Examples of neuronal fate specification: cerebral cortex and retina
Reading: 8th ed 380-400; 9th ed 351-363

Learning Goals
Distinguish between intrinsic differences and signaling interactions that lead cells to their eventual fate in the
nervous system
Compare eye development to cerebral cortex development.
Compare the fate determination process of cortical and retinal cells
Design experiments to test the fate or commitment of different populations of neural crest cells.
Predict the outcome of changing the environment of a population of neural crest cells.

How are the cell fates of the mammalian cerebral cortex determined? (continued from class 10)
Organization of the laminae of the cortex
The cortex is what allows mammals to "process" information. The neurons executing most of the action are located in the cortical plate of the neocortex. The neurons get to this location by migrating along glial processes that extend from the ventricular zone all the way out to the cortical plate. They are organized into 6 layers, and differentiate in a sequential fashion
How are the fates of these cells determined (*ie,* how do they know which layer to go to)?
These questions were addressed with two basic techniques (that are NOT covered well in your book, sorry!)

1) Clonal analysis : allows identification of the types of cells that a single dividing cell can make. Thymidine labeling experiments (replicating DNA takes up the radioactively labeled T) demonstrated that the first layer of neurons to form is the deepest (layer 5/6) (figs 10.10, 10.11). In other words, these layers are formed by an "inside-out" pattern of migration: the cells that leave the ventricular zone first migrate the shortest distance, to layer 5/6, while cells that are born later migrate out to the more superficial layers, 1/2/3.
2) Transplantations: heterochronic: different time (same place) and heterotopic: different place (same time).

In the cortex, the transplantation experiments were heterochronic, and demonstrated several features of laminar determination (see ppt slides):

1. EARLY: "birthdate" determines fate.
--When progenitor cells that would normally migrate to deep layers were transplanted into the same area, but into an older host, these cells were able to take on the fate of the cells surrounding them (changed their fate to migrate to superficial layers). This was only possible **if** they were transplanted before their final S phase, suggesting that it is during the final DNA synthesis that the cell either receives the signal to be committed.

2. LATER, the possible fate of the cells becomes restricted.
--When late stage progenitors that would normally migrate to superficial layers are transplanted into earlier stage hosts [P1 (post-natal day 1) cells transplanted to an E29 (embryonic day 29) host], the transplanted cells cannot change their fate, even if they undergo their final S phase in the new environment.
--They are irreversibly committed to migrate to superficial layers.

Take home: Fate is restricted over time.
Are there certain molecules known to be involved?

What signals are involved in this progressive fate determination? Is there a "default" fate of the cells that is overridden over time?

To test this, cells from early stage ventricular zone (E29) were isolated in culture and put into different environments while they were undergoing their final S phase. They were placed in low density (no contacts), pellet (dissociated and then allowed to re-associate; contacts present, but different from usual), and explants (a chunk of tissue in which all normal architecture preserved). After 6 hours in culture, cells that were not already dissociated were dissociated, and the cell suspension transplanted into late stage host (P1). The finding was quite interesting: when not in contact with each other, E29 cells were unable to take on deep layer fate (low density and many of the pellet cells). Thus, the deep layer fate was not something that was intrinsic and lost over time, but rather a fate that had to be maintained by signaling. The deep layer fate was not the default! This experiment did not prove that the superficial layers were default, but did demonstrate that the fate change taken by E29 cells when put into a later stage host could have been due both to LACK of signals from the normal early stage cells, and/or different signals present at the late stage that helped to signal superficial fate. A very recent paper (Mutch 2009) suggests that levels of b-catenin in the cells could be responsible for this commitment—presumably the b-catenin is activated at a high level in early stage precursors, and no longer activated in late stage precursors. .

Development of the vertebrate eye

Formation of the optic vesicle.

The eye field is defined in the anterior neural plate by combinations of signaling molecules present at that spot (ie, low Wnt, other factors). Outpocketing of the brain itself at the level of the diencephalon makes the primary optic vesicle. Then, the optic vesicle then contacts the ectoderm of the head, inducing the lens placode. The medial optic vesicle narrows to become the optic stalk while the lateral part of the optic vesicle is pushed in, or invaginates to become the optic cup. The outer layer of the optic cup will become the retinal pigmented epithelium (RPE) and the inner layer will become the neural retina. The transcription factor Rx is required for formation of the optic vesicle. Expression is present in two lateral patches in the anterior neural plate and in the optic vesicle. If Rx is not present (due to a loss of function mutation or knockout), the optic tissue cannot form. In addition, the division of the eye field into two halves, so that two separate eyes can be generated, is dependent on inhibition of another important transcription factor, pax-6, in the middle of the eye field. This occurs because Shh from the notochord (right in the center of the animal, below the developing eye field), inhibits pax-6 expression in that area. Finally, FGFs and BMPs play a general role in supporting the differentiation of the retina, and later, neurotrophic factors like brain derived neurotrophic factor (BDNF) are required for maintenance of the retina and the retinal pigmented epithelium.

Differentiation of the neural retina

One of the interesting things about the retina is that a single progenitor can give rise to any cell in the retina. The fates of the cells are mediated by environmental factors including the time of birth and other signals in the environment when they are differentiating. This was demonstrated through the use of retroviral labeling of single progenitor cells. The virus carried a functional copy of the B-gal gene,

and thus all cells derived from the progentior contained B-gal, and the "clone" of cells derived from a single progenitor could be visualized by lacZ staining. Note that this is an interesting difference when compared to the progenitor cells of the cerebral cortex, which have different fates dependent on time (progressive restriction of cell fate rather than pluripotent at any time). Despite this pluripotency, the vertebrate retina develops in a step-wise fashion similar to that of the cortex, with specific cell types differentiating at specific times.

As in the cortex, the determination of these cell types is clearly dependent on both the presence of different transcription factors at different successive times of development, as well as signaling to the dividing cells from already differentiated neurons in the retina. The first cells to differentiate are retinal ganglion cells, then photoreceptors, then bipolar cells.

The first level of control of these cell fates is through the time dependent regulation of translation. Several key transcription factors have already been transcribed in all retinal progenitor cells, but are prevented from being translated by the expression of asset of microRNAs. The microRNAs are highly expressed in the early retinal progenitor cells, and bind to the 3' UTRs of the transcripts required for bipolar differentiation. In this way, the bipolar fate is repressed, until those microRNAs stop being made, and then the biopolar-specific proteins can be made. In addition to this regulation, there are also signaling molecules secreted from the retinal cells as they differentiate that biases what the next set of post-mitotic cells can differentiate into. We'll discuss further some experiments that demonstrated these different molecules are being secreted at different times during retinal development.