

Notch signaling in the mammalian central nervous system: insights from mouse mutants

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The Notch pathway, although originally identified in fruit flies, is now among the most heavily studied in mammalian biology. In mice, loss-of-function and gain-of-function work has demonstrated that Notch signaling is essential both during development and in the adult in a multitude of tissues. Prominent among these is the CNS, where Notch has been implicated in processes ranging from neural stem cell regulation to learning and memory. Here we review the role of Notch in the mammalian CNS by focusing specifically on mutations generated in mice. These mutations have provided critical insight into Notch function in the CNS and have led to the identification of promising new directions that are likely to generate important discoveries in the future.

The role of the Notch pathway during animal neural development is best understood in fruit flies, where Notch has been shown to inhibit differentiation by lateral signaling and regulate cell fate through inductive interactions^{1,2}. Dissection of these processes in flies has relied heavily on loss-of-function genetics, and tremendous progress has been made in understanding both their molecular mechanisms and biological functions. Indeed, to date, the vast majority of known Notch pathway members and molecular interactions were first identified using fly genetics. Similarly, our core understanding of pathway function on a cellular level has emerged almost exclusively from work in flies. In recent years there has been extensive interest in extending our understanding of the Notch pathway from flies to mammals.

The cloning of Notch pathway members in mice has made it possible to use loss-of-function analyses to examine the role of Notch during mammalian neural development. Over the past decade, many laboratories have examined mouse mutants for members of the Notch pathway, including receptors³⁻¹¹, ligands¹²⁻²⁰, modulators²¹⁻³⁴ and effectors³⁵⁻³⁹. In general, these disruptions have resulted in an increase in neuronal differentiation markers and a decrease in progenitor markers, leading to the prevailing view that Notch maintains a progenitor state. While this view of Notch function is consistent with the fly work, it is more limited in scope, suggesting that functions for Notch in the mammalian nervous system remain to be elucidated.

In recent years, our understanding of the Notch signaling cascade and its role in the mammalian CNS have grown more complex. With respect to signaling, for example, although the modulators Numb (encoded by

Numb, also known as *Nmb*) and Numbl (encoded by *Numbl*, also known as *Nbl*) have largely been described as negative regulators of Notch in flies, loss-of-function studies in the mouse have challenged this view^{21,23,24}. In addition, it has become clear that although CBF1 (also called RBP-J or CSL) and the *Hes* genes^{40,41} are critical effectors of the pathway, other previously unappreciated effectors are likely to exist⁴². With respect to pathway function, recent studies have identified previously unknown roles for Notch during glial fate specification^{2,43}, neuronal maturation⁴⁴⁻⁴⁶ and even learning and memory^{33,47-49}. Thus, Notch signaling in the mammalian CNS, already an area of widespread interest, is growing into an even larger topic.

Here, we give a brief overview of the Notch signaling cascade and then review the pathway, with a specific emphasis on mutants generated in the mouse. Loss-of-function analyses are critical for identifying unique and essential functions, and, fortunately, mouse mutants exist for the majority of key Notch pathway components. Although pleiotropy and redundancy have complicated the analyses of these mutants, the versatility of conditional knockouts has created new opportunity for progress. All told, mouse mutants are an invaluable resource for investigating the role of Notch, both in the CNS and in other tissues.

Overview of the Notch pathway

The Notch signaling pathway (Fig. 1) is best characterized as mediating cell-cell signaling between adjacent cells^{1,50}. Both the ligands, members of the Delta and Jagged gene families, and the receptors, of which there are four in mammals, are single-pass transmembrane proteins. Upon ligand binding, the intracellular domain of Notch (NICD) is released from the plasma membrane and translocates into the nucleus, where it converts the CBF1 repressor complex into an activator complex. The NICD/CBF1 activator complex, which includes the co-activator Mastermind⁵¹, among other proteins⁵², upregulates targets such as the *Hes* and *Herp* (*Hes*-related protein) genes^{40,41}, which are basic helix-loop-helix (bHLH) transcriptional regulators that antagonize proneural

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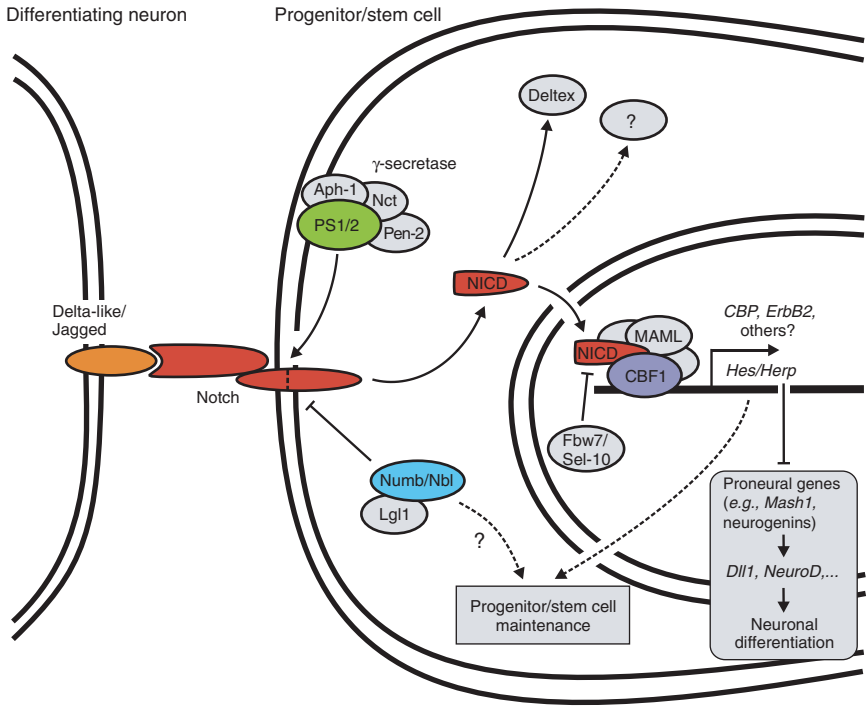


Figure 1 The Notch pathway in mammals. Notch signaling between adjacent cells is a mechanism to generate cellular heterogeneity. Ligand-receptor interactions lead to γ -secretase cleavage of Notch and release of the intracellular domain (NICD). This domain enters the nucleus and together with a complex including CBF1 and Mastermind (MAML) promotes the transcription of targets such as the *Hes* and *Herp* genes. These genes encode bHLH proteins that antagonize proneural genes such as *Mash1* and the neurogenins. This antagonism blocks neuronal gene expression and consequently inhibits differentiation. Notch signaling may also act through other CBF1 targets and through CBF1-independent cascades, which involve Deltex (*Dx*) and pathways yet to be identified. The Numb and Numbl-like (*Nbl*) proteins have generally been considered negative regulators of Notch. However, recent studies of mice with mutations in these genes have suggested that they may be essential for neural progenitor maintenance and may or may not interact with Notch.

genes like *Mash1* (also known as *Ascl1*) and the neurogenins⁵³. This antagonism blocks early neuronal gene expression and is central to the inhibition of neuronal differentiation by Notch.

In addition to the primary components of the linear Delta/Jagged-Notch-CBF1-Hes/Herp signaling cascade, many other genes play a role in modulating Notch signaling. For example, Notch proteins undergo three proteolytic processing events, the last of which is a ligand-dependent intramembraneous cleavage mediated by the presenilin proteins (PS1 and PS2; ref. 50). Other pathway components include the phosphotyrosine binding (PTB) domain-containing proteins Numb and Numbl-like^{54,55}, E3 ubiquitin ligases such as Itch (also known as Suppressor of Deltex (*Dx*), encoded by *Su(dx)*) and Fbw7 (also known as Sel-10; refs. 56,57), and glycosyltransferases of the Fringe family⁵⁸. Although the role of these modulators is only beginning to be understood, it is clear that Notch pathway regulation is far more complex than initially had been appreciated.

Beyond the mechanism of signal transduction, another fundamental challenge is identifying the Notch targets relevant in specific contexts. On the basis of homology to the fly gene Suppressor of Hairless (*Su(H)*), CBF1 was identified as the primary transcriptional effector of Notch in vertebrates^{59,60}. Consistent with such a role, the deletion of *Cbfl* (also known as *Rbpsuh*) has a phenotype similar to the *Notch1* knockout (see below) but more severe, as would be expected for an effector that mediates signaling through all Notch receptors^{35,61}. Similarly, vertebrate CBF1

targets were identified by screening for genes orthologous to *Su(H)* targets of the Enhancer of split (*E(spl)*) complex. Such efforts led to isolation of the *Hes* genes (related to *hairy* and *E(spl)*). Although the identification of *Cbfl* and the *Hes* genes has been critical to mammalian Notch studies, there has been a tendency in the field to focus almost exclusively on these genes when trying to understand pathway output. It is becoming increasingly clear, however, that Notch signaling is mediated by CBF1 targets other than the *Hes* and *Herp* genes^{40,41}, and that some aspects of Notch signaling are likely to be CBF1-independent⁴².

Lessons from the mutants

A conservative estimate of the number of genes in the Notch pathway suggests that there are more than two dozen in mammals, many of which have been mutated in mice (Table 1). Rather than attempting to review them all, we have chosen to focus largely on mutations generated in the core pathway components. The CNS phenotypes of these mutations have been the most carefully studied to date, and they remain an ongoing resource for understanding fundamental aspects of Notch function.

Notch receptor mutations

One of the first Notch pathway genes to be disrupted by homologous recombination was Notch1, which was mutated by two groups independently^{3,4}. Both studies found that mutant embryos died during early embryogenesis (around embryonic day 11 (E11)), although little detail was initially provided regarding neural phenotypes. Subsequently,

a detailed analysis of neural development in *Notch1*^{-/-} mutants was reported⁶¹. That study examined the expression both of pathway components such as *Hes1*, *Hes5* and Delta-like 1 (*Dll1*) and of early differentiation markers such as *Math4A* (also known as *Neurog2*), *NeuroD* (also known as *Neurod2*) and *NSCL-1* (also known as *Nhlh1*). Consistent with the view that Notch activity is needed for progenitor maintenance, the differentiation markers were found to be upregulated in mutants. An interesting though often overlooked finding was that although *Hes5* expression was found by northern blot analysis to be reduced in *Notch1*^{-/-} mutants, *Hes1* expression did not seem to be affected. This latter result is puzzling in light of the *Hes1*^{-/-} mutant phenotype and the extensive literature supporting the notion that *Hes1* is a primary Notch/CBF1 target^{38,40,62,63}. Similar results were obtained with *CBF1*^{-/-} mutants⁶¹, suggesting that while *Hes1* may well be a bona fide Notch/CBF1 target, it is also likely to be regulated by other signaling cascades. This notion is supported by previous findings that *Hes1* can be upregulated in PC12 cells cultured in the growth factors NGF, FGF2 or EGF⁶⁴ and in postnatal cerebellar granule cells cultured in Sonic hedgehog⁶⁵.

After the original *Notch1* deletion studies, alleles of *Notch2* (refs. 5,6), *Notch3* (ref. 7) and *Notch4* (ref. 8) were also generated, as were floxed alleles of *Notch1* (refs. 11,66). Although *Notch3* and *Notch4* do not seem to have significant phenotypes when deleted, *Notch2*^{-/-} mutants, similar to *Notch1*^{-/-} mutants, die around E11 (ref. 5). However, in contrast to

Table 1 Brief description of CNS phenotypes of Notch pathway mutants listed together with references.

Gene(s)	Ref.	CNS phenotype(s)
<i>Notch1</i>	61	Precocious neuronal differentiation. <i>Hes5</i> ↓, <i>Mash1</i> ↑, <i>NeuroD</i> ↑.
	66	Precocious neuronal differentiation. <i>Hes5</i> ↓, <i>Mash1</i> ↑, <i>Dll1</i> ↑.
	10	Neurosphere frequency ↓ at E10.5.
	49	Spatial learning and memory deficits in heterozygotes.
	11	Neural progenitor deletion: Precocious neuronal differentiation.
<i>Notch2</i>	9	Telencephalic deletion: neurosphere frequency ↓ at E12.5.
	5	Widespread neural cell death at E10.
<i>PS1</i>	25	Impaired neurogenesis at E14.5, 'massive neuronal loss' at E16.5.
	27	Loss of Cajal-Retzius cells and cortical dysplasia.
	26	Precocious neuronal differentiation. <i>Hes5</i> ↓, <i>Dll1</i> ↑.
	47	Knockout in postnatal cortex. Spatial memory deficits. No change in <i>Hes1/5</i> , <i>Dll1</i> .
<i>PS1, PS2</i>	10	Neurosphere frequency ↓ at E14.5.
	79	Neural tube disorganization. <i>Hes5</i> 'undetectable,' <i>Dll1</i> ↑.
	10	No neurospheres formed at E14.5.
<i>Adam10</i>	33	Memory and synaptic plasticity deficits. Neurodegeneration. <i>CBP</i> ↓
	28	<i>Hes5</i> ↓ in neural tube, <i>Dll1</i> ↑.
<i>Dll1</i>	19	<i>Hes5</i> ↓, preferential production of early born neurons in telencephalon.
	20	↑ Neurons, ↓ glial cells in differentiated neurosphere cultures
<i>Cbf1</i>	61	Precocious neuronal differentiation. <i>Hes5</i> ↓, <i>Dll1</i> ↑, <i>Mash1</i> ↑, <i>NeuroD</i> ↑, etc.
	10	No neurospheres formed at E8.5.
<i>Hes1</i>	49	Spatial learning and memory deficits in heterozygotes.
	38	Precocious neuronal differentiation. <i>Hes5</i> ↑.
<i>Hes5</i>	67	30-40% reduction in Müller glial cells in retina.
	36	Constitutively active Notch1 unable to inhibit neuronal differentiation.
<i>Hes1, Hes5</i>	37	Neurosphere frequency ↓ at E11.5.
	68	Severe precocious neuronal differentiation.
<i>Numb</i>	21	Precocious neuronal differentiation. MAP2↑, neurofilament↑.
	22	↓ Neuronal differentiation in hindbrain and spinal cord. No change in <i>Hes1</i> , <i>Hes5</i> .
	31	Deficits in granule cell maturation. Reduced Purkinje cell number.
<i>Numb, Numbl</i>	23	Precocious neuronal differentiation along neuraxis.
	30	Cortical disorganization and hyperproliferation. <i>Hes1</i> ↑, <i>Hes5</i> ↑
	24	Precocious neuronal differentiation. <i>Hes5</i> ↓, <i>Mushashi</i> ↓
<i>Lgl1</i>	32	Defects in axonal arborization. Reduced length and branch points.
	73	Cortical disorganization and hyperproliferation. Nestin↑, βIII-tubulin↓
<i>Herp1, Herp2</i>	70	Reduced neural tube thickness.
<i>Fbw7</i>	75	Impaired neural tube closure.

Studies describing non-neural aspects of pathway mutants are not listed.

Notch1^{-/-} mutants, *Notch2*^{-/-} mutants do not display defects in somitogenesis, and they do not show alterations in *Hes5* expression in the CNS. *Notch2*^{-/-} mutants do undergo widespread cell death in the CNS starting around E9, but it is unclear whether this phenotype reflects a role for *Notch2* in the developing CNS or if it is the indirect consequence of other embryonic perturbations.

To circumvent the early lethality of *Notch1* deletion, several studies have addressed the effect of deleting this receptor in specific brain structures. In one case, Cre-loxP-mediated recombination was used to delete *Notch1*

from the medial cerebellar anlage⁶⁶. Consistent with the traditional model of Notch function in the nervous system, the authors found that *Notch1* deletion resulted in upregulation of proneural genes (such as *Mash1* and *Math1*) and precocious neuronal differentiation. More recently, conditional deletion of *Notch1* in the neural progenitor pool (using a floxed allele and the nestin-Cre driver) was also found to result in precocious neuronal differentiation¹¹. Interestingly, deletion of *Notch1* in the telencephalon (using the foxg1-Cre driver) led to reduced neuronal numbers *in vivo* later in development, most likely resulting from precocious neuronal differentiation and earlier progenitor pool depletion⁹. In support of this contention, the telencephalic deletion of *Notch1* led to a reduction in progenitor frequency (assayed as neurospheres) *in vitro*⁹. This result is consistent with reduced neurosphere frequencies observed after widespread deletion of *Notch1*, *Cbf1*, *PS1* and *PS2* (ref. 10), or *Hes1* and *Hes5* (ref. 37). In summary, the conditional deletions of *Notch1* support the canonical view that Notch signaling inhibits neuronal differentiation and maintains the neural progenitor pool.

Ligand mutations

In addition to the receptor mutations, many Notch ligand mutations have been examined in mice. These have mostly been described with respect to their functions during somitogenesis, limb patterning and vascular development¹²⁻²⁰. However, a few studies have examined the effects of deleting Delta-like 1 (*Dll1*) on neural development^{19,20}. One such study found that *Dll1*^{-/-} mutant embryos had decreased *Hes5* expression, consistent with the expected reduction in Notch activation¹⁹. In addition, the study found that *Dll1*^{-/-} mutants showed a decrease in the radial progenitor marker RC2 and an increase in neuronal markers such as βIII-tubulin and GABA. These findings support the traditional view that Notch signaling inhibits neuronal differentiation in the developing CNS. Interestingly, however, on the basis of comparisons of the *Dll1*^{-/-}, *Mash1*^{-/-} and other mutants, the authors suggest that Notch signaling might also regulate the diversification of the progenitor pool into distinct progenitor subtypes. This function would precede the role of Notch in inhibiting

the differentiation of mature cell types (that is, neurons and oligodendrocytes) and could convert a homogeneous proliferative pool into a heterogeneous mixture of stem cells, neuroblasts and glioblasts.

Further evidence that Notch signaling may generate progenitor diversity was obtained by *in vitro* analysis of *Dll1*^{-/-} mutants²⁰. This work suggested that Notch signaling first specifies glial progenitors and then functions in those cells to promote astrocyte versus oligodendrocyte fate. Both this study and the work described above indicate that in mice, Notch influences multiple choice points in the neural progenitor lineage.

While this idea is consistent with what is known about Notch function during fruit fly neural development, it has not received much attention in mammalian studies. However, on the basis of the spatial and temporal concurrence of Notch signaling and progenitor pool diversification in the developing mammalian CNS, it would be surprising if Notch did not influence this process.

Effector mutations

As discussed above, the Notch signaling cascade is primarily transduced through the transcriptional regulator CBF1, when nuclear translocation of NICD converts CBF1 from a repressor to an activator. Consistent with the *Notch1*^{-/-} mutant phenotype, *Cbfl*^{-/-} mutants show altered gene expression suggestive of widespread precocious neuronal differentiation (such as decreased *Hes5* and increased *Dll1* and *NeuroD*)⁶¹. The interpretation of this phenotype is confounded by the fact that these mutants show severe growth retardation by E8.5, a time before neural tube closure. In light of this limitation, and because *Cbfl* appears to be a non-redundant bottleneck in the Notch cascade, conditional deletions of *Cbfl* in the CNS are likely to be highly informative. Of course, *Cbfl* deletion will not uncover purported *Cbfl*-independent Notch signaling, an aspect of the pathway that will remain difficult to address until the relevant molecular mechanisms are more clearly elucidated.

The most widely accepted Notch/CBF1 targets are the *Hes* and recently identified *Herp* gene families^{40,41}. Although there are seven *Hes* genes, not all are clear Notch targets, and studies in the mammalian CNS have focused on *Hes1* and *Hes5*. *Hes1*^{-/-} mutant embryos show severe defects in neural development, including lack of cranial neural tube closure and eventual anencephaly³⁸. However, because these animals die perinatally, it is possible to examine alterations in gene expression much later into development than with *Notch1*^{-/-} or *Cbfl*^{-/-} mutants. Consistent with the canonical model, precocious neurogenesis in *Hes1*^{-/-} mutants was suggested by early expression of markers like *Mash1*, *Nscl* and neurofilament.

Based upon both loss-of-function and gain-of-function studies, it seems likely that to some extent *Hes1* and *Hes5* serve redundant functions in the neural progenitor pool. First, *Hes1*^{-/-} *Hes5*^{-/-} double mutants show a far more severe phenotype than *Hes1*^{-/-} and *Hes5*^{-/-} single mutant phenotypes combined³⁶. Second, elevated *Hes5* expression was detected in *Hes1*^{-/-} mutants, suggesting the existence of compensatory mechanisms between these Notch targets³⁸. Third, although some precocious neuronal differentiation was evident in *Hes5*^{-/-} mutants, these animals were largely normal, suggesting that *Hes1* is capable of almost completely compensating for lack of *Hes5* function. Finally, although a constitutively active form of Notch1 could inhibit neuronal differentiation in either *Hes1*^{-/-} or *Hes5*^{-/-} mutant cells, it could not do so in *Hes1*^{-/-} *Hes5*^{-/-} double mutants³⁶. As an aside, it is interesting to note that *Hes5*^{-/-} mutants exhibit a 30–40% decrease in Müller glial cell number⁶⁷. This loss-of-function data directly supports a role for Notch signaling in promoting glial fate².

The inability of activated Notch1 to inhibit neuronal differentiation in *Hes1*^{-/-} *Hes5*^{-/-} double mutants raises the question of whether *Hes1* and *Hes5* are the only relevant Notch/CBF1 targets in the CNS. This seems unlikely for several reasons. First, recent work has shown that *Hes1*^{-/-} *Hes3*^{-/-} *Hes5*^{-/-} triple mutants show even more precocious neuronal differentiation than *Hes1*^{-/-} *Hes5*^{-/-} double mutants⁶⁸. Second, *Herp1* and *Herp2* (also called *Hey2* and *Hey1*, respectively), which can form heterodimers with the *Hes* proteins⁴¹, are expressed in the embryonic neural progenitor pool and could mediate an essential function for Notch signal transduction in that region⁶⁹. *Herp1*^{-/-} *Herp2*^{-/-} double mutants have recently been described, and although the analysis focused on vascular defects, the authors noted that the neural tube was

substantially thinner in mutants⁷⁰. Third, several reports have identified *ErbB2* as a Notch target that has a role during mammalian neural progenitor maintenance^{71,72}. This finding is intriguing and suggests that other yet-to-be-identified non-canonical targets may exist. Finally, although it is clear that *Hes1* and *Hes5* are required effectors of Notch during neural cell-fate specification, it is unclear to what extent these genes and/or other targets are needed for later roles of Notch in the CNS. For example, recent data suggests that CREB binding protein (*CBP*, also known as *Crebbp*) may be an important Notch/CBF1 target in the postnatal brain (see below)³³.

Signaling modulators and the Numb conundrum

The Notch/CBF1 signaling cascade involves a large number of proteins that transduce and/or modulate the signal from the cell surface to the nucleus (Fig. 1). These include PS1 and PS2 (ref. 50), nicastrin⁵⁰, Numb and Numbl⁵⁵, Lgl1 (ref. 73), Dx⁷⁴, Itch⁵⁷, Fbw7 (ref. 75) and Mastermind⁵¹, among many others (Fig. 1). As mentioned above, PS1 and PS2 mediate ligand-dependent Notch receptor processing as part of the γ -secretase complex, which also includes nicastrin, Aph1, and Pen-2 (ref. 76). Numb and Numbl are PTB domain-containing proteins generally thought to negatively regulate Notch signaling, possibly by promoting receptor turnover. Numb is of particular interest because its asymmetric localization in dividing neural progenitors, which is dependent upon Lgl1, may have a causal role during cell-fate specification in the developing CNS⁷⁷. Itch and Fbw7 are among a growing family of E3 ubiquitin ligases involved in trafficking and/or turnover of Notch pathway components^{56,57}. Interestingly, although both Itch and Fbw7 promote Notch receptor turnover, the former acts at the plasma membrane and the latter acts in the nucleus. Dx also seems to encode an E3 ligase and influence Notch protein localization⁷⁸. It is worth noting that although the mechanism of Dx action remains unclear, this pathway component stands out as a potential mediator of CBF1-independent Notch signaling^{42,78}.

While there are loss-of-function alleles in mice for many of the genes listed above, *PS1*, *PS2*, *Numb* and *Numbl* have had the most extensive CNS phenotypes reported. The *PS1*^{-/-} *PS2*^{-/-} mutant phenotype during neural development both *in vivo*^{26,79} and *in vitro*¹⁰ is similar to that found during disruption of other positive regulators of Notch and supports a role for the pathway in neural progenitor maintenance. In contrast, the *Numb*^{-/-} *Numbl*^{-/-} mutant phenotypes remain the most puzzling aspect of the Notch loss-of-function literature in the mouse CNS, and we will focus on these phenotypes below.

Genetic analysis in flies has clearly demonstrated that Numb can antagonize Notch signaling⁵⁴. However, although some studies have suggested that mammalian Numb and Numbl can antagonize Notch^{45,80,81}, the mouse mutant analyses do not all support such a function. Two different loss-of-function alleles of *Numb* were generated by independent groups^{21,22}. In one case, the first coding exon was deleted²², whereas in the other, exons 5 and 6 were deleted to disrupt the PTB domain, which is essential for function in flies²¹. Unfortunately, both alleles might produce truncated protein products (the exon 1 deletion may initiate translation further downstream, whereas the exon 5,6 deletion can be spliced over to generate a mutant protein lacking exactly 72 residues).

The initial characterization of each of the *Numb* alleles reached different conclusions. In one case (exon 1 deletion), the authors concluded that *Numb* disruption resulted in impaired neuronal differentiation in certain regions of the CNS (such as hindbrain, spinal cord, dorsal root ganglia), but not in others (for example, the forebrain)²². Aspects of their data are consistent with the general view that Numb antagonizes Notch. The authors highlighted this fact, but they also discussed

the possibility that Numb may act in a Notch-independent manner, as they found no alterations in *Hes1* or *Hes5* expression.

In contrast, a second *Numb* deletion study (exon 5,6 deletion) found precocious neuronal differentiation in the forebrain, as evidenced by expression of MAP2 and neurofilament²¹. The authors went on to suggest that these data, together with the apical localization of Numb protein in neocortical progenitors⁸², indicated that Numb was needed to maintain a progenitor state. This result was particularly intriguing because it suggested that if Numb had a role in the Notch signaling cascade, that role was as a positive rather than a negative regulator. It remains unclear how to reconcile these two *Numb* studies until a more detailed characterization of the different alleles is obtained. As discussed below, more recent work has suggested that understanding Numb function in the developing CNS poses an ongoing challenge.

To address the possible redundancy between *Numb* and *Numbl*, several recent studies have examined the effect of Cre-*loxP* mediated deletion of *Numb* in a *Numbl*^{-/-} mutant background^{23,24,30} (*Numbl*^{-/-} mutants are apparently normal²⁴). These studies used three different Cre drivers to delete *Numb*: nestin-Cre²³, Emx1-Cre³⁰ and D6-Cre²⁴, which are reported to delete *Numb* starting at E8.5, E9.5 and E10.5, respectively. Remarkably, although these studies used the same *Numb* and *Numbl* alleles, they reached dramatically different conclusions. One group, using either nestin-Cre or D6-Cre to delete *Numb*^{-/-} in *Numbl*^{-/-} mutants, found that the laminar organization of the neocortex was retained, but that there was widespread precocious neuronal differentiation^{23,24}. The other group used Emx1-Cre and instead found that the laminar neocortical organization was replaced by rosette-like structures, and that the progenitor pool was expanded at the expense of neuronal differentiation³⁰. This study also found widespread upregulation of the Notch targets *Hes1* and *Hes5*.

One possible explanation of the differences obtained using different Cre drivers is that depending upon the timing of the deletion, different phenotypes might be obtained. However, this explanation is unlikely because deletion at E8.5 and E10.5 had essentially the same phenotype, whereas deletion at E9.5 seemed opposite. Furthermore, Cre-mediated deletions are not instantaneous, and there is likely to be substantial overlap between the timing of all three deletions.

An alternative, more plausible explanation is that something specific to the individual Cre drivers is influencing the experimental outcome. Indeed, one group speculated²⁴ that if the Emx1-Cre deletion were inefficient, that study might be characterizing the effects of mosaic and/or hypomorphic *Numb* function. It is not clear why that would result in the observed phenotype, although it may relate to secondary effects caused by severe tissue disorganization. It is worth noting that a mutation in *Lgl1*, a gene essential for Numb subcellular localization, has a phenotype remarkably similar to the Emx1-Cre mediated *Numb* deletion, including hyperproliferation, tissue disorganization and rosetting⁷³. Because Numb protein is not properly localized in *Lgl1*^{-/-} mutants, the *Lgl1*^{-/-} mutant phenotype might mimic a *Numb* hypomorphic phenotype.

On the basis of the mutant analyses, it seems clear that the traditional view of Numb and Notch interaction is oversimplified in the mammalian

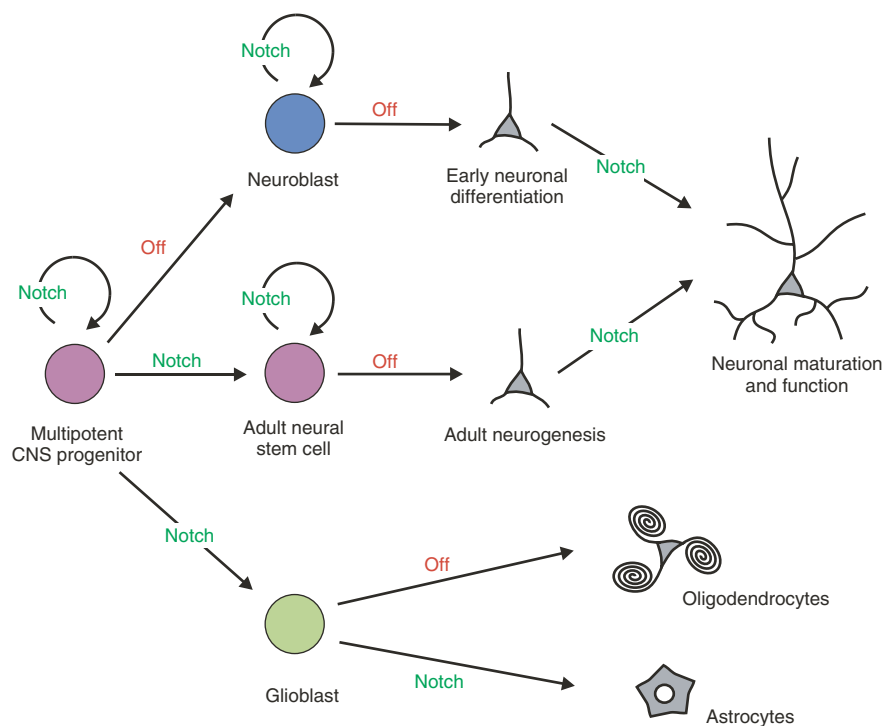


Figure 2 Analyses of mouse mutants have supported many roles for Notch signaling in the developing and adult CNS. Processes that are likely to involve pathway signaling are labeled 'Notch' (green), and those that are likely to require downregulation of Notch signaling are labeled 'Off' (red). Specific details are provided in the text.

CNS. Many issues remain to be addressed, including the function of the four different *Numb* isoforms⁸³ and *in vitro* studies suggesting that Numb or Numlike can indeed antagonize Notch^{45,80,81}. *In vivo* it remains possible that Numb does not interact with Notch to the extent previously assumed but instead functions in parallel toward the same end. It is also possible that the long-standing view of Notch function in maintaining the progenitor pool is what has been oversimplified and is in need of revision. For example, as described above, before influencing the decision to differentiate into neurons or glia, Notch signaling may promote the generation of distinct progenitor subtypes. It is certainly possible that Numb and/or Numlike inhibit Notch during this process, thereby influencing the balance of progenitor subtypes. In any event, ongoing studies of Numb and Numlike will likely precipitate substantial changes in our view of neural progenitor regulation in the mammalian CNS.

Notch beyond cell-fate specification

Literature is lacking on the effects of Notch loss-of-function on post-mitotic neuronal development and function. Until recently, there was little evidence that Notch signaling played any role in the CNS beyond cell-fate specification. Then several years ago, numerous groups showed that Notch could influence neurite development *in vitro*⁴⁴⁻⁴⁶. These studies found that Notch activation reduced neurite extension, but presumed signaling blockade (via expression of *Numb*, *Numbl* or *Dx*) could promote neurite extension. Subsequent studies have found that *Numb* deletion disrupts neuronal maturation in the developing cerebellum³¹, whereas deletion of *Numb* and *Numbl* disrupts axonal arborization in sensory ganglia *in vivo*³². The mechanisms behind these phenomena are unclear, although the latter study indicated that, similar to what has been shown recently in fruit flies⁸⁴, Numb regulates the endocytic trafficking of Notch receptors to promote their degradation. Consistent

with a function in axonal development, Notch is present in the growth cones of extending axons in fruit flies and has been shown to interact with the axonal tyrosine kinase Abl⁸⁵.

Beyond the developmental work described above, several recent studies have sought to address the role of Notch signaling in the adult brain *in vivo*. A gene expression study found that Notch pathway components are expressed in the early postnatal and adult mouse brain, both in germinal zones and in neurons⁸⁶. Subsequently, several groups have used loss-of-function approaches to examine the role of Notch in the adult brain. One such study found that mice heterozygous for mutations in either *Notch1* or *Cbfl* showed deficits in spatial learning and memory⁴⁹. While this finding was intriguing, the authors could not rule out the possibility that subtle developmental defects contributed to the observed phenotype. Furthermore, even if there were no such defects, the study did not determine whether the deficits resulted from loss of Notch1/CBF1 signaling in neurons or in postnatal germinal zones such as the subgranular zone (SGZ) of the dentate gyrus. Consistent with a role for Notch in learning and memory, a transgenic antisense strategy which 'knocked down' *Notch1* levels ~50% found deficits in hippocampal long-term potentiation (LTP)⁴⁸.

An alternative approach that ostensibly addressed the role of Notch signaling in the adult brain was the postnatal deletion of presenilins 1 and 2 (*PS1^{-/-} PS2^{-/-}*)³³. Because proteolytic processing of the Notch receptors by PS1 and PS2 is essential for Notch signaling, deletion of *PS1* and *PS2* is an effective means to disrupt the pathway. In a *PS2* mutant background, the authors used the α CaMKII-Cre driver to delete *PS1* in excitatory neurons beginning 3 weeks after birth. Remarkably, *PS1^{-/-} PS2^{-/-}* mutant animals showed learning and memory deficits and neuronal dysfunction and underwent gradual neurodegeneration. A strength of this work is that the deletion occurred after development was essentially complete. However, the presenilin proteins have numerous substrates^{50,87}, and the Notch pathway itself was not definitively addressed. That said, the authors did identify an optimal CBF1 binding site in the promoter region of *CBP*, a gene known to function during learning and memory. Since *CBP* expression was reduced in *PS1^{-/-} PS2^{-/-}* mutants, this binding site is consistent with a role for Notch in the observed phenotype. This study and those outlined above strongly suggest that Notch functions in the mammalian CNS beyond its role during cell-fate specification (Fig. 2).

Conclusions

Although much remains to be learned about Notch function in the mammalian CNS, mouse mutations have been an essential resource. With respect to the vertebrate nervous system, the Notch field has been dominated by studies of cell-fate specification, where the general view is that Notch maintains the neural progenitor state and inhibits differentiation. Disruption of positive regulators of the pathway has been grossly consistent with this view. However, more detailed analyses have also revealed that Notch is likely to regulate progenitor pool diversification and neuronal maturation. In addition, emerging data suggests that Notch signaling has a role in neuronal function in the adult brain. It will be of great interest to determine which components of the Notch signaling cascade, known or unknown, function in each of these processes. In addition, understanding the perplexing relationship between Notch and Numb function in the mammalian CNS remains a pressing question.

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COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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Erratum: Heterogeneity in synaptic transmission along a *Drosophila* larval motor axon

Giovanna Guerrero, Dierk F Reiff, Gautam Agarwal, Robin W Ball, Alexander Borst, Corey S Goodman & Ehud Y Isacoff
Nat. Neurosci., 8, 1188–1196 (2005)

In the version of this article initially published online, the second author's name was misspelled. The correct spelling is Dierk F Reiff.

Erratum: Notch signaling in the mammalian central nervous system: insights from mouse mutants

Keejung Yoon & Nicholas Gaiano
Nat. Neurosci., 8, 709 – 715 (2005)

The version of this article that was published contained typographical errors in some gene names. On page 710, in the right column, third paragraph, the fourth sentence should have read as follows: "That study examined the expression both of pathway components such as Hes1, Hes5 and Delta-like 1 (Dll1) and of early differentiation markers such as Math4A (also known as Neurog2), NeuroD and NSCL-1 (also known as Nhlh1)." The last sentence of that paragraph should have read as follows: "This notion is supported by previous findings that Hes1 can be upregulated in PC12 cells cultured in the growth factors NGF, FGF2 or EGF64 and in postnatal cerebellar granule cells cultured in Sonic hedgehog⁶⁵." The fourth and fifth sentences in the second paragraph, right column, on page 713 should have read as follows: "These studies found that Notch activation reduced neurite extension, but presumed signaling blockade (via expression of Numb, Numbl or Dx) could promote neurite extension. Subsequent studies have found that Numb deletion disrupts neuronal maturation in the developing cerebellum³¹, whereas deletion of Numb and Numbl disrupts axonal arborization in sensory ganglia *in vivo*³²." In addition, on page 712, in the right column, top line, the authors would like to revise the sentence to read as follows: "Third, several reports have identified ErbB2 as a Notch target that has a role during mammalian radial glial maintenance^{91,92}."

CORRIGENDUM

Corrigendum: Visual field maps and stimulus selectivity in human ventral occipital cortex

Alyssa A Brewer, Junjie Liu, Alex R Wade & Brian A Wandell
Nat. Neurosci., 8, 1102–1109 (2005)

The discussion section contains an incorrect citation. In the 3rd paragraph on page 1107, "Tootell *et al.*¹⁶ (subsequent to Halgren *et al.*)" should read: "Tootell *et al.*¹⁶ (subsequent to Hadjikhani *et al.*)". The authors regret the error