Questions for the julius+ axel paper:

1. explain what is being done in the top image of figure 1 ,"gradient fractionation of choroid plexus RNA". (By explanation I don't mean a simple paraphrasing of what is written on the text, but a real explanation of why they do it)

they centrifuge the rna in a gradient of sucrose so that the heavier RNAs will sink deep and the smaller rnas will float. In this way they can size select the RNA for further experiments.

2. explain the experiment described in figure 8. NIH 373 cells do not have any calcium fluctuations when exposed to serotonin. Neurons that have the serotonin receptor DO hae calcium fluctuations when exposed to serotonin. If you add the 5HT receptor to 3t3 cells, they should have calcium fluctuations. First, they transfected a plasmid that encodes for the 5HT receptor into 3t3 cells. Then they loaded the cells with a chemical that emits a fluorescent signal when calcium levels rise. To measure the changes in fluorescence, they analyze the cells with a fluorescent cell scanner. You can see that only the 3t3 cells that express the 5ht receptor show increases in the fluorescent signal,when exposed to 5ht.

3. Why do they make RNA in vitro (from the cDNA library) to inject into the oocytes?

Because if they inject RNA it can directly be translated into a protein simply by injecting into the cytoplasm. If they inject DNA, they would have to hit the nucleus of the oocyte, which is very difficult.

4. The human (or rat ) genome has approx. 30,000 genes. Why do they make a library that has 1,2 million clones?

To make sure that they have complete coverage of low abundance genes, and to make sure that in some cases they have full length cDNAs

5. the gene that they identified is 3kb, but in the northern in figure 10. They mention that it is really 5.2 KB. Why the discrepancy?

The mrna has untranslated regions (UTRs) that are not part of the protein coding region. In this case, those UTRs account for the extra 2 kb.

6. Fig 10: why is the actin band from the heart smaller than actin in any other tissues?
There are many kinds of forms of actin. The heart, being a contractile organ has a different kind of actin that most other parts of the body. Because heart's actin has a sequence similar to the rest of the body, it is still recognized by the probe. However, because it is encoded by a different gene, it produces a different mRNA which has a different size.

7. Legend of figure 1: "..transcribed in vitro in the presence of the cap precursor GpppG…". Why do they need the GpppG?

The ribosome from the oocyte will not translate an mRNA unless it is capped by the GpppG.

8. A gene is expressed in a particular cell because it is selectively transcribed there. For instance: the serotonin receptor is expressed in the choroid plexus, but not in the liver. How do they manage to express serotonin receptor in the frog oocyte?

As long as they provide the right mRNA (plus the cap and the polyA), the frog oocyte will translate anything that you out it, even if it usually doesn't express that gene endogenously.

9. The oocyte is clearly not an electrically active cell. How come if you put a serotonin receptor now it becomes "neuron-like", and it is responsive to neurotransmitters?

All cells have a membrane potential (which is usually -70 mV). Neurons have, in addition to other ion channels, voltage-gated sodium channels which are responsible for the firing of action potentials. If you put other channels into cells that are usually non-excitable, they will be able to modify their membrane potential.

10. figure 3. They inject a pool of 100,000 clones (!) and they get a electrical signal when the cell is exposed to serotonin. How do they know which of the 100,000 clones is the responsive one? How do they do to eventually isolate the "real" serotonin receptor , if they start with a pool of 100,000?

The key here is that the serotonin receptor is not a channel by itself. Like the olfactory receptor, the activation of the serotonin receptor is coupled to a G-protein, which will activate a channel. Thus, there is an amplification cascade between the serotonin receptor and the channel that is triggers to open. In their experiment, if the serotonin receptor was by itself a chennle, they would not have been able to detect it, because there would be no amplification cascade by the G-protein intermediary.

11. Why do they transfect fibroblasts with DNA but they inject RNA into the oocytes?
Because they want to make a cell line that permanently expresses the serotonin receptor, they want to make sure that the DNA that they transfect will integrate permanently into the genome. If they were to inject RNA into the fibroblast, the RNA will degrade in a few hours, and they would not be have been able to have a cell line that permanently expressed the receptor. For the oocyte, it is a different story: they just want to express protein for a few hours, and after that, they throw away the oocyte. In addition, there is question of injecting into the nucleus versus cytoplasm that we mentioned before (question 3).

12. Why do they put the serotonin receptor in the "..expression vector pMV7" when they want to transfect it into fibroblasts? Why do they have an "independent cassette encoding neomycin.."?.

To express a cDNA in mammalian cells they need a specific promoter, and the pMV7 is such a promoter. As opposed to that, a bacterial promoter would not have worked in that case. They need the neomycin cassette to be able to select for the cells that have incorporated the transfected gene. The plasmid that they transfected has both the serotonin cDNA and neomycicn. They cannot do anything to directly enrich the cells that have serotonin and discard the cells that don't. However, if a cell does not have the neomycin cassette and you grow the cells with neomycin, they will die. In contrast, if a few cells have the neomycin cassette (you grow the cells with neomycin), only those cells will survive. Now, if you have transfected BOTH serotonin receptor and the neomycin cassette, and you are selecting for neomycin, you will indirectly will be selecting for the cells that also have serotonin receptor.

13. figure 4: how do they know that out the 3 kb clone that they have, the protein coding region is only 1400 base pairs?

Because they look for the ORF in the sequence, like you did in the computer exercise, and they realize that there is a lot of UTR before and after the protein coding region of the gene. (see question 5)