Problem Set 7 Answer Key
7.06 Spring 2015

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?

Metaphase arrest

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

Mutate the amino acids in the two Separase cleavage sites

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

Put in a stop codon early in the sequence or mutate the cysteine in the active site.

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

Change the lysine residue to which Ubiquitin is conjugated or delete the D box. [The D box is the recognition motif for Cdc20/APC]

(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*) gain of function
ii) *separase* loss of function
iii) *securin* gain of function

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?

Dynein is a minus-end directed motor and thus promotes poleward movement. This pool of dynein is at the kinetochore.

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

Cenp E is a plus-end directed motor that counteracts dynein in prometaphase. It is necessary so the chromosomes move to the metaphase plate rather than ending up at the poles in prometaphase. Cenp E is at the kinetochore.

(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?

Kinesin 13 is necessary for anaphase A, when the driving force for poleward movement is MT depolymerization.

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.

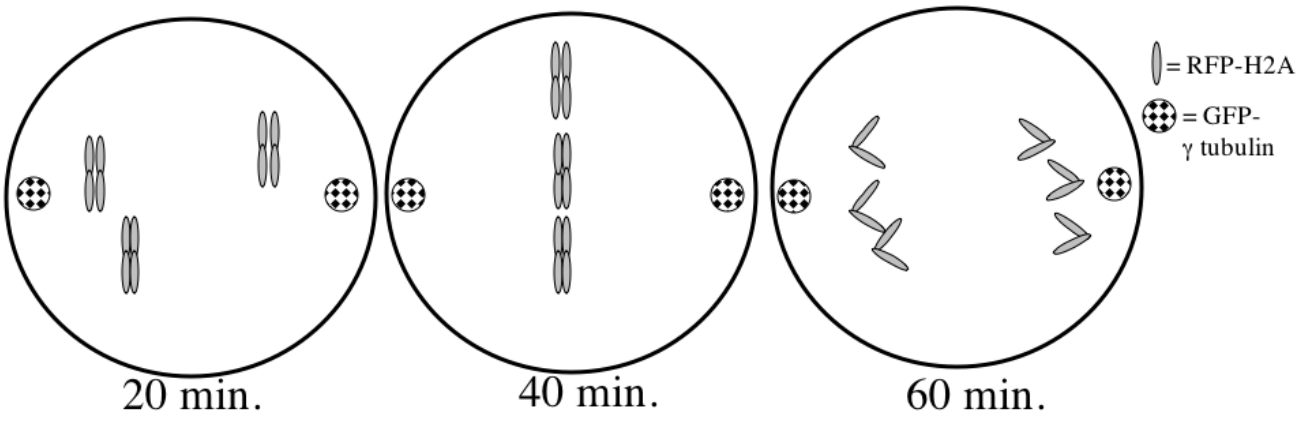
(A) What cellular component contains gamma tubulin? What is the function of gamma tubulin?

the centrosome/MTOC

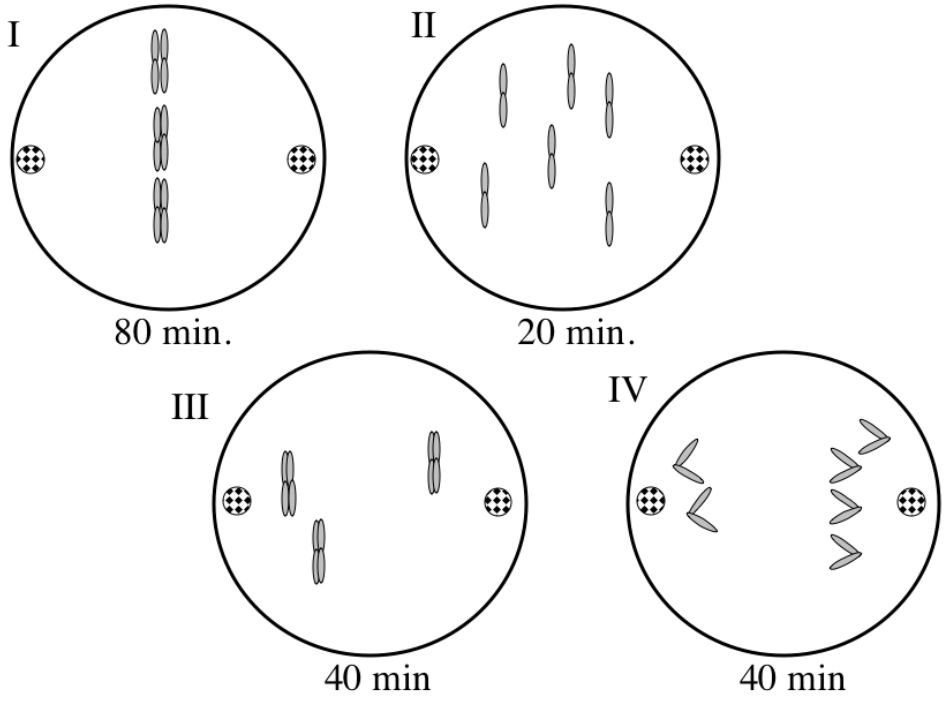
the rings made by gamma tubulin nucleate microtubule polymerization

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.

II, because SMC1 is a subunit of cohesion. Without cohesin the sister chromatids will prematurely separate, so at 20 minutes they already have lost cohesion.

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

III, because CenpE is needed for congression to the metaphase plate. In III, the chromosomes are not on a metaphase plate at 40 minutes.

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

I, because Cdc20/APC activity is required for the metaphase/anaphase transition and this activity requires binding of Cdc20 to the APC. In I the cells are still in metaphase at 80 minutes.

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

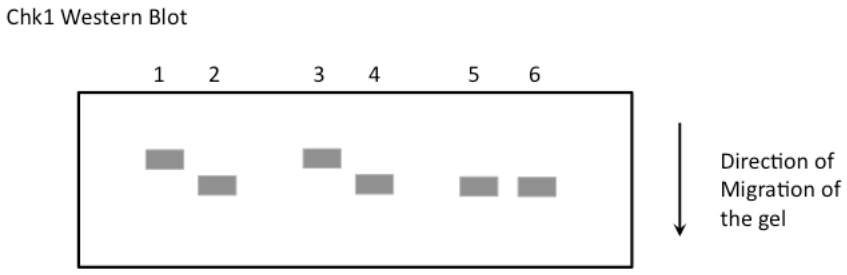
IV, because if Cdc20 cannot bind Mad2 the metaphase/anaphase transition will happen even if bipolar MT-kinetochore attachments have not been made. So chromosome missegregation events such as in IV will occur.
Note: metaphase/anaphase would occur earlier than the normal time point of 40 minutes.

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

Of the possibilities shown, III, because Aurora B will not be present to cause release of MT-Kinetochore attachments, so ones in which both kinetochores attach to the same pole will be present and cause chromosomes to move towards that pole.

Note: the other thing you would see would be something like IV, except occurring earlier than 40 minutes. This is because without Aurora B MT-Kinetochore attachments will be stable, Mad2 will come off the kinetochores, the SAC will be shut off, and Cdc20/APC will be activated. So metaphase/anaphase will occur earlier, and there will be missegregation from chromosomes that did not have bipolar attachments.

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?

1, because Chk1 has a mobility shift consistent with phosphorylation

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

5 and 6, because this blocks phosphorylation of Chk1

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

3 and 4, because Chk1 is still phosphorylated in response to HU treatment

(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?

Atr

This makes sense because it links Atr activation (the sensor) directly to sites of incomplete DNA replication or DNA damage that results in single-stranded DNA

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?

Cortical dyneins act on astral MT during anaphase B to pull on the microtubule organizing center (MTOC or centrosome) which helps pull spindle poles apart.

In dynein mutants, cytokinesis is delayed because poles are not separated during Anaphase B.

(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?

Anaphase B also is driven by a bipolar kinesin-5-dependent sliding filament mechanism. Kinesin-5 slides overlapping interpolar MT away from each other, which helps to push poles apart.

Since both dynein and kinesin-5 have key roles during anaphase B, it suggests that the lack of pole separation during anaphase B is leading to the cytokinesis delay.

(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?

Cyto wild-type function promotes a delay in cytokinesis until anaphase B is completed.

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

kinesin-5 and dynein are both wild type. Hence anaphase B progresses normally. A cyto-mediated delay is not required. The cyto mutant is disrupting a checkpoint, and the cyto gene product itself is not required for anaphase B or cytokinesis.

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

There are 2 sets of proteins:

Motor proteins (eg. dyneins and kinesin-5) for pole separation during anaphase B.

Checkpoint proteins (eg. Cyto) that ensure anaphase B happens properly before cytokinesis can occur.

[Note: this problem is a hypothetical example to illustrate the principle of a checkpoint.]