Chapter 3

DNA Damage and DNA Repair

- DNA continuously damaged
  - Chemical modifications
  - Loss of bases
  - Single strand/double strand breaks
  - Intra- and Inter-strand cross-links
- DNA replication important for incorporation of incorrect bases
  - Oxidative stress important in mutations
  - Spontaneous deamination of cytosine, etc…

Repair Mechanisms

- Glycosylases-
  - remove damaged bases
- Apurinic/apyrimidinic endonucleases-
  - prepare sites lacking bases for short patch or long patch base excision repair
- Nucleotide excision repair systems-
  - Carcinogen adducts and pyrimidine dimers (UV light)
- Metallothioneins & Glutathione transferases-
  - Protect against reactive O_2 species
- Apoptosis-
  - Cell cycle check points (stress, viral infections)

Damage during Replication

Base Excision and Nucleotide Excision Repair

- Most chromosomal alterations and base mutations removed by repair mechanisms
  - If alterations so bad then cells eliminated or prevented from replicating
  - Defects in repair mechanisms important for progression of cancer
- Sites/Types of Problems with DNA replication:
  - Misincorporation of bases
  - Slippage of the replisome in tandem repeat sequences
  - Single-strand breaks being converted into double strand breaks
  - Stalling of the replisome at sequences that have been modified

- Base Misincorporation:
  - Replication of DNA must be precise- DNA polymerase has a "proof-reading" function
  - In a genome of >3X10^9 bp, several hundred mistakes are expected during each replication
    - Most misincorporations are corrected by "base mismatch repair" (MMR) systems
      - » MMR also takes care of single stranded loops that result from slippage of DNA in repeat sequences
    - Mesomeric isoforms of bases- analogs of normal bases but are short lived: they can be stabilized by chemical modification (hydroxylation of Guanine at the 8 position stabilizes a OH-G:A mismatch)
      - » Mispairing are recognized by specific proteins that activate the MMR system
  - Precision of DNA replication dependent upon nucleotide precursor pool
    - » Deregulation of precursor pools may activate cellular checkpoints through the TP53 protein
    - » In cells with low FOLATE levels (or due to methotrexate chemotherapy), thymidine levels are low and uracil bases may be incorporated- these must be removed and removal may cause strand breaks

- Spontaneous chemical reactions by DNA bases:
  - Cytosine deamination- yields uridine which is foreign to DNA and removed by uracil glycohydrolyase (~ 1000 uracils spontaneously generated in each cell per day)
  - Methylation of Guanine- can lead to mispairing and methyl groups removed by specialized enzyme (methyl-guanine methyltransferase- MGMT)
    - » Downregulation of MGMT is found in some cancers
  - Guanine also the base most susceptible to reactive O_2 species

- Glycosylases hydrolyse the N-glycosidic bond between modified bases and deoxyribose leaving ABASIC sites in DNA
  - Abasic sites are filled in by "short-patch repair" that is initiated by endonucleases (AP-nucleases= apurinic/apyrimidinic endonucleases)
    - These cleave the DNA strand with the missing base to provide a free 3'-hydroxyl group for DNA polymerase ß. This removes the deoxyribose and replaces it with the correct nucleotide. DNA ligase closes the gap- only one base replaced in "short-patch repair"
  - "Base excision repair"- the type of DNA repair resulting from the combined action of AP-endonucleases, DNA polymerases, and DNA ligases

- Dilemma during mismatch repair-
  - How is it decided which base is incorrect, or whether a single-strand loop constitutes a deletion or an insertion??
    - In procaryotes the parent strand is methylated and the daughter strand is not until later
    - In eucaryotes the newly synthesized strands are distinguished by the presence of single-stranded breaks that serve as the starting points for nucleotide excision
  - The hydrolytic deamination of cytosine or the oxidation of guanine lead to altered bases with an increased potential for mispairing, BUT they do NOT interfere with DNA replication or transcription

Nucleotide Excision Repair and Crosslink Repair

- UVB wavelengths of light (280-320 nm) absorb into DNA and induces reactions between adjacent pyrimidine bases (T-T dimers, T-C dimers and C-C dimers)
  - These dimers prevent replication and transcription
- "Bulky Adducts"-
  - Aflatoxin or benzopyrene exposure lead to modified bases that are too bulky to fit into a double helix and cannot be recognized by polymerases

- "Bulky adducts" and "photoproducts" are removed by nucleotide excision repair (NER)
  - "global genome" and "transcription-coupled" repair systems are involved in removal and repair of these
    - Transcription-coupled repair is more rapid but is restricted to regions of the genome transcribed by RNA polymerase II.
      - » When RNA polymerase encounters a "bulky adduct" it activates repair through the TFIIH complex
      - » TFIIH complex has ~ 10 proteins including cyclin H
    - Global genome repair is the same basic pathway, but does not involve RNA polymerase- this repair is slower but has a broader specificity
  - Other repair mechanisms as well…….

Diseases: Mutations of genes involved in nucleotide excision repair

- Xeroderma pigmentosum
- Cockayne Syndrome
- Trichothiodistrophy

- Inherited in recessive manner- rare
- Patients of Cockayne Syn & Tricho …
  - show symptoms of premature aging and life expectancy shortened

- Patients with Xeroderma pigmentosum (XP)…
  - Have extreme photosensitivity of skin
  - Abnormal pigmentation
  - 2000 X increase in skin cancer before the age of 30 (often during first ten years of life)
    - All types of skin cancers are increased
  - Have defects in BOTH transcription-coupled and global-genome repair systems

Crosslinks- impediment to replication and transcription

- Mechanism used in G2 cells-
  - Excision made behind the lesion – by XPF/ERCC1
    - A gap is made and one strand is filled in using the homologous sequence as a template
    - Second strand is synthesized following excision of the cross-linked fragment
  - Dependent on FANC proteins

FANC Proteins

- Patients with **Fanc**oni Anemia …
  - Small and have malformations in different organ systems
    - Lower arm, radius and thumb
    - "Café au lait" spots diagnostic
    - Pancytopenia (decreased production of all blood cell types)
    - Patients suffer from pre-neoplastic "myelodysplastic syndrome" and may progress to leukemias (like AML = acute myeloic leukemia)

- FANC proteins
  - Repair of crosslinks
  - Regulation of cytokine synthesis
  - Apoptosis
  - FNACC- cellular defense against oxidative stress
  - Several FANC proteins- A, C, E, F, G
    - These cooperate to "mono-ubiquinate" FANCD2 which activates "repair foci"
  - Defects in FANC proteins associated with defects in B and T cell maturation
    - Involves gene rearrangements and joining of double strand breaks caused by lymphocyte specific recombinases
  - Several commonly used cytostatic drugs are DNA crosslinkers
    - Cis-platinum component of many cancer chemotherapy formulas (miracle drug for testicular cancer) and depending upon whether these drugs work or not is dependent upon expression level of FANC proteins (need to be low so as to prevent excision-repair and stop cancer cell growth)

Strand-Break Repair

- Many single strand breaks are repaired by DNA ligases
- Double strand breaks are more difficult to repair
  - ds breaks separate a fragment of DNA from the centromere and may lead to loss of chromosome fragment during mitosis
  - Associated with activation of cell cycle checkpoints that prevent the cell from entering or preceeding through S phase and mitosis

ds Break Repair Mechanisms

- Non-Homologous Repair System
  - (NHEJ)- non-homologous end joining-
    - Not precise, but most widely used- mech of choice in G1 phase
    - End product of this repair is a restored DNA double helix with a deletion, which is normally kept at a minimum
    - When NHEJ begins it activates cellular check points (TP53 protein phosphorylated by DNA-PK)
- Homologous Repair System
  - (HRR)- homologous recombination repair-
    - Can be performed in an error-free manner
    - Mechanism of choice in G2 phase
    - Most prevalent syndrome is ataxia telangiectasia (AT) caused by recessive inheritance of homozygous mutations in gene coding for ATM protein kinase
    - Lack of ATM function leads to incomplete function (phosphorylation) of TP53, FANCD2, and BRCA1
    - Activation of ATM activates checkpoints that block the cell from proliferating

Defects in Repair and Cancer Susceptibility

- Many conditions in which DNA repair proteins are mutated lead to increased cancer risk (table 3.2)
- Defective DNA repair is sufficient for cancer development, but is it necessary?
  - Can cancer arise just by accumulation of rare alterations that have occurred in spite of functional DNA repair?
    - » Answer- ???????

- Defects in DNA repair may not be required for cancer development, but defective signaling to check points in cell cycle could be necessary
  - Found in most cancers (defects in cell cycle regulation)
    - » Allows cell proliferation to occur despite extensive DNA damage
- DNA repair becomes less effective with age and DNA defects/mutations may accumulate

Cell Protection Mechanisms in Cancer

- Low molecular weight compounds -
  - Used to protect against altered osmolarity , buffer redox state, quench radicals and stabilize macromolecules and membranes
    - Table 3.3
    - Low molecular weight compounds include:
      - » Polyamines, amino acids (taurine), glutathione, and vitamins E and C (tocopherol and ascorbic acid)

- Glutathione
  - Contains thiol group that reacts with radicals
  - Normal oxidized form is disulfide GSSG
    - Glutathione reductase reduces GSSG → GSH
    - Glutathione peroxidase (contains selenium) removes hydrogen peroxide generating GSSG
      - » Lack of glutathione peroxidase (due to lack of selenium) may increase risk of cancer