Name: _____Signature: _____

Perm Number: _____Seat Number (required):_____

Midterm Examination I – April 26, 2012
MCDB 153 – Developmental Neurobiology

100 total points (7 pages; 8 questions)

WRITE LEGIBLY……YOU CAN'T EARN POINTS FOR ANSWERS THAT WE CAN'T READ!!!!!!!!!!

Use the back of the page if you need additional space to answer any of the questions. Be sure to write "see other side" by your answer so we know to look over there; also, put the question number on the back of the page with your additional comments.

Question 1. Astrotactin knockout mice have deficits in their nervous systems, but much of the nervous system is intact. Similarly, RNAi experiments on APP during development show deficits in neuronal migration along radial glia, but much of the nervous system is intact. So, imagine that you make a double knock out of astrotactin and APP.

(6 points) How would you interpret the data if you observed that the double knockout has a completely disrupted nervous system?

I would conclude that:

1. astrotactin and APP are functionally redundant with one another
2. together, they are necessary to mediate radial glial migration.

(6 points) How would you interpret the data if you observed that the double knockout has a more compromised nervous system than when either of the two genes is knocked out/RNAi suppressed by itself but was still largely intact?

I would conclude that astrotactin and APP both contribute to neuronal migration along radial glial cell but some other factor(s) must also be required.

Question 2. Imagine that you engineered an embryo such that it expressed a dominant negative BMP-4 receptor in all relevant cells during the epidermal-neural fate determination phase of development. For the purposes of this question, assume that BMP-4 receptors work via ligand induced dimerization.

(4 points) What would a dominant negative BMP-4 receptor look like? [a diagram with an explanation might be useful here]

This is a polypeptide that includes the entire extracellular domain and transmembrane domain and very little of the intracellular domain.

(4 points) At a molecular level, what would be the effect of expressing a dominant negative BMP-4 receptor on the epidermal-neural fate determination decision? Why?

There would be no BMP-4 signalling despite the presence of the BMP-4 ligand. The reason for this is that no BMP-induced intracellular signal can be initiated unless the intracellular domains of the two dimerized receptors can interact with one another. Thus, a dimerized receptor complex containing one full-length receptor and one truncated receptor has been "poisoned" and is not functional.

(4 points) If you made this embryo, what is the most important biological question that you would be able to ask?

What is the role of BMP-4 signalling in the epidermal-neural fate determination decision?

(4 points) Based on what you know about the BMP-4 story, what would you predict that you would observe?

Without BMP-4 signalling to induce an epidermal fate in the ectodermal cells, I would expect a dramatic increase in the number of ectodermal cells that go to a neural fate.

Question 3. (5 points) What is a GFP-fusion protein? For example, what is GFP-tubulin? [a diagram with an explanation might be useful here]

A GFP-fusion protein is actually two different proteins fused together in a single polypeptide chain, i.e., GFP and some protein of interest. A GFP-tubulin fusion protein would have GFP and tubulin fused together into a single protein chain.

(5 points) How would you make a cell synthesize GFP-tubulin? [a flow diagram with an explanation might be useful here]

There are really two stages in this process. First, you have to build a plasmid. In this plasmid, you first need a promoter, followed by DNA sequences that sequentially, and in frame, encode both GFP and the protein of interest. (the GFP and protein of interest can be in either order). Second, you need to introduce this plasmid into the cells of interest, or animal of interest, generally using transfection.

(5 points) What would this enable you to do?

This would enable you to visualize your protein of interest, in real time and in living cells

Question 4. (6 points) Define the "Universal Mechanism of Animal Development".

This principle states that the same basic strategies and molecules are at play in development across very wide evolutionary distances.

(6 points) Describe, in detail, how it provided important clues for elucidation of the epidermal-neural fate determination decision.

In mammals, people had discovered that chordin acted to promote neuralization of the ectoderm. But the next key step would be to figure out what chordin interacted with, and this was a very difficult question to address. But, because of the Universal Mechanism idea, they considered the fact that chordin has a drosophila homolog called "sog", and genetic analyses had demonstrated that sog interacts with another drosophila protein called "dpp". So, they thought that perhaps chordin might interact with the mammalian homolog of dpp, which was BMP-4. This gave them a good candidate protein that might interact with chordin, which it did. This allowed the connection to be made between chordin and BMP-4.

Question 5. (10 points) If you take an animal cap from a pre-gastrulation embryo and put it into culture, it goes to an epidermal fate. On the other hand, if you take an animal cap from a post-gastrulation embryo and put it into culture, it takes on a neural fate. What happens anatomically and biochemically between the two time points in development to cause the change in fate?

Anatomically, the relevant event that occurs between the time of early gastrulation and late gastrulation is that the mesoderm forms directly beneath a subset of the ectoderm.

Biochemically, the mesoderm releases factors such as chordin and noggin and these serve to block the BMP-4 epidermalization signal, causing the ectoderm with underlying mesoderm to go to its default neural fate.

Question 6. (10 points) Sonic hedgehog (Shh) seems to be a very interesting protein. When it is expressed near the ventricular zone early in development, it tells the cells in the innermost layer of the neural tube to proliferate. However, just a little bit later in development, it is secreted by the notochord and tells the cells at the ventral region of the neural tube to stop proliferating and to become floor plate cells and motor neurons. Suggest 2 plausible/reasonable mechanisms by which the same signal (Shh) can instruct cells to do such different things?

One simple answer might be that the two different groups of cells going to different fates (i.e., proliferation versus differentiation) may have different Shh receptors that use different downstream signaling pathways and therefore send different signals to the nucleus. Another simple possibility is that the Shh receptor is the same in both groups of cells but the cellular context is different, for example, perhaps different signaling machinery is present in each of the two groups of cells, thereby sending different signals to the nucleus albeit initiated by the same receptor. Or perhaps the chromatin structure of the DNA in the two different cell types is different, such that in one cell, the Shh signal activates genes that are mediators of proliferation whereas in the other cell, the genes activated by the Shh signal mediate differentiation.

Question 7. We discussed two experiments that labeled cells at an early stage of an experiment and then examined the progeny of those cells at a later time. These were the ^{3}H-thymidine labeling experiment and the retroviral labeling experiment.

(5 points) Describe the two major experimental differences between these two labeling strategies.

Efficiency of labeling – The tritium strategy will label ALL dividing cells, so it has very high efficiency for dividing cells. The viral strategy will label only a very, very small percentage of the cells, so very low efficiency.

Strength of signal with the passage of time – The signal in the tritium strategy will be progressively lower and lower as the amount of time from the original pulse gets longer and longer. In contrast, there is no reduction in strength of signal as time passes in using the viral strategy because all cells contain the viral genes in their chromosomes.

(5 points) Could retroviral labeling have been used for the experiment actually done with 3H-thymidine, i.e., the demonstration that neurogenesis occurs in the innermost region of the neural tube and newly generated cells migrate to the outermost layer of the neural tube? Why or why not?

Yes, but you would have to start with an embryo that already has several layers of cells in the neural tube. You would then look at the cells at various times after infection and study the resulting clones. What you would see would be labeled cells at the ventricular zone and then a non-labelled region immediately outside of the VZ and then outside of that unlabelled zone, you would see more labeled cells.

(5 points) Could 3H labeling have been used to the experiment actually done with retroviruses, i.e., the demonstration that cells derived from a single progenitor at the innermost layer of the neural tube can give rise to both neurons and glia? Why or why not?

No, because the retroviral experiment requires analysis of clones of cells derived from individual dividing cells at the ventricular zone. Since the tritium will label all dividing cells at the VZ, you wouldn't be able to tell which cells farther out in the neural tube came from which dividing cell at the VZ.