

# LIN-12/Notch signaling: lessons from worms and flies

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LIN-12/Notch proteins function as receptors for intercellular signals during development. Many aspects of LIN-12/Notch-mediated signaling have been elucidated through studies of cell-cell interactions that occur during *Caenorhabditis elegans* and *Drosophila melanogaster* development. The basic principles that operate in these lower organisms have also been shown to apply to vertebrates (for review, see Gridley 1997). Molecular features defined in lower organisms have also been shown to be conserved in vertebrates, including components of the signaling and signal transduction systems (for review, see Weinmaster 1997). The focus of this paper is on what has been learned about LIN-12/Notch signaling from invertebrates. First, a description of roles for LIN-12/Notch proteins in development is given, using different model cell fate decisions to illustrate various features. A discussion of the mechanism of LIN-12/Notch signal transduction follows, including new *in vivo* evidence that favors the direct participation of the intracellular domain of LIN-12/Notch proteins in regulating target gene expression. Finally, other influences on LIN-12/Notch activity are discussed, particularly protein turnover and protein processing.

## The LIN-12/Notch "pathway"

Genetic studies have identified many conserved components that are important for LIN-12/Notch signaling. Table 1 summarizes the *C. elegans* and *Drosophila* genes involved in LIN-12/Notch signaling mentioned here, and their vertebrate counterparts. Of particular note is that transmembrane protein ligands of the conserved Delta/Serrate/LAG-2 (DSL) family activate LIN-12/Notch signal transduction (for review, see Weinmaster 1997) and that many LIN-12/Notch outputs are mediated by a transcription factor named Suppressor of Hairless [Su(H)] in *Drosophila* and LAG-1 in *C. elegans* (see Schweisguth and Posakony 1994; Bailey and Posakony 1995; LeCourtois and Schweisguth 1995; Christensen et al. 1996). One "pathway" involving Su(H) is considered below and is diagrammed in Figure 2 (below). However, there is evidence that certain responses to activated

Notch do not depend on Su(H) (Lecourtois and Schweisguth 1995; Shawber et al. 1996; Wang et al. 1997) and, as described below, there are other potential roles for LIN-12/Notch proteins that may use other signal transduction components.

## LIN-12/Notch proteins mediate cell-cell interactions

The fundamental role inferred from genetic studies of LIN-12/Notch proteins is that they mediate inductive or lateral cell-cell interactions that specify cell fate. Inductive interactions involve signaling between nonequivalent cells. There are numerous examples of inductive interactions mediated by LIN-12/Notch proteins, and in many of these cases, the expression of ligand in the inducing cell or tissue appears to be the critical regulatory step. For example, two inductive interactions mediated by the *C. elegans* LIN-12/Notch protein GLP-1 have been studied in some detail. GLP-1 is expressed during embryogenesis by two equivalent blastomeres, ABa and ABp (Crittenden et al. 1994); the ligand APX-1 is expressed by a nonequivalent blastomere, P<sub>2</sub>, and induces ABp to follow a distinct fate from ABa (Mango et al. 1994; Mello et al. 1994; Mickey et al. 1996). GLP-1 is also expressed in the germ line; the ligand LAG-2 produced by a somatic gonadal cell activates GLP-1 in the germ line to promote mitosis (Crittenden et al. 1994; Henson et al. 1994).

Lateral interactions occur within a population of equivalent cells and result in the generation of cells of different types. This process has been termed "lateral inhibition," because it has been envisaged as a competition between cells for one of the fates, with one cell deciding to adopt a default fate (the fate it would adopt if it were isolated from the other cells) and then inhibiting others from adopting that fate (Wigglesworth 1940; Lawrence 1992). However, results from genetic studies of *lin-12* in *C. elegans* and *Notch* in *Drosophila* suggest that cells communicate with each other prior to commitment to either fate, so that the cells essentially specify each other; hence, I prefer the term "lateral specification" for this process. In some cases, other factors influence the outcomes of signaling between cells that are essentially equivalent.

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**Table 1.** *C. elegans* and *Drosophila* genes featured

Gene	Organism	Protein product	Mammalian
<b>A. Receptors</b>			
<i>lin-12</i>	<i>C. elegans</i>	LIN-12/Notch family	Notch1–Notch4
<i>Notch</i>	<i>Drosophila</i>		
<i>glp-1</i>	<i>C. elegans</i>		
<b>B. Ligands</b>			
<i>apx-1</i>	<i>C. elegans</i>	DSL family	Delta1,2; Jagged1,2
<i>lag-2</i>	<i>C. elegans</i>		
<i>Delta</i>	<i>Drosophila</i>		
<i>Serrate</i>	<i>Drosophila</i>		
<b>C. Cell-autonomous factors that affect LIN-12/Notch activity</b>			
1. <i>numb</i>	<i>Drosophila</i>	novel transmembrane protein	Numb
2. <i>finger</i>	<i>Drosophila</i>	novel secreted protein	Manic, Radical, Lunatic Fringe
<b>D. Downstream effectors that physically interact with LIN-12/Notch</b>			
1. <i>Suppressor of Hairless</i>	<i>Drosophila</i>	transcription factor	CBF1 (RBPJ $\kappa$ )
<i>lag-1</i>	<i>C. elegans</i>	related to yeast chromatin control protein	D79984
2. <i>emb-5</i>	<i>C. elegans</i>		
<b>E. Other genes involved in Notch pathway</b>			
1. <i>mastermind</i>	<i>Drosophila</i>	novel nuclear protein	—
2. <i>neuralized</i>	<i>Drosophila</i>	novel nuclear protein	—
3. <i>strawberry notch</i>	<i>Drosophila</i>	novel nuclear protein	—
4. <i>Hairless</i>	<i>Drosophila</i>	binds Su(H)	—
5. <i>deltex</i>	<i>Drosophila</i>	novel cytoplasmic protein	—
6. <i>dishevelled</i>	<i>Drosophila</i>	novel cytoplasmic protein	Dvl 1–3
7. <i>E(spl)</i> complex	<i>Drosophila</i>	bHLH transcription factors	Hairy/E(spl) (HES)
8. <i>groucho</i>	<i>Drosophila</i>	corepressor	TLE
9. <i>a-c</i> complex	<i>Drosophila</i>	bHLH transcription factors	MASH
10. <i>big brain</i>	<i>Drosophila</i>	resembles ion channel	—
11. <i>scabrous</i>	<i>Drosophila</i>	secreted glycoprotein	—
<b>F. LIN-12/Notch turnover</b>			
1. <i>sel-1</i>	<i>C. elegans</i>	similar to yeast HRD3	IBD2
2. <i>sel-10</i>	<i>C. elegans</i>	F-box/WD40 repeat	SEL-10
<b>G. LIN-12/Notch processing and/or trafficking</b>			
1. <i>kuz</i>	<i>Drosophila</i>	metalloprotease	ADAM10
<i>sup-17</i>	<i>C. elegans</i>	multipass transmembrane protein	presenilin1,2
2. <i>sel-12</i>	<i>C. elegans</i>		
<i>hop-1</i>	<i>C. elegans</i>		

See text for details and references concerning *C. elegans* and *Drosophila* proteins. References for mammalian homologs are given in Weinmaster (1997), except for mammalian Fringe proteins (Johnston et al. 1997), IBD2 (I. Biunno, pers. comm.), SEL-10 (G. Wu and J. Kitajewski, pers. comm.), and ADAM10 (Wolfsberg and White 1996).

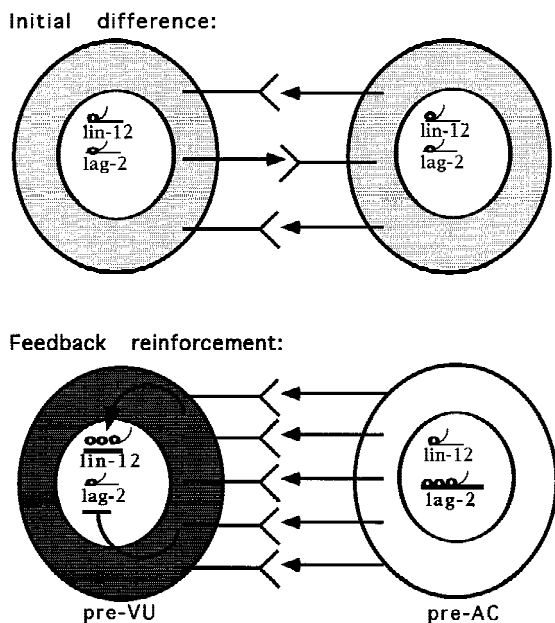
### Feedback mechanisms during lateral specification

In the absence of other influences, lateral specification is stochastic, in that any cell in the population could adopt the default fate. Studies of *lin-12/Notch* activity in *C. elegans* and *Drosophila* have suggested that during some cell fate decisions, differences in *lin-12/Notch* activity are amplified by feedback mechanisms that affect both ligand and receptor expression. These principles are exemplified by a simple decision involving only two equivalent cells in *C. elegans* gonadogenesis and a decision involving a small group of equivalent cells in *Drosophila* sense organ development.

The first evidence that LIN-12/Notch-mediated lateral specification involves the amplification of a stochastic small difference between equivalent cells came from a study of LIN-12-mediated signaling between two cells

of the hermaphrodite gonad of *C. elegans* (see Fig. 1). These gonadal cells, named Z1.ppp and Z4.aaa, are initially equivalent in their developmental potential in that each has an equal chance of becoming the anchor cell (AC), a terminally differentiated cell type, or a ventral uterine precursor cell (VU), which contributes descendants to the ventral uterus (Kimble and Hirsh 1979). However, in any given hermaphrodite, only one of these cells will become the AC, whereas the other becomes a VU, depending on interactions between them (Kimble 1981; Seydoux and Greenwald 1989).

The interactions between Z1.ppp and Z4.aaa are mediated by *lin-12* and *lag-2*, a gene encoding a DSL ligand (Greenwald et al. 1983; Lambie and Kimble 1991; Henderson et al. 1994; Tax et al. 1994). Mutations that constitutively activate *lin-12* have no AC, and mutations that eliminate *lin-12* activity have two ACs



**Figure 1.** A feedback mechanism during the *C. elegans* AC/VU decision. The AC/VU decision is a simple example of lateral specification involving only two equivalent cells. These two cells interact with each other so that one becomes the AC and the other becomes a VU. A stochastic small difference in ligand or receptor activity (*top*) is amplified by a feedback mechanism (*bottom*) that involves differential transcription of genes encoding the ligand (*lag-2*) and receptor (*lin-12*). This same basic mechanism appears to operate during certain other LIN-12/Notch-mediated decisions, including *Drosophila* SOP specification.

(Greenwald et al. 1983). Thus, activation of *lin-12* results in the VU fate; failure to activate *lin-12* results in the AC fate.

Genetic mosaics in which *lin-12(+)* and *lin-12(0)* cells are juxtaposed suggested the existence of a feedback mechanism (Seydoux and Greenwald 1989). In genetic mosaics in which either Z1.ppp or Z4.aaa lacked *lin-12* activity [*lin-12(0)*] while the other cell was *lin-12(+)*, the *lin-12(0)* cell always became an AC, presumably because it could no longer receive the signal. In addition, the *lin-12(+)* cell always became a VU and therefore behaved differently in the genetic mosaic than in wild type, where it has an equal chance of becoming an AC. These observations suggested that a stochastic variation in ligand and/or receptor activity between Z1.ppp and Z4.aaa is amplified by a feedback mechanism in both cells.

Analysis of the patterns of *lin-12* and *lag-2* expression during the AC/VU decision suggested that transcriptional control is a component of the feedback mechanism (Wilkinson et al. 1994). Initially, *lin-12* and *lag-2* are expressed in both Z1.ppp and Z4.aaa. However, the expression patterns change in a reciprocal manner, so that *lin-12* expression becomes restricted to the presumptive VU and *lag-2* expression becomes restricted to the presumptive AC. These changes in gene expression occur prior to commitment. Furthermore, they are influ-

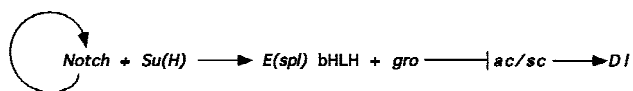
enced by *lin-12* activity, which appears to promote expression of *lin-12* and to repress expression of *lag-2*. If the later phase of *lin-12* expression in the presumptive VU is reduced by deleting a 5' regulatory element, a VU is not made, indicating that positive autoregulation of *lin-12* transcription in the presumptive VU is necessary to specify the VU fate.

Studies in *Drosophila* have suggested that such feedback mechanisms operate in other cell fate decisions (Heitzler and Simpson 1991; see also Huppert et al. 1997), as exemplified by specification of sensory organ precursors (SOPs) that give rise to bristles on the notum. During development of the peripheral nervous system, equivalent proneural cells within a small cluster interact via *Notch* so that one becomes the SOP, giving rise to a neuron and accessory cells, whereas the others become epidermis (Stern 1954; Shellenbarger and Mohler 1978; Hartenstein and Posakony 1990; Simpson 1990).

A feedback mechanism was again inferred by the observation of a bias in cell fate choice in genetic mosaics: When *Notch<sup>+</sup>* cells are juxtaposed to *Notch<sup>-</sup>* cells, the *Notch<sup>-</sup>* cells always become SOPs and the *Notch<sup>+</sup>* cells always become epidermis (Heitzler and Simpson 1991). Furthermore, the opposite bias in cell fate choice was observed in genetic mosaics involving the ligand *Delta*: When *Delta<sup>+</sup>* cells are juxtaposed to *Delta<sup>-</sup>* cells, the *Delta<sup>-</sup>* cells become epidermis and the *Delta<sup>+</sup>* cells become SOPs (Heitzler and Simpson 1991). Support for the involvement of transcriptional control in this feedback mechanism during SOP specification has been suggested by analysis of genetic mosaics involving genes of the *achaete-scute* (*ac-sc*) complex (Heitzler et al. 1996), known transcriptional activators of *Delta* (Kunisch et al. 1994), and the analysis of the pattern of *Delta* expression in the SOP lineage (Parks et al. 1997).

In *Drosophila*, the downstream effects of activating Notch have been intensively studied. Although the circuitry underlying Notch positive autoregulation is not known, the circuitry underlying the feedback loop that represses ligand expression upon receptor activation during SOP specification has been well described (Fig. 2; Heitzler et al. 1996). Su(H) is the key effector upon Notch activation in SOP specification (Schweisguth and Posakony 1994; Bailey and Posakony 1995; Lecourtois and Schweisguth 1995; Schweisguth 1995). Su(H) promotes expression of genes of the *Enhancer of split* [*E(spl)*] complex encoding basic helix-loop-helix (bHLH) proteins (Bailey and Posakony 1995; Lecourtois and Schweisguth 1995), which combine with a protein called Groucho (which maps to the *E(spl)* complex but is not a bHLH protein) to create a repressor (Paroush et al. 1994; Fisher et al. 1996). The E(spl) bHLH-Groucho protein complex represses genes of the *ac-sc* complex, which encodes multiple proteins of a different bHLH class (Oellers et al. 1994; Heitzler et al. 1996). As the Ac-Sc proteins activate expression of *Delta* (Kunisch et al. 1994), this feedback loop operates to repress *Delta* expression in the cell in which Notch has been activated.

There are two additional points pertaining to SOP specification that should be noted here. First, the *ac-sc*



**Figure 2.** A genetic pathway for the repression of *Delta* expression in response to Notch activation during SOP specification. (Arrow) A positive interaction; (blunted arrow) a negative interaction; (circular arrow) positive autoregulation.

genes also act upstream of *Notch*, in that expression of *ac-sc* complex genes establish the proneural clusters. Global positional cues govern the pattern of *ac-sc* expression to establish the proneural clusters that undergo *Notch*-mediated cell-cell interactions (for review, see Skeath and Carroll 1994). The *Notch*-mediated interactions lead to increased expression of *ac-sc* in the presumptive SOP and repression of *ac-sc* in other cells of the cluster. Second, for certain macrochaetes (large bristles) the position of the SOP is not always in the center of the cluster; however, the SOP is generated at a reproducible position within a cluster (Cubas et al. 1991), suggesting that other factors may influence the specification of the SOP.

### Biased LIN-12/Notch-mediated interactions

LIN-12/Notch proteins are also involved in specifying the fates of cells in invariant lineages. In these cases, although each cell of an equivalence group has the potential to adopt either the default or the alternative fate, intrinsic or extrinsic factors lead to a predictable outcome.

#### Asymmetric segregation of modulating factors: *Drosophila Numb*

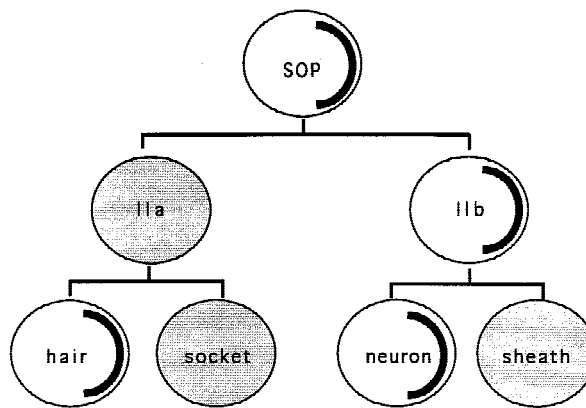
A notable example of a lineage-based mechanism that biases a Notch-mediated interaction occurs during *Drosophila* SOP development, where the asymmetric segregation of Numb modulates Notch activity in one of two daughter cells (Fig. 3). The SOP normally undergoes a defined lineage, producing two daughters: One daughter produces a hair cell and a socket cell; the other daughter produces a neuron and a sheath cell (Hartenstein and Posakony 1989). Temperature-shift experiments have established that *Notch* and *Delta* function at each step of the lineage (Hartenstein and Posakony 1990; Parks and Muskavitch 1993): If *Notch* activity is reduced around the time of the first SOP division, the two daughters each produce a neuron and a sheath cell; if *Notch* activity is reduced at both the first and second divisions in the SOP lineage, four neurons are generated. The involvement of *Notch* and *Delta* at these steps of the SOP lineage imply that cell-cell interactions between the SOP daughters and between SOP granddaughters are important for generating differences within the SOP lineage.

*numb* was identified by its effects on Notch-mediated cell fate decisions (Uemura et al. 1989). *numb* appears to have a cell-intrinsic influence on Notch-mediated sig-

naling in several lineages. For example, at each successive step in the SOP lineage, the Numb protein is believed to be newly synthesized and then preferentially segregated into one of the SOP daughters (Rhyu et al. 1994; Knoblich et al. 1995) (see Fig. 3). Jan and Jan (1995) have suggested that *numb* influences Notch-mediated signaling at successive steps in the SOP lineage by reducing *Notch* activity in the cell that contains Numb, thereby biasing the interaction between sister cells that signal each other. They envisage the *numb*-imposed bias as a mechanism to increase the reliability of Notch-mediated signaling in situations requiring rapid decision making, when there may be insufficient time to activate transcription-based feedback mechanisms (Jan and Jan 1995).

The asymmetric localization of Numb also has been shown to influence a Notch-mediated inductive interaction in a central nervous system (CNS) lineage. Two sister cells, dMP2 and vMP2, adopt different fates during CNS development as a result of an inductive signal emanating from outside the MP2 lineage (Spana and Doe 1996). The preferential segregation of Numb into the dMP2 cell appears to account for the different response of the sister cells to the inductive signal (Spana and Doe 1995, 1996; Spana et al. 1995).

Numb is a novel membrane-associated protein (Uemura et al. 1989). The amino-terminal portion of Numb appears to interact physically with the amino-terminal portion of the Notch intracellular domain (Guo et al. 1996). The mechanism by which Numb binding to Notch inhibits Notch activity is not known; one possibility is that Numb interferes with the interaction of Notch with Su(H) or another transcription factor.

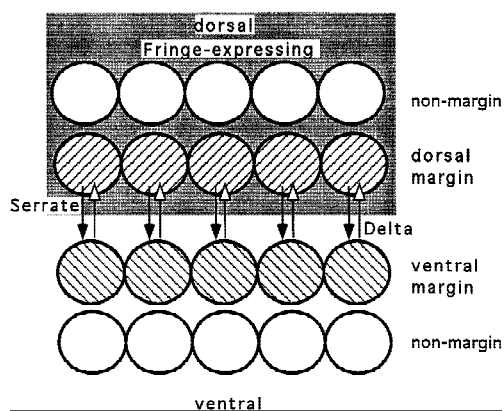


**Figure 3.** Modulation of *Notch* activity by *numb* in the SOP lineage. The SOP undergoes a defined cell lineage, and the activity of *Notch* and *numb* is involved at each step. At each division, asymmetric segregation of Numb (dark crescent) reduces *Notch* activity in the cell that receives it, so that the cell lacking *numb* activity adopts the fate associated with activated *Notch* (shaded circles). If *Notch* activity is absent or if *numb* activity is provided to all SOP descendants by overexpression, four neurons are generated; if *numb* activity is absent or if *Notch* activity is provided to all SOP descendants by overexpression, four socket cells are generated.

### Differential expression of modulating factors: *Drosophila Fringe*

Studies of the *Drosophila fringe* gene suggest that Fringe influences the response of cells to ligands for Notch (Irvine and Wieschaus 1994; Kim et al. 1995; Fleming et al. 1997; Panin et al. 1997; for review, see Irvine and Vogt 1997) (see Fig. 4). The role of Fringe is best understood in the induction of the *Drosophila* wing margin. The dorsal and ventral compartments of the wing primordium are destined to form the dorsal and ventral surfaces of the wing blade. The wing margin forms where these two compartments meet; both dorsal and ventral cells at the interface become specialized margin cells. The wing margin cells secrete a morphogen that organizes wing development and also specifies the distinctive structures of the wing margin, including a highly stereotyped pattern of mechanosensory bristles (for review, see Lawrence and Struhl 1996). Notch-mediated interactions between dorsal and ventral cells establish the wing margin via a complex interplay of regulatory interactions among Notch and Fringe, and the Notch ligands Delta and Serrate.

The role of *fringe* appears to be to cause Notch to respond to Delta rather than Serrate. Serrate has been implicated as a signal from dorsal to ventral cells (Diaz-Benjumea and Cohen 1995; Kim et al. 1995; DeCelis et al. 1996), whereas Delta has been implicated as a signal from ventral to dorsal cells (Diaz-Benjumea and Cohen 1995; DeCelis et al. 1996; Doherty et al. 1996). The patterns of Delta and Serrate expression change over time, dependent on Notch activity. Early in wing develop-



**Figure 4.** The modulation of Notch activity by *fringe* during *Drosophila* wing margin specification. The wing margin forms where the dorsal and ventral compartments are juxtaposed. Formation of the wing margin requires the activity of the Notch (receptor), *Delta* (ligand), and *Serrate* (ligand) genes, and the activity of *fringe* (modulator). Activation of Notch at the boundary between the dorsal and ventral compartments specifies cells on either side of the boundary as the wing margin. The dorsally restricted expression of Fringe is important for limiting the induction of Notch by Serrate and Delta to thin stripes of dorsal and ventral cells that interact across the dorsoventral compartment boundary. (Solid arrow) Serrate; (open arrow) Delta; (hatched cells) Notch is activated.

ment, Serrate and Fringe are expressed in all cells of the dorsal compartment under the control of the dorsal selector gene *apterous*, whereas Delta is expressed in all cells of both the dorsal and ventral compartments. However, Delta expression is dramatically up-regulated in ventral cells along the boundary, in response to Serrate expressed by dorsal cells. Conversely, high levels of Delta induced by Serrate in ventral cells signal back across the boundary and up-regulate Serrate expression in dorsal cells. The end result is a localized activation of Notch in adjacent stripes of cells that abut at the dorsal/ventral compartment boundary, specifying these cells to become wing margin cells.

The presence or absence of Fringe appears to determine to which Notch ligand wing cells respond. Thus, ventral cells (which normally lack Fringe) are primed to respond to Serrate (expressed by dorsal cells). Conversely, wing cells that express Fringe are refractory to Serrate but primed to respond to Delta (expressed at high levels in ventral cells in response to Serrate). As argued by Panin et al. (1997), the dorsally restricted expression of Fringe is therefore responsible for limiting the induction of Notch by Serrate and Delta to thin stripes of dorsal and ventral cells that interact across the dorsoventral compartment boundary. This Fringe-dependent bias is reinforced by an unexpected property of Delta and Serrate signaling in the wing disc: for unknown reasons, cells that express high levels of Delta or Serrate themselves appear unable to receive the same ligand they express and, hence, are further biased to receive the other ligand (see also Micchelli et al. 1997). Panin et al. (1997) point out that this seemingly complicated mechanism may be used to ensure the correct placement of a source of morphogen to pattern a symmetrical structure.

*fringe* encodes a novel protein that is predicted to be secreted but acts cell-autonomously (Irvine and Wieschaus 1994; Panin et al. 1997). It will be of interest to learn if Fringe modifies the interaction of Notch with its ligands or the response of Notch upon the binding of a subset of ligands.

### Cell signaling: *C. elegans* vulval precursor cell fate patterning

The patterning of fates of the vulval precursor cells (VPCs) in *C. elegans* (for review, see Greenwald 1997; Kornfeld 1997) may exemplify the use of a heterologous cell signaling mechanism to superimpose a bias on a LIN-12/Notch-mediated process (Fig. 5). In wild-type hermaphrodites, three hypodermal cells, P5.p, P6.p, and P7.p, are VPCs and generate the cells that form the vulva. These cells lie under the somatic gonad, with P6.p centered under the anchor cell. Although P5.p, P6.p, and P7.p all have similar developmental potential, they always adopt a pattern of fates that may be represented as 2°-1°-2° (Sulston and White 1980; Sternberg and Horvitz 1986). This pattern primarily reflects the activity of two different signaling pathways. One pathway involves an inductive signal from the anchor cell that is transduced

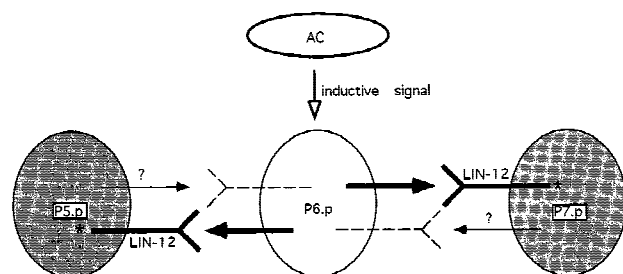
by the LET-23 receptor tyrosine kinase in the vulval precursor cells (for review, see Kornfeld 1997). It is not clear whether the inductive signal is spatially graded (see Katz et al. 1995) or restricted so that only P6.p is induced (see Koga and Ohshima 1995; Simske and Kim 1995). The other pathway involves a LIN-12-mediated lateral signal thought to occur between neighboring vulval precursor cells (Sternberg 1988).

*lin-12* transcription appears to remain uniform during VPC specification, suggesting that a transcription-based feedback mechanism analogous to that used in the AC/VU decision is not used to establish the pattern of vulval fates (Wilkinson and Greenwald 1995). Instead, reception of the inductive signal appears to be an important influence on lateral signaling: In *let-23* genetic mosaics, a *let-23(-)* VPC adopts the 2° fate when adjacent to a *let-23(+)* VPC (Koga and Ohshima 1995; Simske and Kim 1995). This observation suggests that inductive signaling (whether spatially graded or not) appears likely to govern the expression or activity of the ligand for LIN-12. Furthermore, inductive signaling appears to cause a specific down-regulation of LIN-12 protein accumulation (D. Levitan and I. Greenwald, unpubl.). Thus, a combination of ligand up-regulation (transcriptional or post-transcriptional) and receptor down-regulation (post-transcriptional) may bias P6.p to adopt the 1° fate.

It is interesting to note that reduction in the activity of *rhomboid*, which potentiates the activity of the *Drosophila* EGF receptor, causes reduced expression of *Delta* during wing vein development (Sturtevant et al. 1993). This appears to be another example of a linkage of EGF receptor activity to DSL ligand expression, although the effect of EGF receptor activity in this case is the opposite effect of what appears to occur during *C. elegans* VPC specification.

#### Other developmental roles for LIN-12/Notch proteins

Most work on LIN-12/Notch proteins has been concerned with their roles in mediating cell fate decisions. However, in *Drosophila*, *Notch* and its ligand *Delta* have



**Figure 5.** The AC produces a ligand (open arrow), which activates a Ras-mediated signal transduction pathway in P6.p. This biases lateral signaling, so that only P5.p and P7.p attain a threshold value of activated LIN-12 (gray shading). This bias may be achieved by up-regulation of expression or activity of a ligand for LIN-12 as well as by down-regulation of LIN-12 in P6.p.

been implicated in axon extension in defined neurons (Giniger et al. 1993). Recently, Giniger (1998) has shown that axon aberrations associated with reduced *Notch* activity do not appear to reflect underlying changes in cell identity. He also showed that *Notch* is expressed in growth cones in primary *Drosophila* neurons cultured in vitro (Giniger 1998). LIN-12/Notch proteins are also found in mature neurons in *Drosophila* (Fehon et al. 1991) and *C. elegans* (D. Levitan and I. Greenwald, unpubl.), raising the possibility of other functions in neural development.

Another proposed role for Notch is to sequester Dishevelled, a protein that functions in the Wnt/Wingless/Wnt signal transduction pathway (Axelrod et al. 1996). In *Drosophila*, the Wntless (Wnt) and Notch signaling pathways both have input into many of the same cell fate decisions (e.g., Couso and Martinez Arias 1994; Gonzalez-Gaitan and Jaekle 1995; Rulifson and Blair 1995). The *dishevelled* gene displays genetic interactions with *Notch* as well as with *wingless*. Because there is some evidence that Dishevelled may interact with the intracellular domain of Notch, it has been proposed that Notch functions to sequester Dishevelled; this role for Notch is therefore different from that proposed for Notch signal transduction in cell fate decisions (see below).

#### The mechanism of LIN-12/Notch signal transduction

It is now generally accepted that LIN-12/Notch proteins function as receptors that are activated by the binding of DSL ligands. Genetic studies have suggested that receptor self-association may be involved in receptor activation (Greenwald and Seydoux 1990; Heitzler and Simpson 1993).

The mechanism by which activated LIN-12/Notch proteins transduce signals and alter gene expression has been unresolved. Recent results (Schroeter et al. 1998; Struhl and Adachi 1998) support an unusual mechanism for signal transduction described below.

Three important observations are pertinent to models for the mechanism of signal transduction: (1) Expression of just the intracellular domain of LIN-12 and Notch causes phenotypes associated with LIN-12/Notch activation (Lieber et al. 1993; Struhl et al. 1993); (2) the free *Notch* intracellular domain is nuclearly localized (Fortini et al. 1993; Lieber et al. 1993; Struhl et al. 1993; Kopan et al. 1994; Nye et al. 1994); (3) the intracellular domains of LIN-12/Notch proteins physically interact with proteins that function in the nucleus to regulate gene expression. The best characterized of these proteins are the site-specific DNA-binding protein Su(H) in *Drosophila*, LAG-1 in *C. elegans*, and CBF1 (or RBPJ $\kappa$ ) in vertebrates. Genetic studies in *Drosophila* and *C. elegans* have established that Su(H)/LAG-1 is involved in LIN-12/Notch-mediated reception of intercellular signals (see Schweisguth and Posakony 1994; Bailey and Posakony 1995; Lecourtois and Schweisguth 1995; Christensen et al. 1996). There is also evidence that LIN-12 interacts physically and functionally with EMB-5, a

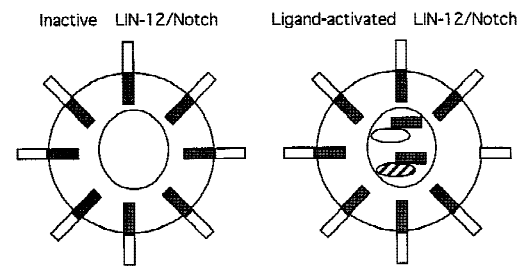
*C. elegans* protein that is similar in sequence to a yeast protein that controls chromatin structure (Hubbard et al. 1996).

Three classes of signal transduction mechanisms have been proposed. One model is that LIN-12/Notch proteins act as a passive tether to keep LAG-1/Su(H) out of the nucleus and that ligand binding induces release and nuclear import of these factors (Fortini and Artavanis-Tsakonas 1994). This model accounts for the physical interaction between Notch and Su(H) as well as evidence that Notch can sequester Su(H) at the membrane under some conditions (Fortini and Artavanis-Tsakonas 1994). However, the passive tether model is incompatible with the genetic data. For example, the *Notch*<sup>-</sup> phenotype is similar to the *Su(H)*<sup>-</sup> phenotype; however, according to the passive tether model, Su(H) should enter the nucleus and be active in the absence of Notch. Furthermore, there is evidence that the subcellular localization of Su(H) does not change upon Notch activation in vivo (Gho et al. 1996).

A more conventional mechanism of signal transduction could also be invoked. The JAK-STAT signaling system, which involves the modification of a transcription factor by a ligand-activated receptor tyrosine kinase, is a useful analogy (Darnell 1997). Thus, LIN-12/Notch may interact with LAG-1/Su(H) at the cell surface; in response to ligand binding, Su(H) (or another associated factor) is modified, dissociates from the receptor, translocates to the nucleus, and regulates expression of downstream target genes. At this time, there is no evidence for any enzymatic activity of the LIN-12/Notch intracellular domain or for a physically associated protein that might provide such a modifying activity.

A different model for the mechanism of signal transduction is that ligand binding leads to cleavage and nuclear translocation of the LIN-12/Notch intracellular domain in a complex with transcription factors (Fig. 6). This model was first proposed to account for the observation that the intracellular domain of Notch is nuclearly localized and behaves like an activated receptor (Lieber et al. 1993; Struhl et al. 1993). It is consistent with the findings that the intracellular domains of LIN-12/Notch interact with transcription factors and studies in cultured cells suggesting that the Notch intracellular domain potentiates the transcriptional activation of target genes by Su(H)/CBF1 (Jarriault et al. 1995; Chen et al. 1997; Eastman et al. 1997). However, under normal in vivo circumstances, the intracellular domain has not been visualized by antibody staining in the nuclei of cells undergoing LIN-12/Notch-mediated signaling.

Recent work in *Drosophila* has provided evidence in support of ligand-dependent nuclear access and a role for the intracellular domain in regulating transcription of target genes in vivo. Struhl and Adachi (1998) reasoned that if the mechanism of Notch signal transduction depends on nuclear import of the intracellular domain, the amount of the intracellular domain that accumulates in the nucleus must be very low (or else it would be visible using conventional methods). They therefore devised a sensitive assay for nuclear access by inserting a GAL4-



**Figure 6.** The ligand-dependent cleavage model for LIN-12/Notch signal transduction. LIN-12/Notch proteins are expressed on the cell surface, where they are free to interact with ligands on the surface of neighboring cells. Binding of ligand causes release of the intracellular domain, which translocates to the nucleus, where facilitates transcriptional activation by DNA-binding proteins such as LAG-1/Su(H)/CBF1. Other specific proteins may also be present in the transcription complex; different complexes may be involved in different decisions.

VP16 transcription factor domain into the intracellular domain of Notch and used this method to demonstrate ligand-dependent nuclear access as assayed by both *UAS-lacZ* reporter gene output and phenotypic rescue. Furthermore, they showed that nuclear access is important for signal transduction by manipulating the subcellular localization of the intracellular domain with sequences that target to the membrane (eliminating activity) or nucleus (potentiating activity). Finally, they showed that the intracellular domain appears to participate in a transcription complex because adding a transcriptional activator domain to the intracellular domain of the full-length receptor promotes *Notch* activity, whereas adding transcriptional repressor domains blocks *Notch* activity.

The results of Struhl and Adachi (1998) provide strong evidence for in vivo nuclear access and action of the intracellular domain of Notch. In principle, nuclear access could be afforded by ligand-dependent cleavage and release of the intracellular domain, analogous to the events that release sterol regulatory element binding proteins (SREBPs) from a transmembrane protein in response to cholesterol depletion (for review, see Brown and Goldstein 1997). Alternatively, an unprecedented trafficking event might enable the intracellular domain of activated Notch to gain access to the nucleus while still attached to the remainder of the protein. Recent work using mammalian tissue culture cells has provided biochemical evidence for a ligand-dependent cleavage event that releases the Notch intracellular domain to function in the nucleus (Schroeter et al. 1998). The cleavage model presented in Figure 6 is a synthesis of the available functional and biochemical data.

#### Other influences on LIN-12/Notch activity

Genetic analysis in *C. elegans* and *Drosophila* has identified many genes that influence LIN-12/Notch activity. Some of these genes have been defined by mutations that result in phenotypes associated with defects in LIN-12/

Notch signaling. Other genes have been defined by mutations that suppress or enhance mutations in the LIN-12/Notch signaling pathway. Some genes have been defined by both approaches.

Many *Drosophila* genes that influence *Notch* activity have been characterized. Several encode nuclear proteins that may be involved in signal transduction, including *mastermind* (Smoller et al. 1990), *neuralized* (Boulianne et al. 1991), *groucho* (see Paroush et al. 1994; Fisher et al. 1996), and *strawberry notch* (Majumdar et al. 1997). The *big brain* gene encodes a protein that resembles an ion channel and potentiates *Notch* activity (Doherty et al. 1997), and *deltex* encodes a cytoplasmic protein that interacts with the intracellular domain of Notch (Matsuno et al. 1995). The *scabrous* gene encodes a secreted glycoprotein that influences Notch activity during eye development (Baker and Zitron 1995; Lee et al. 1996).

As described above, Su(H) is a transcription factor and a key signal transducing element in many cell fate decisions. Hairless influences *Notch* signaling by negatively regulating the activity of the Su(H) (Schweisguth and Posakony 1994; Bang and Posakony 1995). Hairless interacts with Su(H) via a direct protein-protein interaction and interferes with the DNA-binding activity of Su(H) (Brou et al. 1994). The bHLH proteins of the *E(Spl)* complex appear to be direct targets of Su(H) (Bailey and Posakony 1995; Lecourtois and Schweisguth 1995).

In some cases, the results of genetic and molecular characterization suggest other factors that influence cell fate decisions mediated by LIN-12/Notch signaling. Here, potential effects of protein turnover are considered, as suggested by studies of the *C. elegans sel-1* and *sel-10* genes. Also considered are aspects of protein processing and trafficking, based on studies of the *Drosophila kuzbanian (kuz)* gene and its *C. elegans* homolog, *sup-17*, and by studies of the *C. elegans sel-12* and *hop-1* presenilin genes.

#### Protein turnover

Studies of the *C. elegans sel-1* and *sel-10* genes, both negative regulators of *lin-12* activity, have suggested that protein turnover may be important in LIN-12/Notch mediated cell fate decisions (Grant and Greenwald 1996, 1997; Hubbard et al. 1997). SEL-1 is similar to the *Saccharomyces cerevisiae* HRD3 protein, which has been implicated in turnover of the membrane protein HMG CoA reductase (Hampton et al. 1996). SEL-10 is a member of the F-box/WD40 repeat-containing protein family, members of which have been shown to target proteins for ubiquitination and turnover (for review, see Hoyt 1997).

Constitutive turnover or ligand-induced down-regulation of LIN-12/Notch proteins may be important for cell fate decisions to occur normally (discussed in Grant and Greenwald 1997; Hubbard et al. 1997). For example, in the AC/VU decision, Z1.ppp and Z4.aaa initially signal each other: in the absence of turnover or down-regulation of LIN-12, this initial signaling might create sufficient activated receptor so that both cells would achieve

the threshold value of effector activity; in effect, rapid turnover would limit the output from a single ligand-receptor interaction. Furthermore, in this decision, signaling from activated receptor would persist in the absence of down-regulation, masking the effects of the differential transcription underlying the feedback mechanism. Receptor turnover may also be necessary in cases where *Notch* is used for successive decisions, such as in the specification of fates during *Drosophila* eye development (Cagan and Ready 1989), so that activated receptors are cleared before the next signaling event occurs.

#### Protein processing and trafficking

LIN-12/Notch proteins (and their ligands) are transmembrane proteins that must be properly processed, modified, and sorted. LIN-12/Notch proteins appear to be proteolytically cleaved in the extracellular domain to yield a heterodimer composed of the amino- and carboxy-terminal cleavage products (Crittenden et al. 1994; Blaumueller et al. 1997; Pan and Rubin 1997). This proteolytic cleavage event is proposed to occur in intracellular vesicles and to be important for Notch trafficking to the cell surface (Blaumueller et al. 1997).

Several genes that influence LIN-12/Notch activity appear to influence processing and trafficking. With the exception of the putative ligand-induced cleavage event, it is not known whether various steps in LIN-12/Notch processing and trafficking occur constitutively or are points of regulation during cell fate decisions.

A metalloprotease of the ADAM family—Kuz in *Drosophila* and SUP-17 in *C. elegans*—facilitates *lin-12/Notch* signaling (Rooke et al. 1996; Pan and Rubin 1997; Sotillos et al. 1997; Tax et al. 1997; Wen et al. 1997). The genetic interaction between *sup-17/kuz* and *lin-12/Notch* requires the LIN-12/Notch extracellular domain (Pan and Rubin 1997; Sotillos et al. 1997; Wen et al. 1997). Pan and Rubin (1997) have presented evidence that Kuz is required in *Drosophila* for the proteolytic processing of Notch into two fragments consistent with a cleavage event in the extracellular domain. It is not known whether the LIN-12/Notch extracellular domain is a substrate of the SUP-17/Kuz protease or whether SUP-17/Kuz is involved in maturation or activation of LIN-12/Notch proteins.

Proteins of the presenilin family have also been identified as influences on LIN-12/Notch signaling. In humans, presenilins were identified by mutations that cause Alzheimer's disease (for review, see Schellenberg 1995). A connection between presenilin activity and LIN-12/Notch signaling was first suggested by the finding that the *C. elegans sel-12* presenilin facilitates the activity of *lin-12* and *glp-1* (Leviton and Greenwald 1995), and supported by the observation that reducing the activity of both *C. elegans* presenilins causes phenotypes associated with the absence of *lin-12* and *glp-1* activity (Li and Greenwald 1997). The functional relationship between presenilin and Notch activity appears to have been evolutionarily conserved, because targeted

disruption of the mouse *PS1* gene causes striking phenotypes associated with reduced *Notch* activity (Shen et al. 1997; Wong et al. 1997).

Presenilins are multipass transmembrane proteins, and their mechanism of function is not known. A recent study has suggested that PS1 is required for a cleavage event during amyloid precursor protein processing (De Strooper et al. 1998), although whether presenilins promote cleavage per se or a trafficking event necessary for cleavage is not known. The effect of presenilins on amyloid precursor protein processing is intriguing in view of the proteolytic processing events that LIN-12/Notch proteins undergo. Perhaps presenilins facilitate LIN-12/Notch activity by promoting one or more proteolysis events associated with LIN-12/Notch maturation or activation.

### Conservation of LIN-12/Notch form and function in vertebrates

There is every reason to believe that precedents established from studies in invertebrates will be directly applicable to vertebrates. Many of the components that have been identified in invertebrates, principally by genetic methods, have also been found in vertebrates (see Table 1). Furthermore, the available functional and expression data indicate that the vertebrate Notch pathway plays similar roles in mediating cell-cell interactions that specify cell fate (for review, see Gridley 1997). For example, activity of the *Xenopus* ligand X-Delta-1 appears to control lateral specification of neurons, as ectopic X-Delta-1 activity inhibits the formation of primary neurons, and a dominant-negative form of X-Delta-1 promotes the formation of excess primary neurons (Chitnis et al. 1995). It is likely that further identification of components of the signal transduction mechanism and of other influences on LIN-12/Notch signaling in invertebrates will continue to inform studies of Notch signaling in vertebrate development.

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