LIN-12/Notch signaling in *C. elegans*[*]

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Table of Contents

Abstract

Receptors of the LIN-12/Notch family mediate cell-cell interactions during animal development, and aberrations in LIN-12/Notch signaling have been implicated in human disease. Studies in *C. elegans* have been instrumental in defining the basic features of the LIN-12/Notch pathway, the role of LIN-12/Notch proteins as receptors for intercellular signals, the mechanism of signal transduction, and the regulation of LIN-12/Notch signaling during cell fate decisions. This chapter is focused on detailing how the "awesome power of *C. elegans* genetics" has identified many core components and modulators of LIN-12/Notch activity.

[*]Edited by Lisa R. Girard. Last revised July 29, 2005. Published August 8, 2005. This chapter should be cited as: Greenwald, I. LIN-12/Notch signaling in *C. elegans* (August 4, 2005), *WormBook*, ed. The *C. elegans* Research Community, WormBook, doi/10.1895/wormbook.1.10.1, http://www.wormbook.org.

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1. Introduction

Coming soon after the finding of a conserved 'homeobox' in developmental control genes (Laughon and Scott, 1984; McGinnis et al., 1984), the discovery that LIN-12/Notch proteins in *C. elegans* and *Drosophila* contain epidermal growth factor (EGF)-like repeats (Greenwald, 1985; Wharton et al., 1985; Kidd et al., 1986) suggested that the kinds of molecules that underlie common developmental mechanisms shared by all animals would be very broad, encompassing signals and receptors as well as DNA binding proteins, and also that basic signaling systems would be conserved. In subsequent years, systematic analysis in worms and flies has illuminated how LIN-12/Notch proteins transduce signals, and contexts in which this signaling system is used and modulated. New roles for LIN-12/Notch signaling in mammalian development and disease have been identified at a prodigious rate (Gridley, 2003; Harper et al., 2003; Hansson et al., 2004). The focus of this review will be on the contributions of genetic analysis of LIN-12/Notch signaling in *C. elegans* has made to illuminating universal aspects of this essential conserved pathway.

2. Genetic identification and characterization of LIN-12 and GLP-1

C. elegans has two LIN-12/Notch proteins, encoded by the *lin-12* and *glp-1* genes. These genes were identified through genetic screens for developmental mutants, and genetic analysis has established both unique and redundant roles for the two genes in specifying cell fates.

lin-12 was identified in a genetic screen for mutations that have defective vulval development (Greenwald et al.,1983; Ferguson and Horvitz,1985). The first alleles discovered, called *lin-12(d)* alleles, were dominant and hypermorphic, and intragenic reversion yielded null alleles, called *lin-12(0)* (Greenwald et al., 1983). Phenotypic analysis indicated that *lin-12(d)* and *lin-12(0)* alleles cause opposite transformations in many different cell fate decisions, including early gonadogenesis, vulval precursor cell fate specification (see Vulval development), and sex mesoblast specification (see Male development; Greenwald et al., 1983). *lin-12* also is required for π cell specification during hermaphrodite gonadogenesis (see Hermaphrodite cell-fate specification; Newman et al., 1995). Possible roles for *lin-12* activity in differentiation or function, as opposed to cell fate specification per se, have also been described (Eimer et al., 2002; Chao et al., 2005). The requirement for *lin-12* activity for appropriate cell movements that result in a left-right intestinal twist may also be considered as a role in cell function, rather than cell fate, between otherwise identical cells (Hermann et al., 2000).

glp-1 was identified in two different, contemporaneous genetic screens. A screen for sterile mutants revealed that loss of 'zygotic' *glp-1* activity limits germline proliferation and causes germ cells to enter meiosis prematurely (Austin and Kimble, 1987). Subsequently, dominant alleles of *glp-1* that cause the opposite phenotype--continued mitotic proliferation of the germline at the expense of gamete production--were identified (Berry et al., 1997; Pepper et al., 2003). A screen for maternal-effect embryonic lethal mutations revealed that loss of maternal *glp-1* activity prevents induction of the anterior pharynx at the 12-cell stage of embryogenesis (Priess et al., 1987). Subsequent experiments revealed another role for *glp-1* maternal activity in mediating a critical inductive event at the 4-cell stage of embryogenesis (Mango et al., 1994; Mello et al., 1994; Hutter and Schnabel, 1994; Moskowitz et al., 1994), and at least four other interactions in the early embryo (see also Notch signaling in the *C. elegans* embryo).

Double mutants lacking both *lin-12* and *glp-1* activity revealed that the two genes are functionally redundant in some cell fate decisions (Lambie and Kimble, 1991). The distinctive phenotype of the *lin-12 glp-1* double is called Lag, for "Lin-12 and Glp-1". The hallmarks of the Lag phenotype are the absence of a rectum, anus and excretory cell, as well as a duplication of the excretory pore, reflecting defects in cell fate decisions that occur during embryogenesis (Lambie and Kimble, 1991; see also Notch signaling in the *C. elegans* embryo). The functional redundancy inferred from genetic analysis is due to biochemical redundancy of the two receptors, as GLP-1 can substitute for LIN-12 in cell fate decisions (Fitzgerald et al., 1993). *lin-12* and *glp-1* appear to have arisen by gene duplication (Yochem and Greenwald, 1989; Rudel and Kimble, 2002), so it is not surprising that some functions have been conserved.

As described below, genetic screens for other components of the LIN-12 and GLP-1 signaling pathways have exploited the highly penetrant phenotypic defects of single mutants in screens for extragenic suppressors and enhancers, as well as the Lag phenotype of the *lin-12 glp-1* double mutant.

3. Overview of signal transduction by LIN-12 and GLP-1

LIN-12 and GLP-1 have all of the hallmarks that define the LIN-12/Notch family (Yochem and Greenwald, 1989; Yochem et al., 1988; Figure 1). They are type I transmembrane proteins that contain multiple EGF-like motifs commencing just after the signal sequence, followed by three copies of the LIN-12/Notch repeat (LNR) motif. A spacer region between the LNR motifs and the transmembrane domain contains two conserved cysteines. Vertebrate LIN-12/Notch proteins are cleaved by furin in this spacer region during their transit through the secretory system at "site 1" (Brou et al., 2000; Figure 1); it is not known whether the *C. elegans* proteins are also cleaved by furin, but Western blots of GLP-1 are consistent with this possibility (Crittenden et al., 1994). The intracellular domain of LIN-12/Notch proteins contains repeated "CDC10/Ankyrin" motifs, and a canonical PEST protein turnover motif.

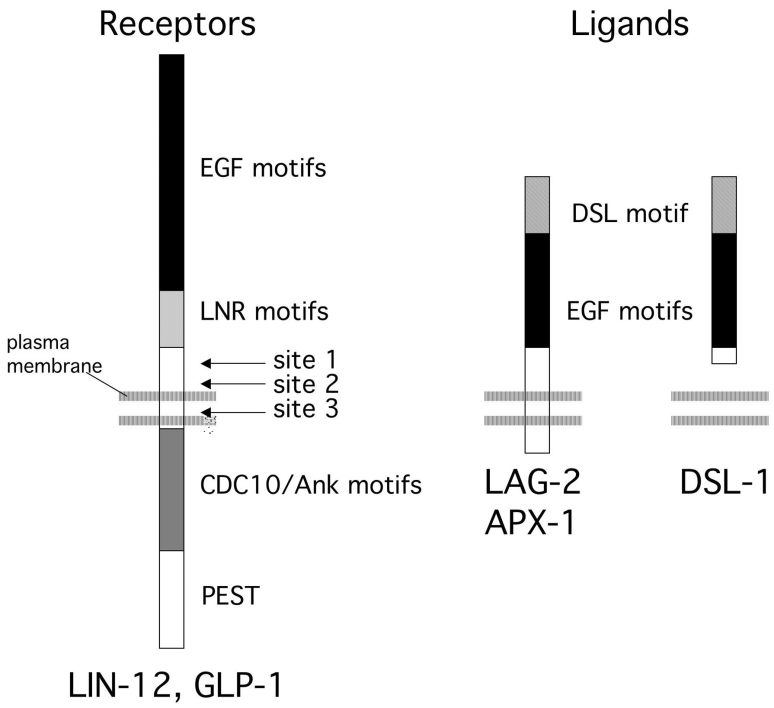

Figure 1. Schematic representation of *C. elegans* LIN-12/Notch and DSL proteins. Conserved motifs: EGF = epidermal growth factor-like motif, LNR = LIN-12/Notch repeat motif, CDC10/Ank = a motif found in yeast CDC10, SWI6, and Ankyrin. There are two *C. elegans* LIN-12/Notch proteins, LIN-12 and GLP-1; both have the same arrangement of motifs, as represented by the single cartoon. Inferred cleavage sites are shown (see also Figure 2). There are ten predicted DSL proteins; representative transmembrane and secreted DSL proteins are shown. All ten DSL proteins have a single amino-terminal DSL domain, followed by one or more EGF motifs.

Ligands for LIN-12 and GLP-1 (Figure 1) are members of the "DSL" family, an acronym derived from canonical ligands from *Drosophila* (Delta, Serrate) and *C. elegans* (LAG-2). These ligands also contain one or more EGF motifs, as well as an amino terminal motif that is unique to the family, the "DSL motif". Binding of DSL ligands to LIN-12/Notch leads to shedding of the LIN-12/Notch ectodomain via cleavage at "site 2" within the spacer region of the extracellular domain (Figure 1 and Figure 2). The remaining transmembrane protein, with only a short extracellular stub, is cleaved constitutively within the transmembrane domain at "site 3" (Figure 1 and Figure 2), releasing the intracellular domain, which translocates to the nucleus (Kopan and Goate, 2000; Schroeter et al., 1998; Struhl and Adachi, 1998; Struhl and Adachi, 2000; Struhl et al., 1993).

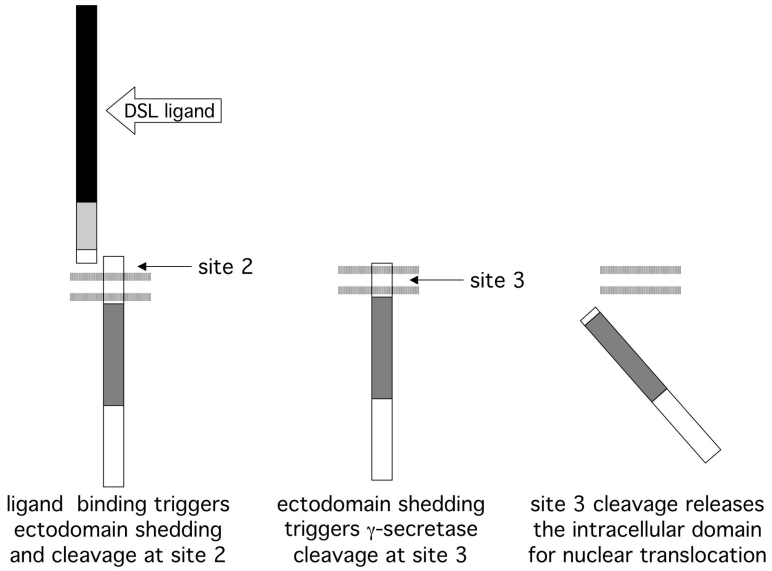

Figure 2. Mechanism of LIN-12/Notch signal transduction. See text for further description.

The intracellular domain contains a block of repeated CDC10/SWI6/Ankyrin motifs, which, along with the juxtamembrane region, mediate the physical interaction with the sequence specific DNA binding protein LAG-1 (Christensen et al., 1996; Roehl et al., 1996). LAG-1 is a member of the "CSL" family, an acronym derived from mammalian CBF1 and *Drosophila* Su(H) as well as LAG-1. Studies of Su(H) and CBF1 suggest that the intracellular domain of LIN-12/Notch proteins convert CSL proteins from repressors into activators, thereby turning on the expression of target genes (Jarriault et al., 1995; Hsieh et al., 1996).

This mechanism of signal transduction was first suggested when expression of the intracellular domains of LIN-12 in *C. elegans* and Notch in *Drosophila* caused constitutive activation, with accumulation of the intracellular domain of Notch observed in the nucleus (Struhl et al.,1993). Then, it was found that the CSL DNA binding protein mediated transcription of Notch target genes and associated physically with the intracellular domain of Notch (Jarriault et al., 1995; Tamura et al., 1995). However, the intracellular domain could not be visualized in the nucleus under normal circumstances, and alternate models to explain these observations did not fall out of favor until compelling in vivo analysis in *Drosophila* (Struhl and Adachi, 1998) and biochemical analysis in mammalian cells (Schroeter et al., 1998) demonstrated ligand-dependent cleavage and nuclear translocation correlated with signal transduction.

4. Identification and characterization of core components

Given the mechanism of signal transduction, the core components of the pathway may be considered to be proteins that are directly involved in liberating the intracellular domain from its membrane tether, and components of the transcriptional activation complex that includes the intracellular domain. Genetic analysis in *C. elegans* has been important in identifying such components, particularly of the site 3 (transmembrane domain) protease complex. The core components and their *Drosophila* and mammalian counterparts are summarized in Table 1. In this section, I will detail how the core components were identified and characterized in *C. elegans*, and briefly summarize the state of understanding about these components from work in *C. elegans* and other systems.

4.1. Ligands

The first identified *C. elegans* ligand, *lag-2*, was recovered in two different genetic screens: null alleles of *lag-2* were identified based on the Lag phenotype they confer (Lambie and Kimble, 1991), and unusual antimorphic alleles were identified as dominant suppressors of the egg-laying defect caused by a *lin-12(d)* mutation (see below; Tax and Thomas, 1994; Tax et al., 1997). Molecular cloning and sequence analysis revealed that *lag-2* encodes a protein homologous to the known *Drosophila* Notch ligands Delta and Serrate (Henderson et al., 1994; Tax and Thomas, 1994). Another *C. elegans* ligand, *apx-1*, was identified in a genetic screen for maternal-effect lethal mutants (Mango et al., 1994; Mello et al., 1994). With the completion of the genomic sequence, it became clear that there are a total of ten genes that encode members of the DSL ligand family (Chen and Greenwald, 2004b). One of

the new predicted ligands, DSL-1, was shown to be a secreted protein that is functionally redundant with *lag-2* and *apx-1* in LIN-12-mediated vulval precursor cell fate specification (Chen and Greenwald, 2004b; see also Vulval development).

4.2. Site 2 (extracellular) cleavage

sup-17 was identified as a suppressor of the 0 AC defect caused by *lin-12(d)* mutations (Tax et al., 1997) and found to encode a metalloprotease of the ADAM family (Wen et al., 1997). Its *Drosophila* homolog, Kuzbanian, was also implicated in Notch signaling (Pan and Rubin, 1997; Rooke et al., 1996; Sotillos et al., 1997). However, in mammals, a different ADAM protein, TACE, has been implicated in the site 2 cleavage (Brou et al., 2000). *sup-17* is a positive factor for *lin-12*/Notch activity and appears to function cell autonomously, but there has not been a direct demonstration of its involvement in the site 2 cleavage. The *C. elegans* TACE ortholog, *adm-4*, also appears to be a positive factor for *lin-12/Notch* activity in some cell fate decisions; *sup-17* and *adm-4* are functionally redundant for at least a subset of LIN-12/Notch mediated decisions in *C. elegans*, but there may also be additional redundant proteases that can mediate the extracellular cleavage (Jarriault and Greenwald, 2005).

4.3. Site 3 (transmembrane) cleavage

The protein complex that mediates the site 3 cleavage of LIN-12/Notch also mediates the transmembrane cleavage of the β-amyloid precursor protein (β-APP), and has been termed "γ-secretase" (Kopan and Goate, 2000). Four proteins are sufficient to reconstitute γ-secretase activity in yeast (Edbauer et al., 2003). Genetic analysis in *C. elegans* has been instrumental in identifying these components and in illuminating their roles.

The first evidence of a link between the LIN-12/Notch and β-APP transmembrane cleavage events came from the recovery of *sel-12* as a suppressor of *lin-12(d)* (Levitan and Greenwald, 1995). *sel-12* encodes a Presenilin, which had, at about the same time, been identified as a major genetic locus in familial early-onset Alzheimer's disease (Sherrington et al., 1995). A second *C. elegans* presenilin, *hop-1*, appears to be functionally redundant with *sel-12*, and genetic analysis of these genes in *C. elegans* established that Presenilin activity is critical for LIN-12/Notch signaling (Li and Greenwald, 1997; Westlund et al., 1999). SEL-12/Presenilin is believed to be the catalytic subunit of the γ-secretase protease complex (Kopan and Goate, 2000). Analysis of SEL-12 revealed what is now generally accepted as the topology of Presenilin: eight membrane-spanning segments with a large cytosolic loop between the sixth and seventh transmembrane domains (Li and Greenwald, 1996, 1998; see Figure 3). These two transmembrane domains contain conserved aspartyl residues that appear to be the catalytic aspartates of the protease (Kopan and Goate, 2000).

Other genetic screens in *C. elegans* yielded the other three components of γ-secretase. Mutations that confer the anterior pharynx (Aph) defect resulting from failure of *glp-1* signaling at the 12 cell stage of embryogenesis yielded two genes, *aph-1* and *aph-2* (Goutte et al., 2000; Goutte et al., 2002). The ortholog of APH-2 is Nicastrin, which was also identified as a genetic locus in familial early-onset Alzheimer's disease (Yu et al., 2000). *aph-1* as well as the fourth component of γ-secretase, *pen-2*, were identified in a screen for mutations that result in synthetic phenotypes when combined with loss of *sel-12* or *aph-2* activity (Francis et al., 2002) Biochemical analysis, principally in mammalian cell culture, has suggested that Nicastrin and Aph-1 form an initial complex, which binds to and stabilizes the Presenilin holoprotein; Pen-2 then associates with this tripartite complex to confer γ-secretase activity (reviewed in Iwatsubo, 2004). Endoproteolysis in the cytosolic loop of SEL-12/Presenilin (Li and Greenwald, 1996; Li and Greenwald, 1998; Thinakaran et al., 1996) is facilitated by Pen-2 and is believed to be essential for γ-secretase activity (reviewed in Iwatsubo, 2004), although deletion of this region does not appreciably abrogate Presenilin activity in *C. elegans* (Levitan et al., 1996).

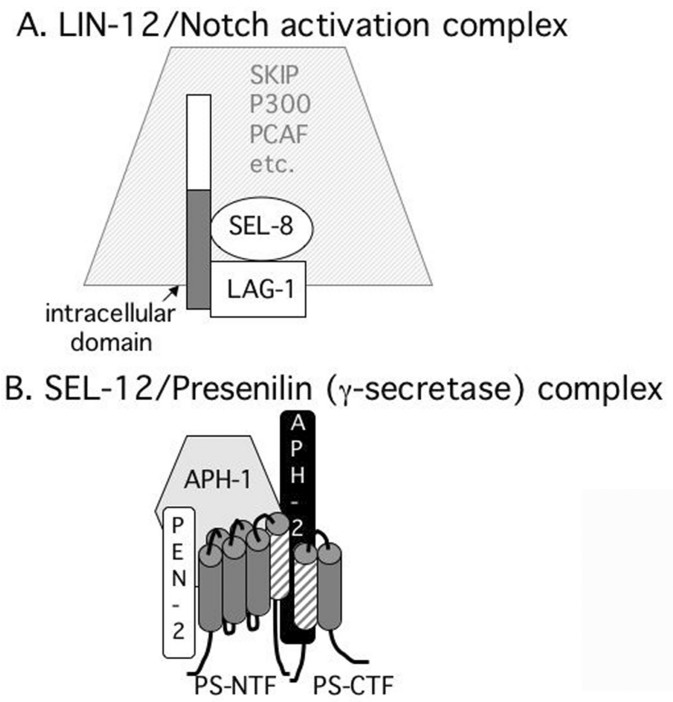

Figure 3. Core component complexes. A. The LIN-12/Notch activation complex. The intracellular domain of LIN-12 or GLP-1 forms a ternary complex with LAG-1 and SEL-8, which are both essential for *lin-12* and *glp-1* activity (Lambie and Kimble, 1991; Tax et al.,1997; Doyle et al., 2000; Petcherski and Kimble, 2000). B. The SEL-12/Presenilin (γ-secretase) complex. SEL-12 and HOP-1, the *C. elegans* Presenilins (PS), adopt an eight transmembrane domain topology and are cleaved into N-terminal (PS NTF) and C-terminal (PS CTF) fragments. Transmembrane domains 6 and 7 contain catalytic aspartyl residues (represented by shading). The contributions of the two-pass transmembrane protein PEN-2, the seven-pass transmembrane protein APH-1, and the single-pass transmembrane protein APH-2/Nicastrin are described in the text.

Table 1. Core components of the LIN-12/Notch pathway

Role	*C. elegans*	*Drosophila*	Mammals
Ligand	LAG-2, APX-1, DSL-1, 7 others	Delta, Serrate	Jagged, Delta-like, 4 others
Receptor	LIN-12, GLP-1	Notch	Notch 1–4
Site 2 cleavage[a]	SUP-17 ADM-4	Kuzbanian (TACE)	(ADAM-10) TACE
Site 3 cleavage	SEL-12, HOP-1 APH-1 APH-2 PEN-2	Presenilin (Aph-1) Nicastrin (Pen-2)	Presenilin 1, 2 APH-1 Nicastrin PEN-2
Nuclear complex	LAG-1 SEL-8[b]	Su(H) Mastermind	CBF1/RBP-J Mastermind-like

[a]SUP-17, Kuzbanian and ADAM-10 appear to be orthologs of each other, and are paralogs of TACE; all are proteases of the ADAM family. Orthology is defined by BLAST searching and phylogenetic analysis (e.g., as in Jarriault and Greenwald, 2005). ADM-4 and TACE also appear to be orthologs. There is evidence for functional redundancy of *sup-17* and *adm-4* (TACE) in *C. elegans*, although *sup-17* appears to have a more significant role (Jarriault and Greenwald, 2005). In *Drosophila* the genetic evidence suggests that Kuzbanian alone may mediate S2 cleavage (Rooke et al., 1996; Pan and Rubin, 1997; Sottilos et al., 1997). We note that placement of SUP-17 at this step is consistent with available genetic information and with the *Drosophila* data. In mammals, TACE has been demonstrated to cleave Notch in biochemical assays (Brou et al., 2000), but genetic analysis is consistent with functionally redundant proteases and ADAM10 involvement.
[b]Official name, first published in Tax et al. (1997); also known as LAG-3.

4.4. The nuclear complex

As mentioned above, CSL proteins are DNA binding proteins that are part of the transcriptional activation complex that forms with the LIN-12/Notch intracellular domain (see also Figure 3). Prior to LIN-12/Notch activation, CSL proteins have transcriptional repressor activity, and genetic analysis in *Drosophila* has established distinct roles for transcriptional repression by CSL proteins in development (e.g., Morel and Schweisguth, 2000). Association with the LIN-12/Notch intracellular domain is believed to lead to transcriptional activation in two steps: first by displacing corepressors, then by recruiting coactivators (Hsieh et al., 1996; Jarriault et al., 1995).

In *C. elegans*, the CSL protein is called LAG-1. Strong hypomorphic and null alleles of *lag-1* were recovered in a screen for mutations that cause a Lag phenotype, suggesting that *lag-1* is a critical component of LIN-12/Notch signaling (Lambie and Kimble, 1991). In addition, weaker alleles of *lag-1* have also been recovered in a screen for alleles that strongly increase the severity of a weak *glp-1* loss-of-function phenotype in the germ line (Qiao et al., 1995) and in screens for suppression of the 0 AC defect caused by a *lin-12(d)* mutation (Katic et al., submitted). *lag-1* was cloned and shown to be orthologous to *Drosophila* Su(H) and mammalian CBF1/RBP-J, leading to the acronym CSL for this family of DNA binding proteins (Christensen et al., 1996) Indeed, LAG-1 appears to have the same binding specificity as its orthologs (Christensen et al., 1996). The crystal structure of LAG-1 complexed with target DNA revealed that the amino and carboxy-terminal domains are strikingly similar to a region oftranscription factors of the NF-κB/Rel family, with the insertion of an unusual beta-trefoil domain (Kovall and Hendrickson, 2004). The beta-trefoil domain appears to have roles in both the recognition of target DNA and in mediating mutually-exclusive interactions with either corepressors or the intracellular domain of LIN-12/Notch, providing a structural basis for the switch between repressor and activator forms (Kovall and Hendrickson, 2004).

The *C. elegans* transcription activation complex also is likely to contain SEL-8 (Figure 3). A single non-null allele of *sel-8* was identified as a suppressor of the 0 AC defect caused by a *lin-12(d)* mutation (Tax et al., 1997). *sel-8* encodes a novel, glutamine rich, nuclear protein (Doyle et al., 2000). SEL-8 was also identified based on the formation of a ternary complex with LAG-1 and the intracellular domain of GLP-1 in yeast; it was referred to as LAG-3 in this study (Petcherski and Kimble, 2000a). Based on fact that both SEL-8 and Mastermind have glutamine-rich stretches, and the observation that both form ternary complexes with the intracellular domains of LIN-12/Notch and CSL proteins, Petcherski and Kimble (2000b) proposed that SEL-8 serves the same function as *Drosophila* and mammalian Mastermind. Mastermind has been well-characterized as an integral part of the complex that binds DNA (Kitagawa et al., 2001; Wu et al., 2000). In addition to contributing to transcriptional activation of target genes, Mastermind may enhance phosphorylation and turnover of the Notch intracellular domain (Fryer et al., 2002).

At this time, these three components--the intracellular domain of LIN-12/Notch, a CSL protein, and SEL-8/Mastermind--are believed to be the core of the transcriptional activation complex formed when LIN-12/Notch is activated (Figure 3; Fryer et al., 2002). Numerous other proteins, which play more general roles in transcriptional activation, are recruited through interactions with this core complex. Among the proteins recruited are CBP/p300, which is recruited by association with Mastermind, and the p300-associated protein PCAF (Fryer et al., 2002), and SKIP, which physically associates with both CBF1 and the Notch intracellular domain (Zhou et al., 2000).

5. Identification of modulators of *lin-12* and *glp-1* activity

Various genetic screens have identified many modulators of *lin-12* and *glp-1* activity. For the purposes of this discussion, I consider a modulator to be any gene whose activity influences *lin-12* and/or *glp-1* activity, but whose gene product has not been established to be a core component. In some cases, new information may at some future date lead to reclassification.

5.1. Suppressors of *lin-12(d)*

In *lin-12(d)* mutants, excess *lin-12* activity alters a cell fate decision during gonadogenesis (see below), leading to the absence of an AC, and consequently to an inability to lay eggs. Extensive, but not saturated, screens for extragenic suppressors of this egg-laying defect have been performed (Greenwald et al., 1983; Tax et al., 1997; Katic et al., submitted). Most genes recovered in these screens, and in the screen for suppressors of a *lin-12* hypomorph (see below), are named "*sel*" (suppressor/enhancer of *lin-12*), because their genetic behavior as a suppressor or enhancer depends on the nature of the *lin-12* allele with which they are combined. Many of the *sel* genes also interact genetically with *glp-1*.

In addition to alleles of the core component genes *lag-2*, *sel-8*, *sup-17* and *lag-1*, the screen for *lin-12(d)* suppressors has yielded mutations in genes for SEL-5, a serine/threonine kinase (Fares and Greenwald, 1999), SEL-7, a novel nuclear protein (Chen et al., 2004a), and SEL-6, a AAA-ATPase (Katic and Greenwald, in preparation; Tables 2 and 3). This screen has also yielded a loss-of-function allele of *bre-5*, which encodes a carbohydrate modifying enzyme that is homologous to *Drosophila* Brainiac (Griffitts et al., 2001), and several additional, as yet uncloned genes (Katic et al., submitted).

Table 2. Modulators of *lin-12* and *glp-1* activity. Genes listed here have displayed a genetic interaction with both *lin-12* and *glp-1* in at least one cell fate decision.

Role	Inferred regulatory role	*C. elegans*	Mammals
Nuclear/ target genes	+	SEL-7	?[a]
	-	SPR-1 SPR-2 SPR-3 SPR-4 SPR-5	CoREST Set/Nap ? ? p110b
ERAD/ quality control	-	SEL-1 SEL-9	Sel-1 p24
Stability	-	SEL-10	Sel-10/Fbw7

[a] ? = no apparent mammalian ortholog based on amino acid sequence identity, but proteins divergent in sequence may perform equivalent functions. SPR-3 and SPR-4 contain zinc fingers (Lakowski et al., 2003). SEL-7 contains no recognizable amino acid sequence motifs and has only nematode orthologs.

5.2. Suppressors of a *lin-12* hypomorph

Reversion of the egg-laying defect caused by partial loss of *lin-12* activity yielded five genes (Sundaram and Greenwald, 1993). Three have been characterized in detail (Table 2). *sel-1* encodes a protein that is involved in endoplasmic reticulum-associated protein degradation (Grant and Greenwald, 1996; Grant and Greenwald, 1997; Urano et al., 2002). *sel-9* encodes a p24 protein involved in quality control of membrane protein trafficking (Wen and Greenwald, 1999). *sel-10* encodes an E3 ubiquitin ligase that forms a complex with the intracellular domain of LIN-12 (Hubbard et al., 1997). Studies of mammalian orthologs have demonstrated that SEL-10 promotes the ubiquitin-mediated turnover of the intracellular domain of LIN-12/Notch proteins via phosphorylation of the PEST domain (Gupta-Rossi et al., 2001; Oberg et al., 2001; Wu et al., 2001).

5.3. Suppressors of *glp-1* hypomorph

Screens for suppressors of the germline defect caused by loss of zygotic *glp-1* activity (Maine and Kimble, 1993) or of the embryonic lethality caused by loss of maternal *glp-1* activity (A.M. Howell and J. Priess, personal communication) identified ten *sog* genes, for suppressor of *glp-1*. None of these have been molecularly characterized. Other suppressors of *glp-1* sterility proved to be genes required for body morphology, which may indicate that the extracellular matrix influences *glp-1* activity in the germ line (Maine and Kimble, 1993).

5.4. Enhancers of a *glp-1* hypomorph

Partial loss of *glp-1* activity provides a sensitized background to search for genes that promote *glp-1* activity, as reducing the activity of such genes will cause a synthetic Glp sterile phenotype. This strategy was used to screen for enhancers of the partial germline proliferation defect of a weak *glp-1* loss-of-function mutation and yielded five *ego* genes, for enhancers of *glp-1* (one), as well as weak alleles of *lag-1* (Qiao et al., 1995; Smardon et al., 2000). *ego-1* plays multiple roles in germline development, and encodes an RNA-directed RNA polymerase (Smardon et al., 2000; Table 2). *ego-1* mutants are resistant to RNAi, suggesting a link between germline development and cellular processes involved in post-transcriptional silencing (Smardon et al., 2000). *ego-1* also promotes heterochromatin assembly, and therefore may influence gene expression at the transcriptional level (E. Maine and

W. Kelly, personal communication). Recent analysis of *ego-5* suggests that regulation of DNA replication proteins may be part of the switch from mitotic to meiotic development (E. Maine, personal communication). The gene *atx-2* was also identified by RNAi as an *ego* gene and found to be required for germline proliferation (Ciosk et al., 2004; Maine et al., 2004). *atx-2* acts independently of GLP-1 signaling and in translational regulation in the germline (Ciosk et al., 2004; Maine et al., 2004).

5.5. Enhancers of *glp-1* gain-of-function

Strong activation of *glp-1* in the germ line results in continued mitotic proliferation without meiosis, called the "tumorous" phenotype. A screen for enhancers of a weak tumorous phenotype caused by mild *glp-1* gain-of-function has identified tumorous enhancers of *glp-1* (*teg* genes), potential negative regulators of *glp-1* activity in the germ line (D. Hansen and T. Schedl, personal communication). The *teg* genes are currently being analyzed in detail.

5.6. Suppressors of Presenilin

sel-12 null alleles are unable to lay eggs because of a defect in *lin-12*-mediated π cell specification (Cinar et al., 2001). *sel-12* null mutants are viable because they retain activity of a second presenilin gene, *hop-1* (Li and Greenwald, 1997). Suppressors of the egg-laying defective phenotype of *sel-12* mutants identified *spr* genes (suppressors of presenilin; Wen et al., 2000), four of which (SPR-1, SPR-3, SPR-4 and SPR-5; Table 2) appear to be members of a CoREST-containing corepressor complex (Eimer et al., 2002; Jarriault and Greenwald, 2002; Lakowski et al., 2003). The CoREST complex may be involved in the balance of repression and activation of *lin-12* target genes or in regulating expression of a limiting component required for *lin-12* activity.

sel-10 also suppresses the egg-laying defect of *sel-12* mutants, and SEL-10 forms a complex with SEL-12, suggesting that *sel-10* may regulate *lin-12* activity through regulating SEL-12 as well LIN-12 levels (Wu et al., 1998).

Table 3. Modulators of *lin-12* or *glp-1* activity. Genes listed here have displayed a genetic interaction with either *lin-12* or *glp-1*. All possible interactions have not necessarily been tested.

Modulator of:	Inferred regulatory role	*C. elegans*	Mammals
glp-1	+	EGO-1	?[a]
lin-12	-	SEL-2	Neurobeachin
lin-12	+	SEL-5	AAK1
lin-12	+	BRE-5	Brainiac glycosyltransferase

[a]? = no apparent ortholog based on amino acid sequence identity, but proteins divergent in sequence may perform equivalent functions.

6. Lateral specification and induction mediated by LIN-12 and GLP-1

LIN-12 and GLP-1 mediate many different cell-cell interactions during *C. elegans* development. Some of these interactions are considered elsewhere in WormBook (see Notch signaling in the *C. elegans* embryo, Vulval development, Male development, Hermaphrodite cell-fate specification, and Germline proliferation and its control) and so have not been catalogued here. The two general categories of cell-cell interactions are lateral specification and induction. Lateral specification (lateral inhibition) occurs when a group of equivalent cells interact among themselves to specify alternative cell fates. Induction occurs when equivalent cells choose alternative fates in response to an external source of ligand, with cells close to the source selecting one fate, and cells further away selecting another.

LIN-12/Notch proteins appear to be unique mediators of lateral specification during animal development. The archetypal example of lateral specification in *C. elegans* involves a cell fate decision between the anchor cell (AC) and ventral uterine precursor cell (VU) fates in early gonadogenesis in hermaphrodites. During the "AC/VU decision", two equivalent cells each have the potential to become an AC or a VU. The two cells interact with each other, via LAG-2 and LIN-12, so that only one becomes the AC. A key concept of general significance that emerged from studies of this decision is the importance of a feedback mechanism that drives the cells to adopt different fates: both Z1.ppp and Z4.aaa initially express both *lin-12* and *lag-2*, but a small initial difference in *lin-12* activity is

amplified by reciprocal feedback loops in the presumptive AC and presumptive VU (Seydoux and Greenwald, 1989; Wilkinson et al., 1994). In the presumptive VU, LIN-12 activation by LAG-2 positively autoregulates *lin-12* transcription and downregulates *lag-2* transcription (Wilkinson et al., 1994). The latter step appears to involve post-transcriptional downregulation of HLH-2, a transcription factor required for *lag-2* transcription, in response to LIN-12 activation (Karp and Greenwald, 2003).

LIN-12/Notch proteins, like many other receptor systems, also mediate inductive interactions during development. In these cases, receptor activity is essentially regulated by where the ligand is expressed or activated. The roles of *glp-1* in germline development (see Specification of the germ line) and pharyngeal induction are canonical inductive interactions: ligands produced in the distal tip cell of the somatic gonad activate GLP-1 in the germ line (Henderson et al., 1994), and ligands produced in one embryonic founder cell activate GLP-1 in descendants of another (Mickey et al., 1996; Shelton and Bowerman, 1996). However, even when ligand originates externally, there appear to be feedback mechanisms that affect receptor expression or activity. During germline development, there appears to be post-transcriptional up-regulation of GLP-1 accumulation (Berry et al., 1997). Another intriguing example is afforded by the *lin-12*-mediated generation of morphological twist of the intestinal tube: twist is initiated when the cells in the left half of the intestinal primordium contact external, LAG-2-expressing cells. and activation of LIN-12 by LAG-2 leads to downregulation of *lin-12* transcription (Hermann etal., 2000).

Regulatory mechanisms integrate LIN-12/Notch signaling with other signaling events during animal development. For example, in *C. elegans*, cross-regulatory interactions between the LET-23/EGF receptor-Ras-MAP kinase pathway and LIN-12 ensure that the central three vulval precursor cells (VPCs) adopt the correct pattern of fates, represented as 2°-1°-2° (see Vulval development). In the presumptive 1° VPC, activation of LET-23 leads to transcription of DSL ligand genes (Chen and Greenwald, 2004b). However, in the presumptive 1° VPC, ligand expression per se is not sufficient to activate LIN-12 in the flanking VPCs; in addition, endocytosis-mediated downregulation of LIN-12, also in response to LET-23 activation, must occur for the DSL ligands to be active (Shaye and Greenwald, 2002). In the presumptive 2° VPCs, activation of LIN-12 leads to transcription of target genes that encode negative regulators of the EGF receptor-Ras-MAP kinase pathway, effectively squelching input from the inductive signaling pathway (Berset et al., 2001; Yoo et al., 2004).

7. Future prospects

With the core signal transduction mechanism largely elucidated, the emphasis has been shifting to understanding how signaling is modulated. One aspect is understanding how signaling is modulated in response to LIN-12/Notch activation itself, i.e. feedback mechanisms. There are likely to be many ways in which feedback modulation is achieved. Transcriptional regulation may involve different transcriptional regulatory circuits (see Notch signaling in the *C. elegans* embryo). Modulation is likely to involve different mechanisms for post-transcriptional regulation as well. Another aspect is understanding how LIN-12/Notch signaling is influenced by, and integrated with, other signaling inputs. This aspect, too, is likely to involve modulation of signaling by transcriptional and post-transcriptional mechanisms. Another important question for the future is how activation of LIN-12/Notch specifies different cell fate choices. One approach to answering this question is the identification and characterization of target genes--and one fruitful approach to identifying target genes is computational analysis to identify genes that contain potential LAG-1 binding sites in regulatory regions (Yoo et al., 2004).

8. Acknowledgements

Thanks to Marty Chalfie, Lisa Girard, Iskra Katic, Bob Johnston, Eleanor Maine, Sophie Jarriault, Jim Priess, Tim Schedl, and Gary Struhl for helpful comments on the manuscript. Work on the identification of genes that influence *lin-12*/Notch activity in my lab is supported by NIH CA095389. I am an Investigator of the Howard Hughes Medical Institute.

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