

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)
2. explain the experiment described in figure 8.
3. Why do they make RNA in vitro (from the cDNA library) to inject into the oocytes?
4. The human (or rat ) genome has approx. 30,000 genes. Why do they make a library that has 1,2 million clones?
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?
6. Fig 10: why is the actin band from the heart smaller than actin in any other tissues?
7. Legend of figure 1: “..transcribed in vitro in the presence of the cap precursor GpppG...”. Why do they need 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?
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?
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?
11. Why do they transfect fibroblasts with DNA but they inject RNA into the oocytes?
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..”?
12. figure 4: how do they know that out the 3 kb clone that they have, the protein coding region is only 1400 base pairs?