The *Caenorhabditis elegans sel-1* Gene, a Negative Regulator of *lin-12* and *glp-1*, Encodes a Predicted Extracellular Protein

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ABSTRACT

The *Caenorhabditis elegans lin-12* and *glp-1* genes encode members of the LIN-12/NOTCH family of receptors. The *sel-1* gene was identified as an extragenic suppressor of a *lin-12* hypomorphic mutant. We show in this report that the *sel-1* null phenotype is wild type, except for an apparent elevation in *lin-12* and *glp-1* activity in sensitized genetic backgrounds, and that this genetic interaction seems to be *lin-12* and *glp-1* specific. We also find that *sel-1* encodes a predicted extracellular protein, with a domain sharing sequence similarity to predicted proteins from humans and yeast. SEL-1 may interact with the LIN-12 and GLP-1 receptors and/or their respective ligands to down-regulate signaling.

THE *lin-12* gene of *Caenorhabditis elegans* encodes a founding member of the *lin-12/Notch* family of putative receptor proteins (reviewed in GREENWALD and RUBIN 1992; ARTAVANIS-TSAKONAS *et al.* 1995). Members of this gene family include another *C. elegans* gene, *glp-1*, Drosophila *Notch*, and vertebrate homologues. Among the vertebrate *lin-12/Notch* genes there are two that have been associated with cancers, murine *int-3* and human *TAN-1* (ELLISEN *et al.* 1991; JHAPPAN *et al.* 1992; ROBBINS *et al.* 1992).

lin-12 mutations affect binary cell fate decisions in many different cell types and at many different developmental stages (GREENWALD *et al.* 1983). Many of these cell fate decisions are known to require cell-cell interactions. The most intensively studied of these cell fate decisions occurs between two cells, Z1.ppp and Z4.aaa, in the somatic gonad of the L2 hermaphrodite. While each of these cells has an equal potential to adopt an anchor cell (AC) or ventral uterine precursor (VU) cell fate, they interact so that only one cell of each type is produced (KIMBLE and HIRSH 1979; KIMBLE 1981; SEYDOUX and GREENWALD 1989).

lin-12 activity controls the decision between the AC and VU fates. In *lin-12* null mutants, both cells become ACs, while in *lin-12(d)* mutants, which by genetic criteria display elevated *lin-12* activity, both cells become VUs (GREENWALD *et al.* 1983). Genetic analysis revealed that in the binary decision between the AC and VU fates, high *lin-12* activity defines the VU fate, and low *lin-12* activity defines the AC fate (GREENWALD *et al.* 1983). In genetic mosaics where one of these two cells was *lin-12(+)* and one was *lin-12(0)*, the *lin-12(0)* cell always became an AC, presumably because it could not receive the signal (SEYDOUX and GREENWALD 1989). Furthermore, the naturally variable fate of the *lin-12(+)* cell became biased, so that it always became a VU (SEYDOUX and GREENWALD 1989). Indeed, cell fate decisions seem to be exquisitely sensitive to the activity level of *lin-12/Notch* signaling. Genetic mosaic analysis of Drosophila *Notch* showed that differences in the number of wild-type *Notch* genes could bias cell fate decisions. If cells containing three copies of wild-type *Notch* were adjacent to cells containing two copies of wild-type *Notch*, the cells with three copies always adopted an epidermal fate, while their neighbors carrying only two copies always adopted a neural fate (HEITZLER and SIMPSON 1991). Transcriptional regulation appears to be at least one component of the feedback mechanism by which initially small differences in *lin-12* activity between equivalent cells is greatly amplified during cell fate determination (WILKINSON *et al.* 1994).

To fully understand how *lin-12* and related genes function to control cell fate, we must identify other genes involved in *lin-12* mediated cell fate decisions. Unlike Drosophila, where several genes with similar null phenotypes to *Notch* have been identified, no genes with phenotypes like *lin-12(0)* or *glp-1(0)* have been found (SEYDOUX *et al.* 1993; J. KIMBLE, personal communication). Two genes that can mutate to display the same "Lag" phenotype as a *lin-12(0) glp-1(0)* double mutant have been identified (LAMBIE and KIMBLE 1991). One gene, *lag-2*, encodes a putative ligand for LIN-12 and GLP-1, while the other gene, *lag-1*, may function downstream (HENDERSON *et al.* 1994; TAX *et al.* 1994; CHRISTIANSEN *et al.* 1996). However, there are likely to be many more than two genes that participate in *lin-12* mediated cell fate decisions.

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These genes may have redundant activities or other developmental roles that mask their involvement with *lin-12* and *glp-1*.

One approach to identify other genes in this pathway, unbiased by predicted null phenotypes, is to identify extragenic suppressors of *lin-12* mutations. We have been studying a gene, *sel-1*, identified as a suppressor of *lin-12* (SUNDARAM and GREENWALD 1993b). Mutations in *sel-1* increase the activity of *lin-12* and *glp-1* (SUNDARAM and GREENWALD 1993b). We show in this report that the *sel-1* null phenotype is wild type, except for an apparent elevation in *lin-12* and *glp-1* activity in sensitized genetic backgrounds. Furthermore, we show that *sel-1* encodes a predicted extracellular protein, possibly associated with the cell surface. We also provide genetic evidence that *sel-1* specifically influences *lin-12* and *glp-1* activity, by showing that *sel-1* mutations do not suppress mutations in other genes encoding membrane bound receptors. SEL-1 may therefore be directly involved in cell-cell interactions and might interact with LIN-12, GLP-1 and/or their ligands.

MATERIALS AND METHODS

General methods and strains: Methods for the handling and culturing of *C. elegans* were essentially as described by BRENNER (1974). The wild-type parent for all strains used was *C. elegans* var. Bristol strain N2 except for those experiments involving GS856 in which part of LGV has been replaced with that of *C. elegans* var. Bergerac strain BO (NIGON 1949; BRENNER 1974; TUCK and GREENWALD 1995; S. TUCK, personal communication; A. TELFER, personal communication). All strains were grown at 20° except where noted.

Mutations used: LG I: *lin-17(n671)* (FERGUSON and HORVITZ 1985). LG II: *let-23(sy1)* (AROIAN and STERNBERG 1991), *let-23(n1045cs)* (FERGUSON and HORVITZ 1985), and *tra-2(b202ts)* (KLASS *et al.* 1976). LG III: *glp-1(e2142ts)* (PRIESS *et al.* 1987), *lin-12(n676n930)* (SUNDARAM and GREENWALD 1993a), *unc-32(e189)* (BRENNER 1974), *unc-36(e251)* (BRENNER 1974), *qC1* (AUSTIN and KIMBLE 1989), *daf-4(e1364)* (RIDDLE 1977), and *daf-4(m63)* (RIDDLE 1977). LG IV: *dpy-20(e1282ts)* (BRENNER 1974), *him-8(e1489)* (HODGKIN *et al.* 1979), and *lin-3(e1417)* (FERGUSON and HORVITZ 1985). LG V: *arDf1* (TUCK and GREENWALD 1995), *lin-25(n545ts)* (FERGUSON and HORVITZ 1985), *lon-3(e2175)* (HODGKIN *et al.* 1988), *rol-4(sc8)* (COX *et al.* 1980), *sel-1(ar23), sel-1(ar29), sel-1(ar75), sel-1(ar77), sel-1(e*1948) (SUNDARAM and GREENWALD 1993b), and *sqt-3(sc63ts)* (COX *et al.* 1980). LGX: *egl-15(n484)* (TRENT *et al.* 1983), and *egl-15(n1477)* (DEVORE *et al.* 1995).

Mutagenesis and screen for new *sel-1* alleles: Worms were mutagenized for 4 hr at 20° in 50 mM ethyl methane sulfonate (EMS) as described in BRENNER (1974). *glp-1(e2142);him-8(e1489);lon-3(e2175) sel-1(e1948)* males were mated to EMS mutagenized *unc-32(e189) glp-1(e2142);sqt-3(sc63)* hermaphrodites at 15°. The adult animals (P_0) were transferred to fresh plates daily for 6 days, and F_1 eggs and larvae remaining on the plates were transferred to 25°. After 3–4 days, plates at 25° were screened for live F_2 progeny, and F_1 Rol (*sqt-3/+*) animals were counted. Individual F_2 Rol animals were picked to their own plates and rescreened after 3 days at 25° for live F_3 animals. A single L1/L2 stage Sqt F_3 animal representative of a F_1 plate was placed at 15° to establish candidate *sel-1* mutant strains. All F_2 Rol plates that produced F_3 animals segregated Sqt animals, indicating that all new suppressor alleles were homozygous viable. After screening ~10,500 F_1 genomes, 20 *sel-1* mutant candidates were isolated. All candidate *sel-1* mutants were then tested for their ability to suppress the Egl phenotype of *lin-12(n676n930)* when placed *in trans* to *sel-1(e*1948). Two mutations, *ar166* and *ar167*, were found to be *sel-1* alleles by this criterion. The 18 other suppressed strains isolated in this screen might contain dominant alleles of previously identified suppressors of *glp-1(e2142)* that do not suppress *lin-12(n676n930)* (A. HOWELL and J. PRIESS, personal communication).

We analyzed the anatomy *sel-1(0)* animals in detail by Nomarski microscopy. We did not find any phenotypic abnormalities. In addition to searching widely for any morphological defects in *sel-1* mutants, we specifically quantified progeny viability, number of anchor cells, and egg laying. Phenotypes were scored in all progeny of animals allowed to lay eggs over a 1-hr period. For *sel-1(e*1948) we found that 57/57 eggs were viable, 57/57 adults were non-Egl, and 52/52 animals had one anchor cell. For *sqt-3(sc63) sel-1(ar167)* we found that 166/169 eggs were viable, 166/166 adults were non-Egl, and 27/27 animals had one anchor cell.

Analysis of double mutants: All strains were constructed by standard methods (BRENNER 1974). *let-23;sel-1*: We found that only 3/50 *let-23(sy1);sel-1(e1948)* animals from homozygous mothers were non-Egl, similar to *let-23(sy1)* alone (AROIAN and STERNBERG 1991). We found similar results with *let-23(n1045cs)* (data not shown). *egl-15;sel-1*: We found that only 3/75 *egl-15(n484);sel-1(e1948)* animals from homozygous mothers were non-Egl, similar to *egl-15(n484)* alone in which we found 4/70 animals were non-Egl. We also found that 0/50 *egl-15(n1477);sel-1(e1948)* animals were non-Egl, and that 0% ($n > 100$) of *egl-15(n1477)* animals were non-Egl. *daf-4;sel-1*: We found that all progeny ($n > 300$) of four *daf-4(m63);sel-1(e1948)* adults placed at 25° became dauer larvae. We also found that all progeny ($n > 1000$) from 20 *unc-32(e189) daf-4(e1364);sqt-3(sc63ts) sel-1(ar167)* mothers became dauer larvae. These results are very similar to *daf-4(m63)* and *daf-4(e1364)* alone (RIDDLE 1977) *tra-2;sel-1*: 25 gravid *tra-2(b202ts) sqt-3(sc63ts) sel-1(ar167)* hermaphrodites raised at 15° were shifted to 25°. All of their progeny ($n > 200$) were sterile and displayed at least partial male tail formation, similar to *tra-2(b202ts)* alone (KLASS *et al.* 1976). *lin-17;sel-1*: We found that 31/45 *lin-17(n671);sqt-3(sc63ts) sel-1(ar167)* animals from homozygous mothers had two vulval protrusions very similar to *lin-17(n671)* alone, in which we found that 46/69 animals had two vulval protrusions.

***sel-1* rescue experiments:** *sel-1* was previously mapped between *rol-4* and *lin-25* (SUNDARAM and GREENWALD 1993b). To map *sel-1* in preparation for cloning we used a Bristol/Bergerac congenic strain, GS856, containing several Tc1 associated polymorphisms: *Tc1(3.7), Tc1(8.6), arP4,* and *arP5,* flanked by *rol-4(sc8)* on the left and *lin-25(n545) him-5(e1467)* on the right (TUCK and GREENWALD 1995). Progeny from heterozygotes of the genotype *rol-4(sc8):BO:lin-25(n545) him-5(e1467)/ sel-1(e1948)* were screened for Rol non-Lin recombinants. The recombinant chromosomes were subsequently assayed for the presence of restriction fragment length polymorphisms (RFLPs) by Southern blot hybridization analysis of *Eco*RI-digested genomic DNA probed with Tc1, and for *sel-1(e1948)* by crossing into a *lin-12(n676n930)* background. The genotypes of the recombinant chromosomes were as follows: 3/15 *rol-4(sc8) sel-1(e1948),* 1/15 *rol-4(sc8) Tc1(3.7) Tc1(8.6) sel-1(e1948),* 6/15 *rol-4(sc8) Tc1(3.7) Tc1(8.6) arP4 arP5 sel-1(e1948),* 5/15 *rol-4(sc8) Tc1(3.7) Tc1(8.6) arP4 arP5.*

Microinjection of DNA into the germ line of *C. elegans* hermaphrodites for transformation was done essentially as described by FIRE (1986) as modified by MELLO *et al.* (1990). Initial rescue experiments were done by injecting *C. elegans*

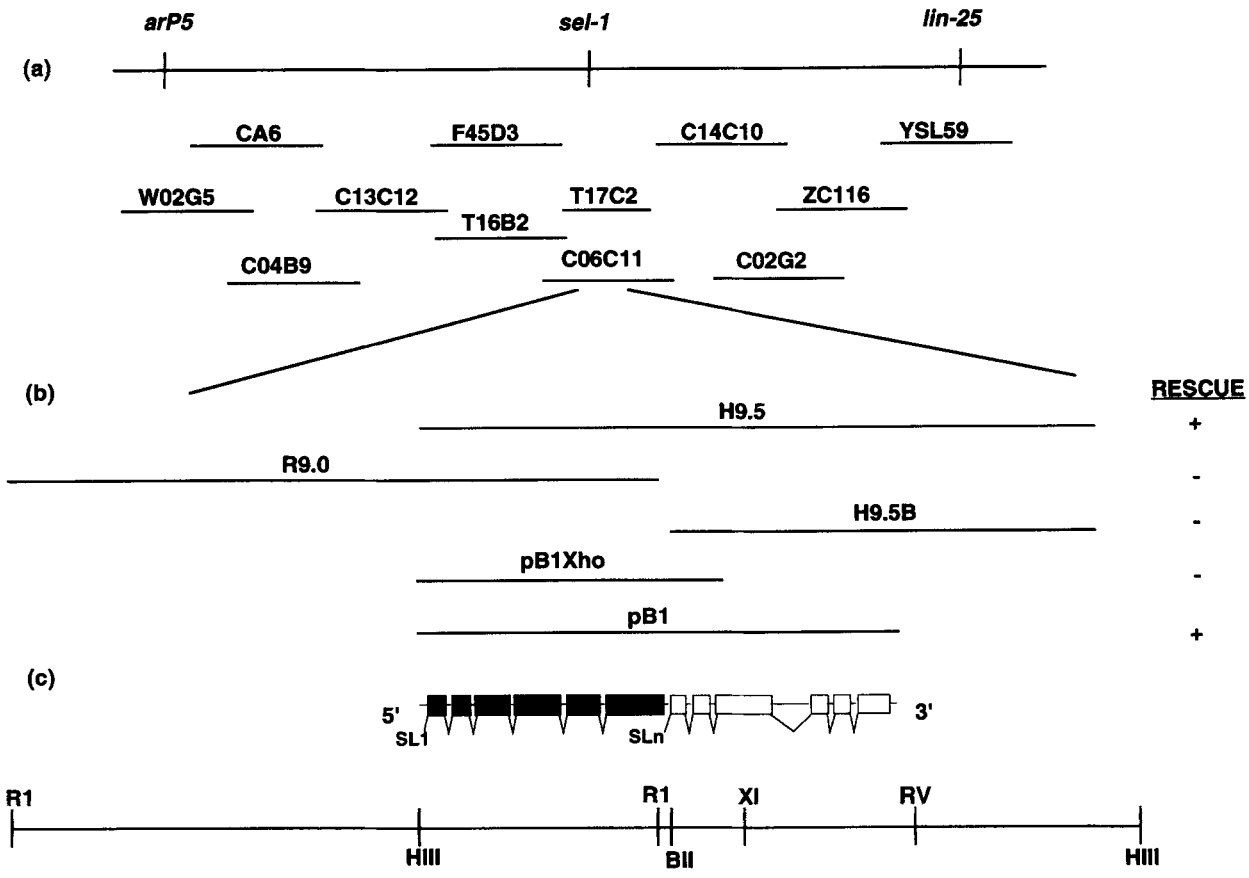

FIGURE 1.—Positional cloning of *sel-1*. (a) Cosmid and lambda clones are shown as lines. *arP5* resides on W02G5 and *lin-25* resides on YSL59 (TUCK and GREENWALD 1995). (b) Lines depict genomic DNA subcloned from C06C11 into pBluescript (Stratagene). +, rescue of *sel-1(e1948)*; −, failure to rescue *sel-1(e1948)* (see MATERIALS AND METHODS). (c) The *sel-1* transcription unit and partial restriction map. Straight lines represent noncoding sequences. Boxes represent coding regions. SL1 and SLn depict *trans*-splice acceptor sites. The orientation of the *sel-1* transcription unit with respect to the *C. elegans* physical map is arbitrary, as we have not determined their relative orientations. RI, *Eco*RI; HIII, *Hin*dIII; BII, *Bgl*II; XI, *Xho*I; and RV, *Eco*RV.

hermaphrodites of the genotype *glp-1(e2142);dpy-20(e1282);sel-1(e1948)* with cosmid DNAs potentially harboring the *sel-1(+)* gene. A mixture of the following cosmids was injected at 5–10 μg/ml each along with the *dpy-20(+)* plasmid pMH86 at 12.5 μg/ml (HAN and STERNBERG 1991): W02G5, CA6, C04B9, C13C12, T16B2, F45D3, C06C11, T17C2, C02G2, and C14C10 (Figure 1) (COULSON *et al.* 1986, 1988). Stable non-Dpy lines were reared at 20°. Individual non-Dpys from stable lines were picked and scored after 3 days at 25° for the number of live progeny and presence of dead eggs. Live broods of ≤10 from half of the non-Dpys in a line was considered indicative of *sel-1(+)* rescuing activity. Two of four lines carrying the 10 cosmid mixture showed rescuing activity; 1/1 lines carrying a 1:10 dilution of the 10 cosmid mix with pMH86 at 12.5 μg/ml and pBS(KS)− (Stratagene) at 80 μg/ml also showed rescuing activity. Similar experiments using the *rol-6(d)* marker pRF4 (MELLO *et al.* 1990), in which >10 lines containing the *sel-1* gene were analyzed, failed to show any rescue in this assay, probably due to lack of germ-line expression. We narrowed the rescuing activity to two overlapping cosmids, C06C11 and T17C2. Individual cosmids were assayed at 5–10 μg/ml each with pBS(KS)− at 75–80 μg/ml, and pMH86 at 12.5 μg/ml. C06C11 rescued 2/3 lines; T17C2 rescued 3/7 lines.

Random subclones from C06C11, cloned into pBS(KS)−, were assayed in mixed pools at DNA concentrations similar to the experiments above. Pools that rescued were then subdivided and re-assayed. Results from rescue assays of single plasmids from rescuing pools were as follows: plasmid H9.5 rescued 1/2 lines, R9.0 rescued 0/2 lines, H9.5B rescued 0/8 lines, pB1 rescued 1/1 lines, pB1Xho rescued 0/6 lines (Figure 1).

Analysis of *sel-1* complementation in a *lin-12* background was done as follows: *unc-36(e251) lin-12(n676n930)/qC1;sel-1(e1948)* animals were injected with *sel-1* plasmids at 5 μg/ml, and pRF4 at 100 μg/ml. Stable Rol lines were generated and homozygous Rol Unc animals were scored for egg laying. Egg laying assays were done by picking L4 animals of the appropriate genotypes grown at 25° to new plates and re-scoring them over the next 2 days for the presence of many eggs near an Unc mother (non-Egl) or severe bloating with a lack of eggs laid (Egl). pB1(RS) rescued 5/5 lines, CSTOP rescued 0/4 lines, FSTOP rescued 4/5 lines, and PB1B rescued 2/3 lines. We also assayed a chromosomally integrated array containing the rescuing plasmid FSTOP (Figure 3) for complementation of *sel-1* activity in the AC/VU decision (GREENWALD *et al.* 1983; SEYDOUX and GREENWALD 1989). We found that 8/11 (72%) animals of the genotype *lin-12(n676n930);sel-1(1948);arIs25[FSTOP]* had two ACs, indicating *sel-1* complementation (SUNDARAM and GREENWALD 1993a,b).

Manipulation of recombinant DNA: Standard molecular biological protocols were performed as described in SAMBROOK *et al.* (1989). DNA sequence was determined using the Sequenase 2.0 kit from U.S. Biochemicals. Most sequencing reactions were done using single stranded DNA templates.

DNA fragments (2–3 kb) from genomic and cDNA clones were subcloned into pBS(KS)− and subsequently used to generate nested sets of deletions using *Exo*III and S1 nuclease (Promega Erase-A-Base System). Genomic DNA from pB1 was sequenced on both strands from position −865 (*Hin*dIII) to 2997 (within the first intron of the downstream gene) and on one strand only from 2998 to the end (*Eco*RV). Only one strand of cDNA clones BB1C and BB1F were sequenced. Sequence was analyzed with computer programs written by STADEN (1986), Genetics Computer Group ver. 7 (DEVEREUX *et al.* 1984), and IBI-MacVector ver. 4.5. Signal sequences were analyzed by the method of VON HEIJNE (1986) using the computer program AnalyzeSignalase ver. 2.03. C-17 and Q-18 received scores of 8.95 and 8.54, respectively, where scores greater than 3.0 are considered indicative of a genuine signal sequence and cleavage site. The predicted protein product of BB1F does not contain any known protein motifs or significant hydrophobic sequences. It does not show significant similarity to any proteins in current databases. Northern analysis was performed on total RNA, typically 15 μg per lane, size separated on a 1.5% formaldehyde gel, and transferred to Nytran (Schleicher and Schuell). Immobilized RNAs were probed either with random primed ^{32}P-dCTP-labeled DNA probes according to membrane manufacturer's instructions, or ^{32}P-ATP-labeled antisense RNA probes as in AUSTIN and KIMBLE (1989).

cDNA cloning and mapping cDNA ends: The entire 7.1-kb insert from pB1 was used to screen a *C. elegans* mixed stage cDNA library (BARSTEAD and WATERSTON 1989). Eight cDNAs were purified from a larger group of positive clones. By sequencing both ends of these cDNA clones, we determined that seven overlapping clones were derived from the 5′ region of pB1 and that one cDNA was derived from nonoverlapping 3′ sequences within pB1. The sequence of the longest 5′ cDNA (BB1C) of 2394 bp, and the 3′ cDNA (BB1F) of 1491 bp, indicated that two genes were lying 102 bp apart, in the same orientation, within this genomic DNA fragment. The 5′ and 3′ ends of mRNAs from the 5′ gene (*sel-1*), were PCR amplified, cloned, and sequenced. PCR-RACE was performed according to manufacturer's instructions (BRL), including cDNA synthesis from total RNA and poly-G tailing. 5′ RACE: cDNA synthesis was primed with primer SEQ#4, and the *sel-1* 5′ end was subsequently amplified with primer SEQ#2 and the anchor primer (BRL). 3′ RACE: cDNA synthesis was primed with the AP primer (BRL), and the *sel-1* 3′ end was amplified with primer F1-FOR and primer UAP (BRL). Two 5′ RACE products containing the entire 22 nucleotide SL1 sequence were cloned and sequenced. Two 3′ RACE products containing poly-A tails were cloned and sequenced.

Sequence analysis of *sel-1* mutants: Analysis of mutant lesions was done by direct sequencing of PCR products, similar to published methods (ALLARD *et al.* 1991; KALTENBOECK *et al.* 1992). PCR was used to amplify the *sel-1* genomic region from each mutant using primers C1-FOR and C1-REV. A second round of PCR was then used to generate single-stranded DNA using only primer C1-FOR. ssDNA was then used as a template for Sequenase v2.0 (USB) with primers SEQ#1-4, NdeGAP1, SEQ#6-10, QE70REV, and SEQ#12. Reactions that identified sequence changes were repeated at least once with completely independent PCR products. The entire coding sequence was covered for *ar23, ar29, ar75, ar77, ar167,* and *e1948.* Partial sequencing of *sel-1(ar166)* shows that it does not have the *e1948* lesion, indicating that is a new allele.

Plasmids used for rescue: *H9.5*: A 9.5-kb pBS− subclone from C06C11, beginning at the same *Hin*dIII site 5′ of *sel-1* (-865) in the sequenced plasmid pB1, but extending another 2.4 kb 3′ of the *Eco*RV site. *R9.0*: A 9-kb pBS− subclone from C06C11, beginning at an *Eco*RI site ~5.6 kb 5′ of the pB1 *Hin*dIII site (−865) and extending to the pB1 *Eco*RI site (2544). *pB1*: The sequenced *sel-1* genomic plasmid in pBS− extending from *Hin*dIII (−865) to *Eco*RV (~6235). *pB1(RS)*: A derivative of pB1 in which pBS− polylinker sequences were removed between *Eco*RV and *Ecl*136II (*Sac*I), destroying both sites. *H9.5Bgl*: A derivative of H9.5 in which all sequences 5′ of a *Bgl*II site (equivalent to pB1 2815) were deleted. *pB1Xho*: A derivative of pB1 in which all sequences 3′ of a *Xho*I site (within the downstream gene) were deleted. *CSTOP*: A derivative of pB1 in which two copies of an *Nhe*I linker (NEB) containing stop codons in all frames were ligated into the *Sty*I site (306). *FSTOP*: A derivative of pB1(RS) in which three copies of an *Nhe*I linker (NEB) containing stop codons in all frames were ligated into the *Bst*XI site (2831). *PB1B*: A derivative of pB1 in which all sequences 3′ of the *Bst*XI site (2831) were deleted.

Oligonucleotides: The position of the last base relative to the genomic sequence is given in parentheses. The A(TG) of the *sel-1* initiator Met codon is +1. C1-FOR: CCG AAC GAT GCT CCG TTA TTA CGC (−172); C1-REV: GGG TCT GGA CAA ATG GCA AAA GTG (+2762); F1-FOR: CCT ACT CGG GTC TAC GCT GTC (+2474); SEQ#1: GCT ATT ACT TAC CTT GG (+306); SEQ#2: CAA CGG GAA TAA TCT CC (+514); SEQ#3: AAA CAC TCA CCA TAG CC (+709); SEQ#4: TGT TTC GTC GGT TAG TC (+919); NdeGAP1: GTC CGA GTC CCA ATT GAG (+1030); SEQ#6: GCC TGT GCA CTA GGA C (+1243); SEQ#7: TTT GTT GGT TGG AAC ACC (+1452); SEQ#8: GCT ATG GGC TTC CAT C (+1655); SEQ#9: TTT CAC GCG AGC AGC CAG C (+1916); SEQ#10: CGG CAT GTA TGC ATC CTG (+2132); QE70REV: AGA TCT ATC CCA TCG TGG TCC AAC (+2237); and SEQ#12: CGT AGA CCC GAG TAG G (+2454).

RESULTS

The *sel-1* null phenotype: *sel-1* mutations were originally isolated as suppressors of the egg laying defect (Egl phenotype) of *lin-12(n676n930),* a hypomorph (SUNDARAM and GREENWALD 1993a,b). These *sel-1* alleles appeared to result in reduced *sel-1* function, and, in an otherwise wild-type background, *sel-1* mutations did not show any detectable phenotypes (SUNDARAM and GREENWALD 1993b). However, because the original suppressor screen identified only egg laying competent (non-Egl) revertants, isolation of lethal, Egl, or sterile *sel-1* alleles could have been precluded.

To determine the null phenotype of *sel-1,* we isolated new *sel-1* mutations in an unbiased noncomplementation screen (see MATERIALS AND METHODS). This screen had the potential to detect *sel-1* alleles with homozygous visible, lethal, or sterile phenotypes. We based this screen upon the observation that, at the restrictive temperature of 25°, animals of the genotype *glp-1(e2142);sel-1(e1948)/arDf1* produce live progeny, while *glp-1(e2142);sel-1(e1948)/sel-1(+)* animals do not (data not shown). After screening ~10,500 mutagenized haploid genomes, two new *sel-1* alleles were isolated, *sel-1(ar166)* and *sel-1(ar167).*

Both new *sel-1* alleles are recessive suppressors of *lin-12(n676n930)* and *glp-1(e2142).* In addition, neither displays any apparent phenotype in a *lin-12(+) glp-1(+)* background (see MATERIALS AND METHODS). Molecular

analysis of *sel-1(ar167)* has revealed that it contains a stop codon very early in the predicted *sel-1* coding region. The truncated product of this mutant (8% of full length) is unlikely to retain any *sel-1* activity (see below). The lack of any phenotype of either new allele obtained in an unbiased screen, taken together with molecular analysis of mutant lesions and previous gene dosage studies, indicates that the *sel-1* null phenotype is wild type. Complete loss of *sel-1* function leads to an apparent increase in *lin-12* or *glp-1* activity when assayed in sensitized genetic backgrounds, but is silent in an otherwise wild-type background.

Cloning the *sel-1* locus: *sel-1* was previously mapped between *rol-4* and the cloned gene *lin-25* on LGV, an interval of ~0.2 map unit (SUNDARAM and GREENWALD 1993b; TUCK and GREENWALD 1995). *lin-25* represented a right-hand marker present on both the genetic and physical maps. We found that *arP5* represented a left-hand marker present on both the genetic and physical maps (see MATERIALS AND METHODS). The *arP5* to *lin-25* interval is ~250 kb and is completely covered by overlapping cosmid clones (COULSON *et al.* 1986, 1988).

We tested cosmid clones from this region of the *C. elegans* physical map for the ability to complement *sel-1*-mediated suppression of the *glp-1* maternal effect lethal (MEL) phenotype at 25° (see MATERIALS AND METHODS). Overlapping cosmid clones C06C11 and T17C2 were found to complement *sel-1* in this assay (Figure 1). We further localized *sel-1(+)* activity to a 7.1-kb subclone of C06C11, contained in plasmid pB1. Smaller subclones failed to rescue *sel-1* in this assay. A pB1 extrachromosomal array that complemented *sel-1* in the *glp-1* based assay was then crossed into a *lin-12;sel-1* background. This extrachromosomal array complemented *sel-1* for its ability to suppress the egg-laying defect of *lin-12* at 25° (see MATERIALS AND METHODS).

The *sel-1* operon: Clones hybridizing to the 7.1-kb complementing genomic fragment were isolated from a *C. elegans* mixed stage cDNA library (BARSTEAD and WATERSTON 1989). The sequence of the longest members of two cDNA classes, BB1C and BB1F, indicated that two genes were lying 102 bp apart, in the same orientation, within this genomic DNA fragment. BB1C represents an mRNA derived from the 5′ most gene (beginning at position 1 of clone pB1), while BB1F was derived from the 3′ genomic region (beginning at position 2753 of clone pB1) (Figure 1). Sequence and Northern blot analysis was consistent with each representing a separate gene transcript rather than alternatively spliced products of the same gene. Additional Northern analysis showed that BB1C exclusively recognized a 2.4-kb RNA species, while BB1F exclusively recognized a 1.5-kb RNA species (data not shown). When the 7.1-kb genomic DNA was used as a probe, it hybridized to both RNA species (Figure 2). The BB1C cDNA 5′ end contained the last nine nucleotides of the SL1 *trans*-spliced leader (KRAUSE and HIRSH 1987). We confirmed the addition of SL1 to mRNAs from the 5′ gene by PCR RACE (see MATERIALS AND METHODS). We determined the 3′ end of the message by PCR RACE and found that poly-A sequences of 15 and 18 nucleotides were added to mRNAs from the 5′ gene 11 nucleotides downstream of a putative poly-A addition signal AACAAA (Figure 5). This sequence has been tentatively identified as a poly-A addition signal in a small percentage of *C. elegans* mRNAs (T. BLUMENTHAL, personal communication). The BB1F cDNA contained a partial *trans*-spliced leader at its 5′ end identical to the last 14 nucleotides of members of the SLn family of *trans*-spliced leaders (L. ROSS and C. S. RUBIN, personal communication). BB1F also contained a 24 nucleotide poly-A sequence at it 3′ end. The orientation and close spacing of these two genes, as well as the use of an alternative *trans*-spliced leader sequence on an mRNA from the 3′ gene, is consistent with known properties of *C. elegans* operons (SPIETH *et al.* 1993; BLUMENTHAL 1995).

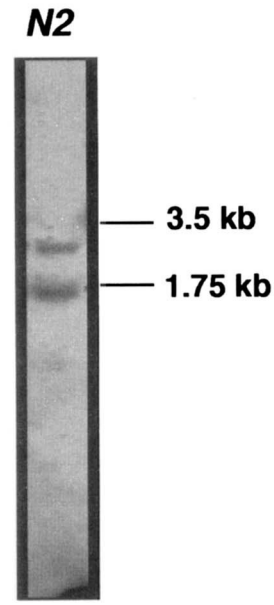

FIGURE 2.—Northern analysis of *sel-1* and its downstream neighbor. Total RNA from mixed stage *C. elegans* populations was size separated by gel electrophoresis, transferred to nitrocellulose, and hybridized with a probe encompassing the entire 7.1-kb *sel-1* complementing genomic DNA fragment. Relative positions of the rRNA bands are shown for size comparison. The top band of 2.4 kb represents mRNAs from the *sel-1* (5′) gene. The bottom band of 1.5 kb represents mRNAs from the down-stream (3′) gene.

To determine which of these genes was *sel-1*, we analyzed the *sel-1* complementing activity of three engineered forms of the rescuing plasmid pB1, the relative abundance of transcripts for each gene in *sel-1* mutant strains, and ultimately the sequence changes present in the 5′ gene in *sel-1* mutant strains (see below). We made two derivatives of the pB1 rescuing plasmid in which the coding sequence of either the 5′ or 3′ gene was interrupted by stop codons (Figure 3). We also constructed a plasmid, PB1B, in which almost all of the 3′

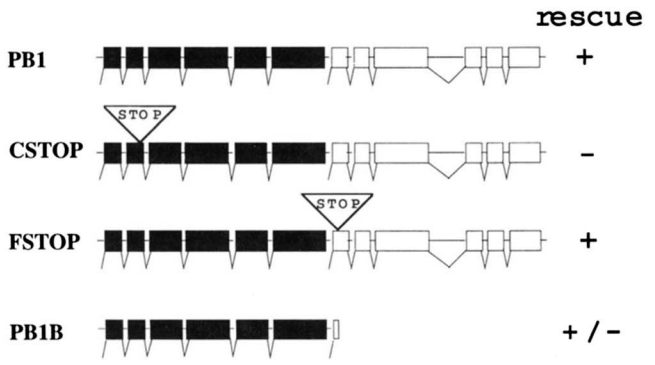

FIGURE 3.—Identification of the 5′ gene as *sel-1*. The gene structure for each construct is shown. Boxes represent coding region and lines represent noncoding region. Constructs were assayed for rescue of *sel-1(e1948)* in a *lin-12(n676n930)* background (see MATERIALS AND METHODS). +, rescue; −, failure to rescue.

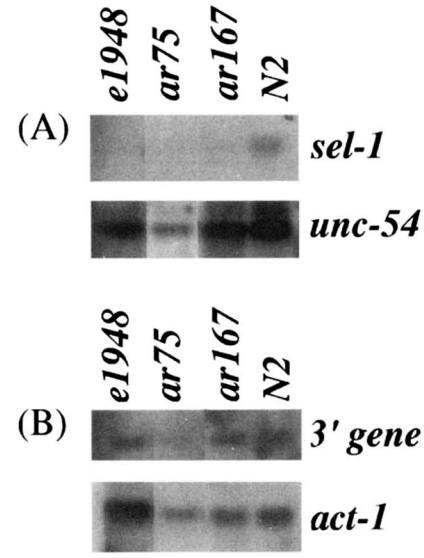

FIGURE 4.—Northern analysis of *sel-1* mutants. Total RNA isolated from *sel-1* mutant strains was size seperated by gel electrophoresis and transferred to nitrocellulose. The name of *sel-1* alleles from which RNA was isolated is listed above each lane. Each panel is a composite of nonadjacent lanes from the same autoradiogram. (A) The top panel shows the results of hybridization to a 5′ gene probe. The bottom panel shows the same filter stripped and rehybridized to an *unc-54* (myosin) cDNA probe. (B) The top panel shows the results of a hybridization to a 3′ gene probe. The bottom panel shows the same filter stripped and rehybridized with an *act-1* (actin) cDNA probe.

gene is deleted but the 5′ gene remains intact (Figure 3). We assayed these constructs for the ability to complement *sel-1* in a *lin-12;sel-1* strain (see MATERIALS AND METHODS). Clones containing stop codons in the 5′ gene failed to rescue, while clones with stop codons interrupting the 3′ gene rescued as well as pB1. The PB1B construct, deleted for most 3′ gene sequences, rescued in this assay, although not as well as pB1. A similar plasmid, pB1Xho, failed to rescue in the *glp-1* based assay mentioned earlier (see MATERIALS AND METHODS; Figure 1). This may indicate that regulatory elements, which enhance transcription of both genes, are present within the 3′ gene. These sequences may be most important for germ-line and/or early embryonic expression of *sel-1*.

As further evidence that the 5′ gene is *sel-1*, we also compared the relative abundance of steady state transcript levels in seven *sel-1* mutant alleles with that of wild type. Northern analysis of total RNA isolated from each strain, probed with either the BB1C or BB1F cDNAs, showed that transcript levels from the 5′ gene were strongly reduced in *sel-1(ar167)*, *sel-1(e*1948), and *sel-1(ar75)* (Figure 4). Transcript levels from the 3′ gene remain unchanged in *sel-1* mutant strains (Figure 4). Strong reductions in steady-state transcript levels are often found in mutants containing stop mutations early within their open reading frames, which lead to unusually long 3′ untranslated regions (HODGKIN *et al.* 1989; PULAK and ANDERSON 1993). Taken together, these rescue experiments and Northern analysis strongly argue that *sel-1* is the 5′ gene corresponding to the BB1C cDNA. We further confirmed the identity of the 5′ gene as *sel-1* by identifying molecular lesions within its coding region for six *sel-1* mutants (see below).

The *sel-1* product: *sel-1* encodes a predicted protein product of 685 amino acids. Hydropathy analysis (KYTE and DOOLITTLE 1982) of the *sel-1* predicted protein revealed two strongly hydrophobic sequences at positions 2–16 and 661–678 (Figure 5). The hydrophobic sequence at positions 2–16 is strongly predicted to be a functional secretory signal sequence when analyzed by the method of VON HEIJNE (1986). Significant scores are given for signal peptidase cleavage after C-17 and Q-18 (see MATERIALS AND METHODS). Cleavage after residue C-17 would produce a mature product of 669 amino acids, with a predicted molecular weight of 74 kD. The hydrophobic sequence at position 661–678 is very near the carboxy terminus. This 16 amino acid hydrophobic sequence is probably too short to span either plasma or organellar membranes, but might serve as a glycosyl-phosphotidylinositol linkage (GPI) signal (FERGUSON and WILLIAMS 1988; TAKEDA and KINOSHITA 1995).

Extensive comparison of the complete predicted SEL-1 sequence with that of all reported gene products revealed a short region of homology between SEL-1 and the predicted products of the human cDNA Ibd2 (Genbank accession number HSU11037) and *S. cerevisiae* predicted gene *L8167.5* (Genbank accession number YSCH8167). This region of SEL-1, residues 563–631, is 54% identical to IBD2. Nearly the same region of SEL-1, residues 569–635, is 41% identical to a similar region of L8167.5 (Figure 6). It may be that this sequence identifies a new structural feature or functional motif. The possibility that these sequences perform similar functions is strengthened by our observation that *L8167.5*, like *sel-1*, is predicted to encode a secreted

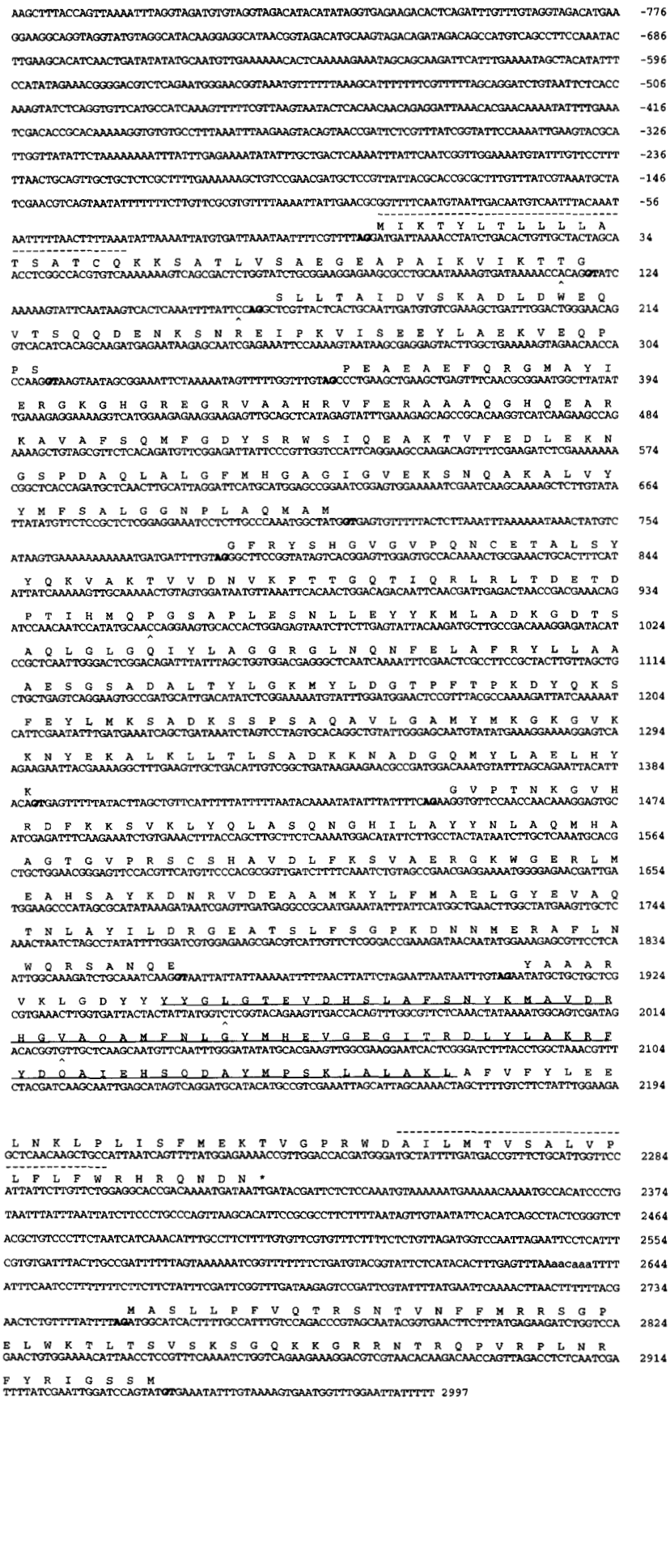

FIGURE 5.—The complete *sel-1* genomic DNA sequence. Bases are numbered such that 1 denotes the predicted translational start of *sel-1*. Amino acid translation is given in the single letter code over the first base of the corresponding codon. Gaps without amino acid sequence denote noncoding sequence identified by comparison of *sel-1* cDNA and genomic sequences. Predicted splice-donors (GT) and splice-acceptors (AG) are shown in bold face type. The first exon and part of the first intron of the gene 3′ of *sel-1* are shown beginning at 2753. Sequences bearing the highest similarity to *Ibd2* and *L8167.5* are underlined. Amino acid residues mutated in *sel-1* mutant strains are marked above (^). The putative polyadenylation signal is shown in lower case type. The Genbank accession numbers are U50828 for the genomic sequence and U50829 for the cDNA sequence.

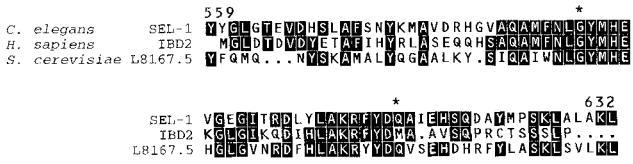

FIGURE 6.—Sequence alignment of SEL-1 with IBD2 and L8167.5. Sequence identities are shown with white type on a black background. Amino acid residues altered in *sel-1* mutant strains are marked with an asterisk above.

protein with a significantly hydrophobic sequence C terminal to the homology region. Since the available Ibd2 sequence is incomplete, derived from an apparently partial cDNA, comparisons are limited. Sequence analysis of *sel-1* mutants underscores the importance of the conserved region for SEL-1 function (Figure 7 and below).

Sequence analysis of *sel-1* mutants: Genomic DNA from *sel-1* mutant strains was PCR amplified and sequenced directly using gene-specific primers across the length of the *sel-1* gene (see MATERIALS AND METHODS). Four of the six *sel-1* mutant lesions identified result in the introduction of premature stop codons within predicted coding sequence (Figure 7). *sel-1(ar167), sel-1(ar75)* and *sel-1(e*1948) introduce stop codons early within the predicted coding sequence. They are therefore predicted to produce severely truncated protein products and are the best candidates for null alleles. The other nonsense mutant, *sel-1(ar77),* introduces a stop codon near the C terminus of the predicted *sel-1* protein, which would lead to deletion of part of the homology domain and the potential GPI linkage signal. *sel-1(ar23)* and *sel-1(ar29)* change the same conserved glycine residue, within the homology domain, to arginine and glutamic acid, respectively (Figure 7).

Gene specificity: Because *sel-1* shows strong genetic interactions with the LIN-12 and GLP-1 receptors, we sought to test the specificity of these interactions. We looked at the ability of *sel-1* mutants to suppress mutations in many different receptor gene families (see Table 1). Because *sel-1* mutations do not affect *lin-12* or *glp-1* null mutants (SUNDARAM and GREENWALD 1993b), we have used nonnull alleles of these receptors where available. We tested hypomorphic alleles of two receptor tyrosine kinases (RTKs), *let-23* (AROIAN *et al.* 1990) and *egl-15* (DEVORE *et al.* 1995), for interactions with *sel-1* mutations. *let-23* is required for VPC cell fate determination, and *egl-15* is required for proper sex myoblast migration. We also tested a hypomorphic allele of *tra-2,* the putative receptor for the *her-1* gene product, a sex determination signal (KUWABARA *et al.* 1992; PERRY *et al.* 1993). Finally, we examined double mutants between *sel-1* and loss of function alleles of *daf-4,* a receptor serine-threonine kinase (ESTEVEZ *et al.* 1993), or *lin-17,* a seven transmembrane domain protein (STERNBERG and HORVITZ 1988; H. SAWA and H. R. HORVITZ, personal communication). None of these receptor mutants was suppressed by loss of *sel-1* function (see Table 1). Over the course of this work we have also made double mutant combinations between *sel-1* and mutations in other genes that produce extracellular products and have noted no interactions between them (data

TABLE 1

Gene specificity of *sel-1* suppression/enhancement

Genotype[a]	Percent mutant[b]
lin-12(n676n930)	96[c]
lin-12(n676n930);sel-1(e1948)	6[c]
glp-1(e2142)	100[c]
glp-1(e2142);sel-1(e1948)	0[c]
let-23(sy1)	97[d]
let-23(sy1);sel-1(e1948)	94 (50)
egl-15(n484)	94 (70)
egl-15(n484);sel-1(e1948)	96 (75)
egl-15(n1477)	100 (>100)
egl-15(n1477);sel-1(e1948)	100 (50)
tra-2(b202)	100[e]
tra-2(b202);sel-1(ar167)	100 (>200)
daf-4(m63)	100[f]
daf-4(m63);sel-1(e1948)	100 (>300)
daf-4(e1364)	100[f]
daf-4(e1364);sel-1(e1948)	100 (>1000)
lin-17(n671)	67 (69)
lin-17(n671);sel-1(ar167)	69 (45)

[a] Full genotypes shown in MATERIALS AND METHODS.
[b] Values in parentheses are number of animals. See MATERIALS AND METHODS.
[c] Data of SUNDARAM and GREENWALD (1993b).
[d] Data of AROIAN and STERNBERG (1991).
[e] Data of KLASS *et al.* (1976).
[f] Data of RIDDLE (1977).

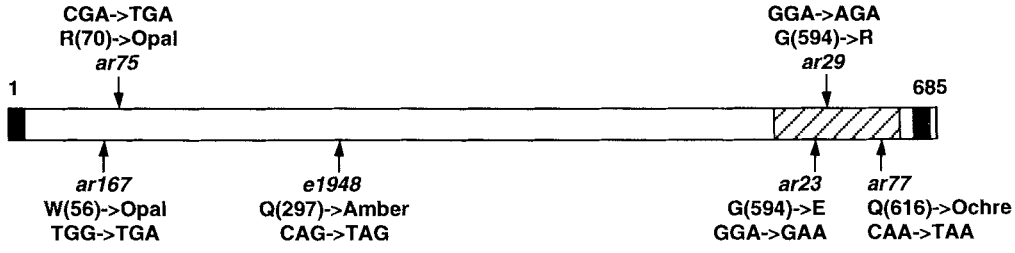

FIGURE 7.—*sel-1* mutant lesions. SEL-1 is shown as a box, amino terminus to the left, carboxy-terminus to the right. The relative positions and sequence changes in *sel-1* mutants are shown. Hydrophobic sequences are shown as black boxes. Sequence most similar to IBD2 and L8167.5 is shown with diagonal stripes.

not shown). These genes include *lin-3*, an EGF-like signal, and *sqt-3*, a collagen (HILL and STERNBERG 1992; VAN DER KEYL *et al.* 1994). Thus it seems that loss of *sel-1* function specifically affects mutations in *lin-12* and *glp-1*, and not mutations in other genes encoding membrane proteins.

DISCUSSION

***sel-1* is a negative regulator of *lin-12* and *glp-1* activity:** We have established here that eliminating *sel-1* function leads to an apparent increase in *lin-12* and *glp-1* activity. Thus we can infer that *sel-1(+)* acts as a negative regulator of *lin-12* and *glp-1* activity or is negatively regulated by them. We have also established that the *sel-1* null phenotype is wild type, except in sensitized genetic backgrounds.

There are a number of reasons why loss of *sel-1* function might have such dramatic phenotypic effects in sensitized genetic backgrounds, but not in otherwise wild-type animals. Genetic redundancy is one likely explanation (reviewed in THOMAS 1993). For example, even if SEL-1 interacts directly with LIN-12 and GLP-1, redundant controls at multiple regulatory levels could produce normal cell fates when only one regulatory step is compromised. In addition, even single regulatory steps can be redundantly controlled. Probably the simplest example of such redundant control would be if *sel-1* were a member of a gene family whose products are themselves interchangeable with SEL-1. In this case, loss of one family member would only reduce the overall "SEL-1" activity level fractionally. Such a mild reduction in SEL-1 activity might be tolerated under normal circumstances, but not when *lin-12* or *glp-1* activity has already been altered. With *lin-12* or *glp-1* activity close to a threshold level, small changes in regulatory control could lead to changes in cell fates.

Another explanation for why *sel-1* mutations have visible effects on weak alleles of *lin-12* and *glp-1*, but not on *lin-12(+)* and *glp-1(+)*, could be that *sel-1(+)* is only capable of modulating *lin-12* and *glp-1* activities within narrow limits. For instance, the function of *sel-1* might be to reduce incrementally *lin-12* and *glp-1* activities during cell fate decisions, such that interactions occur more slowly, perhaps with slightly higher fidelity. Even gene products that contribute to small changes in signal transduction may be important contributors to *lin-12* or *glp-1*-mediated signaling, since in some cases, small differences in *lin-12* activity between cells may be greatly amplified by a feedback mechanism during cell fate decisions (SEYDOUX and GREENWALD 1989; WILKINSON *et al.* 1994). While a gene that has a small effect on cell fate decisions may increase the evolutionary fitness of an animal, its loss might escape detection in simple assays of differentiated cell fates under laboratory conditions.

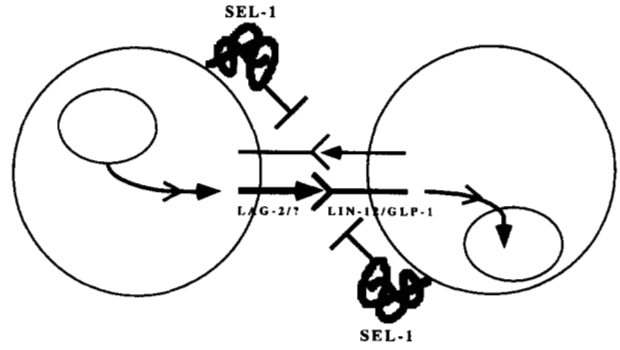

FIGURE 8.—A model for SEL-1 function. The diagram represents a pair of equipotent cells undergoing a *lin-12* or *glp-1* mediated cell fate decision. SEL-1 is shown as a cell-surface protein present on the surface of receptor (*lin-12* or *glp-1*) and/or ligand (*lag-2* or others) expressing cells, down-regulating the interaction. SEL-1 could function to reduce the amount or activities of receptor or ligand molecules. For instance, SEL-1 could function as part of a mechanism to specifically down-regulate the receptor after activation by ligand, by targeting the ligand-receptor complex for degradation or ligand release and recycling.

SEL-1 is an extracellular protein: We have analyzed the predicted protein product of the *sel-1* gene for the presence of hydrophobic sequences, repeats, and known functional motifs. The only notable features identified were hydrophobic sequences near the amino and carboxy-termini of SEL-1 and several repeats of the short sequence LXYY, where X represents any amino acid. The significance of this repeat sequence is unknown at present. The amino-terminal hydrophobic residues comprise part of a predicted secretory signal sequence (VON HEIJNE 1986). Indeed, the SEL-1 amino terminus, when fused to normally cytoplasmic proteins, can direct these proteins into the secretory pathway (B. GRANT and I. GREENWALD, unpublished data). No hydrophobic sequences long enough to form a membrane spanning domain were identified, but the C-terminal hydrophobic sequence might comprise a signal for GPI linkage. If SEL-1 is a GPI-linked protein, then it is likely to associate with the outer membrane of cells in which it is expressed (FERGUSON and WILLIAMS 1988; TAKEDA and KINOSHITA 1995). This does not preclude the possibility of diffusion of SEL-1 to interact with non-expressing cells, however, as recent evidence indicates that GPI-linked proteins can move from one cell surface to another (KOOYMAN *et al.* 1995). Thus, sequence analysis suggests that SEL-1 is probably an extracellular protein, possibly associated with the surface of cells undergoing *lin-12* and *glp-1* mediated cell-cell interactions.

We compared the predicted SEL-1 sequence with all known proteins and predicted gene products. SEL-1 has a region of high similarity with the *S. cerevisiae* predicted gene product L8167.5 and the human predicted gene product IBD2. The region of highest similarity between these predicted proteins (~50% identity over 70 amino acids) may define a new functional or structural motif

that has been conserved over evolution. The importance of this sequence for SEL-1 function is underscored by our finding that *sel-1(ar23)* and *sel-1(ar29)* both contain changes in G-594 within this domain, while *sel-1(ar77)* is predicted to produce a truncated product missing part of this domain.

SEL-1 may be a specific regulator of LIN-12 and GLP-1 activity: There are many possible mechanisms by which loss of a cell surface molecule might lead to increases in apparent receptor activation. We considered the possibility that altering a ubiquitous cellular function, to which SEL-1 contributes, might suppress *lin-12* and *glp-1* mutants when their activities are altered. For instance, changes in the processing, modification, targeting, and recycling of membrane proteins, particularly receptors and/or their respective ligands, could lead to apparent increases in signaling.

We constructed seven double mutant combinations between *sel-1* and receptors that are not related to LIN-12 and GLP-1. These mutations include an allele of *let-23* thought to be sensitive to decreased recycling efficiency (LEE *et al.* 1994). We found that *sel-1* mutations do not suppress or enhance mutations in any of these other receptors. Although these tests are not exhaustive, they may indicate that SEL-1 plays a specific role in LIN-12 and GLP-1 regulation, rather than a broad role in generalized mechanisms required for the proper function of receptors. One possibility is that SEL-1 could interact directly with the LIN-12 and GLP-1 receptors, their respective ligands, and/or other cell surface regulators of LIN-12 and GLP-1 activity, influencing the fates adopted by interacting cells (Figure 8). For instance, LIN-12 and GLP-1 could utilize an extracellular mechanism, mediated by SEL-1, to down-regulate signaling after ligand-mediated activation, much as insulin receptor signaling is down-regulated by dephosphorylation of its intracellular domain (GOLDSTEIN 1993). Further analysis of the localization of SEL-1, and the possible physical interactions between SEL-1 and LIN-12, GLP-1, and/or their ligands should help clarify these issues.

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Note added in proof: sel-1 genomic and cDNA sequences have been submitted to GenBank.

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