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Notch Signaling

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The Notch/Lin-12/Glp-1 receptor family mediates the specification of numerous cell fates during development in *Drosophila* and *Caenorhabditis elegans*. Studies on the expression, mutant phenotypes, and developmental consequences of unregulated receptor activation have implicated these proteins in a general mechanism of local cell signaling, which includes interactions between equivalent cells and between different cell types. Genetic approaches in flies and worms have identified putative components of the signaling cascade, including a conserved family of extracellular ligands and two cellular factors that may associate with the Notch Intracellular domain. One factor, the *Drosophila* Suppressor of Hairless protein, is a DNA-binding protein, which suggests that Notch signaling may involve relatively direct signal transmission from the cell surface to the nucleus. Several vertebrate Notch receptors have also been discovered recently and play important roles in normal development and tumorigenesis.

Multicellular development is governed by the combinatorial and sequential activity of genes that gradually restrict the developmental potential of cell lineages during differentiation. Large-scale mutational analyses performed in *Drosophila* have led to the identification of genetic pathways that control the assembly of a complex multicellular organism from a unicellular oocyte (*1*). The *Drosophila* embryonic axes are established by four distinct groups of maternal-effect genes: the anterior group, the posterior group, the terminal group, and the dorsoventral group. These maternal groups regulate sets of zygotic genes, termed gap, pair rule, and segment polarity genes, which progressively subdivide the embryo into ordered segments. Finally, each segment is assigned a specific identity by the homeotic genes. Whereas these genes provide the blueprint for the overall pattern of the *Drosophila* body plan, the specification of individual cell fates within a tissue is thought to be determined by invariant patterns of cell lineage as well as by regulative events that are dependent on local cell interactions. As discussed below, regulative interactions may occur between cells that are initially equivalent (lateral specification) or nonequivalent (inductive signaling) and result in changes in intracellular physiology in response to extracellular signals.

In this review, we focus on one signaling pathway that plays a central role in the specification of cell fates that occur through local cell interactions in a wide variety of different tissues and organisms. This evolutionarily conserved pathway is mediated by the transmembrane receptor protein encoded by the *Notch* gene of *Drosophila* and its vertebrate homologs, as well as by related proteins that are encoded by the *lin-12* and *glp-1* genes of *C. elegans* (*2*, *3*). Recent studies have demonstrated the pleiotropic nature of Notch activity and its functional requirement throughout development in several species. Notch proteins have been found to function in both types of local cell interactions, namely lateral and inductive signaling. We discuss how these observations, together with data on the effects of constitutive Notch activation in several different developmental contexts, argue for a general function for Notch in the regulation of the competence of a cell to respond to more specific developmental cues. We also summarize data on the interaction of the Notch, Lin-12, and Glp-1 receptor proteins with their putative ligands in both *Drosophila* and *C. elegans*, and with putative intracellular components of the signaling pathway in *Drosophila*. Finally, we present a molecular model for some of the intracellular events in the pathway and their possible connection to nuclear events involved in Notch signaling.

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Lateral Versus Inductive Signaling

During the development of complex multicellular organisms, numerous local cell signaling events are required for proper cell-fate determination. Studies in relatively simple model organisms have distinguished signaling events that involve equivalent cells from those that involve different cell types (Fig. 1). Among a group of initially equivalent cells, a mechanism originally termed lateral inhibition (*4*) could allow an individual cell or a group of cells to be singled out from the surrounding cells. Because signals may be transmitted back and forth between the two emerging cell types, this type of signaling should more properly be termed lateral specification (*5*). The molecular details of lateral specification are still largely hypothetical,

but slight, perhaps random fluctuations in some signaling activity present in the original population of equivalent cells might be amplified in some cells and inhibited in neighboring cells. Examples of cell-fate determinations thought to involve lateral specification include the segregation of neural and epidermal precursor cells in the ventral ectoderm of the *Drosophila* embryo, and the formation of the anchor cell and the ventral uterine precursor cell of the C. *elegans* hermaphrodite gonad (*2*, *3*, *5*).

A second type of regulative signaling, termed inductive signaling, operates between nonequivalent cells. In this case, the signaling cell and the receiving cell begin the process with different properties, including their repertoires of cell-surface receptors and ligands. Under these conditions, the restricted expression of particular receptors and ligands, and the timing of cell-cell contacts, might be important factors that control the inductive interaction. Examples of inductive cell-fate specification that have been studied extensively at single cell resolution include the induction of the R7 cell fate by the R8 cell during *Drosophila* eye development and the induction of the vulval precursor cells by the anchor cell in the C. *elegans* gonad (*2*). As an uncommitted cell progresses toward its terminally differentiated fate, it is presumably subject to numerous local interactions with other cells, which perhaps include both lateral and inductive signaling events that may occur either sequentially or concurrently.

Notch and Notch-Like Proteins in Flies, Worms, and Vertebrates

The *Notch* gene of *Drosophila melanogaster* encodes a 300-kD transmembrane receptor with a large extracellular domain of 36 tandem epidermal growth factor (EGF)–like repeats and three cysteine-rich Notch/Lin-12 repeats, as well as an intracellular domain with 6 tandem Ankyrin repeats and a PEST sequence (*6*) (Fig. 2). Notch participation in local intercellular communication was first appreciated in studies of embryonic neurogenesis in *Drosophila* (*7*). During normal development of the fly central nervous system (CNS), an ectodermal monolayer of ~1800 equipotent cells gradually segregates into two distinct cell populations, namely neuroblasts, which delaminate from the monolayer and migrate dorsally to produce neuronal lineages, and dermoblasts, which remain at the ventral surface of the embryo and give rise to epidermal structures. Within this ectodermal region, so-called proneural cell clusters express transcription factors that are encoded by the *achaete-scute* gene complex (*8*). Local cell interactions mediated by Notch gradually restrict the expression of these factors to one cell, the neuroblast, whereas the surrounding dermoblasts cease to express *achaete-scute* (*8*). This process is considered a classic case of lateral specification, because laser ablation of a delaminating neuroblast in the similarly patterned CNS of the grasshopper causes an adjacent nonneuronal precursor cell to be rerouted into the neuroblast fate, which fulfills the prediction of the lateral signaling model (*9*). In *Drosophila* embryos without a zygotic supply of functional Notch protein, virtually all cells in the ventral ectoderm continue to express *achaete-scute* and adopt the neuroblast cell fate, which leads to a lethal hypertrophy of the nervous system, a phenotype that has been termed neurogenic (*7*). Moreover, genetic mosaic analysis of *Notch* activity during formation of the adult sensory bristles has demonstrated that *Notch* acts cell-autonomously, and that relatively modest differences in the genetic dosage of *Notch* between adjacent cells is sufficient to bias the otherwise stochastic selection of cell fates (*10*). These results reinforce the notion that the Notch protein acts a receptor in lateral signaling events that involve the sorting of epidermal and neural cell types.

Recent data about Notch protein distribution and mutant phenotypes, however,

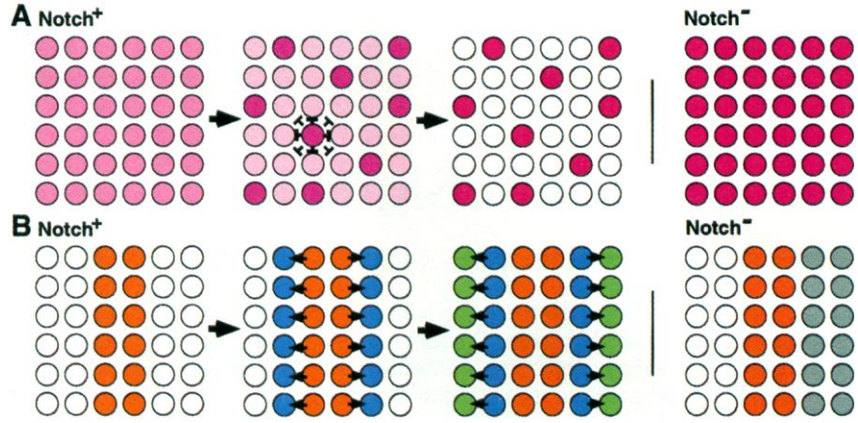

Fig. 1. Notch involvement in cell-fate decisions controlled by lateral specification or inductive signaling. (**A**) Lateral specification. Among a field of initially equivalent wild-type (*Notch*$^+$) cells, random fluctuations in a putative signaling component are amplified cell-autonomously such that differences arise between adjacent cells. These differences are enhanced by the production of a locally acting inhibitory signal, and result in a spaced pattern composed of two distinct cell types. In *Notch*$^-$ cells that lack the proposed receptor for the inhibitory signal (right panel), all cells adopt the same fate. (**B**) Inductive signaling. Cell fates are determined by signals transmitted between nonequivalent cells, with the final pattern dependent on the timing, spatial arrangement, and specificity of the inductive interactions. In *Notch*$^-$ cells (right panel), the absence of Notch function might prevent inductive interactions from occurring (columns at left), or permit aberrant inductive events to occur (columns at right).

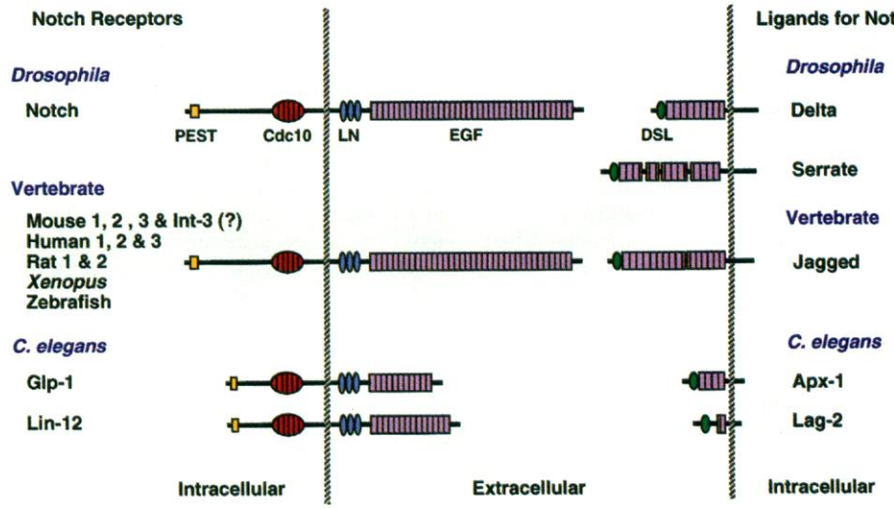

Fig. 2. Structures of Notch and Notch-like proteins (left) and their putative ligands (right) from different species. The mouse and human Notch3 proteins actually contain 34 instead of 36 extracellular EGF-like repeats (not shown) and the exact number of EGF-like repeats in the mouse Int-3 protein is not known (*20*).

reveal that the term neurogenic is an insufficient if not misleading description of Notch activity. The *Notch* gene is very pleiotropic with respect to its expression and functional requirement throughout *Drosophila* development, in contrast to many other tissue-specific or cell type–specific receptors, such as the Sevenless protein (*2*). Notch is broadly expressed in the fly embryo, and continues to be expressed in uncommitted and proliferative cell populations at later stages in the larval and pupal imaginal discs and in the adult ovaries and testes (*11*). Within these tissues, Notch protein is detected in almost all cells and is most highly concentrated at the apical membranes of polarized cells (*11*). Consistent with these expression data, analyses of *Notch* conditional and recessive viable mutant alleles have shown that Notch is required not only for nervous system development, but also for the proper formation of the mesoderm, germ line, ovarian follicle cells, larval Malpighian tubules, adult sensory bristles, and eye structures (*12*) (Fig. 3). These data also implicate Notch in cell-fate decisions mediated by inductive signaling, which shows that Notch is not dedicated exclusively to a lateral specification mechanism. The requirement for Notch function in the developing compound eye is particularly revealing, because ommatidia are assembled by a series of inductive recruitments between cells of different equivalence groups. In fact, induction of the R7 cell by the R8 founder cell of each developing ommatidium, one of the most intensively studied and best understood inductive cell interactions (*13*), requires Notch activity to occur properly and may be blocked by the appropriately timed expression of a constitutively activated form of the Notch receptor (*14*).

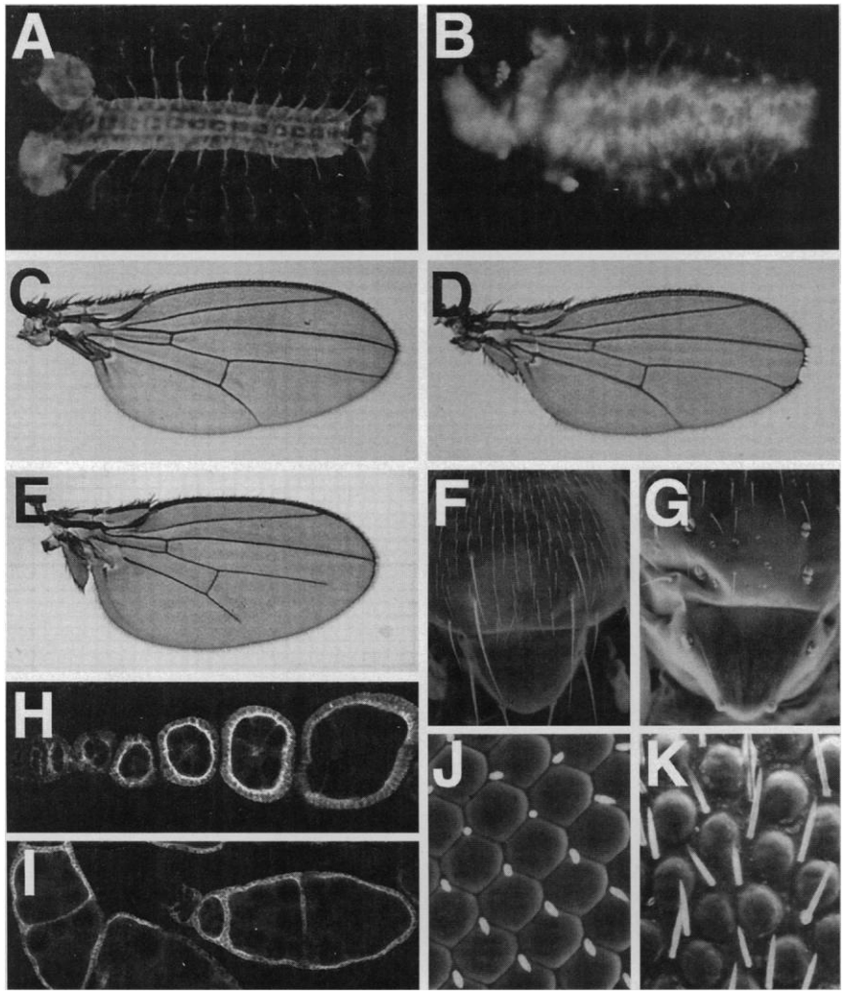

Fig. 3. Pleiotropy of Notch function illustrated by *Notch* gene mutant phenotypes in *Drosophila*. (**A**) Wild-type embryo and (**B**) homozygous *Notch* deficiency embryo stained with horseradish peroxidase antibodies with normal and hypertrophied nervous system structures, respectively. (**C**) Wild-type wing, (**D**) heterozygous *Notch* deficiency wing with dominant notching of the wing blade tip, and (**E**) wing of a homozygous *Abruptex* mutant of *Notch*, showing characteristic shortening of the LIV and LV wing veins. (**F**) Wild-type bristle pattern on the adult thorax and notum and (**G**) bristle group cell-fate alterations on the adult thorax and notum caused by inducible expression of activated Notch in transgenic flies. (**H**) Normal and (**I**) fused egg chambers of temperature-sensitive *Notch*^{ts1} homozygous mutant females cultured at permissive and restrictive temperatures, respectively, and stained with Notch antibodies. (**J**) Wild-type external eye surface and (**K**) eye surface of a homozygous *facet* mutant of *Notch*, showing irregular ommatidial packing and extra lens material deposited between facets. The *Notch* mutants are described in (*27*) and the activated Notch transgenic flies are described in (*44*).

The involvement of Notch and Notch-like proteins in both lateral and inductive signaling events has been demonstrated by studies on the Lin-12 and Glp-1 proteins of *C. elegans*. Lin-12 and Glp-1 are both structurally similar to Notch, although both possess fewer EGF-like repeats in their extracellular domains than Notch (*6*) (Fig. 2). Genetic analyses, combined with laser ablation studies, have shown that Lin-12 is required for the lateral specification of the anchor cell and the ventral uterine precursor cell, and later for lateral specifications among the vulval precursor cells (*15*). Glp-1, on the other hand, participates in the induction of the germ line by the distal tip cell and in the induction of pharyngeal progenitor cells, and is not known to be required for any lateral specification events (*16*, *17*). It is unclear, however, whether Lin-12 and Glp-1 may be easily assigned to the two different types of signaling mechanisms, because phenotypic characterization of *lin-12 glp-1* double mutant animals has uncovered substantial functional redundancy between them, and because the Glp-1 protein can functionally substitute for Lin-12 in transgenic animals and in certain mutants (*18*).

Within the past few years, several homologs of the *Drosophila* Notch protein have been identified in vertebrates, including zebrafish, frogs, mice, rats, and humans (*19*, *20*) (Fig. 2). In mice and men, each of which possesses at least three different Notch proteins, chromosomal rearrangements or retroviral insertions that affect *Notch* or *Notch*-like genes are associated with certain neoplasias (*19*). The vertebrate *Notch* genes are expressed throughout developing tissues at embryonic stages and in proliferative cell layers of mature tissues, in a manner similar to the expression of *Notch* in *Drosophila* (*19*, *20*). Antibodies to two of the three known human Notch proteins have been used to examine Notch protein levels in a sample of human tissues, including certain tumors (*21*). In metaplastic cervical tissues as well as in certain cancerous lesions, Notch proteins are detected at elevated levels relative to the surrounding normal tissue. Whereas further analysis is needed to determine the role of Notch in these neoplastic conditions, the available data support the notion that Notch signaling activity is correlated with the differentiation state of these human tissues. Function-

al studies of two vertebrate Notch homologs have been undertaken in *Xenopus* embryos and in transgenic mice. Expression of a constitutively activated Notch protein in *Xenopus* causes the loss of dorsal structures as well as neural and mesodermal hypertrophy (*22*). Mice defective for one of their *Notch* genes die before 11.5 days of gestation with extensive regions of cell death (*23*). The lethality of these mice implies that the multiple *Notch* genes found in mammals are not completely redundant in function. Although the study of vertebrate Notch proteins is still in the early stages, the data so far indicate that they, like their counterparts in the fly and the worm, are intimately involved with cell-fate specifications in many developmental contexts.

Defining Components of the Notch Pathway

Genetic analyses in *Drosophila* and *C. elegans* have identified several loci that encode putative components of the Notch pathway on the basis of their genetic interactions with *Notch*, *lin-12*, or *glp-1* and with one another, their mutant phenotypes, and in some cases the demonstration of molecular interactions between their products and the receptor protein. These genes include the *Drosophila Delta* and *Serrate* genes and the *C. elegans Lag-2* and *Apx-1* genes, all of which encode putative extracellular ligands, the *Drosophila deltex* gene, which encodes a cytoplasmic protein, and the *Drosophila Hairless*, *Suppressor of Hairless*, *mastermind*, and *Enhancer of split* loci, which all encode potential nuclear factors. This review will focus on these components of the pathway, but it is likely that additional gene products function in Notch signaling. Indeed, genetic and molecular approaches have produced an ever-growing list of candidate molecules. In *Drosophila*, for example, the *strawberry notch*, *vestigial*, *shaggy*, *Star*, *wingless*, and *scabrous* genes all display genetic interactions with *Notch* (*24–26*), and the *neuralized*, *big brain*, *pecanex*, and *almondex* genes display *Notch*-like neurogenic mutant phenotypes (*27*). Ultimately, confirmation that the products of any of these genes actually participate in Notch signaling awaits more extensive molecular and functional data than are presently available.

Ligands for Notch

Genetic and molecular studies have identified a family of structurally related ligands for the *Drosophila* Notch receptor and for the *C. elegans* Lin-12 and Glp-1 receptor proteins. These ligands, encoded by the *Delta* and *Serrate* genes in *Drosophila* (*28*) and by the *lag-2* and *apx-1* genes in C. elegans (*5*, *17*, *29*, *30*), are all membrane-anchored extracellular proteins (Fig. 2). The extracellular domains of all four putative ligands contain a variable number of EGF-like repeats and a second cysteine-rich conserved motif, referred to as the DSL (Delta-Serrate-Lag-2) region (*29*, *30*). Structural, expression, and functional analyses have identified putative vertebrate Notch ligands in *Xenopus*, mouse, rat, chicken, and humans (*31*). These vertebrate molecules have overall structures similar to Delta and Serrate, and all have extracellular regions with EGF-like repeats and the cysteine-rich DSL motif. The DSL region appears to be important for ligand function, because point mutations that affect conserved cysteines in the Lag-2 DSL motif result in strong loss-of-function phenotypes (*30*). In contrast, the intracellular domains of all the putative Notch ligands display no significant sequence similarity, and replacement of most of the Lag-2 intracellular domain with a β-galactosidase fusion protein has no discernible effect on Lag-2 function (*30*).

Cell aggregation assays have shown that both Delta and Serrate bind to the extracellular EGF-like repeat region of Notch and that only two of the extracellular EGF-like repeats of Notch, namely repeats 11 and 12, are necessary and sufficient for this interaction (*32*). Similar binding data have not yet been obtained for Lag-2 and Apx-1, and no such information is available yet for any vertebrate Notch ligands. However, the observation that chimeric *Drosophila* Notch molecules with the 11/12 EGF-like repeats of vertebrate Notch proteins are capable of interacting with insect Delta and Serrate suggests that there is a high degree of functional conservation in the ligand-binding properties of Notch proteins from different species (*32*).

In *Drosophila*, genetic mosaic analysis has demonstrated that Delta acts nonautonomously, consistent with its proposed role as a signaling ligand (*10*). Loss-of-function mutations in *Delta* cause the same embryonic cell-fate transformations as do null mutations in *Notch*, which indicates that the Notch receptor may be activated upon binding of Delta (*2*, *33*). Similar conclusions have been drawn from studies of Notch and Delta activity during adult sensillum development and oogenesis (*10*, *12*, *34*). However, antagonistic genetic interactions between these two genes have also been documented, which suggests that in certain situations, Delta may negatively regulate Notch (*35*, *36*). Mosaic analysis of antagonistic interactions between *Notch* and *Delta* have raised the possibility that Delta acts cell autonomously in these cases (*36*). Antibody patching experiments in transfected S2 cells also provide evidence that Notch and Delta may actually bind to one another when coexpressed at the surface of the same cell (*32*).

Unlike *Delta*, the *Serrate* gene is not associated with any neurogenic loss-of-function phenotypes. However, when expressed at high levels, Serrate can partially compensate for loss of Delta function during embryonic development (*37*). Analysis of Serrate function in wing development suggests that Serrate may act as a cell-autonomous (autocrine) ligand for Notch (*38*). The widespread expression of Notch and its ligands throughout large cell populations raises the question of how the interactions of Notch with Delta and Serrate are regulated in order to coordinate cell-fate specifications (*28*, *38*, *39*). In S2 cell aggregates, Notch-Delta and Notch-Serrate complexes are rapidly internalized in vesicular structures within the Notch-expressing cells, and similar vesicles are also detected in vivo (*11*, *32*). Although Notch and Delta are coexpressed in many cells of the developing eye imaginal disc, the vesicles may be restricted to cells undergoing cell-fate determination (*39*), which suggests that productive Notch binding may require the modification of ligands presented by signaling cells. Many of these unresolved issues might be clarified by further study of the cell-fate alterations that underlie the complex mutant phenotypes and by a more complete description of the subcellular distributions of Notch and its ligands in various *Drosophila* tissues.

In *C. elegans*, Lag-2 is apparently a ligand for both Lin-12 and Glp-1 (*5*, *29*, *30*), whereas the Apx-1 putative ligand is so far only known to be involved in Glp-1-mediated pharyngeal induction (*17*). Genetic and laser ablation data support the notion that binding of these ligands by Lin-12 and Glp-1 results in receptor activation (*5*, *17*, *18*, *29*, *30*). Expression studies in *C. elegans* have provided visualizations of Lin-12 and Glp-1 activities during lateral specification and inductive signaling, respectively. During lateral specification of the anchor cell (AC) and ventral uterine precursor cell (VU) in the hermaphrodite gonad, *lin-12* and *lag-2* are initially expressed in both equipotent cells, but then undergo reciprocal changes in expression such that *lin-12* and *lag-2* expression become restricted to the presumptive VU and the presumptive AC, respectively (*5*). The dynamic changes in *lin-12* and *lag-2* expression in the emerging AC and VU are strong evidence for the existence of a transcriptional feedback mechanism between the two genes in lateral signaling. In contrast, during induction of the germ line by the distal tip cell, Lag-2 protein originates from the signaling distal tip cell whereas Glp-1 is restricted to the mitotic cell region of the germ line (*30*, *40*). Like Delta, receptor-bound Lag-2 is apparently internalized in vesicles within the Glp-1-expressing cells of the germ line (*30*). No expression data are yet available for Apx-1, the second Glp-1 ligand.

The finding that only two of the 36 EGF-like repeats of Notch are necessary and sufficient to promote cell aggregation with Delta and Serrate prompted speculation that the remaining extracellular domain may harbor similar modular binding domains for other ligands, thus making Notch a multifunctional receptor (*32*). This idea could provide an explanation for the pleiotropic action of Notch and a rationale to search for additional genes that interact genetically with Notch and encode molecules that could potentially serve as extracellular ligands. So far, two genes have been found that interact genetically with *Notch* and encode surface proteins, *scabrous* and *wingless* (*25*, *26*). The *scabrous* gene encodes a fibrinogen-related secreted protein that is necessary to establish the spaced pattern of R8 cells in the developing eye disc and for some aspects of the adult bristle pattern, but it is not required for the development of any other tissues (*25*). Molecular interactions between Notch and Scabrous have not yet been detected, however, and careful examination of the pattern of R8 cell determination during the earliest stages of ommatidial formation has demonstrated that the contribution of Scabrous is spatially and temporally separate from that of Notch and Delta (*41*). Thus, the Scabrous protein is unlikely to function as a biochemical ligand for Notch.

The *wingless* gene product is homologous to the mammalian proto-oncoprotein Wnt-1 and is thought to act as the signal in a cell interaction mechanism that specifies differentiation of the embryonic epidermis as well as imaginal structures such as the wing (*42*). Although some components of the Wingless signal transduction pathway have been identified, the receptor for Wingless remains elusive. The ability of certain complex *Notch* mutant combinations to mimic *wingless* mutant phenotypes, and genetic interactions between *Notch* and *wingless*, has prompted speculation that Notch may serve as the long-sought Wingless receptor in spite of major dissimilarities in their expression patterns and primary mutant phenotypes (*26*). Until biochemical data are available to support such an assertion, it would seem prudent to exercise caution before assuming that either Wingless or Scabrous are Notch ligands, especially in view of their mutant phenotypes and their structural dissimilarity to bona fide Notch ligands.

Developmental Effects of Notch Activation

Insight into the developmental role and the general nature of Notch signaling has emerged from studies with truncated, constitutively activated forms of Notch in several species. These engineered Notch forms, which lack extracellular ligand-binding domains, resemble the naturally occurring oncogenic variants of mammalian Notch proteins and are constitutively activated by phenotypic criteria (*3*, *14*, *22*, *43*–*46*). Ubiquitous expression of activated Notch in the *Drosophila* embryo suppresses neuroblast segregation without impairing epidermal differentiation (*43*, *44*). Persistent expression of activated Notch in developing imaginal epithelia likewise results in an overproduction of epidermis at the expense of neural structures (*43*). Neuroblast segregation occurs in temporal waves that are delayed but not prevented by transient expression of activated Notch in the embryo (*43*). Transient expression in well-defined cells of the *Drosophila* eye imaginal disc causes the cells to ignore their normal inductive cues and to adopt alternative cell fates (*14*) (Fig. 4).

Studies utilizing transient expression of activated Notch in either the embryo or the eye disc indicate that once Notch signaling activity has subsided, cells may recover and differentiate properly or respond to later developmental cues (*14*, *43*). In *Xenopus* embryos and mammalian cultures cells, activated Notch interferes with the differentiation of neural and mesodermal cell lineages (*22*, *45*). In *C. elegans*, gain-of-function cell-fate transformations are caused by a similarly truncated intracellular fragment of Lin-12 (*43*), as well as by a Glp-1 polypeptide that consists of little more than the intracellular Ankyrin repeat region (*46*). The cell-fate alterations produced by truncated Notch proteins in different organisms show that Notch possesses an intrinsic signaling activity that cannot be explained easily in terms of cell adhesion or indirect receptor-ligand recruitment models (*47*). The *Xenopus* data suggest that Notch activation regulates the competence of many different cell types to respond to other differentiative cues (*22*), an idea that is supported by the data obtained in *Drosophila* and mammalian cultured cells. Some have argued that these experiments do not support such a model and that the data instead show that Notch activity directly controls binary cell-fate decisions (*3*). It is difficult to reconcile this idea with the fact that neuroblast precursors are only temporarily prevented from selecting their correct cell-fate by transient Notch activation in the *Drosophila* embryo, unless these binary cell-fate decisions are reversible and do not involve commitment.

Intracellular Components of Notch Signaling

Genetic and molecular studies have identified two *Drosophila* genes, *deltex* and *Suppressor of Hairless*, whose products may interact directly with the Ankyrin repeats of Notch (*48*, *49*). The participation of deltex in Notch signaling was realized as a result of the ability of *deltex* mutations to suppress the lethality of certain heteroallelic *Notch* mutant combinations, and supported by the finding of many genetic interactions between *deltex* and other Notch pathway loci (*50*). All known *deltex* mutants are homozygous viable and have *Delta*-like wing phenotypes, although it is unclear whether any of the existing alleles are null mutations (*27*, *48*). The *deltex* gene encodes a previously uncharacterized cytoplasmic protein of 737 amino acids with a ubiquitous tissue distribution throughout development (*48*, *51*). The protein is composed of three domains separated by glutamine-rich stretches. Functional studies in cultured *Drosophila* S2 cells and in yeast have revealed that all three Deltex domains mediate homotypic interactions between Deltex proteins, and that Del-

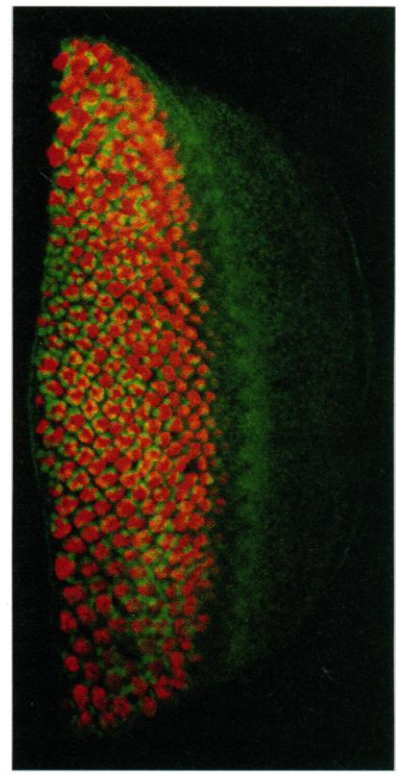

Fig. 4. Expression of a truncated, activated form of Notch transiently blocks neuronal cell-fate determination in the developing *Drosophila* eye. An eye imaginal disc from a transgenic larva in which the intracellular domain of Notch is expressed under the control of *sevenless* gene regulatory sequences shows that this Notch fragment is detected in nuclei (green) and that it alters the pattern of neuronal cell recruitment (red). Red and green nuclei do not overlap, which shows that photoreceptor cell precursors expressing activated Notch (green) are prevented from adopting their proper neuronal fate (red). Posterior is to the left and the morphogenetic furrow of the disc is at the right. See (*14*).

tex domain I binds to the intracellular Ankyrin repeat region of Notch (*48*, *52*).

Experiments in transgenic flies have provided clues regarding the role of Deltex in Notch signaling (*52*). Overexpression of Deltex and activated Notch produce similar adult phenotypes, consistent with the idea that Deltex acts as a positive regulator of the Notch pathway (*48*, *52*). Moreover, *deltex* loss-of-function phenotypes are rescued by activated Notch, which indicates that Notch activation bypasses the requirement for Deltex, and implies that Deltex may function upstream of the Notch receptor (*52*). Overexpression of Deltex domain I alone produces phenotypes that resemble those generated by the full-length Deltex protein, which suggests that Deltex exerts its effects on Notch through its interactions with the Notch Ankyrin repeats.

A second protein that may interact directly with the intracellular Ankyrin repeat region of Notch is the product of the *Suppressor of Hairless* [Su(H)] locus (*49*). Rare, gain-of-function alleles of *Su(H)* have been isolated in a genetic screen for mutations that attenuate Notch signaling in the developing *Drosophila* eye, and numerous allele-specific phenotypic interactions have been uncovered between *Su(H)* and *Notch*, *Delta*, and *deltex* in various tissues and at multiple developmental stages (*49*). In contrast to *deltex*, all known *Su(H)* mutations (>20) are homozygous lethal, and the *Su(H)* gene displays neurogenic phenotypes in the peripheral nervous system (*27*, *53*). Taken together, these genetic observations strongly suggest that the Suppressor of Hairless protein plays a central role in Notch signaling.

The *Drosophila Su(H)* gene encodes a protein of 594 amino acids that is highly related to a family of mammalian transcription factors referred to as RBP-Jκ (recombination signal sequence binding protein for Jκ genes), CBF1 (C-promoter binding factor 1), or KBF2 (kappa binding factor 2) (*53*). The murine Su(H) protein was first isolated by virtue of its ability to bind the recombination recognition sequences of immunoglobulin Jκ genes, which led to the suggestion that it catalyzes immunoglobulin V(D)J gene rearrangements (*54*). However, recent studies indicate that the mammalian Su(H) protein does not bind to Jκ gene recombination sequences, but is instead involved in the Epstein-Barr virus–induced immortalization of B cells, an important event in the etiology of certain human malignancies (*55*). The protein binds to the promoters of several viral and cellular genes and interacts directly with a viral transactivator protein termed Epstein-Barr virus nuclear antigen 2 (EBNA2), which enables the virus to subvert the normal program of B cell differentiation.

Whereas no data yet implicate the mammalian Su(H) homologs in Notch signaling, the *Drosophila* Su(H) protein is sequestered in the cytoplasm when coexpressed with Notch protein in cultured S2 cells, and is translocated to the nucleus when Notch binds to its ligand Delta (*49*). Cytoplasmic retention of Su(H) requires the intracellular Ankyrin repeats of Notch, which associate with the Su(H) protein in the yeast interaction trap assay. Moreover, the Su(H) protein exhibits weak amino-acid similarity to the NH_2-terminal portion of Deltex that binds to the Notch Ankyrin repeat region. This region of similarity may include the putative nuclear localization sequence of Su(H), which raises the possibility that Su(H) may be sequestered in the cytoplasm by direct occlusion of this sequence upon binding to Notch (*49*). If so, Notch signaling may share certain mechanistic features with the regulation of the nuclear import of NF-κB/rel transcription factors by inhibitory IκB proteins. The cytoplasmic proteins of the IκB family contain between five and seven Ankyrin repeats that bind to NF-κB/rel proteins, thereby masking their nuclear localization sequences and resulting in cytoplasmic retention of the NF-κB/IκB complex (*56*). If this analogy is correct, Notch may function as a transmembrane, ligand-responsive IκB-like regulator of the Su(H) DNA-binding protein.

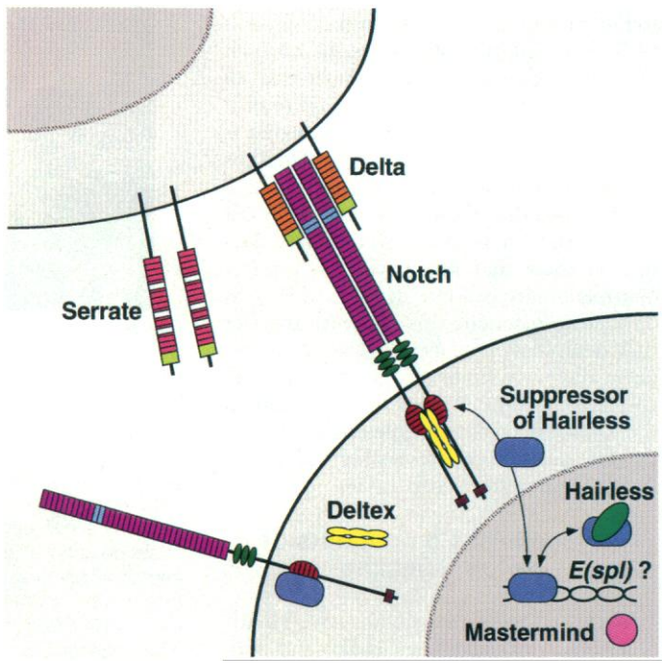

Fig. 5. A speculative model for Notch signaling. The Notch receptor may bind to either Delta or Serrate through its extracellular 11th and 12th EGF-like repeats (blue) (*32*). Ligand binding may result in receptor multimerization (*2*, *3*, *11*, *15*) that is stabilized by interactions between the intracellular Ankyrin repeats of Notch and the cytoplasmic protein Deltex (*48*, *52*). These events might control the putative nuclear translocation of the DNA-binding protein Suppressor of Hairless (*49*) and its known association with the Hairless protein (*59*). The transcriptional induction of the *Enhancer of split [E(spl)]* bHLH genes appears to depend on Notch signaling (*62*) and the molecular role of Mastermind remains to be determined.

Molecular Aspects of Notch Signaling

The ability of two different proteins, Deltex and Suppressor of Hairless, to interact with the intracellular Ankyrin repeats of Notch raises several questions. It is not known whether Deltex and Su(H) compete for Notch binding or if they act cooperatively, what controls the specificity of Notch interactions with each of these proteins, or how these interactions are related to ligand binding and Notch activation. Further work is needed to answer these questions, but a tentative model is worth considering (Fig. 5). Several genetic and molecular studies suggest that upon ligand binding, Notch and Lin-12 undergo receptor multimerization, and that this event is essential for receptor activation (*2*, *3*, *11*, *15*). In cultured S2 cells, binding of Notch to Delta presented on the surface of apposed cells results in the recruitment of Deltex protein to the Notch-Delta complexes and in the nuclear translocation of Su(H) protein, which indicates that Deltex may bind preferentially to Notch multimers, thereby displacing bound Su(H) (*48*, *49*). Similar competitive interactions between Deltex and Su(H) are observed when the two proteins are coexpressed at high levels in Notch-expressing S2 cells in the absence of any Delta-expressing cells (*52*).

Taken together, these observations suggest that Delta and Deltex may act in concert to multimerize Notch proteins and to interfere with the cytoplasmic retention of Su(H) by Notch, thus activating the Notch signaling pathway. This scenario is consistent with the similar embryonic null phenotypes of *Notch* and *Delta*, the similar loss-of-function wing phenotypes of *Delta* and *deltex*, and epistasis data that place both *Delta* and *deltex* genetically upstream of Notch receptor activation (*43*, *52*). More-

over, phenotypes caused by overexpression of Su(H) or Deltex in transgenic flies resemble those generated by activated Notch (*44*, *52*, *57*), as would be expected if excess Su(H) or Deltex saturates the available supply of Notch, resulting in translocation of some Su(H) to the nucleus, and consequent activation of the pathway. This model is undoubtedly an oversimplification because it fails to explain why complete elimination of Notch in vivo does not result in nuclear translocation of Su(H) and the production of activated phenotypes. One possibility is that Su(H) may by modified in some way through its interaction with Notch. Additional studies are required to test and refine this speculative model and to elucidate the complex interactions between Notch, its ligands, and its intracellular partners.

Nuclear Events and Nuclear Notch Proteins

The differentiation state of a particular cell ultimately depends on its transcriptional activity. Presumably, the Notch signaling pathway must control nuclear events in order to influence the progression of uncommitted cells to a more differentiated state. Three loci encoding putative nuclear proteins, *Hairless*, *Enhancer of split*, and *mastermind*, have been implicated in these nuclear events. Genetic studies have shown that *Hairless*, which encodes a novel basic protein (*58*), and *Su(H)* act antagonistically in the specification of adult sensory organs (*57*). Consistent with this genetic relationship, Hairless can associate in vitro with Su(H) and its mammalian homolog RBP-Jκ, thus preventing the transcription factor from binding to DNA (*59*) (Fig. 5). Preliminary analysis suggests that the Hairless protein is found in the nucleus, which links Su(H) directly to another nuclear protein (*60*).

In contrast to direct associations between the Su(H) and Hairless proteins, the *Enhancer of split* genes seem to be regulated by Notch signaling at the transcriptional level. The *Enhancer of split* complex encodes seven small basic Helix-Loop-Helix (bHLH) proteins that are functionally redundant as well as another predominantly nuclear protein containing WD-40 repeats (*61*). These two types of proteins are not homologous but seem to functionally interact with one another (*61*). The expression of at least some of the *Enhancer of split* bHLH genes are dependent on Notch signaling activity, although the mechanism of this transcriptional induction is not yet known (*62*). A putative Su(H) binding site has been found in the regulatory sequences of the *Enhancer of split* genes and might result in Su(H)-dependent transcription of these genes (*55*). If so, other targets for Su(H) must remain to be discovered, for the *Enhancer of split* genes are only expressed in limited subsets of CNS neuronal precursor cells and chromosomal deficiencies that remove the Su(H) binding site and nearby genes are not lethal (*61*, *62*).

The *mastermind* gene encodes a novel ubiquitous nuclear protein whose relationship to Notch signaling has not yet been determined (*63*). However, we believe that it is part of the Notch pathway because it has been recovered repeatedly by many independent genetic screens for Notch modifiers (*49*, *50*, *64*) and because *mastermind* null mutations display a *Notch*-like neurogenic embryonic phenotype (*27*, *33*). Further studies are needed to address the biochemical interactions between these putative nuclear components of Notch signaling and to identify functionally relevant transcriptional targets of the Su(H) protein. Assuming that Notch regulates a common step that allows cells to respond to differentiation signals throughout development, the biochemical nature of this process remains a major unanswered question.

The studies described earlier with truncated, activated forms of Notch reveal that Notch proteins without transmembrane and extracellular domains are translocated to the nucleus in transgenic flies and in transfected mammalian or *Drosophila* cells (*3*, *14*, *43–45*). However, the activated Notch phenotypes may not depend on nuclear translocation of Notch because both membrane-bound and nuclear truncated proteins produce indistinguishable phenotypes in the *Drosophila* eye (*14*). Sequence comparisons and deletion analysis have located two nuclear localization sequences that reside on either side of the Ankyrin repeats (*44*). The presence of these sequences makes it unlikely that the nuclear translocation of the Notch intracellular fragment results from binding of the Ankyrin repeats to a second protein, such as Su(H), which then ferries Notch into the nucleus. However, these observations have prompted speculation that a cleaved fragment of wild-type Notch may participate directly in nuclear processes (*3*, *14*, *43–45*).

Extensive immunohistochemical studies in *Drosophila* have failed to reveal the presence of Notch proteins in nuclei and thus it is unclear if receptor processing is part of normal Notch signaling (*11*, *39*). Nevertheless, protein immunoblot assays of wild-type tissues consistently detect an ~1000 amino acid intracellular Notch fragment in *Drosophila* and humans (*11*, *21*, *32*). Pulse chase experiments in mammalian cells have demonstrated that full-length Notch is rapidly processed into this ~1000 amino acid fragment, which is the major immunoreactive species in the steady state (*21*). It is not yet known whether this Notch species reflects a functionally relevant processing event or is simply a proteolytic breakdown product.

Whereas there are presently no compelling data to support the contention that nonengineered forms of the Notch receptor are ever found in *Drosophila* nuclei, nuclear Notch antigens have been detected in human cervical tissue and rat retina (*21*, *65*). In preliminary studies, this immunoreactivity appears to be associated with cell populations that are considered to be terminally differentiated. It is conceivable that a truncated nuclear form of Notch could result in ligand-independent activation of the pathway in a particular cell. If so, that cell may be frozen in a particular differentiation state. In certain circumstances, activation of Notch might thus be used not to modulate local interactions among uncommitted cells but rather to maintain the differentiated state of cells.

Conclusions

The accumulated data from *Drosophila*, *C. elegans*, and vertebrates suggest that Notch signaling plays a fundamental role in the differentiation of uncommitted cells. It seems that the role of this signaling pathway is not to transmit specific developmental signals but rather to modulate the ability of cells to respond to such signals. An example of how Notch signaling might regulate specific differentiation pathways involves the Ras pathway in the developing *Drosophila* eye, which is used to transmit an inductive signal generated by ligand-induced activation of the Sevenless receptor tyrosine kinase, and may be blocked by appropriately timed activation of the Notch pathway (*14*). This observation suggests that Notch acts in a parallel pathway that might also modulate indirectly a number of other signaling pathways, including those mediated by Wingless and Scabrous (*25*, *26*). Of course, mechanisms must exist that enable an uncommitted cell to integrate information that it receives from numerous different signaling pathways in a coherent manner such that a single cell fate is ultimately expressed.

We anticipate that Notch signaling activity will be important for both normal and abnormal development in mammals, in which most tissues are renewed throughout life from reserves of uncommitted stem cells. Because the execution of these developmental programs presumably involves local cell signaling, stem cells may require the Notch pathway to be either on or off to progress through each stage. The accumulation of somatic mutations in stem cells is thought to promote a variety of pathological conditions, including neoplasias. By analogy to studies in *Drosophila*, in which manipulation of Notch activity in uncommitted cells can force the cells to adopt aberrant cell fates, manipulation of Notch activity in immature

mammalian cell populations might alter their cell fates. Consequently, interfering with Notch activity in mammalian stem cell populations might have important applications for understanding and treating pathological disorders.

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