What is DNA?
DNA/RNA: polynucleotide chains

- **Sugar**: (2’ OH=ribose, 2’ H=deoxyribose)
- **Phosphate**
- **Base**

Nucleotide = sugar + phosphate + base
DNA is a double helix
DNA damage and repair

• How is DNA damaged?
• How is DNA repaired?
• How does the type of damage impact repair?
• Accumulated DNA damage = death (by cancer, or old age)

• “No one here gets out alive”
  – Jim Morrison
Adduct formation

- Nasty chemicals (carcinogens) that adduct to DNA; often to ring Nitrogens in bases
  - E.g. Alkylating agents: reactive carbon containing chemicals (ethylating agents, methylating agents)
Adduct formation

- Not always direct exposure: sometimes carcinogen is toxic product of cellular metabolism
  - Cigarette smoke; benzo-a-pyrene not a big deal…but the break down product is

- Groups are bulky, blocks transcription, replication; can interfere with base pairing, and introduce mutation during replication

MO = monooxygenase
EH = epoxyhydrolase
G = guanine (DNA)
Radiation: UV light

• Non-ionizing radiation (UV light from the sun)
  – Bases absorb energy with peak at 260nM..this is UV
  – Photoactivates base, causes nasty chemistry
  – Result is...covalent bonds between adjacent bases, almost always adjacent pyrimidines
  – Distorts DNA (kink), can block transcription, replication, lead to mutation
Spontaneous Damage: Base loss

- Some times bases just fall off (more often than you might think; 10000/genome/generation)
- Bases gone, but phosphodiester backbone is still intact
- Purines more sensitive than pyrimidines (acid sensitive)
- Causes mutation, can lead to strand breaks
Spontaneous damage:
deamination

1. Converts C to U etc…
2. Altered base has different base pairing rule
   - e.g. U pairs with A (converts CG bp to UA)
3. Unless repaired results in transition mutation
Oxidative stress

- Reactive oxygen species (ROS); things that are or give rise to oxygen with an unpaired electron; a free radical
- E.g hydroxyl radical $\cdot\cdot\cdot$ $\text{H}_2\text{O}$$
- ROS produced by….
  - Respiration
    - “…after all, free radicals are the cost we pay for breathing itself.”
  - Radiation (typically ionizing)
- ROS removed by…
  - Enzymes…SOD, catalase
  - Reducing agents..glutathione, vitamin E, etc.
ROS/IR

• Damages base

• Breaks bonds, any bonds: base (base loss), sugar, phosphodiester/backbone bonds (strand breaks: single strand breaks, and (RARELY) double strand breaks)
Radiation: ionizing

- X-rays, gamma-rays; rarely encountered… except for medical sources
  - Usually damage is secondary consequence of ROS generated after radiolysis of water (DNA rarely a direct target)
  - Damages bases (e.g. 8-oxoG)
  - Strand breaks; often clustered, thus a source of double strand breaks
Cross-linking agents

• Cross-linking agents; a special case where adduct-former is bifunctional (two reactive groups)
  – Intra-strand crosslinks: between adjacent nucleotides, like UV photoproducts
  – Inter-strand crosslinks: between nucleotides on opposite strands of a double helix

• Interstrand cross linkers completely block transcription, replication
Replication errors

• Not DNA damage per se.

• Substitutions
  – Misincorporation: \(<1 \times 10^{-6}\)
  – Improved by proofreading, mismatch repair
  – Made worse by imbalanced nucleotide pools
  – Tautomers

• Slippage
  – Repetitive DNA, secondary structures
Proofreading/Editing

- Some polymerases have extra 3’ to 5’ exonuclease domain (opposite polarity to DNA synthesis; reversal of synthesis)
- Used to “edit” out incorrectly incorporated dNMPs
Tautomers

- Standard tautomers...keto T, G; amino A, C.
- Rare tautomers (enol T, G; iminoA, C)
- Results in non-watson crick base pairs
- Transition mutation
Microsatellite instability

- Slippage of primer or template during replication causes expansion/contraction of microsatellite
DNA damage and repair

• Sources of DNA damage
  – Spontaneous
  – Environmental
    • Radiation
    • Carcinogens
  – Mistakes in replication
DNA damage

- 20,000 abasic sites
- 10,000 oxidized bases
- 7,000 Alkylations
- 10-1000 replication errors?
- 10 double strand breaks

- Per day per cell
DNA is a double helix
DNA is a double helix

Critical (if somewhat obvious) thing to remember for both replication and repair: Symmetry (base pairing) allows for easy and accurate
1. Replication: duplication of information
2. Repair: replacement of damaged information
# DNA REPAIR

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Excision repair=BER, NER, MMR
DSB repair=HR, EJ
Excision repair

• Damage in one strand….remove it, fill in the gap (using un-damaged strand as template), ligate the remaining nick

• Same process…base excision repair, nucleotide excision repair, mismatch repair

• Contrast to double strand break repair (which has no template for repair)
Base excision repair (BER)

- Damage is recognized by glycosylase (different one for each type of damage)
- Common targets are deamination products
- E.g. uracil glycosylase, hypoxanthine glycosylase
Glycosylase recognition
Nucleotide excision repair (NER)

- Recognizes distortion in DNA (more flexible than BER)
- Removes most UV photoproducts, adducts
- Multi-protein machine
NER: recognition of damage

- Cyclobutane dimer
- Excision site
NER: Transcription coupled repair

- NER most efficient in transcribed regions; also template strand more efficiently repaired than non-template strand
- NER machinery part of transcribing RNA polymerase complexes (TFIIH)
- When NOT coupled to transcription, NER can still be targeted, though less efficiently, to damage in “silent” DNA…global genome repair (GGR)
Mismatch repair

- Supresses replication errors; substitutions, slippage (microsatellite instability)
- Unlike NER/BER, not obvious which is damage, which should be used as template
- Need to identify recently synthesized strand
Double strand break repair and recombination

• How do you get double strand breaks?
  – Can be intentional (developmentally programmed)
    • Meiosis, VDJ recombination
  – By accident
    • Ionizing radiation, replication through incompletely repaired damage
  – Therapeutic
    • Chemotherapy, radiation therapy
  – Malicious intent
    • Transposons, retro-elements
How do your repair double strand breaks?

1. Homologous recombination ("HR")
   • Meiotic recombination vs. Mitotic recombination
   • Used almost exclusively in prokaryotes, by far the more important even in yeast

2. End joining ("EJ")
   • Less accurate, but the primary pathway in vertebrates
   • Why use end joining over homologous recombination?
DNA repair

BER/SSBR
- Excision
- Synthesis
- Ligation
  - Accurate
  - Cheap

DSBR
- IR
- HR
  - Accurate
  - Expensive

VDJ
- NHEJ
  - No template
  - Inaccurate?
  - Cheap?

IR
Breast cancer and DSB repair

• DSBs have…
  – Both strands broken
    • Loss of chromosome continuity
    • No intact template
  – Damage in flanking nucleotides
    • More than just ligation
DSB repair

- CtIP, Mre11 etc.
- Ku etc.
- Rad 51, BRCA1, 2 etc.

New pathway?
Pol θ aka Polq aka Mus308

- **Chaos1** (Shima et al., 2004)
  - Pol θ mutation = genetic instability (micronuclei)

- **Mus308** (Chan et al, 2010)
  - Defines Rad51 and Ligase IV independent DSB repair pathway in Drosophila
  - Promotes repair at microhomologies

- **Overexpressed in breast cancer** (Lemee et al, 2010)
  - Strong indicator of poor prognosis
Synthesis across a strand break (part 2)

- Pre-resected Substrate
  - >10 nt 3’ ssDNA tail
  - 4 nt terminal microhomology

David Wyatt
Questions

• Nucleotide excision repair (NER) and Base excision repair (BER) excise damage differently…Why?
• Why couple NER to transcription?
• Nonhomologous end joining (NHEJ) is less accurate than the other double strand break repair pathway: Why do you bother with NHEJ?
• Tumors arising in Hereditary breast cancer are defective in DSB repair – how can this be used as a therapeutic tool?