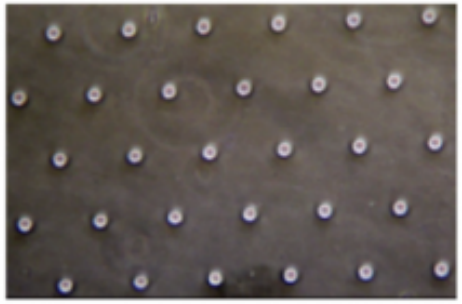
M1D5:
Complete biochemical experiment and apply chemical treatments for sub-nuclear foci assay

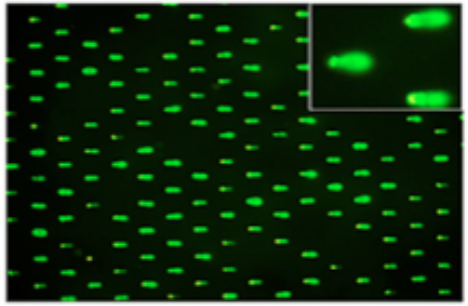
1. Communication lab workshop
2. M1Q1
3. Prelab discussion
4. Benchwork
    - Complete CometChip enzyme treatment
    - Electrophorese CometChips
    - Induce DNA damage for H2AX assay

Overview of Mod1 experiments



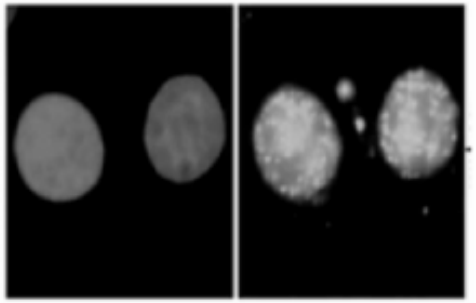
1. Optimize comet chip assay

- Test loading variables



2. Use comet chip assay to measure DNA repair

- Measure effects of MMS and H_2O_2 on BER

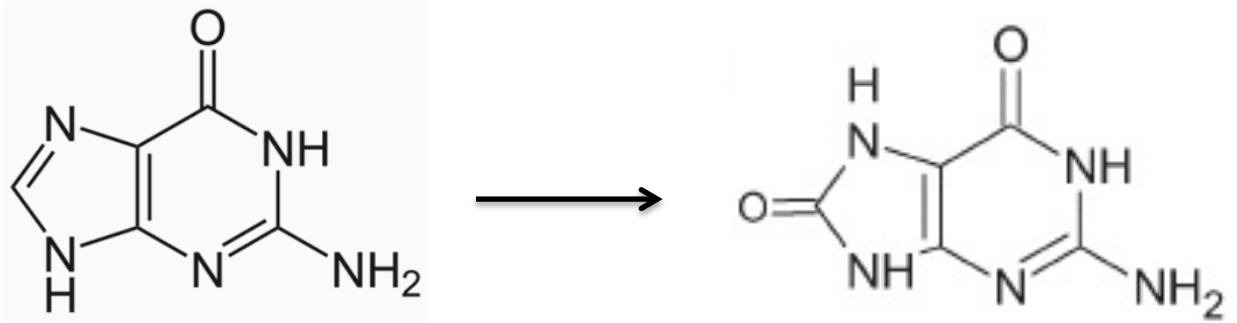


3. Use immuno-fluorescence assay to measure DNA repair

- Examine effect of MMS and H_2O_2 on DSB abundance

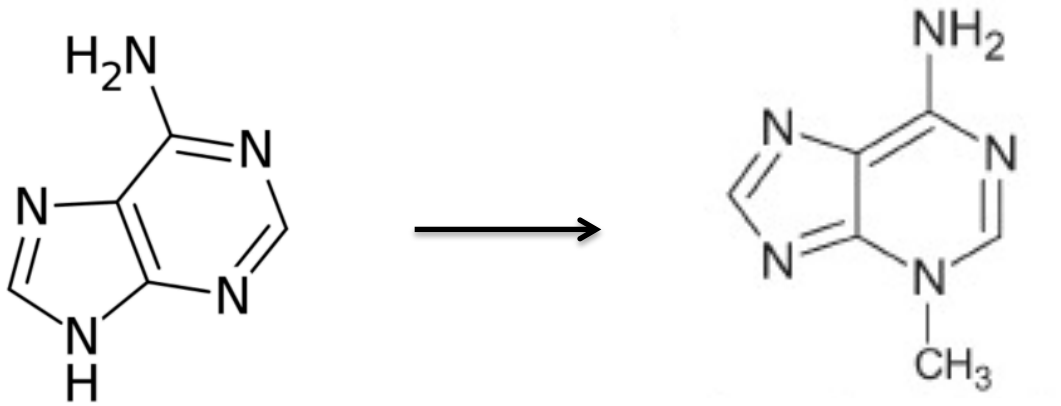
Damaging agents ‘decorate’ bases

Oxidative damage: H_2O_2



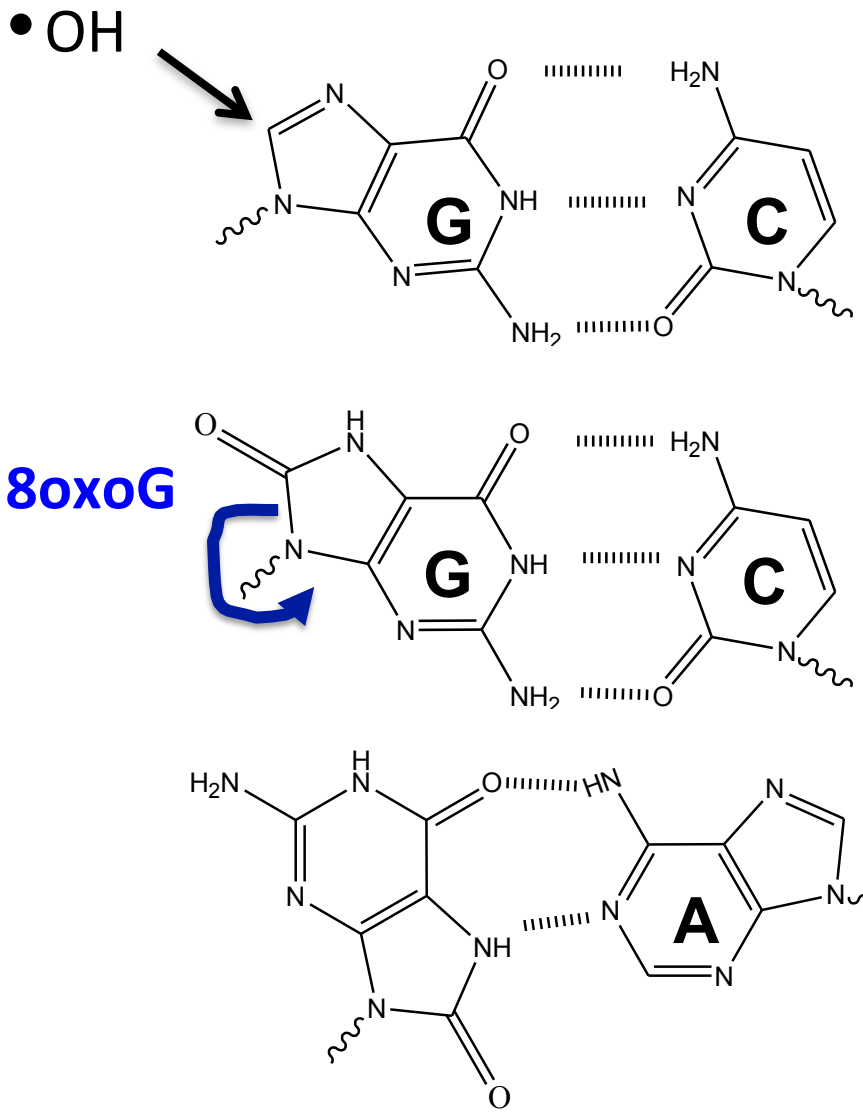
guanine 8oxoG

Alkylating damage: MMS

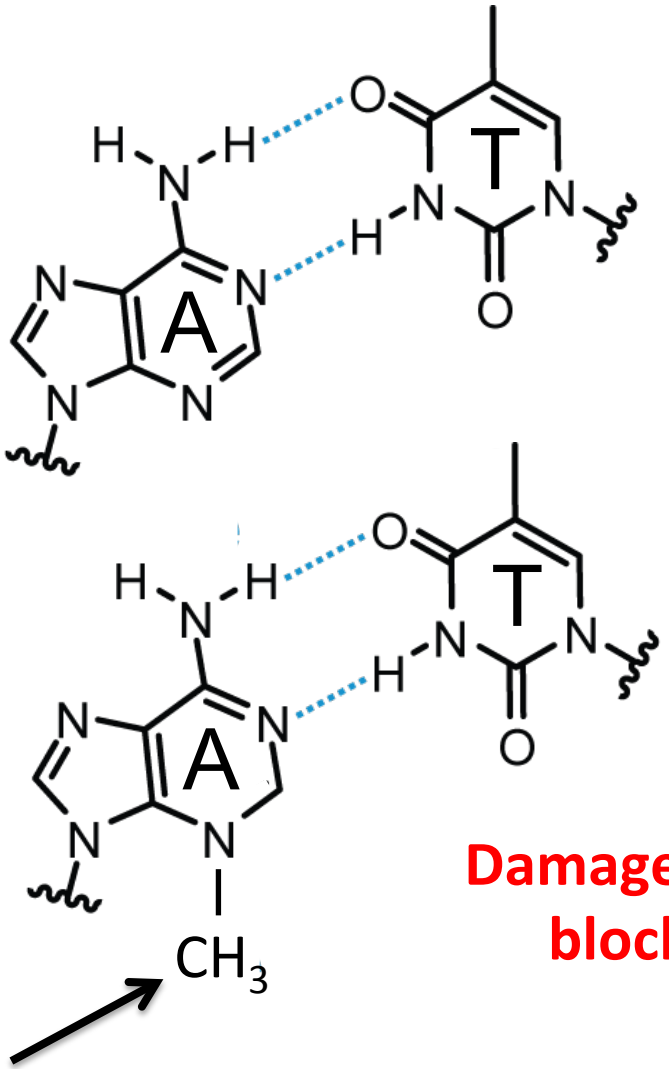


adenine 3meA

Oxidative damage induced by H_2O_2



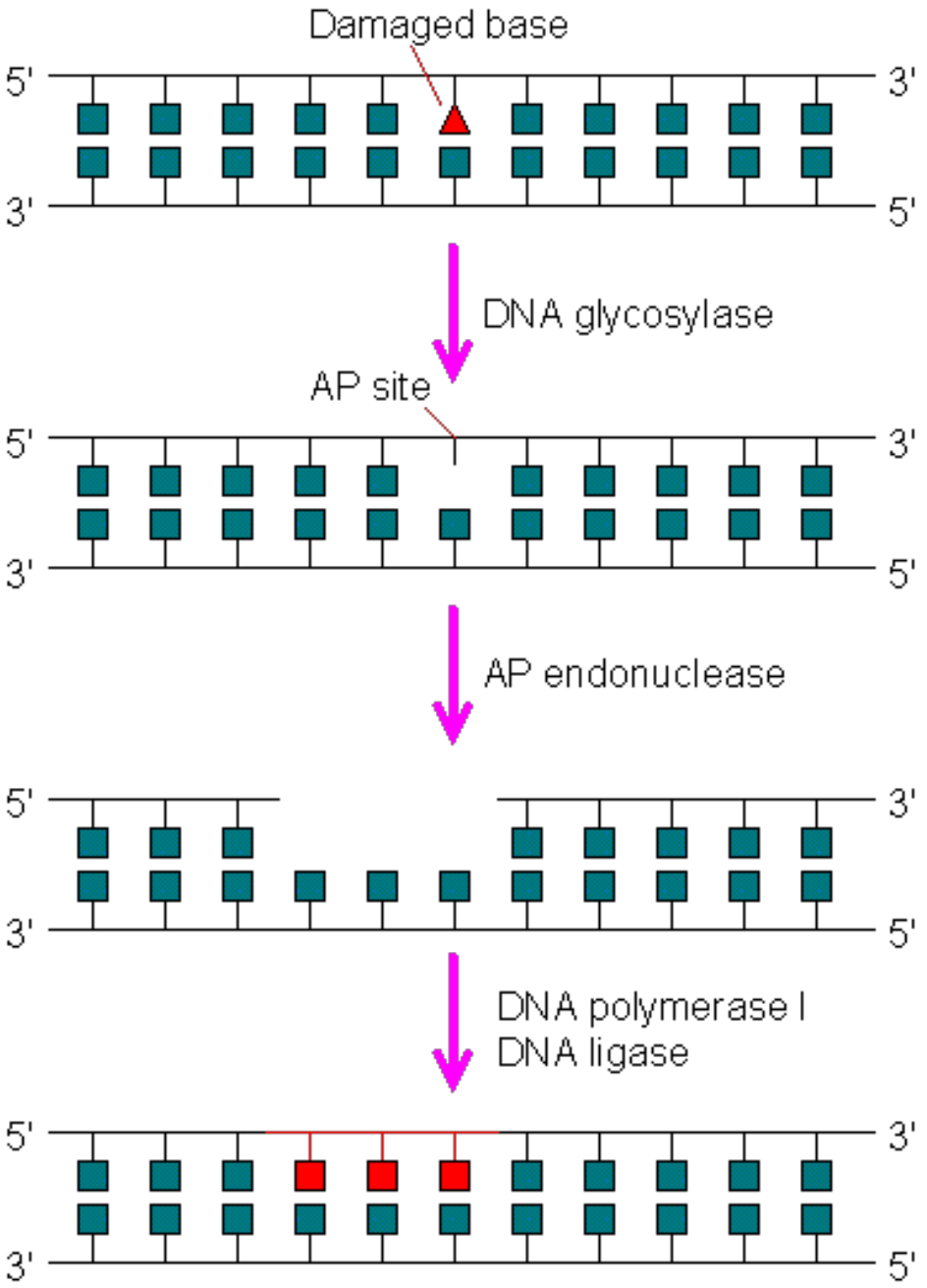
Alkylating damage induced by MMS



Damaged A is a bulky lesion; blocks replication fork

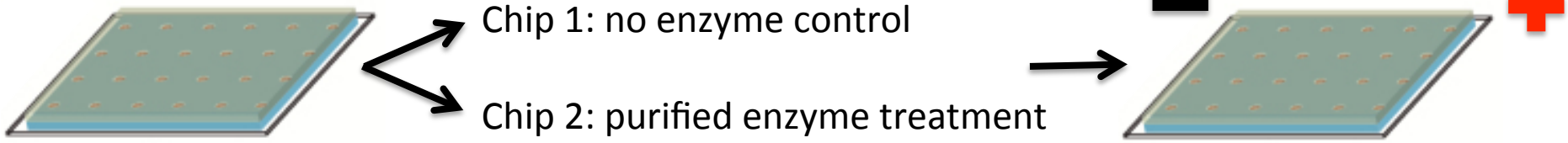
BER pathway corrects damaged bases

- Glycosylases are specific to type of damage
  - Oxoguanine DNA glycosylase (Ogg)
  - Alkyladenine DNA glycosylase (Aag)

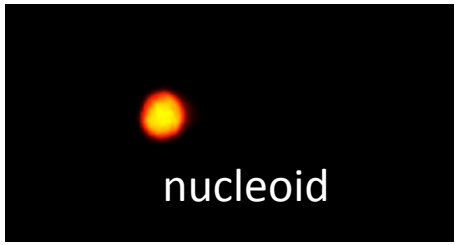


Exp2: Biochemical testing using CometChip

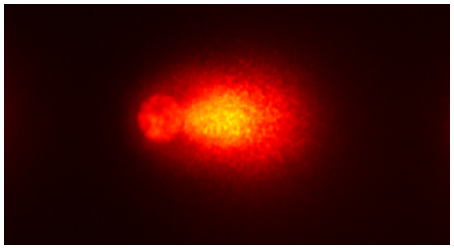
M1D5:



M1D6 and D7:

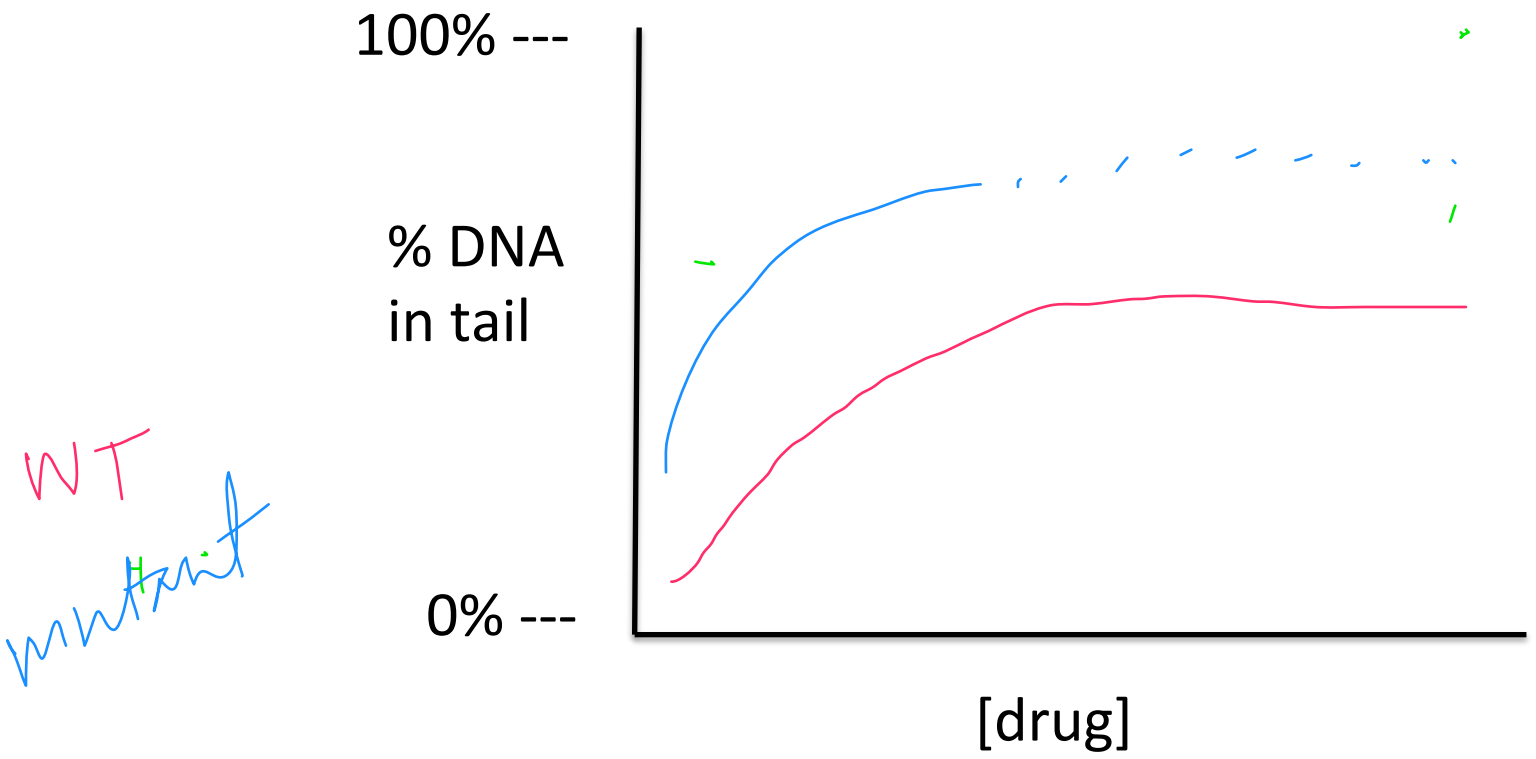


No damage:
supercoiled



Damage:
SSBs, DSBs, abasic sites, alkali labile sites

What result(s) do we expect...

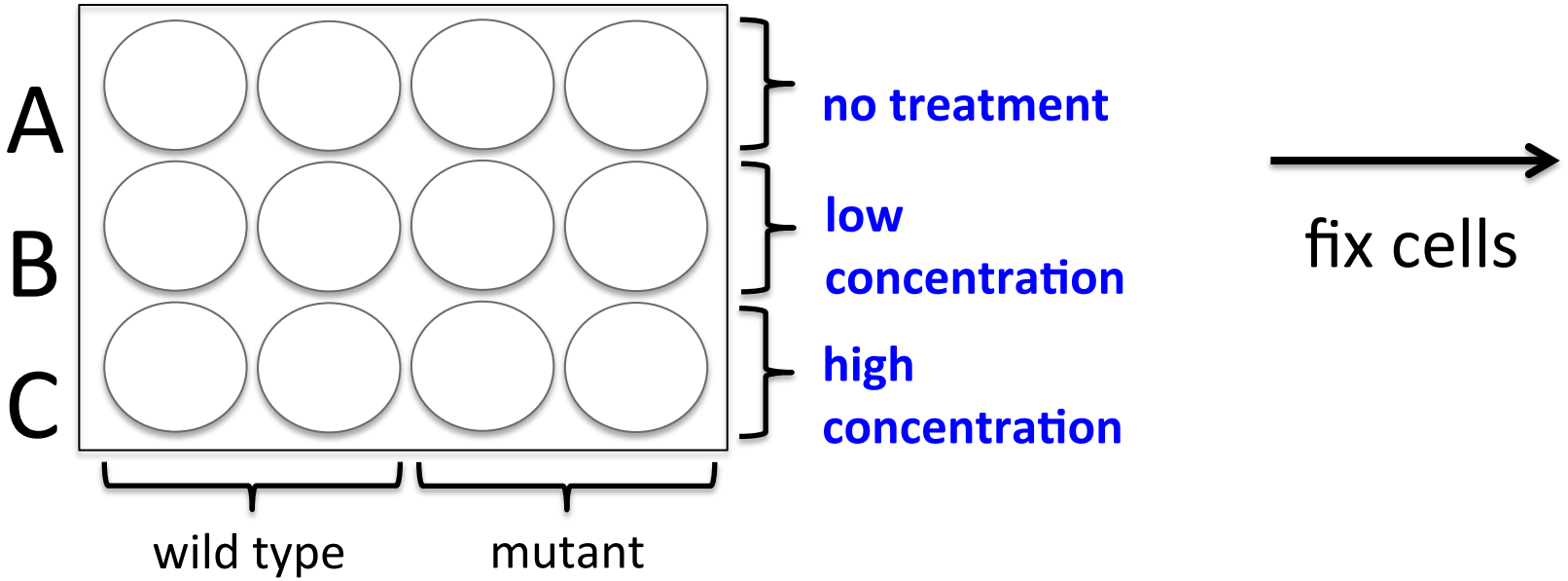


Keep track of the players!

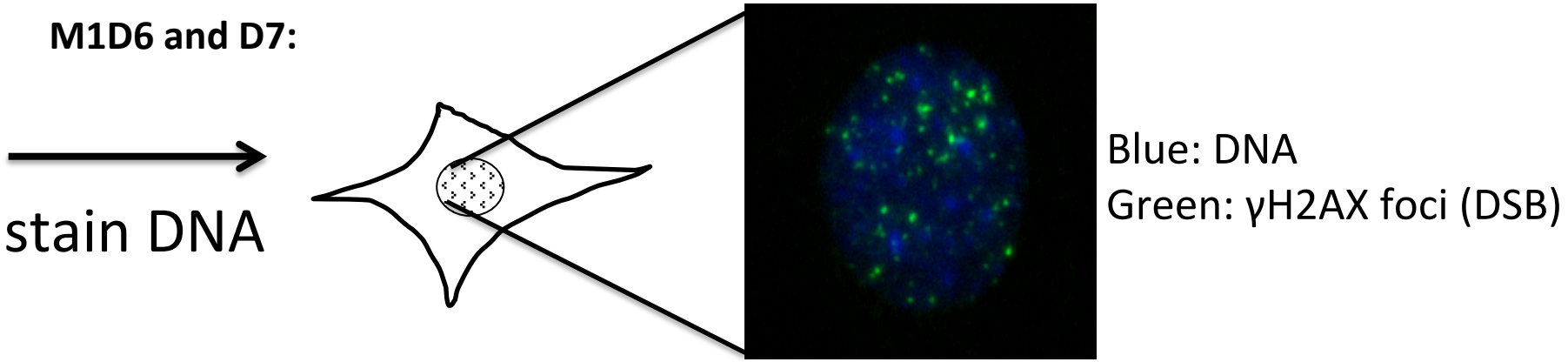
	Oxidative damage	**Alkylating damage**
Chemical treatment:	H_2O_2	MMS
Mutant cell line:	Ogg1-/-	Aag-/-
Enzyme:	Fpg (*E. coli* Ogg1)	hAAG (human Aag)

Exp3: DSB abundance using H2AX

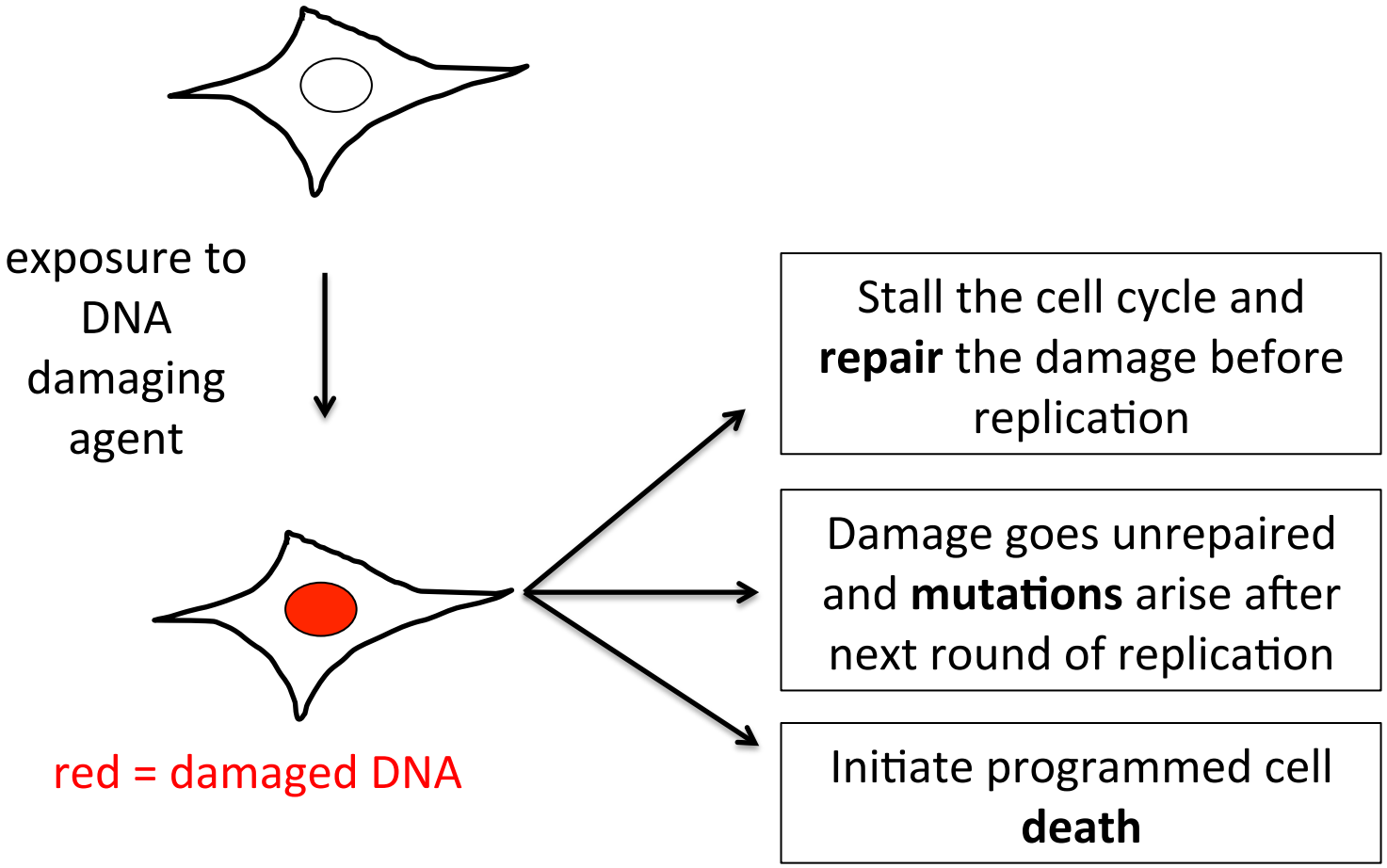
M1D5:



M1D6 and D7:

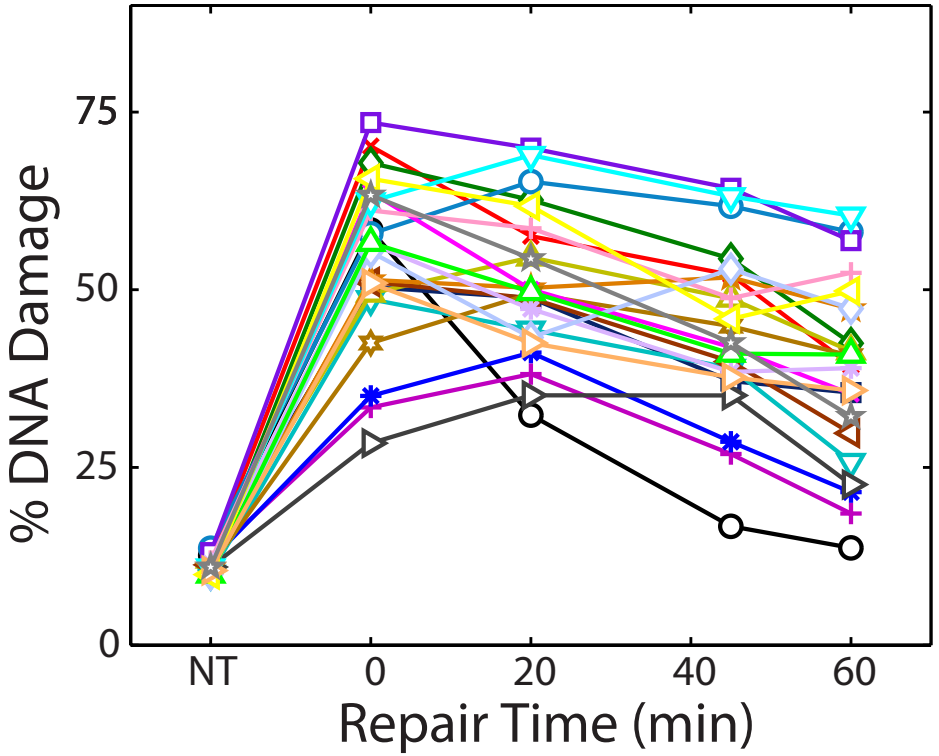


How does a cell respond to DNA damage?

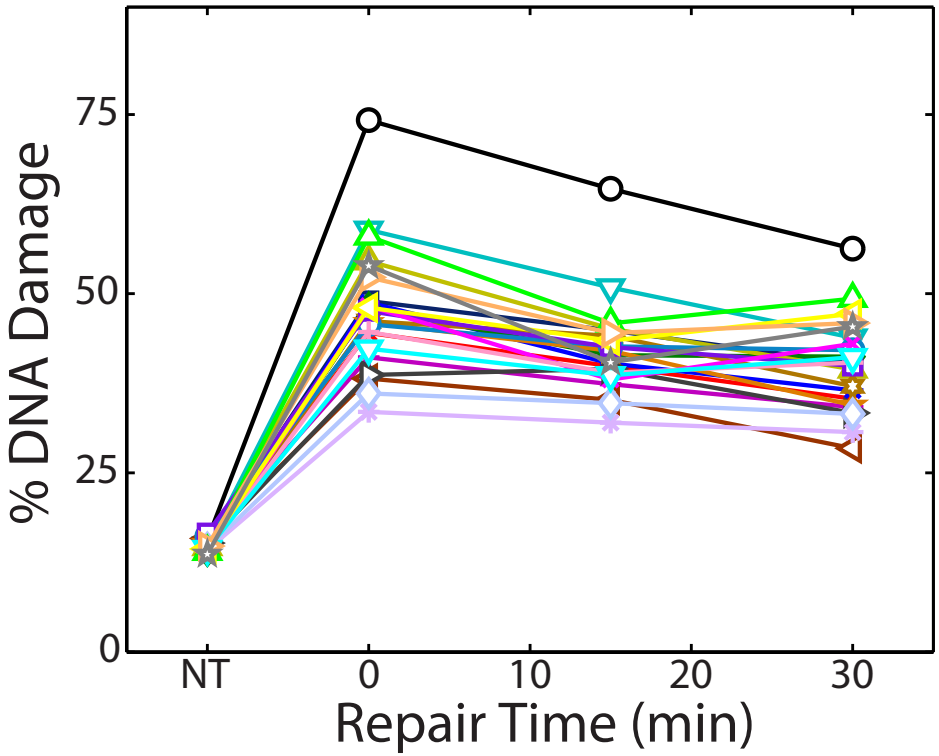


DNA repair capacity varies

Oxidative Damage



Alkylation Damage



from Prof. Engelward

Options to consider: concentrations and recovery time

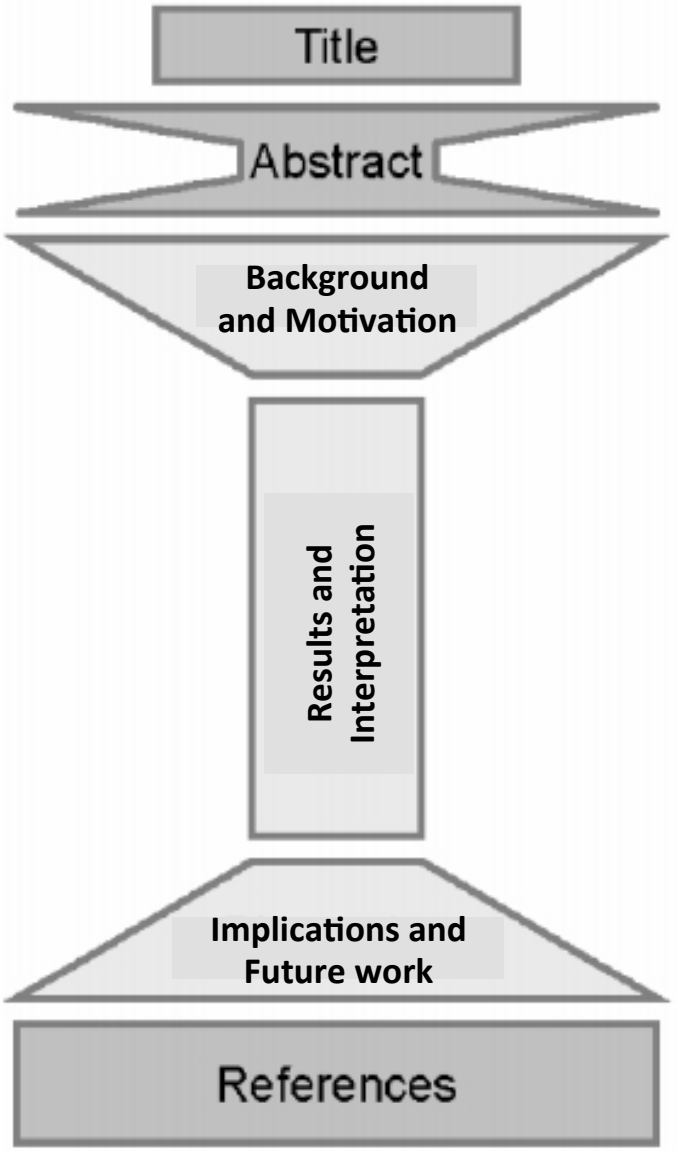
1. Which concentrations will you use to induce DNA damage?
   - H_2O_2: 5, 10, 15, 20, 25 µM
   - MMS: 0.1, 0.2, 0.5, 6, and 8 mM

2. How long will you allow for repair of damaged DNA?
   - 30 min, 60 min, or 24 hr

Data summary due Wed., Oct. 11 at 10p!

- Remember to 'funnel' the information
- Be sure the pieces 'match' throughout

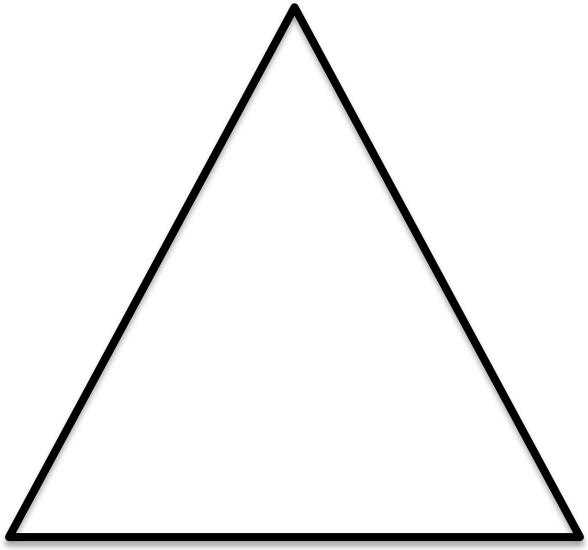
- ONLY the abstract is written as a paragraph, ALL OTHER sections should be in bullets



Writing future works & implications

Implications and Future Work: potential topics [edit]

- ***Topic***: Did your results match your expectations?
  - If no, provide a putative explanation. If yes, how can you further test if your hypothesis is correct?
- ***Topic***: Based on the results, whether they matched your expectations or not, what experiments might you recommend next?
  - Follow-up experiments could distinguish between competing explanations of a given outcome or broaden the sample set for a question you already asked, to give just two examples.
- ***Topic***: How might this assay be improved?
- ***Topic***: How might this assay be used as a research tool? in the clinic? in industry?



Starts with summary of your findings – DO NOT just repeat all of the data

↓

Finishes with tie back to the impact statement (big picture topic)

Other notes on homework due M1D6

- Draft mini-presentation outline
  - Abide by content and time guidelines (see wiki!)
  - Introduce yourself and your research
  - State hypothesis clearly
  - Be quantitative (use placeholders in this assignment)
- Read journal article and prepare for discussion

Today in lab…

- Complete enzyme treatments with CometChips in main laboratory
- Treat cells with DNA damaging agent for H2AX assay in tissue culture
  - Depending on timing, pairs may have to split between rooms
  - If time permits, fix cells
- Electrophorese CometChips