Sources of DNA damage

- Intrinsic
  - Metabolic byproduct (ROS, uric acid)
  - Replication error (nucleotide mismatch, topoisomerase not repaired)
  - rNTP instead of dNTP
  - Spontaneous damage (deamination)
- Extrinsic
  - Ionising radiation (U.V.)
  - Intercalating agent (Disrupt base stacking)
  - Alkylating agent (Disrupt sugar phosphate backbone EtBr)
  - Genotoxic agents (Phthalates)
  - Base anologues (Replace base nucleotides) (5-bromouracil replaces thymine)

Repair mechanisms

Single strand DNA repair

- Post replication mismatch repair (MMR)
  - Mismatched dNTP identified in the polymerase
  - Replication stops, 3' end of daughter strand transferred to exonuclease site
  - Mismatched nucleotide cleaved off, 3' end transferred back to catalysing site
- Mutator protein (Mut) dependent (In E.Coli)
  - Mismatch identified immediately after leaving polymerse
  - Dam methylase hemimethylate the dsDNA GATC sequence, parent strand methylated, not the daughter strand.
  - MutS and MutL bind to mismatch site, read bidirectionally, form loop, until hemimethylated GATC
  - MutS MutL recruit MutH, cleaves unmethylated daughter strand from GATC.
  - Helicase II + Pol I unwind and cleave daughter strand to mismatch
  - Pol III add nucleotide to gap, sealed by ligase
- Base Excision Repair (BER)
  - Glycosylase bind to mismatch, flip out the base, cleave glycosidic bond of nitrogenous base
  - AP endonuclease cleave phosphodiester bond of mismatched nucleotide
  - Repaired by Pol I, sealed by ligase
- Nucleotide excision repair (NER), bulkier than BER
  - Four Uvr proteins (UvrA~D)
  - UvrA and UvrB scan genome, UvrB stays at site of mismatch
  - UvrA leaves, B recruits C to unwind and excise ~15 nucleotide fragment
  - C leaves, D removes the fragment, all leaves
  - Pol I repair, ligase seal
- Direct reversal of modifications
  - Photolyase can reverse UV induced thymine dimers
  - Methyltransferase can reverse methylated nucleotides
  - No DNA excision, no template needed, but costly as enzyme commit suicide afterwards
- Models of transleision synthesis (TLS) (replication across damaged nucleotide)
  - Polymerase switching: High fidelity pol stops at lesion, switch to TLS Pol, then switch back
  - Gap filling: High fidelity Pol does not pair dNTP at lesion, filled by TLS Pol

Double strand DNA repair

- Non-homologous end joining
  - Characteristic: Always available, common in non-dividing cells (G0, G1, S), prone to insertion & deletion
  - Ku70/80 recognise and bind to double stranded breaks
  - Recruit DNA-protein catalytic subunit (DNA-PKcs), form DNA-PK complex
  - Binding of nuclease Artemis, phosphorylated, activated and trims the end of DSB (prone to deletion)
  - Modification of ends by other enzymes (fixing gaps, trimming, prone to insertion)
  - Joining of blunt ends, ligase seal.
- Homology directed repair
  - Characteristic: Available in dividing cells (G2/S stage), less error prone
  - MRN recognise the error sequence
  - MRN recruit ATM to activate array of downstream proteins. exonuclease resect the end and create 3' overhangs.
  - RPA binds to ssDNA, stabilisation, BRCA2 and RAD51 replace RPA ssDNA, forming nucleofilament
  - RAD51 scans for homolgous sequence in sister chromatid, RAD54 direct invasion
  - RAD51 dissociate, Polymerase synthesis of invading strand.
  - Invading strand dissociate, bind to 3' overhang other damaged strand, polymerase + ligase seal.

- Cell cycle checkpoints
  - G1: before S phase, check for nutrient availability and cell size
  - G2: Before mitosis check for complete replication and DNA damage
  - M (Spindle): before anaphase, check if spindles attached to kinetochores
  - S: Check for DNA fidelity and genome stability
- Proteins involved in cell cycle regulation:
  - Cyclin: activate CDK, drive division. Different cyclin signal different cycle events
    - Cyclin dependent kinase (CDK), upregulate cell cycle
    - Cyclin dependent kinase Inhibitor (CDKI), downregulate cell cycle

Sources of DNA damages, intrinsic 3 and extrinsic 4

Single strand repair 5

Models of TLS

Double stranded repairs 2

Cell cycle checkpoints, what is assessed