Questions for the Maclaren et al paper:

1. Figure 2: they figure out that the optimal time for transplantation is from P3 to P7 donors, after the progenitors have completed their proliferation in the donor retina. Could you use select FACS sorting to select specifically the cells that just have completed their division (using brdu labeling) in the donor before transplanting them into the host? Elaborate your answer, by explaining the logic behind FACS and brdu labeling of cells.

No, because you would need to permeabilize the membrane of the cells to be able to do brdu immunostaining, and that would directly kill all cells.

2. Explain in detail what is it gained by doing FACS sorting of the cells from an NRL-GFP transgenic mouse, that can not be achieved with a ubiquitous GFP mouse. Elaborate in your answer by indicating the different outcomes of the experiment if the cells come from the NRLGFP or from the actinGFP mouse.

With the FACS you could select the cells that express the characteristic of interest (expression of NRL in this case), so you get two benefits: a) you discard all the cells that you are not interested in and b) you select the cells that you want at a particular stage in development. If you were to graft cells from the actin-GFP mouse you would get a similar result (some new GFP+ photoreceptors would appear in the host retina), but there are two important differences: a) the number of GFP+ cells would be lower (because by using the FACS you are enriching for the only population of retinal cells that works, and b) Using FACS you KNOW which are the cells that you are transplanting [as opposed to this, if you graft cells from an ubiquitous GFP mouse, and some cells survive as photoreceptors, then you still have no idea WHICH are the cells that have the potential to integrate into the retina after transplantation.

3. Figure 3c: in the inset they claim that the collocalization of green and red suggests that the cells are making synapses.

    a. Describe the logic that they follow to make that assumption. **If one of the fluorescent markers is expressed on a cell (GFP), and the other marker labels a presynaptic protein (red) , there is a good chance that if they are very close to each other, the green cells are receiving synaptic input from the synapses labeled in red. However, this is only a correlation.**
    b. Describe in detail what would you do to prove that these two fluorescent cells make synapses with an electron microscope (NOTE: this is a trick question, and the trick is related to the colors of the cells. It can be done, but it is not trivial. So try to figure out where is the trick here). **You cannot see fluorescence with an electron microscope! What you should do is: a) to see the GFP cell in the EM, you would have to label it with antibodies that are visible at the EM level: for this purpose the**

antibodies can be labeled with large gold particles that will be detectable with the EM. B) once you can see the GFP cell, you can see the apposition of synapses between the GFP+ cell and the axons of the cells that make contacts with the GFP cell.

c. Describe in detail what kind of experiment you would do to prove that the green cell and the red form a functional synapse by using intracellular recording methods. Elaborate on the kind of equipment that you will have to use: microscopes, lasers, electrodes, etc..., and how would you use them.

First, you would cut slices of the retina, and put them flat on the stage of fluorescent microscope. Second, you would identify the GFP+ cells: the cell that receives inputs is labeled in green, and you can see it alive with the microscope. Third, you will impale the green cell with a glass electrode to record its electrical activity. Fourth, you will stimulate cells surrounding the green cell with another electrode, while you record the green cells: if the surrounding cells have synaptic contacts with the green cell, you should be able to detect excitatory or inhibitory postsynaptic potentials (EPPs ar IPPs) in the green cell.

4. Figure 3 a: describe in detail all the stainings that they are using to get each of the colors in the picture, and the steps required to get the final picture.

To get the photoreceptors green they make a transgenic animal with the appropriate promoter in front of GFP. They get the retinal precursors from this animal, and they graft these cells into the retina of host animals. To get the blue, they cut sections of the retina with a microtome, and they just incubate with the dye called Hoechst 22358, which will stain the DNA of the nucleus. To get the red label, the incubate the retinal sections first with an antibody made in rabbit that recognize phosducin. Then they incubate the sections with an antibody that recognizes rabbit antibodies. This antibody is labeled with a fluorescencent red label. To be able to see the different colors, first they image for the green wavelength, and they take a picture. Then, they image for the red wavelength, and they take a picture. Finally, they image for the blue wavelength, and they take a picture. Now, they have three different pictures in the computer, <u>each of the images is in black and white, because the confocal microscope does not "see" colors</u>. You take each computer file, pseudocolor it with red,green or blue, and finally you merged them in photoshop. The yellow will appear simply because that's what you get by merging red and green.

5. Figure 4f: Describe all the steps required to get the pupil to contract as described in this figure.

The lights enters the eye, goes through the whole retina until it gets to the photoreceptors in the back. The photoreceptors (which are usually depolarized and active), become hyperpolarized (get inactivated), and this releases the tonic inhibition that they impart to the bipolar cells. The bipolar cells get activated,

and they activate the retinal ganglion cells. The retinal ganglion cells have axons that go all the way to the back of the brain to the pretectal nucleus, which connects to the edinger-westphal nucleus. The axons from the ed.westphal nucleus activate the cilliary ganglion in the eye. The axons from the cilliary ganglion then activate the sphincter muscle in the pupil.

6. What are the main obstacles for transplanted neurons as a treatment for neurological diseases? List and elaborate on your answers. Use Parkinson's disease as an example to be treated by neuronal transplantation.

- the grafted cells have to become the specific neuronal type that is missing (in the parkisnon's case, you would need dopaminergic neurons from the substantia nigra. It is not understood how to tailor stem cells to become specific neuronal types.
- assuming that the stem cell become dopaminergic neurons, they have to receive synaptic inputs form other cells, and project axons to make synaptic contact with other cells. It is not clear that grafted cells can easily grow dendrites and axons in the adult brain. It is also not clear that neurons in the adult brain can make synapses so easily.

7. Stem cells can give rise to all kinds of cell types. Why not graft stem cells directly into the retina of people that have suffered neuronal losses? At least, mention two limitations and elaborate on them.

Stem cells do not "know" what they are supposed to become if you just graft them into an adult animal. In the embryo, they are receiving all kinds of signals from their neighbors so that they differentiate into a particular cell type. In the adult, these signals are gone, and neighboring cells are not "communicating" with the newcomers. So, it is very likely that the stem cells will not become neurons after being graft. In addition, stem cells keep proliferating and it is likely that they will form tumors in the site of transplantation.