

7.013 Problem Set 5 FRIDAY April 2nd, 2004

Problem sets will NOT be accepted late.

Problem 1

A great medical revolution has occurred with the ability to assist the large number of couples that previously would be incapable of having children. Generally, about half of infertility cases are due to paternal issues.

Congratulations, after inspiration from 7.013 you decided to forsake a lucrative career in computer science and enter the world of medicine. After an additional four years of medical school, and numerous years of training you are fiscally broke, but are now a medical professional treating infertility.

A young and otherwise healthy couple approaches you after experiencing difficulty in conceiving despite over one year of attempts. The woman has had a child from a previous husband. You suspect that the man has infertility issues and begin a work-up to determine the problem.

A. A sample of freshly collected semen is analyzed microscopically for sperm number and activity. Unlike the videos shown in class of vigorous sperm ascending towards the egg, the sperm sample indicates a normal number of sperm swimming rather lazily in a dish.

a. How many sperm are typically present in a human ejaculate? Why are there so many?

b. What (other than an actual defect) might explain the languorous behavior of the freshly ejaculated sperm *in vitro*?

c. Human ejaculate is a complex mixture of many substances. One of the primary ingredients is fructose (a simple disaccharide). What two properties of sperm may explain the presence of fructose in ejaculate?

B. After determining that the number, morphology, and activity of the sperm are normal, you next seek to test whether the sperm are capable of fertilizing an intact egg. However, with human eggs hard to come by you are forced to use hamster eggs. Hamster eggs are easily obtained by hormonal treatment of female hamsters inducing superovulation. A large number of eggs can be recovered by removal and gentle tapping of the uterine horn with eggs dropping out into a dish.

a) What hormone was likely used to induce superovulation of the hamster? What is the normal fate of these extra eggs (without the introduction of hormone)?

b) Have the collected eggs completed meiosis? If not, at what meiotic stage are the eggs frozen? What prompts the completion of meiosis?

c) Would you expect a human sperm to fertilize an intact hamster egg? What component of the hamster egg prevents fertilization from occurring?

C. A simple enzymatic treatment of the hamster eggs can effectively remove the component that you named above. After removal of this component, human sperm can now fertilize the treated hamster egg to form a zygote. Worry not; the resulting zygote cannot actually develop into a human/hamster hybrid.

a) Assuming that the man's sperm can fertilize the treated hamster egg, at what stage of fertilization are the man's sperm possibly failing.

b) Are the couple's plans for children doomed? What mode of ART would you suggest to overcome the man's difficulties?

Problem 2

Teratocarcinoma is a rare form of cancer that typically forms on the testes or ovary. As opposed to most cancers that are composed of accumulations of cells that are similar in appearance, teratocarcinomas are quite heterogenous. Both gross and histologic examination of a teratocarcinoma reveal a variety of differentiated tissues present within the cancerous mass including: hair, teeth, intestinal epithelium, skin, etc..

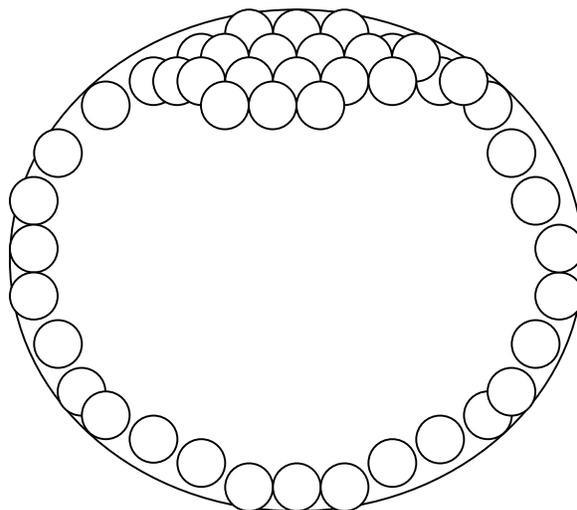
a) Was the cell that gave rise to the teratocarcinoma differentiated?

b) Assuming that the teratocarcinoma cells can give rise to all the tissues present in an adult how would you describe their potency?

Teratocarcinoma cells have proven quite useful *in vitro* (grown in culture) to define both inducers and determinants of cell differentiation. Exposure of undifferentiated teratocarcinoma cells to a given extracellular signal results in their specific differentiation into a given cell fate.

c). Despite their similar potency as embryonic stem cells, teratocarcinoma cells are not used *in vivo* and have no therapeutic potential. Why not?

Below is an illustration showing the cross-section of a blastocyst...



d) Label the section of the blastocyst that gives rise to embryonic stem cells.

Grown in culture, embryonic stem cells form colonies of cells. You would like to use the cells to identify novel determinants of differentiation.

You expose a plate of ES cells to retinoic acid (Vitamin A). Immediately after exposure the ES cells begin to express *Gene N*. One week after exposure you observe that the cells in the dish have the appearance of mature neurons.

e) Please match the items below with all of the terms that apply.

- | | |
|--|-----------------------------------|
| i. ES cell | 1. Inducer |
| ii. Retinoic acid | 2. Undifferentiated Cell |
| iii. Cells after exposure to retinoic acid | 3. Terminally Differentiated Cell |
| iv. Neurons | 4. Determined Cell |

You wish to explore whether the product of *Gene N* is acting as a determinant for neuron formation. Transfection is the process by which a DNA plasmid can be put into a eukaryotic cell (similar to transformation in bacteria).

f) What experiment could you perform to test the hypothesis that the product of *Gene N* acts as a determinant in neuron formation?

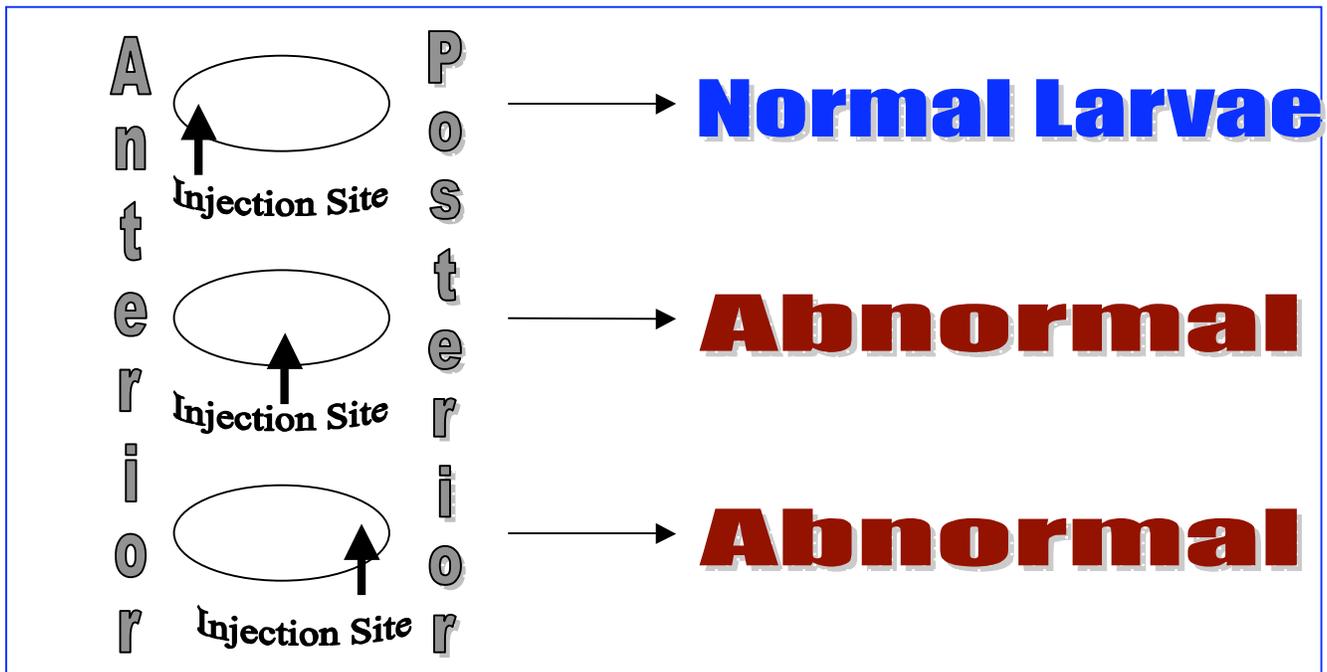
g) Can the nucleus of a differentiated cell, such as a neuron, be used to clone an animal? If so, why?

Problem 3

A large class of mutants in *Drosophila melanogaster* (fruit flies) result in female sterility. The defects in this class of mutants do not result from failure to produce eggs, but due to defects in offspring development. By comparison there are a paucity of mutants that cause male sterility due to similar defects.

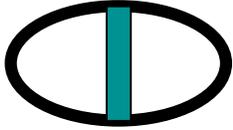
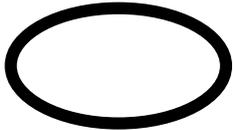
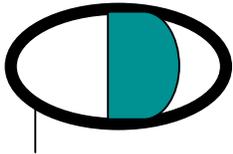
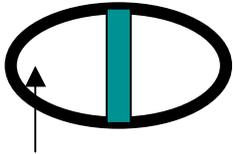
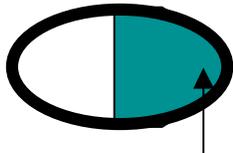
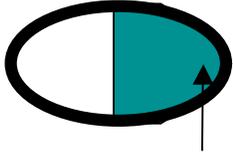
a) Why may there be this discrepancy between the male and female flies? Why can you get a generation of homozygous mutants?

One such mutation is in the gene *bicoid*. Mutation of *bicoid* results in the formation of larvae lacking the anterior segments that normally develop into the head and thorax. To determine the mechanism of Bicoid function your perform the following mRNA injections into *Drosophila* eggs prior to fertilization:



b) What may explain the sensitivity of development to the site of injection? What normally establishes this polarity?

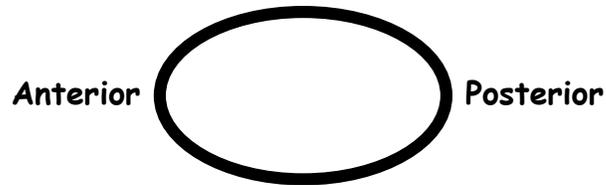
You next look at the expression of a second gene (*Gene B*) that you believe may be regulated by Bicoid. You perform a similar injection experiment, injecting *bicoid* mRNA into either *bicoid* mutant eggs or *wildtype* eggs and look for the pattern of expression of *Gene B*.

Genotype	Injection Site	Gene B Expression
Wildtype	None	
Bicoid Mutant	None	
Wildtype	Anterior	
Bicoid Mutant	Anterior	
Wildtype	Posterior	
Bicoid Mutant	Posterior	

c) Explain the likely function of Bicoid in the expression of *Gene B*?

d) Is the presence of Bicoid on its own sufficient for expression of *Gene B*? Explain.

e) For a wild-type egg, draw the likely pattern of *Gene B* expression if some of the cytoplasm at the posterior of the egg were transferred to the anterior part.



f) For a *bicoid* mutant egg, draw the likely pattern of *Gene B* expression if some of the cytoplasm at the posterior of the egg were transferred to the anterior.

