

M1D2:

Prepare and treat cells for foci experiment

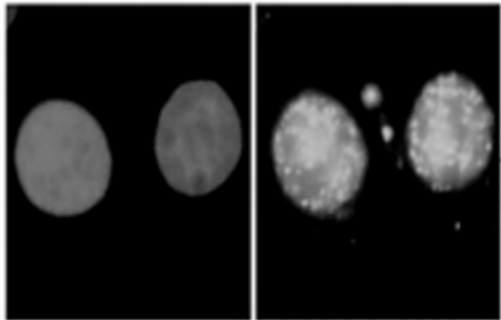
1. Prelab discussion
2. Demonstration of coverslip preparation step
3. Begin gamma-H2AX experiment



Tissue culture

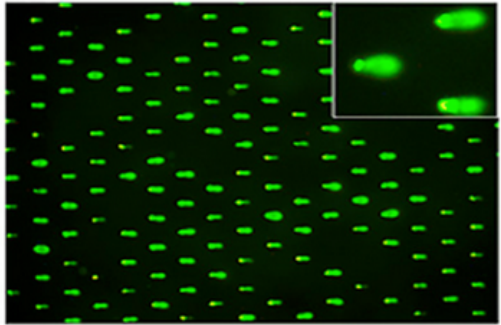
Overview of M1: genomic instability

Research question: Does exposure to As inhibit, or decrease, repair of H₂O₂-induced DNA damage, raising the possibility that combined exposure is an important risk to public health?



1. Use repair foci experiment to measure DNA breaks

- Examine effect of H₂O₂ +/- As on double strand DNA breaks by measuring γ H2AX foci formation



2. Use high-throughput genome damage assay to measure DNA damage

- Measure effects of H₂O₂ +/- As on DNA damage by measuring DNA migration in agarose matrix



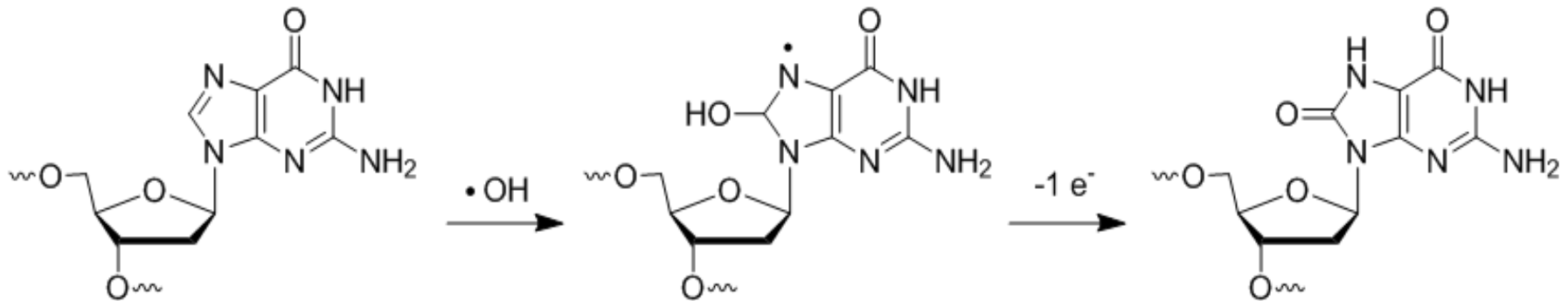
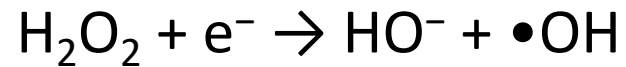
gamma-H2AX assay measures DNA DSB



- ATM kinase phosphorylates H2AX histone at Ser129 in response to DSB
 - H2AX histones near the DSB are phosphorylated
 - Phosphorylated H2AX = gamma-H2AX
- gamma-H2AX can be marked and visualized to measure DSB abundance in cells

How does H₂O₂ cause DNA damage?

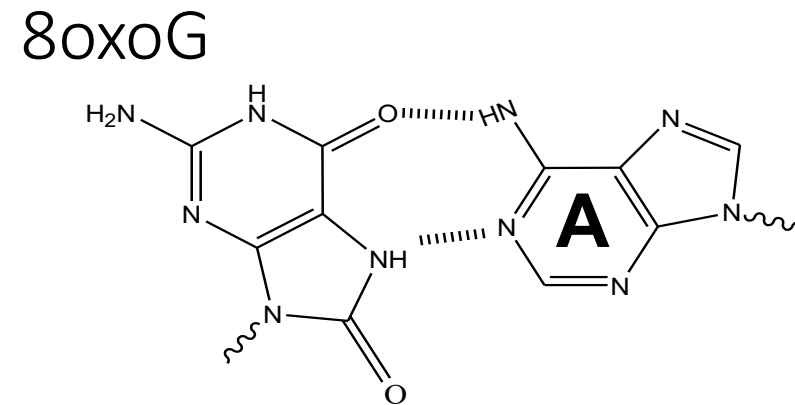
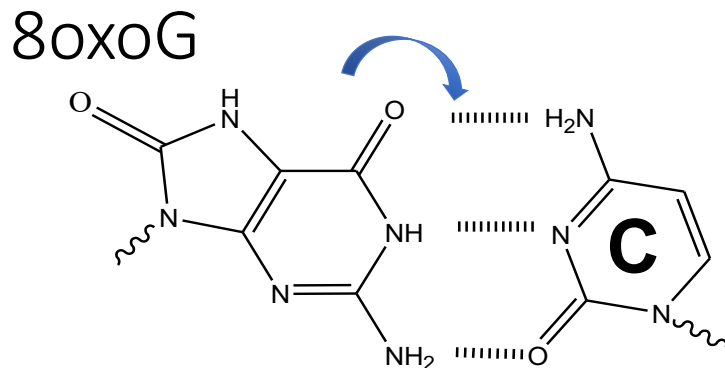
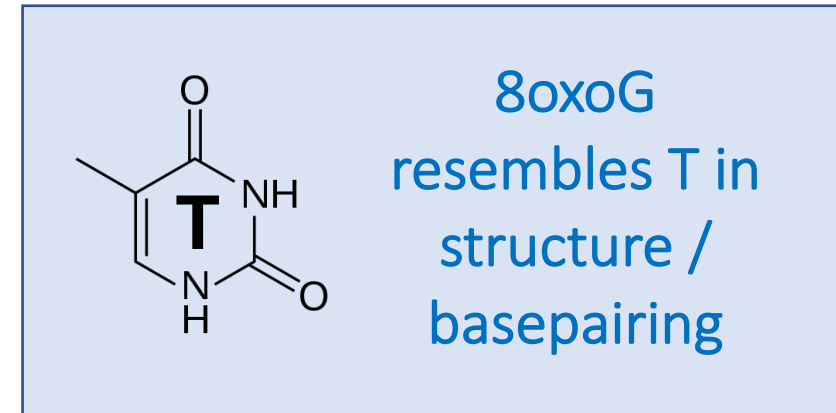
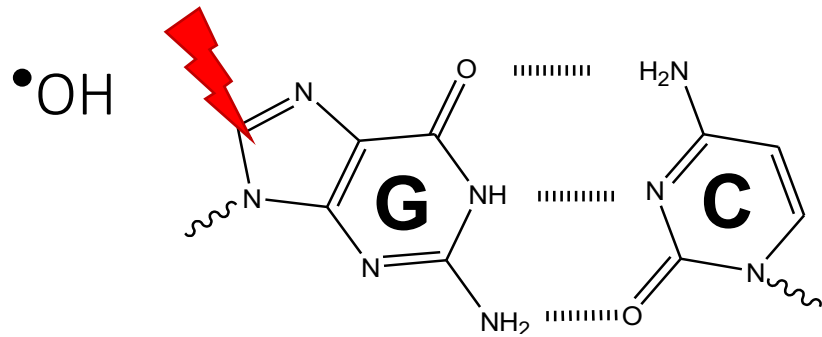
What is an oxidizing agent?



guanine (G)

8-oxo-guanine (8oxoG)

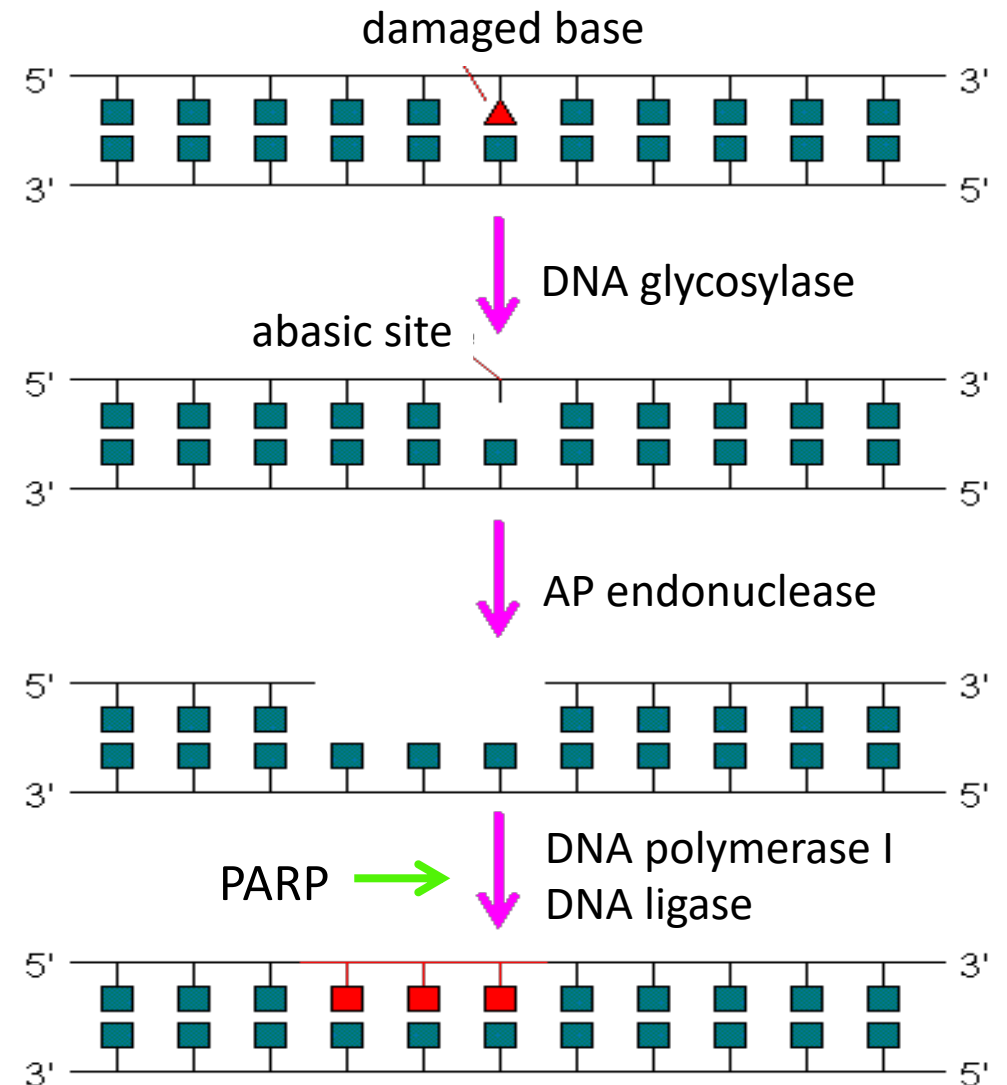
How does H₂O₂ damage lead to mutations?



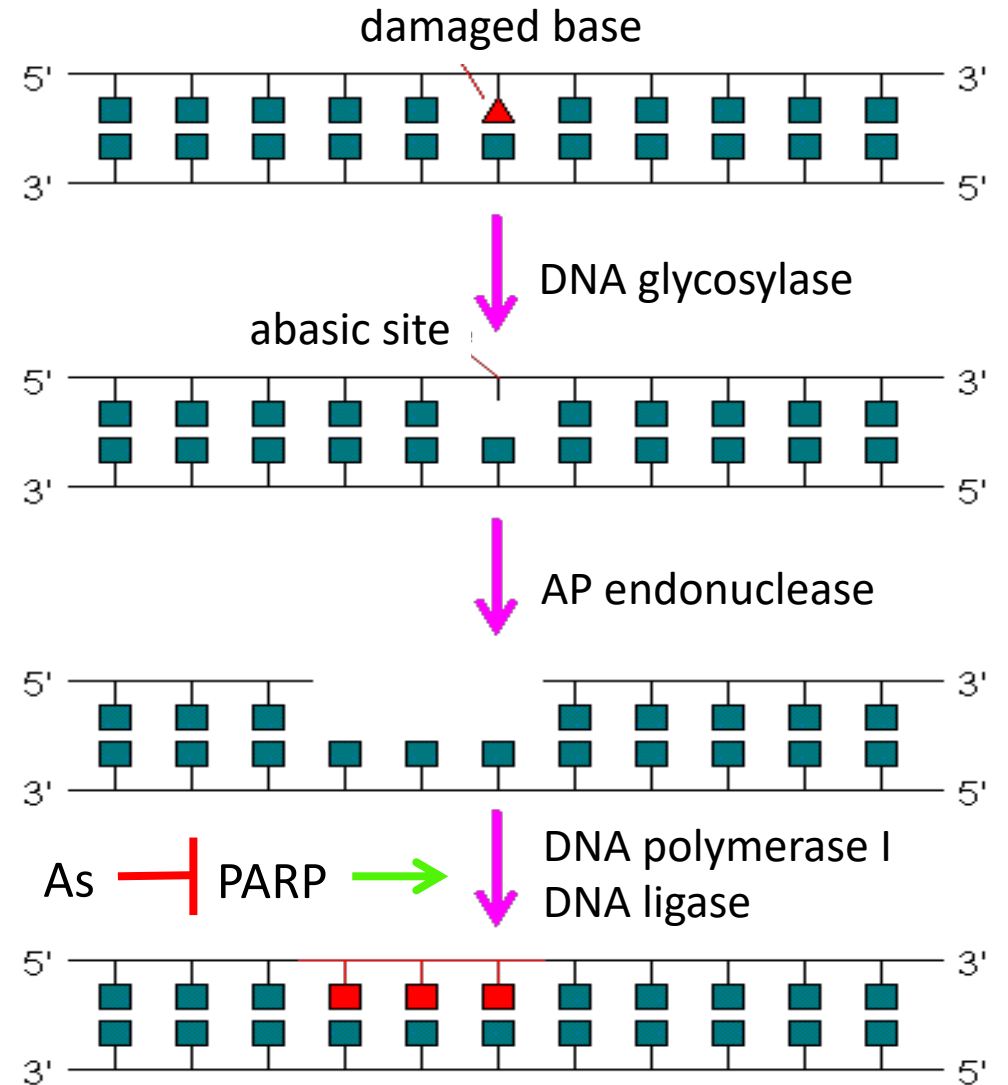
What mutation results if replication occurs before repair?

BER pathway repairs oxidative damage

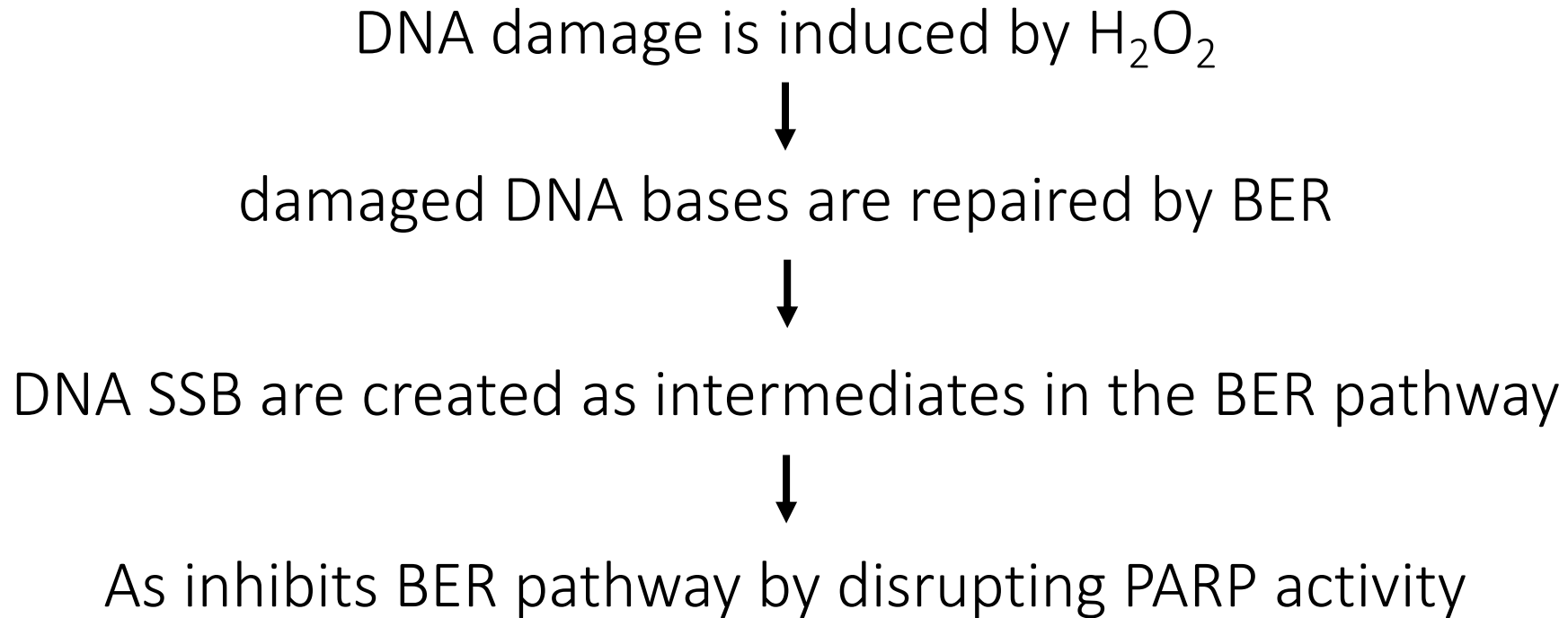
- DNA glycosylase cleaves glycosidic bond to remove damaged base
 - Glycosylases recognize specific lesions
- AP endonuclease cleaves DNA backbone creating SSB
- DNA polymerase incorporates correct bases using template strand
- DNA ligase seals SSB in the backbone
- **PARP recruits enzymes involved in repair pathway**



How does As inhibit the BER pathway?



Taken together...



So why are we using the gamma-H2AX assay to measure DSB?

Experimental notes: treatment conditions

Research question: Does exposure to As exacerbate DNA damage induced by H₂O₂?

Experimental overview: Incubate cells with As then treat with H₂O₂

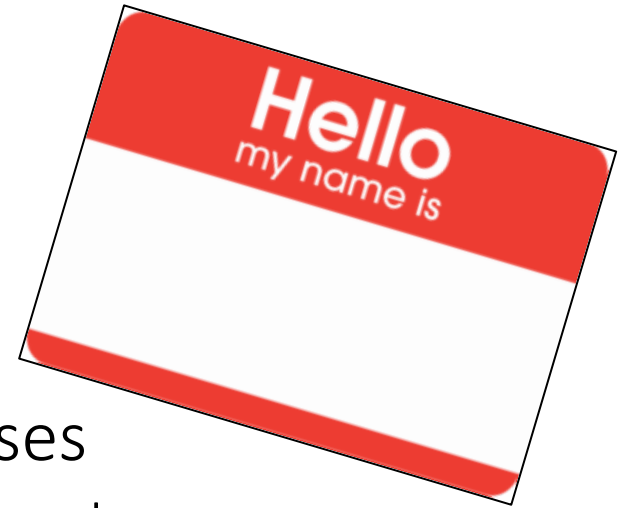
Experimental Condition(s)

Control Condition(s)

Demonstration of coverslip coating procedure

For today...

- What is your team name?
- Respond to Office hours email
- Work through gamma-H2AX procedures / exercises
 - Be sure to record your notes in your laboratory notebook



For M1D3...

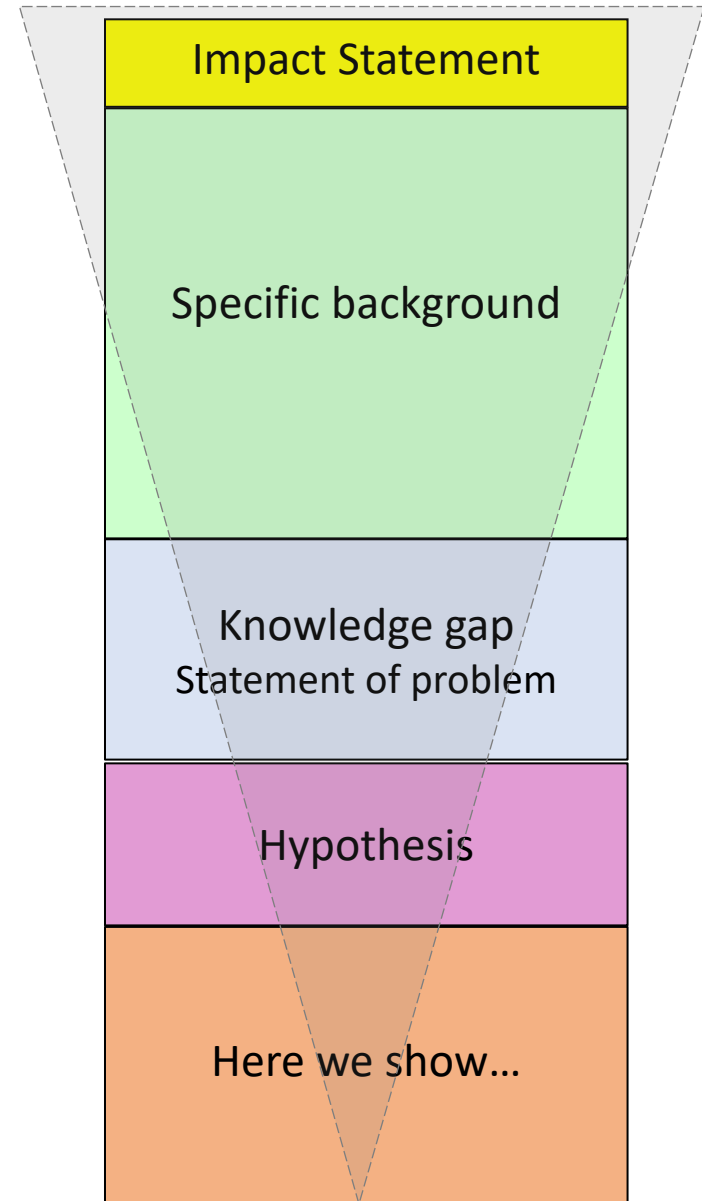
- Write topic sentences for the Background & Motivation section of the Data summary
- Review paper for in-class discussion

Notes on Background & Motivation section...

- Anchor your research in a general topic that is important to a broad audience
 - Focus on describing what is currently known in the field
 - Reference the relevant research in the field
- Connect your research to the general topic
 - Minimum essential information
 - Introduce specific technologies necessary for understanding your specific project
- Address how you will expand on what is currently known
 - Include evidence of incompleteness of current understanding
 - Motivate your investigation
 - **Include a clear hypothesis / research goal**
- Provide a preview of your findings and the implications
 - Tie back to the initial general topic
 - Avoid including extensive methods details

Notes on topic sentences...

- **Topic sentence = first sentence of each paragraph**
- Should 'funnel' from big picture topic to your specific research question / project
 - Provide only the background needed to understand research / problem / goal
 - Clearly state what is not currently known
 - Address how you will fill knowledge gap
 - Provide preview of your results
- Include references!!



How should you introduce your story?

1st paragraph: what is the big picture / problem?

2nd paragraph: what is currently known?

3rd (or 4th) paragraph: what is your research question?

4th (or 3rd) paragraph: how will you address your question?

5th paragraph: here we show...

