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THE LIN-12/Notch SIGNALING PATHWAY AND ITS REGULATION

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ABSTRACT

Notch, LIN-12, and GLP-1 are receptors that mediate a broad range of cell interactions during *Drosophila* and nematode development. Signaling by these receptors relies on a conserved pathway with three core components: DSL ligand, LNG receptor, and a CSL effector that links the receptor to its transcriptional response. Although key functional regions have been identified in each class of proteins, the mechanism for signal transduction is not yet understood. Diverse regulatory mechanisms influence signaling by the LIN-12/Notch pathway. Inductive signaling relies on the synthesis of ligand and receptor in distinct but neighboring cells. By contrast, lateral signaling leads to the transformation of equivalent cells that express both ligand and receptor into nonequivalent cells that express either ligand or receptor. This transformation appears to rely on regulatory feedback loops within the LIN-12/Notch pathway. In addition, the pathway can be regulated by intrinsic factors that are asymmetrically segregated during cell division or by extrinsic cues via other signaling pathways. Specificity in the pathway does not appear to reside in the particular ligand or receptor used for a given cell-cell interaction. The existence of multiple ligands and receptors may have evolved from the stringent demands placed upon the regulation of genes encoding them.

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INTRODUCTION

Signaling by the related receptors *Drosophila* Notch and nematode LIN-12 and GLP-1 controls numerous cell fate decisions during development. In *Caenorhabditis elegans* and *Drosophila*, where cell interactions can be examined at the level of individual cells, the control of cell fates by these receptors has been analyzed in genetic, molecular, and cellular detail. These studies define a conserved pathway for signal transduction and reveal several mechanisms for regulation of the pathway.

The similarities between the *Drosophila* Notch and *C. elegans* LIN-12 and GLP-1 receptors are impressive: All three have a common domain architecture; all three function during development to regulate decisions between alternate cell fates; and all three act via a similar core pathway of control. Nonetheless, these receptors and their regulatory pathways are not identical. For example, Notch has 36 EGF-like repeats, which appear to possess some regional specialization (Kelley et al 1987, Hartley et al 1987, Rebay et al 1991), whereas GLP-1 and LIN-12 have many fewer EGF-like repeats (10 and 13, respectively) with no evidence for regions with specific functions (Kodoyianni et al 1992). Furthermore, in certain cell interactions, signaling by *Drosophila* Notch is intimately linked with the *wingless* pathway (e.g. Blair 1996), but no hint of the same has

been obtained for LIN-12 and GLP-1. Therefore, although common features are broadly recognized, the pathways that transduce and regulate receptor activity are not likely to be superimposable.

This review focuses on signaling by the LIN-12/Notch pathway and its regulation. Due to space constraints, signaling in *C. elegans* and *Drosophila* is stressed. We refer the reader to previous reviews for a more thorough discussion of individual components of the pathway, other views concerning signal transduction and regulatory networks, and a more extensive discussion of the vertebrate pathway (Greenwald 1994, Muskavitch 1994, Simpson 1994, Artavanis-Tsakonas et al 1995, Lewis 1996).

SIGNALING BY THE LIN-12/Notch PATHWAY: AN OVERVIEW

The Notch, LIN-12 and GLP-1 receptors transduce signals by a newly emerging pathway. At the heart of the pathway are three components: DSL ligands (for Delta, Serrate, and LAG-2), LNG receptors (for LIN-12, Notch, and GLP-1), and CSL effectors [for CBF-1, Su(H), and LAG-1] (Figure 1; see legend to Figure 1 for brief description of motif organization). These three constituents are used for most, though perhaps not all, LIN-12/Notch signaling.

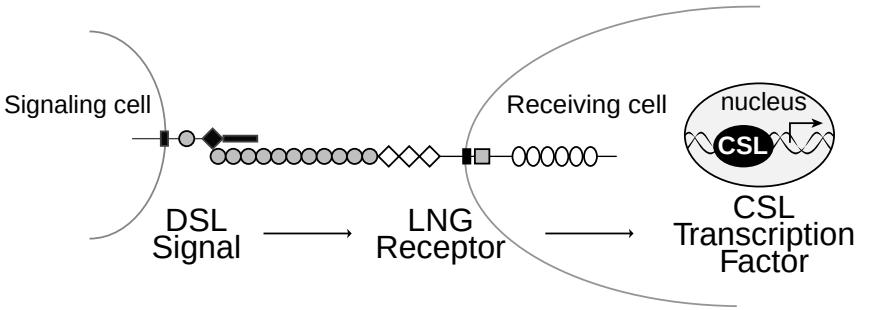

Figure 1 Core components of the LIN-12/Notch signaling pathway. DSL transmembrane protein is shown in a signaling cell; LNG receptor and CSL transcription factor are shown in a receiving cell. Motifs in the DSL protein include a transmembrane domain (*small black rectangle*), one or more complete EGF-like repeats (*gray circle*), a single DSL domain (*black diamond*), and an N-terminal region (*long black rectangle*). Motifs in the LNG receptor include EGF-like repeats (*gray circles*), LNG repeats (*open diamonds*; also known as LIN-12/Notch Repeat repeats), a transmembrane domain (*small black rectangle*), a RAM domain (*gray square*; also known as RAM23 domain), six cdc10/ankyrin repeats (*open ovals*; also known as cdc10/SWI6 repeats and ANK repeats). Each of these three components of the pathway is required for active signaling. Some evidence suggests that both DSL protein and LNG receptor may function as dimers or oligomers, but single proteins are shown here for simplicity. See text for further explanation.

Core Components

LIGANDS DSL transmembrane proteins are likely to be signaling ligands for LIN-12/Notch receptors. Founding members include Delta and Serrate from *Drosophila* (Vässin et al 1987, Kopczynski et al 1988, Fleming et al 1990, Thomas et al 1991) and LAG-2 from *C. elegans* (Lambie & Kimble 1991, Henderson et al 1994, Tax et al 1994). Additional DSL family members include APX-1 in *C. elegans* (Mello et al 1994) and various vertebrate homologues (see Evolutionary Considerations). The role of DSL proteins as ligands is supported by numerous lines of evidence. Genetic mosaic analyses demonstrate clearly that Delta functions in signaling cells (Heitzler & Simpson 1991), and expression studies show that various DSL proteins are expressed specifically in signaling cells (Ghysen et al 1993, Henderson et al 1994, Wilkinson et al 1994, Mickey et al 1996, Moskowitz & Rothman 1996). In tissue culture, Delta-expressing cells adhere to Notch-expressing cells, and Delta and Notch colocalize at the cell junction (Fehon et al 1990, Rebay et al 1991, Lieber et al 1992). Also in tissue culture, Jagged, a vertebrate homologue of Serrate, activates rat Notch1 in myoblasts and prevents muscle differentiation (Lindsell et al 1995). Biochemical evidence for binding rests upon the co-immunoprecipation of Delta with Notch (Fehon et al 1990). The accumulated evidence therefore strongly supports the idea that DSL proteins are ligands for LNG receptors.

Although sequence identity is low among DSL proteins, they share a similar motif organization (Figure 1). Crucial for signaling activity are the N-terminal region and DSL domain (Lieber et al 1992, Muskavitch 1994, Henderson et al 1994, Fitzgerald & Greenwald 1995, Henderson et al 1997). The N-terminal region exhibits little sequence conservation, no motif, and extends between the predicted signal sequence and DSL domain; the DSL domain is a family-specific motif related to EGF-like repeats. Although signaling can be achieved by a minimal fragment composed of the N-terminal and DSL domains, membrane association appears to be critical for full activity, perhaps by localizing or concentrating ligand (Fitzgerald & Greenwald 1995, Henderson et al 1997).

RECEPTORS The LNG receptors are transmembrane proteins; founding members are LIN-12 of *C. elegans* and Notch of *Drosophila* (Greenwald 1985, Wharton et al 1985). Their role as receptors is supported by genetic mosaic analyses (Austin & Kimble 1987, Seydoux & Greenwald 1989, de Celis et al 1991, Heitzler & Simpson 1991), expression studies (Fehon et al 1991, Kooh et al 1993, Crittenden et al 1994, Evans et al 1994, Wilkinson et al 1994, Moskowitz & Rothman 1996), and identification of the intracellular domain as essential for signaling activity (Lieber et al 1993, Rebay et al 1993, Roehl & Kimble 1993, Struhl et al 1993, Roehl et al 1996). Within this intracellular

domain resides a RAM23 domain that binds the CSL effector (Tamura et al 1995, Hsieh et al 1996, Roehl et al 1996), six cdc10/ankyrin (ANK) repeats that are key for receptor activity (Kodoyianni et al 1992, Roehl & Kimble 1993, Shawber et al 1996), and a potential PEST domain. Analysis of LNG receptors on Western blots indicates proteolytic processing (Aster et al 1994, Crittenden et al 1994, Kopan et al 1996, Shawber et al 1996), but the functional significance of that processing has not been determined.

EFFECTOR CSL proteins include CBF1 (also known as RBP-Jκ and KBF2) in vertebrates (Matsunami et al 1989, Grossman et al 1994, Henkel et al 1994), Su(H) in *Drosophila* (Ashburner 1982, Schweisguth & Posakony 1992), and LAG-1 in *C. elegans* (Lambie & Kimble 1991, Christensen et al 1996). CSL proteins carry no known DNA-binding motif; however, a stretch of about 400 conserved amino acids binds DNA with similar sequence specificity in all known CSL proteins (Brou et al 1994, Chun et al 1994, Tun et al 1994, Christensen et al 1996, Roehl et al 1996). They can act as either transcriptional activators (Grossman et al 1994, Henkel et al 1994, Waltzer et al 1994, Zimber-Strobl et al 1994, Bailey & Posakony 1995, Jarriault et al 1995, Lecourtois & Schweisguth 1995) or repressors (Dou et al 1994, Hsieh & Hayward 1995).

Regulation of Receptor by Ligand

The mechanism by which DSL proteins promote receptor activity is not understood. In Notch, two EGF-like (EGFL) repeats, EGFL11 and EGFL12, are both necessary and sufficient for adhesion between Delta-expressing and Notch-expressing tissue culture cells (Rebay et al 1991). However, in vivo, EGFL11 and EGFL12 are not sufficient to mediate signaling (Lieber et al 1993, Rebay et al 1993). Furthermore, lesions in EGFL24 to 29 lead to hyperactive, but ligand-dependent, receptor activity (Kelley et al 1987, Heitzler & Simpson 1993). Activation of receptor by ligand is thought to be associated with a change in the dimerization state of both ligand and receptor (Kelley et al 1987, Kidd et al 1989, Greenwald & Seydoux 1990, Lieber et al 1993, Muskavitch 1994; L Seugnet, P Simpson & M Haenlin, unpublished observation). Missense mutations in the family-specific LNG repeats and in the region between the LNG repeats and the transmembrane domain lead to ligand-independent receptor activity, perhaps by altering the self-association of receptor (Greenwald & Seydoux 1990, Lieber et al 1993, Berry et al 1997). However, it is important to note that in-frame deletions that specifically remove most of the LNG repeats eliminate receptor activity even though the protein remains present (Kodoyianni et al 1992; H Roehl, S Crittenden & J Kimble, unpublished observation). Finally, active signaling is accompanied by uptake of both ligand and receptor into the receiving cell (Henderson et al 1994, Parks

et al 1995, Henderson et al 1997). Clones of cells doubly mutant for truncated constitutively active Notch and *shibire*, a dynamin mutant, show that endocytosis is not necessary for transduction of the signal downstream of Notch (L Seugnet, P Simpson & M Haenlin, unpublished observation). However, in the absence of endocytosis, Notch-mediated lateral signaling is not maintained, possibly because of the accumulation of inactive ligand-receptor complexes at the cell surface.

Signal Transduction by Receptor and CSL Transcription Factor

Two models have been proposed for the regulation of CSL proteins by receptor. Others clearly exist, but given the paucity of data, we discuss only those most hotly debated at the current time. Model one proposes that receptor activation leads to transport of the CSL protein from cytoplasm to nucleus. In tissue culture, Notch retains Su(H) in the cytoplasm before being activated; activation by Delta leads to transport of Su(H) into the nucleus (Fortini & Artavanis-Tsakonas 1994). The most parsimonious interpretation, that Notch represses Su(H), is too simple, because Su(H) is not active in the absence of Notch. The idea that Notch may repress Su(H) can be saved, however, by invoking an additional activity for the receptor that promotes CSL activity (e.g. stabilization, or providing a scaffold for association with other proteins). Model two proposes that the intracellular domain of the receptor is cleaved upon activation and enters the nucleus to work with the CSL protein as a co-activator of transcription. In support of this model, the intracellular domain of mammalian Notch can act together with CBF1 to drive transcription in tissue culture cells (Jarriault et al 1995, Hsieh et al 1996). Furthermore, a truncated form of mammalian Notch, which lacks most of its extracellular domain, is cleaved in tissue culture cells to generate an intracellular fragment that enters the nucleus (Kopan et al 1996). Finally, all LNG receptors tested to date carry a nuclear localization signal within their intracellular domains (Lieber et al 1993, Rebay et al 1993, Struhl et al 1993, Roehl et al 1996).

Model one predicts that CSL proteins should colocalize with receptor in unactivated cells and then appear in the nucleus of activated cells. However, this is not the case: CSL proteins are primarily nuclear in both activated and unactivated cells (Gho et al 1996; V Kodoyianni & J Kimble, unpublished data). Model two predicts that the receptor's intracellular domain will become nuclear upon activation. However, antibodies directed against the intracellular domain do not detect antigen in nuclei of either *Drosophila* or *C. elegans* (Fehon et al 1991, Crittenden et al 1994). Intriguingly, antibodies against the intracellular domain of mammalian Notch detect antigen in the nuclei of differentiated rat neurons, but the state of LIN-12/Notch signaling in these cells is unknown

(Ahmad et al 1995). Therefore, despite numerous clues about the mechanism of signal transduction, no solid evidence yet exists to support one mechanism over another. Other models, of course, remain plausible, including those that combine aspects of the two described above.

In thinking about a mechanism of signal transduction, the idea that CSL proteins can act as either transcriptional activators or repressors (see above for references) is intriguing. Perhaps key CSL binding sites are complexed in a repressed state prior to signaling, and then transformed into an active complex by an activated receptor. Such a mechanism would achieve a profound switch in the transcriptional state of target genes. Furthermore, such a mechanism would not predict a difference in the amount of nuclear CSL protein before and after signaling.

Other Components of the Pathway

Many other genes have been implicated in the regulation of cell fate by the LIN-12/Notch pathway. To conserve space, these are summarized in Table 1. Most are clearly linked to the LIN-12/Notch pathway in only one organism, although homologues of some have now been identified in other organisms as well. An important question to be addressed over the next few years is how extensively the pathway has been conserved beyond the three core components emphasized here.

CELL INTERACTIONS CONTROLLED BY THE LIN-12/Notch PATHWAY

Signaling by the LIN-12/Notch pathway controls numerous cell-cell interactions (Table 2). These interactions are classically divided into two fundamental types. Induction occurs between nonequivalent cells: A cell of one type signals to a neighboring cell of a different type to adopt one of two alternative fates. In contrast, lateral signaling occurs between equivalent cells, each of which adopts one of two alternate fates as a result of the interaction. In this section, we review those cell fate decisions mediated by the LIN-12/Notch pathway in *C. elegans* and *Drosophila* that have been subjected to most intensive analyses.

Induction of Germline Mitosis in C. elegans

A somatic gonadal cell, called the distal tip cell, signals the neighboring germline to divide mitotically (Kimble & White 1981). This proliferative signal induces growth of the germline during larval development and maintains a population of germline stem cells in the adult. Furthermore, localization of this signal establishes polarity in the germline tissue. Signaling by the distal tip cell is required continually during larval development and adulthood for germline growth; in its

Table 1 Other components of the LIN-12/Notch signaling pathway

Gene	D.m.[a]	C.e.[a]	vert.[a]	Molecular identity/Function in pathway	Reference[b]
bearded	+	?	?	Gain-of-function alleles interfere with Notch signaling	Leviten & Posakony (1996)
big brain	+	?	?	Membrane protein; may act upstream of receptor	Rao et al (1992)
deltex	+	?	+	RING finger; SH3-binding domain; positive regulator	Matsuno et al (1995)
fringed	+	+	+	Glycosyl transferase; positive regulator of pathway	Yuan et al (1997)
E(spl)-C	+	?	+	bHLH transcription factors; downstream targets in epidermal/neural cells	see text
groucho	+	+	+	Nuclear protein; WD-40 repeats; homology with Tupl; co-repressor with E(Spl)-C	Paroush et al (1994)
Hairless	+	?	+	Novel basic protein; negative regulator of pathway	Bang et al (1995)
master mind	+	?	?	Nuclear protein; one Zn finger; may act upstream of receptor	Smoller et al (1990)
neuralized	+	?	?	Nuclear protein; may act upstream of receptor	Zhou & Boulianne (1994)
numb	+	?	+	PTB domain; SH3-binding domain; negative regulator of Notch	see text
scabrous	+	?	?	Fibrogen-related secreted protein; Ser/Thr protein kinase; part of positive feedback loop?	Lee et al (1996)
shaggy	+	+	+		see text
strawberry Notch	+	+	?	Nuclear protein; downstream of receptor; positive regulator in inductive interactions	Coyle-Thompson & Banerjee (1993)

vestigial	+	?	?	Downstream target in *Drosophila* wing	Kim et al (1996)
ego-1–ego-5	?	+	?	Enhancers of *glp-1*; none cloned	Qiao et al (1995)
emb-5	?	+	+	Acidic nuclear protein; homology to yeast SPT6; positive downstream effector of LIN-12/GLP-1	Hubbard et al (1996)
glp-4	?	+	?	Enhancer of *glp-1*; not cloned	Qiao et al (1995)
sel-1	?	+	?	Found in intracellular vesicles; negative regulator of LIN-12/GLP-1	Grant & Greenwald (1997)
sel-9, sel-10, sel-11	?	+	?	Suppressors/enhancers of LIN-12; none cloned	Sundaram & Greenwald (1993)
sel-12	?	+	+	Membrane protein; homology to human S182; facilitates LIN-12/GLP-1 signaling	Levitan & Greenwald (1995)
sog-1–sog-10	?	+	?	Suppressors of *glp-1*; none cloned	Maine & Kimble (1993)

[a]D.m., *Drosophila melanogaster*; C.e., *Caenorhabditis elegans*; vert., vertebrate.

[b]Referencing is not complete; instead, a recent reference is provided to facilitate entry into the literature.

Table 2 Cell interactions controlled by the LIN-12/Notch pathway

Interaction	Type	DSL protein	LNG receptor	CSL protein	Target gene
DTC/germline	Induction	LAG-2	GLP-1	LAG-1	?
P2/ABp	Induction	APX-1	GLP-1	LAG-1	?
MS/ABara and ABalp	Induction	?	GLP-1	LAG-1	?
IntraAB interactions[a]	Induction	LAG-2	LIN-12/GLP-1	LAG-1	
D → V wing margin[b]	Induction	Serrate	Notch	Su(H)	*vg*
V → D wing margin	Induction	Delta	Notch	Su(H)	*vg*
AC/VU	Lateral signaling	LAG-2	LIN-12	?	?
VPCs	Lateral signaling	?	LIN-12	?	?
LC/VD	Lateral signaling	LAG-2	LIN-12	?	?
Neural/non-neural fates[c]	Lateral signaling	Delta	Notch	Su(H)	*E(spl)-C*
Specification of SOP progeny	Lineally biased lateral signaling?	Delta	Notch	Su(H)	*E(spl)-C*

[a]This category includes induction of ABplaa and ABplpa.

[b]D, dorsal cells; V, ventral cells.

[c]This category encompasses neurons versus epidermis decision in embryos, specification of sensory organ precursor cells (SOP) in bristles, specification of SOP in chordotonal organs, and specification of R8 photoreceptors in eye.

absence, germline cells enter meiosis and complete gametogenesis. Induction of germline mitosis requires LAG-2 in the signaling cell (Lambie & Kimble 1991, Henderson et al 1994, Tax et al 1994) and GLP-1 and LAG-1 in the receiving cell (Austin & Kimble 1987, 1989, Yochem & Greenwald 1989, Lambie & Kimble 1991, Crittenden et al 1994, Christensen et al 1996).

Blastomere Specification in the Early C. elegans Embryo

A cascade of GLP-1-mediated inductions regulates blastomere specification in the early *C. elegans* embryo. At the 4-cell stage, the P2 blastomere induces the ABp blastomere to adopt a fate distinct from its sibling ABa (Bowerman et al 1992, Hutter & Schnabel 1994, Mango et al 1994, Mello et al 1994, Moskowitz et al 1994). Maternal *apx-1* and *glp-1* are required for the P2/ABp interaction (Mango et al 1994, Mello et al 1994). Therefore, GLP-1 is used for both germline induction and ABp induction, but it responds to LAG-2 in the first case and to APX-1 in the second. Cell contact is required for the P2 induction of ABp (Mello et al 1994). At the 12-cell stage, the MS blastomere induces two descendants of ABa, ABalp and ABara, to generate pharyngeal tissue (Priess et al 1987). The ligand is not known for this second interaction; maternal *glp-1* supplies the receptor (Priess et al 1987, Evans et al 1994) and LAG-1 is likely to serve as the downstream effector (V Kodoyianni & J Kimble,

unpublished data). The two *lag* genes as well as zygotic *lin-12* or *glp-1* regulate two subsequent inductive interactions, one of which induces epidermis at the expense of neurons (Moskowitz & Rothman 1996).

Induction of the Wing Margin in Drosophila

Establishment of the dorsal-ventral (D/V) wing margin relies on a reciprocal induction in the wing imaginal disc: Dorsal cells induce ventral cells to express *wingless* (*wg*), and ventral cells similarly induce dorsal cells to express *wg* (Diaz-Benjumea & Cohen 1995, Rulifson & Blair 1995, Doherty et al 1996, Couso et al 1995). The resultant stripe of *wingless*-expressing cells controls both growth and patterning of the wing. Both inductive signals at the wing margin rely on the Notch receptor. However, dorsal cells signal with Serrate, whereas ventral cells use Delta. Serrate is expressed in all dorsal cells but is only required along the border (Kim et al 1995, Diaz-Benjumea & Cohen 1995, Couso et al 1995, Doherty et al 1996). Delta, by contrast, is expressed at a high level only in ventral cells near the boundary (Doherty et al 1996, de Celis et al 1995). Positive feedback loops may reinforce both signals (Doherty et al 1996; see below).

The AC/VU Decision in the C. elegans Gonad

During normal development, two somatic gonad cells interact to select one of two alternate fates: one becomes an anchor cell (AC) and the other a ventral uterine precursor cell (VU) (Kimble 1981). In this cell interaction, LAG-2 is the signal (Lambie & Kimble 1991, Wilkinson et al 1994) and LIN-12 is the receptor (Greenwald et al 1983). The role of LAG-1, if any, is not known. In the absence of signaling, both cells differentiate as ACs, whereas unregulated signaling leads to both becoming VUs (Greenwald et al 1983). Contact between the AC/VU precursor cells appears to be essential for signaling: When the two cells fail to meet in a mutant affecting cell movement, both adopt the AC fate (Hedgecock et al 1990).

Development of Sensory Bristles in Drosophila

The *Drosophila* epidermis is covered with sensory bristles. At least two stages in the development of these bristles depend on signaling by the LIN-12/Notch pathway. First, lateral signaling regulates the selection of a sensory organ precursor cell (SOP) from a surrounding group of equivalent cells with neural potential, so-called proneural cells (Heitzler & Simpson 1991). Second, the SOP undergoes two rounds of division to generate four different descendants by an invariant lineage that nonetheless depends on Notch for correct specification of progeny fates (Hartenstein & Posakony 1990, Parks & Muskavitch 1993). Delta and Notch act as signal and receptor, respectively, in this cascade of interactions that controls bristle development (reviewed in Muskavitch 1994).

Other Cell Interactions Mediated by Notch, LIN-12, or GLP-1

Signaling by Notch, LIN-12, and GLP-1 controls many other cell fates in both *Drosophila* and nematodes. Notch regulates the embryonic decision between epidermal and neural fates (Lehmann et al 1983), as well as specification of fate in the somatic gastric nervous system (Gonzalez-Gaitan & Jäckle 1995), Malpighian tubule tip cells (Hoch et al 1994), muscle founder cells (Bate et al 1993), specialized follicle cells in the ovary (Ruohola et al 1991), R8 photoreceptors and most other cell types in the eye (Baker & Zitron 1995, Cagan & Ready 1989), and midgut cells (Tepass & Hartenstein 1995). Nematode LIN-12 regulates lateral signaling among numerous pairs or groups of cells including the vulval precursor cells (VPCs) (Greenwald et al 1983); furthermore, LIN-12 mediates the induction of certain uterine cells (Newman et al 1995). Finally, either LIN-12 or GLP-1 regulates other cell interactions during mid-embryogenesis (Lambie & Kimble 1991, Moskowitz & Rothman 1996).

REGULATION OF SIGNALING BY THE LIN-12/Notch PATHWAY

Signaling by the LIN-12/Notch pathway can be regulated at a remarkable number of levels. In this section, we discuss the best known controls of the pathway that lead to induction, lateral signaling, and invariant cell lineage. In addition, we touch upon interactions between LIN-12/Notch signaling and other signal transduction pathways.

Induction: Cell-Specific Expression of Ligand and Receptor

Cells participating in an inductive interaction express either a DSL protein or an LNG receptor (Figure 2*A*). For example, in germline induction, LAG-2 is expressed in the signaling distal tip cell, whereas GLP-1 is restricted to the receiving germline tissue (Henderson et al 1994, Crittenden et al 1994). A similarly tight regulation of both ligand and receptor is seen in the early nematode embryo. Here the APX-1 signal is limited to the P2 blastomere (Mickey et al 1996), and GLP-1 receptor is restricted to AB and its daughters (Evans et al 1994). In the *Drosophila* wing margin, Delta and Serrate are expressed in ventral and dorsal compartments, respectively, whereas Notch is expressed in both compartments (Speicher et al 1994, Kim et al 1995, Doherty et al 1996). Therefore, in inductive interactions, control of gene expression appears to dictate the signaling and receiving partners of the interaction.

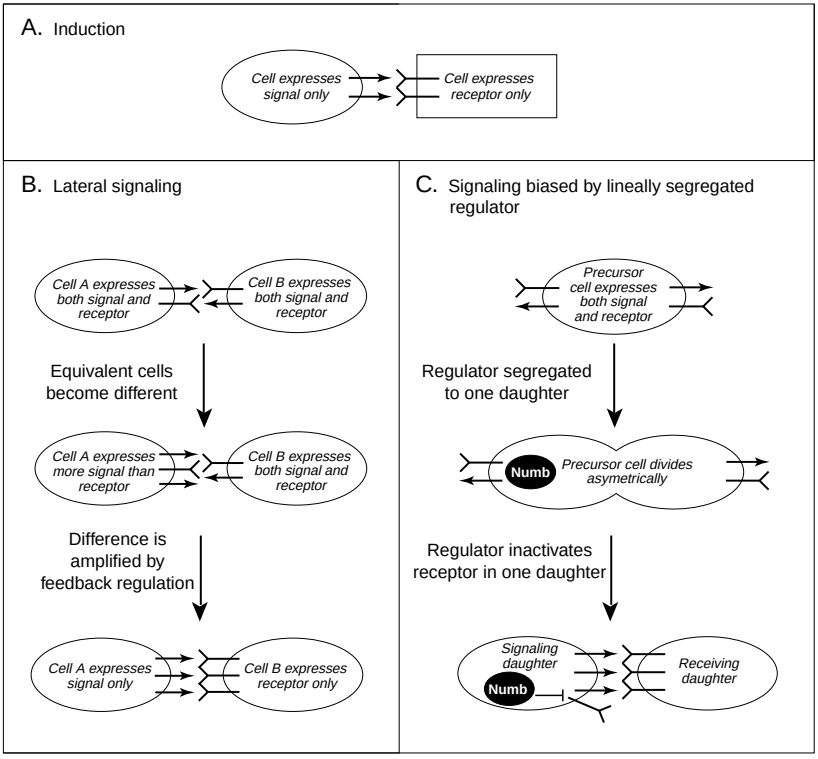

Figure 2 Regulation of the LIN-12/Notch signaling pathway. (*A*) Induction: Cells are not equivalent and are depicted as an oval (*left*) and a rectangle (*right*). Only the signaling cell expresses the DSL protein (*arrow*), whereas only the receiving cell expresses the LNG receptor (*Y-shape*). (*B*) Lateral signaling: Cells are initially equivalent and are both depicted as *ovals*. Owing to lateral signaling, each cell resolves to a signaling cell (*left*) or receiving cell (*right*). See text for further explanation. (*C*) Signaling biased by a lineally segregated regulator: A precursor cell divides asymmetrically to generate two distinct daughter cells. One of these daughters carries a negative regulator of receptor activity and therefore becomes a signaling cell. The other daughter may become the receiving cell. See text for further explanation.

Lateral Signaling and Feedback Regulation

Lateral signaling appears to require three regulatory steps (Figure 2*B*) (Seydoux & Greenwald 1989, Heitzler & Simpson 1991, Wilkinson et al 1994). First, the interacting cells express both ligand and receptor. Second, one of the cells acquires a bias toward either the signaling or receiving fate—perhaps by a stochastic fluctuation in the relative amounts of ligand and receptor. And finally,

the bias is amplified so that one cell expresses ligand and not receptor and the other expresses receptor and not ligand. This final situation resembles induction in that one cell is transformed into a signaling cell and the other into a receiving cell (compare Figures 2*A* and 2*B*).

One prediction of this three-step model involves a change in the expression of ligand and receptor during lateral signaling. In the nematode, both AC/VU precursor cells initially express both *lag-2::lacZ* and *lin-12::lacZ* reporter genes; then shortly before commitment, *lag-2* and *lin-12* expression becomes restricted to future AC and VU cells, respectively (Wilkinson et al 1994). Therefore, cells that initially express both ligand and receptor are transformed into one signaling and one receiving cell. Although this has not been clearly demonstrated in the case of the SOP of *Drosophila,* Ghysen et al (1993) have observed a progressive restriction of Delta from all cells of the proneural cluster to the emerging SOP.

A second prediction is that the relative amounts of ligand and receptor in the signaling cells will influence the cell fate decision. Genetic mosaic experiments in both nematodes and *Drosophila* support this prediction. Normally in nematodes, the choice of which cell adopts the AC or VU fate is random. By contrast, when a wild-type AC/VU precursor lies adjacent to a mutant AC/VU precursor that lacks LIN-12, the wild-type cell invariably adopts the VU fate (Seydoux & Greenwald 1989). Therefore, a cell lacking receptor always becomes a signaling cell and the normally random choice adopts a strong bias. The importance of the relative abundance of ligand and receptor has been dramatically underscored by manipulating the dosage of Notch and Delta in *Drosophila* (Heitzler & Simpson 1991). When cells carrying two copies of wild-type Notch were juxtaposed to cells carrying either three copies or one copy, those cells with less receptor than their neighbors adopted the neural fate. Therefore, a cell expressing two copies of Notch becomes the signaling cell when adjacent to a cell with three copies of Notch, but it becomes the receiving cell when adjacent to a cell with only one copy of Notch. In analogous experiments with Delta, those cells with more Delta than their neighbors became neural. The major conclusion is that a small relative difference between adjacent cells in levels of either ligand or receptor can determine fate: A cell with less receptor or more ligand dominates the interaction.

The amplification of an initially small relative difference in ligand and receptor to a much larger difference may rely on feedback regulation. A rather simple feedback loop has been suggested in the case of the AC/VU decision (Wilkinson et al 1994). Here, receptor activation not only down-regulates expression of ligand in the receiving cell, it also maintains receptor expression in that same cell. Positive feedback upon receptor may occur at the transcriptional level: Deletion of a conserved sequence in the 5′-flanking region of *lin-12*,

called LCS1, eliminates receptor expression in the receiving cell of the AC/VU decision (Wilkinson et al 1994). Nine consensus binding sites for the CSL protein LAG-1 occur in the 5′-flanking region of *lin-12*, including one in LCS1 (Christensen et al 1996). These binding sites may be used to promote *lin-12* transcription.

A more complex, but perhaps more complete, regulatory network has been suggested in *Drosophila*. This circuit includes the *Enhancer of Split* and *Achaete/Scute* gene complexes, *E(spl)-C* and *AS-C*, which encode batteries of bHLH transcription factors (Alonso & Cabrera 1988, Knust et al 1992). Before SOP selection, all cells of the proneural cluster, or PNC, express *AS-C* genes promoting neural development (Cubas et al 1991, Skeath & Carroll 1991, Skeath et al 1992). Subsequently, lateral signaling among the PNCs permits resolution of the group into one signaling cell, the SOP, and adjacent receiving cells. The future SOP maintains *AS-C* expression at a high level, whereas other PNC cells cease *AS-C* expression and adopt the epidermal fate. In mutants lacking Notch or Delta, all PNC cells continue to express *AS-C* and adopt the neural fate (Hartenstein & Posakony 1990, Heitzler & Simpson 1991). Both *E(spl)-C* and *AS-C* are integral to feedback in flies (Heitzler et al 1996). In signaling cells, AS-C proteins probably activate Delta at the transcriptional level (Künisch et al 1994, Heitzler et al 1996). In receiving cells, *E(spl)-C* genes are transcriptionally activated by Su(H) (Bailey & Posakony 1995, Lecourtois & Schweisguth 1995). E(spl)-C proteins appear to act together with the Groucho protein to repress transcription of the *achaete/scute* (*ac/sc*) genes (Delidakis et al 1991, Schrons et al 1992, Oellers et al 1994, Tata & Hartley 1995, Nakao & Campos-Ortega 1996); in this way, activation of *E(spl)-C* by Su(H) leads to repression of *AS-C* and a loss of Delta expression.

Thus during lateral signaling, receptor activation leads to the down-regulation of signal—both for Delta/Notch and LAG-2/LIN-12. However, the regulatory circuitry may not be the same. Although homologues of *E(spl)* and *ac/sc* may have been identified in nematodes (Zhao & Emmons 1995, Wrischnik & Kenyon 1997), they have not been linked to LIN-12 or GLP-1 signaling.

Intriguingly, regulation similar to that found in lateral signaling may also affect other interactions. The best case is found in the early *C. elegans* embryo where maternal *glp-1* represses zygotic *lag-2* expression and activates zygotic *lin-12* (Moskowitz & Rothman 1996). This regulatory relationship is similar to that proposed to occur during lateral signaling but has the added twist that activity in one interaction under maternal control influences that of subsequent zygotic interactions to generate a cascade of induction. In addition, positive feedback regulation may amplify GLP-1 activity during control of germline proliferation (Kodoyianni et al 1992) and may lead to ligand-independent induction in the proximal germline (Seydoux et al 1990).

Induction and Feedback Regulation at the Drosophila Wing Margin

During induction of the wing margin in *Drosophila,* Notch activity leads to up-regulation of Serrate in dorsal cells and up-regulation of Delta in ventral cells (Doherty et al 1996). Induction at the margin is thereby amplified in both dorsal and ventral cells, albeit by up-regulation of different ligands in different cells. This positive regulatory loop contrasts with the negative loop observed during lateral signaling, where LIN-12/Notch activation down-regulates expression of *lag-2/Delta.* Therefore, tissue-specific factors appear to play a critical role in determining how individual cells react to signaling.

Asymmetric Segregation of a Pathway Regulator

Activity of the LIN-12/Notch pathway can also be regulated by segregation of a signaling component or regulator of the pathway to one of two daughters of a cell division (Figure 2*C*). The best example of this regulatory mechanism is observed during development of sensory organs in *Drosophila*. Typically, a SOP generates four distinct daughters by two rounds of division (Lawrence 1966). In the absence of Notch, this simple lineage gives rise to four neurons instead of its four typical fates, of which only one is a neuron (Hartenstein & Posakony 1990, Parks & Muskavitch 1993). Therefore, the generation of specific cell types by this invariant lineage relies on the LIN-12/Notch pathway and presumably upon cell-cell interactions.

The *numb* gene controls LIN-12/Notch signaling in both the peripheral and central nervous systems of flies (Uemura et al 1989, Rhyu et al 1994, Spana et al 1995, Guo et al 1996). Numb acts cell autonomously (Spana et al 1995) and functions upstream of Notch to control cell fate (Guo et al 1996). The effect of Numb is opposite that of Notch, suggesting that Numb is a negative regulator. Consistent with this idea, when assayed in tissue culture cells, Numb inhibits the translocation of Su(H) to the nucleus upon receptor activation (Frise et al 1996). The regulation of Notch by Numb may be direct because Numb binds directly to the receptor's intracellular domain (Guo et al 1996).

The current model is that asymmetric segregation of Numb leads to the down-regulation of Notch in one daughter cell, which creates a bias in the signaling potential of the Numb-bearing cell (Jan & Jan 1995). That bias may be amplified by feedback regulation as has been found for lateral signaling (Wilkinson et al 1994). Thus the down-regulation of Notch in the Numb-bearing cell may lead to up-regulation of Delta and the subsequent transformation of that cell into a signaling cell.

A role for asymmetric segregation in the control of the LIN-12/Notch pathway has also been suggested in other cases. For example, during mammalian

neurogenesis, Notch1 is segregated to the basal cell of an asymmetric division in neural ectoderm (Chenn & McConnell 1995). In the 2-cell embryo of *C. elegans*, the asymmetric distribution of GLP-1 may result from the differential segregation of a translational regulator of GLP-1 (Evans et al 1994).

Interactions with Other Signaling Pathways

Signaling by the LIN-12/Notch pathway can be influenced by extrinsic as well as intrinsic cues (e.g. *numb*). In this section we consider two examples: interactions with the *wingless* and EGF pathways.

INTERACTIONS WITH THE WINGLESS (WG) PATHWAY Genetic interactions between the Notch and *wingless* (*wg*) pathways are impressive, and the two pathways must be linked at some level (Couso et al 1995, Blair 1996). In certain cases, Notch and wingless activities are antagonistic (Gonzalez-Gaitan & Jäckle 1995, Axelrod et al 1996), whereas in others they are synergistic (Neumann & Cohen 1996). Therefore, the relationship between these pathways is subject to the particular cells interacting. In certain cells, transcription of *wg* is induced by Notch and Su(H) (Neumann & Cohen 1996). In those cells where *wg* expression depends on Notch signaling, the two pathways induce the same developmental fate and therefore are not antagonistic. In other cases, where the two pathways act in opposition to each other, cross-talk may occur at any of several levels. For example, *disheveled* (*dsh*), which acts downstream of *wg*, may inhibit Notch activity post-translationally (Axelrod et al 1996). Similarly, SGG/GSK-3 kinase, which is inactivated by phosphorylation upon Wg signaling, may inhibit Notch (Cook et al 1996). During sensory organ development, certain cells are first signaled by Wg to maintain *ac-sc* expression in the proneural cluster and then are signaled by Notch to repress *ac-sc* expression in those cells that are not destined to become the precursor. *Wingless* acts by inactivating *shaggy* (*sgg*) to allow *ac-sc* expression, but *sgg* is then required for the later repression of *ac-sc*, suggesting that it is re-activated by Notch signaling (Simpson et al 1993, Ruel et al 1993). Finally, Wg protein may influence Notch activity by binding its extracellular domain (Couso et al 1995; C Wesley & M Young, unpublished observations). The generality of the *Notch/wg* interactions is not known: No link has yet been forged in *C. elegans* between LIN-12/GLP-1 and pathways homologous to *wingless*.

INTERACTIONS WITH EGF PATHWAY During development of the nematode vulva, the fates of six vulval precursor cells (VPCs) are specified by the combined action of a tyrosine kinase/ras signaling pathway and the LIN-12 pathway (Horvitz & Sternberg 1991). In wild-type animals, the somatic gonadal anchor cell (AC) signals the underlying VPCs to embark on vulval differentiation. The AC signal, called LIN-3, is a TGF-α homologue that acts through a receptor

similar to the EGF receptor to direct a 1° vulval fate in the closest VPC (Horvitz & Sternberg 1991). Lateral signaling by way of LIN-12 between the 1° VPC and flanking VPCs inhibits them from adopting the same fate; instead, those neighbors adopt a secondary fate (2°). The AC signal may bias the lateral signal by, for example, up-regulating ligand in the closest VPC, perhaps as one consequence of the 1° fate, or up-regulating the LIN-12 receptor in the flanking VPCs. Indeed, lin-25, which appears to act upstream of receptor in the LIN-12/Notch pathway, may be regulated by the MAPK pathway operating downstream of LIN-3 (Tuck & Greenwald 1995). In the *Drosophila* eye, signaling by both Notch and EGF is required for successive steps of cell fate specification (Cagan & Ready 1989, Freeman 1996), but the question of how these two pathways relate to one another is not known.

WHERE IS THE SPECIFICITY?

Cells respond differently to LIN-12/Notch activation depending on their developmental history, on their specific complement of ligand, receptor, and regulators, and presumably on other proteins expressed. Cells at the *Drosophila* wing margin express vestigial, wingless, and cut in response to signaling, whereas those in proneural clusters express E(spl)-C. Clearly, these two cell types must possess distinct regulatory factors to achieve such different responses.

How do the core components of the LIN-12/Notch pathway control individual choices of cell fate? One possibility in organisms with multiple ligands or receptors might be that individual ligands or receptors impose specificity on particular decisions. However, in those cases tested, the multiple ligands and receptors do not appear to have specificity in directing particular fates. In *C. elegans*, GLP-1 can replace LIN-12 to specify specific cell fates (Mango et al 1991, Lambie & Kimble 1991, Fitzgerald et al 1993). Most compelling is the result that a *glp-1* cDNA, placed under control of *lin-12* gene regulatory sequences, can rescue a null *lin-12* mutant (Fitzgerald et al 1993). Similarly, an *apx-1* cDNA rescues a *lag-2* null mutant when placed under *lag-2* regulation (Fitzgerald & Greenwald 1995, Gao & Kimble 1995), and Serrate, when placed under control of the heat shock promoter, can replace Delta for at least one interaction (Gu et al 1995).

The most straightforward conclusion is that specificity does not reside in the ligand/receptor interaction per se, but instead is delegated to the regulation of these proteins, mostly at the level of transcription, or to downstream factors. An interesting twist on this simple conclusion is posed by the action of Delta and Serrate at the wing margin. Delta activates Notch in dorsal but not ventral cells, and similarly, Serrate activates Notch in ventral but not dorsal cells. No satisfying explanation for this paradox has been put forward, although certain

aspects have been addressed. For example, Doherty et al (1996) propose that the presence of both Delta and Notch in ventral cells inhibits them from responding to Delta, and that Serrate in dorsal cells may overcome this inhibition by the production of *fringed*. An analogous situation might occur in dorsal cells: Activation of Notch by Serrate may be inhibited in cells expressing both, but may be overcome in ventral cells by the production of Delta plus some other regulator.

To address the lack of specificity among core components of the LIN-12/Notch pathway, it has been argued that signaling by this pathway may simply inhibit differentiation instead of specifying fates (e.g. Artavanis-Tsakanos et al 1995). Evidence supporting this model includes the block to muscle differentiation observed upon activation of rat Notch by Jagged in tissue culture (Lindsell et al 1995). However, Notch directly induces *vg*, a gene that promotes wing differentiation (Kim et al 1996), and it also induces *wg* at the dorsal/ventral wing margin, which is key for establishment of that boundary (Neumann & Cohen 1996). In the epidermis, Notch induces expression of *E(spl)-C* genes, which repress neural differentiation, but E(spl)-C proteins are not general repressors and are not used, for example, during Notch signaling at the wing margin (de Celis et al 1996). Therefore, conflicting data exist, and a single mechanism for LIN-12/Notch signaling may be overly simplistic.

EVOLUTIONARY CONSIDERATIONS

LIN-12/Notch Pathway in Other Organisms

DSL, LNG, and CSL proteins have been found in most animals where sought: DSL homologues (*Xenopus*: Chitnis et al 1995; chick: Henrique et al 1995, Myat et al 1996; rat: Lindsell et al 1995; mouse: Bettenhausen et al 1995), LNG homologues (sea urchin: D McClay, personal communication; *Xenopus*: Coffman et al 1993; zebrafish: Bierkamp & Campos-Ortega 1993; rat: Weinmaster et al 1991; mouse: Franco del Amo et al 1992, Robbins et al 1992, Lardelli & Lendahl 1993, Lardelli et al 1994; and humans: Ellisen et al 1991, Larsson et al 1994, Milner et al 1994), and CSL homologues (referenced in CSL section above). The progress made with the mammalian signaling pathway has been tremendous, relying in part on injection of dominant-negative components in *Xenopus* (Coffman et al 1993, Dorsky et al 1995, Chitnis et al 1995), in part on mouse gene knock-outs (Swiatek et al 1994, Conlon et al 1995, Oka et al 1995), and in part on tissue culture manipulations (Aster et al 1994, Kopan et al 1994, Austin et al 1995, Jarriault et al 1995, Hsieh et al 1996, Kopan et al 1996, Shawber et al 1996). A description of work on the vertebrate pathway is beyond the scope of this review.

A comparison of LIN-12/Notch pathways in various organisms reveals a striking similarity between *Drosophila* and vertebrate pathways. Vertebrate

ligands fall into Delta-like and Serrate-like classes (Nye & Kopan 1995), and most vertebrate receptors have 36 EGF-like repeats, as does *Drosophila* Notch (Artavanis-Tsakonas et al 1995). Furthermore, individual EGF-like repeats along the length of the protein are more similar between fly and vertebrate homologues than they are to other EGF-like repeats in the same protein. CBF1, the vertebrate CSL protein, activates transcription of HES-1, which is an E(spl) homologue (Jarriault et al 1995), and HES-1 in turn down-regulates MASH-1, a homologue of Ac/Sc (Ishibashi et al 1995). Therefore, in both flies and vertebrates, conservation embraces not only the core components, but also downstream target genes and their targets. The *C. elegans* pathway, by contrast, appears to be more distant with respect to individual components as well as regulatory circuitry. For each of the core components, specific sequences and number of motifs are more highly diverged between *C. elegans* and both fly and vertebrates than between fly and vertebrate homologues. In addition, although putative homologues of E(spl) and Ac/Sc have been found (see above), no link has yet emerged with LIN-12/GLP-1 signaling.

Evolution of Multiple Ligands and Receptors

Why does a single organism possess multiple ligands and receptors for signaling by the LIN-12/Notch pathway? Duplicated ligands and receptors may have evolved to achieve the distinct spatial and temporal regulation of individual components. The existence of unique regulatory circuits for individual ligands or receptors that function in the same organism is clearly a fact (e.g. Delta versus Serrate in *Drosophila*, and APX-1 versus LAG-2 and LIN-12 versus GLP-1 in *C. elegans*). These separate types of regulation may provide versatility for generating a pattern in the complex tissues of multicellular organisms. Consistent with this idea, vertebrates possess more receptors than either nematodes or *Drosophila*, which may underlie the generation of their greater body complexity. Alternatively, duplicated ligands and receptors may have evolved from a need for distinct ligands or receptors. Although the bulk of current evidence indicates that duplicated ligands/receptors are functionally interchangeable (see section on Specificity), differences may have been masked by the experimental conditions used to explore their activities.

Conservation of Biological Roles

The biological roles of LIN-12/Notch signaling are diverse. Only one role has emerged that appears to be common among various organisms. In flies, Notch plays a prominent role in selection of neural or nonneural fates; indeed, genes of the Notch pathway were dubbed neurogenic because of this role (Lehmann et al 1983 and references therein). It is striking that LIN-12 and GLP-1 are required for an inductive interaction in the embryo that leads to epidermal

fates at the expense of neural ones (Moskowitz & Rothman 1996), that LAG-2 and LIN-12 are expressed in many cells during development of the ventral nerve cord (S Henderson & J Kimble, unpublished observation; Wilkinson & Greenwald 1995), and that the LIN-12/Notch pathway plays a major role in neural specification in vertebrates (for review see Artavanis-Tsakonas et al 1995).

Other common roles for LIN-12/Notch signaling are not well established; however, distinct biological roles have been found. For example, GLP-1 signaling is required for specification of germline stem cells in *C. elegans*, but Notch signaling does not have the same function in flies. Over the next few years, both the conservation and divergence of biological roles will be highlighted as more is learned about the functions of these signaling pathways in diverse organisms.

CONCLUSIONS AND PROSPECTS

Our understanding of signal transduction by the LIN-12/Notch pathway has increased dramatically over the past few years. A major advance has been the identification of a conserved core pathway consisting of DSL ligand, LNG receptor, and CSL transcription factor. In addition, four mechanisms for regulation of the pathway have been discovered. First is the tightly regulated gene expression of ligand and receptor. These components are expressed in specific cells at precise times of development. Their finely tuned pattern of expression underscores the importance of both the presence of those components and their absence. Second is feedback regulation within the pathway. During lateral signaling, this regulatory circuit ensures the adoption of two alternative fates by cells within a field of equivalent precursors. Also, during induction such feedback regulation may ensure maintenance of cell fate decisions. Third is the asymmetric segregation of a pathway component, a mechanism that partially explains the specification of cell fates by some invariant cell lineages. Fourth is the interaction between the LIN-12/Notch signaling pathway with other pathways. The latter two mechanisms may be variants of lateral signaling, with either intrinsic or extrinsic cues biasing the pathway to achieve a pattern.

The tremendous progress over the last few years opens the door for a more in-depth understanding of how signals are transduced by this pathway. What is the molecular mechanism of signal transduction? Is the core pathway outlined in this review the primary pathway for LIN-12/Notch signaling, or are there parallel pathways of equal importance? The possibility of alternative ligands (e.g. Couso et al 1995) and alternative transducers (Lecourtois & Schweisguth 1995, Christensen et al 1996, Shawber et al 1996) has been suggested. Is there some branch of the pathway that inhibits differentiation generally, while another branch induces specific fates? How conserved are the regulators of the pathway

and the downstream target genes? We look forward with anticipation to the surprises that are likely to emerge from the burgeoning data obtained in studies directed at unraveling the details of this pathway in an ever-increasing variety of organisms.

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Literature Cited

Ahmad I, Zagouras P, Artavanis-Tsakonas S. 1995. Involvement of Notch-1 in mammalian retinal neurogenesis: association of Notch-1 activity with both immature and terminally differentiated cells. *Mech. Dev.* 53:73–85

Alonso MC, Cabrera C. 1988. The *achaete-scute* gene complex of *Drosophila melanogaster* comprises four homologous genes. *EMBO J.* 7:2585–91

Artavanis-Tsakonas S, Matsuno K, Fortini ME. 1995. Notch signaling. *Science* 268:225–32

Ashburner M. 1982. The genetics of a small autosomal region of *Drosophila melanogaster* containing the structural gene for alcohol dehydrogenase. III. Hypomorphic and hypermorphic mutations affecting the expression of Hairless. *Genetics* 101:447–59

Aster J, Pear W, Hasserjian R, Erba H, Davi F, et al. 1994. Functional analysis of the *TAN-1* gene, a human homolog of *Drosophila Notch. Cold Spring Harbor Symp. Quant. Biol.* 59:125–36

Austin CP, Feldman DE, Ida JA, Cepko CL. 1995. Vertebrate retinal ganglion cells are selected from competent progenitors by the action of Notch. *Development* 121:3637–50

Austin J, Kimble J. 1987. *glp-1* is required in the germ line for regulation of the decision between mitosis and meiosis in C. elegans. *Cell* 51:589–99

Austin J, Kimble J. 1989. Transcript analysis of *glp-1* and *lin-12*, homologous genes required for cell interactions during development of C. elegans. *Cell* 58:565–71

Axelrod JD, Matsuno K, Artavanis-Tsakonas S, Perrimon N. 1996. Interaction between wingless and Notch signaling pathways mediated by Dishevelled. *Science* 271:1826–32

Bailey AM, Posakony JW. 1995. Suppressor of Hairless directly activates transcription of *Enhancer of split* Complex genes in response to Notch receptor activity. *Genes Dev.* 9:2609–22

Baker NE, Zitron AE. 1995. *Drosophila* eye development: *Notch* and *Delta* amplify a neurogenic pattern conferred on the morphogenetic furrow by *scabrous. Mech. Dev.* 49:173–89

Bang AG, Bailey AM, Posakony JW. 1995. *Hairless* promotes stable commitment to the sensory organ precursor cell fate by negatively regulating the activity of the *Notch* signaling pathway. *Dev. Biol.* 172:479–94

Bate M, Rushton E, Frasch M. 1993. A dual requirement for neurogenic genes in

Drosophila myogenesis. *Development* Suppl. pp. 149–61
Berry LW, Westlund B, Schedl T. 1997. Germline tumor formation caused by activation of *glp-1,* a *Caenorhabditis elegans* member of the *Notch* family of receptors. *Development* 124:925–36
Bettenhausen B, Hrabé de Angelis M, Simon D, Guénet JL, Gossler A. 1995. Transient and restricted expression during mouse embryogenesis of *Dll1*, a murine gene closely related to *Drosophila Delta. Development* 121:2407–18
Bierkamp C, Campos-Ortega JA. 1993. A zebrafish homologue of the *Drosophila* neurogenic gene *Notch* and its pattern of transcription during early embryogenesis. *Mech. Dev.* 43:87–100
Blair S. 1996. Notch and Wingless signals collide. *Science* 271:1822–23
Bowerman B, Tax FE, Thomas JH, Priess JR. 1992. Cell interactions involved in the development of the bilaterally symmetrical intestinal valve cells during embryogenesis in *Caenorhabditis elegans. Development* 116:1113–22
Brou C, Logeat F, Lecourtois M, Vandekerckhove J, Kourilsky P, et al. 1994. Inhibition of the DNA-binding activity of *Drosophila* Suppressor of Hairless and of its human homolog, KBF2/RBP-Jκ by direct protein-protein interaction with *Drosophila Hairless. Genes Dev.* 8:2491–503
Cagan RL, Ready DF. 1989. *Notch* is required for successive cell decisions in the developing *Drosophila* retina. *Genes Dev.* 3:1099–112
Chenn A, McConnell SK. 1995. Cleavage orientation and the asymmetric inheritance of Notch1 immunoreactivity in mammalian neurogenesis. *Cell* 82:631–41
Chitnis A, Henrique D, Lewis J, Ish-Horowicz D, Kintner C. 1995. Primary neurogenesis in *Xenopus* embryos regulated by a homologue of the *Drosophila* neurogenic gene *Delta. Nature* 375:761–66
Christensen S, Kodoyianni V, Bosenberg M, Friedman L, Kimble J. 1996. *lag-1,* a gene required for *lin-12* and *glp-1* signaling in *Caenorhabditis elegans,* is homologous to human CBF1 and *Drosophila* Su(H). *Development* 122:1373–83
Chung C-N, Hamaguchi Y, Honjo T, Kawaichi M. 1994. Site-directed mutagenesis study on DNA binding regions of the mouse homologue of Suppressor of Hairless, RBP-Jκ. *Nucleic Acids Res.* 22:2938–44
Coffman CR, Skoglund P, Harris W, Kintner C. 1993. Expression of an extracellular deletion of *Xotch* diverts cell fate in Xenopus embryos. *Cell* 73:659–71
Conlon RA, Reaume AG, Rossant J. 1995. *Notch1* is required for the coordinate segmentation of somites. *Development* 121:1533–45
Cook D, Fry MJ, Hughes K, Sumathipala R, Woodgett JR, Dale T. 1996. Wingless inactivates glycogen synthase kinase-3 via an intracellular signalling pathway which involves a protein kinase C. *EMBO J.* 15:4526–36
Couso JP, Knust E, Martinez-Arias A. 1995. Serrate and Wingless cooperate to induce *vestigial* gene expression and wing formation in *Drosophila. Curr. Biol.* 5:1437–48
Coyle-Thompson CA, Banerjee U. 1993. The *strawberry notch* gene functions with *Notch* in common developmental pathways. *Development* 119:377–95
Crittenden SL, Troemel ER, Evans TC, Kimble J. 1994. GLP-1 is localized to the mitotic region of the *C. elegans* germ line. *Development* 120:2901–11
Cubas P, de Celis J-F, Campuzano S, Modolell J. 1991. Proneural clusters of *achaete/scute* expression and the generation of sensory organs in the *Drosophila* imaginal wing disc. *Genes Dev.* 5:996–1008
de Celis J-F, de Celis J, Ligoxygakis P, Preiss A, Delidakis C, Bray S. 1996. Functional relationships between *Notch, Su(H)* and the bHLH genes of the *E(spl)* complex: the *E(spl)* genes mediate only a subset of *Notch* activities during imaginal development. *Development* 122:2719–28
de Celis J-F, Garcia-Bellido A, Bray S. 1995. Activation and function of *Notch* at the dorsoventral boundary of the wing imaginal disc. *Development* 122:359–69
de Celis J-F, Mari-Beffa M, Garcia-Bellido A. 1991. Cell autonomous role of *Notch,* an epidermal growth factor homologue in sensory organ differentiation in *Drosophila. Proc. Natl. Acad. Sci. USA* 88:623–36
Delidakis C, Priess A, Hartley DA, Artavanis-Tsakonas S. 1991. Two genetically and molecularly distinct functions involved in early neurogenesis reside within the *Enhancer of split* locus of *Drosophila melanogaster. Genetics* 129:803–23
Diaz-Benjumea FJ, Cohen S. 1995. Serrate signals through Notch to establish a Wingless-dependent organizer at the dorso/ventral compartment boundary of the *Drosophila* wing. *Development* 121:4215–25
Doherty D, Feger G, Younger-Shepherd S, Jan LY, Jan YN. 1996. Delta is a ventral to dorsal signal complementary to Serrate, another Notch ligand, in *Drosophila* wing formation. *Genes Dev.* 10:421–34
Dorsky RI, Rapaport DH, Harris WA. 1995. *Xotch* inhibits cell differentiation in the *Xenopus* retina. *Neuron* 14:487–96
Dou S, Zeng X, Cortes P, Erdjument-Bromage H, Tempst P, et al. 1994. The recombina-

tion signal sequence-binding protein RBP-2N functions as a transcriptional repressor. *Mol. Cell Biol.* 14:3310–19

Ellisen LW, Bird J, West DC, Soreng AL, Reynolds TC, et al. 1991. *TAN-1*, the human homolog of the Drosophila *Notch* gene, is broken by chromosomal translocations in T lymphoblastic neoplasms. *Cell* 66:649–61

Evans TC, Crittenden SL, Kodoyianni V, Kimble J. 1994. Translational control of maternal *glp-1* mRNA establishes an asymmetry in the C. elegans embryo. *Cell* 77:183–94

Fehon RG, Johansen K, Rebay I, Artavanis-Tsakonas S. 1991. Complex cellular and subcellular regulation of Notch expression during embryonic and imaginal development of *Drosophila*: implications for Notch function. *J. Cell Biol.* 113:657–69

Fehon RG, Kooh PJ, Rebay I, Regan CL, Xu T, et al. 1990. Molecular interactions between the protein products of the neurogenic loci *Notch* and *Delta*, two EGF-homologous genes in Drosophila. *Cell* 61:523–34

Fitzgerald K, Greenwald I. 1995. Interchangeability of *Caenorhabditis elegans* DSL proteins and intrinsic signalling activity of their extracellular domains in vivo. *Development* 121:4275–82

Fitzgerald K, Wilkinson HA, Greenwald I. 1993. *glp-1* can substitute for *lin-12* in specifying cell fate decisions in *Caenorhabditis elegans*. *Development* 119:1019–27

Fleming RJ, Scottgale TN, Diederich RJ, Artavanis-Tsakonas S. 1990. The gene *Serrate* encodes a putative EGF-like transmembrane protein essential for proper ectodermal development in *Drosophila melanogaster*. *Genes Dev.* 4:2188–201

Fortini ME, Artavanis-Tsakonas S. 1994. The Suppressor of Hairless protein participates in Notch receptor signaling. *Cell* 79:273–82

Franco del Amo F, Smith DE, Swiatek PJ, Gendron-Maguire M, Greenspan RJ, et al. 1992. Expression pattern of *Motch*, a mouse homolog of *Drosophila Notch*, suggests an important role in early post-implantation mouse development. *Development* 115:737–44

Freeman M. 1996. Reiterative use of the EGF receptor triggers differentiation of all cell types in the Drosophila eye. *Cell* 87:651–60

Frise E, Knoblich JA, Younger-Shepherd S, Jan LY, Jan YN. 1996. The *Drosophila* Numb protein inhibits signaling of the Notch receptor during cell-cell interaction in sensory organ lineage. *Proc. Natl. Acad. Sci. USA* 93:11925–32

Gao D, Kimble J. 1995. APX-1 can substitute for its homolog LAG-2 to direct cell interactions throughout *Caenorhabditis elegans* development. *Proc. Natl. Acad. Sci. USA* 92:9839–42

Gho M, Lecourtois M, Géraud G, Posakony J, Schweisguth F. 1996. Subcellular localization of Suppressor of Hairless in *Drosophila* sense organ cells during Notch signalling. *Development* 122:1673–82

Ghysen A, Dambly-Chaudière C, Jan LY, Jan Y-N. 1993. Cell interactions and gene interactions in peripheral neurogenesis. *Genes Dev.* 7:723–33

González-Gaitán M, Jäckle H. 1995. Invagination centers within the *Drosophila* stomatogastric nervous system anlage are positioned by *Notch*-mediated signaling which is spatially controlled through *wingless*. *Development* 121:2313–25

Grant B, Greenwald I. 1997. Structure, function, and expression of SEL-1, a negative regulator of LIN-12 and GLP-1 in *C. elegans*. *Development* 124:637–44

Greenwald I. 1985. *lin-12*, a nematode homeotic gene, is homologous to a set of mammalian proteins that includes epidermal growth factor. *Cell* 43:583–90

Greenwald I. 1994. Structure/function studies of lin-12/Notch proteins. *Curr. Opin. Genet. Dev.* 4:556–62

Greenwald IS, Seydoux G. 1990. Analysis of gain-of-function mutations of the *lin-12* gene of *Caenorhabditis elegans*. *Nature* 346:197–99

Greenwald IS, Sternberg PW, Horvitz HR. 1983. The *lin-12* locus specifies cell fates in Caenorhabditis elegans. *Cell* 34:435–44

Grossman SR, Johannsen E, Tong X, Yalamanchili R, Kieff E. 1994. The Epstein-Barr virus nuclear antigen 2 transactivator is directed to response elements by the Jκ recombination signal binding protein. *Proc. Natl. Acad. Sci. USA* 91:7568–72

Gu Y, Hukriede NA, Fleming RJ. 1995. *Serrate* expression can functionally replace *Delta* activity during neuroblast segregation in the *Drosophila* embryo. *Development* 121:855–65

Guo M, Jan LY, Jan YN. 1996. Control of daughter cell fates during asymmetric division: interaction of Numb and Notch. *Neuron* 17:27–41

Hartenstein V, Posakony JW. 1990. A dual function of the *Notch* gene in *Drosophila* sensillum development. *Dev. Biol.* 142:12–30

Hartley D, Xu T, Artavanis-Tsakonas S. 1987. The embryonic expression of the *Notch* locus of *Drosophila melanogaster* and the implications of point mutations in the extracellular EGF-like domain of the predicted protein. *EMBO J.* 6:3407–17

Hedgecock EM, Culotti JG, Hall DH. 1990. The *unc-5*, *unc-6* and *unc-40* genes guide

circumferential migrations of pioneer axons and mesodermal cells on the epidermis in *C. elegans. Neuron* 2:61–85
Heitzler P, Bourouis M, Ruel L, Carteret C, Simpson P. 1996. Genes of the *Enhancer of split* and *achaete-scute* complexes are required for a regulatory loop between Notch and Delta during lateral signaling in *Drosophila. Development* 122:161–71
Heitzler P, Simpson P. 1991. The choice of cell fate in the epidermis of Drosophila. *Cell* 64:1083–92
Heitzler P, Simpson P. 1993. Altered epidermal growth factor-like sequences provide evidence for a role of *Notch* as a receptor in cell fate decisions. *Development* 117:1113–23
Henderson ST, Gao D, Lambie EJ, Kimble J. 1994. *lag-2* may encode a signaling ligand for the GLP-1 and LIN-12 receptors of *C. elegans. Development* 120:2913–24
Henderson ST, Gao D, Christensen S, Kimble J. 1997. Functional domains of *C. elegans* LAG-2, a putative signaling ligand for LIN-12/GLP-1 receptors. *Mol. Biol. Cell.* In press
Henkel T, Ling PD, Hayward SD, Peterson MG. 1994. Mediation of Epstein-Barr Virus EBNA2 transactivation by recombination signal-binding protein Jκ. *Science* 265:92–95
Henrique D, Adam J, Myat A, Chitnis A, Lewis J, Ish-Horowicz D. 1995. Expression of a *Delta* homologue in prospective neurons in the chick. *Nature* 375:787–90
Hoch M, Broadie K, Jäckle H, Skaer H. 1994. Sequential fates in a single cell are established by the neurogenic cascade in the Malpighian tubules of *Drosophila. Development* 120:3439–50
Horvitz HR, Sternberg PW. 1991. Multiple intercellular signalling systems control the development of the *Caenorhabditis elegans* vulva. *Nature* 351:535–41
Hsieh JJ-D, Hayward SD. 1995. Masking of the CBF1/RBP-Jκ transcriptional repression domain by Epstein-Barr virus EBNA2. *Science* 268:560–63
Hsieh JJ-D, Henkel T, Salmon P, Robey E, Peterson MG, Hayward SD. 1996. Truncated mammalian Notch1 activates CBF1/RBP-Jκ-repressed genes by a mechanism resembling that of Epstein-Barr virus EBNA2. *Mol Cell. Biol.* 16:952–59
Hubbard EJ, Dong Q, Greenwald I. 1996. Evidence for physical and functional association between EMB-5 and LIN-12 in *Caenorhabditis elegans. Science* 273:112–15
Hutter H, Schnabel R. 1994. *glp-1* and inductions establishing embryonic axes in *C. elegans. Development* 120:2051–64
Ishibashi M, Ang S-L, Shiota K, Nakanishi S, Kageyama R, Guillemot F. 1995. Targeted disruption of mammalian *hairy* and *Enhancer of split* homolog-1 (*HES-1*) leads to up-regulation of neural helix-loop-helix factors, premature neurogenesis, and severe neural tube defects. *Genes Dev.* 9:3136–48
Jan YN, Jan LY. 1995. Maggot's hair and bug's eye: role of cell interactions and intrinsic factors in cell fate specification. *Neuron* 14:1–5
Jarriault S, Brou C, Logeat F, Schroeter EH, Kopan R, Israel A. 1995. Signaling downstream of activated mammalian Notch. *Nature* 377:355–58
Kelley MR, Kidd S, Deutsch WA, Young MW. 1987. Mutations altering the structure of epidermal growth factor-like coding sequences at the Drosophila *Notch* locus. *Cell* 51:539–48
Kidd S, Baylies MK, Gasic GP, Young MW. 1989. Structure and distribution of the Notch protein in developing *Drosophila. Genes Dev.* 3:1113–29
Kim J, Irvine KD, Carroll SB. 1995. Cell recognition, signal induction, and symmetrical gene activation at the dorso-ventral boundary of the developing Drosophila wing. *Cell* 82:795–802
Kim J, Sebring A, Esch JJ, Kraus ME, Vorwerk K, et al. 1996. Integration of positional signals and regulation of wing formation and identity by *Drosophila vestigial* gene. *Nature* 382:133–38
Kimble J. 1981. Alterations in cell lineage following laser ablation of cells in the somatic gonad of *Caenorhabditis elegans. Dev. Biol.* 87:286–300
Kimble J, White JG. 1981. On the control of germ cell development in *Caenorhabditis elegans. Dev. Biol.* 81:208–19
Knust E, Schrons H, Grawe F, Campos-Ortega JA. 1992. Seven genes of the *Enhancer of split* complex of *Drosophila melanogaster* encode helix-loop-helix proteins. *Genetics* 132:505–18
Kodoyianni V, Maine E, Kimble J. 1992. Molecular basis of loss-of-function mutations in the *glp-1* gene of *Caenorhabditis elegans. Mol. Biol. Cell* 3:1199–213
Kooh PJ, Fehon RG, Muskavitch MAT. 1993. Implications of dynamic patterns of Delta and Notch expression for cellular interactions during *Drosophila* development. *Development* 117:493–507
Kopan R, Nye JS, Weintraub H. 1994. The intracellular domain of mouse Notch: a constitutively activated repressor of myogenesis directed at the basic helix-loop-helix region of MyoD. *Development* 120:2385–96
Kopan R, Schroeter EH, Weintraub H, Nye JS. 1996. Signal transduction by activated mNotch: importance of proteolytic processing and its regulation by the extracellular domain. *Proc. Natl. Acad. Sci. USA* 93:1683–88

Kopczynski CC, Alton AK, Fechtel K, Kooh PJ, Muskavitch MAT. 1988. *Delta*, a *Drosophila* neurogenic gene, is transcriptionally complex and encodes a protein related to blood coagulation factors and epidermal growth factor of vertebrates. *Genes Dev.* 2:1723–35

Künisch M, Haenlin M, Campos-Ortega JA. 1994. Lateral inhibition mediated by the *Drosophila* neurogenic gene *Delta* is enhanced by proneural proteins. *Proc. Natl. Acad. Sci. USA* 91:10139–43

Lambie EJ, Kimble J. 1991. Two homologous regulatory genes, *lin-12* and *glp-1*, have overlapping functions. *Development* 112:231–40

Lardelli M, Dahlstrand J, Lendahl U. 1994. The novel *Notch* homologue mouse *Notch 3* lacks specific epidermal growth factor repeats and is expressed in proliferating neuroepithelium. *Mech. Dev.* 46:123–36

Lardelli M, Lendahl U. 1993. *Motch A* and *Motch B*—two mouse *Notch* homologues coexpressed in a wide variety of tissues. *Exp. Cell Res.* 204:364–72

Larsson C, Lardelli M, White I, Lendahl U. 1994. The human NOTCH1, 2, and 3 genes are located at chromosome positions 9q34, 1p13-p11, and 19p13.2-p13.1 in regions of neoplasia-associated translocation. *Genomics* 24:253–58

Lawrence PA. 1966. Development and determination of hairs and bristles in the milkweed bug, *Oncopeltus fasciatus. J. Cell Sci.* 1:475–98

Lecourtois M, Schweisguth F. 1995. The neurogenic Suppressor of Hairless DNA-binding protein mediates the transcriptional activation of the *Enhancer of split* Complex genes triggered by Notch signaling. *Genes Dev.* 9:2598–608

Lee EC, Hu XX, Yu SY, Baker NE. 1996. The *scabrous* gene encodes a secreted glycoprotein dimer and regulates proneural development in *Drosophila* eyes. *Mol. Cell. Biol.* 16:1179–88

Lehmann R, Jimenez F, Dietrich U, Campos-Ortega JA. 1983. On the phenotype and development of mutants of early neurogenesis in *Drosophila melanogaster. Wilhelm Roux Arch. Dev. Biol.* 192:62–74

Levitan D, Greenwald I. 1995. Facilitation of *lin-12*-mediated signaling by *sel-12*, a *Caenorhabditis elegans* S182 Alzheimer's disease gene. *Nature* 377:351–54

Leviten MW, Posakony JW. 1996. Gain-of-function alleles of *Bearded* interfere with alternative cell fate decisions in *Drosophila* adult sensory organ development. *Dev. Biol.* 176:264–83

Lewis J. 1996. Neurogenic genes and vertebrate neurogenesis. *Curr. Opin. Neurobiol.* 6:3–10

Lieber T, Kidd S, Alcamo E, Corbin V, Young MW. 1993. Antineurogenic phenotypes induced by truncated Notch proteins indicate a role in signal transduction and may point to a novel function for Notch in nuclei. *Genes Dev.* 7:1949–65

Lieber T, Wesley CS, Alcamo E, Hassel B, Krane JF, et al. 1992. Single amino acid substitutions in EGF-like elements of Notch and Delta modify *Drosophila* development and affect cell adhesion in vitro. *Neuron* 9:847–59

Lindsell CE, Shawber CJ, Boulter J, Weinmaster G. 1995. Jagged: a mammalian ligand that activates Notch1. *Cell* 80:909–17

Maine EM, Kimble J. 1993. Suppressors of *glp-1*, a gene required for cell communication during development in *Caenorhabditis elegans*, define a set of interacting genes. *Genetics* 135:1011–22

Mango SE, Maine EM, Kimble J. 1991. Carboxy-terminal truncation activates the *glp-1* protein to specify vulval fates in *Caenorhabditis elegans. Nature* 352:811–15

Mango SE, Thorpe CJ, Martin PR, Chamberlin SH, Bowerman B. 1994. Two maternal genes, *apx-1* and *pie-1*, are required to distinguish the fates of equivalent blastomeres in the early *Caenorhabditis elegans* embryo. *Development* 120:2305–15

Matsunami N, Hamaguchi Y, Yamamoto Y, Kuze K, Kangawa K, et al. 1989. A protein binding to the Jκ recombination sequence of immunoglobulin genes contains a sequence related to the integrase motif. *Nature* 342:934–37

Matsuno K, Diederich RJ, Go MJ, Blaumueller M, Artavanis-Tsakonas S. 1995. Delta acts as a positive regulator of Notch signaling through interactions with the Notch ankyrin repeats. *Development* 121:2633–44

Mello CC, Draper BW, Priess JR. 1994. The maternal genes *apx-1* and *glp-1* and establishment of dorsal-ventral polarity in the early C. elegans embryo. *Cell* 77:95–106

Mickey KM, Mello CC, Montgomery MK, Fire A, Priess JR. 1996. An inductive interaction in 4-cell stage *C. elegans* embryos involves APX-1 expression in the signalling cell. *Development* 122:1791–98

Milner LA, Kopan R, Martin DI, Bernstein ID. 1994. A human homologue of the *Drosophila* developmental gene, *Notch*, is expressed in CD34+ hematopoietic precursors. *Blood* 83:2057–62

Moskowitz IPG, Gendreau SB, Rothman JH. 1994. Combinatorial specification of blastomere identity by *glp-1*-dependent cellular interactions in the nematode *Caenorhabditis elegans. Development* 120:3325–38

Moskowitz IPG, Rothman JH. 1996. *lin-12*

and *lgp-1* are required zygotically for early embryonic cellular interactions and are regulated by maternal GLP-1 signaling in *Caenorhabditis elegans. Development* 122: 4105–17

Muskavitch MAT. 1994. Delta-Notch signaling and *Drosophila* cell fate choice. *Dev. Biol.* 166:415–30

Myat A, Henrique D, Ish-Horowicz D, Lewis J. 1996. A chick homologue of *Serrate*, and its relationship with *Notch* and *Delta* homologues during central neurogenesis. *Dev. Biol.* 174:233–47

Nakao K, Campos-Ortega JA. 1996. Persistent expression of genes of the *Enhancer of split* complex suppresses neural development in *Drosophila. Neuron* 16:275–86

Neumann CJ, Cohen SM. 1996. A hierarchy of cross-regulation involving *Notch, wingless, vestigial* and *cut* organizes the dorsal/ventral axis of the *Drosophila* wing. *Development* 122:3477–85

Newman AP, White JG, Sternberg PA. 1995. The *Caenorhabditis elegans lin-12* gene mediates induction of ventral uterine specialization by the anchor cell. *Development* 121:263–71

Nye JS, Kopan R. 1995. Developmental signaling. Vertebrate ligands for Notch. *Curr. Biol.* 5:966–69

Oellers N, Dehio M, Knust E. 1994. bHLH proteins encoded by the *Enhancer of split* complex of *Drosophila* negatively interfere with transcriptional activation mediated by proneural genes. *Mol. Gen. Genet.* 244:465–73

Oka C, Nakano T, Wakeham A, de la Pompa JL, Mori C, et al. 1995. Disruption of the mouse *RBP-Jκ* gene results in early embryonic death. *Development* 121:3291–301

Parks AL, Muskavitch MAT. 1993. *Delta* function is required for bristle organ determination and morphogenesis in *Drosophila. Dev. Biol.* 157:484–96

Parks AL, Turner FR, Muskavitch MAT. 1995. Relationships between complex Delta expression and the specification of retinal cell fates during *Drosophila* eye development. *Mech. Dev.* 50:201–16

Paroush Z, Finley RL, Kidd T, Wainwright SM, Ingham PI, et al. 1994. Groucho is required for Drosophila neurogenesis, segmentation, and sex determination and interacts directly with Hairy-related bHLH proteins. *Cell* 79:805–15

Priess JR, Schnabel H, Schnabel R. 1987. The *glp-1* locus and cellular interactions in early C. elegans embryos. *Cell* 51:601–11

Qiao L, Lissemore JL, Shu P, Smardon A, Gelber MB, Maine ME. 1995. Enhancers of *glp-1*, a gene required for cell-signaling in *Caenorhabditis elegans*, define a set of genes required for germline development. *Genetics* 141:551–69

Rao Y, Bodmer R, Jan LY, Jan YN. 1992. The *big brain* gene of *Drosophila* functions to control the number of neuronal precursors in the peripheral nervous system. *Development* 116:31–40

Rebay I, Fehon RG, Artavanis-Tsakonas S. 1993. Specific truncations of Drosophila Notch define dominant activated and dominant negative forms of the receptor. *Cell* 74:319–29

Rebay I, Fleming RJ, Fehon RG, Cherbas L, Cherbas P, Artavanis-Tsakonas S. 1991. Specific EGF repeats of Notch mediate interactions with Delta and Serrate: implications for Notch as a multifunctional receptor. *Cell* 67:687–99

Rhyu MS, Jan LY, Jan YN. 1994. Asymmetric distribution of numb protein during division of the sensory organ precursor cell confers distinct fates to daughter cells. *Cell* 76:477–91

Robbins J, Blondel BJ, Gallahan D, Callahan R. 1992. Mouse mammary tumor gene *int-3*: a member of the *notch* gene family transforms mammary epithelial cells. *J. Virol.* 66:2594–99

Roehl H, Bosenberg M, Blelloch R, Kimble J. 1996. Roles of the RAM and ANK domains in signaling by the *C. elegans* GLP-1 receptor. *EMBO J.* 15:7002–12

Roehl H, Kimble J. 1993. Control of cell fate in *C. elegans* by a GLP-1 peptide consisting primarily of ankyrin repeats. *Nature* 364:632–35

Ruel L, Bourouis M, Heitzler P, Pantesco V, Simpson P. 1993. *Drosophila shaggy* kinase and rat glycogen synthase kinase 3 have conserved activities and act downstream of *Notch. Nature* 362:557–60

Rulifson EJ, Blair SS. 1995. *Notch* regulates *wingless* expression and is not required for reception of the paracrine *wingless* signal during wing margin neurogenesis in *Drosophila. Development* 121:2813–24

Ruohola H, Bremer KA, Baker D, Swedlow JR, Jan LY, Jan YN. 1991. Role of neurogenic genes in establishment of follicle cell fate and oocyte polarity during oogenesis in Drosophila. *Cell* 66:433–49

Schrons H, Knust E, Campos-Ortega JA. 1992. The *Enhancer of split* complex and adjacent genes in the 96F region of *Drosophila melanogaster* are required for segregation of neural and epidermal progenitor cells. *Genetics* 132:481–503

Schweisguth F, Posakony JW. 1992. *Suppressor of Hairless*, the Drosophila homolog of the mouse recombination signal-binding protein

gene, controls sensory organ cell fates. *Cell* 69:1199–212
Seydoux G, Greenwald I. 1989. Cell autonomy of *lin-12* function in a cell fate decision in C. elegans. *Cell* 57:1237–45
Seydoux G, Schedl T, Greenwald I. 1990. Cell-cell interactions prevent a potential inductive interaction between soma and germline in C. elegans. *Cell* 61:939–51
Shawber C, Nofziger D, Hsieh JJ-D, Lindsell C, Bögler O, et al. 1996. Notch signaling inhibits muscle cell differentiation through a CBF1-independent pathway. *Development* 122:3765–73
Simpson P. 1994. *The Notch Receptors.* Austin, TX: Landes. 121 pp.
Simpson P, Ruel L, Heitzler P, Bourouis M. 1993. A dual role for the protein kinase *shaggy* in the repression of *achaete-scute. Development* pp. 29–39 (Suppl.)
Skeath JB, Carroll SB. 1991. Regulation of *achaete-scute* gene expression and sensory organ pattern formation in the *Drosophila* wing. *Genes Dev.* 5:984–95
Skeath JB, Panganiban G, Carroll SB. 1992. Gene regulation in two dimensions: the proneural *achaete* and *scute* genes are controlled by combinations of axis patterning genes through a common intergenic control region. *Genes Dev.* 6:2606–19
Smoller D, Friedel C, Schmidt A, Bettler D, Lam L, Yedrobnick B. 1990. The *Drosophila* neurogenic locus *mastermind* encodes a nuclear protein unusually rich in amino acid homopolymers. *Genes Dev.* 4:1688–700
Spana EP, Kopczynski C, Goodman CS, Doe CQ. 1995. Asymmetric localization of numb autonomously determines sibling neuron identity in the *Drosophila* CNS. *Development* 121:3489–94
Speicher SA, Thomas U, Hinz U, Knust E. 1994. The *Serrate* locus of *Drosophila* and its role in morphogenesis of the wing imaginal discs: control of cell proliferation. *Development* 120:535–44
Struhl G, Fitzgerald K, Greenwald I. 1993. Intrinsic activity of the Lin-12 and Notch intracellular domains in vivo. *Cell* 74:331–45
Sundaram M, Greenwald I. 1993. Suppressors of a *lin-12* hypomorph define genes that interact with both *lin-12* and *glp-1* in *Caenorhabditis elegans. Genetics* 135:765–83
Swiatek PJ, Lindsell CE, Franco del Amo F, Weinmaster G, Gridley T. 1994. *Notch1* is essential for post-implantation development in mice. *Genes Dev.* 8:707–19
Tamura K, Taniguchi Y, Minoguchi S, Sakai T, Tun T, Furukawa T, Honjo T. 1995. Physical interaction between a novel domain of the receptor Notch and the transcription factor RBP-Jκ/Su(H). *Curr. Biol.* 5:1416–23
Tata F, Hartley D. 1995. Inhibition of cell fate in *Drosophila* by *Enhancer of split* genes. *Mech. Dev.* 51:305–15
Tax FE, Yeargers JJ, Thomas JH. 1994. Sequence of *C. elegans lag-2* reveals a cell-signalling domain shared with *Delta* and *Serrate* of *Drosophila. Nature* 368:150–54
Tepass U, Hartenstein V. 1995. Neurogenic and proneural genes control cell fate specification in the *Drosophila* endoderm. *Development* 121:393–405
Thomas U, Speicher SA, Knust E. 1991. The *Drosophila* gene *Serrate* encodes an EGF-like transmembrane protein with a complex expression pattern in embryos and wing discs. *Development* 111:749–61
Tuck S, Greenwald I. 1995. *lin-25*, a gene required for vulval induction in *Caenorhabditis elegans. Genes Dev.* 9:341–57
Tun T, Hamaguchi Y, Matsunami N, Furukawa T, Honjo T, Kawaichi M. 1994. Recognition sequence of a highly conserved DNA binding protein RBP-Jκ. *Nucleic Acids Res.* 22:965–71
Uemura T, Shepherd S, Ackerman L, Jan LY, Jan YN. 1989. *numb*, a gene required in determination of cell fate during sensory organ formation in Drosophila embryos. *Cell* 58:349–60
Vässin H, Bremer KA, Knust E, Campos-Ortega JA. 1987. The neurogenic gene Delta of *Drosophila melanogaster* is expressed in neurogenic territories and encodes a putative transmembrane protein with EGF-like repeats. *EMBO J.* 6:3431–40
Waltzer L, Logeat F, Brou C, Israel A, Sergeant A, Manet E. 1994. The human Jκ recombination signal sequence binding protein (RBP-Jκ) targets the Epstein-Barr virus EBNA2 protein to its DNA responsive elements. *EMBO J.* 13:5633–38
Weinmaster G, Roberts VJ, Lemke G. 1991. A homolog of *Drosophila Notch* expressed during mammalian development. *Development* 113:199–205
Wharton KA, Johansen KM, Xu T, Artavanis-Tsakonas S. 1985. Nucleotide sequence from the neurogenic locus Notch implies a gene product that shares homology with proteins containing EGF-like repeats. *Cell* 43:567–81
Wilkinson HA, Fitzgerald K, Greenwald I. 1994. Reciprocal changes in expression of the receptor *lin-12* and its ligand *lag-2* prior to commitment in a C. elegans cell fate decision. *Cell* 79:1187–98
Wilkinson HA, Greenwald I. 1995. Spatial and temporal patterns of *lin-12* expression during *C. elegans* hermaphrodite development. *Genetics* 141:513–26
Wrischnik LA, Kenyon CJ. 1997. The role of

lin-22, a *hairy/Enhancer of split* homolog, in patterning the peripheral nervous system of *C. elegans*. *Development*. In press

Yochem J, Greenwald I. 1989. *glp-1* and *lin-12*, genes implicated in distinct cell-cell interactions in *C. elegans*, encode similar transmembrane proteins. *Cell* 58:553–63

Yuan YP, Schultz J, Mlodzik M, Bork P. 1997. Secreted fringe-like signaling molecules may be glycosyltransferases. *Cell* 88:9–11

Zhao C, Emmons SW. 1995. A transcription factor controlling development of peripheral sense organs in *C. elegans*. *Nature* 373:74–78

Zhou L, Boulianne GL. 1994. Comparison of the neuralized genes of *Drosophila virilis* and *D. melanogaster*. *Genome* 37:840–47

Zimber-Strobl U, Strobl LJ, Meitinger C, Hinrichs R, Sakai T, et al. 1994. Epstein-Barr virus nuclear antigen 2 exerts its transactivating function through interaction with recombination signal binding protein RBP-Jκ, the homologue of *Drosophila Suppressor of Hairless*. *EMBO J.* 13:4973–82