and receptor-desensitization mechanisms. Several studies have shown that receptor phosphorylation on the COOH-terminus is associated with decreases in receptor affinity and receptor internalization. Cells expressing tailless CCR2B, fMLP, or cAR1 receptors or cAR1 receptors where all of the serines in the COOH-terminal domain were substituted with alanine residues show altered desensitization responses (decrease in affinity, internalization) but are still able to carry out chemotaxis [H. Arai, F. S. Monteclaro, C. L. Tsou, C. Franci, I. F. Charo, *J. Biol. Chem.* **272**, 25037 (1997); M. H. Hsu, S. C. Chiang, R. D. Ye, E. R. Prossnitz, *ibid.*, p. 29426; J. Y. Kim *et al.*, *ibid.*, p. 27313].
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36. We thank S. van Es and J. Silverman for their excellent comments and stimulating discussions; G. Servant, O. D. Weiner, and H. R. Bourne for providing panel B of Fig. 2; and R. A. Firtel for panel C of Fig. 4. Supported by NIH grant GM57874 and GM28007.

REVIEW

Notch Signaling: Cell Fate Control and Signal Integration in Development

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Notch signaling defines an evolutionarily ancient cell interaction mechanism, which plays a fundamental role in metazoan development. Signals exchanged between neighboring cells through the Notch receptor can amplify and consolidate molecular differences, which eventually dictate cell fates. Thus, Notch signals control how cells respond to intrinsic or extrinsic developmental cues that are necessary to unfold specific developmental programs. Notch activity affects the implementation of differentiation, proliferation, and apoptotic programs, providing a general developmental tool to influence organ formation and morphogenesis.

The building of an organism from a single cell to a multicellular three-dimensional structure of characteristic shape and size is the result of coordinated gene action that directs the developmental fate of individual cells. The acquisition of different cell fates orchestrates an intricate interplay of cell proliferation, migration, growth, differentiation, and death, elaborating and bringing together cellular ensembles in a precise manner. Intrinsic, cell-autonomous factors as well as nonautonomous, short-range and long-range signals guide cells through distinct developmental paths. Frequently, an organism uses the same signaling pathway within different cellular contexts to achieve unique developmental goals. How intrinsic and extrinsic factors are integrated in ontogeny to specify cell fates defines the central question of developmental biology.

Notch signaling is an evolutionarily conserved mechanism that is used by metazoans to control cell fates through local cell interactions. The realization that this signaling mechanism controls an extraordinarily broad spectrum of cell fates and developmental processes (in organisms ranging from sea urchins to humans) resulted in a veritable explosion of Notch-related studies in the past decade. Our intention here is not to review all the systems and cellular events that depend on this mechanism, because several reviews adequately cover these many issues (*1*–*7*). Instead, we present some general developmental features emerging from collective studies in vertebrate and invertebrate experimental systems as well as consider certain mechanistic aspects of Notch signaling. These studies make it apparent that signals transmitted through the Notch receptor, in combination with other cellular factors, influence differentiation, proliferation, and apoptotic events at all stages of development. Thus, Notch signaling appears to function as a general developmental tool that is used to direct cell fate and, consequently, to build an organism.

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Elements of Notch Signaling

The gene encoding the Notch receptor was discovered in flies almost 80 years ago by virtue of the fact that partial loss of function (haploinsufficiency) results in notches at the wing margin (*8*). Notch received its notoriety as a result of classic embryonic analyses of lethal loss-of-function mutations, which were conducted by Poulson (*9*). These mutations produce a "neurogenic" phenotype, where cells destined to become epidermis switch fate and give rise to neural tissue (*10*). The *Notch* gene, first characterized in *Drosophila melanogaster*, encodes a 300-kD single-pass transmembrane receptor. The large extracellular domain contains 36 tandem epidermal growth factor (EGF)–like repeats and three cysteine-rich Notch/LIN-12 repeats. Six tandem ankyrin repeats, a glutamine-rich domain (opa), and a PEST sequence are found within the intracellular domain (*11*). Notch-like proteins have been identified and extensively characterized in *Caenorhabditis elegans* (LIN-12 and GLP-1) (*2, 3*), sea urchins, and many different vertebrates, including humans (*4, 12*). In all animal models tested, mutations in the Notch receptor invariably result in developmental abnormalities and thus, not surprisingly, human pathologies (*13*–*15*).

Genetic and molecular interaction studies resulted in the identification of a number of proteins that may participate in transmitting or regulating Notch signals (Fig. 1). From this increasing array of proteins, whose direct relation to Notch signaling is often unclear, a small group of elements emerges as forming the core of this signaling pathway. In *Drosophila*, the two single-pass transmembrane proteins, Delta and Serrate, have been identified as partially redundant Notch ligands (Delta and Jagged in vertebrates, LAG-2 and APX-1 in *C. elegans*) (*2, 4, 16*). The transcription factor Suppressor of Hairless [Su(H)] (CBF1/RJBk in mammals, LAG-1 in *C. elegans*) appears to function as the major downstream effector of Notch signaling, and the genes of the *Enhancer of split* [*E(spl)*] locus, which encode nuclear basic helix-loop-helix (bHLH) proteins, are primary targets of Notch signaling (*1, 2*).

The basic picture emerging from many different studies has the extracellular domain of the ligands, expressed on the surface of one cell, interacting with the extracellular domain of the Notch receptor on an adjacent cell. As a result of receptor activation, Su(H) binds to regulatory sequences of the *E(spl)* genes and up-regulates expression of their encoded bHLH proteins (*17, 18*). The bHLH factors, in turn, affect the regulation of downstream target genes. One well-defined target is the *Achaete-Scute* complex, which contains proneural genes that encode proteins involved in the segregation of neuronal and epidermal lineages (*19*), a process affected by mutations in *Notch*. There is no doubt that this linear picture is only a skeleton, as we know that each step is embellished with additional elements and features that modulate the activity and efficacy of the signals transmitted through the Notch receptor.

At the extracellular level, the action of the ligands can be influenced by at least one molecule, Fringe (*20*), but it is quite possible that other extracellular factors capable of in-

fluencing the action of Notch ligands exist. Although genetic studies in *Drosophila* have raised the possibility that the extracellular proteins Wingless and Scabrous directly interact with the Notch receptor, compelling evidence is still lacking (*21, 22*).

Posttranslational proteolytic events seem to regulate the activities of the Notch receptor and the ligands. The Notch receptor is cleaved in the trans-Golgi network, apparently by a furin-like convertase, and presented on the cell surface as a heterodimer (*23, 24*). Two additional proteins have been implicated in posttranslational events that modulate Notch signals. Mutations in the ADAM metalloprotease Kuzbanian (Kuz) and the presenilins, proteins that are associated with β-amyloid precursor processing and the development of Alzheimer's disease, have been identified as modulators of Notch signaling (*25, 26*). Their exact involvement in Notch biochemistry is not clear but it appears that they are directly involved in the generation or trafficking (or both) of Notch and Delta fragments that are crucial for receptor signaling. The genetic evidence from *Drosophila* indicates that normal development is exceptionally sensitive to *Notch* and *Delta* gene dosage, suggesting that the quantity of the receptor or the ligands present on the surface of a cell is an important and, perhaps, tightly regulated parameter of Notch signaling. We therefore expect any gene affecting, directly or indirectly, the maturation or the trafficking of the receptor and its ligands to be an effective modulator of signaling.

At the intracellular level, the Notch region encompassing the ankyrin repeats has been shown to be necessary for the transmission of Notch signals (*27, 28*). Homotypic and heterotypic interactions involving the ankyrin repeats have been documented (*29*). Ankyrin repeat-interacting proteins include EMB 5, a *C. elegans* protein that is related to the regulator of chromatin structure Stp6 of *Saccharomyces cerevisiae* (*30*), Deltex, and Su(H). Deltex is a pioneer protein that contains a ring zinc-finger motif and putative SRC homology 3 binding domains (*31*). In *Drosophila*, Deltex acts as a positive, albeit nonessential, regulator of Notch signaling (*32*). In mammalian cells, both negative and positive regulation by the Deltex homolog has been documented (*33, 34*). Su(H) interacts with two distinct sites in the intracellular domain of Notch, one that encompasses the ankyrin repeats and another that does not (*29, 35*). Other proteins reported to interact with Notch in *Drosophila* are Numb, a factor critical for the elaboration of the peripheral nervous system (*36*); Disheveled, an element of the Wingless pathway; and Disabled, an accessory protein of the Abl kinase (*37*). In mammalian systems, Bcl3 [a member of the IkB family (*38*)] and Nur77 (a protein involved in lymphoid development) have also been shown to interact with the intracellular domain of Notch (*39*).

Notwithstanding the fact that experimental evidence has been gathered to suggest that Notch signals may be transmitted independently of Su(H) (*18, 29, 33, 39, 40*), this protein is clearly the major effector of Notch signaling. The activity of Su(H) can be antagonized through its interaction with the Hairless protein, a potent negative regulator of Notch (*41*).

The proteins that are capable of molecular interactions with Notch certainly do not represent the entire array of Notch interactions, for the list of proteins that interact with core pathway elements revealed by genetic studies is considerably larger. Although the core elements may be ubiquitous, cell- or tissue-specific factors are likely to exist. Given the multitude of documented interactions between Notch and other cellular elements, it may, perhaps, be more useful to view Notch signaling from the perspective of a "network" rather than a linear "pathway."

Notch-Ligand Interactions

Genetic mosaic studies have revealed that the action of Delta is nonautonomous and short-range, consistent with the notion that Delta is a transmembrane ligand that only affects the activity of adjacent cells expressing the receptor (*42*). Cell aggregation studies of *Drosophila* cultured cells have revealed that receptor-ligand interactions are mediated by specific EGF repeats of the Notch receptor and the conserved extracellular region of the ligand, referred to as the Delta:Serrate:LAG-2 (DSL) domain (*6, 7*). These observations reinforce the simple model in which a transmembrane ligand on one cell interacts with the receptor on a neighboring cell. However, several observations that have attracted less attention indicate that Notch-ligand interactions are far more complex.

Examination of Notch and Delta expression indicates that individual cells often express both the receptor and the ligand (*7, 42, 43*). Colocalization studies in cultured cells suggest that Notch and Delta can interact in cis (*43*). Such interactions may reflect the in vivo situation, because genetic analyses demonstrate that the ligands can exert cell-autonomous effects on Notch-

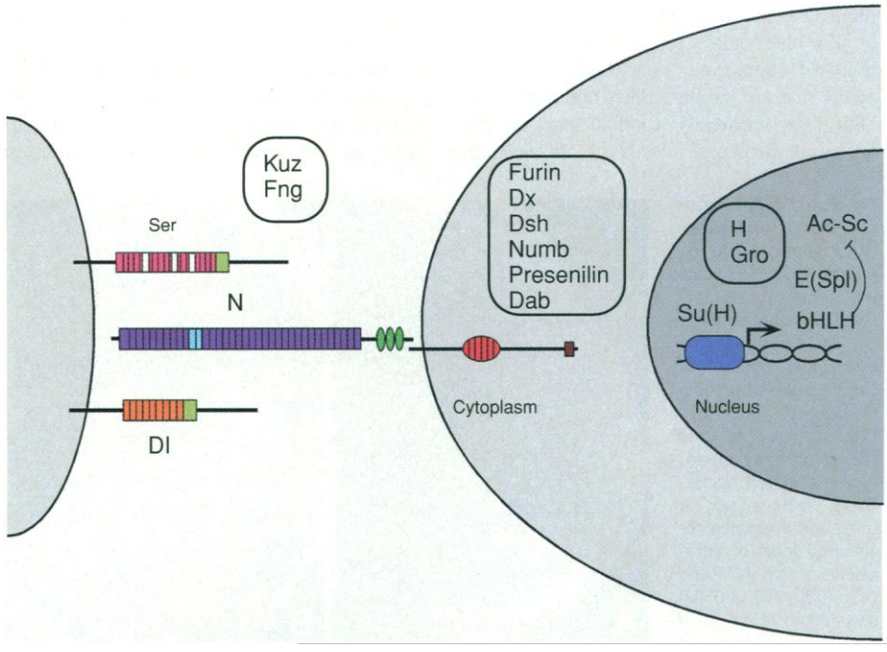

Fig. 1. Elements of Notch signaling. A nonmechanistic schematic of various elements that have been shown to modulate Notch activity. Extracellular regions of Notch (N) and Delta (Dl) interact to activate the receptor. As a result of activation, the Supressor of Hairless [Su(H)] transcription factor eventually binds to regulatory sequences of the *Enhancer of split* [E(Spl)] complex genes, which encode bHLH proteins. bHLH products, together with Groucho, can repress the expression of the *Achaete-Scute* (Ac-Sc) proneural genes. Several additional factors that influence signaling through these core elements and that display molecular interactions are also shown. These include the ligand Serrate (Ser) and its negative regulator Fringe (Fng); the metalloprotease Kuzbanian (Kuz), which acts as a Delta- and potentially as a Notch-processing enzyme; the trans-Golgi convertase Furin, which cleaves Notch; Presenilin, which may cleave Notch in the membrane; and the Notch intracellular domain interacting proteins Deltex (Dx), Disheveled (Dsh), Disabled (Dab), and Numb; and in the nucleus, the two regulators Hairless (H) and Groucho (Gro). Structural elements of Notch and Delta are represented as follows: Purple and orange boxes represent EGF repeats, light-blue boxes represent EGF 11-12 of Notch, the yellow box represents the DSL domain, green ovals represent Notch and Lin-12 repeats, the red oval represents the six ankyrin repeats, and the brown box represents the pest sequence.

dependent signals (*44*). Another level of complexity is implied by the fact that Delta molecules mediate homotypic cell adhesion (*45*). Furthermore, depending on the level of ligand expression, their action may be either agonistic or antagonistic (*44*).

In considering how the ligands interact with the receptor, the picture is further complicated by recent observations showing that the entire extracellular domain of Delta (DlEC) is found as a soluble product in the supernatant of Delta-expressing *Drosophila* cultured cells. The same fragment is detected in *Drosophila* extracts, and its appearance, both in vivo and in cell cultures, can be inhibited by inactivating the ADAM metalloprotease Kuz (*46, 47*). Loss of Kuz function also results in phenotypes that are similar to those associated with loss of Notch signaling (*48–50*), suggesting that Delta cleavage is essential for Delta function. Consistent with this is the observation that additional gene copies of *Delta* suppress partial loss-of-function kuz phenotypes (*46*). These findings raise the possibility that the cleaved DlEC may act as a soluble ligand. However, given the mosaic data, which demonstrates that Delta acts on adjacent cells, DlEC activity must be confined to its immediate cellular environment.

The observations regarding the in vivo activity of engineered, truncated soluble fragments that are similar, albeit nonidentical, to DlEC are apparently contradictory. Soluble forms of *Drosophila* Delta or Serrate were found to act as antagonists of the pathway in vivo (*51*). On the other hand, soluble extracellular fragments expressed in *C. elegans* or ligand fragments added to human hematopoietic cell cultures appear to act as agonists (*52*). Importantly, when the purified, naturally cleaved DlEC is added to a preparation of cortical neurons, which express endogenous Notch receptor, they retract their processes. An identical response is displayed when these neurons are transfected with constitutively active forms of Notch, suggesting that DlEC can activate the endogenous Notch receptor on the neurons, acting thus as an agonist (*46*). Presently, it is not certain why the soluble forms of ligand seem to have both agonistic and antagonistic behavior. The various soluble forms of ligand that have been tested differ at their COOH-termini and, thus, cannot be formally compared. Therefore, further structure and function studies are necessary to answer these questions. Moreover, it is worth considering that both agonistic and antagonistic (*53*) activities may hinge upon a cell's sensitivity to levels of soluble ligand or the context in which Notch signaling is occurring.

Although it has now been demonstrated that production of the heterodimeric form of the Notch receptor depends on the activity of a furin-like convertase rather than Kuz, as had been originally postulated, Kuz may still be involved in Notch receptor function (*24, 49*). Studies in mammalian culture cells have implicated a second site of cleavage that may be triggered by an interaction between the Notch receptor and its ligands (*24, 54*). It has been proposed that this cleavage event is dependent, either directly or indirectly, on Kuz (*24*).

By analogy to the action of other ADAM metalloproteases, Kuz is presumed to act on the cell surface (*55*). However, in the absence of concrete evidence regarding the subcellular localization of Kuz, other possibilities should not be excluded. It is conceivable that both Delta cleavage and the putative Notch cleavage may occur inside the cell, especially because both extracellular and intracellular domains of Delta can be internalized in Notch-expressing cells (*47*). Details of the mechanism by which the ligand elicits activation of the receptor are unclear. We know, however, that expression of only the transmembrane, COOH-terminal half of the Notch heterodimer (N^{TM}) results in constitutive activation. This allows us to consider that the NH_2-terminal part of the receptor acts as a suppressor of activation and, accordingly, suggests a mechanism whereby the extracellular domain is shed upon interaction with the ligand, resulting in receptor activation.

The importance of a functional analysis of soluble extracellular modulators of Notch activity is not confined to gaining mechanistic insights into Notch signaling. The cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) syndrome is an inherited disease associated with point mutations in the extracellular domain of the human Notch 3 receptor (*13*). Furthermore, the Alagille syndrome is associated with human Jagged 1 mutations that predict truncated extracellular fragments of the ligand (*14, 15, 56*). In each case, it is not clear whether the associated mutations reflect a loss or gain of function. Nonetheless, it is likely that the mutant activity could be influenced by extracellular soluble molecules. Such molecules may therefore be useful in addressing, therapeutically, these two pleiotropic and potentially lethal human syndromes.

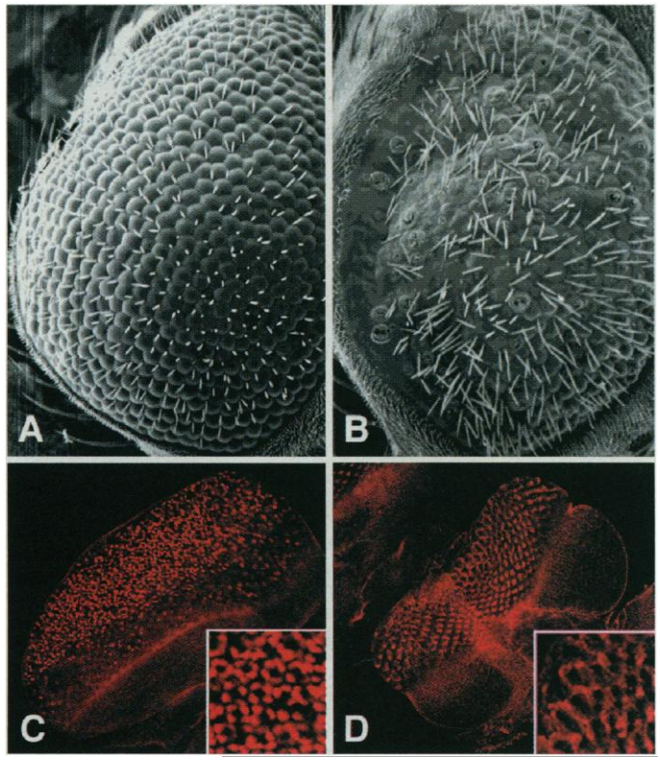

Fig. 2. Activated Notch phenotypes and nuclear localization: The expression of a truncated, constitutively active form of the receptor in the developing eye under control of the sevenless promoter elicits rough-eye phenotypes. **(A)** and **(B)** show the phenotype associated, respectively, with a nuclear form and a membrane-tethered form of intracellular Notch [N^{nuc} and N^{act} (*92*)]. **(C)** and **(D)** show confocal images of the corresponding eye discs, depicting the subcellular localization of Notch (red) revealed by a Notch antibody raised against the intracellular domain. The expression of N^{nuc} correlates with almost complete nuclear localization of the protein (C) and is associated with a weak rough-eye phenotype (A), whereas the more severe phenotype generated by N^{act} expression (B) corresponds to membrane and cytoplasmic staining (D). Insets in (C) and (D) show images at a higher magnification.

Participation of Notch in Nuclear Events

How Notch signaling modulates nuclear processes has been one of the most challenging problems of Notch biology. A model for Notch signaling was offered on the basis of the conserved, nuclear localization signals that lie within the intracellular domain of the Notch receptor and the nuclear accumulation of activated forms of Notch consisting of truncated proteins lacking the extracellular and transmembrane domain (*26, 27, 57*). These observations, even early on, raised the obvious but intriguing possibility that Notch may be directly involved in nuclear activities (*58*). The simplest scenario has the intracellular domain of Notch

proteolytically cleaved in a ligand-dependent fashion and subsequently translocated into the nucleus. Once in the nucleus, the intracellular domain of Notch could directly modulate nuclear events. However, with the exception of certain transformed and terminally differentiated mammalian cells, extensive immunocytochemical analyses have consistently failed to detect Notch in nuclei of developing animals (*59, 60*). Nevertheless, several recent studies provide a rationale for how Notch could function in the nucleus, despite the continuing lack of compelling immunocytochemical evidence.

A direct role for Notch in transcription is suggested by studies of transfected cultured cells, where, in the presence of Su(H)/CBF1, transcription from reporter genes containing Su(H)/CBF1-binding elements was stimulated by the addition of intracellular forms of Notch (*61*). Given that Su(H)/CBF1 and the intracellular domain of Notch physically interact and that the intracellular domain of mouse Notch 1 interacts with CBF1 when the latter is bound to DNA, these observations support a model in which the two proteins form a complex acting as a transcriptional activator (*61*). Transgenic flies carrying chimeric proteins composed of full-length Notch and the Gal4 DNA-binding domain reveal ligand-dependent expression of a sensitive reporter gene carrying several Gal 4 binding sites (*62*). This implies that the Notch protein is cleaved and translocated to the nucleus, where it activates transcription of the reporter gene. Experiments in cultured cells involving increasing amounts of transfected plasmids encoding a membrane-tethered, activated Notch protein eventually allow Notch to be detected in the nucleus. Parallel monitoring of the activity from a reporter construct shows that expression occurs before nuclear Notch immunoreactivity is observed. These results led to the conclusion that only small amounts of nuclear Notch, below the limit of detection, are sufficient for in vivo Notch signaling (*63*).

An intracellular mouse Notch 1 cleavage product derived from an activated, membrane-tethered form has been isolated from transfected cultured cells, and the site of cleavage identified. In this cell culture system, cleavage occurs at a conserved valine that lies either within or close to the transmembrane domain (*63*). However, mutating this residue attenuates but does not eliminate the ability of a membrane-tethered, activated Notch protein to stimulate transcription in cultured cells, and therefore, its relevance for the in vivo function of Notch remains to be determined. Although the protease responsible for intracellular cleavage has not been identified, genetic and biochemical data indicate that the transmembrane presenilin proteins may be involved in this event (*25, 26*). It should be mentioned, however, that protein immunoblot analysis of Notch with different antibodies in different systems always reveals the existence of a whole range of proteolytic products derived from full-length Notch [for example, (*23*)]. It is not known if any of these products reflect degradation intermediates of the receptor or fragments that are essential for signaling.

Despite the recent evidence supporting a nuclear translocation mechanism, some additional caveats need to be considered. Most importantly, the argument that only a small amount of nuclear Notch is necessary for signaling is challenged by the fact that nuclear localization does not correlate with the phenotypes elicited by the expression of constitutively activated forms of Notch. In *Drosophila*, expression of activated nuclear Notch has shown that the severity of a phenotype is influenced by the site of insertion or the transgene copy number and, hence, the level of nuclear Notch expressed. A similar range of phenotypic severity is also observed with membrane-tethered, activated Notch. This indicates that the range of responses to Notch signaling in vivo is quite broad and not readily saturated. Additionally, both membrane-tethered and nuclear forms appear to elicit similar, physiological responses. This notion is supported by the observation that both forms of activated Notch can rescue cuticle defects in Notch null embryos (*27, 62*). Similarly, in transgenic mice, the phenotypes resulting from the expression of either membrane-bound or nuclear forms of Notch are indistinguishable (*64*). Therefore, it seems unlikely that the extent of pathway activation can simply be explained on the basis of regulated nuclear entry, because the phenotypes associated with the expression of nuclear Notch, which shows large amounts of nuclear immunoreactivity, can be less severe than those resulting from membrane-tethered forms, which do not show nuclear immunoreactivity (Fig. 2). Indeed, if one assumes that nuclear entry is a pivotal condition for signal transduction, then one would expect the phenotypes resulting from nuclear Notch to be always more severe than those generated with membrane-tethered Notch; this, however, is not the case.

Therefore, although in the literature the simple proteolysis–nuclear translocation model of signaling is often accepted, many questions still remain. Experiments with *Drosophila* cell lines show that the normally nuclear Su(H) protein is sequestered in the cytoplasm when coexpressed with full-length Notch protein in cultured S2 cells but is translocated to the nucleus when Notch binds to its ligand, Delta (*65*). Although this observation suggests a potential regulatory mechanism, the complete elimination of Notch in vivo does not result in the production of activated phenotypes. Furthermore, immunocytochemical analysis of Su(H) during bristle development in cells known to undergo Notch signaling failed to provide evidence in support of a Su(H) cytoplasm-to-nucleus translocation model, implied by the cell culture assay (*66*). It is conceivable that an association between Notch and Su(H) in the cytoplasm is necessary for a posttranslational modification that is essential for signaling, but so far, there is

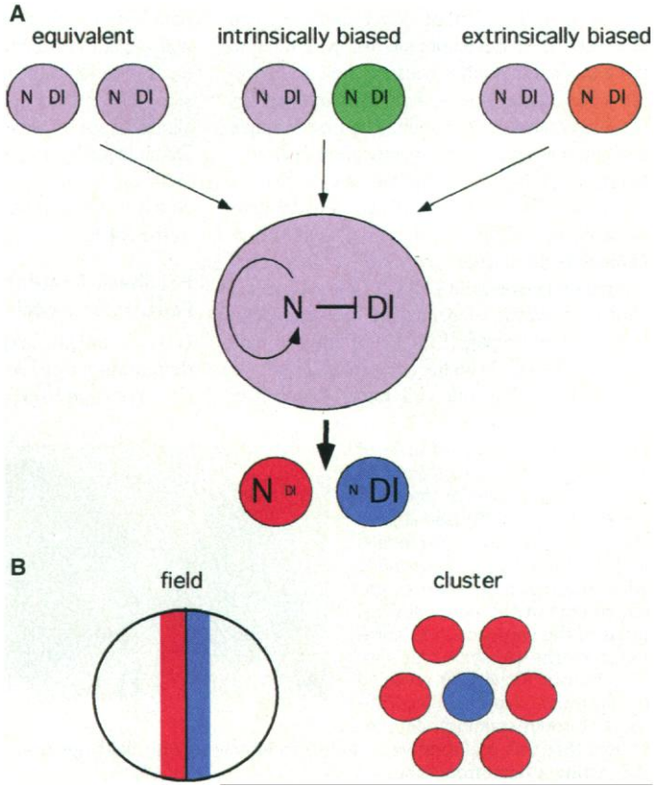

Fig. 3. Interaction between cellular neighbors. **(A)** Neighboring cells in developing tissues communicate through Notch signals to direct cell fate decisions. Neighbors may be equivalent, or in response to an intrinsic or extrinsic signal, may be biased toward a particular developmental fate. Feedback amplifies and consolidates the differences between Notch and Delta, causing one cell to enter the receiving mode and another cell to enter the signaling mode. **(B)** Such Notch-mediated interactions segregate specific cell lineages from clusters of cells and help define borders between fields of cells.

little experimental evidence supporting this notion (*6, 65*). In vivo analysis of the putative Notch cleavage site and biochemical analysis of posttranslational modifications of the various Notch pathway elements should help define more precisely the biochemical nature of Notch signaling.

Controlling Interactions Between Cellular Neighbors

Phenotypic analyses in both invertebrate and vertebrate systems indicate that the fundamental role of Notch in controlling cell fate choices occurs between adjacent cells that may or may not be developmentally equivalent. Lateral specification events (*2*) are often responsible for the segregation of specific lineages from clusters of precursor cells as well as for defining borders between fields of cells. As development proceeds, differences between neighbors caused by stochastic events and intrinsic or extrinsic factors are stabilized or amplified through Notch and Delta signals, eventually guiding the elaboration of distinct biochemical events that dictate final cell fates (Fig. 3). The essential quality of Notch-mediated cell communication depends on the differential expression of ligand and receptor in apposing cells. The juxtaposition of cells expressing differing amounts of ligand and receptor suggest that a cell can adopt a "signaling" mode simply by expressing more ligand relative to its neighbor (*42*). Thus, among apparently equivalent neighbors expressing both Delta and Notch, a small increase in ligand in one cell relative to the other could favor its adopting the signaling role.

As an illustration of Notch-mediated cell fate control in development, we will refer to three distinct examples because they are representative of the type of precursor interactions that are controlled by Notch. The first example is a paradigmatic lateral specification event between developmentally equivalent cells that involves the differentiation of two gonadal cells in *C. elegans*. Either of these cells can differentiate to an anchor cell (AC) or a ventral uterine precursor cell (VU) (*2*). The AC or VU choice, however, depends on the interaction between the receptor (LIN-12) of one cell with its ligand (LAG-2) on the adjacent cell. Activation of LIN-12 in one cell forces that cell to adopt the VU fate, whereas loss of LIN-12 function or failure of activation results in AC differentiation.

The next two examples are of nonequivalent precursor cells that communicate through Notch to progress to the next differentiation stage. In such cases, intrinsic or extrinsic factors confer a bias to one of two neighbors, which is then consolidated by Notch-Delta interactions. Thus, the second example involves the differentiation of the sensory organs of the peripheral nervous system. The sensory organ precursors (SOP) divide once to produce two cells (IIa and IIb), which each divide one more time, giving rise to a hair-and-socket cell pair and a neuron-and-sheath pair. Each step depends on Notch signaling and the presence of Numb (*67, 68*). This protein is expressed in SOPs and is asymmetrically segregated after each division, so that only one of the two daughter cells receives Numb. Cells that receive Numb antagonize Notch activity, whereas those that do not will adopt the fate associated with Notch activation. Thus, in two neighboring cells, both of which may express Notch and Delta, the intrinsic factor Numb can influence the activity of the receptor so that only one of the neighbors becomes responsive to Notch stimulation.

In the third example, Notch-dependent cell fate acquisition between nonequivalent precursor cells is influenced by an extrinsic signal (*69*). In the developing eye disc of *Drosophila*, the adjacent R3 and R4 photoreceptor precursor cells exist in what appears to be a gradient of an unknown signal, which emanates from the equator of the imaginal disc. This signal, ultimately transmitted through the Wingless signaling pathway elements Frizzled and Dishevelled, is capable of up-regulating Delta expression. The precursor cell that is closer to the signaling source, R3, expresses higher levels of Delta acquiring the R3 fate. The high levels of Delta on the surface of R3 succeed in activating Notch in the adjacent R4 precursor, guiding it to the R4 fate.

Feedback Regulation: An Essential Feature of Notch Signaling?

Genetic mosaic experiments involving the development of sensory organs in *Drosophila* first suggested the existence of a feedback mechanism between Notch and Delta, but the first direct experimental evidence came from studies with *C. elegans* (*42, 70*). With reporter constructs, dynamic changes of LIN-12 and LAG-2 expression in the emerging AC and VU had been observed, consistent with the existence of a transcriptional feedback mechanism .

In *Drosophila*, perhaps the best illustrative example of feedback regulation is offered during the development of the wing veins (*71*). Within the provein anlage, the central presumptive vein cells express high levels of Delta, whereas the more lateral cells, which give rise to intervein tissue, express high levels of Notch. Accumulation of Notch in the presumptive intervein cells appears to depend on Delta activity, because loss-of-function Delta mutations down-regulate Notch expression in this region. Conversely, increasing Delta expression in the presumptive intervein cells increases Notch expression in these cells. On the other hand, the level of Delta expression may be influenced by Notch activity, because increasing Notch activity through the expression of an activated Notch protein reduces Delta expression.

The factors that are responsible for Delta-dependent up-regulation of Notch expression during wing-vein morphogenesis are not known, nor is it known how general such a regulation mechanism may be. In *Drosophila*, there is evidence to suggest that the down-regulation of Delta expression upon Notch activation occurs through Su(H)-mediated up-regulation of the *E(spl)* genes. The bHLH proteins encoded by this locus function as transcriptional repressors and, in conjunction with the co-repressor Groucho, down-regulate expression of the *Achaete-Scute* complex genes (*17, 18, 72*). The proteins encoded by the proneural genes of the Achaete-Scute complex appear to be necessary for Delta expression (*19, 73*).

If the essence of Notch and Delta signals is to consolidate differences between adjacent cells, feedback regulation provides a plausible mechanism to amplify what may initially be small differences in the levels of Delta and Notch expression (Fig. 3). Evidence for the existence of Notch-Delta feedback regulation is accumulating from studies involving invertebrates and vertebrates, but there is no doubt that the scheme of feedback regulation (Fig. 3A) is a simplification. Precursor cells may be biased by extrinsic or intrinsic factors, such that the critical levels of either Notch or Delta needed to influence a particular fate may differ, depending on cell type or context (*74*). Irrespective of how general Notch-Delta feedback will turn out to be, the additional level of com-

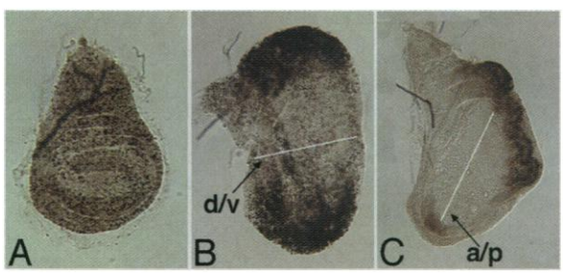

Fig. 4. Proliferative effects of Notch signaling. Expression of an activated, intracellular form of Notch along the (**B**) dorsal-ventral (d/v) or (**C**) anterior-posterior (a/p) boundary of *Drosophila* wing imaginal disc produces enlargements in the peripheral regions of the wing pouch as compared to the (**A**) wild-type disc (*78*). Mitotic activity, as revealed by bromodeoxyuridine incorporation (brown staining), demonstrates that this proliferative response to Notch activity does not overlap with Notch expression, suggesting a nonautonomous effect.

plexity it can add to the interpretation of genetic interactions must be considered (*75*).

The Cellular Spectrum of Notch Action

Extensive analyses of loss- and gain-of-function *Notch* mutations that have been carried out in *Drosophila* over the years and similar studies in *C. elegans*, sea urchins, frogs, fish, chickens, mice, and humans indicate a remarkable conservation of function. The analysis of loss of Notch function in vertebrate and invertebrate systems has demonstrated the extraordinary extent to which metazoan development relies on Notch signaling. In vertebrates, Notch malfunction has been associated with solid and lymphatic tumors and has been shown to disrupt aspects of neurogenesis, somite formation, angiogenesis, and lymphoid development (*1, 4*). The list of specific cell types affected by Notch in all these systems is growing rapidly, and their discussion is beyond the scope of this review. We will, however, briefly mention some developmental studies that have been conducted with *Drosophila* to convey the broad developmental role of Notch.

In *Drosophila*, one can state with confidence that there is hardly a tissue that is not affected by Notch. With antibodies against specific antigens as well as enhancer- and promoter-trap lacZ lines, which permit the labeling of most embryonic tissues, it was demonstrated that a loss of Notch signaling results in abnormalities in tissues derived from all three germ layers (*76*). Post-embryonically, Notch signaling is needed for the elaboration of the central and peripheral nervous systems, as well as for spermatogenesis, oogenesis, myogenesis, heart formation, and imaginal disc development. In imaginal discs, a requirement for Notch function was demonstrated at many different levels and at successive stages during the elaboration of a given lineage.

For instance, Notch appears to be involved in most, if not all, stages of eye development. Early in development, the expression of Notch antagonists driven by the eyeless promoter and enhancer, which is expressed in the eye primordia, abolishes eye formation (*77, 78*). Consistent with an involvement of Notch in establishing the entire program of eye development is the finding that Notch signaling is necessary and sufficient to trigger the expression of eyeless, a gene that is capable of triggering eye formation. Furthermore, depending on the genetic background, ectopic activation of Notch can induce the formation of ectopic eyes (*77*). Later in the development of the eye disc, the assembly of ommatidia relies on a series of successive cell interactions, almost all of which depend on Notch signaling (*79*).

Apart from controlling the fate of various cell types in the eye, Notch signaling can influence the overall patterning of that organ. Notch mutations were demonstrated to affect early, dorsal and ventral patterning of the eye (*80*), as well as the planar polarity displayed by the differentiated eye disc. The eye disc is an exquisite example of epithelial patterning with a highly polarized organization, in which ommatidial clusters display mirror-image symmetry in relation to the imaginal disc equator. The establishment of this pattern relies on the differentiation of the R3 and R4 photoreceptors. Both loss- or gain-of-function *Notch* mutations do not allow the R3 and R4 precursors to respond differentially to a signal emanating from the equator of the eye disc, and consequently, the planar polarity of the eye is lost (*69*).

Notch signaling may, in fact, play a general role in the establishment of asymmetry. Feather primordia show a polarized expression of Notch pathway elements, and mutations in the Notch homolog of the Australian sheep blowfly, *Lucilia cuprina*, show asymmetry phenotypes (*81*). In addition to Notch mutations, certain alleles of *Delta* and *Deltex* produce cuticular asymmetries (*82*). The notion that Notch signaling is used to amplify and thus stabilize the differential response of neighboring cells positioned within a signal gradient may, in an analogous fashion to the R3 and R4 situation in the eye, reflect a broadly used mechanism to establish chirality in development.

In order to influence so many different developmental decision, Notch must obviously interact with intrinsic cellular factors as well as other signaling pathways. Modifier screens have demonstrated that Notch activity can be modulated, for example, by mutations in elements of the Wingless and EGF signaling pathways (*22, 75*). The definition of borders between cellular fields is a crucial event during the formation of appendages, and many studies have established a role for Notch in the development of the dorsal-ventral border of the wing. A brief consideration of the involvement of Notch in wing margin development provides a good example of how Notch interacts with other signaling pathways and cell-intrinsic factors (*78, 83, 84*). Wing margin formation requires the coordination of Wingless and Notch signaling as well as Vestigial gene expression. Disheveled is a component of the Wingless pathway that can directly interact with the intracellular portion of Notch (*85*). The *Vestigial* gene contains Su(H) binding elements and, thus, is a target of Notch signaling. Nubbin is a nuclear protein that binds to regulatory sequences of the *Vestigial* gene and influences its availability as a Notch target (*86*). All of these activities must be properly integrated in space and time to effect the formation of the dorsal-ventral wing border.

It is now clear that the control of cell fates by Notch is highly dependent on developmental context. In many precursor cells, activation of the Notch receptor can inhibit the progression of a cell to the next differentiation state (*6*). However, it appears that cells are not "frozen" into one particular state by Notch activity but can respond to certain signals. In this respect, it is worth noting that Notch proteins have been detected in postmitotic neurons, cells that are considered terminally differentiated . The role of Notch in terminally differentiated tissue is not known, but it has been speculated that Notch may confer onto these cells some degree of developmental plasticity (*59*).

Notch Influences Apoptosis and Proliferation

A series of recent studies revealed that apart from the well-documented involvement of Notch in differentiation, both proliferation and apoptotic events can be affected by Notch signaling. In mice, receptor activation in the thymus renders thymomas resistant to glucocorticoid-induced apoptosis (*87*). Likewise, Notch activation appears to inhibit apoptosis in murine erythroleukemia cells (*88*). Two-hybrid assays demonstrated that the intracellular domain of Notch interacts with Nur77, a nuclear receptor that participates in apoptotic events (*39*). When constitutively activated forms of the receptor, analogous to the oncogenic forms of the vertebrate Notch homolog TAN1 (*64*), are overexpressed in a T hybridoma cell line, these cells are protected from Nur77-dependent apoptosis. Given these observations, it is possible that the oncogenic nature of TAN1 may reflect an inhibition of cell death rather than a stimulation of proliferation. On the other hand, the involvement of Notch activation of mammary tumors in mice is most likely the result of abnormal proliferation (*1*).

A link between proliferation events and Notch has been seen in several instances. In *Drosophila*, Notch, together with Wingless, induces cell cycle arrest within the so-called nonproliferative region, located at the dorsal-ventral boundary of the developing wing (*89*). The genetic analysis that was performed indicates that the involvement of Notch in the cell cycle regulation is indirect and results from modulating Wingless and *Achaete-Scute* complex gene activity.

In contrast to cell cycle arrest, Notch activation can also induce proliferation (*78, 79, 90*). In *C. elegans*, hermaphrodites and males homozygous for a constitutively active form of GLP-1 have germ cells that never exit the mitotic cycle (*91*). Additionally, the expression of an activated form of the Notch receptor along the dorsal-ventral or the anterior-posterior boundary of the *Drosophila* wing induces mitotic activity. This effect of Notch is, however, indirect, because the regions of the highest Notch activity do not coincide with the regions of the highest mitotic activity (Fig. 4) (*78*). The elements mediating the nonautonomous effect of Notch on prolifera-

tion are unknown; is it is also unknown how general such effects are, although similar nonautonomous effects on proliferation have also been documented in the leg disc (*90*).

The examination of receptor activation in different imaginal discs demonstrates that the ability of Notch to influence cell proliferation is the result of synergistic effects between Notch and other genes and depends on developmental context. For instance, the simultaneous expression of activated Notch and Vestigial in the eye disc results in an extreme overgrowth of this imaginal disc (*78*), whereas other discs remain relatively unaffected.

Conclusion

Notwithstanding the complexity of the developmental action of Notch, some general principles underlying the action of this fundamental cell-interaction mechanism have emerged. Developing animals use Notch signaling to amplify and consolidate molecular differences between adjacent cells. The implementation of a particular developmental program modulated by Notch depends, however, on how Notch integrates its activity with other cellular factors. A developing metazoan uses many different means to modulate Notch activity. Direct interactions with Notch signaling or interference with the synthesis and maturation of Notch signaling elements are strategies used to modulate Notch signals. The fundamental nature of this signaling mechanism and its ability to influence many specific developmental events in a context-dependent manner may find medical applications. It is conceivable that appropriate manipulation of Notch signaling may become a useful tool in addressing a variety of human dysplastic conditions as well as tissue regeneration.

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