WIKI Part 2: Treat cells for γH2AX assay

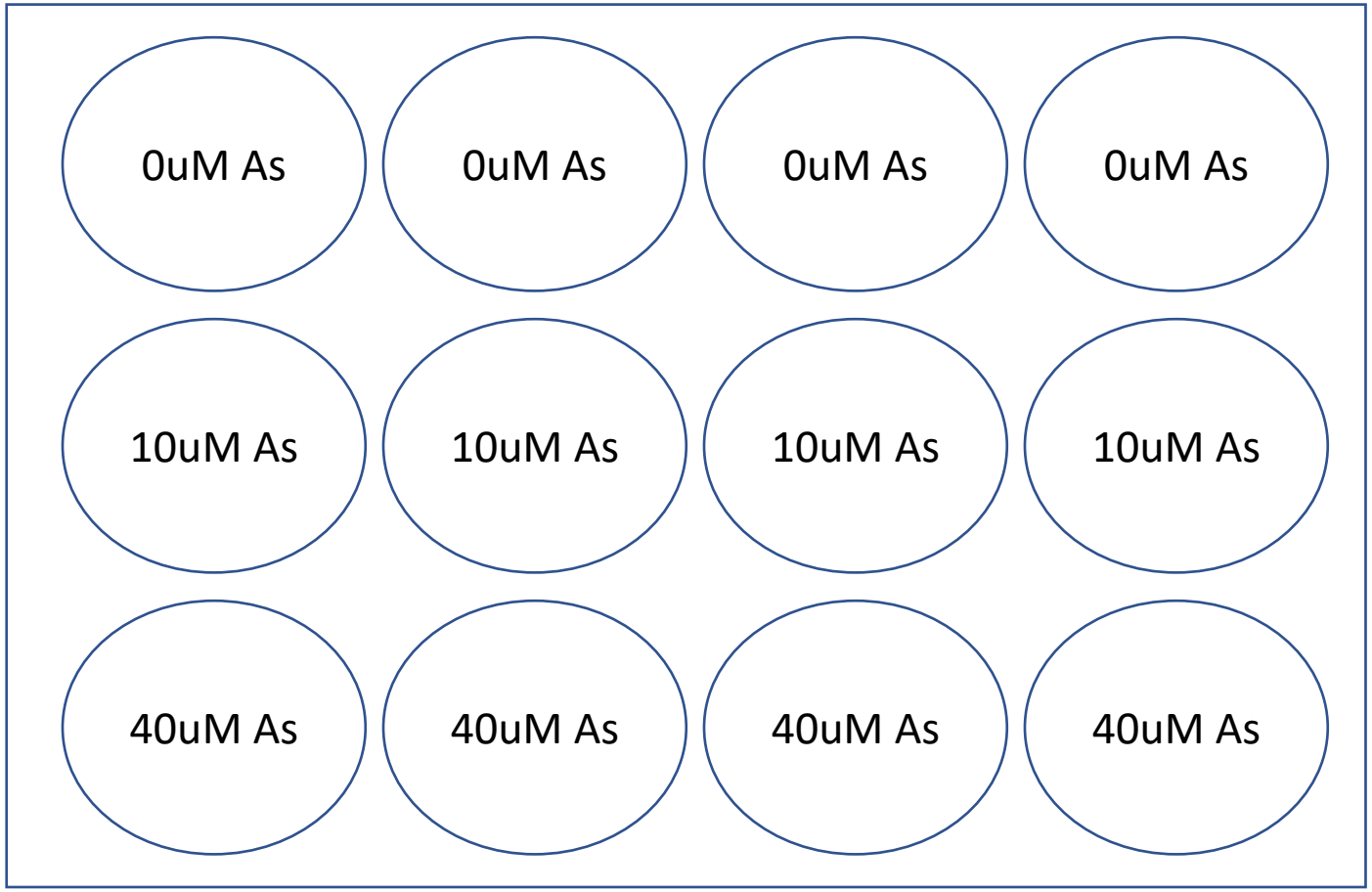
Expose cells to As

Before going into TC,

•Calculate the dilution of As stock needed to prepare a final concentration of 10 µM and 40 µM in 5 mL of media.

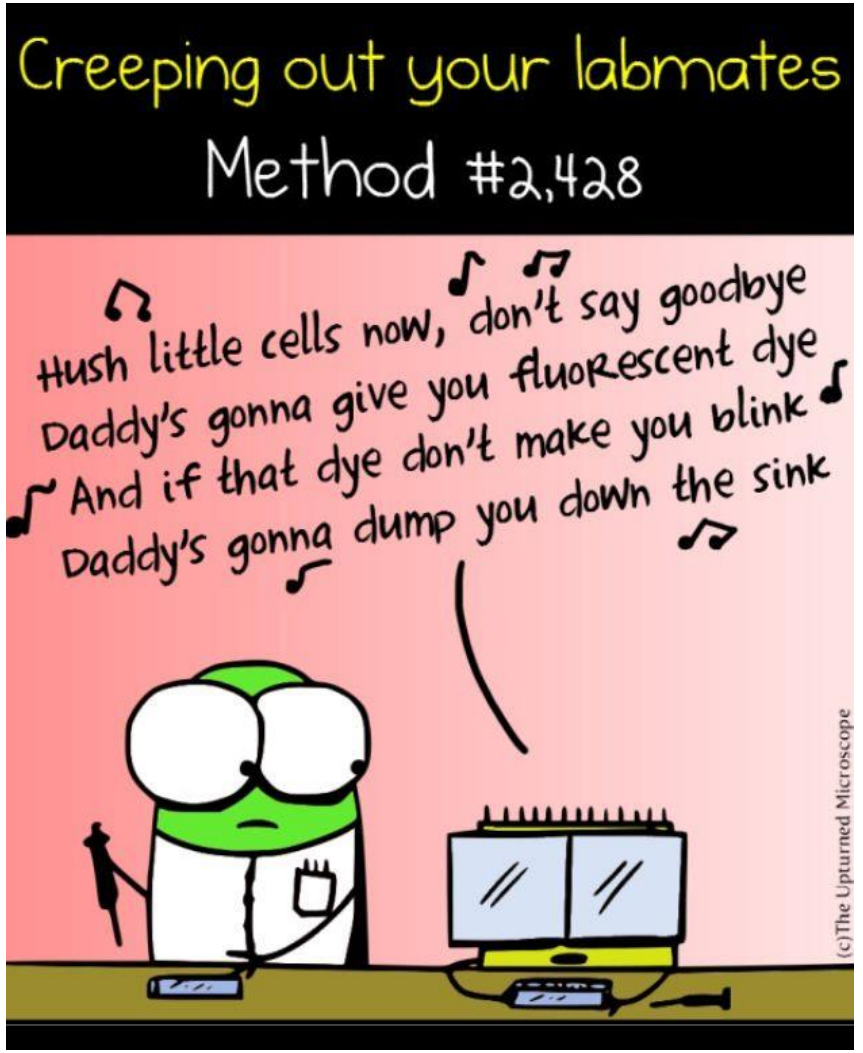
- Stock concentration of As is 10 mM.

- In TC, aspirate the spent media from your coverslips and replace it with media containing arsenic according to the chart to the side (1ml for each well)



M1D2: Prepare and treat cells for γ-H2AX experiment

1. Prelab
2. Treat and fix primary MEF cells for H2AX assay
3. Work on Background and Motivation section



Admin Notes:

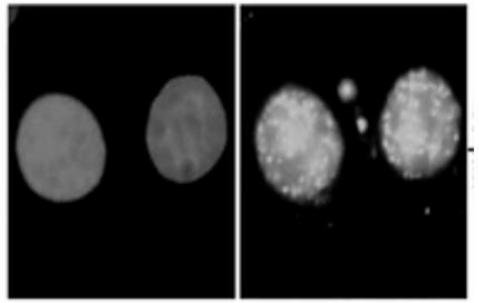
- Sign EHS training roster for lab-specific training
- If you haven't already, respond to Noreen's office hours email

Mod1 Overview

Last lab: Seeded cells on coverslips for gamma H2AX experiment

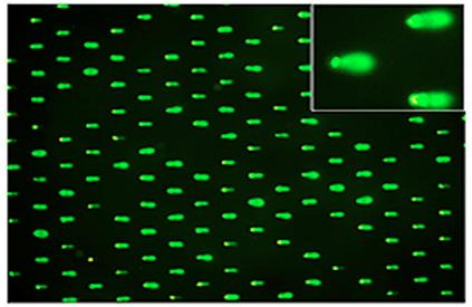
This lab: Treat cells with As and H2O2, then fix cells.

Next lab: Immunofluorescence staining for gamma H2AX



1. Use repair foci experiment to measure DNA breaks

- Examine effect of H_2O_2 +/- As on double strand DNA breaks by measuring γH2AX foci formation



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

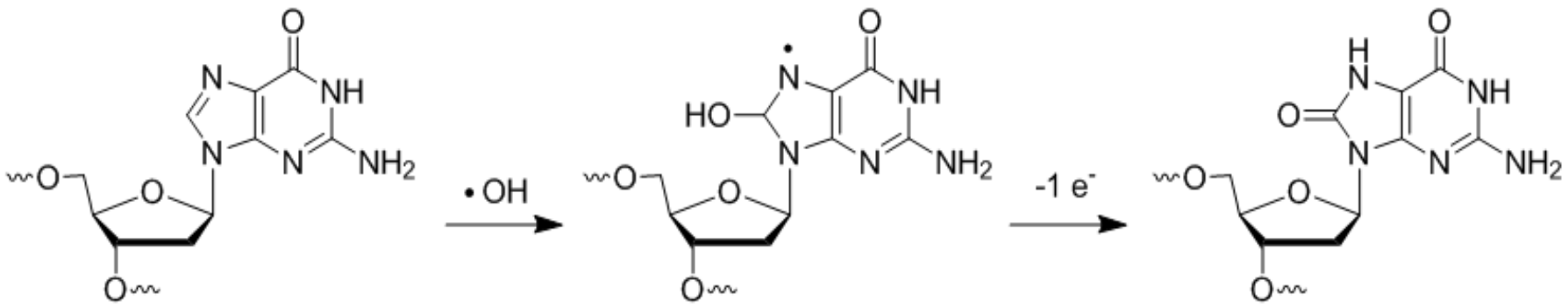
- Measure effects of H_2O_2 +/- As on DNA damage by measuring DNA migration in agarose matrix

How does H_2O_2 damage DNA?

By producing Reactive Oxygen Species

$H_2O_2 + e^- \rightarrow HO^- + \bullet OH$

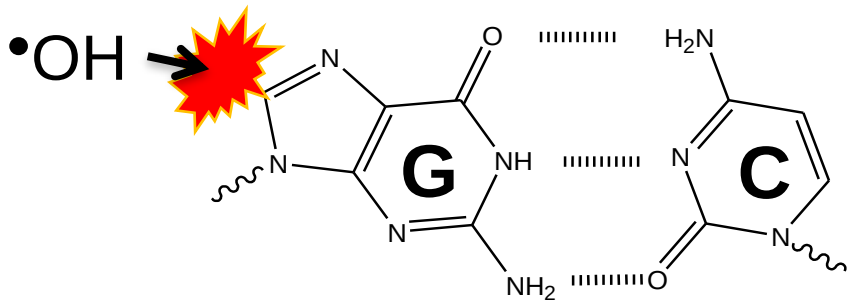
$2\ H^+ + 2\ e^- + H_2O_2 \rightarrow 2\ H_2O$

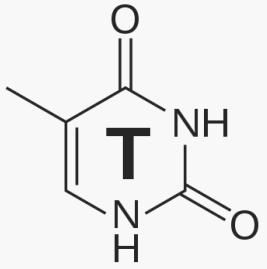


Guanine

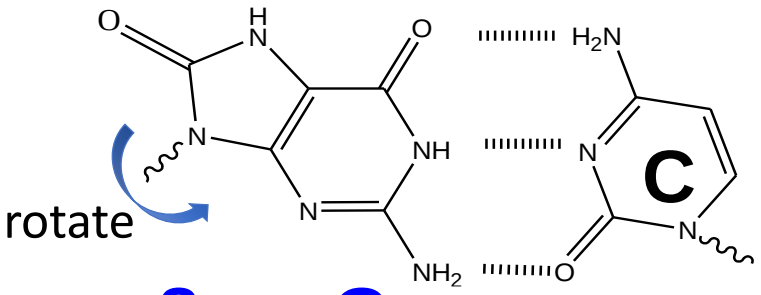
8-oxo-Guanine

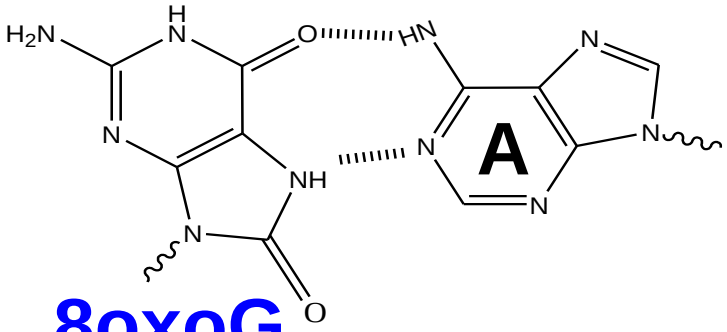
How does H_2O_2 damage DNA?





Damaged G now looks like T



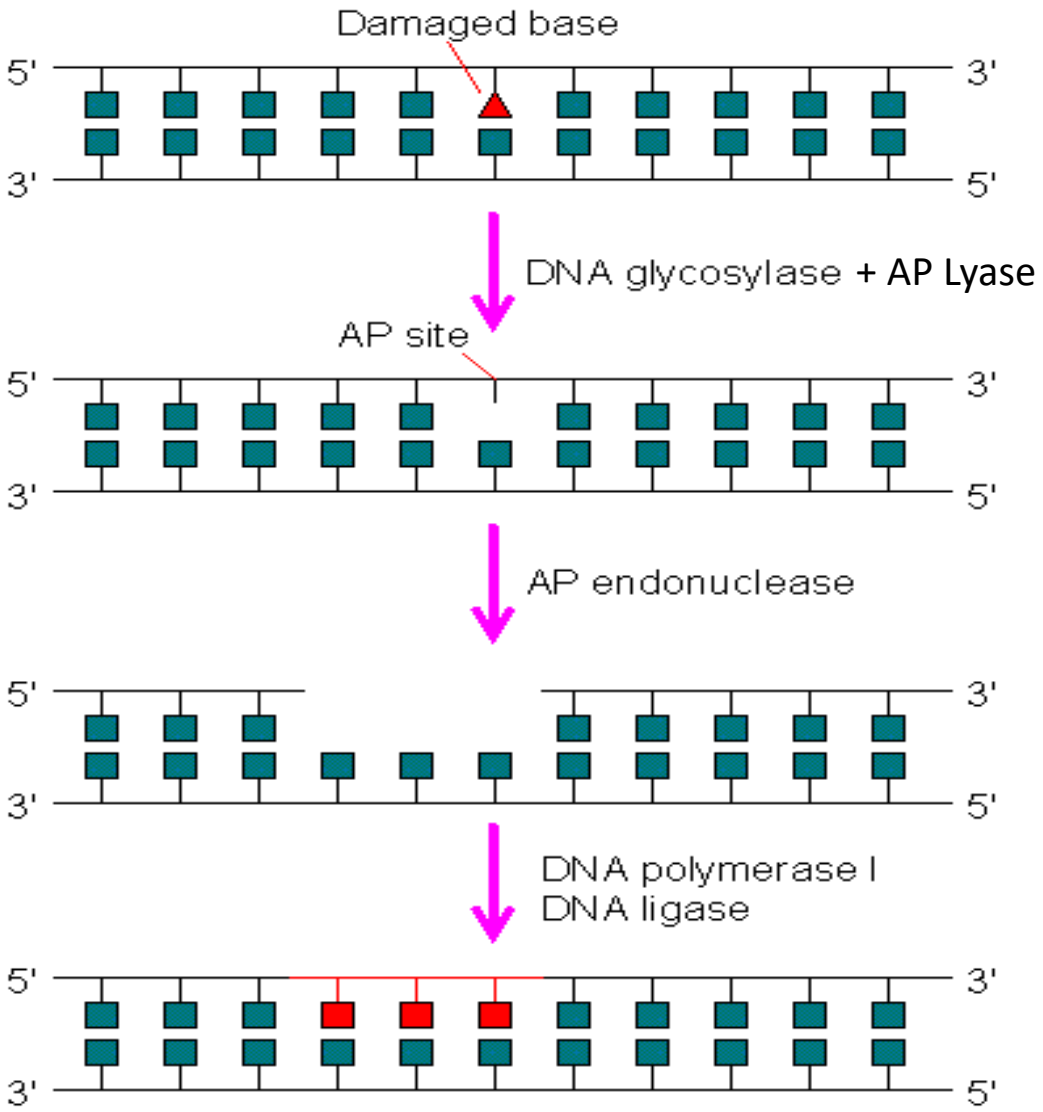


Mutation if replicated

GC → AT

How do cells repair oxidative DNA Damage?

The BER pathway



As

http://www.web-books.com/MoBio/Free/Ch7G.htm

How do we look at DNA damage in intact cells?

Look for γ -H2AX foci

H2AX = a histone

H_2O_2 can cause damage to DNA, resulting in a damaged base

↓

That damage causes a single strand break as cell tries to repair the DNA

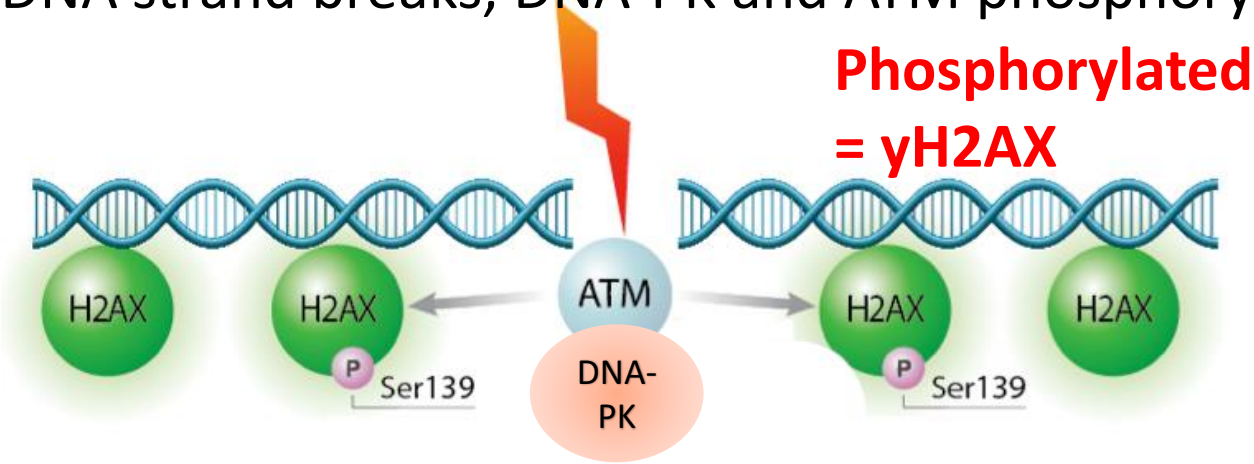
↓

Multiple single strand breaks cause double strand breaks

↓

At DNA strand breaks, DNA-PK and ATM phosphorylate the histone H2AX

Phosphorylated H2AX = yH2AX



We can identify the frequency of these breaks as a measure of DNA damage

Increasing γ -H2AX foci are a **correlate** of DNA Damage

Why is this important to keep in mind?

Correlates have confounding aspects
Might need to either *validate* results
OR
Rule out alternate explanations

Treatment conditions for this experiment

- Goal: Identify potential additive/synergistic effect pretreatment with As has on H_2O_2 induced DNA damage
  - Treat cells with As for 2 hours, then treat cells with H_2O_2 for 30 minutes

Experimental Condition

As + H_2O_2

Control Conditions

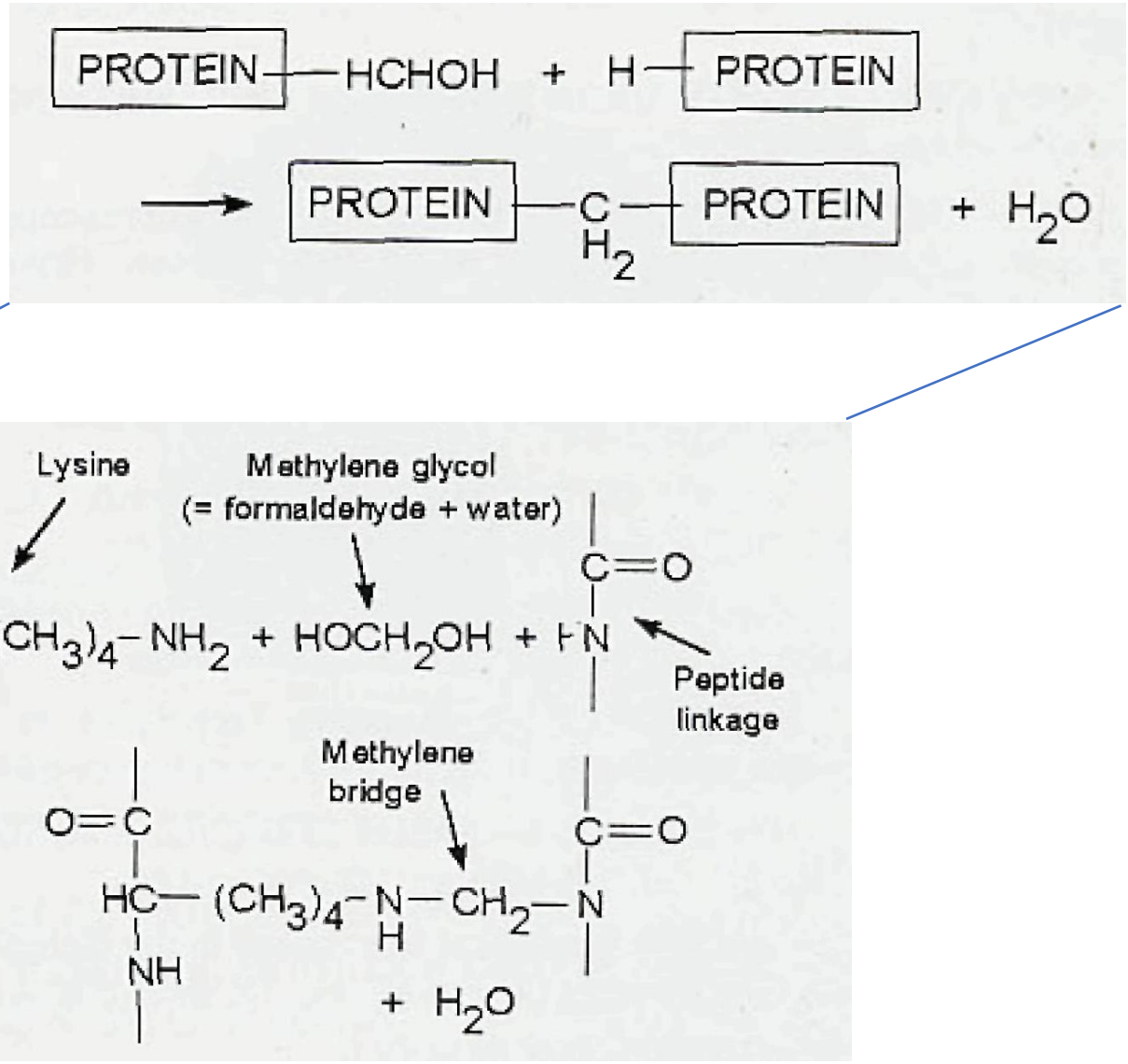
No additions

As alone

H_2O_2 alone

Fixing cells with paraformaldehyde

- Formaldehyde is a chemical crosslinker
  - Paraformaldehyde is the polymer version– depolymerize into formaldehyde with heat or basic conditions
- Commonly used in cell and tissue microscopy
  - Crosslinking of proteins will “fix” samples in place for static imaging
  - Can also trap nucleic acids, etc… between crosslinked proteins



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In lab today

1. Expose As-treated cells to H_2O_2 and fix them for IF staining
2. Talk about purpose and structure of background and motivation during As incubation

DO NOT use the aspirator to remove cell media with Arsenic.
It needs to be collected as hazardous waste!

M1D3HW

1. Work **with your lab partner** to write methods for M1D1 and M1D2
2. Schedule appointment with BE Comm Fellow before M1D5

Pro tips for writing a methods section

Include enough information to replicate the experiment

- List manufacturer's name (Company)
- Be **concise and clear** in your description

Use subsections with descriptive titles

- Put in logical order, rather than chronological order
- Begin with topic sentence to introduce purpose / goal of each experimental procedure

Use clear and concise full sentences

- NO tables or lists, all information should be provided in full sentences and paragraphs
- Write in passive voice and use past tense

Use the most flexible units

- Write concentrations (when known) rather than volumes

Eliminate 20.109 specific details

- Example "labeled Row A, Row B…"
- Do not include details about tubes and water!
- Assume reader has some biology experience
- Include parts of the protocol that the teaching faculty completed, but do not say "completed by teaching faculty."

How can you improve this example?

"Cells were grown in 12 mL of DMEM supplemented with FBS. We spun down the cells and counted them with a hemocytometer. Flasks were incubated in 37 C incubator."

How can you improve this example?

What cells? From where were the cells attained?

How much? What else was added to the media?

"Cells were grown in 12 mL of DMEM supplemented with FBS. We spun

Volume here does not have context as based on the flask used. When might flask / plate size be helpful??

Define all abbreviations and include supplier / manufacturer.

Use passive voice and avoid jargon!

down the cells and counted them with a hemocytometer. Flasks

Be specific about the purpose of each of the steps used…cells were harvested using centrifugation (be sure to include speed and time) then counted using a hemocytometer. And what else was used? At what final concentration / percent?

Be specific about the subject of each action / step.

were incubated in 37 C incubator."

Specific location / equipment used is not important, just the temperature conditions. What other growth conditions were maintained?

Revised example…

Maintaining primary MEF cells

Primary Mouse Embryonic Fibroblasts (MEF) cells (gift of Engelward Laboratory, MIT) were grown in Dulbecco's… (DMEM) (Manufacturer) supplemented with 20% fetal bovine serum (FBS) (Manufacturer) and 100 U / mL of penicillin and streptomycin (Manufacturer). To harvest, cells were trypsinized, collected in fresh media, and counted using 10% (v/v) trypan blue and a hemocytometer. Cultures were maintained at 37 C, 5% CO_2, and 95% relative humidity.

What is the purpose of the Background & Motivation section?

What *specific* information should be included in the Background & Motivation section?

Notes on Background & Motivation section…

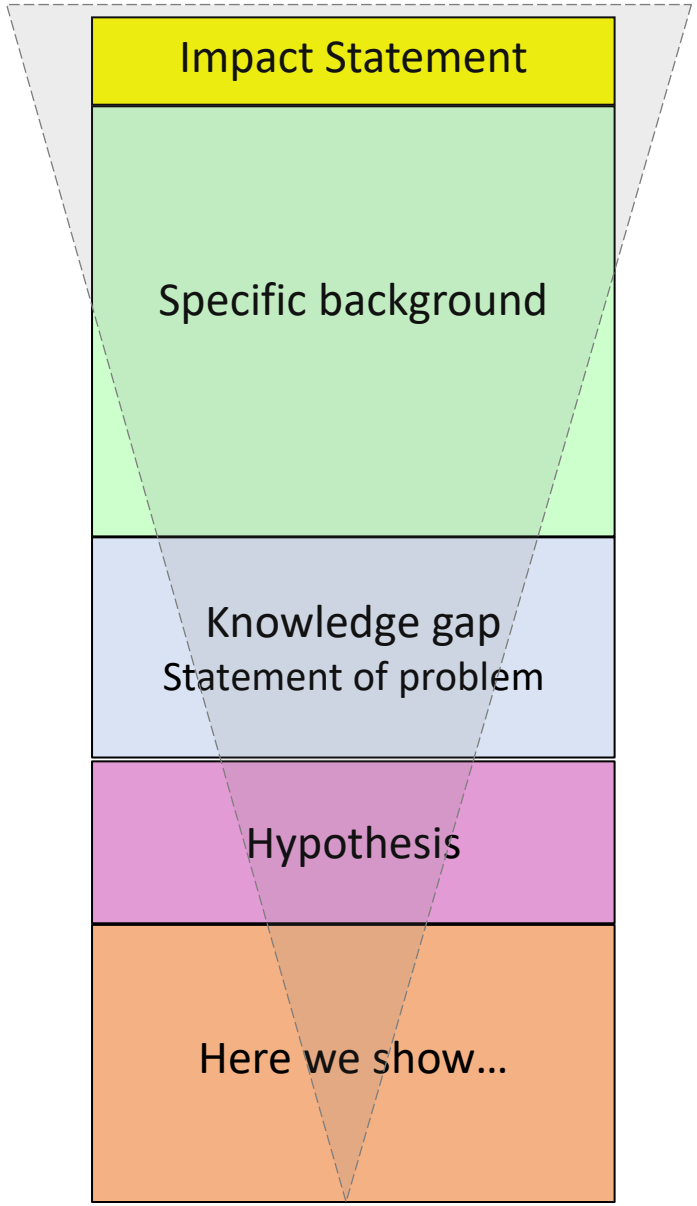
- Anchor your research in a general topic that is important to a broad audience
  - Focus on describing what is currently known in the field
  - Reference the relevant research in the field
- Connect your research to the general topic
  - Minimum essential information
  - Introduce specific technologies 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…