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Notch signaling and neural connectivity

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Abstract

The cell surface receptor Notch contributes to the development of nearly every tissue in most metazoans by controlling the fates and differentiation of cells. Recent results have now established that Notch also regulates the connectivity of the nervous system, and does so at a variety of levels, including specification of neuronal identity, division, survival and migration, as well as axon guidance, morphogenesis of dendritic arbors and weighting of synapse strength. To these ends, Notch engages at least two signal transduction pathways, one that controls nuclear gene expression and another that directly targets the cytoskeleton. Coordinating the many functions of Notch to produce neural structure is thus a pivotal aspect of building and maintaining the nervous system.

Introduction

It often seems that there is no cell type that can be made in a metazoan without the involvement of the receptor, Notch, and its associated signaling pathway. Notch signaling defines the borders of developmental compartments [1], controls what fraction of cells in a developmental equivalence group will enter a specific lineage [2, 3], discriminates the fates of sibling cells at asymmetric divisions [4] and mediates the switch from proliferative progenitor to differentiated offspring [5]. It oversees the survival of stem cells and their differentiating progeny [6] and is a central piece of the clock that generates somites in vertebrates [7]. For many years, it seemed that the hegemony of Notch finally ended with the terminal differentiation of a cell [8]. Recent discoveries, however, have revealed that Notch also has roles to play in mature cells. In the nervous system, for example, once a definitive neuron has been produced, Notch controls axon growth and guidance, dendritic morphogenesis and synaptic function [9, 10]. In this review, we will discuss some of the many ways in which Notch signaling controls the formation and function of neural circuits.

Consider what is required to establish neural connectivity (Figure 1). Neurons are born and acquire their identities. In general, they should be in a particular place in the animal, and sometimes they must migrate to get there. A neuron sends out an axon with a specific trajectory, while its presumptive synaptic partners establish dendritic arbors that spatially overlap that trajectory. Finally, synapses are formed and their functional strengths adjusted to match the needs of the circuit. All of these steps are subject to regulation by Notch.

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Direct and indirect effects of Notch on axon and dendrite patterning

Most neurons have a defined identity long before axonal and dendritic projections develop, and, in general, the identity determines the projection pattern by controlling the constellation of cell surface and signaling proteins that the neuron expresses [11–13]. Inasmuch as Notch controls cell identity at the time of cell birth, it is not surprising that neural connectivity is regulated by Notch in the indirect sense that transforming cell identity concurrently transforms the projection pattern. There are many examples of Notch determining neural connectivity in this sense. For example, in the adult *Drosophila* olfactory system, nearly every sensillum in the antenna includes a pair of olfactory receptor neurons (ORNs) that are siblings derived from a common precursor, and it is differential Notch signaling that makes their identities different: one is a Notch OFF cell (by virtue of accumulating the Notch antagonist protein, Numb), the other has Notch ON (by virtue of lacking Numb) [14](Figure 2A). Each of the sibling neurons expresses a different olfactory receptor protein, and each projects to a different glomerulus in the antennal lobe. Forced activation of Notch, however, causes both neurons to express the olfactory receptor of the Notch ON fate and project together to the cognate glomerulus. Inactivation of Notch also equalizes the receptor complement and projection pattern, but in the Notch OFF direction.

Control of connectivity by control of identity is a straightforward consequence of the familiar cell fate function of Notch, but some effects of Notch on neural connectivity do not fit into this simple pattern. Experiments in *Drosophila* first suggested that Notch might control axon growth and guidance directly [9]. It was found that embryonic peripheral axons grow along a cellular substratum that bears the Notch ligand, Delta, while the axons and growth cones express Notch [15]. Inactivation of *Notch* or *Delta* at the time of axon growth led to stalling or misrouting of those axons, consistent with Notch acting in the axon to promote axon extension. Another striking example is the dorsal cluster neurons (DCNs) of the adult *Drosophila* brain. Here, reducing Notch activity leads to overgrowth of DCN axons. However, the effects of Notch are executed after differentiation of the DCNs is complete, at the time their axons are growing [16]. Analyses of mammalian neurons in a variety of primary culture and cell line paradigms have provided insights into mechanisms by which Notch may control neurite growth [17–19]. These include stabilization of microtubules[20], turnover of varicosities and filopodia on neurites, and changes in the expression of a variety of cytoskeletal and signaling proteins that modulate neurite growth[21]. Consistent with *in vitro* studies, Notch and its ligands are present on both axons and dendrites of mammalian neurons *in vivo* [22, 23]. Notch activation acutely limits the number and/or length of neurites [24], and in *Drosophila*, absence of Notch cell-autonomously decreases the number of filopodia on identified CNS pioneer growth cones [25].

Just as for axons, dendritic development is profoundly sensitive to Notch activity [26] (Figure 2B). In cultures of dissociated mammalian cortical neurons, artificial activation of Notch reduces the length of dendritic branches and increases branch number, while conversely, inhibition of Notch leads to longer dendrites with fewer branches [27]. This may reflect a Notch-dependent input to dendritic “tiling”, since the same pattern is seen when comparing cortical neurons plated in culture at high density (whereupon they bear short, dense arbors) vs low density (generating larger, sparser arbors) [23]. This led to the idea of a process akin to classic “lateral inhibition”, but acting to limit the overlap of the arbors of adjacent neurons. It is imagined that contact between the arbors of two neurons, each of which expresses both Notch and Notch ligand, mutually inhibits further growth of the dendritic trees. Control of dendritic architecture by Notch has been observed *in vivo* in the mouse dentate gyrus, using conditional knockouts and overexpression methods [28], and in *Drosophila* sensory neurons (M. Shivalkar, N. Sweeney, F-B. Gao and EG, unpublished).

Sculpting a dendritic tree is an elaborate process, requiring integration of many developmental and functional signals, and consistent with this, the suppression of neurite growth by Notch is modulated in a complex way by interaction with signals from other receptors. For example, the p75 neurotrophin receptor also regulates dendritic arborization, in part by cooperating with Notch to control expression of the canonical Notch target Hes5. The interaction between Notch and p75 is complex, however, as their effects on dendrites can be either synergistic or antagonistic, depending on growth conditions [29].

The nuclear and cytoplasmic pathways of Notch signaling: mechanisms and implications

Notch controls the morphogenesis of neuronal projections in differentiated neurons -- but how? Clearly, much of this activity occurs at the level of regulating genes that are important in axo- and dendrogenesis. The conserved molecular mechanism by which Notch directly controls gene expression is well established [30](Figure 3A). When Notch binds its ligand, either Serrate (in vertebrates called Jagged) or Delta, ligand internalization exerts tension on the Notch extracellular domain, allowing ADAM family metalloproteases to cleave Notch close to the plasma membrane. This permits the membrane-bound gamma secretase complex to cleave Notch adjacent to the inner edge of the membrane, releasing the Notch intracellular domain (NICD) to transit to the nucleus, forming a transcriptional regulatory complex with at least two other components, the DNA-binding protein Suppressor of Hairless (Su(H) in *Drosophila*, CBF-1/RBP-J κ in vertebrates and LAG-1 in *C. elegans*; collectively known as the CSL proteins) and the transcriptional co-activator Mastermind. This complex associates with the promoters of genes bearing CSL-binding sites and directly activates their expression. Regulation of mammalian dendritogenesis by the transcriptional action of Notch has been clearly demonstrated [23, 27], as has its regulation of DCN axons in *Drosophila* [16]. In both cases, constitutive activation of Notch nuclear signaling is sufficient to mimic the effect of applying Notch ligand. Moreover, the transcriptionally active intracellular domain of Notch can be detected in the nuclei of differentiated neurons in the mouse cortex [27, 28].

Canonical transcriptional signaling, however, is not the only way that Notch controls neuronal morphogenesis. For over a decade, there have been persistent hints of the existence of one or more “non-canonical” mechanisms of Notch action [15, 31–35]. Much Notch-dependent axon patterning in *Drosophila*, for example, arises from direct cytoplasmic control over the activity of the Abl tyrosine kinase signaling pathway [15, 25, 36, 37]. Abl defines a key nexus in the machinery of axon and dendrite patterning that links multiple guidance receptors to actin structure [38], controlling the relationship between filopodia, lamellipodia and substratum adhesion in the growth cone [25, 39, 40]. Genetic experiments showed that Notch is a cell-autonomous negative regulator of Abl signaling [25, 36] (Figure 3B). Biochemically, Notch binds two core components of the Abl pathway *in vivo*, the adaptor protein Disabled, and Trio, a guanine exchange factor (GEF) for Rho GTPases[37]. Construction of modified Notch derivatives that function selectively in either the Su(H)-pathway, or the “Notch/Abl pathway”, respectively, demonstrated molecularly that while the Su(H) pathway accounted for control of cell fate by Notch, the ability to regulate axon patterning was specifically associated with the Abl pathway [25, 37]. The interaction of Notch with Abl signaling is conserved in mammals. Several labs have shown that mNotch1 binds to mDisabled in developing mouse neurons [41–43], just as dNotch binds dDisabled in *Drosophila*. This binding is essential for Notch-dependent signal transduction from Reelin receptors to control radial migration of cortical neurons. Given that radial migration also requires mEnabled [44], the key, conserved downstream target of Abl, it seems plausible that Abl itself (and/or its paralog, Arg) may lie between mDisabled and mEnabled in this Notch-dependent process, though that remains to be established.

The existence of two Notch signaling pathways impinging on neural connectivity immediately raises the question of whether these act independently or in concert. Evidence is now beginning to accumulate suggesting the latter to be the case. In some instances, this reflects different, but coordinated, forms of Notch action in adjacent cells. In the pioneering of CNS longitudinal axon tracts in *Drosophila* [25](Figure 4), Notch signals through Su(H) in a row of specialized glial cells, inducing them to construct a continuous substratum for longitudinal pioneer axons. Simultaneously, Notch acts autonomously in the growth cones of those longitudinal pioneers to suppress Abl pathway signaling. This drives axon growth along the glial-associated substratum by promoting filopodial extension and modulating substratum adhesion. In this developmental context, a common source of ligand coordinates substratum morphogenesis with pioneer axon growth. Both are induced by contact with a fascicle of Delta-decorated commissural axons that crosses the critical portion of the nascent longitudinal pathway shortly prior to the onset of longitudinal axon extension.

In other developmental contexts, there is growing evidence that both Notch signaling pathways operate within a single cell, allowing Notch to coordinate nuclear gene regulation (*via* the canonical pathway) with cytoplasmic morphogenesis (*via* Abl). For example, while various experiments show the preferential action of cytoplasmic Notch/Abl pathway activity in patterning of CNS longitudinal axons and ISNb motor axons in *Drosophila*, that preference is not absolute. There is also evidence for a contribution from canonical Notch signaling [36, 37, 45]. Coordinate use of canonical and non-canonical Notch signaling is also employed in the cranial neuroepithelium in zebrafish to regulate mitosis and apicobasal polarization of the cells, respectively [35]. Moreover, while canonical Notch signaling can account for some of the effects of Reelin during radial migration of mouse cortical neurons, it does not do so completely[41]. This suggests that another Notch signal, perhaps through the Abl pathway, also contributes to cortical lamination. Finally, Notch regulates dendritic morphogenesis in *Drosophila* sensory neurons, and ongoing experiments suggest that this involves parallel contributions from both Notch signaling mechanisms (M. Shivalkar, F-B. Gao and EG, unpublished).

Notch in adult neural function

Once axons and dendrites are constructed and neural circuits established, their proper function depends on keeping synaptic activity balanced within narrow tolerances. The involvement of Notch at the level of neural function was first suggested by a spate of papers showing that Notch activity is required for memory in *Drosophila* and mice [46–48]. More recently, potential molecular correlates of this role have been uncovered, including induction by Notch of the expression of a cell adhesion molecule that is required for long term memory in flies [49]. In mammals, Notch and its ligands are localized at synapses [22], and elegant experiments reveal Notch to be both an essential contributor to synaptic plasticity and a marker of synaptic function [50, 51]. Gaiano and coworkers demonstrated that the activity-regulated protein ARC/ARG3.1 stimulates Notch signaling in the mouse hippocampus in response to synaptic activity, and that Notch, in turn, is required for induction of both LTP and LTD, and also for proper morphology of dendritic spines [22]. Meanwhile, in *Drosophila* olfactory neurons, Struhl and colleagues showed that the combination of odorant binding and olfactory neuron synaptic signaling led cell-autonomously to Presenilin-mediated cleavage of Notch, and thus, presumably, to Notch-mediated alterations in neuronal gene expression [52]. While the behavioral implications of Notch activation were not investigated, it is noteworthy that the activation of Notch in individual olfactory neurons was modulated in a complex way by inputs from other glomeruli, perhaps offering a mechanism for the known interaction between percepts of different odors. These investigations are just in their infancy, but it seems certain that we have not heard the last from Notch in the arena of neuronal activity and brain function.

Conclusion

Perhaps it was naïve to expect that once early embryonic patterning is established, nature would deploy an entirely separate set of machinery to build tissues out of cells, and yet another to keep the organism alive and functioning in adulthood. We have now learned that many of the ubiquitous signaling pathways that organize the early embryo are reused many times and many ways throughout the life of the organism to perform the molecular calculations to which each is so exquisitely adapted. Studies of Notch have contributed greatly to this realization, but we still have much to learn about the many faces of Notch in the development and function of neural circuits. What is the significance of neurons using two Notch signaling mechanisms, one that targets the nucleus and another targeting the cytoskeleton? How much of the effect of Notch on behavior is a consequence of its developmental roles and how much reflects acute inputs to neuronal activity? To the extent that effects are acute does that reflect influences on synaptic physiology or on the structure of axons and dendrites? Does Notch act primarily as an instructive factor that directs the formation of circuits, or as a homeostatic factor that limits their spread? And perhaps most mysterious of all, how does such a simple signaling mechanism produce such a bewildering array of downstream consequences, including completely different effects in the same cell at different times? These and other questions will be certain to keep the community of Notch researchers busy for some time to come.

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Figure 1. Notch-regulated events in the development of neural connections

A and B represent two neurons with different identities and projection patterns. Green oblongs represent substratum cells for the axon of "B", blue neuron represents a synaptic target of "B", gray bars represent synapses.

Figure 2. Indirect and direct control of neuronal projections by Notch

A) Axon targeting as a feature of cell identity. Most olfactory sensillae in the *Drosophila* antenna include a pair of sibling neurons, one of which has active Notch signaling at birth (orange) and one that does not (cyan). These cells express different olfactory receptors and project to different glomeruli (lightly shaded oblongs) in the antennal lobe of the brain. Inactivating Notch signaling causes both neurons to project to the glomerulus appropriate for the Notch-OFF cell, activation of Notch causes both to project to the target of the Notch-ON cell. Olfactory receptor expression is switched in parallel with the projection pattern. (Unlike in vertebrates, the *Drosophila* olfactory receptor protein is not responsible for axon targeting.) Figure adapted from [14].

B) Direct modulation of dendritic branching. Mammalian cortical neurons growing at low or high cell density are depicted [23]. For clarity, one cell in each panel is shown in dark blue and axons are not shown. All of the cells express both Notch and a Notch ligand, allowing mutual activation of Notch when two cells come into contact. At low density, where there are initially few contacts between adjacent cells, Notch signaling is weak (light green nuclei) and the dendritic tree is sparse, with long branches. At high cell density, multiple contacts between dendrites of adjacent cells induce strong Notch signaling (dark green nuclei), and dendrites are short, and highly branched.

A Canonical Notch signaling mechanism

B “Non-canonical” Notch/Abl signaling pathway

Figure 3. Notch signaling mechanisms

A) Canonical Notch signaling. After binding of Notch to its ligand (Delta or Serrate/Jagged), ligand endocytosis (1) exerts tension on the Notch extracellular domain, allowing sequential cleavage, first by (2) an ADAM metalloprotease (such as Kuzbanian), and then by (3) the γ -secretase complex. This frees the Notch intracellular domain (NICD) to translocate to the nucleus (4), where it forms a transcriptional activator complex, together with a DNA-binding unit (“CSL” protein: CBF1/RBP-J κ ; Suppressor of Hairless; Lag-1) and a coactivator (Mastermind). The complex binds promoters of target genes bearing CSL-binding sites (5) and stimulates their transcription.

B) Notch/Abl cytoplasmic signaling. Notch binds to and suppresses the effects of two cofactors of Abl tyrosine kinase, the adaptor protein, Disabled and the Rac guanine exchange factor (GEF), Trio [36]. Disabled is an upstream regulator of Abl kinase and its target, Enabled [53], Trio is an upstream regulator of Rac GTPase [54]. Enabled and Rac, in turn, control actin structure and dynamics in the growth cone [25].

Figure 4. Two forms of Notch activity in CNS longitudinal axon growth in *Drosophila*

A) Notch plays two parallel, but separate, roles to promote growth of longitudinal axons in the *Drosophila* CNS [25]. Canonical, nuclear Notch signaling in glial cells (green) promotes morphogenesis of the substratum for approaching pioneer axons (dMP2, vMP2; black). “Non-canonical”, cytoplasmic Notch signaling in the growth cones of the pioneer axons drives their extension alongside the glia. Ligand for both Notch functions is provided by the axons of commissural interneurons that bear Delta protein (pink), and that cross the longitudinal pathway. It is likely that there is also some level of canonical Notch signaling occurring in the pioneer neurons (light gray nuclei). CNS midline is indicated to the right; Delta⁺ commissural interneurons are located laterally in the nerve cord (left).