The *let-60* Locus Controls the Switch Between Vulval and Nonvulval Cell Fates in *Caenorhabditis elegans*

Min Han, Raffi V. Aroian and Paul W. Sternberg

Howard Hughes Medical Institute, Division of Biology, California Institute of Technology, Pasadena, California 91125

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ABSTRACT

During induction of the *Caenorhabditis elegans* hermaphrodite vulva by the anchor cell of the gonad, six multipotent vulval precursor cells (VPCs) have two distinct fates: three VPCs generate the vulva and the other three VPCs generate nonspecialized hypodermis. Genes that control the fates of the VPCs in response to the anchor cell signal are defined by mutations that cause all six VPCs to generate vulval tissue (Multivulva or Muv) or that cause all six VPCs to generate hypodermis (Vulvaless or Vul). Seven dominant Vul mutations were isolated as dominant suppressors of a *lin-15* Muv mutation. These mutations are dominant alleles of the gene *let-60*, previously identified only by recessive lethal mutations. Our genetic studies of these dominant Vul recessive lethal mutations, recessive lethal mutations, intragenic revertants of the dominant Vul mutations, and the closely mapping semidominant multivulva *lin-34* mutations suggest that: (1) loss-of-function mutations of *let-60* are recessive lethal at a larval stage, but they also cause a Vul phenotype if the lethality is rescued maternally by a *lin-34* gain-of-function mutation. (2) The dominant Vul alleles of *let-60* are dominant negative mutations whose gene products compete with wild-type activity. (3) *lin-34* semidominant Muv alleles are either gain-of-function mutations of *let-60* or gain-of-function mutations of an intimately related gene that elevates *let-60* activity. We propose that *let-60* activity controls VPC fates. In a wild-type animal, reception by a VPC of inductive signal activates *let-60*, and it generates into a vulval cell type; in absence of inductive signal, *let-60* activity is low and the VPC generates hypodermal cells. Our genetic interaction studies suggest that *let-60* acts downstream of *let-23* and *lin-15* and upstream of *lin-1* and *lin-12* in the genetic pathway specifying the switch between vulval and nonvulval cell types.

VULVAL development in *Caenorhabditis elegans* has been studied intensively as a model system to understand the mechanism by which cell-cell interactions specify the formation of a pattern of cell types during animal development (for recent reviews see HORVITZ 1988; STERNBERG 1990). During postembryonic development of the *C. elegans* hermaphrodite, each of six vulval precursor cells (VPCs) has the potential to generate either vulval cells or hypodermal cells. During vulval induction, however, only three of the six VPCs are specified to become the two VPC types, 1° and 2°, by a graded signal from the anchor cell of the gonad (Figure 1). 1° and 2° precursor cells divide further to form the vulva. The other three VPCs remain in the ground state (3° cell type) and generate progeny that fuse with a large syncytial hypodermal cell (SULSTON and HORVITZ 1977; KIMBLE 1981; STERNBERG and HORVITZ 1986; STERNBERG 1988). The relative positions of the VPCs with respect to the anchor cell determine which of them are induced to 1° or 2° cells (STERNBERG and HORVITZ 1986). Besides the inductive signal from the anchor cell, a "lateral signal" acts between the VPCs to prevent the immediate neighbors of a presumptive 1° cell from also becoming 1° cells (STERNBERG 1988).

Mutations that result in misspecification of VPC fates have defined genes necessary for the normal patterning process (HORVITZ and SULSTON, 1980; SULSTON and HORVITZ 1981; GREENWALD, STERNBERG and HORVITZ 1983; FERGUSON and HORVITZ 1985, 1989; FERGUSON, STERNBERG and HORVITZ 1987). Vulvaless (Vul) mutations cause fewer than three VPCs to generate vulval cells, often resulting in an egg-laying defect (Figure 1). Multivulva (Muv) mutations cause more than three VPCs to generate vulval cells and undergo morphogenesis to produce additional vulval-like structures (Figure 1). These mutations define three major classes of genes: (1) "Vul" genes are necessary for 1° and 2° cell fates. (2) "Muv" genes promote the 3° cell fate. (3) *lin-12* is necessary for determining 2° cell fates. Genetic interactions among these three classes of mutants suggest that there are two interacting genetic pathways that specify the fates of VPCs (STERNBERG and HORVITZ 1989): Vul and Muv genes act in a pathway that determines whether a VPC becomes a 3° (nonvulval) or a non-3° (vulval) cell, and the *lin-12* gene functions in a separate pathway that determines whether a VPC becomes a 2° or non-2° cell. The sites of action of the Muv and Vul genes are not known, but based on

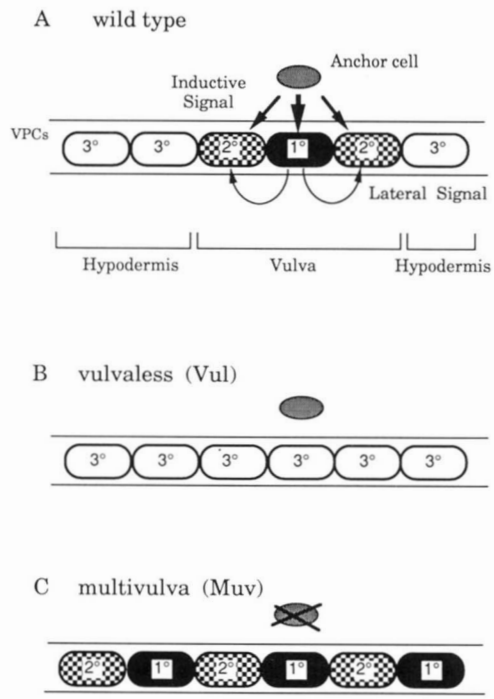

FIGURE 1.—Vulval induction in wild type and mutant *C. elegans* hermaphrodites. (A) The six multipotent vulval precursor cells (VPCs), P3.p, P4.p, P5.p, P6.p, P7.p and P8.p, are located just ventral to the gonad. Their fates are regulated by the anchor cell of the gonad. According to the current model (STERNBERG and HORVITZ 1986; STERNBERG 1988), a graded signal from the anchor cell induces the central three cells (P5.p, P6.p and P7.p) to generate vulval sublineages (1° or 2°) as opposed to a nonvulval sublineage (hypodermal cell, 3°). The 1° VPC (P6.p in wild type) prevents neighboring VPCs from also becoming 1° via a lateral signal. (B) In vulvaless mutant hermaphrodites, all six VPCs adopt the nonvulval fate (3°) even in presence of the inductive signal. (C) In multivulva mutant hermaphrodites, all six VPCs generate vulval sublineages (1° or 2°) even if anchor cell is ablated.

gonadal ablation and epistasis experiments, most do not act only in the anchor cell (FERGUSON, STERNBERG and HORVITZ 1987). Recent genetic mosaic experiments indicate that *lin-15*, a Muv gene, can act in cells other than the VPCs (R. HERMAN and E. HEDGECOCK, personal communication). However, since the outcome of the signaling pathway is the control of VPC fates, many of the Vul and Muv genes are expected to act within the VPCs in the response to the inductive signal. Understanding how these Muv and Vul genes interact with each other to specify VPC fates is the key to understanding the molecular genetics of this process.

Given the expected complexity of such a cellular regulatory pathway, we predicted that all essential components were not yet identified. To further dissect this pathway, we have taken the approach of isolating mutations that suppress existing Muv mutations. This approach might not only improve the efficiency at which the mutations that are directly involved in VPC induction are isolated but also might indicate how the new and existing genes interact in the pathway. In this paper we describe the isolation and characterization of dominant extragenic suppressors of the Muv mutation *lin-15(n309)*. These dominant suppressor mutations result in a dominant vulvaless phenotype, and are dominant negative ("antimorphic") alleles of *let-60*, previously identified only by recessive mutations with a lethal phenotype. We show, by analysis of the dominant and recessive alleles of *let-60*, that *let-60* function is essential for specifying 1° and 2° vulval cell types, since reduction or elimination of the gene activity results in a vulvaless phenotype. We also present suggestive evidence that the *lin-34* Muv mutations are gain-of-function alleles of *let-60*; the Muv phenotype (where more than three VPCs become vulval cell types) might be caused by *let-60* hyperactivity. Our study of the genetic interactions of *let-60* and other genes in the vulval induction pathway indicates that *let-60* acts downstream of *let-23* and *lin-15* but upstream of *lin-1* and *lin-12*.

MATERIALS AND METHODS

General methods: Methods for culturing, handling, mutagenesis, and genetic manipulation of *C. elegans* were as described by BRENNER (1974). All experiments were performed at 20°. The standard *C. elegans* cellular and genetic nomenclature, defined by SULSTON and HORVITZ (1977) and HORVITZ *et al.* (1979), respectively, is followed in this paper. "VPCs" (vulval precursor cells) are the six cells (P3.p, P4.p, P5.p, P6.p, P7.p and P8.p) that have the potential to participate in vulval development.

Strains: The standard wild type strain N2 and most other strains with various genetic markers were from BRENNER (1974) or the Caenorhabditis Genetics Center. The alleles of various mutants used in the paper are listed below. The source of strains other than BRENNER (1974) or the Genetics Center are also indicated.

LGI: *dpy-5(e61)*.

LGII: *rol-6(e187)*; *unc-4(e120)*; *let-23(mn23)* and *mnC1[dpy-10(e128) unc-52(e444)](II)*(HERMAN 1978).

LGIII: single mutations: *unc-36(e251)*; *unc-32(e189)*.

Linked double mutations: *lin-12(n137) dpy-19(e1259)* (FERGUSON and HORVITZ 1985) and *unc-32(e189) lin-12(n676 n909)* (GREENWALD, STERNBERG and HORVITZ 1983).

LGIV: single mutations: *dpy-20(e1282)*; *unc-22(s7)* (MOERMAN and BAILLIE 1979); *nT1(IV;V)* (FERGUSON and HORVITZ 1985); *lin-34(n1046)* (FERGUSON and HORVITZ 1985); *sDf8* (MOERMAN and BAILLIE 1981); *nDf27* (ELLIS and HORVITZ 1986). *lin-1(e1275)* (HORVITZ and SULSTON 1980).

Linked multiple mutations: *unc-24(e138) mec-3(e1338)dpy-20(e1282)* (provided by M. CHALFIE's laboratory), *dpy-20(e1362) unc-31(e169)*, *dpy-20(e1282) unc-22(s7)* (provided by D. BAILLIE's laboratory); *lin-3(n1059) dpy-20(e1282)* (provided by R. HILL); *unc-8(e49) dpy-20(e1362)*; *let-60(s59) unc-22(s7) unc-31(e169)* and *let-65(s254) unc-22(s7)* (ROGALSKI, MOERMAN and BAILLIE 1982), *let-100(s1160) unc-22(s7) unc-31(e169)*, *let-60(s1124) unc-22(s7) unc-31(e169)* and *let-60(s1155) unc-22(s7) unc-31(e169)* (CLARK *et al.* 1988).

LGV: *dpy-11(e224)*; *him-5(e1490)* (HODGKIN, HORVITZ and BRENNER 1979).

LGX: *lon-2(e678)*; *unc-3(e151)*; *lin-15(n765)* and *lin-15(n309)* (FERGUSON and HORVITZ 1985).

Analysis of vulval developmental defects: Criteria for recognition of egg-laying defect (Egl) and multivulva (Muv) phenotype were previously described by HORVITZ and SULSTON (1980). For counting percentage Muv, adult animals with one or more pseudovulvae (ventral protrusions) in addition to a vulva were classified as Muv. The vulvaless (Vul) phenotype is examined by observing L3 and L4 larvae under Nomarski optics. The percentage of VPC induction is determined as the percentage of VPCs induced to vulval cell type relative to wild type. In a completely vulvaless animal, each of the six VPCs divide once to generate daughters that fuse with the syncytial hypodermis (the 3° fate). The induction in these animals is said to be 0%. In a wild-type hermaphrodite, three of the six VPCs are induced to divide further than the first round of division, producing the progeny characteristic of 1° and 2° VPCs (STERNBERG and HORVITZ 1986). The induction of these further divisions is said to be 100%. Animals with fewer than three cells induced to further division have less than 100% induction (Vul); animals with more than three VPCs induced have more than 100% induction (Muv). According to this definition, if only one of the two daughters of a VPC divided further to generate vulval tissue, the induction is one-half-cell. Therefore, an individual animal with 50% VPC induction would have one and "one-half" VPCs induced. See STERNBERG and HORVITZ (1986) for a discussion of such "hybrid" lineages.

To eliminate the signal producing anchor cell, we ablated the somatic gonad precursor cells during the L1 larval stage (SULSTON and WHITE 1980). The laser microbeam system used for ablation was described previously (AVERY and HORVITZ 1987; STERNBERG 1988).

Isolation of *lin-15(n309)* suppressors: Strain MT309 [*lin-15(n309)*] was mutagenized with ethylmethane sulfonate (EMS) and the F_2 progeny were screened for non-Muv revertants. In most cases, candidates were picked with an egg-laying defect (Egl) phenotype. Each candidate was transferred to a new plate and those that gave viable non-Muv progeny were characterized further. Ten revertants were isolated after screening about 100,000 F_1 mutagenized gametes. All revertants have an Egl plate phenotype and are defective in VPC induction. The dominant nature of seven alleles was established as follows. For each of these seven revertants, fewer than one-fourth of the healthy progeny of an individual non-Muv animal were Muv (other Muv progeny exploded during adulthood). Muv animals were individually picked to agar plates, and found to segregate only Muv progeny as the original parent *lin-15* strain, indicating loss of the suppressor. In addition, any suppressed non-Muv animals always segregate a small portion of Muv animals along with the majority of non-Muv progeny. These results indicate (1) the suppressor mutations in these strains are heterozygous; (2) these mutations are recessive lethal; (3) the suppressor and Egl phenotypes are dominant. All seven revertants were crossed with wild-type males and the suppressor mutations were recovered without the *lin-15* mutations in the background. We refer to these alleles as *let-60(dn)*, where *dn* is dominant negative (see RESULTS).

Genetic mapping of the *let-60(dn)* mutations: The seven dominant suppressor alleles were mapped by crossing the hermaphrodite mutants with males carrying genetic markers on different linkage groups and following the Egl phenotype (the plate phenotype of vulvaless animals observable under the dissecting microscope) in the F_1 progeny. All of the dominant alleles proved to be linked to linkage group IV. The results of three point mapping with markers on chromosome *IV* are shown in Table 1. A genetic map with *let-60*, *lin-34* and relevant nearby genes is shown in Figure 2.

TABLE 1

Genetic three-point mapping of *let-60(dn)* on chromosome *IV*

Markers			Recombinants with *let-60*/total Recombinants[a]	
A	B	*let-60* allele	A non-B[b]	B non-A[c]
unc-24	*dpy-20*	*sy100*	6/6	0/8
		sy92	7/7	0/3
		sy93	3/3	0/14
		sy94	7/7	0/3
		sy95	15/15	0/5
		sy99	19/19	0/11
		sy101	11/11	0/6
dpy-20	*unc-31*	*sy99*	0/7	8/8
dpy-20	*unc-22*	*sy100*	0/20	2/2
		sy99	0/4	2/2
let-65	*unc-22*	*sy93*		1/3
lin-3[d]	*dpy-20*	*sy100*		2/129
		sy93		0/45
		sy94		0/30

In each mapping experiment, *let-60(dn)* alleles were placed in *trans* to two linked markers on chromosome *IV*. Recombinants resulting from recombination between the two markers were selected and scored for the *let-60(dn)* phenotype.

[a] Number of recombinant animals that retain the *let-60* alleles out of total recombinants homozygous for one marker gene.

[b] Recombinants with genotype homozygous for marker A but not for marker B.

[c] Recombinants with genotype homozygous for marker B but not for marker A.

[d] The recessive lethal allele *n1059* is used for *lin-3*.

Isolation of intragenic revertants of *let-60(dn)*: The dominant Vul phenotype of *let-60(dn)* was reverted by screening for the appearance of non-Egl F_1 animals following EMS mutagenesis of *let-65* + + *unc-22*/+ *let-60(sy101dn)* *dpy-20* + hermaphrodites. F_2 eggs were picked from each plate with nonEgl F_1 animals. Candidate F_2 non-Egl animals were picked and analyzed further by crossing with marker strains. *sy101 sy163*, isolated by this method, suppresses the dominant Vul phenotype of *sy101* completely. The suppressor is tightly linked to *sy101dn*.

The dominant suppression phenotype of *let-60(dn)* was reverted by screening for the reappearance of the Muv phenotype of *lin-15(n309)* in a *let-60(dn)* background. Two *let-60(dn)* alleles, *sy94* and *sy101*, were used to construct strains with genotypes of the form *unc-24* + *let-60(dn)* +/+ *lin-3* + *dpy-20(e1282)*; *lin-15(n309)/lin-15(n309)*. The *lin-3* mutation used (*n1059*) is a recessive lethal allele. These strains were constructed by crossing *lin-3 dpy-20*/++ males to +*let-60(dn)*+/*unc-24*+*dpy-20*; *lin-15* hermaphrodites. F_1 cross progeny and F_2 progeny were picked and the animals with desired genotype were selected. Hermaphrodites homozygous for *lin-15* were identified by the Muv phenotype of the viable Dpy recombinants (resulting from recombination between *lin-3* and *let-60*). The *lin-15* Muv phenotype is completely suppressed in these strains, except for the rare Dpy recombinants, which can be easily distinguished from nonrecombinants. These strains were mutagenized with EMS and F_1 progeny were screened for nonDpy Muv animals resulting from new suppressor mutations. Since the experiment was designed to isolate intragenic loss-of-function mutations, only F_1 animals were screened. *sy127* was isolated from a mutagenized culture containing both the strain with *sy94dn* and the strain with *sy101dn*, which had

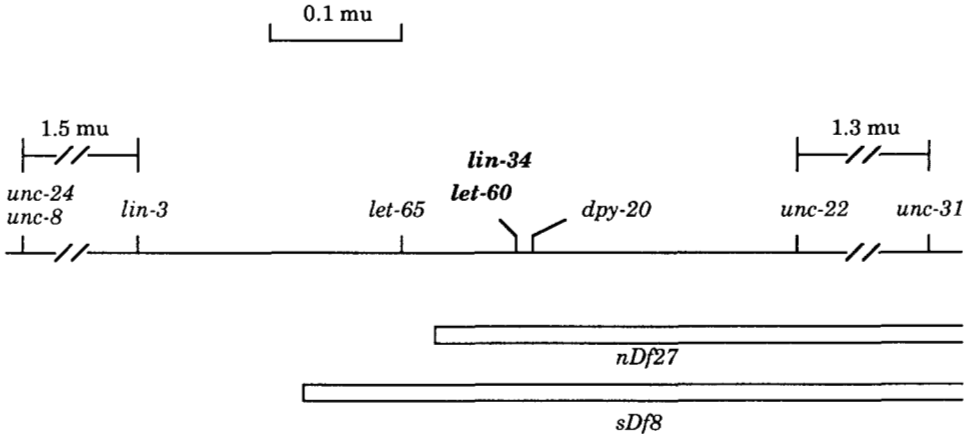

FIGURE 2.—Genetic map of relevant genes near *let-60* on chromosome *IV*. *let-60-lin-34* and seven other genes are shown along with the left breakpoints of two deficiencies. Relative distance between the genes other than *let-60-lin-34* are based on the current genetic map (EDGLEY and RIDDLE 1989). The relative distance between *let-60* and other genes were determined based on the genetic data given in Table 1 and MATERIAL AND METHODS. mu, map unit.

been inadvertently mixed. Therefore, the precise genotype of the new strain is not clear, and is designated as *syXdn sy127*. A dominant Muv mutation, *sy130gf*, was also isolated from the strain with *sy94dn* as the *let-60* allele; *sy130gf* was localized in *trans* on the *lin-3 dpy-20* chromosome. Both *syXdn sy127* and *sy130* were characterized further by crossing out the *lin-15* mutation.

Intragenic revertants should be recovered at similar frequency to that of recovering loss-of-function mutations in a wild-type strain (typically, between 1/2000 and 1/5000 EMS-mutagenized gametes; BRENNER 1974; GREENWALD and HORVITZ 1980). The frequency of isolating intragenic suppressors in this screen is tenfold lower: approximately 1/35,000 EMS-mutagenized gametes. One likely explanation is that *lin-15(n309)* animals are slow growing; the intragenic revertant (*let-60(lf/+; lin-15*) may be less viable or fecund than the parental strain (*let-60(dn)/+; lin-15*). In this screen, we have picked more than 15 Muv animals as candidates for harboring suppressor mutations, but only two gave viable progeny.

Genetic mapping of *let-60(dn)* revertants: All three new mutations, *sy127*, *sy163* and *sy130*, were mapped with respect to nearby markers. *sy127* was mapped relative to *unc-8*, *unc-31* and *dpy-20* (see Figure 2). All three Unc-8 nonDpy-20 recombinants from a strain of genotype *unc-8 + + dpy-20(e1362)/+ unc-24 sy127* +segregated *sy127*. All four Unc-31 nonUnc-22 nonLet recombinants from *unc-8 + sy127+ +/+ let-100 + unc-22 unc-31* heterozygotes picked up *sy127*. Therefore, *sy127* is located between *unc-8* and *unc-31* and close to *dpy-20* and *unc-22*. The distance between *unc-8* and *unc-31* is approximately 3 map units. *sy163* was mapped relative to *unc-24* and *unc-31*. All nine Unc-24 nonUnc-22 recombinants from a strain of genotype *unc-24 ++ unc-22/ + sy163 dpy-20 +* segregated *sy163* and *dpy-20*. All ten Unc-31 nonLet recombinants from strains of genotype *+let-100 ++ unc-22 unc-31/unc-24 + sy163 dpy-20 ++* and *let-100 ++ unc-22 unc-31/+ sy163 dpy-20 ++* segregated *sy163*. Therefore, *sy163* is located between *unc-24* and *unc-31* and close to *unc-22*.

Four-point mapping for *sy130* was done by constructing a *let-65 ++ unc-22/+ lin-34(sy130gf) dpy-20+* heterozygote and screening for Unc nonLet recombinants. *lin-34(sy130gf)* confers a semidominant Muv phenotype (see Table 2). Among 35 Unc recombinants selected, 23 segregated Dpy and Muv progeny, 12 segregated neither Dpy nor Muv progeny, and none segregated Muv nonDpy progeny. Therefore *sy130* maps between *let-65* and *unc-22* and close to *dpy-20*. We also isolated two animals of genotype *lin-34(n1046gf) dpy-20/let-60(sy100) dpy-20* as recombinants from *lin-34(n1046gf) + unc-22/let-60(sy100) dpy-20 +* heterozygotes, placing *lin-34* to the left of *dpy-20* (Figure 2). Similar data placing *lin-34* just left of *dpy-20* in the region of *let-60* have been obtained by G. BEITEL and R. HORVITZ, and by G. JONGEWARD (both personal communications).

Complementation tests: The following tests were performed.

let-60(dn) with deficiencies: For *sDf8* and *nDf27*, *+let-60(sy100) dpy-20+/lin-34(n1046) ++ unc-22*; *him-5* males were crossed to hermaphrodites carrying deficiencies in *trans* to *nT1* (a chromosomal translocation between linkage groups IV and V that balances the right half of IV). The presence of Unc cross progeny (*Df/unc-22*) indicated that the mating was successful. Since these deficiencies uncover the *dpy-20* mutation, the absence of viable Dpy progeny indicates the failure of *let-60* to complement the deficiencies.

let-60(dn) with let-60(dn): For *sy92* and *sy95*, *++ let-60(sy100) dpy-20/unc-24 lin-34(n1046) ++* males were crossed with *unc-24 + let-60(dn) + /unc-24 mec-3 +dpy-20* hermaphrodites. Only rare nonUnc nonDpy animals are found among the cross progeny (*e.g*, two nonDpy nonUnc hermaphrodites among more than 20 Dpy nonUnc hermaphrodites from one cross). These rare nonDpy nonUnc F_1 animals were recombinants (*unc-24 + dpy-20(e1282)/+ lin-34(n1046)+*) because they all segregated both Dpy Unc (*unc-24 dpy-20*) and Muv nonUnc (*lin-34(n1046gf)*) F_2 hermaphrodites. Neither *+ sy100 dpy-20/unc-24 sy95 +* nor *+sy100 dpy-20/unc-24 sy92 +* animals were found among the cross progeny, and thus these genotypes were inferred to be lethal. For *sy94*, a similar result was obtained with *unc-24 + let-60(sy100) dpy-20(e1282)/+ lin-34(n1046gf) ++ unc-22* males crossed with *unc-24 ++ let-60(sy94dn) +/+ lin-3 lin-34(sy130gf) +dpy-20* hermaphrodites.

let-60(dn) with let-60(lf): let-60(s1124) and *let-60(s59)*, previously isolated in a screen for recessive lethal mutations (CLARK *et al.* 1988), are loss-of-function alleles (see RESULTS). Males of genotype *let-60(s1124) + unc-22unc-31/+ dpy-20(e1282) ++*; *him-5/+* were crossed with *++ let-60(sy100dn) dpy-20/lin-3 lin-34(sy130gf) +dpy-20* hermaphrodites. Phenotypically nonDpy F_1 hermaphrodites were examined for vulval induction and further analyzed. Among more than 50 nonDpy F_1 progeny examined, half were egg-laying competent (nonEgl) and were determined to be *++ let-60(s1124) + unc-22 unc-31/lin-3 lin-34(sy130gf) + dpy-20 ++*. (Their progeny were used to examine the vulval induction of *s1124/s1124* progeny). The other half of the F_1 progeny were Egl and they segregated only dead larvae as the F_2. The genotype of this latter class must be *let-60(s1124) + unc-22 unc-31/let-60(sy100) dpy-20 ++*, and they were rescued by maternal activity of *lin-34(sy130gf)* (see Figure 5). Therefore, *sy100* fails to complement *s1124*. A similar

analysis was carried out for *let-60(s59)*: *let-60(s59) unc-22/ dpy-20(e1362); him-5* males were crossed with *lin-34(sy130gf) dpy-20/let-60(sy100) dpy-20*. As a control, we crossed N2 males with *let-60(sy100dn) dpy-20/lin-34(sy130gf)dpy-20* hermaphrodites and nonDpy F_1 hermaphrodites were examined for vulval induction and their genotype inferred by segregation as above. In addition, *s1124, s59* and *s1155*, the third previously isolated *let-60(lf)* allele, were tested for complementation with *sy100* for lethality in separate experiments. *let-60(lf) + unc-22/+dpy-20+* males were crossed with *let-60(sy100dn) dpy-20 +/lin-3(n1046gf) + unc-22* hermaphrodites. F_1 Egl hermaphrodites among nonDpy nonUnc cross progeny were picked and found to generate only dead larvae as F_2 progeny. *s1124* also fail to complement *sy99dn* and *sy101dn* in similar tests. *sy93dn* also failed to complement *let-60(s1124)*. However, from the cross between *let-60(s1124)/+* males and *sy93dn/sy93dn* hermaphrodites, a low percentage of *sy93dn/s1124* animals (4 out of more than 100) have been found among cross progeny. This observation is not surprising since *sy93dn* homozygotes are viable even though they grow slowly and display uncoordinated movement in addition to being vulvaless.

let-60(dn) revertants with let-60(dn): Both *cis*-dominant revertants of *let-60(dn), sy101dn sy163* and *syXdn sy127*, were tested for complementation for viability with *let-60(sy100)*. For *sy101dn sy163, let-60(sy-100) dp-20 +/+ lin-34(n1046gf) unc-22* males were crossed with *let-100 ++ unc-22 unc-31/+ sy163 dpy-20 + unc-31* hermaphrodites; no viable Dpy animals were found among cross progeny. For *syXdn sy127, unc-24 let-60(sy100dn)dpy-20 +/+ lin-34(n1046gf) + unc-22* males were crossed with *unc-24 syXdn sy127 +/+ lin-34(n1046gf) unc-22; lin-15(n765)* hermaphrodites; only rare recombinant Unc-24 homozygotes were found among the cross progeny. Therefore, *sy101dn sy163* and *syXdn sy127* fail to complement *let-60(sy100)* for viability.

let-60(dn) revertants with deficiency: syXdn sy127 ++/++ dpy-20 unc-22; him-5 males were crossed with *sDf8/unc-24 mec-3 dpy-20* hermaphrodites. F_1 nonDpy animals were picked and tested for a twitching phenotype in 1% nicotine solution (indicating a genotype of *unc-22/+* or *sDf8/+*). It was found among more than 30 F_1 nonDpy animals tested, only one hermaphrodite shows the twitching phenotype and it is sterile. Therefore, *syXdn sy127/sDf8* is lethal.

***trans*-Heterozygotes:** The following tests were performed.

lin-34 with lin-34: sy130 was isolated as a dominant suppressor of the dominant suppression phenotype of *let-60(dn)*. *sy130* was identified as a putative *lin-34gf* allele by crossing *lin-34(n1046) unc-22(s7)/++* males into the revertant hermaphrodites of genotype *unc-24 + let-60(sy94dn)/+ lin-3 + sy130 dpy-20(e1282)*. Ninety-eight percent of the F_1 progeny with genotype *sy130/n1046* were found to be Muv.

lin-34 with deficiency: lin-34(n1046gf) + unc-31/+ dpy-20+ males were mated with *++ sDf8/unc-24 mec-3 dpy-20* hermaphrodites, which are Dpy. NonDpy F_1 hermaphrodites which should be *lin-3(n1046gf) unc-31/sDf8 +*, were picked for analysis. The percentage of Muv animals among nonUnc-31 adult animals was counted under a dissecting microscope. Seven percent (of 512 animals) were Muv. A strain of genotype *+lin-34(n1046gf) unc-22+ /unc-24 mec-3 + dpy-20+* was constructed for a *lin-34/+* control; 11% (of 467 heterozygous adult animals) were Muv.

lin-34 and let-60(dn): lin-34(n1046gf) was placed in *trans* to each of six *let-60(dn)* alleles (the recessive viable allele *sy93* was not tested). For *let-60(sy100dn)* and *let-60(sy101dn)*, strains with genotype *lin-34(n1046gf) + unc-22/ let-60(dn)dpy-20+* were constructed and analyzed. For *let-60(sy92dn), let-60(sy94dn)* and *let-60(sy95dn)*, strains with genotype *+ lin-34(n1046)unc-22/unc-24 let-60(dn) +* were constructed and analyzed. *lin-34(sy130gf) dpy-20* was placed in *trans* to *let-60(sy100dn) dpy-20, let-60(sy99dn) unc-31* and *let-60(s1124) unc-22 unc-31*. When each of three *let-60(dn)* alleles, *sy100, sy95* or *sy92* was placed in *trans* to *lin-34*, approximately 1/6 to 1/4 the progeny of the heterozygous parents are homozygous *let-60(dn)/let-60(dn)*. These homozygotes are Vul and segregate only dead larvae as their progeny. We have also constructed similar *lin-34(gf)/let-60(dn)* heterozygotes with *him-5* in background so that we could examine the mating ability of the male animals (HODGKIN 1983). Individual L4 males of these strains was placed in a plate containing three to four hermaphrodites with either Unc (*unc-24*) or Dpy Unc (*dpy-20* and *unc-31*) phenotypes. Among four *let-60(dn)* allele examined, some of the males containing *sy100* (6 of 32), *sy94* (5 of 26), or *sy92* (13 of 22) were able to mate when in *trans* to *lin-34(n1046gf)*; none of the *sy101/n1046* heterozygous males were able to mate (none of 20). By contrast, 12 of 38 *lin-34(n1046gf) + unc-22 +/+ dpy-20 + unc-31; him-5* males were able to mate.

Construction and analysis of double mutants: The following methods were used.

let-60(s1124) with lin-15(n309): Heterozygous *+ let-60(s1124) + unc-22(s7) unc-31(e169)/unc-8(e49) + dpy-20(e1362) ++; him-5/+* males were mated with *dpy-20(e1282); lin-15(n309)/lin-15(n309)* hermaphrodites. NonDpy F_1 hermaphrodites were picked to new plates. Each F_1 segregated Muv F_2 animals, which continued to propagate all Muv progeny. These Muv animals are heterozygous for the *unc-22* mutation but segregate mostly *unc-22* homozygotes as dead larvae, indicating that the genotype of the Muv animals is *let-60(s1124) + unc-22 unc-31/+ dpy-20 ++; lin-15*. Unc-22 animals that survive (L4 larvae or young sterile adults) were examined under Nomarski optics, and no vulval induction were found among 10 animals examined.

let-60(sy100dn) with lin-1(e1275): We first constructed a strain with *lin-1* linked to *unc-24* and *unc-22(s7)*. We then crossed *lin-1 unc-24 unc-22/+++* males with *+let-60(sy100dn) dpy-20 +/lin-34(n1046gf) ++ unc-22* hermaphrodites. The heterozygous cross progeny [*++ let-60(sy100dn) dpy-20/lin-1 unc-24 ++ unc-22*] were individually picked and their progeny were screened for Muv recombinants. Since *let-60(sy100dn)* and *dpy-20* are very tightly linked to each other [about 0.01 map units (m.u.)] and far from *lin-1* (>10 m.u.), nonUnc Muv animals almost exclusively resulted from recombination between *lin-1* and *unc-24*. More than six independent nonUnc Muv recombinants (*lin-1 + let-60(sy100) dpy-20 +/lin-1 unc-24 ++ unc-22*) were picked, and found to segregate Dpy Muv progeny [*lin-1 let-60(sy100) dpy-20*)]. The *lin-1* mutation suppresses both the lethal and Vul phenotype of *let-60(sy100dn)* homozygotes (see RESULTS).

let-60(s1124) with lin-1(e1275): A strain with *lin-1* linked to *dpy-20(e1282)* was constructed. We then crossed *lin-1 dpy-20/++* males with *let-60(s1124) + unc-22 unc-31/+dpy-20(e1362) + unc-31* hermaphrodites. The heterozygous *lin-1 +dpy-20 ++/+ let-60(s1124) + unc-22 unc-31* progeny were picked, and their progeny were screened for nonDpy Muv recombinants. Again, since *let-60* is very close to *dpy-20* and far away from *lin-1*, the nonDpy Muv animals all resulted from recombination between *lin-1* and *let-60* and gave rise to animals of genotype *lin-1 let-60(s1124) + unc-22 unc-31/lin-1 + dpy-20(e1282) ++*. The progeny of these recombinants were examined. Only a small number of Unc-22 animals were found on each plate (about 1/4 of Dpy Muv animals); all these animals were Muv and sterile. *let-*

60(s1124) homozygotes from heterozygous mothers often yield occasional survivors, but these survivors are Vul.

let-60(sy100dn) with lin-12(n137): A strain with genotype *dpy-19 + lin-12(n137)/+ unc-32 lin-12(n676 n909); him-5* (MT2375; P. STERNBERG and R. HORVITZ, unpublished) was used for the construction. *n137* is a dominant allele of *lin-12. n676 n909* is a *lin-12(d)* mutant plus an intragenic revertant resulting in loss of *lin-12* function (GREENWALD, STERNBERG and HORVITZ 1983). MT2375 males were mated to *++ let-65 + unc-22/unc-24 mec-3 + dpy-20+* hermaphrodites. The male cross progeny (showing Lin-12(d) phenotype) were picked and mated to *unc-36; + let-60(sy100dn) dpy-20/lin-34(n1046gf)++* hermaphrodites. Hermaphrodite progeny heterozygous for the *unc-22* mutation were selected with 1% nicotine (MOERMAN and BAILLIE 1979). Hermaphrodites with the Lin-12(d) phenotype (Egl with five small ventral protrusions) were picked. Those broods segregating *unc-36; let-60(s100dn) +/let-65 + unc-22* animals were identified, and their genotype was determined to be *+dpy-19 lin-12(n137)/unc-36 ++; +let-60(sy100) dpy-20 +/let-65 ++ unc-22.* Animals with this genotype display the Lin-12(d) phenotype. Analogous experiments were done with *let-60(sy99dn)* and *let-60(sy94dn)* with similar results.

let-60(s1124) with lin-12(n137): MT2375 males (see above) were crossed with *+ let-60(s1124) + unc-22 unc-31/unc-8 + dpy-20 ++* hermaphrodites. F_1 hermaphrodites heterozygous for *unc-22* (show twitching phenotype in 1% nicotine) were picked at the L4 stage. Egl adults with *lin-12(d)* phenotype (with five small ventral protrusions and Egl) were picked. These animals [*dpy-19 lin-12(n137)/++; let-60(s1124) unc-22 unc-31/+++*] segregated a small number of sterile Unc-22 F_2 animals which were homozygous for *s1124* (and had escaped from larval lethality). Visualized under Nomarski optics, seven out of ten of these Unc-22 animals had Lin-12(d) phenotype (all six VPCs are 2°). Only 3/4 of the Unc-22 animals were expected to be either heterozygous or homozygous for *lin-12(d).*

lin-34(n1046gf) with let-23(mn23): Heterozygous *let-23(mn23)unc-4(e120)/mnC1* males were crossed with *lin-34(n1046gf)* hermaphrodites. F_1 nonMuv hermaphrodites were individually picked. Animals with a genotype of *let-23 unc-4/++; lin-34/+* were identified by analyzing the F_2 progeny of these broods. The F_2 homozygous Unc-4 animals were picked from the above double heterozygous F_1 mothers and examined for phenotypes. Twenty-six nonrecombinant Unc-4 animals were all sterile adults, and 23 of them were Muv. Therefore, the lethal, but not the sterile, phenotype of *let-23(mn23)* is suppressed by *lin-34(n1046gf).*

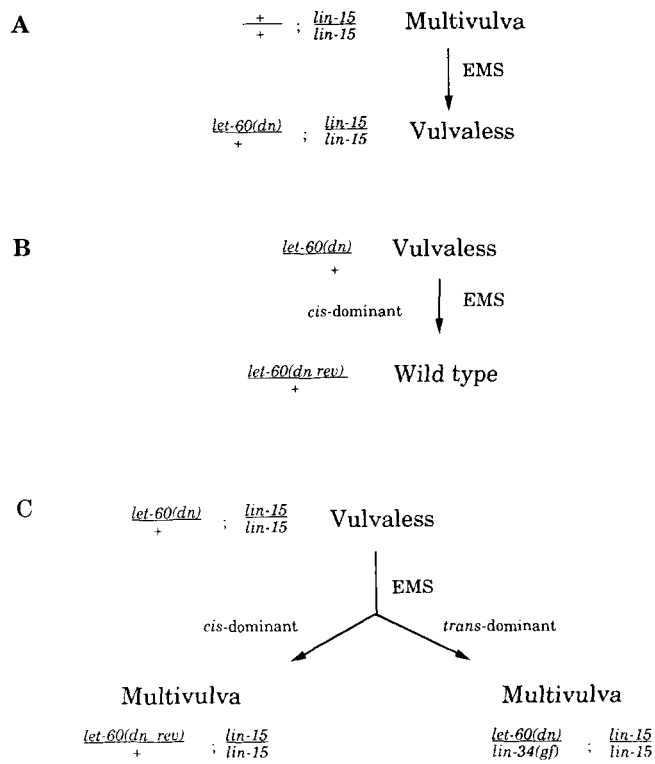

FIGURE 3.—Schematic illustration of the isolation of *let-60* and *lin-34* mutations as dominant suppressors. (A) Isolation of *let-60(dn)* mutations as dominant suppressors in strains with a *lin-15* mutation. Seven dominant *let-60* vulvaless mutations were isolated after screening about 100,000 EMS-mutagenized gametes for revertants of *lin-15* Muv phenotype. All these mutations were actually obtained in screening the F_2 progeny, although they were dominant and should be present in F_1. These mutations are referred to as *let-60(dn)* (*dn* for dominant negative, see text). (B) A loss-of-function allele of *let-60* was isolated as a *cis*-dominant suppressor of the vulvaless phenotype of *let-60(dn)/+*. The revertants have wild-type vulvae. (C) Isolation of dominant revertants of the dominant suppression phenotype of *let-60(dn).* The suppressed *lin-15* Muv phenotype was re-isolated when the *let-60(dn)* dominant phenotype is suppressed *cis*-dominantly by a new mutation in *let-60* (putative loss-of-function (*lf*) allele, indicated by "rev" for revertant), or *trans*-dominantly by a new mutation in *lin-34.* Without *lin-15* in background, *let-60(dn rev)* has no dominant phenotype (see Figure 4H); *let-60(dn)/lin-34* has a weak Muv phenotype.

RESULTS

Isolation of dominant Vul mutations as suppressors of a *lin-15* Muv mutation: *lin-15* mutations cause all six VPCs to become 1° or 2° (multivulva, or Muv, see Figure 4B) regardless of whether the inductive signal is present (FERGUSON and HORVITZ 1985; FERGUSON, STERNBERG and HORVITZ 1987; STERNBERG 1988; Sternberg and HORVITZ 1989). Mutations of genes whose products interact with *lin-15* gene product or of genes acting downstream of *lin-15* in the pathway might be expected to suppress the Muv phenotype of *lin-15.* We have isolated such suppressor mutations by mutagenizing *lin-15(n309)* animals with EMS and screening for phenotypically nonMuv revertants in the F_2 (Figure 3).

After screening approximately 100,000 EMS-mutagenized gametes, we isolated ten extragenic suppressors of the Muv phenotype of *lin-15(n309).* Two of them are recessive, Vul mutations of *let-23* (R. V. AROIAN and P. W. STERNBERG, manuscript in preparation). Another suppressor also proved to be a recessive Vul mutation. This mutation, *sy96*, maps to the left of *unc-24* on chromosome *IV* and defines a new gene, *lin-45* (M. HAN and P. W. STERNBERG, unpublished results). The other seven mutations, which we analyze in this paper, have a dominant suppressor phenotype (Table 2) and all map to the same region on chromosome *IV.* They were located about 0.01 m.u. to the left of *dpy-20* by three-point mapping with a *lin-3* lethal allele and a *dpy-20* allele as markers (see MATERIALS AND METHODS, Table 1 and Figure 2). These seven mutations result in a dominant Vul phe-

TABLE 2

Phenotypes of *let-60* and *lin-34* alleles

Class	Allele (*m*)	Hermaphrodite phenotype[a]				%Egl[c] (*m*/+)	Male mating[d] (*m*/+)	Mutant source[e]
		m/*m*	*m*/*m*; *n309*[b]	*m*/+	*m*/+; *n309*			
WT	+	WT	Muv	WT	Muv	0	+	
Deficiency	*sDf8*	Let	ND	WT	ND	0	+	(1)
Loss-of-function [*let-60*(*lf*)]	*sy101 sy163*	Let	Let	WT	Muv	0	+	This study
	syX sy127	Let	ND	WT	ND	0	+	This study
	s1124	Let	Let[f]	WT	Muv	0	+	(2)
	s1155	Let	ND	WT	ND	0	+	(2)
	s59	Let	ND	WT	ND	0	+	(3)
Dominant negative [*let-60*(*dn*)]	*sy93*	Vul	Vul	Vul	Vul	>99	−	This study
	sy99	Let	Let	Vul	Vul	97	−	This study
	sy101	Let	Let	Vul	Vul	97	−	This study
	sy94	Let	Let	Vul	Vul	93	−	This study
	sy100	Let	Let	Vul	Vul	89	−	This study
	sy95	Let	Let	Vul	Vul	59	−	This study
	sy92	Let	Let	Vul	Vul	42	−	This study
Gain-of-function [*lin-34gf*]	*n1046*	Muv	Muv	wMuv[g]	Muv	ND	+	(4)
	sy130	Muv	ND	wMuv	Muv	ND	+	This study

The mutations isolated and studied in this paper are divided into three different classes. The two gain-of-function mutations (*n1046* and *sy130*) are alleles of *lin-34* and the rest of the mutations are alleles of *let-60*.

[a] The phenotype of each *let-60* allele is described as "Vul" for vulvaless, "Muv" for multivulva, or "Let" for lethal. ND, not determined.

[b] Genotypes of *let-60*, *lin-34* and *lin-15*. "*m*" indicates the mutation in *let-60* or *lin-34*; "+" indicates the wild type allele. *n309* is an allele of *lin-15*. *m*/*m*; *n309* indicates the strain is homozygous for the *let-60* mutation on chromosome *IV* and homozygous for the *lin-15* mutation on chromosome *X*.

[c] The percentage of hermaphrodites that fail to lay eggs (only tested for *m*/+ strains). To score %Egl for the *let-60*(*dn*) alleles, strains with genotype *unc-24* + *let-60*+/+*let-65* + *unc-22* were used except *sy93*, which was not linked to *unc-24* in the test. For each of the *let-60*(*dn*) alleles, more than 200 F_1 progeny of Egl parents were scored. Fewer than 1% of the hermaphrodites were sterile.

[d] Male mating indicates the capability of males of *m*/+ genotype to mate with hermaphrodites. More than 30 L4 or young adult males were used in tests for each *let-60*(*dn*) allele. "−" indicates no cross-progeny were found in a mating test. Defects in male spicules were found in *let-60*(*dn*)/+ animals for all the *let-60*(*dn*) alleles.

[e] The references for previously isolated alleles: (1) MOERMAN and BAILLIE (1981); (2) CLARK *et al.* (1988); (3) ROGALSKI, MOERMAN and BAILLIE (1982); (4) FERGUSON and HORVITZ (1985).

[f] Animals that escape the early larval lethality are Vul, and die as young adults.

[g] "wMuv" indicates a weakly penetrant Muv phenotype. For *lin-34*(*n1046*) and *lin-34*(*sy130*), about 10–20% of the heterozygous animals are Muv, compared to greater than 90% Muv among homozygotes.

notype with or without *lin-15*(*n309*) in the background (Figures 3E and 4C). All of these mutations have cell lineage defects: fewer than three VPCs adopt the vulval cell fates (1° or 2°). Although all seven alleles suppress completely the Muv phenotype of *lin-15*(*n309*), they differ with respect to the penetrance of the Vul phenotype (Table 2). Males heterozygous for these mutations (*m*/+) have defective copulatory spicules and fail to mate (Table 2). Six of the seven dominant alleles are recessive lethal, arresting at larval developmental stages (L1-L2). Animals homozygous for the seventh allele, *sy93*, are viable and are Vul with or without *lin-15* in the background (Table 2).

The similar map locations and similar phenotypes of these seven mutations suggested that they define a single locus. Complementation tests for the recessive lethal phenotype indicate that these mutations are indeed alleles of one locus. We found *sy92*, *sy95* and *sy94* fail to complement *sy100* for viability (see MATERIALS AND METHODS).

Deficiencies of the region spanning this locus (Figure 2) do not have a dominant Vul phenotype (Table 2). Therefore, these suppressors are not loss-of-function mutations. As described below, we have determined that these dominant Vul mutations are dominant negative (designated *dn*) alleles of the gene *let-60*.

Isolation of intragenic revertants and determinants of the dominant Vul mutations as *let-60* alleles: To ascertain the wild-type function of the gene identified by the dominant Vul mutations, it was necessary to obtain and characterize intragenic revertants. A *lf*/+ (*lf* for loss-of-function) heterozygote is expected to exhibit a phenotype similar to that of a *deficiency*/+ animal and should not exhibit any dominant phenotype. Adding a loss-of-function mutation in *cis* to a dominant *let-60* allele should thus suppress the dominant phenotype caused by the allele.

To revert the dominant phenotype of the *let-60*(*dn*) alleles, we carried out two different screens. In one screen, we sought to isolate revertants of the Vul phenotype of *let-60*(*dn*) (Figure 3B). One tightly

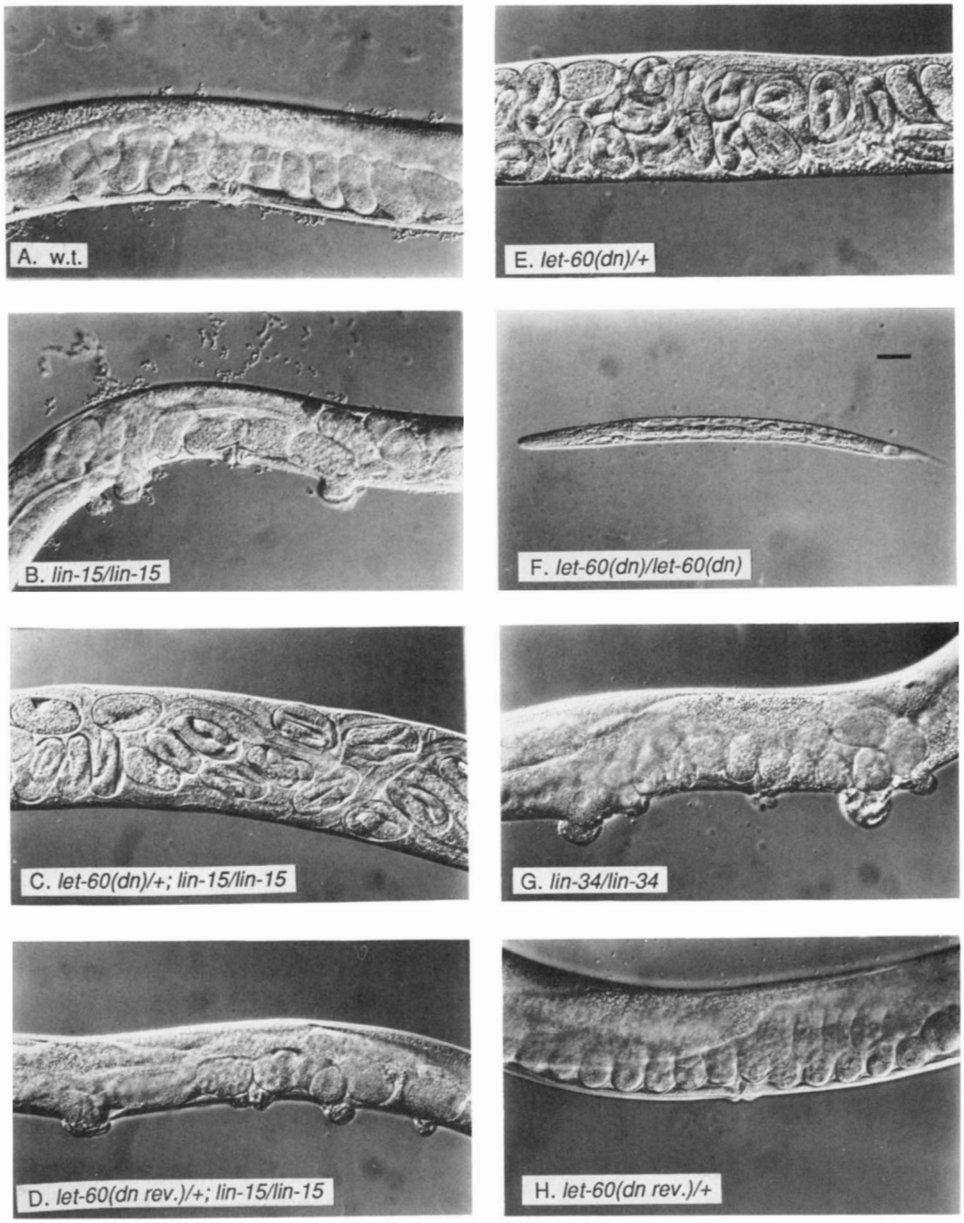

FIGURE 4.—Phenotypes in vulval development of hermaphrodites with mutations in *lin-15*, *let-60* and *lin-34*. Photomicrographs were taken with Nomarski optics (Plan Neofluor 40X dry lens with Kodak 2415 film). All animals are positioned with ventral side to the bottom of the micrograph. (A) and (H) show hermaphrodites with wild-type vulva. (B), (D) and (G) show multivulva (Muv) hermaphrodites. Additional vulval cells were induced in these Muv animals and gave rise two to three pseudo-vulval structures in the ventral hypodermis. (C) and (E) show vulvaless (Vul) hermaphrodites. The eggs hatch inside these Vul animals due to the defect in egg-laying. (F) shows a dead early larval hermaphrodite. The complete genotype for each of the animals is: (A), N2; (B), *lin-15(n309)*; (C), *unc-24 let-60(sy94dn) +/+lin-3 + dpy-20; lin-15*; (D), *unc-24 let-60(sy127) ++/++ dpy-20 unc-22; lin-15*; (E), *+let-60(sy100dn) dpy-20+/let-65 ++ unc-22*; (F), *let-60(sy100dn) dpy-20/let-60(sy100dn) dpy-20*; (G), *lin-34(sy130gf) dpy-20*; (H), *+unc-24 let-60(sy127) + /unc-8 ++ dpy-20*. Scale bar is 20 μm.

linked dominant suppressor of *let-60(sy101dn)*, *sy163* (Table 2), was isolated after screening approximately 9000 EMS-mutagenized gametes. *sy163* suppresses the *let-60(dn)* dominant phenotypes completely, and the double mutant alleles (*sy101dn sy163*) remain recessive lethal at a young larval stage. *sy101dn sy163/+* hermaphrodites have a wild-type level of vulval induction.

In another screen, we sought to restore the Muv phenotype to a *lin-15(n309)* strain which is dominantly suppressed by a *let-60(dn)* and hence Vul (Figure 3C). From a screen of approximately 35,000 mutagenized gametes, we isolated two new mutations that suppress the suppressor phenotype of *let-60(dn)*. In both isolates, the Muv phenotype of *lin-15(n309)* reappears [being no longer suppressed by the *let-60(dn)* mutation]. The dominant Vul phenotype of *let-60(dn)* is also completely suppressed by two new alleles in the absence of the *lin-15* mutation. In one of the revertants, *syXdn sy127* (*syX* is either *sy94dn* or *sy101dn*, see MATERIALS AND METHODS for explanation), the new mutation is also tightly linked to the dominant negative allele. *syXdn sy127* is also recessive lethal at an early larval stage (L1-L2) and fails to complement both *sy100dn* and a deficiency for its lethal phenotype. The *syXdn sy127/+* heterozygous strain has a wild-type level of vulval induction (see Figure 4, D and H), and males of this genotype mate normally.

The two linked revertants, *sy101dn sy163* and *syXdn sy127*, are most likely intragenic revertants and loss-of-function mutations of the *let-60* gene (also see Table 2 for comparison). Both revertants behave like deficiencies uncovering the region and the recessive lethal *let-60* alleles [loss-of-function mutations (*lf*); discussed below] isolated independently of *let-60(dn)*

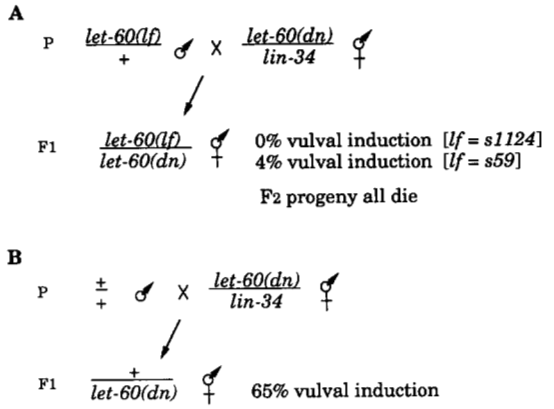

FIGURE 5.—Genetic interactions between *let-60(dn)* and *let-60(lf)*. The complete genotypes of the parent strains are described in MATERIALS AND METHODS. *dn*, dominant negative; *lf*, loss-of-function; *gf*, gain-of-function. The allele for *let-60(dn)* is *sy100*, and the allele for *lin-34(gf)* is *sy130*. *s1124* and *s59* were used as loss-of-function mutations. See Figure 6 for the maternal effect of *lin-34(gf)*. Only hermaphrodite F_1 and F_2 progeny were analyzed. Compared to 65% vulval induction in +/*let-60(dn)* animals (B), the 0% and 4% vulval induction phenotype of *let-60(lf)*/*let-60(dn)* animals (A) indicates the *let-60(lf)* alleles fail to provide function in vulval induction.

(CLARK *et al.* 1988). It is unlikely that our "*cis*" revertants (*rev*) and the dominant Vul alleles (*dn*) are in different, nearby genes: the phenotype of *let-60(dn rev)*/+ is wild type and thus is distinct from the lethal phenotype of *let-60(dn)*/*let-60(lf)*.

Previously, three *let-60* larval lethal mutations were isolated by screening recessive lethal alleles in the region on chromosome *IV* (ROGALSKI, MOERMAN and BAILLIE 1982; CLARK *et al.* 1988) (Table 2). As described above, we carried out complementation tests between these previously isolated alleles and our dominant Vul recessive lethal mutations, and found they fail to complement for viability (see MATERIALS AND METHODS, also Figure 5). The three previously isolated lethal alleles as well as our two *cis* revertant alleles, *sy101dn sy163* and *syXdn sy127*, are recessive and behave in complementation tests similar to a deficiency uncovering the *let-60* locus. The three alleles were isolated in relatively high frequency (3 out of an equivalent of 6500 EMS-mutagenized gametes, ROGALSKI, MOERMAN and BAILLIE 1982; CLARK *et al.* 1988) [see MATERIALS AND METHODS for discussion of relative frequencies of obtaining *let-60(lf)*]. Based on all these results, we believe that the five recessive lethal alleles, including the two tightly linked *let-60(dn)* revertants and the three previously isolated alleles, represent loss-of-function mutations of *let-60*.

***let-60* function in vulval development and the nature of the dominant Vul mutations:** To determine the function of the *let-60* gene in vulval development, it is critical to know the phenotype of a loss-of-function mutation. We have already discussed above that the loss-of-function mutations are recessive lethal prior to the L3 stage and have no dominant phenotype. It is thus difficult to study the phenotype of loss-of-function mutations in vulval development, which occurs during the L3 stage. However, some of the *s1124lf/s1124lf* progeny from a *s1124lf/+* heterozygote can surpass the larval lethal stage to survive to the early adult stage. We have examined the vulval induction in such "survivor" animals of genotype *let-60(s1124lf)/let-60(s1124lf)* from a *let-60(s1124lf)/lin-34(sy130gf)* mother [see below for analysis of *lin-34(sy130gf)*]. Under Nomarski optics, we found that these survivors have 0% VPC induction (14 animals examined). Failure of vulval induction in these animals is not due to the fact that the animals are sick or dying, since the Vul phenotype of the surviving *let-60(s1124lf)* animals can be completely suppressed by *lin-1* (see below). This result indicates that Vul is also a loss-of-function phenotype.

We also performed a genetic interaction analysis to overcome the recessive lethal problem of *let-60(lf)* and determine the phenotype of loss-of-function alleles in vulval induction. Two previously isolated recessive lethal alleles, *s1124* (CLARK *et al.* 1988) and *s59* (ROGALSKI, MOERMAN and BAILLIE 1982), were placed in *trans* to our dominant Vul, recessive lethal allele *let-60(sy100dn)*. A *let-60(sy100dn)* homozygote from a *lin-34(gf)/let-60(dn)* mother is viable for one generation (it normally would be larval lethal from a *let-60(dn)*/+ mother) (see below and Figure 6). We took advantage of this maternal effect of *lin-34(gf)* Muv mutations to examine interactions between *let-60(sy100dn)* and *let-60(lf)*. A *let-60(dn)/let-60(lf)* heterozygote from a *let-60(dn)/lin-34(gf)* mother is also expected to live for one generation and hence allows us to examine the phenotype in vulval induction (Figure 5). We crossed *let-60(lf)*/+ males with *let-60(sy100dn)/ lin-34(sy130gf)* hermaphrodites, we found that *let-60(lf)* fails to provide any function in vulval induction when lethality is rescued. Examined with Normarski opitics, *let-60(lf)/let-60(sy100dn)* animals from a *let-60(sy100dn)/lin-34(sy130gf)* mother have nearly no vulval induction (0% VPC induction among 16 *s1124lf/sy100dn* heterozygous hermaphrodites and 4% VPC induction among eight *S591f/sy100dn* heterozygous hermaphrodites.) By contrast, *sy100dn/+* animals (18 examined) from a cross between wild-type (N2) males and hermaphrodites *let-60(sy100dn)/lin-34(sy130gf)* display about 65% VPC induction (Figure 5). This result confirms that loss-of-function results in a vulvaless phenotype. We thus conclude that *let-60* is necessary for vulval development.

Since the *let-60(dn)* mutations act in the same phenotypic direction (Vul and Let) as *let-60(lf)*, these dominant Vul mutations of *let-60* are "dominant negative" (*dn*) mutations ("antimorphic mutations"). In a

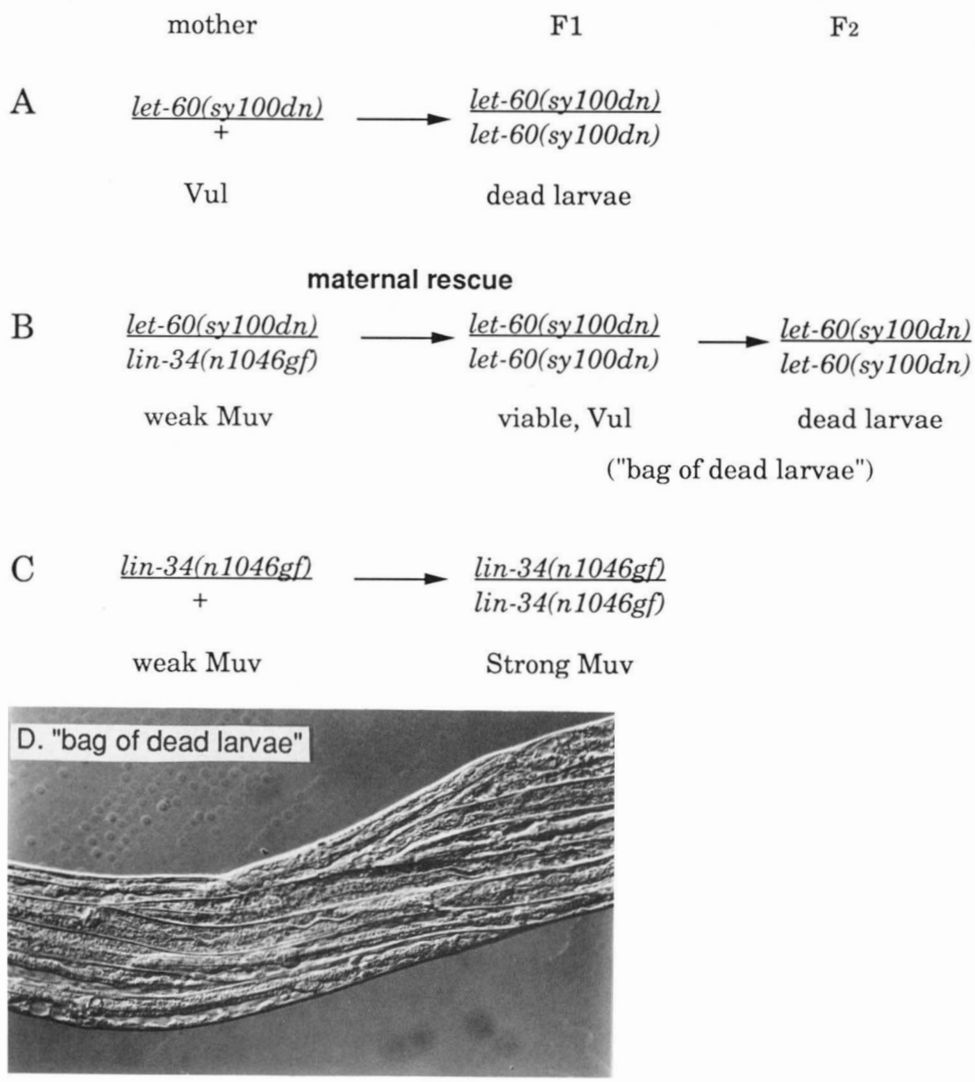

FIGURE 6.—Dominant suppression of *let-60(dn)* by semidominant Muv mutations of *lin-34gf*. (A) *let-60(sy100dn)* is dominantly vulvaless and recessively lethal at an early (L1-L2) larval stage (Table 2). (B) A *lin-34(gf)* allele (*n1046* or *sy130*), in *trans* to *let-60(sy100dn)*, completely suppresses the Vul phenotype of *let-60(sy100dn)*. The lethality of *sy100/sy100* is also suppressed through the dominant maternal effect of the *lin-34* mutation. The F_1 *sy100/sy100* progeny are viable and completely Vul, and their progeny (F_2) are all lethal at larval stages. Most of these F_2 larvae die in their mother's body so that a "bag of larvae" phenotype results. (C) *lin-34* mutations show a strong Muv phenotype (above 95% penetrant) as homozygotes and a weak Muv phenotype (less than 40%) as heterozygotes. (D) A maternally rescued hermaphrodite ("bag of dead larvae") described in (B). The genotype of the hermaphrodite is *let-60(sy100dn) dpy-20/let-60(sy100dn) dpy-20* and the genotype of its parent is *let-60(sy100dn)dpy-20 +/lin-34(n1046gf) + unc-22*. The photomicrograph was taken under Nomarski optics as in Figure 4 (same scale as in Figure 4).

let-60(dn)/+ heterozygote, there is less wild-type gene activity than that in a *let-60(lf)*/+ heterogyzgote. The dominant Vul phenotype of *let-60(dn)* is the result of this reduction of gene activity.

***lin-34* Muv mutations, tightly linked to *let-60*, suppress *let-60(dn)* phenotypes:** *lin-34* was previously defined by the semidominant Muv mutation *n1046* (FERGUSON and HORVITZ 1985). This mutation confers a "strong Muv" phenotype (defined here as greater than 80% penetrance) in homozygotes and a "weak Muv" phenotype (defined here as less than 30% penetrance) in heterozygotes. Additional semidominant Muv alleles of *lin-34* have been isolated as suppressors of mutations in *lin-10* (D. PARRY, S. KIM and R. HORVITZ, personal communication), *let-23* (G. JONGEWARD and P. W. STERNBERG, UNPUBLISHED RESULTS) AND *let-341* (S. CLARK and R. HORVITZ, personal communication). We have also isolated a semidominant Muv allele (*sy130*), as a dominant suppressor of the dominant suppressor phenotype of *let-60(dn)* (see above and Figure 3C). *sy130/sy130* animals are Muv (about 95%) (Figure 4G and Table 2). *sy130*/+ animals have a weak Muv phenotype (about 10%). *sy130* interacts in *trans* with *lin-34(n1046)* to produce a highly penetrant (strong) Muv phenotype (>95% *sy130/n1046* heterozygotes are Muv, see MATERIALS AND METHODS). Based on this result and similar mapping data for *sy130* and *lin-34(n1046)* (MATERIAL AND METHODS; G. BEITEL and R. HORVITZ, personal communication), we suggest that *sy130* is also an allele of *lin-34*. As *Df*/+ animals do not have a semidominant Muv phenotype, *sy130* is a gain-of-function (*gf*) mutation in *lin-34*. *sy130gf* also maps between *dpy-20* and *let-65*, very close to the *dpy-20* gene, as do all the *let-60* alleles (see MATERIALS AND METHODS and Figure 2). The fact that both *lin-34* and *let-60* are located in the same small chromosome interval suggests that the *lin-34* and *let-60* mutations might be different alleles of the same gene. This possibility is consistent with our observations of the genetic interactions between *let-60* and *lin-34*.

As described above, *lin-34(sy130gf)* was isolated as

a *trans*-dominant suppressor of *let-60(dn)*, indicating a close relationship between these two classes of mutations. We have further examined the interactions of *let-60* alleles with other *lin-34* Muv alleles. Three types of results demonstrate that the *lin-34* Muv mutations strongly suppress the *let-60* mutations (Figure 6). (1) The *lin-34* mutations dominantly suppress the dominant Vul phenotype of *let-60(dn)*: *let-60(dn)/lin-34* animals show the weakly penetrant Muv phenotype of *lin-34/+* rather than the Vul phenotype of *let-60(dn)/+*. Specifically, between 5% and 20% of animals of genotypes *lin-34(n1046)* or *lin-34(sy130)* in *trans* to each of six *let-60(dn)* alleles are Muv (the remaining 80–95% are wild type, data not shown). The suppression of the Vul phenotype of *let-60(dn)* by *lin-34* mutations is complete, even though the majority (80–90%) of *lin-34/+* animals are not Muv. (2) *lin-34* mutations suppress maternally the lethality of some *let-60(dn)* alleles (*sy100*, *sy92* and *sy95*). This maternal effect is also dominant. For example, homozygous *let-60(sy100dn)* F_1 progeny from a *let-60(sy100dn)/+* mother are normally lethal at a larval stage. However, the *let-60(sy100dn)/let-60(sy100dn)* F_1 progeny from a *let-60(sy100dn)/lin-34(n1046)* parent are viable for one more generation; the F_2 progeny are all dead larvae (Figure 6). *lin-34* mutations do not rescue maternally the defect in vulval induction in *let-60(dn)/let-60(dn)* animals. The *sy100dn* homozygotes rescued by the *lin-34* maternal effect have 0% VPC induction (none of 10 animals examined under Nomarski optics had any VPCs induced to vulval cell types). (3) *lin-34* Muv mutations can partially overcome the male mating defect of some *let-60(dn)/+* animals; *sy92dn*, *sy95dn*, *sy100dn* and *sy94dn* males can mate at low efficiency if placed in *trans* to *lin-34(n1046)* (see MATERIALS AND METHODS).

A *lin-34* mutation in *trans* to a deficiency (*e.g.*, *lin-34(n1046)/sDf8*) displays a weak Muv phenotype (about 8% of animals are Muv), similar to *lin-34/+* (about 10–20%, see MATERIAL AND METHODS; also see FERGUSON and HORVITZ 1985). This observation suggests that the *lin-34* mutations are not loss-of-function mutations, because otherwise, the *lin-34/Df* hemizygotes should display a Muv phenotype of equal or greater penetrance than *lin-34/lin-34* homozygotes (above 90% Muv). Moreover, *lin-34(gf)*, which are most likely alleles of *let-60*, have a phenotype (Muv) opposite to that of *let-60(lf)* (Vul). Therefore, all the *lin-34* Muv alleles are likely to be gain-of-function mutations. A simple explanation for our results is that the activity of *let-60* is elevated by the presence of a *lin-34(gf)* mutation, either because *lin-34(gf)* mutations are gain-of-function alleles of *let-60*, or that *lin-34(gf)* mutations are gain-of-function alleles of another gene that acts positively in the same signaling pathway as *let-60*.

TABLE 3

Mutual suppression of *let-60(sy100dn)* and *lin-15(n309)*

Genotype		Phenotype			
let-60	*lin-15*	%Egl[b]	%Muv	%Induction[c]	%Induction without signal[d]
+/+;	+/+	<1	<1	100	0
+/+;	*n309/n309*	<1	100	200	200
sy100/+;	+/+[a]	87	<1	57	ND
sy100/+;	*n309/n309*	21	<1	88	0

[a] The complete genotype on chromosome *IV* is + + *let-60(sy100dn)* + */unc-24 mec-3* + *dpy-20*.
[b] Egl stands for egg-laying defective, which, in this case, results from an animal being vulvaless. More than 200 animals were scored.
[c] Percentage VPCs induced to vulval cells relative to wild type, scored with Nomarski optics (see MATERIALS AND METHODS).
[d] The signal is eliminated by ablation of gonad cells during the first larval stage (MATERIALS AND METHODS). Data for wild type are from SULSTON and WHITE (1980), and for *lin-15(n309)* from STERNBERG and HORVITZ (1989) and STERNBERG (1988).

Genetic interactions of *let-60* with other genes in the vulval induction pathway: To understand the role of *let-60* in the genetic pathway specifying the VPC fates, we constructed and analyzed several double mutant strains carrying a *let-60(dn)* mutation and Muv mutations in *lin-1*, *lin-12* and *lin-15*. In addition, we examined the interaction between *let-23* and *lin-34(gf)*. Our results suggest that *let-60* acts downstream of *let-23* and *lin-15* but upstream of *lin-1* and *lin-12* in the pathway specifying the VPC fates.

lin-15 acts upstream of let-60: The seven dominant negative *let-60* Vul mutations were isolated as suppressors of *lin-15(n309)*. This suppression is not specific to the *n309* allele because another *lin-15* allele, *n765*, can also be dominantly suppressed by *let-60(dn)* mutations. We have also examined the interaction between *lin-15(n309)* and a loss-of-function mutation of *let-60*, *s1124*. This analysis was possible, because as mentioned above, a small percentage of animals homozygous for the recessive lethal allele *let-60(s1124lf)* can grow to an early adult stage. While the Muv phenotype of *lin-15(n309)* is fully displayed in a *let-60(s1124lf)/+* background, the Muv phenotype is changed to a completely Vul phenotype in "survivors" of genotype *let-60(s1124lf)*; *lin-15(n309)*. This suppression itself suggests that the *let-60* gene acts downstream of *lin-15* in the genetic pathway that specifies VPC types. Furthermore, we have observed that the Vul and Muv phenotypes are mutually suppressed in a *let-60(sy100dn)/+*; *lin-15(n309)* double mutant; not only is the Muv phenotype of *lin-15* suppressed by *let-60(sy100dn)*, but the Vul phenotype of *let-60(sy100dn)/+* is also partially suppressed by the presence of the *lin-15* mutation. The level of VPC induction is close to wild type in a *sy100/+*; *n309/n309* double mutant (88% VPC induction; 21% Egl) in contrast to 57% VPC induction (87% Egl) in the strain with *sy100dn/+* only, Table 3). More impor-

tantly, although the Muv phenotype of *lin-15(n309)* is independent of the inductive signal from the gonad anchor cell (FERGUSON, STERNBERG and HORVITZ 1987; STERNBERG 1988), the induction of VPCs depends absolutely on the inductive signal in the mutually suppressed *let-60(sy100dn)/+; lin-15(n309)* double mutant. We have ablated all the gonad cells and hence the signal-producing anchor cell of ten *let-60(sy100dn)/+; lin-15(n309)* double mutants at the L1 larval stage; all VPCs generated hypodermal cells in these animals (Table 3). These results suggest that *let-60* and *lin-15* may function antagonistically in the pathway specifying VPC fates, and that the *let-60(sy100dn)* mutation can compensate to some degree for the *lin-15(n309)* defect and restore the relative normal output of the signal response pathway. One possibility is that *lin-15* is a negative regulator of *let-60* activity. Reduction of *lin-15* activity could then result in a higher level of *let-60*, which is no longer subject to the regulation by the upstream signal. This view is supported by the fact that the gain-of-function *lin-34* mutations also display a signal-independent Muv phenotype. Specifically, an average of 120% VPC induction was found among five *lin-34(gf)* animals whose gonad primordia were ablated at an early larval stage (100% is wild type, 200% is maximal for Muv; see MATERIALS AND METHODS). *lin-34(gf)* animals with intact gonads display an average of 165% induction (13 animals).

lin-1 acts after let-60: lin-1 is another Muv gene that acts in the genetic pathway specifying VPC fates (HORVITZ and SULSTON 1980; SULSTON and HORVITZ 1981; FERGUSON and HORVITZ 1985; FERGUSON, STERNBERG and HORVITZ 1987). The Muv phenotype of *lin-1* is epistatic to the Vul phenotype of many Vul genes in the pathway (FERGUSON, STERNBERG and HORVITZ 1987), and the *lin-1* phenotype is coexpressed with *lin-12* phenotypes in double mutants (P. W. STERNBERG, unpublished observation). These results lead to a hypothesis that *lin-1* acts downstream of Vul genes (*e.g.*, *let-23*) and other Muv genes (*e.g.*, *lin-15*) and, as a negative regulator of 1°- and 2°-specific functions. To further characterize the position of *let-60* in the pathway, we constructed double mutants with *lin-1(e1275)* and the loss-of-function mutation of *let-60, s1124*. We found that *lin-1(e1275)* does not rescue the lethality of *let-60(s1124lf)/let-60(s1124lf)*: the typical double homozygous animals are larval lethal, but a small percentage of them survive to reach adulthood stage and are sick and sterile. However, those small number of surviving adult animals are all Muv, indicating the Vul phenotype of the *s1124* mutation is suppressed by the *lin-1* mutation. We have also found that the Vul phenotype of *let-60(sy100dn)* is suppressed by the *lin-1* Muv mutation. The *lin-1* Muv phenotype is fully expressed even in a double homozygote. We could observe this phenotype because the lethality of *let-60(sy100dn)/let-60(sy100dn)* is suppressed by *lin-1(e1275)*. The homozygous double mutant is viable and can be continuously propagated. These results suggest that *lin-1* acts downstream of *let-60* in the vulval induction pathway, and that *lin-1* interacts with *let-60* in a pathway required for larval growth.

lin-12 acts after let-60 in 2° fate specification: One of many *lin-12* functions is to distinguish between 2° and non-2° (1° or 3°) VPC types during vulval induction (GREENWALD, STERNBERG and HORVITZ 1983; STERNBERG and HORVITZ 1989). *lin-12* dominant mutations (*lin-12(d)*) cause all six VPCs to be 2°, while *lin-12* loss-of-function mutations cause all six VPCs to be non-2°. It has been proposed that *lin-12* is involved in the lateral signaling which prevents the neighbors of a presumptive 1° from also becoming 1°, and that *lin-12* acts downstream of most Muv and Vul genes whose function is to specify the choice between 3° and non-3° cell fates (STERNBERG and HORVITZ 1989). For example, a *let-23* Vul mutation causes all six VPCs to adopt the 3° cell type. In a *lin-12(d); let-23* double mutant, all six VPCs are 2°. To order the action of *let-60* with respect to *lin-12*, we constructed and examined a double mutant with a *lin-12(d)* allele *n137* and each of four *let-60* alleles (dominant negative alleles *sy100dn*, *sy99dn*, *sy94dn* and a loss-of-function allele *s1124*) (see MATERIALS AND METHODS). We found that the *lin-12(d)* phenotype (five ventral protrusions and egg-laying defective) is fully expressed in all *lin-12(d)/+; let-60(dn/+)* strains, and in survivors of genotype *lin-12(d)/+; let-60(s1124)/let-60(s1124)*. In other words, all six VPCs are 2° in the double mutants. Therefore, *lin-12* hyperactivity bypasses the need for *let-60* function for promoting 2° fate, suggesting that *lin-12* acts after *let-60* in 2° fate specification.

lin-34 acts after let-23: let-23 is another essential gene with a function in vulval induction (FERGUSON and HORVITZ 1985; FERGUSON, STERNBERG and HORVITZ 1987). Some recessive mutations of *let-23* cause a Vul phenotype. However, loss-of-function of *let-23* results in a larval lethal phenotype (R. V. AROIAN and P. W. STERNBERG, manuscript in preparation). A *lin-34(gf)* Muv mutation has been isolated as a suppressor of the *let-23* Vul phenotype (G. JONGEWARD and P. STERNBERG, unpublished results), suggesting that *lin-34* acts downstream of *let-23* during vulval induction. We constructed a double mutant with *lin-34(n1046gf)* and a loss-of-function, recessive lethal mutation of *let-23, mn23* (HERMAN 1978). We found that *let-23(mn23); lin-34(n1046gf)* hermaphrodites were sterile adults and showed a Muv phenotype (88% of the animals are Muv). Sterility is another phenotype associated with some *let-23* muta-

tions (R. V. AROIAN and P. W. STERNBERG, manuscript in preparation) and was not suppressed by *lin-34(n1046gf)*. However, the *let-23* lethal and Vul phenotypes were clearly suppressed by the *lin-34(gf)*. Therefore, we conclude that *lin-34* acts after *let-23* in the genetic pathways involved both in vulval induction and larval growth.

DISCUSSION

Dominant negative mutations of *let-60*: We have exploited the properties of dominant vulvaless (Vul) mutations in the *let-60* gene to analyze its role in vulval induction. Loss of *let-60* activity results in death at an early larval stage, prior to vulval induction. These dominant Vul mutations were isolated as extragenic suppressors of a *lin-15* multivulva mutation, in effect selecting for vulvalessness and viability. These mutations thus allowed us to conclude that *let-60* plays an important role in vulval induction. We used the dominant Vul mutations to obtain both recessive lethal loss-of-function alleles of *let-60* as well as semidominant multivulva *lin-34* mutations that behave as gain-of-function alleles of *let-60* (see below). Analysis of these mutations has allowed us to understand the role of *let-60* in the switch between vulval and nonvulval VPC fates during vulval induction, as detailed below.

We have found that these dominant Vul mutations are dominant negative, *i.e.*, they result in a *let-60* product that appears to compete with the wild-type product ("antimorphic," MULLER 1932). In *let-60(dn)*/+ heterozygotes, *let-60* activity is reduced more than in a heterozygote carrying one copy of a loss-of-function mutation (*lf*/+), indicating that its function in vulval induction is disrupted. There are many possible ways that a mutant gene product can compete with a wild-type gene product and cause the dominant negative effects (reviewed by HERSKOWITZ 1987). For example, a *let-60* gene product may normally form multimers, and the multimeric complex containing wild-type and mutant products could be defective in vulval induction.

A key component of a developmental switch: *let-60* has the properties of a component of a developmental switch because its activity determines which of two alternative fates the six VPCs have. We propose that, in wild-type animals, *let-60* activity is increased by the inductive signal. Mutations with opposite effects on *let-60* activity have opposite consequences for VPC fates (Table 4). Loss or significant reduction of *let-60* activity causes the VPCs to become the nonvulval cell type (3°) even in the presence of inductive signal. In contrast, in *lin-34* Muv mutants, all six VPCs become vulval cell types. Based on mapping results and genetic interactions between mutations of *let-60* and *lin-34*, *lin-34* Muv mutations appear to be either gain-of-function mutations of *let-60* or gain-of-function mutations of an intimately related gene that elevates *let-60* activity. In either case, *lin-34(gf)* mutations apparently result in elevation of *let-60* activity. Thus, an increase of *let-60* activity causes all six VPCs to become vulval cell types compared to the three in wild type, even in the absence of the inductive signal (Table 4). The site of *let-60* action is unknown; however, we hypothesize that *let-60* acts in the VPCs in the pathway of response to inductive signal because this is the simplest interpretation of existing data.

TABLE 4

Illustration of the function of *let-60* activity in controlling the fate of each VPC in response to inductive signal

let-60 genotype	+/− signal	*let-60* activity	VPC fate
Wild type	+	High	Vulval [1° or 2°]
	−	Low	Nonvulval [3°]
Mutants	+ or −	Always high	Vulval [1° or 2°]
	+ or −	Always low	Nonvulval [3°]

We propose that in each of the six VPCs, inductive signal indirectly regulates the *let-60* activity which controls VPC fates. In the column marked "+/− signal", "+" means the individual VPC receives the signal from anchor cell, "−" means the individual VPC does not receive the signal either due to the position of the cell or due to elimination of the signal source by ablation of gonad cells (MATERIALS AND METHODS). *let-60* activity levels are defined genetically: *lin-34(gf)* causes "high" activity (hyperactive), and *let-60(lf)* or *let-60(dn)* cause "low" activity.

If *let-60* and *lin-34* are the same gene, changes of the gene activity caused by dominant negative ("antimorphic") mutations *let-60(dn)* and gain-of-function ("hypermorphic") mutations *lin-34(gf)* may be the consequence of qualitatively different changes in protein structure. For example, the *let-60* product might contain a functional domain and a regulatory domain. The *let-60(dn)* Vul phenotype may result from defects in the functional (*e.g.*, catalytic) domain, while the *lin-34(gf)* Muv phenotype may be caused by defects in the regulatory domain. The regulatory domain could be a site for interacting with a negative regulator, which would keep *let-60* inactive until the VPC receives inductive signal.

let-60 appears to act in more than one aspect of *C. elegans* development. We have described that all the putative loss-of-function mutations and most of the dominant negative mutations are recessive lethal at an early larval stage. We have also described that the *let-60(dn)* mutations result in defects in male spicules and mating. The spicule defect of *let-60(dn)* males is due to at least one alteration in cell fate (H. CHAMBERLIN and P. W. STERNBERG, unpublished results). These observations suggest that *let-60* acts in multiple cells during development.

***let-60* function is regulated by *let-23* and *lin-15*:** Vulval induction is a complicated and multistep process. Along with other Muv and Vul genes, *let-60* functions in one of the key steps in distinguishing

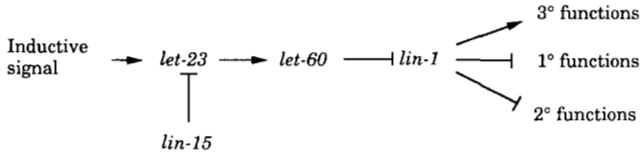

FIGURE 7.—Functional relationship between *let-60* and some other genes in the genetic pathway of vulval fate specification. Based on known genetic interactions (FERGUSON, STERNBERG and HORVITZ 1987; STERNBERG and HORVITZ 1989) we propose the functional relationships between *let-60* and some other genes in the pathway. The arrows indicate the positive regulation of one gene by another. "T" bars indicate the negative regulation of one gene by another. The arrows and bars do not necessarily indicate a direct interaction. We propose that *let-60* activity is positively regulated by inductive signal through *let-23* and negatively controlled by *lin-15* via *let-23*. *let-60* controls the 1°- and 2°-specific functions through inhibition of *lin-1*. *lin-12* could act either in combination with *lin-1* or downstream of *lin-1* to specify 2° functions. The interaction between *lin-12* pathway and *let-60* pathway might involve intercellular or autocrine signals (STERNBERG and HORVITZ 1989).

whether a VPC becomes a vulval cell types (1°, 2°) or a nonvulval cell type (3°) in response to an inductive signal. By studying genetic interactions between *let-60* and other Muv or Vul genes, we can start to elucidate the functional relationship between these genes. The relationship between *let-60* and other Vul and Muv genes is proposed as shown in Figure 7. Since the ordering of gene action is based on dominant mutations [*lin-12(d)*, *lin-34(gf)*, *let-60(dn)*] and possibly non-null recessive mutations (*lin-15*, *lin-1*), we regard these conclusions, which represent the simplest interpretations of our data, as tentative.

We propose that *let-60* activity is positively controlled by *let-23* activity. Again, this is based on our conclusion that *lin-34(gf)* are either gain-of-function alleles of *let-60* or gain-of-function mutations of an intimately related gene that activates *let-60*. Both the lethal and vulvaless phenotypes of *let-23* are suppressed by *lin-34(gf)* mutations (G. JONGEWARD and P. W. STERNBERG, unpublished results; this study), and *lin-34(gf)* mutations result in a signal-independent Muv phenotype. In other words, a *lin-34(gf)* mutation bypasses the need for either inductive signal or *let-23*.

lin-15 is proposed to be a negative regulator of the vulval induction pathway acting before *let-60*, since a decrease in *let-60* activity suppresses the Muv phenotype of *lin-15*. However, *lin-15* could exert its negative effect on *let-60* via *let-23*, since the *lin-15* Muv phenotype is also suppressed by *let-23* Vul mutations. If *lin-15* interacts with *let-60* via *let-23* as proposed in Figure 7, the mutual suppression between *lin-15(n309)* and *let-60(sy100dn)* (Table 3) could be explained by an increase in *let-23* activity in the *lin-15(n309)* background which compensates for the reduction in *let-60* activity. It is known that to some extent, there is also mutual suppression between particular *lin-15* and *let-23* mutations (STERNBERG and HORVITZ 1989). This mutual suppression could result from partial defects in the *lin-15* and *let-23* gene products, which either have antagonistic regulatory effects on *let-60* gene activity, or directly interact with each other. We do not believe that the controlling effect of the inductive signal on *let-60* is exerted via *lin-15*, because the dependence on inductive signal is not relieved by the *lin-15* mutation in a *lin-15(n309)*; *let-60(sy100dn)*/+ double mutant. Moreover, although a *lin-15* mutation alone causes a signal-independent Muv phenotype, the exact pattern of VPC fates in a *lin-15* mutant can be responsive to the inductive signal (STERNBERG 1988). Furthermore, *lin-15* most likely acts in cells other than the VPCs (R. HERMAN and E. HEDGECOCK, personal communication).

***let-60* controls VPC fates via *lin-1* and *lin-12*:** *lin-1* is proposed to act downstream of the *let-60* gene because *lin-1* mutations are epistatic to *let-60* mutations (Figure 7). *lin-1* mutations cause a Muv phenotype, and *lin-1* might act as a negative regulator of the expression of 1°- and 2°-specific functions. *lin-12* is proposed to act downstream of the *let-60* gene in promoting the 2°-specific functions because dominant *lin-12* mutations are epistatic to *let-60* mutations with respect to the 2° cell fate. *lin-12* is a component of a developmental switch specifying 2° *vs.* non-2° (1° or 3°) VPC fates (GREENWALD, STERNBERG and HORVITZ 1983; STERNBERG and HORVITZ 1989). In contrast, *let-60* is a component of a developmental switch specifying 3° *vs.* non-3° VPC fates (1° or 2°). The Vul/Muv pathway is likely to control, at least in part, the activity of *lin-12* (STERNBERG and HORVITZ 1989). It is not known whether the interaction of these pathways occurs within the same VPC or via intercellular signals. The precise pattern of VPC fates is established by the combined action of these two pathways. The activity states of *let-60* and *lin-12* define the action of each pathway.

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