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

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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 seZ(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 mouseproto-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).
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 methanesulfonate (EMS) mutagenesis of nematodes have been previously described (Brenner 1974). Most experiments were done at 25°C, 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 Baille (1991). Other mutations are described in Brenner (1974) unless otherwise indicated.

**LG I:** dpy-3(e61), dpy-14(e188), unc-38(x20) (Lewis et al. 1983), dpy-40(e430), df4 (Howell et al. 1987), hdp20 (McKim and Rose 1990). The translocation kT(eV) (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(n137e232), lin-12(n137n720), lin-12(n302), lin-12(n379), lin-12(n676), lin-12(n676n909), lin-12(n676n930), lin-12(n941), lin-12(d269), lin-12(d48), n-1(e1865) (H. Hedgecock, unpublished data; Herman 1989, unc-32(e189), unc-36(e251), eT1(III;V) (Rosenthal and Baille 1981), qdB3 (Austin and Kimble 1987).

**LG V:** daf-17(m47) (Riddle, Swanson and Albert 1981), dpy-11(e224), eg-10(e692) (Trent, Tsung and Horvitz 1983), him-5(e1467) (Hodgkin, Horvitz and Brenner 1979), let-334(e908), let-340(e1022), let-409(e823), let-412(e5759), let-416(e830), let-434(e1040), let-446(e1504), lin-25(n545) (Ferguson and Horvitz 1985), lon-3(e2175), myo-3(e378) (Waterston 1989), rol-3(e754), rol-3(e754), rol-4(e48) (COX et al. 1980), sma-1(e30), spo-1(e63) (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) (Rosenthal and Baille 1981), arDf1 (S. Tuck, unpublished data), ctdf1 (Rogalski, Bullerjahn and Riddle 1988), mDf1 and mDf3 (Brown 1984), dDf29, dDf35, dDf47, dDf57, dDf71, dDf11 (Hunter and Wood 1992), mmDp26 (HERMAN, MADD and KARI 1979), DnT1 (E. Ferguson, unpublished data) is a derivative of the translocation n7(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) (Rosenthal and Baille 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°C 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 F2 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 mutagen-
ized 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-1/++; [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 (ar23, 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-1/++; [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°C. 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°C 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°C 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°C, 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°C, 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, ar64). 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°C 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°C unless sel(arX) is also present (Table 2 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°C (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-3 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+et1 Df(s)] were scored for egg laying. Males of genotype unc-32 lin-12(n676n930)et1; dpy-11 sel+ et1 ++ were crossed to hermaphrodites of genotype unc-32 lin-12(n676n930)et1; dpy-11 Df(s)/et1, and the Dpy Unc hermaphrodite cross progeny [of genotype unc-32 lin-12(n676n930); sel+et1 Df(s)] 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+et1 Df(s)] appeared normal. mdf1, sDf97, sDf71: see "Complementation tests between recessive sel mutations on LG V and existing mutations."

sDc29, 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...
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-14/+/dpy-5; unc-32(n676n930)/+++ were mated to heterozygotes of genotype dpy-5 pep-4; unc-32(n676n930)/++; dpy-5 pep-4; unc-32(n676n930)/+++; hdp20. Unc sel progeny of such animals were picked to establish both wild-type cross progeny and sel progeny. For each strain, the entire broods (including both Unc and non-Unc animals) of at least five heterozygotes were scored for egg laying and number of ACs at 25°C.

**TABLE 1**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Genotype of heterozygous parent</th>
<th>Phenotype of selected recombinants</th>
<th>sel genotype of selected recombinants</th>
</tr>
</thead>
<tbody>
<tr>
<td>sel-1</td>
<td>rol-4 + lin-25/+/ sel-1 +</td>
<td>Rol</td>
<td>3/8 sel-1/+/</td>
</tr>
<tr>
<td></td>
<td>sma-1 rol-4/+/ + sel-1</td>
<td>Rol</td>
<td>5/8 sel-1/+</td>
</tr>
<tr>
<td>sel-9</td>
<td>dpy-11 rol-3/+/ + sel-9</td>
<td>Dpy</td>
<td>27/27 sel-9/+</td>
</tr>
<tr>
<td></td>
<td>rol-3 + unc-42/+/ sel-9 +</td>
<td>Rol</td>
<td>5/5 sel-9/+</td>
</tr>
<tr>
<td>sel-10</td>
<td>sqt-3 + unc-76+/+ sel-10 +</td>
<td>Sqt</td>
<td>5/5 sel-10/+</td>
</tr>
<tr>
<td></td>
<td>unc-42 + sma-1/+/ sel-11 +</td>
<td>Unc</td>
<td>15/14 sel-11/+</td>
</tr>
<tr>
<td></td>
<td>(daf-11+) sma-1(+ sel-11) +</td>
<td>Sma</td>
<td>1/1 +/+</td>
</tr>
<tr>
<td></td>
<td>+ sma-1 myo-3/ sel-11 + +</td>
<td>Smal</td>
<td>2/2 sel-11/+</td>
</tr>
<tr>
<td>sel(ar40)</td>
<td>+ dpy-5 unc-40/sel(ar40) + +</td>
<td>Dpy</td>
<td>14/14 +/+</td>
</tr>
<tr>
<td></td>
<td>unc-38 + dpy-5/+ sel(ar40) +</td>
<td>Dpy</td>
<td>1/57 sel(ar40)/+</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>36/37 +/+</td>
</tr>
</tbody>
</table>

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.

The table includes information on the genetic crosses and the phenotypic outcomes of these crosses. The table is structured in a tabular format, with columns indicating the gene, genotype of the heterozygous parent, phenotype of selected recombinants, and sel genotype of selected recombinants. The data are derived from various genetic experiments, including crosses and complementation tests, and are used to map the genetic loci on LG V.
and Unc non-Dpy hermaphrodite cross progeny [of genotype un-12(n676n930); sel/sel'] were picked. Unc self progeny of these hermaphrodites (of genotype un-32 lin-12(n676n930); dpy-11 sel') were viable in both cases) were picked. Unc-32 non-Dpy self progeny (which were viable in all cases). Unc-32 lin-12(n676n930); dpy-11 sel+ was transferred to 25°C for scoring, as described, for all mutations except mDf3, which was verified by crossing in an sDfDs marker line. The presence of the mDf3 line was verified by crossing in an sDfDs marker line. The presence of the mDf3 line was verified by crossing in an sDfDs marker line.

The Egl defect was partially suppressed (see Tables 8 and 9).

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For all mutations except mDf3, greater than 95% of Unc-32 non-Dpys were Egl, indicating complementation (n = 20). sel-9 vs. sel-10 and sel-11: unc-32 lin-12(n676n930); sel/sel' were scored for egg laying. All such animals had 2 ACs, as described. sel-9 vs. sel-11: unc-32 lin-12(n676n930); sel/sel' were scored for egg laying. All such animals had 2 ACs, as described. sel-9 vs. sel-11: unc-32 lin-12(n676n930); sel/sel' were scored for egg laying. All such animals had 2 ACs, as described. sel-9 vs. sel-11: unc-32 lin-12(n676n930); sel/sel' were scored for egg laying. All such animals had 2 ACs, as described. sel-9 vs. sel-11: unc-32 lin-12(n676n930); sel/sel' were scored for egg laying. All such animals had 2 ACs, as described. sel-9 vs. sel-11: unc-32 lin-12(n676n930); sel/sel' were scored for egg laying. All such animals had 2 ACs, as described. sel-9 vs. sel-11: unc-32 lin-12(n676n930); sel/sel' were scored for egg laying. All such animals had 2 ACs, as described. sel-9 vs. sel-11: unc-32 lin-12(n676n930); sel/sel' were scored for egg laying. All such animals had 2 ACs, as described.

Topics:
- suppressors of lin-12 and glp-1
- Deficiency mapping data
- TABLE 2

<table>
<thead>
<tr>
<th>Relevant genotype</th>
<th>sel(+)</th>
<th>sel-1(d948)</th>
<th>sel-9(ar22)</th>
<th>sel(ar40)</th>
<th>sel-1(ar39)</th>
<th>sel genes removed by deficiency*</th>
</tr>
</thead>
<tbody>
<tr>
<td>lin-12(n676n930); sel/sel'</td>
<td>32 (108)</td>
<td>0 (42)</td>
<td>0 (55)</td>
<td>0 (36)</td>
<td>0 (38)</td>
<td>-</td>
</tr>
<tr>
<td>lin-12(n676n930); sel/4</td>
<td>32 (40)</td>
<td>36 (36)</td>
<td>4 (123)</td>
<td>18 (21)</td>
<td>14 (28)</td>
<td>-</td>
</tr>
<tr>
<td>lin-12(n676n930); sel/dpJ3</td>
<td>39 (59)</td>
<td>33 (49)</td>
<td>0 (44)</td>
<td>37 (8)</td>
<td>28 (18)</td>
<td>sel-9, (sel-11)</td>
</tr>
<tr>
<td>lin-12(n676n930); sel/mdP3</td>
<td>26 (23)</td>
<td>22 (9)</td>
<td>3 (63)</td>
<td>31 (16)</td>
<td>22 (9)</td>
<td>sel-9</td>
</tr>
<tr>
<td>lin-12(n676n930); sel/dpJ4</td>
<td>28 (26)</td>
<td>18 (34)</td>
<td>3 (115)</td>
<td>ND</td>
<td>ND</td>
<td>None</td>
</tr>
<tr>
<td>lin-12(n676n930); sel/dpJ5</td>
<td>ND</td>
<td>ND</td>
<td>3 (30)</td>
<td>ND</td>
<td>ND</td>
<td>None</td>
</tr>
<tr>
<td>lin-12(n676n930); sel/df29</td>
<td>40 (5)</td>
<td>ND</td>
<td>33 (58)</td>
<td>30 (23)</td>
<td>ND</td>
<td>None</td>
</tr>
<tr>
<td>lin-12(n676n930); sel/df57</td>
<td>36 (14)</td>
<td>ND</td>
<td>3 (30)</td>
<td>ND</td>
<td>ND</td>
<td>None</td>
</tr>
<tr>
<td>lin-12(n676n930); sel/df14</td>
<td>37 (30)</td>
<td>30 (30)</td>
<td>ND</td>
<td>35 (40)</td>
<td>31 (45)</td>
<td>(sel-11)</td>
</tr>
<tr>
<td>lin-12(n676n930); sel/rdf15</td>
<td>18 (54)</td>
<td>0 (73)</td>
<td>ND</td>
<td>14 (77)</td>
<td>ND</td>
<td>sel-1, (sel-10)</td>
</tr>
<tr>
<td>lin-12(n676n930); sel/tiDf2</td>
<td>100 (4)</td>
<td>ND</td>
<td>ND</td>
<td>43 (7)</td>
<td>ND</td>
<td>(sel-10)</td>
</tr>
</tbody>
</table>

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, 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.

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

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. Results are puzzling and suggest that dpJ29 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 dpJ29 chromosome contains a duplication of the sel-9 region. The Egl defect was partially suppressed (see Table 7).
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 selv or selv(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 selv 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); 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-36 animals from heterozygous mothers were examined.

**lin-12(n676n909); sel:**

Unc-32 lin-12(n676n909)/unc-36 lin-12(n676n930); rol-3 selv(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 selv(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 selv(v)+++, from which independent Roller lines were established. Rol non-Dpy non-Unc animals were scored for the 0 A-Egl phenotype at 25°. dpy-5++; unc-36 lin-12(n379)/++ males were mated to selv(ar40); unc-32 hermaphrodites, and Egl semi-Dpy non-Unc cross progeny [of genotype dpy-5 selv(ar40); unc-36 lin-12(n379)] picked and used to establish a true-breeding non-Dpy line. Non-Unc animals from this line were scored for the 0 A-Egl phenotype at 25°.

**lin-12(n379); sel and lin-12(n302); sel:**

Unc-36 lin-12 unc-32 lin-12(n676n930); m selv 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:**

glep-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 20° (PRIES, 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 selv(++) hermaphrodites were selfed and Roller progeny [of genotype unc-36 glp-1/unc-32 lin-12; rol-3 selv] picked to 25°. Unc-36 self-progeny of such Roller animals were picked and scored for number of live progeny per 3 days. If homozygous unc-36 glp-1; rol-3 selv 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 selv 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° (PRIES, 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-32 glp-1 unc-32 lin-12; rol-3 selv(+) hermaphrodites were selfed, and Roller progeny [of genotype unc-36 glp-1/unc-32 lin-12; rol-3 selv] picked to 25°. Unc-36 self-progeny of such Roller animals were picked and scored for the Glp phenotype.

**glp-1(q231); sel:**

glep-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 selv(+) 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 selv, 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 selv hermaphrodites at 25°, and Non-Unc hermaphrodite cross progeny [of genotype unc-36 glp-1+/+ glp-1; rol-3 selv](25°) 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).
Suppressors of lin-12 and glp-1

TABLE 3

Summary of relevant phenotypes of lin-12 mutants

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<tbody>
<tr>
<td>lin-12(d) strong</td>
<td>0</td>
<td>2*</td>
<td>2*</td>
<td>2*</td>
<td>2*</td>
<td>2*</td>
<td>2*</td>
<td>Muv</td>
<td>0 AC-Egl</td>
<td>0</td>
</tr>
<tr>
<td>lin-12(d) weak</td>
<td>0</td>
<td>3*</td>
<td>3*</td>
<td>3*</td>
<td>3*</td>
<td>3*</td>
<td>3*</td>
<td>Vul</td>
<td>0 AC-Egl</td>
<td>0</td>
</tr>
<tr>
<td>lin-12(+)</td>
<td>1</td>
<td>3*</td>
<td>3*</td>
<td>2*</td>
<td>1*</td>
<td>2*</td>
<td>3*</td>
<td>WT</td>
<td>WT</td>
<td>0</td>
</tr>
<tr>
<td>lin-12(n676n930)</td>
<td>1 or 2</td>
<td>3*</td>
<td>3*</td>
<td>variable</td>
<td>Variable</td>
<td>1*</td>
<td>3*</td>
<td>Egl</td>
<td>Sterile</td>
<td>100</td>
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<tr>
<td>lin-12(0)</td>
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<td></td>
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</table>

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

<table>
<thead>
<tr>
<th>lin-12(n676n930)</th>
<th>lin-12(d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>h</td>
<td>d</td>
</tr>
</tbody>
</table>

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°C (SUNDARAM and GREENWALD 1993). After EMS-mutagenesis, we obtained 27 non-Egl revertants of lin-12(n676n930) at a total frequency of 5 × 10⁻³. 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 AND METHODS).
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.

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

The percentage of gonadal arms containing proximal mitotic germ nuclei.

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

Percentage of gonadal arms containing proximal mitotic germ nuclei.

See text and MATERIALS AND METHODS for explanation of sel(arX).

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

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 dF35 and cDf1 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 arDf2 since both those deficiencies complement sel-10, but it remains possible that either of these deficiencies removes sel-10.

Table 4

<table>
<thead>
<tr>
<th>Genotype</th>
<th>% Egl</th>
<th>% 2 AC</th>
<th>VPC fate</th>
<th>% proximal mitosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>lin-12(n676n930)</td>
<td>95(112)</td>
<td>35(40)</td>
<td>Mutant</td>
<td>55(31)</td>
</tr>
<tr>
<td>lin-12(n676n930); sel-1(e1948)</td>
<td>10(128)</td>
<td>0(42)</td>
<td>Suppressed</td>
<td>2(27)</td>
</tr>
<tr>
<td>lin-12(n676n930); sel-9(ar22)</td>
<td>7(151 )</td>
<td>0(57)</td>
<td>Suppressed</td>
<td>2(29)</td>
</tr>
<tr>
<td>lin-12(n676n930); sel-10(ar28)</td>
<td>20(153)</td>
<td>0(36)</td>
<td>Suppressed</td>
<td>0(18)</td>
</tr>
<tr>
<td>lin-12(n676n930); sel-10(ar41)</td>
<td>97(255)</td>
<td>3(29)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>lin-12(n676n930); sel-11(ar39)</td>
<td>7(248 )</td>
<td>0(38)</td>
<td>Suppressed</td>
<td>0(32)</td>
</tr>
<tr>
<td>sel(ar40); lin-12(n676n930)</td>
<td>19(129)</td>
<td>0(21)</td>
<td>Mutant</td>
<td>0(25)</td>
</tr>
</tbody>
</table>

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, how-
ever, 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 hypoder-

mis 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°C: At the permissive temperature of 15°C, 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°C (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/+/+. 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 1982). 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-10* 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°C (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. PLESS 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


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<td>TTTT</td>
<td>NTTT</td>
<td>SS</td>
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<tr>
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<td>SS</td>
<td>LTTT</td>
<td>TTTT</td>
<td>NTSS</td>
<td>SS</td>
</tr>
<tr>
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<td>SS</td>
<td>LTTT</td>
<td>TTTT</td>
<td>NTSS</td>
<td>SS</td>
</tr>
<tr>
<td>lin-12(n576n930); sel-1(a1948)</td>
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<td>LOTT</td>
<td>TTTT</td>
<td>NTLL</td>
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<tr>
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<tr>
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<td>S</td>
<td>LTTT</td>
<td>TTTT</td>
<td>NTLL</td>
<td>SS</td>
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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; STN, ETT, GTO (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

<table>
<thead>
<tr>
<th>sel mutations enhance the 0 AC-Egl phenotype caused by lin-12(n676n930) at 15°C</th>
<th>Relevant genotype</th>
<th>% 0 AC-Egl</th>
</tr>
</thead>
<tbody>
<tr>
<td>lin-12(n676n930); +</td>
<td>7 (204)</td>
<td></td>
</tr>
<tr>
<td>lin-12(n676n930); sel-1(e1948)</td>
<td>72 (449)</td>
<td></td>
</tr>
<tr>
<td>lin-12(n676n930); sel-9(ar22)</td>
<td>55 (554)</td>
<td></td>
</tr>
<tr>
<td>lin-12(n676n930); sel(arX) sel-10(ar41)</td>
<td>40 (135)</td>
<td></td>
</tr>
<tr>
<td>lin-12(n676n930); sel-10(ar41)</td>
<td>56 (164)</td>
<td></td>
</tr>
<tr>
<td>lin-12(n676n930); sel-11(ar39)</td>
<td>64 (303)</td>
<td></td>
</tr>
<tr>
<td>sel(ar40); lin-12(n676n930)</td>
<td>50 (107)</td>
<td></td>
</tr>
<tr>
<td>sel(ar40); lin-12(n676n930); sel-1(e1948)</td>
<td>97 (243)</td>
<td></td>
</tr>
</tbody>
</table>

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

*Complete genotype: unc-12 lin-12(n676n930); lin-3 sel-10(ar41).

However, since the phenotype of sel-11/Df is not known, this result does not rule out alleleism 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 specificifications. (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 reversal of the Egl defect of lin-12(oz48) animals have so far yielded only apparent intragenic revertants (Wakelk 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°C (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 (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 1983; 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(d) 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
partial loss-of-function allele that causes maternal-effect embryonic lethality but does not significantly affect germinal 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 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.

### Table 7

**sel-1 dosage studies**

<table>
<thead>
<tr>
<th>Relevant genotype</th>
<th>% 2 AC</th>
<th>% Egl</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>lin-12(n676n930); +/+&lt;sup&gt;1&lt;/sup&gt;</td>
<td>32 (108)</td>
<td>96 (49)</td>
<td>sel-1 suppresses the 2 AC and Egl defects.</td>
</tr>
<tr>
<td>lin-12(n676n930); sel-1/sel-1&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0 (42)</td>
<td>6 (198)</td>
<td></td>
</tr>
<tr>
<td>lin-12(n676n930); sel-1/+&lt;sup&gt;3&lt;/sup&gt;</td>
<td>31 (68)</td>
<td>91 (43)</td>
<td>sel-1 is a recessive suppressor.</td>
</tr>
<tr>
<td>lin-12(n676n930); sel-1/Df&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0 (73)</td>
<td>59 (39)</td>
<td>Reducing the dosage of sel-1(+) causes a more suppressed phenotype.</td>
</tr>
<tr>
<td>lin-12(n676n930); +/Df&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18 (54)</td>
<td>100 (70)</td>
<td>Increasing the dosage of sel-1(+) causes a less suppressed phenotype.</td>
</tr>
<tr>
<td>lin-12(n676n930); sel-1/sel-1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0 (41)</td>
<td>8 (36)</td>
<td></td>
</tr>
<tr>
<td>lin-12(n676n930); sel-1/sel-1/+&lt;sup&gt;c&lt;/sup&gt;</td>
<td>15 (46)</td>
<td>100 (55)</td>
<td></td>
</tr>
<tr>
<td>lin-12(n676n930); +/+&lt;sup&gt;d&lt;/sup&gt;</td>
<td>32 (47)</td>
<td>100 (27)</td>
<td></td>
</tr>
<tr>
<td>lin-12(n676n930); +/+&lt;sup&gt;e&lt;/sup&gt;</td>
<td>51 (73)</td>
<td>100 (59)</td>
<td></td>
</tr>
</tbody>
</table>

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

<sup>1</sup> unc-32 lin-12(n676n930); +.  
<sup>2</sup> unc-32 lin-12(n676n930); sel-1(e1948).  
<sup>3</sup> unc-32 lin-12(n676n930); dpy-11 sel-1(e1948)/++.  
<sup>4</sup> unc-32 lin-12(n676n930); dpy-11 sel-1(e1948)/+ arDf1.  
<sup>5</sup> unc-32 lin-12(n676n930); dpy-11 arDf1 arDf1.  
<sup>6</sup> unc-32 lin-12(n676n930); sel-1(e1948) (from ctDp1-bearing mothers).  
<sup>7</sup> unc-36 lin-12(n676n930); sel-1(e1948); ctDp11.  
<sup>8</sup> unc-36 lin-12(n676n930); + (from ctDp11-bearing mothers).  
<sup>9</sup> unc-36 lin-12(n676n930); +; ctDp11.

### Table 8

**sel-9 dosage studies**

<table>
<thead>
<tr>
<th>Relevant genotype</th>
<th>% 2 AC</th>
<th>% Egl</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>lin-12(n676n930); +/+&lt;sup&gt;1&lt;/sup&gt;</td>
<td>32 (108)</td>
<td>96 (49)</td>
<td>sel-9 suppresses the 2 AC and Egl defects.</td>
</tr>
<tr>
<td>lin-12(n676n930); sel-9/sel-9&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0 (55)</td>
<td>5 (154)</td>
<td></td>
</tr>
<tr>
<td>lin-12(n676n930); sel-9/+&lt;sup&gt;3&lt;/sup&gt;</td>
<td>4 (123)</td>
<td>96 (97)</td>
<td>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.</td>
</tr>
<tr>
<td>lin-12(n676n930); sel-9/Df&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3 (85)</td>
<td>61 (95)</td>
<td></td>
</tr>
<tr>
<td>lin-12(n676n930); +/Df&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26 (23)</td>
<td>100 (45)</td>
<td></td>
</tr>
<tr>
<td>lin-12(n676n930); sel-9/sel-9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0 (70)</td>
<td>19 (54)</td>
<td>Increasing the dosage of sel-9(+) antagonizes sel-9 suppression of the Egl defect but not sel-9 suppression of the 2 AC defect.</td>
</tr>
<tr>
<td>lin-12(n676n930); sel-9/sel-9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2 (92)</td>
<td>78 (71)</td>
<td></td>
</tr>
<tr>
<td>lin-12(n676n930); +/+&lt;sup&gt;d&lt;/sup&gt;</td>
<td>29 (76)</td>
<td>100 (16)</td>
<td></td>
</tr>
<tr>
<td>lin-12(n676n930); +/+&lt;sup&gt;e&lt;/sup&gt;</td>
<td>19 (85)</td>
<td>100 (24)</td>
<td></td>
</tr>
</tbody>
</table>

The number of animals scored is given in parentheses. Similar results were obtained with sel-9(ar29) (data not shown).

<sup>1</sup> unc-32 lin-12(n676n930); +.  
<sup>2</sup> unc-32 lin-12(n676n930); sel-9(ar29).  
<sup>3</sup> unc-32 lin-12(n676n930); dpy-11 sel-9(ar29)/++.  
<sup>4</sup> unc-32 lin-12(n676n930); dpy-11 sel-9(ar29)/+; mDf3.  
<sup>5</sup> unc-32 lin-12(n676n930); dpy-11 mDf3.  
<sup>6</sup> unc-32 lin-12(n676n930); dpy-11 (from mDf26-bearing mothers).  
<sup>7</sup> unc-32 lin-12(n676n930); dpy-11; mDf26.  
<sup>8</sup> unc-32 lin-12(n676n930); dpy-11 (from mDf26-bearing mothers).  
<sup>9</sup> unc-32 lin-12(n676n930); dpy-11; mDf26.
Table 9

<table>
<thead>
<tr>
<th>Relevant genotype</th>
<th>% 0 AC-Egl</th>
</tr>
</thead>
<tbody>
<tr>
<td>lin-12(n676n930); sel-9/+</td>
<td>39 (77)%</td>
</tr>
<tr>
<td>lin-12(n676n930); sel-9/93</td>
<td>14 (61)</td>
</tr>
<tr>
<td>lin-12(n676n930); +/+3</td>
<td>3 (59)</td>
</tr>
<tr>
<td>lin-12(n676n930); +/+4</td>
<td>7 (61)</td>
</tr>
</tbody>
</table>

The number of animals scored is given in parentheses.

### 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 10^3 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
alleles, and thus require some mutation isolated by J. PRIES and data not shown). 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 ratio (73) 9 (43) Increasing the dosage of sel-11(+) causes a less suppressed phenotype.

### Table 10

<table>
<thead>
<tr>
<th>Relevant genotype</th>
<th>% 2 AC</th>
<th>% Egl</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>lin-12(n676n930); +/+</td>
<td>32 (108)</td>
<td>96 (49)</td>
<td>sel-11 suppresses the 2 AC and Egl defects.</td>
</tr>
<tr>
<td>lin-12(n676n930); sel-11(+)</td>
<td>0 (38)</td>
<td>10 (124)</td>
<td></td>
</tr>
<tr>
<td>lin-12(n676n930); +/+</td>
<td>14 (28)</td>
<td>95 (21)</td>
<td>sel-11 is a recessive suppressor of the Egl defect, but weakly semidominant for suppression of the 2 AC defect.</td>
</tr>
<tr>
<td>lin-12(n676n930); sel-11(+)</td>
<td>0 (73)</td>
<td>9 (43)</td>
<td>Increasing the dosage of sel-11(+) causes a less suppressed phenotype.</td>
</tr>
<tr>
<td>lin-12(n676n930); sel-11(+)</td>
<td>11 (133)</td>
<td>100 (56)</td>
<td></td>
</tr>
<tr>
<td>lin-12(n676n930); +/+</td>
<td>32 (47)</td>
<td>100 (27)</td>
<td></td>
</tr>
<tr>
<td>lin-12(n676n930); +/+</td>
<td>51 (73)</td>
<td>100 (50)</td>
<td></td>
</tr>
</tbody>
</table>

The number of animals scored is given in parentheses. Similar results were obtained with sel-11(ar40) (data not shown).

### Table 11

<table>
<thead>
<tr>
<th>Relevant genotype</th>
<th>% 2 AC</th>
<th>% Egl</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>lin-12(n676n930); +/+</td>
<td>32 (108)</td>
<td>96 (49)</td>
<td>sel(ar40) suppresses the Egl and 2 AC defects.</td>
</tr>
<tr>
<td>lin-12(n676n930); sel(ar40)</td>
<td>1 (106)</td>
<td>18 (35)</td>
<td></td>
</tr>
<tr>
<td>lin-12(n676n930); sel(ar40)</td>
<td>5 (40)</td>
<td>23 (177)</td>
<td>sel(ar40) is a dominant suppressor of both the Egl and 2 AC defects.</td>
</tr>
<tr>
<td>lin-12(n676n930); sel(ar40)</td>
<td>4 (24)</td>
<td>100 (23)</td>
<td>Increasing the dosage of mutant sel(ar40) does not affect suppression of the 2 AC defect. Increasing the dosage of sel(ar40)&quot; causes a less suppressed phenotype.</td>
</tr>
<tr>
<td>lin-12(n676n930); sel(ar40)</td>
<td>50 (16)</td>
<td>100a</td>
<td></td>
</tr>
</tbody>
</table>

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. PRIES and A. M. HOWELL (unpublished data; see text). a This strain could not be grown at 25° and was very sickly at 20°.

The 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 allele besides lin-12(n676n930), all strongly enhance the gain-of-function 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...
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-lO(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; YOCHEN 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-
complete genotype is were done at 25° and complete genotypes are unr-32; roi-3 sel.

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-

tect) 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/glpl 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-

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

<table>
<thead>
<tr>
<th>Relevant genotype</th>
<th>Average brood size ± standard deviation</th>
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GLP-1 suppression by sel mutations

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

The identification of suppressors of lin-12 and glp-1 mutations makes no assumptions about null pheno-

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); rol-3+/+ him-5.
4 sel(ar40); unc-36 glp-1(e2142); rol-3+/+ him-5.
5 unc-36 glp-1(e2142); rol-3 sel*/+ him-5.
6 unc-36 glp-1(e2142); rol-3 sel*/+ him-5.
7 unc-36 glp-1(e2142); rol-3 sel*/+ him-5.
8 unc-36 glp-1(e2142); rol-3 sel*/+ him-5.
9 unc-36 glp-1(e2142); rol-3 sel*/+ him-5.
10 unc-36 glp-1(e2142); rol-3 sel*/+ him-5.
11 unc-36 glp-1(e2142); rol-3 sel*/+ him-5.
12 unc-36 glp-1(e2142); rol-3 sel*/+ him-5.
13 unc-36 glp-1(e2142); rol-3 sel*/+ him-5.
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36 unc-36 glp-1(e2142); rol-3 sel*/+ him-5.
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49 unc-36 glp-1(e2142); rol-3 sel*/+ him-5.
50 unc-36 glp-1(e2142); rol-3 sel*/+ him-5.
51 unc-36 glp-1(e2142); rol-3 sel*/+ him-5.
52 unc-36 glp-1(e2142); rol-3 sel*/+ him-5.
53 unc-36 glp-1(e2142); rol-3 sel*/+ him-5.
types, 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 Lambe 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 Lambe 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.

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