

7.22

2004 FINAL – FOR STUDY FALL 2005

Name: _____

7.22 Final Exam
200 points

12.13.04

1. **140 points.** The title and abstract of a paper in press in the journal *Gene Expression Patterns* is copied below. Two figures from the paper follow.

Abstract removed due to copyright reasons. Please see:

Wanner, S. J., M. C. Danos, J. L. Lohr, and J. R. Miller. "Molecular cloning and expression of *Ena/Vasp-like (Evl)* during *Xenopus* development." *Gene Expr Patterns* 5, no. 3 (February, 2005): 423-8.

Terms you may not know:

Isoform: different form of the same thing- in this case, differentially spliced mRNAs derived from the *Evl* gene.

Cement gland: a mucus secreting organ

Myotome: the part of the somite that forms the skeletal muscle

Placodes: cells adjacent to the neural tube that form organs like the ears (otic placode), lateral line (a sense organ) and olfactory system (nose)

St. 20, st.25: tailbud, somites have begun to form

St.30, st. 35: hatching stages, organogenesis and differentiation is well underway

Notes about figure 1 from Prof. Sive:

a dot indicates that there is no matching amino acid in the other transcripts. The actual amino acids do not matter- so do not worry if you cannot clearly read them. If there is an amino acid in all three positions, it is the same in all three.

Figure 1 removed due to copyright reasons. Please see:

Wanner, S. J., M. C. Danos, J. L. Lohr, and J. R. Miller. "Molecular cloning and expression of Ena/Vasp-like (Evl) during *Xenopus* development." *Gene Expr Patterns* 5, no. 3 (February, 2005): 423-8.

Notes about figure 2 from Prof. Sive: look at “terms you may not know” for help with abbreviations.

Figure 2 removed due to copyright reasons. Please see:

Wanner, S. J., M. C. Danos, J. L. Lohr, and J. R. Miller. "Molecular cloning and expression of Ena/Vasp-like (Evl) during *Xenopus* development." *Gene Expr Patterns* 5, no. 3 (February, 2005): 423-8.

- a. **10 points** The technique of in situ hybridization was used to examine the expression of the Evi gene. Explain the principal of this technique. Indicate how the different Evi RNA isoforms were detected.

This paper describes expression patterns of Evi transcripts. Now, design a research plan incorporating both gain of function and loss of function to study the function of Xenopus Evi. You should do the following:

- b. Describe a gain of function approach to understanding the function of the Evi gene, as indicated below.

5 points What hypothesis are you testing? Be as specific as you can.

10 points What experiment(s) would you perform to test this? Try to use techniques that are appropriate for Xenopus.

5 points Describe a positive control you would perform and the rationale (reason) for this.

5 points Describe a negative control you would perform and the rationale for this.

5 points Describe a result consistent with your hypothesis.

5 points Describe a result inconsistent with your hypothesis.

5 points Unfortunately, the experimental results you obtained from performing the gain of function experiment you outlined above did not support your hypothesis. Offer an alternate hypothesis to be tested in gain of function experiments.

10 points Describe an experiment you would perform to address this alternate hypothesis.

- c. Describe a loss of function approach to understanding the function of the Xenopus Evi gene, as indicated below.

5 points What hypothesis are you testing? Be as specific as you can.

10 points What experiment(s) would you perform to test this? Try to use techniques that are appropriate for *Xenopus*.

5 points Describe a positive control you would perform and the rationale for this.

5 points Describe a negative control you would perform and the rationale for this.

5 points Describe a result consistent with your hypothesis.

5 points Describe a result inconsistent with your hypothesis.

5 points Unfortunately, the experimental results you obtained from performing the loss of function experiment you outlined above did not support your hypothesis. Offer an alternate hypothesis that can be tested in loss of function assays.

10 points Describe an experiment you would perform to address this alternate hypothesis.

d. **10 points** Would you perform the gain-of function or the loss-of-function experiment first? Explain clearly why your choice is logical.

- e. **10 points** How might the multiple transcripts from the *Evl* gene complicate designing or interpreting your experiments?
- f. **10 points** The *Evl* gene is expressed in the myotome of the somites only fairly late during development, at stage 30. Propose an experiment to find out when cells of the future somite become determined to express this gene?

2. **60 points.** This is a question about building structure.

a. **5 points** What is the embryological term for the creation of structure?

b. **10 points** Mesenchyme is a cell state. Give two major characteristics of this cell state.

Another cell state is epithelium. Give two characteristics of the epithelial cell state that distinguish it from mesenchyme?

c. **5 points** Elongation of cell sheets is essential for normal development. In a vertebrate embryo, give a clear example of when cell sheet elongation contributes to normal development. Indicate which organ or process you are describing, and be as specific as you can.

d. **10 points** Two ways to elongate a cell sheet are epiboly and convergent extension. Define each. Use diagrams if you like.

- Epiboly

- Convergent extension

e. **10 points** A recent publication has shown that oriented cell division plays a role in elongating the zebrafish embryo. Use a diagram to show how regulated cell division could elongate a sheet of cells.

f. **10 points** Wnt signaling plays a key role in many developmental processes. Indicate what role each of the following factors plays in this pathway.

<u>Ligand</u>	<u>receptor</u>	<u>intracellular signaling factor</u>	<u>downstream transcription factor</u>
---------------	-----------------	---------------------------------------	--

β -catenin

Frizzled7

Wnt3A

Dsh

GSK3

g. **10 points** Down Syndrome is a human birth defect caused by trisomy (three copies of) for chromosome 21. In this syndrome multiple structural defects are observed, particularly in the heart and brain. A couple who have previously had a Down Syndrome baby wish to have another child, but want to make sure they do not have another child with the same Syndrome. Describe a protocol by which preimplantation genetic diagnosis be used to ensure this does not happen?