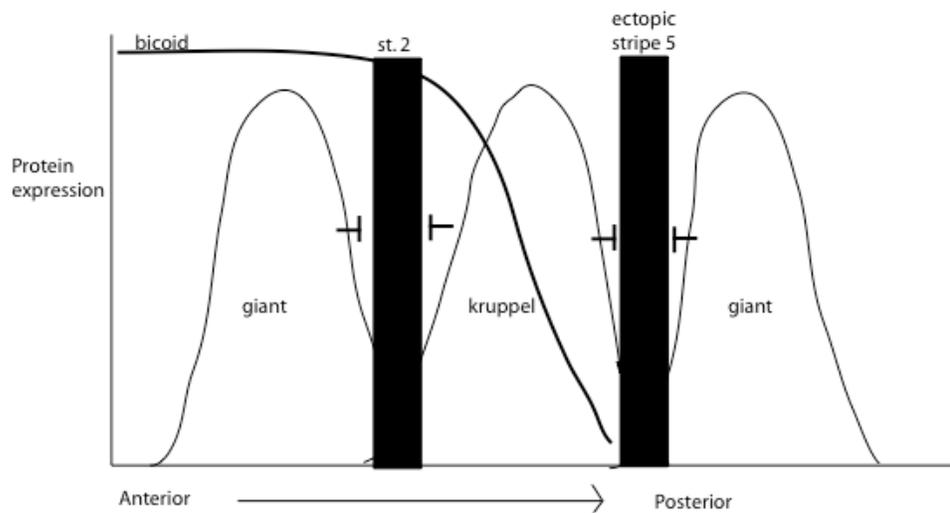


Question 1 (6 points). Predict the expression pattern (in the fly embryo) of a transgene driven by a defective Sog enhancer lacking Snail binding sites.

The transgene will be expressed in the neurogenic ectoderm and in the mesoderm. The enhancer driving the transgene is activated by high and low levels of nuclear Dorsal but is no longer capable of being repressed by Snail as the Snail binding sites are mutated.

Question 2. (6 points) Predict the expression pattern (in the fly embryo) produced by a modified eve stripe 2 enhancer that contains binding sites for a ubiquitous activator (present throughout the early embryo) in place of the normal Bicoid binding sites.



Eve stripe 2 is activated by Bicoid and repressed by the anterior Giant stripe and by Kruppel. The extent of the Bicoid gradient limits activation of the eve stripe 2 enhancer to the anterior half of the embryo. If you replace the Bicoid binding sites with those of an ubiquitous activator you will get both stripe 2 and an ectopic stripe in the position of eve stripe 5. The mutated eve stripe 2 enhancer can in theory be activated everywhere along the A/P axis, but the two Giant domains and the Kruppel domain carve out two stripes of expression. eve stripe 5 is normally activated by caudal, and repressed by kruppel and the posterior giant stripe – so this explains why the ectopic stripe from the mutated eve stripe 2 enhancer is in the stripe 5 position. A case could also be made for some additional expression at the posterior pole (or not), so points should not be taken off for that.

Question 3. 10 pts. Consider the *Mesp* enhancer of the sea squirt, *Ciona intestinalis*, which directs gene expression in the presumptive heart. It is activated at the point of intersection between two localized maternal determinants: beta-catenin and Macho-1. These determinants define the gut and tail muscles, respectively. They activate a homeobox transcription factor, *Lhx3*, and a T-box factor, *Tbx6*, respectively. Propose a model for the localized activation of *Mesp* at the junction between the gut and tail muscles.

Beta catenin activates *Lhx3* in the beta-catenin expression domain, and Macho-1 activates *Tbx6* in the Macho-1 expression domain. The *Mesp* enhancer contains binding sites for both *Lhx3* and *Tbx6*. One of these two transcription factors recruits HAT or Swi/Snf, the other recruits mediator. When either transcription factor alone binds to the *Mesp* enhancer, it is insufficient to activate transcription. So the *Mesp* enhancer is off in the gut and tail muscles, which have only *Lhx3* or *Tbx6* alone. When both *Lhx3* and *Tbx6* are bound to the *Mesp* enhancer, they act synergistically to activate transcription of the *Mesp* gene by remodeling the chromatin and then recruiting mediator. The only place both *Tbx6* and *Lhx3* are present is at the intersection of the beta-catenin and Macho-1 domains, so this is the region where *Mesp* gets transcribed.

Question 4. 8 pts. Name that phenotype: mutant fly embryo lacking both the Polycomb repressor and the entire Bithorax gene complex.

In a polycomb mutant background all the Hox genes are de-repressed and expressed throughout the anterior-posterior axis. Because of posterior dominance, the most posterior Hox gene expressed is the one that will determine segment identity. As the entire BX-C complex has been deleted in this mutant, in addition to one of the polycomb genes, the most posterior hox gene remaining is Antennapedia, so all the body segments will be transformed into T2.

Question 5. (12 points for the two parts)

Part 1: By writing the appropriate letter in the space, put the following four events in order as they occur in early chick development:

 C
 A
 D
 B

occurs first
occurs second
occurs third
occurs fourth

- A.** As the blastodisc thins down to a single-layered epiblast, it sheds cells into the fluid space between it and the yolk mass. The shed cells adhere weakly to each other, and to the underside of the epiblast, to form the hypoblast.
- B.** The primitive streak forms in the part of the epiblast directly above the endoblast.
- C.** The fertilized egg rotates in the oviduct, keeping the blastodisc displaced to one side. The uppermost edge of the blastodisc is biased to become the posterior marginal zone.
- D.** Endoblast cells migrate from the posterior marginal zone, displacing the hypoblast under the epiblast.

Part 2: Explain i) why the primitive streak forms above the endoblast and not above the hypoblast and ii) why the anterior neural plate forms above the hypoblast and not the endoblast.

For full credit, make use of such terms as Nodal signals, Nodal antagonists [such as Cerberus], endo-mesoderm induction, ectoderm, Bmp antagonists, Wnt antagonists, neural induction, and posteriorization.

The hypoblast releases Cerberus, which is an antagonist of Nodal, Wnt, and Bmp signals. The endoblast does not release Cerberus. In the region of epiblast over the hypoblast, Cerberus blocks Nodal signals and hence blocks endo-mesoderm induction and primitive streak formation. This region remains as ectoderm. In the region of epiblast over the endoblast, endo-mesoderm induction is not blocked, epiblast cells become endoderm and mesoderm, and the primitive streak forms. Furthermore, in the ectodermal epiblast over the hypoblast, Cerberus also blocks Bmps, thereby opposing epidermis formation, and blocks Wnts, thereby opposing posteriorization of neural tissue. These conditions favor anterior neural plate formation. (The actual signals for neural induction may come from the Node and anterior endomesoderm.)

Question 6 (12 points)

As you know, the *Xenopus* early gastrula normally develops to a single full sized bilateral tadpole at hatching. However, the late blastula can be cut on the bilateral plane, splitting the organizer in half, and each of the two halves will close its cut surface, start gastrulation, and continue to develop. By the hatching stage, each half develops to a bilateral half-sized tadpole, not to a left or right half of a tadpole.

Explain this result of two bilateral tadpoles from the halves in terms of: A) the arrangement of the organizer and competence zones before and after cutting and healing, and B) in terms of the release of antagonists by the organizer to cells of the competence groups.

Part A: This can be answered with drawings or words. A word" answer is given here:

The normal late blastula has bilateral symmetry with the plane running through the organizer and three competence groups. Their locations can be roughly described as: ectodermal competence group above the organizer (animal pole up), the mesodermal competence group to the left and right of the organizer and encircling the midzone, and the endodermal competence group beneath the organizer. When the late blastula is cut in half on the plane, and when the halves heal, the ventral part of each competence group closes in and adheres to the cut side of the half organizer. At the late blastula stage, each competence group is still uniform over its entirety (in its responsiveness to inducers from the organizer) and so the healing restores bilateral symmetry to each half.

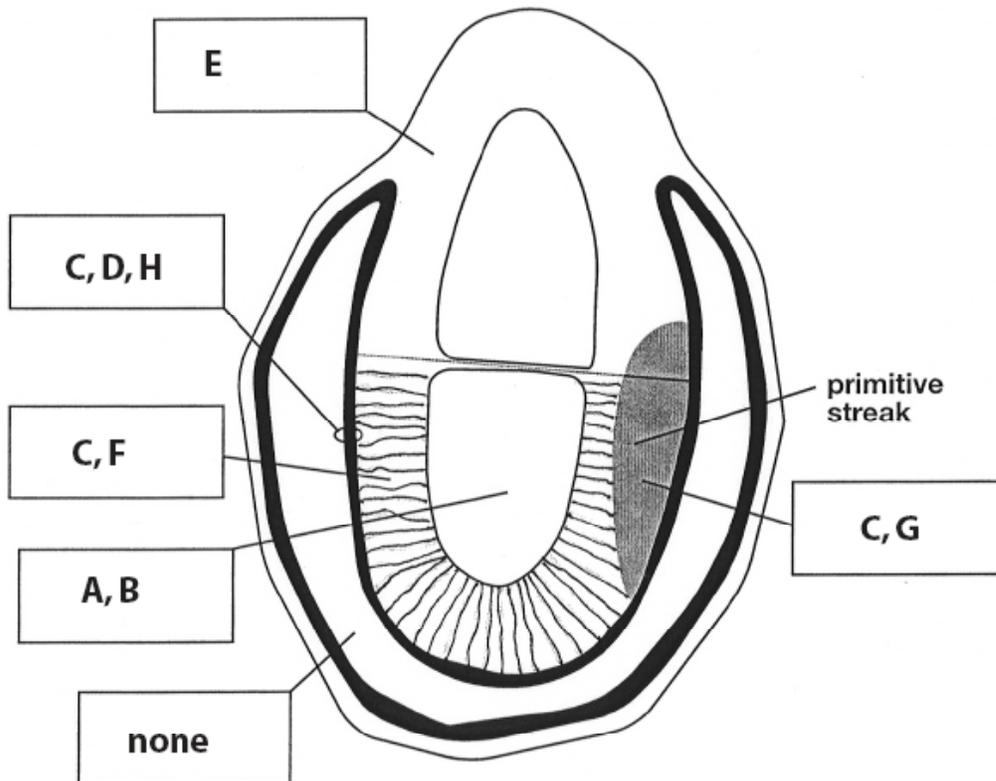
Part B: When each half heals, its half-sized competence groups are arranged with bilaterally symmetry around its half organizer. As said above, each competence group is uniform over its entirety in its responsiveness to inducers from the organizer. The organizer releases inducers (anti-Bmps and anti-Wnts) to both sides, that is, with bilateral symmetry. Thus, in both healed halves, the left side forms to the left of the half-organizer, and the right side forms to the right of the half organizer. A bilateral tadpole, half-sized, develops.

Question 7 (8 points)

A schematic cross section is shown of a 6.5 day mouse embryo, implanted in the uterine wall. Cells of the epiblast are shown, and the primitive streak has begun to form on one side.

Using the list below, put each letter into the appropriate box or boxes to identify regions of the embryo. (Some letters may go correctly in one or more boxes; boxes may have zero, one or more letters.)

- A. pro-amniotic or amniotic cavity
- B. cells underwent programmed death at this location
- C. descended from interior cells of the 64-cell compacted embryo
- D. site at which antagonists of Nodal, Bmp, and Wnts are secreted.
- E. descended from outer cells of the 64-cell compacted embryo
- F. will become anterior neural plate of the embryo
- G. site of mesoderm induction
- H. site of the anterior visceral endoderm



Question 8 (12 points) During development of a tadpole, cells of the nervous system must differentiate in order to produce reflex behaviors, but if all the cells differentiated immediately, there would be no neural precursors left to permit further growth of the nervous system. Explain what signaling pathway regulates the decision to withdraw from the cell cycle and differentiate into a neuron. Provide an example of an experiment that shows the consequences of activating this pathway.

The lateral inhibition pathway mediated by Notch signaling prevents differentiation. Thus absence of Notch signaling permits withdrawal from the cell cycle, and differentiation.
Experiment.

To activate Notch signaling, provide the Notch Intracellular domain in a subset of cells. A notch cDNA is deleted to remove the N-terminus, and a synthetic mRNA is made for injection. To track the injected region, a tracer such as lacZ mRNA is injected with the notch ICD mRNA into a two cell Xenopus embryo. About half the neural plate will inherit the mRNA and because Notch ICD protein will be in excess, this region will be subject to activation of Notch Signaling. Notch ICD goes to the nucleus and along with Suppressor of hairless DNA binding protein activates Notch targets.

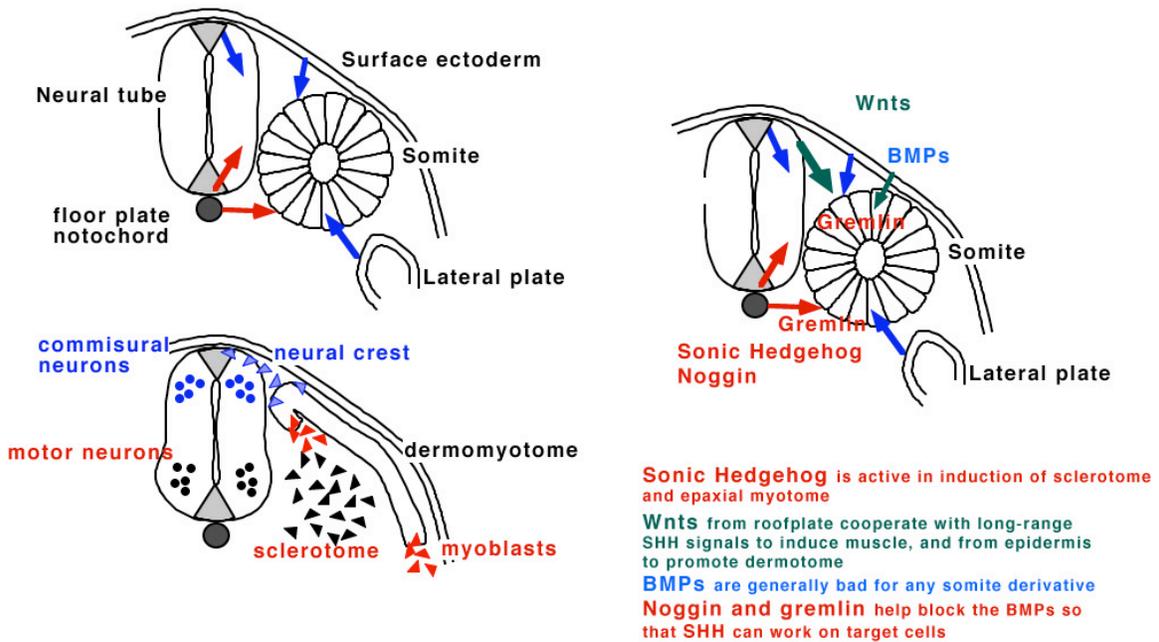
The embryos are then fixed at neural plate stage and stained for lacZ to show where the mRNA was inherited. The embryos are also stained for differentiating neurons by in situ hybridization. On the injected (lacZ) side, the differentiation of neurons is suppressed, while on the control, uninjected side, the three stripes of neurons are still able to differentiate.

Therefore, activation of Notch signaling suppresses Differentiation.

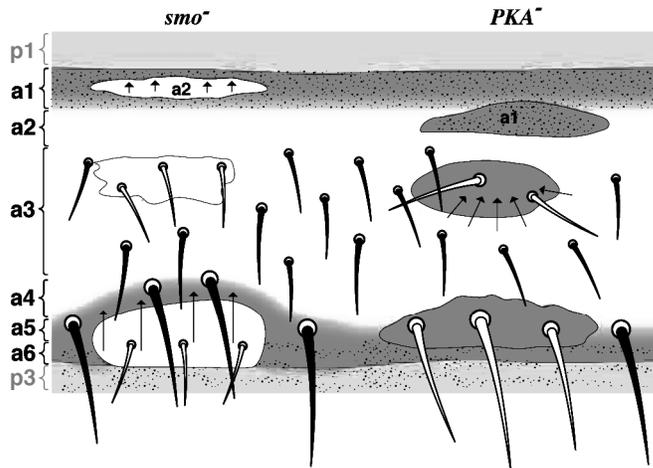
Question 9 (12 Points) Draw one or more pictures of a cross section of an early chick embryo at the “rosette” stage of somite formation (trunk level). Draw one or more pictures of the embryo once the sclerotome has segregated.

Indicate on the diagram the various molecular signals and sources of signals that are thought to act during somite patterning. Label the parts of the somite that differentiate as a result of signals.

Hedgehog, Wnts and BMP antagonists participate in neural tube and somite patterning



Question 10 (22 Points) This figure is taken from a paper where the morphogen properties of Hedgehog were examined. Mutant clones for smoothened, or PKA, were made in the abdomen of *Drosophila*. The dark pigment is considered to be a direct readout of Hedgehog function. a1-a6 mark distinguishable regions of the anterior part of the segment. Hedgehog is known to be expressed in the pale grey regions p1 and p3



The clone of *smo*⁻ cells in the a1 region of the segment is unpigmented and developing the detailed cuticle anatomy of the a2 region (you can't see this anatomy here, so take their word for it).

a. Why is this clone unpigmented? 6 points

Since pigment is a readout of HH signaling, the absence of signaling means no HH signaling. Smoothened receptor is the transducing/signaling component which is necessary and sufficient for signaling, in the absence of *smo* there is no signaling in the HH pathway.

b. Suggest what the consequence of the PKA mutation may be on the hedgehog signaling pathway. 6 points

Since the clone is pigmented, the pathway must be activated. I.e. PKA normally needed to suppress signaling, and in its absence HH signals are activated.

Question 10 c. Why is there dark pigment anterior to the *smo*^{-/-} clone? For this part of the question, consider the various aspects of the result that relate to hedgehog acting as a morphogen, and of negative feedback mechanisms that may be initiated in normal cells in response to hedgehog. 10 points

There is dark pigment, indicating HH signaling anterior to the smoothed clone, beyond the region that would be pigmented in the wild type region. Therefore **signaling is active over a greater distance**. Since ***smo*- cells cannot transduce a HH signal**, this rules out the possibility that HH signaling is passed from cell to cell in a **relay mechanism**, and instead means that HH must **diffuse efficiently through the *smo*- clone**, to activate signaling beyond the clone. One aspect of a morphogen's property is that it should **diffuse over a range** from its source, and this experiment therefore shows this property. The experiment does not go far in showing the second aspect, that different doses of morphogen **elicit multiple different fates**, since we only have two fates visible by pigmentation (brown or not), therefore could be just a switch between two fates.

The mechanism by which HH diffuses further in the case of the *smo*⁻ clone is because *smo* activity **activates patched expression**. Patched is the **negative regulator** of HH signaling, so if activated Ptc soaks up HH and **limits its range**. In the absence of Smo, **no ptc is made**, so **HH goes further** than in the wild type region. The result also suggests that normally there would be a **negative feedback** mechanism, set up by *smo* activation of Ptc, to **set up and sharpen the graded distribution** of HH.

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Question 11. (8 points) Cells are usually in either a proliferative, non-differentiating mode, or a non-proliferating, mode where they can differentiate. Discuss a molecular switch which enables a cell to make a clear choice as to which pathway to follow.

Example: **MyoD** activity modulated by **Cyclin dependent kinase 4**. When the cell is receiving **proliferative signals**, CDK4 is **active**, and **phosphorylates** MyoD protein, **inactivating** it.

When cell cycle stimuli are low, and CDK activity drops, **MyoD** activity **activates p27** a **negative regulator of CDK4**, this suppresses residual CDK4 activity and allows MyoD to be fully active (**non phosphorylated**) sharpening the difference between active and inactive MyoD. The p27 inhibition of CDK also forces the cell to **stop the cell cycle**, so that it withdraws from the cycle and differentiates.

Question 12 (6 points) Provide an example of redundant/overlapping gene activity during development. What is the phenotype of mutants in each and of both of the components.

MyoD and **Myf5** have overlapping activity in the early phases of muscle cell development.

Mutations in either **myoD** or **myf5** develop muscle, while **myoD/myf5** double mutants develop no muscle.

There are some differences in the **myoD** and **myf5** mutants, since these factors are expressed in slightly different populations of cells, and therefore different subsets or are missing at early stages in the two individual mutants. Nevertheless, when **myoD** is mutant, **myf5** expressing cells compensate for the absence of **myoD** and vice versa.

Question 13 (10 points) In a classic experiment, the optic cup was grafted to the flank of an embryo, and a lens developed. This led to the idea that all parts of the epidermis had the ability to develop as lens. However, in modern times, and due to new experiments, this interpretation is thought to be incorrect. Suggest why the original experiments might have been an artifact, and what technique should be (and was) used to show that grafted optic cup cannot induce lens from any ectoderm, but rather can only induce lens in a limited “lens field” in the head.

The old experiments lacked a lineage tracer to show that the lens was induced from the ectoderm. Instead, the lens that developed was carried over as a contaminating piece of ectoderm during the surgery from the grafted optic cup.

Therefore the experiment should be carried out with lineage tracer, so that the grafted tissue and host tissue can be distinguished.

Inject donors with tracer such as fluorescent dextran, use these to take optic cup and graft in different regions of the host.

It is found that in a restricted region of the head, the lens field, the graft can elicit production of a lens that lacks lineage tracer, and is therefore from the host.

In other regions, if lens develops, it is found to contain lineage tracer, and therefore came along with the graft, and was not induced from the host epidermis.

Question 14 (12 points)

Classic experiments in the chick embryo have shown that a notochord implanted next to the neural tube can induce ectopic floor plate and motor neurons.

However, when Sonic hedgehog soaked beads, or sonic hedgehog expressing cells were implanted next to the neural tube, they had almost no effect.

In contrast, when similar beads or cells were placed next to an explant of intermediate neural tube, they strongly induced floorplate and motor neurons.

Make a hypothesis for why the implants did not induce floor plate and motor neurons in the context of the embryo. What change would you make to the experiment to test your idea?

Hypothesis: The explant is not subject to BMP signaling as the neural tube would be in the context of the embryo. Therefore the explant is sensitive to Shh signaling, which induces floor plate and motor neurons. In the embryo, BMP signals come from lateral plate, roof plate, epidermis, and together would suppress ventral neural tube development in response to SHH. In the notochord graft, the graft produces not only SHH, but also Bmp antagonists like noggin and chordin, which suppress BMP activity and allow the SHH to signal to induce floorplate and motor neurons.

Experiment: To test whether the addition of BMP antagonists would allow the Shh implant to induce floor plate and motor neurons, the beads would be soaked in SHH plus noggin, or the expressing cells would be made to express both Shh and noggin. These implants would be expected to induce floorplate or motor neurons, in contrast to the SHH alone.

Question 15 (8 points)

“Two negatives make a positive”

Give two examples of developmental contexts where this may be true.

Neural induction:

Bmp antagonists inhibit BMPs BMPs inhibit neural induction, and promote epidermal development.

Therefore BMP antagonists induce neural development.

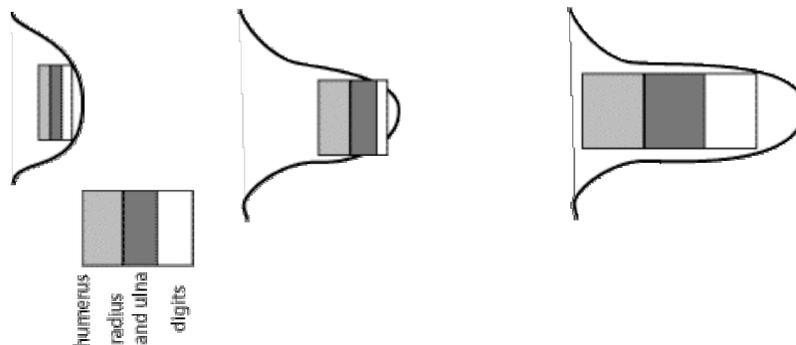
AER maintenance

BMPs in the limb bud inhibit AER maintenance, Gremlin inhibits BMPs, Therefore Gremlin promotes maintenance of the AER.

Etc.

Question 16 (10 points)

When the apical ectodermal ridge of the developing limb bud is removed, cell death occurs in about a 200 micron depth of the underlying mesenchymal region. If the AER is removed at progressively later stages of development, progressively greater amounts of proximo distal pattern of the limb are differentiated. Describe and interpret this result in light of the “early specification” model of limb development.



In the diagram three stages of limb bud development are shown, early, middle and late, with the fate map of the full limb indicated in the boxes. In the early specification model, these regions are already specified, and removal of each region will remove that structure from the final limb. When the AER is removed early, the 200 microns would include both digit and radius/ulnar regions of the specification map, thus removal will result in only the humerus being developed.

At the second stage, just the white, digit region would die, leaving humerus and radius/ulna. In the third, all regions would already be grown out, and removal of 200 microns by death would have little consequence.

Question 17 (16 points)

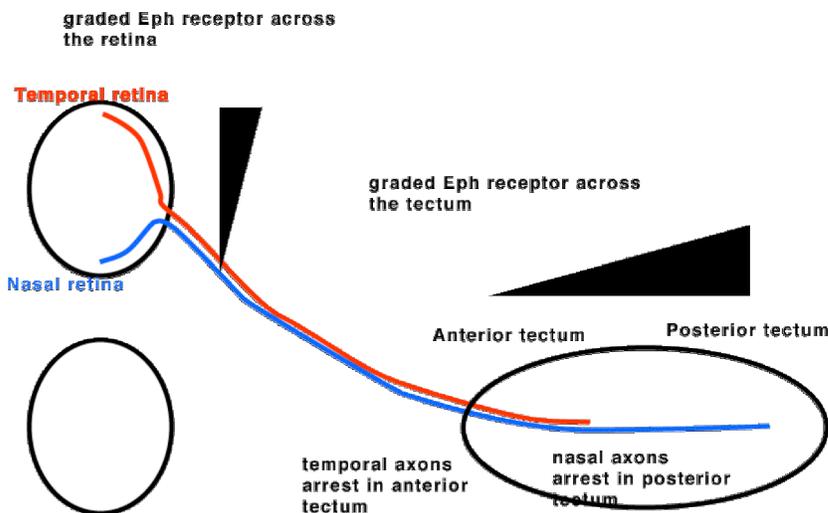
Describe the mechanism by which neural crest cells aggregate locally into ganglia along the sides of the spinal cord. What are the molecular similarities with the projection of retinal ganglion cell axons to the optic tectum? (5 points)

Neural crest cells emerge uniformly along the dorsal spect of the spinal cord, but as they migrate out, they are repelled from the posterior somite, which expresses ephrin. The NC cells express Ephrin receptor, so are sensitive to this, and local cytoskeletal collapse induced by activation of the Ephreceptor causes the cells to avoid this region, and therefore aggregate next tot he anterior of the somite.

This is similar to the projection of retinal ganglion cell axons, where cells in the **temporal** part of the **retina** express **high** amounts of the **Eph receptor**. When these axons arrive at the **tectum**, they encounter **ephrin** ligand, and as the **growth cone** navigates along the tectum, the ephrin causes local growth cone **collapse**, and the migration **arrests** in the **anterior tectum**. **Nasal RGCs** extend axons that express **less Eph receptor**, and therefore can migrate **further** over the tectum before being repelled by **high** amounts of ephrin in the posterior.

Thus there is **similarity** in the molecular cue being used, and in the phenomenon that **different doses of ligand provides specificity** in local aggregation of cells/axons of a particular type.

Draw out a scheme whereby axons projecting from one source (e.g. the retina) might find specific targets in the region to which they project (e.g. the tectum). (6 points)
How would you illustrate the properties of this mechanism in a culture situation? (5 points)



to demonstrate in culture, use the **stripe assay**, with stripes of **anterior** and **posterior tectal membranes** deposited on a permissive substrate, such as **fibronectin**. **Nasal RGCS** extend axons over **both**, but **temporal** axons are **repelled** from the **posterior** membranes.

Question 18 (10 points) Provide five contexts where the Wnt/beta catenin signaling pathway is used to make a developmental decision

1. In conjunction with *macho1* to specify muscle in the ascidian embryo
2. In early frog development, in conjunction with *veg1* to induce the organizer
3. From the lateral ventral regions of the gastrula, to induce posterior neural development
4. From the roof plate, working with Shh on the somite, to induce the muscle forming region (epaxial myotome)
5. From the surface ectoderm, to induce dermomyotome.
6. Wnts in the roofplate of the dorsal neural tube, patterning dorsal neurons
7. Wingless signaling in the A/P segment patterning in *Drosophila*

Provide five contexts where BMP antagonists are used to influence a developmental decision.

Organizer mediated neural induction

Keeping the ventral neural tube free of BMP to allow Shh mediated induction of ventral cell types

In the epaxial myotome region, to permit development of muscle

In the limb, to allow maintenance of the Apical ectodermal ridge.

In the cartilage of the skeleton, to prevent excessive cartilage condensation

Etc..

Question 19 (12 points) Why do motor neurons only develop in a limited domain of the spinal cord? Use at least twelve underlined key words in your answer.

The spinal cord is subject to signaling from the midline **notochord**, which is adjacent to the **ventral spinal cord**. The notochord secretes **SHH** and **BMP antagonists**, which permit ventral types of development. The Bmp antagonist restrict **dorsally derived BMPs** from acting in the ventral domain. Shh **diffuses** away from the midline forming a **graded distribution** of signal, which is steep due to the induction of the **patched receptor**, which works as a **negative feedback** regulator by binding Shh protein.

Shh signaling **turns on multiple different genes** in a dose dependent way, thus acting as a **morphogen**, active signaling causes **active gli** to be made, while in the absence of shh, **repressor gli** is made. The **ratio** of these activators and repressors is interpreted at **gli target** sites, which presumably have different **affinities** for the gli factor to account for the different strengths of induction. The initial targets are a series of **transcriptional repressors**, which have **cross repressive properties**. Thus **Irx3** is turned on at a distance from the notochord, and represses expression of **olig2**, and thus determines the **dorsal limit** of the **motor neuron domain**. The **ventral boundary** is set by repression of olig2 by the **nkx2** repressor, which turns on only at **high levels** of Shh. In turn olig2 represses irx3 and nkx2, **sharpening** the boundary of expression, and mediating a narrow zone of motor neuron development.