DNA damage and repair summary

1. Defects in repair cause disease
2. Common types of DNA damage
3. DNA repair pathways
   - Direct enzymatic repair
   - Base excision repair
   - Nucleotide excision repair
   - Mismatch repair
   - Double-strand break repair
     - Non-homologous end joining
     - Homologous recombination

### TABLE 23.1 Some Human Hereditary Diseases and Cancers Associated with DNA-Repair Defects

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<th>Disease</th>
<th>DNA-Repair System Affected</th>
<th>Sensitivity</th>
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<th>Symptoms</th>
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<td>DNA mismatch repair</td>
<td>UV irradiation, chemical mutagens</td>
<td>Colon, ovary</td>
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<td>Nucleotide excision repair</td>
<td>UV irradiation, point mutations</td>
<td>Skin carcinomas, melanomas</td>
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<td>Bloom’s syndrome</td>
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<td>Fanconi anemia</td>
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<td>Hereditary breast cancer, BRCA-1 and BRCA-2 deficiency</td>
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<td>Breast and ovarian cancer</td>
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</tr>
</tbody>
</table>

Common types of DNA damage -- 1

1. Depurination: A, G
2. Deamination: C $\rightarrow$ U, A $\rightarrow$ Hypoxanthine

Common types of DNA damage -- 2

Pyrimidine dimers (UV induced).

Repair pathways
Common types of DNA damage -- 3

Two carcinogens that mutate (the P53 gene) by base alkylation

+ Mismatches (mistakes in DNA synthesis)
Interstrand cross-links,
Double-strand DNA breaks

Total damage from all mechanisms: $10^4 - 10^6$ lesions/day!

Diverse DNA repair systems

- Augment DNA polymerase proofreading
- Mostly characterized in bacteria
- General mechanisms shared in eukaryotes
  1. Direct repair, e.g. pyrimidine dimers
  2. Base excision repair
  3. Nucleotide excision repair
  4. Mismatch excision repair
  5. Double-strand break repair and recombination
Base excision repair pathway (BER).
(a) A DNA glycosylase recognizes a damaged base and cleaves between the base and deoxyribose in the backbone.
(b) An AP endonuclease cleaves the phosphodiester backbone near the AP site.
(c) DNA polymerase I initiates repair synthesis from the free 3’ OH at the nick, removing a portion of the damaged strand (with its 5’→3’ exonuclease activity) and replacing it with undamaged DNA.
(d) The nick remaining after DNA polymerase I has dissociated is sealed by DNA ligase.

AP= apurinic or apyrimidinic
(a=without)

Examples of bases cleaved by DNA glycosylases:
Uracil (deamination of C)
8-oxoG paired with C (oxidation of G)
Adenine across from 8-oxoG (misincorporation)
Thymine across from G (5-meC deamination)
Alkyl-adenine (3-meA, 7-meG, hypoxanthine)

Human alkyl-adenine DNA glycosylase

DNA bent & modified base flipped out of duplex -- “Non-Watson-Crick” structure
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Two pathways of increasing complexity

Base Excision repair

Nucleotide Excision repair

Comparison of two major DNA repair pathways: (A) base excision repair. This process starts with a 5'-3' exonuclease. Then the nuclease activity of the DNA polymerase removes an adduct directly damaged in the DNA, after the action of the nuclease. The newly synthesized DNA is then added by a polymerase. The base excision repair (B) is more complex. In this pathway, the DNA is first unwound, then the DNA polymerase adds nucleotides to fill in the gap, and the enzyme ligates the gap. In contrast, base excision repair only removes the adduct and leaves the DNA intact.
Nucleotide excision repair

(a) Two excinucleases (excision endonucleases) bind DNA at the site of bulky lesion. (b) One cleaves the 5’ side and the other cleaves the 3’ side of the lesion, and the DNA segment is removed by a helicase. (c) DNA polymerase fills in the gap and (d) DNA ligase seals the nick.

Nucleotide excision repair -- eukaryotes

Mutations in any of at least seven genes, XP-A through XP-G, cause an inherited sensitivity to UV-induced skin cancer called xeroderma pigmentosum. The XP proteins are among >30 required for nucleotide excision repair.
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Mismatch repair

Which strand is new and which is the parent?

Mut S binds mismatch
Mut L links S to H
Mut H recognizes the parental strand
Mismatch repair

Which strand is new and which is the parent?
The mutation is in the new strand!
-CH3 marks the parental strand!

MutH - Binds 7-meGATC
MutS - Binds mismatch
MutL - links MutH and MutS

Mismatch repair -- Recognition

Which strand is new and which is the parent?
The mutation is in the new strand!
A-CH3 marks the parental strand!

MutS - Binds mismatch
MutL - links MutH and MutS
MutH - Binds G\textsuperscript{me}ATC

DNA is threaded through the MutS/MutL complex. The complex moves simultaneously in both directions along the DNA until it encounters a MutH protein bound at a hemimethylated GATC sequence. MutH cleaves the unmethylated strand on the 5' side of the G in the GATC sequence.
Mismatch repair -- Resolution

1. The combined action of DNA helicase II, SSB, and one of many different exonucleases (only two are labeled) removes a segment of the new strand between the MutH cleavage site and a point just beyond the mismatch.

2. The resulting gap is filled in by DNA polymerase III, and the nick is sealed by DNA ligase.

Mismatch repair -- Hereditary Non-Polyposis Colon Cancer (HNPCC) gene (Humans)

HNPCC results from mutations in genes involved in DNA mismatch repair, including:
- several different MutS homologs
- MutL homolog
- other proteins: perhaps they play the role of MutH, but not by recognizing hemi-methylated DNA (no 6meA GATC methylation in humans, no dam methylase)
Mismatch repair -- MSH proteins -- eukaryotes

Defects in mismatch excision repair lead to colon and other cancers.

1. MSH2:MSH6 complex binds the mismatch and identifies newly synthesized strand.

2. MLH1 endonuclease and other factors such as PMS2 bind, recruiting a helicase and exonuclease, which together remove several nucleotides including the lesion.

3. The gap is filled by Pol δ and sealed by DNA ligase.

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Double-strand break repair

Two basic mechanisms: End-joining and Recombination

The end-joining pathway of ds break repair is mutagenic, because it removes several base pairs at the break site.

Mediated by Ku proteins.
Double-strand break repair -- Homologous recombination pathways

RecBCD

RecA

Strand exchange with nicks

RecBCD helicase/nuclease in bacteria

RecBCD recognizes ends and unwinds and degrades DNA until it encounters a chi site. Nuclease activity is suppressed on that strand, generating a ssDNA 3’ overhanging end that initiates recombination.
RecA mediates strand exchange

Model for 3-strand strand exchange reaction.

(a) RecA protein forms a filament on the single-stranded DNA.

(b) A homologous duplex incorporates into this complex.

(c) One of the strands in the duplex is transferred to the single strand originally bound in the filament.

(d) The other strand of the duplex is displaced.

Double-strand break repair -- Homologous recombination pathways

RecBCD

Strand exchange with nicks
Branch migration extends heteroduplex

Resolution -- cleavage separates chromosomes

Arrows show two possible cleavage geometries

Ends exchanged

100% chance of some heteroduplex
50% chance of recombinant ends (exchange of chromosome arms)

Ends unchanged

OR
Double-strand break repair -- Homologous recombination in eukaryotes

1. Ds break

2. dsDNA activates ATM kinase, which activates exo-nucleases that create ss 3’ ends. In a reaction that depends on BRCA 1 & 2, Rad51 coats the ss 3’ ends.

3. Rad51 and friends pair the 3’ end with the sister chromatid.

4. DNA polymerase elongates.

5. Pairing of the new DNA bridges the gap.

6. The gap is filled and ligated.

Crossing over (recombination) is common

5 crossovers in a pair of grasshopper meiotic chromosomes
Three mechanisms of Loss of Heterozygosity

1. Spontaneous second mutation (not shown),
2. Mis-segregation and

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