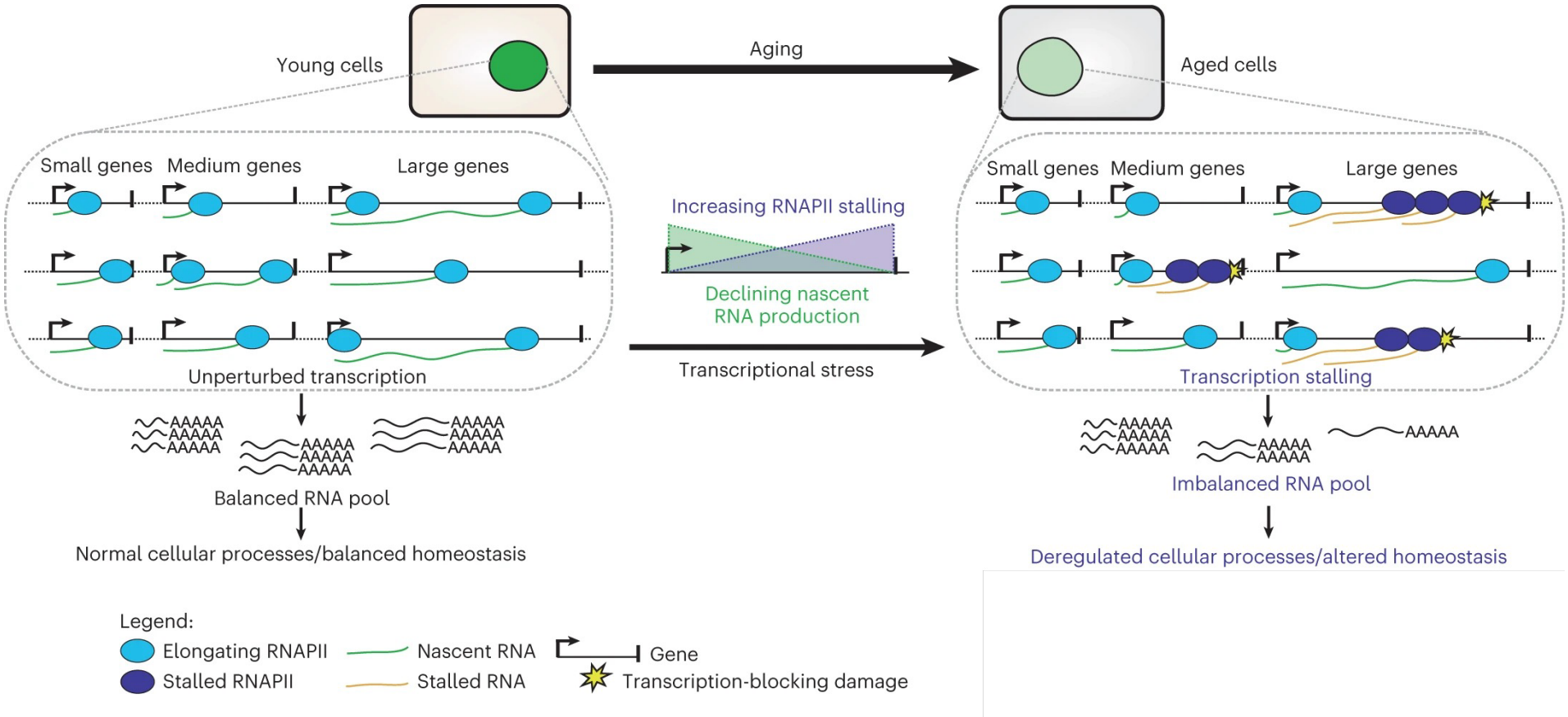
Unit 4: DNA Repair

We all get old...partly because cells can't deal with all the DNA damages that accumulate with age, which block RNA polymerases transcribing (long) genes...



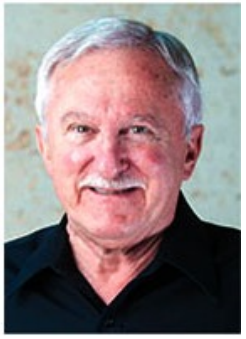
Gyenis et al. Nature Genetics 2023

DNA Damage Tolerance and Repair

1: Dealing with problems that occur during DNA replication

- Ribonucleotides incorporated during DNA replication
- Mutations resulting from errors made during DNA replication
  - Mismatch Repair Pathway

(P.Modrich/Nobel Prize Chemistry 2015)



2: Dealing with problems caused by DNA damage unrelated to DNA replication

- Non-exhaustive list of damages
- Strategies and mechanisms of DNA damage tolerance and repair:
  - Bypass/Translesion DNA polymerases
  - Direct Reversal

- Base Excision Repair (T.Lindahl/Nobel Prize Chemistry 2015)

- Nucleotide Excision Repair

(A.Sancar/Nobel Prize Chemistry 2015)

We will not cover:
Double-stranded break repair

Learning outcomes:
What you need to know/understand after this unit

Understand the errors that can occur during replication and the mechanisms involved in fixing these errors

Know the major type of damages studied in lecture and their consequences on DNA polymerases (block/mutations)

Understand how translesion DNA polymerases ensure replication over damaged DNA

Understand and know the 3 type of mechanisms that correct DNA damages and what type of damages they typically correct

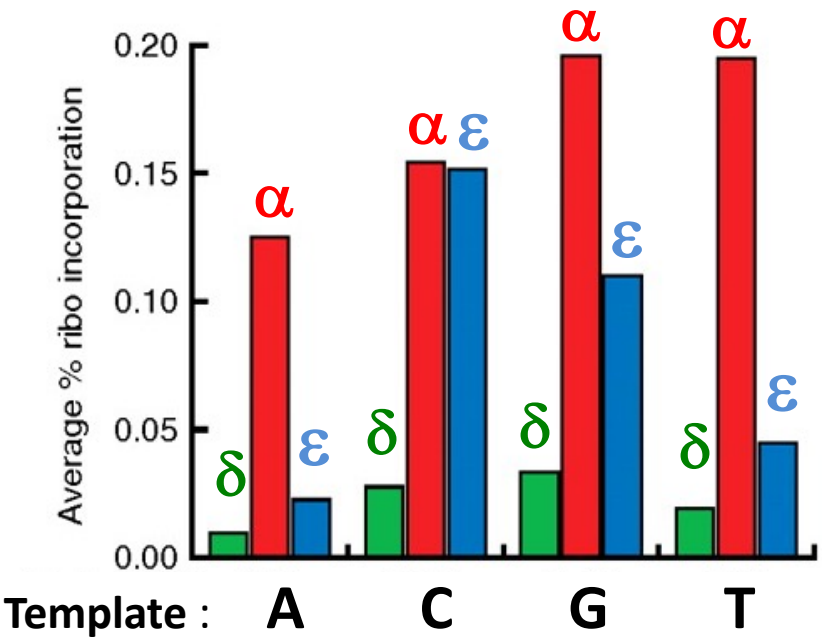
Understand how DNA repair enzymes recognize damaged DNA: General principles and examples studied

The problem of ribonucleotide incorporation by DNA polymerases

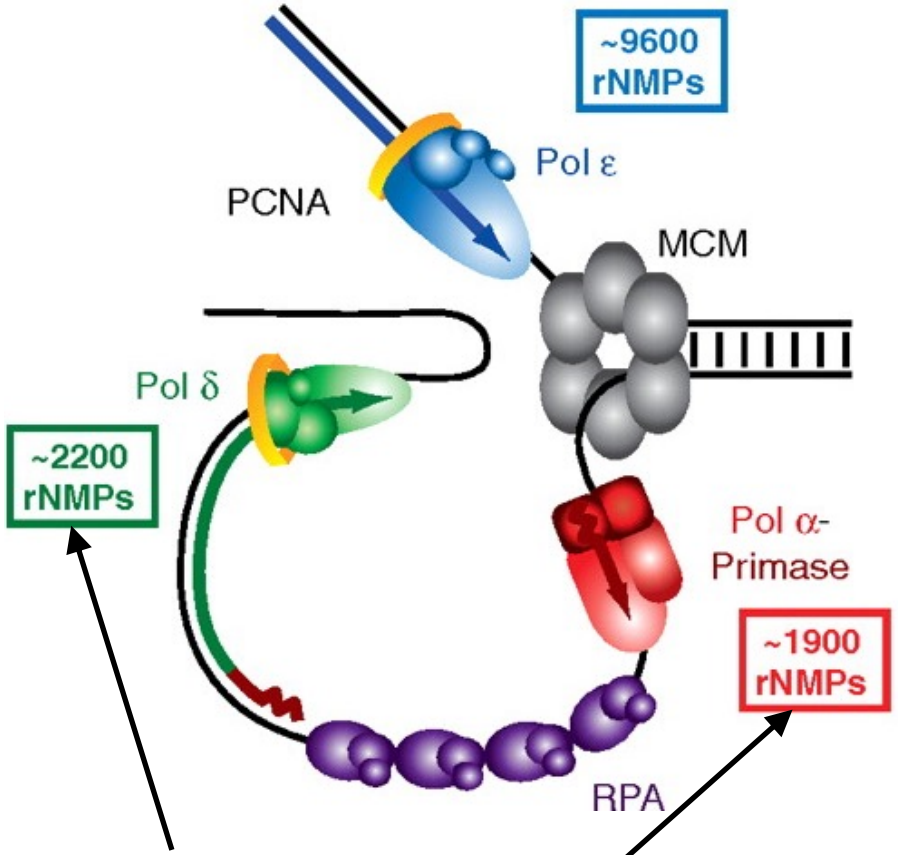
Why wouldn’t we want rNTPs incorporated during DNA replication?

The problem of ribonucleotide incorporation by DNA polymerases

- **DNA polymerases discriminate deoxy vs riboNTP, but not at 100%**
- **[rNTPs] >> [dNTPs] in vivo → this leads to some riboNTP incorporation in newly synthesized DNA (sugar discrimination by DNA polymerases is not 100%)**

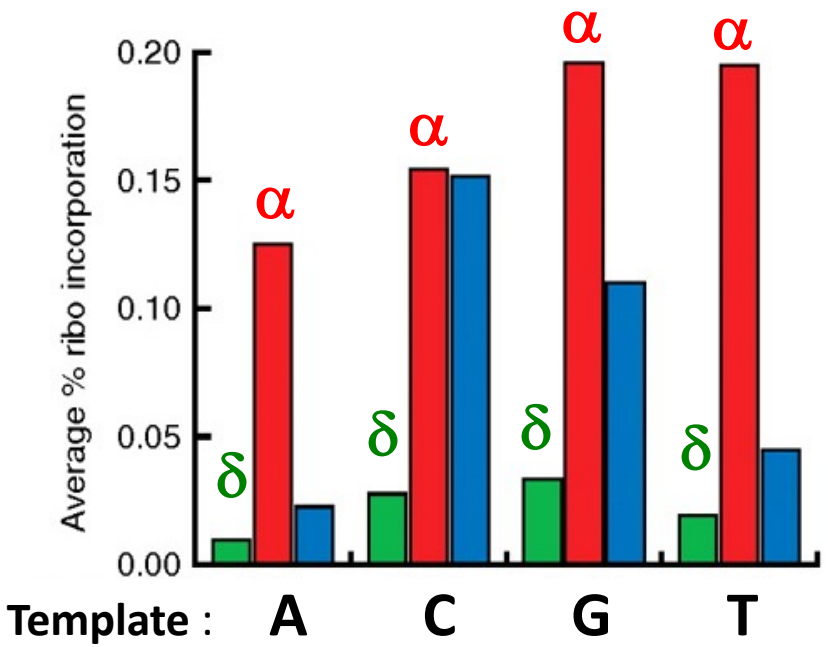


(PNAS 107, p4950, 2010)



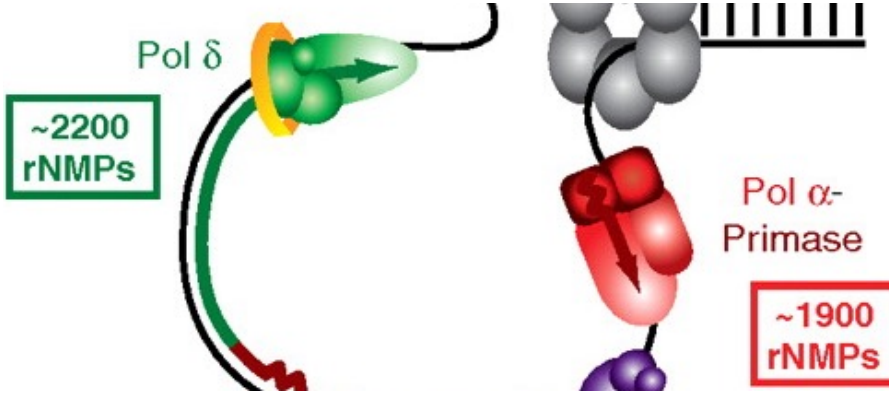
Total number of rNMPs incorporated when replicating the whole yeast genome

Why is Pol δ incorporating more rNMPs than α while its avg % rNTP incorporation is lower?



A: α synthesizes primers which are then removed by processing of Okazaki fragments so less rNMPs overall

B: Rates of misincorporation measured in vitro don´t reflect polymerases activities in vivo

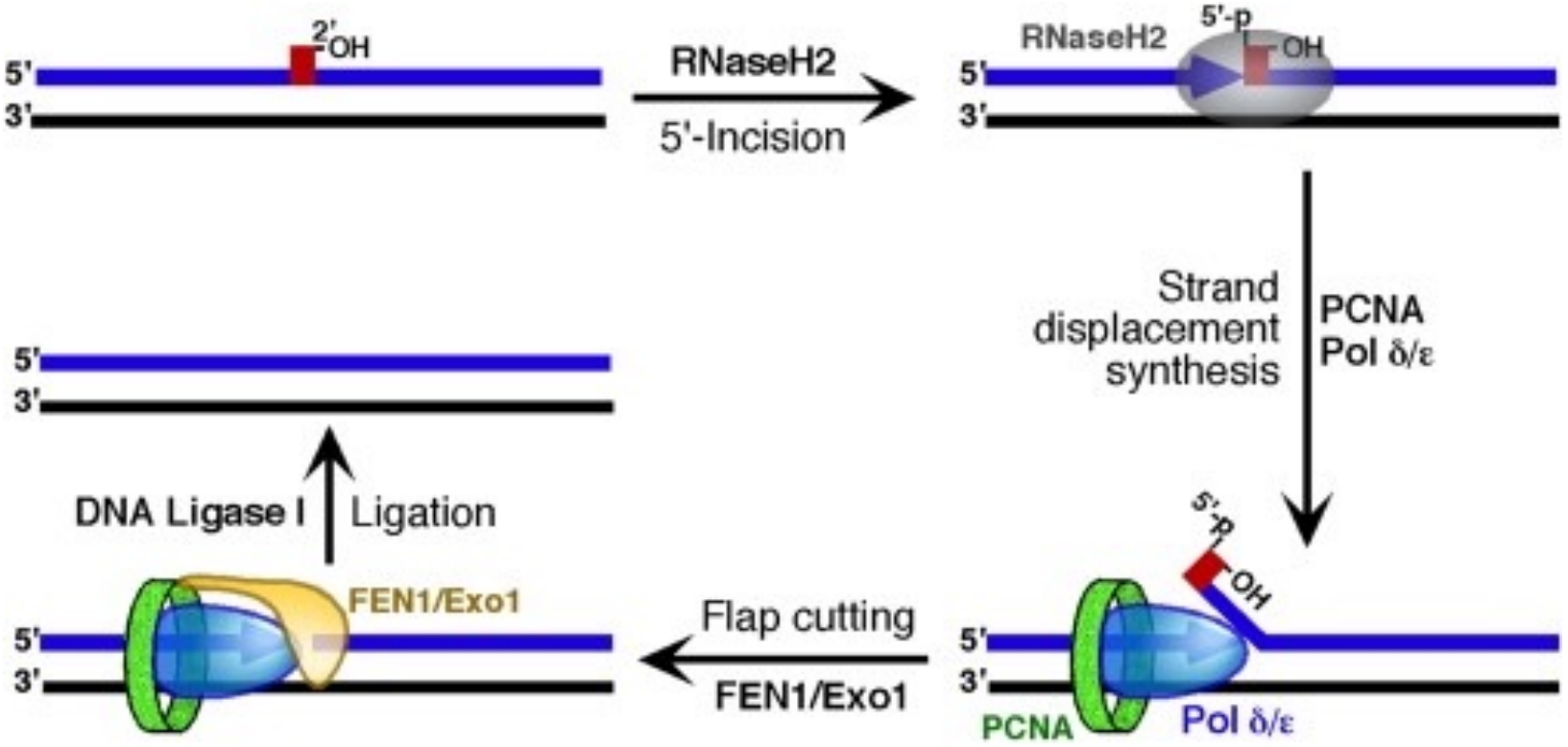


C: α has a riboNMP proofreading activity which delta does not have

D: δ synthesizes more DNA length so this results in more rNMP per round of replication despite lower %

Enzymes that deal with removal of RNA primers in Okazaki fragments also remove riboNTPs *mis*incorporated into DNA:

Mainly RNase H + Enzymes that process Okazaki fragments

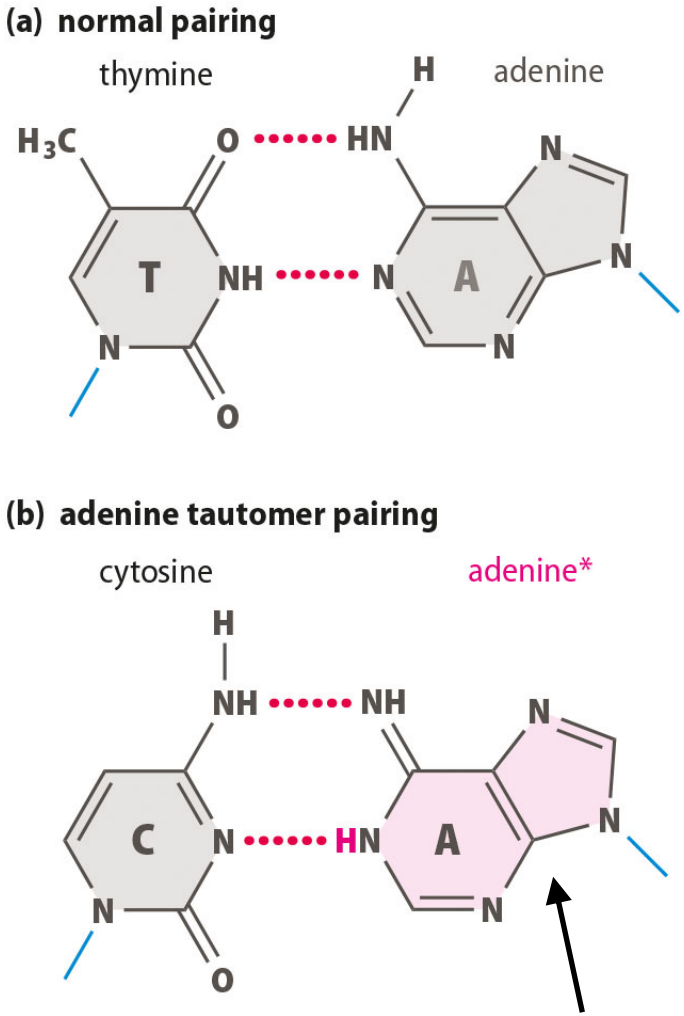


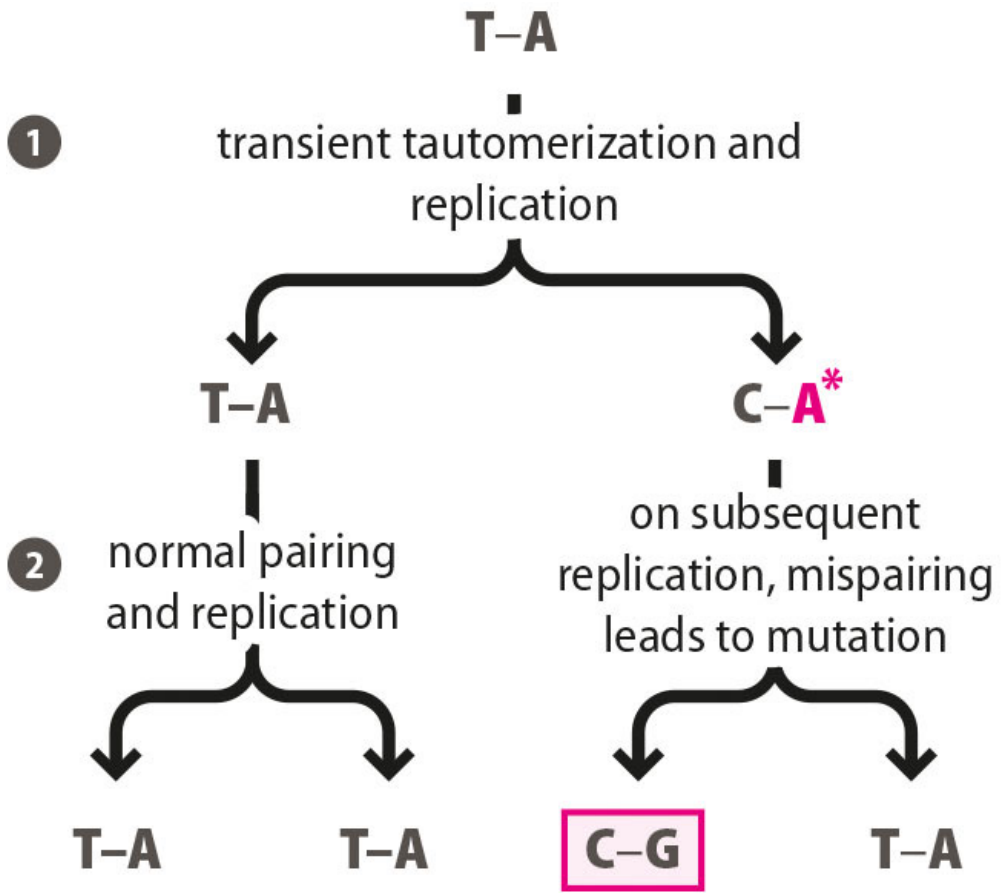
Sparks et al. Mol. Cell 2012

Why might a DNA polymerase make a mistake?

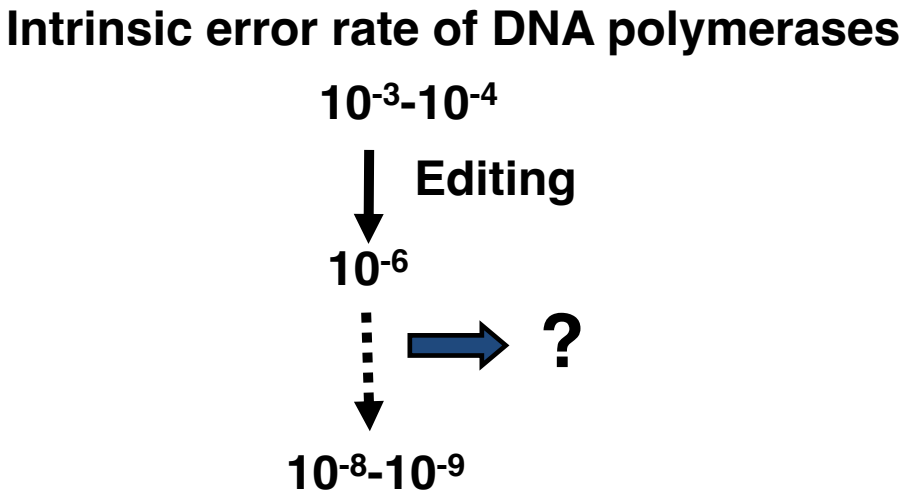
Mistakes can occur due to tautomerization

Transient tautomerization happens with probability of ~10^{-3}-10^{-5}





How do we get to the 10^{-8}-10^{-9} error rates observed for DNA replication?



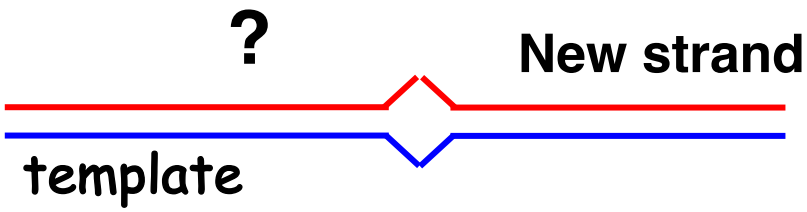
Increasing replication fidelity by mismatch repair
(2015 Nobel Prize: Paul Modrich)

Suppose a mistake is made:

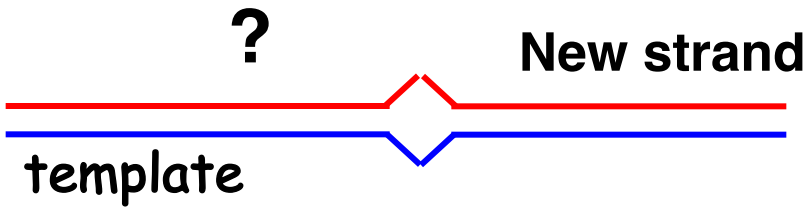
How would a cell know that there is a mistake?

Once we identify that there's a mismatch, how do we know which of the two nucleotides is correct?

How do we differentiate between the template strand and the new strand?

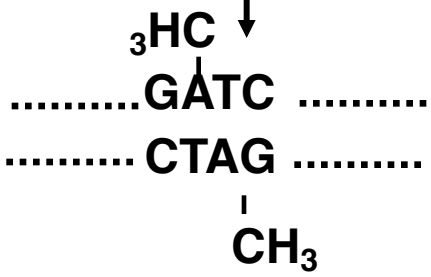


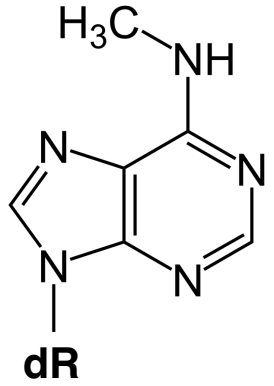
Increasing replication fidelity by mismatch repair in bacteria



1) DNA is methylated at specific palindromic sequences (adenosine N6)

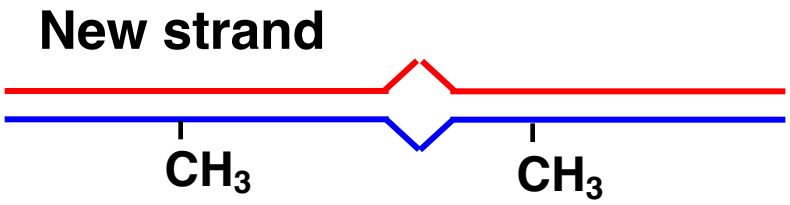
Dam methylase





Dam methylase methylates adenosines using a methyl group from S-adenosyl methionine (SAM)

2) DNA methylation is delayed after replication



How frequently are hemimethylated GATC sites encountered on average in chromosomes (assuming 25% A, T, G, C)?

A: every 256 base pairs (4^4)

B: every 64 base pairs (4x16)

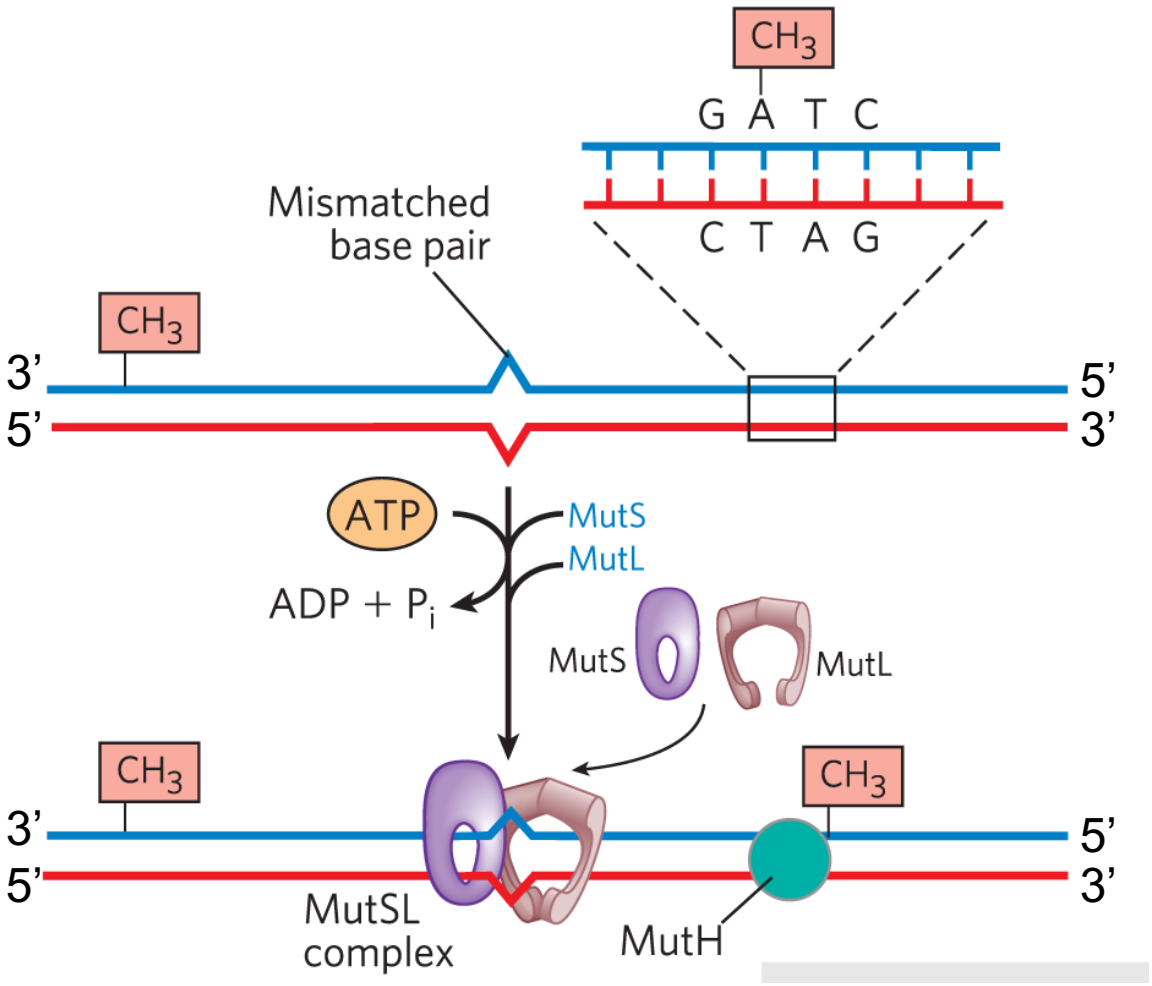
C: every 16 base pairs (2^4)

D: every 65536 base pairs (16^4)

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: binds MutS**
  - **(3) MutH: an endonuclease that nicks the new DNA strand**

Mismatch repair in *E. coli*

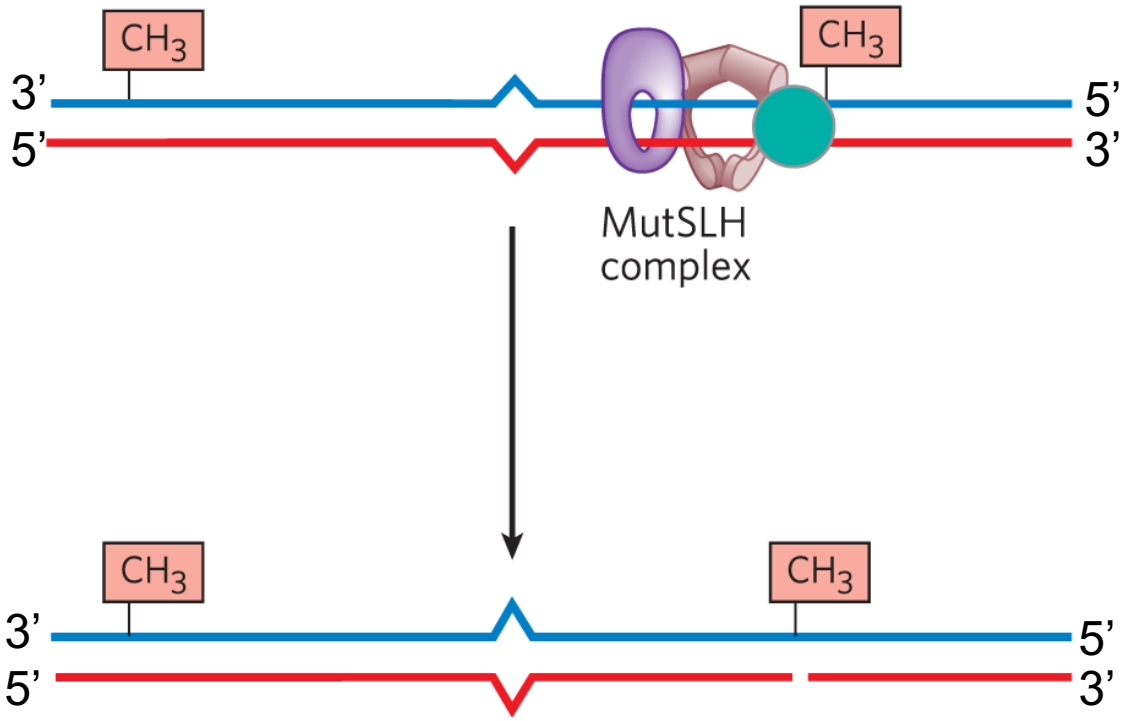


Step 1: MutH binds to hemimethylated GATC

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

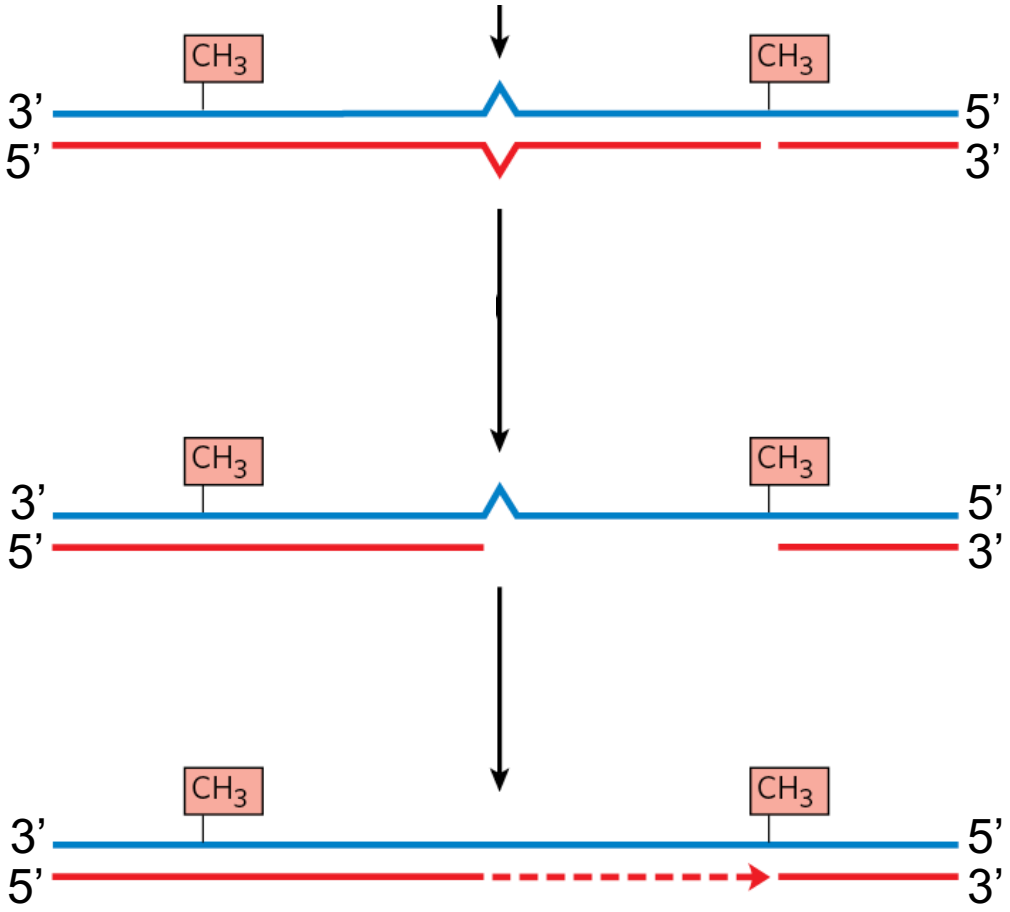
Mismatch repair in *E. coli*



<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

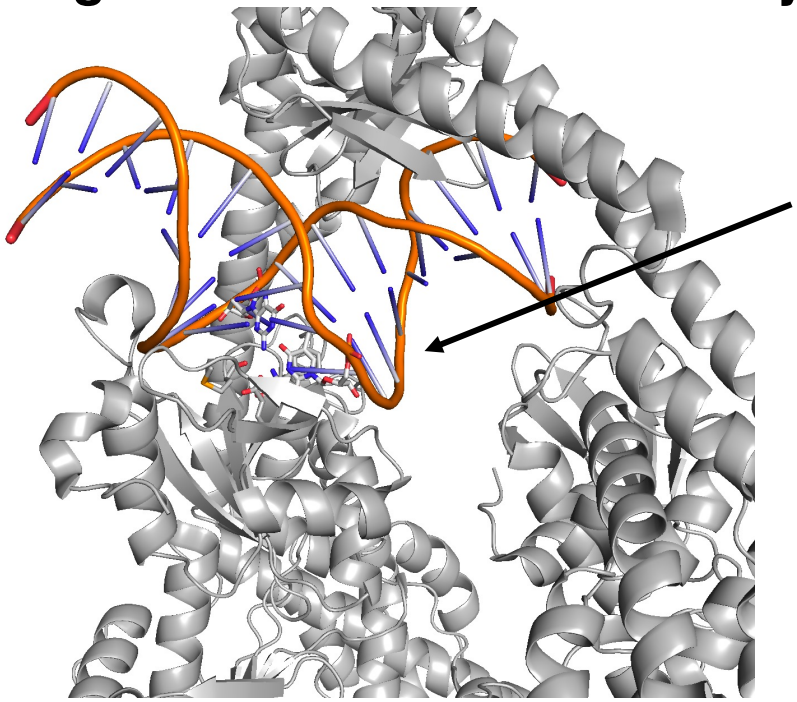
Step 8: DNA pol III fills in the gap with new DNA

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

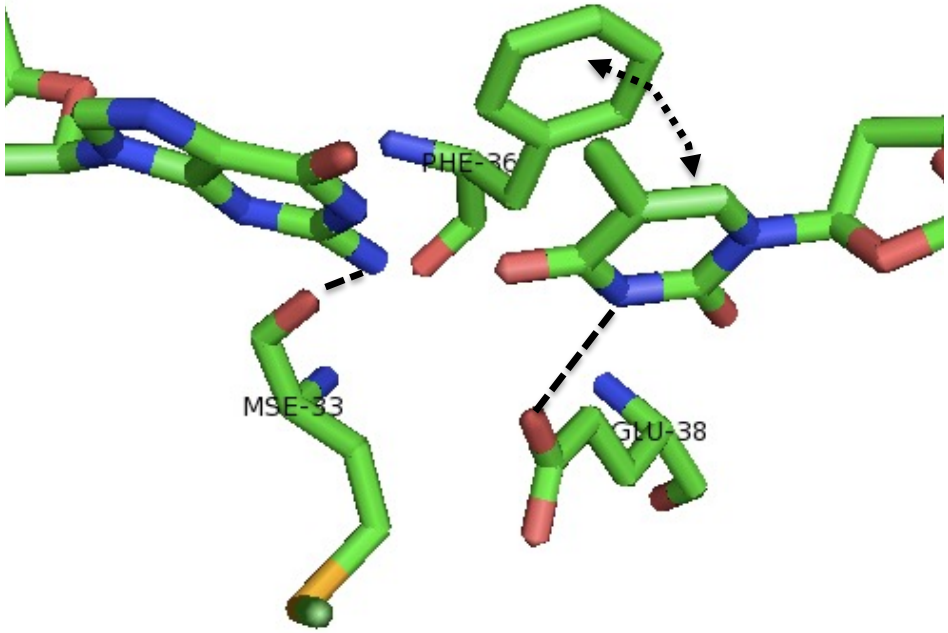


- DNA is kinked at site of mismatch

- 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**

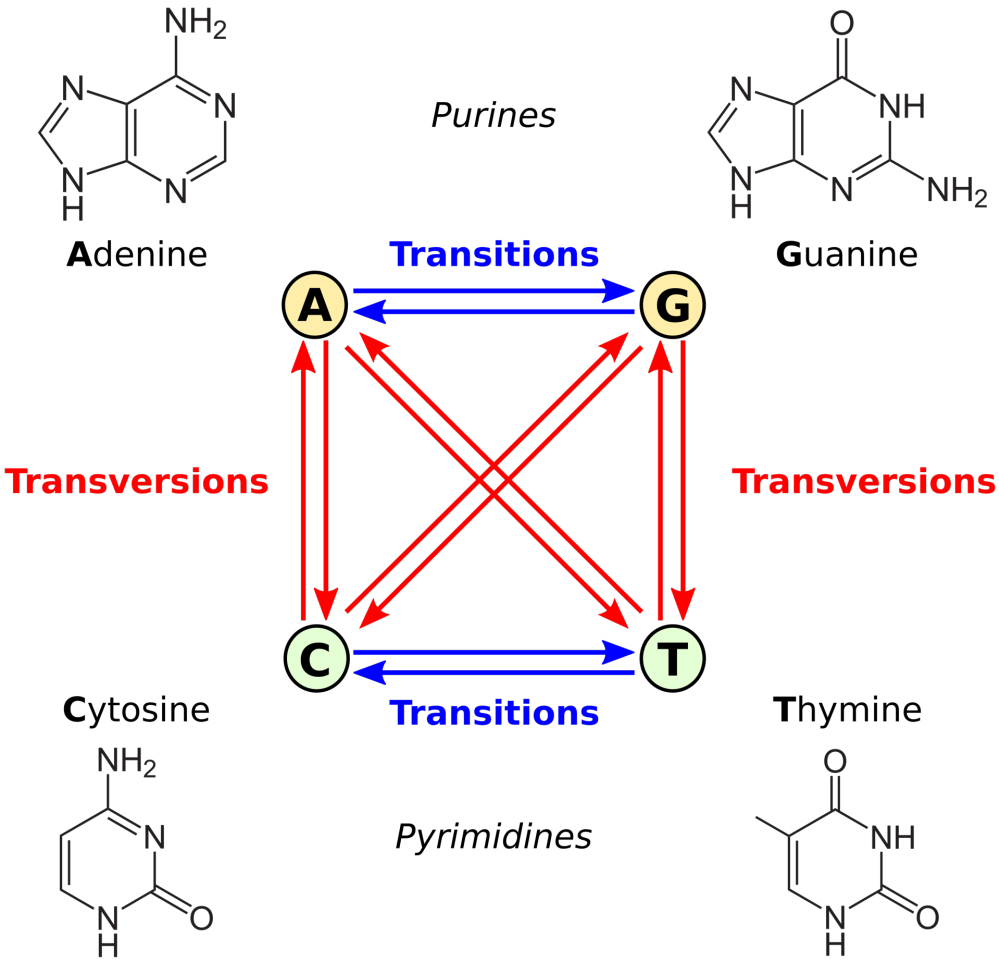
6. Bulky DNA adducts

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



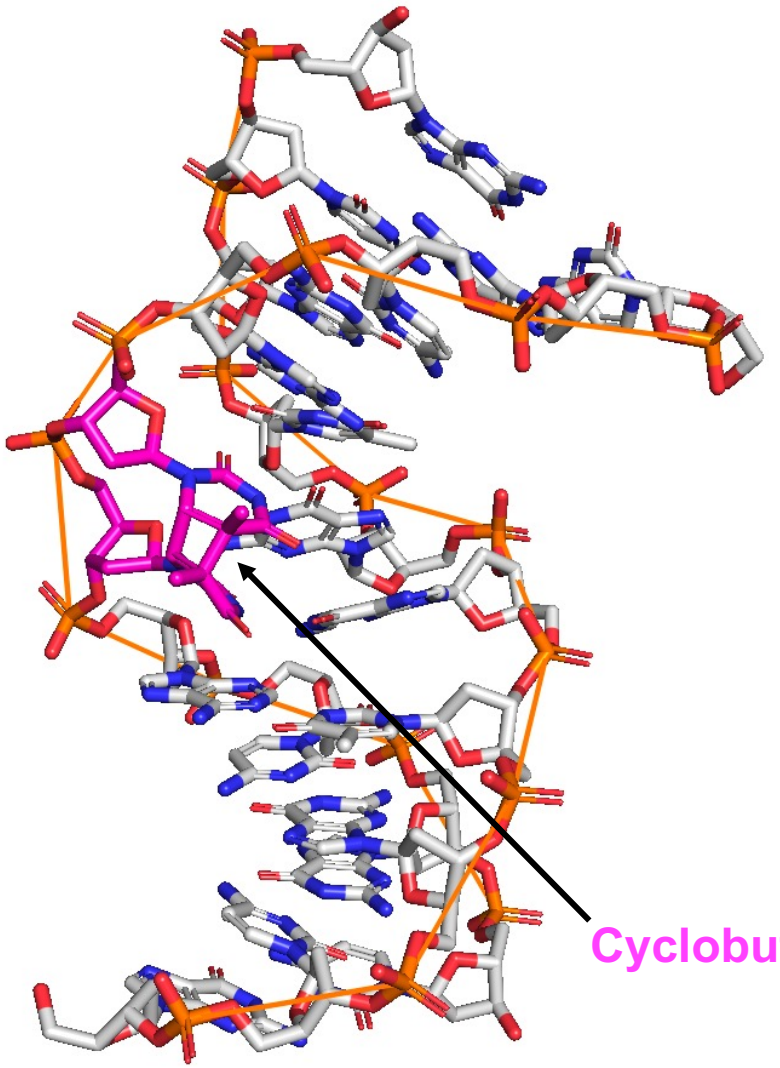
Some examples:

<u>Transition</u>: G-C → A-T

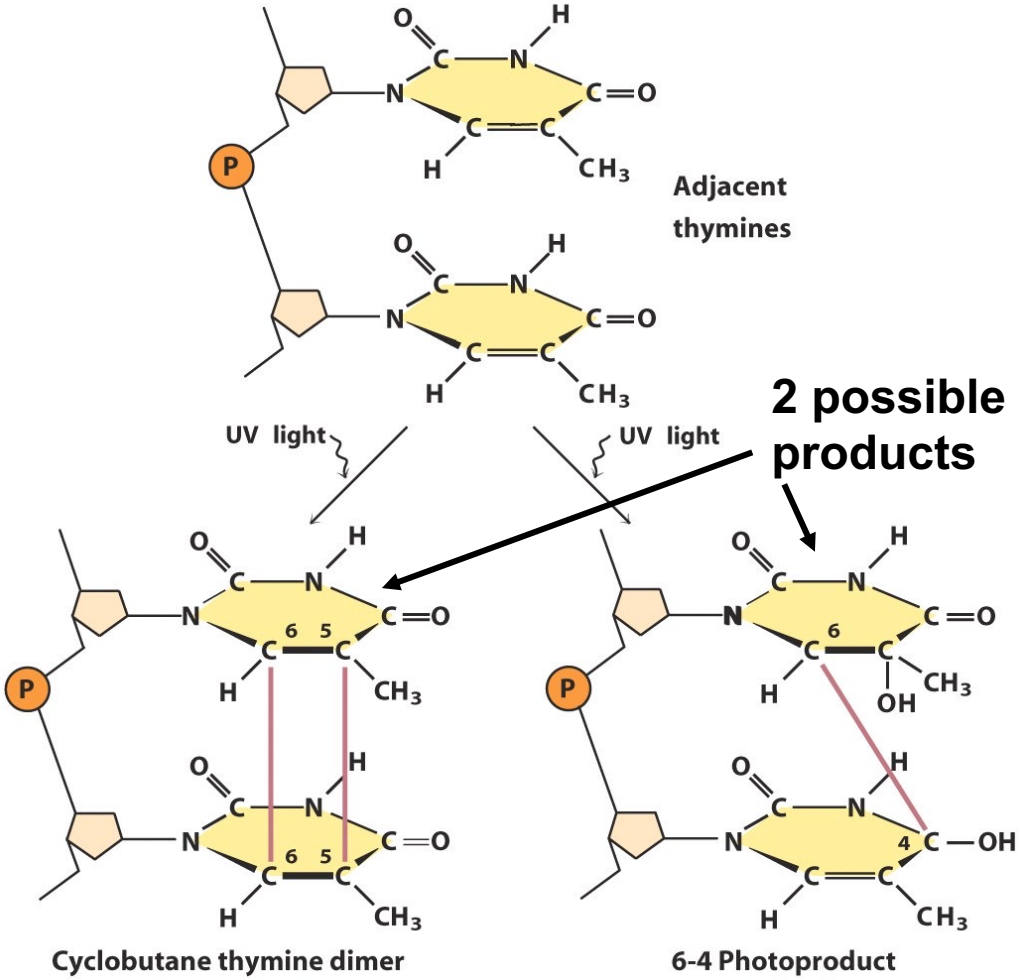
<u>Transversion</u>: G-C → T-A

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

Induction of pyrimidine dimers by UV light



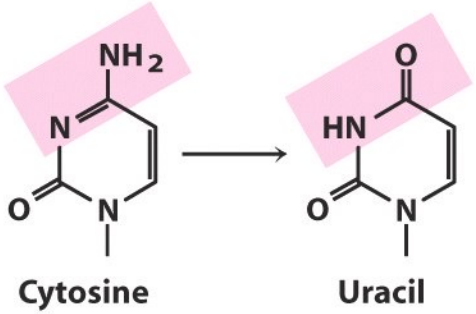
Cyclobutane thymine dimer



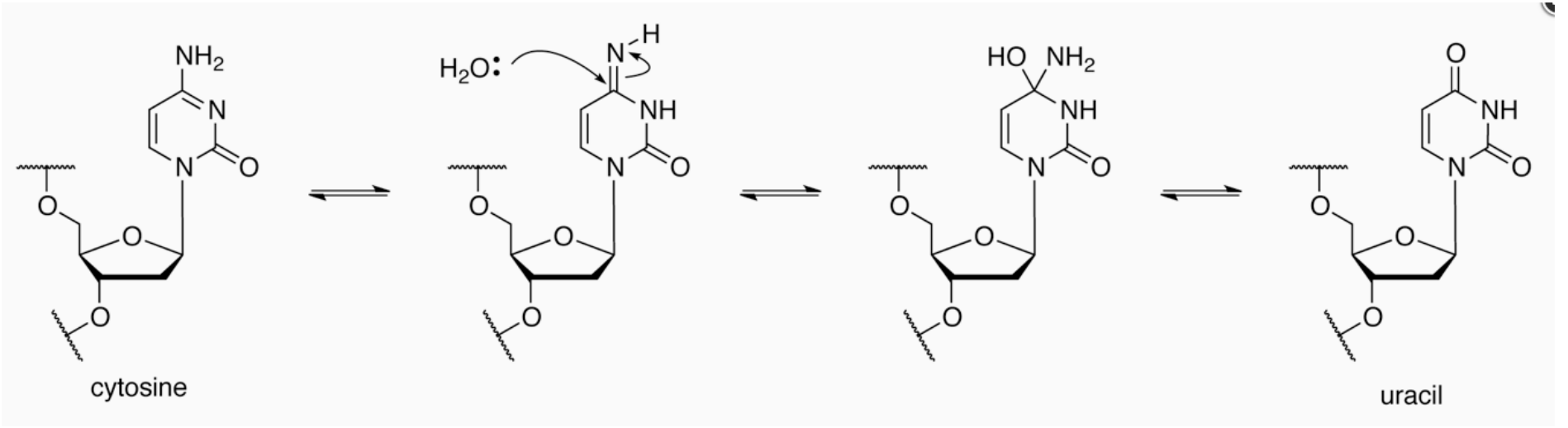
Will a DNA polymerase be able to replicate through this?

PyMol: thymidinedimer.pse

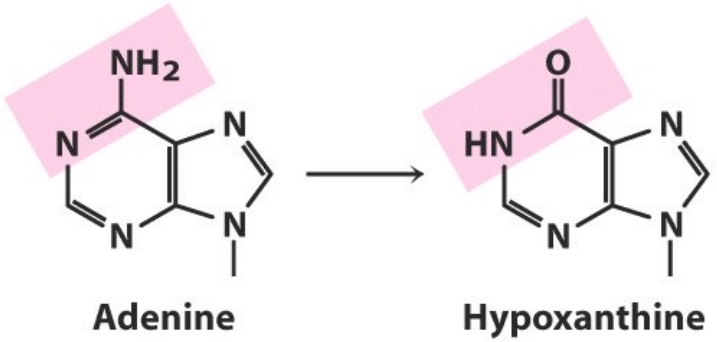
Spontaneous Deaminations



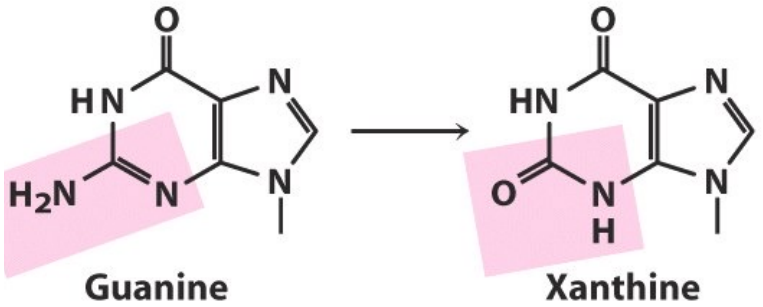
C --> U: 10^{-8}/24 hours:
~ 100 events/day for a mammalian cell genome



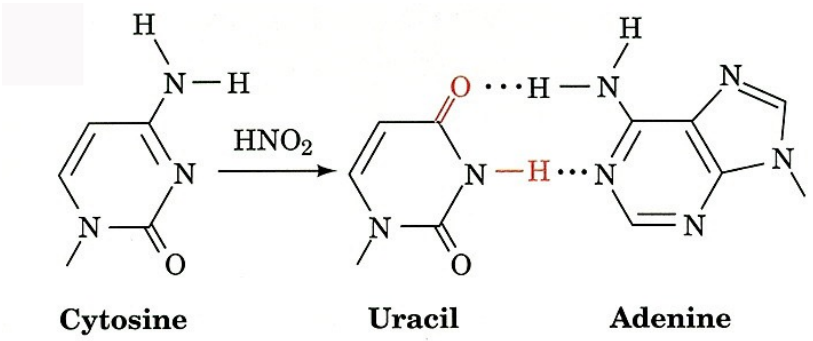
A --> H
10^{-9}/24 hours

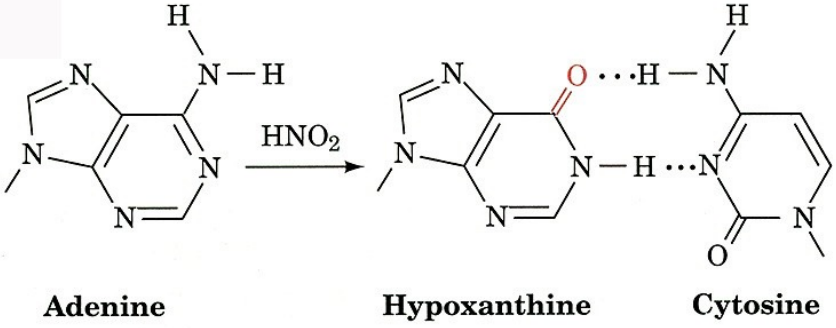


G --> X
10^{-9}/24 hours



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

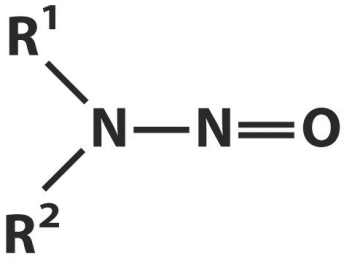
C: Yes because they can´t be recognized by the proofreading active site of DNA pols. and stall in the exo active site

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

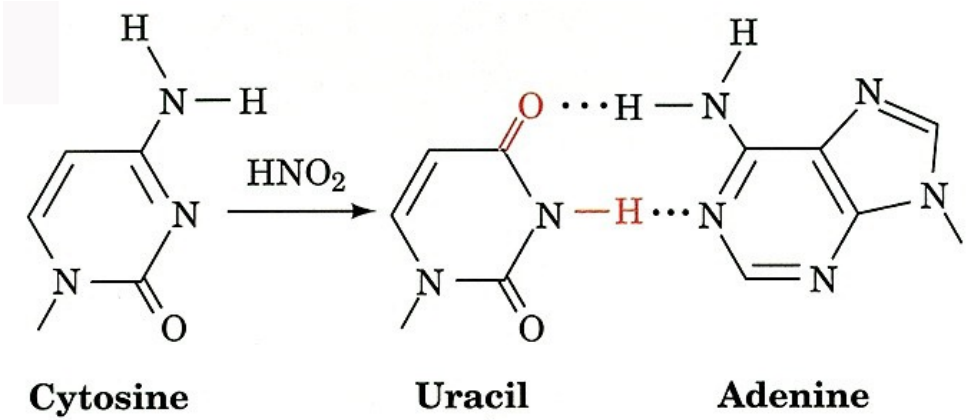


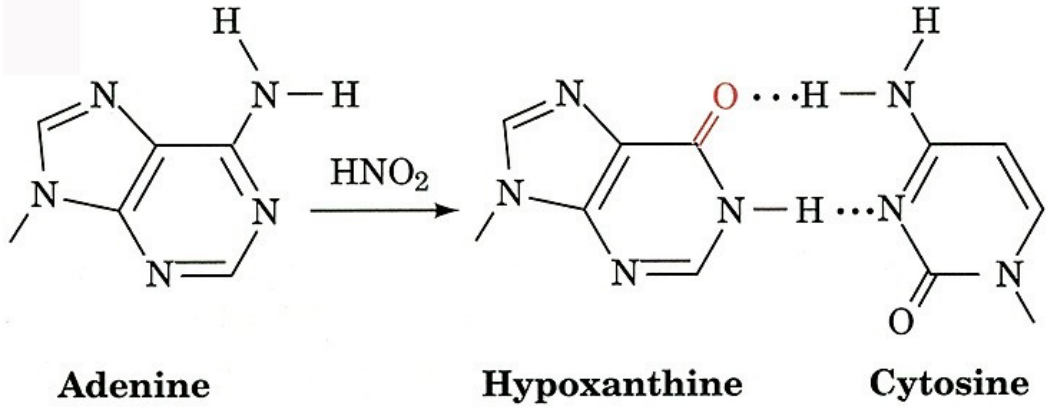
Nitrosamine
(by-product of rubber production)

Nitrous acid precursors

(Nitrous acid: HNO_2)

Genetic Consequences of Deaminations



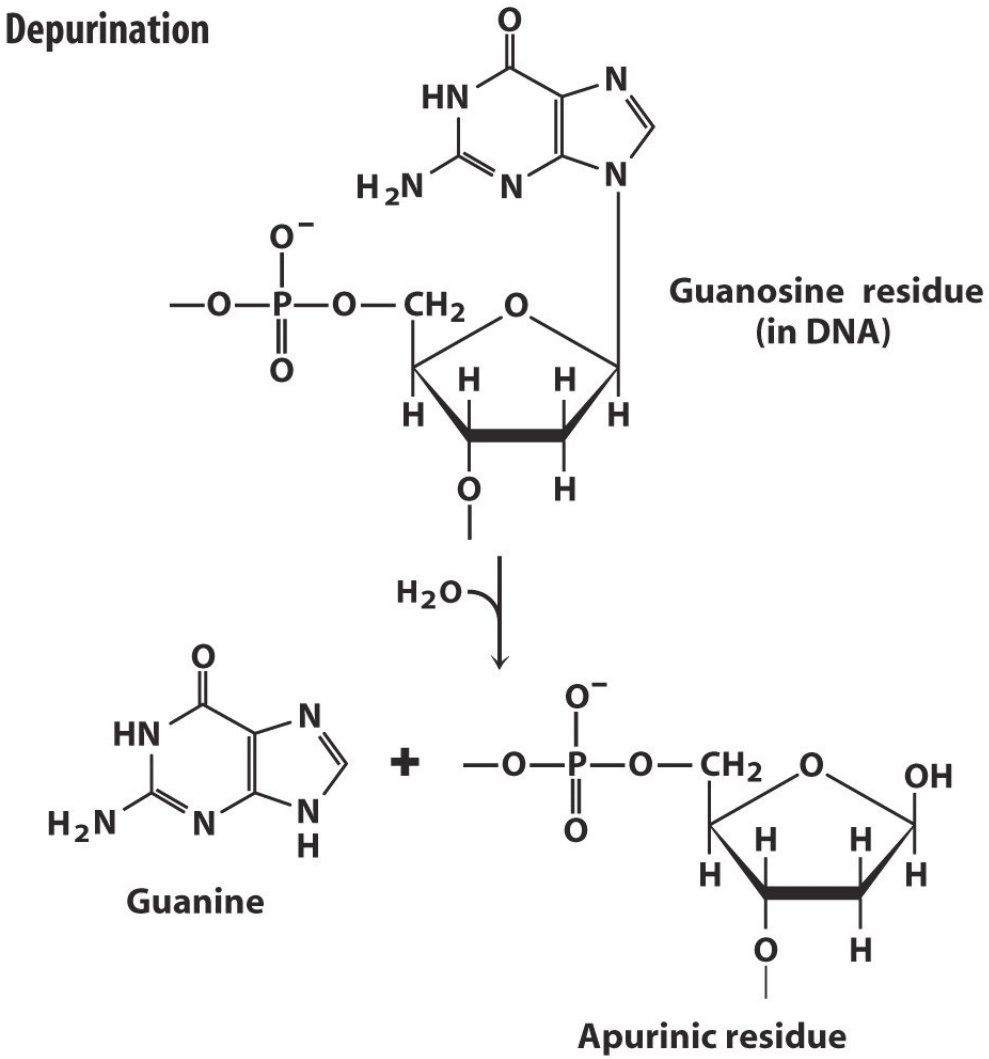


- Xanthine also base pairs with C

Spontaneous depurinations and depyrimidinations

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

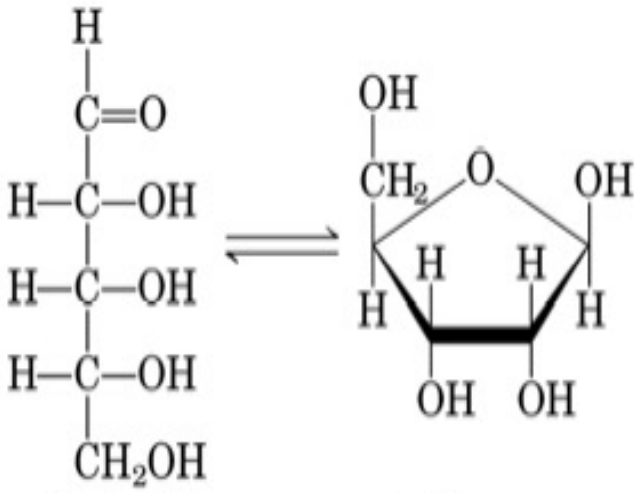
Depyrimidination: 500 events/day for a mammalian cell



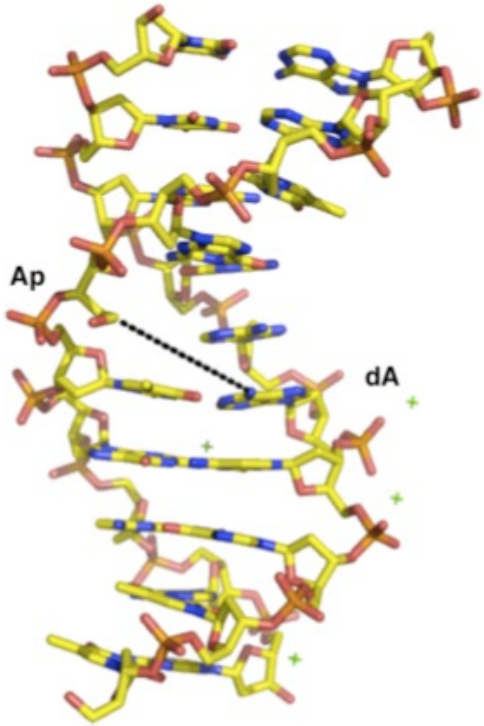
Nomenclature:
Abasic = No Base
Apurinic = no purine base
Apyrimidinic = no pyrimidine base
Generally called an abasic site or AP site.

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.



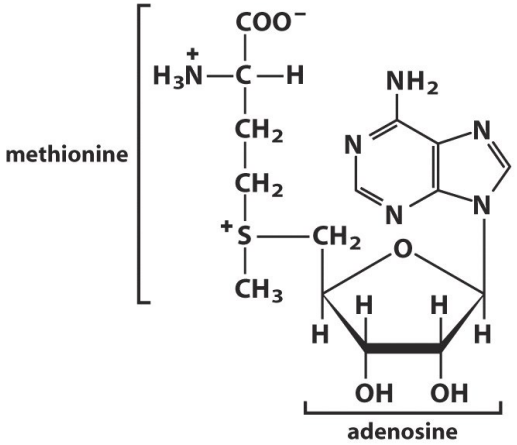
Interstrand crosslinks are bad for replication/transcription etc…

PyMol: view_AP_crosslink.pse

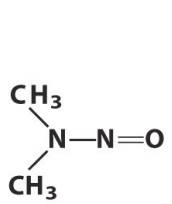
J. Am. Chem. Soc. 2014, 136, 3483−3490

Alkylations of bases: Chemical Sources and mechanism

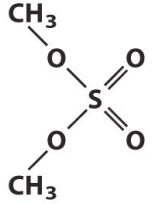
Base alkylation: Addition of methyl, ethyl, etc., group onto a base



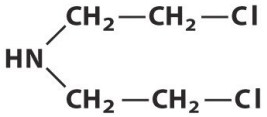
S-Adenosylmethionine



Dimethylnitrosamine



Dimethylsulfate



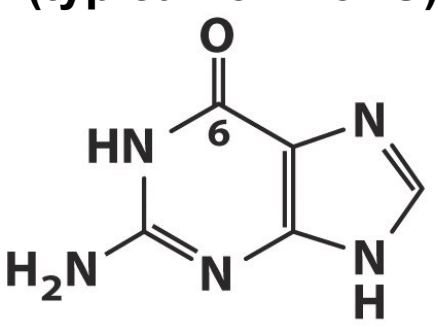
Nitrogen mustard

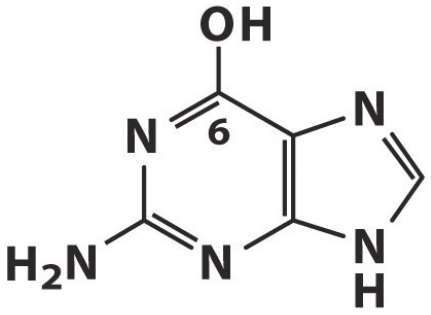
Alkylating agents

Used as a chemical weapon in WWI

Guanine tautomers

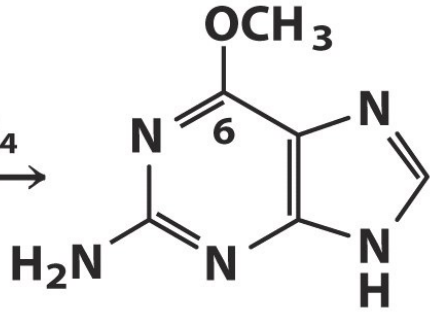
Keto
(typical form of G)





Enol
(rare)

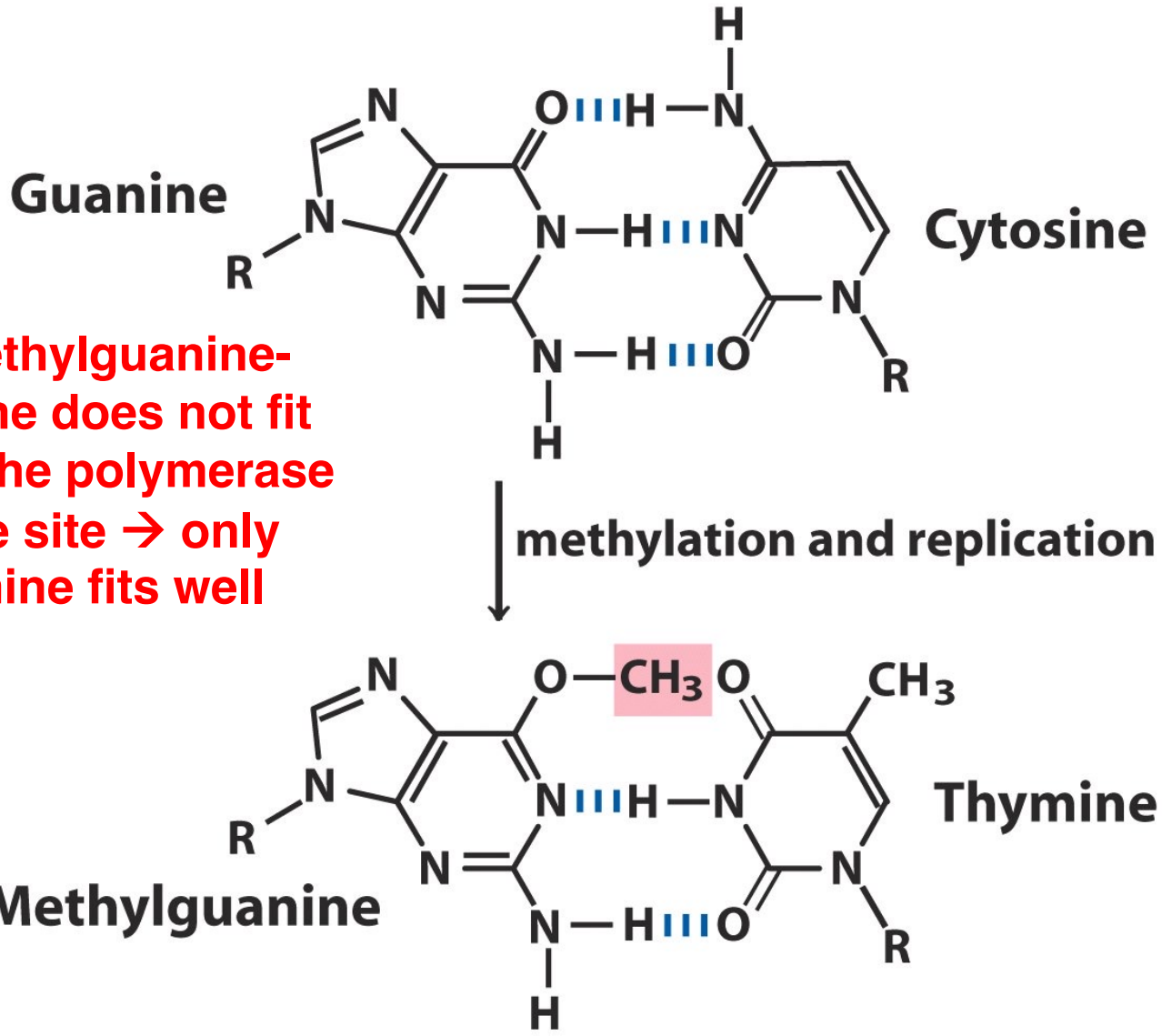
$(CH_3)_2SO_4$



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

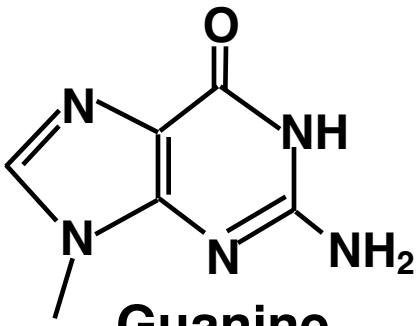
G-C → O^6meG-T → A-T

Oxidative damage of DNA

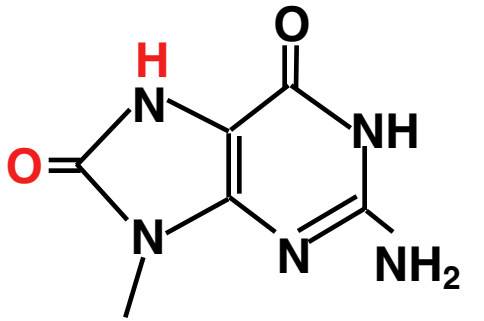
Major cause of oxidative damage: hydroxyl radicals produced via activity of the electron transport chain

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

Consequences for Nucleotides:



Guanine



8-oxoguanine

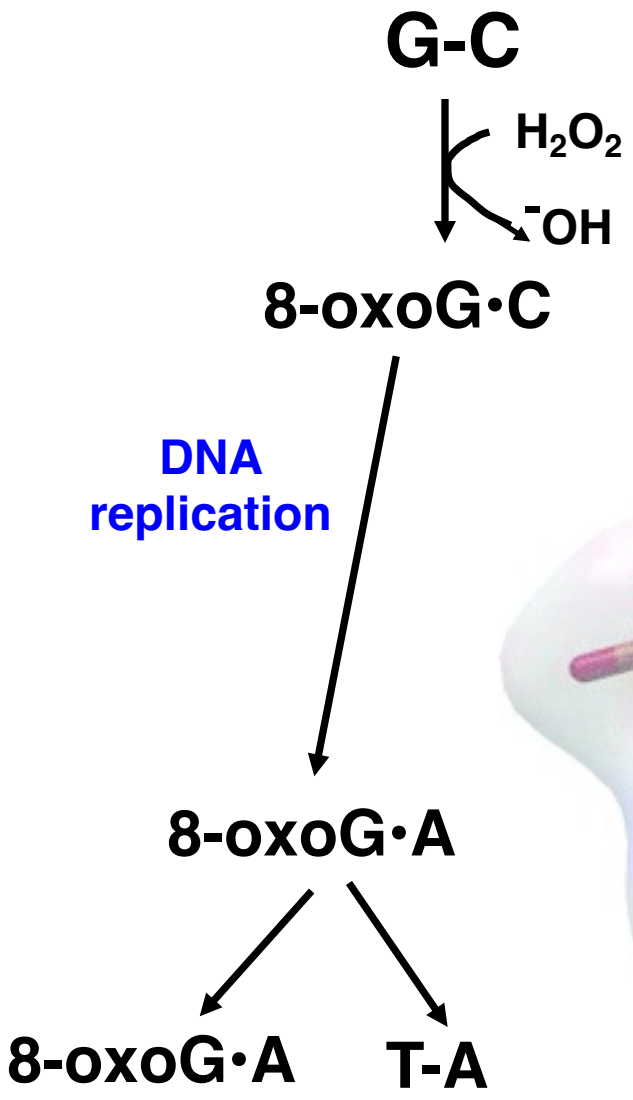
Thymine → **5-formyl Uracil**

Deoxyribose → **Ribose**

Consequences for Nucleic Acids:

Strand Breaks
(bad for DNA replication)

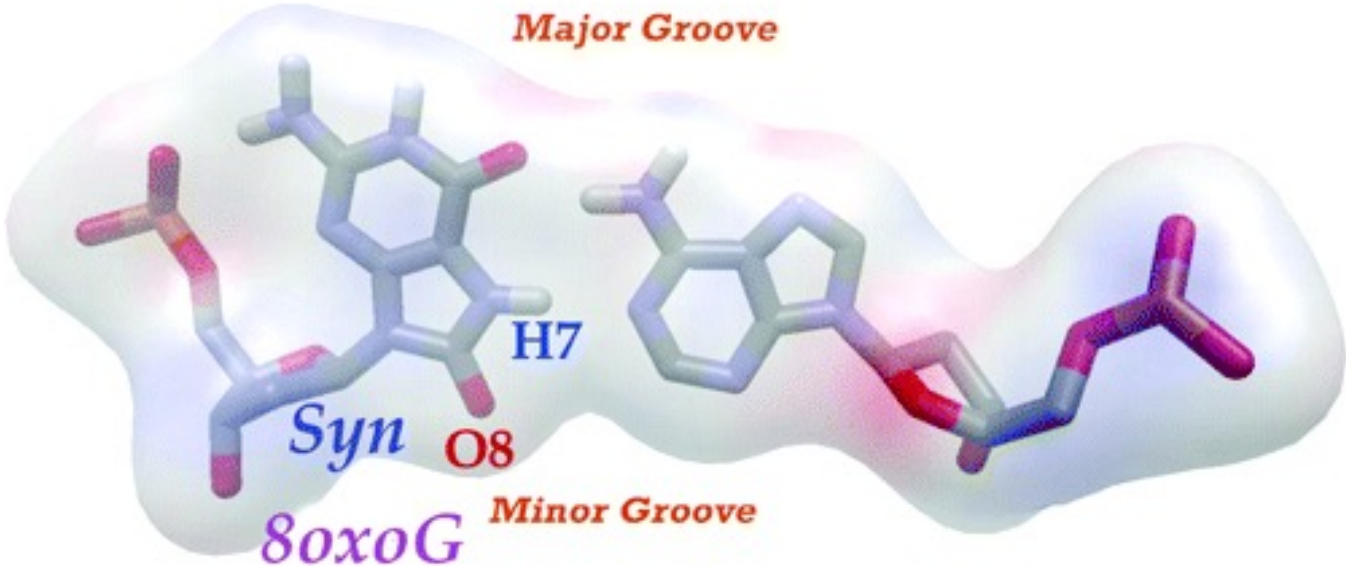
8-oxoguanine generates replication blocks or G-C → T-A transversions after DNA replication



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

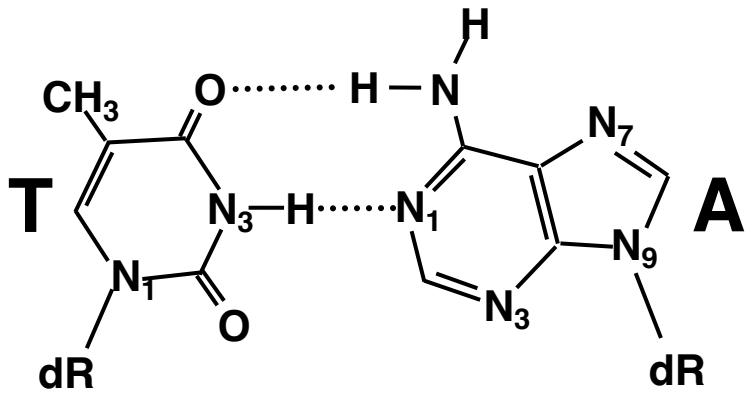
→ DNA Polymerases tend to incorporate A opposite to 8-oxoG

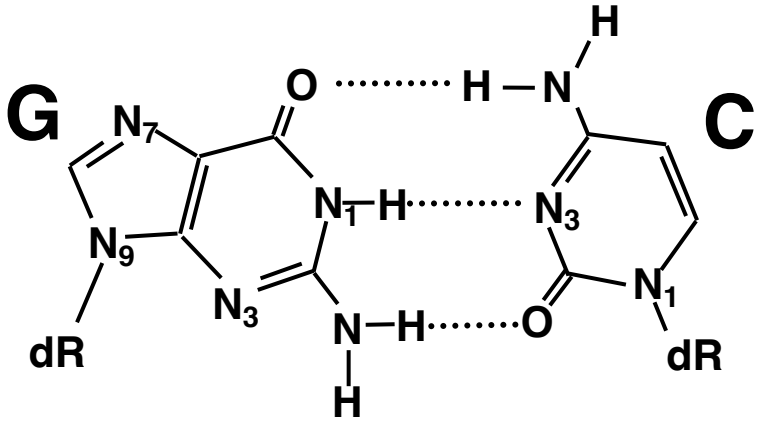


J Am Chem Soc.
2005 Oct 12;127(40):13906-18

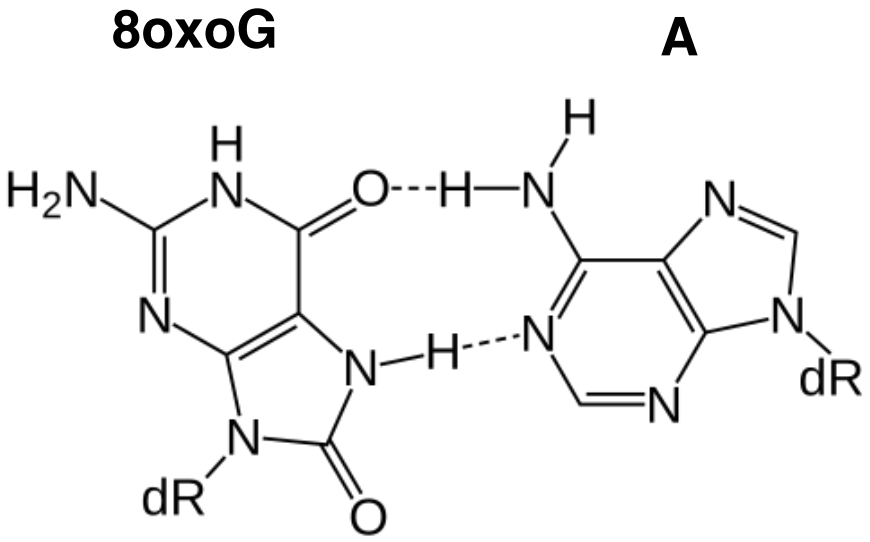
Recall normal Watson-Crick base pairing

Watson-Crick base pairs





Hoogsteen base pair



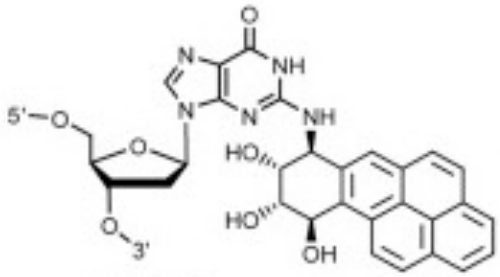
Bulky DNA adducts caused by:

cigarette smoke

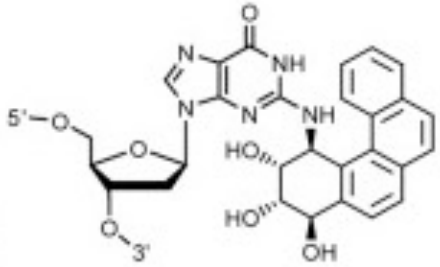
bulky DNA adducts

diesel engine exhaust

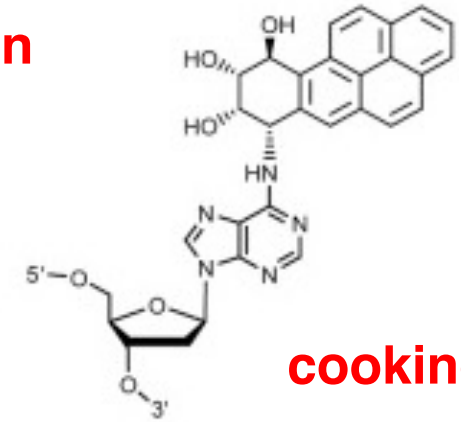
block DNA Replication



[BP]-dG

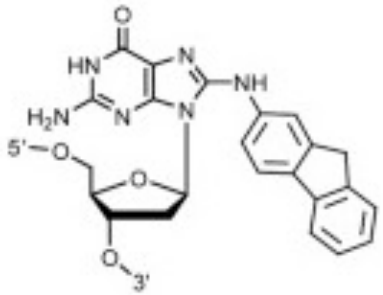


B[*c*]Phe DE-dG

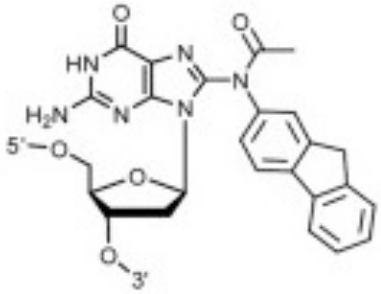


[BP]-dA

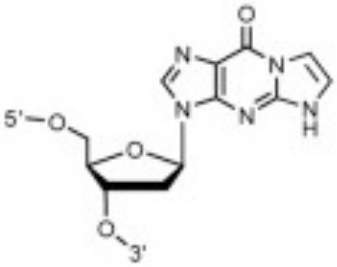
cooking/broiling of food



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

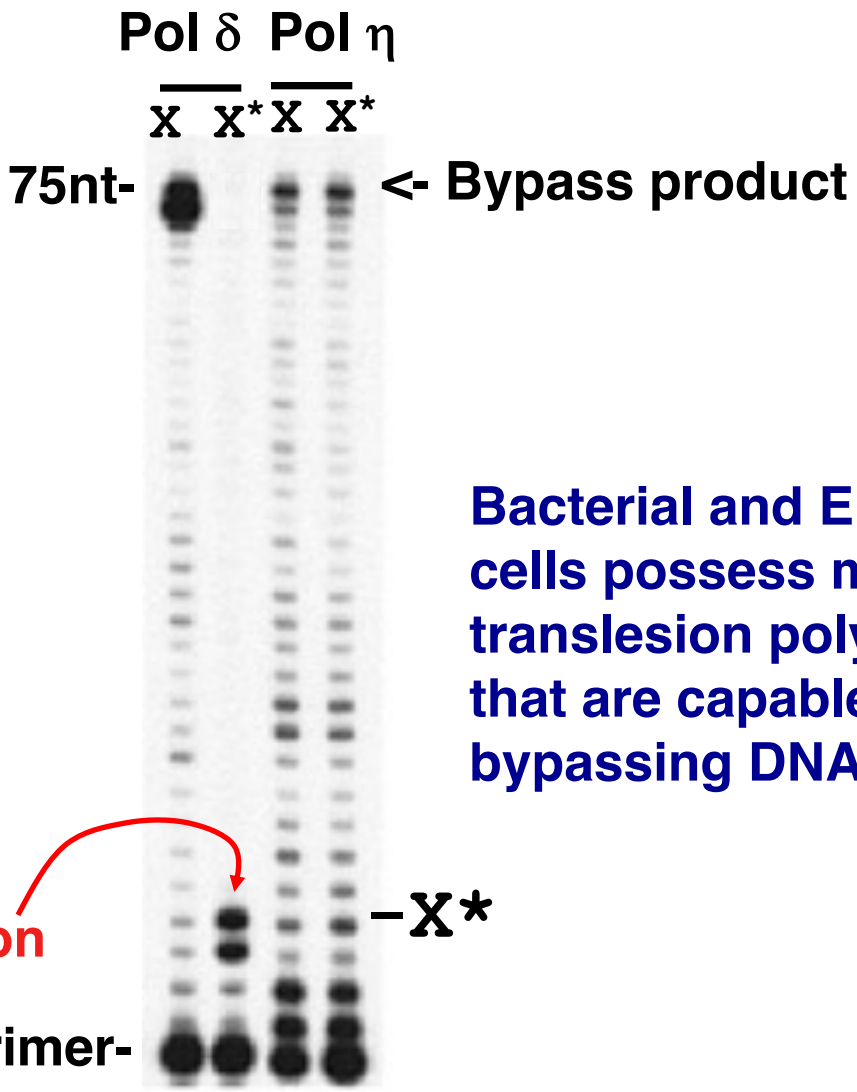
Recall from polymerase unit: Different Families of DNA polymerases

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



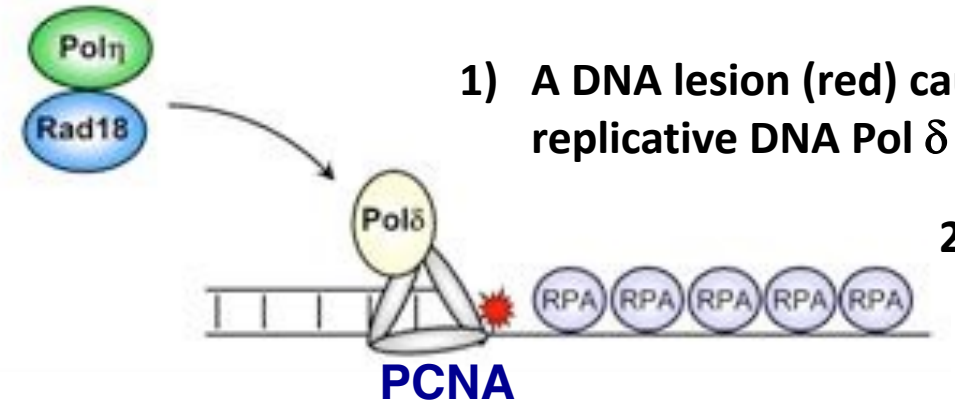
Primer Extension Assay to map template replication by the two DNA polymerases

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

Block of polymerization at 8-oxoG

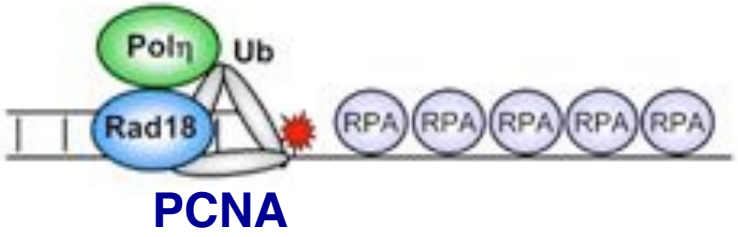
```
Primer    (41nt)  5´-AGG
Template  (75nt)  3´-TCCGTA(X/X*)AATG--5´
```

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



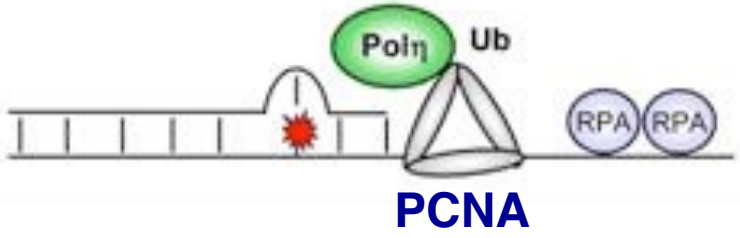
1) A DNA lesion (red) causes stalling of the replicative DNA Pol δ

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)



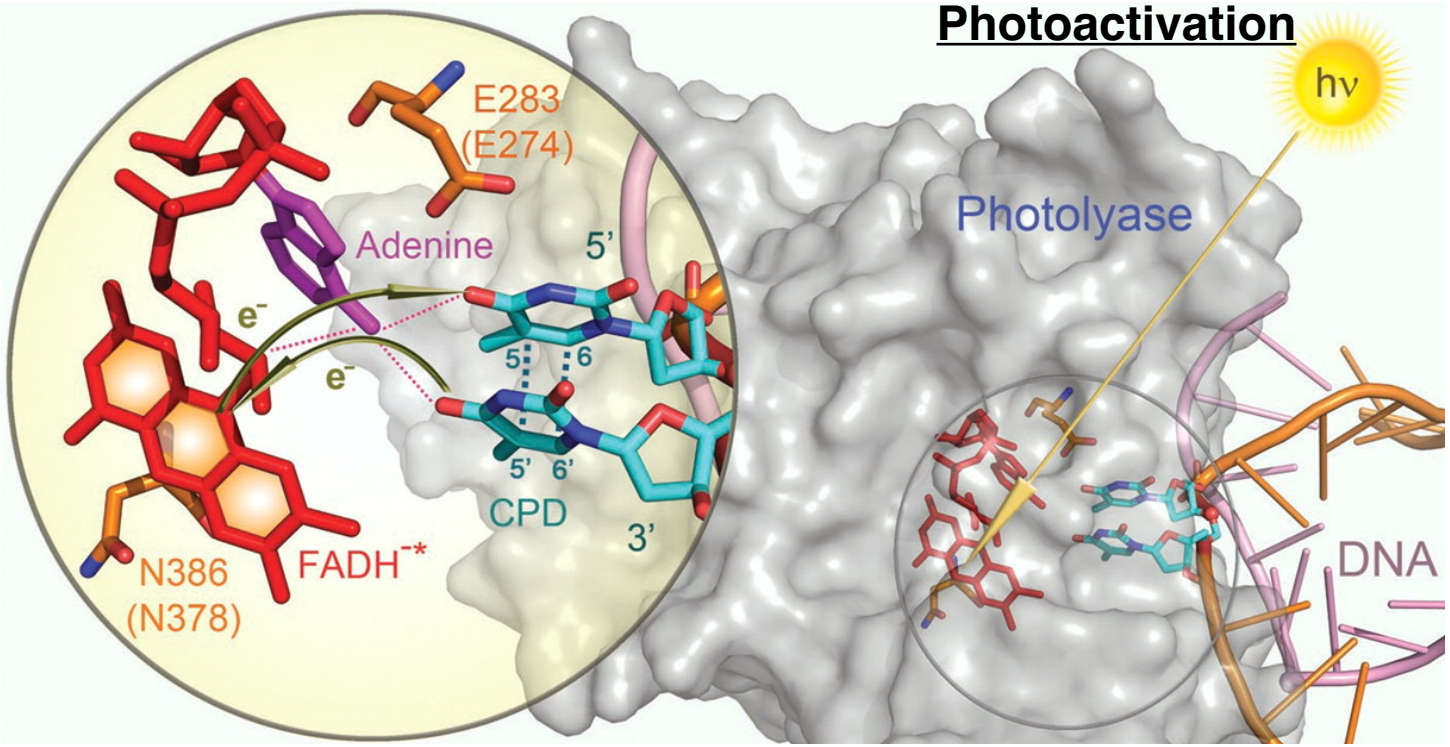
(4) Pol η binds to mono-ubiquitinated PCNA and performs replicative bypass of damaged DNA, preserving replication fork movement.

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

Strategy #2: Direct repair

Direct Reversal of pyrimidine dimers by Photolyase

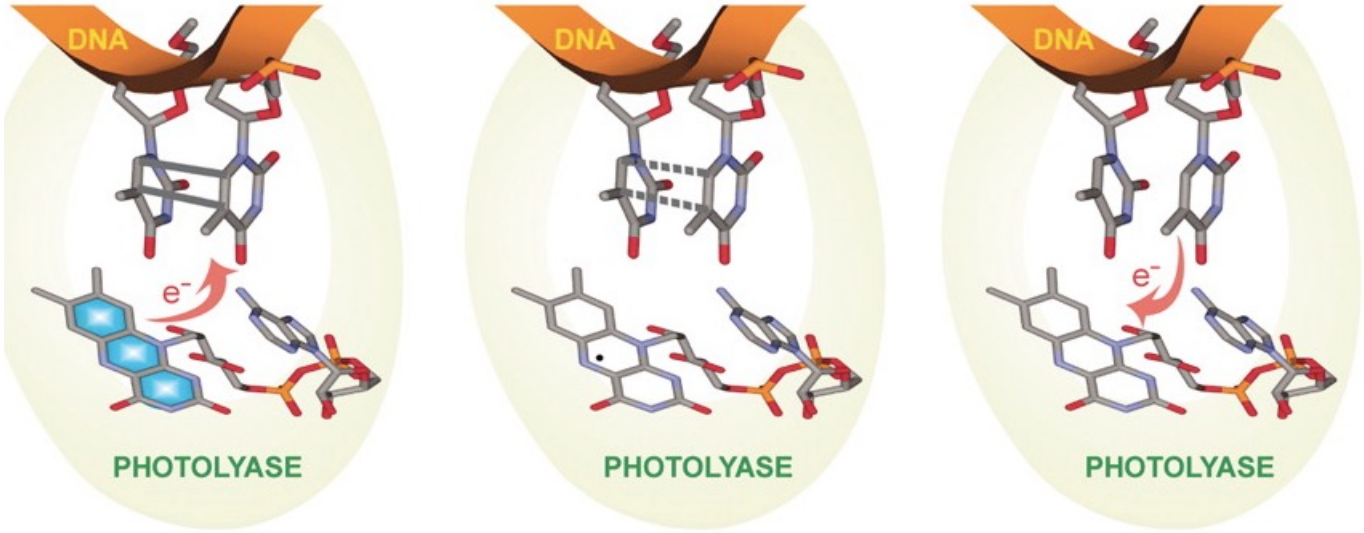
light activates the FADH- cofactor, which transfers an electron to the pyrimidine dimer for splitting of the ring.



Liu Z et al. PNAS 2011

Found in bacteria, archaea, plants and some animals

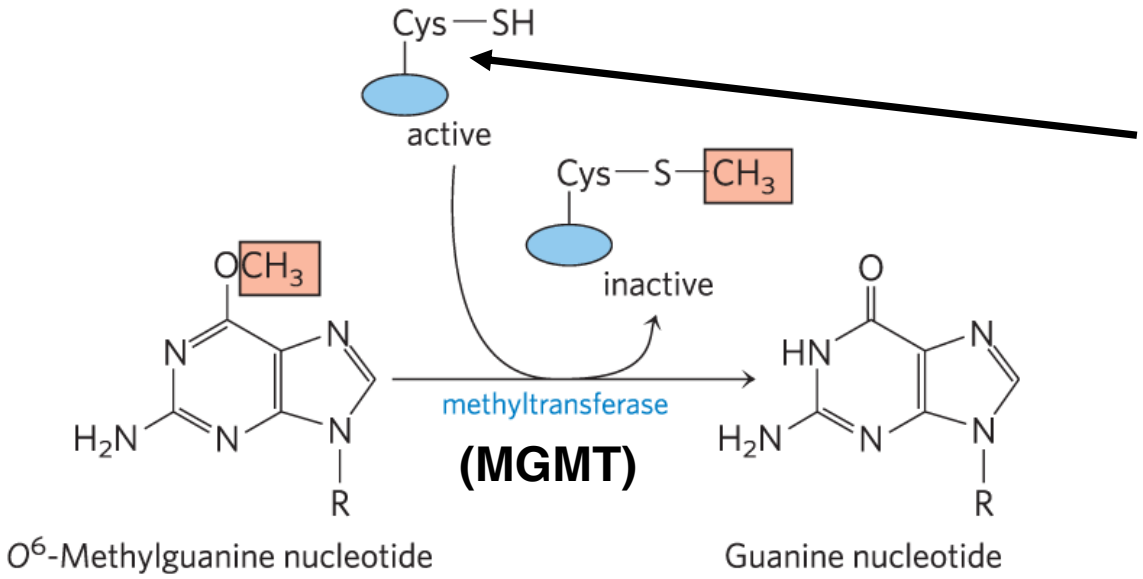
Absent in placental mammals including humans



Kao et al. PNAS 2005

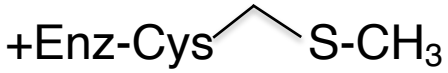
$$FADH^{-*} + T<>T \xrightarrow{k_1} FADH^{\bullet} + T<>T^{\bullet -} \xrightarrow{k_2} FADH^{-} + T + T$$

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

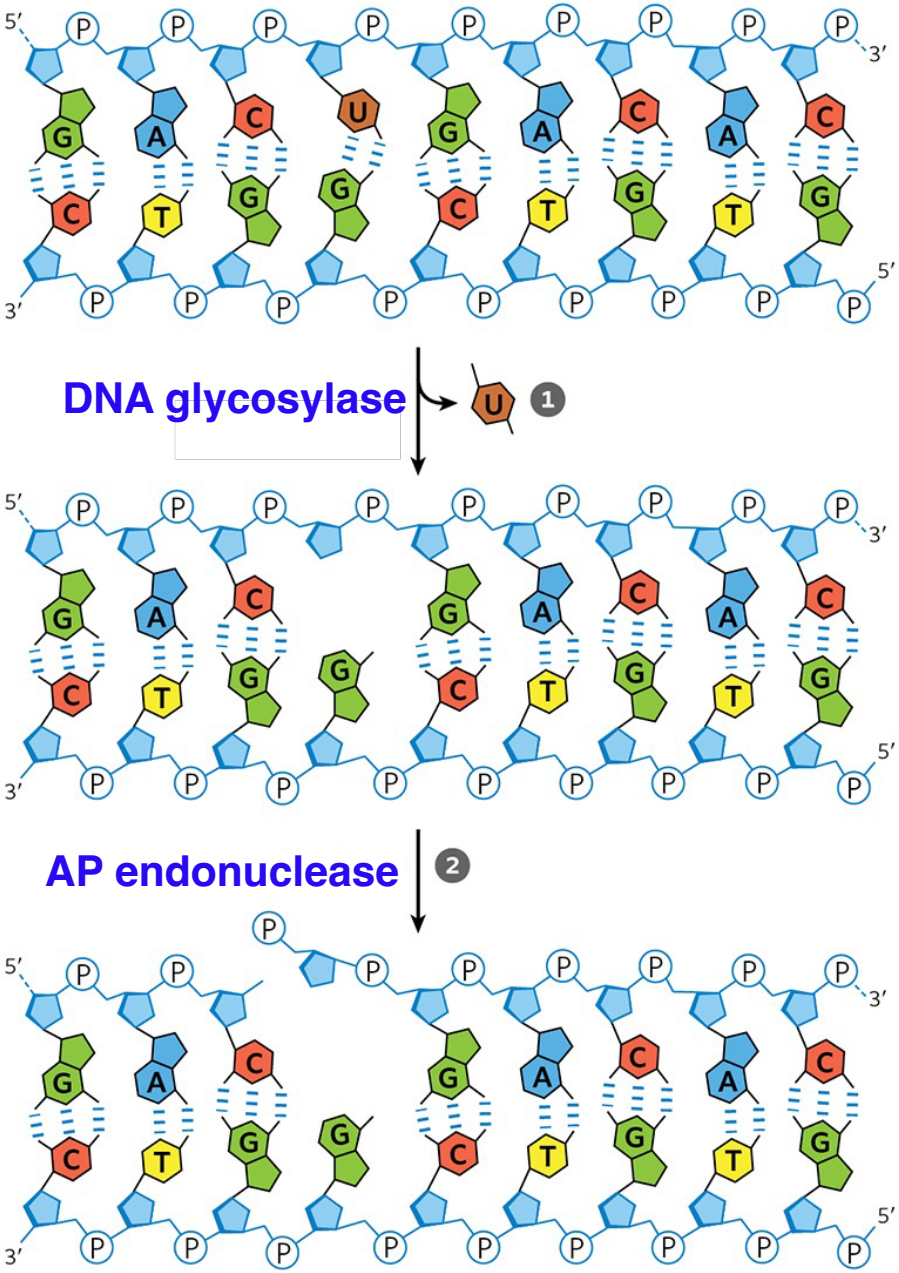
2) AP endonuclease = Cuts strand at AP site (A[urinic or Apyrimidic)

AP endonucleases also take care of spontaneous depurination events

3) DNA Pol + DNA ligase

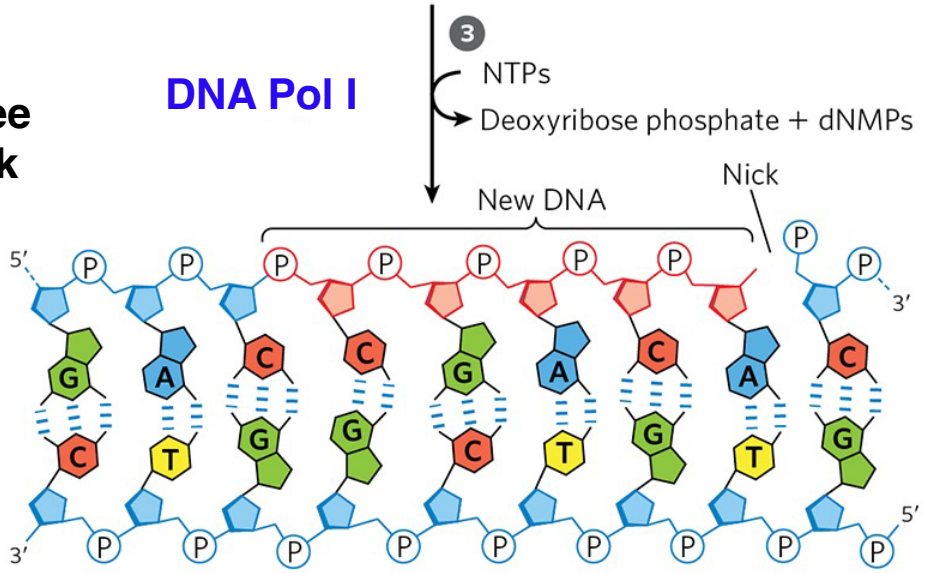
DNA glycosidase and AP endonuclease activities can sometimes be performed by the same protein, e.g. OGG1

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

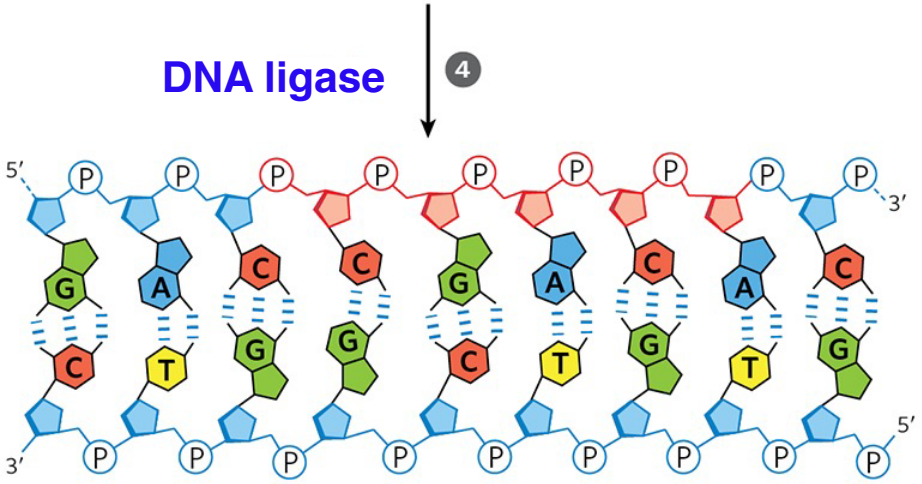


Step 2: an AP endonuclease cleaves the phosphodiester backbone near the AP site

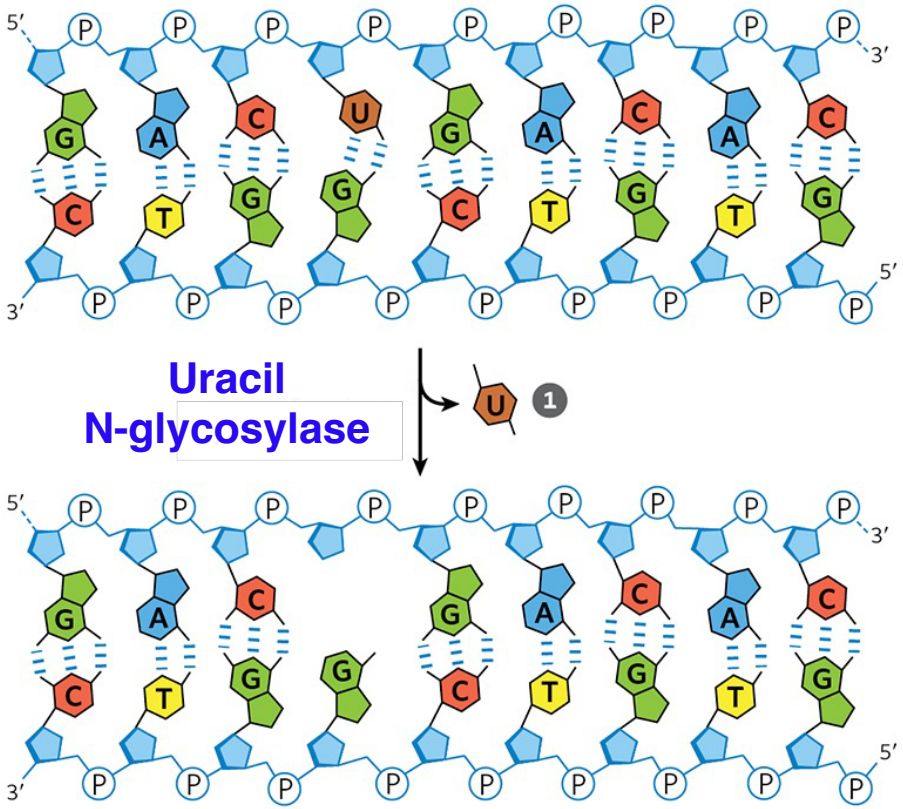
<u>Step 3</u>: DNA Pol I initiates repair synthesis from the free 3' OH at the nick (nick translation)

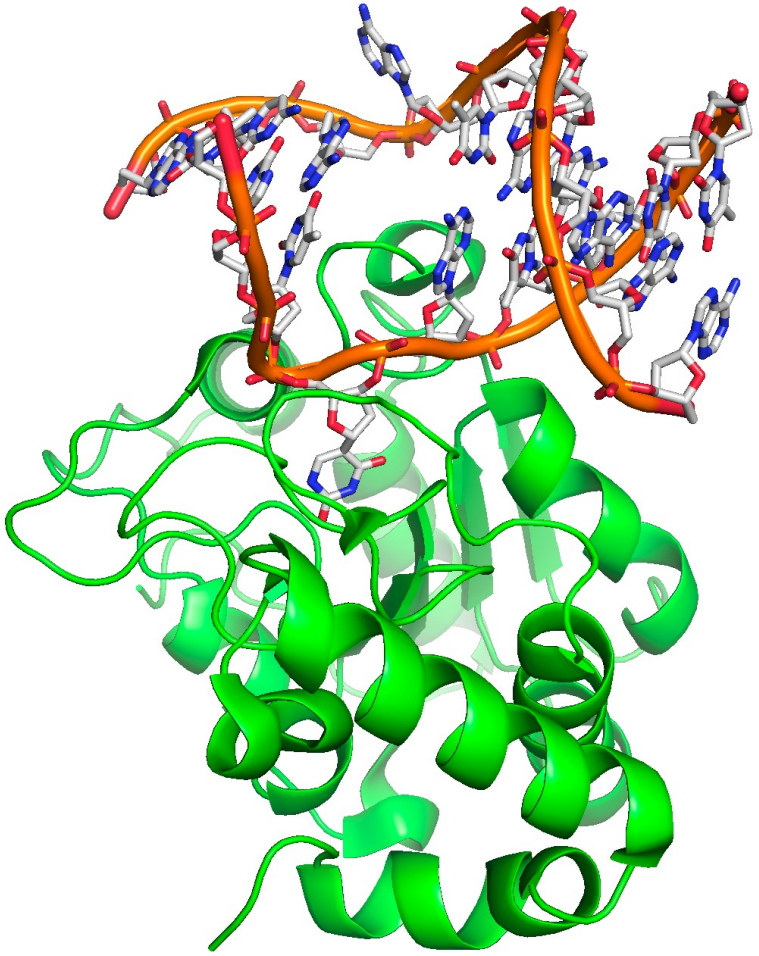


<u>Step 4</u>: DNA ligase seals the nick



Structure and activity of uracil N-glycosylase





PDB ID = 1EMH

PyMol: deoxyUrecognitionbyUNG.pse
UNG_DNAComplex-2.pse

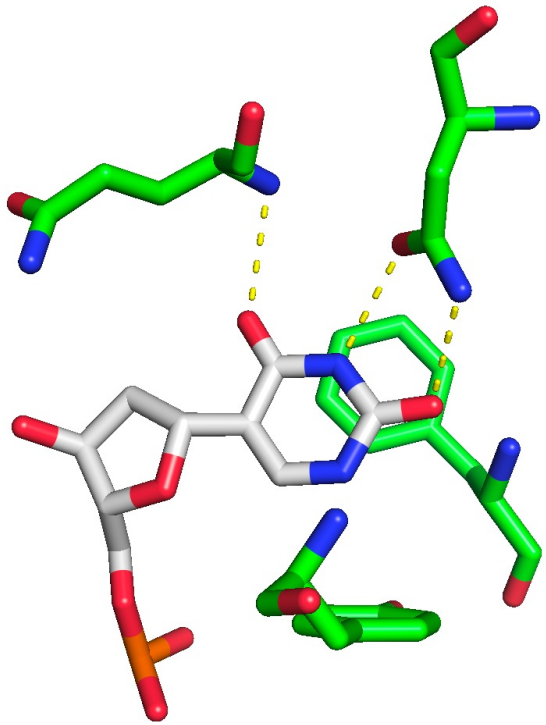
Why is Uracil-N-Glycosidase not catalyzing the reaction in this crystal?

A: It does not have the metal ions required to activate the reaction

B: The deoxyribose doesn't have the required 3'OH

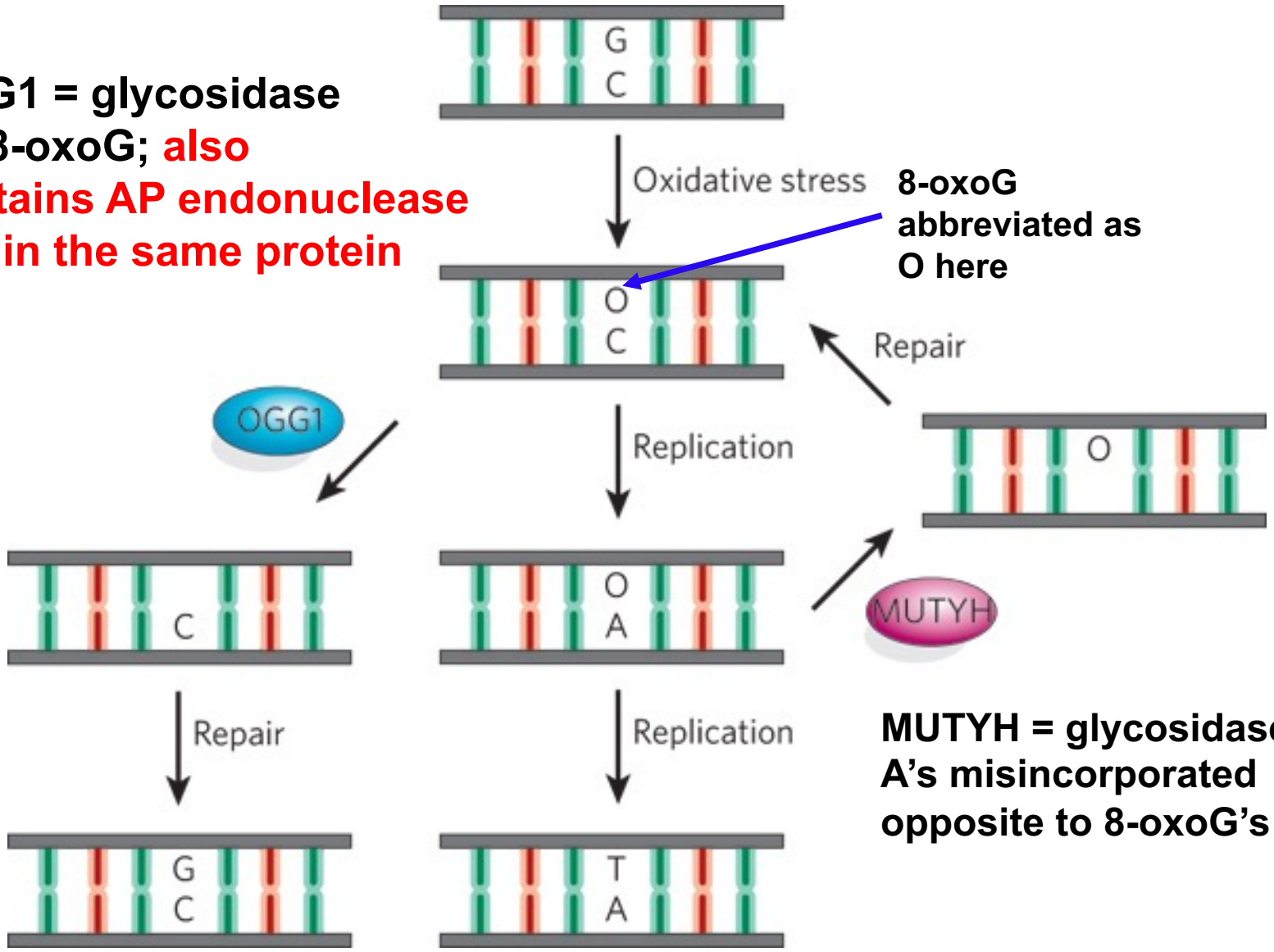
C: An amino acid of the active site is mutated so the enzyme cannot promote catalysis

D: The base is a pseudouracil



DNA repair strategies for 8-oxoG damage

OGG1 = glycosidase for 8-oxoG; also contains AP endonuclease within the same protein

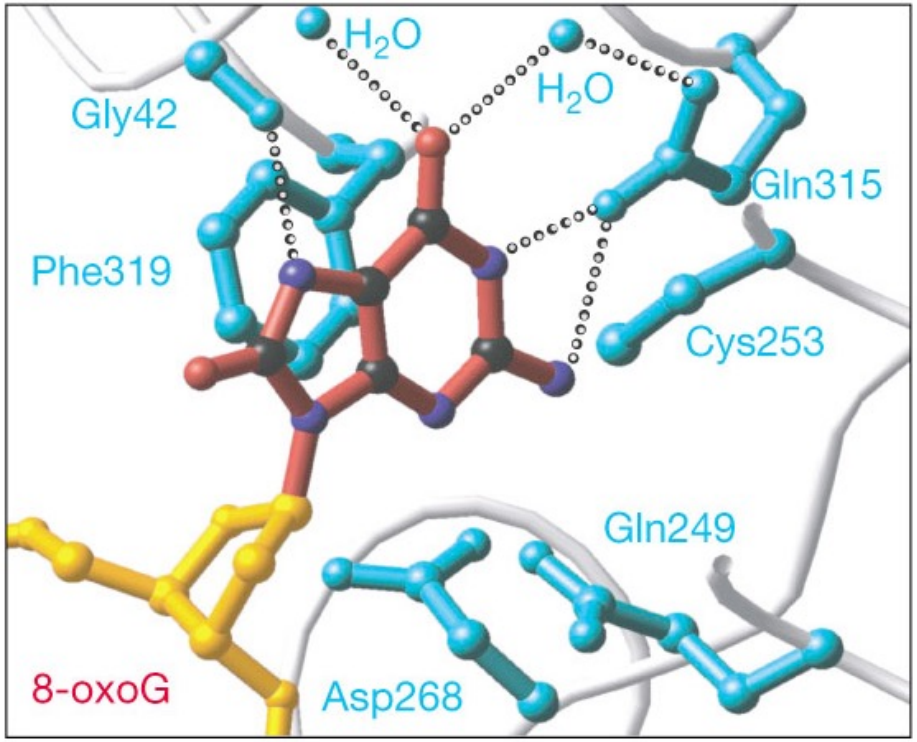


MUTYH = glycosidase for A's misincorporated opposite to 8-oxoG's

David et al., *Nature* **447**, 941 - 950 (2007)-Figure 1

How does hOGG1 recognize 8-oxoG?

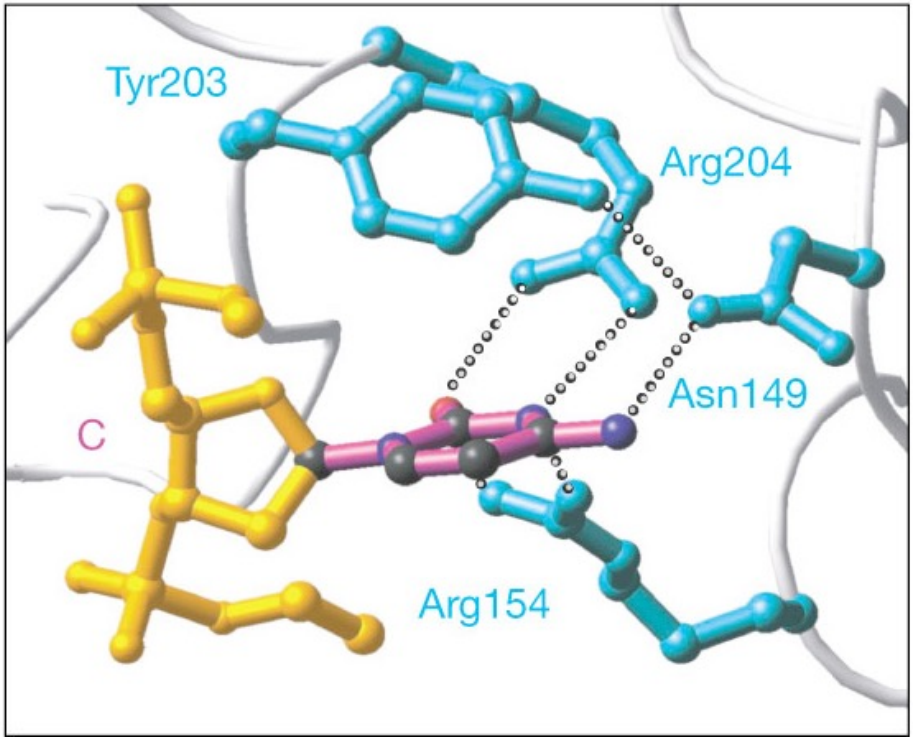
Recognition of 8-OxoG



The oxoG base is stacked between Phe 319 and Cys 253. Residues Gly 42, Gln 315 and two water molecules hydrogen bond to the Watson–Crick and Hoogsteen faces of the lesion base.

PDB ID = 1EBM

Recognition of unpaired C



The cytosine paired opposite oxoG is recognized by H-bonding interactions with Arg 154 and Arg 204, and an additional H bond with Asn 149.

PyMol: OGG1.pse
8oxoG_recognition.pse

Bruner et al., *Nature* **403**, 859 - 866 (24 February 2000)-Figure 6

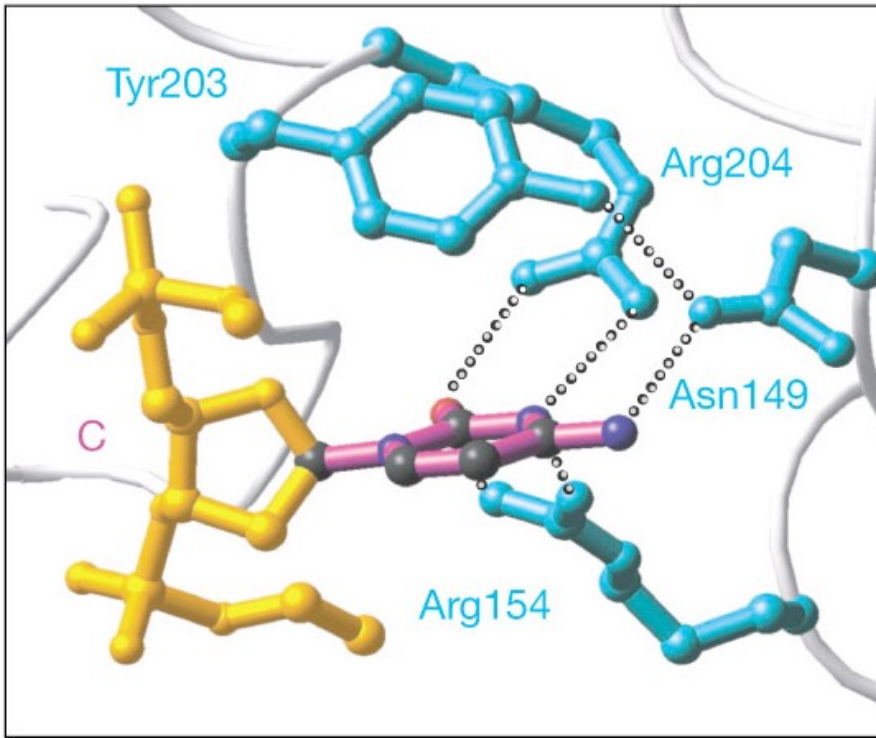
Why do you think OGG1 recognizes the unpaired C?

A: It provides a second mechanism for indirect recognition of 8-oxoG since 8oG are in syn and no longer interact with Cs

B: It increases binding to the DNA substrate such that the enzyme affinity for 8oxoG DNA increases

C: It prevents the binding of OGG1 to G-C base pairs

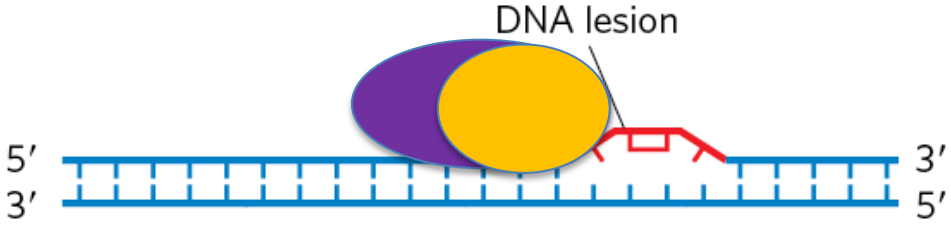
D: It allows OGG1 to distinguish between 8oxoGs that have not yet been replicated vs. 8oxoGs that have already been replicated



Strategy #4: Nucleotide excision repair

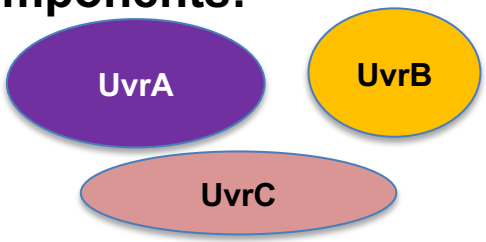
Nucleotide excision repair in bacteria

DNA lesions that cause large distortions DNA structure are generally repaired via nucleotide excision repair



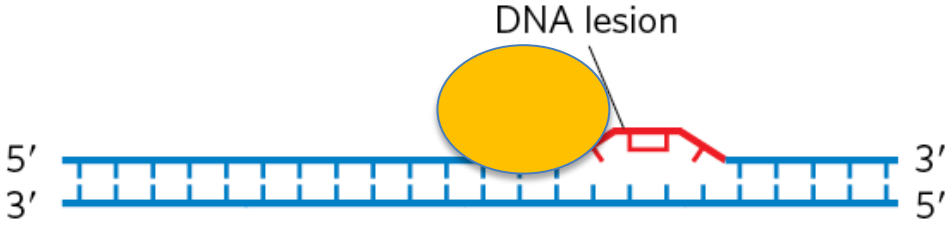
Key enzymatic complex: <u>ABC excinuclease</u> which contains 3 protein components:

- **UvrA**
- **UvrB**
- **UvrC**



"<u>Excinuclease</u>": catalyzes 2 endonucleolytic cleavages

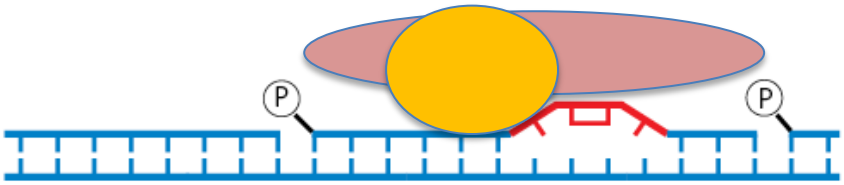
<u>Step 1</u>: UvrA scans the DNA and binds to the site of a lesion. UvrB binds to UvrA either before or after encountering the lesion.



<u>Step 2</u>: UvrA dissociates, leaving a UvrB-DNA complex

Uvr = UV Resistance; bacteria with deficiency or mutations in genes coding for Uvr proteins show a decrease in UV resistance

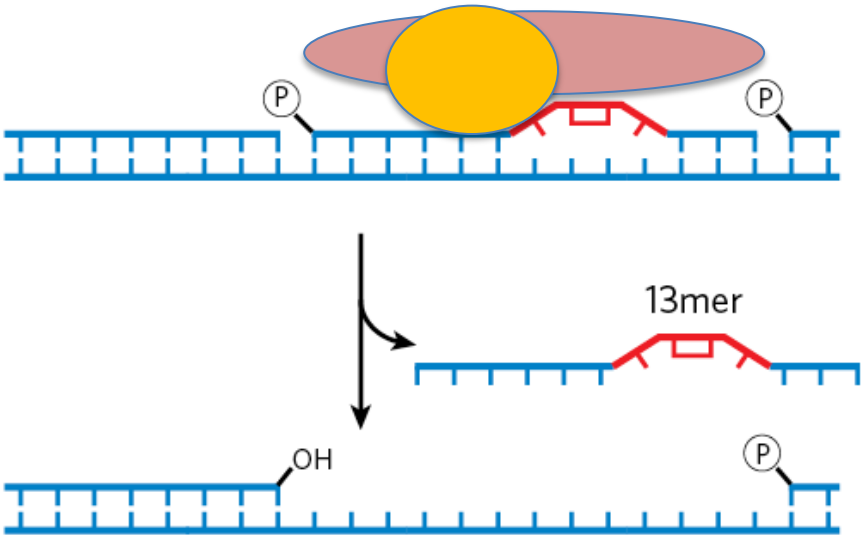
Nucleotide excision repair in bacteria



<u>Step 3</u>: UvrC binds to UvrB and UvrB makes an incision at the 5th phosphodiester bond on the 3' side of the lesion

<u>Step 4</u>: UvrC makes an incision at the 8^{th} phosphodiester bond on the 5' side of the lesion

Nucleotide excision repair in bacteria

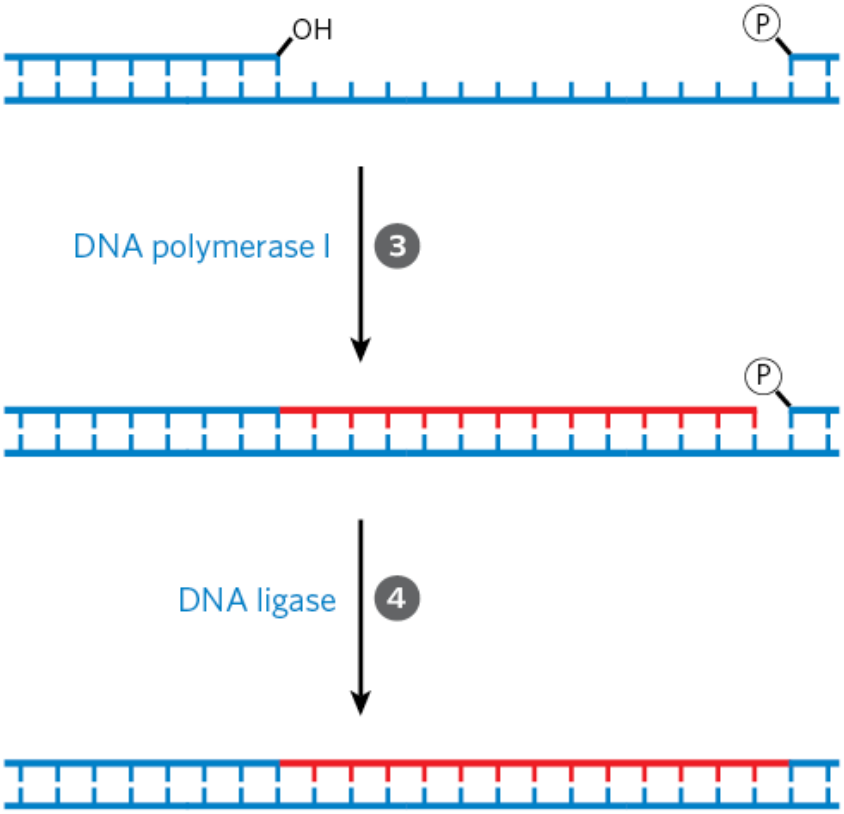


<u>Step 3</u>: UvrC binds to UvrB and UvrB makes an incision at the 5th phosphodiester bond on the 3' side of the lesion

<u>Step 4</u>: UvrC makes an incision at the 8^{th} phosphodiester bond on the 5' side of the lesion

<u>Step 5</u>: UvrD helicase removes the resulting fragment

Nucleotide excision repair in bacteria



<u>Step 6</u>:DNA Pol I fills in the gap

<u>Step 7</u>:DNA ligase seals the nick

*** Note: NER can also be facilitated by arrests in transcription due to DNA lesions = transcription coupled repair**

Where have we seen UvrD before?

Step 5: UvrD helicase removes the resulting fragment

A: It's the helicase involved in base excision repair

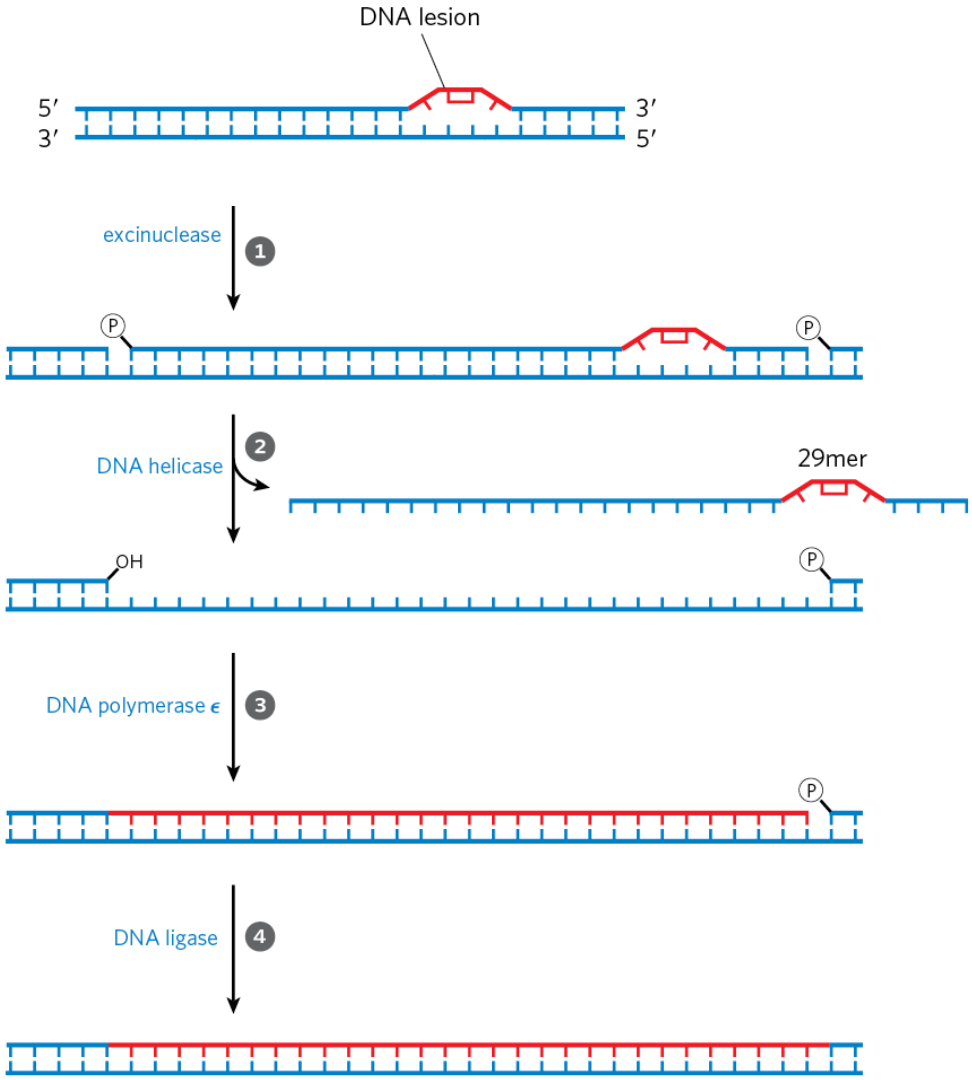
B: It unwinds DNA containing Thymine dimers to help photolyase insert these in their active site

C: It's the helicase that unwinds the leading and lagging strand templates during replication

D: It's the helicase involved in mismatch repair

Nucleotide excision repair in eukaryotes

Pathway is similar, factors involved are different



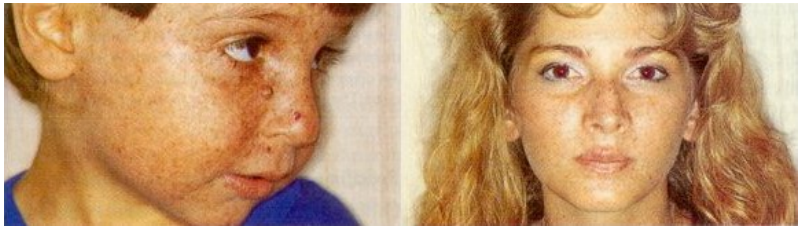
Nucleotide Excision Repair Pathways in Eukaryotes

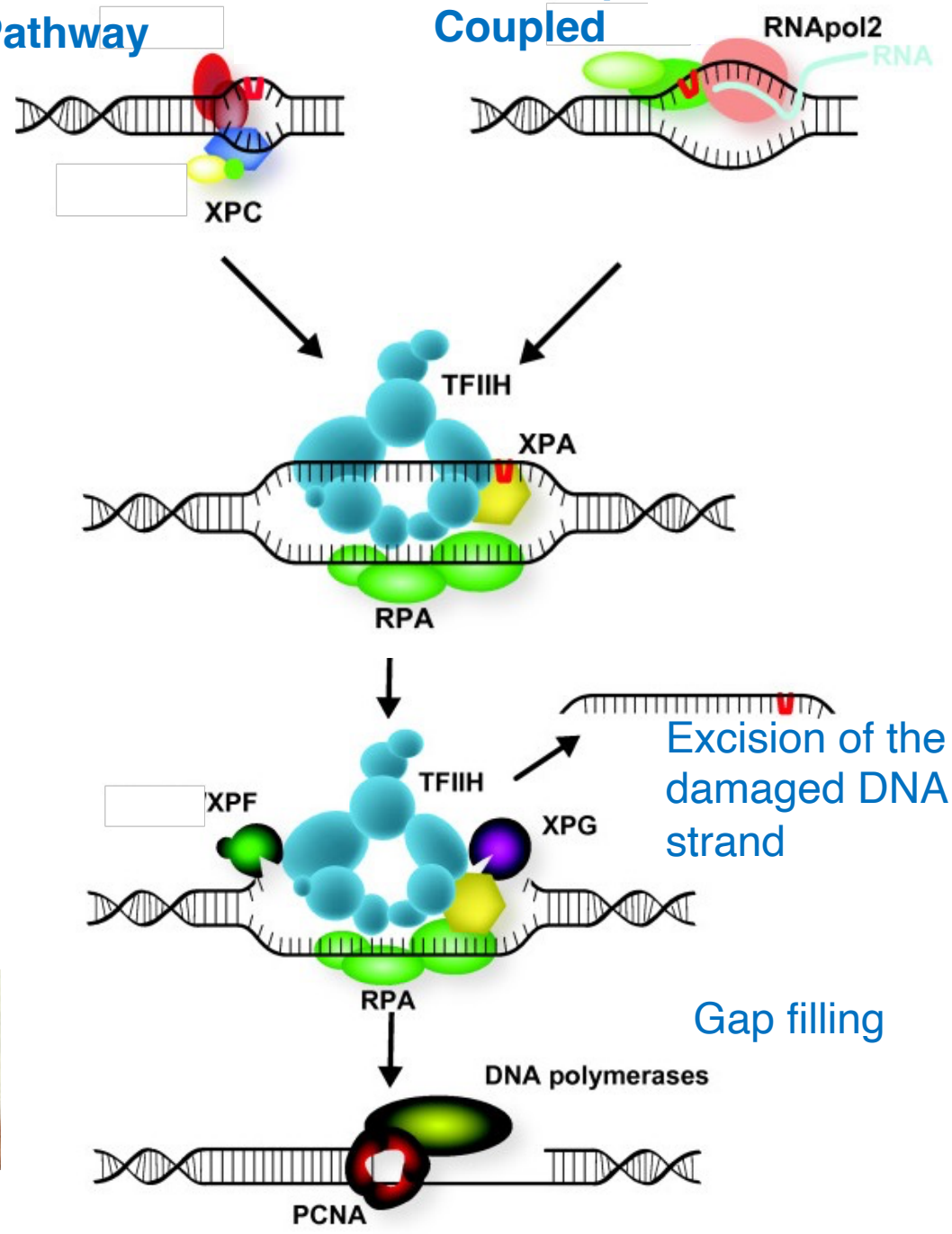
(Y dimer, bulky adducts, crosslinks)

involves:
XPC = damage recognition
XPA, TFIIH, RPA = DNA unwinding
XPG, XPF = Endonucleases

TFIIH = basal RNA Polymerase II transcription factor H; contains XPB & XPD subunits

XP = Xeroderma pigmentosum





Summary of DNA damage and repair mechanisms

Damage	Repair mechanisms
Pyrimidine dimers	Direct repair by photolyase (only in some organisms); Nucleotide excision repair
Deamination of bases	Base excision repair
Depurinations and depyrimidinations	Base excision repair (skip 1st glycosylase step)
Interstrand crosslinks	Nucleotide excision repair (mechanism is more complicated than discussed in class); homologous recombination (not discussed
Alkylations of bases	Direct repair by MGMT for O^{6}methylG
Oxidative damage	Base excision repair (OGG1 for 8-oxoguanine)
Bulky DNA adducts	Nucleotide excision repair

*** All types of damage in this table can be bypassed by translesion DNA synthesis**