

Name: _____

TA Name and Recitation Time:

7.06 Cell Biology QUIZ #2

This is an **open book** exam, and you are allowed access to books and notes, but not computers or any other types of electronic devices.

Please write your answers to questions in **pen** (not pencil) in the space allotted.

Please write only on the **FRONT SIDE** of each sheet.

And be sure to put your **name on each page** in case they become separated.

There are **9 pages** to the quiz, make sure you have a complete copy!

Remember that we will Xerox all of the quizzes.

Good Luck!

Question 1. 30 pts _____

Question 2. 30 pts _____

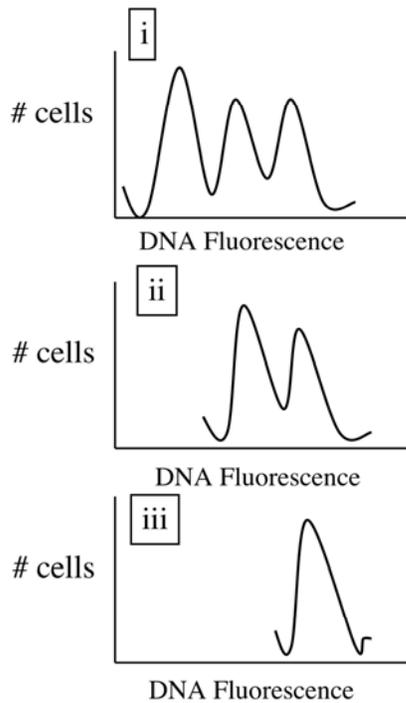
Question 3. 20 pts _____

Question 4. 20 pts _____

Question 1. (30 points)

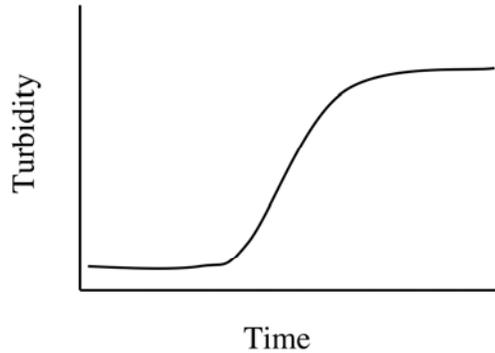
The questions below ask you to evaluate experimental data.

1a. (6 points) You use FACS to measure the DNA content of three samples: a cell preparation from mouse testis with all the testis cell types, isolated prophase I arrested mouse oocytes, and mammalian cell culture. Unfortunately you forgot to label the tubes. **Which of the three FACS profiles below is most likely to correspond to each of the three samples? Explain your reasoning.**

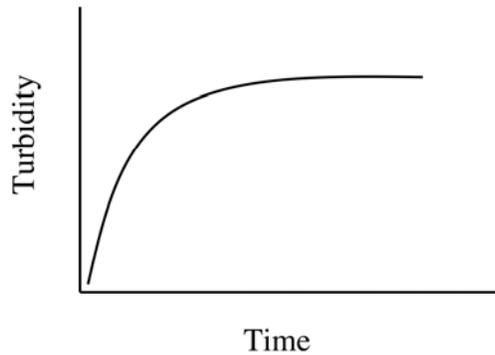


1b. (6 points) You analyze the kinetics of polymerization of α - β tubulin dimers in vitro. You use turbidity as an assay for polymerization because this increases with polymerization and find the following:

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If you add γ -tubulin rings you observe the following



Explain the effect of γ -tubulin. How does this account for the role of γ -tubulin in vivo and its cellular location?

1c. (10 points) You did an experiment in which you incubated the cohesin complex with Separase alone or Securin and Separase in vitro then ran a gel, prepared a western blot and probed it with an antibody against the Scc1 subunit. In a separate in vitro experiment you incubated CyclinB protein with Cdh1/APC with or without the 26S proteasome, ran a gel, made a western blot and probed it with antibodies against CyclinB. Not having learned from failing to label your tubes in the FACS experiment, you failed to label the two western membranes. In each experiment, however, you were careful to include the untreated proteins as a control. Label below which lane is most likely to contain which sample.

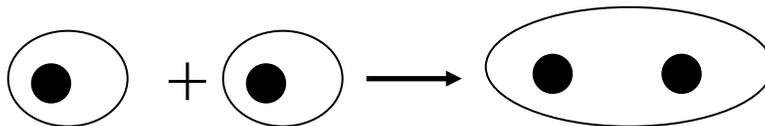
Explain the protein mobility and the effect of each treatment on Cdk1.

Question 2. (30 points)

Two types of classical cell biology experiments uncovered the principles of cell cycle regulation. We now understand the observations made in these experiments in molecular terms, because we can explain the results by the action of the cell cycle regulators we discussed in class.

Rao and Johnson did a series of experiments in which they fused a cell in one phase of the cell cycle with a cell in another phase of the cell cycle. In the fused cell both nuclei could be observed.

2a. (2 points) One experiment done by Rao and Johnson was to fuse a mammalian cell in G1 with a mammalian cell in S phase. In the fused cell the two nuclei are visible, and it was observed that the fusion caused the G1 nucleus to enter S phase.



G1 cell

S phase cell

Fused Cell

How could they show the G1 nucleus entered S phase?

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2b. (6 points) From your knowledge about the cell cycle control of the initiation of DNA replication, **what components present in the G1 nucleus and the S phase cell were responsible for the G1 nucleus initiating DNA replication in the fused cell?**

2c. (6 points) When Rao and Johnson fused a G2 cell with an S phase cell the G2 nucleus did not undergo DNA replication. **Why can't a replication origin in a G2 nucleus be made to fire when fused to an S phase cell?**

2d. (3 points) Fusing a cell in mitosis with an S phase cell caused the S phase cell to immediately enter mitosis, condensing even the unreplicated segments of the chromosomes. **What cell cycle regulators in the M phase cell triggered mitosis in the S phase nucleus?**

2e. (7 points) In the fusion of the M phase cell to the S phase cell why didn't the incomplete DNA replication checkpoint block the onset of mitosis in the S phase nucleus?

2f. (6 points) The other type of experiment that provided great insights into cell cycle regulation was cytoplasmic transfer from *Xenopus* eggs. Remember that mature *Xenopus* eggs are arrested at metaphase II of meiosis. If cytoplasm is taken from mature eggs and injected into interphase cells in embryos they immediately go into mitosis. **What active cell cycle component must have been present in the *Xenopus* egg and transferred in the cytoplasm to cause the interphase cells to go into mitosis?**

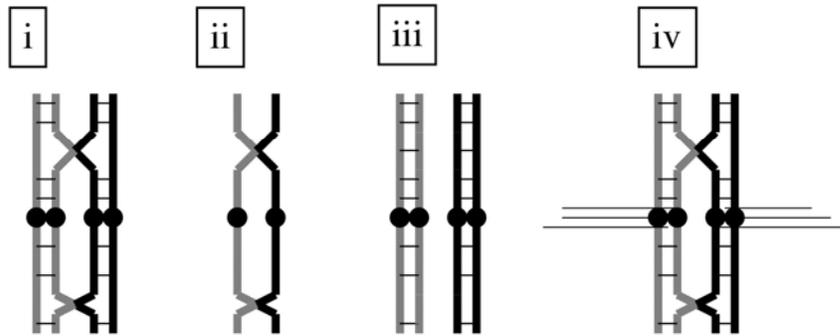
Question 3. (20 points)

Humans have extremely high rates of meiotic nondisjunction. It is estimated that at least one chromosome has undergone inaccurate chromosome segregation in 20% of oocytes and 3-4% of spermatocytes.

3a. (2 points) Why might the rate of nondisjunction be higher in oocytes than spermatocytes?

3b. (4 points) Circle the diagram below that properly shows the configuration of the sister chromatids and homologs for one chromosome pair in the prophase I arrest in oocytes.

(The thick vertical lines represent DNA duplexes, the short horizontal lines cohesion, and the long horizontal lines microtubules. The black circles are kinetochores.)



3c. (6 points) Circle all of the components that maintain attachments between homologs in meiosis I.

condensin

cohesin

chiasmata

centrosome

Sgo (MEI-S332)

kinesin

kinetochore

microtubules

dynein

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3d. (8 points) Which of the following models explain the human maternal age effect on meiotic nondisjunction? **Explain your reasoning for your choice or choices.**

- i.) the proteins of the pre-RC break down over time
- ii.) securin is not stable over prolonged periods
- iii.) the microtubules of the spindle have a short half life
- iv.) the cohesin complex breaks down

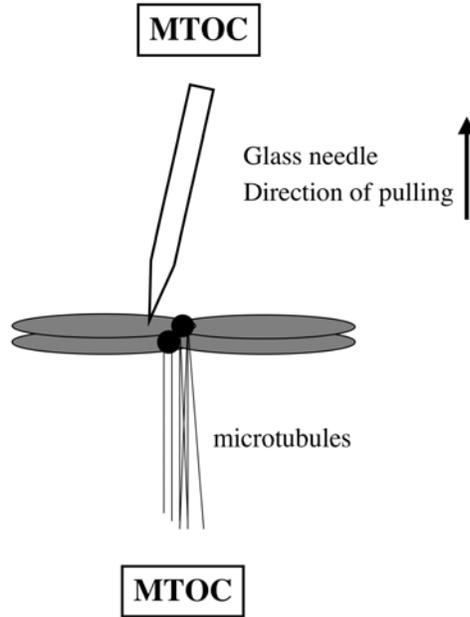
Question 4. (20 points)

Some mitotic cells are large enough and have big enough chromosomes that it is possible to move the chromosomes with a glass needle. The glass needle can be used to detach the kinetochores from the microtubules or to pull on the chromosomes.

4a. (3 points) If one kinetochore is detached from microtubules in a metaphase cell, the cell does not go into anaphase until microtubules reattach. **Why does the cell delay going into anaphase?**

4b. (4 points) If one kinetochore of a bioriented metaphase chromosome is detached from microtubules and reattached to microtubules from the same spindle pole as its sister kinetochore the cell does not go into anaphase. **Explain the delay. What does this experiment show beyond the experiment in 4a?**

4c. (5 points) The same manipulation is done to attach both sister-chromatid kinetochores from one chromosome to microtubules from the same pole, but this time the glass needle is used to pull one sister chromatid in the direction of the opposite pole (see the drawing below). The cell proceeds readily into anaphase. **Explain why pulling the sister chromatid towards the opposite pole causes the onset of anaphase. What does this experiment show beyond the experiments in 4 a and b?**



4d. (5 points) In the diagram above draw the location of the Mad2 protein. Explain the molecular basis for the observations in the experiments in parts 4 (a) and (c).

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4e. (3 points) From your knowledge of anaphase, explain why the drug taxol that stabilizes microtubules is toxic to dividing cells.