Problem Set 7
7.06 Spring 2015
Due May 1, 2015 in recitation section

Question 1. You decide to analyze cell division in human HeLa cells and plan to make mutant forms of key mitotic regulators. You will express these in cells in which the endogenous protein levels have been ablated by RNAi. Assume the endogenous protein is missing in all parts of this question.
(A) What would be the terminal phenotype of cells that express Scc1(Rad21) that cannot be cleaved?

(B) How could you mutate the *scc1* (*rad21*) gene such that the protein is always full length?

(C) How could you mutate the *separase* gene such that the Scc1 (Rad21) protein is always full length?

(D) How could you mutate the *securin* gene such that the Scc1 (Rad21) protein is always full length?

(E) For each of the three mutations above, state whether the mutation is a gain-of-function mutation, a dominant-negative mutation, or standard loss-of-function mutation.

i) *scc1* (*rad21*)
ii) *separase*
iii) *securin*

Question 2. Microtubule motors are critical for mitosis.

(A) What role does dynein play in prometaphase? Where is the pool of dynein required for this localized?

(B) Which motor counteracts dynein in prometaphase and why is this necessary? Where is this motor localized?

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(C) Kinesin 13 promotes depolymerization of microtubules from the plus ends. What role do you think kinesin 13 plays in mitotic chromosome movement and why?

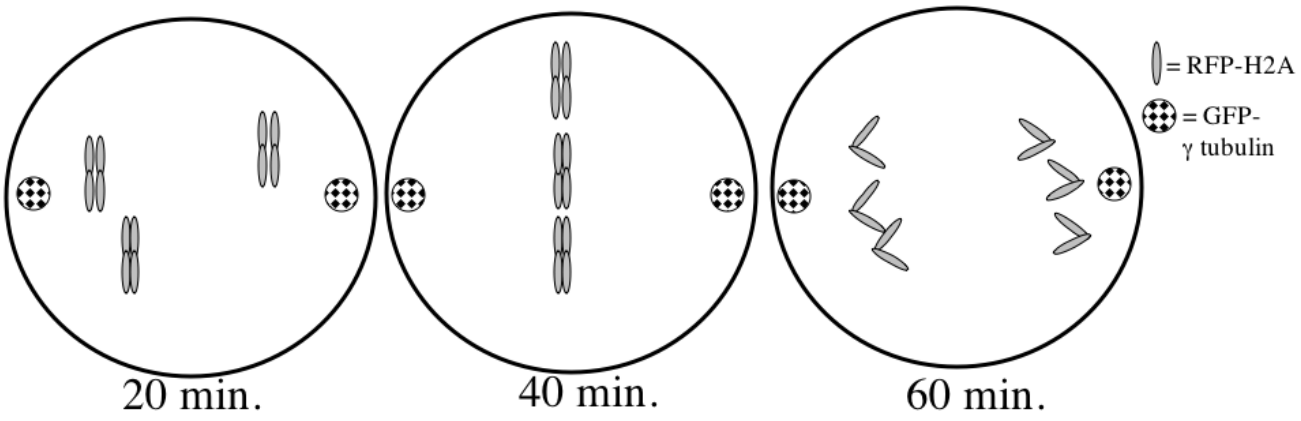
Question 3

You want to investigate the functions of several proteins during mitotic chromosome segregation in human cells. You use RNAi to knock down the levels of these proteins and follow the consequences by live imaging of cells expressing GFP-gamma tubulin and RFP-histone H2A, which permits you to visualize chromosome behavior. In another set of experiments you replace the wild-type protein with a mutant form described below.

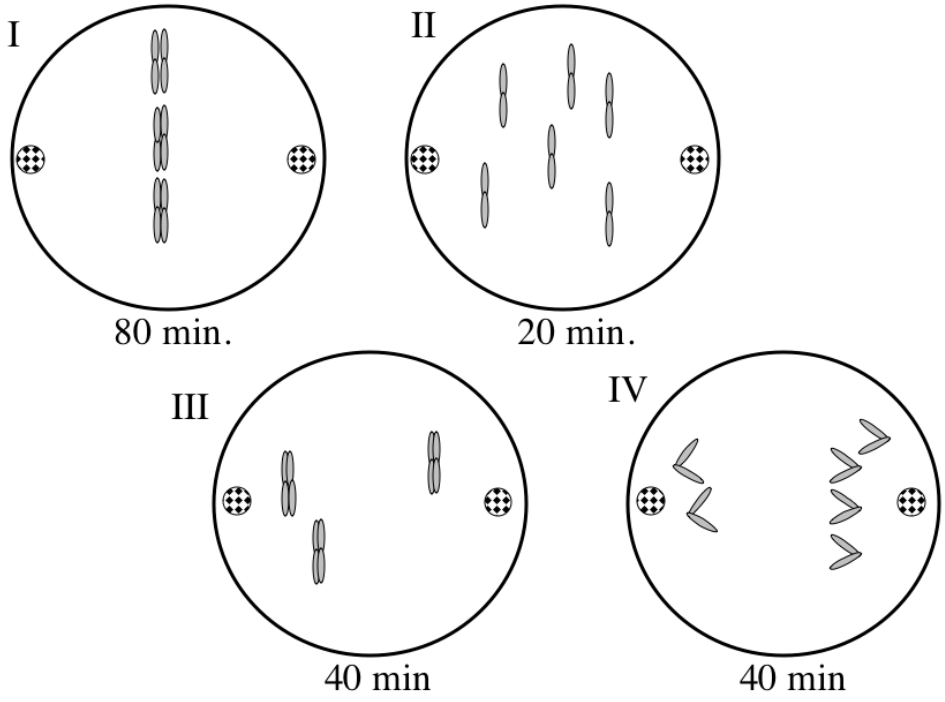
(A) What cellular component contains gamma tubulin? What is the function of gamma tubulin?

You analyze the configuration of chromosomes relative to the spindle poles at defined time points after nuclear envelope breakdown. The diagrams below show what you observe, although for simplicity only three chromosomes are shown.

Control RNAi- time points after nuclear envelope breakdown



Defects observed with the different RNAi targets at the indicated time points after nuclear envelope breakdown



(B) Which defect (I-IV) do you expect to observe with RNAi against SMC1? Briefly explain.

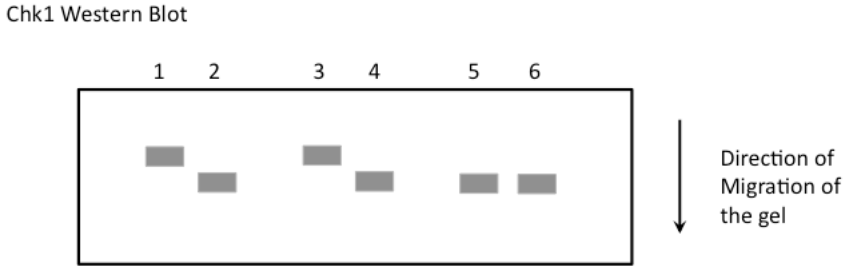
(C) Which defect to you expect to observe with RNAi against CenpE? Explain.

(D) Which would you see if you replaced Cdc20 with a form that cannot bind the APC? Explain.

(E) Which would you see if you replaced Cdc20 with a form that cannot bind Mad2? Explain.

(F) Which would you see if you did RNAi against Aurora B kinase? Explain.

Question 4. HU depletes pools of dNTPs. The lanes on the Chk1 Western below are pairs of HU treated and untreated control cells.



(A) Is lane 1 or 2 most likely to be from cells treated with HU?

(B) Which of the pairs (3 and 4 or 5 and 6) are from cells treated with RNAi against Atr?

(C) Which of the pairs (3 and 4 or 5 and 6) are from cells treated with RNAi against Atm?

(D) RPA is a protein that binds to single-strand DNA. It is known to be able to recruit a DNA damage signaling kinase via an adaptor protein. Which do you expect it to recruit? Why does it make sense that activation of this kinase requires interaction with RPA?

Question 5. The fission yeast *S. pombe* elongates as it grows, and cytokinesis splits the rod-shaped cell symmetrically. During cytokinesis a cleavage furrow composed of microfilaments forms at the site of the central spindle microtubules after anaphase B.

(A) You obtain a temperature-sensitive dynein mutant and find that at the nonpermissive temperature, cytokinesis is delayed. From your knowledge about the role of dynein in anaphase B, why do you think cytokinesis is delayed?

(B) You observe the same delay in cytokinesis in a kinesin 5 mutant. Taking into account the phenotype of the dynein mutant, what does this suggest?

(C) You mutagenize the temperature-sensitive *dynein* mutant and recover double mutants in which at the nonpermissive temperature the cells undergo cytokinesis without a delay. Frequently the DNA is caught at the cleavage furrow resulting in a cut phenotype. You pursue one of these mutants, *cyto*. In combination with *kinesin 5* mutant alleles *cyto* mutants also have a cut phenotype. In contrast, the *cyto* mutant alone has no cytokinesis defect.

Mutant	*dynein*	*kinesin 5*	*dynein, cyto*	*kinesin 5, cyto*	*cyto*
Phenotype	Cytokinesis delay	Cytokinesis delay	cut	cut	No defect

i) Based on these phenotypes what is the normal function of the gene defined by the *cyto* mutation?

ii) Why does the *cyto* mutant alone have no defect?

iii) What does this tell you about the regulation of cytokinesis?