Questions for Serafini et al paper

1. What would you do to separate the nuclear, membrane and cytosolic fractions of a cell?

Open up the cells and spin them in an ultracentrifuge with a gradient, so that the nuclei go down, then the microsomal fraction, and finally the cytoplasm will be floating on top.

2. Describe what is the "microsomal" fraction. The netrins are secreted proteins: why do they find them in the microsomal fraction, and not in the extracellular fluid of the embryo?

The microsomal fraction is the membranous intracellular compartment: golgi apparatus, endoplasmic reticulum, etc…Secreted proteins will accumulate in the microsomal fraction. The extracellular fluid is tiny, and very difficult to purify. The microsomal fraction is much easier to purify.

Why can they guess that the netrins are secreted proteins based on their sequence? Does it matter if the sequence that hey are analyzing is the cDNA sequence or the protein sequence that they got from the band that they purified? Why?

Proteins that are going to be secreted have a Nterminus sequence called signal peptide. The sequence is cleaved off once the protein is being directed for secretion. You can see the signal peptide in the cDNA of a protein, but not in the final protein once secreted.

3. What experiment can you do to test unambiguously that the netrins are really secreted proteins?

Put a myctag on its cDNA, transfect them into cells, collect the supernatant, and check by western blot if the supernatant contains the protein.

4. why do they bother to put a myc tag into the recombinant netrins that they generate?

Because they don't have an antibody that recognizes netrin they need to put a tag with an antibody that recognizes that tag. Once they get specific antibodies that recognize native netrin, they could use those (for instance they could use them to solve the previous question)

5. What could you do to test that inhibiting the netrins in the *in vitro* test that they use, actually blocks the directed growth of the axons?

Once you have an antibody that recognizes native netrin, you can first, put the two explants of floor and roof plate next to each other and see that

axons go from one explant to the other. In contrast, in the presence of the blocking antibody against netrin, the axons will not go there anymore.

6. What could you do to test that inhibiting the netrins in *in vivo* (in the animal) actually blocks the directed growth of the axons?

Get a mutant animal that does not have netrins or make a KO animal in which you disrupt netrin.

7. why do you think that they go through the trouble of making the recombinant netrins by transfecting COS cells, instead of the much simpler way of growing bacteria that carry a plasmid encoding netrin?

Bacteria do not produce secreted proteins well, in part because they are not glycosylated.

8. Imagine that you are very interested in investigating the existence of an extracellular molecule that could trigger the release of neurotransmitter from neurons.

8a. Describe in detail the in vitro assay that you would use to test the existence of that molecule.

Get neurons in vitro. Incubate them with FM dye. Test baseline release. Add extracts from cells in the brain to the neurons in vitro and check if the extract increase FM uptake.

8b. how could you test if the molecule is a protein, lipid or sugar? **Use a protease, lipase or glycosidase to inhibit the Prot, Lipid or sugar.**

8c. how could you identify if the molecule is a low molecular weight compound? **If it is small, you could pass the extract though a filter that will retain molecules bigger than X Daltons, so that only small stuff will go through.**

8d. Describe in detail the strategy that you would follow to identify that molecule. **This is more a chemistry problem than a biology problem, because you will have to use the methods of analytical chemistry (mass spectroscopy etc..)**

9. Imagine that for your experiment in 8, you would need 2 different proteins to be present simultaneously to do the job, and that each one of them by themselves will not work. How would you modify your strategy to look for these molecules?

You try the whole extract and it works. You start fractionating and it works. You keep fractionating and it does not work anymore. You start combining fractions, and now it works again.

0. Dissecting the brain of a chick embryo takes approximately 5 minutes. If you have to dissect 25,000 of these, and that's all you do in the lab with not interruption, working 60 hours a week, that would take you some 8 months. How could you manage to get other people to help you to finish this task earlier? [Note: this is a serious question. Sometimes the most difficult part of a project is to figure out a way of making it feasible]. **You could get high school students to do it as a small science project (but you will need a -80C freezer to keep the samples intact). You can get an army of undergraduates to help you in the lab. You can offer all the other people in the lab authorship in the paper and a fraction of the patent if the gene has commercial value.**