Multiple Functions of *let-23*, a *Caenorhabditis elegans* Receptor Tyrosine Kinase Gene Required for Vulval Induction

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

The *let-23* gene, which encodes a putative tyrosine kinase of the epidermal growth factor (EGF) receptor subfamily, has multiple functions during *Caenorhabditis elegans* development. We show that *let-23* function is required for vulval precursor cells (VPCs) to respond to the signal that induces vulval differentiation: a complete loss of *let-23* function results in no induction. However, some *let-23* mutations that genetically reduce but do not eliminate *let-23* function result in VPCs apparently hypersensitive to inductive signal: as many as five of six VPCs can adopt vulval fates, in contrast to the three that normally do. These results suggest that the *let-23* receptor tyrosine kinase controls two opposing pathways, one that stimulates vulval differentiation and another that negatively regulates vulval differentiation. Furthermore, analysis of 16 new *let-23* mutations indicates that the *let-23* kinase functions in at least five tissues. Since various *let-23* mutant phenotypes can be obtained independently, the *let-23* gene is likely to have tissue-specific functions.

INDUCTION of the six vulval precursor cells (VPCs) in *Caenorhabditis elegans* to differentiate into mature vulval tissue provides an opportunity to study mechanisms of signal transduction and cell-type determination. The six VPCs in wild type adopt the following fates (from anterior to posterior): 3° 3° 2° 1° 2° 3°, where 3° fate is an unspecialized, hypodermal fate and 2° and 1° are specialized, vulval fates (SULSTON and HORVITZ 1977; SULSTON and WHITE 1980; STERNBERG and HORVITZ 1986). This pattern of cell fate is established primarily by an inductive signal generated by the anchor cell in the gonad (SULSTON and WHITE 1980; KIMBLE 1981; STERNBERG and HORVITZ 1986; THOMAS, STERN and HORVITZ 1990). In addition, lateral signalling between VPCs prevents the adoption of adjacent 1° fates (STERNBERG 1988).

Genetic and molecular analyses are being combined to study the different aspects of vulval induction. Mutations have been identified that prevent induction of the VPCs (all 3° fate; vulvaless or Vul phenotype), that cause signal-independent induction (all 1° and 2° fates; multivulva or Muv phenotype), that are necessary for determination of 2° vulval fate, and that perturb the execution of vulval fates (HORVITZ and SULSTON 1980; SULSTON and HORVITZ 1981; GREENWALD, STERNBERG and HORVITZ 1983; FERGUSON and HORVITZ 1985 1989; FERGUSON, STERNBERG and HORVITZ 1987; STERNBERG and HORVITZ 1989; KIM and HORVITZ 1990; HAN, AROIAN and STERNBERG 1990; BEITEL, CLARK and HORVITZ 1990). The *lin-12* gene, necessary for the 2° fate (GREENWALD, STERNBERG and HORVITZ 1983), encodes a putative transmembrane receptor similar to the *Drosophila Notch* product and may be a receptor for the lateral signal between VPCs (YOCHEM, WESTON and GREENWALD 1988; SEYDOUX and GREENWALD 1989; STERNBERG and HORVITZ 1989). The gene *let-60*, required for vulval induction (HAN, AROIAN and STERNBERG 1990; BEITEL, CLARK and HORVITZ 1990), encodes a member of the *ras* family (HAN and STERNBERG 1990), and the gene *lin-11*, which is required for vulval fate execution (FERGUSON, STERNBERG and HORVITZ 1987), encodes a putative transcription factor (FREYD, KIM and HORVITZ 1990). The *let-23* gene encodes a tyrosine kinase of the EGF receptor subfamily (AROIAN *et al.* 1990) and functions via *let-60 ras* (HAN, AROIAN and STERNBERG 1990; HAN and STERNBERG 1990). The structure of the *let-23* gene suggests it might be the receptor for the inductive signal (AROIAN *et al.* 1990).

The *let-23* gene was originally defined by a larval lethal mutation (HERMAN 1978), but initial observations of the subviable allele *n1045* indicated that *let-23* plays a key role in vulval induction (FERGUSON and HORVITZ 1985; FERGUSON, STERNBERG and HORVITZ 1987; STERNBERG and HORVITZ 1989). At 15° *n1045* hermaphrodites are Vul (*i.e.*, result in no vulval induction and cannot lay eggs), but at 25° they are egg-laying proficient (Egl^+) and sometimes display one or two ectopic vulva-like structures reminiscent of Muv mutations (FERGUSON and HORVITZ 1985). Here we characterize the properties of *let-23* mutations in the vulva and other tissues. We demonstrate that complete loss of *let-23* function results in no vulval induction. Furthermore, we show that the "weak Muv"

appearance of *n1045* hermaphrodites grown at 25° results from VPCs that still require, but seem hypersensitive to, inductive signal. It is therefore distinct from previously described multivulva mutations, which are signal-independent. This *n1045* phenotype apparently results from a partial reduction in *let-23* function. These data suggest that the *let-23* gene is involved in two opposing pathways: stimulation of vulval fates and inhibition of vulval fates. The multiplicity of roles assumed by the *let-23* gene again surfaces during the development of other *C. elegans* tissues. The *let-23* gene displays mutant phenotypes in multiple tissues, and we find that these phenotypes are, to a large extent, independently mutable.

MATERIALS AND METHODS

General methods: Methods for culturing, handling, mutagenesis and genetic manipulation of *C. elegans* were performed as described by BRENNER (1974). Unless otherwise noted, we performed all experiments 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" 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. Measurements of dead larvae were obtained using a calibrated ocular micrometer.

Strains: The standard wild-type strain N2 and marker strains were from BRENNER (1974) and the Caenorhabditis Genetics Center. Below is a list of mutants and alleles used throughout the paper; references are given where appropriate. If only one allele of a gene is used (*e.g.*, *dpy-10*), the allele is given only in this section. Unless otherwise stated, *him-5* is *him-5(e1490)*.

LGII single mutations: dpy-10(e128), vab-9(e1744), rol-6(e187), unc-4(e120), the balancer *mnC1[dpy-10(e128) unc-52(e444)]* (II) (HERMAN 1978), *mnDf61, mnDf67 unc-4(e120), mnDf68 unc-4(e120)* (all SIGURDSON, SPANIER and HERMAN 1984).

LGII linked double mutations: dpy-10(e128) rol-6(e187), dpy-10(e128) unc-4(e120), rol-6(e187) unc-4(e120).

let-23 mutations: let-23(mn23) unc-4(e120), let-23(mn216) unc-4(e120), let-23(mn224) unc-4(e120) (HERMAN 1978; SIGURDSON, SPANIER and HERMAN 1984), *let-23(sy97); him-5(e1490) V* (from H. CHAMBERLIN). Ten other lethal alleles not specifically mentioned and linked to *rol-6(e187): sy5, sy6, sy7, sy9, sy11, sy13, sy14, sy16, sy17* and *sy18* (all this paper).

LGV: him-5(e1467), him-5(e1490): (HODGKIN, HORVITZ and BRENNER 1979).

LGX: lin-15(n309): (FERGUSON and HORVITZ 1985).

Unlinked triple mutant: let-23(sy97) II; him-5(e1490) V; lin-15(n309) X (from H. CHAMBERLIN).

Isolation of *let-23* alleles as suppressors of *lin-15*: At 15°, *n1045* hermaphrodites display a Vul phenotype (all six VPCs often execute the 3° nonvulval fate) and are egg-laying incompetent or Egl due to the lack of a vulva (FERGUSON and HORVITZ 1985). Conversely, Muv *lin-15* hermaphrodites all have multiple ventral protrusions and are egg-laying competent. When grown at 15°, the double mutant, *let-23(n1045); lin-15,* is often Vul and Egl with no ventral protrusions. We therefore sought to isolate new *let-23* mutations as suppressors of *lin-15*. Since this was an F2 screen, only viable, fertile *let-23* alleles could be isolated. Homozygous *lin-15* hermaphrodites were mutagenized with ethyl methanesulfonate (EMS) and allowed to self for two generations. We then picked nonmultivulva hermaphrodites that were retaining eggs or had formed "bags of worms." (The larvae are trapped within the cuticle of the Egl mother, subsequently eating their way out.) The phenotypes viewed under the dissecting microscope for Egl and Muv hermaphrodites were described by HORVITZ and SULSTON (1980). If these Egl, non-Muv hermaphrodites bred true, we mated them with *rol-6/mnC1* males, tested for linkage by examining segregation, and performed complementation tests. For *let-23(sy1); lin-15* this proved easy. While *let-23(sy97)* hermaphrodites have never been observed to copulate with males, the presence of *lin-15* in the original isolate allowed copulation. Although the *let-23(sy97); lin-15* double mutant is most often Vul (36% average induction; $n = 27$; 13/27 had no induction; see Figure 2 for description of induction), it still displays greater vulval differentiation than that of *sy97* hermaphrodites. Since *lin-15* mutants are 100% Muv (200% induction, all six VPCs are induced), these data suggest that *let-23* is epistatic to *lin-15*. In over 100,000 mutagenized gametes screened (see HAN, AROIAN and STERNBERG 1990), we have found only these two *let-23* alleles.

We linked these alleles to *dpy-10* and *unc-4* by placing each of them in *trans* to the double mutant *dpy-10 unc-4*, picking Dpy non-Unc and Unc non-Dpy recombinant progeny, and then selecting for progeny carrying the *let-23* mutation. We similarly constructed a *rol-6 cis* double mutant using the double *rol-6 unc-4*.

Noncomplementation screen against *sy1*: In the second screen for new alleles, we made use of the fact that *sy1* hermaphrodites are 100% viable but completely Vul, even in *trans* to the deficiency *mnDf68*. Thus, any hypomorphic *let-23* allele should be completely viable in *trans* to *sy1*, even if that allele eliminates function of the locus. We screened for new mutations that failed to complement *sy1* for the Vul phenotype and expected that these would include homozygous inviable *let-23* alleles. We mutagenized *rol-6* hermaphrodites and set up crosses of 3–4 hermaphrodites with 4–5 *sy1; him-5* males. The parents were transferred onto new plates after 36 hours and both sets of F_1 progeny were scored for non-Rol Egl hermaphrodites that retained eggs or hermaphrodites that had formed bags (it is often not possible to score Rol in bags of worms). These worms were picked and allowed to self. The absence of Rol progeny but presence of Egl progeny in their F_2 brood indicated the presence of a lethal *let-23* allele. All alleles were selected from independent matings except the pairs *sy13* and *sy10*, and *sy14* and *sy12*. Since in each case the pair behave differently (*sy13* and *sy14* are 100% lethal but *sy10* and *sy12* are not), we believe they are independent mutations. These alleles were subsequently recovered by mating F_3 non-Rol males, which can be *rol-6 let-23(new)/sy1; him-5* or *sy1/sy1; him-5* at roughly a 2:1 ratio, with *rol-6 unc-4* hermaphrodites, and selecting for Rol non-Uncs. We balanced these alleles over *mnC1* and screened for both *him-5* and non-*him-5* strains.

The 12 lethal alleles recovered in this screen were isolated because they fail to complement *sy1* for the vulval defect. We further tested these alleles by: (1) mating *rol-6 let-23(n1045)/mnC1* males with each of the strains and scoring hermaphrodites under the dissecting microscope and spicules of several of the males under Nomarski optics, (2) mating *rol-6 let-23(sy8)/mnC1; him-5* males with each lethal and looking for F_1 Rols, and (3) mating males from each lethal (*e.g.*, *rol-6 let-23(sy8)/mnC1; him-5*) with the deficiency *mnDf67* and looking for F_1 Rols (*mnDf67* deletes *let-23* and *rol-6 /mnDf67* is Rol).

We linked *dpy-10* to *sy10* and *sy12* by picking Dpy non-Unc recombinants from the heterozygote *rol-6 let-23(sy10 or sy12)/ dpy-10 unc-4* and selected recombinants that segregated the *let-23* mutation but not the *rol-6* mutation. Since *dpy-10* is epistatic to *rol-6*, we determined the absence of *rol-6* by complementation.

The original *rol-6 let-23(sy12)/mnC1* strain (PS227) is 100% sterile. During maintenance of the strain *dpy-10 let-23(sy12)/ rol-6 unc-4* (derived from PS227; see above), we fortuitously picked a recombinant which resulted in the strain *let-23(sy12)/ rol-6 unc-4*. We then linked *rol-6* and *unc-4* to this *sy12* by picking Rol non-Unc and Unc non-Rol recombinants, which were then balanced in *trans* to *mnC1*. The new *sy12 unc-4/mnC1* strain is 100% sterile like its parent. The new *rol-6 sy12/mnC1* strain (PS716) was, however, approximately 30% fertile. This partially fertile *rol-6 let-23(sy12)/mnC1* strain, PS716, was used for additional experiments since it has undergone the most back-crossing. We cannot detect any differences between PS227 and PS716 for the other phenotypes. We were also able to pick a similar recombinant for *sy10* and linked it to *rol-6* and *unc-4* as for *sy12*. This *rol-6 sy10* strain is still, as the parent, 100% sterile.

Complementation matrix: Since homozygous *let-23* hermaphrodites have difficulty mating with males, and since most homozygous *let-23* alleles produce males that cannot mate, all allele combinations were made using analogous sets of hermaphrodites and male strains balanced in *trans* to a *let-23*(+) chromosome. This protocol also maintains internal consistency for the comparison of results among different allele combinations. We mated N2 males with *dpy-10 rol-6* hermaphrodites and picked wild-type males (*dpy-10 rol-6/++*). These males were mated with *dpy-10 let-23(X)/ rol-6 unc-4* hermaphrodites where *let-23(X)* is any *let-23* allele. In the next generation we picked Dpy L4 hermaphrodites, many of which are cross progeny *dpy-10 let-23(X)/ dpy-10 rol-6*. Self progeny, homozygous *dpy-10 let-23(X)*, are also Dpy but can be eliminated in the next cross since they do not segregate Rol. Individual Dpy hermaphrodites were mated with 4 to 5 *(rol-6 let-23(Y))/mnC1* males where *let-23(Y)* is any *let-23* allele (*rol-6 let-23/mnC1* male strains are well balanced and are easily propagated). These parents were transferred to new plates first after 2 days, then every day for a total of four plates per cross. This procedure allows for synchrony among the progeny on a plate. Self progeny are all Dpy hermaphrodites. Cross progeny are hermaphrodites and males, either Dpy, (*dpy-10 let-23(X)/mnC1*[*dpy-10 unc-52*] and *dpy-10 rol-6/mnC1*), Rol (*rol-6 let-23(Y)/dpy-10 rol-6*), or non-Dpy non-Rol (*dpy-10 let-23(X)/rol-6 let-23(Y)*). A wild-type chromosome is introduced for *let-23(X)*, by substituting *dpy-10* hermaphrodites for *dpy-10 let-23(X)/ rol-6 unc-4*. To substitute a wild-type chromosome for *let-23(Y)*, we replace *rol-6 let-23(Y)/mnC1* males with *rol-6/mnC1* males. Since *mnDf68* deletes *rol-6* and *let-23*, *mnDf68 unc-4/ mnC1* males were directly used in place of *rol-6 let-23(Y)/ mnC1* males.

We controlled for recombinants as follows. Loss of *rol-6* in *dpy-10 rol-6/++* males are detected by the lack of Rol animals segregating in the final set of crosses (this is why we used individual hermaphrodites in each cross). Since *dpy-10 let-23(X)/rol-6 unc-4* hermaphrodites are maintained clonally, we verified the mother's genotype by segregation. The only troublesome recombinant would be loss of *let-23(X)* from *dpy-10*. This recombinant picks up *unc-4* and thus segregates Dpy Uncs in the final crosses.

Two other recombinants could affect our data. Class I recombinants result from recombination between *rol-6*(+) and *let-23(X)* in the mother [*dpy-10 let-23(X)/dpy-10 rol-6*]. This *dpy-10 rol-6*(+) *let-23*(+) gamete can give rise to a non-Dpy non-Rol animal that lacks *let-23(X)*, namely *dpy-10/rol-6 let-23(Y)*. Class II recombinants result from breakdown of the *mnC1* balancer which removes *let-23(Y)* from *rol-6*, potentially giving rise to a non-Dpy non-Rol progeny that lacks *let-23(Y)*, namely *rol-6 /dpy-10 let-23(X)*. The other recombinant, which removes *rol-6* from *let-23(Y)*, would pick up *dpy-10* from the *mnC1* chromosome and thus not give a false non-Dpy non-Rol.

Recombinant classes I and II both effectively separate *rol-6* and *let-23*. To ascertain the frequency of such recombinants, we mapped *rol-6* relative to *let-23* in the strain *rol-6 let-23(sy15)/vab-9(e1744)*. We found 11 Rol nonlethal recombinants out of a total of 7232 Vab and wild-type animals. The calculated *rol-6* to *let-23* distance is 0.23 ± 0.08 map units. Therefore, if *let-23(X)/let-23(Y)* is 100% viable, we would expect about 1/400 of non-Dpy non-Rol animals to be class I recombinants. In the cases where *let-23(X)/let-23(Y)* is 10% viable, this ratio becomes 1/40, which is about what we found. We therefore do not expect these recombinants to seriously affect our results. We expect class II recombinants to be even more rare since in these cases a recombination event between *rol-6* and *let-23* needs to occur in addition to the breakdown of the balancer *mnC1*. We have indeed found this class of recombinants to be rare except in the presence of homozygous *him-5*, which increases recombination in this region in the presence of *mnC1*. (We do not know if recombination is increased in the absence of *mnC1*.) For this reason, we only used non-*him-5* male strains in our crosses. In many cases, we recovered hermaphrodites that had wild-type vulvae and verified that they were nonrecombinant by segregation. It is not practical to do this with the males that have wild-type spicules, since individual matings and scoring of subsequent segregation is involved. It is thus possible there is a slight underestimation of the penetrance of the spicule defect shown in Table 1C. Assuming a viability of 10%, recombinants would introduce a 3% (1/40) error. Since the viability of most allele combinations is greater than 10%, the error introduced for most combinations is less than 3%.

Temperatures of 15°, 20° and 25° were kept within 0.5°. Crosses with *mn224* were performed differently since *mn224* is linked to *unc-4* and not *rol-6*. In the first cross we mated N2 males with *dpy-10 unc-4* and carried out all other crosses as above, in which *let-23(Y)* is introduced via *let-23(mn224) unc-4/mnC1*, and the Unc-4 phenotype replaces Rol-6 in the scoring.

Since *dpy-10* and *rol-6* both affect the cuticle and such genes can have unusual interactions (Cox *et al.* 1980), we mated *dpy-10 rol-6* hermaphrodites with *rol-6 unc-4/mnC1* males. We found 373 Dpy males, 384 Rol males, and 381 Rol hermaphrodites, the 1:1:1 ratio as expected. Therefore, ignoring *unc-4*, *dpy-10 rol-6/dpy-10* + animals are Dpy and *dpy-10 rol-6/+ rol-6* animals are Rol. For the *dpy-10* +/+ *rol-6* control, see +/+ results in Table 1A–E.

Data points for all phenotypes were often taken over more than one day to avoid age-related biases. We have not seen any age-effects for either vulval induction, male spicules or fertility.

Since all crosses were performed with balanced *let-23(mutant)/let-23*(+) hermaphrodites, we tested for maternal rescue of any of the phenotypes. Larval lethality is not maternally rescued in any of our lethal alleles. Homozygous *sy1*, *sy97* and *n1045* mutations result in no quantitative differences in vulval, lethal, male tail, or sterile phenotypes whether coming from homozygous or heterozygous mothers (see below). There is no maternal rescue of the fully pene-

trant *sy10* sterility. The P12 transformation is, however, partly rescued maternally (see RESULTS).

We chose *sy15* as our standard lethal allele in these crosses at random from fourteen possible null lethal alleles. All lethals were tested in *trans* to *sy1* and *n1045(20°)* and fail to complement vulval and male tail defects (except *mn224*). Furthermore, all lethals isolated in this paper fail to complement the lethal allele *let-23(sy8)* and are lethal in *trans* to *mnDf67*, a deficiency which deletes *let-23* (SIGURDSON, SPANIER and HERMAN 1984; AROIAN *et al.* 1990).

Measuring vulval induction/hybrid lineages: Non-Dpy non-Rol L4 hermaphrodites were placed live on pads of 5% Noble agar (as described by SULSTON and HORVITZ 1977; STERNBERG and HORVITZ 1981) and examined for their extent of vulval induction. The final induction pattern was checked for internal consistency in both the number of syncytial nuclei and the number of nonsyncytial VPC progeny. To check the genotype of hermaphrodites, we pulled individual animals off slides in S Basal with a capillary and suction apparatus and transferred them to a Petri plate, and examined their genotype by segregation. To eliminate the anchor cell in *n1045* hermaphrodites, we laser ablated somatic gonad precursor cells during the L1 larval stage (SULSTON and WHITE 1980; STERNBERG and HORVITZ 1981).

Hybrid vulval lineages in *let-23* mutant hermaphrodites appear to arise from VPCs that have a reduced levels of signal transduction since they are associated with reduction of function *let-23* alleles which lower induction below wild type but do not eliminate it. This conclusion is supported by induction patterns seen in eighty homozygous *sy97* hermaphrodites (Table 1A and other data not shown). Only four of these hermaphrodites had any VPC induction and in all cases a single VPC executed a hybrid lineage (all other VPCs executed 3° fate). Hybrid lineages also correlate with the lower induction levels seen in Table 1A. Collating our data from the genotypes with the lowest induction (<5%; 108 animals), we see that 8/11 instances of VPC induction were hybrid (compare this to 72/156 instances from non-hyperinduced genotypes with average induction >30%; 106 animals).

Hybrid lineages also show a strong polarity bias in *let-23* mutants, which correlates with the position of the anchor cell relative to the VPC daughters. When P5.p executed a hybrid fate, the anchor cell-proximal daughter, P5.pp, executed the vulval fate in 49/53 instances. When P7.p executed a hybrid fate, the anchor cell-proximal daughter, P7.pa, executed the vulval fate in 41/46 instances. For P6.p, the anterior daughter executed the vulval fate 20/54 times, and the posterior daughter 34/54 times. Although these data are collected from all *let-23* allele combinations, these trends do not significantly differ among the various alleles.

Last, the decision for a given VPC to execute a hybrid lineage does not show any obvious correlation with fates of neighboring VPCs. P5.p can execute a hybrid lineage whether P6.p executes a hypodermal fate (24/53), a hybrid fate (11/53), or a vulval fate (18/53). Similarly, P7.p can execute a hybrid lineage whether P6.p executes a hypodermal fate (14/46), a hybrid fate (18/46), or a vulval fate (18/46). P6.p can execute a hybrid lineage when both neighbors execute hypodermal fates (19/54), when one neighbor executes a nonhypodermal (*i.e.*, hybrid or vulval) fate (21/54), and when both neighbors execute a non-hypodermal fate (14/54). These data do not rule out a possible role for neighbor-neighbor interactions in establishing hybrid fate, but they do suggest that neighboring VPCs alone do not cause a given VPC to adopt a hybrid fate.

Measuring survival: We expect the ratio of Dpy:Rol:non-Dpy non-Rol males to be 2:1:1 if there is no lethality associated with *let-23(X)/let-23(Y)*. An approximate sample size of the total number of non-Dpy non-Rol males, both viable and inviable, is therefore the number of Dpy males plus the number of Rol males divided by three. We counted all males on all the plates in a set and calculated the percent survival (s) as

$$s = 3 * (\text{No. non-Dpy non-Rol males})/(\text{No. Dpy males} + \text{No. Rol males}).$$

The 95% confidence limits are $3 * 1.96 * \sqrt{s * (1 - s)/N}$, where N is the number of Dpy plus Rol males divided by three. For example, *n1045/n1045* at 20° is 42% ± 13% viable, *n1045/sy10* is 56% ± 17% viable, *n1045/mn224* is 15% ± 12% viable, and *sy97/sy15* is 0.4% ± 1.7% viable. Additionally, in all but one case (*n1045/mnDf68* at 20°), we found that the ratio of Dpy:Rol males was, within 95% confidence limits, consistent with the expected 2:1 ratio (assuming lethality is recessive).

Measuring wild-type spicules: We examined both spicules of live non-Dpy non-Rol adult males usually within 1–2 days of adulthood under Nomarski optics. We have also noted defects in the male gubernaculum, but these may be a consequence of spicule disorganization.

Measuring fertility: Due to the demands of scoring all the phenotypes, most fertility data points were collected in a separate set of crosses identical to those used for the other phenotypes. We picked L4 and young adult hermaphrodites over several days. These hermaphrodites were checked every 12 hours for four days. Any worm that was Egl (turned into a "bag of worms") was removed from the plate; if an Egl$^+$ worm was found, all the other hermaphrodites were transferred to a new plate. This allowed unambiguous confirmation of whether or not a given hermaphrodite was fertile. Hermaphrodites left after the four days were counted as sterile. They were also identifiable by mottled uteri.

We found two classes of fertile hermaphrodites. Class A fertiles had healthy brood sizes (including dead larvae) and healthy looking uteri; class B fertiles had small brood sizes (<6 including dead larvae) and mottled uteri typical of *let-23* sterile hermaphrodites (see RESULTS). Class B fertiles are counted as fertile in Table 1D, but the results are not substantially altered if these are classified as sterile: *sy1* and *sy97* hermaphrodites in *trans* to any allele give class A fertiles, and the same allelic series holds true (see Table 4). However, the fertile hermaphrodites counted for both *sy15* and *mn224* in *trans* to *n1045* at 20° are mostly class B (10/15 and 1/1, respectively). If these class B fertiles are classified as sterile, then the percent fertility for *sy15* and *mn224* in *trans* to *n1045* changes to 12% and 0% respectively. Thus, these alleles are not as different from *mnDf68* as they appear.

Studies on the oocyte basis of sterility were carried out as follows. We took eleven individual *dpy-10 n1045/mn224 unc-4* hermaphrodites from the matrix cross and put them each on a plate with five N2 males. No progeny were produced on any of the plates. We also took seven individual *dpy-10 n1045/mn224 unc-4* males and mated them with several *dpy-10 unc-4* (2 plates) and several *unc-4* (5 plates) hermaphrodites. We found cross-progeny on four of the seven plates.

Measuring wild-type P12: We assessed the fate of P12 in the same hermaphrodites in which we measured vulval induction. Maternal rescue of the P12 phenotype was checked as follows. Penetrance of the P12 transformation was measured in the Unc progeny of *sy97 unc-4* and *sy97 unc-4/mnC1* mothers. Likewise, the penetrance was meas-

ured in the Unc progeny of *n1045 unc-4* mothers grown at 15° and *n1045 unc-4/mnC1* mothers grown at 15°. In the case of *sy12*, the penetrance was measured in F_1 progeny of the complementation crosses (mother = *dpy-10 sy12/dpy-10 rol-6*) and in their F_2 progeny (mother = *dpy-10 sy12/rol-6 sy12*).

Since in *let-23(n1045)* males grown at 15° there is a transformation both of P12.p to P11.p and of P12.aap to P11.aap (P. W. STERNBERG and R. HORVITZ, unpublished results), and since P11 and P12 form an equivalence group in males (SULSTON and HORVITZ 1980), we believe that the lack of P12.pa and presence of an extra P11.p-like cell (presumably P12.p) represents a transformation of P12 to P11. This inference assumes that the male and hermaphrodite behavior of these cells are the same.

Maternal rescue and marker controls: We have examined vulval induction in the following strains: *sy1, sy1 unc-4* from homozygous and heterozygous mothers, *sy97, sy97 unc-4* from homozygous and heterozygous mothers, *n1045 unc-4* 20° from homozygous and heterozygous mothers, and *sy10 unc-4* from heterozygous mothers. We have seen no substantial differences in vulval induction from homozygous and heterozygous mothers in any of these strains. For example, vulvae in *n1045 unc-4* hermaphrodites at 20° (this strain behaves like the original *n1045* strain; see legend to Table 3) were hyperinduced in 6/19 animals, wild-type in 11/19 animals, and Vul in 2/19 animals when coming from a homozygous mother (see RESULTS and Figure 2 for a description of the hyperinduced phenotype). Similarly, when coming from a heterozygous *n1045 e120/mnC1* mother, vulvae in *n1045 unc-4* hermaphrodites at 20° were hyperinduced in 7/19 animals, wild type in 9/19 animals, and Vul in 3/19 animals. With the exception of *n1045* (see legend to Table 3), the vulval induction seen in *let-23* mutants is roughly the same as that seen in the complementation matrix crosses. That similar vulval induction occurs in both our controls and complementation scheme also suggests that our sample sizes for each data point in the complementation scheme are sufficient to show general trends in induction.

We observed that *sy1/sy1; him-5* males roughly mate as well as *him-5* males, *n1045/n1045him-5(e1467) 20°* males mate poorly, and *sy97/sy97; him-5* males do not mate at all (>40 males attempted). These data are consistent with our complementation results. Qualitatively, the lethality associated with *sy1/sy1*, *n1045/n1045* and *sy97/sy97* is as observed in the complementation matrix. The percent *let-23* survival in the strains *rol-6 sy10/mnC1* (20%), *rol-6 sy12/mnC1* (11%), and *sy97 unc-4/mnC1* (15%) (in all cases the number of animals scored is less than in the matrix) is consistent with complementation data. Mating *rol-6 let-23(Z)/mnC1; him-5* males (where *let-23(Z)* = *sy10*, *sy12* and the lethal *sy14*) into *dpy-10 n1045* hermaphrodites at 20° and measuring percent survival yields similar results as found in the matrix (56, 53 and 20%, respectively), further arguing against maternal rescue of larval lethality.

RESULTS

Isolation of new *let-23* alleles and characterization of *let-23* phenotypes: To understand the role of *let-23* in the VPCs and other cells, we isolated and genetically characterized new *let-23* alleles. Prior to this study, there were four known alleles: *n1045* (FERGUSON and HORVITZ 1985) and the larval lethal alleles *mn23*, *mn216* and *mn224* (HERMAN 1978; SIGURDSON, SPANIER and HERMAN 1984). We isolated two viable Vul *let-23* alleles, *sy1* and *sy97*, as *lin-15(n309)* suppressors (see MATERIALS AND METHODS). We also isolated fourteen *let-23* alleles in an F_1 noncomplementation screen against *sy1*. Twelve of these 14 alleles are 100% penetrant larval lethals; the other two alleles, *sy10* and *sy12*, have a partially penetrant larval lethality.

Two of the new alleles, *sy97* and *sy10*, revealed novel defects in the male tail and hermaphrodite fertility. Previously, *let-23* mutations have been associated with vulval defects (Figure 1, A–D; FERGUSON and HORVITZ 1985), larval lethality (HERMAN 1978), and a loss of the cell P12.pa with concomitant apparent duplication of P11.p in the hermaphrodite tail (Figure 1, E and F; FIXSEN *et al.* 1985), which may actually represent a transformation of P12 to P11 (see MATERIALS AND METHODS). During attempted crosses with hermaphrodites, we found that *sy97/sy97* males could not produce cross progeny. Comparison of wild-type (Figure 1G) with mutant males (Figure 1H) revealed abnormal spicules in the tail that vary from slightly shortened and broken to severely crumpled and disorganized. We also found that, although 15% of *sy10/sy10* hermaphrodites survive past the first larval stage, these survivors are sterile. These sterile adults appear not to fertilize their oocytes, which subsequently degenerate in the uterus. As these adults age, the degenerate oocytes accumulate, giving the uterus a mottled appearance. Sterility is likely caused by oocyte and not sperm defects: *n1045/mn224* hermaphrodites (which often have wild-type vulvae) are sterile whereas *n1045/mn224* males are fertile, and sperm from wild-type males cannot rescue *n1045/mn224* hermaphrodite sterility.

To characterize the larval lethal phenotype, we examined the cellular anatomy of *let-23* mutant dead larvae. These larvae appear to arrest at the mid-late L1 stage: they have the "lateral alae" characteristic of wild-type L1 larvae, and their gonads contain between 8 and 16 cells (KIMBLE and HIRSH 1979; KIMBLE and WHITE 1981). The dead larvae homozygous for the lethal alleles isolated in this study arrest at approximately the same length (average length 320 μm), indicating growth arrest at a similar time of development.

The *let-23* vulva and P12 defects involve a transformation of cell fate, as does the male spicule defect (H. CHAMBERLIN, personal communication). We do not know the cellular basis for either *let-23* lethality or sterility. However, since neither the vulva nor P12 nor male spicules are required for viability or hermaphrodite fertility (SULSTON and HORVITZ 1977; SULSTON and WHITE 1980) and since the lethal and sterile phenotypes appear distinct and are somewhat separa-

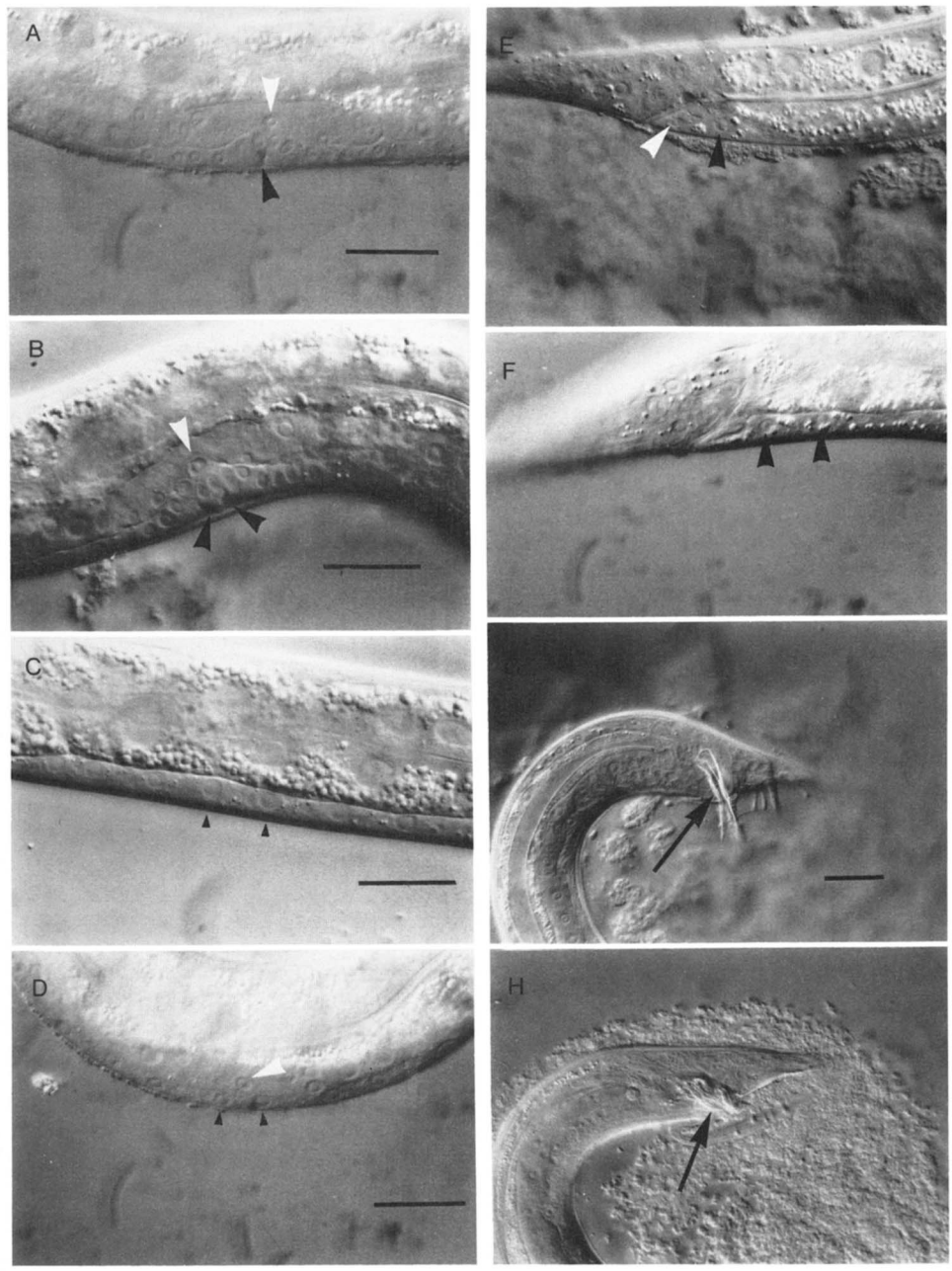

FIGURE 1.—Gallery of Nomarski photomicrographs illustrating *let-23* phenotypes. **A**, Induction in a wild-type L3 lethargus hermaphrodite. Black arrow points to the single vulval invagination; white arrow points to the anchor cell nucleus. **B**, L3 molt *n1045* animal grown at 25° displaying more vulval induction than wild type (phenotype = hyperinduced; see text). The lineages of the VPCs in this animal were observed to execute (anterior to posterior) 3° 3° 2° 1° 1° 2° fates. Black arrows point to the double vulval invagination caused by the extra primary cell; white arrow points to the anchor cell nucleus. **C**, Induction in an L3 molt *n1045* animal grown at 25° with gonad ablated. Note the lack of an anchor cell. All cells executed the 3° fate, demonstrating that hyperinduction is anchor-cell dependent. The two P6.p daughters are indicated with black arrows. **D**, Induction in a Vul *let-23(sy1)* animal at the L3 molt. Despite the presence of an anchor cell (white arrow), all VPCs executed 3° fate. The two P6.p daughters are indicated with black arrows. **E**, Wild-type P11.p (large nucleus; black arrow) and P12.pa (small nucleus; white arrow). **F**, Absence of P12.pa in a *let-23(sy97)* hermaphrodite and appearance of two P11.p-like nuclei (two large nuclei; black arrows). **G**, Wild-type spicules in a *him-5(e1490)* male. The spicule on the right side of this animal (black arrow) is in focus in this photomicrograph and partly projects out from the tail of the animal. It is long and straight. **H**, Crumpled spicules in a *let-23(sy97); him-5(e1490)* male (black arrow). Scale bar = 20 μm. Scale in E and F is same as in A; scale in H is same as in G. For A, C and D, anterior is right and posterior left. For B, anterior is left, posterior right. E and G printed in reverse orientation for ease of comparison with F and H.

ble (see below), we infer that all phenotypes arise from defects in different cells.

***let-23* null phenotype:** Complete loss of *let-23* function results in larval lethality based on the following criteria. First, larval lethals arose at a frequency typical for null mutations (1/1600) in our F_1 noncomplemen-

tation screen against *sy1* (BRENNER 1974; GREENWALD and HORVITZ 1980). This screen allows recovery of mutations that completely eliminate *let-23* activity, and larval lethals were the most common allele obtained. Second, larval lethality is the most severe phenotype associated with *let-23* mutations. Third, an allelic series can be made with larval lethals retaining least *let-23* function. Fourth, larval lethals behave like deletions in *trans* to other alleles. Lastly, one larval lethal is due to a premature stop codon located in the tyrosine kinase domain (AROIAN *et al.* 1990). As discussed below, the larval lethal *mn224* is an exception to the third and fourth criteria. The phenotype associated with complete loss of *let-23* function is probably not more severe than larval lethality since a *mnDf61/mnDf67* heterozygote, which deletes the *let-23* gene and a small region around it (SIGURDSON, SPANIER and HERMAN 1984), results in larval lethality. Thus no zygotic embryonic lethal gene resides in the overlap of these two deficiencies.

Loss of *let-23* in the vulva leads to the vulvaless phenotype: To study the role of the *let-23* gene in vulval induction, we made all possible *trans* heterozygotes using our five viable and subviable alleles, *n1045* (20°), *sy1*, *sy10*, *sy12* and *sy97*, and determined the percent vulval induction (a measure of vulval differentiation; see Figure 2) for each allele combination (Table 1A). We included in this analysis a representative null allele, *sy15*, the deficiency *mnDf68*, and the lethal allele *mn224*, which is phenotypically distinct from other lethal alleles. We also determined vulval induction in *n1045* hermaphrodites grown at 25° (see Table 3 and below) as homozygotes, as heterozygotes in *trans* to a *let-23*(+) chromosome, and in *trans* to *mnDf68*, a deficiency that deletes the *let-23* gene and the nearest genes on either side (SIGURDSON, SPANIER and HERMAN 1984).

Wild-type vulval induction is invariant (Figure 2A). The three VPCs closest to the anchor cell, P5.p, P6.p and P7.p, execute 2°, 1° and 2° fates, respectively. The other three VPCs, P3.p, P4.p and P8.p, divide once and fuse with the hypodermal syncytium, executing a "lower," 3° fate (see Figure 2 for definition of the 1°, 2° and 3° fate hierarchy). For comparison, induction is shown for *let-23* vulvaless animals (Figure 2, B and C) and for *let-23* hyperinduced animals (Figure 2D; see below).

Loss of *let-23* function in the vulva leads to a completely Vul phenotype (all VPCs 3°) based on the data in Table 1A and the following arguments. First, vulval induction of either *sy1* or *n1045* is lowered in *trans* to either a deletion or the *sy15* null, and in some cases decreases to 0% (*e.g.*, *sy1/mnDf68*). Second, we can infer an allelic series of decreasing *let-23* vulval activity, and lack of induction is consistent with least *let-23* function. Thus, in *trans* to any given allele, *n1045* at

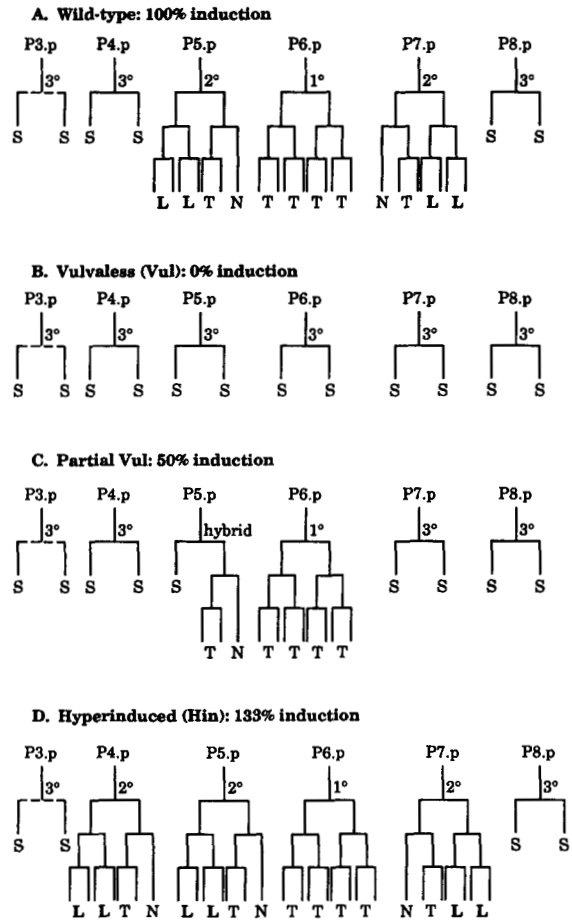

FIGURE 2.—Vulval induction in wild-type and *let-23* mutants. **A**, In wild-type animals, three VPCs, P5.p, P6.p and P7.p, divide more than once and execute vulval fates 2°, 1°, 2°, respectively. The other three VPCs, P3.p, P4.p and P8.p, do not form vulval tissue but rather divide once and fuse with the hypodermal syncytium (S); this fate is designated 3°. While the location of the anchor cell is not fixed during the induction period, it is generally centered over P6.p. In 1° lineages, the first two divisions are longitudinal in the ventral cord producing four progeny all of which subsequently divide transversely (T). In 2° lineages, the first two divisions are also longitudinal. The four progeny then execute three different patterns of divisions: longitudinal (**L**, bold face indicates adhesion to the cuticle), transverse (T), and no division (N). For more details see STERNBERG and HORVITZ (1986). The designation 1°, 2° and 3° arise because either P5.p or P7.p, which normally execute a 2° lineage, will execute a 1° lineage if P6.p has been ablated in an otherwise wild-type animal (SULSTON and WHITE 1980; STERNBERG and HORVITZ 1986). Similarly, P4.p will execute a 2° lineage if P5.p has been ablated and P8.p will execute a 2° lineage if P7.p is ablated. This "hierarchy" of fates is also indicated in experiments in which all but one VPC is ablated. This isolated VPC will execute a 1°, 2° or 3° fate depending on its distance from the anchor cell. The closer the cell is to the anchor cell, the higher the fate it will execute (STERNBERG and HORVITZ 1986). **B**, In completely Vul *let-23* animals, all VPCs execute 3° or non-vulval fate. Percent induction is 0 (0/3 * 100). **C**. In partially Vul animals, some vulval differentiation occurs, but less than in wild-type. In these animals, hybrid lineages often occur. In the example given here, P5.p executes half a 3° and half a 2° fate, and percent induction in this animal is 50 (1.5/3 * 100). Partial induction can range from 17% (0.5/3 * 100) to 83% (2.5/3 * 100). **D**, In hyperinduced animals, induction in greater than 100%. In this example, percent induction is 133 (4/3 * 100).

TABLE 1

Penetrance of different *let-23* phenotypes for different allele combinations

	♂								
♀	+	*sy1*	*n1045* (20°)	*sy97*	*sy10*	*sy12*	*sy15*	*Df*	*mn224*
A. Percent vulval induction									
+	100							100	100[a]
	(21)							(29)	(34)
sy1	100	14	33	36	8.7	6.5	0	0	101[a]
	(20)	(30)	(32)	(31)	(21)	(36)	(14)	(23)	(30)
n1045 (20°)	100		44	8.7	23	18	1.9	4.4	105[a]
	(20)		(42)	(25)	(26)	(30)	(26)	(30)	(21)
sy97	100			0	0	0	0		
	(20)			(21)	(19)	(21)	(1)		
sy10	100				1.6	0	—[b]		
	(20)				(21)	(24)			
sy12	100					0.6	—[c]		
	(20)					(31)			
B. Percent survival									
+	100							92	ND
	(334)							(257)	
sy1	103	108	100	106	94	104	ND	118	100
	(271)	(243)	(161)	(263)	(232)	(349)		(170)	(214)
n1045 (20°)	97		42	37	56	49	18	25	15
	(341)		(502)	(238)	(303)	(241)	(332)	(211)	(305)
sy97	97			11	21	19	0.4		
	(289)			(389)	(302)	(379)	(453)		
sy10	105				14	17	0		
	(257)				(373)	(442)	(344)		
sy12	106					19	0.3		
	(354)					(280)	(301)		
C. Percent wild-type spicules									
+	100							100	ND
	(20)							(20)	
sy1	100	100	96	90	95	92	ND	95	95
	(21)	(22)	(24)	(20)	(20)	(24)		(20)	(21)
n1045 (20°)	100		24	0	52	59	4.6	0	92
	(21)		(38)	(22)	(21)	(22)	(22)	(17)	(20)
sy97	100			0	5.6	0	0		
	(20)			(19)	(18)	(21)	(2)		
sy10	100				4.8	0	—[b]		
	(20)				(21)	(20)			
sy12	100					0	0		
	(21)					(21)	(1)		
D. Percent fertile hermaphrodites									
+	ND							100[d]	ND
								(18)	
sy1	100	100		100	100	100	100	100	ND
	(23)	(24)		(30)	(30)	(31)	(18)	(30)	
n1045 (20°)	100	100	100	100	71	100	36	0	8
	(23)	(27)	(26)	(36)	(34)	(30)	(42)	(30)	(13)
sy97	100			95	100	100	100		
	(22)			(22)	(31)	(31)	(1)		
sy10	100				0	15	0		
	(23)				(45)	(53)	(1)		
sy12	100					28	0		
	(24)					(47)	(1)		

20° displays the most vulval induction followed by *sy1*, *sy10*, *sy12*, *sy97* (except in *trans* to *sy1*; see below), and then *sy15*. The allele *sy97* has 0% induction in *trans* to *sy10*, *sy12*, or itself. The data also indicates that a reduction but not elimination of *let-23* function can lead to a partly Vul phenotype.

Characterization of the phenotype of *let-23(n1045)* at 25°: As mentioned in the introduction, *let-23(n1045)* hermaphrodites grown at 25° display a phenotype reminiscent of multivulva mutations (FERGUSON and HORVITZ 1985). To characterize this phenotype further, we analyzed vulval induction patterns

TABLE

♀	♂ +	*syl*	*n1045* (20°)	*sy97*	*sy10*	*sy12*	*sy15*	*Df*	*mn224*
E. Percent wild-type P12									
+	100							100	ND
	(21)							(29)	
syl	100	100	88	94	95	97	ND	100	97
	(20)	(30)	(32)	(31)	(20)	(36)		(23)	(30)
n1045 (20°)	100		91	92	80	86	77	97	90
	(20)		(44)	(25)	(25)	(29)	(22)	(30)	(21)
sy97	100			95	95	76	100		
	(20)			(20)	(20)	(21)	(1)		
sy10	100				90	80	—[b]		
	(20)				(21)	(25)			
sy12	100					83	0		
	(20)					(29)	(1)		

Each row represents the *let-23* chromosome inherited from the mother and each column represents the *let-23* chromosome inherited from the father. See MATERIALS AND METHODS for details. A, The percent vulval induction and the number of hermaphrodites scored (in parentheses) for each heterozygote. B, The percent survival of each *trans*-heterozygote and the number of Dpy plus Rol males divided by three (in parentheses) as a measure of the sample size (see MATERIALS AND METHODS). Survival greater than 100% is statistically possible due to the method of calculation. C, The percentage of males with both spicules wild type. It is possible for a given male to have one wild-type spicule and one mutant spicule, and we classify these as mutant. The number in parentheses is the number of males scored. D, The percentage of hermaphrodites for a given allele combination that are fertile. The number of hermaphrodites scored is given in parentheses. See MATERIALS AND METHODS for a description of *sy15* and *mn224* fertility. E, The percent wild-type P12 scored for different allele combinations. Number in parentheses is the number of hermaphrodites scored. ND = not determined; *Df* = *mnDf68*.

[a] For *mn224/+*, *mn224/syl* and *mn224/n1045* 0/34, 3/30 and 3/21 hermaphrodites were hyperinduced, respectively.

[b] Cross was carried out but no *let-23/let-23* cross-progeny survived. Since *sy97*, *sy10* and *sy12* are nearly inviable in *trans* to *sy15*, *mn224* and *mnDf68*, only *sy15 trans*-heterozygotes were made with these three alleles.

[c] The one hermaphrodite that survived was picked up as an adult and not scored for this phenotype.

[d] Two of these 18 hermaphrodites were sickly with low broods. This is probably a function of the deletion and not the *let-23* locus since lethal alleles in *trans* to a wild-type chromosome are healthy.

and cell lineages in *n1045* hermaphrodites grown at 25° with and without an anchor cell (Table 2). In the presence of an anchor cell, induction is often greater than wild type; more than the three wild-type VPCs can differentiate into vulval fates, and the VPCs that take on 2° fates in wild-type hermaphrodites, P5.p and P7.p, can take on the "higher" 1° fate. These induction patterns are distinct from the induction patterns in Muv hermaphrodites in two ways (*e.g.*, STERNBERG 1988). First, there is often a breakdown in the lateral inhibition that normally prevents the formation of adjacent 1° fates. Second, in *n1045* hermaphrodites grown at 25°, induction is generally centered around the anchor cell: VPCs that execute 3° fates are farther away from the anchor cell than VPCs that execute 2° fates, which are in turn farther away than VPCs that execute 1° fates. However, in Muv hermaphrodites, the most distal VPCs often execute higher fates than their more anchor cell-proximal neighbors (*e.g.*, from anterior to posterior 2° 1° 2° 1° 2° 1°). Thus in *n1045* hermaphrodites, VPCs seem to still respond to the anchor-cell signal in a graded fashion. This conclusion is supported by the finding that in *n1045* animals grown at 25°, there is no induction in the absence of the anchor cell. Again, this is unlike induction in Muv hermaphrodites, which occurs even in the absence of an anchor cell (STERNBERG 1988). We designate this vulval phenotype as the "*h*yper*in*duced" or "Hin" phenotype. This *n1045* 25° phenotype appears to result from hypersensitive VPCs since we do not believe that the *let-23* gene acts in the anchor cell. In particular, the *let-23* gene is unlikely to act in the anchor cell because of its epistasis to the Muv gene *lin-15* (see MATERIALS AND METHODS). A *lin-15(n309)* hermaphrodite lacking an anchor cell still has a Muv phenotype, but a *let-23(sy97); lin-15(n309)* hermaphrodite, which retains only slight *let-23* vulval activity, is most often Vul. Thus, lack of the *let-23* product is not the same as a lack of the anchor cell, and we infer that the *let-23* gene does not act in the anchor cell. Since *lin-15* might act in cells other than the VPCs or the anchor cell (HERMAN and HEDGECOCK 1990), it is also possible that the *let-23* gene may act in cells other than the VPCs, such as those of the surrounding hypodermis.

Genetic basis for hyperinduction: Hyperinduction in *n1045* hermaphrodites is recessive and dosage-sensitive (Table 3). Heterozygote *n1045/+* hermaphrodites have wild-type vulvae at 25°. In addition, whereas hermaphrodites with two copies of *n1045* grown at 25° often have more than wild-type induction (phenotypically Hin), hermaphrodites with only one copy of *n1045* (*n1045/mnDf68*) grown at 25° have less than wild-type induction (phenotypically Vul or partly Vul).

Hyperinduction appears to result from a partial

TABLE 2

Hyperinduction in *n1045* hermaphrodites grown at 25°

	AC						
	P3.p	P4.p	P5.p	P6.p	P7.p	P8.p	No.
A. Wild-type	S or S S	S S	LLTN	TTTT	NTLL	S S	
n1045/n1045 (25°)	S	LLON	OTTT	TTTT	NTLL	S S	1
	S	S OO	LLTN	TTTT	NTLL	S S	1
	S	LLON	OOTT	TTTT	NTOL	NOLL	1
B. Wild-type	3°	3°	2°	1°	2°	3°	
n1045/n1045 (25°)	3°	2°	1°	1°	1°	2°	1
	3°	2°	1°	1°	2°	3°	7
	3°	2°	2°	1°	2°	3°	7
	3°	3°	2°	1°	2°	2°	2
	3°	3°	2°	1°	2°	3°	6
	3°	2°	1°	2°	3°	3°	2
C. *n1045/n1045* (25°) no anchor cell	3°	3°	3°	3°	3°	3°	7

The relative placement of the anchor cell (AC) during the induction period (late L2-early L3 stage) is indicated. Below each VPC is the fate executed in *n1045* 25° hermaphrodites. The right-most column (No.) indicates the number of hermaphrodites seen with a given induction pattern. Nomenclature is as given in Figure 2 except where noted. A, The lineages of three *n1045* hermaphrodites grown at 25°. Bold-face indicates adhesion to the cuticle and is diagnostic of 2° lineages, as is the presence of an N cell. Oblique axes of nuclear division (O) are acceptable as part of either 1° (here OOTT or OTTT) or 2° (here **LLON**, **NTOL**, or **NOLL**) lineages (STERNBERG 1988). For clarity, 1° lineages are enclosed in heavy boxes and 2° lineages in thin boxes. The lineage [S OO] is hybrid (see DISCUSSION for more on hybrid lineages). B, The fates of the six VPCs determined by anatomical examination of 25 hermaphrodites. Fates shown here are consistent with those lineages shown in A. When examining many animals, this anatomical method is more practical than lineaging due to ease and speed. It is reliable if performed in early-mid L4 hermaphrodites since all divisions are complete and the progeny retain their relative positions and orientations. Subsequently, scoring induction by anatomy becomes more difficult due to the movement of the progeny cells during vulval morphogenesis. C, Anatomically determined VPC fates in gonad-ablated (and therefore anchor-cell deficient) L1 *n1045* hermaphrodites grown at 25° (see RESULTS and MATERIALS AND METHODS).

decrease in *let-23* function. Since *n1045/mnDf68* animals grown at 25° are partially Vul and *+/mnDf68* animals grown at 25° have wild-type vulvae, one copy of *n1045*(25°) has less vulval function than one copy of a *let-23*(+) chromosome. Extrapolating, we infer that at 25°, two copies of *n1045* has less vulval function than two copies of *let-23*(+), even though the former has more vulval induction than the latter. Hyperinduction therefore correlates with a *reduction* of some aspect of *let-23* vulval function. However, this reduction is only partial since a more severe or complete loss *let-23* function results in a partly or completely Vul phenotype, respectively. That the Hin phenotype correlates with more *let-23* function than the Vul phenotype is further supported by the fact that *n1045/n1045* at 25° is Hin but *n1045/mnDf68*, which should have less function, is Vul.

Hyperinduction is not restricted to *n1045*, suggesting that it is not merely an unusual property of the *n1045* allele. The lethal allele *mn224* displays the Hin phenotype in *trans* to both *syl* and *n1045* at 20° (Table 1A). Both *syl* and *n1045(20°)* have less than wild-type *let-23* vulval function since they display a Vul or partly Vul phenotype in *trans* to other alleles. The *mn224* Hin phenotype therefore also correlates with lowering *let-23* vulval function since *mn224/+* has wild-type vulval induction. That is, reducing the *let-23* vulval activity from *mn224/+* to *mn224/syl* or *mn224/n1045* can result in Hin vulvae. Unfortunately, we cannot determine the extent of vulval induction of *mn224* in *trans* to other alleles since these combinations are lethal (Table 1B). The *n1045* and *mn224* data are summarized in Figure 3 as a plot of induction versus inferred *let-23* activity.

***let-23* tissue specificity:** The allele *mn224* is defective in an essential function but retains some *let-23* function in the vulva, suggesting that the *let-23* phenotypes in different tissues are separable. Two general models could account for this separability of phenotypes. First, different tissues could be differentially sensitive to *let-23* dosage. For example, the *let-23* product might phosphorylate factor a in tissue A and factor b in tissue B, with factor a being less prevalent in tissue A than factor b in tissue B. Therefore, tissue A would be more sensitive to a decrease in *let-23* dosage than tissue B. This model predicts alleles that reduce *let-23* function would "uncover" mutant phenotypes in a specific order: *let-23* mutations could result in an A^-B^+ or A^-B^- phenotype (depending on the severity of the allele) but not an A^+B^- phenotype. Second, *let-23* itself might have independently mutable domains and encode tissue-specific functions. For

TABLE 3

Genetic basis of *n1045* hyperinduction

let-23 genotype	No. of hermaphrodites at 25° with induction: <100% (phenotypically Vul of partly Vul)	=100% (phenotypically wild-type[a])	>100% (phenotypically Hin)	Average % induction
+/+	0	All	0	100
n1045/n1045	2	12[b]	9	107
n1045/+	0	21	0	100
n1045/mnDf68	19[c]	0	0	15
+/mnDf68	0	21	0	100

All data were generated at 25° using the same complementation scheme as in Table 1. Average induction seen with *n1045* is generally less at all temperatures when generated with this scheme than when using the original *n1045* isolate. For example, induction in the original *n1045* strain at 25° is 127% (data from Table 2) versus 107% here. Induction of *n1045* homozygotes at 15° is 11% using the complementation scheme and 25% in the original strain. Nonetheless, for the data generated by either method we have determined that at 25° *n1045* displays the Hin phenotype, this hyperinduction is recessive, and *n1045/mnDf68* animals are Vul or partly Vul but never Hin. There are three possible explanations for this difference in *n1045* induction. First, since the complementation scheme uses mothers which are belanced over a *let-23*(+) chromosome, it is possible that *n1045* is maternally rescued. However, we were unable to detect differences in vulval induction of *n1045/n1045* progeny of either homozygous (*n1045/n1045*) or heterozygous (*n1045/+*) mothers (see MATERIALS AND METHODS). Second, it is possible that the linked markers (*rol-6* and *dpy-10*) used in the complementation scheme have dominant effects on induction, although such an effect has not been seen with any other allele (see MATERIALS AND METHODS). Last, the difference could be due to the elimination by recombination of a tightly linked modifier during marker addition. Precedent for this may exist with the allele *sy12* (see MATERIALS AND METHODS).

[a] Except where noted, animals with 100% induction displayed wild-type induction patterns.

[b] Two of these 12 hermaphrodites did not have wild-type induction patterns, although the wild-type number of VPCs (*i.e.*, three) divided.

[c] Twelve of these 19 animals had 0% induction.

example, the *let-23* gene might interact differently with factor a and b such that it is possible to disrupt the interactions of the *let-23* product with factor a only (A^-B^+ phenotype), factor b only (A^+B^- phenotype), or with both (A^-B^- phenotype).

We distinguished between these models by quantitating the penetrances of five *let-23* phenotypes associated with the allele combinations used to measure vulval induction (see above) and ask whether or not these phenotypes are separable by *let-23* dosage. The penetrances of the five phenotypes are given in Table 1 such that 100% is wild type in all cases: percent vulval induction (Table 1A); percent survival (Table 1B); percent wild-type male spicules (Table 1C); percent fertile hermaphrodites (Table 1D); and percent wild-type P12 (Table 1E).

We find that for any given phenotype but P12, we can determine a loss of function phenotype and can order the alleles from most to least *let-23* activity (Table 4A). All alleles were recessive for all phenotypes. These results suggest that the *let-23* mutant phenotypes arise from reduction or elimination of *let-23* function and that the *let-23* gene is needed for the wild-type development of these different tissues. Only a few allele combinations are inconsistent with the ordering of alleles given in Table 4A. The vulval phenotype of the *sy1/sy97* heterozygote is less severe than expected (Table 1A). This is perhaps best demonstrated as percent of hermaphrodites which can lay eggs: 8% of *sy1/sy1* ($n = 98$) and 0% of *sy97/sy97* ($n > 100$) hermaphrodites are Egl^+ whereas 53% of *sy1/sy97* hermaphrodites are Egl^+ ($n = 30$). The simplest interpretation of this intragenic complementation is that the *let-23* product acts as a multimer and that these mutations are in protein coding sequence (reviewed in WHITEHOUSE 1969). Also, homozygous *n1045* hermaphrodites at 20° display more penetrant essential and male spicule defects than expected based on other *trans* heterozygous combinations (Table 1, B and C). This increased penetrance might result from some slight dominant negative effects of *n1045* for these phenotypes, or from *n1045* partially complementing the defects in *sy10*, *sy12* and possibly *sy97* for these two phenotypes .

Although our mutations display tissue-specific effects, we cannot order the phenotypes with respect to their sensitivity to *let-23* dosage (Table 4B). Rather, the phenotypes are independently mutable and the *let-23* gene appears to encode tissue-specific functions. The allele *sy1* would indicate that the vulva is the most sensitive tissue to *let-23* dosage. This conclusion, however, is contradicted by *n1045* and *mn224*. The allele *n1045* is less severe than *sy1* in the vulva but more severe in all other tissues. The allele *mn224* is less severe than *sy1* in the vulva but displays fully penetrant larval lethality and sterility. Unpredictably, *mn224* is also nearly wild type for spicule function. This allele therefore suggests that it is possible to eliminate *let-23* function in some tissues and not others. Lastly, the allele *sy97*, which is wild type for hermaphrodite fertility but severe in all other tissues, would suggest that defects in fertility are the least sensitive phenotype to *let-23* dosage and contradicts sensitivities inferred from *n1045* and *sy10*. These results indicate that the tissue-specific effects associated with different *let-23* alleles result not from differences in dosage sensitivity but from independently mutable domains.

Although this conclusion is based primarily on three alleles (*sy1*, *sy97* and *mn224*), we believe these findings are significant. First, these alleles represent half of our non-null alleles (14 out of 20 alleles are nulls). Second, with few exceptions (see above), these alleles consistently and quantitatively behave as outlined, despite the fact that we can otherwise assign loss of function phenotypes. Third, these results are taken from alleles which were generated in only two types

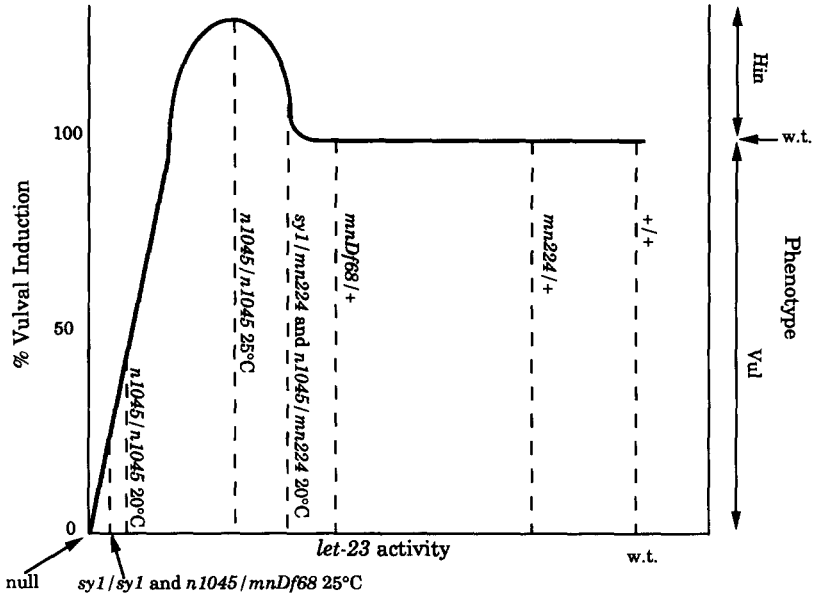

FIGURE 3.—Hypothetical dose response curve for *let-23* vulval induction. Since *let-23*(+)/*mnDf68* is wild type, we assume that initial decreases in *let-23* do not affect induction. Therefore, starting from wild type, the curve is flat. We infer that homozygous *n1045* at 25° has less function than wild type but is hyperinduced so the curve rises as activity drops. A further decrease in function (*e.g.*, *n1045*/*mnDf68* at 25°) results in the Vul phenotype so the curve falls off until at no *let-23* activity, induction is 0%. Since *mn224* is lethal, we cannot infer its *let-23* activity as a homozygote relative to the other alleles but it appears to have significantly more activity than *mnDf68*, and has wild-type induction in *trans* to *let-23*(+). Hence, we put *mn224*/+ far to the right of *mnDf68*/+. Both homozygous *sy1* and *n1045* at 20° are Vul, but in *trans* to *mn224* are Hin. As discussed in the text, this is consistent with our *n1045* results, and we have plotted the graph accordingly. w.t. = wild type.

of screens: for vulvaless (17/20 alleles) and lethal (3/20) mutations.

The P12 to P11 transformation in *let-23* mutants differs from the other phenotypes in that it exhibits partial maternal rescue (Table 5). This maternal rescue explains the weak penetrance seen in Table 1E since the data are generated from balanced *let-23(mutant)/let-23*(+) mothers. We have found no instance of completely penetrant transformation, and we cannot assign a loss of function phenotype. The allele *sy1* has 100% wild-type P12 even from a homozygous *sy1* mother (data not shown).

DISCUSSION

We initiated studies of *let-23* to understand the genetic basis for the *h*yper*in*duced (Hin) phenotype associated with one allele. In Hin hermaphrodites, the VPCs are often induced to a higher fate than in wild-type hermaphrodites (*i.e.*, 3° and 2° cells in wild type can become 2° and 1°, respectively, in a Hin animal). Our results suggest that in a Hin hermaphrodite, the VPCs are hypersensitive to the inductive signal. Furthermore, our data suggest that hyperinduction results from a partial reduction of *let-23* function and not from gain of function or neomorphic function. This conclusion is surprising given that a strong reduction or elimination of *let-23* function results in reduced or no induction of the VPCs. In addition, we measured the penetrance of five *let-23* phenotypes for many allele combinations. These phenotypes show a remarkable degree of independence of mutability (Table 4B). For example, it is possible to preferentially eliminate the *let-23* vulval function alone (allele *sy1*), to preferentially eliminate the essential and fertile functions (allele *mn224*), and to preferentially retain the fertile function (allele *sy97*).

Models for *let-23* function in the vulva: Our data suggest that proper determination of vulval fate requires two opposing pathways, both regulated by the *let-23* receptor tyrosine kinase. Since a complete loss of *let-23* function leads to no induction of vulval fate, the *let-23* gene is required for a *stimulatory* pathway essential for any vulval differentiation to occur. On the other hand, since some mutations that reduce *let-23* function result in greater than wild-type induction of vulval fate (Hin phenotype), the *let-23* gene is also apparently involved in an *inhibitory* pathway that normally acts negatively to limit the amount of induction that occurs. This inhibitory pathway seems to modulate the stimulatory pathway and not vice versa since a loss of *let-23* function leads to no induction and since VPCs in a Hin animal require the anchor cell signal and respond to it in a graded fashion, suggesting that stimulation of induction itself is functional but not properly regulated. These two pathways may or may not be separately controlled by the *let-23* gene itself. For example, the *let-23* product might activate two cascades, one that results in induction and the other that results in negative regulation. Alternatively, the *let-23* product might activate only one cascade that later splits into two pathways, stimulatory and inhibitory. In addition, other genes are likely to act with *let-23* in both these pathways since, for example, mutations in *lin-2* and *lin-7* can result in both the Vul and Hin phenotypes (FERGUSON and HORVITZ 1985; G. JONGEWARD and P. STERNBERG, in preparation).

We propose that hyperinduction results from *let-23* mutations that compromise the inhibitory pathway but not the stimulatory pathway. In these animals, induction occurs but is not properly limited or regulated, resulting in higher than wild-type VPC fates. We envision two models for how *let-23* mutations can lead to hyperinduction (Figure 4A). These models are parallel to the possible models for separable tissue-

TABLE 4

Summary of complementation analysis

A. Ordering of *let-23* alleles for different *let-23* phenotypes

	let-23 phenotype			
	Defective vulval induction	Lethality	Defective male spicules	Hermaphrodite sterility
Increasing severity ↓	*mn224* *n1045* (20°) *sy1* *sy10* *sy12* *sy97* *sy15, mnDf68*	*sy1* *n1045* (20°) *sy10, sy12, sy97* *mn224, sy15, mnDf68*	*sy1, mn224* *n1045* (20°) *sy10* *sy12* *sy97, sy15, mnDf68*	*sy1, sy97* *n1045* (20°) *sy12* *sy10* *mn224, sy15* *mnDf68*
Null phenotype	Vulvaless	Larval lethal	Crumpled spicules	Sterile

B. Inferred defects in *let-23* functions for different alleles

	let-23 function				
Allele	Vulval induction	Essential	Male spicules	Hermaphrodite fertility	P12
Wild type	+	+	+	+	+
n1045	(+)	(+)	(+)	(+)	(+/−)
sy10	(−)	(−)	(−)	(−)	?
sy15, mnDf68	−	−	−	−	?
sy1	(−)	+	+	+	+
mn224	(+)	−	+	−	?
sy97	(−)	(−)	(−)	+	(+/−)

A, For each *let-23* phenotype, we have ordered the alleles from least severe to most severe and have assigned a complete loss of function phenotype as follows. Complete loss of vulval function correlates with no vulval induction (see text). A complete loss of *let-23* essential function likely results in larval lethality. First, both the deletion *mnDf68* and the canonical null *sy15* in *trans* to all alleles but *sy1* decrease survival. In particular, the alleles *sy10, sy12* and *sy97* are virtually inviable in *trans* to a null. There is thus a critical threshold of *let-23* dosage for survival since any combination of two of these alleles results in 10–20% survival, but only one copy of any of them is <1% viable. Second, in an allelic series, 0% survival is consistent with least *let-23* essential function (e.g. the canonical lethal *sy15* and the lethal *mn224* behave like *mnDf68* for essential function and are inviable). As discussed above, a *let-23* null also results in larval lethality. This distinction between loss of essential function and the null phenotype is necessary because of the allele *mn224*, which is a loss-of-function for essential activity but not a loss-of-function for the entire gene, since it retains substantial vulval and male spicule activities. Complete loss of *let-23* spicule activity likely results in a completely penetrant crumpled spicule phenotype. First, *n1045* at 20° in *trans* to null alleles has a higher penetrance of mutant phenotype. Second, in an allelic series, low function levels correlate with a highly penetrant crumpled spicule phenotype (see especially *sy97*). We have found that *let-23* males with defective spicules cannot mate (see MATERIALS AND METHODS). Complete loss of *let-23* fertile activity likely results in a completely penetrant sterile phenotype. First, *n1045* at 20° in *trans* to null alleles has a higher penetrance of the mutant phenotype. Second, in an allelic series, low activity levels correlate with complete sterility (see especially *sy10*). B, This table summarizes inferred impairment in the different *let-23* tissue-specific functions associated with each *let-23* allele. Inferred impairment for each entry is based on the severity of the defects seen in that allele relative to wild-type and the other alleles within that phenotype. From least to most impaired: + refers to wild-type or close to wild-type function; (+) refers to reduced function; (−) refers to low function; − refers to no function as defined by a deficiency.

specific phenotypes (see RESULTS), except that the separable phenotypes (Hin and Vul) now affect the same tissue. If the inhibitory pathway is more sensitive to *let-23* dosage than the stimulatory pathway, then a moderate reduction in *let-23* function would preferentially affect the inhibitory pathway, resulting in a higher than wild-type induction (model I). Alternatively, the inhibitory pathway could be controlled by a region of *let-23* distinct from the stimulatory pathway; mutations that have a Hin phenotype could then result from a mutation preferentially affecting this inhibitory pathway region (model II). This latter possibility is intriguing given that the *let-23* protein is a member of the EGF receptor tyrosine kinase subfamily (AROIAN *et al.* 1990) and that one of our Hin alleles, *n1045*, is amber-suppressible (FERGUSON and HORVITZ 1985). This mutation may therefore result in a truncated receptor lacking some C-terminal sequence (a truncation starting too far from the C terminus, such as in the kinase domain, would presumably have more severe phenotypes than seen for *n1045*). It is known that the C terminus of the EGF receptor is needed for its proper negative regulation because of the presence of autophosphorylation sites (BERTICS and GILL 1985) and sequences required for down-regulation of receptor (RIEDEL *et al.* 1989; CHEN *et al.* 1989). Furthermore, both mutations that eliminate autophosphorylation sites (HONEGGER *et al.* 1988) and truncations of the receptor C terminus (WELLS *et al.* 1990) can result in cells hypersensitive

TABLE 5

Maternal rescue of P12 phenotype

let-23 genotype of mother	*let-23* genotype	Percent wild-type P12
sy97/sy97	*sy97/sy97*	40 ± 9
sy97/+	*sy97/sy97*	78 ± 8
sy12/sy12	*sy12/sy12*	39 ± 18
sy12/+	*sy12/sy12*	83 ± 14
n1045/n1045 15°	*n1045/n1045* 15°	44 ± 8
*n1045/+*15°	*n1045/n1045* 15°	55 ± 7

We scored P12 phenotype in hermaphrodites from mothers that were homozygous and heterozygous for *let-23*. Given is the percent wild-type P12 and two standard deviations. The maternal rescue with the amber allele *n1045* is less than in *sy97* or *sy12* hermaphrodites. The incomplete penetrance of the rescue might indicate a need for some *let-23* zygotic product.

to ligand. Thus, for example, the *n1045* mutation might result in a receptor competent to transduce signal but defective in down-regulation. As a consequence of receptors recycling to the cell surface, the VPCs might become hypersensitive to the inductive signal. The absence of hyperinduction in *n1045* hemizygotes could be explained if one copy of defective receptor provided insufficient stimulatory function. Similarly, the recessive nature of this mutation may be due to the limiting effects of one copy of defective receptor or restoration of proper regulation by the wild-type copy.

The inferred inhibitory pathway might operate intercellularly or intracellularly (Figure 4B). The anchor cell inductive signal might act to stimulate a VPC, which then negatively signals its neighbors to inhibit their induction (intercellular inhibition; model III). If the inhibitory signal transmitted by a VPC was proportional to the inductive signal the VPC received, then this would serve to reinforce the graded anchor cell signal: P6.p would inhibit P5.p and P7.p from executing too high a fate, and P5.p and P7.p would similarly inhibit P4.p and P8.p respectively. Precedent for intercellular VPC interactions (*e.g.*, 1°–1° lateral inhibition) exists (STERNBERG 1988; THOMAS, STERN and HORVITZ 1990). However, since the Hin phenotype can include the execution of 2° fate by VPCs that normally execute a 3° fate in addition to the presence of adjacent 1° cells, then this inter-VPC inhibition would lower the extent of induction in general and not only prevent the formation of adjacent 1° cells. Alternatively, both the stimulatory and inhibitory pathways regulated by *let-23* could operate within a given VPC (model IV). For example, the inhibitory pathway could involve intracellular down-regulation of the receptor for inductive signal, which might be *let-23* itself. Failure of this process could result in VPCs that do not properly negatively regulate the signal transduction, resulting in the hyperinduced phenotype.

***let-23* tissue specificity:** Mutations in the *let-23* gene can, to a large extent, independently affect the different *let-23* phenotypes, suggesting that the *let-23* gene behaves differently in different tissues and that the *let-23* gene has tissue-specific functions. Several possible mechanisms could account for this tissue-specificity: promoter elements specific for different tissues, tissue-specific alternatively spliced transcripts, or differential interactions with tissue-specific factors. There is some evidence that three of our *let-23* mutations are in protein coding sequence and not promoter elements. First, the *n1045* mutation is amber-suppressible. Second, the alleles *sy1* and *sy97* show striking allele-specific complementation for the vulval function. In addition, although several alternatively spliced transcripts could account for independent mutability, initial molecular characterization of the *let-23* gene suggests that there are not enough transcript species (AROIAN *et al.* 1990).

Another possibility is that there is one *let-23* product that interacts with different factors in different tissues. These factors could act to modify, stabilize, or destabilize the *let-23* product in different tissues, or they could be tissue-specific ligands or substrates of the *let-23* receptor itself. The independent mutability of the *let-23* functions might then be due to differential interactions of the *let-23* product with these tissue-specific factors. As noted above, a multiple substrate mechanism might be responsible for the stimulatory and inhibitory pathways regulated by the *let-23* gene in the vulva. That tissue-specificity is independently mutable as opposed to strictly dosage-sensitive prompts us to at least consider that the vulval stimulatory and inhibitory pathways are directly and separately controlled by the *let-23* gene.

The multiplicity of *let-23* function is perhaps not surprising given what is known about the mammalian EGF receptor. The receptor has diverse effects in the cell (reviewed in ULLRICH and SCHLESSINGER 1990), and it and its subfamily members are believed to behave differently in different cell types (KHAZAIE *et al.* 1988; DIFIORE *et al.* 1990). Moreover, the Drosophila EGF receptor locus (DER), known by *faint-little-ball*, *torpedo*, and *Ellipse* mutations (SCHEJTER and SHILO 1989; PRICE, CLIFFORD and SCHUPBACH 1989; BAKER and RUBIN 1989), displays a similar multiplicity of phenotypes and partial independence of mutability of the gene functions associated with the phenotypes (CLIFFORD and SCHUPBACH 1989). As with the *let-23* gene, there probably are too few DER transcripts for an alternative splicing model to account for the differential mutability.

Role of the *let-23* gene in determination and possibly proliferation of vulval fate: That the *let-23* gene encodes an EGF receptor-like molecule raises the question of how such a molecule might function in nematode development. In mammalian systems,

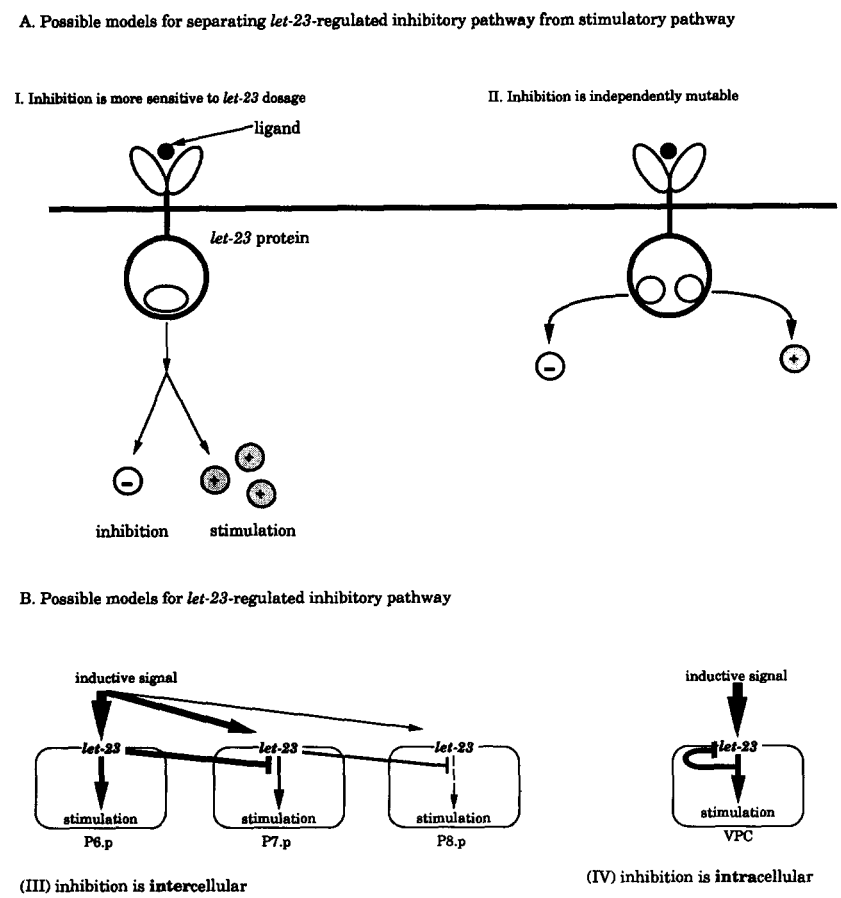

FIGURE 4.—Models for *let-23* function during vulval induction. **A**, Model for the separability of the stimulatory and inhibitory pathways regulated by *let-23* . This separation can be achieved by either assuming that the inhibitory pathway is more sensitive to *let-23* levels than the stimulatory pathway (model I) or that the stimulatory pathway and the inhibitory pathway are separately controlled by *let-23* and are somewhat independently mutable (model II). In model I, the two pathways may or may not be separately controlled by the *let-23* gene; they could branch at or downstream of the *let-23* product and still have different dosage sensitivities. As noted in the text, other genes, such as *lin-2* and *lin-7* which also display the Vul and Hin phenotypes, are likely to act with *let-23* in both of these pathways (G. JONGEWARD and P. STERNBERG, in preparation). **B**, Models for *let-23*-regulated inhibitory pathway. The inhibitory pathway which is defective in hyperinduced animals could act intercellularly (model III) or intracellularly (model IV). In model III, *let-23* is needed for stimulation of vulval fates and negative signalling between VPCs. If the *let-23*-regulated inhibitory pathway is compromised, then a given VPC would become hypersensitive to inductive signal because it is not inhibited by neighboring VPCs. This neighboring VPC inhibition may either be passive (*i.e.*, VPCs proximal to the anchor cell normally prevent high signal levels from reaching more distal VPCs by simply removing signal from the extracellular space between the anchor cell and the VPCs) or active. In model IV, hypersensitivity results from alleviation of internal inhibition. Normally, the *let-23* gene acts to signal a VPC to adopt vulval fate and also negatively regulates transduction in the same VPC to ensure proper response levels. In the example given, negative regulation occurs upstream of *let-23*, but it may occur downstream. For simplicity, we assume that both of the *let-23*-regulated pathways act in the VPCs, but, until we know where *let-23* is expressed, we cannot exclude that the stimulatory and/or inhibitory pathway originate in other cells, such as the surrounding hypodermis.

ligands of EGF receptor, EGF and TGF-α, can elicit proliferation, can inhibit proliferation, or can have other unrelated effects (reviewed in SPORN and ROBERTS 1988). Examination of vulval development in *let-23* mutants indicates that the *let-23*-directed pathways are involved in cell-type determination and possibly also cell proliferation.

Evidence for a proliferative role comes from hybrid vulval-hypodermal lineages found in some *let-23* mutant animals (see Figure 2C). These non-wild-type lineages result in one daughter of a VPC executing a vulval fate and the other daughter executing a hypodermal fate; these lineages are not unique to *let-23* (SULSTON and WHITE 1980; KIMBLE 1981; SULSTON and HORVITZ 1981; GREENWALD, STERNBERG and HORVITZ 1983; STERNBERG and HORVITZ 1986, 1989). Our *let-23* data suggest that hybrid lineages appear to arise from VPCs in which there are lower than wild-type levels of signal transduction. We also found that the polarity of hybrid lineages correlates with the location of the anchor cell. For either P5.p or P7.p, the VPC daughter closer to the anchor cell has a nine times greater chance of executing the vulval fate than the daughter farther from the anchor cell, whereas for P6.p, whose daughters are equidistant from the anchor cell, the chance is significantly less biased. In addition, the decision by a given VPC to execute a hybrid lineage does not show any obvious

correlation with the fate of the neighbor(s) of that VPC (see MATERIALS AND METHODS for data on hybrid lineages). Based on these data, we speculate that the anchor-cell signal is causing a bias in the distribution of some factor in the VPCs necessary for their daughters to divide. Therefore, in VPCs with lowered levels of induction, for example due to reductions in *let-23* or *let-60 ras* activity (both of which can result in hybrid lineages; this paper and M. HAN, personal communication), the anchor-cell distal daughter is less likely to divide than the anchor-cell proximal daughter. This model suggests that hybrid lineages arise from a defect in proliferation of some VPC daughter cells and is consistent with a growth factor receptor-directed pathway. A specific version of this model is that the *let-23* product coupled to its hypothetical ligand itself might be the factor whose distribution is influenced by the anchor-cell signal, since in mammalian systems ligand induces clustering of receptor (reviewed in SCHLESSINGER *et al.* 1983). Other models unrelated to proliferation are also possible to explain hybrid fates; for example, these lineages may represent determination of a novel VPC fate.

Nonetheless, the *let-23* gene does appear to function directly in cell-type determination independent of proliferation. This conclusion is best demonstrated by the transformation of 2° fate to 1° fate in some hyperinduced animals in which there is no change in the number of rounds of mitosis involved (except for the "N" cell; see Figure 2A).

The overall functioning of the *let-23* gene is therefore intricate. The *let-23* gene appears to function differently in the different tissues where it acts. In the vulva, the data further suggest that *let-23* functions in two antagonistic pathways (indeed, mutations in the gene provided the opportunity to discern these different pathways), and these pathways seem to control cell-type determination and possibly also proliferation.

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