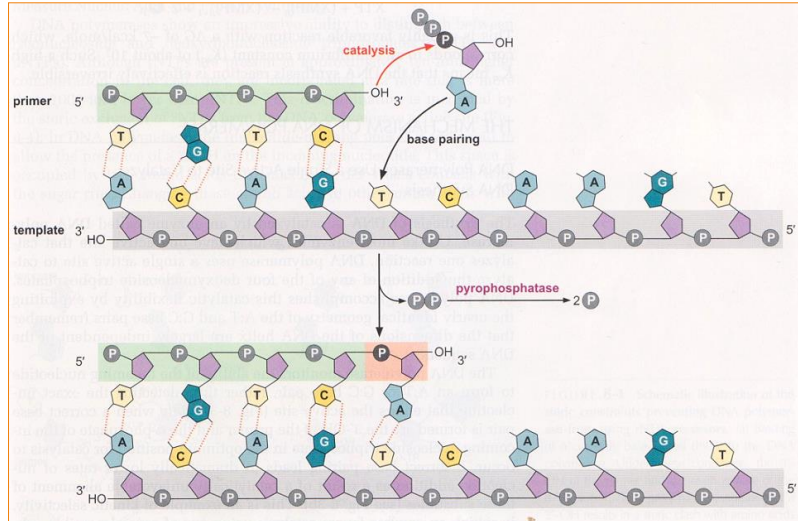
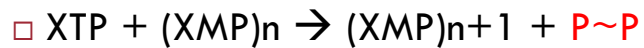




## DNA is synthesized by extending the 3' end of the primer



## What is the driving force for DNA synthesis?



( $\Delta G$  -3.5kCal/mole)



( $\Delta G$  -7kCal/mole)

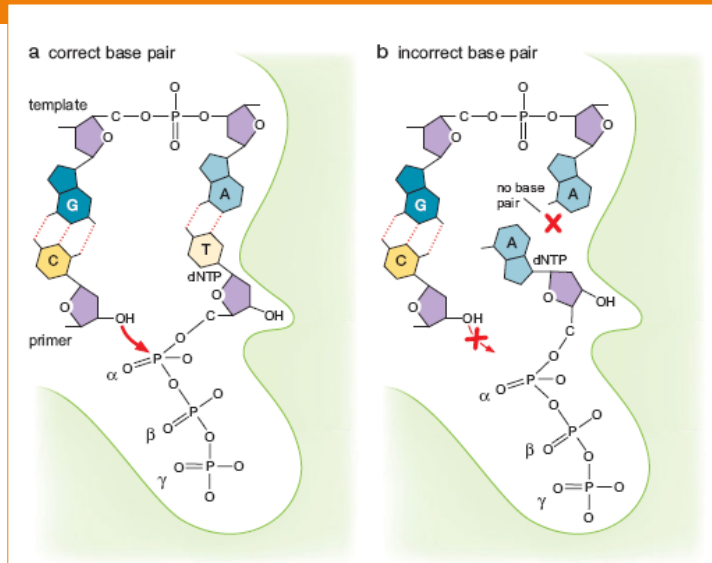
## The Mechanism of DNA Polymerase

- Unlike most enzymes, DNA Pol uses a single active site that catalyze one reaction, i.e, the addition of any of four dNTPs, HOW?
- DNA Pol explores the nearly identical geometry of A:T and G:C base pairs, or it monitors the ability of incoming nt to form an A:T or G:C base pair, rather than detecting the exact that enters the active site
- Only when the correct base pair is formed are the 3'-OH or primer and 5''-P of incoming dNTP are in optimum position for catalysis to occur.

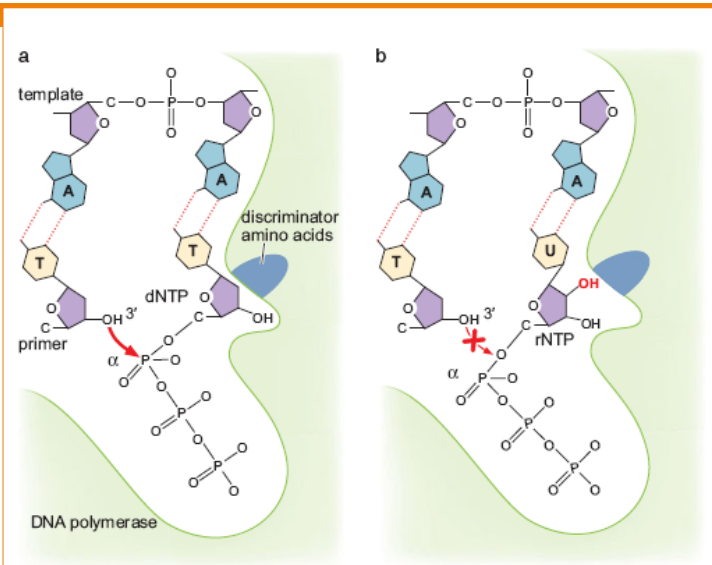
## The Mechanism of DNA Polymerase (cont'd)

- The rate of incorporation of incorrect nt is as much as 10,000-fold slower than incorporation of the correct nt
- DNA pol can distinguish between dNTPs and rNTPs, although rNTPs are present at approx 10-fold higher conc in the cell. This is accomplished by steric exclusion of rNTPs from DNA Pol active site. The nt pocket is too small to allow the presence of 2'-OH of rNTP and this space is occupied by 2 amino acids that make van der Waals contact with sugar ring??

## Correctly paired bases are required for DNA Polymerase-catalyzed nt addition



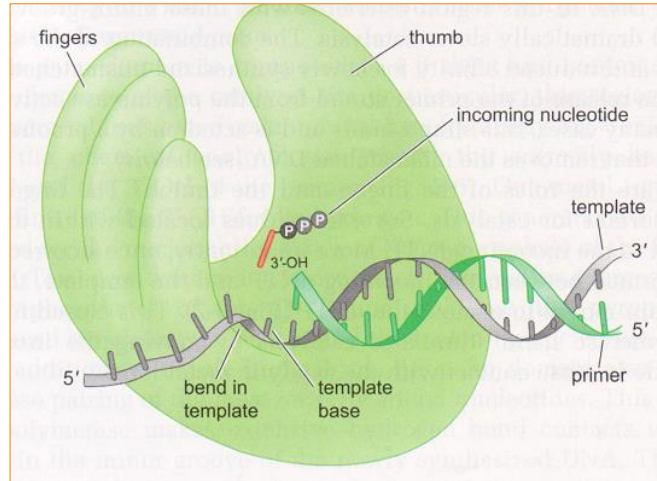
## Steric constraints preventing DNA polymerase from using rNTP precursors



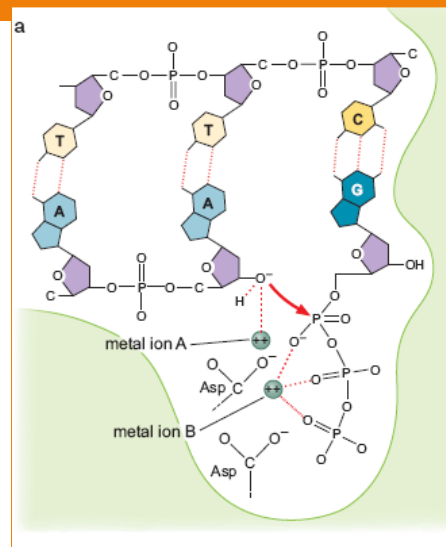
## DNA polymerase resemble a Hand that Grips the Primer: Template junction

Three domains of DNA Pol:

- Thumb,
- Fingers,
- Palm

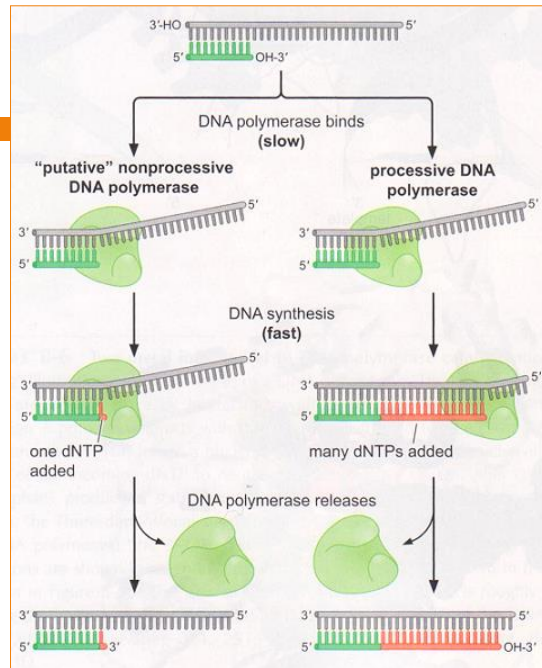


## Two metal ions bound to DNA Polymerase catalyze nt addition

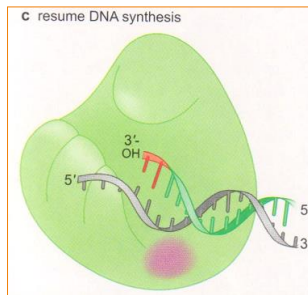
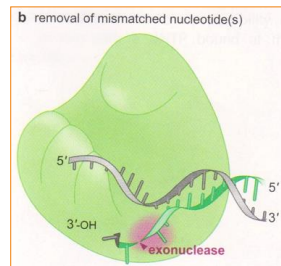
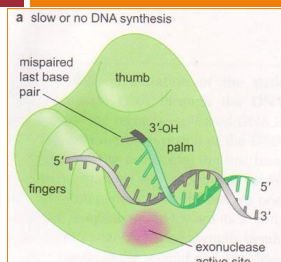


## DNA Polymerases are processive enzymes

- Processivity: average number of nts added each time the enzyme binds a primer:template junction
- Catalysis rate: can be up to 1000 nt/second
- Processivity: depending on the specific enzyme, it can range from few nts to >50,000 bases added per binding event

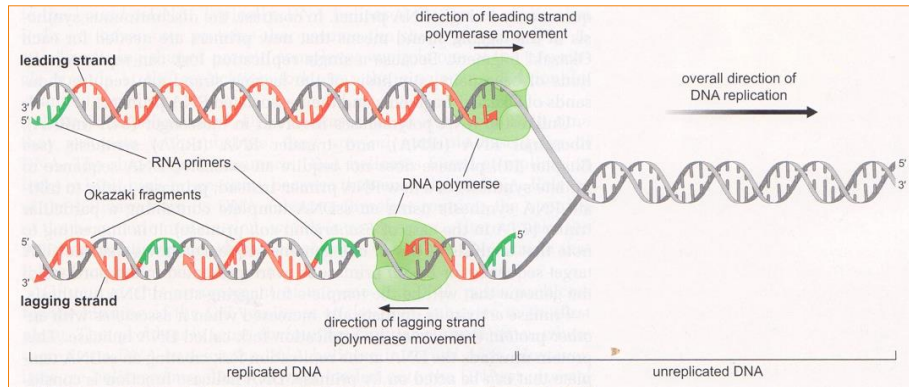


## Exonucleases proofread newly synthesized DNA



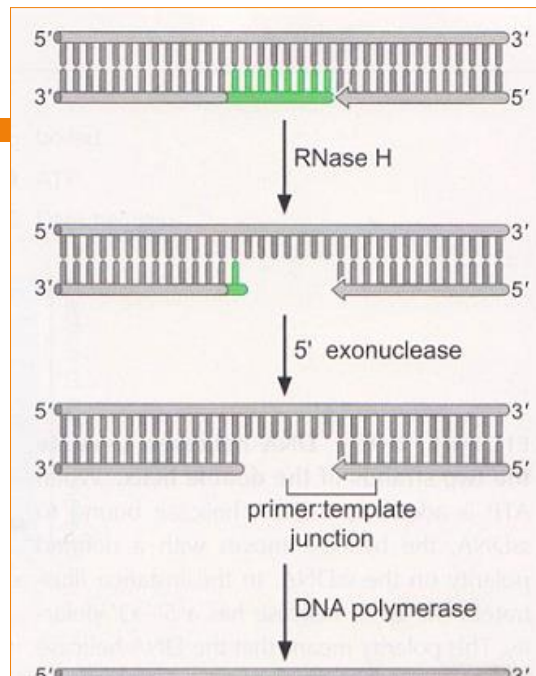
DNA Pol inserts 1 incorrect nt every  $10^5$  nts  
 Proofreading exonucleases decrease the error rate to 1 in every  $10^7$  nts  
 Postreplication mismatch repair process reduces error rate to 1 in  $10^{10}$ ,

**Replication fork:** both strands of DNA are synthesized together at same replication fork



Okazaki fragments length: 1000-2000 bp in bacteria and 100-400 nts in eukaryotes.  
RNA primers: 5-10 nts.

RNA primers must be removed to complete DNA replication



## DNA polymerases are specialized for different roles in the cell

**TABLE 8-2** Activities and Functions of DNA Polymerases

| Prokaryotic ( <i>E. coli</i> )     | Number of Subunits | Function                                |
|------------------------------------|--------------------|-----------------------------------------|
| Pol I                              | 1                  | RNA primer removal, DNA repair          |
| Pol II (Din A)                     | 1                  | DNA repair                              |
| Pol III core                       | 3                  | Chromosome replication                  |
| Pol III holoenzyme                 | 9                  | Chromosome replication                  |
| Pol IV (Din B)                     | 1                  | DNA repair, translesion synthesis (TLS) |
| Pol V (UmuC, UmuD' <sub>2</sub> C) | 3                  | TLS                                     |

Only Pol I & III have proofreading activity and function in DNA replication

## DNA polymerases are specialized for different roles in the cell

| Eukaryotic     | Number of Subunits | Function                                                          |
|----------------|--------------------|-------------------------------------------------------------------|
| Pol $\alpha$   | 4                  | Primer synthesis during DNA replication                           |
| Pol $\beta$    | 1                  | Base excision repair                                              |
| Pol $\gamma$   | 3                  | Mitochondrial DNA replication and repair                          |
| Pol $\delta$   | 2–3                | Lagging-strand DNA synthesis; nucleotide and base excision repair |
| Pol $\epsilon$ | 4                  | Leading-strand DNA synthesis; nucleotide and base excision repair |
| Pol $\theta$   | 1                  | DNA repair of cross-links                                         |
| Pol $\zeta$    | 1                  | TLS                                                               |
| Pol $\lambda$  | 1                  | Meiosis-associated DNA repair                                     |
| Pol $\mu$      | 1                  | Somatic hypermutation                                             |
| Pol $\kappa$   | 1                  | TLS                                                               |
| Pol $\eta$     | 1                  | Relatively accurate TLS past <i>cis-syn</i> cyclobutane dimers    |
| Pol $\iota$    | 1                  | TLS, somatic hypermutation                                        |
| Rev1           | 1                  | TLS                                                               |

## Class activity??

- Both the leading and lagging strands requires primase to initiate DNA synthesis, but the frequency of primase function on the two strands is dramatically different. WHY??
- Why the primase adds an RNA primer, not a DNA primer??

## Class discussion

- Life and biodiversity depend on a happy balance between mutation and its repair!

## Mutability & DNA Repair:

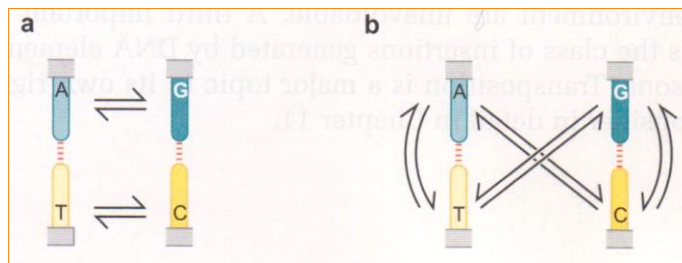
### Sources of mutations

(Chapter 9)

- Important sources
  - ▣ Inaccuracy in DNA replication
  - ▣ Chemical damage to DNA
  - ▣ Insertions generated by mobile DNA elements or transposons
- Consequences of DNA sequence change
  - ▣ Permanent change to DNA (mutation)
  - ▣ Chemical alteration to DNA and prevent its use as a template
- Cell response to mutations: it must scan the genome to detect errors & mend the lesions and if possible restores original sequence

## Replication errors & their repair

- Mutations include almost every conceivable change in DNA sequence
- Switches of one base to another
  - ▣ Transitions: pyrimidine-to-pyrimidine or purine-to-purine
  - ▣ Transversions: pyrimidine-to-purine or purine-to-pyrimidine



## Replication errors & their repair

- Insertions & deletions: either small or drastic changes
- Mutations can arise spontaneously at any given site and ranges from  $10^{-8}$  to  $10^{-11}$  per round of DNA replication
- Some sites are hot spots: simple sequence repeats of di- or tri- or tetra-nucleotide sequences (DNA microsatellites)
- Slippage occurs during replication of DNA microsatellites (repetitive DNA sequences), resulting in increase or decrease of repeats >> generates polymorphisms
- Examples: the “CA” repeat sites are highly polymorphic in the population

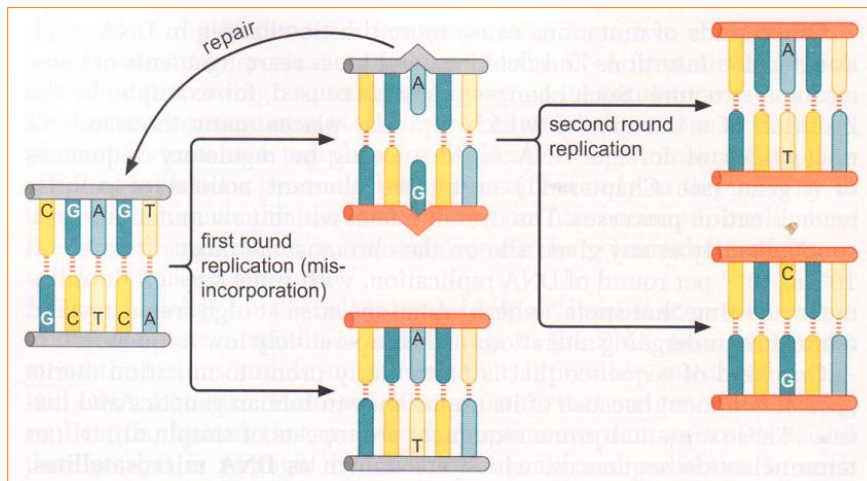
## Expansion of Triple repeats causes Disease

- Huntington Disease
  - Caused by expansion of the “CAG” repeat
  - CAG is the codon for Gln, & its expansion results in extended stretch of Gln in the mutant protein
  - Normal individuals carry 9-35 CAG repeats (average 18-19); affected individuals have >40 repeats; borderline repeats 36-39 repeats have low penetrance
  - The poly-Gln interferes with normal interaction of protein with transcription factor Sp1 & the corresponding Gln-rich patch in the TAFII130 ( a subunit of the transcription factor TFIIID) > impairs transcription in neurons and brain

## Expansion of Triple repeats causes Disease

- Other diseases caused by triplet expansion includes
  - adult muscular (myotonic) dystrophy
    - ▣ Caused by expansion of CTG repeat in 3' UTR of a protein kinase gene or DMPK
    - ▣ Normal range of repeats is from 5 to 30
- fragile X syndrome
  - ▣ Caused by expansion of CGG repeat in 5' UTR of FMR1 gene)
  - ▣ Normal range of repeats is up to 60

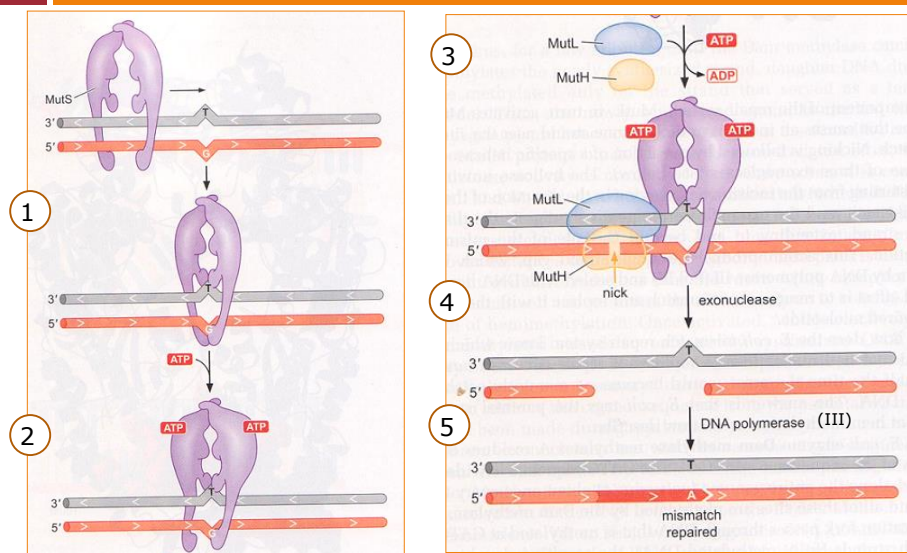
## Some replication errors escape proofreading



## Mismatch repair removes errors that escape proofreading

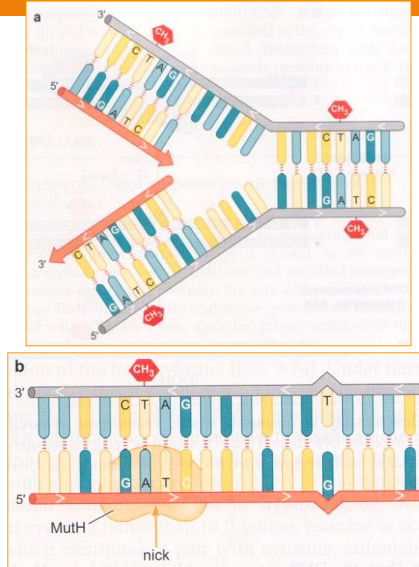
- Mismatch repair system: responsible for the fidelity of DNA replication
- Functions:
  - ▣ It must scan the genome for mismatches, mismatches are transient
  - ▣ It must correct the mismatch accurately
  - ▣ In *E. coli* mismatches are detected by the MR protein: MutS (has ATPase activity)
  - ▣ MutS embraces the mismatch, induces a kink in DNA, recruits MutL. The MutL activates MutH (endonuclease)
  - ▣ The UvrD Helicase unwinds the DNA starting at incision site

## Mismatch repair removes errors that escape proofreading: mismatch repair system



How does the E. coli mismatch repair system recognize which of the 2 mismatched nts to repair?

- E. coli Dam methylase, methylates A residues on both strands of the sequence 5'-GATC-3'
- MutH makes incision in unmethylated daughter strand



## DNA damage:

by Spontaneous Hydrolysis, Deamination & depurination

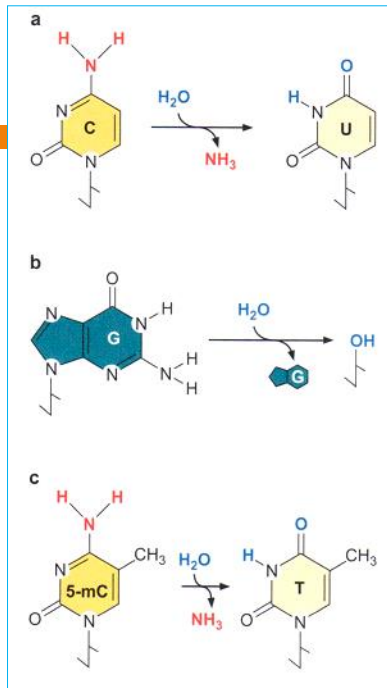
- Other Causes of damage:
  - ▣ Environmental factors like radiation
  - ▣ Chemical mutagens
- Examples:
  - ▣ Spontaneous deamination of C >> U (U pairs with A rather than G)
  - ▣ Spontaneous deamination of A or G >> hypoxanthine (pairs with C) Or xanthine (continues to pair with C but with 2 H-bonds), respectively
  - ▣ Depurination by spontaneous hydrolysis of N-glycosyl linkage >> abasic nt

## Mutations due to hydrolytic damage

- (a) Deamination of C to U
- (b) Depurination of G to abasic nt
- (c) Deamination of 5mC to T (natural nt??)

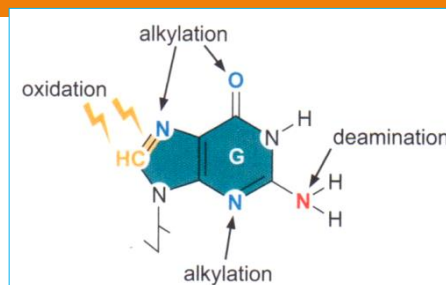
Why DNA contains T instead of U ??

5mC are generated by DNA methylases > 5mC are hot spots for spontaneous mutations?

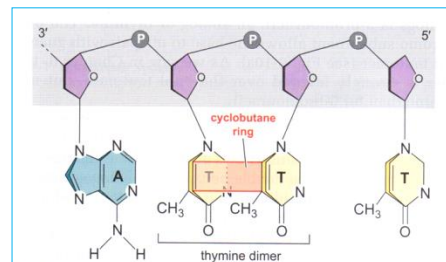


## DNA damage by Alkylation, oxidation and radiation

G modification



Thymine dimers



### DNA damage by Alkylation, oxidation and radiation

- **Alkylation:** methyl or ethyl groups are transferred to reactive sites on bases & phosphates in DNA backbone
- Example: Nitrosamines, which methylates G to O<sup>6</sup>-methylguanine which mispairs with T (G:C > A:T)
- **Oxidation:** via ROS (O<sub>2</sub><sup>-</sup>, H<sub>2</sub>O<sub>2</sub>, OH.) generated from ionizing radiation & chemicals
- Oxidation of G > oxoG which pairs with A (G:C to A:T) & C
- G:C to A:T transversion is very common in human cancers

### DNA damage by Alkylation, oxidation and radiation

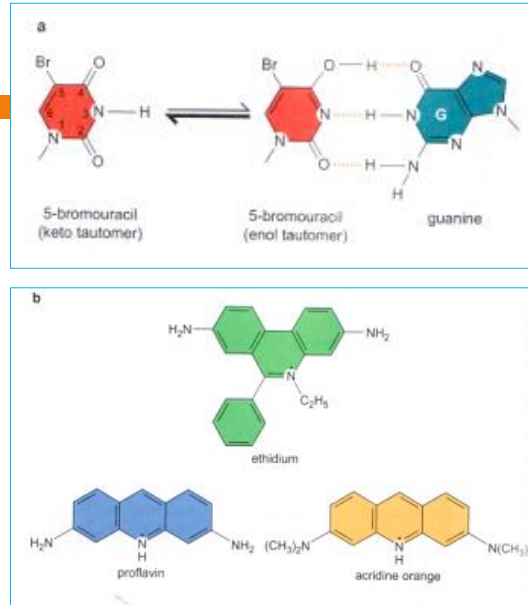
- **Radiation with UV light:** forms T-dimers
- Gamma radiation & X-rays (ionizing radiation): cause double-strand breaks and can generate free radicals
- Many anticancer drugs like Bleomycin cause breaks in DNA (clastogenic agents)

Mutations are caused by:

(a) base analogs

&

(b) intercalating agents



## Repair of DNA damage

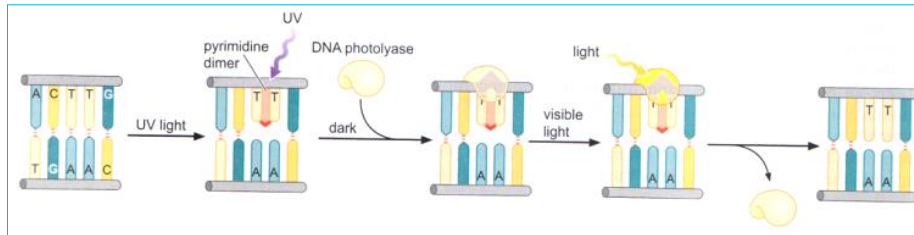
### DNA repair systems

| Type                       | Damage                                    | Enzyme                                                           |
|----------------------------|-------------------------------------------|------------------------------------------------------------------|
| Mismatch repair            | Replication errors                        | MutS in <i>E. coli</i> , MSH in humans                           |
| Photoreactivation          | Pyrimidine dimers                         | DNA photolyase                                                   |
| Base excision repair       | Damaged bases                             | DNA glycosylase                                                  |
| Nucleotide excision repair | Pyrimidine dimers, bulky adducts on bases | UvrA/B/C/D in <i>E. coli</i> , XPC/A/D, ERCCI-XPF, XPG in humans |
| Double-strand break repair | Double-strand breaks                      | RecA & RecBCD in <i>E. coli</i>                                  |
| Translesion DNA synthesis  | Pyrimidine dimers or apurinic site        | Y-family DNA polymerases, such as UmuC in <i>E. coli</i>         |

## Direct reversal of DNA damage:

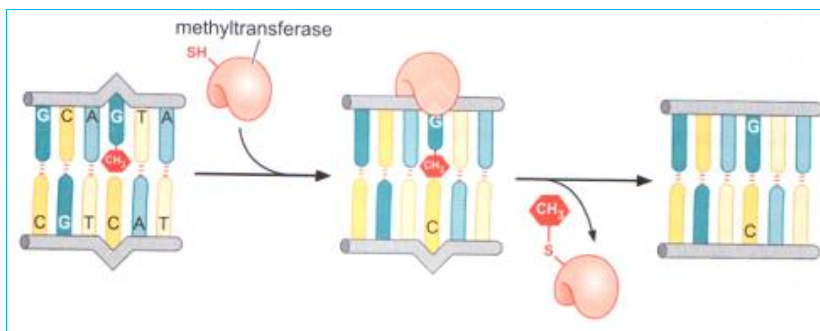
### Photoreactivation

Photoreactivation cause direct reversal of DNA damage



## Direct reversal of DNA damage:

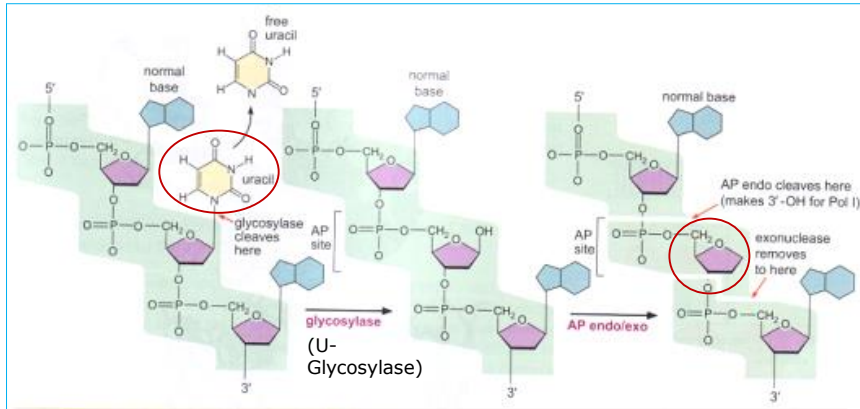
### removal of CH<sub>3</sub> group from O<sup>6</sup>-mG



## Repair of DNA damage:

### Base excision repair pathway

Glycosylase: U-glycosylase; AP: apurinic or apyrimidinic



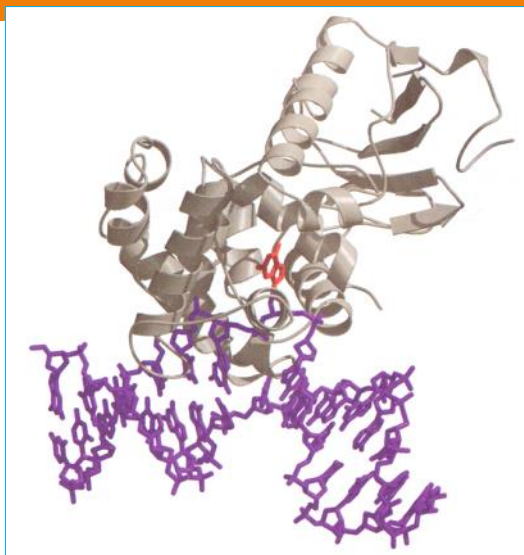
After the damaged nt is removed a repair DNA Pol and DNA ligase restore the intact strand

## Structure of a DNA Glycosylase complex

How the glycosylase recognize the damaged base buried in the helix?

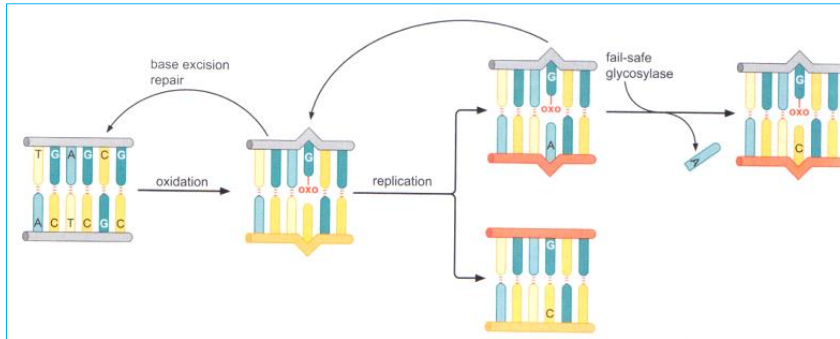
Damaged base is flipped out of the helix (?)

Cells have 8 different glycosylases, & DNA glycosylases are lesion-specific:  
e.g. U- and oxoG-glycosylases



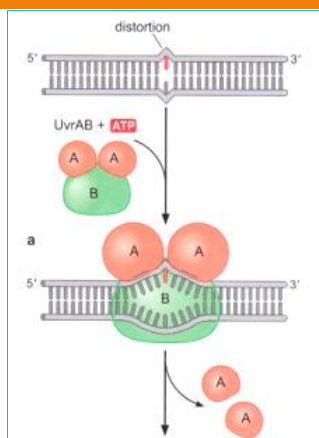
## oxoG:A repair and the Fail-safe Glycosylase

oxoG Glycosylase removes the undamaged incorrect A opposite oxoG

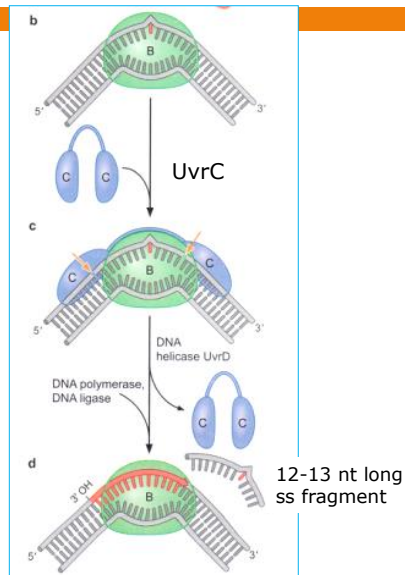


Another Fail-safe glycosylase removes T opposite to G, that results from deamination of 5mC to T

## Repair of DNA damage: Nucleotide excision repair pathway



Nucleotide excision repair in eukaryotes is similar but more complex, involving 25 or more polypeptides



Clinical correlations:

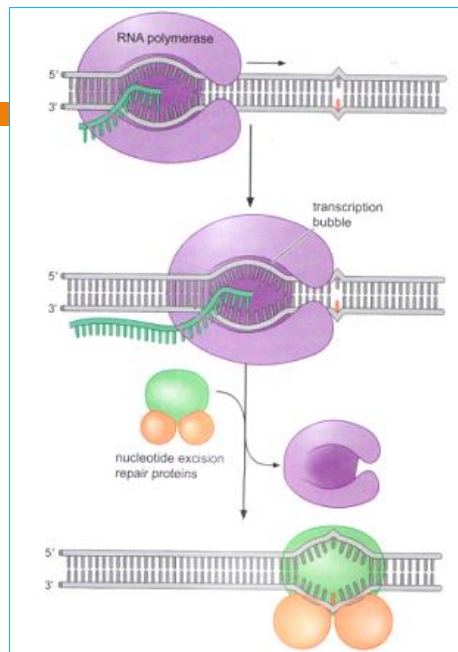
### UVR proteins & Xeroderma pigmentosum

- Mutants of the uvr genes are sensitive to UV light and lack the capacity to remove T-dimers
- In human seven XP genes (XPA-G) are responsible for the Nucleotide excision repair system
- Defects in the XP genes give rise to Xeroderma pigmentosum (sun-light induced skin cancer)

### Transcription-coupled repair >

RNA polymerase serves as another damage-sensing protein. It recruits the Nucleotide excision repair proteins and backs up or dissociates from DNA

In eukaryotes, the TFIIH is crucial for this process with its subunits XPA & XPD



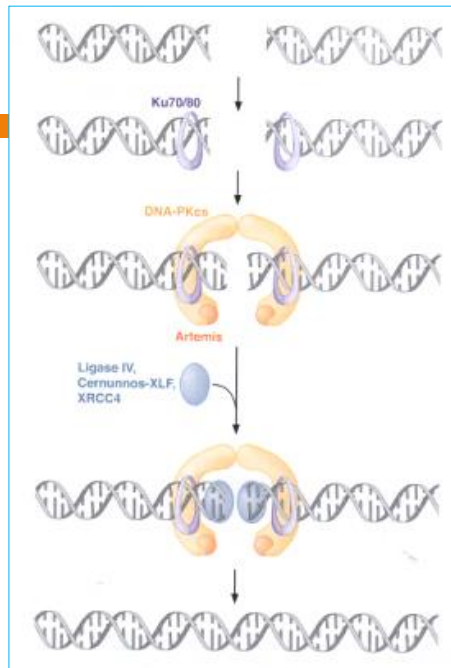
## Recombination repairs DNA breaks

- How do cells repair double-strand breaks (DSB) in DNA in which both strands of the duplex are broken?
- >>By the DSB repair pathway> retrieves sequence information from undamaged sister chromosome
- Alternatively and under some conditions, SBs are repaired by direct joining of broken ends> non-homologous end joining (NHEJ)
- NHEJ protects and process broken ends then join them
- NHEJ is mutagenic

## Mammalian pathway for NHEJ

In mammalian cells, 7 components of NHEJ pathway are known: Ku70, Ku80, DNA-PKs, Artemis, Ligase IV, Cernunnos-XLF & XRCC4.

Artemis has a 5' to 3' exonuclease and endonuclease activities that are activated by phosphorylation by DNA-PKcs.



## Translesion DNA synthesis:

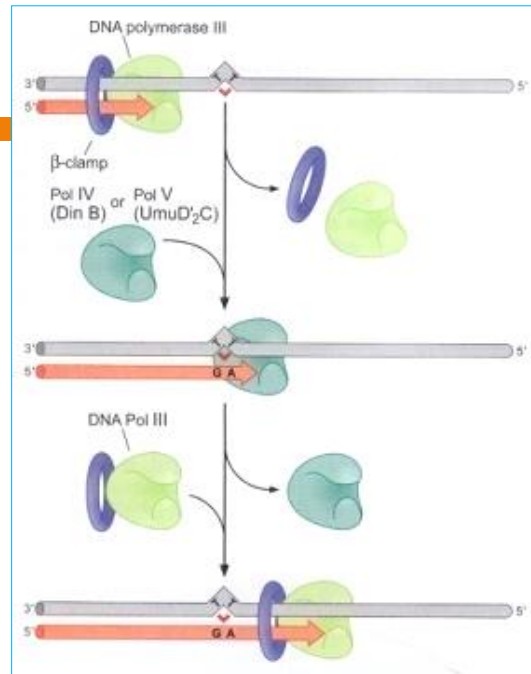
It enables Replication to proceed across DNA damage

TLS is highly error-prone!!

In E.coli, TLS is carried out by UmuC & UmuD' >> a member of Y family of DNA Polymerases

Nucleotide synthesis is not guided by base pairing but may not be random!!

In E. coli Translesion Polymerase is not present under normal conditions



## Translesion DNA synthesis:

- In TLS Nucleotide synthesis is not guided by base pairing but may not be random!! For example, in human a member of Y family of translesion DNA Polymerases correctly inserts two A residues opposite a thymine dimer!
- In E. coli Translesion Polymerase is not present under normal conditions. The genes encoding translesion polymerases are expressed as part of a pathway known as the SOS response