

**Mechanisms of Genomic Rearrangements:  
How Hexavalent Chromium, a Major Environmental Concern,  
Induces Chromosome Instability**

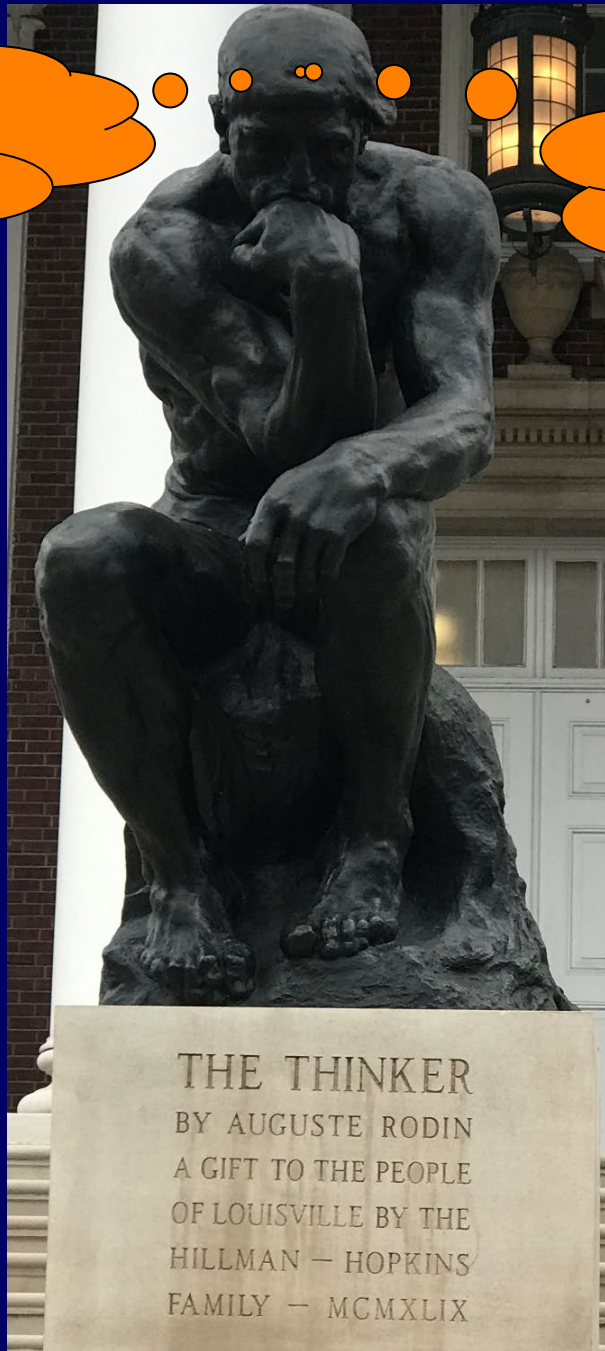
John Pierce Wise, Sr.

Wise Laboratory of Environmental and Genetic Toxicology  
Department of Pharmacology and Toxicology  
University of Louisville

Annual Genetic Toxicology Association Meeting  
May 4<sup>th</sup>, 2023

Why study chromium?

What are the key outcomes and lesions in Cr(VI) carcinogenesis?



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# Chromium: Global Environmental Pollutant

- ◆ Name derived from the Greek word *chroma* meaning *color*
- ◆ Exposure is widespread:
  - Wide industrial use for over ~200 years
  - Pigment, rust inhibitor, antifouling agent
  - Enters air, soil and water from many routes



[hotspotoutdoors.com](http://hotspotoutdoors.com)



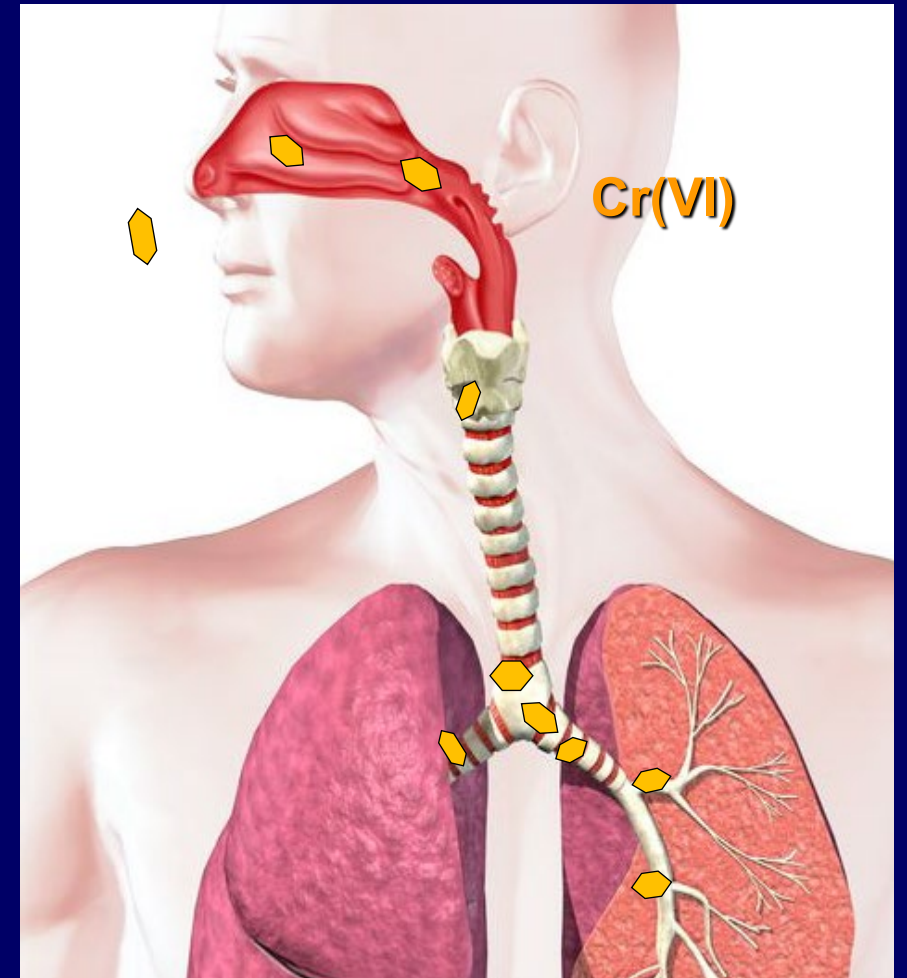
<http://www.vmmv.org/tanktk/hetzer/hetzer.htm>



<http://www.nontoxicprint.com/safepainting.htm>

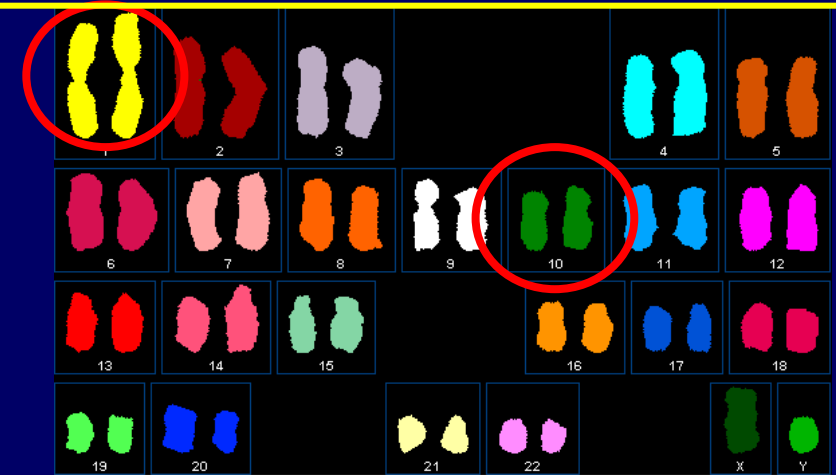
# Chromium (VI)-Induced Carcinogenesis

- ◆ Particulate Cr(VI) is the potent form of Cr(VI) as a human lung carcinogen
- ◆ Cr(VI) particles accumulate at lung bifurcation sites resulting in the formation of tumors



# Chromosome Instability (CIN): A Driving Mechanism in Lung Cancer

- ◆ Alteration in the number or structure of chromosomes
- ◆ Hallmark of lung cancer
- ◆ Proposed as an early event in carcinogenesis
- ◆ Cr(VI) induces numerical and structural chromosome instability

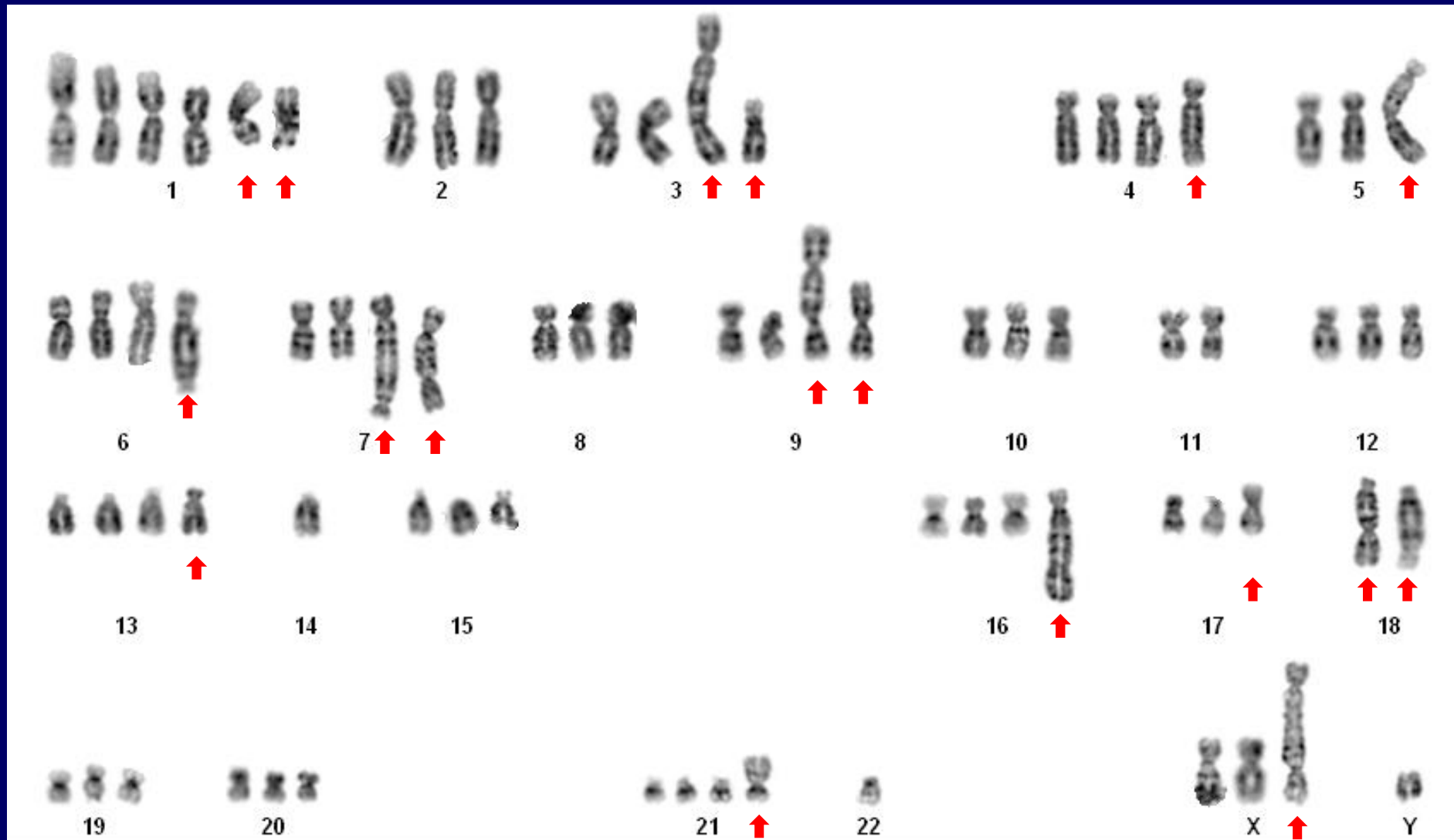


Normal human lung cell karyotype

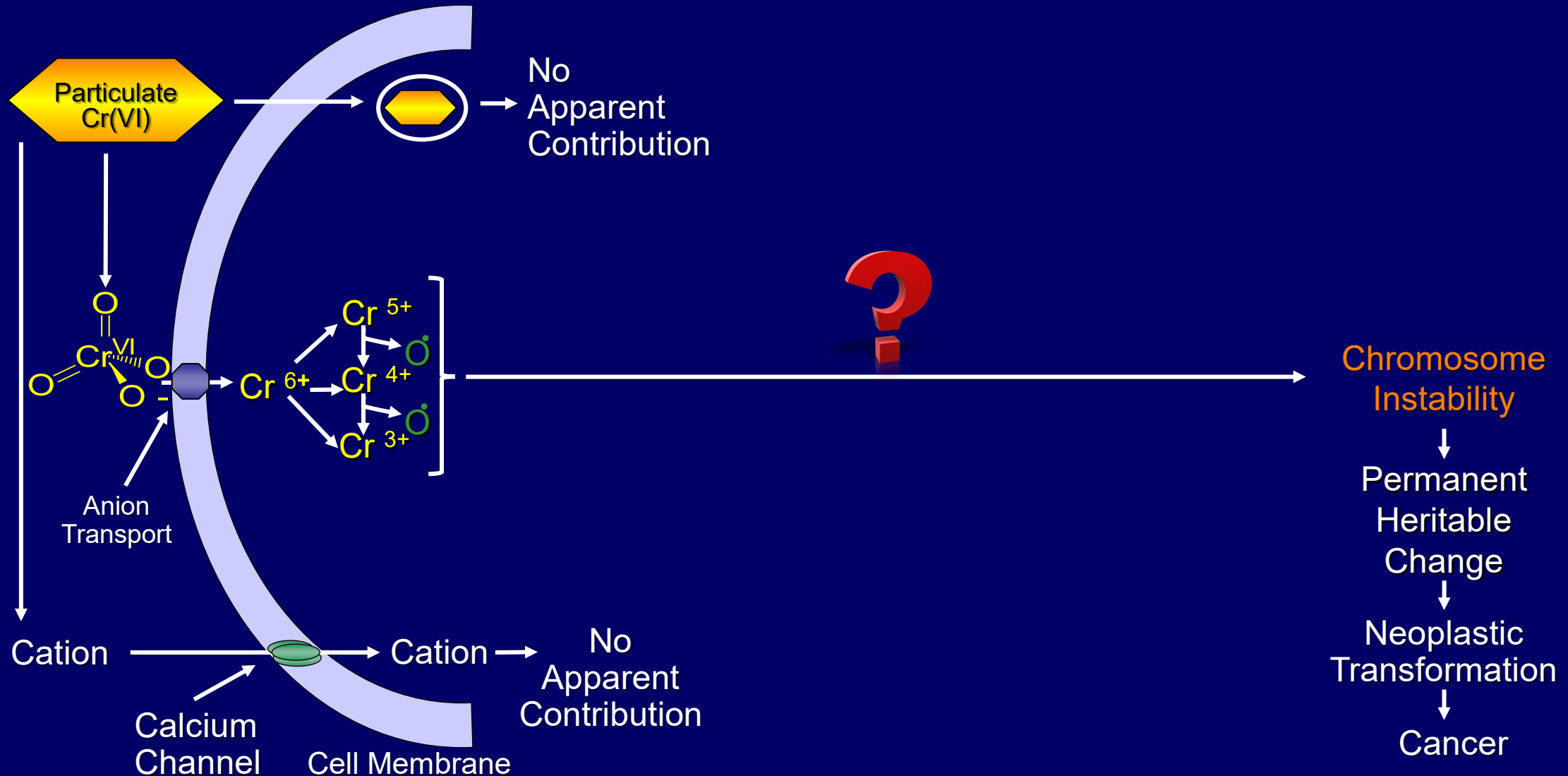


Cancer cell karyotype

# Particulate Cr(VI)-Induced Chromosome Instability in a Human Lung Cell

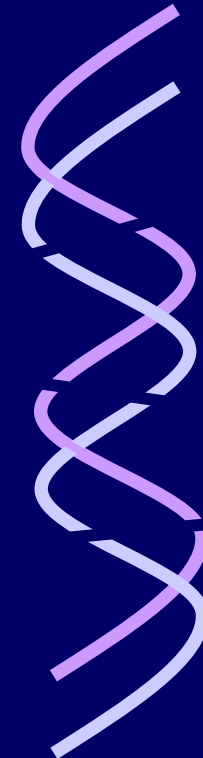


# Chromosome Instability Is a Key Driving Mechanism for Particulate Cr(VI)-Induced Carcinogenesis in Human Lung Cells



# DNA Double Strand Breaks Are the Key Lesion Responsible for Structural Chromosome Instability

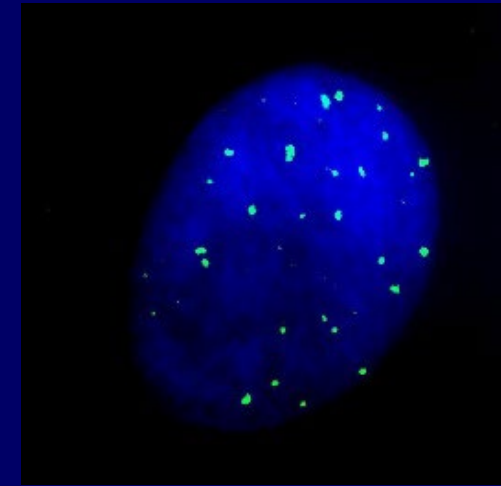
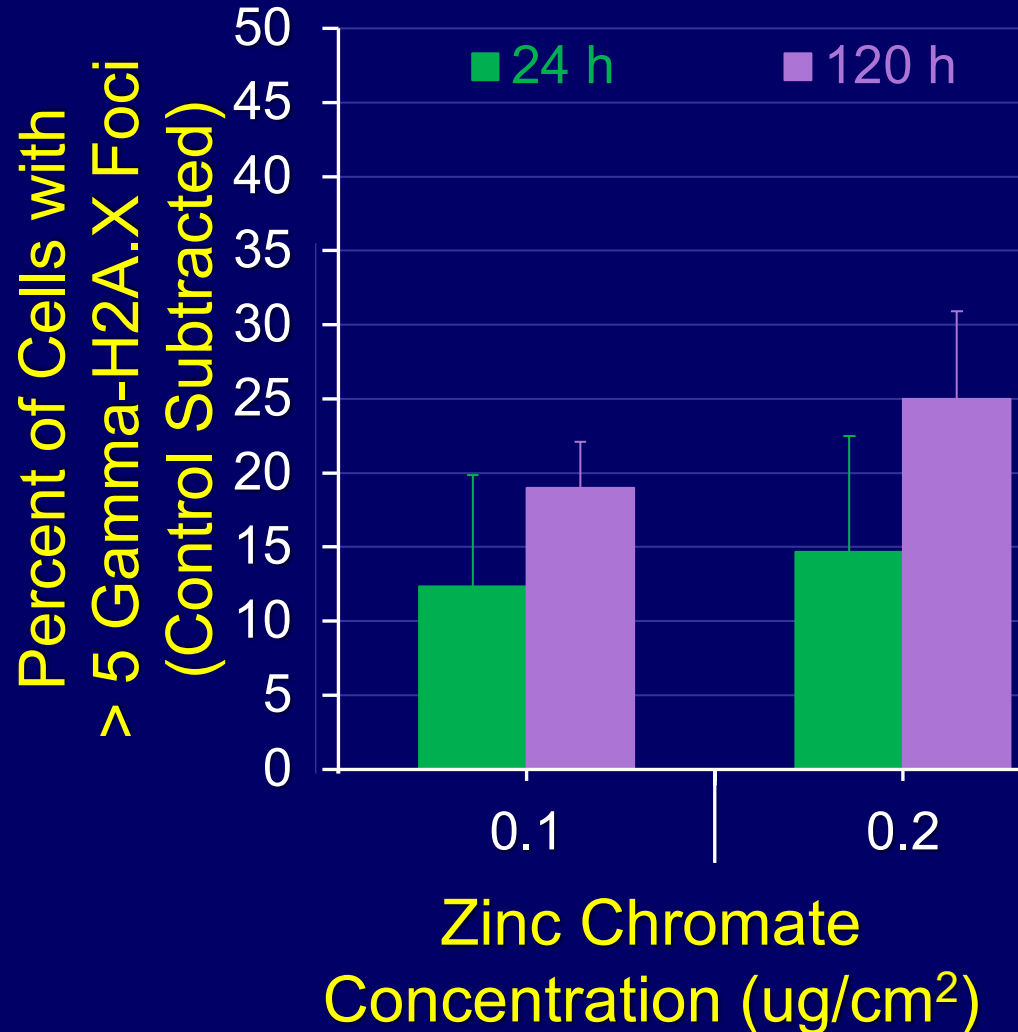
- ◆ Both DNA strands are broken
- ◆ The loss of both strands could cause genomic material loss
- ◆ Double strand breaks must be repaired to prevent chromosomal instability
- ◆ Induced by Cr(VI) exposure



DNA Double Strand Breaks



# The Key Lesion: Prolonged Particulate Cr(VI) Exposure Induces DNA Double Strand Breaks in Human Lung Cells



Gamma-H2A.X Foci



Qin Qin

Why study chromium?

What are the key outcomes and lesions in Cr(VI) carcinogenesis?

Ok  
Cr(VI) is common in the environment, causes lung cancer and chromosome instability and DNA double strand breaks are driving events



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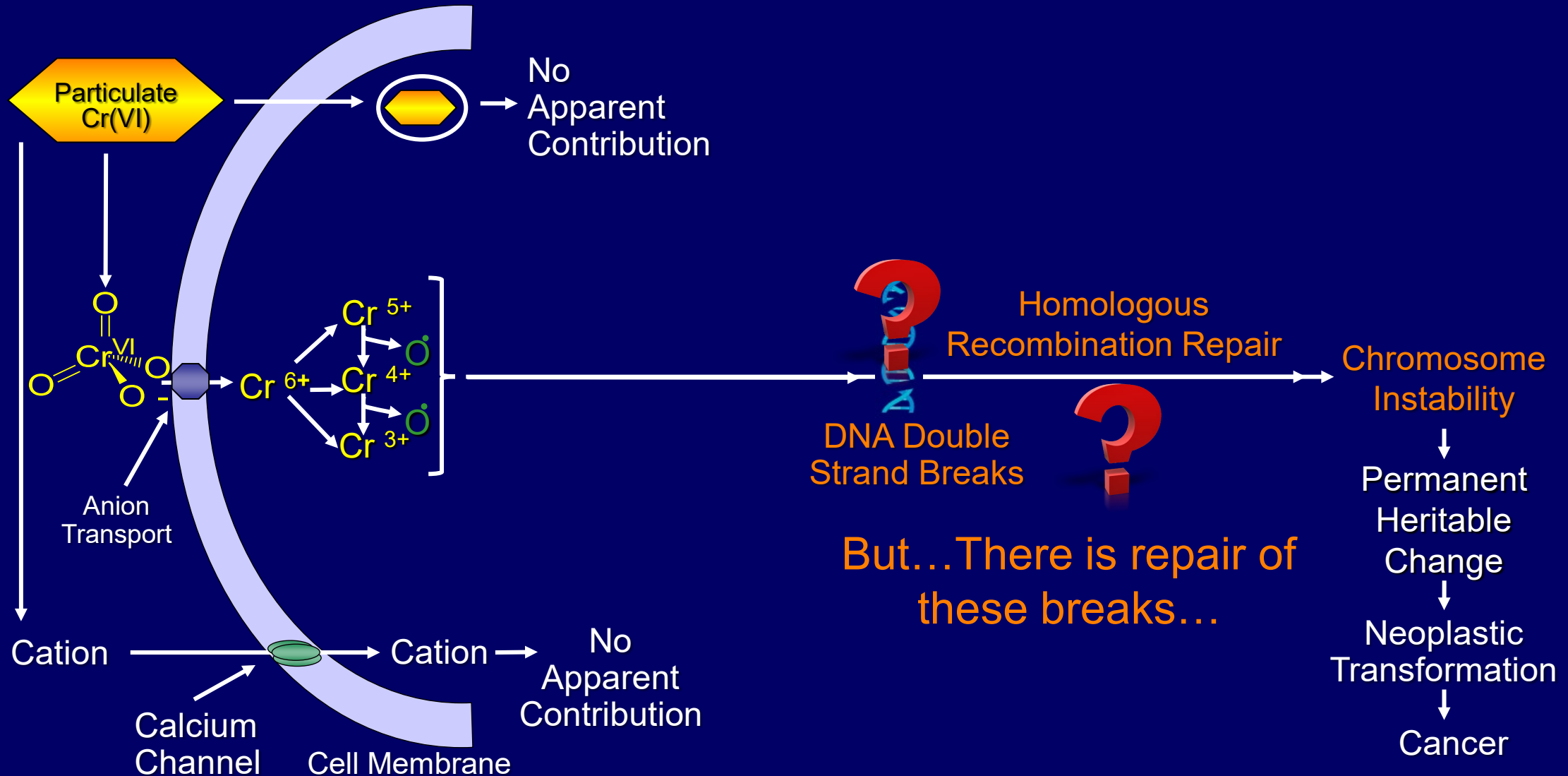
Why isn't repair preventing breaks from inducing chromosome instability?

Hypothesis:  
Particulate Cr(VI) can  
inhibit repair

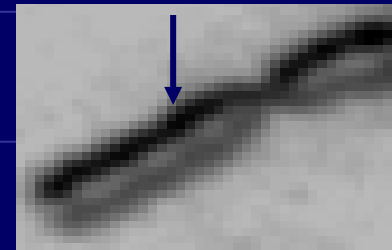
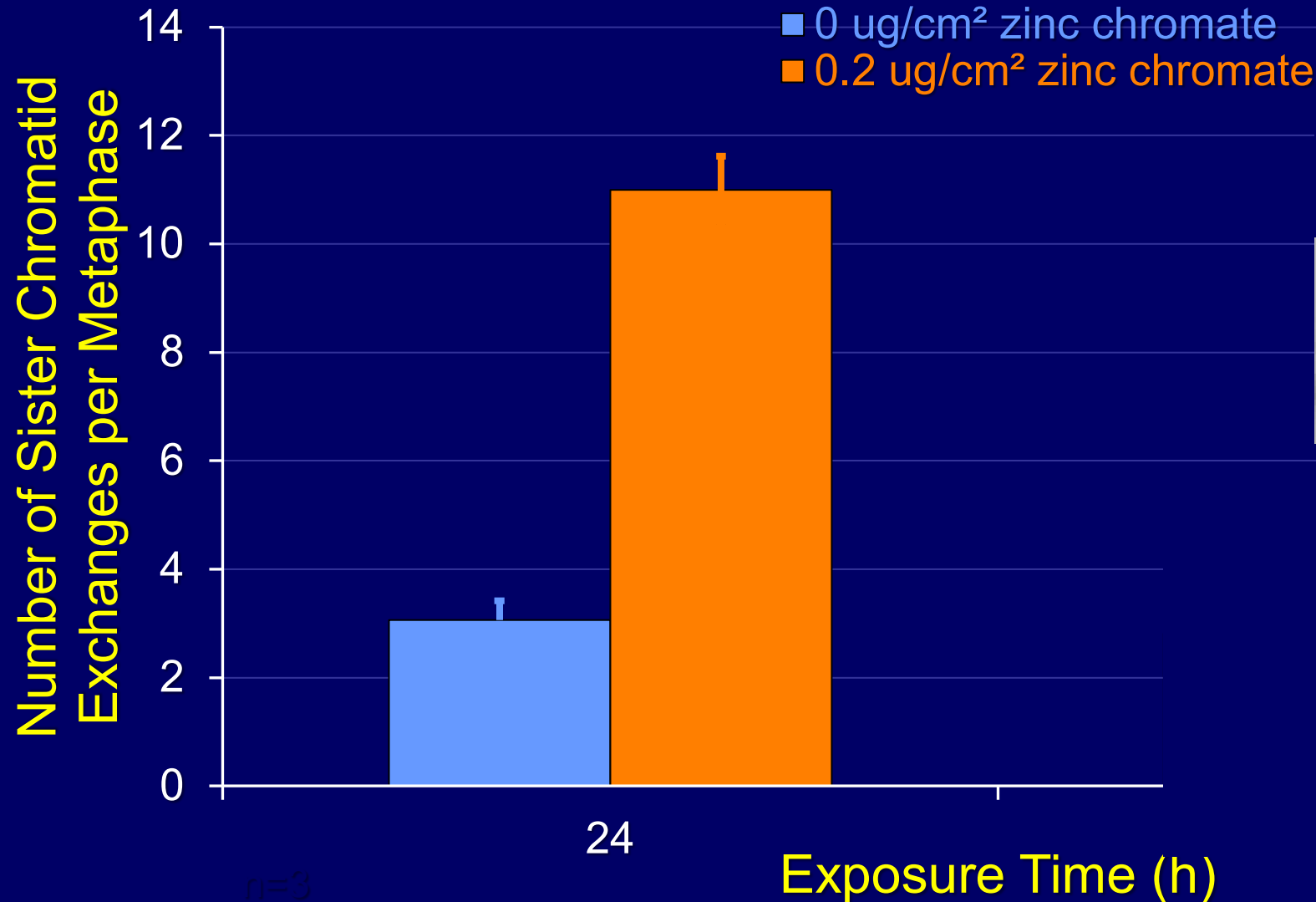


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# Chromosome Instability Is a Key Driving Mechanism for Particulate Cr(VI)-Induced Carcinogenesis in Human Lung Cells



# Prolonged Particulate Cr(VI) Exposure Inhibits Homologous Recombination Repair (Sister Chromatid Exchange Formation)



Cynthia Browning

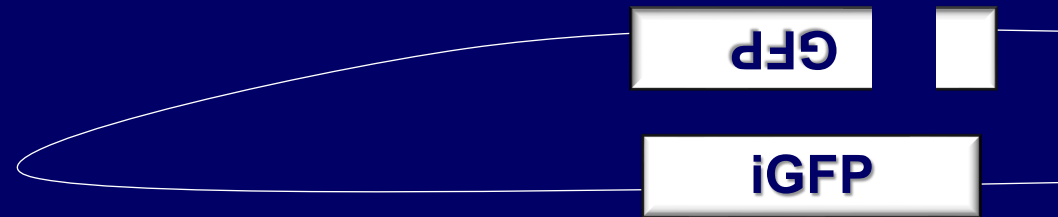
# DR-GFP Homology Directed Repair Reporter

Before HR repair

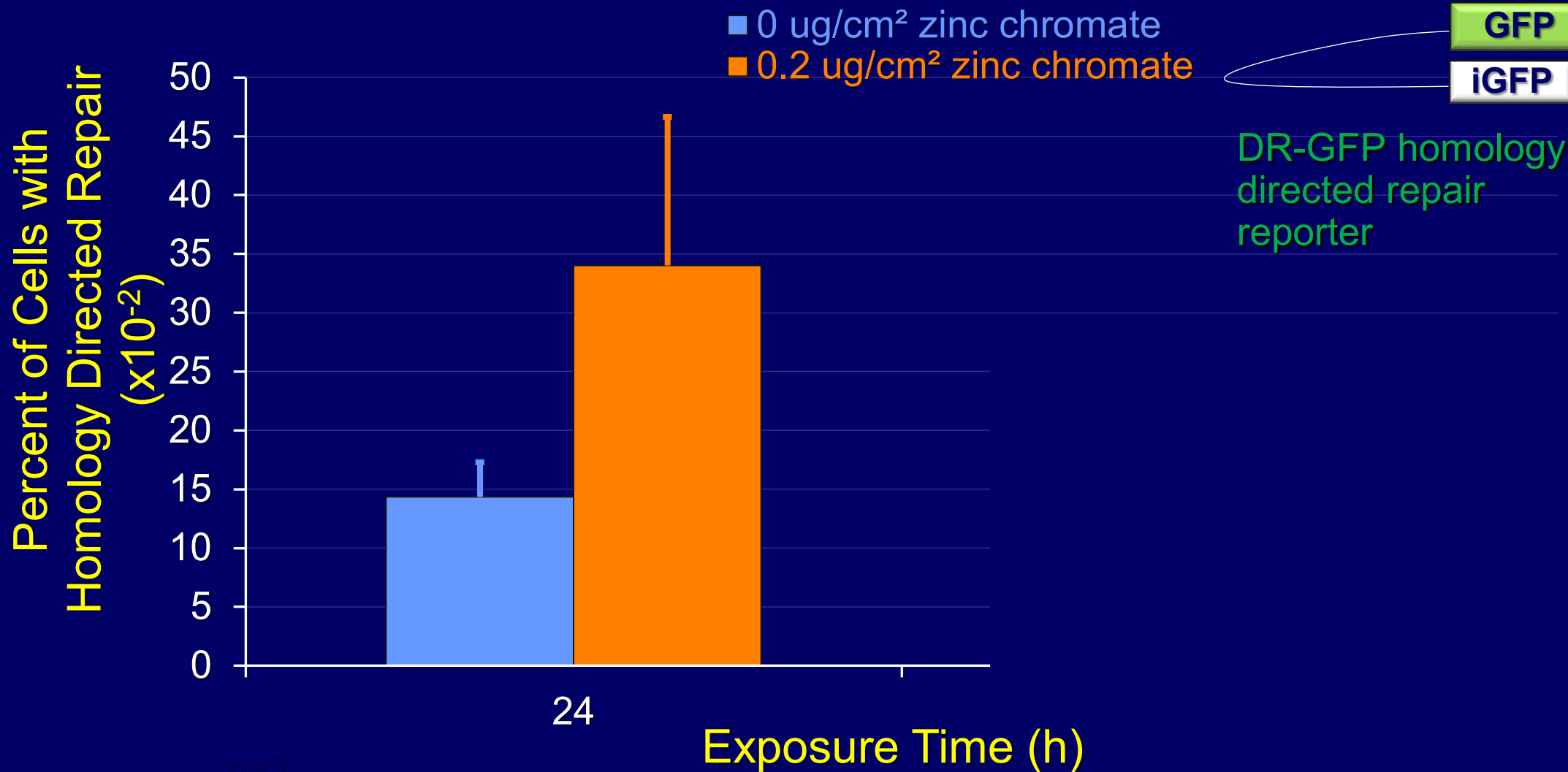


# DR-GFP Homology Directed Repair Reporter

After HR repair



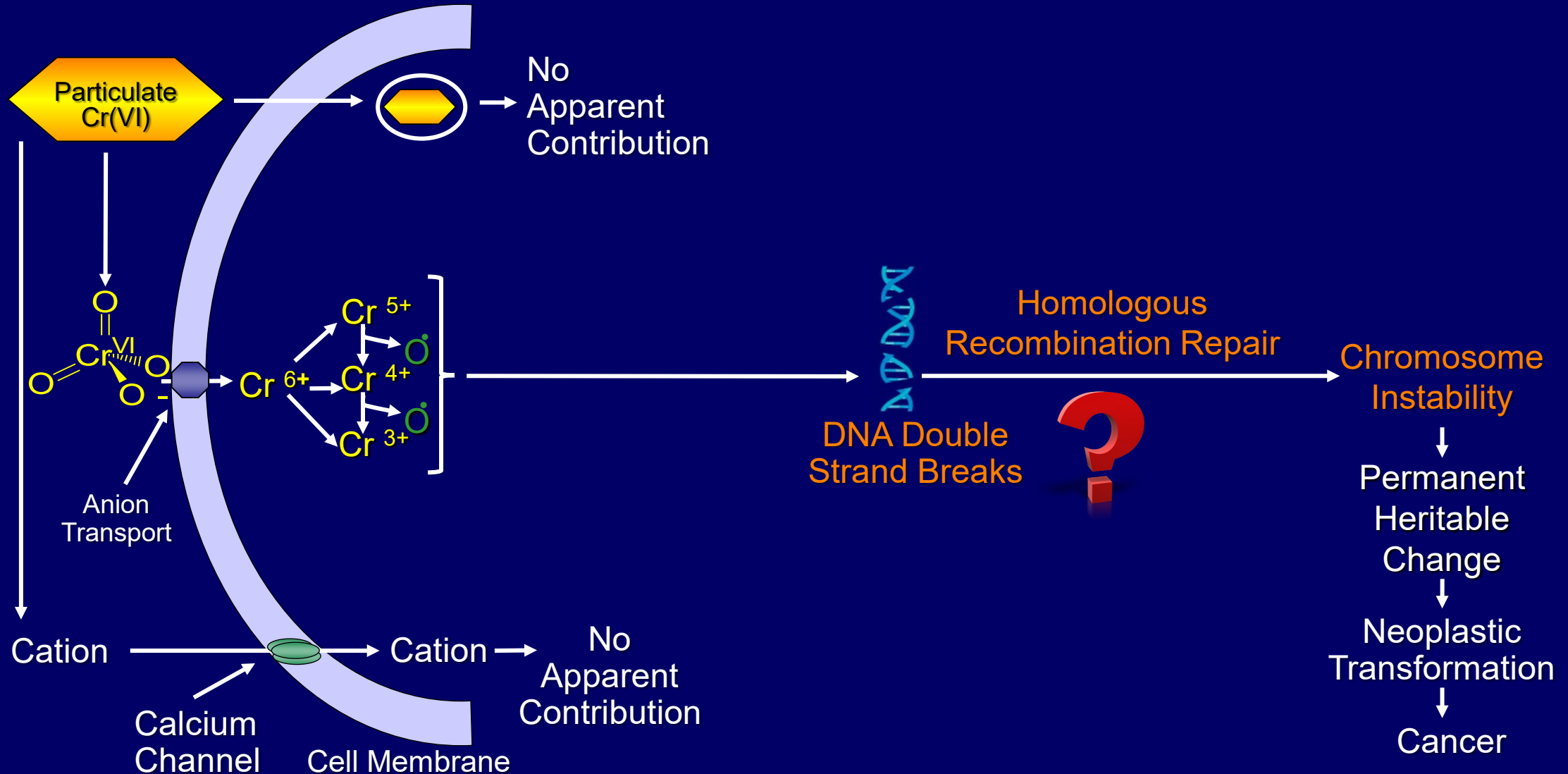
# Prolonged Particulate Cr(VI) Exposure Inhibits Homologous Recombination Repair (DR-GFP Homology Repair Reporter)



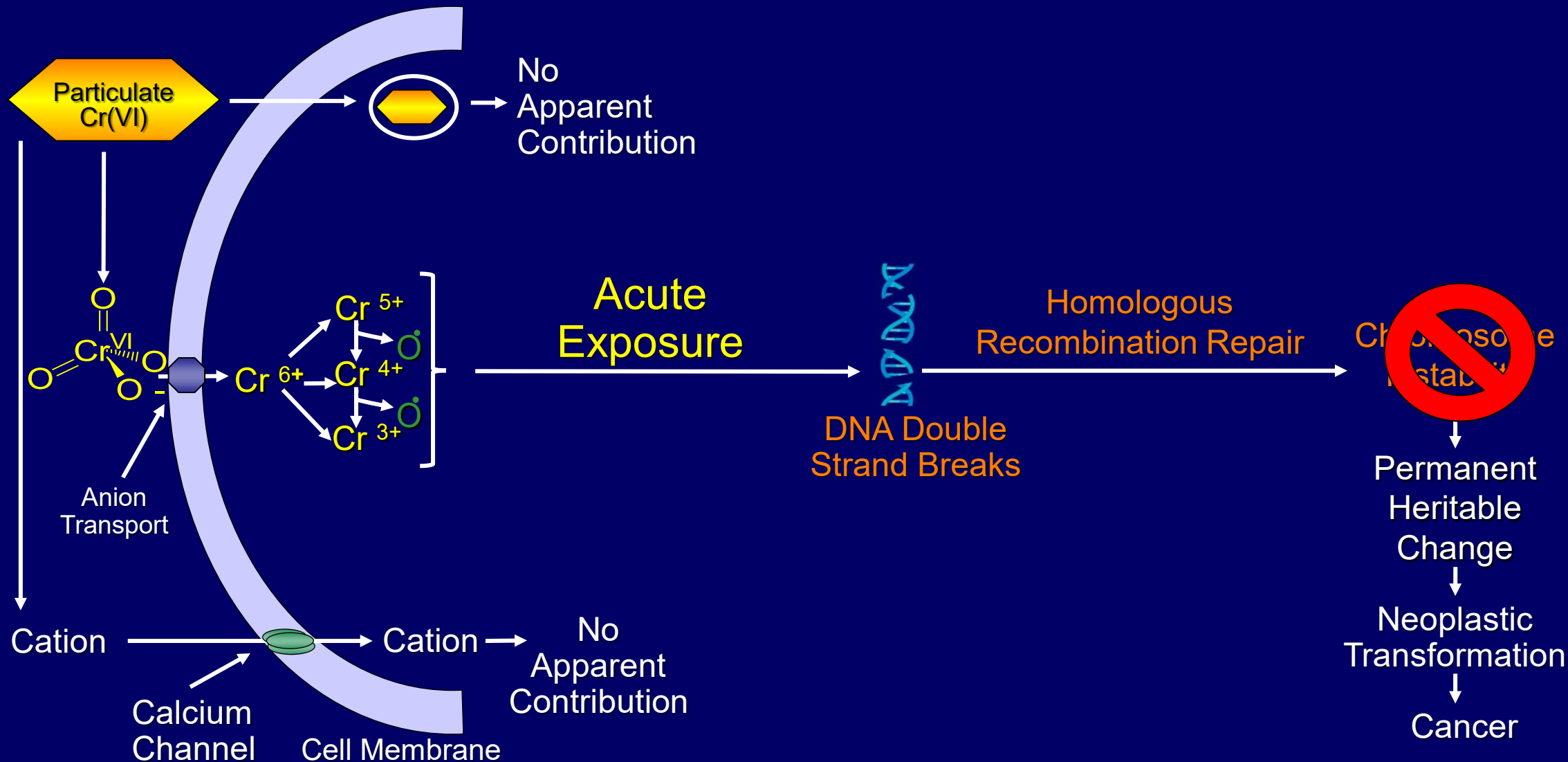
Cynthia Browning



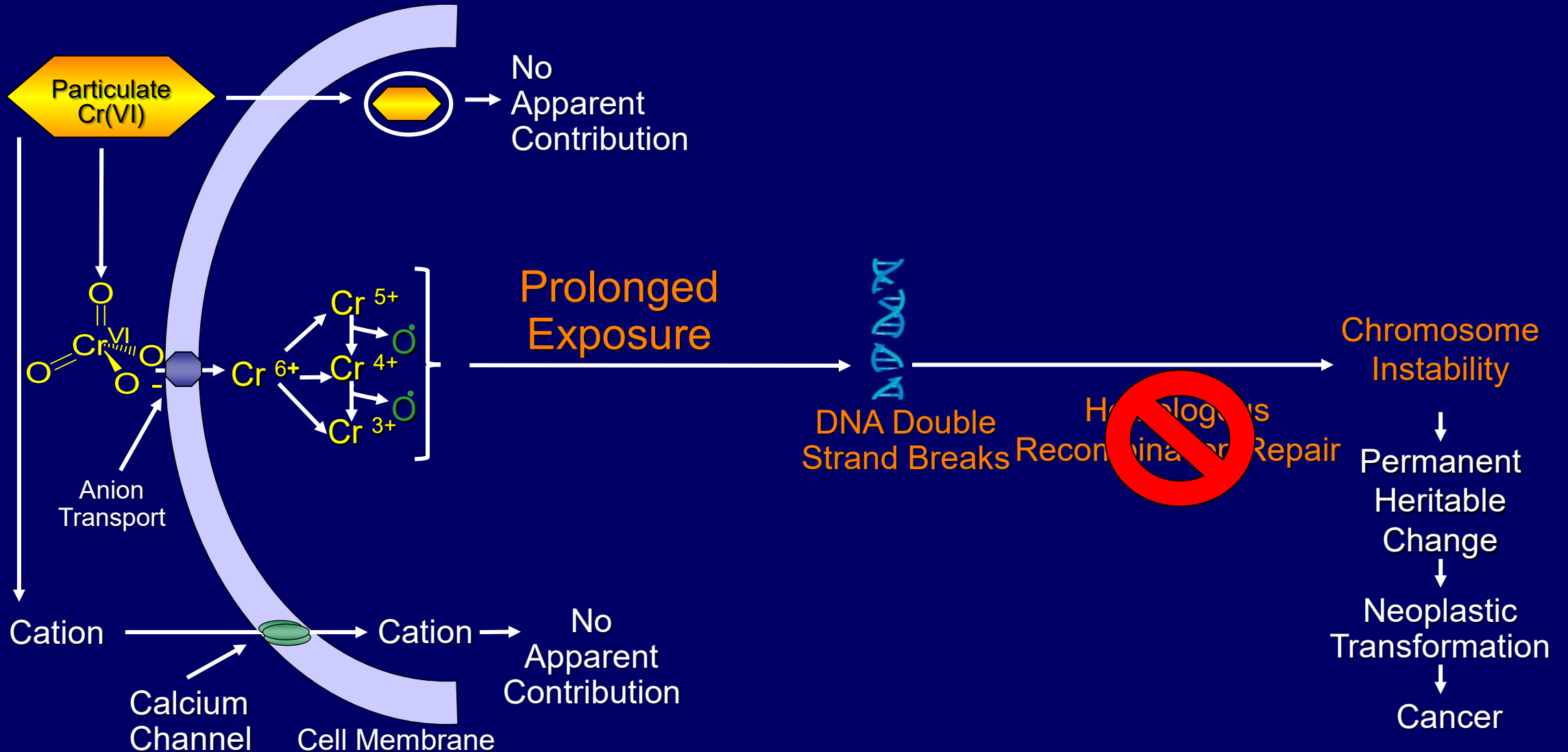
# Chromosome Instability Is a Key Driving Mechanism for Particulate Cr(VI)-Induced Carcinogenesis in Human Lung Cells



# Physico-Chemical-Biological Particulate Cr(VI) Mechanism in Human Lung Cells



# Physico-Chemical-Biological Particulate Cr(VI) Mechanism in Human Lung Cells



Why isn't repair preventing breaks from inducing chromosome instability?

Hypothesis:  
Particulate Cr(VI) can inhibit repair

**YES**  
Particulate Cr(VI)  
inhibits homologous  
recombination repair



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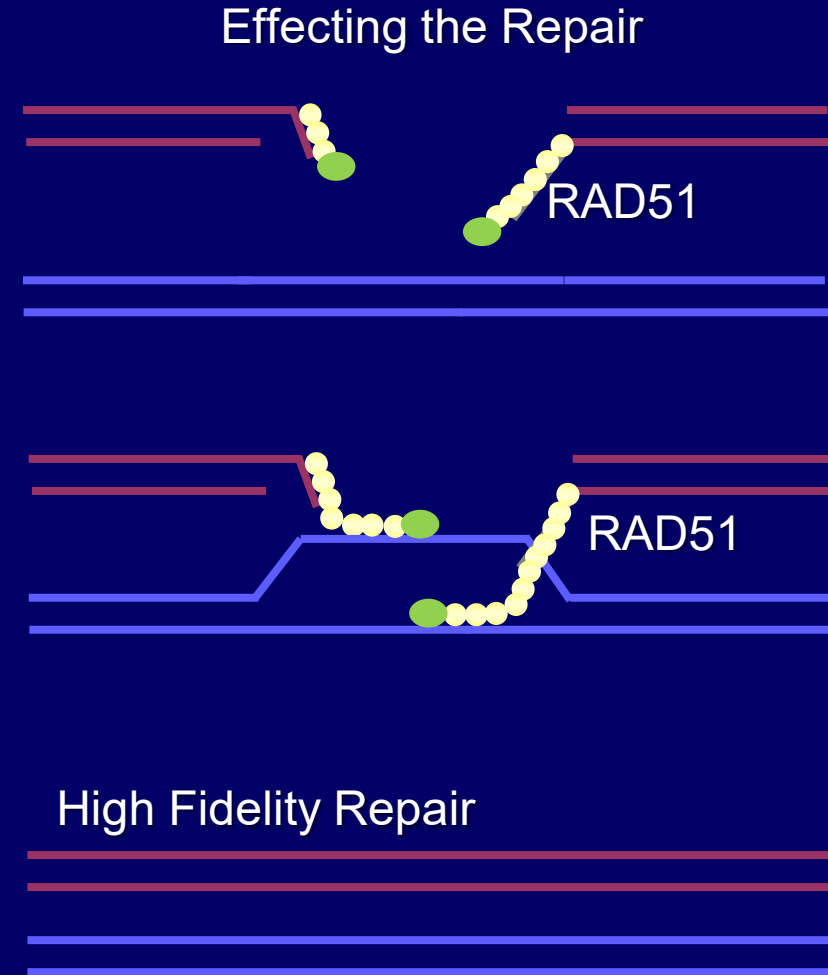
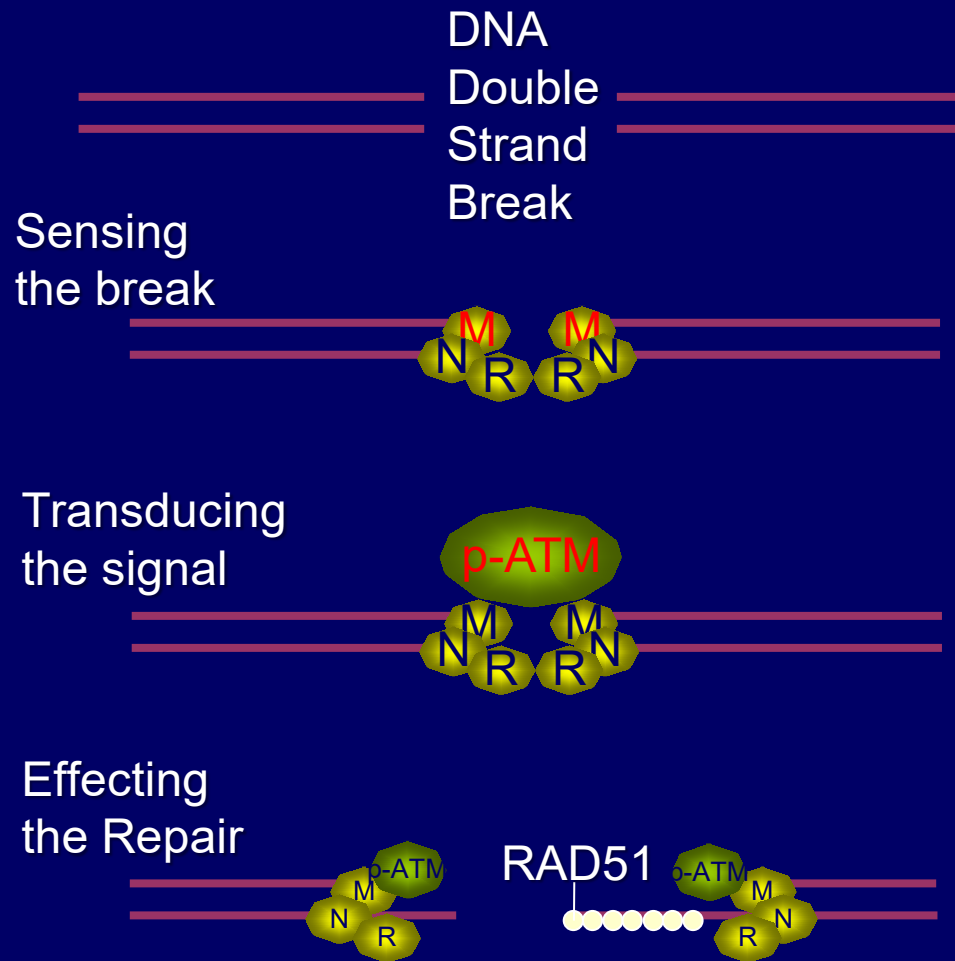
How does Cr(VI) target homologous recombination repair?



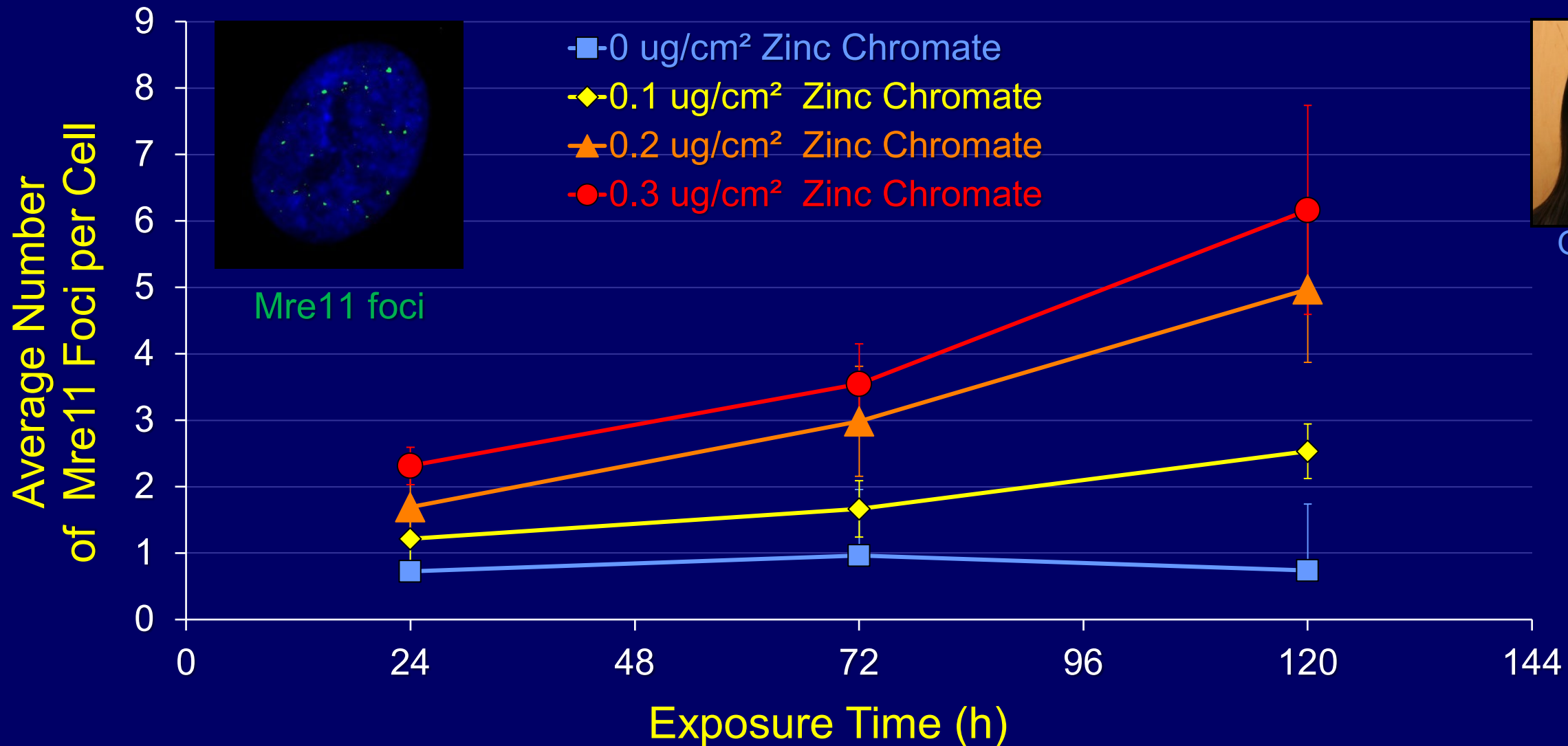
Hypothesis:  
Particulate Cr(VI)  
targets a specific  
repair step

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# Simplified Schematic of Homologous Recombination Repair

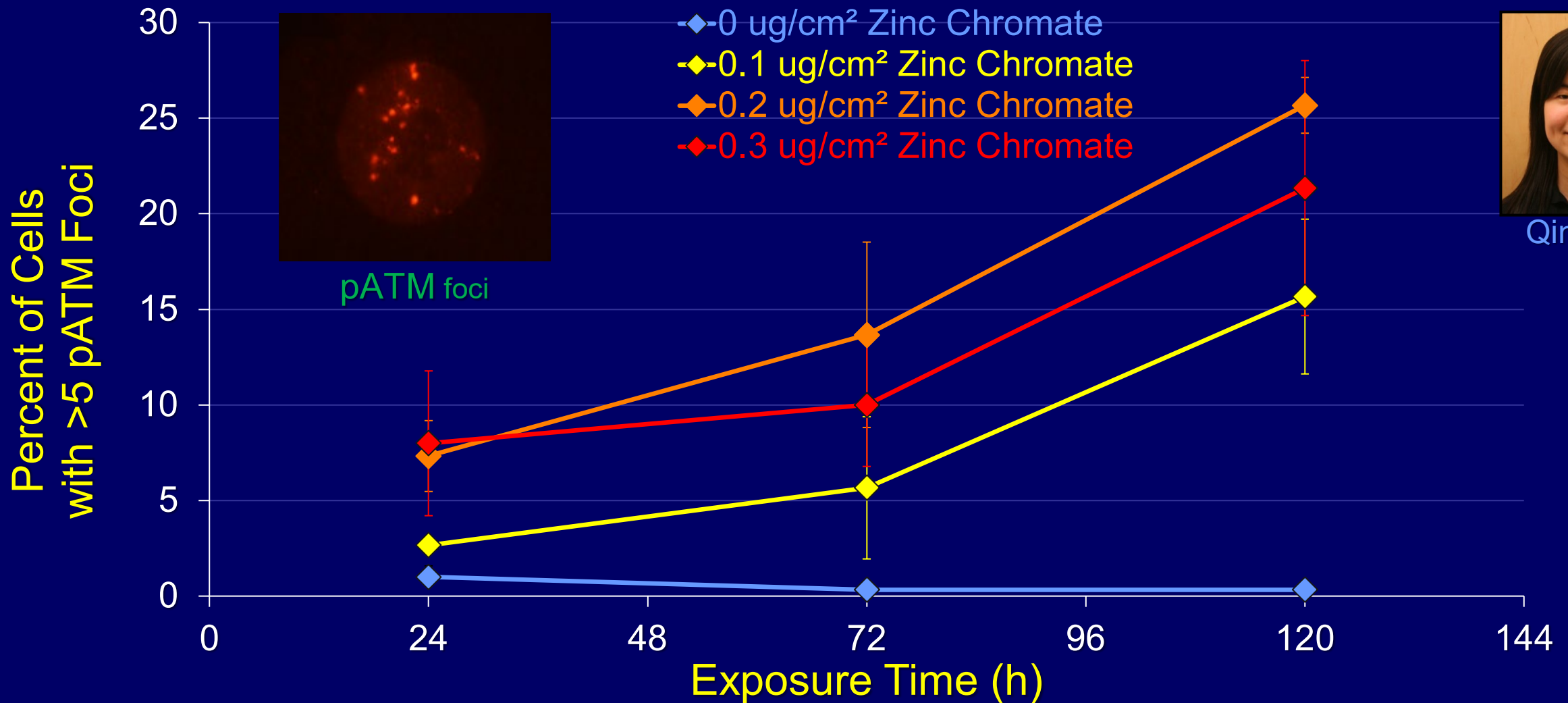


# Particulate Cr(VI) Exposure Does Not Affect the Sensor Step



Qin Qin

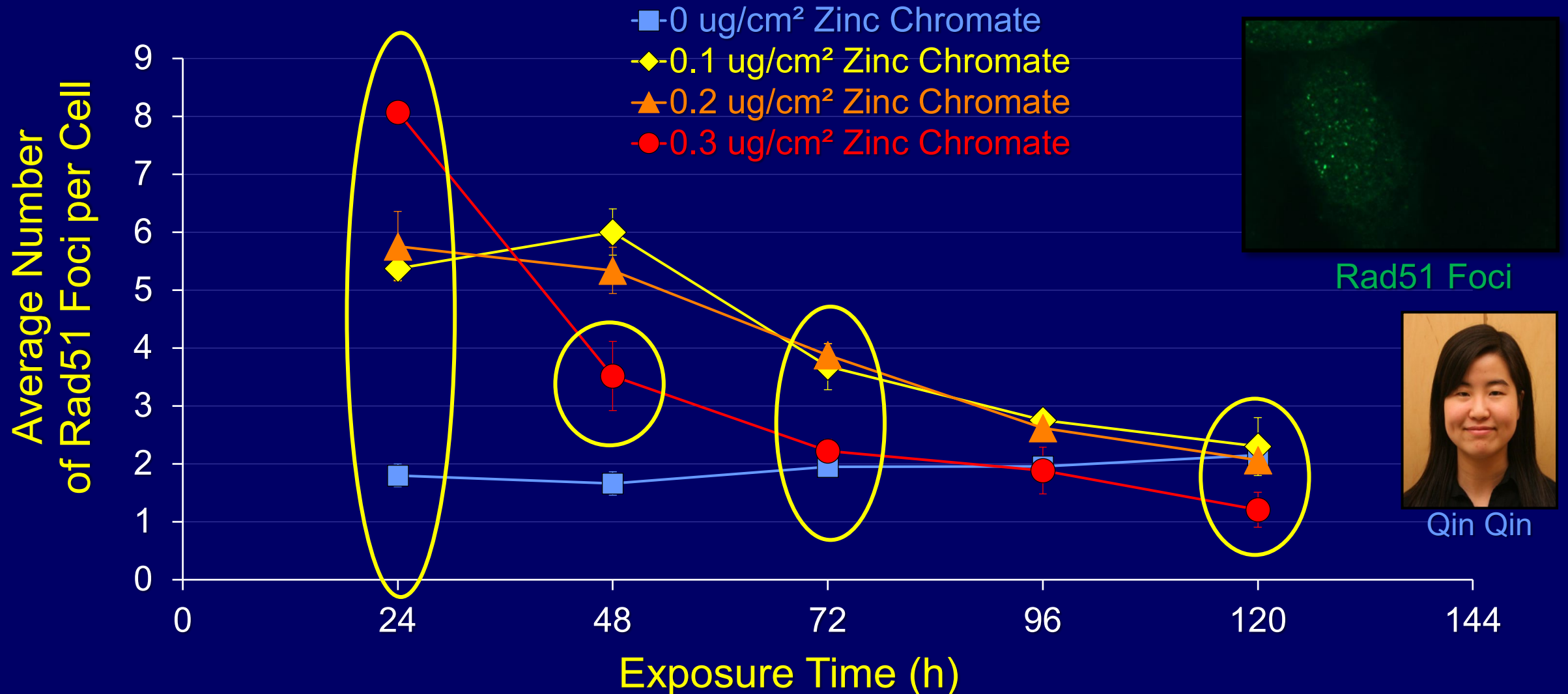
# Particulate Cr(VI) Exposure Does Not Affect the Transducer Step



Qin Qin

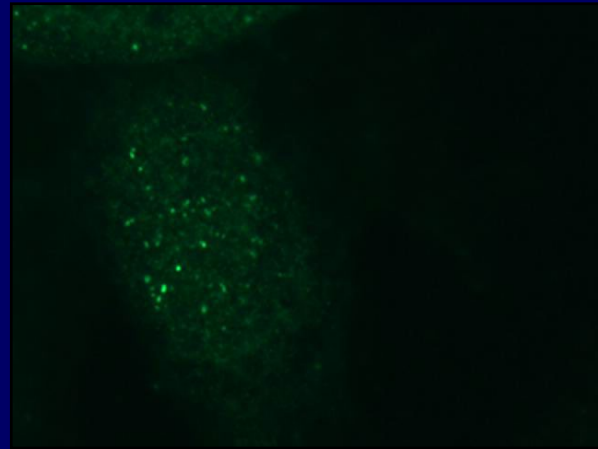


# Prolonged Particulate Cr(VI) Exposure Inhibits Homologous Recombination Repair by Targeting RAD51

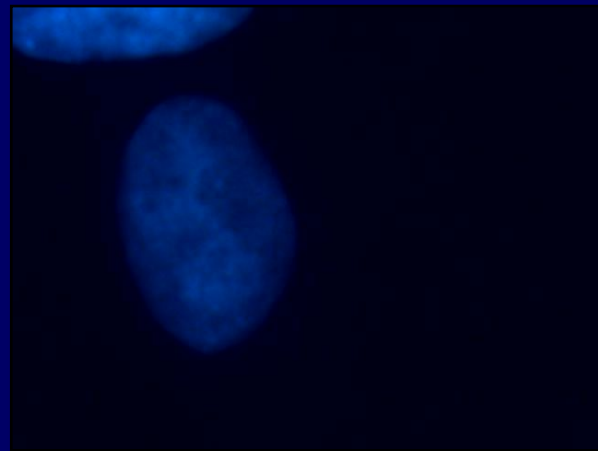
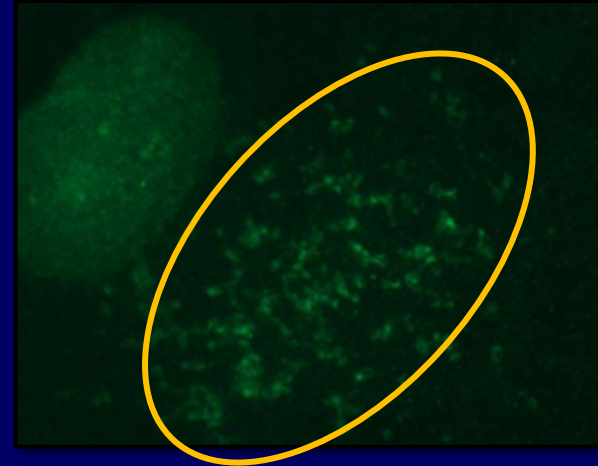


# Prolonged Particulate Cr(VI) Exposure Alters Rad51 Intracellular Localization

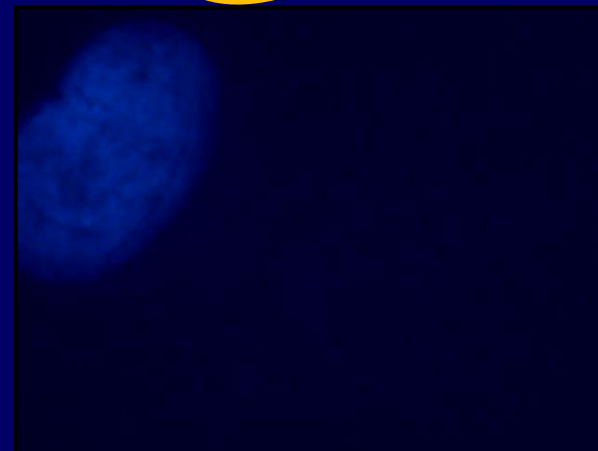
Nuclear Localization



Cytoplasmic Accumulation



24 h zinc chromate



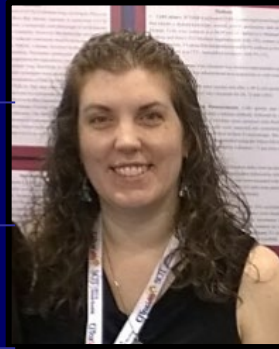
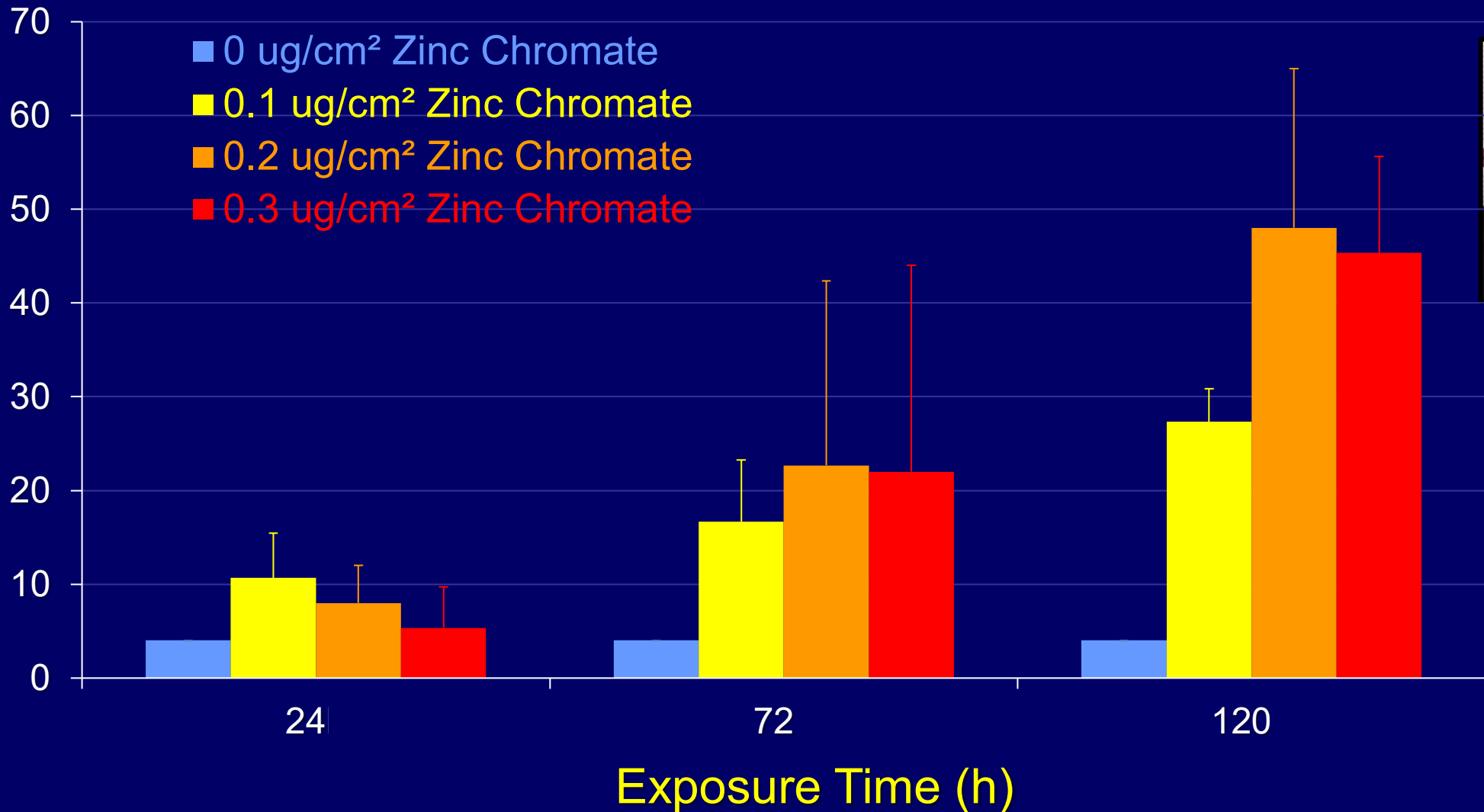
120 h zinc chromate



Qin Qin

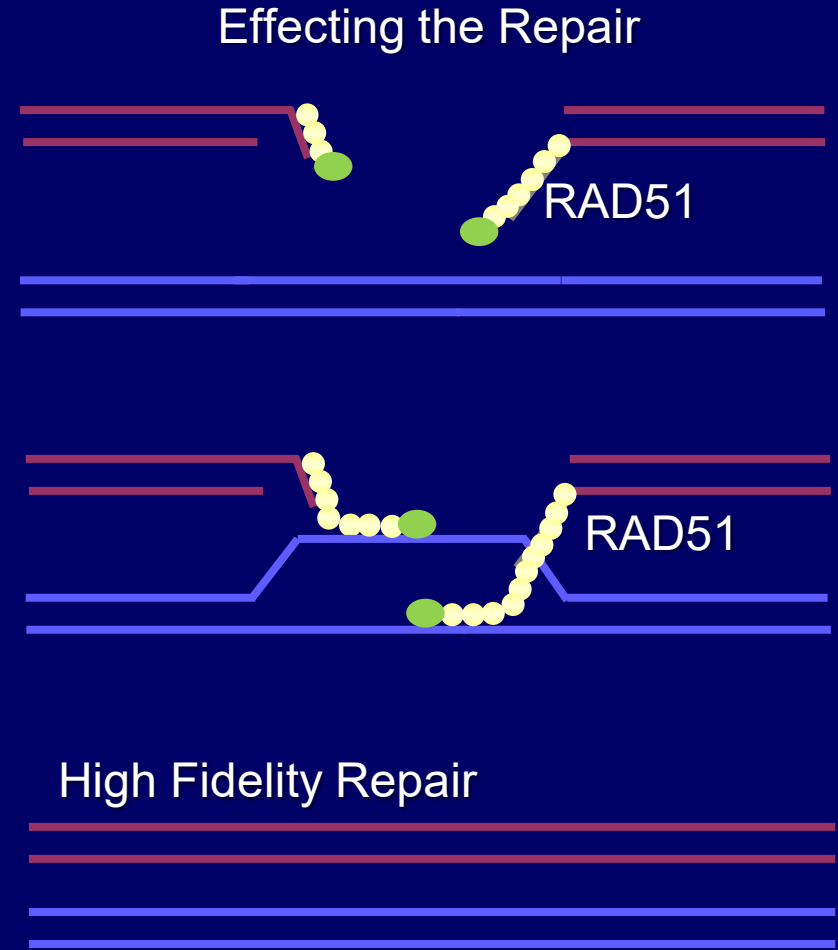
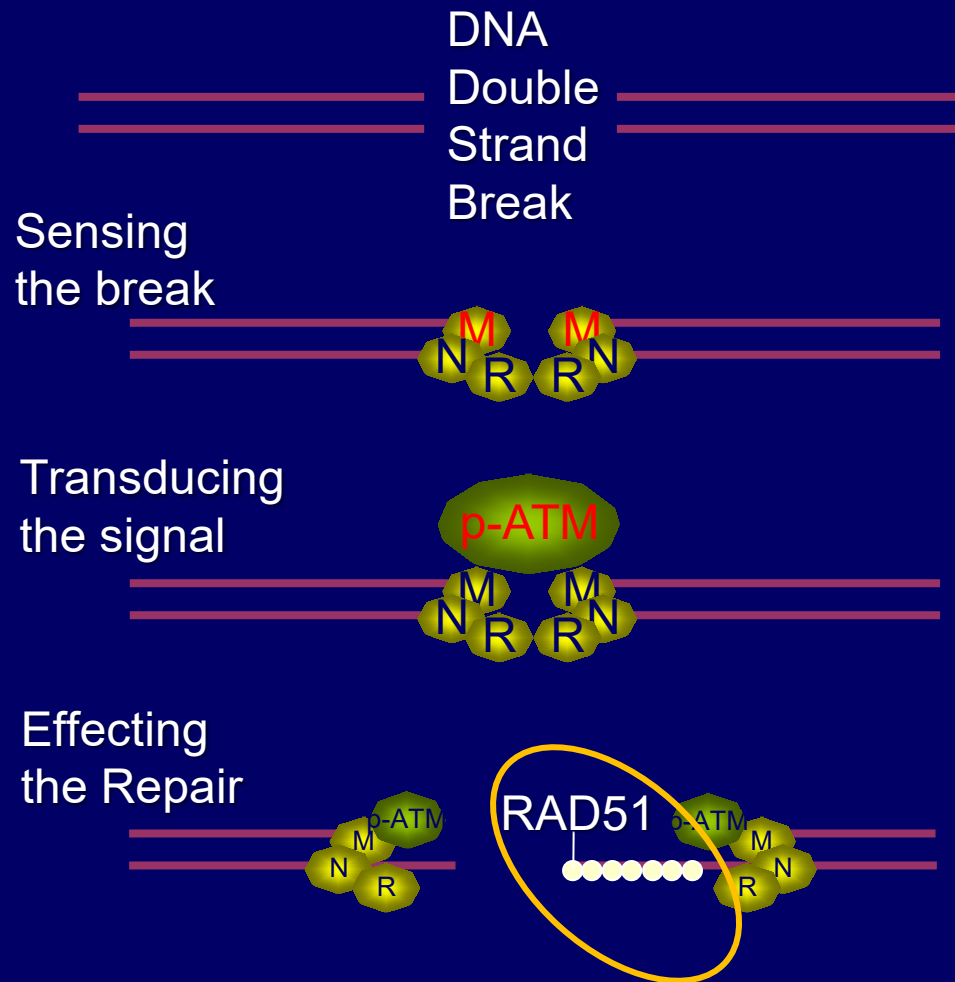
# Prolonged Particulate Cr(VI) Exposure Induces Inappropriate Cytoplasmic RAD51 Accumulation

Percent of Cells with RAD51  
Cytoplasmic Accumulation

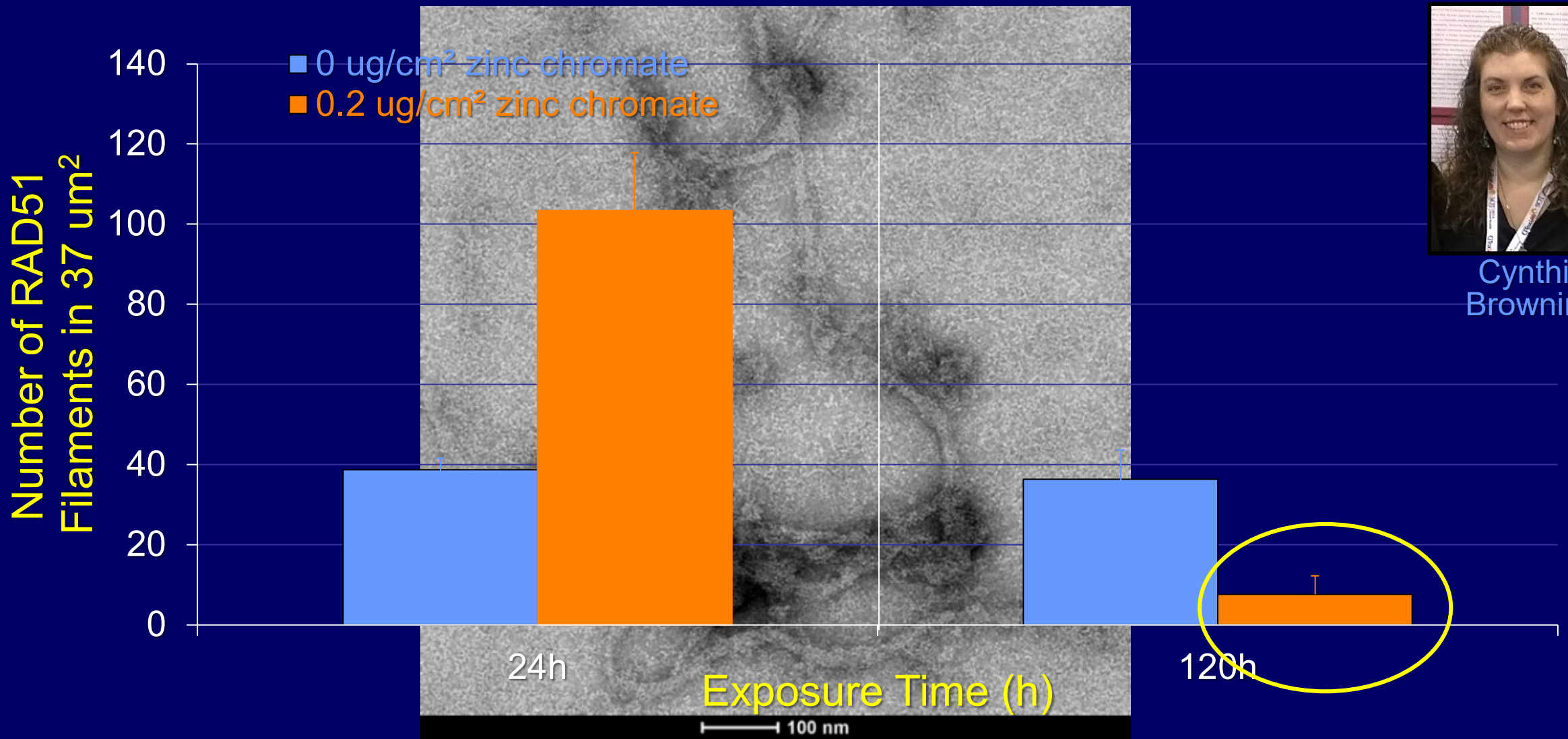


Cynthia  
Browning

# Simplified Schematic of Homologous Recombination Repair

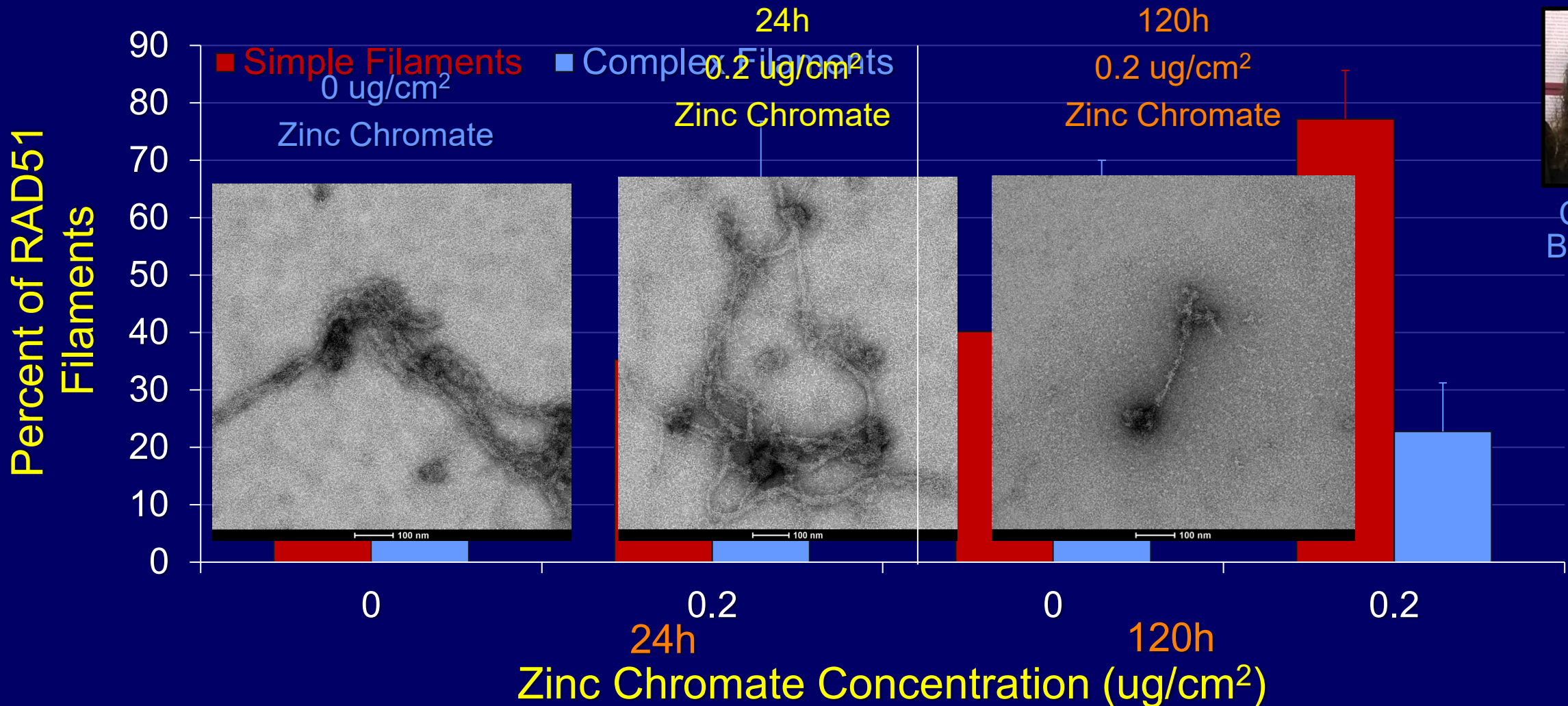


# Prolonged RAD51 Filamentation (WIS) Exposure Inhibits RAD51 Transient Monofilament Formation



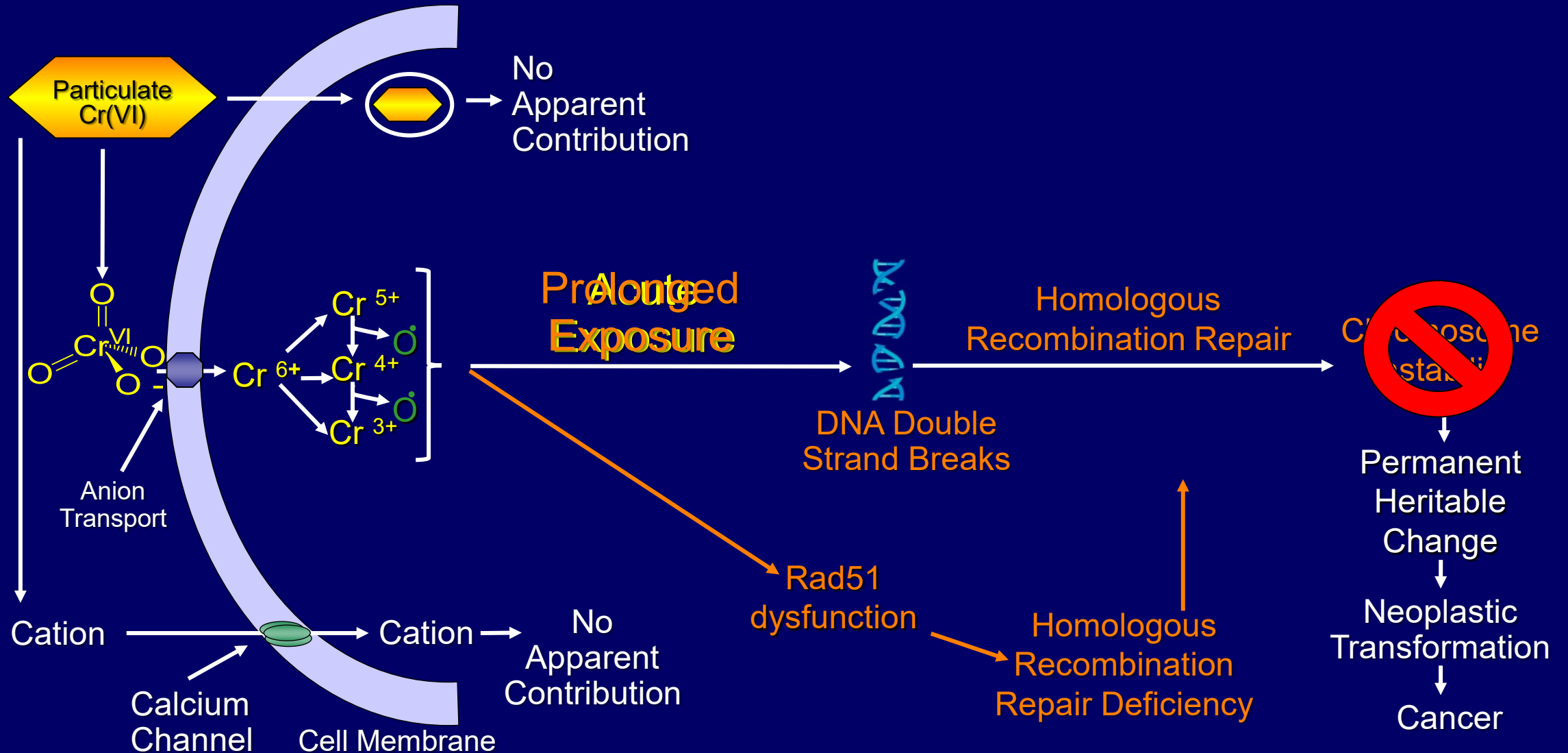
Cynthia Browning

# Prolonged Particulate Cr(VI) Exposure Alters RAD51 Filament Structure



Cynthia Browning

# Physico-Chemical-Biological Particulate Cr(VI) Mechanism in Human Lung Cells



How does Cr(VI) target homologous recombination repair?



Hypothesis:  
Particulate Cr(VI)  
targets a specific  
repair step

YES  
Particulate Cr(VI)  
targets RAD51 in the  
effector step

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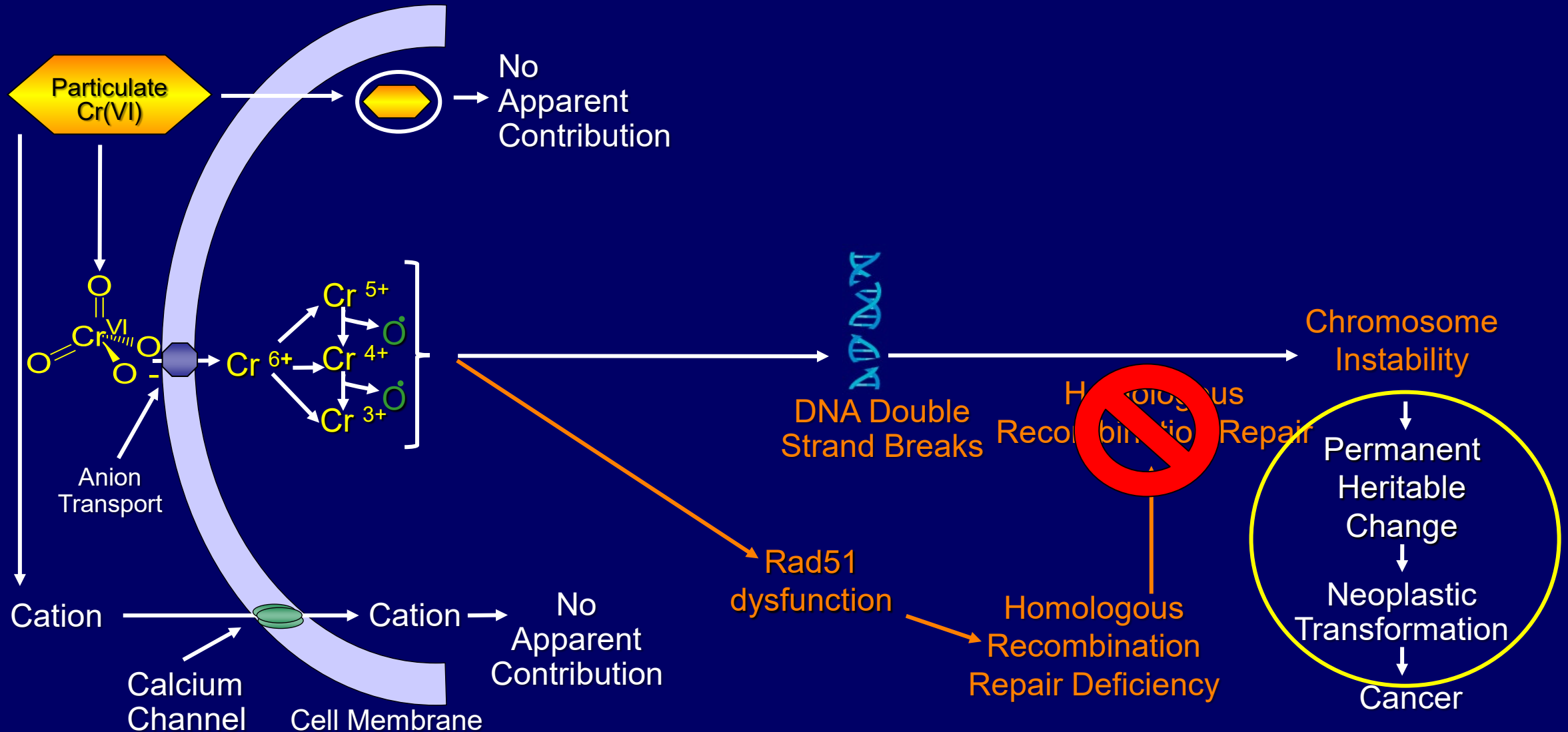
Does this matter? Do  
Cells with Cr(VI)-  
Induced Repair  
Deficiency and  
Chromosome Instability  
Survive



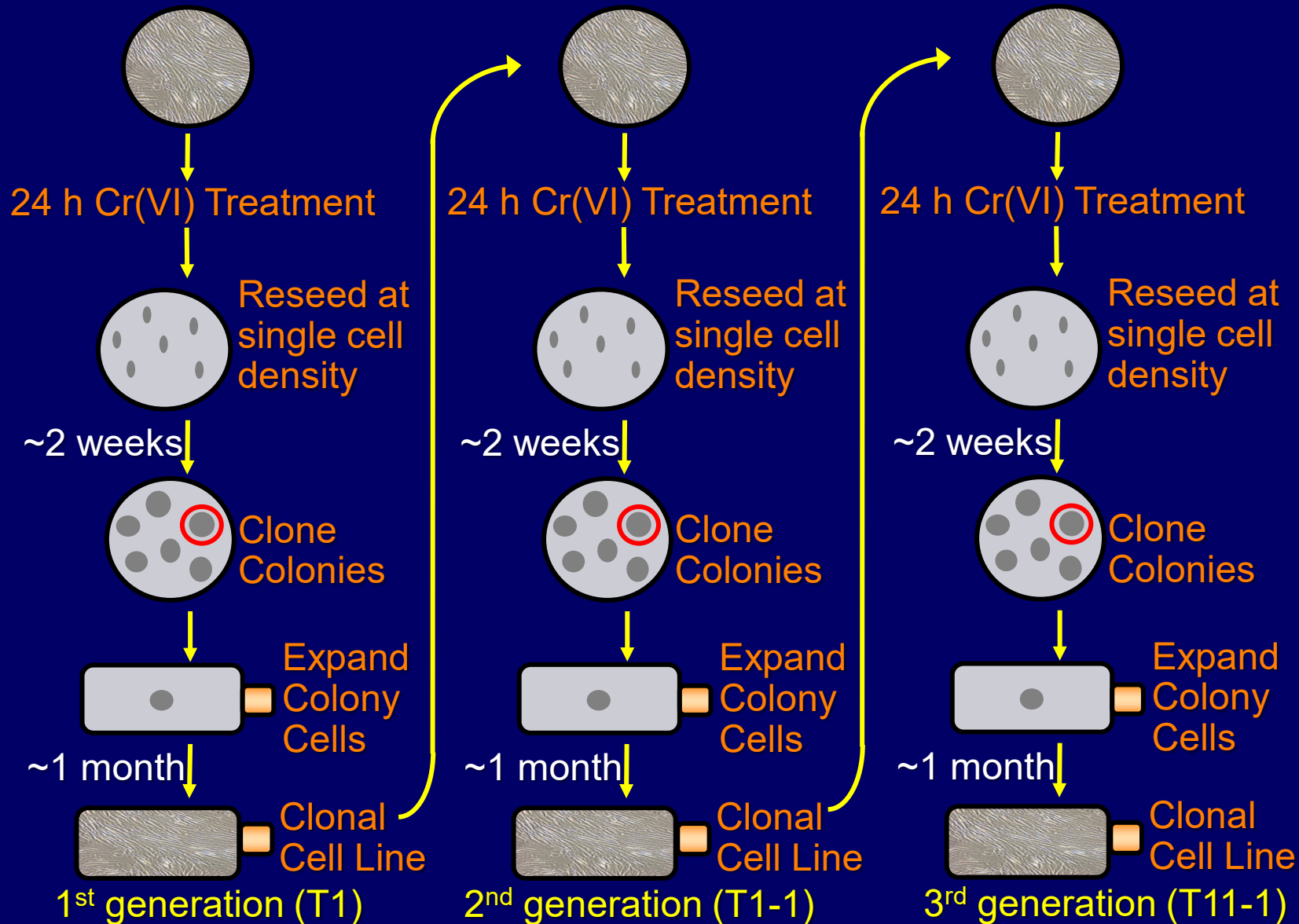
Hypothesis:  
Particulate Cr(VI)  
induces a permanent  
phenotypic change

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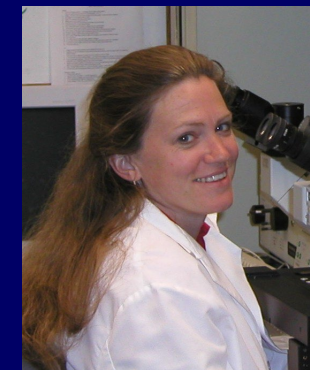
# Physico-Chemical-Biological Particulate Cr(VI) Mechanism in Human Lung Cells



# Clonal Expansion Experiment

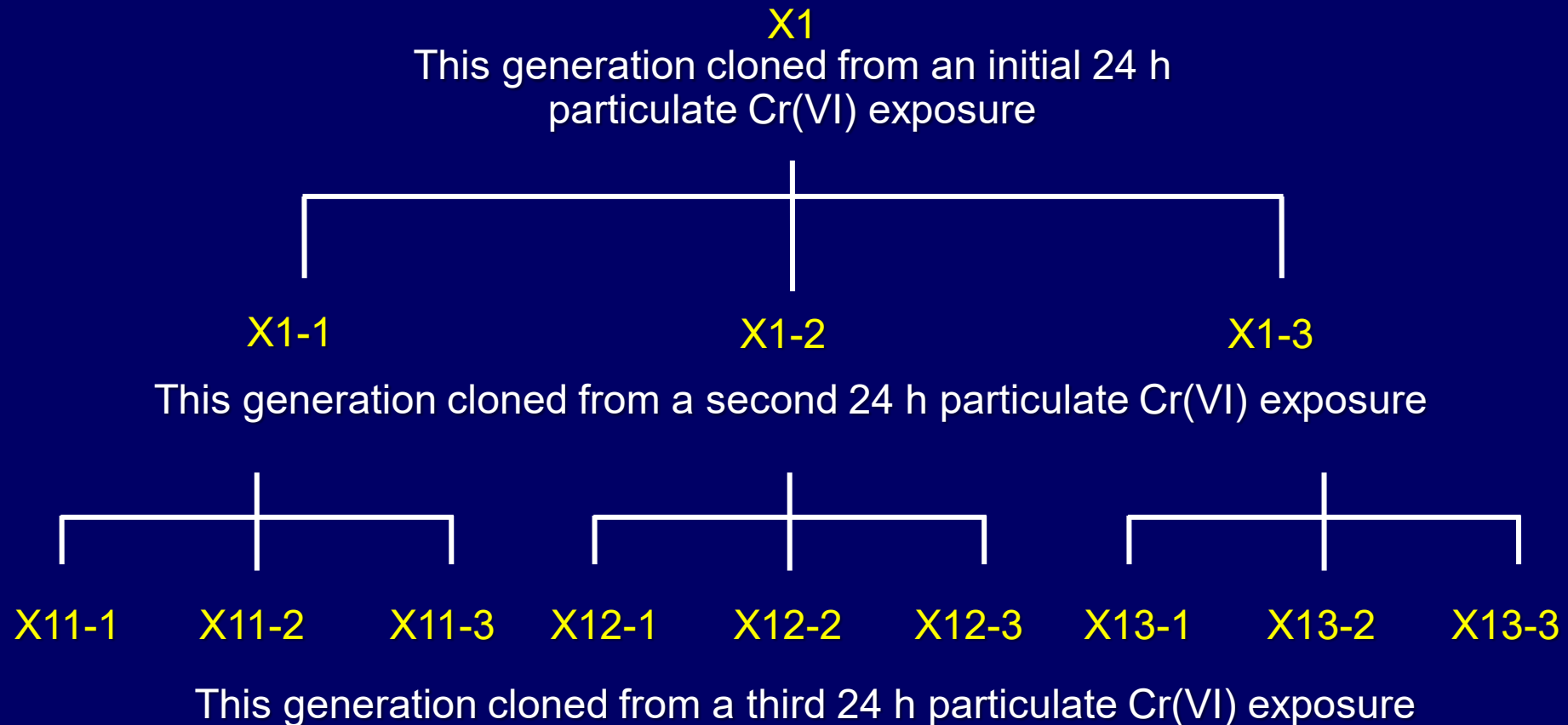


- ◆ Both treated and untreated for a 'treated clone' set & an untreated 'control clone' set
- ◆ Since each cell line emerges from an individual cell – phenotypes reflect permanent changes
- ◆ 91 Control Clones & 62 Treated Clones
- ◆ Characterize each generation for chromosomal & other changes

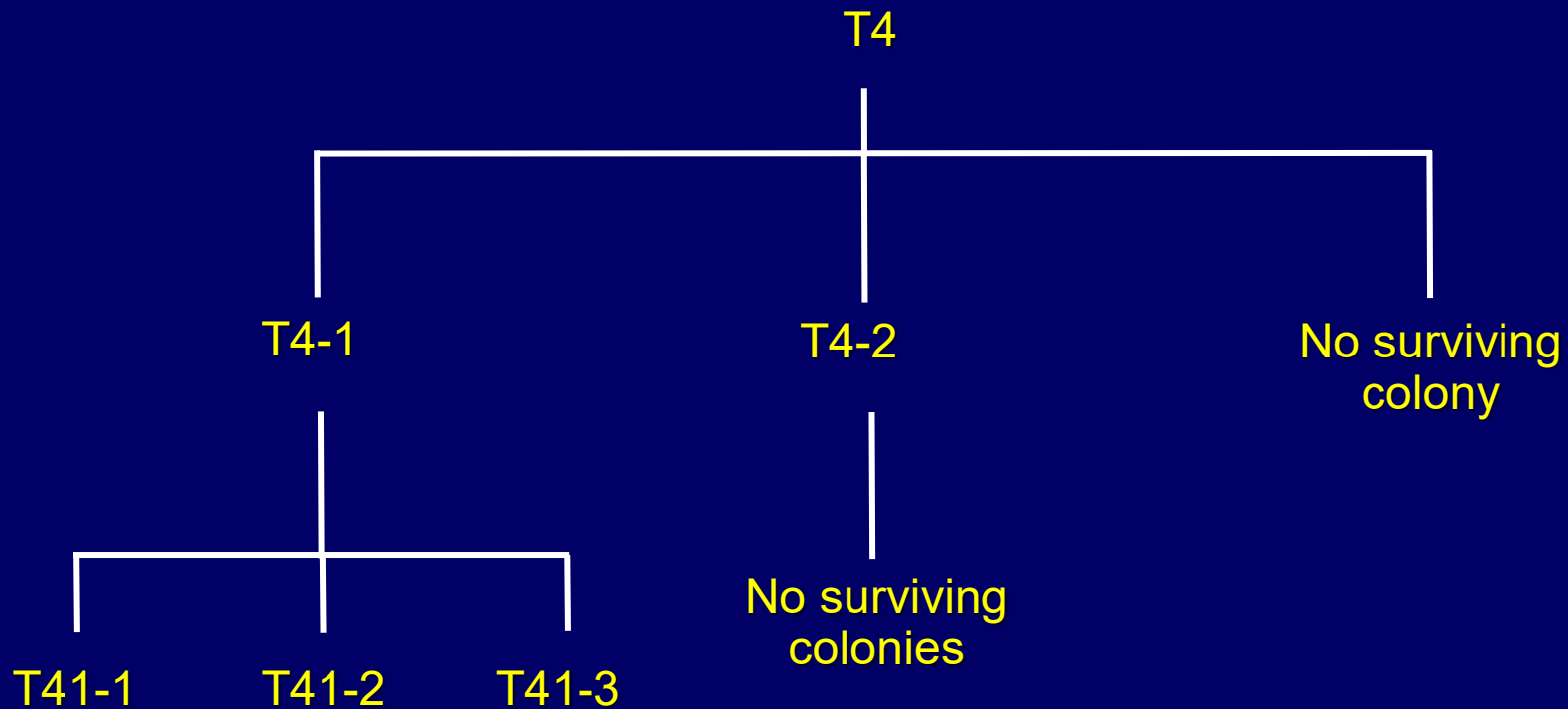


Sandra Wise

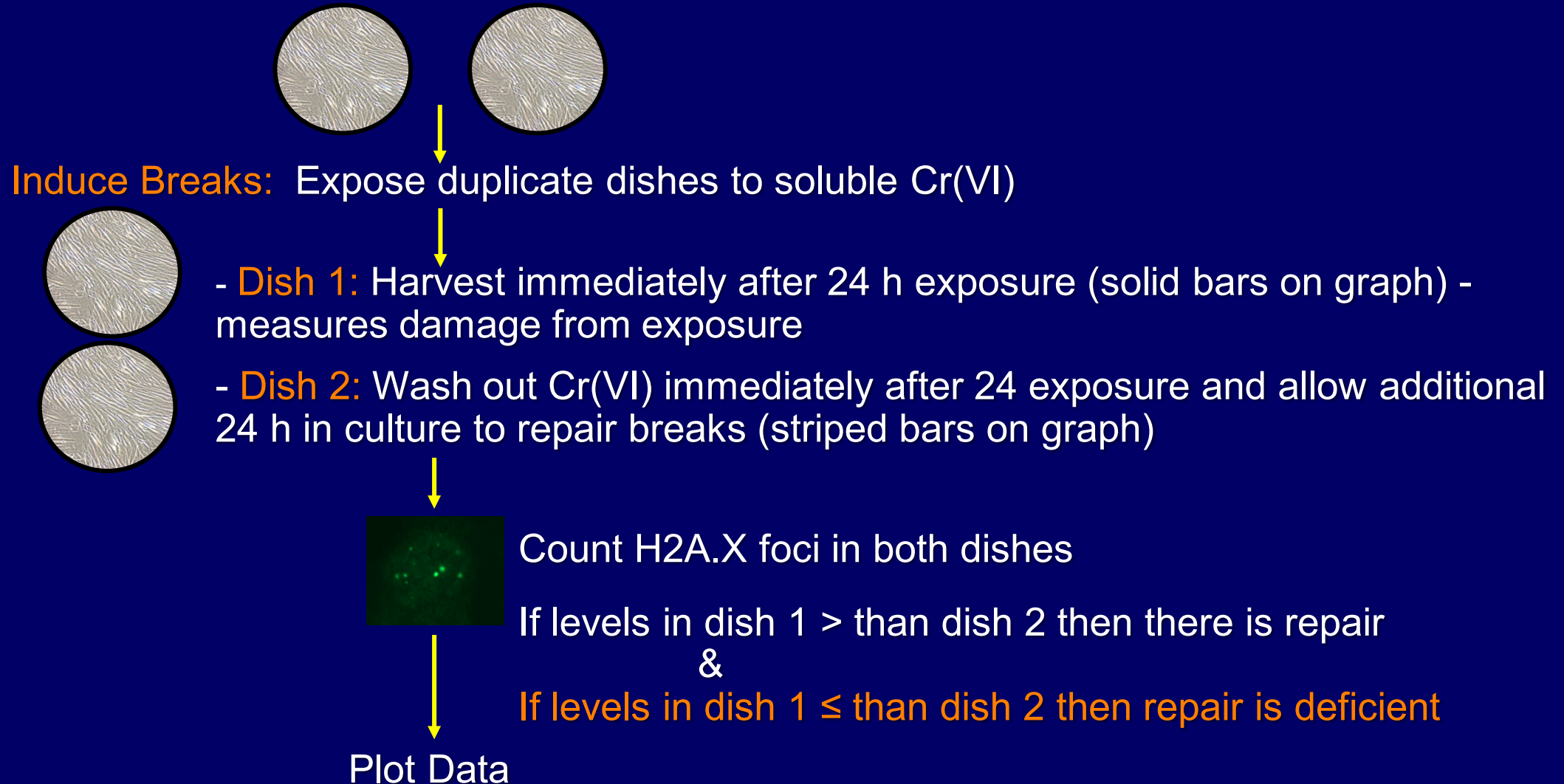
# Pedigree of Treatment Protocol



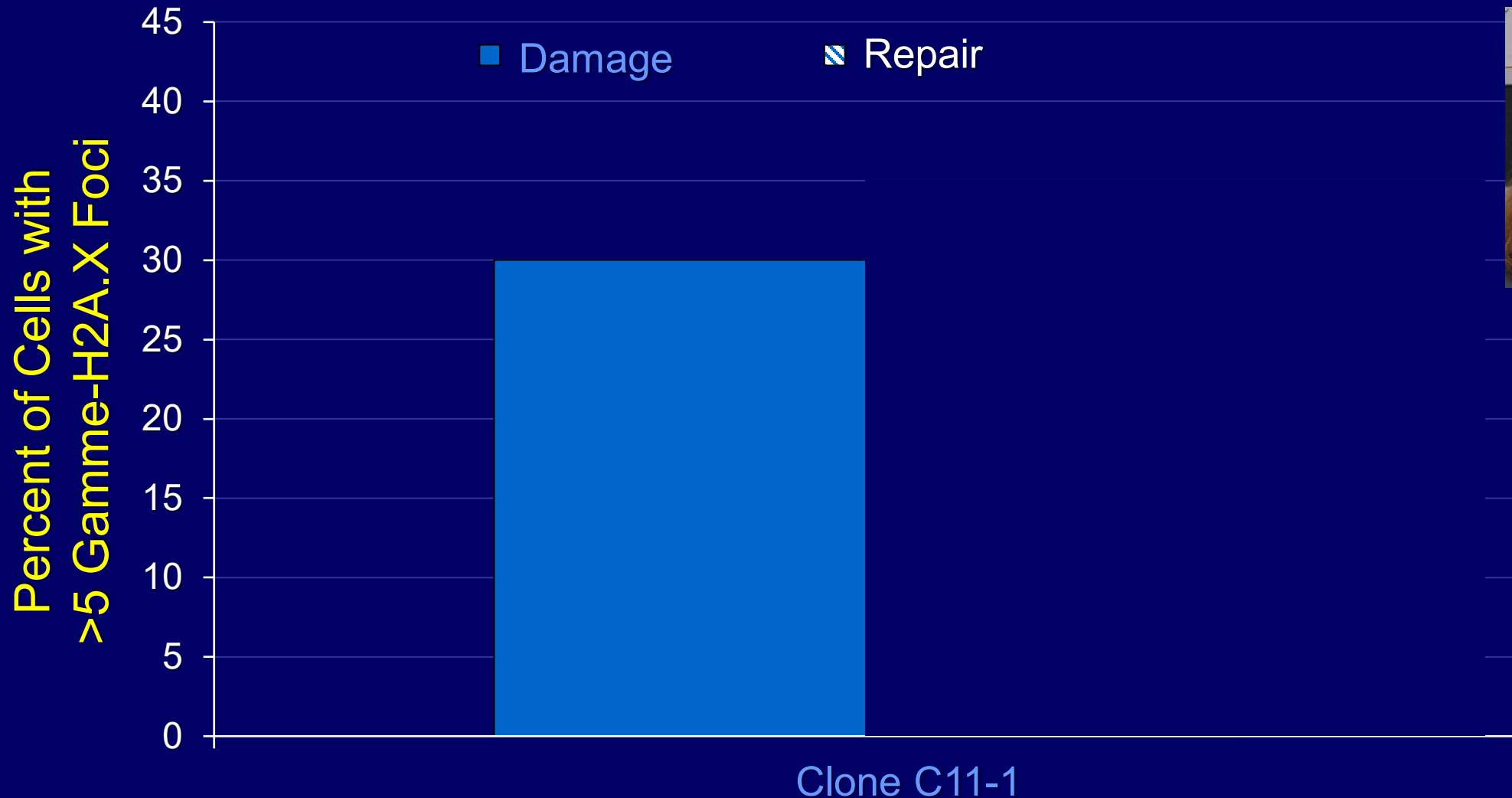
# Pedigree of Treated Clone Family T4 (1 of 7 Treated Clone Families)



# Measuring DNA Double Strand Break Repair in the Clones



# Representative Example of Repair in a Control Clone



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Wise

# Representative Example of Repair in a Treated Clone with Deficient Repair

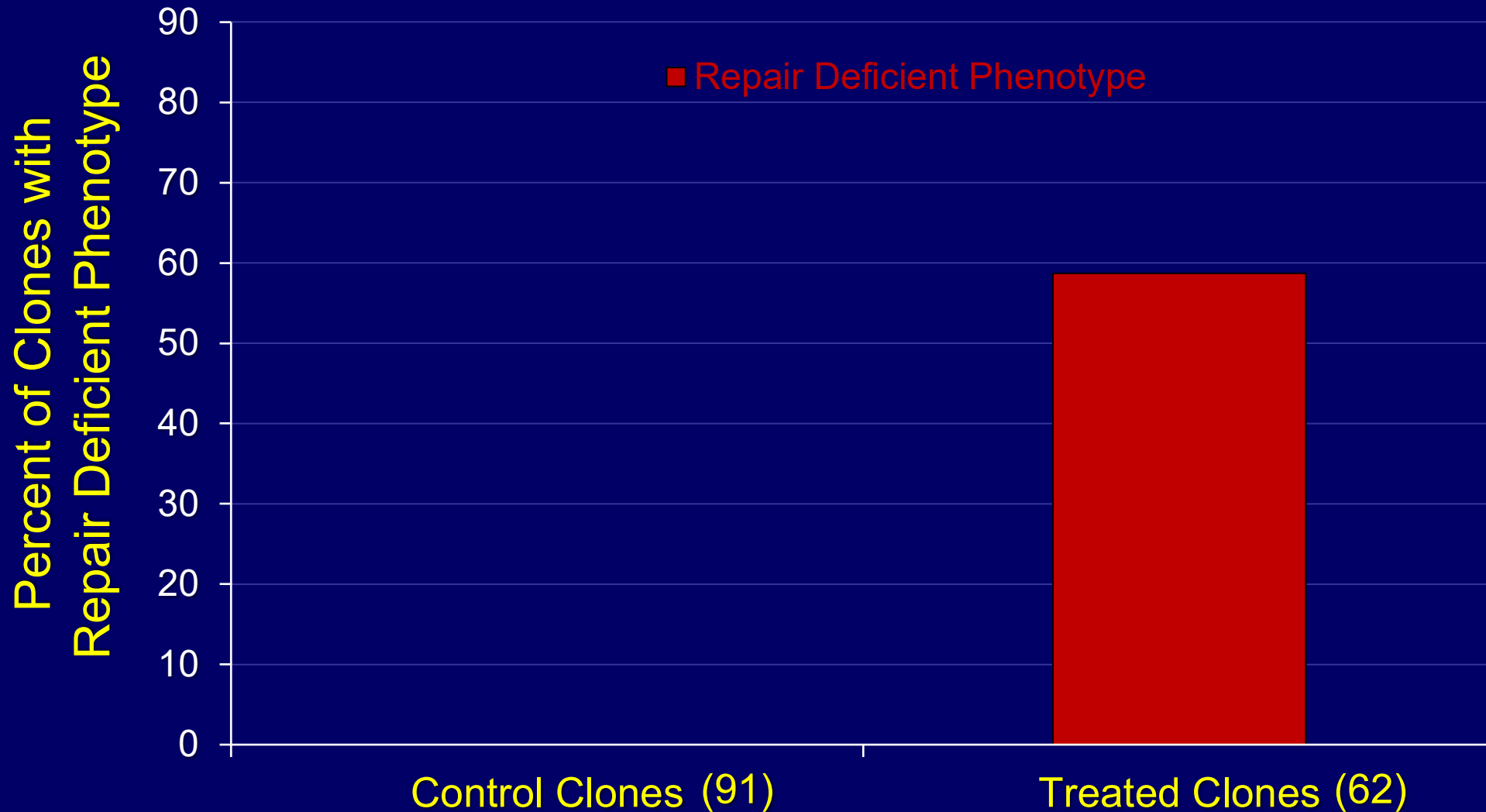


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Clone T41-2

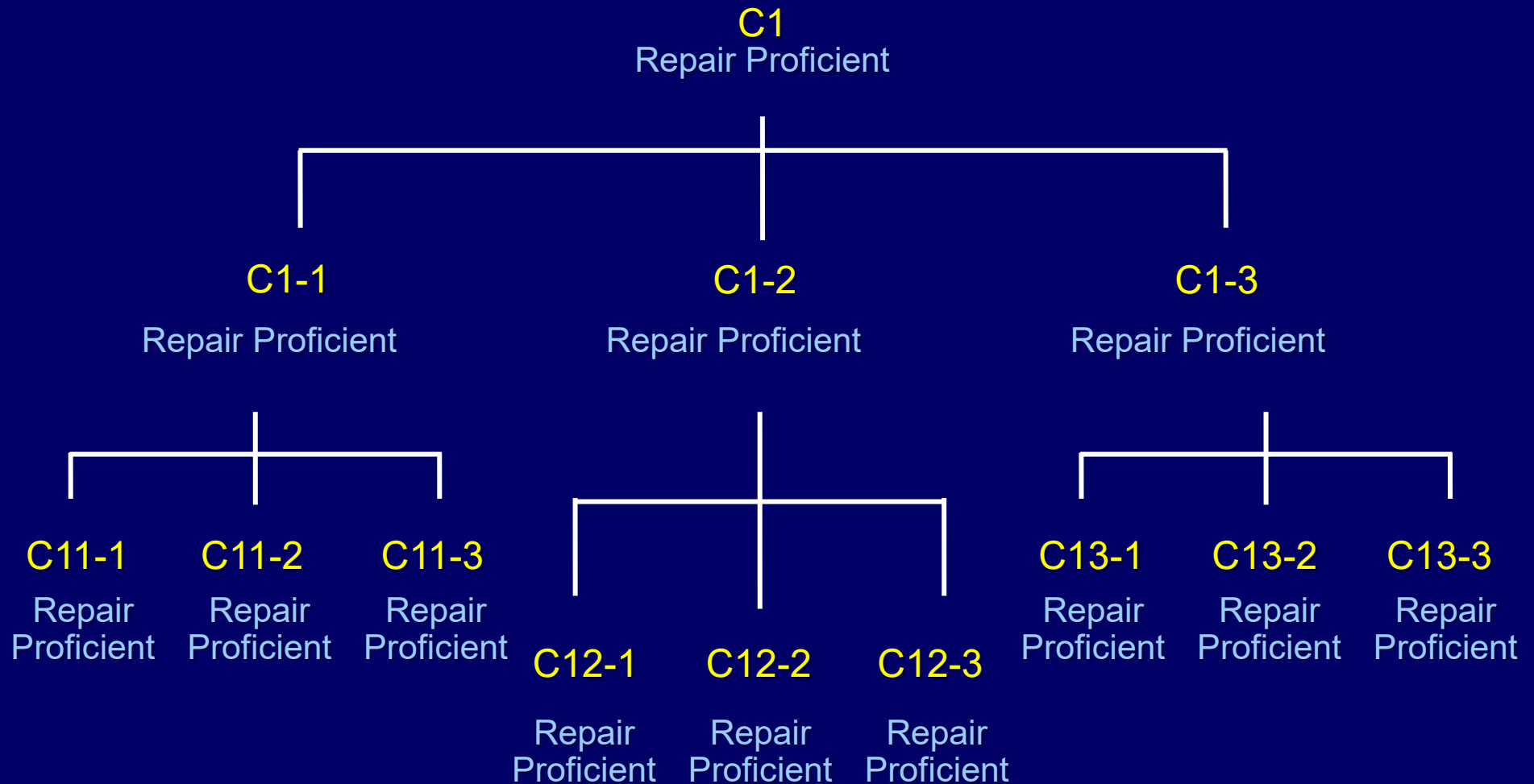


# Cr(VI) Induces Permanent DNA Repair Deficiency

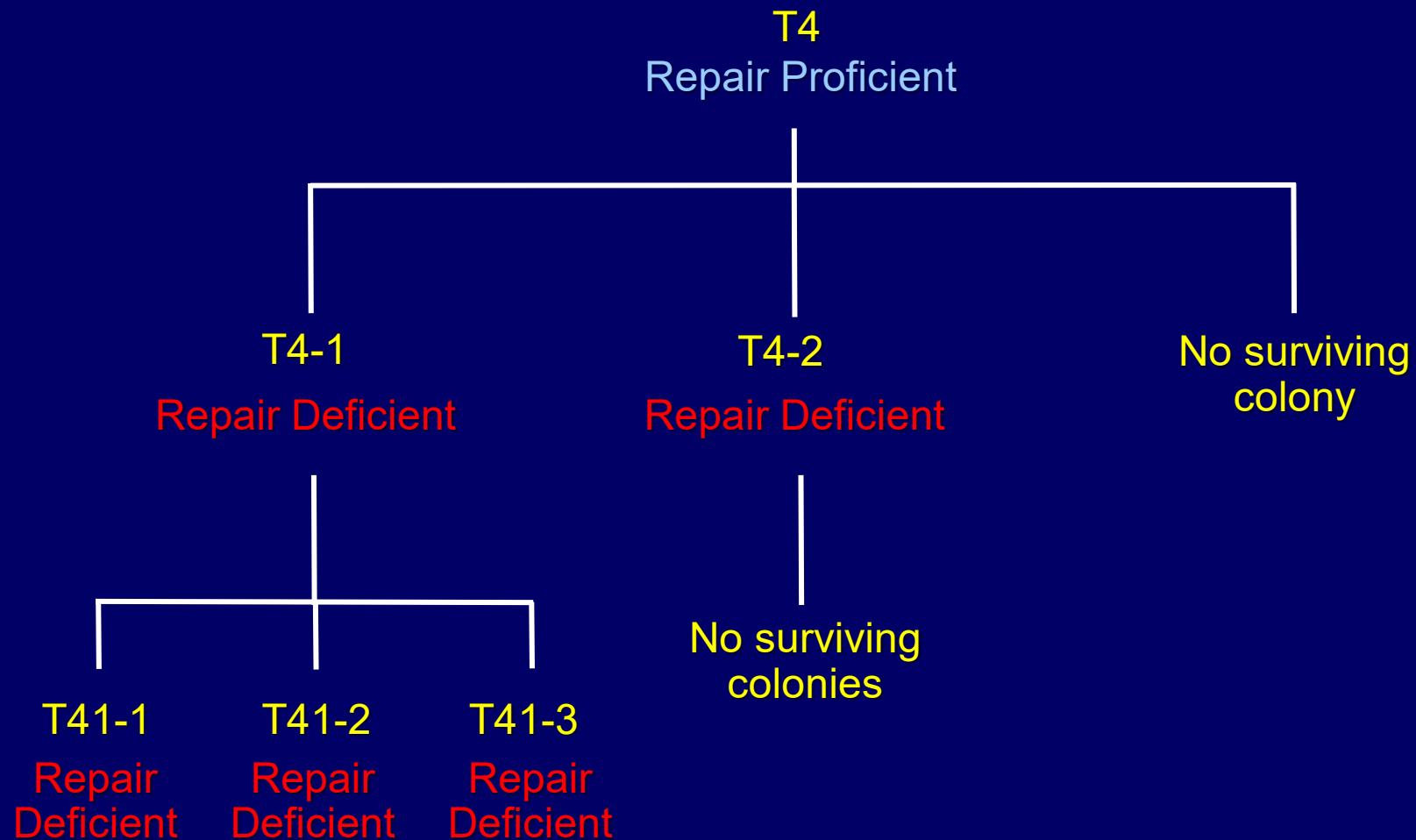


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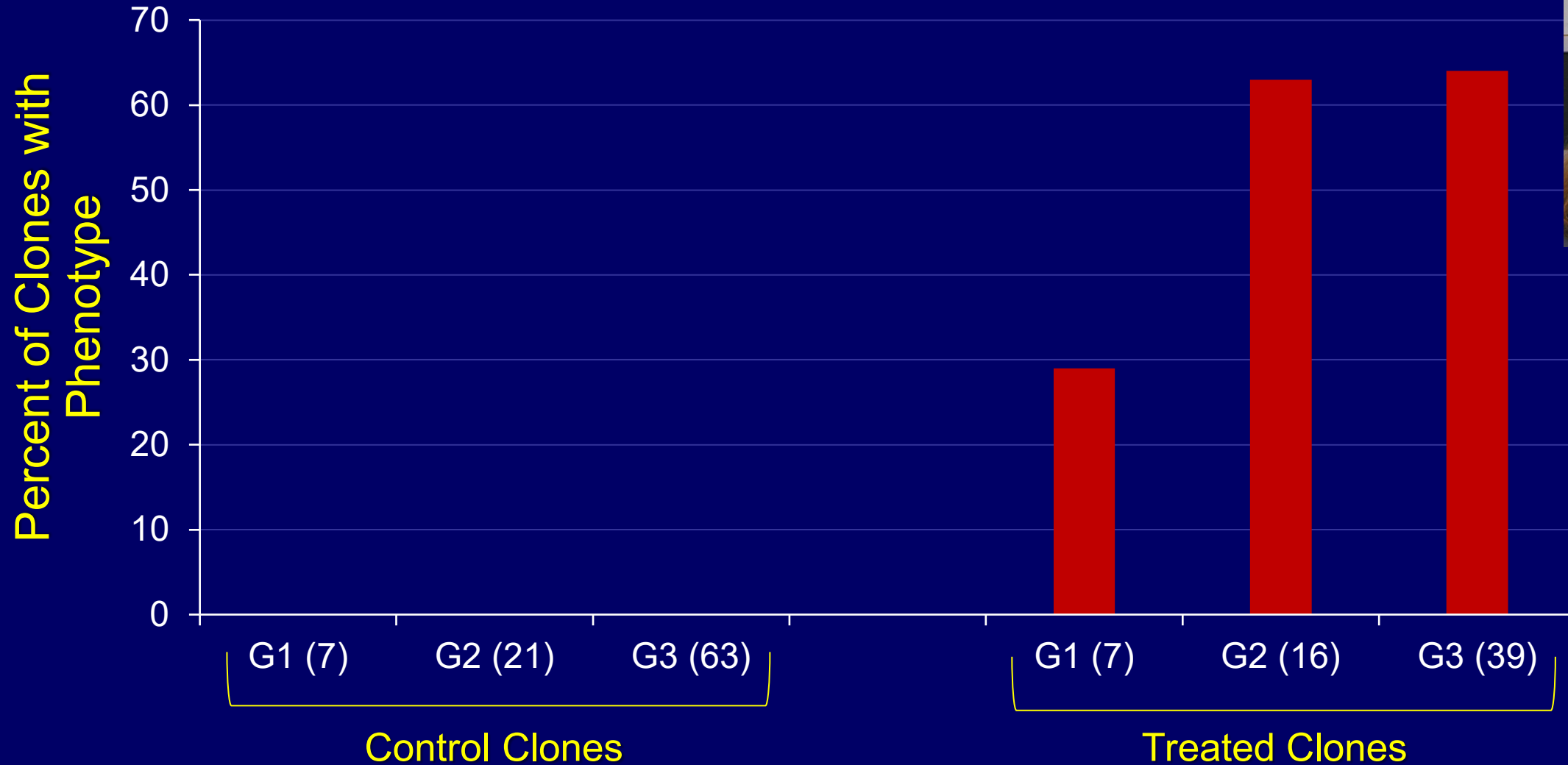
# Pedigree of Control Clone Family C1 (1 of 7 Control Clone Families)



# Pedigree of Treated Clone Family T4 (1 of 7 Treated Clone Families)

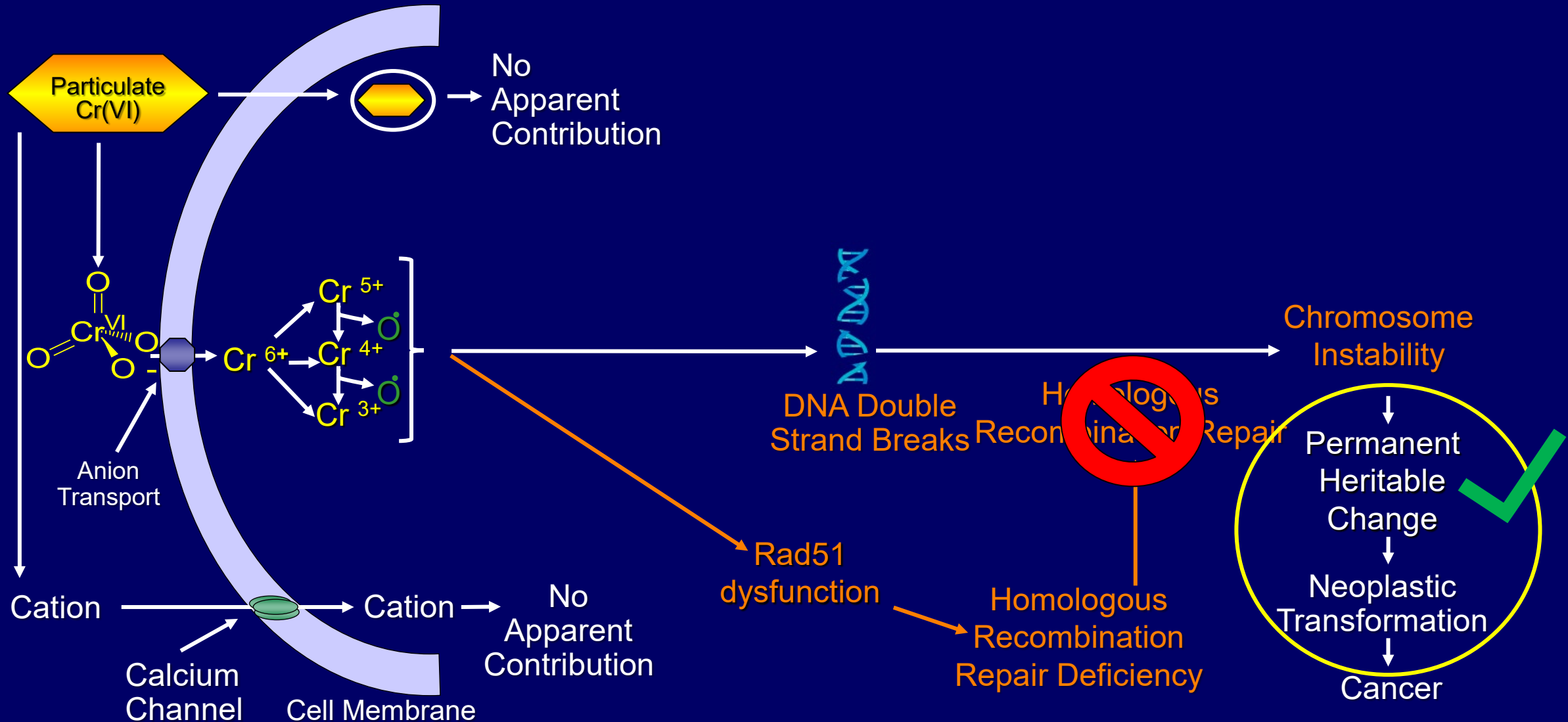


# Cr(VI) Induces a Heritable Phenotype of Repair Deficiency



Sandra  
Wise

# Physico-Chemical-Biological Particulate Cr(VI) Mechanism in Human Lung Cells



Does this matter? Do  
Cells with Cr(VI)-  
Induced Repair  
Deficiency and  
Chromosome Instability  
Survive

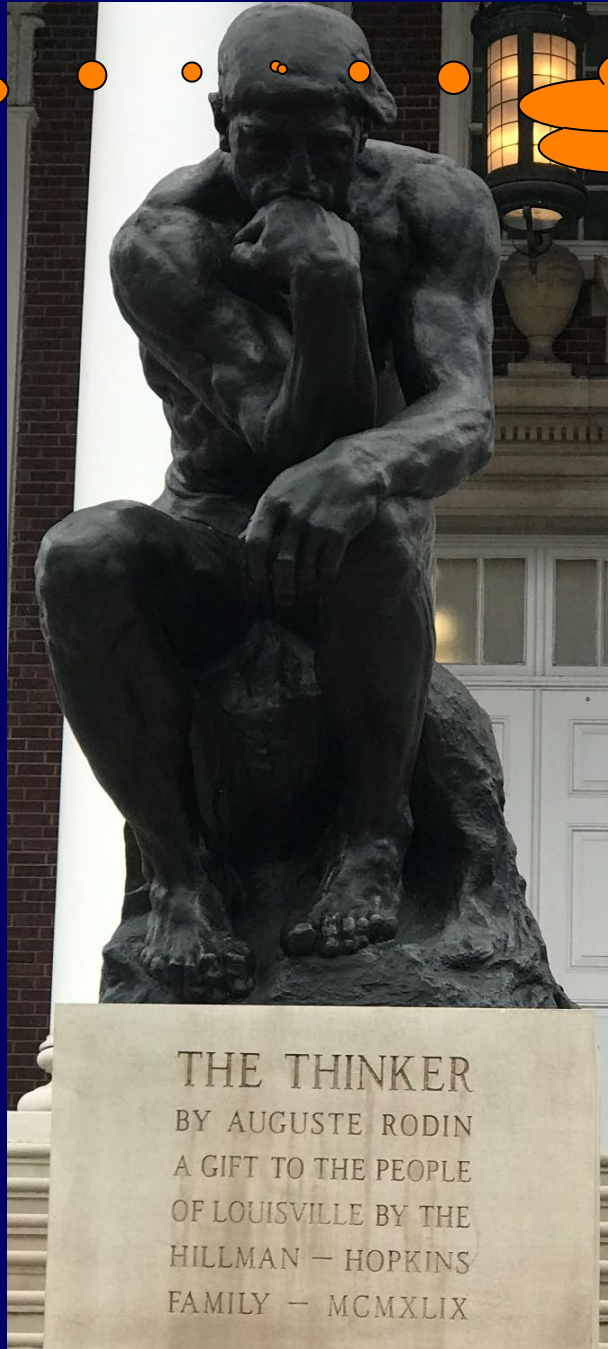


Hypothesis:  
Particulate Cr(VI)  
induces a permanent  
phenotypic change

YES

- ✓ YES - permanent changes in:
- Ability to Repair DNA Breaks
  - HR Repair Function
  - Chromosome Number
  - Chromosome Structure

What's next?



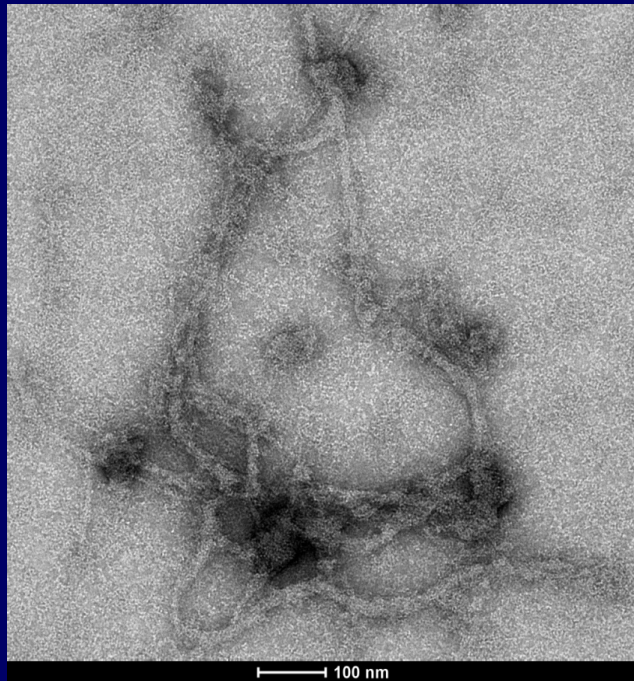
Who helped with all of this work?

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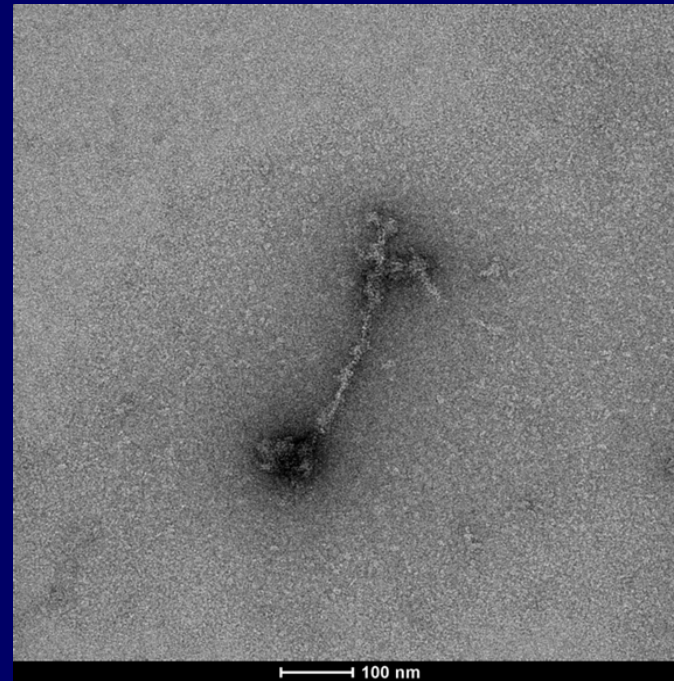
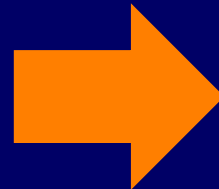
## Future Course

Investigate what chromium is doing to cause these effects. Questions like:

- What does Cr bind to?
- How does it displace RAD51 from the nuclear protein filament?
- Many others!



Cr





## Future Course

Translate these outcomes from cells to animals and people

### Poster 12

“Translating Particulate Hexavalent Chromium-Induced Genetic Impacts from Human Lung Cells to Lung Tissue”



Dr. Haiyan Lu

# Acknowledgements

## Wise Laboratory of Environmental & Genetic Toxicology

### Faculty

Calvin Joseph Kouokam  
Sandra Wise

### Postdoctoral Fellow

Haiyan Lu

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Qin Qin  
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Kentucky Lung Cancer Research Program {JPWSr}

## Animal Use Protocol

Work was conducted under UofL IACUC approval #18272

## Images

All photos were either taken by the Wise Laboratory or credit is provided with the individual photo and is available upon request. Rat cartoon was drawn by Chunlei Qian

“The content is solely the responsibility of the presenters and does not necessarily represent the official views of the National Institute of Environmental Health Sciences or the National Institutes of Health or any of our other funders.”

Questions?



[www.WiseLaboratory.org](http://www.WiseLaboratory.org)

Exposure Time (h)	Amount of Inhaled Cr(VI) - Ambient Air (ug)	Amount of Inhaled Cr (VI)- Occupational Air (ug)	Amount of Cr(VI) added to Cell Cultures (ug)
24	0.06 – 6	19	0.06 – 1.8
120	0.3 – 30	96	0.06 – 1.8