Identification and Characterization of Genes That Interact With *lin-12* in *Caenorhabditis elegans*

Frans E. Tax,*[,1] James H. Thomas,*[,†] Edwin L. Ferguson[†,2] and H. Robert Horvitz[†,‡]

**Department of Genetics, University of Washington, Seattle, Washington 98195 and ‡Howard Hughes Medical Institute, Department of Biology and †Massachusetts Institute of Technology, Cambridge, Massachusetts 02139*

Manuscript received October 24, 1996
Accepted for publication August 21, 1997

ABSTRACT

We identified and characterized 14 extragenic mutations that suppressed the dominant egg-laying defect of certain *lin-12* gain-of-function mutations. These suppressors defined seven genes: *sup-17, lag-2, sel-4, sel-5, sel-6, sel-7* and *sel-8*. Mutations in six of the genes are recessive suppressors, whereas the two mutations that define the seventh gene, *lag-2*, are semi-dominant suppressors. These suppressor mutations were able to suppress other *lin-12* gain-of-function mutations. The suppressor mutations arose at a very low frequency per gene, 10–50 times below the typical loss-of-function mutation frequency. The suppressor mutations in *sup-17* and *lag-2* were shown to be rare non-null alleles, and we present evidence that null mutations in these two genes cause lethality. Temperature-shift studies for two suppressor genes, *sup-17* and *lag-2*, suggest that both genes act at approximately the same time as *lin-12* in specifying a cell fate. Suppressor alleles of six of these genes enhanced a temperature-sensitive loss-of-function allele of *glp-1*, a gene related to *lin-12* in structure and function. Our analysis of these suppressors suggests that the majority of these genes are part of a shared *lin-12/glp-1* signal transduction pathway, or act to regulate the expression or stability of *lin-12* and *glp-1*.

CELL interactions play a major role in the development of the nematode *Caenorhabditis elegans* (reviewed by HORVITZ and STERNBERG 1991; LAMBIE and KIMBLE 1991a). One type of cell interaction in *C. elegans* occurs when two bipotential cells interact and as a consequence express two distinct developmental fates; such cells are said to compose an equivalence group (KIMBLE *et al.* 1979). This type of cell interaction is well illustrated by the two cells Z1.ppp and Z4.aaa, named for their cell lineage origins (SULSTON and HORVITZ 1977; KIMBLE and HIRSH 1979). These two cells are physically adjacent, and one becomes the anchor cell (AC) while the other becomes a ventral uterine precursor cell (VU). Although either cell can acquire either the AC or the VU fate, each animal always generates just one AC and one VU (KIMBLE 1981), implying that these cells somehow communicate to decide their fates. If either Z1.ppp or Z4.aaa is killed with a laser microbeam, the remaining cell invariably becomes an AC (KIMBLE 1981). Thus, the AC fate is termed primary (1°) and the VU fate is termed secondary (2°). Even when surrounding gonadal cells are killed, Z1.ppp and Z4.aaa interact correctly to produce one AC and one VU cell (SEYDOUX and GREENWALD 1989). These results indicate that the cell that will form the AC (either Z1.ppp or Z4.aaa) sends a signal to the other cell, causing it to become a VU. When one of these cells is killed (KIMBLE 1981) or physically displaced (HEDGECOCK *et al.* 1990), this signal is missing, so the remaining cell acquires the 1° fate.

A number of cell interactions in diverse tissue types are affected by mutations in the gene *lin-12* (GREENWALD *et al.* 1983). The effect of *lin-12* mutations on the cells Z1.ppp and Z4.aaa is typical. Dominant alleles of *lin-12*, called *lin-12(d)*, cause both Z1.ppp and Z4.aaa to adopt the 2° (VU) fate. Conversely, null mutations of *lin-12* cause both cells to adopt the 1° (AC) fate. Gene dosage studies show that the cell-fate transformations caused by *lin-12(d)* mutations are enhanced by additional copies of the *lin-12(+)* gene, suggesting that *lin-12(d)* mutations cause increased gene function (GREENWALD *et al.* 1983). Thus, in most cases, high *lin-12* activity causes a cell to adopt the 2° fate, and low *lin-12* activity causes a cell to adopt the 1° fate.

Molecular analysis indicated that *lin-12* plays a direct role in cell interactions. *lin-12* encodes a protein with a domain containing a repeated peptide motif that is homologous to epidermal growth factor (GREENWALD 1985), and an overall structure similar to that of the Drosophila Notch protein (YOCHEM *et al.* 1988). Genetic mosaic analysis of the role of *lin-12* in the Z1.ppp/Z4.aaa cell interaction indicated that *lin-12* function is required only in the signal-receiving cell (the presumptive VU) for normal development (SEYDOUX and

Corresponding author: James H. Thomas, Department of Genetics, SK-50, University of Washington, Seattle, WA 98195.
E-mail: jht@genetics.washington.edu
[1]*Present address:* Department of Molecular and Cellular Biology, University of Arizona, Tucson, AZ 85721.
[2]*Present address:* Department of Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL 60637.

GREENWALD 1989). These results together suggest that *lin-12* encodes the receptor for intercellular signaling between these cells. *glp-1* is closely related to *lin-12* in DNA and protein sequence (YOCHEM and GREENWALD 1989), and *glp-1* is implicated as a receptor for cell interactions that mediate germ line proliferation and early embryonic cell fate (AUSTIN and KIMBLE 1987; PRIESS *et al.* 1987; MELLO *et al.* 1994). *lin-12* and *glp-1* appear to function redundantly in controlling other cell fates in later embryogenesis (LAMBIE and KIMBLE 1991b).

Members of the *lin-12/Notch* family in several other species have been reported (Xenopus, COFFMAN *et al.* 1990; mice, ROBBINS *et al.* 1992; rats, WEINMASTER *et al.* 1991, 1992; and humans, ELLISEN *et al.* 1991), and analysis of these genes has suggested that they function in a similar manner. These observations coupled with the effects of experimentally introduced deletions indicated that the extracellular domains of *lin-12/Notch* family members function as negative regulatory domains (Xenopus, COFFMAN *et al.* 1993; Drosophila, LIEBER *et al.* 1993; REBAY *et al.* 1993; STRUHL *et al.* 1993; and *C. elegans*, ROEHL and KIMBLE 1993; STRUHL *et al.* 1993). Interestingly, two chromosomal rearrangements causing truncation or partial loss of the extracellular domains of *Notch* family genes are correlated with tumor formation (mice, ROBBINS *et al.* 1992; humans, ELLISEN *et al.* 1991). In *C. elegans*, the effect on the AC/VU equivalence group caused by introducing *lin-12* and *glp-1* genes deleted for extracellular protein sequences is similar to the phenotype caused by *lin-12(d)* alleles (GREENWALD *et al.* 1983; ROEHL and KIMBLE 1993; STRUHL *et al.* 1993). The *lin-12(d)* alleles have been hypothesized to encode interaction-independent receptors and have been shown to have missense changes in the extracellular domain (GREENWALD and SEYDOUX 1990).

The cell interactions affected by *lin-12* and *glp-1* must utilize other genes responsible for the generation, reception and interpretation of intercellular signals. In addition, there must be genes that regulate the expression of *lin-12*. *lag-1* has recently been shown to encode a protein similar to the Drosophila *Su(H)* gene and is believed to function downstream of *lin-12/glp-1* receptor activation (CHRISTENSEN *et al.* 1996). Here we report the identification of extragenic suppressor mutations of *lin-12(d)* alleles. Such suppressors should identify other genes that regulate or respond to *lin-12*. We also report a more detailed genetic analysis of two of these suppressor genes, *sup-17* and *lag-2*. *lag-2* has been cloned (HENDERSON *et al.* 1994; TAX *et al.* 1994), and its predicted protein structure resembles the products of other *lin-12/Notch* family ligands in *C. elegans*, Drosophila, and mammals (REBAY *et al.* 1991; MELLO *et al.* 1994; LINDSELL *et al.* 1995). Thus, our screen resulted in the identification of at least one gene that is likely to interact directly with *lin-12* and *glp-1*.

MATERIALS AND METHODS

General genetic methods: Methods for culturing, handling, mutagenesis, and genetic manipulation of *C. elegans* were as described (BRENNER 1974). Unless stated, genetic manipulations and experiments were performed at 20°. The standard wild-type strain N2 and most other strains used in this work were obtained from BRENNER (1974) or the Caenorhabditis Genetics Center, University of Missouri. This article follows the standard *C. elegans* nomenclature (HORVITZ *et al.* 1979), and most mutants are described in HODGKIN *et al.* (1988).

Marker mutations used: *LGI: dpy-5(e61), unc-13(e1091)* or *unc-13(e51), fer-1(hc1), unc-29(e1072), nDf23, nDf24, nDf25, nDf29, nDf30, unc-75(e950), lin-11(n566).*

LGII: sqt-2(sc3), dpy-10(e128), unc-4(e120), mnC1 dpy-10(e128) unc-52(e444), nDf3.

LGIII: dpy-17(e164), lon-1(e185), ncl-1(e1865), unc-36(e251), dpy-19(e1259ts), sma-2(e502), unc-32(e189), eT1(III,V), unc-93(e1500), dpy-18(e364), dpy-1(e1), yDf10, unc-79(e1068), ced-4(n1162), emb-1(hc57ts), emb-2(hc58ts), emb-7(hc66ts), emb-8(hc69ts), emb-32(g58ts).

LGIV: unc-5(e53), dpy-9(e12), nT1(IV,V).

LGV: unc-34(e566), unc-60(e677), sDf20, sDf26, sDf27, sDf28, sDf31, sDf32, sDf33, sDf34, sDf40, sDf41, sDf46, sDf48, sDf49, sDf51, sDf70, let-330(s573), let-338(s1020), unc-62(s472), lag-2(s1486), unc-46(e177), dpy-11(e224), unc-42(e270), him-5(e1467) or him-5(e1490), unc-76(e911), dpy-21(e428), unc-51(e369), rol-9(sc148), ste(q265), fog-2(q71), yDp1.

LGX: lon-2(e678), sup-7(st5), unc-9(e101), unc-3(e151), lin-15(n765), mnDf1, mnDf5, mnDf7, mnDf9, mnDf10, mnDf11, mnDf19, sup-20(n821), and *let-4(mn105).*

The following strains were used for the analysis of *lin-12(d)* suppression: JT5210 *sel-6(sa44); lin-12(n302),* JT5211 *sel-6(sa44); lin-12(n952),* JT5212 *sel-6(sa44); lin-12(n950),* JT5218 *sup-17(sa38); lin-12(n302),* JT5222 *sup-17(sa45); lin-12(n302),* JT5224 *lin-12(n950); sel-7(n1253),* JT5226 *lin-12(n950); sel-6(n1256),* JT5254 *sel-4(n1259); lin-12(n950),* JT5258 *sel-5(n1254) lin-12(n950),* JT5260 *lin-12(n950); lag-2(sa37),* JT5264 *lin-12(n302); lag-2(sa37),* JT5277 *sel-8(sa54) lin-12(n302),* JT5278 *sel-8(sa54) lin-12(n952),* JT5292 *sel-5(n1250) lin-12(n302),* JT5316 *sel-8(sa54) lin-12(n950),* MT137 *lin-12(n137),* MT2191 *sup-17(n316); lin-12(n952),* MT2756 *lin-12(n952); lag-2(n1255),* MT2757 *sup-17(n1260); lin-12(n302),* MT2763 *lin-12(n952); sel-7(n1253),* MT2776 *lin-12(n137), lag-2(n1255);* MT2782 *lin-12(n302); lag-2(n1255),* MT2898 *sup-17(n1260); lin-12(n952),* MT2982 *sel-4(n1259); lin-12(n302),* MT3000 *sel-5(n1254) lin-12(n302),* MT3011 *lin-12(n950 n1328),* MT3017 *lin-12(n302); sel-6(n1256),* MT3048 *sel-5(n1254) lin-12(n952),* MT3050 *sel-4(n1259); lin-12(n952),* MT3052 *lin-12(n952); sel-6(n1256),* MT3063 *lin-12(n950),* MT3069 *lin-12(n950 n1328); lag-2(n1255),* MT3604 *lin-12(n950); lag-2(n1255),* and MT3605 *sup-17(n1260); lin-12(n950).*

Mutant isolation and distinguishing intragenic revertants from extragenic suppressors of the *lin-12(d)* allele: The suppressor mutations *n1250, n1253, n1254, n1255, n1256, n1259,* and *n1260* were isolated after mutagenesis with EMS of MT2565 *unc-29(e1072); lin-12(n302).* Mutagenized fourth larval stage (L4) hermaphrodites were picked two per plate and allowed to self-fertilize for two generations. During this time the plates were inspected daily for eggs on the plate. Any eggs that were found were picked together to a new plate and allowed to grow to adults. If any of these animals made a vulva (non-Vul), they were confirmed as revertants, and single animals were picked to establish a revertant stock. Only one revertant was retained from each plate, to ensure independence of mutations. One *sup-17* allele, *n1258,* was isolated from a similar screen after EMS mutagenesis of MT302 *lin-12(n302).* Another *sup-17* allele, *n316,* was identified in a simi-

lar screen after EMS mutagenesis of MT177 *lin-12(n177)* animals. The mutations *sa37, sa38, sa44, sa45,* and *sa54* were isolated in a similar screen after EMS mutagenesis of MT3063 *lin-12(n950).* Since subsequent analysis showed that each of the mutations suppresses all *lin-12(d)* alleles tested, any of these mutations might in principle have been isolated in any of the screens.

No immediate attempt was made to be sure that the revertant stock was homozygous for the suppressor mutation (*lin-12(null)* mutations, a major class of revertants, are dominant suppressors, but cannot be made homozygous since they confer recessive sterility and some inviability). For each revertant stock, MT2644 *dpy-19(e1259) lin-12(n302); him-5(e1490)* males were mated with one or more non-Vul animals (which therefore must carry the suppressor mutation) and several F_1 cross progeny were picked, as recognized by the segregation of *dpy-19* animals. From an F_1 that segregated non-Vul animals, 20–50 Dpy progeny were picked to test linkage of the suppressor to *lin-12* (*dpy-19* maps ~0.2 mu from *lin-12*). If any of the Dpy progeny segregated non-Vul animals, these were picked to establish a *sup*-carrying strain with a *dpy-19*-marked *lin-12(n302)* chromosome. For the mutagenesis experiment in which *unc-29* was present in the parent, the segregation of *unc-29* was also followed to determine its possible linkage to any extragenic *sup*'s. (*sup-17* was already known at the time of this experiment to map ~0.2 mu from *unc-29.*) Many of the suppressors that mapped close to *lin-12* were kept and frozen as heterozygotes (*dpy-19(e1259) lin-12(n302)/lin-12(n302) sup*), because they were homozygous sterile or lethal. A few linked revertants were homozygous viable and were frozen as homozygotes. In later experiments, most such linked revertants were discarded.

Mapping suppressor genes: Most initial mapping was done by crossing males bearing a *dpy* mutation together with *lin-12(n302)* and *him-5(e1467)* to a *sup; lin-12(n302)* isolate. *dpy* markers from the centers of each of the five autosomes were used: LGI, *dpy-5(e61);* LGII, *dpy-10(e128);* LGIII, *dpy-19(e1259);* LGIV, *dpy-13(e184);* LGV, *dpy-11(e224).* Linkage was assessed as described for *dpy-19* above, except that in many cases non-Vul animals were also picked and the segregation of Dpy animals scored. For LGX a transmission test was used: *lin-12(n302); him-5(e1467)* males were mated with *sup; lin-12(n302)* hermaphrodites. Male cross progeny were mated with MT2646 *dpy-17(e164) lin-12(n302)/dpy-17(e164) unc-32(e189)* hermaphrodites. Cross progeny that did not segregate Unc-32 animals (genotype *lin-12(n302)/dpy-17 lin-12(n302)*) were tested for segregation of non-Vul animals. The *sup* mutation was inferred to be autosomal if approximately half of the progeny failed to segregate the suppressor.

Three-factor crosses were generally performed by balancing *lin-12(n302)* with *unc-32(e189)* and identifying *lin-12(n302)* homozygotes by failure to segregate *unc-32,* allowing the construction of animals of genotype *marker1 marker2/sup; lin-12(n302)/lin-12(n302).* For the mutations on the left arm of chromosome III, a *marker1 marker2/sup lin-12(n302)* heterozygote was constructed and *marker1 non-marker2* animals were picked, and the recombinant chromosome was made homozygous to assess the presence or absence of the suppressor mutation.

Deficiency mapping of *sel-7(n1253) X* was performed by assessing the penetrance of the suppression of the *lin-12(n302)/+* phenotype in an *n1253/Df* genetic background. N2 males were mated with hermaphrodites of genotype *mnDp1/+; Df/Df* (*mnDp1* is an attached duplication that complements all of the deficiencies used), and the male progeny of genotype *mnDp1/+; Df/0* (since the *Dfs* are on the *X* chromosome and cause recessive lethality) were mated with hermaphrodites of genotype *dpy-19 lin-12(n302); sel-7(n1253) unc-3.* The Unc non-Dpy progeny of this cross must be of the genotype *dpy-19 lin-12(n302)/+; Df/sel-7 (n1253) unc-3,* since each *Df* used either deletes *unc-3* or is linked in *cis* to an *unc-3* mutation. In each case complementation was indicated by the generation of >50% Vul animals, while failure to complement was indicated by the generation of fewer than 10% Vul animals (Table 1).

sel-8(sa54), which causes an incompletely penetrant maternal-effect lethal phenotype when in a *lin-12(+)* background, failed to complement *yDf10* for suppression of *lin-12(d)* phenotypes and for the maternal-effect lethal phenotype. Interestingly, the maternal-effect lethality appears to be suppressed by the presence of *lin-12(d)* alleles (F. TAX and J. THOMAS, unpublished data).

Determination of quantitative suppression: To quantify the suppression of the Vul phenotype in strains containing *lin-12(d)* alleles and various suppressor allele combinations, random L4 progeny from animals growing at 20° were picked to fresh plates. After 1 or 2 days the animals were checked, and if they were laying eggs, the parent was removed; if the animal had formed a bag of worms, it was left alone. This procedure reduced any bias for selecting suppressed or unsuppressed parents and produced a partially synchronized brood of progeny. All progeny from each parent were counted, thus avoiding any bias caused by differential growth rate of animals of different genotypes. When the progeny matured to become young adults, the induction of their vulval lineages was assessed by inspection with the dissecting microscope. Induction of a structurally normal vulva was taken as an indication of the presence of an anchor cell, while no vulval induction indicated the absence of an anchor cell (KIMBLE 1981; GREENWALD *et al.* 1983). Whether the parent of each counted brood was suppressed or not was not correlated with the degree of suppression of the progeny (data not shown).

Temperature-shift experiments: Egg-laying competent animals of the strain to be tested were identified and picked in groups of several animals to fresh plates at the appropriate temperature. Care was taken to pick young adults to achieve good egg-laying synchrony. After 1 hr of egg-laying at 25° or 2 hr at 15°, the parents were picked to a fresh plate. This procedure was repeated several times for each set of parents. This protocol produced a set of plates with synchronous eggs of various ages from the same parent animals. For each time point to be tested, a plate was selected at the appropriate time and animals that were in the molt were selected and transferred to another plate at the new temperature. This procedure served to reinforce synchrony among the tested animals. When the animals reached young adulthood, their degree of vulval induction was determined as described above.

Identification of lethal *sup-17* alleles: Potential null *sup-17* alleles were isolated as follows: MT2961 *unc-29(e1072); lin-12(n952); him-5(e1490)* L4 males were treated with EMS, and 10 males were mated with five young MT2953 *sup-17(n1260); lin-12(n952); unc-3(e151)* hermaphrodites per plate. The parents were transferred to fresh plates daily for 4 days. Three days after the parents were removed, the progeny on each plate were screened for non-Vul hermaphrodite cross progeny (non-Unc). Any non-Vul hermaphrodites were picked to fresh plates and their progeny were observed for the presence of more than half non-Vul animals. The presence of non-Vul *unc-29* animals confirmed the identification of a new *sup-17* allele. The *unc-3* mutation, on the *X* chromosome, was included both to mark self-progeny and to prevent cross-progeny males from mating (*unc-3* males are Unc and cannot mate). Two *sup-17* alleles (*n1305* and *n1306*) were isolated by a similar screen, except that mutagenized MT2639 *unc-29(e1072); lin-12(n302); him-5(e1490)* males were mated with

MT2908 *sup-17(n1258); unc-32(e189) lin-12(n137)* hermaphrodites, and the screen was conducted at 15°. A pilot cross of *nDf23/unc-13 lin-11* males to *sup-17(n1260)* or *sup-17(n1258)* hermaphrodites showed that *nDf23/n1260* animals at 20° and *nDf23/n1258* animals at 15° were viable and fertile (*nDf23* deletes *sup-17*).

Test for *lin-12* maternal effect: Eggs laid by *lin-12(n302 n865)/+* parents were collected. None of these eggs arrested development before hatching, and 244 non-Lin progeny developed to adulthood, indicating that ~81 *lin-12(n302 n865)* progeny should have been present. Of these *lin-12(n302 n865)* progeny, only 10 developed into adults, and most of the rest arrested development in mid to late larval stages. Four of the 10 adults were sterile, and the other six produced a total of 142 progeny. Of these 142 homozygous *lin-12(n302 n865)* progeny of a homozygous parent, none arrested development before hatching, and 23 developed to adulthood. The remainder arrested development in mid to late larval stages with no apparent differences from the progeny of heterozygous parents.

Identification of revertants of *lag-2(n1255)*: Potential intragenic revertants of the semi-dominant suppressor phenotype of *lag-2(n1255)* were identified as follows. MT2835 *lin-12(n302); lag-2(n1255) unc-46(e177)* hermaphrodites were mutagenized with EMS, and two L4 hermaphrodites were picked per plate. The parents were transferred to fresh plates daily for 3 days. Two or three days after removal of the parents, the progeny were screened visually for the absence of a vulva. Since *lag-2(n1255)* is ~98% penetrant as a homozygous suppressor of *lin-12(n302)*, several hundred candidates were picked. Each candidate was picked to a fresh plate, and their progeny were screened for those that segregated a substantial fraction of Vul progeny. Three candidates passed this progeny test, and of these two contained newly induced mutations that were linked to *unc-46* and subsequently shown to be intragenic *lag-2* revertants. The third was unlinked and presumably carried a dominant Vul mutation.

Linkage of *lag-2* mutations and allelism of putative intragenic *lag-2* revertants: Although *n1255* and *sa37* map to the same region and cause similar phenotypes, they are both semi-dominant, precluding a conclusive complementation test between them. We assessed whether they were tightly linked by examining the progeny of *n302/n302; n1255/sa37* animals. Of ~1500 total progeny, 76 were Vul and were picked individually to test whether they might be recombinant between *n1255* and *sa37* (*sa37/+* or *n1255/+*). The progeny of all 76 animals picked were at least 90% suppressed (nonVul), which suggests these animals were of genotype *sa37/sa37, n1255/n1255,* or *sa37/n1255,* and not *n1255/+* or *sa37/+*, which are able to lay eggs ~30% of the time (Table 2). Thus, no recombinants were detected and we estimate an upper limit of 0.2% for the recombination frequency between *sa37* and *n1255*.

To determine the linkage of the putative intragenic revertant *n1323* and *lag-2(n1255)* we used the following strategy. MT1329 *lin-12(n302); him-5(e1467)* males were mated with MT2874 *lin-12(n302)/eT1(III); lag-2(n1255) n1323 unc-46(e177)/eT1(V)* hermaphrodites. We picked many progeny and identified cross-progeny that did not segregate *eT1*. These animals were of genotype *lin-12(n302)/lin-12(n302); lag-2(n1255) n1323 unc-46(e177)/+*. Since *n1255* alone causes a 30% penetrant semi-dominant suppression of *lin-12(n302)*, recombinants that carried *n1255* without *n1323* in *cis* (recombinants between the two mutations) would have a non-Vul phenotype ~30% of the time (see Table 6). We screened the progeny of 23 parents of the above genotype for non-Vul animals over the following two generations. Animals of this genotype produced an average brood of 35 viable progeny. Based on this brood size and the penetrance of expected recombinants, we calculated that this method allowed the examination of ~160 potential recombinants per plate. Of the 23 plates screened, five non-Vul potential recombinants were found and none bred true (this was close to the expected background caused by the incomplete penetrance of the *lin-12(n302)* mutation). Therefore, we did not obtain any recombination events between *n1255* and *n1323* in ~3600 chromosomes. This result placed the two mutations within ~0.05 map units of each other. Since the *lag-2* region of the genome has a low gene density per map unit (EDGLEY and RIDDLE 1987), this small distance suggests that the two mutations map to the same gene.

Determination of amber suppressibility of *lag-2* and *sup-17* mutations: Males of genotype *+/eT1(III); lag-2(n1255 mut) unc-46(e177)/eT1*(V) (where *mut* is either *n1322* or *n1323*) were mated with MT2989 *eT1(III)/eT1(III); eT1(V)/eT(V); sup-7(st5) dpy-7(e1324)* hermaphrodites. Non-Unc hermaphrodite progeny (*eT1* homozygotes are Unc) were picked. These animals were of genotype *+/eT1(III); lag-2(n1255 mut) unc-46(e177)/eT1(V); sup-7(st5) dpy-7(e1324)/+*. Their progeny were tested both for the presence of viable *unc-46* animals (the Unc-46 phenotype is readily distinguished from the *eT1* Unc) and for the absence of the distinctive rod-like arrested L1 *lag-2(n1255 mut)* homozygotes. Both *n1322* and *n1323* produced no Unc-46 progeny and produced the expected numbers of rod-like arrested L1 animals. Therefore *sup-7(st5)* in either one or two copies does not suppress *n1322* or *n1323*. For *sup-17* mutations, each of the lethal alleles and each of the viable alleles with mutant phenotypes when not in a *lin-12(d)* strain were tested. For the lethal alleles, hermaphrodites of genotype *sup-17(mut) unc-29/+; sup-7(st5) dpy-7(e1324)/+* were constructed, and their *unc-29* progeny were observed for suppression of the phenotypes caused by the *sup-17* alleles.

Cell lineage analysis: The P(3–8).p cell lineages of seven *sup-17(n1258)* animals were determined as described (SULSTON and HORVITZ 1977). Parents were grown at 15° and allowed to lay eggs. The eggs were incubated at 15° for ~16 hr when they are in the late stages of embryogenesis, then were shifted to the nonpermissive temperature of 25°. The animals were mounted for Nomarski observation as early L3 larvae and, except for frequent brief periods of observation at 20°, were kept at 25° throughout the lineage analysis. Each animal produced a single anchor cell.

Analysis of other cell fates: The fates of other cell types were determined based on the observations of anatomy made with a dissecting microscope and with Nomarski microscopy; cell lineages were not determined. With the exception of *sup-17(n1258)* animals (see above), the cell fates of P(3–8).p were determined less rigorously on the basis of the presence of a normal vulva and ectopic ventral protrusions. In all cases, the ectopic protrusions were small and appeared characteristic of those associated with the 2° cell fate (GREENWALD *et al.* 1983). The *Y* cell in the male was distinguished from DA9 by the presence of a larger nucleus with a small visible nucleolus in the appropriate position (SULSTON *et al.* 1980; GREENWALD *et al.* 1983). The fates of the SM cells and coelomocytes were inferred by scoring L4 larvae for the presence of ectopic coelomocytes in the posterior dorsal body region (GREENWALD *et al.* 1983). The presence of a linker cell was scored in the L4 male by observing the morphology of the gonad and of the cell at the leading tip of the gonad (SULSTON *et al.* 1980). The fates of P(9–11).p were inferred from the presence or absence of ectopic hook structures just anterior to the normal hook as observed by Nomarski microscopy of young adult males (GREENWALD *et al.* 1983). The presence of an AC was

inferred by the presence of a normal vulva (KIMBLE 1981), since an anchor cell induces a normal vulva in both *lin-12(+)* and *lin-12(d)* animals (GREENWALD *et al.* 1983).

Isolation and genetic analysis of *lin-12(n950 n1328)*: The mutation *n1328* was identified as a revertant of *lin-12(n950)* by M. HERMAN (personal communication). Subsequent analysis indicated that *n1328* is probably an intragenic revertant in *lin-12* because it is a *cis*-dominant suppressor of certain *lin-12* phenotypes (see RESULTS) and because it maps very close to *lin-12*. Specifically, from *n950 n1328/+ +* parents, 36/36 progeny that were homozygous for *n950* were also homozygous for *n1328*. In addition, from *dpy-19 unc-69/n950 n1328* heterozygotes, among 19 Unc non-Dpy recombinants and 17 Dpy non-Unc recombinants, none segregated *n950* without the Vul suppressor activity characteristic of *n1328*.

Genetic mosaic analysis: The parental strain used for mosaic analysis of *sel-5* was *sel-5(n1254) ncl-1(e1865) unc-36(e251) lin-12(n302); sDp3*, and this strain was grown at 20°. *sDp3* carries wild-type copies of each of these genes except *lin-12* (AUSTIN and KIMBLE 1987). Non-Unc late L3 animals were mounted for observation using Nomarski optics. Animals were screened for vulval induction as judged by observation of the cell lineage progeny of P5.p, P6.p, and P7.p. In such induced animals the Ncl phenotypes of the following cells were recorded: ASKL, ASKR, ADLL, ADLR, ASIL, ASIR, ASHL, ASHR, AWCL, AWCR, I3, M4, both distal tip cells, the anchor cell, left and right coelomocytes, PVR, DVC, the three anterior-most pairs of dorsal body-wall muscle cells on each side (derived from C), and the dorsal body wall muscle cells on each side near the posterior bulb of the pharynx (derived from D). The presence of *sDp3* was assessed in the germ line by recovering the mosaic animals and observing the classes of progeny they generated. Vulval induction was confirmed by the capacity of animals to lay eggs as adults. From the known lineal origins of each of these cells the position of the duplication loss could be assigned fairly accurately. Many losses later in the lineage could not be assigned to an exact cell division, but assignment was sufficiently accurate to allow interpretation of these experiments. A total of 16 mosaic animals with losses in the precursor cells to the anchor cell were analyzed. All 16 could be interpreted as simple single-loss events, with losses in the positions shown in Figure 4.

Construction of suppressor strains with *glp-1* alleles: The two *glp-1* alleles tested were *glp-1(e2141)* and *glp-1(e2142)* (PRIESS *et al.* 1987). Males heterozygous for one of the *glp-1* alleles were crossed with hermaphrodites containing one or more markers close to or flanking the suppressor mutation (to act as a balancer chromosome). The resulting males were crossed with hermaphrodites homozygous for the suppressor. Cross progeny were confirmed by the segregation of the visible markers and the *glp-1* mutation. Animals homozygous for the suppressor were identified in the next generation by the absence of the balancer markers. For unlinked suppressor mutations the following markers were used: *lag-2, unc-34(e566) unc-60(m35); sup-17, unc-29(e1072); sel-4, sqt-2(sc3); sel-6, dpy-21(e428) unc-51(e369); sel-7, unc-3(e151)*. For *sel-8(sa54)*, which is linked to *glp-1*, the following heterozygote was constructed: *sel-8(sa54) lin-12(n302)/dpy-17(e164) unc-32(e189) glp-1(e2142)*, then Unc non-Dpy recombinants were picked. Almost all should have been heterozygous for *sel-8(sa54)*, and homozygous progeny of genotype *sel-8(sa54) dpy-17(e164) glp-1(e2142)* could be obtained but were sterile. *sel-8(sa54)*, when crossed out of the *lin-12(d)* background, caused a cold-sensitive maternal-effect lethality (F. TAX and J. THOMAS, unpublished results). The terminal phenotype of *sel-8(sa54)* embryos has not been characterized further.

Analysis of strains for enhancement of *glp-1* phenotypes: Strains were grown at 15° (the permissive temperature for both *glp-1* alleles). L3 animals were picked to individual plates and transferred to fresh plates for several days at 20° or 25° if they were fertile and could lay eggs (most strains were fertile). The progeny were allowed to develop for a day, and the animals on each plate were scored for abnormal phenotypes using a dissecting microscope. The number of sterile hermaphrodites varied widely from plate to plate. The strains analyzed were as follows: JT37 *lag-2(sa37)*, JT5243 *glp-1(e2141) unc-16(e109)*, JT5274 *sup-17(n316); glp-1(e2141) unc-16(e109)*, JT5356 *glp-1(e2141) unc-16(e109); sel-6(sa44)*, JT5357 *sup-17(n1258)/+p-1(e2141) unc-16(e109)*, JT5358 *glp-1(e2141) unc-16(e109); lag-2(n1255)*, JT5359 *glp-1(e2141) unc-16(e109); lag-2(sa37)*, JT5413 *sel-6(sa44)*, JT5423 *glp-1(e2141) unc-16(e109); sel-6(n1256)*, JT5446 *glp-1(e2141) unc-16(e109); sel-7(n1253)*, JT5481 *sel-7(n1253)*, JT5482 *sel-4(n1259)*, JT5483 *sel-6(n1256)*, JT5521 *sel-4(n1259)/+p-1(e2141) unc-16(e109)*, MT316 *sup-17(n316)*, MT2769 *sup-17(n1258)*, MT2964 *lag-2(n1255)*, and N2.

RESULTS

Identification of suppressors of *lin-12(d)*: In hermaphrodites carrying *lin-12(d)* mutations the presumptive anchor cell (AC) is transformed to a ventral uterine precursor cell (VU) (GREENWALD *et al.* 1983). Since the anchor cell is required for the induction of vulval development (KIMBLE 1981), *lin-12(d)* animals fail to induce a vulva, and hence they are vulvaless (Vul) and cannot lay eggs. Animals mutant for a subset of *lin-12(d)* alleles have bumps known as ectopic pseudovulvae along their ventral sides (called Muv for Multivulvae, GREENWALD *et al.* 1983). *lin-12(d)* Vul hermaphrodites are fertile, because they produce eggs that develop and hatch within the parent's body and eventually escape. On the surface of a Petri plate containing *lin-12(d)* animals there are no eggs, since the eggs all develop inside the parent animal. It is therefore simple to identify revertants of the *lin-12(d)* phenotype by searching for eggs on a plate. Previous studies had shown that loss-of-function *lin-12* alleles and extragenic suppressors could be identified by reverting *lin-12(d)* alleles (GREENWALD *et al.* 1983; FERGUSON and HORVITZ 1985).

Animals homozygous for the *lin-12(d)* alleles *n302, n950,* or *n177* were mutagenized with EMS (BRENNER 1974). Plates with the mutagenized animals were screened for the presence of laid eggs for two generations, to allow possible recessive suppressors to become homozygous. Only a single egg-laying competent revertant was kept from each plate to ensure independence of suppressor mutations. In total, ~150,000 mutagenized genomes were screened, and 123 egg-laying competent revertants were identified. Linkage analysis (see MATERIALS AND METHODS) indicated that 109 of these revertants were tightly linked to the *lin-12* locus. Most or all of the tightly linked revertants are likely to be *lin-12* alleles, since most share the phenotypic characteristics caused by *lin-12* reduction-of-function mutations. However, many of these linked revertants

were not tested for failure to complement a *lin-12* loss-of-function mutation, so they might include extragenic suppressors in genes closely linked to *lin-12*. The tightly linked revertants were not further analyzed. The isolation and mapping of these extragenic suppressor mutations is described in greater detail in MATERIALS AND METHODS.

The 14 extragenic suppressor mutations were mapped to seven genes located on five chromosomes (Figure 1; map data, Table 1), named *sup-17, lag-2, sel-4, sel-5, sel-6, sel-7,* and *sel-8* (*sel* for suppressor/enhancer of *lin-12*, see SUNDARAM and GREENWALD 1993). All of the suppressor mutations except for *sa37* and *n1255* were recessive or nearly recessive for their suppression of *lin-12(d)* (Table 2). Mapping and complementation tests (Tables 1 and 2) indicated that these screens identified one allele each of *sel-4, sel-7* and *sel-8;* two alleles of *sel-3, sel-5,* and *sel-6;* and five alleles of *sup-17*. We assigned both *sa37* and *n1255* to the *lag-2* gene for reasons described below. The other six genes were tentatively identified as new loci. Each suppressor gene was mapped accurately (Table 1 and Figure 1), in most cases with respect to the closest known adjacent genes. The genetic region in each case was inspected for previously identified genes that might reasonably be expected to correspond to our *lin-12(d)* suppressor genes (for example, any *sup* gene, *lin* gene, or *let* gene). Candidates were tested for complementation of the *lin-12* suppressor phenotype. Table 3 summarizes our findings.

Mechanisms of suppression: Induction of a functional vulva requires an anchor cell (KIMBLE 1981; STERNBERG and HORVITZ 1986). It therefore seemed likely that suppression of the egg-laying defects caused by *lin-12(d)* alleles would involve the restoration of an anchor cell and the development of a normal vulva. However, in principle reversion could be caused by other mechanisms, such as the induction of vulval development by a different cell or vulval development independent of induction. For a representative mutation in each suppressor gene, we used Nomarski microscopy to confirm by direct observation that the anchor cell was restored. In each case, a single anchor cell that appeared morphologically normal was found in some animals of the suppressed strain. We presume that this anchor cell is formed by Z1.ppp or Z4.aaa, as it is normally, but we have not directly observed cell lineages to confirm this hypothesis.

At least one mutation in each of the seven suppressor genes was constructed in combination with at least three *lin-12(d)* alleles, to test for allele specificity of suppression (Table 4). Different *lin-12(d)* mutations cause differing degrees of increased *lin-12* gene activity (GREENWALD *et al.* 1983). *lin-12(d)* mutations can be arranged in an allelic series on the basis of phenotypic strength, in which *n302* is one of the weaker alleles, *n950* is one of the stronger alleles, and *n952* is intermediate in strength (see first three lines, Table 4 and GREENWALD *et al.* 1983). We constructed strains that carried a representative allele of each of the suppressor genes in combination with the three *lin-12(d)* alleles *n302, n952,* and *n950*. For each genotype we determined the fraction of hermaphrodites that generated a normal vulva, indicative of suppression of the *lin-12* defect (Table 4). For each suppressor gene we observed a correlation between the degree of suppression and the strength of the *lin-12(d)* allele used. The degree of suppression decreased as the *lin-12* allele became stronger, or in a few cases did not significantly change. Some degree of suppression was observed in all cases. These results indicate that none of the suppressor mutations tested displayed clear allele specificity for *lin-12(d)* mutations. For *lag-2(n1255)* and the *sup-17* alleles *n316, n1260,* and *n1258,* combinations with other *lin-12(d)* alleles were constructed (including at least one suppressor allele from each gene with *n137, n177,* and *n769*), and these combinations also all showed suppression to an extent expected from the strengths of the *lin-12* alleles (data not shown).

***lin-12(d)* extragenic suppressor mutations are rare:** In the search for extragenic suppressor mutations, we isolated 109 probable intragenic *lin-12* revertants, about one per 1500 mutagenized genomes screened. This frequency of *lin-12* mutations is close to that expected for null mutations in most *C. elegans* genes (about one per 2000 mutagenized genomes, BRENNER 1974) and similar to the frequency observed previously for *lin-12* (about one per 1500 mutagenized genomes, GREENWALD *et al.* 1983). However, in the same screen we isolated a total of only 14 extragenic suppressor mutations, which defined seven different genes. Thus, for each of the identified suppressor genes, mutations causing suppression of *lin-12(d)* arise at an average frequency of one per 75,000 mutagenized genomes, some 30-fold rarer than expected for typical null mutations. We conclude that the suppressor mutations in each of these genes are unlikely to be null mutations. There are several possible explanations for this low frequency, including the following: (1) null alleles do not suppress, (2) null alleles suppress but also confer lethality, or (3) the suppressor genes are small mutagenic targets. We have determined the cause of the low frequency of suppressor mutations for two of the genes, *sup-17* and *lag-2* (see below). For *sup-17,* we found that null mutations are suppressors of the *lin-12(d)* phenotype, but they are also lethal. For *lag-2* we found that null mutations cause lethality and are not suppressors, and that the *lag-2* suppressor mutations are rare altered-function mutations.

Further analysis of *sup-17*: Like suppressor mutations in several other genes that we identified, *sup-17* suppressor mutations displayed a very weak semi-dominance in

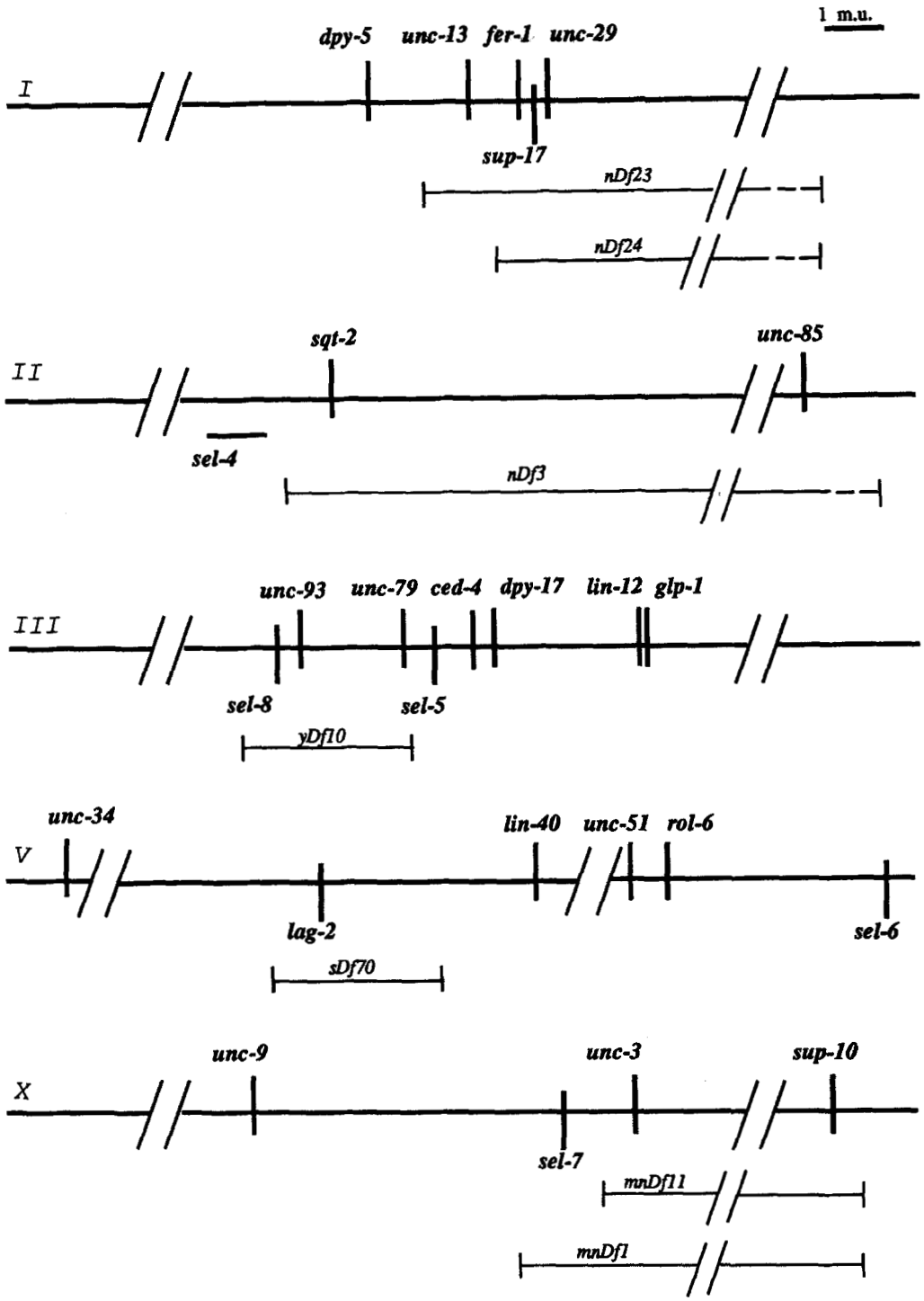

FIGURE 1.—Map positions of the seven suppressor genes. Only a few nearby genes are shown. In the region around the suppressor loci the nearest known genes are indicated.

suppressing *lin-12(d)* (Tables 2 and 5). Two possible explanations for this weak semi-dominance are that the suppressor mutations produce an altered gene product that acts dominantly or that loss of one functional copy of the gene causes suppression because of reduced gene dosage. Since genetic deficiencies exist that delete the *sup-17* locus, we were able to test the cause of this semi-dominance. First, two *lin-12* genotypes were identified that displayed the weak semi-dominance clearly. Each genotype was heterozygous for a weak *lin-12(d)* mutation (*n676* or *n302*) and a wild-type *lin-12* gene (Table 5). These heterozygotes were only weakly transformed for the AC/VU cell fates and thus were very sensitive to suppression. The effect of two deficiencies (*nDf23*

TABLE 1

Mapping and complementation data for extragenic suppressor loci

Gene	Heterozygote	Results
sup-17	*dpy-5 unc-75/sup-17(n316)*	5/17 Dpy non-Unc segregated *n316*
	sup-17(n316) unc-75/lin-11	0/5 Unc non-Sup segregated *lin-11*
	dpy-5 unc-29/sup-17(n316)	9/9 Dpy non-Unc segregated *n316*
		2/12 Unc non-Dpy segregated *n316*
	fer-1 unc-29/sup-17(n316)	6/9 Unc non-Fer segregated *n316*
	fer-1 unc-29/sup-17(n1258)	4/13 Unc non-Fer segregated *n1258*
	sup-17(n1258)/nDf23	nDf23 failed to complement *n1258*
	sup-17(n1258)/nDf24	nDf24 failed to complement *n1258*
	sup-17(n1258)/nDf29	nDf29 complemented *n1258*
	sup-17(n1258)/nDf30	nDf30 complemented *n1258*
lag-2	*lin-12(n302); dpy-11/lag-2(n1255)*	15/53 *dpy-11* segregated *n1255*
	lin-12(n302); dpy-11 him-5/lag-2(n1255)	1/10 Dpy non-Him segregated *n1255*
	lin-12(n302); unc-60/lag-2(n1255)	5/30 *n1255* segregated *unc-60*
	lin-12(n302); unc-34 lag-2(n1255) dpy-11/+	12/24 Dpy non-Unc were *n1255* homozygotes
		9/19 Unc non-Dpy were *n1255* homozygotes
	lin-12(n302); dpy-11 unc-60/lag-2(n1255)	6/10 Dpy non-Unc segregated *n1255*
	unc-46 sDf27/unc-46 lag-2(n1255 n1323)	*sDf27* failed to complement *n1323* lethality
	unc-46 sDf28/unc-46 lag-2(n1255 n1323)	*sDf28* failed to complement *n1323* lethality
	unc-46 sDf20/unc-46 lag-2(n1255 n1323)	*sDf20* complemented *n1323* lethality
	unc-46 sDf31/unc-46 lag-2(n1255 n1323)	*sDf31* failed to complement *n1323* lethality
	unc-46 sDf34/unc-46 lag-2(n1255 n1323)	*sDf34* failed to complement *n1323* lethality
	unc-46 s1486/unc-46 lag-2(n1255 n1323)	*s1486* failed to complement *n1323* lethality
	unc-46 sDf48/unc-46 lag-2(n1255n1323)	*sDf48* failed to complement *n1323* lethality
	unc-46 sDf49/unc-46 lag-2(n1255n1323)	*sDf49* failed to complement *n1323* lethality
	unc-46 sDf50/unc-46 lag-2(n1255n1323)	*sDf50* failed to complement *n1323* lethality
	unc-46 sDf51/unc-46 lag-2(n1255n1323)	*sDf51* failed to complement *n1323* lethality
	unc-46 sDf70/unc-46 lag-2(n1255n1323)	*sDf70* failed to complement *n1323* lethality
	lin-12(n302); lag-2(n1255 n1323)/+	0/3600 progeny segregated *n1255*
	lin12(n302); dpy-11/lag-2(sa37)	12/44 *dpy-11* segregated *sa37*
sel-4	*lin-12(n302); sqt-2/sel-4(n1259)*	1/26 *sqt-2* segregated *n1259*
		155/155 *sqt-2/sqt*(+) segregated *n1259*
		0/6 Sel segregated *sqt-2*
	n302/+; sel-4(n1259)/nDf3	53/62 non-Vul (complemented)
sel-5	*dpy-17 unc-32/sel-5(n1250) lin-12(n302)*	0/5 Dpy non-Unc segregated *n1250*
	sel-5(n1250) lin-12(n302)/unc-32 dpy-18	6/6 Dpy non-Unc segregated *n1250*
	sel-5(n1254) lin-12(n302)/sel-5(n1250) lin-12(n302)	67/67 progeny carried a suppressor
	unc-93 dpy-17/sel-5(n1254) lin-12(n302)	9/10 Unc non-Dpy segregated *n1254*
	unc-79 ced-4 dpy-17/sel-5(n1250) lin-12(n302)	6/11 Unc non-Dpy segregated *n1250* and no *ced-4*
		4/11 Unc non-Dpy segregated no *n1250* and no *ced-4*
		2/11 Unc non-Dpy segregated *n1250* and *ced-4*
sel-6	*lin-12(n302); dpy-11/sel-6(n1256)*	4/21 *dpy-11* segregated *n1256*
	lin-12(n302); dpy-21/sel-6(n1256)	4/19 *dpy-21* segregated *n1256*
		3/43 *sel-6* segregated *dpy-21*
	lin-12(n302); unc-51/sel-6(n1256)	2/40 *sel-6* segregated *unc-51*
	lin-12(n302); dpy-11 unc-42/sel-6(n1256)	3/4 Dpy non-Unc segregated *n1256*
		1/4 Unc non-Dpy segregated *n1256*
	lin-12(n302); unc-51 fog-2/sel-6(n1256)	4/20 Sel segregated Unc Fog
	lin-12(n302); dpy-21 unc-51/sel-6(n1256)	24/32 Sel segregated no Unc and no Dpy
		4/32 Sel segregated Unc and Dpy
		4/32 Sel segregated Dpy and no Unc
	lin-12(n302); rol-9/unc-51 sel-6(sa44)	6 non-Vul non-Unc segregated *rol-9*
sel-7	*lin-12(n302); lon-2/sel-7(n1253)*	10/23 Lon segregated *n1253*
	lin-12(n302); unc-9/sel-7(n1253)	2/21 Unc segregated *n1253*
		4/11 Sel segregated *unc-9*
	lin-12(n302); lon-2 unc-9/sel-7(n1253)	0/8 Unc non-Lon segregated *n1253*
		5/5 Lon non-Unc segregated *n1253*
	lin-12(n302); unc-3/sel-7(n1253)	2/105 *unc-3* segregated *n1253*
	lin-12(n302)/+; sel-7(n1253)/+	58/84 non-Vul
	lin-12(n302)/+; sel-7(n1253)/mnDf1	52/57 non-Vul (failed to complement)

TABLE 1

Continued

Gene	Heterozygote	Results
	lin-12(n302)/+; sel-7(n1253)/mnDf5	29/66 non-Vul (complemented)
	lin-12(n302)/+; sel-7(n1253)/mnDf7	47/69 non-Vul (complemented)
	lin-12(n302)/+; sel-7(n1253)/mnDf9	55/86 non-Vul (complemented)
	lin-12(n302)/+; sel-7(n1253)/mnDf10	48/54 non-Vul (failed to complement)
	lin-12(n302)/+; sel-7(n1253)/mnDf11	39/98 non-Vul (complemented)
	lin-12(n302)/+; sel-7(n1253)/mnDf19	82/131 non-Vul (complemented)
	lin-12(n302)/+; sel-7(n1253)/sup-20	37/62 non-Vul (complemented)
	lin-12(n302)/+; sel-7(n1253)/let-4	63/98 non-Vul (complemented)
sel-8	*dpy 19 lin-12(n302)/sel-8(sa54) lin-12(n302)*	6/42 *dpy-19* segregated *sa54*
	unc-32 dpy-18/sel-8(sa54) lin-12(n302)	12/12 Dpy non-Unc segregated *lin-12* and *sa54*
	dpy-1 unc-93/sel-8(sa54) lin-12(n302)	57/58 Dpy non-Unc segregated *sa54*
	unc-79 dpy-17/sel-8(sa54) lin-12(n302)	0/10 Unc non-Dpy segregated *sa54*
	unc-93 dpy-17/sel-8(sa54) lin-12(n302)	0/17 Unc non-Dpy segregated *sa54*
	dpy-1 unc-79/sel-8(sa54) unc-32 lin-12(n302)	1/26 Unc non-Dpy segregated *sa54* 30/31 Dpy non-Unc segregated *sa54*
	sel-8(sa54) lin-12(n302)/unc-32 yDf10	73/73 non-Vul (failed to complement)
	sel-8(sa54) unc-32/yDf10 unc-32	*sa54* failed to complement *yDf10* for maternal-effect lethality
	sel-8(sa54) unc-32/emb-1(hc57ts)	*sa54* complemented *emb-1(hc57ts)* for maternal-effect lethality
	sel-8(sa54) unc-32/emb-2(hc58ts)	*sa54* complemented *emb-2(hc58ts)* for maternal-effect lethality
	sel-8(sa54) unc-32/emb-7(hc66ts)	*sa54* complemented *emb-7(hc66ts)* for maternal-effect lethality
	sel-8(sa54) unc-32/emb-8(hc69s)	*sa54* complemented *emb-8(hc69ts)* for maternal-effect lethality
	sel-8(sa54) unc-32/emb-32(g58ts)	*sa54* complemented *emb-32(g58ts)* for maternal-effect lethality

and *nDf25*) that delete *sup-17* were tested, and both were found to produce a weak semi-dominant suppression of *lin-12* that was not significantly different from that caused by the *sup-17* suppressor mutations *n316* and *n1258* (Table 5). Both deficiencies delete genes to the right and to the left of *sup-17* (EDGLEY and RIDDLE 1987) and are therefore presumed to lack the *sup-17* gene entirely. We conclude that the weak semi-dominance of *sup-17* mutations is caused by reduced gene activity.

Although *sup-17* suppressor mutations arose at a higher frequency than did mutations at the other suppressor loci, their frequency was still well below that expected for null mutations. To isolate putative *sup-17* null mutations, we performed a non-complementation screen for new *sup-17* mutations. *unc-29*, which maps ~0.2 map units to the right of *sup-17*, was used to mark the new mutations in *cis*. To determine whether it was possible to isolate null mutations of *sup-17* in our screen, we performed a pilot cross using a genetic deficiency of *sup-17*. *nDf23/+* males were mated with marked *sup-17(n1260); lin-12(n952)* hermaphrodites. As expected, about half of the cross progeny were suppressed for the *lin-12(d)* Vul phenotype, indicating that *nDf23/sup-17(n1260)* animals were suppressed. These *nDf23/sup-17(n1260)* animals were picked and produced about 3/4 viable progeny, indicating that *nDf23/sup-17(n1260)* animals were fully viable even when segregated from parents of the same genotype. We conclude that, since a genetic deficiency of *sup-17* could be recovered from this cross, *sup-17(null)* point mutations could also be isolated in the screen described next.

The non-complementation screen was performed with EMS as the mutagen. From an estimated 20,000 mutagenized genomes screened, we isolated 10 *sup-17* mutations. Of these 10, four could be maintained as viable homozygotes (*n1305ts, n1313, n1314, n1317*), and the remaining six caused lethality (*n1306, n1315, n1316, n1318, n1319am*, and *n1320*). The viable alleles appeared similar to *n1260* both in strength of the *lin-12(d)* suppressor phenotype and in conferring a weak dumpy (Dpy) and a protruding vulva phenotype; alleles of this sort clearly could be isolated in the *lin-12(d)* suppressor screens described earlier. *n1305* also displayed a temperature sensitivity for suppression similar to that of *n1260*, and it is possible that it is *n1260* itself, recovered from an *unc-29* recombinant. The six suppressors that caused lethality might do so either because of the loss of *sup-17* gene function or because of a linked lethal mutation. Several experiments indicate that the lethality is caused by *sup-17*. First, each lethal mutation mapped very close to *unc-29*: each suppressor mutation was induced on a chromosome bearing an *unc-29* mutation, and few or no Unc non-Let recombinants were observed in subsequent crosses. Second, the lethal phenotype of each of the six mutations was distinctive and similar for all the alleles (see below). Third, the lethality of *n1258* and *n1306* failed to complement. We conclude that the phenotype conferred by *sup-17* putative null mutations is lethal. For convenience, we will refer to

TABLE 2

Recessiveness and complementation tests

Suppressor genotype	% suppressed (non-Egl)	Number
+/+	0	Many
sup-17(n316)/+	1	104
sup-17(n1260)/+	4	621
sup-17(sa38)/+	1	108
sup-17(sa45)/+	3	153
sup-17(n316)/sup-17(n1260)[a]	46	35
sup-17(n1260)/sup-17(sa38)	68	105
sup-17(n1260)/sup-17(sa45)	73	103
lag-2(n1255sd)/+[b]	50	380
lag-2(sa37sd)/+[b]	27	109
lag-2(sa37)/lag-2(n1255)	98	115
sel-4(n1259)/+	1	77
sel-5(n1254)/+	1	106
sel-5(n1250)/+	3	147
sel-5(n1254)/sel-5(n1250)	27	210
sel-6(n1256)/+	6	245
sel-6(sa44)/+	10	118
sel-6(n1256)/sel-6(sa44)	82	131
sel-7(n1253)/+	4	100
sel-8(sa54)/+	2	108

All tests were at 20° and were performed in the background of a homozygous *lin-12(n302)* mutation. Data for strains homozygous for these suppressors can be found in Table 4.

[a] *sup-17(n1260)* animals at 25°, *sup-17(n1258)* animals at 20°, and *sup-17(n316)* animals at all temperatures also displayed fully recessive, slightly Dpy, Egl, and pVul phenotypes themselves (independent of the *lin-12* genotype), and all pairwise combinations between them failed to complement for these phenotypes.

[b] From TAX *et al.* (1994), plus additional data.

the six lethal mutations collectively as *sup-17(0)*. All 13 *sup-17* mutations, including the seven viable ones, were tested for suppressibility by the amber suppressor *sup-7(st5)* (see MATERIALS AND METHODS). The lethal alleles were assessed for suppression of lethality, while the viable alleles were assessed for suppression of their Dpy and protruding vulva phenotypes. Only one mutation, *sup-17(n1319)*, was found to be suppressed. *n1319* is one of the lethal alleles of *sup-17*, and *sup-17(n1319); sup-7(st5)/+* animals were viable. This finding supports our identification of the lethal alleles as putative nulls for *sup-17*.

The *sup-17* lethal phenotype showed partial maternal rescue: *sup-17(0)/sup-17(0)* progeny of *sup-17(0)/+* hermaphrodites were usually viable and fertile, but the *sup-17(0)/sup-17(0)* progeny of *sup-17(0)/sup-17(0)* mothers arrested development during late embryogenesis and do not hatch. The *sup-17(0)* mutations also caused several zygotic abnormalities: *sup-17(0)* homozygotes segregating from a heterozygous hermaphrodite were suppressed for *lin-12(d)* phenotypes, slightly Dpy and uncoordinated (Unc), usually vulvaless (Vul, see below), slow-growing, and some failed to reach adulthood. If wild-type males were mated with these *sup-17* homozygotes, some viable progeny were produced, indicating some zygotic rescue of the *sup-17(0)* phenotype.

The *sup-17(0)* animals arrested development during late embryogenesis, a phenotype more severe than that conferred by any *lin-12* mutation (*lin-12(d)* animals are viable and fertile, while *lin-12(null)* animals range from late larval lethal to sterile adults, GREENWALD *et al.* 1983). Thus, it seems likely that *sup-17*, in addition to interacting with *lin-12*, has an additional function during embryogenesis that does not involve *lin-12*. However, this conclusion is not compelling since *lin-12(0)* results in zygotic sterility, thus precluding tests for a

TABLE 3

Summary of extragenic suppressor genes

Gene	Phenotype	No. of alleles isolated as suppressors	No. of other alleles
sup-17 I	Nearly recessive suppression caused by loss of function. Probable null phenotype is embryonic lethal.	5	10[a]
lag-2 V	Semi-dominant suppression caused by gain of function. Probable null phenotype recessive L1 lethal	2	9[b]
sel-4 II	Nearly recessive suppression, wild-type alone	1	0
sel-5 III	Nearly recessive suppression, wild-type alone, cold-sensitive alleles	2	0
sel-6 V	Nearly recessive suppression, wild-type alone	2	0
sel-7 X	Nearly recessive suppression, wild-type alone	1	0
sel-8 III	Nearly recessive suppression, maternal-effect lethal alone (cs)	1	0

[a] Includes six maternal-effect lethal and four viable suppressor alleles isolated in noncomplementation screens (see text).

[b] Two of the alleles are recessive lethal mutations made by isolating intragenic revertants of the *n1255* suppressor allele (see text), one is from a screen for lethal mutations in the region (JOHNSEN and BAILLIE 1991), and six are from LAMBIE and KIMBLE (1991a). The latter seven alleles do not carry the suppressor mutation in the background.

TABLE 4

Quantitation of suppression of three *lin-12(d)* alleles for AC/VU

Suppressor mutation	*lin-12(d)* genotype	% not transformed (non-Egl)	No. counted
+	*n302*	0	Many
+	*n952*	0	Many
+	*n950*	0	Many
+	*n302/lin-12(0)*[a]	78	419
+	*n952/lin-12(0)*[a]	39	496
+	*n950/lin-12(0)*[a]	28	397
sup-17(n1260)	*n302*	52	353
sup-17(n1260)	*n952*	46	99
sup-17(n1260)	*n950*	3	189
lag-2(n1255)	*n302*	94	189
lag-2(n1255)	*n952*	76	292
lag-2(n1255)	*n950*	12	156
sel-4(n1259)	*n302*	31	286
sel-4(n1259)	*n952*	8	206
sel-4(n1259)	*n950*	7	271
sel-5(n1254)	*n302*	31	203
sel-5(n1254)	*n952*	46	165
sel-5(n1254)	*n950*	<1	219
sel-6(n1256)	*n302*	61	168
sel-6(n1256)	*n952*	28	279
sel-6(n1256)	*n950*	15	188
sel-6(sa44)	*n302*	90	281
sel-6(sa44)	*n952*	46	383
sel-6(sa44)	*n950*	33	270
sel-7(n1253)	*n302*	53	162
sel-7(n1253)	*n952*	49	128
sel-7(n1253)	*n950*	8	226
sel-8(sa54)	*n302*	25	238
sel-8(sa54)	*n952*	17	431
sel-8(sa54)	*n950*	2	263

All numbers are from animals grown at 20°. The first three lines are reprinted with permission from GREENWALD *et al.* (1983).

[a] The *lin-12(0)* allele analyzed was *lin-12(n137 n720)*.

TABLE 5

The weak semi-dominance of *sup-17* mutations is due to haploinsufficiency

sup-17 genotype	*lin-12* genotype	% not transformed (non-Egl)	No. counted
+/+[a]	*n302*/+	55	125
n1258/+[a]	*n302*/+	74	122
nDf23/+[b]	*n302*/+	74	95
+/+	*n676*/+	40	149
n316/+	*n676*/+	86	203
nDf23/+	*n676*/+	94	87
nDf25/+	*n676*/+	91	67

Using Fisher's exact test, $P = 0.003$ that +/+ differed from *n1258*/+, $P < 0.0001$ that +/+ differed from *n316*/+, and $P > 0.05$ that *nDf23* and *nDf25* differed from *n316*/+.

[a] The marker mutation *unc-29(e1072)* was homozygous.

[b] The marker mutation *unc-29(e1072)* was hemizygous since the deficiency *nDf23* deletes *unc-29*. *unc-29(e1072)* was not present in the experiments involving *n676*.

possible *lin-12* maternal affect. In an attempt to find a *lin-12* maternal-effect phenotype, we used a strong reduced-function *lin-12* allele, *lin-12(n302 n865)*, which produces a small number of progeny (this mutation causes cell lineage defects similar to those caused by strong *lin-12(null)* mutations but is slightly leaky for the sterile phenotype). These progeny were observed for evidence of embryonic lethality or any other increase in severity of phenotype in comparison with *lin-12(n302 n865)* animals from a heterozygous parent (see MATERIALS AND METHODS). No dead embryos were formed, and no other phenotypic differences were observed compared to the homozygotes segregating from a heterozygous parent. This result suggests that *lin-12* does not have a maternal-effect function during embryogenesis. It remains possible, of course, that there is some maternal-effect *lin-12* function, but that it is less sensitive to reduction of *lin-12* function than are any of the postembryonic phenotypes.

The cell lineages of the P(3–8).p cells were determined in seven *sup-17(n1258ts)* animals grown at 25°, and the lineages for P(5–7).p are shown in Figure 2. Each animal generated one fairly normal 1° lineage, always formed by the P(6).p, the cell closest to the anchor cell. However, the cell lineages displayed by P(5).p and P(7).p, which normally would express a 2° cell lineage, were often abnormal. Five of 14 such cells generated the typical 3° cell lineage. Six additional cells generated abnormal cell lineages that might be best described as hybrids of the 2° and 3° cell lineages (see Figure 2). Only three of 14 cells generated normal 2° cell lineages. These results suggest that loss of *sup-17* function causes defects in 2° cell fate, possibly resulting in transformation toward the 3° fate. *lin-12(0)* mutations completely prevent expression of the 2° cell fate (GREENWALD *et al.* 1983). We also found that these seven animals produced only a single anchor cell, suggesting that the *lin-12*-controlled AC/VU cell interaction was not disrupted.

Further analysis of *lag-2*: Two semi-dominant mutations *n1255* and *sa37* have been tentatively assigned as alleles of a single gene for the following reasons. First, both mutations are strongly semi-dominant in their suppression. Second, both mutations suppress the AC/VU cell fate transformation caused by *lin-12(d)* mutations but have no effect on the P(3–8).p cell fate transformation (all of the other suppressor mutations at least partially suppress both transformations, F. TAX and J.

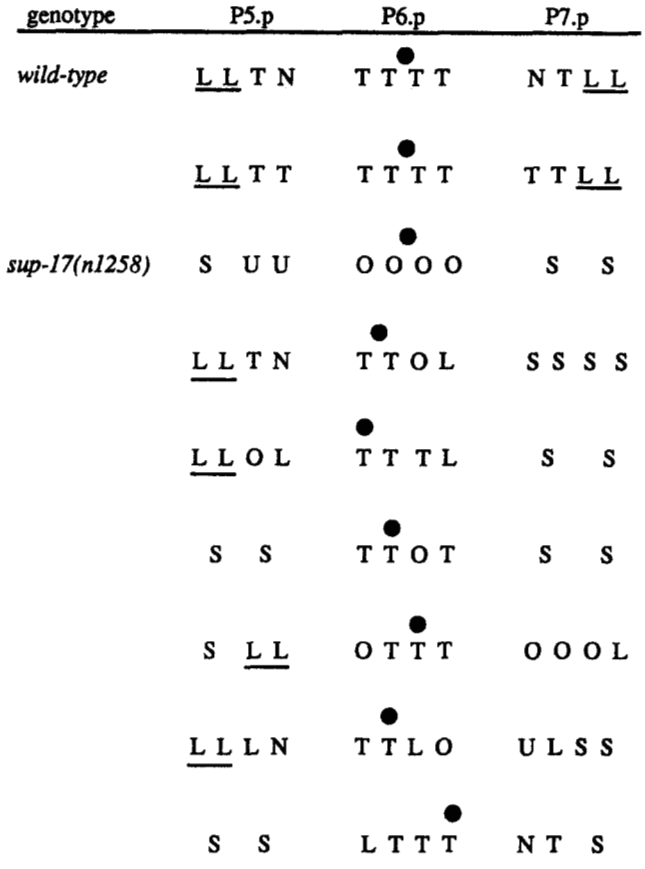

FIGURE 2.—Cell lineages of P(5–7).p in *sup-17(n1258)* animals at 25°. Cell lineages were determined as described in MATERIALS AND METHODS and are depicted in the manner of STERNBERG and HORVITZ (1986). ●, position of the anchor cell in each animal. A capital letter designates the terminal division in each precursor lineage. L, a longitudinal division axis; T, a transverse division axis; O, an oblique division axis (usually a minor variant of the T axis); U, means that a cell that would normally be expected to divide failed to; S, the cell divided and the progeny appeared to fuse with the hypodermal syncitium; N, a cell that normally fails to divide. The underlines refer to cells that remain attached to the cuticle after the terminal division. The patterns LLTN, LLTT (and their mirror images from P7.p) are both wild-type 2° patterns. TTTT and variants in which T is replaced by O are normal 1° patterns. S S is a normal 3° pattern. Other patterns are not seen in the wild type and may represent hybrid or defective cell lineages.

THOMAS, unpublished results). Third, these two mutations map within ~0.2 μ of each other and no recombinants between them have been detected (see MATERIALS AND METHODS and TAX *et al.* 1994). Fourth, *n1255/sa37* heterozygotes display strong synergy for suppressing *lin-12(d)* mutations (Table 2). Conclusive evidence for allelism has been demonstrated by our analysis of the DNA sequence from both *lag-2* semi-dominant alleles, which showed the same single nucleotide change in both mutants (TAX *et al.* 1994; F. TAX and J. THOMAS, unpublished results).

The strong semi-dominance of *n1255* and *sa37* was unique among the suppressor mutations (Table 2). We tested whether this semi-dominance was caused by a reduction-of-function or by a gain-of-function activity. In the region in which *n1255* and *sa37* mapped, there is a small deficiency (*sDf70*) that deletes *lag-2* (JOHNSEN and BAILLIE 1991; F. TAX and J. THOMAS, unpublished results). *sDf70* showed no semi-dominant suppression of the *lin-12(n302)* mutation (Table 6), indicating that the semi-dominance of *n1255* and *sa37* is caused by altered or increased gene function.

To identify null mutations of *lag-2*, we reverted the suppressor phenotype of *n1255*. We mutagenized *lin-12(n302); lag-2(n1255)* animals and screened their progeny for loss of suppression (see MATERIALS AND METHODS). Pilot experiments using *sDf27* and *sDf28*, which delete the *lag-2* locus, showed that it would be possible to isolate null mutations by this procedure, even if they were homozygous lethal. From a screen of 4500 mutagenized genomes, we identified two revertants of *n1255* that had lost suppressor activity. The revertant mutations were named *n1322* and *n1323*. Both *n1322* and *n1323* cause a recessive L1 lethal phenotype, which was closely linked to the revertant locus, and *n1322* and *n1323* failed to complement for the lethal phenotype.

The revertants *n1322* and *n1323* could be intragenic mutations in *lag-2*, or they could be in a closely linked suppressor locus. To help distinguish between these possibilities we tested the linkage of the revertant lesion

TABLE 6

Gene dosage studies of *lag-2(n1255)* and *lag-2(sa37)* semi-dominance

lag-2 genotype	% suppressed (non-Egl)	No. counted
+/+	0	>500
sDf70/+	1	131
lag-2(n1255)/+	34	112
lag-2(sa37)/+	31	315
lag-2(n1255)	95	175
lag-2(sa37)	98	277
lag-2(n1255)/*sDf70*	51	93
lag-2(sa37)/*sDf70*	51	370
lag-2(n1255)/*lag-2(n1255 n1322)*	43	164
lag-2(sa37)/*lag-2(n1255 n1322)*	36	119
lag-2(sa37)/*lag-2(s1486)*	27	94
lag-2(n1255)/*lag-2(n1255 n1323)*	4	107
lag-2(sa37)/*lag-2(n1255 n1323)*	6	216
lag-2(sa37)/+/+	18	259
lag-2(sa37)/*lag-2(sa37)*/+	85	219

All strains were homozygous for *lin-12(n302)* and were grown at 20°. *unc-32(e189)* and *unc-46(e177)* were present as markers in many of these strains and had no effect on suppression (data not shown). Extra copies of *lag-2(+)* were provided by *yDp1*.

n1323 to *n1255*. Since *n1323* abolished the suppressor phenotype of *n1255*, we screened the progeny of *n1255 n1323/+* for suppressed animals (see map data, Table 1 and MATERIALS AND METHODS). This analysis indicated that *n1323* was within 0.05 map units of *n1255*. Thus, since both revertants of *n1255* arose in the same gene and were <0.05 map units from *n1255*, it is very likely that *n1322* and *n1323* are intragenic revertants of *n1255* that reduce or eliminate *lag-2* gene function. Neither was amber suppressible for its lethal phenotype. *n1322* and *n1323* failed to complement lethal alleles of *lag-2* isolated by others (LAMBIE and KIMBLE 1991b; TAX *et al.* 1994), and *n1255, sa37, n1322* and *n1323* were thus assigned as *lag-2* alleles.

We further characterized the properties of the *lag-2(d)* alleles *n1255* and *sa37* by analyzing the effects of varying wild-type and mutant *lag-2* gene dosage on suppression of *lin-12(d)* (Table 6). We found that *lag-2(d)/Df* was a stronger suppressor than *lag-2(d)/+*, indicating that the dominant alleles do not encode a more active wild-type function. Analysis of strains containing an extra copy of *lag-2(+)* on *yDp1*, a free duplication carrying *lag-2(+)*, showed that the ratio of *lag-2(+)* and *lag-2(d)* copies influences the amount of suppression. The dosage analysis is most consistent with *lag-2(d)* alleles acting as altered-function alleles with competition between dominant and wild-type gene products. However, *lag-2(sa37)* and *lag-2(n1255)* must also confer some wild-type function, as they resulted in a grossly wild-type phenotype in a *lin-12(+)* background (TAX *et al.* 1994).

Differences in suppression were also observed when either of the dominant alleles was placed in *trans* to various loss-of-function mutations in *lag-2*. Strains in which the *trans* allele was *lag-2(n1255 n1323)* were suppressed very poorly compared to the other lethal alleles (Table 6). The *lag-2(d)* alleles in *trans* to *sDf70* suppressed slightly better than when in *trans* to any of the point mutations tested. We have confirmed molecularly that the DNA encoding *lag-2* is present in *n1322* and *n1323* animals (F. TAX and J. THOMAS, unpublished results). The result that *lag-2(n1255 n1323)* can reduce the level of suppression exhibited by *lag-2(d)* more than does a deficiency of the locus suggests that the proteins encoded by the two alleles of *lag-2* can interact, directly or indirectly.

Temperature sensitivity of suppression: The degree of suppression of *lin-12(d)* mutations for vulval induction was determined for each suppressor mutation at 15°, 20° and 25°, as shown in Table 7. In all but three cases, the *lin-12(d)* allele used was *n302*. *lin-12(n137)* was used with *lag-2(n1255)*, and *lin-12(n952)* was used with *sup-17(n316)* and *sel-6(sa44)*, as these combinations of the strongest suppressors with stronger *lin-12(d)* alleles show more spread in the degree of suppression at different temperatures. The *lin-12(d)* mutations themselves showed only small differences at different temperatures (all *lin-12(d)* mutations were slightly more penetrant for certain lineage transformations tested at 15°, data not shown, and I. GREENWALD, personal communication). Six suppressor mutations showed dramatic differences at different temperatures: *sup-17(n1260), lag-2(n1255), lag-2(sa37), sel-6(sa44), sel-5(n1250)*, and *sel-5(n1254)*. In addition we observed that a second *sup-17* mutation, *n1258*, displayed temperature sensitivity: it was a stronger suppressor at 20° than at 15° and caused highly penetrant lethality at 25° (data not shown). *lag-2(sa37), sel-6(sa44), sup-17(n1260)* and

TABLE 7

Temperature sensitivity of suppression of *lin-12(d)* suppressors

Suppressor genotype	Temperature	% not transformed (non-Egl)	No. counted
sup-17(n1260)	15	9	390
	20	52	353
	25[a]	96	36
sup-17(n316)	15	74	121
	20	92	146
	25	89	185
lag-2(n1255)	15	2	484
	20	28	454
	25	82	230
sel-4(n1259)	15	43	297
	20	31	286
	25	71	207
sel-5(n1254)	15	82[b]	195
	20	31	203
	25	3	288
sel-5(n1250)	15	94	279
	20	31	245
	25	10	231
sel-6(n1256)	15	45	164
	20	61	168
	25	75	107
sel-6(sa44)	15	25	193
	20	46	329
	25	91	243
sel-7(n1253)	15	53	105
	20	53	162
	25	46	162

The data in each case are for suppression of homozygous *lin-12(n302)*, with the exception of *sup-17(n316), lag-2(n1255)*, and *sel-6(sa44)*. *sup-17(n316)* and *sel-6(sa44)* data are for suppression of homozygous *lin-12(n952)*. For *lag-2* the data are for suppression of homozygous *lin-12(n137)*, since this combination best showed the temperature sensitivity of *lag-2(n1255)*. With all other *lin-12* alleles tested (including *n952* and *n302*) the suppression by *lag-2(n1255)* was also temperature sensitive (data not shown).

[a] *sup-17(n1260)* was subviable in homozygotes at 25°. The suppression at 25° was further complicated by the fact that *sup-17* itself caused a Vul defect when gene activity was sufficiently low.

[b] There was significant variation from brood to brood in this case. The percentage given is the average of four broods, but all broods were significantly more suppressed than at 20°.

lag-2(n1255) were stronger suppressors at higher temperatures (henceforth called heat-sensitive), while *sel-5(n1250), sel-5(n1254)* and *sel-8(sa54)* were stronger at low temperature (cold-sensitive). In the case of *sup-17(n1258)* and *sup-17(n1260)*, other alleles of this gene showed little difference in suppression at different temperatures. We conclude that *n1260* and *n1258* most likely encode heat-sensitive *sup-17* gene products or that *n1260* and *n1258* synthesis is heat-sensitive. In contrast, both alleles of *sel-5, n1250* and *n1254*, were cold-sensitive, while both alleles of *lag-2, n1255* and *sa37*, were strongly heat-sensitive. The mutations in *lag-2* and *sel-5* were isolated in separate rounds of mutagenesis and are therefore clearly independent. It is possible that cold sensitivity is characteristic of reduced or loss-of-function mutations in the *sel-5* gene. However, we have not determined whether the two *sel-5* mutations are loss-of-function or gain-of-function alleles.

To determine the temperature-sensitive periods for suppression by *sup-17* and *lag-2*, we performed temperature-shift experiments. For each mutation, *n1260* and *n1255*, we constructed a double mutant with an appropriate *lin-12(d)* allele. The *lin-12(d)* allele was chosen so as to produce the maximal difference between the degree of suppression of AC/VU at 15° and at 25°. To study *sup-17(n1260)* we used *lin-12(n302)*, and to study *lag-2(n1255)* we used *lin-12(n137)*. Double mutant animals were grown at 25°, and egg-laying proficient parents were picked to plates preincubated at either 15° or 25°. Every 2 or 3 hr for the next 12 hr the parents were moved to a fresh plate. Temperature shifts were performed at hatching and at each larval molt. To improve synchrony, the plates were screened at the times appropriate for each molt, and animals actually in the molt were selected for temperature shift (generally more than half of the animals were in the molt). The animals were allowed to mature at their new temperature, and their frequency of vulval induction was scored as an indicator of the suppression of the AC/VU cell fate transformation of *lin-12*. The results are presented in Figure 3.

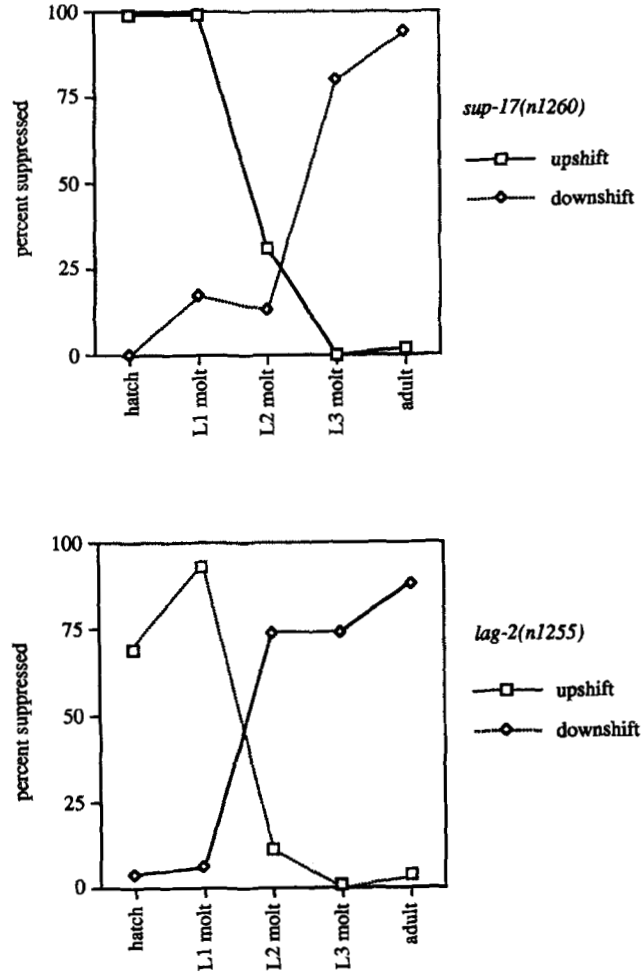

FIGURE 3.—Temperature shifts experiments of *sup-17(n1260)* and *lag-2(n1255)*. Suppression was indicated by production of a vulva. Genotypes analyzed were *sup-17(n1260); lin-12(n302)* and *lin-12(n137); lag-2(n1255)*.

For each gene there was a temperature-sensitive period at about the L2 molt. Both the upshift and downshift curves showed a similar transition time, suggesting that slow imposition or relief of the conditional defect was not a problem for these mutations. This time period coincides with the time that the AC/VU cell fate is being determined (KIMBLE 1981) and the time at which *lin-12* is acting to specify the AC/VU cell fate (GREENWALD *et al.* 1983).

Lineage specificity of suppressor action: While constructing strains with the suppressor mutations and some of the *lin-12(d)* alleles, we noticed that the *lag-2(d)* alleles *sa37* and *n1255* failed to suppress the Pn.p cell fate transformation of strong *lin-12(d)* alleles such as *n950* (phenotypically Muv, for Multivulvae, GREENWALD *et al.* 1983). Mutations in the other six suppressor genes partially or completely suppressed the Muv phenotype of strong *lin-12(d)* alleles, despite suppressing AC/VU more weakly than *lag-2(sa37)* and *lag-2(n1255)* (data not shown). These results suggested that the *lag-2(d)* alleles may be lineage-specific suppressors. To examine this possibility, we studied the suppression of four other *lin-12(d)* cell-fate transformations (Table 8). *lag-2(n1255)* suppressed the transformation of AC/VU in the hermaphrodite and of LC/VD and P(9–11).p in the male but did not suppress the transformations of P(5–7).p, SM/CC and Y/DA9.

As shown in Table 8, the same lineage specificity of suppression was observed in an apparent intragenic partial revertant of *lin-12(n950)*, called *n1328* (M. HERMAN, personal communication). As observed with *lag-2(n1255), lin-12(n950 n1328)* suppressed the transformation of AC/VU in the hermaphrodite and of LC/VD and P(9–11).p in the male but did not significantly suppress the transformations of P(5–7).p, SM/CC and Y/DA9. All other intragenic revertants isolated as *lin-*

12(n950) suppressors did not appear to be lineage-specific, suggesting that *n1328* is a rare, non-null revertant allele. Since the *lin-12(n950n1328)* double mutant retained some *lin-12(d)* character for all of the cell lineage transformations, we suspected that the *n1328* mutation could be an intragenic revertant. We were unable to detect any recombinants between *n950* and *n1328* (see MATERIALS AND METHODS), which supports our hypothesis that *n1328* is an intragenic revertant of *lin-12(n950)*. Suppression of *lin-12(d)* phenotypes in the strain *lin-12(n950 n1328); lag-2(n1255)* remained similarly lineage specific (Table 8). These animals showed suppression of the transformation of AC/VU in the hermaphrodite and of LC/VD and P(9–11).p in the male, but did not show suppression of the transformations of P(5–7).p and Y/DA9. There may be slight suppression of the SM/CC transformation. These results are consistent with the idea that *lag-2* is expressed within only a subset of the cells affected by *lin-12*. Alternatively, *lag-2* may be coexpressed with *lin-12*, but the *lag-2(d)* alleles may be able to suppress only the transformations caused by *lin-12(d)* in specific cells (see DISCUSSION).

Genetic mosaic analysis of *sel-5*: The *sel-5* gene maps in a position favorable for genetic mosaic analysis. To determine whether *sel-5* functions in signaling cells or signal-receiving cells, animals genetically mosaic for the *sel-5* gene were generated as described in detail in MATERIALS AND METHODS. In brief, animals mosaic for *sel-5* function were generated using a free duplication bearing the *sel-5(+)* gene present in a strain homozygous for *sel-5(n1254)* and *lin-12(n302)*. The nucleolar marker *ncl-1* was used to determine the point of duplication loss during development (SULSTON *et al.* 1983; HEDGECOCK and HERMAN 1995). The mosaics were analyzed for the AC/VU cell interaction, for which the focus of *lin-12* function has been determined to be in the signal-receiving VU cell (SEYDOUX and GREENWALD 1989). Animals bearing the free duplication in all of their somatic cells invariably lacked an AC and were thus Vul. Animals in which the free duplication was lost from cells in which *sel-5* functions were suppressed and produced an AC, which induced a normal vulva. The relevant parts of the *C. elegans* lineage are shown in Figure 4. Cells scored for their nucleolar phenotype are listed according to their known positions in the lineage (SULSTON *et al.* 1983). The inferred position of duplication loss in each mosaic animal that produced an AC is marked with an asterisk. Six losses occurred late in the lineages that generate Z1 and Z4 and the precise division at which the loss occurred was not determined (indicated by dashed lines). Another six losses occurred after the generation of Z1 and Z4. Because of the small number of cells affected by these six losses and the indeterminacy of which cell (Z1.ppp or Z4.aaa) gives rise to the AC, we did not determine whether the loss occurred in the Z1 or Z4 lineage (as indicated by arrows in Figure 4). One loss occurred in P1 and the resulting animal had an AC and a normal vulvae, indicating that *sel-5* function in the AB lineage does not affect the AC/VU interaction. The remaining 15 losses occurred at various stages after the separation of Z1 and Z4 in the cell lineage (at the MS division). In all 15 cases, the AC that resulted from *sel-5* suppression of *lin-12(d)* was Ncl. This indicates that suppression results only from loss of the *sel-5(+)*-bearing duplication in the presumptive AC. We interpret this result to indicate that loss of *sel-5* function in the presumptive AC can block the constitutively active *lin-12(d)* signaling in that cell, permitting it to adopt the nonsignal-receiving fate (AC). We conclude that *sel-5(+)* functions cell-autonomously to promote *lin-12* activity in the presumptive VU cell.

Interactions of suppressors with *glp-1*: The *glp-1* gene encodes a second *Notch/lin-12* family member in *C. elegans* (YOCHEM and GREENWALD 1989) and also acts as a receptor in specific cell interactions during development (AUSTIN and KIMBLE 1987; PRIESS *et al.* 1987). *lin-12* and *glp-1* appear to function redundantly in specific cell interactions during embryogenesis (LAMBIE and KIMBLE 1991b). Our initial goal was to identify additional components of a *lin-12*-mediated signaling pathways, and it is possible some of these components might be shared with *glp-1*-mediated cell signaling pathways. Since our screens for revertants of *lin-12(d)* alleles resulted in the isolation of extragenic suppressors that act as if they reduce *lin-12* function (see DISCUSSION), we asked whether these suppressors could also act to reduce *glp-1* function. We tested this by constructing strains that were homozygous for *lin-12(d)* suppressor mutations and *glp-1(e2141)* or *glp-1(e2142)*. These two *glp-1* alleles are heat-sensitive and result in full viability at 15° (PRIESS *et al.* 1987). At 25°, *glp-1(e2142)* affects only embryos, while *glp-1(e2141)* affects both embryos and distal tip cell regulation of the germline. *sel-8(sa54)* acted as a dominant enhancer of *glp-1(e2142)*, and the strain could not be maintained (data not shown). All of the other strains homozygous for suppressor mutations and *glp-1(e2141)* were viable and fertile at 15° (see MATERIALS AND METHODS). Each strain was tested for three characteristics: frequency of the *glp-1* embryonic lethal phenotype, brood size, and the frequency of *glp-1*-like sterile animals among the brood. The *glp-1*-like sterile phenotype and the reduction in brood size are interpreted as being caused by absence or reduction of the distal tip cell (dtc) regulation of germ cell proliferation (AUSTIN and KIMBLE 1987). Each suppressor mutation was also examined for causing these phenotypes in a *lin-12(+) glp-1(+)* background to see if there were any single-mutant defects that might contribute to any of these three *glp-1* phenotypes.

sup-17(n1258) and *sel-7(n1253)* were the only mutations tested that enhanced the *glp-1* embryonic defect (Table 9). Neither suppressor alone under these condi-

TABLE 8

The suppression by *lag-2(n1255)* and *lin-12(n950 n1328)* of different cell types transformed by *lin-12(d)*

Cell lineage	Genotype	% cells with *lin-12(d)* phenotype[a]	No. tested
AC/VU	*lin-12(n137)*	100	500
	lin-12(n137); lag-2(n1255)	19	230
	lin-12(n950)	100	500
	lin-12(n950 n1328)	79	205
	lin-12(n950 n1328); lag-2(n1255)	2.5	363
SM/CC	*lin-12(n137)*	45	47
	lin-12(n137); lag-2(n1255)	44	57
	lin-12(n950)	49	73
	lin-12(n950 n1328)	47	40
	lin-12(n950 n1328); lag-2(n1255)	32	53
P(5-7).p	*lin-12(n137)*	94[b]	225
	lin-12(n137); lag-2(n1255)	94[c]	175
	lin-12(n950)	86	245
	lin-12(n950 n1328)	88	335
	lin-12(n950); lag-2(n1255)	90[c]	141
	lin-12(n950 n1328); lag-2(n1255)	93[c]	258
LC/VD male	*lin-12(n137)*	32	31
	lin-12(n137); lag-2(n1255)	4	45
	lin-12(n950)	17	48
	lin-12(n950 n1328)	0	95
	lin-12(n950 n1328); lag-2(n1255)	0	77
Y/DA9 male	*lin-12(n950)*	80	5[d]
	lin-12(n950); lag-2(n1255)	81	27
	lin-12(n950 n1328)	68	19
	lin-12(n950 n1328); lag-2(n1255)	84	19
P(9-11).p male	*lin-12(n950)*, 25°	40	30
	lin-12(n950); lag-2(n1255), 25°	4	23
	lin-12(n950), 20°	73	22
	lin-12(n950 n1328), 20°	28	39
	lin-12(n950 n1328); lag-2(n1255), 20°	6	17

Cell fates were scored as described in MATERIALS AND METHODS. AC/VU, P(5-7).p and SM/CC fates were scored in hermaphrodites. Y/DA9, P(9-11).p and LC/VD fates were scored in males (with *him-5(e1490)* in the strain).

[a] Using Fisher's exact test, $P < 0.001$ that *lin-12(n137)* differed from *lin-12(n137); lag-2(n1255)*, and that *lin-12(n950)* differed from *lin-12(n950 n1328)* for the AC/VU phenotypes. For the SM/CC phenotypes, $P > 0.2$ that *lin-12(n137)* differed from *lin-12(n137); lag-2(n1255)* and that *lin-12(n950)* differed from *lin-12(n950 n1328)*; $P = 0.07$ that *lin-12(n950 n1328); lag-2(n1255)* differed from *lin-12(n950 n1328)*. For the p(5-7).p phenotypes, $P > 0.2$ that *lin-12(n137)* differed from *lin-12(n137); lag-2(n1255)*, that *lin-12(n950)* differed from *lin-12(n950 n1328)* and $P = 0.12$ that *lin-12(n950 n1328); lag-2(n1255)* differed from *lag-2(n950)*. For the LC/VD phenotype, $P = 0.002$ that *lin-12(n137)* differed from *lin-12(n137); lag-2(n1255)*, and $P < 0.001$ that *lin-12(n950 n1328)* and *lin-12(n950 n1328); lag-2(n1255)* differed from *lin-12(n950)*. For the Y/DA9 phenotypes, $P > 0.2$ that *lin-12(n950)* differed from *lin-12(n950); lag-2(n1255)* and that *lin-12(n950 n1328)* differed from *lin-12(n950 n1328); lag-2(n1255)*. For the P(9-11).p phenotypes, $P = 0.003$ that *lin-12(n950)* differed from *lin-12(n950); lag-2(n1255)*, and $P < 0.001$ that *lin-12(n950 n1328)* and *lin-12(n950 n13280; lag-2(n1255)* differed from *lin-12(n950)*.

[b] The maximal number of ectopic pseudovulvae observed in *lin-12(d)* animals without an anchor cell is five, since six P(3-8).p cells form 2° lineages, but two of these cells organize together to form a single pseudovulva (I. GREENWALD and P. STERNBERG, personal communication). The percentage of cells with the *lin-12(d)* phenotype in the table is derived from the number of pseudovulvae observed per animal divided by five.

[c] For these genotypes, the large majority of animals had an anchor cell (see earlier lines in table). The presence of an anchor cell reduces the maximal number of pseudovulvae observed to three because the

TABLE 8

Continued

anchor cell induces one P(5-7).p cell to the 1° fate, and the cells from this lineage and from the two adjacent 2° lineages organize together to form a single functional vulva (GREENWALD *et al.* 1983). Thus, for these cases, the percentage cell fate transformation in the table is the number of pseudovulvae per animal divided by three.

[d] This class proved extremely difficult to assess in greater numbers, because the *lin-12(n950); him-5(e1490)* strain produced very small broods and invariably formed a bag of worms. The Y/DA9 cell fate transformation was tested in young L1s, which were almost always still trapped in the maternal cuticle, rendering the optical image insufficiently clear to score these cells. An additional eight of 10 animals of the genotype *lin-12(n137)* were reported by GREENWALD *et al.* (1983). Since *lin-12(n137)* is very similar in strength to *n950* (probably slightly stronger), this result confirms no suppression by *lag-2* or *n1328* for the Y/DA9 transformation of *lin-12(n950)* or *lin-12(n137)*.

tions produced dead embryos, so the phenotype was caused by an interaction with *glp-1* (Table 9). This result suggests that *sel-7* and *sup-17* affect germline or early embryonic functions of *glp-1*.

All suppressors tested had an effect on the brood size and production of hermaphrodites with a clear gonad containing no oocytes or embryos (Glp sterile, for germ line proliferation defective, Table 9). These phenotypes reflect disruption of the signaling from the distal tip cell (dtc) to the germline (AUSTIN and KIMBLE 1987). The presence of *sup-17(n1258), sel-7(n1253),* or either *lag-2* allele caused a significant reduction in the brood size of *glp-1(e2141)* animals (Table 9). *sup-17(n1258)* animals had a reduced brood size in a *glp-1(+)* background, which might contribute to the reduction of the brood size in *sup-17(n1258); glp-1(e2141)* double mutant animals.

sup-17(n1258), sup-17(n316), lag-2(sa37), lag-2(n1255), sel-7(n1253), sel-6(n1256), sel-6(sa44), and *sel-4(n1259)* all caused at least 10% of *glp-1(e2141)* hermaphrodites grown at 20° to develop with clear gonads and to be sterile (Table 9). The strength of enhancement of the sterile Glp phenotype was correlated with the strength of suppression of the *lin-12(d)* alleles. For example, the *lag-2(d)* alleles were the strongest *lin-12(d)* suppressors and also the strongest *glp-1(e2141)* enhancers (Tables 4 and 9). In contrast, the weaker *lin-12(d)* suppressors *sel-4(n1259)* and *sel-7(n1253)* also had a weaker effect on *glp-1(e2141)*. These results support the idea that the enhancement of *glp-1* and the suppression of *lin-12(d)* are caused by mutations in genes that are shared components in their signaling pathways.

Several suppressor mutations also caused some unusual phenotypes in a *glp-1* background. Approximately 20–30% of *glp-1(e2141); sel-6(sa44)* animals had an everted vulva phenotype (a large bump can be found in the normal position of the vulva, see SEYDOUX *et al.* 1993) when grown at 25°, similar to the phenotype of *lin-12(0)* animals (F. TAX and J. THOMAS, unpublished results). A similar phenotype was seen in *glp-1(e2141); sel-7(n1253)* animals, but the phenotype was not as strong or as penetrant as in the case of *sel-6(sa44)*. Occasional *sel-7(n1253)* single mutant animals grown at 25° had similar vulval defects.

DISCUSSION

Reversion screens of *lin-12(d)* alleles: We have conducted screens for extragenic mutations that suppress the egg-laying defects caused by two *lin-12(d)* mutations and identified 14 suppressors that defined seven different genes. Suppression of *lin-12(d)* phenotypes could be due either to a gain-of-function mutation, or due to a reduction-in-function or loss-of-function. We examined whether the effects of each suppressor were dominant or recessive. The two suppressor alleles of *lag-2* that we identified are clearly semi-dominant in their suppression of *lin-12(d)* (see Table 2 and Table 6). The suppressor alleles that define the other six genes display between 1% and 10% dominant suppression in *lin-12(n302)* homozygotes, one of the weaker *lin-12(d)* alleles (Table 2). We believe the weak semi-dominance observed probably reflects some dosage-sensitive properties of the *lin-12(d)* mutations, which are thought to encode cell-interaction-independent receptors. We have observed that several *sup-17* alleles suppress the *lin-12(d)* allele *n302* in a weakly dominant manner (Table 2). These *sup-17* alleles convey their own phenotypes in a *lin-12(+)* background, but these defects are recessive (F. TAX and J. THOMAS, unpublished results). Similarly, our dosage experiments demonstrated that deficiencies that remove *sup-17* can weakly suppress the phenotypic effects caused by *lin-12(d)* alleles (Table 5). These deficiencies in a *lin-12(+)* background do not cause noticeable phenotypes. Thus, it appears that these *lin-12(d)* strains are more sensitive than *lin-12(+)* to reductions in the dosage of genes such as *sup-17*. We hypothesize that the weak semi-dominance of some of the other suppressors is due to reduced dosage in the sensitized *lin-12(d)* background. It is also possible that this weak semi-dominance is due to gain-of-function activity of these suppressor mutations. Interestingly, several recessive suppressors of reduction-of-function alleles of *lin-12* and *glp-1* were found to have gain-of-

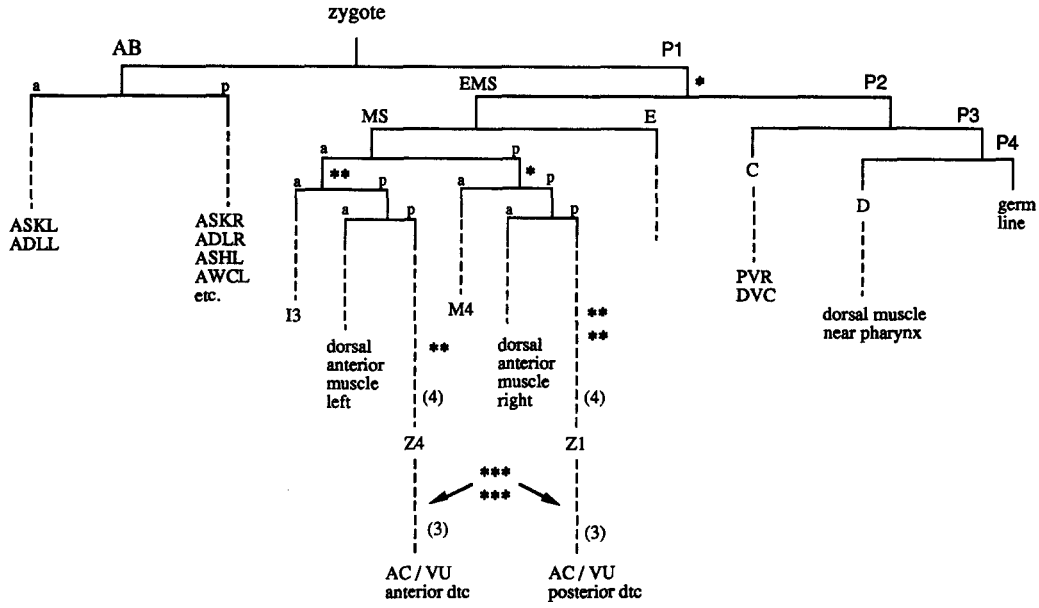

FIGURE 4.—Mosaic analysis of *sel-5(n1254)*. Relevant cell lineages are diagrammed and the names of relevant blastomeres are given. Cells scored to determine position of duplication loss are shown at the bottom. Dashed lines indicate several cell divisions occur before the generation of the indicated cells. a and p indicate the orientation of some cell divisions. Each duplication loss is indicated by an asterisk; multiple asterisks indicate the number of independent losses found. Asterisks next to dashed lines indicate that the exact position of the loss was not determined. The arrows indicate that these six losses could have occurred in either the Z1 or Z4 lineage.

function activity (MAINE and KIMBLE 1993; SUNDARAM and GREENWALD 1993).

The mechanism of *lin-12(d)* suppression: The correlation between the strength of the *lin-12(d)* mutation and the penetrance of suppression suggests that each mutant suppressor gene acts by quantitatively antagonizing *lin-12* function. We hypothesize that each *lin-12(d)* allele is antagonized in a similar way by a given suppressor mutation, and that the differing degrees of suppression result from the different strengths of the *lin-12(d)* mutations. The behavior of the suppressors is consistent with their acting to reduce the gene activity of the *lin-12(d)* alleles. All *lin-12(d)* mutations tested have been shown to be phenotypically enhanced in their dominant phenotypes by one or two added copies of the *lin-12(+)* gene (GREENWALD *et al.* 1983). It seems likely, therefore, that the extragenic suppressor mutations are acting to reduce *lin-12* gene activity, although we have not directly adressed this hypothesis in this work.

We suggest three ways our suppressor mutations might act to affect *lin-12* receptor synthesis or ability to receive or transmit signal. First, a suppressor mutation might interfere with the proposed receptor dimerization and interaction-independent signaling of the *lin-12(d)* receptor (GREENWALD and SEYDOUX 1990). A mutation in a ligand or in a membrane protein could have such an effect, although such a mutation would probably have dominant effects (see below for *lag-2* discussion). Second, a suppressor mutation might reduce the expression of the *lin-12* gene, and the reduced expression could partially compensate for the increase in *lin-12* function caused by *lin-12(d)* mutations. Reducing the dosage of a *lin-12(d)* mutation by a factor of two does reduce the *lin-12(d)* phenotypes (Table 4 and GREENWALD *et al.* 1983), although the effect of most of our suppressors is stronger than this effect. Third, a suppressor mutation could affect a step in the intracellular pathway through which the *lin-12* receptor acts to determine cell fate. We suppose that such a mutation might partially reduce the efficacy of the *lin-12* receptor in activating the AC to VU cell-fate transformation.

Interestingly, all six suppressor mutations we tested also enhanced a *glp-1* reduction-of-function allele. This observation suggests that these suppressors act to reduce both *lin-12* and *glp-1* function and supports the idea that the wild-type function of these suppressors is in a shared *lin-12/glp-1* signal transduction pathway. This observation also demonstrates that these suppressors do not specifically antagonize the proteins encoded by the *lin-12(d)* alleles, but interfere with normal signaling. These observations predict that these suppressors would also affect reduction-of-function *lin-12* alleles, but we did not test this class of *lin-12* allele.

***sup-17* and *lag-2* are essential for *C. elegans* development:** We have identified suppressor mutations in two genes, *sup-17* and *lag-2*, for which probable null alleles are lethal. Probable *sup-17* null mutations caused maternal-effect embryonic lethality and resulted in some zygotic defects, including cell lineage defects within the

TABLE 9

Interactions between *lin-12(d)* suppressors and *glp-1(e2141)*

Genotype	% dead embryos	Brood size[a]	% steriles[b]	Lags or novel phenotypes
glp-1(e2141)	<1	193 (6)	<1	None
Wild type	<1	334 (6)	<1	None
glp-1(e2141); lag-2(sa37)	<2	115 (6)	52 (213)	None
glp-1(e2141); lag-2(n1255)	<2	85 (6)	52 (155)	None
lag-2(sa37)	<1	275 (6)	<1	None
lag-2(n1255)	<1	260 (5)	<1	None
sup-17(n316); glp-1(e2141)	<2	153 (8)	18 (154)	None
sup-17(n1258); glp-1(e2141)	22	85 (6)	30 (102)	2% L1 lethal
sup-17(n316)	<2	170 (7)	<1	See text
sup-17(n1258)	<2	155 (5)	<1	See text
glp-1(e2141): sel-6(n1256)	<1	157 (6)	20 (151)	None
glp-1(e2141); sel-6(sa44)	<1	133 (6)	40 (257)	Everted vulvae at 25°
sel-6(n1256)	<1	265 (6)	<1	None
sel-6(sa44)	<1	247 (6)	<1	Sterile at 25°
sel-4(n1259); glp-1(e2141)	<1	193 (6)	26 (394)	None
sel-4(n1259)	<1	334 (5)	<1	None
glp-1(e2141); sel-7(n1253) vulvae, see text	15	62 (6)	10 (159)	Everted vulvae or protruding
sel-7(n1253)	<1	334 (6)	<1	See text

Phenotypes were assessed as described in MATERIALS AND METHODS. All tests were performed at 20° unless otherwise indicated. *unc-16(e109)* was present in all strains.

[a] Number of broods in parentheses.

[b] Number counted in parentheses.

P(5–7).p cells that resembled *lin-12* null phenotypes. Thus, the *sup-17* gene appears to act with *lin-12* in several cell-fate decisions as well as in at least one process for which *lin-12* does not appear to be needed, as evidenced by the maternal-effect lethality of the *sup-17* null mutant.

We identified dominant *lag-2* mutations that strongly suppressed *lin-12(d)* mutations. Both *lag-2(d)* alleles enhanced the germ-line defect caused by *glp-1(e2141)* (Table 9). This enhancement could be caused either by a direct effect of *lag-2(d)* on *glp-1* or by a reduced activity of *lag-2(d)* that requires an impaired *glp-1* allele to observe. Revertants of the dominant suppressor activity caused larval lethality and were allelic to other mutations in *lag-2* that cause a phenotype similar to that of the *lin-12* and *glp-1* double mutants (LAMBIE and KIMBLE 1991b). Thus, *lag-2* may be required for both *lin-12*- and *glp-1*-mediated cell interactions. Strong support for this notion was provided by the molecular cloning of *lag-2*, which revealed that the *lag-2* protein is similar to Notch family ligands (HENDERSON *et al.* 1994; TAX *et al.* 1994).

The molecular characterization of *lag-2(d)* alleles and the genetic dosage analysis provide some insight into the specific mechanism of suppression. The same base substitution was found in both alleles, which argues that a highly specific alteration in the protein is required for the suppression. The mutations cause the substitution of a conserved Gly in an extracellularly located EGF repeat (TAX *et al.* 1994; F. TAX and J. THOMAS, unpublished results). The extracellular location of this mutation is consistent with the idea that the dominant *lag-2* alleles encode altered ligands that suppress by interfering with the *lin-12(d)* encoded receptors. However, *lag-2(sa37)* and *lag-2(n1255)* must also contain some wild-type function, as they cause a grossly wild-type phenotype in a *lin-12(+)* background. The results of the gene dosage studies of *lag-2(+)* and *lag-2(d)* alleles indicate that the suppression of *lin-12(d)* by the *lag-2* alleles does not result from reduced *lag-2* activity.

The suppressors can affect *lin-12(d)* in other cell-fate decisions: The suppressor mutations we have identified were selected on the basis of their abilities to suppress the AC/VU cell fate transformation. Certain *lin-12(d)* alleles cause transformations in other sets of cells, such as the vulval precursor cells P(3–8).p. Mutations in six of the seven genes suppressed the Muv phenotype seen in strong *lin-12(d)* mutants (data not shown). In the case of the seventh gene, *lag-2*, the dominant suppressor alleles did not affect the *lin-12(d)* transformations of the P(3–8).p cells. However, we found that the *lag-2(d)* alleles suppressed *lin-12(d)* transformations in two other lineages. Therefore, mutations in all seven suppressor genes can suppress *lin-12(d)* in at least two sets of cells. We conclude that the mutations we have identified interact with *lin-12* in more than one tissue.

The lineage-specific suppression of the *lag-2(d)* alleles suggests the possibility that *lag-2* does not function in all of the interactions in which *lin-12* is needed. One explanation for this result is that *lag-2* is not the ligand for *lin-12* in these cells. There is at least one other *lin-12/Notch* family ligand in *C. elegans* (*apx-1*, MELLO *et al.* 1994), and it is not known whether it is expressed in these specific cells. Another possibility is that *lag-2(d)* is present but does not suppress *lin-12(d)* in certain cell interactions because of an unknown mechanistic difference in these cell interactions. The lineage specificity of *lin-12(n950n1328)* suggests that *lin-12* functions differently in different cell interactions. *n1328* could affect intracellular signal transduction within only a subset of interactions affected by *lin-12(d)*. Another possiblity is that the *n1328* protein affects the specific interaction of *lin-12* with *lag-2*, thus resulting in a pattern of suppression similar to that of *lag-2(d)*.

Relationships between our *lin-12* suppressors and other *lin-12/Notch* family suppressors: A class of dominant alleles of the Drosophila *Notch* gene, called *Abruptex (Ax)*, disrupt cell-fate transformations in a manner similar to *lin-12(d)* mutations (PALKA *et al.* 1990). Screens for dominant extragenic *Ax* suppressors identified mutations in three genes: *Delta*, *mastermind* and *deltex* (XU *et al.* 1990). *Delta* and *lag-2* both encode *lin-12/Notch* family ligands (HENDERSON *et al.* 1994; TAX *et al.* 1994). Since other *lin-12(d)* suppressors have not been cloned, we do not know whether they correspond to any of the other loci found in the *Ax* screen. Other screens in *C. elegans* have also identified enhancers and suppressors of *lin-12* and *glp-1*. A screen for enhancers of reduced function *lin-12* alleles yielded mutations in five genes, all of which also enhanced *glp-1* alleles (SUNDARAM and GREENWALD 1993). Several groups have isolated suppressors of reduced function alleles of *glp-1* (MAINE and KIMBLE 1989, 1993; QIAO *et al.* 1995; A. M. HOWELL and J. PRIESS, personal communication). None of these enhancers or suppressors appear to be allelic to the genes described here. Thus, there is now a large set of genes available for the genetic and molecular analysis of *lin-12* and *glp-1*-mediated cell-signaling in *C. elegans*.

We thank I. GREENWALD, P. STERNBERG, J. PRIESS, B. BOWERMAN, J. SCHUMACHER, S. KIM, M. FINNEY and L. AVERY for helpful discussions during the lengthy course of this work and writing. We are grateful for the comments and suggestions of two anonymous reviewers. We thank M. HERMAN for isolating *lin-12(n950 n1328)*; R. JOHNSEN and D. BAILLIE for providing us with their lethal allele of *lag-2*; and ERIC LAMBIE, J. KIMBLE and T. SCHEDL for other strains. Some nematode strains were provided by the Caenorhabditis Genetics Center, which is funded by the National Institutes of Health (NIH) National Center for Research Resources. This work was supported by a Monsanto Fellowship of the Life Sciences Research Foundation to J.H.T., Public Health Service research grant GM-39868 to J.H.T., a Searle Scholars Program/The Chicago Community Trust award to J.H.T., and by Public Health Service research grants to H.R.H. F.E.T. was supported by an NIH Genetics predoctoral training grant.

LITERATURE CITED

AUSTIN, J., and J. KIMBLE, 1987 *glp-1* is required in the germ line for regulation of the decision between mitosis and meiosis in C. elegans. Cell **51:** 589–599.

BRENNER, S., 1974 The genetics of *Caenorhabditis elegans*.. Genetics **77:** 71–94.

CHRISTENSEN, S., V. KODOYIANNI, M. BOSENBERG, L. FRIEDMAN and J. KIMBLE, 1996 *lag-1*, a gene required for *lin-12* and *glp-1* signaling in *Caenorhabditis elegans*, is homologous to human *CBF1* and *Drosophila Su(H)*. Development **122:** 1373–1383.

COFFMAN, C., W. HARRIS and C. KINTNER, 1990 *Xotch*, the *Xenopus* homologue of *Drosophila Notch*. Science **249:** 1438–1441.

COFFMAN, C., P. SKOGLUND, W. HARRIS and C. KINTNER, 1993 Expression of an extracellular deletion of *Xotch* diverts cell fate in Xenopus embryos. Cell **73:** 659–671.

EDGLEY, M., and D. L. RIDDLE, 1987 The nematode *Caenorhabditis elegans*, pp. 351–365 in *Genetic Maps 1987*, edited by S. J. O'BRIEN. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

ELLISON, L. W., J. BIRD, D. C. WEST, A. L. SORENG, T. C. REYNOLDS *et al.*, 1991 *TAN-1*, the human homolog of the Drosophila *Notch* gene, is broken by chromosomal translocations in T lymphoblastic neoplasms. Cell **66:** 649–661.

FERGUSON, E., and H. R. HORVITZ, 1985 Identification and characterization of 22 genes that affect the vulval cell lineages of the nematode *Caenorhabditis elegans*. Genetics **110:** 17–72.

GREENWALD, I., 1985 *lin-12*, a nematode homeotic gene, is homologous to a set of mammalian proteins that includes epidermal growth factor. Cell **43:** 583–590.

GREENWALD, I., and G. SEYDOUX, 1990 Analysis of gain-of-function mutations in the *lin-12* gene of *C. elegans*. Nature **346:** 197–199.

GREENWALD, I., P. STERNBERG and H. R. HORVITZ, 1983 The *lin-12* locus specifies cell fates in Caenorhabditis elegans. Cell **34:** 435–444.

HEDGECOCK, E. M., J. G. CULOTTI and D. H. HALL, 1990 The *unc-5*, *unc-6* and *unc-40* genes guide circumferential migrations of pioneer axons and mesodermal cells on the epidermis in C. elegans. Neuron **4:** 61–85.

HEDGECOCK, E. M., and R. K. HERMAN, 1995 The *ncl-1* gene and genetic mosaics of *Caenorhabditis elegans*. Genetics **141:** 989–1006.

HENDERSON, S. T., D. GAO, E. J. LAMBIE and J. KIMBLE, 1994 *lag-2* may encode a signalling ligand for the GLP-1 and LIN-12 receptors of *C. elegans*. Development **120:** 2913–2924.

HODGKIN, J., M. EDGLEY, D. L. RIDDLE and D. G. ALBERTSON, 1988 Genetic nomenclature, list of mapped genes, genetic map, physical maps, pp. 491–584 in *The Nematode Caenorhabditis elegans*, edited by W. B. WOOD. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

HORVITZ, H. R., and P. W. STERNBERG, 1991 Multiple intercellular signalling systems control the development of the *Caenorhabditis elegans* vulva. Nature **351:** 535–541.

HORVITZ, H. R., S. BRENNER, J. HODGKIN and R. K. HERMAN, 1979 A uniform genetic nomenclature for the nematode *Caenorhabditis elegans*. Mol. Gen. Genet. **175:** 129–133.

JOHNSEN, R. C., and D. BAILLIE, 1991 Genetic analysis of a major segment [LGV(left)] of the genome of *Caenorhabditis elegans*. Genetics **129:** 735–752.

KIMBLE, J., 1981 Alterations in cell lineage following laser ablation of cells in the somatic gonad of *C. elegans*. Dev. Biol. **87:** 286–300.

KIMBLE, J., and D. HIRSH, 1979 The postembryonic cell lineages of the hermaphrodite and male gonads in *Caenorhabditis elegans*. Dev. Biol **70:** 396–417.

KIMBLE, J., J. SULSTON and J. WHITE, 1979 Regulative development in the post-embryonic lineages of Caenorhabditis elegans, pp. 59–68 in *Cell Lineage, Stem Cells and Cell Determination*, edited by N. LEDOUARIN. Elsevier, New York.

LAMBIE, E., and J. KIMBLE, 1991a Genetic control of cell interactions in nematode development. Annu. Rev. Genet. **25:** 411–436.

LAMBIE, E., and J. KIMBLE, 1991b Two homologous regulatory genes,

lin-12 and *glp-1*, are functionally redundant. Development **112:** 231–240.

LIEBER, T., S. KIDD, E. ALCAMO, V. CORBIN and M. W. YOUNG, 1993 Anti-neurogenic phenotypes induced by truncated Notch proteins indicate a role in signal transduction and may point to a novel function for Notch in nuclei. Genes Dev. **7:** 1949–1965.

LINDSELL, C. E., C. J. SHANKER, J. BOUTEN and G. WEINMASTER, 1995 JAGGED: a mammalian ligand that activates Notch 1. Cell **80:** 909–917.

MAINE, E., and J. KIMBLE, 1989 Identification of genes that interact with *glp-1*, a gene required for inductive cell interactions in *Caenorhabditis elegans*. Development **105:** 133–143.

MAINE, E. M., and J. KIMBLE, 1993 Suppressors of *glp-1*, a gene required for cell communication during development in *Caenorhabditis elegans*, define a set of interacting genes. Genetics **135:** 1011–1022.

MELLO, C. C., B. W. DRAPER and J. R. PRIESS, 1994 The maternal genes *apx-1* and *glp-1* and establishment of dorsal-ventral polarity in the early C. elegans embryo. Cell **77:** 95-106.

PALKA, J., M. SCHUBIGER and H. SCHWANNIGER, 1990 Neurogenic and antineurogenic effects from modifications at the *Notch* locus. Development **109:** 167–175.

PRIESS, J. R., H. SCHNABEL and R. SCHNABEL, 1987 The *glp-1* locus and cellular interactions in early C. elegans embryos. Cell **51:** 601–611.

QIAO, L., J. L. LISSEMORE, P. SHU, A. SMARDEN, M. B. GELBER *et al.*, 1995 Enhancers of *glp-1*, a gene required for cell-signaling in *Caenorhabditis elegans*, define a set of genes required for germline development. Genetics **141:** 551–569.

REBAY, I., R. G. FEHON and S. ARTAVANIS-TSAKONAS, 1993 Specific truncations of Drosophila *Notch* define dominant activated and dominant negative forms of the receptor. Cell **74:** 319–329.

REBAY, I., R. J. FLEMING, R. G. FEHON, L. CHERBAS, P. CHERBAS *et al.*, 1991 Specific EGF repeats of *Notch* mediate interactions with *Delta* and *Serrate*: implications for *Notch* as a multifunctional receptor. Cell **67:** 687–699.

ROBBINS, J., B. J. BLONDEL, D. GALLAHAN and R. CALLAHAN, 1992 Mouse mammary tumor gene *int-3*: a member of the *Notch* gene family transforms mammary epithelial cells. J. Virol. **66:** 2594–2599.

ROEHL, H., and J. KIMBLE, 1993 Control of cell fate in *C. elegans* by a GLP-1 peptide consisting primarily of ankyrin repeats. Nature **364:** 632–635.

SEYDOUX, G., and I. GREENWALD, 1989 Cell autonomy of *lin-12* function in a cell fate decision in C. elegans. Cell **57:** 1237–1245.

SEYDOUX, G., C. SAVAGE and I. GREENWALD, 1993 Isolation and characterization of mutations causing abnormal eversion of the vulva in *Caenorhabditis elegans*. Dev. Biol. **157:** 423–436.

SIMON, M. A., D. L. BOWTELL, G. S. DODSON, T. R. LAVERTY and G. M. RUBIN, 1991 *Ras1* and a putative guanine nucleotide exchange factor perform crucial steps in signaling by the *sevenless* protein tyrosine kinase. Cell **67:** 1–16.

STERNBERG, P., and H. R. HORVITZ, 1986 Pattern formation during vulval development in C. elegans. Cell **44:** 761-772.

STRUHL. G., K. FITZGERALD and I. GREENWALD, 1993 Intrinsic activity of the LIN-12 and NOTCH intracellular domains *in vivo*. Cell **74:** 331–345.

SULSTON, J., and H. R. HORVITZ, 1977 Postembryonic cell lineages of the nematode *Caenorhabditis elegans*. Dev. Biol. **56:** 110-156.

SULSTON, J. E., D. G. ALBERTSON and J. N. THOMSON, 1980 The *Caenhorhabditis* male: postembryonic development of non-gonadal structures. Dev. Biol. **78:** 542–576.

SUNDARAM, M., and I. GREENWALD, 1993 Suppressors of a *lin-12* hypomorph define genes that interact with both *lin-12* and *glp-1* in *Caenorhabditis elegans*. Genetics **135:** 765–783.

TAX, F. E., J. J. YEARGERS and J. H. THOMAS, 1994 Sequence of *C. elegans lag-2* reveals a cell signalling domain shared with *Delta* and *Serrate* of *Drosophila*. Nature **358:** 150-154.

WEINMASTER, G., V. J. ROBERTS and G. LEMKE, 1991 A homolog of *Drosophila Notch* expressed during mammalian development. Development **113:** 199–205.

WEINMASTER, G., V. J. ROBERTS and G. LEMKE, 1992 *Notch2*, a second mammalian *Notch* gene. Development **116:** 931–941.

YOCHEM, J., and I. GREENWALD, 1989 *glp-1* and *lin-12*, genes implicated in distinct cell-cell interactions in C. elegans, encode similar transmembrane proteins. Cell **58:** 553–563.

YOCHEM, J., K. WESTON and I. GREENWALD, 1988 *C. elegans lin-12* encodes a transmembrane protein with overall similarity to Drosophila *Notch*. Nature **335:** 547-550.

XU, T., I. REBAY, R. J. FLEMING, T. N. SCOTTGALE and S. ARTAVANIS-TSAKONAS, 1990 The *Notch* locus and the genetic circuitry involved in early *Drosophila* neurogenesis. Genes Dev. **4:** 464–475.

Communicating editor: I. GREENWALD