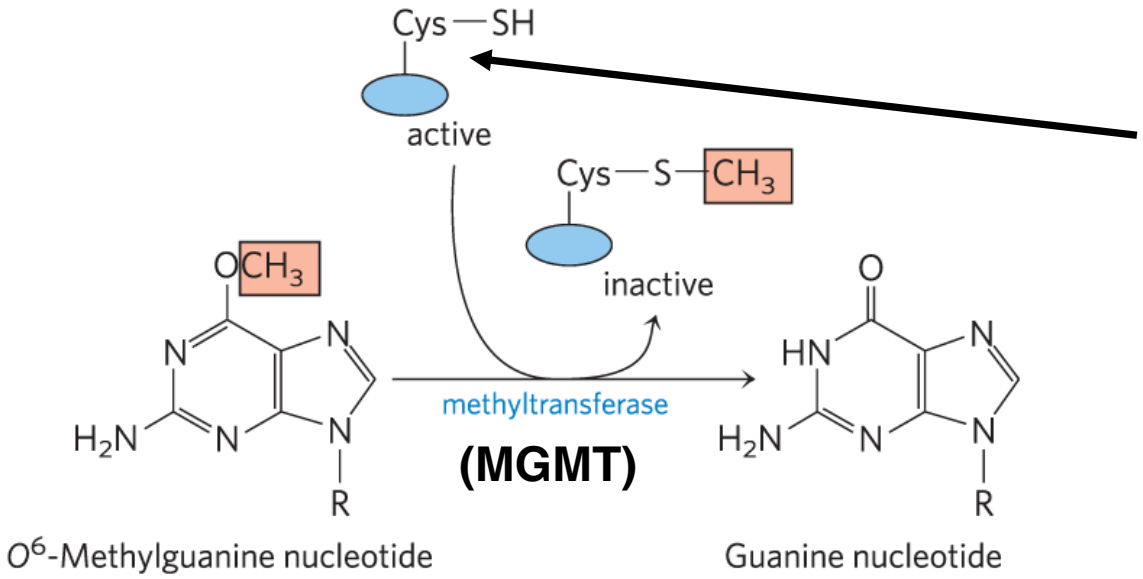
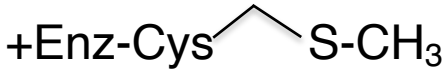
Repairing O^6-methylG: Dealkylation of guanines by Methyl Guanine Methyl Transferase (MGMT)



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

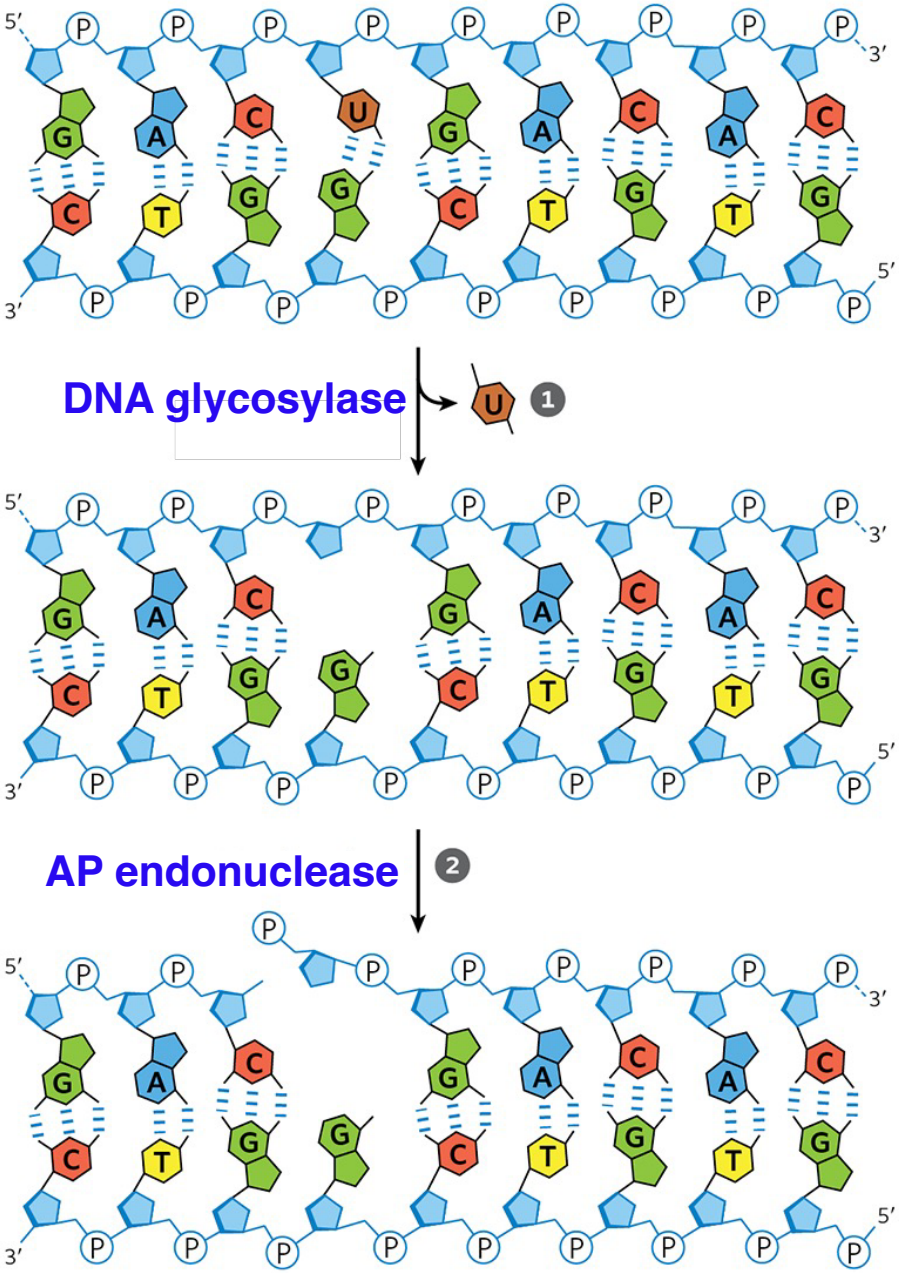
2) AP endonuclease = Cuts strand at AP site (A[urinic or Apyrimidic)

AP endonucleases also take care of spontaneous depurination events

3) DNA Pol + DNA ligase

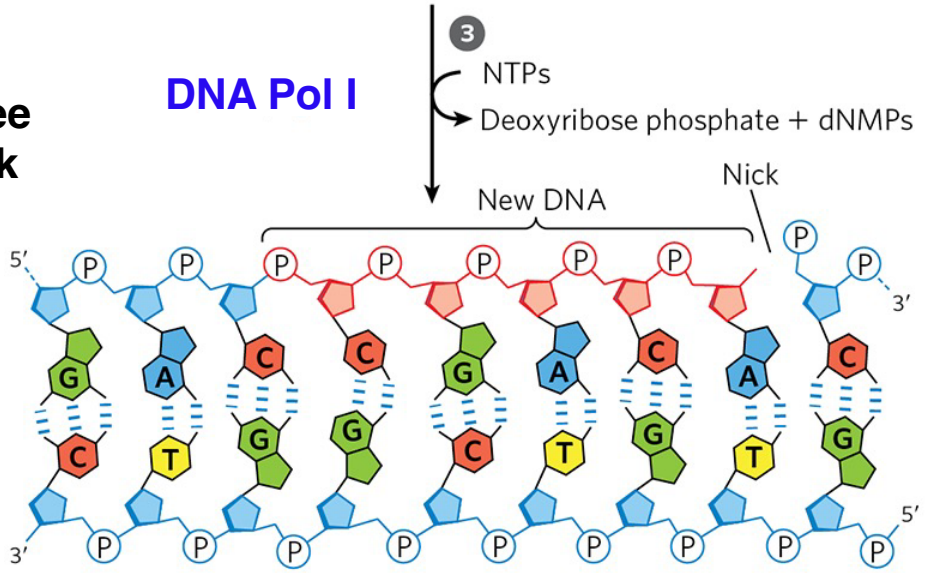
DNA glycosidase and AP endonuclease activities can sometimes be performed by the same protein, e.g. OGG1

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

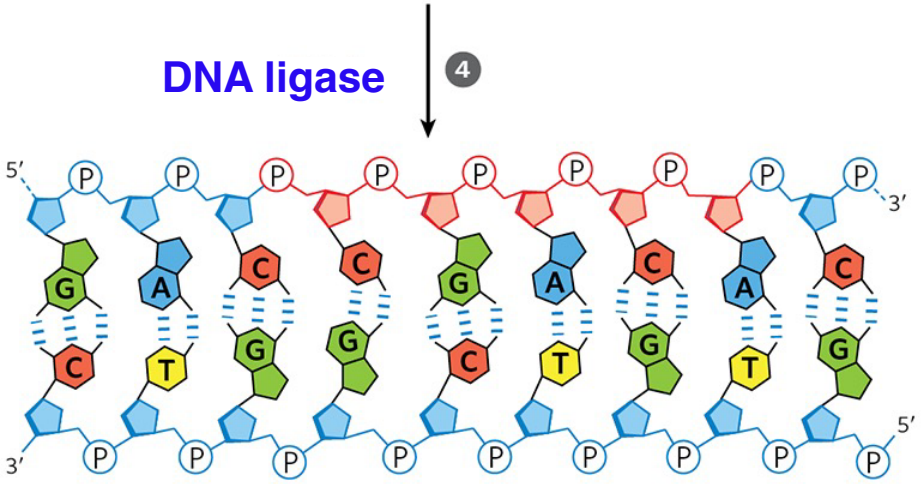


Step 2: an AP endonuclease cleaves the phosphodiester backbone near the AP site

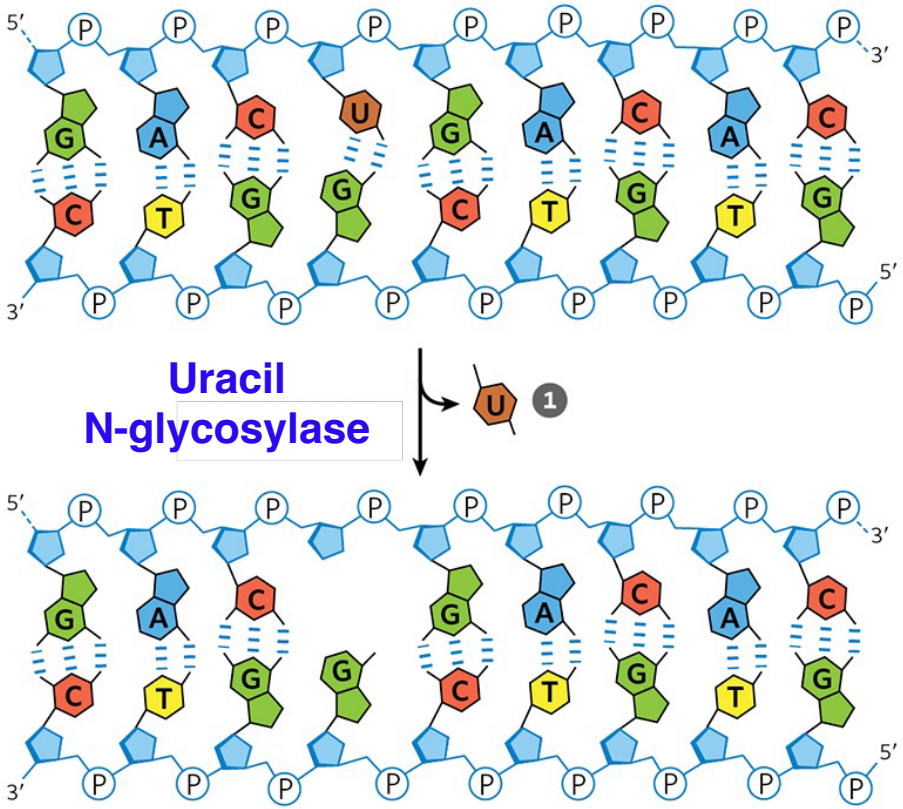
<u>Step 3</u>: DNA Pol I initiates repair synthesis from the free 3' OH at the nick (nick translation)

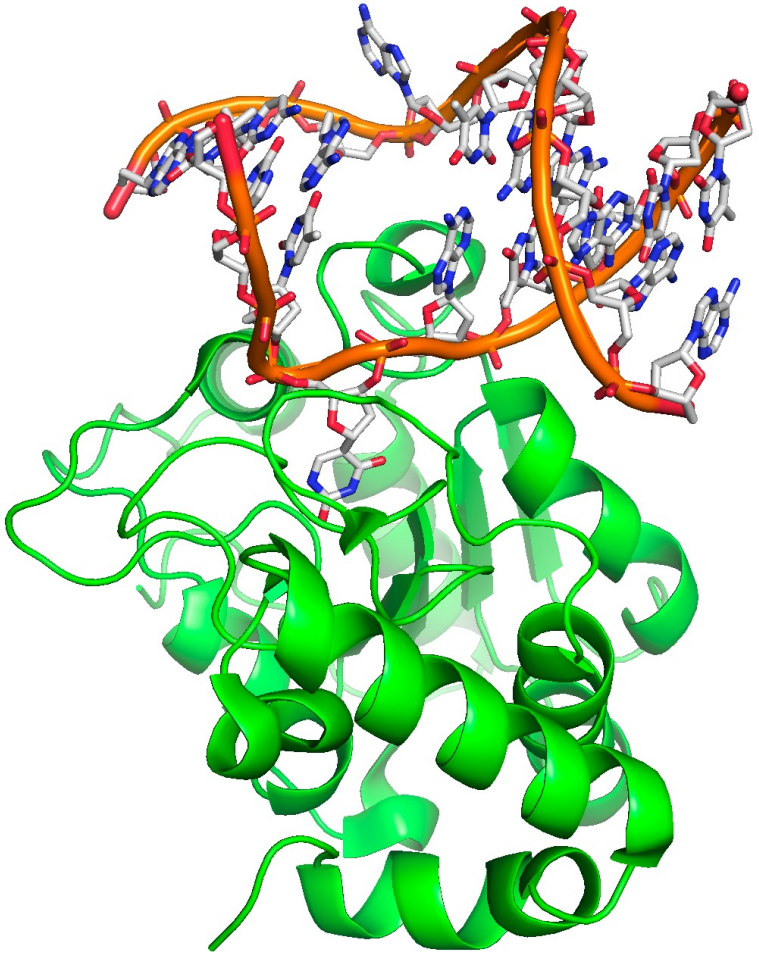


<u>Step 4</u>: DNA ligase seals the nick



Structure and activity of uracil N-glycosylase





PDB ID = 1EMH

PyMol: deoxyUrecognitionbyUNG.pse
UNG_DNAComplex-2.pse

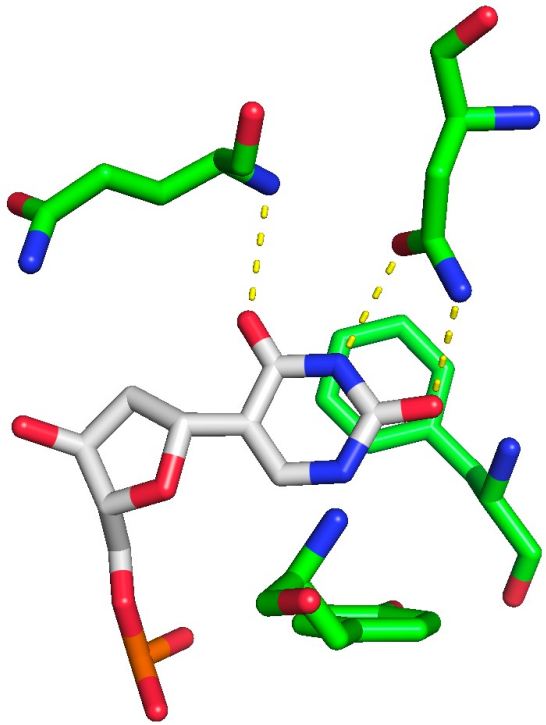
Why is Uracil-N-Glycosidase not catalyzing the reaction in this crystal?

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

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

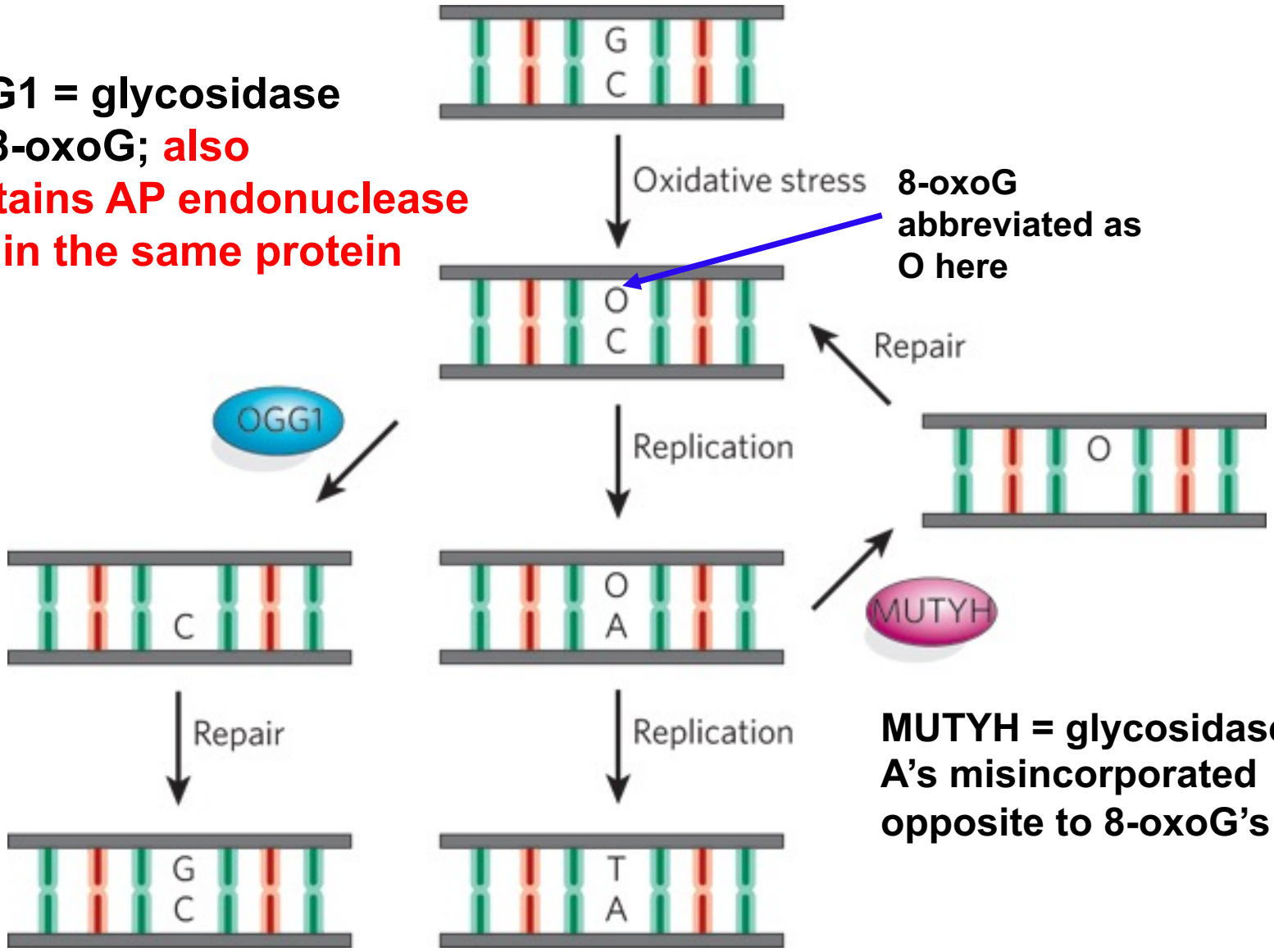
C: An amino acid of the active site is mutated so the enzyme cannot promote catalysis

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

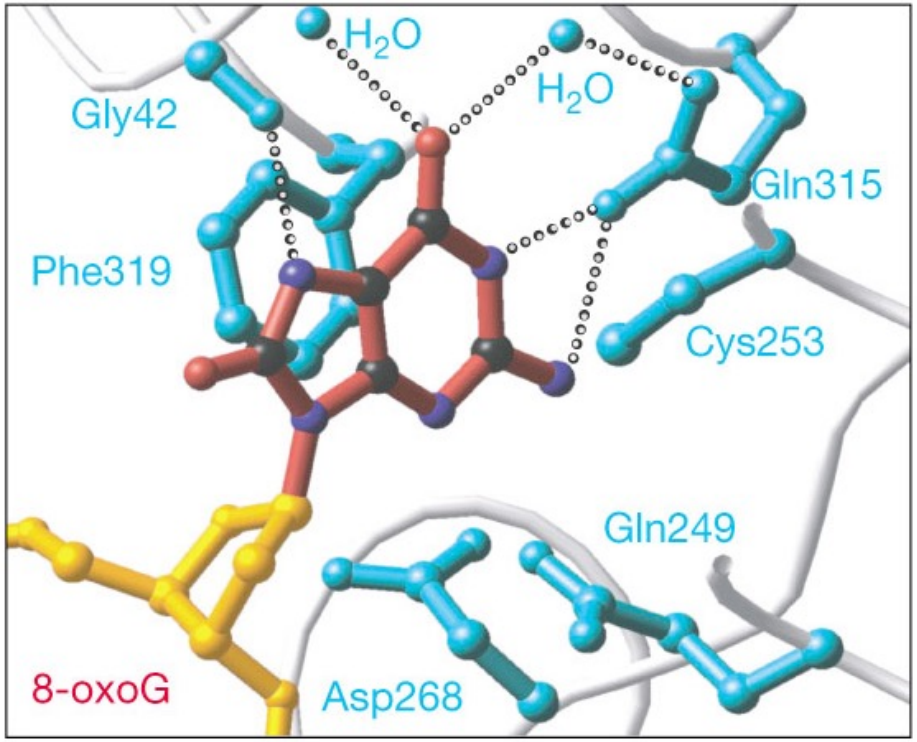


MUTYH = glycosidase for A's misincorporated opposite to 8-oxoG's

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

How does hOGG1 recognize 8-oxoG?

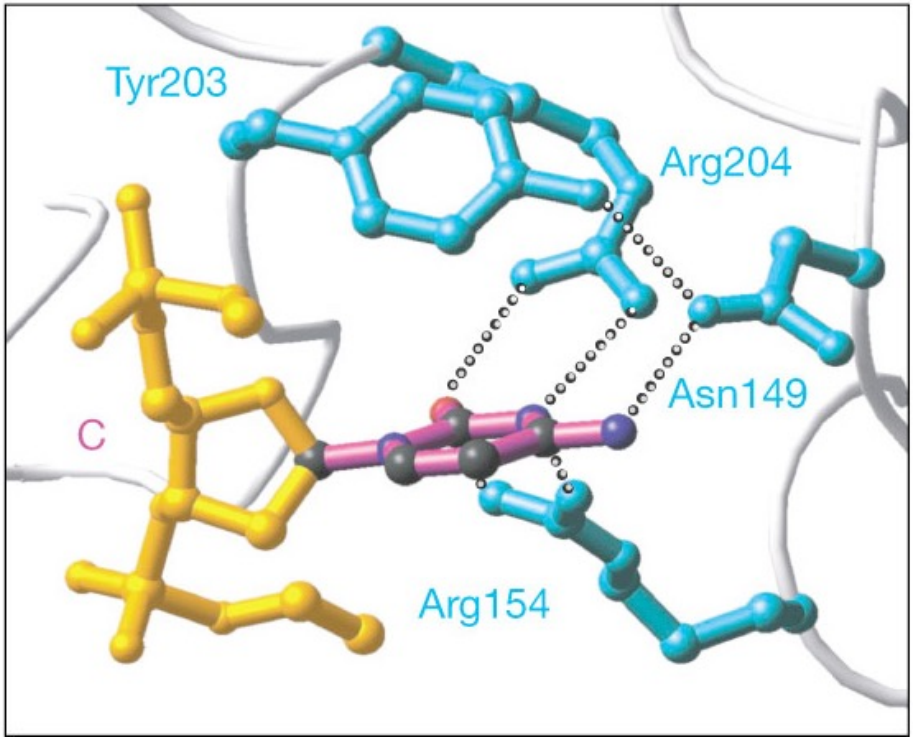
Recognition of 8-OxoG



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

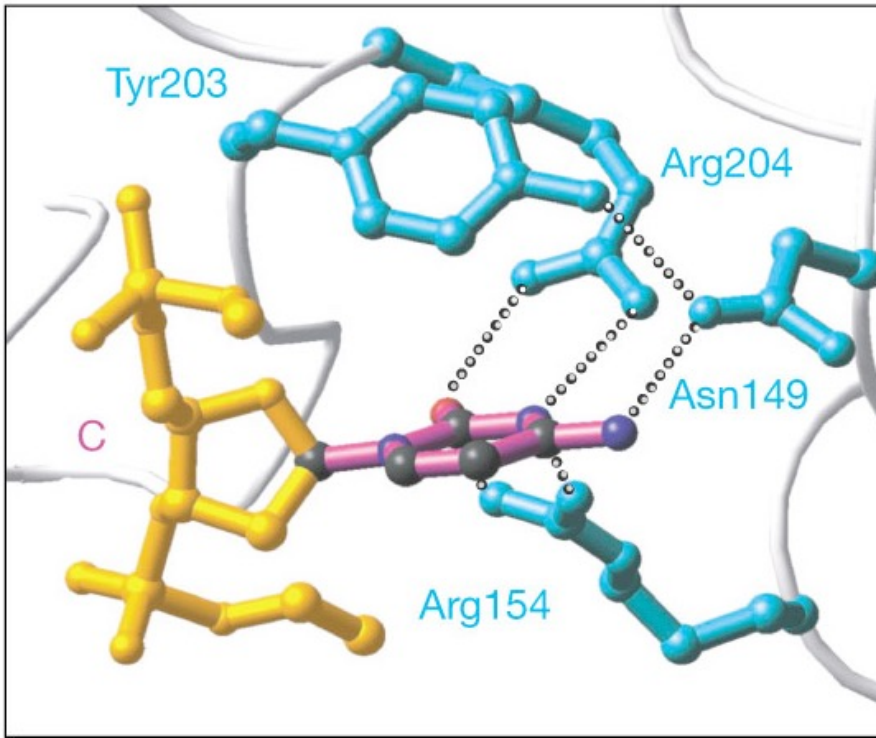
Why do you think OGG1 recognizes the unpaired C?

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

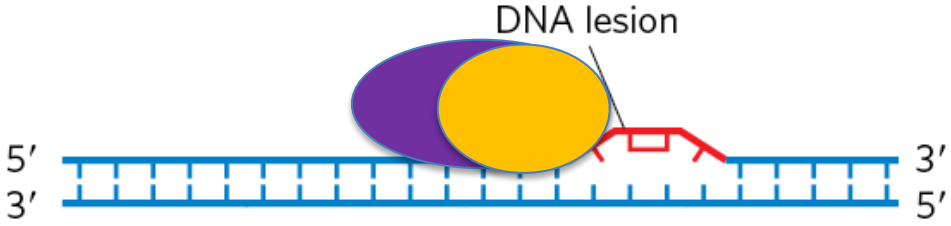
D: It allows OGG1 to distinguish between 8oxoGs that have not yet been replicated vs. 8oxoGs that have already been replicated



Strategy #4: Nucleotide excision repair

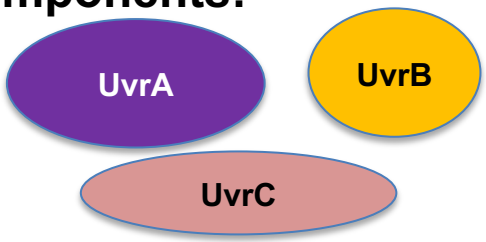
Nucleotide excision repair in bacteria

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



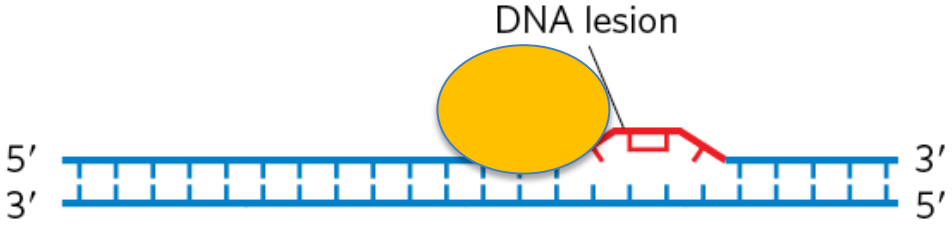
Key enzymatic complex: <u>ABC excinuclease</u> which contains 3 protein components:

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



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

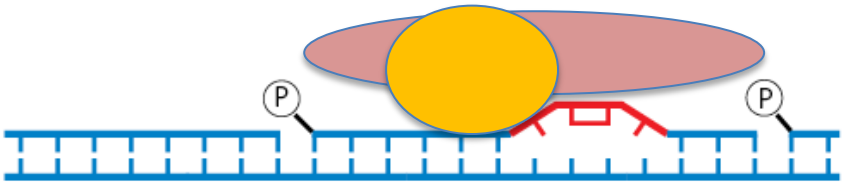
<u>Step 1</u>: UvrA scans the DNA and binds to the site of a lesion. UvrB binds to UvrA either before or after encountering the lesion.



<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

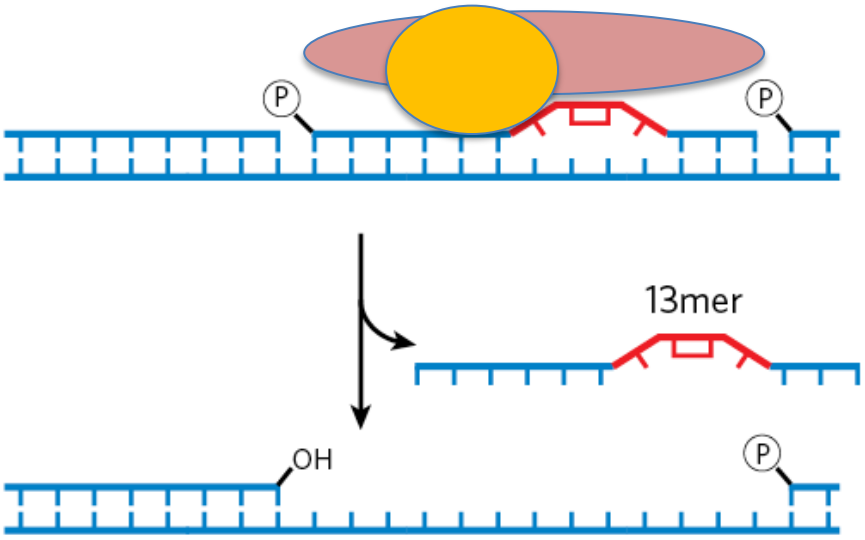
Nucleotide excision repair in bacteria



<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

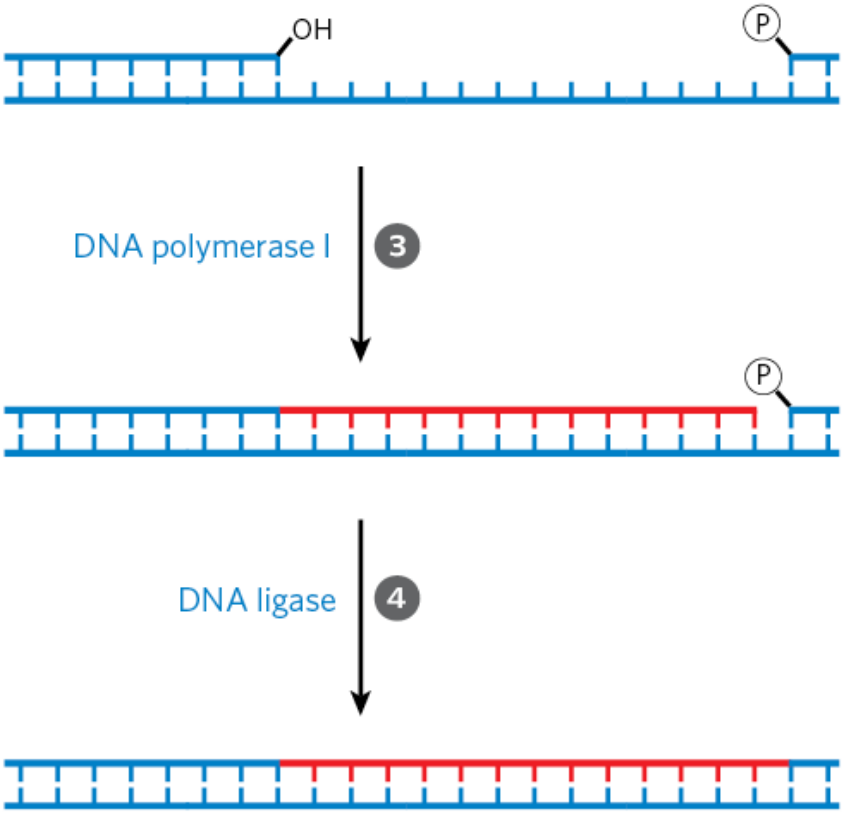


<u>Step 3</u>: UvrC binds to UvrB and UvrB makes an incision at the 5th phosphodiester bond on the 3' side of the lesion

<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

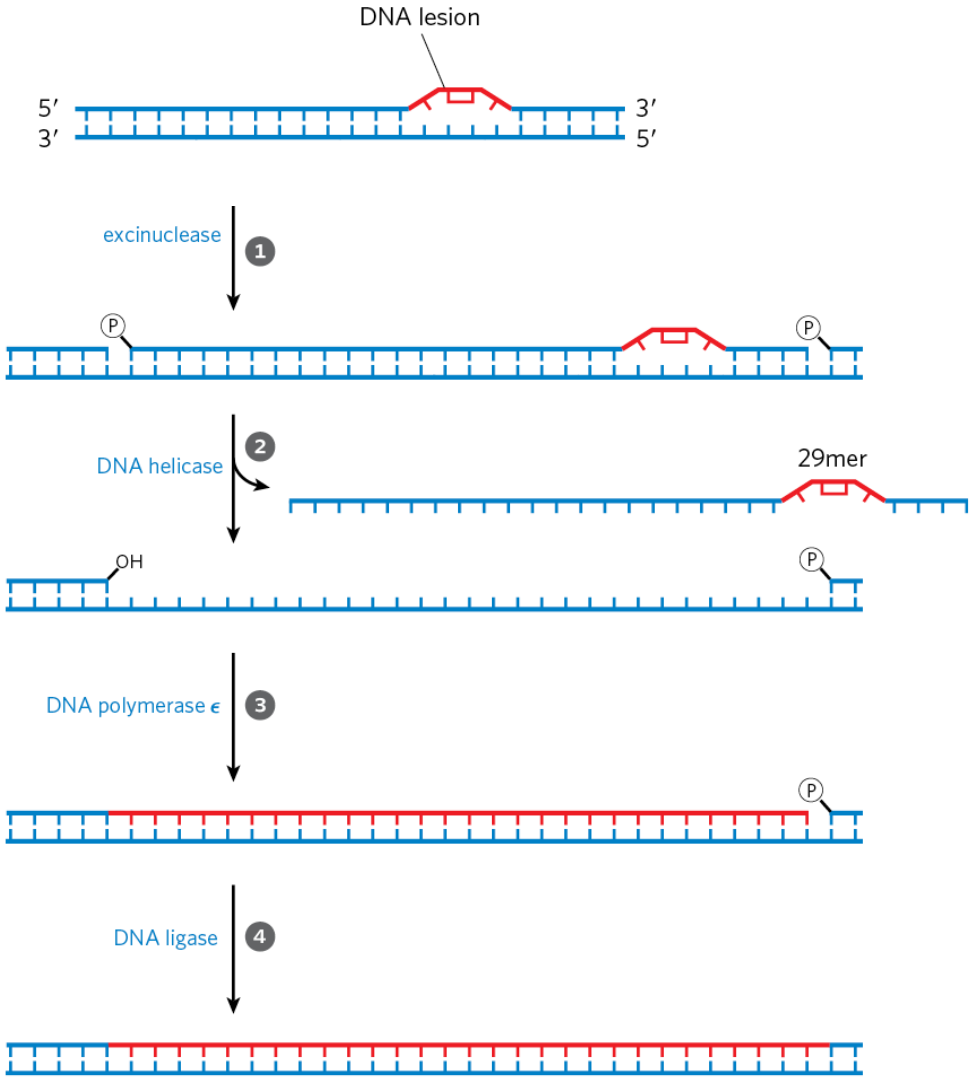
B: It unwinds DNA containing Thymine dimers to help photolyase insert these in their active site

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



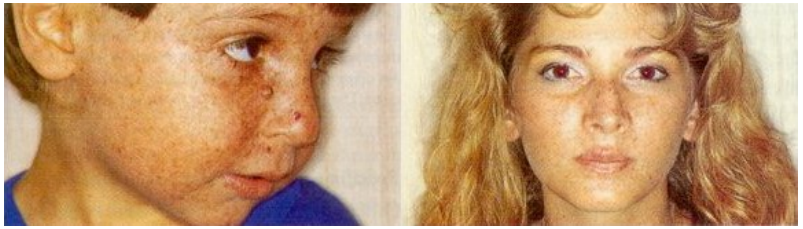
Nucleotide Excision Repair Pathways in Eukaryotes

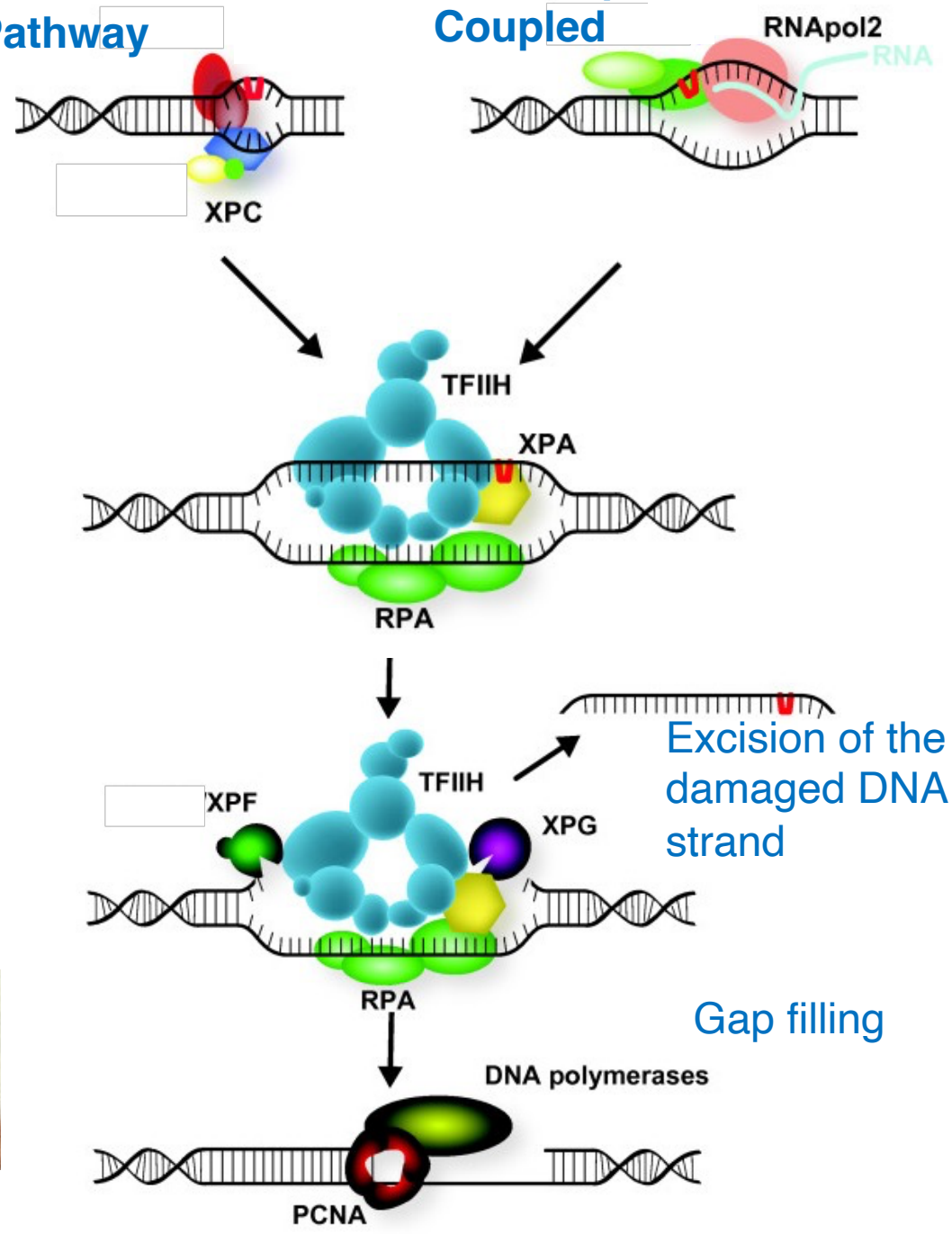
(Y dimer, bulky adducts, crosslinks)

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**