

Effects of SEL-12 presenilin on LIN-12 localization and function in *Caenorhabditis elegans*

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Accepted 3 July; published on WWW 25 August 1998

SUMMARY

Presenilins have been implicated in the development of Alzheimer's disease and in facilitating LIN-12/Notch activity. Here, we use genetic methods to explore the relationship between *C. elegans* LIN-12 and SEL-12 presenilin. Reducing *sel-12* activity can suppress the effects of elevated *lin-12* activity when LIN-12 is activated by missense mutations but not when LIN-12 is activated by removal of the extracellular and transmembrane domains. These results suggest that SEL-12 does not function downstream of activated LIN-12. An active SEL-12::GFP hybrid protein accumulates in the perinuclear region of the vulval precursor cells (VPCs) of living hermaphrodites, consistent with a localization in endoplasmic reticulum/Golgi membranes; when *sel-12* activity is

reduced, less LIN-12 protein accumulates in the plasma membranes of the VPCs. Together with the genetic interactions between *lin-12* and *sel-12*, these observations suggest a role for SEL-12 in LIN-12 processing or trafficking. However, SEL-12 does not appear to be a general factor that influences membrane protein activity, since reducing *sel-12* activity does not suppress or enhance hypomorphic mutations in other genes encoding membrane proteins. We discuss potential parallels for the role of SEL-12/presenilin in facilitating LIN-12/Notch activity and in amyloid precursor protein (APP) processing.

Key words: *Caenorhabditis elegans*, LIN-12, SEL-12, Alzheimer's disease, Presenilin, Notch

INTRODUCTION

Alzheimer's disease is a devastating neurological disorder, which afflicts millions of people each year. Several genes have been identified by studies of familial Alzheimer's disease (FAD) (Chartier-Harlin et al., 1991; Goate et al., 1991; Levy-Lahad, 1995a,b; Rogaev et al., 1995; Sherrington et al., 1995). Approximately half of people afflicted with early onset FAD bear mutations in either the presenilin 1 (PS1) or presenilin 2 (PS2) genes (Sherrington et al., 1995; Levy-Lahad, 1995a,b; Rogaev et al., 1995). While mutations in these genes are known to result in increased processing of the amyloidogenic A β 42(43) peptide from amyloid precursor protein (Borchelt et al., 1996; Duff et al., 1996; Scheuner et al., 1996), the mechanism by which this occurs is not known.

Receptors of the LIN-12/Notch family mediate cell-cell interactions that specify cell fate during development (reviewed in Greenwald, 1998). Genetic studies in *Caenorhabditis elegans* and mice have provided evidence that presenilins influence LIN-12/Notch activity (Levitan and Greenwald, 1995; Li and Greenwald, 1997; Shen et al., 1997; Wong et al., 1997). This influence was first suggested by the finding that the *C. elegans sel-12* gene facilitates the activity of *lin-12* and *glp-1*, two *C. elegans* LIN-12/Notch genes, and encodes a protein with about 50% amino acid sequence identity to human PS1 (Levitan and Greenwald, 1995). SEL-12 was

established as a bona fide presenilin by the demonstration that human PS1 and PS2 can functionally substitute for *sel-12* in *C. elegans* (Levitan et al., 1996). The functional relationship between presenilin and Notch activity appears to have been evolutionarily conserved, because targeted disruption of the mouse *PS1* gene causes striking phenotypes associated with reduced *Notch* activity (Shen et al., 1997; Wong et al., 1997). Further support for a functional relationship between presenilin and LIN-12/Notch activity comes from the finding that a second *C. elegans* presenilin, *hop-1*, also facilitates *lin-12* and *glp-1* activity (Li and Greenwald, 1997).

We previously showed that *sel-12* functions in the cell in which LIN-12 has been activated, the 'receiving' cell during cell-cell interactions (Levitan and Greenwald, 1995). This finding suggests several possible roles for *sel-12*. *sel-12* might be a component of the *lin-12* pathway, for example by functioning in signal transduction. SEL-12 might be involved in activation of LIN-12, for example by increasing the efficiency with which LIN-12 binds to or is activated by ligand. Alternatively, *sel-12* might affect LIN-12 activity by influencing the processing and/or trafficking of LIN-12 within the cell.

In this study, we have used genetic methods available in *C. elegans* to explore potential contributions of presenilin function to LIN-12/Notch activity. First, we found that reducing *sel-12* activity can suppress the effects of elevated *lin-*

12 activity when LIN-12 is activated by missense mutations but not when LIN-12 is activated by the removal of the extracellular and transmembrane domains. These results suggest that *sel-12* does not function downstream of *lin-12* and instead are consistent with a role for SEL-12 in the process of LIN-12 activation or the trafficking of LIN-12 to the surface. We therefore investigated whether reducing *sel-12* activity has a detectable effect on LIN-12 protein level or subcellular localization; in the vulval precursor cells, which appear to be particularly sensitive to reduced *sel-12* activity, we found evidence that *sel-12* activity influences LIN-12 protein accumulation in the apical membranes. We also investigated where in the cell SEL-12 might encounter LIN-12, and found evidence that the major site of SEL-12 accumulation is in the ER/Golgi. These observations are consistent with a role for *sel-12* in LIN-12 trafficking, and raised the issue of whether *sel-12* has a general effect on membrane protein trafficking. We therefore examined whether *sel-12* activity influences the activity of other membrane proteins by studying genetic interactions with mutations in other genes encoding secreted or transmembrane proteins, and obtained results suggesting that SEL-12/presenilin is not a general protein trafficking factor. The potential parallels between the effects of SEL-12/presenilin on LIN-12/Notch and amyloid precursor protein suggest that studies of the role of presenilin in normal development will have direct bearing on the role of presenilin in the genesis of Alzheimer's disease.

MATERIALS AND METHODS

Genetics

Standard genetic methods are described in Brenner (1974). Strains were grown at 20°C unless otherwise noted. Mutations used in this study were:

LG I: *daf-8(e1393)*, *daf-8(sa233)* (T. Inoue and J. Thomas, personal communication), *lin-17(n671)*, *lin-17(sy277)*, *lin-17(n698)* (Sternberg and Horvitz, 1988; Sawa et al., 1996).

LG II: *let-23(sy1)*, *let-23(n1045)* (Ferguson and Horvitz, 1985; Aroian et al., 1990), *sma-6(e1482)*, *sma-6(wk7)* (S. Krishna and R. Padgett, personal communication).

LG III: *daf-4(m592)*, *daf-4(e1364)*, *daf-4(m72)* (Riddle et al., 1981; Estevez et al., 1993; C. Gunther and D. Riddle, personal communication), *daf-7(e1372)*, *daf-7(m70)*, *daf-7(m62)*, *daf-7(n696)* (Ren et al., 1996), *arIs12[lin-12(intra)]* (Struhl et al., 1993), *lin-12(n302)* (Greenwald et al., 1983), *lin-12(e2621)* (Hodgkin and Doniach, 1997), *lin-12(oz48)* (Sundaram and Greenwald, 1993a).

LG IV: *daf-1(m40)*, *daf-1(m213)*, *daf-1(p168)*, *daf-1(m42)* (Riddle et al., 1981; Georgi et al., 1990; C. Gunther and D. Riddle, personal communication), *daf-14(sa340)*, *daf-14(m77)* (Riddle et al., 1981; T. Inoue and J. Thomas, personal communication), *lam-1(rh219)* (G. Kao, W. Wadsworth and E. Hedgecock, personal communication), *lin-3(e1417)*, *lin-3(n378)*, *lin-3(n1058)* (Ferguson and Horvitz, 1985; Hill and Sternberg, 1992).

LG X: *egl-15(n484)*, *egl-15(n1447)* (DeVore et al., 1995), *sel-12(ar171)*, *sel-12(ar131)* (Levitan and Greenwald, 1995), *unc-1(e538)* (Brenner, 1974), *unc-6(rh46)* (Ishii et al., 1992).

Strain constructions were performed using standard genetic techniques (Brenner, 1974). Briefly, to construct *sel-12(ar171)* double mutants, *sel-12(ar171) unc-1(e538)/0*; *mnDp68* or *unc-1(e538)/0*; *mnDp68* males (for the control strains) were crossed to *gene-x* hermaphrodites. F₂ *gene-x* non-Unc homozygotes were cloned from the F₁ cross progeny of genotype *gene-x/+*; *sel-12(ar171) unc-1(e538)/++* or *gene-x/+*; *unc-1(e538)/+*. Unc-1 hermaphrodite

progeny of *gene-x*; *sel-12(ar171) unc-1(e538)/++* or *gene-x*; *unc-1(e538)/++* hermaphrodites were picked to generate the strains *gene-x*; *sel-12(ar171) unc-1(e538)* or *gene-x*; *unc-1(e538)*.

To make *sel-12(ar131)* double mutants, *sel-12(ar131)/0* males were crossed to *gene-x*; *unc-1(e538)* hermaphrodites. Non-Unc cross progeny were of genotype *gene-x/+*; *sel-12(ar131)/unc-1(e538)*. *Gene-x* hermaphrodites that failed to segregate Unc progeny were isolated to make the strain *gene-x*; *sel-12(ar131)*.

Scoring dauer formation

All strains with *Daf-c* mutations were maintained at 15°C. Single hermaphrodites were picked to plates at 15°C and their F₁ L4 progeny were shifted to 20°C to score enhancement of the *Daf-c* phenotype in the F₂ progeny. Experimental and control animals were shifted at the same time on plates from the same batch to control for small, random fluctuations in temperature or dryness of the plate. For strains that were egg-laying competent, the parent hermaphrodite was transferred daily. All progeny on a plate were scored 5-6 days later for the number of animals with the characteristic morphology of a dauer larva – thin, dark, fast-moving, non-pumping animals.

Construction of SEL-12::GFP

The gene encoding green fluorescent protein (GFP) (Chalfie et al., 1994) was amplified by PCR from the plasmid pPD95.67 (A. Fire, S. Xu, J. Ahnn and G. Seydoux; personal communication) using the primers GFP-fRI 5'AGGAATTCAGCTCCAAAGAAGAAGCG 3' and GFP-rRI 5' GAATTCCTTTGTAATAGTTCATCCATGCCA 3'. The PCR product was digested with *EcoRI* and ligated to Bluescript KS- also digested with *EcoRI* to make the plasmid pmlGFPRI (X. Li; personal communication). The insert was released from pmlGFPRI by digesting with *EcoRI* and ligated to a *EcoRI* digested 6.0 kb *SacI/XhoI* genomic fragment containing the *sel-12* coding sequences plus 2.8 kb upstream sequences, to generate the plasmid pGsel-12GFP. In this plasmid, GFP is located in the large intracellular loop after transmembrane domain six (see Li and Greenwald, 1996).

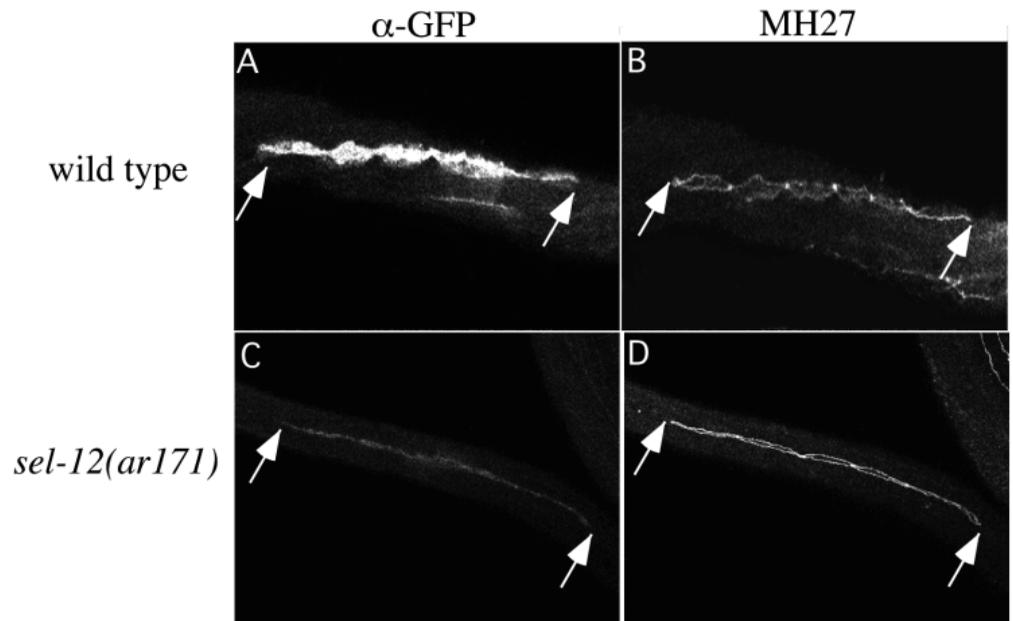
pGsel-12GFP was coinjected at a concentration of 20 µg/ml with pRF4 [*rol-6 (su1006)*] (Mello et al., 1991) at a concentration of 100 µg/ml into *sel-12(ar131)* hermaphrodites. Nine stable lines were obtained and all were scored for rescue of the *sel-12(ar131)* egg-laying defect as in Levitan et al. (1996). All nine lines exhibited rescuing activity. GFP expression was observed in living hermaphrodites in eight of the lines. All lines exhibited similar patterns of expression; the strongest signals were observed with lines carrying the arrays *arEx183* and *arEx184*. For documentation, *arEx183* or *arEx184* animals were mounted on a 2% agarose pad in 2 µl of 1% formaldehyde and viewed with a Zeiss Axiophot LSM 410 confocal microscope.

Indirect immunofluorescence

Animals were fixed as described in Bettinger et al. (1996). Fixed worms were incubated with a polyclonal anti-GFP antibody (Clontech) diluted 1:200 and monoclonal antibody MH27 (Priess and Hirsh, 1986) diluted 1:1000 in PTB (1× PBS, 1% BSA, 0.5% Triton X-100), overnight at 4°C. Worms were washed with 1× PBS, 0.1% BSA, 0.5% Triton-X 100 with several changes of buffer for 4 hours at room temperature. Cy3-conjugated goat anti-rabbit secondary antibody and FITC-conjugated goat anti-mouse secondary antibody (both from Jackson Immunoresearch) were diluted 1:300 in PTB and incubated with the fixed worms overnight at 4°C. Worms were washed as before, for 4 hours at room temperature. Worms were mounted on a 2% agarose pad with 3 µl of 10% N-propyl gallate and viewed with a Zeiss Axiophot LSM 410 confocal microscope.

At least seven independent staining experiments were performed and, in every case, LIN-12::GFP accumulation appeared markedly reduced in *sel-12* as opposed to wild type. The hermaphrodites shown in Fig. 1 are representative of these experiments. In two cases, we attempted to quantify the relative accumulation of LIN-12::GFP in the

Fig. 1. Confocal images of hermaphrodites fixed and stained with anti-GFP antibody to examine LIN-12::GFP accumulation and monoclonal antibody MH27, which outlines tight junctions including the VPCs (Priess and Hirsh, 1996). All strains contain *arIs41*[LIN-12::GFP] (Levitan and Greenwald, 1998). (A,B) wild-type hermaphrodites; (C,D) *sel-12(ar171)* hermaphrodites. All views are ventral; anterior is to the left. (A,C) Anti-GFP staining; (B,D) MH27 staining. The images shown are representative of the majority of hermaphrodites visible in each sample; see Materials and Methods for more information about the analysis of the pattern of LIN-12::GFP accumulation.



plasma membranes of VPCs in wild type and *sel-12* mutants by measuring the amount of anti-GFP immunofluorescence relative to anti-MH27 fluorescence using the public domain NIH Image program (available at <http://rsb.info.nih.gov/nih-image/>), and the results support the interpretation that *sel-12* generally reduces LIN-12::GFP accumulation in the apical membranes of the VPCs (data not shown).

RESULTS

Background: the role of *lin-12* in cell fate decisions

Much of the work on *lin-12* has focused on its role in two cell fate decisions during *C. elegans* development.

The AC/VU decision

The anchor cell (AC)/ventral uterine precursor cell (VU) decision occurs during development of the hermaphrodite gonad. Two cells, named Z1.ppp and Z4.aaa, are initially equivalent in their developmental potential, in that each has an equal chance of becoming the AC or a VU. However, in any given hermaphrodite, only one of these cells will become the AC, while the other becomes a VU (Kimble, 1981). The AC is a differentiated cell that is required for vulval development (see below). The VU gives rise to descendant cells that form part of the ventral uterus.

The AC/VU decision depends on *lin-12*-mediated interactions between Z1.ppp and Z4.aaa (Kimble, 1981; Greenwald et al., 1983; Seydoux and Greenwald, 1989). Elevating *lin-12* activity causes both Z1.ppp and Z4.aaa to become VUs (the '0 AC defect'), while reducing *lin-12* activity causes both Z1.ppp and Z4.aaa to become ACs (the '2 AC defect'). Initially both Z1.ppp and Z4.aaa express *lin-12* and *lag-2*, which encodes a ligand for LIN-12 (Henderson et al., 1994; Tax et al., 1994). The AC/VU decision is determined after a stochastic event, probably a small variation in ligand and/or receptor activity, becomes amplified by a feedback mechanism that influences *lin-12* and *lag-2* transcription (Seydoux and Greenwald, 1989; Wilkinson et al., 1994). The pattern of LIN-12 protein accumulation in this

decision appears to reflect the pattern of transcriptional regulation (Levitan and Greenwald, 1998).

VPC specification

C. elegans hermaphrodites have six vulval precursor cells (VPCs), consecutively numbered P3.p-P8.p, which have the potential to generate vulval cells (Sulston and White, 1980; Sternberg and Horvitz, 1986). Each VPC has the potential to adopt one of three fates, 1°, 2° or 3° (Sulston and White, 1980; Sternberg and Horvitz, 1986). In wild-type hermaphrodites, P3.p-P8.p always adopt the same pattern of fates: 3°-3°-2°-1°-2°-3°.

The pattern of VPC fates appears to be the outcome of three different signalling events (reviewed in Greenwald, 1997). One signalling event is an inductive signal from the AC that promotes expression of the 1° fate. There is also a *lin-12*-mediated lateral signal that promotes expression of the 2° fate. Elevating *lin-12* activity causes all VPCs to adopt the 2° fate, resulting in a 'Multivulva' phenotype, while reducing *lin-12* activity prevents any VPC from adopting the 2° fate (Greenwald et al., 1983). The regulation of *lin-12* activity during VPC fate specification does not appear to involve the regulation of *lin-12* transcription (Wilkinson and Greenwald, 1995). However, LIN-12 protein accumulation is specifically downregulated in P6.p in response to inductive signal (Levitan and Greenwald, 1998).

sel-12 suppresses only one of two constitutively active forms of LIN-12

There are two ways to achieve ligand-independent constitutive *lin-12* activity. One way is by using *lin-12(d)* mutations, a group of missense mutations in the extracellular domain of the full-length receptor (Greenwald et al., 1983; Greenwald and Seydoux, 1990). Another way is to express the intact intracellular domain of LIN-12, using the *lin-12(intra)* transgene (Struhl et al., 1993). By either method, constitutive *lin-12* activity can affect the AC/VU decision, causing the 0 AC defect, and VPC specification, causing a Multivulva phenotype.

Table 1. *sel-12(ar171)* does not suppress all activated forms of *lin-12*

Relevant genotype	% 0 AC (n)	%Muv (n)*
<i>lin-12(n379)</i>	93.3 (30)	0
<i>lin-12(n379); sel-12(ar171)</i>	16.7 (24)	0
<i>lin-12(n302)</i>	100	0
<i>lin-12(n302); sel-12(ar171)</i>	26.3 (38)	0
<i>lin-12(n950)</i>	100	100
<i>lin-12(n950); sel-12(ar171)</i>	89.5 (57)	14.3 (147)
<i>lin-12(n137)</i>	100	100
<i>lin-12(n137); sel-12(ar171)</i>	81.9 (46)	8.4 (143)
<i>lin-12(intra)</i>	89 (27)	87.6 (137)
<i>lin-12(intra); sel-12(ar171)</i>	87.5 (32)	81.3 (118)

Number of anchor cells was scored by examining L3 animals with Nomarski microscopy. *lin-12(d)* alleles are listed in order of increasing activity. The Muv phenotype was scored in the dissecting microscope. All strains contain *unc-1(e538)* except *lin-12(n302); sel-12(+)*. *lin-12(intra)* refers to the *arIs12* transgene (see Materials and Methods).

*Three or more pseudovulvae.

We previously found that reducing *sel-12* activity leads to a cell-autonomous reduction in the constitutive activity of *lin-12(n950)*, a *lin-12(d)* allele, suggesting the possibility that SEL-12 is a downstream effector of LIN-12 (Levitan and Greenwald, 1995). To explore this possibility, we examined the effect of reducing *sel-12* activity on several different *lin-12(d)* alleles and on *lin-12(intra)* (Table 1). *lin-12(intra)* displays both the 0 AC and Multivulva defects; all four *lin-12(d)* alleles display the 0 AC defect, and the two stronger alleles also display the Multivulva defect. As shown in Table 1, *sel-12(ar171)* suppresses the phenotypic defects caused by all four *lin-12(d)* alleles. In contrast, *sel-12(ar171)* does not appreciably suppress either the 0 AC or Multivulva defects caused by *lin-12(intra)*. The ability of *sel-12(ar171)* to suppress *lin-12(d)* but not *lin-12(intra)* does not appear to be due simply to differences in the degree of constitutive activity: *lin-12(intra)* appears to have lower constitutive activity than, for example, *lin-12(n950)*, as evinced by the lower penetrance of the 0 AC defect and more variable expressivity of the Muv defect of *lin-12(intra)* (Table 1). These genetic interactions suggest that *sel-12* does not act in signal transduction by activated LIN-12 and, instead, suggest a role for SEL-12 prior to or during activation of the transmembrane receptor by ligand.

Effects of reducing *sel-12* activity on LIN-12::GFP localization and accumulation

The finding that *sel-12* is likely to function prior to or during receptor activation led us to examine whether reducing *sel-12* activity affects LIN-12 protein level or subcellular localization. LIN-12 may be visualized by a LIN-12::GFP reporter protein that functions like a LIN-12(+) protein (Levitan and Greenwald, 1998). We used the LIN-12::GFP reporter protein to investigate the effect of reducing *sel-12* activity on LIN-12 localization and accumulation.

It should be noted here that *sel-12* mutants do not display any strong phenotypes associated with loss of *lin-12* activity, so we would not expect to see a profound difference in LIN-12::GFP accumulation between wild type and *sel-12* mutants. We therefore concentrated on the VPCs, because these cells seem to be more sensitive to reduced *sel-12* activity (Levitan

and Greenwald, 1995). Furthermore, the VPCs are relatively large, and strongly express the LIN-12::GFP reporter protein, making it easier to detect differences between wild type and *sel-12* mutants.

In wild-type hermaphrodites, LIN-12::GFP can be visualized in the perinuclear region and on the apical surface of all VPCs before VPC fate specification; the level of LIN-12::GFP is downregulated specifically in P6.p in response to the inductive signal from the AC but remains uniform in the other VPCs (Levitan and Greenwald, 1998). We found that *sel-12(ar171)* causes a reliable reduction in LIN-12::GFP accumulation at the apical surface relative to wild-type controls (Fig. 1). There is no obvious difference in the subcellular localization of LIN-12::GFP in a *sel-12* mutant background; as in wild type, LIN-12::GFP appears to be restricted to the apical surface. It is difficult to say with certainty if there is any effect on perinuclear accumulation; however, if there is an effect, it is not as striking or as reproducible as the effect on plasma membrane accumulation. These results are consistent with a role for SEL-12 in LIN-12 trafficking or processing.

We also examined LIN-12::GFP accumulation in various somatic gonadal cell types, including Z1.ppp and Z4.aaa, and did not see any reliable difference in the level of LIN-12::GFP accumulation between wild type and *sel-12* mutant backgrounds (data not shown).

Subcellular localization of SEL-12::GFP

Given the potential effect of SEL-12 on LIN-12 trafficking or processing, we wanted to know where in the cell SEL-12 might encounter LIN-12. This information would inform possible models as to how *sel-12* influences LIN-12 protein accumulation or localization in the plasma membrane. Most immunolocalization and cell fractionation studies have placed presenilin in the ER/Golgi. For example, endogenous PS1 has been detected in the ER and Golgi in brain cells (Huynh et al., 1997) and, when overexpressed in mammalian cells, presenilins appear to be primarily localized to the ER and Golgi (Kovacs et al., 1996; DeStrooper et al., 1997). However, J. Li et al. (1997) report that antibodies to PS1 and PS2 principally stain the nuclear membrane, interphase kinetochores and the centrosomes in cultured lymphocytes and fibroblasts that express endogenous presenilins.

To visualize the SEL-12 protein in living worms, we engineered a SEL-12::GFP reporter protein (see Materials and Methods). The SEL-12::GFP protein appears to function normally: when expressed in transgenic worms using *sel-12* regulatory sequences, it is able to rescue the egg-laying defect of a *sel-12* reduction-of-function mutant, and the transgenic worms do not display any detectable abnormalities in viability, development or behavior (see Materials and Methods).

In the VPCs of transgenic worms expressing the SEL-12::GFP reporter protein, the SEL-12::GFP protein is functioning in its normal context. In the VPCs, SEL-12::GFP is visualized exclusively in the perinuclear region, consistent with a location in the ER/Golgi (Fig. 2). Although it is formally possible that there is an undetectable amount of SEL-12::GFP in other membranes, the simplest interpretation is that SEL-12 encounters LIN-12 in the ER/Golgi. This result is consistent with a role for SEL-12 in LIN-12 trafficking or processing.

We also see SEL-12::GFP accumulation spanning the invagination of the L4 vulval cells and along the extent of

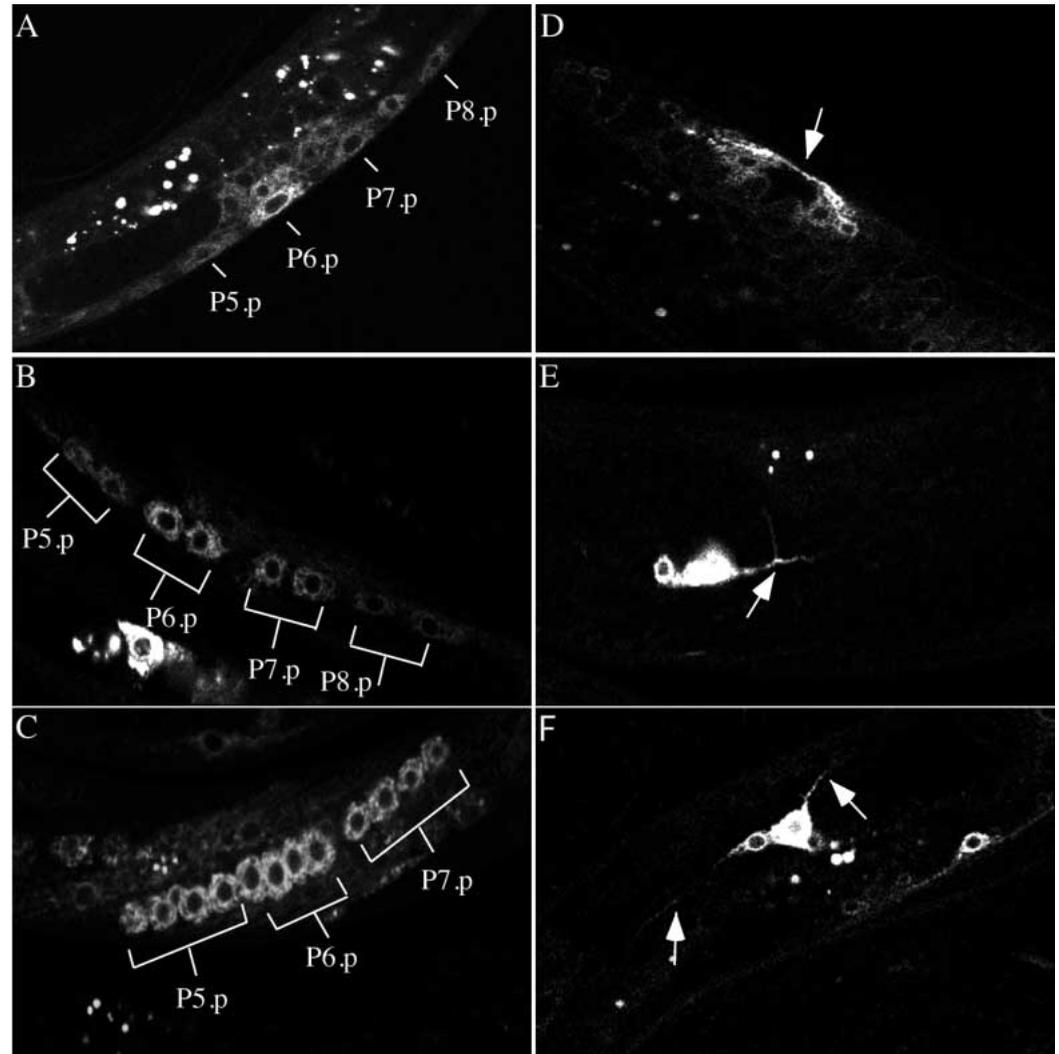


Fig. 2. Confocal images of hermaphrodites expressing SEL-12::GFP. Anterior is to the left. (A) SEL-12::GFP is expressed in all six VPCs at the time of VPC fate specification. Because these animals are rollers, P3.p and P4.p are not visible in this plane of focus; however, they also express SEL-12::GFP. (B) Pn.pxx stage. VPCs have divided once and SEL-12::GFP is expressed in all twelve descendants. P3.p and P4.p descendants are not in this plane of focus. (C) Pn.pxx stage. VPCs have divided twice and SEL-12::GFP is restricted to the progeny of P5.p-P7.p. (D) SEL-12::GFP is seen spanning the invagination formed by vulval cells in the L4 larva. (E,F) SEL-12::GFP is also seen in axons (arrows).

axons (Fig. 2). It is possible that this accumulation is in the plasma membrane, or, alternatively, that it is in smooth ER present in the axons.

Genetic interactions between *sel-12* and genes encoding other membrane proteins

The above observations using LIN-12::GFP and SEL-12::GFP raise the issue of whether SEL-12 is a general protein trafficking factor. The use of sensitized genetic backgrounds resulting from partial loss-of-function mutations is a powerful way to probe for functional interactions between genes. We therefore explored the issue of whether *sel-12* generally influences the activity of membrane proteins by constructing double mutants between *sel-12(ar171)* and hypomorphic mutations in a variety of genes encoding secreted or membrane proteins. If *sel-12* activity facilitates the activity of other membrane proteins, then the *gene-X; sel-12* double mutant would display an enhanced mutant phenotype; if *sel-12* is a negative influence, then the double mutant would display a suppressed mutant phenotype. The potential sensitivity of the genetic approach is underscored by our observation that the *lin-12(hypomorph); sel-12* double mutants display an enhanced 2 AC defect even though *sel-12(ar171)* does not cause a 2 AC defect (Levitan and Greenwald,

1995) and does not cause a reliable difference in LIN-12::GFP accumulation in Z1.ppp and Z4.aaa (data not shown).

Mutations in seven different genes did not display any genetic interactions with *sel-12(ar171)* (Table 2). These genes encode a serpentine receptor (LIN-17, a Frizzled homolog), a transmembrane ligand (LIN-3, an EGF-like ligand), two receptor tyrosine kinases (LET-23, EGL-15), a type II TGF- β receptor (SMA-6), and two extracellular matrix proteins (LAM-1, a laminin B chain, and UNC-6, a netrin) (see Table 2 legend for references).

However, mutations in a group of genes comprising a TGF- β signalling pathway displayed strong genetic interactions with *sel-12* (Table 3). These genes encode a TGF- β ligand (DAF-7), a type I TGF- β receptor (DAF-1), a type II TGF- β receptor (DAF-4), and two SMAD genes (DAF-8 and DAF-14) (see Materials and Methods for references). Loss-of-function mutations in any one of these *daf* genes cause a dauer-constitutive phenotype, characterized by the entry of worms into diapause even under favorable growth conditions. Mutations in *sel-12* enhance the dauer-constitutive phenotype of hypomorphic mutations of all of these *daf* genes (Table 3). However, *lin-12* hypomorphic alleles also enhance the dauer-constitutive phenotype of apparent null alleles of these genes (Table 4). Since

Table 2. Mutations which were unaffected by *sel-12(ar171)*

Gene	Protein	Allele	% null phenotype (n)	
			<i>sel-12(+)</i>	<i>sel-12(ar171)</i>
<i>lin-17*</i>	Frizzled	<i>n671</i>	86.4 (88)	89.6 (96)
		<i>sy277</i>	84.7 (72)	85.7 (56)
		<i>n698</i>	39 (100)	32.9 (97)
<i>lin-3†</i>	EGF	<i>e1417</i>	0 (192)	0 (217)
		<i>n378</i>	0 (143)	0 (197)
		<i>n1058</i>	1.9 (206)	1.7 (57)
<i>let-23‡</i>	EGF receptor	<i>sy1</i>	5 (203)	1.3 (151)
		<i>n1045</i>	56 (142)	63 (165)
<i>egl-15§</i>	FGF receptor	<i>n484</i>	0 (229)	0 (139)
		<i>n1447</i>	3 (129)	6.8 (73)
<i>lam-1¶</i>	Laminin B	<i>rh219</i>	0 (84)	0 (125)
<i>unc-6 </i>	Netrin	<i>rh46</i>	71 (38)	63 (38)
<i>sma-6**</i>	Type II TGF- β receptor	<i>e1482</i>	0 (30)	0 (32)
		<i>wk7</i>	76 (25)	85 (20)

All phenotypes were scored at 20°C. All strains contain *unc-1(e538)*. *sma-6* strains also contain *him-5(e1467)*.

*Phenotype scored was DiO filling of phasmids as in Sawa et al., 1996.

†Phenotype scored was larval lethality as in Hill and Sternberg, 1992.

‡Phenotype scored was larval lethality as in Aroian and Sternberg, 1991.

§Phenotype scored was larval lethality as in DeVore et al., 1995.

¶Phenotype scored was larval lethality (G. Kao, W. Wadsworth, and E. Hedgecock, personal communication).

||Phenotype scored was Unc and gonad migration defect as in Ishii et al., 1992.

**Phenotype scored was ray transformations in the male tail (S. Krishnan and R. Padgett, personal communication).

Table 3. *sel-12* mutations enhance the Daf-c phenotype of many Daf alleles

Gene	Allele	% DAF (n)		
		<i>sel-12(+)</i>	<i>sel-12(ar171)</i>	<i>sel-12(ar131)</i>
+	+	0 (many)	0 (157)	0 (203)
<i>daf-1</i>	<i>m40</i>	72.1 (457)	97.4 (420)	99.3 (278)
	<i>m213</i>	12.8 (382)	98.0 (275)	57.3 (483)
	<i>p168</i>	0 (596)	11.2 (178)	ND
	<i>m42</i>	31.0 (809)	81.2 (363)	71.8 (401)
<i>daf-4</i>	<i>m592</i>	2.4 (167)	1.4 (210)	ND
	<i>e1364</i>	29.0 (455)	81.7 (82)	95.5 (45)
	<i>m72</i>	35.9 (434)	95.5 (70)	96 (100)
<i>daf-7</i>	<i>e1372</i>	4 (530)	0.7 (434)	ND
	<i>m70</i>	11.9 (326)	97.5 (203)	95.4 (65)
	<i>m62</i>	23.4 (893)	72.6 (354)	91.2 (159)
	<i>n696</i>	62.4 (364)	99.1 (236)	100 (178)
<i>daf-8</i>	<i>e1393</i>	6 (159)	1.1 (180)	ND
	<i>sa233</i>	0 (244)	26.6 (109)	16.9 (219)
<i>daf-14</i>	<i>sa340</i>	0 (196)	0 (386)	ND
	<i>m77</i>	13.3 (391)	66.7 (126)	76.6 (141)

Dauer formation was scored at 20°C, except *daf-1(m40)* which were scored at 25°C as described in Materials and Methods. All strains with either *sel-12(+)* or *sel-12(ar171)* also contain *unc-1(e538)*. Alleles in **bold** are the strongest available alleles. ND, not done.

the decision to form a dauer larva is sensitive to growth conditions, we wanted to ensure that these genetic interactions are not merely due to the fact that larvae are retained internally due to the egg-laying defects of *sel-12* and *lin-12* mutants; we therefore showed that an egg-laying defective allele of *egl-15* does not enhance the *daf* mutations (Table 4). We note also that

Table 4. *lin-12* hypomorphic mutations enhance the Daf-c phenotype of Daf alleles

Gene	allele	% DAF (n)			
		<i>lin-12(+)</i>	<i>lin-12(oz48)</i>	<i>lin-12(e2621)</i>	<i>egl-15(n484)</i>
+	+	0 (many)	0 (107)	0 (211)	ND
<i>daf-1</i>	<i>m213</i>	22.7 (163)	82.4 (142)	ND	22.4 (247)
	<i>m42</i>	34.2 (386)	ND	57.0 (249)	ND
<i>daf-4</i>	<i>m72</i>	26.9 (156)	ND	82.8 (134)	ND
<i>daf-7</i>	<i>n696</i>	61.7 (162)	95 (72)	94.4 (307)	ND

Dauer formation was scored at 20°C as described in Materials and Methods. Alleles in **bold** are the strongest available alleles. ND Not Done.

we did not detect any difference in DAF-4::GFP accumulation in a *sel-12(ar171)* background (data not shown). The simplest interpretation of all the data is that the genetic interaction between *sel-12* and the *daf* genes reflects the reduction of *lin-12* activity by *sel-12*. The interaction between *lin-12* or *sel-12* and the *daf* genes may reflect as yet unknown *lin-12*-mediated cell fate transformations or other effects on neurons that provide input into the decision to form a dauer larva.

In summary, the double mutant combinations presented here have not identified any membrane-associated protein other than LIN-12 or GLP-1 whose activity depends on the level of *sel-12* activity. The failure to observe genetic interactions between *sel-12* and mutations in other genes does not prove that the effects of SEL-12 are restricted to LIN-12 or GLP-1. However, it does suggest that if there are other proteins that are influenced by *sel-12* activity, they may constitute a relatively small set, and perhaps have special features that define them. This inference also is supported by the available genetic data concerning phenotypes resulting from loss of presenilin activity. In the mouse, *presenilin 1* null mutants were found to resemble mouse *Notch1* null mutants (Shen et al., 1997; Wong et al., 1997). In *C. elegans*, concomitant loss of both SEL-12 presenilin and HOP-1 presenilin causes phenotypes associated with concomitant loss of both *lin-12* and *glp-1* activity (Li and Greenwald, 1997). However, in both *C. elegans* and the mouse, there is still the possibility that further analysis will reveal other consequences of loss of presenilin activity.

DISCUSSION

In this study, we have used genetic methods available in *C. elegans* to explore potential contributions of presenilin function to LIN-12/Notch activity. We found that *sel-12* does not act downstream of *lin-12*. This raised the possibility that SEL-12 functions in LIN-12 processing or trafficking to the surface, analogous to the postulated role for presenilin in amyloid precursor protein trafficking or processing (see below). We then examined the effects of reducing *sel-12* activity on LIN-12 subcellular localization and accumulation, and determined the subcellular localization of SEL-12 in living worms using a functional SEL-12::GFP fusion protein. We found that a *sel-12* loss-of-function mutation reduces the amount of LIN-12 accumulation in the apical membranes of the vulval precursor cells, which are particularly sensitive to reduced *sel-12* activity (Levitan and Greenwald, 1995). Furthermore, in the vulval precursor cells, we detected the

SEL-12::GFP protein in the perinuclear region, suggesting its major subcellular location is in the ER/Golgi. These observations are consistent with a role for SEL-12 in promoting the stability, production or trafficking of LIN-12 to the surface. However, SEL-12 may not play a general role in membrane protein trafficking, since we were unable to detect a genetic interaction between *sel-12* and other genes encoding secreted or transmembrane proteins (see also Results).

Most work on presenilin function has focused on its effects on amyloid precursor protein (APP). APP is a transmembrane protein that is processed into two different secreted peptide forms, A β 40 and A β 42(43). The A β 42(43) form is enriched in amyloid deposits associated with Alzheimer's disease (Sisodia et al., 1990; Haass et al., 1992; Seubert et al., 1992). Both peptides share the same N terminus, processed by an activity termed β -secretase; they differ in their C termini, where A β 40 and A β 42(43) result from processing by an activity termed γ -secretase. The protease(s) associated with the β - or γ -secretase activities is not known, but the processing event is believed to occur in the endoplasmic reticulum (Selkoe, 1994). FAD mutations in both PS1 and PS2 result in an increased production of the A β 42 peptide (Borchelt et al., 1996; Duff et al., 1996; Scheuner et al., 1996), and the absence of PS1 activity results in reduced γ -secretase activity (De Strooper et al., 1998).

Many different models have been proposed for the role of presenilins in APP processing. The most recent data favor two models (see De Strooper et al., 1998). In view of the effects on APP processing, it has been proposed that presenilins might activate the γ -secretase activity that cleaves APP, analogous to the way that the multipass membrane protein SCAP activates cleavage of SREBP (Brown and Goldstein, 1997). Alternatively, it has been proposed that presenilins might facilitate the intracellular trafficking of APP and/or the secretases, so that APP is placed into the correct compartment to undergo γ -secretase cleavage.

Our data suggest that the effect of SEL-12/presenilin on LIN-12/Notch is analogous to its effect on APP. LIN-12/Notch proteins are transmembrane proteins with hallmark epidermal growth factor-like, LIN-12/Notch repeat, and cdc10/SWI6 (ankyrin) motifs (reviewed in Weinmaster, 1997). Like APP, LIN-12/Notch proteins must be correctly sorted and transported to the cell surface, and undergo proteolytic cleavage events. There appears to be at least one constitutive proteolytic cleavage event that occurs in the extracellular domain during the transport to the plasma membrane; the cleaved form produced by this constitutive cleavage event may be the major species present at the cell surface (Blaumueller et al., 1997; Pan and Rubin, 1997). In addition, binding of ligand appears to induce a cleavage event in or near the transmembrane domain; this apparent cleavage event enables the intracellular domain to translocate to the nucleus, where it participates directly in regulating downstream gene expression (Schroeter et al., 1998; Struhl and Adachi, 1998).

It is conceivable that SEL-12/presenilin is involved in promoting one or more of these cleavage events, either by activating protease(s) or promoting trafficking of either LIN-12 or proteases to an appropriate compartment. The strong accumulation of SEL-12::GFP in the ER/Golgi is consistent with a role for SEL-12 in a constitutive cleavage event involved in maturation of LIN-12/Notch proteins. The fact that we observed less LIN-12::GFP at the cell surface in a *sel-12* mutant

background could be explained in the context of this model by proposing that abnormal processing of LIN-12 leads to its failure to be transported to the plasma membrane or to its degradation.

The putative ligand-dependent cleavage of activated LIN-12 might occur at the plasma membrane or in internalized vesicles. The failure to observe SEL-12::GFP in the plasma membranes of the VPCs does not preclude a role for SEL-12 in ligand-dependent cleavage. It is possible that SEL-12::GFP is present at low abundance in the plasma membrane; the ligand-induced event appears to affect a very small proportion of receptor molecules, suggesting that the agent that promotes the cleavage may not be very abundant.

Although the issue of the biochemical mechanism of presenilin function is not resolved in any system, the parallels between APP and LIN-12/Notch trafficking and processing suggest that a common mechanism is involved. An important challenge for the future will be to identify the primary effect of SEL-12/presenilin on APP and LIN-12/Notch, since proteolytic processing, intracellular trafficking and degradation are intimately linked, and altering one process can affect another. The ability to use genetic approaches in *C. elegans* as well as biochemical approaches in mammalian cell culture systems provide complementary means to attack this difficult problem.

We thank Don Riddle and Cathy Guenther, T. Inoue and J. Thomas, G. Kao, W. Wadsworth and E. Hedgecock, S. Krishna and R. Padgett, and the Caenorhabditis Genetics Center (which is funded by the National Center for Research Resources of the NIH) for strains. We thank Xiajun Li for a plasmid containing GFP inserted into a *sel-12* cDNA, Andy Fire for a useful GFP construct, and Ilya Temkin for integrating an extrachromosomal SEL-12::GFP array. We are grateful to Gary Struhl and members of our laboratory for helpful comments on the manuscript. We thank Theresa Swayne for help with data analysis and confocal microscopy, which was performed at a central facility that is supported by NIH Shared Instrument Grant 1S10 RR10506 and NIH grant #5 P30 CA13696 as part of the Herbert Irving Cancer Center at Columbia University. This work was supported by NIH grant NS35556 (to I. G.). I. G. is an Associate Investigator of the Howard Hughes Medical Institute.

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