7.22 **2004 FINAL – FOR STUDY FALL 2005**

Name: _____

7.22 Final Exam **12.13.04**
200 points

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.

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 Evl gene. Explain the principal of this technique. Indicate how the different Evl RNA isoforms were detected.

The principle of the technique involves nucleic acid hybridization. It assays RNA expression. A RNA probe, complementary to the RNA of the gene of interest is labeled and incubated with fixed tissue believed to be expressing the gene. After a period of allowing the probe to hybridize to RNA in the tissue, the unhybridized probe is washed out and the hybridized probe visualized through the label.

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

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

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

I hypothesize that Evl is sufficient to promote cell-cell adhesion.

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

I would ask whether over expression of Evl is sufficient to promote adhesion. In particular, I would ask whether cells that normally do not express Evl proteins will stick to cells that normally do express these proteins when Evl proteins are overexpressed. In order to test this, I would inject a mix of RNA encoding Xevl and related proteins into the two cell stage frog embryo, along with a lineage tracer such as RNA encoding Green Fluorescent Protein (GFP). At the gastrula stage, I would remove animal caps (ectoderm) from injected embryos and dissociate these into single cells by incubation in Ca/Mg free medium. I would also remove animal caps from embryos injected only with control RNA (encoding red Fluorescent Protein, RFP) and dissociate these. I would then mix the two cell populations together, add back Ca/Mg and ask whether the GFP labeled and RFP labeled cells sort out as the cells re-adhere.

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

I would test a gene with known function in mediating cell sorting, in a similar assay. This could include N-cadherin, or paraxial protocadherin both of which are known to promote homotypic cell adhesion.

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

In order to ensure that any phenotype I observed was not a result of non-specific RNA effects, I would inject RNA that should not cause changes in adhesion, for example globin RNA injected in the same amounts as the Evl Rnas.. I would coinject this with GPF RNA, and perform a dissociation/reassociation experiment as described.

5 points Describe a result consistent with your hypothesis.

Cells in regions of the embryo not normally expressing Evl now begin to stick to the somite.

5 points Describe a result inconsistent with your hypothesis.

No effect is observed, that is the embryo looks normal after Evl injection.

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.

I would postulate that Evl can mediate cell type determination, and does not alter adhesion.

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

As described above, I would overexpress Evl proteins, by injecting RNA encoding Xevl and related proteins into the early embryo along with GFP as a lineage tracer. When embryos reached hatching stages, I would analyze expression of muscle differentiation and neural differentiation markers. In particular, I would ask whether any regions outside the myotome or neural tube express these markers, and have therefore been determined as nervous or nyotome tissues.

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

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

I hypothesize that Evl proteins are required for adhesion of the myotome.

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

I would ablate Evl gene function by injecting one or more antisense morpholino-modified oligonucleotides into the early embryo at the two cell stage along with GFP RNA as a lineage tracer. Oligos will be directed to the 5' UTR and translational start site of the gene. I will ensure that oligos designed target all three alternately spliced Evl transcripts. At hatching stage, I would examine the somites and ask whether the somites are intact by staining for a myotome-specific RNA (other than Evl).

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

In order to ensure that were there a phenotype after ablation of Evl gene function I would observe this, I would inject antisense ogligos to another gene that is known to give a phenotype.

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

In order to ensure that any phenotype I see is not sue to non-specific effects, I would inject a control oligo, that is known to cause no phenotype. I would inject the same amount of this as the test Xevl oligo.

5 points Describe a result consistent with your hypothesis.

The somites fall apart, or fail to form properly.

5 points Describe a result inconsistent with your hypothesis.

The somites appear normal in every way.

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.

Evl is required for cell type specification, rather than adhesion. In particular, I hypothesize that Evl is required for neural, cement gland and myotome differentation.

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

A similar antisense experiment would be performed as described above, but I would assay expression of neural, cement gland and muscle differentiation markers.

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

I would perform the loss of function assay first. If it yields conclusive data, this would be more important than the gain of function, that only indicates what can happen, and not what does happen. Both sets of experiments are quite difficult, and there is no advantage to doing one or the other first from that point of view.

e. **10 points** How might the multiple transcripts from the Evl gene complicate designing or interpreting your experiments?

Not all transcripts need have the same function. If one wanted to address this, one would have to test the activity and requirement for each transcript separately. This could be done by injecting RNAs encoding transcripts individually in gain of function experiments. In loss of function experiments, this could be done by designing antisense oligos specific to each transcript. Alternately, after inhibiting activity of all Evl proteins, one could try to rescue the phenotype with individual transcripts, thus showing which was most important.

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?

I would figure out which cells in the early embryo were going to give rise to the myotome by fate mapping. A non-diffusible dye could be injected into the blastula stage embryo and the cell(s) that give rise to the myotome mapped. Subsequently, after dye injection into the correct cells, these cells could be removed at different times of development and transplanted into a different region of a host embryo at the same (or a different) stage. The age of the donor cells that went on to express Evl RNAs after transplant would be a measure of their determination.

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

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

morphogenesis

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

Single cells, migratory

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

Cell sheet, tight junctions

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.

Elongation of the axial mesoderm (notochord) is essential for elongation of the body along the anteroposterior axis.

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

- Epiboly

Cells spread out (to become squamous) and increase their surface area. They are thus able to cover a larger area than when cuboidal or columnar.

- Convergent extension

Cells in a broad sheet intercalate or interdigitate with one another, effectively "lining up". This decreases the width of the sheet and therefore elongates it.

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.

(hard to do electronically) Mitotic spindles are oriented such that the cleavage plane divides all cells in the same plane. This leads to elongation of the sheet if the cleavage plane is at right angles to the direction the sheet will elongate.

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.

	Ligand	receptor	intracellular signaling factor	downstream transcription factor
β-catenin				**x**
Frizzled7		**x**		
Wnt3A	**x**			
Dsh			**x**	
GSK3			**x**	

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?

Eggs would be obtained from the mother by superovulation after hormonal treatment, and surgical removal of the eggs. These would be used for in vitro fertilization with the father's sperm. Embryos would be incubated in vitro until the eight cell stage at which time one blastomere would be removed. The chromosome content of this cell (arrested in mitotic metaphase by addition of cytochalasin) would be examined by Fluorescence In Situ Hybridization (FISH) analysis with a probe specific to chromosome 21. If three signals for chromosome 21 are observed, the corresponding embryo would be positive for Down Syndrome. A cell with only two signals for chromosome 21 would have come from a normal embryo, which would be introduced into the mother's uterus for further development.