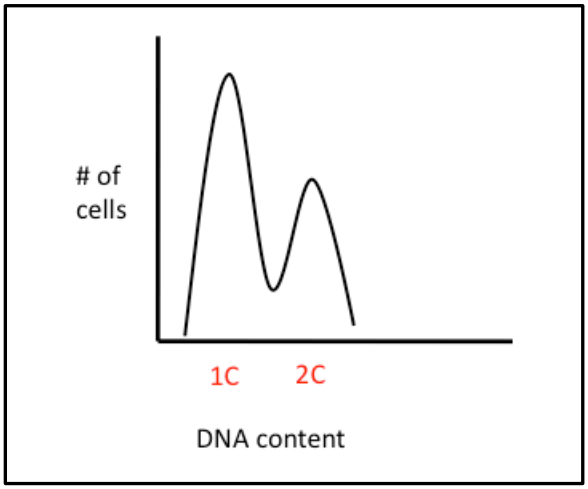
Problem Set 5

7.06 Spring 2015
Due April 3, 2015

1. You are interested in studying the cell cycle in budding yeast, *S. cerevisiae*. This organism is haploid.
A growing culture yields the following FACS plot:



A) **What would the two values be on the X axis for the DNA content?**

1C and 2C

Write these on the axis in the figure.

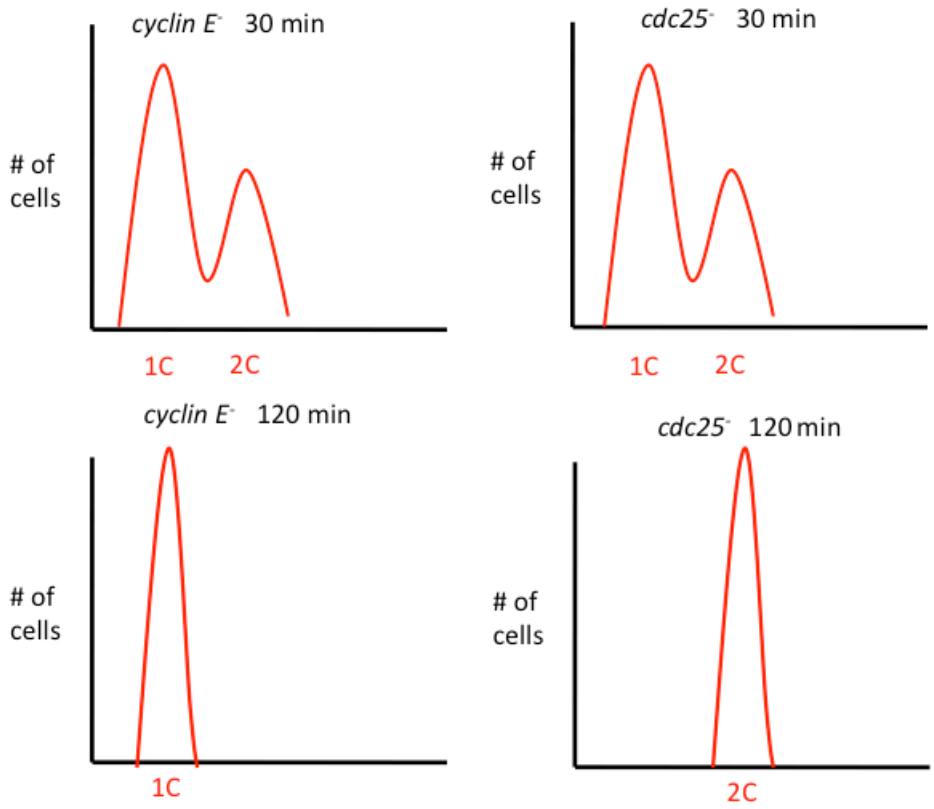
B) You decide to isolate loss-of-function cell cycle mutants in budding yeast. **Why will these alleles need to be conditional?**

The mutants will arrest the cell cycle, so the cells will not grow and divide and ultimately will die. To be able to grow a culture or a colony you will need a conditional allele that can grow at the permissive temperature and then be examined for arrest at the nonpermissive temperature.

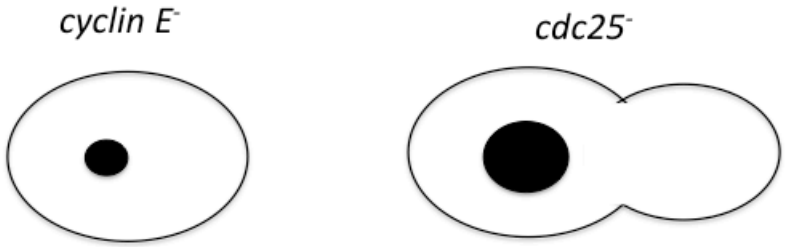
C) You isolate a conditional allele of:

1. The budding yeast equivalent of Cyclin E
2. The budding yeast equivalent of Cdc25

The cell cycle in budding yeast takes 90 minutes. **For each mutant draw what the FACS profile will look like for a culture after 30 minutes at the nonpermissive temperature and what it will look like 120 minutes after the shift to the nonpermissive temperature. Be sure to label the X axis.**

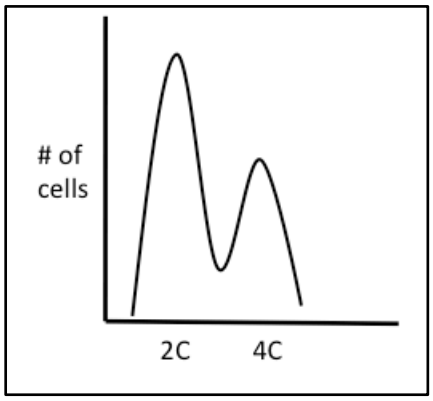


D) **Draw what the cells would look like for each of the two mutants after 120 minutes at the nonpermissive temperature.**



2. You decide to study the effects of drugs on the cell cycle in mammalian cell culture. Hydroxyurea (HU) inhibits ribonucleotide reductase, leading to a reduction in levels of dNTPs. Colchicine disrupts the microtubule cytoskeleton that is necessary for chromosome segregation in mitosis. The cell cycle takes about 24 hours in these cells. 10 hours of this is taken up by S phase and 1 hour by mitosis.

This is the FACS profile for untreated cells.

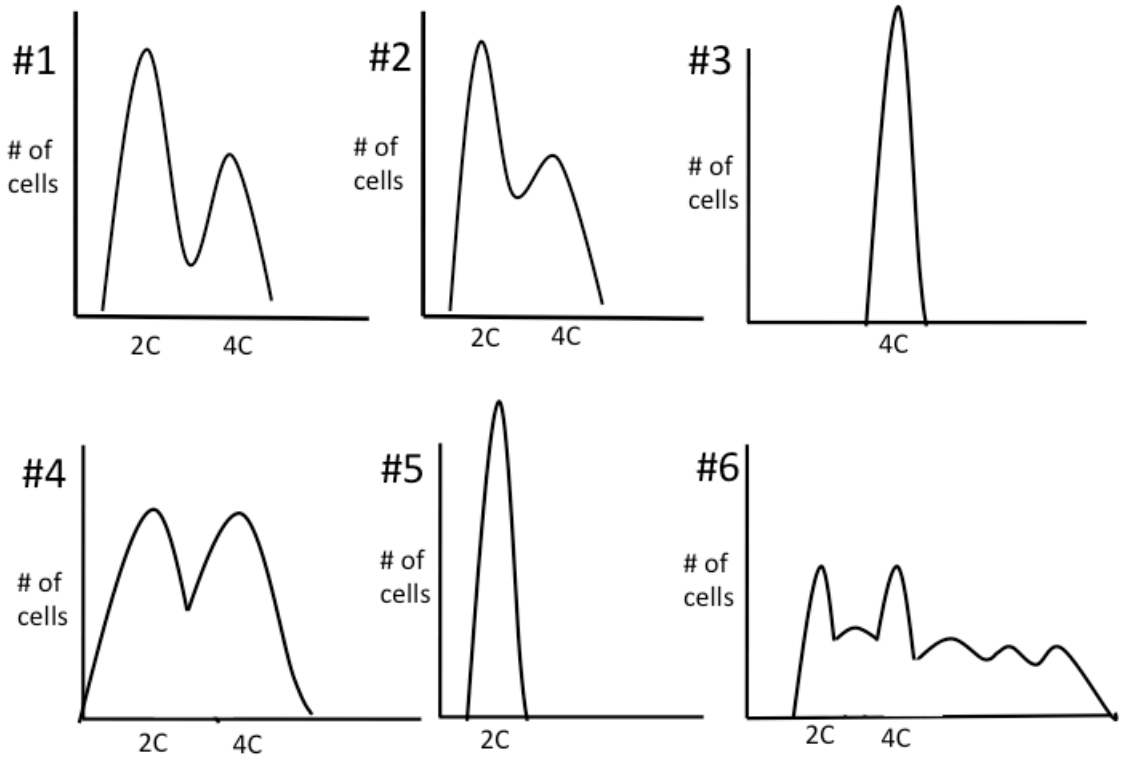


A) **Which of the FACS profiles below (#1-6) you would observe after treating the culture with HU for five hours?**
#2, because the cells in the population that were in S phase would arrest.

B) **Which would you observe after treating the cells with colchicine for five hours?**
#1, because less that 5% of the cells are in mitosis, which is when colchicine would cause arrest.

C) **Which would you observe after treating the cells with colchicine for 30 hours?**
#3. After 30 hours of treatment all the cells, no matter where they were in the cell cycle when the drug was added will have reached mitosis and arrested with a 4C DNA content.

Possible FACS profiles

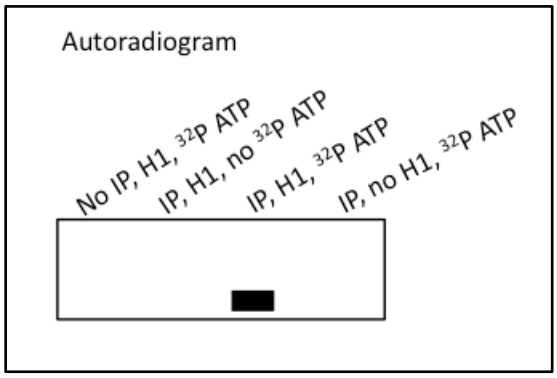


3. You want to assay the activity of CyclinB/Cdk1 during the cell cycle in mammalian cell culture.

A) **Will you need to synchronize your cell culture? Why or why not?**

Yes, the cell cultures will have to be synchronized. In an asynchronous culture each cell can be at a different place in the cell cycle, so when you do the kinase assay on the total culture you won't be able to determine when during the cell cycle it is active. You can synchronize mammalian cell cultures by removing growth factors to arrest all the cells in G1 before the restriction point or by removing thymidine to arrest them at the G1/S boundary. If you then add back the growth factors or thymidine the cell population will synchronously progress through the cell cycle. You can do the assay at specific time points after release of the arrest to know when the kinase is active. By BrdU labeling you can tell when they go into S phase. By examining the cells you can tell when they enter mitosis.

B) All Cyclin/Cdk complexes readily phosphorylate histone H1, making this a good substrate to assay Cdk activity. These assays are done by immunoprecipitating (IP) the kinase using a bead coupled antibody. ATP with a radioactive gamma ^{32}P phosphate group, Mg^{+2} and histone H1 are added to the immunoprecipitate and incubated. The sample is boiled, run on an SDS protein gel, and visualized by autoradiography to detect phosphorylation of histone H1.
This is the result you would see, with appropriate controls.



If you want to look at Cyclin B/Cdk1 activity, does it matter whether you immunoprecipitate the Cyclin B or the Cdk1 subunit? Why or why not?

Yes, you must do the IP with an antibody against Cyclin B. If you IP Cdk1, it can bind to either Cyclin A or Cyclin B, so you won't know whether the kinase activity you are assaying is Cyclin B/Cdk1 or Cyclin A/Cdk1.

4. You examine Cyclin B/Cdk1 activity in the fission yeast cell cycle mutants below, comparing it to wild-type controls. **For each indicate whether the mutant:wild-type activity ratio would be equal to 1, less than 1, or greater than 1. Briefly explain why.**

A) wee1-

>1
Because Wee1 in an inhibitor of Cdk1 kinase activity; it places inhibitory phosphates on T14 and Y15.

B) cdc25-

1<
Because Cdc25 is an activator of Cdk1 kinase activity; it removes the inhibitory phosphates at T14 and Y15.

C) CAK-

<1
Because CAK must phosphorylate T161 on Cdk1 to fully remove the inhibition of the T-loop that blocks substrate binding.

D) wee1-, cdc25-

>1

Because if Wee1 does not place an inhibitory phosphate on Cdk1, then the kinase will be more active, regardless of the presence or absence of Cdc25 whose purpose is only to remove the inhibitory phosphate introduced by Wee1.

E) Cdk1 with threonine 161 changed to alanine

<1
Because CAK cannot phosphorylate the alanine residue and allow removal of T-loop inhibition of substrate binding.

F) CAK-, Cdk1T161D (threonine 161 changed to aspartic acid)

>1
Because the T161D mutation acts like a phospho-mimic and the T-loop inhibition of Cdk1 substrate binding is alleviated, allowing Cdk1 to be more active than wild-type kinase. CAK activity is unnecessary