sli-1, a Negative Regulator of *let-23*-Mediated Signaling in *C. elegans*

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ABSTRACT

By screening for suppressors of hypomorphic mutations of *let-23*, a receptor tyrosine kinase necessary for vulval induction in *Caenorhabditis elegans*, we recovered ≥12 mutations defining the *sli-1* (suppressor of lineage defect) locus. *sli-1* mutations suppress four of five phenotypes associated with hypomorphic alleles of *let-23* but do not suppress *let-23* null alleles. Thus, a *sli-1* mutation does not bypass the requirement for functional *let-23* but rather allows more potent LET-23-dependent signaling. Mutations at the *sli-1* locus are otherwise silent with respect to vulval differentiation and cause only a low-penetrance abnormal head phenotype. Mutations at *sli-1* also suppress the vulval defects but not other defects associated with mutations of *sem-5*, whose product likely interacts with LET-23 protein during vulval induction. Mutations at *sli-1* suppress *lin-2*, *lin-7* and *lin-10* mutations but only partially suppress *lin-3* and *let-60* mutations and do not suppress a *lin-45* mutation. The *sli-1* locus displays dosage sensitivity: severe reduction of function alleles of *sli-1* are semidominant suppressors; a duplication of the *sli-1(+)* region enhances the vulvaless phenotype of hypomorphic mutations of *let-23*. We propose that *sli-1* is a negative regulator that acts at or near the LET-23-mediated step of the vulval induction pathway. Our analysis suggests that *let-23* can activate distinct signaling pathways in different tissues: one pathway is required for vulval induction; another pathway is involved in hermaphrodite fertilty and is not regulated by *sli-1*.

VULVAL induction in *Caenorhabditis elegans* is an example of proto-oncogene mediated signal transduction. The anchor cell of the somatic gonad produces an inductive signal (KIMBLE 1981). This signal is encoded by the *lin-3* gene and is a member of the epidermal growth factor (EGF) family of growth factors (HILL and STERNBERG 1992; R. HILL, W. KATZ, T. CLANDININ and P. STERNBERG, unpublished observations). In response to this signal, three of the six vulval precursor cells (VPCs) undergo three rounds of mitosis and form vulval tissue. This response is mediated by the products of: *let-23*, an EGF receptor homolog (AROIAN *et al.* 1990), *sem-5*, an SH2/SH3 "adaptor" (CLARK *et al.* 1992a), *let 60 ras* (HAN and STERNBERG 1990), *lin-45 raf* (HAN *et al.* 1993) and *sur-1/mpk-1* (LACKNER *et al.* 1994; WU and HAN 1994) as well as several other genes, including *lin-10* (KIM and HORVITZ 1990). Reduction-of-function mutations at any of these loci cause most or all of the VPCs to remain uninduced, undergoing a single round of mitosis and forming nonspecialized epidermis. Thus, an animal homozygous for one of these mutations is vulvaless (Vul).

Little is known about the negative regulation of this process. The *lin-15* gene prevents signal independent activation of vulval fates (FERGUSON *et al.* 1987; FERGUSON and HORVITZ 1989; CLARK *et al.* 1994; HUANG *et al.* 1994) most likely acting in the nearby syncytial epidermis hyp7 (HERMAN and HEDGECOCK 1990). *lin-1* is required for epidermal fates and acts at a late step in the signal transduction pathway (HORVITZ and SULSTON 1980; FERGUSON *et al.* 1987; HAN *et al.* 1990, 1993; CLARK *et al.* 1992b). In the absence of either the *lin-1* or *lin-15* gene products, VPCs assume vulval fates in a signal-independent manner, resulting in the formation of ventral pseudovulvae (the multivulva or Muv phenotype). Evidence for negative regulation of the response to the inductive signal has come from analysis of hypomorphic alleles of several genes necessary for vulval induction. These genes, *let-23, lin-2* and *lin-7*, are required for response to the inductive signal as well as negative regulation of this response (FERGUSON and HORVITZ 1985; AROIAN and STERNBERG 1991; G. JONGEWARD and P. STERNBERG, unpublished observations). Rare alleles of these loci display apparent hypersensitivity to the inductive signal. This apparent hypersensitivity is inferred from their hyperinduced phenotype (Hin), defined as greater than wild-type vulval differentiation that is signal dependent.

let-23 functions in several sets of cells during *C. elegans* development and is required for inductive signaling in at least two of these cases (HERMAN 1978; FERGUSON and HORVITZ 1985; FERGUSON *et al.* 1987; AROIAN and STERNBERG 1991; CHAMBERLIN and STERNBERG 1993,

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1994). To define genes that function to regulate the activity or expression of *let-23*, we have recovered and characterized mutations that suppress hypomorphic vulvaless mutations of *let-23*. The screens used to recover these suppressors were designed to identify intragenic revertants, as well as loci that transduce the inductive signal after *let-23*, interact with *let-23* or are involved in negative regulation of the response to the inductive signal. This approach was successful, as we have identified mutations of at least three of the expected classes. We identified an intragenic revertant of *let-23* that restores a nearly wild-type phenotype. One gain-of-function mutation of *let-60 ras* was recovered; this mutation is epistatic to a *let-23* mutation for vulval differentiation. We also recovered mutations at loci which appear to function as negative regulators of *let-23*, including *unc-101* (LEE *et al.* 1994). Here we describe our analysis of another of these loci, *sli-1* (suppressor of cell lineage defect), that suggests it might function as a negative regulator of *let-23*.

MATERIALS AND METHODS

Strain maintenance and construction: Ethyl methane sulphonate (EMS) mutagenesis, strain maintenance and genetic manipulations were as described by BRENNER (1974). X-ray mutagenesis was performed using 1500 rads. Strains are from BRENNER (1974) unless otherwise indicated.

PS267 [*let-238(mn229) let-23(sy1) + sqt-1(e1350) / + mnDf67 unc-4(e120) +*] was constructed as follows: *let-238 unc-4/ mnC1*[*dpy-10(e128) unc-52(e444)*] males (SP713; SIGURDSON *et al.* 1984) were mated to *rol-6(e187) let-23(sy1)* hermaphrodites (PS66). Non-Rol cross progeny hermaphrodites were allowed to self-fertilize on individual plates. Animals segregating dead larvae but no Dpy Unc self-progeny (Dpy Unc being the phenotype of *mnC1* homozygotes) were selected (genotype: + *let-238* + *unc-4/ rol-6* + *let-23(sy1)* +). From the progeny of these animals, we selected rare Vul non-Rol recombinants that segregate dead larvae (genotype: + *let-238 let-23(sy1) / rol-6* + *let-23*) and the *let-238 let-23* chromosome was balanced in *trans* to *mnC1* by mating rare egg-laying competent non-Rol progeny of the original recombinant to *rol-6 unc-4/ mnC1* males. This strain, PS94 *let-238 let-23/ mnC1* was heat shocked to obtain male self-progeny. These males were mated to *unc-4 sqt-1* (*e1350*) hermaphrodites. *e1350/ e1350* animals are Dpy, while *e1350/ +* animals are Rol (CB1350; COX *et al.* 1980). Cross-progeny L4 hermaphrodites (Rol non-Unc) were selected and allowed to self-fertilize on individual plates. From animals that segregated dead larvae but no Dpy Unc-52 self-progeny (thus of genotype: *let-238 let-23 ++ / ++ unc-4 sqt-1*, rare Dpy non-Unc recombinants (genotype: *let-238 let-23* + *sqt-1/ ++ unc-4 sqt-1* were selected and immediately mated to *mnDf67 unc-4/ mnC1* males. Vul Rol non-Unc cross progeny were selected (genotype: *let- 238 let-23* + *sqt-1/ + mnDf67 unc-4/ +*) and allowed to self-fertilize to establish PS267. Rare non-Rol progeny were discarded throughout the time that this strain was in use.

Animals bearing homozygous *let-23* mutant alleles and carrying the deficiency *meDf3* were constructed as follows: *rol-6 let-23(sy97)/ mnC1* males were mated to hermaphrodites of genotype *meDf3/ unc-1(e538) dpy-3(e27)* hermaphrodites (AV24; VILLENEUVE 1994). Non-Unc non-Dpy L4 hermaphrodite cross progeny were selected, placed on individual plates, and allowed to self-fertilize. Animals that segregated dead eggs, rare males (both are indicative of the presence of the deficiency), Rolling (Rol) animals and no Dpy Uncs (the phenotype of both balancer chromosomes) were identified [genotype *rol-6 let-23(sy97) / ++ meDf3/ +*]. From these animals, self progeny were scored to determine the number of Rol and non-Rol progeny, the ratio of which was used to determine the ability of this deficiency to dominantly suppress the lethality of this *let-23* allele. The genotype of the Rol animals was confirmed by examining their self-progeny. In parallel, wild-type males were mated to *let-23(sy97) unc-4; sli-1(sy143)* hermaphrodites. L4 non-Unc hermaphrodite cross progeny were selected and allowed to self-fertilize on individual plates. All progeny from these animals were scored as Unc or non-Unc to assess the ability of the allele *sli-1(sy143)* to dominantly suppress the lethality of *let-23(sy97)*.

let-23(sy1); sli-1(sy143) / meDf3 was constructed by mating *let-23(sy1) / +; sli-1(sy143) / O* males to *meDf3/ unc-1 dpy-3* hermaphrodites. Non-Unc non-Dpy L4 hermaphrodite cross progeny were selected, placed on individual plates and allowed to self-fertilize. Animals that segregated dead eggs, rare males and Hin (or Vul) animals but no Dpy Unc animals [therefore of genotype *let-23(sy1) / +; meDf3/ sli-1*] were identified. From the progeny of these animals, hermaphrodites displaying vulval abnormalities were selected and allowed to self-fertilize on individual plates [these animals will be of genotype *let-23(sy1); sli-1/ meDf3* or *let-23(sy1); sli-1*]. The genotype of these animals was determined by scoring for the presence of dead eggs and males among their self-progeny.

let-23(sy97) unc-4; sli-1(sy143) / meDf3 animals were constructed by mating *let-23(sy97) unc-4/ mnC1; sli-1(sy143) / O* males to *meDf3/ unc-1 dpy-3* hermaphrodites. Non-Unc non-Dpy L4 hermaphrodite cross progeny were selected, placed on individual plates and allowed to self-fertilize. Animals that segregated dead eggs, rare males, Unc-4 (Unc-4 is distinguishable from both Unc-1 and Unc-52) but no Dpy Unc progeny (the phenotype of the balancer chromosomes *mnC1* and *unc-1 dpy-3*) were identified [these animals were therefore of genotype *let-23(sy97) unc-4/ ++; sli-1/ meDf3*]. From the progeny of these animals, Unc-4 L4 hermaphrodites were selected and placed on individual plates these animals are of genotypes *let-23(sy97) unc-4; sli-1/ meDf3* and *let-23(sy97) unc-4; sli-1*. Animals bearing the deficiency were identified by the presence of males among their self-progeny. L4 hermaphrodite progeny of these deficiency-bearing animals were individually examined under Nomarski optics for the extent of vulval differentiation, placed on individual plates and allowed to self-fertilize. Animals that segregated males and dead eggs [and thus were of genotype *let-23(sy97) unc-4; sli-1/ meDf3*] were identified; animals not bearing the deficiency served as controls.

let-23(sy97) unc-4; sli-1(sy102) / meDf3 and *let-23(sy97) unc-4; sli-l(sy112) / meDf3* strains were constructed by mating *let-23(sy97) unc-4/ ++; sli-1(sy102* or *sy112) / O* males to *meDf3/ unc-1 dpy-3* hermaphrodites. Non-Unc non-Dpy L4 hermaphrodite cross progeny were selected, placed on individual plates and allowed to self-fertilize. Animals that segregated dead eggs, rare males and Unc-4s were retained [these animals were therefore of genotype *let-23(sy97) unc-4/ ++; sli-1(sy102* or *sy112) / meDf3*]. Vul Unc-4 progeny of these animals were selected and allowed to self-fertilize on individual plates. From animals which segregated males and dead eggs, L4 hermaphrodite self-progeny were examined individually under Nomarski optics for the extent of vulval differentiation, placed on individual plates and allowed to self-fertilize. Animals that segregated males and dead eggs [and thus were

of genotype *let-23(sy97) unc-4*; *meDf3/ sli-1 (sy102* or *sy112)*] were identified; animals not bearing the deficiency were discarded.

let-23(sy97) unc-4; *meDf3/+* animals were constructed by mating *let-23(sy97) unc-4/++* males to *meDf3/ unc-1 dpy-3* hermaphrodites. Non-Unc non-Dpy L4 hermaphrodite cross progeny were selected, placed on individual plates and allowed to self-fertilize. Animals that segregated dead eggs, rare males and Vul Unc-4 progeny were retained. From the progeny of these animals, Vul Unc-4 hermaphrodites were selected and allowed to self-fertilize on individual plates (most of these animals are genotypically *let-23 unc-4*; *meDf3/+*; others are *let-23 unc-4*; *+*). Animals bearing the deficiency were identified by the segregation of male self-progeny and dead eggs. L4 hermaphrodite self-progeny of these deficiency bearing animals were examined individually under Nomarski optics for the extent of vulval differentiation, placed on individual plates and allowed to self-fertilize. Animals that segregated male self-progeny and dead eggs (and thus were of genotype *let-23 unc-4*; *meDf3/+*) were scored; animals not bearing the deficiency were discarded.

The *let-23(sy1)*; *sli-1(sy143)* strain was constructed as follows: *let-23(sy1)*; *him-5(e1490)* males were mated to *sli-1(sy143)* hermaphrodites. Cross progeny (*sli-1* hemizygous) males were picked and mated to L4 *sli-1(sy143)* hermaphrodites. L4 hermaphrodite progeny (apparent cross-progeny) from this mating were selected and allowed to self fertilize. Animals bearing the *let-23(sy1)* mutation segregated animals displaying vulval abnormalities and were used to establish *let-23(sy1)*; *sli-1(sy143)* stocks.

Other strains were constructed by standard methods.

Mapping: The following double mutant strains were used for mapping to linkage groups. *I*: PS592 *dpy-5(e61)*; *let-23(sy1)*, *II*: PS79 *dpy-10(e128) let-23(sy1)*, *III*: PS512 *let-23(sy1)*; *unc-32(e189)*, *IV*: PS262 *let-23(sy1)*; *dpy-20(e1282)*, *V*: PS593 *let-23(sy1)*; *dpy-11(e224)*, *X*: PS594 *let-23(sy1)*; *lon-2(e678)*.

Subsequent mapping was done with the following strains: *let-23(sy1)*; *unc-3(e151)*, *let-23(sy1)*; *unc-1(e719) dpy-3(e27)*, *che-2(e1033)* (CB1033; LEWIS and HODGKIN 1977), *egl-17(e1313)* (CB1313; TRENT *et al.* 1983), *let-23(sy1)*; *sli-1(sy143) unc-1(e719)*, and *egl-17(e1313) sli-1(sy143) unc-1(e719)* (PS1104; this work).

Mapping crosses were performed as follows: *let-23(sy1)*; *him-5(e1490)*; *sli-1(sy102)* males were mated to hermaphrodites of the mapping strains above [general genotype of *let-23(sy1)*; *dpy-y*]. Cross progeny [non-Dpy genotypically *let-23(sy1)/let-23(sy1)*; *him-5/+*; *dpy-y/+*; *sli-1(sy102)/+*] hermaphrodites were selected and allowed to self-fertilize. Twelve to 24 suppressed (Hin) non-Dpy hermaphrodites were removed to individual plates and allowed to self-fertilize. Linkage was determined based on the fraction of these animals which segregated Dpy progeny. Subsequent mapping was done similarly with animals bearing the *X*-linked markers *unc-3(e151)* and the double mutant *unc-1(e719) dpy-3(e27)*.

Three factor crosses were performed by mating males of genotype *let-23(sy1)*; *him-5*; *sup-x* (where *sup-x is* a candidate *sli-1* allele) to hermaphrodites of genotype *let-23(sy1)*; *unc-1 dpy-3*. Cross progeny (non-Unc non-Dpy) L4 hermaphrodites were selected and allowed to self-fertilize. Recombinant (Dpy non-Unc and Unc non-Dpy) hermaphrodites were selected and allowed to self-fertilize. Progeny of these animals were scored for the presence of Hin animals (indicative of homozygous *sli-1*). *sli-1* maps to the left of *unc-1*, and thus this cross allows *sli-1* to be distinguished from *suv-1*, defined by extragenic suppressors of *lin-10*, which maps to the right of *unc-1* (A. VILLENEUVE and S. KIM, personal communication). All candidate *sli-1* alleles from the PS267 screen were mapped using this cross. Additional three-factor crosses were performed using animals of genotypes *let-23(sy1)*; *sli-1(sy143) unc-1* and *egl-17 sli-1(sy143) unc-1*. These strains were constructed as follows: *let-23(sy1)/let-23(sy1)*; *sli-1(sy143)+/+ unc-1* hermaphrodites were allowed to self-fertilize. Unc animals were selected and placed on plates where they were allowed to self-fertilize. Rare Hin Unc recombinant animals were selected. By standard crosses, animals of genotype *let-23(+)*; *sli-1(sy143) unc-1* were recovered. *egl-17* males were mated to these *sli-1(sy143) unc-1* hermaphrodites. Cross progeny non-Unc L4 hermaphrodites were selected and allowed to self-fertilize. Egg-laying defective (Egl) animals were selected and allowed to self-fertilize. Rare Unc Egl progeny were selected. The *sli-1* genotype of these recombinants was scored after reintroducing the *let-23(sy1)* mutation via mating.

Complementation tests: Candidate *sli-1* alleles from the *let-23(sy97) unc-4* reversion screen were tested for complementation of the *sli-1* reference allele *sy102* in the following manner: wild-type (N2) males were mated to egg-laying competent hermaphrodites of genotype *let-23(sy97) unc-4*; *sup-x*, where *sup-x* is a candidate *sli-1* allele. Cross progeny males were selected and mated to hermaphrodites of the genotype *unc-54(e190)*; *let-23(sy97) unc-4*; *sli-1(sy102)*. Unc-4 non-Unc-54 L4 hermaphrodite cross progeny were selected (*unc-54/+*; *let-23 unc-4*; *sy102/ sup-x*), placed on individual plates and scored for their ability to lay eggs.

Nomarski microscopy and cell ablation: *Vulval differentiation:* The extent of vulval differentiation was measured as described by HAN and STERNBERG (1990). The anatomy of L3 lethargus or L4 hermaphrodites were examined under Nomarski optics and the fate of individual VPCs inferred from the anatomy. Wild-type animals have three VPCs generating vulval progeny; vulvaless animals have fewer than three VPCs generating vulval progeny; multivulva or hyperinduced animals have more than three VPCs generating vulval progeny. In some cases, a VPC generates one daughter that makes vulval progeny whereas the other daughter is nonvulval epidermis; such VPCs are scored as one-half VPC differentiating into vulval tissue.

P12 vs. P11: L3 and L4 animals were scored for the lack of a P12.pa cell and the presence of two P11.p-like cells (AROIAN and STERNBERG 1991), but presumably the transformation is actually P12 to P11, based on a more detailed analysis of the mutations in the pathway (FIXSEN *et al.* 1985).

Male tail abnormalities: Male tail phenotypes were scored by HELEN CHAMBERLIN by examining young adult males for the "crumpled spicule" phenotype associated with several *let-23* mutations (AROIAN and STERNBERG 1991).

Ablation procedure: Ablation of the gonad (including anchor cell) precursor cells was performed as described by AVERY and HORVITZ (1987) and SULSTON and WHITE (1980).

Other measurements of suppression of lethality: *lin-3:* The strain + *lin-3(n378)* + *unc-22(e66)/unc-24(e138) lin-3(n1059) dpy20(e1282)* +; *sli-1(sy143)* was constructed by standard methods. No Dpy Unc-24 progeny were observed in several generations of maintaining this strain. Thus, the lethality associated with the allele *lin-3(n1059)* is not suppressed.

let-23: let-23(mn224 or *mn23) unc-4/mnC1* males were mated to *sli-1*(sy143) hermaphrodites. Possible cross-progeny L4 hermaphrodites were selected, placed on individual plates, and allowed to self-fertilize. Several animals heterozygous for the *let-23 unc-4* chromosome were found, but no Unc-4 animals were found in their progeny other than rare recombinants derived from breakdown of the balancer.

let-60: Using standard methods, we constructed a strain of gen-

otype + *let-60(n1046gf)* + *unc-22(s7)* / *unc-24 let-60(sy100dn) dpy-20* +; *sli-1(sy102)*. Approximately 100 Unc-24 Dpy animals were selected and placed on a single plate; these animals are viable due to maternal rescue of *sy100dn* (HAN *et al.* 1990). None of these *let-60(sy100dn); sli-1(sy102)* animals produced viable progeny.

lin-45: Rare non-Egl animals of genotype *lin-45(sy96) unc-24; sli-1(sy143)* were allowed to lay a cohort of eggs on a plate. These eggs were counted. Two days later, viable animals were counted.

Suppression of hermaphrodite sterility: Strains for these experiments were constructed by standard methods. The following mutations were used: *let-60(n1046)*, *lin-1(e1275ts)*, *lin-1(e1777)*, and *lin-3(n1058)*. In all experiments, *let-23(sy10)* and *let-23(sy12)* were marked in *cis* with *unc-4*, and *lin-3(n1058)* was marked in *cis* with *unc-24(e138)*. *let-23(sy10)* and *let-23(sy12)* result from identical base changes within the *let-23* coding sequence (AROIAN *et al.* 1994) and were used interchangeably for these experiments.

RESULTS

Isolation of suppressors of the *let-23* vulval defect: We screened for suppressors of the vulvaless phenotype of viable, hypomorphic alleles of *let-23* to identify genes that interact with this EGF-receptor homolog. We used two *let-23* genotypes as starting strains (Figure 1, A and B). One of these strains, PS267, balances a weak *let-23* allele in *trans* to a deficiency, the other is homozygous for a more severe allele. From these screens we recovered partially overlapping sets of suppressor mutations (Figure 2).

The first screen takes advantage of *sy1*, a tissue-specific allele that truncates six amino acids from the carboxyl-terminus of LET-23. Animals homozygous for *sy1* display abnormal vulvae but are essentially wild type in all other tissues that require *let-23* function (AROIAN and STERNBERG 1991; AROIAN *et al.* 1994). The strain PS267 balances this allele in *trans* to a deficiency of the locus (Figures 1A and 3). Animals of this genotype almost never lay eggs (0/3600 in contrast to 7% of *sy1* homozygotes; AROIAN and STERNBERG 1991; G. D. JONGEWARD, unpublished data) but are otherwise wild type. Approximately 75,000 ethyl methane sulfonate (EMS) mutagenized PS267 gametes were screened by examining F_2 grandprogeny of mutagenized animals for their ability to lay eggs. A total of 16 mutations were recovered in this screen, defining several loci (Figure 2). A single *let-60(gf)* allele, *sy103*, was recovered. *sy103*, like the other four *let-60(gf)* alleles, is semidominant (data not shown). We recovered *sy103* as an F1 (dominant) suppressor of *let-23* and mapped it to the *let-60* region of linkage group *IV* (data not shown). *sy103* was sequenced by BEITEL *et al.* (1990) and shown to be identical to the other *let-60 ras(gf)* alleles. Two new *unc-101* alleles were also recovered. Analysis of these two alleles and the *unc-101* locus is described elsewhere (LEE *et al.* 1994). Several alleles that define a new locus, *sli-1*, were recovered. These mutant alleles all act as silent suppressors of mutations at *let-23*. Generally, animals of genotype *let-23; sli-1* either displayed wild-type vulval development or displayed greater than the wild-type number of VPCs generating vulval progeny (Hin; Figure 4, Table 1). The allele *sy102* was chosen for further analysis because it causes a slightly more penetrant suppression of the *let-23(sy1)* vulval defect than the other alleles recovered in this screen. Ten suppressor mutations (*sy102, sy104, sy106, sy112, sy114, sy115, sy159, sy160, sy162,* and *sy185*) define one locus, *sli-1*, based on their mapping to the left end of the *X* chromosome, left of *dpy-3*. Characterization of the other three mutations (*sy105*, *sy107* and *sy113*) was limited to determining that they are neither intragenic *let-23* revertants, *sli-1, unc-101,* nor *let-60* alleles.

We also mutagenized PS267 with 1500 rads of X-rays in a screen for suppressors. Approximately 36,000 mutagenized gametes were screened for F_1 (dominant) or F_2 (recessive) suppressors and an additional 650,000 mutagenized F_1 chromosome sets were screened for F_1 (dominant) suppressors only. No true-breeding suppressors were recovered from these screens.

A more severe allele of *let-23, sy97,* was used as the parent stock for the second screen. Approximately 85% of *sy97* homozygotes die; escapers are egg-laying defective (Egl) and display little vulval differentiation (100% Egl and Vul). Approximately 14,000 mutagenized F_1 chromosome sets were screened for suppressors. Twenty-five mutations were recovered. One of these, *sy122,* is a dominant suppressor of *let-23* that is tightly linked to *let-23*. This allele has been shown to be an intragenic revertant of *let-23* by direct sequencing (AROIAN *et al.* 1994). Four mutations (*sy137, sy142, sy147,* and *sy149*) did not suppress *sy97* in trans to *sy102* and are not discussed further here. We were unable to use complementation tests to assign allelism as some *sli-1* alleles are slightly semidominant suppressors of *let-23(sy97)*. We mapped four strong suppressors of the remaining 20 mutations (*sy120, sy121, sy123, sy125, sy126, sy128, sy129, sy131, sy133, sy134, sy135, sy136, sy140, sy141, sy143, sy144, sy145, sy146,* and *sy148*). *sy143* and *sy129* map to the left of *dpy-3*, and thus are *sli-1* alleles (see below). *sy120* and *sy133* do not map in this interval.

Three factor crosses place *sli-1* in the interval between *unc-1* and *egl-17* (Figure 3). Specifically, five of 12 Unc non-Egl recombinant chromosomes from an *egl-17(e1313) sli-1(sy143) unc-1(e719)* / + + + heterozygous hermaphrodite carried the *sli-1* mutation, placing *sli-1* in the interval between *egl-17* and *unc-1*. Deficiencies that delete flanking markers as well as *sli-1* uncover the suppression phenotype of the locus (Table 1). Duplications that include flanking loci complement the suppression phenotype (Table 1). All of the *sli-1* alleles isolated as PS267 suppressors also fail to complement the allele *sy102* except for *sy112* (Table 1). Because

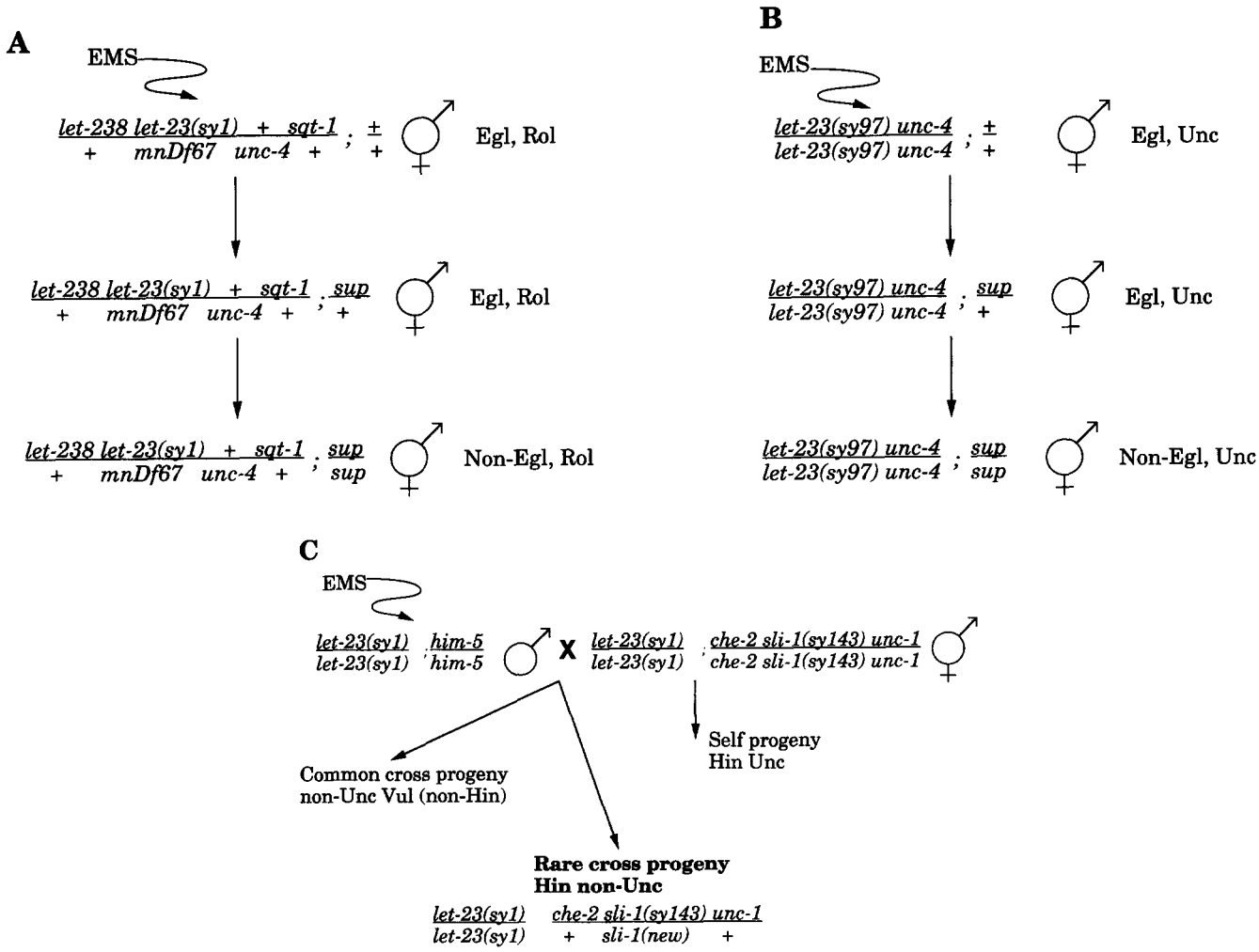

FIGURE 1.—Screens for mutations described in this study. (A) Reversion of PS267. The *sqt-1* allele used confers a semidominant Roller phenotype, thus all genotypes can be scored for this marker and many recombinant chromosomes can be discarded. Sup, suppressor mutation; Egl, egg-laying defective; Rol, roller. *let-238* and *Df* homozygous animals are inviable. (B) Reversion of *let-23(sy97)*. Eighty-five percent of animals homozygous for this allele die as L1 larvae. (C) Noncomplementation screen to recover new alleles of *sli-1*. *him-5* is included to obtain males from the vulvaless *sy1* strain. *che-2* and *unc-1* are flanking markers for *sli-1*.

sy112 fails to complement other *sli-1* mutations and maps to the left of *unc-1*, we conclude it is an allele of *sli-1*. *sy112* may not be a loss-of-function mutation: the phenotype of *sy112* is less severe in *trans* to a deficiency: *sy112* homozygotes suppress *sy97* to wild type while *sy112* hemizygotes suppress only 32% of *sy97* animals to wild type (Table 1).

Loss-of-function phenotype: To characterize the *sli-1* locus genetically, we attempted to recover complete loss-of-function alleles of the locus. A deficiency of the locus is viable in *trans* to the allele *sli-1(sy143)* in both a wild-type background and in a *let-23(sy1)* background. Animals of the genotype *let-23(sy1); sli-1(sy143)/meDf3* display a phenotype similar to that of *let-23(sy1); sli-1(sy143)*, in that most animals of either genotype are competent to lay eggs and have prominent pseudovulvae. Therefore it was possible to recover complete loss-of-function alleles in *trans* to this *sli-1* allele. We recovered one *sli-1* allele in a non-complementation screen of 5200 mutagenized haploid genomes (Figure 1C). This allele, *sy263*, is a weaker suppressor of *let-23(sy1)* than the reference allele *sy143*: an average of 2.5 VPCs generate vulval cells in *let-23(sy1); sli-1(sy263)* animals.

Analysis of deficiencies of the region suggest that the phenotypes of the stronger alleles are indeed strong reduction-of-function phenotypes of the locus. As mentioned above, the *trans* heterozygote [*let-23(sy1); meDf3/sli-1(sy143)*] is suppressed for the *let-23* vulval defect. These animals are similar to *let-23(sy97); sli-1(sy143)* double homozygotes. Other, weaker *sli-1* alleles are enhanced by *meDf3* (Table 1). Thus, the allele *sy143* is about as severe as *meDf3* and the alleles *sy102* and *sy112* are weaker. The allele *sy143* behaves similarly to *meDf3* when measured in *trans* to *sli-1(sy102)* in a *let-23(sy97)* background (Table 1), suggesting that *sli-*

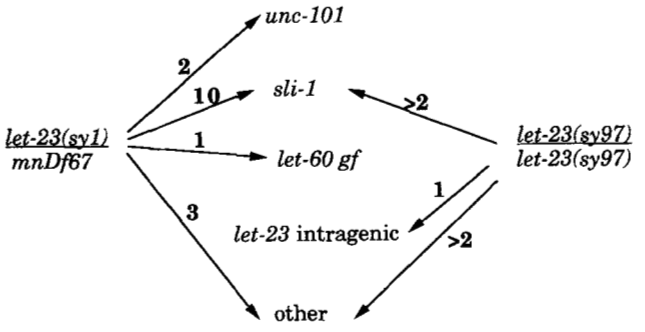

FIGURE 2.—Mutations recovered as *let-23* suppressors. Frequency of recovery is only relevant from the reversion of PS267; subviability complicates any estimation of frequency in the other screen. The estimated frequency of intragenic reversion is by extrapolation assuming a 10-fold bias in recovery due to dominant suppression of *let-23(sy97)* lethality. Numbers above the arrows indicate the number of alleles isolated.

1(sy143) is nearly as severe as the deficiency. This comparison holds partially true for heterozygous animals. *let-23(sy97); meDf3/+* heterozygotes are occasionally egg-laying competent and display 43% vulval differentiation. *let-23(sy97); sli-1(sy143)/+* animals display significantly less vulval differentiation than *Df/+* animals but more than *+/+* animals (Table 1). Thus, *meDf3* is more severe than *sy143*. Similarly, the allele *sy143* is a strong suppressor of *let-23(sy97)* lethality. Approximately 80% of animals of genotype *let-23(sy97); meDf3/+* or *let-23(sy97); sli-1(sy143)/+* are viable, whereas 15% of animals of the genotype *let-23(sy97); sli-1(+)* are viable (see Table 4), suggesting that loss of one copy of the locus is sufficient to suppress the lethality of weak alleles of *let-23*. Thus, *meDf3* and *sli-1(sy143)* are equal in their ability to suppress this phenotype.

We conclude that the alleles we have characterized are strong reduction-of-function alleles at the *sli-1* locus based on four criteria. First, the allele *sy143* behaves similarly to the deficiency *meDf3*, although *sy143* is weaker than the deficiency for suppression of the vulvaless phenotype of *let-23*. Second, *sy143* is the most severe allele in an allelic series defined by the alleles recovered as suppressors of *let-23* mutations, but is not exceptional when compared with the other alleles (Table 1). Third, in a noncomplementation screen that could have recovered null alleles of *sli-1*, we recovered an allele similar to the previously identified alleles. Fourth, the screen for suppressors of *let-23(sy97)* should allow recovery of *sli-1* null alleles as dominant suppressors of *let-23* because a null allele at this locus should suppress the lethality of this *let-23* allele and should semidominantly suppress the vulval defect of this allele. *Df/+* is viable and suppresses *let-23* lethal and vulvaless phenotypes. We did not recover recessive lethal, weak dominant suppressors of the vulval defect. Therefore, *sli-1* is not an essential gene.

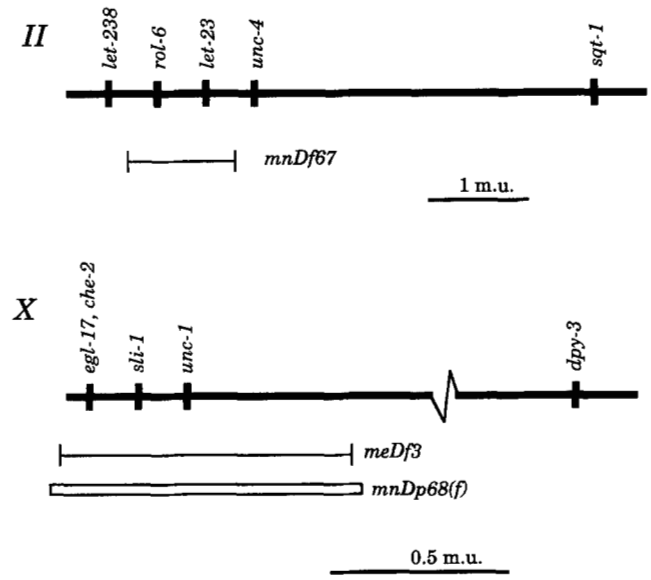

FIGURE 3.—Partial genetic maps of the markers and rearrangements used in this study. Cluster of chromosome *II* and left arm of the *X* chromosome are shown. Deficencies are represented by lines with bars at ends. The free duplication *mnDp68* is represented by a hollow bar. m.u., map units.

***sli-1* phenotypes:** All of the *sli-1* alleles that we have analyzed display no obvious phenotype in the absence of another mutation (Table 1) except for a low penetrance abnormal head phenotype viewed in the dissecting microscope (*e.g.* for *sy143/sy143*; <5%; 11/294 L1 larvae; 2/210 L2 larvae). This phenotype is present at similar low levels in all *sli-1* genotypes examined to date. Five of 24 L1 *sy143/sy143* larvae (20%) had abnormal head morphology viewed in Nomarksi optics (Figure 5).

Because *sli-1* mutations increase the extent of vulval differentiation in various mutant backgrounds, we tested whether *sli-1* mutations result in vulval differentiation in the absence of the inductive signal. We examined six *sli-1* animals whose gonads were ablated during the L1 stage and observed no vulval differentiation (Table 2).

***sli-1* mutations suppress hypomorphic alleles of *let-23*:** The vulvaless phenotypes of all partial reduction-of-function *let-23* mutations examined are suppressed by a *sli-1* mutation. The weak allele *let-23(sy1)* is suppressed by all of the *sli-1* alleles tested. These *let-23(sy1); sli-1* animals frequently display a Hin phenotype and only rarely do animals display less than wild-type vulval differentiation (Table 1). The excessive vulval differentiation of *sy1; sy143* animals is gonad-dependent (Table 2).

The *let-23(n1045)* allele is suppressed at 15° but not at 25° by the *sli-1* allele *sy143*. *let-23(n1045); sli-1 (sy143)* animals are not temperature sensitive (Table 3): *let-23(n1045)* hermaphrodites display less than wild-type vulval differentiation at 15°; and greater than wild-type vulval differentiation at 25°. By contrast, *let-*

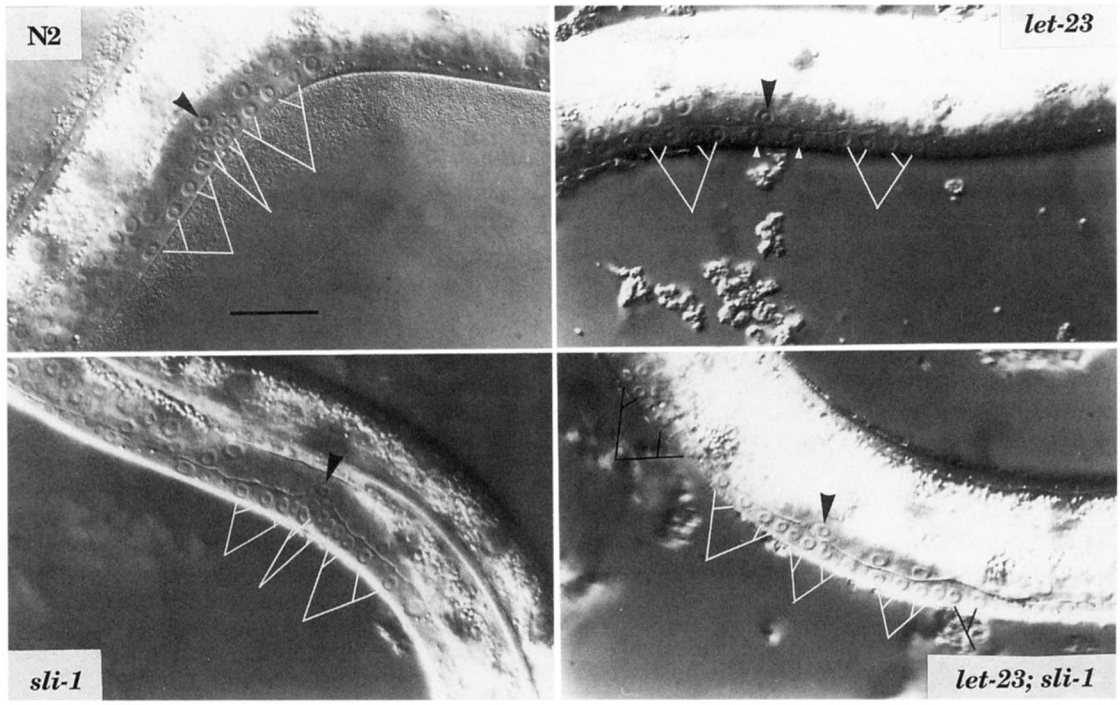

FIGURE 4.—Nomarski photomicrographs of left lateral views of L3 lethargus/young L4 hermaphrodites of various *let-23(sy1)* and *sli-1(sy143)* genotypes. Large black arrowhead marks the anchor cell, white trees show presumed lineage relationships among cells, black trees show presumed lineage relationships among cells that do not normally form vulval tissue. In the *let-23(sy1); sli-1(sy143)* animal, two of the progeny cells of one of the induced VPCs are out of the focal plane; the siblings of these P8.p progeny are marked by a partial tree. Anterior to the left and ventral at the bottom. Scale bar, 20 μm.

23(n1045); *sli-1(sy143)* double mutant animals (at 15°, 20° or 25°) resemble *let-23(n1045)* animals at 25°. *let-23(sy12)*, an allele similar in severity to *let-23(sy97)*, is also suppressed to an apparent Hin phenotype by the *sli-1* allele *sy143* (Table 3).

Animals bearing the *let-23* alleles *sy1, sy12,* or *n1045* in combination with a *sli-1* mutation are frequently Hin (Table 3). Animals bearing certain weak *sli-1* alleles (such as *sy115* or *sy263*) in combination with *let-23(sy1)* display less than wild-type vulval differentiation (Table 1). Most animals of these genotypes are Vul. Suppression to wild type is uncommon; only animals bearing the *let-23* allele *sy97* and a subset of *sli-1* mutations (the severe alleles *sy129* and *sy143,* but not *sy102* or *sy112*) approach wild type (Table 1). Hin animals are not seen in any animal bearing the *let-23* allele *sy97,* regardless of the *sli-1* genotype. The approximately normal vulval differentiation of *sy97; sy143* is dependent on the presence of the gonad. The hyperinduced phenotype therefore seems to be more dependent on the *let-23* genotype than the *sli-1* genotype [*i.e.,* mutations that suppress *let-23(sy97)* to wild type or nearly wild type are sufficient to cause the Hin phenotype in combination with other *let-23* alleles, but no *sli-1* alleles are apparently sufficient to cause the Hin phenotype in an animal of genotype *let-23(sy97); sli-1*]). We hypothesize that many *let-23* alleles, but not *sy97*, are defective in some LET-23-dependent SLI-1-independent negative regulation of the extent of vulval differentiation; two defects in negative regulation are necessary to obtain a Hin phenotype, one defect due to the *let-23* allele, the other due to a *sli-1* mutation (see also AROIAN and STERNBERG 1991; G. JONGEWARD and P. STERNBERG, unpublished data).

***sli-1* mutations suppress *let-23* mutations in a tissue-specific manner:** Mutations in *let-23* can affect several different developmental processes, disrupting vulval development, P12 neuroectoblast determination, male tail development, larval growth and hermaphrodite fertility (FERGUSON and HORVITZ 1985; AROIAN and STERNBERG 1991; CHAMBERLIN and STERNBERG 1994). We examined the ability of *sli-1* mutations to suppress the other *let-23* phenotypes by examining a number of allelic combinations of hypomophic *let-23* mutations and the strong *sli-1* allele *sy143*. We find that *sli-1(sy143)* suppresses all of the identified *let-23* defects associated with weak alleles of *let-23* except for sterility.

Lethality: The lethality of *sy97* is suppressed semidominantly by the allele *sli-1(sy143)* and by the deficiency of the region *meDf3* (Table 4). *sli-1(sy143)* suppresses *sy97* to complete viability. Similarly, the subviability of the *let-23* allele *n1045* is also suppressed by *sli-1(sy143)*. However, neither null alleles such as *mn23* and *sy15* nor the tissue-preferential lethal allele *mn224* are measurably suppressed (MATERIALS AND METHODS). Mutations at the *sli-1* locus are apparently

TABLE 1

Extent of vulval differentiation in various *let-23; sli-1* double mutant animals

	let-23(sy1)					*let-23(sy97)*					*let-23*(+)		
sli-1 Genotype	%<3	%=3	%>3	*n*	Avg/animal	%<3	%=3	%>3	*n*	Avg/animal	%=3	*n*	Avg/animal
+/+	95	5	0	29	0.8	100	0	0	20	0.0	100	20	3.0
+/+/+	100	0	0	22	0.0	—	—	—	—	—	100	13	3.0
sy143/sy143/+	100	0	0	20	0.2	—	—	—	—	—	—	—	—
meDf3/+	—	—	—	—	—	74	26	0	19	1.3	100	20	3.0
sy143/+	91	9	0	23	1.0	100	0	0	11	0.05	—	—	—
sy102/+	100	0	0	10	0.6	—	—	—	—	—	—	—	—
sy112/+	100	0	0	13	0.7	—	—	—	—	—	—	—	—
sy143/sy143	0	5	95	20	4.3	3	97	0	72	2.9	100	20	3.0
sy143/meDf3	—	—	—	—	—	0	100	0	17	3.0	100	13	3.0
sy102/sy102	17	28	55	29	3.7	24	76	0	17	2.5	100	20	3.0
sy102/meDf3	—	—	—	—	—	17	83	0	18	2.7	—	—	—
sy102/sy143	—	—	—	—	—	12	88	0	17	2.9	—	—	—
sy102/sy112	85	11	4	27	1.1	—	—	—	—	—	—	—	—
sy112/sy112	5	73	23	22	3.2	0	100	0	20	3.0	100	20	3.0
sy112/meDf3	—	—	—	—	—	68	32	0	22	1.2	—	—	—
sy129/sy129	0	25	75	20	4.3	10	90	0	21	2.9	100	22	3.0
sy114/sy114	0	55	45	20	3.4	—	—	—	—	—	—	—	—
sy115/sy115	35	35	30	20	2.7	—	—	—	—	—	—	—	—
sy263/sy263	20	80	0	20	2.5	—	—	—	—	—	—	—	—

Heterozygous animals were either generated by direct matings or examined under Nomarski optics, removed to Petri dishes, allowed to self-fertilize and from progeny phenotypes the maternal genotype was determined. %<3, percent of animals examined that had fewer than three VPCs generating vulval progeny; %=3, percent of animals with exactly three VPCs generating vulval progeny; %>3, percent of animals examined that had greater than three VPCs generating vulval progeny (Hin or Muv); avg/animal, average number of VPCs generating vulval progeny per animal. Partial triploids were constructed using a free duplication (see MATERIALS AND METHODS).

not sufficient to bypass the requirement for *let-23* for viability. This observation is consistent with the role of *sli-1* in vulval differentiation: *let-23; sli-1* doubly mutant animals are still dependent on the gonad, and thus a *sli-1* mutation does not bypass the requirement for *let-23* in the vulva. Instead, *sli-1* mutations seem to modify the function of *let-23*.

Male copulatory spicules: Although all *sy97* males have abnormal spicules, 68% of *let-23(sy97); sli-1(sy143)/O* males ($n = 19$) have normal spicules.

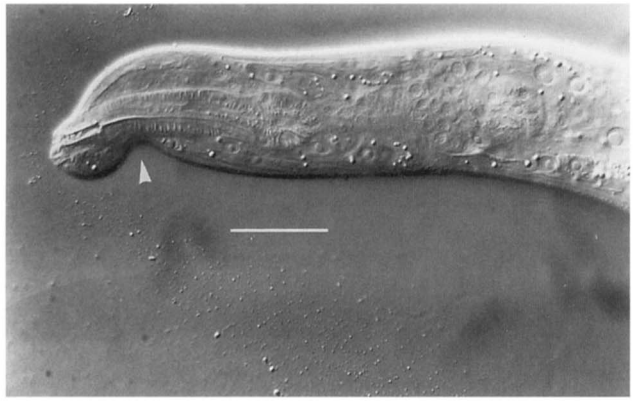

FIGURE 5.—The visible phenotype of *sli-1(sy143)*. Nomarski photomicrograph of a L2 *sli-1(sy143)* animal displaying the head phenotype present in *sli-1* homozygous larvae.

P12: sli-1(sy143) suppresses the transformation of the P12 cell to P11 in *let-23(sy97)* animals, although both the transformation by *let-23(sy97)* and the suppression by *sli-1* are incomplete (Table 4). This suppression is semi-dominant: essentially all *let-23(sy97); sli-1(sy143)* and *let-23(sy97); sli-1(sy143)/+* animals displayed a wild-type P12 phenotype, compared with 35 of 48 *let-23(sy97); +* animals.

Hermaphrodite sterility: The sterility of the allele *let-23(sy12)* is not suppressed. Most animals of the genotype *let-23(sy12)* are inviable; escapers are sterile and Vul (AROIAN and STERNBERG 1991). *let-23(sy12); sli-1(sy143)* animals are also sterile ($n = 24$) but are fre-

TABLE 2

Gonad dependence of *sli-1* suppression

Genotype	Gonad +	Gonad −
+; +	3 (20)	0 (*)
+; *sli-1(sy143)*	3 (20)	0 (6)
let-23(sy1); +	1 (20)	0 (7)
let-23(sy1); sli-1(sy143)	4 (20)	0 (5)
let-23(sy97); sli-1(sy143)	3 (72)	0 (5)

Gonad dependence of vulval differentiation in *let-23; sli-1* animals. Wild type is from SULSTON and WHITE (1980) and our observations. Values in parentheses are number of animals; *, many.

TABLE 3

Suppression of *let-23* alleles by *sli-1(sy143)*

Genotype	Average number of VPCs undergoing vulval differentiation	
	sli-1(+)	*sli-1(sy143)*
let-23(+)	3.0 (20)	3.0 (20)
let-23(n1045) 15°	1.1 (20)	3.1 (20)
let-23(n1045) 20°	2.5 (20)	3.3 (20)
let-23(n1045) 25°	3.4 (20)	3.4 (20)
let-23(sy12) 20°	0.018 (31)	4.0 (20)

Extent of vulval differentiation in animals of other *let-23; sli-1* genotypes. The data for *let-23(sy12); +* is from AROIAN and STERNBERG (1991). Values in parentheses are number of animals.

TABLE 4

***sli-1*-mediated suppression of *let-23* mutant phenotypes**

Genotype		Phenotype	
let-23	*sli-1*	Viability	P12
+	+/+	100 (*)	100 (*)
sy97	+/+	15 (*)	73 (48)
sy97	*meDf3*/+	79 (191)	83 (18)
sy97	*sy143*/+	83 (952)	100 (22)
sy97	*sy143/sy143*	99 (880)	86 (22)
n1045	+/+	56 (75)	ND
n1045	*sy143/sy143*	100 (27)	ND

Dominant suppression of *let-23(sy97)* lethality by *meDf3* and *sli-1(sy143)* was estimated by comparing the number of animals homozygous for a marker in *trans* to *let-23(sy97) (either unc-4* or *rol-6)* to the number of non-Unc or non-Rol animals. Suppression of the P12 transformation by either *sli-1(sy143)/ +* or *meDf3/+* was determined under Nomarski optics followed by confirmation of the genotype of the animal by segregation. Values are percent of animals wild type; *, many, ND, not determined.

quently Hin (Table 3 and data not shown), indicating that *sli-1(sy143)* suppresses the vulvaless phenotype of *sy12*. This observation suggests that *let-23* mediates two genetically distinct functions and that *sli-1* only regulates one of these two activities. Two additional lines of evidence are consistent with this possibility. First, *sli-1* displays a similar tissue specific pattern of suppression with *lin-3(n1058)*. As discussed below, *sli-1(sy143)* weakly suppresses the vulval defects associated with hypomorphic *lin-3* mutations. *lin-3(n1058); sli-1(+)* animals are sterile (FERGUSON and HORVITZ 1985) and Vul (1.6 Avg. induction, $n = 31$); *lin-3(n1058); sli-1(sy143)* animals display increased vulval induction (2.8 VPCs induced per animal, $n = 30$) but remain sterile ($n = 30$). Second, mutations known to bypass the requirement for *let-23* function in the vulva are unable to bypass *let-23* function in the gonad. Specifically, the gain of function *let-60(n1046)* mutation causes animals lacking *let-23* activity in the vulva to display excess vulval differentiation (HAN *et al.* 1990); in addition, *let-23(sy10); let-60(n1046)* animals are Muv and sterile ($n = 20$). Similarly, loss of function mutations in *lin-1* can result in a Muv phenotype; *let-23(sy10); lin-1(e1275ts)* animals are sterile and Muv ($n = 11$) as are *let-23(sy10); lin-1(e1777)* animals ($n = 35$). Taken together, these experiments suggest that *sli-1* is a negative regulator of only one of the two genetically separable activities of *let-23*.

Dosage sensitivity: The *sli-1* locus shows dosage sensitivity for suppression of *let-23* phenotypes in at least two tissues. For instance, a higher percentage of animals of genotype *let-23(sy97) / let-23(sy97); sli-1(sy143) / +* animals are viable than are *let-23(sy97) / let-23(sy97); +/+* control animals (Table 4). This is also true of *let-23(sy97) / let-23(sy97); meDf3/+* animals (Table 4). These *let-23(sy97); meDf3/+* animals also display suppression of the vulval defect (Table 1). Thus, this locus is haplo-insufficient for suppression of *let-23* lethal and vulvaless phenotypes. We have used a free duplication of the *sli-1* region, *mnDp68* to increase the copy number of *sli-1(+)*. Animals of genotype *let-23(sy1) / let-23(sy1); mnDp68[X; f] sli-1(sy143) / sli-1(sy143)* or *let-23(sy1) / let-23(sy1); mnDp68[X; f]* display a more penetrant vulval defect than do *let-23(sy1)* homozygotes (Table 1). It is possible that the equivalence of *sy143/ sy143/ Dp* to *+/+/Dp* is due to *sy143* being a nonnull allele.

***sli-1* suppression of mutations of other genes required for vulval induction:** To determine whether suppression by *sli-1* was limited to *let-23*, we examined the ability of *sli-1* to suppress mutant phenotypes associated with mutations in other genes in the vulval induction pathway. This analysis suggests that *sli-1* acts as a negative regulator of either *let-23* or other genes acting at about the same step in the vulval induction pathway.

lin-3: The Vul phenotypes caused by certain hypomorphic mutations of *lin-3* are partially suppressed by *sli-1* mutations. Although animals bearing a mutation at both *lin-3* and *sli-1* are frequently as defective in vulval development as are *lin-3; +* animals, some animals display a less penetrant vulval defect than do control animals not mutant for *sli-1*. A strong *lin-3* genotype (a reduction of function allele *n378* in *trans* to a putative null allele *n1059*) is suppressed from an average of 0.1–0.6 VPCs forming vulval tissue per animal as compared with 3.0 VPCs forming vulval tissue per animal in wild type (Table 5; R. HILL and P. STERNBERG, unpublished data). Thus *sli-1* partially suppresses the vulval defect of a severe *lin-3* allelic combination. This may reflect residual or suppressible *lin-3* activity, because vulval differentiation in *sli-1* animals requires the presence of the gonad, the source of *lin-3* (Table 2). Homozygous *lin-3(n1059)* progeny of this *trans* heterozygote are inviable, indicating that this *sli-1* allele is not suffi-

TABLE 5

***sli-1*-mediated suppression of mutations in genes required for vulval induction**

Vul genotype	Average number of VPCs undergoing vulval differentiation per animal	
	sli-1(+)	*sli-1(sy143)*
+	3.0 (20)	3.0 (20)
lin-3(e1417rf)	0.8 (20)	1.4 (20)
lin-3(n378rf)	0.8 (22)	2.0 (16)
lin-3(n3784rf)/lin-3(n1059lf)	0.1 (20)	0.6 (10)
lin-2(e1309lf)	0.5 (20)	4.3 (21)
lin-2(n768rf)	2.9 (20)	3.6 (20)
lin-7(e1413lf)	1.0 (20)	3.3 (20)
lin-7(n308rf)	2.6 (20)	4.1 (20)
lin-10(e1439lf)	0.4 (17)	4.1 (20)
sem-5(n2019rf)	0.5 (20)	2.6 (23)
let-60(n2021rf)	2.4 (22)	2.96 (27)
let-60(s1124rf)	0.0 (20)	0.02 (20)
lin-45(sy96rf)	0.9 (24)	1.1 (20)

Extent of vulval differentiation in doubly mutant animals bearing a *sli-1* mutation and mutations in other genes required for vulval induction. rf, partial reduction-of-function allele; lf, complete loss-of-function allele. Values in parentheses are number of animals.

cient to suppress a *lin-3* null. Suppression of *lin-3* does not result in hyperinduced animals: there is an increase in the number of VPCs forming vulval tissue to a maximum of three.

lin-2, lin-7 and lin-10: lin-2, lin-7 and *lin-10* are defined by recessive, loss-of-function mutations that reduce but do not eliminate vulval differentiation (HORVITZ and SULSTON 1980; FERGUSON and HORVITZ 1985; KIM and HORVITZ 1990); the residual vulval differentiation is gonad-dependent (STERNBERG and HORVITZ 1989). All allelic combinations of *sli-1* and *lin-2, lin-7,* and *lin-10* mutations tested result in frequent Hin animals (Table 5). *lin-2, lin-7,* and *lin-10* are the only known genes required for vulval differentiation in which putative null alleles are suppressed by *sli-1* mutations. It is unclear whether this suppression occurs because animals bearing these null alleles are viable (and the Vul phenotype of animals homozygous for null alleles of other genes would be suppressed if these animals were viable) or whether it reflects the ability of *sli-1* mutations to specifically suppress putative null alleles of *lin-2, lin-7,* and *lin-10*. Animals homozygous for *lin-2, lin-7,* or *lin-10* null alleles (*e1309, e1413* and *e1439*, respectively) still display significant residual vulval differentiation (Table 5; FERGUSON and HORVITZ 1985; STERNBERG and HORVITZ 1989; KIM and HORVITZ 1990), in contrast to what is believed to be the case for null alleles of *lin-3, let-23,* or *let-60*. It is possible that *sli-1* suppresses these null alleles indirectly, by increasing whatever activity is responsible for residual differentiation in the absence of *lin-2, lin-7* or *lin-10* gene products. A *sli-1* mutation can also interact with hypomorphic mutations in *lin-2* or *lin-7* increasing the penetrance of the Hin phenotype of animals homozygous for either *lin-2(n768)* or *lin-7(n308)*. Animals homozygous for these alleles frequently display a Hin phenotype in the absence of a *sli-1* mutation (FERGUSON and HORVITZ 1985; G. JONGEWARD and P. STERNBERG, unpublished data). These double mutant animals are very similar in phenotype to double mutant animals bearing putative null alleles at these loci and *sli-1* mutations.

sem-5: The vulval differentiation defect associated with *n2019*, a hypomorphic mutation at *sem-5* (CLARK *et al.* 1992a) is suppressed to a nearly wild-type phenotype by *sli-1(sy143)* (Table 5). Phenotypically, these *sli-1 sem-5* animals are similar to *let-23(sy97)*; *sli-1* animals, in that no Hin animals are observed. A *sli-1* mutation does not suppress the sex myoblast phenotype associated with a *sem-5* mutation (data not shown). The lethality associated with this *sem-5* mutation is partially suppressed; the brood size of *sem-5* animals is ~21 (n = 5 hermaphrodites). The brood size of *sli-1 sem-5* animals averages 44 (n = 2 hermaphrodites). This may reflect a slight ability on the part of the *sli-1 sem-5* animals to lay eggs, but it seems unlikely that these animals lay enough eggs to double the number of progeny generated.

let-60: A weak hypomorphic allele of *let-60, n2021*, is slightly suppressed by a *sli-1* mutation. In contrast to *let-60(n2021)* animals, which have an average of 2.4 VPCs undergoing vulval differentiation (13 of 22 wild type; Table 5), an average of 2.96 VPCs undergo vulval differentiation in *let-60(n2021); sli-1(sy143)* animals (Table 5; 25 of 27 wild type). Animals bearing a homozygous dominant negative *let-60* allele [*let-60(sy100dn)*] and the *sli-1* allele *sy102* display no vulval differentiation (n = 16), as does the *let-60(dn); sli-1*(+) (HAN *et al.* 1990). *let-60(dn)* homozygotes segregate only dead larvae; this phenotype is not suppressed by *sli-1(sy102)*. Because the dominant negative effect of *let-60* is likely due to an effect on an activator of LET-60 ras (HAN and STERNBERG 1991), we also examined the ability of *sli-1(sy143)* to suppress the vulval defects caused by the lethal allele *s1124*. The majority of *s1124* homozygotes die early in larval development; a small fraction survive to the adult stage before dying. None of 20 *s1124; sy143* hermaphrodites that survived to adulthood displayed any vulval differentiation; these animals were inviable, indicating that the lethality is not suppressed by *sli-1(sy143)*.

lin-45: lin-45 is not significantly suppressed by *sli-1*. The extent of vulval differentiation in *lin-45(sy96); sli-1(sy143)* double mutants is not significantly different than that of *lin-45(sy96)*; + animals (Table 5). In addition, the lethality associated with this allele is not suppressed: very few *lin-45(sy96); sli-1(sy143)* animals are

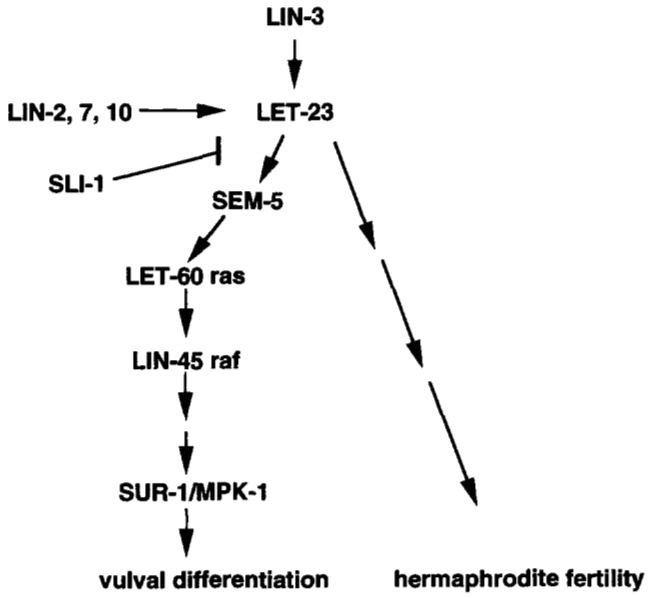

FIGURE 6.—Formal genetic pathway showing the proposed activity of SLI-1 as a negative regulator of LET-23/SEM-5 mediated signaling during vulval differentation but not the pathway leading to hermaphrodite fertility. The dependence of fertility on SEM-5 is not known rigorously. See text and STERNBERG (1993) for discussion of the pathway analysis and references.

viable (only six of 96 eggs) as are *lin-45(sy96)*; *sli-1* (+) animals. By contrast, a similarly subviable allele of *let-23* is suppressed to complete viability by *sli-1(sy143)*.

Summary: Mutations at *sli-1* are sufficient to suppress the vulval defect of mutations of *let-23*, *sem-5*, *lin-2*, *lin-7*, and *lin-10* mutations, but not mutations at *lin-45* and only partially mutations at *let-60* or *lin-3*. We conclude that *sli-1* acts as a negative regulator of the vulval induction pathway at a step prior to the activities of *lin-45 raf*, most likely at the LET-23/SEM-5 step (Figure 6).

Moreover, the lethal phenotypes of mutations at *lin-3*, *let-60*, and *lin-45* are not suppressed by mutations at *sli-1*. In addition to the observations described above, during maintenance of strains defective in *sli-1* and heterozygous for lethal alleles of either *lin-3*, *let-60* and *lin-45*, we did not observe any viable animals homozygous for the lethal alleles.

DISCUSSION

Suppressors of hypomorphic *let-23* mutations define the *sli-1* locus: We have recovered and characterized suppressors of hypomorphic mutations of *let-23*, an EGF-receptor homolog required for *C. elegans* vulval differentiation. These suppressors were recovered in two screens that impose different selective constraints. Reversion of PS267 (carrying a very weak *let-23* allele balanced in *trans* to a deficiency of the locus) selected solely for suppression of a severe *let-23* Vul phenotype. In contrast, reversion of *let-23(sy97) unc-4* selected strongly for suppression of the lethal as well as of the Vul phenotype, and thus not surprisingly, the alleles recovered as suppressors of the vulval defect of *let-23(sy97)* were also dominant suppressors of the lethality of this allele. Of the four loci that we have characterized, mutations were common at one locus *(sli-1)* and rare at the other three. The rare class included mutations at two loci, which were expected to function as suppressors of *let-23*, these being an intragenic revertant and a *let-60 ras (gf)* mutation. The fourth class comprised *unc-101*, which is described elsewhere (LEE *et al.* 1994).

sli-1 alleles were recovered frequently in both screens. Reversion of PS267 yielded *sli-1* alleles at a frequency of 1/7500. *sli-1* alleles were recovered at this frequency or higher as suppressors of *let-23(sy97)*; we did not characterize fully most of these suppressors. Because *sli-1* mutations dominantly suppress the lethality of the *let-23(sy97)* allele and >80% of animals homozygous for the *let-23(sy97)* allele die, any mutation able to suppress this lethality dominantly will be preferentially represented in the F_2 (any F_1 animal bearing a putative dominant suppressor of lethality will be viable, whereas most animals not bearing such a mutation will die). Thus, this screen is more extensive for dominant suppressors of lethality provided that these mutations are also able to suppress the vulval defect. In addition, because we picked F_3 eggs, this screen selects for recessive suppression of the lethal phenotype as we are demanding viability of the F_2 animals.

***sli-1* normally acts antagonistically to *let-23*:** We believe the suppression of *let-23* and other vulvaless mutations occurs because *sli-1* encodes a negative regulator of LET-23-mediated vulval induction for three reasons. First, we do not believe that *sli-1* is an informational suppressor because *sli-1* mutations suppress a number of alleles that share little in common based on molecular data. Two classes of informational-suppressor mutations have been characterized in *C. elegans* (reviewed by HODGKIN *et al.* 1987). *sli-1* is clearly not an amber suppressor, as it suppresses several alleles that are not amber suppressible, based on characterization of the molecular lesions associated with these mutations, and fails to suppress several mutations that are suppressed by amber suppressors [such as *let-60(n1046)* and *unc-24(e138)*]. A second class of informational suppressor is the *smg* class of genes (HODGKIN *et al.* 1989; PULAK and ANDERSON 1993). *sli-1* is unlike these genes, as it does not interact with *dpy-5* in a manner consistent with these mutations, nor does it display the male abnormal phenotypes associated with these mutations (HODGKIN *et al.* 1989). We can exclude another possibility, that *sli-1* alters splicing: while *sli-1* mutations suppress two mutations of 3' splice acceptor sites [*let-23(sy97)* and *let-23(n1045)*], two other such mutations are not suppressed [*lin-45(sy96)* and *dpy-10(e128)*; AROIAN *et al.*

1993, 1994; HAN *et al.* 1993]. If *sli-1* is an informational suppressor, it represents a novel class. Second, the *sli-1* locus displays dosage sensitivity. Deficiencies of the *sli-1* region or strong *sli-1* mutations in *trans* to a wild-type copy of the *sli-1* locus result in strong suppression of the lethality of a subviable *let-23* allele, as well as partial suppression of the vulval defect of this allele. In addition, a duplication of *sli-1*(+) enhances the vulvaless phenotype of *let-23*(*sy1*), suggesting that the wild-type activity of *sli-1* is antagonistic to the wild-type activity of *let-23*. Third, mutants defective in both *sli-1* and *unc-101* display excessive vulval induction, supporting our hypothesis that *sli-1* functions as a negative regulator (G. JONGEWARD and P. STERNBERG, unpublished data).

Role of *sli-1* in vulval induction: Mutations at the *sli-1* locus are capable of suppressing mutations in several genes required for vulval induction. Because *sli-1* mutations do not suppress severe loss-of-function mutations in *lin-3*, *let-23*, *let-60* or *lin-45*, we believe that *sli-1* is not the sole target of the LIN-3/LET-23 signaling pathway, but rather functions as a regulatory branch. To determine the targets of this regulatory input, we analyzed additional double mutant combinations. Mutations at the *sli-1* locus strongly suppress reduction-of-function mutations in *let-23*, *sem-5*, *lin-2*, *lin-7* and *lin-10*, but only weakly suppress partial reduction-of-function mutations in *lin-3*, and *let-60*. Furthermore, because the lethality of strong hypomorphic mutations of *let-60* or *lin-45* are not suppressed, *sli-1* mutations appear to suppress *lin-3*, *let-60* and *lin-45* much less than they suppress *let-23*, *sem-5*, *lin-2*, *lin-7*, and *lin-10*. We therefore hypothesize that *sli-1* is a negative regulator, acting after *lin-3* and before the activity of *let-60 ras* and *lin-45 raf* (Figure 6).

Possible mechanisms of SLI-1 action: It is not clear if SLI-1 directly interacts with components of the vulval induction pathway. We believe that the strength of suppression by *sli-1* mutations indicates the closeness of the interaction: SLI-1 is more likely to interact with LET-23 and SEM-5 than with LIN-3 or LIN-45. Moreover, mutations at *sli-1* are competent to suppress *let-23* mutations in several tissues. It is difficult to postulate a simple model for SLI-1 interacting with LIN-10, for instance, because this protein is required neither for viability nor male tail development (FERGUSON and HORVITZ 1985; KIM and HORVITZ 1990), all of which are processes involving distinct cells in which *sli-1* functions. It is possible that *sli-1* interacts with different genes in different tissues to control the functions of this signal transduction pathway. For instance, *sli-1* could interact with *lin-10* in the vulva, and with other (unidentified) tissue-specific genes elsewhere. However, the simplest model is that SLI-1 is a negative regulator of LET-23.

The *sy97* mutation most likely results in LET-23 protein that is truncated for one-half of its carboxyl terminal tail and thus would be missing putative SEM-5 binding sites (SONYANG *et al.* 1993; AROIAN *et al.* 1994). SEM-5 binds to human EGF-receptor *in vitro*, and a human SEM-5 homolog GRB-2 rescues a *sem-5* mutant in transgenic *C. elegans* (STERN *et al.* 1993); thus it is likely that SEM-5 binds to a phosphorylated YXN motif in the carboxyl tail of LET-23. This defect in signal transduction is suppressed by the *sli-1* mutation. We hypothesize that in a *sli-1* mutant, activation of LET-60 ras and LIN-45 raf occurs via different signaling components. Specifically, SLI-1 might negatively regulate a SEM-5-independent pathway from LET-23 to LET-60. One caveat to this hypothesis is that we have not analyzed null mutations of SEM-5 because of their lethality.

Negative regulation of vulval differentiation: Mutations at the *sli-1* locus cause animals homozygous for a viable mutation in *let-23*, *lin-2*, *lin-7*, or *lin-10* to differentiate excessive vulval tissue in a signal-dependent manner (the hyperinduced phenotype). As discussed above, one allele of *let-23*, *sy97*, does not display this excessive differentiation when combined with a *sli-1* mutation. We believe that this reflects an inherent ability of the *sy97* allele to activate a *sli-1*-independent pathway of negative regulation. This pathway might normally function to refine the pattern of induced cells, perhaps by activating lateral inhibition among cells that receive inductive signal or by activating an intracellular pathway of negative regulation (see AROIAN and STERNBERG 1991 for further discussion). Other *let-23* alleles (such as *sy1*, *n1045*, and *sy12*) are unable to activate this regulation. Normally, this defect would be masked by the failure in signal transduction (because inability to negatively regulate would be irrelevant to a cell that cannot respond to inductive signal).

Mutations at the *sli-1* locus also suppress putative null alleles of *lin-2*, *lin-7*, and *lin-10*. If these alleles are indeed null (HORVITZ and SULSTON 1980; FERGUSON and HORVITZ 1985; KIM and HORVITZ 1990), then these loci are not entirely essential for vulval induction, because animals homozygous for putative null alleles of these loci display significant residual vulval differentiation (SULSTON and HORVITZ 1981; FERGUSON *et al.* 1987; STERNBERG and HORVITZ 1989). Suppression of these null alleles by *sli-1* mutations may therefore take place as a result of either suppression of the null alleles directly, or increased activation of a "bypass pathway" normally responsible for the residual induction seen in these animals. Defects in *lin-2*, *lin-7* and *lin-10* as with the *let-23* alleles such as *sy1*, result in a failure to activate a negative regulatory pathway that is independent of *sli-1*. Hence, in combination with a *sli-1* mutation, these mutations that alone cause a vulvaless phenotype, result in a hyperinduced phenotype.

Tissue-specific action of *let-23* and *sli-1*: The tissue-specific suppression displayed by mutations at the *sli-1*

locus is striking. *sli-1* mutations are capable of suppressing defects caused by hypomorphic *let-23* alleles in four of the five developmental processes known to require *let-23* function. Specifically, *sli-1* mutations can suppress defects in vulval development, larval development, P12 neuroectoblast determination and male tail development, but are unable to suppress the sterile phenotype displayed by *let-23(sy12)* animals. Two models are consistent with this observation. One possibility is that *let-23* activates a single signaling pathway and different tissues require different levels of this activity for normal function. In this model, gonad function requires the most *let-23* activity; *sli-1* mutations are unable to suppress the gonad defects caused by *let-23* mutations because they cannot stimulate the downstream signaling pathway sufficiently. Another explanation for this suppression spectrum is that *let-23* utilizes two distinct signaling pathways; one pathway mediates *let-23* function in the gonad while a distinct pathway regulated by *sli-1* mediates *let-23* function in the other tissues. Five lines of evidence are more consistent with this second model. First, certain *let-23* mutations are tissue preferential and define distinct domains of the receptor required for its function in different tissues (AROIAN and STERNBERG 1991; AROIAN *et al.* 1994). Second, loss-of-function mutations in genes that act downstream of *let-23* in the vulval induction pathway often cause larval lethality and defects in P12 neuroectoblast determination and male tail development but do not appear to cause a sterile phenotype similar to that seen in *let-23(sy12)* animals (BEITEL *et al.* 1990; HAN *et al.* 1990, 1993; CLARK *et al.* 1992a,b; E. LAMBIE, personal communication; T. CLANDININ and P. STERNBERG, unpublished observations). Third, mutations known to bypass the requirement for *let-23* function in the vulva do not bypass the requirement for *let-23* function in the gonad (HAN *et al.* 1990; RESULTS). Fourth, *sli-1* suppresses *lin-3(n1058)*, a sterile mutation affecting the presumptive ligand for *let-23*, with a tissue specific pattern similar to that seen with *let-23(sy12)*, that is, the vulval defects caused by *lin-3(n1058)* are suppressed while the gonad defects are not. Finally, we have recently identified second site suppressors of the sterility associated with *let-23(sy12)* that do not suppress the lethality or vulval defects associated with this *let-23* mutation (T. CLANDININ and P. STERNBERG, unpublished observations). We therefore believe that *let-23* mediates at least two genetically separable activities; one pathway employs known effectors and regulators (including *sli-1*) to mediate *let-23* function in many developmental processes while an alternate pathway mediates *let-23* function in the gonad and uses distinct downstream components.

Silent suppressors and gene number: Screens such as those described here will be useful in identifying new loci that regulate tyrosine kinase mediated signaling but that are silent in wild-type backgrounds. *sli-1* would never have been identified in a standard screen for mutations as the only phenotype associated with *sli-1* mutation in a wild-type background is a low penetrance head defect. Current estimates of gene number based on genomic sequencing are much higher than those based on genetic screens (SULSTON *et al.* 1992; WATERSTON *et al.* 1992; WILSON *et al.* 1994). Like *sli-1*, some of these uncharacterized loci are likely redundant or modifiers of other genes, and will only be identified mutationally as enhancers or suppressors of specific phenotypes (see also GREENWALD and HORVITZ 1980).

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