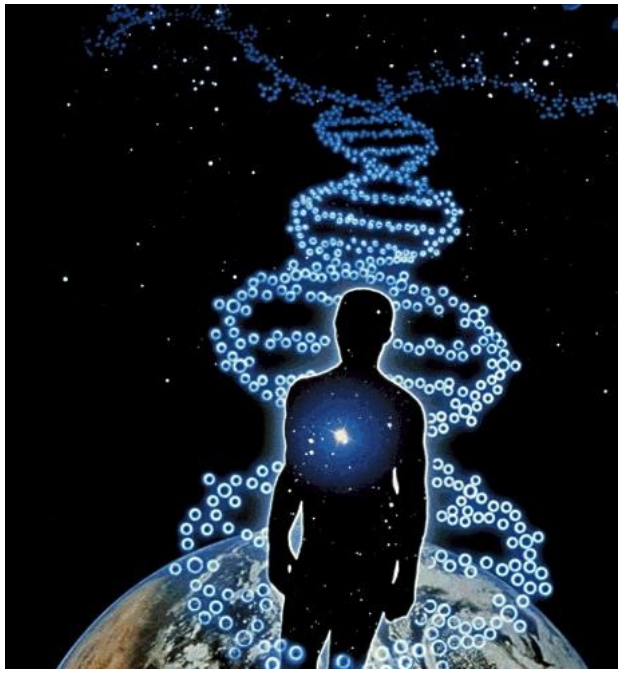
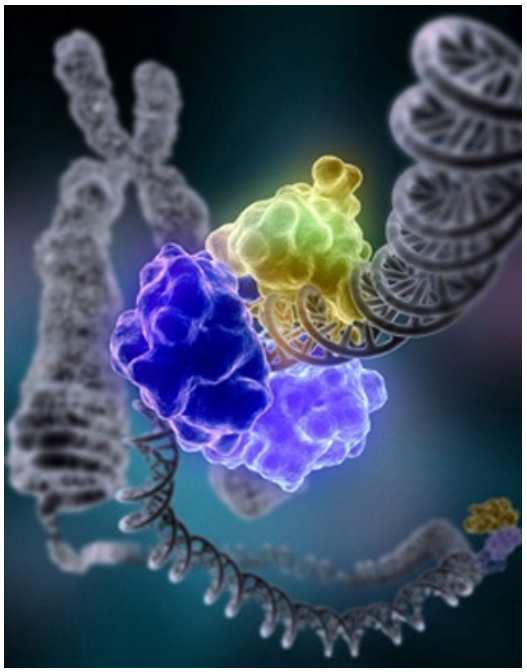
DNA (damage) Repair





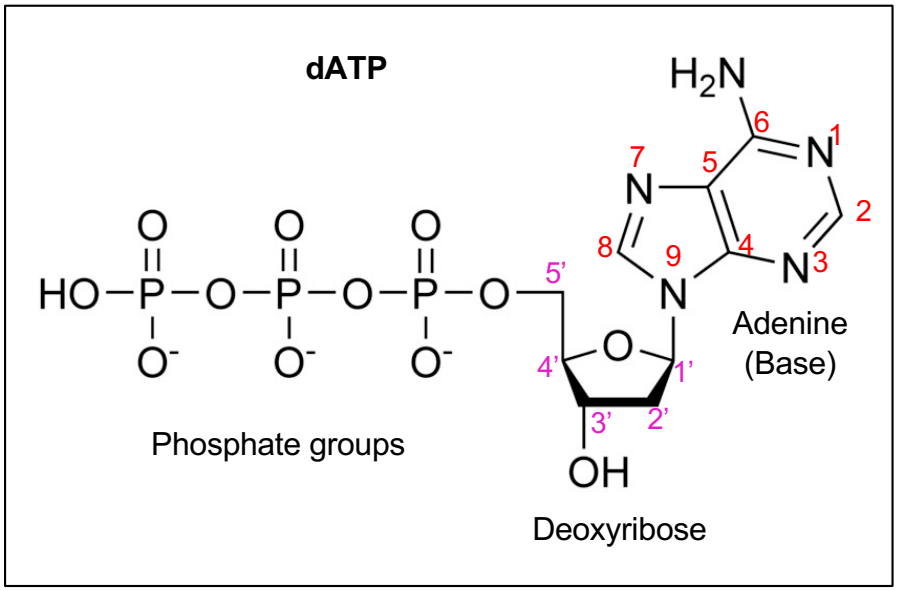
Part 1: DNA is biochemically unstable. Large numbers & many types of DNA lesions occur daily.

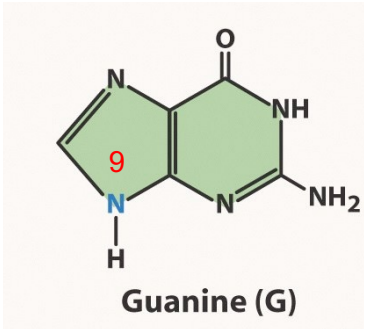
Part 2: Multiple repair mechanisms cope with different types of DNA lesions.

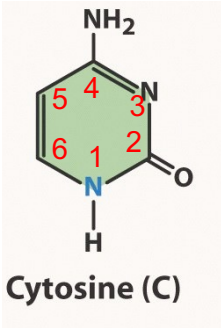
- **Overview of four classical DNA repair pathways**
- **Three examples of DNA repair pathways.**
- **Outstanding questions.**

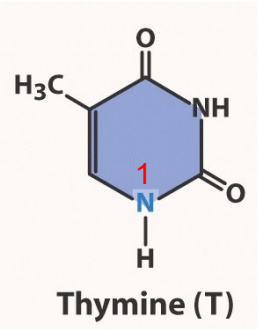
- **DNA repair is intimately connected with many other processes (replication, transcription, epigenetics, cell cycle, cell types, signaling, development, innate immunity, metabolism……..).**
- **DNA repair defects cause hundreds of human diseases.**
- **DNA repair is both cancer-suppressive and treatment target**

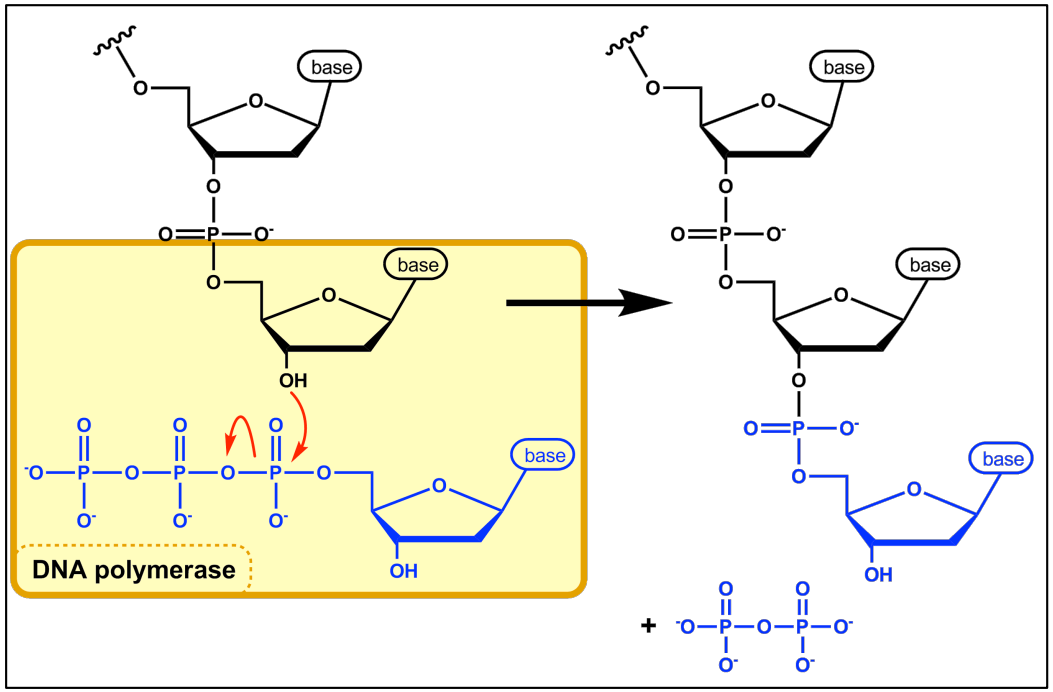
A quick recap: chemical composition of DNA



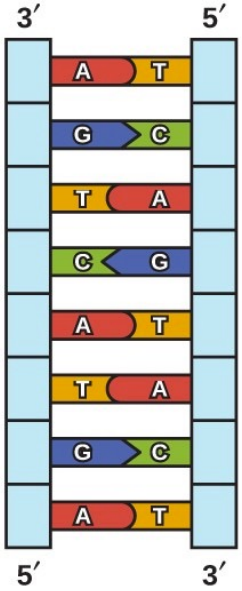


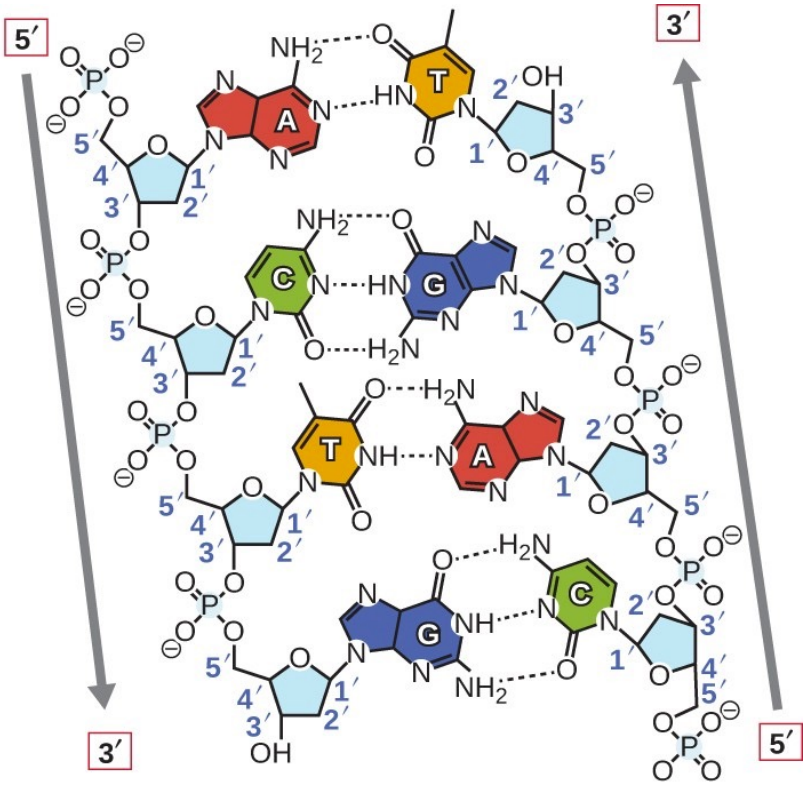






Where do lesions occur on DNA?

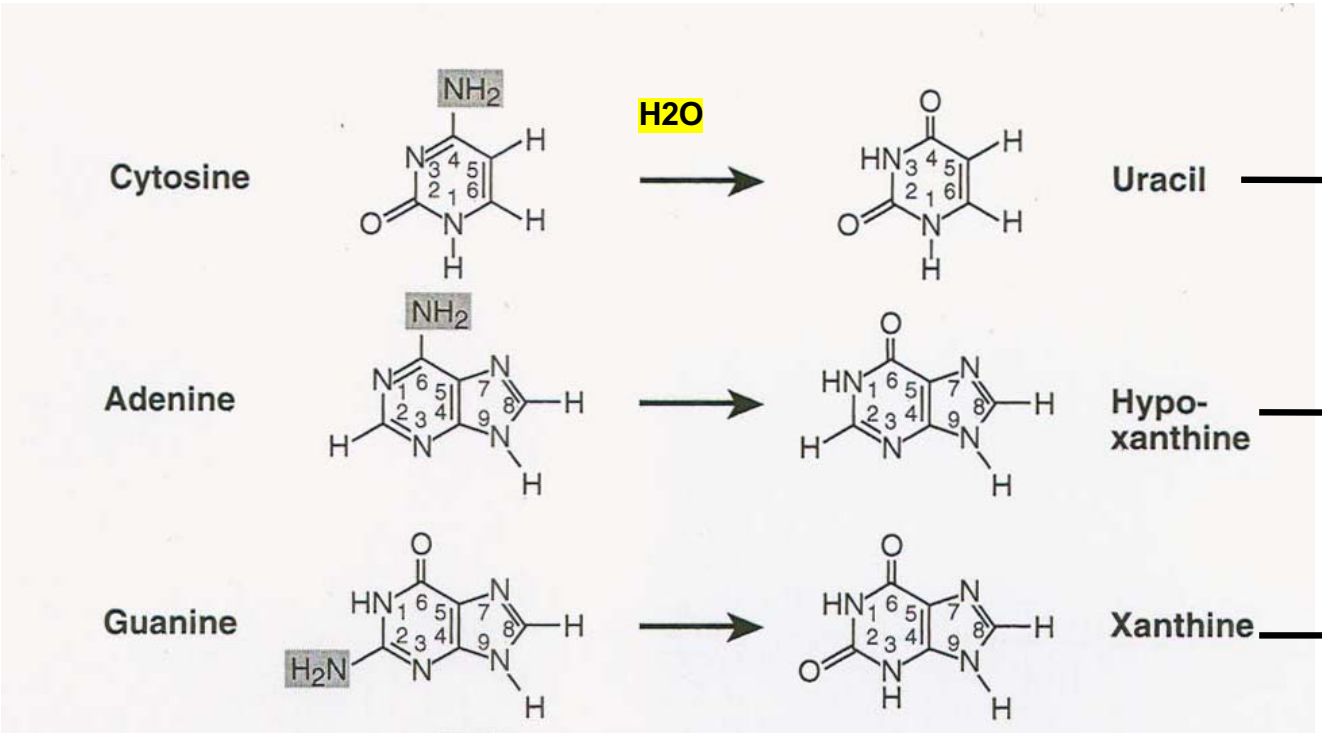




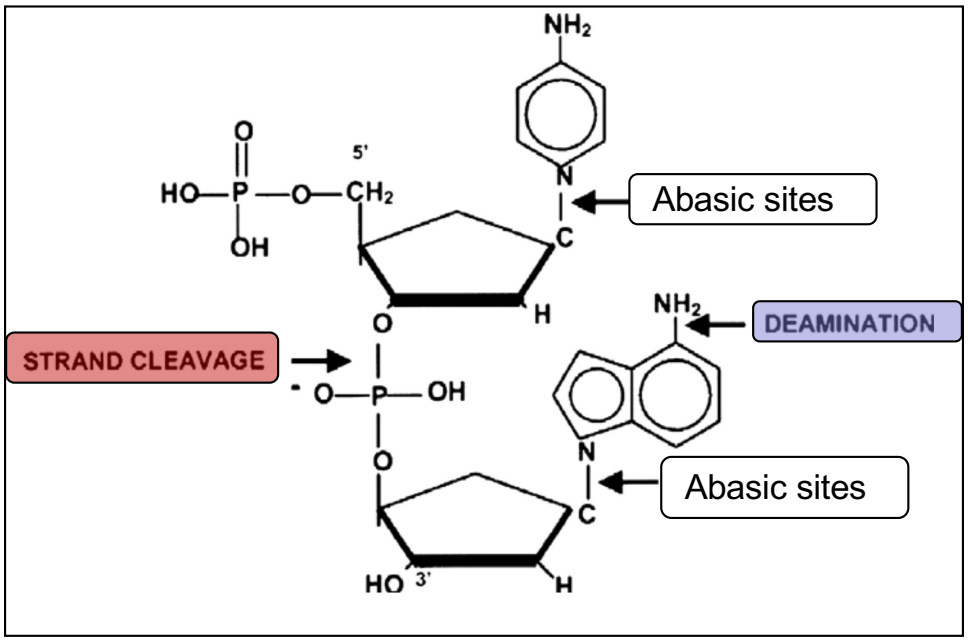
3 billion base pairs of DNA in human genome

Hydrolysis - loss of small chemical groups

Deamination can occur at 3 bases



Hydrolysis - Loss of entire base or ssDNA break

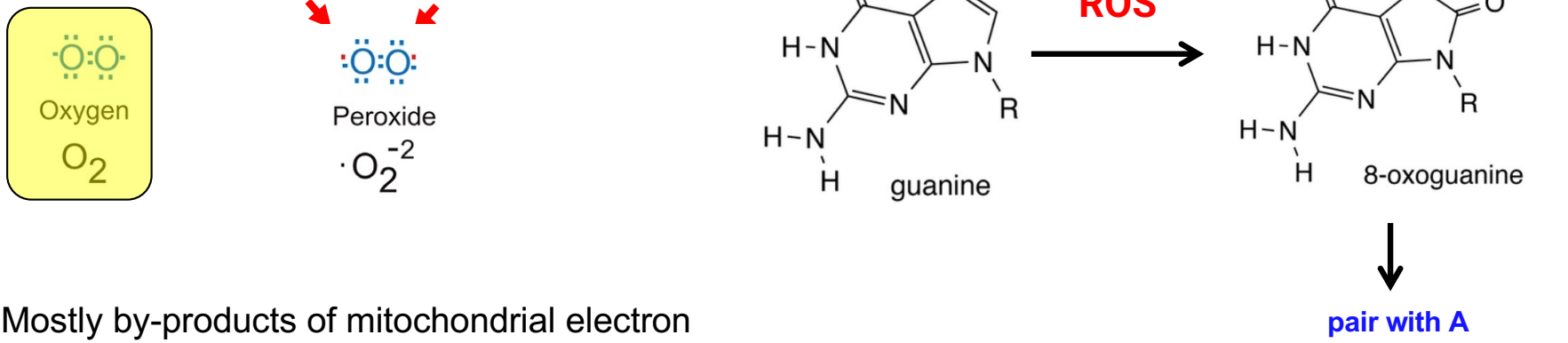


Abasic sites are generated when a base is lost by
Hydrolysis of the N-glycosyl bonds by water.

Absic sites are not stable and can lead to ssDNA breaks.

Oxidation: react with small chemical groups & alter base structures

Oxygen and Reactive oxygen species (ROS)

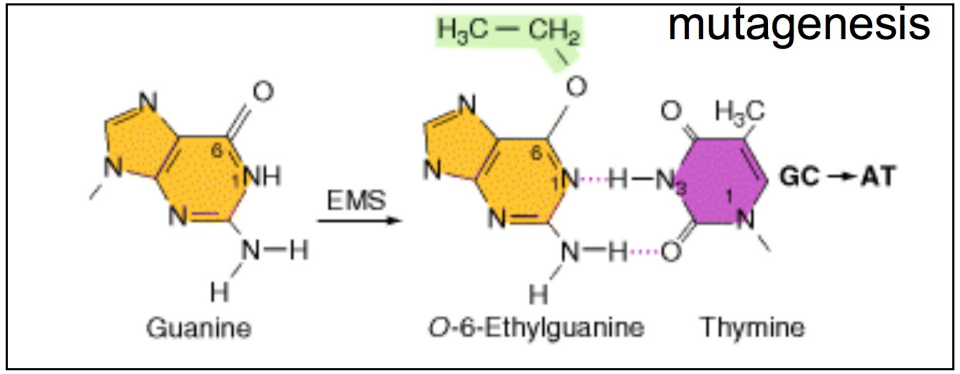


Mostly by-products of mitochondrial electron transport & metal catalyzed oxidation reactions.

Methylation – gain of small chemical groups on the bases

(mediated by **chemicals or enzymes**)

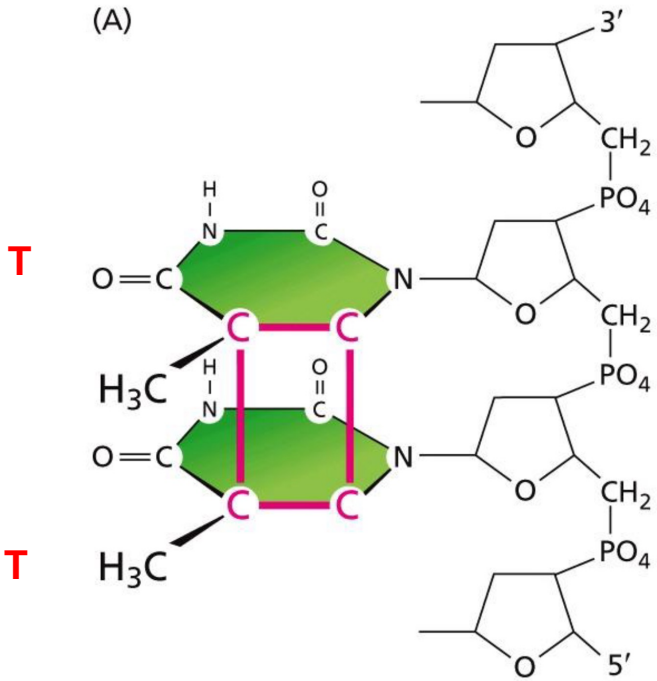
Methylation can occur at several places on the 4 bases



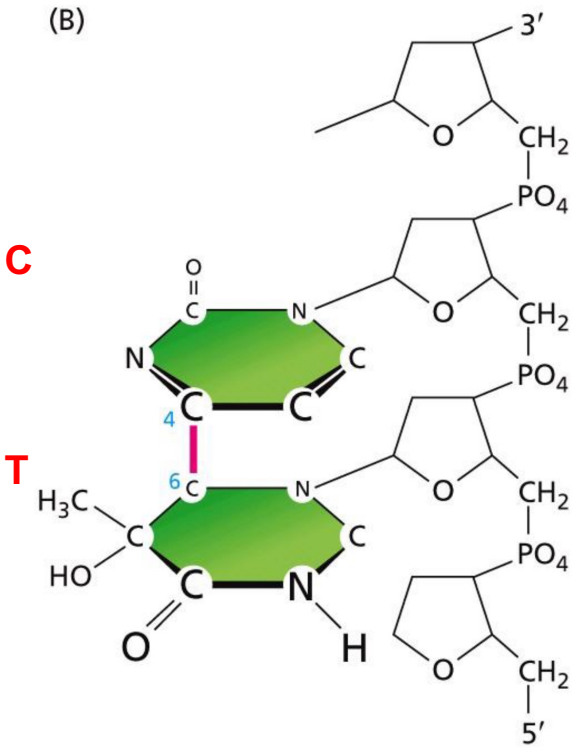
Covalent linkages between bases on the same strand

UV photoproducts

The most frequent photoproducts are covalent linkages between adjacent Pyrimidines: cytosine and thymine



CPD
(cyclobutane pyrimidine dimer)



(6-4)-PP
(6-4)-photoproduct

Crosslink agents and different types of DNA lesions

cis-Platinum leads to several types of crosslinks on DNA

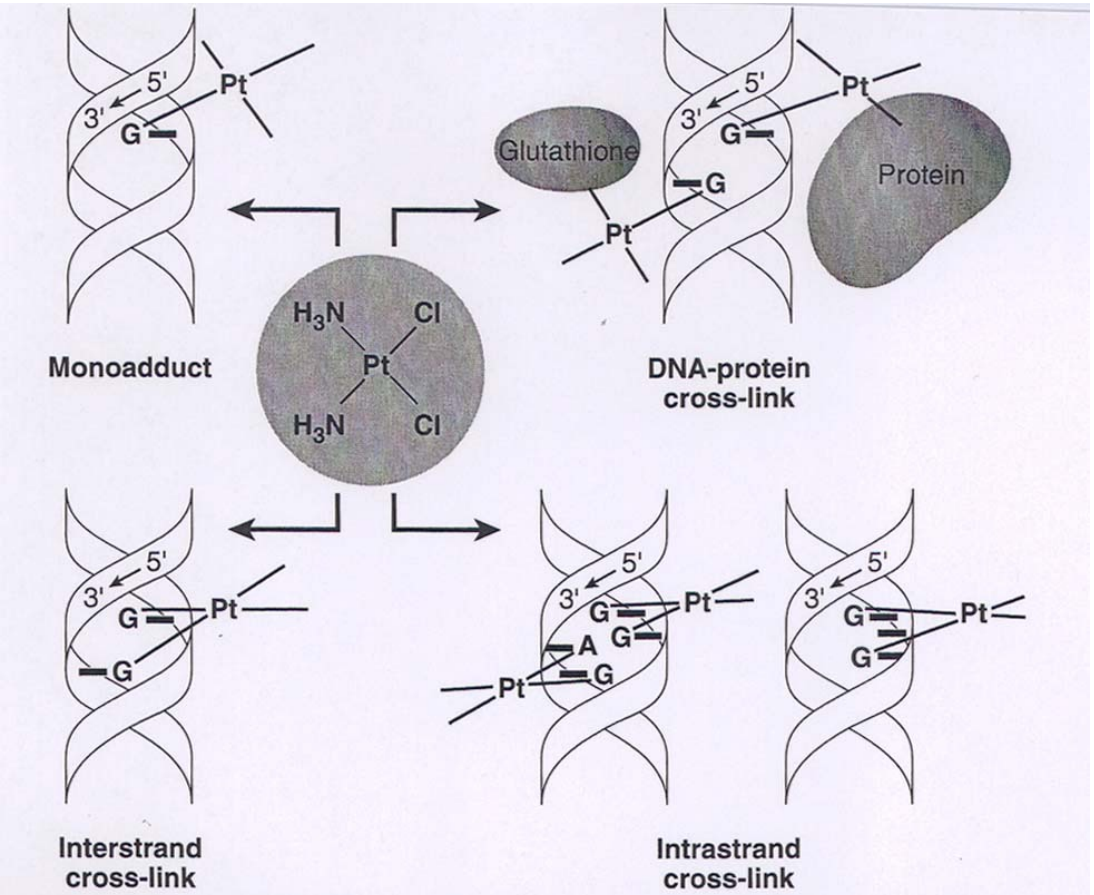


Figure 1–34 *cis*-Platinum can form monoadducts with DNA, interstrand cross-links, intrastrand cross-links, and protein-DNA cross-links, such as glutathione-DNA cross-links. (*Adapted from Eastman [99] with permission.*)

Estimated amounts of base lesions

Table 2–1 Endogenous DNA lesions arising and repaired in a diploid mammalian cell in 24 h

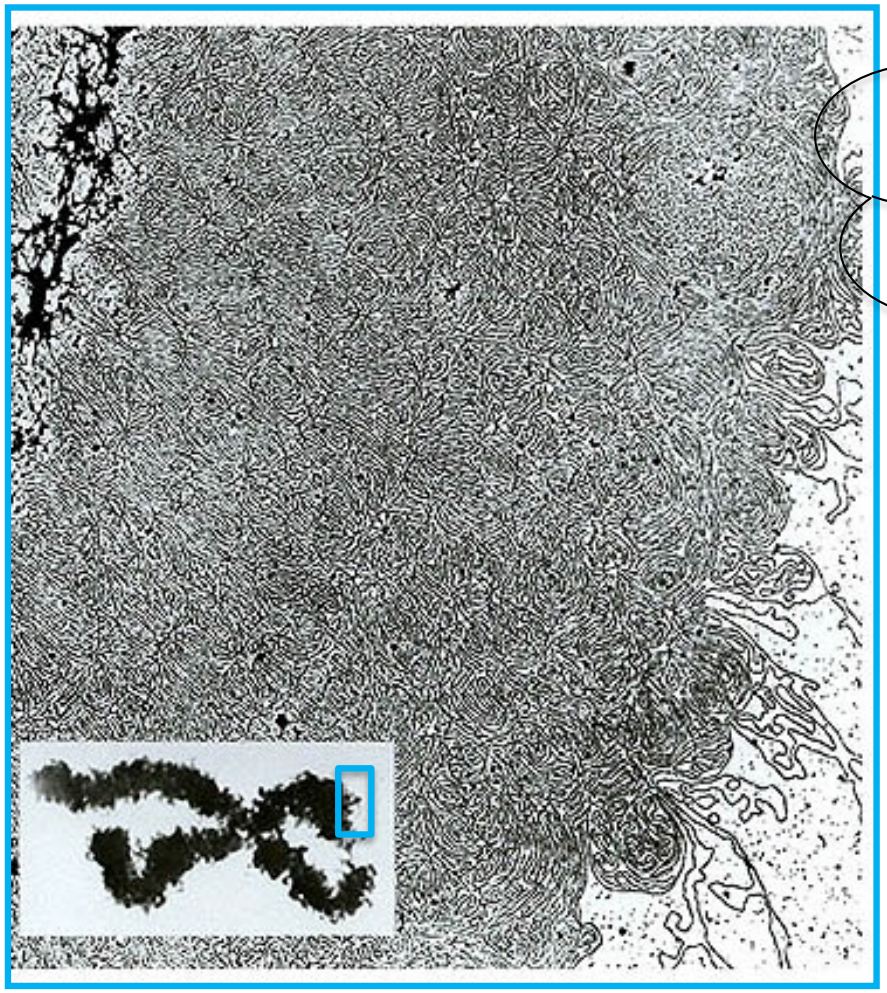
Endogenous source	No. of lesions: 100% double-stranded DNA
Hydrolysis	
Depurination (Abasic site)	18,000
Depyrimidination (Abasic site)	600
Cytosine deamination	100
5-Methylcytosine deamination	10
Oxidation	
8-oxoG	~1,000–2,000
Ring-saturated pyrimidines (thymine glycol, cytosine hydrates)	~2,000
Lipid peroxidation products (M_1G, etheno-A, etheno-C)	~1,000
Nonenzymatic methylation by *S*-adenosylmethionine	
7-Methylguanine	6,000
3-Methyladenine	1,200
1-Methyladenine and 3-methylcytosine	ND[c]
Nonenzymatic methylation by nitrosated polyamines and peptides	
O^6-Methylguanine	20–100

[a]Data from reference 297.

[b]Estimates are for two 3×10^9 -bp genomes per cell.

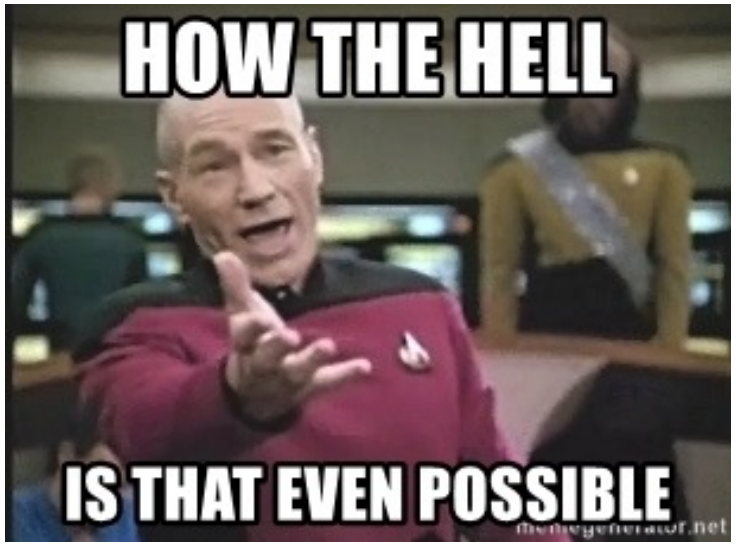
[c]ND, none detected

Repair of eukaryotic genome is challenging

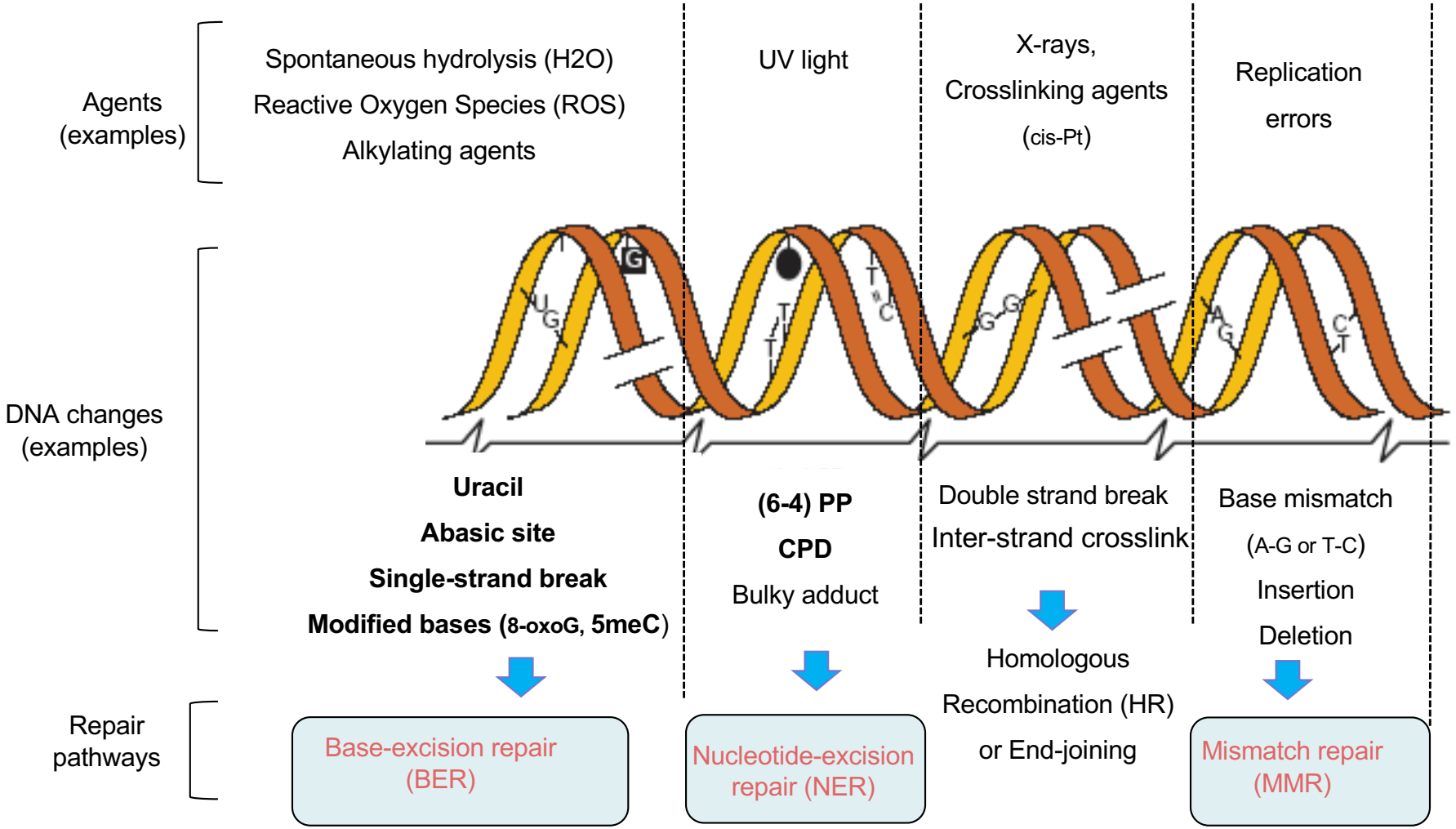


Electron micrograph of DNA from a small part of a chromosome

How can cell fix the huge numbers of DNA lesions in the human genome to minimize deleterious mutations that can lead to hundreds of types of diseases?!



DNA lesions and repair pathways (partial)



Several new types of DNA repair pathways

Common features of DNA repair pathways

Stage I

Detecting DNA lesions

- Proteins recognize specific modified bases (BER)
- Proteins recognize DNA strand distortion (NER)
- Proteins bind to to mismatched bases (MMR)
- Proteins recognize broken ends (HR, EJ)

Stage II

Removing lesions and clean-up

- Different types of **nucleases** & **other enzymes** to remove different types of lesions
- lesion recognizing proteins + scaffold proteins: recruit lesion removal enzymes

Stage III

Synthesizing new DNA

- More than a dozen of DNA polymerases to be deployed for repair tasks
- These polymerases are less precise and require tight regulations.
- Other proteins required: DNA ligase and more clean-up enzymes.

Important Considerations:

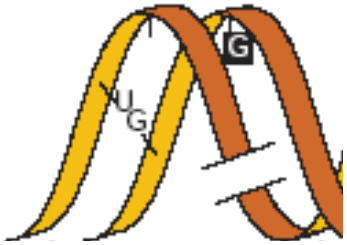
- Processivity of the multi-step repair processes.
- Cope with chromatin contexts and restore epigenetic states afterwards.
- DNA repair efficiency in response to genotoxins.
- Coordinate with other processes, such as transcription.

Base Excision Repair (BER)

Spontaneous hydrolysis (H2O)

Reactive Oxygen Species (ROS)

Alkylating agents (MMS, EMS, SAM)



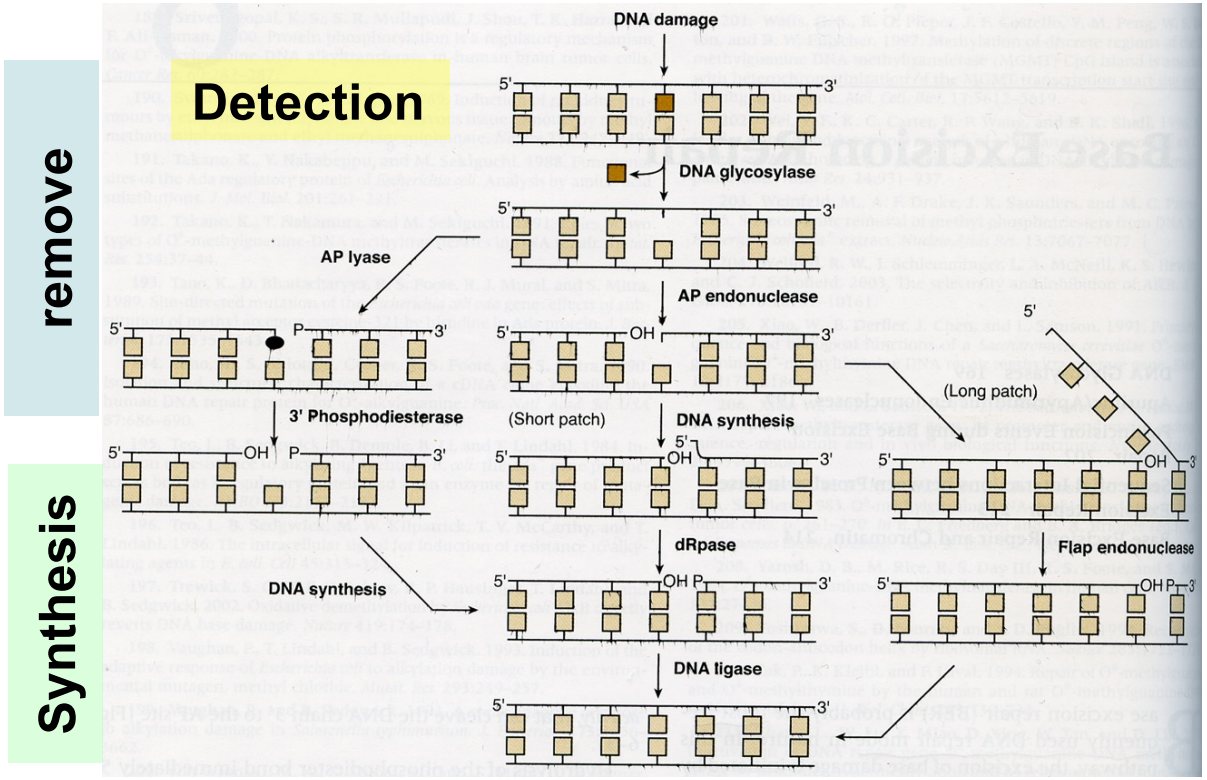
Uracil

Abasic site

Single-strand break

Modified bases (8-oxoG, 5meC)

Base-excision repair (BER)

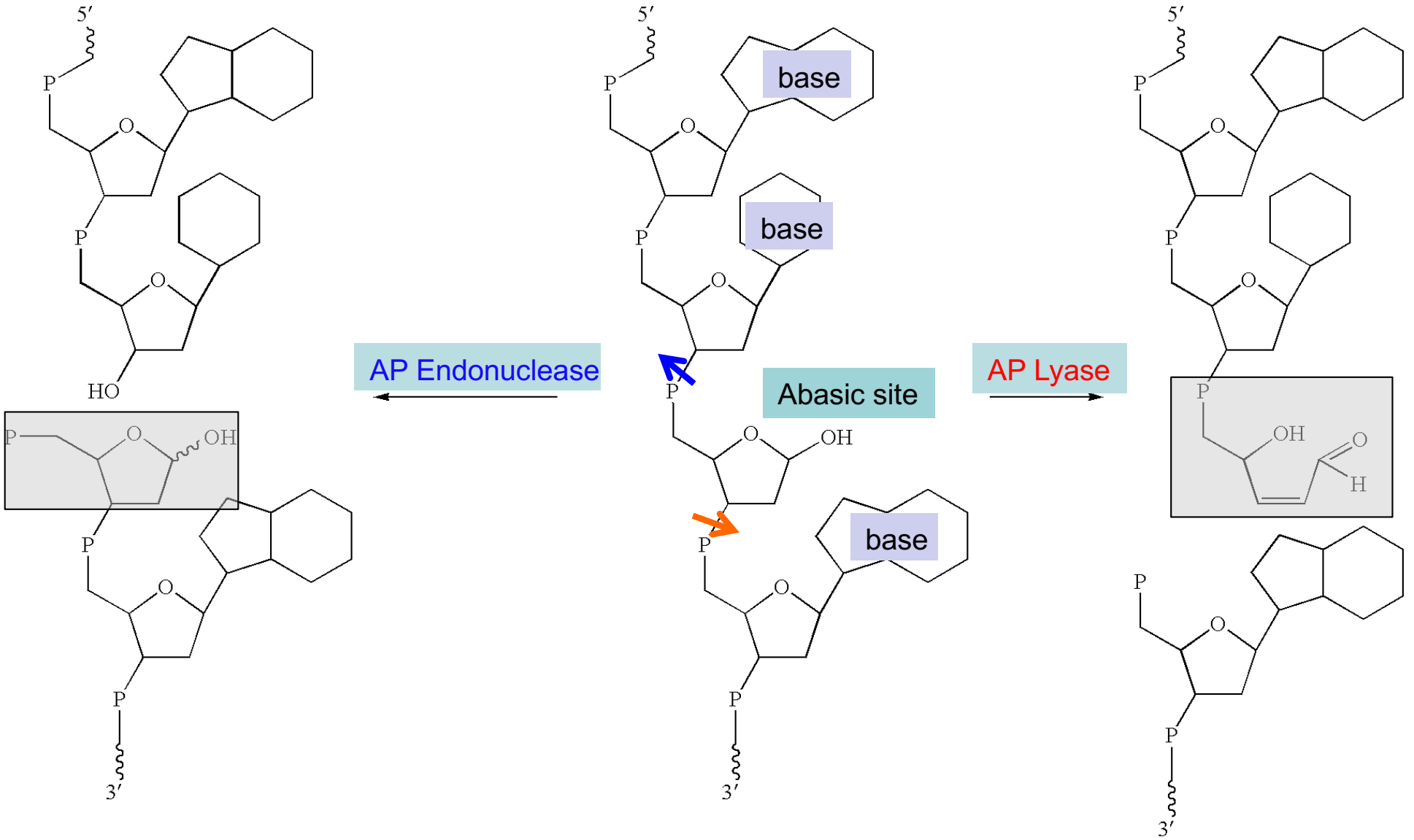


Damage Recognized:

Base deamination,
Base oxidation,
Base methylations

Gene Products Required (7):

1. Glycosylases (>11 types in human)
2. AP lyase +3' Phosphodiesterase
3. AP endonuclease + dRpase (Deoxyribo-phosphodisterase)
4. DNA polymerase
5. Flap nuclease
6. DNA Ligase

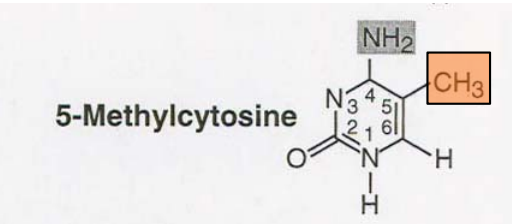


Deoxyribo-phosphodiesterase (dRpase)

3’ Phosphodiesterase

BER in development

Base modification: a means for functional variability, while maintaining genetic information

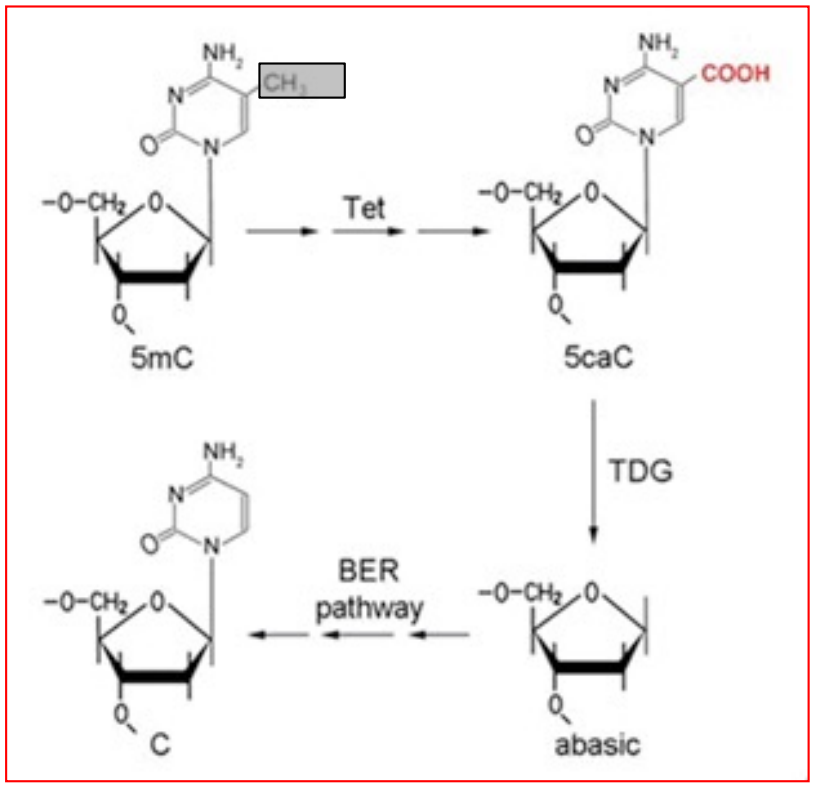


- In mammalian genomes, 5mC exists mostly in CpG context (~80% CpGs methylated) by DNMTs.
- 5mC - transcription repression, used widely for control lineage-specific genes, X-chr & mobile elements inactivation.

Demethylation is observed:

1.early in development, some posterior epiblast cells become primordial germ cells wherein loss of 5mC and prepares them for germ-cell-specific processes.

2.At specific loci in response to stimuli.

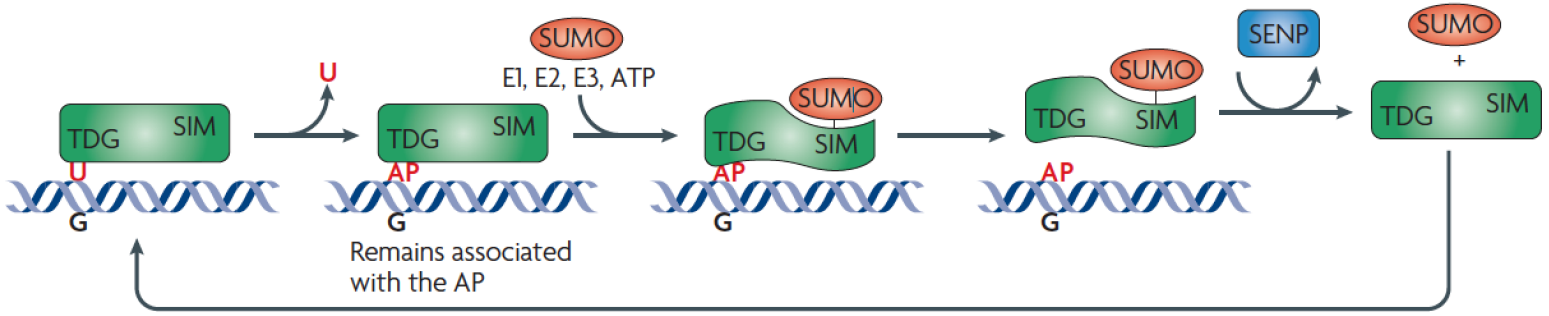


How are BER enzymes coordinated?

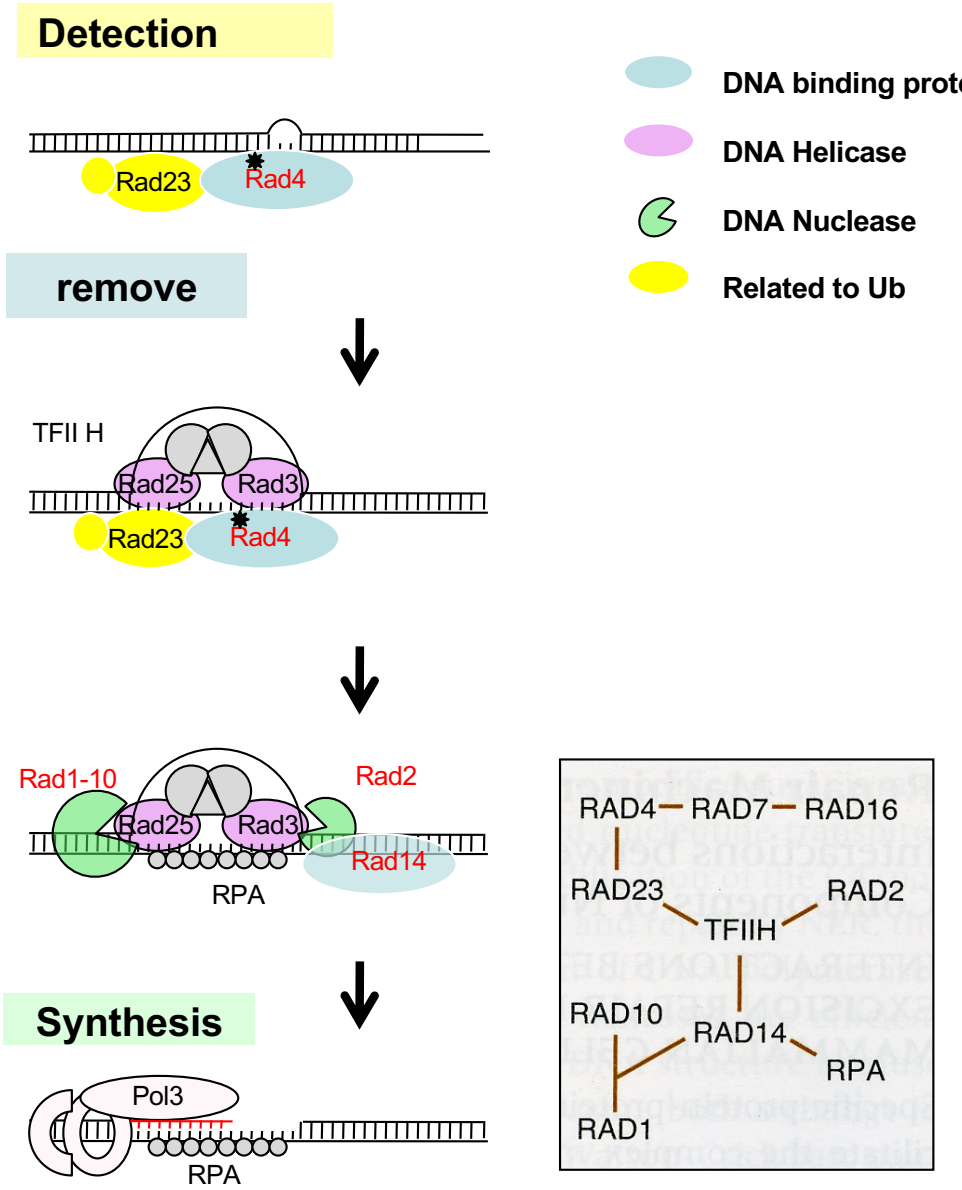
Hands-off Model: proceed by the sequential action of factors facilitated by pair-wise interactions

- Protect reaction intermediates & ensure the completion of the reaction once initiated.
- Reaction accuracy and specificity.

TDG requires sumoylation and desumoylation for each catalytic cycle.



Key proteins & steps in Nucleotide Excision Repair (NER)



DNA binding proteins

DNA Helicase

DNA Nuclease

Related to Ub

Damage Recognized:

Large changes in the nucleotide – eg. CPD

Model of the Global Genomic NER (GG-NER) mechanism.

(A) A lesion induces DNA helix distortion;

(B) **Rad4-23** detects helix distortion & stabilizes DNA bend;

(C) **Rad4-23** recruits **TFIIH** at the site of the lesion;

(D) **TFIIH** (10 subunits, txc component, +ATP) unwinds DNA, until the Rad3 helicase encounters a modified base; the other helicase Rad25 goes on unwinding DNA to create a 20-bp "bubble" structure;

(E) Rad14, Rad2, and RPA are recruited

(F) Rad1-Rad10 joins the complex to enable dual incision (5' cut by Rad1-10 and 3' cut by Rad2).

(G) RPA remains on ssDNA to facilitates transition to repair synthesis by Pol III with the help of RFC and PCNA; ligase I finally seals the nick.

NER in human diseases

<u>Diseases associate with NER</u>

XP (Xeroderma Pigmentosum): UV^s , multiple skin disorders, skin ***cancer***, neurological abnormalities.

XPC(**Rad4**)-XPD(Rad3)-XPE(DDB2)-XPF(Rad1)-XPG(Rad2); XPA (**Rad14**)-XPB (Rad25)

CS (Cockayne's Syndrome): UV^s, mental and growth ***retardation***.

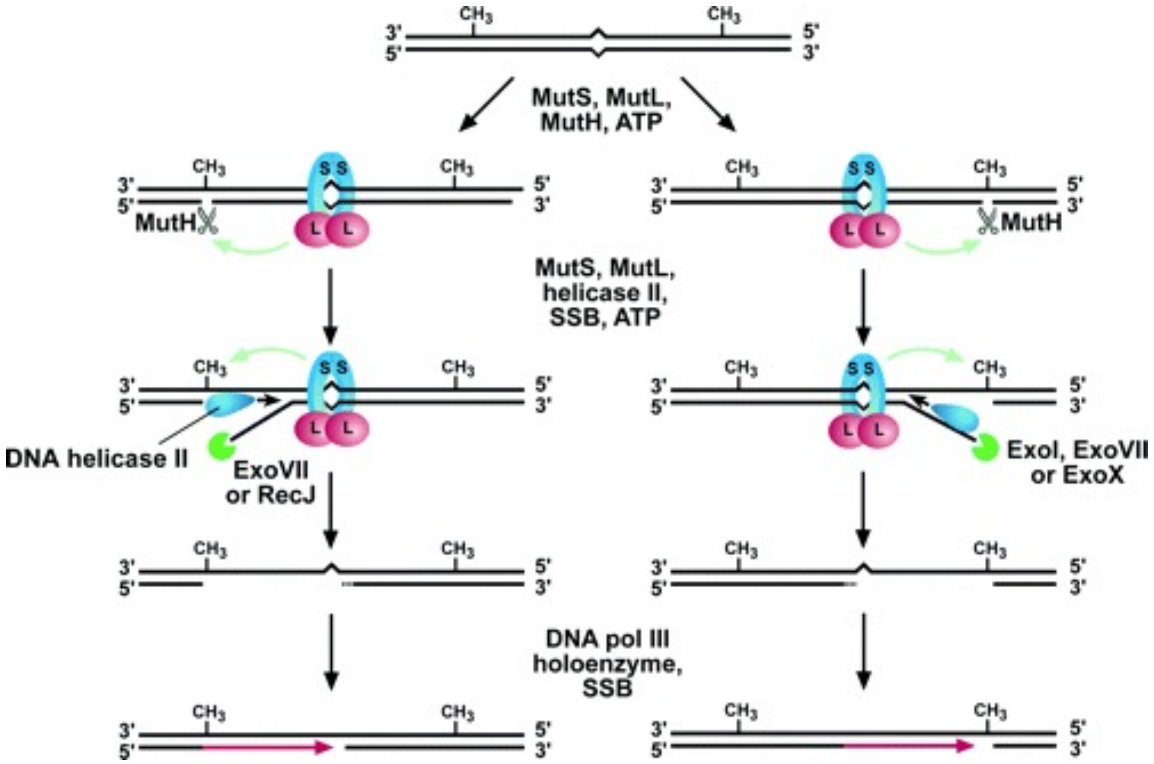
CSA (Rad28), CSB (Rad26) – Transcription coupled NER

TTD (Trichothio-dystrophy): mental and growth retardation, brittle hair.

XPB (Rad25), XPD(Rad3) – TFIIH subunits

Structural and genetic studies to understand the differences among the three syndromes are lacking.

Mechanism of E. coli methyl-directed MMR



Green arrows = MutS- and MutL-dependent signaling between the 2 DNA sites involved in the reaction.

Damage Recognized:
Base-base mismatch
Small insertion/deletion loops

MMR proteins:

- **MutS** (damage recognition)
- **MutL** (recruit of MutH and MutU)
- **MutH** (endonuclease cut at unmodified strand at GTAC)
- **MutU** (DNA helicase II)
- **Exonucleases** (ExoI, etc)
- **DNA Pol III -**fills in the gap
- **SSB** (Single strand binding protein)
- **DNA Ligase**

Mismatch Repair in Eukaryotic cells (yeast and human)

MutSα (Msh2/Msh6) - recognizes mismatch or 1 bp insertion or deletion
MutSβ (Msh2/Msh3) - recognizes 2-12 bp insertion or deletion

MutLα (Mlh1/Pms1) - "match maker" that recruit downstream factor

Discrimination between parent and daughter strand may be accomplished by the presence of nick in daughter strands or by ribonucleotides?

MMR in human diseases

.

- Defects lead to **Lynch syndrome or** Hereditary Nonpolyposis Colon Cancer (HNPCC). A common cancer predisposition disease. It is nearly always associated with "microsatellite instability" (variations # repeat units of short tandemly repeats).

- MMR affects triplet repeat stabilities implicated in Huntington, fragile X etc.

Base Excision Repair

- removal of oxidative and alkylating damage; also involved in SSB repair.
- damaged bases are removed as free bases.
- intimately linked with the SUMO system.
- have an important role in development and aging.

Nucleotide Excision Repair

- removal of UV-induced damage and bulky adducts & ~ 20% oxidative damage
- damaged bases are removed as oligonucleotides (~20nt)
- Most proteins participate in other cellular processes.
- intimately linked with the ubiquitination system.
- deficient in human disorders (XP, CS and TTD)

Mismatch repair

- removal of nucleotide errors or small insertion/deletion generated by DNA polymerases.
- wrong bases are removed by generating a ssDNA gap.
- Most proteins also affect other cellular processes (HR)
- deficient in human disorders: Lynch syndrome and neurodegeneration disorders.

Many puzzles regarding DNA repair

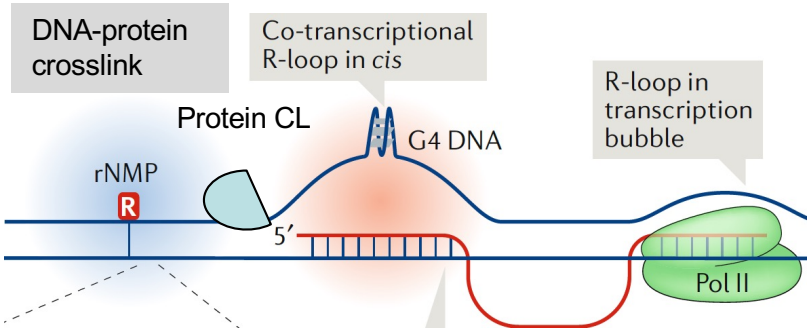
1. **Mechanisms**. How each step & handover occur accurately; majorities of repair proteins are modified by SUMO, Ub, Phos - how modifications aid repair? New repair proteins are continuing to be discovered.

2. **Chromatin environments**: DNA repair foci - phase-separation; epigenetic markers - silenced vs activate chromatin regions?

3. **Cross-talks**: intimately linked with other cellular functions DNA replication, checkpoints, transcription, innate immunity, metabolism, etc.

4. **Disorders** - How defects in DNA repair processes can lead to different human disorders?

New DNA repair pathways



Examples:

- Protein-DNA **crosslink** repair
- RER – **R**ibonucleotide **E**xcision **R**epair
- R-loop repair
- G4 structure removal
- Repair during DNA replication
- Repair during mitosis (MiDAS)

Connections between DNA repair & human diseases

Review

DNA Damage and Cancer Immunotherapy: A STING in the Tale

Review

CellPress

DNA damage and innate immunity: links and trade-offs

Georgia Chatzinikolaou[1], Ismene Karakasilioti[1,2], and George A. Garinis[1,2]

Interplay between Cellular Metabolism and the DNA Damage Response in Cancer

Feature Review

DNA Damage Triggers a New Phase in Neurodegeneration

DNA repair, damage tolerance, and signaling