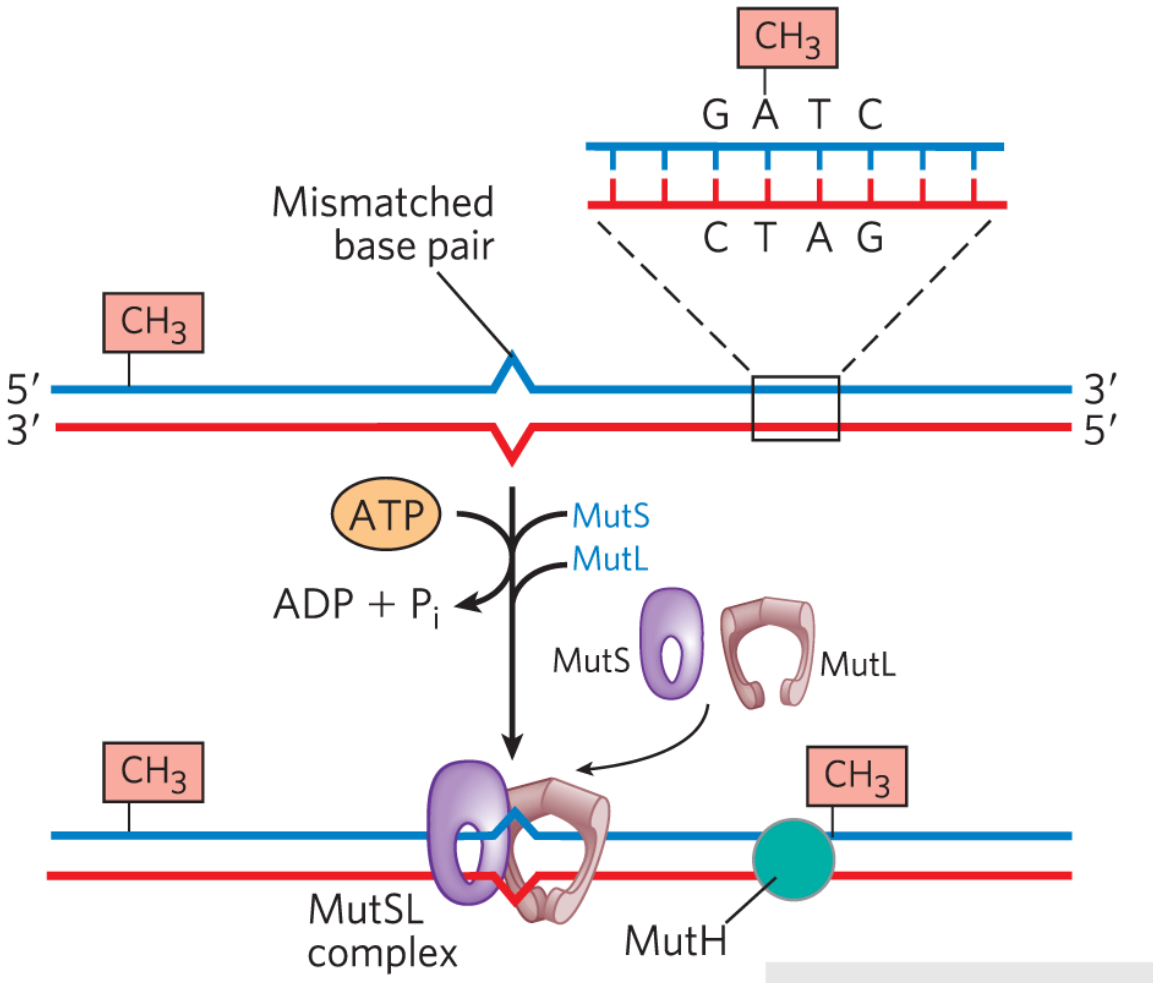
Mismatch repair in *E. coli*

- **The proteins involved in mismatch repair are called Mut proteins, where Mut stands for mutator**
  - **Inactivation of Mut proteins causes a high mutation rate (a "mutator phenotype")**
- **3 key proteins:**
  - **(1) MutS: recognizes and binds mismatched base pairs**
  - **(2) MutL:**
  - **(3) MutH: an endonuclease that nicks the new DNA strand**

Mismatch repair in *E. coli*

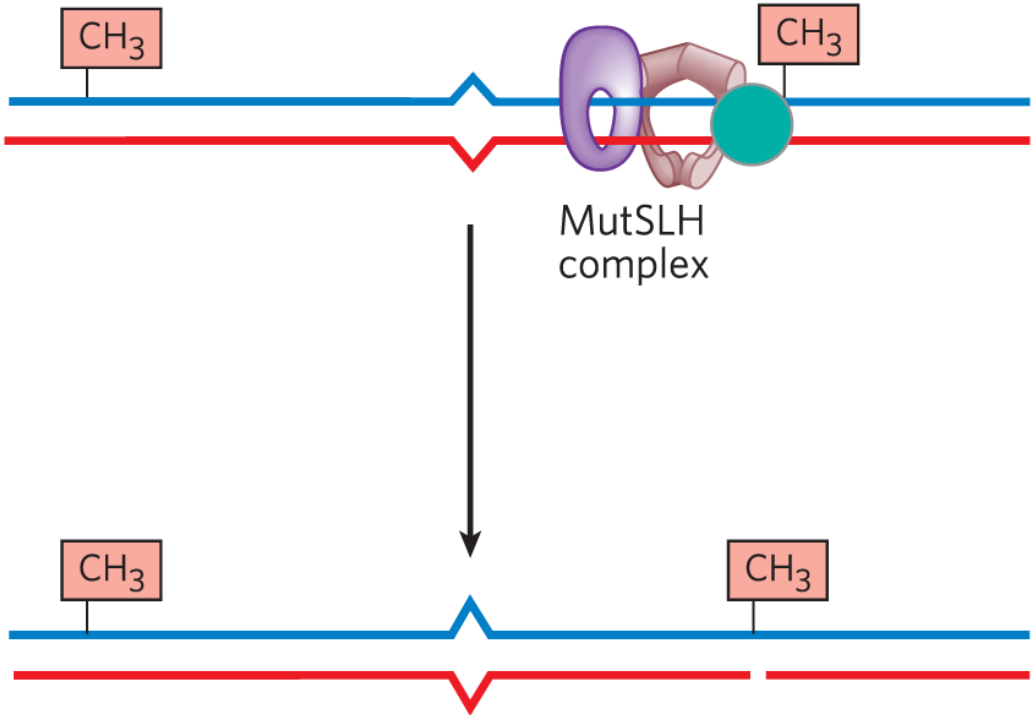


Step 1: MutH binds to hemimethylated GATC

Step 2: MutS scans the DNA and forms a clamplike complex upon encountering a mismatch (detects all but C-C)

Step 3: MutL binds to MutS → MutSL complex

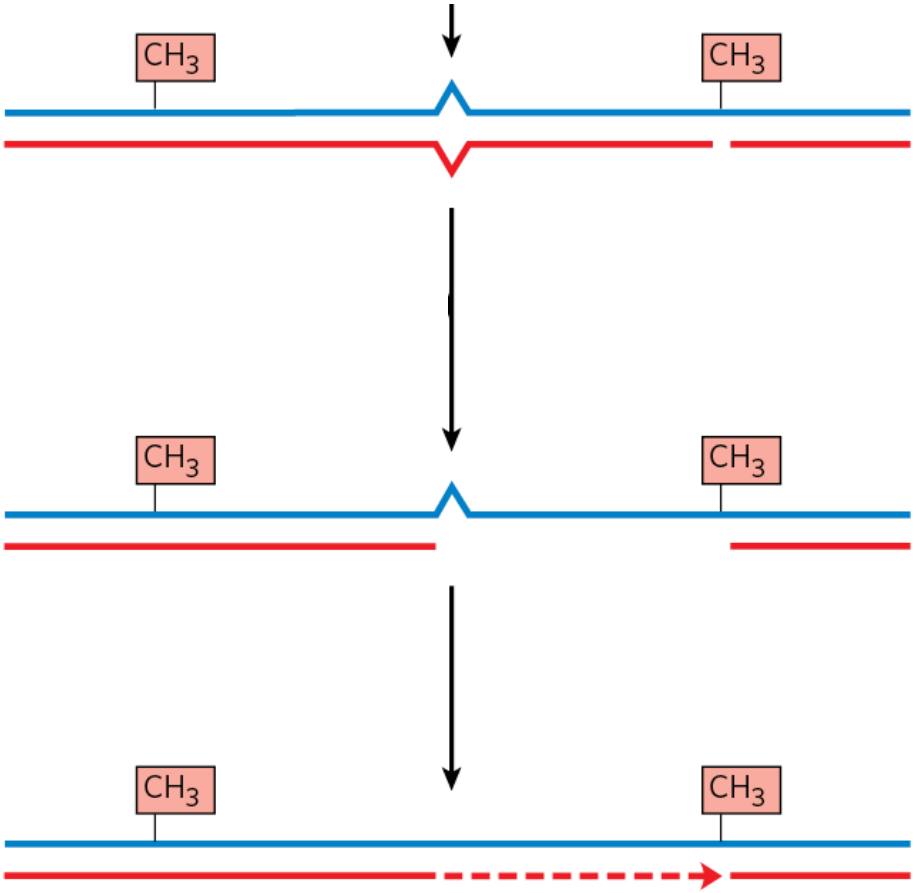
Mismatch repair in *E. coli*



<u>Step 4</u>: MutSL complex slides along DNA to MutH

<u>Step 5</u>: MutH catalyzes cleavage of the unmethylated strand on the side of the G in GATC

Mismatch repair in *E. coli*



Step 6: DNA helicase II (UvrD helicase) unwinds the unmethylated DNA, SSB binds the ssDNA

Step 7: An exonuclease degrades the unwound unmethylated ssDNA

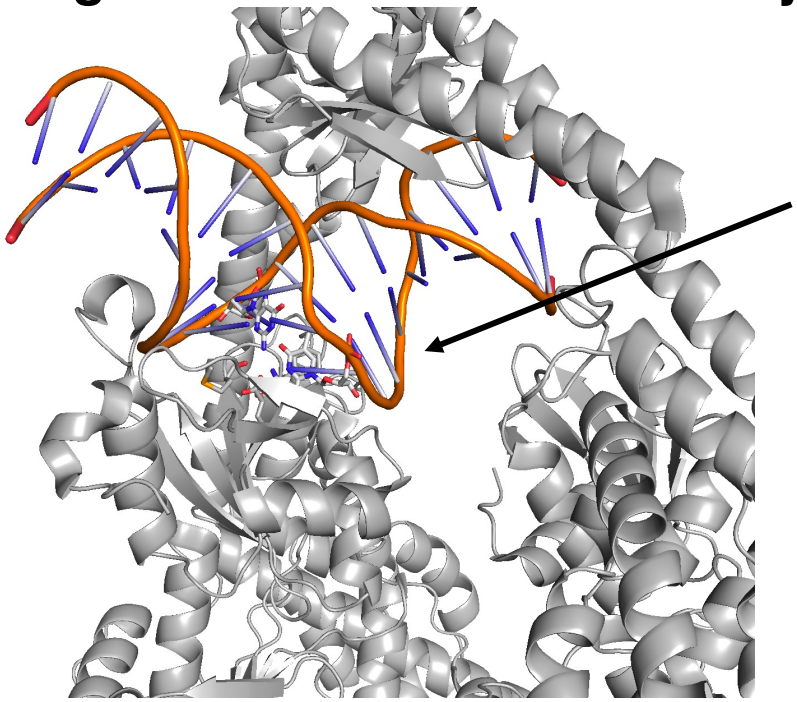
Step 8: DNA pol III fills in the gap with new DNA

Step 9: DNA ligase seals the nick

Mismatch repair in other organisms

- Key steps in mismatch repair are the same in prokaryotes and eukaryotes
  - (1) Recognition of the mismatched base pair
  - (2) Removal of the incorrect nucleotide on the newly synthesized strand
  - (3) Resynthesis using the parental DNA strand as a template
- What differs between organisms:
  - (1) Strategies for recognition of the newly synthesized strand
  - (2) Cleavage around the incorrect nucleotide

Recognition of a G-T mismatch by MutS in prokaryotes

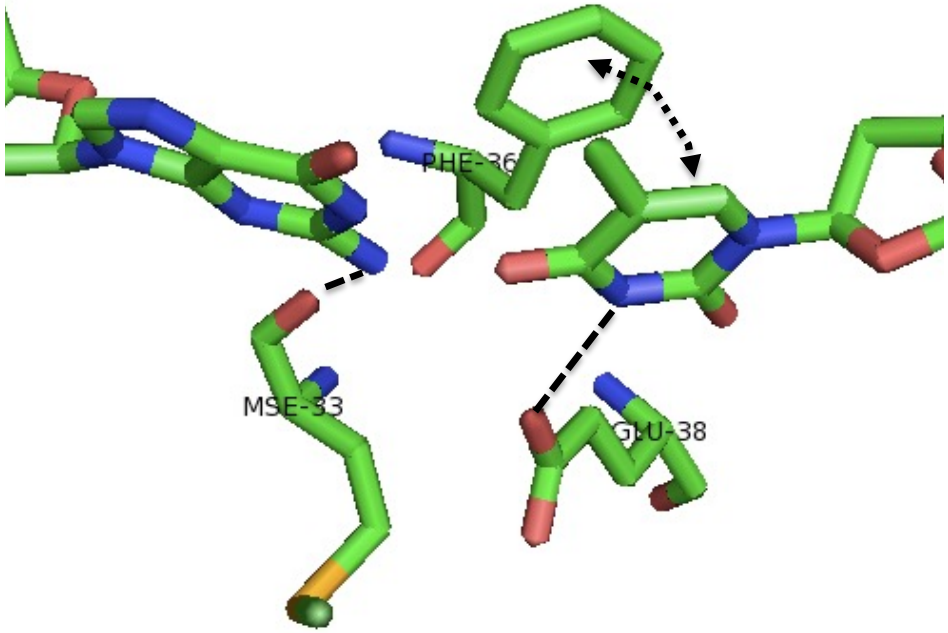


- DNA is kinked at site of mismatch

- Recognition of mismatch by:

- H bonding of MutS residues with mismatched bases (MET33 with G, GLU38 with T)

- stacking on extruded base (PHE36)



PyMol: MismatchRecognitionbyMutS-2.pse

Lamers et al. Nature 2000

A non exhaustive list of post-replicative DNA damages that need to be repaired or dealt with:

1. Pyrimidine dimers
(UV light)

2. Deamination of bases
- **Spontaneous**
- **Chemically induced**
  - **C->U**
  - **5 meC -> T**
  - **A->HX**

3. Hydrolysis of glycosidic bond
(Depurination & depyrimidination)

4. Alkylation of bases
Methylation of guanine N6
G-> O^6methylG

5. Oxidative damages
- **G -> 8 oxoguanine**
- **Strand Breaks**

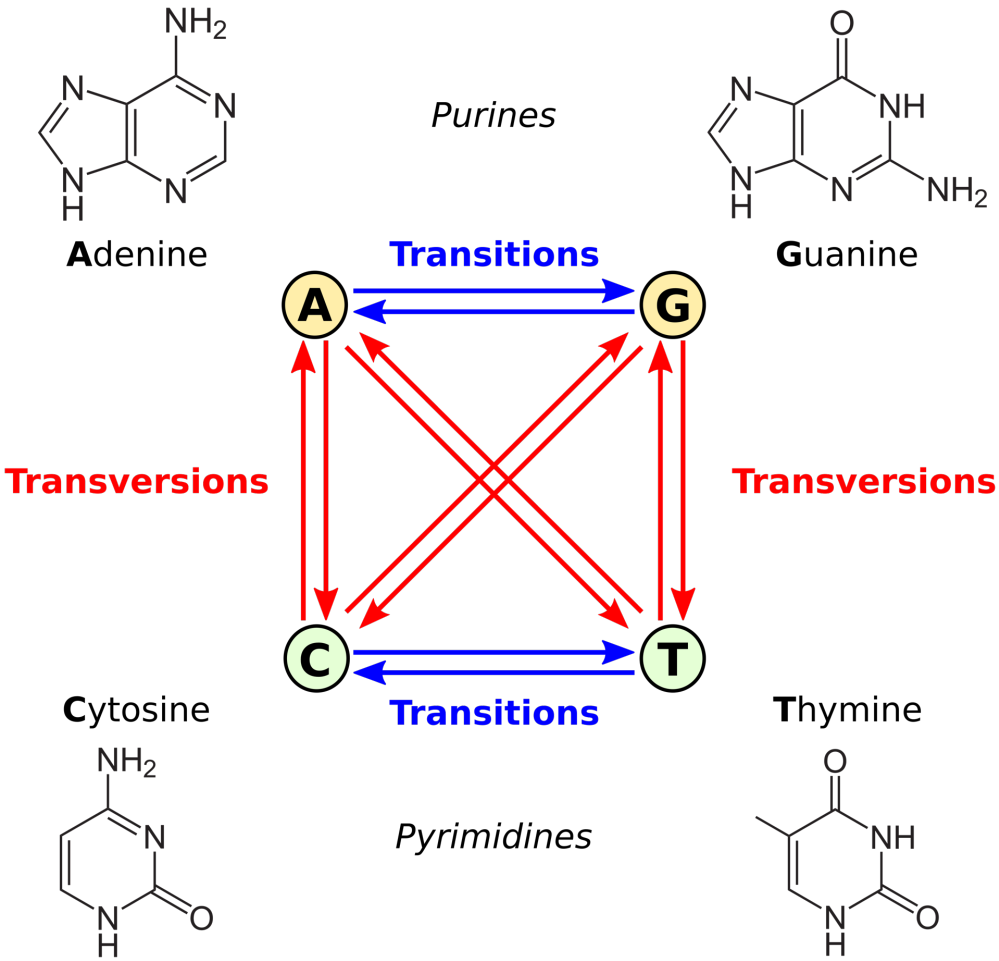
6. Bulky DNA adducts

Things that need to be considered for each damage:

- **Do they block replication by DNA polymerases?**

- **If polymerases can replicate these damages, do they induce a mutagenic event?**

First, some nomenclature: transitions and transversions



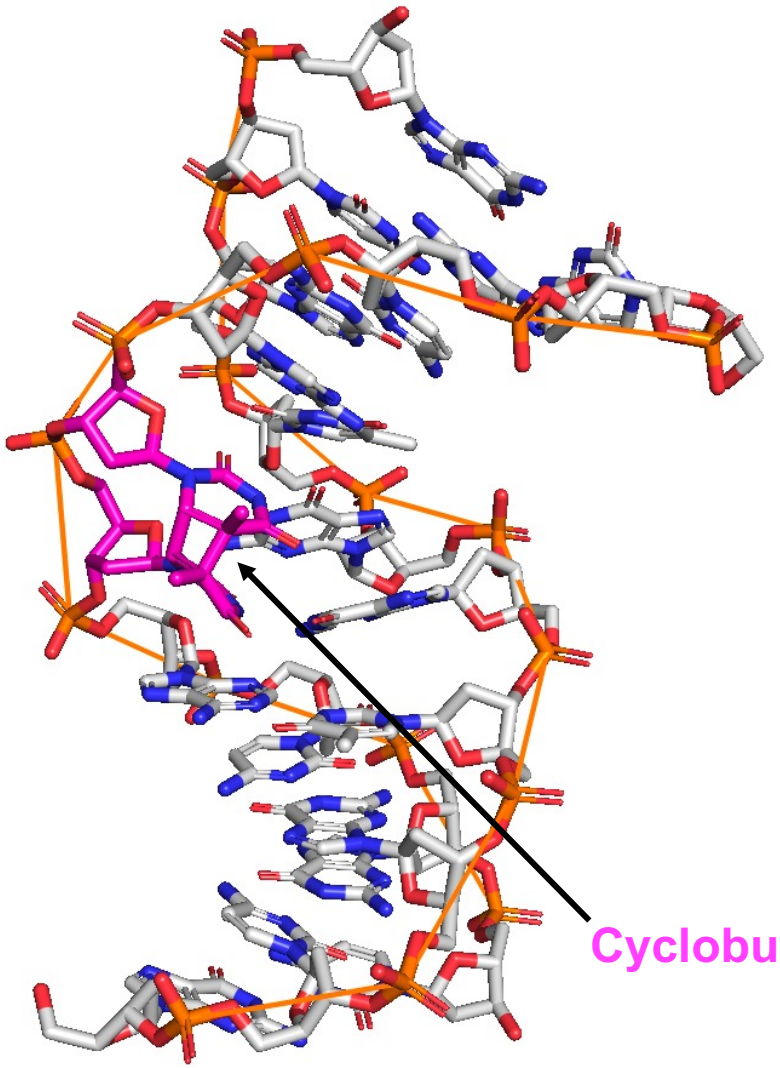
Some examples:

<u>Transition</u>: G-C → A-T

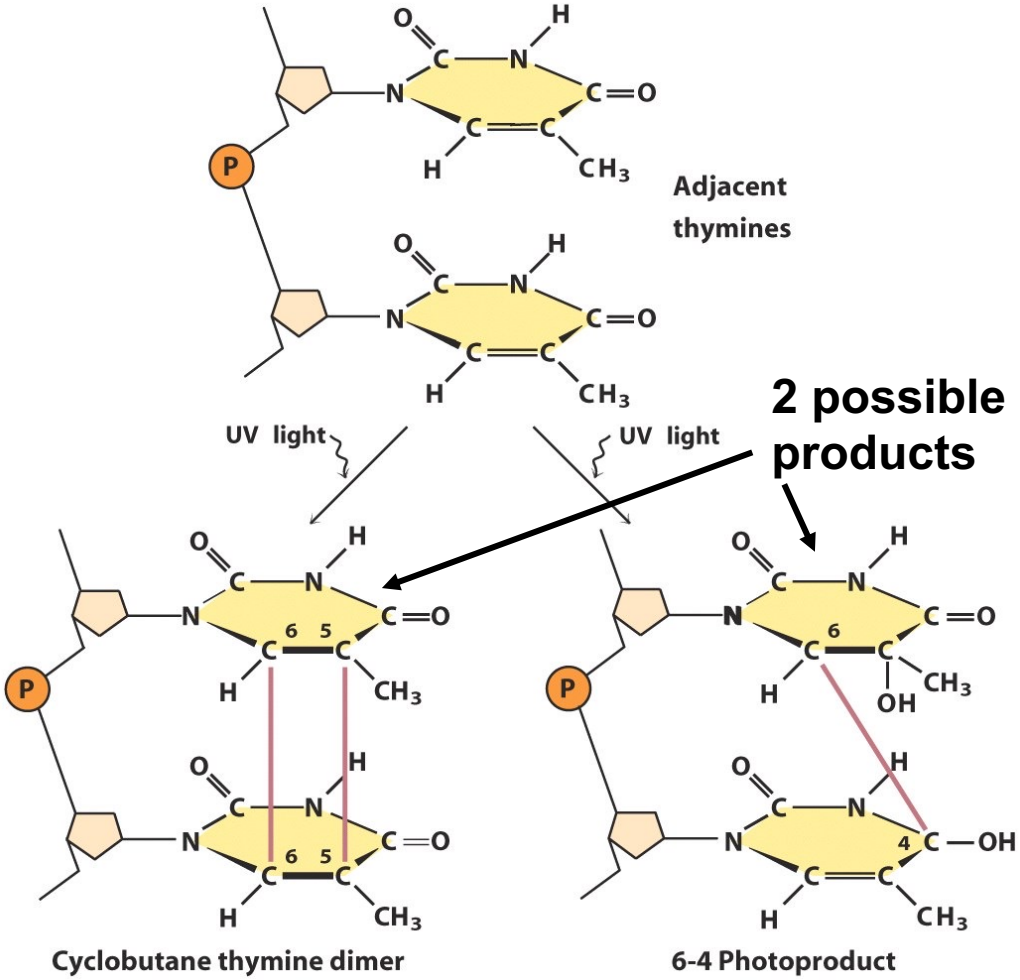
<u>Transversion</u>: G-C → T-A

https://en.wikipedia.org/wiki/Transversion

Induction of pyrimidine dimers by UV light



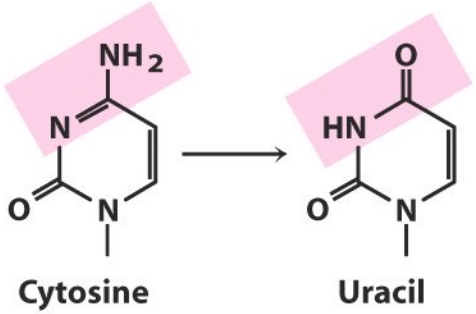
Cyclobutane thymine dimer



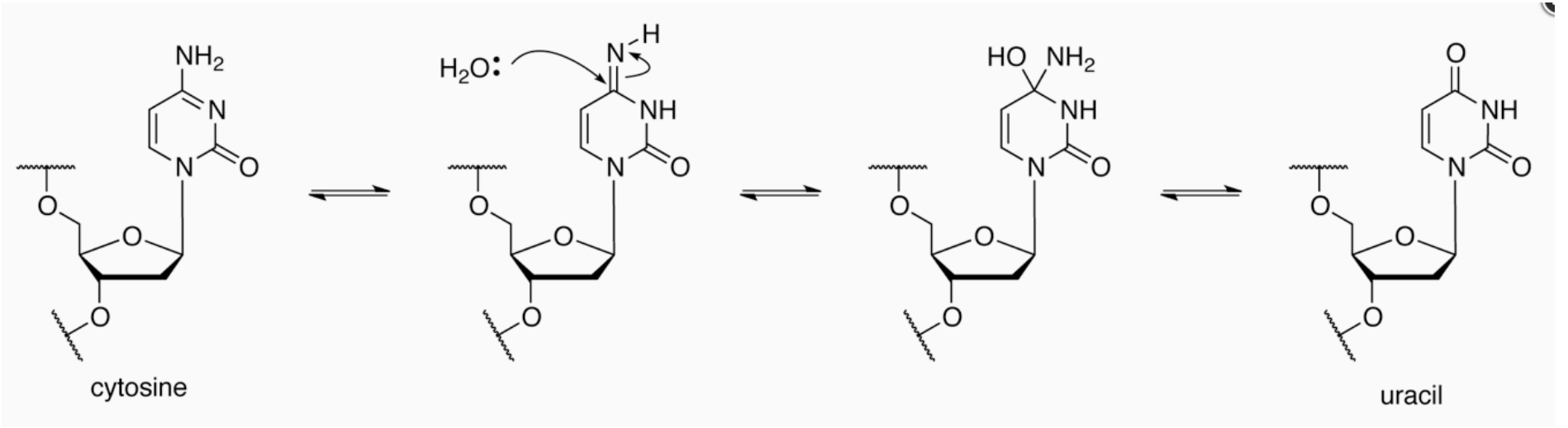
Will a DNA polymerase be able to replicate through this?

PyMol: thymidinedimer.pse

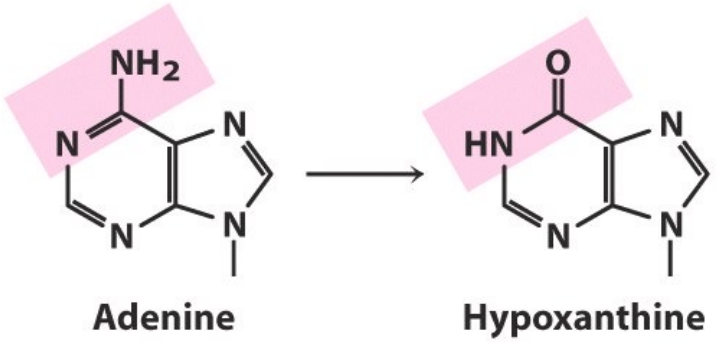
Spontaneous Deaminations



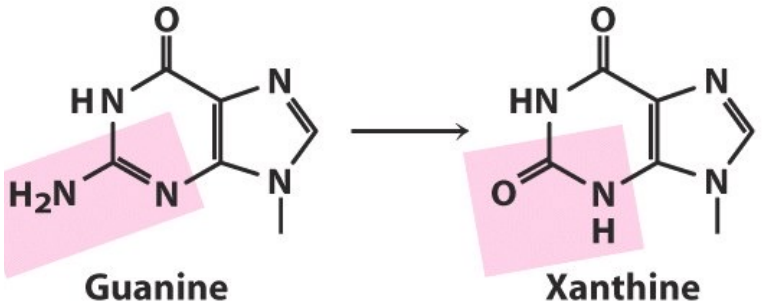
C --> U: 10^{-8}/24 hours:
~ 100 events/day for a mammalian cell genome



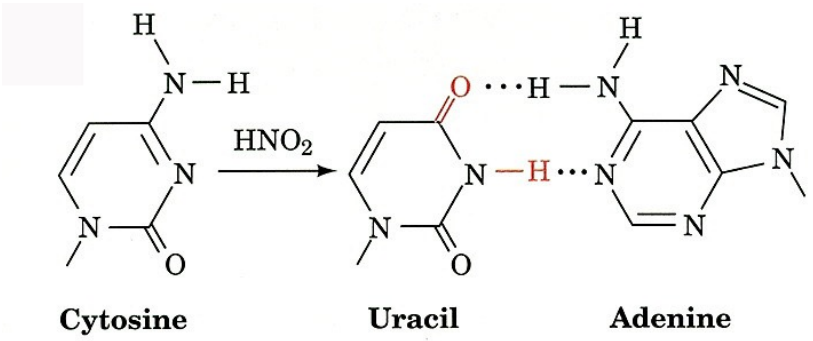
A --> H
10^{-9}/24 hours

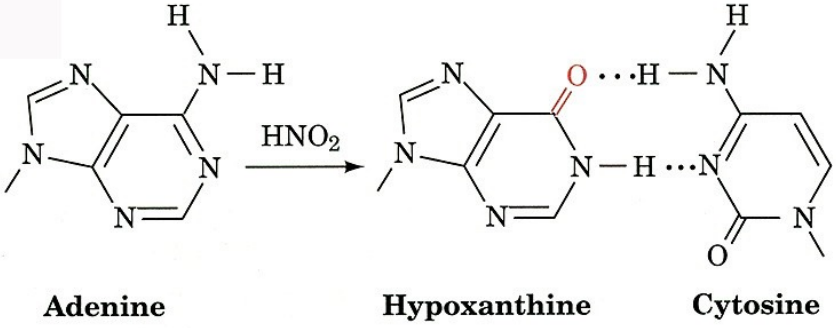


G --> X
10^{-9}/24 hours



Do you think deaminations of bases in the DNA template can cause DNA replication blocks?





A: Yes because the geometry of U-A and X-C base pairs is different from ‘normal´ ones

B: No because deaminated bases are eliminated so quickly that they don’t interfere with replication

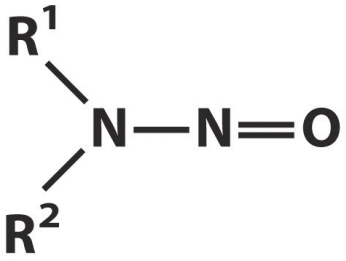
C: Yes because they can´t be recognized by the proofreading active site of DNA pols. and stall in the exo active site

D: No because the active site of DNA Pols. can accommodate deaminated bases

Chemical Sources of Deaminations

$NaNO_2$ Sodium nitrite

$NaNO_3$ Sodium nitrate

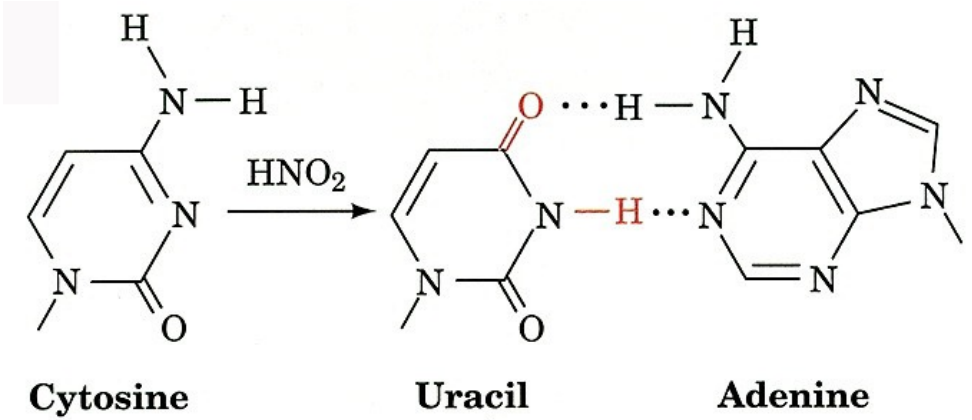


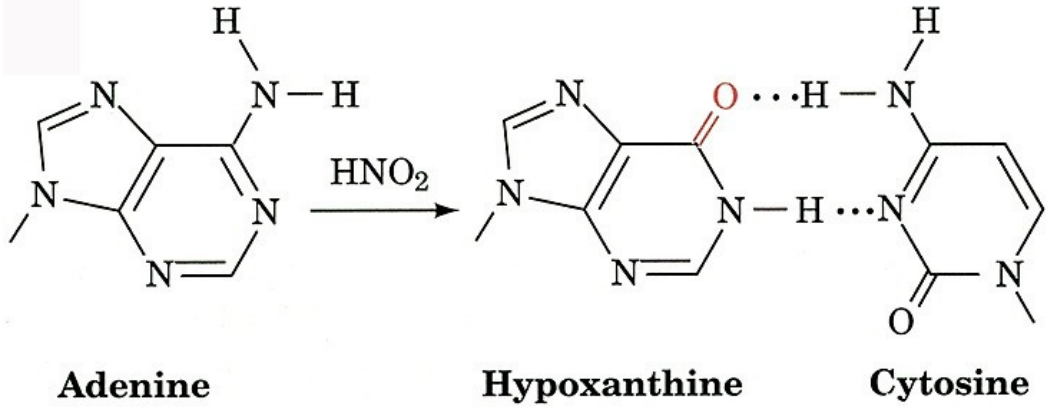
Nitrosamine
(by-product of rubber production)

Nitrous acid precursors

(Nitrous acid: HNO_2)

Genetic Consequences of Deaminations





- Xanthine also base pairs with C

Spontaneous depurinations and depyrimidinations

Depurination: 10,000 events/day for a mammalian cell

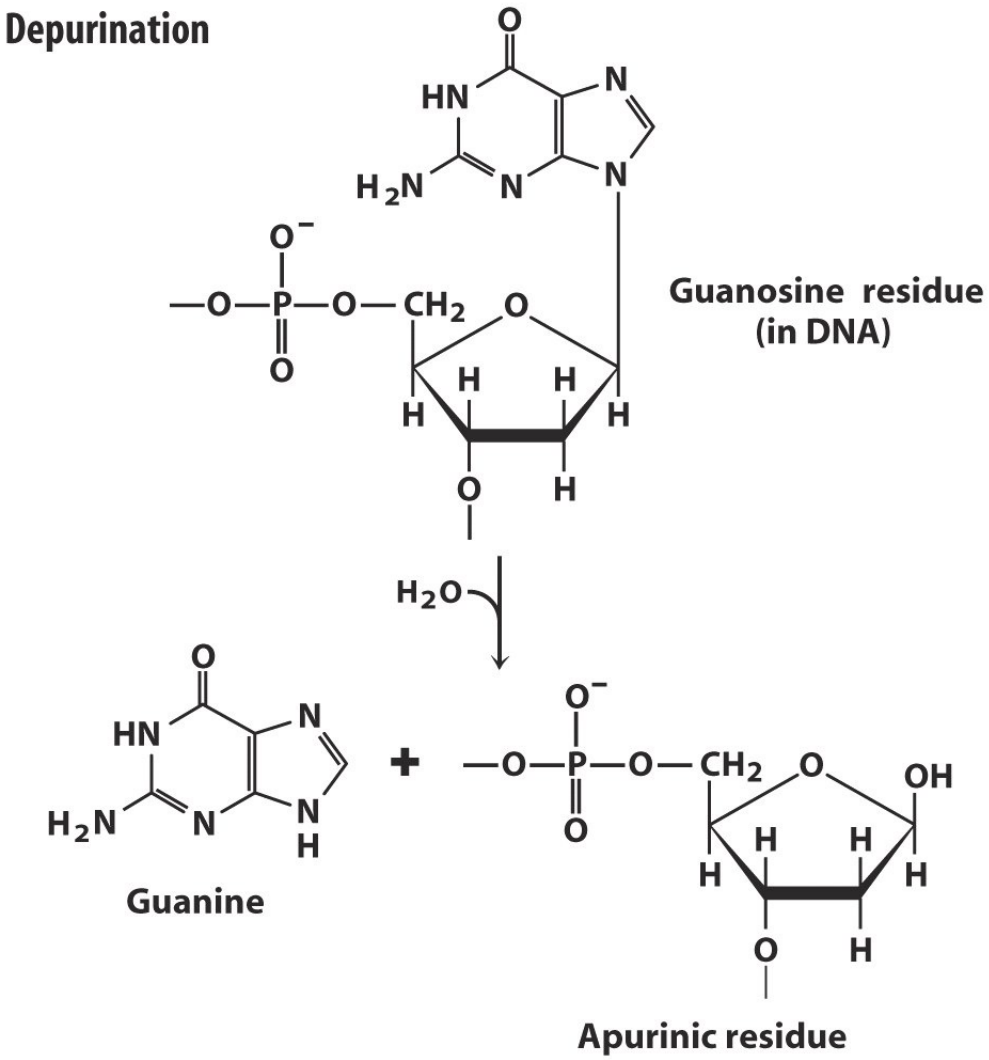
Depyrimidination: 500 events/day for a mammalian cell

Nomenclature:

Abasic = No Base

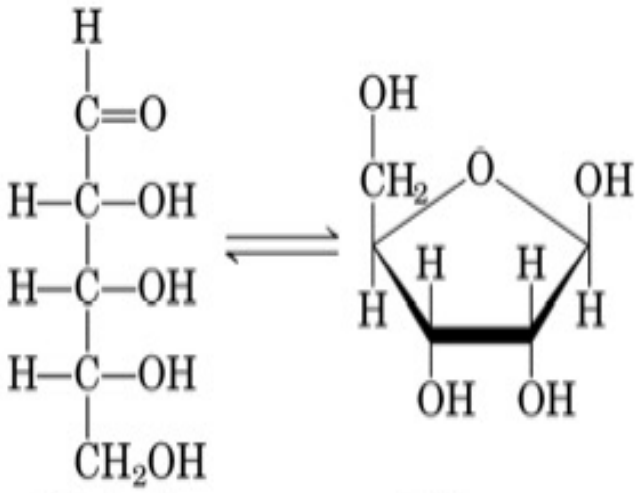
Apurinic = no purine base

Apyrimidinic = no pyrimidine base

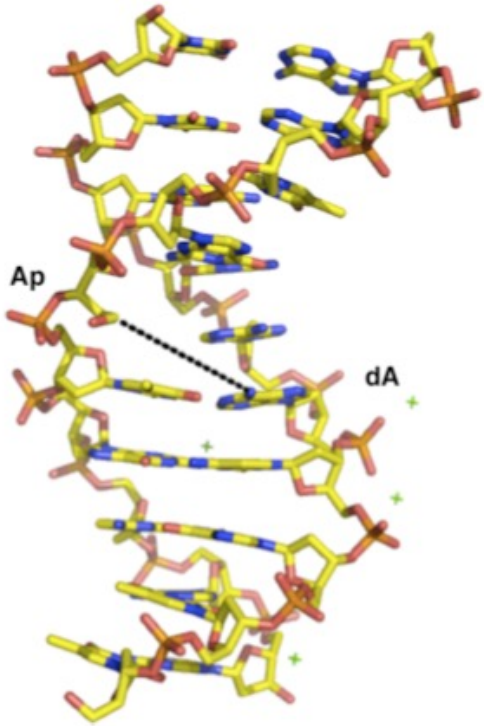


Abasic sites: More than just empty space: potential reactivity

Abasic sites exist as an equilibrating mixture of a cyclic and linear forms (aldehyde)



Aldehydes are electrophilic functional groups that can form covalent adducts with nucleophilic sites in DNA, in particular with the N6-amino group of adenine residues on the opposing strand at high yields (15−70%) under physiologically relevant conditions.



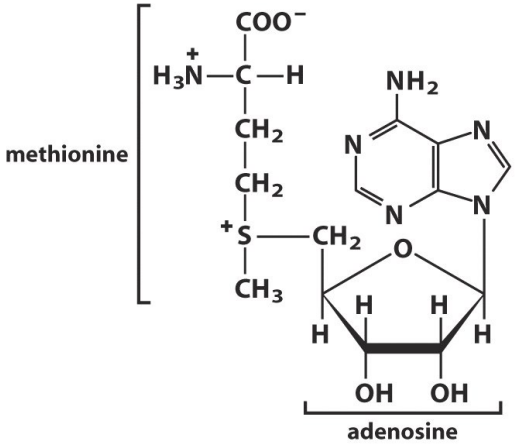
Interstrand crosslinks are bad for replication/transcription etc…

PyMol: view_AP_crosslink.pse

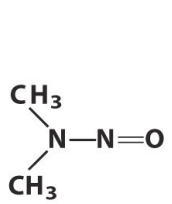
J. Am. Chem. Soc. 2014, 136, 3483−3490

Alkylations of bases: Chemical Sources and mechanism

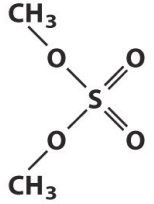
Base alkylation: Addition of methyl, ethyl, etc., group onto a base



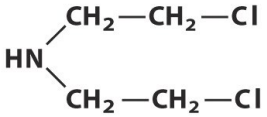
S-Adenosylmethionine



Dimethylnitrosamine



Dimethylsulfate



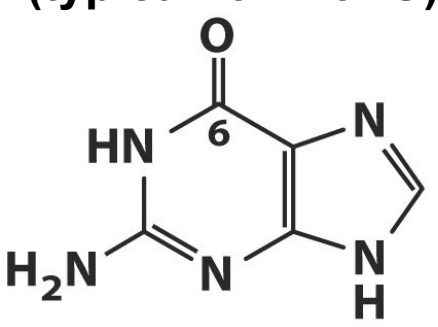
Nitrogen mustard

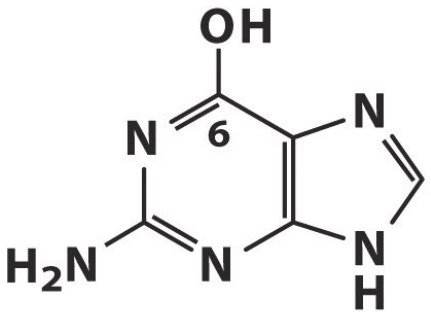
Alkylating agents

Used as a chemical weapon in WWI

Guanine tautomers

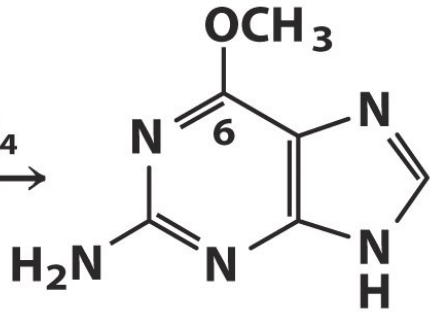
Keto
(typical form of G)





Enol
(rare)

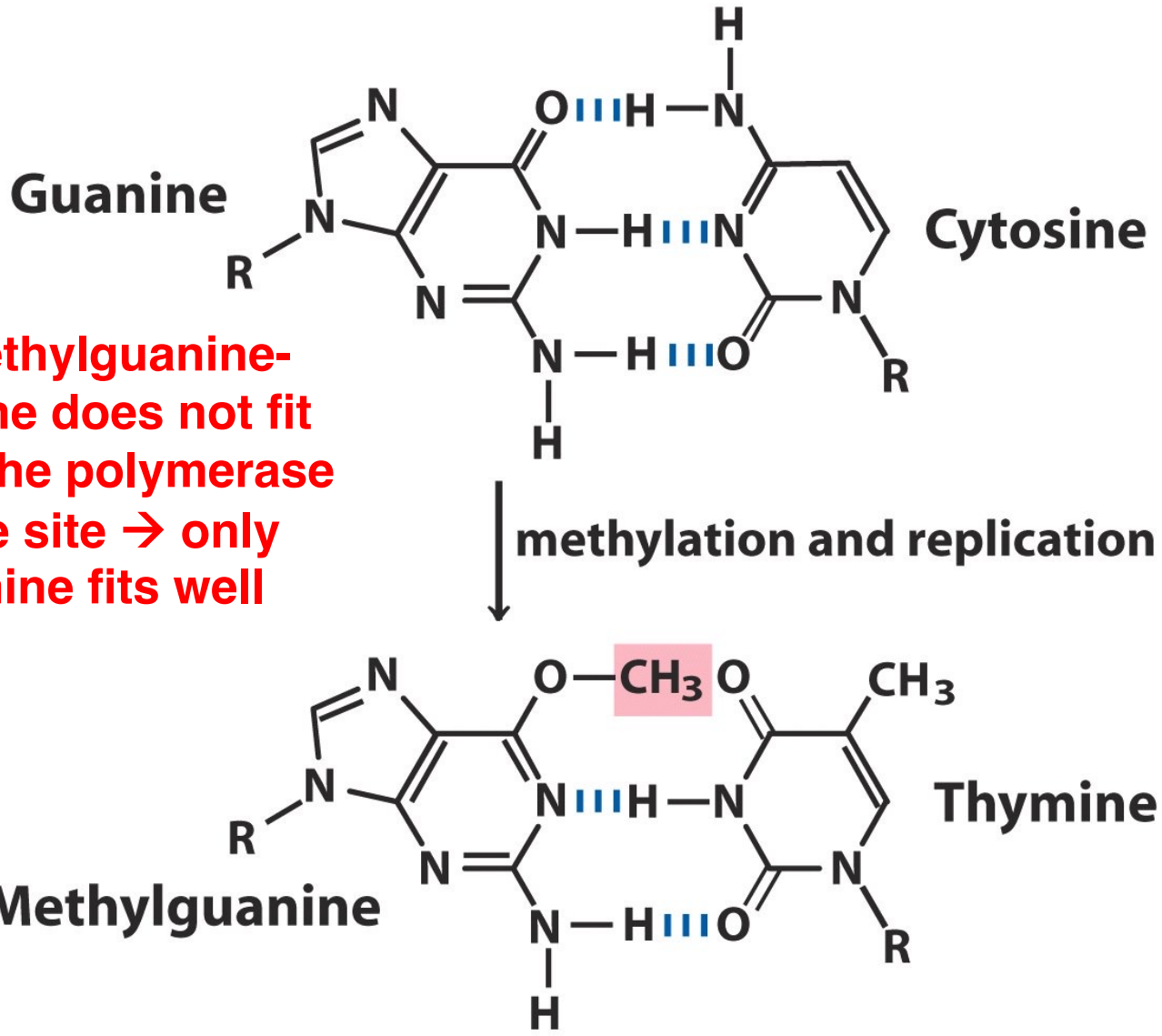
$(CH_3)_2SO_4$



O^6-Methylguanine

One of the most frequent damages: methylation of guanine

Consequences of O^6-meG for replication



O^6-methylguanine-cytosine does not fit well in the polymerase active site → only thymine fits well

G-C → O^6meG-T → A-T