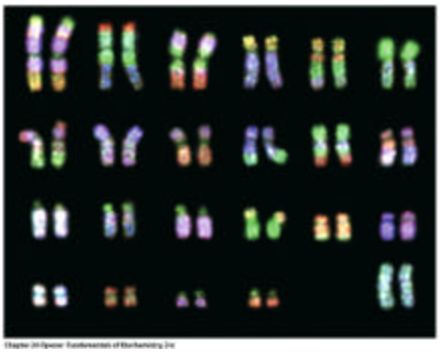
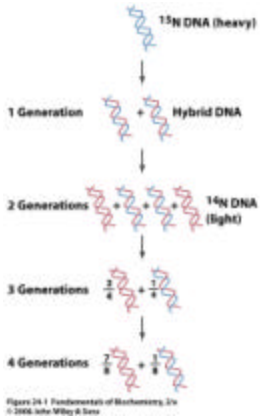
18 DNA Replication and Repair

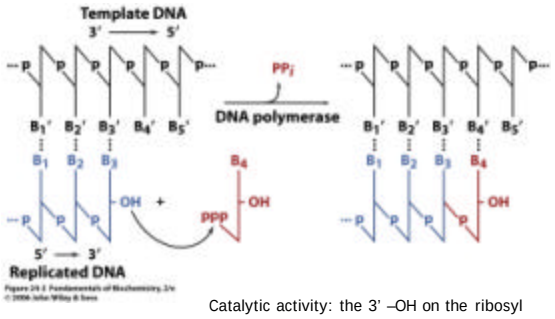


Meselson/Stahl Experiment

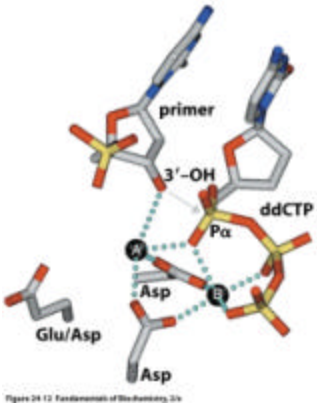
Used labelled nucleotide precursors to follow the synthesis of DNA strands in successive generations.

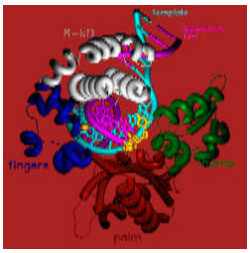
Semiconservative Replication



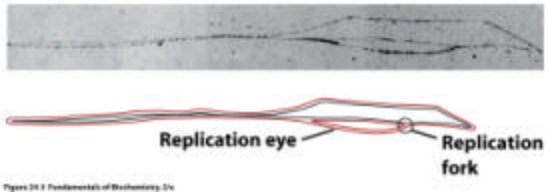


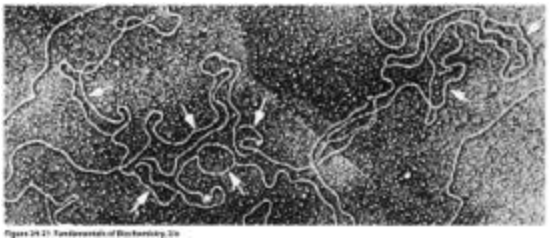
Catalytic activity: the 3' –OH on the ribosyl Group acts as a nucleophile to attack the Alpha-phosphate group. Ppi cleavage makes The reaction irreversible



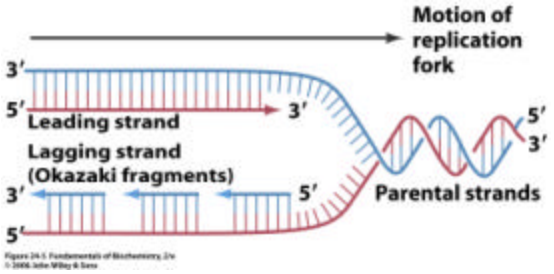


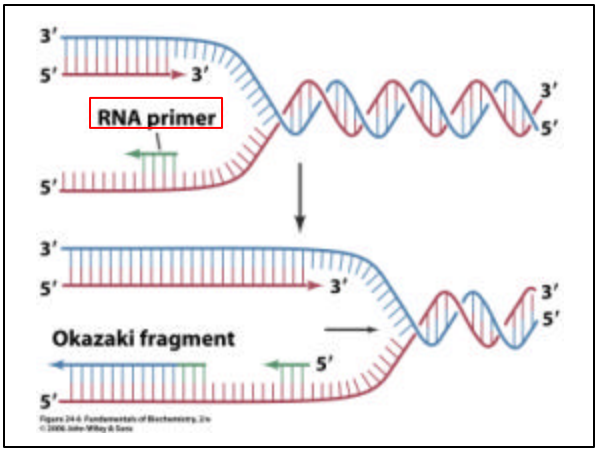
http://chem-faculty.ucsd.edu/kraut/cover.html

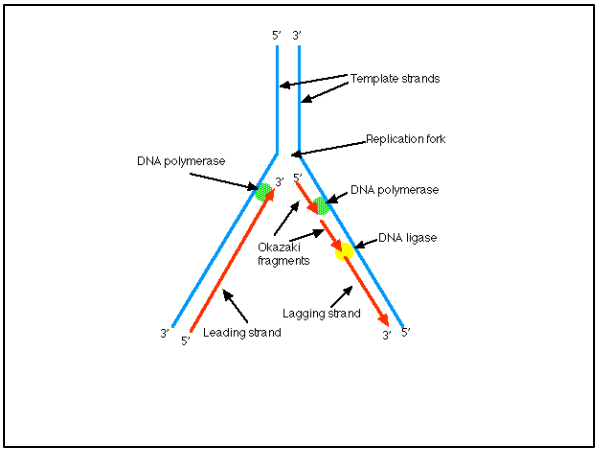


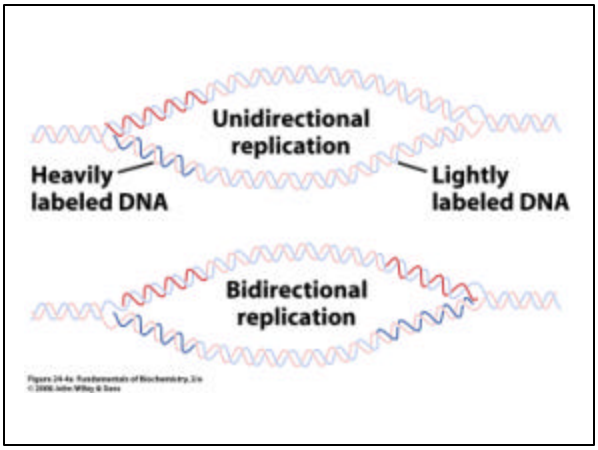


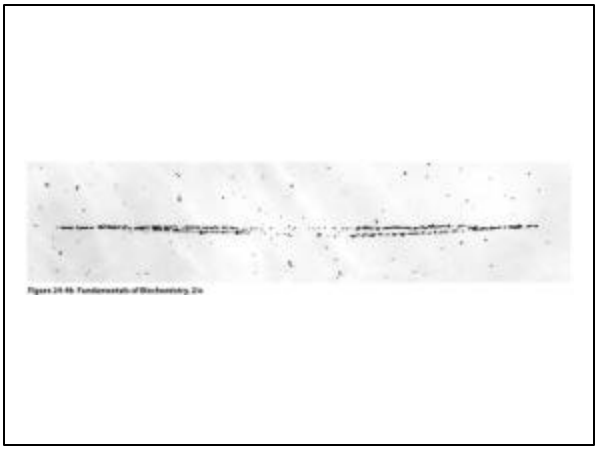
Semidiscontinuous Replication:

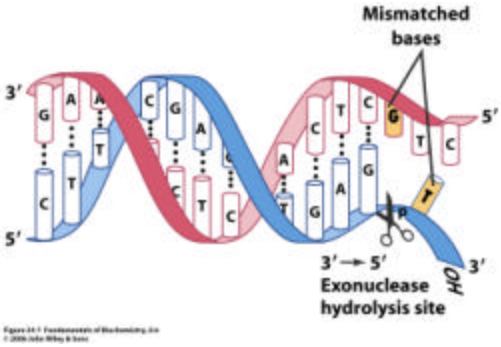



3′
5′
3′
RNA primer
5′
3′
5′
3′
5′
3′
Okazaki fragment
5′
5′
3′
5′

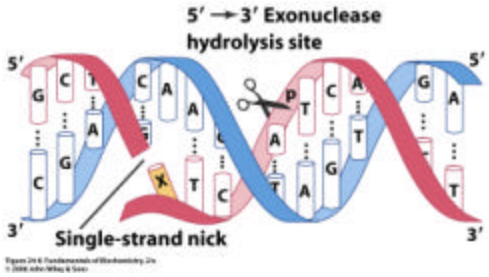

5′ 3′
Template strands
Replication fork
DNA polymerase
3′ 5′
DNA polymerase
DNA ligase
Okazaki fragments
Lagging strand
3′ 5′
Leading strand
3′ 5′


Unidirectional replication
Heavily labeled DNA
Lightly labeled DNA
Bidirectional replication

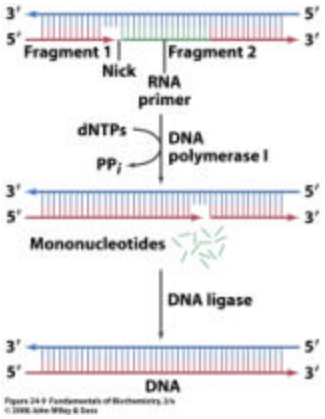




The 3’ to 5’ exonuclease function of DNA POL-I removes mismatched nucleotides at the 3’ position.



The 5’ to 3’ exonuclease function of DNA POL-I removes opens the DNA strand ~10 bp downstream from nick-site.



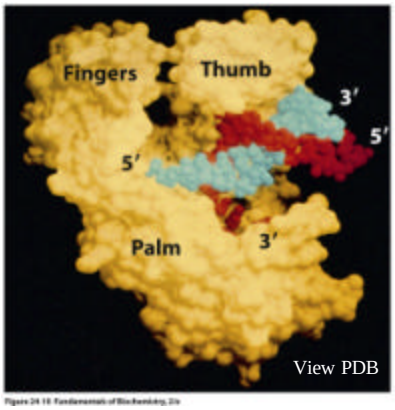


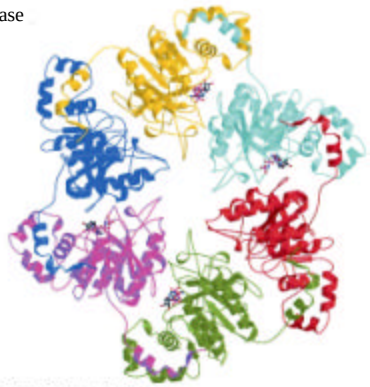
Table 24-1 Properties of *E. coli* DNA Polymerases

	Pol I	Pol II	Pol III
Mass (kD)	103	90	130
Molecules/cell	400	?	10–20
Turnover number[a]	600	30	9000
Structural gene	*polA*	*polB*	*polC*
Conditionally lethal mutant	+	–	+
Polymerization: 5′ → 3′	+	+	+
Exonuclease: 3′ → 5′	+	+	+
Exonuclease: 5′ → 3′	+	–	–

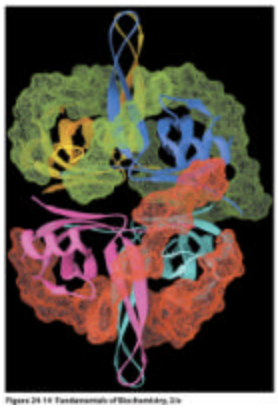
[a]Nucleotides polymerized $min^{-1} \cdot molecule^{-1}$ at 37°C.

Source: Kornberg, A. and Baker, T.A., *DNA Replication* (2nd ed.), p. 167, Freeman (1992).

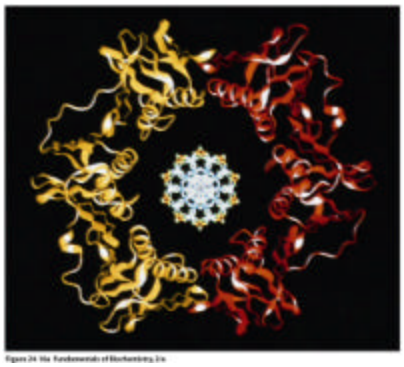
Helicase

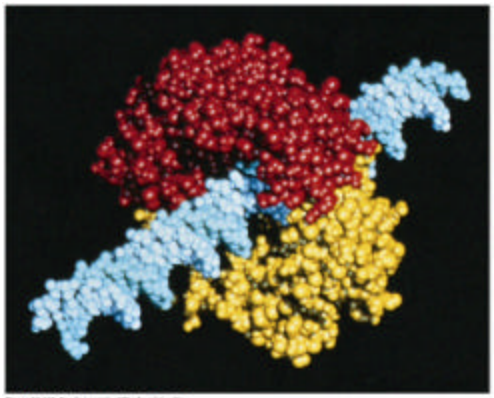


Single-strand DNA binding Protein: SSB

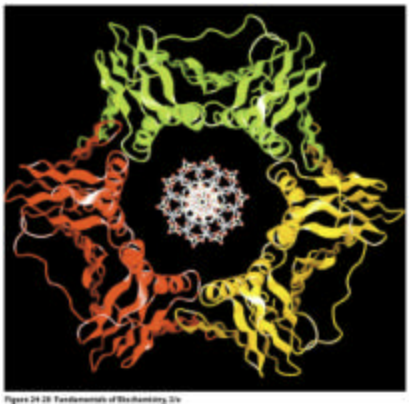


B-clamp

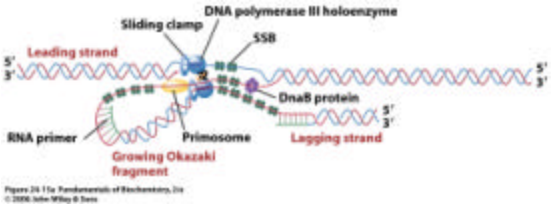




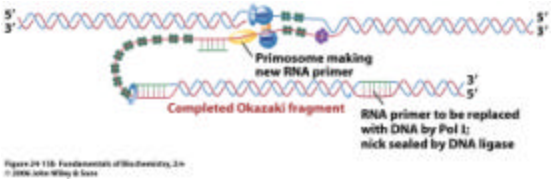
PCNA: proliferating cell nuclear antigen

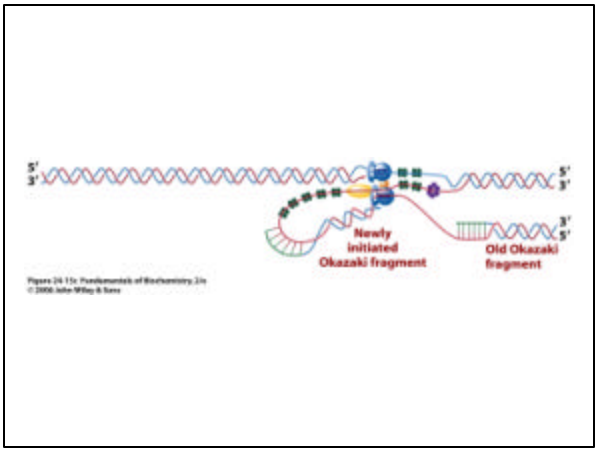


Replisome: 2 DNA pols
Both directions are synthesized simultaneously

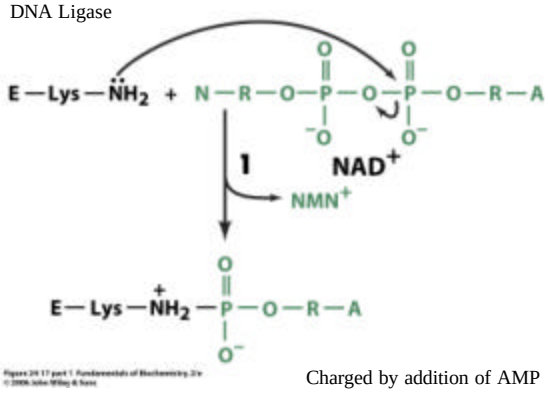


Primosome: complex to sythesize RNA primer fragment

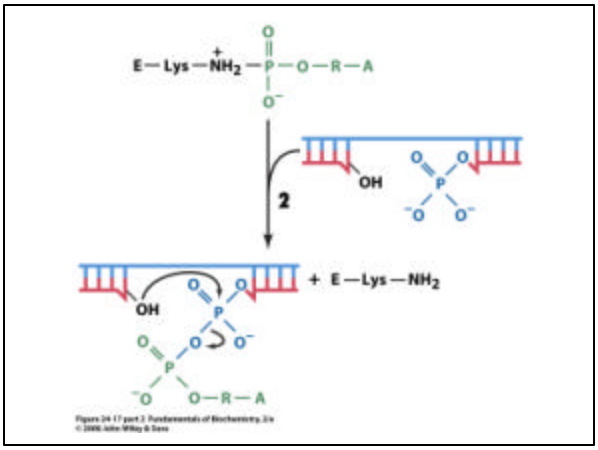




DNA Ligase



Charged by addition of AMP



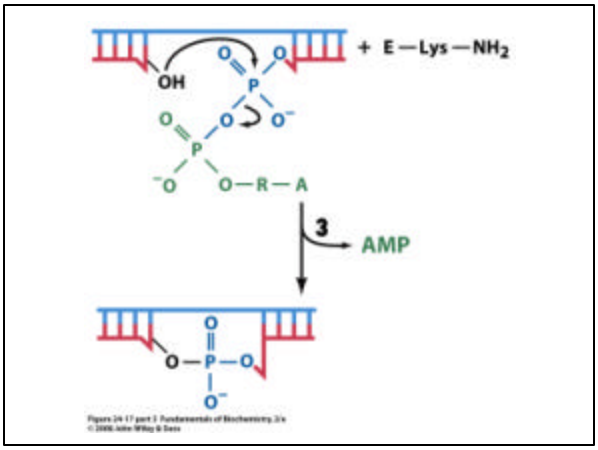


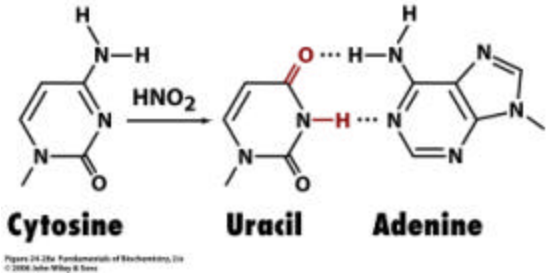
Table 24-2 Properties of Some Eukaryotic DNA Polymerases

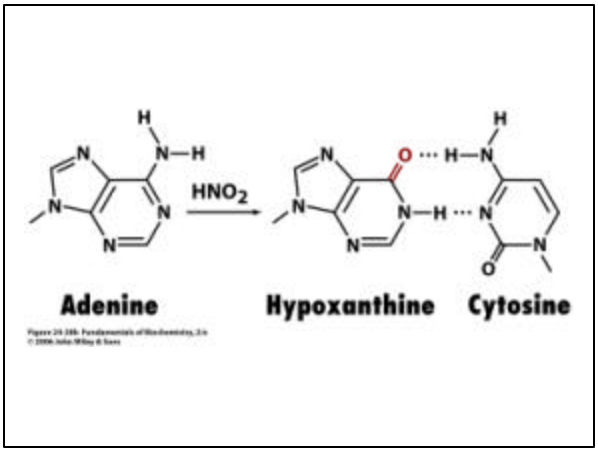
	α	δ	ε
3′ → 5′ Exonuclease	no	yes	yes
Associates with primase	yes	no	no
Processivity	moderate	high	high
Requires PCNA	no	yes	no

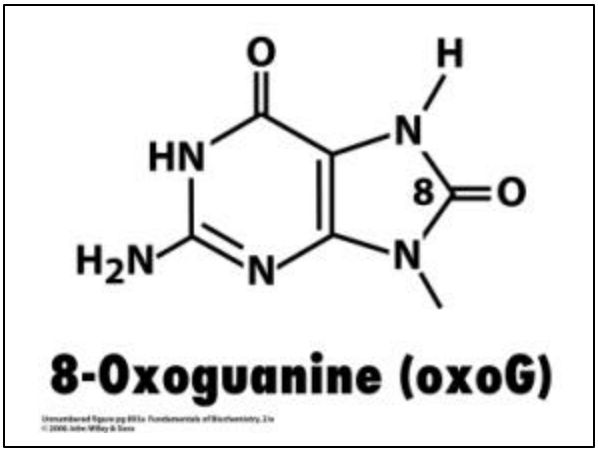
DNA Repair Mechanisms:

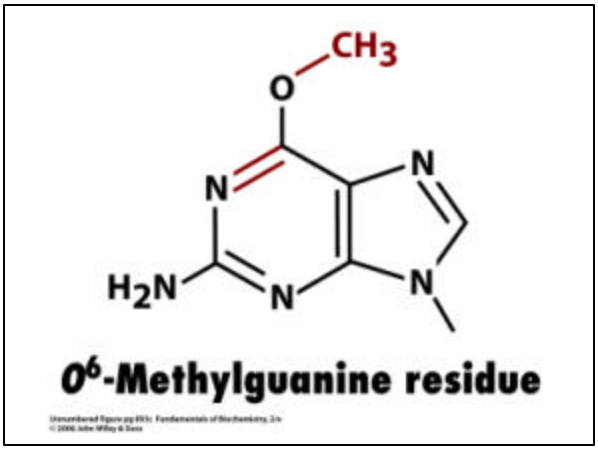
1. Direct Reversal Repair
2. Base Excision Repair
3. Nucleotide Excision Repair
4. Mismatch Repair
5. Error-Prone Repair

UV light induces
Thymine dimerization

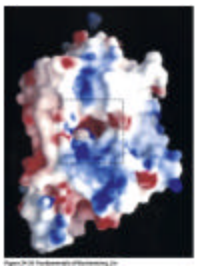






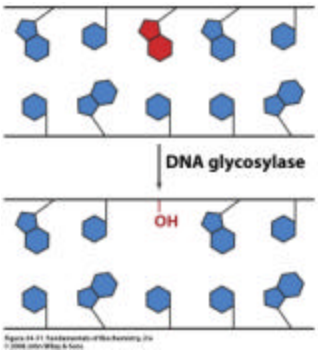


Photolyase:
Direct
DNA
Repair

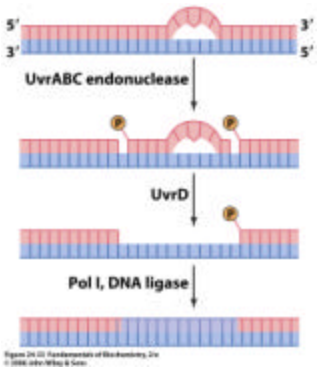


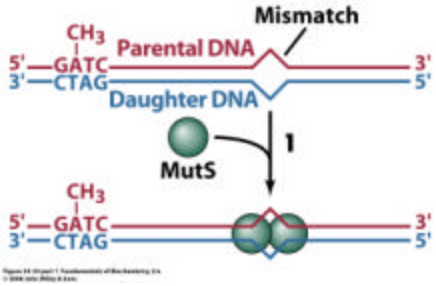
Light repair
phr gene - codes for deoxyribodipyrimidine photolyase that, with cofactor folic acid, binds in dark to T-dimer. When light shines on cell, folic acid absorbs the light and uses the energy to break bond of T dimer; photolyase then falls off DNA

Base repair keeps the sugar backbone intact and employs glycosylases to modify the nucleoside base attached to the ribosyl unit.

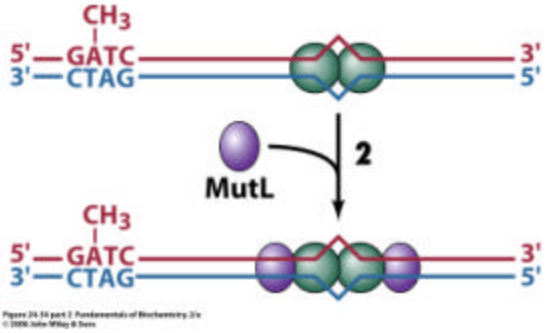


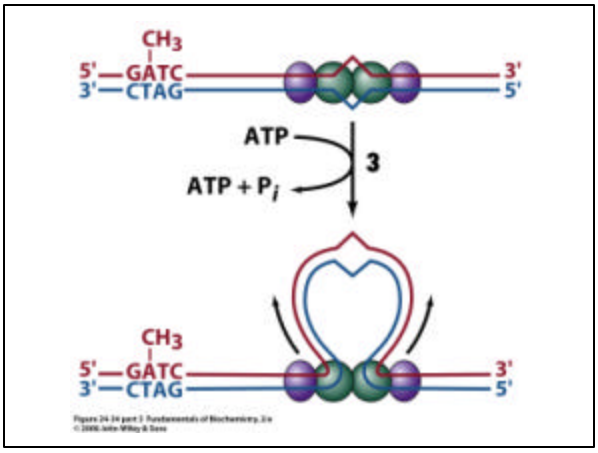
Nucleotide Excision

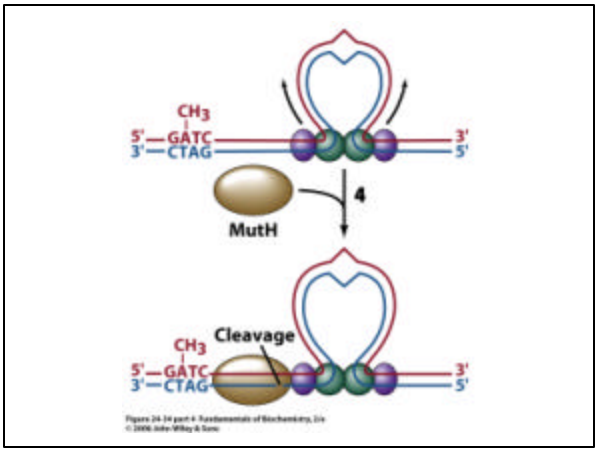


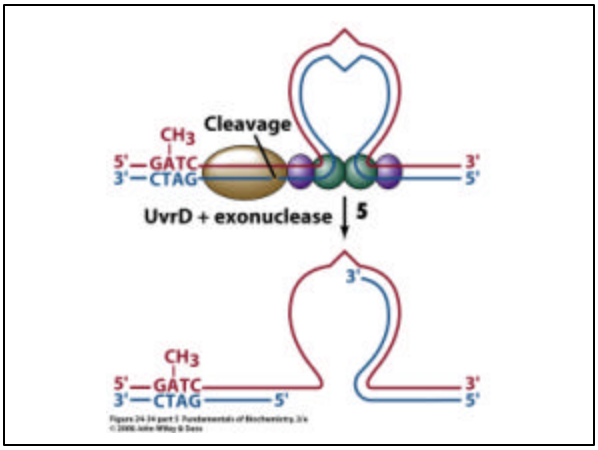


Accounts for 99% of all repairs
Follows behind replication fork.
Two ways to correct mistakes made during replication:
1) 3'>5' exonuclease - proofreading
2) Mismatch repair




CH3
5'—GATC—3'
3'—CTAG—5'
ATP
ATP + Pi
3
CH3
5'—GATC—3'
3'—CTAG—5'


CH3
5'—GATC—3'
3'—CTAG—5'
4
MutH
Cleavage
CH3
5'—GATC—3'
3'—CTAG—5'


Cleavage
CH3
5'—GATC—3'
3'—CTAG—5'
UvrD + exonuclease
5
3'
CH3
5'—GATC—3'
3'—CTAG—5'