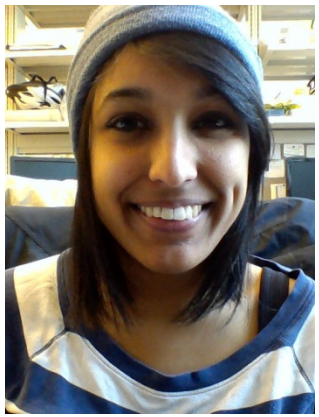
20.109 Spring 2014 Module 2
System Engineering and Protein Foundations





Agi Stachowiak

Shannon Hughes

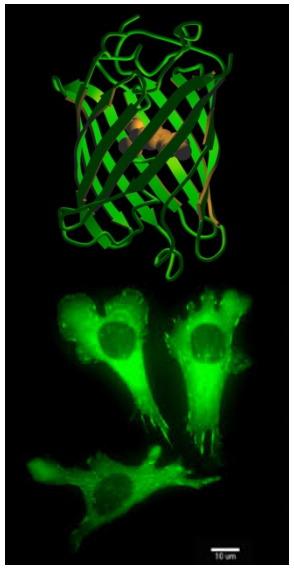
Aneesh Ramaswamy

Suhani Vora (TA)

Leona Samson (Lectures)

Zachary Nagel (help with development)

What experimental question will you ask in Module 2?

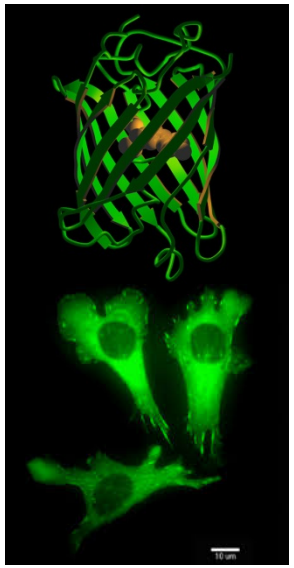


How efficiently does DNA repair by the Non Homologous End Joining (NHEJ) pathway act on DNA damage with different topologies?

This raises the following questions

- How does DNA get damaged?
- What is DNA repair?
- Why does DNA repair exist?
- Why do we care about how efficient DNA repair is?
- How does one actually measure DNA repair efficiency?

Key Experimental Methods for Module 1



- Mammalian tissue cell culture
- Monitoring protein level by Western blot
- Generating plasmids with DNA damage
- Transfecting plasmids into mammalian cells
- Using fluorescent proteins as reporters of biological processes
- Flow cytometry to measure DNA repair
- Statistical analysis of biological data

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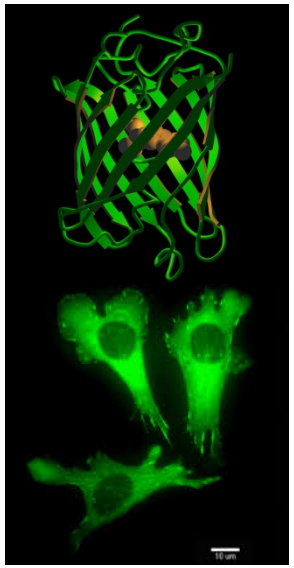

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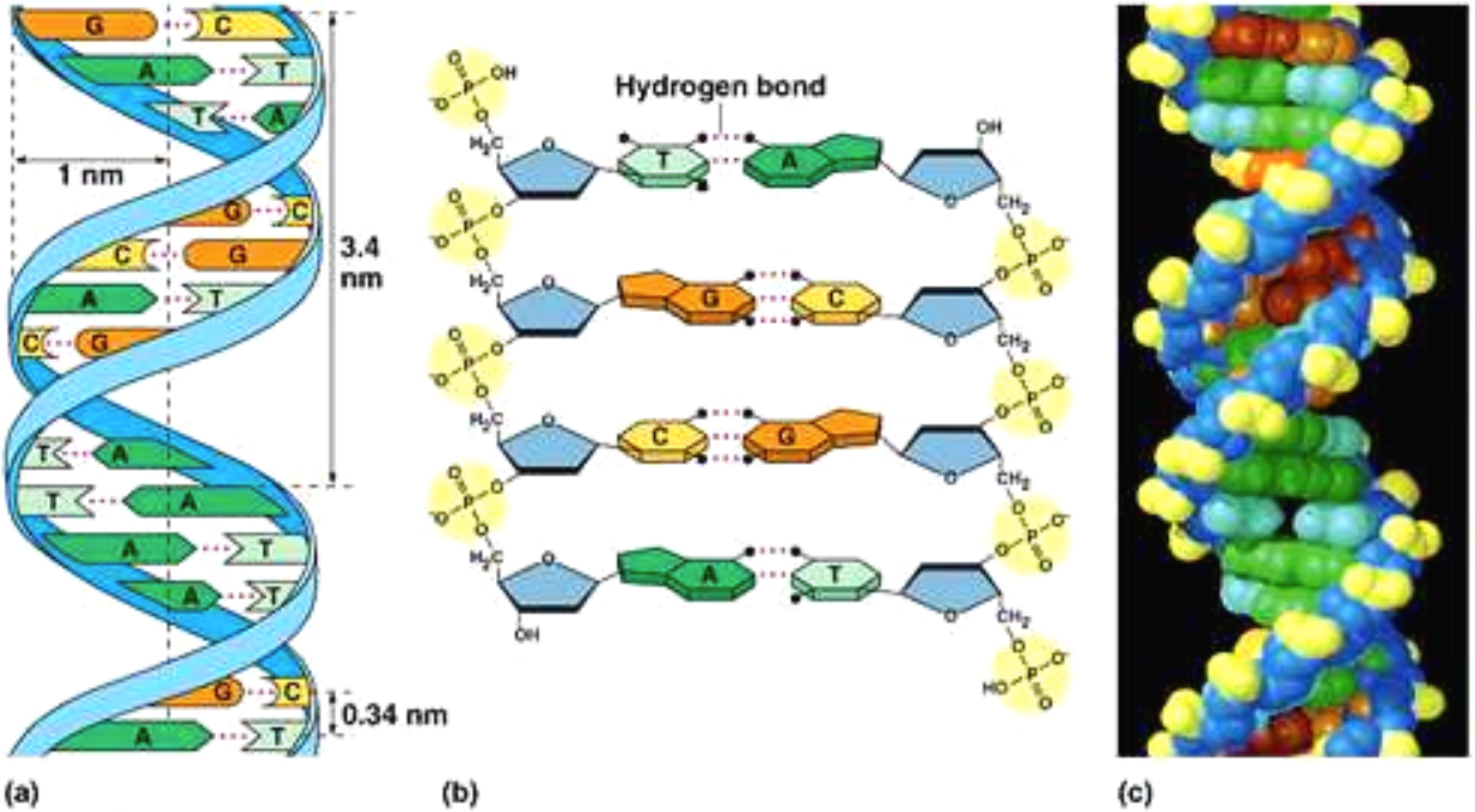
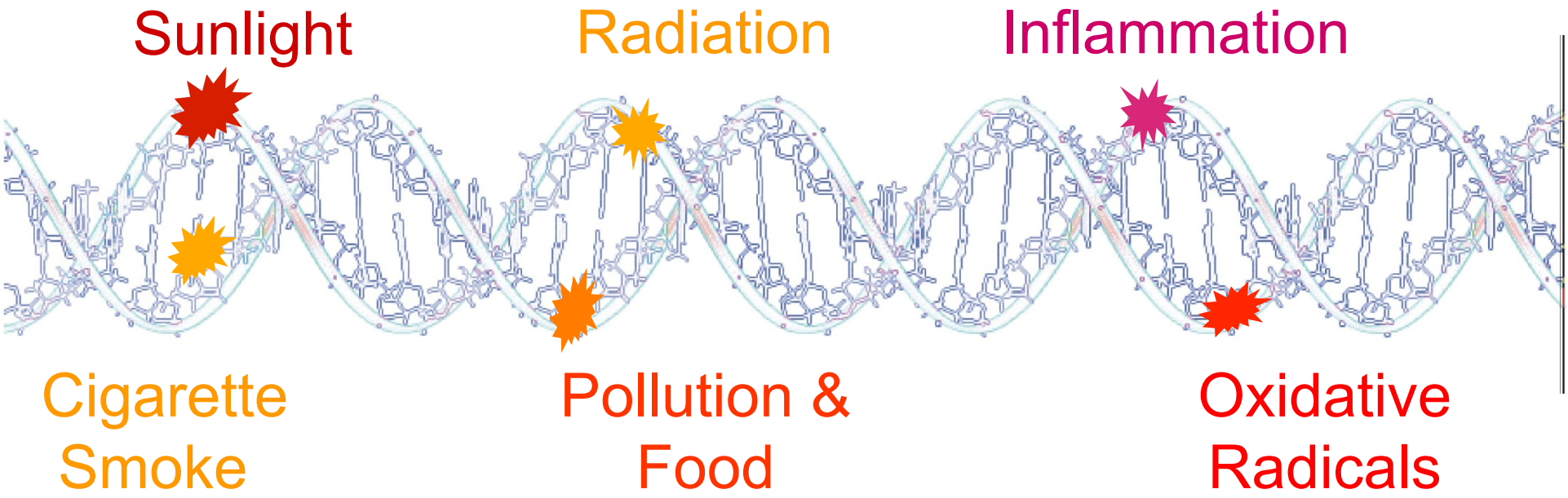
The Structure of DNA



DNA is constantly being damaged by endogenous and exogenous agents



Courtesy of Bevin Engelward

Central Dogma

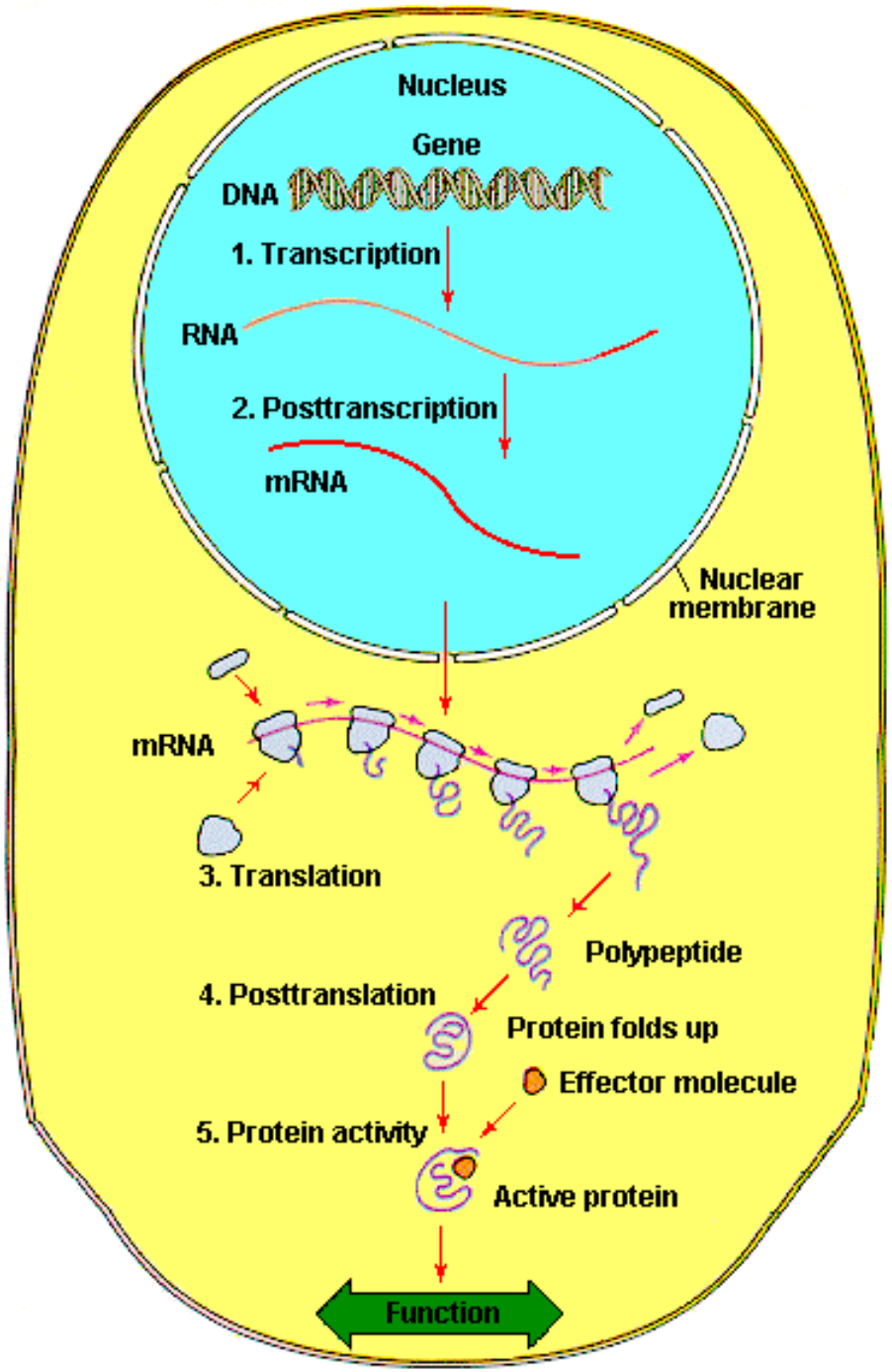
DNA

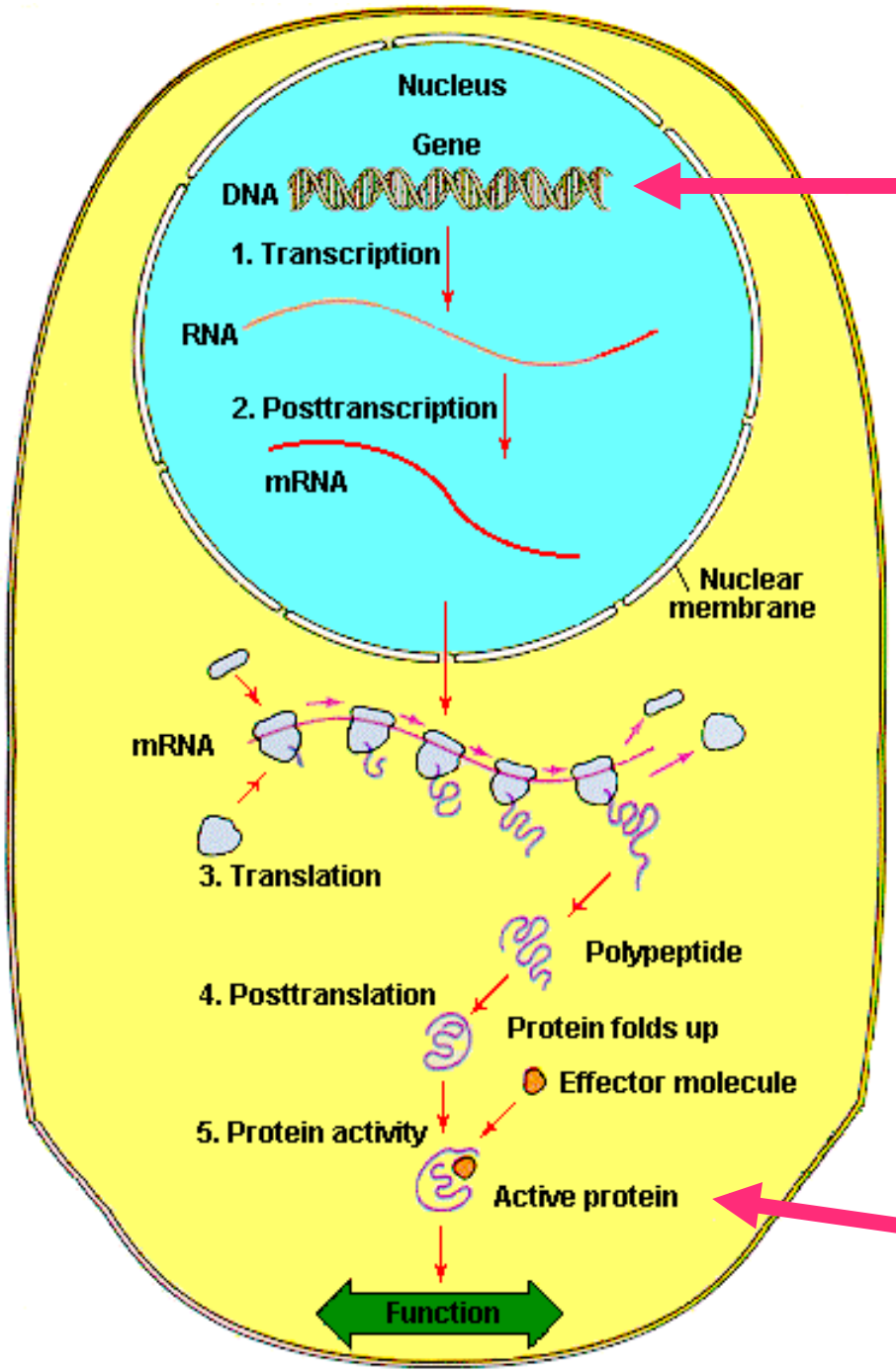
makes

RNA

makes

Protein





Damage to DNA can creates permanent changes in the genetic information (mutations)

Inactive proteins or proteins with altered function are produced

Some cancer Chemotherapy agents and all Radiotherapies CAUSE DNA DAMAGE

Environmental exposures to potentially harmful agents

Harmful agents


Eat


Medicine


Drink


Absorbed


Breathe


Infection

People have different exposures

People have different responses

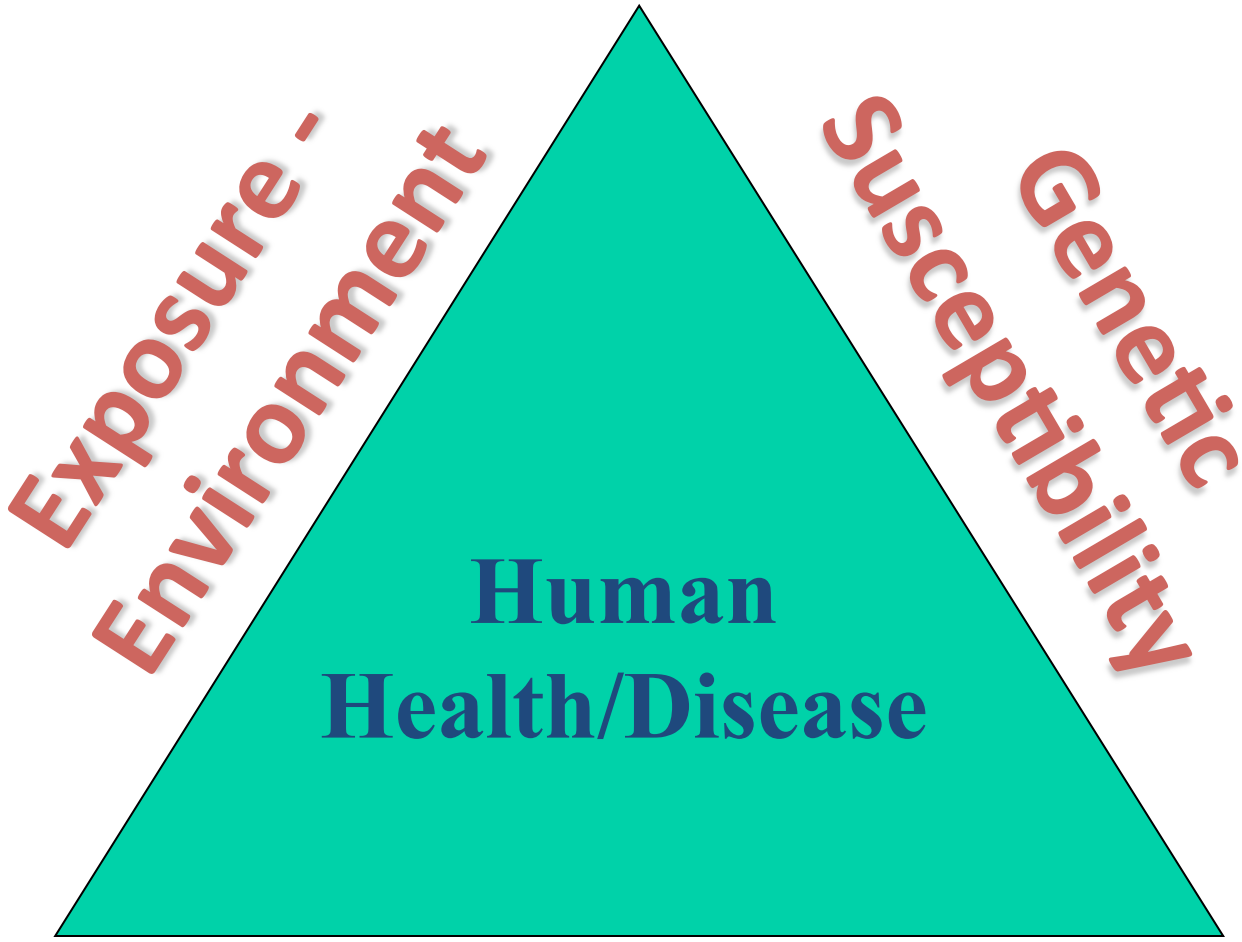
2007 – Breakthrough of the year

Natural sequence variation

single nucleotide polymorphisms (SNPs) every 1000 base pairs.

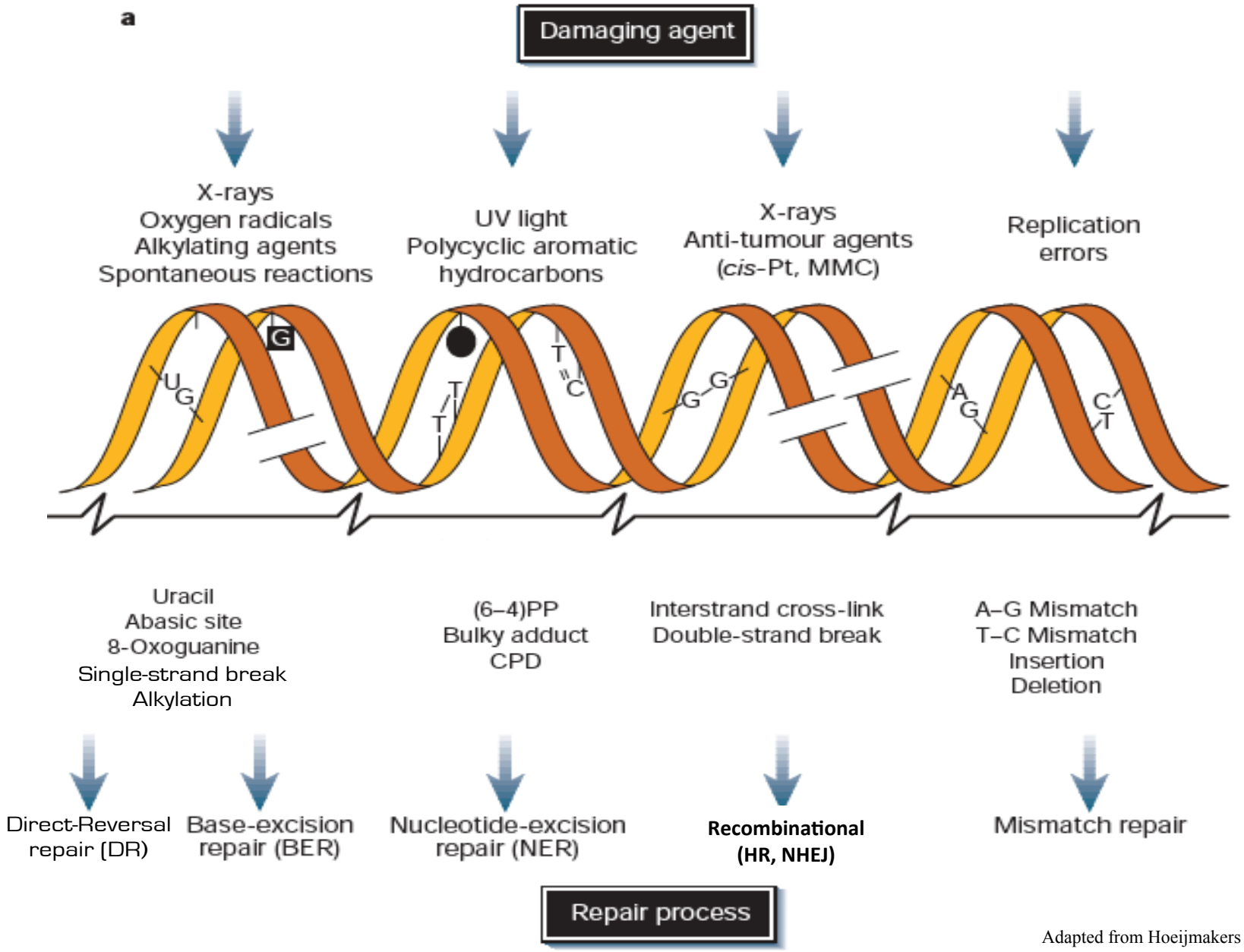
Compare two people – have about ~ 3 million SNP variants!

Toxic agents in our environment
Gene-Environment Interaction



Time/Age/Behavior

DNA Damage and Repair



Adapted from Hoeijmakers **2001** *Nature*

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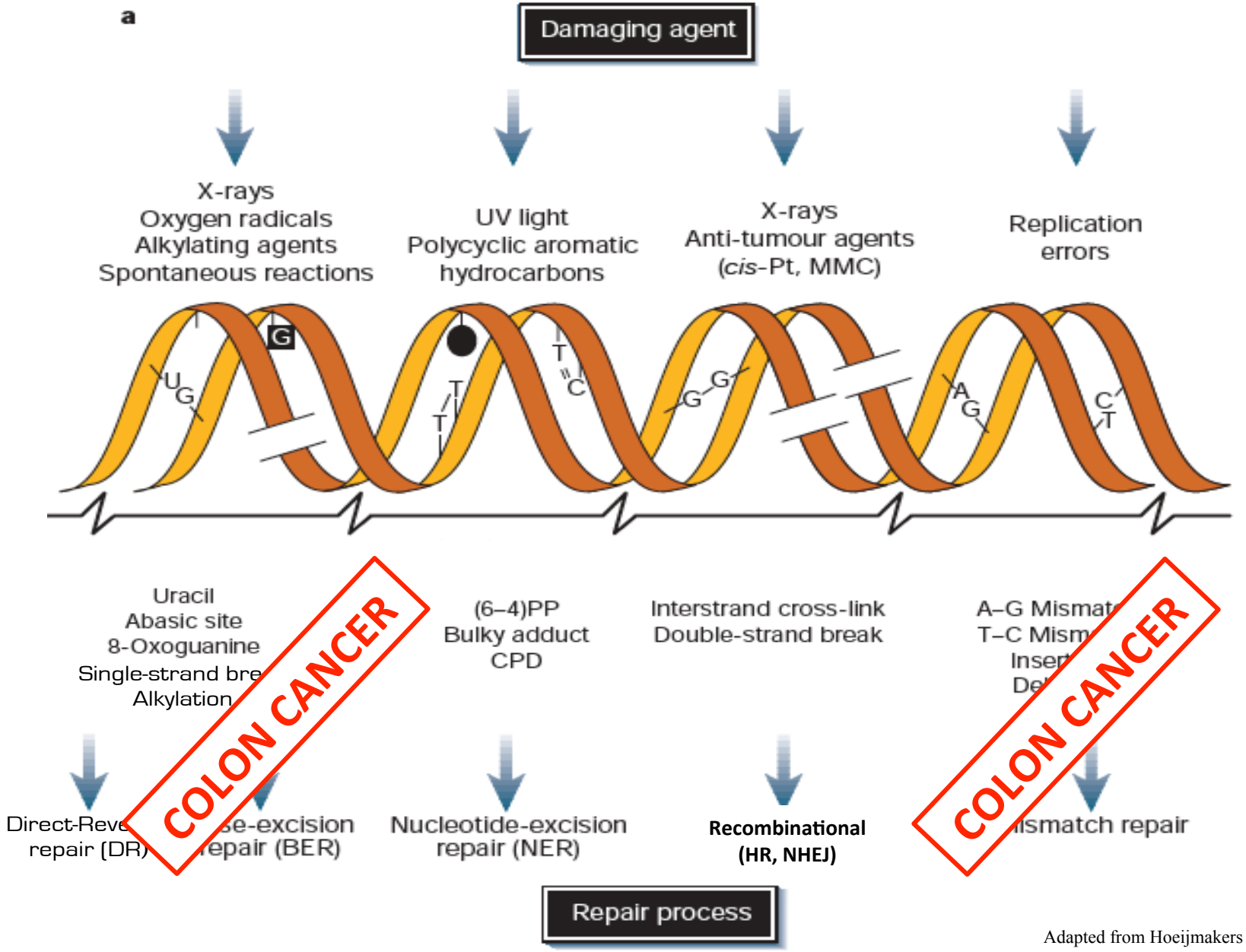

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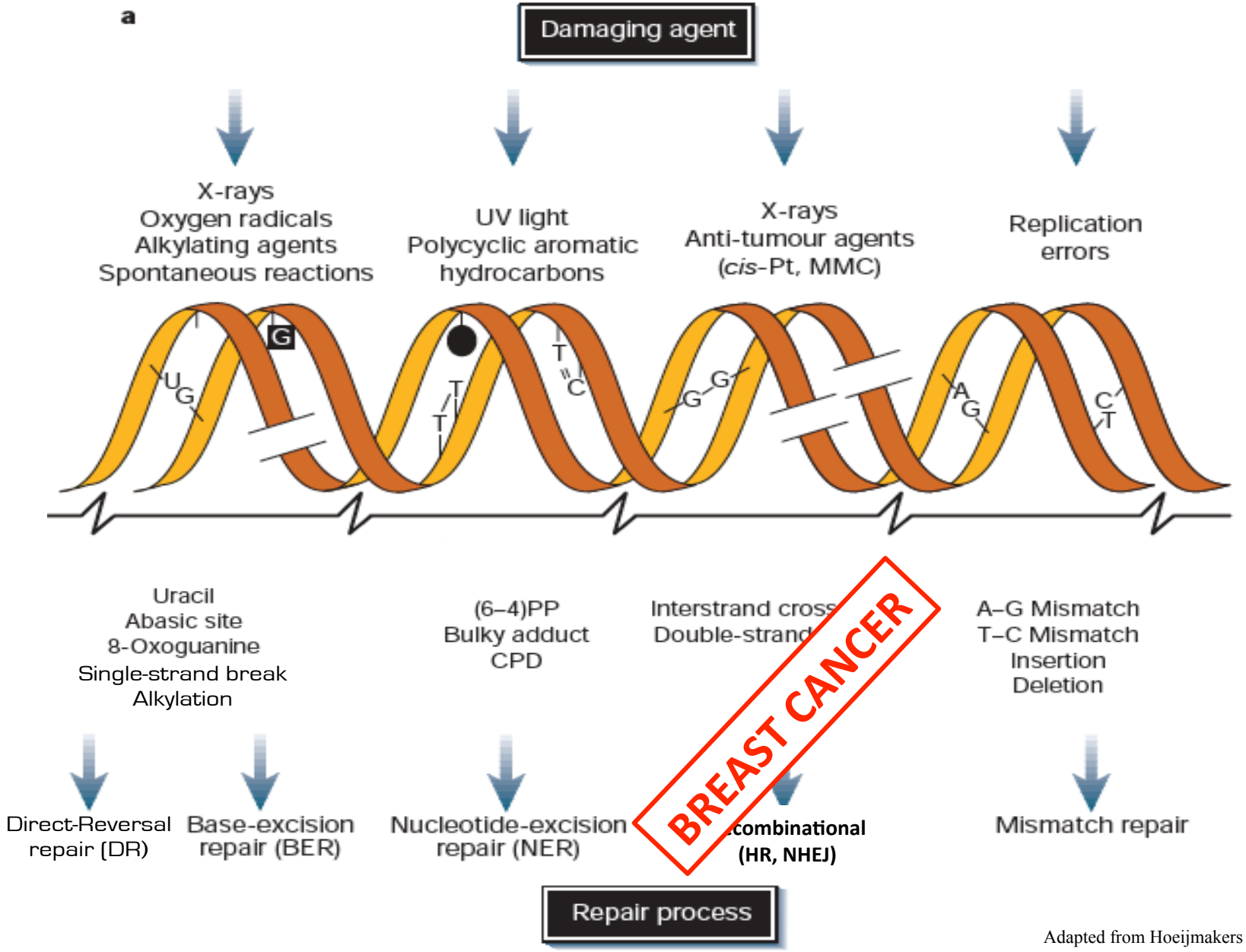

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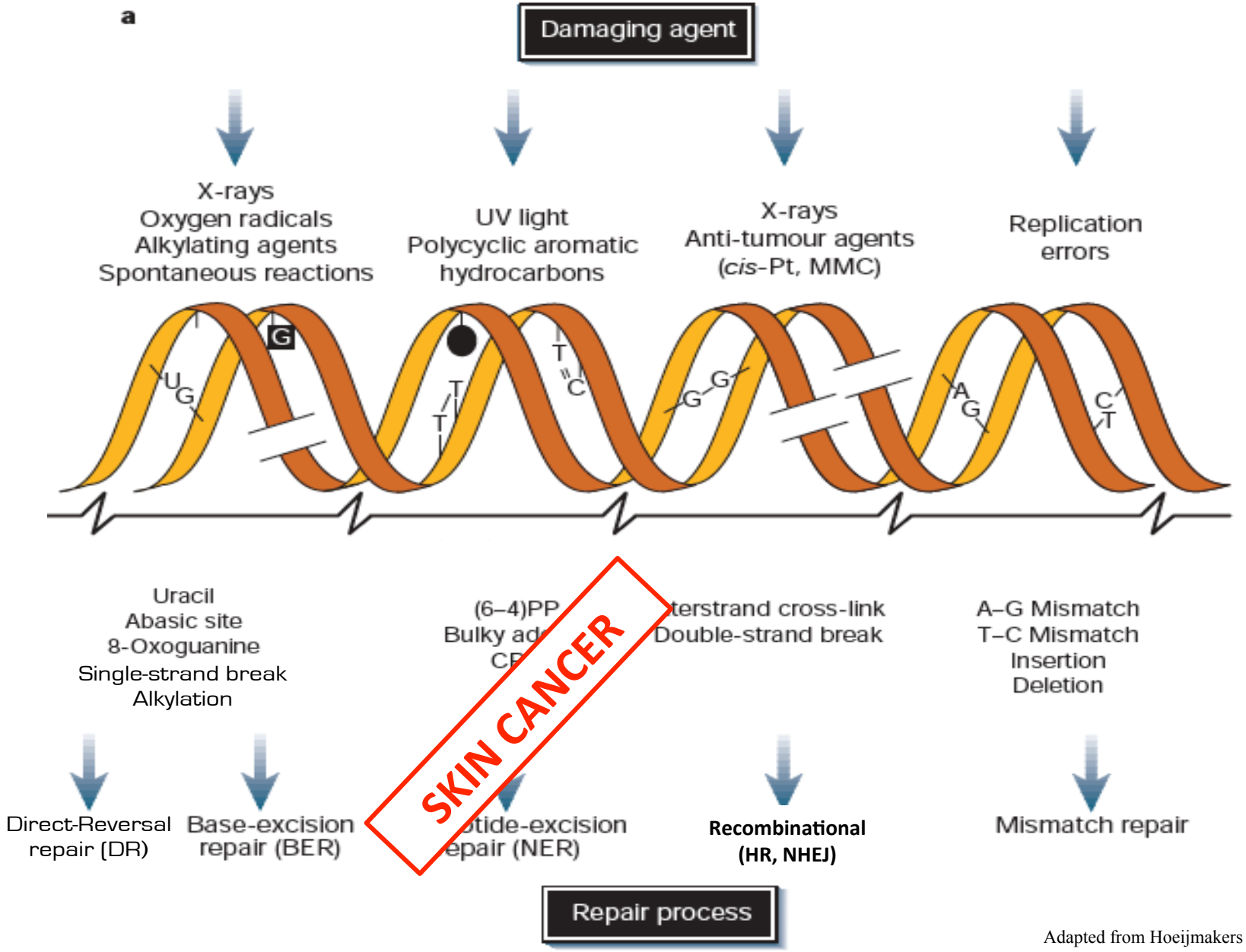

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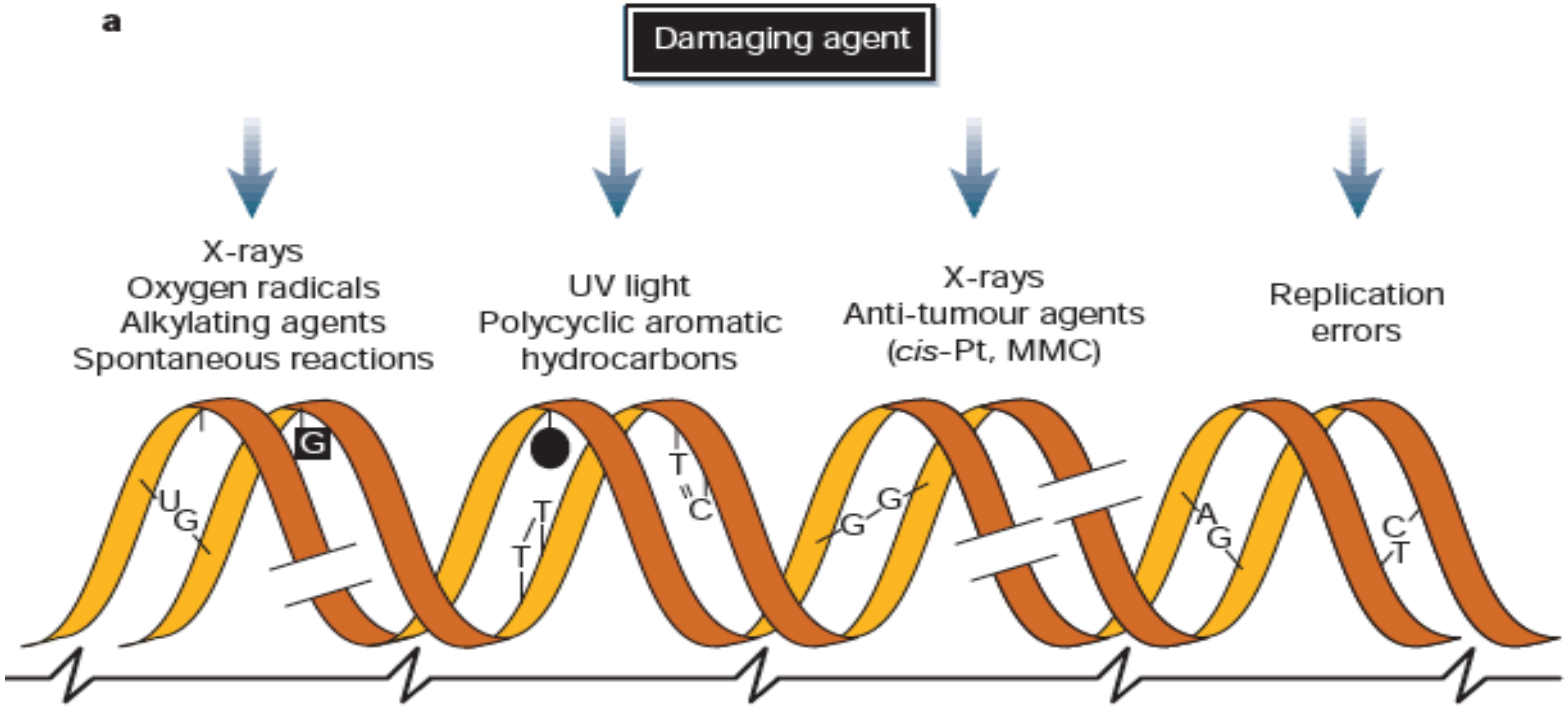
DNA Damage and Repair



RESPONSES of TUMOR and NON-TUMOR CELLS to CANCER RADIOTHERAPY and CHEMOTHERAPY

Direct-Reversal repair (DR)

Base-excision repair (BER)

Nucleotide-excision repair (NER)

Recombinational (HR, NHEJ)

Mismatch repair

Repair process

Adapted from Hoeijmakers **2001** *Nature*

DNA Repair Strategies

- Direct Reversal

  Methyltransferase, Oxidative demethylase

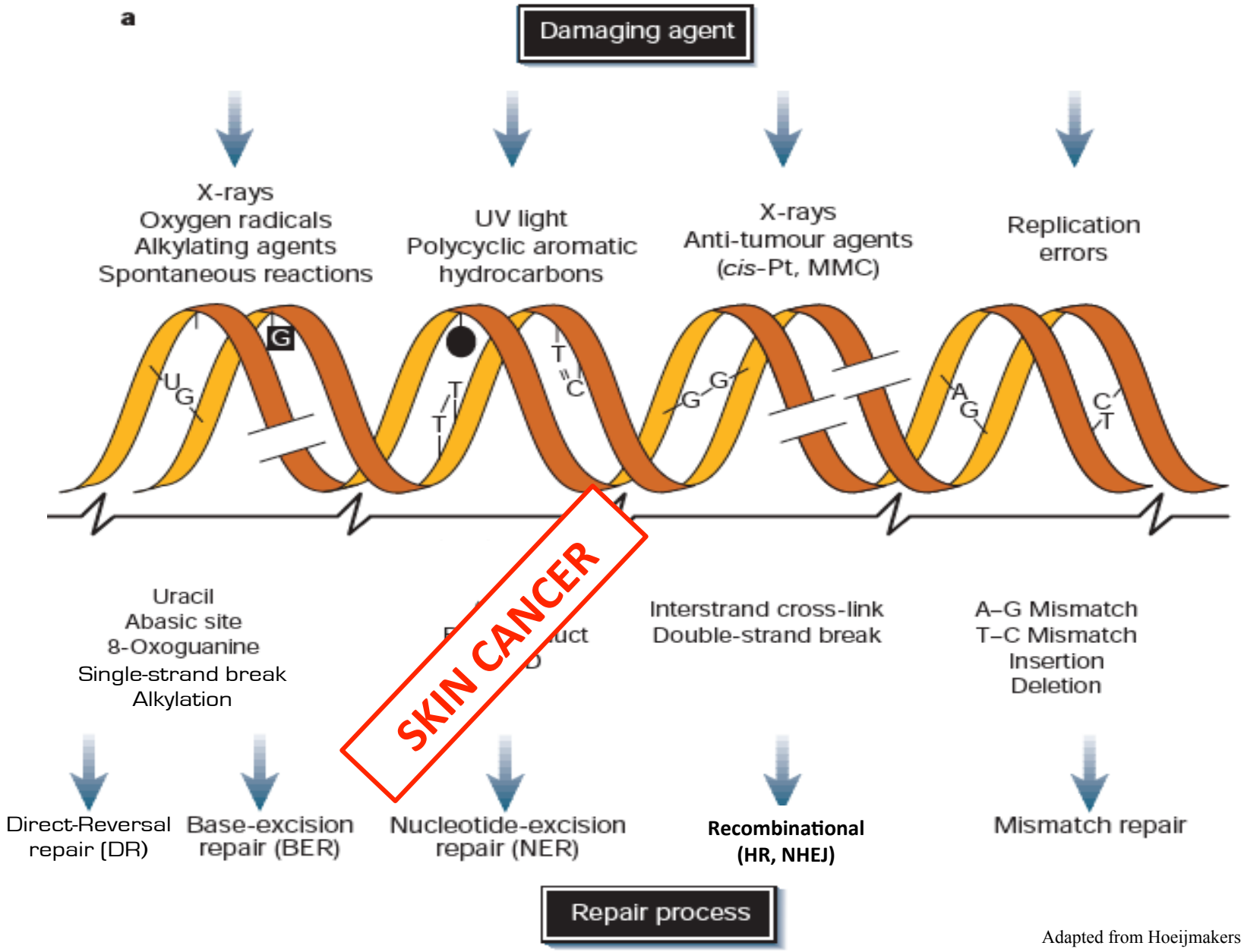
- Excision Repair

  Base excision, nucleotide excision, mismatch repair

- Double strand break repair

  Homologous recombination, Non-homologous end joining

DNA Damage and Repair



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What are the known risk factors for Skin Cancer?

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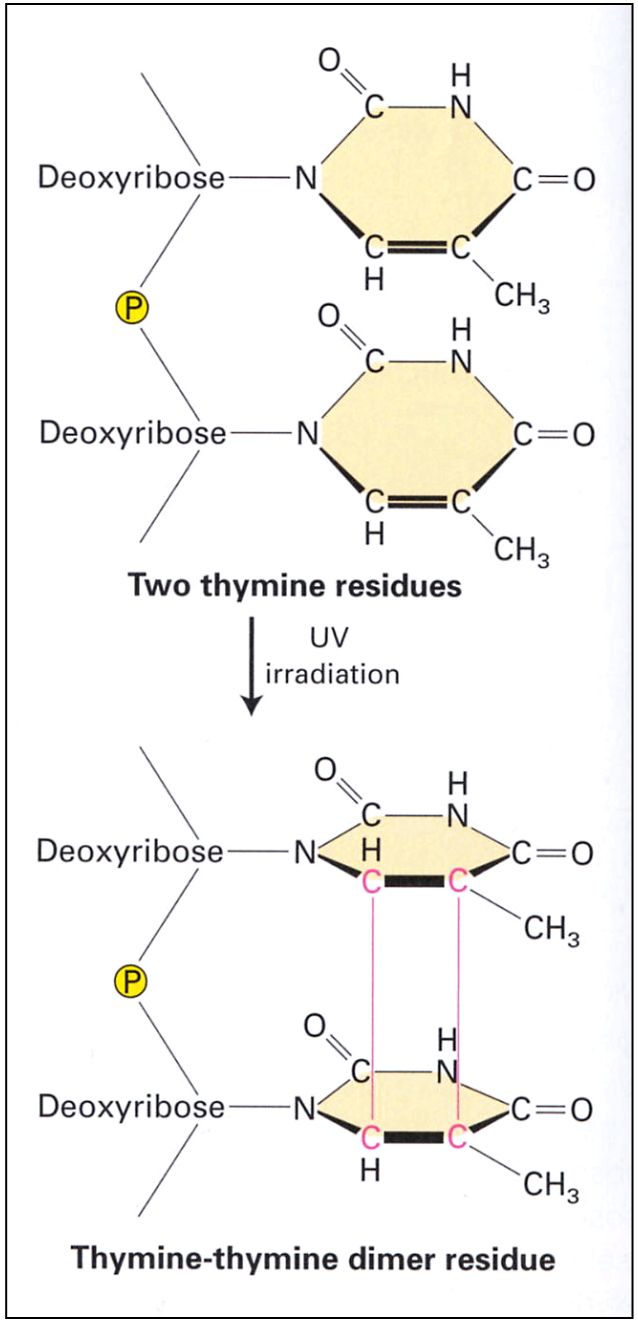
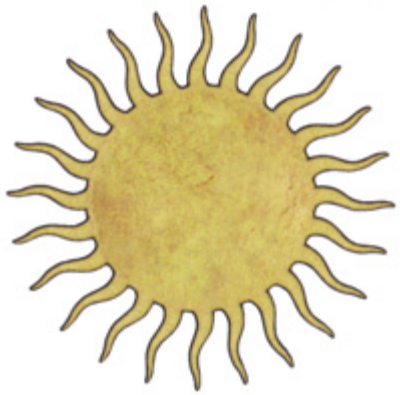
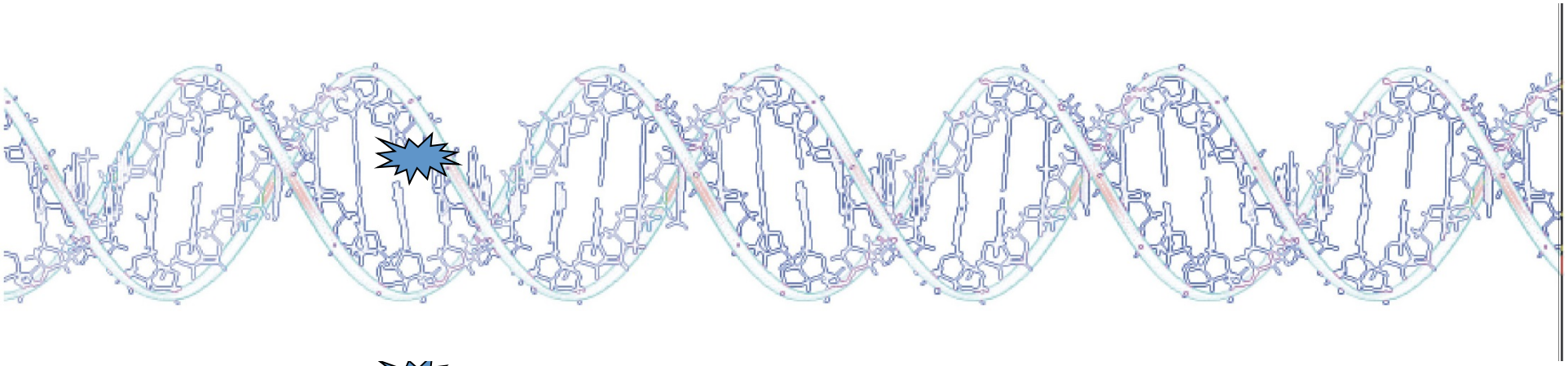
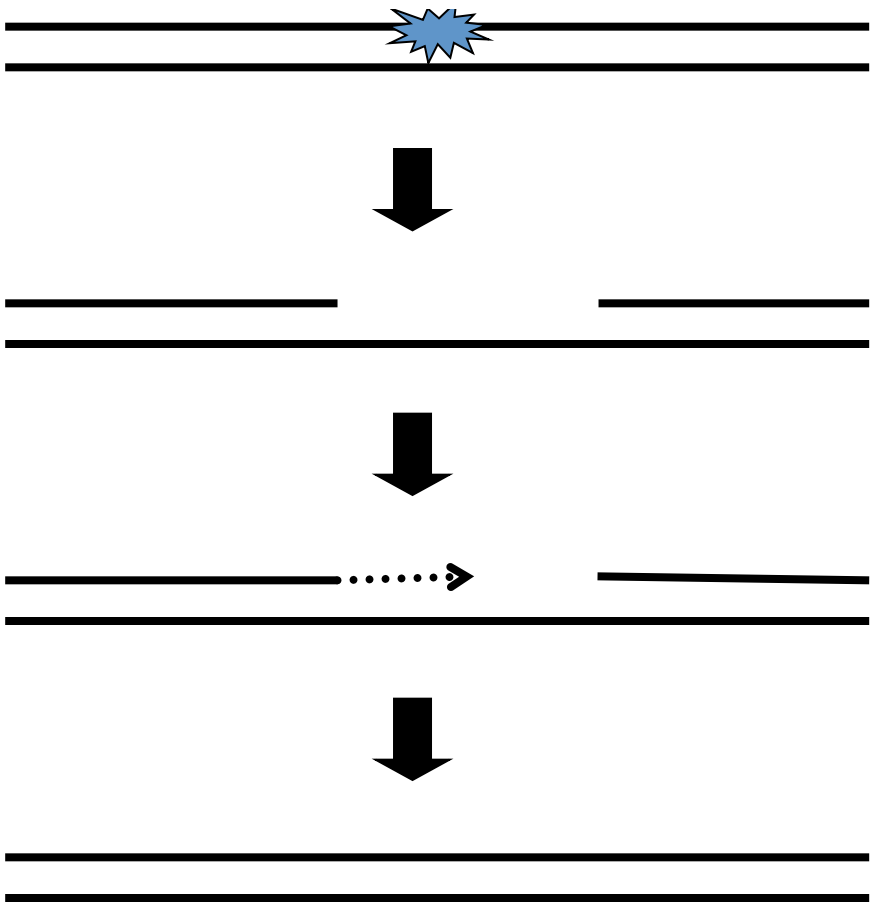




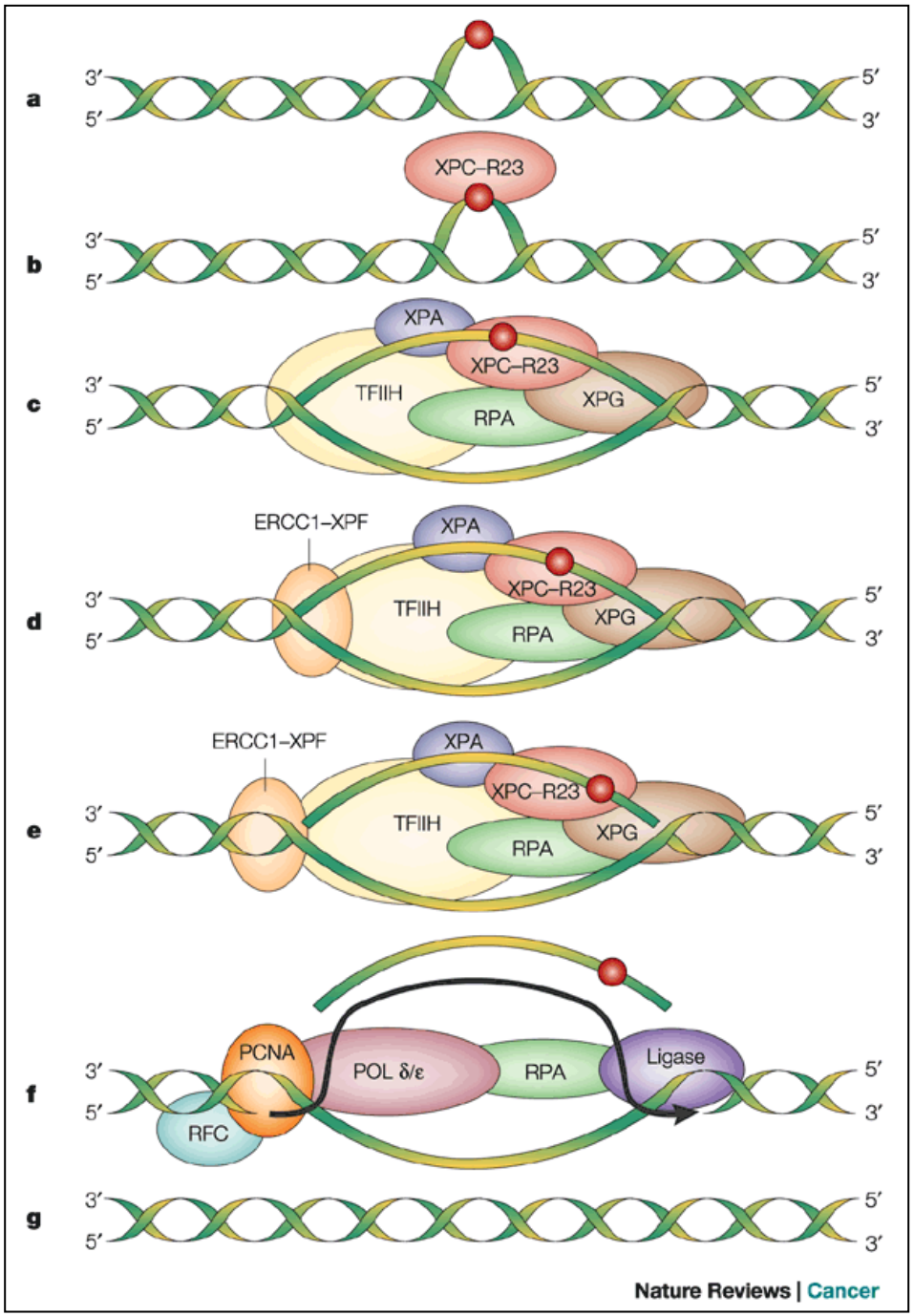
Before

After





Nucleotide Excision Repair



Nucleotide Excision Repair

XPA
XPB
XPC
XPD
XPE
XPF
XPG

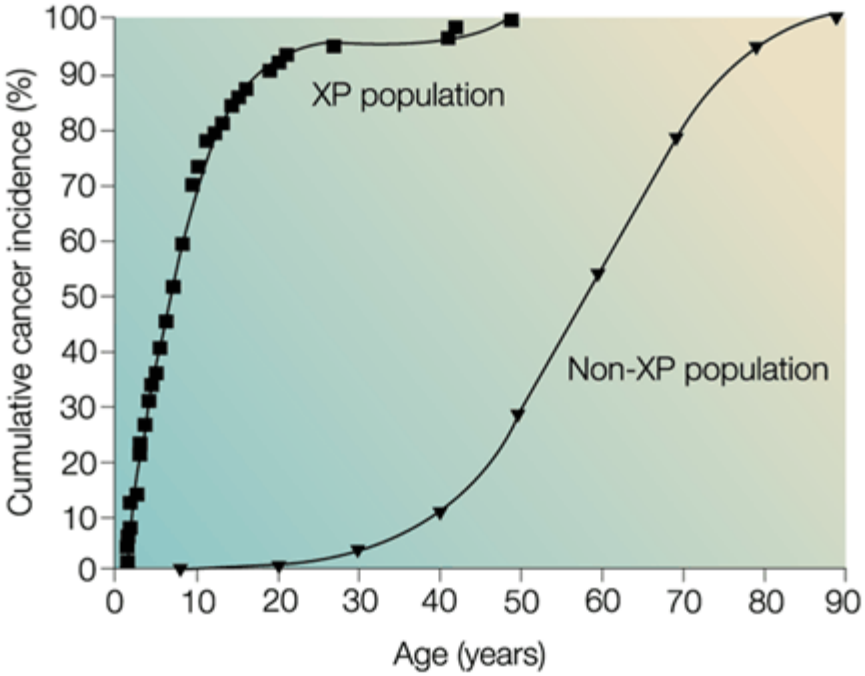
Errol C. Friedberg
Nature Reviews Cancer 1, 22-33 (2001)

Xeroderma Pigmentosum

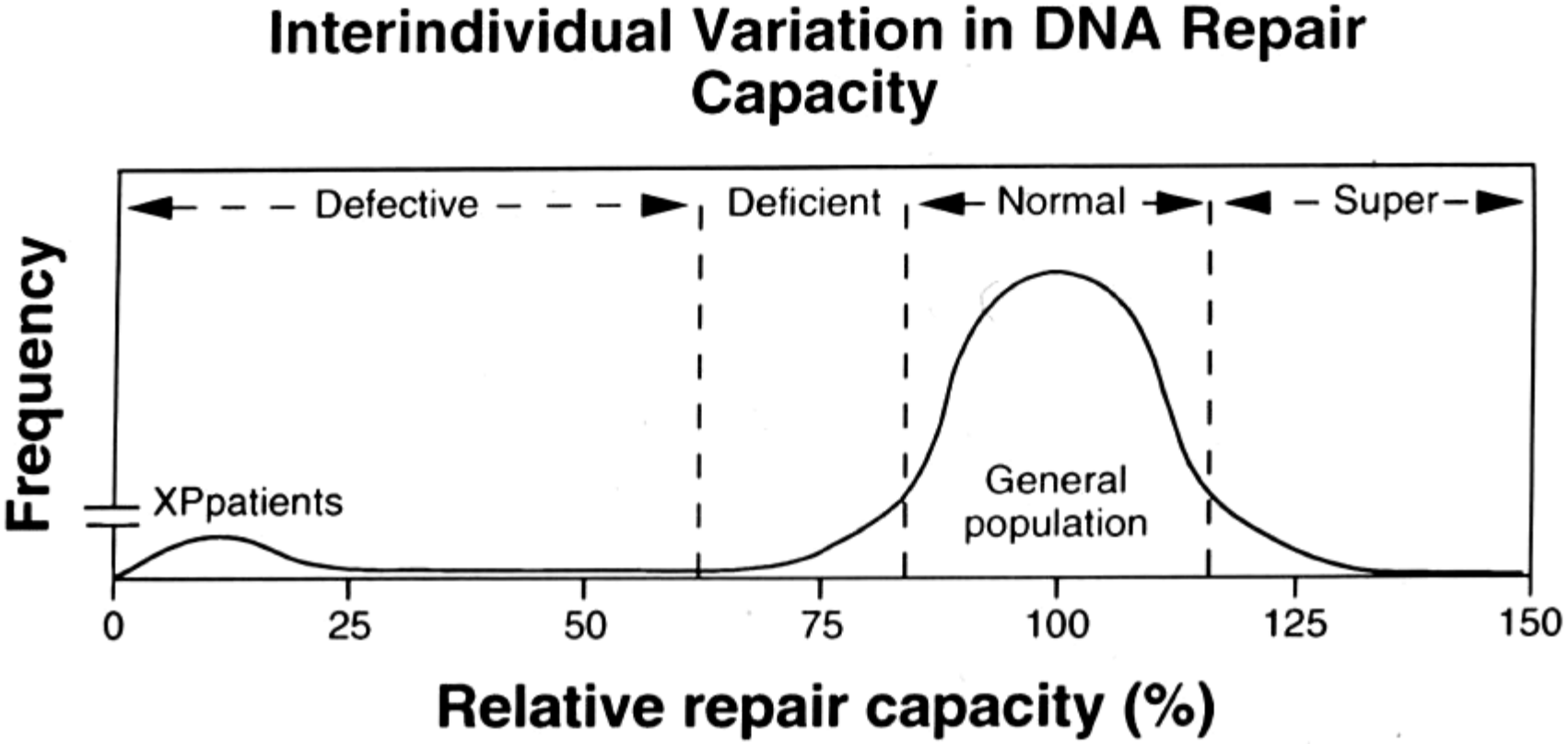
Grossly Deficient in Nucleotide Excision Repair

2000-fold increased risk of skin cancer

Lack of DNA repair accelerates the onset of cancer



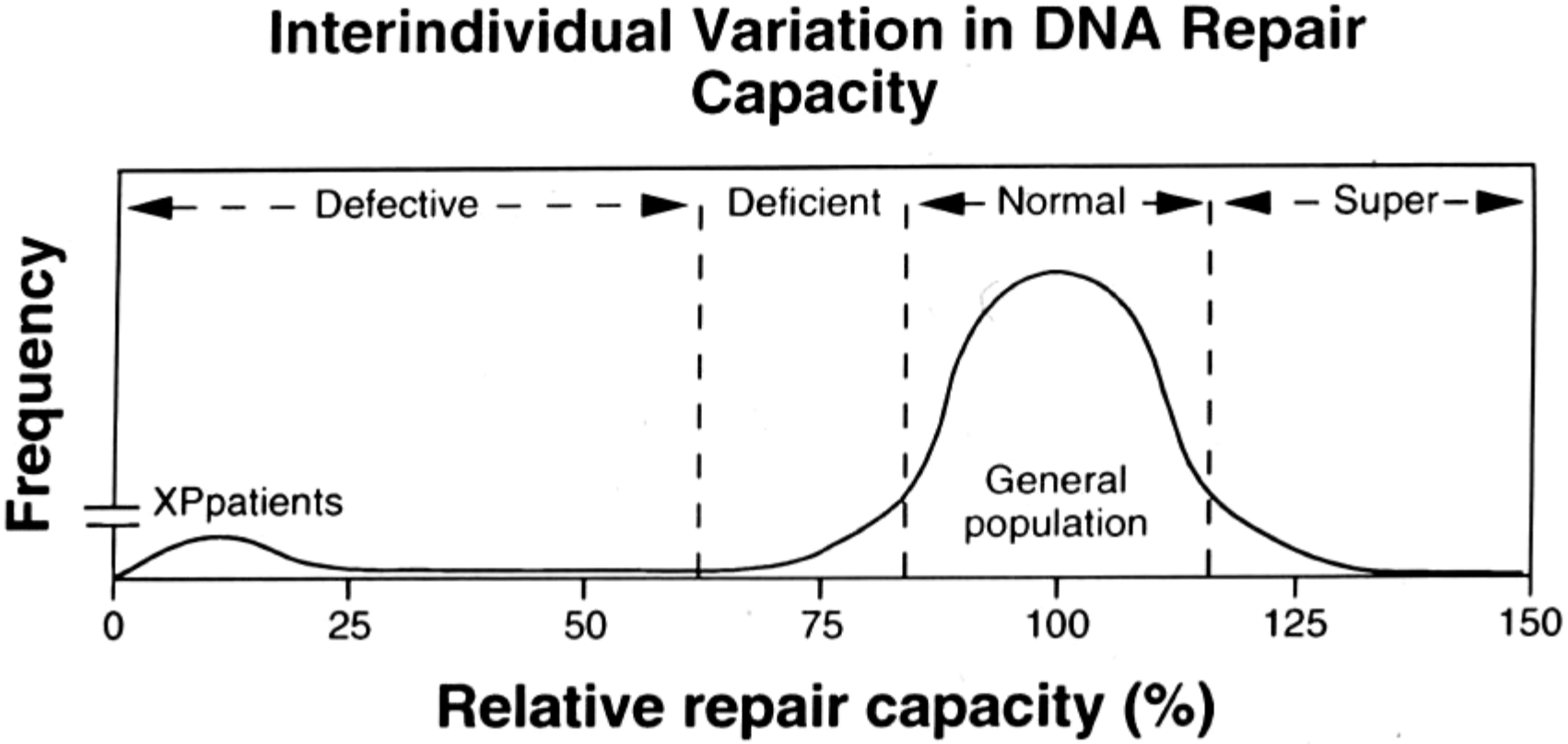
Nature Reviews |



Adapted from **GROSSMAN and Wei (1995)** Clinical Chem 41: 1854-1863

XP frequency = ~1:250,000 giving a theoretical maximum of how many cases worldwide with 2,000-fold increased risk

Even if just 1% of the population is relatively repair deficient, could have how many with several-fold increased risk



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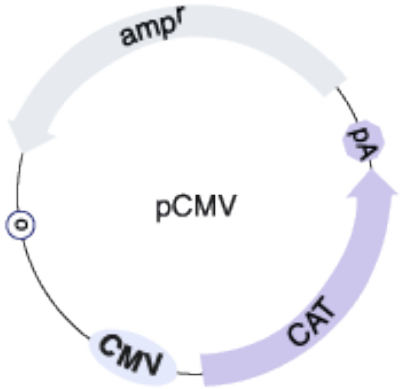
XP frequency = ~1:250,000 giving a theoretical maximum of ~28,000 cases worldwide with 2,000-fold increased risk

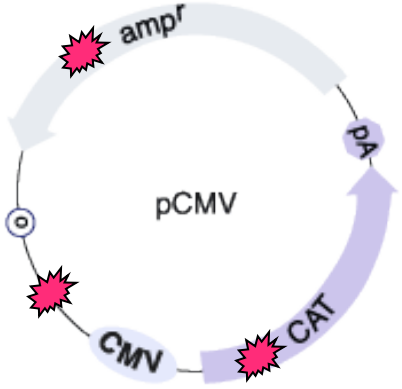
Even if just 1% of the population is relatively repair deficient, could have tens of millions with several-fold increased risk

Reactivation of UV damaged DNA by Host cell Reactivation (HCR)

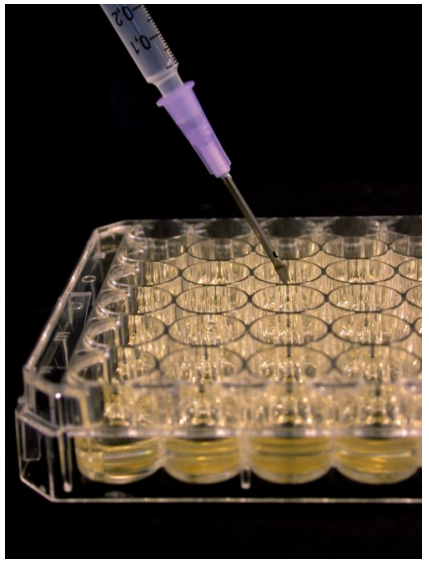
Athas & GROSSMAN
Cancer Res. 1991

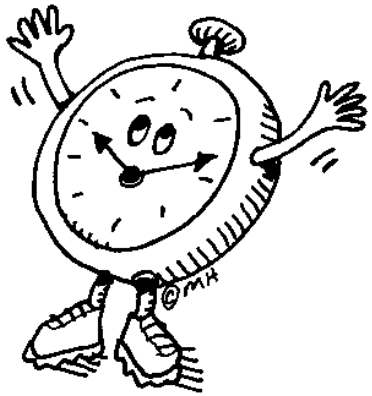


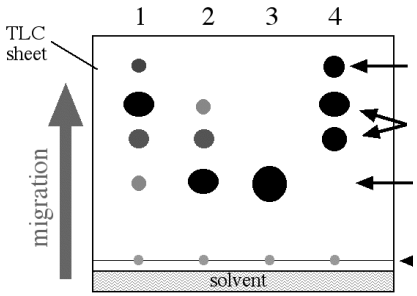
+ UV light



Transient transfection peripheral blood lymphocytes



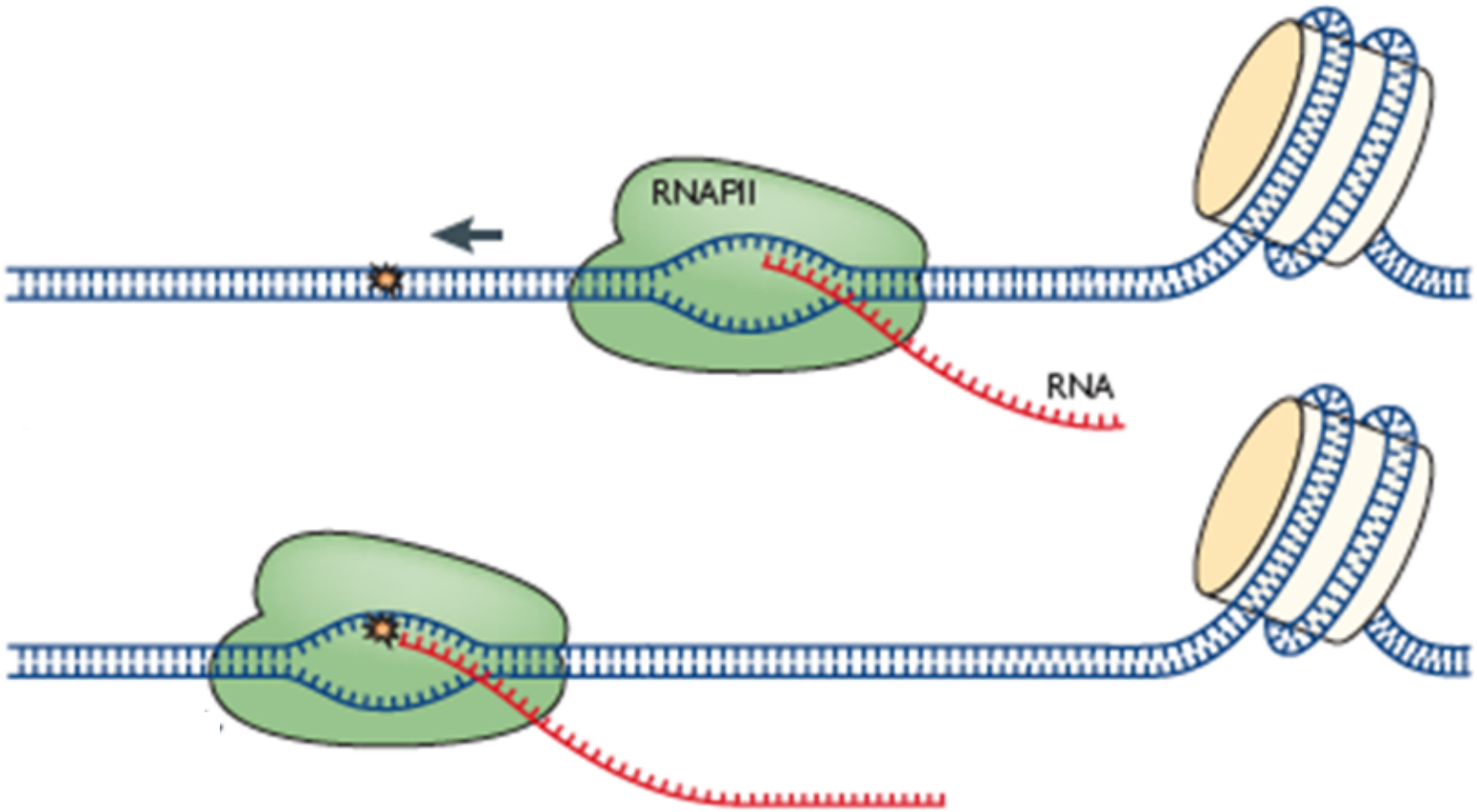




CAT Assay

Time to repair

RNA Polymerase II is exquisitely sensitive to DNA lesions



Nature Reviews Molecular Cell Biology **9**, **958-970**

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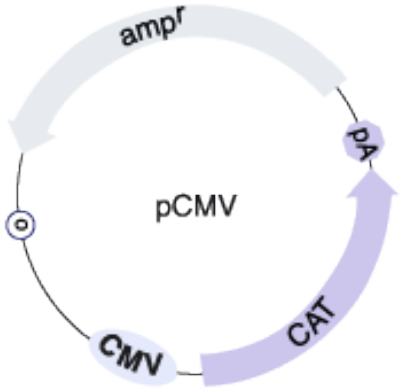

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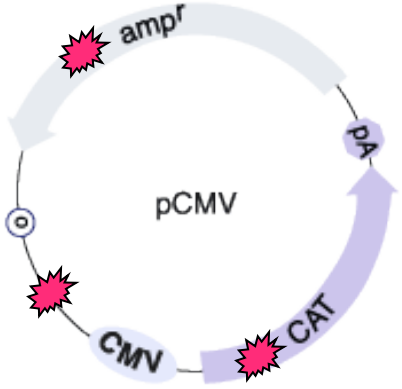

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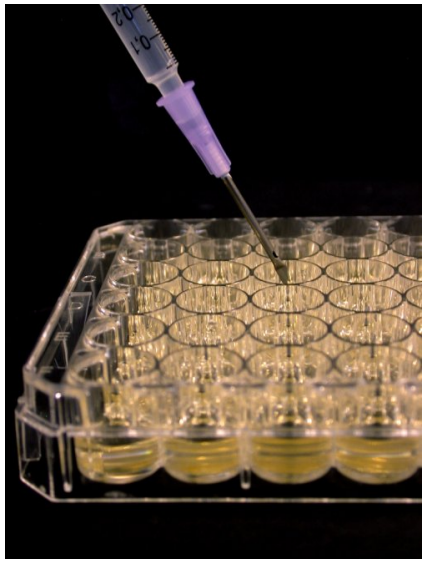

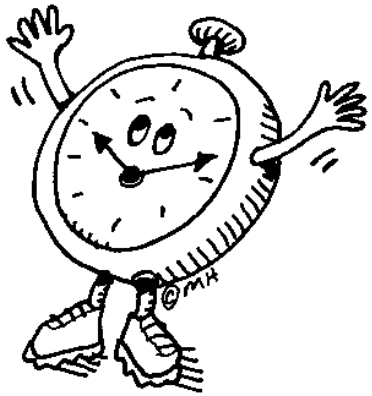

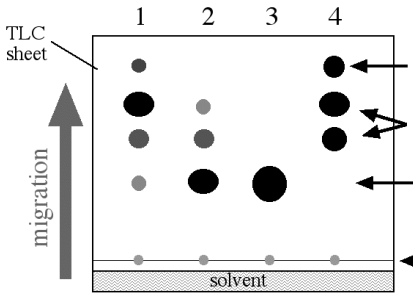

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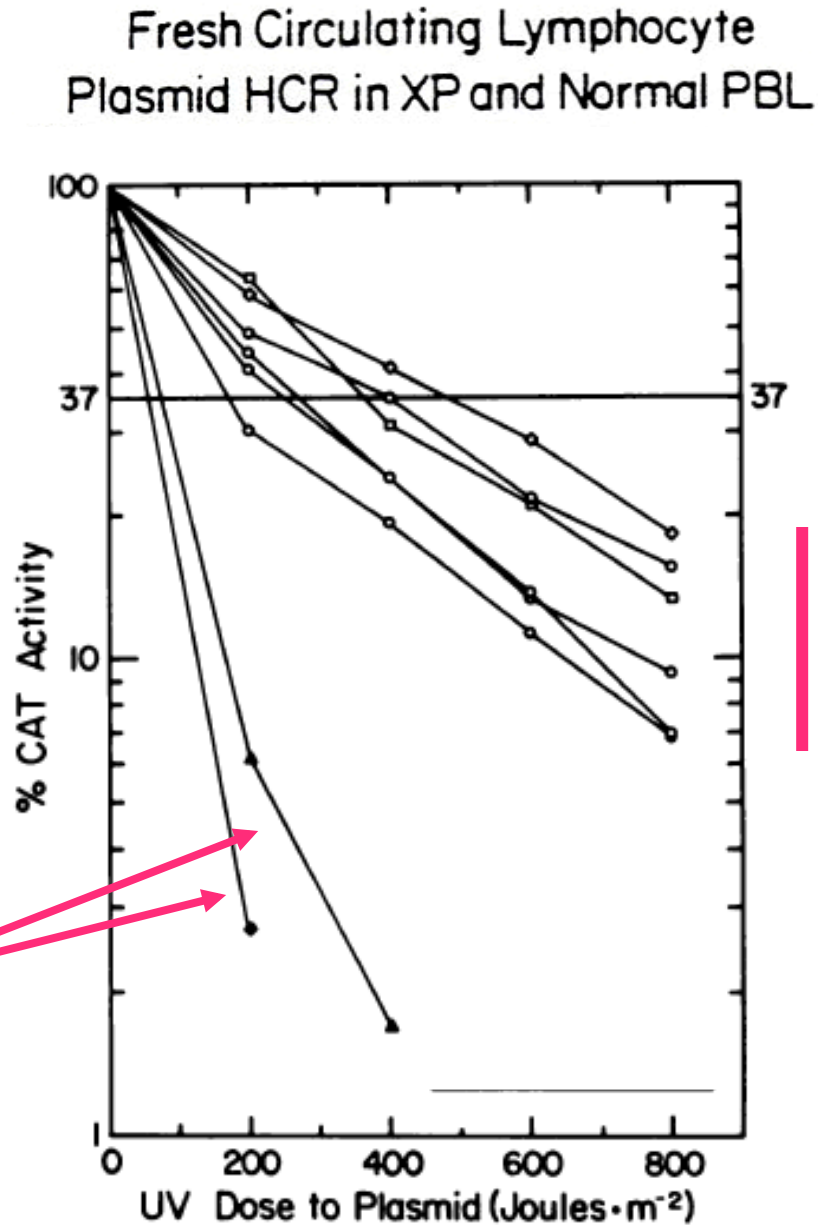
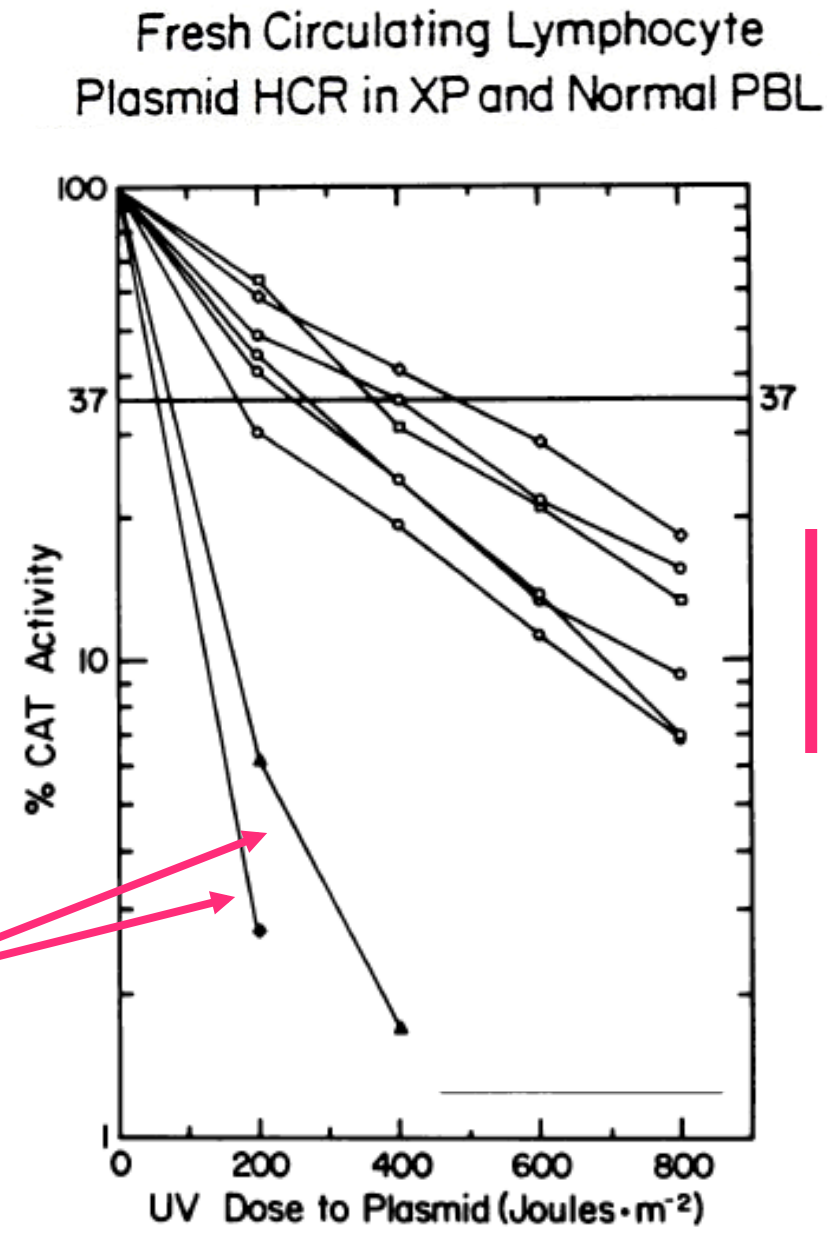


Cells from ‘healthy” people

Cells from XP patients

Athas & GROSSMAN

[CANCER RESEARCH 51, 5786–5793, November 1, 1991]



Cells from XP patients

Relatively HIGH repair

Relatively LOW repair

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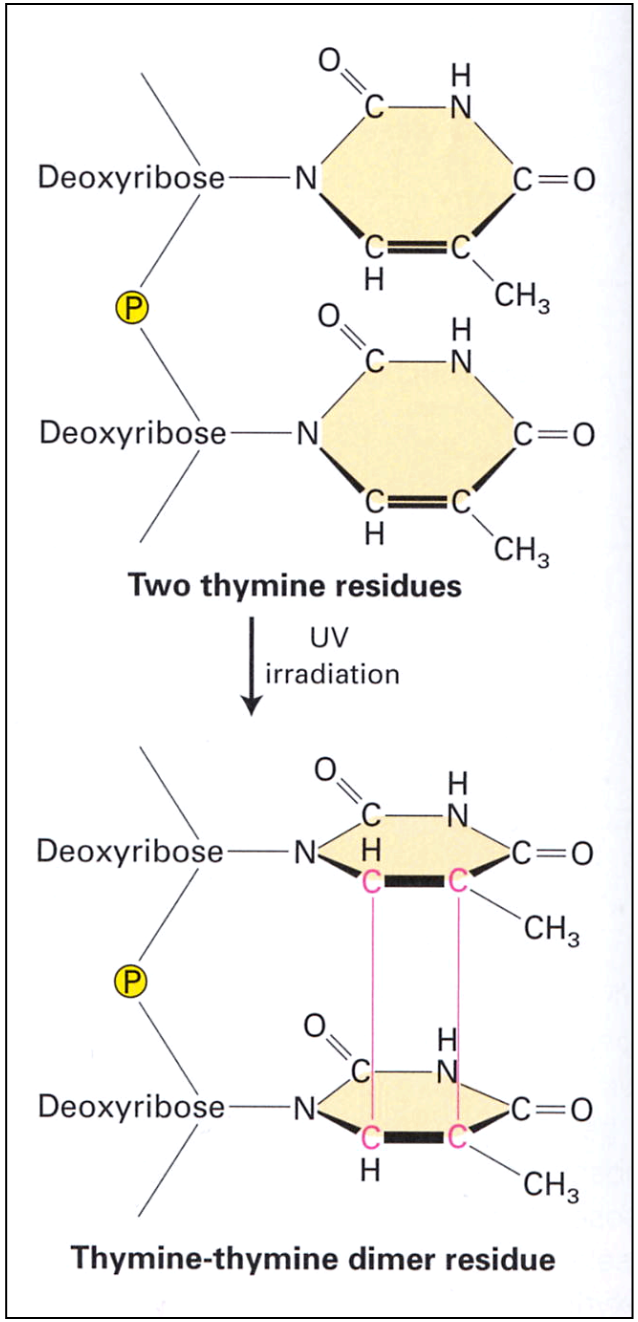

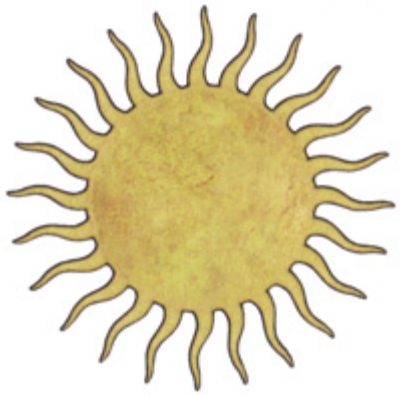

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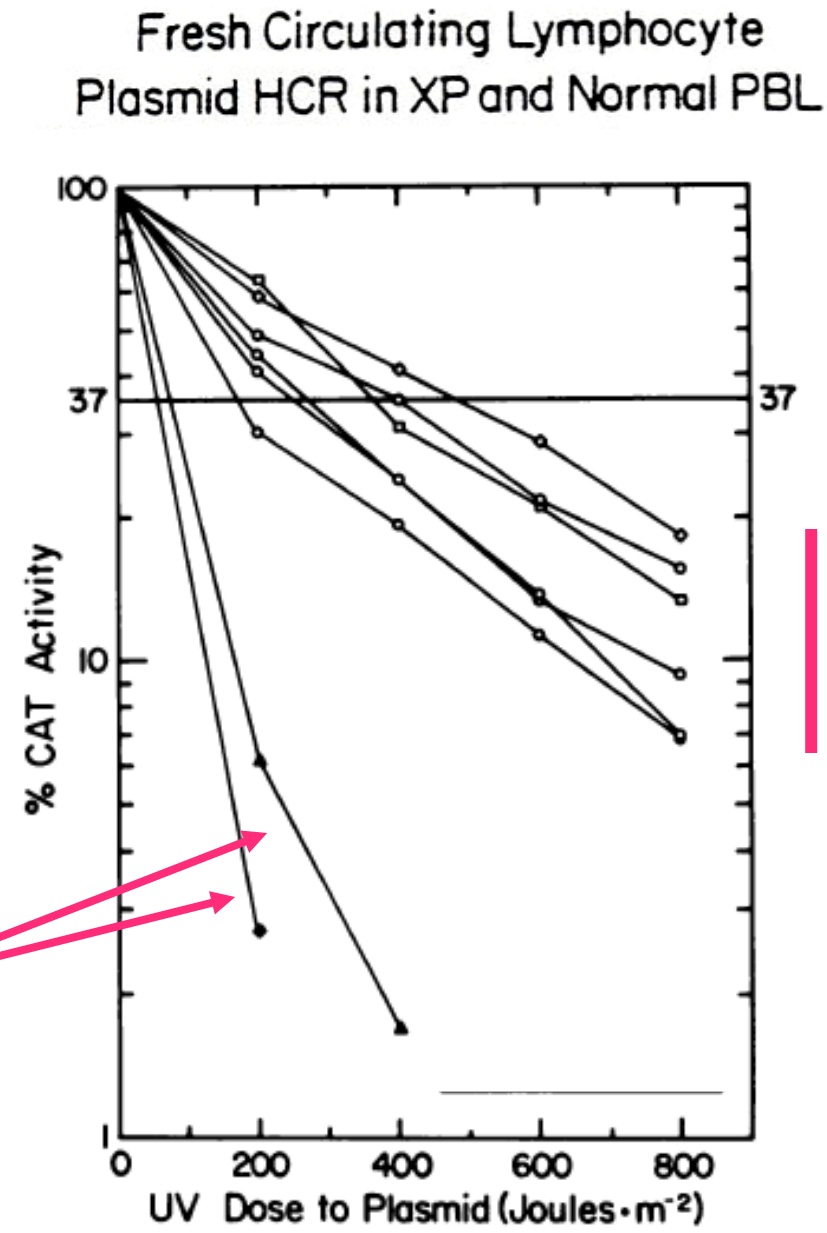

Cells from XP patients

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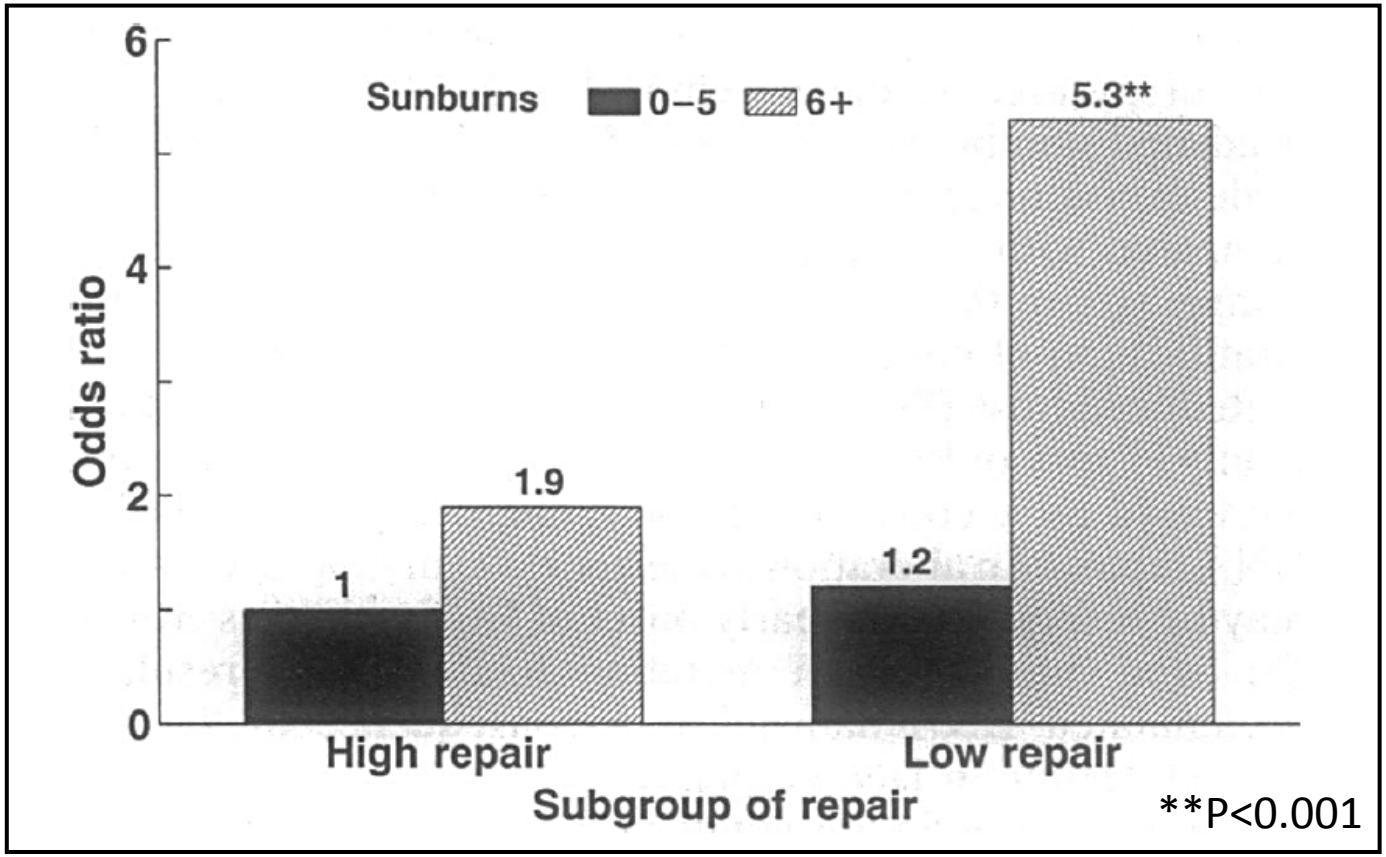
Low NER status combined with excessive sun exposure is very dangerous



Wei Q, Matanoski GM, Farmer ER, Hedayati MA, **GROSSMAN L.** Proc Natl Acad Sci U S A. 1993 90:1614-8.

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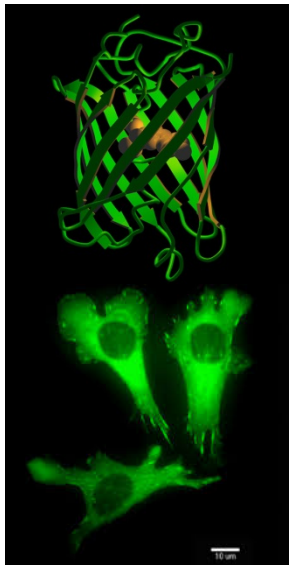

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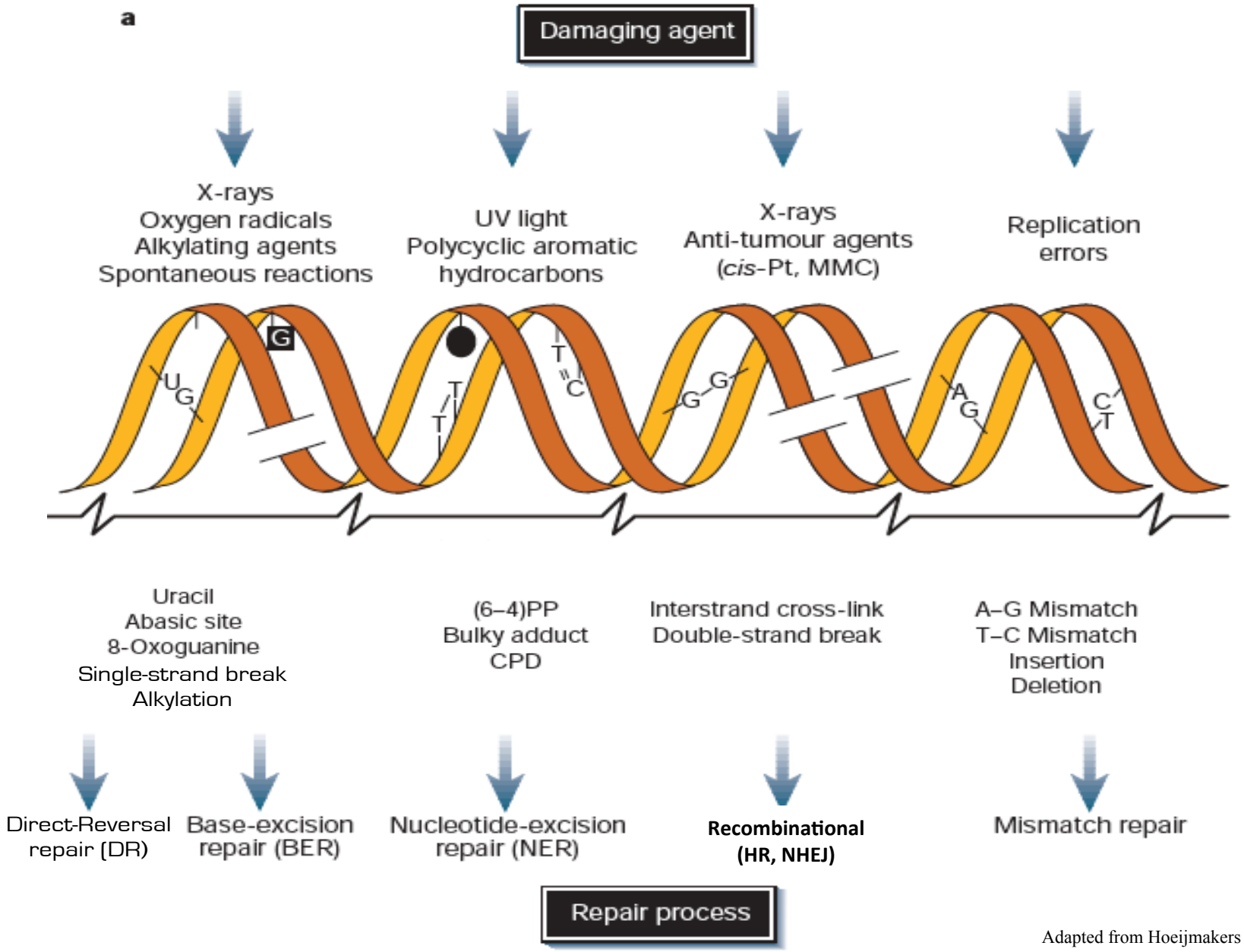
- Excision Repair

  Base excision, nucleotide excision, mismatch repair

- Double strand break repair

  Homologous recombination, Non-homologous end joining

DNA Damage and Repair



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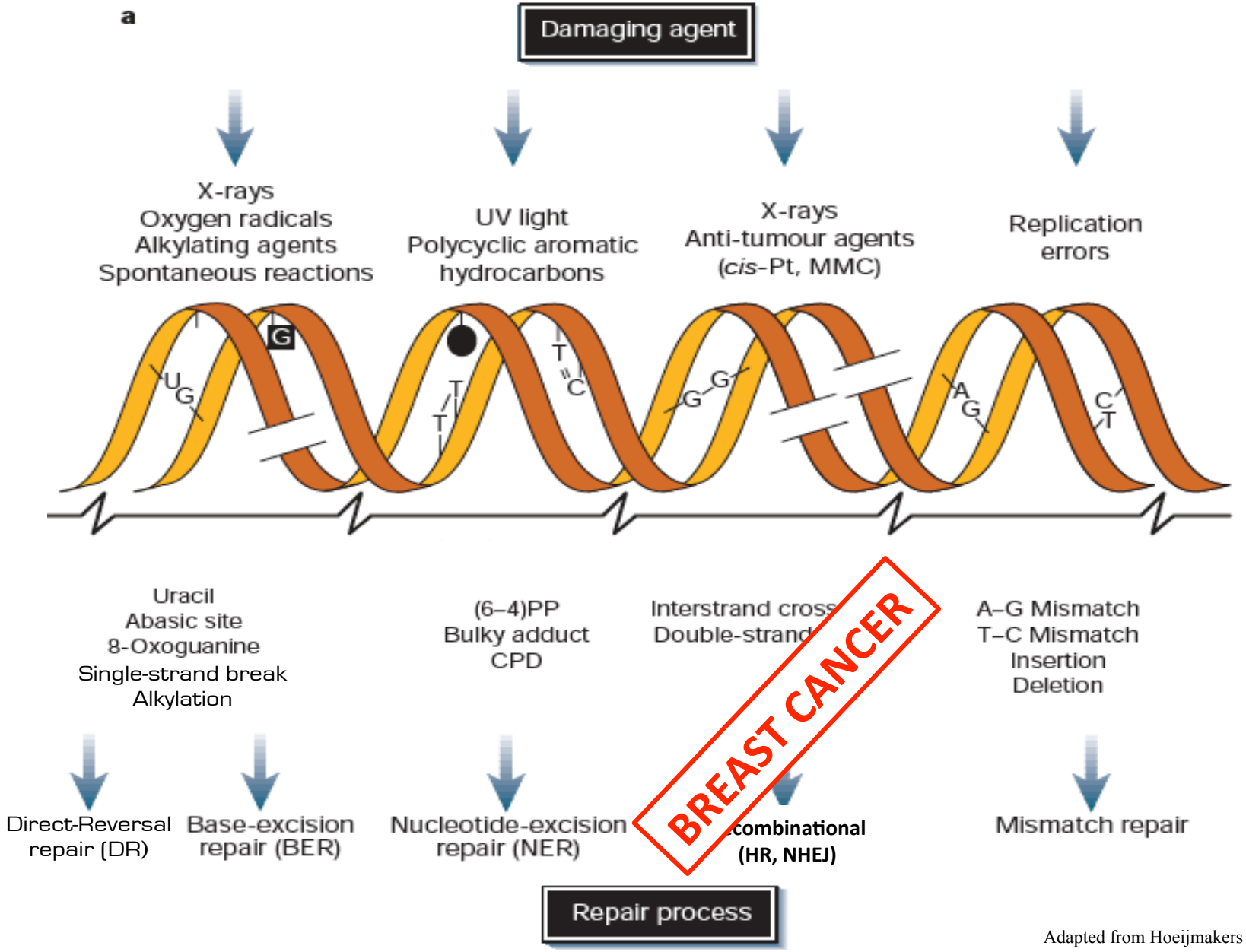

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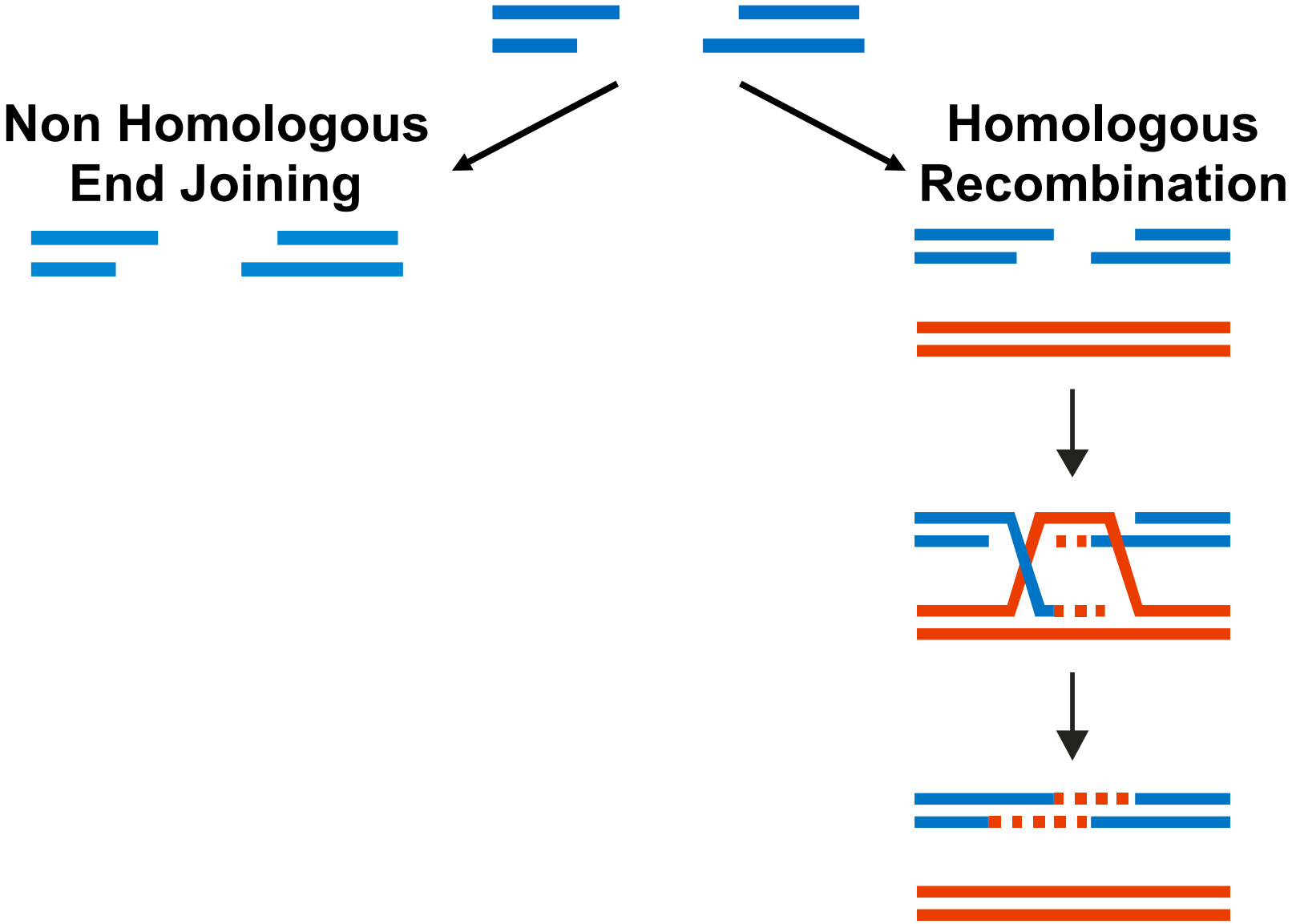
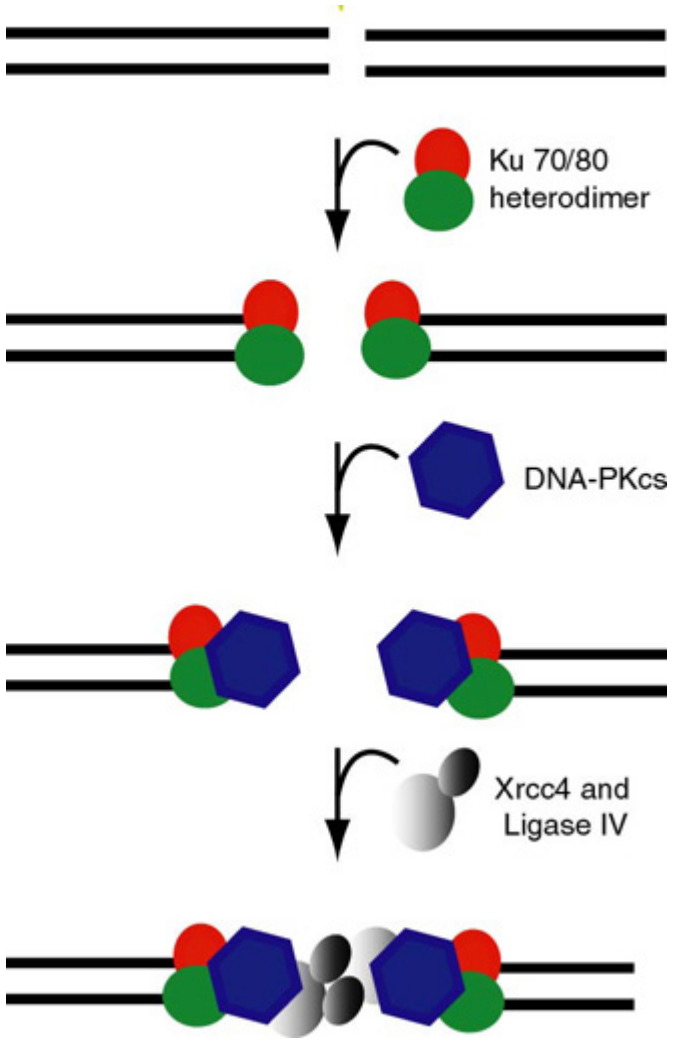
DNA double-strand break repair



Non-Homologous End Joining (NHEJ)

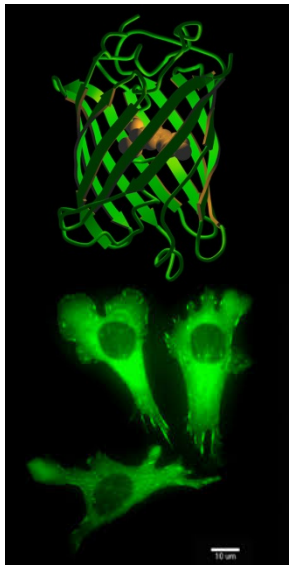


Ku70
Ku80

DNA-PKcs

Xrcc4
Ligase IV

Key Experimental Methods for Module 1



- Mammalian tissue cell culture
- Monitoring protein level by Western blot
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- Transfecting plasmids into mammalian cells
- Using fluorescent proteins as reporters of biological processes
- Flow cytometry to measure DNA repair
- Statistical analysis of biological data

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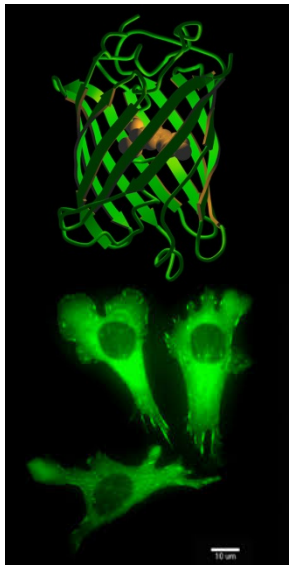

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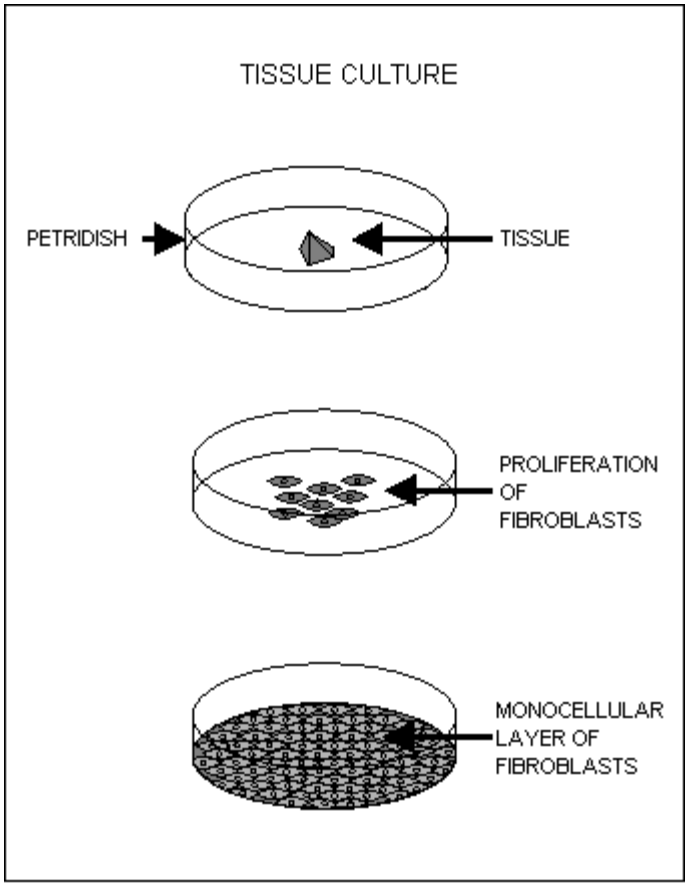
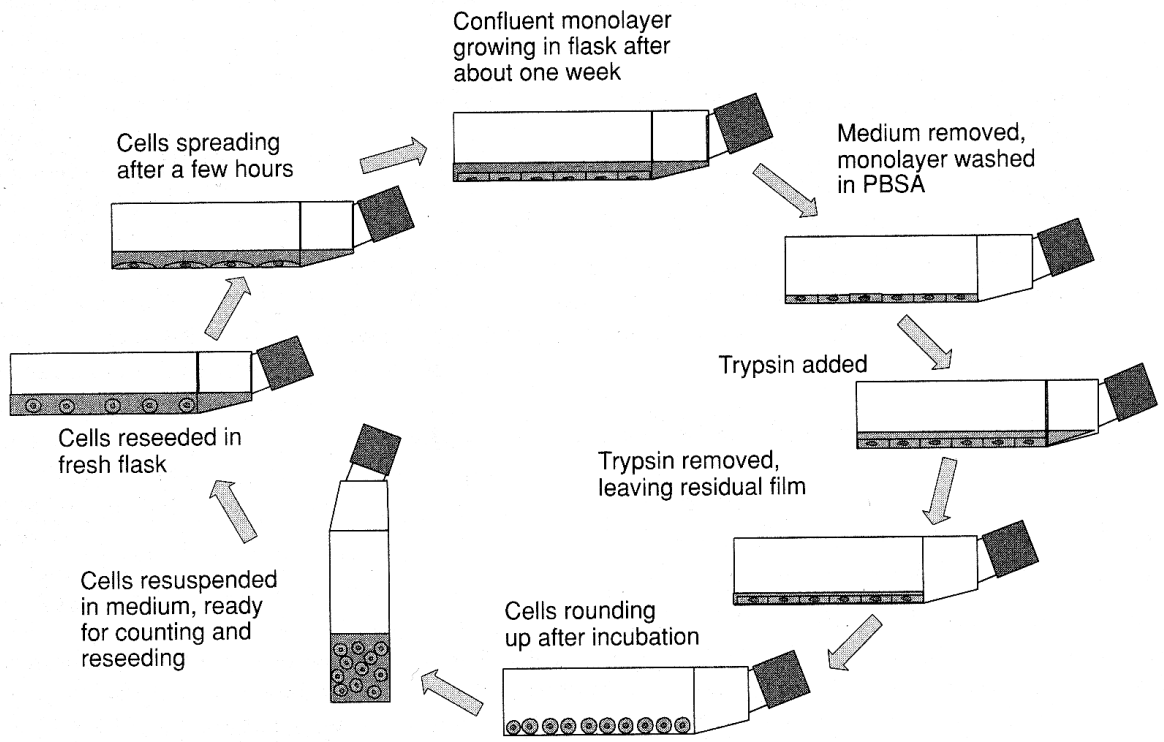
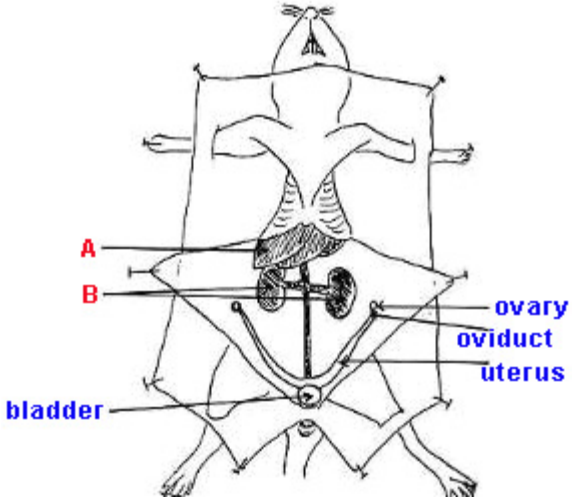
How do you grow mammalian cells?



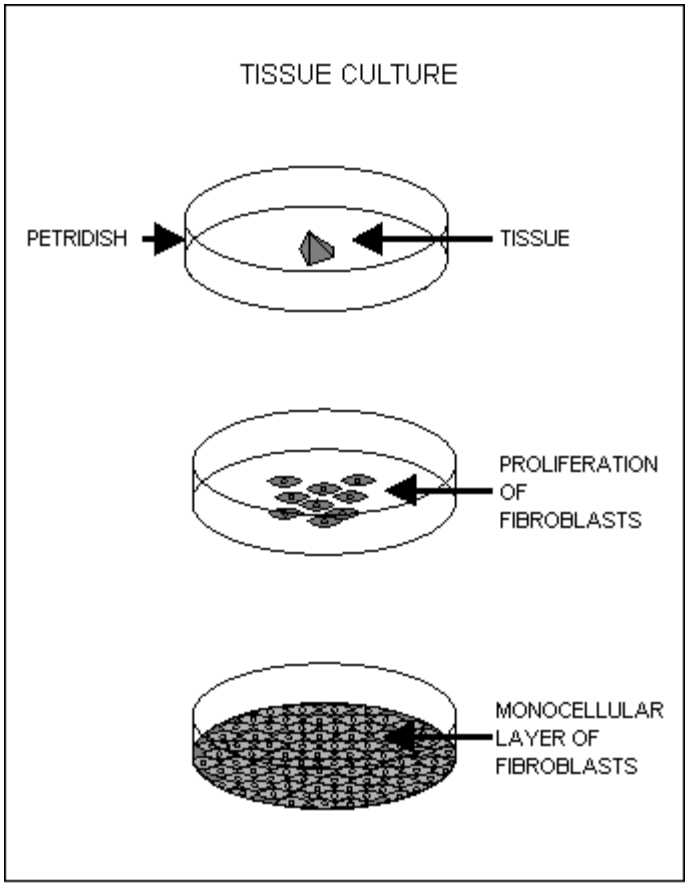


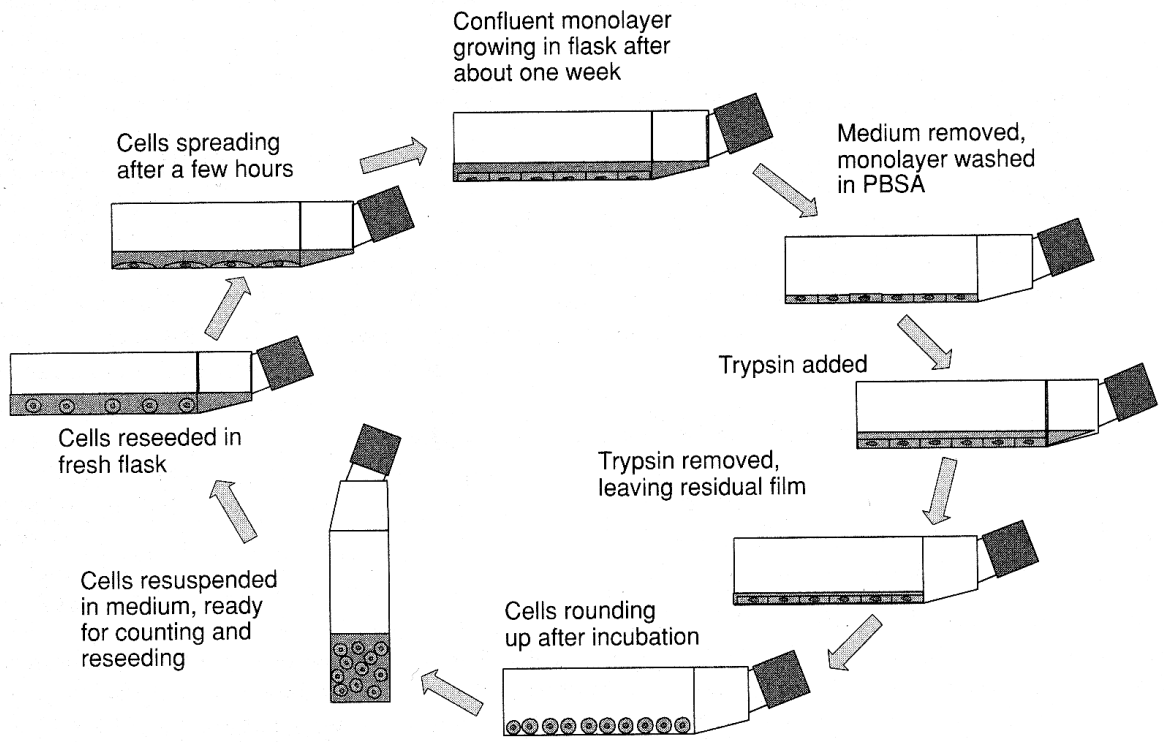
From Freshney's "Culture of Mammalian Cells"

Chinese Hamsters



How do you grow mammalian cells?

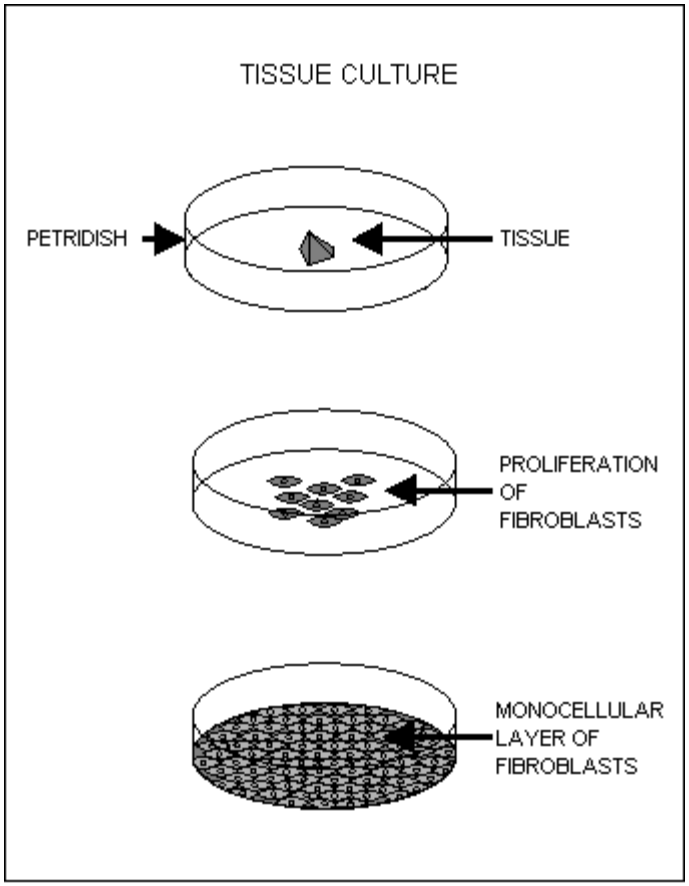


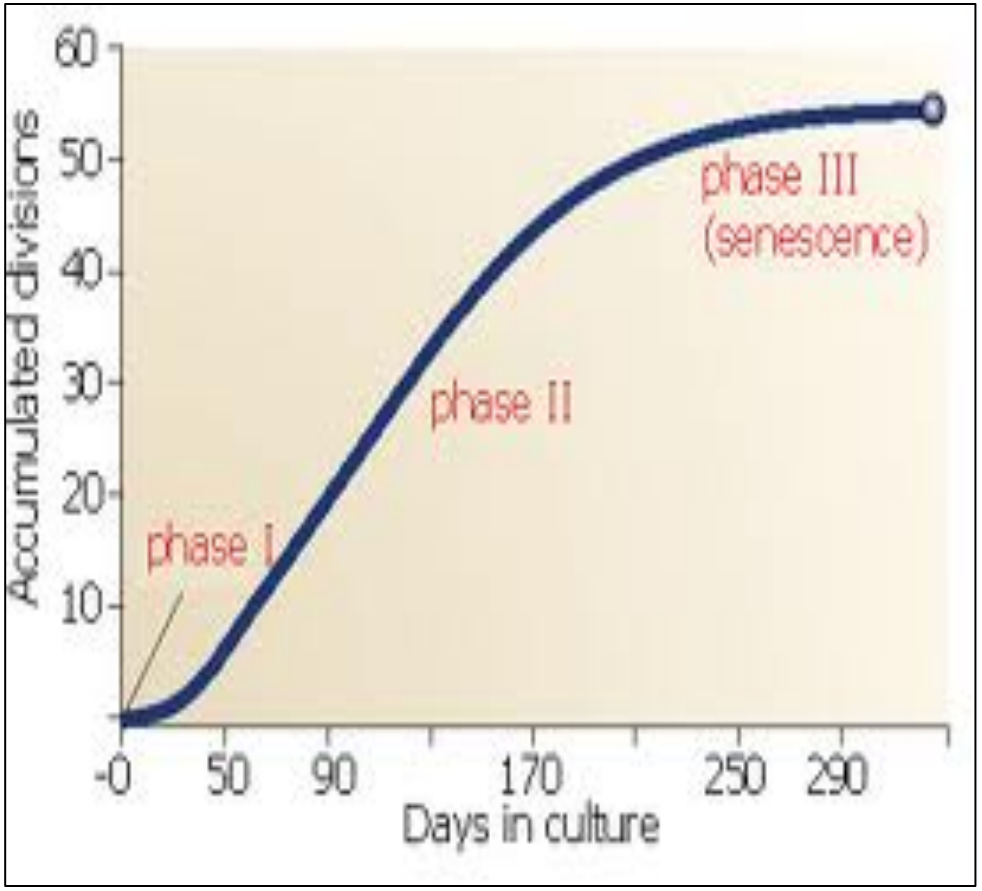


"Sub-Culturing"

From Freshney's "Culture of Mammalian Cells"

How do you grow mammalian cells?



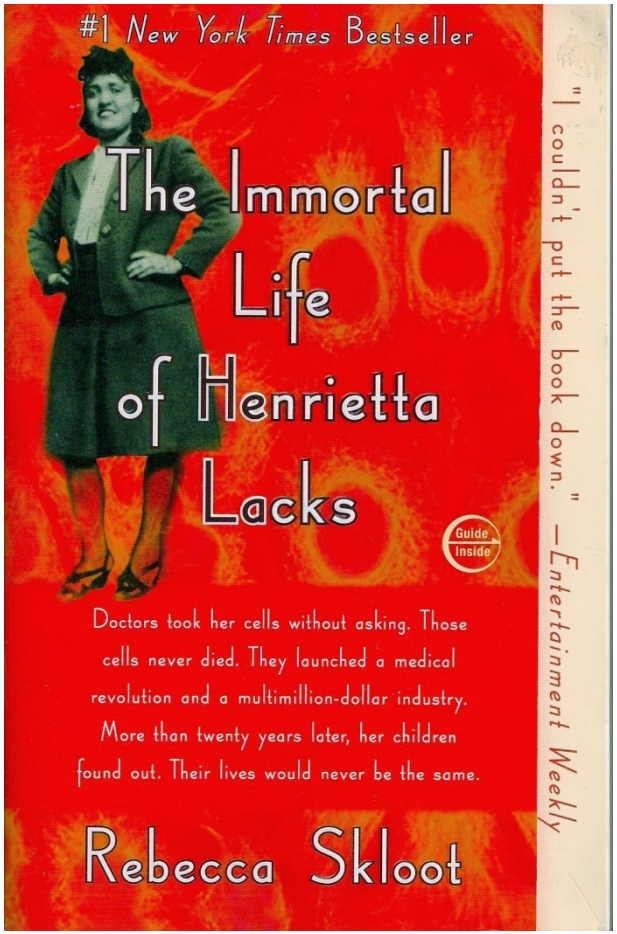


From Freshney's "Culture of Mammalian Cells"

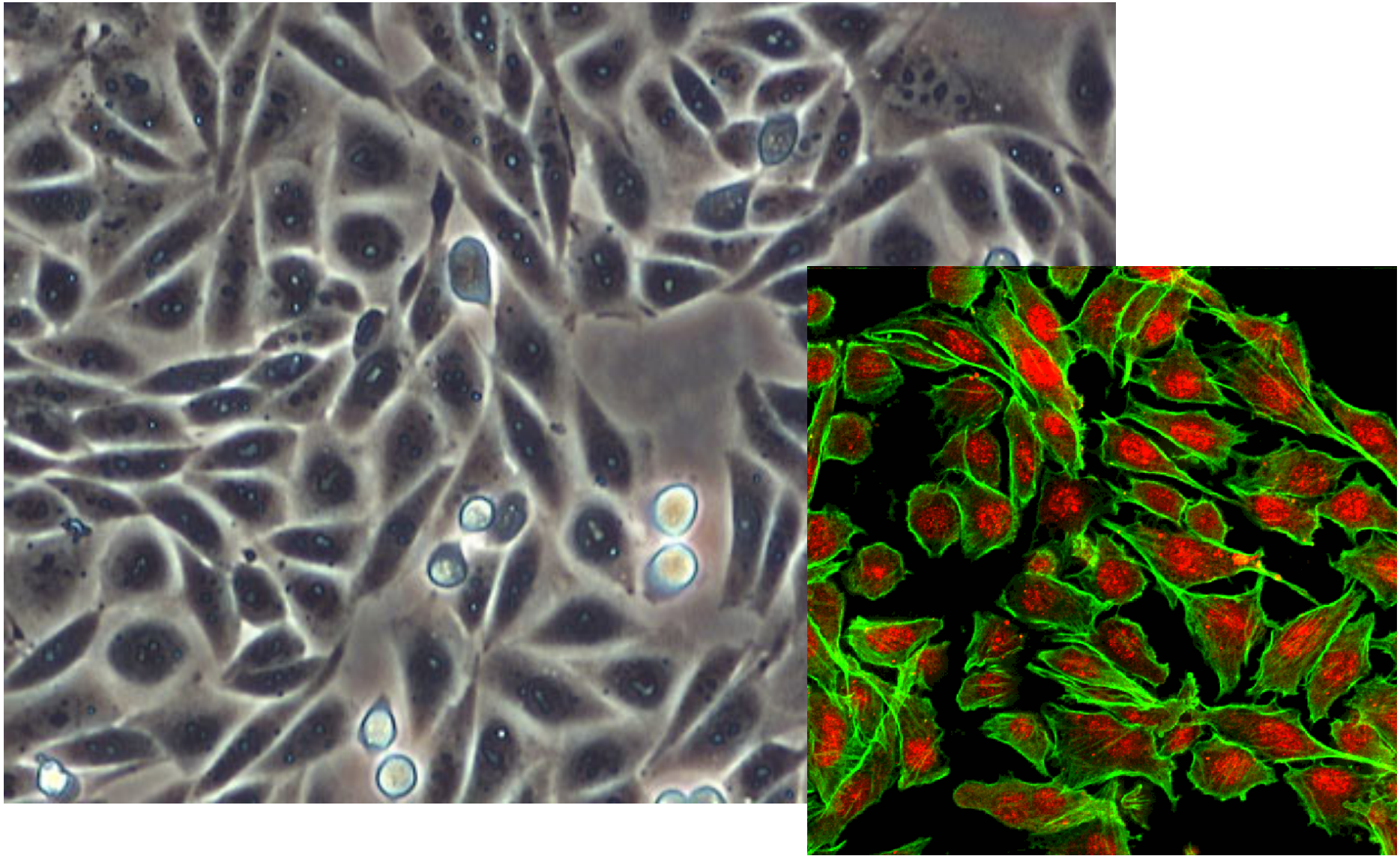
Phase Contrast

HeLa cells from the Nikon microscope web site

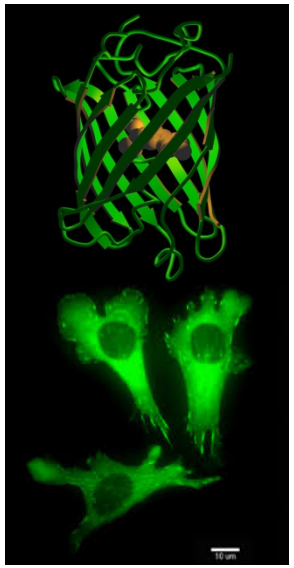
HeLa cells have been cultured continuously for scientific use since they were first taken from the ovarian tumor of **Henrietta Lacks** suffering from cervical cancer in the 1950s. They have been utilized for many purposes, including the development of a polio vaccine, the pursuit of a cure for diseases such as leukemia and cancer, and the study of the cellular effects of drugs and radiation.



Chinese Hamster Ovary (CHO) cells are immortal – they can grow indefinitely



Key Experimental Methods for Module 1

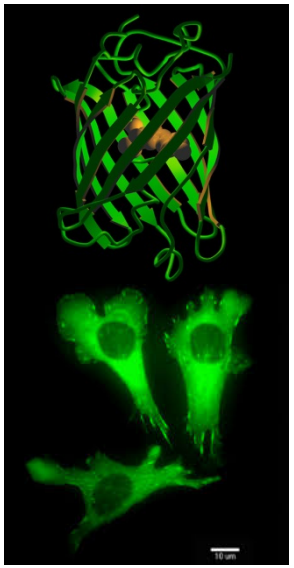


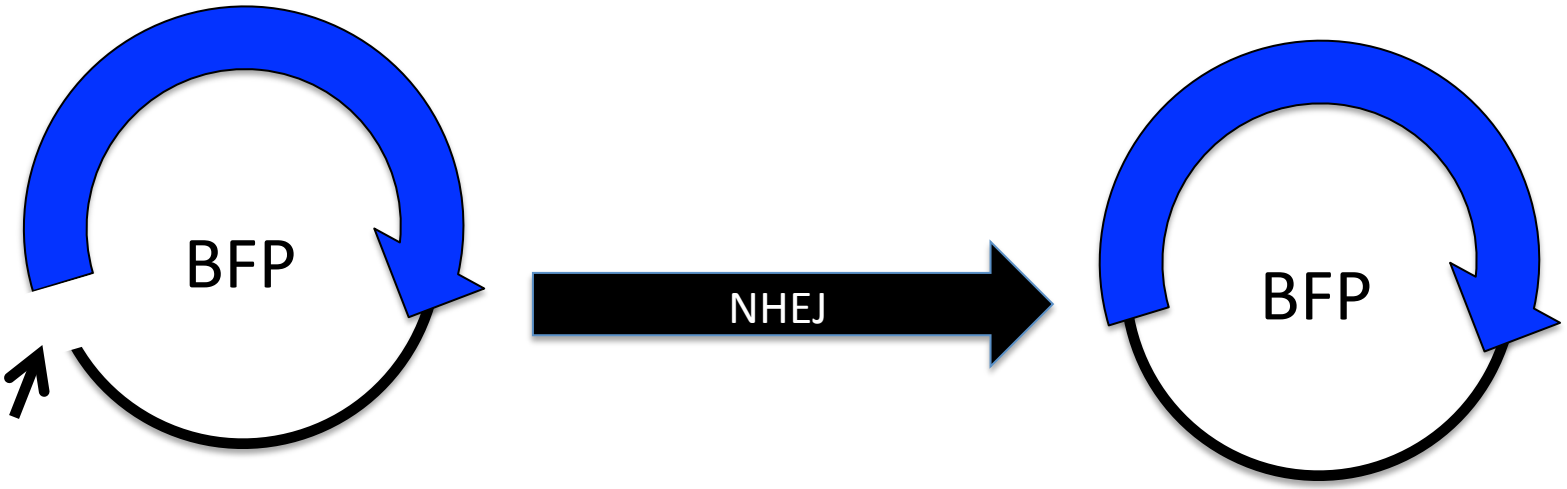
- Mammalian tissue cell culture
- Monitoring protein level by Western blot
- Generating plasmids with DNA damage
- Transfecting plasmids into mammalian cells
- Using fluorescent proteins as reporters of biological processes
- Flow cytometry to measure DNA repair
- Statistical analysis of biological data

Chinese Hamster Ovary (CHO) cells are immortal – they can grow indefinitely

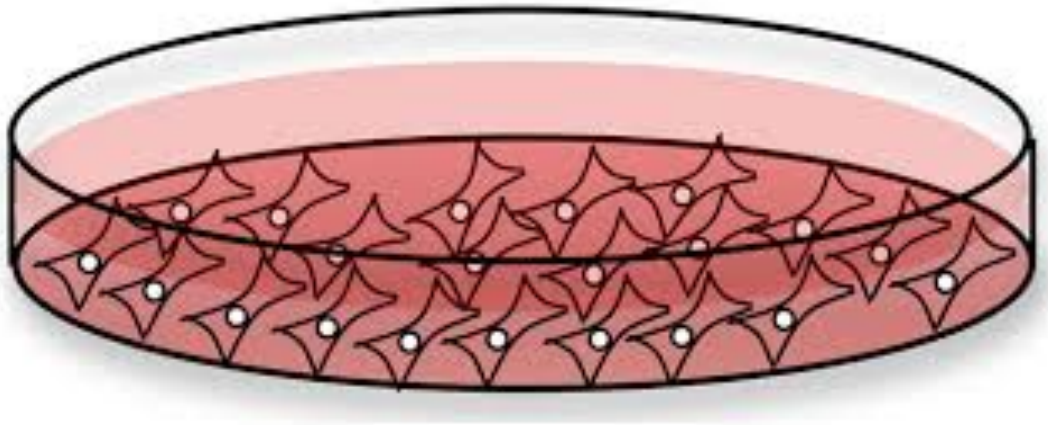
NEXT LECTURE

- Isolating X-ray-sensitive (xrs) CHO cells
- Xrs cells are deficient in NHEJ
- Detecting NHEJ proteins by Western
- Measuring NHEJ activity
- Using fluorescent proteins to measure biological processes….



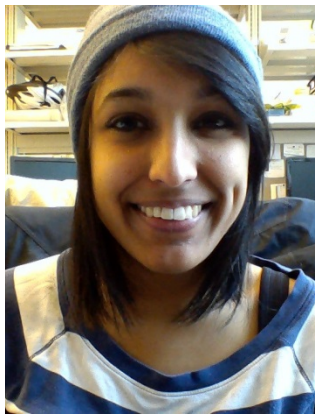


Substrate contains a DNA double strand break



20.109 Spring 2014 Module 2
System Engineering and Protein Foundations





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