DNA Repair
Biochemistry 302

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Summary of types of DNA damage

- Depurination
- Deamination
- Alkylation
- UV photoproducts
- Oxidation (maybe most important)
  - ROS, reactive oxygen species (H$_2$O$_2$, hydroxyl radicals, and superoxide radicals)
  - ROS generated during irradiation or as byproducts of aerobic metabolism
  - Defense systems (e.g. catalase and superoxide dismutase)
  - Oxidants escaping cellular defense can promote...
    - Deoxyribose oxidation
    - Base oxidation
    - Strand breaks
## Summary of DNA Repair Systems

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Environmental DNA damage: Reactive chemical agents

- Direct action vs damage cause by metabolic by-products
- Precursors of nitrous acid (HNO₂) e.g. NaNO₂, NaNO₃, and nitrosamine
  - Accelerate deamination
  - Food preservatives
- Alkylating agents
  - Replace H atom
  - Methylation of purines results in altered base-pairing (O₆-methylguanine cannot pair with cytosine)
  - Used experimentally as DNA modifying agents (mutagens)
DNA repair systems I: Direct change of a modified base

- **$O^6$-Alkylguanine alkyltransferase (methyltransferase)**
  - Catalyzes transfer of alkyl to Cys residue resulting in protein turnover
  - Alkylated form self-regulates its own transcription
  - Energetically costly- an entire protein consume to fix 1 base

- **MutT nucleotide hydrolase**
  - Accumulates in $O_2$-stressed cells (8-oxo-G can bp w/A)
  - Cleaves 8-oxo-dGTP prior to incorporation in DNA

- **Photoreactivation**

*Fig. 25.11*
Environmental DNA damage: Radiation and pyrimidine dimers

- One of the first forms of DNA damage discovered
- Irradiation of bacteria w/ 260 nm light $\rightarrow$ condensation of adjacent ethylene groups
- Human skin cells particularly susceptible
- Ionizing radiation (x-rays and gamma rays)
  - Ring opening and base fragmentation
  - Breaks in DNA backbone
- UV + ionizing radiation exposure accounts $\sim$10% of all DNA damage caused by environmental agents

Lehninger Principles of Biochemistry, 4th ed., Ch 8
DNA repair systems I: Direct repair of thymine dimers by DNA photolyases

- Enzyme (*E. coli* and yeast) uses a “photosynthesis-like” free-radical-dependent mechanism.
- Enzyme binds to lesion in the dark and breaks C5-C5 and C6-C6 bonds in the light.
- Enzyme contains two cofactor chromophores that absorb light at specific $\lambda$s (photoantenna MTHF-polyGlu transmits light energy to FADH$^-$).
- Excited FADH$^+$ transfers an electron to the dimer. Electronic rearrangement restores thymine monomers.
- Enzyme is not found in mammalian cells.

Fig. 25.10
DNA repair systems II: Base Excision Repair (BER)

- Damaged bases repaired
  - Products of deamination (uracil), depurination, alkylation, and oxidation
  - Thymine dimers (phage T4 specific mechanism)
- Four-step mechanism
  - Removal of damaged base via specific DNA-N-glycosylase
  - Nicking of abasic strand by AP endonuclease
  - Gap repair synthesis by DNA polymerase I
  - Nick repair by DNA ligase
- AP endonucleases types
  - Cut 5’ to abasic site
  - Cut 3’ to abasic site
Base Excision Repair: Removal of 8-oxo-guanine by hOGG1 (glycosylase/β-lyase)

- OGG1 recognizes oxoG opposite C.
- Active site Lys of hOGG1 attacks the C1' of deoxyribose resulting in the extrusion of oxoG.
- BER apparatus restores correct G/C base-pairing.

(Example of a modified base-specific DNA glycosylase)
Molecular basis of oxoG recognition
(What’s peculiar about these structures?)

Multiple enzyme systems ensure that 8-oxoguanine is excluded from DNA

Fig. 25.14
Mechanisms to prevent GC→AT or AT→GC conversions depending upon route of entry (BER, base excision repair)

• MutM = *E. coli* analog of hOGG1
• MutT = 8-oxo-dGTP nucleotide hydrolase
• MutY = adenine DNA glycosylase (only) works for adenine opposite oxoG
• Redundancy built into the system.
DNA repair systems III: Nucleotide Excision Repair (NER)

- Typically occurs with bulky lesions that distort the DNA helix
  - pyrimidine dimers not removed by BER
  - Intra-strand G crosslinks (cisplatin-induced)
  - alkylation
- *E. coli* machinery (similar in yeast and mammals)
  - UvrA, B, C excinuclease (catalyzes two specific endonucleolytic cleavages)
  - UvrD helicase II
  - DNA polymerase and DNA ligase

a) Intra-strand crosslink ~ 90%
b) Inter-strand crosslink ~ 5%
c) Monofunctional adducts ~ 2%

Cisplatin = cis-diaminedichloroplatinum (cancer chemotherapeutic agent but highly toxic, only short-term efficacy)

http://www.md.huji.ac.il/courses/bioorganic/cisplatin_3.ppt
Cisplatin-DNA adducts

H-bonding between N7 of G & cisplatin

1,2-d(G*pG*)  1,3-d(G*pT*pG*)  d(G*pC)/d(G*pC)

Note how guanines become de-stacked.
Excision repair by *E. coli* UvrABC excinuclease system

Step 1: Dimeric UvrA binds UvrB and tracks along DNA.

Step 2: UvrAB complex sees lesion and with energy provided by ATP hydrolysis bends DNA.

Step 3: UvrA dissociates and UvrC associates with UvrB.

Steps 4 & 5: UvrBC complex cuts on both sides of the lesion (UvrB nicks at the 5\textsuperscript{th} PDE bond on 3′ side, UvrC at the 8\textsuperscript{th} PDE bond on the 5′ side).

Step 6: UvrD (helicase II) removes 12-13 nt fragment. DNA polymerase I fills in missing piece.

Step 7: Nick sealed by DNA ligase.

Fig. 25-12
Similarity of NER repair in humans

• Human excinuclease
  – 16 polypeptides: no homology to UvrABC subunits
  – NER sole repair pathway for pyrimidine dimers in humans

• Xeroderma pigmentosum (XP)
  – Deficiency in one or more XP NER proteins (XPA→XPG)
  – Extreme sensitivity to light and high incidence of skin cancers
  – Neurological abnormalities: Due to high rate of oxidative metabolism in neurons.

• Translesional bypass
  – DNA polymerase η (eta): inserts two A residues opposite T-T
  – XP-variant (XP-V) afflicted people lack Pol η function

Lehninger Principles of Biochemistry, 4th ed., Ch 25
Site-specific DNA methylation....

• **Bacteria**
  – Restriction & modification
  – Mismatch error correction

• **Eukaryotes**
  – Tissue-specific inactivation of genes during development
  – Suppression of transposon migration
  – Formation of Z-DNA

• **Occurrence**
  – *E. coli*: $N^6$ of adenine (major), $N^4$ of cytosine (minor), GATC restriction sites
  – Eukaryotes: 3-5% of cytosines (C5), mainly in CpG islands (animal cells and higher plants, absent in insects)
DNA repair systems IV: Mismatch Repair

- Corrects mismatched bases arising from: 
  - Replication errors 
  - Deamination of 5-mC to T 
  - Nonhomologous recombination 
  - Specificity G-T > others > C-C 

- Strand recognition system *E. coli* specific 
  - Hemi-methylation 
  - Certain mismatch repair factors use DNA methylation to identify the newly replicated strand with mismatched nucleotide.

- Enzymatic Components 
  - MutS, MutL (motor proteins) 
  - MutH (endonuclease), 
  - MutU or DNA helicase II (also known as UrvD) 
  - DNA polymerase III, DNA ligase

Lehninger Principles of Biochemistry, 4th ed., Ch 25
Mechanism of mismatch repair in *E. coli*

1. Dimeric MutS motor protein scans DNA and binds to the site of mismatch.
3. MutH nicks DNA when unmethylated strand on 5’ side of CTAG is encountered (could be 1000 or more bp)
4. Helicase and exonuclease unwinds and degrades unmethylated segment with mismatched base.
5. Gap filled by polymerase III and nick sealed by ligase.

Note similarities to NER: scanning $\rightarrow$ conformational change $\rightarrow$ helicase/exo action $\rightarrow$ gap filling.
Exonuclease used depends on location of MutH site relative to mismatch

Mismatch on 3′ side of MutH cleavage site: 5′→3′ exo degradation

Mismatch on 5′ side of MutH cleavage site: 3′→5′ exo degradation

Note the requirement for SSB as well. Why?

Lehninger Principles of Biochemistry, 4th ed., Ch 25
Mismatch repair in eukaryotes

- Recognition mechanism does not seem to involve methylation and/or GATC sequences (no MutH)
- Different MutS homologs (MSH2, MSH3, MSH6)
  - Heterodimerize with different mismatch specificities
  - MSH2/MSH6 (single-base mismatches, bind less well to longer mispaired loops)
  - MSH2/MSH3 (longer mismatches: 2 to 6 bp)
- MutL homologs
  - Heterodimer of MLH1 and PMS1 (post meiotic segregation)
- Mutations in mismatch repair genes confer increased cancer risk
  - hMLH1 and hMSH2 mutations most prevalent
  - Early onset HNPCC (hereditary nonpolyposis colon cancer)
Summary

• DNA damage induces base modifications that are mutagenic due to effects on base-pairing.
• Direct repair: Reverses modification w/o excision and resynthesis of base or nucleotide.
• Base excision: Removes & replaces individual nucleotides: diverse and specific
• Nucleotide excision repair: Removes larger and bulkier lesions.
• Mismatch repair: Corrects errors in replication through MutS/LH complex.
• Methylation: Required for genome protection (prokaryotes) and gene regulation (eukaryotes).