p24 Proteins and Quality Control of LIN-12 and GLP-1 Trafficking in *Caenorhabditis elegans*

Chenhui Wen* and Iva Greenwald*‡

*Department of Biochemistry and Molecular Biophysics, ‡Howard Hughes Medical Institute, Columbia University, College of Physicians and Surgeons, New York, New York 10032

Abstract. Mutations in the *Caenorhabditis elegans sel-9* gene elevate the activity of *lin-12* and *glp-1*, which encode members of the LIN-12/NOTCH family of receptors. Sequence analysis indicates SEL-9 is one of several *C. elegans* p24 proteins. Allele-specific genetic interactions suggest that reducing *sel-9* activity increases the activity of mutations altering the extracellular domains of LIN-12 or GLP-1. Reducing *sel-9* activity restores the trafficking to the plasma membrane of a mutant GLP-1 protein that would otherwise accumulate within the cell. Our results suggest a role for SEL-9 and other p24 proteins in the negative regulation of transport of LIN-12 and GLP-1 to the cell surface, and favor a role for p24 proteins in a quality control mechanism for endoplasmic reticulum–Golgi transport.

Key words: LIN-12 • SEL-9 • Notch • p24 • Emp24p

THE *lin-12* and *glp-1* genes of *Caenorhabditis elegans* encode members of the LIN-12/NOTCH family of receptor proteins. LIN-12/NOTCH proteins mediate cell–cell interactions to specify cell fate during animal development (Greenwald, 1998). The extracellular domains of LIN-12/NOTCH proteins contain multiple tandem EGF-like motifs and three LIN-12/NOTCH repeat (LNR) motifs. The intracellular domains of LIN-12/NOTCH proteins contain tandem *cdc10*/SWI6 (also known as ankyrin) motifs. LIN-12/NOTCH proteins are activated by ligands of the Delta/Serrate/LAG-2 family (Weinmaster, 1997). Binding of ligand is believed to induce a cleavage event in or near the transmembrane domain; this apparent cleavage event enables the intracellular domain to translocate to the nucleus, where it participates directly in regulating downstream gene expression (Schroeter et al., 1998; Struhl and Adachi, 1998).

Genetic screens in *C. elegans* and *Drosophila* have identified factors that influence *lin-12/Notch* activity. Many genes have been identified in sensitized genetic backgrounds, by suppressing or enhancing mutations in *lin-12* (Sundaram and Greenwald, 1993b; Tax et al., 1997). Most of these genes have been named *sel* genes, for suppressor/enhancer of *lin-12*. The suppressor/enhancer approach mitigates potential difficulties arising from possible functional redundancy of mechanisms that control receptor activity as well as gene redundancy in each step. Several *sel* genes that have been characterized are involved in basic cell biological processes. Two *sel* genes, *sel-12* (presenilin) and *sup-17* (ADAM10/Kuzbanian), appear to affect processing of LIN-12 and GLP-1 (Wen et al., 1997; Levitan and Greenwald, 1998). Two other *sel* genes, *sel-1* and *sel-10*, are likely to affect LIN-12 and GLP-1 turnover (Grant and Greenwald, 1997; Hubbard et al., 1997). The *sel* genes, and their interactions with *lin-12* and *glp-1*, therefore may illuminate connections between basic cell biological processes and signaling.

In this study, we show that *sel-9* functions by affecting LIN-12 and GLP-1 trafficking. SEL-9 is a member of the p24 family of proteins, and reducing *sel-9* activity increases the level of *lin-12* and *glp-1* activity. We have identified other genes encoding *C. elegans* p24 proteins, and shown that reducing the activity of one of these genes also increases the level of *lin-12* and *glp-1* activity. Members of the p24 family have been implicated in cargo selectivity of ER to Golgi transport. The genetic interactions of *sel-9* with *lin-12* and *glp-1*, and the effect of *sel-9* on the subcellular localization of mutant GLP-1, are consistent with a role for SEL-9 and other p24 proteins in cargo selection during trafficking to the cell surface.

Materials and Methods

General Methods and Strains

General methods are described by Brenner (1974). The wild-type parent for all strains was *C. elegans* var. Bristol strain N2. Strains were grown at 20°C unless otherwise noted. Mutations used were: LG I: *arIs12 [lin-12(intra)]* (Struhl et al., 1993); LG III: *unc-36(e251)* (Brenner, 1974), *unc-*

Address correspondence to Iva Greenwald, HHMI/Biochemistry, 701 West 168th Street, Room 720, New York, NY 10032. Tel.: (212) 305-6928/6931. Fax: (212) 305-1721. E-mail: greenwald@cuccfa.ccc.columbia.edu

32(e189) (Brenner, 1974), *lin-12(n676n930ts)* (Sundaram and Greenwald, 1993a), *lin-12(n302)* (Greenwald et al., 1983), *lin-12(ar170)* (Hubbard et al., 1996), *glp-1(e2141ts, e2142ts, q231)* (Austin and Kimble, 1987; Priess et al., 1987), *glp-1(q415)* (Kodoyianni et al., 1992); LG V: *dpy-11(e224)* (Brenner, 1974), *rol-3(e754)* (Brenner, 1974), *unc-23(e25)* (Brenner, 1974), *sel-9(ar22, ar26)* (Sundaram and Greenwald, 1993b), *mom-2(ne141)* (Rocheleau et al., 1997); and extrachromosomal array *arEx29[lin-12(+)]* (Fitzgerald et al., 1993).

Mutagenesis and Screen for New sel-9 Alleles

At 25°C, *glp-1(e2142)* hermaphrodites produced inviable progeny; this phenotype is suppressed by *sel-9(ar22)* (Sundaram and Greenwald, 1993b). Furthermore, *glp-1(e2142); sel-9(ar22)/Df* hermaphrodites also produce viable progeny (data not shown), suggesting that null alleles in principle may be obtained by complementation screening.

EMS mutagenesis was performed as described by Brenner (1974). *glp-1(e2142); him-8(e1489); rol-3(e754) sel-9(ar26)/sel-9(ar22) unc-23(e25)* males were mated to EMS mutagenized *glp-1(e2142); dpy-11(e224)* hermaphrodites at 15°C. The parents were transferred to fresh plates daily for 5 d. F1 progeny were grown at 15°C until the L4 stage. Non-Dpy cross progeny were picked to fresh plates and transferred to 25°C. 10 F1 animals were put on each plate and the total number of F1 cross progeny was counted while picking. After 4 d, plates at 25°C were screened for live F2 progeny. Eventually only one animal from each plate was kept as a candidate. Dpy animals were backcrossed at least twice before further analysis.

Genetic Mapping of sel-9

sel-9 was previously mapped between *rol-3* and *unc-42* (Sundaram and Greenwald, 1993b). We mapped *sel-9* between *rol-3* and *unc-23*: 2/10 Rol non-Unc recombinants from heterozygotes of the genotype *rol-3 unc-23/ sel-9* segregated *rol-3 sel-9*. *sel-9* was further mapped between *rol-3* and *mom-2*: from heterozygotes of the genotype *rol-3 sel-9 unc-23/mom-2*, 1/23 Rol non-Unc recombinants harbored a *rol-3 sel-9 mom-2* recombinant chromosome.

sel-9 Cloning by an Antisuppression Assay

Transgenic lines were generated by microinjecting *lin-12(n676n930); sel-9(ar22)* hermaphrodites with cosmid or plasmid DNA at a concentration of 10 μg/ml, along with the dominant *rol-6* marker pRF4 at a concentration of 100 μg/ml (Mello et al., 1991). Stable Rol lines were reared at 25°C, and individual Rol hermaphrodites from each line were analyzed for the Egl defect. A line is considered rescued if >50% of the Rol hermaphrodites were Egl. Initial rescue was obtained with each of two overlapping cosmids, F21F8 and W02D7. The 20-kb overlapping region was further subcloned into plasmid vector pBS(SK^+) (Stratagene). Plasmid pSX2.8 contains the 2.8-kb DNA fragment that contains *sel-9(+)* activity in the antisuppression assay; this plasmid was also shown to be able to rescue the morphological defects caused by *sel-9(ar173)*.

Sequence Analysis of sel-9 and lin-12

Standard molecular biology protocols were performed as described in Sambrook et al. (1989). The DNA sequences of F21F8 and W02D7 were obtained from the *C. elegans* genome sequencing project (Waterston et al., 1997). The exons of *sel-9* were predicted by GENEFINDER (Edgley et al., 1997); we confirmed this prediction by sequencing a cDNA clone, yk371h2 (generously provided by Dr. Yuji Kohara, National Institute of Genetics, Japan).

The lesions associated with all *sel-9* mutations were found by sequencing the *sel-9* coding region of the mutants. We amplified the *sel-9* genomic region by PCR reactions from individual *sel-9* mutant hermaphrodites. For each mutation, two independent PCR products were cloned into Bluescript(SK^+) (Stratagene). A lesion was considered confirmed if it appeared in two independent clones.

Lesions associated with *lin-12(n941), lin-12(n676n930), lin-12(ar170),* and *lin-12(oz48)* were found by sequencing most of the coding region of the mutants, as for *sel-9*. *lin-12(n941)* corresponds to W400STOP. The *n930* lesion corresponds to C138T. The *ar170* lesion corresponds to G270R. The *oz48* lesion corresponds to G449R.

SEL-9::GFP

A PCR product containing the coding sequence of GFPS65T was cloned into a PmeI site at the end of the *sel-9* coding region in pSX2.8 (see above). As a result, the stop codon of *sel-9* was changed to Ser. The resulting SEL-9::GFP hybrid protein is nonfunctional in the antisuppression assay, but, as it is expressed under the control of *sel-9* regulatory sequences, it was useful for determining that all somatic cells express SEL-9 (data not shown).

RNA-mediated Interference

Double-stranded RNA (dsRNA)[1] was prepared using the RNA transcription kit (Stratagene) and injected without dilution according to Fire et al. (1998).

Laser Microsurgery

lin-12(n676n930); sel-9(ar26) hermaphrodites, along with unoperated control animals, were kept at 15°C except for the period of laser microsurgery (20°C for ~5 min). The nucleus of Z4 was ablated in newly hatched L1 larvae. The presence of an anchor cell (AC) was scored during the late L3 stage.

Immunofluorescence

Anti–GLP-1 staining of dissected hermaphrodite gonads was performed as described in Crittenden et al. (1994). Phalloidin staining was performed as described in Strome (1986). Worms were mounted on a 2% agarose pad with 3 μl of 10% *N*-propyl gallate and viewed with a Zeiss LSM410 laser scanning confocal attachment on a Zeiss Axiovert 100 microscope.

Results

Relevant Properties of lin-12 and glp-1 Mutations

Our analysis of *sel-9* depends on genetic interactions between *sel-9* and the *lin-12* or *glp-1* genes. Here, before we describe the genetic analysis of *sel-9*, we summarize the relevant properties of mutations in *lin-12* and *glp-1*, which both encode receptors of the LIN-12/Notch family (Yochem et al., 1988; Yochem and Greenwald, 1989). We note that *lin-12* and *glp-1* are functionally redundant in some cell fate decisions (Lambie and Kimble, 1991), and that GLP-1 can fully substitute for LIN-12 when expressed under the control of *lin-12* regulatory sequences (Fitzgerald et al., 1993). In our analysis, we have made consistent observations using both genes.

Three morphological characteristics influenced by *lin-12* were used for our analysis of *sel-9* (Table I): the number of ACs, vulval morphology, and egg laying. Wild-type hermaphrodites have one AC and a normal vulva, and are able to lay eggs. Reduced *lin-12* activity causes an extra AC to be produced (the 2 AC defect), a variably abnormal vulva, and a defect in egg laying (Egl) (Greenwald et al., 1983; Sundaram and Greenwald, 1993a). In this study, we focus on genetic interactions between *sel-9* and two alleles that reduce, but do not eliminate, *lin-12* activity: *lin-12(n676n930)* and *lin-12(ar170)*.

Constitutive, elevated *lin-12* activity causes the AC to be missing (the 0 AC defect) and, as a consequence, an egg-laying defect (the 0 AC-Egl defect) which is different from the Egl defect associated with reduced *lin-12* activity (Greenwald et al., 1983). A greater elevation of *lin-12* activity also causes extra vulval cells to be made (the Multivulva defect). Constitutive activity may result from mis-

1. *Abbreviations used in this paper:* AC, anchor cell; ds, double-stranded; Egl, Egg-laying defective; Muv, Multivulva; RNAi, RNA-mediated interference; VU, ventral uterine precursor cell.

Table I. Relevant Features of lin-12 Mutations Used in This Study

Allele	Nature of mutation	No. of AC	Multivulva	Egg-laying defect
+	Wild-type	1 AC	No	None
n941	Null	2 AC	No	N/A*
ar170	Hypomorph	2 AC	No	None‡
n676n930§	25°C hypomorph	2 AC	No	Egl
	15°C constitutive, hypermorph	0 AC‡	No	0 AC-Egl‖
n302	Constitutive, hypermorph	0 AC	No	0 AC-Egl
lin-12(intra)	15°C constitutive, hypermorph	0 AC‡	Yes	0 AC-Egl¶

The *n930* lesion [C138T] appears to be a heat-sensitive partial loss-of-function mutation. At 25°C, *lin-12(n676n930)* behaves like a hypomorph (partial loss-of-function allele), and displays variably penetrant defects associated with loss of *lin-12* activity, as shown; at 15°C, this allele behaves like a very weak hypermorph.
*Egg laying cannot be assessed because *lin-12(0)* mutants are sterile.
‡Low penetrance Egl defect.
‖Incompletely penetrant gain-of-function defect.
§*lin-12(n676n930)* was derived by reverting the *lin-12(d)* allele *lin-12(n676)* [G884D).
¶*arIs12[lin-12(intra)]* also has an egg-laying defect that may be unrelated to *lin-12* activity, possibly due to the insertion site.

sense mutations in the extracellular domain, collectively termed *lin-12(d)*, or by removing the extracellular domain, as in the *lin-12(intra)* transgene (Greenwald and Seydoux, 1990; Struhl et al., 1993).

The absence of *glp-1* activity causes defects in germline proliferation and maternal effect lethality (Austin and Kimble, 1987; Priess et al., 1987). The *glp-1(e2142)* allele used extensively in this study displays only the maternal effect lethal defect at 25°C (Priess et al., 1987). Other *glp-1* mutations used are temperature-sensitive for both defects, and their properties are described where relevant below.

Isolation and Classification of New sel-9 Alleles

Two *sel-9* mutations were isolated as suppressors of the egg laying defect (Egl) of the partial loss-of-function (hypomorphic) allele *lin-12(n676n930)* (Sundaram and Greenwald, 1993b). Genetic analysis suggested that these two *sel-9* alleles, *sel-9(ar22)* and *sel-9(ar26)*, are not null alleles (Sundaram and Greenwald, 1993b).

We isolated new *sel-9* mutations in noncomplementation screens that in principle could have yielded null alleles (see Materials and Methods). Five *sel-9* alleles (*ar173*, *ar174*, *ar175*, *ar176*, and *ar178)* were isolated after screening 12,000 mutagenized haploid genomes. We classified the existing *sel-9* mutations into two groups, group A (weaker) and group B (stronger), based on their interactions with *lin-12* hypomorphic alleles (Table II). Mutations in group B appear to increase *lin-12* activity to a greater extent than mutations in group A. This inference is based on two observations: in combination with *lin-12(n676n930)*, alleles of group B result in a Multivulva phenotype (Table II), and in combination with *lin-12(ar170)*, alleles of group B suppress the 2 AC defect (Table II). We note that the alleles in group B were all isolated in noncomplementation screens and could not have been isolated as suppressors of *lin-12(n676n930)*, since they cause *lin-12(n676n930)* to lack an AC, which is necessary for vulval development and egg laying.

All *sel-9* alleles show genetic interactions with several different missense mutations affecting the LIN-12 extracellular domain (see below). However, none suppress defects caused by *lin-12(n941)*, a *lin-12* null allele associated with a stop codon at position 400 in the extracellular domain (Materials and Methods).

Genetic analysis suggests that all *sel-9* mutations are antimorphic (dominant-negative) (Sundaram and Greenwald, 1993b). A heterozygous deficiency is unable to suppress the 2 AC defect of *lin-12(n676n930)* or the maternal effect lethality of *glp-1(e2142)*. In contrast, *sel-9/+* heterozygosity suppresses these defects, implying that *sel-9* alleles have gain-of-function character. The gain-of-function character appears to be antimorphic, because addition of wild-type alleles can reverse the suppression of *lin-12(n676n930)* and *glp-1(e2142)* by mutations in *sel-9* (Sundaram and Greenwald, 1993b; Wen, C., and I. Greenwald, unpublished observations). In addition, we have evidence that all *sel-9* mutations cause reduction of *sel-9(+)* activity (see below).

Cell Autonomy of sel-9 Function in the AC/Ventral Uterine (VU) Decision

To examine the cell autonomy of *sel-9* function, we examined its effect on the decision of two cells, Z1.ppp and Z4.aaa, between the AC and VU precursor cell fates. Normally, *lin-12*–mediated interactions between Z1.ppp and Z4.aaa causes one to become the AC (see Greenwald,

Table II. sel-9 Combinations with lin-12 Hypomorphs

With *lin-12(n676n930)*				
Relevant genotype*	*sel-9* group‡	*n*	% 0 AC§	Multivulva defect‖
lin-12(n676n930)	+	40	0	None
lin-12(n676n930); sel-9(ar22)	A	37	0	None
lin-12(n676n930); sel-9(ar26)	A	Many	(None)	None
lin-12(n676n930); sel-9(ar178)	A	Many	(None)	None
lin-12(n676n930); sel-9(ar173)	B	16	75 ± 10.8	Muv
lin-12(n676n930); sel-9(ar174)	B	17	100	Muv
lin-12(n676n930); sel-9(ar175)	B	Many	(All)	Muv
lin-12(n676n930); sel-9(ar176)	B	Many	(All)	Muv

With *lin-12(ar170)*			
Relevant genotype*	*sel-9* group‡	*n*	% 2 AC
lin-12(ar170)	+	41	83 ± 5.9
lin-12(ar170); sel-9(ar22)	A	67	78 ± 5.1
lin-12(ar170); sel-9(ar178)	A	36	81 ± 6.5
lin-12(ar170); sel-9(ar173)	B	13	7.7 ± 7.4
lin-12(ar170); sel-9(ar174)	B	31	32 ± 8.4
lin-12(ar170); sel-9(ar175)	B	66	49 ± 6.1
lin-12(ar170); sel-9(ar176)	B	40	50 ± 7.9

All experiments were done at 25°C. Sequence analysis subsequently established that *sel-9(ar22)* is the same alteration as *sel-9(ar178)*, and that *sel-9(ar175)* the same alteration as *sel-9(ar174)*. Standard variance is shown for the AC data. *sel-9* alleles also suppress the hypomorphic allele *lin-12(oz48)* (data not shown).
*All strains also contain *unc-32(e189)*.
‡*sel-9* mutations were placed in group B if they cause the 0 AC and Multivulva (Muv) defects in combination with *lin-12(n676n930)*.
§In cases where the number of AC was not scored directly, the absence of an AC could be inferred from the characteristic egg-laying defect (Greenwald et al., 1983). *sel-9* class A alleles suppress the 2 AC defect of *lin-12(n676n930)* (Sundaram and Greenwald, 1993b; and data not shown).
‖A hermaphrodite is considered Muv if it has three or more ventral pseudovulvae.

1998). When *lin-12* is constitutively active, hermaphrodites lack an AC, because both Z1.ppp and Z4.aaa become VUs. The penetrance of the 0 AC phenotype reflects the degree of elevated activity (Greenwald et al., 1983). For example, *lin-12(n676n930)* behaves like a weakly activated allele at 15°C, in that ~5% of hermaphrodites lack an AC. In the presence of *sel-9(ar26)*, the proportion of *lin-12(n676n930)* hermaphrodites lacking an AC is increased to 78%, suggesting that *lin-12* activity is elevated by *sel-9(ar26)*.

When either Z1 or Z4 (the precursors to Z1.ppp or Z4.aaa) is ablated with a laser microbeam, the fate of the remaining cell reflects its intrinsic level of *lin-12* activity in the absence of signaling. In wild-type hermaphrodites, if Z1 (or Z1.ppp) is ablated, Z4.aaa always becomes an AC (Kimble, 1981) because *lin-12* is not activated in the absence of ligand. However, in *lin-12(d)* hermaphrodites, if Z1 (or Z1.ppp) is ablated, Z4.aaa becomes a VU because of the ligand-independent activation of LIN-12(d) (Greenwald and Seydoux, 1990; Grant and Greenwald, 1997). If *sel-9(+)* functions in the receiving end of *lin-12*–mediated cell–cell interactions, we would expect to see that *sel-9(ar26)* increases the intrinsic *lin-12* activity in the absence of the signaling cell.

The results of ablation experiments suggest that *sel-9* can affect *lin-12* activity cell autonomously (Table III). As mentioned above, 78% of *lin-12(n676n930); sel-9(ar26)* hermaphrodites lack an AC. Similarly, in operated *lin-12(n676n930); sel-9(ar26)* hermaphrodites, when Z4 was removed at early L1 stage, Z1.ppp became a VU in 77% of the cases. These results demonstrate that the effect of *sel-9* on *lin-12* activity does not depend on the signaling cell, since *sel-9(ar26)* can increase the intrinsic level of *lin-12* activity in the absence of the signaling cell. Although this experiment does not rule out an additional role for *sel-9* in the signaling cell, the extent of enhancement of *lin-12(n676n930)* can be completely accounted for by *sel-9* function in the receiving cell.

Molecular Cloning of sel-9

sel-9(ar22) suppresses the Egl phenotype caused by *lin-12(n676n930ts)* at 25°C. Adding a copy of *sel-9(+)* allele *[lin-12(n676n930); sel-9(ar22)/sel-9(ar22)/sel-9(+)]* can partially reverse the suppression (Sundaram and Greenwald, 1993b). Therefore, we were able to use reversal of suppression to assess the *sel-9(+)* activity of microinjected DNAs (Fig. 1).

We mapped *sel-9* between *rol-3* and the cloned gene *mom-2*, an interval of <0.1 map unit. The *mom-2* gene resides on cosmid clone F38E1 (Rocheleau et al., 1997; Thorpe et al., 1997). We tested cosmid clones to the left of F38E1 for their ability to complement *sel-9(ar22)* in a *lin-12(n676n930); sel-9(ar22)* background (see Materials and Methods). Arrays containing either one of two overlapping cosmid clones, F21F8 and W02D7, gave rescue in the antisuppression assay. The overlapping region was further subcloned and the 2.8-kb fragment in pSX2.8 was determined to contain *sel-9(+)* activity. The sequence of the 2.8-kb fragment is predicted to encode a single gene (by GENEFINDER; see Edgley et al., 1997). A cDNA clone, yk371h2 (generously provided by Dr. Yuji Kohara), contains the DNA sequence of all predicted exons contained in pSX2.8. This predicted gene was confirmed to be *sel-9* by finding that all *sel-9* mutations contain molecular lesions in the coding region (Fig. 2).

sel-9 Encodes a Member of the p24 Family of Proteins

The predicted SEL-9 protein sequence reveals that SEL-9 belongs to the p24 family of proteins (Figs. 2 and 3). Multiple members of the p24 family are found in all eukaryotes, from yeast to mammals. Members of the p24 family are type I membrane proteins with a signal peptide at the amino terminus, a lumenal (extracytosolic) domain, a single transmembrane domain, and a short cytoplasmic tail. p24 proteins have a predicted lumenal coiled-coil domain, conserved amino acids in the transmembrane domain and cytoplasmic tail, and similar overall size and organization (Blum et al., 1996; Fiedler and Rothman, 1997). They may be grouped into at least three subfamilies based on primary sequence (Blum et al., 1996). One subfamily comprises yeast Emp24p and mammalian p24A; SEL-9 appears to be a member of this subfamily. Another subfamily comprises yeast Erv25p and mammalian Tmp21, and the third subfamily comprises mammalian gp25L proteins.

We searched the *C. elegans* genomic sequence database for additional p24 proteins and identified at least four

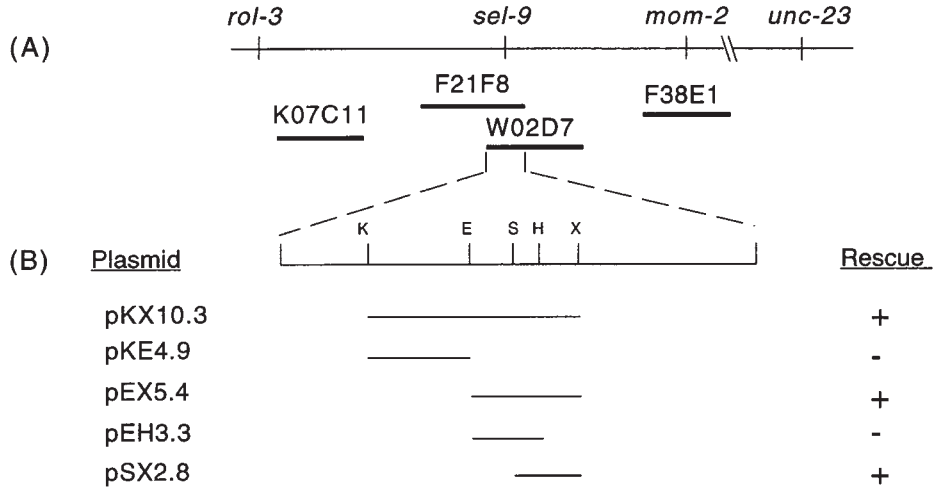

Figure 1. Molecular cloning of *sel-9*. Lines depict genomic DNA subcloned from the overlapping region of two cosmid clones, F21F8 and W02D7. The ability of these plasmid clones to rescue *sel-9(ar22)* is indicated; + indicates a clone was able to rescue, − indicates a clone was unable to rescue. See Materials and Methods for rescue assays. K, KpnI; E, EcoRV; S, SacI; H, HindIII; X, XhoI.

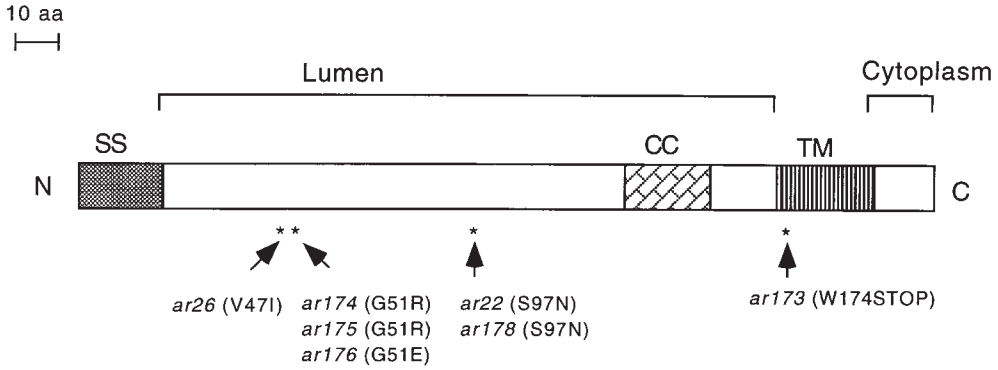

Figure 2. Schematic depiction of SEL-9. All p24 proteins are predicted to be type I membrane proteins with the amino terminus in the lumenal region of the ER and the carboxy terminus in the cytoplasm; in some cases, this prediction has been confirmed (see Blum et al., 1996). Sequence alterations associated with all *sel-9* mutations are shown. We cannot guarantee that *sel-9(ar178)* and *sel-9(ar175)* are independent isolates of *sel-9(ar22)* and *sel-9(ar174)*, respectively. SS, signal sequence; CC, heptad repeats predicted to form a coiled-coil structure; TM, transmembrane domain.

other proteins with the hallmarks of p24 proteins (Fig. 3). As described below, we have also performed a functional test of one of these, F47G9.1, a member of the Erv25/Tmp21 subfamily (Fig. 3).

p24 proteins are major membrane components of COPI- and COPII-coated vesicles (Schimmoller et al., 1995; Stamnes et al., 1995; Sohn et al., 1996). Genetic studies in yeast and biochemical studies in yeast and mammalian cells have led to proposals for p24 protein function in cargo selectivity of ER to Golgi transport (Schimmoller

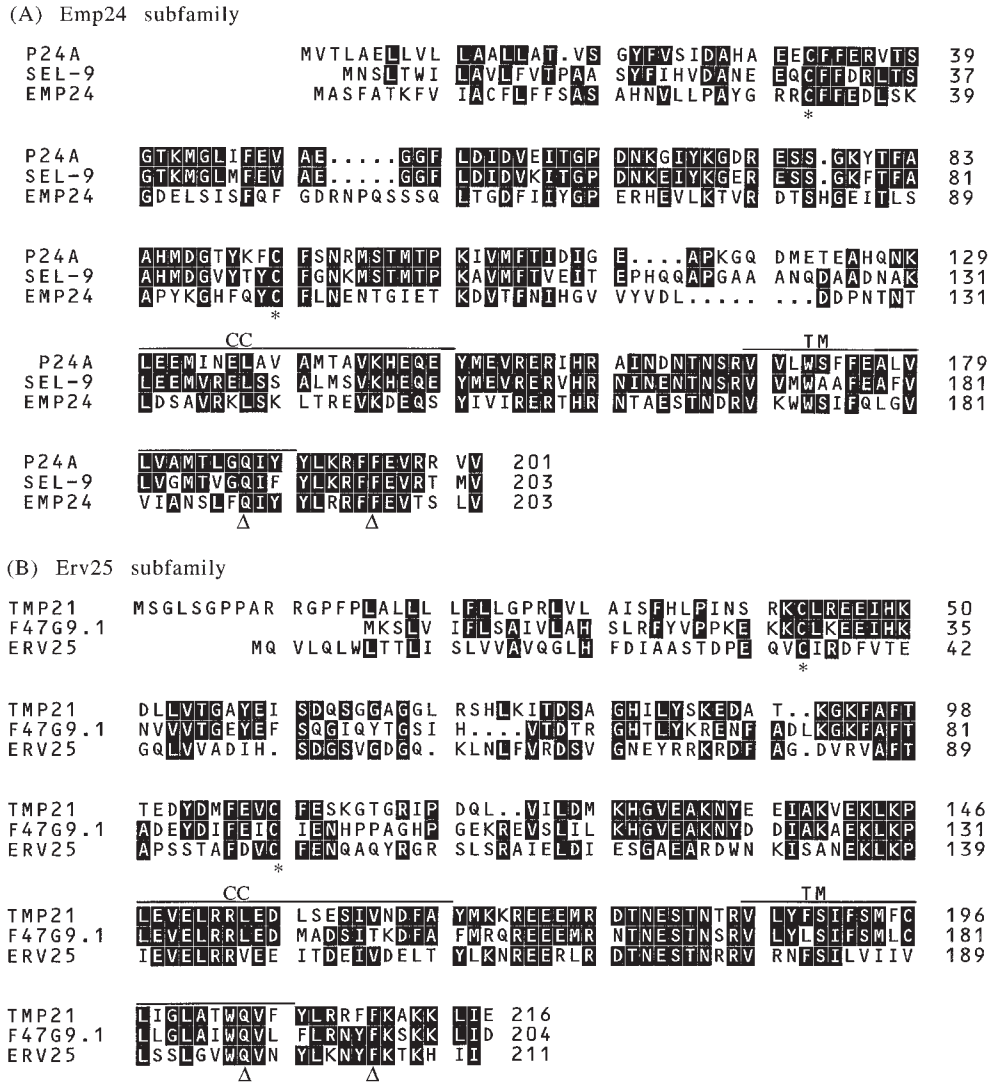

Figure 3. p24 protein subfamilies. EMP24, yeast Emp24p; P24A, human p24A; ERV25, yeast Erv25p; TMP21, human Tmp21. Sequences of other members of the p24 family can be found in Blum et al. (1996) and Stamnes et al. (1995). The transmembrane (TM) and predicted coiled-coil (CC) domains are overlined. A signal sequence is present at the amino terminus of each protein but is not indicated. Residues that are identical in at least two of the three proteins within a subfamily are shown in reverse contrast letters. Conserved cysteines in the lumenal domain are indicated with asterisks and residues identified as functionally important for coat protein binding in the transmembrane/cytosolic domain are indicated with open triangles. The genomic DNA sequences of *sel-9* (accession number AF014940) and of F47G9.1 (accession number Z74035) are in GenBank. Two other *C. elegans* sequences, F57B10.5 (accession number AF039713) and K08E4.6 (accession number Z68316), also display hallmarks of p24 proteins, as does another *C. elegans* sequence present in the unfinished sequence database at the time of submission of this manuscript (data not shown).

et al., 1995; Belden and Barlowe, 1996; Elrod-Erickson and Kaiser, 1996). Our analysis of the interaction of *sel-9* with *lin-12* and *glp-1* is consistent with a role in cargo selectivity, as described further below and in the Discussion.

We constructed a SEL-9::GFP hybrid protein in which green fluorescent protein (GFP) was fused in frame to the carboxy terminus of SEL-9 (see Materials and Methods). The hybrid protein appeared to accumulate intracellularly in all somatic cells and at all developmental stages (data not shown). This observation implies that SEL-9 is present in cells undergoing *lin-12*–mediated cell fate decisions. Unfortunately, this SEL-9::GFP hybrid protein does not appear to function normally, as it was unable to reverse the suppression of *lin-12(n676n930)* by *sel-9* at 25°C (data not shown), so we cannot meaningfully analyze its subcellular distribution.

Sequence Analysis and the Nature of sel-9 Mutations

We sequenced all existing *sel-9* mutations and found that none creates an early stop codon or deletion that would be a clear molecular null allele (Fig. 2). *sel-9(ar173)* creates a stop codon at the beginning of the predicted transmembrane domain, and is predicted to result in a truncated SEL-9 protein lacking most of the TM domain and the entire cytoplasmic tail. All other *sel-9* mutations contain missense changes in the predicted lumenal region of SEL-9.

Six of the seven *sel-9* alleles, corresponding to four different missense mutations, do not cause any obvious defects in an otherwise wild-type background. In contrast, *sel-9(ar173)* homozygous hermaphrodites are dumpy (Dpy), uncoordinated (Unc), and slightly roller (Rol) and egg-laying defective (but have normal vulval lineages; data not shown). These phenotypes are all complemented by an extrachromosomal array containing multiple copies of the *sel-9(+)* gene, and hence appear to result from the *sel-9(ar173)* mutation. However, *sel-9(ar173)/sel-9(ar173)/mnDp26 [sel-9(+)]* hermaphrodites still display a similar spectrum of phenotypes as *sel-9(ar173)*, although they are less abnormal than *sel-9(ar173)/sel-9(ar173)*, suggesting that *sel-9(ar173)* is an antimorph (data not shown), perhaps interfering with the secretion of proteins other than LIN-12 and GLP-1. We postulate that the presence of the lumenal portion of SEL-9 interferes with secretion when an absent or nonfunctional carboxy terminus prevents association with vesicle coat proteins. In support of this interpretation is the observation that fusion of GFP in frame at the carboxy terminus causes the same spectrum of phenotypes as *sel-9(ar173)* (data not shown).

Although we do not have demonstrable *sel-9* null alleles, we note that in yeast, deletion of either *emp24* or *erv25*, or of both genes, has measurable effects on specific aspects of secretion without any significant deleterious effects (Schimmoller et al., 1995; Belden and Barlowe, 1996; Elrod-Erickson and Kaiser, 1996). Thus, it is conceivable that the *sel-9* null phenotype may, at a gross level, be wild-type. Below, we provide evidence suggesting that available *sel-9* alleles, in addition to being antimorphs, also reduce or eliminate *sel-9* activity.

Reducing sel-9 and F47G9.1 Activity Increases lin-12 and glp-1 Activity

The technique of RNA-mediated interference (RNAi) may be used to investigate the effects of reducing gene activity (Rocheleau et al., 1997). RNAi is based on the observation that injection of RNA, and, in particular, dsRNA (Fire et al., 1998), can produce specific phenotypes similar to loss or reduction of function of the target gene. We have used this method to investigate the effects of reducing *sel-9* and F47G9.1 activity.

The assay we used depends on the observation that at 15°C, *lin-12(n676n930)* behaves like a weak gain-of-function allele, and a small proportion of *lin-12(n676n930)* hermaphrodites lacks an AC and displays the 0 AC-Egl phenotype. We examined the ability of dsRNA to enhance the 0 AC-Egl phenotype of *lin-12(n676n930)* at 15°C. Double-stranded *sel-9* or F47G9.1 RNA was injected into *lin-12(n676n930)* homozygous L4 hermaphrodites grown at 15°C, and injected hermaphrodites and mock-injected control hermaphrodites were maintained at 15°C. All hermaphrodites injected with *sel-9* dsRNA or F47G9.1 dsRNA produced a markedly greater proportion of 0 AC-Egl progeny than did control hermaphrodites (see Table IV).

We also examined the ability of dsRNA to suppress the maternal effect lethal phenotype of *glp-1(e2142)* at 25°C, since maternal gene activity seems to be particularly sensitive to inhibition by this method. Double-stranded *sel-9* or F47G9.1 RNA was injected into *glp-1(e2142ts)* homozygous L4 hermaphrodites grown at the permissive temperature. The injected hermaphrodites and uninjected control hermaphrodites were shifted to 25°C. Control hermaphrodites laid only dead eggs. In contrast, all hermaphrodites injected with *sel-9* dsRNA or F47G9.1 dsRNA produced live progeny (see Table IV). The suppression of *glp-1(e2142)* by *sel-9* or F47G9.1 RNAi appears to be incompletely heritable for at least one additional generation, as has been observed in RNAi experiments for certain other genes.

The *sel-9* and F47G9.1 RNAi effects on *lin-12(n676-n930)* and *glp-1(e2142)* indicate that a reduction in p24 activity appears to elevate *lin-12* and *glp-1* activity. These results also suggest that available *sel-9* alleles reduce *sel-9* activity, since all *sel-9* mutations enhance *lin-12(n676n930)* and suppress the maternal effect lethality of *glp-1(e2142)* (Sundaram and Greenwald, 1993b; see Table VI).

We also injected F47G9.1 dsRNA into *sel-9(ar174)*, and did not observe any reduced viability or obvious effects on the egg-laying ability or vulval morphology of adult progeny (see Table IV). This result is consistent with the observation that the phenotype of a *Δemp24 Δerv25* strain is no more severe than either single mutant (Belden and Barlowe, 1996).

Allele Specificity of sel-9 Suppression/Enhancement

sel-9 mutations do not suppress/enhance all *lin-12* or *glp-1* mutations. Rather, they appear to be specific for alleles of *lin-12* and *glp-1* that cause alterations in the extracellular domain (Fig. 4 and Tables II–VI).

sel-9 appears to increase the activity of the partial loss-of-function mutations *lin-12(n676n930)*, *lin-12(ar170)*, and *lin-12(oz48)*, all of which have missense mutations in the extracellular domain (Table II, Fig. 4, and data not shown). *sel-9* also appears to increase the activity of gain-of-function *lin-12(d)* mutations such as *lin-12(n302)*: the

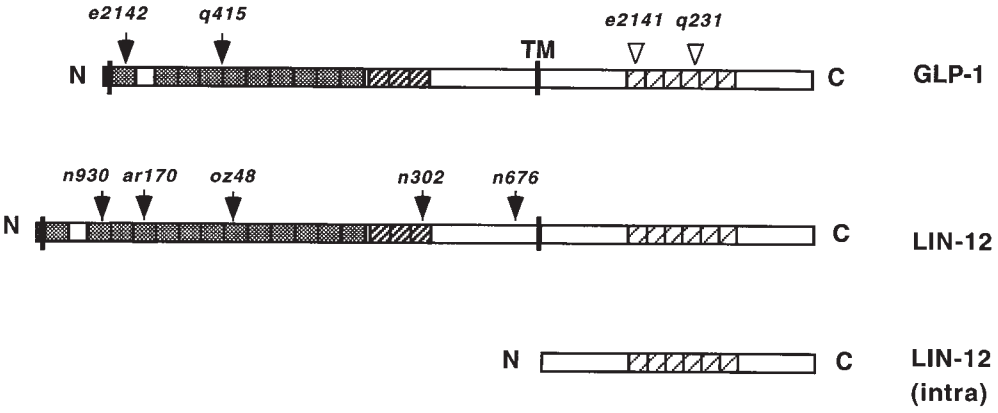

Figure 4. Allele specificity of *sel-9* suppression/enhancement. Schematic drawing of LIN-12 and GLP-1, showing EGF-like motifs (shaded) and LNR motifs (narrowly striped) in the extracellular domains and cdc10/SWI6 (ankyrin) motifs (widely striped) in the intracellular domain (see Weinmaster, 1997). The locations of *lin-12* and *glp-1* mutations are indicated (Greenwald and Seydoux, 1990; Kodoyianni et al., 1992; this study). Black arrowheads indicate mutations that can be suppressed or enhanced by *sel-9*; open arrowheads indicate mutations that are not suppressed or enhanced by *sel-9*. The position of the *n930* lesion is shown, but it was always present in the double mutant *lin-12(n676n930)*. Also shown is LIN-12(intra) (Struhl et al., 1993), which is not enhanced by *sel-9*.

penetrance of the Multivulva phenotype is increased in the presence of *sel-9* mutations (Table V). In contrast, *sel-9* does not appear to increase the activity of *arIs12[lin-12(intra)]* (Table V): the penetrance of the Multivulva phenotype is not increased in the presence of *sel-9(ar174)*. These results suggest that the interaction between *sel-9* and *lin-12* requires the extracellular domain of LIN-12 or that LIN-12 be a transmembrane protein. The ability of *sel-9* to enhance the weak gain-of-function phenotype caused by a multicopy *lin-12(+)* transgene (Table V) is consistent with this inference.

We further investigated the possibility that *sel-9* suppression is specific for mutations in the extracellular domain by taking advantage of a variety of available, sequenced alleles of *glp-1* (Kodoyianni et al., 1992). All *sel-9* alleles can suppress the maternal effect lethality caused by *glp-1(e2142)*, a missense mutation in the first EGF-like motif of the extracellular domain, but cannot suppress either the germline defect or the embryonic lethality caused by *glp-1(e2141)* and *glp-1(q231)*, missense mutations in the cdc10/SWI6 domain of GLP-1 (Kodoyianni et al., 1992) (Table VI). Since *glp-1(e2141)* and *glp-1(e231)* appear to lower *glp-1* activity more than *glp-1(e2142)* (Kodoyianni et al., 1992), the ability of *sel-9* alleles to suppress *glp-1(e2142)* but not *glp-1(e2141)* and *glp-1(q231)* may be explained in three different ways: *sel-9* does not function in the germline; *sel-9* mutations can only suppress mild loss of *glp-1* activity; or *sel-9* mutations interact only with specific *glp-1* alleles.

Table III. Cell Autonomy of sel-9 Function in the AC/VU Decision

Genotype	Cells ablated	0 AC	1 AC
lin-12(n676n930)	None	1/15 (7%)	14/15 (93%)
lin-12(n676n930)	Z4	1/12 (8%)	11/12 (92%)
lin-12(n676n930); sel-9(ar26)	None	55/70 (78%)	15/70 (22%)
lin-12(n676n930); sel-9(ar26)	Z4	10/13 (77%)	3/13 (23%)

Hermaphrodites were grown at 15°C except for the short period of laser microsurgery (see Materials and Methods).

To distinguish among these possibilities, we examined the effect of *sel-9* on *glp-1(q415)* (Table VI). *glp-1(q415)* contains a missense mutation (Gly226→Glu) in the fourth EGF-like repeat (Kodoyianni et al., 1992). At 15°C, the germline of *glp-1(q415)* proliferates normally but all embryos are dead; at 25°C, the germline proliferation of *glp-1(q415)* is defective (Kodoyianni et al., 1992). We found that *sel-9(ar26)*, a weaker *sel-9* allele, can suppress the germline proliferation defect but not the maternal effect lethality of *glp-1(q415)* at 25°C. However, *sel-9(ar174)*, a stronger *sel-9* allele, can suppress both the germline defect and the maternal effect lethality caused by *glp-1(q415)* at 25°C. Thus, *sel-9* does function in the germline. In the germline, *glp-1(q415)* probably has a *glp-1* activity level similar to that of *glp-1(e2141)* and *glp-1(q231)*, since all three mutants produce similar numbers of germ cells at the restrictive temperature (Austin and Kimble, 1987; Priess et al., 1987; Kodoyianni et al., 1992). Thus, the ability of *sel-9(ar26)* to suppress *glp-1(q415)* but not *glp-1(e2141)* and *glp-1(q231)* suggests that the interaction between *sel-9* and *glp-1* depends on alterations in the extracellular domain of GLP-1.

sel-9 Affects the Subcellular Localization of GLP-1(q415)

To determine if the effect of *sel-9* is on LIN-12/GLP-1 trafficking in *C. elegans*, we examined the level or subcellular localization of wild-type and mutant GLP-1 proteins in a *sel-9(+)* and *sel-9* mutant background using an antibody cocktail that recognizes GLP-1 in dissected gonads (Crittenden et al., 1994; Fig. 5). In the germline of wild-type hermaphrodites, GLP-1 is visible mainly in the plasma membrane in the distal region in a honeycomb pattern corresponding to the membranes surrounding each germline nucleus (Crittenden et al., 1994). We saw no evidence for a change in the level or subcellular localization of GLP-1(+) in a *sel-9(ar174)* mutant background (data not shown). In the germline of *glp-1(q415)* hermaphrodites, the membranes are present in their typical honeycomb pattern, as shown by phalloidin staining; however, GLP-

Table IV. RNAi Experiments

Genotype	dsRNA	Average % 0 AC-Egl progeny*
Enhancement of *lin-12(n676n930)* at 15°C		
lin-12(n676n930)	None	5.4
lin-12(n676n930)	*sel-9*	60.3
lin-12(n676n930)	F47G9.1	62.9

Genotype	Injected dsRNA	No. of hermaphrodites yielding live progeny‡	Morphology of adult progeny§
Suppression of *glp-1(e2142)* at 25°C			
glp-1(e2142)	None	0/4	—
glp-1(e2142)	*sel-9*	12/12	Normal
glp-1(e2142)	F47G9.1	10/10	Normal
glp-1(e2141)	None	0/10	—
glp-1(e2141)	*sel-9*	0/10	—
glp-1(q231)	None	0/9	—
glp-1(q231)	*sel-9*	0/10	—

Genotype	Injected dsRNA	Yielding live progeny	Morphology of adult progeny§
Additional experiments			
sel-9(ar174)	F47G9.1	10/10	Normal
sel-9(ar173)	*sel-9*	6/6	No difference‖
Wild-type (N2)	*sel-9* + F47G9.1		Normal

Double-stranded *sel-9* or F47G9.1 RNA was synthesized in vitro and injected into recipient L4 hermaphrodites. For *lin-12(n676n930)* recipients, mock (water)-injected hermaphrodites and dsRNA-injected hermaphrodites were maintained at 15°C. For *glp-1* hermaphrodites, noninjected and dsRNA-injected L4 hermaphrodites were shifted to and then maintained at 25°C.

*Injected hermaphrodites were allowed to lay eggs at 15°C for several hours, and then were removed. Their progeny were scored for the ability to lay eggs. For mock-injected hermaphrodites, two broods were scored: 1/24 and 2/31 were Egl. For *sel-9* dsRNA, four broods were scored: 11/17, 14/22, 12/21, and 7/13 were Egl. For F47G9.1 dsRNA, four broods were scored: 15/23, 10/19, 12/17, and 14/22 were Egl.

‡*glp-1(e2142)* hermaphrodites produce no live progeny; after certain dsRNA injections, hermaphrodites produced virtually no dead eggs, and numerous (>100) live progeny.

§No egg-laying or vulval defects were observed.

‖Dpy, Unc, Rol phenotypes associated with *sel-9(ar173)*.

1(q415) does not display the honeycomb pattern, and instead accumulates within the cell, consistent with a defect in its transport to the plasma membrane. In contrast, in the germline of *glp-1(q415); sel-9(ar174)* hermaphrodites, plasma membrane accumulation of GLP-1(q415) is at least partially restored, as a honeycomb pattern is evident. These results suggest that the absence of *sel-9* activity relieves the block on trafficking of mutant GLP-1(q415) protein to the plasma membrane.

Table V. sel-9 Combinations with Alleles or Transgenes Resulting in Elevated lin-12 Activity or Expression

Relevant genotype	% hermaphrodites with ≥1 pseudovulva (*n*)*	Average No. of pseudovulvae (*n*)
lin-12(n302)	11 ± 2.3 (189)	0.2 (189)
lin-12(n302); sel-9(ar26)	46 ± 2.0 (199)	1.2 (199)
lin-12(n302); sel-9(ar173)	100 (55)	4.4 (55)
lin-12(n302); sel-9(ar174)	100 (47)	3.9 (47)
arIs12[lin-12(intra)]	75 ± 3.6 (145)	1.9 (145)
arIs12[lin-12(intra)]; sel-9(ar174)	75 ± 4.5 (92)	2.1 (92)
arEx29[lin-12(+)]‡	5.9 ± 2.5 (85)	0.1 (85)
arEx29[lin-12(+)]; sel-9(ar174)	81 ± 4.3 (81)	3.1 (81)

Hermaphrodites were grown and scored at 20°C.

*At least one pseudovulva in addition to a normal vulva, or two or more pseudovulvae. The standard variance is indicated.

‡*arEx29* appears to cause a slight elevation in *lin-12* activity, probably due to overexpression of LIN-12(+) (Fitzgerald et al., 1993).

Table VI. sel-9 Combinations with glp-1 Mutations

Relevant genotype*	Germline proliferation‡	Embryonic viability§
Wild-type	+	+
glp-1(e2142)	+	−
glp-1(e2142); sel-9(ar26)	+	+
glp-1(e2142); sel-9(ar174)	+	+
glp-1(q415)	−	−
glp-1(q415); sel-9(ar26)	+	−
glp-1(q415); sel-9(ar174)	+	+
glp-1(e2141)	−	−
glp-1(e2141); sel-9(ar26)	−	−
glp-1(e2141); sel-9(ar174)	−	−
glp-1(q231)	−	−
glp-1(q231); sel-9(ar26)	−	−
glp-1(q231); sel-9(ar174)	−	−

All experiments were done at 25°C, the restrictive temperature for the *glp-1* alleles.

**glp-1(q415)* and *glp-1(q231)* were marked with *unc-32(e189)*. *sel-9(ar26)* was marked with *rol-3(e754)*.

‡Germline proliferation was scored as + if a normal L4-adult germline anatomy (mitosis distal, meiosis proximal) was seen and numberous (>50) fertilized eggs were produced and − if a Glp L4-adult germline anatomy (no mitosis) was seen and no eggs were produced.

§Embryonic viability was scored as + if all progeny grew to adulthood and − if all progeny arrested in embryonic or early larval stages.

Discussion

In this study, we have shown that SEL-9 is a member of the Emp24/p24A subfamily of p24 proteins. Reducing the activity of *sel-9* and F47G9.1, which encodes a member of the Erv25/Tmp21 subfamily of p24 proteins, can increase the activity of certain mutations in *lin-12* or *glp-1*. The interaction between *sel-9* and *lin-12* appears to be cell autonomous. The common feature of the alleles that are affected by *sel-9* is that they are missense mutations in the extracellular domain of LIN-12 or GLP-1; in contrast, the mutations that are not affected include an activated form of LIN-12 caused by truncation of the extracellular domain and transmembrane domain, and point mutations of GLP-1 that alter the intracellular domain. We examined the subcellular localization of the protein encoded by *glp-1(q415)*, one of the suppressed mutations, and found that it accumulates within the cell; however, a mutation in *sel-9* enables GLP-1(q415) to accumulate in the plasma membrane. We discuss specific aspects of these results in the previous section. Here, we discuss how these results are consistent with a function for SEL-9 in cargo selection during transport of LIN-12 and GLP-1 to the cell surface.

In eukaryotic cells, secretory protein trafficking is mediated by transport vesicles, which bud from a donor membrane of one compartment and fuse with a recipient membrane of a different compartment. Distinct vesicle coat protein complexes mediate different budding/fusion events. Anterograde transport from ER to Golgi is mediated by COPII-coated vesicles (Bednarek et al., 1996; Schekman and Orci, 1996). Bidirectional transport between the ER and Golgi, and intra-Golgi transport, is me-

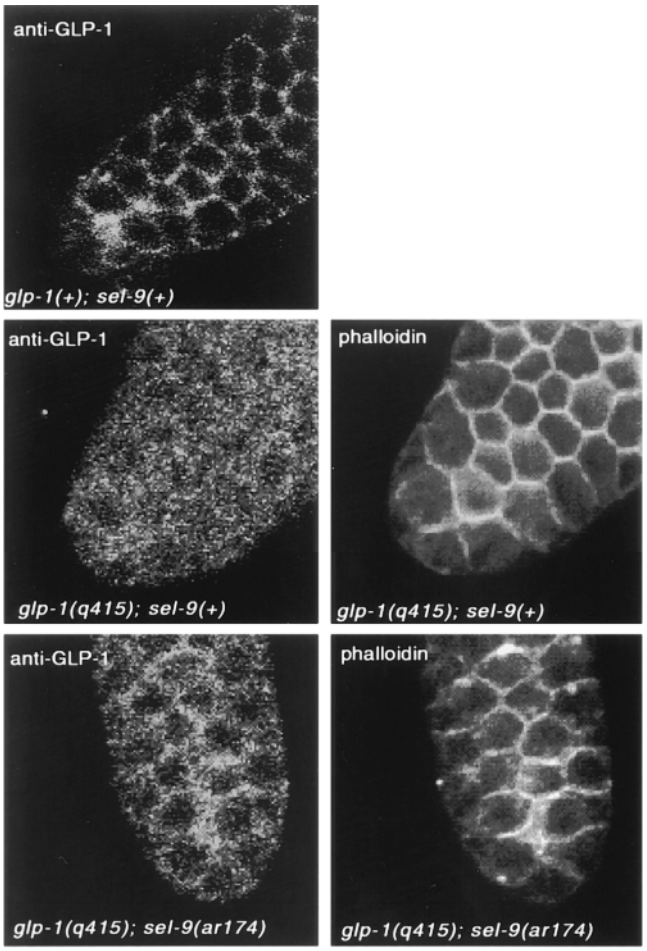

Figure 5. Subcellular distribution of GLP-1. All panels show the distal regions of hermaphrodite gonads. The F-actin–specific probe Alexa488-phalloidin (phalloidin; Molecular Probes A-12379) stains plasma membranes that surround the germline nuclei (Strome, 1986). An antibody cocktail that recognizes GLP-1 (anti–GLP-1; Crittenden et al., 1994) is used to visualize GLP-1 localization. For each genotype, the F-actin and GLP-1 staining is shown in the same focal plane. Hermaphrodites were grown at 15°C until adulthood, and then were shifted to 25°C for 24 h before staining. At the time of staining, the morphology of the germline looked normal in all cases (data not shown).

diated by COPI-coated vesicles (Rothman and Wieland, 1996; Orci et al., 1997). Endocytic trafficking is mediated by clathrin-coated vesicles (Robinson, 1994).

A key feature of vesicle-mediated trafficking is the net transfer of cargo from one compartment to another, while components of the donor compartment are selectively excluded from vesicles and/or recycled. Furthermore, there appears to be a quality control mechanism, so that misfolded or mutant proteins cargo proteins are not transferred (Hammond and Helenius, 1995). Little is known about how selective packaging or quality control occurs. Signals on cargo and the coat proteins appear to influence assembly of the COPII coat complex (Springer and Schekman, 1998). However, other factors appear to influence selectivity: for example, null mutations which bypass the anterograde secretion block associated with the absence of Sec13p (one component of the COPII coat complex) also cause leakage of ER resident proteins and mutant invertase (Elrod-Erickson and Kaiser, 1996).

One gene identified as a bypass suppressor of *Δsec13* was *EMP24* (Elrod-Erickson and Kaiser, 1996), a defining member of the p24 subfamily to which SEL-9 belongs. p24 proteins are transmembrane protein components of COPI- and COPII-coated vesicles (Schimmoller et al., 1995; Belden and Barlowe, 1996; Elrod-Erickson and Kaiser, 1996; Sohn et al., 1996), and interact with coat proteins via their transmembrane/carboxy-terminal domains (Fiedler et al., 1996; Sohn et al., 1996; Dominguez et al., 1998). Strains lacking the p24 proteins Emp24p or Erv25p have similar secretion defects: there is reduced ER to Golgi transport of a subset of secretory proteins and leakage of ER resident proteins (Schimmoller et al., 1995; Belden and Barlowe, 1996; Elrod-Erickson and Kaiser, 1996). Two different roles for p24 proteins have been proposed. One possibility is that p24 proteins are receptors/adaptors for lumenal cargo (Kirchhausen et al., 1997). Alternatively, Elrod-Erickson and Kaiser (1996) have proposed that the proteins encoded by *EMP24* and other *Δsec13* bypass suppressors are part of a quality control mechanism that prevents the premature formation of vesicles that have not properly segregated cargo from ER-resident proteins.

The genetic interactions between *sel-9* and *lin-12* or *glp-1* are consistent with an Emp24p-like role for SEL-9 in the transport of LIN-12 and GLP-1. SEL-9 and F47G9.1 may act during the sorting process to keep misfolded or mutant LIN-12 and GLP-1 proteins from transport vesicles, or, as proposed by Elrod-Erickson and Kaiser (1996), as a general block to the progress of vesicles containing aberrant proteins. Our genetic data are more consistent with a role for p24 proteins in a quality control mechanism as opposed to a role in cargo reception. In our functional interactions, *sel-9* behaves as a negative regulator. If *sel-9* were principally to function as a LIN-12/GLP-1 cargo receptor, we might have expected it to behave as a positive factor: loss or reduction of a cargo receptor should reduce the amount of LIN-12 or GLP-1 at the cell surface. Instead, loss or reduction of *sel-9* activity increases the amount of *lin-12* or *glp-1* activity, enhances the weak gain-of-function activity associated with overexpression of an essentially wild-type LIN-12 protein, and demonstrably increases the amount of a mutant GLP-1 protein at the cell surface. Our results, like those of Elrod-Erickson and Kaiser (1996), are therefore more consistent with a major role for p24 proteins in quality control as opposed to cargo reception.

All of the mutations that were affected by reducing *sel-9* activity alter the extracellular domain of LIN-12 or GLP-1. These mutations may lead to general structural defects in the extracellular domain, since the mutations affect different subregions (Fig. 4) and have different effects (some elevate and some reduce activity). SEL-9(+) may directly or indirectly recognize the abnormal extracellular domains of the mutant LIN-12 or GLP-1 proteins and block their transport, thus effectively functioning to negatively regulate the amount of LIN-12/GLP-1 in the plasma membrane. In *sel-9* mutants, however, abnormal LIN-12/GLP-1 proteins may instead be transported to cell surface, where they may be able to function. This inference is supported

by our examination of the cell biology underlying these genetic interactions. When *sel-9* activity is normal, the mutant GLP-1(q415) protein appears to be retained within the cell, and the hermaphrodites display a *glp-1* mutant phenotype. In contrast, when *sel-9* is mutant, the GLP-1(q415) mutant protein is found in the plasma membrane, and the hermaphrodites display a wild-type phenotype.

We postulate that the effect of *sel-9* on mutant LIN-12 or GLP-1 reflects a role for SEL-9(+) in the transport of LIN-12(+) and GLP-1(+). SEL-9(+) may inhibit the transport of aberrant LIN-12(+) and GLP-1(+) proteins, which may occur at some frequency due to misfolding or misprocessing. The finding that *sel-9* mutations enhance the weak gain-of-function defect associated with overexpression of a tagged LIN-12 protein with a wild-type extracellular domain is consistent with this postulated role.

One issue that deserves comment is the lack of a severe phenotype associated with reduced *sel-9* activity. In yeast, *Δemp24* causes only a moderate reduction of secretion of a select group of proteins and does not cause a marked visible phenotype (Schimmoller et al., 1995; Belden and Barlowe, 1996; Elrod-Erickson and Kaiser, 1996). This lack of a visible phenotype might be attributable to functional redundancy among the multiple p24 proteins in yeast; however, particularly if p24 proteins depend on each other for stability (Belden and Barlowe, 1996; Dominguez et al., 1998), then perhaps elimination of all p24 protein activity might not result in a deleterious phenotype. In *C. elegans*, there also appear to be multiple p24 proteins. The *sel-9* alleles we isolated appear to reduce *sel-9* activity, but we do not know the *sel-9* null phenotype with certainty: none of the existing mutations cause early stop codons or deletions of the coding region. Like Emp24p, SEL-9 may be involved in the transport of a select group of proteins, including LIN-12 and GLP-1. We note that if *sel-9* activity were essential for all secretory protein transport, we might reasonably have expected to see some evidence for a phenotype caused by RNAi. The definitive answer to the question of the phenotype caused by a lack of p24 proteins will be most readily addressed in yeast, where it will be feasible to construct strains lacking multiple genes for p24 proteins.

Our characterization of *sel-9* emphasizes a link between the secretory apparatus and cell signaling during development. The characterization of other developmental genes is providing other linkages between secretion and cell signaling processes. For example, the establishment of dorsoventral polarity occurs during oogenesis and involves a signal from the oocyte to the follicle cells and a second signal from follicle cells back to the oocyte (Ray and Schupbach, 1996). The gene *windbeutel*, which is required for proper dorsoventral polarity, acts in the follicle cells and encodes an ER protein that has been proposed to chaperone a secreted signal produced in the follicle cells (Konsolaki and Schupbach, 1998). Whether the linkages that have been found between the secretory apparatus and cell signaling processes reflect constitutive secretory functions or serve as points of regulation will be an issue for future study.

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