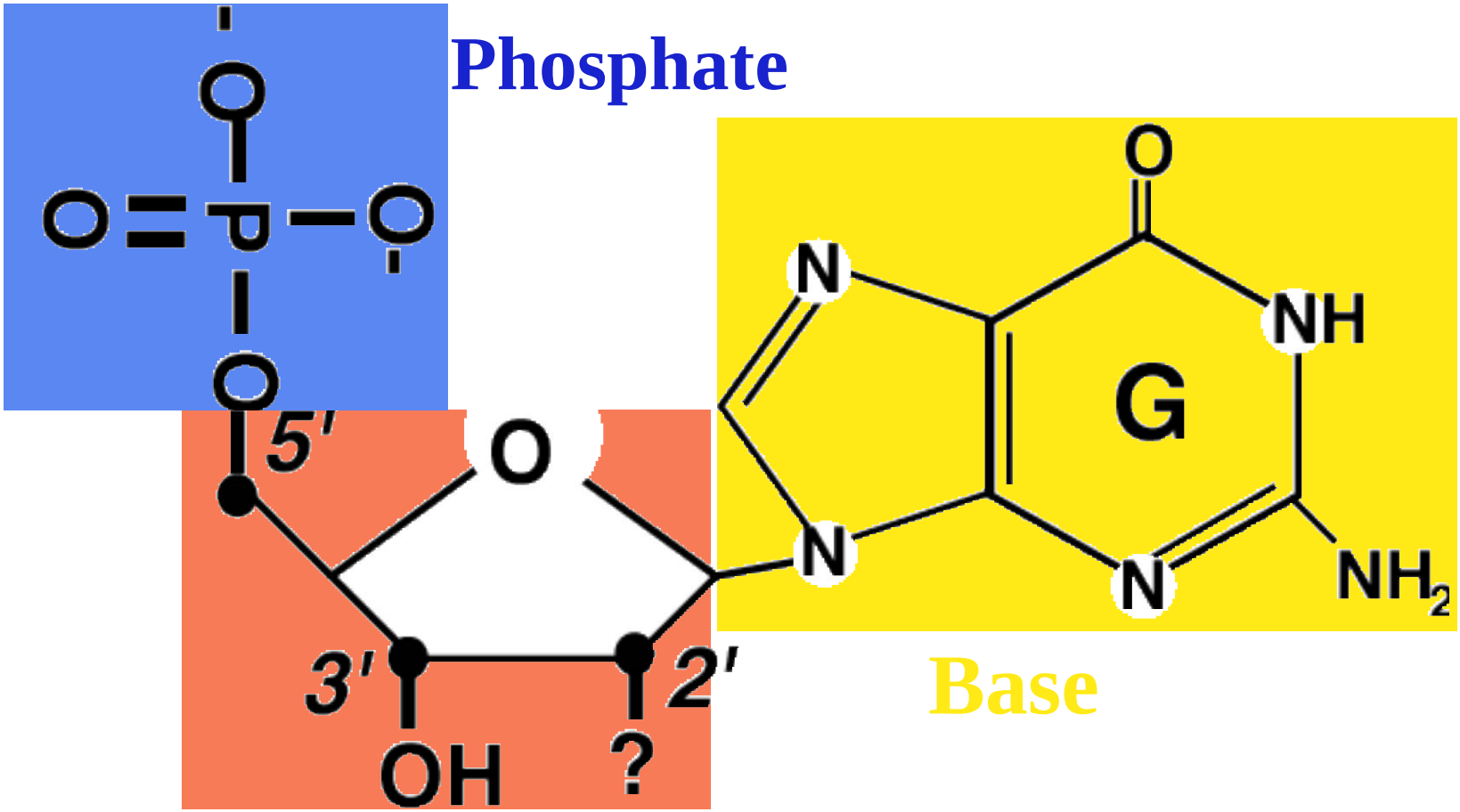
What is DNA?

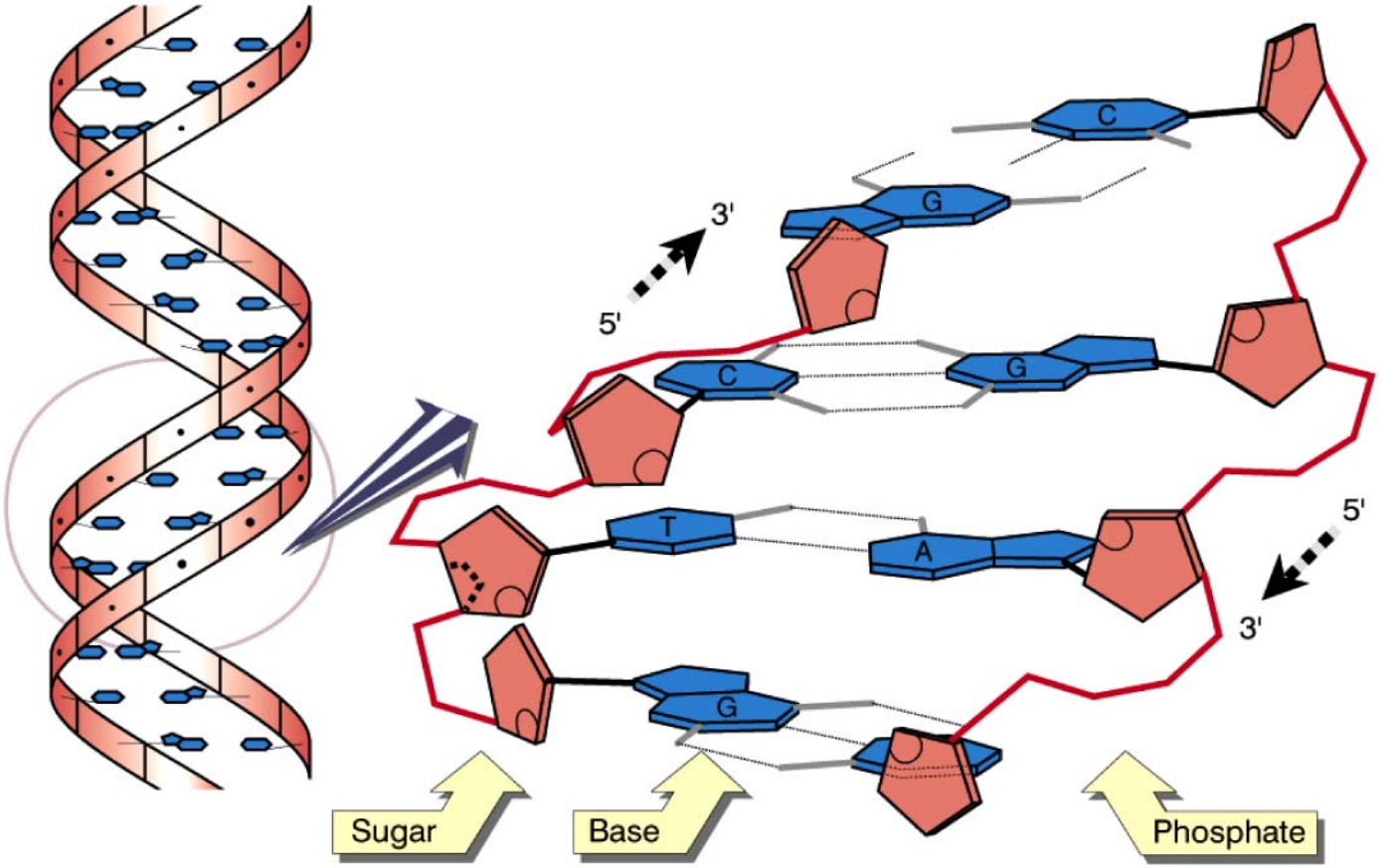
DNA/RNA: polynucleotide chains



Sugar (2’ OH=ribose, 2’ H=deoxyribose)

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”

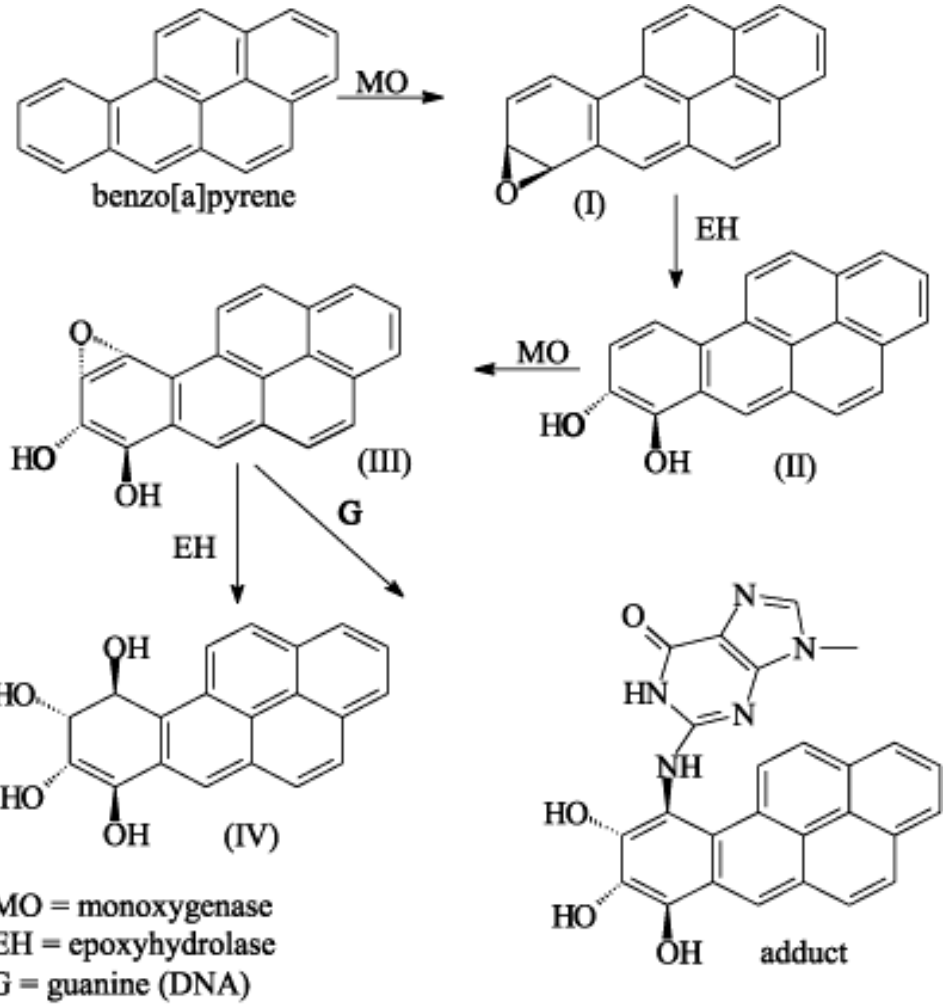
  –Jim Morrison

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)

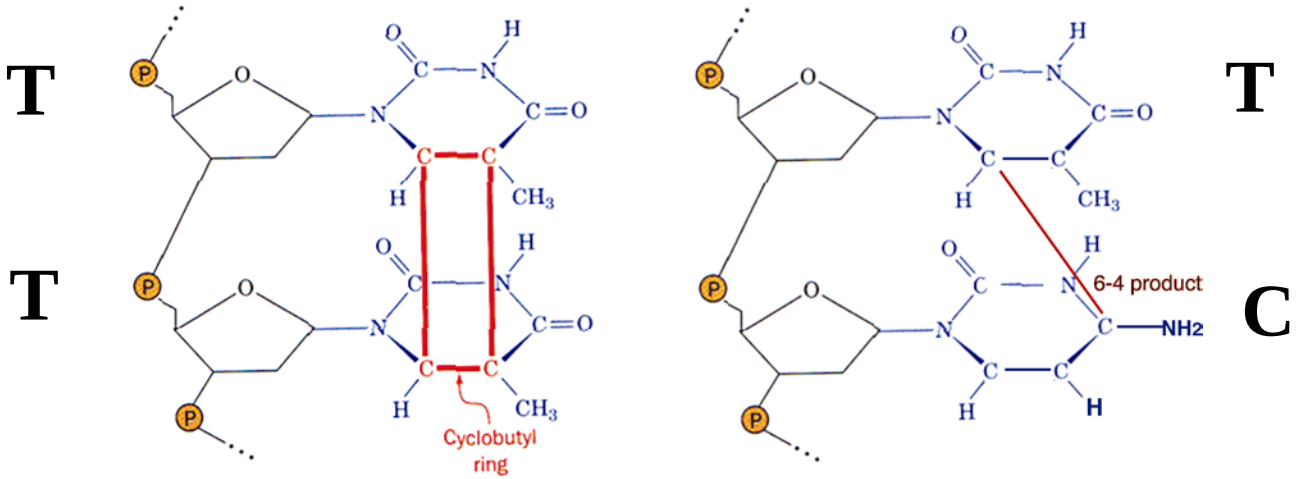
Adduct formation

- 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



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 adajacent bases, almost always adjacent pyrimidines
  - Distorts DNA (kink), can block transcription, replication, lead to mutation

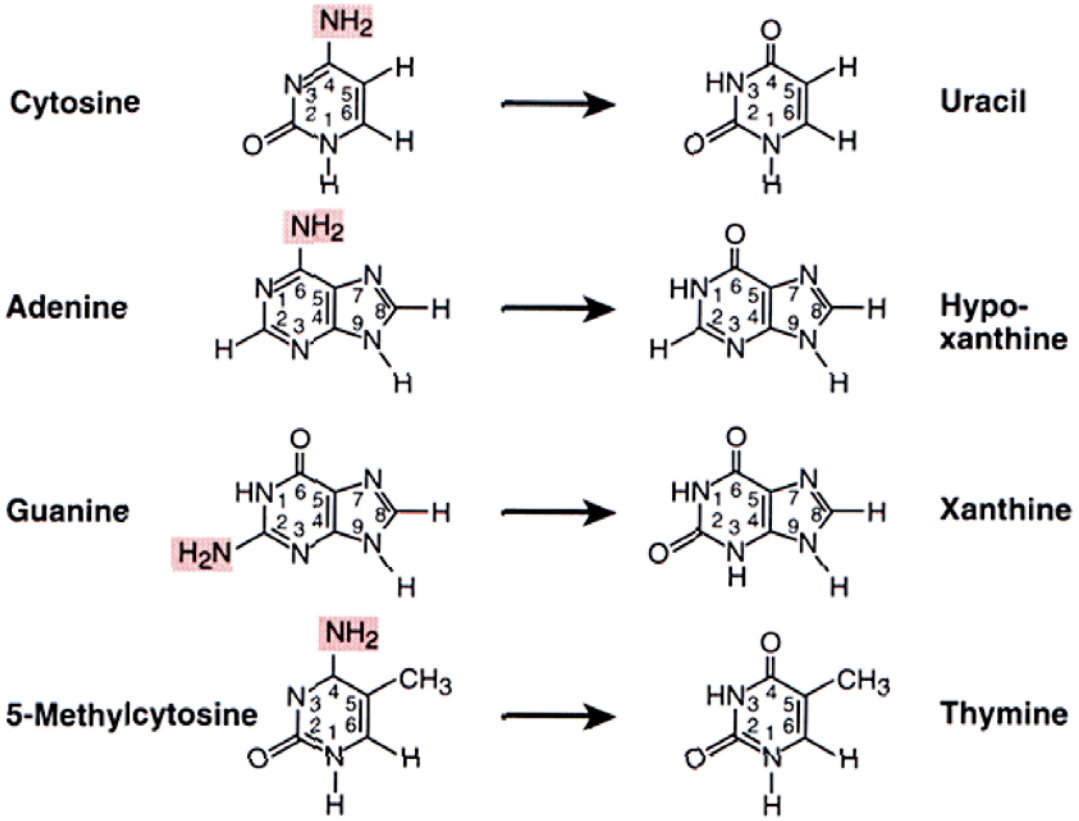


Spontaneous Damage: Base loss

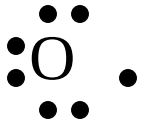
- Some times bases just fall off (more often than you might think; 10000/genome/generation)
- Bases gone, but phosphodiester backbone is still intact
- Purines more sensitive than pyrimidines (acid sensitive)
- Causes mutation, can lead to strand breaks

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

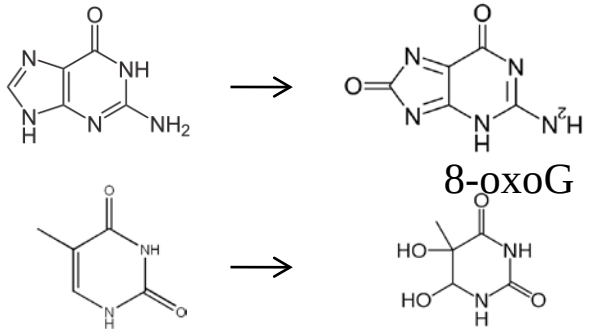


Oxidative stress

- Reactive oxygen species (ROS); things that are or give rise to oxygen with an unpaired electron; a free radical
- E.g hydroxyl radical 
- 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

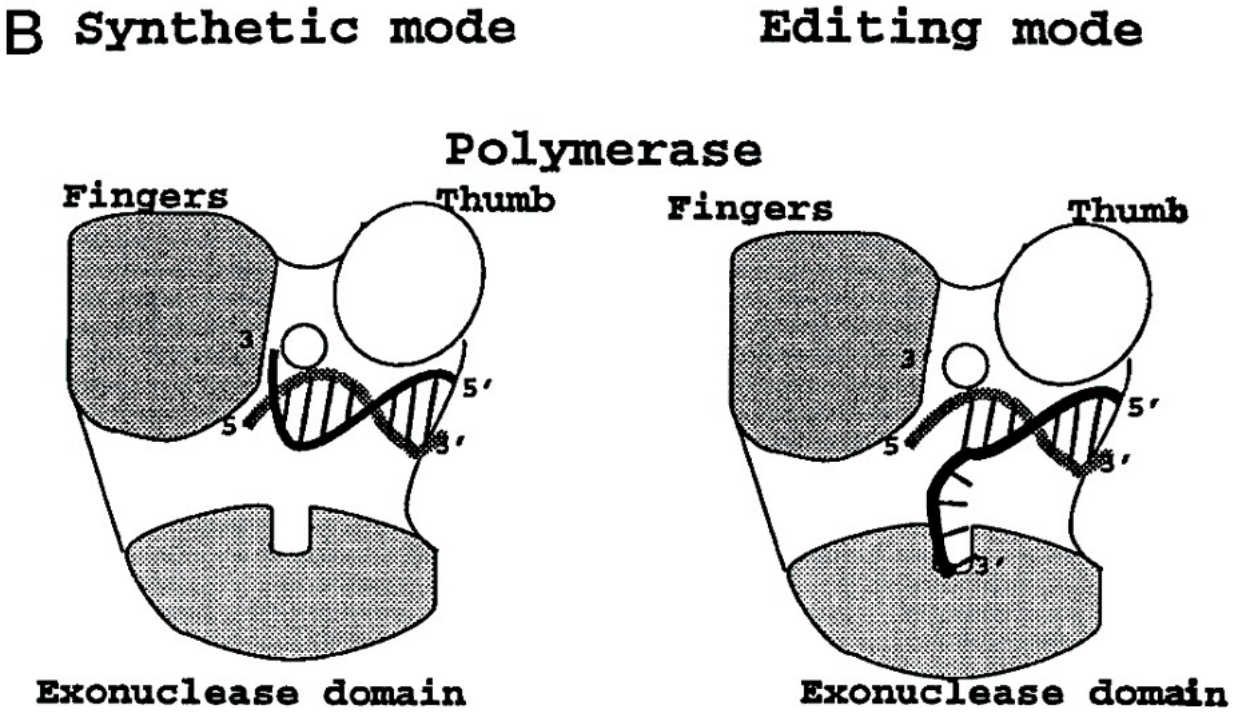
- Cross-linking agents; a special case where adduct-former is bifunctional (two reactive groups)
  - Intra-strand crosslinks: between adjacent nucleotides, like UV photoproducts
  - Inter-strand crosslinks: between nucleotides on opposite strands of a double helix
- Interstrand cross linkers completely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

Replication errors

- Not DNA damage per se.
- Substitutions
  - Misincorporation: $<1X10^{-6}$
  - Improved by proofreading, mismatch repair
  - Made worse by imbalanced nucleotide pools
  - Tautomers
- Slippage
  - Repetitive DNA, secondary structures

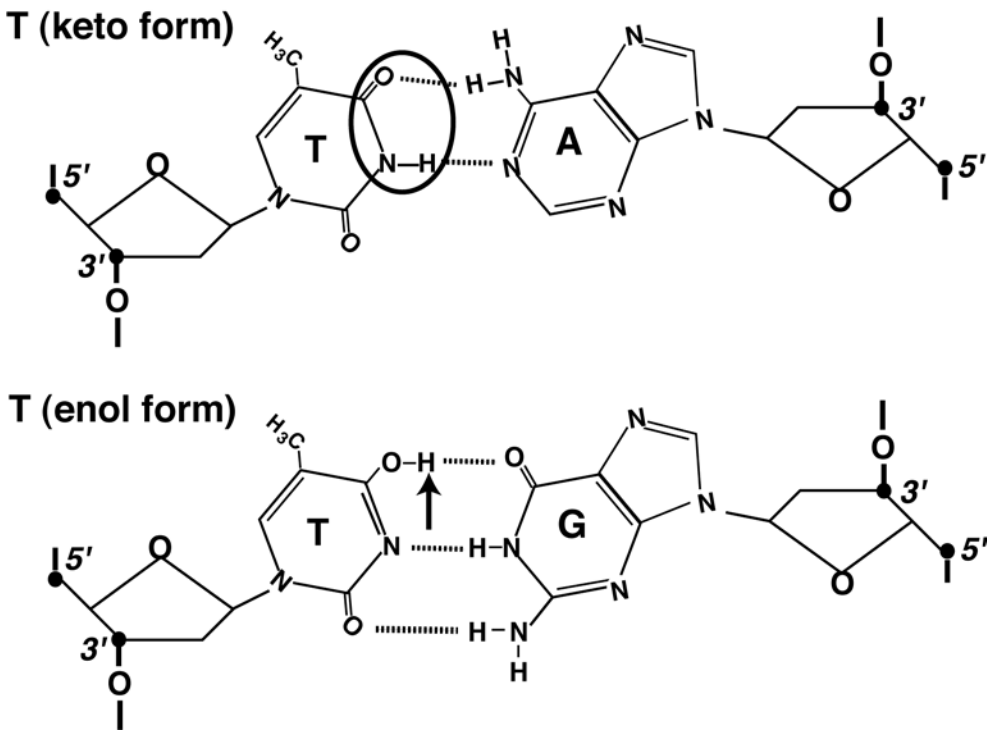
Proofreading/Editing

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



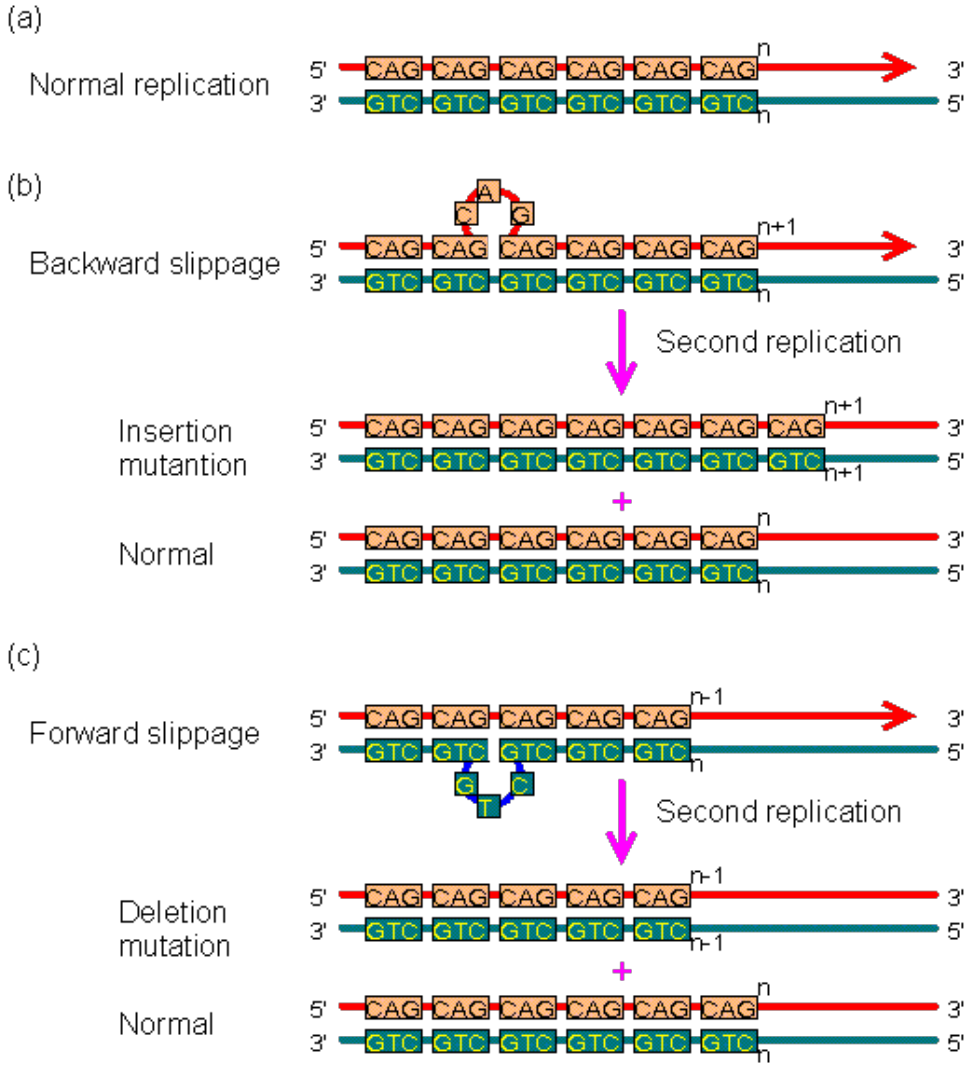
Tautomers

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



Microsattelite instability

- Slippage of primer or template during replication causes expansion/contraction of microsattelite



http://www.web-books.com/MoBio/Free/images/Ch7F3.gif

DNA damage and repair

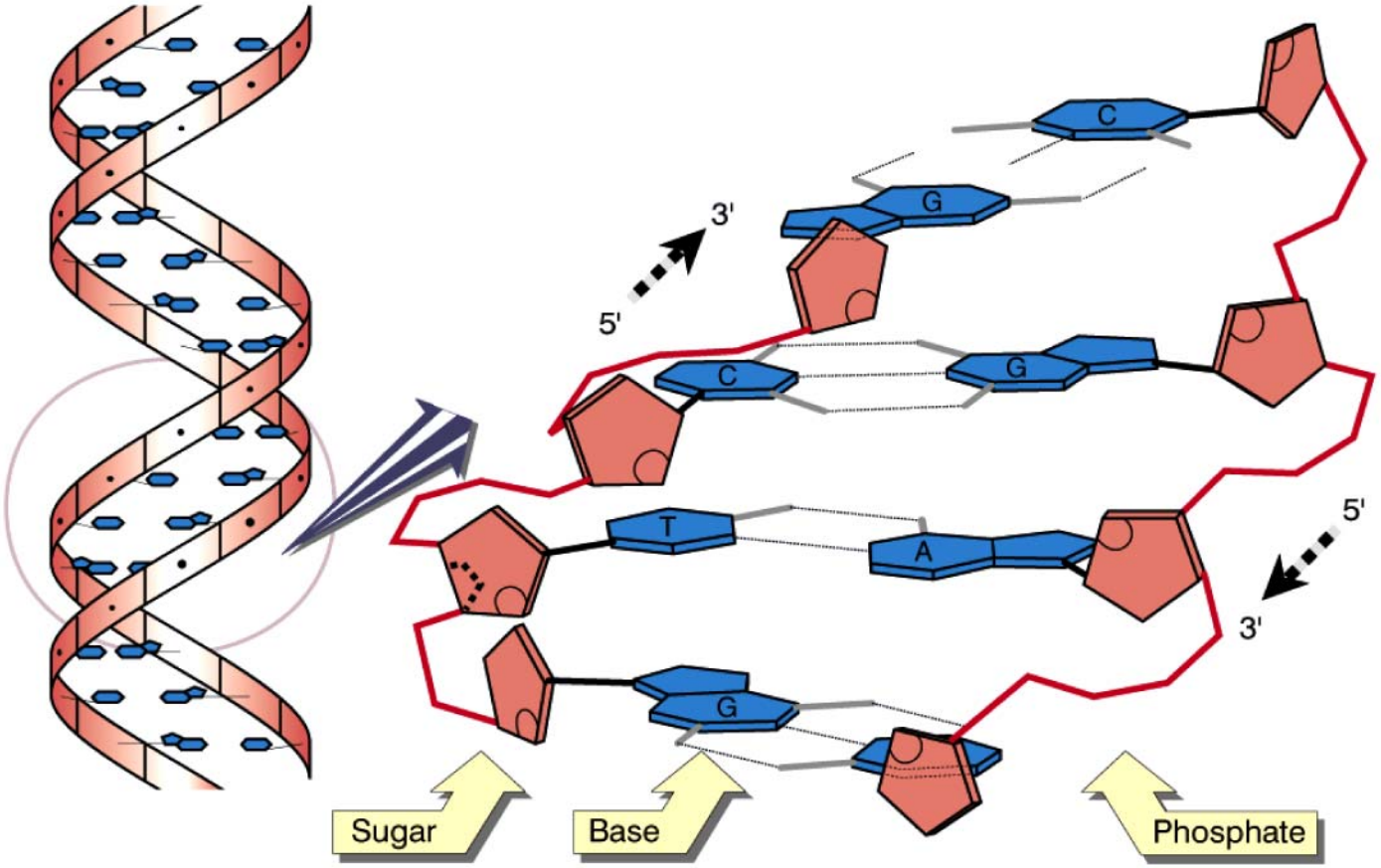
- Sources of DNA damage
  - Spontaneous
  - Environmental
    - Radiation
    - Carcinogens
  - Mistakes in replication

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

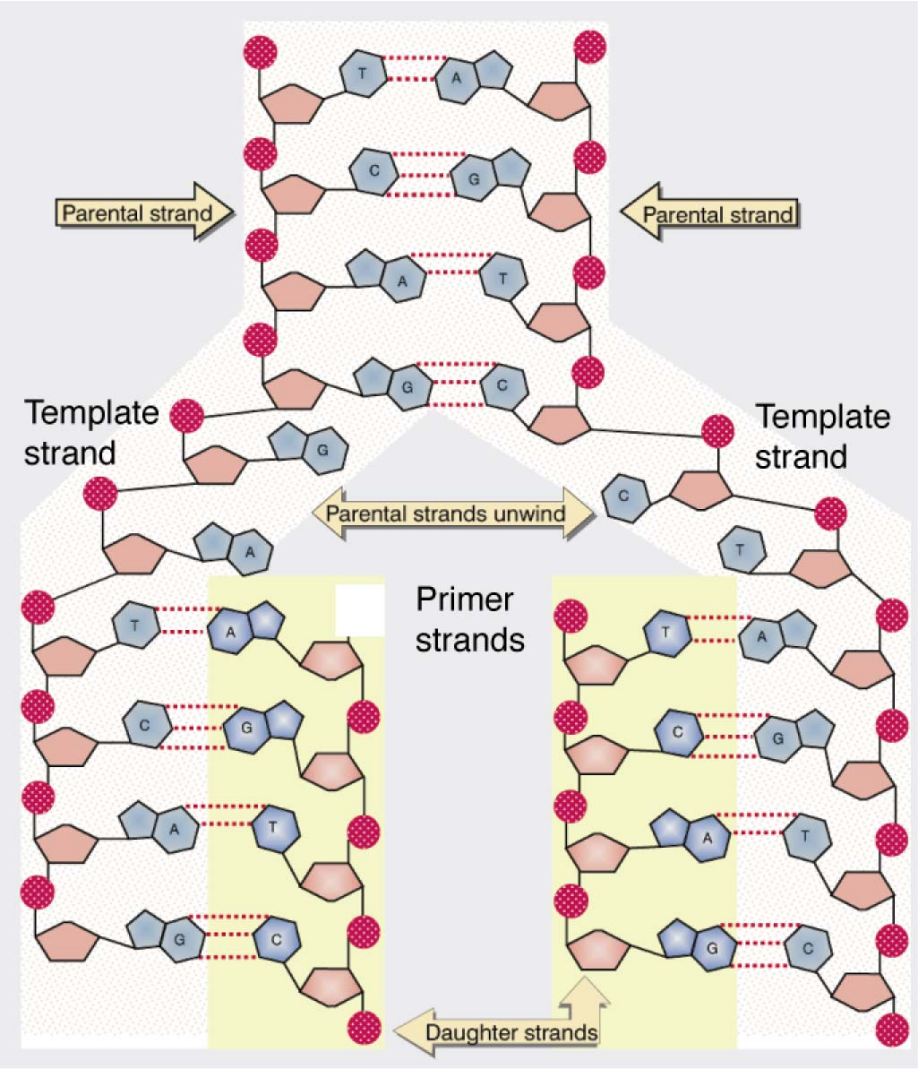
DNA is a double helix



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

Repair Pathway	Major functions
Base Excision Repair (BER)	Deaminations, Depurinations
Nucleotide Excision Repair (NER)	UV photoproducts, Adducts, Cross-links
Translesion synthesis (TLS)	Bypass all of the above
Mismatch Repair (MMR)	Replication errors
Homologous Recombination (HR)	Double strand breaks, Adducts, Cross-links
End joining (EJ)	Double strand breaks

Excision repair=BER, NER, MMR

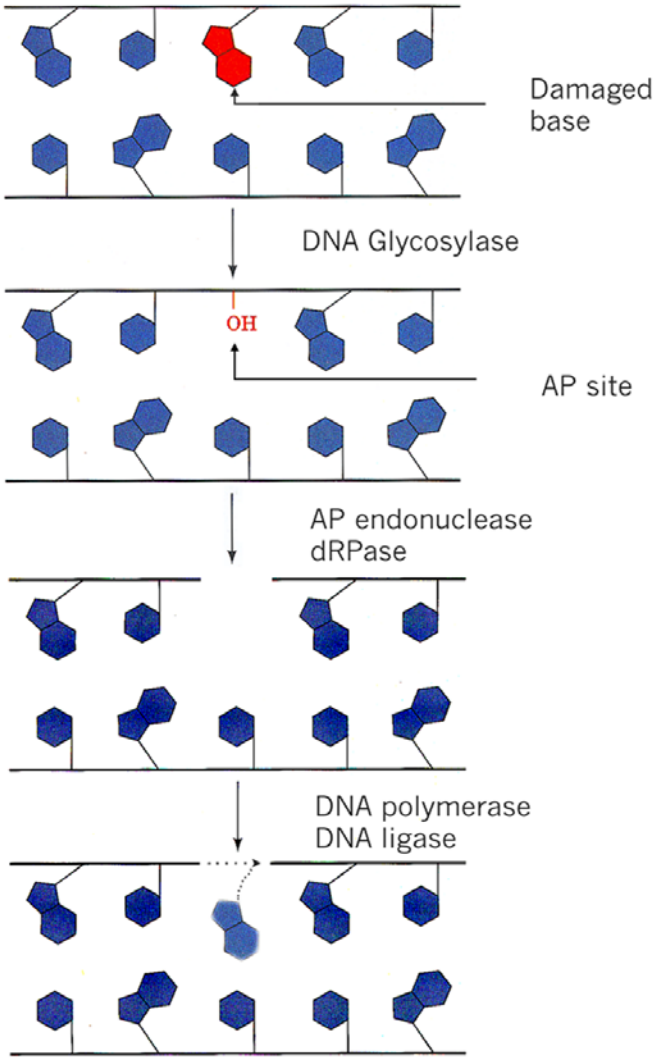
DSB repair=HR, EJ

Excision repair

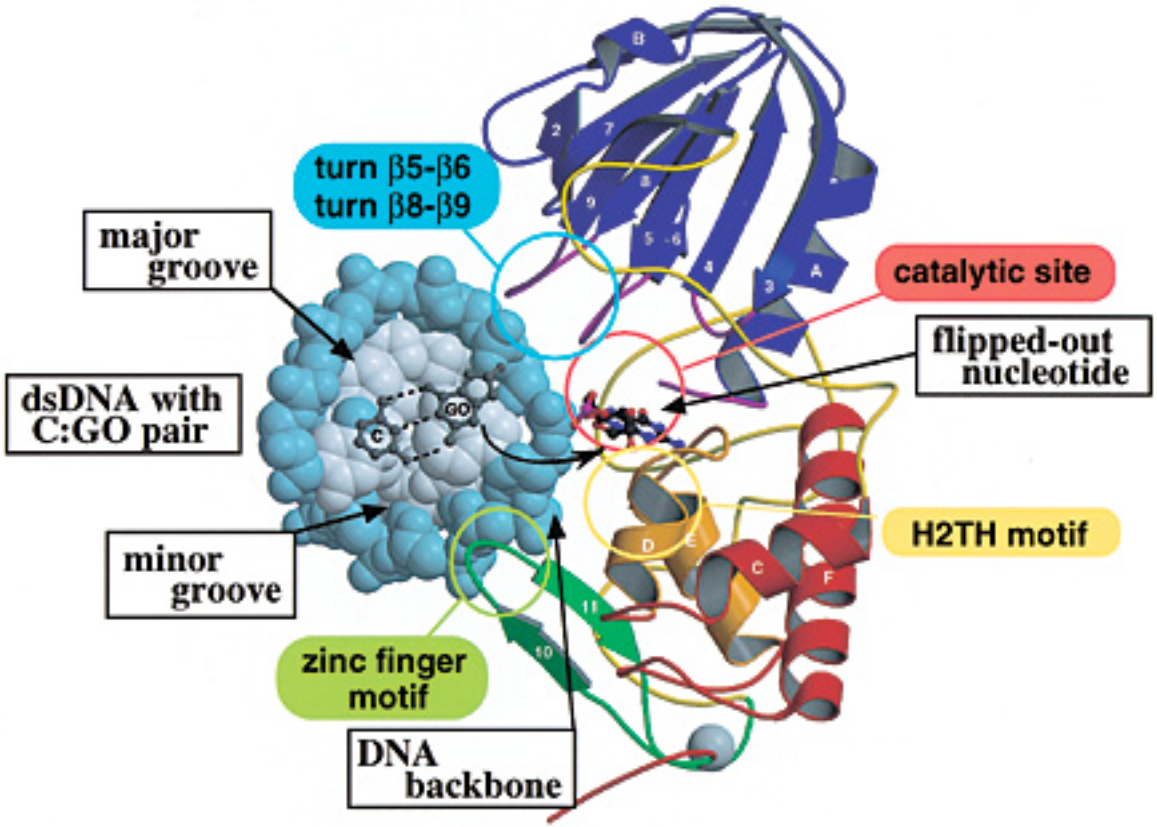
- Damage in one strand….remove it, fill in the gap (using un-damaged strand as template), ligate the remaining nick
- Same process…base excision repair, nucleotide excision repair, mismatch repair
- Contrast to double strand break repair (which has no template for repair)

Base excision repair (BER)

- Damage is recognized by glycosylase (different one for each type of damage)
- Common targets are de-amination products
- E.g. uracil glycosylase, hypoxanthine glyosylase

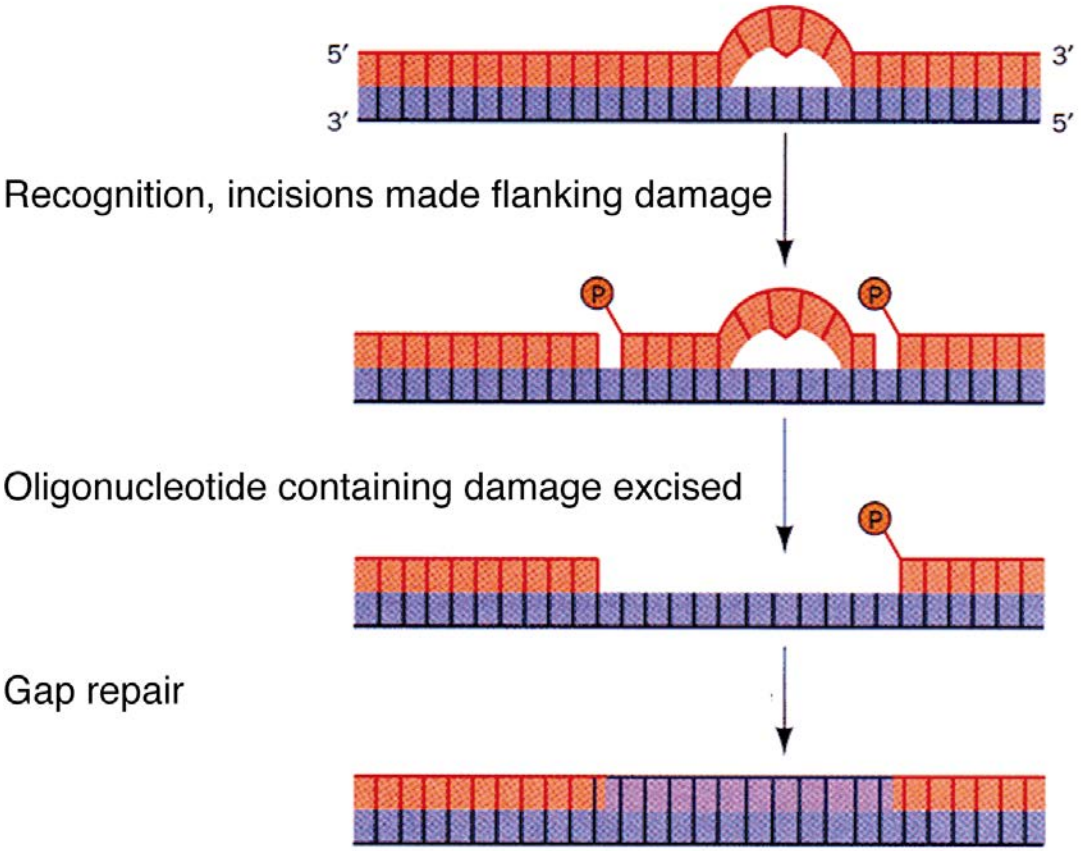


Glycosylase recognition



Nucleotide excision repair (NER)

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

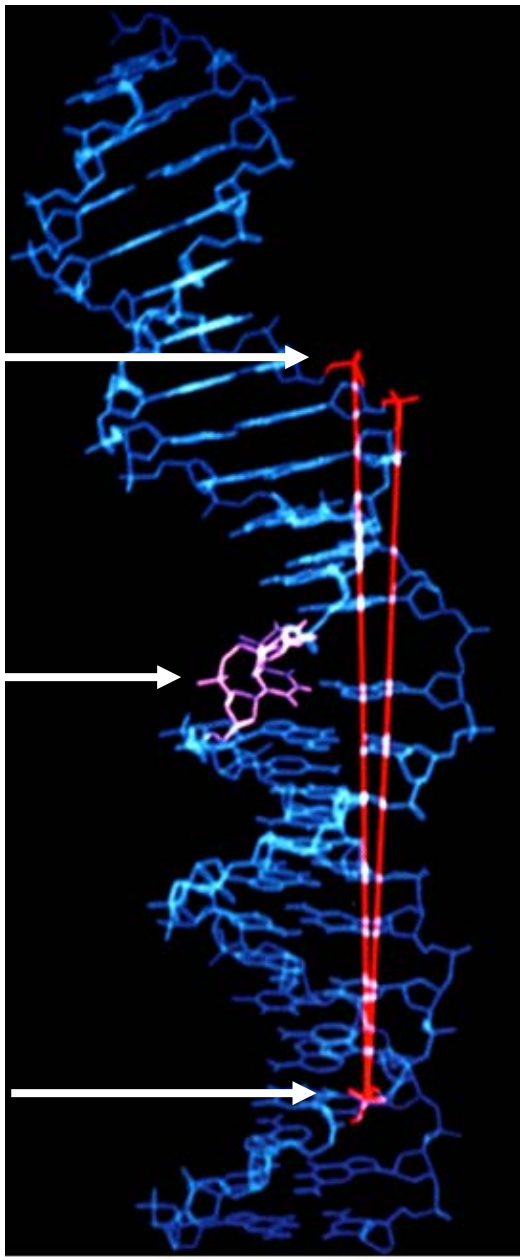


NER: recognition of damage

Excision site

Cyclobutane dimer

Excision site

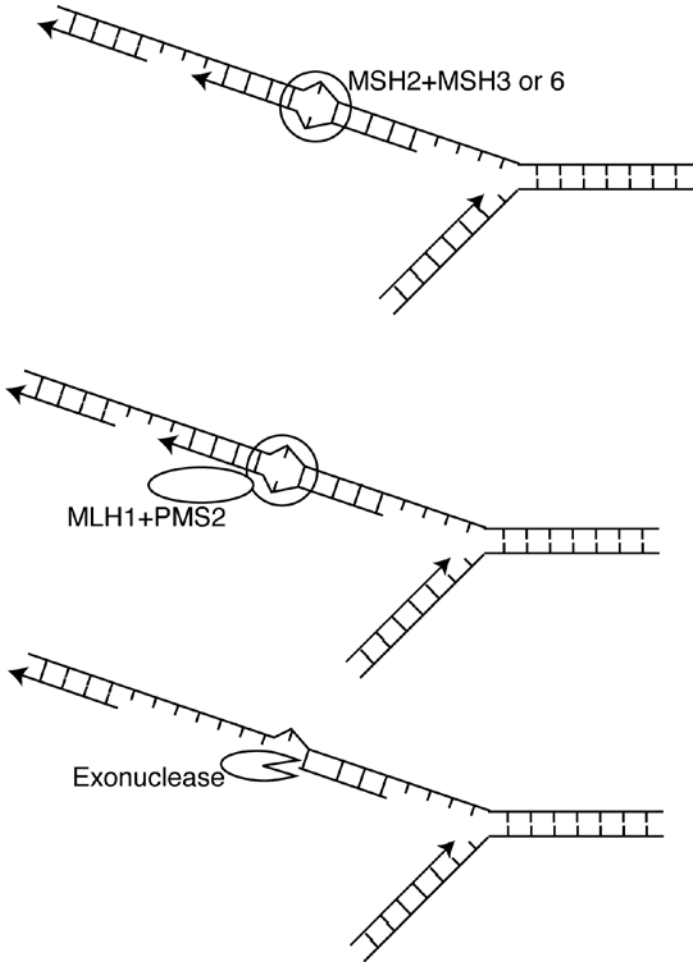


NER: Transcription coupled repair

- 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 (microsattelite 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

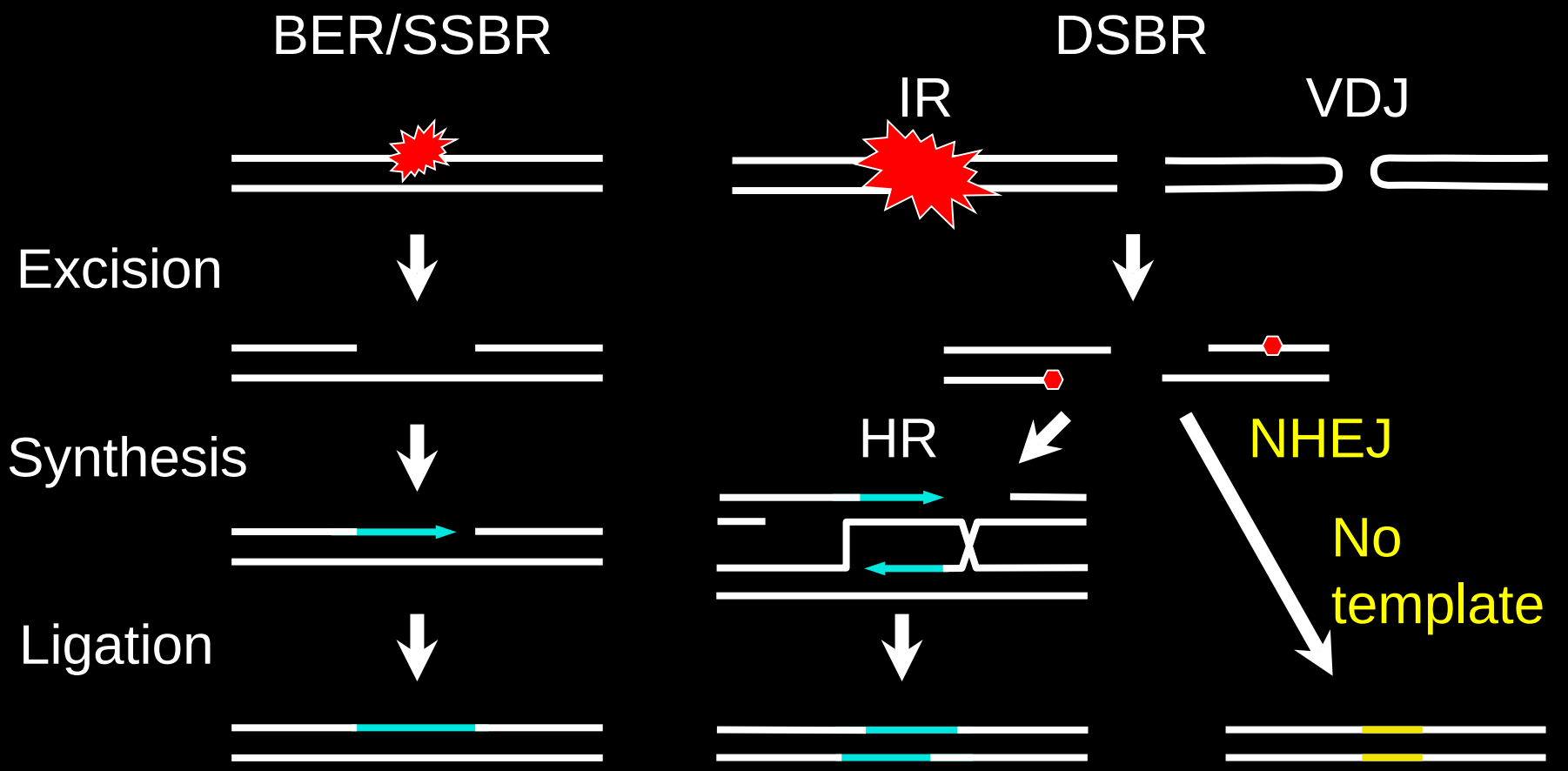
- How do you get double strand breaks?
  - Can be intentional (developmentally programmed)
    - Meiosis, VDJ recombination
  - By accident
    - Ionizing radiation, replication through incompletely repaired damage
  - Therapeutic
    - Chemotherapy, radiation therapy
  - Malicious intent
    - Transposons,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

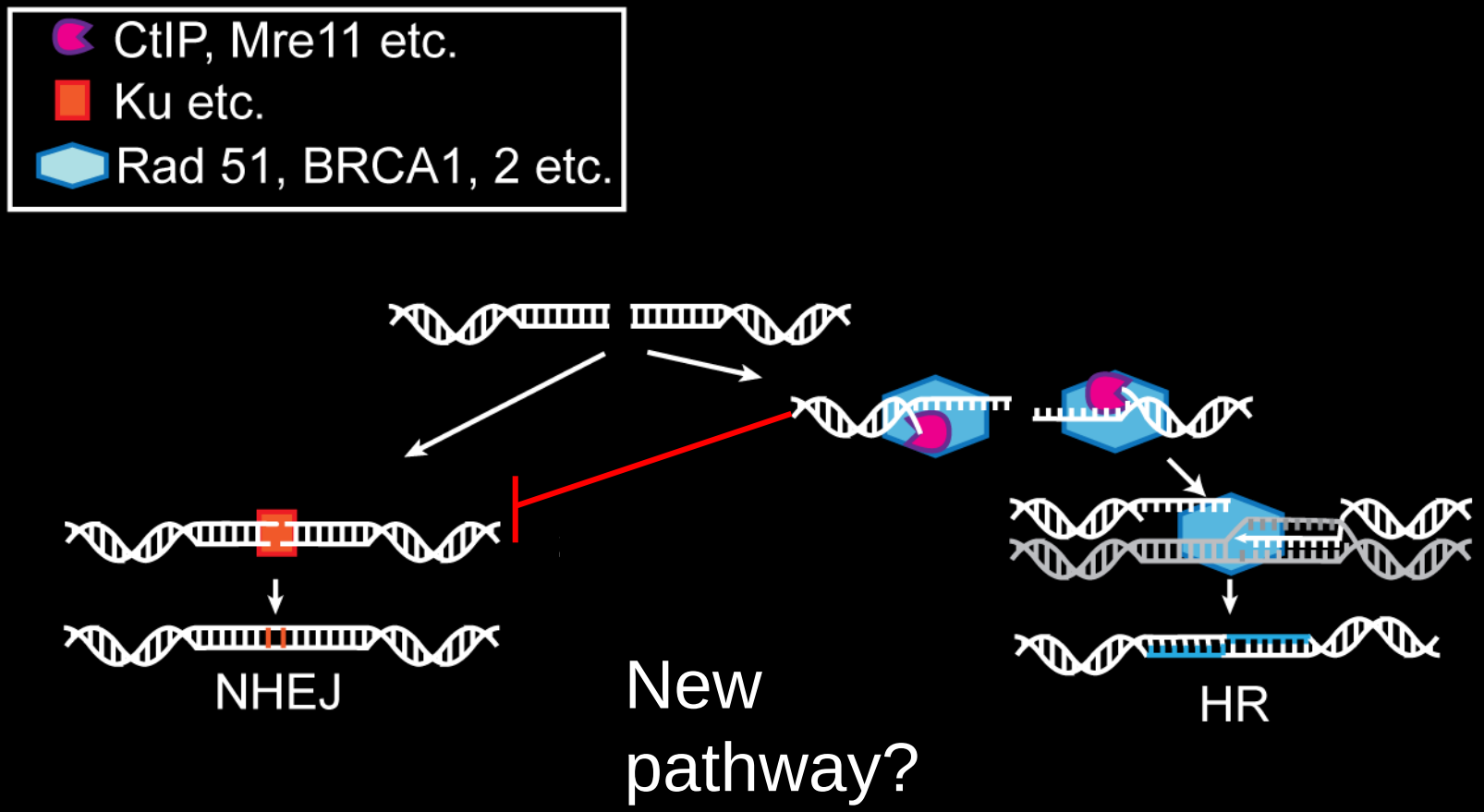
DNA repair


BER/SSBR
DSBR
IR
VDJ
Excision
Synthesis
Ligation
HR
NHEJ
No
template
Accurate
Cheap
Accurate
Expensive
Inaccurate?
Cheap?

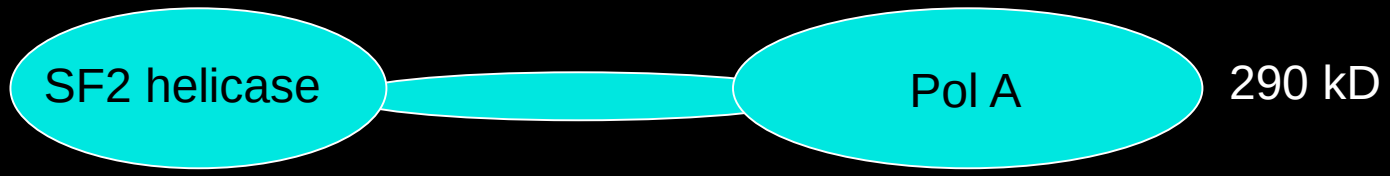
Breast cancer and DSB repair

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

DSB repair



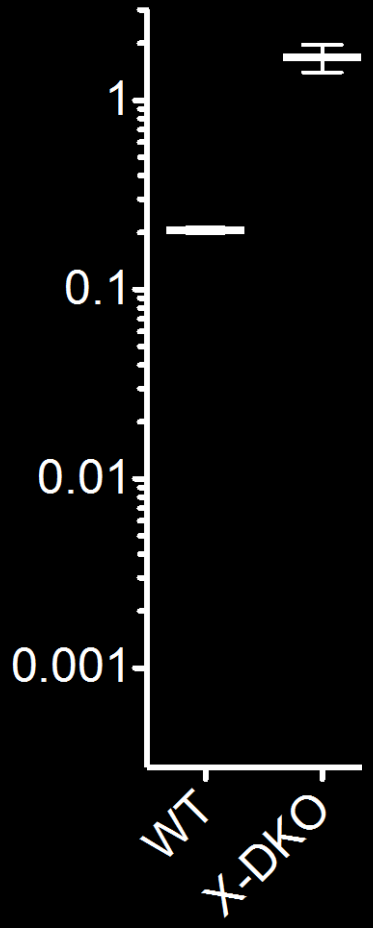
Pol θ aka Polq aka Mus308



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