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Neuron
Previews

Expanding Neuronal Layers by the Local Division of Committed Precursors

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DOI 10.1016/j.neuron.2007.11.003

A general strategy in vertebrate neural development is for neuronal precursors to stop dividing, to begin to differentiate, and then to migrate to their final destination. In this issue of *Neuron*, however, Godinho et al. provide evidence that some neuronal precursors undergo a terminal symmetric cell division at their final destination to form an entire neuronal layer.

Over a century ago, classic observations by Ramon y Cajal revealed that several regions of the central nervous system (CNS) are organized into layers containing neurons of similar morphology and projection patterns. Since then, many experiments have shown that neuronal cell body position is often linked to the pattern of neuronal connections, highlighting the functional importance of laminar organization. But how are neuronal layers formed during development? The current view, supported by a large body of evidence, is that neural progenitor cells give rise to neurons in a proliferative zone located some distance away from the final position adopted by the neurons. Then, through a process of radial and tangential migration, the newly born neurons reach their resting position within the layered structure. Because of the importance of neuronal migration in the establishment of neuronal layers, much experimental work has focused on the mechanisms that regulate directed cell migration in the process of neuronal layer formation (Ayala et al., 2007). But, in addition to neuronal migration, could there be other mechanisms that contribute to layer formation? In this issue of *Neuron,* Godinho et al. (2007) now report that, in the zebrafish retina, some neurons are generated within their final laminar location, thereby avoiding the need to migrate to this location. These results point to an interesting novel strategy in the establishment of neuronal layers and could influence our understanding of developmental CNS diseases such as lissencephaly and double cortex.

The vertebrate retina is a typical laminated structure. It contains six major classes of neurons and two types of glia, organized into three distinct cellular layers. The outer nuclear layer (ONL) contains the cone and rod photoreceptors. The inner nuclear layer (INL) lies just inside the ONL and contains the cell bodies of Müller glial cells and the retinal interneurons—horizontal cells, amacrine cells, and bipolar cells. The innermost ganglion cell layer (GCL) contains the only projection neurons of the retina, the retinal ganglion cells, as well as displaced amacrines and some astrocytes. Within the INL, some neurons are located at sublaminar positions, where they can readily establish their connections to other types of neurons: the horizontal cells, for example, are conveniently located in the outermost part of the INL, where they contact the presynaptic photoreceptor cells in the adjacent layer.

Pioneering cell-lineage analyses have indicated that at least some of the earliest retinal progenitor cells (RPCs) are multipotent and can give rise to all the different retinal cell types (reviewed in Livesey and Cepko, 2001), except for the astrocytes, which migrate into the retina from the optic nerve head (Watanabe and Raff, 1988). Similar to other layered structures of the CNS, RPCs are located in their own proliferative zone—the retinal neuroepithelium—where they undergo DNA synthesis at the basal surface and mitosis at the apical surface. Consequently, postmitotic neurons born at the apical surface have to migrate radially, often through previously generated neurons, to reach their final position within the retinal layers located basally (Figure 1). In contrast to other laminated structures, however, the newborn retinal neurons appear to migrate on each other, apparently using the basal process inherited at mitosis, rather than on radial glial fibers (Saito et al., 2003).

Although the vast majority of RPC mitoses occur at the apical surface of the retinal neuroepithelium, some mitotic figures have been observed within the developing INL of the human and cat retina (Rapaport et al., 1985; Rapaport and Vietri, 1991; Robinson et al., 1985; Smirnov and Puchkov, 2004), raising the possibility that some retinal neurons might be generated there, thereby contributing to the formation of the INL. Taking advantage of the rapid development and transparency of the zebrafish retina, Godinho et al. (2007) performed in vivo imaging experiments to study these divisions and the cell types they generate. The authors first showed that, just like in the cat and human retina, a significant number of mitotic cells in the zebrafish retina are located in the developing INL. Using a transgenic

fish line that expresses green fluorescent protein (GFP) under the control of the regulatory elements of the *pancreas transcription factor 1* (*ptf1a*) gene, which drives expression of GFP in amacrine and horizontal cells, they showed that some GFP+ cells also expressed a mitotic cell marker. These GFP+ mitotic cells were located in the outermost part of the INL, where horizontal cells normally reside, whereas they were not found in the amacrine cell layer, suggesting that the INL mitoses might generate horizontal cells.

To test this possibility, they injected fertilized zebrafish eggs with a construct expressing yellow fluorescent protein (YFP) under the control of the promoter elements of the *Connexin 55.5* (*Cx55.5*) gene, which had been shown previously to specifically drive gene expression in horizontal cells. This approach generated fish that contained only a few cells expressing YFP in the retina, which could be followed by time-lapse imaging. As expected, these cells were located at the outer margin of the INL, which is the laminar location of horizontal cells, and displayed the morphology of immature horizontal cells. Following the fate of these cells over time, Godinho et al. found that some of the cells rounded up and divided in the outer INL. The two daughter cells then migrated laterally, away from each other, and adopted the unique morphology of horizontal cells (Figure 1).

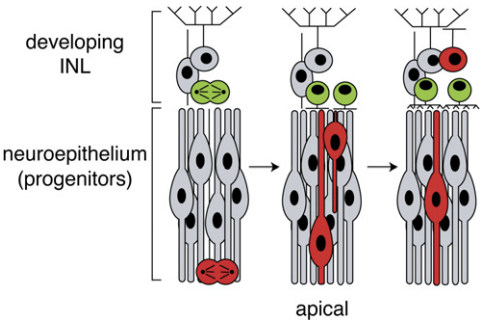

Figure 1. Two Distinct Modes of Neuronal Layer Formation in the Developing Zebrafish Retina

Neural progenitors in the retinal neuroepithelium undergo mitosis at the apical surface (red). The neuronal daughter cell of this division then migrates toward the inner nuclear layer (INL), where it will take up its final position, whereas the progenitor daughter remains in the neuroepithelium, where it will continue to divide. Apical divisions can also give rise to two postmitotic daughters or two proliferating progenitors (not shown). In the Godinho et al. (2007) study, it is shown that some committed precursor cells undergo mitosis in the developing INL and give rise exclusively to two horizontal cells, which will remain in the same laminar location as their mother cell (green). For clarity, the developing ONL is not shown.

These results showed that some precursors in the developing zebrafish retina undergo mitosis at the laminar position of mature horizontal cells and give rise exclusively to horizontal cells. It remained unclear, however, to what extent INL divisions contribute to horizontal cell layer formation. To address this question, the authors recorded the development of the horizontal cell layer in the *ptf1a:GFP* transgenic zebrafish by time-lapse confocal imaging. Consistent with their previous results on both fixed sections of these fish and live imaging on *Cx55.5:M-YFP*-expressing cells, they found that many GFP+ cells were undergoing mitosis in the outer INL. Importantly, by counting the number of mitoses recorded and the total number of horizontal cells generated in the imaged area, they estimated that INL mitoses generate almost 90% of the horizontal cells in this region, indicating that INL division is the primary mode of horizontal cell layer formation, at least in the zebrafish.

But what could be the advantage of generating a specific type of neuron at the position of their final laminar location? Godinho et al. (2007) suggest that the close proximity of horizontal cell precursors to differentiated horizontal cells might be a way of controlling the number of horizontal cells generated via negative feedback inhibition. In addition, they suggest that such INL divisions might increase the efficiency and rate of neural circuit formation, as both presynaptic photoreceptors and postsynaptic horizontal cells are generated simultaneously. It is also tempting to speculate that INL divisions might facilitate the cell-cell interactions required for horizontal cell tiling, in which the horizontal cell bodies and dendrites end up regularly distributed in the retina; in support of this possibility, Godinho et al. (2007) observed that, upon mitosis in the INL, the two daughter cells always migrate apart laterally to take up their final position.

An important question raised by this study is whether the concept of layer-specific mitosis could apply to other organisms and other regions of the nervous system. The reports of nonapical divisions in the cat and human retina suggest that layer-specific mitosis might contribute to layer formation in the retinas of many species, although further studies will be required to provide direct evidence. Nonapical divisions have also been observed outside of the retina. In the developing mouse cortex, for example, progenitor cells undergo mitosis in two distinct areas: the apical surface of the ventricular zone (VZ) and the subventricular zone (SVZ), which is positioned superficial to the VZ. The INL divisions observed by Godinho et al. (2007) are, in some respects, similar to the divisions in the cortical SVZ. Both INL and SVZ divisions are symmetric, neurogenic, and terminal, and the dividing cells display a multipolar morphology without apical or basal attachments (Noctor et al., 2004). Nonetheless, there is an important difference: the daughters of INL divisions remain in the INL and contribute to its formation (Godinho et al., 2007), whereas neurons produced by SVZ progenitors migrate to the superficial layers of the cortex (Noctor et al., 2004). Interestingly, a recent study showed that, in the mouse cerebral cortex, another population of proliferating cells, capable of generating neurons and glia, is located in the marginal zone (MZ), the future layer 1 of the cortex (Costa et al., 2007). Whether MZ mitoses actually contribute to layer 1 formation is still unclear, but the results of Godinho et al. (2007) suggest that this is an interesting possibility. It will be important in the future to determine whether layer-specific mitoses contribute to neuronal layer formation in other parts of the nervous system, and to what extent they are involved in the formation of a specific layer

compared with the classic mode of layer formation.

The finding that some progenitor cells in the retina undergo a single terminal symmetric division in the INL to exclusively generate two horizontal cells is itself important. The apparent random composition of the clones obtained in pioneering RPC lineage studies have led to the widely accepted view that there are no committed precursor cells for any particular retinal cell type, with the possible exception of rod photoreceptors, and that cell-fate decisions in the developing retina are largely lineage independent (Livesey and Cepko, 2001). Increasing evidence, however, suggests that intracellular developmental programs may play an important part in cell-fate decisions in the retina, as well as in other parts of the nervous system (reviewed in Cayouette et al., 2006). In the developing mouse cortex, for example, recent experiments have shown that the timing of neurogenesis is preprogrammed in individual progenitor cells (Shen et al., 2006). Time-lapse imaging studies in the intact developing zebrafish retina have shown that some RPCs expressing a particular transcription factor stereotypically undergo an asymmetric cell division to generate a retinal ganglion cell and another RPC (Poggi et al., 2005), suggesting that these cells are programmed to undergo the same pattern of cell division over and over again. Similarly, the experiments by Godinho et al. (2007) have identified a precursor cell committed to the horizontal cell lineage that divides in a stereotyped pattern, undergoing a terminal symmetric cell division that produces two horizontal cells. Although it remains unclear whether these horizontal cell precursors originate from multipotent RPCs, these results suggest that fate commitment in these cells occurs before the last mitosis. Interestingly, Godinho et al. showed that these horizontal cell precursors express RPC markers such as Pax6 and Prox1 and are, at the same time, able to express fluorescent proteins driven by the regulatory elements of genes normally expressed in postmitotic horizontal cells (*Cx55.5* and *ptf1a*). This unique combination of gene expression should now prove useful in identifying the molecular mechanisms regulating the lineage commitment and differentiation program operating in these cells. Importantly, these results open new avenues of research on the mechanisms of cell-fate decisions in the retina specifically and in the nervous system more generally, as they suggest that cell lineage might be more important in these decisions than currently believed. The advance of live imaging technologies should help determine whether there are other reproducible cell lineages in the retina and other parts of the nervous system.

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