

## BICH/GENE 431 KNOWLEDGE OBJECTIVES

### Chapter 9 – Mutations and DNA Repair

Three major causes for mutations in DNA: replication errors, chemical or environmental damage, insertion of transposable elements

Replication errors at frequency of about 1 in 100,000; accuracy increased about 100-fold by proofreading; DNA repair improves accuracy another 100-1000-fold

DNA mismatch repair to catch replication errors

- MutS, MutL, MutH proteins in *E. coli*; know this repair pathway
- How is incorrect base (strand) recognized in *E. coli*?
- Eukaryotic mismatch repair: MSH is homolog of MutS, interacts with sliding clamp, repairs strand with nick before ligation, mutations in genes lead to higher incidence of colon cancer

Spontaneous DNA damage (in water)

- deamination: know examples, 5-methylC becomes T (mutagenic hotspot)
- depurination

Chemical mutagens

- alkylating agents (DMS, nitrosamines, MNNG); common product is O<sup>6</sup>-methylguanine
- reactive oxygen species (hydrogen peroxide, hydroxide radicals); common product is oxoG

UV light causes pyrimidine dimers, such as thymine dimers

Ionizing radiation (x rays, gamma rays) cause ds DNA breaks

Bleomycin (anti cancer drug) causes ds breaks

Base analogs – what are they? A common example is 5-bromouracil (can base pair sometimes with G)

Intercalating agents – know examples; insert between bases in DNA to cause insertions or deletions during replication

Direct reversal of damage

- DNA photolyase to remove thymine dimers (plants, bacteria, not humans)
- Methyltransferase enzyme to repair O<sup>6</sup>-methylguanine (single turnover)

Base excision repair – removes base, leaving AP site

- glycosylase enzymes are specific for altered base
- know pathway of repair
- base flipping mechanism used by glycosylases
- fail-safe glycosylase for removing A replicated opposite unrepaired oxoG

Nucleotide excision repair – removes nucleotide(s) containing abnormal base

- UvrABCD pathway in *E. coli*
- XP proteins, plus many others, in humans (XP denotes Xeroderma pigmentosum, a genetic disease caused by defects in nucleotide excision repair)
- transcription and nucleotide excision repair are coupled in order to direct repair to genes that are being expressed – TFIIH in eukaryotes is a general transcription factor and a nucleotide excision repair enzyme

### Double-strand break repair – two major pathways

- recombination repair mainly used in bacteria and lower eukaryotes (yeast): details covered in next chapter; uses homologous recombination
- NHEJ (nonhomologous end joining) is major pathway in higher eukaryotes – by nature it is mutagenic; identifier proteins are Ku70 and Ku80 that bind to broken ends of DNA

### Translesion DNA synthesis

- bypass synthesis when replication fork approaches unrepaired DNA damage
- highly mutagenic, so a method of last resort for cells
- know basic mechanism in *E. coli*
- special DNA polymerases in Y family (bacteria to humans)
- SOS response in *E. coli* used to induce TLS enzymes and other DNA repair