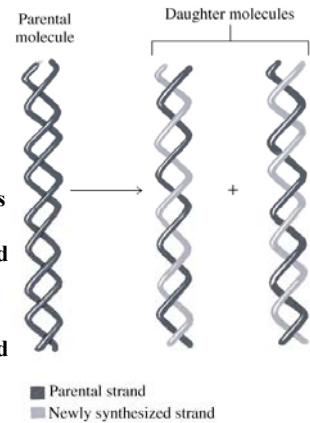


BCMB 3100 - Chapter 20 DNA Replication

- [Semi-conservative DNA replication](#)
- [DNA polymerase](#)
- DNA replication
- Replication fork; Okazaki fragments
- Sanger method for DNA sequencing
- DNA repair
- PCR

Fig 20.1

- 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



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 unsuccessful they turned to *E. coli*

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

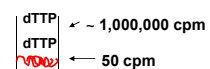
Results of Kornberg experiments (1955-58)

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

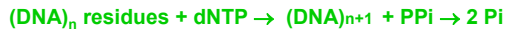
Enzyme purification → DNA Polymerase I

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

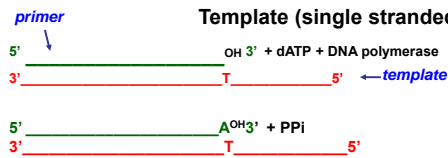


Requirements: dATP, dTTP, dGTP, dCTP

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 strand.

DNA Polymerase I is moderately processive (~20 residues)

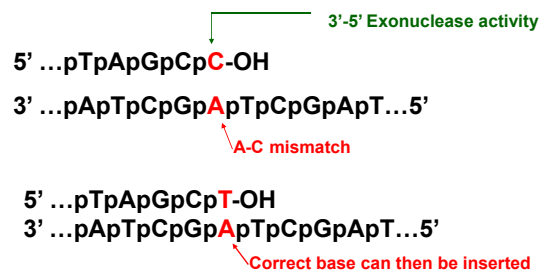
*Polymerization is in the 5' → 3' direction

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



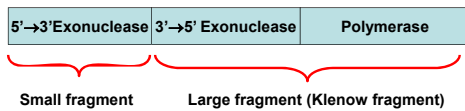
*DNA polymerase I examines the result of each polymerization before proceeding to the next.

Editing: 5' → 3' Exonuclease Activity

5' ...pApCpTpApGpCpC-3'
3' ...pTpGpApTpCpGpG-5'

Editing

5' pTpApGpCpC-3'
3' ...pTpGpApTpCpGpG-5'



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 Polymerase 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*

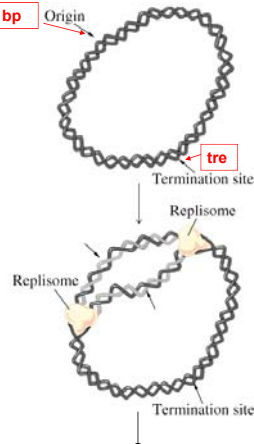
BCMB 3100 - Chapter 20 DNA Replication

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

Fig 20.2

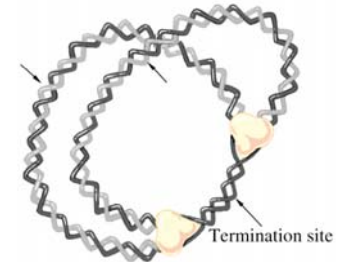
Ori C; 245 bp

- *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



- 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 **tre** (**tus: terminator utilization substance binds**)

Fig 20.2 (cont)

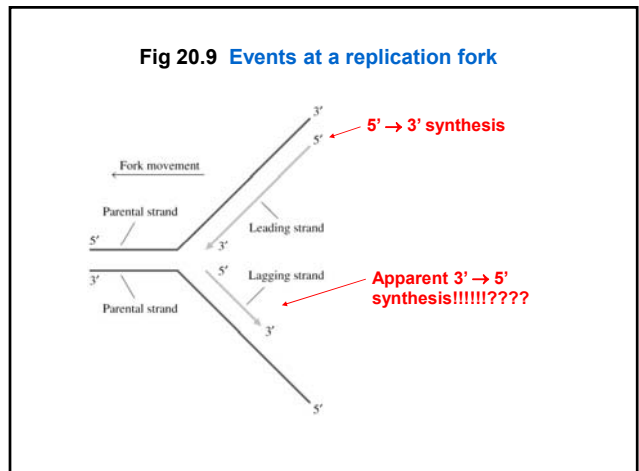
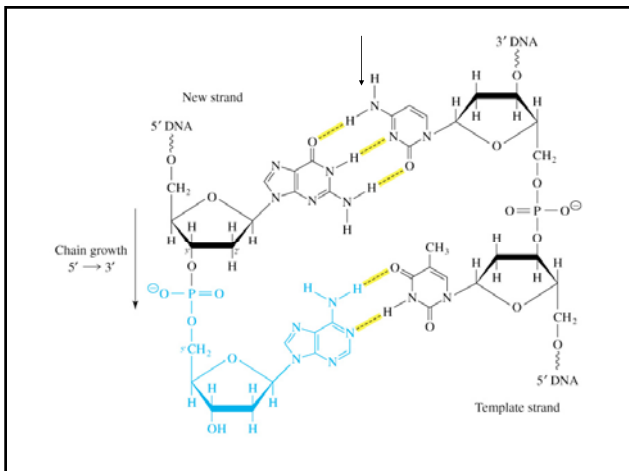
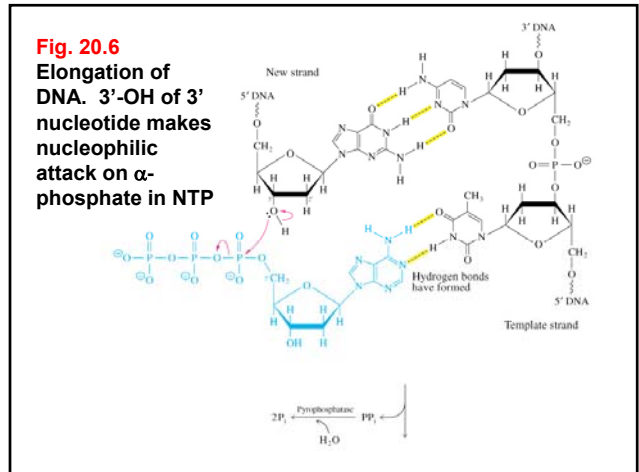
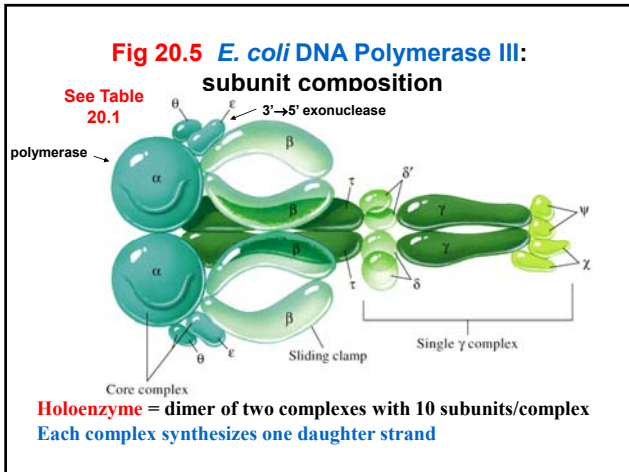


Eukaryotic replication

- Eukaryotic chromosomes are large linear, double-stranded DNA molecules
- Replication is **bidirectional**
- 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

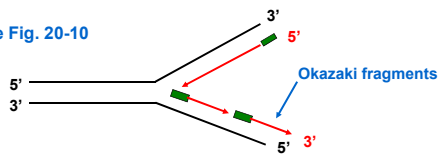


Is there a 3'→5' DNA polymerase that can account for the apparent 3'→5' synthesis? **No!!**

There is discontinuous DNA synthesis

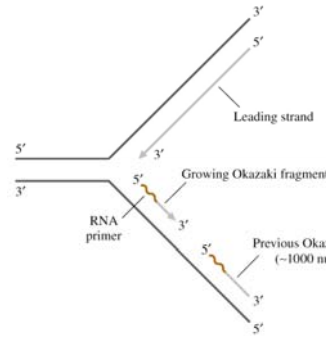
Reiji **Okazaki** (1968) showed that a significant amount of newly synthesized DNA exists as small (~1000 nucleotides) fragments called _____

See Fig. 20-10



DNA ligase joins _____ together

Fig 20.11 Diagram of lagging-strand synthesis



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

RNA primer synthesized by **primase**

Fig 20.13 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.

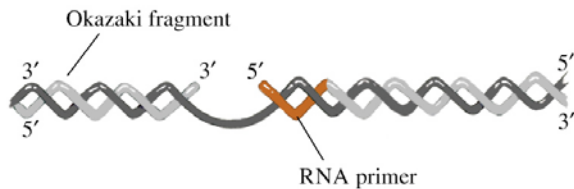


Fig 20.13 (continued)

(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.

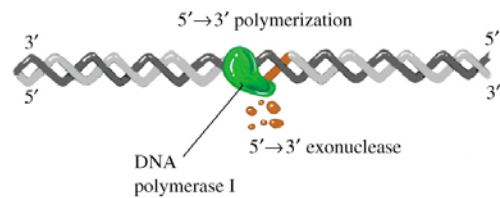


Fig 20.13 (continued)

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

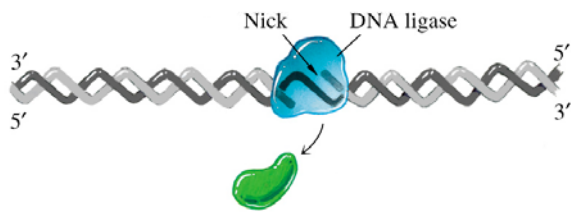
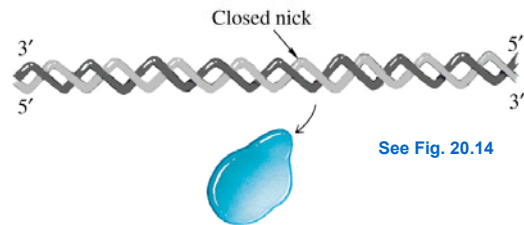


Fig 20.13 (continued)

(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)

Fig 20.15 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.

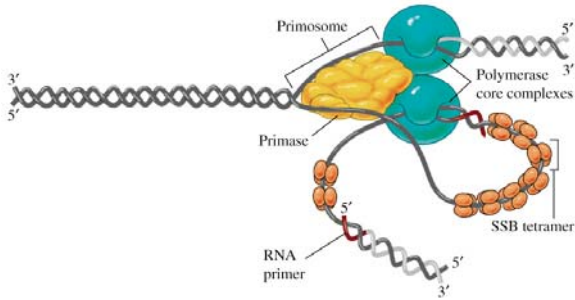


Fig 20.15 (continued)

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

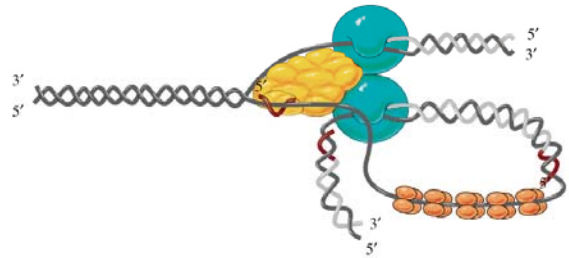


Fig 20.15 (continued)

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

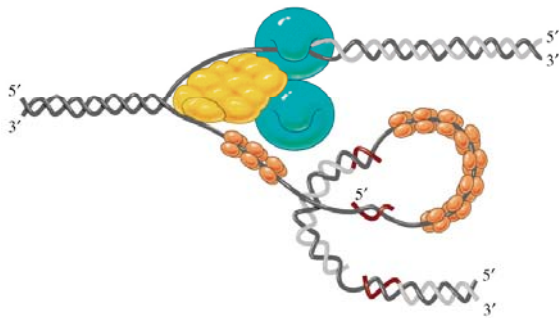
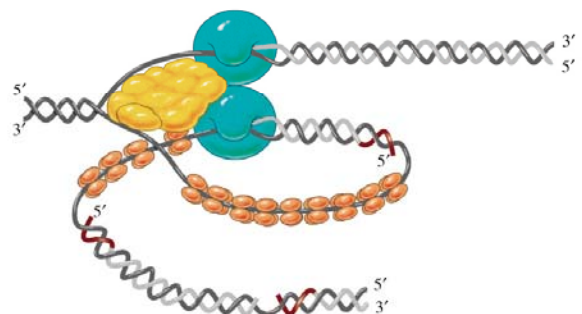


Fig 20.15 (continued)

(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

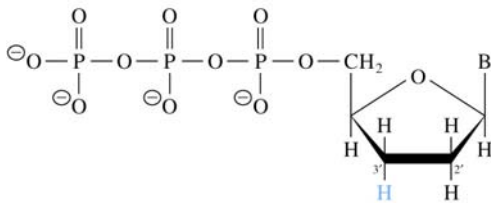
Differences

- 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

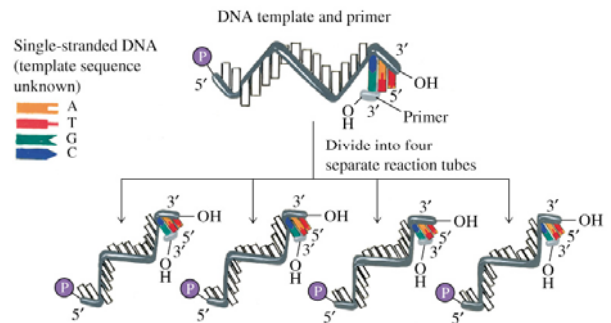
**BCMB 3100 - Chapter 20
DNA Replication**

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

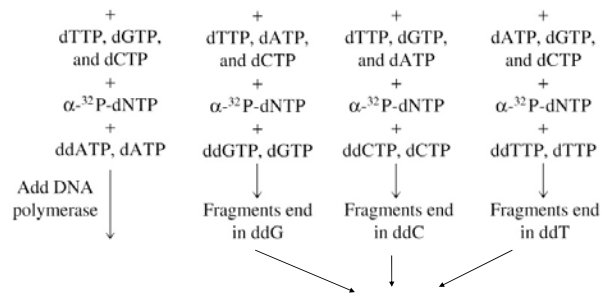
**Dideoxynucleotide Sequencing:
the Sanger Method**



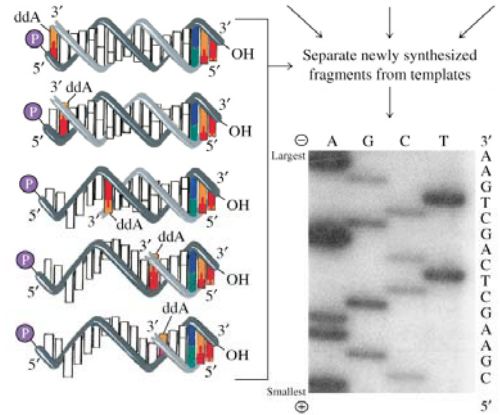
Box 20.1 Sanger method for sequencing DNA



Box 20.1 (continued)



Box 20.1 (cont)



BCMB 3100 - Chapter 20 DNA Replication

- Semi-conservative DNA replication
- DNA polymerase
- DNA replication
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- PCR

Error rate for nucleotide insertion in eukaryotes is 10^{-9} to 10^{-11}

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

<i>E.coli</i> 5'→3' DNA Polymerase	10^{-5}
DNA Pol III 3'→5' exonuclease	10^{-7}
DNA repair enzymes	10^{-9} to 10^{-10}

Fig 20.19 Photodimerization of adjacent thymines induced by UV light

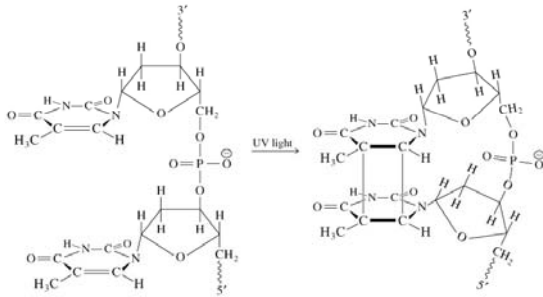


Fig 20.20

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

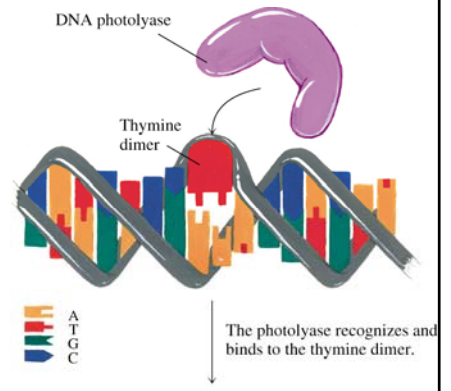
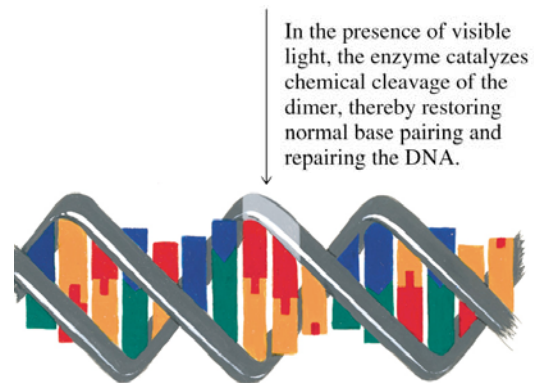


Fig 20.20 (cont)



Fig 20.20 (cont)



Excision Repair Pathway (Figs. 20.23-20.26)

- 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 - Chapter 20 DNA Replication

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

Polymerase Chain Reaction (PCR)

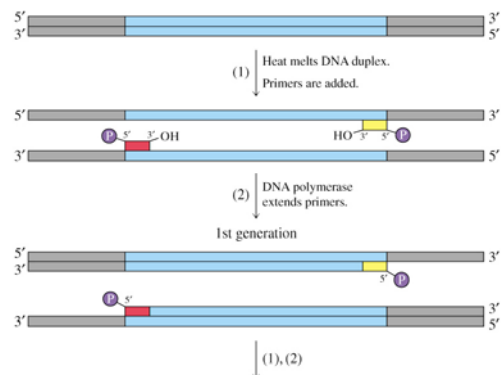
Kary Mullis (1984)

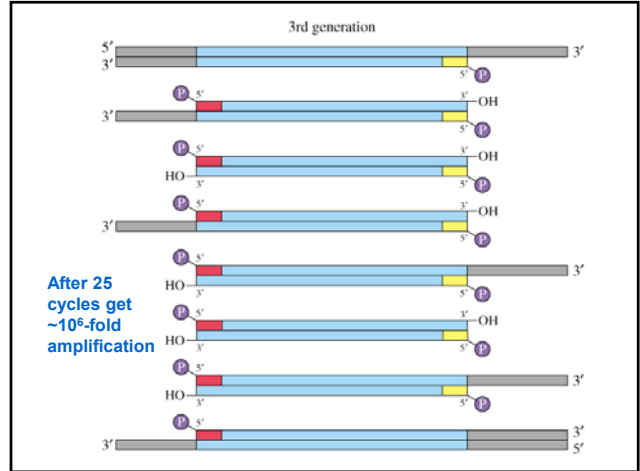
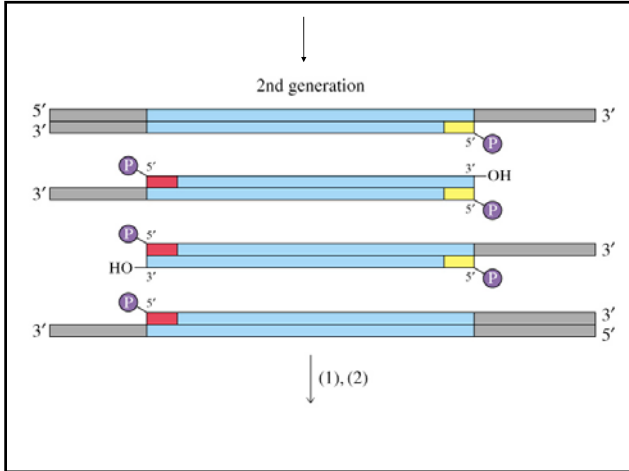
A repetitive method that yields a $\sim 10^6$ -fold amplification of a specific DNA sequence.

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

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

Figure 23.17 Polymerase Chain Reaction (PCR)





Extra Information

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

Fig 20.10

- Okazaki's experiment
- Demonstration of discontinuous DNA synthesis

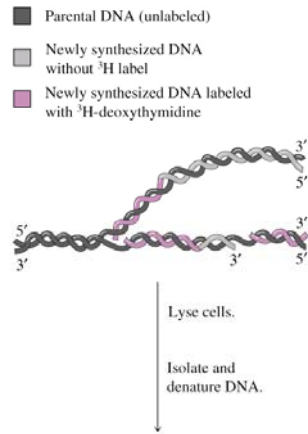


Fig 20.10 (cont)

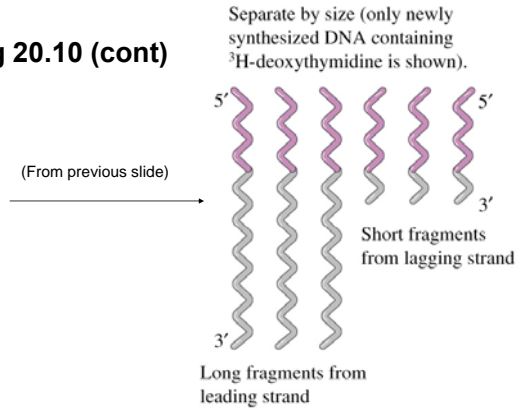


Fig 20.14 Mechanism of DNA ligase in *E. coli*

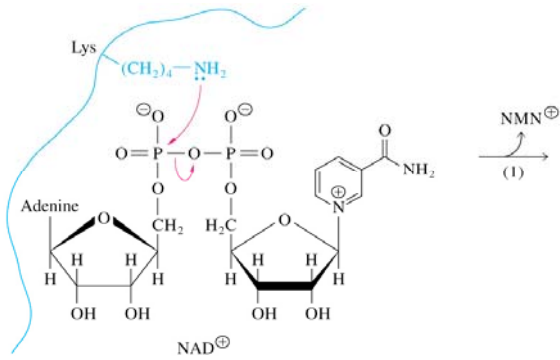
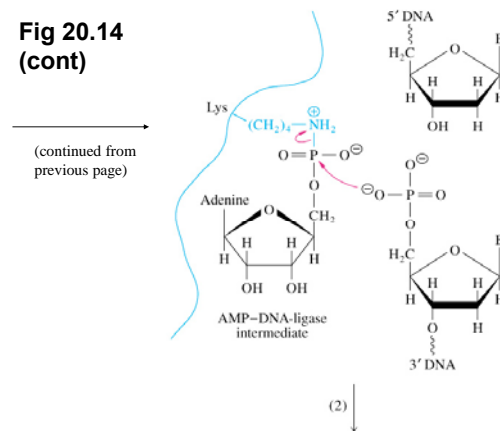
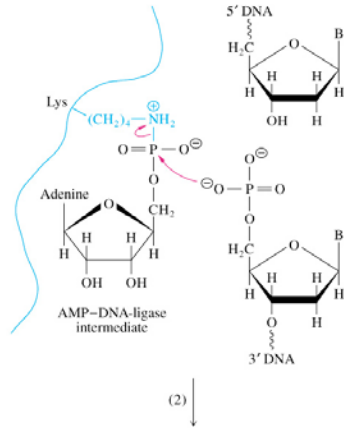


Fig 20.14 (cont)



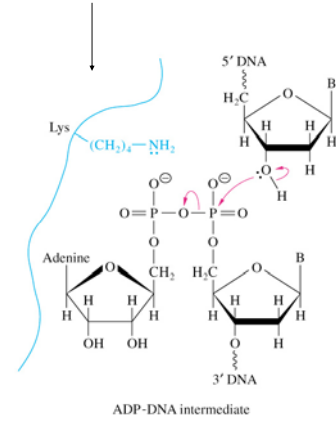
**Fig 20.14
(cont)**

(continued from previous page)



**Fig 20.14
(cont)**

(3)
AMP



**Fig 20.14
(cont)**

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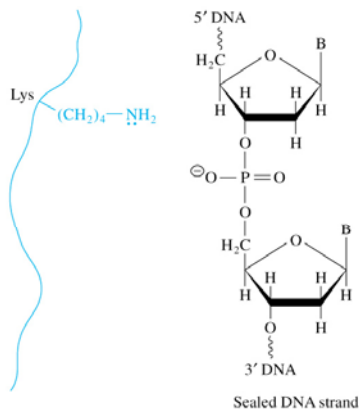


Fig 20.23

- General excision-repair pathway

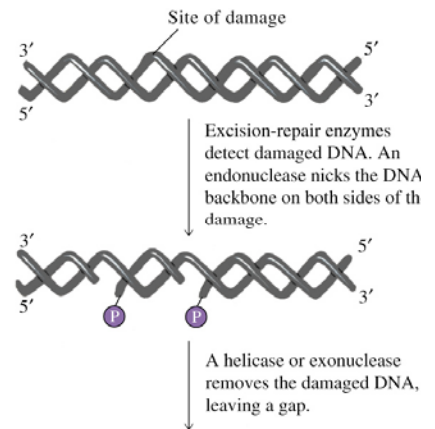


Fig 20.23 (cont)

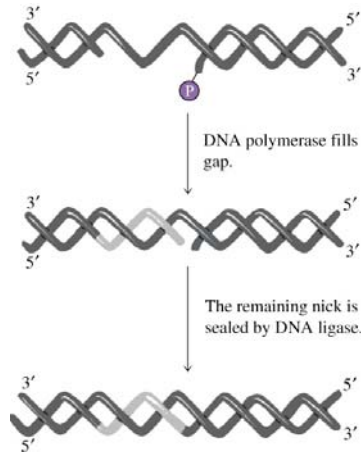


Fig 20.24

- 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

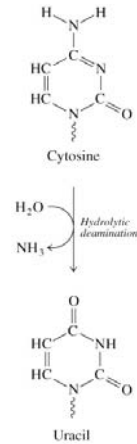


Fig 20.25 Uracil N-glycosylase (human mitochondria)

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

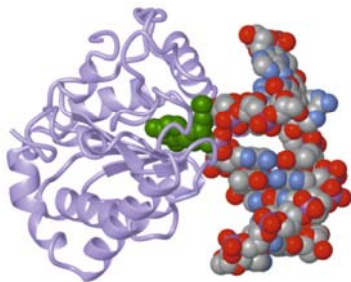


Fig 20.26

- Repair of damage from deamination of cytosine

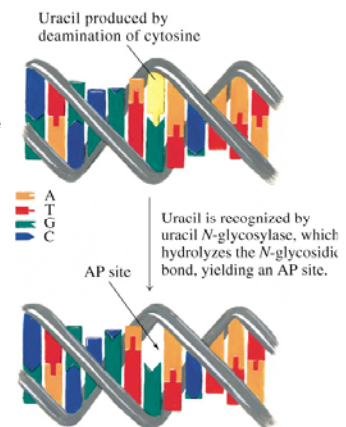


Fig 20.26
(cont)

