7.06 Recitation Section 4

Main concepts from Lecture #6:

1) The cell cycle consists of four phases: G1, S, G2 and M. G1 is primarily a growth phase. During S phase the cells replicate their DNA. G2 is another growth phase, during which time preparations are made for mitosis. During M phase cells segregate their chromosomes and then undergo cytokinesis.

2) The cell cycle progresses because of the different substrates of the ser/thr kinase activity of cyclin-CDK complexes. Cyclin-CDK complexes are regulated in many different ways:

a) At the post-translational level by phosphorylation (both activating and inhibitory).
b) At the level of synthesis, primarily transcription
c) By binding of the CKI inhibitors,
d) By regulated proteolysis.

3) CDK activity is controlled by cyclin binding and phosphorylation. Cyclin binding alone is not sufficient to activate the CDK enzyme. First, CAK must phosphorylate the cyclin on Thr 160. Second, Cdc25, a phosphatase, must remove the inhibitory phosphorylation on Tyr15 that is added by the Wee1 kinase.

4) In mammalian cells different cyclin-CDK complexes are active at different points during the cell cycle, driving different events.

G1 – CycC/CDK4,6
Early S – CycE/CDK2
Late S – CycA/CDK2
G2/M – CycA,B/CDK1

5) Ubiquitin Ligases, or E3s, are enzymes that attach poly-ubiquitin chains to proteins, which targets them for degradation by the proteasome. There are two major E3s involved in cell cycle control: the SCF (Skp1/Cullin/F-box), and the anaphase-promoting complex (APC). Substrate specificity in the SCF is provided by F-box proteins, while substrate specificity for the APC is provided by the adapter proteins Cdh1 and Cdc20. The SCF is important in the G1/S transition, while the APC is important during mitosis.

Experiments discussed in Lectures #9, 10:

Experiment	What this experiment tells you	How to perform this experiment
FACS (fluorescence activated cell sorting)		
BrdU Pulse Experiments		
Cell Division Cycle Screen		

Questions based on Lectures #9, 10:

1. Textbooks often draw chromosomes like this:

-- What does this drawing represent?

-- When do chromosomes look like this and when do they not look like this?

2. What would the FACS profile of the following mutants look like, and why?

-- A mutant in the SCF that renders it inactive

-- A CDK1 mutant

3) How would the activity of CycB/CDK1 be affected if all of its Lys side chains were mutated to Ala (assume that it otherwise functions normally)?

-- What type of mutant in another cell cycle component would give a similar phenotype (w.r.t CycB-CDK activity)? Why?

-- What type of mutant would give the same phenotype (w.r.t CycE-CDK activity) as a CycE mutant that lacks all of its Lys side chains? Why?