

Out-Foxing Fate: Molecular Switches Create Neuronal Diversity in the Retina

Progenitor cells in the mammalian retina generate at least 55 distinct kinds of neurons. Two reports in this issue of *Neuron* reveal two transcription factors (Foxn4 and Bhlhb4) that contribute to the development of this remarkable cellular diversity.

Anyone trying to understand how the brain works will not get far before confronting one of the nervous system's most remarkable features: its cellular diversity. The profusion of neuronal cell types—varying by morphology, connectivity, and function—is one of the most awe-inspiring, if daunting, aspects of the brain's wiring diagram. For developmental neurobiologists, this cellular diversity presents a tremendous challenge: what is the molecular blueprint for generating this neuronal cornucopia? There has been significant progress in recent years toward understanding the choice of cell fate and subsequent differentiation of broad cell classes—motor neurons in the spinal cord, for example, or retinal ganglion cells (Mu and Klein, 2004; Shirasaki and Pfaff, 2002). But each of these classes comprises multiple subtypes, so the race is now on to discover molecules that specify even more specific subsets of neurons. In this issue of *Neuron*, two papers address how neuronal diversity is generated in the mammalian retina: one describing a gene that acts in retinoblasts to direct cell fate decisions, and one describing a gene that acts during neuronal differentiation to impart subtype-specific neuronal characteristics.

The retina has long been a favored model system for studying neuronal cell fate (Cepko et al., 1996). Light is transduced by rod and cone photoreceptors, which pass their information to retinal ganglion cells via an excitatory interneuron, the bipolar cell. Inhibitory interneurons, horizontal and amacrine cells, filter and sharpen the signal. Thus, only six neuronal classes form the circuitry underlying vision. However, even in this apparently simple system things get quite complex. There are parallel circuits for light transduced by rods versus cones, which require separate bipolar and amacrine cell subtypes. There are also parallel circuits detecting light onset and offset, a separate circuit for blue light, specializations for motion detection, and more. All this adds up to at least 55 neuronal subtypes in the mammalian retina and perhaps double that number in other vertebrates (Masland, 2001; Wagner and Wagner, 1988).

How does the retina make all these different cells? Lineage tracing studies show that individual retinoblast progenitor cells are competent to give rise to multiple cell classes (Holt et al., 1988; Wetts and Fraser, 1988). But they do not do so all at once—each cell class is

born mainly during a fixed time window. These observations led to the hypothesis that retinoblasts pass through a series of different “competence states,” during which they sequentially produce different types of neurons (Cepko et al., 1996). Although this model is generally accepted, it has been difficult to identify the molecular substrate of competence states or to explain molecularly why a retinoblast might be limited to producing certain neurons at a given time. The paper by Li et al. (2004) in this issue addresses both of these questions. First, the authors provide evidence that the transcription factor Foxn4 controls the competence state of a certain progenitor subpopulation in the mouse retina, and second, they suggest a molecular mechanism by which Foxn4 might act to limit the fates available to progenitors.

The retinoblasts at the center of the Foxn4 story are identified by their expression of the syntaxin protein. Initially, these progenitors preferentially generate amacrine and horizontal cells, but over time they begin making rod photoreceptors instead (Alexiades and Cepko, 1997). Li et al. (2004) show that the syntaxin-positive retinoblasts also express Foxn4, but only until around the time of birth—which, coincidentally, is about the time when these progenitors switch to making rods. When they knocked out the mouse *Foxn4* gene, Li et al. found that horizontal cells and the vast majority of amacrine cells were missing, replaced by an excess of rods. This finding reveals, first, that the syntaxin/Foxn4-positive retinoblasts are the major (or only) source of amacrine and horizontal cells in the mouse retina. And second, it suggests that Foxn4 expression might define the early, amacrine-generating competence state for these progenitors, whereas its absence might define the late, rod-generating state. To test this idea, the authors virally misexpressed *Foxn4* in the neonatal retina, a time when Foxn4 expression is normally decreasing and photoreceptor genesis predominates. Misexpression of *Foxn4* dramatically increased amacrine cell production at the expense of rods, further indicating that expression of this transcription factor defines the amacrine-generating competence state.

The authors next explored how Foxn4 expression might limit a retinoblast's fate. One possibility is that, by regulating expression of genes that promote one fate over another, Foxn4 might act like a set of weighted dice, biasing progenitors that decide to differentiate while Foxn4 is expressed toward becoming amacrine or horizontal cells. The transcription factors NeuroD1 and Math3 are together essential for amacrine cell specification (Inoue et al., 2002), while Prox1 is required for horizontal cell genesis (Dyer et al., 2003). Loss of Foxn4 function reduced NeuroD1 and Math3 expression, eliminated Prox1 expression, and upregulated expression of Crx, which promotes rod and cone photoreceptor fate. It therefore appears that Foxn4 controls progenitor competence by regulating genes involved in fate specification. The transcription factor expression profile of a Foxn4-positive retinoblast seems to encourage ama-

crine/horizontal fates, while the same progenitor without *Foxn4* assumes a rod-friendly expression profile.

Now that *Foxn4* expression has been linked to specific progenitor competence states, the mystery becomes: why does *Foxn4* go away when it does? Or, put another way, what mechanism controls the timing of the competence state switch? One hint comes from the observation by Li et al. that, in *Foxn4* mutants, *Foxn4* expression persists much longer than it normally would. This suggests that *Foxn4* itself normally limits the temporal window of its own expression. It will be interesting to see how this is accomplished—for example, whether it is a cell-intrinsic effect of *Foxn4* function or a cell-extrinsic effect of losing amacrine cell-derived signals—and what other mechanisms participate in timing competence state switches.

While the study of Li et al. sheds light on the mechanism by which retinoblasts switch from making one cell class to the next, the downstream genes that build each of the 55 retinal cell subtypes are still waiting to be discovered. Bramblett et al. (2004) report in this issue of *Neuron* that they have found one—a transcription factor of the basic helix-loop-helix (bHLH) family that is required for differentiation of a particular subtype of bipolar cell. The cell type in question receives input specifically from rod photoreceptors and is thus part of the specialized circuitry devoted to vision under low light conditions. Bramblett et al. find that the gene *Bhlhb4* has a remarkably precise expression pattern in the adult mouse retina, showing complete and nearly exclusive overlap with markers of rod bipolar cells. Developmental regulation of *Bhlhb4* expression is equally precise—it is expressed only during the brief period when bipolar cells are born and begin forming synapses with their pre- and postsynaptic partners. Bramblett et al. generated a *Bhlhb4* knockout and found that, while rod bipolar cells are initially born, they fail to differentiate in critical ways, including a failure to form synapses with rods. Eventually all the cells die. Electrophysiological and anatomical analyses show that the defect is specific to the rod pathway. These findings demonstrate that *Bhlhb4* is required for rod bipolar cell differentiation and survival.

Bhlhb4 mutants show one of the most subtype-specific phenotypes yet reported in the retina. Only *Vsx1*, which is required for the differentiation of a subset of cone bipolar cells (Chow et al., 2004; Ohtoshi et al., 2004), and *Prox1*, which controls development of the single horizontal cell type in the rodent retina (Dyer et al., 2003), affect comparably small neuronal subclasses. It will be interesting to see which genes are regulated by these transcription factors, as their targets will likely be responsible for imparting subtype-specific identity. The finding that single genes are required for differentiation of these neuronal subclasses is intriguing, because combinatorial transcription factor codes are thought to be crucial for determining neuronal subtypes in the spinal cord (Shirasaki and Pfaff, 2002). The importance of combinatorial codes for retinal cell fate remains to be determined. In any case, it is noteworthy that, so far, the molecules instructing class or type specificity are all transcription factors and thus intrinsic to the cell, while extrinsic signals, such as Notch, Hedgehog, Wnt, FGF, and BMP, seem to play less of a cell type-specific role during the production of retinal neurons.

To reach their goal of understanding the generation of neuronal diversity, developmental neurobiologists will no doubt continue finding new genes that help build specific subtypes of neurons. But they will also have to figure out how subtype-specific differentiation factors like *Bhlhb4* fit into the competence state model and how they are regulated by the fate determination machinery. By identifying key molecular players for both differentiation and fate specification, the articles in this issue lay the groundwork for exciting discoveries to come.

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Steering Growth Cones with a CaMKII/Calcineurin Switch

Calcium can regulate and induce both attractive and repulsive turnings by growth cones. In this issue of *Neuron*, Wen et al. report that differential activations of CaMKII and calcineurin (CaN) act as the read out for distinct patterns of intracellular calcium signals and a switch between attraction and repulsion.