Notch signaling: control of cell communication and cell fate

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Summary

Notch is a transmembrane receptor that mediates local cell-cell communication and coordinates a signaling cascade present in all animal species studied to date. Notch signaling is used widely to determine cell fates and to regulate pattern formation; its dysfunction results in a tremendous variety of developmental defects and adult pathologies. This primer describes the mechanism of Notch signal transduction and how it is used to control the formation of biological patterns.

Introduction

The ability to form biological patterns is key to the orderly and reproducible development of all multicellular life. Pattern formation is made possible by molecular mechanisms of cell-cell signaling, which permit cells to influence each other's fate and behavior. One of the most important mechanisms of cell signaling is mediated by Notch, a transmembrane receptor that coordinates a signaling system known as the Notch pathway. *Notch* was identified genetically almost 100 years ago by a mutant fly with 'notches' in its wings (Morgan, 1917), which indicated its requirement in wing outgrowth. Notch has since been found to be crucial for patterning in a great number of other developmental settings throughout the animal kingdom, from worms to humans.

This primer describes the key molecular features of Notch signaling and some representative biological processes that it controls by first introducing the core players and mechanism of Notch signal transduction; Notch is an unusual protein in that it functions both at the cell surface to receive extracellular signals and in the nucleus to regulate gene expression. In addition, some of the many biological settings where Notch signaling regulates cell fates and pattern formation are discussed, together with molecular strategies that influence Notch signaling in specific developmental locations. Finally, how the knowledge of Notch function and biology from model organism studies has lent insight into human diseases caused by aberrant Notch signaling is also considered.

Notch signaling: key players and mechanism

Core components of Notch signaling

Key components of Notch signaling were originally recognized genetically through mutant animals whose phenotypes resembled those of *Notch* mutants. In flies, *Notch* was the founding member of a collection of 'neurogenic' mutants (see Box 1), so named because they produce a remarkable excess of neurons at the expense of epidermis (Poulson, 1945; Lehmann et al., 1983). Nematodes have two homologs of Notch (LIN-12 and GLP-1), which were identified by mutations that affect cell lineages and germ-line proliferation. The *lin-12*/*glp-1* double mutant displays an aggregate phenotype that constitutes the full loss of Notch activity in the worm; this phenotype is characteristic of a small class of 'LIN and GLP' or 'LAG' mutants in worms (Lambie and Kimble, 1991).

These mutants laid the foundation for genetic, molecular and biochemical studies that established the core Notch signaling apparatus. At its heart lies a Delta-type ligand, a Notch-type receptor and a transcription factor of the CBF1/Su(H)/LAG1 (CSL) family (Fig. 1). All metazoan organisms studied to date contain one or more orthologs of each of these proteins, and these are summarized in Table 1. Delta- and Notch-related proteins are all single-pass transmembrane proteins that contain extracellular arrays of epidermal growth factor (EGF) repeats; specific EGF repeats mediate direct contact between ligand and receptor (Rebay et al., 1991). CSL proteins are sequence-specific DNA-binding proteins (Henkel et al., 1994) that function downstream of Notch. Because almost all locations of Notch signaling involve this ligand-receptor-transcription factor trio, they are generally considered as the 'core' components of Notch signaling.

Box 1. Neurogenic genes

The field of Notch signaling originated with the study of 'neurogenic' fly embryos, which exhibit excessive neuronal differentiation. The term 'neurogenic' has persisted over the decades: partly out of deference to history; and partly out of the efficacy of the neurogenic phenotype in continuing to identify new genes that are functionally connected to Notch signaling, even to this day. However, the term 'neurogenic' has also been the source of some continuing confusion, as it might reasonably be assumed to refer to a gene that promotes neurogenesis and/or functions exclusively during neurogenesis. Therefore, it is important to understand that: (1) 'neurogenic' describes a loss-of-function condition (thus, 'neurogenic' genes actually serve to repress neurogenesis); and (2) 'neurogenic' genes do not function exclusively during neurogenesis (rather, they usually operate throughout development).

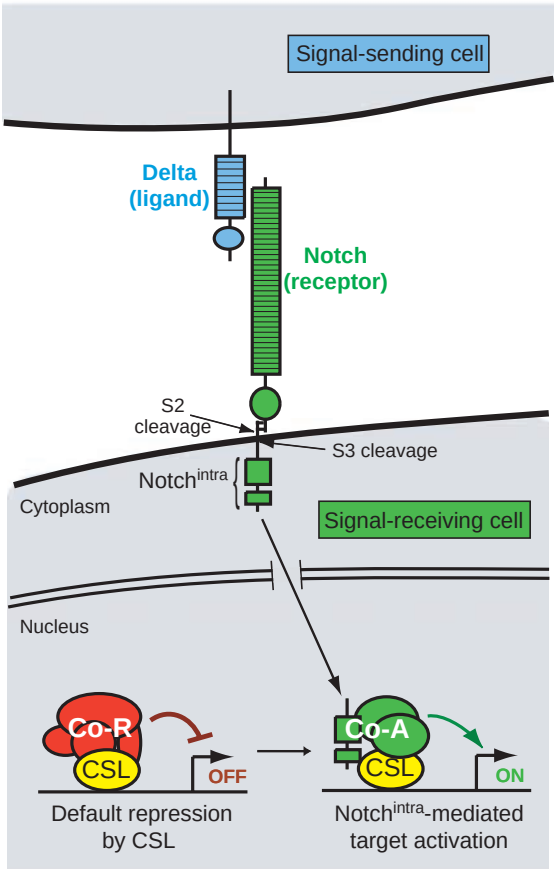

Fig. 1. Basic operation of the Notch pathway. The key players are a Delta-type ligand, the receptor Notch and the CSL transcription factor (see Table 1). Delta and Notch are transmembrane proteins containing extracellular arrays of EGF repeats (depicted by rectangles). Activation of Notch by its ligand triggers two proteolytic cleavages of Notch (S2 and S3, see also Box 2). S3 cleavage releases the Notch intracellular domain (Notch^{intra}), which translocates to the nucleus. Notch^{intra} activates CSL. The CSL co-repressor complex is displaced by a co-activator complex containing Notch^{intra} (Co-A, green icons), which mediates Notch target gene activation. In the absence of nuclear Notch^{intra}, CSL associates with a co-repressor complex (Co-R, red icons), which actively represses the transcription of Notch target genes.

Two homes for the receptor Notch

Some signaling cascades are truly cascades, and involve a complicated sequence of proteins that pass a message from the outside of the cell into the nucleus. At the opposite end of the spectrum lies Notch signaling, which operates by a remarkably direct mechanism. The route towards understanding how Notch works, however, has not been so direct.

For many different types of signal-activated cell-surface receptors, removal of the extracellular domain creates a mutant receptor that is permanently in the active mode. This is the case for Notch: an artificial, truncated Notch protein consisting of only its intracellular domain (Notch^{intra}) has strong constitutive activity in flies and worms (Lieber et al., 1993; Struhl et al., 1993). Interestingly, these engineered Notch^{intra} proteins localized to the nuclei of transgenic animals, which indicated that the transmembrane receptor Notch might have a nuclear function. Consistent with this model, a direct protein-protein interaction has been observed between Notch^{intra} and the CSL transcription factor (Fortini and Artavanis-Tsakonas, 1994). However, a competing model based upon tissue culture data proposed that the purpose of Notch-CSL binding was to hold CSL in the cytoplasm until receptor activation, at which point CSL would be released and travel to the nucleus (Fortini and Artavanis-Tsakonas, 1994).

Both models were challenged by the locations of the natural proteins in tissues engaged in Notch signaling: endogenous nuclear Notch is essentially never seen, while CSL appears constitutively nuclear. Eventually, the evidence came together to support strongly the Notch nuclear translocation model. The key findings were: (1) that Notch is proteolyzed in response to its interaction with ligand, which releases a soluble intracellular fragment (a natural Notch^{intra} molecule; see Box 2) (Kopan et al., 1996; Schroeter et al., 1998; Struhl and Adachi, 1998); (2) that Notch^{intra} is a transcriptional co-activator (Jarriault et al., 1995; Hsieh et al., 1996); and (3) that exceedingly small, histochemically invisible, amounts of Notch^{intra} suffice to activate target genes (Schroeter et al., 1998; Struhl and Adachi, 1998). The current ‘canonical’ view of Notch signaling is that ligand-induced activation of Notch triggers the cleavage and liberation of a small amount of Notch^{intra}, which then translocates to the nucleus and serves as a CSL transcriptional co-activator (Fig. 1, but see Box 3 for some examples of ‘non-canonical’ Notch signaling).

Table 1. Names of core components of Notch signaling (ligand, receptor and transcription factor) in different species

Core component	*C. elegans*	*D. melanogaster*	Mammals
Ligand	LAG-2 APX-1 ARG-2 F16B12.2	Delta Serrate	Delta-like1 (DLL1) Delta-like2 (DLL2) Delta-like3 (DLL3) Jagged 1 (JAG1) Jagged 2 (JAG2)
Receptor (Notch)	LIN-12 GLP-1	Notch	Notch1 Notch2 Notch3 Notch4
Transcription factor (CSL)	LAG-1	Suppressor of Hairless [Su(H)]	CBF1/RBPJκ RBPL

Notch^{intra} flips a CSL transcriptional switch

If CSL proteins reside in the nucleus, do they do anything when Notch is at the cell surface? CSL function was initially perplexing; vertebrate CSL proteins were first characterized as transcriptional repressors (Dou et al., 1994), but genetic tests in flies showed that CSL activated target genes during Notch signaling (Bailey and Posakony, 1995; Lecourtois and Schweisguth, 1995). How can the same protein be both a repressor and an activator?

Insight into this puzzle came from a virus. The EBNA2 protein from Epstein-Barr Virus (EBV) is a transcriptional co-activator that binds to and hijacks CSL in infected B cells. Interestingly, EBNA2 converts CSL from a default repressor into an activator of transcription (Hsieh and Hayward, 1995; Waltzer et al., 1995). Notch^{intra} was later found to use the same

Box 2. Notch proteolytic processing

Following the suggestion that Notch is cleaved during Notch signaling in the early 1990s, the search for the 'Notch-ase' was on. Notch proteolysis turned out to be more complicated than anticipated, and involves successive cleavage events termed S1, S2 and S3 (Fig. 1, note that S1 is not shown) (reviewed by Fortini, 2002). Vertebrate Notch is constitutively cleaved in the Golgi complex (S1) by a furin convertase and is reassembled into a functional heterodimeric receptor at the cell surface, although the evidence for similar processing of invertebrate Notch is equivocal. Physical interactions between specific EGF repeats of the ligand and Notch then trigger the second cleavage of Notch (S2), which releases the majority of the extracellular domain (see Fig. 1). This is mediated by the metalloproteases TACE in vertebrates, and possibly Kuzbanian/SUP-17 in invertebrates. Truncated Notch is then a substrate for γ-secretase, a multicomponent complex that cleaves Notch within its transmembrane domain (S3) and releases its intracellular domain ($Notch^{intra}$) (Fig. 1). Functional γ-secretase has four principal transmembrane components: presenilin, nicastrin, Aph1 and Pen2 (reviewed by De Strooper, 2003). Presenilin is a putative aspartyl protease whose dysfunction also underlies the abnormal cleavage of the transmembrane β-amyloid precursor protein in Alzheimer's disease.

strategy (Jarriault et al., 1995; Hsieh et al., 1996). The basis of this switch involves distinct CSL co-repressor and co-activator complexes (Fig. 1, 'nucleus'). In the absence of Notch signaling, CSL associates with transcriptional co-repressors that actively keep target gene expression switched off (Kao et al., 1998). Following Notch activation, the CSL co-repressor complex is replaced by a co-activator complex coordinated by $Notch^{intra}$. Active repression of targets in the absence of Notch signaling allows cells to tightly control signaling outputs (see Box 4 for unexpected consequences of this), and this dual mode of regulation is now understood to be a common feature of most of the major signaling cascades (reviewed by Barolo and Posakony, 2002).

Although engagement of the $Notch^{intra}$/CSL activator complex is normally necessary for a target gene to respond to Notch signaling, it is not always sufficient. Indeed, no Notch target gene is expressed in every location where Notch itself is activated. This specificity is due to co-regulation of Notch target genes by other transcription factors and/or signaling pathways (reviewed by Bray and Furriols, 2001). For example, cone cell-specific expression of *pax2* (*sv* – FlyBase) during *Drosophila* eye development is achieved through coordinated regulation by at least three inputs – Notch signaling, EGF receptor signaling and Lozenge (Flores et al., 2000). Combinatorial regulation allows for a transcriptional response that is appropriate for each different developmental setting (see also Box 4 for consequences of complex gene regulation).

Regulation of development by Notch signaling

Notch mutant fly embryos are so strongly affected that Donald Poulson, who pioneered the use of mutants to study fly development, was compelled to write that 'All in all, a kind of hopeless monster is produced which can not develop beyond the embryonic stage' (Poulson, 1945). We now know that Notch is likely to be involved in the development of most tissues in species throughout the animal kingdom, with myriad effects on cell fate specification, proliferation and cell death (Table 2). The following examples are a brief glimpse into the repertoire of Notch, and illustrate some different types of Notch-regulated patterning events.

Notch signaling restricts cell fates

Contingency plans are part and parcel of development. Often, more cells than necessary have the opportunity to become a specialized cell type, a scheme that allows for backups. An important role for Notch signaling is to prevent these 'extras' from actually taking on such specialized fates (Fig. 2A,B).

The classic example of this is the *Drosophila* neural-epidermal choice. Special groups of cells known as proneural clusters have neural potential because of their expression of basic helix-loop-helix (bHLH) transcriptional activators, also known as proneural proteins. Notch signaling restricts neural differentiation by repressing the expression of proneural genes (Parks et al., 1997). The failure of Notch signaling causes all proneural cluster cells to have high levels of proneural proteins and become neural, manifest as the 'neurogenic' phenotype (Fig. 2C,D). Constitutive Notch signaling has the opposite effect and suppresses neural differentiation.

Many proteins that mediate neural repression in *Drosophila* are encoded by the *Enhancer of split* Complex [*E(spl)*-C]. This genomic region contains multiple genes that are directly turned on by Notch signaling via CSL-binding sites in their promoters (Bailey and Posakony, 1995; Lecourtois and Schweisguth, 1995), including several genes that encode related bHLH repressor proteins. Forced expression of E(spl)bHLH repressors is sufficient to inhibit neural development (Nakao and Campos-Ortega, 1996), possibly by directly inhibiting the expression of bHLH proneural activators (Heitzler et al., 1996).

The same E(spl)bHLH repressors are deployed in other settings where Notch signaling restricts the number of progenitor cells during *Drosophila* development, including those of the visceral and somatic musculature, midgut and intestine, heart and a host of other internal organs (Hartenstein et al., 1992). Notch signaling in vertebrates also represses neurogenesis and myogenesis (Fig. 2E,F and Table 2) via homologous Hairy/E(spl)-related bHLH repressors known as HES (mammal), HER (fish) or ESR (frog) proteins (Chitnis et al., 1995) (reviewed by Artavanis-Tsakonas et al., 1999). Activation of bHLH repressors by Notch signaling is therefore a general strategy for preventing equipotent cells from all acquiring the same fate, a role sometimes referred to as 'inhibitory Notch signaling'.

Notch signaling specifies cell fates and creates boundaries

A second general role of Notch is to promote the development of a given cell type or body region, often by inducing the expression of positively acting regulatory molecules. In many of these cases, Notch signaling creates a new cell type as a result of cell-cell interactions at the boundary between distinct cell populations (Fig. 3A,B). This is sometimes referred to as 'inductive' Notch signaling, and contrasts with the 'inhibitory' role of Notch where Notch signaling represses a given cell fate among equipotent cells.

A well-understood example of this occurs during *Drosophila*

Table 2. A non-exhaustive list of developmental processes that are regulated by Notch signaling in different species

C. elegans	*D. melanogaster*	Vertebrates
Regulation of early blastomere specification	Inhibition of neurogenesis	Inhibition of neurogenesis
Regulation of AC/VU decision	Regulation of gliogenesis, neural lineage fates	Regulation of fate choices in the inner ear
Regulation of vulval precursor fates	Inhibition of wing venation	Inhibition of non-neural ectodermal derivates
Induction of left-right asymmetry	Inhibition of myogenesis, cardiogenesis	(*Xenopus* ciliated cells, chick feather buds)
Induction of germline proliferation	Inhibition of midgut precursors	Inhibition of myogenesis, cardiogenesis
	Induction of mesectoderm	Induction of left-right asymmetry
	Induction of wing margin	Regulation of limb bud development
	Induction of leg segments	Regulation of somitogenesis
	Induction of dorsoventral eye polarity	Regulation of lymphopoiesis
	Induction of cone cells in the eye	Regulation of vascular development
	Regulation of hematopoiesis	Regulation of kidney development

This is only a sampling of the spectrum of Notch functions; new examples continue to be discovered.

wing development. Notch signaling between the dorsal and ventral compartments of the future wing specifies the wing margin, a line of cells that organizes the outgrowth of the wing. A loss of Notch signaling eliminates the wing margin and wing tissue (visible as the eponymous wing 'notching'), while ectopic Notch signaling results in extra wing tissue (Fig. 3C-E). In this setting, a key output of Notch activation is to directly turn on *vestigial* in the presumptive wing margin (Kim et al., 1996). Vestigial is a transcriptional co-activator that is essential for wing development, and ectopic Vestigial will direct the formation of wing-like outgrowths in inappropriate locations.

Notch signaling also works at boundaries during vertebrate somitogenesis. The segmented vertebrate body plan is founded upon regularly spaced blocks of mesoderm known as somites. Somites split off progressively from the presomitic mesoderm, a process driven by periodic oscillations in gene expression that are called the segmentation 'clock'. Notch signaling appears to be central to the segmentation clock, as the expression of many Notch pathway components oscillates within the presomitic mesoderm, and mutation of members of the Notch pathway causes defects in clock oscillation and segmentation (Fig. 3F,G) (Conlon et al., 1995; Palmeirim et al., 1997) (reviewed by Bessho and Kageyama, 2003). Oscillating gene expression involves an auto-repressive activity of the Notch-activated bHLH repressors Hes1 and Hes7, which turn their own expression off. As these repressor proteins are short lived, their rapid degradation permits a new cycle of *Hes1/7* transcription to begin (reviewed by Bessho and Kageyama, 2003).

It remains to be fully understood, though, how the segmentation clock physically leads to somitogenesis, and whether or not Notch/CSL or Hes proteins directly regulate any genes that mediate somite partitioning. More generally, there are many 'inductive' Notch-regulated developmental processes, in which Notch signaling promotes rather than represses a cell type or behavior, for which the relevant target genes remain to be identified. For example, it is not well understood how Notch promotes germline proliferation in *C. elegans* (Berry et al., 1997) (Fig. 3H,I) or specifies cell fates such as mammalian astrocytes, fly glia and early worm blastomeres (Table 2). Although bHLH repressors are expressed in some 'Notch-inductive' settings, most of the well-characterized examples also involve the direct activation of genes that encode positively acting transcriptional regulators or proteins specific to terminally differentiated cells. Whether this is generally true or not will be revealed by a more detailed understanding of genes directly regulated by Notch^{intra}/CSL during normal development.

Giving direction to Notch signaling

As we have now seen, the major biological role of Notch signaling is to control the developmental fates of cells and to make cells different from one another. Therefore, cells become distinguished from one another according to whether they

Box 3. 'Non-canonical' Notch signaling

In the vast majority of developmental settings, Notch signaling involves the activation of the receptor Notch by a Delta-type ligand, which leads to changes in gene expression via the CSL transcription factor. However, every rule has its exception, and Notch signaling does not always operate as this canonical trio.

Other ligands?

Although Delta proteins are the major in vivo ligands for Notch, other proteins have been suggested to act as Notch ligands. Convincing evidence indicates that F3/contactin, a member of the immunoglobulin superfamily, is also a Notch ligand. Like Delta-type ligands, F3 binds Notch directly and induces Notch cleavage, nuclear translocation and a specific target gene response (Hu et al., 2003). Notch signaling via F3 specifically promotes oligodendrocyte maturation and myelination in the vertebrate central nervous system.

Do Notch and CSL have other signaling partners?

The existence of CSL-independent Notch signaling is controversial, but genetic and physical interactions between members of the Notch and Wingless signaling pathways have suggested an alternate path for Notch signal transduction via components of the Wingless pathway (Axelrod et al., 1996; Ramain et al., 2001). The converse situation of Notch-independent gene activation by CSL occurs during EBV infection (and CSL co-option by EBNA2) (Henkel et al., 1994). In addition, Notch-independent CSL auto-activation occurs in socket cells of *Drosophila* peripheral sense organs, where it is required for socket cell physiology (Barolo et al., 2000).

Non-nuclear mechanisms?

Notch is present on growth cones and can regulate axon guidance. It has been proposed that Notch may directly regulate the actin cytoskeleton via components of protein complex that includes the tyrosine kinase Abl (Giniger, 1998).

predominantly send or receive Notch signals. In some cases, this is easily explained by the exclusive distribution of ligand and receptor. For example, during *C. elegans* gonadal development, the somatic distal tip cell signals to adjacent germline cells to induce their division, which generates the pool of germline cells. The directionality of signaling is due to the fact that the distal tip cell expresses only the ligand LAG-2, while the germline cells express only the Notch receptor GLP-1 (Henderson et al., 1994). Notch ligands often show spatially patterned expression, so this is a general focal point for controlling Notch signaling. However, there are many situations where all of the cells involved in Notch signaling express both ligand and receptor. How is signaling made directional in these cases?

Box 4. Notch signaling defies expectation

The concept of a 'Notch pathway' implies a straightforward signaling cascade with straightforward consequences on gene expression. However, things are always more intricate in reality, and it is worth considering some non-intuitive behaviors of Notch target genes in response to experimental manipulations.

First, the dual function of CSL as both a repressor and activator of target gene transcription complicates experimental interpretation. The observation that mutation of CSL can have milder effects than mutation of Notch was initially consistent with the idea that Notch might regularly operate via other transcription factors. In retrospect, the slightly milder phenotype of CSL mutation is likely to reflect the canceling effects of removing both its repressor and activator function, while *Notch* mutants selectively affect the activator function of CSL .

Second, the combinatorial regulation of Notch target genes produces unexpected consequences. For example, it might be reasonably supposed that a loss of Notch signaling should result in a loss of Notch target gene expression. However, Notch target genes in the *Drosophila E(spl)*-Complex are instead expressed at higher levels in CSL mutants (Bailey and Posakony, 1995). This is a result of their direct regulation by both CSL and proneural bHLH proteins. As proneural bHLH protein levels increase when Notch signaling is compromised, the expression of these Notch target genes persists without Notch signaling.

Third, some Notch target genes are expressed ectopically in response to forced expression of $Notch^{intra}$. However, other Notch target genes require that multiple criteria be satisfied in order to be expressed (such as the presence of other transcription activators or the absence of specific repressors). Therefore, it is neither the case that all Notch target genes are 'off' in Notch mutants, or 'on' in the presence of constitutive Notch signaling.

Feedback regulation in Notch signaling

Notch signaling sometimes shows a remarkable ability to amplify small differences in the signaling capacities of different cells. One tactic is for signaling to regulate receptor and/or ligand transcription. In this way, the degree to which a cell activates Notch signaling has a dynamic effect on how well it sends and/or responds to Notch signals in return.

A clear example of this occurs during the anchor cell/ventral uterine cell (AC/VU) decision in *C. elegans* gonadal development. Although the destiny of almost all nematode cells is predetermined by lineage, AC and VU acquire their identity through a Notch-mediated discussion between two cells, referred to as Z1.ppp and Z4.aaa. Initially, LIN-12 (Notch) and LAG-2 (ligand) are expressed by both cells, and they engage in 'mutual' bi-directional Notch signaling. However, random fluctuation in expression of LIN-12 and LAG-2 is amplified by positive feedback; activation of LIN-12 promotes its own expression and inhibits the expression of LAG-2 by the cell. In the end, one cell expresses only the ligand and differentiates as AC, while the other cell expresses only receptor and turns into VU (Wilkinson et al., 1994; Christensen et al., 1996). A directional signaling situation like this is sometimes called 'lateral' signaling.

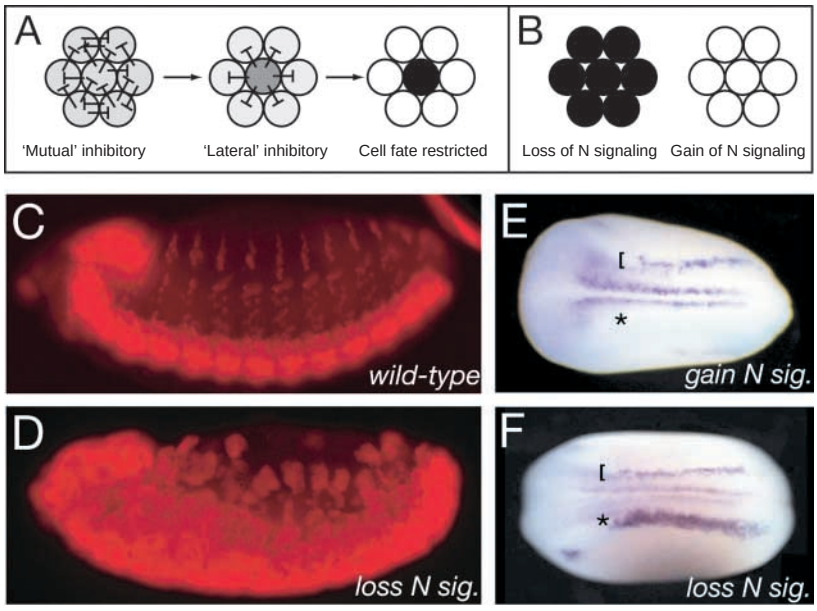

Fig. 2. Inhibitory Notch signaling restricts cell fates. (A) A proposed dynamic of Notch signaling among a group of equipotent cells. Initially, cells that share a special cell fate potential (gray) both send and receive Notch signals, known as 'mutual' inhibitory Notch signaling. Later, one cell commits to the specialized fate (black) and inhibits surrounding cells (white) from adopting this fate, a situation known as 'lateral' inhibitory Notch signaling. (B) The failure of Notch signaling results in extra cells adopting the special cell fate, while excessive Notch signaling prevents the differentiation of these cells. (C,D) Notch signaling inhibits neurogenesis in the *Drosophila* embryo. (C) A wild-type embryo stained for the neural marker ELAV (red). (D) An embryo that completely lacks Su(H), the fly CSL transcription factor, displays a strong excess of neurons – the classic 'neurogenic' phenotype. (E,F) Notch signaling inhibits neurogenesis in *Xenopus* (images courtesy of Elise Lamar). Staining for a neural form of tubulin (purple) reveals neuronal differentiation. (E) The lower half of this embryo expresses constitutively active $Notch^{intra}$, which inhibits neuronal differentiation [compare the number of neurons in the bracketed region in wild type (top) with the starred region in the mutant tissue (bottom)]. (F) The lower half of this embryo expresses an inhibitor of the Notch co-activator complex (dominant negative form of Mastermind). This leads to a failure of Notch signaling and a strong neurogenic phenotype (star).

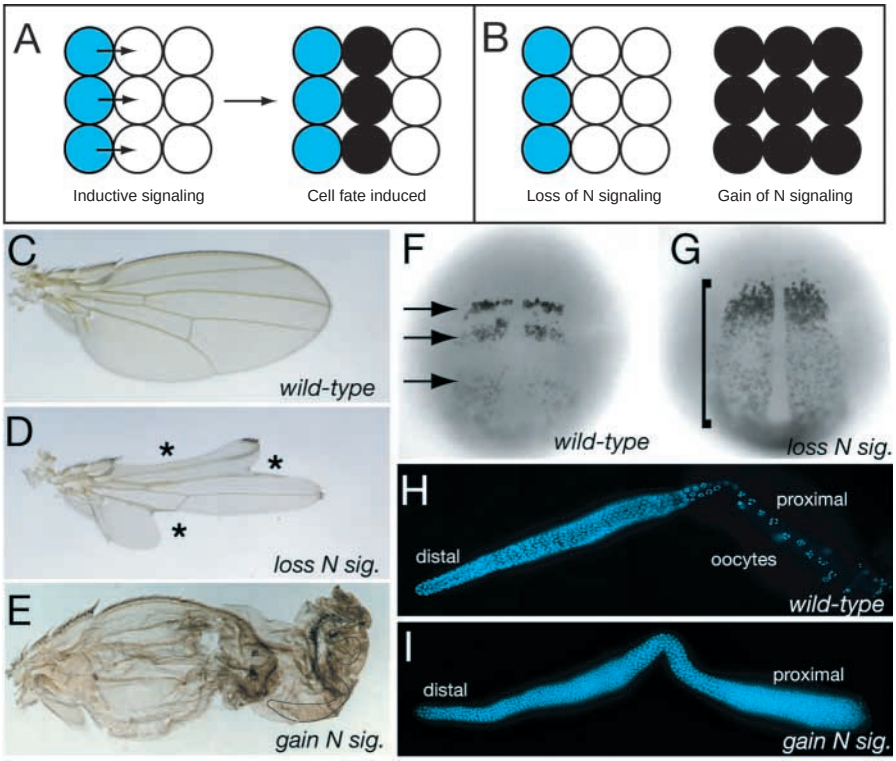

Fig. 3. Notch signaling specifies cell fate and behavior. (A) A schematic of inductive Notch signaling, which typically occurs between non-equivalent cell populations. In this case, the blue cells signal to adjacent white cells to induce a new cell fate or change their behavior (black cells). (B) The failure of inductive Notch signaling results in the absence of this cell fate or behavior, whereas excessive Notch signaling has the reciprocal effect. (C-E) Notch signaling promotes *Drosophila* wing growth. (C) Wild-type adult wing. (D) Wing containing large *Su(H)* mutant clones in which loss of the CSL transcription factor causes notching (stars), owing to the failure to specify the wing margin during earlier development. (E) Wing containing clones of cells that misexpress Delta and that induce a large wing overgrowth (image courtesy of Jose F. de Celis). (F,G) Notch signaling is involved in the segmentation clock. Shown are stage 3S zebrafish embryos stained for the Notch ligand *DeltaC* (images courtesy of Clarissa Henry). (F) In a wild-type embryo, *DeltaC* oscillates in stripes (arrows) that correlate with the partitioning of somites. (G) An embryo in which the bHLH repressor-encoding Notch target genes *her1* and *her7* have been inhibited by injection of morpholinos. The oscillatory pattern of *DeltaC* expression is lost (bracket), and such embryos develop abnormal somites. (G,H) Notch signaling promotes germline proliferation. Shown are *C. elegans* gonads stained with DAPI (blue) to reveal nuclei (images courtesy of Tim Schedl). (H) In the wild-type gonad, mitotic nuclei are localized to the distal region, while the remainder of the gonad is meiotic and produces germ cells (oocytes). (I) A gain-of-function mutant in the GLP-1 receptor (Notch) shows a 'tumorous' phenotype in which mitotic cells are found throughout the gonad and germ cells fail to differentiate.

Similar 'mutual' to 'lateral' Notch signaling is seen during the selection of *Drosophila* neural precursors from groups of equipotent proneural cluster cells discussed earlier (Fig. 2A), a process known to be very sensitive to the levels of ligand and receptor in each individual cell (Heitzler and Simpson, 1991). A proposed mechanism for Notch-mediated regulation of ligand incorporated the finding that the expression of *Delta* can be activated by proneural proteins, which are, in turn, inhibited by Notch-activated bHLH repressors (Heitzler et al., 1996). However, this regulatory chain does not fully explain how signaling becomes directional, as increased levels of proneural proteins in neural precursors and decreased levels of proneural proteins in the inhibited cells are not well correlated with changes in Delta or Notch levels during normal development (Parks et al., 1997). Thus, post-transcriptional mechanisms to amplify differences in Delta-Notch signaling must also exist, some of which are discussed in the next section.

Modulating ligand and receptor activity

A second way to influence Notch signaling among cells that co-express ligand and receptor involves post-translational modifications. Two molecules that target the Delta ligand are Neuralized and Mindbomb. Although they are not related to one another structurally, both are RING-type E3 ubiquitin ligases that directly ubiquitinate Delta and cause it to be internalized from the plasma membrane. Recent studies show that the effect of Neuralized and Mindbomb is actually to increase the ability of Delta to activate Notch signaling in neighboring cells (Itoh et al., 2003) (reviewed by Le Borgne and Schweisguth, 2003). One way in which this might work is if the extracellular domain of Notch is co-internalized with Delta into the Delta-expressing cell, which might facilitate the 'S2' cleavage of Notch in the Notch expressing cell (Fig. 1). The expression of these ubiquitin ligases is initiated in a subset of Notch-dependent processes, including neurogenesis, and may be responsible for making Notch signaling directional during the neural/epidermal fate decision.

Notch activity is regulated by sequential glycosylation of particular EGF repeats (reviewed by Schweisguth, 2004). Notch modification by the glycosyltransferase Fringe has complex effects. Fringe inhibits the ability of Notch to be activated by Serrate- and Jagged-type ligands, but stimulates the response of Notch to Delta-type ligands (Panin et al., 1997). In vitro data suggest that Fringe enhances Notch-Delta binding and decreases Notch-Serrate binding. Fringe is often deployed in a spatially restricted pattern to position or create directional Notch signaling during cell fate-inductive events. Some examples of this occur during development of the fly wing margin, at the dorsoventral boundary of the fly eye, at the dorsoventral limb borders of vertebrate limbs and in developing vertebrate somites (reviewed by Irvine, 1999).

Inherited factors bias inhibitory Notch signaling

A special type of directional inhibitory Notch signaling occurs during many instances of asymmetric cell divisions (Fig. 4A).

In this situation, although both sister cells are capable of sending and receiving Notch signals, directionality is imposed by the asymmetric segregation of factors that influence Notch signaling. Such factors localize to a crescent centered at one end of the mitotic spindle in the mother cell (Fig. 4B).

A crucial Notch-inhibitory factor is Numb (Rhyu et al., 1994), a phosphotyrosine binding (PTB) domain adaptor protein that binds directly to Notch and prevents the Notch pathway from becoming activated in that cell. This is well illustrated during *Drosophila* sensory lineages, in which asymmetric cell division is the consequence of Numb being inherited by only one daughter cell, and Notch signaling being suppressed in that daughter cell (reviewed by Posakony, 1994). For example, one cell in the adult bristle lineage divides to give rise to one socket cell and one shaft cell (Fig. 4A,C). Notch signaling is activated only in the socket cell and prevents it from becoming a shaft cell; the presumptive shaft cell is immune to Notch signaling because it inherits Numb (Fig. 4A,B). If both cells have activated Notch signaling – either through loss of Numb or constitutively active Notch – they both turn into socket cells (Fig. 4D). Conversely, if neither cell has activated Notch signaling, a double-shaft structure results (Fig. 4E). Therefore, both Numb and Notch signaling are needed to ensure that the daughter cells of this asymmetric cell division adopt different fates.

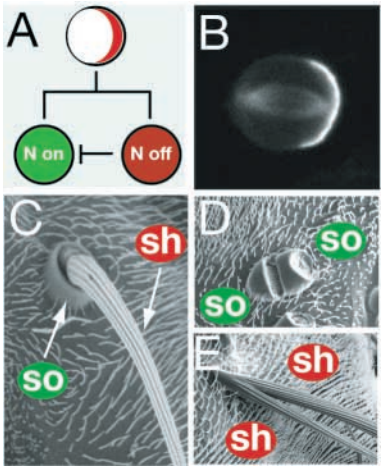

Fig. 4. Notch-mediated asymmetric cell divisions are influenced by localized determinants. (A) An asymmetric cell division in which one cell divides to give two daughters that adopt different fates; Notch signaling is 'on' in the green cell and 'off' in the red cell. Although both cells express Notch and Delta, signaling is directional because the red cell inherits determinants [such as Numb (red crescent in the mother cell)] that inhibit Notch signaling in that cell. (B) Crescents are centered on one end of the mitotic spindle and segregated into only one daughter cell. Here, a crescent of Partner of Numb-GFP is visualized along with the spindle, which is labeled with tau-GFP (image courtesy of Fabrice Roegiers). (C) The two exterior cells of a *Drosophila* mechanosensory bristle are produced by the lineage shown in A. The shaft cell (sh) activates Notch signaling in its sister, the socket cell (so); the shaft cell does not have activated Notch because it inherits Numb. Activation of Notch in both cells results in two sockets (D), whereas a failure to activate Notch in either cell results in two shafts (E) (images courtesy of Scott Barolo).

Aberrant Notch signaling in human disease

Given the profound and widespread roles of Notch signaling across a range of tissues, it is perhaps no surprise that deviant Notch signaling underlies some human diseases. Consistent with the roles of Notch signaling in development and neural behavior, relevant heriditary conditions include Alagille syndrome (where mutations in the Notch ligand *JAG1* affect the development of many organs, including that of the liver, skeleton, heart and eye) (Li et al., 1997; Oda et al., 1997), certain forms of spondylocostal dysostosis (where mutations in the ligand *DLL3* result in rib fusions and trunk dwarfism) (Bulman et al., 2000) and cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) (where mutations in the extracellular EGF repeats of *NOTCH3* predispose individuals to dementia, migraines and strokes) (Joutel et al., 1996). In addition, a mild decrease in Notch signaling in mice causes specific defects in spatial learning and memory, which might make Notch a more general culprit in cognitive deficits (Costa et al., 2003).

Aberrant Notch signaling is also intimately involved in several human cancers. This was first demonstrated in a recurrent t(7;9) translocation that is associated with certain pre-T cell acute lymphoblastic leukemias (T-ALL) (Ellisen et al., 1991). This chromosomal rearrangement results in constitutive Notch activity in T cells. Conversely, cells that are mutant for *Notch1* form skin and corneal tumors in mice, indicating that Notch also suppresses tumorigenesis (Nicolas et al., 2003). Many other human and murine cancers, including certain neuroblastomas, and mammary, skin, cervical and prostate cancers, are correlated with alterations in expression of Notch proteins and/or ligands. Although causal relationships in many cases await better characterization, these observations suggest broad roles for Notch dysfunction in cellular transformation.

The CSL transcription factor is a particular victim of viruses that redirect CSL function towards their own ends. Multiple proteins from Epstein-Barr virus (EBV), Kaposi's sarcoma-associated herpesvirus (KSHV), and adenovirus type 5 bind CSL directly and modulate CSL-dependent transcription of both viral and host target genes in a Notch-independent fashion (reviewed by Allenspach et al., 2002). A majority of humans are latently infected with EBV and adenovirus, but, as is the case for KSHV, initial infection is usually asymptomatic. A serious problem arises in immunocompromised individuals, though, where opportunistic behavior of EBV and KSHV causes oncogenic transformation and malignancy.

The molecular elucidation of these diseases is now the basis of diagnostic tools. However, it is hoped that knowledge of the mechanism of Notch signaling will be relevant for therapeutic design. This hope remains to be realized, but one can imagine that conditions that arise from Notch pathway gain-of-function might be alleviated by small molecule antagonists of genetic inhibitors of Notch processing/Notch^{intra}-co-activator function, while conditions that arise from Notch pathway loss-of-function might be treated with targeted delivery of soluble Notch ligands or other strategies that either locally activate Notch activity or suppress CSL co-repressor activity. A variety of relevant mouse disease models are now available to help test these strategies. Of course, given the very general functions of the Notch pathway, minimization of collateral or non-specific effects will be necessary to make such therapies clinically useful.

Concluding remarks

A clear theme of developmental and evolutionary biology is that nature is frugal: useful proteins and signaling pathways are reused in diverse settings. Notch signaling is an example of a particularly successful signaling cascade that is used

repeatedly throughout the development of all metazoan organisms. Many Notch-mediated affairs are highly analogous; for example, Notch signaling mediates the inhibition of many different cell fates through the same bHLH repressor proteins. However, the redeployment of this pathway during evolution has resulted in diverse outcomes to Notch activation in different situations. Depending on the setting, Notch can inhibit, delay or induce differentiation, and can variously promote apoptosis, cell division or a static state (Table 2). Things become more complicated when one considers how signaling pathways interact with one another. For example, Notch signaling and EGF receptor signaling can work in parallel or in series to regulate a given process, and can either cooperate or antagonize each other during transcriptional regulation of a given target. Notch signaling is also influenced by a great number of other factors in specific settings, only some of which have been addressed in this primer (reviewed by Greenwald, 1998; Schweisguth, 2004). All of these considerations mean that although one can be guided by previous studies, it is imperative to evaluate each new example of Notch signaling carefully.

Given the pervasive use of the Notch pathway in animal development, how was this signaling cascade originally assembled during evolution? Although only metazoans are known to use Notch signaling, certain 'prospective' components of the Notch pathway are found in other types of eukaryotes. A striking example is the definitive existence of CSL homologs in some fungi. If these species never had a Notch pathway, they may shed light on ancestral functions of CSL transcription factors. Will they be found to be transcriptional activators or repressors, or both? And are there any species in which both Notch and CSL are present, but are not associated in a common signaling process? It will be a great future challenge to understand when and how functional connections arose between components of a presumptive Notch pathway during evolution.

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