7.06 Spring 2015
Problem Set 8
Due in Recitation May 8, 2015

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?

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

(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?

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.

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

(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?

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

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

Question 3

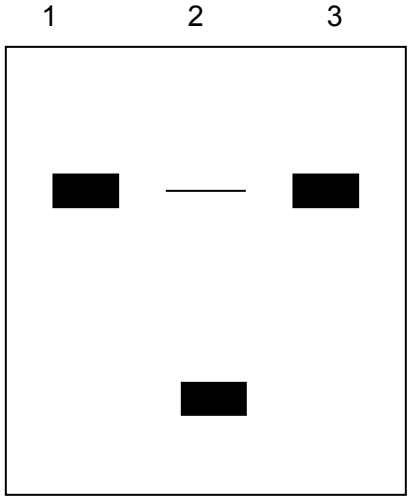
You want to study the properties of embryonic stem (ES) cells. ES cells are normally grown in the presence of fibroblast feeder cells, but being inexperienced you did not know this fact and instead you grew the ES cells in standard tissue culture medium over the weekend. To your great dismay you discover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

(A) Describe <u>two microscopy assays</u> 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.

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.

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
Lane 2: ES cells in standard tissue culture medium
Lane 3: ES cells in medium previously used to culture 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.

Question 4

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

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

(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.

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

(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.