20.109 MOD1
Measuring Genomic Instability

Fall 2022
Day 3

Bevin P. Engelward, *Sc.D.*
Professor of Biological Engineering

Previous lecture -

Cancer is caused by acquired traits; mutations make new traits possible

Overview of the steps of BER

Chemistry of nucleotide addition (on the blackboard & in handout)

Story of water contamination and arsenic

How PARP helps BER

A careful look at the major steps of BER

γH2AX as a Marker of DNA Damage

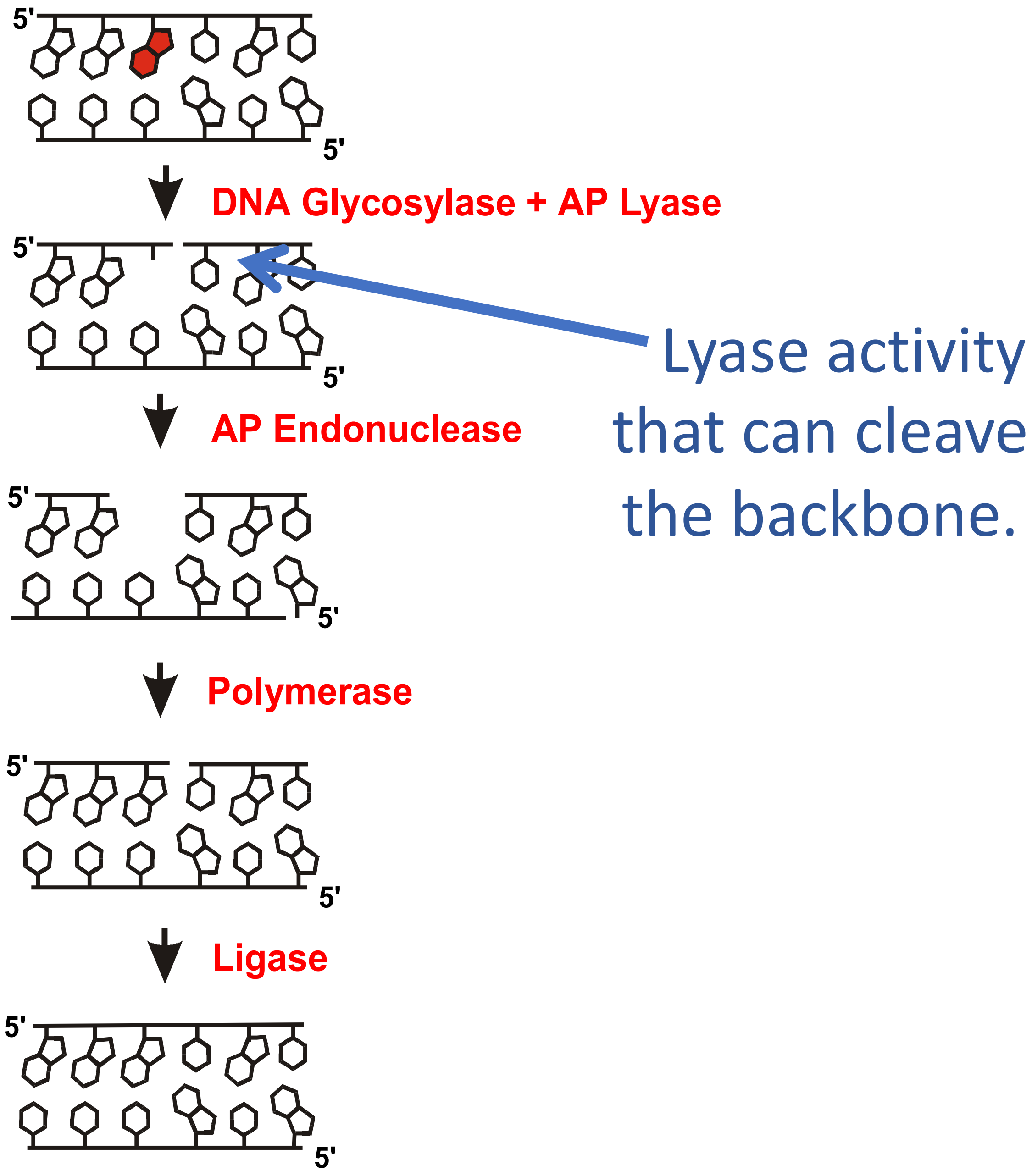
Interlude

Base Excision Repair
(BER)

Base Excision Repair

8-oxoguanine
DNA Glycosylase
(OGG1)

Removes the damaged base by cleaving the glycosylic bond.

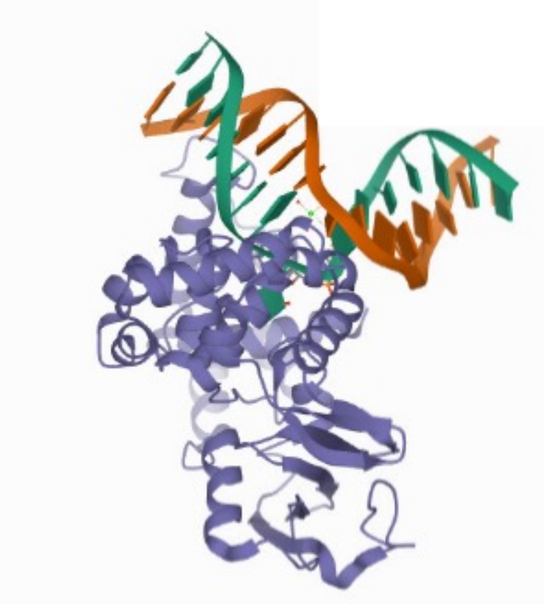


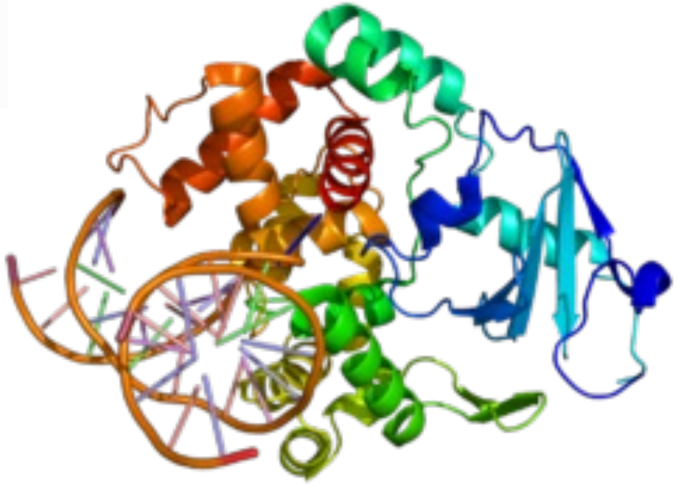
8-oxoguanine DNA Glycosylase (Ogg1)

Removes the damaged base.

Cleaves the backbone

Leaves behind an abasic site with a a nick.





Mutations in OGG1 are Associated with Increased Risk of Breast Cancer

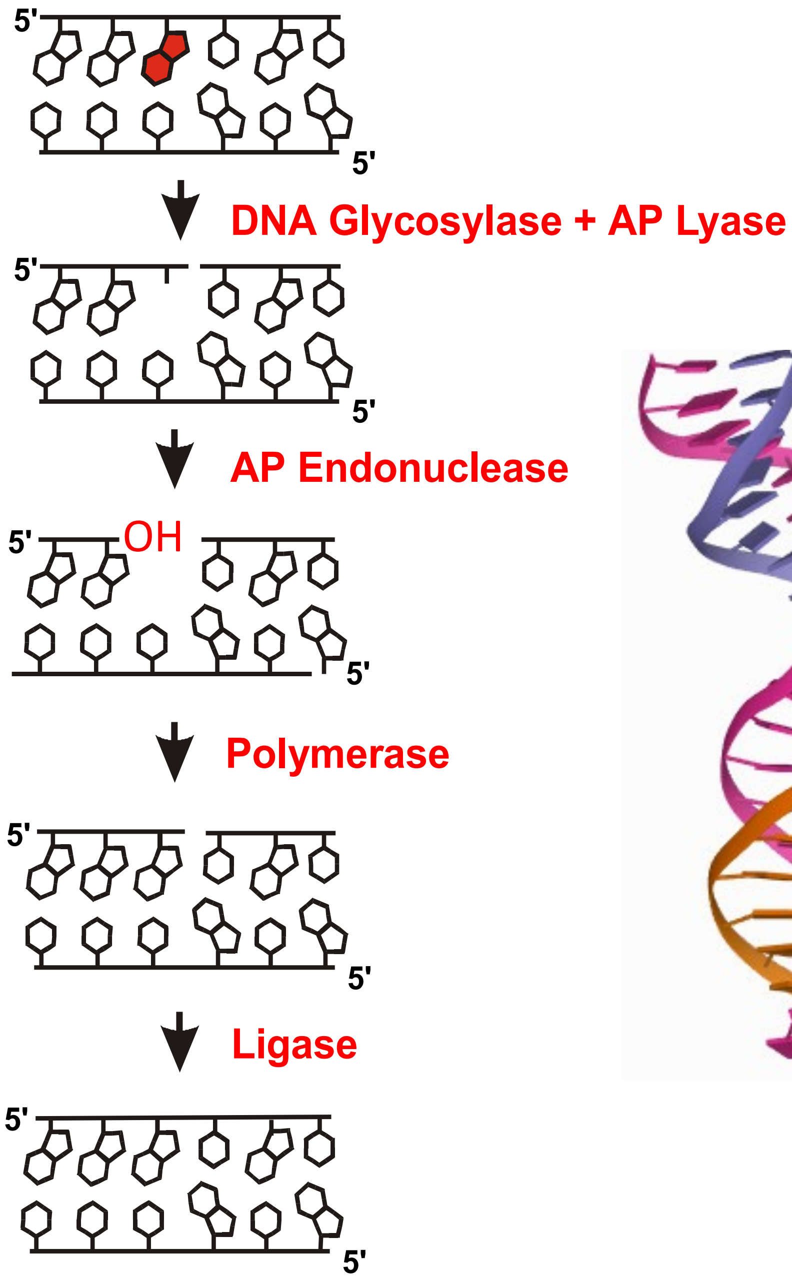
In some cases, the risk is > 15X Higher

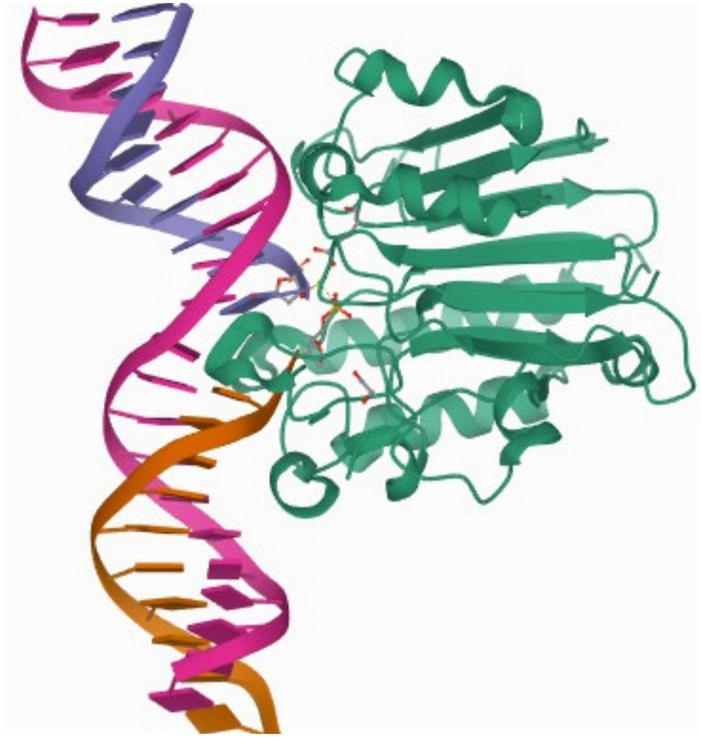
Base Excision Repair

AP Endonuclease

"Cleans the end" (removes the abasic sugar)

Creates a 3'OH that can be extended.

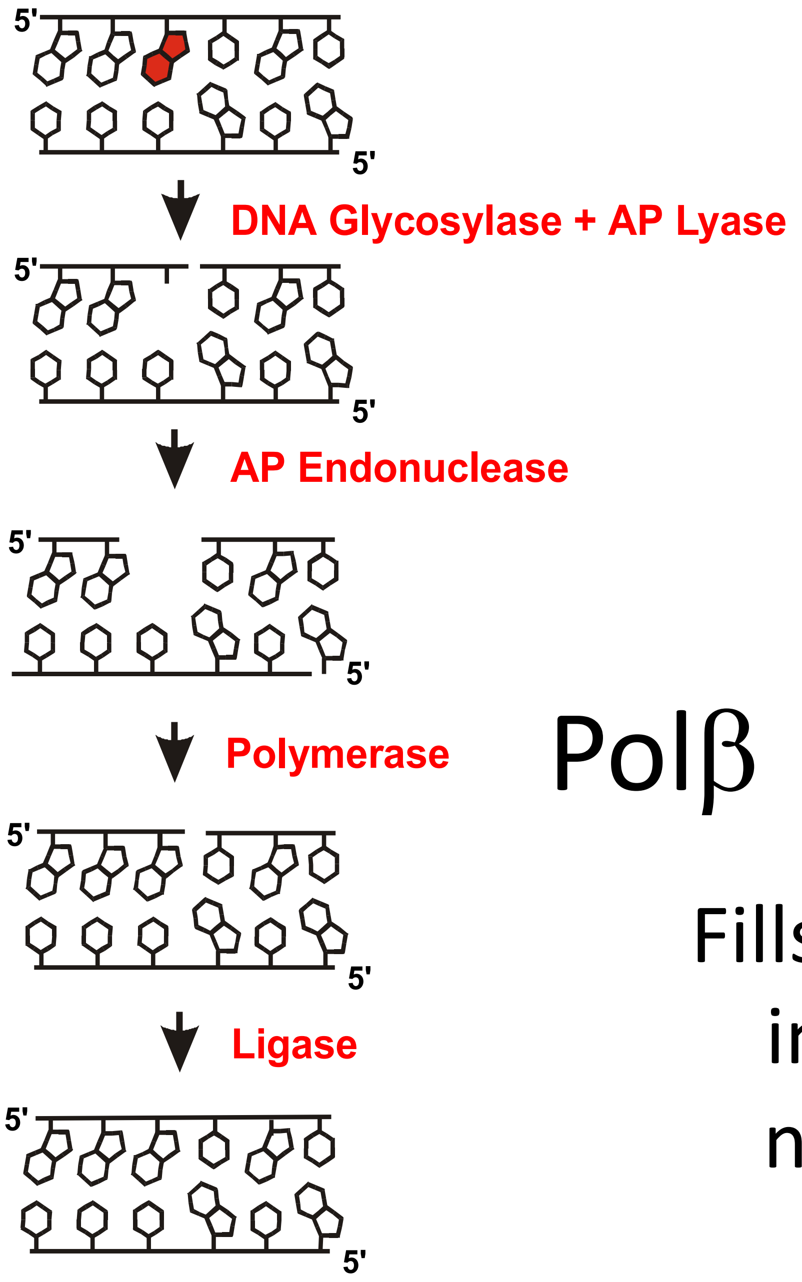




Single Nucleotide Addition by Polβ

Base Excision Repair

DNA Polymerase Beta

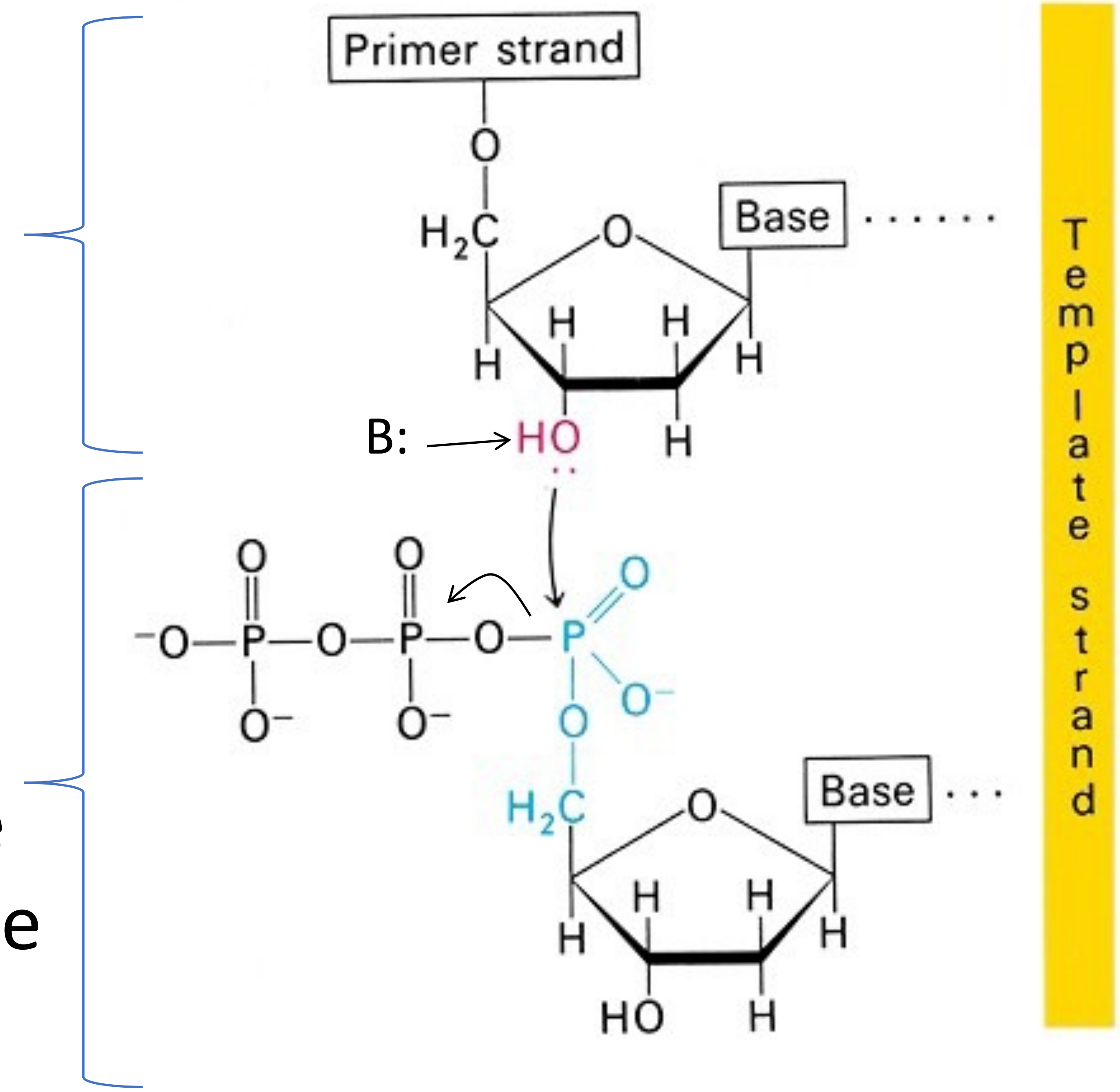


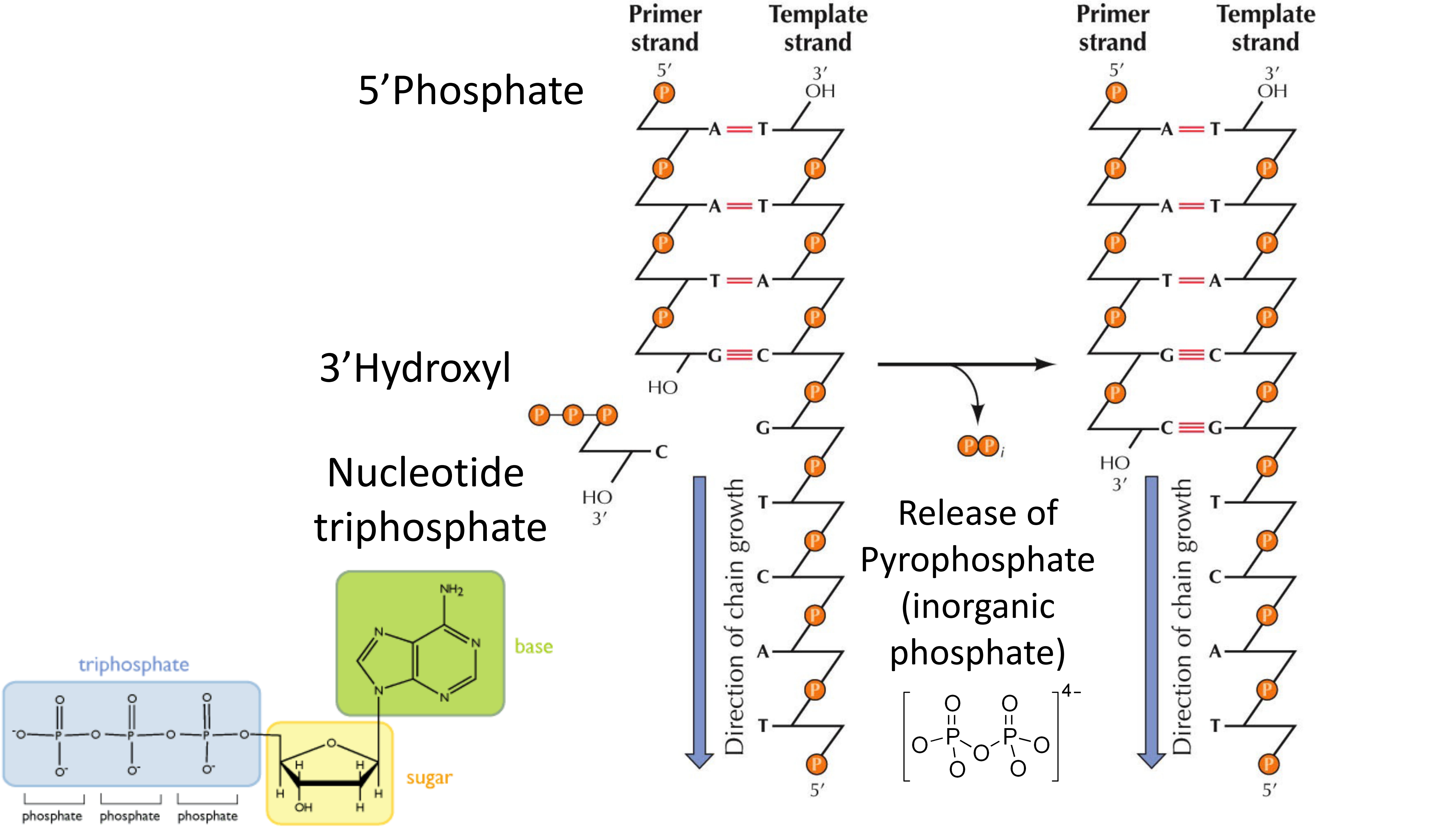
Polβ

Fills the gap by inserting a nucleotide

3’ End

Incoming Nucleotide Triphosphate





5'Phosphate
3'Hydroxyl
Nucleotide triphosphate
Release of Pyrophosphate (inorganic phosphate)
Primer strand
Template strand
5'
3'
OH
HO
A
T
G
C
P
PP_i
Direction of chain growth
$4-$
triphosphate
base
sugar
phosphate
phosphate
phosphate
NH_2
N
OH
H

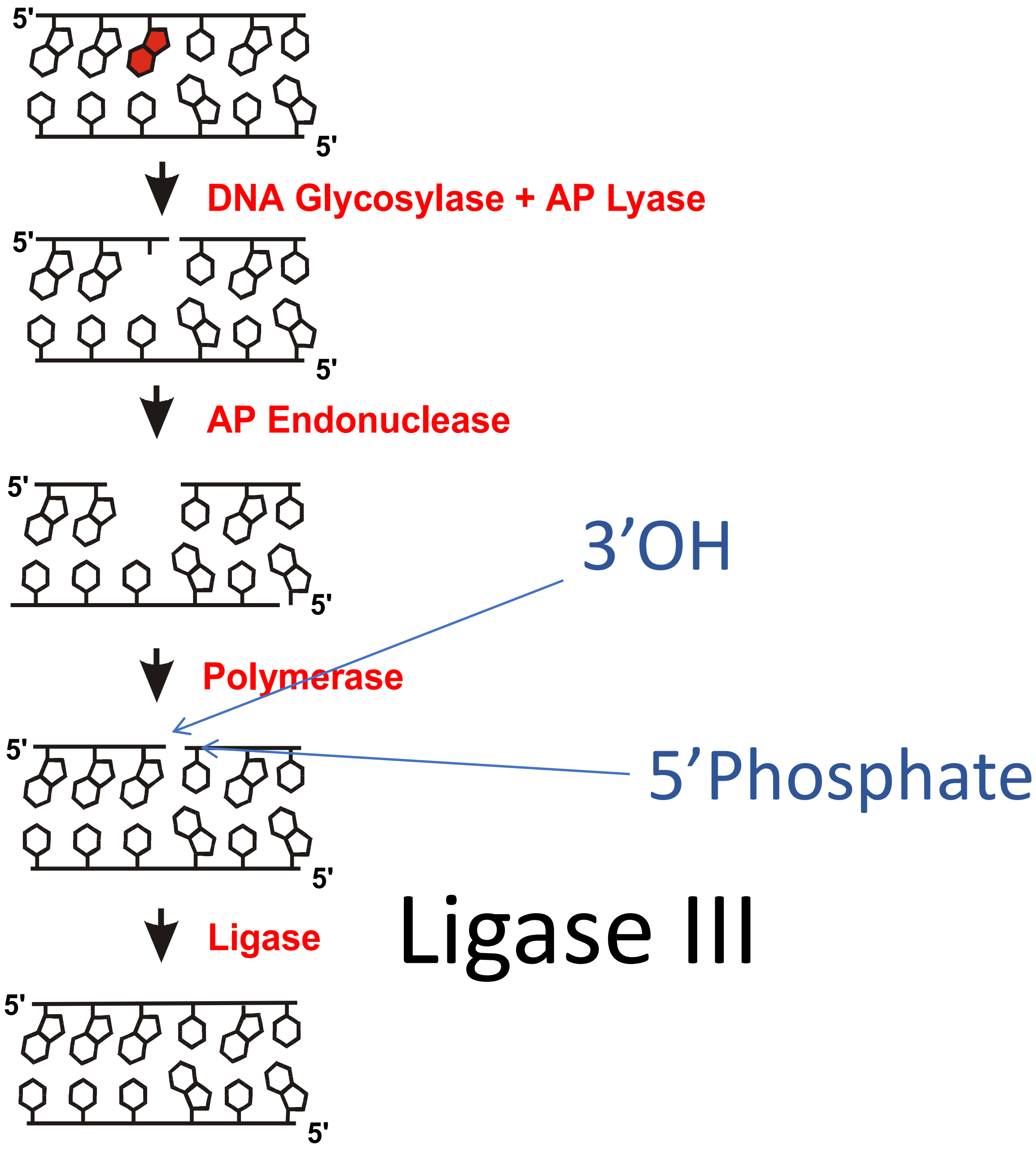
DNA Polymerase Beta (Polβ)

Mutations in Polβ in Mice cause Lupus-Like Symptoms – Possible association with Lupus in People but still ***Unknown***

- Autoimmune disease
- Fatigue
- Fever
- Joint pain, stiffness and swelling
- Butterfly-shaped rash on the face
- Skin lesions that appear or worsen with sun exposure
- Fingers and toes that turn white or blue when exposed to cold or during stressful periods
- Shortness of breath
- Chest pain
- Dry eyes
- Headaches, confusion and memory loss

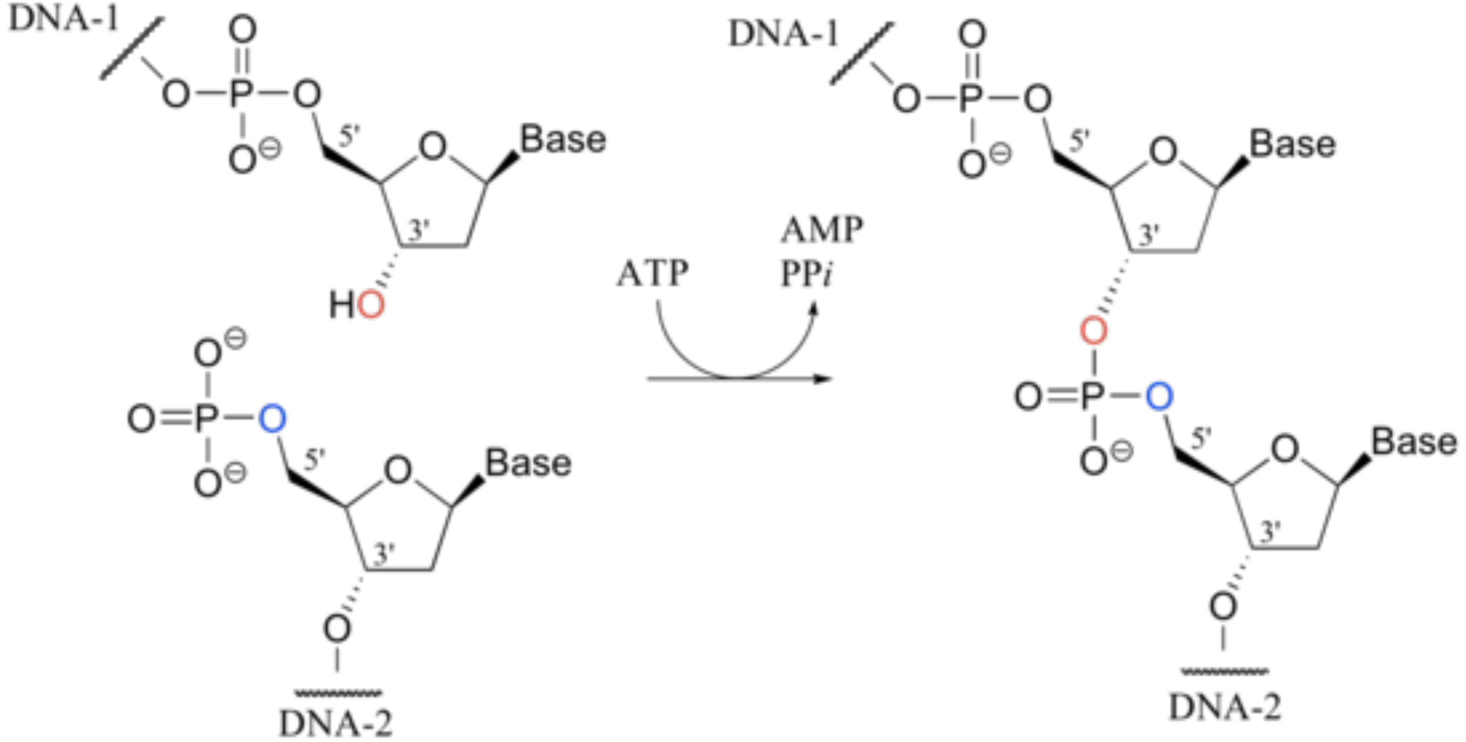
DNA Ligase III

Base Excision Repair

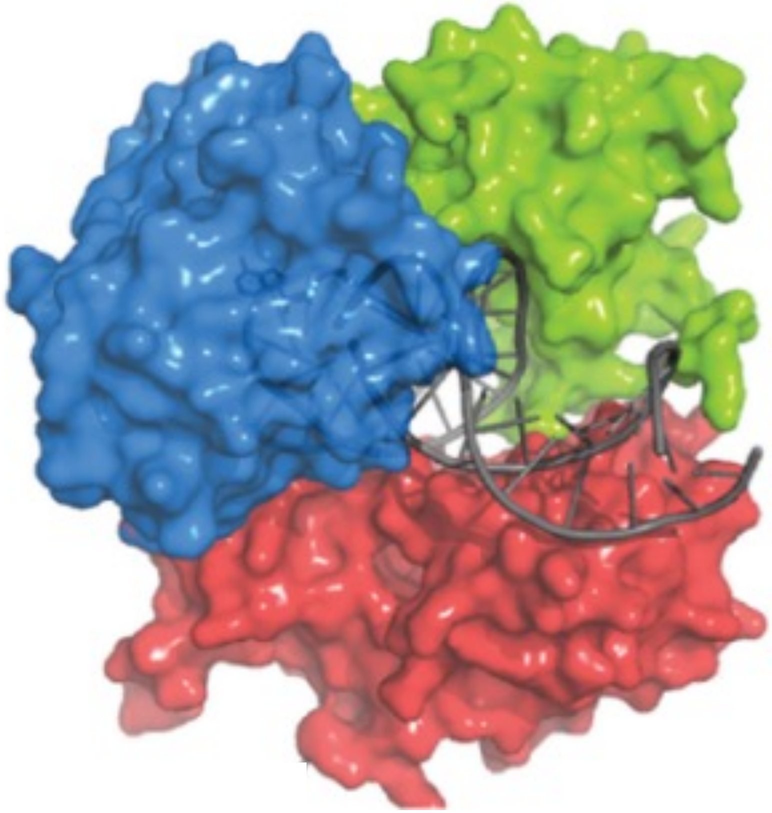


DNA Ligase III

Seals the nick by linking the 3'OH with the 5'Phosphate


DNA-1
Base
3'
5'
HO
Base
5'
3'
DNA-2
ATP
AMP
PPi
DNA-1
Base
5'
3'
Base
5'
3'
DNA-2

Ligase III

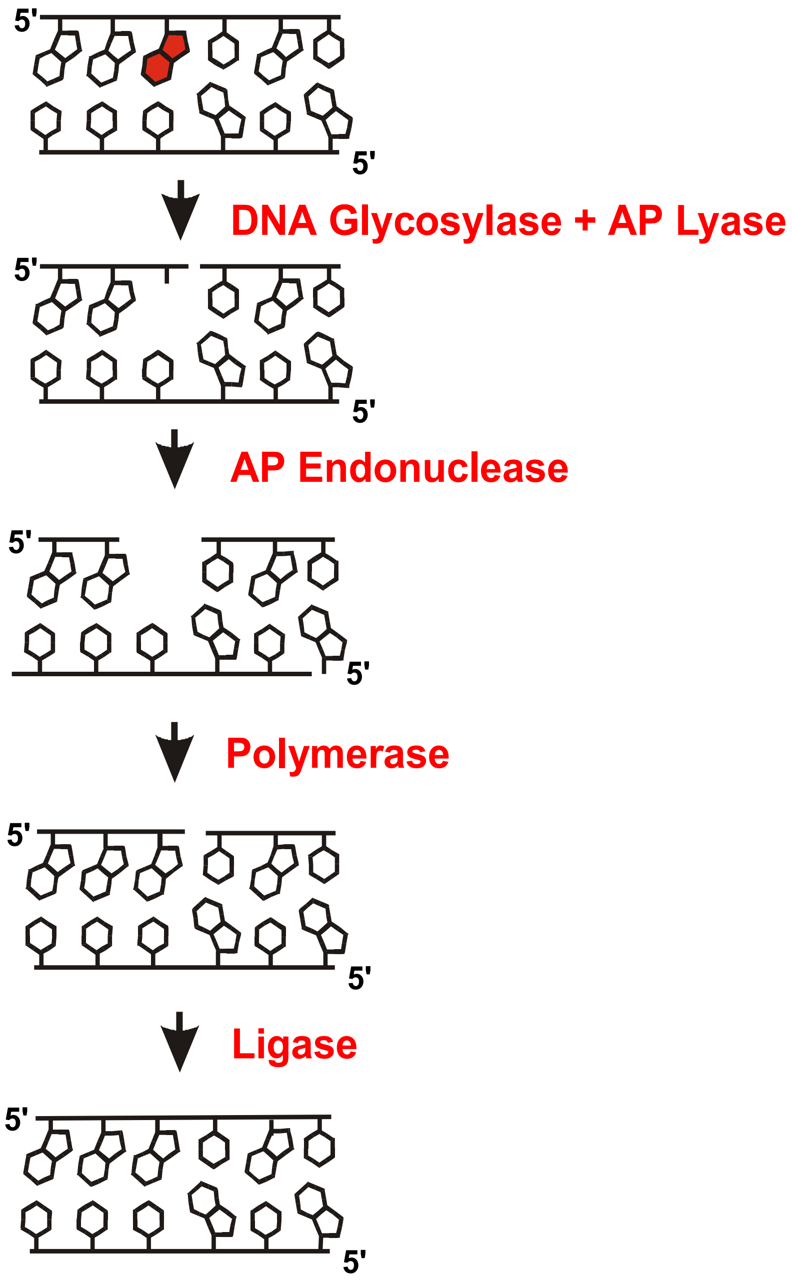


Pascali, O' Brien, Tomkinson, and Ellenberger, Nature 432: 473-478.

Mutations in Ligase III can cause Bloom's Syndrome

- Autosomal recessive
- Growth retardation
- Butterfly rash
- Defective immune system
- Increased risk of cancer

Base Excision Repair



Beautiful Pristine DNA!

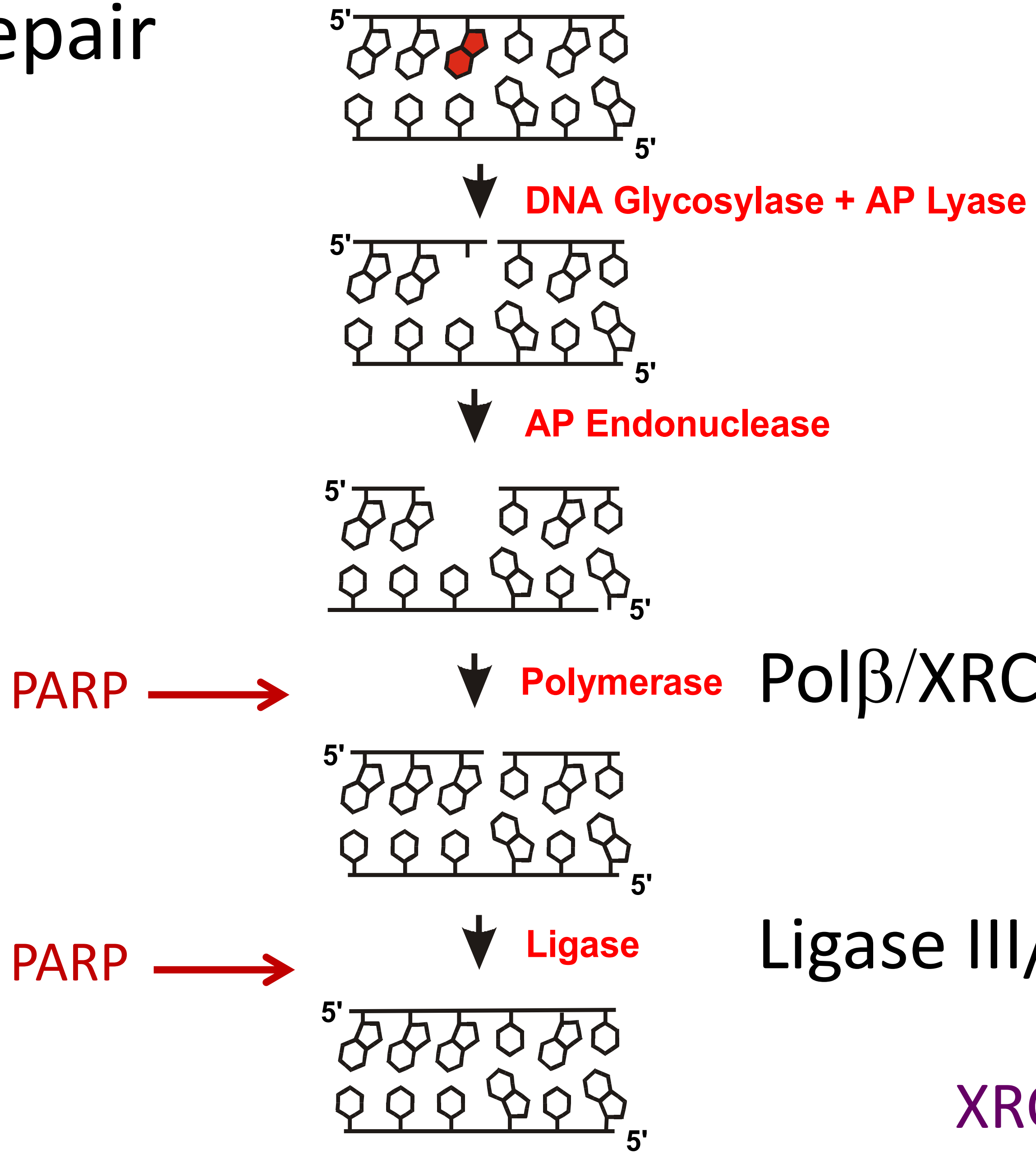
PARP

Poly(ADP-Ribose) Polymerase

PARP is a BER “Helper”

Accelerates BER

Base Excision Repair

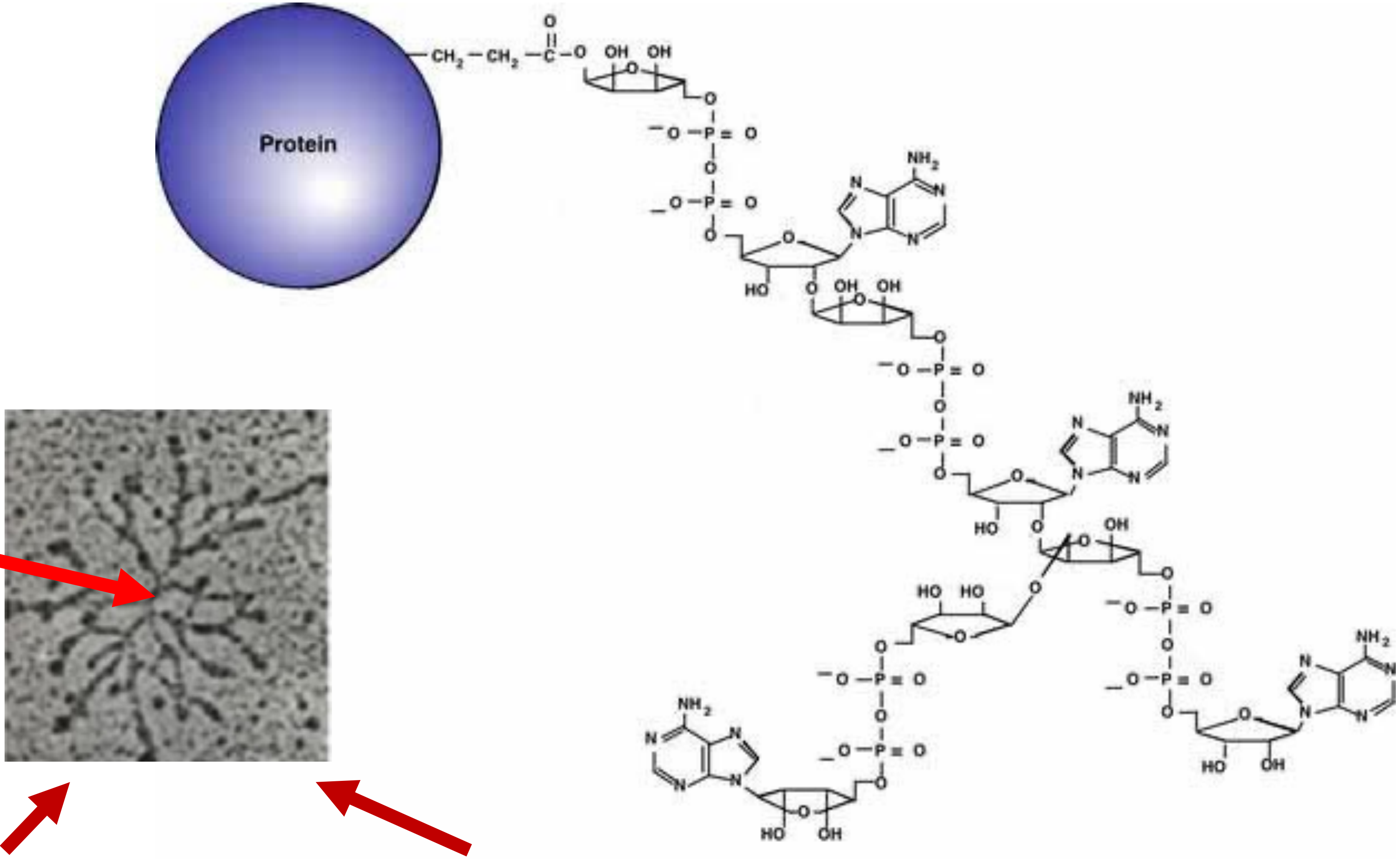


Polβ/XRCC1

Ligase III/XRCC1

XRCC1 = A Scaffold

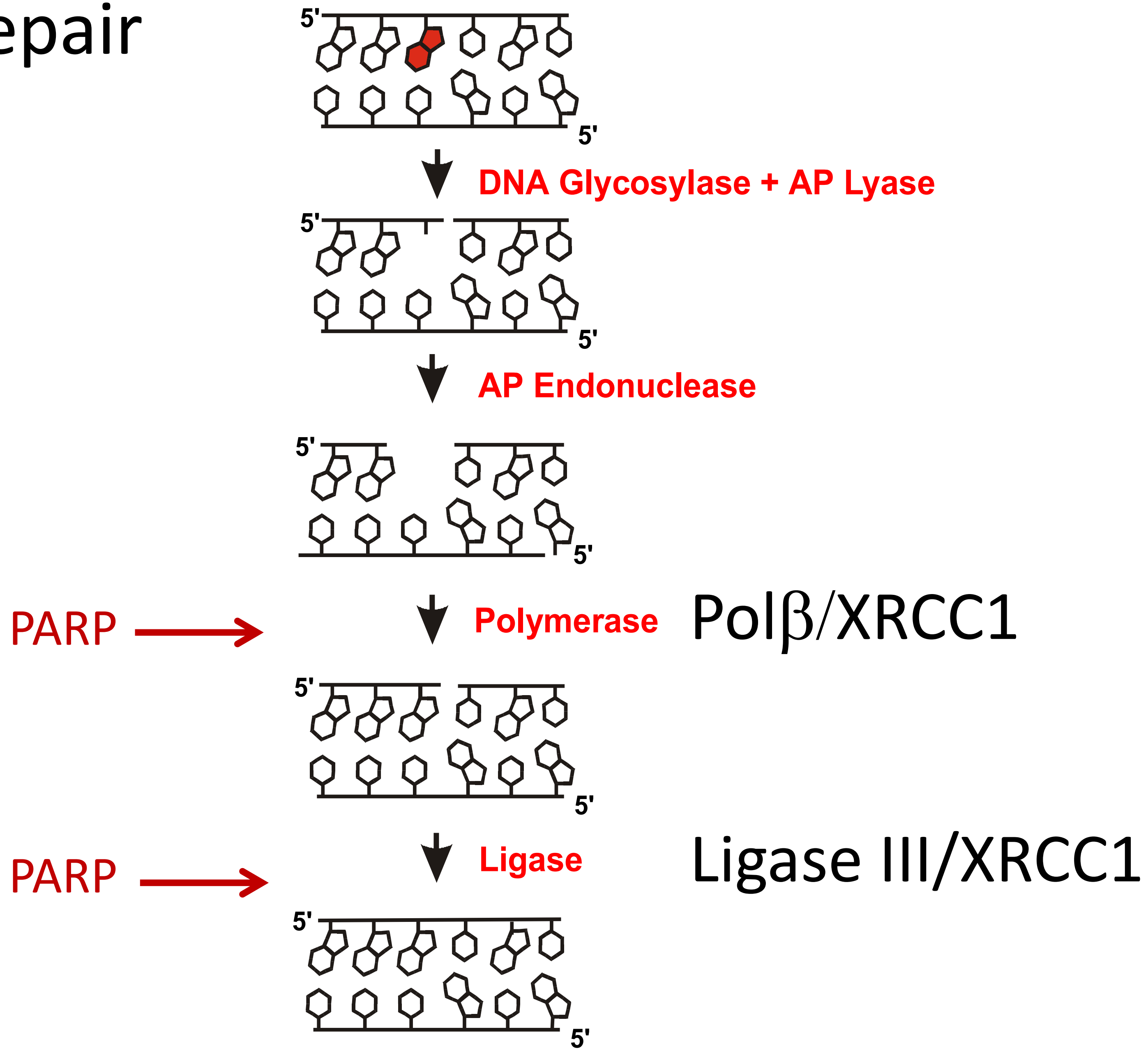
PARP Automodification Creates a Branched Structure



PARP is in the middle

Polβ/XRCC1 Ligase III/XRCC1

Base Excision Repair

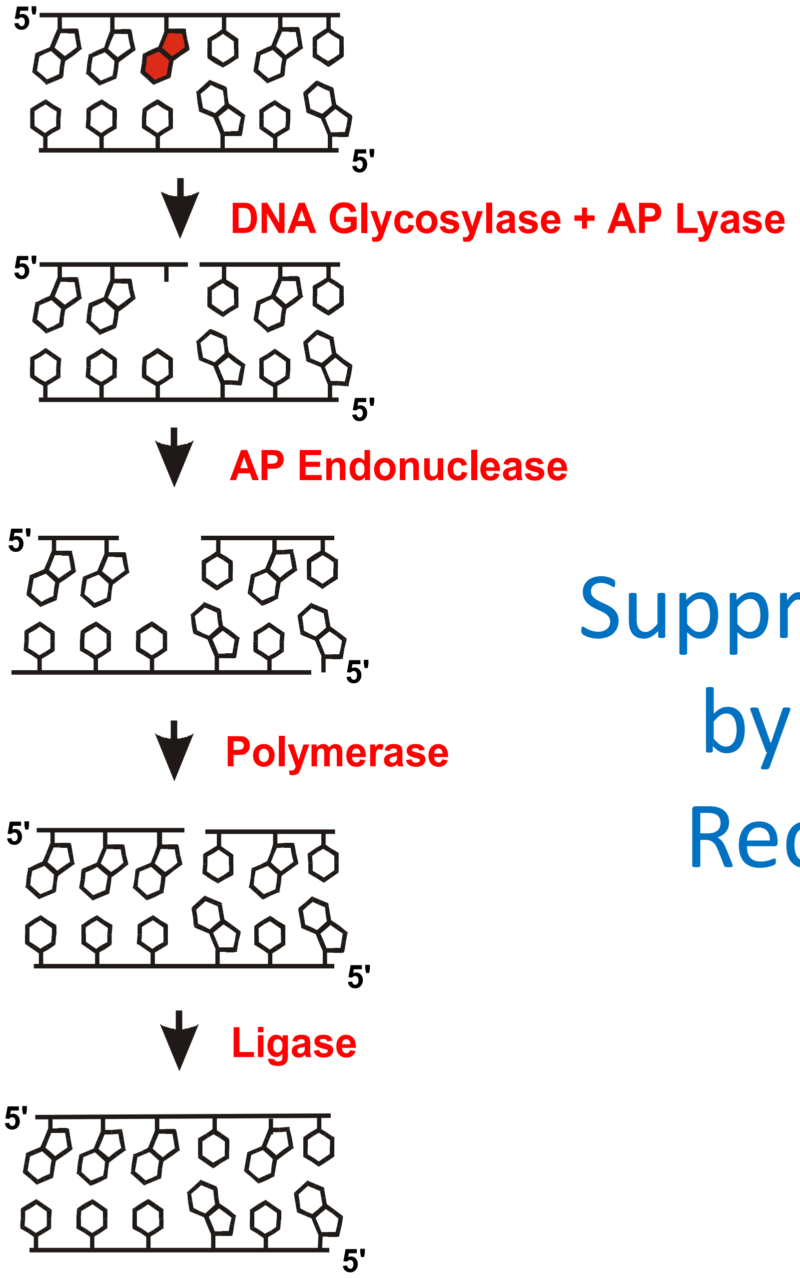


As Inhibits SSB Repair

As displaces zinc, disrupting zinc fingers in PARP

As ⊣ PARP →

As ⊣ PARP →



Suppression of PARP by As Reduces Recruitment of DNA Repair Proteins

As Inhibition of PARP leads to Increased Single Strand Breaks

Closely Opposed Single Strand Breaks lead to Double Strand Breaks

Summary

- Base excision repair requires multiple steps
- Key enzymes in BER are DNA glycosylase (OGG1), AP Endonuclease, Polymerase, and Ligase

- Polymerase requires a 3'OH
- Ligase requires a 3'OH and a 5'Phosphate

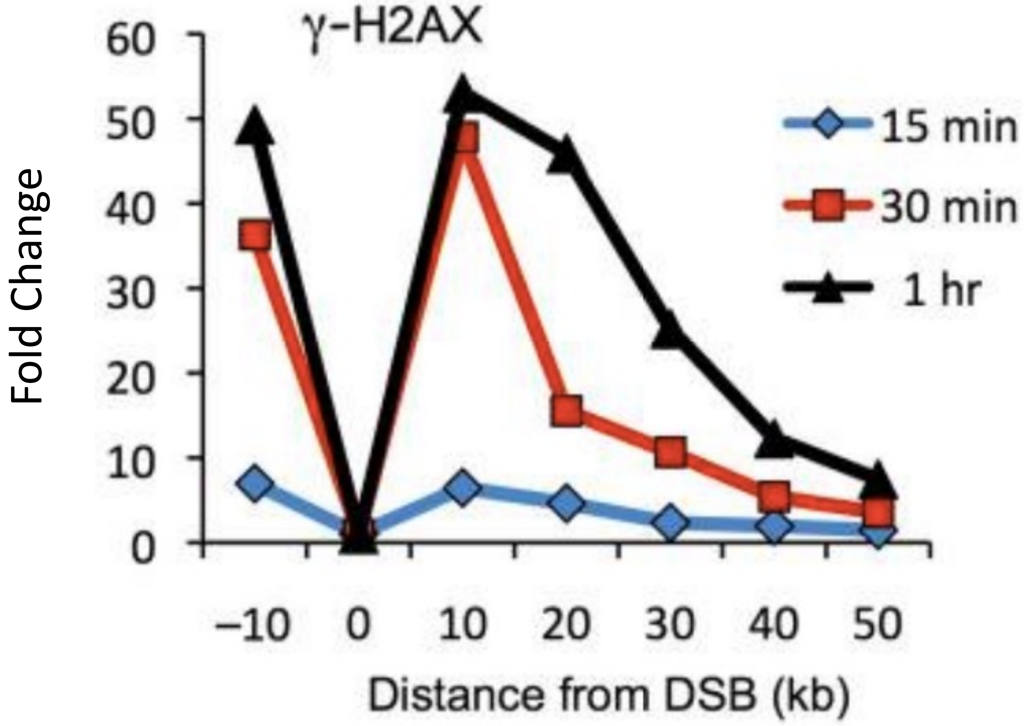
- PARP serves as a beacon to recruit BER enzymes
- PARP has a zinc finger and is inhibited when As replaces Zn
-

A careful look at the major steps of BER

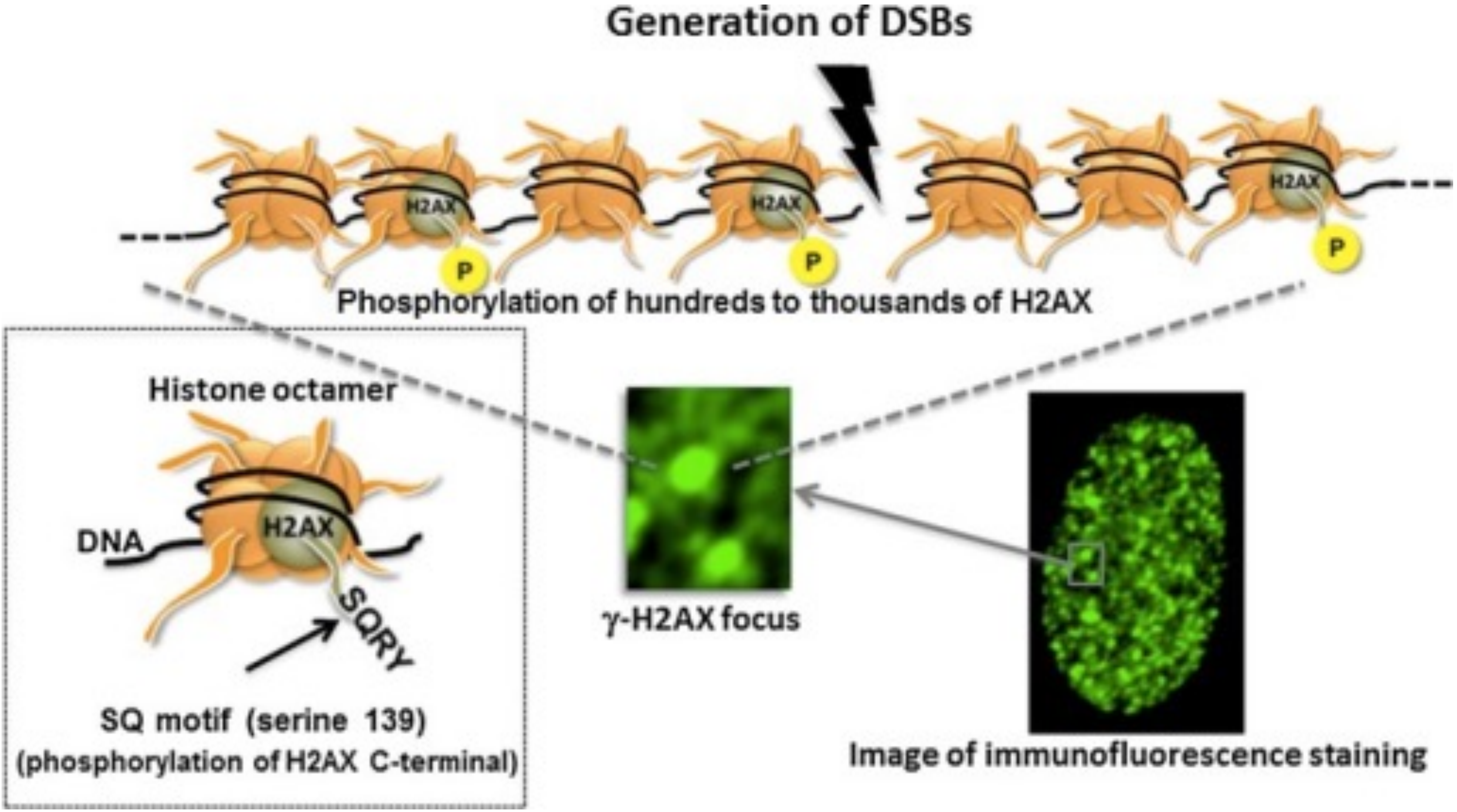
γH2AX as a Marker of DNA Damage

Interlude

Sensing DNA Damage with Antibodies



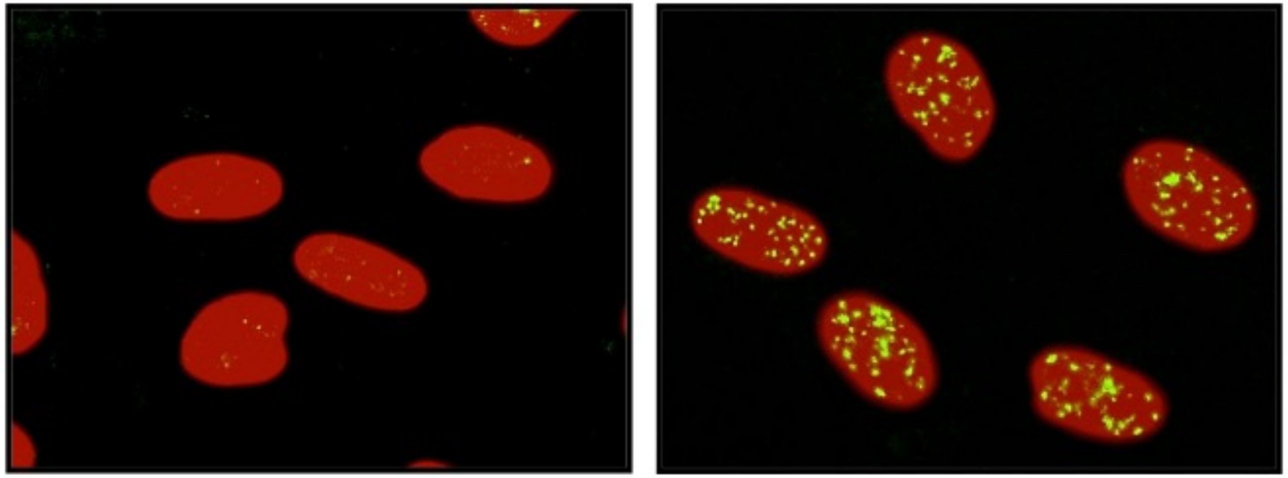
Study performed in *S. cerevisiae*
http://www.nature.com/nsmb/journal/v21/n1/fig_tab/nsmb.2737_F1.html



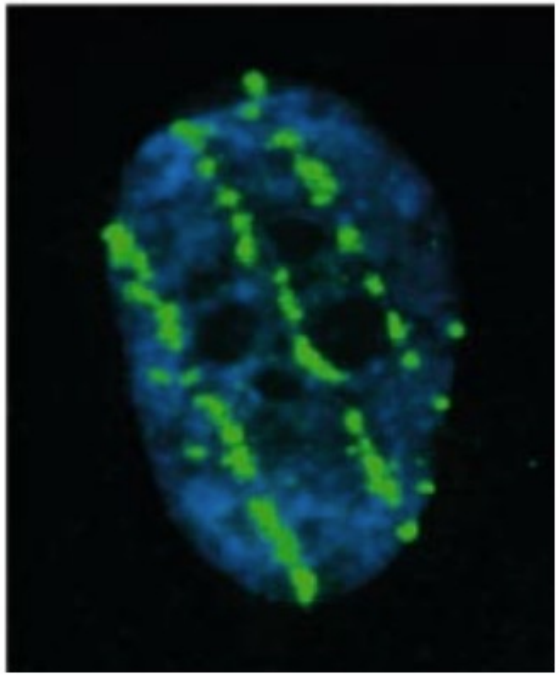
https://openi.nlm.nih.gov/detailedresult.php?img=PMC4380052_rru10502&req=4

γH2AX for Low versus High LET radiation

0 Gy 2 Gy



Low LET

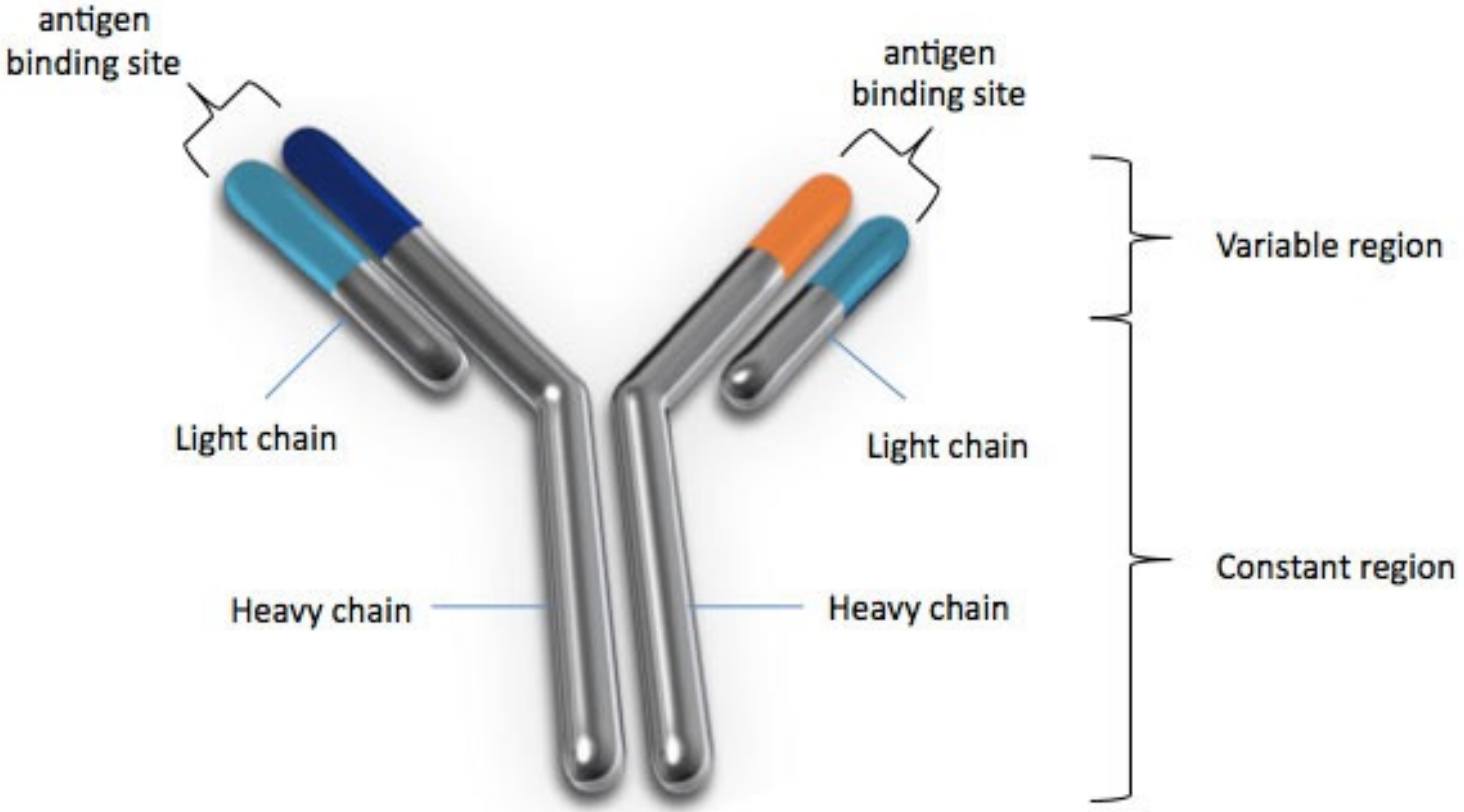


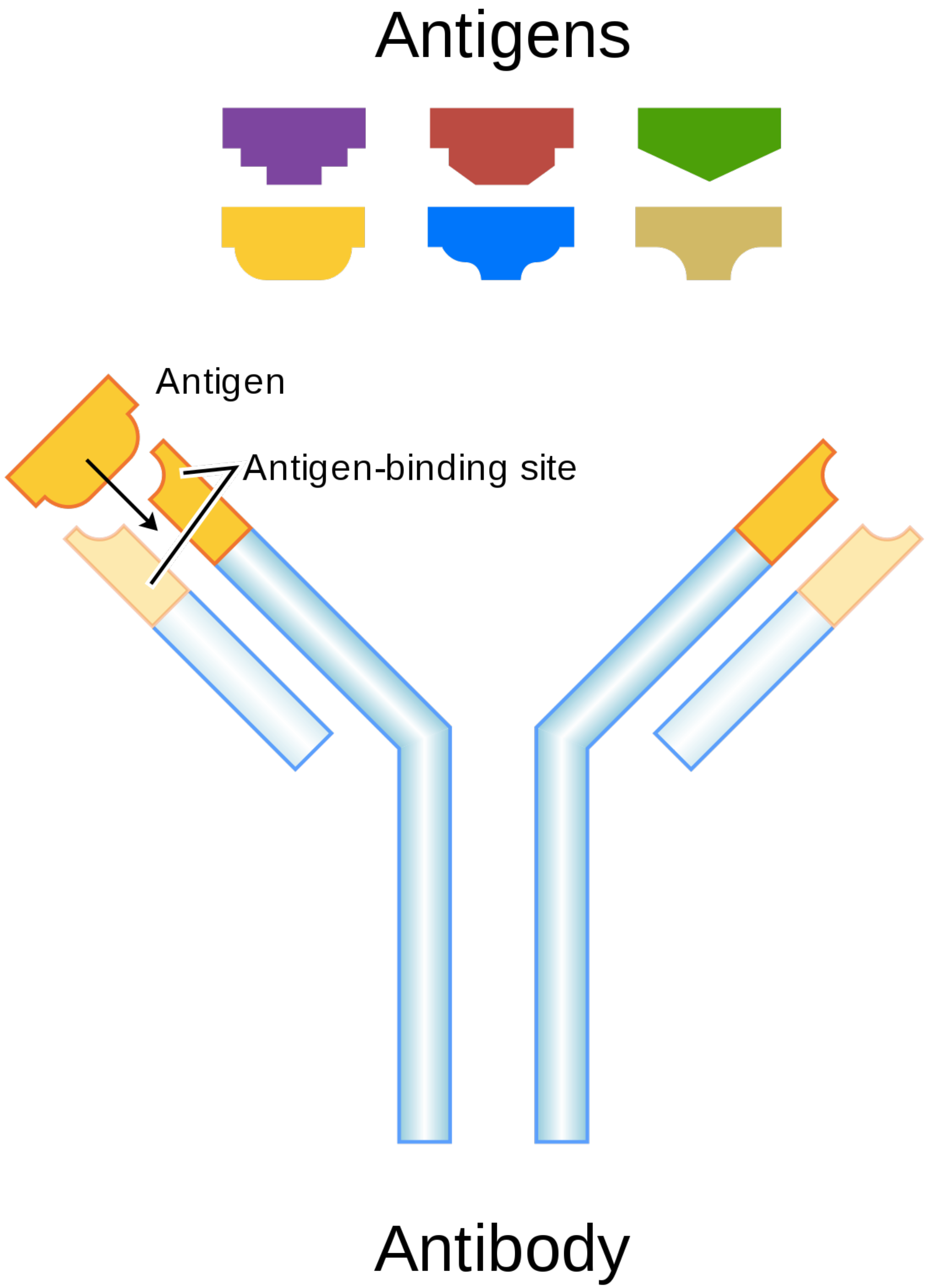
High LET

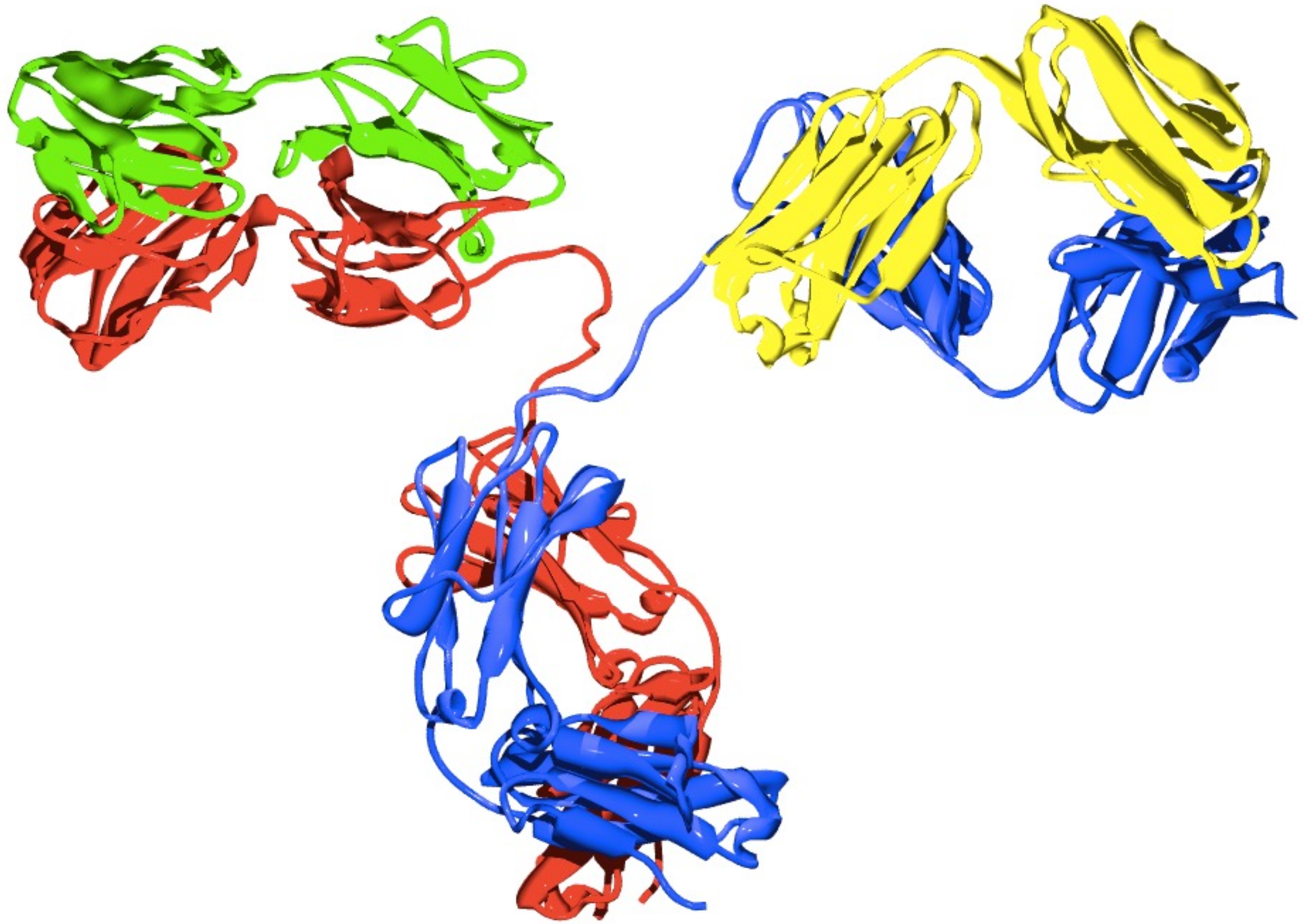
D Anderson et al 2013 JINST 8 C06008

Desai et al., Rad. Res. 2005

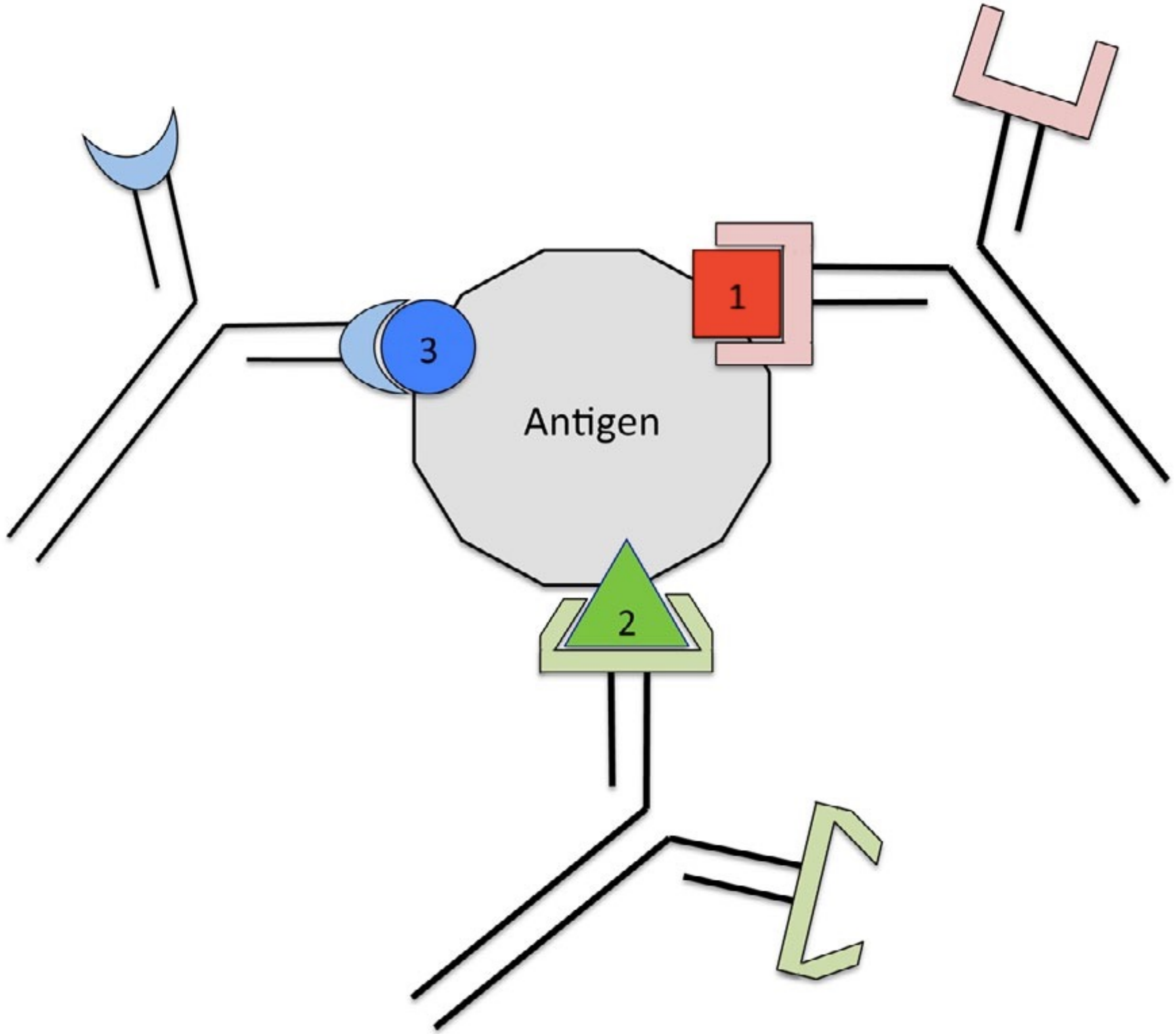
Antibody Fundamentals


antigen
binding site
antigen
binding site
Variable region
Light chain
Light chain
Constant region
Heavy chain
Heavy chain


Antigens
Antigen
Antigen-binding site
Antibody



Three Different Epitopes



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- Polymerase requires a 3’OH
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- PARP serves as a beacon to recruit BER enzymes
- PARP has a zinc finger and is inhibited when As replaces Zn

- H2AX gets phosphorylated when near DSBs to create γH2AX
- γH2AX serves as a beacon to recruit DNA repair enzymes

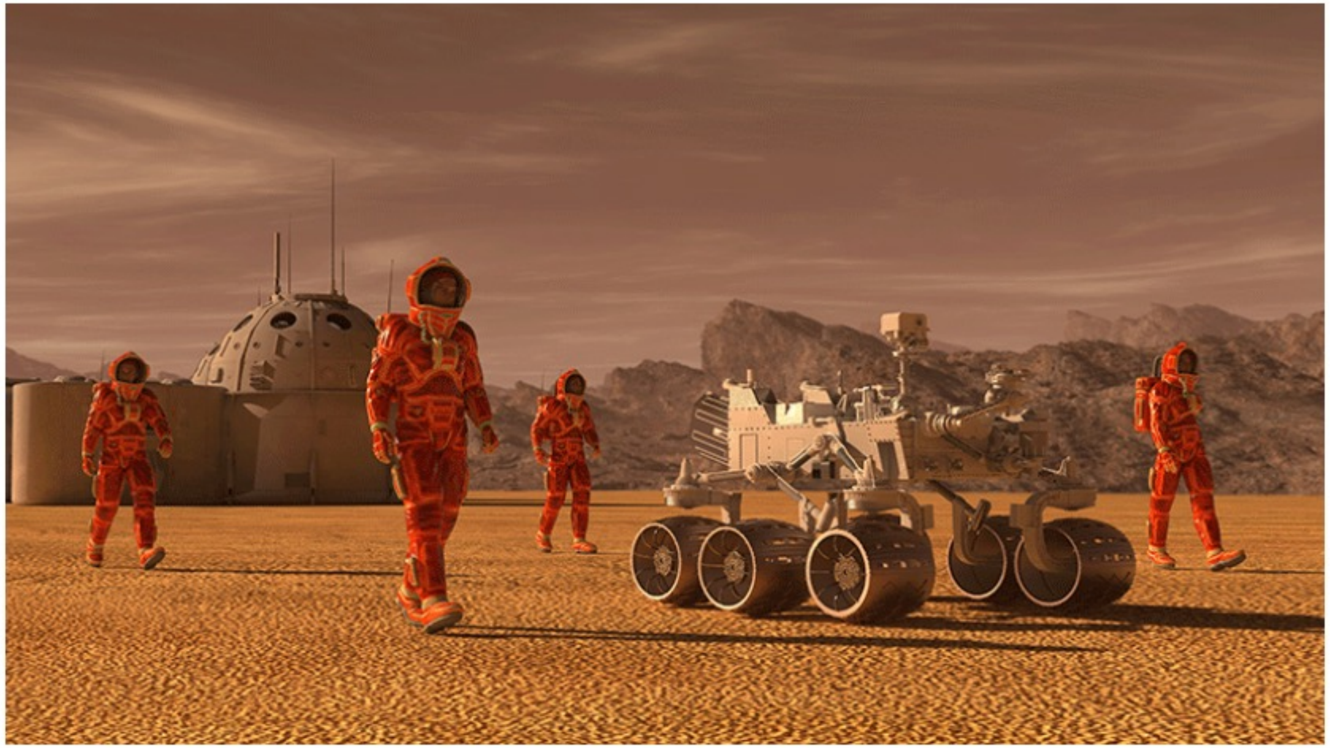
A careful look at the major steps of BER

γH2AX as a Marker of DNA Damage

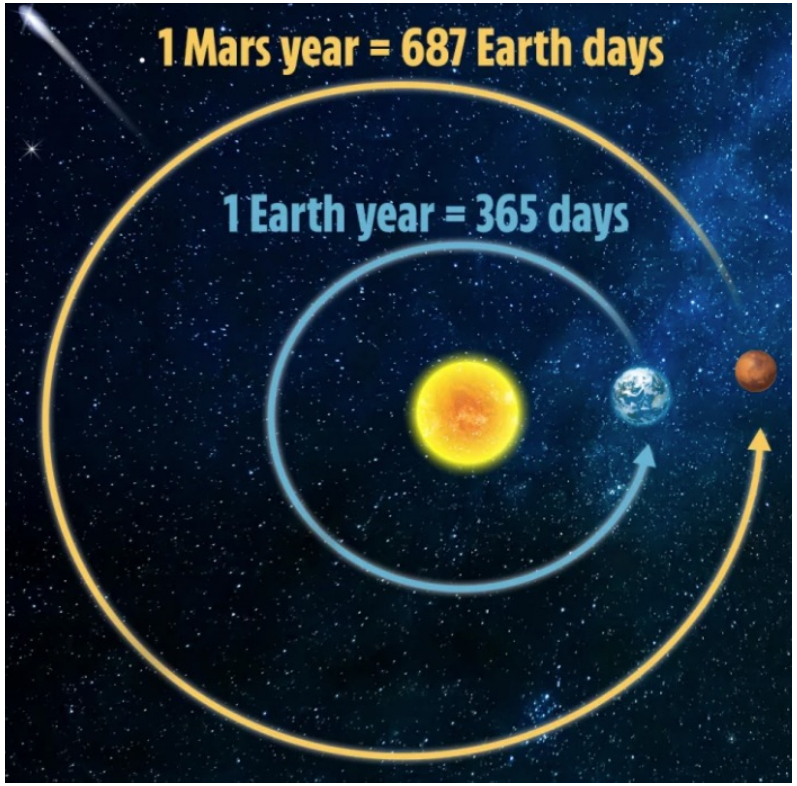
Interlude

Interlude

How what you are learning in class relates to a trip to Mars



The trip to Mars is ~34 million miles and will take about seven months.

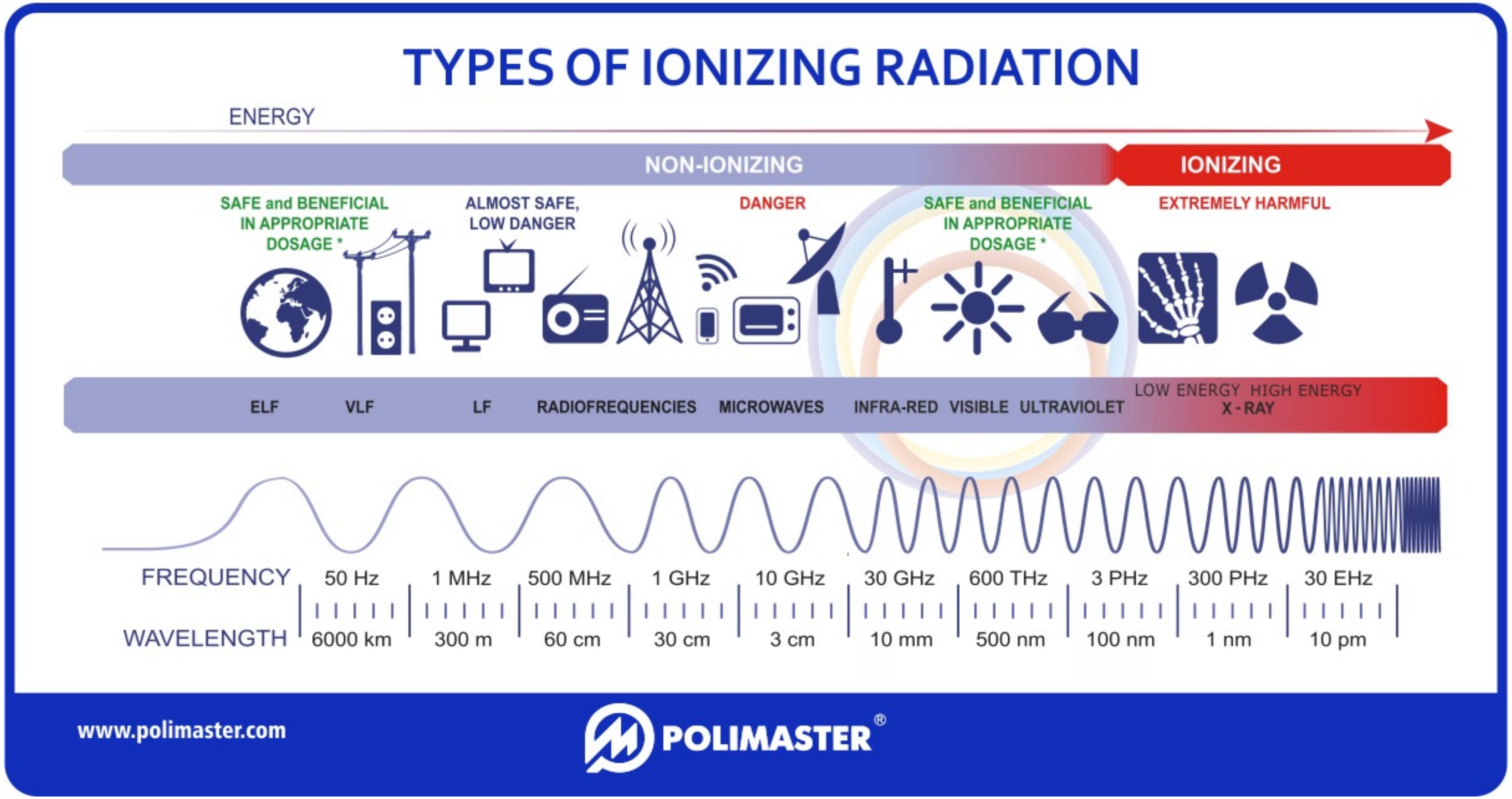


Goals of Elon Musk and SpaceX:

- **Fully reusable launch vehicles**
- **Human-rated spacecraft**
- **On-orbit propellant tankers**
- **Rapid-turnaround launch/landing mounts**
- **Local production of rocket fuel on Mars**

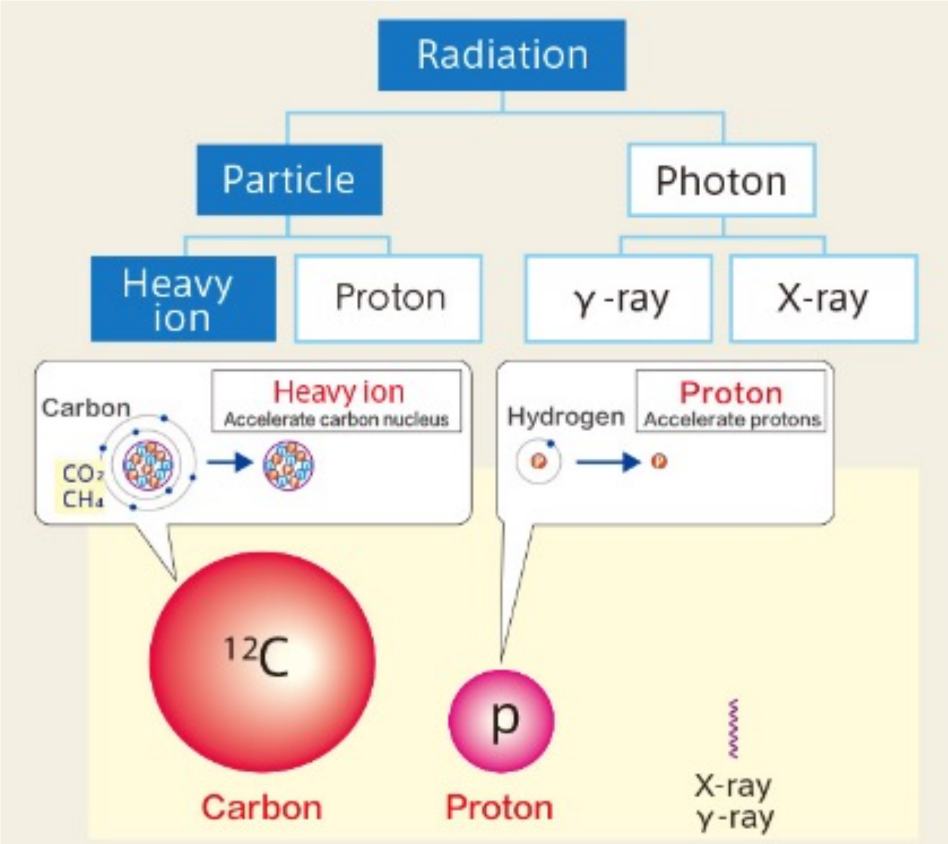
What Biological Issue is an Obstacle to Space Travel?

Ionizing Radiation Breaks Bonds



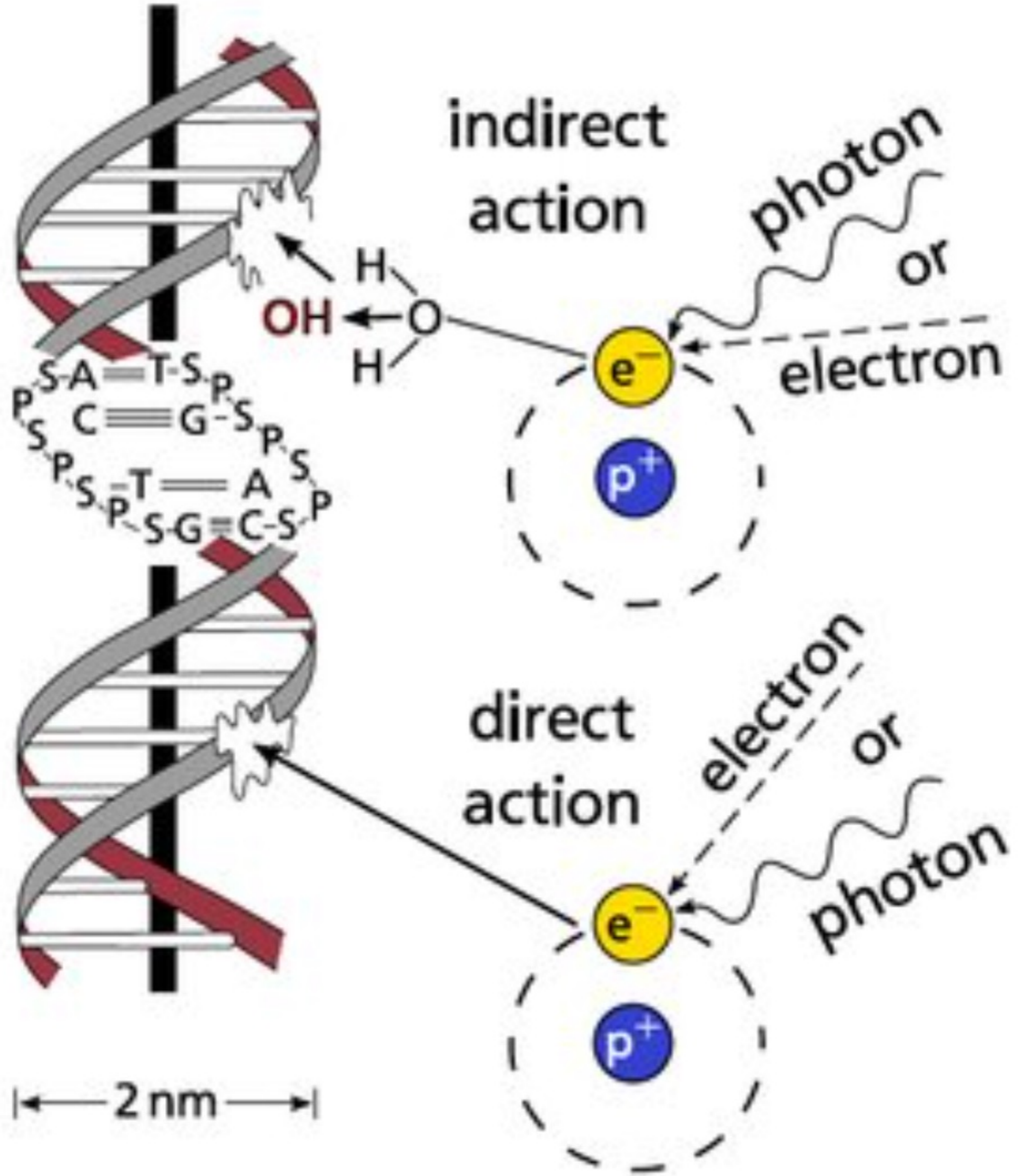
From Polimaster

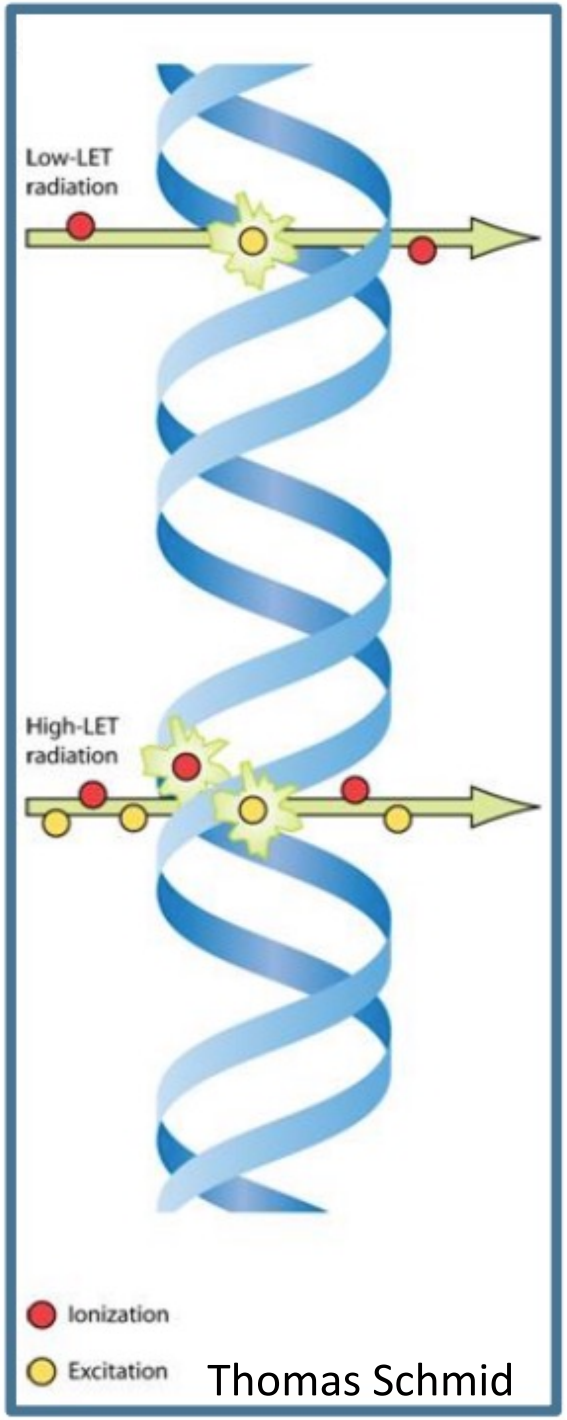
Carbon ion therapy is an emerging cancer treatment.



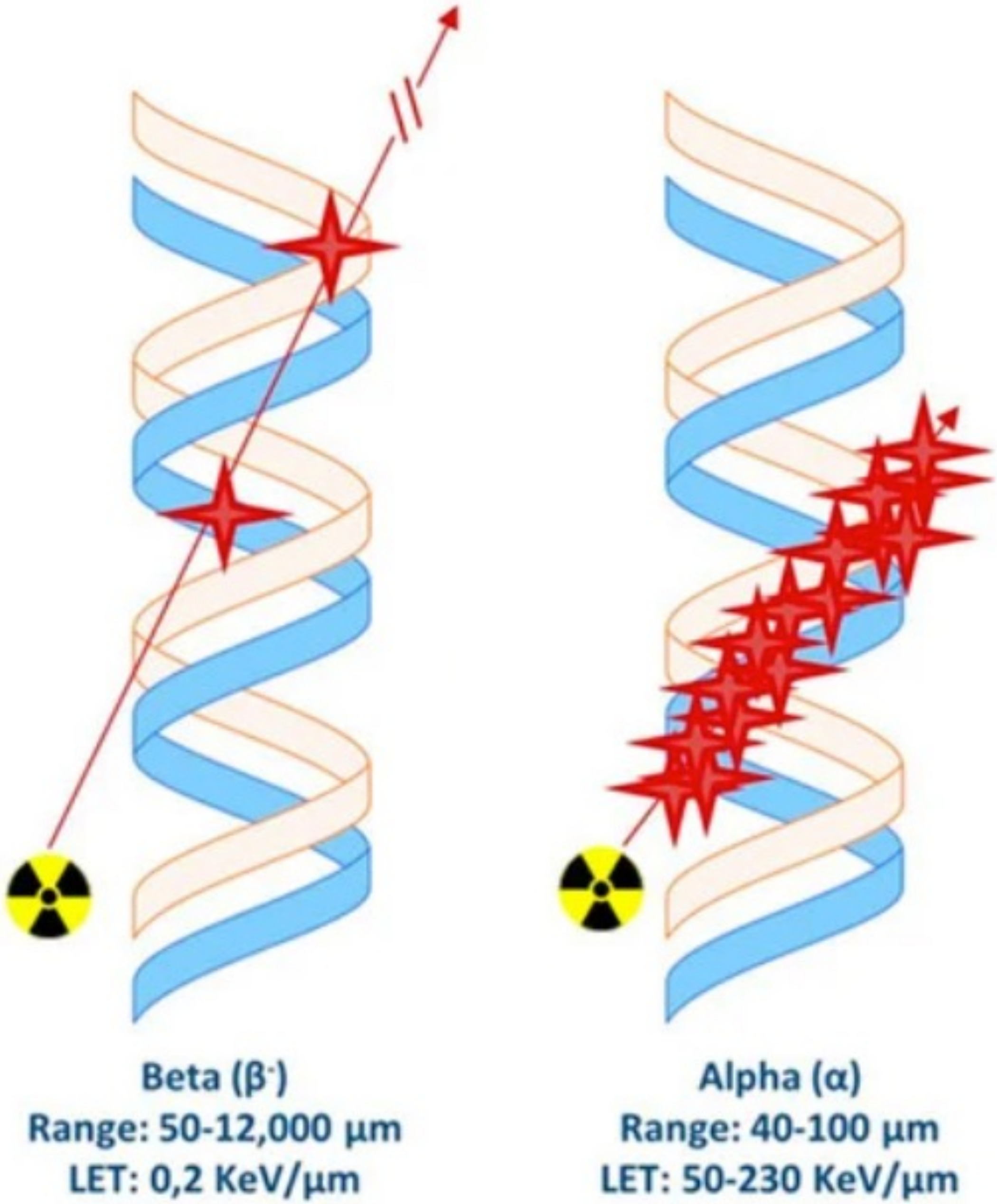
From Osaka

X-rays and Gamma rays are low LET (linear energy transfer)





Thomas Schmid



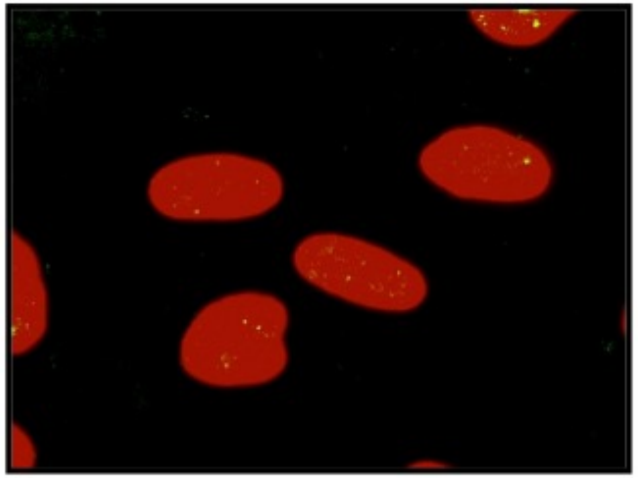
Galactic Cosmic Rays include heavy ion radiation.

One type of radiation comes from iron atoms stripped of their electrons and flying near the speed of light.

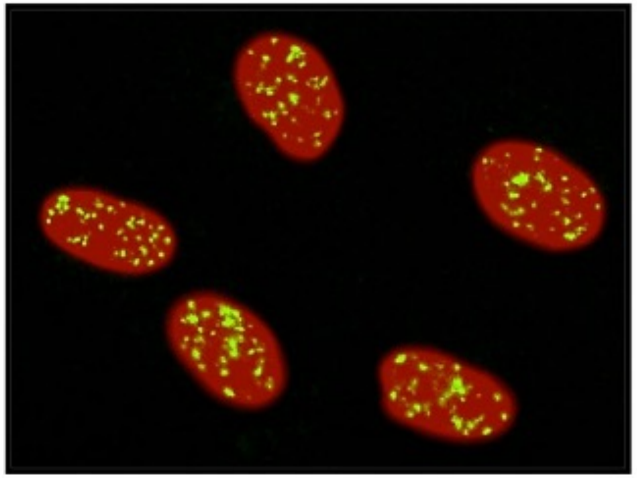
Ruigrok et al., Phramaceuticals 2019

γH2AX for Low versus High LET radiation

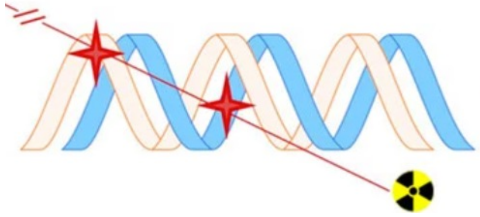
0 Gy

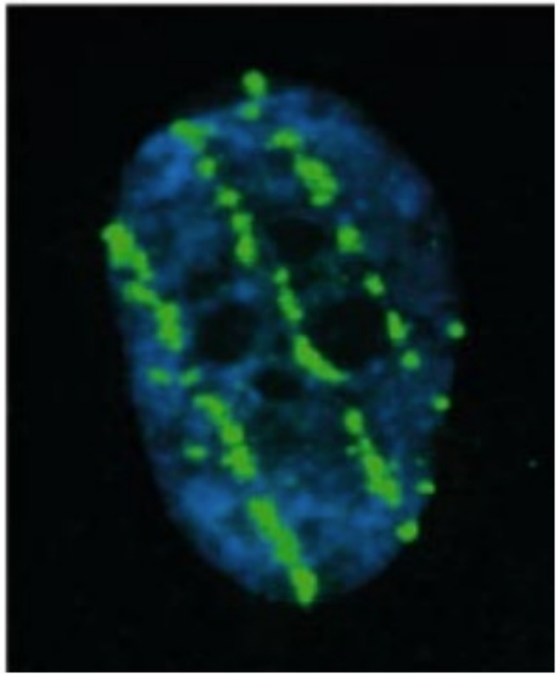


2 Gy

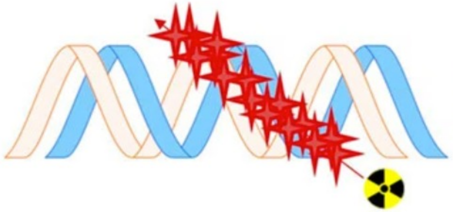


Low LET



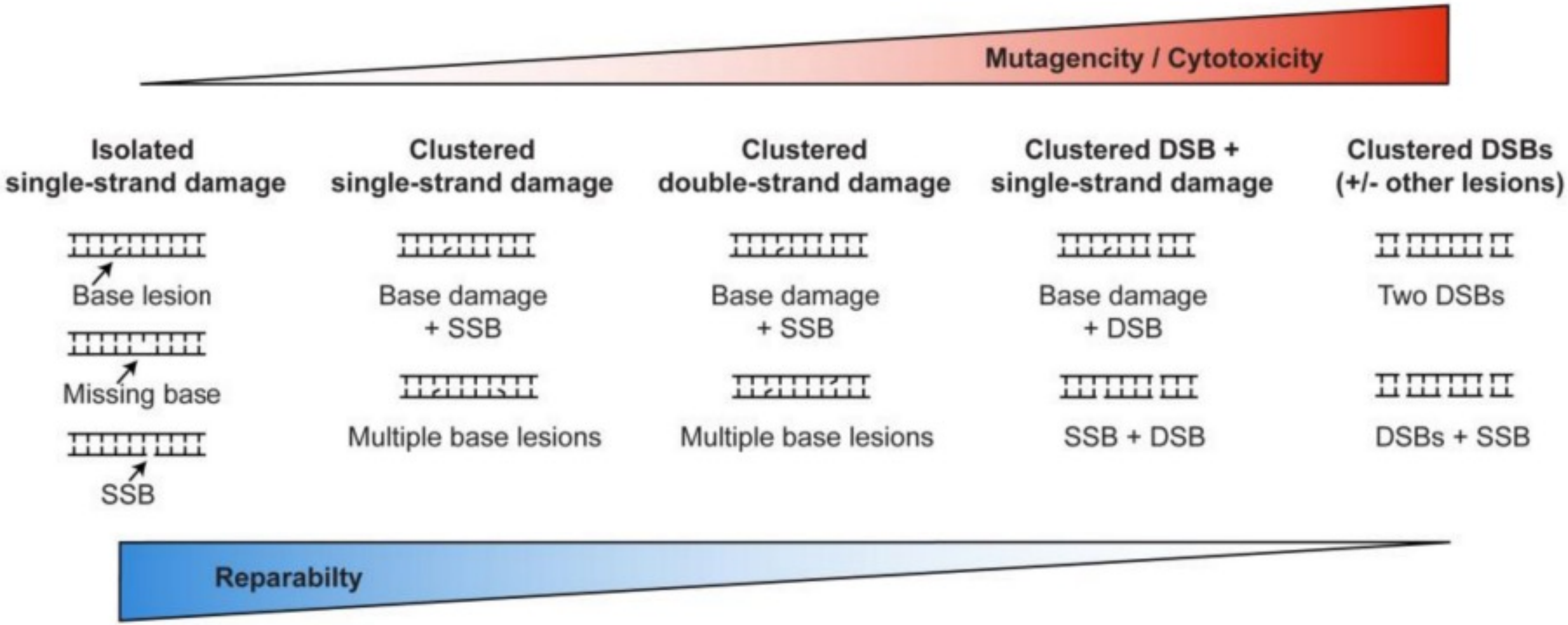


High LET



D Anderson et al 2013 JINST 8 C06008

Desai et al., Rad. Res. 2005



Jac Nickoloff, Genes 2020

What are some types of radiation that you are exposed to?

Chest x-ray

~0.1 mSv

Flight

~0.035 mSv

Ambient Radiation on Earth

~0.007 mSv/day (up to ~0.3 mSv/day)

Food

Carbon-14
Potassium-40

Banana = 1% of daily dose

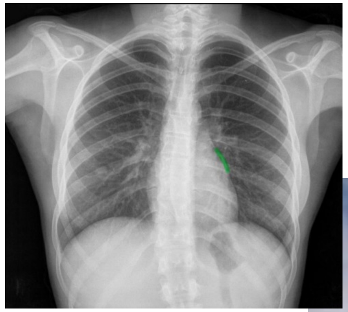
~0.00007 mSv

Airport Scanner

0.0001 mSv

~70 fold less than normal ambient radiation

About equal to ~2 bananas.



How much radiation do you think the astronauts will be exposed to on a trip to Mars?

Chest x-ray	Flight	Ambient Radiation on Earth	Food	Airport Scanner
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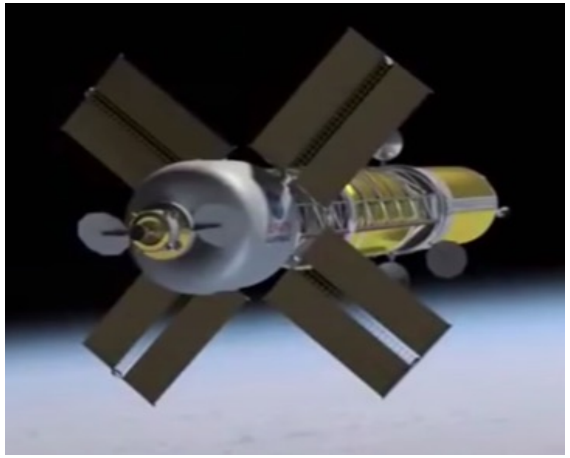
Mars

1.3 mSv/day
(~200X more than on earth)

360-day trip = ~450 mSv
(4,500X more than a chest x-ray)



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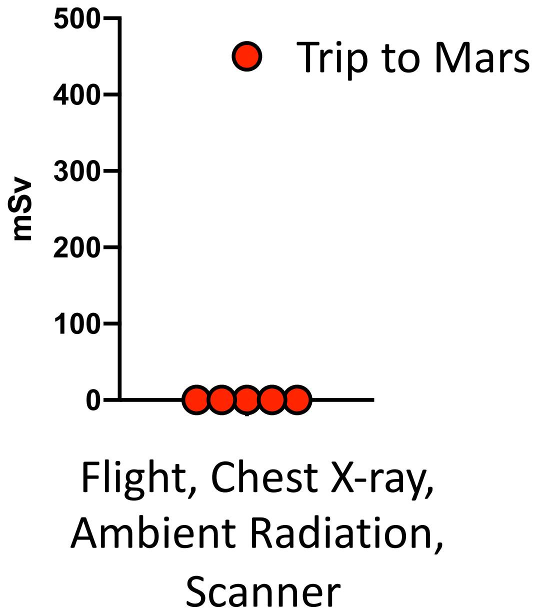
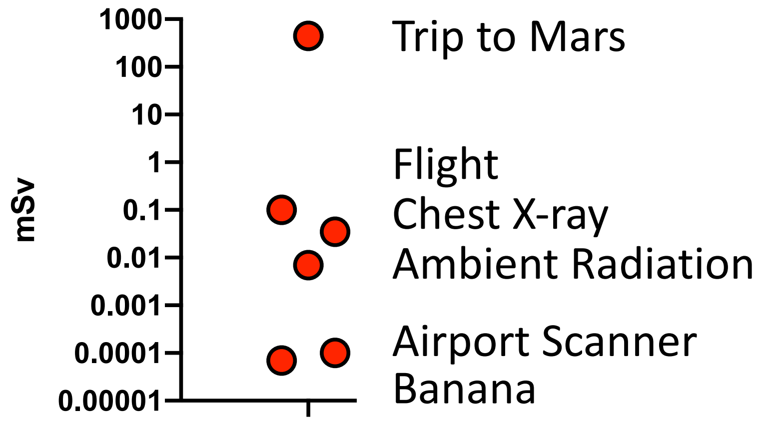


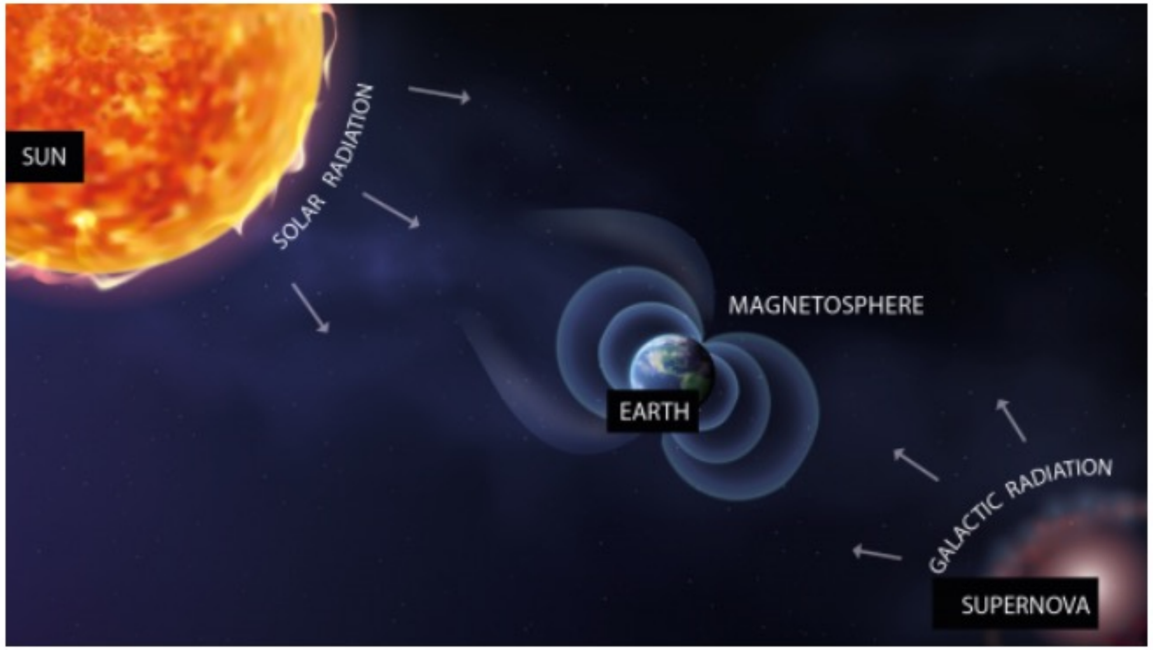


Simulated galactic cosmic rays causes neurological problems in animals.

Male mice:

- anxiety
- reduced social interaction
- impaired memory

Female mice were largely protected from these effects.



Astronauts on the space station benefit from the magnetosphere.

95% of radiation in space is **Galactic Cosmic Rays**

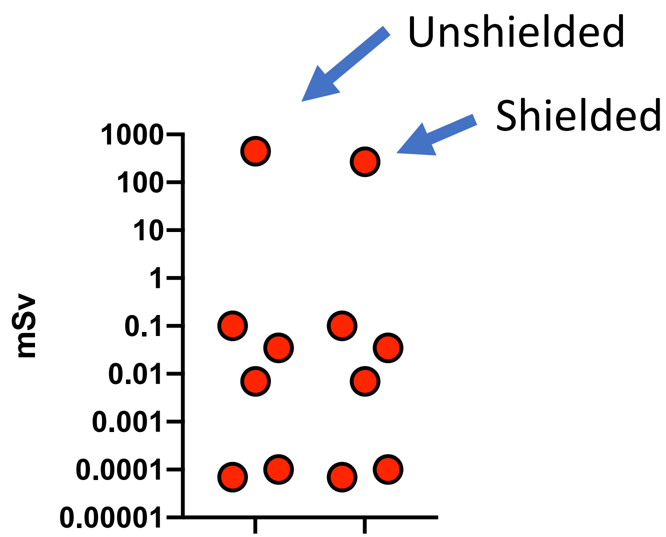
Hard to shield galactic cosmic rays without prohibitively heavy shielding mass.

Feasible shielding does not stop most of the iron particles.

Partially blocking galactic cosmic rays leads to high secondary radiation that is easily absorbed.

Lightweight plasic (FRX1) is made of carbon and hydrogen. It is better than aluminum (50% better at shielding solar flares and 15% better at shielding galactic cosmic radiation).

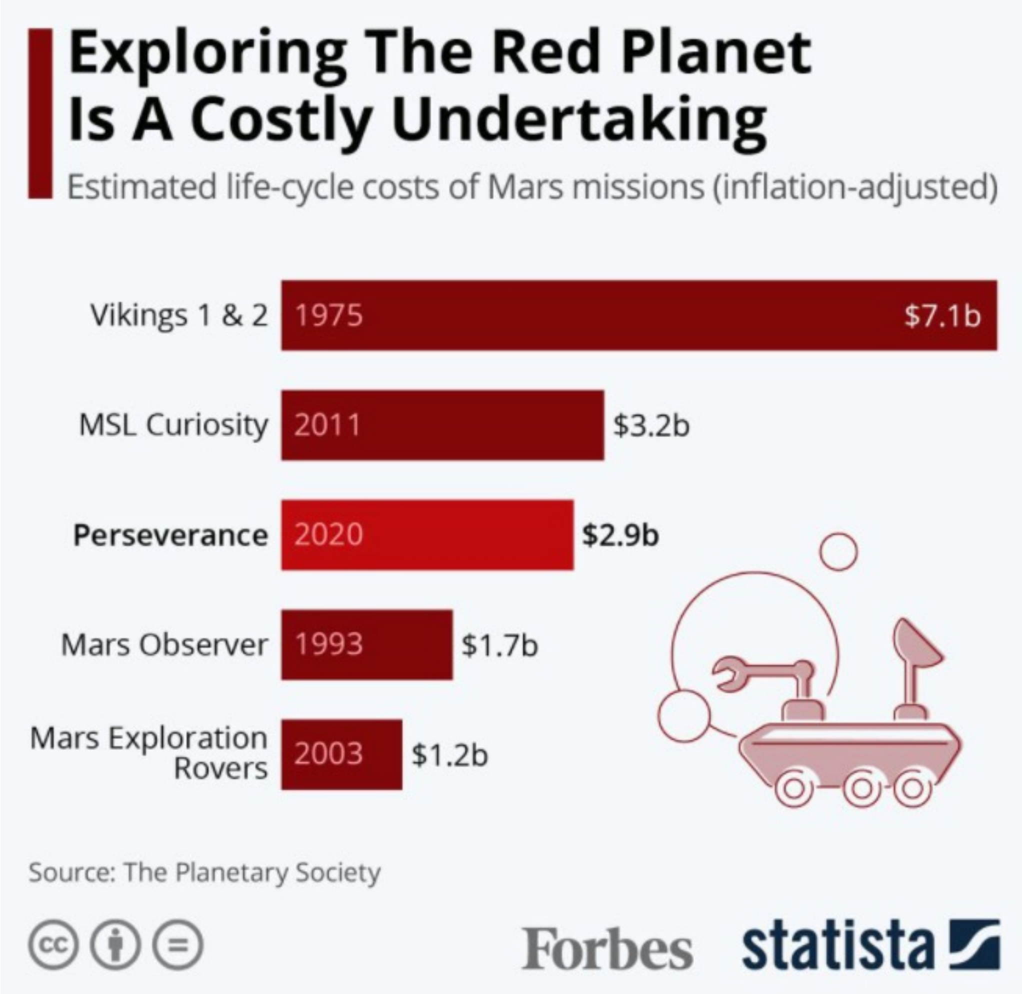
But, shields ~6 cm thick block only 30-35% of the radiation astronauts will experience going to Mars.



NASA is investigating medical and dietary supplements to mitigate ionizing radiation.

Some say that radiation is a major hurdle when it comes to going to Mars.

How does the idea of going to Mars affect our society?



Some say that going to Mars is out of the question due to the radiation problem.

How does the idea of going to Mars affect public health?

Mars One estimates the cost of bringing the first four people to Mars at US$ 6 billion.

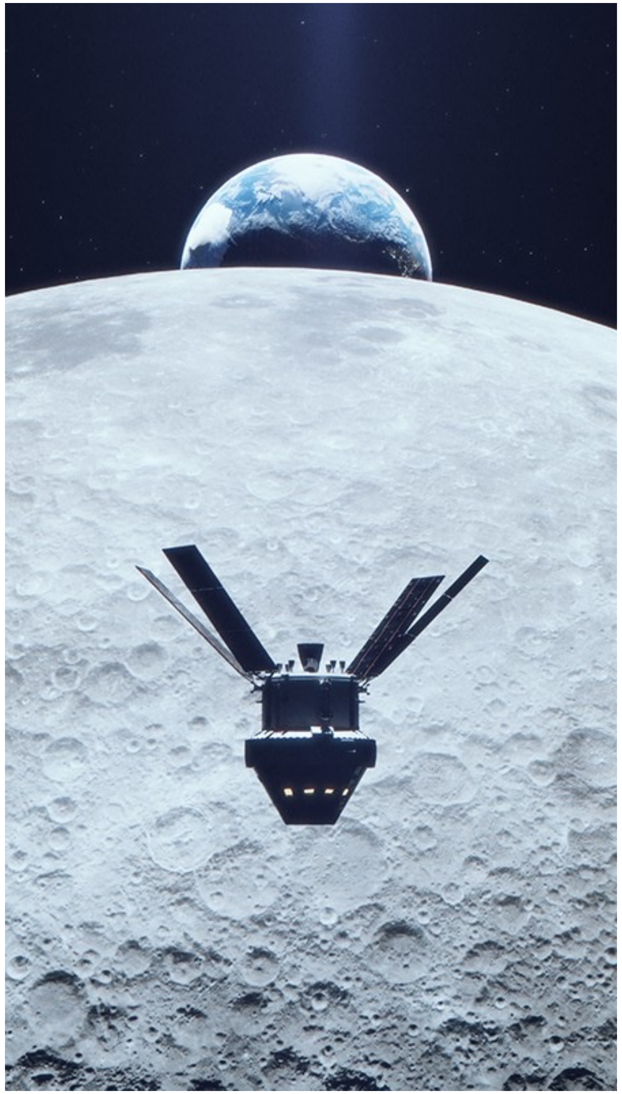
Washington, DC—For its second major grant announcement of fiscal year 2021, the National Endowment for the Arts announces **more than $88 million** in recommended grants to organizations in all 50 states and jurisdictions.

This is 70X less than what we are spending to go to Mars.

NEWS FEATURE | 11 May 2022

The $93-billion plan to put astronauts back on the Moon

The world's most powerful rocket will make a trip around the Moon in 2022 – a step towards landing people there in 2025, and part of the US Artemis programme.



Today’s News!

NASA's Artemis 1 moon rocket launch hinges on critical fueling test on Sept. 21

By Tariq Malik published about 17 hours ago

The test will check fuel leak fixes and a "kinder and gentler" fueling process.

NASA’s Space Launch System (SLS) rocket is seen at Launch Pad 39B Thursday, Sept. 8, 2022, at NASA’s Kennedy Space Center in Florida as teams work to replace the seal on an interface, called the quick disconnect, between the liquid hydrogen fuel feed line on the mobile launcher and the rocket. (Image credit: NASA/Chad Siwik)

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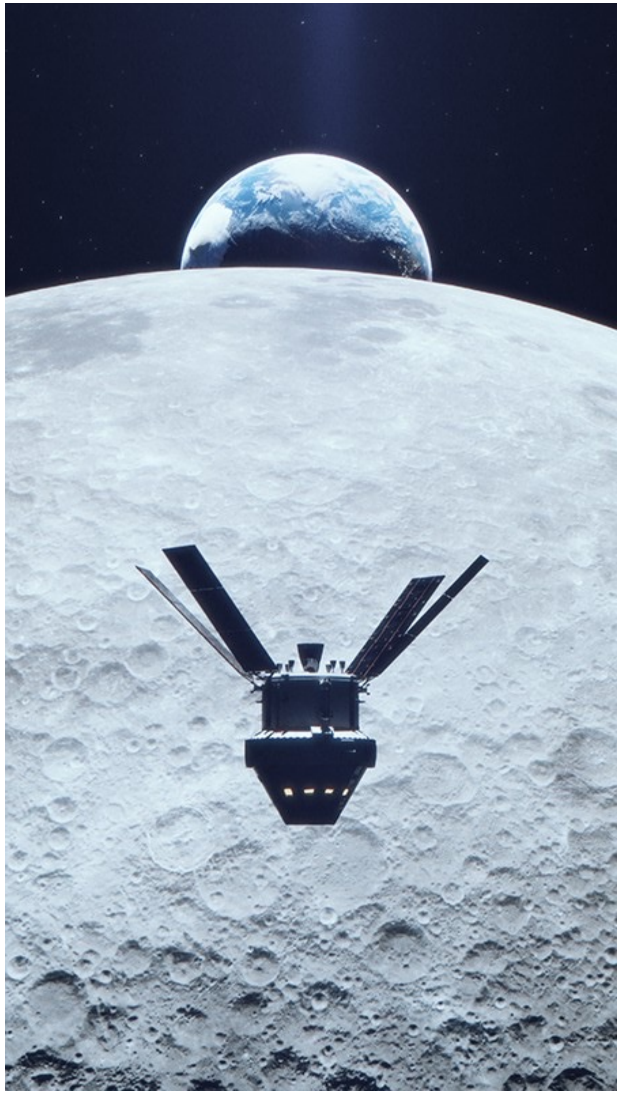

President Joe Biden speaks during an event at the White House.

Biden's budget calls for $56 billion to fight climate change.

Dreams of living on Mars are having a direct impact on Public Health

A careful look at the major steps of BER

γH2AX as a Marker of DNA Damage

Interlude