Today we discuss DNA repair mechanisms - Note:
a) DNA is irreplaceable unlike proteins and RNA  
b) DNA can be damaged  
- environmental stress: UV, ionizing radn, chem. agents,  
- cellular process of replicn *  
c) DNA can be repaired - very varied and complex repair systems.  
Severe lesions (DNA damage) can’t be repaired, leads to cell death.  
Sometimes repair is imperfect leading to mutation.  
Mutation = change in base sequence leading to permanent DNA change  
Can be silent or nonsilent. (usu. nonsilent mutations - detrimental)  
a permanent change in base sequence:  
a) substitution; b) addition; c) deletion  
Multicellular animals:  
Mutation noticed often only in germ line cells (sperm or egg)  
For somatic cells (differentiated cells) no effect beyond the cell except when it leads to malignant transformation: cancer.  

Review of genetic terminology:  
Genetic map = identifies relative positions of genes on chromosomes  
Linkage map = based on recombination frequencies  
Physical map = based on actual physical locations of genes on a chromosome  
Phenotype = any characteristics of an organism that can be detected by appearance, structure, or some measurable property  
Genotype = genetic composition of an individual  
Allele = particular form of a gene  
Marker = any allele whose frequency can be determined quantitatively  
Copy number = # copies of a gene or other DNA sequence per cell  
Diploid = meaning the copy # for most of the genes is 2 (most are diploid)  
Haploid = copy of each allele is 1 (most proks are haploid)  
Extra-chromosomal DNA have higher copy #s. (eg euk organelle DNA or bacterial plasmid DNA)  
Mutation = a change in one of genes which leads to a mutant phenotype  
Reversion = a second mutation in a gene which restores the wild type  
Wild-type = a normal phenotype  
Typical diploid has at least 1 functional allele of each gene  
Suppression is (eg “second site mutation”)  
Many mutations are “silent”  
Conjugation = transfer of DNA from a donor cell to a recipient cell; allowed mapping by using markers and interrupting conjugating cultures at intervals; whole process in E coli is about 100 minutes.  
by determining minutes it takes to transfer the marker to the recipient, can map the DNA.  
Lesions = damage to DNA  
Germ cells = egg or sperm  
Somatic cells = differentiated cells  

To start discussion of repair mechanisms, we first note what happens when DNA is NOT repaired: mutation occurs  
If nonsilent mutation, it may lead to cancer in multicellular organisms!  
Carcinogen detection is complex and expensive.  
But carcinogens in multicellular organisms are often found to be mutagens also in bacteria. > 90% correlation.  
It is quick and inexpensive to see if substances cause mutations in bacteria: Ames Test.  

Ames test for carcinogens: convenient way to detect mutagens.  
Uses a - His pathway defective enzyme strain.  
Disk in center contains decreasing doses of mutagen. Explain the results.  

How serious is spontaneous mutation?  
In a typical mammalian cell: 1000s of lesions /24 hours.  
But DNA repair results in < 1/1000 mutation.  
DNA repair makes life possible.  
Basic principle is DNA is double stranded:  
i.e. complementary strands  
thus undamaged strand can be used to recreate new strand!  
There are many repair mechanisms. They overlap. They are energetically expensive.
4 Types of repair mechanisms

1. Mismatch repair - mismatch can arise during replication
2. Base excision repair - abnormal bases can form spontaneously
3. Nucleotide excision repair - DNA lesions resulting in large structural changes
4. Direct repair - Uses UV light to revert back to repair base.

Protection of DNA is an imperative for the cell!
Many factors can damage DNA. In response, cell has repair mechanisms.

DAM methylase
“BER”

<table>
<thead>
<tr>
<th>TABLE 25-5</th>
<th>Types of DNA Repair Systems in E. coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzymes/proteins</td>
<td>Type of damage</td>
</tr>
<tr>
<td>Mismatch repair</td>
<td>DNA mismatches</td>
</tr>
<tr>
<td>DNA polymerase I</td>
<td>Direct repair</td>
</tr>
<tr>
<td>DNA ligase</td>
<td>Base excision repair</td>
</tr>
<tr>
<td>ABC exonuclease</td>
<td>Nucleotide excision repair</td>
</tr>
<tr>
<td>DNA polymerase I</td>
<td>DNA lesions that cause large structural changes (e.g., pyrimidine dimers)</td>
</tr>
<tr>
<td>DNA ligase</td>
<td>Pyrimidine dimers</td>
</tr>
<tr>
<td>DNA methyltransferase</td>
<td>O6-Methylguanine</td>
</tr>
<tr>
<td>AKB protein</td>
<td>1-Methylguanine, 3-methylcytosine</td>
</tr>
</tbody>
</table>

Methylation and mismatch repair

For a short period following replication, the template strand is methylated and the new strand is not.

For a few minutes the new strand is methylated and the base excision repair, “BER” becomes available.

For a short period following replication, the template strand is methylated and the new strand is not.
Repair #1: Mismatch Repair

When there is mismatch, MutS, MutL, and MutH participate (actually about 12 proteins involved).

MutH detects “nearest” methylated base.

How common is GATC palindrome?

Why is this energetically expensive?

2 alternatives; exonucleases (I or X: 5‘→3’; VII or RecJ: 3’→5’) depends on position of mismatch relative to cleavage site. Note length of repair can be extensive. (depends on the frequency of methylation in the DNA)
Repair mech #2: Base excision repair (BER): involves DNA glycosylases, then AP endonuclease, then Pol I. Defective bases removed by specific glycosylases. (e.g. U gly’ase)

Repair #3: Nucleotide-excision Repair: for damage causing large distortion of DNA. Uses Pol I
Repair#4: Direct Repair: Repair of pyrimidine cyclobutane dimers with photolyase: energy derived from UV light is used to reverse the photoreaction that caused the lesion.
Repair of $O^6$-methylG involves inactivation of methyltransferase!

Other example: AlkB protein also inactivated.

DNA damage its effect on DNA replication: recombinational, error-prone repair. SOS response.

SOS response. Error prone. Allows a few to survive.