The Multivulva Phenotype of Certain *Caenorhabditis elegans* Mutants Results From Defects in Two Functionally Redundant Pathways

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ABSTRACT

We previously identified *Caenorhabditis elegans* mutants in which certain of the six vulval precursor cells adopt fates normally expressed by other vulval precursor cells. These mutants define genes that appear to function in the response to an intercellular signal that induces vulval development. The multivulva (Muv) phenotype of one such mutant, CB1322, results from an interaction between two unlinked mutations, *lin-8(n111) II* and *lin-9(n112) III*. In this paper, we identify 18 new mutations, which are alleles of eight genes, that interact with either *lin-8(n111)* or *lin-9(n112)* to generate a Muv phenotype. None of these 20 mutations alone causes any vulval cell lineage defects. The "silent Muv" mutations fall into two classes; hermaphrodites carrying a mutation of each class are Muv, while hermaphrodites carrying two mutations of the same class have a wild-type vulval phenotype. Our results indicate that the Muv phenotype of these mutants results from defects in two functionally-redundant pathways, thereby demonstrating that redundancy can occur at the level of gene pathways as well as at the level of gene families.

THE cellular anatomy and the developmental patterns of cell divisions and cell fates of the nematode *Caenorhabditis elegans* are essentially invariant (SULSTON and HORVITZ 1977; DEPPE *et al.* 1978; KIMBLE and HIRSH 1979; SULSTON, ALBERTSON and THOMSON 1980; SULSTON *et al.* 1983). We are attempting to understand the genetic specification of a particular aspect of *C. elegans* development, the cell lineages that generate the vulva of the hermaphrodite. The vulva is formed by the descendants of three of six tripotent hypodermal cells. Previous studies have indicated that the fate of each of these six precursor cells is determined at least in part in response to an intercellular signal generated by the anchor cell of the somatic gonad (SULSTON and HORVITZ 1977; SULSTON and WHITE 1980; KIMBLE 1981; STERNBERG and HORVITZ 1986; STERNBERG 1988). The three precursor cells that are near the anchor cell are induced to generate the vulva. The precursor cell nearest the anchor cell adopts the 1° vulval fate, whereas the two adjacent precursor cells adopt a 2° vulval fate. The remaining three precursor cells adopt a 3° nonvulval fate.

We have previously identified eleven genes that by the criteria of mutant phenotype, site of action, and time of action appear to act within the vulval precursor cells in the determination of the fates of these cells in response to the anchor cell signal (FERGUSON and HORVITZ 1985; FERGUSON, STERNBERG and HORVITZ 1987). Mutations in these genes cause certain of the vulval precursor cells to adopt fates normally expressed by other precursor cells. In vulvaless (Vul) mutants, all six precursor cells express the nonvulval fate. In multivulva (Muv) mutants, all six precursor cells express vulval (1° or 2°) fates, dividing to generate multiple vulval-like protrusions along the ventral side of the animal.

Based on differences in cell lineage and morphology, the Muv strains can be divided into groups. In one group of Muv mutants, comprising mutants defective in any of three genes—*lin-15*, *lin-34* and *lin-13*—the six vulval precursor cells generally express an alternating pattern of 1° and 2° vulval fates (FERGUSON, STERNBERG and HORVITZ, 1987; STERNBERG, 1988). A fourth Muv strain, CB1322, has a phenotype similar to that of mutants defective in any of these three genes. However, the Muv defect in this strain results from an interaction between two unlinked mutations, *lin-8(n111) II* and *lin-9(n112) III*, neither of which alone results in any vulval cell lineage abnormalities (SULSTON and HORVITZ 1981; FERGUSON, STERNBERG and HORVITZ 1987).

In this paper, we analyze the genetic basis for the synthetic Muv phenotype of CB1322. We identify 18 additional mutations that result in a Muv phenotype in the presence of a mutation in either *lin-8* or *lin-9* but alone cause no defects in the vulval cell lineages. The patterns of interactions among these mutations suggest that the Muv phenotype of CB1322, as well

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as the phenotypes of the other members of this group of Muv mutants, result from defects in two functionally redundant pathways.

MATERIALS AND METHODS

Strains and genetic nomenclature: *Caenorhabditis elegans* var. Bristol strain N2 and most of the mutant strains used for mapping and strain construction were obtained from BRENNER (1974) or from the Caenorhabditis Genetics Center, which is supported by contract number N01-AG-9-2113 between the National Institutes of Health and the Curators of the University of Missouri. Except where noted, these genes have been described by BRENNER (1974) and SWANSON, EDGLEY and RIDDLE (1984). The alleles used are either the reference alleles listed in the above publications or alleles that result in similar phenotypes. N2 is the wild-type parent of all nematode strains used in this work.

LGI: *unc-11(e47); dpy-5(e61); dpy-14(e188); unc-13(e1091).*

LGII: *bli-2(e768); dpy-10(e128); rol-1(e91); unc-52(e444); mnC1 dpy-10(e128) unc-52(e444). mnC1 dpy-10 unc-S5* is an LGII chromosomal abnormality that balances the right half of the chromosome (HERMAN 1978).

LGIII: *dpy-17(e164); lon-1(e185); sma-3(e491); lin-16(e1743); daf-4(e1364); unc-36(e251); unc-86(el507); dpy-19(e1259); sup-5(e1464); sma-2(e502); unc-32(e189).*

LGIV: *unc-5(e53); unc-22(e66).*

LGV: *dpy-11(e224).*

LGX: *lon-2(e678); sup-7(st5); dpy-7(e1324); unc-3(e151); mnDp1; mnDf43; mnDf4.*

This paper follows the standardized *C. elegans* genetic nomenclature (HORVITZ *et al.* 1979). The genes identified by silent Muv mutations were assigned *lin*, for *lin*eage abnormal, gene names.

General techniques: Methods for the culturing, handling and genetic manipulation of *C. elegans* have been described (BRENNER 1974).

Mutagenesis of hermaphrodites of genotypes *lin-8(n111)* and *lin-9(n112)*: Phenotypically wild-type L4 hermaphrodites homozygous for either *lin-8(n111)* or *lin-9(n112)* were mutagenized with ethyl methanesulfonate (EMS) as described by BRENNER (1974). Individual mutagenized hermaphrodites were put on separate 100 mm NGM plates (BRENNER 1974) and allowed to produce 20–40 progeny. The progeny of 3000 F_1 hermaphrodites from mutagenized *lin-8(n111)* animals and 4000 F_1 hermaphrodites from mutagenized *lin-9(n112)* animals were screened for Muv animals, each of which was a candidate for carrying a mutation that interacted with either *lin-8(n111)* or *lin-9(n112)* to produce a Muv phenotype. Muv hermaphrodites were picked onto separate plates and allowed to self-fertilize. If a Muv animal appeared to be self sterile, it was mated with wild-type males and, if progeny were produced, Muv hermaphrodites were reisolated from the F_2 progeny. Only one Muv strain from each parental plate was characterized to ensure that each isolate carried an independently derived mutation.

The Muv phenotypes of 12 of the 17 strains that were isolated during these mutageneses were shown to be dependent on the presence of two mutations, the parental mutation and a newly induced mutation (see RESULTS). We will refer to these newly isolated mutations (as well as the preexisting *lin-8* and *lin-9* mutations) as "silent Muv" mutations, as they result in a Muv phenotype only in certain pairwise combinations (see RESULTS). The remaining five strains carried mutations that result in a Muv phenotype independent of the silent mutation in the parental strain. Specifically, these strains defined three alleles of *lin-1*—*n753*, *n757*, and an allele that was subsequently lost—and one allele of *lin-15*—*n765ts*; studies of these strains have been described elsewhere (FERGUSON and HORVITZ 1985). The mutation responsible for the Muv phenotype of the remaining strain, MT665, which was isolated after mutagenesis of *lin-8(n111)* hermaphrodites, displayed linkage to *unc-3 X* but not to *bli-2 II*, which is linked to *lin-8*, suggesting that the Muv phenotype of this strain may result from a *lin-15* mutation.

Isolation and characterization of Muv strains after mutagenesis of the strain MT1312: Five silent Muv strains were isolated after mutagenesis of the strain MT1312. This strain displays a highly penetrant vulvaless (Vul) phenotype. Neither hermaphrodites nor males of this strain can mate. To characterize the mutation that caused the Vul phenotype of MT1312, we attempted to isolate extragenic suppressors that would allow hermaphrodites of this strain to mate. We mutagenized MT1312 hermaphrodites with EMS and examined the F2 progeny of these animals for egg-laying-competent individuals. Although no phenotypically wild-type hermaphrodites were found among the progeny of approximately 12,500 F1 progeny of mutagenized hermaphrodites, 16 fertile Muv strains were isolated. Five of these 16 Muv strains were further characterized; the remaining 11 strains were lost. These five Muv strains had multiple ventral pseudovulvae, but were not egg-laying competent. However, occasional Muv hermaphrodites ruptured at the vulva, and although dying, could be fertilized by wild-type males. These matings enabled the isolation and subsequent characterization both of the mutation responsible for the Vul phenotype of MT1312, *lin-12(n676)*, and of the mutations that resulted in the Muv phenotypes of these strains. Studies of *lin-12(n676)* have been described elsewhere (GREENWALD, STERNBERG and HORVITZ 1983; FERGUSON and HORVITZ 1985).

When the mutations responsible for the Muv phenotypes of these strains were mapped, they were found to display linkage to two linkage groups (see RESULTS). As one of the mutations responsible for the Muv phenotype of each strain displayed linkage to *unc-3 X*, it is likely that the same *X*-linked mutation is present in all five of these strains and was present in the original strain, MT1312, prior to the reversion experiments. We isolated this mutation, *n767*, from the Muv strain MT1643.

Construction of strains homozygous for a single silent Muv mutation: A recessive marker in *trans* was used to identify the chromosome carrying the silent Muv mutation to be isolated. From hermaphrodites of genotype *b*/+; *a*+/+ *r*, where *a* is the silent Muv mutation to be separated from the parental silent Muv mutation *b* and *r* is a recessive marker (or a number of closely linked recessive markers), wild-type hermaphrodites were picked. Those that segregated R animals but no Muv animals were of putative genotype +/+; *a*+/+ *r*. Progeny hermaphrodites of genotype *a*/*a* were obtained by picking wild-type hermaphrodites that did not segregate any R animals. These genotypes were confirmed by mating *b* males with hermaphrodites of putative genotypes *a*/*a*, picking at least eight F_1 progeny, and observing the presence of Muv hermaphrodites among the progeny of each F_1 hermaphrodite. In these experiments, *dpy-5 unc-13* was used in *trans* to *lin-35(n745)*; *lon-1 unc-86 dpy-19* was used in *trans* to *lin-36(n766)*; *lon-1* was used in *trans* to *lin-37*; *unc-52* was used in *trans* to *lin-38(n751)*; and *unc-3* was used in *trans* to *lin-15(n767)*.

Strains homozygous for either of two *X*-linked silent Muv mutations, *lin-15(n433)* or *lin-15(n744)*, were constructed

by mating males of genotype *b*/+; *a*/0, where *a* is the *X*-linked silent Muv mutation and *b* is the second silent Muv mutation in the strain, with hermaphrodites of genotype *b*; *a*. The non-Muv progeny of genotype *b*/+; *a*/*a* segregated hermaphrodites of genotype *a*/*a*.

Construction of class A-class A double mutants: To construct double mutants between *lin-15(n767) X* and either *lin-8(n111) II* or *lin-38(n751) II*, males of genotypes *a r*/++ were mated with *lin-15* hermaphrodites (*a r* was either *lin-8 dpy-10 or rol-1 lin-38 unc-52*). The resulting males were mated with *lin-15* hermaphrodites and the F_1 progeny of putative genotype *a r*/++; *lin-15* were identified on the basis of segregation of R progeny. Most R progeny of hermaphrodites of genotype *a r*/++; *lin-15* were of the desired genotype. The genotype of the *lin-8 dpy-10*; *lin-15* strain was confirmed by mating males of genotype *lin-35 unc-13*/++ with these hermaphrodites and observing that the great majority of the F_2 Dpy Unc progeny were Muv.

A strain carrying the linked mutations *lin-8(n111)* and *lin-38(n751)* was constructed as follows. Three of the 39 Dpy segregants from hermaphrodites of genotype *lin-8 dpy-10* +++/++ *rol-1 lin-38 unc-52*; *lin-9*/+ were of putative genotype *lin-8 dpy-10* +++/*lin-8 dpy-10 rol-1 lin-38 unc-52*; *lin-9*/+, as 1/4 of both classes of progeny (Dpy hermaphrodites and Dpy Rol Unc hermaphrodites) were Muv. Dpy non-Rol non-Unc non-Muv progeny from each of the three hermaphrodites were mated with *lin-8* males. Cross-progeny hermaphrodites that did not segregate any Muv animals were of genotype *lin-8 dpy-10 rol-1 lin-38 unc-52*/*lin-8*++++ From their progeny, the non-Dpy Rol Unc animals that did not segregate any Dpy animals were of the desired genotype *lin-8* + *rol-1 lin-38 unc-52*.

Construction of class B-class B double mutants: Strains containing two class B mutations were obtained by constructing hermaphrodites that were heterozygous for both of the class B mutations and linked markers either in *cis* or in *trans* to each of the class B mutations. In some constructions, these hermaphrodites were also heterozygous for a class A mutation, which was removed by segregation in the next generation. Animals were obtained that were homozygous for each of the chromosomes carrying a class B mutation. In these experiments, for *lin-35(n745)*; *lin-9(n112)*, *unc-13* was in *cis* to *lin-35*, and *dpy-17* was in *cis* to *lin-9*; for *lin-35(n745)*; *lin-36(n766)*, *unc-13* was in *cis* to *lin-35*, and *dpy-19 sma-2* was in *trans* to *lin-36*; for *lin-35(n745)*; *lin-37(n758)*, *unc-13* was in *cis* to *lin-35*, and *lon-1* was in *cis* to *lin-37*.

To construct double mutants between *lin-15(n744)* and other class B mutations, *lin-15* progeny were picked from among the segregants of hermaphrodites of genotype *r b*/++; *lin-15*/+ grown at 25°, where *r* is a marker in *cis* to the second class B mutation, *b*. (At 25°, *lin-15(n744)* animals are thinner and less fertile than the wild type.) The R segregants of these animals were of the desired genotypes. *dpy-17* was in *cis* to *lin-9*, *unc-13* was in *cis* to *lin-35*, *unc-32* was in *cis* to *lin-36*, and *lon-1* was in *cis* to *lin-37*.

***lin-15* intragenic recombination:** To determine the frequency of recombination between the *X*-linked class B silent Muv mutation *n744* and the *lin-15* Muv allele *n309*, Muv hermaphrodites of genotype *lin-8(n111)*; *unc-3 lin-15(n309)*/*n744* were constructed. The progeny of these animals were examined for rare non-Muv animals: one such animal was obtained among 3,168 progeny. This hermaphrodite segregated 1/4 Muv animals, 1/2 phenotypically wild-type animals and 1/4 Unc non-Muv animals, indicating that *n744* maps about 0.03% to the left of *n309*.

To determine the frequency of recombination between the *X*-linked class A silent Muv mutation *n767* and *n309*, the progeny of Muv hermaphrodites of genotype *lin-9(n112)*; *unc-3 lin-15(n309)*/*n767* were examined. No non-Muv hermaphrodites were found among 1117 progeny, indicating that *n767* maps within 0.09% of *n309*.

Construction of hermaphrodites of genotypes *lin-8*; *lin-34*; *sup-7* and *lin-9*; *lin-34*; *sup-7*: To construct a strain of genotype *lin-8(n111)*; *lin-34(n1046) unc-22; sup-7 dpy-7*, wild-type males were mated with hermaphrodites of genotype *lin-8(n111)*; +*lin-9(n942) unc-32*/*sma-2*++. Progeny males were of two genotypes, *lin-8(n111)*/+; *lin-9(n942) unc-32*/++ and *lin-8(n111)*/+; *sma-2*/+, and were mated with hermaphrodites of genotype *lin-34(n1046) unc-22; sup-7 dpy-7*. Forty-five cross-progeny hermaphrodites were picked; nine were of genotype *lin-9(n942) unc-32*/++; *lin-34(n1046) unc-22*/++; *sup-7 dpy-7*/++ and were used to obtain hermaphrodites of genotype *lin-9(n942) unc-32*; *lin-34(n1046) unc-22; sup-7 dpy-7* (see below). One of the other 36 hermaphrodites was of genotype *lin-8(n111)*/+; *lin-9(n942) unc-32*/++; *lin-34(n1046) unc-22*/++; *sup-7 dpy-7*/++. From the progeny of that hermaphrodite, two of 55 phenotypically wild-type hermaphrodites picked were of genotype *lin-8(n111)*; *lin-9(n942) unc-32*/++; *lin-34(n1046) unc-22*/++; *sup-7 dpy-7*/++, as they segregated Dpy progeny, Muv Unc-22 progeny, Muv Unc-32 progeny but no non-Muv Unc-32 progeny. From the progeny of these two animals, seven of 44 phenotypically wild-type hermaphrodites picked were of the genotype *lin-8(n111)*; *lin-34(n1046) unc-22*/++; *sup-7 dpy-7*/++. From the progeny of these hermaphrodites, three isolates of genotype *lin-8(n111)*; *lin-34(n1046) unc-22*; *sup-7 dpy-7* were established and were tested at 15° to confirm the presence of *sup-7*. [*sup-7* results in sterility at 15° (WATERSTON 1981).]

To obtain hermaphrodites of genotype *lin-9(n942) unc-32*; *lin-34(n1046) unc-22*; *sup-7 dpy-7*, Dpy non-Unc hermaphrodites were picked from the progeny of the hermaphrodites of genotype *lin-9(n942) unc-32*/++; *lin-34(n1046) unc-22*/++; *sup-7 dpy-7*/++. From the four Dpy hermaphrodites that were heterozygous for both *lin-9(n942) unc-32* and *lin-34(n1046) unc-22*, the phenotypes of the progeny hermaphrodites of genotype *lin-9(n942) unc-32*; *lin-34(n1046) unc-22*; *sup-7 dpy-7* were examined.

***lin-9* non-complementation screen:** To obtain mutations that failed to complement the silent Muv mutation *lin-9(n112)*, L4 males of genotype *lin-8(n111)* were mutagenized with EMS and mated at 20° with L4 hermaphrodites of genotype *lin-8(n111)*; *dpy-19 lin-9(n112)*; *unc-3*. The parents from each mating (six hermaphrodites and six males) were transferred to a new plate every day, and the F_1 progeny of the mating were examined for the presence of Muv non-Dpy non-Unc hermaphrodites, which were candidates for carrying a mutation that failed to complement *lin-9(n112)*. However, such Muv hermaphrodites could also carry either of two additional classes of mutations. First, such hermaphrodites could carry a mutation that resulted in a dominant Muv phenotype. Second, as hermaphrodites of genotype *lin-8(n111)*; *lin-9(n112)*/+; *b*/+ (*b* is a recessive silent Muv mutation in certain other genes) can sometimes be Muv (see RESULTS), such hermaphrodites could also carry a silent Muv mutation in a gene other than *lin-9*. Approximately 10,000 cross progeny were examined, and five Muv hermaphrodites were isolated. Three Muv hermaphrodites carried new alleles of *lin-9*. Two alleles, *n942* and *n943*, result in sterility as homozygotes and are maintained in balanced strains of genotypes *lin-8*; *lin-9*/*unc* and *lin-9*/*unc*, where *unc* is either *unc-32* or *unc-36*. The other allele, which was subsequently lost, resulted in a viable phenotype as a homozygote and interacted with *lin-8(n111)* to generate a Muv phenotype. The other two Muv hermaphrodites car-

ried silent Muv mutations that were not alleles of *lin-9*. One of these mutations, *n1138*, is linked to *unc-3 X* and may be an allele of *lin-15*; the other mutation, *n1137*, has not been characterized further.

RESULTS

Identification of additional Muv strains that have phenotypes dependent on two mutations: The phenotype of the Muv strain CB1322 is synthetic, as it depends on the presence of two mutations, *lin-8(n111) II* and *lin-9(n112) III*; hermaphrodites homozygous for either of these two mutations have wild-type vulval cell lineages (SULSTON and HORVITZ 1981; FERGUSON, STERNBERG and HORVITZ 1987). We sought to identify other mutations that would interact with either *lin-8(n111)* or *lin-9(n112)* to generate a Muv phenotype. As described in MATERIALS AND METHODS, we mutagenized hermaphrodites homozygous for either *lin-8(n111)* or *lin-9(n112)*, obtaining twelve Muv strains from the mutagenesis of *lin-8(n111)* hermaphrodites and five Muv strains from the mutagenesis of *lin-9(n112)* hermaphrodites. For 12 of these 17 strains, the Muv phenotype displayed linkage both to the linkage group of the mutation present in the parental strain, *lin-8(n111)* or *lin-9(n112)*, and to a second linkage group, indicating that the Muv phenotype of the strain was caused by two unlinked mutations, one of which was the mutation in the parental strain (Table 1). The remaining five Muv strains contained mutations that resulted in a Muv phenotype whether or not the mutation in the parental strain was present (see MATERIALS AND METHODS).

In addition, as described in MATERIALS AND METHODS, five other Muv strains were fortuitously isolated after mutagenesis of the strain MT1312. As the Muv phenotype of each of these strains displayed linkage both to the *X* chromosome and to a marker on another chromosome (Table 1), it is likely that a single *X*-linked mutation, which we call *n767*, was present in the strain MT1312 prior to the mutagenesis.

In the rest of this paper, we refer to the mutations in these Muv strains as "silent Muv" mutations, and the Muv phenotype that results from the presence of two silent Muv mutations as a "synthetic Muv" phenotype.

Complementation and mapping: Two silent Muv mutations, *a1* and *a2*, that mapped to the same chromosome were considered to be allelic if and only if hermaphrodites of genotype *a1/a2*; *b* (where *b* is a third silent Muv mutation with which both *a1* and *a2* interact to give a Muv phenotype) were Muv and produced all Muv progeny. It proved necessary to examine the progeny of Muv hermaphrodites of putative genotype *a1/a2*; *b* for two reasons: (1) at elevated temperatures (22.5° or 25°) certain of these mutations display partially dominant phenotypes, *i.e.*, some hermaphrodites of genotype *a1/+*; *b* are Muv; and (2) certain pairs of nonallelic recessive mutations partially fail to complement, *i.e.*, some hermaphrodites of genotype *a1/+*; *a2/+*; *b* display a Muv phenotype of reduced expressivity compared to the Muv phenotypes of the strains *a1*; *b* and *a2*; *b* (E. FERGUSON, unpublished observations). Using the criteria described above, it was possible to assign each silent Muv mutation unambiguously to a single complementation group.

As described below, six silent Muv mutations were shown to be alleles of *lin-15*, a gene previously defined by five visible Muv mutations (FERGUSON and HORVITZ 1985). Nine other silent Muv mutations defined four new genes involved in vulval development—*lin-35*, *lin-36*, *lin-37* and *lin-38*. Multiple-factor crosses (Table 2) were performed to position each of these four genes on its linkage group (Figure 1). Three additional silent Muv mutations (*n770*, *n771* and *n833*) were each mapped to a linkage group and shown to complement all previously characterized silent Muv mutations on that linkage group. However, we have not positioned the genes defined by these three mutations on their linkage groups, and since these mutations could be alleles of previously defined genes, they have not been assigned separate gene names.

General characteristics of the newly isolated Muv strains: The Muv strains share two characteristics. First, some aspect of the phenotype of each strain is heat sensitive (Table 3). (The one strain that does not display an obvious heat-sensitive phenotype, *lin(n833)*; *lin-15(n767)*, has a heat-sensitive maternal effect phenotype; see below.) At 15°, most strains display incompletely penetrant Muv phenotypes; at 20°, most strains have a completely penetrant Muv phenotype; and at 25°, all strains have a completely penetrant Muv phenotype (Table 3 and legend). In addition, most Muv strains display a heat-sensitive decrease in viability. Such Muv strains display three common phenotypes when grown at high temperature: a generation time that is longer than that of the wild type; a decrease in the size of the adult hermaphrodites compared to the wild type; and a high incidence of sterility. This heat-sensitivity most likely reflects a heat-sensitive process revealed or induced by a reduction or loss of function of the silent Muv genes. (Similar genetic analyses indicate that certain other *C. elegans* processes are also heat sensitive; GOLDEN and RIDDLE 1984; FIXSEN 1985.)

The second general characteristic of the Muv strains is that the Muv phenotypes of most strains display a maternal effect; *i.e.*, the penetrance of the Muv phenotype in hermaphrodites of genotype *a*; *b* that are the progeny of heterozygous hermaphrodites of genotype *a/+*; *b/+* is lower than the penetrance of the

TABLE 1

Origins and chromosomal linkages of silent Muv strains

Parental mutation	Strain	New mutation	Genotype of F_2 Muv hermaphrodites with respect to the chromosomal marker linked to the: Parental mutation	New mutation
lin-8(n111) II				
	MT664	*lin-15(n374) X*	4/22 *bli-2 II/+*	5/17 *lon-2 X/+*
	MT1622	*lin-15(n743) X*	1/11 *bli-2 II/+*	0/13 *unc-3 X/+*
	MT1623	*lin-15(n744) X*	1/10 *bli-2 II/+*	0/19 *unc-3 X/+*
	MT663	*lin-35(n373) I*	0/7 *bli-2 II/+*	1/7 *dpy-5 I/+*
	MT1624	*lin-35(n745) I*	2/12 *bli-2 II/+*	0/12 *dpy-5 I/+*
	MT1626	*lin-36(n747) III*	1/16 *bli-2 II/+*	0/11 *unc-32 III/+*
	MT1629	*lin-36(n750) III*	1/12 *bli-2 II/+*	0/14 *unc-32 III/+*
	MT1635	*lin-37(n758) III*	1/10 *bli-2 II/+*	0/10 *unc-32 III/+*
lin-9(n112) III				
	MT990	*lin-15(n433) X*	0/14 *unc-32 III/+*	6/17 *lon-2 X/+*
	MT1628	*lin-15(n749) X*	0/10 *unc-32 III/+*	7/17 *lon-2 X/+*
	MT1630	*lin-38(n751) II*	0/15 *unc-32 III/+*	0/11 *mnC1 II/+*
	MT1638	*lin-38(n761) II*	0/24 *unc-32 III/+*	0/11 *mnC1 II/+*
lin-15(n767) X				
	MT1643	*lin-36(n766) III*	1/35 *unc-3 X/+*	1/14 *unc-32 III/+*
	MT1646	*lin-36(n772) III*	0/16 *unc-3 X/+*	0/12 *unc-32 III/+*
	MT1648	*lin(n770) III*	2/22 *unc-3 X/+*	2/12 *unc-32 III/+*
	MT1645	*lin(n771) III*	10/24 *lon-2 X/+*	1/28 *unc-32 III/+*
	MT1753	*lin(n833) I*	0/8 *unc-3 X/+*	2/10 *dpy-5 I/+*

The mutations responsible for the Muv phenotypes of each strain were mapped to linkage groups by mating males carrying these mutations with hermaphrodites of genotypes *dpy-5 I; bli-2 II; unc-32 III* or *unc-5 IV; dpy-11 V; lon-2 X* (TRENT, TSUNG and HORVITZ 1983), picking Muv animals from the F_2 progeny, and noting the frequency of segregation of each of the chromosomal markers. If the chromosomal marker is linked to the mutation(s) responsible for the Muv phenotype, 2p of the Muv hermaphrodites should segregate the marker (where p is the recombination frequency between the marker and the Muv mutation). Conversely, if the chromosomal marker is unlinked to the mutation(s) responsible for the Muv phenotype, 2/3 of the Muv hermaphrodites should segregate the marker. Only those markers listed displayed linkage to the mutations in the Muv strains. Mutations in two genes—*lin-38 II* and *lin-15 X*—did not display tight linkage to any of the above six markers. Using the protocol described above, some *lin-15* mutations were confirmed to map to LGX by establishing linkage to *unc-3 X*. Similarly, mutations in *lin-38* were confirmed to map to LGII by establishing linkage to *mnC1 dpy-10 unc-52*.

Muv phenotype in hermaphrodites of genotype *a; b* that are the progeny of *a; b* homozygous parents. This maternal effect was observed during the initial characterization of many of these strains, and subsequently was quantified for eight strains (Table 4). For the three strains for which it was tested, the degree of maternal rescue was also temperature-sensitive. For example, although the strain *lin-8(n111); lin-37(n758)* displays a completely penetrant Muv phenotype at both 15° and 20°, the penetrance of the Muv phenotype of *lin-8; lin-37* animals derived from heterozygous parents is higher at 20°, 44% Muv, than at 15°, 0% Muv.

We have not determined whether these maternal effects are the result of a synthetic interaction between the two silent mutations in the strain or whether this characteristic results from one of the two mutations in the strain. However, there are some results that suggest that the heat-sensitive decrease in viability observed in the synthetic Muv strains may not be caused by an interaction between the two mutations in the strain, as some silent Muv mutations have a heat-sensitive effect on viability independent of a second such mutation (see below).

Phenotypes resulting from isolated silent Muv mutations: To determine the phenotype of hermaphrodites carrying a silent Muv mutation in only one gene, we constructed six strains each homozygous for one of the mutations *lin-15(n744)*, *lin-15(n767)*, *lin-35(n745)*, *lin-36(n766)*, *lin-37(n758)*, and *lin-38(n751)* (see MATERIALS AND METHODS). At 20°, hermaphrodites of all six strains (as well as *lin-8(n111)* and *lin-9(n112)* hermaphrodites) have a wild-type phenotype as viewed with the dissecting microscope. However, at 25°, hermaphrodites of genotypes *lin-9(n112)*, *lin-15(n744)*, *lin-35(n745)*, or *lin-37(n755)* are thinner and less fertile than the wild type but are not Muv.

There are two classes of silent Muv mutations: To determine the pattern of interactions among the silent Muv mutations, we constructed a series of double mutant strains, each carrying two silent Muv mutations (see MATERIALS AND METHODS). The phenotypes of these double mutant strains (Table 5) indicate that the silent Muv mutations fall into two classes, which we have named "A" and "B." A double mutant strain carrying one class A mutation and one class B mutation is Muv, while a double mutant strain carrying two class A or two class B mutations is not Muv. (Most non-Muv strains had a wild-type phenotype at 20°;

TABLE 2

Two-factor crosses

Gene	Genotype of heterozygote	Segregants	Map distance (p)
lin-38	*lin-8*; + *lin-38 unc-52/rol-6* ++	138 wild type 76 Rol 71 Muv Unc 7 Unc 7 Muv	4.7%

Three-, four-, and five-factor crosses

Gene	Genotype of heterozygote	Phenotype of selected recombinants	Genotype of selected recombinants (with respect to unselected markers)
lin-35			
	++ *lin-35/unc-11 dpy-5* +; *lin-8*	Dpy	0/11 *lin-35*/+
	+ *lin-35* +/*dpy-5* + *unc-13*; *lin-8*	Dpy	4/11 *lin-35*/+
		Unc	10/18 *lin-35*/+
	+ *dpy-14* +/*lin-35* + *unc-13*; *lin-8*	Unc	2/5 *dpy-14*/+
		Muv	2/4 *dpy-14*/+
lin-36			
	lin-36 ++/+ *unc-36 dpy-19*; *lin-15(n767)*	Dpy	3/3 *lin-36*/+
	+ *lin-36* +/*dpy-17* + *unc-36*; *lin-15(n767)*	Dpy	8/8 *lin-36*/+
		Unc	1/11 *lin-36*/+
	+ *lin-13* ++/*lon-1* + *lin-36 unc-32*; *lin-15(n767)*	Lon	14/17 *lin-13*/+
	+ *daf-4* ++/*lon-1* + *lin-36 unc-32*; *lin-15(n767)*	Lon	2/10 *daf-4*/+
	+ *lin-36* +/*lin-13* + *unc-36*; *lin-15(n767)*	Unc	2/9 *lin-36*/+
lin-37			
	lin-8; *lin-37* ++/+ *unc-86 dpy-19*	Unc	0/7 *lin-37*/+
	lin-8; + *lin-37* + *unc-86* +/*lon-1* + *lin-36* + *unc-32*	Unc-86	2/7 *lon-1 lin-36*/++
			5/7 *lon-1*/+
		Lon	11/18 *lin-37 unc-86*/++
	lin-8; + *sma-3* + *lin-37 unc-86*/*lon-1* + *lin-16* +	Sma	2/4 *lin-16*/+
		Muv Unc-86	1/4 *lon-1 lin-16*/++
	lin-8; *lin-37* ++ *unc-86*/+ *lin-13 unc-36* +	Unc-86	6/15 *lin-13*/+
		Muv	2/5 *lin-13 unc-36*/++
			3/5 *unc-36*/+
lin-38			
	rol-1 ++/+ *lin-38 unc-52*; *lin-9*	Muv	1/11 *rol-1*/+
		WT	1/11 +++/*rol-1* ++
			2/11 +++/+ *lin-38 unc-52*

Two-, three-, four-, and five-factor crosses were performed essentially as described by BRENNER (1974). Because the silent Muv mutations result in a wild-type phenotype, these mutations were scored in the presence of a second silent Muv mutation. For example, from heterozygotes of genotype *r1* + *r2*/+ *a* +; *b* (where *r1* and *r2* are chromosomal markers, *a* is the silent Muv mutation to be mapped with respect to those markers, and *b* is a second silent Muv mutation with which *a* interacts to generate a Muv phenotype), R1 non-R2 and R2 non-R1 hermaphrodites were picked. The progeny of each recombinant hermaphrodite were examined for the presence of Muv animals. In one cross involving the mapping of *lin-38*, wild-type hermaphrodites were picked and scored for the segregation of the *cis* markers. The silent Muv genes *lin-36* and *lin-37* map adjacent to *lin-13*, which results in a sterile Muv phenotype. The segregation of *lin-13* from recombinant Muv hermaphrodites homozygous for either *lin-36* or *lin-37* was scored by determining whether progeny homozygous for the recombinant chromosome were sterile.

however, almost all animals of genotype *lin-35*; *lin-9* were sterile.) Mutations in *lin-8* and *lin-38* are class A mutations, while mutations in *lin-9*, *lin-35*, *lin-36* and *lin-37* are class B mutations; as described below, some of the *lin-15* mutations are class A mutations, while others are class B mutations.

***lin-15* is a complex locus with two independently mutable activities:** Six silent Muv mutations displayed linkage to the right arm of LGX (Table 1). Complementation tests established that the three *X*-linked class B mutations isolated after mutagenesis of *lin-8(n111)* hermaphrodites, *n744*, *n374* and *n743*, were allelic. Similarly, complementation tests established that *n767*, the class A mutation present in MT1312, was allelic with two *X*-linked class A mutations, *n433* and *n749*, isolated after mutagenesis of *lin-9(n112)* hermaphrodites.

A series of deficiencies of the right arm of LGX (MENEELY and HERMAN 1979; 1981) were used to map further *n744* and *n767* (Figure 1). *mnDf4* failed to complement both *n767* and *n744*, as hermaphrodites of genotypes *lin-8*; *n744/mnDf4* and *lin-36*; *n767/mnDf4* were Muv. Conversely, *mnDf43* complemented both mutations, as hermaphrodites of genotypes *lin-8*; *n744/mnDf43* and *lin-36*; *n767/mnDf43* had a wild-type phenotype. Thus, *n744* and *n767* both mapped

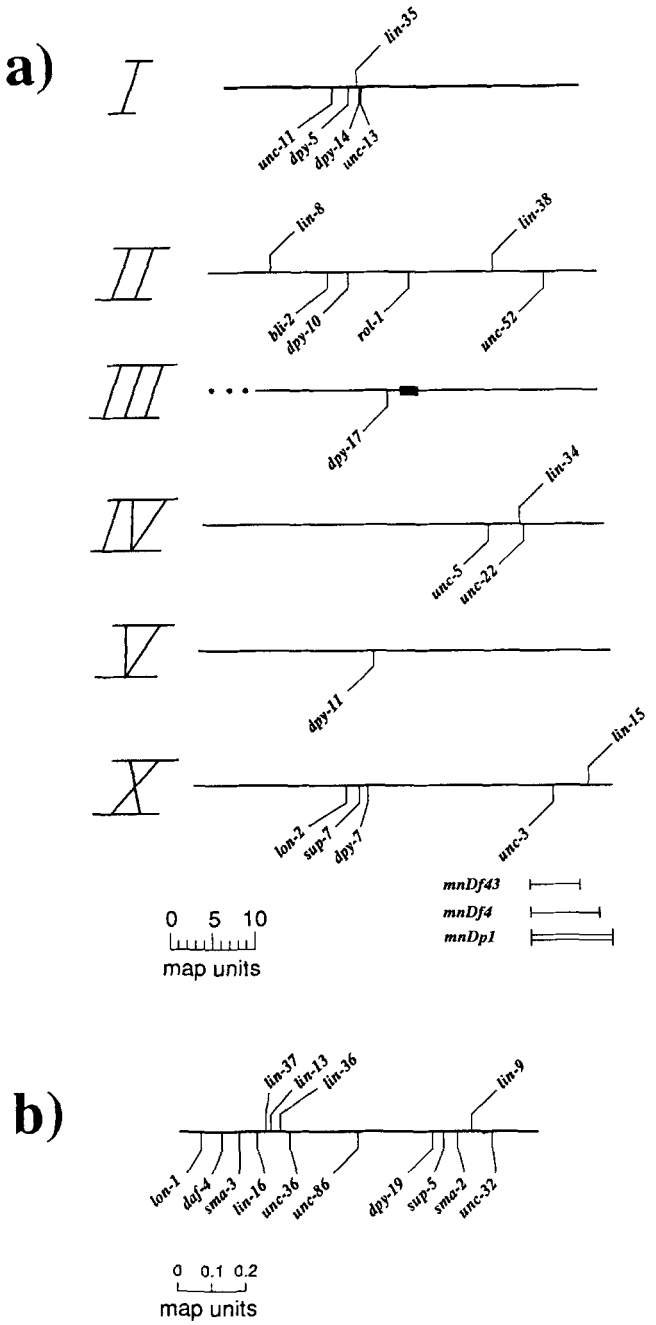

FIGURE 1.—a, Partial genetic map of *C. elegans* indicating the markers used in this study. Genes with silent Muv alleles or with alleles that confer Muv phenotypes similar to those of the synthetic Muv strains are drawn above the lines representing the *C. elegans* linkage groups. The extents of a duplication (*mnDp1*) and of two deficiencies (*mnDf4* and *mnDf43*) are indicated below these lines. The region of LGIII indicated by the rectangle is shown in more detail in b. b, Expanded genetic map showing the region of LGIII extending from *lon-1* to *unc-32*.

to the same region of LGX as *lin-15*, a gene previously defined by mutations that result in a Muv phenotype (FERGUSON and HORVITZ 1985).

Complementation tests and mapping experiments were performed to determine whether the *X*-linked class A and class B mutations were alleles of *lin-15* (Table 6). The canonical Muv allele of *lin-15*, *n309*, failed to complement the silent alleles of both classes, as hermaphrodites of genotypes *lin-8*; *n744/lin-15(n309)* and *lin-36*; *n767/lin-15(n309)* had Muv phenotypes. In addition, S. KIM (personal communication) mapped *n744* about 0.03 map unit left of *lin-15(n309)* and mapped *n767* to within 0.09 map unit of *lin-15(n309)* (see MATERIALS AND METHODS). These map distances are similar to intragenic distances reported for other *C. elegans* genes (MOERMAN and BAILLIE 1979; ROSE and BAILLIE 1980; WATERSTON, SMITH and MOERMAN 1982; ROGALSKI and BAILLIE 1985; BULLERJAHN and RIDDLE 1988). Taken together, these results lead us to conclude that *n767* and *n744* are alleles of *lin-15*.

The two classes of *lin-15* silent Muv mutations complement, as hermaphrodites of genotypes *lin-8*; *lin-15(n744)/lin-15(n767)* and *lin-36*; *lin-15(n744)/lin-15(n767)* have a wild-type phenotype (Table 6). Thus, these experiments indicate that *lin-15* is a complex locus with two activities, which can be mutated either independently or coordinately, and that mutations that affect one *lin-15* activity complement mutations that affect the second activity.

One of the Muv alleles of *lin-15*, *n765*, results in a heat-sensitive Muv phenotype (FERGUSON and HORVITZ 1985). To determine whether *lin-15(n765)* restores both class A and class B aspects of *lin-15* activity at 15°, a series of double mutants was constructed between *lin-15(n765)* and various class A and class B mutations. At 15°, the double mutants containing *lin-15(n765)* and a class A allele—*lin-8(n111)* or *lin-38(n751)*—were Muv, while the double mutants containing *lin-15(n765)* and a class B allele—*lin-9(n112)*, *lin-35(n745)* or *lin-36(n766)*—had a wild-type phenotype. These observations indicate that at 15°, *lin-15(n765)* results in a class B phenotype and that *lin-15(n765)* is temperature-sensitive for class A activity.

All other visible Muv mutants that have a phenotype similar to *lin-8*; *lin-9* hermaphrodites also have defects in both class A and class B functions: Hermaphrodites carrying either the recessive mutation *lin-13(n387)* or the partially dominant mutation *lin-34(n1046)* have a Muv phenotype that reflects abnormalities in the vulval cell lineages that are similar to those of *lin-8*; *lin-9* hermaphrodites (FERGUSON, STERNBERG and HORVITZ 1987). However, under certain conditions, both *lin-13(n387)* and *lin-34(n1046)* result in nearly wild-type phenotypes (FERGUSON and HORVITZ 1985). As we describe below, under these conditions both mutations result in a class B silent Muv phenotype (Table 7). Thus, the behavior of the *lin-13* and the *lin-34* mutations is similar to that of *lin-15(n765ts)*, which suggests that the Muv phenotypes of these two mutations may be the result of alterations in both class A and class B activities. As

TABLE 3

Temperature-sensitivity of silent Muv strains

Genotype	Penetrance of Muv phenotype		25° Viability of strain
	15°	20°	
lin-8(n111); lin-9(n112)	100% (*n* = 225)	100% (*n* = 165)	Slow growing
lin-8(n111); lin-15(n374)	3% (*n* = 176)	100% (*n* = 213)	wt
lin8(n111); lin-15(n743)	100% (*n* = 198)	100% (*n* = 191)	Lethal
lin-8(n111); lin-15(n744)	100% (*n* = 201)	100% (*n* = 240)	Lethal
lin-35(n373); lin-8(n111)	100% (*n* = 139)	100% (*n* = 153)	Lethal
lin-35(n745); lin-8(n111)	100% (*n* = 201)	100% (*n* = 209)	Lethal
lin-8(n111); lin-36(n747)	7% (*n* = 223)	98% (*n* = 177)	wt
lin-8(n111); lin-36(n750)	0% (*n* = 231)	75% (*n* = 357)	wt
lin-8(n111); lin-36(n766)	9% (*n* = 284)	98% (*n* = 207)	wt
lin-8(n111); lin-37(n758)	100% (*n* = 227)	100% (*n* = 161)	Lethal
lin-9(n112); lin-15(n433)	3% (*n* = 282)	100% (*n* = 214)	Slow growing
lin-9(n112); lin-15(n749)	5% (*n* = 155)	98% (*n* = 278)	Lethal
lin-38(n751); lin-9(n112)	100% (*n* = 167)	100% (*n* = 165)	Lethal
lin-38(n761); lin-9(n112)	3% (*n* = 224)	50% (*n* = 357)	Slow growing
lin-36(n766); lin-15(n767)	32% (*n* = 302)	100% (*n* = 226)	wt
lin-36(n772); lin-15(n767)	4% (*n* = 296)	99% (*n* = 369)	wt
lin(n770); lin-15(n767)	58% (*n* = 243)	95% (*n* = 230)	Slow growing
lin(n771); lin-15(n767)	94% (*n* = 278)	100% (*n* = 211)	wt
lin(n833); lin-15(n767)	100% (*n* = 165)	100% (*n* = 116)	wt

The penetrance of the Muv phenotype in each strain was determined after growing that strain at the indicated temperature for at least two generations. At 25°, all animals from all strains were Muv, and many strains were less viable than the wild type. To quantitate this reduction in viability, we measured the amount of time in which a strain consumed a given amount of bacteria. Two fertile hermaphrodites of each strain were grown at 20° and allowed to lay eggs for four hours on each of four plates. Each plate contained a bacterial lawn of roughly equivalent size (approximately 2 cm in diameter) and density. The plates were put at 25° and were checked at intervals to determine when all the bacteria on the plate had been consumed. Wild type, the strain consumed the bacterial lawn as rapidly as the wild type (5 days); slow growing, the strain consumed the bacterial lawn in 10–20 days; lethal, the strain did not grow at 25° or took at least 30 days to consume the bacterial lawn.

lin-13 mutations cause reduction of gene function, it is likely that the wild-type *lin-13* gene product has both class A and class B activity; however, because the *lin-34* mutation is semidominant, we cannot be certain whether the wild-type product of *lin-34* normally has either activity.

Mutations in *lin-13* result in a heat-sensitive Muv phenotype (FERGUSON and HORVITZ 1985): at 25° *lin-13* hermaphrodites that segregate from a heterozygous parent are sterile and Muv, whereas at 15° such *lin-13* hermaphrodites are phenotypically wild type but generate progeny that are sterile and not Muv. We constructed a double mutant between *lin-13(n387)* and a class A *lin-15* allele, *n767*. At 15°, the double mutant hermaphrodites of genotype *unc-36 lin-13(n387); lin-15(n767)* that were the progeny of hermaphrodites of genotype *unc-36 lin-13(n387)* +/ ++ *unc-32; lin-15(n767)* were fertile and Muv, demonstrating that at 15° *lin-13(n387)* behaves as a class B silent Muv mutation.

The mutation *lin-34(n1046)* causes a partially dominant, incompletely penetrant Muv phenotype. The penetrance of the Muv phenotype of *lin-34* is reduced from 56% to 13% by the amber suppressor tRNA mutation *sup-7* (WATERSTON 1981; WILLS *et al.* 1983), indicating that *n1046* is an amber mutation (FERGUSON and HORVITZ 1985). To determine whether the *lin-34; sup-7* double mutant could have defects in either class A or class B function, we constructed triple mutants carrying *lin-34, sup-7* and either a class A or a class B silent Muv mutation (see MATERIALS AND METHODS). At 20°, the penetrance of the Muv phenotype in hermaphrodites of genotype *lin-8(n111); lin-34; sup-7* (59%, *n* = 224) was greater than the penetrance of the Muv phenotype in *lin-34; sup-7* hermaphrodites (13%). In contrast, the penetrance of the Muv phenotype in hermaphrodites of genotype *lin-9(n942); lin-34(n1046); sup-7* was low (16%, *n* = 25). Because the penetrance of the Muv defect of the *lin-34; sup-7* hermaphrodites was increased in the presence of a class A mutation, but not in the presence of a class B mutation, hermaphrodites of genotype *lin-34; sup-7* are preferentially defective in class B function.

The null phenotype of *lin-9*: To determine the phenotype that results from lack of *lin-9* activity, we performed a screen, described in MATERIALS AND METHODS, to identify other *lin-9* mutations that failed to complement the silent Muv phenotype of *lin-9(n112)*. Two of the three newly induced alleles of *lin-9, n942* and *n943*, resulted in sterility and interacted with *lin-8(n111)* to generate a sterile Muv phe-

TABLE 4

Rescue of the Muv phenotype by maternal activity

Progeny genotype	Parental genotype	Penetrance of the Muv phenotype 15°	Penetrance of the Muv phenotype 20°
lin-8(n111); lin-9(n112)	Homozygous		100% (n = 165)
	Heterozygous		22% (n = 123)
lin-35(n745); lin-8(n111)	Homozygous	100% (n = 201)	
	Heterozygous	0% (n = 82)	
lin-8(n111); lin-36(n766)	Homozygous		98% (n = 207)
	Heterozygous		13% (n = 112)
lin-8(n111); lin-37(n758)	Homozygous	100% (n = 227)	100% (n = 161)
	Heterozygous	0% (n = 56)	44% (n = 57)
lin-38(n751); lin-9(n112)	Homozygous	100% (n = 167)	
	Heterozygous	20% (n = 71)	
lin-36(n766); lin-15(n767)	Homozygous	32% (n = 302)	100% (n = 226)
	Heterozygous	3% (n = 78)	77% (n = 74)
lin-38(n751); lin-36(n766)	Homozygous		98% (n = 300)
	Heterozygous		41% (n = 89)
lin(n833); lin-15(n767)	Homozygous	100% (n = 165)	100% (n = 116)
	Heterozygous	0% (n = 86)	100% (n = 16)

To quantitate the degree of maternal rescue of the Muv phenotype, hermaphrodites of genotype *a*/+; *b r*/++ (*a* and *b* are silent Muv mutations and *r* is a recessive marker closely linked to *b*) were transferred daily to separate plates. The penetrance of the Muv phenotype in progeny of genotype *a*; *br* was determined by multiplying the percentage of phenotypically R progeny that were Muv by four. *dpy-17* was used to mark *lin-8*; *lin-9* segregants. *unc-13* was used to mark *lin-37*; *lin-8* segregants. *unc-32* was used to mark *lin-8*; *lin-36* segregants. *lon-1* was used to mark *lin-8*; *lin-37* segregants. *rol-1 unc-52* was used to mark *lin-38*; *lin-9* segregants. *unc-84 unc-3* was used to mark *lin(n833)*; *lin-15* segregants. *lon-1 unc-32* was used to mark *lin-36*; *lin-15* segregants. *unc-32* was used to mark *lin-38*; *lin-36* segregants. The penetrance of the Muv phenotypes in hermaphrodites that were the progeny of homozygous *a*; *b* parents was measured in strains without *cis* markers. n, for the progeny of homozygous parents, n equals the number of animals observed; for the progeny of heterozygous parents, n is estimated as 1/4 of the number of phenotypically R progeny.

TABLE 5

Phenotypes of double mutants carrying two silent Muv mutations

		Class A		Class B				
		lin-38 (n751)	*lin-15 (n767)*	*lin-9 (n112)*	*lin-35 (n745)*	*lin-36 (n766)*	*lin-37 (n758)*	*lin-15 (n744)*
Class A	*lin-8(n111)*	WT	WT	Muv	Muv	Muv	Muv	Muv
	lin-38(n751)		WT	Muv	Muv	Muv	Muv	Muv
	lin-15(n767)			Muv	Muv	Muv	Muv	ND
Class B	*lin-9(n112)*				ST	ND	ND	WT
	lin-35(n745)					WT	WT	WT
	lin-36(n766)						ND	WT
	lin-37(n758)							WT

Double mutants were constructed as described in MATERIALS AND METHODS. On the basis of the phenotypes of the double mutant strains at 20°, these mutations are of two classes, A and B. WT, phenotypically wild type; Muv, multivulva; ST, sterile not multivulva; ND, not determined because of close linkage between the two mutations.

notype. [The remaining allele, which was subsequently lost, had a phenotype similar to that of *lin-9(n112)*.] As the mutations that cause both the silent Muv phenotype and the sterility of the *lin-9(n942)* and *lin-9(n943)* strains fail to complement, it is likely that both defects (sterility and the class B phenotype) are caused by mutations in *lin-9*. The frequency with which these three EMS-induced *lin-9* mutations were obtained, 3.3×10^{-4} per mutagenized F_1 haploid genome, is comparable to the frequency, 5×10^{-4} per mutagenized F_1 haploid genome, with which EMS induces mutations that eliminate the activity of an average gene (BRENNER 1974; GREENWALD and HORVITZ 1980; MENEELY and HERMAN 1981), suggesting that the two severe *lin-9* mutations may result in the loss of *lin-9* activity. However, as no deficiencies of the *lin-9* region exist, we do not know whether hermaphrodites of genotype *lin-9(n112)/Df* are viable, and thus we cannot be confident that null mutations of *lin-9* would be recovered using our protocol.

TABLE 6

Interactions among *lin-15* alleles

Genotype	Phenotype	Genotype	Phenotype
a; lin-15(b)/+	WT	*b; lin-15(a)/+*	WT
a; lin-15(Muv)/+	WT	*b; lin-15(Muv)/+*	WT
a; lin-15(b)/lin-15(b)	Muv	*b; lin-15(a)/lin-15(a)*	Muv
a; lin-15(b)/lin-15(Muv)	Muv	*b; lin-15(a)/lin-15(Muv)*	Muv
a; lin-15(b)/lin-15(a)	WT	*b; lin-15(a)/lin-15(b)*	WT

Hermaphrodites homozygous for a silent Muv mutation of either class and heterozygous for different *lin-15* alleles.

a, the class A silent mutation *lin-8(n111)*; *b*, the class B silent mutation *lin-36(n766)*; *lin-15(Muv)*, *lin-15(n309)*; *lin-15(a)*, *lin-15(n767)*; *lin-15(b)*, *lin-15(n744)*. WT, wild-type. Animals of the different genotypes were obtained as described below. *lin-8(n111); lin-15(n744)/+*: Males of genotype in *lin-8(n111)* were mated at 25° with hermaphrodites of genotype *lin-8(n111); unc-32; lin-15(n744). lin-8(n111); lin-15(n309)/+*: Males of genotype *lin-8(n111)* were mated at 25° with hermaphrodites of genotype *dpy-10 lin-8(n111); unc-3 lin-15(n309). lin-8(n111); lin-15(n744)/lin-15(n309)*: Males of genotype *lin-8(n111); lin-15(n744)/0* were mated at 25° with hermaphrodites of genotype *lin-8(n111) dpy-10; unc-3 lin-15(n309). lin-8(n111); lin-15(n744)/lin-15(n767)*: Males of genotype *lin-8(n111) +/lin-8(n111) dpy-10; lin-15(n767)/0* were mated at 25° with hermaphrodites of genotype *lin-8(n111); unc-32; lin-15(n744). lin-36(n766); lin-15(n767)/+*: Males of genotype *lin-36(n766); him-5* were mated at 25° with hermaphrodites of genotype *lin-36(n766) dpy-19; lin-15(n767). lin-36(n766); lin-15(n309)/+*: Males of genotype *lin-36(n*766*); him-5* were mated at 20° with hermaphrodites of genotype *lon-1 lin-36(n766) unc-32; unc-3 lin-15(n309). lin-36(n766); lin-15(n767)/lin-15(n309)*: Males of genotype *lin-36(n766); him-5/+; lin-15(n767)/0* were mated at 20° with hermaphrodites of genotype *lon-1 lin-36(n766) unc-32; unc-3 lin-15(n309). lin-36(n766); lin-15(n767)/lin-15(n744)*: Males of genotype *lin-36(n766); him-5/+; lin-15(n767)/0* were mated at 20° with hermaphrodites of genotype *lin-36(n766) unc-32; lin-15(n744)*.

DISCUSSION

In our effort to understand the complete genetic hierarchy that promotes vulval development, we investigated the basis for the synthetic nature of the Muv phenotype of the strain CB1322 *lin-8(n111); lin-9(n112)*. We obtained 18 additional mutations that alone result in a wild-type vulval phenotype but interact with either *lin-8* or *lin-9* to generate a Muv phenotype. Twelve of these "silent Muv" mutations were obtained by mutagenizing hermaphrodites homozygous for either *lin-8* or *lin-9*; the remaining mutations were isolated by mutagenizing a strain that carried the silent Muv mutation *lin-15(n767)*. We examined the pattern of interactions among the silent Muv mutations by constructing double mutants. Our analysis indicated that these mutations fall into two classes, A and B. Strains containing a mutation of each class are Muv, while strains containing two mutations of the same class have a wild-type vulval phenotype. Two genes—*lin-8* and *lin-38*—are defined by class A mutations, four genes—*lin-9*, *lin-35*, *lin-36* and *lin-37*—are defined by class B mutations, and one gene—*lin-15*—has both class A and class B mutations. In addition, we demonstrated that the visible Muv mutants defective in the genes *lin-13*, *lin-15* or *lin-34*, which have phenotypes similar to those of *lin-8*; *lin-9* hermaphrodites, also have defects in both class A and class B activity.

Our findings suggest that the phenotypes of this group of Muv mutants are caused by defects in two functionally redundant sets of genes, each of which has multiple nonredundant components. Each such set could either encode products that act in a sequential pathway or encode products that form a multi protein complex. The activity of one pathway (or complex) is disrupted by class A mutations, while the activity of the second pathway is disrupted by class B mutations. The pathways are functionally redundant because the activities of both pathways must be disrupted for a Muv phenotype to be manifest. The activity of each gene within the two pathways is unique because in the background of a class A or class B mutation the presence of any mutation of the opposite

TABLE 7

Genes with silent Muv alleles

Gene	No. of alleles	Classes of alleles		
		Class A silent	Multivulva	Class B silent
lin-8 II	1	*n111*		
lin-38 II	2	*n751, n761*		
lin-9 III	3			*n112, n942, n943*
lin-35 I	2			*n745, n373*
lin-36 III	4			*n766, n772, n747, n750*
lin-37 III	1			*n758*
lin-15 X	11	*n767, n433, n749*	*n309, n765*ts 25°, *e1763, n377, n1139*	*n744, n765*ts 15°, *n743, n374*
lin-13 III	2		*n387*ts 25°, *n388*ts 25°	*n387*ts 15°
lin-34 IV	1		*n1046*	*n1046; sup-7*

Mutations in each gene result in one of three phenotypes: a defect in class A activity with no resulting Muv phenotype (Class A silent), a defect in class B activity with no resulting Muv phenotype (Class B silent); or a defect in class A and class B activity with a resulting Muv phenotype (Muv). The phenotypes resulting from the three mutations, *lin-13(n387)*, *lin-15(n765)*, and *lin-34(n1046)*, can differ depending on temperature or genetic background (see RESULTS for details). ts, temperature sensitive.

class will result in a Muv phenotype. We cannot determine whether these pathways specify a similar function (*e.g.*, alternate biochemical pathways leading to the production of a single end product) or specify different functions, the presence of either one of which is sufficient to generate a wild-type phenotype (*e.g.*, two intracellular signaling systems that have different second messengers).

These observations extend the concept of genetic redundancy from redundancy at the level of gene families to redundancy at the level of gene pathways. Previous reports have demonstrated that for certain gene families mutant phenotypes can be generated by null mutations only when all members of the gene family have been mutated. For example, strains carrying a null mutation in either of the two yeast histone H2B genes are viable, while the elimination of the activities of both genes is lethal (RYKOWSKI *et al.* 1981). Similarly, the elimination of the activities of either of the two members of the family of yeast *ras* genes results in viability, while the double mutant is lethal (TATCHELL *et al.* 1984; KATAOKA *et al.* 1984). This report demonstrates that although the activity of each gene in the class A and class B pathways is unique, the pathways specified by these two sets of genes are functionally redundant. Because genes that function in redundant pathways are not likely to mutate to generate a visible phenotype, such genes, and by inference, the existence of such pathways, are not likely to be identified in genetic screens that saturate for mutations of a given phenotype.

Because we do not know the null phenotypes of any of the genes specified by these mutations, we cannot unambiguously define which genes are active in both pathways and whether some genes are specific to a single pathway. Two genes, *lin-13* and *lin-15*, are likely to act in both pathways. The cell lineage abnormalities associated with reduction-of-function Muv alleles in these genes are similar to those observed in Muv hermaphrodites of genotype *lin-8*; *lin-9*. In this paper, we demonstrate that the temperature-sensitive Muv phenotype of *lin-13(n387)* results from a temperature-sensitive defect in the class A pathway and a non-temperature-sensitive defect in the class B pathway, indicating that the *lin-13(n387)* mutant is defective in both pathways. For *lin-15*, we show that the Muv alleles of *lin-15* fail to complement both the class A and class B alleles of the gene, demonstrating directly that the *lin-15* Muv mutants are defective in both pathways. The *lin-15* class A and class B alleles fully complement, which indicates that each of the *lin-15* activities is separately mutable; perhaps *lin-15* encodes multiple protein products, *e.g.*, by alternative splicing of the *lin-15* mRNA.

It is likely that some of the six genes defined solely by class A or class B mutations function in only one of the two pathways. We have the most information about *lin-9*. We attempted to determine the null phenotype of this gene by identifying new mutations that failed to complement the silent Muv phenotype of the class B allele *lin-9(n112)*. Two of the three *lin-9* alleles we obtained conferred a sterile, class B phenotype, while the third allele conferred a fertile class B phenotype. The fact that we obtained three additional *lin-9* mutations that resulted in a class B phenotype but did not obtain any *lin-9* alleles that alone resulted in a Muv phenotype, suggests (but does not prove; see RESULTS) that *lin-9* encodes an activity specific to the class B pathway. A second argument suggests that the remaining five genes—*lin-8, lin-35, lin-36, lin-37* and *lin-38*—may encode activities specific to only one of the two pathways. If the product of any of these genes functioned in both pathways, and if the gene, like *lin-15*, could mutate to a Muv phenotype without resultant lethality, it is likely that we would have isolated Muv alleles of at least some of these genes during our previous screen for vulval cell lineage mutations (FERGUSON and HORVITZ 1985). In that screen, we isolated 95 mutants, defining 22 genes, with either a Muv or Vul phenotype. We isolated multiple alleles of all the genes with activities that appear to be specific to these lineages, as well as multiple alleles of many genes with null phenotypes that are likely to be lethal (including five alleles of *lin-15* and two alleles of *lin-13*). Thus, our data suggest that we have identified most genes for which either reduction or loss of gene activity results in a Muv or Vul phenotype. Our failure to identify Muv mutants defective in any of the five genes above suggests that these genes are not able to mutate to a Muv phenotype, either because the genes function in only one of the two pathways or because they function in both pathways but cannot mutate to a Muv phenotype without resultant lethality.

Although the class A and class B pathways are functionally redundant in the vulval tissues, mutations in the class B pathway can affect viability and/or fertility in the presence of a functional class A pathway, suggesting that the genes encoding the class B pathway may have a unique function in certain nonvulval tissues. Specifically, in a noncomplementation screen, we isolated new alleles of *lin-9* that confer a sterile phenotype. In addition, strains carrying any of three class B mutations—*lin-9(n112)*, *lin-35(n745)* and *lin-37(n758)*—are less fertile and have a smaller body size than the wild type at 25°. Last, a double mutant carrying the two class B mutations *lin-9(n112)* and *lin-35(n745)* is sterile. In contrast, class A mutations do not have any effect on viability or fertility even at high temperatures or in any pairwise combinations. However, because we do not know the null phenotypes of any of the genes specified by class A mutations, we cannot determine whether the genes in

the class A pathway function in nonvulval tissues.

In earlier studies we have shown that the set of genes identified by the silent Muv mutations is likely to function within the vulval precursor cells in mediating the response to the intercellular signal that induces vulval development (FERGUSON, STERNBERG and HORVITZ 1987). This signal causes the six tripotent vulval precursor cells to adopt one of three fates in a position-dependent manner. The three precursor cells nearest the signalling cell adopt one of two vulval fates, and the remaining three cells adopt a nonvulval fate. In these Muv mutants, however, all six cells adopt vulval fates in the absence of the inducing signal. Thus, since the silent Muv mutations appear to result in reduction or loss of gene function, the genes that encode the components of the class A and class B pathways act to promote the nonvulval fate. Since mutations in both pathways cause vulval precursor cells to adopt vulval fates, it is possible that the extracellular signal induces vulval development by inactivating these pathways. Perhaps the signal acts by negatively regulating a gene or genes that, like *lin-13* and *lin-15*, function in both pathways. A molecular analysis of these genes and their products should help reveal why these pathways are functionally redundant and how these genes act to control vulval cell fates.

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