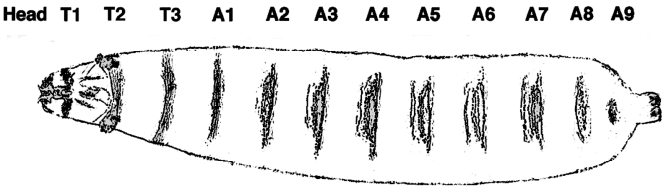
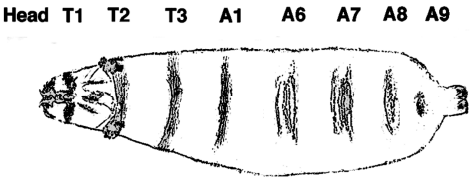
Question #X (13 points)

Below is a drawing of a wild-type Drosophila cuticle



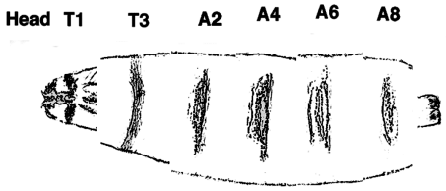
Briefly describe and sketch a cuticle that is representative of each of the following general categories of mutants

a) gap-mutant (3 pts)



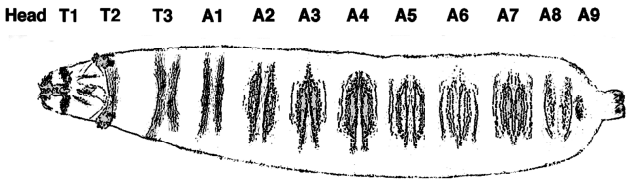
Gap mutants have the deletion of a contiguous series of segments
[any drawing showing at least three contiguous segments deleted is acceptable]

b) pair-rule mutant (3 pts)



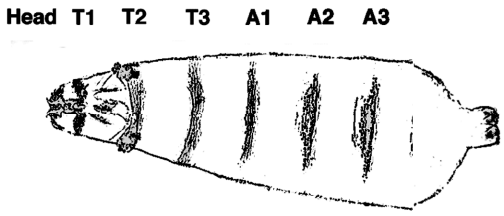
Pair rule mutants show deletions with a two segment periodicity
[any drawing showing every other segment deletion is acceptable]

c) segment polarity mutant (4 pts)



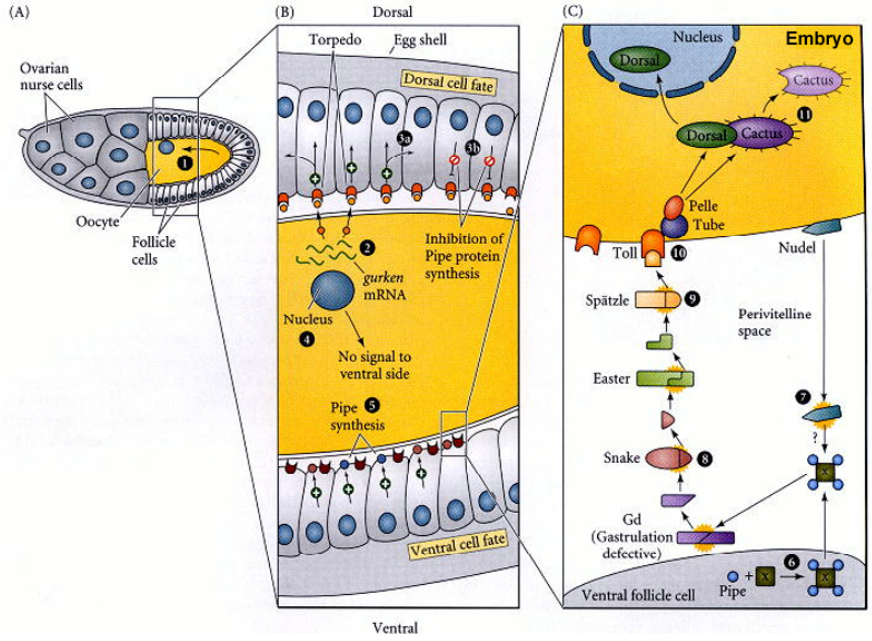
Segment polarity mutants show deletions within every segment (usually with replacement of deleted regions with mirror image replacements)
[any drawing show deletion within every segment, but still showing normal number of segments, is acceptable for 3 pts. Full credit (4 pts) if they either show or mention mirror image duplication]

d) draw the cuticle expected for an embryo derived from a female homozygous mutant for a null mutation in nanos. (3 pts)



Deletion of roughly the posterior half (deletion of entire abdomen is also acceptable). Deletion of posterior with expansion of anterior is acceptable. Note that question asked for just a sketch, so no written explanation is necessary.

Question #Y (20 points)
We discussed the pathway that establishes the initial dorsal/ventral polarity of the fly embryo (see below).



Predict the phenotype of embryos laid by females of the following genotypes (assume all "–" alleles are complete lack of function alleles.). No explanations needed for a-c.

a) cactus^{-}, cactus^{-} (2 pts)
Ventralized

b) dorsal^{-}, dorsal^{-} (2 pts)
Dorsalized

c) dorsal^{-}, dorsal^{-} ; cactus^{-}, cactus^{-} (2 pts)

Dorsalized

d) Provide an explanation for your answer to (c). This should include an explanation of the phenotype seen for dorsal^{-}, dorsal^{-} alone; cactus^{-}, cactus^{-} alone, and the double mutant combination (7 pts).

Cells in which dorsal protein enters the nucleus take on a ventral fate, thus when no dorsal protein is present, the entire embryo is dorsalized. Cactus protein normally holds dorsal protein in the cystoplasm, until cactus is acted upon (could also say phosphorylated or degraded) by tube/pelle, and this normally only occurs in the more ventral parts of the embryo (and not dorsally). Thus, when there is no cactus protein, dorsal can enter the nucleus everywhere, and the entire embryo is ventralized.

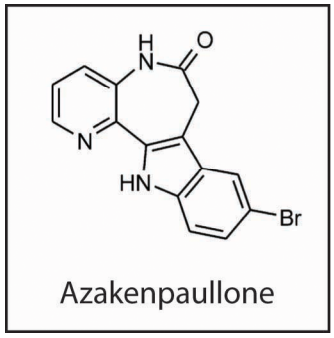
In the double mutant, there is no cactus to keep dorsal protein in the cytoplasm, but at the same time there is no dorsal protein. Thus, there is no dorsal protein anywhere (it makes no difference if cactus is or is not present) and the entire embryo is dorsalized.

e) How do you distinguish between a maternal-effect mutation and a zygotic-effect mutation (assume that in both cases we are talking about recessive mutations)? (7 pts)

For zygotic-effect mutations, the phenotype is seen in those animals which are homozygous mutant. For maternal-effect mutations, the phenotype is seen in animals that come from females that are homozygous mutant, and it makes no difference whether or not the father contributed a mutant allele. [Could also draw out crosses]

Final Exam Key 2010

1. (9 points) In their search for new drugs affecting the Wnt signaling pathway, pharmaceutical chemists have recently synthesized azakenpaullone (structure to the right), a strong inhibitor of GSK3, the kinase component of the pathway that phosphorylates beta-catenin protein, marking it for breakdown. The agent penetrates cells quickly, imposing inhibition, and can be washed out quickly to relieve inhibition.



Azakenpaullone

A. (5 points) A *Xenopus* embryo is treated with azakenpaullone in the period from the 2-cell stage until the late blastula stage, at which time the inhibitor is washed out. Predict the location and extent of the organizer in the subsequent gastrula, and predict the eventual phenotype of the hatching embryo. Briefly explain your predictions.

Organizer: predict that it occupies the entire equatorial level (360°) of the early gastrula.

Phenotype at hatching: predict a radial dorsalized embryo (or called cylindrically symmetric dorso-anterior embryo), with a large heart, anterior neural tissue, eye around the circumference, sometimes lots of notochord, but missing tail and most or all somites.

Explanation: azakenpaullone inhibits GSK3, leading to a buildup of beta-catenin protein throughout the embryo (2-cell to late blastula). Beta-catenin/Tcf and Nodals work together to induce and derepress organizer formation around the entire equatorial zone. The enlarged organizer induces excessive neural tissue and heart, and sometimes forms notochord. (Somites are reduced because the mesoderm is composed entirely of organizer mesoderm).

B. (4 points) A *Xenopus* embryo is treated with azakenpaullone in the period of neural induction and neurulation, at which time the inhibitor is washed out. Predict the eventual phenotype of the hatching embryo, and briefly explain your prediction.

Predict a hatching embryo with reduced anterior neural structures (forebrain and misbrain), and expanded posterior neural structures (hindbrain and spinal cord).

Explanation: azakenpaullone inhibits GSK3, leading to a buildup of beta-catenin protein throughout the embryo (during neural induction and neurulation), including in the anterior parts of the embryo where normally Wnts are less abundant and Wnt antagonists are present (secreted from head organizer). At these stages the neural ectoderm undergoes posteriorization due to Wnts, that is, due to high beta-catenin combining with Tcf. Azakenpaullone increases beta-catenin protein in cells even where Wnts are absent, causing posteriorization in anterior neural ectoderm as well as posterior.

2. (15 points)
The **notochord** is a distinguishing trait of members of the chordate phylum. Summarize your knowledge about notochord development by writing T (true) or **F** (false) next to each of the following statements:

F	The notochord will develop in a *Xenopus* embryo depleted for maternal beta-catenin mRNA.
F	The notochord will develop in a *Xenopus* embryo depleted for maternal VegT mRNA.
T	The notochord will not develop in a *Xenopus* embryo injected with anti-sense morpholinos to Nodals (anti-xnr1,2,4,5,6, and derriere).
T	A second notochord will develop in a *Xenopus* embryo injected on the ventral side with Wnt mRNA at the 4 cell stage.
T	The trunk-tail organizer of the amphibian embryo is composed of notochord precursor cells.
F	The head organizer of the amphibian embryo is composed of notochord precursor cells.
T	Hensen's node of the chick embryo contains notochord precursor cells.
T	Notochord precursor cells release Bmp antagonists such as Chordin, Noggin, and Follistatin in the *Xenopus* embryo.
F	Notochord precursor cells release Wnt antagonists such as Frzb, Dkk, and Crescent in the *Xenopus* embryo.
T	Notochord precursor cells engage in convergent extension morphogenesis.
F	Notochord precursor cells engage in spreading migration morphogenesis.
T	The notochord of the chick embryo is laid down behind Hensen's node as it regresses.
F	Roofplate formation in the *Xenopus* neural tube depends on signals released by the notochord.
T	Floorplate formation in the *Xenopus* neural tube depends on signals released by the notochord.
T	Sclerotome formation from the somite depends on signals released by the notochord.

3. **(9 points)**
A. At the egg cylinder stage of the newly implanted mouse embryo, the anterior visceral endoderm (AVE) region of the hypoblast layer determines the site at which the primitive streak will form and the site of future anterior neural tissue, and hence the orientation of the anterior-posterior axis of the embryo.
To indicate the correct spatial relationships, circle one of the letters in the list of statements below:
Answer C is correct.

a. The primitive streak and anterior neural tissue form on the same side of the cup as the AVE.
b. The primitive streak forms on the same side of the cup as the AVE, and the anterior neural tissue forms on side opposite the AVE.
c. The primitive streak forms on the side of the cup opposite the AVE, and the anterior neural tissue forms on the same side as the AVE.
d. The primitive streak and anterior neural tissue form on the side of the cup opposite the AVE.

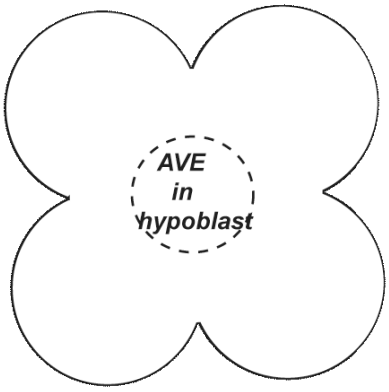
B. Briefly explain why these spatial relationships hold (noting the appropriate signaling ligands and antagonists).

The AVE releases Nodal antagonists such as Cerberus and lefty. Primitive steak formation requires Nodal signaling. It occurs on the side of the cup opposite the AVE, where antagonist concentrations are lowest.

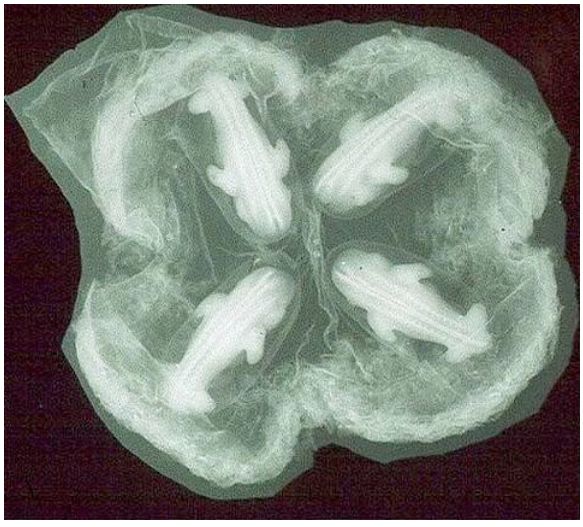
The AVE releases Wnt antagonists such as Dkk that block the Wnt-mediated posteriorization of neural ectoderm in the region close to the AVE, that is, on the same side of the cup.

C. The nine-banded armadillo normally gives birth to monozygotic (genetically identical) quadruplets, the only mammal to do this. The fertilized egg develops to a single blastocyst with an unusually large epiblast composed of four cup-shaped quadrants, all underlain by a continuous hypoblast layer (visceral endoderm). Four primitive streaks then develop in the epiblast, one per quadrant, followed by four embryonic bodies, as shown in the image on the right below.
Draw on the left diagram to indicate where the AVE or AVEs would have been located in the hypoblast layer at the time the four primitive streaks were forming, and explain your answer briefly. Assume we can see through the epiblast layer to the underlying hypoblast layer.

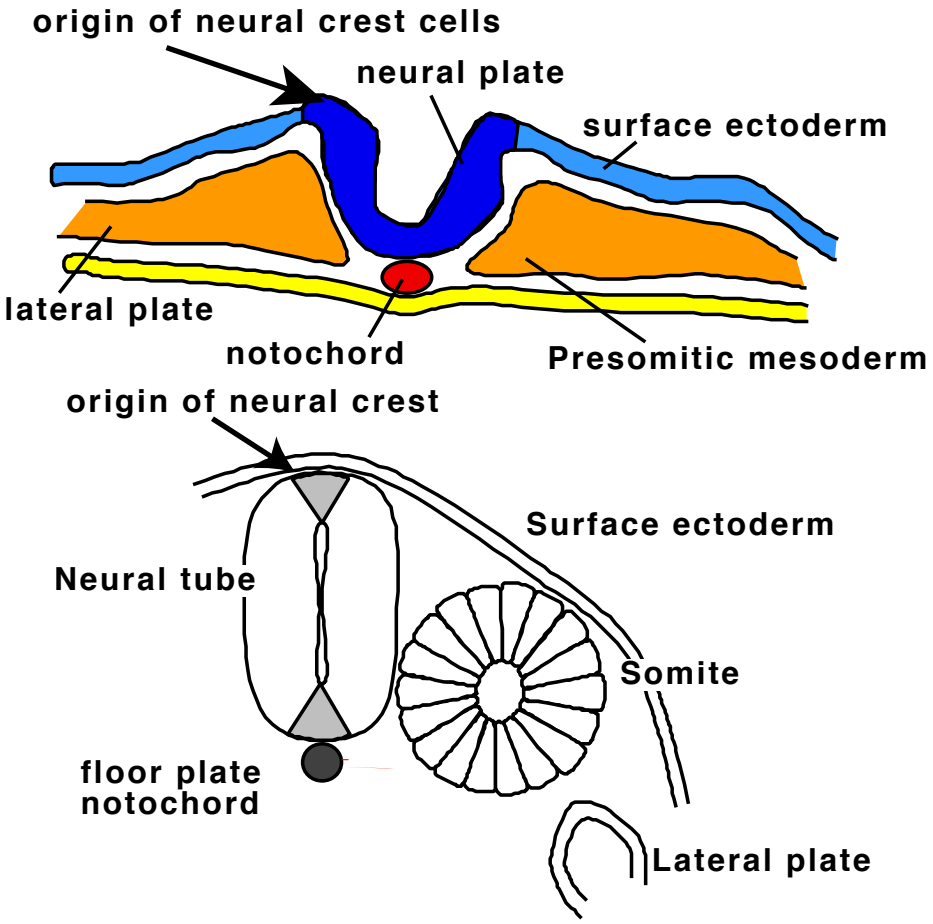
Explanation: AVE releases Nodal antagonists and primitive streak formation in the center. It occurs at epiblast sites most distant from AVE, at the outer edge of each quadrant. Anterior neural structures form closest to the AVE (the four heads are pointed inward).



Top view of 4-cupped epiblast.
AVE is underneath.



6a (5 points) Where do neural crest cells form? Draw a cross section through a chick embryo at a stage before neural tube closure and after neural tube closure indicating where they will be/ are formed.



6b (2 points) Name one molecular signal that contributes to their formation.

BMP (Wnt, FGF)

6c (6 points) We discussed experiments that showed neural crest cells are not committed to a particular fate as they begin their emigration. Describe the critical experiment.

Lineage tracing of individual neural crest cells: Different chick **embryos were injected** with a **lineage tracer- Rhodamine dextran**- into **single cells** as the neural crest cells **were emigrating from the neural tube**. The chick was **later analyzed for where the tracer is found**. The observation was that individual neural crest cells can **contribute to several cell types in any one embryo**, **e.g.** melanocytes, Dorsal root ganglion neurons; sympathetic ganglion and enteric nervous system. **This shows that individual cells can contribute to multiple cell types** and hence that they are not committed to a particular fate as they emigrate.

Question 6d (5 points) What kinds of signals might neural crest cells be exposed to as they migrate? Name one of the molecular signals, where it comes from, what kind of cellular fate results, and in what tissue.

They may be subjected to many **secreted signals** from different sources
e.g. neural crest cells **migrate past the aortic precursors**, and **BMPs** from the wall of the developing **aorta** influence cells to become **neurons** of the **sympathetic ganglion**

Question 6e (4 points) What experiment shows that this signal is required for the formation of the cell you cited above?

In a **control** explant culture, pieces of neural tube were put near **explants** of the **aorta**, such that **neural crest cells were near the aorta**, and it was found that they became **neurons**. To test whether the **BMP** is required for the fate, the culture was done in the presence of a **BMP antagonist**, such as **Noggin**, and this **prevented** the cells from becoming neurons, and they adopted **different fates**. This shows that the **BMP is required** for the formation of sympathetic neurons

Question 6f (6 points) Provide another six examples of neural crest derivatives.

trunk: melanocytes, dorsal root ganglion sensory neurons, glial cells, smooth muscle cells (of the heart), hormone secreting cells, such as chromaffin cells of the adrenal gland, enteric nervous system neurons

cranial, branchial arches, skeleton of the jaw and skull, connective tissue and muscles of the head

Question 7

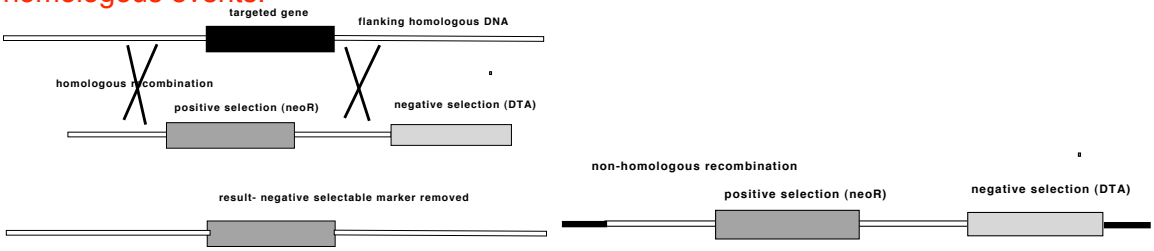
7a (6 points) What would you predict would be the effect of making a transgenic mouse where the Notch ICD (intracellular domain) gene fragment was placed under the control of an Olig2 enhancer, and why?

The Olig2 enhancer would **drive expression of Notch ICD** in the domain of the **spinal cord** that normally forms **motor neurons**. Notch ICD **activates** the Notch pathway by binding to the Su(H) transcription factor. This activates the **lateral inhibition** pathway, **preventing neuronal differentiation**. The motor neurons would therefore **not differentiate** and the animal would be **paralyzed**.

7b (4 points) What is the reason for using a positive selectable marker, and negative selectable marker in a knockout construct for ES cell manipulation?

Positive selection: When the construct is **electroporated into ES cells**, only a **small minority** of the cells take up and **integrate the DNA**, so that it is expressed. In order to **grow these specifically,** there needs to be a positive selection for these cells, to **distinguish** them from all the **background**/non-electroporated cells.

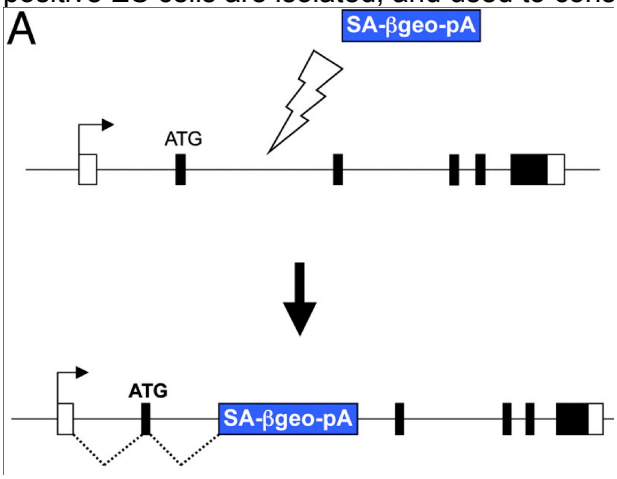
Negative selection: When DNA integrates by non-homologous recombination, it usually integrates as a complete molecule, while in homologous recombination, the ends of the construct do not integrate. This can be exploited to reduce the background of non-homologous recombinants, by using the negative selection marker at the end of the knockout construct. This will kill the cells that integrate teh DNA by non-homologous recombination, reducing the background of unwanted events/iincreasing the signal to noise ratio of homologous to non-homologous events.



7c (4 points) What method would you employ to take ES cells carrying a knocked out gene of interest and generate a fertile mouse carrying the mutated gene?

The ES cells would be **injected** into the **blastocyst** of a mouse with a **different coat color**. The blastocyst would be **reimplanted** into the **uterus** of a pseudopregnant mother, and brought to term. **Chimeric mice** would be chosen by the contribution of the **ES cell coat color** (e.g. agouti on a black background). These mice would then be **mated**, and the pups **tested for transmission of the ES cell with the targeted gene through the germline**. If the targeted ES cell is transmitted , then the resulting mouse would also be **fertile and carry the mutated gene**.

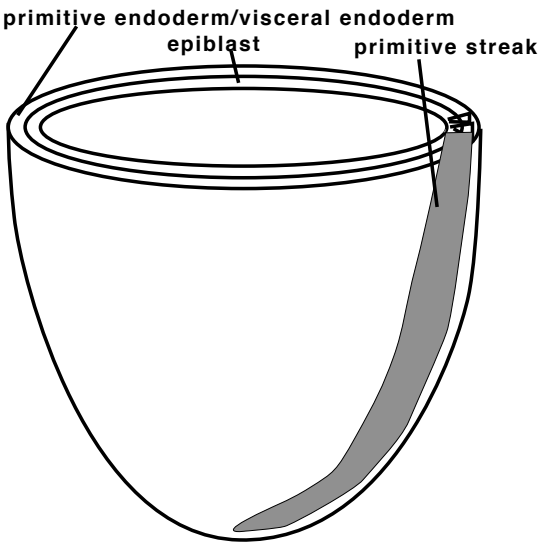
7d (5 points) In addition to the knockouts and transgenics we discussed in class, people have used a gene trap method, where a modified neomycin resistance gene is fused to beta galactosidase (βgeo) and this construct has a splice acceptor (SA) that often puts the construct in frame when the construct integrates randomly into the genome. Resistant and beta-gal positive ES cells are isolated, and used to construct mice.



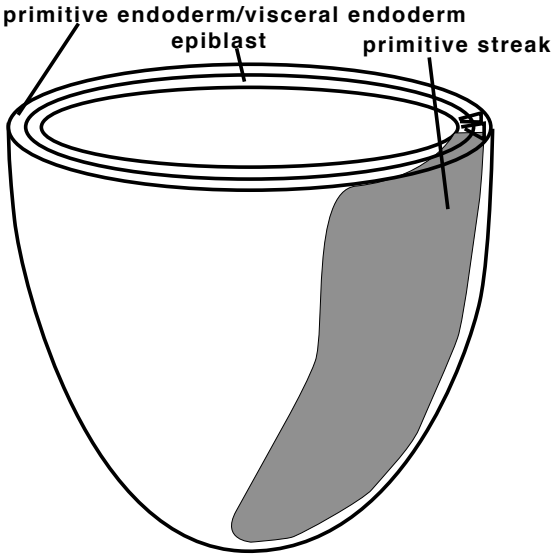
(from Skarnes, w.c.)

Suppose the gene trap has integrated at a single site, as in the diagram, and therefore generates a cell line from which you manage to generate a mouse for breeding. Although viable as a heterozygous animal, the gene trap is lethal when homozygous. When you stain a 6.5 day embryo for lacZ, you find that the entire primitive streak stains, but surprisingly is much more extensive than a normal primitive streak. Draw how stained embryos might look when heterozygous or homozygous for the gene trap. Label the parts of the embryo that are visualized by staining, or morphology.

Heterozygous



Homozygous

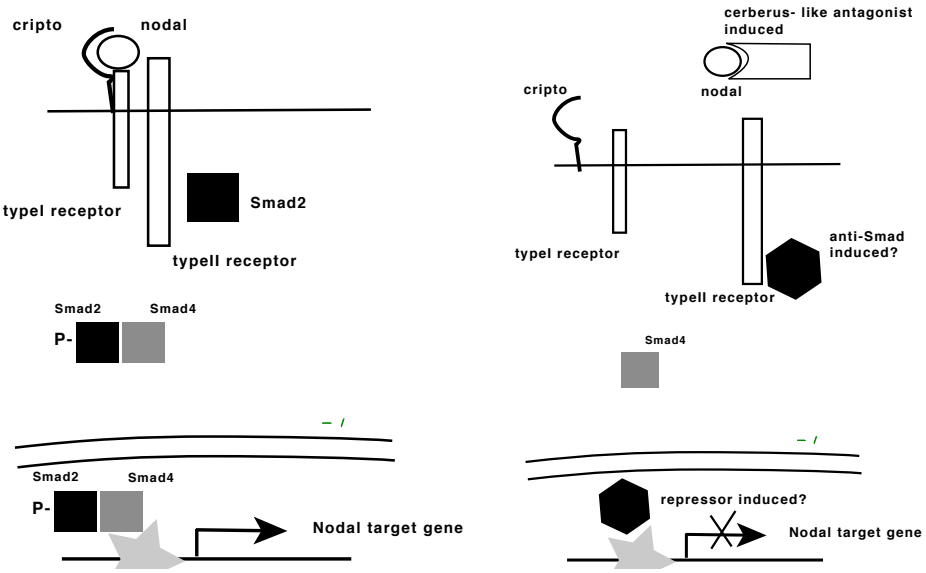


7e (4 points) What gene is the trap likely to have integrated into? What is the normal activity of the protein product of this gene?

Likely to be integrated into **lefty** (lefty2). Normal activity of the protein is to **antagonize nodal** signaling by **binding to the co-receptor** cripto/oep needed for nodal signaling

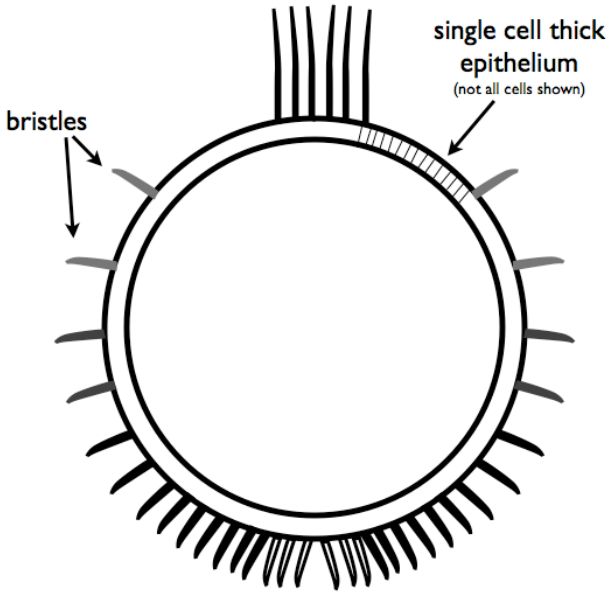
7f (5 points) If it turns out not to be the gene you predict, describe or invent a gene whose protein product might have the same ultimate effect, but work in a different way. Explain this with a drawing

Could be a different extracellular inhibitor that binds to ligand (like cerberus) that is either **induced** by nodal signaling, or **already expressed**
Could be an intracellular anti Smad, like Smad6/7 that is induced by nodal signaling
Could be an antagonistic transcription factor that inhibits nodal signaling



Question 8

A new marine worm that lives in the mud has been discovered. The worm is similar to a fly larva, in that it has belts of bristles, but unlike the fly, these extend in a graded pattern all around the circumference from ventral to dorsal. The ventral bristles are unpigmented, and dorsal bristles have a different shape. Also unlike the fly embryo (but like the frog or others) embryo develops by compete cleavage.



The organisms live in the mud, but can be induced to ovulate and be fertilized at any time of year by exposure to light. The large egg and embryo is easy to inject with a variety of materials. As with Xenopus embryos, injected mRNAs are slow to diffuse after injection, while fluorescent dextrans diffuse fast, and lipophilic dyes permanently mark the membranes of the cells into which they are injected.

It is found that a hedgehog gene is expressed at high level all along the ventral midline, a patched gene is expressed in a ventral to dorsal gradient and a smoothened gene is expressed ubiquitously. All of these genes are expressed starting at a blastoderm stage

8a (3 points) What manipulation would you do to inhibit function of the hedgehog gene?

Inject a Morpholino oligonucleotide that wouldb ind to the hh mRNA and inhibit its translation

8b (3 points) Supposing that this kind of manipulation is very effective, you find that all of the bristles except the long ones are lost from manipulated embryos. What does this tell you?

This shows that most of the bristles are dependent on HH signaling for their formation, while the long bristles do not depend on HH/their pattern is independent of HH

8c (4 points) What experiment would you do to see whether the long bristles are dependent on another signal? Name your favorite two candidate signals, and a precedent for why they may be expressed locally.

BMP -precedent is that is is expressed opposite hh in the vertebrate neural tube/ expressed at the dorsal side of a fly embryo. Could inject noggin mRNA to block BMP signaling, and ask whether this inhibits long bristle formation

Wnt -Precedent is that it is expressed opposite *hh* in the vertebrate neural tube. Could inject dkk1 mRNA to block its function

FGF expressed in ventral side of lfy embryo in a localized way, so why not in the dorsal side here? Inject sprouty mRNA to block its function
etc

8d (2 points) You now increase hedgehog expression- how would you do this?
Inject hh mRNA into the fertilzied egg or cleavage stage embryo

8e (4 points) The result of doing this manipulation at the 16-cell stage in one particular embryo is that a patch on the right hand side now has a very dense array of unpigmented bristles, with dense dark bristles adjacent to those. What do you think may be going on, and how would you modify the experiment to make this more easily interpreted?

We have a localized ectopic source of HH, since we injected the 16-cell stage. The injected cell may have ended up on the right side, where it causes a local HH source. At peak HH levels, the unpigmented bristles form, while at lower levels of HH the adjacent fate - dense bristles that are pigmented, forms. i.e. the HH is acting as a morphogen inducing these different fates at different concentrations

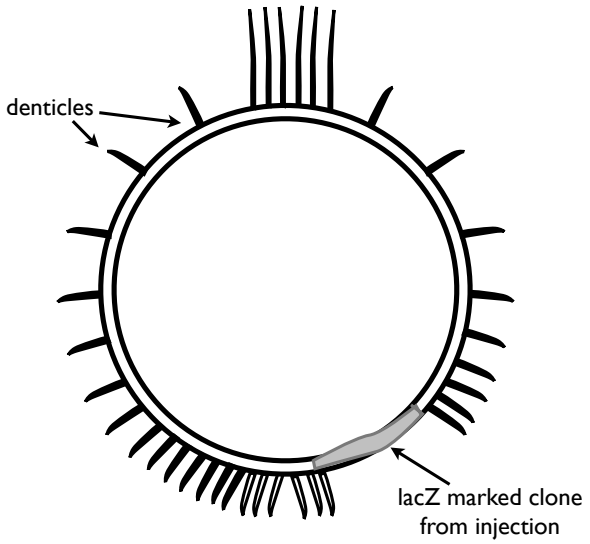
To interpret the effect, I would **mix in GFP/lacZ mRNA** into the HH mRNA so that we can **trace** where the HH is being overexpressed. Also **inject more embryos** so we can look for **consistency** of effect. We can then **correlate** the **high point of HH** with the **bristle phenotype**, and expect the **unpigmented** bristles to form at the point of **lacZ stain/GFP fluorescence**, with the dark dense bristles **adjacent** to that.

8f (10 points) What would be the most important two additional experiments would you do to test whether the graded distribution of bristles is due to a graded distribution of the hedgehog ligand? Don't forget to specify the expected outcome and interpretation, including what interpretation might be excluded by the result.

1. Descriptive experiment to ask whether HH protein is **present** is a graded way. **Stain** the HH with an **antibody**/express a GFP fusion protein (!) and ask whether it is **present in a concentration gradient with a high point at the ventral midline**, **consistent** with it acting as a **morphogen**. This result does not rule out that the HH may act in a relay.

2. Test of the **relay** vs **morphogen** effect of HH. Morphogen model states that HH diffuses and at different threshold concentrations induces different phenotypes. We know that patched is expressed in a graded way, consistent with it being a HH target, as in other cases (e.g. vertebrate spinal cord). Also smoothened transducer is present and zygotically expressed.
So we can make a **patch** of cells where **Smo function is blocked** (smo-) using an injected MO against zygotic smo expression, by injecting the MO at the **cleavage stage** (e.g. 16-c ell stage) along with a **GFP/lacZ lineage tracer.**
If HH acts in a relay, the clones of smo- cells adjacent to the HH source would **block the relay**, so that bristles w**ould not form** in the smo- clone and **not on the distal side**. However, based on precedents in the spinal cord, we expect that the HH protein could **diffuse through the smo- region** and although the smo- **GFP/lacZ marked cells would not make bristles,** the HH would **activate bristle formation on the distal side**. Indeed, we may expect a **denser than normal distribution of bristles on that side**, consistent with results in the spinal cord, where the smo- clones do not activate the feedback inhibitor/receptor PTC, a **HH target**. Normally **Ptc inhibits the diffusion** of HH, so if not expressed, the HH would **diffuse readily over the smo- clone**, activating a higher HH threshold (denser bristles) on the distal side. The results would be most striking and **informative when the marked clone is adjacent to the HH source**. This **outcome would support the model** that HH is acting an a graded way after diffusing through the field of cells, to activate different cell fates at different concentrations. If a long way from the Hh source, it does not address the relay model so clearly, since the relay may already have been activated.

A **drawing** would be nice



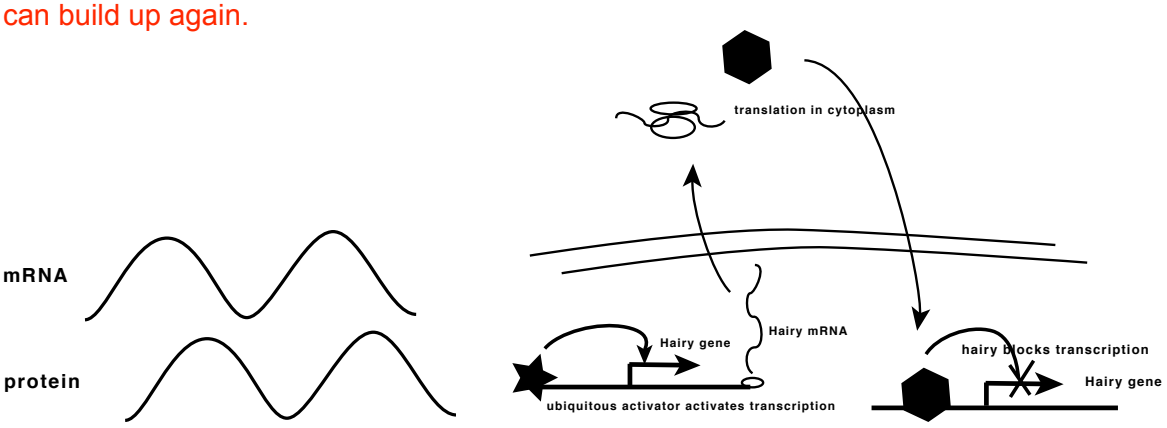
Question 9

Strong signals in the embryo usually evoke a feedback response.

9a (6 points) Provide an example where the negative feedback causes an oscillating expression of mRNA. What properties of the mRNA and protein product are necessary for this oscillation?

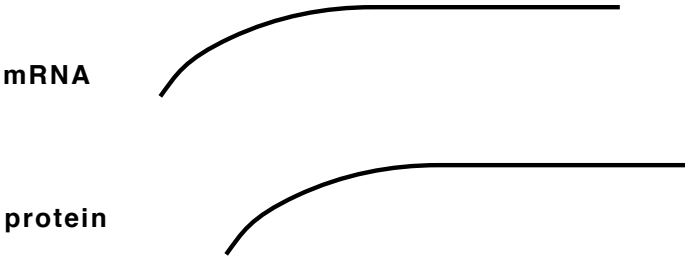
Draw out a scheme showing how this oscillation works, you can draw as many diagrams to explain this as you wish to illustrate the main points.

negative feedback in somite formation due to hairy oscillation.
mRNA is unstable, protein is unstable. Protein requires mRNA synthesis transport to cytoplasm, translation, import to nucleus, providing a dealy in the production of protein after transcription. they oscillate out of phase, as mRNA builds up it makes more proteins, then the protein represses mRNA expression. mRNA decays, makes less protein, repression stops, so mRNA can build up again.



9b (4 points) Suppose you eliminated these properties, what would you expect to happen to the oscillation and why?

If we eliminate the **instability** of hairy **mRNA** and or **protein**, then there would be **no rapid decay** of the mRNA /protein, and the cycle would be damped and **stop oscillating**



9c (4 points) An oscillator like this can be part of a clock and wavefront. Explain how the wavefront in this case is thought to work and what the consequence is.

The wavefront is based on expression of **FGF** in the **tailbud**. Away from the tailbud the mRNA and the protein **decay**, leading to a **graded activation** of the FGF pathway. The tailbud is **growing** and therefore **moving away** from the zone of differentiation, so a wavefront of activity moves along the axis. Below a **threshold** of activation the somitic cells will **initiate their differentiation**, and depending one whether hairy is **high** (posterior) or **low** (anterior) they will form the **anterior and posterior parts of the somite.** They then initiate their program of differentiation into somite blocks.

9d (3 points) What would be an example of a positive feedback mechanism in a developing embryo, and the consequence of its function?

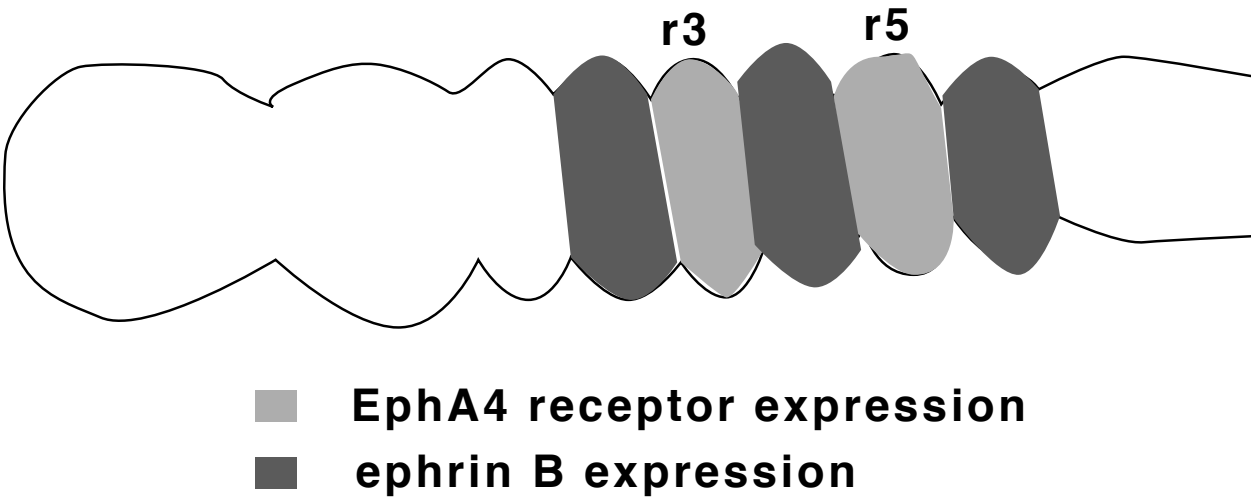
The positive feedback by MyoD, where MyoD has been shown in cultured cells to turn on other myogenic factors, and maintain its own expression. This reinforces and executes the muscle differentiation program

Question 10

10a.(4 points) In the spinal cord, ephrin signaling restricts the migration of neural crest cells to the anterior part of each somite, leading ultimately to periodic outgrowth of spinal nerves corresponding to the arrangement of somites. From these observations, it makes sense that periodic spinal nerves are extrinsically signaled by the somites. What other experiment shows that there is no intrinsic segmentation in the spinal cord, and how does this result differ from what is found in the hindbrain?

Lineage tracing of the fates of individual cells in the neural tube. Individual cells were injected with a tracer- rhodamine dextran, and the distribution of their progeny was observed at a later time. It was found in the spinal cord that when comparing many clones from many embryos, the cells can spread anterior and posterior without regard for any putative segmental boundary. In contrast, in the hindbrain, the clone of cells respects the rhomobomere boundary and by observing many clones, consistent barriers of lineage restriction are seen, over which they cannot cross (at lest for a period of development)

10b (4 points) In the hindbrain, ephrinB and Eph tyrosine kinase receptors have also been found to be expressed and have been implicated in the formation of sharp boundaries between rhombomeres. Receptor EphA4 is a transcriptional target of the krox20 transcription factor, which is expressed in rhombomeres 3 and 5. Based on this, how would you expect the ephrinB and its receptor to be expressed?



Question 11 (9 points) When Spemann grafted an optic cup from a developing newt embryo underneath the epidermis on the side of a host, he found that a lens formed. This happened wherever the optic cup was grafted. How would you interpret this experiment, and what might you do to make the observations and interpretation more authoritative? What would that interpretation be?

The simple interpretation is that the **optic cup** is able to **induce a lens wherever it is grafted**, and hence that **any epidermis is competent to become lens**.

However, he did not have lineage tracers available, and when the experiment was repeated, it was found that when the graft comes from an embryo carrying a fluorescein dextran lineage tracer, the lens in many of the grafts is also labeled, indicating that it was accidentally grafted along with the optic cup. SO the crucial **improvement** in the experiment is to graft from a **lineage tracer filled donor**, into an **unlabelled** host, and repeat **many grafts** at **different sites**. this will show that there is only a **limited field of epidermal cells, restricted to the region near where the eye forms**, that are **competent** to become **lens** tissue.

Question 12

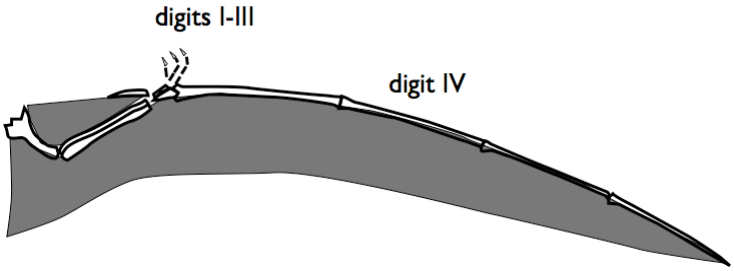
12a (2 points) What would be the consequence of placing a bead soaked in BMP protein onto the apical ectodermal ridge of the early limb bud of a chick, and why?

This woul dcause the AER to regress. Normally the AER is maintained by a feedback loop that involves gremlin antagonist working on endogenous BMPs. If excess BMP is added, then this overwhelms the gremlin and causes the AER to regress.

12b (2 points) What would be the consequence of placing a similar bead under the ectoderm at the position where the radius and ulna are beginning to condense, and why?

This would cause an excess of mesenchymal cells to condense into the precursor of the cartilage model, i.e. differentiate as chondrocytes, resulting in a massive excess of cartilage near the bead in both radius and ulna. this is becuase the dosage of BMP affects the decision of mesenchymal cells whether to differentiate as cartilage or remain as mesenchymal precursors of other cells.

12c (6 points) In a triumph of embryology and paleontology, you collaborate with Steven Spielberg to establish a line of pterosaurs (adult wing shown below). Using these, you examine the developing wing of the embryo, to see why the tissue behind digit IV makes a wing membrane. What kind of gene expression might you expect in this area in the embryo, and what precedents might there be for your **two** favored predictions?



As found in the bat wing: there may be a large amount of FGF8 expression in this region of the wing, that protects the interdigital mesenchyme from apoptosis, and preserves the wing membrane

As found in the duck foot, there may be gremlin expression in the region where cell death would occur in other related animals like the chick, and the gremlin would protect the tissue from BMP-induced cell death.

12 d (5 points) How would you test the function of one of these and what outcome do you expect?

Take the gremlin case: We could infect with a retrovirus that expresses a mutated and dominant active BMP type I receptor (with a juxtamembrane phosphomimetic glutamic acid residue)
Orinfect with a retrovirus that carries a BMP gene
Or transplant a bead soaked in BMP into the region of wing membrane

In all of these cases we expect to see apoptosis at the site of applicaiton, and a remodeling to remove the wing membrane from that region.

12e (3 points) What would you predict would occur if a (big) bead soaked in Sonic Hedgehog protein was grafted onto the anterior edge of the limb bud

A mirror image Wing duplication would result with series IV,III, II, I I,II,III, IV