7.06 Spring 2015
Problem Set 8 Answer Key

Question 1

You may have learned in 7.03 that *Drosophila melanogaster* males are unusual because they do not undergo meiotic recombination, rather they evolved a mechanism for homologous chromosome segregation that involves pairing and attachment at specific sites in each of the homolog pairs. You decide that for a UROP project you will study meiotic chromosome segregation in *Drosophila ananassae*, in which meiotic recombination does occur in males as well as females.

In *Drosophila ananassae* normally females are XX and males are XY. There is a dominant marker on the Y chromosome that makes the body brown rather than yellow. This lets you detect the Y chromosome, which is important because there are exceptional flies with just an X chromosome, X0, that are males with yellow bodies. Flies with three sex chromosomes, XXY, are females with brown bodies.

You are given a mutant that does not undergo crossing over (*rec1*) and a *rec8* mutant.

(A) You cross a *rec1* mutant male to a wild-type female. Some of the female progeny are yellow, but others are brown. Some of the male progeny are brown, but others are yellow. Explain how this differs from a cross between a wild-type male and a wild-type female. How does the defect in the *rec1* mutant male to explain the progeny recovered?

In a cross between a wild-type male (XY, brown) and a wild-type female (XX, yellow) all the progeny will be either brown males or yellow females.
In the *rec1* mutant meiotic recombination does not occur, so there are chromosome segregation errors in meiosis I. The mutant males can produce sperm that have no sex chromosomes, giving rise to X0 male progeny that are yellow. They also can produce sperm with both an X and Y chromosome, yielding XXY brown female progeny. Due to random segregation in the absence of recombination, at some frequency the X and Y chromosomes will segregate in meiosis I, producing sperm with either an X or a Y chromosome and normal brown male and yellow female progeny.

(B) In a *rec8* mutant male would you predict that there would be meiosis I or II segregation defects? Why?

You would predict both divisions to be affected. In meiosis I loss of rec8 cohesin will cause failure to maintain chiasmata, so homolog segregation will be defective. In meiosis II, loss of cohesin at the centromere will cause defective sister chromatid segregation.

(C) You recover a mutant in which the levels of meiotic recombination are normal, the kinetochores and spindle are normal, and yet there is meiosis I chromosome missegregation. What function could be defective in the mutant?

One possibility is sister-chromatid cohesion, so that although chiasmata are formed they are not maintained.

The other possibility is that the synaptonemal complex is defective so that even though the total number of recombination events is normal they are not distributed properly to ensure each chromosome has at least one crossover in meiosis.

Question 2

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.

(A) You recover a mutant that is hypersensitive to Xrays. Xrays induce double-strand breaks in the DNA, but 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.

The mutant must be defective in repair of double-strand DNA breaks. Mitotically growing cells are okay unless exposed to Xrays that cause double-strand breaks. The mutant cells are unable to repair these breaks and die, thus are hypersensitive to Xrays compared to wild-type cells.
Recombination is required for accurate homolog segregation in meiosis I, and meiotic recombination is initiated by the formation of double-strand breaks. So the mutant would be unable to undergo meiotic recombination and repair meiotic double-strand breaks. This is sensed by a checkpoint, leading to arrest in prophase I.

(B) Why is mitosis unaffected in the mutant cells?

Mitotic chromosome segregation does not require recombination; the homologous chromosomes do not pair or segregate.

(C) 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?

The *spo11* mutant cells complete meiosis because double-strand breaks are not formed, thus unrepaired breaks are not detected by the checkpoint and meiosis is not arrested.

(D) Do you predict the nondisjunction events in the *spo11* mutant to occur in meiosis I, meiosis II, or both? Why?

Recombination via double-strand break repair is required for segregation of the homologs in meiosis I. The nondisjunction will be in meiosis I, and sister chromatid segregation in meiosis II will not be affected.

(E) What do you predict to be the meiotic phenotype of a double mutant with your mutant and the *spo11* mutant?

The *spo11* phenotype will be observed in the double mutant: completion of meiosis with high levels of meiosis I nondisjunction. This is because if double-strand breaks are not made the mutant defect of being unable to repair them will not be detected.

Question 3

You want to study the properties of embryonic stem (ES) cells. ES cells are normally grown in the presence of fibroblast feeder cells, but being inexperienced you did not know this fact and instead you grew the ES cells in standard tissue culture medium over the weekend. To your great dismay you discover on Monday that all the cells had died.

(A) Describe two microscopy assays that you could perform that would allow you to determine that the cells had died by apoptosis. Describe what you would expect to see in the event that cells indeed underwent apoptosis.

TUNEL assay: In this assay addition of the enzyme deoxyterminal transferase and fluorescent nucleotides will label apoptotic nuclei because they have a lot of 3' DNA ends. In apoptotic cells you would expect to see many fluorescent foci.

DAPI staining: In this assay add DAPI to cells to visualize the DNA. Apoptotic cells will have highly uniformly condensed DNA.

General cell Morphology: Apoptotic cells will exhibit blebbing membranes, pyknotic nuclei, and a reduced cell volume.

Immunofluorescence of Annexin V to show that lipid asymmetry has been lost and phosphatidylserine is on the outside of the bilayer.

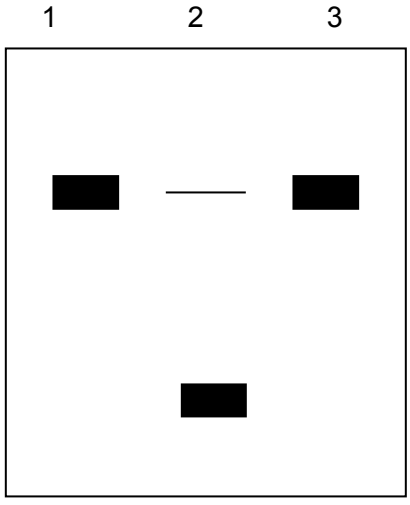
Having determined that the ES cells die by apoptosis you now wish to characterize the apoptotic events in more detail. You first want to determine whether cytochrome C release is occurring in the ES cells that are grown in standard tissue culture medium rather than in the presence of feeder cells.

(B) Suggest an experiment that would show that Cytochrome C is released from mitochondria in ES cells grown in standard tissue culture medium (assume you have all the tools necessary to do the experiment). Be sure to include appropriate controls.

You could perform an immunofluorescence experiment to detect the protein in the cytoplasm rather than inside the mitochondria.

In the experiment you would grow the ES cell in standard tissue culture medium and then examine cytochrome C. To be sure that you can detect cytochrome C in the cytoplasm you need controls. A positive control could be generated by treating cells in a manner that you know creates apoptosis, for example you could treat cells with DNA damaging agents or UV known to induce apoptosis. As a negative control (to be sure that the antibody is not cross-reacting with a cytoplasmic protein) you could use ES cells grown on feeder cells. These cells should not undergo apoptosis. You also need to label mitochondria with a fluorescence marker to distinguish mitochrondrial from cytoplasmic localization of cytochrome C.

Having convinced yourself that these cells are indeed undergoing apoptosis and do so by releasing Cytochrome C from mitochondria you want to understand what it is about these feeder cells that maintain the ES cells alive. Is cell-cell contact critical or do the feeder cells secrete a trophic factor into the medium that promotes ES cell survival?
To distinguish between these possibilities you examine Caspase 9 protein by western blot analysis under three different conditions. This is the result you obtain:



Caspase 9 Western

Lane 1: ES+Feeder cells

(C) What does this western blot tell you about how feeder cells inhibit apoptosis in ES cells? Provide a hypothesis that explains this result.

The western blot results shows that the medium previously used to grow feeder cells is sufficient to prevent apoptosis. This result indicates that feeder cells secret a soluble factor that prevents apoptosis.

Hypothesis: It is most likely that the feeder cells produce a soluble trophic factor (i.e. an ES cell growth hormone) that is required to maintain the apoptotic program in an inactive state.

Question 4

(A) Explain two advantages of a protease cascade versus transcriptional control to induce apoptosis.

Three possibilities: rapid activation; amplification of signal; irreversible

(B) What are two proteins of distinct types released from the mitochondria following expression of Bad?

Cytochrome C
Anti-IAPs (can give names as Diablo or Smac)

(C) In mammals killer lymphocytes can impede tumor growth.
i. What do you predict to be the consequence for tumor growth if the gene for a secreted protein that binds and blocks the Fas ligand is amplified? Explain.

Increased growth
This is because extrinsic signaling for apoptosis will be blocked

ii. What do you predict to be the consequence for tumor growth if the Fas receptor becomes mutated such that it constitutively trimerizes? Explain.

reduced tumor growth
This is because there will be constitutive activation of the extrinsic cell death pathway

(D) What experiment could you do to determine whether in mammalian cells the extrinsic pathway for inducing apoptosis feeds into the intrinsic pathway? Be certain to say how you would assay for activation of the intrinsic pathway.

Take a cell line, add Fas ligand to activate Fas receptor and extrinsic apoptosis pathway. Use an antibody against Cytochrome C to see if Fas activation leads to release of Cytochrome C from the mitochondria as an assay for activation of the intrinsic pathway.