



Cranking It Up a Notch

Anjen Chenn and Christopher A. Walsh

Neuroscience is all about competition: competition for space, competition for support, competition to be heard. The brain's job of making choices—on a macroscale between opposing sensations, or on a microscale between conflicting neural impulses—is achieved through

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constant competition between neurons. In this game (perhaps as in science) success is measured by the number and strength of a neuron's connections, which in turn depend on the size of the neuron's axons and dendrites (collectively called neurites) that it uses to talk to and listen to its nervous neighbors. For inexplicable reasons the high-minded competition between neurons sometimes gets ugly, leaving some neurons in smoldering ruins, with their neurites tangled and twisted. An elegant study by Šestan *et al.* (1) on page 741 of this issue and a related paper by Berezovska *et al.* (2) in *Neuroscience* now identify the Notch receptor as the latest weapon in this tribal warfare. It appears that the Notch signaling pathway (defined originally as the gatekeeper controlling the birth of neurons) perpetually mediates the competition between neurons for new territory. This suggests that the Notch pathway may be intimately involved throughout the life of neurons, not only at their birth, and may even be critically important for the untimely death of neurons that occurs in degenerative brain diseases such as Alzheimer's disease.

Notch was originally identified in *Drosophila* through loss-of-function mutations that produced too much nervous system at the expense of other structures (3). Notch mediates "lateral inhibition" in which undifferentiated cells basically battle it out with their neighbors to decide who will become neural progenitors (4, 5). Each cell tries to inhibit its neighbors by secreting proteins with knife-edge names such as Jagged, Serrate, and Delta, which bind to the Notch receptor and activate a

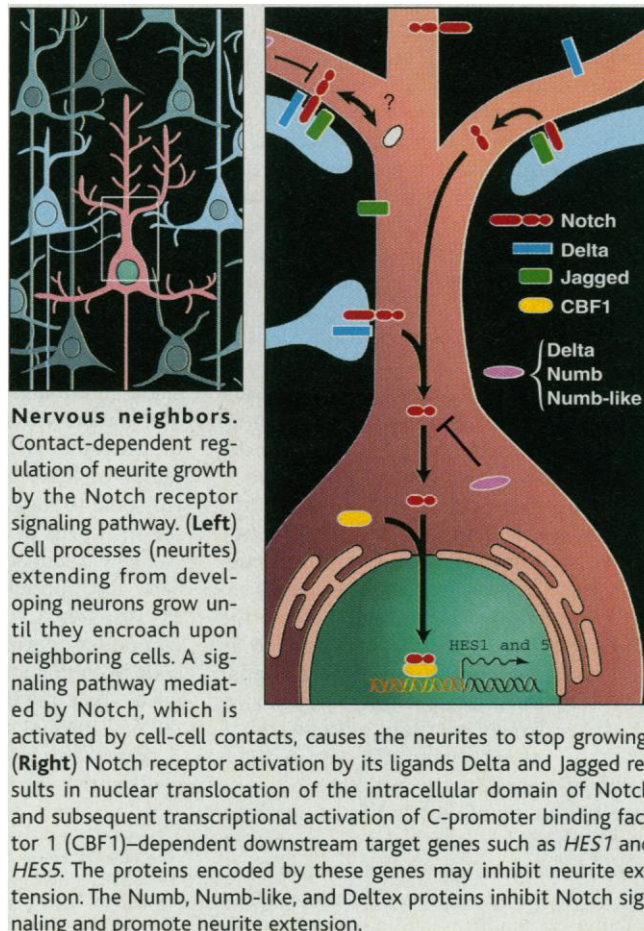
downstream signaling pathway that prevents neighboring cells from becoming neural progenitors (see the figure). Meanwhile, the secreting cell is being bombarded with the same compounds secreted by its neighbors (6), and has to find a way to overcome activation of its own Notch receptor if it wants to become a neuron. Whichever cell is most successful at quashing its rivals and itself resists being inhibited becomes the neural progenitor. An even broader view of Notch function has recently emerged with the proposal that Notch activation serves to prevent cells from responding to signals that would otherwise cause them to become more differentiated (7–9).

The latest twist to Notch's manifold activities relates to decisions of neuronal adolescence or middle age that are made long after the birth of neurons. During development neuronal axons traverse long distances to establish communication with remote targets. The first hint of Notch involvement in later events was a study by Giniger suggesting that Notch activity was important in this axon pathfinding (10, 11). *Drosophila* that harbored temperature-sensitive mutations in Notch demonstrated abnormalities in axon pathfinding in the intersegmental nerve, but the identity of the pathfinding neurons was not altered (10).

Now, the Šestan and Berezovska papers (1, 2) demonstrate that Notch signaling regulates neurite growth in neurons of the developing mammalian cerebral cortex. Their work suggests that Notch's activities in developing neurons are conserved among species and are found in many different brain regions. Šestan *et al.* begin by showing quite beautifully that a wide variety of Notch receptors

and ligands (including Notch1 and 2 as well as their ligands Delta1 and Jagged2) are present in maturing and even in adult neurons of the mouse cerebral cortex. What are they doing there? Data about Notch signaling indicate that proteolytic cleavage and nuclear translocation of the intracellular portion of the Notch receptor mediate transcriptional activation through the C-promoter binding factor-1/Suppressor of Hairless/LAG-1 (CSL) family of transcription factors (9). Šestan *et al.* now present impressive evidence that Notch1 and 2 are found in the nucleus, suggesting that the Notch receptors are highly active in cortical neurons, particularly during the postnatal period of dendritic growth.

To examine the role of Notch signaling in cortical neurons, *in vitro* cultures were investigated under conditions of low- or high-density growth that increases or decreases the number of cell-cell contacts, respectively. It is well established that neurite growth is inhibited by high neuronal density. Šestan and colleagues demonstrated that most neurons in low-density cultures did not exhibit nuclear localization of Notch, whereas high-density neurons did, suggesting that cell-cell contacts activate Notch. The investigators confirmed (using Notch reporter constructs) that cell-



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cell contacts stimulated Notch activation. These findings suggest that contact-dependent Notch activation correlates with the inhibition of neurite outgrowth.

To investigate whether activation of Notch could directly inhibit neurite growth, neurons were transfected with plasmids encoding truncated forms of Notch1 and 2 comprising the entire intracellular domain. These forms of Notch lack the extracellular ligand-binding domain and are constitutively activated. Šestan *et al.* showed that transfection of the Notch intracellular domain stimulated transcription from reporter constructs that are normally activated by Notch, but inhibited the growth of neurites even in low-density cultures. In similar experiments in postmitotic primary mouse hippocampal and cortical neurons, Notch1 transfection caused the regression of preexisting neurites (2). In high-density cultures that already had a high level of Notch activity, the introduction of the Notch intracellular domain caused a retraction of neurites. Šestan and co-workers also showed that the exogenous addition of Notch ligands (Delta or Jagged) mimicked reporter gene activation and the inhibition of neurite growth.

Finally, inhibitors of Notch signaling were used to demonstrate that the effects observed were specific for Notch activation. Numb, Numb-like, and Deltex are thought to modulate Notch signaling and are also expressed in the developing cortex. It was observed that these mediators inhibited neurite extension, promoted by the Notch intracellular domain, and transcriptional activation to different degrees. But, when transfected into high-density cortical neuronal cultures, Numb, Numb-like, and Deltex promoted the extension of neurites, causing the neurons to behave as if they were in a low-density environment.

These findings suggest that Notch activation can alert neurons to the proximity of their neighbors' neurites. If there are lots of neurons around, neurites retract, becoming short, dense, and bushy. On the other hand, if neurons are isolated, they spread their neurites until they find somebody out there to compete with. These capabilities may be important because the whole nervous system is characterized by the careful spacing of neurons of similar type into mosaics, so that neighboring dendrites barely overlap, assuring even coverage by each neuronal type without holes or unnecessary overlap (12). The regular spacing of cell bodies can perhaps be attributed to the activity of Notch genes in controlling neuronal birth (13), but one can speculate that the regular spacing of dendrites and axons might re-

flect the operation of Notch signaling genes later on.

These studies raise many further questions about how and where Notch activation is regulated. Do all neurites (axons or dendrites) require Notch activity *in vivo*, and how is the activation of Notch limited? Does Notch signaling mediate activity-dependent neurite remodeling? Is Notch signaling influenced by synaptic activity? How does Notch interface with the neurotrophins that regulate neurite growth and neuronal survival?

The importance of Notch in maintaining normal adult neuronal function has been previously hinted at by a number of human neurological syndromes with derangements in Notch signaling. The CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy) syndrome is associated with point mutations in Notch3 (14), and individuals with Alagille syndrome (where mild mental retardation is associated with multiple developmental disorders) have Jagged1 mutations (15). Moreover, genes that predispose to early Alzheimer's disease, called presenilins, are required for the normal intracellular

processing and activation of Notch (16–19). The role of the Notch pathway in neurite outgrowth may ultimately lead to a better understanding of the abnormal neurite patterns that characterize Alzheimer's disease.

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PERSPECTIVES: MOLECULAR EVOLUTION

Do Proteins Predate DNA?

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The central dogma of molecular biology—that DNA makes RNA, which in turn makes protein—begs the question of the order in which these three fundamental biopolymers arose. The proposition that RNA came first has achieved wide popularity (1, 2). However, the concept of a primordial RNA world does not identify which molecule came next: Was it DNA (a more stable information storage medium) or protein (a more versatile catalyst)? An appraisal of the diverse and sophisticated catalytic potential of RNA oligomers (ribozymes) has led some to suggest that proteins came last, the final twist to a nucleic acid world (3–6). But new findings corroborate the view of an early RNA/protein environment with DNA evolving last. Establishing the correct chronology defines the context of genetic code evolution and allows predictions to be made about the distribution of RNA within the fundamental machinery of life.

Parsimony favors the notion that life first evolved through a single biological macro-

molecule that both stored genetic information and catalyzed the reactions required for self-replication. Today proteins perform sophisticated catalysis and DNA stores information, whereas RNA can do both. Intuitive early speculations (7) that RNA dominated some primordial biosphere reached mainstream theory (1) through two avenues of research. First, investigators have produced a diverse array of ribozymes that catalyze fundamental metabolic reactions and bind specific ligands. Second, identification of putative “molecular fossils” in extant metabolism (8) has inspired the “palimpsest” model of evolution (3) in which modern protein enzymes are postulated to have incompletely replaced earlier ribozyme equivalents. Indeed, patterns within present-day metabolism support the RNA-first model over any alternative. DNA probably arose as an RNA derivative because all organisms make deoxyribonucleotides by reducing ribonucleotides, and make thymine by methylating uracil (9). Proteins-first models cannot explain the presence of functional RNA in processes such as translation in extant organisms: The 20 “natural” amino acids are more chemically diverse than the four nucleotides, which suggests that proteins have greater

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Science, New Series, Vol. 286, No. 5440. (Oct. 22, 1999), pp. 689-690.

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