7.22 Final Exam December 17, 2001

Answer all questions in the books provided.
Exams not handed in at the end of the exam period will not be graded.

Please note that random exams will be photocopied.

200 points total
4 questions

Question 1. (60 points)

Differentiation in the Drosophila eye imaginal disc initiates in the posterior region of the disc and then progresses as a wave that sweeps anteriorly across the disc. This movement is marked by progression of the so-called morphogenetic furrow (MF). Anterior, or ahead of the MF, cells are unpatterned and proliferating. In and posterior to the MF, cells stop dividing and begin to differentiate as photoreceptor neurons. If the MF fails to initiate or is halted in its progression across the eye imaginal disc, all subsequent eye development is blocked. Refer to the figure below for a diagrammatic representation of these events. Although MF initiation and movement across the fly eye imaginal disc is a unique mechanism for establishing A/P pattern, it involves the same set of secreted signaling molecules that are used in many different contexts throughout development including *decapentaplegic* (*dpp = TGF_/BMP* homolog), *hedgehog* and *wingless* (*WNT* homolog).

Figure Legend: *Initiation and progression of the MF are illustrated. a) An early eye imaginal disc with the morphogenetic furrow (MF), depicted as a solid black line, initiating in the posterior and beginning to progress anteriorly. b) Depicts a slightly older eye disc where the MF has already traveled half way across the eye disc. Anterior to the MF cells are proliferating; posterior to the furrow, the photoreceptors are differentiating, depicted by the hatched shading. c) The fully differentiated eye. Discs are oriented with anterior(A) to the left, posterior(P) to the right, dorsal(D) up and ventral(V) down.*

a) (10 points) It has been proposed that *dpp* is required for initiation and propagation of the MF. Describe three experiments, the expected results if this hypothesis is correct, and the conclusions you would draw from each result.

1. LOF – no initiation; dpp necessary
2. Overexpress – initiate and propagate secondary MF; dpp sufficient
3. Where is it normally expressed – posterior of eye disc and in/ahead of MF as it moves; right place at right time

b) (10 points) At the time the MF initiates, dpp is expressed along both the posterior and dorsal/ventral margins of the disc. However, MF initiation occurs only in the posterior and not in the dorsal or ventral regions of the eye disc. Wingless is expressed along the dorsal/ventral margins of the eye disc, but not in the posterior. Propose a model to explain why MF initiation is posteriorly restricted and describe two experiments and the expected results that would support your hypothesis.

Model- Wg inhibits Dpp from initiating a MF on the lateral(D/V) margins
1. Express Wg in posterior – should inhibit furrow initiation
2. LOF Wg – ectopic furrows initiate from lateral margins

c) (10 points) Eye-specific LOF mutations in *hedgehog* have small eyes that have normally differentiated posterior tissues but lack all anterior tissues. What does this phenotype suggest about the normal function of hedgehog with respect to the MF?

MF must initiate properly because end up with some normally differentiated eye tissue in posterior, but MF doesn't propagate because lack anterior eye tissue. Thus hh required for propagation but not initiation of MF.

d) (10 points) Because *hedgehog* is required in many contexts throughout development, LOF mutations are generally embryonic lethal.

i) If you didn't have the eye-specific allele of *hedgehog* described in part (c), how would you analyze the LOF *hedgehog* phenotype during MF initiation and propagation?

Somatic mosaic analysis using FLP/FRT system. Generate patches of homozygous mutant tissue in otherwise heterozygous animal. Since hh recessive, animal is alive. Need to mark clones so can distinguish wild type from mutant tissue.

ii) Describe the anticipated phenotype from this experiment that is consistent with your answer to part (c). An annotated picture may be the simplest way to explain your answer.

Furrow can't move through a hh -/- clone but will continue moving normally through rest of tissue. End up with "scars" of undifferentiated patches in eye.

e) (10 points) Hedgehog expression is detected in differentiating cells immediately posterior to the MF. Propose a positive regulatory feedback loop between hedgehog and

dpp that would explain MF initiation and directional movement across the eye disc. You may include additional signaling pathways in your model. Include an explanation for why the MF never retreats posteriorly.

dpp expressed in MF. Passage of MF or dpp signaling itself induces hh expression in cells posterior to MF. hh signaling would then induce dpp expression in cells anterior to MF which in a sense "initiate" a new furrow which is the same as propagating it.

Either the receptors are localized asymmetrically wrt the MF or else there is an inhibitory mechanism, perhaps wingless expression, induced posterior to the MF that ensures forward direction.

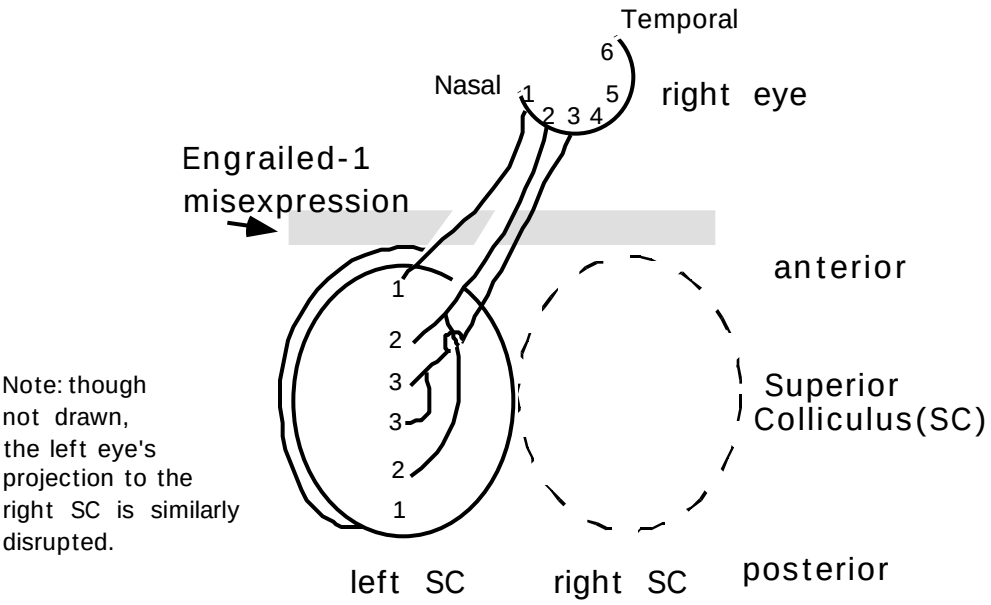
f) (10 points) Overexpression of the gene *eyeless* (*Pax6* in vertebrates) can induce the formation of extra eyes. However, these extra eyes arise only in certain regions of the animal (for example head, wing, and leg; but not thorax or abdomen) suggesting that not all regions of the body are competent to become eye tissue. Based on what you have learned about fly eye development from this question, propose an explanation for this observation and describe two experiments and the expected results that would prove your hypothesis.

Need dpp and hh to drive MF in order to get eye differentiation; can only induce ectopic eyes in tissues where dpp and hh are normally expressed.

1. **Overexpress eyeless in dpp or hh mutants – no ectopic eyes anywhere**
2. **Overexpress dpp and hh and eyeless in regions of fly where normally don't get ectopic eye induction, now get eye induction**
3. **Look at expression of endogenous dpp and hh proteins – won't see it in tissues where can't induce eyes; will be present in tissue that is competent to form eye**

Question 2. (55 points)

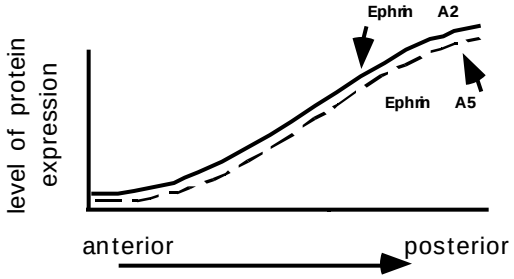
Engrailed is a transcription factor first identified in *Drosophila* embryos. Its vertebrate homologue, Engrailed-1, is expressed in the isthmus region of the brain (at the mesencephalon/myelencephalon border). Suppose you misexpressed Engrailed-1 in a transgenic mouse so that it was expressed in front of, as well as, behind the superior colliculus. You found the following disruption of the retinal projection to the superior colliculus when you labeled small regions of retina with an anterograde tracer in the



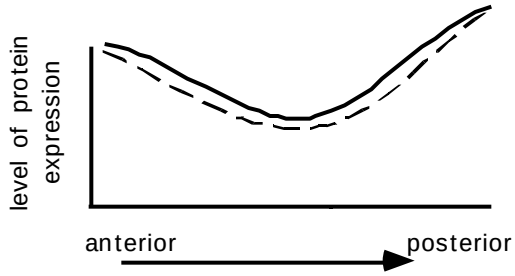
postnatal period.

Given this disruption of the retinocollicular map, Ephrin A2 and Ephrin A5 are likely to have had their distributions altered by the misexpression of Engrailed-1 anterior to the superior colliculus.

a) (10 points) Draw the normal distribution of Ephrin A2 and Ephrin A5 along the anterior -posterior axis of the superior colliculus.



b) (10 points) Draw the redistribution of these molecules in the Engrailed-1 misexpressing transgenic that would explain the disruption of the retinocollicular projection.



c) (10 points) Why don't temporal retinal axons project to the superior colliculus in the Engrailed-1 misexpressing transgenic?
Because the double gradient raised the Ephrin A5 and A2 levels, which are repulsive molecules for high Eph receptor expressing temporal axons, to such a level that temporal axons cannot enter the colliculus at all.

d) (20 points) You remove the anterior 1/3rd of the superior colliculus and the posterior 1/3rd of the superior colliculus in the Engrailed-1 misexpressing transgenic and distribute the membranes from these 2 regions in alternating stripes in a tissue culture assay.

(i) What do you predict would be the growth pattern of axons from normal nasal retina?

They would not distinguish stripe boundaries just as they do in normal animals.

(ii) What do you predict would be the growth pattern of normal temporal axons?

Temporal axons would not grow at all because they are repulsed by both poles.

e) (5 points) If you found, in the transgenic mouse described above, that Engrailed-1 did indeed stop its expression at the anterior border of the superior colliculus, would you expect to find a binding site for Engrailed-1 in the regulatory region of the Ephrin A2, Ephrin A5 genes? Explain.

No, Engrailed-1 is a transcription factor so it would act on binding sites within the nucleus of the same cell. (The Ephrin A2 and A5-expressing cells are not the same cells as the Engrailed-1 expressing cells.)

Question 3. (55 points)

Neural crest cells will migrate off of a trunk neural tube onto a substrate covered with fibronectin and laminin where they divide to form small colonies with each colony consisting of a wide and variable range of neural crest derivatives.

a) (10 points) How would you prove that each colony was actually a clone of one of the neural crest cells that migrated off of the neural tube?

One could label a small subset of neural crest with a retrovirus carrying some reporter gene..a vital reporter like GFP would be ideal in this case. After the colonies had developed you would have to visualize the reporter and score the colonies. If they are the progeny of one cell you should find that the vast majory of reporter positive colonies have all cells expressing the reporter. You could also watch each cell over time but this would probably involve sophisticated time lapse imaging of multiple cells over the course of ~ 1 week.

Neural crest cells treated as described above will form clones that are mostly pure melanocytes when cultured in the presence of steel factor that is given off by epidermal cells. However such neural crest cells will form clones in which 50% of the clones are all neurons, 25% of the clones are all smooth muscle cells and the remaining clones consist of neurons and smooth muscle cells if BMP-2, instead of steel factor, is present in the culture medium.

b) (10 points) What additional analyses would have to be conducted on these cultured cells to assure that these data indicate that the factors are actually inducing a fate change in the neural crest progenitor cells?

Would have to watch them continuously to assure that a significant number of cells are not dying in the presence of the particular factor. Tritiated thymidine or bromodeoxyuridine incorporation might also be applied to assure that all cells were dividing. Failure of some cells to take up thymidine or and/or significant cell death would be expected if the "growth"factors were promoting the selective survival and/ or selective proliferation of one type of already determined progenitor for a particular derivative of the neural crest.

Nitrocellulose microcarriers were implanted, for a brief period, in *Axolotl* (salamander) host embryos in pathways of neural crest migration prior to the migration of host neural crest. These microcarriers were then used as *in vitro* substrates for neural crest migrating off of a second donor's neural tube as in the first experiment described above. Progenitors that came to rest on microcarriers that had resided in the ventral pathway of neural crest migration produced colonies in which all cells were neurons. Progenitors that stopped migrating on the laminin/fibronectin produced colonies showing a wide range of neural crest derivatives.

c) (10 points) What does this tell you about the induction of neural crest phenotypes?
The final phenotype can be altered by molecules in the ECM.

d) (5 points) What type of neural crest derivative would you expect to differentiate if the progenitor cells on the microcarrier described above were exposed to glucocorticoids?
adrenal chromaffin cells or adrenaline secreting cells

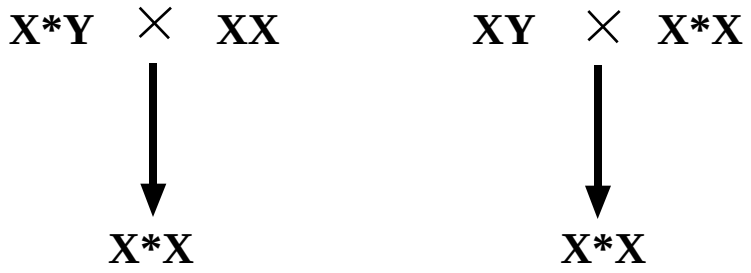
e) (10 points) What type of neural crest phenotype would you predict when neural crest cells migrated onto a microcarrier that had resided in the dorsal pathway of a host?
melanocytes

f) (10 points) Given that the extracellular matrix (ECM) in neural crest pathways changes with time, how might you use the microcarrier explant technique to determine if age, not position, of the ECM were responsible for the inducing function of the ECM?
Alter the age of the host when the microcarrier is placed in a neural crest pathway

Question 4. (30 points)

You are studying a human disease caused by mutations in an X-linked gene. The gene mutated in this recessive, inherited disease encodes a metabolic enzyme that is normally expressed in all tissues of the body and whose activity can be measured in a reliable and quantitative assay.

Female patients with one normal copy of the gene and one mutated copy of the gene may still exhibit the disease phenotype. This is true regardless of whether the patient inherits the mutation from her father or from her mother: (* denotes the mutation)



a) (10 points) Explain why you could still see a phenotype in a heterozygous female, given that the trait is recessive. Include in your answer a discussion of the autonomous/nonautonomous nature of action of the gene product.

Since this is an X-linked gene, some of the cells will have by chance inactivated the wt copy of the gene, and some by chance the mutant copy of the gene. The heterozygous female is essentially a mosaic. If the gene product acts autonomously (each cell lacking the gene product cannot be rescued by other cells in the same individual which have a functional copy), the individual could show a phenotype from the cells which inactivated the functional copy.

You study a pair of identical male twins (monozygous; i.e, they came from the same zygote) with a mutation in this gene and measure the amount of enzymatic activity. You also study a pair of identical female twins (monozygous), each with one copy of the same mutation that the male twins have.

Below is table summarizing your results, including results from two normal controls lacking the mutation.

	Enzymatic activity (relative units)
Male twin A	30
Male twin B	30
Female twin A	40
Female twin B	80
Male control	100
Female control	100

b) (10 points) Given that the female twins are genetically identical, provide a plausible explanation for why female twin A is more severely affected than female twin B. Exclude the possibility of any new mutation arising and discount environmental factors.
X-inactivation is a random stochastic process, with each cell in the early female embryo making its own decision of which X to inactivate. In female twin A, by chance, more cells probably inactivated the wt copy of the gene than in female twin B. This difference would account for the difference in clinical outcome.

c) (10 points) Suppose someone were to clone male twin A by transferring the nucleus from one of his fully differentiated cells into an enucleated egg. If you performed the same assay on the clone, would you expect exactly the same level of enzymatic activity in the clone as in male twin A? Explain.
No. Although levels are exactly the same between the two male twins, a clone generated by nuclear transfer is not exactly the same thing. The differentiated nucleus being transferred has undergone epigenetic changes. In order for the clone to develop exactly the same as the original individual, all these changes must be reset and reprogrammed such that all genes are expressed in the same pattern and at the same time(s) as in normal development. It is highly unlikely that this would happen for all of the genes involved in this metabolic pathway.