DNA Repair

- Since many mutations are deleterious, DNA repair systems are vital to the survival of all organisms
  - Living cells contain several DNA repair systems that can fix different type of DNA alterations

- DNA repair mechanisms fall into 2 categories
  - Repair of damaged bases
  - Repair of incorrectly basepaired bases during replication

- In most cases, DNA repair is a multi-step process
  - 1. An irregularity in DNA structure is detected
  - 2. The abnormal DNA is removed
  - 3. Normal DNA is synthesized
Damaged Bases Can Be Directly Repaired

- Called DIRECT REPAIR

- In a few cases, the covalent modifications of nucleotides can be reversed by specific enzymes
  - Photolyase can repair thymine dimers induced by UV light
    - It splits the dimers restoring the DNA to its original condition
  - O⁶-alkylguanine alkyltransferase repairs alkylated bases
    - It transfers the methyl or ethyl group from the base to a cysteine side chain within the alkyltransferase protein
Direct repair of damaged bases in DNA

(a) Direct repair of a thymine dimer

(b) Direct repair of a methylated base
Base Excision Repair System

- **Base excision repair (BER)** involves a category of enzymes known as DNA-N-glycosylases
  - These enzymes can recognize a single damaged base and cleave the bond between it and the sugar in the DNA
  - Removes one base, excises several around it, and replaces with several new bases using Pol adding to 3’ ends then ligase attaching to 5’ end

- Depending on the species, this repair system can eliminate abnormal bases such as
  - Uracil; Thymine dimers
  - 3-methyladenine; 7-methylguanine
Depending on whether a purine or pyrimidine is removed, this creates an apurinic and an apyrimidinic site, respectively. Nick replication would be a more accurate term.
An important general process for DNA repair is nucleotide excision repair (NER)
- Nicks DNA around damaged base and removes region
- Then fills in with Pol on 3’ends, and attaches 5’ end with ligase

This type of system can repair many types of DNA damage, including
- Thymine dimers and chemically modified bases

NER is found in all eukaryotes and prokaryotes
- However, its molecular mechanism is better understood in prokaryotes
DNA REPAIR of damaged base:
Nucleotide Excision Repair fixes errors created by mutagens

- Excision repair enzymes release damaged regions of DNA.

- Single strand released

- Repair is then completed by DNA polymerase and DNA ligase
Several human diseases have been shown to involve inherited defects in genes involved in NER.

- These include xeroderma pigmentosum (XP) and Cockayne syndrome (CS).
  - A common characteristic of both syndromes is an increased sensitivity to sunlight.
- Xeroderma pigmentosum can be caused by defects in seven different NER genes.
Skin lesions of Xeroderma Pigmentosum
Mistakes during replication alter genetic information

- Errors during replication are exceedingly rare, less than once in $10^9$ base pairs
- Proofreading enzymes correct errors made during replication
  - DNA polymerase has 3’ – 5’ exonuclease activity which recognizes mismatched bases and excises them
  - If errors slip through proofreading:
    - In bacteria, methyl-directed mismatch repair finds these errors on newly synthesized strands and corrects them
    - In euks, mismatch repair finds these errors on newly synthesized strands and corrects them
DNA polymerase proofreading
Mismatch Repair System

- If proofreading fails, the methyl-directed mismatch repair system comes to the rescue.
- This repair system is found in all species.
- In humans, mutations in the system are associated with particular types of cancer.
- Methyl-directed mismatch repair recognizes mismatched base pairs, excises the incorrect bases, and then carries out repair synthesis.
Methyl-directed mismatch repair in Prokaryotes
Mismatch Repair in Eukaryotes

- Eukaryotes also have mismatch repair, but it is not clear how old and new DNA strands are identified.
  - Four genes are involved in humans, \textit{hMSH2} and \textit{hMLH1}, \textit{hPMS1}, and \textit{hPMS2}
  - All of these are mutator genes
  - mutation in any one of them confers hereditary predisposition to hereditary nonpolyposis colon cancer (HNPCC: OMIM 120435).
Transpositions

- Cytologically invisible sequence rearrangement:
  - movement of a segment of DNA from one location to another in the genome.
  - Not a translocation....

- This may be a transfer of DNA or a duplication of DNA.

- The sequences that cause transpositions
  - are called transposable elements,
  - have specific characteristics,
  - notably the potential to propagate themselves.

- Transposable elements are found in virtually all organisms.
Types of Transposable Elements

- **Transposons:**
  - Move their DNA directly without the requirement of an RNA intermediate.

- **Retroposons:**
  - Copy and then move the copied DNA
  - via reverse transcription of an RNA intermediate.
Transposons

- Encode an enzyme called Transposase.

- Rather than converting RNA to DNA, this enzyme:
  - directly removes the DNA sequence and
  - inserts it in another location.

- Transposons usually have inverted repeats (IR) on either side upstream and downstream.
Transposons encode transposase enzymes that catalyze events of transposition.
Transposons

- Transposase excises the sequence between the inverted repeats and inserts it into another region of the genome.

- The gap created is widened by exonucleases.

- The gap is filled in by repair enzymes that use the sister chromatid or homologous chromosome as a template to fill the gap.
  - If copying from a sister chromatid (also containing the transposon) it will reappear in the original location
    - And hence be copied in the genome
  
  - If the homologous chromosome is used to replace missing transposon (did not have transposon) it will not be replaced
    - And hence not be copied in the genome
Transposons

Transposition of P element to new location

P element in original genomic position

P element excised

Excision of P element leaves a gap at its original location

Exonucleases widen the gap

Repair of gap using a sister chromatid or homologous chromosome containing a P element

Transposon remains in original position

Repair of gap using a homologous chromosome lacking a P element

Transposon no longer at original position
Retroposons

- The DNA sequence in a retroposon codes for a reverse transcriptase,
  - which catalyzes the formation of DNA from an RNA template.
- They always copy DNA and cause a duplication
- Many retroposons also have other polypeptide coding sequences.
- Many retroposons have a poly A tail.
- Others have direct repeat sequences on either side,
  - these are generated because of the way the DNA sequence has been inserted.
Retroposons: The process of LTR transposition

Fig. 13.23
Example of Transposable Elements Found in the Human Chromosome

Types of transposable elements in the human genome

<table>
<thead>
<tr>
<th>Element</th>
<th>Transposition</th>
<th>Structure</th>
<th>Length</th>
<th>Copy number</th>
<th>Fraction of genome</th>
</tr>
</thead>
<tbody>
<tr>
<td>LINEs</td>
<td>Autonomous</td>
<td>ORF1 ORF2 (pol)</td>
<td>1–5 kb</td>
<td>20,000–40,000</td>
<td>21%</td>
</tr>
<tr>
<td>SINEs</td>
<td>Nonautonomous</td>
<td>AAA</td>
<td>100–300 bp</td>
<td>1,500,000</td>
<td>13%</td>
</tr>
<tr>
<td>DNA transposons</td>
<td>Autonomous</td>
<td>transposase</td>
<td>2–3 kb</td>
<td>300,000</td>
<td>3%</td>
</tr>
<tr>
<td></td>
<td>Nonautonomous</td>
<td></td>
<td>80–3000 bp</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Transposable elements move around the genome and are not susceptible to excision or mismatch repair

Why?

They are not damaged bases and they are not mismatches
Homework Problems

- Chapter 15
- # 15
- DON’T forget to take the online QUIZ
- DON’T forget to submit the online iActivity
  - “Overview B”