Strange Bedfellows: Reelin and Notch Signaling Interact to Regulate Cell Migration in the Developing Neocortex

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DOI 10.1016/j.neuron.2008.10.009

Neuronal cell migration in the developing neocortex relies on the Reelin signaling cascade to establish the characteristic "inside out" lamination pattern. In this issue of *Neuron*, Rakic and colleagues report that Notch signaling, long known to regulate neural stem cells, also plays a critical role in mediating the effects of Reelin on neuronal cell migration.

The construction of the mammalian neocortex during development is perhaps the most complex biological process that occurs in nature. A pool of seemingly homogenous neural stem cells first undergoes proliferative expansion and diversification and then initiates the production of successive waves of neurons. As those neurons are generated, they migrate out of the germinal zone to take up residence in the nascent cortical plate where they integrate into the developing neocortical circuitry. The spatial and temporal coordination of neuronal generation, migration, and differentiation is tightly regulated and of paramount importance to the creation of a mature brain capable of processing and reacting to sensory input from the environment and of conscious thought.

A dizzying array of pathways has been implicated in the regulation of neocortical development. What has lagged behind, however, has been an understanding as to how those pathways may work together or in opposition to achieve the final outcome. Prominent among the many signaling cascades known to regulate neocortical development are those of Notch and Reelin. These pathways have largely been relegated to opposite ends of the embryonic neocortex, with Notch thought to function primarily in the germinal zone adjacent to the ventricle and Reelin thought to function primarily near the pial or outer surface of the brain. The prospect for these pathways to interact has existed, at least in principle, in that Notch functions in radial glial progenitors, which extend processes to the pial surface, and also in neurons present in the cortical plate, which are near Reelin-expressing Cajal-Retzius cells in the marginal zone. Consistent with the latter, a recent study by Rakic and colleagues (Hashimoto-Torii et al., 2008), published in this issue of *Neuron*, now provides evidence that Reelin and Notch signaling do indeed interact in neocortical neurons to regulate migration and laminar position.

Notch in the Developing Neocortex: Regulation of Progenitors…and Neurons

Notch signaling is well known to play a central role in neural stem cell regulation (Yoon and Gaiano, 2005). Activation of Notch receptors (Notch1-4, in mammals), by ligands such as Delta-like-1 and Jagged-1, leads to nuclear localization of the intracellular domain of Notch (NICD) and activation of targets such as the *Hes* and *Hey* genes, via the transcriptional regulator RBP-J. This signaling event is essential for the maintenance of the neural stem/progenitor cell pool, as disruption of the Notch cascade leads to precocious neurogenesis and depletion of the germinal pool (Yoon and Gaiano, 2005). More specifically, as neocortical neural stem cells are radial glia, Notch activation by ligand-expressing neurons or intermediate progenitors maintains radial glial character while inhibiting neurogenesis (Yoon et al., 2008).

In addition to the traditional role for Notch during neocortical development, numerous studies have provided evidence that Notch also functions in neurons as they undergo differentiation in the cortical plate (Redmond et al., 2000; Sestan et al., 1999). Notch1 immunoreactivity was shown to be present in the nucleus of newly generated neurons, and Notch was found to regulate neurite extension in neocortical neurons. That work also raised the interesting possibility that Notch signaling in neurons might serve other yet to be identified functions. Now Rakic and colleagues have shown that one such function is as a downstream mediator of the Reelin signaling pathway (Hashimoto-Torii et al., 2008).

Reelin, Neuronal Migration, and Neocortical Lamination: How Does It Work?

Reelin is a matrix-associated secreted glycoprotein that binds to its transmembrane receptors ApoER2 and VLDLR, leading to phosphorylation of Dab1 and *src*-family kinases (i.e., Src and Fyn) and, through poorly understood intracellular signaling mechanisms, regulates neuronal migration (Cooper, 2008). Normally, the first cortical neurons generated migrate out of the germinal zone and form what is called the "preplate" (Kawauchi and Hoshino, 2008). Neurons generated subsequently then migrate into the preplate, causing it to split into two layers: the Cajal-Retzius layer, which expresses Reelin and will remain the most superficial layer of the neocortex; and the subplate, which will remain the deepest layer. Each successive wave of neurons migrates past the subplate, and any other previously generated neurons, but not past the Cajal-Retzius layer. This pattern of migration results in the "inside-out" generation of the neocortical neuronal layers.

In the absence of Reelin signaling, neuronal migration is perturbed and the neocortical lamination pattern is severely disrupted. The preplate does not split, and each successive wave of migratory neurons "piles up" behind (i.e., not superficial to) the previously generated neurons. This results in what amounts to an inversion of the neocortical layers, a characteristic feature of the *reeler* mutant mouse, from which Reelin was identified. Interestingly, the mislocated layers are able to wire in the mutant neocortex in a manner that is remarkably functional considering the extent to which the neurons are aberrantly positioned.

Many questions remain unresolved regarding how Reelin signaling functions to influence neuronal migration (Cooper, 2008). Fundamental clues emerged about a decade ago, when disruption of Dab1 (in the mouse mutants *scrambler* and *yotari*) and disruption of the lipoprotein receptors ApoER2 and VLDLR (also Reelin receptors) were found to have the same phenotype as the *reeler* mutant and to play a role in Reelin signaling. More recently, studies have suggested that the Reelin cascade can interact with a wide variety of intracellular signaling molecules, including Akt, GSK3β, Crk family kinases, Cdk5, and Lis1, among others (Kawauchi and Hoshino, 2008). However, the exact mechanism by which Reelin signaling regulates neuronal migration and laminar position remains ill-defined. Even the exact purpose of Reelin signaling with respect to cell behavior remains unclear. Common models of Reelin function suggest that it might be a chemo-attractant for migrating neurons, or it might play a role in splitting the preplate so that inside-out lamination can begin, or that it provides a "stop signal" instructing neurons to detach from radial glial processes and to undergo differentiation.

Reelin Signaling Is Mediated by Notch during Neuronal Migration

By connecting Reelin function to the Notch cascade, Hashimoto-Torii et al. (2008) have brought together two of the most heavily studied pathways in neocortical development, resulting in a sizable step forward for both. To do so, they first showed that Reelin deficiency leads to reduced Notch signaling in neurons. For example, *reeler* mutants exhibited a dramatic reduction in Notch1 immunoreactivity in the nuclei of neocortical neurons. In addition, they found much lower levels of NICD1 (the activated form of the receptor) both by immunohistochemistry and western blot, and reduced expression of the target genes *Hes1* and *Hes5*. While interesting, these data did not demonstrate that disrupted Notch signaling plays a causal role in generating the *reeler* phenotype. However, the study also showed that deletion of *Notch1* and *Notch2* in neocortical neurons, using Cre-loxP-mediated recombination, resulted in a migration defect surprisingly similar to what is observed in the *reeler* neocortex. This result is consistent with the idea that reduced Notch signaling in the neurons of *reeler* mutants contributes significantly to the observed migration phenotype.

To strengthen the finding that Notch signaling functions downstream of Reelin in the embryonic neocortex, Hashimoto-Torii et al. (2008) examined the effect of forced Notch1 activation (via mosaic NICD1 overexpression) in *reeler* mutants in vivo. Remarkably, they found that NICD1 could largely rescue the migration defect, suggesting that the primary role of Reelin signaling during neuronal migration is to activate Notch. Furthermore, the authors showed that the migration defect caused by overexpression of a dominant-negative form of Dab1 could be suppressed by coexpression of NICD1 or of a constitutively active form of RBP-J. These findings, together with the loss-of-function data described above, suggest that not only is Notch signaling necessary for Reelin function, it is also sufficient to replace Reelin function. In some sense, the apparent simplicity of this finding is difficult to accept at face value. It raises questions regarding the relevance of the many other signaling molecules previously implicated in mediating the Reelin signal. However, neuronal migration is likely regulated by the integration of multiple signals, and it will be interesting to determine how Notch works in concert or in parallel with other molecular cascades during neuronal migration.

In an effort to identify a molecular link between the Reelin cascade and Notch signaling, Hashimoto-Torii et al. (2008) examined the effect of Reelin signaling on the degradation of Notch1. Recent work of Giniger and colleagues in *Drosophila* has shown that Notch can directly bind to Disabled (from which the mouse protein Dab1 derives its name) (Le Gall et al., 2008), lending support to the notion that the Reelin and Notch pathways may physically interact. Hashimoto-Torii et al. (2008) contend that activated Dab1 inhibits the Fbxw7-mediated degradation of NICD1, an interaction that would be expected to strengthen Notch signaling. Such an interaction was supported by molecular manipulations in COS-7 cells, where Dab1 suppressed the ability of Fbxw1 to reduce NICD1 levels. In addition, the authors examined the ubiquitination of Notch1 in slice cultures derived from *reeler* mutant and nonmutant embryos and provided evidence consistent with Notch1 being more highly polyubiquitinated in *reeler* mutants. While the biochemical analysis is not the focus of this study, it does establish a valuable framework upon which subsequent mechanistic studies between the Reelin and Notch cascades can be examined.

Reelin and Notch in Neurons…and in Radial Glia?

The work of Hashimoto-Torii et al. (2008) clearly suggests that Reelin and Notch signaling work together to regulate neuronal migration in the neocortex. The authors assert that they are characterizing interactions between Reelin and Notch in neurons, and based upon their data this is a reasonable assertion. For example, in *reeler* mutants they observe vastly reduced Notch signaling in neurons present in the cortical plate. Their in vivo molecular manipulations, which are quite clear in terms of the phenotypic outcomes, are driven using the Tubulin α1 promoter, which is expressed primarily in neurons. The work is unique not only in that it connects Reelin and Notch signaling but also in that it identifies what appears to be a novel function for Notch in neurons.

All that said, many of the in vivo experiments presented are complicated by the fact that Notch signaling is highly active in radial glial cells, and radial glial processes extend into the Cajal-Retzius layer, where they are adjacent to Reelin-expressing cells. If Reelin and Notch can interact in neurons, there is no reason to think that they can't interact in radial glia. Both Notch and Reelin receptors are expressed in radial glia, and activation of both pathways promotes radial glial

character, including expression of the radial glial marker BLBP (Gaiano et al., 2000; Hartfuss et al., 2003). Furthermore, as radial glia not only give rise to neocortical neurons (Anthony et al., 2004; Noctor et al., 2002), but also serve as their primary migratory scaffold, perturbations in radial glia could result in aberrant neurogenesis and/or neuronal migration. Since the Tubulin α1 promoter element Hashimoto-Torii et al. (2008) used for their molecular manipulations in vivo can drive expression in the germinal zone (Gal et al., 2006), it is difficult for them to rule out entirely that altered Notch and/or Reelin signaling in the progenitor pool might have contributed indirectly to effects on the morphology and migratory properties of the neurons generated. However, as many previous Reelin studies suggest that the cascade is activated in neurons (Cooper, 2008), it is reasonable to believe that most if not all of the Notch interactions observed by Hashimoto-Torii et al. (2008) are occurring in neurons.

Interestingly, further evidence that Reelin interacts with Notch signaling in radial glia has come from very recent work in a human neural progenitor cell line (Keilani and Sugaya, 2008). That study showed that Reelin treatment led to elevated NICD1 levels and enhanced radial glial characteristics. In addition, and consistent with *Drosophila* work mentioned above (Le Gall et al., 2008), Keilani and Sugaya (2008) used coimmunoprecipitation to show that Dab1 can bind to Notch1. Overall, this study supports the idea that Reelin and Notch signaling interact, and that the latter acts downstream of former.

The observation that Reelin-Notch interactions are present in human cells raises interesting possibilities regarding the use of human stem cells to establish in vitro models for the study of human neuronal migration. For example, similar to what has recently be done with induced pluripotent stem (iPS) cells derived from the skin of an ALS patient (Dimos et al., 2008), it should be possible to generate iPS cell-derived neurons from patients with lissencephaly caused by *RELN* mutations. Such cells would greatly facilitate the study of potential interactions between RELN and NOTCH signaling in humans, and this overall approach is likely to contribute a great deal to our understanding of human neurological disorders.

Conclusions

The work of Hashimoto-Torii et al. (2008) represents and exciting new step in the study of neocortical development. The authors have made the somewhat startling observation that Notch plays a key role downstream of Reelin signaling. As with most groundbreaking studies, many interesting questions need to be answered before this finding is fully understood. First, how does Notch signaling interact with other known signaling elements downstream of Reelin, and how exactly does it influence the migratory behavior of neurons? In addition, how does the effect of Notch on neuronal migration relate to its previously described function in regulating dendritic arborization? It will also be important to determine the role of Notch ligands during neuronal migration and to identify the relevant target genes. By investigating the molecular regulation of neuronal migration and neocortical development, we will enhance our understanding of basic biological principles and will gain insight relevant to the treatment of human disease.

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