Molecular Disease Mechanisms

Lecture 2: Genotoxic Carcinogenesis

Dr. Hailey Gahlon
ETH Laboratory of Toxicology
hailey.gahlon@hest.ethz.ch

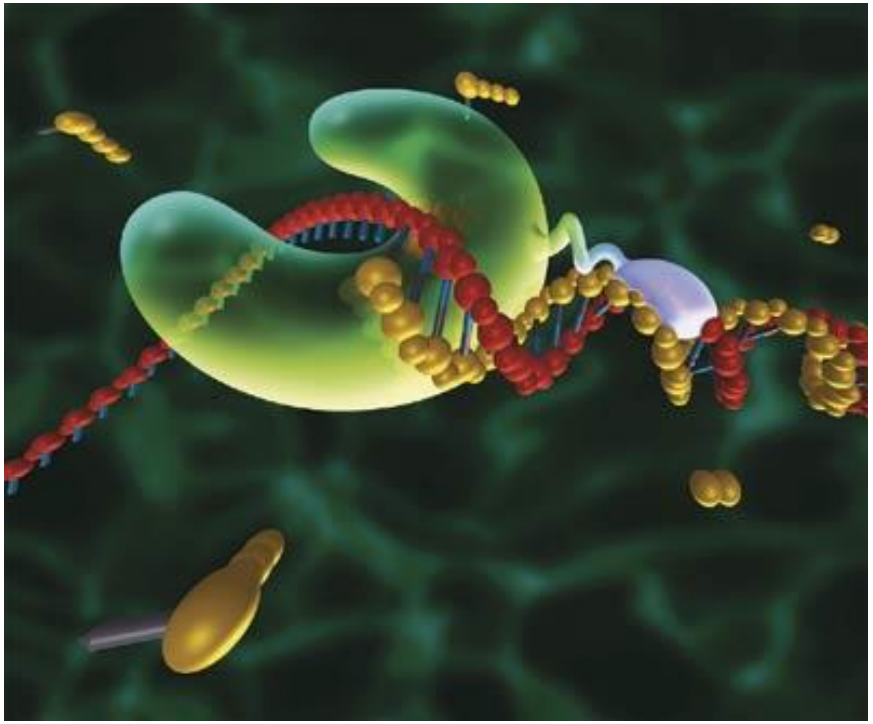
Lecture 2, Part 3

MUTAGENESIS (TRANSLESION DNA SYNTHESIS)

DNA Polymerases

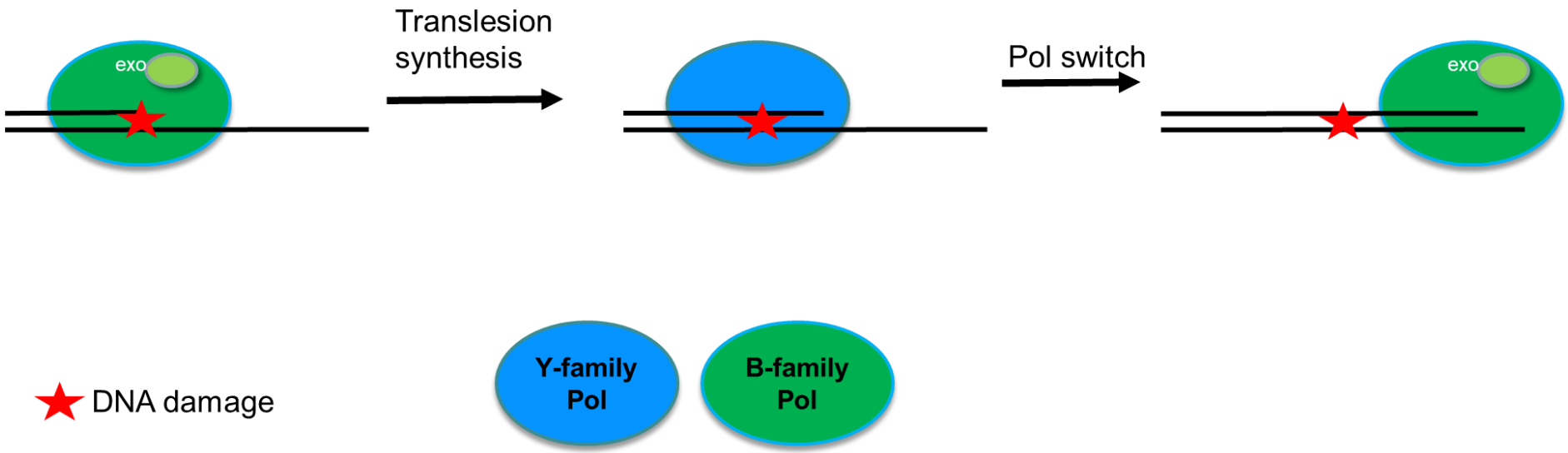
- Role of replicative Pols
- TLS Pols characteristics
  - rates and fidelity
  - active site properties
  - proofreading
  - processivity

DNA replication movie



TLS: Translesion DNA Synthesis

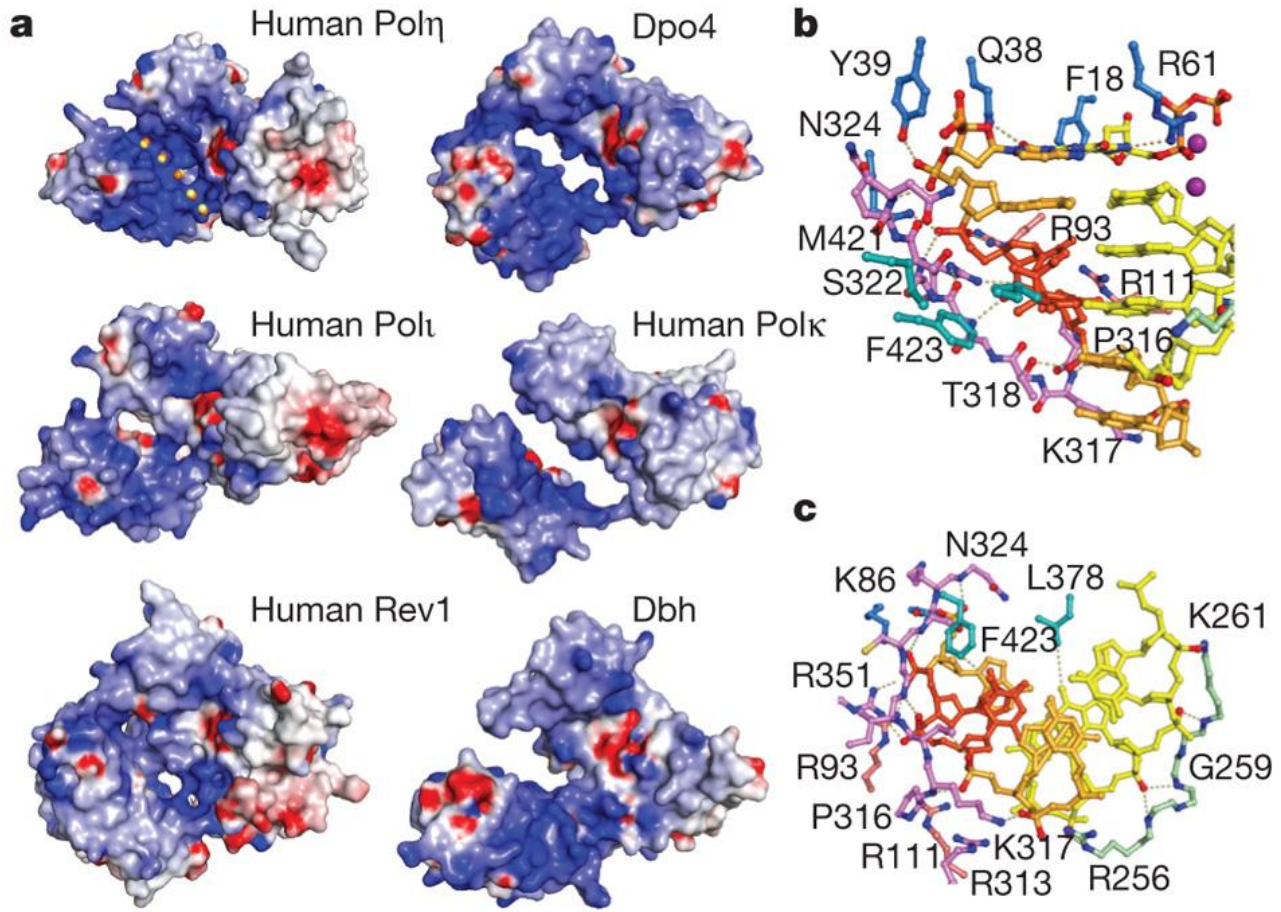
Y-family polymerases catalyze translesion DNA synthesis



DNA binding of Y-family DNA polymerases

Red (-)

Blue (+)



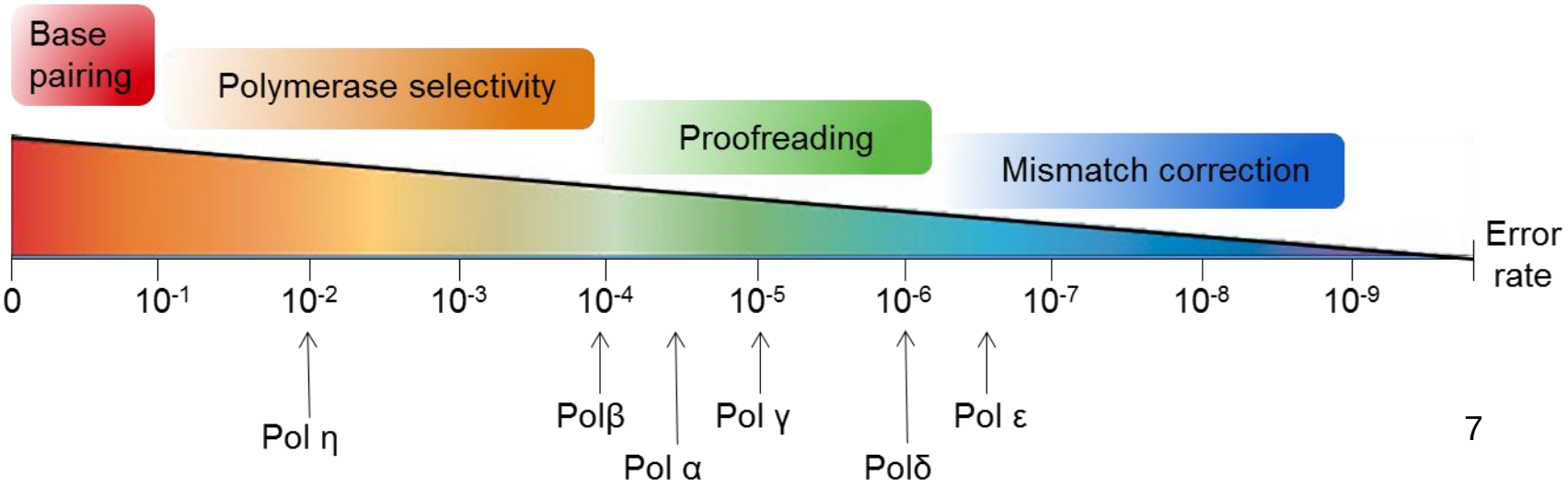
C Biertümpfel *et al. Nature* **465**, 1044-1048 (2010) doi:10.1038/nature09196

The Current Roster of DNA Polymerases

Greek Name	Human Name	Yeast Name	Proposed Function
α	POLA	*POL1*	Replication
β	POLB	—	BER; ss break repair
γ	POLG	*MIP1*	Mitochondrial replication; Mt BER
δ	POLD1	*POL3*	Replication
ϵ	POLE	*POL2*	Replication
ζ	POLZ	*REV3*	Bypass synthesis
η	POLH	*RAD30*	Bypass synthesis
θ	POLQ	—	Bypass synthesis
ι	POLI	—	Bypass synthesis (?)
κ	POLK	—	Bypass synthesis
λ	POLL	*POL4*	NHEJ
μ	POLM	—	NHEJ (?)
ν	POLN	—	Bypass synthesis
—	REV1	*REV1*	Bypass synthesis

Comparing Polymerases

Characteristic	B Family Polymerases (δ, ε)	Y Family Polymerases (η, ι, κ)
Error Rate	Low (< 10^{-10}/bp)	High (10^{-2} to 10^{-4})
Fidelity	High	Low
Proofreading	3’ exonuclease	None
Processivity	High	Low

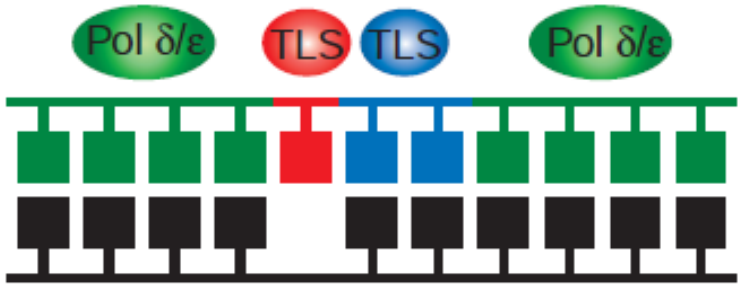


Polymerase Switching Models

- Basic DNA Pol Switch Model

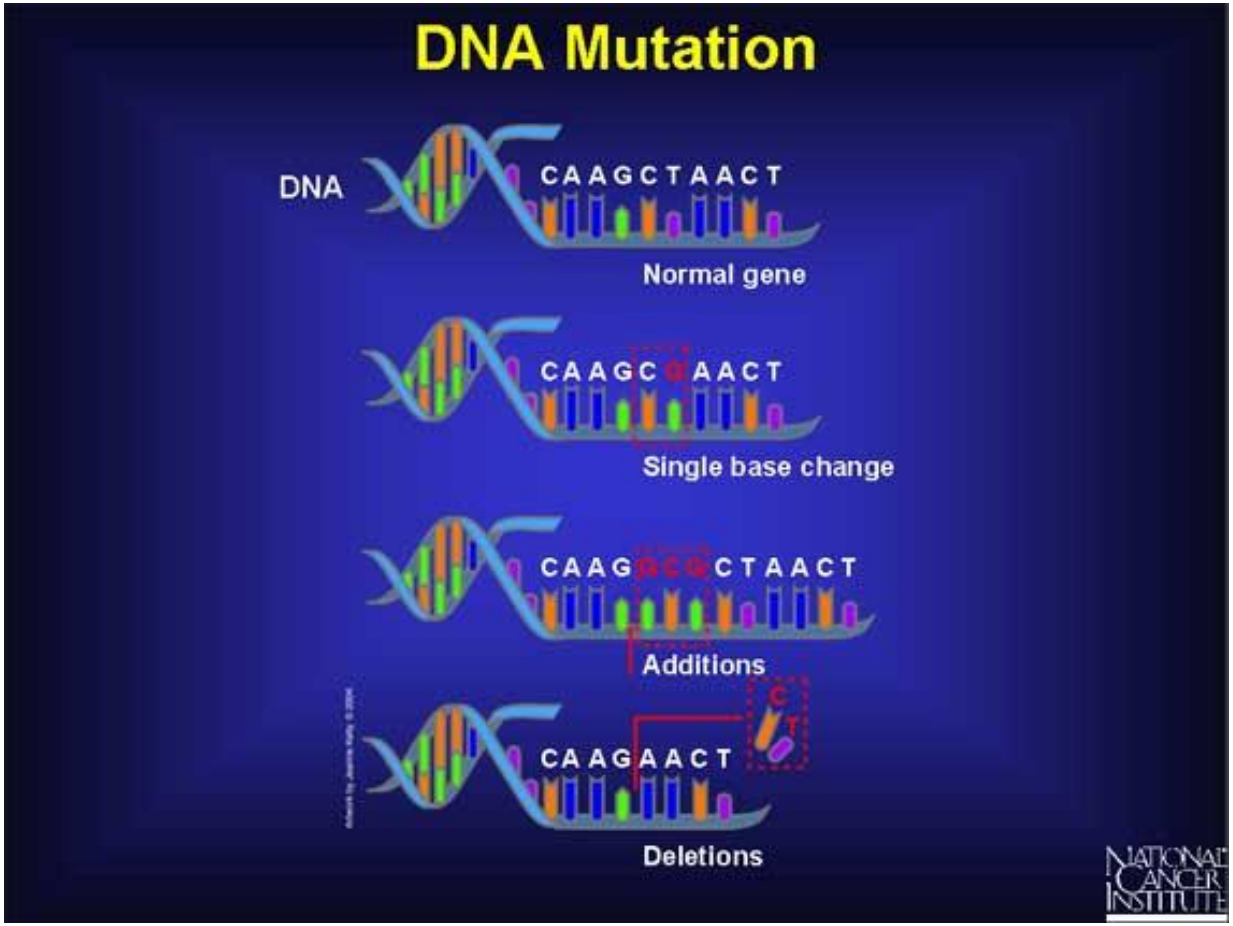
- 2 relevant mechanisms for TLS, depending on the lesion structure and active enzymes

One TLS polymerase (red) responsible for insertion and extension.



Two TLS polymerases (red and blue) responsible for insertion and extension.

Types of mutations induced by genotoxins



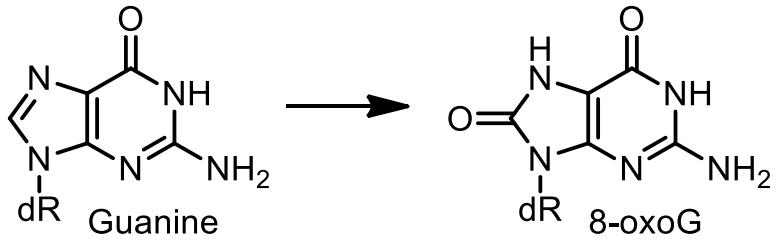
- Point/Single Base Change
  - transversion
  - transition
- Frame shift-causing
  - Deletion
  - Insertion/Addition

Outcomes of mutations

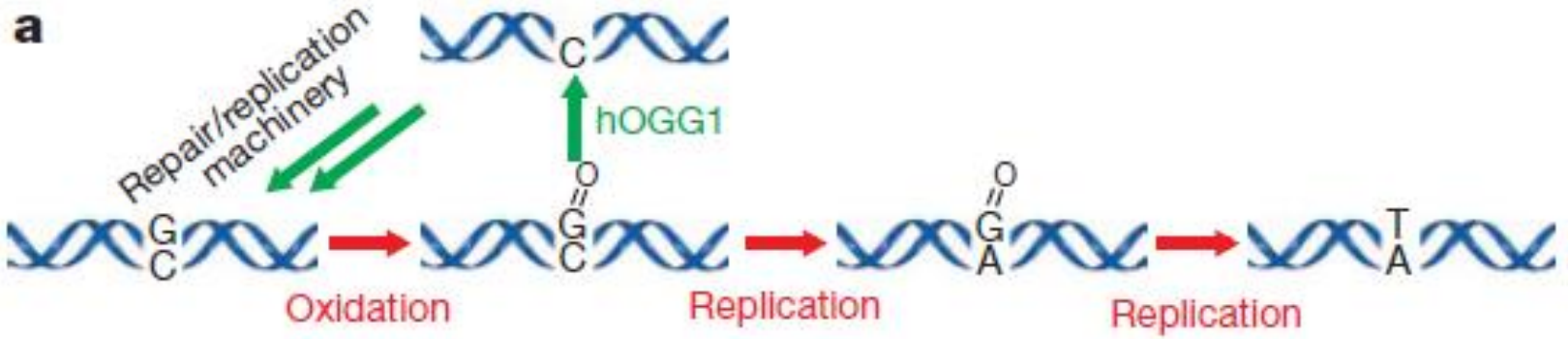
- Translation into mutant proteins
- Lethality
- Mutant can be recognized and repaired
- Cells expressing mutant proteins can be recognized and eliminated
- Mutant proteins can impart cancer phenotype

Mutagenesis Example: Oxidation-Induced Mutagenesis

- Polymerase-mediated DNA replication of 8-oxoG often incorporates an A
- This mismatch (8oxoG:A) is not proofread efficiently
- The poor proofreading can be attributed to the geometry of 8-oxo-G:A, which is similar to that of the correct base pair

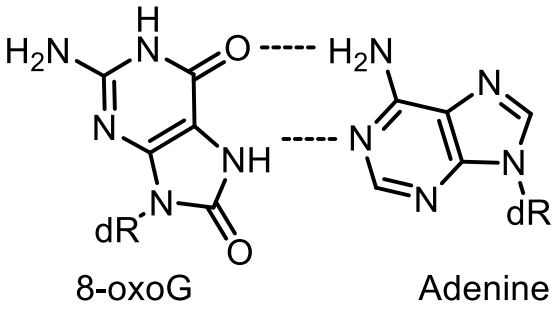


Replication of the Lesion 8oxoG



8oxoG causes G -> T transversions

8oxoG-A base pair is geometrically Similar to the natural G-C base pair



Bruner, S. *et al.* Nature. 2000. 403, 859-866

Determinants of Replication Fidelity and Mutation Rates

- Biochemical Factors related to the polymerase enzyme
  - Expression levels
  - Regulation
  - Kinetic behavior/accuracy
- Chemical Factors
  - Structure/size of the modification

    (this also dictates which polymerase is active)
  - Watson-Crick H-bonding
  - Base Stacking Interactions with neighboring bases as well as aromatic residues on the enzyme
  - A-Rule (DNA polymerases insert Adenine opposite abasic sites)
- Elimination of damage by Repair!

menti.com – TLS question

Lecture 2, Part 4

DNA REPAIR

Why is DNA repair important and why do we want to understand it?

- Mitigate damaging effects of endogenous DNA damage that is formed at high rates
  - oxidative lesions
  - Abasic sites
- Protect against carcinogenesis from xenobiotic agents
- Is a significant source of drug resistance in cancer chemotherapy

2015 Nobel Prize in Chemistry

Tomas Lindahl (Swedish), Francis Crick Institute, UK
Paul Modrich (American), Duke University, USA
Aziz Sancar (Turkish), Univ North Carolina, Chapel Hill, USA

“mechanistic studies of DNA repair"

http://www.nobelprize.org/nobel_prizes/chemistry/

NOBEL PRIZE IN CHEMISTRY 2015

The Nobel Prize in Chemistry 2015 was awarded to **Tomas Lindahl**, **Paul Modrich**, and **Aziz Sancar** for having mapped how cells repair damaged DNA.

DNA DAMAGE

BASES: A PAIRS WITH T C PAIRS WITH G

DNA damage occurs regularly, due to UV radiation, carcinogenic substances, & copying errors. The prize is for the discovery of the mechanisms that repair this damage.

BASE EXCISION REPAIR

1. C loses amino group to form U. U can't pair with G.
2. Enzymes remove U and its section of the DNA strand.
3. The correct base is inserted and the strand is sealed.

DNA is an unstable molecule. Lindahl showed that base excision repair prevents its decay. Without this mechanism, development of life would have been impossible.

NUCLEOTIDE EXCISION REPAIR

1. UV radiation can cause two Ts to bind to each other.
2. Enzyme cuts a 12 nucleotide strand, removing damage.
3. The resulting gap in the DNA is filled and then sealed.

Sancar explained how DNA is repaired after damage from UV and mutagenic substances. People with defects in this repair system are at higher risk of developing cancer.

MISMATCH REPAIR

1. Sometimes the nucleotides in copied DNA don't match.
2. Enzymes remove a section containing the faulty nucleotide.
3. The resulting gap in the DNA is filled and then sealed.

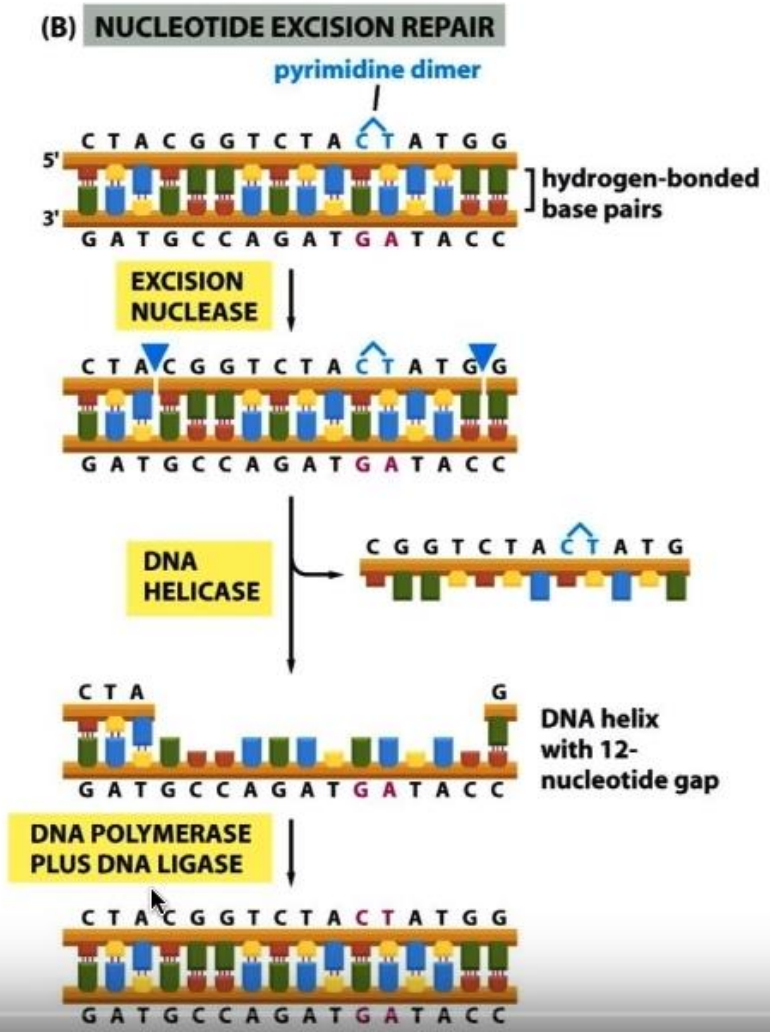
Modrich showed how errors produced when cells divide and DNA is replicated are repaired. This reduces the error rate of DNA replication by a factor of 1000.

DNA Repair Pathways

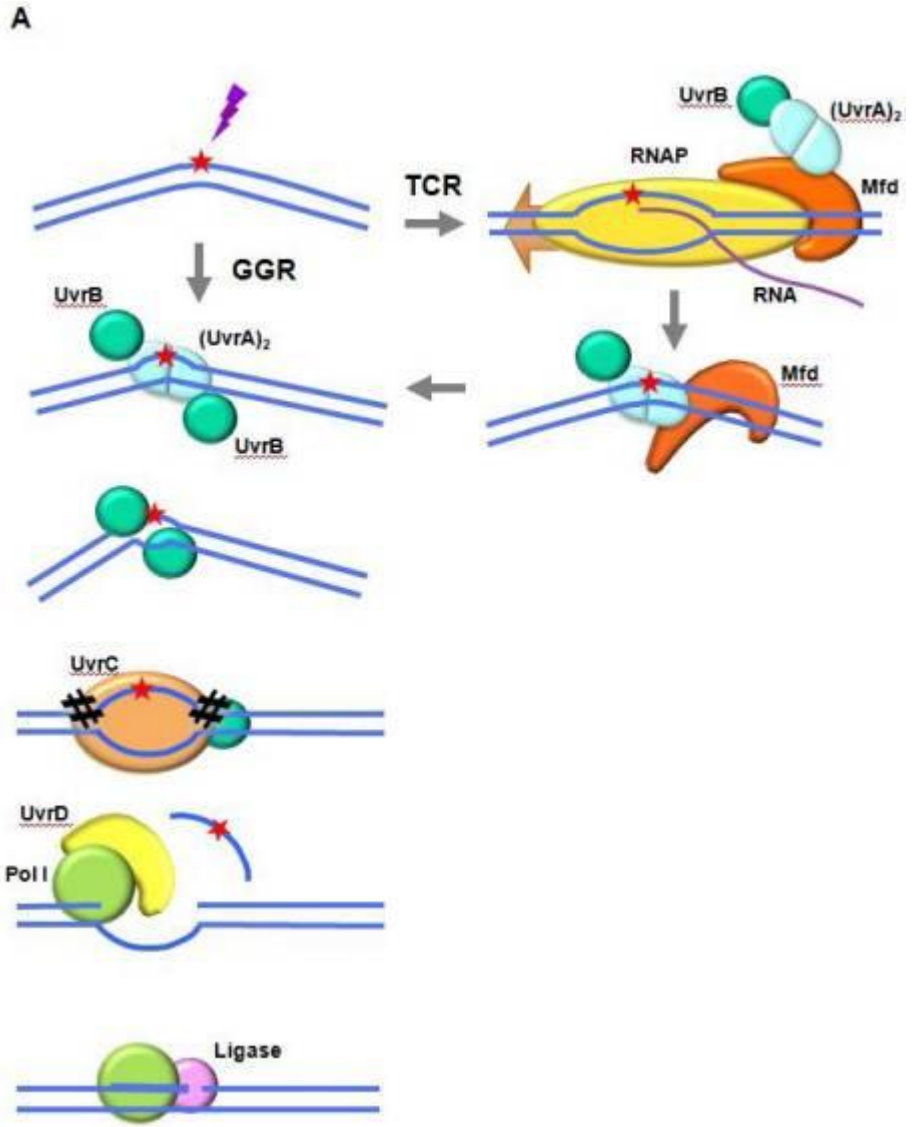
1. Nucleotide excision repair
2. Base excision repair
3. Mismatch repair
4. Homologous recombination
5. Non-homologous end-joining
6. Dealkylation (Direct reversion)

Nucleotide excision repair pathway

1. damage recognition
2. cut into strand at both ends of lesion
3. removal of oligonucleotide created by cuts
4. synthesis of new DNA to replace DNA
5. Ligation of remaining nick

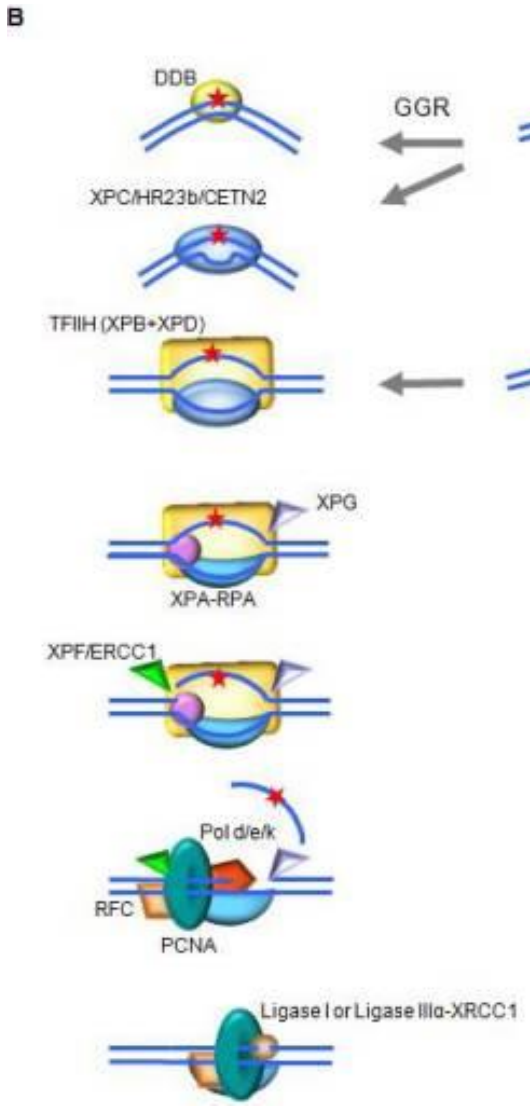


NER in Prokaryotes



Nucleotide excision repair in humans. DNA Repair. 2015

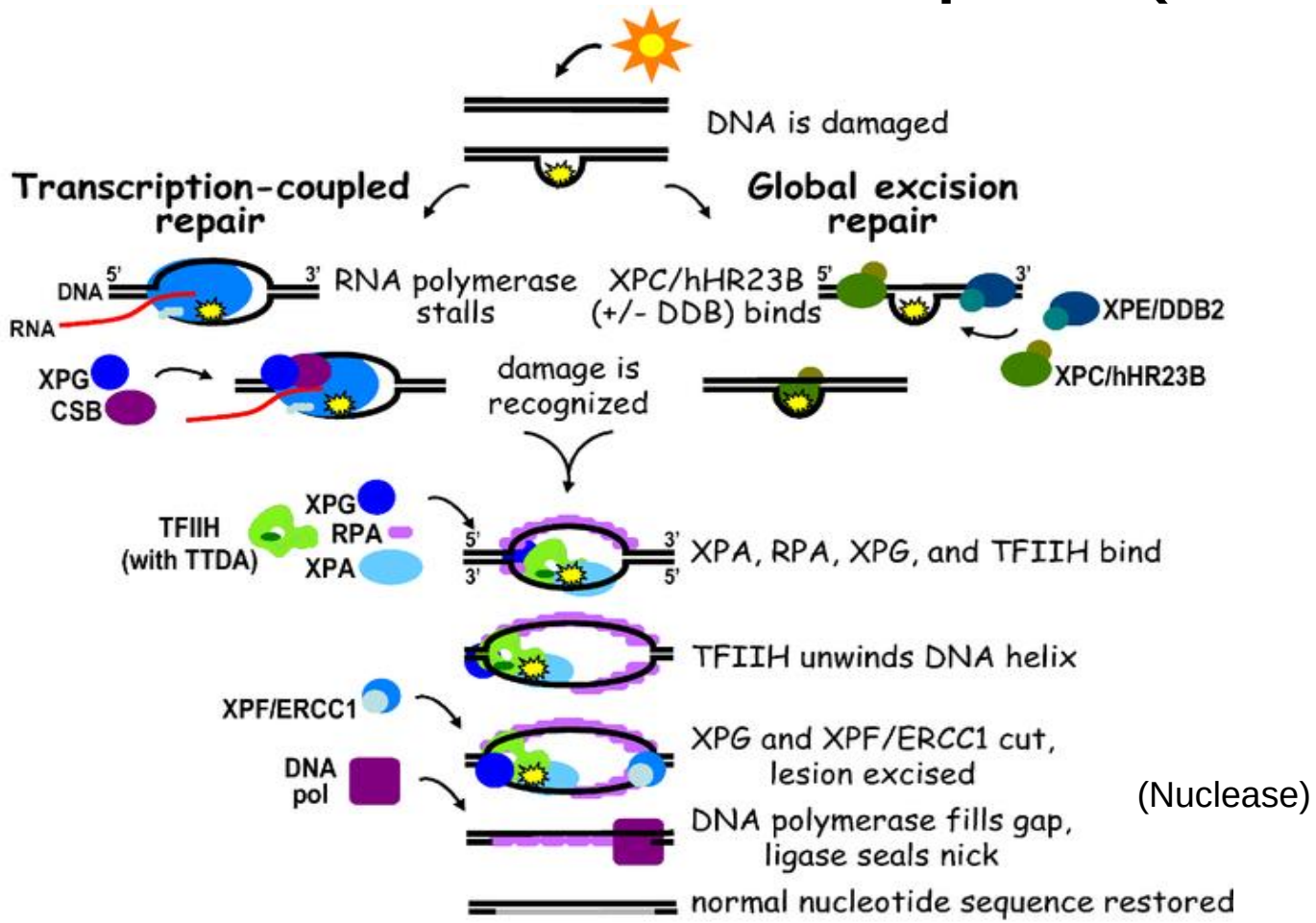
NER in Eukaryotes



NER movie

Nucleotide excision repair in humans. DNA Repair. 2015

1. Nucleotide excision repair (NER)

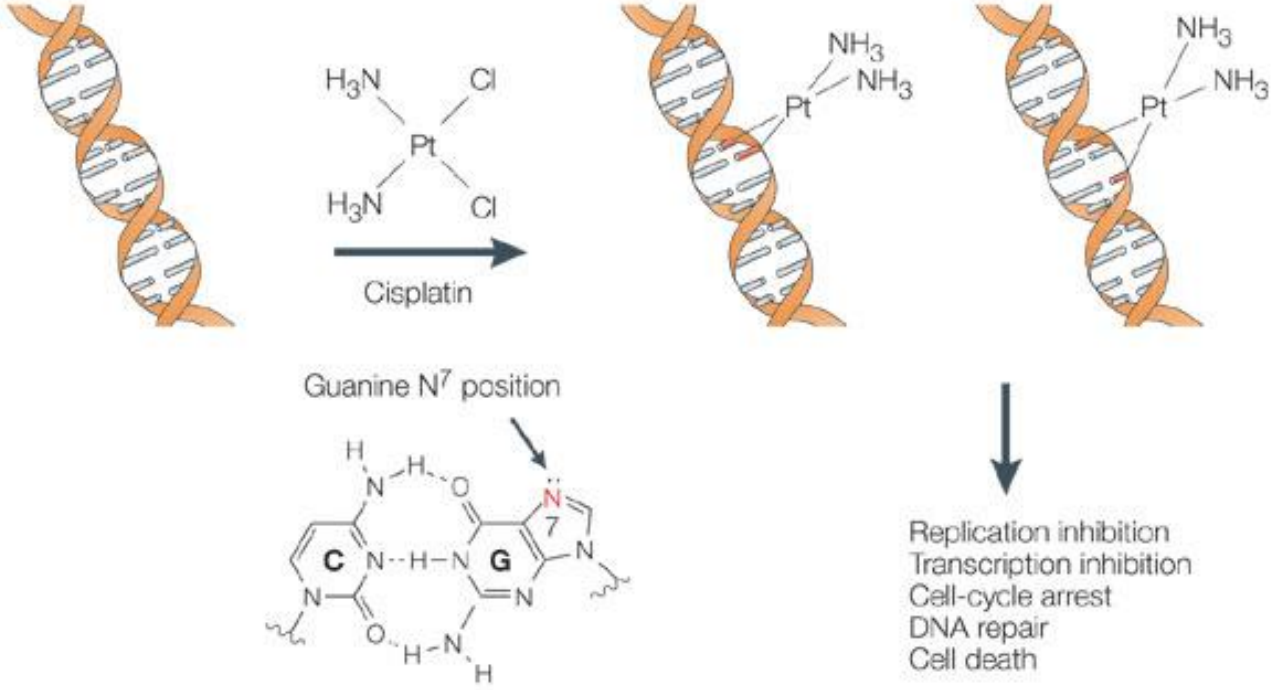


(Nuclease)

1. Recognition; 2. Assembly of multi-protein complex for remodeling and cutting; 3. removal of short single-stranded DNA segment containing lesion; 4. DNA synthesis (polymerase); 5. ligation (ligase)

NER substrates

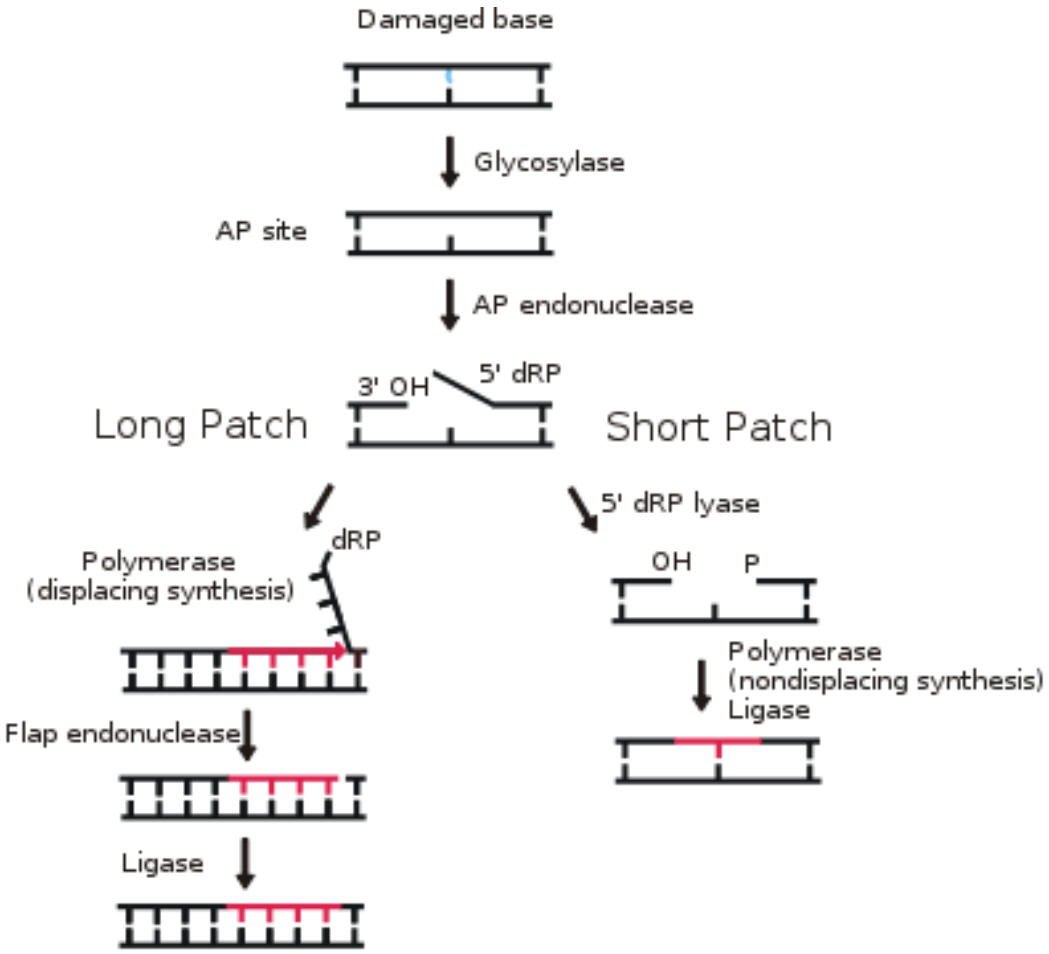
- bulky, helix-distorting DNA damage
  - thymine dimers and 6,4-photoproducts (UV exposure)
  - Chemotherapy-induced DNA crosslinks (Pt drugs as shown below)



http://www.nature.com/nrd/journal/v4/n4/fig_tab/nrd1691_F3.html

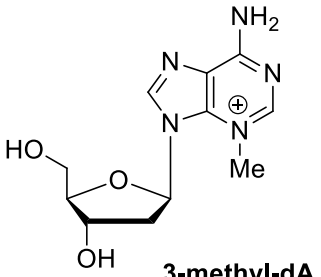
2. Base excision repair (BER)

- small, non-helix-distorting base adducts
- Initiation: DNA <u>glycosylases</u>
  - recognize damage
  - remove altered bases
  - form AP sites
- AP sites cleaved by AP <u>endonuclease</u>
- New DNA synthesized by polymerase
  - In short-patch single nucleotide is replaced
  - In long-pach 2-10 nucleotides are synthesized and flap endonuclease trims displaced strand
- Strand sealed by <u>ligase</u>

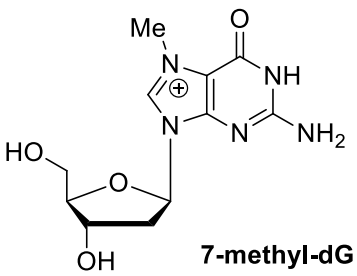


BER substrates

- Alkylated bases:
  - 3-methyladenine
  - 7-methylguanine



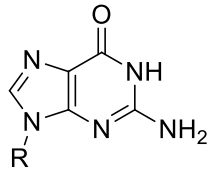
3-methyl-dA



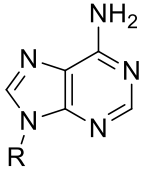
7-methyl-dG

- Oxidized bases:
  - 8-oxoguanine

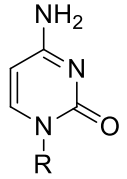
- Deaminated bases:
  - xanthine
    - deamination of G
  - hypoxanthine
    - deamination of A
  - uracil
    - deamination of C



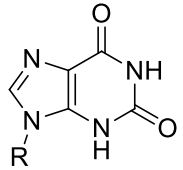
guanine



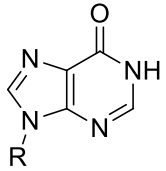
adenine



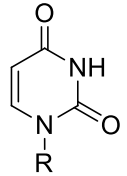
cytosine



xanthine



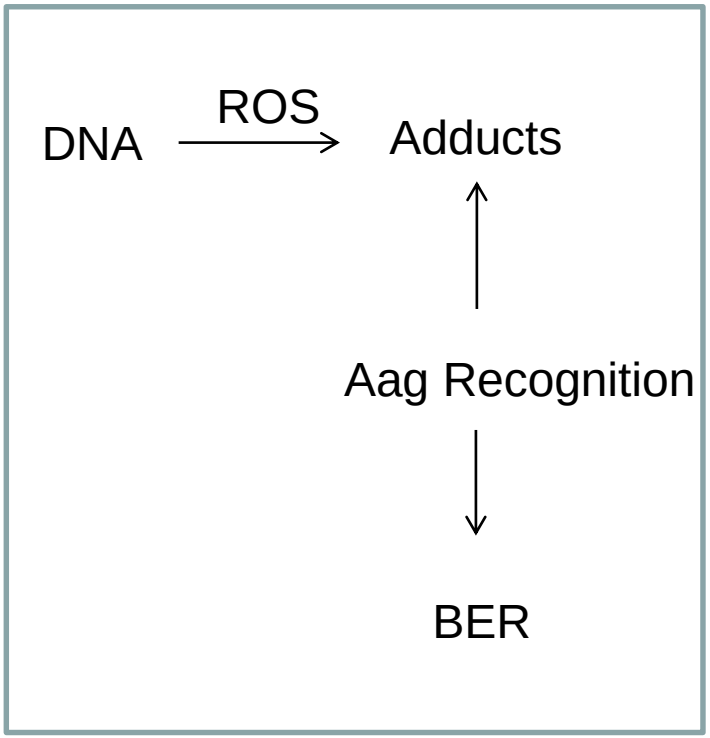
hypoxanthine



uracil

DNA damage induced by chronic inflammation contributes to colon carcinogenesis

- chronic inflammation increases cancer risk
- ROS induce DNA damage recognized by alkyladenine DNA glycosylase (Aag)
- Aag recognition of adduct initiates base excision repair



J Clin Invest. 2008 July 1; 118(7): 2516–2525. doi: 10.1172/JCI35073

Importance of BER in protection against colon carcinogenesis

- Impacts of Aag-mediated DNA repair (+Aag mice)
  - prevents colonic epithelial damage
  - reduces the severity of chemical–induced colon tumorigenesis
- Accumulation of DNA adducts in Aag-deficient mice following stimulation of colonic inflammation

3. Mismatch repair

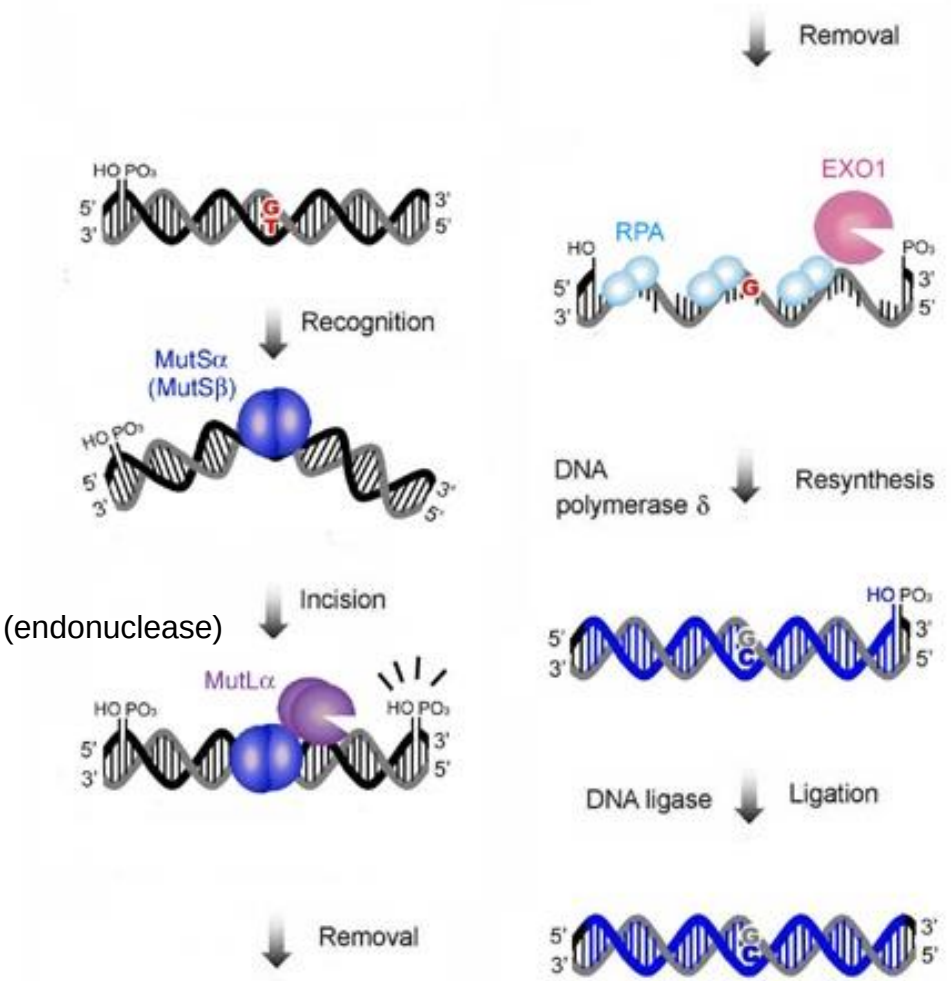
- *DNA mismatch repair* repairs mis-incorporated bases during DNA replication.

- MutS recognizes and binds mismatch
- MutL makes incision, DNA contains nicks not yet sealed by DNA ligase

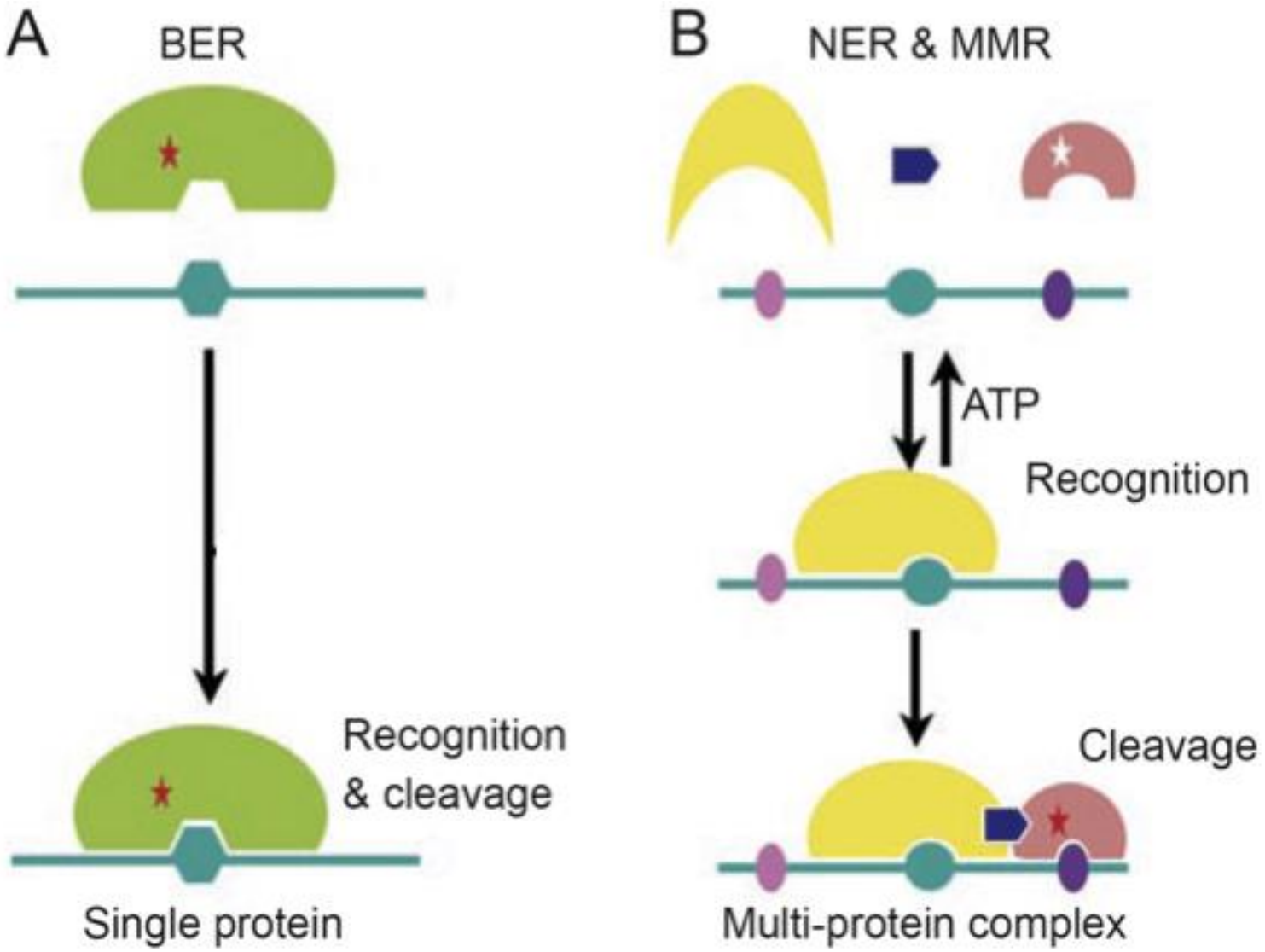
- Patch is removed (RPA, replication protein A; EXO1, exonuclease 1)

- DNA synthesis (polymerase) and ligation (ligase)

- Hereditary nonpolyposis colorectal cancers attributed to mutations in the genes encoding MSH2 and MLH1 respectively
  - MSH2 and MLH1 = tumour suppressor genes.



STRATEGIES USED FOR DAMAGE RECOGNITION



Yang, Cell Res. *18*, 184-197 (2008)

Study Tip: What to know for repair pathways

- Similarities and differences in processes for recognition, removal, replacement synthesis, ligation
- Understand basic process and relevant enzyme types Example:
  - ➢ in BER, small alkyl adduct is recognized and excised by a glycosylase
  - ❑ In BER, 3-methyl adenine is recognized and excised by alkyladenine DNA glycosylase (Aag)
- Characteristics of damage – what pathway is relevant?

DNA Repair Pathways

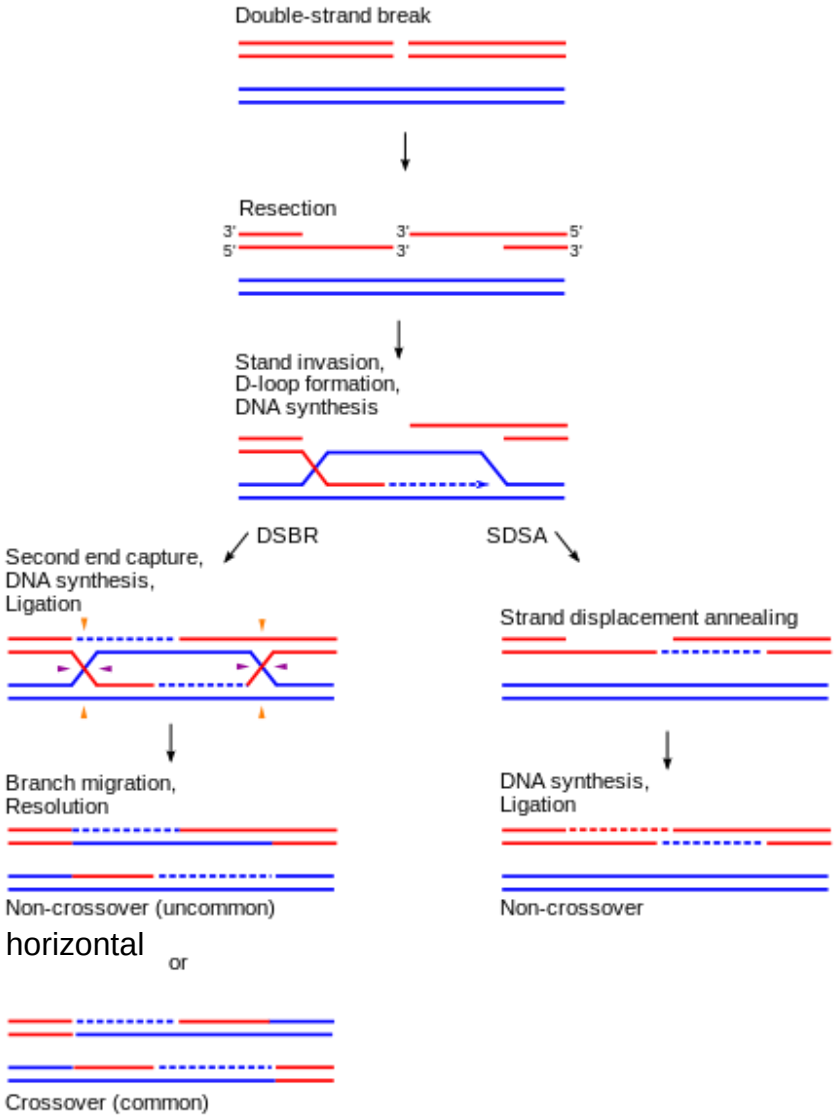
- ✓ Nucleotide excision repair
- ✓ Base excision repair
- ✓ Mismatch repair
4. Homologous recombination
5. Non-homologous end-joining
6. Dealkylation (Direct reversion)

4. Homologous Recombination

- After double-strand break
- sections of DNA around the 5' ends of the break are cut away in a process called *resection*.
- In the *strand invasion* step that follows, an overhanging 3' end of the broken DNA molecule then "invades" a similar or identical DNA molecule that is not broken (sister chromatid in mitosis).

- After strand invasion:
  - DSBR (double-strand break repair) or
  - SDSA (synthesis-dependent strand annealing)

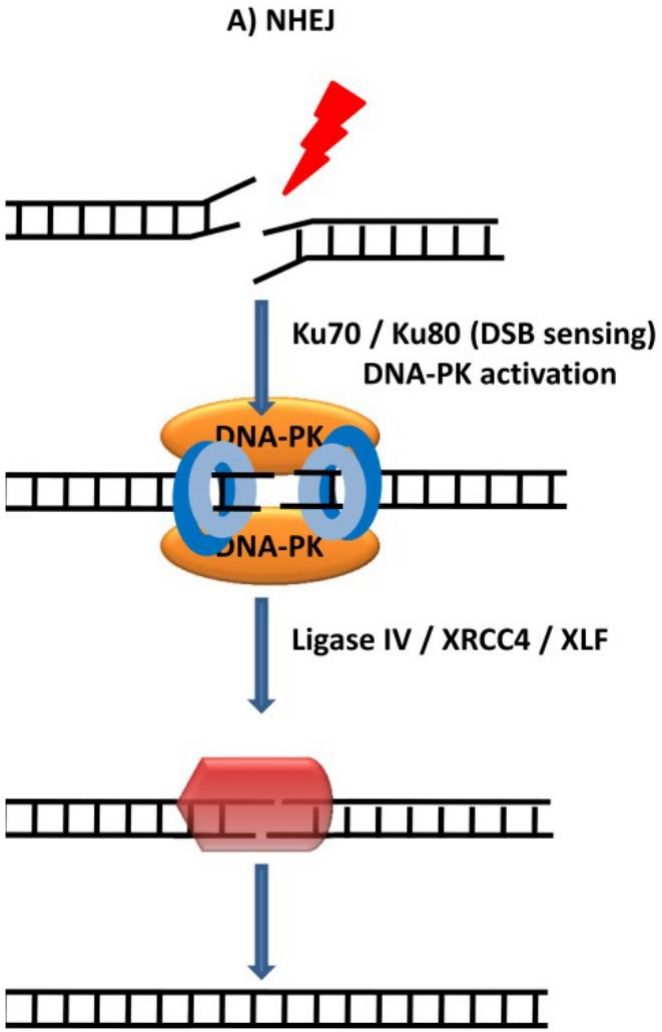
VIDEO ON HR REPAIR



http://en.wikipedia.org/wiki/File:HR_schematic_diagram.svg

5. Non-homologous end-joining (NHEJ)

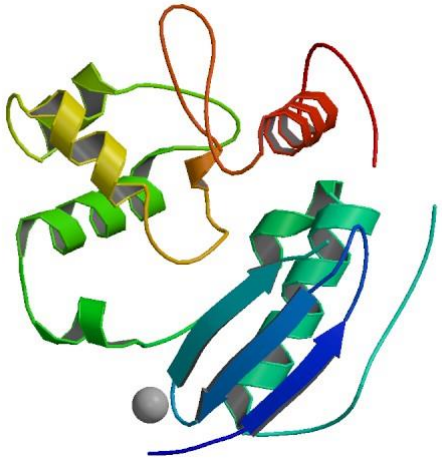
- Recognition: Ku70/Ku80 encircles duplex DNA at DSB
- Ku70/Ku80 stabilizes/structurally aligns the two DNA ends and recruits DNA-Protein Kinase (PK).
- DNA-PK phosphorylates and activates the NHEJ effector complex (ligase IV/XRC44/XLF) that ligates broken DNA.



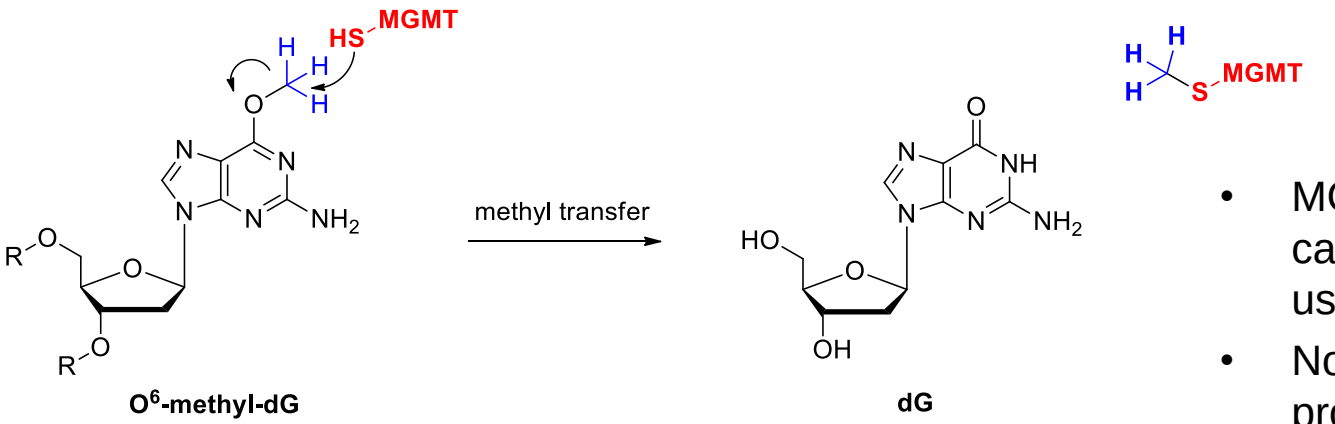
-NHEJ more common than HR
(NHEJ fast 30 min, HR slow 7 hrs)
-But more mutagenic

http://www.intechopen.com/books/protein-phosphorylation-in-human-health/signalling-dna-damage

6. Direct reversion/Dealkylation repair



O-6-methylguanine methyltransferase (MGMT)



O^6-methyl-dG

dG

- MGMT is consumed; can no longer be used.
- Non-enzymatic process

MGMT and cancer

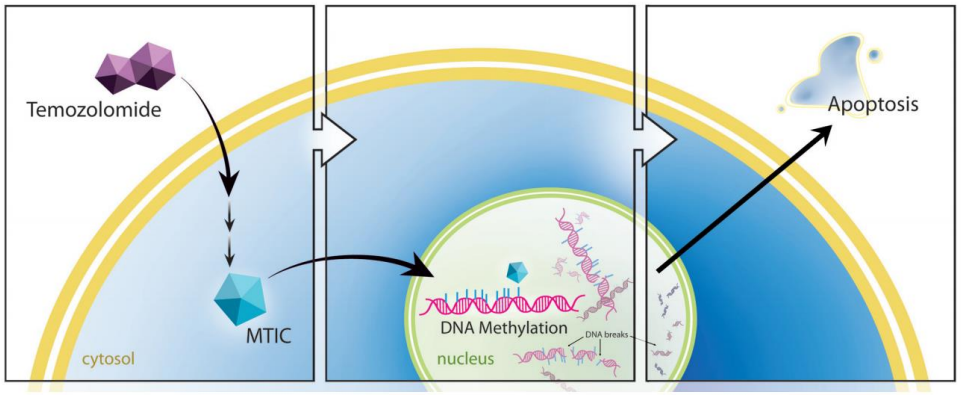


Fig 1. Schematic illustration of the proposed mechanism of temozolomide. Temozolomide is converted intracellularly into MTIC, which methylates DNA. Cellular repair mechanisms cannot adjust, resulting in DNA nicks and ultimately apoptosis.

-Temzolomide is an alkylating cancer drug

-MGMT inhibitors sensitize cancer cells to temzolomide therapy (targeting DNA repair proteins in cancer therapy)

-438 known polymorphisms of MGMT:

-*Gly160Arg.* Here, Gly160 residue lies nearby the Cys145 active site (rare variant found in less than 1% of Caucasians, but it is expressed in approximately 15% of Japanese).

-This polymorphism has generated interest due to its strong resistance to the MGMT inhibitor O^6-BG. Patients with this MGMT polymorphsims are not good candidates for O^6-BG therapy combined with alkylating agent treatment.

Anticancer Research October 2009 vol. 29no. 10 3759-3768

DNA Repair Overview