DNA REPLICATION, DNA MUTABILITY & DNA REPAIR

Course: Molecular Biology (02022312)

Instructor: Dr. M A Srour

Textbook:
Watson J, Baker TA, Bell SP, Gann A, Levine M, Losick R (2008). Molecular Biology of the Gene, 6th ed. Chap 8 / pp.195-256; Chap 9 /pp.257-82

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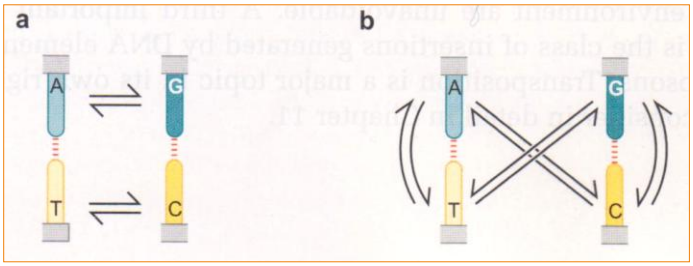
Mutability & DNA Repair:

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



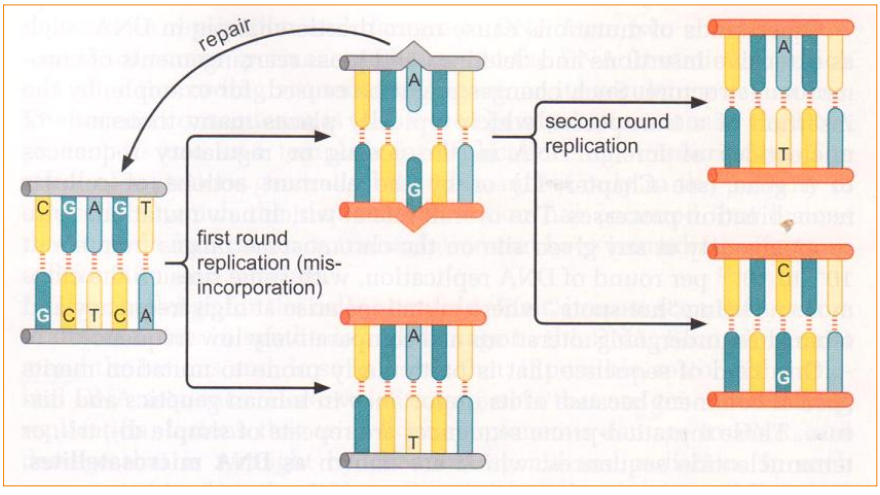
Replication errors & their repair

- 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
  - 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 TFIID) > impairs transcription in neurons and brain
- Other diseases caused by triplet expansion unclues adult muscular (myotonic) dystrophy & fragile X syndrome

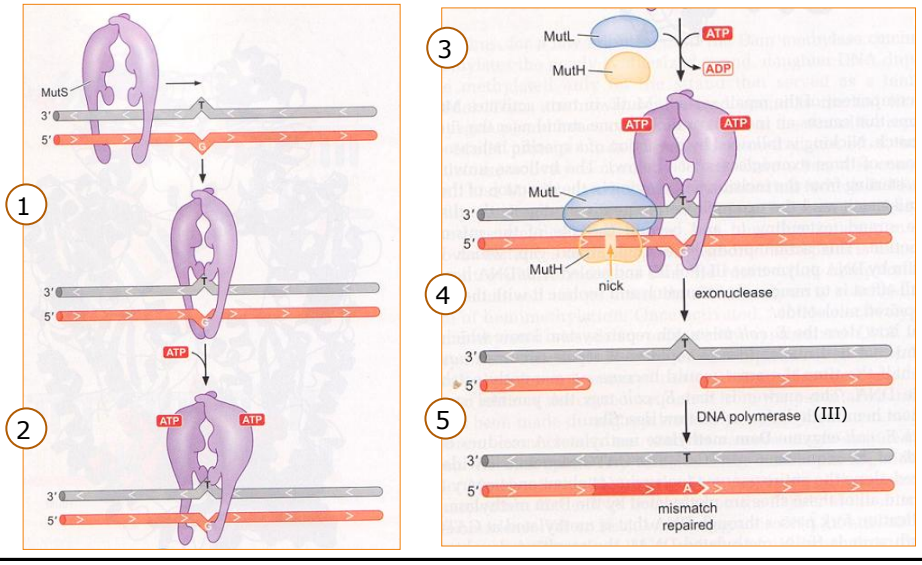
Some replication errors escape proofreading



Mismatch repair removes errors that escape proofreading

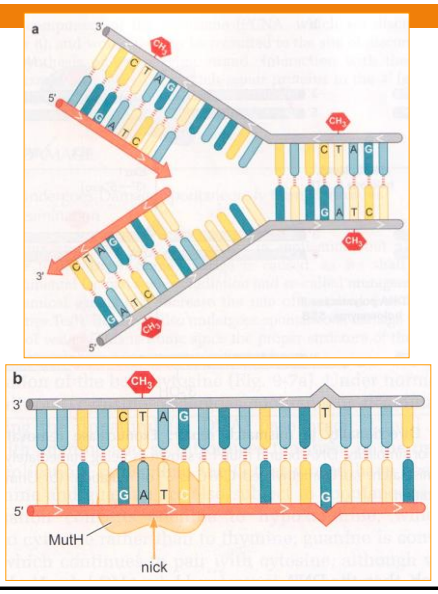
- Mismatch repair system: responsible for the fidelity of DNA replication
- Functions:
  - It must scan the genome for mismatches, mismatches are transient
  - It must correct the mismatch accurately
  - In E. coli mismatches are detected by the MR protein: MutS (has ATPase activity)
  - MutS embraces the mismatch, induces a kink in DNA, recruits MutL. The MutH 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 recognizes 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

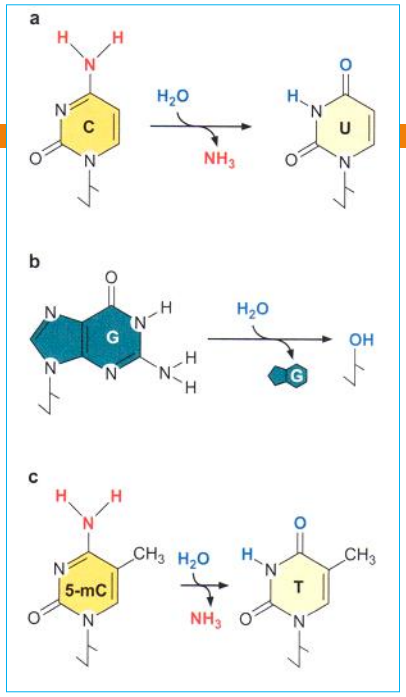
- Other Causes of damage:
  - Environmental factors like radiation
  - Chemical mutagens
- Examples:
  - Spontaneous deamination of C >> U (U pairs with A rather than G)
  - Spontaneous deamination of A or G >> hypoxanthine (pairs with C) Or xanthine (continues to pairs with C but with 2 H-bonds), respectively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

Mutation 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??)

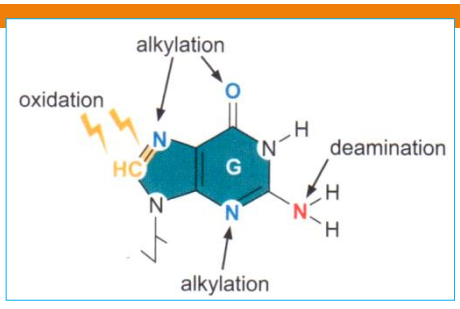
Why DNA contains T instead of U ??

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

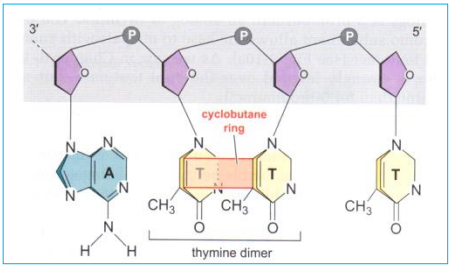


DNA damage by Alkylation, oxidation and radiation

G modification



Thymine dimers



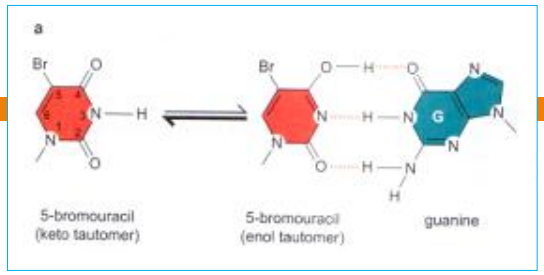
DNA damage by Alkylation, oxidation and radiation

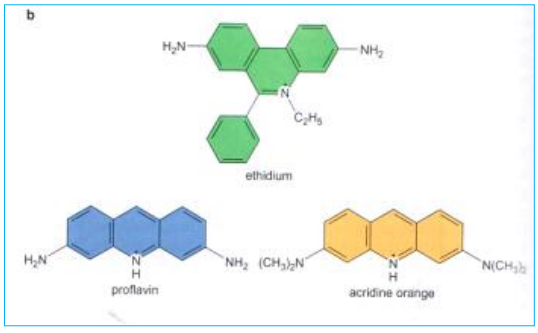
- Alkylation: methyl or ethyl groups are transferred to reactive sites on bases & phosphates in DNA backbone
- Example: Nitrosamines, which methylates G to O^6-methylguanine which mispairs with T (G:C > A:T)
- Oxidation: via ROS (O2-, H2O2, OH.) generated from ionizing radiation & chemicals
- Oxidation 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





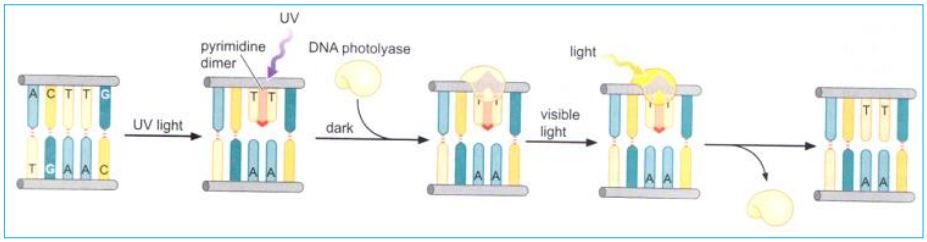
Repair of DNA damage

DNA repair systems

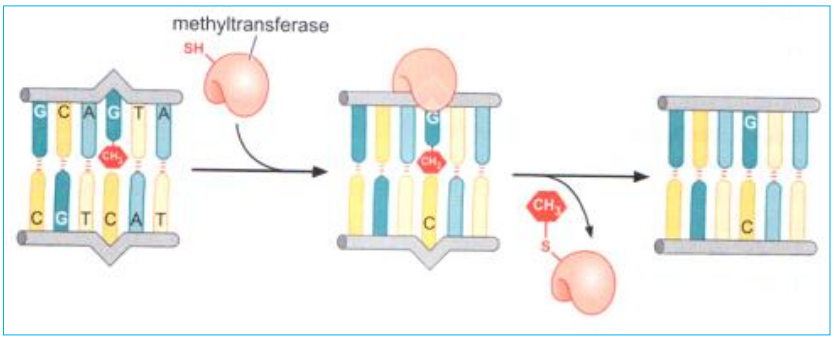
Type	Damage	Enzyme
Mismatch repair	Replication errors	MutS in E. coli, MSH in humans
Photoreactivation	Pyrimidine dimers	DNA photolyase
Base excision repair	Damaged bases	DNA glycosylase
Nucleotide excision repair	Pyrimidine dimers, bulky adducts on bases	UvrA/B/C/D in E. coli XPC/A/D, ERCCI-XPF, XPG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

Direct reversal of DNA damage:
Photoreactivation

Photoreactivation cause direct reversal of DNA damage

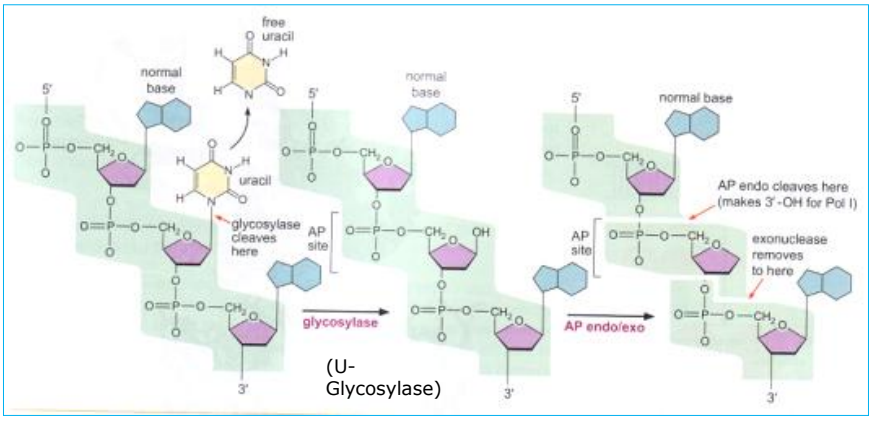


Direct reversal of DNA damage:
removal of CH3 group from O^6-mG



Repair of DNA damage:
Base excision repair pathway

Glycosylase: U-glycosylase
AP: apurinic or apyrimidinic

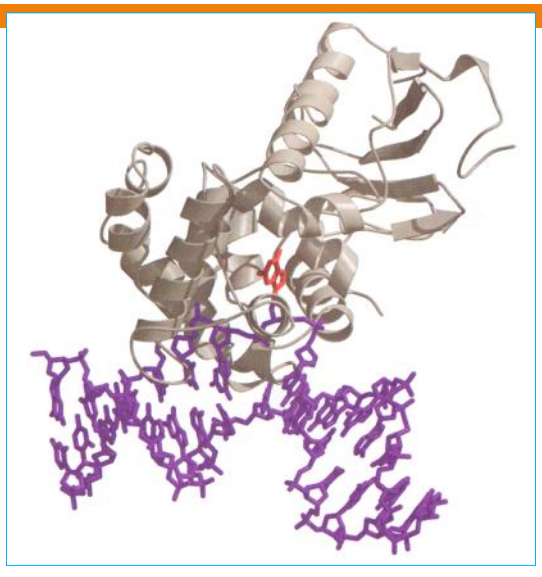


Structure of a DNA Glycosylase complex

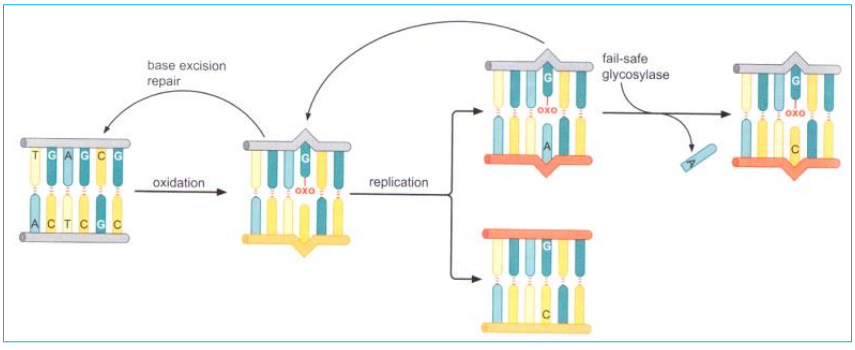
How the glycosylase recognize the damaged base buried in the helix?

Damaged base is flipped out of the helix (?)

Cells have 8 different glycosylases, & DNA glycosyalses are lesion-specific

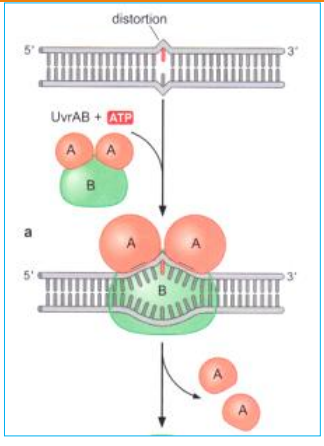


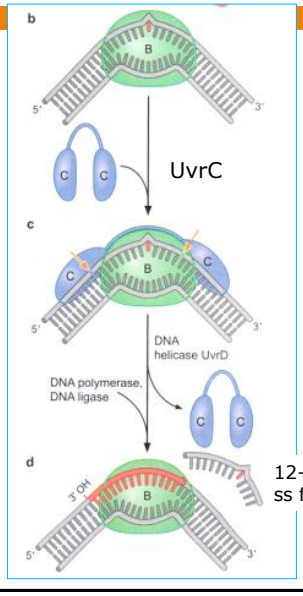
oxoG:A repair and the Fail-safe Glycosylase



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

Repair of DNA damage:
Nucleotide excision repair pathway





12-13 nt long ss fragment

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

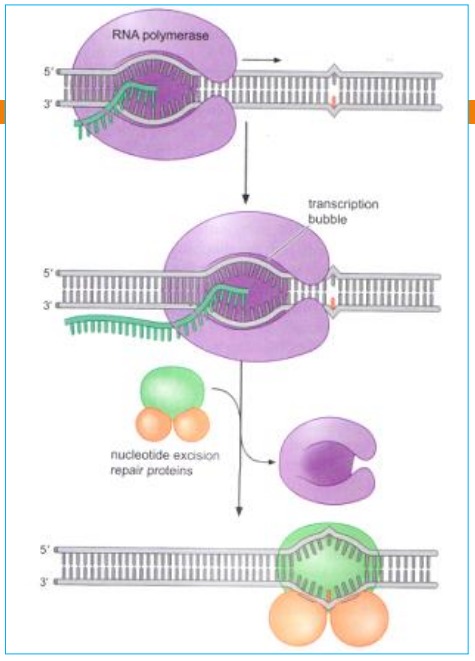
Clinical correlations:
UVR proteins & Xeroderma pigmentosum

- 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

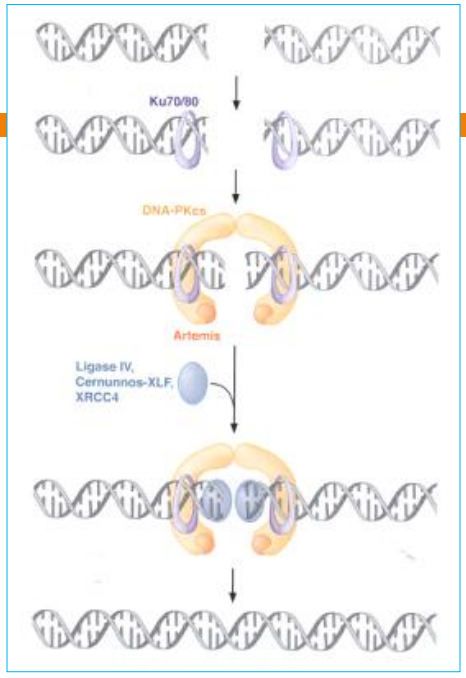


Recombination repairs DNA breaks

- 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
- DSBs are also 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

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!!

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

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