Notch signaling: genetics and structure[*]

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Abstract

Receptors of the Notch family mediate cell-cell interactions during animal development, and aberrations in Notch signaling have been implicated in human disease. Studies in *Caenorhabdits elegans* have made essential contributions towards understanding the biological roles and molecular mechanism of this fundamental signaling system. A major development in the field since the original version of this chapter (LIN-12/Notch signaling in *C. elegans*) has been an explosion in information about the structural biology of Notch signaling; crystallographic determinations of structures, including structures of *C. elegans* components, have contributed much to the current understanding of molecular mechanism. Thus, here, we not only cover the genetics of Notch in *C. elegans*, focusing on conserved core components and modulators, we have also included structural information about these components, describing the key events occurring during ligand binding and transcriptional control of target genes. In addition to text, we include Tables listing core components and key modulators of the signaling pathway along with their orthologs in *Drosophila* and mammals, a Table listing validated target genes in various processes in *C. elegans*, and animated features to illustrate structural attributes.

1. Introduction

Notch is a fundamental signaling system that mediates cell-cell interactions in animal development, and aberrations in Notch signaling or components of the signaling system underlie human disease. Genetic analysis of Notch signaling in *C. elegans* has illuminated universal aspects of this essential conserved pathway. Although the two *C. elegans Notch* genes, *lin-12* and *glp-1*, are best known for their roles in cell fate decisions during development, recent studies have expanded the roles of these genes in *C. elegans* beyond cell fate to other types of events including the regulation of tubular morphogenesis (Rasmussen et al., 2008), dauer maintenance and recovery (Ouellet et al., 2008), the response to anoxia (LaRue and Padilla, 2011), behavior (Singh et al., 2011), oocyte growth (Nadarajan et al., 2009) and cell fate reprogramming (Jarriault et al., 2008). Thus, ongoing investigations into Notch signaling in *C. elegans* have implications for many different aspects of development and physiology.

This chapter contains five additional sections. In Section 2, we briefly summarize the key events during signal transduction by Notch. In Section 3, we describe the conserved core components of the Notch signaling system and their genetic identification in *C. elegans*. In Section 4, we consider detailed aspects of the mechanism illuminated by structural studies of the *C. elegans* and mammalian core components individually and in complexes. In Section 5, we list target genes in *C. elegans* and comment on a potential difference in binding site architecture between *C. elegans* and other animals. In Section 6, we summarize genetic screens for modulators of *Notch* signaling in *C. elegans*.

2. Summary of key events during signal transduction by Notch

All Notch proteins have the arrangement of amino acid sequence motifs illustrated in Figure 1. The intracellular domain of Notch is essentially a membrane-tethered transcription factor whose release is regulated by ligand binding. Release of the intracellular domain involves two proteolytic cleavage steps (Figure 1). The first cleavage is regulated: binding of a ligand of the DSL family exposes a cleavage site ("site 2") in the ectodomain, making it accessible to an ADAM family protease. The second cleavage is constitutive: the remaining transmembrane protein, with only a short extracellular stub, is a substrate for γ-secretase, which cleaves within the transmembrane domain at "site 3". The intracellular domain, released from its transmembrane tether, translocates to the nucleus. In the nucleus, the intracellular domain forms a complex with a sequence-specific DNA binding protein of the CSL family and a co-activator of the Mastermind family to promote target gene expression.
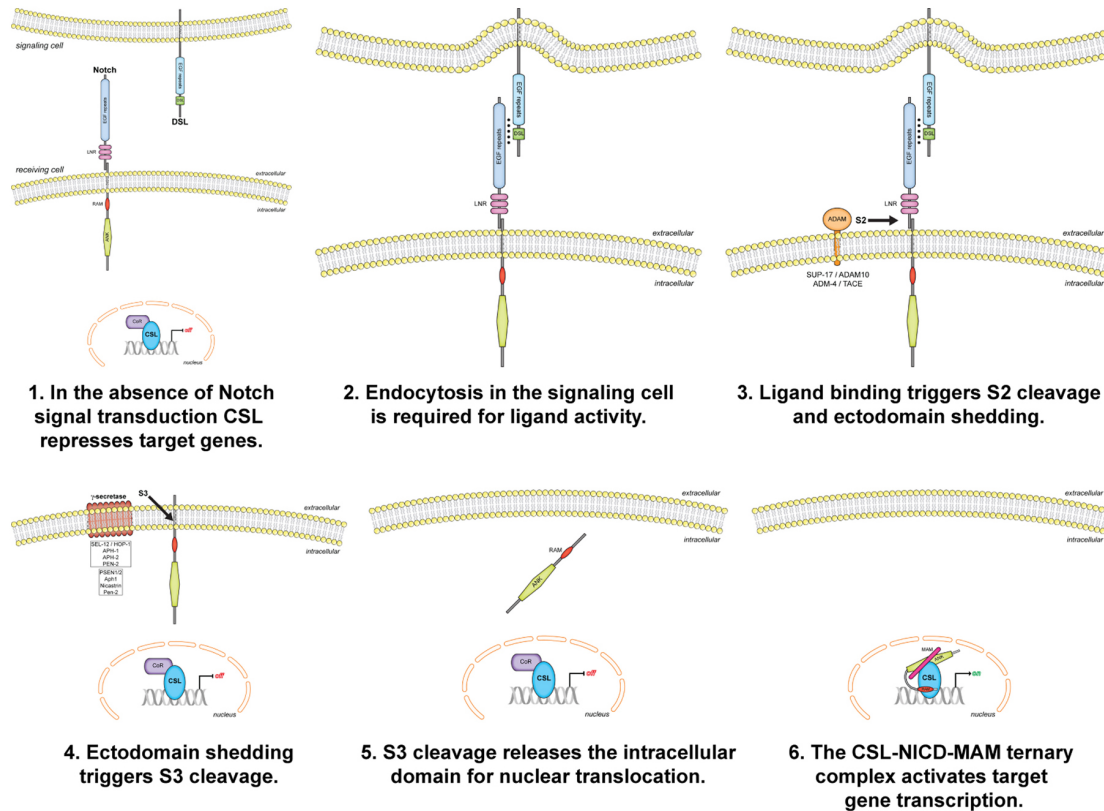
1. In the absence of Notch signal transduction CSL represses target genes.

2. Endocytosis in the signaling cell is required for ligand activity.

3. Ligand binding triggers S2 cleavage and ectodomain shedding.

4. Ectodomain shedding triggers S3 cleavage.

5. S3 cleavage releases the intracellular domain for nuclear translocation.

6. The CSL-NICD-MAM ternary complex activates target gene transcription.

Figure 1. Overview of signal transduction.

This basic model was first proposed after observing that expression of the intracellular domains of LIN-12 in *C. elegans* and Notch in *Drosophila* caused phenotypes associated with constitutive activation of the receptors (Struhl et al., 1993). For a detailed discussion of how the cleavage model was supported, challenged, and finally accepted, as well as further information about the central role *C. elegans* played in understanding the role of Notch signaling in lateral specification (lateral inhibition), please see (Greenwald, 2012).

3. Genetic identification of core components

All of the core components of the Notch signaling system were identified through genetic analysis in *C. elegans* and *Drosophila*. Genetic studies in *C. elegans* first linked γ-secretase activity to Notch signaling and defined all four γ-secretase core components. Ligands and nuclear complex components were first identified in *Drosophila*, but genetic and structural studies of the *C. elegans* components led to significant insights into their functions (see below and Section 4). The domain organization of LIN-12/Notch, the canonical ligand LAG-2, and the nuclear complex components is shown in Figure 2. Core components are also listed in Table 1, along with their *Drosophila* and mammalian orthologs.
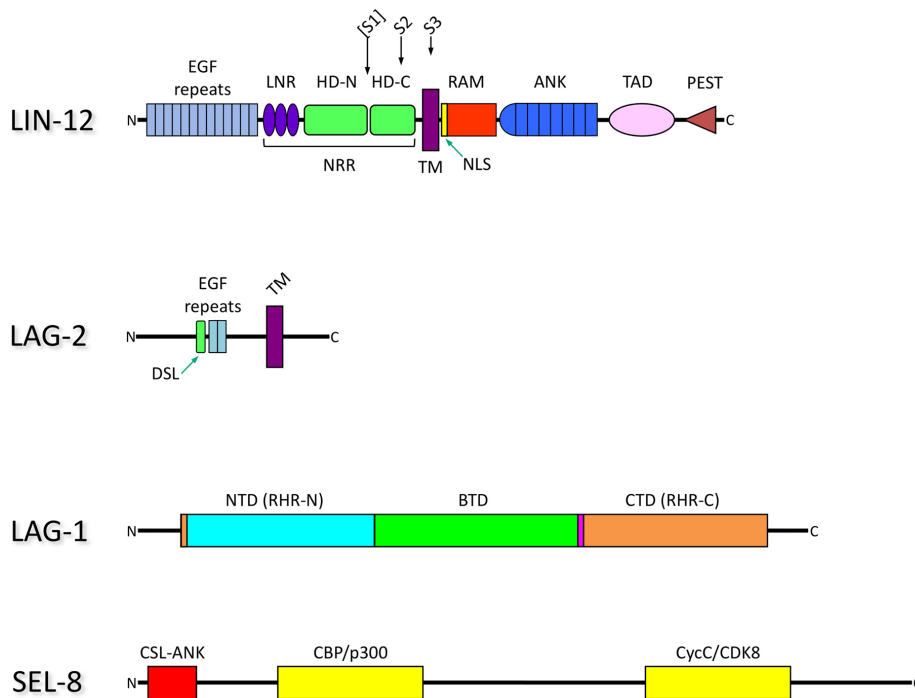
Figure 2. Domain organization of core components from *C. elegans*.

LIN-12: The ectodomain is followed by a single transmembrane (TM) region and the intracellular domain (NICD). The S2 (ADAM protease) and S3 (γ-secretase) cleavage sites are indicated. The potential, but not formally demonstrated, S1 cleavage site in LIN-12 is shown in parentheses. GLP-1 has a similar domain organization.

EGF, Epidermal Growth Factor (EGF)-like motif.

LNR, LIN-12/Notch Repeat.

HD, Heterodimerization Domain; shown as if Furin cleavage occurs at site 1, resulting in HD-N, amino terminal segment, HD-C, carboxy terminal segment. Cleavage at NRR, Negative Regulator Region. The NRR encompasses the LNR repeats and the HD domain.

TM, TransMembrane domain.

NLS, Nuclear Localization Sequence.

RAM, a region that mediates interactions with LAG-1.

ANK, Ankyrin repeat motifs.

TAD, Transcriptional Activation Domain.

LAG-2: The DSL ligand LAG-2 is composed of an ectodomain, a single transmembrane domain, and a short intracellular domain.

DSL, Delta Serrate LAG-2 domain.

EGF, EGF-like motif.

LAG-1: The CSL DNA-binding transcription factor consists of three domains.

NTD, Amino Terminal Domain.

CTD, Carboxy Terminal Domain. The NTD and CTD are often referred to as Rel Homology Region (RHR) -N and -C domains, due to their structural similarity to Rel proteins, such as NF-kappaB.

BTD. β-Trefoil Domain.

SEL-8: This component appears to be the counterpart of *Drosophila* or mammalian Mastermind, but shares little primary sequence similarity with them. Domains other than CSL-ANK are inferred based on Mastermind.

CSL-ANK, N-terminal domain that interacts with CSL and the ANK domain of NICD (CSL-ANK).

CBP/p300, domain that recruits the ubiquitous coactivator CBP/p300.

CycC/CDK8, domain that recruits the kinase CycC/CDK8.

Table 1. Core components of the Notch signaling system

Gene	allele type	Notch phenotype	Suppress/ enhance[§]	Mammalian	*C. elegans* references
Receptors					
lin-12	hypermorph*	0 AC-Egl, Muv, other*		Notch1-4	(Greenwald et al., 1983; Ferguson and Horvitz, 1985)
	null	2 AC, Evl, other**			(Greenwald et al., 1983)
glp-1	null (zygotic)	Glp***		Notch1-4	(Austin and Kimble, 1987)
	loss (maternal)	Mel (Aph or Apx)***			(Priess et al., 1987; Mello et al., 1994)
	hypermorph	Tum, Pro			(Berry et al., 1997; Pepper et al., 2003)
Ligands[§§]					
lag-2 (sel-3)	null	Lag****		(DSL)	(Lambie and Kimble, 1991)
	neomorph		Sup *lin-12(d)*		(Tax and Thomas, 1994; Tax et al., 1997)
apx-1	loss	Mel (Apx)		(DSL)	(Mango et al., 1994; Mello et al., 1994)
arg-1				(DSL)	(Mickey et al., 1996)
dsl-1	null			(DSL)	(Chen and Greenwald, 2004)
Ligand activity					
epn-1	RNAi	Glp		Epsin	(Tian et al., 2004)
Site 2 protease					
sup-17	loss	Mel	Sup *lin-12(d)*	ADAM10	(Tax et al., 1997; Wen et al., 1997)
adm-4				ADAM17/ TACE	(Jarriault and Greenwald, 2005)
Site 3 protease					
sel-12	null	Egl	Sup *lin-12(d)*	Presenilin 1, 2	(Levitan and Greenwald, 1995; Cinar et al., 2001)
hop-1	null	none		Presenilin 1, 2	(Li and Greenwald, 1997; Westlund et al., 1999)
aph-1	loss	Mel (Aph), Egl	Pen*****	Aph1	(Francis et al., 2002; Goutte et al., 2002)
aph-2	loss	Mel (Aph), Egl		Nicastrin	(Goutte et al., 2000; Levitan et al., 2001)
pen-2	loss		Pen	Pen2	(Francis et al., 2002)
Nuclear complex					
lag-1	null	Lag	Sup *lin-12(d)*	CBF1/RBP-J	(Lambie and Kimble, 1991)
sel-8 (lag-3)	rare	Mel	Sup *lin-12(d)*	Mam	(Tax et al., 1997; Doyle et al., 2000)
	RNAi	Lag			(Petcherski and Kimble, 2000)

Alternate, unofficial gene names are given in parentheses.

[§]identified in a suppressor or enhancer screen.

[§§]Ligands are members of the DSL family; a similar domain structure is seen in Drosophila and mammalian ligands, but orthology is difficult to assign.

lin-12(d) alleles, see text. 0 AC-Egl, lack of an anchor cell prevents vulval induction. Muv, multivulva due to ectopic induction of the 2° vulval fate. For additional phenotypes, see Greenwald et al. (1983) and Wormbase.

**lin-12(0)* mutants have 2 anchor cells (2AC) and abnormal vulval eversion (Evl); for other phenotypes, see Greenwald et al. (1983) and Wormbase.

***Glp, germline proliferation defective; Mel, maternal effect lethal; Aph, anterior pharynx defective; Apx, anterior pharynx excess. See listed references and Wormbase for more details.

****larval arrest with cell fate transformations seen in the *lin-12 glp-1* double mutant; see Lambie and Kimble (1991) and Wormbase for details.

*****Pen, presenilin enhancer (Francis et al., 2002)

3.1. Receptors: LIN-12 and GLP-1

LIN-12 and GLP-1 both have the hallmark arrangement of amino acid sequence motifs present in all Notch proteins (Yochem et al., 1988; Yochem and Greenwald, 1989) (Figure 1).

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 isolated, called *lin-12(d)* alleles, were dominant and hypermorphic. Intragenic reversion of these dominant mutations 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 cell fate transformations in many different cell fate decisions, establishing the binary nature of Notch-mediated cell fate decisions (Greenwald et al., 1983). Further analysis suggested that *lin-12(d)* mutations result in ligand-independent constitutive activation and affect a negative regulatory domain or higher order structure (Greenwald and Seydoux, 1990); now, with structural information, the region affected by these mutations has been termed the "negative regulatory region" (NRR) (see Section 4). These *lin-12(d)* mutations are the prototype for a class of mutations that have been found to cause T- cell acute lymphoblastic leukemia (T-ALL) (Weng et al., 2004).

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; see also WormBook chapters Germline proliferation and its control and Notch signaling in the *C. elegans* embryo). Other screens based on germline aspects of *glp-1* function yielded dominant alleles altering the NRR and causing continued mitotic proliferation of the germline at the expense of gamete production (Berry et al., 1997; Pepper et al., 2003). An allele that deletes the PEST domain also has increased activity (Mango et al., 1991) and resembles another class of T-ALL mutations (Weng et al., 2004).

A screen for maternal-effect embryonic lethal mutations revealed that loss of maternal *glp-1* activity prevents cell fate induction at the 12-cell stage of embryogenesis (Priess et al., 1987); subsequently, several additional embryonic cell-cell interactions have been shown to require *glp-1* or *lin-12* activity (Priess, 2005).

The existence of two Notch genes in *C. elegans* allowed for a test of whether different Notch proteins are functionally redundant, which is of additional interest because vertebrates have four Notch proteins (and *Drosophila* has only one). Lambie and Kimble (Lambie and Kimble, 1991) showed that double mutants lacking zygotic *lin-12* and *glp-1* activity die as L1 larvae with various discrete defects, defining the "Lag" phenotype, for "*L*in-12 *a*nd *G*lp-1." Their results suggested that the two Notch proteins, though divergent in sequence, are functionally redundant (Lambie and Kimble, 1991). GLP-1 is able to substitute for LIN-12 in cell fate decisions, providing further evidence for true functional redundancy (Fitzgerald et al., 1993).

The Lag phenotype of the *lin-12 glp-1* double mutant served as the basis of a genetic screen that identified two additional core components, *lag-1* and *lag-2* (Lambie and Kimble, 1991) (Table 1). These two genes, along with other core components and modulators, also emerged in genetic screens for extragenic suppressors and enhancers (Section 6).

3.2. Ligands: DSL proteins

"DSL" is an acronym derived from three canonical ligands for Notch: *Drosophila D*elta and *S*errate, and *C. elegans L*AG-2. DSL proteins all contain one or more EGF motifs, as well as an amino terminal motif that is unique to the family, the "DSL motif" (Fig. 2).

The first *C. elegans* ligand identified, *lag-2*, was defined genetically in two ways: by null alleles that confer the Lag phenotype (Lambie and Kimble, 1991) and by neomorphic alleles that act as suppressors of *lin-12(d)* mutations (Tax and Thomas, 1994; Tax et al., 1997). The *apx-1* (Mango et al., 1994; Mello et al., 1994), *arg-1*(Mello et al., 1994; Kostas and Fire, 2002) and *dsl-1* (Chen and Greenwald, 2004; Hoyos et al., 2011) genes also encode ligands that have been genetically linked to discrete Notch-mediated events in *C. elegans* development.

C. elegans DSL proteins have two interesting differences as compared to ligands in other organisms. First, it is possible to engineer secreted forms that can activate LIN-12 or GLP-1 (Fitzgerald and Greenwald, 1995), forms that in other organisms generally act as dominant-negatives. In addition, there appear to be naturally secreted forms (Chen and Greenwald, 2004). However, it is not known whether these secreted forms function in association with membranes or require endocytosis for ligand activity (see below). Second, sequence differences at the amino termini of *C. elegans* ligands include the lack of a pair of variant EGF-like repeats, termed the "Delta and OSM-11" (DOS)

motif, which is present in other DSL proteins (Komatsu et al., 2008) (see also Kovall and Blacklow, 2010). Instead, *C. elegans* contains genes that encode "DOS-only" proteins; the canonical member of the DOS-only family, OSM-11, appears to facilitate ligand activity in Vulval Precursor Cells (VPCs) and has been proposed to act as a co-ligand in this context (Komatsu et al., 2008). The generality of DOS-only protein function remains to be determined.

Extensive analysis in *Drosophila* and mammals has established Epsin as a core component required for ligand activity (e.g., Wang and Struhl, 2004). The role of Epsin-mediated endocytosis is an active area of research in the Notch field. RNAi experiments suggest that EPN-1, *C. elegans* Epsin, influences ligand activity (Tian et al., 2004), but an absolute requirement for Epsin in *C. elegans* (or for endocytosis per se) has not yet been assessed.

3.3. Nuclear complex: LAG-1 and SEL-8

The intracellular domain of Notch, a CSL protein, and Mastermind form a transcriptional activation complex that promotes expression of target genes. Numerous other proteins, which play more general roles in transcriptional activation, are also recruited through interactions with this core complex.

CSL, an acronym for "CBF1/Su(H)/LAG-1", is the sequence-specific DNA binding protein with which the intracellular domain of Notch proteins interact to promote target gene expression. Mammalian CBF1 (also called RBP-J) had been independently identified numerous times as a DNA binding protein in association with different promoters; the key connection of this protein to Notch was made through genetic studies of *Drosophila Suppressor of Hairless* [*Su(H)*]. In the absence of Notch activation, CSL functions as a transcriptional repressor, in a complex with co-repressors.

In *C. elegans*, *lag-1* was defined by null mutations that cause a Lag phenotype, suggesting that *lag-1* is an essential component for Notch signaling (Lambie and Kimble, 1991). Weaker alleles of *lag-1* were subsequently also recovered in modifier screens (Qiao et al., 1995; Katic et al., 2005). Molecular cloning revealed that LAG-1 is a CSL protein and further analysis substantiated that it has the same binding site specificity as its *Drosophila* and mammalian orthologs (Christensen et al., 1996) (see below).

sel-8 was initially defined by neomorphic mutations that suppress *lin-12(d)* (Tax et al., 1997) (Doyle et al., 2000) and the SEL-8 protein (under the provisional name "LAG-3") was also independently identified through a yeast two-hybrid screen for proteins that interact in a ternary complex with LAG-1 and the GLP-1 intracellular domain (Petcherski and Kimble, 2000). These interactions suggested that SEL-8 serves the same function as *Drosophila* and mammalian Mastermind (Petcherski and Kimble, 2000). Mastermind is an integral part of the transcriptional activation complex (Wu et al., 2000; Kitagawa et al., 2001) and may enhance phosphorylation and turnover of the Notch intracellular domain (Fryer et al., 2004).

3.4. Proteases: ADAM proteases and γ-secretase

Three proteolytic processing events have been defined for mammalian Notch. Proteolysis at site 1 by Furin during its trafficking to the surface is constitutive (Blaumueller et al., 1997; Logeat et al., 1998). It is not known whether LIN-12 and GLP-1 are processed at site 1, but Western blot analysis of GLP-1 is consistent with the possibility that it is (Crittenden et al., 1994). *Drosophila* Notch does not undergo this cleavage (Kidd and Lieber, 2002), suggesting that it is not pertinent to signal transduction *per se*.

Proteolysis of Notch at sites 2 and 3 leads to release of the intracellular domain for signal transduction. Site 2 is located in the ectodomain and is cleaved in a ligand-dependent manner. The site 2 protease is a member of the ADAM (A Disintegrin And Metalloprotease domain) protease family. In *C. elegans*, genetic analysis suggests that the main site 2 protease is encoded by *sup-17*. This gene was defined by hypomorphic alleles that suppress *lin-12(d)* mutations (Tax et al., 1997); molecular cloning and sequence analysis (Wen et al., 1997) established it as an ADAM protease orthologous to *Drosophila* Kuzbanian, also implicated in Notch signaling (Rooke et al., 1996; Pan and Rubin, 1997). The mammalian ortholog of SUP-17 and Kuzbanian is called ADAM10. Biochemical studies originally implicated a different mammalian ADAM protein, called TACE or ADAM17, in S2 cleavage of Notch (Brou et al., 2000). However, further studies in *C. elegans* and mammals suggest that both SUP-17/ADAM10 and ADM-4/TACE may function as S2 proteases and that there may be some functional redundancy between them (Jarriault and Greenwald, 2005).

Site 3 is located within the transmembrane domain. The site 3 protease, γ-secretase, can cleave within the transmembrane domain of virtually any type 1 membrane protein substrate with a short ectodomain (Struhl and Adachi, 2000). Thus, Notch becomes a substrate for γ-secretase as a result of the ligand-induced S2 cleavage. γ-secretase is a protein complex that consists of Presenilin, the catalytic subunit, and three other proteins that contribute to substrate recognition and stability: APH-2/Nicastrin, APH-1, and PEN-2. Expression of these four components together in yeast are sufficient to reconstitute γ-secretase activity (Edbauer et al., 2003).

Genetic analysis in *C. elegans* was critical for elucidating the components of the complex and the requirement of γ-secretase for Notch activity. Presenilin was initially connected to Notch when null alleles of *sel-12* were identified as suppressors of *lin-12(d)* (Levitan and Greenwald, 1995). These null alleles are viable because of functional redundancy with a second Presenilin-encoding gene, *hop-1*. The essential requirement for Presenilin in Notch signaling was revealed by showing that concomitant loss of both *sel-12* and *hop-1* cause hallmark Lin-12, Glp-1 and Lag phenotypes, demonstrating that *Notch*-mediated signaling events throughout development depend on Presenilin activity (Li and Greenwald, 1997; Westlund et al., 1999).

In vivo topological studies in *C. elegans* defined the seventh transmembrane domain (Li and Greenwald, 1996; Li and Greenwald, 1998), which had not been identified by traditional topological studies or sequence analysis, and included what proved to be a key catalytic aspartyl residue. Definitive evidence that Presenilin has a catalytic function came from subsequent aspartyl protease inhibitor studies in mammalian cells.

The three other core components of γ-secretase were also identified genetically in *C. elegans*. APH-1 and APH-2 were identified by maternal-effect lethal mutations that confer the Anterior PHarynx defect resulting from failure of *glp-1* signaling at the 12 cell stage of embryogenesis (Priess, 2005; Goutte et al., 2000; Goutte et al., 2002) (Table 1). Homozygous mutants are viable when maternally rescued. The mammalian ortholog of APH-2, Nicastrin, was independently identified through its physical association with Presenilin and initially assessed functionally in *C. elegans* by RNAi, revealing a role in Notch signaling (Yu et al., 2000). *aph-1* as well as the fourth component of γ-secretase, *pen-2*, were identified in a "Presenilin enhancer" (Pen) screen for Notch loss-of-function phenotypes in a *sel-12* mutant background (Francis et al., 2002) (Table 1). 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; Thinakaran et al., 1996; Li and Greenwald, 1998) is facilitated by PEN-2 and is believed to be important 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).

4. Structural studies of Notch signaling

Following the identification of the core signaling components from genetic studies in *C. elegans* and *Drosophila*, there has been much interest in determining high-resolution structures of these components, and the complexes they form, in order to provide further molecular insights into the mechanism of signal transduction. In particular, there have been three areas in which significant progress has been made: (1) the structures of the Notch and DSL EGF repeats that are involved in receptor-ligand complexation; (2) the structure of the NRR (negative regulatory region), which is involved in sequestering the S2 cleavage site of Notch until interactions are formed with a DSL ligand; and (3) the structures of CSL, Notch, and Mastermind transcription complexes. In this section, we give a brief overview of the structures that have been determined and the functional information that was born out of these studies. For a more comprehensive review of this subject matter, please see Kovall and Blacklow (2010).

4.1. Notch and DSL ectodomain structures

Both Notch and DSL are multidomain membrane proteins that contain a single transmembrane spanning region. The ectodomains of all Notch proteins contain multiple EGF repeats. LIN-12 has 13 repeats and GLP-1 has 10, in both cases fewer than found in the *Drosophila* (36) and mammalian Notch proteins (28-36). The EGF repeats of mammalian Notch proteins are heavily modified by O-linked glycans, and only a subset of these repeats is important for interacting with a DSL ligand. Similarly, the extracellular domains of DSL proteins also contain multiple EGF repeats, as well as the DSL domain that gives this family of ligands its name.

EGF repeats are small modular domains that typically contain three disulphide bonds and are primarily composed of β-strand, forming an extended rod-like structure. Some, but not all, EGF repeats bind calcium, which

has been shown to be important for receptor-ligand complexation. Hanford and coworkers (Cordle et al., 2008) have determined several structures of EGF repeats using both NMR and crystallographic methods; these include (1) the EGF repeats 11-13 from human Notch1, which are necessary for interacting with ligand, and (2) the DSL domain followed by EGF repeats 1-3 from human Jagged1 (Figs. 3 and 4). While previous studies have shown that these regions of Notch1 and Jagged1 are important for receptor-ligand complexation and signaling *in vivo*, the isolated fragments interact only weakly *in vitro* (Cordle et al., 2008). Nonetheless, the EGF repeat structures revealed conserved interfaces on both Notch1 and Jagged1 that were shown to be important for both trans-signaling and cis-inhibition by *in vivo* assays in *Drosophila* (Cordle et al., 2008a; Cordle et al., 2008b). We note that the first two EGF-like motifs of Jagged1 correspond to the "DOS domain," and that the structural information suggests that they are bona fide EGF repeats rather than a structurally distinctive domain.
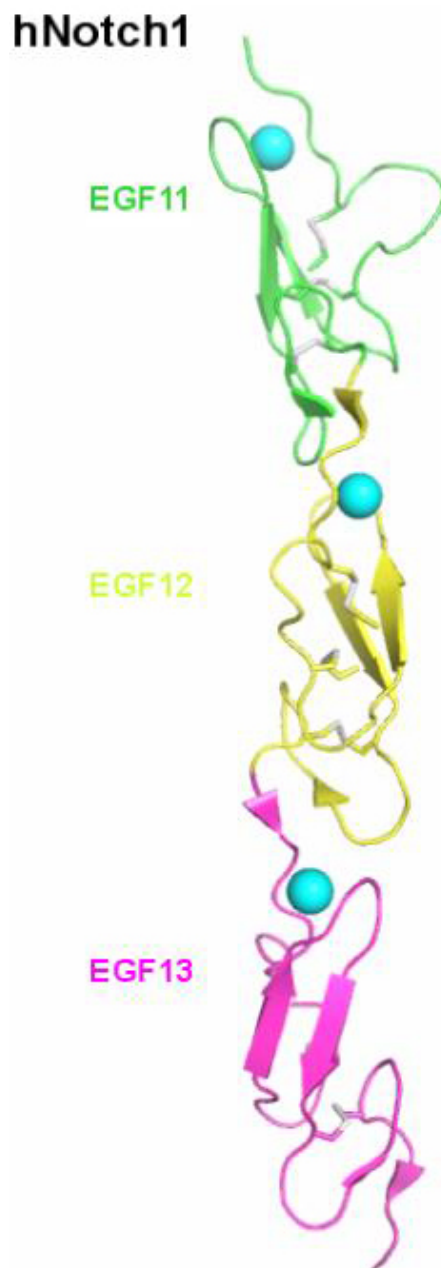

Figure 3. EGF repeats structure from human Notch1. Ribbon diagram of EGF repeats 11-13 from human Notch1 (PDB ID: 2VJ3). Repeats 11, 12, and 13 are colored green, yellow, and magenta, respectively. Disulfide bonds are represented as grey sticks and bound calcium ions are cyan spheres.
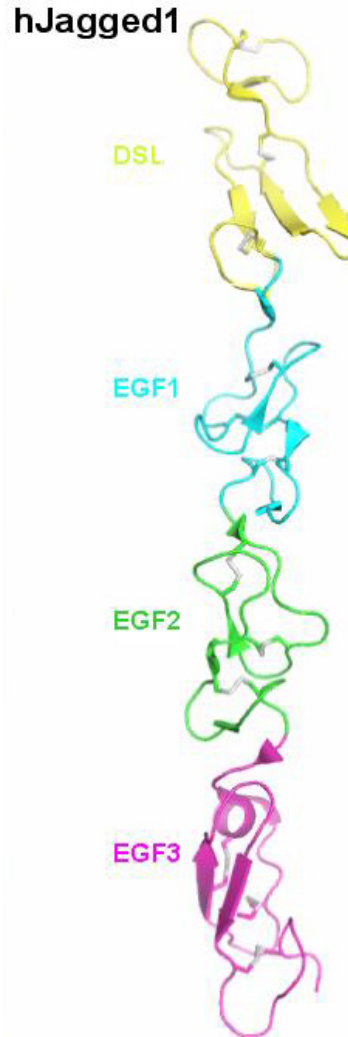
Figure 4. Structure of the DSL domain and EGF repeats from the human ligand Jagged1. Ribbon diagram of DSL domain and EGF repeats 1-3 from human Jagged1 (PDB ID: 2VJ2). The DSL domain is colored yellow, and EGF repeats one, two, and three are colored cyan, green, and magenta, respectively. Disulfide bonds are shown as grey sticks.

The EGF repeats of Notch are followed by the NRR (negative regulatory region). The NRR functions to keep Notch in an off state until it interacts with a DSL ligand. Interestingly, constitutively activating mutations in the NRR were first identified in *lin-12* and *glp-1*, and then later leukemia-causing mutations were found to cluster at the NRR of human Notch1; these mutations destabilize the structure of the NRR, causing the Notch receptor to be constitutively active (Greenwald and Seydoux, 1990; Berry et al., 1997; Pepper et al., 2003; Weng et al., 2004). Blacklow and coworkers determined the structure of the NRR in its auto-inhibited form (Figure 5) (Gordon et al., 2007; Gordon et al., 2009). It is composed of three LIN-12/Notch Repeat (LNR) domains and a heterodimerization domain (HD). Each LNR domain is disulphide bonded and binds calcium. Collectively, the tertiary structure is such that the three LNR domains protect the S2 site contained within the HD from cleavage. The structure of the NRR suggests that some sort of conformational change or unfolding event must occur to expose the S2 site for cleavage by ADAM; however, the molecular details of this process are unclear. One current model in the field is that endocytosis of the ligand-receptor complex in the signaling cell creates a mechanical force that exposes the NRR, or that an allosteric change occurs in the NRR upon formation of the DSL-Notch complex (reviewed in Kopan and Ilagan, 2009).
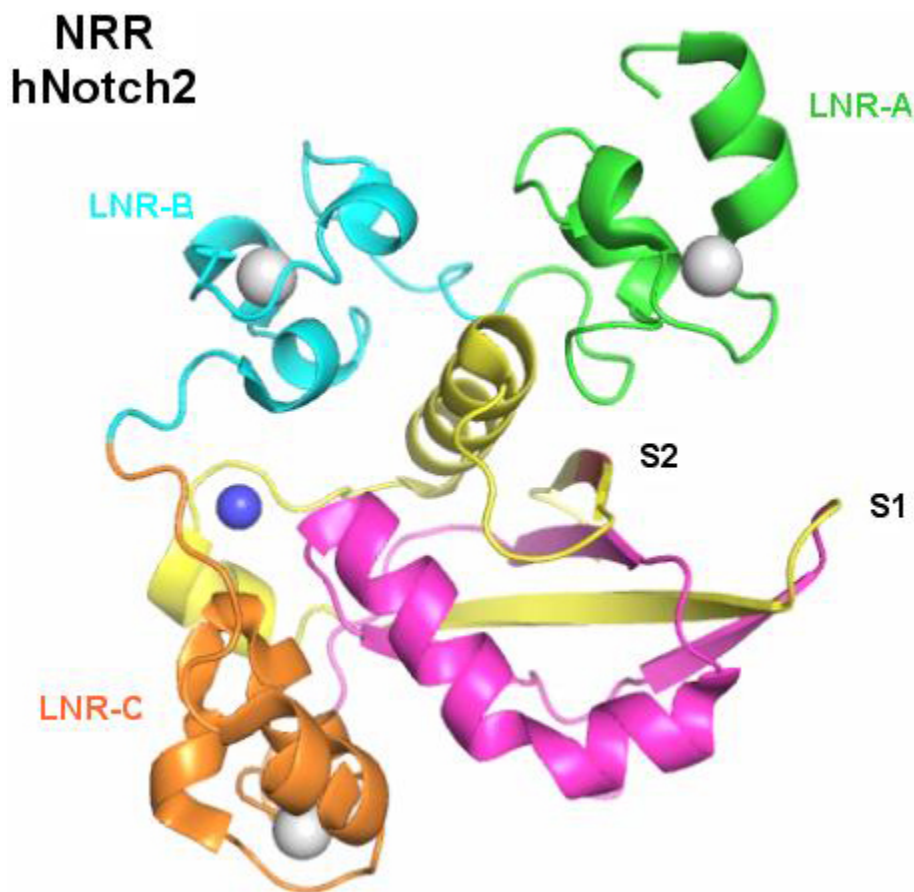
Figure 5. NRR structure from human Notch2. Ribbon diagram of the NRR from human Notch2 (PDB ID: 2OO4). LNR-A is colored green, LNR-B is cyan, and LNR-C is orange. Calcium and Zinc ions are represented as grey and blue spheres, respectively. The HD domain is colored magenta for the region N-terminal to the S1 cleavage site and yellow for the region C-terminal to the S1 cleavage site. The S2 cleavage site is colored red.

4.2. Structures of Notch transcription complexes

Perhaps the most extensive progress to date has been made in solving the structures of the proteins that are involved in regulating transcription from Notch target genes. These include structures of the DNA binding protein CSL, the RAM and ankyrin domains (ANK) of the receptor Notch, and the transcriptional coactivator Mastermind (MAM) (Kovall and Blacklow, 2010). Furthermore, several of these structures have been determined for components from *C. elegans*, *Drosophila*, and mammals, which illustrate the high degree of structural conservation between organisms. The first structure of CSL bound to a cognate DNA was determined using the *C. elegans* ortholog LAG-1 (Figure 6) (Kovall and Hendrickson, 2004). The structure revealed that all CSL proteins have a highly conserved core that is composed of three domains: an N-terminal domain (NTD), a β-trefoil domain (BTD), and a C-terminal domain (CTD). The NTD and CTD are Rel homology domains that are structurally similar to other DNA binding transcription factors, such as NF-kappaB and NFAT. The BTD derives its name from the pseudo three-fold arrangement of β-strands that compose this domain. The NTD and BTD of CSL form a continuous electropositive surface that makes specific contacts in the major and minor grooves, respectively, to recognize DNA (Kovall and Hendrickson, 2004). As discussed below in the context of CSL-NICD-MAM ternary complex structures, the BTD and CTD, as well as the NTD, make protein-protein interactions with transcriptional coregulators.
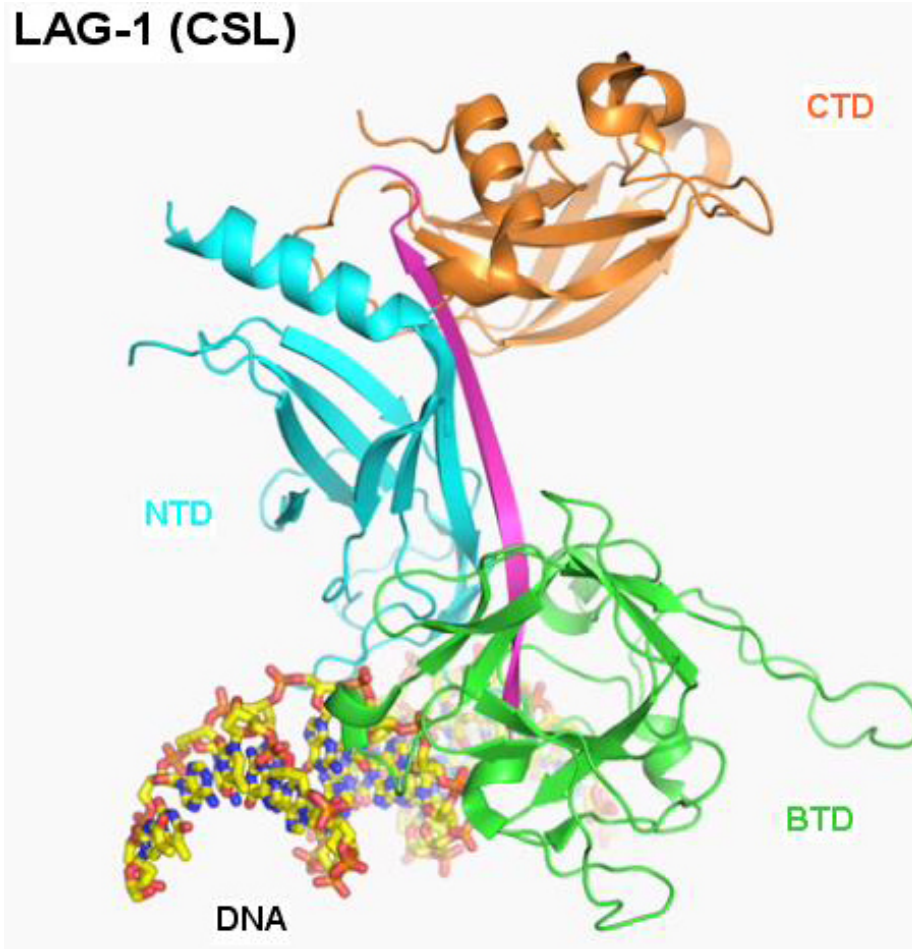
Figure 6. Structure of LAG-1 (CSL) bound to DNA. Ribbon diagram of CSL from *C. elegans* (PDB ID: 1TTU). The NTD, BTD, and CTD are colored cyan, green, and orange, respectively. A β-strand that bridges interactions with all three domains is colored magenta. The DNA is shown in a stick representation with carbon, oxygen, nitrogen, and phosphorous atoms colored yellow, red, blue, and orange, respectively.

Following the structure of LAG-1 bound to DNA, the structure of the transcriptionally active CSL-NICD-MAM ternary complex bound to DNA was determined for Notch components from both *C. elegans* (LAG-1/LIN-12/SEL-8) and humans (RBP-J/NICD1/MAML1) (Nam et al., 2006; Wilson and Kovall, 2006) (Figures 7, 8). Remarkably, despite the evolutionary sequence divergence between nematodes and mammals, the overall architecture of these two complexes is well conserved. The structures show that the RAM domain of NICD, which is intrinsically disordered prior to complexation (Nam et al., 2003; Bertagna et al., 2008), binds the BTD of CSL. The ANK domain of NICD, which primarily binds the CTD of CSL, consists of seven iterative ankyrin repeats, as well as a poorly conserved N-terminal capping repeat that was observed in the *C. elegans* complex structure. MAM adopts a strikingly bent helical conformation, which binds to a continuous groove formed by the CTD-ANK interface and the NTD of CSL. It should also be mentioned that there were several structural differences observed between the *C. elegans* and human CSL-NICD-MAM ternary complexes (Kovall, 2007). Most notable was the compact arrangement of domains observed in CSL for the *C. elegans* complex structure, but not the human structure. The functional significance of this observation has yet to be determined.
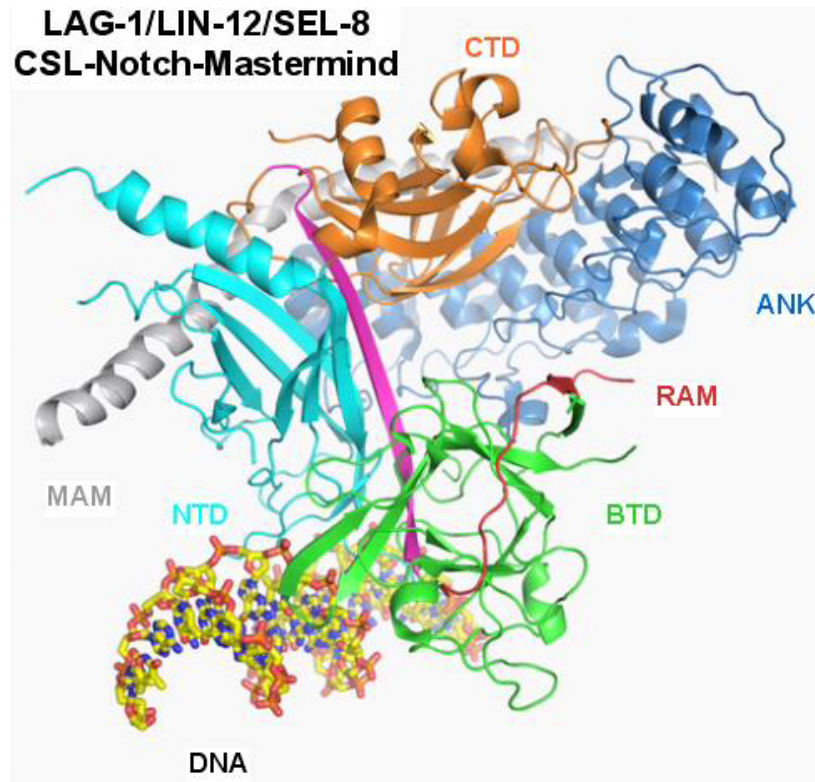
Figure 7. Structure of the *C. elegans* LAG-1/LIN-12/SEL-8 ternary complex structure bound to DNA. Ribbon diagram of the CSL-NICD-MAM ternary complex from *C. elegans* (PDB ID: 2FO1). CSL (LAG-1) and DNA are colored as in Figure 6. The RAM and ANK domains of NICD (LIN-12) are colored red and blue, respectively. MAM (SEL-8) is colored grey.
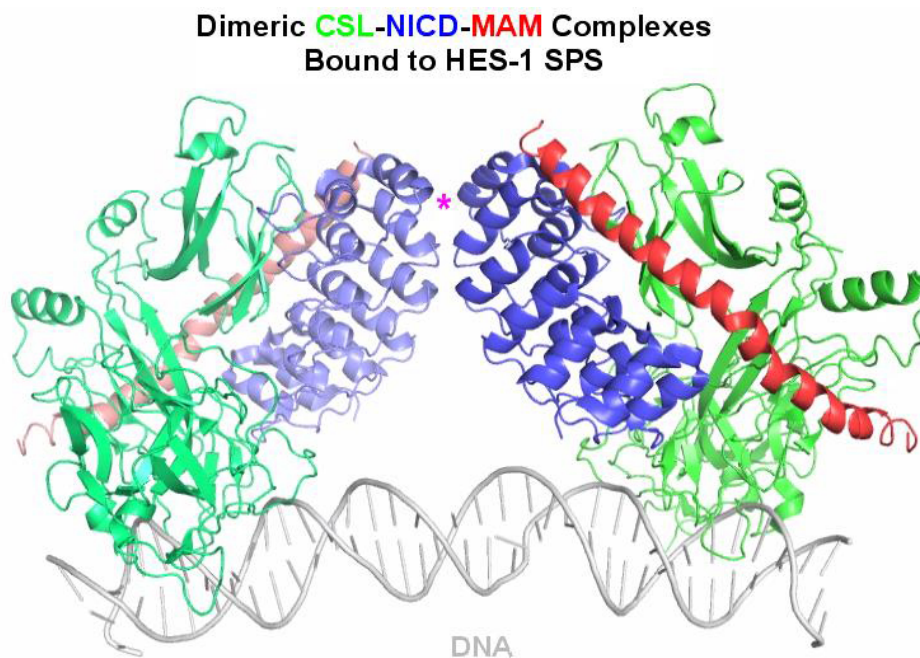

Figure 8. Structure of dimeric human CSL-NICD-MAM ternary complexes bound to HES-1 DNA. Figure shows two CSL-NICD-MAM ternary complexes bound to the HES-1 SPS for Notch components from humans. CSL, NICD, and MAM are colored green, blue, and red, respectively. The DNA is colored grey. A magenta asterisk denotes the site of cooperative interactions between the ANK domains of NICD.

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A number of additional structures have been determined for the individual components, as well as complexes that represent assembly intermediates. These include Notch ANK structures from *Drosophila* and mammals (Zweifel et al., 2003; Nam et al., 2006), CSL-RAM-DNA complex structures from *C. elegans* (Friedmann et al., 2008), and a structure of murine CSL bound to DNA (Friedmann et al., 2008). In parallel, several biochemical and biophysical studies were published that provided a detailed mechanism for how the transcriptionally active CSL-NICD-MAM ternary complex assembles. The RAM domain of NICD forms a high affinity interaction with the BTD of CSL. This targets NICD to CSL and has three important functional ramifications: (1) RAM has been shown to be important for displacing transcriptional corepressors from CSL; (2) the tethering of NICD to CSL via RAM greatly increases the local concentration of ANK (Bertagna et al., 2008), which is necessary to facilitate the very weak CTD-ANK interaction (Del Bianco et al., 2008; Friedmann et al., 2008); and (3) it has been proposed that RAM induces a conformational change in CSL that facilitates assembly of the ternary complex. It should be mentioned, however, that the degree of allosteric coupling varied between Notch components from different organisms. Following the complexation of CSL-NICD, Mastermind binds the complex (Nam et al., 2003). Similar to the ANK domain of NICD, MAM also has very weak intrinsic affinity for either CSL or Notch, though when all three factors are present, a stable high affinity ternary complex is formed (Nam et al., 2003).

Another point worth noting is that originally it was thought that CSL had high affinity for DNA (~1nM K_d) and therefore was constitutively bound at target genes (Hamaguchi et al., 1989); thus, exchange of coregulators would take place on DNA-bound CSL. However, it was later determined that CSL only has moderate affinity for DNA (~100nM K_d), which is at least 10-fold weaker than the affinity of CSL for the RAM domain of NICD (Friedmann and Kovall, 2010). Interestingly, for both *C. elegans* and mammalian components, the ternary complex of CSL with NICD and MAM neither changes the specificity nor the affinity of CSL for DNA (Del Bianco et al., 2008; Friedmann et al., 2008). Furthermore, to date, none of the various corepressors that interact with CSL affect its affinity for DNA (Maier et al., 2011; VanderWielen et al., 2011). (Note: DIN-1 is the *C. elegans* ortholog of the mammalian corepressor MINT, but the *Drosophila* corepressor Hairless is not found in *C. elegans*). Taken together, these observations suggest that there may be some CSL-coregulator complexes in the nucleus that are bound to DNA and others that are free in the nucleoplasm. However, as all of these aforementioned binding studies were done with purified proteins produced in bacteria, the possibility that post-translational modifications affect the DNA binding properties of CSL cannot be excluded at this time.

5. Target genes

LAG-1 appears to have the same binding site preference as its *Drosophila* and mammalian orthologs: LAG-1 protein binds to ATGGGAA *in vitro*, a sequence that is required *in vivo* for LIN-12 autoregulation (Wilkinson et al., 1994), and binding is specifically competed by the oligonucleotides containing the consensus RTGGGAA sequence (Christensen et al., 1996). Both YRTGRGAA and YGTGDGAA binding motifs have been identified in validated target genes in *C. elegans*.

In *C. elegans*, targets of the activation complex have been identified through forward computational screens based on the presence of binding site consensus sequences. Targets have also been recognized retrospectively by scanning genes with expression patterns or functional characteristics suggesting a role in Notch-mediated processes for the presence of potential binding sites. Showing that mutation of the potential binding sites abolishes expression can be taken as strong evidence that the gene is a direct target for the activation complex *in vivo*, as cell fate transformations in mutant backgrounds may confound the interpretation of a change in expression of a potential target. Table 2 summarizes target genes identified to date.

Table 2. Validated targets of LIN-12 or GLP-1

Target gene	Context	Product	References
lin-12	VU*	LIN-12/Notch	Wilkinson et al. 1994
mir-61/250	VU*	microRNA	Yoo and Greenwald, 2005
lip-1	VPC**, germline	ERK phosphatase	Berset et al., 2001; Lamont et al., 2004; Lee et al., 2006
lst-1	VPC**, germline	novel nematode-specific	Yoo et al., 2004; Singh et al., 2011
lst-2	VPC**	FYVE finger protein	Yoo et al., 2004
lst-3	VPC**	SAP domain/DNA binding?	Yoo et al., 2004

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Target gene	Context	Product	References
lst-4	VPC**	SNX9 sorting nexin	Yoo et al., 2004
fbf-1	germline	RNA binding protein	Lamont et al., 2004; Lee et al., 2006
pkg-1 (egl-4)	neurons	cGMP-dependent protein kinase	Singh et al. 2004

*VU = Ventral Uterine precursor cell of the somatic gonad

**VPC = Vulval Precursor Cell (specifically, P5.p and P7.p).

A list of other potential targets, predicted computationally using the same crtieria that led to the validated targets *lst-1-4*, is given in the Supplemental Material of Yoo et al., 2004.

Interestingly, none of the identified *C. elegans* target genes contain paired CSL binding sites. Originally identified within the *Enhancer of split locus* in *Drosophila* and termed SPS (SuH paired site), an SPS consists of two CSL binding sites in a head-to-head arrangement separated by approximately 16 base pairs (Cave et al., 2005). In mammals, the well-established Notch target gene *hes-1* contains a prototypical SPS (Jarriault et al., 1995). The SPS binds two CSL-NICD-MAM ternary complexes in a cooperative manner, which lowers the amount of NICD necessary to activate a target gene (Nam et al., 2007). Blacklow and colleagues determined the structure of dimeric human Notch transcription complexes bound at the *hes-1* SPS promoter element (Arnett et al., 2010). The structure showed that the cooperativity was mediated by interactions between the ANK domains of NICD, which induced a modest bending in the DNA. The ANK residues required for cooperativity are not conserved in the *C. elegans* orthologs LIN-12 and GLP-1 (Nam et al., 2007), which raises the question as to whether the SPS motif is utilized in nematodes to regulate transcription from Notch target genes.

6. Modulators of *lin-12* and *glp-1* activity

In *C. elegans*, genetic suppressor and enhancer screens have yielded candidate modulators as well as core components. Modulators are not essential for Notch signaling but can influence Notch activity. They may function generally or only in specific cellular contexts. They may act "directly", through modifying or otherwise physically interacting with Notch, leading to altered Notch level, activity or stability. Or they may act "indirectly", by affecting cell physiology in ways that synergize with or antagonize Notch activity. The kind of modifiers identified seems heavily influence by the particular starting allele and the cellular context that form the basis of a given screen.

Here, we summarize some of the suppressor and enhancer screens that have been performed, starting with mutations that elevate or reduce *Notch* activity. Such screens have been done on large scales starting with specific alleles of the *Notch* genes *lin-12* or *glp-1*, and with loss-of-function mutations in two components of γ-secretase, *sel-12* and *aph-1*. The simple predictions for recovery of loss-of-function mutations in modulators in these starting conditions are given below, as well as a cross-reference to the section below in which such screens are detailed as in the table:

starting condition	loss of a positive modulator	loss of a negative modulator
elevated *Notch*	suppress (section 6.1 below)	enhance (section 6.3)
reduced *Notch*	enhance (section 6.2)	suppress (section 6.4)

Classifying a gene as a "modulator" rather than a core component is somewhat provisional. If a null allele of the gene results in a Notch phenotype, a good case can be made that it is a core component; however, maternal rescue (e.g., as for *aph-1* and *aph-2*) or functional redundancy (e.g., as for *sel-12* and *hop-1*) may obscure an essential role. Thus, a gene may shift from being classified as a "modulator" to a "core component" with further study. In addition, some modulators may be more general, i.e., function in multiple cellular contexts, whereas others may be more restricted. Some modulators may show allele-specificity within the same cellular context, which may be indicative of a role in an aspect of cell physiology or protein homeostasis rather than in Notch signaling *per se*. Thus, the category of "modulator" is quite heterogeneous and does not have distinct boundaries.

Genes provisionally classified as modulators through such genetic interactions are listed in conjunction with a brief description of the main screens used to identify them. Selected genes identified in candidate gene assays are also shown. Some modulators have been characterized in numerous assays; not all assays are shown for a given modulator, so for more details about individual genes please use the hyperlinks to Wormbase.

We note that we have not listed all of the genetic interactions associated with a given modulator. Furthermore, we note that even if a modulator is named after the screen in which it was identified, its function is not necessarily restricted to a particular context. For example, many genes described 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; furthermore, many *sel* genes also interact genetically with *glp-1*.

6.1. Positive modulators: suppressors of elevated *Notch* activity

Suppressors of *lin-12(d)*. The excess activity of *lin-12(d)* mutants prevents the specification of the anchor cell of the gonad, resulting in the absence of vulval induction and hence an egg-laying defect (see WormBook chapter Vulval development). Suppression of this defect has yielded null or hypomorphic mutations in many core components and positive modulators (Tax and Thomas, 1994; Katic et al., 2005) (Table 3) and neomorphic alleles of *lag-2* (Tax and Thomas, 1994; Tax et al., 1997). In addition to lacking a functional vulva, "strong" *lin-12(d)* alleles also have a "Multivulva" phenotype because all six Vulval Precursor Cells (VPCs), instead of just two, adopt the particular fate resulting from LIN-12 activation (see Vulval development); suppression of this defect yielded *sel-12* (Levitan and Greenwald, 1995).

Table 3. Suppressors of *lin-12(d)*

Gene	Product	*Drosophila*	Mammals	Role/effect	Worm refs
*lag-2 (sel-3)**	DSL protein	DSL	DSL	Ligand	(Tax and Thomas, 1994; Tax et al., 1997)
sel-5	Kinase	NAK	AAK1	modulate Notch endocytosis**	(Fares and Greenwald, 1999)
*emb-4 (sel-6)***	nuclear ATPase	AAQ22465	Aquarius	unknown	(Katic and Greenwald, 2006)
sel-7	Novel, nuclear	n/a	n/a	unknown	(Chen et al., 2004)
*sel-8 (lag-3)****	Glutamine-rich	Mastermind	Mastermind	Nuclear complex	(Doyle et al., 2000)
sel-12	Presenilin	Presenilin	Presenilin	g-secretase	(Levitan and Greenwald, 1995)
bre-5	galactosyl-transferase	Brainiac	B3gnt4-001	protein and lipid modification	(Katic et al., 2005)
*lag-1****	DNA binding protein	Su(H)	CBF1	binding to target gene regulatory sequences	(Katic et al., 2005)

Alternate, unofficial names are listed in parentheses.

bold, core component

*Suppressor alleles are neomorphic.

**see (Gupta-Rossi et al., 2011)

***Suppressor alleles are partial loss-of-function. Unless otherwise designated, others are null alleles, although we note that *sel-12* null mutants are viable because of functional redundancy with *hop-1*.

Suppressors of *glp-1* gain-of-function. *glp-1(ar202)* results in elevated *glp-1* activity but remains significantly sensitive to ligand (Pepper et al., 2003). At 25°C, *glp-1(ar202)* mutants have ectopic proximal proliferation that results in sterility and a late-onset tumorous phenotype. This allele has both a complicated phenotype and complicated genetic behavior, which have consequences for the types of modifiers that interact with it, as discussed by Hubbard (2011).

Loss of either of two tetraspanin-encoding genes, *tsp-12* and *tsp-14*, suppress *glp-1(ar202)* sterility; further characterization in *C. elegans* and human cell culture suggested that a subset of Tetraspanins modulate γ-secretase activity (Dunn et al., 2010). Null alleles of *rsks-1*, which encodes the sole worm homolog of mammalian p70S6K, also suppress *glp-1(ar202)* (Korta et al., 2012). Suppression of *glp-1(ar202)* has also been used to evaluate *C. elegans* orthologs of mammalian proteins found in physical association with γ-secretase; (M. Vargas and I. Greenwald, unpublished observations).

6.2. Positive modulators: enhancers of reduced *Notch* activity

Enhancers of a *glp-1* hypomorph. Five *ego* genes, for *e*nhancers of *glp-one*, were identified by enhancing the partial germline proliferation defect of a weak *glp-1* loss-of-function mutation (Qiao et al., 1995; Smardon et al., 2000). Three of these genes have been cloned, and additional candidate genes that display similar genetic interactions have also been identified (Table 4).

Table 4. Enhancers of a *glp-1* hypomorph

Gene	Product	Mammalian	Role/effect	Worm refs
ego-1	RNA-directed RNA polymerase	n/a	many aspects of germline development	(Smardon et al., 2000)
ego-2	Bro1-domain		protein trafficking	(Liu and Maine, 2007)
atx-2 (ego-4)	Ataxin 2-like	Ataxin-2	translational regulation	(Ciosk et al., 2004; Maine et al., 2004)
lag-1	CSL	CBF1	core component	(Qiao et al., 1995; Katic et al., 2005)
Candidate assay				
epn-1	Epsin	Epsin	ligand endocytosis	(Tian et al., 2004)
csr-1	nematode-specific Argonaut	n/a	many	(She et al., 2009)
ekl-1	Tudor domain	n/a	many	(She et al., 2009)
drh-3	DEAH/D box helicase		many	(She et al., 2009)
cye-1	Cyclin	IF1H1	cell cycle regulation	(Fox et al., 2011)

Alternate, unofficial names are listed in parentheses.

bold, core component. Note that assigning *epn-1* as a core component is provisional, as discussed in Section 3.

It is notable that EGO-2 is one of two Bro1-domain proteins in *C. elegans*; the other, ALX-1, was isolated in a yeast two-hybrid screen with the intracellular domain of LIN-12 and was found to be required for LIN-12 trafficking and degradation in the VPCs (Shaye and Greenwald, 2005) (see Section 6.3). However, *ego-2* and *alx-1* appear to have distinct roles: *ego-2,* like *epn-1*, appears to function mainly in the signaling cell (Tian et al., 2004; Liu and Maine, 2007), and *ego-2* and *alx-1* can either synergize or antagonize depending on context (Liu and Maine, 2007). The cellular focus of *ego-2* and *epn-1* and their interactions with *glp-1* suggest that endocytosis of DSL proteins influences their activity in *C. elegans*, as in other organisms, despite unusual aspects of the structure.

Enhancers of reduced γ-secretase activity. Different synthetic phenotypes are obtained when null alleles of *sel-12* and *hop-1* are combined. The *hop-1; sel-12* double mutants segregating from *hop-1* heterozygous mothers display a Glp-1 sterile phenotype (Westlund et al., 1999). Screens for other genes that cause Glp-1 sterility in combination with *sel-12* identified the novel gene *pen-2* ("*Presenilin ENhancer*") as well as the *aph-1* and *aph-2* genes that had been identified in a phenotype-based screen (Francis et al., 2002) (Table 1).

6.3. Negative modulators: enhancers of increased *Notch* activity

Enhancers of *lin-12(d)*. *sel-2* was found serendipitously in a screen for suppressors of the egg-laying defect of a weak *lin-12(d)* allele, but instead proved to be a strong enhancer in the VPCs: the activity of the weak allele is enhanced to result in the Multivulva phenotype associated with strong alleles (de Souza et al., 2007). SEL-2 is orthologous to mammalian Neurobeachin/LRBA. Loss of *sel-2* activity affects the trafficking of LIN-12 in the VPCs and appears to affect trafficking in other polarized epithelial cells as well (de Souza et al., 2007).

Two other genes, *alx-1* (see also Section 6.2) and *wwp-1*, which encodes the WWP-1/Su(dx)/Itch ubiquitin ligase, were found in a yeast two hybrid screen and found to facilitate LIN-12 trafficking and degradation in the VPCs (Shaye and Greenwald, 2005). Although these two genes were not assessed for their effects on *lin-12(d)* activity, aberrant trafficking of LIN-12 can lead to gain-of-function phenotypes (Shaye and Greenwald, 2005), a phenomenon also observed in *Drosophila* (e.g., Wilkin et al., 2004).

sel-10, identified as a suppressor of reduced *lin-12* activity (see 6.4), also enhances weak *lin-12(d)* alleles to cause a Multivulva phenotype (Sundaram and Greenwald, 1993b; Hubbard et al., 1997). Ongoing screens have identified many additional genes that can enhance *lin-12(d)* activity (Y. Deng and I. Greenwald, unpublished observations).

Enhancers of *glp-1* gain-of-function. Activation of *glp-1* in the germ line promotes mitotic proliferation and a "tumorous" phenotype (Berry et al., 1997). A screen for enhancers of a weak tumorous phenotype caused by mild *glp-1* gain-of-function identified four tumorous enhancers of *glp-1* (*teg* genes), potential negative regulators of *glp-1* activity in the germ line, three of which have been cloned (Table 5).

Table 5. Enhancers of *glp-1* gain-of-function

Gene	Product	Mammalian	Role/effect	Worm refs
teg-1	splicing factor	CD2BP2	pre-mRNA splicing	(Wang et al., 2012)
teg-4	splicing factor	SAP130	downstream of *glp-1*	(Mantina et al., 2009)
prp-17*	splicing factor	PRP17	downstream of *glp-1*	(Kerins et al., 2010)
puf-8 (teg-2)	Pumilio-related	Pumilio homolog 2	translational control	(Racher and Hansen, 2012)
pas-5	proteasome subunit	PSMA5	protein stability	(Macdonald et al., 2008)
mett-10**	methyltransferase	METTL16	nuclear protein	(Dorsett et al., 2009)
him-17	THAP domains	N/A	localizes to chromatin	Bessler et al., 2007
Candidate assay				
dlc-1	dynein light chain	DYNLL2	dynein motor complex	(Dorsett and Schedl, 2009)
dhc-1	dynein heavy chain	DNAH9	dynein motor complex	(Dorsett and Schedl, 2009)

Alternate, unofficial names are listed in parentheses.

*see (Kerins et al., 2010) for a long list of many different splicing factor orthologs that show similar behavior.

**mett-10 was originally identified by a rare Tumorous allele; loss of function mutations enhance a weak tumorous allele of *glp-1*. DLC-1 and DHC-2 interact with METT-10 in a yeast two-hybrid assay (discussed in Dorsett and Schedl, 2009).

6.4. Negative modulators: suppressors of reduced *Notch* activity

Suppressors of mutations that reduce γ-secretase activity. Two different screens have been done in this category. One was based on the egg-laying defect of *sel-12* null mutants, which cannot lay eggs because of a *lin-12*-mediated gonadal defect (Cinar et al., 2001), but are viable because they retain *hop-1*/Presenilin activity. Suppressors of the egg-laying defect of *sel-12* mutants identified null or hypomorphic alleles of five *spr* genes (*suppressors of presenilin) (Wen et al., 2000). One gene, *spr-2*, encodes the ortholog of the likely chromatin factor SET (Wen et al., 2000). Four genes appear to act together: *spr-1*/CoREST and *spr-5*/LSD1, members of the mammalian CoREST co-repressor complex, and *spr-3* and *spr-4*, which encode two zinc-finger proteins that may function in a complex with CoREST (Eimer et al., 2002; Jarriault and Greenwald, 2002; Lakowski et al., 2003). Loss of CoREST activity has been proposed to result in increased expression of *hop-1* (Eimer et al., 2002; Lakowski et al., 2003) or an altered balance of repression and activation of *lin-12* target genes (Jarriault and Greenwald, 2002). Interestingly, in murine T-ALL cells, there may be some association between the binding sites for CSL and REST, a transcription factor that recruits CoREST to repress target genes (Wang et al., 2011).

Another screen in this category was based on suppression of the maternal effect lethal phenotype of a hypomorphic allele of the *aph-1* component of γ-secretase (Hale et al., 2012). This screen identified *sao-1*, which physically interacts with SEL-10. SEL-10 is an E3 ubiquitin ligase that directly affects the stability of LIN-12 and GLP-1 (Hubbard et al., 1997; Welcker and Clurman, 2008) and has also been implicated in SEL-12/Presenilin stability (Wu et al., 1998). *sao-1* displays numerous additional genetic interactions with other components of the Notch signaling system, all consistent with the proposal that SAO-1 may act with SEL-10 in downregulating one or more components that influence *Notch* activity (Hale et al., 2012).

Suppressors of *lin-12* hypomorphic alleles. Partial loss-of-function (hypomorphic) mutants of *lin-12* have a functional vulva but are egg-laying defective (Sundaram and Greenwald, 1993a). Suppression of the egg-laying defect has been used as the basis of a screen for suppressors (Sundaram and Greenwald, 1993b) and to characterize other interactors (Table 6).

Table 6. Suppressors of *lin-12* hypomorphs

Gene	Product	Mammalian	Role/effect	Worm refs
sel-1	Extracellular, GPI-linked	HRD3, SEL1L	Unfolded Protein Response	(Grant and Greenwald, 1996, 1997)
sel-9	p24 ER protein	p24	ER quality control	(Wen and Greenwald, 1999)
sel-10	E3 ubiquitin ligase	Fbw7	Directs turnover of Notch(intra)	(Hubbard et al., 1997)
sel-11 (hrd-1)	E3 ubiquitin ligase	HRD1	Unfolded Protein Response	(Choi et al., 2010)

Alternate, unofficial names are listed in parentheses.

*For null alleles of *sel-10* to suppress the egg-laying defect of *lin-12(n676n930)*, an as-yet-uncloned second mutation, *arX*, must be present (Sundaram and Greenwald, 1993b).

sel-1, *sel-9*, and *sel-11* exert their effects through physiological processes. In contrast, SEL-10 directly binds to the LIN-12 intracellular domain through a "Cdc4 phosphodegron" sequence and regulates its stability (Hubbard et al., 1997; de la Cova and Greenwald, 2012). This role is conserved in the mammalian ortholog, Fbw7, which also acts as a tumor suppressor in many contexts (Welcker and Clurman, 2008). In particular, Fbw7 function as a tumor suppressor appears to be mediated through its effect on Notch in T-ALL (Thompson et al., 2007).

Suppressors of *glp-1* hypomorphic alleles. 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. Additional suppressors of *glp-1*sterility proved to be in genes

required for body morphology, which may indicate that the extracellular matrix influences *glp-1* activity in the germ line (Maine and Kimble, 1989).

7. Concluding remarks

Studies of *lin-12* and *glp-1* in *C. elegans* have continued to yield new insights into the *Notch* signaling system and its regulation. Between now and the next update, we expect to see further progress towards a structural understanding of *Notch* signaling; potentially, predictions from structural studies could be tested *in vivo* in *C. elegans*. We also expect new modulators to be defined through genetic screens, and that the human orthologs of some of these modulators will be connected to human disease processes.

8. Acknowledgements

9. References

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