I. Cellular responses to DNA damage

repair or **reversal** of damage

bypass – DNA polymerase is blocked by many types of damage; special bypass polymerases can systhesize past apurinic sites or pyrimidine dimers; these are typically very error-prone (they just make a "best guess" as to what nucleotides to incorporate.

cell cycle arrest – replication or mitosis in the presence of damage can be disastrous; most cells will arrest progression through S phase or entry into M phase until damage is repaired.

apoptosis – in metazoans, cells experiencing too much damage can kill themselves

II. Overview of some DNA repair strategies

DNA repair mechanisms are named according to when they occur (*e.g.*, postreplication repair), what type of damage they act on (*e.g.* double-strand break repair), or actual mechanism (*e.g.* nucleotide excision repair).

A. When damage is limited to one strand, repair pathways use the complementary strand:

Base excision repair (BER) corrects damaged and missing bases

1) **DNA glycosylase** removes base by cleaving nucleoside linkage (also called glycosidic bond); leaves an apurinic or apyrimidinic (AP) site; specialized glycosylases recognize bases damaged by reactive oxygen species, uracil, etc.
2) **AP endonuclease** (APE) sugar-phosphate backbone at AP sites (created by glycosylases or by spontaneous depurination
3) exonucleases remove a short stretch of bases, DNA polymerase β synthesizes across the gap, and ligase seals the nick

Nucleotide excision repair (NER) corrects UV-induced damage and helix-disorting lesions

1) specialized recognition proteins bind to regions where the double-helix is distorted; stalled RNA polymerase II, which cannot get past damage may also trigger repair.
2) helicases unwind DNA to make a bubble
3) structure-specific endonucleases nick the damaged strand 5' and 3' of the lesion to remove a segment of 20-25 nt containing the lesion
4) DNA polymerase synthesizes across the gap and ligase seals the nick

Mismatch repair (MMR) fixes mismatches that arise during replication – misincorporated bases and small insertion and deletion errors (as in microsatellites)

1) MutS / MutL recognizes mismatched bases or small loop due to insertion/deletion
2) MutH nicks the strand with the error, which is then degraded and resynthesized.

How can the cell tell the good strand from the bad? Methylation.

In *E. coli*, adenine methylase methylates A whenever the sequence GATC occurs.

Before replication: 5'-G A^m T C -3'
3'-C T A^m G-5'

After replication: 5'-G A^m T C -3' (old, template strand)
3'-C T A G-5' (new strand, temporarily not methylated)

MutH nicks at a CTAG that is "hemi-methylated", on the unmethylated strand.

Eukaryotes have up to six MutS-like proteins and and up to four MutL-like proteins. There is no MutH-like protein in eukaryotes, and no Dam methylase – mechanism of old vs. new strand discrimination is unknown.

B. When damage includes both strands, homologous recombination is often used.

Double-strand break repair

1) Homologous recombination: broken ends used to search for homologous sequence (*e.g.*, sister chromatid); complex process involving new DNA synthesis to copy sequences from donor. If the donor is the homologous chromosome (or other template), an allele on the broken chromosome can be replaced with an allele from the homologous chromosome, a process called **gene conversion**.

   Meiotic recombination begins with a double-strand break, and uses many of the double-strand break repair proteins.

   A key step in homologous recombination is the **homology search** and **strand invasion**, catalyzed by RecA (*E.coli*) or homologous proteins (Rad51 and up to four additional structurally related proteins).

2) Alternatively, some repair of DSBs occurs by **non-homologous end joining** (NHEJ), but sequences around the break site can be lost. Many NHEJ proteins play a key role in V(D)J recombination, the process by which genes encoding antibodies are rearranged to generate diversity in the immune system.

Interstrand crosslink repair – mechanism not known, but involves recombination, because proteins related to Rad51 are required

IV. Consequences of repair defects

Repair defects often create a **mutator** phenotype – with higher rates of mutation and **genome instability**. Genome instability is a hallmark of cancer. Thus, defects in DNA repair frequently cause diseases associated with increased incidence of cancer.

BER is essential – mutations in BER genes cause lethality. Some recombination genes, like Rad51, are essential.

MMR defects are associated with hereditary nonpolyposis colon cancer (HNPCC) and increased microsatellite instability.

NER defects cause xeroderma pigmentosum (XP), which includes severe sensitivity to sunlight and early onset of skin cancer (median age = 8 years). XP variant is caused by mutations in the pyrimidine dimer bypass polymerase. Cockayne syndrome, which includes developmental and neurological defects, is caused by inability to couple NER and BER to transcriptional blockage.

NHEJ defects result in severe combined immunodeficiency, due to inability to rearrange antibody genes and T cell receptors.

Mutations in *Atm*, which encodes a protein that senses DNA damage and signals cell cycle arrest, cause ataxia telangiectasia. AT is associated with progressive loss of motor skills during childhood, lymphoma, and other symptoms.

p53 encodes a protein that signals apoptosis after DNA damage. *p53* mutations are extremely frequent in malignant cells. Inherited *p53* mutations cause Li-Fraumeni syndrome, characterized by extreme predisposition to cancer.

Many other genes that are associated with predisposition to cancer encode proteins that function in DNA repair in some undefined way.