

## 7.06 Spring 2007 PROBLEM SET 2

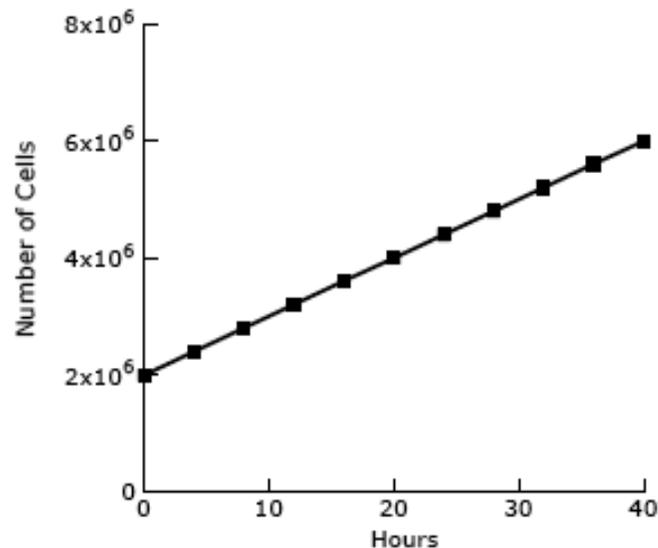
### Question 1.

You are studying the cell division cycle of a mammalian cell line grown in culture. Your goal is to determine how long cells spend in each part of the cell cycle. The culture is not synchronized: each cell in the culture is going through a cell cycle independent of the other cells. Therefore, at any point in time, a range of cell cycle states is present.

You pulse the cells for 30 minutes with BrdU, wash it out, and then add an excess of cold thymidine (in what is called a pulse-chase experiment). Following the Pulse-Chase treatment, you remove an aliquot of cells every hour and perform the following types of analysis.

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**Analysis 1:** First you count the number of cells in each aliquot and calculate the total number cells in the culture flask at various times. Your results are shown in figure 1.



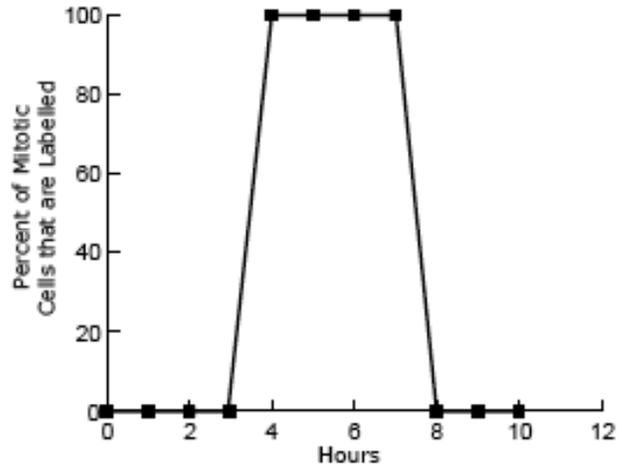
**Figure 1:** Total number of cells in the culture at various times.

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**Analysis 2:** Next, you stain the cells with fluorescent anti-tubulin antibodies and (using a microscope) determine the percentage of cells that are undergoing mitosis. At each time point, you consistently find that approximately 5% of the cells are undergoing mitosis.

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**Analysis 3:** From the hourly aliquots you isolate the mitotic cells. You use autoradiography to determine what fraction have been labeled with BrdU. Your results are shown in Figure 2.



**Figure 2: Fraction** of mitotic cells labeled with Brd U

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**1a. Draw a simple representation of the cell cycle showing the relationship of G1, mitosis, S-phase and G2. Indicate the position of START (the restriction point).**

**1b. What is the division time for this cell line? What data lead you to this conclusion?**

**1c. What is the duration of mitosis in the cell line used in analyses 1-3? What data lead you to this conclusion?**

**1d. What is the duration of S phase, G1 and G2 in the cell line in analyses 1-3? What data lead you to this conclusion?**

**Question 2.**

You are a graduate student in the laboratory of Paul Nurse and you have obtained several *S. pombe* strains that are mutant in various aspects of cdc2 regulation. You would like to determine the effect that each of the mutations has on cdc2 activity. (Note *S. pombe* cdc2 is the same cdk as cdk1)

**2a. How will you assay the activity of cdc2/cdk1 in the various mutants?**

**2b.** Indicate the activity of cdc2/cdk1 protein in the following mutants by comparing to cdc2/cdk1 activity that would be observed in a wild-type strain. **Would the mutant:wild type activity ratio be equal to 1, less than 1, or greater than 1? Why?**

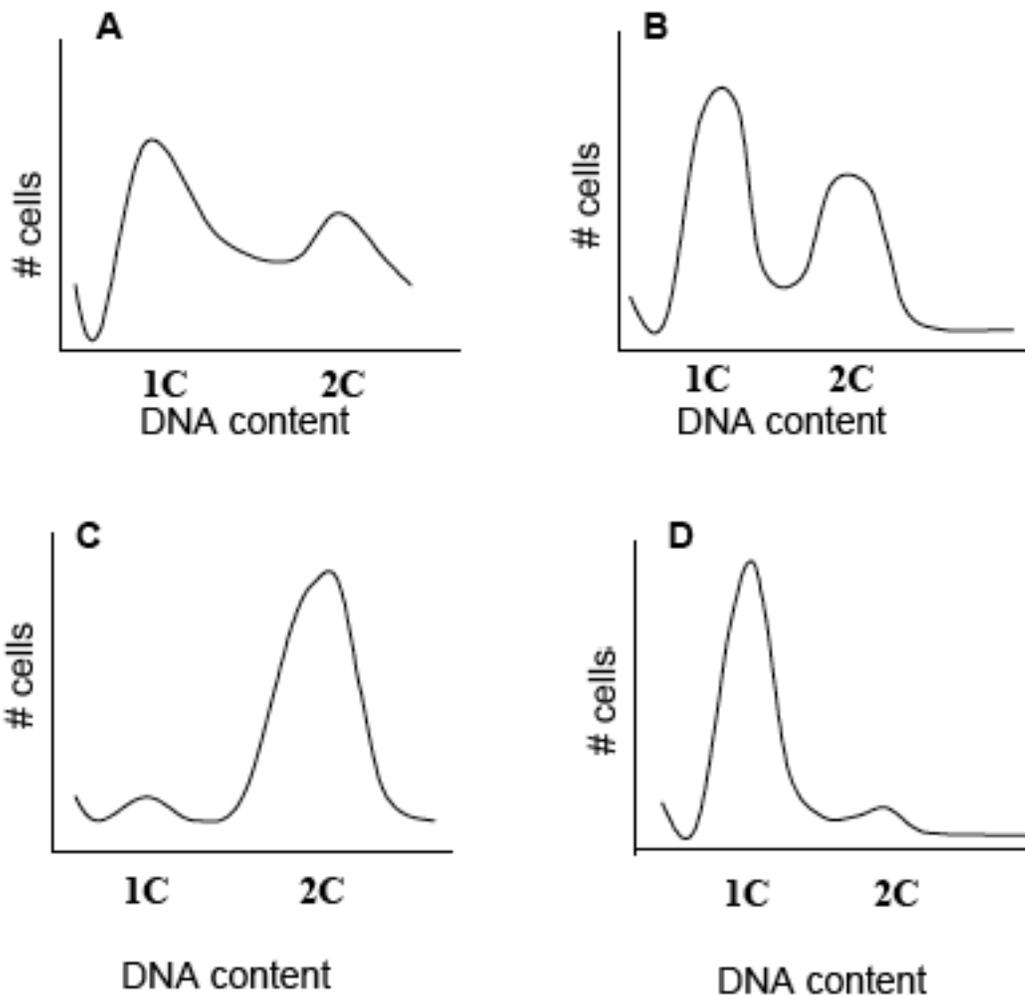
- 1) wee1-
- 2) cdc25-
- 3) wee1-, cdc25-
- 4) CAK-
- 5) Cdc2T161A (Thr changed to alanine)
- 6) CAK-, cdc2T161D (Thr changed to aspartic acid)

**2c.** Your baymate in the Nurse lab, a sincere but somewhat dim-witted grad student, has obtained a DNA-damage checkpoint mutant in a screen for mutants that are hypersensitive to  $\gamma$ -irradiation. The mutant progresses through the cell cycle in a wild-type manner in the absence of DNA-damage agents. He suspects that the mutant has a problem in activating the chk1 and chk2 kinases known to be involved in propagating the DNA-damage response. However, he finds that both kinases are able to be activated to a wild-type level in the presence of  $\gamma$ -irradiation. Completely stumped, he asks you for your help. You hypothesize that in this mutant, cdc25 may be unresponsive to the DNA-damage checkpoint activation. **How can you test your hypothesis? Give two experiments you could do and the expected results.**

### Question 3.

Your first task as new UROP in a *S.cerevisiae* budding yeast lab is to look at the cell cycle profiles of three temperature-sensitive mutant haploid yeast strains using FACS (fluorescence activated cell sorting).

**3a.** You incubate wild-type cells and your three temperature sensitive mutant cells at the permissive temperature, and then shift to the restrictive temperature for at least one cell cycle. You then stain the DNA of these cells with a fluorescent DNA stain. You then perform FACS analysis on the strains, and obtain the FACS profiles below. **Which profile is from wild-type cells, and what are the defects in the three mutant strains? Explain your answers.**



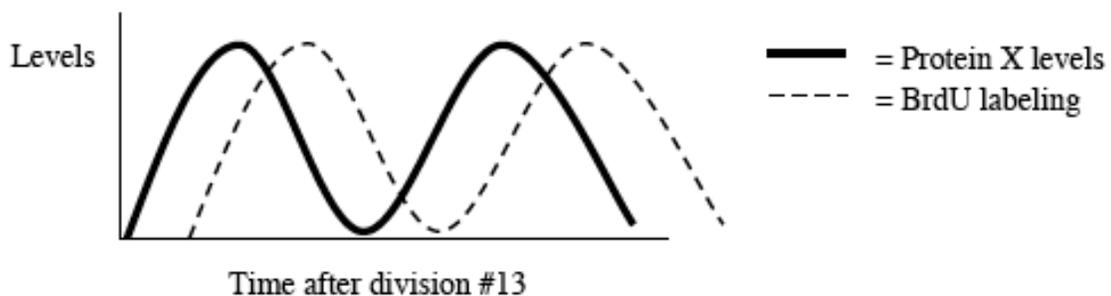
**3b.** You grow the four strains described above at the permissive temperature, and then incubate the strains at the restrictive temperature for the length of at least one cell cycle. You then stain the DNA and examine the cells under a microscope. **Draw the “terminal phenotypes” (what the cells look like under a microscope once they are frozen at the point in the cell cycle at which they become arrested or delayed) you would expect to see for each of the strains based on the defects you assigned above.**

**3c.** Some of the FACS profiles above could also have represented wild-type yeast strains that were treated with different compounds, such as  $\alpha$ -factor, HU (hydroxyurea) or nocodazole. **Which profiles in part a could represent such treated wild-type strains, and why?**

**3d. Describe an approach you could use to identify the genes mutated in these strains.**

**Question 4.**

You are interested in studying cell cycle regulation in *Drosophila* embryogenesis and decide to use genetics to identify critical cell cycle proteins. In the *Drosophila* embryo the first 13 divisions are rapid S-M cycles driven by maternal stockpiles of cell cycle regulators. Subsequent divisions occur in a normal cell cycle with gap phases and require gene expression from the embryo genome. You can visualize these divisions easily within the embryo. You identify a mutant in which homozygous embryos die. The first 13 divisions occur in these mutants but no BrdU incorporation is detected in the later divisions. You identify the gene mutated in this organism, you make antibodies against the corresponding wild-type protein (Protein X), and you track levels of the protein in the wild-type embryo at progressive times of embryogenesis relative to BrdU labeling.



**4a. Name two specific proteins that we learned about in class that Protein X could be, given everything you know about it.**

**4b.** *Drosophila* embryos normally survive low doses of Xrays. You screen for mutants and recover one (mutant Z) that, when homozygous, causes embryos to die even with low doses of Xrays. The homozygous embryos are normal in the absence of irradiation. You identify the gene mutated in this organism, and the corresponding protein (Protein Z). Given what you currently know, it is possible that Protein Z falls into one of two very different classes of proteins. **Name the two specific classes of proteins that Protein Z could fall into.**

**4c.** You find that, if you treat mutant Z embryos with doses of hydroxyurea that slow S phase but do not arrest the cell cycle, these embryos are now better able to survive X rays. **Into which proposed class of proteins from part (b) does protein Z fall? Explain why this result means that Protein Z falls into the one class you chose, and not the other.**

**4d.** You make an antibody against Protein Z. You treat wild-type embryos with a low dose of Xrays, make extracts from these embryos, and use your antibody to immunoprecipitate Protein Z from the extracts. You run an aliquot of the immunoprecipitation on a SDS-PAGE gel, and stain with Coomassie.  
**How is this experiment different from the co-immunoprecipitation technique in terms of:**

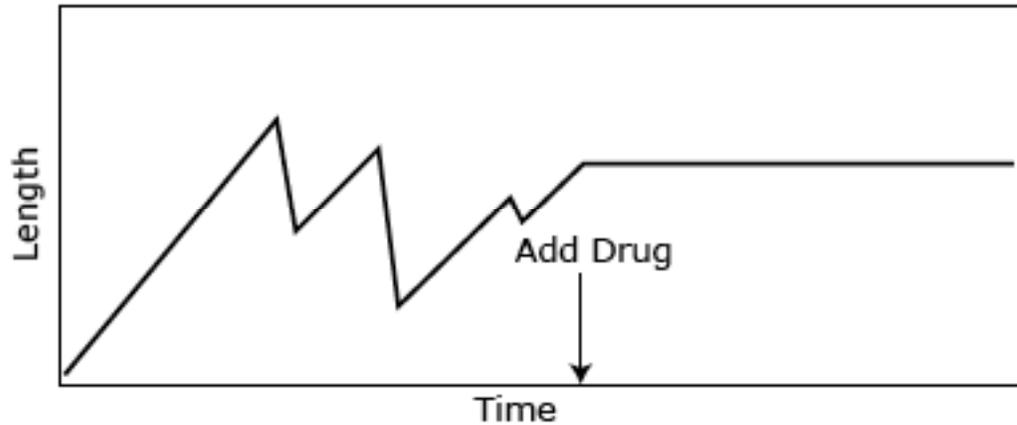
**(i) how the experiment was conducted**

**(ii) what the goal of the experiment is**

**4e.** You see multiple bands in your stained gel. **If you sequenced the protein found in one of those bands and it was a CDK, what would you propose regarding the mechanism of how Protein Z functions (given everything you know about it)?**

**Question 5.**

**5a.** You are provided purified  $\alpha$ - $\beta$  tubulin heterodimers and follow the length of microtubule filaments they form in vitro. Your advisor asks you to investigate the effects of a drug. You examine one of these fibers and follow its length over time, as plotted in the graph below.

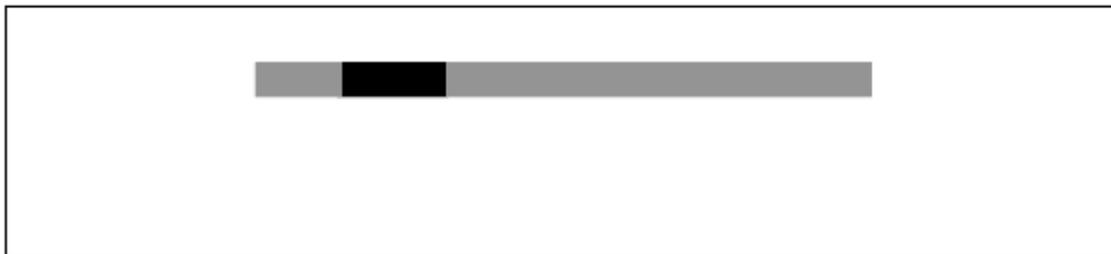


**i.) Explain the behavior of the filament prior to adding the drug.**

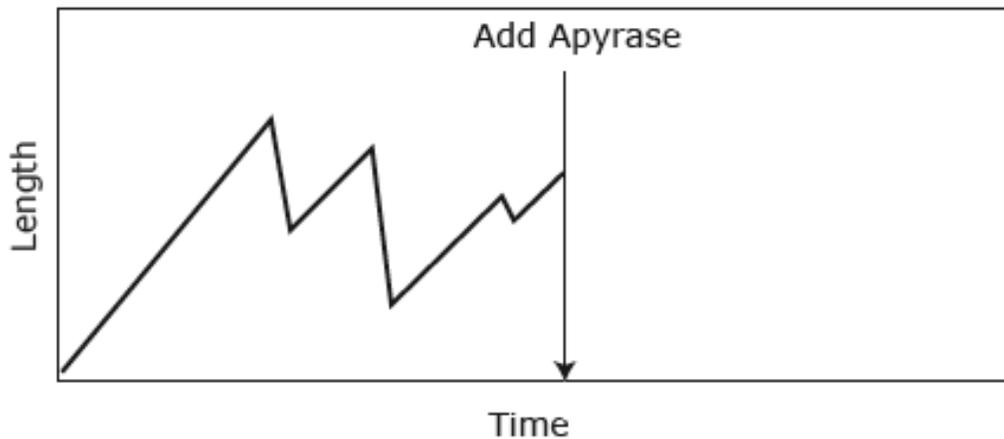
**ii.) You add the drug and cells stop dividing. How does this drug affect the microtubule protofilament?**

**5b.** Next, you assemble tubulin polymers *in vitro*, using two batches of tubulin labeled to differing extents with a fluorescent tag (one with 0.5 mol of Rhodamine per mol of tubulin and the other with 3 mol of Rhodamine per mol of tubulin). First you make a region with highly labeled tubulin. Then, you add the highly labeled seed to the dimly labeled tubulin. The assembled fibers are shown below. **Label the two ends of the polymer with (+)/(-) and describe why the dimly labeled region on one end is shorter than on the other end.**

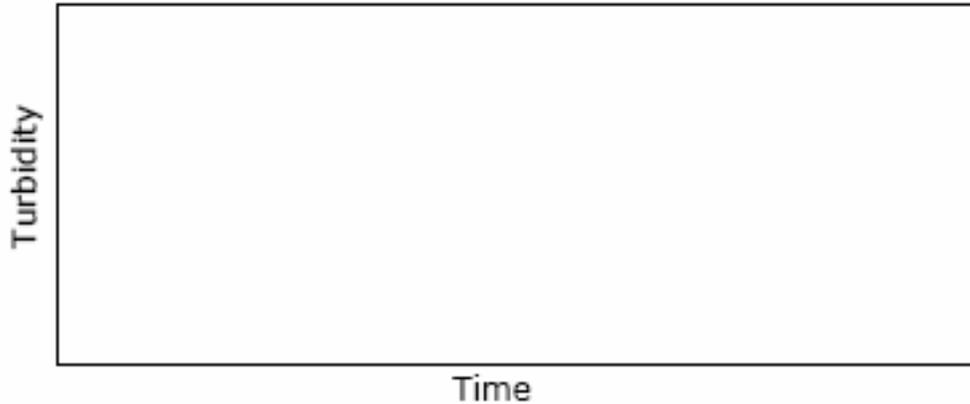
	Fiber made of 3 mol rhodamine/mol tubulin
	Fiber made of 0.5 mol rhodamine/mol tubulin



**5c.** Again, you examine a single tubulin microtubule and follow its length over time, as plotted in the graph below. It is very similar to what is seen *in vivo*. **Draw a line representing what happens to the length of the polymer after you add apyrase, an enzyme that hydrolyzes ATP, GTP and other high energy nucleoside triphosphates.**



**5d.** You have a solution of tubulin without any high energy nucleoside triphosphates. At time=0, you add a high concentration of GTP and ATP. **Draw on the graph below how the turbidity of the solution of tubulin will change over time.** (Turbidity increases with increasing filament length.) **Explain the shape of your graph.**



**Question 6.**

The fungus *Coprinus* undergoes meiosis synchronously as part of its developmental cycle. It is possible to isolate meiotic mutants in *Coprinus* and to examine the effects on meiotic chromosome segregation by staining the cells with a DNA stain and an antibody against tubulin.

**6a.** You recover a mutant that is hypersensitive to Xrays. Although Xrays induce double-strand breaks in the DNA, normally they are readily repaired and viability is not affected. In this mutant the cells die after exposure to Xrays. Although the mutant *Coprinus* cells grow fine during mitosis (provided they are not exposed to Xrays), mutant cells fail to complete meiosis and arrest in prophase I. **Provide a model for the defect in this mutant, explaining the hypersensitivity to Xrays and the prophase I arrest.**

**6b. Why is mitosis unaffected in the mutant cells?**

**6c.** You have a mutant in which the *Coprinus spo11* gene is nonfunctional. Spo11 encodes the enzyme that makes double-strand breaks in meiosis. The *spo11* mutant completes meiosis but there is massive chromosome nondisjunction. **Why does the *spo11* mutant complete meiosis but your mutant arrest in prophase I?**

**6d. Do you predict the nondisjunction events in the *spo11* mutant to occur in meiosis I, meiosis II, or both? Why?**

**6e. What do you predict to be the meiotic phenotype of a double mutant with your mutant and the *spo11* mutant?**