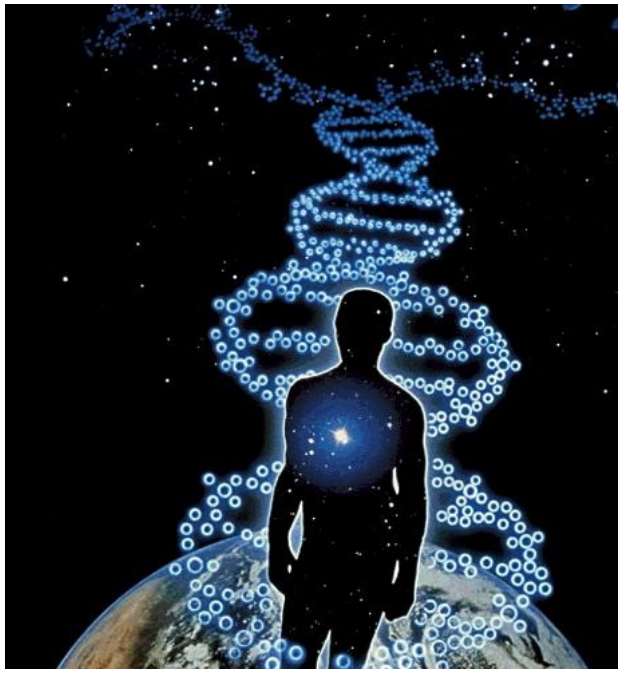
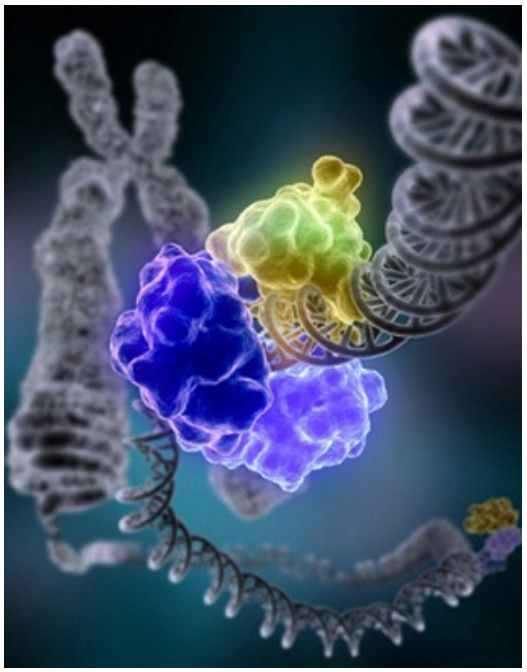
DNA (damage) Repair





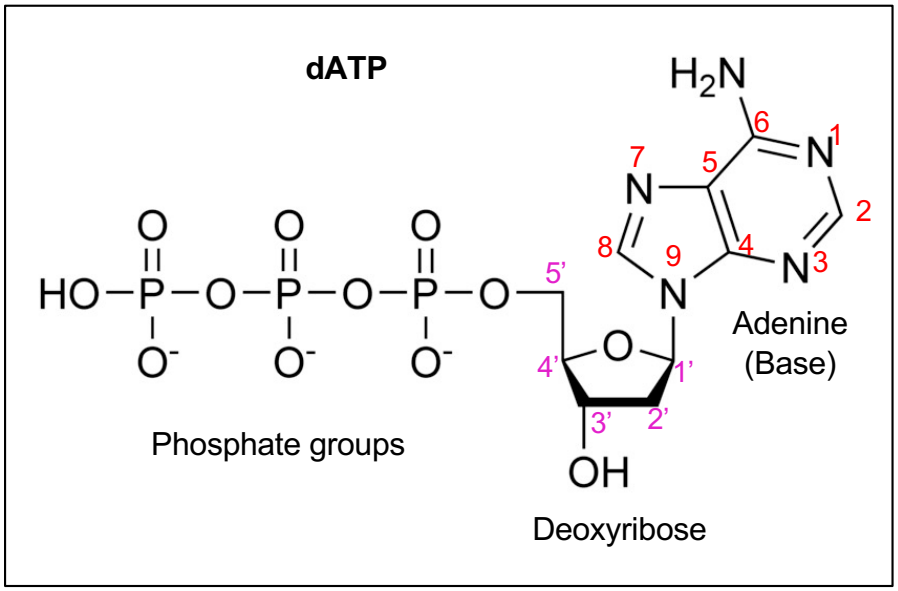
Part 1: DNA is biochemically unstable. Large numbers & many types of DNA lesions occur daily.

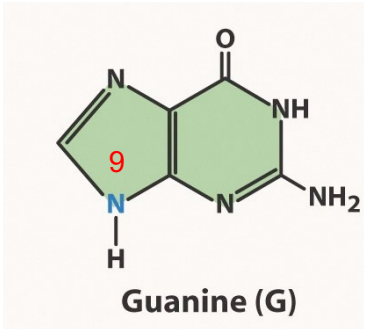
Part 2: Multiple repair mechanisms cope with different types of DNA lesions.

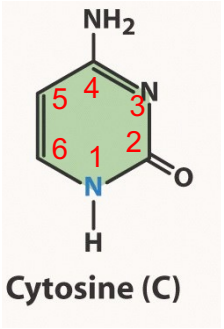
- **Overview of four classical DNA repair pathways**
- **Three examples of DNA repair pathways.**
- **Outstanding questions.**

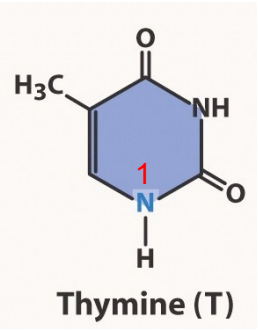
- **DNA repair is intimately connected with many other processes (replication, transcription, epigenetics, cell cycle, cell types, signaling, development, innate immunity, metabolism……..).**
- **DNA repair defects cause hundreds of human diseases.**
- **DNA repair is both cancer-suppressive and treatment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

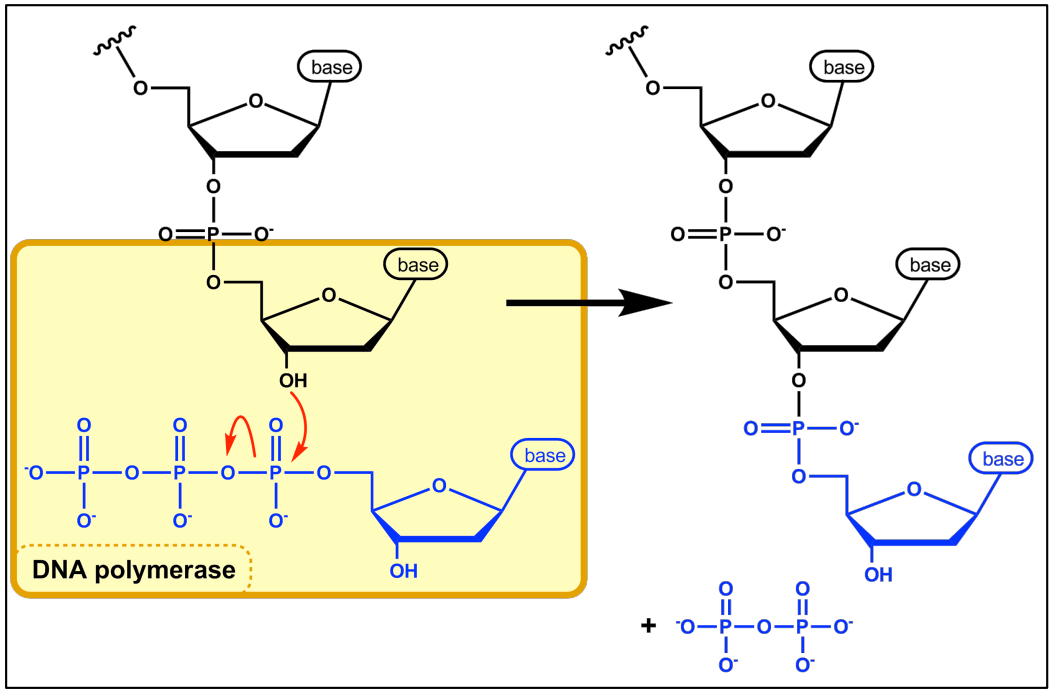
A quick recap: chemical composition of DNA



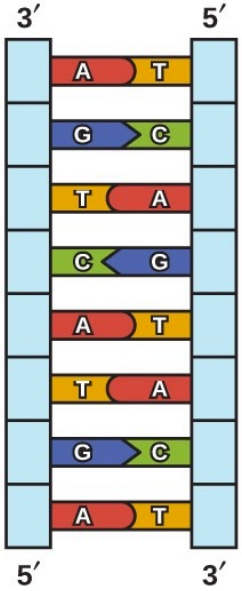


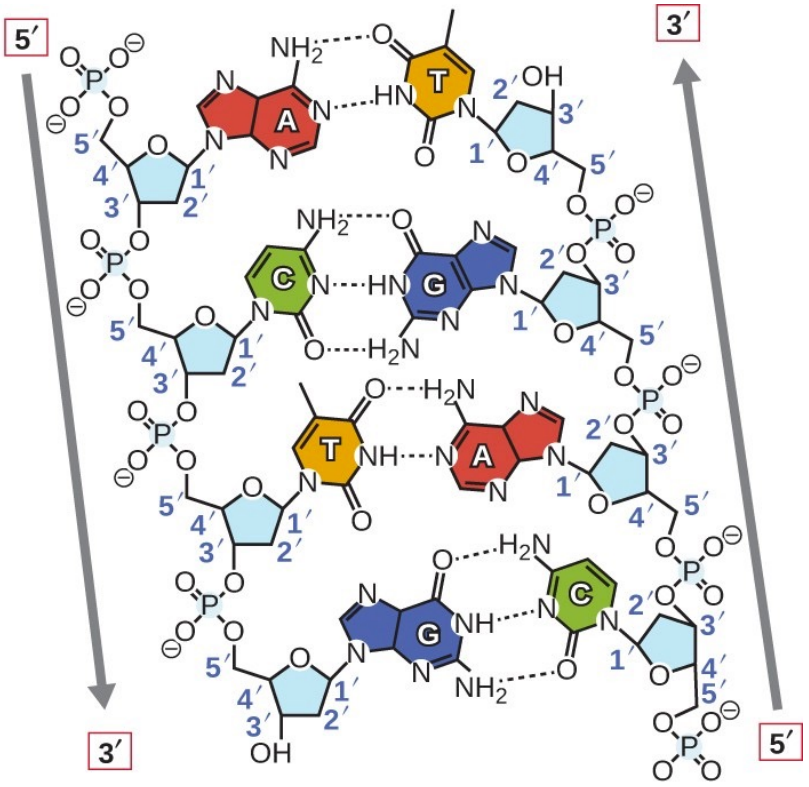






Where do lesions occur on DNA?



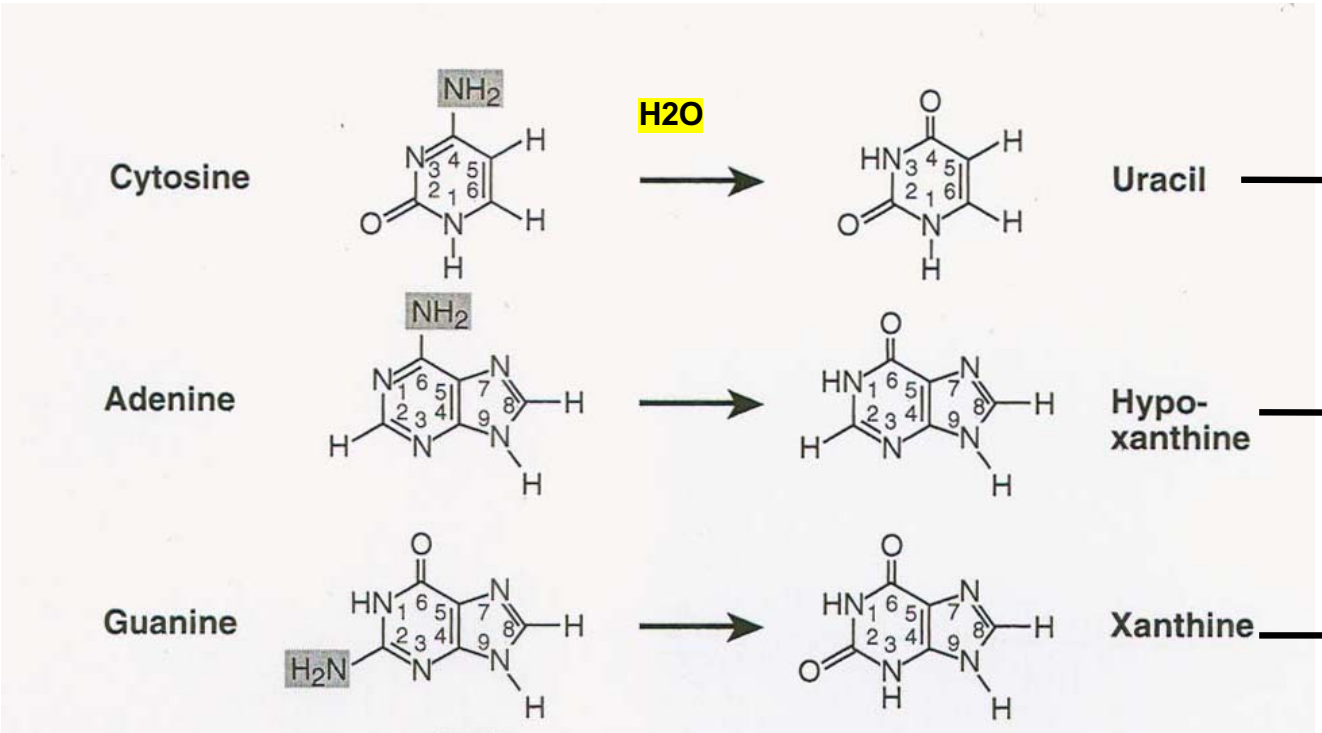




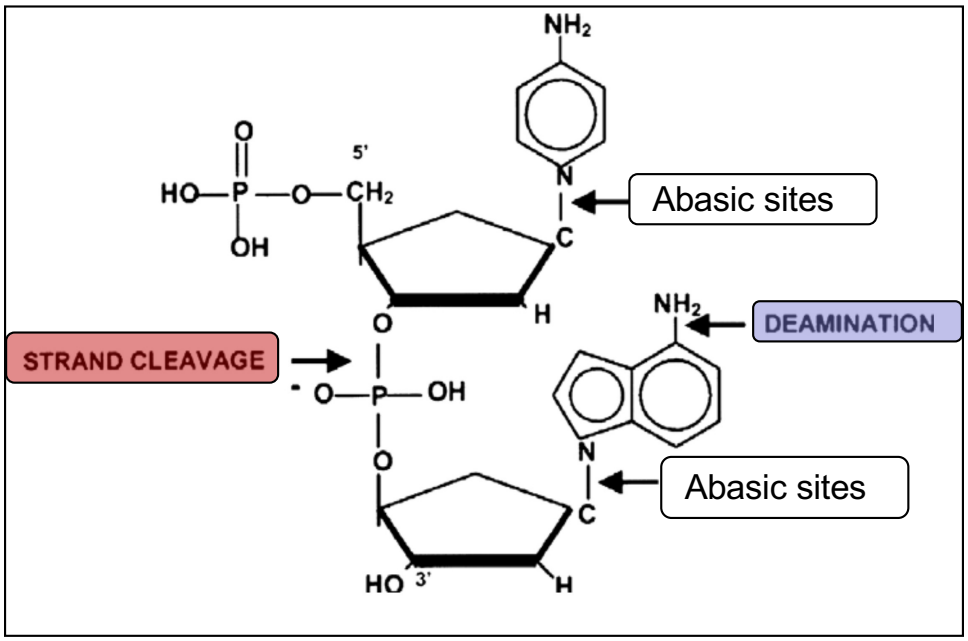
3 billion base pairs of DNA in human genome

Hydrolysis - loss of small chemical groups on bases

Deamination can occur at 3 bases



Hydrolysis - Loss of an entire base or generating ssDNA breaks

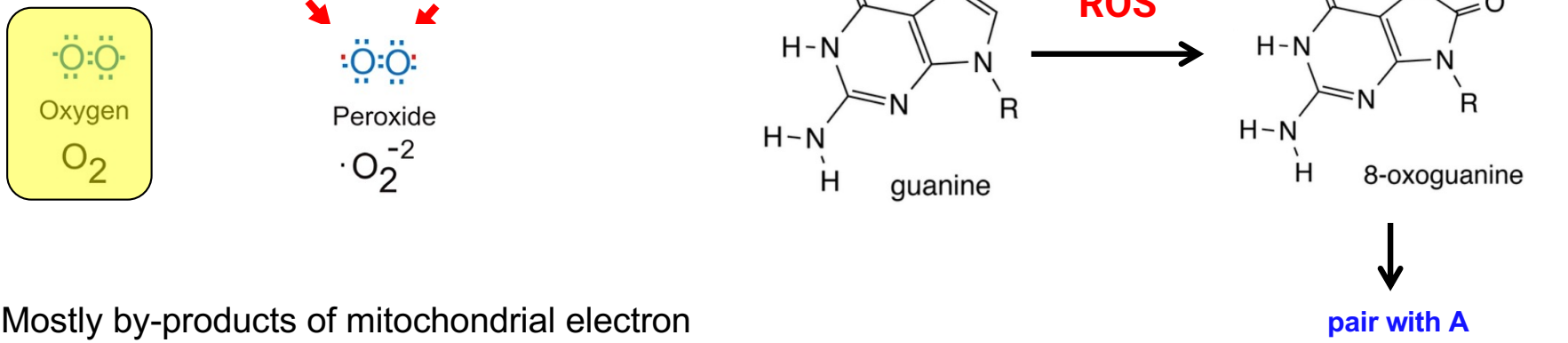


Abasic sites are generated when a base is lost by Hydrolysis of the N-glycosyl bonds by water.

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

Oxidation: react with small chemical groups & alter bases

Oxygen and Reactive oxygen species (ROS)

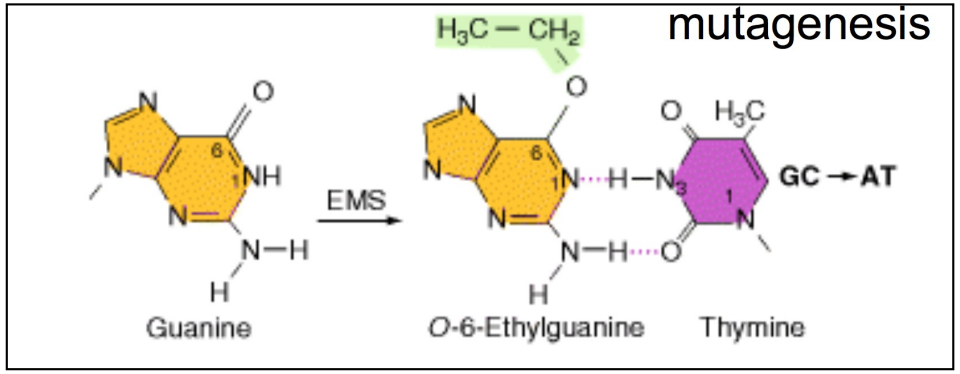


Mostly by-products of mitochondrial electron transport & metal catalyzed oxidation reactions.

Methylation – gain of small chemical groups on the bases

(mediated by **chemicals or enzymes** –covered in Dirk's lecture)

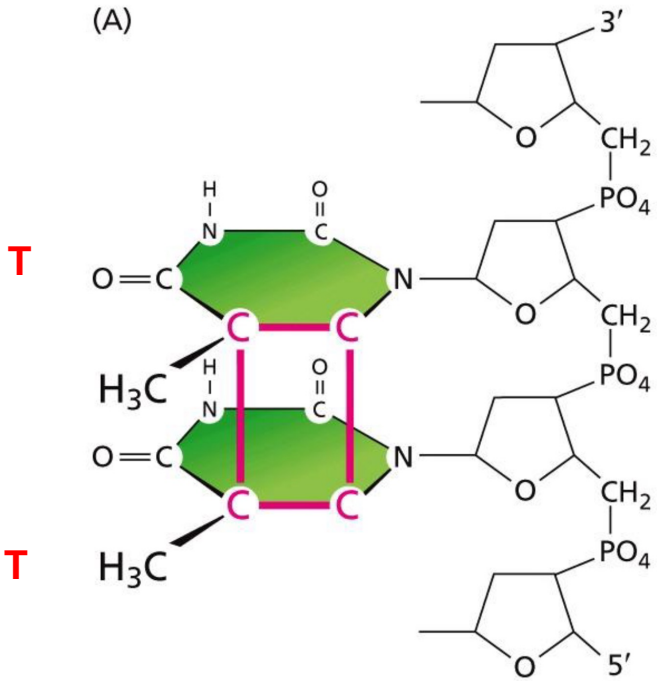
Methylation can occur at several places on the 4 bases



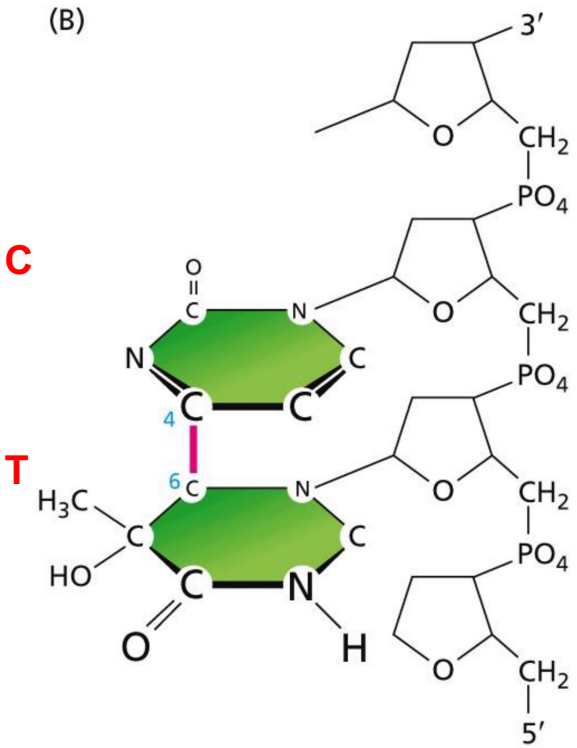
Covalent linkages of two bases on the same strand

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 - generate multiple types of DNA lesions

cis-Platinum leads to several types of crosslinks on DNA

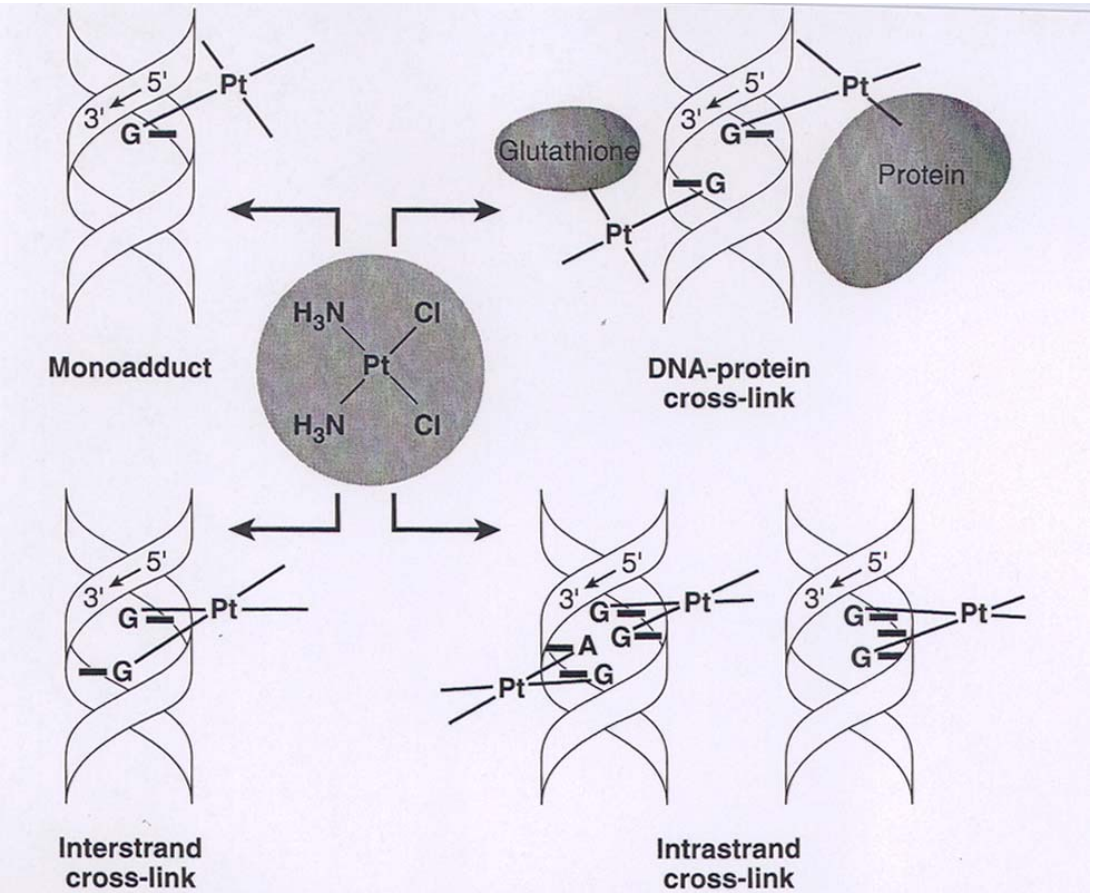


Figure 1–34 *cis*-Platinum can form monoadducts with DNA, interstrand cross-links, intrastrand cross-links, and protein-DNA cross-links, such as glutathione-DNA cross-links. (*Adapted from Eastman [99] with permission.*)

All can block RNA and DNA polymerases

Estimated amounts of base lesions

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

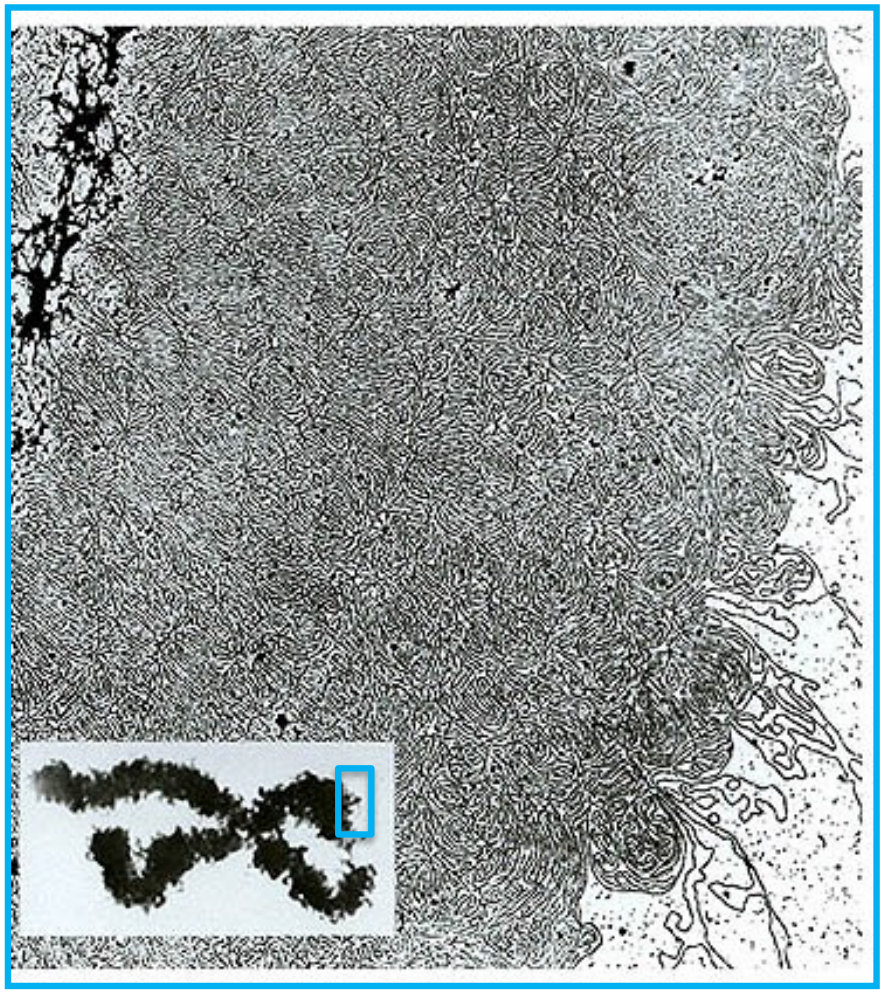
Endogenous source	No. of lesions: 100% double-stranded DNA
Hydrolysis	
Depurination (Abasic site)	18,000
Depyrimidination (Abasic site)	600
Cytosine deamination	100
5-Methylcytosine deamination	10
Oxidation	
8-oxoG	~1,000–2,000
Ring-saturated pyrimidines (thymine glycol, cytosine hydrates)	~2,000
Lipid 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.

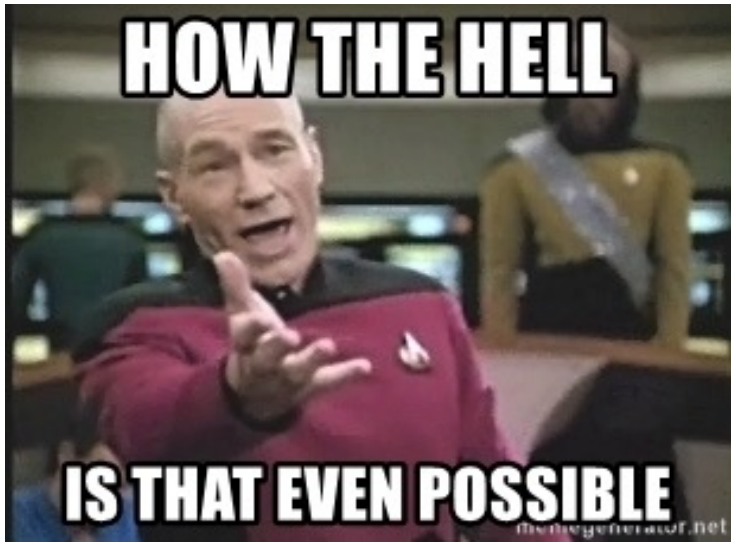
[c]ND, none detected

Repair of eukaryotic genome is challenging

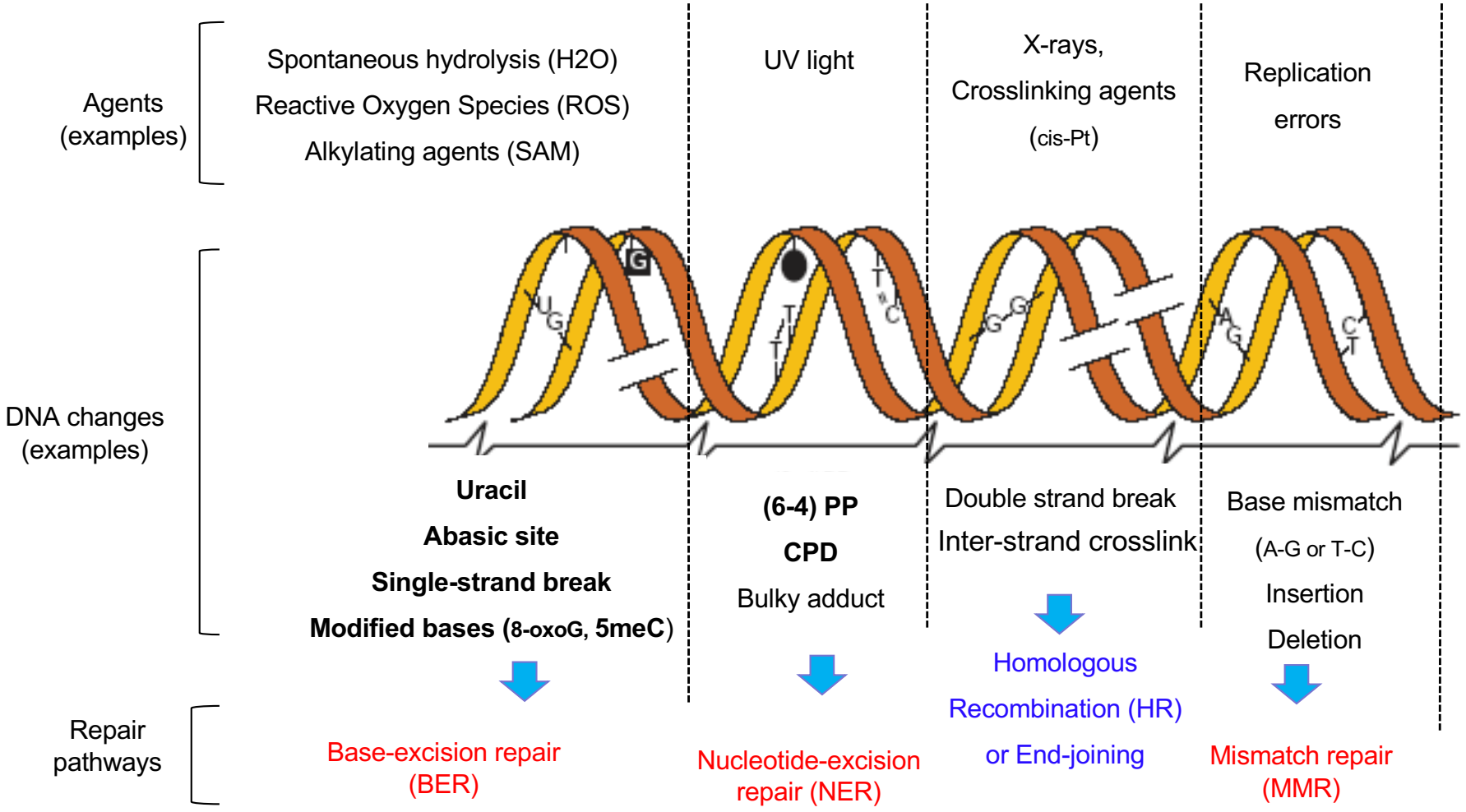


Electron micrograph of DNA from a small part of a chromosome

How can cell fix the huge numbers of DNA lesions & minimize disease-causing mutations ?!



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

- **Nucleases, helicase, other enzymes** to remove different types of lesions
- lesion recognizing proteins + scaffold proteins: recruit lesion removal enzymes

Stage III

Synthesizing new DNA

- DNA polymerases to be deployed for repair tasks
- Some polymerases are less precise and require tight regulations.
- DNA ligase and more clean-up enzymes.

Important Considerations:

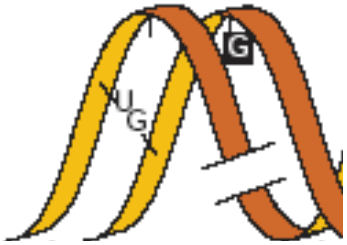
- Processivity of the multi-step repair processes.
- Cope with chromatin contexts and restore epigenetic states afterwards.
- DNA repair efficiency in response to genotoxins.
- Coordinate with other processes, such as transcription.

Base Excision Repair (BER)

Spontaneous hydrolysis (H2O)

Reactive Oxygen Species (ROS)

Alkylating agents (MMS, EMS, SAM)



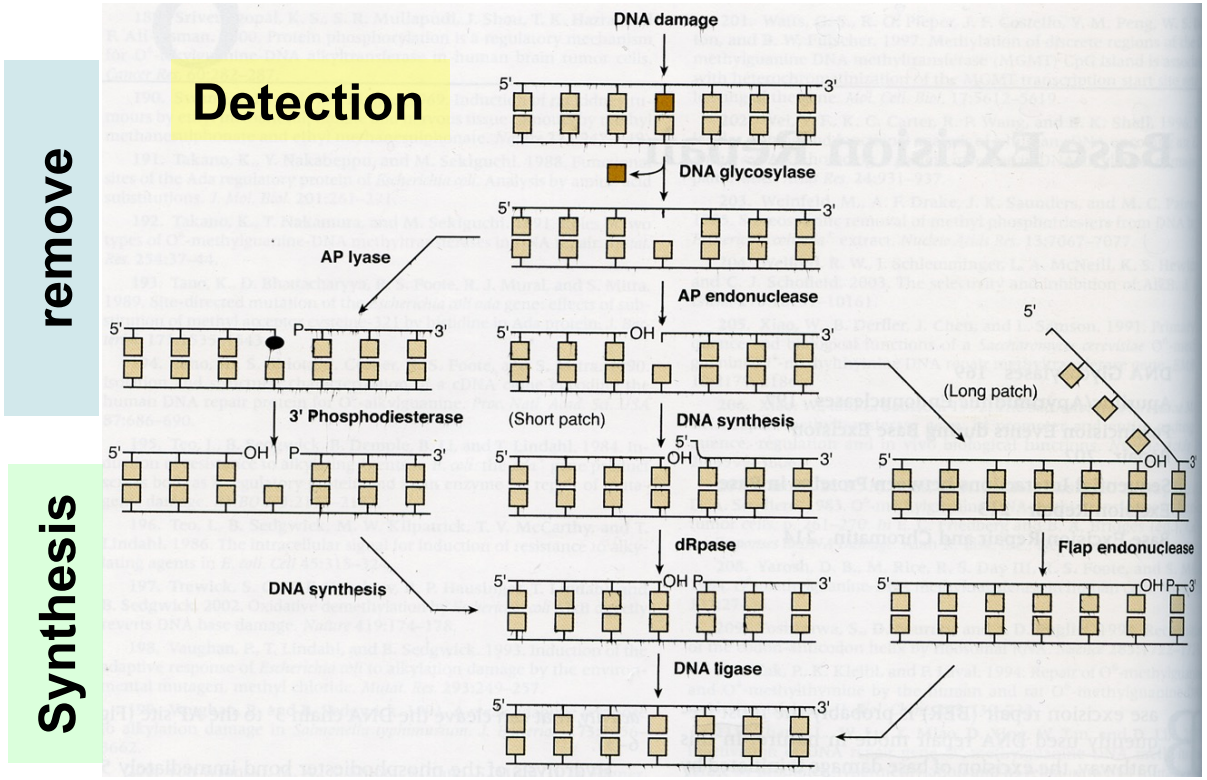
Uracil

Abasic site

Single-strand break

Modified bases (8-oxoG, 5meC)

Base-excision repair (BER)

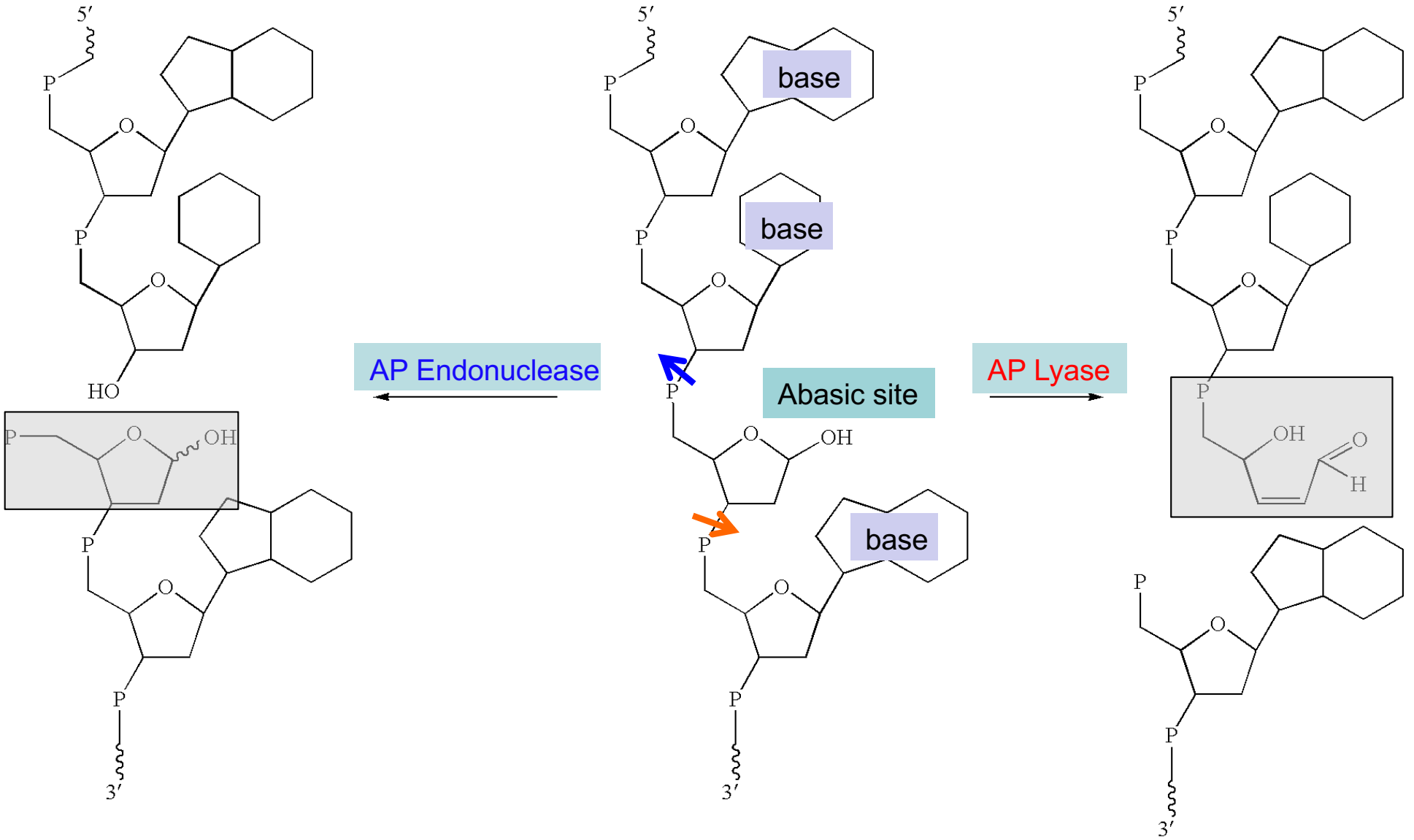


Damage Recognized:

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

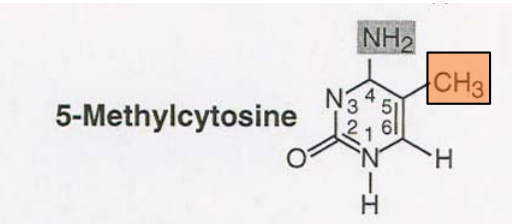


Deoxyribo-phosphodiesterase (dRpase)

3’ Phosphodiesterase

BER in development

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



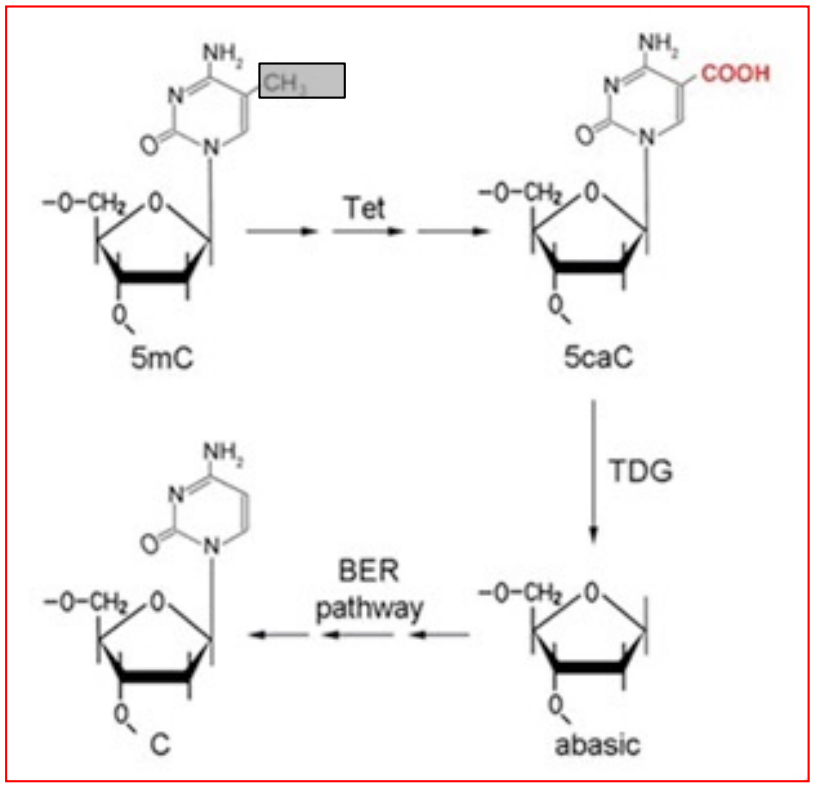
- In mammalian genomes, 5mC exists mostly in CpG context (~80% CpGs methylated) by DNMTs.
- 5mC - transcription repression, used widely for control lineage-specific genes, X-chr & mobile elements inactivation.

Covered a bit by Dirk

Demethylation is observed:

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

2.At specific loci in response to stimuli.

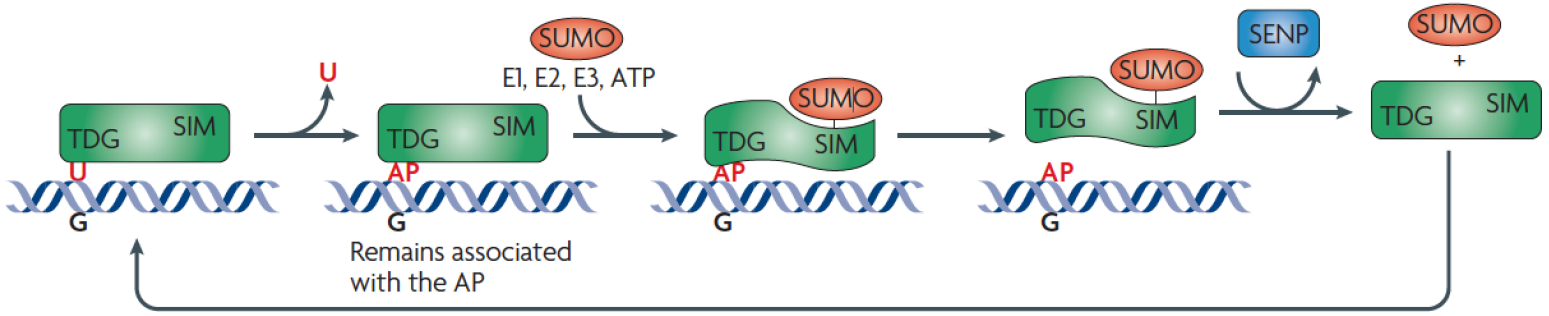


How are BER enzymes coordinated?

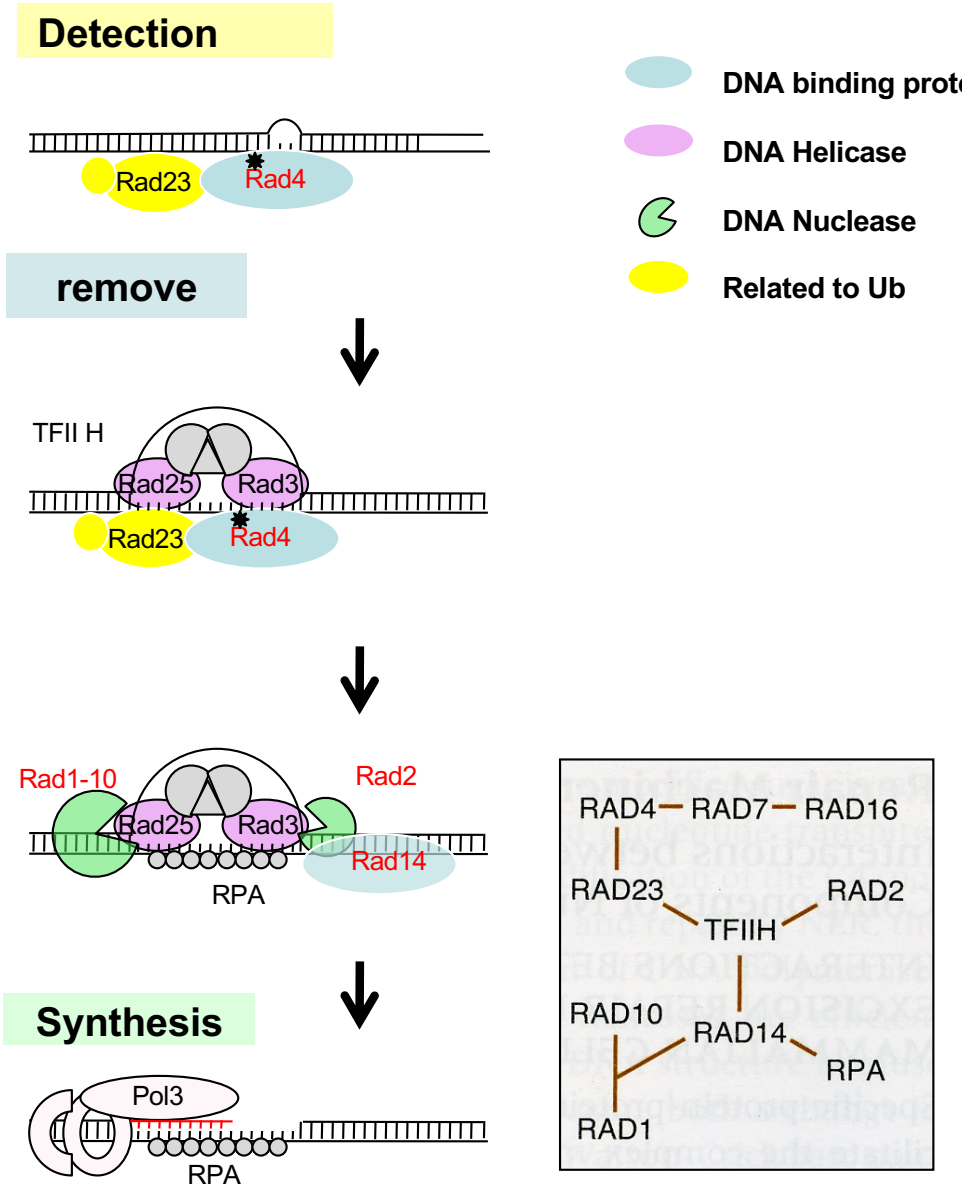
Hands-off Model: proceed by the sequential action of factors facilitated by pair-wise interactions

- 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

Diseases associate with NER

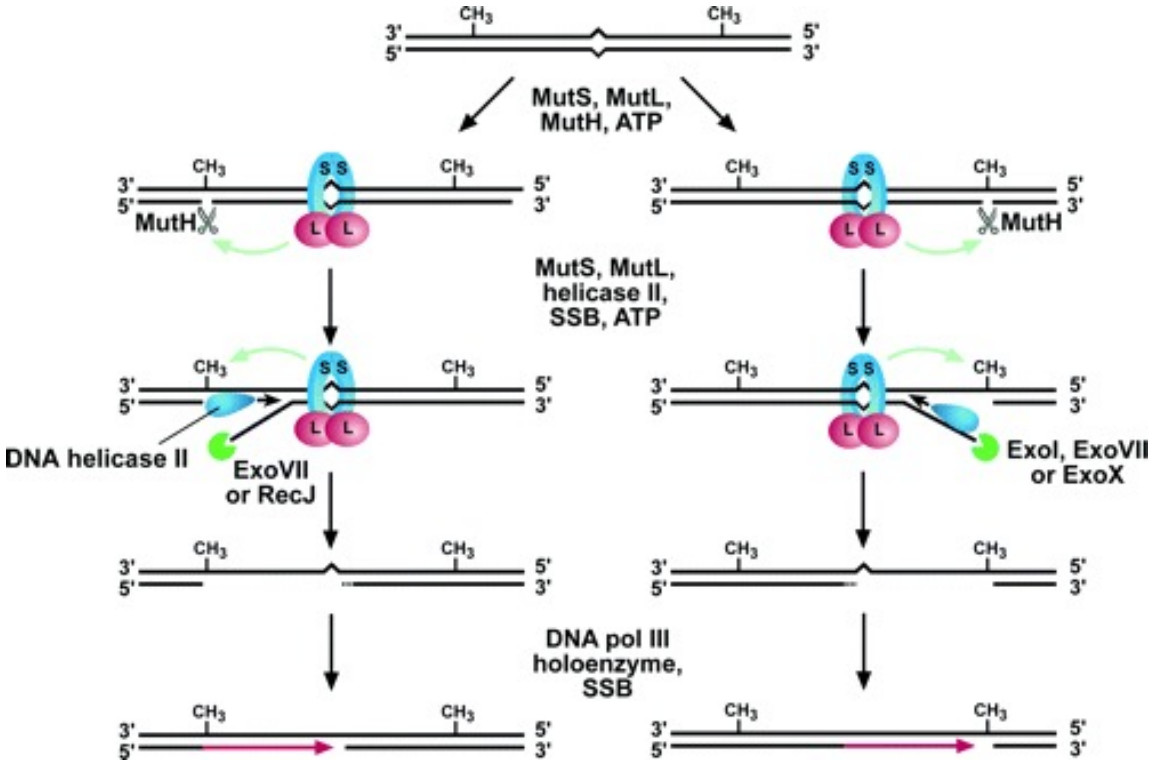
- XP (Xeroderma Pigmentosum): UV^s , multiple skin disorders, skin ***cancer***, neurological abnormalities. XPC (Rad4) – XPD (Rad3)- XPE (DDB2)-XPF (Rad1) –XPG (Rad2), XPA **(Rad14)-XPB (Rad25).**

- 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; single strand break 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 POL.
- wrong bases are removed by generating a ssDNA gap.
- Most proteins also affect other cellular processes (HR).
- deficient in human disorders (Lynch syndrome & neurodegeneration disorders).

Many puzzles regarding DNA repair

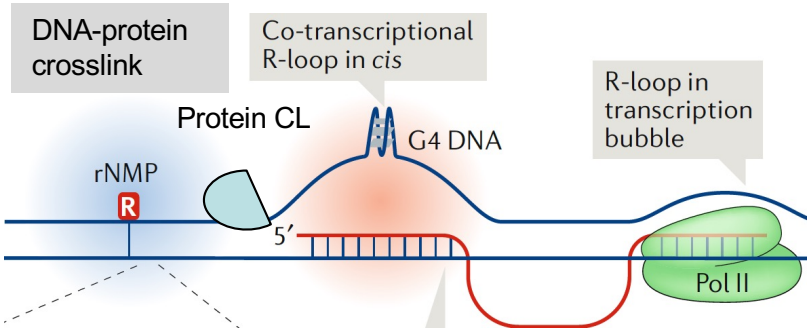
1. **Mechanisms**. How each step & handover occur accurately; majorities of repair proteins are modified by SUMO, Ub, Phos - how modifications aid repair? New repair proteins are continuing to be discovered.

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