Suppressors of a *lin-12* Hypomorph Define Genes That Interact With Both *lin-12* and *glp-1* in *Caenorhabditis elegans*

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Manuscript received April 14, 1993
Accepted for publication July 29, 1993

ABSTRACT

The *lin-12* gene of *Caenorhabditis elegans* is thought to encode a receptor which mediates cell-cell interactions required to specify certain cell fates. Reversion of the egg-laying defective phenotype caused by a hypomorphic *lin-12* allele identified rare extragenic suppressor mutations in five genes, *sel-1, sel-9, sel-10, sel-11* and *sel(ar40)* (*sel* = suppressor and/or enhancer of *lin-12*). Mutations in each of these *sel* genes suppress defects associated with reduced *lin-12* activity, and enhance at least one defect associated with elevated *lin-12* activity. None of the *sel* mutations cause any obvious phenotype in a wild-type background. Gene dosage experiments suggest that *sel-1* and *sel(ar40)* mutations are reduction-of-function mutations, while *sel-9* and *sel-11* mutations are gain-of-function mutations. *sel-1, sel-9, sel-11* and *sel(ar40)* mutations do not suppress amorphic *lin-12* alleles, while *sel-10* mutations are able to bypass partially the requirement for *lin-12* activity in at least one cell fate decision. *sel-1, sel-9, sel-10, sel-11* and *sel(ar40)* mutations are also able to suppress the maternal-effect lethality caused by a partial loss-of-function allele of *glp-1*, a gene that is both structurally and functionally related to *lin-12*. These *sel* genes may therefore function in both *lin-12* and *glp-1* mediated cell fate decisions.

CELL-CELL interactions specify the fates of many cells during *Caenorhabditis elegans* development (for recent reviews see HORVITZ and STERNBERG 1991; LAMBIE and KIMBLE 1991; GREENWALD and RUBIN 1992). The *lin-12* gene plays a central role in several different cell fate decisions requiring cell interactions (GREENWALD, STERNBERG and HORVITZ 1983), and may encode a receptor for intercellular signals (GREENWALD 1985; YOCHEM, WESTON and GREENWALD 1988; SEYDOUX and GREENWALD 1989). *lin-12* belongs to a growing gene family whose other members include *C. elegans glp-1* (AUSTIN and KIMBLE 1989; YOCHEM and GREENWALD 1989), Drosophila *Notch* (WHARTON *et al.* 1985; KIDD *et al.* 1986), Xenopus *Xotch* (COFFMAN, HARRIS and KINTNER 1990), rat *Notch* (WEINMASTER, ROBERTS and LEMKE 1991), rat *Notch2* (WEINMASTER, ROBERTS and LEMKE 1992), mouse *Motch* (FRANCO DEL AMO *et al.* 1992; REAUME *et al.* 1992), the mouse proto-oncogene *int-3* (JHAPPEN *et al.* 1992; ROBBINS *et al.* 1992) and the human proto-oncogene TAN-1 (ELLISEN *et al.* 1991). All members of this family encode large transmembrane proteins containing a similar arrangement of three types of repeated amino acid sequence motifs: epidermal growth factor-like (EGFL) motifs, *lin-12/Notch* repeat (LNR) motifs, and cdc10/SWI6 motifs. The *C. elegans glp-1* and Drosophila *Notch* genes share functional as well as structural similarities with *lin-12*: both are also required for cell fate decisions involving cell interactions, and both have been found to function in cells that receive intercellular signals (AUSTIN and KIMBLE 1987; PRIESS, SCHNABEL and SCHNABEL 1987; HOPPE and GREENSPAN 1986, 1990; HEITZLER and SIMPSON 1991). While the functions of the remaining family members are not yet known, the striking conservation of structure among these genes implies that the vertebrate and invertebrate family members have similar functions. Information learned from studying *lin-12* will therefore be relevant to understanding the roles of family members in the development of many different organisms, including humans.

Further understanding of the role of *lin-12* in cell fate decisions requires a knowledge of other genes also acting in these decisions. For example, if *lin-12* encodes a receptor, what is its ligand? What is its downstream target? What factors regulate its expression? There are two standard genetic approaches that are used to identify additional genes acting in the same process as an existing gene. One approach is to isolate mutations causing the same phenotype as those in the original gene; the power of this type of approach has been demonstrated in numerous systems. A second approach is to isolate extragenic suppressors or enhancers of mutations in the original gene; such an approach has been used extensively in microbial systems to define interacting genes (HARTMAN and ROTH 1973) and is also quite feasible in *C. elegans* (HODGKIN, KONDO and WATERSTON 1987).

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Genetics 135: 765–783 (November, 1993)

In Drosophila, several additional genes (the "neurogenic loci") have been identified that have the same null phenotype as *Notch*, and that therefore probably function in the same cell fate decision-making processes as *Notch* (LEHMANN *et al.* 1983). However, this approach has not been successful in *C. elegans*: no other genes have been found that can mutate to either a Lin-12- or Glp-1-like phenotype (SEYDOUX, SAVAGE and GREENWALD 1993; J. KIMBLE, unpublished data). One reason for this failure appears to be that some genes act in conjunction with both *lin-12* and *glp-1*. LAMBIE and KIMBLE (1991) have identified two such genes, *lag-1* and *lag-2*, which have the same null phenotype as a *lin-12 glp-1* double mutant (lag = lin-12 and *glp-1*).

There are likely to be many more genes which function in *lin-12*- and/or *glp-1*-mediated processes, but do not mutate to Lin-12, Glp-1 or Lag phenotypes for any of the following reasons. (1) There may be genes that function in some *lin-12*- and/or *glp-1*-mediated cell fate decisions but not in others; eliminating the activity of such a gene would cause only a subset of the defects seen in the *lin-12*, *glp-1* or *lag* mutants. (2) Some genes that act in *lin-12*- and/or *glp-1* mediated processes may be functionally redundant; eliminating the activity of only one such gene might not cause any phenotype. (3) A gene that functions in one *lin-12*- and/or *glp-1*-mediated process might have additional roles as well; eliminating the activity of such a gene would therefore cause a novel phenotype. Screens for extragenic suppressors or enhancers of *lin-12* or *glp-1* mutations make no assumptions about the nature of the null phenotypes of interacting genes and therefore could in principle circumvent some of the above problems.

Here we describe the results of a screen for suppressors of the egg-laying (Egl) defect caused by a partial loss-of-function (hypomorphic) *lin-12* allele. We screened approximately 500,000 haploid mutagenized genomes and identified extragenic suppressor mutations in five genes, *sel-1*, *sel-9*, *sel-10*, *sel-11* and *sel(ar40)* (*sel* = suppressor and/or enhancer of lin-12). These mutations also suppress a partial loss-of-function *glp-1* allele, suggesting that, like *lag-1* and *lag-2*, the *sel-1*, *sel-9*, *sel-10*, *sel-11* and *sel(ar40)* genes may function in both *lin-12*- and *glp-1*-mediated processes.

MATERIALS AND METHODS

General methods: General methods for the handling, culturing and ethyl methane sulfonate (EMS) mutagenesis of nematodes have been previously described (BRENNER 1974). Most experiments were done at 25°, except as noted.

Strains and genetic nomenclature: *C. elegans* var. Bristol strain N2 is the wild-type parent for all strains used in this work. The mutations used are listed below. *lin-12* mutations are described in GREENWALD, STERNBERG and HORVITZ (1983), SEYDOUX, SCHEDL and GREENWALD (1990) and SUNDARAM and GREENWALD (1993). Linkage group (LG) V deficiencies and *let* mutations are described in JOHNSEN and BAILLIE (1991). Other mutations are described in BRENNER (1974) unless otherwise indicated.

LG I: dpy-5(e61), dpy-14(e188), unc-38(x20) (LEWIS *et al.* 1980), *unc-40(e430), sDf4* (HOWELL *et al.* 1987), *hDp20* (MCKIM and ROSE 1990). The translocation *hT1(I;V)* (MCKIM, HOWELL and ROSE 1988) causes recessive lethality and suppresses recombination on the left halves of linkage groups I and V.

LG III: dpy-17(e164), dpy-18(e364), dpy-19(e1259), lin-12(n137), lin-12(n137e2032), lin-12(n137n720), lin-12(n302), lin-12(n379), lin-12(n676), lin-12(n676n909), lin-12(n676n930), lin-12(n676n927), lin-12(n941), lin-12(q269), lin-12(oz48), ncl-1(e1865) (E. HEDGECOCK, unpublished data; HERMAN 1989), *unc-32(e189), unc-36(e251), eT1(III;V)* (ROSENBLUTH and BAILLIE 1981), *qDp3* (AUSTIN and KIMBLE 1987).

LG V: daf-11(m47) (RIDDLE, SWANSON and ALBERT 1981), *dpy-11(e224), egl-10(n692)* (TRENT, TSUNG and HORVITZ 1983), *him-5(e1467)* (HODGKIN, HORVITZ and BRENNER 1979), *let-334(s908), let-340(s1022), let-409(s823), let-412(s579), let-416(s113), let-433(s1904), let-434(s1904), let-464(s1504), lin-25(n545)* (FERGUSON and HORVITZ 1985), *lon-3(e2175), myo-3(st378)* (WATERSTON 1989), *rol-3(e754), rol-3(s742), rol-4(sc8)* (COX *et al.* 1980), *sma-1(e30), sqt-3(sc63)* (COX *et al.* 1980), *srf-8(dv38)* (LINK *et al.* 1992), *srf-9(dv4)* (LINK *et al.* 1992), *unc-42(e270), unc-46(e177), unc-76(e911), eT1(III;V)* (ROSENBLUTH and BAILLIE 1981), *arDf1* (S. TUCK, unpublished data), *ctDf1*(ROGALSKI, BULLERJAHN and RIDDLE 1988), *mDf1* and *mDf3* (BROWN 1984), *sDf29, sDf35, sDf47, sDf57, sDf71, ctDp11* (HUNTER and WOOD 1992), *mnDp26* (HERMAN, MADL and KARI 1979). *DnT1* (E. FERGUSON, unpublished data) is a derivative of the translocation *nT1(IV;V)* (FERGUSON and HORVITZ 1985) containing both recessive lethal and dominant visible markers such that homozygotes are inviable and heterozygotes are uncoordinated (Unc). *eT1(III;V)* (ROSENBLUTH and BAILLIE 1981) is a reciprocal translocation that suppresses recombination on the right arm of linkage group III and the left arm of linkage group V, and which causes markers in these two regions to appear linked.

Cell lineage and anatomical analysis: General methods for Nomarski differential interference contrast microscopy of living animals have been described (SULSTON and HORVITZ 1977). All anatomical analyses were carried out in a 25° constant temperature room. Cell fate transformations and egg-laying defective (Egl) phenotypes associated with *lin-12* mutations are described in the text. However, a detailed description of criteria and methods used to score the Egl phenotype of *lin-12(n676n930)* and other hypomorphic *lin-12* mutants, the 2 AC and proximal mitosis phenotypes associated with reduced *lin-12* activity, and the 0 AC-Egl and Multivulva (Muv) phenotypes associated with elevated *lin-12* activity, are described in the accompanying paper (SUNDARAM and GREENWALD 1993).

Isolation of suppressors of *lin-12(n676n930)*: *unc-32 lin-12(n676n930)* hermaphrodites raised at 15° or 20° were mutagenized with 50 mM EMS (BRENNER 1974) and allowed to self-fertilize for two generations. The F_2 generation was screened at 25° for non-Egl animals; only one candidate was kept per plate. Candidates were self-fertilized in order to establish revertant strains. All revertant strains were outcrossed to N2 twice before being analyzed. The proportion of Unc non-Egl animals segregating from heterozygous *unc-32 lin-12(n676n930)/++; sel/+* mothers in such outcrosses was used as an initial indicator of the recessiveness or dominance of *sel* mutations.

After screening an estimated 368,000 haploid mutagenized genomes, we obtained two alleles of *sel-1*, two alleles of *sel-9*, one allele of *sel-10*, one allele of *sel-11* and eight apparent alleles of *sel(ar40)*. Additional alleles were isolated in similar screens from which frequencies could not be reliably calculated.

Assignment of recessive *sel* alleles to linkage group V: Heterozygotes of genotype *unc-32 lin-12(n676n930)/++; sel/+; dpy/+* [or *sel/dpy*] were self-fertilized, and Unc non-Egl non-Dpy progeny [of genotype *unc-32 lin-12(n676n930); sel*] were picked and scored for the segregation of the *dpy* marker in the next generation. The fraction of animals segregating the marker is 2/3 for unlinked markers, and 2p (where p = recombination frequency) for closely linked markers. Most recessive *sel* mutations showed loose linkage to *dpy-11 V* (data not shown). Three recessive *sel* mutations (*ar25*, *ar27* and *ar37*) were incompletely penetrant and unlinked to *dpy-11*, and have not been further characterized.

Assignment of dominant *sel* alleles to linkage group I: Heterozygotes of genotype *unc-32 lin-12(n676n930)/++; sel/+; dpy/+* [or *sel/dpy*] were self-fertilized, and Unc non-Dpy progeny were picked. The fraction of such animals not segregating non-Egl progeny is 1/4 for unlinked *dpy* markers, and 2p for closely linked markers. All 13 dominant *sel* mutations showed tight linkage to *dpy-5 I* (data not shown). Since these mutations are dominant, we cannot perform complementation tests to establish their allelism. Although it is formally possible that these *sel* mutations define multiple, closely linked genes, we think this is unlikely, and so we have tentatively assigned them to one locus, for which *sel(ar40)* is the canonical allele. The other putative alleles, which have not been further characterized, are: *ar24*, *ar35*, *ar36*, *ar38*, *ar42*, *ar74*, *ar76*, *ar80*, *ar81*, *ar82*, *ar83* and *ar86*.

Three-factor crosses (Table 1): Hermaphrodites of genotype *unc-32 lin-12(n676n930)/++; ab/sel* were constructed, and A non-B or B non-A recombinant progeny were picked at 20°. Recombinants were tested for the presence or absence of the *sel* allele in one of three ways: (1) If recombinants were *unc-32 lin-12(n676n930)/++*, Unc A or Unc B progeny were picked to 25° and scored for segregation of non-Egl progeny. For all *sel-10* mapping experiments (see below), and in some other cases where marker effects made it difficult to evaluate the Egl phenotype, Unc A or Unc B progeny were also picked to 15° and scored for the segregation of "0 AC-Egl" progeny. (2) If recombinants were not *unc-32 lin-12(n676n930)/++*, homozygous *a* or *b* recombinant lines were established, and an *unc-32 lin-12(n676n930)* chromosome was then crossed in. Unc A or Unc B animals were then scored as in (1). (3) In cases where the Egl phenotype of homozygous marked animals could not be scored reliably at either 15° or 25°, recombinant chromosomes were tested for the presence of the relevant *sel* allele by complementation. For example, all Unc non-Sma non-Dpy progeny from *unc-32 lin-12(n676n930)/++; sma-1 (sel)/dpy-11 sel* mothers were scored for egg laying, and the *sel* mutation was judged to be present if a high percentage of such animals were non-Egl compared with controls.

Complementation tests among recessive *sel* mutations on linkage group V: Recessive *sel* mutations on LG V were originally checked for complementation of suppression of the Egl defect by crossing *unc-32 lin-12(n676n930)/+; sel/+* males to *unc-32 lin-12(n676n930); dpy-11 sel′* hermaphrodites at 25°, and scoring Unc non-Dpy hermaphrodite cross progeny for egg laying. Mutations were scored as failing to complement if approximately half of such animals were non-Egl. These tests defined four different complementation groups: *sel-1* (five alleles: ***e1948***, *ar23*, *ar29*, *ar75*, *ar77*), *sel-9* (two alleles: ***ar22***, *ar26*), "*sel-10*" (two alleles: *ar28*, ***ar41***; but see below), and *sel-11* (two alleles: ***ar39***, *ar84*). Unless otherwise stated, the canonical alleles of each locus (shown in bold) were used in all experiments.

***sel-10* and *arX*:** During three-factor mapping experiments, it became apparent that for both "*sel-10*"-containing revertant strains (as originally defined by two-factor map data and complementation tests), suppression of the Egl phenotype caused by *lin-12(n676n930)* at 25° is actually dependent on two different loosely linked mutations, one that we call *sel-10* and another that we will call here "*sel(arX)*." Neither *sel-10(ar28)* nor *sel-10(ar41)* suppresses the *lin-12(n676n930)* Egl phenotype at 25° unless *sel(arX)* is also present (Table 5 and data not shown). However, in the absence of *sel(arX)*, either *sel-10(ar28)* or *sel-10(ar41)* can enhance the 0 AC-Egl phenotype caused by *lin-12(n676n930)* at 15° (Table 6 and data not shown). *sel-10(ar28)* and *sel-10(ar41)* fail to complement each other for this enhancer phenotype, showing that they are indeed allelic. All *sel-10* three-factor map data were obtained by scoring this enhancer phenotype.

Since the original *sel-10(ar28)* and *sel-10(ar41)*-containing chromosomes fail to complement for suppression of the *lin-12(n676n930)* Egl phenotype, both must contain allelic *sel(arX)* mutations. *sel(arX)* may have been present in the background of the *lin-12(n676n930)* strain prior to mutagenesis, or it may have arisen during our screen for non-Egl revertants. Therefore, we do not know if *sel(arX)* is identical between the *sel-10(ar41)* and *sel-10(ar28)*-containing strains. It is possible that *sel(arX)* is also present in the background of our other revertant strains (although it is not required for suppression in those cases). Since the only known phenotype associated with *sel(arX)* is cooperation with *sel-10* mutations to suppress the *lin-12(n676n930)* Egl phenotype, *sel(arX)* is difficult to work with and has not been extensively characterized. *sel(arX)* appears to map to the left of *sma-1 V*, and may be responsible for the partially non-Egl phenotype of *lin-12(n676n930); sel(arX) sel-10/mDf1* hermaphrodites (data not shown).

In many experiments involving *sel-10* that are described in this paper *sel(arX)* is also present; this is always indicated. In experiments done in the absence of *sel(arX)*, we have found that *sel-10(ar41)* is capable of suppressing the 2 AC defect in *lin-12(n676n930)* and *lin-12(n941)* animals, and causing a strong Muv phenotype and maternal-effect lethality in *lin-12(n302)* animals (Table 4, Figure 2, and data not shown).

sel dosage experiments and deficiency mapping (Table 2)

***sDf35*:** Males of genotype *unc-32 lin-12(n676n930)/eT1; sel/eT1 him-5* were crossed to hermaphrodites of genotype *unc-32 lin-12(n676n930)/eT1; dpy-11 sDf35/eT1*, and Unc-32 hermaphrodite cross progeny [of genotype *unc-32 lin-12(n676n930); sel/dpy-11 sDf35*] were scored for egg laying. Males of genotype *unc-32 lin-12(n676n930)/++; dpy-11 sel/++* were crossed to hermaphrodites of genotype *unc-32 lin-12(n676n930)/eT1; dpy-11 sDf35/eT1*, and the Dpy Unc hermaphrodite cross progeny [of genotype *unc-32 lin-12(n676n930); dpy-11 sel/dpy-11 sDf35*] were scored by Nomarski optics for number of ACs. Dpy non-Unc hermaphrodite cross progeny [of genotype *++/unc-32 lin-12(n676n930); dpy-11 sel/dpy-11 sDf35*] appeared normal.

***mDf1*, *sDf57*, *sDf71*:** see "Complementation tests between recessive *sel* mutations on LG V and existing mutations."

***mDf3*, *sDf29*, *sDf47*:** Males of genotype *unc-32 lin-12(n676n930)/eT1; dpy-11 sel/eT1 him-5* were crossed to hermaphrodites of genotype *unc-32 lin-12(n676n930)/eT1; Df/eT1*, and the Unc-32 hermaphrodite cross progeny [of

TABLE 1

Three-factor map data

Gene	Genotype of heterozygous parent	Phenotype of selected recombinants	sel genotype of selected recombinants
sel-1	*rol-4* + *lin-25*/+ *sel-1* +	Rol	5/8 *sel-1*/+
			3/8 +/+
	sma-1 rol-4 +/+ + *sel-1*	Rol	7/7 +/+
sel-9	*dpy-11 rol-3* +/+ + *sel-9*	Dpy	3/3 *sel-9*/+
		Rol	27/27 +/+
	rol-3 + *unc-42*/+ *sel-9* +	Rol	1/31 *sel-9*/+
			30/31 +/+
		Unc	5/5 *sel-9*/+
sel-10	*sqt-3* + *unc-76*/+ *sel-10* +	Sqt	5/8 *sel-10*/+
			3/8 +/+
		Unc	3/5 *sel-10*/+
			2/5 +/+
	lon-3 lin-25 +/+ + *sel-10*	Lon	5/5 *sel-10*/+
	lin-25 + *unc-76*/+ *sel-10* +	Lin-25	2/4 *sel-10*/+
			2/4 +/+
		Unc	1/1 *sel-10*/+
sel-11	+ *sma-1 rol-4*/*sel-11* + +	Rol	11/11 *sel-11*/+
	unc-42 + *sma-1*/+ *sel-11* +	Unc	13/14 *sel-11*/+
			1/14 +/+
	(*daf-11*+) *sma-1*/(+ *sel-11*) +	Sma	2/2 *sel-11*/+
	+ *sma-1 myo-3*/*sel-11* + +	Sma	5/5 +/+
sel(ar40)	+ *dpy-5 unc-40*/*sel(ar40)* + +	Dpy	14/14 +/+
	unc-38 + *dpy-5*/+ *sel(ar40)* +	Dpy	1/37 *sel(ar40)*/+ 36/37 +/+

Data are for the canonical alleles of each locus, except in the cases of *sel-9*, where data from the two alleles *ar22* and *ar26* are pooled, and *sel-10*, where data from the two alleles *ar41* and *ar28* are pooled. Parentheses indicate that the relative order of two genes is unknown.

genotype *unc-32 lin-12(n676n930); dpy-11 sel/Df*] were scored for their egg-laying ability. Non-Dpy self progeny of these hermaphrodites were scored by Nomarski optics for number of ACs. Some *unc-32 lin-12(n676n930); dpy-11 sel/mDf3* animals were also obtained by the method described under "Complementation tests between recessive *sel* mutations on LG V and existing mutations."

***arDf1*, *ctDf1*:** *unc-32 lin-12(n676n930)/eT1; dpy-11 sel/eT1 him-5* males were crossed to *Df/DnT1* hermaphrodites to generate *unc-32 lin-12(n676n930)/++; dpy-11 sel/Df* hermaphrodite cross progeny. These were selfed, and Unc non-Dpy progeny [*unc-32 lin-12(n676n930); dpy-11 sel/Df*] scored for egg laying. The Unc non-Dpy progeny of at least three of these animals were then scored for number of ACs.

***sDf4*:** Because +/*sDf4* animals are unhealthy, Egl, and have a very low brood size, it was not feasible to obtain enough *sel(ar40)/sDf4; lin-12(n676n930)* animals to reliably check suppression of *lin-12(n676n930)* defects. When *sel(ar40) dpy-5*/++ males were mated to *sDf4/hT1* hermaphrodites, and L3 or L4 Dpy cross progeny [of genotype *sel(ar40) dpy-5/sDf4*] were examined by Nomarski optics, all looked normal ($n = 8$), although (like control animals) they were quite sick and sterile as adults.

***mnDp26*:** *unc-32 lin-12(n676n930)/eT1; dpy-11 sel-9/eT1 him-5* males were crossed to *rol-3; mnDp26* hermaphrodites to generate *unc-32 lin-12(n676n930)/++; dpy-11 sel-9/rol-3; mnDp26* hermaphrodite cross progeny. WT self progeny of these hermaphrodites were individually picked to identify those that were *unc-32 lin-12(n676n930)/++; dpy-11 sel-9; mnDp26*, from which Unc and Non-Unc strains were established. Non-Unc animals appeared normal. The entire broods (including both Dpy and Non-Dpy animals) of at least five Unc hermaphrodites were scored for egg laying and number of ACs at 25°.

***ctDp11*:** *ctDp11* (Hunter and Wood 1992) is a fusion between *ctDp8* (which complements markers on the right arm of linkage group V, including *her-1*) and *sDp3* (which complements markers on linkage group III, including *unc-36*, but does not complement *lin-12*). *dpy-17 ncl-1 unc-36; him-8; her-1 unc-42; ctDp11* males were mated to *unc-36 lin-12(n676n930); sel* hermaphrodites to generate non-Unc cross progeny of genotype *dpy-17 ncl-1 unc-36/unc-36 lin-12(n676n930); him-8/+; her-1 unc-42/sel*. Wild-type self progeny of such hermaphrodites were picked to identify those that were *unc-36 lin-12(n676n930); sel; ctDp11*. For each such strain, the entire broods (including both Unc and non-Unc animals) of at least five hermaphrodites were scored for egg laying and number of ACs at 25°.

***hDp20*:** Males of genotype *sel(ar40) dpy-14/dpy-5; unc-32(n676n930)*/++ were mated to hermaphrodites of genotype *dpy-5 dpy-14; hDp20*, and wild-type cross progeny were picked to identify those of genotype *sel(ar40) dpy-14/dpy-5 dpy-14; unc-32(n676n930)/++; hDp20*. Unc non-Dpy progeny of such animals were picked to establish both *sel(ar40) dpy-14; unc-32 lin-12(n676n930); hDp20* and *dpy-5 dpy-14; unc-32 lin-12(n676n930); hDp20* strains. For each strain, all non-Dpy progeny of at least five hermaphrodites were scored for egg laying and number of ACs at 25°.

Complementation tests between recessive *sel* mutations on LG V and existing mutations

***sel-1* vs. *egl-10*:** *egl-10(n692)* is slightly semi-dominant at 25° (Trent, Tsung and Horvitz 1983). *unc-32 lin-12(n676n930)/++; dpy-11 sel-1/egl-10* hermaphrodites (3/4 of which were non-Egl) were selfed, and Unc non-Dpy

TABLE 2

Deficiency mapping data

Relevant genotype	% 2 AC *sel(+)*	*sel-1(e1948)*	*sel-9(ar22)*	*sel(arX)* *sel-10(ar41)*	*sel-11(ar39)*	*sel* genes removed by deficiency*
lin-12(n676n930); sel/sel[1]	32 (108)	0 (42)	0 (55)	0 (36)	0 (38)	—
lin-12(n676n930); sel/+[2]	32 (40)	36 (36)	4 (123)	18 (51)	14 (28)	—
lin-12(n676n930); sel/sDf35[3]	39 (36)	33 (49)	0 (44)	37 (8)	28 (18)	*sel-9*, (*sel-11*)
lin-12(n676n930); sel/mDf3[4]	26 (23)	22 (9)	3 (63)[a]	31 (16)	22 (9)	*sel-9*
lin-12(n676n930); sel/sDf47[4]	28 (26)	18 (34)	3 (115)	ND	ND	None
lin-12(n676n930); sel/sDf71[4]	ND	ND	3 (30)	ND	ND	None
lin-12(n676n930); sel/sDf29[4]	40 (5)	ND	33 (58)[b]	30 (23)	ND	None
lin-12(n676n930); sel/sDf57[4]	36 (14)	ND	3 (30)	ND	ND	None
lin-12(n676n930); sel/ctDf1[4]	37 (30)	30 (30)	ND	35 (40)	31 (45)	(*sel-11*)
lin-12(n676n930); sel/arDf1[5]	18 (54)	0 (73)[c]	ND	14 (77)	ND	*sel-1*, (*sel-10*)
lin-12(n676n930); sel/itDf2[4]	100 (4)	ND	ND	43 (7)	ND	(*sel-10*)

The number of animals scored is given in parentheses. Unless otherwise indicated, the Egl defect was not suppressed. ND, no data.

* For genes indicated in parentheses, neither the three-factor map data or deficiency mapping data allow us to determine unambiguously whether or not the gene is removed by a given deficiency. Because *sel-10* and *sel-11* mutations may be gain-of-function mutations, the phenotype of *sel/Df* cannot be predicted; a finding that *sel/Df* does not suppress does not prove that the deficiency does not remove the relevant *sel* gene.

[a] The Egl defect was partially suppressed (see Table 8).

[b] This result is puzzling and suggests that *sDf29* in some way antagonizes *sel-9* suppressor activity. Since additional copies of *sel-9*(+) also antagonize *sel-9* suppressor activity (Tables 8 and 9), one hypothesis is that the *sDf29* chromosome contains a duplication of the *sel-9* region.

[c] The Egl defect was partially suppressed (see Table 7).

[1] *unc-32 lin-12(n676n930).*

[2] *unc-32 lin-12(n676n930); dpy-11 sel/++.*

[3] *unc-32 lin-12(n676n930); dpy-11 sel/dpy-11 sDf35.*

[4] *unc-32 lin-12(n676n930); dpy-11 sel/Df.*

[5] *unc-32 lin-12(n676n930); dpy-11 sel/unc-42 arDf1.*

progeny scored for egg laying. Greater than 95% of such animals were Egl, indicating complementation (*n* = 31).

***sel-9* vs. *let-334*, *let-340*, *let-412*, *let-433*, *let-434*, *let-464*, *rol-3*, *sDf57*, *sDf71*, *mDf1* and *mDf3*:** *unc-32 lin-12(n676n930)/eT1; dpy-11 sel-9/eT1 him-5* males were crossed to *dpy-18/eT1; unc-46 let/eT1* hermaphrodites, and wild-type progeny were picked to identify those that were *unc-32 lin-12(n676n930)/dpy-18; dpy-11 sel-9/unc-46 let* (which were viable in all cases). Unc-32 non-Dpy self progeny of these hermaphrodites [of genotype *unc-32 lin-12(n676n930); dpy-11 sel-9/let*] were scored for egg laying. For all mutations except *mDf3*, greater than 95% of Unc-32 non-Dpys were Egl, indicating complementation (*n* > 20).

***sel-9* vs. *let-409* and *let-416*:** *unc-32 lin-12(n676n930)/eT1; dpy-11 sel-9/eT1* males were mated to *dpy-18/eT1; dpy-11 let/eT1* hermaphrodites, and Dpy cross progeny (which were viable in both cases) were picked. Unc self progeny of these hermaphrodites were of two genotypes: *unc-32 lin-12(n676n930); dpy-11 sel* (1/3) or *unc-32 lin-12(n676n930); dpy-11 sel/dpy-11 let* (2/3). For both *let-409* and *let-416*, more than 2/3 of such animals were Egl, indicating complementation (*n* > 50).

***sel-9* vs. *srf-9*:** *srf-9* enhances the VPC fate defects of *lin-12(d)* mutants (LINK *et al.* 1992), and causes a 0 AC-Egl phenotype in *lin-12(n676n930)* hermaphrodites at 25° [40/44 *unc-32 lin-12(n676n930); srf-9* animals had 0 AC]. While it therefore seemed possible that *sel-9* and *srf-9* could be allelic, this does not appear to be the case. *unc-32 lin-12(n676n930)/eT1; srf-9 him-5/eT1 him-5* males were mated to *unc-32 lin-12(n676n930); dpy-11 sel-9* hermaphrodites, and Unc non-Dpy hermaphrodite cross progeny [of genotype *unc-32 lin-12(n676n930); srf-9 him-5/dpy-11 sel-9*] were scored for egg laying. Greater than 95% of such animals were Egl, indicating complementation (*n* = 21). All such animals had 1 AC (*n* = 16), which is comparable to *sel-9/+* controls, but different from *srf-9/+* controls [6/23 *unc-32 lin-12(n676n930); srf-9 him-5/dpy-11* animals had 2 AC].

***sel-11* vs. *srf-8*:** *srf-8* also enhances the VPC defects of *lin-12(d)* mutants (LINK *et al.* 1992) and causes a 0 AC-Egl phenotype in *lin-12(n676n930)* hermaphrodites at 25° [12/12 *unc-32 lin-12(n676n930); srf-8* animals had 0 AC]. *unc-32 lin-12(n676n930)/++; dpy-11 sel-11/srf-8* hermaphrodites (which were wild-type) were selfed, and Unc non-Dpy non-Srf progeny scored for egg laying. Greater than 95% of such animals were Egl, indicating complementation (*n* = 43).

***sel-11* vs. *daf-11*:** *unc-32 lin-12(n676n930)/++; dpy-11 sel-11/daf-11* hermaphrodites (which were wild type) were selfed, and Unc non-Dpy non-Daf progeny scored for egg laying. All such animals were Egl, indicating complementation (*n* = 19).

lin-12 allele-specificity experiments

Strains were constructed by standard methods which are briefly summarized below. The designation *m* (for marker) is used to refer to *rol-3* in the case of strains containing LG V *sel* mutations, or *dpy-5* in the case of strains containing *sel(ar40)* mutations. All strains were constructed at 20°; certain strains were then transferred to 25° for scoring, as noted. For each strain, the entire broods of at least two hermaphrodites were scored for egg laying as well as for number of ACs and/or vulval phenotypes, as described above.

***lin-12(+); sel*:** True breeding non-Unc non-M lines were established from the progeny of *unc-32 lin-12(n676n930)/++; sel/m* mothers. The presence of the *sel* mutation in each line was verified by crossing in an *unc-32 lin-12(n676n930)* chromosome and checking for segregation of Unc non-Egl animals.

lin-12(n941); sel, lin-12(q269); sel* and *lin-12(oz48); sel: *unc-36 lin-12/unc-32 lin-12(n676n930); m sel(V)* or *sel(ar40); unc-36 lin-12/unc-32 lin-12(n676n930)* strains were constructed by standard methods. Unc-32 progeny were transferred to 25° to verify the presence of the *sel* mutation. Unc-36 progeny were selfed to establish homozygous strains where possible; otherwise, Unc-36 animals from heterozygous mothers were examined. *lin-12(oz48); sel* strains were scored at 25°.

lin-12(n676n927); sel: *unc-32 lin-12/unc-36 lin-12(n676n930); m/sel* hermaphrodites were selfed to obtain true-breeding non-M strains. Unc-36 progeny were transferred to 25° to verify the presence of the *sel* mutation. Unc-32 progeny were selfed to establish homozygous strains. All strains were scored at 25°.

lin-12(n137n720); sel(arX) sel-10: *unc-32 lin-12(n137n720)/++; m sel(arX) sel-10/++* hermaphrodites were selfed to obtain M non-Unc progeny, which were then selfed to obtain M Unc progeny, which were then selfed to establish homozygous lines. For *sel-10(+)* controls, Unc animals from heterozygous mothers were examined.

lin-12(n676n909); sel(arX) sel-10: *unc-32 lin-12(n676n909)/unc-36 lin-12(n676n930); rol-3/sel(arX) sel-10* hermaphrodites were selfed to obtain non-Rol non-Unc progeny that did not segregate Rol. Unc-36 progeny from such plates were picked to establish homozygous lines [even *sel-10(+)* control lines were weakly fertile and could be propagated].

lin-12(n379)/+; sel: *dpy-17 lin-12(n379)/unc-36 lin-12(n676n930); rol-3 sel(V)/him-5* males were mated to *unc-32* hermaphrodites, and Egl non-Unc cross progeny picked to identify those that were *dpy-17 lin-12(n379)/unc-32; rol-3 sel(V)/++*, from which independent Roller lines were established. Rol non-Dpy non-Unc animals were scored for the 0 AC-Egl phenotype at 25°. *dpy-5/+; unc-36 lin-12(n379)/++* males were mated to *sel(ar40); unc-32* hermaphrodites, and Egl semi-Dpy non-Unc cross progeny [of genotype *dpy-5/sel(ar40); unc-36 lin-12(n379)/unc-32*] picked and used to establish a true-breeding non-Dpy line. Non-Unc animals from this line were scored for the 0 AC-Egl phenotype at 20°.

lin-12(n379); sel* and *lin-12(n302); sel: *unc-36 lin-12/unc-32 lin-12(n676n930); m/sel* hermaphrodites were selfed to establish true-breeding non-M strains. Unc-32 progeny were transferred to 25° to verify the presence of the *sel* mutation. Unc-36 progeny were selfed to establish homozygous strains where possible; otherwise, Unc-36 animals from heterozygous mothers were scored.

glp-1; sel experiments

glp-1(e2142); sel: *glp-1(e2142)* hermaphrodites are essentially wild type when grown at 15°, and have relatively normal germline proliferation but produce only dead embryos when grown at 25° (PRIESS, SCHNABEL and SCHNABEL 1987). At the semipermissive temperature of 20°, *glp-1(e2142)* hermaphrodites produce a few live progeny.

unc-36 glp-1/unc-32 lin-12; rol-3 sel/++ hermaphrodites were selfed and Roller progeny [of genotype *unc-36 glp-1/unc-32 lin-12; rol-3 sel*] picked to 25°. Unc-36 self-progeny of such Roller animals were picked and scored for production of live progeny after 3 days. If homozygous *unc-36 glp-1; rol-3 sel* strains could be established at 25°, L4 animals were then picked directly from these strains and scored for number of live progeny generated after 3 days.

To score suppression of *glp-1(e2142)* at 20°, homozygous *unc-36 glp-1; rol-3 sel* animals grown at 15° were shifted to 20° as L4 larvae and scored for number of live progeny after 4 days. This "shifting" method was also used for other experiments at 25° (data not shown) and seems to give rather variable results.

glp-1(e2144): *glp-1(e2144)* hermaphrodites are essentially wild type when grown at 15° and have few germ cells and are sterile when grown at 25° (PRIESS, SCHNABEL and SCHNABEL 1987). Sterile *glp-1* animals have a distinctive "clear uterus" phenotype (referred to here as the Glp phenotype) that can be seen under the dissecting microscope. Small increases in the number of germ cells (not resulting in the production of zygotes) would not affect this dissecting microscope phenotype and therefore weak suppression of the germline proliferation defect might not have been detected in our experiments.

unc-36 glp-1/unc-32 lin-12; rol-3 sel/++ hermaphrodites were selfed, and Roller progeny [of genotype *unc-36 glp-1/unc-32 lin-12; rol-3 sel*] picked to 25°. Unc-36 self-progeny of such Roller animals were picked and scored for the Glp phenotype.

glp-1(q231); sel: *glp-1(q231)* hermaphrodites are essentially wild type when grown at 15°, have many germ cells but produce only dead embryos when grown at 20° and have few germ cells and are sterile when grown at 25° (MAINE and KIMBLE 1989). *unc-32 glp-1/++; rol-3 sel/++* hermaphrodites were selfed and Roller non-Unc progeny picked. Such Rollers were allowed to lay eggs at 20° for 36 hr before being transferred to 25°. From plates on which Rollers were of genotype *unc-32 glp-1/++; rol-3 sel*, Unc-32 progeny were picked and scored for the Glp phenotype (25°) or for number of live progeny (20°) as described above.

Testing *sel* mutations for dominant suppression of *glp-1(e2142)*: For 25° data, *glp-1; him-5* males grown at 15° were mated to *unc-36 glp-1; rol-3 sel* hermaphrodites at 25°, and non-Unc hermaphrodite cross progeny [of genotype *unc-36 glp-1/+ glp-1; rol-3 sel/him-5*] were scored for number of live progeny generated after 3 days. For 20° data, *glp-1; him-5* males were mated to *unc-36 glp-1; rol-3 sel* hermaphrodites at 15°, and non-Unc hermaphrodite cross progeny [of genotype *unc-36 glp-1/+ glp-1; rol-3 sel/him-5*] were picked to 20° as L4 larvae and scored for number of live progeny generated after 4 days.

RESULTS

Relevant phenotypes caused by different *lin-12* mutations (background): Three types of *lin-12* mutations are discussed in this paper: (1) recessive amorphic [*lin-12(0)*] mutations, which appear to eliminate *lin-12* activity (GREENWALD, STERNBERG and HORVITZ 1983); (2) recessive hypomorphic [*lin-12(h)*] mutations, which appear to reduce but not eliminate *lin-12* activity (SUNDARAM and GREENWALD 1993); and (3) dominant hypermorphic [*lin-12(d)*] mutations, which appear to elevate *lin-12* activity (GREENWALD, STERNBERG and HORVITZ 1983). The relevant dissecting microscope phenotypes and specific cell fate transformations caused by these different types of *lin-12* mutations are described in this section and in Table 3. More details about these *lin-12* mutant phenotypes and a description of other mutant phenotypes can be found in GREENWALD, STERNBERG and HORVITZ (1983), LAMBIE and KIMBLE (1991) and in the accompanying paper (SUNDARAM and GREENWALD 1993).

TABLE 3

Summary of relevant phenotypes of *lin-12* mutants

Genotype	No. of ACs[a]	VPC fates						Vulval phenotype	Egg-laying phenotype	% proximal germline mitosis
		P3.p	P4.p	P5.p	P6.p	P7.p	P8.p			
lin-12(*d*) strong	0	2°	2°	2°	2°	2°	2°	Muv	0 AC-Egl	0
lin-12(*d*) weak	0	3°	3°	3°	3°	3°	3°	Vul	0 AC-Egl	0
lin-12(+)	1	3°	3°	2°	1°	2°	3°	WT	WT	0
lin-12(*n676n930*)	1 or 2	variable[b]						Variable[b]	Egl	35
lin-12(*0*)	2	3°	3°	1°	1°	1°	3°	Evl	Sterile	100

Muv, Multivulva; Vul, Vulvaless; Evi, abnormally everted vulva.

[a] ACs derived from Z1.ppp and/or Z4.aaa. Additional ACs derived from other cells are sometimes found in *lin-12(0)* and *lin-12*(*h*) mutants (GREENWALD, STERNBERG and HORVITZ 1983; SEYDOUX, SCHEDL and GREENWALD 1990; SUNDARAM and GREENWALD 1993).

[b] See Table 5 and SUNDARAM and GREENWALD (1993) for a detailed description of VPC fates and other vulval defects in *lin-12*(*n676n930*) mutants.

A phenotype caused by both *lin-12(d)* and *lin-12(h)* mutations is the inability to lay eggs (Egl = egg-laying defective). Wild-type hermaphrodites lay eggs through the vulva, a ventral hypodermal structure that forms an opening between the uterus and the cuticle. When development of the vulva or other components of the egg-laying system is abnormal, eggs cannot be laid properly and instead accumulate inside the body cavity (HORVITZ and SULSTON 1980). Larvae may then begin to hatch internally. If no vulval opening is present, as in *lin-12(d)* mutants, the larvae devour their mother from the inside, creating a "bag of worms." If some sort of opening is present, as in *lin-12(h)* mutants (SUNDARAM and GREENWALD 1993) the larvae may swim through to the outside, and the mother appears bloated but does not always turn into a bag of worms.

Although the Egl phenotypes of *lin-12(d)* and *lin-12(h)* mutants are somewhat similar as seen under the dissecting microscope, the underlying causes of these Egl phenotypes, as assessed by Nomarski microscopy, are very different. The Egl phenotype of *lin-12(d)* mutants results from the absence of an anchor cell (AC) (GREENWALD, STERNBERG and HORVITZ 1983), which in wild type is necessary for vulval induction and morphogenesis (KIMBLE 1981); in this paper, we will use the term "0-AC Egl" to denote the *lin-12(d)* Egl phenotype. In contrast, *lin-12(h)* mutants have at least one AC, and their Egl phenotype appears to result from a combination of several different incompletely penetrant defects, including the presence of extra ACs ("2 AC" phenotype), abnormal vulva precursor cell (VPC) fate specification and vulval morphogenesis, and a "late defect" perturbing some unknown aspect of egg-laying system development (SUNDARAM and GREENWALD 1993).

Egg laying is a convenient dissecting microscope phenotype for routine genetic manipulations and screens for extragenic suppressors (see below). However, because many different factors influence egg-laying ability, the Egl phenotype of *lin-12(h)* mutants is not always a sensitive or reliable indicator of defects in specific cell fate decisions, which must therefore be scored directly by Nomarski microscopy. In this paper, we have scored three particular cell fate decisions: the decision of the somatic gonad cells Z1.ppp and Z4.aaa between the AC and ventral uterine precursor cell (VU) fates, the decision of vulval precursor cells (VPCs) between 1° and 2° fates, and the decision of proximal germline cells between mitosis and meiosis. The phenotypes resulting from defects in each of these decisions in *lin-12* mutants are summarized in Table 3.

Isolation of extragenic suppressors of the Egl defect of *lin-12(n676n930)* hermaphrodites: *lin-12(n676n930)* is a temperature-sensitive *lin-12(h)* allele that causes a highly penetrant Egl phenotype at 25° (SUNDARAM and GREENWALD 1993). After EMS-mutagenesis, we obtained 27 non-Egl revertants of *lin-12(n676n930)* at a total frequency of 5×10^{-5}. Thirteen revertants contain dominant suppressors that map near *dpy-5* on linkage group I (MATERIALS AND METHODS; Table 1; Figure 1); these suppressor mutations have been tentatively assigned to one locus defined by the canonical allele *sel(ar40)*. Nine revertants contain recessive suppressors of the *lin-12(n676n930)* Egl defect; these suppressor mutations define three different complementation groups (*sel-1*, *sel-9* and *sel-11*; see MATERIALS AND METHODS) all mapping to linkage group V (Table 1; Figure 1). Two revertants contain mutations which only suppress the *lin-12(n676n930)* Egl defect in combination with another mutation, "*sel(arX)*," but which have strong effects on several specific cell fate decisions in *lin-12* mutants (see below and MATERIALS AND METHODS); these mutations also map to linkage group V and define the *sel-10* locus (Table 1; Figure 1). Three additional revertants contain recessive suppressor mutations that are of low penetrance, map elsewhere and have not been further characterized (see MATERIALS

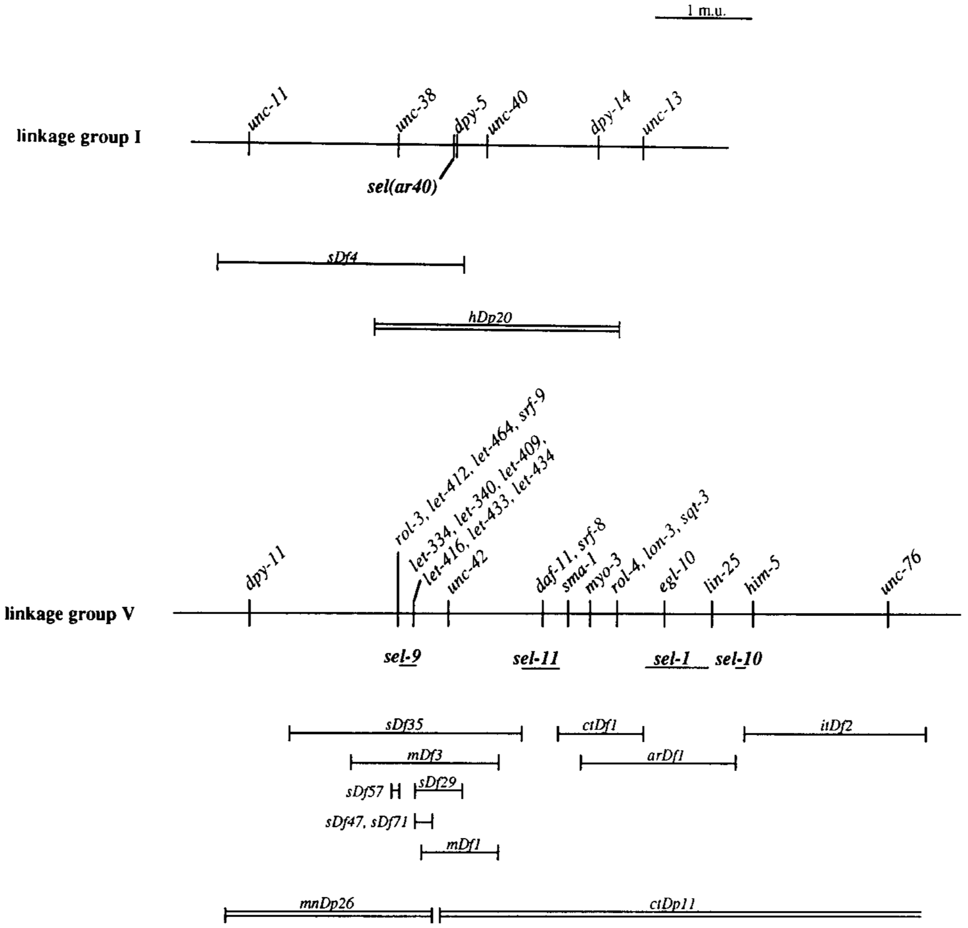

FIGURE 1.—Genetic map positions of *sel* mutations. Map positions are based on the results of three-factor crosses (Table 1) and complementation tests with deficiencies (Table 2). Both *sDf35* and *ctDf1* complement *sel-11* (Table 2); therefore, *sel-11* has been placed between the breakpoints of these two deficiencies. However, because there is some indication that *sel-11* mutations may be gain-of-function mutations, the phenotype of *sel-11/Df* cannot be predicted, and it is still possible that either of these deficiencies removes *sel-11*. Similarly, *sel-10* has been tentatively placed between the breakpoints of *arDf1* and *itDf2* since both those deficiencies complement *sel-10*, but it remains possible that either of these deficiencies removes *sel-10*.

TABLE 4

***sel* mutations suppress defects caused by *lin-12(n676n930)* at 25°**

Genotype	% Egl	% 2 AC	VPC fates[a]	% proximal mitosis[b]
lin-12(n676n930)	95 (112)	35 (40)	Mutant	35 (31)
lin-12(n676n930); sel-1(e1948)	10 (128)	0 (42)	Suppressed	2 (27)
lin-12(n676n930); sel-9(ar22)	7 (151)	0 (37)	Suppressed	2 (29)
lin-12(n676n930); sel(arX) sel-10(ar28)[c]	20 (153)	0 (36)	Suppressed	0 (18)
lin-12(n676n930); sel-10(ar41)[d]	97 (255)	3 (29)	ND	ND
lin-12(n676n930); sel-11(ar39)	7 (248)	0 (38)	Suppressed	0 (32)
sel(ar40); lin-12(n676n930)	19 (129)	0 (21)	Mutant	0 (25)

The number of animals scored is given in parentheses. No additional markers were present in these strains.

[a] See Table 5 for exact lineages of mutant and suppressed VPCs.

[b] Percentage of gonadal arms containing proximal mitotic germ nuclei.

[c] See text and MATERIALS AND METHODS for explanation of *sel(arX)*.

[d] Complete genotype: *unc-32 lin-12(n676n930); lon-3 sel-10(ar41)*.

AND METHODS). We have named these loci "*sel*" genes for suppressor and/or enhancer of *lin-12*, because *sel* mutations suppress defects associated with reduced *lin-12* activity but enhance at least one defect associated with elevated *lin-12* activity (see below).

In the remainder of this paper, data are given for the canonical alleles of each locus, unless otherwise stated. These canonical alleles are: *sel(ar40)*, *sel-1(e1948)*, *sel-9(ar22)*, *sel-10(ar41)* and *sel-11(ar39)*. In many experiments involving *sel-10* that are described in this paper, *sel(arX)* is also present; this is always indicated.

***sel* mutations suppress loss-of-function defects caused by *lin-12(n676n930)* at 25°:** The Egl defect caused by *lin-12(n676n930)* at 25° is suppressed to varying extents in the different *lin-12(n676n930)*; *sel* revertant strains (Table 4). An important criterion of our suppressor screen was that mutations suppress the late defect of *lin-12(n676n930)* animals, since the late defect is largely responsible for the Egl phenotype of such animals (SUNDARAM and GREENWALD 1993). The *sel-1*, *sel-9*, *sel-11* and *sel(ar40)* mutations must suppress the late defect since they suppress the *lin-12(n676n930)* Egl phenotype. *sel-10* mutations, however, may not suppress the late defect since they do not suppress the *lin-12(n676n930)* Egl phenotype. *sel-10* mutations were only isolated in our screen due to the presence of some other background mutation(s), referred to here as *sel(arX)*, which cooperate(s) with *sel-10* mutations to cause strong suppression of the *lin-12(n676n930)* Egl phenotype (see MATERIALS AND METHODS for a further discussion of *sel-10* and *arX*).

We examined the somatic gonad, ventral hypodermis and germline in revertant animals to determine which, if any, of the known cell fate transformations caused by *lin-12(n676n930)* were suppressed. We found that all of the *sel* mutations suppress the 2 AC and proximal mitosis phenotypes (Table 4). The *sel-1*, *sel-9*, *sel(arX) sel-10* and *sel-11* mutations also relieve the VPC and early vulval morphogenesis defects (Table 5 and data not shown). *sel(ar40)* apparently does not suppress the VPC lineage defects (Table 5), indicating that suppression of the VPC lineage defects is not required for proper egg laying. *sel(ar40)* does, however, improve early vulval morphogenesis (data not shown).

***sel* mutations enhance the gain-of-function 0 AC-Egl defect caused by *lin-12(n676n930)* at 15°:** At the permissive temperature of 15°, a small percentage of *lin-12(n676n930)* hermaphrodites display a 0 AC-Egl phenotype (Table 6); because no AC is present, we infer that both Z1.ppp and Z4.aaa adopted the VU fate. This phenotype probably results from residual *lin-12(d)* activity of the original *lin-12(n676)* allele from which *lin-12(n676n930)* is derived (see SUNDARAM and GREENWALD 1993). A high percentage of *lin-12(n676n930); sel* revertant hermaphrodites have a 0 AC-Egl phenotype when grown at 15° (Table 6). Thus, the *sel* mutations apparently enhance the AC to VU fate transformation associated with elevated levels of *lin-12* activity (but see also below).

Gene dosage studies of *sel* mutations

The low frequency at which the *sel* mutations were obtained suggested that they might not be null mutations. To determine the nature of the *sel* mutations, we performed genetic dosage studies in which animals with different doses of mutant and wild-type *sel* alleles were compared with respect to suppression of the Egl and 2 AC defects caused by *lin-12(n676n930)* (Tables 7–12). If a *sel* mutation results in a loss of function, then it should act like a deficiency in such studies: *lin-12(n676n930); sel/+* and *lin-12(n676n930); Df/+* should have similar phenotypes, and *sel/Df* should suppress as well or better than *sel/sel* (unless it causes a novel phenotype). A different outcome would indicate that a *sel* mutation is a gain-of-function mutation, and comparison of other genotypes would indicate if gene activity were elevated or altered (MULLER 1932). It should be noted that, in these experiments, suppression of the 2 AC defect is a more reliable assay of *sel* gene activity than is suppression of the Egl defect since many deficiencies and duplications can themselves cause an Egl phenotype. In addition, the duplication *ctDp11* simultaneously increases the dosage of *sel-1(+)*, *sel-10(+)* and *sel-11(+)* (see Figure 1), possibly complicating some of our results. The key results for each gene are summarized below. The interpretation of *sel-10* dosage experiments was complicated by the presence of *sel(arX)*, and will not be presented here.

***sel-1* mutations may be loss-of-function mutations** (Table 7): *sel-1* mutations are recessive suppressors of both the Egl and 2 AC defects. *sel-1/Df* and *sel-1/sel-1* suppress the 2 AC defect to a similar extent, consistent with *sel-1* mutations resulting in reduced gene function. The 2 AC defect appears weakly suppressed in *+/Df* heterozygotes, suggesting that the *sel-1* gene may be weakly haploinsufficient. If so, *sel-1* mutations must not be complete loss-of-function mutations, since *sel-1/+* does not suppress the 2 AC defect.

***sel-9* mutations are gain-of-function mutations** (Table 8):*sel-9* mutations are recessive suppressors of the Egl defect, but semidominant suppressors of the 2 AC defect. Deficiencies of the region do not show this dominant effect; therefore, *sel-9* mutations are gain-of-function mutations. With respect to suppression of the 2 AC defect, *sel-9* mutations appear neomorphic since *sel-9/+*, *sel-9/Df*, *sel-9/sel-9* and *sel-9/sel-9/+* all suppress to a similar extent. However, with respect to suppression of the Egl defect, *sel-9(+)* appears to antagonize *sel-9* suppressor activity, since *sel-9/Df* suppresses better than *sel-9/+* (but not as well as *sel-9/sel-9*), and *sel-9/sel-9* suppresses better than *sel-9/sel-9/+*. *sel-9(+)* also antagonizes the ability of *sel-9(ar22)* to enhance the 0 AC-Egl phenotype of *lin-12(n676n930)* animals at 15° (Table 9).

***sel-11* mutations may be gain-of-function mutations or haploinsufficient loss-of-function mutations (Table 10):** *sel-11* mutations are recessive suppressors of the Egl defect, but appear to be weakly semidominant suppressors of the 2 AC defect [the dominant activity of *sel-11(ar39)* is more apparent in combinations with *glp-1(e2142)*; see below]. Either *sel-11/Df* does not suppress or no deficiency exists that removes *sel-11* (see Table 2, Figure 1). Although we cannot rule out the possibility that *sel-11* is haploinsufficient, *sel-11/sel-11/+* does not suppress the 2 AC defect as well as *sel-11/sel-11*, but slightly better than *sel-11/+*, suggesting that *sel-11* mutations could be antimorphic gain-of-function mutations.

***sel(ar40)* may be a haploinsufficient loss-of-function mutation** (Table 11): *sel(ar40)* is a dominant suppressor of both the Egl and 2 AC defects. Dominance may result from either a gain-of-function activity, or from haploinsufficiency. Possible alleles of *sel(ar40)* were isolated by J. PRIESS and A. M. HOWELL (unpublished data; see below), who have evidence that such mutations are haploinsufficient suppressors of a *glp-1* mutation. Unfortunately, we were unable to test

TABLE 5

Suppression of *lin-12(n676n930)* Vulval Precursor Cell (VPC) fate defects by *sel* mutations

Genotype	P3.p	P4.p	P5.p	P6.p	P7.p	P8.p
Wild type	S S	S S	LLTN	TTTT	NTLL	S S
	(3°)	(3°)	(2°)	(1°)	(2°)	(3°)
*lin-12(n676n930)**	S S	S LL	LLTT	TTTT	NT S	S S
	S S	LLLN	TTTT	TTLL	S S	S S
	S S	S LN	TOTT	TTTT	NTLL	S S
lin-12(n676n930); sel-1(e1948)	S S	LOTN	TTTT	NTLL	S S	S S
	S S	S S	LLTN	TTOT	NTLL	S S
	S S	LLTN	TTTT	NTOL	S S	S S
	S S	LLTN	TT?T	NTLL	S S	S S
	S S	LLTN	TLTT	TTTT	?L?L	S S
	S S	LLTN	TTTT	NTLL	S S	S S
lin-12(n676n930); sel-9(ar22)	S	S S	LLTN	TTTT	NTLL	S S
	S S	LLTN	TTT?	?TTT	N?LL	S S
	S	S S	LLTN	TTTT	NTLL	S S
	S S	S S	LLTN	TTTT	NTLL	S S
	S S	LLTN	TTTT	NTLL	S S	S S
	S S	LLTN	TTTT	NTLL	S S	S S
	S NN	LLTN	TTTT	NTLL	S S	S S
	S S	LLTN	TTTT	NTLL	S S	S S
lin-12(n676n930); sel(arX) sel-10(ar28)	S S	LLTN	TTTT	NTLL	NN S	S S
	S S	S S	LLTN	TTTT	NTLL	S S
lin-12(n676n930); sel-11(ar39)	S S	S S	LLTN	TTTT	NTLL	S S
	S	LLTN	TTTT	NTLL	S S	S S
	S	S SS	LLTN	TTTT	NTLL	S S
sel(ar40); lin-12(n676n930)	S S	S NN	TTTT	TTTT	NL S	S S
	S S	S S	S ON	TTTT	NT S	S S
	S	S S	S TN	TTTT	NT S	S S
	S	S TN	TTTL	?TTT	S S	S S
	S	S S	LLTT	TTTT	NT S	S S
	S S	S S	TTTT	TTTT	TT S	S S

Each line represents the vulva lineages of an individual animal. All animals shown had 1 AC. Nomenclature for describing VPC fates follows that of STERNBERG and HORVITZ (1986), in which fates are described in terms of the axes of the last nuclear divisions of the lineage; each letter refers to the axis of a single division. S, no division, fused with hypodermal syncitium; N, no division, compact nuclear morphology; L, lateral division; T, transverse division; O, oblique division; ?, division not observed; underline indicates that the resulting descendants remained adherent to the ventral cuticle. S and SS are considered 3° fates; LLTN, LLTT, LLTO (or their mirror images) are considered 2° fates (light box), and TTTT (or any lineage in which all four cells divide and generate descendants which do not adhere to the ventral cuticle) is considered a 1° fate (heavy box).

* The VPC lineages of only three representative animals are shown here. See SUNDARAM and GREENWALD (1993) for additional lineages.

directly for haploinsufficiency due to technical difficulties in working with *sDf4* (see MATERIALS AND METHODS). However, *sel(ar40)/+* and *sel(ar40)/sel(ar40)/+* suppress the 2 AC defect to a similar extent [but not as well as *sel(ar40)/sel(ar40)*], consistent with *sel(ar40)* being a loss-of-function mutation.

Tests for allelism of recessive *sel* mutations to existing loci: For both *sel-1* and *sel-9*, *sel/Df* suppresses partially the Egl defect caused by *lin-12(n676n930)* at 25°, while *sel/+* does not suppress. Therefore, existing mutations mapping in the same region as either *sel-1* or *sel-9* could be tested for failure to complement these *sel* mutations for suppression (see MATERIALS AND METHODS). *egl-10* complemented *sel-1*; and *let-334*, *let-340*, *let-409*, *let-412*, *let-433*, *let-434*, *let-416*, *let-464*, *rol-3* and *srf-9* all complemented *sel-9*. Thus, the *sel-1* and *sel-9* mutations do not appear to correspond to any previously identified loci. *daf-11* and *srf-8*, both of which map in the same region as *sel-11*, also complemented *sel-11* by this same test.

TABLE 6

***sel* mutations enhance the 0 AC-Egl phenotype caused by *lin-12(n676n930)* at 15°**

Relevant genotype	% 0 AC-Egl
lin-12(n676n930); +	7 (204)
lin-12(n676n930); sel-1(e1948)	72 (449)
lin-12(n676n930); sel-9(ar22)	55 (554)
lin-12(n676n930); sel(arX) sel-10(ar41)	46 (153)
lin-12(n676n930); sel-10(ar41)[a]	56 (164)
lin-12(n676n930); sel-11(ar39)	64 (303)
sel(ar40); lin-12(n676n930)	50 (107)
sel(ar40); lin-12(n676n930); sel-1(e1948)	97 (243)

The number of animals scored is indicated in parentheses. All *lin-12(n676n930)* chromosomes were marked with *unc-32*.

[a] Complete genotype: *unc-32 lin-12(n676n930); lon-3 sel-10(ar41)*.

However, since the phenotype of *sel-11/Df* is not known, this result does not rule out allelism between *sel-11* and *daf-11* or *srf-8*.

Effects of *sel* mutations on different *lin-12* alleles

To test the allele-specificity of suppression, we crossed the *sel* mutations into various *lin-12* backgrounds and observed their effects on egg laying as well as on the number of ACs and/or VPC fate specification. (Figures 2–4). The results are summarized below.

***lin-12(+)*:** All *lin-12(+); sel* homozygotes lay eggs normally, and examination by Nomarski optics did not reveal any cell fate transformations affecting the AC or VPCs (data not shown).

***lin-12(0)* alleles:** *sel-1*, *sel-9*, *sel-11* and *sel(ar40)* mutations do not affect the *lin-12(0)* allele *lin-12(n941)* or the near null allele *lin-12(q269)* (Figure 2A) and thus cannot bypass the requirement for *lin-12* activity. In contrast, *sel(arX) sel-10* mutations partially suppress the 2 AC defect caused by any of three *lin-12(0)* alleles tested, including *lin-12(n941)* (Figure 2). In addition, *sel(arX) sel-10* mutations increase the fertility of these ordinarily sterile *lin-12(0)* strains such that they can be readily propagated, although all animals are Egl (data not shown). *sel-10(ar41)* can also suppress partially the sterility and 2 AC phenotypes of *lin-12(n941)* animals in the absence of *sel(arX)* (Figure 2B and data not shown).

***lin-12(h)* alleles:** *sel-1*, *sel-9*, *sel(arX) sel-10* and *sel-11* mutations suppress the 2 AC defect caused by the hypomorphic mutations *lin-12(n676n927)* or *lin-12(oz48)* (Figure 3A); in most cases, however, the *sel* mutations do not significantly affect the Egl defect caused by these alleles (Figure 3B). Screens for reversion of the Egl defect of *lin-12(oz48)* animals have so far yielded only apparent intragenic revertants (Wakelee 1992). The observation that the *sel-1*, *sel-9*, *sel(arX) sel-10* and *sel-11* mutations suppress the 2 AC defect caused by *lin-12(oz48)* demonstrates that interactions between these *sel* mutations and *lin-12* do not require the presence of a *lin-12(d)* mutation.

sel(ar40) suppresses both the 2 AC and Egl defects caused by *lin-12(n676n927)* but does not significantly affect *lin-12(oz48)* (Figure 3).

***lin-12(d)* alleles:** Although *sel-1*, *sel-9*, *sel(arX) sel-10*, *sel-11* and *sel(ar40)* mutations all greatly enhance the 0 AC-Egl phenotype of *lin-12(n676n930)* hermaphrodites grown at 15° (Table 6), only *sel(arX) sel-10* mutations significantly enhance the 0 AC-Egl phenotype caused by the *lin-12(d)* allele *lin-12(n379)* (Figure 4A). *sel(ar40)* actually weakly suppresses the 0 AC-Egl phenotype caused by the *lin-12(d)* allele *lin-12(n302)*: although *lin-12(n302)* hermaphrodites never have an AC, rare *sel(ar40); lin-12(n302)* animals have 1 AC and are egg-laying competent (1/165 was non-Egl, and occasional non-Egl animals are consistently seen on stock plates).

sel-1, *sel-9*, *sel(arX) sel-10* and *sel-11* mutations each enhance the vulval precursor cell (VPC) fate defects of *lin-12(d)* mutants; each causes normally Vulvaless *lin-12(n379)* and *lin-12(n302)* hermaphrodites to exhibit a Multivulva (Muv) phenotype (Figure 4B; see Table 1 for explanation of Vul and Muv phenotypes). This effect is strongest with *sel(arX) sel-10* mutations. *sel-10(ar41)* has a similar effect in the absence of *sel(arX)* (data not shown). *sel(ar40)*, which does not suppress the VPC fate defects caused by *lin-12(n676n930)*, also does not affect VPC fates in *lin-12(d)* mutants.

sel-10 has a striking additional effect on *lin-12(d)* alleles: many (6/31) *lin-12(n379); sel(arX) sel-10* and most (47/57) *lin-12(n302); sel(arX) sel-10* hermaphrodites are sterile; those animals that are fertile have very low brood sizes (data not shown). Closer examination revealed that dying embryos are present inside such animals; these embryos appear to undergo many rounds of cell division, but begin to degenerate without any morphogenesis occurring (data not shown). *sel-10(ar41); lin-12(n302)* is also a maternal-effect lethal in the absence of *sel(arX)* (data not shown).

***sel* mutations also suppress a partial loss-of-function allele of *glp-1*:** The *lin-12* and *glp-1* genes encode similar proteins (Greenwald 1985; Yochem, Weston and Greenwald 1988; Austin and Kimble 1989; Yochem and Greenwald 1989) and have both distinct and overlapping functions during *C. elegans* development. The *glp-1* gene product is required zygotically for germline proliferation and maternally for early embryonic development (Austin and Kimble 1987; Priess, Schnabel and Schnabel 1987). In a *lin-12(0)* background, *glp-1* activity is also required zygotically for larval viability (Lambie and Kimble 1991). Several lines of evidence suggest that the *lin-12* and *glp-1* products may be functionally interchangeable (see DISCUSSION).

The *glp-1(e2142)* allele is a temperature-sensitive

TABLE 7

***sel-1* dosage studies**

Relevant genotype	% 2 AC	% Egl	Interpretation
lin-12(n676n930); +/+[1]	32 (108)	96 (49)	*sel-1* suppresses the 2 AC and Egl defects.
lin-12(n676n930); sel-1/sel-1[2]	0 (42)	6 (198)	
lin-12(n676n930); sel-1/+[3]	31 (68)	91 (43)	*sel-1* is a recessive suppressor.
lin-12(n676n930); sel-1/Df[4]	0 (73)	59 (39)	Reducing the dosage of *sel-1*(+) causes a more suppressed phenotype.
lin-12(n676n930); +/Df[5]	18 (54)	100 (70)	
lin-12(n676n930); sel-1/sel-1[6]	0 (41)	8 (36)	Increasing the dosage of *sel-1*(+) causes a less suppressed phenotype.
lin-12(n676n930); sel-1/sel-1/+[7]	15 (46)	100 (55)	
lin-12(n676n930); +/+[8]	32 (47)	100 (27)	
lin-12(n676n930); +/+/+[9]	51 (73)	100 (59)	

The number of animals scored is given in parentheses. Similar results were obtained with *sel-1(ar29)* (data not shown).

[1] *unc-32 lin-12(n676n930); +.*

[2] *unc-32 lin-12(n676n930); sel-1(e1948).*

[3] *unc-32 lin-12(n676n930); dpy-11 sel-1(e1948)/++.*

[4] *unc-32 lin-12(n676n930); dpy-11 sel-1(e1948)/+ arDf1.*

[5] *unc-32 lin-12(n676n930); dpy-11 +/+ arDf1.*

[6] *unc-32 lin-12(n676n930); sel-1(e1948)* (from *ctDp11*-bearing mothers).

[7] *unc-36 lin-12(n676n930); sel-1(e1948); ctDp11.*

[8] *unc-36 lin-12(n676n930); +* (from *ctDp11*-bearing mothers).

[9] *unc-36 lin-12(n676n930); +; ctDp11.*

TABLE 8

***sel-9* dosage studies**

Relevant genotype	% 2 AC	% Egl	Interpretation
lin-12(n676n930); +/+[1]	32 (108)	96 (49)	*sel-9* suppresses the 2 AC and Egl defects.
lin-12(n676n930); sel-9/sel-9[2]	0 (55)	5 (154)	
lin-12(n676n930); sel-9/+[3]	4 (123)	96 (97)	*sel-9* is a recessive suppressor of the Egl defect, but a semidominant suppressor of the 2 AC defect. *sel-9* is not haploinsufficient, and therefore is a gain-of-function mutation.
lin-12(n676n930); sel-9/Df[4]	3 (63)	61 (95)	
lin-12(n676n930); +/Df[5]	26 (23)	100 (45)	
lin-12(n676n930); sel-9/sel-9[6]	0 (70)	19 (54)	Increasing the dosage of *sel-9*(+) antagonizes *sel-9* suppression of the Egl defect but not *sel-9* suppression of the 2 AC defect.
lin-12(n676n930); sel-9/sel-9/+[7]	2 (92)	78 (71)	
lin-12(n676n930); +/+[8]	29 (76)	100 (16)	
lin-12(n676n930); +/+/+[9]	19 (83)	100 (24)	

The number of animals scored is given in parentheses. Similar results were obtained with *sel-9(ar26)* (data not shown).

[1] *unc-32 lin-12(n676n930); +.*

[2] *unc-32 lin-12(n676n930); sel-9(ar22).*

[3] *unc-32 lin-12(n676n930); dpy-11 sel-9(ar22)/++.*

[4] *unc-32 lin-12(n676n930); dpy-11 sel-9(ar22)/+ mDf3.*

[5] *unc-32 lin-12(n676n930); dpy-11 +/+ mDf3.*

[6] *unc-32 lin-12(n676n930); dpy-11 sel-9(ar22)* (from *mnDp26*-bearing mothers).

[7] *unc-32 lin-12(n676n930); dpy-11 sel-9(ar22); mnDp26.*

[8] *unc-32 lin-12(n676n930); dpy-11* (from *mnDp26*-bearing mothers).

[9] *unc-32 lin-12(n676n930); dpy-11; mnDp26.*

partial loss-of-function allele that causes maternal-effect embryonic lethality but does not significantly affect germline proliferation (PRIESS, SCHNABEL and SCHNABEL 1987). J. PRIESS and A. M. HOWELL (unpublished data) screened for dominant suppressors of *glp-1(e2142)* [*sog* mutations] and obtained *sog(zu28)* and many other mutations that appear to be allelic to *sel(ar40)*. The *sel(ar40)* and *sog(zu28)* mutations both map 0.01 map units left of *dpy-5* on linkage group I (Table 1; A. M. HOWELL and J. PRIESS, unpublished data), and both are dominant suppressors of the Egl and 2 AC defects caused by *lin-12(n676n930)* (Table 11 and data not shown; A. M. HOWELL and J. PRIESS, unpublished data) and of the maternal effect embryonic lethality caused by *glp-1(e2142)* (Table 12; A. M. HOWELL and J. PRIESS, unpublished data). Like *sel(ar40)*, *sog(zu28)* causes no obvious phenotype in a wild-type background (A. M. HOWELL and J. PRIESS, unpublished data). Since both *sel(ar40)* and *sog(zu28)* are dominant suppressors, we cannot do complementation tests to confirm their allelism; it therefore remains possible, but we think unlikely, that *sel(ar40)* and *sog(zu28)* define two different genes.

Since *sel(ar40)* was able to suppress both *lin-12(n676n930)* and *glp-1(e2142)*, we wondered if any of our other *sel* mutations might also suppress *glp-1(e2142)*; such mutations might not have been identified in the screen of PRIESS and HOWELL (unpublished

TABLE 9

***sel-9*(+) antagonizes the ability of *sel-9(ar22)* to enhance the 0 AC-Egl phenotype caused by *lin-12(n676n930)* at 15°**

Relevant genotype	% 0 AC-Egl
lin-12(n676n930); sel-9/sel-9[1]	39 (77)[a]
lin-12(n676n930); sel-9/sel-9/+[2]	14 (81)
lin-12(n676n930); +/+[3]	3 (38)
lin-12(n676n930); +/+/+[4]	7 (61)

The number of animals scored is given in parentheses.

[1] *unc-32 lin-12(n676n930); dpy-11 sel-9(ar22)* (from *mnDp26*-bearing mothers).

[2] *unc-32 lin-12(n676n930); dpy-11 sel-9(ar22); mnDp26.*

[3] *unc-32 lin-12(n676n930); dpy-11* (from *mnDp26*-bearing mothers).

[4] *unc-32 lin-12(n676n930); dpy-11; mnDp26.*

[a] This number is lower than expected for this genotype, suggesting that *mnDp26* may have a maternal effect.

data), as they are not strongly dominant. We found that, indeed, *sel-1*, *sel-9*, *sel(arX) sel-10* and *sel-11* mutations all suppress the maternal effect lethality caused by *glp-1(e2142)* (Table 13). *sel-1(e1948)* and *sel(arX) sel-10(ar41)* are relatively weak, recessive suppressors of *glp-1(e2142)*, while *sel-9(ar26)* and *sel-11(ar39)* are stronger suppressors, and are slightly semidominant. *sel-10(ar41)* is not able to suppress *glp-1(e2142)* in the absence of *sel(arX)*.

Allele-specificity of *glp-1* suppression: *glp-1(q231)* and *glp-1(e2144)* are two temperature sensitive reduction-of-function alleles that cause both the germline proliferation and maternal-effect lethal phenotypes at 25° (PRIESS, SCHNABEL and SCHNABEL 1987; MAINE and KIMBLE 1989). We found that *sel-1*, *sel-9*, *sel(arX) sel-10* and *sel-11* mutations fail to suppress the germline proliferation defect caused by either *glp-1(q231)* or *glp-1(e2144)* at 25° (Table 13), although very weak suppression may not have been detected by our methods (see MATERIALS AND METHODS). However, at the semipermissive temperature of 20°, *sel(arX) sel-10(ar41)* does weakly suppress the maternal-effect lethal phenotype caused by *glp-1(q231)* (Table 13).

DISCUSSION

We reverted the Egl phenotype caused by the hypomorphic allele *lin-12(n676n930)*, and identified extragenic suppressor mutations in five genes, *sel-1*, *sel-9*, *sel-10*, *sel-11* and *sel(ar40)*. These *sel* genes appear to interact with *lin-12* in multiple cell fate decisions since the *sel* mutations each suppress several different defects associated with reduced *lin-12* activity. *sel-1*, *sel-9*, *sel(arX) sel-10*, *sel-11* and *sel(ar40)* also appear to interact with *glp-1* since mutations in these genes suppress the maternal-effect lethality caused by the reduction-of-function allele *glp-1(e2142)*. However, none of the *sel* mutations causes any phenotype in a wild-type background. Two possible explanations for this absence of a phenotype are: (1) some *sel* mutations may be non-null alleles of genes which have visible null phenotypes; (2) some *sel* mutations may define genes whose null phenotype is wild type.

***sel-9* and *sel-11* mutations behave as antimorphic gain-of-function mutations:** *sel-9* and *sel-11* mutations were isolated at very low frequency (<1 in 10^5 per locus) and appear to be gain-of-function mutations, although haploinsufficiency has not been ruled out for *sel-11*. Both are recessive suppressors of the Egl defect but semidominant suppressors of the 2 AC defect caused by *lin-12(n676n930)* at 25°.

The suppressor activity of *sel-9* mutant alleles is antagonized by addition of *sel-9(+)* alleles. Classically, dominant gain-of-function alleles whose effects are lessened by the addition of wild-type alleles are termed antimorphic (MULLER 1932). Some antimorphic alleles interfere with wild-type gene activity and have also been termed dominant-negative mutations (HERSKOWITZ 1987). However, our data do not suggest that *sel-9* suppressor alleles exert their effects by poisoning *sel-9(+)* activity; rather, *sel-9(+)* appears to antagonize *sel-9* suppressor activity. One possible molecular model to explain this behavior would be that the *sel-9* and *sel-9(+)* gene products compete for some interaction, with the *sel-9* interaction leading to suppression. In this case, *sel-9* might be recessive for suppression of the Egl defect because it is a relatively poor competitor. A similar type of model has been proposed to explain recessive gain-of-function alleles of the *Drosophila* gene *cactus* (ROTH *et al.* 1991).

The suppressor activity of *sel-11* mutations is also antagonized by addition of the corresponding wild-type allele [or possibly by the presence of the extra copy of *sel-1(+)* and/or *sel-10(+)* also present on the duplication used in this experiment]. If *sel-11* is not haploinsufficient, such mutations also appear antimorphic, and similar models could explain their actions.

***sel-1* and *sel(ar40)* mutations behave as partial or complete loss-of-function mutations:** *sel-1* and *sel(ar40)*-like mutations were found at somewhat higher frequencies than *sel-9* and *sel-11* mutations (*sel-1*: 1 in 10^5; *sel(ar40)*-like: 1 in 40,000), but were still relatively rare compared with the average mutation frequency in *C. elegans* (1 in 2,000; BRENNER 1974). Gene dosage studies are consistent with *sel-1* and *sel(ar40)* mutations reducing gene activity, but do not clearly distinguish between a partial or complete loss of function. Since both *sel-1/Df* and *sel(ar40)/Df* have no phenotype in a *lin-12(+)* background, either *sel-1* and *sel(ar40)* mutations are not null mutations, or the null phenotypes of these genes are wild type. Genes with redundant functions might be expected to have wild-type null phenotypes. If the normal *sel-1* and *sel(ar40)* gene activities were redundant with each other, then a *sel(ar40); sel-1* double mutant might have

TABLE 10

sel-11 dosage studies

Relevant genotype	% 2 AC	% Egl	Interpretation
lin-12(n676n930); +/+[1]	32 (108)	96 (49)	*sel-11* suppresses the 2 AC and Egl defects.
lin-12(n676n930); sel-11/sel-11[2]	0 (38)	10 (124)	
lin-12(n676n930); sel-11/+[3]	14 (28)	95 (21)	*sel-11* is a recessive suppressor of the Egl defect, but weakly semidominant for suppression of the 2 AC defect.
lin-12(n676n930); sel-11/sel-11[4]	0 (73)	9 (43)	Increasing the dosage of *sel-11*(+) causes a less suppressed phenotype.
lin-12(n676n930); sel-11/sel-11/+[5]	11 (133)	100 (56)	
lin-12(n676n930); +/+[6]	32 (47)	100 (27)	
lin-12(n676n930); +/+/+[7]	51 (73)	100 (59)	

The number of animals scored is given in parentheses. Similar results were obtained with *sel-11(ar84)* (data not shown).

[1] *unc-32 lin-12(n676n930).*

[2] *unc-32 lin-12(n676n930); sel-11(ar39).*

[3] *unc-32 lin-12(n676n930); dpy-11 sel-11(ar39)/++.*

[4] *unc-36 lin-12(n676n930); sel-11(ar39)* (from *ctDp11*-bearing mothers).

[5] *unc-36 lin-12(n676n930); sel-11(ar39); ctDp11.*

[6] *unc-36 lin-12(n676n930)* (from *ctDp11*-bearing mothers).

[7] *unc-36 lin-12(n676n930); ctDp11.*

TABLE 11

sel(ar40) dosage studies

Relevant genotype	% 2 AC	% Egl	Interpretation
lin-12(n676n930); +/+[1]	32 (108)	96 (49)	*sel(ar40)* suppresses the Egl and 2 AC defects.
lin-12(n676n930); sel(ar40)/sel(ar40)[2]	1 (106)	18 (345)	
lin-12(n676n930); sel(ar40)/+[3]	5 (40)	23 (177)	*sel(ar40)* is a dominant suppressor of both the Egl and 2 AC defects.
lin-12(n676n930); sel(ar40)/sel(ar40)/+[4]	4 (24)	100 (23)	Increasing the dosage of mutant *sel(ar40)* does not affect suppression of the 2 AC defect. Increasing the dosage of *sel(ar40)*$^+$ causes a less suppressed phenotype.
lin-12(n676n930); +/+/+[5]	50 (16)	100[a]	

The number of animals scored is given in parentheses. Similar results were obtained with *sog(zu28)* (data not shown), an apparent allelic mutation isolated by J. PRIESS and A. M. HOWELL (unpublished data; see text).

[a] This strain could not be grown at 25° and was very sickly at 20°.

[1] *unc-32 lin-12(n676n930).*

[2] *sel(ar40); unc-32 lin-12(n676n930).*

[3] *sel(ar40)/+; unc-32 lin-12(n676n930); dpy-11/+.*

[4] *sel(ar40) dpy-14; unc-32 lin-122°; hDp20.*

[5] *dpy-5 dpy-14; unc-32 lin-12(n676n930); hDp20.*

some phenotype in a *lin-12(+)* background; however, this is not the case (data not shown). *sel-1* and *sel(ar40)* mutations do have additive effects on the AC/VU decision in *lin-12(n676n930)* animals (Table 6 and data not shown).

***sel-1*, *sel-9*, *sel-11* and *sel(ar40)* mutations elevate the level or effect of *lin-12* activity:** *sel-1*, *sel-9*, *sel-11* and *sel(ar40)* mutations do not suppress *lin-12(0)* alleles, and thus require some *lin-12* activity in order to exert their effects. All such mutations suppress reduction-of-function defects caused by at least one other *lin-12(h)* allele besides *lin-12(n676n930)*, all strongly enhance the gain-of-function 0 AC-Egl phenotype caused by *lin-12(n676n930)* at 15°, and some enhance the VPC fate defects caused by *lin-12(d)* alleles. These *sel* mutations therefore appear to act by either increasing the level of *lin-12* activity or by increasing the response of downstream components to *lin-12* activity. Since *sel-1* and *sel(ar40)* mutations appear to be reduction-of-function mutations, the wild-type products of these genes may be negative regulators of *lin-12* activity or negatively regulated by *lin-12* activity. Since *sel-9* and *sel-11* mutations appear to be gain-of-function mutations, the wild-type products of these genes may be positive regulators of *lin-12* activity or positively regulated by *lin-12* activity.

***sel-10* mutations appear to bypass the need for *lin-12* activity:** *sel-10* mutations are able to suppress partially the 2 AC defect caused by several genetically defined *lin-12(0)* alleles: in approximately one-third of *lin-12(0); sel-10* hermaphrodites, either Z1.ppp or Z4.aaa does not become an AC and presumably becomes a VU. *sel-10* mutations therefore appear to bypass the need for *lin-12* activity, allowing *lin-12(0)* animals to express a cell fate that is normally dependent on *lin-12* activity.

One possible explanation of the above result is that all of the *lin-12(0)* alleles used may encode products

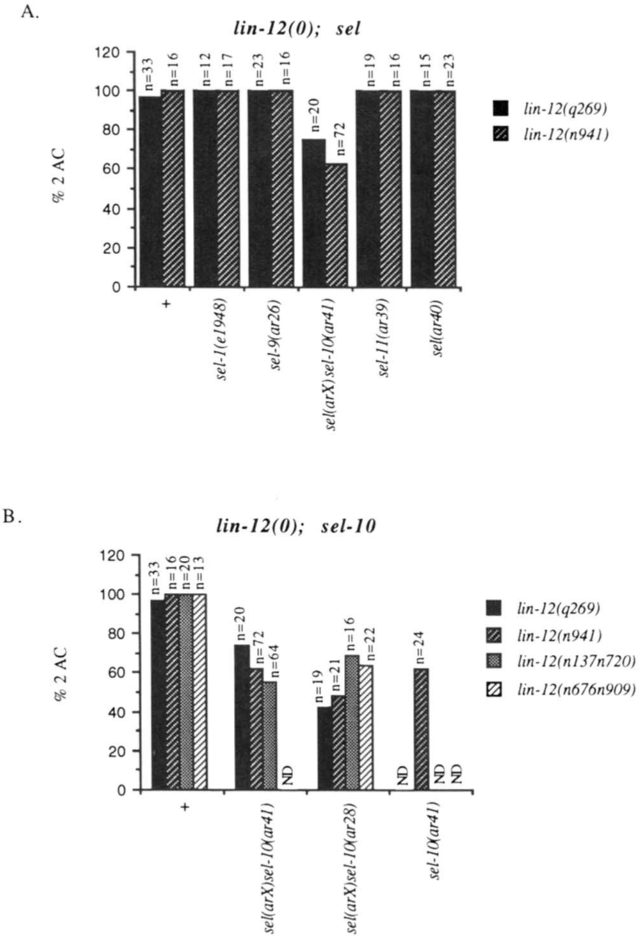

FIGURE 2.—Allele specificity experiments: *lin-12(0)* alleles. Percentage of animals having two or more ACs. All experiments were done at 20°. (A) *lin-12(0); sel*. For *sel* mutations on LG V and controls, complete genotypes are *unc-36 lin-12(0); rol-3 sel*. For *sel(ar40)*, complete genotype is *sel(ar40); unc-36 lin-12(0)*. (B) *lin-12(0); sel-10*. Complete genotypes are as in A, except that for *sel-10(ar41)* in the absence of *sel(arX)*, the complete genotype is *unc-36 lin-12(0); lon-3 sel-10*. Data for *lin-12(q269)* and *lin-12(n941)* are the same as in A.

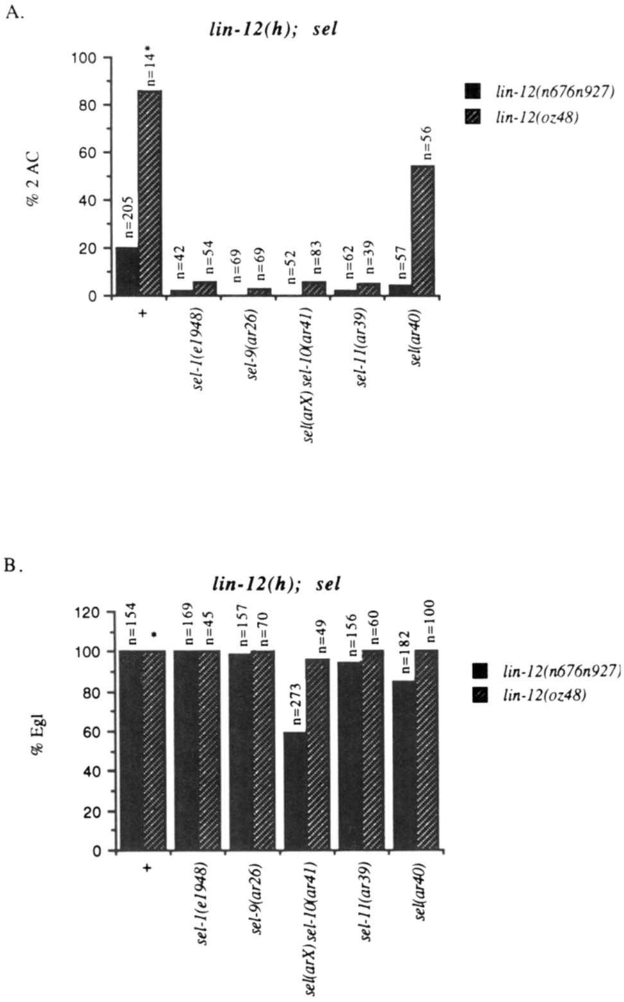

FIGURE 3.—Allele specificity experiments: *lin-12(h)* alleles. All experiments were done at 25°. For *sel* mutations on LG V and controls, complete genotypes are *unc-32 lin-12(n676n927); sel* and *unc-36 lin-12(oz48); rol-3 sel*. For *sel(ar40)*, complete genotypes are *sel(ar40); unc-32 lin-12(n676n927)* and *sel(ar40); unc-36 lin-12(oz48)*. (A) Percentage of animals having two or more ACs. (B) Percentage of animals expressing an Egl phenotype. *The presence of the *rol-3* marker mutation increased the penetrance of the 2 AC defect in *unc-36 lin-12(oz48)* mutants from 62% ($n = 62$) to 86% ($n = 14$), and also greatly reduced the fertility and vigor of such animals.

that can be stabilized or rendered functional by *sel-10*. However, assuming this is not the case, there are two general mechanisms by which *sel-10* could be acting. (1) *sel-10* mutations might act downstream of *lin-12* in a linear genetic pathway to trigger the VU fate; in this case *sel-10(+)* might be a normal target of *lin-12* activity. (2) *sel-10* mutations might allow specification of the VU fate through an alternative, parallel pathway. The second possibility is supported by laser ablation experiments showing that the VU fate in *lin-12(n941); sel(arX) sel-10(ar41)* animals still requires cell interactions (possibly with the presumptive AC), as it does in wild type (M. Sundaram and I. Greenwald, unpublished data).

***sel* genes may interact with both *lin-12* and *glp-1*:** The *glp-1* gene is structurally similar to *lin-12* and functions in similar ways in distinct cell fate decisions (AUSTIN and KIMBLE 1987, 1989; PRIESS, SCHNABEL and SCHNABEL 1987; YOCHEM and GREENWALD 1989). We have found that all of the mutations identified in our screen for suppressors of reduced *lin-12* activity also suppress the reduction-of-function allele *glp-1(e2142)*. Thus, *sel(ar40)*, *sel-1*, *sel-9*, *sel(arX) sel-10* and *sel-11* may function in both *lin-12* and *glp-1*-mediated cell fate decisions.

This result is consistent with several lines of evidence suggesting that the *lin-12* and *glp-1* products may be biochemically interchangeable. (1) *lin-12(−) glp-1(−)* double mutants have defects not seen in either single mutant, suggesting that *lin-12* and *glp-1* activities are redundant in some cell fate decisions or processes (LAMBIE and KIMBLE 1991). (2) A study of abnormal germline development suggested that the AC-to-VU signal (an inferred ligand for the *lin-12* prod-

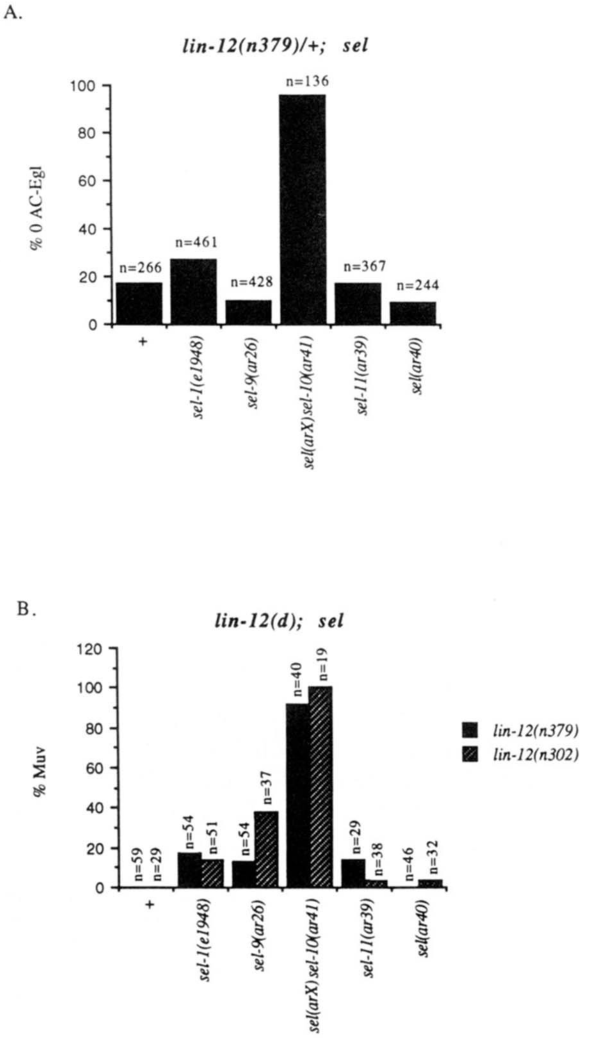

FIGURE 4.—Allele specificity experiments: *lin-12(d)* alleles. (A) *lin-12(n379)/+; sel.* Percentage of animals expressing a 0 AC-Egl phenotype. For *sel* mutations on LG V and controls, experiments were done at 25° and complete genotypes are *dpy-17 lin-12(n379)/unc-32; rol-3 sel.* For *sel(ar40)*, experiment was done at 20° and complete genotype is *sel(ar40); unc-36 lin-12(n379)/unc-32.* (B) *lin-12(d); sel.* Percentage of animals expressing a Muv phenotype (see MATERIALS AND METHODS). Complete genotypes are *unc-36 lin-12(d); sel.* All experiments were done at 20°.

TABLE 12

sel mutations suppress the maternal-effect lethality caused by _glp-1(e2142)_

Relevant genotype	Average brood size ± standard deviation	
	25°	20°
glp-1(e2142)[1]	0 (33)	12 ± 6 (5)
glp-1(e2142)[2]	ND	4 ± 4 (25)
sel(ar40); glp-1(e2142)[3]	151 ± 18 (10)	324 (1)
sel(ar40)/+; glp-1(e2142)[4]	38 ± 22 (12)	251 ± 5 (2)
glp-1(e2142); sel-1(e1948)[5]	17 ± 11 (17)	219 ± 22 (6)
glp-1(e2142); sel-1(e1948)/+[6]	0.2 ± 1 (25)	2 ± 2(10)
glp-1(e2142); sel-9(ar26)[5]	119 ± 29 (13)	191 ± 23 (5)
glp-1(e2142); sel-9(ar26)/+[6]	1 ± 1 (12)	119 ± 16 (5)
glp-1(e2142); sel(arX) sel-10(ar41)[5]	5 ± 7 (10)	29 ± 16 (7)
glp-1(e2142); sel(arX) sel-10(ar41)/+[6]	ND	15 ± 4 (12)
glp-1(e2142); sel-10(ar41)[7]	0 (25)	23 ± 12 (10)
glp-1(e2142); sel-11(ar39)[5]	46 ± 20 (10)	142 ± 9 (5)
glp-1(e2142); sel-11(ar39)/+[6]	3 ± 4(18)	50 ± 30 (12)

The number of animals scored is given in parentheses. 25° and 20° experiments were done by slightly different methods (see MATERIALS AND METHODS).

[1] *unc-36 glp-1(e2142); rol-3.*

[2] *unc-36 glp-1(e2142)/+ glp-1(e2142); rol-3 +/+ him-5.*

[3] *sel(ar40); unc-36 glp-1(e2142).*

[4] *sel(ar40)/+; unc-36 glp-1(e2142)/+ glp-1(e2142); him-5/+.*

[5] *unc-36 glp-1(e2142); rol-3 sel.*

[6] *unc-36 glp-1(e2142)/+ glp-1(e2142); rol-3 sel +/+ + him-5.*

[7] *unc-36 glp-1(e2142); lon-3 sel-10(ar41).*

TABLE 13

Allele-specificity of _glp-1_ suppression by _sel_ mutations

Relevant genotype	% sterile Glp at 25°	Average brood at 20°
glp-1(q231); +[1]	100 (31)	0 (36)
glp-1(q231); sel-1(e1948)[1]	100 (77)	0 (24)
glp-1(q231); sel-9(ar26)[1]	100 (37)	0.2 (15)
glp-1(q231); sel(arX) sel-10(ar41)[1]	100 (79)	1.5 (15)
glp-1(q231); sel-11(ar39)[1]	100 (54)	0 (45)
glp-1(e2144); +[2]	100 (58)	ND
glp-1(e2144); sel-1(e1948)[2]	100 (45)	ND
glp-1(e2144); sel-9(ar26)[2]	100 (53)	ND
glp-1(e2144); sel(arX) sel-10(ar41)[2]	100 (55)	ND
glp-1(e2144); sel-11(ar39)[2]	100 (13)	ND

The number of animals scored is given in parentheses.

[1] *unc-32 glp-1(q231); rol-3 sel.*

[2] *unc-36 glp-1(e2144); rol-3 sel.*

uct) can inappropriately interact with the *glp-1* product under some circumstances (SEYDOUX, SCHEDL and GREENWALD 1990). (3) An unusual allele of *glp-1* can substitute for *lin-12* in cell fate decisions in the vulva (MANGO, MAINE and KIMBLE 1991). (4) Recent studies of chimeric *lin-12/glp-1* proteins have directly shown that the *glp-1(+)* protein expressed under *lin-12* promoter regulation can substitute for *lin-12* in several different cell fate decisions (FITZGERALD, WILKINSON and GREENWALD 1993). In the extreme case it is possible that the *lin-12* and *glp-1* products are completely interchangeable, and that the two genes vary only in expression patterns (AUSTIN and KIMBLE 1989; YOCHEM and GREENWALD 1989). Alternatively, there may be slight functional differences between the *lin-12* and *glp-1* proteins that are more important in some cell fate decisions than others.

Multiple alleles of only two genes, *lag-1* and *lag-2*, were identified by LAMBIE and KIMBLE (1991) in their screen for mutants having the same phenotype as a *lin-12(−) glp-1(−)* double mutant. Therefore, while it seems likely that additional genes also interact with both *lin-12* and *glp-1*, these other genes probably have different null phenotypes (see introductory section). The identification of suppressors of *lin-12* and *glp-1* mutations makes no assumptions about null phenotypes, and hence has been able to identify other genes that may interact with both *lin-12* and *glp-1* (A. M. HOWELL and J. PRIESS, unpublished data; this work).

The *sel(ar40)*, *sog(zu28)* (A. M. HOWELL and J. PRIESS, unpublished data), *sel-1*, *sel-9*, *sel(arX) sel-10* and *sel-11* mutations are the first examples of mutations that have been found to suppress both *lin-12* and *glp-1* alleles. Screens for suppressors of *lin-12(d)* alleles (FERGUSON and HORVITZ 1985, and personal communication; F. TAX and J. THOMAS, unpublished data) and for suppressors of partial loss-of-function *glp-1* alleles (MAINE and KIMBLE 1989, 1993; J. PRIESS and A. M. HOWELL, unpublished data) have so far identified completely distinct sets of genes from each other and from those reported here [with the likely exception of *sel(ar40)* and *sog(zu28)*]. In most cases it is not yet clear whether these other *sel* and *sog* genes interact with only *lin-12* or only *glp-1*, or whether they might also interact with both *lin-12* and *glp-1*. However, in one case a suppressor of *lin-12(d)* mutations ("*sel-3*") turned out to be a gain-of-function allele of *lag-2* (F. TAX and J. THOMAS, unpublished data; cited in LAMBIE and KIMBLE 1991).

Constraints imposed by the suppressor screen: Our screen for extragenic suppressors was based on reversion of the Egl phenotype caused by the hypomorphic allele *lin-12(n676n930)*. The nature of this screen imposed several constraints on the suppressor mutations that could be isolated. First, because the highly penetrant Egl phenotype of *lin-12(n676n930)* hermaphrodites results from the cumulative effects of several different defects (SUNDARAM and GREENWALD 1993), we may have been selecting for suppressor mutations that could compensate for lowered *lin-12* activity in multiple cell fate decisions or processes. Indeed, we found that suppressor mutations in *sel-1*, *sel-9* and *sel-11* each suppress the 2 AC, VPC, vulval morphogenesis, proximal mitosis, and late defects caused by *lin-12(n676n930)*. *sel(ar40)* also suppresses most of these defects, with the exception of the VPC lineage defects, which apparently do not significantly affect egg laying. On the other hand, *sel-10* mutations apparently do not suppress the late defect and were only isolated in our screen in combination with another mutation, *sel(arX)*, which cooperates with *sel-10* mutations to cause suppression of the *lin-12(n676n930)* Egl phenotype.

Our screen also required that suppressor mutations not cause a lethal, sterile or other phenotype that would preclude egg laying, at least in the presence of the *lin-12(n676n930)* mutation. For example, one might imagine that many mutations capable of suppressing *lin-12(n676n930)* defects would overcompensate and cause a *lin-12(d)*-like 0 AC-Egl phenotype; such mutations would not have been isolated as suppressors of the Egl phenotype. Therefore, our screen demanded that suppressor mutations subtly readjust the level or effect of *lin-12(n676n930)* activity back to a more wild-type situation. The apparent non-null nature of many of the *sel* mutations we isolated, and the fact that the *sel* mutations do not cause any phenotype in a *lin-12(+)* background may be a direct consequence of these constraints.

Similar constraints associated with other suppressor screens may explain the fact that many of the suppressors of *lin-12* or *glp-1* isolated to date cause no phenotype other than suppression (MAINE and KIMBLE 1993; J. PRIESS and A. M. HOWELL, unpublished data; F. TAX and J. THOMAS, unpublished data). Some of these suppressor mutations are gain-of-function mutations, while others appear to be loss-of-function mutations but are not necessarily complete null mutations. An important next step in the characterization of the *sel* and *sog* genes is the isolation of null mutants and the characterization of their phenotypes. In the one case where the null phenotype of a suppressor of *lin-12* is known (*sel-3/lag-2*), that phenotype has been very informative (F. TAX and J. THOMAS, unpublished data; cited in LAMBIE and KIMBLE 1991). Genetic mosaic analysis and the molecular characterization of the *sel*, *sog*, and *lag* genes will help to further distinguish among possible models for the roles of these genes in *lin-12* and/or *glp-1* mediated processes.

We thank ANN MARIE HOWELL and JIM PRIESS for sharing their initial observations on the similarities between *sel(ar40)* and *sog(zu28)*, and for generously providing us with their *sog(zu28)* allele, members of the BAILLIE and ROSE laboratories for providing numerous strains, and BARTH GRANT, KEVIN FITZGERALD, GAUTAM KAO, DIANE LEVITAN, SIMON TUCK and JOHN YOCHEM for helpful comments on the manuscript. Some nematode strains were provided by the Caenhorhabditis Genetics Center, which is funded by the NIH National Center for Research Resources (NCRR). This material is based upon work supported by a National Science Foundation Graduate Fellowship and a grant from the National Institutes of Health (GM37602).

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Communicating editor: R. K. HERMAN