SEL-5, A Serine/Threonine Kinase That Facilitates *lin-12* Activity in *Caenorhabditis elegans*

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ABSTRACT

Ligands present on neighboring cells activate receptors of the LIN-12/Notch family by inducing a proteolytic cleavage event that releases the intracellular domain. Mutations that appear to eliminate *sel-5* activity are able to suppress constitutive activity of *lin-12(d)* mutations that are point mutations in the extracellular domain of LIN-12, but cannot suppress *lin-12(intra)*, the untethered intracellular domain. These results suggest that *sel-5* acts prior to or during ligand-dependent release of the intracellular domain. In addition, *sel-5* suppression of *lin-12(d)* mutations is tissue specific: loss of *sel-5* activity can suppress defects in the anchor cell/ventral uterine precursor cell fate decision and a sex myoblast/coelomocyte decision, but cannot suppress defects in two different ventral hypodermal cell fate decisions in hermaphrodites and males. *sel-5* encodes at least two proteins, from alternatively spliced mRNAs, that share an amino-terminal region and differ in the carboxy-terminal region. The amino-terminal region contains the hallmarks of a serine/threonine kinase domain, which is most similar to mammalian GAK1 and yeast Pak1p.

DURING development, cells with equivalent potential adopt different fates as a consequence of cell-cell interactions. Many such interactions are mediated by receptors of the LIN-12/Notch family (reviewed in Greenwald 1998) binding to transmembrane protein ligands of the Delta/Serrate/LAG-2 (DSL) family (Greenwald 1998). Ligand binding appears to induce cleavage in or near the transmembrane domain to release the intracellular domain (Lieber *et al.* 1993; Struhl *et al.* 1993; Kopan *et al.* 1994; Schroeter *et al.* 1998; Struhl and Adachi 1998). The intracellular domain of LIN-12/Notch proteins translocates to the nucleus, where it complexes with transcription factors of the CBF1/Suppressor of Hairless/LAG-1 family and participates in transcriptional regulation of target genes (Jarriault *et al.* 1995; Kopan *et al.* 1996; Chen *et al.* 1997; Eastman *et al.* 1997; Struhl and Adachi 1998).

Other members of the LIN-12/Notch pathway, and factors that influence the activity of the LIN-12/Notch pathway, have been conserved evolutionarily. Some of these components have been identified in genetic screens based on **s**uppression or **e**nhancement of *lin-12* mutations (*sel* genes). Screens that rely on suppressing missense mutations that cause constitutive LIN-12 activity have yielded at least seven genes. Three of these genes have been characterized molecularly and have been found to be conserved components that are important for LIN-12/Notch activity. *lag-2*, a ligand gene, was identified in such screens by antimorphic alleles (Tax *et al.* 1994, 1997). *sel-12*, a presenilin gene, was defined by loss-of-function mutations (Levitan and Greenwald 1995). Presenilin, which was identified independently by mutations that cause familial early-onset Alzheimer's disease by altering the processing of β-amyloid precursor protein (Selkoe 1998), appears to be critical for the transmembrane proteolytic processing event that constitutes LIN-12/Notch signal transduction (DeStrooper *et al.* 1999; Struhl and Greenwald 1999). Finally, *sup-17*, which encodes a transmembrane disintegrin/metalloprotease of the ADAM family, was defined by loss-of-function mutations (Tax *et al.* 1997; Wen *et al.* 1997); the Drosophila homolog of this gene, *kuzbanian*, was identified independently in genetic screens on the basis of its *Notch*$^-$ phenotype (Rooke *et al.* 1996).

We report the characterization of another gene, *sel-5*, which was identified in a screen for suppressors of missense mutations that activate LIN-12 (Tax *et al.* 1997). Tax *et al.* (1997) identified two alleles of *sel-5* in their suppressor screen and performed genetic mosaic analysis, which suggested that *sel-5* suppression is cell autonomous. Here, we have performed additional genetic analysis and have shown that *sel-5* encodes two proteins containing a common serine/threonine kinase domain. We discuss possible ways *sel-5* activity may influence *lin-12* activity.

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MATERIALS AND METHODS

General *Caenorhabditis elegans* methods and strains: General methods for the handling and maintenance of *C. elegans* are as described previously (Brenner 1974). The wild-type parent for all strains was *C. elegans* var. Bristol N2 (Brenner 1974). The alleles used in this work are:

Linkage group (LG) *I*: *smg-1(r861)* (Hodgkin *et al.* 1989), *unc-54(r293)* (Pulak and Anderson 1993).

LG *III*: *unc-79(e1068)* (Sedensky and Meneely 1987), *mab-21(bx53)* (Baird *et al.* 1991), *dpy-17(e164)* (Brenner 1974), *ncl-1(e1865)* (Hedgecock and Herman 1995), *unc-36(e251)* (Brenner 1974), *unc-32(e189)* (Brenner 1974), *lin-12(n302)* (Greenwald *et al.* 1983), *lin-12(n676)* (Greenwald *et al.* 1983), *lin-12(n950)* (Greenwald *et al.* 1983), *lin-12(n137)* (Greenwald *et al.* 1983), *lin-12(ar170)* (Hubbard *et al.* 1996), *glp-1(e2141)* (Priess *et al.* 1987), *glp-1(e2142)* (Priess *et al.* 1987), *glp-1(ar202)* (J. Hubbard and I. Greenwald, unpublished observations). *sDp3* is a free duplication of part of this chromosome (Rosenbluth *et al.* 1985). *sDf121(s2098)* is a homozygous lethal deletion, which is rescued by *sDp3*, of part of this chromosome (Stewart *et al.* 1998).

LG *IV*: *dpy-20(e1282)* (Hosono *et al.* 1982).

LG *V*: *him-5(e1467)* and *him-5(e1490)* (Hodgkin *et al.* 1979).

LG *X*: *sel-12(ar131)* (Levitan and Greenwald 1995).

Transgenes: *arIs12* [*lin-12(intra)*] (Struhl *et al.* 1993) expresses the intracellular domain of LIN-12 under the control of *lin-12* regulatory sequences and is marked with the dominant marker *rol-6(su1006)*. *arIs13* [*lag-2::lacZ*] (Wilkinson *et al.* 1994) carries the reporter *lag-2::lacZ* and is marked with *rol-6(su1006)*. *arIs41* (Levitan and Greenwald 1998) expresses a functional LIN-12::GFP fusion protein under the control of *lin-12* regulatory sequences and is marked with *rol-6(su1006)*. *arEx29* (K. Fitzgerald and I. Greenwald, unpublished results) is an extrachromosomal array carrying multiple copies of the *lin-12(+)* genomic region and is marked with *rol-6(su1006)*.

Genetic mapping of the *sel-5* locus: To map *sel-5* relative to *mab-21*, we examined recombinants segregating from *unc-79(e1068) mab-21(bx53) dpy-17(e164)*/*sel-5(n1254) lin-12(n302); him-5(e1490)* hermaphrodite parents. Of the 34 Unc non-Dpy hermaphrodites picked, 16 had the recombinant chromosome *unc-79(e1068) sel-5(n1254) lin-12(n302)*, 16 had the chromosome *unc-79(e1068) mab-21(bx53) lin-12(n302)*, and 2 had the chromosome *unc-79(e1068) lin-12(n302)*. This last class of recombinants places *sel-5* to the left of the cloned gene *mab-21* (Chow *et al.* 1995; Figure 1).

To map *sel-5* relative to *sDf121*, we mated *sel-5(n1254) lin-12(n302); him-5(e1490)* males to *dpy-17(e164) sDf121(s2098) unc-32(e189)*/*dpy-17(e164) ncl-1(e1865) unc-36(e251)* hermaphrodites. Non-Dpy F_1 hermaphrodite progeny were picked to individual plates and scored for egg laying, and their genotype was deduced from markers present in their progeny (F_2).

Determining the left endpoint of *sDf121*: The left endpoint of *sDf121* was determined using the polymerase chain reaction (PCR) with test primers in that region on unhatched eggs laid by the strain *dpy-17(e164) sDf121(s2098) unc-32(e189); sDp3*, essentially as was previously done for the right endpoint of this deletion (Stewart *et al.* 1998). The main difference is that in every reaction, we included a positive control primer pair (known to be outside the region of the deletion; GCGAT TGGGCGAACTGGTAACCACAG and CCGTCGAGCCAGC CAAGCGACAACCATCGC) and a negative control primer pair (from a region known to be deleted in *sDf121* and is covered by *sDp3*, GGTGGTATTATTGTATCCATAAACGC and AGTATTGACACCCAAAGAATATAAC), in addition to the test primer pair (TGATTACTGTAAGTTGCTACAAGATA and AATGTCTTCAGTATGTAGTTTGTTAC for F35G12; CTAACATCATTCTATTGAGCTGCTTG and TTGGACAAT GTGCCGAAAGTTCAGAC for F56F3). More than 10 eggs were scored for the presence of the test primer pair expected band (~400 bp) on an agarose gel when the expected band (~200 bp) was detected for the positive control primer pair (signifying the presence of DNA in the reaction) and no band of the expected size (~600 bp) was detected for the negative control primer pair (signifying that the unhatched egg did not have *sDp3*).

Analysis of mutant phenotypes: Two independent isolates of each genotype were tested; in every case, they gave similar results, and the data are pooled in the tables. The presence of *sel-5* mutations was confirmed by sequencing and/or complementation analysis (see Table 1) in strains in which these alleles did not have a visible effect. For all assays, Egl^+ hermaphrodites were allowed to lay eggs for a timed interval (usually overnight) and all progeny produced during that interval were analyzed; for egg-laying defective (Egl) hermaphrodites, all progeny were analyzed. Similar results were seen with both methods. To score egg laying, L4 hermaphrodites were placed on a single seeded plate and checked for 2 (20°) or 4 (15°) more days. To check the anchor cell, the presence of a vulva, or the existence of dorsal coelomocytes, worms at the appropriate stage were checked at 630× magnification with a Zeiss Axiophot compound microscope. The number of pseudovulvae in hermaphrodites and males and the presence of ectopic hooks in male tails were all scored with a dissection microscope.

Molecular analysis: Standard methods were used for the manipulation of recombinant DNA (Sambrook *et al.* 1989), unless otherwise indicated. All enzymes were from New England Biolabs (Beverly, MA), unless otherwise indicated. PCR was done using the Expand long template PCR system (Boehringer Mannheim, Mannheim, Germany) according to the manufacturer's instructions.

***sel-5* cDNA and mutant alleles sequence analysis:** To determine the 5′ end of *sel-5*, we performed PCR on a *C. elegans* cDNA library (Barstead and Waterston 1989) using the primers PstSL1 (ACACTGCAGGGTTTAATTACCCAAGTTT GAG), containing a *Pst*I site (underlined) followed by the SL1 *trans*-spliced leader; (Krause and Hirsh 1987) and HindF35N (ACAAAGCTTCTACAGTTCCAGAACCGGATGGCA TTG), containing a *Hin*dIII site (underlined) followed by *sel-5* sequence from an exon in the cDNA clone yk7a7. The 1.1-kb PCR fragment was digested with *Pst*I and *Hin*dIII and subcloned into the same sites in the pBluescript SK^+ vector (Stratagene, La Jolla, CA) to give plasmid pJF93.

To determine the complete *sel-5A* cDNA sequence, we linked the overlapping sequences of the yk7a7 (generously provided by Y. Kohara) and pJF93 inserts. To determine the complete *sel-5B* cDNA sequence, we linked the overlapping sequences of the yk13e1 (generously provided by Y. Kohara) and pJF93 inserts; we also sequenced the insert in plasmid pJF99 (see below).

The lesions associated with *sel-5(n1250)* and *sel-5(n1254)* were determined by sequencing several PCR products from single-stranded templates (Allard *et al.* 1991; Kaltenboeck *et al.* 1992), by use of internal primers to cover all exons and exon/intron boundaries. To determine the extent of the deletion in *sel-5(ok149)*, we did single worm PCR (Williams *et al.* 1992) on these worms using the primers internal to *sel-5* (except for the added restriction sites) PstIR1 (ACACTG CAGTTTCTTCCAGGTGGATTTGC; the added *Pst*I site is underlined) and BamIL1 (ACAGGATTCCAAACACATCATC CACCACC; the added *Bam*HI site is underlined). The 1.3-kb PCR fragment was digested with *Pst*I and *Bam*HI, subcloned

TABLE 1

***sel-5* suppresses the 0 AC-Egl defect caused by *lin-12(d)* alleles**

Relevant genotype	Percentage of Egl[a] hermaphrodites	
	15°	20°
+	0 (n = many)	0 (n = many)
lin-12(n302)	100 (n = many)	>99.9 (n = many)
sel-5(n1250) lin-12(n302)	30.8 (n = 130)	71.7 (n = 113)
sel-5(n1254) lin-12(n302)	32.6 (n = 138)	58.4 (n = 142)
sel-5(ok149) lin-12(n302)	27.4 (n = 135)	56 (n = 134)
lin-12(n676)	100 (n = many)	100 (n = many)
sel-5(n1250) lin-12(n676)	59.7 (n = 109)	66.7 (n = 105)
sel-5(n1254) lin-12(n676)	26.2 (n = 122)	77.3 (n = 110)
lin-12(n950)	100 (n = 94)	100 (n = 118)
sel-5(n1250) lin-12(n950)	94.9 (n = 196)	97.9 (n = 145)
sel-5(n1254) lin-12(n950)	94.7 (n = 114)	99.1 (n = 110)
sel-5(ok149) lin-12(n950)	95.8 (n = 142)	97.5 (n = 80)
lin-12(n137)	100 (n = many)	100 (n = 138)
sel-5(n1254) lin-12(n137)	100 (n = 185)	99.3 (n = 139)
arEx29[lin-12(+)]	45.2 (n = 168)	82.9 (n = 105)
sel-5(n1254); arEx29	15.2 (n = 184)	37.5 (n = 80)
+ lin-12(n302)[b]	ND	55 (n = 100)
+ +		
sel-5(n1254) lin-12(n302)[c]	ND	41.7 (n = 60)
+ +		
sel-5(n1254) lin-12(n302)[d]	ND	0 (n = 75)
sel-5(n1250) +		
sel-5(n1254) lin-12(n302)[e]	ND	1.6 (n = 190)
sel-5(n1254) +		
sel-5(n1254) lin-12(n302)[f]	ND	0 (n = 120)
sel-5(ok149) +		
sel-5(n1254) lin-12(n302)[g]	ND	2 (n = 47)
sDf121(s2098) +		

n, number of hermaphrodites scored.
[a] Egl, egg-laying defective.
[b] Actual genotype: *lin-12(n302)/dpy-17(e164) unc-32(e189).*
[c] Actual genotype: *sel-5(n1254) lin-12(n302)/dpy-17(e164) unc-32(e189).*
[d] Actual genotype: *sel-5(n1254) lin-12(n302)/sel-5(n1250) unc-32(e189).*
[e] Actual genotype: *sel-5(n1254) lin-12(n302)/sel-5(n1254) unc-32(e189).*
[f] Actual genotype: *sel-5(n1254) lin-12(n302)/sel-5(n1254) unc-32(e189).*
[g] Actual genotype: *sel-5(n1254) lin-12(n302)/dpy-17(e164) sDf121(s2098) unc-32(e189).*

into the same sites of pBluescript SK$^+$, and sequenced. *sel-5(ok149)* has 1.977 kb deleted and the sequence AAATT CAAATGACGAGAT inserted in its place.

Sequence comparisons and alignments were obtained using the Blast program (Altschul *et al.* 1990) through the NCBI Web site and GCG version 8 programs (Devereux *et al.* 1984).

Plasmid constructions: The vector litmus38D1 was made by digesting litmus38 (New England Biolabs) with *Mfe*I and *Eco*RI and religating the compatible ends, thus removing all restriction sites between them. The vector PIN2 drives inserted sequences under the control of *sel-12* regulatory sequences; it contains unique *Bam*HI and *Not*I sites inserted at the second amino acid of a *sel-12* rescuing genomic fragment containing 2.8 kb of 5′ flanking region (D. Levitan and I. Greenwald, unpublished observations).

Plasmid pJF98 is the 13.5-kb *Nhe*I fragment from cosmid F35G12 (generously provided by A. Coulson) subcloned into the same site in litmus38D1. This plasmid contains a genomic fragment of *sel-5* with 6.237 kb of 5′ flanking sequence and 0.589 kb of 3′ flanking sequence (downstream of the *sel-5A* polyadenylation site).

pJF99 is the 2.08-kb PCR fragment amplified from a *C. elegans* cDNA library (Barstead and Waterston 1989) using primers F35A1 (ACACGGCCG**ATG**CCTCTAGGGCTTTTCA GCTCTGGAAAAG; the added *Eag*I site is underlined and is followed by the initiation codon of *sel-5* shown in boldface) and F35B1(ACACGGCCG**CTA**AACTTGAAAACCACGAGAAG TGGTTC; the added *Eag*I site is underlined and is followed by the stop codon of *sel-5B* shown in boldface type), restriction digested with *Eag*I, and inserted into the same site in litmus38D1. The pJF99 insert was completely sequenced to confirm it contains the *sel-5B* cDNA. pJF99 was digested with *Sna*BI and *Apa*I (sites present only in the vector), blunt ended with T4 DNA polymerase, and religated, thus removing all the restriction sites in between and creating plasmid pJF101.

The 2.08-kb *Eag*I fragment from pJF99 was subcloned into the *Not*I site of PIN2 yielding plasmid pJF120 (*sel-5B* under the control of *sel-12*). PCR was done on plasmid KSGFPS65T (Levitan and Greenwald 1998) using primers SpeGFP1 (ACAACTAGTCCCATGAGTAAAGGAGAAGAACTTTTCAC TGG; the added *Spe*I site is underlined) and SpeGFP2 (ACAACTAGTTTTGTATAGTTCATCCATGCCATGTC; the

TABLE 2

sel-5* does not suppress the 0 AC defect caused by *lin-12(intra)

Relevant genotype	Percentage of hermaphrodites with 0 AC
Wild type	0 (n = many)
arIs12[lin-12(intra)][a]	72.7 (n = 33)
sel-5(n1250); arIs12[b]	78.0 (n = 59)
sel-5(n1254); arIs12[c]	77.5 (n = 80)
sel-5(ok149); arIs12[d]	78.4 (n = 51)

Assays were done at 15°. n, number of hermaphrodites scored.

[a] Actual genotype: *unc-32(e189); dpy-20(e1282); arIs12*. For this genotype and those that follow, *dpy-20* was included to faciliate scoring of ACs by minimizing rolling due to the transgene marker.

[b] Actual genotype: *sel-5(n1250) unc-32(e189); dpy-20(e1282); arIs12*.

[c] Actual genotype: *sel-5(n1254) unc-32(e189); dpy-20(e1282); arIs12*.

[d] Actual genotype: *sel-5(ok149) unc-32(e189); dpy-20(e1282); arIs12*.

added *Spe*I site is underlined) yielding a 0.7-kb fragment. This fragment was digested with *Spe*I and inserted into the same site of pJF101, in frame, to get a translational fusion, thus yielding plasmid pJF104. The 2.7-kb *Eag*I fragment from pJF104 was subcloned into the *Not*I site of PIN2 yielding plasmid pJF110, which therefore has SEL-5B::GFP(S65T) under the control of *sel-12* sequences.

pJF103 is the 1.64-kb *Eag*I-*Spe*I fragment from pJF101 ligated with the 5.4-kb *Eag*I-*Spe*I fragment from pyk7a7 (circularized plasmid from yk7a7), thus reconstituting the *sel-5A* cDNA (ATG to stop codon with 3′ untranslated sequences). PCR was done using pJF103 as template and the primers F35A1 and F35A2 (ACACGGCCG**TTA**CAAGTCGGTTGGATCAT CATGATCTTCC; the added *Eag*I site is underlined and is followed by the stop codon of *sel-5A* shown in boldface type) and yielded a 3.2-kb fragment. This fragment was digested with *Eag*I and inserted into the same site in litmus38D1 yielding plasmid pJF105, which contains the *sel-5A* cDNA (ATG to stop codon). pJF106 is pJF105 digested with *Sna*BI and *Apa*I (sites in vector), blunt ended with T4 DNA polymerase, and recircularized, thus removing the restriction sites between these two enzymes.

The 3.2-kb *Eag*I insert from pJF106 was inserted into the *Not*I site of PIN2, thus yielding plasmid pJF113, which has *sel-5A* cDNA under the control of *sel-12* regulatory sequences. pJF109 is the 0.7-kb PCR fragment (template KSGFPS65T, primers SpeGFP1 and SpeGFP2, see above) carrying GFP-(S65T), digested with *Spe*I, and inserted into the same site in pJF106, in frame. The 3.9-kb *Eag*I fragment from pJF109 was subcloned into the *Not*I site of PIN2 to make plasmid pJF111, which therefore has SEL-5A::GFP(S65T) under the control of *sel-12*.

To place GFP at the C terminus of SEL-5A, PCR was done on KSGFPS65T using primers BamGFP1 (ACAGGATCCCAT GAGTAAAGGAGAAGAACTTTTCACTGG; the added *Bam*HI site is underlined) and BamGFP2 (ACAGGATCCTTTGTA TAGTTCATCCATGCCATGTG; the added *Bam*HI site is underlined) yielding a 0.7-kb fragment. This fragment was digested with *Bam*HI and used to replace the 0.178-kb *Bam*HI fragment in pJF105 to give plasmid pJF107. The 3.7-kb *Eag*I insert from pJF107 was subcloned into the *Not*I site of PIN2, thus making plasmid pJF108, which has SEL-5A::GFP(S65T) (GFP at the C terminus; see results) under the control of *sel-12*.

Worm transformation: Microinjection of DNA into the germ line of *C. elegans* hermaphrodites was done essentially as previously described (Fire 1986; Mello *et al.* 1991). Plasmid DNA was injected at 1–50 μg/ml. For most experiments, pRF4 (Mello *et al.* 1991), carrying *rol-6(su1006)*, was injected at 150 μg/ml as a cotransformation marker. For the SEL-5::GFP localization experiments, pMH86 (Han and Sternberg 1991), carrying wild-type *dpy-20*, was injected at 20 μg/ml into a *dpy-20(e1282)* background as a cotransformation marker.

Double-stranded RNA synthesis and microinjection: Double-stranded RNA was synthesized *in vitro* essentially as described previously (Fire *et al.* 1998). Briefly, RNA was synthesized from phagemid clones using an RNA transcription kit (Stratagene) in which both the T3 and the T7 polymerase were added to the same reaction. The RNA was purified on RNeasy columns (Qiagen, Valencia, CA), eluted in water, diluted in injection buffer (Fire *et al.* 1998), and allowed to anneal at 37° for 10–30 min.

The RNA was microinjected into the pseudocoelomic space of young adult hermaphrodites. Injected worms were placed on individual seeded plates and the phenotype(s) of their F_1 progeny was checked.

RESULTS

The AC/VU decision and *lin-12* genetics (background): Two gonadal cells, named Z1.ppp and Z4.aaa,

TABLE 3

***sel-5* does not enhance mutations that reduce *lin-12* activity**

Genotype	Percentage of hermaphrodites with one AC	
	15°	20°
+	100 (n = many)	100 (n = many)
sel-5(n1250)	100 (n = 56)	100 (n = 85)
sel-5(n1254)	100 (n = 59)	100 (n = 56)
sel-5(ok149)	100 (n = 69)	100 (n = 86)
lin-12(ar170)	93 (n = 40)	60 (n = 47)
sel-5(n1254) lin-12(ar170)	93.8 (n = 48)	63 (n = 49)

Hermaphrodites had either one or two ACs. The percentage is expressed as the number of hermaphrodites with one AC divided by the total number of hermaphrodites scored (n).

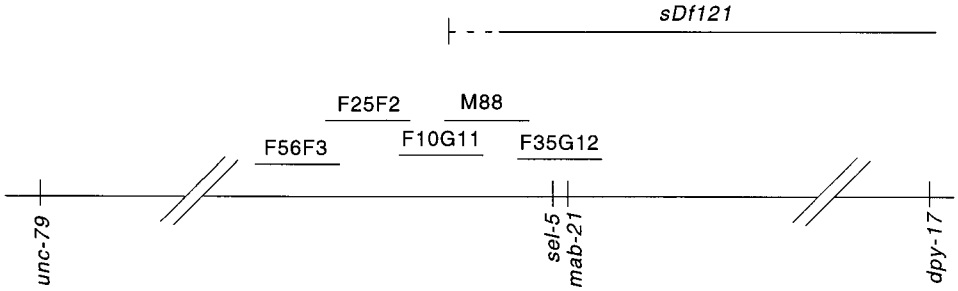


Figure 1.—The *sel-5* region of LG *III*. Only the relevant genes, cosmids, and rearrangements mentioned in the text are shown. Both *sel-5* and *mab-21* are contained on cosmid F35G12 (30, 709 kb). *unc-79* is ~0.5 cM to the left of *sel-5*, while *dpy-17* is ~1.5 cM to the right of *sel-5*. The left endpoint of *sDf121* was found to lie between sequences in F35G12 and F10G11 (see text).

are initially equivalent in their developmental potential in that each has an equal chance of becoming the anchor cell (AC), a terminally differentiated cell type, or a ventral uterine precursor cell (VU), which contributes descendants to the ventral uterus (Kimble and Hirsh 1979). However, in any given hermaphrodite, only one of these cells will become the AC, while the other becomes a VU, depending on *lin-12*-mediated interactions between them (Kimble 1981; Greenwald *et al.* 1983; Seydoux and Greenwald 1989). This process is termed the "AC/VU decision." Studies of genetic mosaics and reporter genes have suggested that a stochastic variation in *lag-2* (ligand) and *lin-12* (receptor) activity between Z1.ppp and Z4.aaa is amplified by a feedback mechanism in both cells, so that *lag-2* expression becomes restricted to the presumptive AC and *lin-12* expression becomes restricted to the presumptive VU (Seydoux and Greenwald 1989; Wilkinson *et al.* 1994).

Mutations that eliminate *lin-12* activity result in two ACs (Greenwald *et al.* 1983). Mutations that constitutively activate *lin-12* cause the absence of an AC and hence such mutants lack a vulva (Greenwald *et al.* 1983); this phenotype is referred to below as "0 AC-Egl." There are two different types of constitutively activated *lin-12* alleles: *lin-12(d)* mutations, a set of missense mutations in the extracellular domain (Greenwald and Seydoux 1990), and *lin-12(intra)*, a transgene that encodes just the intracellular domain (Struhl *et al.* 1993). The key difference between these activated forms is that LIN-12(d) proteins are transmembrane proteins that presumably must undergo proteolytic processing to release the intracellular domain for signal transduction, whereas LIN-12(intra) does not.

TABLE 4

Identification of *sel-5* sequences by candidate gene RNAi

dsRNA	ORF	No. of suppressed F_1 broods/ No. of worms injected
P25[a]	*lin-12*	9/9
yk45f5	*F25F2.2*	0/12
yk20f5	*M88.5*	0/9
yk7a7[b]	*F35G12.3*	9/11
yk13e1	*F35G12.3*	5/15
yk53f6	*F35G12.4*	0/8

Experiments were performed at 20°; similar results were obtained at 15°. *lin-12(n302)* hermaphrodites were injected with dsRNA, and their progeny were examined for the presence of Egl^+ hermaphrodites. Suppressed broods gave ~5–75% egg-laying F_1 hermaphrodites.

[a] All F_1 hermaphrodites checked had one AC. When wild-type (N2) hermaphrodites were injected with P25 dsRNA, occasional hermaphrodites had two ACs, but most appear phenotypically wild type.

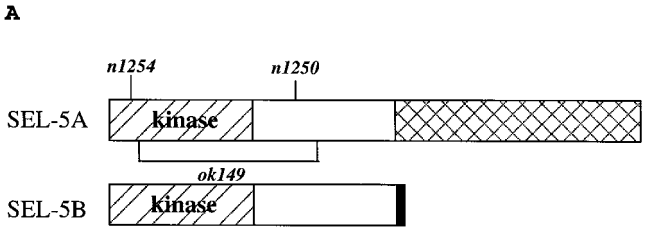
[b] When wild-type (N2) hermaphrodites were injected with yk7a7 dsRNA, most F_1 hermaphrodite progeny had one AC and no visible defects.

Genetic analysis of *sel-5* in the AC/VU decision: Two alleles of *sel-5*, *sel-5(n1250)* and *sel-5(n1254)*, were identified in screens for suppressors of the 0 AC-Egl defect caused by the *lin-12(d)* allele *lin-12(n950)* (Tax *et al.* 1997). A new allele, *sel-5(ok149)*, was generated by the *C. elegans* gene knockout consortium when the coding region was identified (as described below). The *sel-5* mutations are essentially recessive to *sel-5(+)*, but *sel-5/sDf121* and heteroallelic combinations display suppressor activity (Table 1), suggesting that *sel-5* mutations are loss-of-function. This inference has been confirmed by sequence analysis of *sel-5* mutations, as described below.

sel-5 mutations appear to reduce *lin-12* activity, as all three *sel-5* alleles suppress the 0 AC-Egl phenotype of *lin-12(d)* alleles by restoring one AC (Table 1). The proportion of hermaphrodites with one AC depends on two factors:

1. Temperature: All three *sel-5* alleles also appear to be cold sensitive, *i.e.*, suppression of *lin-12(d)* is greater at low temperature (15°). As *sel-5(n1254)* and *sel-5(ok149)* are likely to be molecular null alleles, this observation suggests that the process in which *sel-5* functions is cold sensitive.
2. The starting level of *lin-12* activity: *sel-5* mutations are more efficient suppressors when the level of constitutive *lin-12* activity is lower. For example, *sel-5* is an efficient suppressor of the 0 AC-Egl defect of *lin-12(n302)*, a "weaker" activated allele, than *lin-12(n137)*, a "stronger" activated allele. In addition, *sel-5* homozygotes, and heteroallelic combinations, are more efficient suppressors of *lin-12(n302)/+* than *lin-12(n302)*.

To gain insight into the interactions between *sel-5* and *lin-12* in the AC/VU decision, we examined the interaction between *sel-5* and *lin-12(intra)*. *lin-12(intra)*,



B

```
                ▼ SL1
tttactaatttcaggcgaaaagccATGCCTCTAGGGCTTTTCAGCTCTGGAAAAGCACAAGTTCTGTGTGATGAA   51
                        M  P  L  G  L  F  S  S  G  K  A  Q  V  L  C  D  E     17

AAAATTCCTGGAGGCAAGAAAAAGGAACCGAAACAATTAAGTGAGAATAAATGCAAAGgtat-40 nt-tcagGT  159
K  I  P  G  G  K  K  K  E  P  K  Q  L  S  E  N  K  C  K  G                   37

GTCACATTGAAGTTAGACCATACACGAGTCACTATAGAGAAGCAAATCGCCGAAGgtag-75 nt-ccagGTGGA  302
V  T  L  K  L  D  H  T  R  V  T  I  E  K  Q  I  A  E  G                  G     57

TTTGCAATCGTCTACGTTGCGAGCGATAGAAAAAACAATAAATTCGCCTTGAAACGTCAATTCACCAAAGACAAT  377
F  A  I  V  Y  V  A  S  D  R  K  N  N  K  F  A  L  K  R  Q  F  T  K  D  N     82

                                                        a→n1254
GAGAAACAGCTGGAGGCATGTTGCAGAGAGCACAGTTTTCTGgtga-39 nt-tcagAAACAATGTATTGGTCAT  484
E  K  Q  L  E  A  C  C  R  E  H  S  F  L                  K  Q  C  I  G  H     102

AAAAATATTGTGGAATTCGTTGACAGTTATACAAATTGCCTTGGAAATGGTATTTGGGAATGCATGCTGTTGACA  559
K  N  I  V  E  F  V  D  S  Y  T  N  C  L  G  N  G  I  W  E  C  M  L  L  T     127

GAATATCATCAAAgtga-80 nt-ccagAAAACGTCTTGCAATTGATGAACGAAAGAATATCTCAAAACCAATAT  707
E  Y  H  Q  K                 N  V  L  Q  L  M  N  E  R  I  S  Q  N  Q  Y     147

TTAACAAACGACGAGATTCTCTCAATTTTCACGGATCTCTGTGAAGCCGTATCCTTCATTCATAATCGGCCACAA  782
L  T  N  D  E  I  L  S  I  F  T  D  L  C  E  A  V  S  F  I  H  N  R  P  Q     172

CCTATCATTCATCGCGATTTAAAAGTTGAAAATGTACTCATTTCTTCCCATAAACCACCACATTACGTTCTCTGC  857
P  I  I  H  R  D  L  K  V  E  N  V  L  I  S  S  H  K  P  P  H  Y  V  L  C     197

GATTTTGGAAGTGCTACCACTCAAATTTTATCTGTGGAAAAGTATGGAGTAGAATATGTGAAGTCAGAAGTGGAA  932
D  F  G  S  A  T  T  Q  I  L  S  V  E  K  Y  G  V  E  Y  V  K  S  E  V  E     222

AGGAATACGACTATGTGTTATCGATCGCCTGAAATGATTGATTTTTACAGTGGATTAGAGATTGgttc-57 nt-  1057
R  N  T  T  M  C  Y  R  S  P  E  M  I  D  F  Y  S  G  L  E  I  G              244

tcagGCCTAAAATCTGATATCTGGGCACTGGGCGTTCTTCTATACAGGCTATGCTTTTTCTGTGTTCCTTTCGAA  1132
      L  K  S  D  I  W  A  L  G  V  L  L  Y  R  L  C  F  F  C  V  P  F  E     267

GAATCACCGTTAGCAATTCAATCTGTCAATTATCAATTTCCATCCGTTCCAAATATTCCCGACGAGATCAAAGTT  1207
E  S  P  L  A  I  Q  S  V  N  Y  Q  F  P  S  V  P  N  I  P  D  E  I  K  V     292

TTGATTTgtaa-39 nt-ctagACATGCTTCTCGATATCGACGTAAATCGGCGACCATCGATATATCAAACATCA  1314
L  I  Y                 M  L  L  D  I  D  V  N  R  R  P  S  I  Y  Q  T  S     312

GTGTTGGCGTTTGAAGCGAATCATCGGAAACCACTTTCAGAAGAGATTCAAAATAAGAAAgtta-93 nt-tcag  1475
V  L  A  F  E  A  N  H  R  K  P  L  S  E  E  I  Q  N  K  K                   332
                                 ▲ end of kinase domain homology

TGCACCGACGCAGTTCCTTCATTAAAATCATGTATTCAATTGATGAGAGATGGATCGAATCCACGTAACAAAAGA  1550
C  T  D  A  V  P  S  L  K  S  C  I  Q  L  M  R  D  G  S  N  P  R  N  K  R     357

GATTCATCACCACGAAATCCTGAAGCTCCGCCAATTCAATCAAGCTCCAAAATGGCTTCTTTGTCTCAACAAGTC  1625
D  S  S  P  R  N  P  E  A  P  P  I  Q  S  S  S  K  M  A  S  L  S  Q  Q  V     382
```

Figure 2.—*sel-5* predicted proteins and mutant alterations. (A) Schematic representation of the two SEL-5 proteins. Similar shading indicates regions of identical amino acid sequence. The positions of the various mutations for the different alleles are indicated (see text for details). (B) Relevant genomic sequence of *sel-5* and predicted protein sequences. Coding nucleotide (nt) sequence is shown in uppercase letters; noncoding sequence is shown in lowercase letters. Numbering of the nucleotide sequence starts at the A (+1) of the initiation codon. The positions of the SL1 *trans*-spliced leader sequence and the polyadenosine tails for the alternatively spliced mRNAs are indicated by arrowheads above the nucleotide sequence. The nucleotide changes in *sel-5(n1250)* and *sel-5(n1254)* are also shown above the nucleotide sequence. The nucleotides underlined are deleted in *sel-5(ok149)* and replaced by the sequence AAATTCAAATGAC GAGAT. The predicted SEL-5A protein sequence is shown below the nucleotide sequence. SEL-5B has the same amino terminus; the divergent SEL-5B predicted sequence is shown above the nucleotide sequence. The predicted end of the putative kinase domain is indicated. The double underlined amino acids (929–987) were replaced by GFP in the SEL-5::GFP construct (see text).

like *lin-12(d)* alleles, results in constitutive *lin-12* activity (Struhl *et al.* 1993). However, *sel-5* is unable to suppress the 0 AC defect associated with *arIs12* [*lin-12(intra)*] (Table 2). We think that the failure of *sel-5* to suppress *lin-12(intra)* is not likely due simply to differences in the degree of constitutive activity (point 2 above), as *lin-12(intra)* has a lower penetrance of the 0 AC defect and hence appears to have lower constitutive activity than *lin-12(n302)* and *lin-12(n676).* As described above, LIN-12(intra) is a cytosolic protein that is not associated with the plasma membrane, whereas LIN-12(d) mutations are transmembrane proteins. The interactions between *sel-5* and different activated forms therefore suggest that *sel-5* does not act in signal transduction by activated LIN-

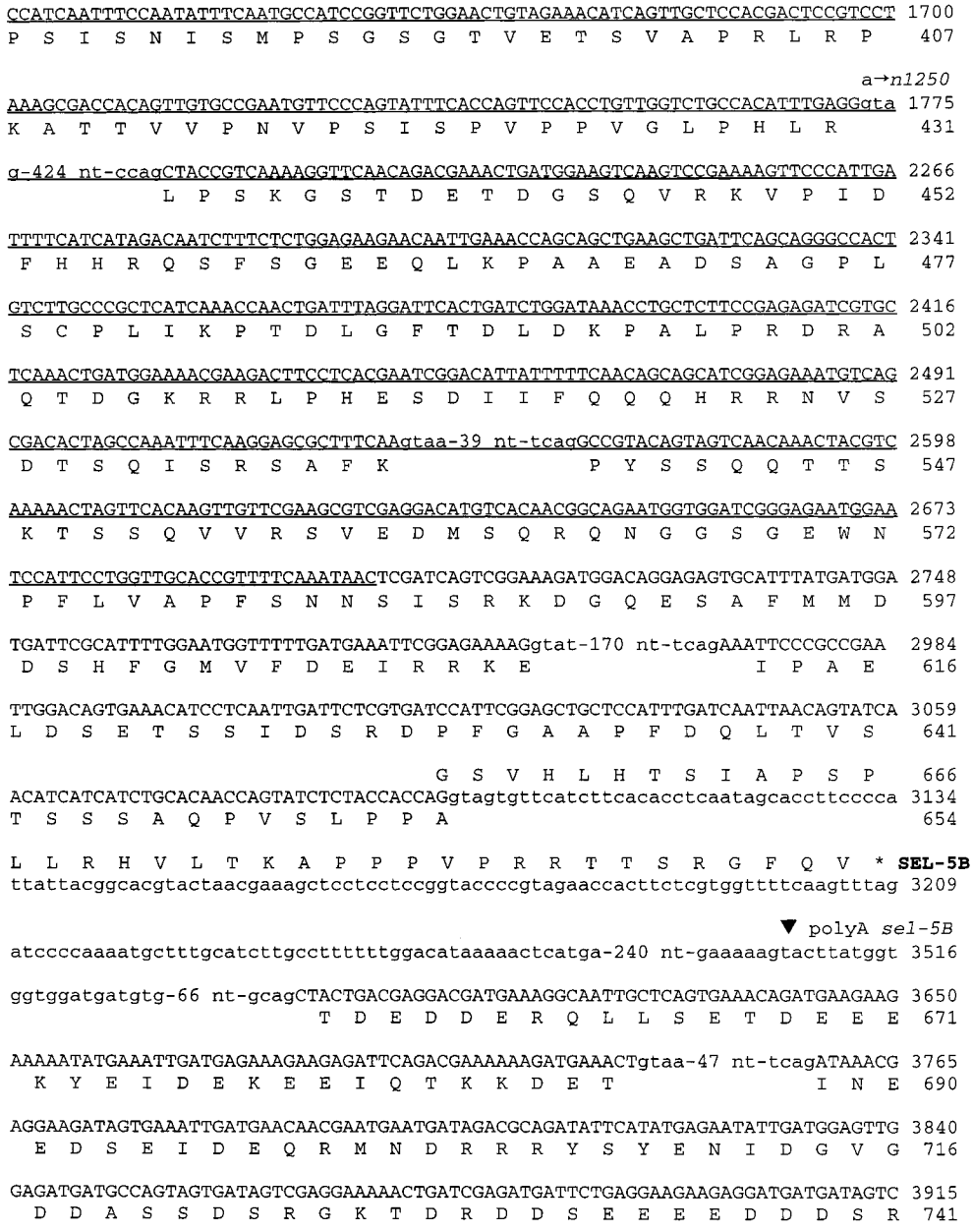


Figure 2.—*Continued.*

12 and instead suggest a role for SEL-5 prior to or during ligand-dependent release of the intracellular domain.

None of the *sel-5* mutations cause defects in the number of anchor cells (Table 3). Mutations that reduce *lin-12* activity might enhance the 2 AC defect of *lin-12* hypomorphic alleles, or might cause a synthetic 2 AC defect when combined with mutations in genes encoding other factors that facilitate *lin-12* activity. However, we have not seen any evidence for such interactions for *lin-12(ar170),* a *lin-12* hypomorphic mutation (Hubbard *et al.* 1996) (Table 3). We do not know if these negative results mean that full-length LIN-12 must be constitutively active for *sel-5* to influence its activity, as in *lin-12(d)* mutants or when LIN-12(+) is overexpressed, or whether there is a difference in the degree to which *lin-12* activity must be lowered to see an effect [*i.e.*, *lin-12(d)* suppression may require less of a reduction in *lin-12* activity than causing a 2 AC phenotype under these conditions].

We also investigated the potential involvement of *sel-5* in transcriptional control of *lag-2* and *lin-12.* In wild-type hermaphrodites, *lag-2::lacZ* and *lin-12::lacZ* transcriptional reporter genes are initially expressed in both Z1.ppp and Z4.aaa, and a stochastic fluctuation is amplified by a feedback mechanism so that only the presumptive anchor cell expresses LAG-2, while the presumptive VU expresses only LIN-12 (Wilkinson *et al.* 1994). A similar result is seen with a *lin-12::gfp* translational reporter (Levitan and Greenwald 1998). We examined the expression of the *lag-2::lacZ* and *lin-12::gfp* in *sel-5(n1254)* hermaphrodites and saw no change in transcription patterns as compared to *sel-5(+)* (data not

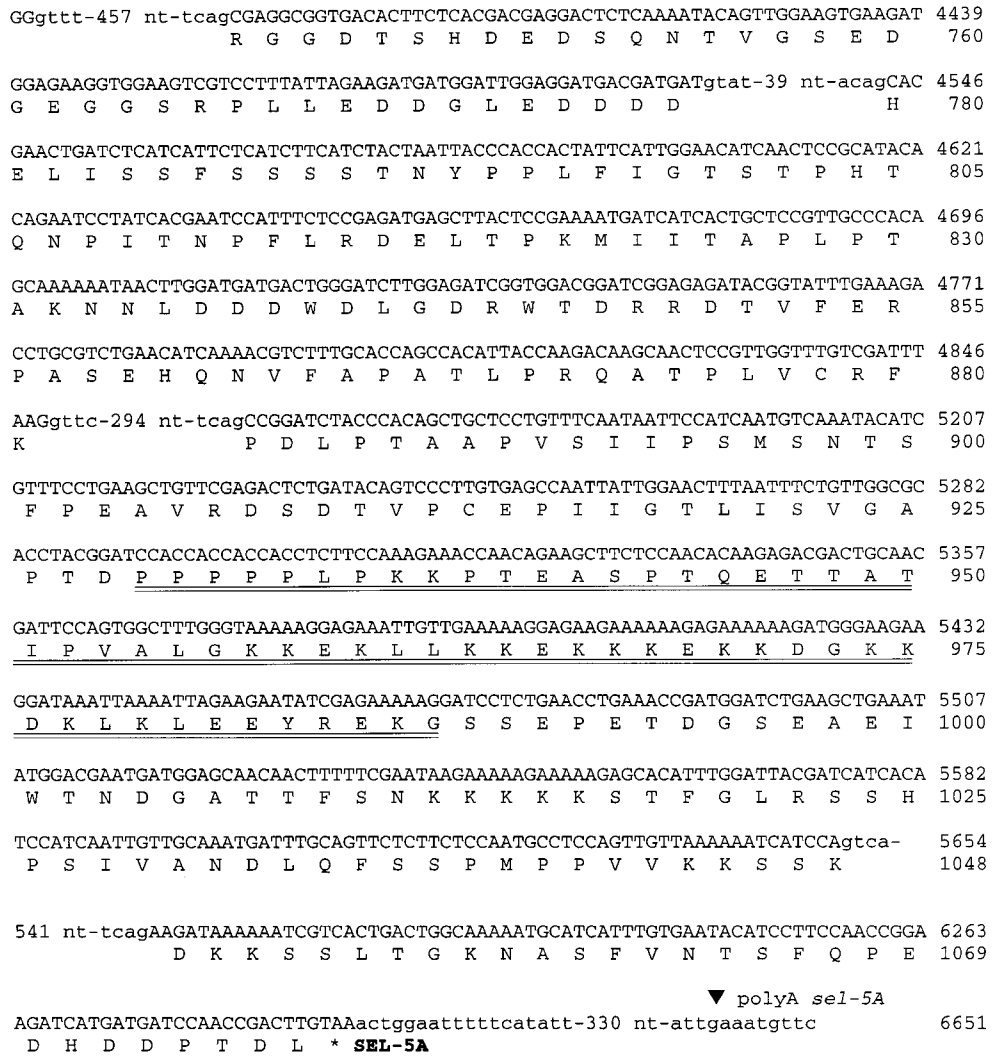


Figure 2.—*Continued.*

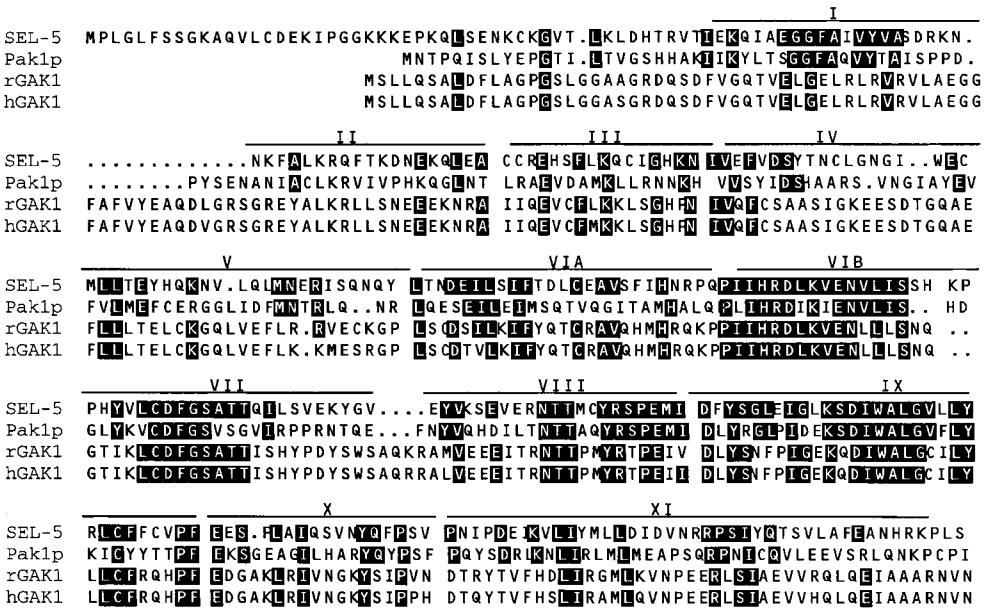
shown). These observations suggest that *sel-5* is not involved in the transcriptional feedback mechanism that operates during the AC/VU decision.

Molecular cloning of *sel-5*: *sel-5* had been mapped previously to chromosome III between *ced-4* and *dpy-17* (Tax *et al.* 1997). We obtained additional map data placing *sel-5* to the left of *mab-21* and within the deficiency *sDf121* (see Table 1 and materials and methods). To correlate the genetic and physical maps in this area, we determined that the left endpoint of *sDf121* deleted sequences within cosmid F35G12 (see materials and methods) but did not delete sequences in cosmid F56F3 (see materials and methods) or F10G11 (Stewart *et al.* 1998), thus placing *sel-5* within a few open reading frames (ORFs) to the left of *mab-21* (Figure 1).

We were not successful in our attempts to identify *sel-5* sequences by an antisuppression assay: transgenic lines carrying cosmids and yeast artificial chromosomes (YACs) were generated in a *sel-5(n1254) lin-12(n302)* background, and the presence of egg-laying-defective hermaphrodites would have been an indication that the transgenes carried *sel-5(+)*. Since *sel-5* mutations appear to be loss-of-function mutations, we tried an alternative approach, RNA-mediated interference (Fire *et al.* 1998), to deplete the activity of candidate genes. We injected double-stranded RNA (dsRNA) corresponding to various ORFs in the region into *lin-12(n302)* adult hermaphrodites and looked for F_1 progeny that were able to lay eggs. Injection of dsRNA corresponding to two independent cDNA clones, yk13e1 and yk7a7, yielded a high percentage of such egg-laying F_1 progeny (Table 4).

yk13e1 and yk7a7 both correspond to the same ORF, *F35G12.3*, of which there are two splice variants sharing a common N terminus (see below). To confirm that *F35G12.3* is *sel-5*, we sequenced the predicted exons and exon/intron boundaries of *F35G12.3* in the two original *sel-5* alleles, *sel-5(n1250)* and *sel-5(n1254)*, and found a single base change in each allele (see below). In addition, a deletion within F35G12.3 was generated by the *C. elegans* gene knockout consortium and behaves in complementation tests as a *sel-5* allele [designated *sel-5(ok149)*] (materials and methods). These results indicate that F35G12.3 corresponds to *sel-5*.

A



B

Protein	Organism	% Identity (Similarity)
Pak1p	*S. cerevisiae*	32.1 (39.4)
YNL020C	*S. cerevisiae*	32.6 (40.3)
YBR059C	*S. cerevisiae*	30.6 (37.4)
SPBC6B1.02	*S. pombe*	30.6 (37.8)
AF052296	*D. heteroneura*	41.2 (47.7)
rGAK-1	Rat	35.9 (42.3)
hGAK-1	Human	33.5 (40.6)
Cdc28p	*S. cerevisiae*	18.5 (24.8)
PKCα	Rat	22.6 (29.1)
c-src	Human	21.5 (29.7)

Figure 3.—SEL-5 kinase domain similarity. (A) Sequence alignment of the SEL-5 kinase domain to that from *Saccharomyces cerevisiae* Pak1p (GenEmbl no. U24167), rat rGAK1 (GenEmbl no. D38560), and human hGAK1 (GenEmbl no. D88435). The 12 conserved subdomains of the kinase catalytic site are numbered above the sequences. Identical amino acids are reverse contrasted. (B) The percentage relatedness of the different proteins to SEL-5 was calculated by dividing the number of identical (or identical plus similar) amino acids by the longer of the two sequences. The following amino acids were considered similar: I/L/V, S/T, R/K, N/Q, and D/E. (Top) The proteins most similar to SEL-5. These include *S. cerevisiae* Pak1p, *S. cerevisiae* YNL020C (GenEmbl no. Z71296) and YBR059C (GenEmbl no. Z35928), *Schizosaccharomyces pombe* SPBC6B1.02 (GenEmbl no. AL021838), *Drosophila heteroneura* partial sequence AF052296 (GenEmbl no. AF052296), rat GAK1 and human GAK1. (Bottom) A comparison of SEL-5 to representative members from other families of ser/thr kinases: *S. cerevisiae* Cdc28p (GenEmbl no. X00257), rat PKCa (GenEmbl no. X07286), and human c-src (GenEmbl no. 59932).

Molecular analysis of the *sel-5* coding region and *sel-5* mutations: We sequenced yk7a7, yk13e1, and PCR products (materials and methods) and found that all the exons and introns were as predicted by AceDB (Eeckman and Durbin 1995) and reported in sequence database (GenEmbl no. Z46242). The cDNA analysis indicated that there are two alternatively spliced variants of *sel-5*; yk7a7 corresponds to the longer cDNA (referred to as *sel-5A*) and yk13e1 corresponds to the shorter cDNA (referred to as *sel-5B*). The presence of these two splice variants was also confirmed by the appearance of two equally abundant bands of the appropriate sizes (3.7 and 2.4 kb) on a Northern blot of total N2 (wild-type) RNA probed with the complete *sel-5* gene (data not shown). In the mRNA, SL1 is transpliced 10 bp upstream of the initiating AUG; a polyadenosine tail is added 352 bp after the stop codon in *sel-5(A)* and 298 bp after the stop codon of *sel-5B*.

sel-5A and *sel-5B* encode predicted products of 1077 and 690 amino acids, respectively. The first 653 amino acids of these products are identical and at the amino termini include a region of 325 amino acids that is homologous to the catalytic site of serine/threonine kinases (Figure 2; Hanks and Hunter 1995). The putative kinase domain of SEL-5 is most homologous to the GAK1 kinase in rats and humans and their homologues in budding yeast (Figure 3). No other significant similarity to any known protein was detected outside of the kinase domain.

The *sel-5(n1254)* G-to-A transition destroys the acceptor splice site at the end of intron 3, and the *sel-5(n1250)* G-to-A transition destroys the donor splice site at the beginning of intron 8 (Figure 2). Both mutations are predicted to result in premature termination of both SEL-5A and SEL-5B. In the absence of cryptic alternative splicing, *sel-5(n1254)*, which behaves by genetic criteria as a null allele (Table 1), is predicted to terminate prior to the kinase domain, while *sel-5(n1250)* is predicted to terminate after the kinase domain. *sel-5(ok149)* contains a deletion of sequences from within exon 5 to sequences

TABLE 5

Complementation of *sel-5(n1254)* by SEL-5A and SEL-5B

Insert	Rescued lines/total lines
None[a]	0/2
SEL-5A	2/2
SEL-5B	5/7
SEL-5A::GFP	1/1

PIN2-derivative plasmids, in which inserted cDNAs are expressed under the control of *sel-12* regulatory sequences, were injected at 5 μg/ml along with marker plasmids into hermaphrodites of genotype *smg-1(r861); sel-5(n1254) lin-12(n302)* (see materials and methods for details). Lines were scored as displaying rescue if a majority of hermaphrodites were egg-laying defective (Egl) and/or vulvaless (Vul) (typically ~97–100% Egl and/or Vul). Lines were scored as nonrescued if they displayed ~50–70% Egl and/or vulvaless (Vul), similar to the parental strain. Assays were done at 20°.

[a] PIN2 expression vector without an inserted cDNA.

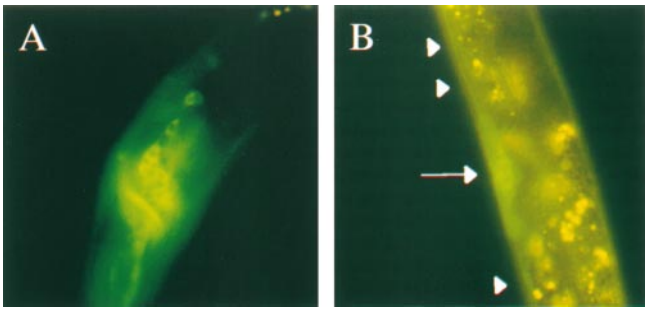


Figure 4.—Intracellular distribution of SEL-5::GFP protein. Shown are epifluorescent micrographs of L4 (A) or L3 (B) stage *dpy-20(e1282)* hermaphrodites with an extrachromosomal array carrying the wild-type *dpy-20* gene and the PIN-2::SEL-5A plasmid (*sel-5A::gfp* under the control of *sel-12* sequences). (A) Cytoplasmic staining (nuclear excluded) in head neurons. (B) Cytoplasmic staining (nuclear excluded) in the VPC (arrow) and neuronal processes in the ventral midcord (arrowheads).

within exon 10 that would remove amino acids 153–582 of both proteins. Translation of the predicted mRNA results in a premature stop codon due to a shift in the ORF in the kinase domain (the alternative amino acids QIQMTRFDQSERWTGECIYDG are predicted before the stop codon).

Both SEL-5A and SEL-5B can complement *sel-5(n1254)*: *sel-5(n1254) lin-12(n302)* hermaphrodites carrying transgenes corresponding to the *sel-5* gene (complete ORF with 6.277-kb upstream sequences and 0.589-kb downstream sequences) remained egg-laying proficient; we observed what appears to be transient rescue in some lines in early generations that was lost in later ones (data not shown). Cosmid F35G12, which contains *sel-5(+)*, also shows this behavior (data not shown), so we believe the lack of antisuppression reflects low expression of *sel-5*, perhaps because of some property of extrachromosomal arrays carrying *sel-5* sequences.

To assess the function of SEL-5A and SEL-5B in the AC/VU decision, we therefore used heterologous regulatory sequences, from the *sel-12* gene, to drive their expression (see materials and methods). Hermaphrodites of genotype *sel-5(n1254) lin-12(n302)* carrying extrachromosomal arrays expressing either SEL-5A or SEL-5B under the control of *sel-12* regulatory sequences were egg-laying defective (Table 5). This reversal of suppression by both products indicates that both SEL-5A and SEL-5B can function in the AC/VU decision and that the sequences unique to SEL-5A are not necessary for this function of *sel-5*.

SEL-5A subcellular localization: To investigate the subcellular localization of SEL-5A, we replaced 59 amino acids at its carboxyl-terminus with GFP (Chalfie *et al.* 1994) and expressed the resulting SEL-5A::GFP hybrid protein under the control of *sel-12* regulatory sequences. The SEL-5A::GFP protein is functional, as *sel-5(n1254) lin-12(n302)* hermaphrodites carrying transgenes expressing SEL-5A::GFP are egg-laying defective (Table 5). We examined the subcellular localization of the hybrid protein in *sel-5(+) lin-12(+)* hermaphrodites. The hybrid protein seems to localize predominantly to the cytoplasm of various cells (including Z1.ppp and Z4.aaa; data not shown) and is excluded from the nucleus (Figure 4); several independent transgenic lines showed a similar pattern of subcellular localization.

***sel-5* displays tissue-specific interactions with *lin-12*:** We assessed the ability of *sel-5* mutations to suppress phenotypes other than the 0 AC-Egl phenotype associated with *lin-12(d)* mutations (Table 6). In hermaphrodites, strong *lin-12(d)* mutations cause a highly penetrant Multivulva phenotype, because the cells P3.p-P8.p, also called the vulval precursor cells (VPC), adopt a particular vulval fate, termed "2°", and generate pseudovulvae. In addition, hermaphrodites carrying strong *lin-12(d)* mutations are missing dorsal coelomocytes, because the cells M.dlpa and M.drpa instead become sex myoblasts. In males, strong *lin-12(d)* mutations cause ectopic hooks (sensory structures), because the cells P9.p-P11.p adopt the male equivalent of the "2°" fate, and cause P3.p-P6.p to generate pseudovulvae inappropriately. We saw no effect on the Multivulva phenotype of *lin-12(d)* hermaphrodites or the generation of ectopic hooks or pseudovulvae in males. However, we did see suppression of the transformation in fate of dorsal coelomocytes to sex myoblasts.

We also explored potential interactions between *sel-5* and *glp-1*, another *C. elegans lin-12/Notch* gene (Yochem and Greenwald 1989). *lin-12* and *glp-1* are functionally redundant for certain cell fate decisions (Lambie and Kimble 1991), and GLP-1 can efficiently substitute for LIN-12 when expressed under the control of *lin-12* regulatory sequences (Fitzgerald *et al.* 1993). We combined *sel-5(n1254)* with *glp-1(e2141)* and *glp-1(e2142)*, which are partial loss-of-function alleles at 15° (Austin

TABLE 6

Cell-type-specific suppression of *lin-12(d)* by *sel-5*

Genotype[a]	Average no. of pseudovulvae in hermaphrodites	% males with pseudovulvae	% males with ectopic hooks	% hermaphrodites with dorsal coelomocytes[c]
+	0 (n = many)	0 (n = many)	0 (n = many)	100 (n = many)
lin-12(n950)	5.2 (n = 80)	92.6 (n = 94)	95.7 (n = 94)	ND
sel-5(n1250) lin-12(n950)	5.1 (n = 80)	97.0 (n = 105)	99 (n = 105)	ND
sel-5(n1254) lin-12(n950)	5.3 (n = 80)	96.1 (n = 98)	98 (n = 98)	ND
sel-5(ok149) lin-12(n950)	4.9 (n = 94)	96.0 (n = 75)	100 (n = 75)	ND
lin-12(n137)	5.09 (n = 44)[b]	ND	ND	6.8 (n = 74)
sel-5(n1254) lin-12(n137)	4.79 (n = 42)[b]	ND	ND	77.6 (n = 67)

Strains were grown at 15° unless otherwise indicated; similar results were obtained with strains grown at 20°.
[a] Males were obtained from strains carrying *him-5(e1490)* in addition to the *lin-12* or *sel-5* allele shown.
[b] Scored hermaphrodites grown at 20°.
[c] The presence of at least one dorsal coelomocyte was confirmed by Nomarski microscopy.

and Kimble 1987; Priess *et al.* 1987), and saw no effect on total brood size, maternal effect lethality, or fertility (data not shown). We also injected *glp-1(ar202)* hermaphrodites with *sel-5* yk7a7 dsRNA and did not see any suppression of the gain-of-function mitotic proliferation defect of this allele (data not shown).

These observations suggest that *sel-5* influences *lin-12* activity in a tissue-specific manner. We do not know whether *sel-5* is expressed in a tissue-specific manner or whether *sel-5* activity influences *lin-12* activity only under certain conditions.

RNA-mediated interference analysis of selected genes pertaining to GAK1 or GAK1 interacters: The GAK1 family of proteins has been shown to interact either functionally or physically with numerous other genes and proteins, most notably, cyclin G and p53 (Thiagalingam *et al.* 1995; Kanaoka *et al.* 1997). The similarity of the SEL-5 kinase domain to the GAK1 family suggested that other GAK1-interacting proteins might play a role in the AC/VU decision. To test this hypothesis, we used RNA-mediated interference (RNAi) to reduce endogenous gene activity of *C. elegans* relatives of HMG1, p53-binding protein, Rb, c-abl, Ref-1, cyclin G, Cdk5, and PP2A. We injected dsRNA for these genes into *lin-12(n302)* adult hermaphrodites and looked for suppression of the egg-laying defect in the F_1 progeny. We saw no evidence for suppression (Table 7), in contrast to the efficient suppression observed when *sel-5* dsRNA is injected (Table 4).

DISCUSSION

Previous work suggested that *sel-5* facilitates *lin-12* activity cell autonomously (Tax *et al.* 1997). In this study, we have performed additional genetic analysis to explore the effect of *sel-5* on *lin-12* activity, and below, we speculate about the possible function of *sel-5* in *lin-12*-mediated cell fate decisions.

We have also cloned the *sel-5* gene and showed that it encodes two alternatively spliced products, which share a serine/threonine kinase domain. The kinase domain of SEL-5 is most similar to that of mammalian

TABLE 7

RNAi with selected genes pertaining to GAK1 or GAK1 interacters

dsRNA	ORF	Closest homologue	No. of suppressed F_1 broods/ no. of worms injected
yk456a4	*F47D12.4*	HMG1	0/10
yk486h12	*F46F3.3*	p53-binding protein	0/10
yk13c4	*lin-35*	Rb	0/11
yk12e6	*abl-1*	c-abl	0/10
yk198g4	*R09B3.d*	Ref-1	0/8
yk401b10	*R0F2.1*	Cyclin G (rat)	0/12
yk171d8	*T27E9.3*	Cdk5	0/12
yk422f10	*C13G3.3*	Phosphatase 2A β′ subunit	0/10

lin-12(n302) hermaphrodites were injected with dsRNA as described in materials and methods, and the presence of egg-laying-competent progeny was assessed. Assays were done at 20°.

GAK1 and yeast Pak1p. GAK1 was isolated as a cyclin G-interacting protein and was also shown to co-immunoprecipitate with CDK5 (Kanaoka *et al.* 1997). Pak1p was isolated in yeast as a multicopy suppressor of a yeast mutation defective in p53-mediated transcriptional activation (Thiagalingam *et al.* 1995). p53 is known to transcriptionally regulate the expression and/or activity of several genes and to interact with some others (reviewed by Ko and Prives 1996; Levine 1997), including cyclin G, CDK5, phosphatase 2A β′ subunit, and HMG1. p53 is also thought to exert part of its effect on the cell cycle through its regulation of the phosphorylation of Rb (reviewed by Ko and Prives 1996; Levine 1997). However, we have not been able to detect any obvious links between *sel-5* or *lin-12* and the p53 or Rb pathway in the AC/VU decision.

We have shown that loss of *sel-5* activity can suppress the AC fate transformation associated with constitutive activity of *lin-12(d)* mutations, which are point mutations in the extracellular domain of LIN-12, but not of *lin-12(intra)*, the untethered intracellular domain. This result suggests that *sel-5* acts prior to or during ligand-dependent release of the intracellular domain.

The genetic interactions between *sel-5* and constitutively active *lin-12* alleles are in many ways reminiscent of the interactions between *lin-12* and *sel-12*, a presenilin (Levitan and Greenwald 1995, 1998), and between *lin-12* and *sup-17*, a metalloprotease homolog of ADAM10/Kuzbanian (Wen *et al.* 1997). SEL-12/presenilin has been implicated in the ligand-dependent release of the intracellular domain (DeStrooper *et al.* 1999; Struhl and Greenwald 1999), and SUP-17/ADAM10 has been proposed to be involved in a cleavage event that occurs upon ligand binding (Logeat *et al.* 1998). The similar genetic interactions raise the possibility that *sel-5* is involved in modulating one of these proteolytic processing events, perhaps by activating some component by phosphorylation. The genetic interactions between *sel-5* and the two classes of constitutively active *lin-12* alleles would be consistent also with a role for SEL-5 in the trafficking of LIN-12 (or processing factors) to the cell surface. However, in this context, we note that we see no evidence for a change in the subcellular distribution or accumulation of a LIN-12::GFP hybrid protein in a *sel-5* mutant background (H. Fares, unpublished observations).

In contrast to *sup-17* and *sel-12*, the ability of *sel-5* to suppress *lin-12(d)* mutations is tissue specific: loss of *sel-5* activity can suppress defects in the AC/VU decision of the somatic gonad and the sex myoblast/coelomocyte decision, but cannot suppress defects in ventral hypodermal cell fate decisions. It is possible that *sel-5* is not expressed in ventral hypodermal cells or that its function in ventral hypodermal cells is masked by a redundant activity or process in these cells. Alternatively, it is possible that a more interesting biological difference underlies the tissue specificity. For example, one difference between the *lin-12*-mediated cell fate decisions that are not affected by *sel-5* activity (in the ventral hypodermis) *vs.* the cell fate decisions that are affected by *sel-5* activity (the AC/VU decision and sex myoblast/coelomocyte decision) is that the unaffected decisions involve ectodermal derivatives, whereas the affected decisions involve mesodermal derivatives. Another obvious difference is that the unaffected cells are epithelial cells with a well-defined apical/basolateral axis of polarity. Where the epithelial cell polarity machinery is not active, perhaps contact between LIN-12 and its ligand(s) results in cell polarization or specific membrane microdomains; if so, then *sel-5* might be involved in defining the axis of polarity or might influence the transport to or modification of a component at the region of cell contact.

Loss of *sel-5* activity does not cause any cell fate transformations associated with loss of *lin-12* (or *glp-1*) activity. The absence of a visible phenotype has been a characteristic of many genes recovered in suppressor/enhancer screens for genes that influence *lin-12* activity in *C. elegans*, and in principle might reflect functional redundancy due to related genes or of different mechanisms that influence receptor activity. In the case of *sel-12*, null mutants lack the hallmark *lin-12* cell fate transformations affecting the AC and VPC (Levitan and Greenwald 1995) because a strict requirement for presenilin activity is masked by the presence of a second, functionally redundant, presenilin gene, *hop-1* (Li and Greenwald 1997). At this time, there is no obvious candidate for a gene that may be affording functional redundancy for *sel-5*, as there is no gene with high similarity in the *C. elegans* genome sequence database. We therefore favor the view that the lack of a *sel-5* null mutant phenotype reflects a relatively small effect on *lin-12* activity, possibly because other mechanisms afford some redundant influence.

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