Repair of DNA Damage
Biochemistry 302

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Overview of DNA Restructuring

- **Restriction and Modification** (protective mechanisms in prokaryotes, useful in recombinant DNA techniques)
- **Repair** (in response to DNA damage)
- **Recombination** (redistribution of genomic contents, reproduction, repair)
- **Transposition and Amplification** (developmental processes and/or responses to external stress)

Fig. 25.1
Consequences of DNA damage.....

DNA Damage

DNA Repair

X

DNA Replication

<table>
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<th>Mutations</th>
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<td>Replication Errors</td>
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<td>Persistent Damage</td>
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<td>Genomic Instability</td>
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Cancer

Aging
Maintenance of DNA stability
(Ensuring that information content is transmitted w/o error)

• High accuracy replication/editing
  – 3’ exonuclease-mediated
  – Uracil-DNA-N-glycosylase pathway (for removal of uracil arising due to mis-incorporation of dUMP (rare) or from cytosine deamination)

• Mechanisms that correct “damaged” DNA arising from….
  – Replicative errors not corrected by proofreading activity of DNA Polymerases
  – Environmental damage (chemical or photochemical)
## Summary of DNA Repair Systems

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<td>DNA glycosylases, AP endonuclease, UvrABC nuclease (Helicase, DNA Pol I, DNA ligase)</td>
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<td>Cross-linked bases, Intercalation sites</td>
<td>UmuC, UmuD, RecA</td>
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<td>Pyrimidine dimers, ds breaks</td>
<td>RecA and other factors</td>
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</table>
DNA damage: Pyrimididine dimers

- One of the first forms of DNA damage discovered
- Irradiation of bacteria with ~260 nm light
- Thymine dimers not recognized by DNA Pol III

Fig. 25.9
Overview of types of DNA damage

- **UV photoproducts**
  - Low intensity UV produces pyrimidine dimers
  - Ionizing radiation produces base cleavage, strand breaks

- **Depurination**
  - Protonation of N7 weakens glycosidic bond → breakage
  - Catalyzed by acid *in vitro*

- **Deamination**
  - C → U most common (1/10^5 per 24 h), purines less common
  - Chemically induced by nitrates, hydroxylamine, nitrous acid

- **Alkylation (replace H atom)**
  - Methylation of purines (G or A) results in altered base-pairing

- **Oxidation**
  - Induced by superoxide radicals generated from metabolic rxns

*Alkylating agents*
DNA Damage: Chemical structure of bases that are mutagenic (i.e. produce altered, non-Watson-Crick base-pairs)

Cytosine

\[
\begin{align*}
\text{Cytosine} & \quad \text{Uracil} \\
\text{Adenine} & \quad \text{Hypoxanthine}
\end{align*}
\]

Deamination

Base analogs: used experimentally as mutagens

5-Bromouracil

5-Azacytosine

bp w/T & G

bp w/T & C

2-Aminopurine

6-thioguanine
DNA repair systems I: Direct change of a modified base

- **$O^6$-Alkylguanine alkyltransferase**
  - Catalyzes transfer of alkyl to Cys residue resulting in protein turnover (not really an enzyme)
  - Alkylated form self-regulates its own transcription
- **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**

![Diagram of DNA repair systems](image.png)
DNA repair systems I: Direct repair of thymine dimers by DNA photolyase

- Ubiquitous enzyme that functions via a photosynthesis like mechanism.
- Enzyme contains two cofactor chromophores that absorb light at specific $\lambda$s (light harvesting cofactor 5,10-methenyl-THF transmits energy to FADH$^-\,$).
- Enzyme binds to lesion in the dark and breaks C5-C5 and C6-C6 bonds in the light.
- FADH$^-\,$ serves as the reaction center transferring an electron to the dimer.
- Enzyme is not found in human cells.

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

• Damaged bases typically repaired
  – Uracil, hypoxanthine, 3-methyl and 7-methylated purines, hydroxymethyl uracil
  – Thymine dimers

• Proceeds by three-step mechanism
  – Cleavage of glycosidic bond of damaged base via DNA-N-glycosylase (host of enzymes with different substrate specificities in cells)
  – Removal of ribose by AP (abasic) endonuclease
  – Gap repair by DNA Pol I and DNA ligase

• Classification
  – Cleaved bond (5’ or 3’ to abasic site)
  – Products (3’ OH, 5’ phosphate)
Base Excision Repair: N-glycosylases

- Base excision process requires three activities
  - N-glycosylase
  - AP (abasic) endonuclease
  - Deoxyribophosphodiesterase (5’ exonuclease activity of DNA Pol I)
- Some enzymes include first two activities (e.g. T4 endonuclease V)
- Process leaves a gap of 1-3 nt which is repaired by template-dependent Pol I/ligase

Fig. 25.13

Phage T4 BER of thymine dimer

Fig. 24.35
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

- 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.

Mechanisms to prevent GC→AT or AT→GC conversions depending upon route of entry (BER, base excision repair)
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 crosslinks (cisplatin-induced)
  - alkylation
- *E. coli* machinery (similar in yeast and mammals)
  - UvrA, B, C enzyme system
  - UvrD Helicase II
  - DNA Pol I and DNA Ligase

a) Intra-strand crosslink ~ 90%

b) Inter-strand crosslink ~ 5%

c) Monofunctional adducts ~ 2%

http://www.md.huji.ac.il/courses/bioorganic/cisplatin_3.ppt

Cisplatin = *cis*-diaminedichloroplatinum
Cellular uptake of cisplatin and its targets

Cisplatin = cis-diaminedichloroplatinum

Cisplatin-DNA adducts

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 leaving a 12 to 13 nt gap.

Step 6: UvrD (DNA helicase II) unwinds/removes cut section and DNA Pol I fills in missing piece.

Step 7: Nick repair by DNA ligase

Fig. 25-12
Consequences of defects of excision repair systems in mammals

• Xeroderma pigmentosum (XP)
  – Deficiency in one or more enzymes (two human excinucleases for 5’ and 3’ cutting, −22 to +6 relative to crosslink) in NER pathway
  – Extreme sensitivity to light and high incidence of skin cancers (no known treatment)

• Cockayne’s syndrome and Cancer?
  – Linked to transcription-coupled repair (specialized NER process requiring additional proteins)
    • Stalled RNA polymerase acting as a signal for repair
    • Mechanism for ensuring integrity of genes that get used
  – Cockayne’s syndrome: skin sensitivity, growth failure, neurological disorders but no increased risk of cancer
  – BRCA1 implicated in transcription-coupled NER
DNA Repair/Restructuring
Biochemistry 302

Bob Kelm
February 2, 2004
Importance of DNA methylation in….

• Mismatch error correction
• Restriction & modification
  – Protection of host cell from viral infection
  – Recombinant DNA technology
• Gene regulation
  – Tissue-specific inactivation of genes during development
  – Genomic imprinting
• Occurrence
  – *E. coli*: $N^6$ of adenine (major), $N^4$ of cytosine (minor), GATC restriction sites
  – Eukaryotes: 3-5% of cytosines ($C^5$), mainly in CpG islands (animal cells, absent in insects)
Methylation and its role in *E. coli* defense against infection: DNA restriction and modification

- Discovered by Werner Arber, 1960s: λ phage grow rapidly in *E. coli* K12 but poorly in *E. coli* B (“restriction”).
- Isolate rare phage emerging from strain B → now infect strain B with high efficiency but no longer infect K12 (“modification”).

Fig. 25.5
Type II restriction endonucleases (work horses of recombinant DNA technology)

- Recognize simple symmetrical sequences
- Cut unmethylated DNA generating 3’OH & 5’-phosphate termini with either blunt or overhanging ends
- Most are homodimers with subunits of 30-40 kDa and require a divalent cation for catalysis
- Counterpart methylase: recognizes un- and hemi-methylated DNA, AdoMet

**Table 25-2**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Bacterial Source</th>
<th>Restriction and Modification Site*</th>
</tr>
</thead>
<tbody>
<tr>
<td>BamHI</td>
<td><em>Bacillus amyloquefaciens</em> H</td>
<td>GATCC</td>
</tr>
<tr>
<td>BglII</td>
<td><em>B. globigii</em></td>
<td>A/GATCT</td>
</tr>
<tr>
<td>EcoRI</td>
<td><em>Escherichia coli</em> RF13</td>
<td>G/AATTC</td>
</tr>
<tr>
<td>EcoRII</td>
<td><em>E. coli</em> R245</td>
<td>CCGGG</td>
</tr>
<tr>
<td>HaeIII</td>
<td><em>Haemophilus aegyptius</em></td>
<td>GGCGC</td>
</tr>
<tr>
<td>Hpal</td>
<td><em>H. gallinarum</em></td>
<td>GACGCNNNNNNNNNNN</td>
</tr>
<tr>
<td>HpaII</td>
<td><em>H. haemolyticus</em></td>
<td>GGGGAT</td>
</tr>
<tr>
<td>HinIII</td>
<td><em>H. influenzae</em> Rd</td>
<td>GTPyPuAC</td>
</tr>
<tr>
<td>HinI</td>
<td><em>H. influenzae</em> Ri</td>
<td>GANTC</td>
</tr>
<tr>
<td>HpAI</td>
<td><em>H. parainfluenzae</em></td>
<td>GTTTAAC</td>
</tr>
<tr>
<td>Hpall</td>
<td><em>H. parainfluenzae</em></td>
<td>CCGG</td>
</tr>
<tr>
<td>MspI</td>
<td><em>Mesorilla sp.</em></td>
<td>CCGG</td>
</tr>
<tr>
<td>NorI</td>
<td><em>Nocardia rubra</em></td>
<td>GCGCGGC</td>
</tr>
<tr>
<td>PdiI</td>
<td><em>Pseudononas denitrificans</em></td>
<td>GAGCCTNNNNNNNNNNNN</td>
</tr>
<tr>
<td>PstI</td>
<td><em>Proteus skii</em></td>
<td>CTGCAAG</td>
</tr>
<tr>
<td>SalI</td>
<td><em>Streptomyces albus</em> G</td>
<td>GTCGAC</td>
</tr>
<tr>
<td>Smal</td>
<td><em>Serratia marcescens</em> Sb</td>
<td>CCGG</td>
</tr>
<tr>
<td>XbaI</td>
<td><em>Xanthomonas badriae</em></td>
<td>TCTAGA</td>
</tr>
</tbody>
</table>

*The methylated base in each site, where known, is identified with the letter m. All sequences read 3′ to 5′, left to right. The cleavage on the opposite strand in each case is inferred from the symmetry of the site (except for *HpaII* and *PdiI*, each of which has an asymmetric site). Pu = purine. Py = pyrimidine. N = any base.
Maintenance methylase ensures epigenetic methylation of daughter DNA.

5-azadeoxycytidine (N instead of C in 5 position) can be incorporated but not methylated.

Fig. 25.3 Copyright © 2000 Benjamin/Cummings, an Imprint of Addison Wesley Longman, Inc.
DNA repair systems IV: Mismatch Repair Systems

• Corrects mismatched bases arising from:
  – Replication errors (if not correct by proofreading)
  – Deamination of 5-methylcytosine to yield thymine
  – Non-homologous recombination
  – Specificity G-T > others > C-C

• Strand recognition system in *E. coli*
  – A of GATC sequence is methylated by DAM enzyme (DNA adenine methylase) soon after replication.
  – Repair enzymes use DNA methylation to identify the daughter strand with mismatched nucleotide.

• Enzymatic Players
  – MutS, MutL (motor proteins) MutH (endonuclease),
  – MutU or DNA helicase II (also known as UrvD)
  – DNA Pol III, DNA ligase
Mechanism of mismatch repair in *E. coli*

1. Dimeric MutS motor protein scans DNA and binds to the site of mismatch.
2. MutL and MutH co-associate with MutS activating ATP-dependent motor activity to pull DNA.
3. MutH nicks unmethylated strand 5′- of CTAG.
5. Gap filled by Pol III complex.

Note similarities to NER: scanning → conformational change → helicase/exo action → gap filling.
Features of mismatch repair in eukaryotes

- Similar system to *E. coli*
- Three different MutS homologs (MSH proteins – MSH2, MSH3, MSH6)
  - Heterodimerize with different mismatch specificities
  - MSH2-MSH6 (single-base mismatches, insertions, and deletions)
  - MSH2-MSH3 (insertion or deletions of two to four nt)
  - Mutations in MSH proteins confer increased cancer risk (microsatellite instability of tumor cells due to DNA Pol copying or skipping short repeating sequence)
- Recognition mechanism does not seem to involve methylation.
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 MutSHL complex.
• Methylation: Required for genome protection (prokaryotes) and gene regulation (eukaryotes).
Meselson up to his old tricks…1961

• Infect *E. coli* with heavy and light λ phage
• Centrifuge λ phage to equilibrium in CsCl
• Note λ phage subtypes (recombinants) of intermediate size
• Recombinants arise by the breaking and rejoining of DNA from both parents.

**Heteroduplex DNA**

**Progeny DNA**

• Different genotypes arise from replication of heteroduplex DNA containing a mismatch.
Classes of recombination processes

<table>
<thead>
<tr>
<th>Type</th>
<th>Sequence homology</th>
<th>RecA protein or counterpart</th>
<th>Sequence-specific enzyme</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homologous</td>
<td>Yes</td>
<td>Yes</td>
<td>No&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Site-specific</td>
<td>Yes (about 15 bases)</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Transposition</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Illegitimate</td>
<td>No</td>
<td>No</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

<sup>a</sup>The Chi site or its counterpart determines cutting sites (see page 951), but initial recognition of pairing sites occurs by sequence homology.
Homologous recombination: Why is it advantageous to a cell?

• Recombinatorial Repair
  – Post-replicative mechanism to repair DNA using the “good” non-mutated strand

• Prokaryotic Recombination
  – Information exchange during bacterial mating between donor F+ and recipient F- cells (Sex factor plasmid, F, carries genes required for conjugation of E. coli.)

• Meiotic/Mitotic Recombination
  – Information exchange (crossing over) between paired sister chromatids during Prophase I
  – Restarting stalled replication forks
  – Telomere length maintenance in cells lacking telomerase
Holliday Model

Nicking

- Nicking at same location on homologous strands of two chromosomes

Strand invasion

- Partial unwinding and strand invasion of other duplex

Ligation

- Nick ligation to generate a cross-strand intermediate: Holliday junction

Branch migration

- Branch migration (moves by unwinding & rewinding)

“Resolution” of junction: Isomerization → strand breaking & rejoining yields two possible outcomes:
- Nonrecombinant or recombinant heteroduplexes (different strands from those 1st nicked)

Parallel geometry

Antiparallel geometry

Fig. 25-20
Core interactions at junction crossover
($A_6C_7C_8$ or $R_6$-$C_7$-$Y_8$ motif)

Parallel stacked-X
Open-X (+ protein)
Antiparallel stacked-X

B.F. Eichman et al. (2000) *PNAS* 97:3971
F.A. Hays et al. (2003) *JBC* 278:49663