes synthesizing end of linear DNA difficult. The ends of linear chromosomes are protected by likely duplex loop formation
- Eukaryote chromosomes have **telomeres** at ends
- Telomere has single-stranded G-rich strand extending from end; makes end more stable by, possibly, duplex loop

Figure 34.22
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Telomere formation by Telomerase
(in vertebrates)

- **Telomere**: hundreds of tandem repeats of hexanucleotide sequence
- Telomere synthesized by **telomerase** (special ribonucleoprotein reverse transcriptase polymerase with own RNA template that acts as template to extend leading strand)
- Telomerase activity low in most cells (high in cancer cells)

See Fig. 34.20

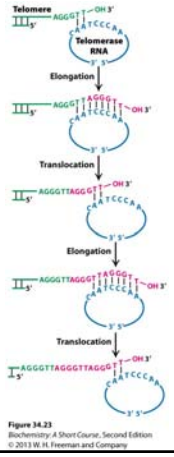

Figure 34.23
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DNA Replication and Repair

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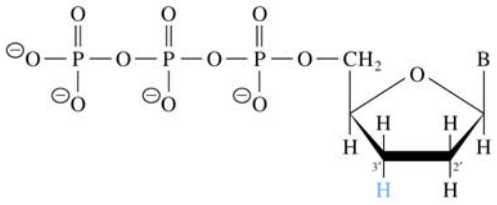
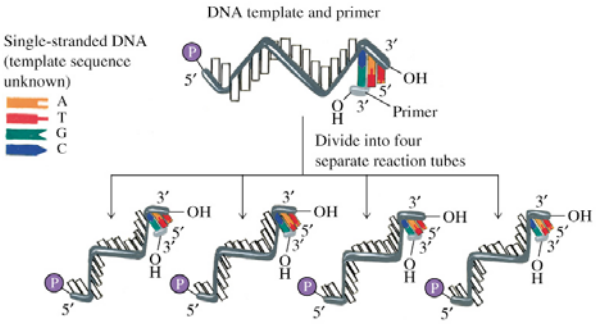
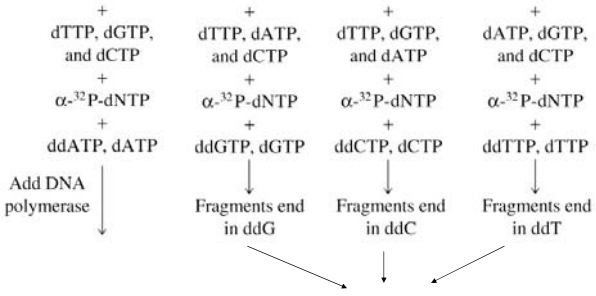
- **DNA polymerase**
- **DNA replication**
- **Replication fork; Okazaki fragments**
- **Sanger method for DNA sequencing**
- **DNA repair**
- **PCR**

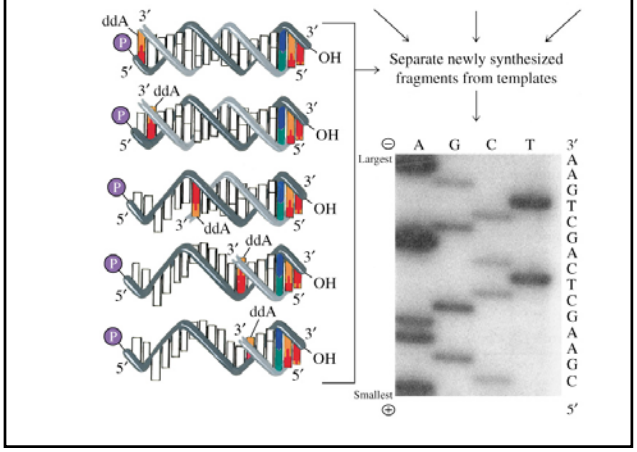
Dideoxynucleotide Sequencing: the Sanger Method



Sanger method for sequencing DNA







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DNA Replication and Repair

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Error rate for nucleotide insertion in eukaryotes is 10^{-9} to 10^{-11} (recall Human DNA has 3.2 billion base pairs (*bp*))

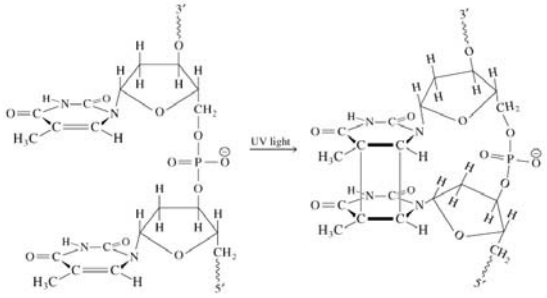
Due to good repair system

DNA is only biological molecule that is repaired

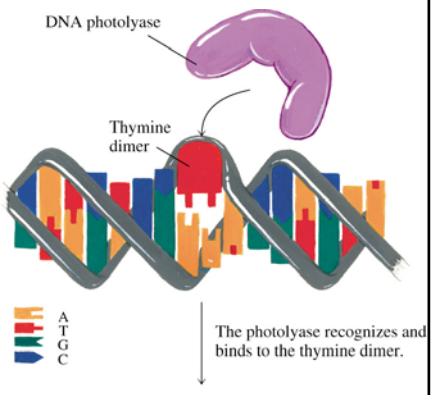
DNA is damaged by UV light, ionizing radiation & chemicals

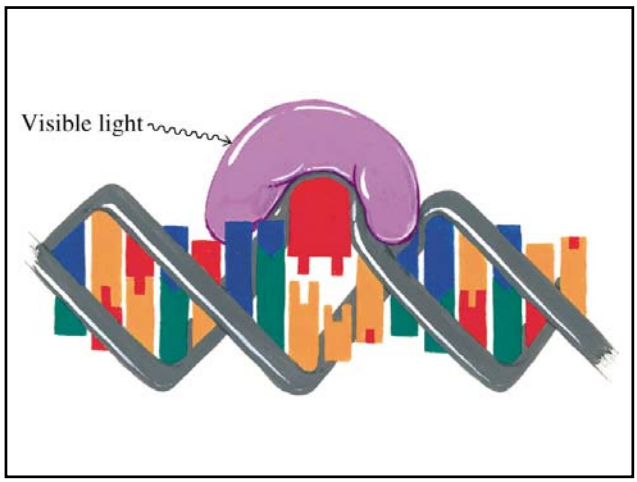
	Combined Error Rate
E.coli 5'→3' DNA Polymerase	10^{-5}
DNA Pol III 3'→5' exonuclease	10^{-7}
DNA repair enzymes	10^{-9} to 10^{-10}

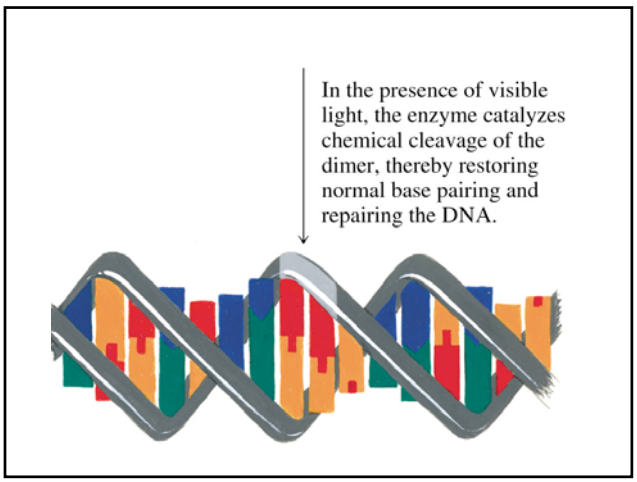
Photodimerization of adjacent thymines induced by UV light



- Repair of thymine dimers by DNA photolyase
- **Direct DNA Repair**







Excision Repair Pathway

- DNA can be damaged by alkylation, methylation, deamination, loss of heterocyclic bases (depurination or depyrimidization)
- General excision-repair pathway can repair many of these defects
- Overall pathway is similar in all organisms

1) Damaged DNA cleaved by endonuclease
2) A 12-13 nucleotide ssDNA gap results
3) Gap is filled by DNA Pol I (prokaryotes) or repair DNA Pol (eukaryotes) and nick is ligated by DNA ligase

BCMB 3100 - Chapters 34 & 35
DNA Replication and Repair

- Semi-conservative DNA replication
- DNA polymerase
- DNA replication
- Replication fork; Okazaki fragments
- Sanger method for DNA sequencing
- DNA repair
- PCR

Polymerase Chain Reaction (PCR)

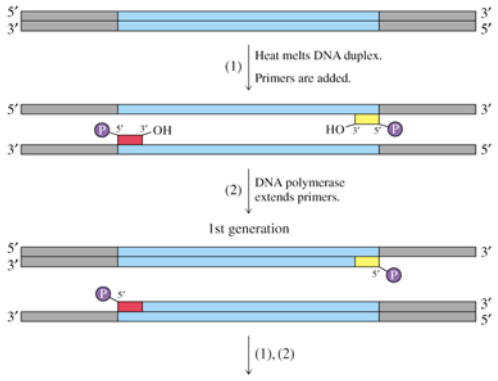
Kary Mullis (1984)
(see Figs. 41.14 & 41.15)

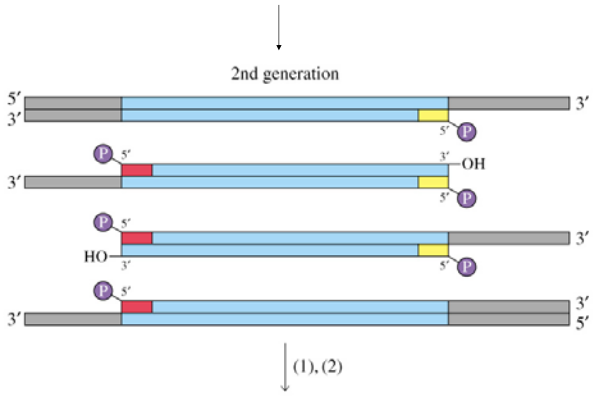
A repetitive method that yields a ~10^6-fold amplification of a specific DNA sequence.

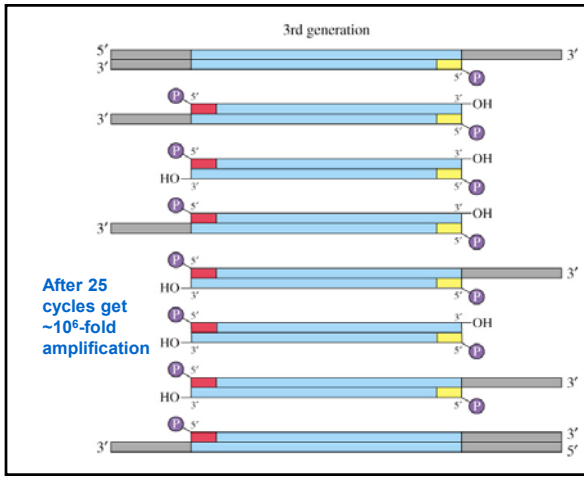
Can detect as little as one DNA molecule!!!!

This means you can get DNA sequence from mummies, mammoths, at crime scenes, etc.

Polymerase Chain Reaction (PCR)



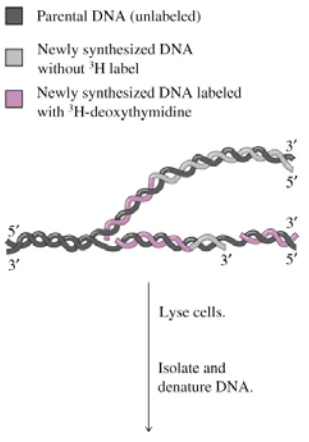


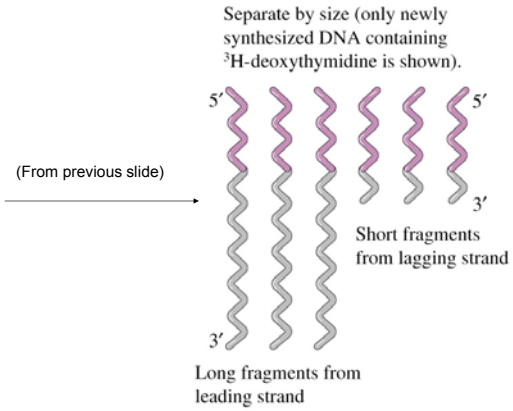


Extra Information

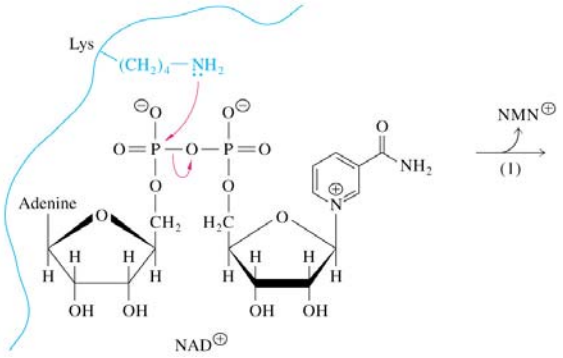
How is the DNA code deciphered to allow the synthesis of proteins and of other catalytic/information molecules????

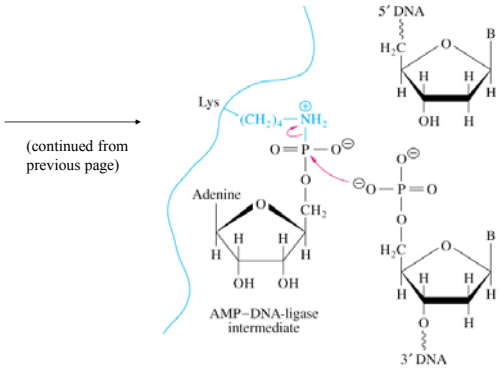
- Okazaki's experiment
- Demonstration of discontinuous DNA synthesis

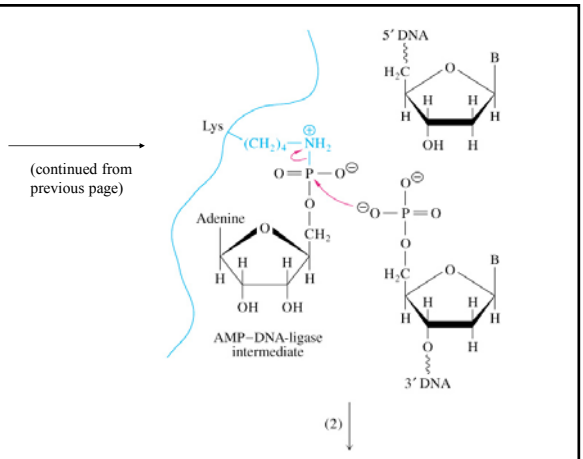


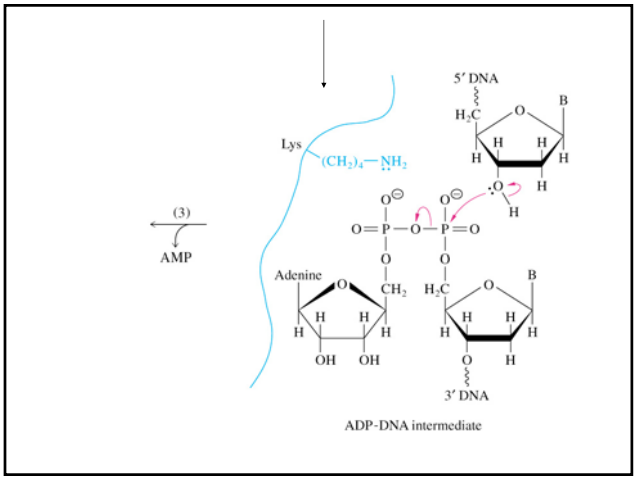


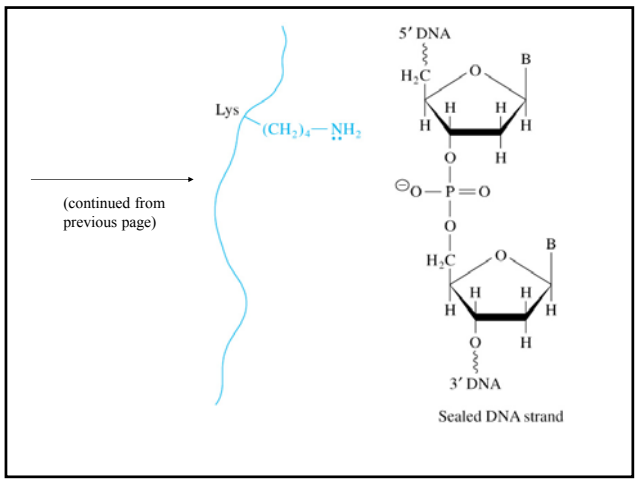
Mechanism of DNA ligase in *E. coli*

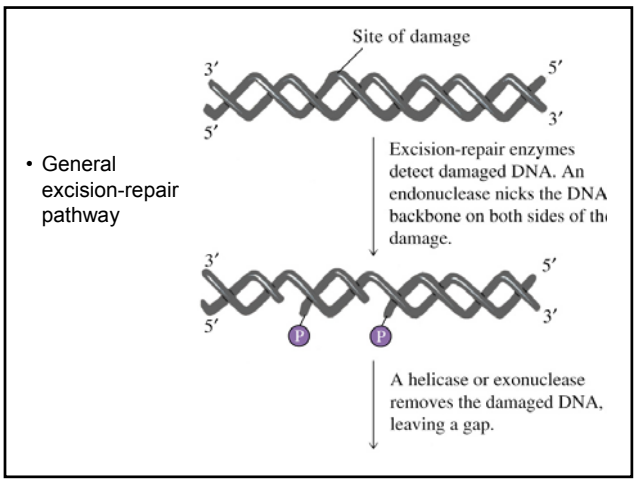




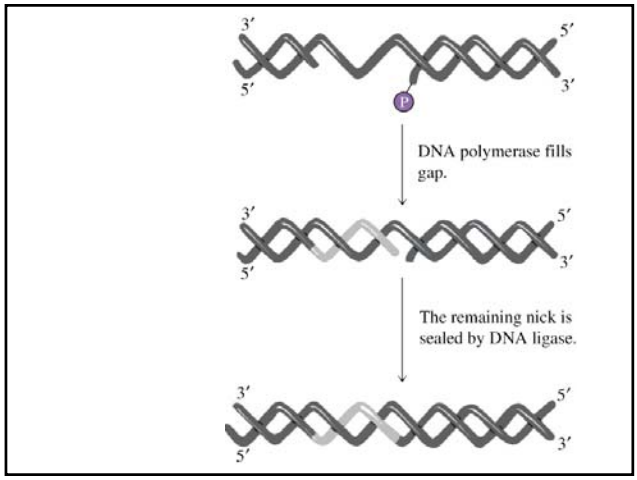




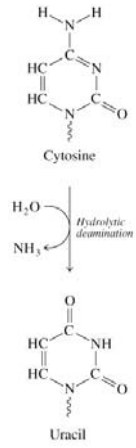




- General excision-repair pathway

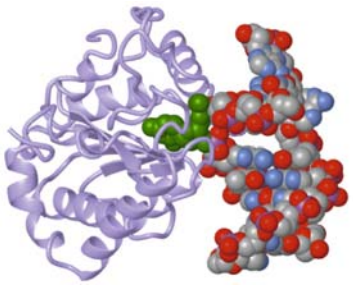


- Hydrolytic deamination of cytosine to uracil
- Uracil in place of cytosine causes incorporation of an incorrect base during replication
- DNA glycosylases hydrolyze base-sugar N-glycosidic bonds
- Deaminated bases are then removed and replaced



Uracil N-glycosylase (human mitochondria)

- Enzyme is bound to a uracil-containing nucleotide (green) that has been flipped out of the stacked region of DNA



- Repair of damage from deamination of cytosine