

M1D2: Prepare and treat cells for foci experiment

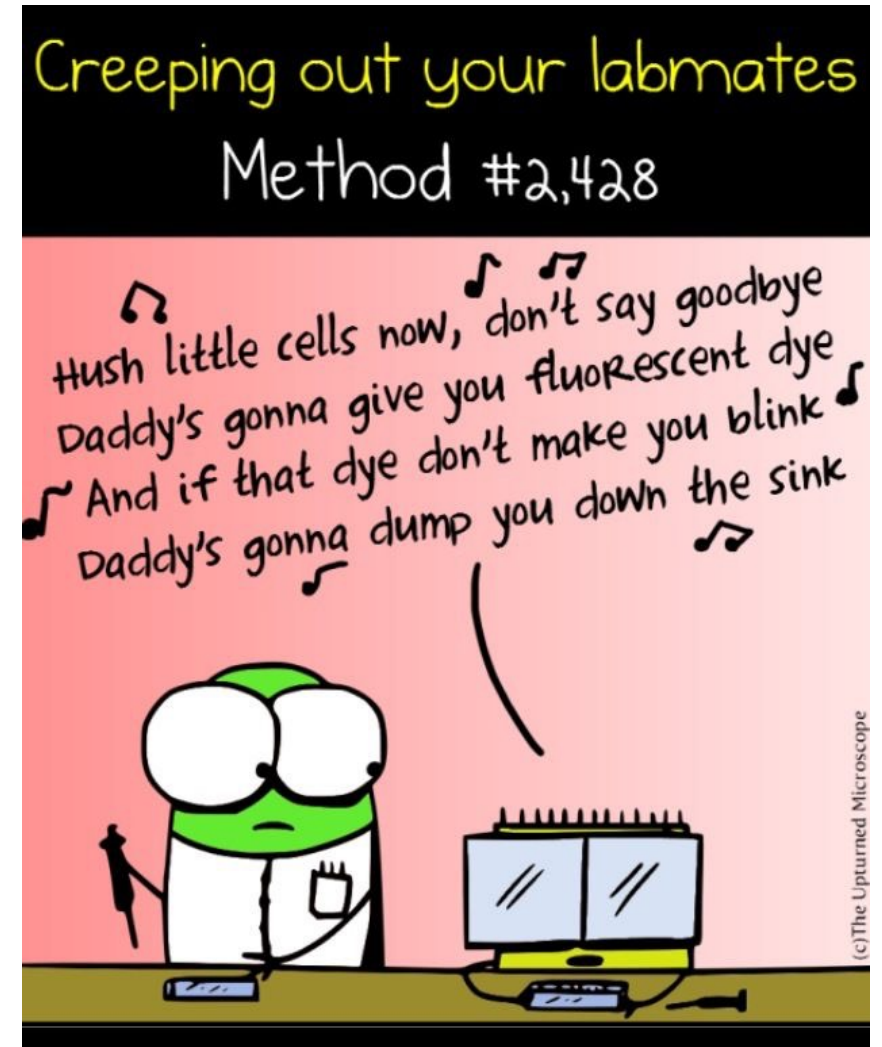


1. Prelab
2. Experiments for today:
 - Adhere cells to coverslips
 - Treat and fix MCL-5 cells for H2AX assay

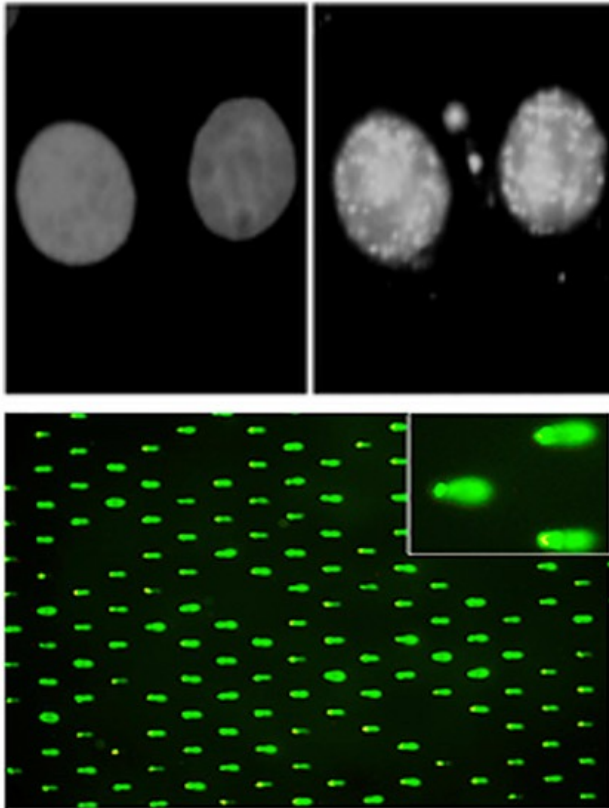
Admin Notes:

Sign EHS training

If you haven't already, respond to Noreen's office hours email



Mod1 Overview



γ H2AX

1. Use repair foci experiment to measure DNA breaks

- Examine effect of H_2O_2 +/- As on double strand DNA breaks by measuring γ H2AX foci formation

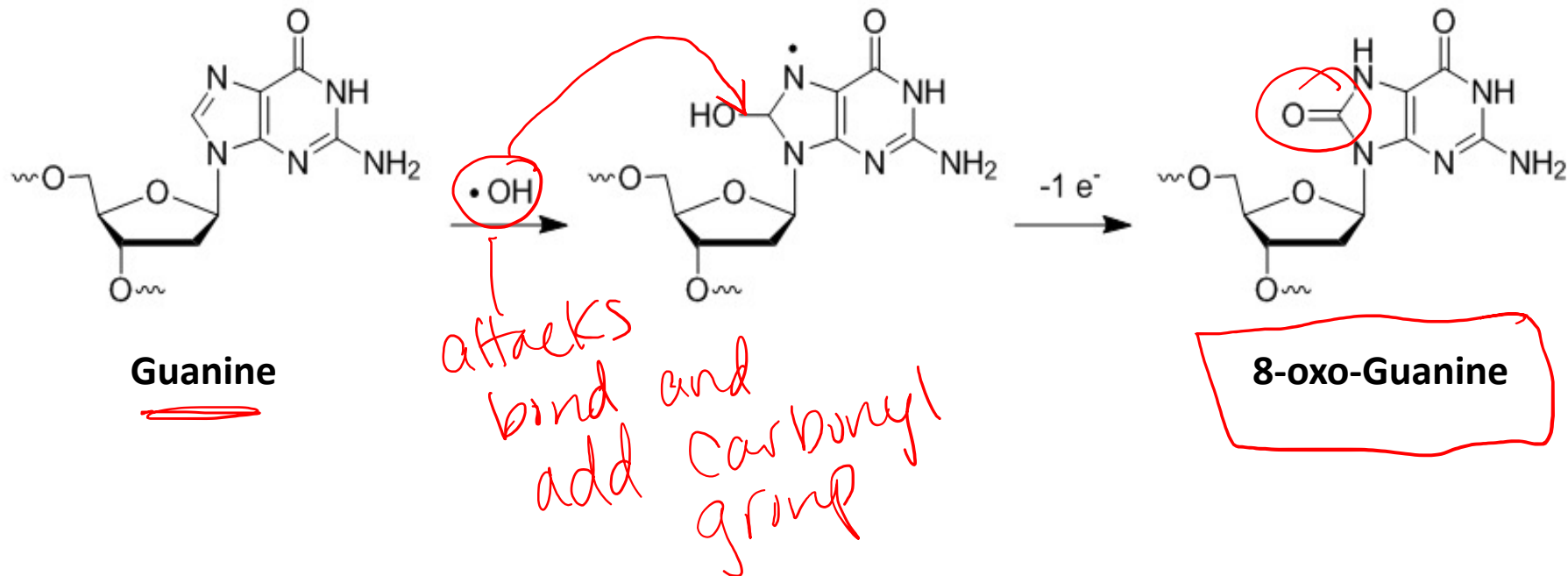
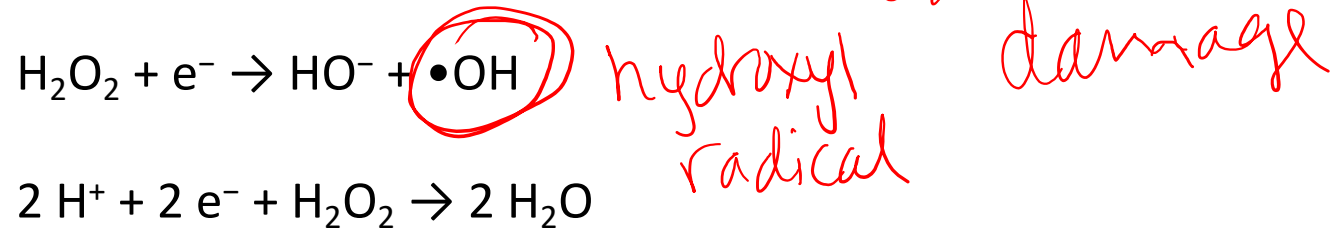
2. Use high-throughput genome damage assay to measure DNA damage

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

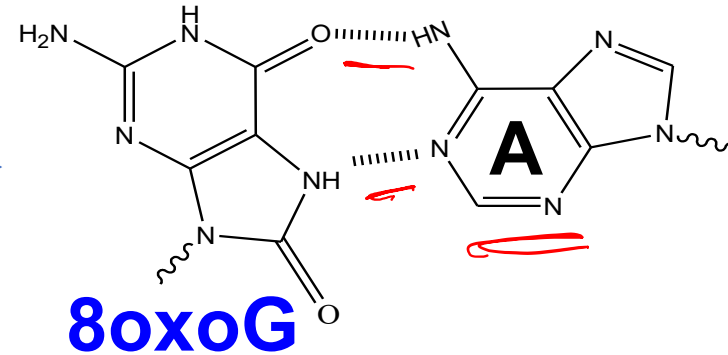
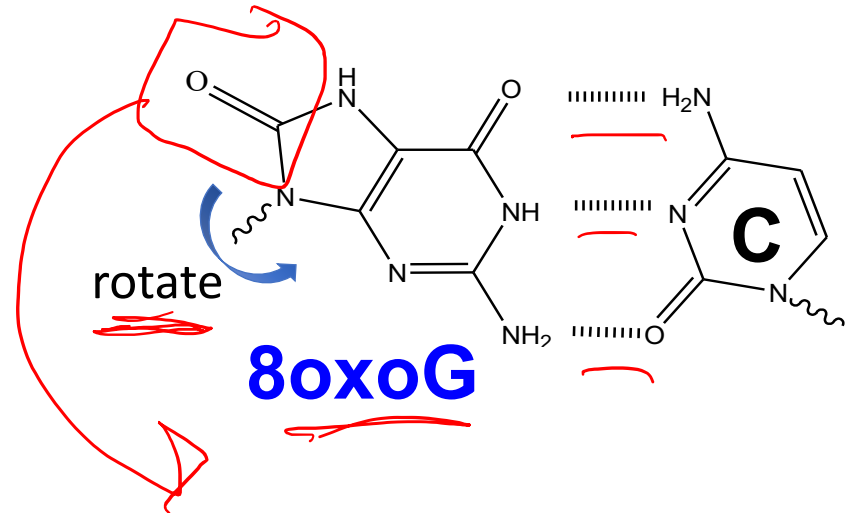
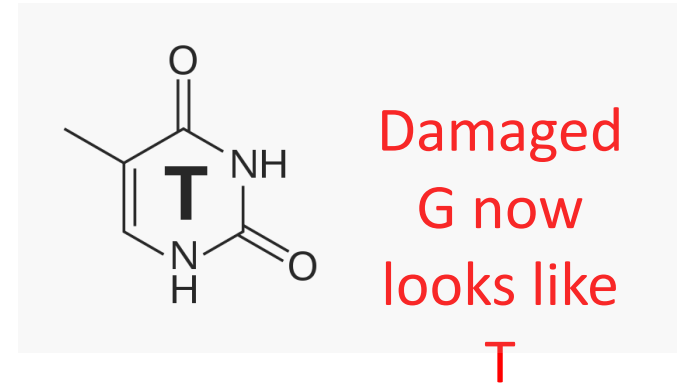
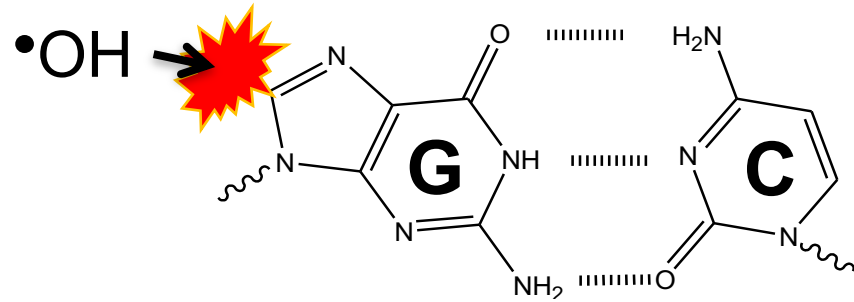
H_2O_2 = oxidative damage — base problems
As = preventing repair

How does H_2O_2 damage DNA?

ROS = Reactive O₂ Species



How does H_2O_2 damage DNA?

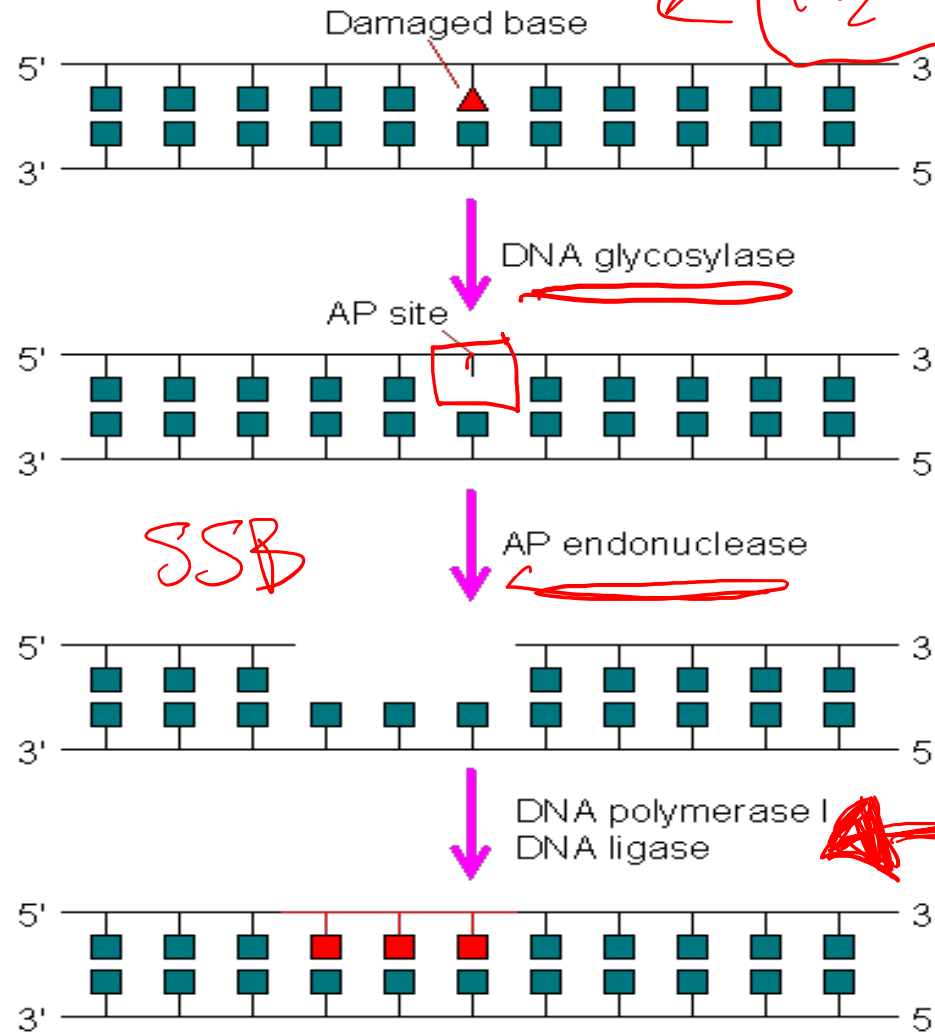


→ Mutation if replicated
GC → AT

How do cells repair oxidative DNA Damage?

Typically, the BER pathway

Base
Excision
Repair



AP = apasiz
site

PAPP
recruits

AS

How do we look at DNA damage in intact cells?

Look for γ -H2AX foci = DSB

H₂O₂ can cause damage to DNA, resulting in a damaged base

↓ SSB

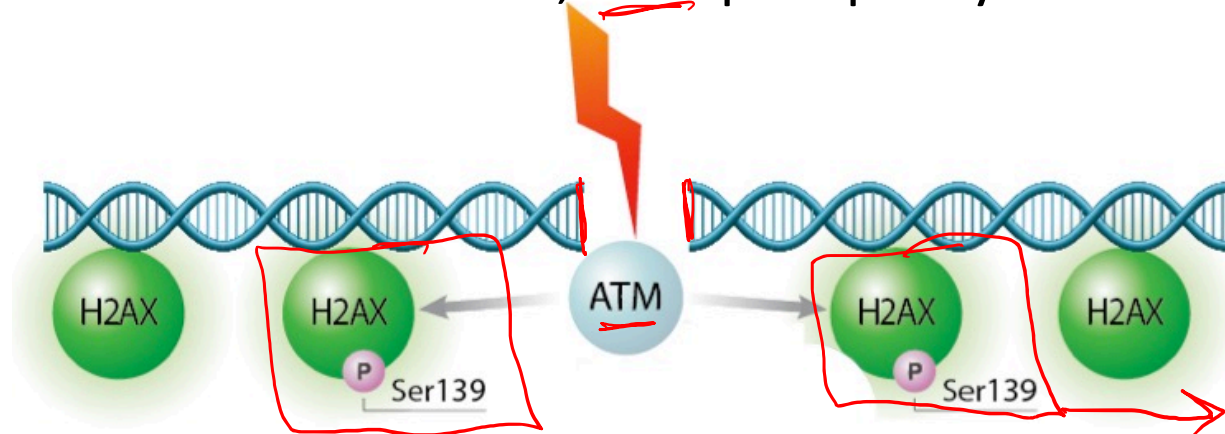
That damage causes a single strand break as cell tries to repair the DNA

↓ DSB

Multiple single strand breaks cause double strand breaks

↓

At double strand breaks, ATM phosphorylates the histone H2AX



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

Histone
phos. H2AX = γ H2AX

Treatment conditions for this experiment

- Goal: identify any additive effect pretreatment with As has on H_2O_2 induced DNA damage
 - Treat cells with As for 24 hours, then treat cells with H_2O_2 for 30 minutes

Experimental Condition

As + H_2O_2

Control Conditions

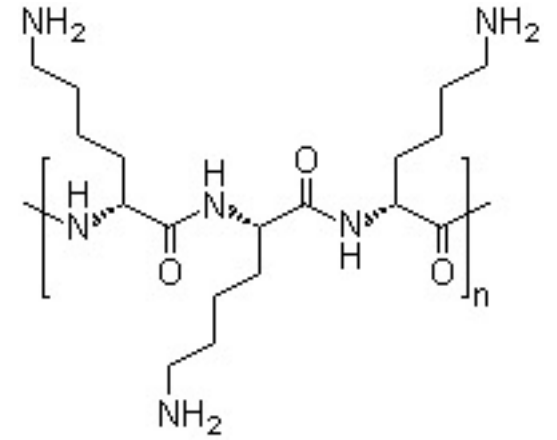
As
 H_2O_2
No treatment

Our imaging protocol requires cells to adhere to glass coverslips in monolayer

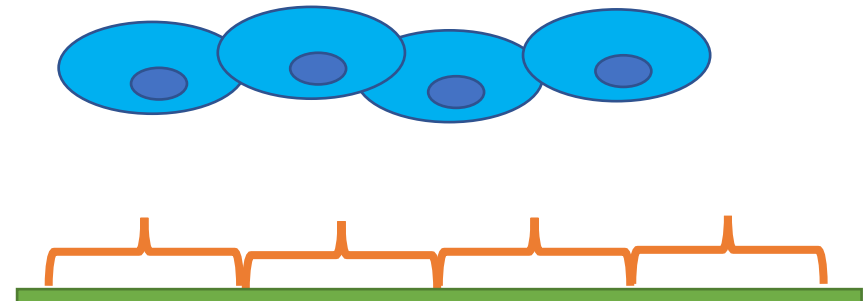
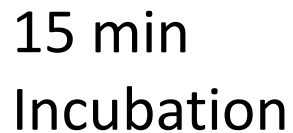
- We have non-adherent cells!
- Must adhere them to coverslips prior to imaging

(coated coverslips)

- Many ways to get cells to adhere to glass or plastic



- neurons, 1° cells



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

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

create
waste
container
- 50ml
conical

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

methods = paragraphs

→ 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 → cells were incubated...

Ⓟ 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."

do not write M1D1

How can you improve this example?

what sys
"Cells were grown in 12 mL of RPMI supplemented with FBS. We spun
colloidal
volume
abbreviations
culture medium active
down the cells and counted them with a hemocytometer. Flasks
specific conditions
how long
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 RPMI 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...

gift

topic sentence

Maintaining MCL-5 cell line

Human lymphoblastoid cells (MCL-5) cells (gift of Engelward Laboratory, MIT) were grown in Roswell... (RPMI) (Manufacturer) supplemented with 10% fetal bovine serum (FBS) (Manufacturer) and 100 U / mL of penicillin and streptomycin (Manufacturer). To harvest, cells were centrifuged for 5 minutes at 300g and pelleted cells were resuspended in fresh media. Cells were counted using 10% (v/v) trypan blue and a hemocytometer. Cultures were maintained at 37 C, 5% CO₂, and 95% relative humidity.

Coating coverslip demo!

What is the **purpose** of the Background & Motivation section?

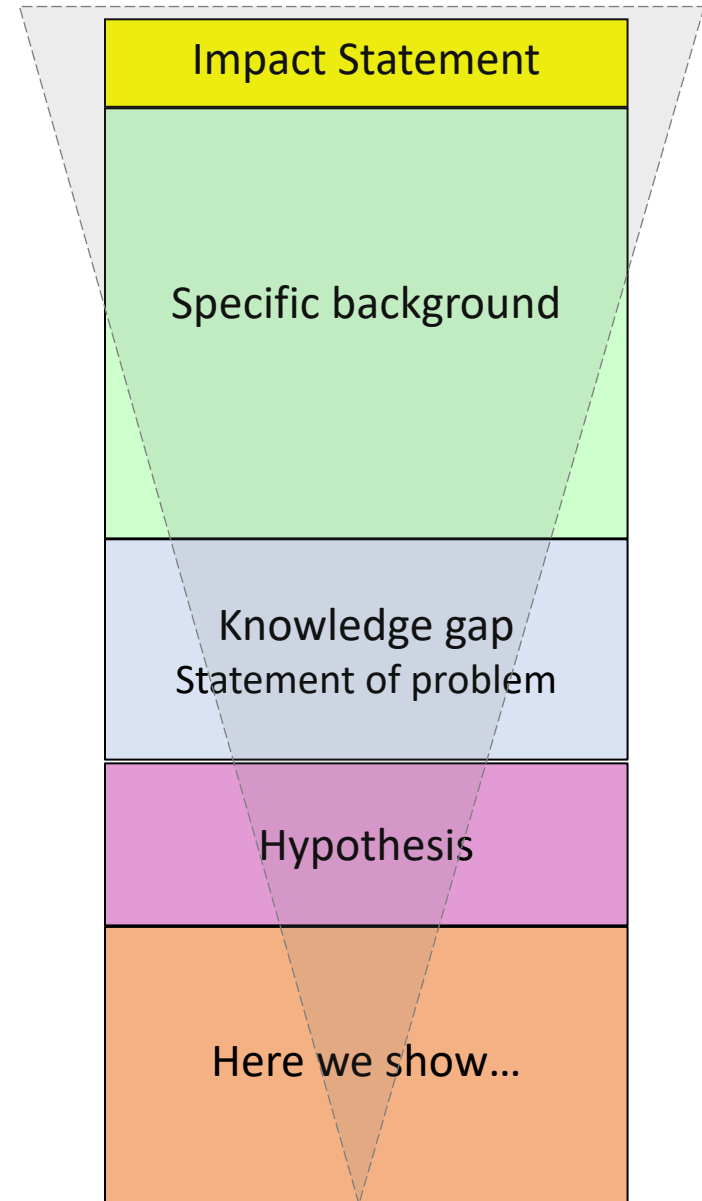
What *specific information* should be included in the Background & Motivation section?

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...

