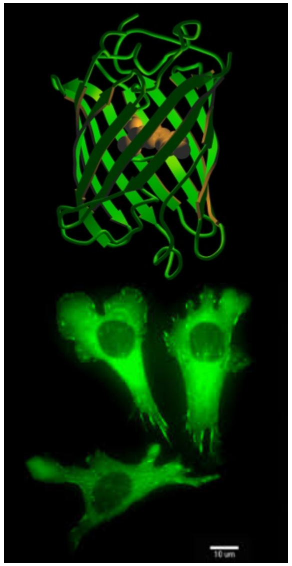
Module 1: DNA Engineering

Leona Samson – Lecture 5

Experiments and lectures based upon research in Prof. Bevin Engelward's laboratory

What experimental question will you ask in Module 1?

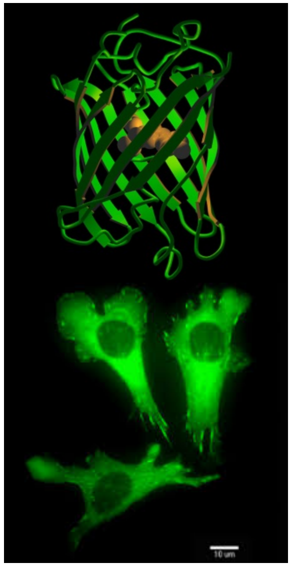


What conditions affect the frequency of DNA repair by homologous recombination in mouse embryonic stem cells?

This raises the following questions

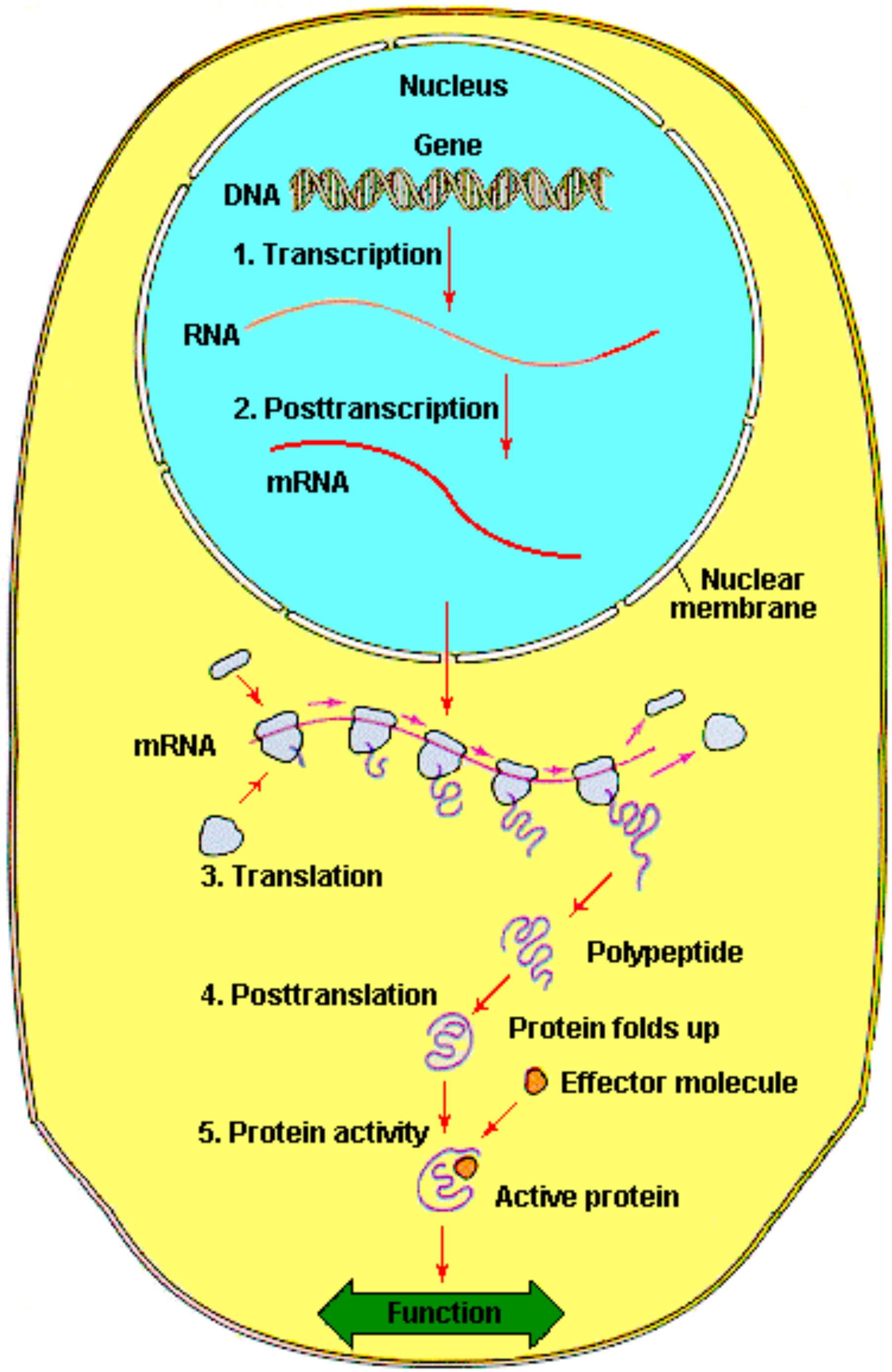
- How does DNA get damaged?
- What is DNA repair?
- Why does DNA repair exist?
- Why do we care about how efficient DNA repair is?
- How does one actually measure DNA repair?

Key Experimental Methods for Module 1



- Construction of truncated eGFP gene – Cloning
    - PCR, Restriction Enzymes, Ligation/Transformation, Bacterial culture
- Mammalian tissue cell culture
- Transfecting plasmids into mammalian cells
- Flow cytometry to measure DNA repair
- Statistical analysis of biological data

Central Dogma



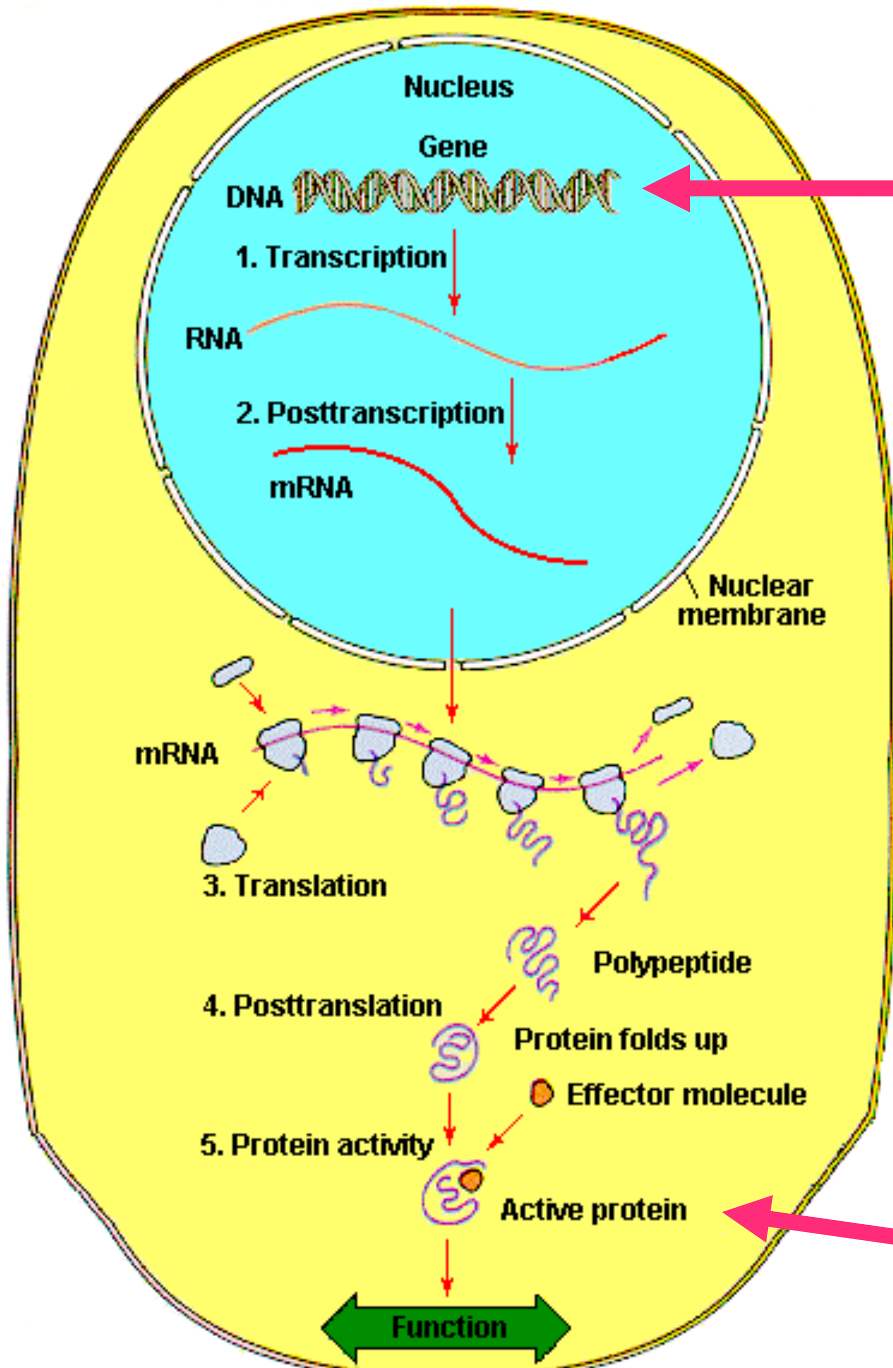
DNA

makes

RNA

makes

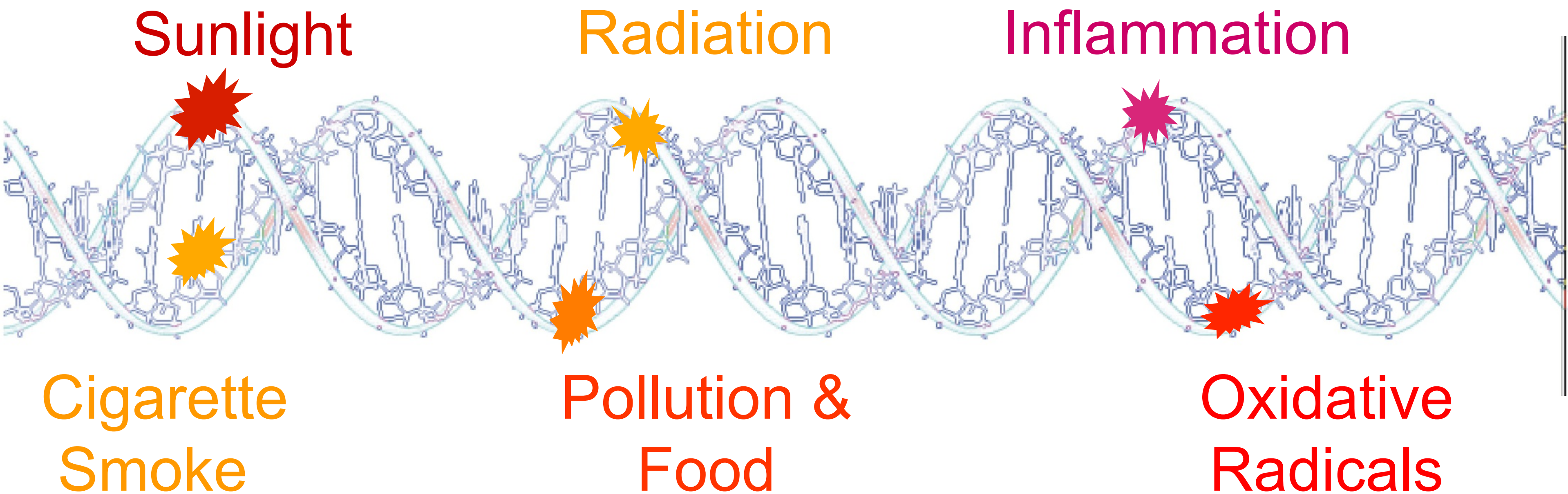
Protein



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

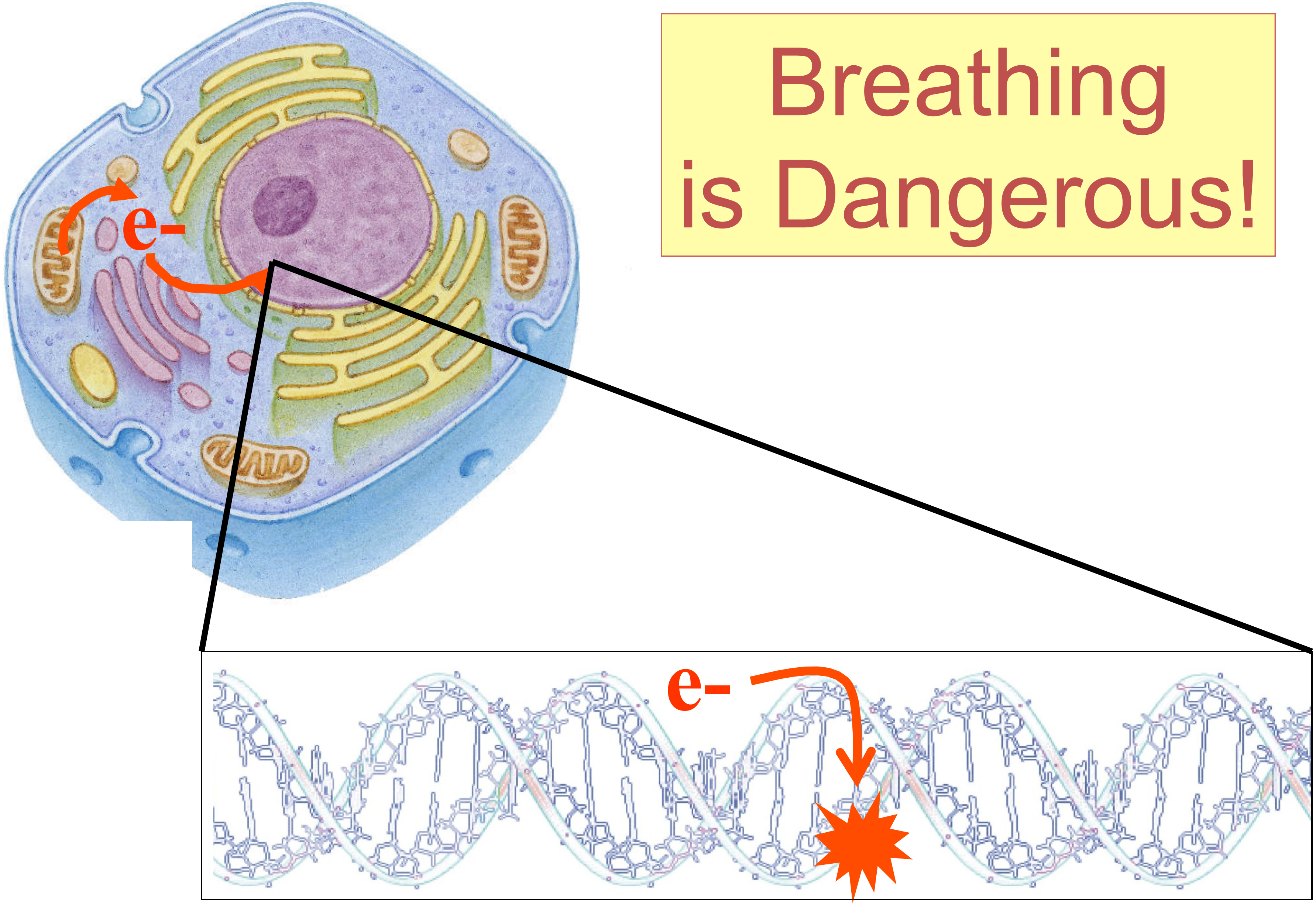
Inactive proteins, or proteins with altered function, are produced

DNA is constantly being damaged by endogenous and exogenous agents

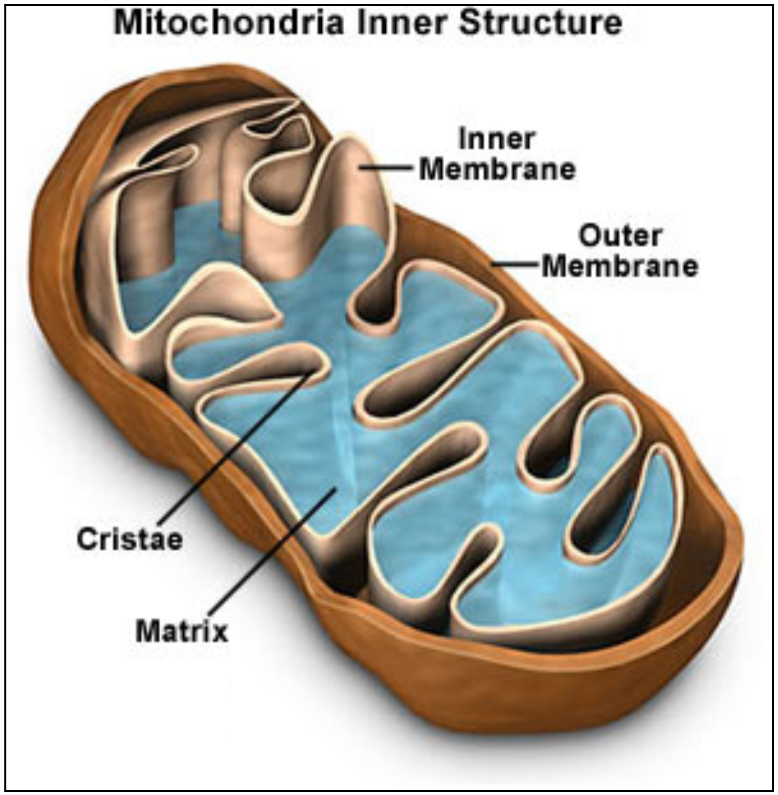


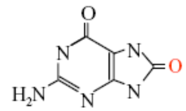
Courtesy of Bevin Engelward

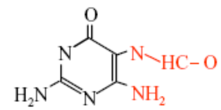
Breathing is Dangerous!

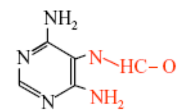


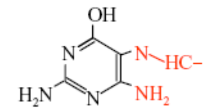
Breathing leads to DNA Damage: Reactive Oxygen Species (ROS)

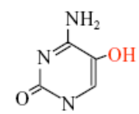


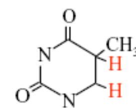

8-oxoguanine

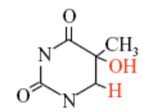

2,5-amino 5-formamidopyrimidine

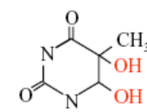

4,6-diamino 5-formamidopyrimidine

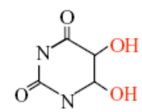

2,6-diamino 4-hydroxy-5-formamidopyrimidine

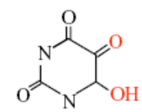

5-hydroxycytosine

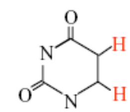

5,6-dihydrothymine

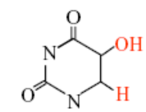

5-hydroxy-5,6-dihydrothymine

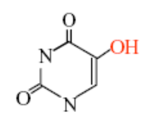

Thymine glycol

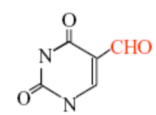

Uracil glycol

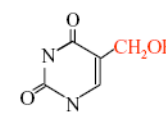

Isodialuric acid

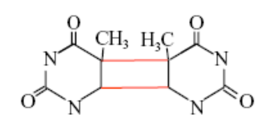

5,6-dihydrouracil

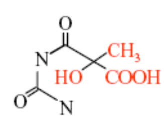

5-hydroxy-5,6-dihydrouracil

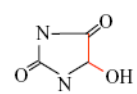

5-hydroxyuracil

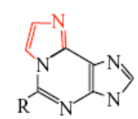

5-formyluracil

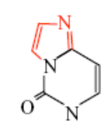

5-hydroxymethyluracil

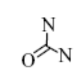

Cyclobutane pyrimidine dimer


Methyltartronylurea


5-hydroxyhydantoin

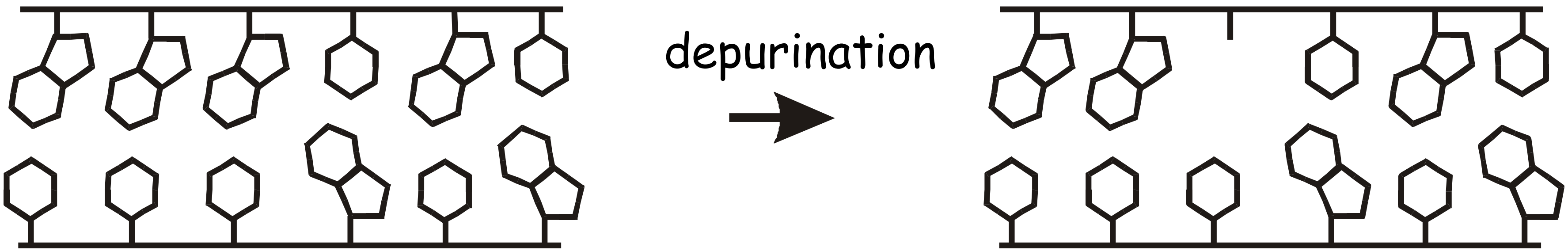

1, N6-ethenoadenine


3,N4-ethenocytosine


Urea

From Slupphaug, Kavli and Krokan

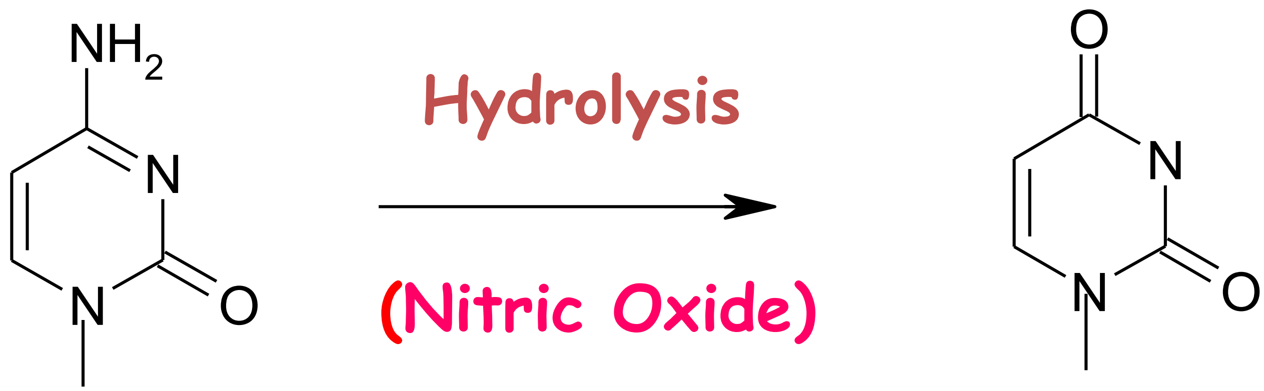
DNA Reacts also with Water - Hydolysis



Hydrolytic events/human cell/per day

Depurinations	10,000/day/cell
Depyrimidinations	500/day/cell
Deaminations	
C to U	160/day/cell
5MeC to T	12/day/cell
A to Hx	1/day/cell

Deamination



Cytosine **Uracil**

Uracil from cytosine deamination in DNA causes G:C to A:T Transition mutations

Take the case of the deamination of cytosine:

$T_{1/2}$ of this bond is 30,000 years

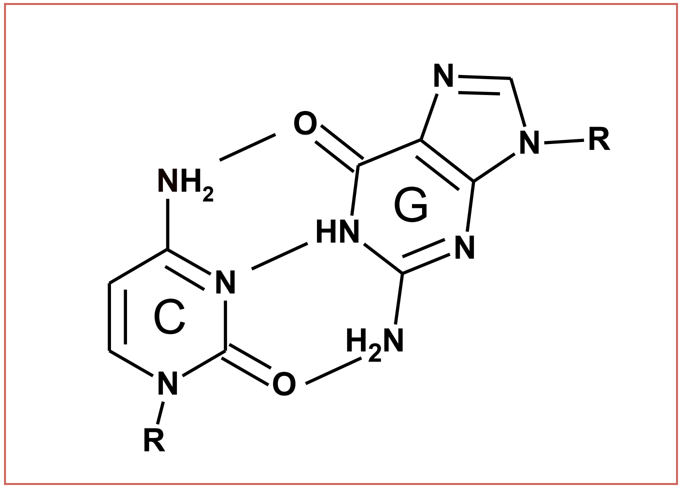
6×10^9 bp per cell

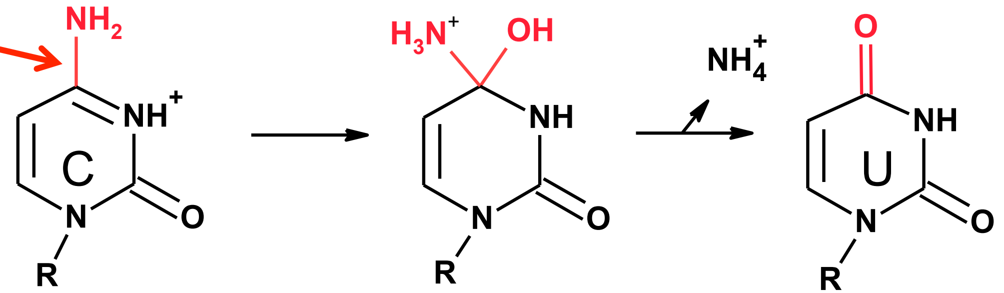
~ 3×10^9 are G:C

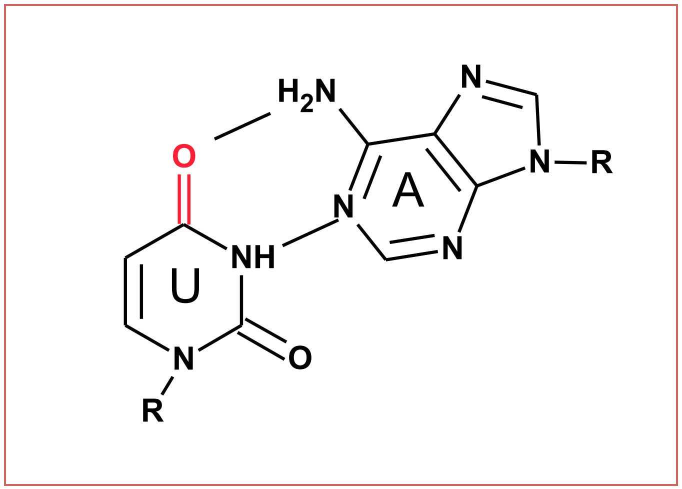
~ 1.5×10^9 C's deaminate every 30,000 years

~ 1.5×10^5 deaminate every 3 years (~1000 d)

~ 150 times per day per cell!!!!







Most cancer Chemotherapy agents and all Radiotherapies CAUSE DNA DAMAGE

Environmental exposures to potentially harmful agents

Harmful agents

People have different exposures

People have different responses

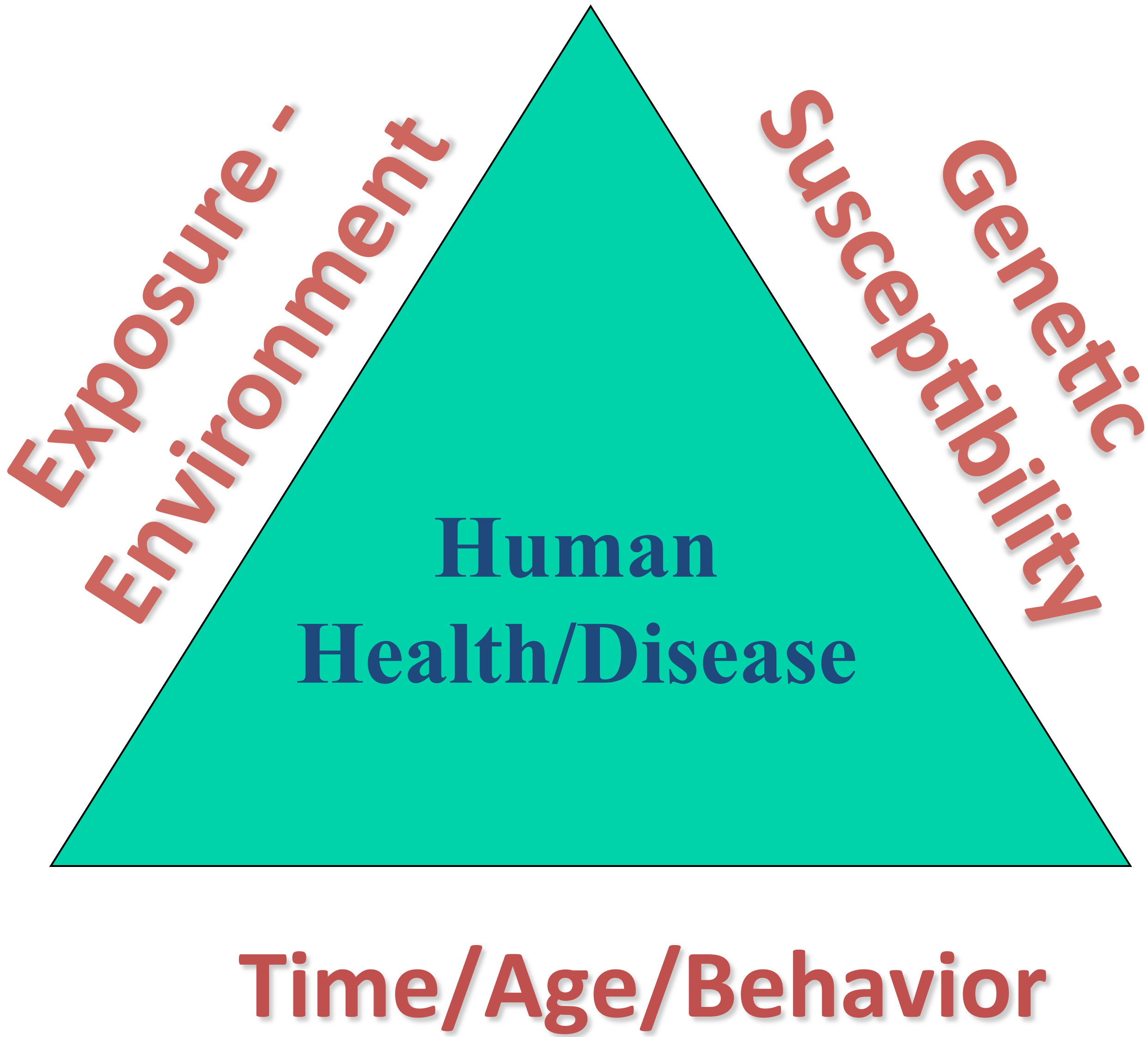
2007 - Breakthrough of the year

Natural sequence variation

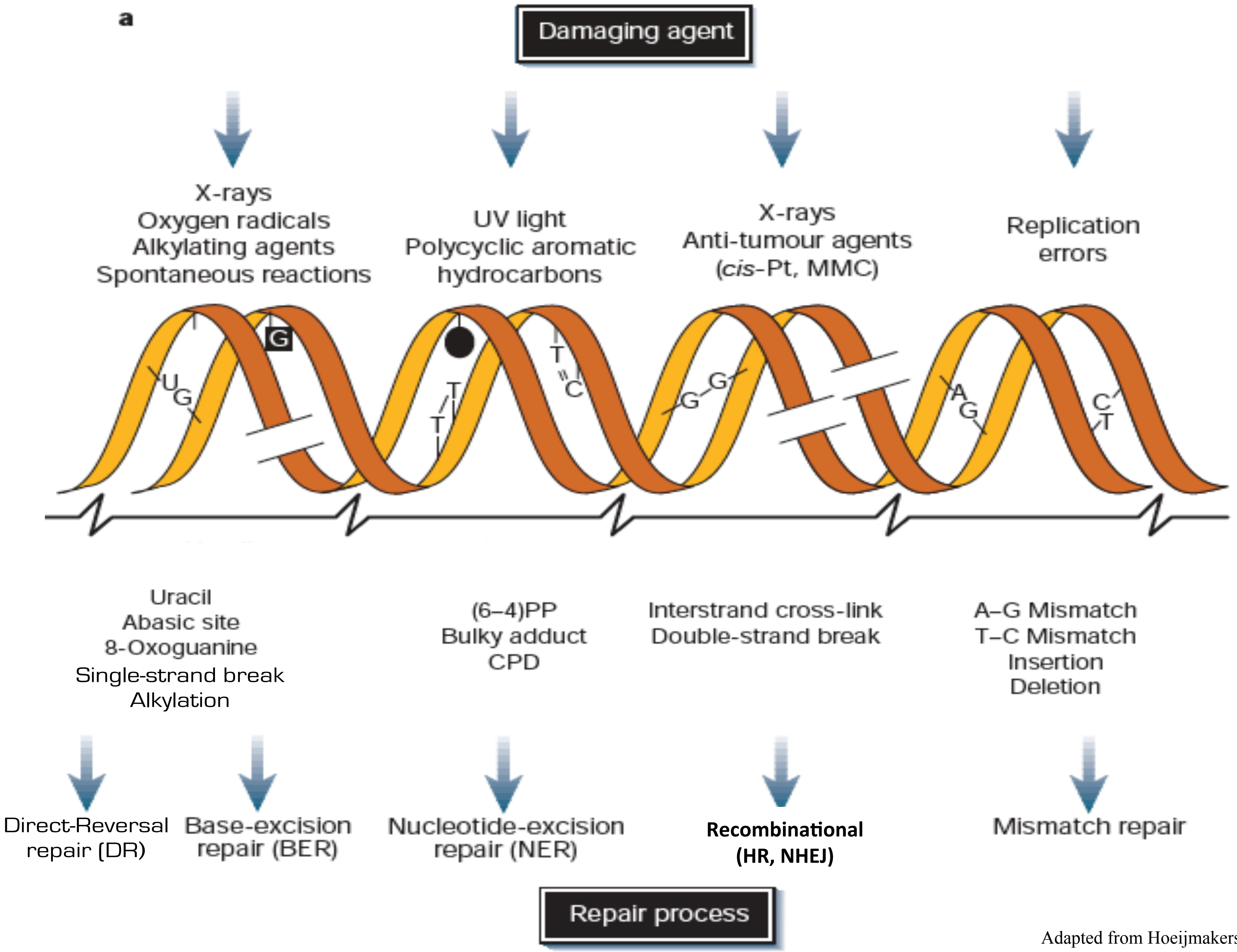
single nucleotide polymorphisms (SNPs) every 1000 base pairs.

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

Toxic agents in our environment
Gene-Environment Interaction

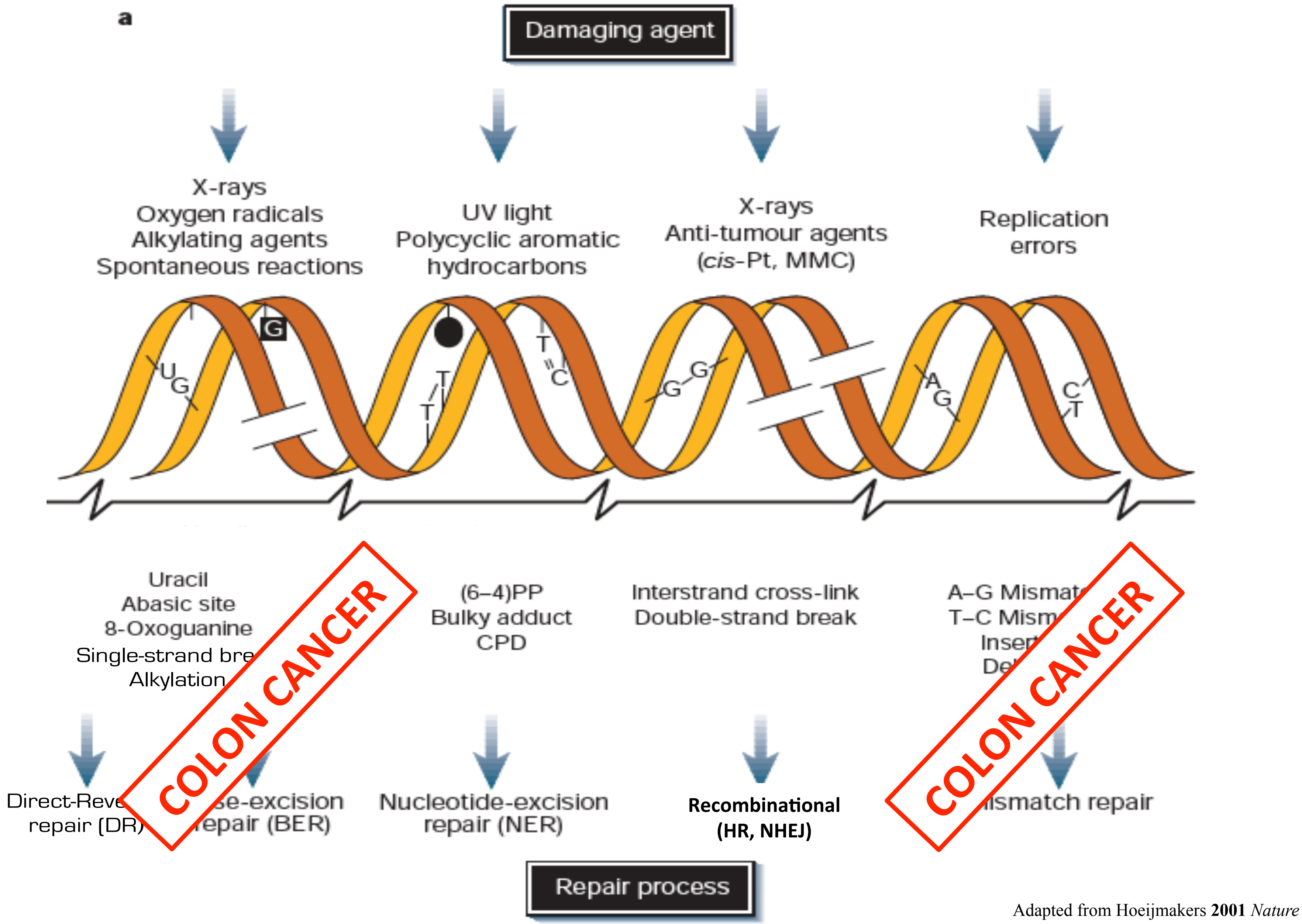


DNA Damage and Repair



Adapted from Hoeijmakers **2001** *Nature*

DNA Damage and Repair



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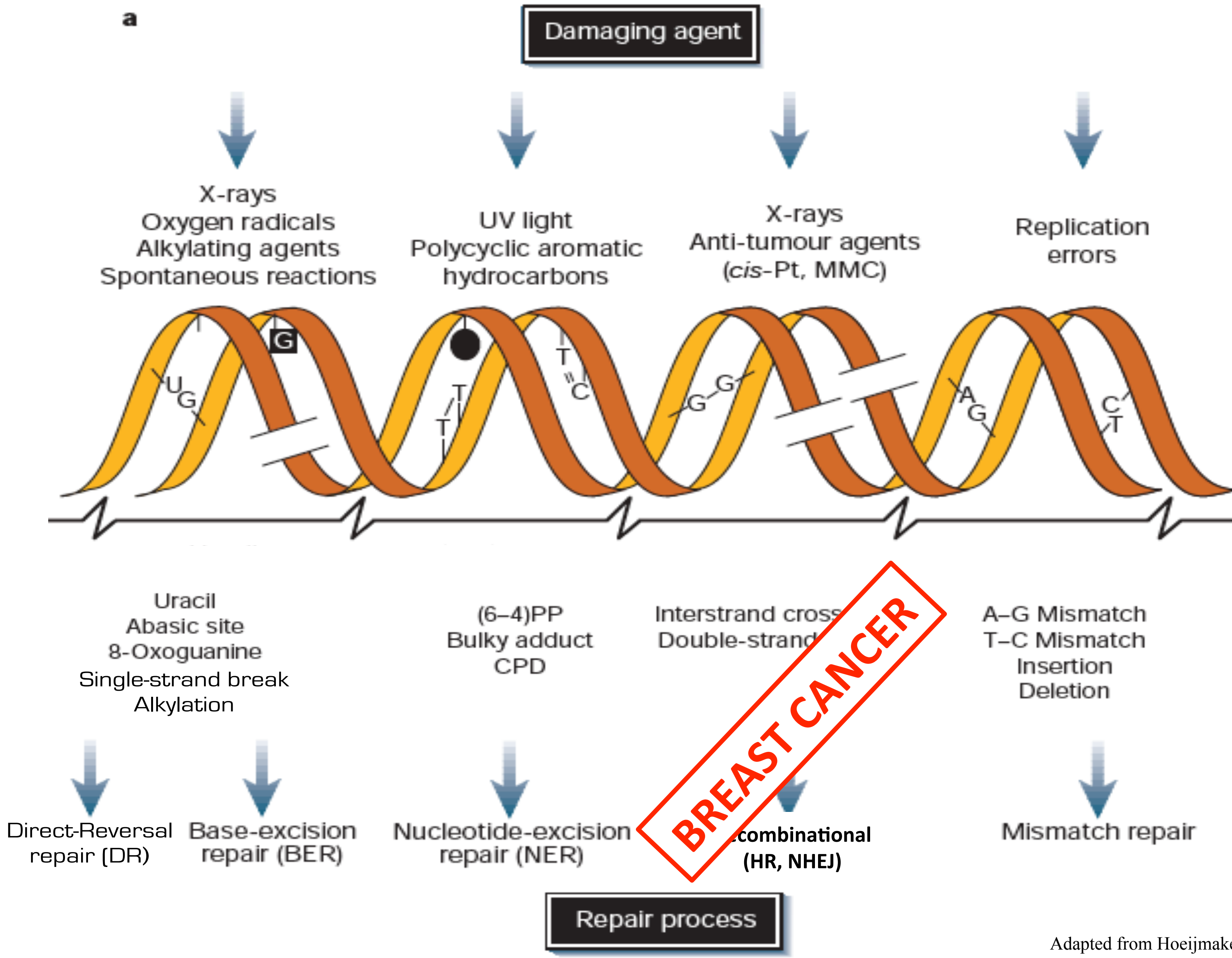
DNA Damage and Repair



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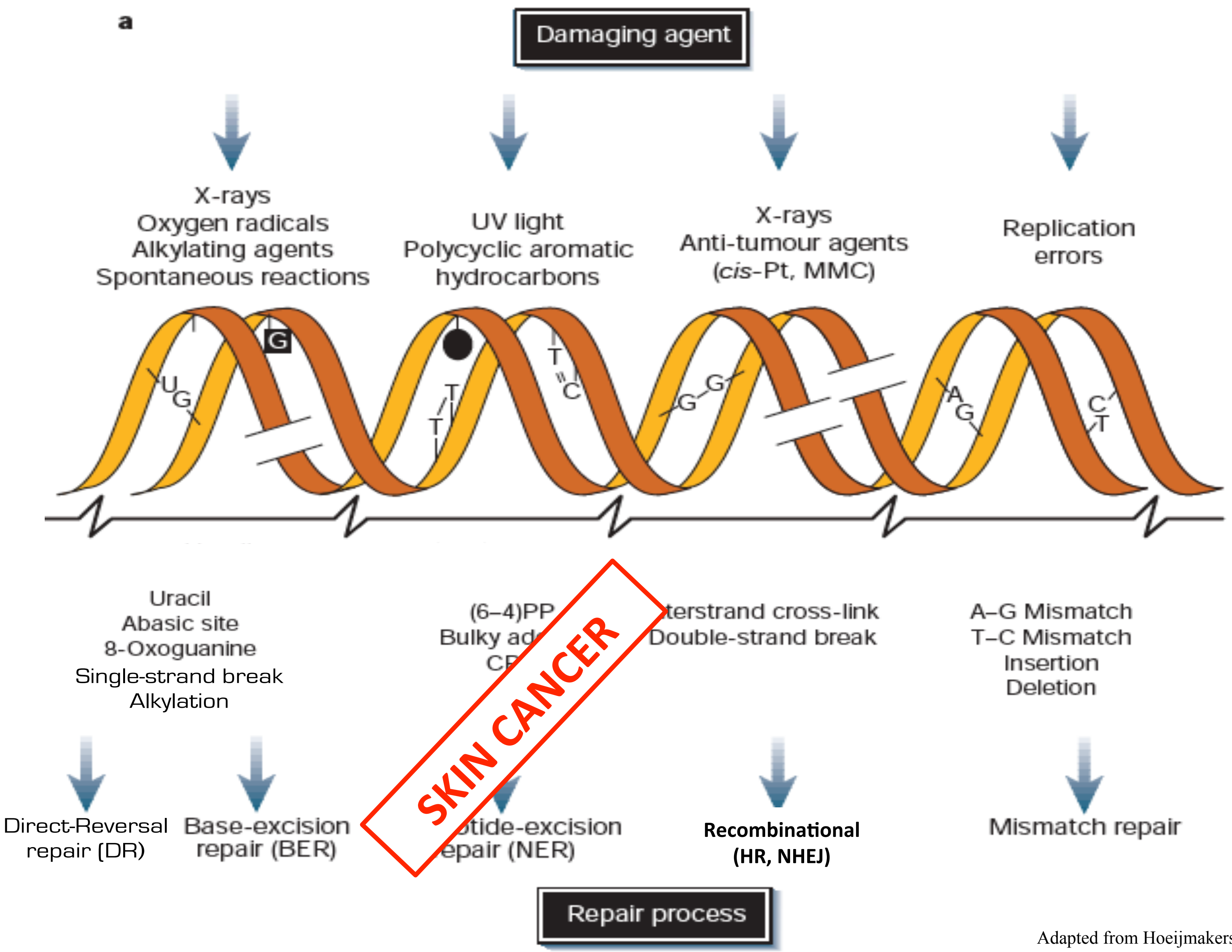
DNA Damage and Repair



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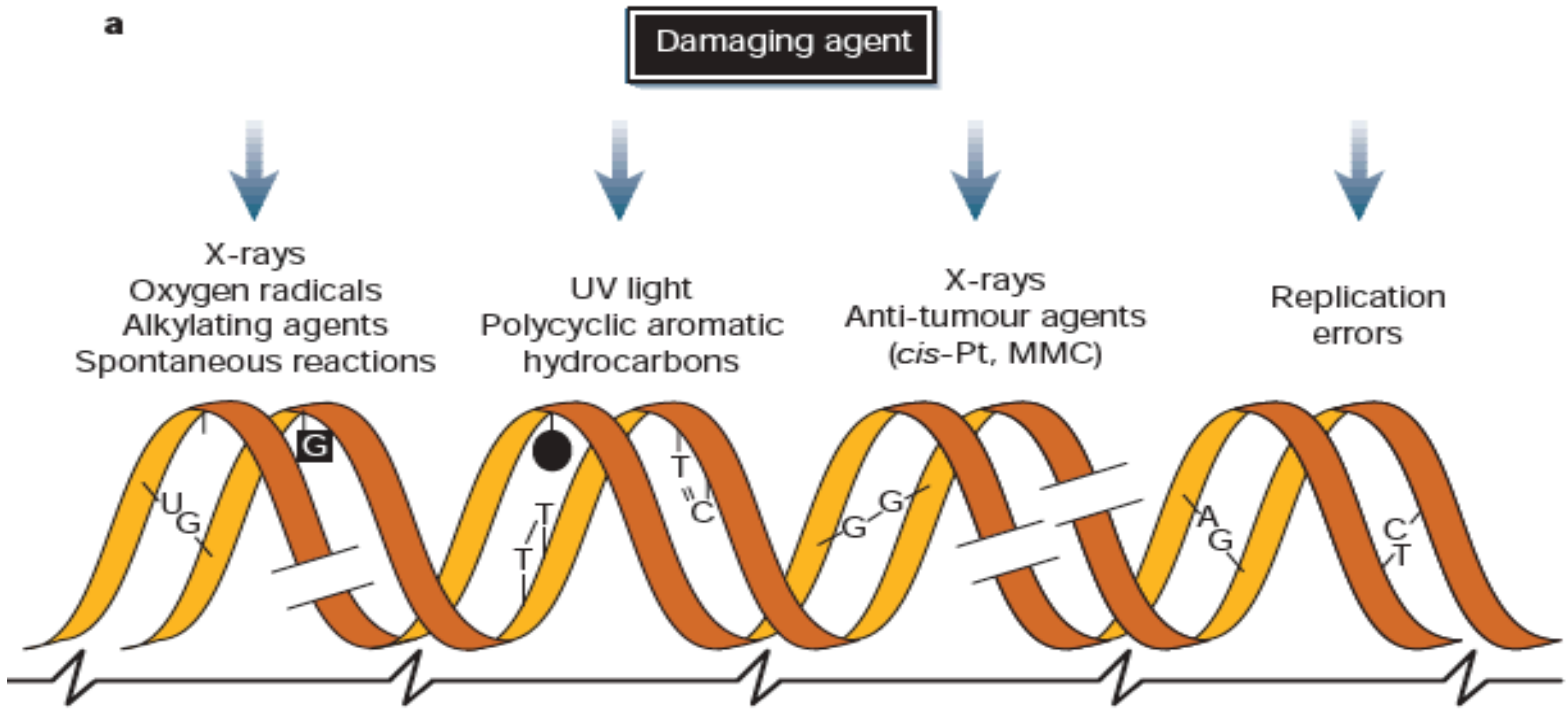
DNA Damage and Repair



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

Adapted from Hoeijmakers **2001** *Nature*

DNA Repair Strategies

- Direct Reversal

  Methyltransferase, Oxidative demethylase

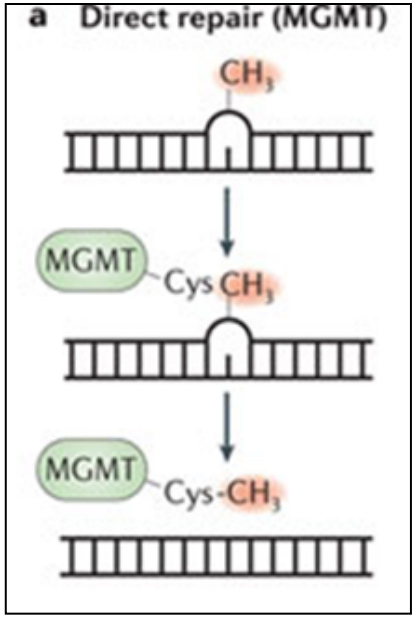
- Excision Repair

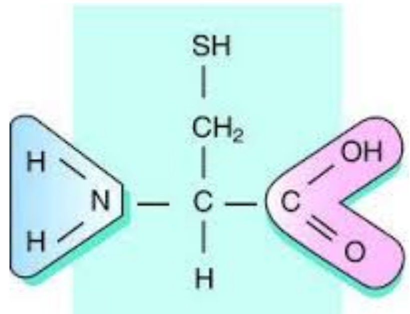
  Base excision, nucleotide excision, mismatch repair

- Double strand break repair

  Homologous recombination, Non-homologous end joining

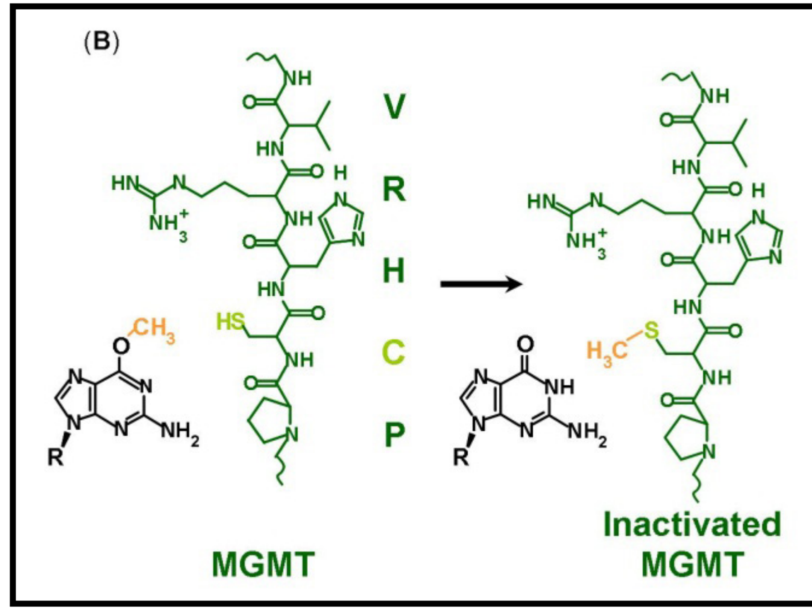
DIRECT REVERSAL OF DNA DAMAGE

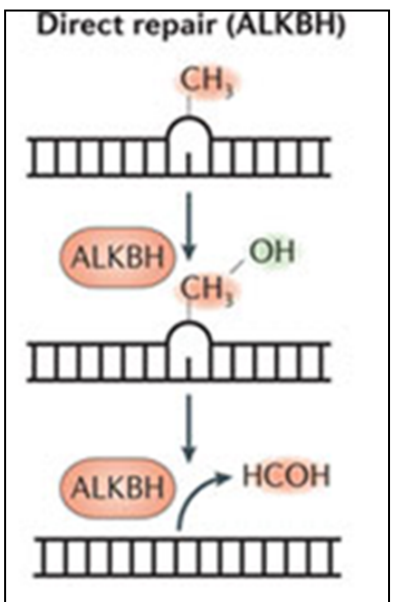


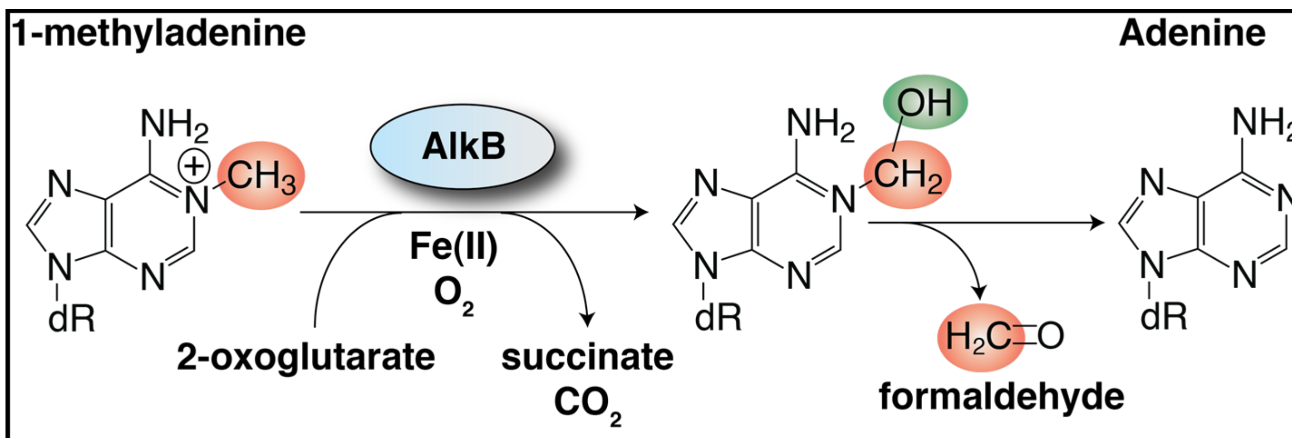


Cysteine

Methylguanine Methyltransferase (MGMT)







Oxidative demethylation via the AlkB dioxygenase enzyme

DNA Repair Strategies

- Direct Reversal

  Methyltransferase, Oxidative demethylase

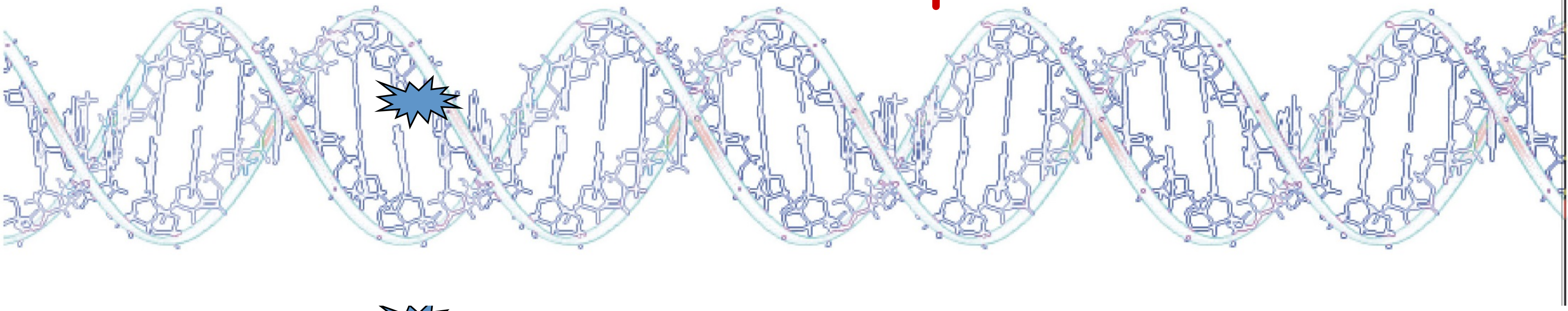
- Excision Repair

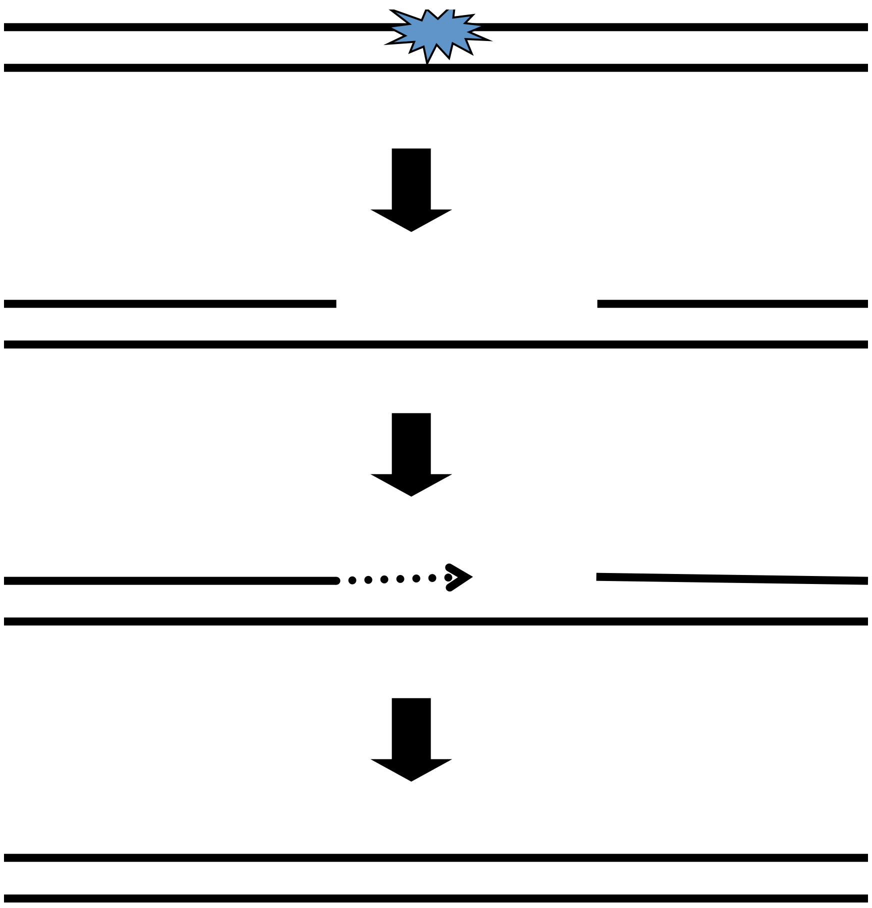
  Base excision, nucleotide excision, mismatch repair

- Double strand break repair

  Homologous recombination, Non-homologous end joining

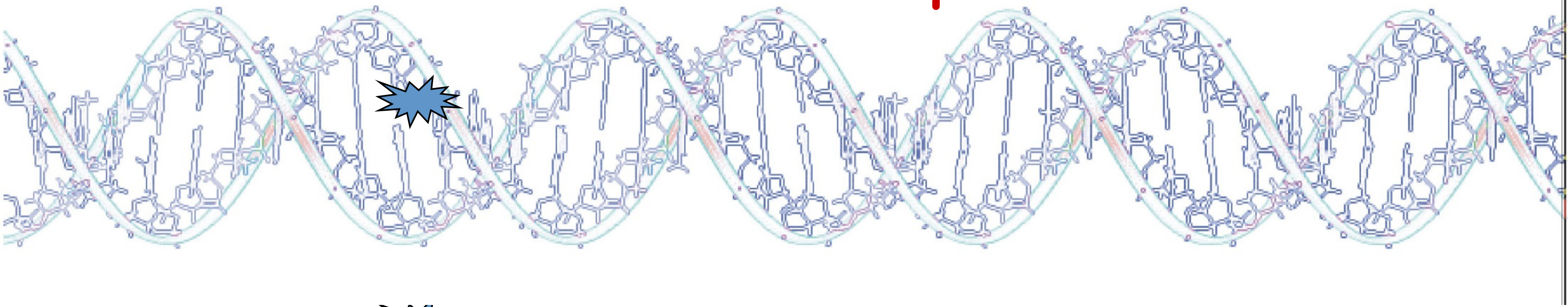
Excision Repair

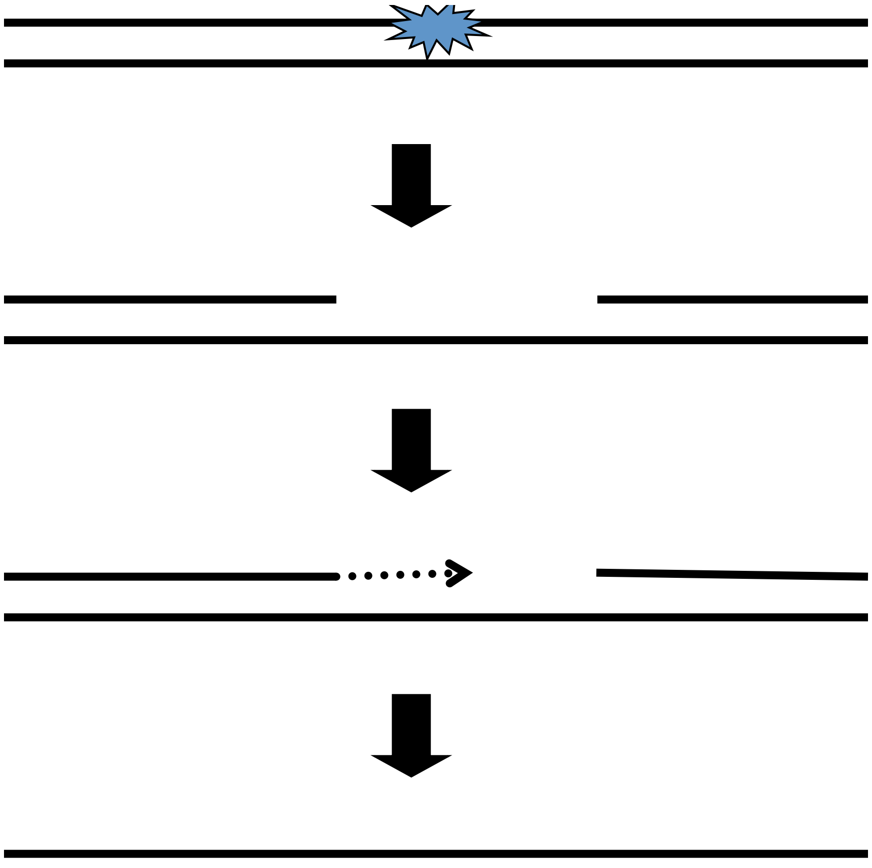




- Base Excision Repair
- Nucleotide Excision Repair
- Mismatch Repair

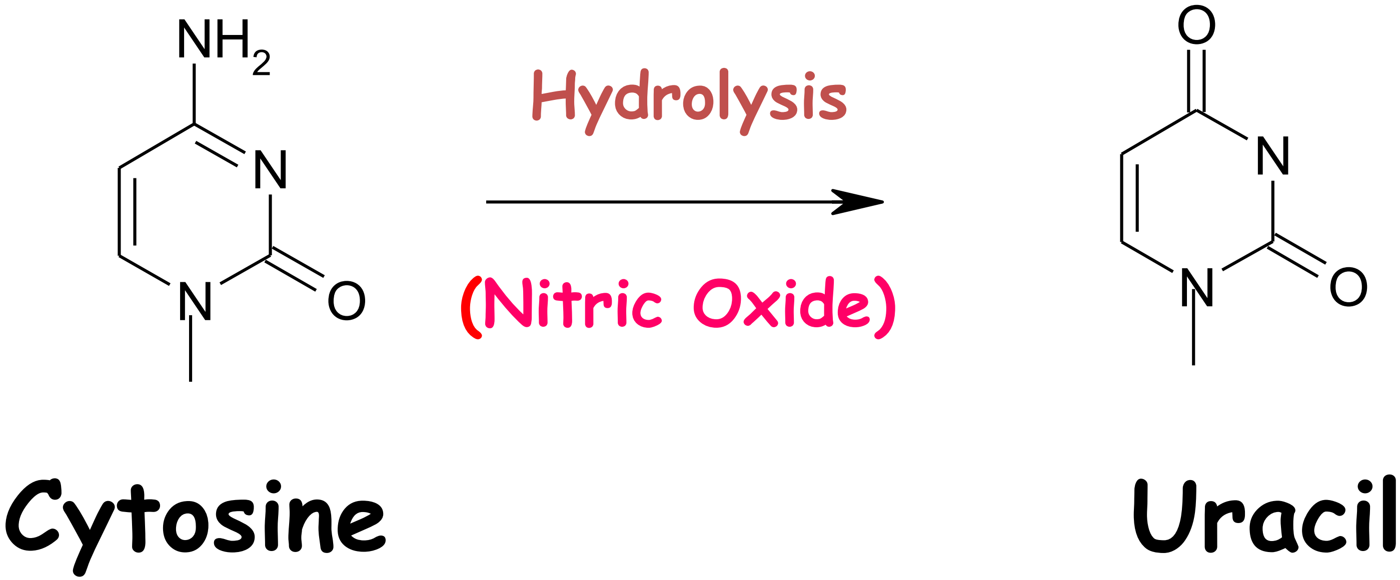
Excision Repair



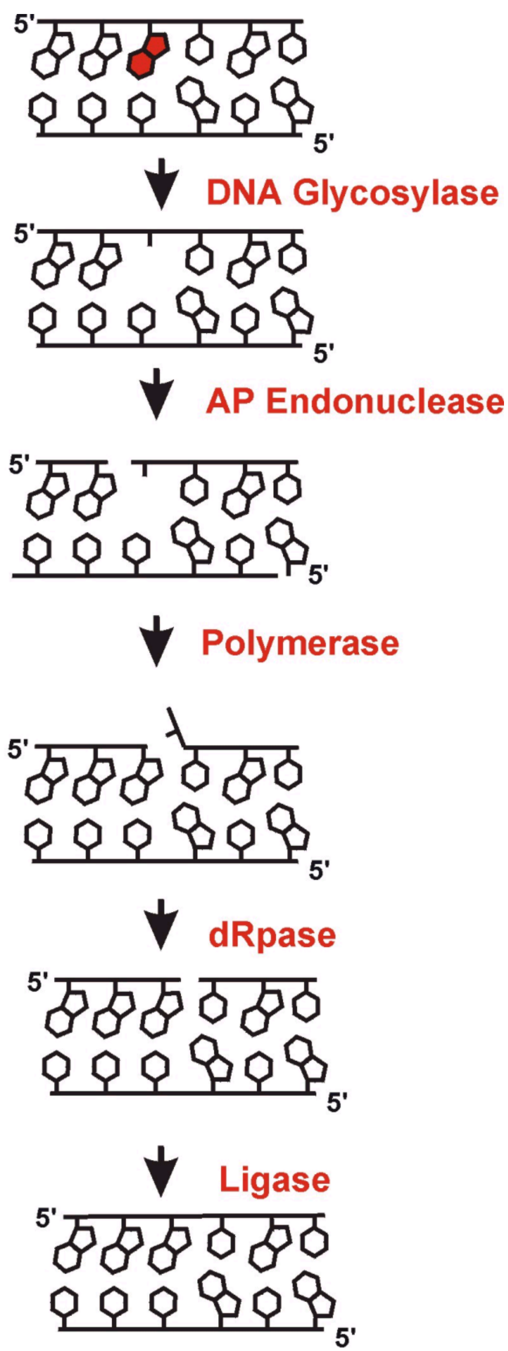


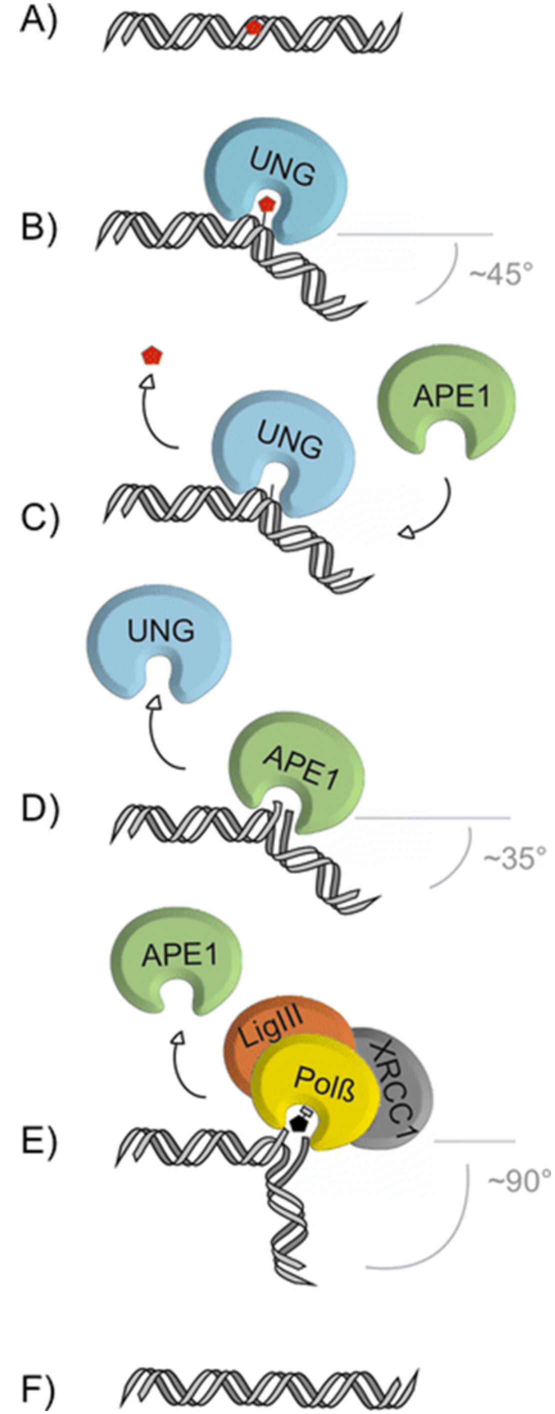
- Base Excision Repair
- Nucleotide Excision Repair
- Mismatch Repair

Deamination



Uracil from cytosine deamination in DNA causes G:C to A:T Transition mutations





Uracil DNA Glycosylase

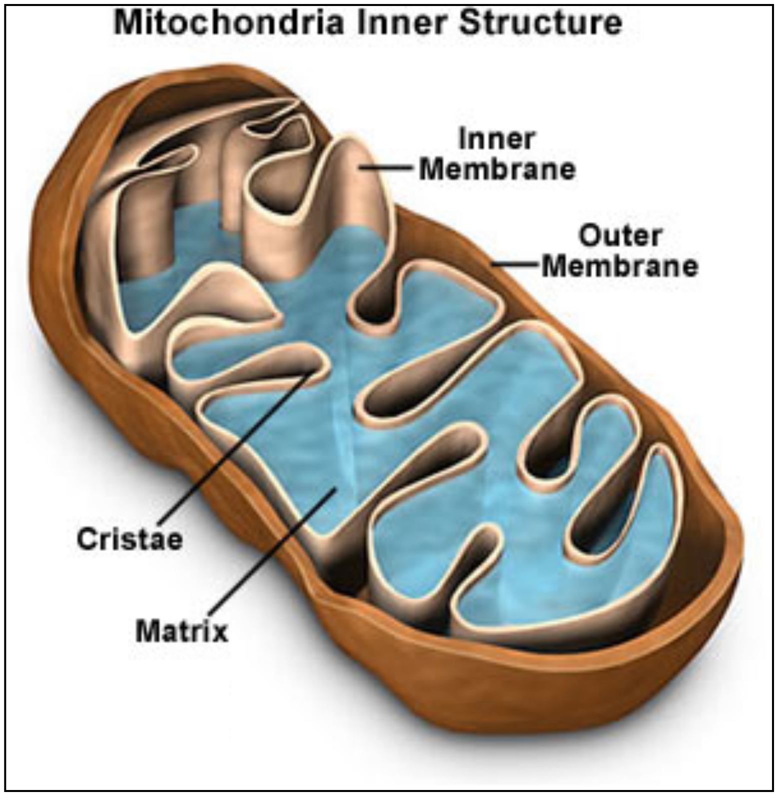
AP endonuclease

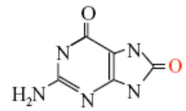
DNA polymerase

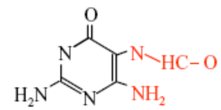
5′ dRPlyase

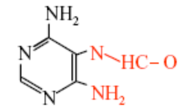
DNA Ligase

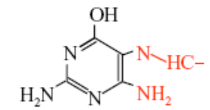
Breathing leads to DNA Damage: Reactive Oxygen Species (ROS)

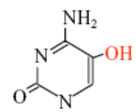


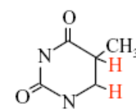

8-oxoguanine

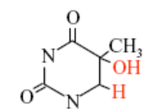

2,5-amino 5-formamidopyrimidine

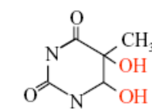

4,6-diamino 5-formamidopyrimidine

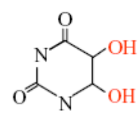

2,6-diamino 4-hydroxy-5-formamidopyrimidine

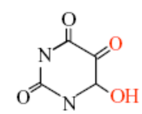

5-hydroxycytosine

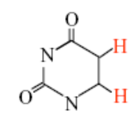

5,6-dihydrothymine

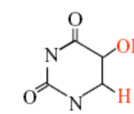

5-hydroxy-5,6-dihydrothymine

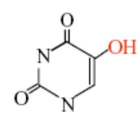

Thymine glycol

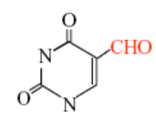

Uracil glycol

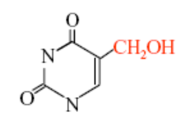

Isodialuric acid

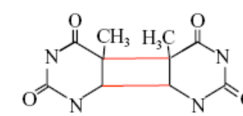

5,6-dihydrouracil

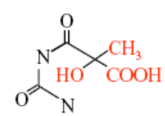

5-hydroxy-5,6-dihydrouracil

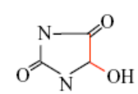

5-hydroxyuracil

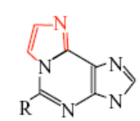

5-formyluracil

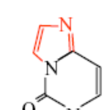

5-hydroxymethyluracil

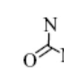

Cyclobutane pyrimidine dimer


Methyltartronylurea


5-hydroxyhydantoin

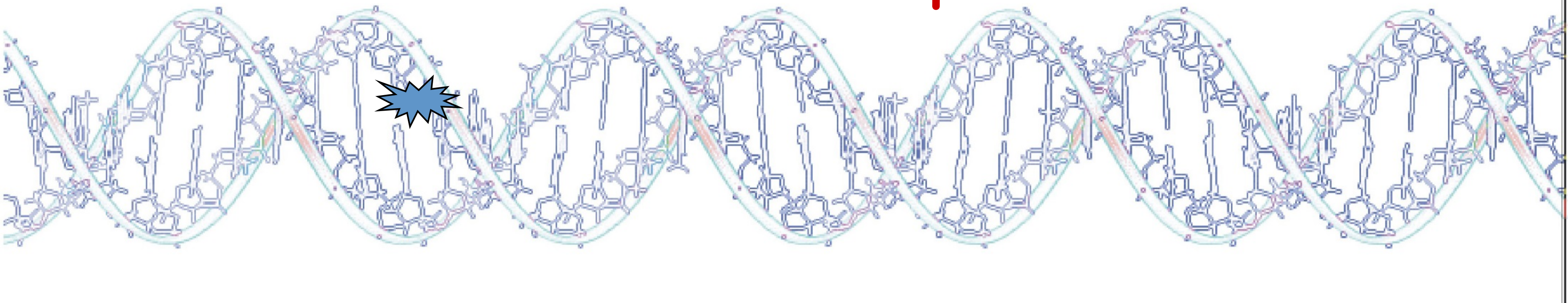

1, N6-ethenoadenine

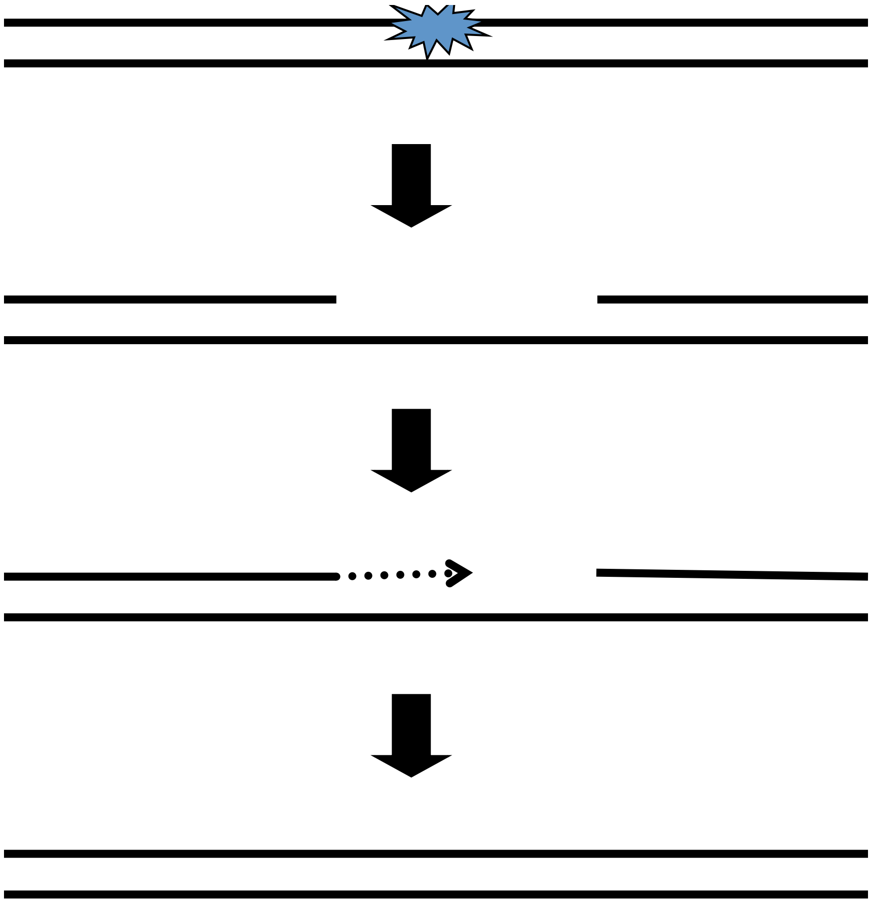

3,N4-ethenocytosine


Urea

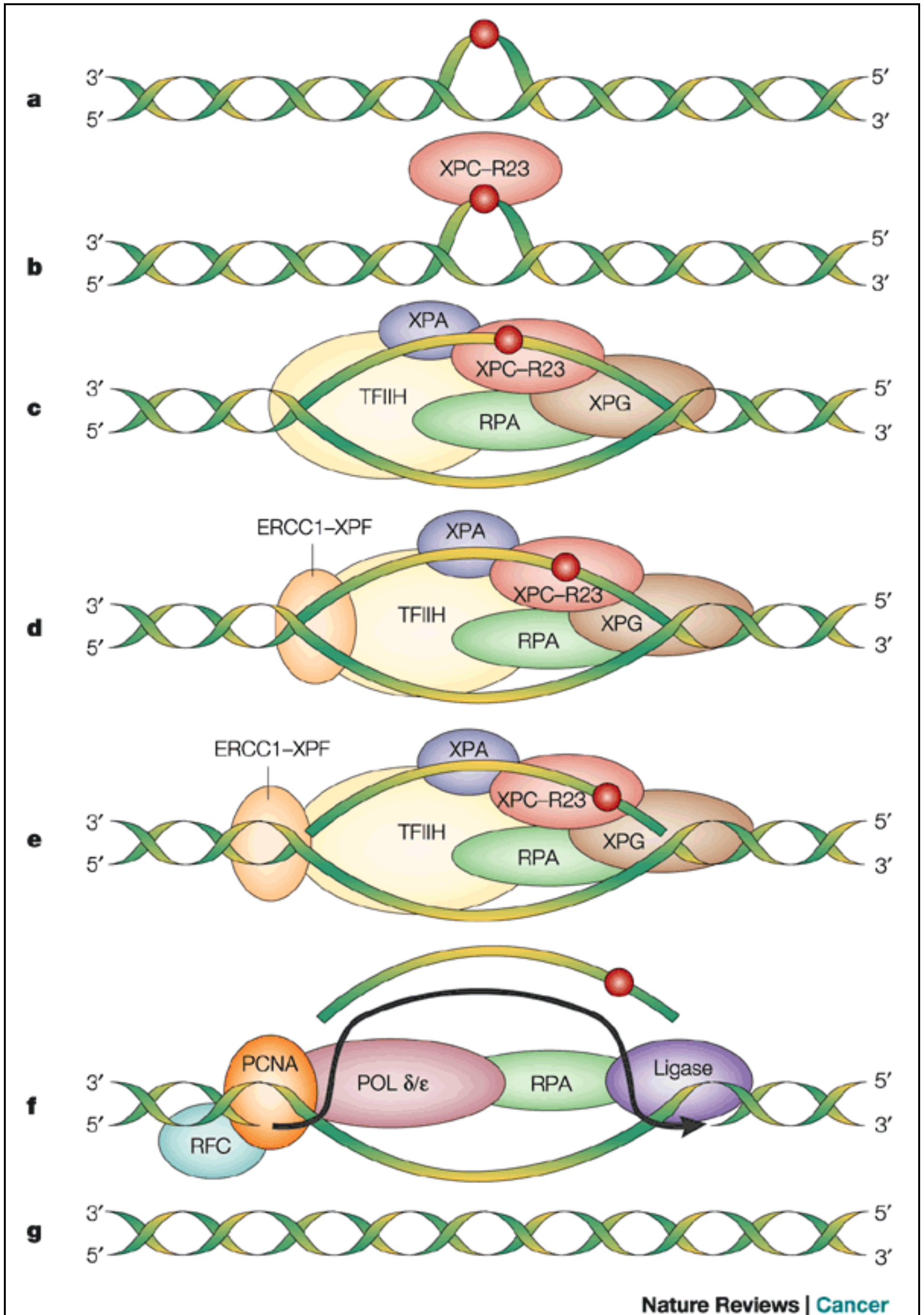
From Slupphaug, Kavli and Krokan

Excision Repair





- Base Excision Repair
- Nucleotide Excision Repair
- Mismatch Repair



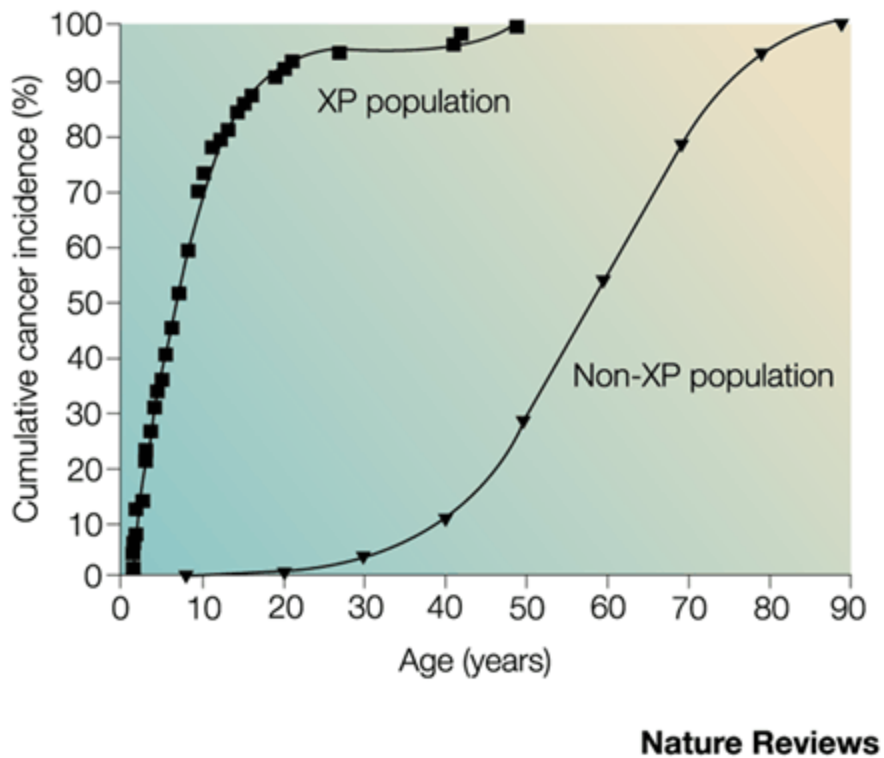
Nucleotide Excision Repair

XPA
XPB
XPC
XPD
XPE
XPF
XPG

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

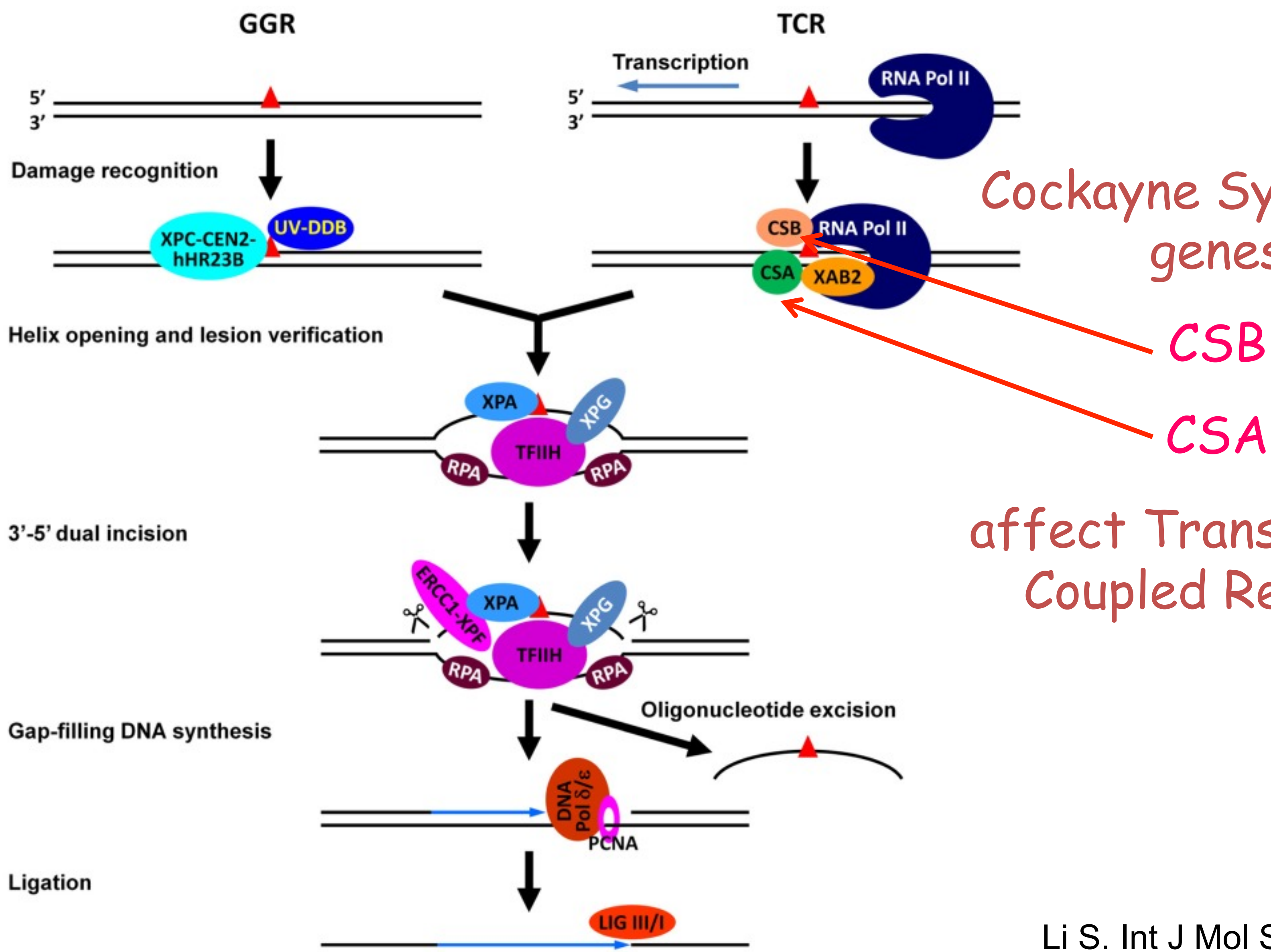
Lack of DNA repair accelerates the onset of cancer

Xeroderma pigmentosum (XP)



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

There are two kinds of NER – Global Genome Repair (GGR) and Transcriptions Coupled Repair (TCR)

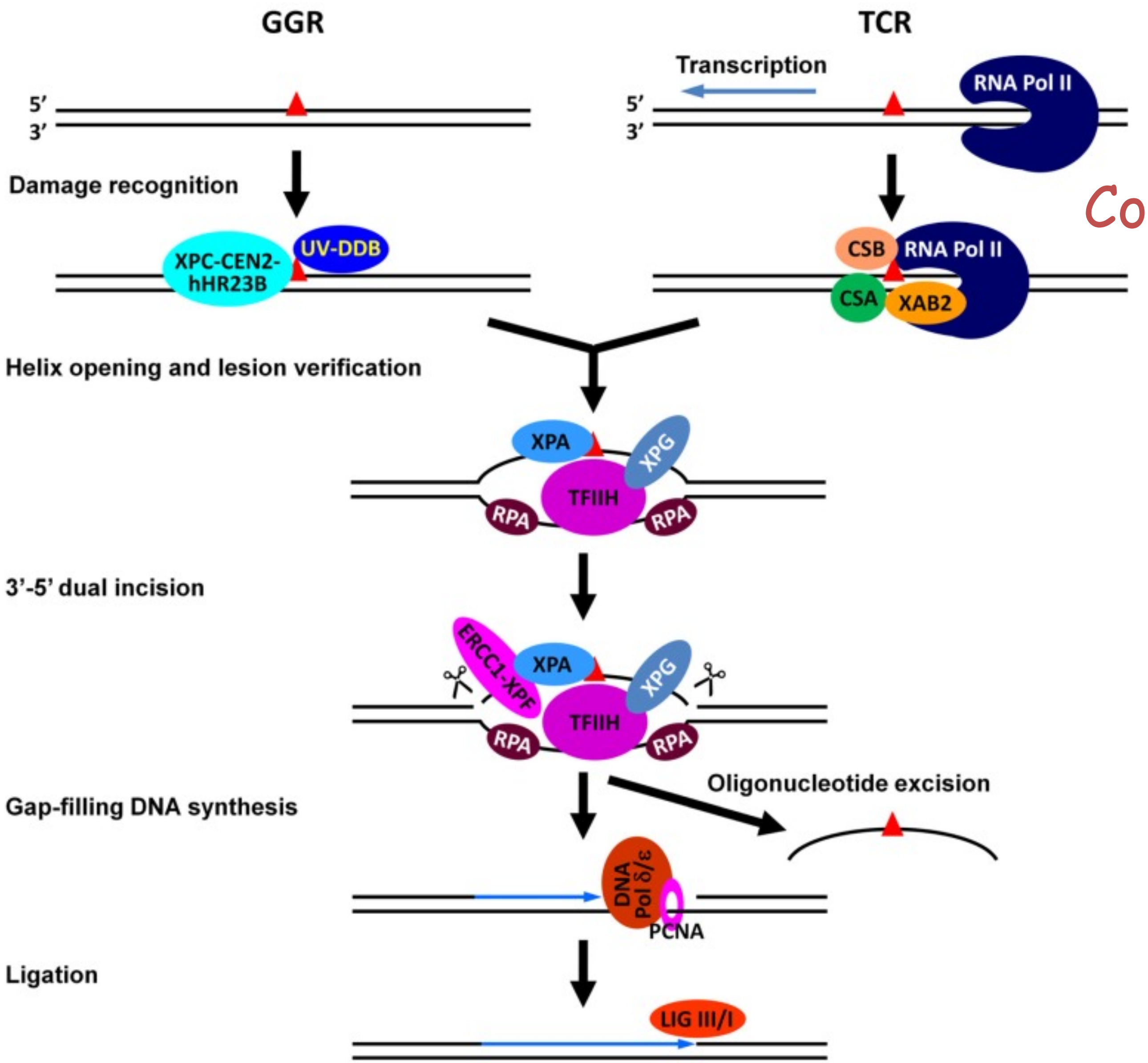


Cockayne Syndrome genes

affect Transcription Coupled Repair…

Li S. Int J Mol Sci. 2012 Sep 28;13(10):12461-86

There are two kinds of NER – Global Genome Repair (GGR) and Transcriptions Coupled Repair (TCR)



Cockayne Syndrome

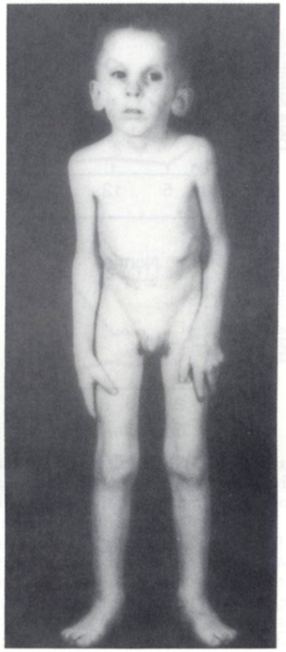
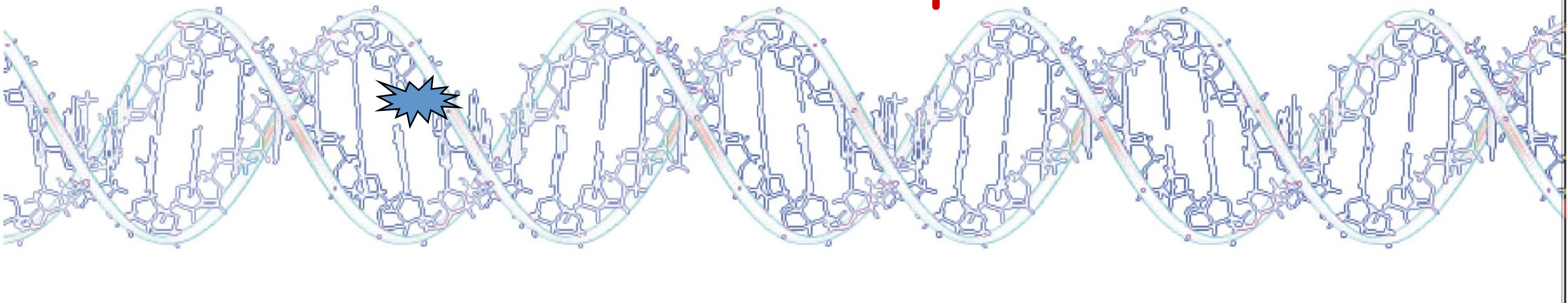
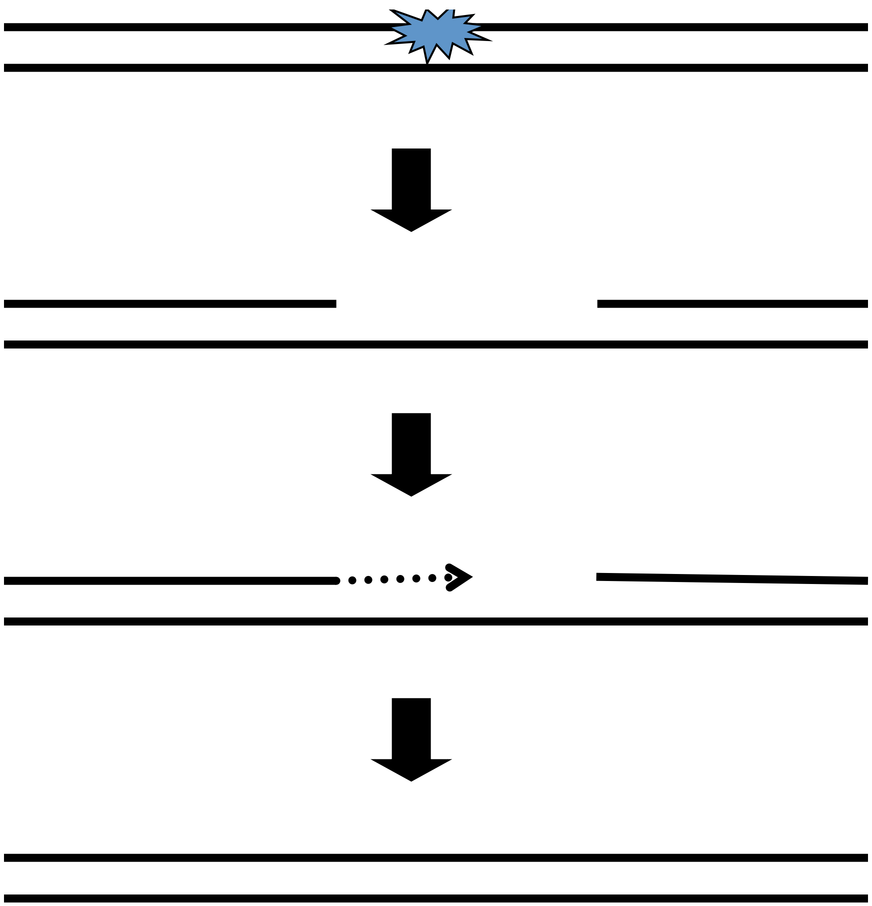


Figure 14–18 Individuals with CS have arrested growth and development, resulting in a dwarfed appearance. The sunken eyes and disproportionately long arms and legs are also very characteristic. (*Courtesy of James German.*)

Li S. Int J Mol Sci. 2012 Sep 28;13(10):12461-86

Excision Repair





- Base Excision Repair
- Nucleotide Excision Repair
- Mismatch Repair

Environmental exposures to potentially harmful agents

Harmful agents

People have different exposures

People have different responses

Every time a cell replicates its genome mistakes can be made to alter the sequence – MUTATIONS

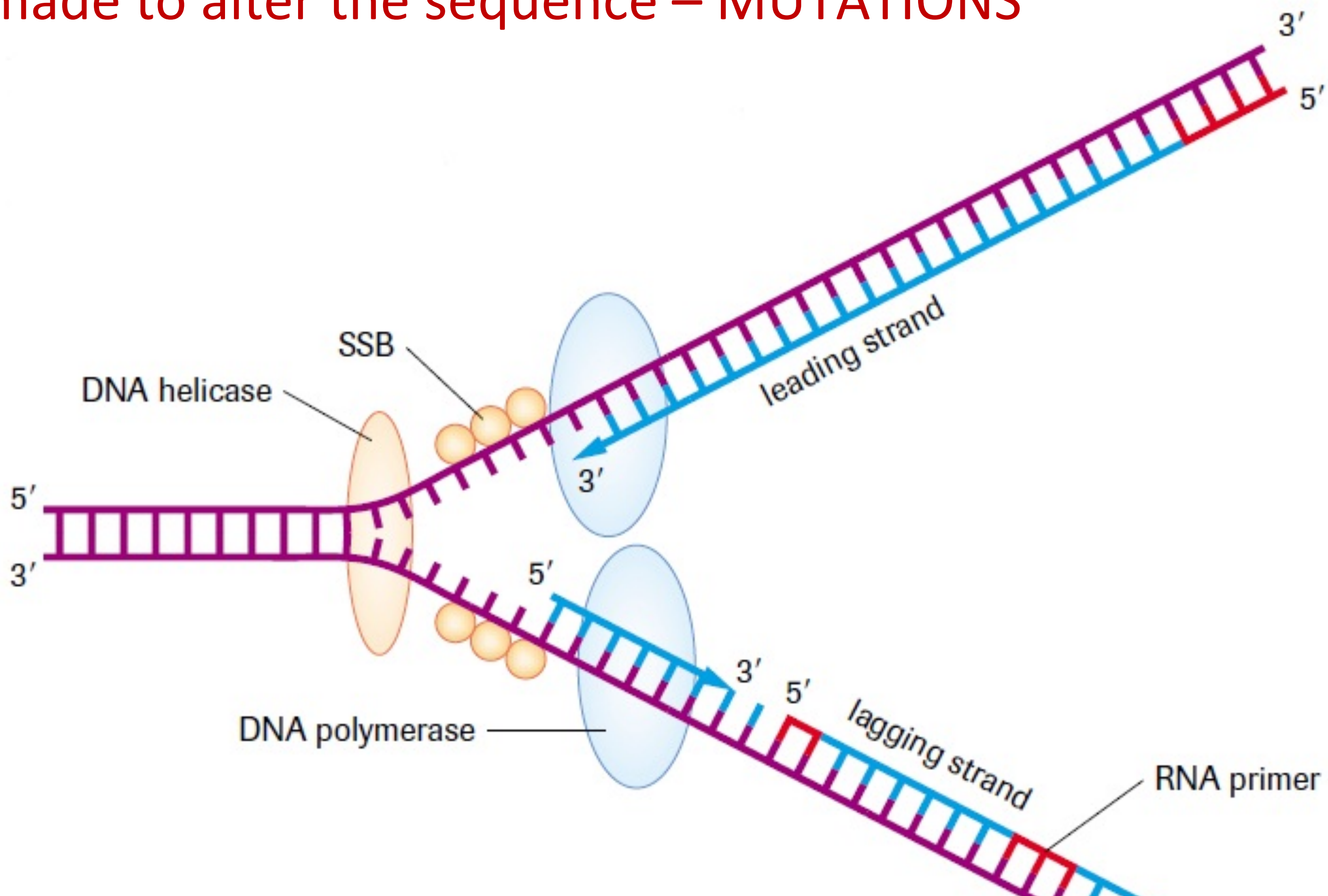
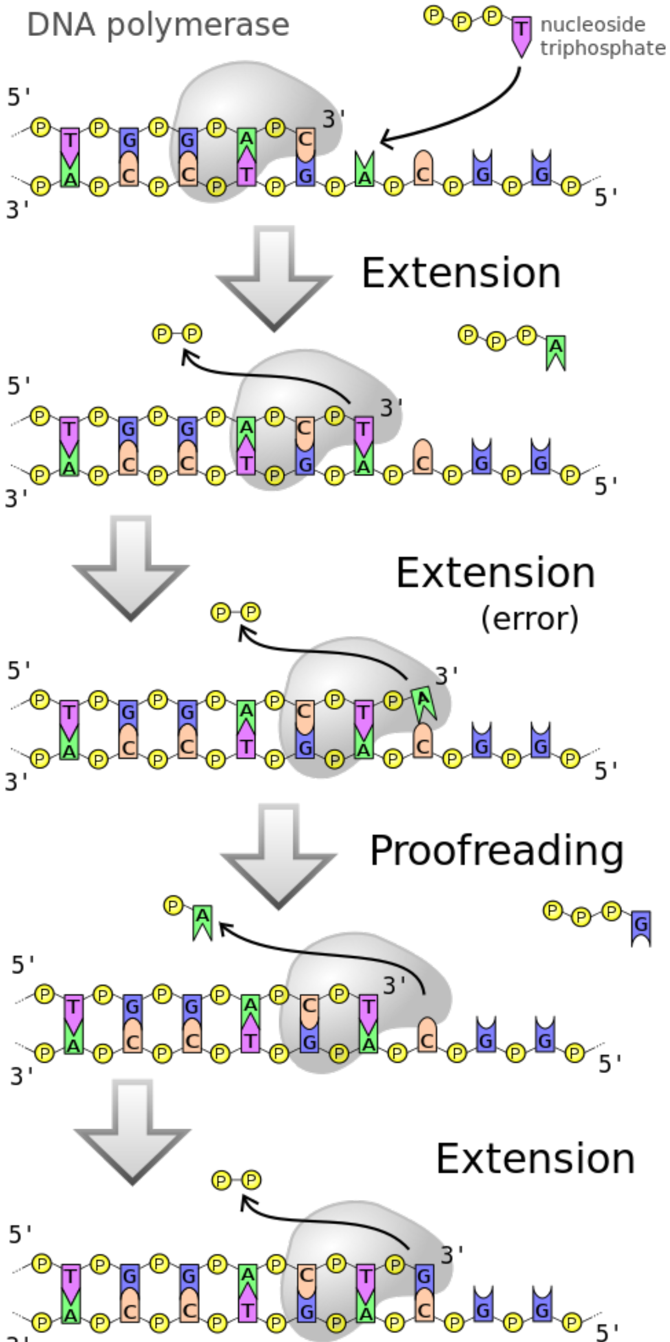


Figure 5
DNA polymerase III adds complementary nucleotides in the 5′ to 3′ direction, using RNA primers as starting points.

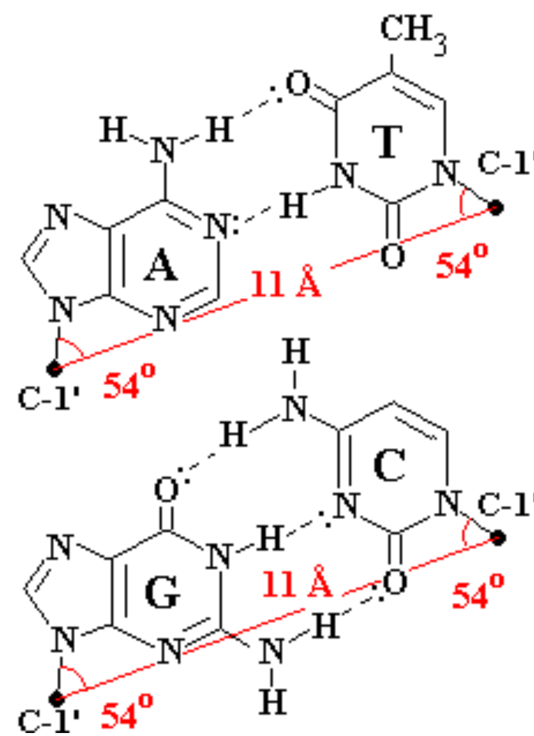
http://biochemhelp.com/dna-replication-in-prokaryotes.html

REPLICATION FIDELITY

- How many times does DNA polymerase have to choose the correct nucleotide during one cell division???
- Is one mistake in a million choices acceptable?
- How is fidelity achieved?



DNA Polymerases have different ways of increasing fidelity

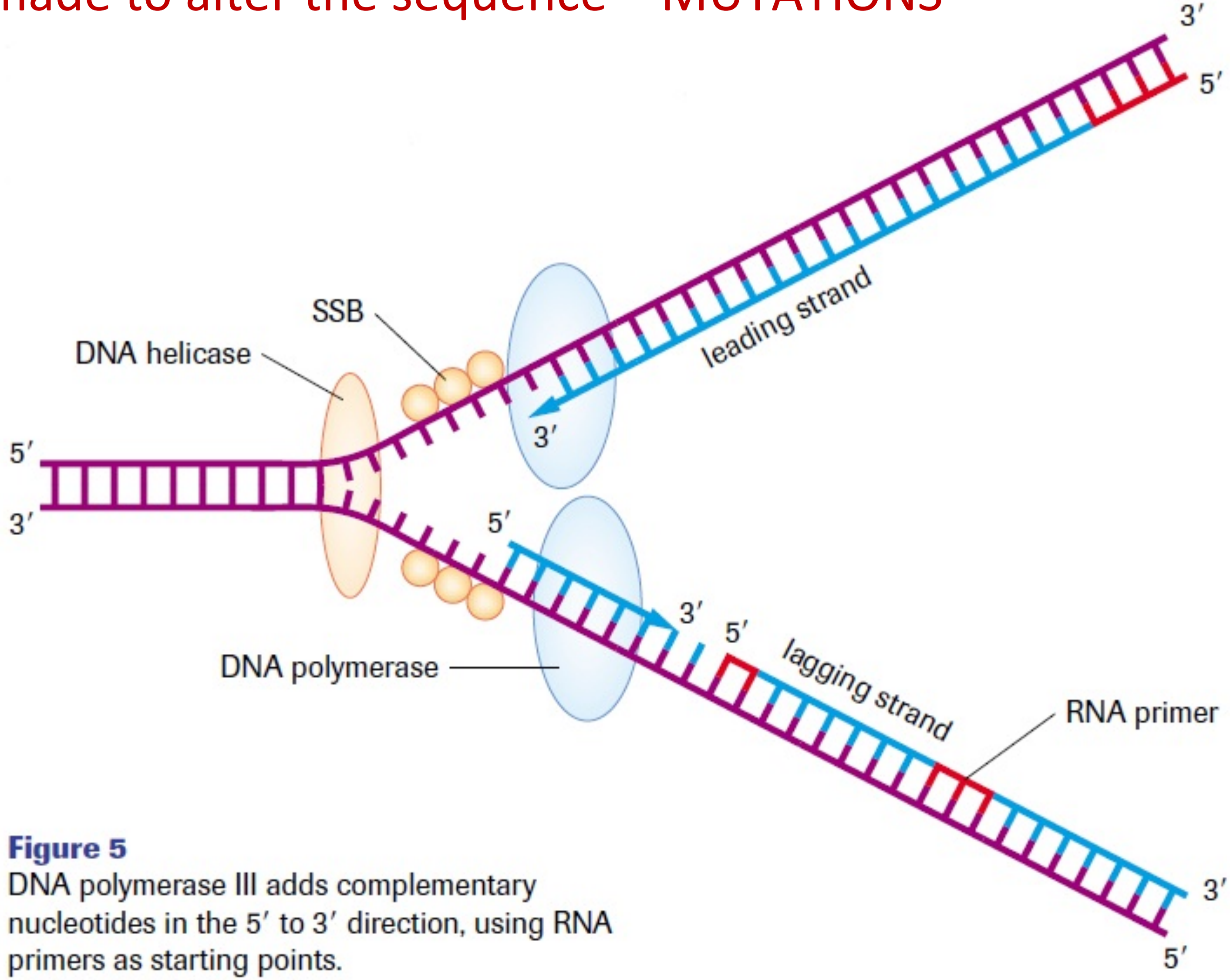


Mechanisms for maintaining genetic stability associated with DNA replication in *E. coli*

Mechanism	Cumulative Error Frequency
Base pairing	$\sim 10^{-1}$ to 10^{-2}
DNA polymerase actions (including base selection and $3' \rightarrow 5'$ proofreading exonuclease)	$\sim 10^{-5}$ to 10^{-6}
Accessory proteins (e.g., single-strand binding protein)	$\sim 10^{-7}$
Post-replicative mismatch correction	$\sim 10^{-10}$

After M. Radman et al., ref. 17.

Every time a cell replicates its genome mistakes can be made to alter the sequence – MUTATIONS



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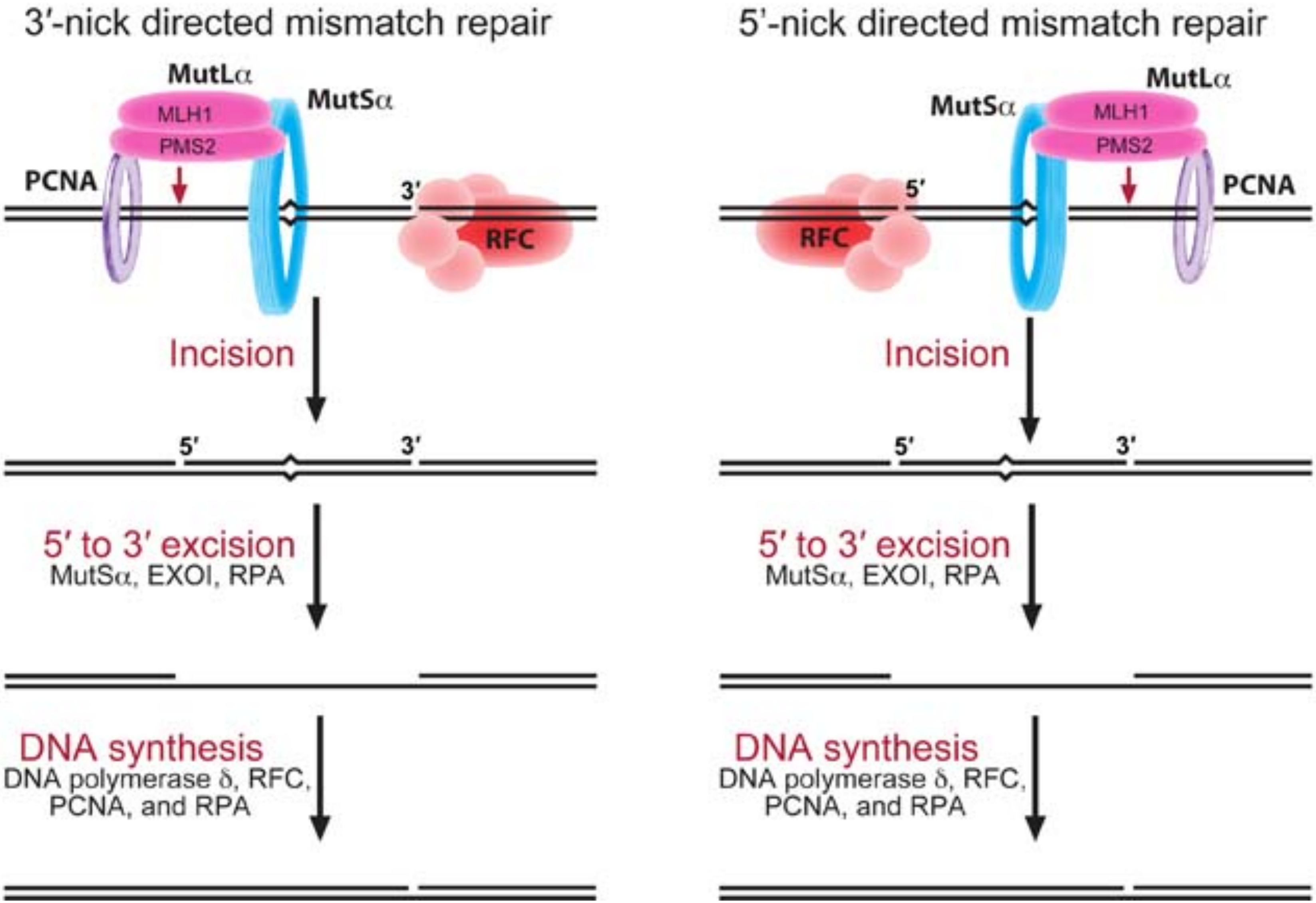
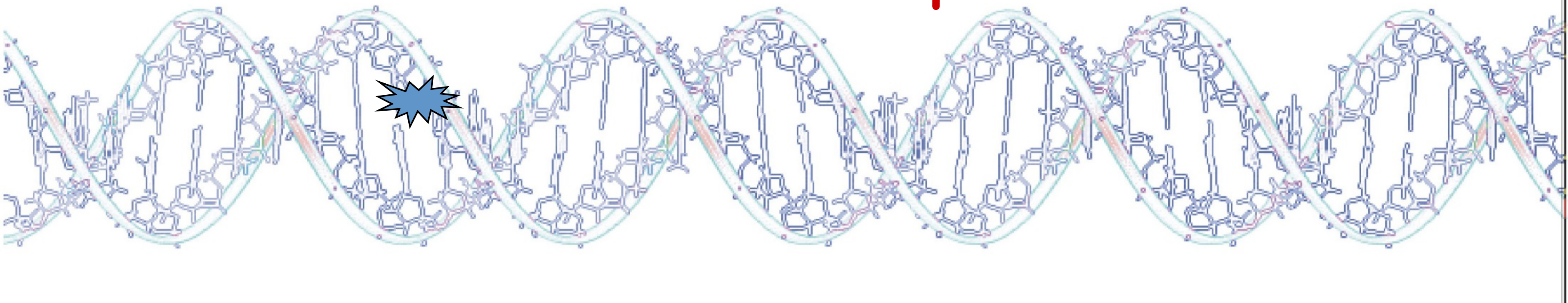
After M. Radman et al., ref. 17.

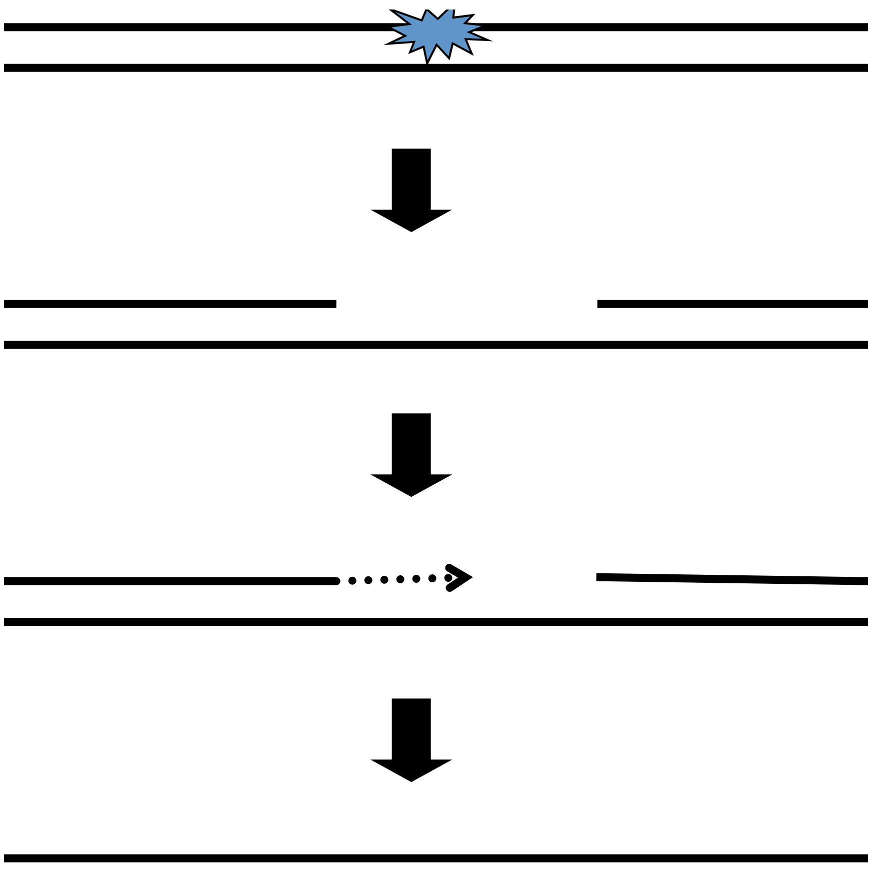
Human mismatch repair



MMR deficiency leads to early onset Colorectal Cancer

Excision Repair





- Base Excision Repair
- Nucleotide Excision Repair
- Mismatch Repair

DNA Repair Strategies

- Direct Reversal

  Methyltransferase, Oxidative demethylase

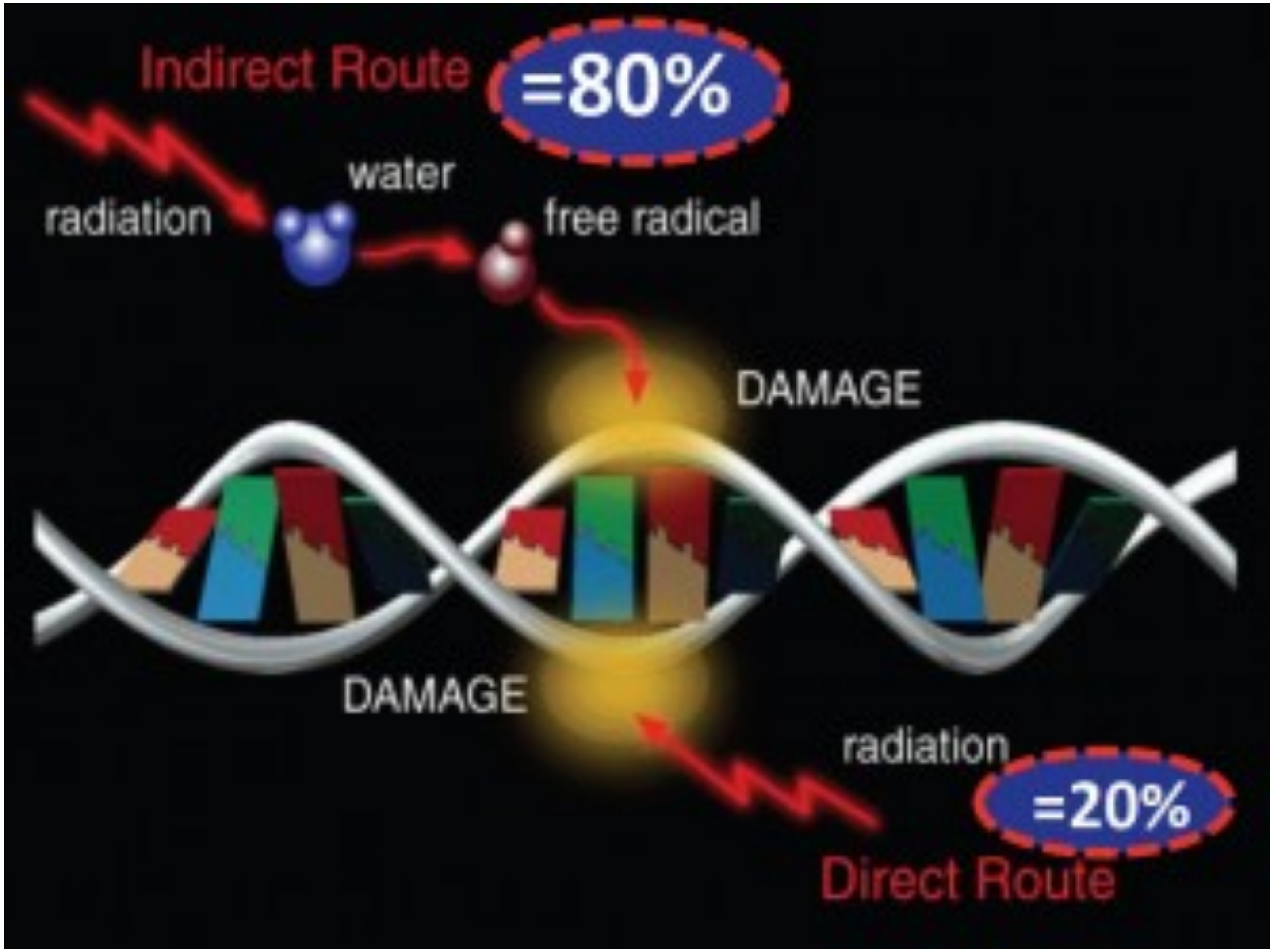
- Excision Repair

  Base excision, nucleotide excision, mismatch repair

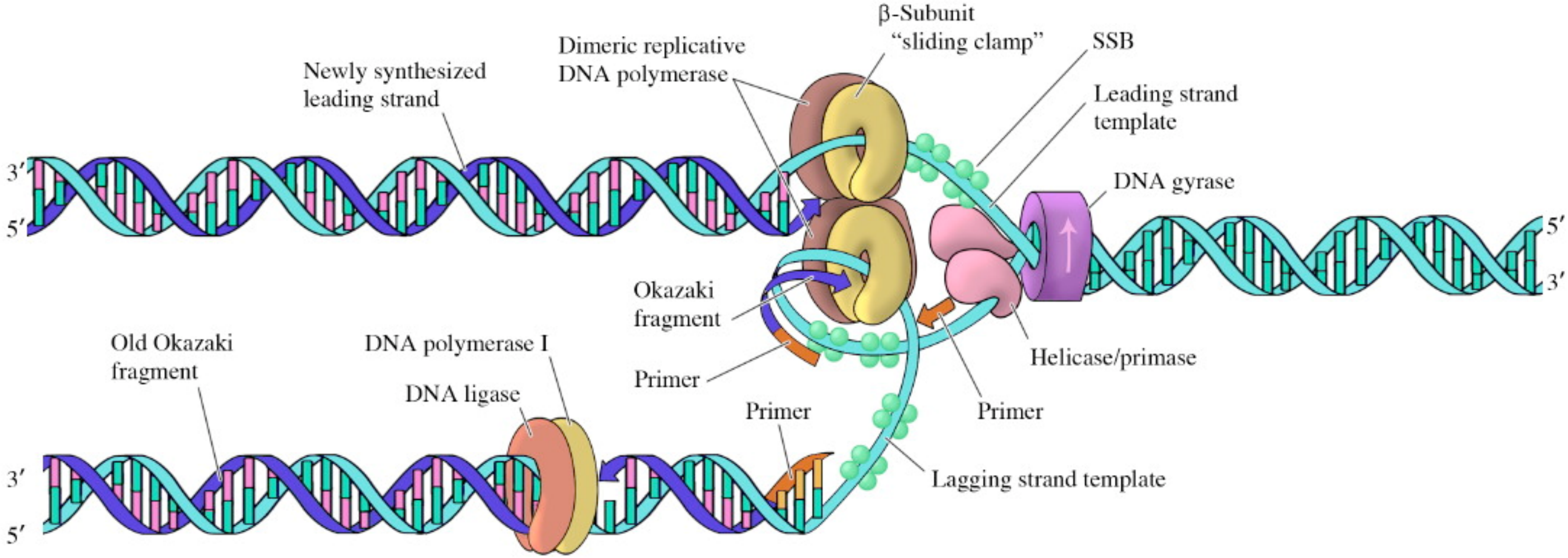
- Double strand break repair

  Homologous recombination, Non-homologous end joining

X-rays induce DNA double strand breaks. How?

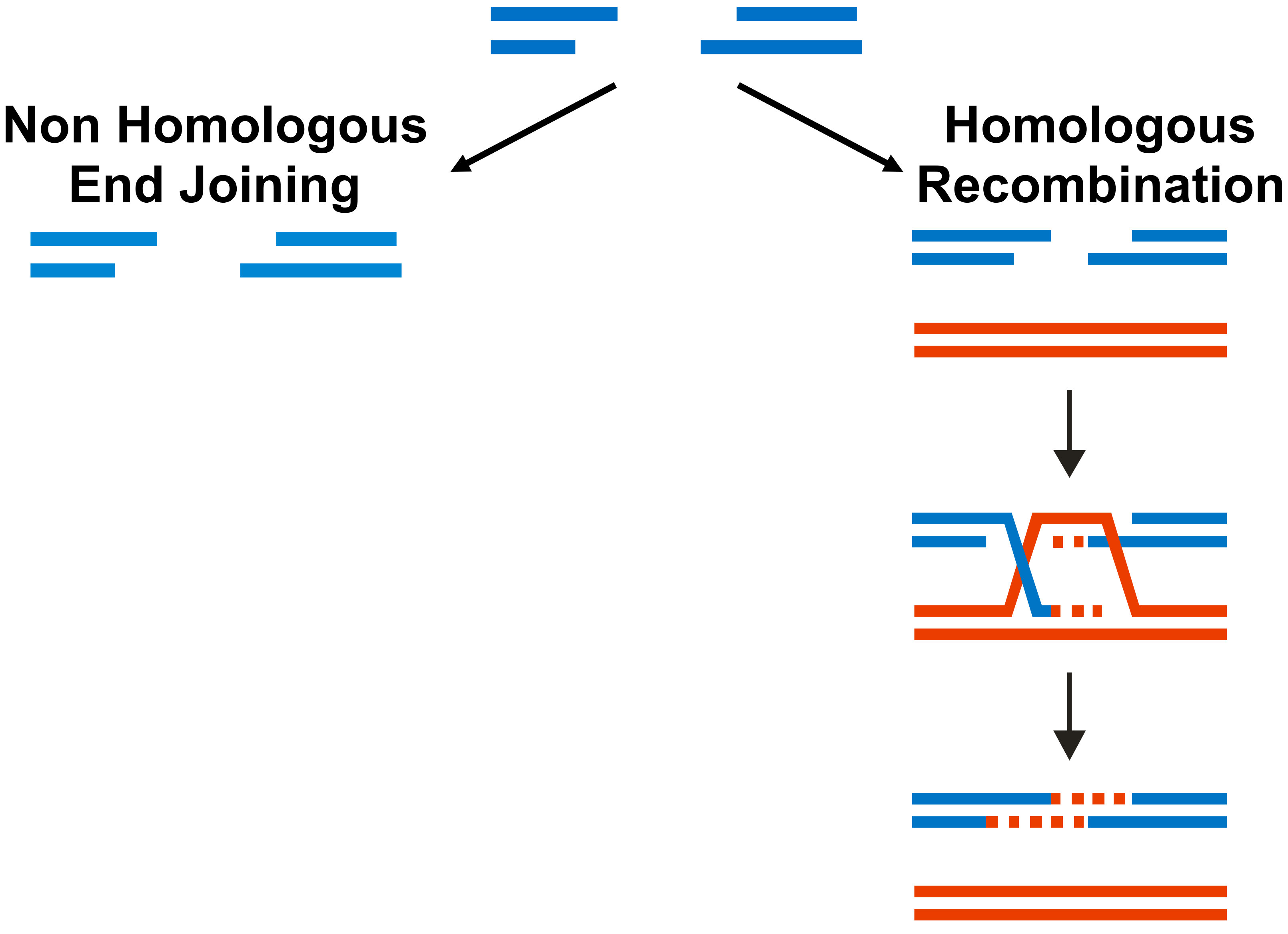


Replicated versus non-replicated DNA

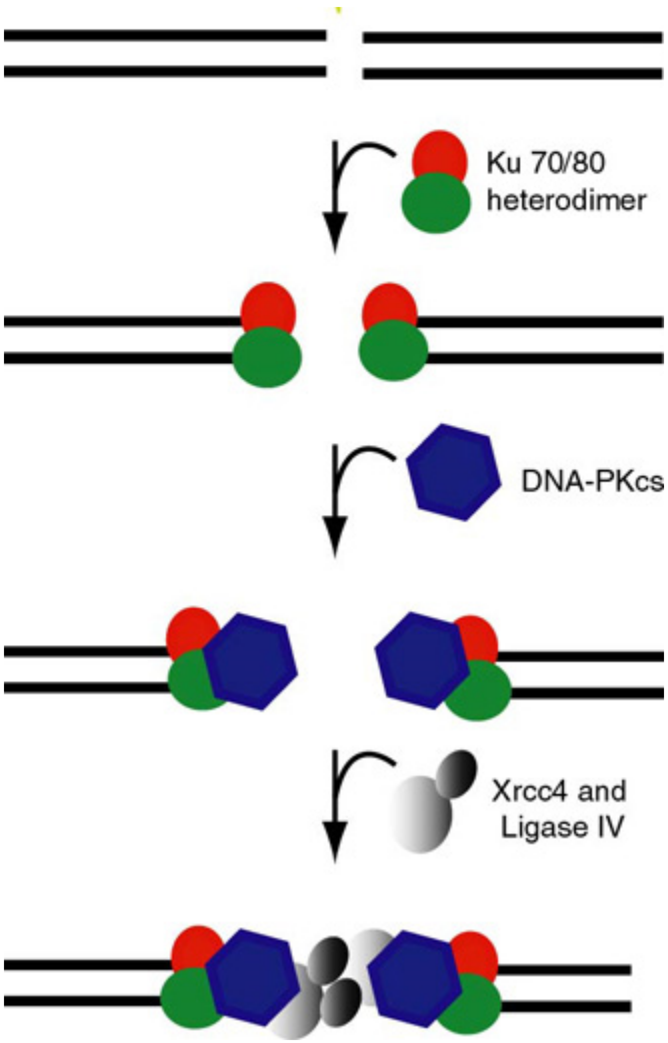


http://oregonstate.edu/instruct/bb350/ahernmaterials/a10/10p18.jpg

DNA double-strand break repair



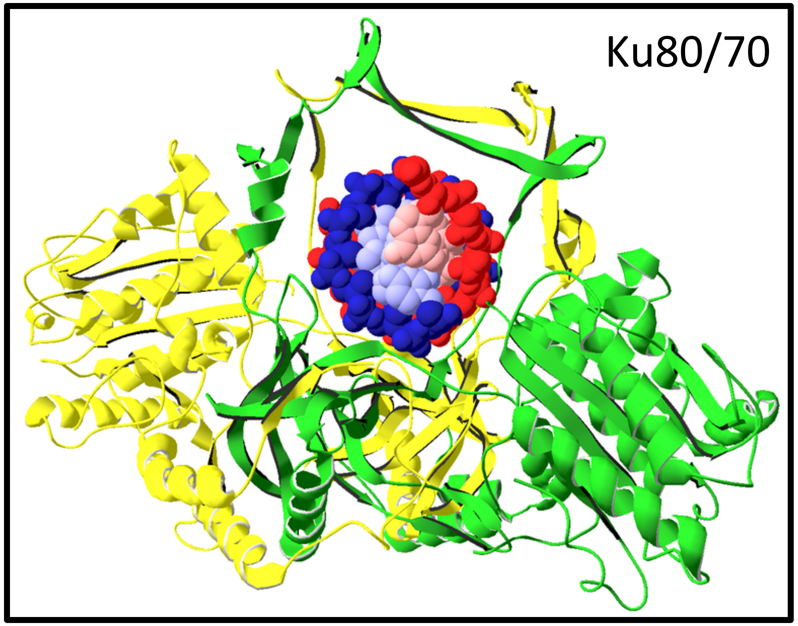
Non-Homologous End Joining (NHEJ)

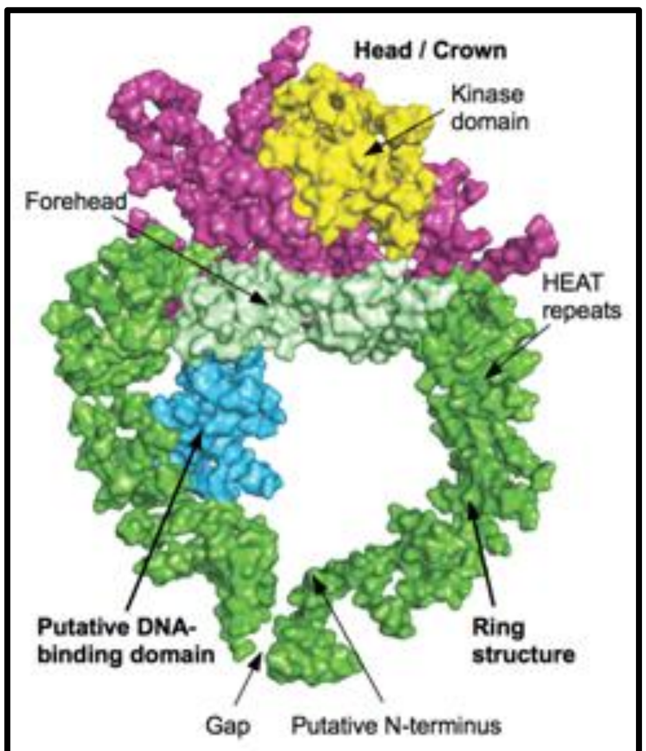


Ku70
Ku80

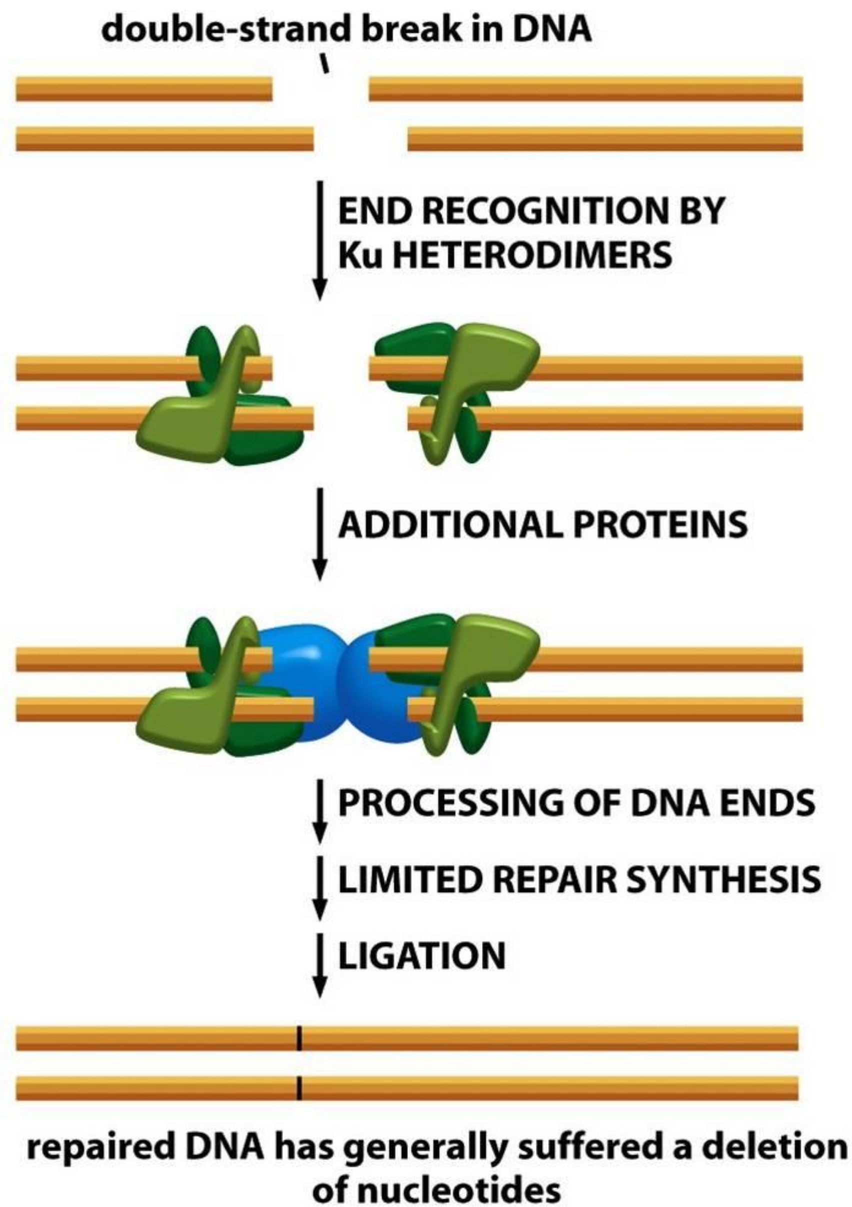
DNA-PKcs

Xrcc4
Ligase IV

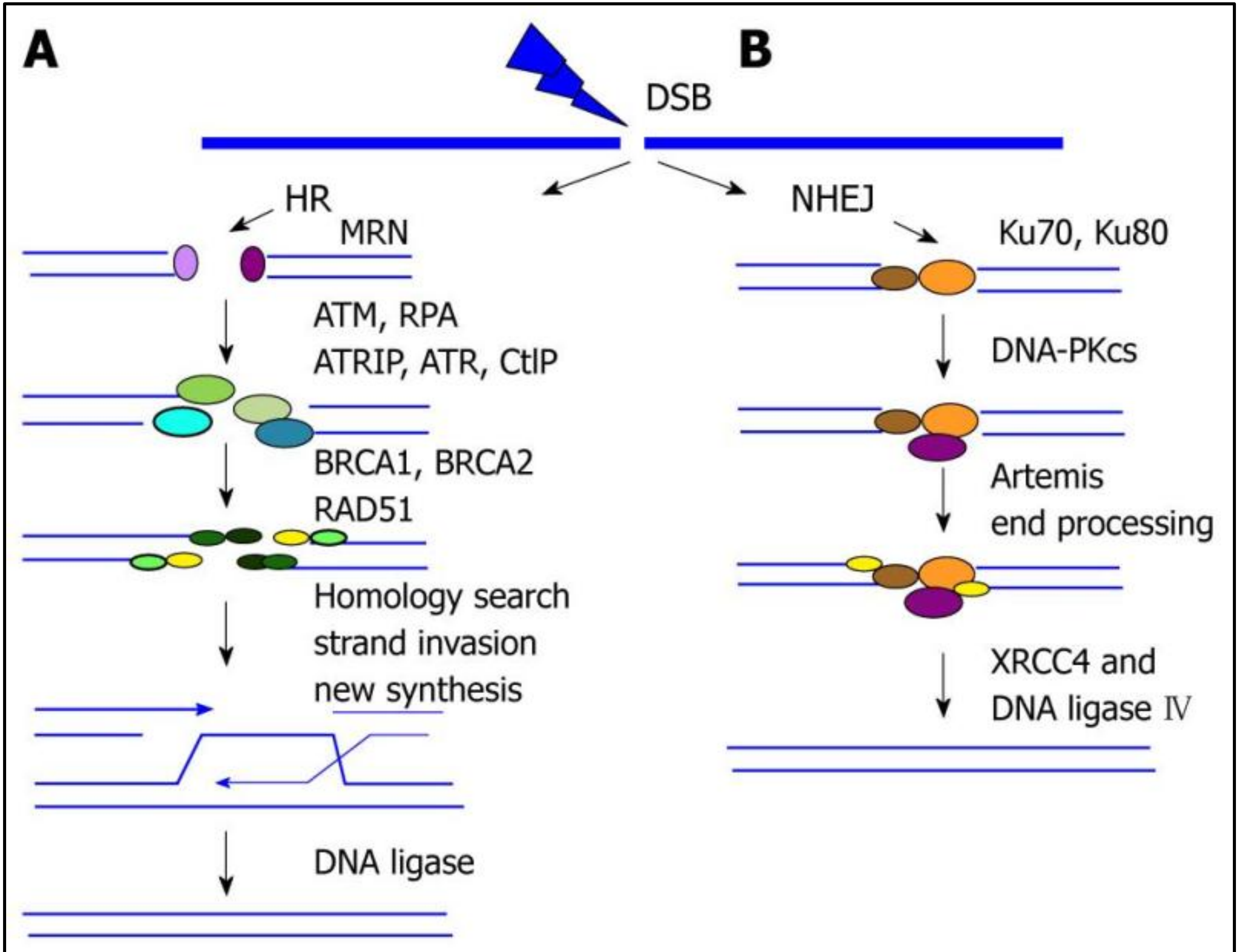

Ku80/70


Head / Crown
Kinase domain
Forehead
HEAT repeats
Putative DNA-binding domain
Ring structure
Gap
Putative N-terminus

DNA-PKcs


double-strand break in DNA
END RECOGNITION BY Ku HETERODIMERS
ADDITIONAL PROTEINS
PROCESSING OF DNA ENDS
LIMITED REPAIR SYNTHESIS
LIGATION
repaired DNA has generally suffered a deletion of nucleotides

DNA Double Strand Break (DSB) Repair

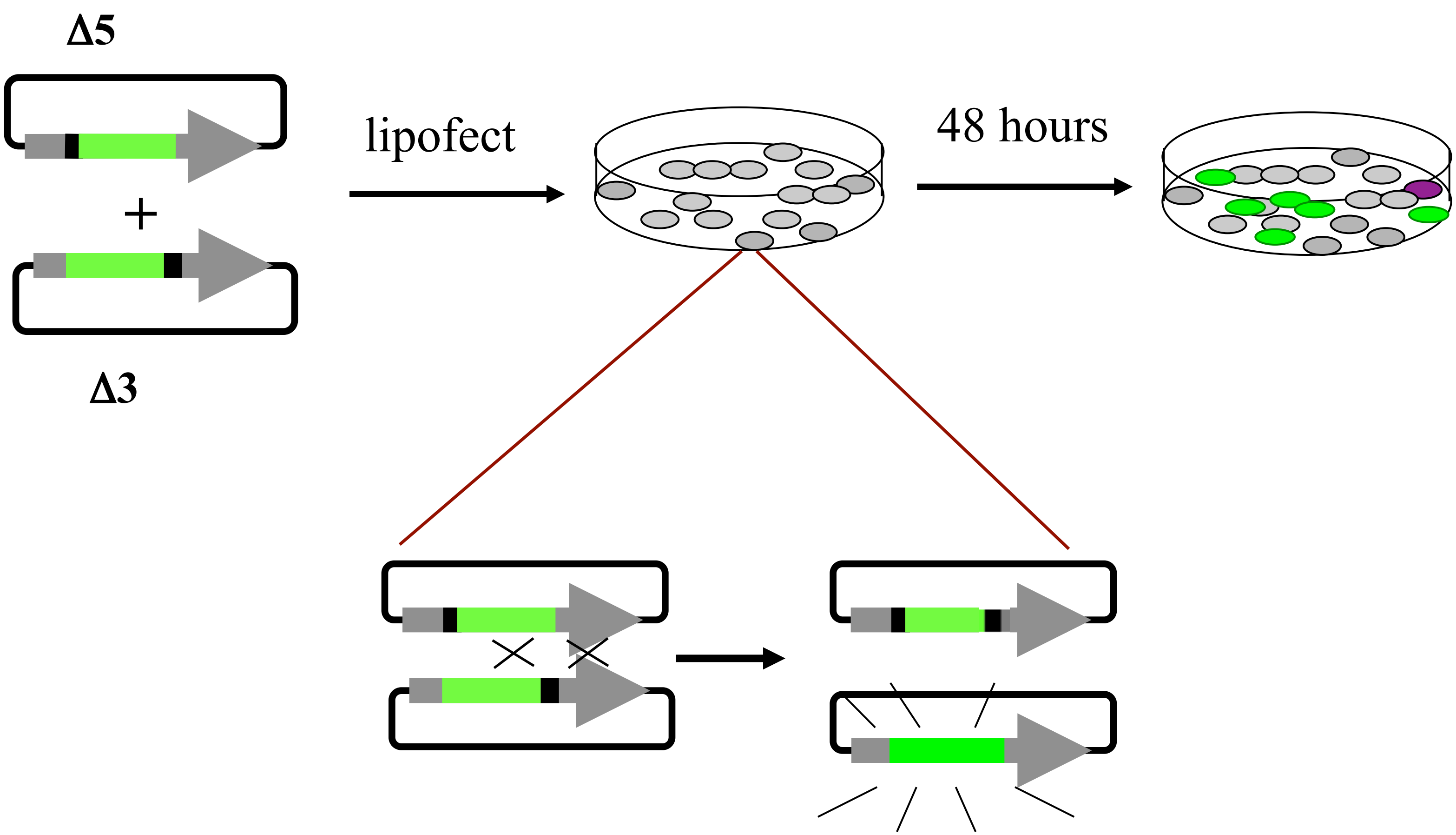


http://web.mit.edu/engelward-lab/animations/NHEJ.html
Non Homologous End Joining

http://web.mit.edu/engelward-lab/animations/SDSA.html
DSB repair using Homologous Recombination

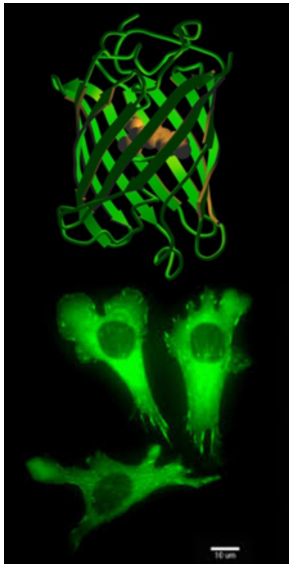
http://web.mit.edu/engelward-lab/animations/forkHR.html
Repair of a collapsed Replication Fork

A Plasmid-Based Assay for Homologous Recombination in Mammalian Cells



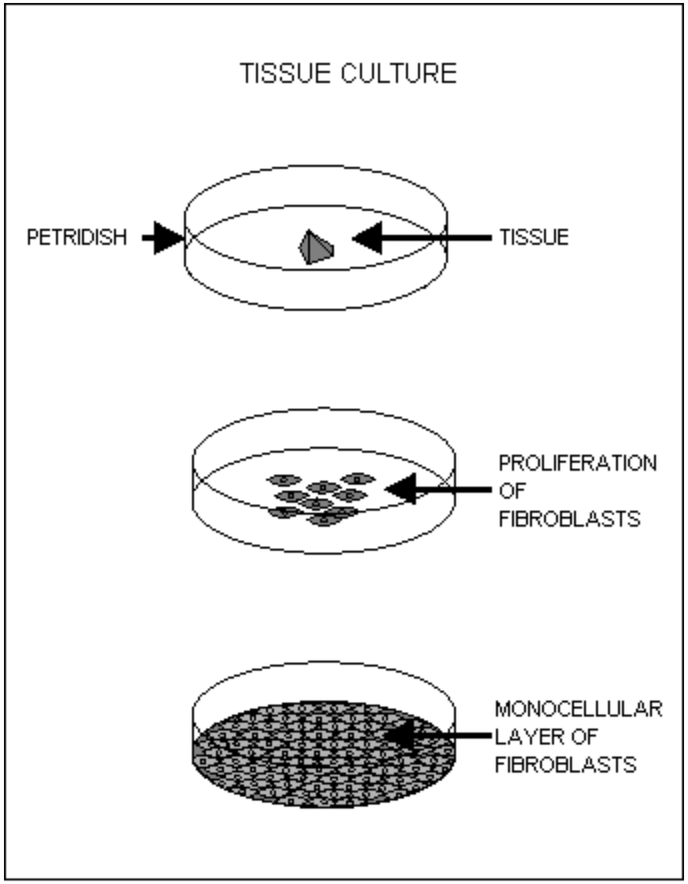
Bevin Engelward

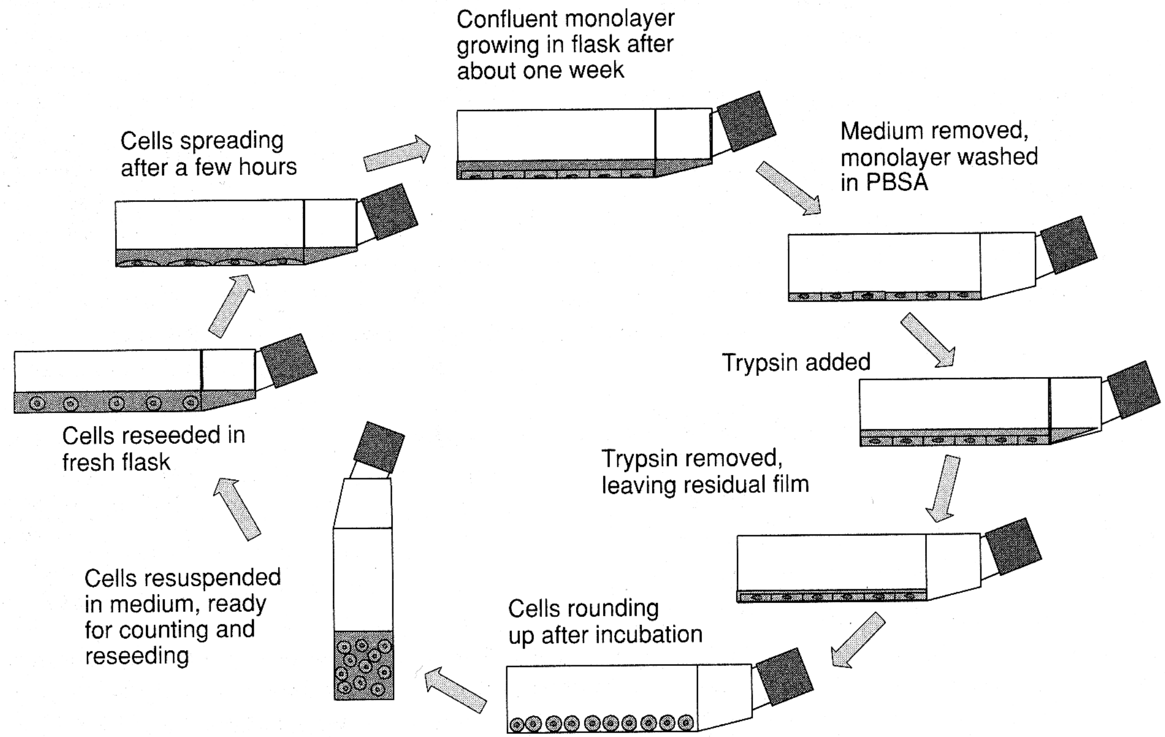
Key Experimental Methods for Module 1



- Construction of truncated eGFP gene – Cloning
  - PCR, Restriction Enzymes, Ligation/Transformation, Bacterial culture
- Mammalian tissue cell culture
- Transfecting plasmids into mammalian cells
- Flow cytometry to measure DNA repair
- Statistical analysis of biological data

How do you grow mammalian cells?

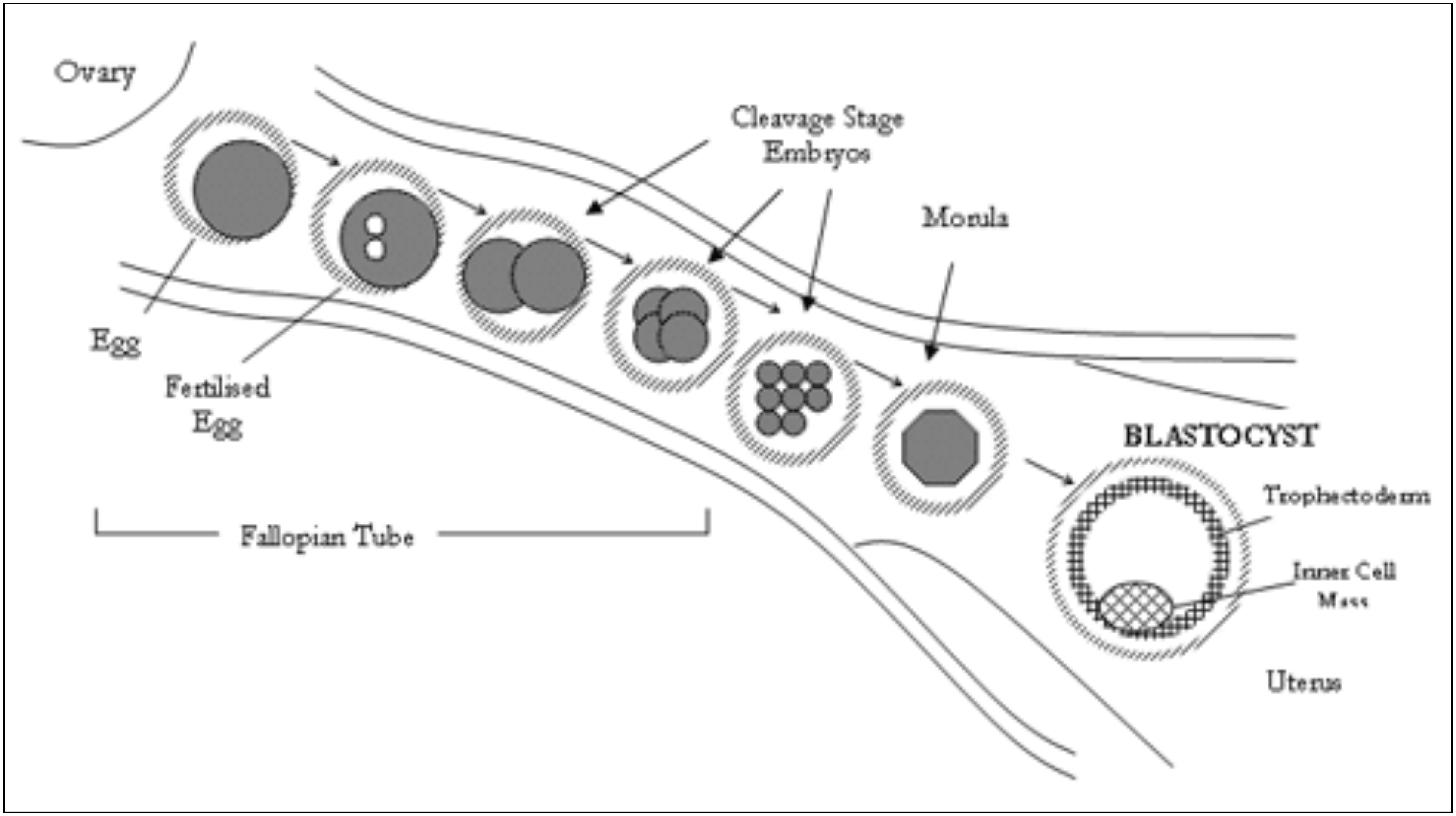


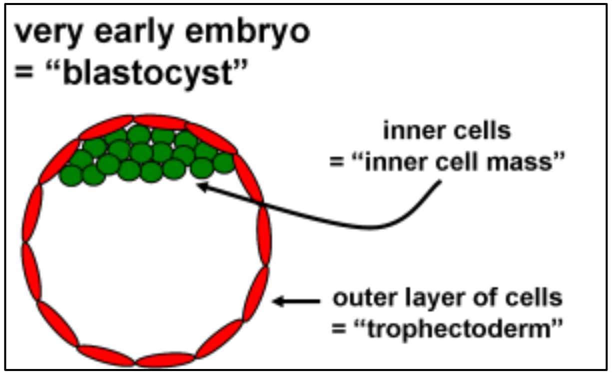


"Sub-Culturing"

From Freshney's "Culture of Mammalian Cells"

You will work with Mouse Embryonic Stem Cells mES cells





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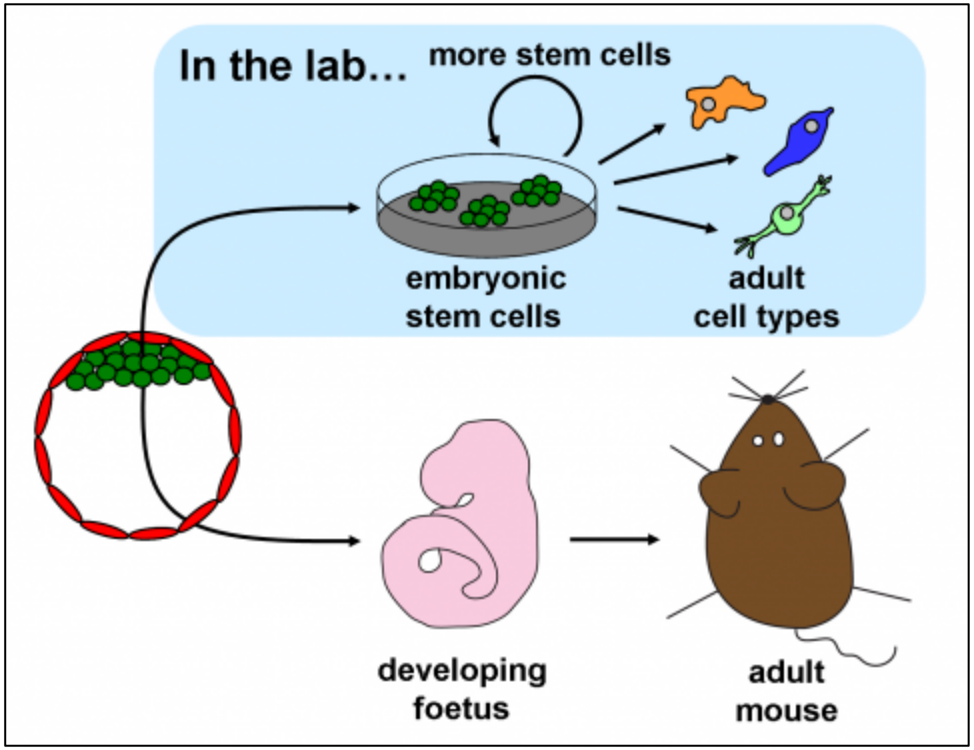
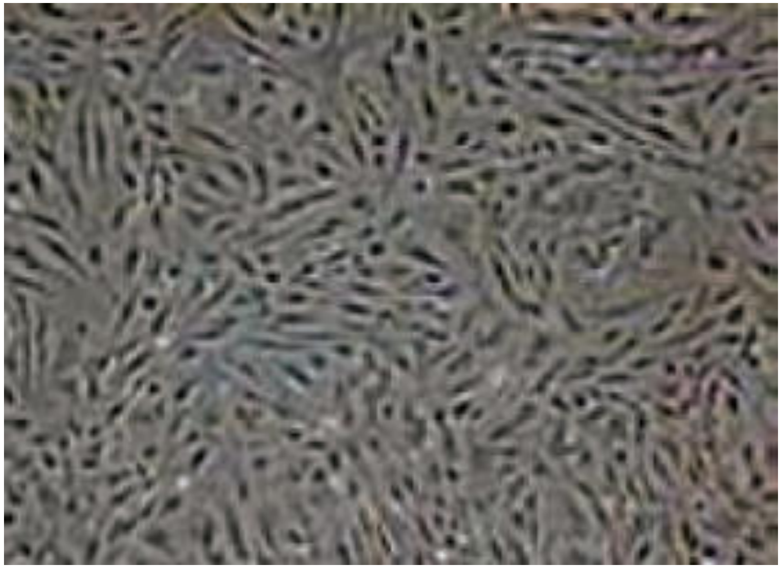
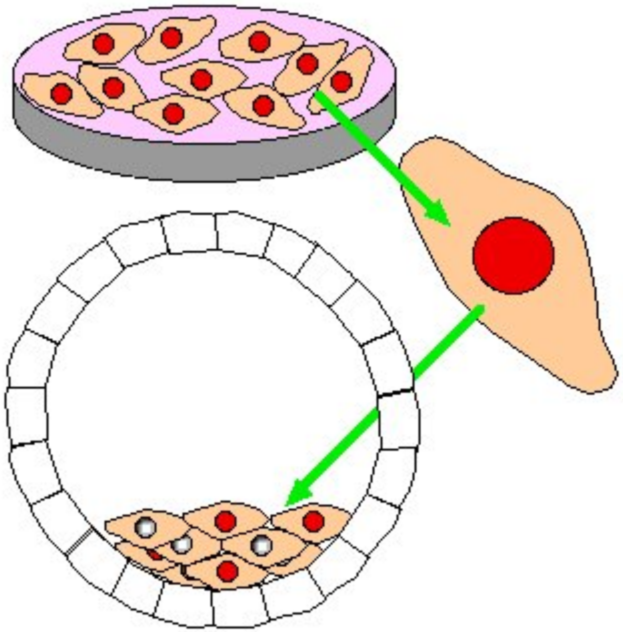
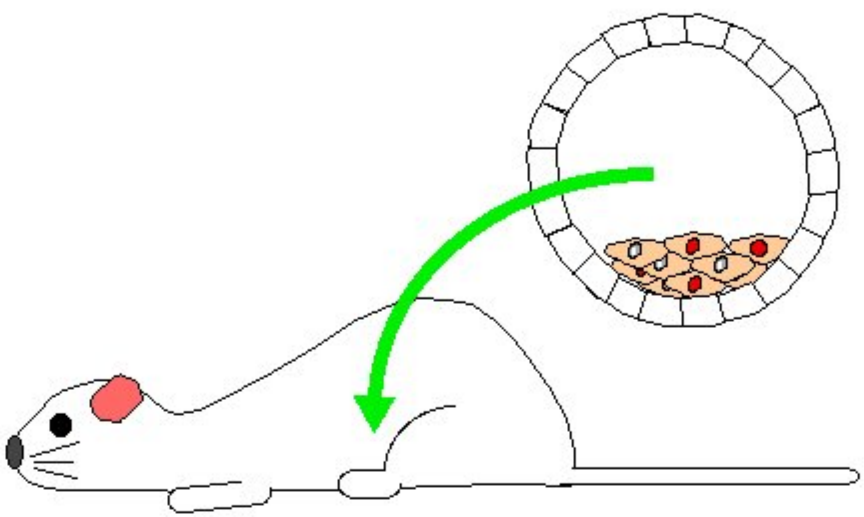


mES cells grown in the lab

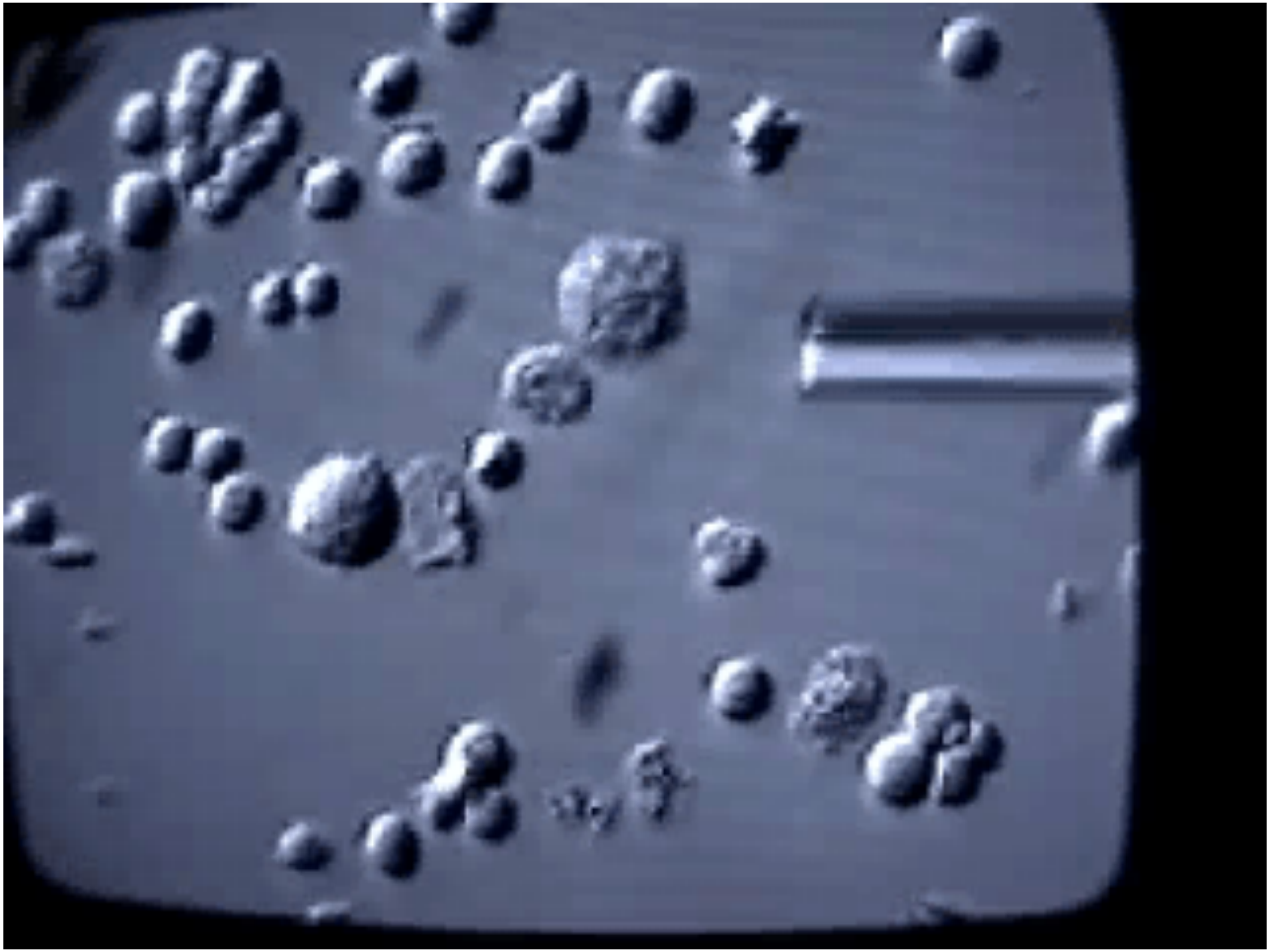


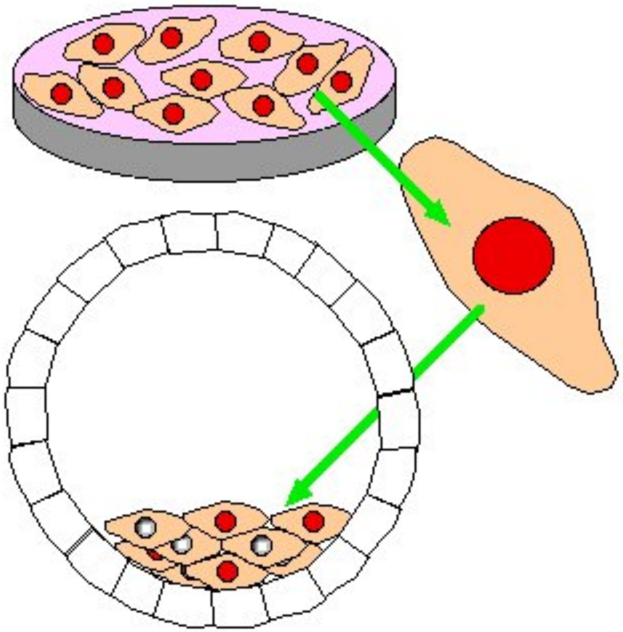


If you inject the cultured ES cells (originally from a blastocyst for a mouse with GREY FUR) into a new blastocyst that would normally give rise to a mouse with WHITE FUR



The blastocyst, now containing two types of totipotent embryonic stem cells, is implanted into a foster mother; she will give birth the chimeric offspring





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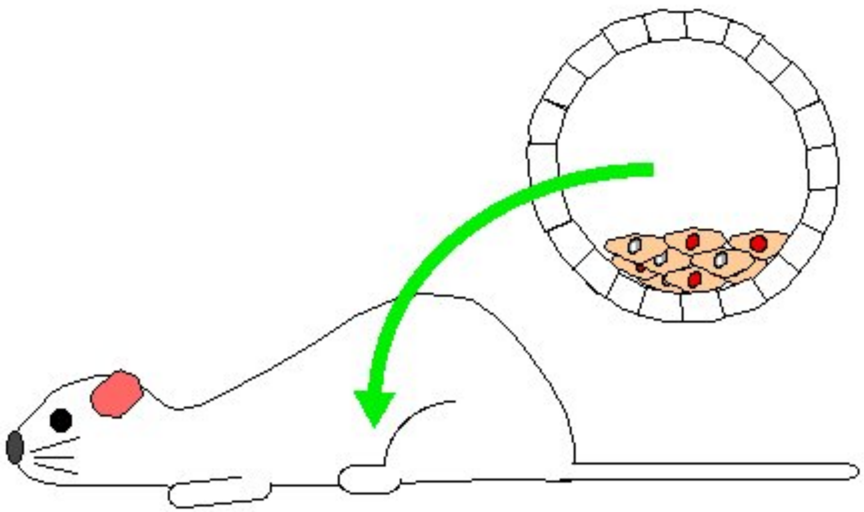

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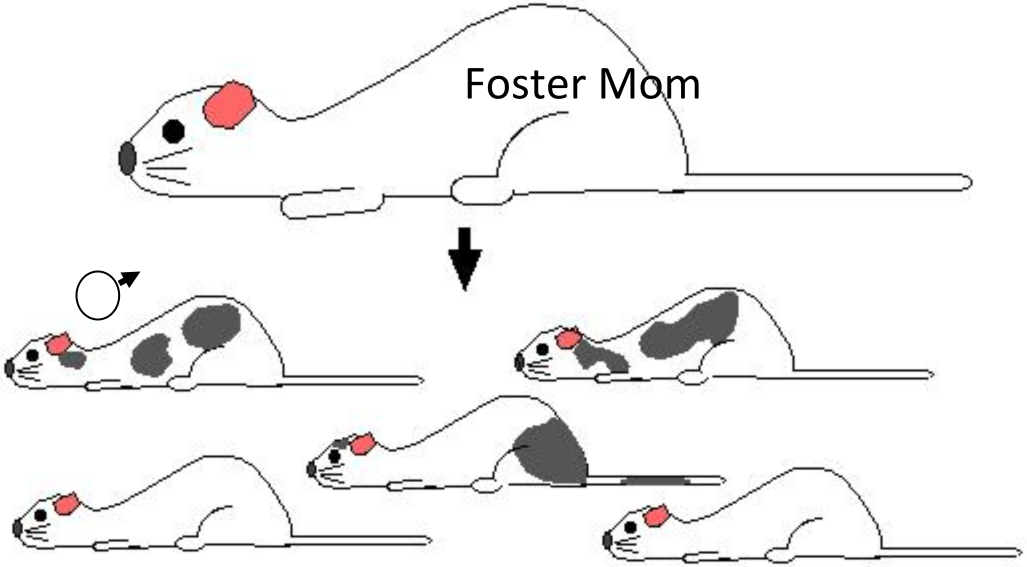
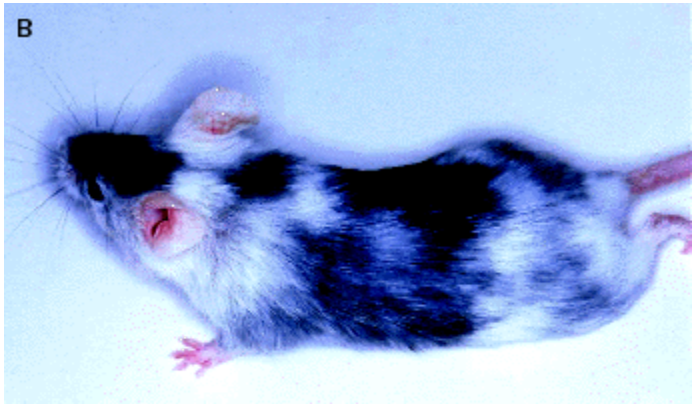
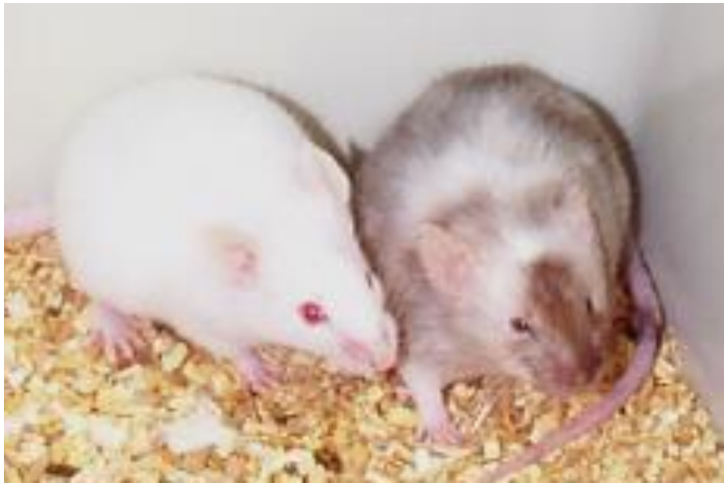
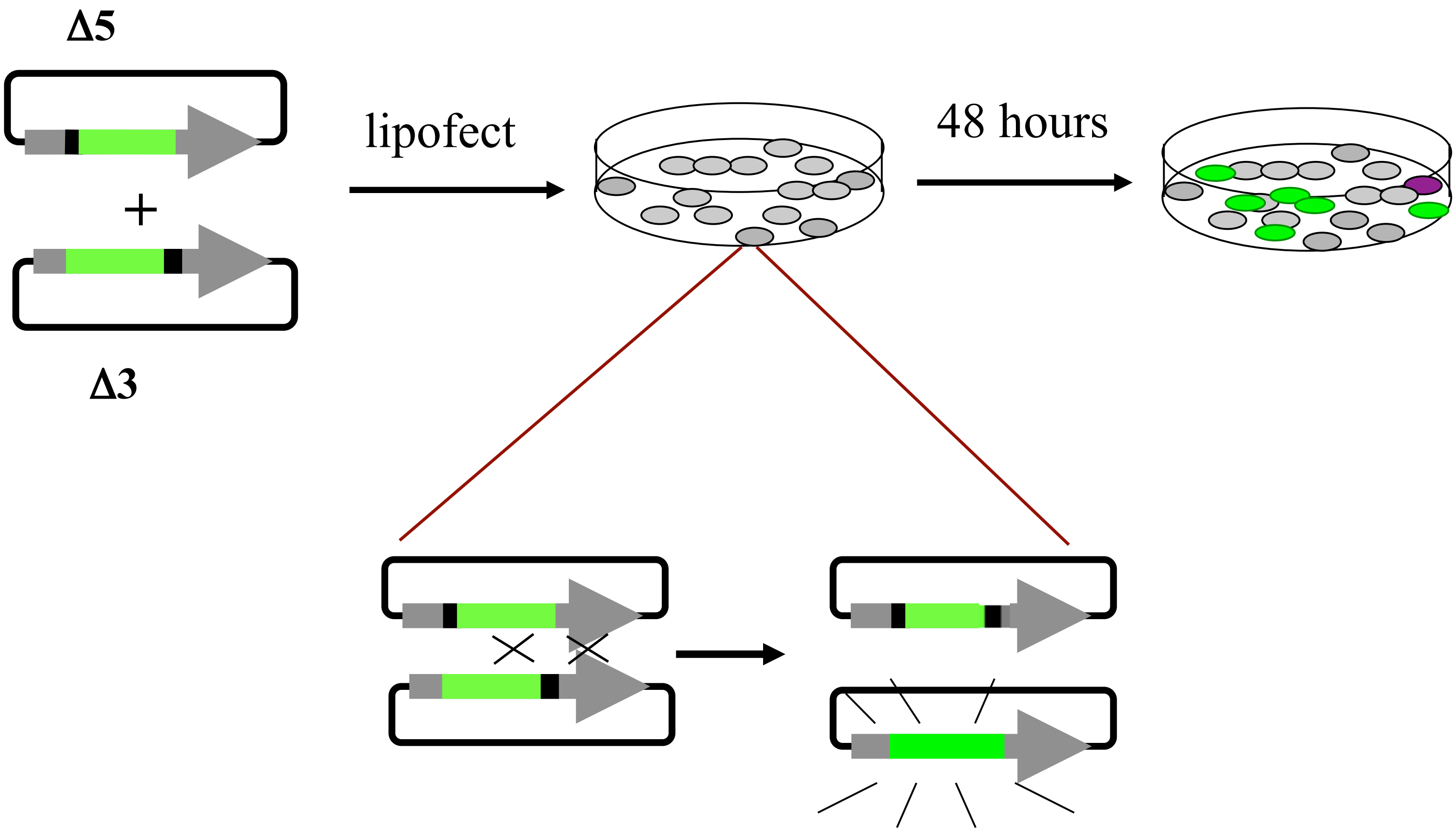


Some mice are Chimeric





A Plasmid-Based Assay for Homologous Recombination in Mammalian Cells



Bevin Engelward