

Module 2: Measuring gene expression

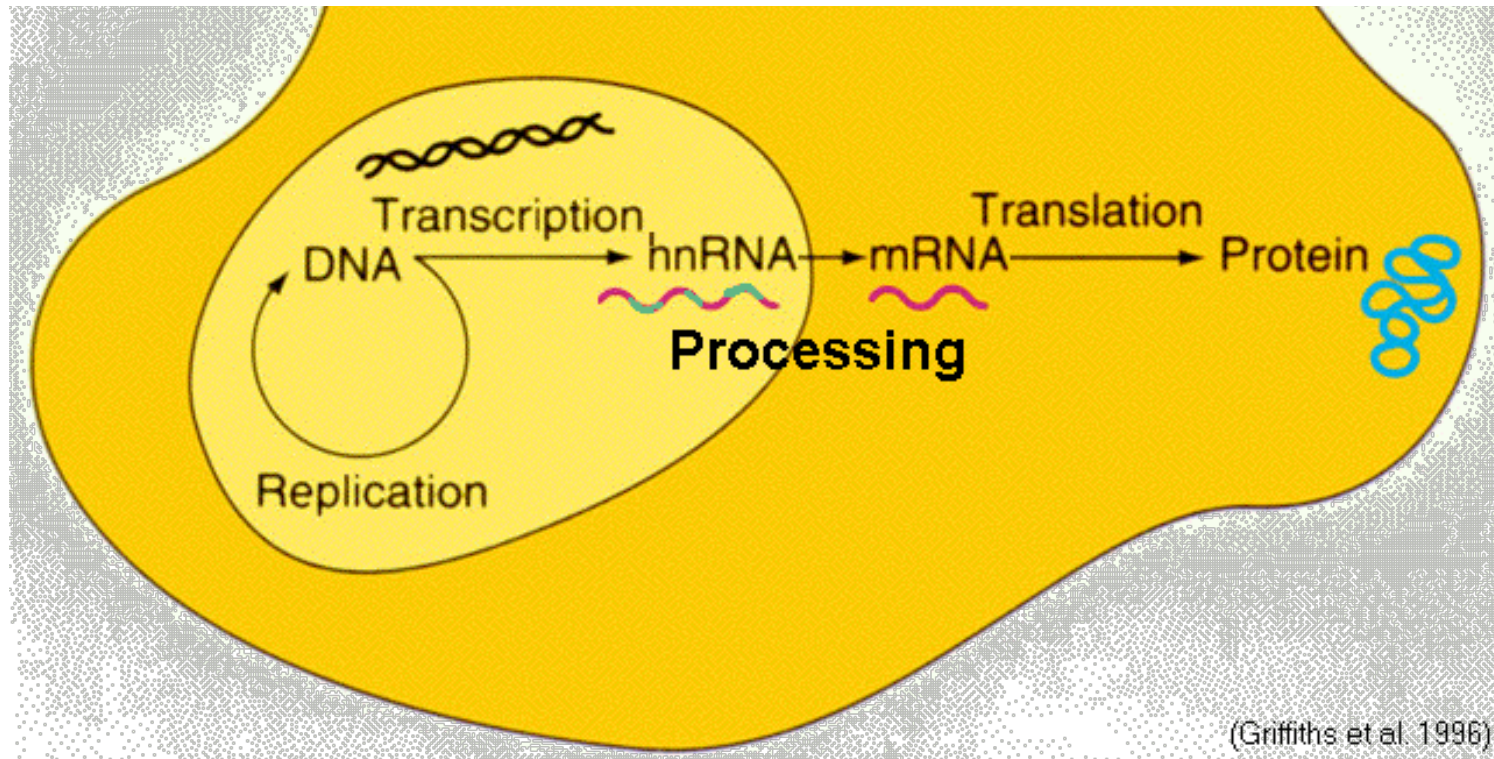
DNA damage and repair pathways

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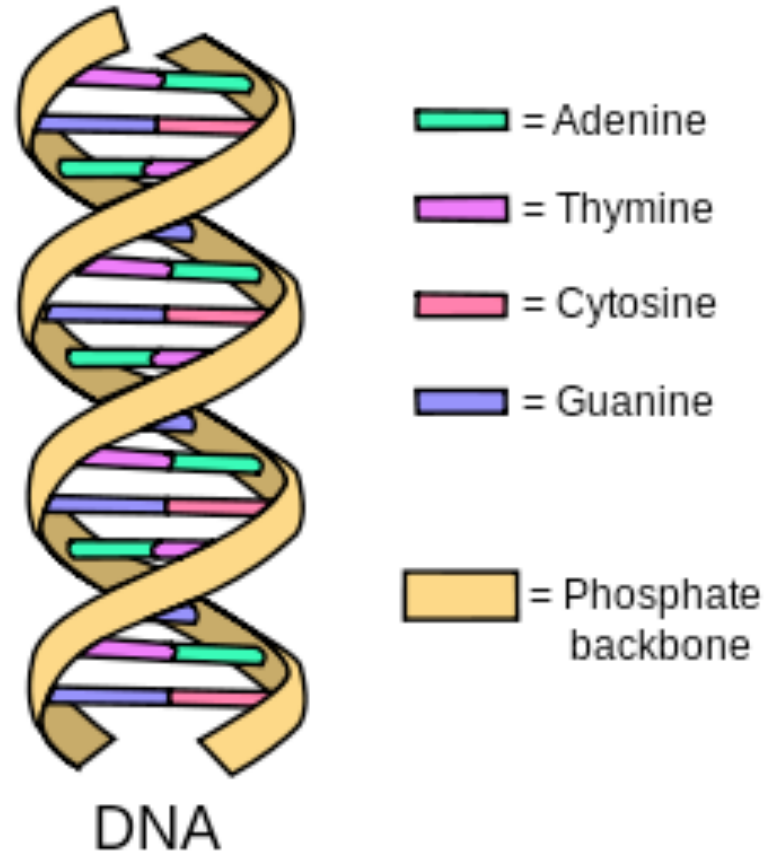
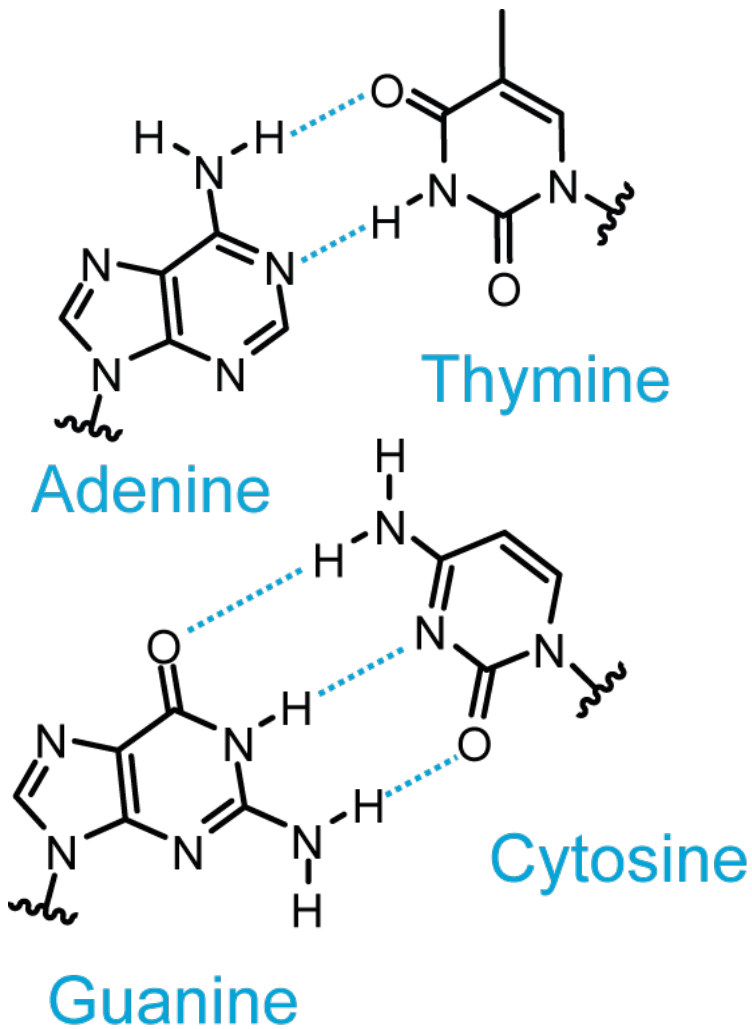
What will we study in Mod 2?

1. DNA damage and repair pathways
2. Role of BRCA2 in DNA repair and cancer
3. Pathway addiction in cancer treatment
4. Differential gene expression in cancer cell lines
5. Laboratory skills:
 - Mammalian cell tissue culture procedures
 - Big data analysis methods
 - Molecular biology techniques

DNA is the hereditary material in all known organisms



Each human cell contains 6 billion bp

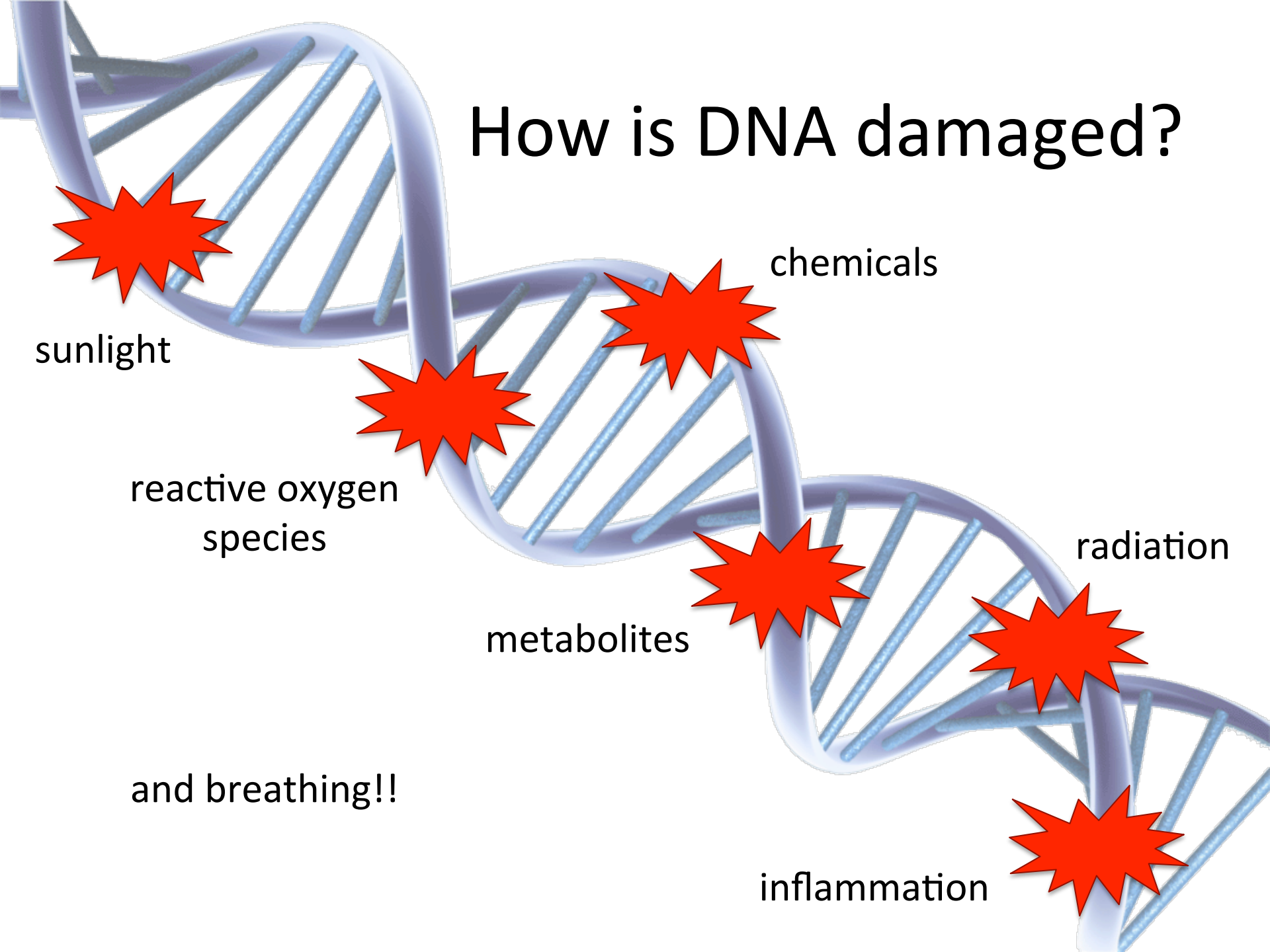


In the time it takes you to read this sentence, your cells will accumulate ~10 trillion DNA lesions throughout your body!

Assumptions:

20,000 lesions / cell / day, 10^{13} cells in body, 4 sec to read

How is DNA damaged?



sunlight

reactive oxygen
species

and breathing!!

chemicals

metabolites

inflammation

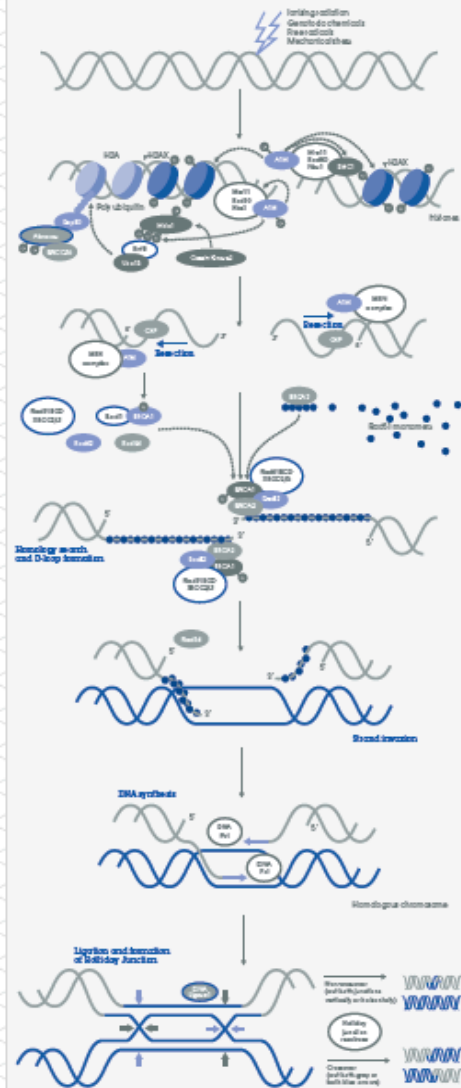
radiation

DNA damage \neq mutation

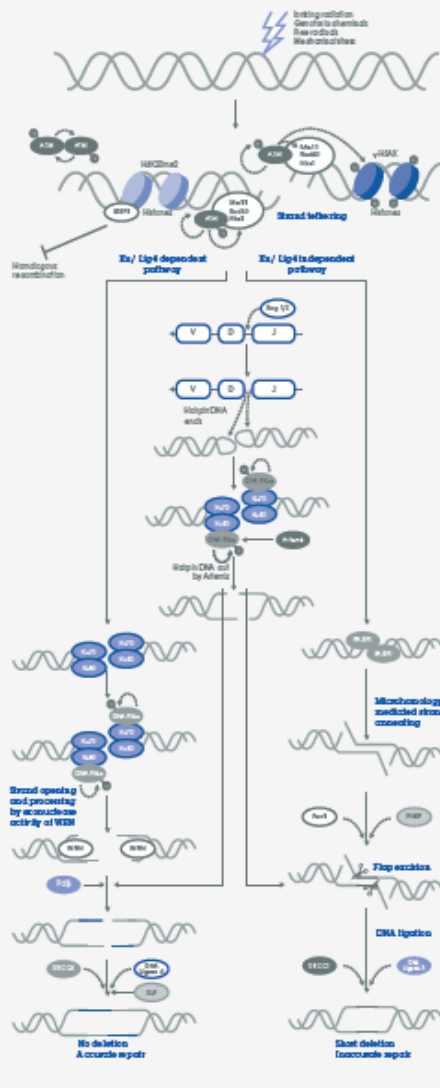
- Damage is the creation of a DNA lesion
 - Basepair ‘decorations’
 - Strand breaks
- Mutations occur when the damage is ‘copied’ during replication and becomes ingrained in the genetic code

How is DNA damage repaired?

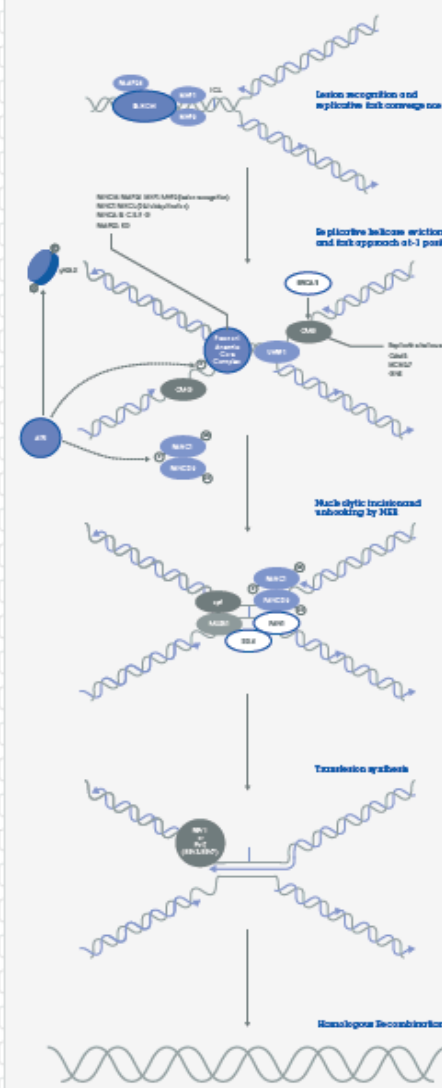
Homologous recombination



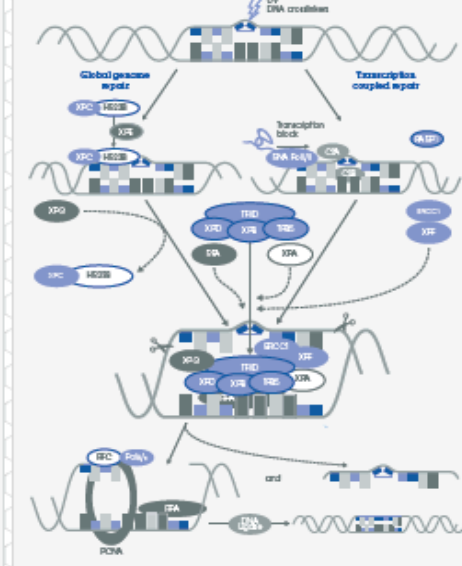
Nonhomologous end joining



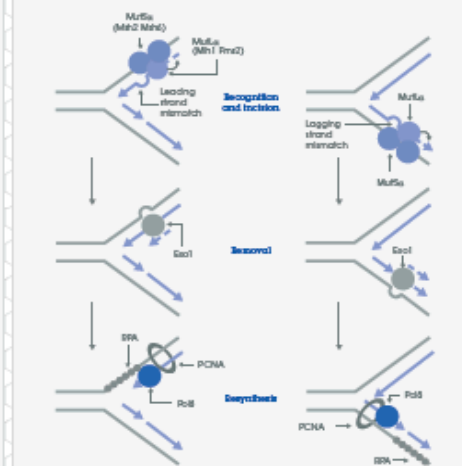
Interstrand cross-link repair



Nucleotide excision repair



Mismatch repair



The Nobel Prize in Chemistry 2015



Photo: A. Mahmoud

Tomas Lindahl

Prize share: 1/3



Photo: A. Mahmoud

Paul Modrich

Prize share: 1/3

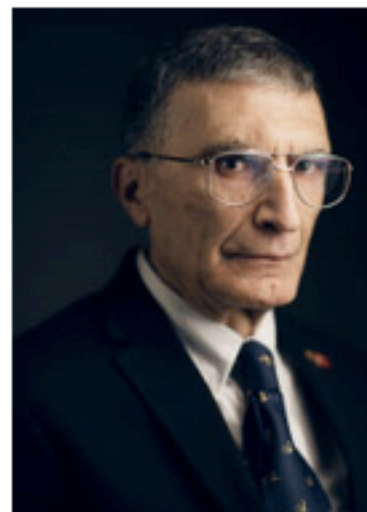


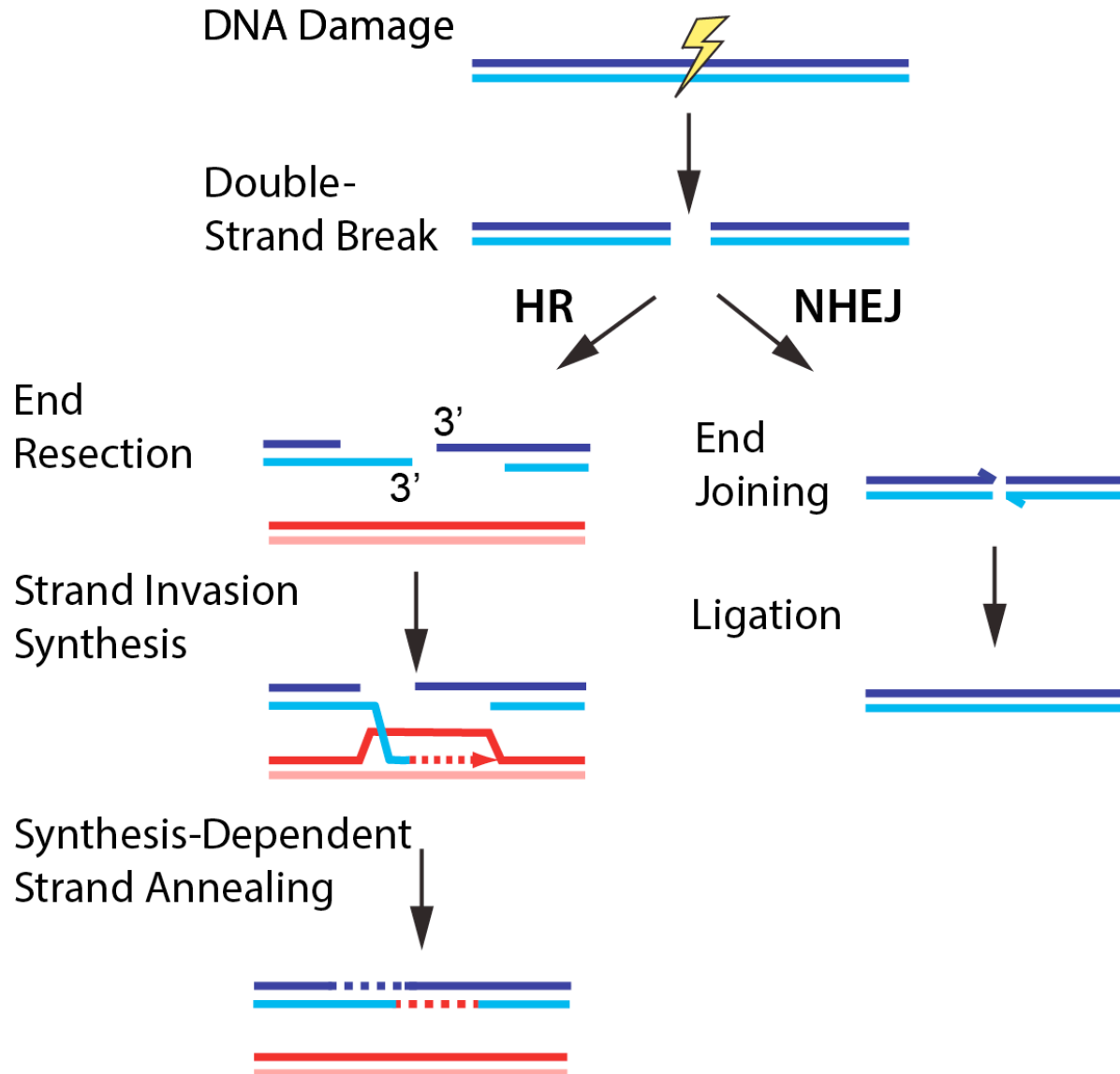
Photo: A. Mahmoud

Aziz Sancar

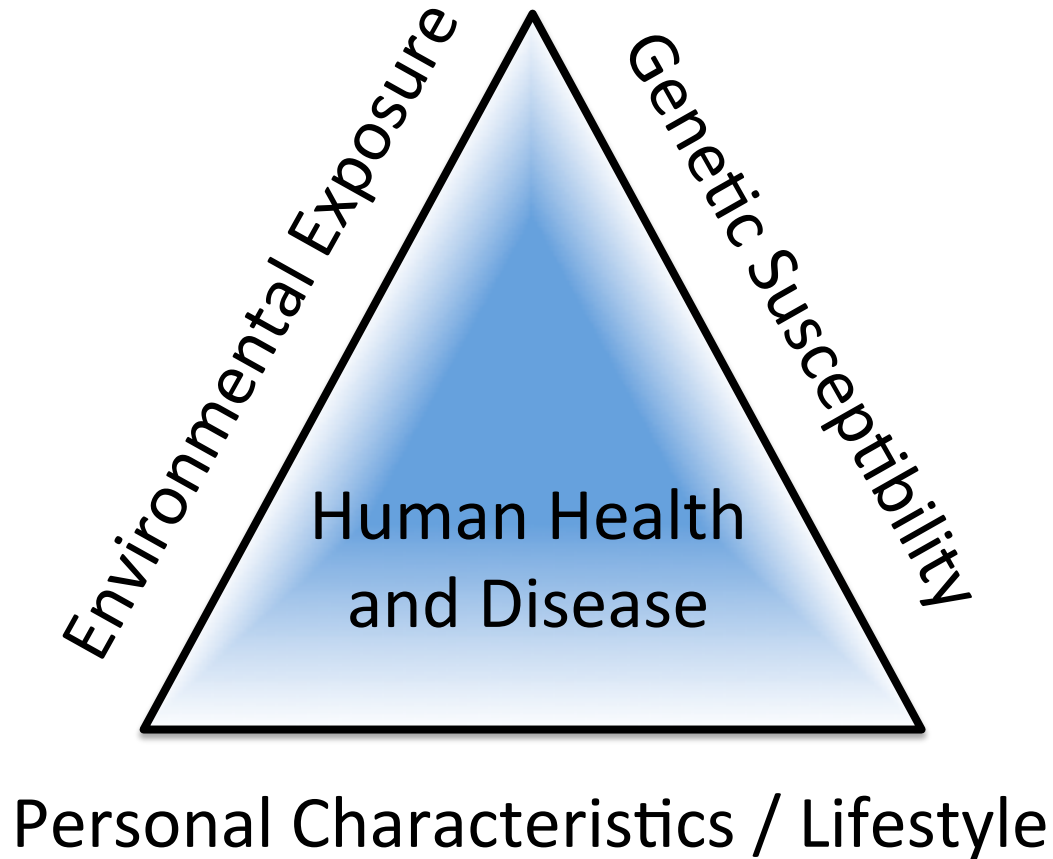
Prize share: 1/3

The Nobel Prize in Chemistry 2015 was awarded jointly to Tomas Lindahl, Paul Modrich and Aziz Sancar *"for mechanistic studies of DNA repair"*.

HR and NHEJ repair double-strand breaks

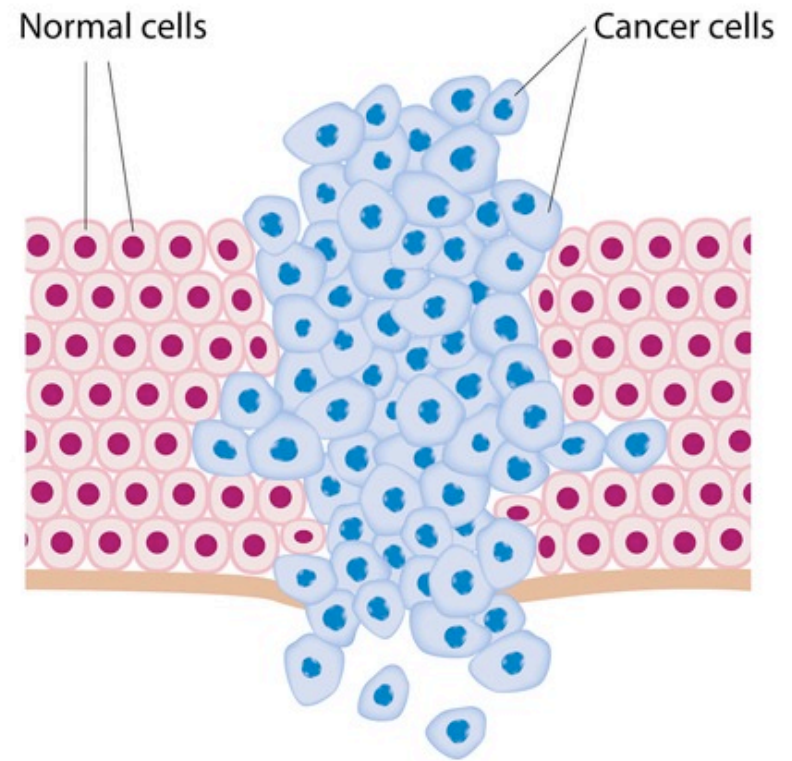


How does this relate to cancer?



Last year, ~600K cancer deaths in US

- Abnormal cell growth that invades nearby tissues
 - Metastasis is the spread from original site
- Undifferentiated and unresponsive to cell signaling cues



Xeroderma pigmentosum increases risk of skin cancer by 2,000-fold

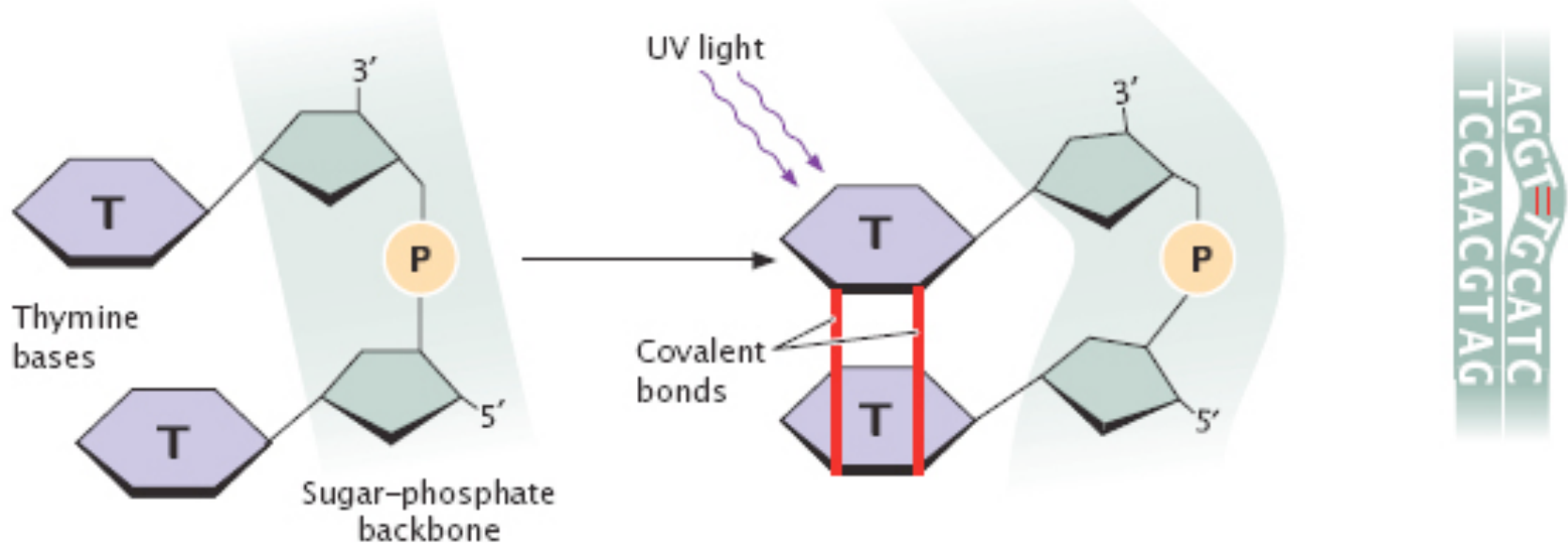
- Rare autosomal recessive genetic disorder in DNA repair pathway
 - Nucleotide excision repair (NER)



- Deficient in ability to correct damage caused by ultraviolet light

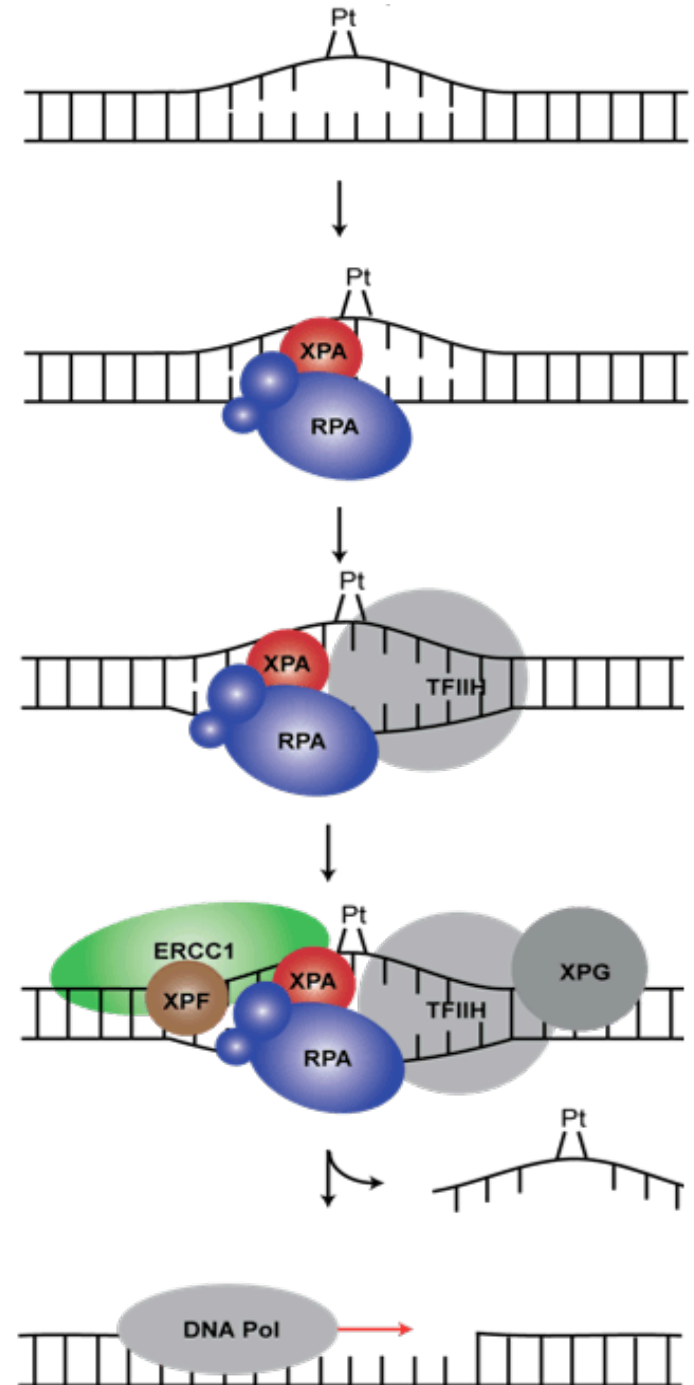
UV light induces pyrimidine dimers

- Neighboring thymines covalently bind
- Results in DNA lesion that prohibits replication

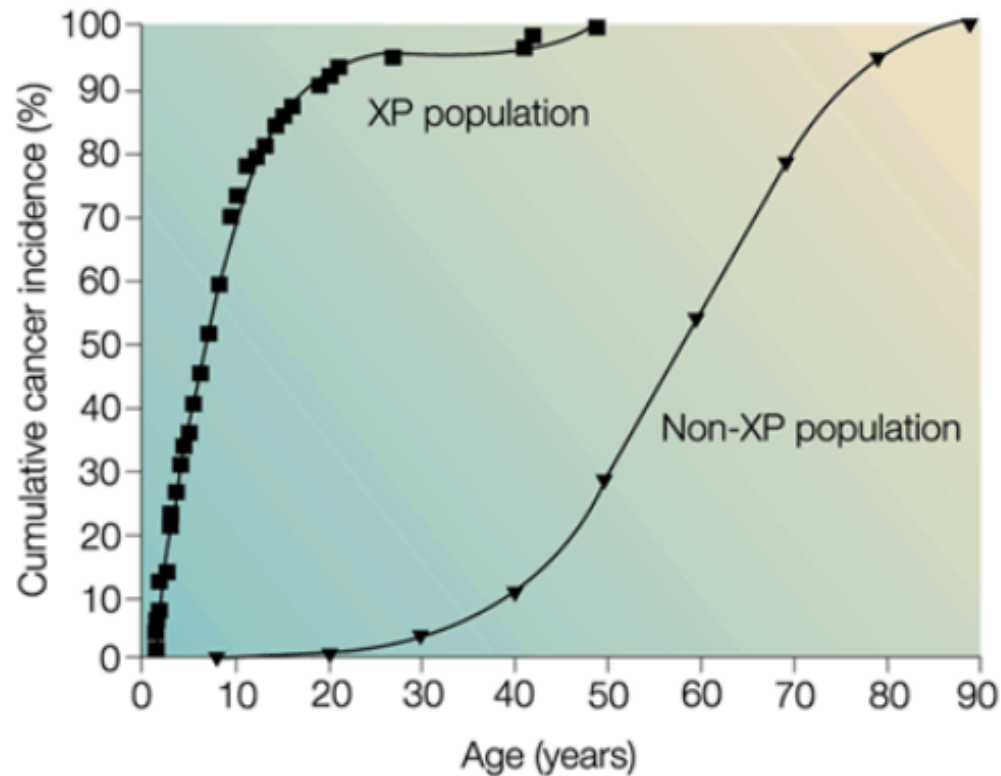


NER corrects thymine dimers

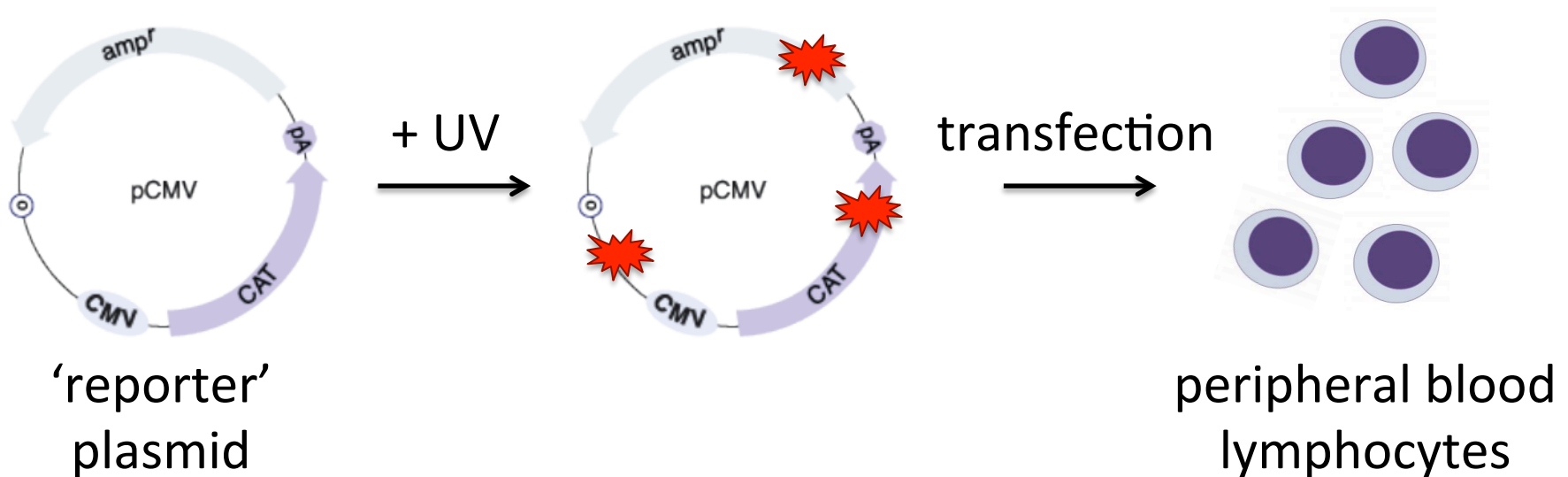
- Segment of DNA containing the lesion is removed
- New DNA is synthesized by polymerase
 - Undamaged strand used as template



Mutations in NER enzymes accelerate cancer onset

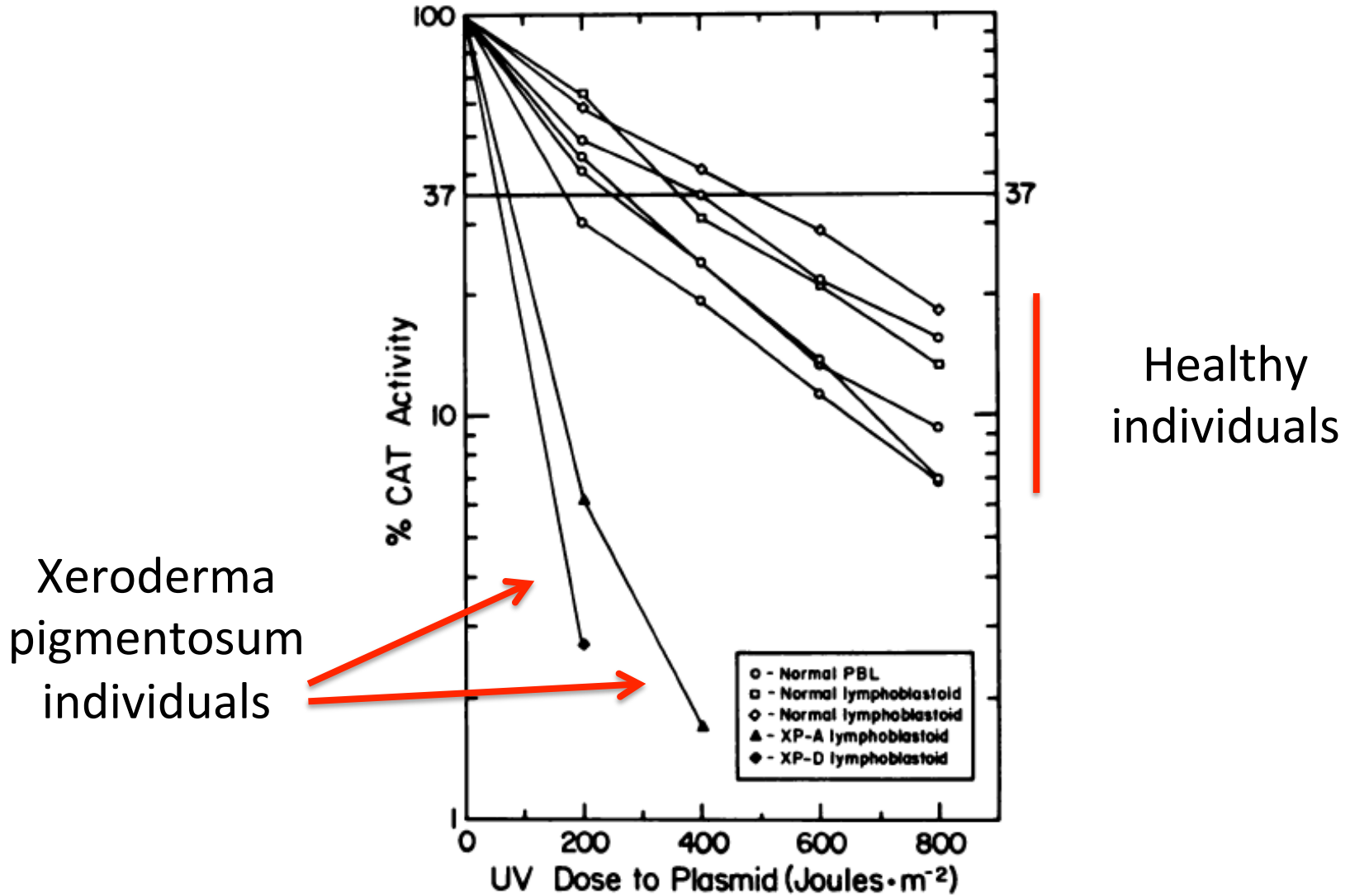


Does variation exist in the 'normal' population?



Then, measure Cat enzymatic activity to determine repair capacity

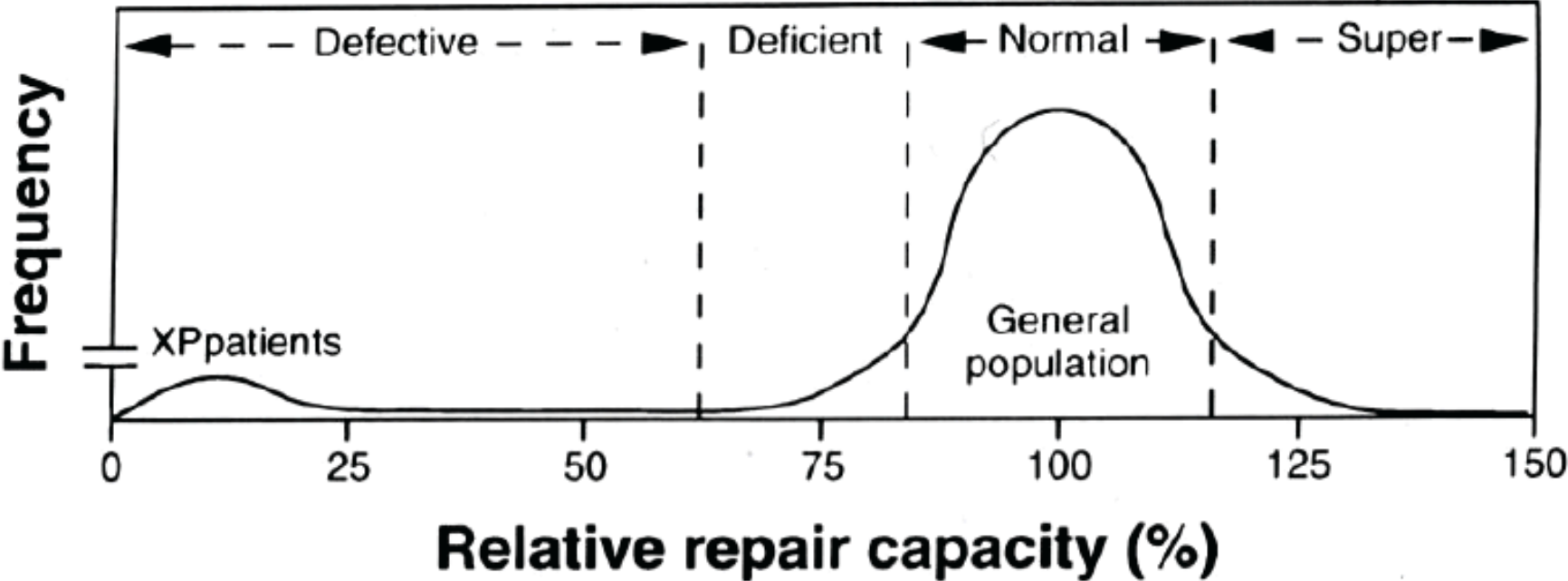
Yes!



Healthy individuals

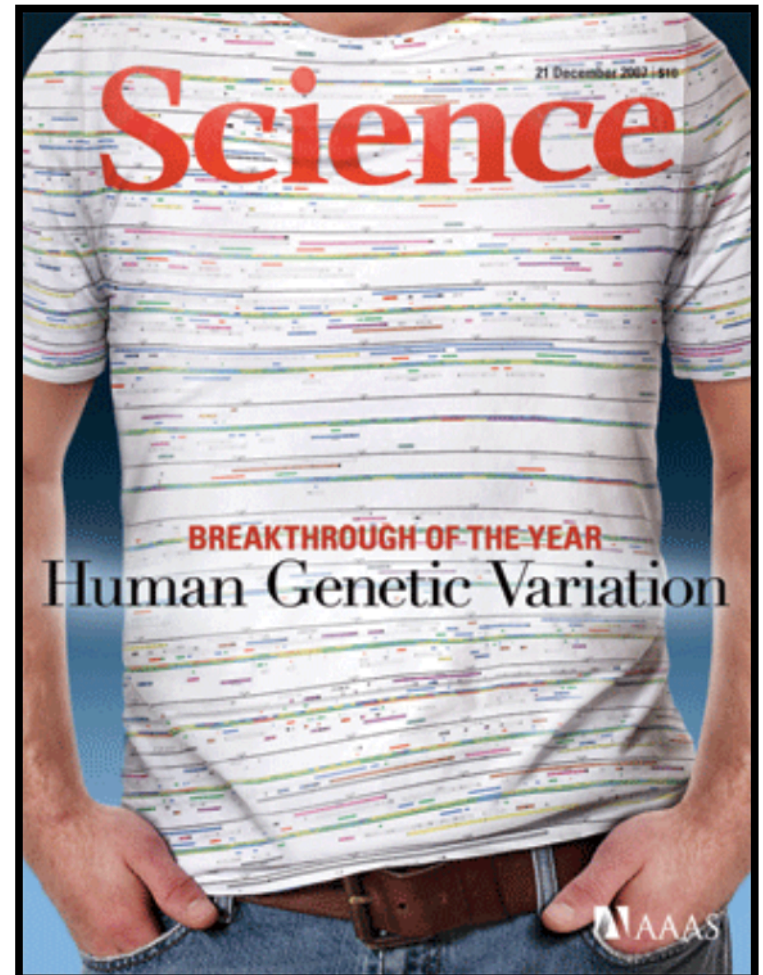
Xeroderma pigmentosum individuals

Normal population includes deficient and super repairers

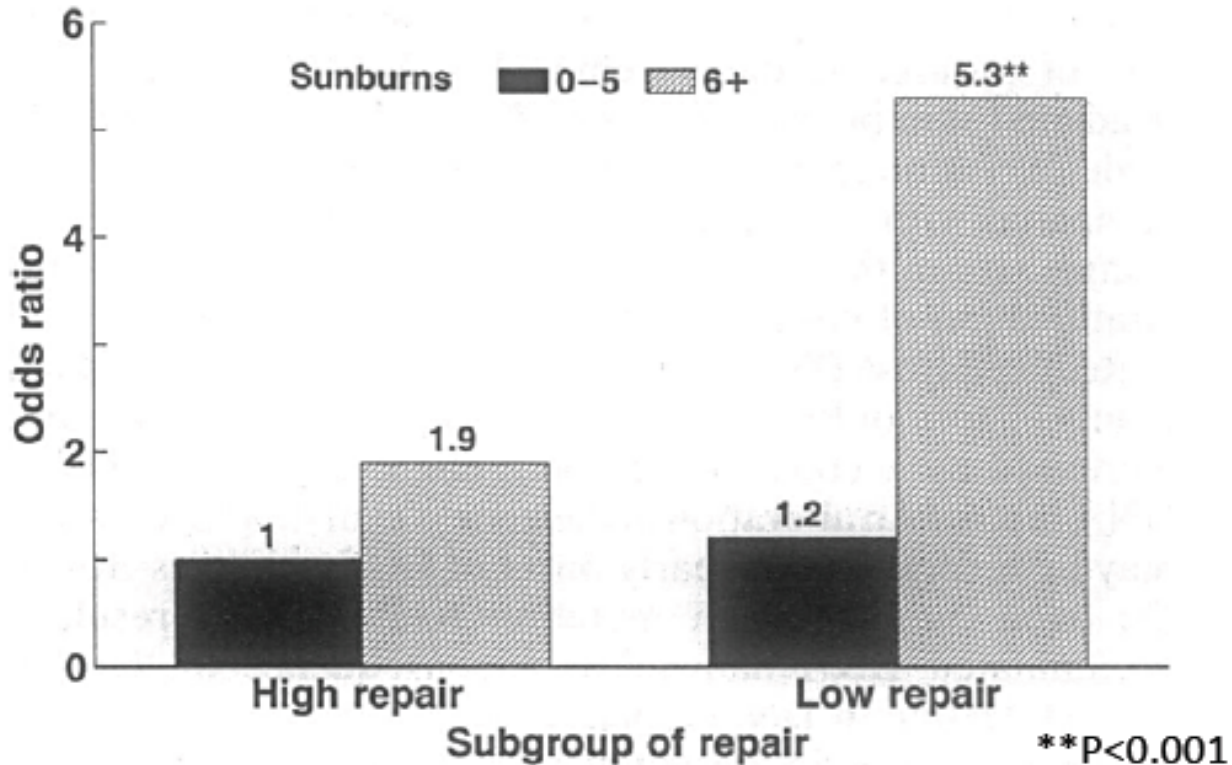


Natural sequence variation exists throughout the genome

- Single nucleotide polymorphisms (SNPs) occur every $\sim 1,000$ bp
 - If you were to compare two individuals, would find ~ 6 million SNP variants
- Each SNP represents single bp change (difference) in DNA sequence

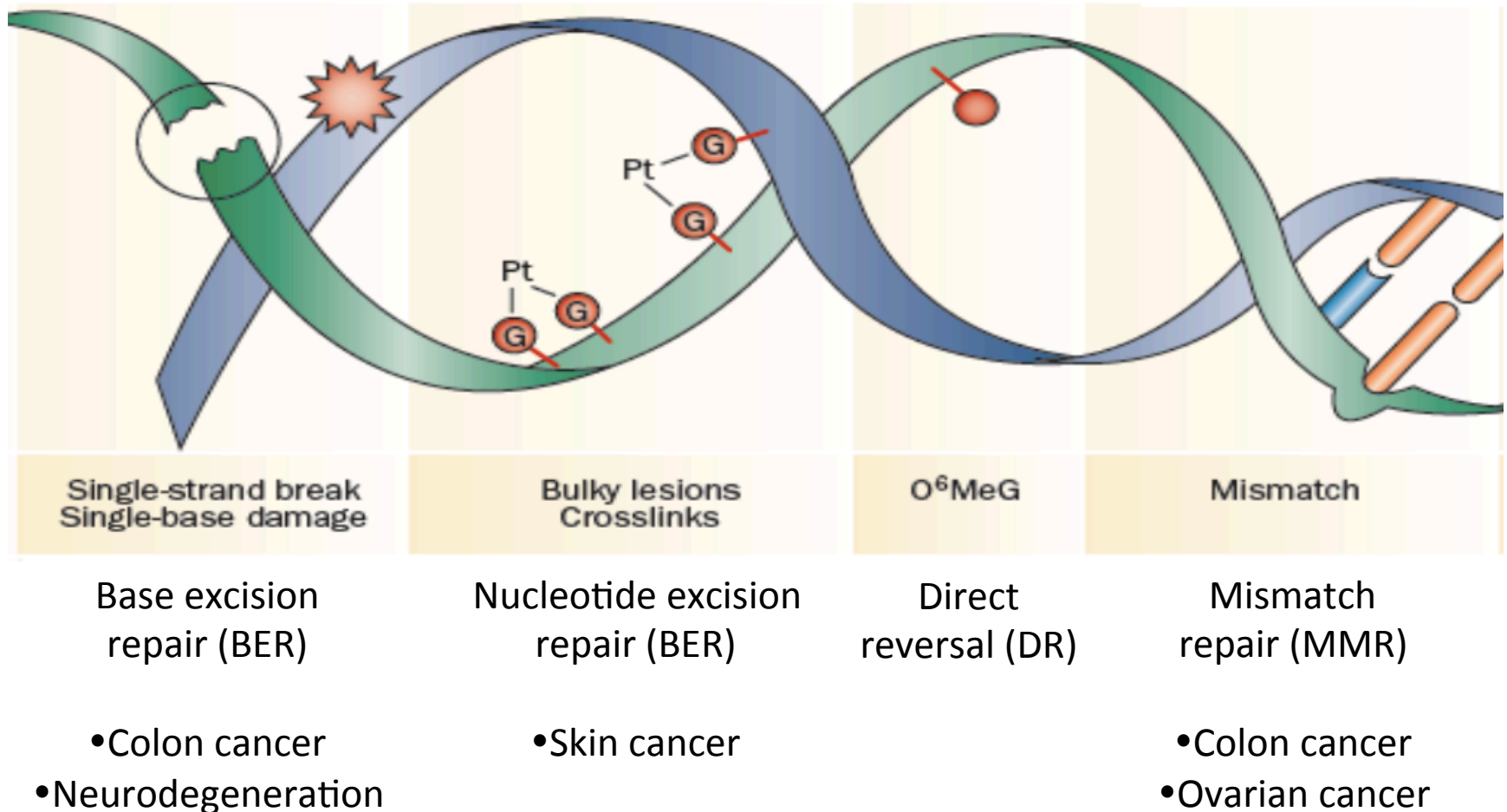


What does this mean?

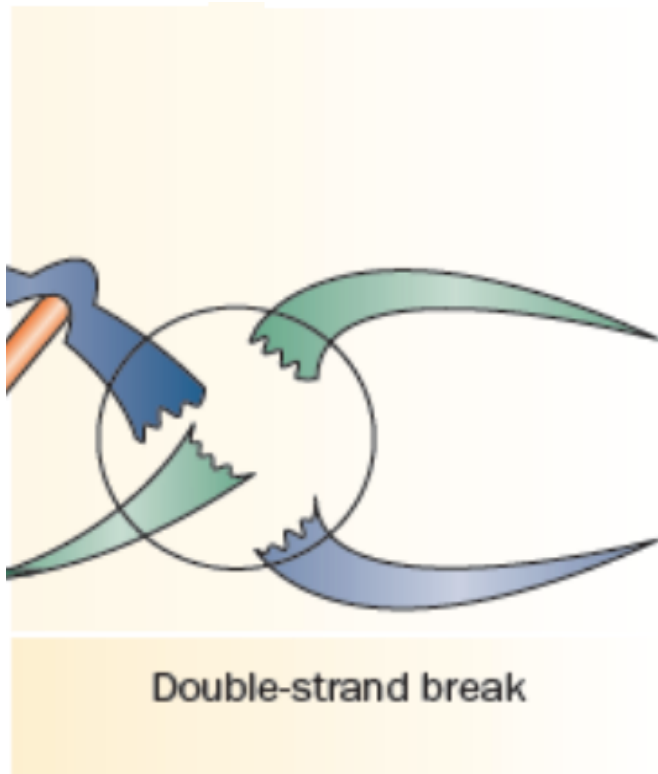


genetic susceptibility + environmental exposure
= 5.3x more likely to develop skin cancer

Mutations in DNA repair pathways linked to several cancers / diseases



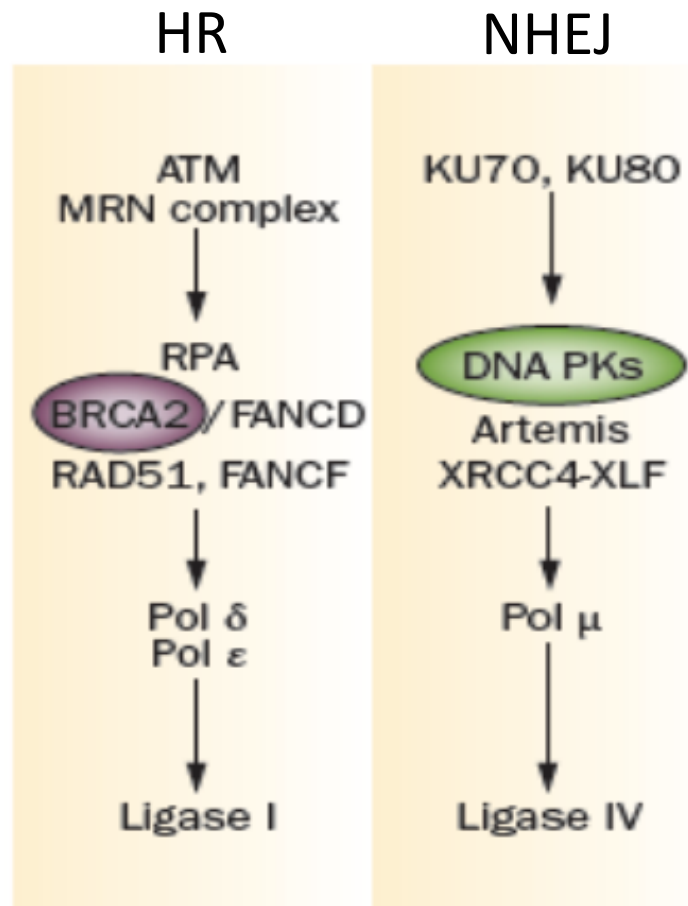
Our research will focus on double-strand break repair pathways



- Homologous recombination (HR)
 - Breast cancer
 - Ovarian cancer
 - Pancreatic cancer
- Non-homologous end joining (NHEJ)
 - Immune deficiency

Mutations in repair pathways can be exploited in cancer therapies

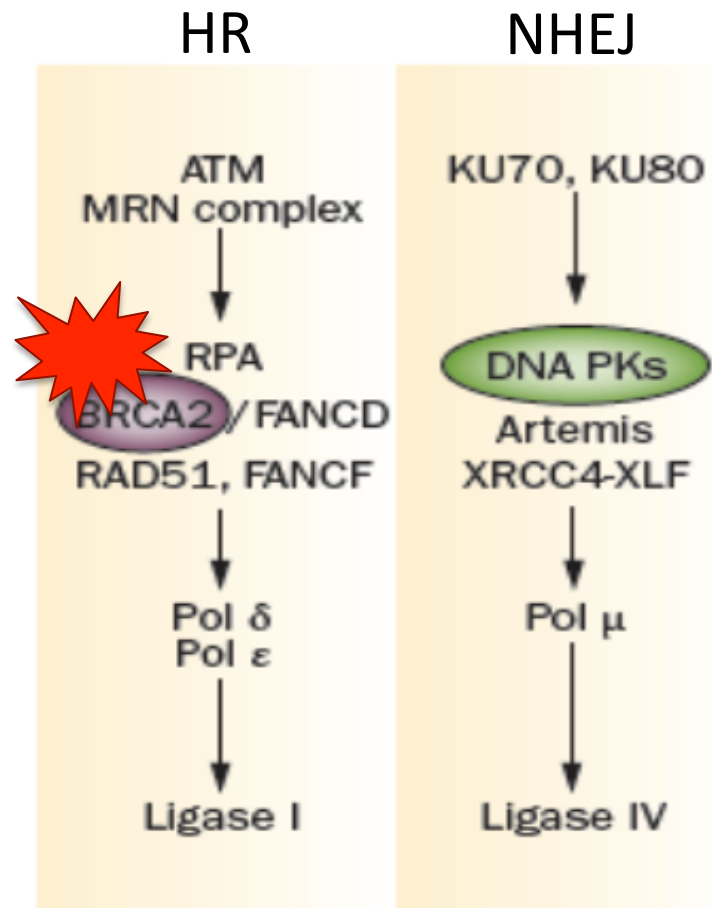
BRCA2 recruits enzymes involved in homology searching, strand exchange, and Holliday junction formation



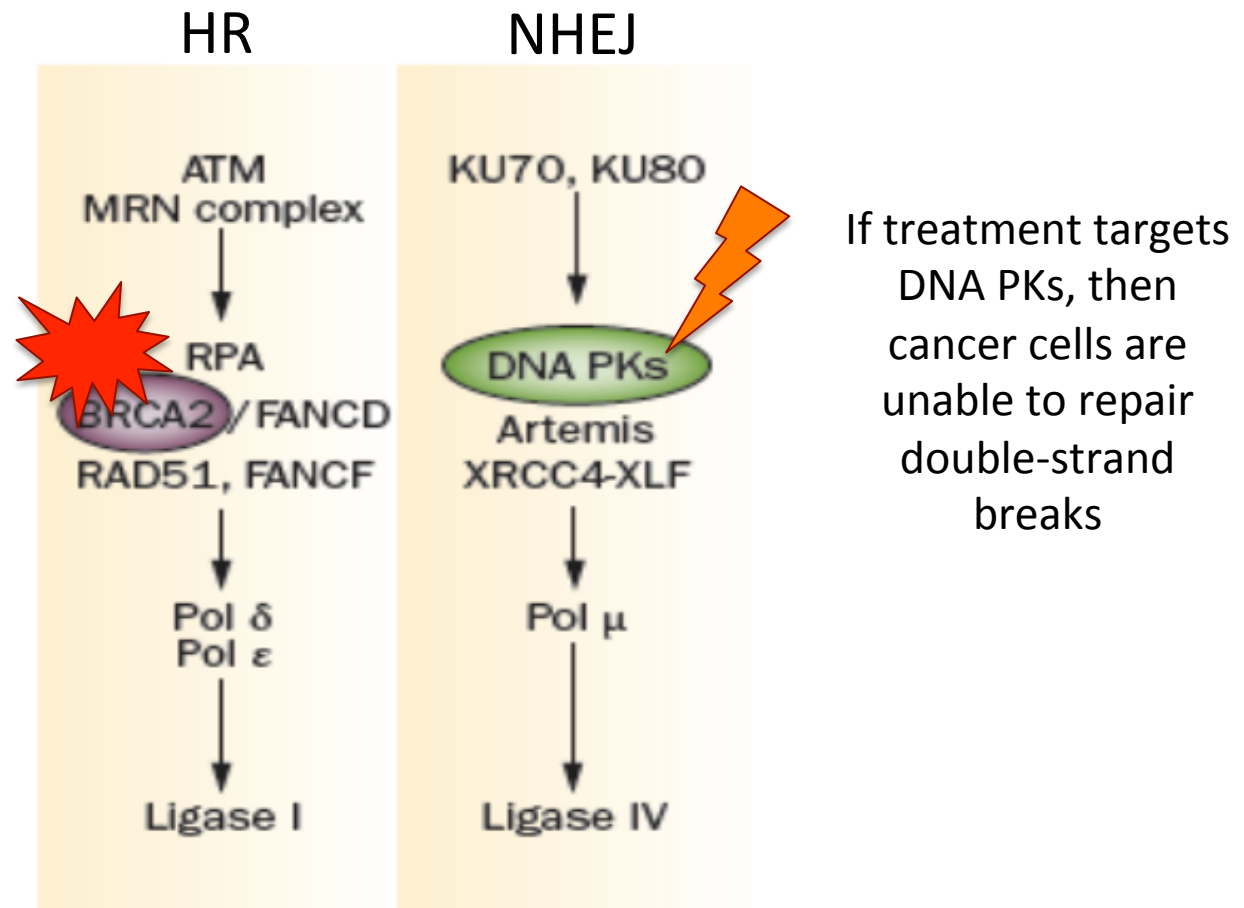
DNA PKs kinase activity required for re-ligating broken DNA ends

Cancer cells rely on same repair pathways as normal cells

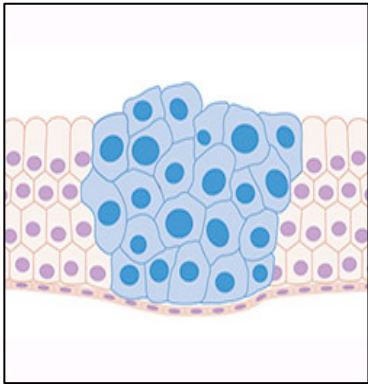
If cancer cells carry a mutation in *BRCA2*, then cells are dependent on NHEJ for double-strand break repair



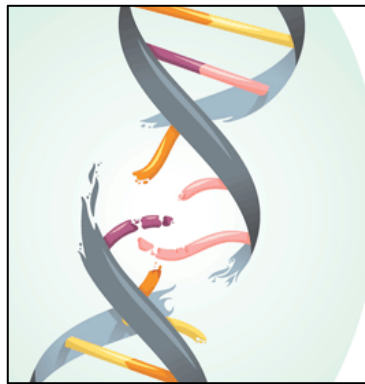
Targeting active repair pathway may preferentially kill cancer cells



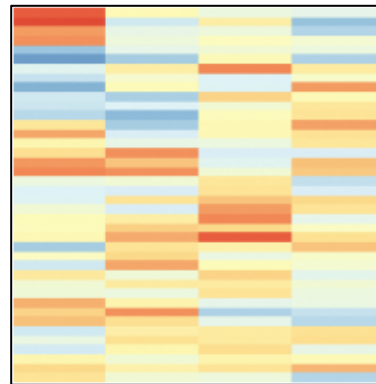
Overview of Mod 2



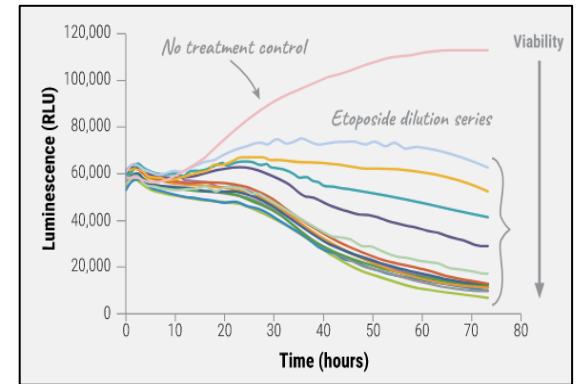
cancer biology



DNA repair



gene expression



cell viability

Background Information
(published literature)

Experimental Data
(your results!)

Your goal is to synthesize the information / data
into a single, coherent story