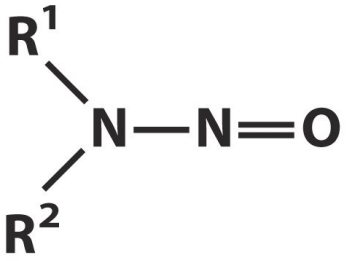
Chemical Sources of Deaminations

$NaNO_2$ Sodium nitrite

$NaNO_3$ Sodium nitrate

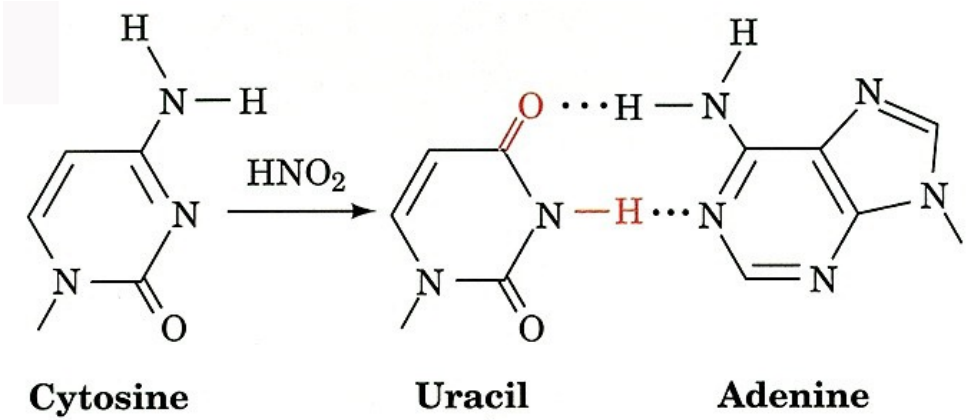


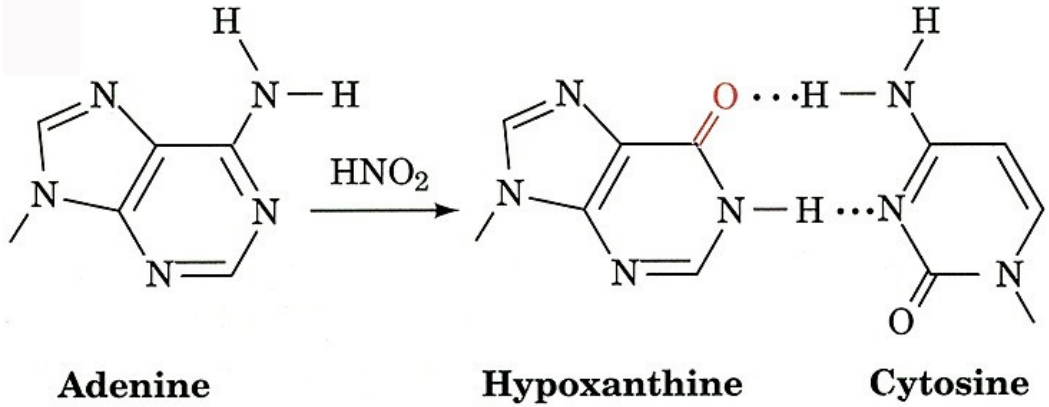
Nitrosamine
(by-product of rubber production)

Nitrous acid precursors

(Nitrous acid: HNO_2)

Genetic Consequences of Deaminations



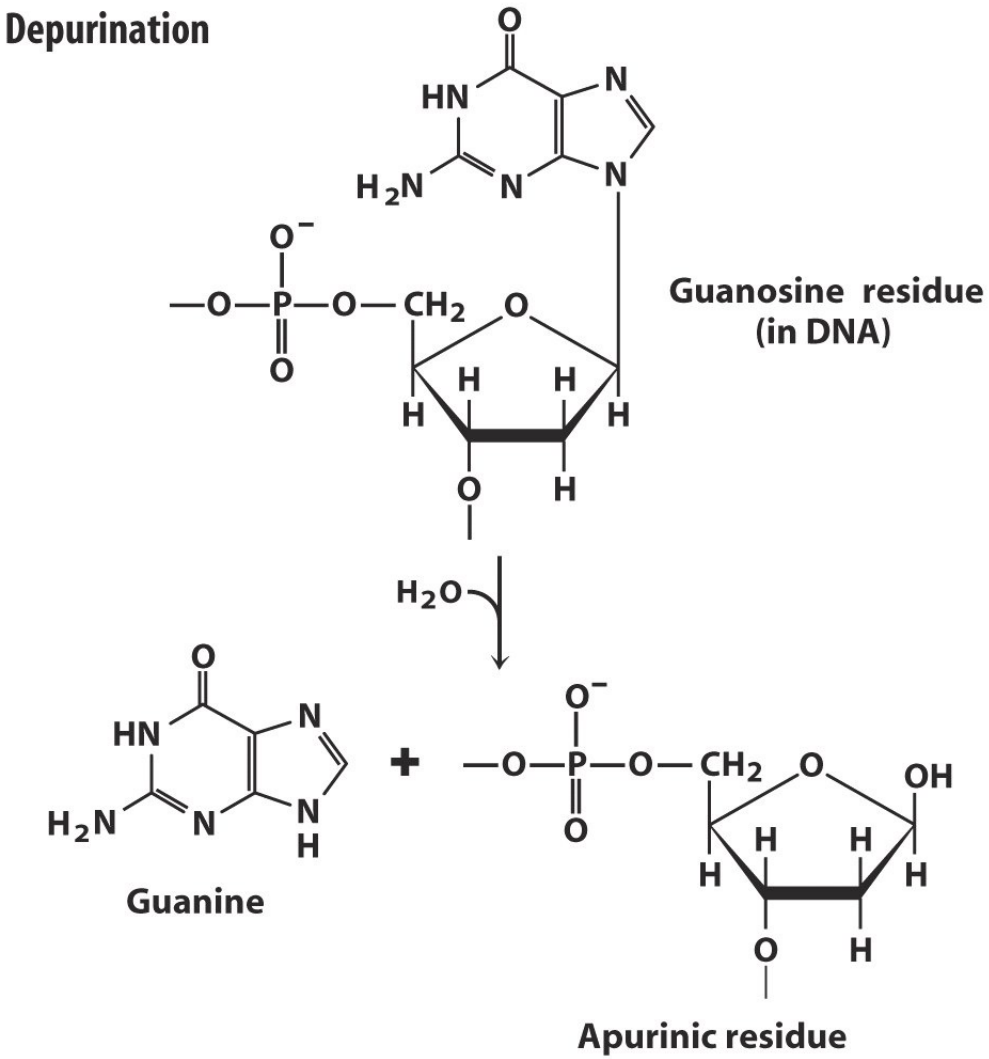


- Xanthine also base pairs with C

Spontaneous depurinations and depyrimidinations

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

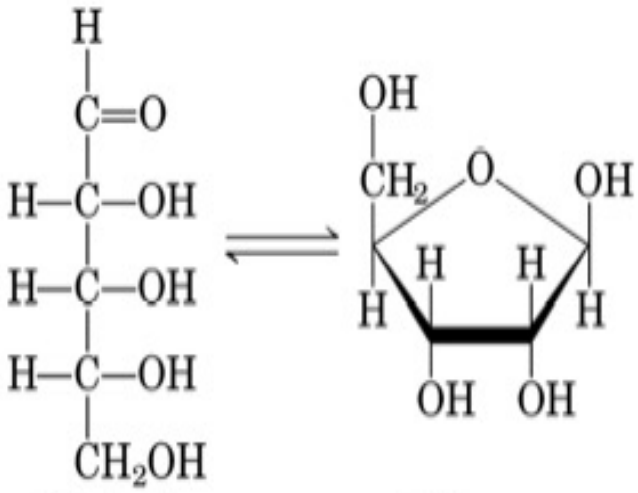
Depyrimidination: 500 events/day for a mammalian cell



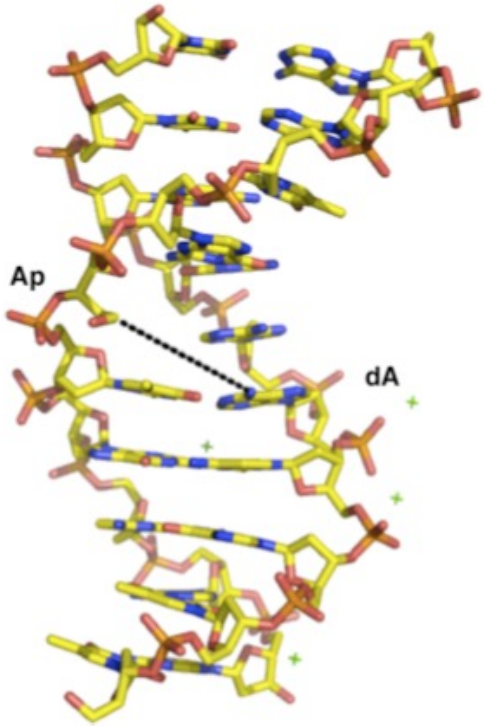
Nomenclature:
Abasic = No Base
Apurinic = no purine base
Apyrimidinic = no pyrimidine base
Generally called an abasic site or AP site.

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.



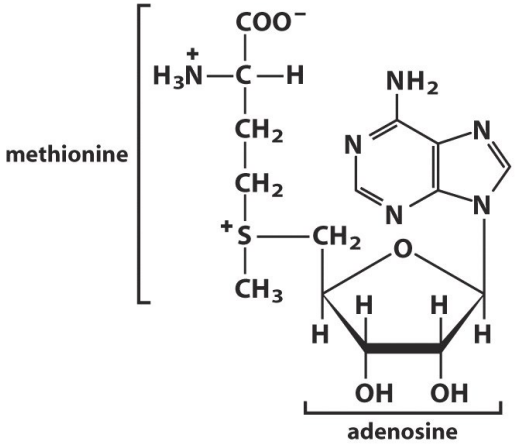
Interstrand crosslinks are bad for replication/transcription etc…

PyMol: view_AP_crosslink.pse

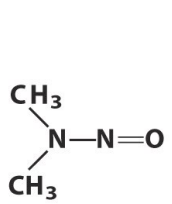
J. Am. Chem. Soc. 2014, 136, 3483−3490

Alkylations of bases: Chemical Sources and mechanism

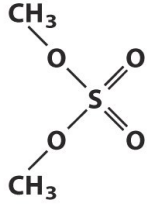
Base alkylation: Addition of methyl, ethyl, etc., group onto a base



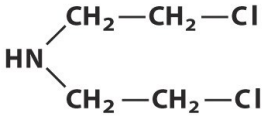
S-Adenosylmethionine



Dimethylnitrosamine



Dimethylsulfate



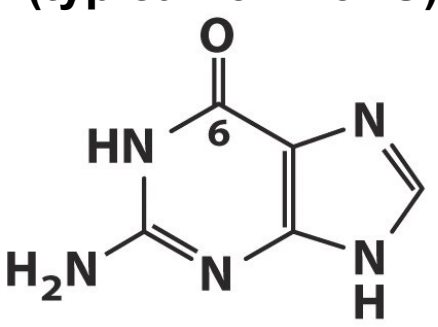
Nitrogen mustard

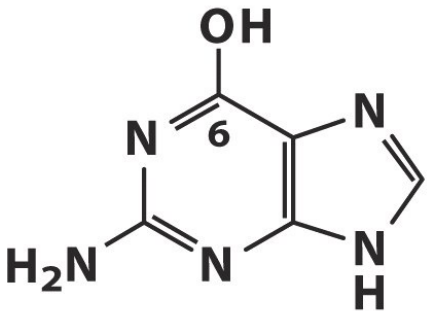
Alkylating agents

Used as a chemical weapon in WWI

Guanine tautomers

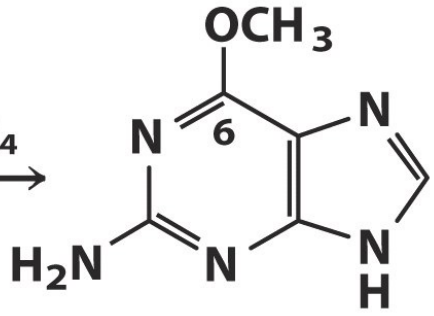
Keto
(typical form of G)





Enol
(rare)

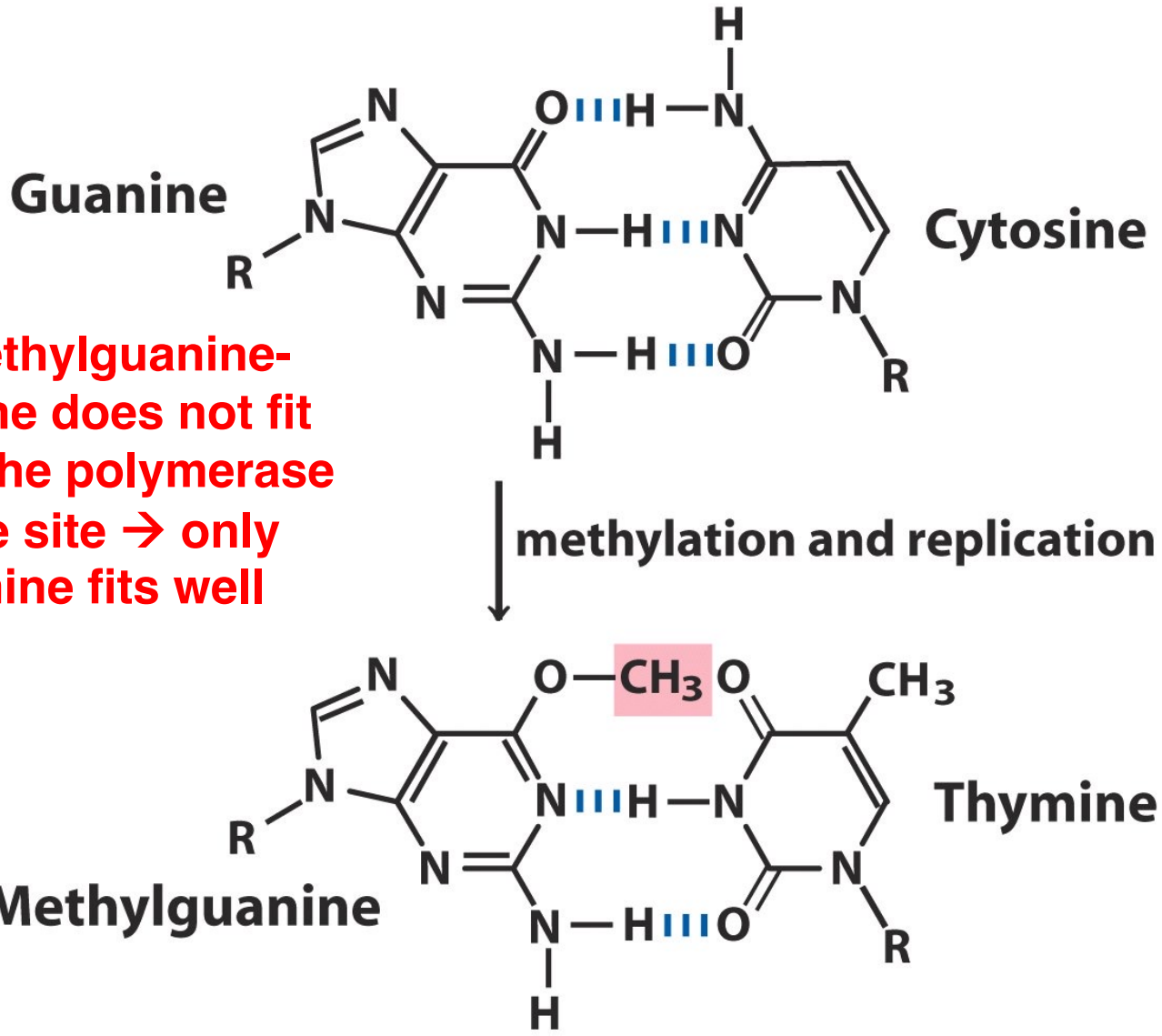
$(CH_3)_2SO_4$



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

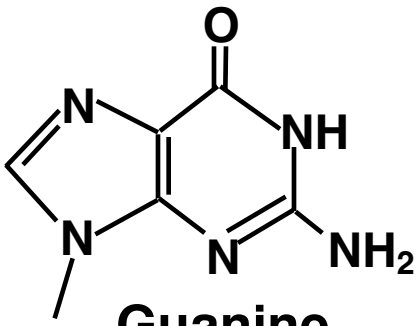
G-C → O^6meG-T → A-T

Oxidative damage of DNA

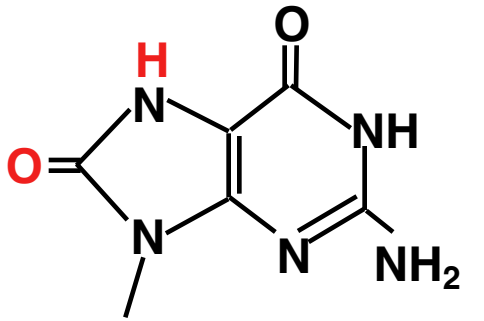
Major cause of oxidative damage: hydroxyl radicals produced via activity of the electron transport chain

Cells don't have any enzymes that can neutralize hydroxyl radicals.

Consequences for Nucleotides:



Guanine



8-oxoguanine

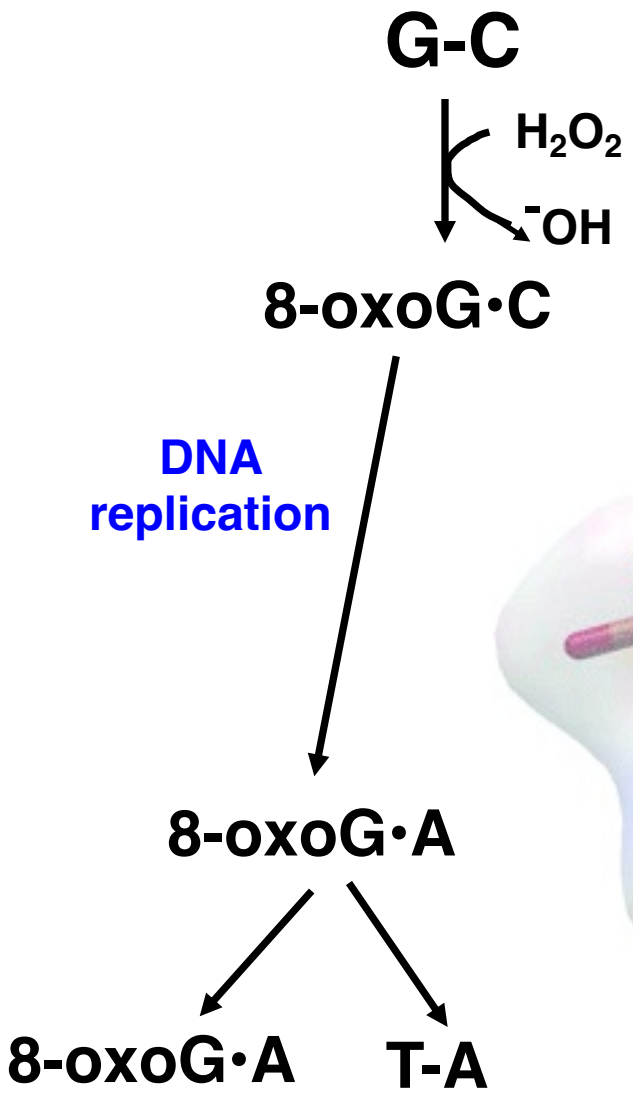
Thymine → **5-formyl Uracil**

Deoxyribose → **Ribose**

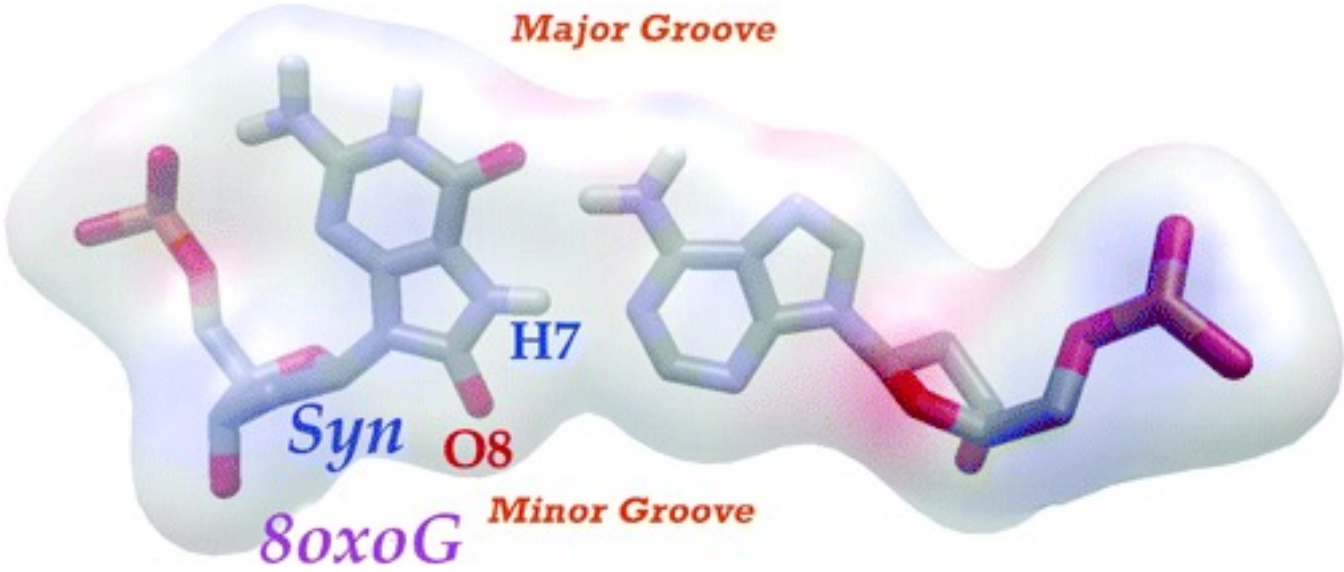
Consequences for Nucleic Acids:

Strand Breaks
(bad for DNA replication)

8-oxoguanine generates replication blocks or G-C → T-A transversions after DNA replication



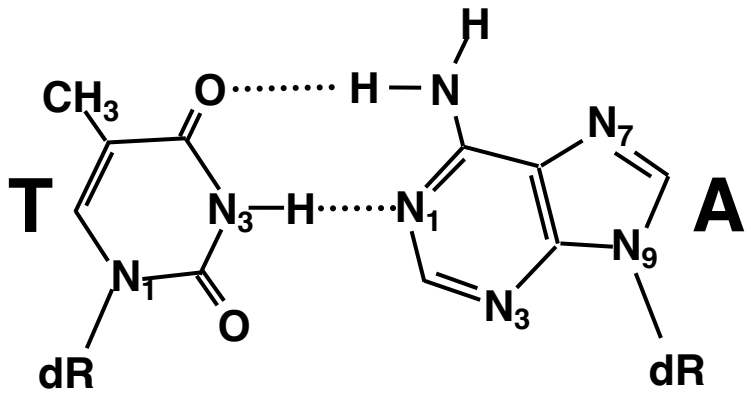
8-oxoG tends to adopt the syn conformation → A is the only nt that can pair with syn 8-oxoG to form a base pair whose geometry resembles that of a Watson-Crick base pair → DNA Polymerases tend to incorporate A opposite to 8-oxoG

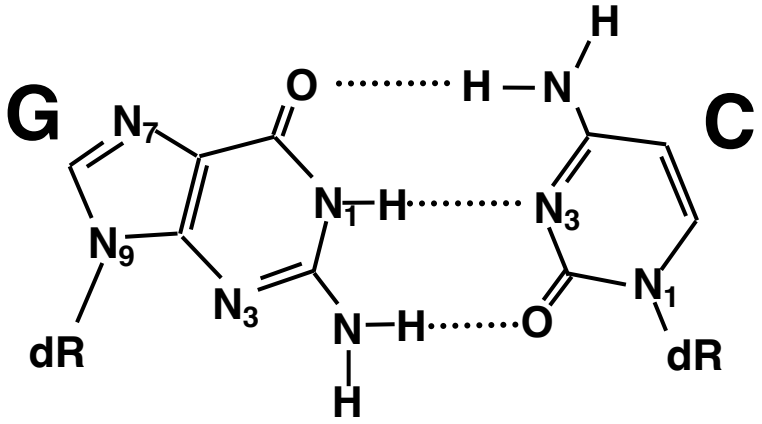


J Am Chem Soc.
2005 Oct 12;127(40):13906-18

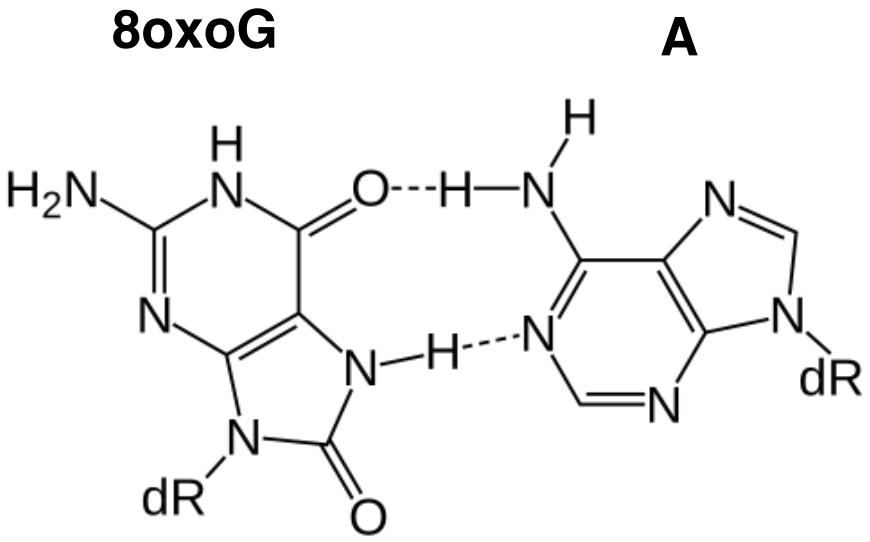
Recall normal Watson-Crick base pairing

Watson-Crick base pairs





Hoogsteen base pair



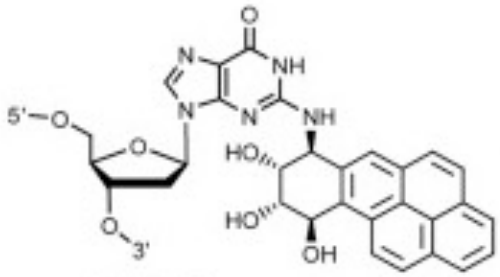
Bulky DNA adducts caused by:

cigarette smoke

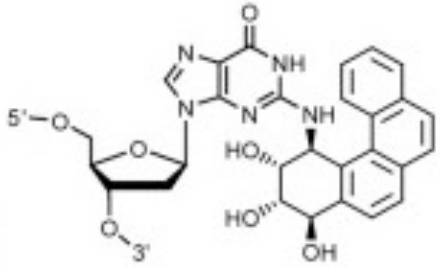
bulky DNA adducts

diesel engine exhaust

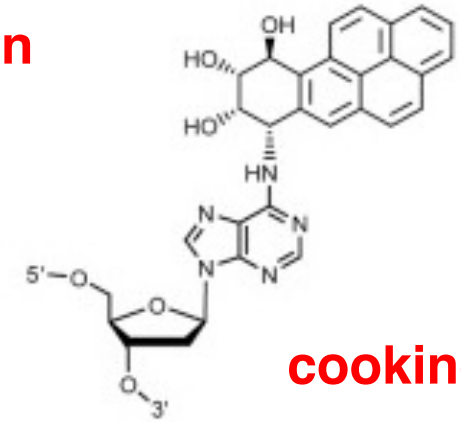
block DNA Replication



[BP]-dG

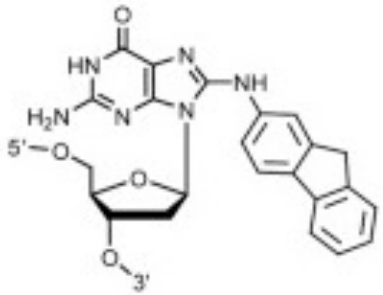


B[*c*]Phe DE-dG

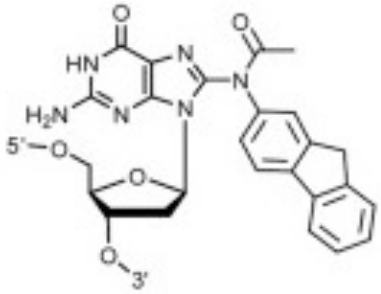


[BP]-dA

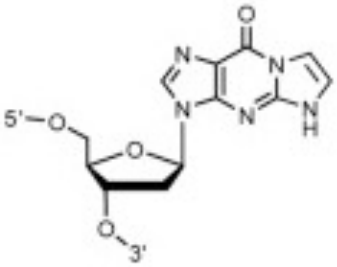
cooking/broiling of food



AF-dG



AAF-dG



1,N^2-ε-dG

What type of DNA damage would you expect your DNA to suffer after consuming charbroiled hot dogs at the Rose Bowl on a sunny day?

A: Pyrimidine dimers

B: Deaminated bases

C: Bulky adducts

D: All the above

DNA repair and tolerance strategies & enzymes

1- Bypass of lesions: avoids DNA replication stalls

- bypass of DNA damage by *translesion* DNA Polymerases

-> not a “repair” but is used to prevent DNA replication blocks

2- Direct Reversal of Damage

(alkylation of bases, pyrimidine dimers)

- Photolyase reversion of Y dimers
- Dealkylation of guanines by suicidal MGMTase
- Dealkylation of 1mA and 3mC by AlkB (not shown)

3- Base excision repair (T.Lindahl/Nobel 2015)

(deamination, alkylation, oxidation of bases)

- Uracil-N glycosylase
- 8-oxoG glycosylase

4- Nucleotide excision repair (A.Sancar/Nobel 2015)

(pyrimidine dimers, bulky DNA adducts)

- Bacteria: UvrA, UvrB, UvrC, Helicase II (UvrD)
- DNA pol. I, DNA ligase
- Eukaryotes : Xeroderma pigmentosum proteins, TFIIH

Strategy #1: Bypass of lesions

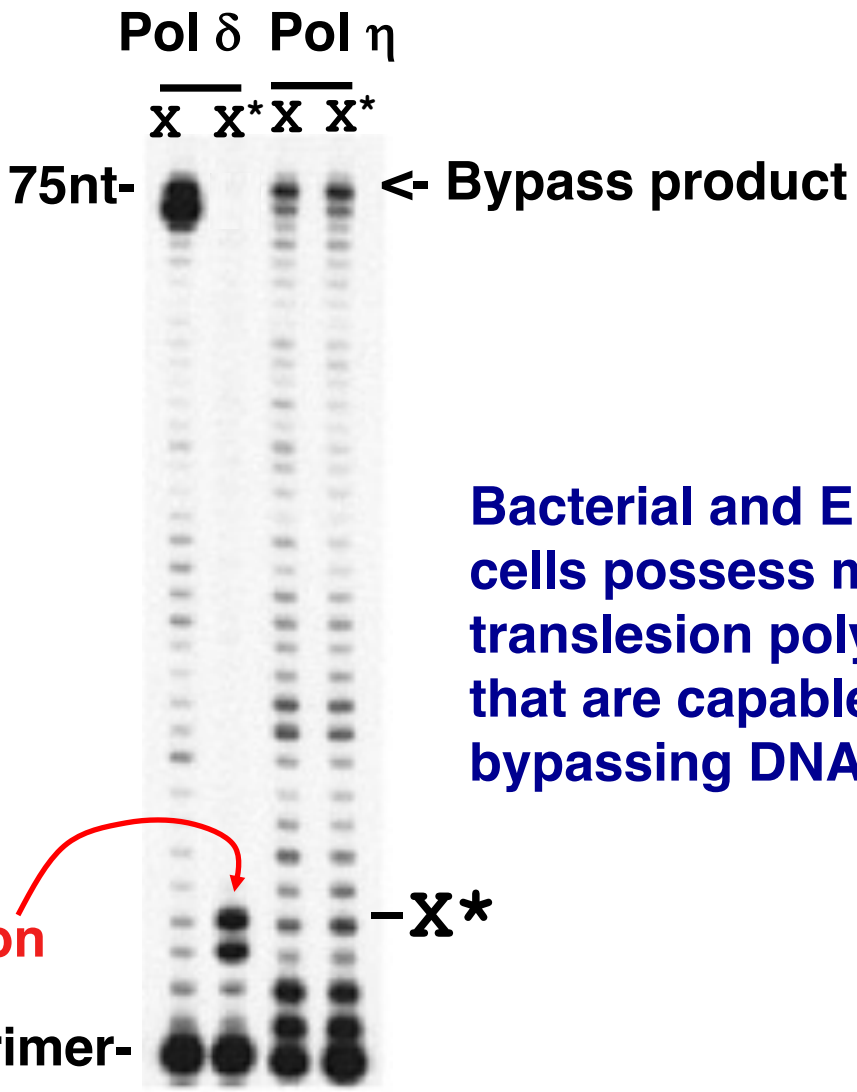
Recall from polymerase unit: Different Families of DNA polymerases

A Family	bacteriophage	T7	DNA Replication
	Bacteria	Pol. I	DNA Repair/Replication
B Family	Eukaryotes	Pol.α	DNA Replication
		Pol.δ	DNA Replication
		Pol.ε	DNA Replication
		Pol.ζ	Translesion / bypass
	Bacteria	Pol. II	
C Family	Bacteria	Pol. III	DNA Replication
Y Family	Eukaryotes	Pol.η Pol.κ	Translesion/ bypass
	Bacteria	Pol.IV, V	
Reverse Transcriptases		Telomerase	Chromosomes Ext.
	Retrovirus	RTs	Viral Replication
D Family		Pol.D	Not Covered
X Family		Pols β,λ,μ,δ	

Bypass of 8-oxoG lesions by a specialized eukaryotic DNA polymerase: Pol η (eta)

X = Guanosine

X * = 8-Oxo Guanosine



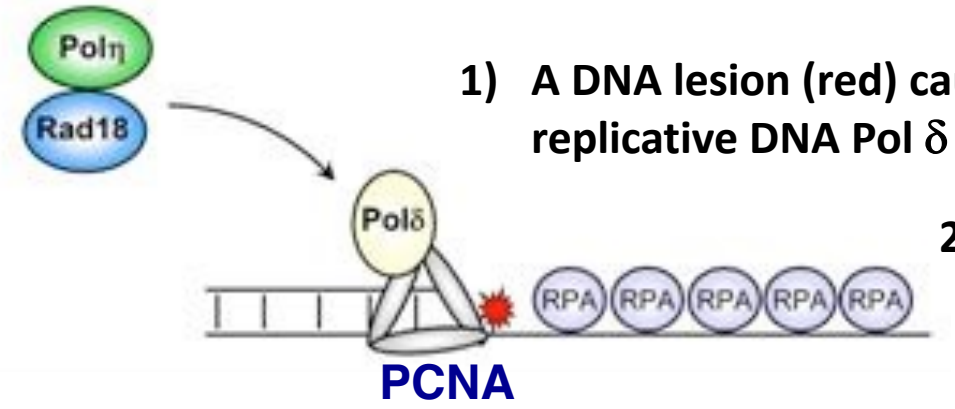
Primer Extension Assay to map template replication by the two DNA polymerases

Bacterial and Eukaryotic cells possess multiple translesion polymerases that are capable of bypassing DNA lesions

Block of polymerization at 8-oxoG

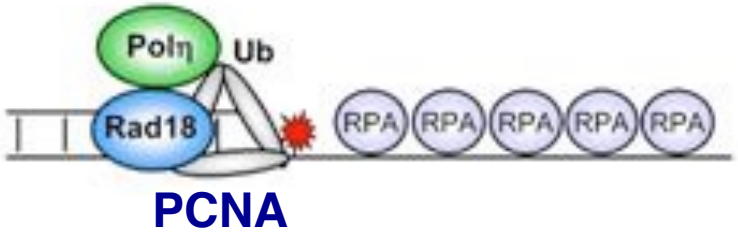
```
Primer    (41nt)  5´-AGG
Template  (75nt)  3´-TCCGTA(X/X*)AATG--5´
```

Switch between Replicative and Translesion DNA polymerases involves PCNA Ubiquitination and prevents stalling of replication at the sites of DNA damage



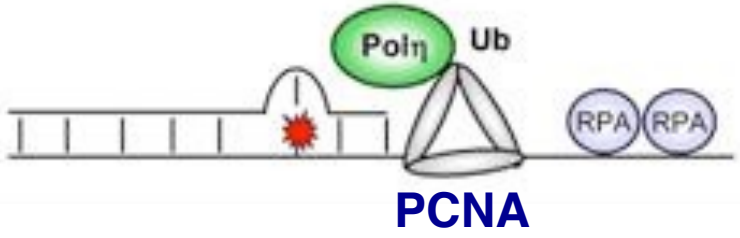
1) A DNA lesion (red) causes stalling of the replicative DNA Pol δ

2) The E3 ubiquitin ligase Rad18 guides Pol η (a TLS DNA polymerase) to stalled replication forks



3) Rad18 adds a single ubiquitin protein group (monoubiquitinates) to stalled PCNA

(Monoubiquitination =/= polyubiquitination which typically triggers protein degradation)



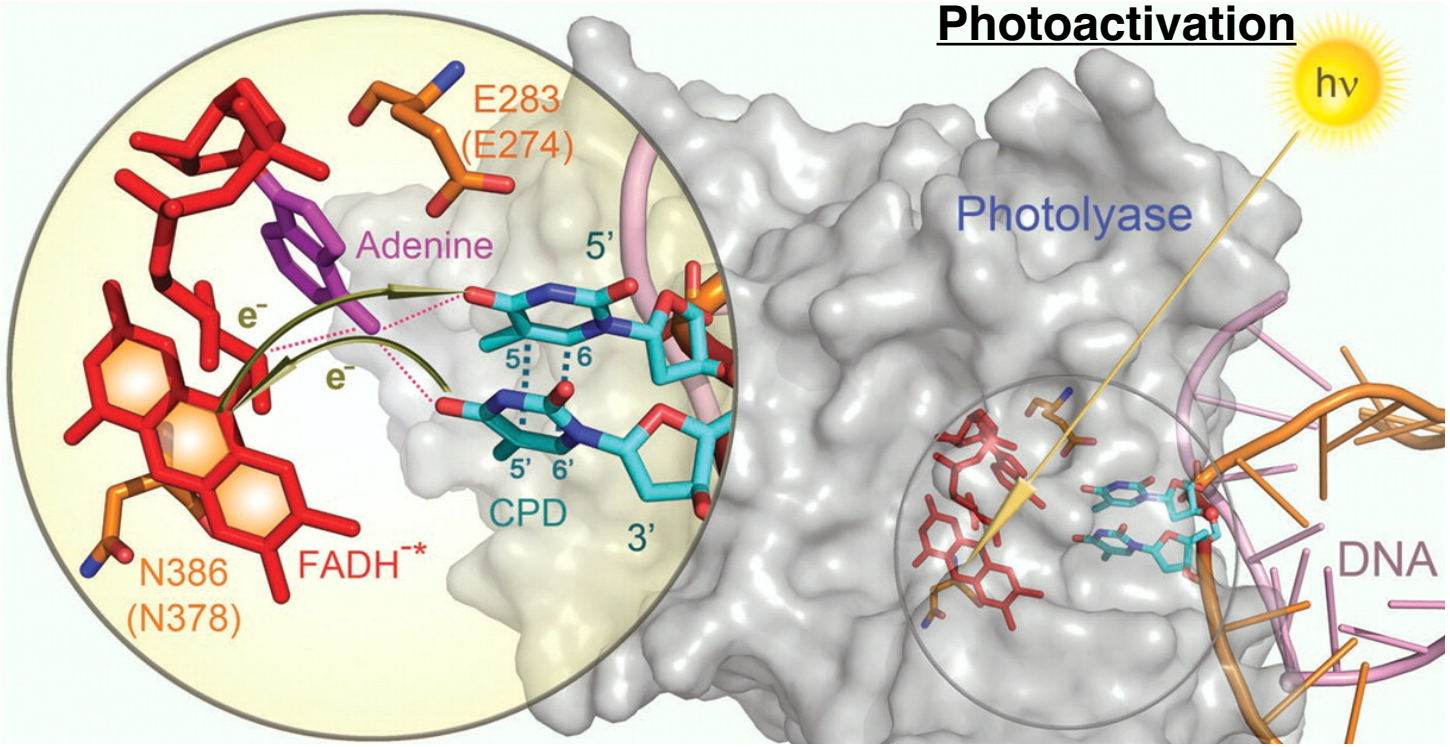
(4) Pol η binds to mono-ubiquitinated PCNA and performs replicative bypass of damaged DNA, preserving replication fork movement.

http://www.pathology.unc.edu/faculty_labs/vaziri_lab/image012.jpg

Strategy #2: Direct repair

Direct Reversal of pyrimidine dimers by Photolyase

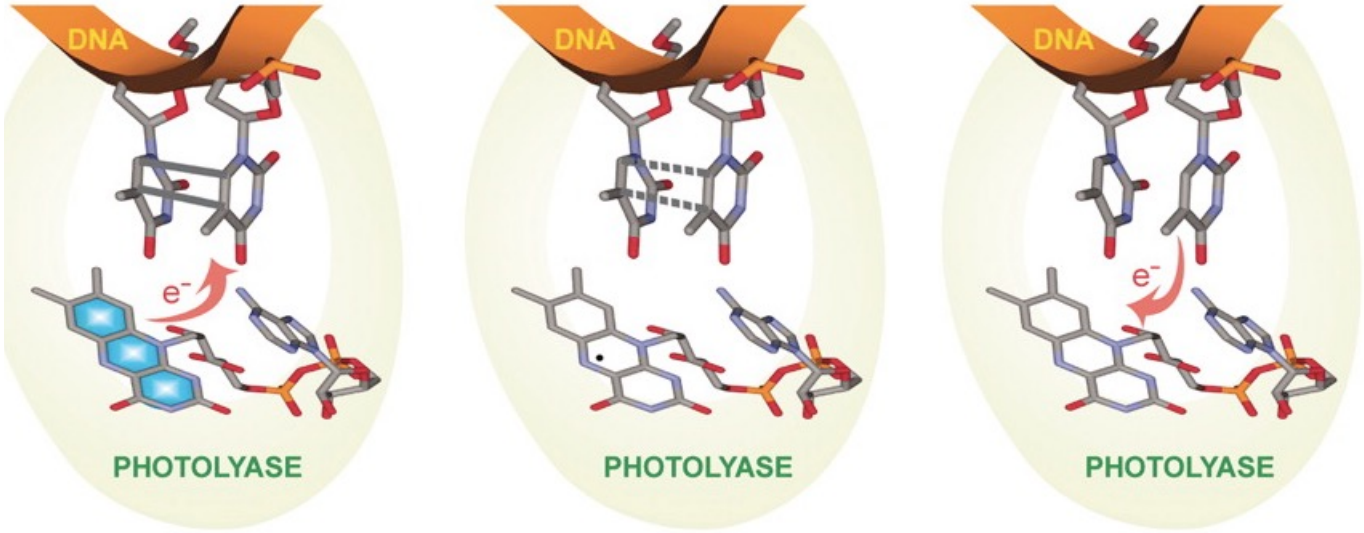
light activates the FADH- cofactor, which transfers an electron to the pyrimidine dimer for splitting of the ring.



Liu Z et al. PNAS 2011

Found in bacteria, archaea, plants and some animals

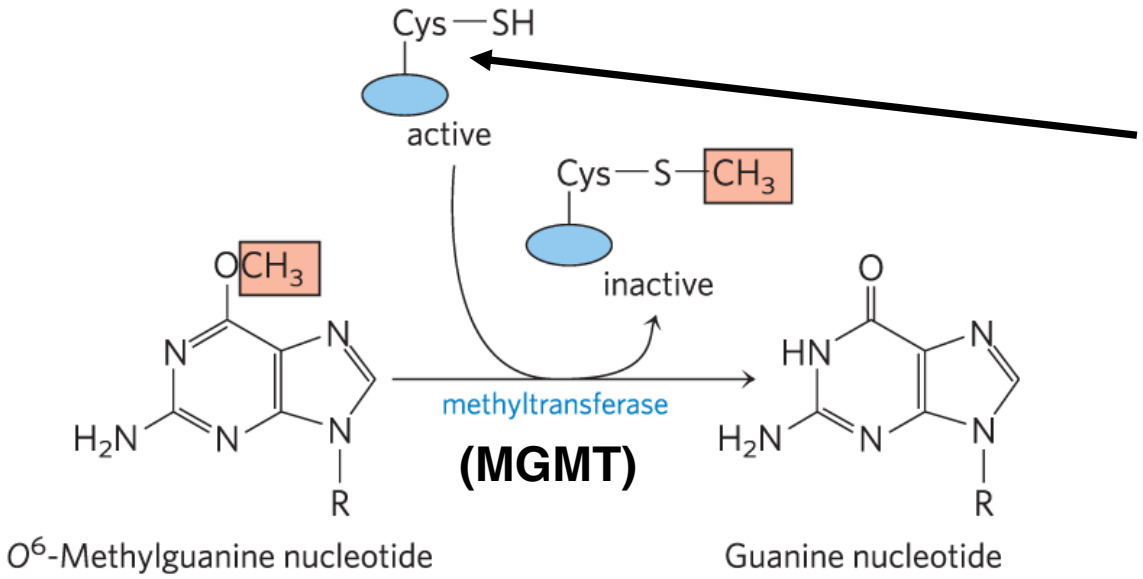
Absent in placental mammals including humans



Kao et al. PNAS 2005

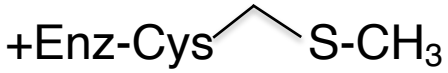
$$FADH^{-*} + T<>T \xrightarrow{k_1} FADH^{\bullet} + T<>T^{\bullet -} \xrightarrow{k_2} FADH^{-} + T + T$$

Repairing O^6-methylG: Dealkylation of guanines by Methyl Guanine Methyl Transferase (MGMT)



A cysteine residue in MGMT is the methyl acceptor.

MGMT is a suicide enzyme (it can only perform this reaction once):



- **Mutations of human MGMT linked to cancer:**
  => maintaining DNA information is required for tumor suppression

- **The "inactivated" enzyme serves as a transcription factor to induce expression of DNA repair genes -> amplifies the cellular response to DNA damage**

Strategy #3: Base excision repair

Base Excision Repair: General strategy

Key components:

1) DNA glycosylase/ glycosidase = Cleaves glycosidic bond at damaged base
Uracil, 8-oxoG, etc...

Glycosidases are specialized to recognize 1 type of damaged base

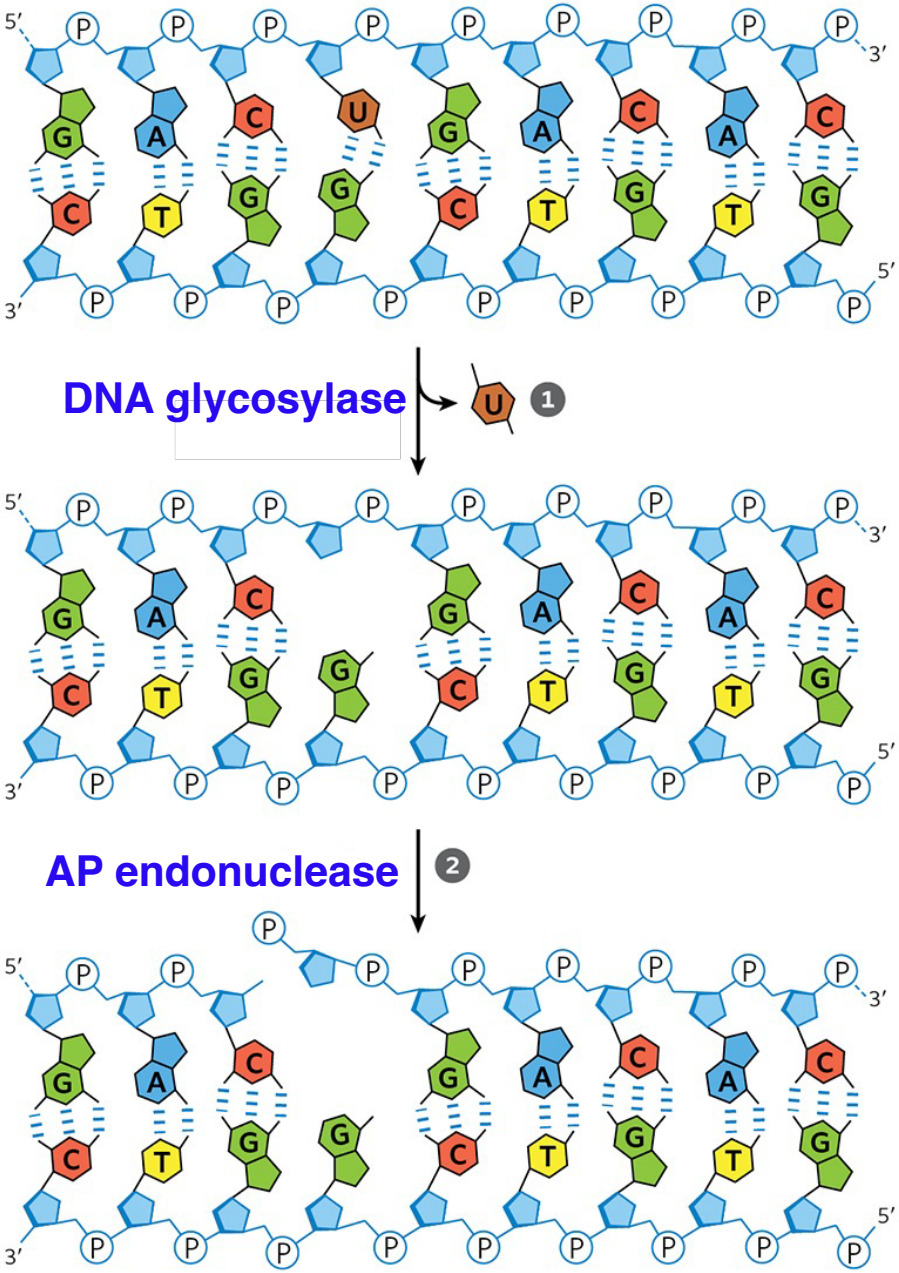
2) AP endonuclease = Cuts strand at AP site (A[urinic or Apyrimidic)

AP endonucleases also take care of spontaneous depurination events

3) DNA Pol + DNA ligase

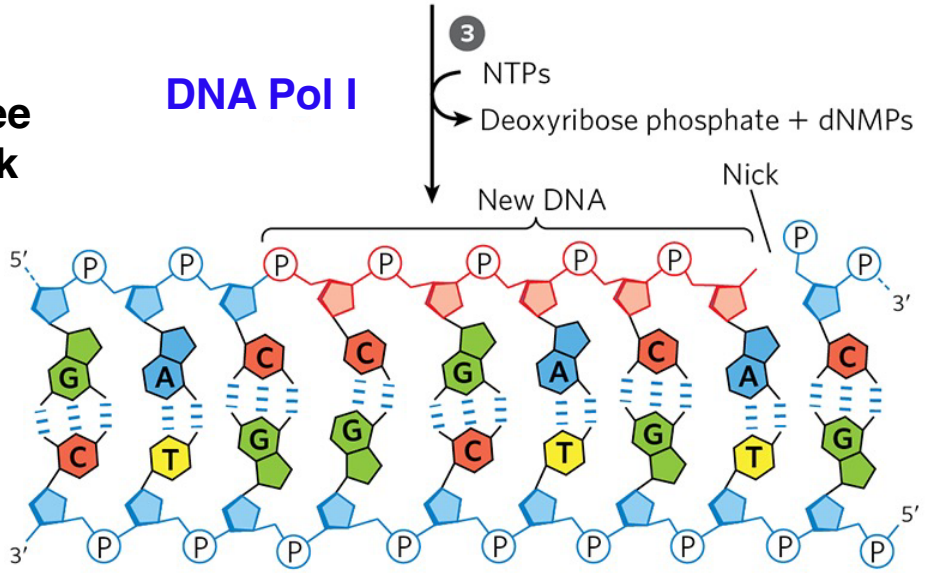
DNA glycosidase and AP endonuclease activities can sometimes be performed by the same protein, e.g. OGG1

Step 1: a DNA glycosylase (here uracil glycosylase) recognizes the damaged base and cleaves between the base and deoxyribose



Step 2: an AP endonuclease cleaves the phosphodiester backbone near the AP site

<u>Step 3</u>: DNA Pol I initiates repair synthesis from the free 3' OH at the nick (nick translation)



<u>Step 4</u>: DNA ligase seals the nick