20.109 MOD1
Measuring Genomic Instability

Fall 2023
Day 3

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Professor of Biological Engineering

Previous lecture -

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

Overview of the steps of BER

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

How Double Strand Breaks are Shaping History

Drawing the DNA Bases

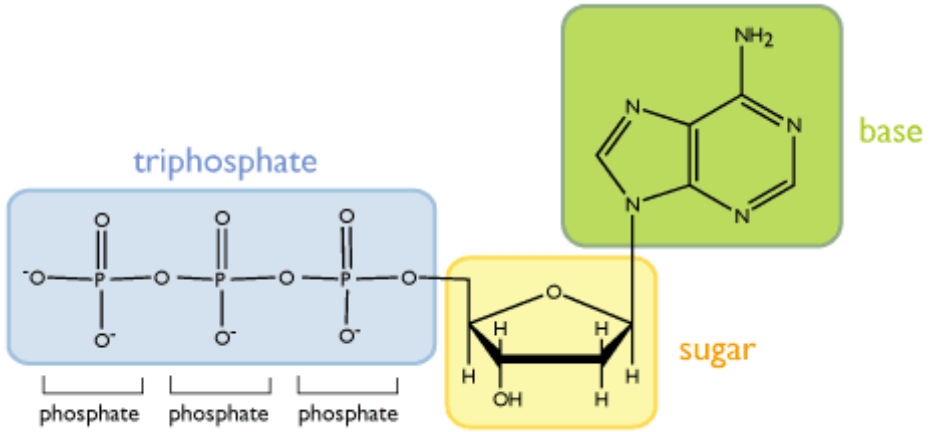
(On the board)

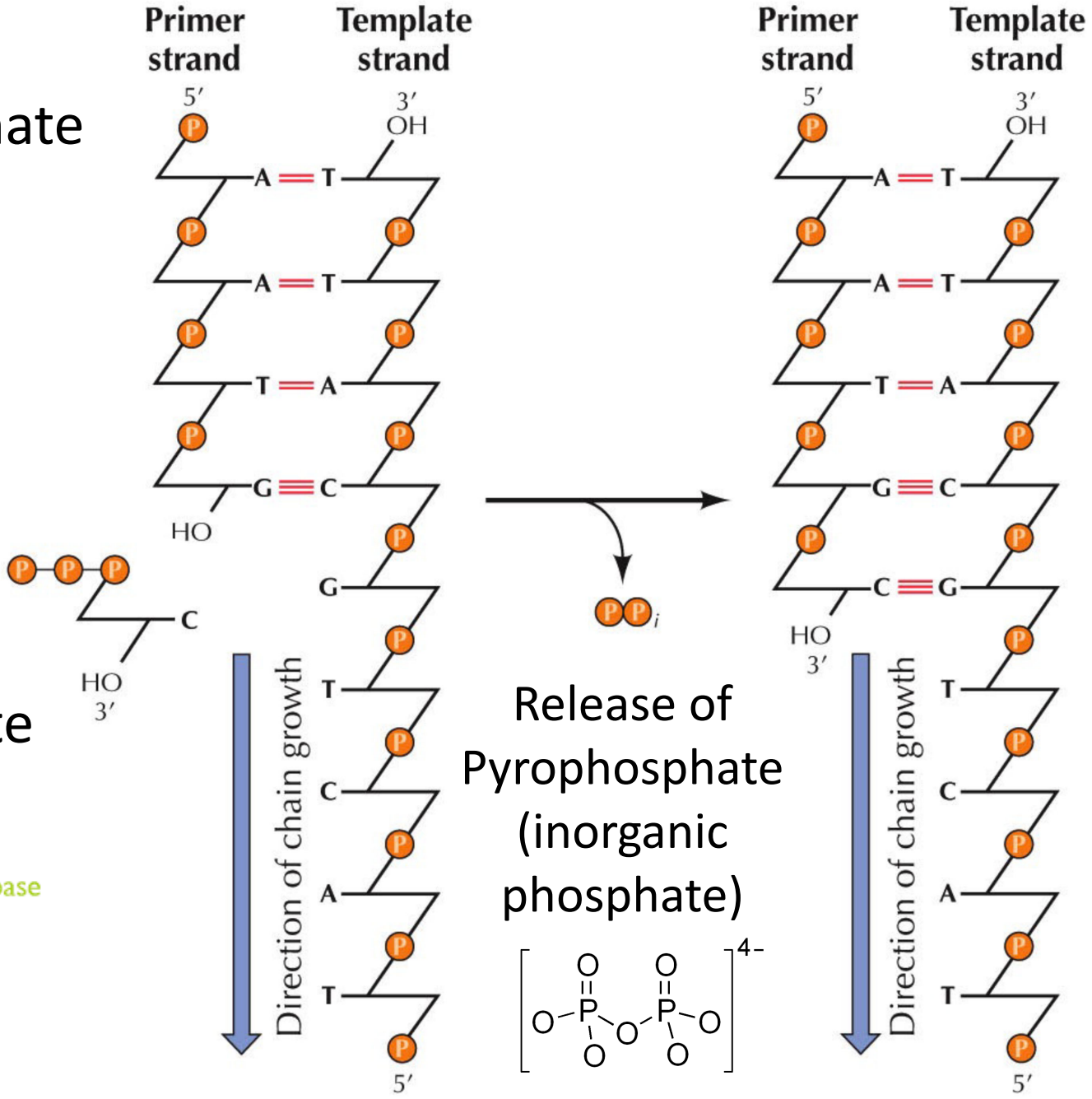
Chemistry of Nucleotide Addition

5’Phosphate

3’Hydroxyl

Nucleotide triphosphate



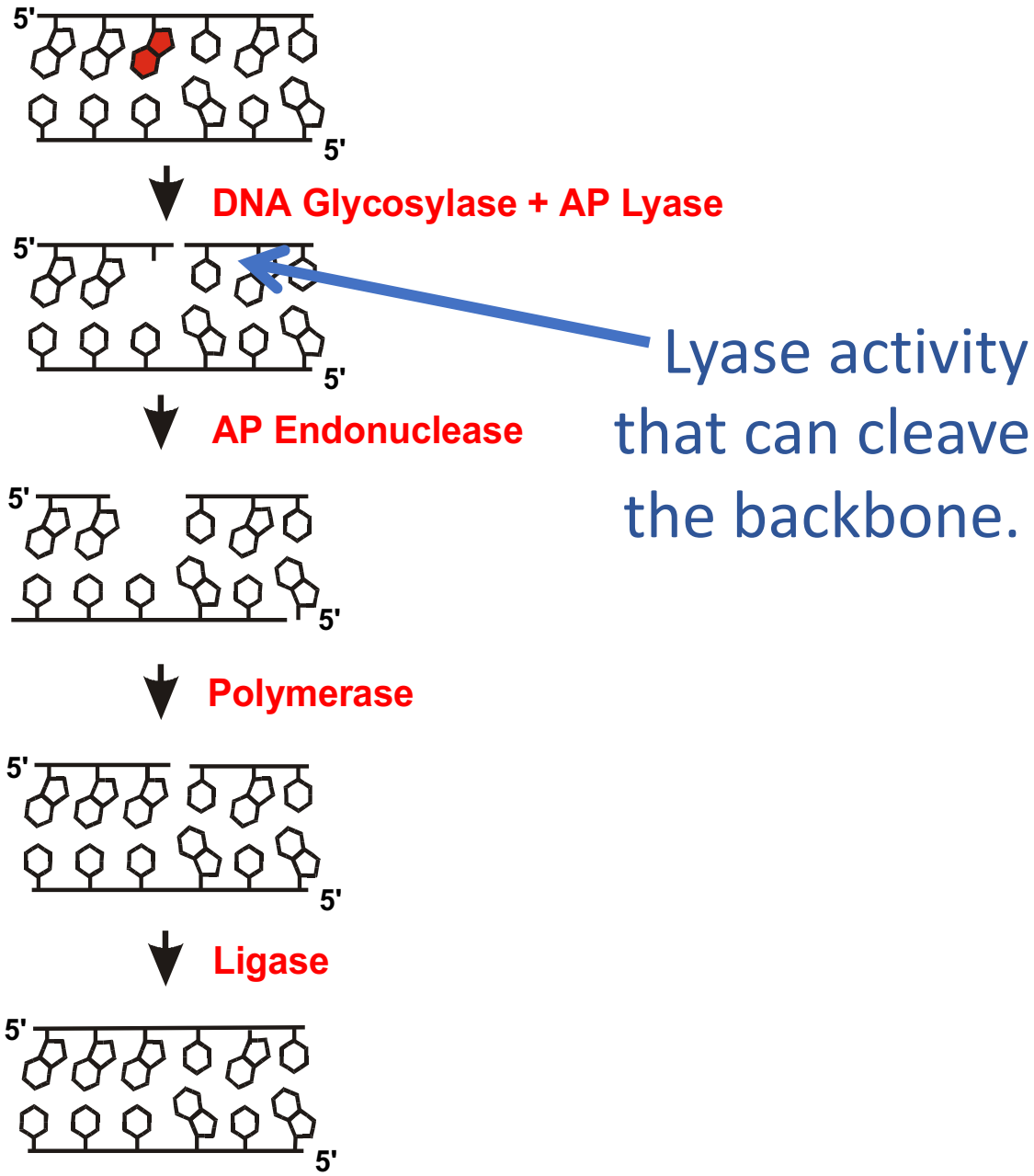


Base Excision Repair (BER)

Base Excision Repair

8-oxoguanine DNA Glycosylase (OGG1)

Removes the damaged base by cleaving the glycosylic bond.

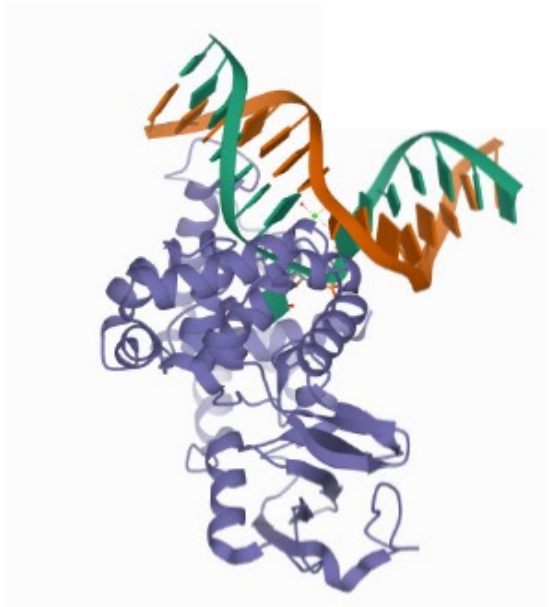


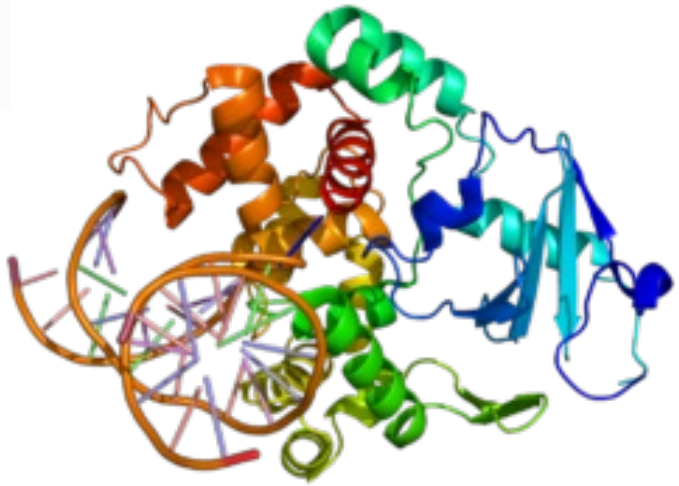
8-oxoguanine DNA Glycosylase (Ogg1)

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

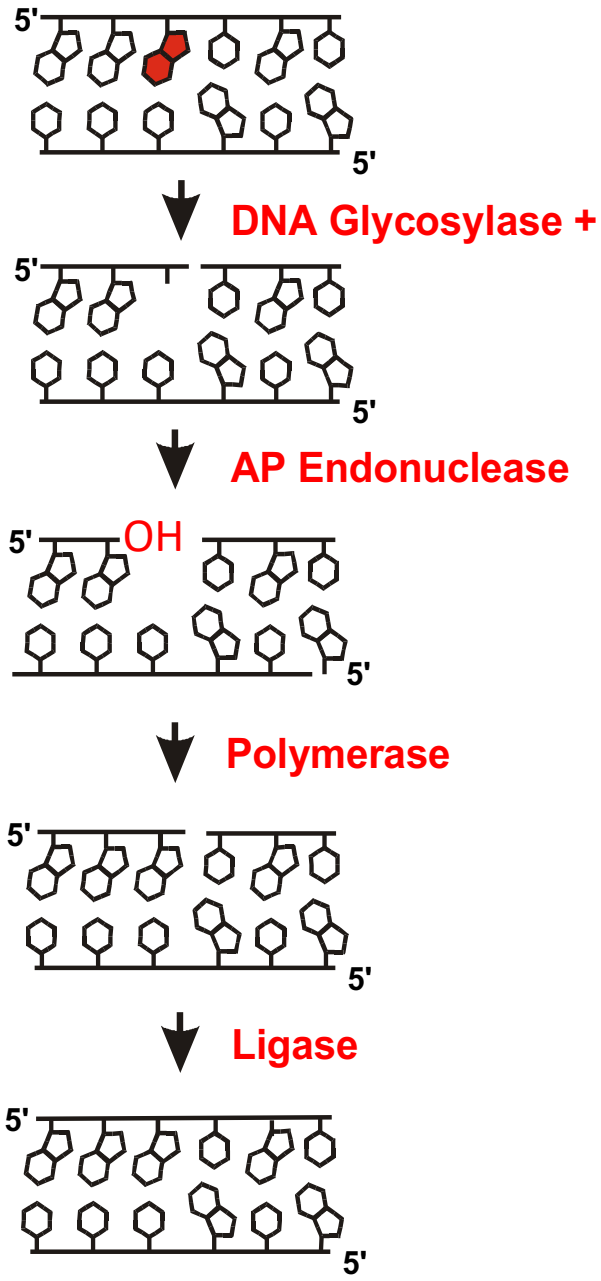
In some cases, the risk is > 15X Higher

Base Excision Repair

AP Endonuclease

"Cleans the end" (removes the abasic sugar)

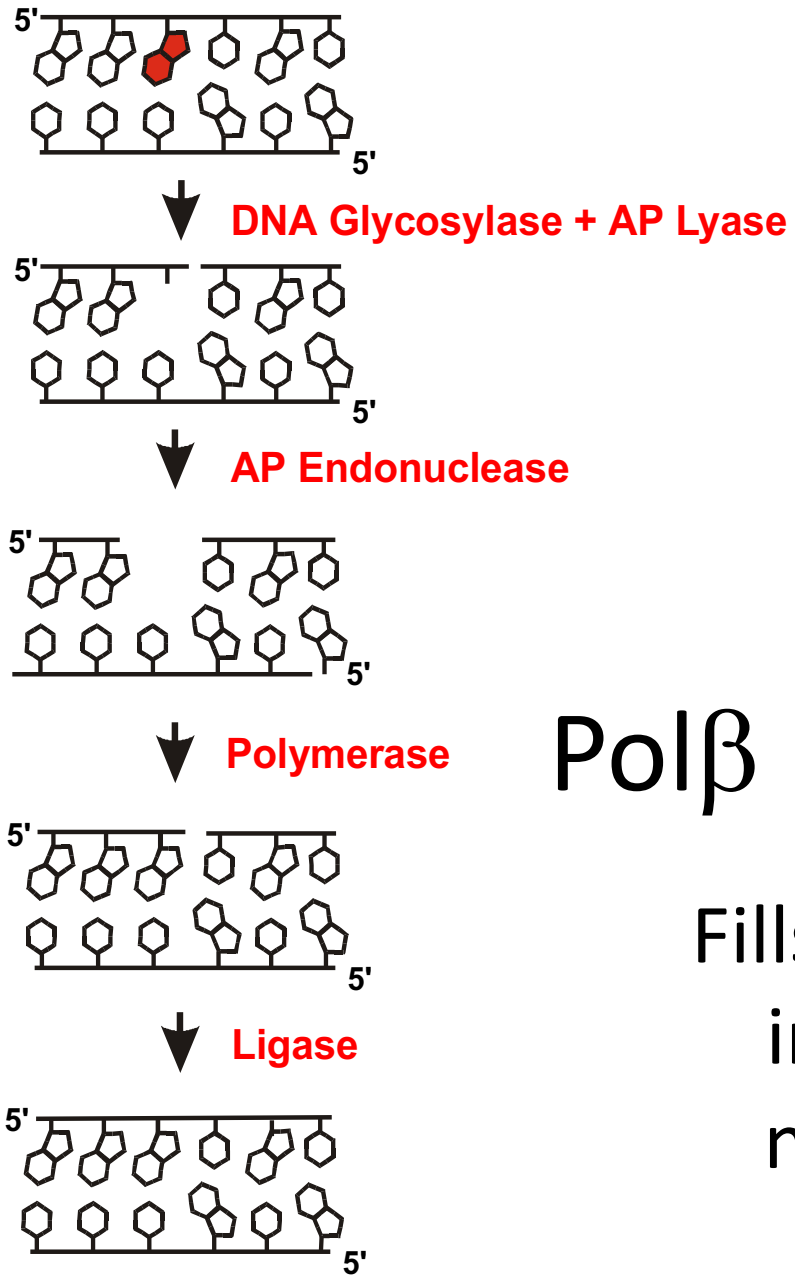
Creates a 3'OH that can be extended.



Single Nucleotide Addition by Polβ

Base Excision Repair

DNA Polymerase Beta

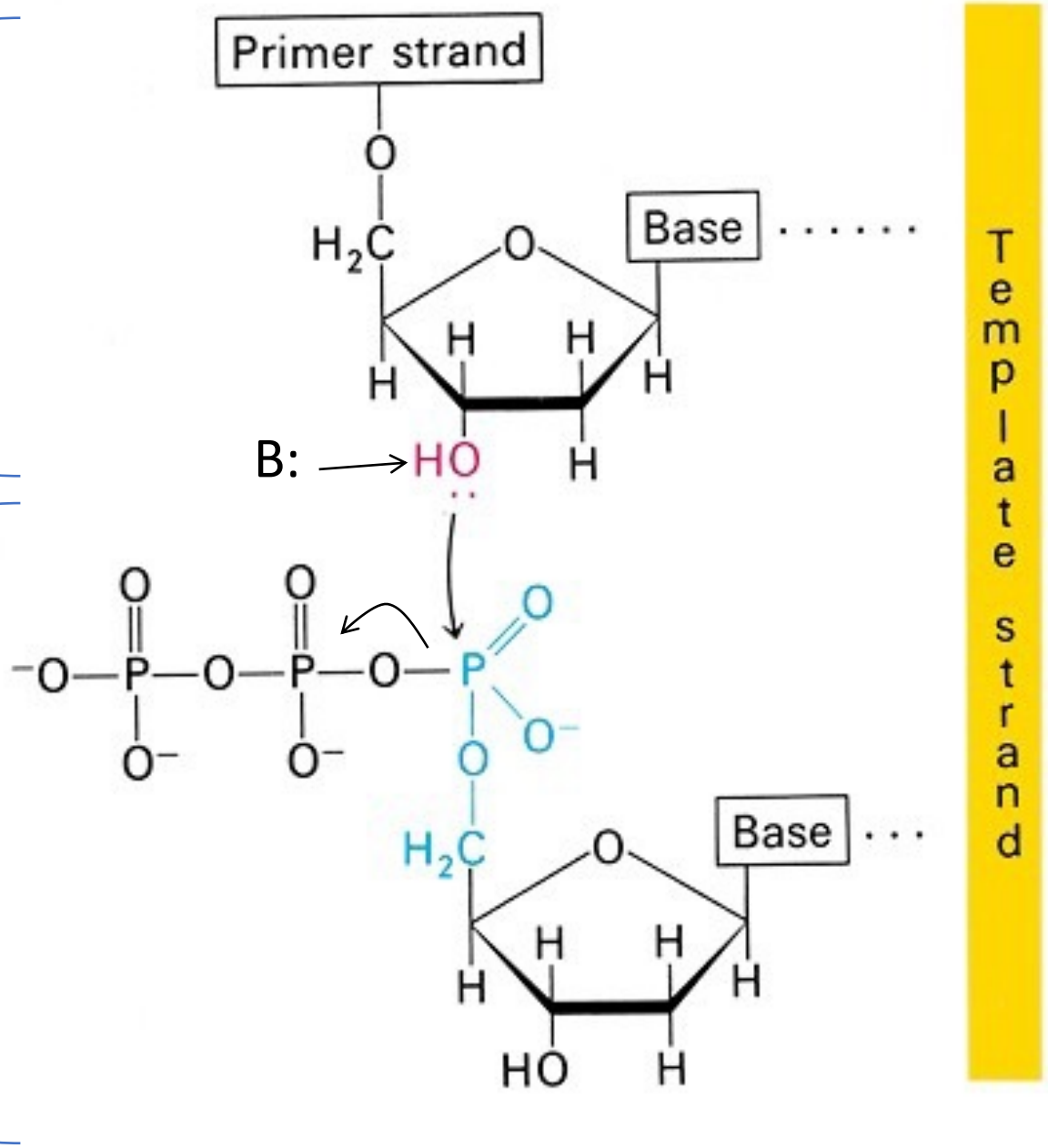


Polβ

Fills the gap by inserting a nucleotide

3’ End

Incoming Nucleotide Triphosphate



DNA Polymerase Beta (Polβ)

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

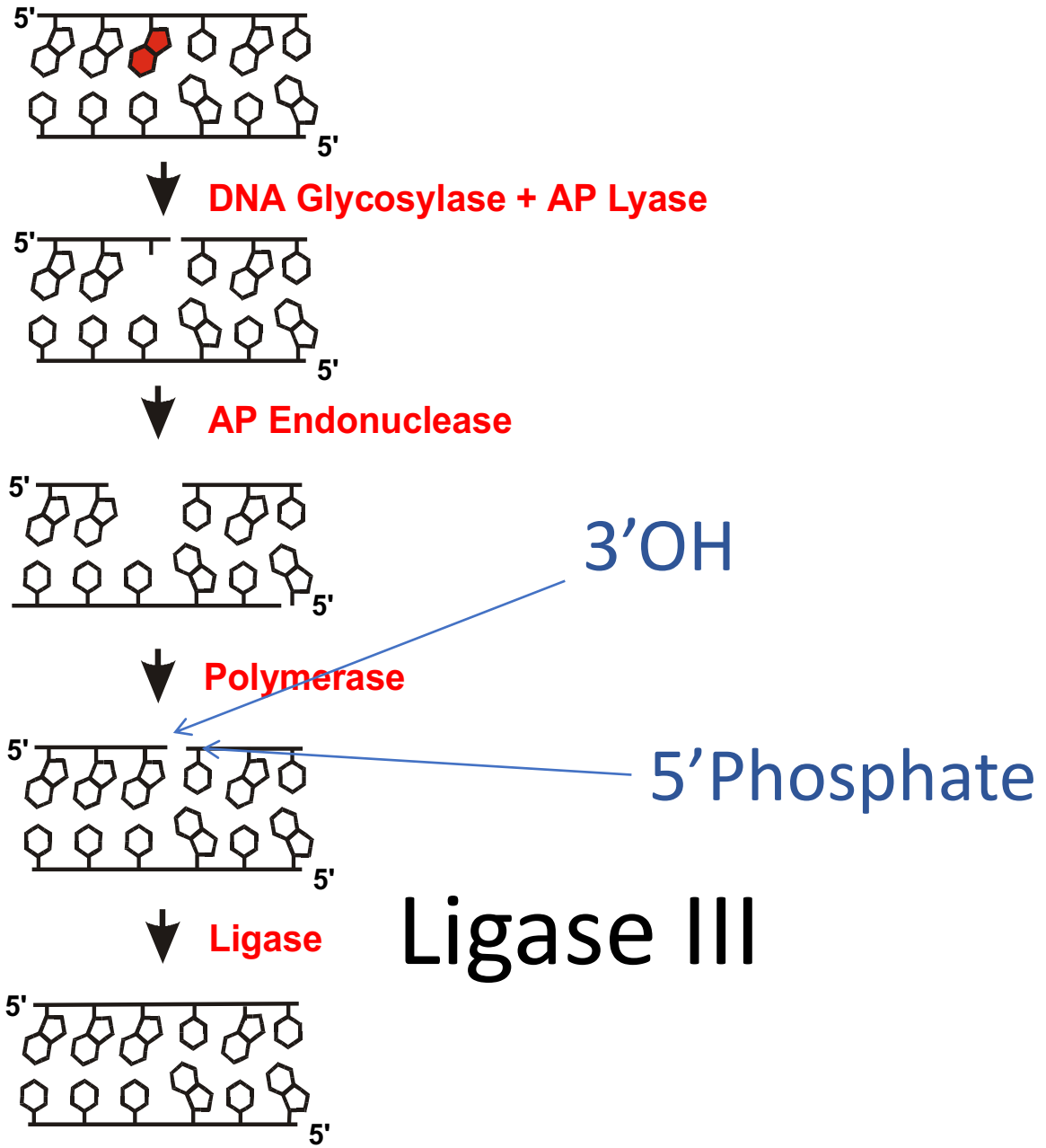
- Autoimmune disease
- Fatigue
- Fever
- Joint pain, stiffness and swelling
- Butterfly-shaped rash on the face
- Skin lesions that appear or worsen with sun exposure
- Fingers and toes that turn white or blue when exposed to cold or during stressful periods
- Shortness of breath
- Chest pain
- Dry eyes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

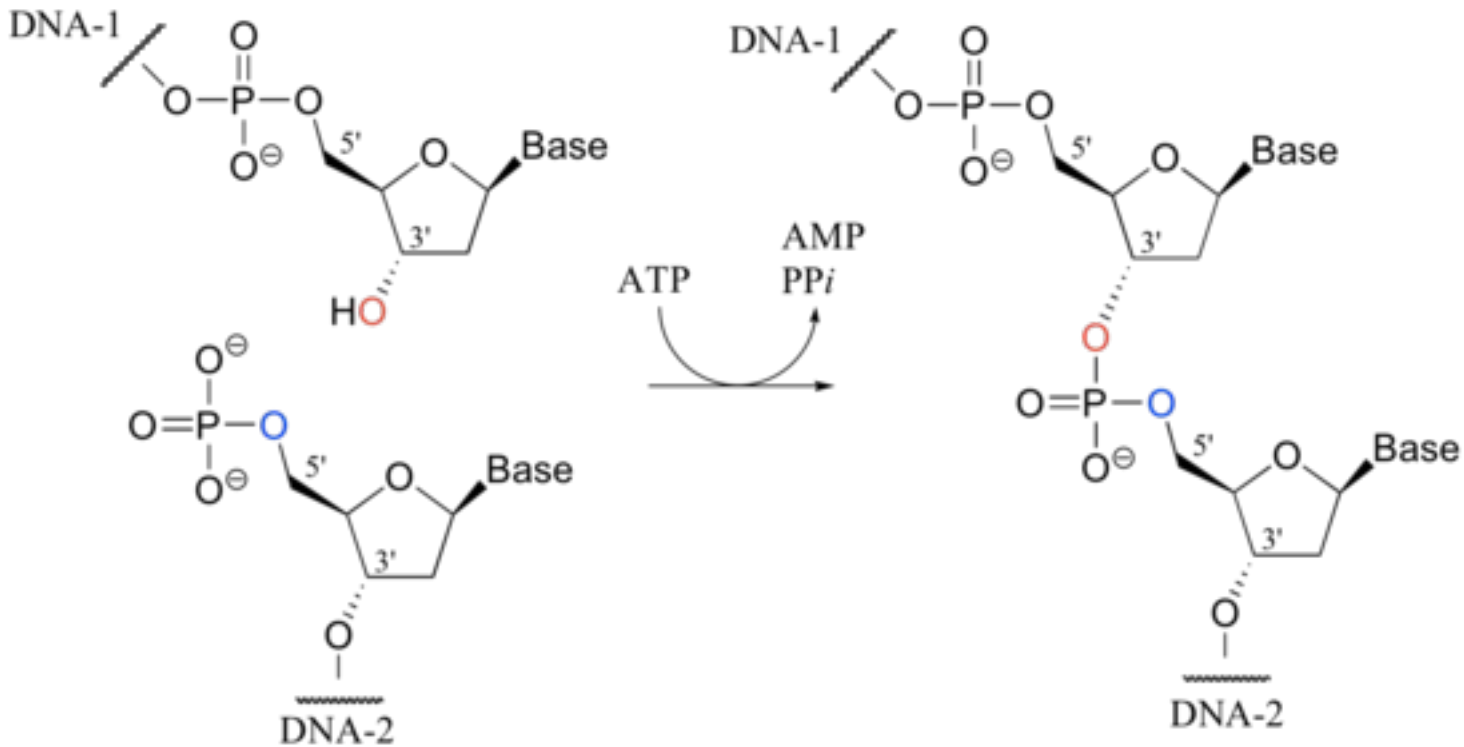
DNA Ligase III

Base Excision Repair

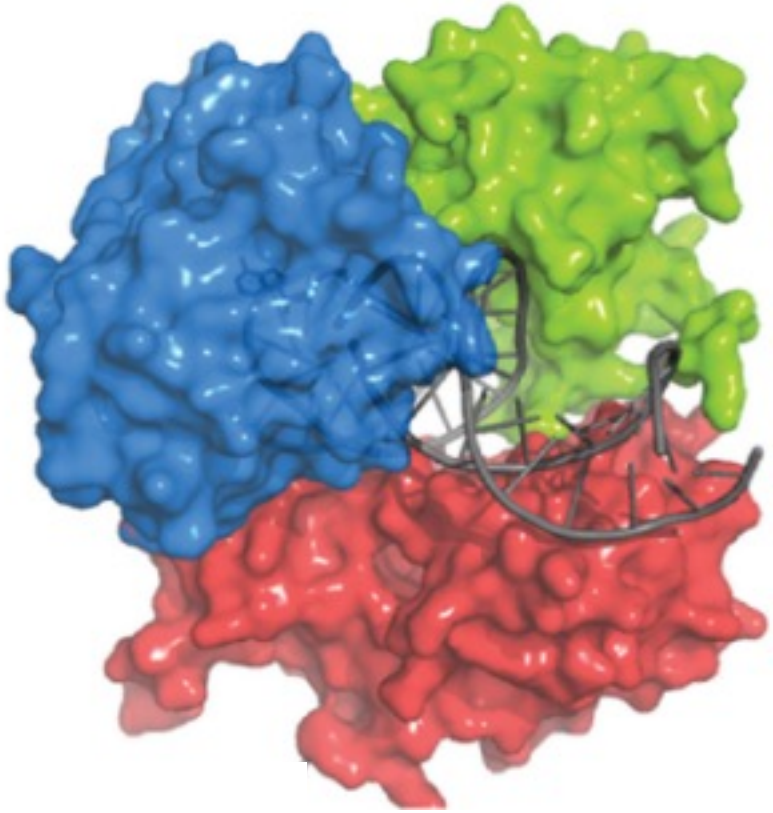
DNA Ligase III

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



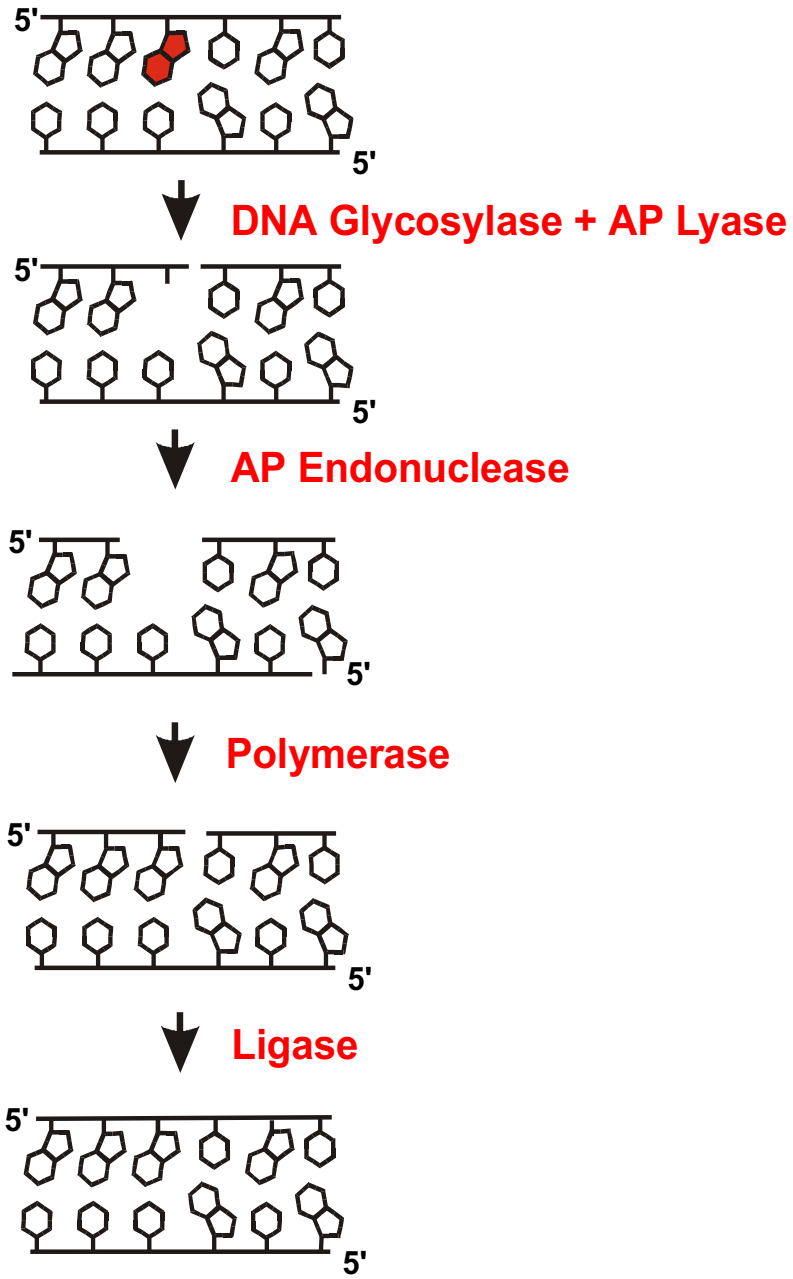

DNA-1
O
O–P–O
O⊖
5'
O
Base
3'
HO
O⊖
O=P–O
O⊖
5'
O
Base
3'
O
DNA-2
ATP
AMP
PPi
DNA-1
O
O–P–O
O⊖
5'
O
Base
3'
O
O=P–O
O⊖
5'
O
Base
3'
O
DNA-2

Ligase III



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

Base Excision Repair



Beautiful Pristine DNA!

PARP

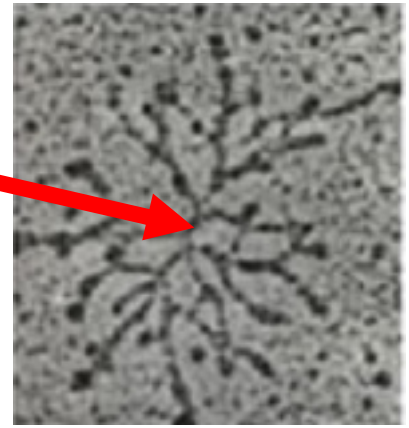
Poly(ADP-Ribose) Polymerase

PARP is a BER “Helper”

Accelerates BER

PARP Automodification Creates a Branched Structure

PARP is in the middle

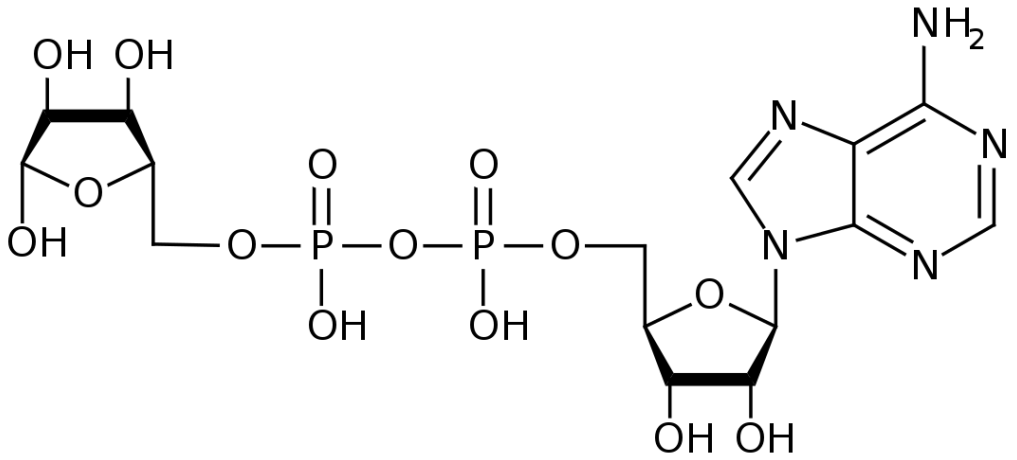


PARP Binds to Single Strand Breaks

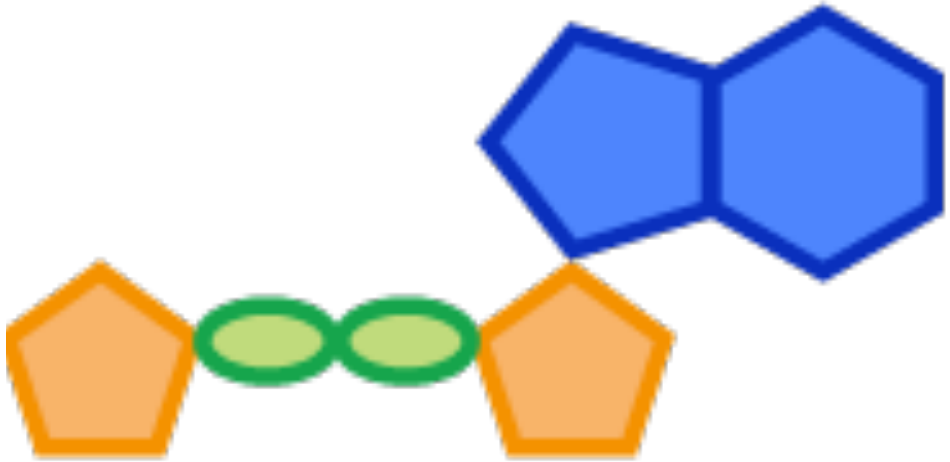
Once bound, it is activated to create PAR

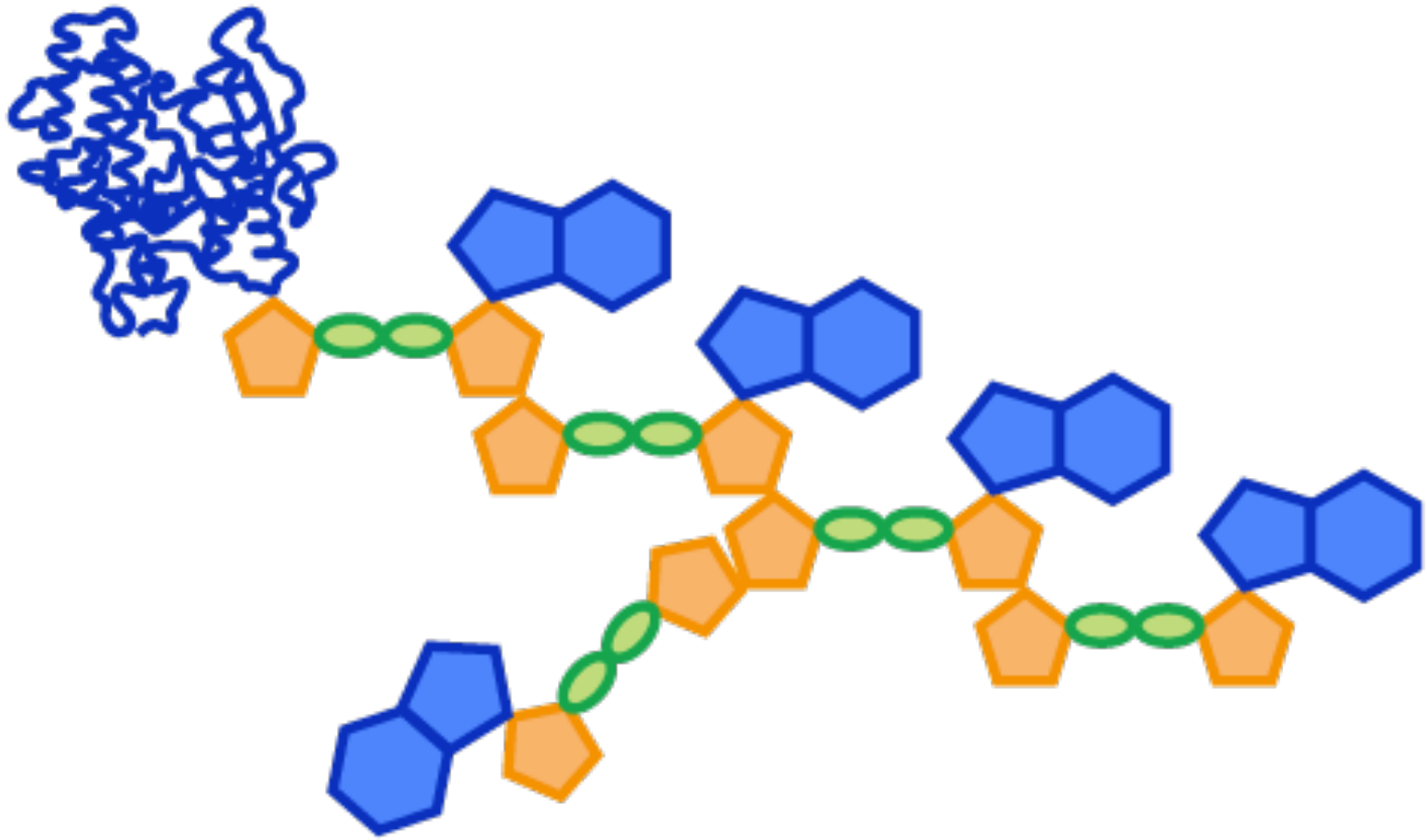
Poly(ADP)-Ribose is made from ADP-ribose

ribose ADP

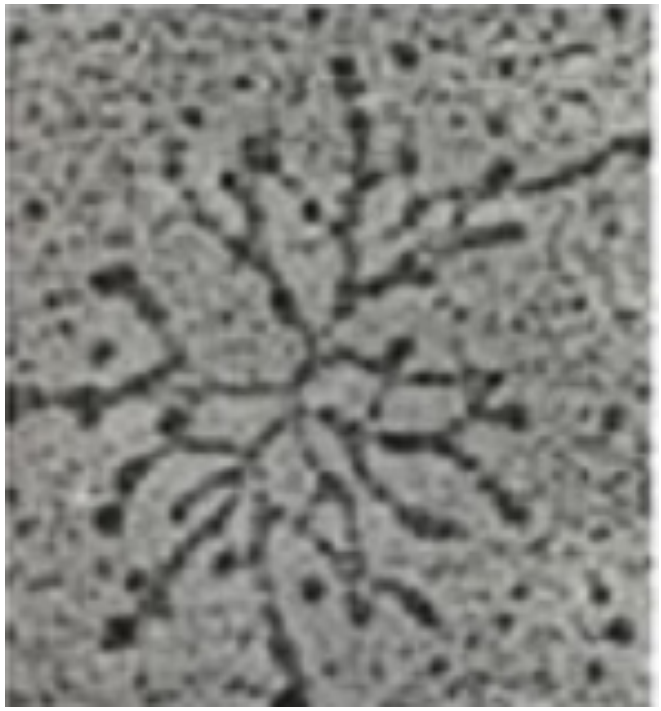


ADP-ribose





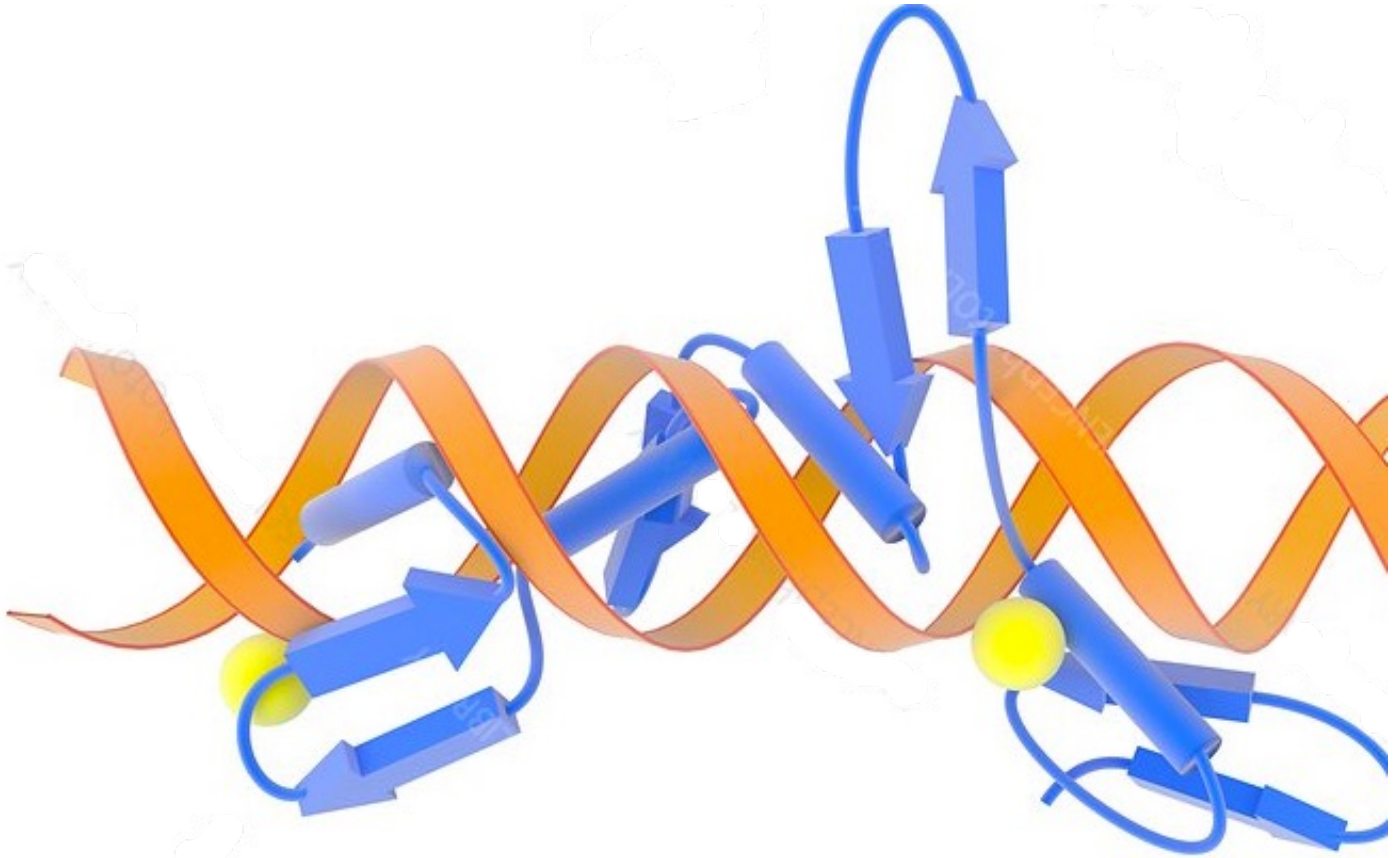
SSB-induced Poly(ADP-Ribose) [Parylation]



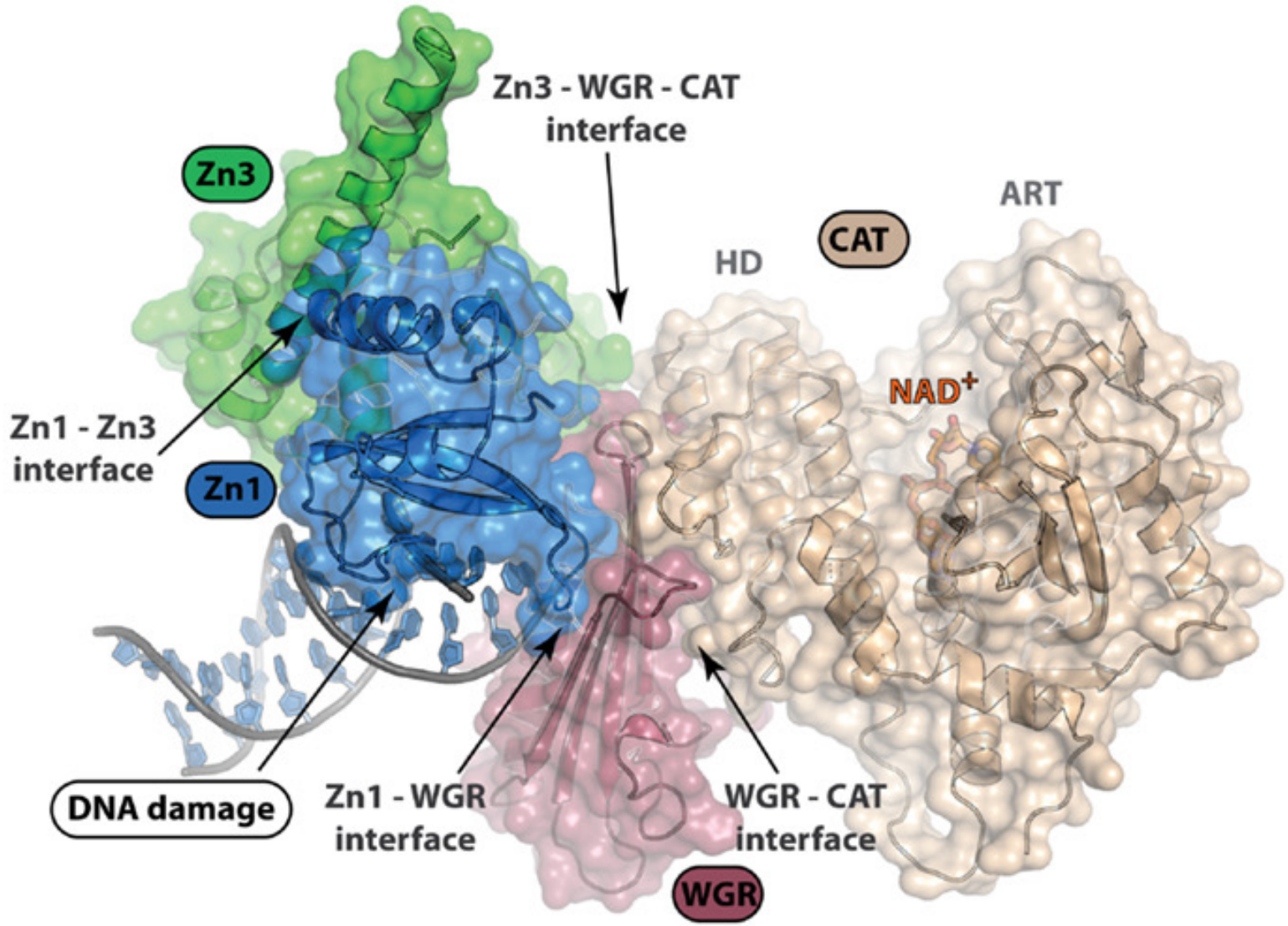
BER Components
Interact with PAR

PAR Recruits
XRCC1 – Scaffold
Polβ
Ligase III

Zinc Fingers Interact Tightly with DNA

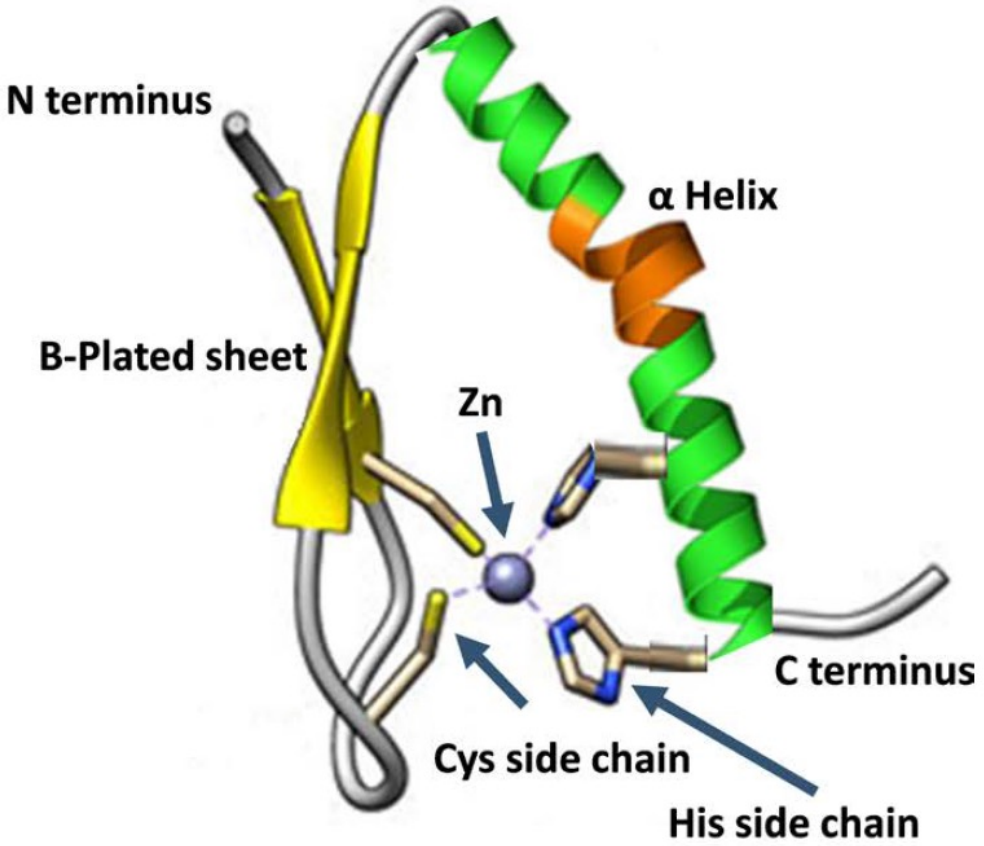


PARP has Zinc Fingers

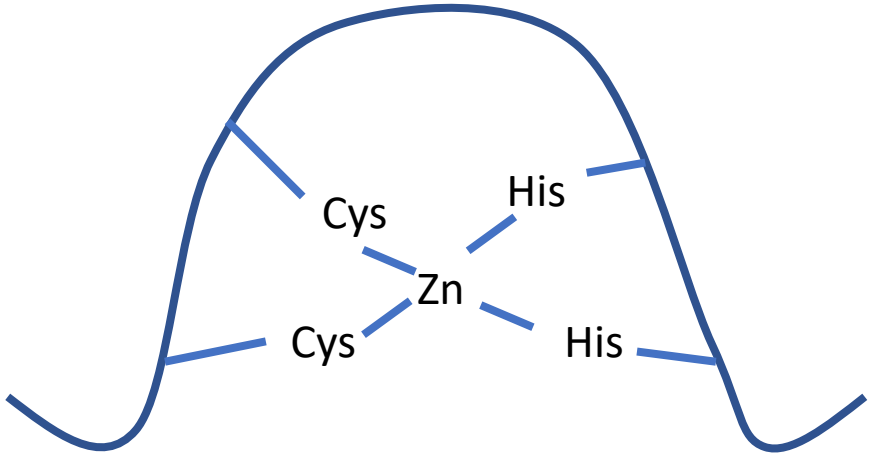


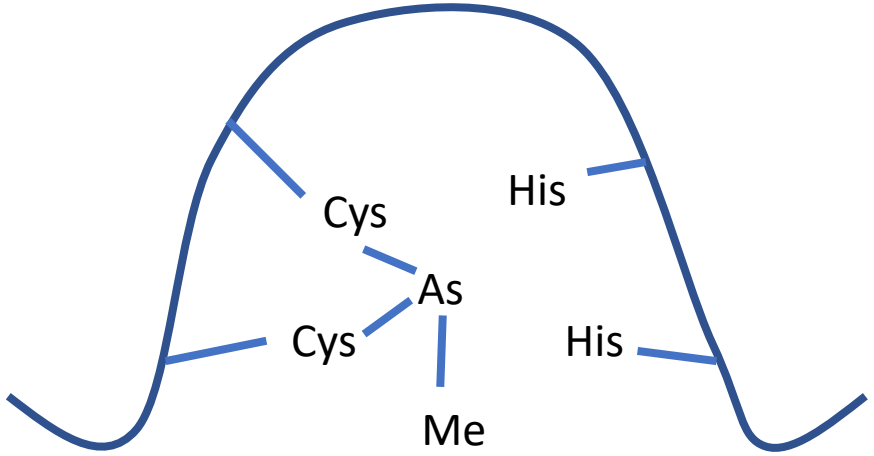
Jamin, Brody, Armen, Pascal

Zinc Fingers have Amino Acids that Bind Zinc



Arsenic Disrupts Zinc Fingers

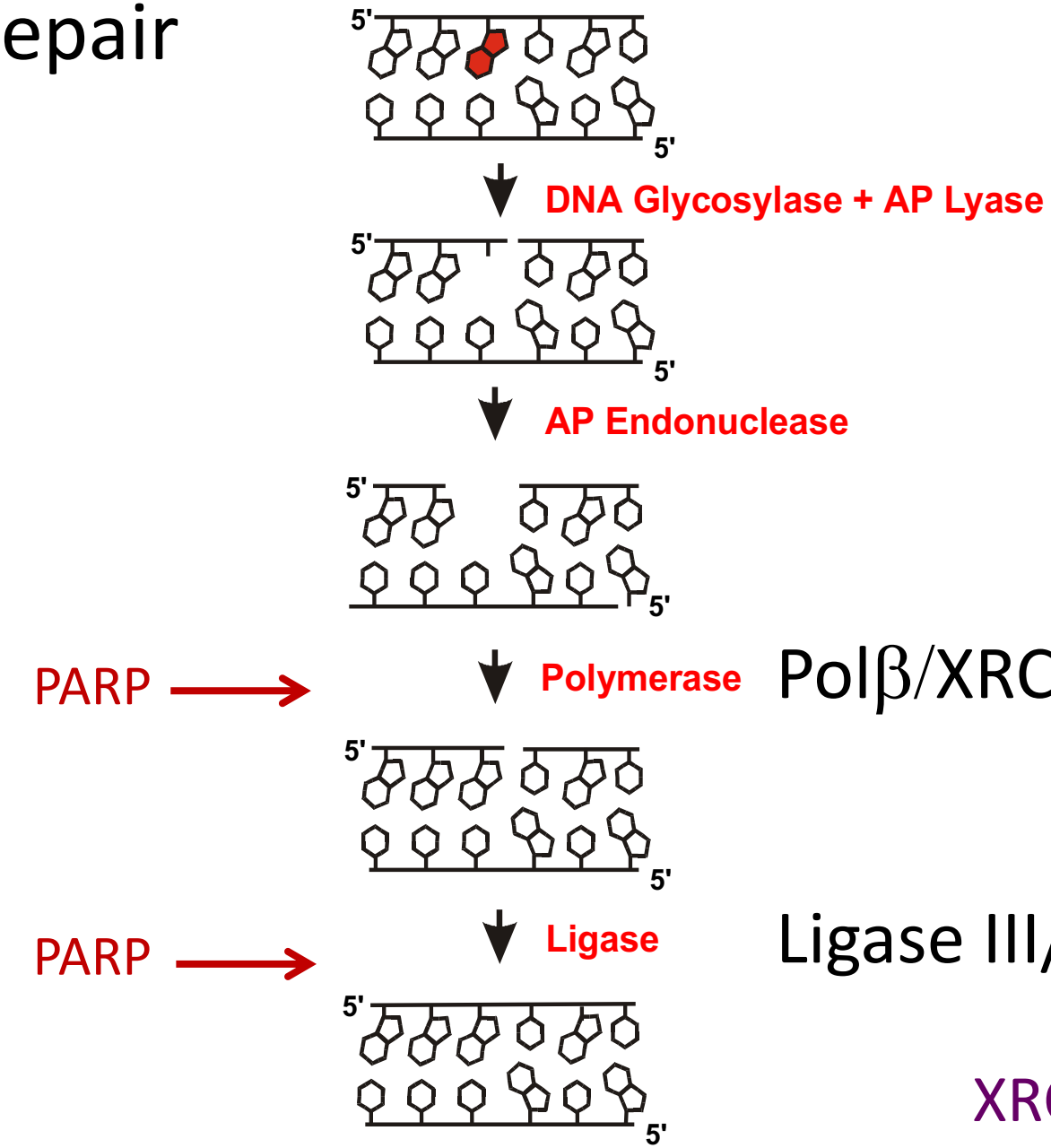




Base Excision Repair

For you to figure out:

Which BER components bind directly to PAR?



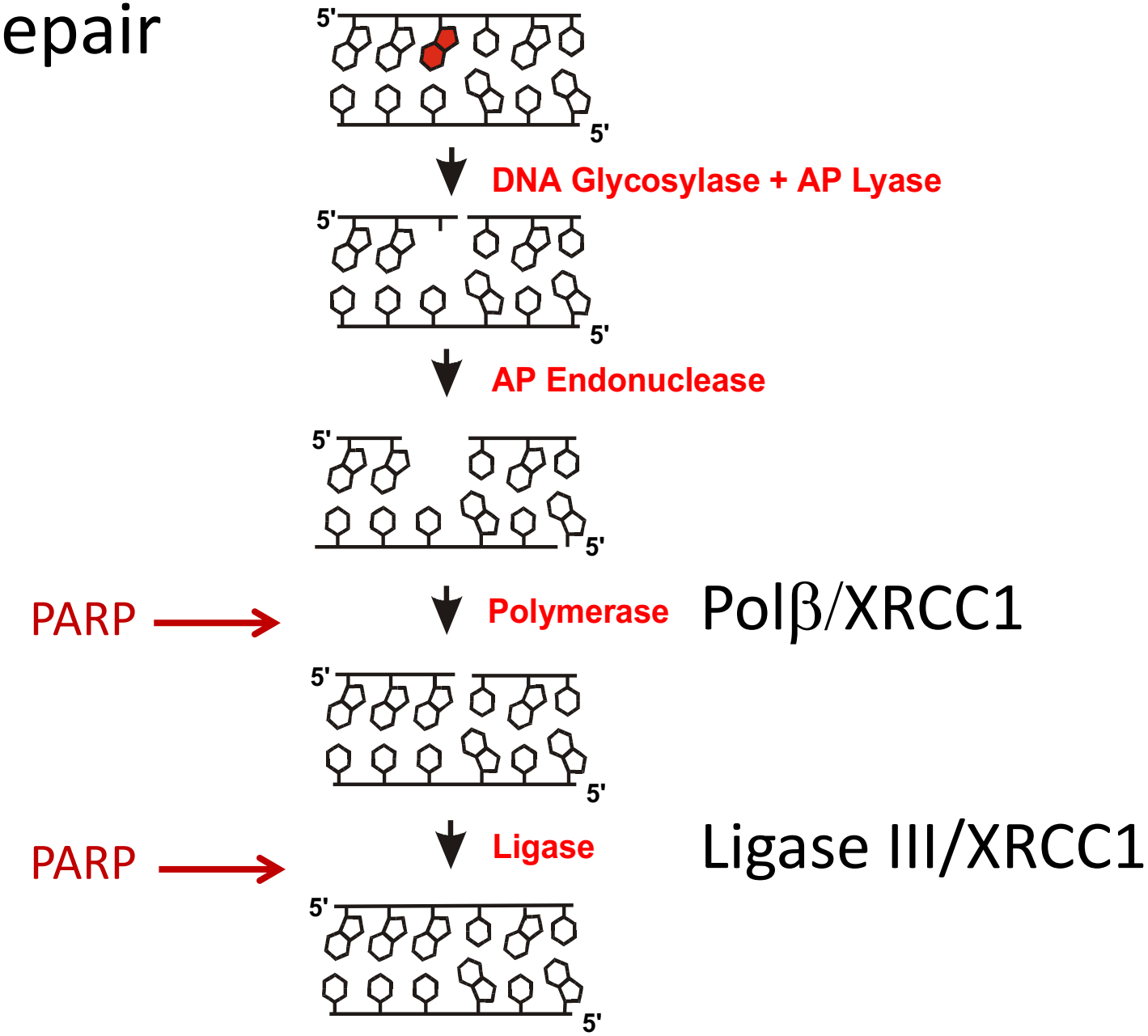
XRCC1 = A Scaffold

Replacement of Zinc with Arsenic Changes the Structure of PARP

Arsenic leads to PARP inhibition

PARP inhibition slows BER

Base Excision Repair

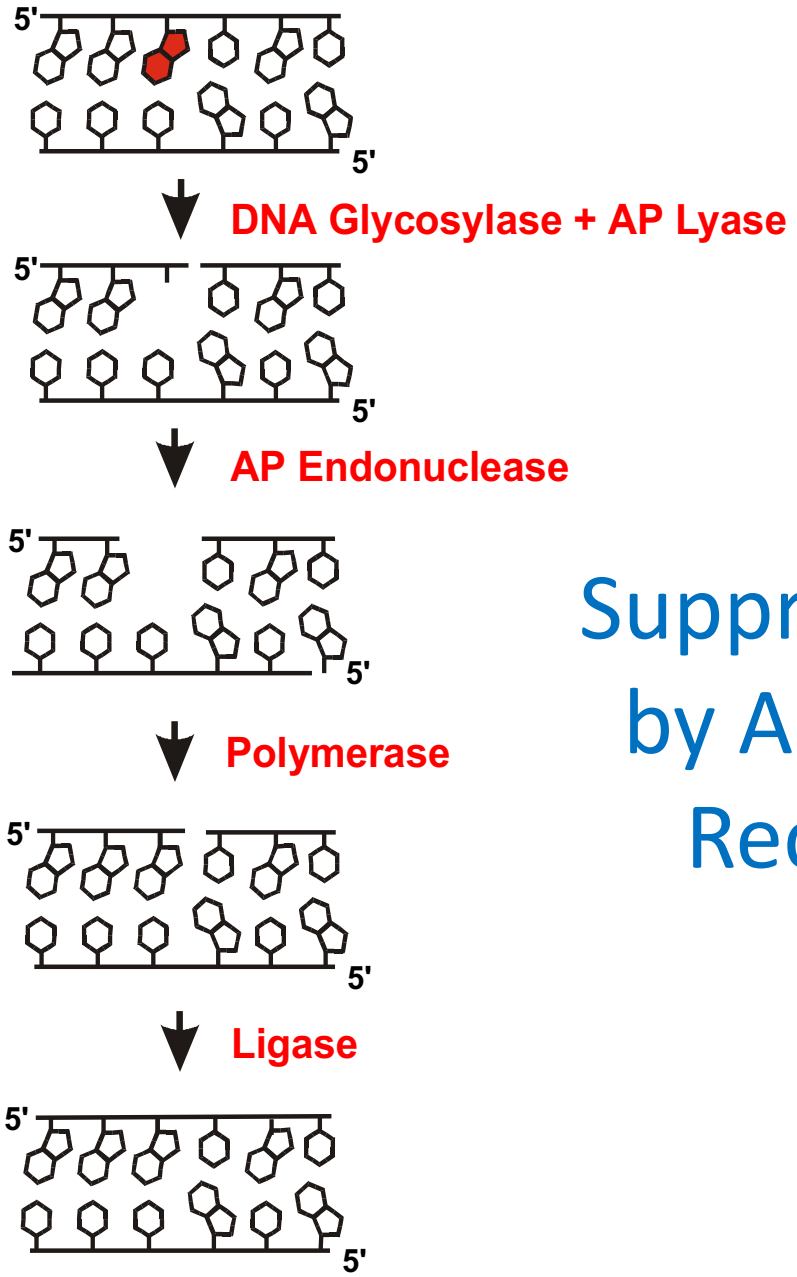


As Inhibits SSB Repair

As displaces zinc, disrupting zinc fingers in PARP

As ⊣ PARP →

As ⊣ PARP →



Suppression of PARP by As May Reduce 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 beta, Ligase III and XRCC1

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

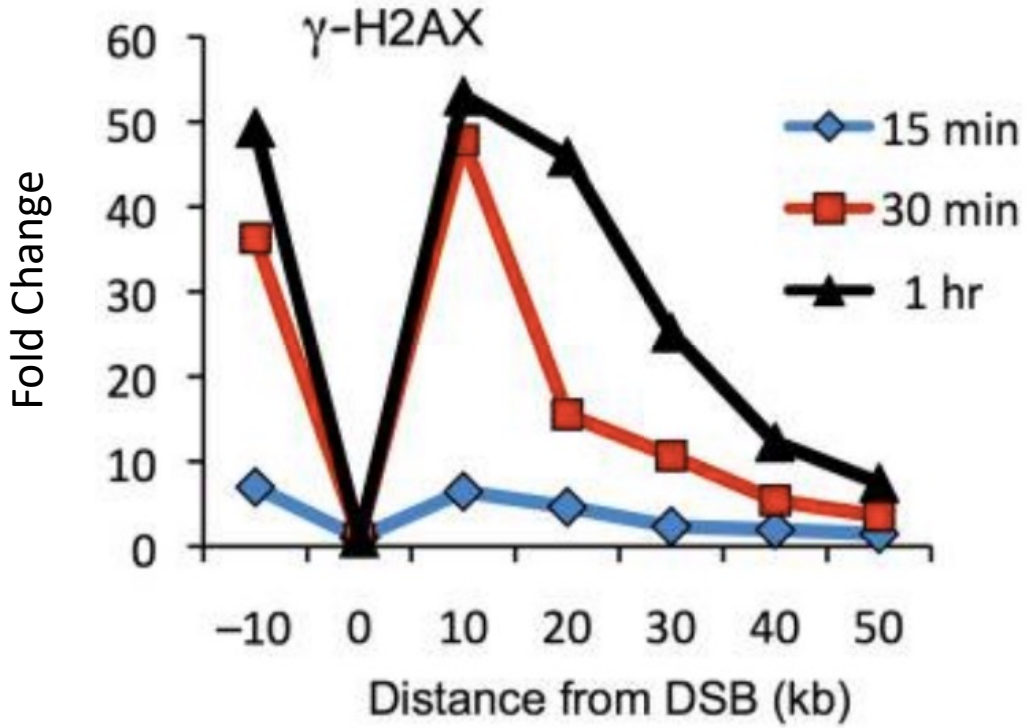
- PARP serves as a beacon to recruit BER enzymes
- PARP has a zinc finger and is inhibited when As replaces Zn

A careful look at the major steps of BER

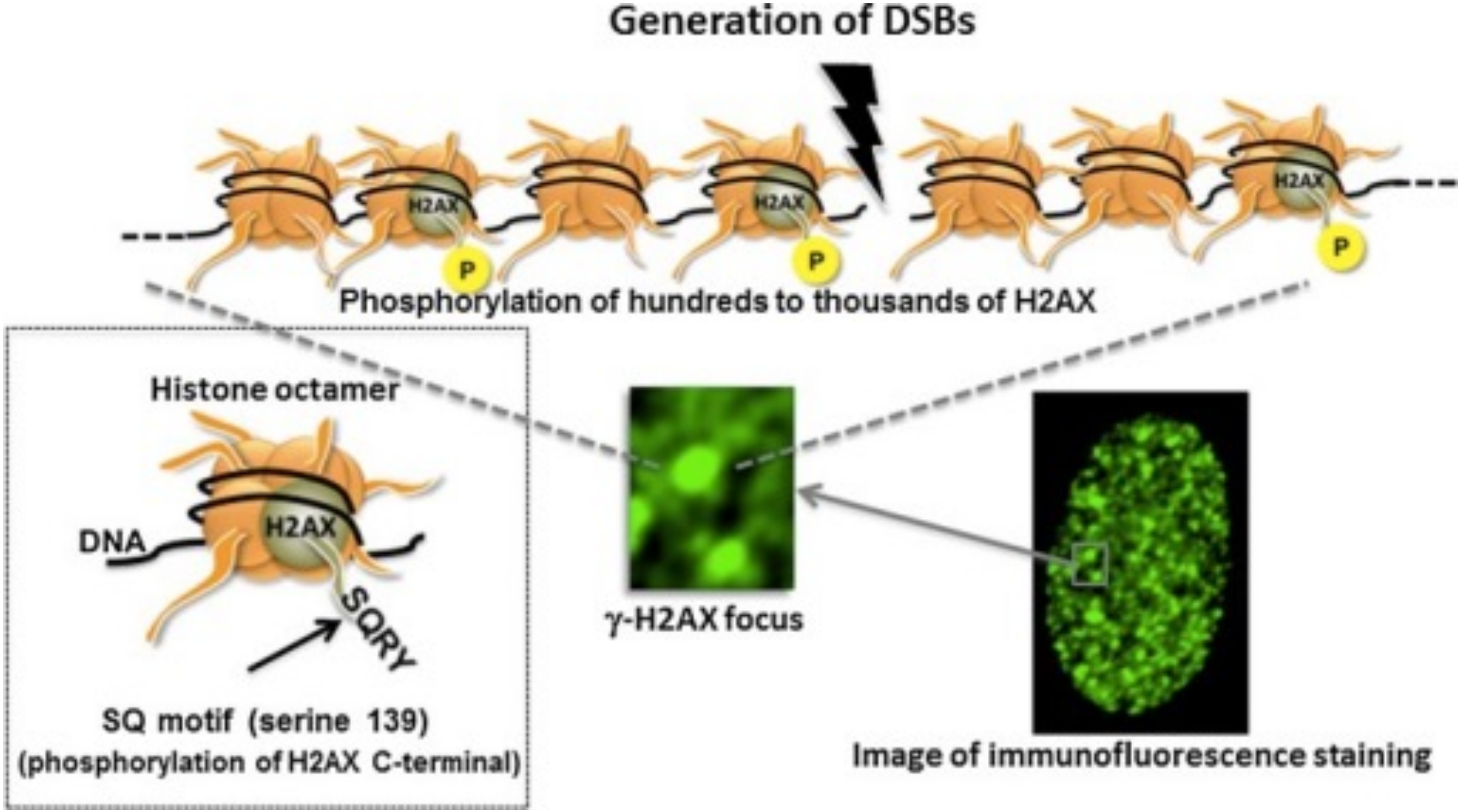
γH2AX as a Marker of DNA Damage

Interlude

Sensing DNA Damage with Antibodies



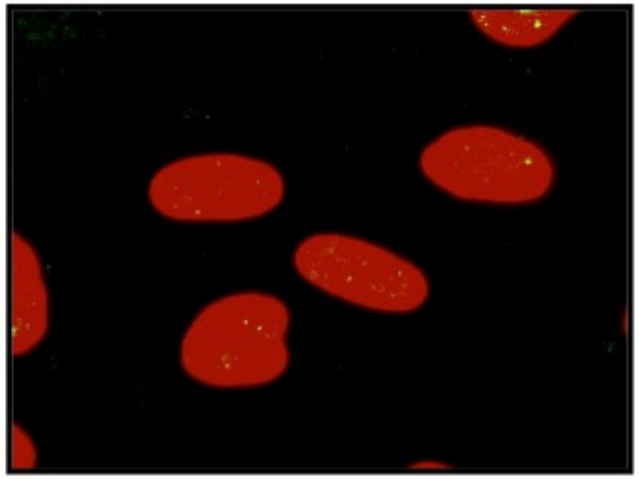
Study performed in *S. cerevisiae*
http://www.nature.com/nsmb/journal/v21/n1/fig_tab/nsmb.2737_F1.html



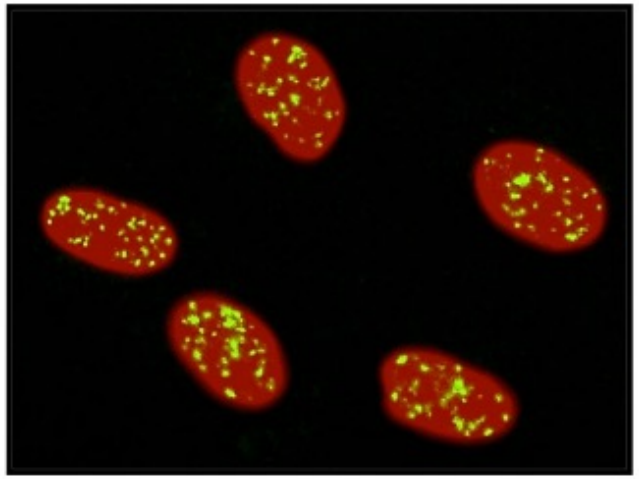
https://openi.nlm.nih.gov/detailedresult.php?img=PMC4380052_rru10502&req=4

γH2AX for Low versus High LET radiation

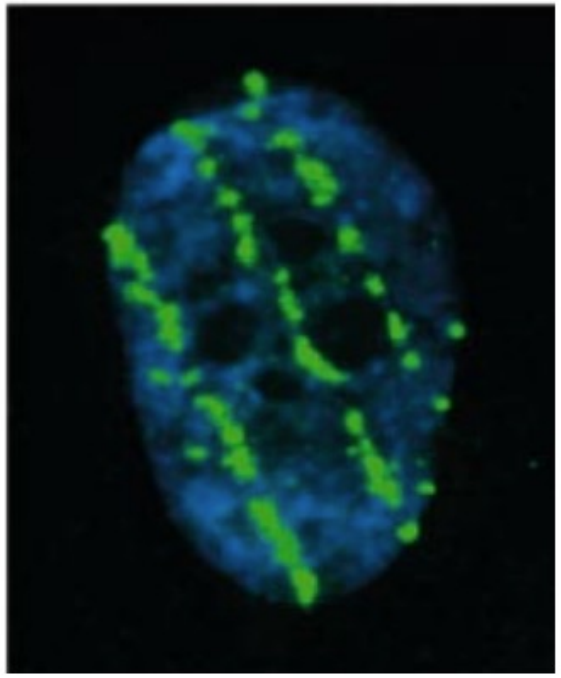
0 Gy



2 Gy



Low LET



High LET

D Anderson et al 2013 JINST 8 C06008

Desai et al., Rad. Res. 2005

Antibody Fundamentals

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

- 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

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

Flip Side: Space exploration research yields many benefits

1. **Cell phone cameras.**
2. **Wireless headphones.**
3. **Infrared ear thermometers.**
4. **Water purification systems.**
5. **Solar energy.**
6. **Memory foam.**
7. **Fitness Heart Rate Monitors.**
8. **Scratch-resistant lenses.**
9. **LED medical technology.**
10. **Dustbuster vacuums.**

A careful look at the major steps of BER

γH2AX as a Marker of DNA Damage

Interlude