SEL-8, a nuclear protein required for LIN-12 and GLP-1 signaling in *Caenorhabditis elegans*

Timothy G. Doyle*, Chenhui Wen†, and Iva Greenwald*†‡§

*Integrated Program of Cellular, Molecular, and Biophysical Studies, †Howard Hughes Medical Institute, and ‡Department of Biochemistry and Molecular Biophysics, Columbia University College of Physicians and Surgeons, New York, NY 10032

Communicated by Wayne A. Hendrickson, Columbia University, New York, NY, May 12, 2000 (received for review April 17, 2000)

LIN-12 and GLP-1 are members of the LIN-12/Notch family of receptors that mediate cell–cell interactions during development. The *sel-8* gene had been identified previously in a screen for suppressors of a mutation that constitutively activates LIN-12. Here, we report that *sel-8* is essential for *lin-12*- and *glp-1*-mediated signaling, and that SEL-8 is a glutamine-rich nuclear protein. We postulate that SEL-8 serves as a transcriptional coactivator or as an assembly factor for transcription complexes that contain the LIN-12 or GLP-1 intracellular domains.

Notch | transcription | coactivator

Cell–cell interactions specify distinct fates for cells that have equivalent developmental potential. These interactions ensure that the correct numbers and types of cells are generated. Many cell–cell interactions during development are mediated by receptors of the LIN-12/Notch family, which are activated by transmembrane protein ligands of the Delta/Serrate/LAG-2 (DSL) family (1). Ligand binding appears to trigger a proteolytic processing event in the extracellular domain, near the membrane (2, 3), causing "shedding" of the ectodomain. Ectodomain shedding leads to cleavage in or near the LIN-12/Notch transmembrane domain, releasing the intracellular domain for signal transduction (4, 5).

The released intracellular domain [LIN-12(intra)/Notch(intra)] is found in the nucleus, where, in a complex with the transcription factor of the CBF1/Suppressor of Hairless [Su(H)] family, it directly participates in transcriptional activation of target genes. CBF1 (originally called RBP-Jκ) was isolated based on its sequence-specific DNA binding properties (6). A connection with LIN-12/Notch signaling was first made when *Drosophila Su(H)*, a gene implicated in Notch signaling based on its mutant phenotype, was shown to encode a CBF1 homolog (7). Subsequently, the *Caenorhabditis elegans lag-1* gene was shown to encode the Su(H)/CBF1 ortholog (8). CBF1, Su(H), and LAG-1 all have been shown to bind to the same DNA sequence *in vitro* and to interact physically with the intracellular domains of LIN-12/Notch proteins (8–10). In addition, CBF1 and Su(H) have been shown to function as repressors in the absence of coactivators such as the intracellular domain of Notch (11–13); it is very likely that LAG-1 does as well.

Genetic screens in *C. elegans* and *Drosophila* have been effective in identifying conserved components of the LIN-12/Notch pathway and factors that influence the activity of LIN-12/Notch proteins. In *C. elegans*, genetic screens, based on *s*uppression or *e*nhancement of *lin-12* mutations, have defined many new "*sel*" genes. Screens based on the suppression of missense mutations that cause constitutive LIN-12 activity [*lin-12(d)* mutations] (14, 15) have led to the recovery of partial or complete loss-of-function mutations in several *sel* genes subsequently found to be involved in signal transduction.

Two genes that appear to be involved in ligand-induced activation of LIN-12 were defined by alleles that suppress *lin-12(d)* mutations. One gene, *sup-17*, encodes a metalloprotease of the ADAM family, and is known as Kuzbanian in *Drosophila* and mammals (16). Although the role of SUP-17/Kuzbanian has been the subject of some controversy, a plausible role is in the proteolytic processing that leads to ectodomain shedding upon ligand-binding. Another gene, *sel-12* (14), encodes a "presenilin," so named because mutations in human presenilin cause Alzheimer's disease. SEL-12/presenilin is critical for the transmembrane proteolytic processing event of LIN-12/Notch signal transduction (17, 18).

Tax *et al.* (15) recovered a single allele of *sel-8* in a screen for suppressors of a *lin-12(d)* mutation. Here, we report a genetic and molecular characterization of *sel-8*. Our analysis demonstrates that *sel-8* is essential for the activity of the two *C. elegans* LIN-12/Notch genes, *lin-12* and *glp-1*, and that SEL-8 is a highly glutamine-rich nuclear protein. We propose models for how SEL-8 might function in nuclear events of LIN-12 and GLP-1 signaling.

Materials and Methods

Genetic Materials and Methods. Standard methods were used for handling, maintenance, and genetic analysis; the wild-type parent for all strains used in this study is *C. elegans* var. Bristol strain N2 (19). Experiments were conducted at 20°C unless otherwise indicated. Information about incidental markers used for mapping or facilitating genetic analysis can be obtained from the *Caenorhabditis Genetics Center* web site (http://biosci.umn.edu/CGC/CGChomepage.htm). Additional information about the relevant mutations used in this study can be found in the following references: *sel-8(sa54)* (15), *lin-12(n302)*, *lin-12(n952)*, and *lin-12(n950)* (20, 21) (all LG *III*). The *arIs50* [SEL-8::GFP (green fluorescent protein)] transgene is described below.

Molecular Methods. Standard molecular biology methods are described in (22). PCR-rapid amplification of cDNA ends (RACE) was performed according to the manufacturer's instructions (GIBCO/BRL), including cDNA synthesis from total RNA and poly(G) tailing. For 5′ RACE, cDNA synthesis was primed with the primer C2 (GCTGGTGTTGGGACTGAACGAATA), and then subsequently amplified with primer DT4 (GTGGTTGCTGCTGACCCATCCAA) and the anchor primer (GIBCO/BRL).

Transgenic Lines. DNA was injected into the germline of *C. elegans* hermaphrodites (23). pRF4 [*rol-6(su1006)*] (24) at 100 μg/ml or pMH86 [*dpy-20(+)*] (25) at 20 μg/ml [along with 80 μg/ml pBluescript (Stratagene)] were used as cotransformation markers, the latter used for recipient strains that contained the chromosomal marker *dpy-20(e1282)*.

Abbreviations: RNAi, RNA-mediated interference; dsRNA, double-stranded RNA; GFP, green fluorescent protein; RACE, rapid amplification of cDNA ends.

§To whom reprint requests should be addressed. E-mail: greenwald@cuccfa.ccc.columbia.edu.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

We wanted to test whether C32A3.1 sequences are able to reverse the suppression of the Egl defect of *sel-8(sa54) lin-12(n302)* hermaphrodites. As we were unable to obtain unrearranged sequences from available cosmids, we generated a PCR product corresponding to C32A3.1 by using a fusion approach described in ref. 26. Two independent PCRs were performed. One reaction, using primers S8Gen-J (TCTATGTATAAAACTTACGGCTCGTCTTCGG) and S8–4RB (GAAGGACACCTGGCTATCGTATCCATA), amplified a 5.2-kb fragment from N2 genomic DNA. The other reaction, using primers S8Gen-1B (CTACCACGACGGAACCATCAAACCGCTTGC) and S8-N (ATGAAACCGTCGACAAGCAAGACG), amplified a 7.8-kb fragment from N2 genomic DNA. Together, the two fragments spanned the five exons of the predicted ORF C32A3.1 as well as 6.3 kb of upstream promoter sequence and 2.0 kb of downstream sequence. The two fragments contained 115 bp of sequences that overlapped with each other. The fragments then were fused by combining 1 μl of each PCR and then amplifying them with a set of nested primers, S8Gen-4R (CAGAACTCTCCATCGGTCAGATTAACC) at the beginning of the promoter, and S8Gen-1 (GAACTCTCATGAATCACGGATCCCGCGA) at the end of the downstream sequence. The fusion of the PCR product was confirmed on an agarose gel and then injected at ≈10 μg/ml into *sel-8(sa54) lin-12(n302)* hermaphrodites with pRF4 as a cotransformation marker.

The pS8GFP plasmid encodes a SEL-8::GFP protein and contains all genomic sequences from C32A3.1 described above, with GFP inserted in-frame after codon 446; the resulting protein would lack the last 22 aa of SEL-8. Details of the construction are available on request. pS8GFP was injected at 50 μg/ml into *sel-8(sa54) lin-12(n302); dpy-20(e1282)* hermaphrodites with pMH86 [*dpy-20(+)*] (25) as a cotransformation marker. Five transgenic lines carrying extrachromosomal arrays were generated, and one was integrated by using standard methods (23). The integrated transgene *arIs50* was used primarily for analyzing the expression pattern.

RNA-Mediated Interference (RNAi). Double-stranded RNA (dsRNA) was synthesized *in vitro* from PCR products with T3 and T7 polymerase binding sites incorporated into the ends from the primers. For *sel-8*, the PCR product included the sequences from the entire second exon of *sel-8* (C32A3 base pairs 20,082–20,680). The primer sequences used, with the T3 and T7 sites italicized, were 32.1.DT3 (*AATTAACCCTCACTAAAGGG*GCCGAAAAATGCCAAAAAAAAAAGC) and 32.1.DT7 (*GTAATACGACTCACTATAGGGC*CTGTAGTTTTCGCTACGAGATATTTTGCGC). dsRNAs were purified on RNeasy columns (Qiagen, Chatsworth, CA), eluted in water, diluted in injection buffer (27), and allowed to anneal at 37°C for 30 min. dsRNA was microinjected into the pseudocoelomic space of young adult hermaphrodites. Injected worms were cultured individually. The phenotypes of arrested larvae and adult escapers were examined for phenotypes associated with reduction of *lin-12* and/or *glp-1* activity at different times after RNA injection using Nomarski microscopy.

To score embryonic arrest phenotypes, hermaphrodites were allowed to lay eggs overnight after injection of dsRNA, and the eggs laid during this period were discarded. The hermaphrodites were transferred to fresh plates and allowed to lay eggs for 1 h. The terminal phenotype of these eggs generally was scored after 6 h of development, as time-course experiments established that affected embryos appeared normal after 3 h, but arrested after 6 h, in comparison with wild-type embryos staged in parallel (data not shown).

There was some variability in the results, depending on the injected hermaphrodite; this kind of variability is inherent in RNAi experiments. Some hermaphrodites gave few affected offspring; these were discarded without further examination. Other injected hermaphrodites gave a mixture of arrested embryos and affected larvae. In some cases, arrested embryos constituted over 90% of the brood.

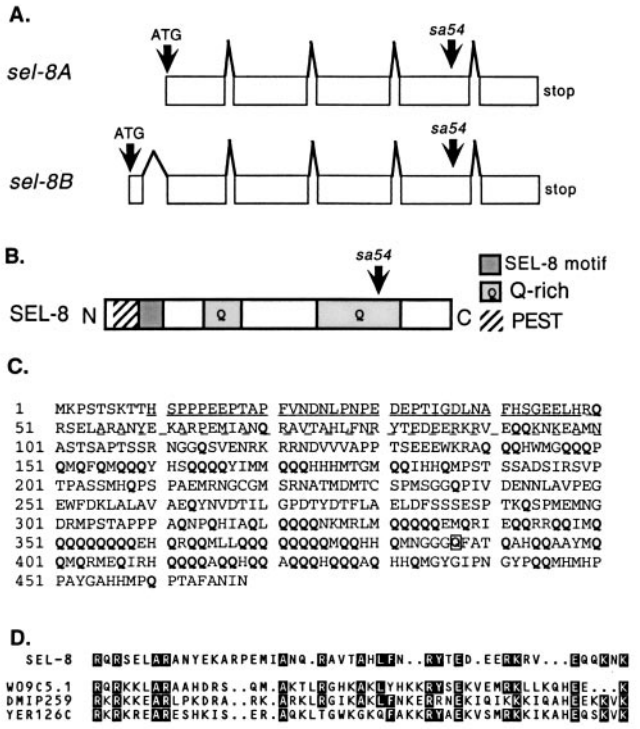

Fig. 1. Sequence analysis. (*A*) Schematic representation of transcripts from the *sel-8* gene. RACE analysis revealed two potential transcripts, *sel-8A* and *sel-8B*. The *sel-8(sa54)* C-to-T mutation is indicated (nucleotide 17,295 of cosmid C32A3, GenBank accession no. Z48241). Introns are not drawn to scale. (*B*) Schematic representation of the SEL-8 protein. The ''SEL-8 motif'' that is found in W09C5.1 and its orthologs, the PEST sequence, and the two glutamine-rich regions are indicated and described further in the text. (*C*) Amino acid sequence of SEL-8. The sequence based on the *sel-8A* RACE product is shown. The PEST sequence is underlined with a solid line, and the motif shared with W09C5.1 is underlined with a dashed line. Glutamine (Q) residues are indicated in bold type. The boxed glutamine corresponds to codon 387 of *sel-8A*, which is changed to ''stop'' in *sel-8(sa54)*. (*D*) Sequence motif found in SEL-8 and W09C5.1 (GenBank accession no. T26298) and its apparent yeast (YER126c, GenBank accession no. S43218) and *Drosophila* (DMIP259, GenBank accession no. AAF52940) orthologs. Reverse-contrast lettering indicates amino acids that are identical in SEL-8 and at least two of the other proteins.

Results

Molecular Cloning and Sequence Analysis of *sel-8*. *sel-8(sa54)* previously had been mapped close to the left of *unc-93* (15). We refined the map position of *sel-8* to the interval between the cloned genes *ben-1* and *unc-93*: 5/9 Ben-1 recombinants from heterozygotes of genotype *ben-1(u116) unc-93(e1500)/sel-8(sa54)* contained *sel-8(sa54)*. As the genetic region corresponded to a physical interval containing only seven predicted genes, we sequenced the exons of the seven predicted genes (*C54C6.5*, *C54C6.4*, *C48D5.1*, *C48D5.2*, *C32A3.1*, *C32A3.2*, and *C32A3.3*) as well as a possible pseudogene (*C54C6.3*), and found a single C-to-T base change in only one predicted gene, *C32A3.1* (Fig. 1). We showed that this change, affecting codon 387 of the putative SEL-8 protein (see below), cosegregated with *sel-8(sa54)* in mapping experiments. From heterozygotes of genotype *ben-1 unc-93/sel-8(sa54) lin-12(n302)*, 3/6 Ben-1 non-Unc-93 recombinants contained *sel-8* and C387T and 3/6 recombinants did not contain *sel-8* and had the wild-type sequence.

Furthermore, from heterozygotes of genotype *daf-2 unc-93/sel-8(sa54) lin-12(n302)*, 2/7 Unc-93 non-Daf recombinants contained *sel-8* and C387T and 5/7 recombinants did not contain *sel-8* and had the wild-type sequence. We also note that the cold-sensitive, maternal effect lethality described previously (15) cosegregated with *sel-8* suppressor activity in these experiments, providing evidence that both the lethality and suppression are caused by a mutation in the same gene.

To confirm that C32A3.1 corresponds to *sel-8*, we used PCR to amplify a genomic fragment corresponding to sequence coordinates14,860–27,930 of the cosmid C32A3 (GenBank accession no. Z48241). This PCR product is predicted to encompass 6.3 kb of 5′ flanking sequence and 2.0 kb of 3′ flanking sequence, using the C32A3.1 product as predicted by GENEFINDER (28). We generated transgenic lines carrying extrachromosomal arrays expressing SEL-8A (see Fig. 1 and description of SEL-8A below) in a *sel-8(sa54) lin-12(n302)* background and found a significant reduction in the proportion of Egl^+ (suppressed) hermaphrodites: four independent lines ranged from 0 to 4% Egl^+, whereas three independent control lines (marker only) ranged from 27% to 31% Egl^+. This antisuppression assay, in conjunction with the tight linkage of the C-to-T change to *sel-8(sa54)* and the absence of any other sequence changes in other candidate coding regions establishes that C32A3.1 is *sel-8*.

The initiator methionine codon of the C32A3.1 coding region was predicted to be at position 21759 by GENEFINDER. To determine the 5′ end of the C32A3.1 mRNA, we performed RACE analysis and obtained two RACE products. One RACE product conformed to the 5′ end predicted by GENEFINDER: this product had a spliced leader, GGTTTTAATTACCCAAGTTTGAG, joined to nucleotide 21770 of C32A3; this transcript would have a 5′ untranslated region of 11 bases. We term the predicted protein encoded by this transcript "SEL-8A." Another RACE product had a different, novel spliced leader, GGTTTAACCCAGTTAATTGAG, joined to nucleotide 30379 of C32A3; this transcript would be produced by splicing out an intron of about 9 kb and would have a 5′ untranslated region of 22 bases if the first potential initiator methionine codon at nucleotide position 30357 of C32A3 is used. We termed this predicted protein "SEL-8B."

SEL-8A is expected to contain 468 aa, and SEL-8B is expected to contain 490 aa. Hydropathy analysis reveals that these proteins are extremely hydrophilic, with no potential signal sequence or membrane spanning domains (data not shown). The most striking feature of the sequence is the presence of two highly glutamine-rich regions. The *sel-8(sa54)* C-to-T transition changes codon 387 of *sel-8A* into a premature stop codon (Fig. 1). The predicted premature termination product would lack part of the second glutamine-rich region, suggesting that this region contributes to the action of SEL-8(+).

The predicted SEL-8 proteins do not contain any amino acid sequence motifs detectable by the programs SMART (http://smart.embl-heidelberg.de/) or INTERPRO (http://www.ebi.ac.uk.interpro/). Although BLAST searches did not identify any SEL-8 orthologs in other species, we did identify a potential motif near the amino terminus that is present near the amino terminus of another *C. elegans* protein, W09C5.1, and its apparent yeast and *Drosophila* orthologs (Fig. 1). The yeast protein that contains this "SEL-8 motif" is nuclear (29). Amino-terminal to this motif in SEL-8 is a likely PEST sequence, as ascertained by the program PESTFIND (http://bioweb.pasteur.fr/seqanal/interfaces/pestfind-simple.html). There is also a potential nuclear localization sequence, RKRR, following the SEL-8 motif.

SEL-8 Expression and Subcellular Localization. We inserted GFP between codons 446 and 447(see *Materials and Methods*) and found that the resulting protein appears to be functional, as

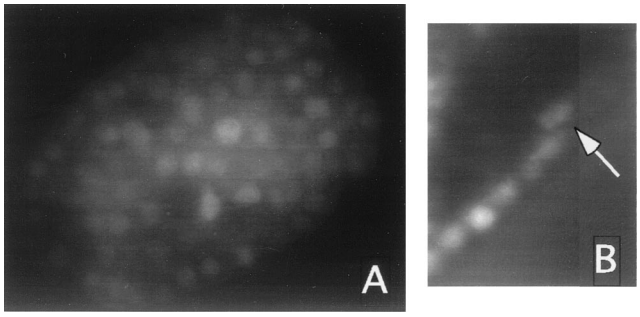

Fig. 2. SEL-8::GFP localization. Epifluorescence micrographs of living *arIs50* individuals show nuclear localization of SEL-8::GFP. (*A*) Early embryo. (*B*) L2 hermaphrodite, somatic gonad. The arrow indicates Z1.ppp (Z4.aaa is in a different focal plane). The other fluorescent nuclei visualized in this focal plane are in the ventral cord.

sel-8(sa54) lin-12(n302) hermaphrodites carrying transgenes expressing SEL-8::GFP are more egg-laying defective (five independent lines ranged from 7% to 14% Egl^+) than control hermaphrodites carrying transgenes expressing only the marker gene (three independent lines ranged from 21% to 27% Egl^+).

We generated integrated lines (23) and examined the subcellular localization of SEL-8::GFP in living animals by using the integrated line *arIs50*. In all cells where fluorescence is visible, SEL-8::GFP is nuclear. SEL-8::GFP appears to accumulate in most, if not all, nuclei of embryos before morphogenesis (Fig. 2). In larvae, SEL-8::GFP is visible throughout development in head, tail, and ventral cord neurons. In addition, there is a dynamic and complex expression pattern in other tissues. We focused our analysis of SEL-8::GFP expression on two sets of cells that undergo *lin-12*-mediated decisions. In the somatic gonad, SEL-8::GFP is expressed in Z1.ppp and Z4.aaa during the L2 stage, at the time that these cells are deciding between the anchor cell and ventral uterine precursor cell fates (Fig. 2). SEL-8::GFP is also visible, albeit faintly, in the vulval precursor cells in the L3 stage, the time at which they undergo a LIN-12-mediated cell fate decision, and is expressed in a dynamic pattern during the vulval cell lineages (data not shown).

RNAi and *sel-8* Loss of Function. In principle, if *sel-8* activity is required for all LIN-12/Notch signaling events during development, then elimination of *sel-8* activity should cause the same phenotype as eliminating all LIN-12/Notch activity. As *sel-8(sa54)* appeared unlikely to be a molecular null allele (based on its sequence and cold-sensitivity), we used RNAi to examine the requirement for *sel-8* activity during development. RNAi is accomplished by injection of a dsRNA into the pseudocoelom of *C. elegans* hermaphrodites. This procedure causes specific depletion of maternal and zygotic gene activity, and in many cases can phenocopy null mutations (27).

We examined the progeny of N2 hermaphrodites that were injected with *sel-8* dsRNA for several different phenotypes associated with the loss of LIN-12/Notch activity in different cell fate decisions. In *C. elegans*, there are two LIN-12/Notch genes, *lin-12* and *glp-1*. Characteristic anatomical defects have been described for loss-of-function mutations in either *lin-12* or *glp-1*, and additional defects, collectively termed "Lag," have been described when the activity of both genes are reduced concomitantly (20, 30–32). Loss-of-function mutations in genes necessary for *lin-12* and *glp-1* signaling, such as *lag-1*, also cause a Lag phenotype (32).

When we injected N2 wild-type hermaphrodites with *sel-8* ds RNA, we observed viable progeny with characteristic Lin-12(−), Glp-1(−), or Lag defects (Fig. 3). These phenotypes included a large vulval protrusion characteristic of *lin-12* null mutant her-

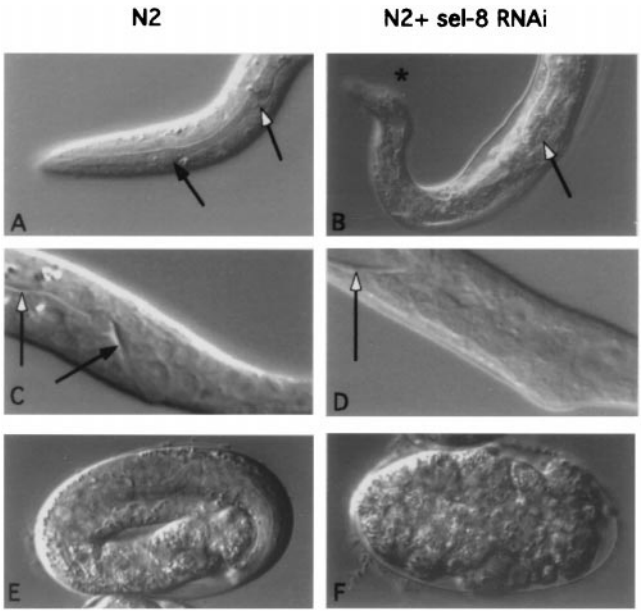

Fig. 3. RNA-mediated interference. Nomarski photomicrographs of the progeny of untreated or mock-injected N2 hermaphrodites (*Left*) and the progeny of hermaphrodites that had been injected with *sel-8* dsRNA (*Right*). (*A* and *B*) Head region, L1 larvae. Black arrow indicates the anterior bulb of the pharynx (missing in *B*), and the white arrow indicates the posterior bulb. (*C* and *D*) Tail region, L1 larvae. Black arrow indicates the rectum (missing in *D*), and the white arrow indicates the intestine. Note the distension of the intestine in *D* resulting from the absence of the rectum. (*E* and *F*) Embryos 6–7 h after egg laying. (*E*) The embryo has reached the 3-fold stage. (*F*) The embryo has arrested without undergoing elongation. We also observed embryos with the same overall morphology as in *F* when we injected N2 hermaphrodites with a mixture of *glp-1* and *lin-12* dsRNAs, or with *lag-1* dsRNA.

maphrodites (20); progeny with severely reduced number of germ cells and premature differentiation of germ cells as sperm, the hallmarks of reduced zygotic *glp-1* activity in the germ line (30), and arrested larvae lacking an anterior pharynx, a defect associated with reduced maternal *glp-1* activity (31). We also saw arrested larvae lacking a rectum and/or having a twisted nose, characteristics of the Lag phenotype resulting from concomitant depletion of the zygotic activity of both *lin-12* and *glp-1* (32). These results indicate that *sel-8* activity is required for many different, and perhaps all, *lin-12*- and *glp-1*-mediated cell fate decisions.

However, the major *sel-8(RNAi)* phenotype we observed is embryonic arrest around the onset of morphogenesis (Fig. 3). We wondered whether this *sel-8(RNAi)* embryonic arrest phenotype represented the absence of all LIN-12/Notch signaling in *C. elegans*, or another role for *sel-8*. The phenotype obtained by removing all LIN-12/Notch activity—i.e., both maternal and zygotic activity—has not been described, as zygotic *glp-1* activity is necessary for the development of the germ line (30, 31). We therefore depleted the activity of both *lin-12* and *glp-1*, or the activity of *lag-1*, using RNAi. In both cases, we observed a terminal phenotype similar to that caused by *sel-8(RNAi)*, suggesting that the *sel-8(RNAi)* embryonic arrest phenotype reflects the absence of all LIN-12/Notch signaling.

We note that the more severe *sel-8(RNAi)* phenotype implies that *sel-8(sa54)* is not a null allele. *sel-8(sa54)* appears to be a partial loss-of-function mutation, as *sel-8(sa54)* progeny segregating from homozygous *sel-8(sa54)* mothers at 15°C have incompletely penetrant phenotypes associated with the absence of *sel-8* activity, including embryonic arrest before morphogenesis and larval arrest with a missing anterior pharynx.

We also have examined whether the nuclear localization of SEL-8::GFP is perturbed in arrested embryos when *lin-12*, *glp-1*, and *lag-1* are concomitantly reduced by RNAi. We saw no evidence for an alteration in the localization or level of nuclear SEL-8::GFP fluorescence (data not shown), suggesting that SEL-8 is stable and properly localized in the absence of LIN-12 and GLP-1 signaling; however, a caveat to this approach is that overexpression from the *arIs50* [SEL-8::GFP] transgene might obscure detection of an effect on SEL-8::GFP stability.

Discussion

We have shown that *sel-8* activity is critical for both *lin-12*- and *glp-1*-mediated cell fate decisions, as depletion of *sel-8* activity by RNA-mediated interference causes phenotypes associated with loss of *lin-12* activity, loss of *glp-1* activity, or the concomitant loss of both *lin-12* and *glp-1* activity. The predominant phenotype associated with depletion of *sel-8* activity by RNA-mediated interference is early embryonic arrest at around the onset of morphogenesis. We also have found the same phenotype when the activity of both *lin-12* and *glp-1*, or of *lag-1*, is depleted by RNA-mediated interference. As the terminal phenotypes of *sel-8(RNAi)* animals can be accounted for by loss of *lin-12* and *glp-1* signaling, it seems likely that *sel-8* functions only in this process. However, it remains possible that *sel-8* has other functions, which are perhaps redundant in other contexts or which are obscured by the terminal phenotype.

We also have identified the *sel-8* coding region and the deduced SEL-8 protein products. At this time, we have not identified an obvious ortholog of SEL-8 in another organism. This result is surprising, as other essential components of the LIN-12/Notch signaling system are highly conserved. The failure to detect apparent orthologs may be caused by the incompleteness of available genome sequence databases or a high degree of divergence at the level of primary amino acid sequence.

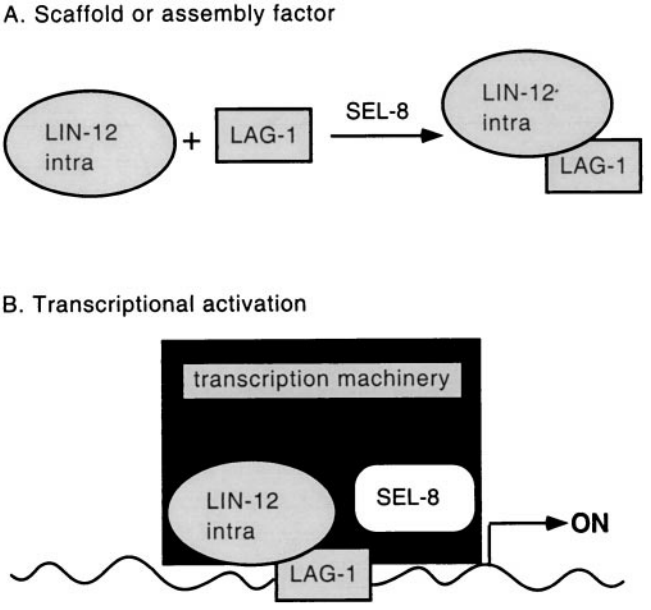

Fig. 4. Models for SEL-8 function. SEL-8 is a nuclear protein that is required for LIN-12 and GLP-1 signaling. Although several models would be consistent with these basic observations, we feature two here. (*A*) Scaffold/assembly factor. SEL-8 may serve to stabilize or to facilitate the assembly of a complex that contains LIN-12(intra)/GLP-1(intra) and LAG-1, and perhaps other proteins. (*B*) Transcriptional activation. The glutamine-rich regions of SEL-8 may function as a transcriptional activation domain that interacts with proteins in the RNA polymerase II transcription initiation complex.

We have found that the major site of accumulation of a functional SEL-8::GFP protein is in the nucleus. This observation suggests that SEL-8 functions in the nucleus to facilitate *lin-12* and *glp-1* signaling. Although at this time we do not know the molecular mechanism of SEL-8 function, we consider here three plausible models that are consistent with our findings.

One model is that SEL-8 facilitates the nuclear import of LAG-1, LIN-12(intra)/GLP-1(intra), or another component of the LIN-12/GLP-1 transcription complex. We have not observed any change in the nuclear accumulation of a GFP-tagged LIN-12(intra) in *sel-8(RNAi)* arrested embryos (C.W. and I.G., unpublished observations), suggesting that SEL-8 is not required for nuclear import of LIN-12(intra). However, SEL-8 may be required for nuclear import of LAG-1 or another component, or overexpression of LIN-12(intra) from the transgene may overcome the need for SEL-8 for nuclear import of LIN-12(intra).

Another model, schematically depicted in Fig. 4, is that SEL-8 is important for the assembly or stability of the transcription complex that contains LAG-1 and LIN-12(intra)/GLP-1(intra). Although LAG-1 and its relatives have been shown to interact physically with LIN-12(intra)/Notch(intra) (8–10), it is conceivable that *in vivo*, scaffolding or assembly factors might be necessary to facilitate association of these components with each other, or with other components of the transcription complex.

Finally, we consider a model in which SEL-8 acts in the transcription complex with LIN-12(intra) and LAG-1 in the transcriptional activation of target genes (Fig. 4). We favor this model because it takes into account the most striking sequence feature of SEL-8, the two long glutamine-rich regions. These glutamine-rich regions of SEL-8 are reminiscent of characterized glutamine-rich transcriptional activation domains (33). The glutamine-rich regions appear to contribute to the essential function of SEL-8, as *sel-8* activity is reduced by partial truncation of the carboxyl-terminal glutamine-rich region in *sel-8(sa54)*.

Transcription of target genes in response to LIN-12/Notch signaling has been studied in several systems. *Drosophila* Su(H) and human CBF1 have been shown to function as repressors in the absence of Notch signaling (11–13, 34). The mechanism of repression has been well studied for CBF1, which appears to tether a corepressor complex that includes the proteins SMRT and histone deacetylases (35). According to a model proposed by Hsieh *et al.* (34), the LIN-12/Notch intracellular domain overcomes this repressor activity first by displacing the corepressor complex and then serving as a coactivator. In the context of this model, SEL-8 might help displace the corepressor complex or serve as a coactivator for a complex that contains LAG-1 and LIN-12(intra).

Note Added in Proof. A. Petcherski and J. Kimble (36) have shown that SEL-8 (which they call "LAG-3") forms a ternary complex containing LAG-1 and the GLP-1 intracellular domain, and obtained RNAi and nuclear localization data consistent with our results.

We thank Ilya Temkin for expert assistance with microinjection experiments and Richard Ruiz for other technical assistance, Jane Hubbard for help with assessing the Glp phenotype of *sel-8(RNAi)*, Xantha Karp for assistance in identifying gonadal cells, and Oliver Hobert, Rhett Kovall, Gary Struhl, and Johnny Fares for comments on this manuscript. The molecular identity of *sel-8* as C32A3.1 and the *sel-8(RNAi)* phenotype were first reported in the abstracts for the 1999 *C. elegans* meeting. T.G.D. was supported by Training Grant AG0189 from the National Institute of Aging. I.G. is an Investigator of the Howard Hughes Medical Institute.

1. Greenwald, I. (1998) *Genes Dev.* **12,** 1751–1762.
2. Brou, C., Logeat, F., Gupta, N., Bessia, C., LeBail, O., Doedens, J. R., Cumano, A., Roux, P., Black, R. A. & Israel, A. (2000) *Mol. Cell* **5,** 207–216.
3. Mumm, J. S., Schroeter, E. H., Saxena, M. T., Griesemer, A., Xu, T., Pan, D. J., Ray, W. J. & Kopan, R. (2000) *Mol. Cell* **5,** 197–206.
4. Schroeter, E. H., Kisslinger, J. A. & Kopan, R. (1998) *Nature (London)* **393,** 382–386.
5. Struhl, G. & Adachi, A. (1998) *Cell* **93,** 649–660.
6. Hamaguchi, Y., Matsunami, N., Yamamoto, Y. & Honjo, T. (1989) *Nucleic Acids Res.* **17,** 9015–9026.
7. Schweisguth, F. & Posakony, J. W. (1992) *Cell* **69,** 1199–1212.
8. Christensen, S., Kodoyianni, V., Bosenberg, M., Friedman, L. & Kimble, J. (1996) *Development (Cambridge, U.K.)* **122,** 1373–1383.
9. Brou, C., Logeat, F., Lecourtois, M., Vandekerckhove, J., Kourilsky, P., Schweisguth, F. & Israel, A. (1994) *Genes Dev.* **8,** 2491–5203.
10. Tun, T., Hamaguchi, Y., Matsunami, N., Furukawa, T., Honjo, T. & Kawaichi, M. (1994) *Nucleic Acids Res.* **22,** 965–971.
11. Dou, S., Zeng, X., Cortes, P., Erdjument-Bromage, H., Tempst, P., Honjo, T. & Vales, L. D. (1994) *Mol. Cell. Biol.* **14,** 3310–3319.
12. Hsieh, J. J. & Hayward, S. D. (1995) *Science* **268,** 560–563.
13. Morel, V. & Schweisguth, F. (2000) *Genes Dev.* **14,** 377–388.
14. Levitan, D. & Greenwald, I. (1995) *Nature (London)* **377,** 351–354.
15. Tax, F. E., Thomas, J. H., Ferguson, E. L. & Horvitz, H. R. (1997) *Genetics* **147,** 1675–1695.
16. Wen, C., Metzstein, M. M. & Greenwald, I. (1997) *Development (Cambridge, U.K.)* **124,** 4759–4567.
17. De Strooper, B., Annaert, W., Cupers, P., Saftig, P., Craessaerts, K., Mumm, J. S., Schroeter, E. H., Schrijvers, V., Wolfe, M. S., Ray, W. J., *et al.* (1999) *Nature (London)* **398,** 518–522.
18. Struhl, G. & Greenwald, I. (1999) *Nature (London)* **398,** 522–525.
19. Brenner, S. (1974) *Genetics* **77,** 71–94.
20. Greenwald, I. S., Sternberg, P. W. & Horvitz, H. R. (1983) *Cell* **34,** 435–444.
21. Greenwald, I. & Seydoux, G. (1990) *Nature (London)* **346,** 197–199.
22. Sambrook, J., Fritch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*. (Cold Spring Harbor Lab. Press, Plainview, NY), 2nd Ed.
23. Mello, C. & Fire, A. (1995) in *Methods in Cell Biology. Caenorhabditis elegans: Modern Biological Analysis of an Organism.*, eds. Epstein, H. F. & Shakes, D. C. (Academic, San Diego), pp. 451–482.
24. Mello, C. C., Kramer, J. M., Stinchcomb, D. T. & Ambros, V. A. (1991) *EMBO J.* **10,** 3959–3970.
25. Han, M. & Sternberg, P. W. (1991) *Genes Dev.* **5,** 2188–2198.
26. Hobert, O., Tessmar, K. & Ruvkun, G. (1999) *Development (Cambridge, U.K.)* **126,** 1547–1562.
27. Fire, A., Xu, S., Montgomery, M. K., Kostas, S. A., Driver, S. E. & Mello, C. C. (1998) *Nature (London)* **391,** 806–811.
28. Edgley, M. L., Turner, C. A. & Riddle, D. L. (1997) in *C. elegans II*, eds. Riddle, D. L., Blumenthal, T., Meyer, B. J. & Priess, J. R. (Cold Spring Harbor Lab. Press, Plainview, NY), pp. 1059–1062.
29. Rout, M. P., Aitchison, J. D., Suprapto, A., Hjertaas, K., Zhao, Y. & Chait, B. (2000) *J. Cell Biol.* **148,** 635–652.
30. Austin, J. & Kimble, J. (1987) *Cell* **51,** 589–599.
31. Priess, J. R., Schnabel, H. & Schnabel, R. (1987) *Cell* **51,** 601–611.
32. Lambie, E. & Kimble, J. (1991) *Development (Cambridge, U.K.)* **112,** 231–240.
33. Gerber, H.-P., Seipel, K., Georgiev, O., Hoefferer, M., Hug, M., Rusconi, S. & Schaffner, W. (1994) *Science* **263,** 808–811.
34. Hsieh, J. J., Henkel, T., Salmon, P., Robey, E., Peterson, M. G. & Hayward, S. D. (1996) *Mol. Cell. Biol.* **16,** 952–959.
35. Zhou, S., Fujimoro, M., Hsieh, J. J., Chen, L., Miyamoto, A., Weinmaster, G. & Hayward, S. D. (2000) *Mol. Cell. Biol.* **20,** 2400–2410.
36. Petcherski, A. & Kimble, J. (2000) *Nature (London)* **405,** 364–368.