THE GENETIC ANALYSIS OF THE VULVAL CELL
LINEAGES OF CAENORHABDITIS ELEGANS

by

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To my parents

ABSTRACT

The cell lineages of the nematode Caenorhabditis elegans are essentially invariant among individuals. We have begun an attempt to understand the genetic specification of one particular cell lineage, that of the hermaphrodite vulva. During vulval development, six equipotential cells of the ventral hypodermis respond to an inductive signal from the gonadal anchor cell to adopt one of three fates dependent upon position. Thus, the vulva is a model system to study the processes of induction and pattern formation at the level of individual cells.

We have isolated over 100 mutants, defining 26 genes, that are abnormal in the vulval cell lineages. These mutants have one of two phenotypes: Vulvaless hermaphrodites lack a vulva; Multivulva hermaphrodites have supernumerary pseudovulvae. Our analysis suggests that we may have identified most, or all, genes that can mutate to a Multivulva or Vulvaless phenotype.

We have constructed a genetic pathway of vulval development. Three genes affect the production of vulval precursor cells; one gene affects the formation of the anchor cell; 15 genes are involved in the determination of the fates of the vulval precursor cells in response to the inductive signal; and three genes are necessary for the execution of the vulval cell fates.

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CHAPTER I

Introduction

This thesis describes experiments aimed at the elucidation of the pathway of genetic control of vulval development in the nematode Caenorhabditis elegans. To introduce this work, I will first briefly review aspects of the development and the genetics of C. elegans that have facilitated this analysis. I will then briefly describe the types of experiments which are available to determine a pathway of gene action. Lastly, I will summarize the development of the vulva in wild type hermaphrodites (Horvitz and Sulston, 1977; Sulston and White 1980; Kimble, 1981; Sternberg and Horvitz, 1985a).

The development of C. elegans

C. elegans, a small (1mm) free living soil nematode, has two sexes: self-fertilizing hermaphrodites and males. Hermaphrodites have five pairs of autosomes and two sex chromosomes, while males have five pairs of autosomes and a single sex chromosome. Adults have a tubular body, consisting of an external cuticle and an underlying layer of musculature, which surrounds the gonad and digestive apparatus. At 20^o, the generation time is approximately 3 1/2 days,

during which time the animal develops from an egg through four larval stages (L1 through L4) to an adult. C. elegans can be cultured in the laboratory by growing the animals on Petri plates that have been seeded with bacteria, which are consumed as food.

The small size of C. elegans allows living animals to be examined using a light microscope equipped with Nomarski differential interference contrast optics (Sulston and Horvitz, 1977). Using Nomarski optics, which permit visualization of differences in refractive index, changes in the appearance of individual nuclei during cell division, cell migration, cell death and cell differentiation can be followed in living animals. The use of these techniques have shown that the cell lineages of C. elegans are essentially invariant among individuals throughout development (Sulston and Horvitz, 1977; Deppe et al., 1978; Kimble and Hirsh, 1979, Sulston and White, 1980; Sulston et al., 1983). [Throughout this thesis, the term "cell lineage" refers to a pattern of cell division and the expression of specific fates by the descendent cells (e.g., Figure 1-1f).]

The newly hatched male and hermaphrodite are very similar (Sulston and Horvitz, 1977); the majority of differences between the two sexes occur as a result of sex-specific cell divisions that occur during, later larval development. Such differences include the expression of sex specific lineages by some ventral hypodermal cells that generate the secondary sexual structures of both sexes: the vulva of the hermaphrodite and the copulatory apparatus of the male tail.

To determine whether the invariant cell lineages of C. elegans are primarily cell autonomous, or whether cells respond to positional information during development, cell ablation experiments using a laser microbeam were performed upon animals of the wild type strain (Sulston and White, 1980; Sulston et al., 1983). The results of these experiments, although not exhaustive, suggest that the majority of cell lineages in C. elegans are cell autonomous (i.e., the fates of individual cells depend on their ancestry, not upon their position). However, certain cells, including those that generate the hermaphroditic vulva, respond to positional information to express one of a number of intrinsically determined lineages.

One of the striking features of the cell lineage of C. elegans is that many cells undergo lineages that result in similar patterns of cell division and cell fate (e.g., each of the 12 Pn.a cells undergoes a similar pattern of cell division and cell fate to generate the post-embryonic additions to the ventral nerve cord, Figure 1-1b). Repetition of cell lineage patterns by different cells is also observed in other nematode species (e.g., Panagrellus redivivus) and may result from the repeated execution of a similar developmental subprogram (Sternberg and Horvitz, 1981; 1982). The repeated cell division patterns are known as "sublineages." It is possible (and Chapter 3 provides some evidence) that the execution of a sublineage requires the expression of a common genetic program.

The genetics of C. elegans

A number of characteristics of C. elegans makes the organism amenable for genetic analysis. Hermaphroditic self-fertilization allows the isolation and propagation of mutant strains without the necessity of mating. Mutations can be induced by treatment with a variety of mutagens. The F1 progeny of mutagenized animals are heterozygous for newly-induced mutations. Because hermaphrodites self-fertilize, 1/4 of the progeny of the F1 hermaphrodites bearing a new mutation will be homozygous for the mutation and display a mutant phenotype. Thus, hermaphrodites homozygous for a recessive mutation can be isolated without the necessity of mating among siblings. In addition, because mating is not required for propagation, mutations that result in gross morphological or behavioral abnormalities which prevent mating can be maintained as homozygous strains. Many of the vulval cell lineage mutants described in this thesis have abnormalities in vulval anatomy that render hermaphrodites unable to mate; hermaphroditic self-fertilization has greatly facilitated the isolation and characterization of these mutant strains.

Two facts suggest that it is feasible to identify all genes that affect a particular process in C. elegans. (1) The large brood size of a C. elegans hermaphrodite (approximately 300 progeny) and the small size of the animal facilitates the screening of large numbers of animals for the appearance of rare genetic events. Approximately 10^5 animals can be grown on a single Petri dish (100 x 20 mm) and examined using a dissecting microscope. (2) C. elegans has a small genome, approximately 8×10^7 base pairs per haploid genome (Sulston and Brenner, 1974), encoding approximately 2000 essential genes (Brenner

1974). Mutagenesis with ethyl methanesulfonate (EMS) disrupts the function of an average gene with a frequency of one of every 2000 mutagenized chromosomes (Brenner, 1974; Greenwald and Horvitz, 1980). Thus, the combination of efficiency of screening, coupled with the frequency of inducing mutations in individual genes, may make it possible to identify all genes in which loss of gene function confers a phenotype of interest. For example, it is likely that many or all of the genes involved in the mechanosensory response to gentle touch (Chalfie and Sulston, 1981; M. Chalfie personal communication) have been identified, as have many of the genes involved in dauer formation (Riddle, Swanson and Albert, 1981), and vulval development (Chapter 2, this thesis).

Other genes that affect a process may not confer a visible phenotype when mutated. Such genes may sometimes be identified as enhancers or suppressors of mutations in previously identified genes. For example, three genes encode different forms of acetylcholinesterase (Johnson et al., 1981; Culotti et al., 1981; Johnson and Russell, 1983). The lack of any one form of the enzyme does not result in a visible phenotype. However a double mutant lacking two of the three forms of the enzyme has an Uncoordinated phenotype and an animal lacking all three forms of the enzyme does not survive. In addition, Chapter 4 reports the identification of mutations in four genes involved in vulval development which alone confer a wild-type phenotype, but act in concert with other such mutations to result in a visible phenotype.

Approximately 500 genes have been identified by mutation (Swanson, Edgley and Riddle, 1984). These genes affect a variety of aspects of the biology of C. elegans. Approximately 50 genes have been identified that affect the gross morphology of the animal: mutations in dpy (dumpy) genes cause the animal to have a shorter and fatter body shape than wild type, mutations in lon genes cause the animal to have a longer body shape than wild type, and mutations in bli (blister) genes cause the cuticle to separate from the body. Many mutations also result in abnormalities in behavior: mutations in over 100 unc (uncoordinated) genes alter the normal pattern of movement. Mutations that alter other behaviors of the animal have also been identified; e.g., thermotaxis (Hedgecock and Russell, 1975), male mating (Hodgkin, 1983b), and egg laying (Horvitz and Sulston, 1980; Trent, Tsung and Horvitz, 1983; C. Desai, personal communication).

Mutations in over fifty genes result in alterations in the invariant cell lineage of C. elegans. Most, but not all of these genes have been named lin, for lineage abnormal. Cell lineage mutants were isolated in either of two general ways. Most cell lineage mutants were isolated by screening the F2 progeny of mutagenized hermaphrodites using a dissecting microscope for individuals defective in either anatomy or behavior. Mutant animals were subsequently examined using Nomarski optics to ascertain if any cell lineages were defective. The majority of cell lineage mutants isolated using this protocol were isolated as mutants defective in the behavior of egg-laying (Horvitz and Sulston, 1981; Trent, Tsung and Horvitz, 1983; Ambros and Horvitz, 1984; C. Desai, personal communication; this

thesis, Chapter 2). Subsequent analysis of the egg-laying defective mutants has revealed some mutants with abnormalities in the cell lineages that generate each of the three important components of the egg laying system: the vulva itself, the vulval muscles which contract to extrude eggs, and a pair of neurons innervating the vulval muscles, the HSN's (hermaphrodite specific neurons).

Other cell lineage mutants were identified by direct observation of the progeny of mutagenized hermaphrodites using Nomarski optics. Such loci include one gene, ced-3, that is involved in the control of programmed cell death (Ellis and Horvitz, 1985). Mutants defective in ced-3 block programmed cell deaths and allow cells that normally would die to survive and differentiate.

Mutants defective in the postembryonic cell lineages are of two general classes. Some mutants have defects in most or all postembryonic cell lineages (e.g., lin-5 or lin-6) and likely have defects in genes involved in basic processes of cell division (Sulston and Horvitz, 1981). However, in the majority of the cell lineage mutants that have been analyzed, one cell or set of cells express a fate (i.e., undergoes a cell lineage) characteristic of another cell or set of cells. Thus, as reviewed by Sternberg and Horvitz (1984), the genes defective in these mutant strains may control the expression of developmental sublineages.

Three types of transformations in cell fate have been observed in mutant strains: transformations in space, in sex and in time. For

example, in mab-5 males, three posterior ventral hypodermal cells, P(9-11).p, adopt fates characteristic of the anterior hypodermal cells P(1-8).p (C. Keynon, personal communication). Similarly, in lin-22 males, the lateral hypodermal cells V2 through V4 adopt fates characteristic of a posterior hypodermal cell, V5 (W. Fixsen, personal communication). Many of the vulval cell lineage mutations are also of this class (Sulston and Horvitz, 1981; Chapter 3, this thesis). Mutations in three genes -- lin-4, lin-14, lin-28 -- transform the fates of ventral and lateral hypodermal cells in time (Ambros and Horvitz, 1984); in these heterochronic mutants the hypodermal cells adopt the fate of the analogous ancestral or descendent cell present in a different larval stage.

Mutations in two classes of genes cause sexual transformations in cell lineages. Mutations in four genes -- tra-1, tra-2, tra-3 and her-1 -- cause animals of one genotypic sex to develop as animals of the opposite sex. As described below, these genes are likely to be involved in the pathway of sex determination. However, mutations in other genes cause sexual transformations in specific cell lineages. For example, dominant mutations in the gene egl-1 cause the HSNs to undergo programmed cell death, the fate that normally befalls the lineally equivalent cells in males. In addition, some vulval cell lineage mutations, which cause ectopic vulval-like divisions in hermaphrodites, cause the corresponding cells in males to express vulval-like fates (Sulston and Horvitz, 1981; P. Sternberg and E. Ferguson, unpublished observations).

The examples above illustrate how, using Nomarski optics, the effects of cell lineage mutations can be observed at the level of individual cells. Thus, an alteration in the lineage of a particular cell is the quantum unit of measurement with which we observe the effects of these mutations upon development.

Construction of a genetic pathway

Among the first genetic experiments to deduce a pathway of gene action were the experiments of Beadle and Ephrussi (1937), which elucidated the order of synthesis of some of the eye pigments of *Drosophila*, and those of Srb and Horowitz (1944), which elucidated the biosynthetic pathway of arginine in *Neurospora*. Since these beginnings, increasingly complex pathways have been analyzed. The genetic pathway of morphogenesis of the phage T4 was elucidated in a series of classic experiments (e.g., Edgar and Lielausis, 1968), while Hartwell et al. (1971, 1974) identified and characterized the action of many of the genes involved in the control of the cell cycle in yeast. More recently, genes controlling the determination of sex in two organisms, *Drosophila* and *C. elegans*, have been identified and the patterns of interactions among these genes have been examined (Baker and Ridge, 1980; Hodgkin, 1980; Doniach and Hodgkin, 1984).

The construction of a genetic pathway can be greatly facilitated by examining, in as much detail as possible, the functioning of the pathway in the wild type. The identification of mutants defective in the pathway permits the identification of the genes involved in the

pathway and serves to delineate different functional steps in the pathway. The determination of the wild-type function of each gene requires both an analysis of how the mutation affects gene function (i.e., reduction or elimination of gene activity, increase or ectopic expression of normal activity, or acquisition of novel activity) and a characterization of the time and site of wild-type gene action. Lastly, it is necessary to determine the order of gene function.

In addition to the pathway of vulval development described in this thesis, two other genetic pathways have been elucidated in C. elegans: the pathways of dauer formation and sex determination. During dauer formation, C. elegans, in response to at least two environmental cues, chooses to enter one of two developmental states, a dauer larva or the normal L3 stage. The genetic pathway of dauer formation encompasses both genes necessary for the processing of environmental signals and genes that alter the global development of the organism in response to this signal. In the genetic pathway of sex determination, the measurement of the ratio of the number of X chromosomes to autosomes controls the action of a series of genes that determine the phenotypic sex of the animal. In both of the above pathways, while the initial inputs to the pathways are known, the individual steps in the pathway can only be defined genetically by mutations that disrupt the pathway. However, as described below, in vulval development, many of the individual steps in the pathway can be ascertained by observation of the wild type.

Genetic pathway of dauer formation

Under adverse conditions, such as the lack of food or overcrowding, a developmentally arrested, non-feeding form of C. elegans, called a dauer larvae, is formed at the second molt. Dauer larvae have a cuticle structure different from any other larval stage. The cuticle completely encloses the animal, which appears relatively thin due to radial shrinkage. The animal stops pharyngeal pumping, and often remains motionless unless mechanically stimulated. An animal will remain in the dauer state until food becomes available. At this time pumping resumes, and ultimately the dauer larva will molt and resume normal development as an L4 larva.

Recent studies (Golden and Riddle, 1982; 1984) have identified three environmental cues involved in dauer formation. A small fatty-acid like pheromone is released by C. elegans under crowded conditions and both promotes dauer formation and inhibits recovery from the dauer state. A second signal is derived from bacteria and stimulates recovery from the dauer state. This food signal appears to act antagonistically to the dauer pheromone over a wide range of concentrations. In addition, the process of dauer formation and recovery is temperature-sensitive; at high temperatures ($>22^{\circ}$) the sensitivity of the wild type to dauer inducing pheromone is markedly higher. This temperature-sensitivity of the wild-type to dauer pheromone has allowed the determination of the temperature-sensitive period (TSP) of dauer formation in the wild-type (Golden and Riddle, 1984). The TSP for dauer formation is during the first larval molt, a full stage prior to the time of dauer formation. Thus, experiments performed on the wild-type have determined the environmental inputs

into dauer formation, and the time of commitment to the dauer stage, but have not revealed any of the cellular mechanisms which mediate this developmental decision.

To genetically dissect the pathway of dauer formation, two types of mutants defective in the process of dauer formation were obtained: dauer constitutive and dauer defective (Riddle, 1977; Riddle, Swanson and Albert, 1981). Both classes of mutants were named daf (for dauer formation). Temperature-sensitive dauer constitutive mutants were identified as mutants that formed dauers in the presence of food. Thirty-eight such mutants, defining seven genes, were isolated. Each of the seven genes are represented by multiple alleles, suggesting that most or all of the genes that can mutate to a dauer constitutive phenotype have been identified. Mutants that lack the ability to enter into the dauer state were obtained after mutagenesis of the wild type. The frequency of such dauer defective mutants was extremely high (5% of the F2 animals were dauer defective) suggesting that many genes can mutate to a dauer defective phenotype.

Upon examination, some of the dauer defective mutants, but none of the dauer constitutive mutants, were found to respond abnormally to other environmental signals such as chemoattractants and osmotic stress. Thus, some dauer defective mutants are likely to be defective in their ability to sense chemosensory signals from the environment. Examination using an electron microscope revealed defects in the amphids (two groups of chemosensory nerves near the mouth of the animal) in two dauer defective strains, daf-10 and daf-6 (Albert,

Brown and Riddle, 1981). This suggests that these groups of cells are likely to be involved in the sensing of an environmental signal during dauer formation. In general, dauer defective mutants might be abnormal in either lacking a response to dauer pheromone or constitutively sensing the presence of food (perhaps by the abolition of an inhibitory circuit).

None of the dauer defective mutants respond to dauer pheromone (Golden and Riddle, 1984); however, at low temperatures all but one, daf-2, of the dauer constitutive mutants has an increased sensitivity to the dauer pheromone. The increased sensitivity could be caused by either the inability to sense the presence of food, or by an increased sensitivity to the dauer pheromone.

Except for dauer constitutive mutations in one gene, daf-2, the epistatic relationships among the dauer constitutive and dauer defective mutations define a linear pathway of 12 distinct steps (Riddle, Swanson, and Albert, 1981), suggesting that the majority of these genes could be involved in a single functional pathway. Riddle, Swanson and Albert (1981) have postulated that the epistatic order of these genes may reflect the order of neuronal processing of environmental signal(s), with the dauer constitutive mutations generating a "false" signal for dauer entry and the dauer defective mutations blocking that signal at different points in the pathway. However, the order of gene function within the pathway can not be deduced from the epistatic relationships between these mutations, since the interrelationships of the functions specified by each of

the genes has not been determined. (See Chapter 3 for further discussion of this caveat).

Genetic pathway of sex determination

During development of C. elegans, the assessment of the ratio of the number of X chromosomes to the number of autosomes determines the phenotypic sex of the animal. By examining the phenotypic sex of triploid and tetraploid animals, Madl and Herman (1979) determined that animals with an X to autosome ratio greater than 0.75 develop as hermaphrodites (wild-type hermaphrodites have an X to autosome ratio of 1), while animals that have an X to autosome ratio less than 0.67 develop as males (wild-type males have an X to autosome ratio of 0.5).

Mutations in seven genes (tra-1, tra-2, tra-3, her-1, fem-1, fem-2 and fem-3) cause the phenotypic sex of the animal to be independent of its chromosomal composition, suggesting that these genes function in a pathway that determines the sex of the organism in response to the X to autosome ratio (Hodgkin and Brenner, 1977; Hodgkin, 1980; Doniach and Hodgkin, 1984; Kimble, Edgar and Hirsh; 1984, J. Hodgkin, personal communication). Mutants defective in these genes were obtained either from mutagenesis of the wild type, or as suppressors of mutants defective in previously identified sex-determining genes. All of these genes have at least four recessive alleles; many of the genes have 20 or more alleles. Thus, it is likely that most, if not all, of the genes that determine sex have been identified.

Recessive loss of function mutations in these genes confer one of three general phenotypes. Mutations in the tra genes cause animals of genotype XX to develop as phenotypic males but have no effect on XO animals. Thus, the tra gene products appear to promote hermaphrodite development but are not needed for male development. Mutations in her-1 cause XO animals to develop as fertile hermaphrodites but have no effect on XX animals. The her gene product appears to be required for male development but not for hermaphrodite development. Mutations in the three fer genes cause XX and XO animals to develop as females. Thus, the fer gene products are required both for development of the male soma and for spermatogenesis in both males and hermaphrodites.

The epistatic interactions among mutations in these seven genes define a single linear pathway in the soma.

$$\underline{\text{her-1}} > \underline{\text{tra-2,tra-3}} > \underline{\text{fem-1,fem-2,fem-3}} > \underline{\text{tra-1}}$$

(The epistatic interactions in the germline are more complex.) If, as postulated by Hodgkin (1980) and Doniach and Hodgkin (1984) each of these genes negatively regulates the activity of the succeeding gene in the pathway, then the epistatic relationship among these genes is indicative of the order of gene function.

This interpretation of the interrelationship of gene function is supported by the existence of dominant mutations in four of these genes. These dominant mutations confer phenotypes opposite to

recessive mutations in the same genes and may represent constitutive activation of the genes. For example, dominant mutations in tra-1 cause XX and XO animals to develop as females, similar to the phenotype caused by recessive mutation in the fem genes. The phenotypes conferred by the two classes of tra-1 mutations are consistent with a model in which the fem genes (at least in the soma) negatively regulate the activity of tra-1. Thus, the existence in a gene of both dominant and recessive mutations that confer opposing phenotypes strongly suggests that the activity of the gene has two states in the wild type, high and low, and that each state of the gene results in the expression of a different developmental program.

Pathway of vulval development

The vulva of the *C. elegans* hermaphrodite is formed from the descendants of three of six equipotential cells of the ventral hypodermis, P(3-8).p, in response to an inductive signal from the anchor cell of the gonad. The pathway of vulval development in the wild type has been described elsewhere (Sternberg and Horvitz, 1984; 1985a) but is summarized here (Figure 1-1).

Based upon observations on wild type hermaphrodites and perturbations of wild type development, a pathway of vulval development consisting of five temporally separate steps has been proposed (Sulston and Horvitz, Sulston and White, 1980; Kimble 1981; Sternberg and Horvitz, 1984). The first two steps in vulval development are the formation of both the potential vulval precursor

Figure 1-1 The pathway of vulval development in the wild type. (a) Generation of the potential vulval precursor cells P(3-8).p. During the early portion of the first larval stage (L1), the nuclei of P(1-12) migrate into the ventral nerve cord. The P cells divide during the L1; the anterior daughter of each cell, Pn.a, is a neuroblast that generates the postembryonic additions to the ventral nerve cord. The posterior daughter of each cell, Pn.p, becomes a hypodermal cell. P(1,2,9-11).p join the hypodermal syncytium without dividing. P12.p divides once; its posterior daughter dies, and its anterior daughter becomes a unique class of hypodermal cell. The potential vulval precursor cells, P(3-8).p, do not divide until the third larval stage (L3). (b) Generation of the anchor cell and production of the inductive signal. The anchor cell of the gonad is formed during the early portion of the second larval stage (L2) from one of the two cells Z1.aaa or Z4.ppp. An inductive signal from the anchor cell induces the formation of the vulva; if the anchor cell is ablated with a laser microbeam prior to the early L3, none of the cells P(3-8).p express vulval cell lineages. However, if the anchor cell is ablated during the early L3, the vulval precursor cells will still express vulval fates, suggesting that the inductive signal is no longer required at this time. (c) Reception of the inductive signal by the vulval precursor cells and determination of P(3-8).p cell fate. Prior to the early L3, P(3-8).p are equipotential cells; each of these cells has been observed to express each of three sublineages -- 1^o, 2^o, and 3^o -- after experimental perturbations of development in the wild type. During the early L3, the fates of each of the cells P(3-8).p are determined in response to the graded inductive signal of

the anchor cell. The anchor-cell proximal cells, P(5-7).p, are determined to undergo lineages that generate vulval cells; P6.p is determined to undergo a 1⁰ sublineage, P5.p and P7.p are determined to undergo 2⁰ sublineages. The anchor-cell distal cells, P(3,4,8).p, are not induced; these three cells are determined to undergo 3⁰ sublineages. If any of the daughters of P(3-8).p are ablated with a laser microbeam, no changes are observed in the lineages or fates of the remaining vulval cells, suggesting that the fates of the vulval precursor cells are determined prior to the divisions of P(3-8).p.

(d) Execution of the vulval sublineages. During the mid to late L3, P(3-8).p express their respective fates. P(3,4,8).p adopt a 3⁰ fate; these cells divide once and join the hypodermal syncytium, represented by [S S]. (In approximately 50% of wild-type hermaphrodites, P3.p joins the hypodermal syncytium without dividing, represented by the lineage pattern [S].) P6.p adopts a 1⁰ fate. The four P6.p granddaughter cells, P6.pxx, are formed by two rounds of anterior-posterior divisions. The P6.pxx cells subsequently divide transversely, represented by [TTTT]. P5.p and P7.p undergo 2⁰ lineages. P5.p undergoes two rounds of anterior-posterior divisions. The two P5.pax cells divide longitudinally, represented by [LL]. P5.ppa divides transversely, represented by [T], and P5.ppp fails to divide, represented by [N]. P7.p undergoes a symmetric pattern of division represented by [NTLL]. During the L3 molt and early L4, the vulval cells that are proximal to the anchor cell detach from the ventral cuticle and move dorsally. The vulval cells that are distal to the anchor cell remain attached to the ventral cuticle. The anchor cell nucleus subsequently disappears and, at the L4 molt, the vulva

everts. A more detailed description of wild-type vulval development is given by Sternberg and Horvitz (1985a).

a) ANATOMY OF L1 HERMAPHRODITE

b) FORMATION OF VULVAL PRECURSOR CELLS

c) FORMATION OF ANCHOR CELL

d) ANATOMY OF LATE L2 HERMAPHRODITE

e) DETERMINATION OF P(3-8).p CELL FATE

f) EXECUTION OF VULVAL FATES

cells, P(3-8).p, and the anchor cell. P(3-8).p are formed in the L1 as the posterior daughters of six of the twelve P cells, which are blast cells that generate the post-embryonic additions to the ventral nerve cord and hypodermis (Figure 1-1a and 1-b). The anchor cell is formed during the L2 (Figure 1-1a and 1-c) from one of two cells in the somatic gonad. The inductive stimulation by the anchor cell on vulval development was ascertained by ablation of the anchor cell with a laser microbeam prior to the early L3; subsequently none of the cells P(3-8).p expressed vulval cell lineages.

The third and central step in vulval development is the reception of the anchor cell signal and the determination of the fates of P(3-8).p (Figure 1-1d and 1e). The results of three experiments suggest that the determination of the fates of the vulval precursor cells occurs in the P(3-8).p cells during the early L3 (Sternberg and Horvitz, 1985a). (1) Prior to the early L3, P(3-8).p are equipotential cells; each of these cells has been observed to express each of the three vulval fates -- 1^0 , 2^0 , and 3^0 -- after experimental perturbation of development in the wild type. (2) The inductive signal from the anchor cell ends prior to the divisions of P(3-8).p; if the anchor cell is ablated just prior to the divisions of P(3-8).p in the early L3, the vulval precursor cells will express their normal lineages. (3) Cell ablation experiments performed on the daughters of P(3-8).p did not reveal any ability of the remaining vulval cells to regulate.

The cells P(3-8).p are determined to express one of three fates

dependent upon position. The cells proximal to the anchor cell, P(5-7).p are determined to express vulval fates; P6.p, the cell closest to the anchor cell, is determined to express a 1^o fate and P5.p and P7.p, the cells adjacent to P6.p, are determined to express 2^o fates. The cells distal to the anchor cell, P(3,4,8).p are uninduced. These cells are determined to express 3^o, or non-vulval, fates. A series of additional experiments (Sternberg and Horvitz, 1985a) suggest that the determination of P(3-8).p cell fate results from of a passive interpretation of the inductive signal by the P(3-8).p cells and does not depend upon any interaction among these cells.

The fourth step in the pathway of vulval development is the expression of the vulval fates during the mid to late L3. Each of the vulval fates can be distinguished by the numbers and types of progeny that are produced (Figure 1-1e, Sternberg and Horvitz, 1984; this thesis, Chapter 3). The fifth step in vulval development is the morphogenesis of the vulva. During the L3 molt and early L4, the vulval cells that are proximal to the anchor cell detach from the ventral cuticle and move dorsally. The vulval cells that are distal to the anchor cell remain attached to the ventral cuticle. The anchor cell nucleus subsequently disappears, and, at the L4 molt, the vulva everts.

The development of the vulva of C. elegans represents a system where the processes of induction and pattern formation can be studied at the level of single cells. This thesis is an attempt to identify

all the genes that are involved in the control of the vulval cell lineages and to determine how these genes interact during vulval development. Chapter 2 describes the identification of 22 genes that affect the vulval cell lineages and analyzes how mutations in each of these genes affect gene function. (The characterization of four additional genes involved in vulva development, which were identified by mutations that enhance the phenotype resulting from other vulval cell lineage mutations, is reported in Chapter 4.) Chapter 3 describes the experiments that assigned a majority of these genes to specific steps in the pathway of vulval development. Eleven genes appear to be involved in the determination of P(3-8).p cell fate. Chapter 4 contains a further genetic analysis of mutations in four of these 11 genes.

Chapter II

Identification and characterization of 22 genes that affect the
vulval cell lineages of the nematode Caenorhabditis elegans

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ABSTRACT

Ninety-five mutants of the nematode Caenorhabditis elegans altered in the cell lineages of the vulva have been isolated on the basis of their displaying one of two phenotypes, Vulvaless or Multivulva. In Vulvaless mutants, which define 12 genes, no vulva is present. In Multivulva mutants, which define ten genes, one or more supernumerary vulva-like protrusions are located along the ventral side of the animal. A single recessive mutation is responsible for the phenotypes of most, but not all, of these strains. Fifteen of these 22 genes are represented by multiple alleles. We have shown by a variety of genetic criteria that mutations that result in a Vulvaless or Multivulva phenotype in six of the 22 genes most likely eliminate gene function. In addition, Vulvaless or Multivulva mutations in seven of the other genes most likely result in a partial reduction of gene function; the absence of the activity of any of these genes probably results in lethality or sterility. Our results suggest that we may have identified most, or all, genes of these two classes.

INTRODUCTION

The nematode Caenorhabditis elegans is well suited for studies concerning the genetic control of cell lineage. C. elegans consists of relatively few cells (e.g., there are only 959 somatic nuclei in the adult hermaphrodite) of many different types (SULSTON and HORVITZ 1977; KIMBLE and HIRSH 1979; SULSTON et al. 1983). The cellular anatomy and patterns of cell divisions and cell fates of C. elegans are essentially invariant among individuals from the single-celled zygote to the adult (SULSTON and HORVITZ 1977; DEPPE et al. 1978; KIMBLE and HIRSH 1979; SULSTON, ALBERTSON and THOMSON 1980; SULSTON et al. 1983). A number of mutations that alter this normally invariant cell lineage have been isolated and characterized (e.g., HORVITZ and SULSTON 1980; SULSTON and HORVITZ 1981). Some of these mutations affect many cell divisions; others are more specific.

We have begun an attempt to identify all genes that affect a particular set of cell lineages in C. elegans, those involved in the development of the vulva of the hermaphrodite. We hope to determine the number of such genes, their patterns of interaction, whether or not each of these genes also affects other cell lineages and what other features are shared by the set of lineages affected by a particular gene. We have chosen to study the cell lineages that generate the vulva for three major reasons. First, these lineages are technically easy to study: they involve relatively few, highly visible precursor cells that undergo three rounds of divisions over a

time interval of only about 5 hr (SULSTON and HORVITZ 1977). Second, the vulval precursor cells are involved in four generally interesting developmental phenomena (SULSTON and HORVITZ 1977; SULSTON and WHITE 1980; KIMBLE 1981; P. STERNBERG, personal communication): (1) determination -- each of the six initially multipotential vulval precursor cells is determined to express one of three distinct fates, (2) induction -- the gonadal anchor cell induces the formation of the vulva, (3) pattern formation -- the six potential vulval precursor cells are of equivalent developmental potential but nonetheless express three distinct fates in a precise spatial pattern defined by their distances from the anchor cell, (4) regulation -- if a vulval precursor cell is ablated, another cell can replace it. Our goal is to identify the genes and, ultimately, the molecules involved in these developmental processes.

The third reason that we chose to focus our study on the vulval cell lineages is that a number of mutants abnormal in vulval cell divisions had already been identified (HORVITZ and SULSTON 1980; SULSTON and HORVITZ 1981). These mutants were both viable and fertile and could be easily recognized with a dissecting microscope. Two classes of mutants had been characterized. In Multivulva (Muv) mutants, three ventral hypodermal cells that normally produce nonvulval progeny instead undergo vulval like lineages to produce multiple vulva-like protrusions along the ventral side. In Vulvaless (Vul) mutants, three other ventral hypodermal cells that normally generate the cells of the vulva fail to do so. Because a vulva is not formed, the fertilized eggs of a Vul hermaphrodite are not laid.

Consequently, the eggs hatch inside the body of the parent, and the young larvae eat their parent (they later escape from the parental cuticle). Vul mutants can be recognized with a dissecting microscope by the presence of "bags of worms," in which each parental cuticle encloses its progeny larvae.

This paper reports the isolation and genetic characterization of 95 mutants that display a Multivulva or Vulvaless phenotype.

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: bli-3(e767); lin-6(e1466); unc-11(e47); dpy-5(e61); unc-13(e1091); sDf5; lin-28(n719) (AMBROS and HORVITZ 1984); unc-56(e403); sup-17(n316); unc-29(e1072); nDf23; nDf24; nDf25; unc-75(e950). sup-17(n316) was obtained by phenotypically reverting lin-12(n177) (E. FERGUSON, unpublished results). nDf23, nDf24 and nDf25 were obtained by crossing N2 males that had been mutagenized with gamma-rays (GREENWALD and HORVITZ 1980) to hermaphrodites of genotype dpy-5 unc-29. F1 Unc non-Dpy hermaphrodites, selected by their resistance to the cholinergic agonist levamisole (LEWIS et al. 1980), were candidates for carrying a deficiency of the unc-29 region. These hermaphrodites were then tested to determine whether they were heterozygous for a deficiency of the unc-29 region, i.e., whether they

carried a recessive lethal mutation that failed to complement mutations in one or more genes linked to unc-29. nDf23 fails to complement lin-28, unc-56, sup-17 and unc-29. nDf24 and nDf25 fail to complement lin-10, lin-28, unc-56, sup-17 and unc-29.

LGII: cat-2(e1112); sup-9(n180); nDf3; unc-85(e1414);
bli-2(e768); dpy-10(e128); tra-2(n196); mnDf88; let-253(mn184);
let-236(mn88); lin-5(e1348); mnDf68; vab-9(e1744); rol-6(e187);
unc-4(e120); him-9(e1487); unc-52(e444); C1 dpy-10(e128) unc-52(e444).
C1 dpy-10 unc-52 is a chromosomal abnormality of LGII that balances the right half of the chromosome (HERMAN 1978). mnDf88, let-253, let-236 and mnDf68 are described by SIGURDSON, SPANIER, and HERMAN (1984).

LGIII: unc-93(e1500); dpy-17(e164); lon-1(e185); daf-4(e1364);
lin-16(e1743); unc-36(e251); unc-86(e1416,n848); nDf16 (V. AMBROS and M. FINNEY, personal communication); dpy-19(e1259); sup-5(e1464);
sma-2(e502); unc-32(e189); unc-69(e587); tra-1(e1099); eT1(III;V) (ROSENBLUTH and BAILLIE 1981); let(n886). n848 is a heat-sensitive allele of unc-86 (M. FINNEY, personal communication). let(n886) is a mutation that confers a recessive lethal phenotype and that is linked to eT1(III;V) (M. FINNEY, personal communication).

LGIV: dpy-9(e12); unc-17(e245); dpy-13(e184); unc-5(e53);
unc-8(e49,n491); dpy-20(e1362); unc-22(e66); nDf27 (H. ELLIS, personal communication); dpy-26(n199); unc-31(e169); unc-30(e191); ced-3(n717) (H. ELLIS, personal communication); dpy-4(e1166); unc(n752). n491 is

a dominant allele of unc-8 (J. PARK, personal communication). unc(n752), which confers a recessive Unc phenotype and is linked to the translocation nT1(IV;V), was identified after ethyl methanesulfonate (EMS) mutagenesis of hermaphrodites of genotype nT1(IV)/unc-8(n491); nT1(V)/unc-60; individual F1 hermaphrodites were picked and their progeny were examined to determine whether any mutation cosegregated with, and thus was linked to, the Vul phenotype of nT1(IV;V) (E. FERGUSON, unpublished results).

LGV: unc-60(e677); dpy-11(e224); unc-42(e270); sma-1(e30); him-5(e1467); unc-76(e911); unc-51(e369); unc(n754). unc(n754) is a mutation that confers a dominant Unc phenotype and that is linked to nT1(IV;V) (E. FERGUSON, unpublished results).

LGX: unc-78(e1217); dpy-23(e840); lon-2(e678); sup-7(st5); dpy-7(e1324); unc-84(e1410); unc-3(e151); let-15(mn127); let-40(mn150); let-18(mn122); let-38(mn141); sup-10(n183); mnDp1; mnDf1; mnDf4; mnDf11; mnDf19; mnDf43.

This paper follows the standardized C. elegans genetic nomenclature (HORVITZ et al. 1979). All vulval cell lineage mutations except n300 and n1045 were assigned lin, for lineage abnormal, gene names. As described in Results, it is not clear whether n300 is simply a mutation present on the translocation nT1(IV;V) or whether n300 is a result of the translocation itself. For this reason we have not assigned a gene name to n300, although it defines a separate complementation group. Because n1045 was shown to be an allele of the

previously defined gene let-23 (see Results), it was not assigned a lin gene name.

General techniques: Methods for the culturing, handling and genetic manipulation of C. elegans have been described (BRENNER 1974). Most experiments were done at 20^o, except those involving dpy-19, sup-5, sup-7 or lin-18, which were done either at 20^o or 25^o, and lin-12, lin-13 or let-23, which were done at 15^o, 20^o or 25^o.

Photography: Brightfield photomicrographs were taken with Kodak Technical Pan film 2415 using a Zeiss Universal microscope equipped with a Neofluar 6.3 objective and a Zeiss microflash illuminator. For most photographs, animals were first placed on an NGM plate (BRENNER 1974) with no bacteria for approximately 5 min and then transferred to a slide containing 10-15 ul of 20% (w/v) Ficoll (Sigma) in M9 buffer (BRENNER 1974). Sephadex beads G-200-120 (Sigma) with a particle size of 40-120 um were then placed in the solution and an 18 x 18 mm coverslip was lowered gently onto the liquid. The Ficoll increased the viscosity of the buffer solution and prevented the animals from thrashing while allowing apparently normal movement. The combination of the Sephadex beads and the viscosity of the solution supported the weight of the coverslip and prevented the animals from being crushed.

Sources of vulval cell lineage mutants: Most of the new mutants described in this manuscript were isolated by N. TSUNG during a general screen for egg-laying defective mutants (TRENT, TSUNG and HORVITZ 1983). Other mutants were isolated either by ourselves or by

the following members of our laboratory: V. AMBROS, C. DESAI, H. ELLIS, W. FIXSEN, I. GREENWALD, J. PARK and C. TRENT. lin-1(e1026, e1275, n176), lin-2(e1309, e1424, e1453), lin-3(e1417), lin-4(e912), lin-7(e1413) and lin-8(n111); lin-9(n112) were described previously by HORVITZ and SULSTON (1980) and SULSTON and HORVITZ (1981). S. BRENNER isolated lin-18(e620); M. CHALFIE, lin-15(n377); E. HEDGECOCK, lin-31(e1750); J. HODGKIN, lin-15(e1763) and J. PLENEFISCH, lin-1(n1140).

EMS was used as a mutagen (BRENNER 1974) to generate almost all of the mutants abnormal in vulval cell lineages. lin-4(e912) was obtained after P-32 decay (HORVITZ and SULSTON 1980; BABU and BRENNER 1981). lin-7(n701) was obtained after gamma-ray mutagenesis (V. AMBROS, personal communication). The origin of n300 is unclear. An egg-laying defective hermaphrodite strain obtained after EMS treatment of the wild type was crossed with wild-type males, and strains with two distinct egg-laying defective phenotypes were isolated from the F2 progeny. These strains carried two different mutations, unc-86(n306) III and n300, which is associated with the reciprocal translocation nT1(IV;V) (see Results). Upon reexamination, the original strain proved to carry the unc-86 mutation but not n300 or nT1(IV;V).

Complementation: Because some mutations affecting vulval cell lineages are not expressed in males, complementation tests were scored in hermaphrodites. Males carrying the unknown mutation were mated with hermaphrodites homozygous for both a known vulval cell lineage mutation and a recessive marker used to distinguish self from cross

progeny. If the unknown mutation was recessive and if lin cross-progeny hermaphrodites were observed, the lineage mutations failed to complement. [Because lin-11(n389) hermaphrodites are unable to mate and lin-13(n387) hermaphrodites are sterile, complementation tests involving these two mutations were performed using hermaphrodites homozygous for a recessive marker and heterozygous for the lin mutation and for a closely linked unc mutation. unc-32 was used to balance lin-13, and unc-29 was used to balance lin-11.]

Suppression studies: The cell lineage mutations were tested for suppression by one of two amber suppressors, sup-5 III or sup-7 X (WATERSTON and BRENNER 1978; WATERSTON 1981; WILLS et al. 1983). All alleles of genes without suppressible alleles were tested. For two genes with suppressible alleles, only some alleles were tested; specifically, nine of 16 lin-1 alleles and eight of 13 lin-7 alleles were tested. Some mutations had been tested previously by HORVITZ and SULSTON (1980).

To help score the presence of sup-7 in these suppression experiments, we constructed the linked double mutant sup-7 dpy-7 X. The presence of sup-5 was scored using the linked double mutants lon-1 sup-5 III (HORVITZ and SULSTON 1980) or dpy-19 sup-5 III (M. FINNEY, personal communication). Table 2-1 describes the protocol used in testing most mutations and presents data for those mutations suppressed by a single copy of one of the suppressors. Three mutations -- lin-24(n1057), lin-34(n1046), and let-23(n1045) -- were better suppressed by two copies of one of the suppressors than by a

Table 2-1. From the progeny of hermaphrodites of genotype lin/+; sup dpy/+ +, Lin animals and Dpy animals were picked (dpy-19 was used in cis to sup-5; dpy-7 was used in cis to sup-7). In most cases the lin mutation was not suppressed, and about 2/3 of the Lin hermaphrodites segregated Dpy animals and about 2/3 of the Dpy hermaphrodites segregated Lin animals. Dpy Lin animals were picked, for example, to establish a putative lin; sup-7 dpy-7 strain, which was placed at 15° . If the strain became sterile (sup-7 homozygotes are sterile at 15°; WATERSTON 1981), the strain was confirmed to be homozygous for sup-7 and the mutation was considered to be nonsuppressed. [For some Muv strains, the sickness of the Muv mutant prevented the establishment of the lin; sup-7 dpy-7 strain. In these cases, lin/+; sup-7 dpy-7 hermaphrodites were placed at 15°; the presence of Lin hermaphrodites among the sterile progeny of these animals indicated that the lin mutation was not suppressed. The alleles of lin-3 and let-23 that result in lethality or sterility were determined not to be suppressed based on the observations that hermaphrodites of genotype unc-8 lin-3/+ +; sup-7 dpy-7 (or let-23 unc-4/+ +; sup-7 dpy-7) segregated Unc Lin (or Unc Let) progeny.]

If a lin mutation was suppressed by a single copy of the amber suppressor, none or few of the Lin hermaphrodites segregated Dpy animals (line 1), and none or few of the Dpy hermaphrodites segregated Lin animals (line 2). (The Dpy hermaphrodites that segregated Lin animals and the Lin hermaphrodites that segregated Dpy animals were recombinants between the dpy and sup mutations. The distance between sup-7 and dpy-7 is approximately 4 map units. However, because sup-7

dpy-7 hermaphrodites grow more slowly than hermaphrodites of genotype sup-7 dpy-7/+ dpy-7, the frequency of Dpy non-Sup recombinants among the Dpy animals picked tended to be higher than expected from the recombination frequency between these two genes.) In these cases, from the same parent, about 30 phenotypically wild-type progeny were also picked. Of these animals, approximately 2/11 were of genotype lin/lin; sup dpy/+ +, as they segregated 1/4 Lin non-Dpy, 1/4 Dpy non-Lin and 1/2 wild-type progeny (line 3). From the progeny of phenotypically wild-type hermaphrodites of putative genotype lin/lin; sup dpy/+ +, three classes of animals were picked, Lin, wild-type, and Dpy. None or few of the Lin animals segregated Dpy progeny (line 4). All of the wild-type hermaphrodites were of parental genotype, as evidenced by the phenotypes of their progeny (line 5). The Dpy progeny were of putative genotype lin; sup dpy and were tested at 15° for sterility to confirm the presence of the suppressor (line 6). To confirm the presence of the lin mutation in the putative lin; sup dpy strain, Lin hermaphrodites were reisolated from the strain after crossing with wild-type males. The Lin hermaphrodites were confirmed to be of genotype lin; + + by noting the absence of Dpy animals among their progeny (E. FERGUSON, unpublished observations). ND, not determined.

Table 2-1

Dominant suppression of some vulval cell lineage mutations by the amber suppressors sup-5 and sup-7

	Progeny of hermaphrodites of genotype					
	<u>lin-1(e1777)/+;</u> <u>sup-7 dpy-7/+ +</u>	<u>lin-1(n431)/+;</u> <u>sup-7 dpy-7/+ +</u>	<u>lin-7(e974)/+;</u> <u>sup-7 dpy-7/+ +</u>	<u>lin-7(e1449)/+;</u> <u>sup-7 dpy-7/+ +</u>	<u>dpy-19 sup-5/+ +;</u> <u>lin-18(n1051) +/+ lon-2</u>	
(1) Fraction of Lin progeny that segregated Dpy animals	0/3	1/7	1/16	0/7	0/11	
(2) Fraction of Dpy progeny that segregated Lin animals	2/11	1/10	0/7	0/8	ND	
(3) Fraction of phenotypically wild-type progeny that were of putative genotype <u>lin/lin; sup dpy/+ +</u>	6/30	2/20	3/33	2/29	12/65	
Progeny of hermaphrodites of putative genotype						
	<u>lin-1(e1777);</u> <u>sup-7 dpy-7/+ +</u>	<u>lin-1(n431);</u> <u>sup-7 dpy-7/+ +</u>	<u>lin-7(e974);</u> <u>sup-7 dpy-7/+ +</u>	<u>lin-7(e1449);</u> <u>sup-7 dpy-7/+ +</u>	<u>dpy-19 sup-5/+ +;</u> <u>lin-18(n1051)</u>	
(4) Fraction of Lin progeny that segregated Dpy animals	1/9	0/4	0/17	0/15	0/13	
(5) Fraction of wild-type progeny that were of parental genotype	8/9 (1/9 was <u>lin; + +</u>)	9/9	18/21 (3/21 were <u>lin; + +</u>)	14/14	36/36	
(6) Fraction of Dpy non-Lin progeny of putative genotype <u>lin; sup dpy</u> that were sterile at 15°	3/3	2/2	4/4	3/3	3/3	

single copy. Details of these three suppression experiments are described below (also see Results).

lin-24(n1057): As lin-24(n1057) results in a Vul phenotype only when heterozygous to a wild-type allele of the locus, we performed suppression experiments to examine the phenotype of lin-24(n1057)/+ hermaphrodites. These experiments were complicated by the fact that, at 20°, some hermaphrodites of genotype dpy-19 sup-5 can have an egg-laying defective (Egl) phenotype similar to that of lin-24(n1057)/+ hermaphrodites. However, no dpy-19 sup-5 hermaphrodites are Vul; as viewed with a dissecting microscope, all dpy-19 sup-5 hermaphrodites have a recognizable vulval structure. To compare the penetrance of the Vul defect of hermaphrodites of genotype unc-22 lin-24(n1057)/+ + with the penetrance of the Vul defect of hermaphrodites of genotype dpy-19 sup-5; unc-22 lin-24(n1057)/+ +, L4 hermaphrodites of both genotypes were picked. Those hermaphrodites that became Egl were examined either with a dissecting microscope or in a few cases with Nomarski optics, to determine whether a recognizable vulval structure was present. Hermaphrodites that either lacked a vulva or had a protrusion with no recognizable vulval characteristics were considered to have a Vul phenotype. The penetrance of the Vul phenotype of hermaphrodites of genotype dpy-19 sup-5; unc-22 lin-24(n1057)/+ + (3%, n = 279) was much lower than the penetrance of the Vul phenotype of hermaphrodites of genotype unc-22 lin-24(n1057)/+ + (22%, n = 301), demonstrating that sup-5 suppresses lin-24(n1057)/+. sup-5 was demonstrated not to be a dominant suppressor of lin-24(n1057)/+ as 13 of 21 Vul hermaphrodites that were

the progeny of hermaphrodites of genotype dpy-19 sup-5/+ +; unc-22 lin-24(n1057)/+ + segregated Dpy animals. [Hermaphrodites of genotype dpy-19 sup-5; unc-22 lin-24(n1057)/+ + were constructed by mating dpy-19 sup-5/+ + males with hermaphrodites of genotype dpy-19 sup-5; unc-22 lin-24(n1057) and picking Dpy non-Unc cross progeny. Hermaphrodites of genotype dpy-19 sup-5; unc-22 lin-24(n1057) were constructed by picking Dpy Unc progeny from animals of genotype dpy-19 sup-5/+ +; unc-22 lin-24(n1057)/+ +. These strains were sterile at 15^o, confirming the presence of sup-5. To confirm the presence of lin-24(n1057), hermaphrodites of putative genotype dpy-19 sup-5; unc-22 lin-24(n1057) were mated with wild-type males and Vul hermaphrodites were observed among the F1 progeny.]

lin-34(n1046): From the progeny of hermaphrodites of genotype lin-34(n1046) unc-22/+ +; sup-7 dpy-7/+ +, Unc, Dpy and Dpy Unc hermaphrodites were picked. The six Dpy Unc hermaphrodites of putative genotype lin-34(n1046) unc-22; sup-7 dpy-7 were not Muv and segregated very few Muv progeny. Of the 35 Unc hermaphrodites that were picked, 17 segregated Dpy Unc progeny, very few of which were Muv. Ten of the 13 Dpy hermaphrodites that were picked segregated Dpy Unc hermaphrodites. On five of the plates the Dpy Unc hermaphrodites were mostly non-Muv; on the other five plates most of the Dpy Unc hermaphrodites were Muv. All ten Dpy isolates were subsequently tested at 15^o for the presence of the suppressor, and only the first five isolates were sterile and thus homozygous for sup-7. To confirm these results, the suppressibility of n1046 was tested using a dpy-19 sup-5 strain. sup-5 also is a recessive suppressor of n1046 (E.

FERGUSON, unpublished results).

let-23(n1045): The suppression experiments of let-23(n1045) were complicated by the temperature-dependent phenotypes of both n1045 and sup-7; at the temperatures at which sup-7 animals are fertile, 22.5° and 25°, the majority of n1045 hermaphrodites appear wild-type, whereas at the temperature at which the Vul phenotype of n1045 hermaphrodites is most penetrant, 15°, sup-7 hermaphrodites are sterile. From parental hermaphrodites of genotype let-23(n1045) unc-4/+ +; sup-7 dpy-7/+ +, five Dpy Unc hermaphrodites were picked and grown at 25° and 20 Unc hermaphrodites were picked and grown at 15°. (The cis marker unc-4 was used to identify animals homozygous for n1045.) Four of the five Dpy Unc hermaphrodites of putative genotype let-23(n1045) unc-4; sup-7 dpy-7 segregated very few arrested larvae (5%, n = 95) and were subsequently shown to be homozygous for sup-7 (by the criterion of sterility at 15°). The fifth Dpy Unc hermaphrodite segregated a normal number of arrested larvae (29%, n = 60) but generated descendants fertile at 15° and thus not homozygous for sup-7. The presence of n1045 in two of the first four Dpy Unc isolates was confirmed by mating the Dpy Unc hermaphrodites with wild-type males and reisolating Unc Vul hermaphrodites in the F2. Fifteen of the 20 Unc hermaphrodites that were grown at 15° were heterozygous for sup-7 (as they segregated approximately 1/4 sterile progeny) and segregated very few arrested larvae and many egg-laying competent progeny. In contrast, all of the progeny of the five Unc hermaphrodites that did not segregate sup-7 either arrested during larval development or were Vul, suggesting that at 15° sup-7/+

suppresses both the lethality and the vulval lineage defects that result from n1045.

Male mating: Male mating experiments were carried out essentially as described by HODGKIN, HORVITZ and BRENNER (1979). Six L4 males and six L4 dpy-11 hermaphrodites were placed on a Petri dish containing a 1-cm spot of bacteria. The males were removed after 24 hr, and the hermaphrodites were transferred to a fresh plate every 12 hr thereafter until no further cross progeny were produced. Most males were obtained using him-5. n300 males were generated using him-9. lin-13 males were obtained from the progeny of fertile lin-13; him-5 hermaphrodites, which were obtained at 15° from the balanced heterozygote strain lin-13 +/+ unc-32; him-5. Heterozygous lin-12(n137) males were obtained as non-Unc males from the strain + unc-32 lin-12(n137)/unc-36 + +; him-5.

lin-3 complementation screen: To obtain mutations that failed to complement the Vulvaless mutation lin-3(e1417), wild-type L4 males were mutagenized with EMS and mated with L4 hermaphrodites of genotype dpy-20 lin-3(e1417)/nT1(IV); +/nT1(V). A recessive Unc mutation, unc(n752), was also present on nT1(IV;V). [The complementation screen was performed using heterozygous lin-3(e1417) hermaphrodites because 90% of lin-3(e1417) hermaphrodites are Vul and thus not able to mate.] The parents (six males and six hermaphrodites) were transferred to a fresh plate every day and the F1 progeny of the mating were examined for the presence of non-Dpy, non-Unc Vulvaless hermaphrodites, which could carry a mutation that failed to complement lin-3(e1417).

Ninety-five matings were done, and approximately 40,000 F1 progeny were examined, 20,000 of which were heterozygous for lin-3(e1417). When this protocol was used, in addition to six partially dominant Vul or Egl mutations -- including lin-24(n1057), lin-33(n1043), and lin-33(n1044) --, two additional alleles of lin-3, n1058 and n1059, were obtained.

Construction of the double mutant lin-24(n432) ced-3(n717): From the progeny of hermaphrodites of genotype lin-24(n432) unc-31 +/+ + ced-3, many Vul non-Unc hermaphrodites were picked. As lin-24(n432) results in a partially dominant Vul phenotype, most hermaphrodites were of the parental genotype; however, a few hermaphrodites were recombinants of genotype lin-24(n432) unc-31 +/lin-24(n432) + ced-3 and segregated very few egg-laying competent progeny. The progeny of these hermaphrodites that failed to segregate Unc progeny were of putative genotype lin-24(n432) ced-3. Three such putative lin-24 ced-3 animals were saved, and the presence of ced-3 in these strains was confirmed using Nomarski optics (H. ELLIS, personal communication).

RESULTS

Isolation of mutants: We have identified 95 independently derived mutants abnormal in the vulval cell lineages. These mutants fall into two general classes, Multivulva (Muv) and Vulvaless (Vul), both of which have been described previously (HORVITZ and SULSTON 1980; SULSTON and HORVITZ 1981). In a Muv hermaphrodite, one or more supernumerary vulva-like protrusions are located along the ventral side of the animal. Some Muv hermaphrodites lack a functional vulva. The ventral protrusions are readily visible when viewed with a dissecting microscope and have been used as the basis for the isolation of the Muv strains described in this manuscript. Like those Muv mutants obtained previously (SULSTON and HORVITZ 1981), the majority of these new Muv mutants generate supernumerary vulval-like structures as a consequence of the expression of vulval cell lineages by cells not normally involved in vulval development (P. STERNBERG, personal communication). However, in Muv mutants defective in two newly identified genes, lin-17 and lin-18, an abnormality in the lineage of the posterior-most of the three vulval precursor cells generates a single supernumerary vulva-like structure immediately posterior to the vulva (P. STERNBERG, personal communication).

In a Vul hermaphrodite, no vulva is present. Some Vul hermaphrodites express some vulval cell divisions resulting in a

single ventral protrusion at the normal location of the vulva. A Vul hermaphrodite does not lay eggs and its progeny hatch internally, causing the parent to become a "bag of worms." (A bag of worms comprises the cuticle of the adult hermaphrodite enclosing its progeny larvae.) Most of the new Vul mutants reported in this manuscript initially were identified on the basis of their defects in egg laying when viewed with a dissecting microscope, i.e., (1) these Vul strains produced many bags of worms and (2) individual Vul hermaphrodites generally laid no eggs. Egg-laying defective mutants with these characteristics were examined using Nomarski optics for the presence of a vulva. Those strains in which the vulva was clearly absent in a majority of hermaphrodites were designated Vul mutants and studied further to establish that they were defective in vulval cell lineages (P. STERNBERG, personal communication).

Our classification of a strain as either a Muv strain or a Vul strain implies that most, but not necessarily all, of the animals of that strain have either a Muv or a Vul phenotype. In some incompletely penetrant Muv or Vul strains, individual hermaphrodites can be phenotypically wild-type or can have the superficially opposite phenotype. Specifically, in certain Vul strains, some hermaphrodites lack a vulva but have more than one ventral protrusion at or near the site of the vulva; other hermaphrodites can have a functional vulva and one or possibly two supernumerary ectopic vulva-like structures. In certain Muv strains, some hermaphrodites lack a functional vulva and have a single ventral protrusion at the normal site of the vulva. Thus, the terms "Muv" and "Vul" refer to the most common phenotype

resulting from a mutation in a strain but do not necessarily accurately describe the phenotype of all individual hermaphrodites of that strain.

Complementation and mapping: To manipulate genetically Muv and Vul mutations, it is necessary to be able to mate either hermaphrodites or males carrying these mutations. A hermaphrodite without a vulva can reproduce, but it cannot mate with males. However, most of the vulval cell lineage mutants we have studied are of incomplete penetrance, i.e., some hermaphrodites form a functional vulva and are able to mate. In these cases genetic studies could be performed by mating males with egg-laying competent hermaphrodites. In contrast, in some strains the vulval defect is 100% penetrant. Males of such strains were induced by heat shock (HODGKIN 1983). If these males were able to mate, a doubly mutant strain containing him-5 was constructed, and the males produced by this strain were used for subsequent genetic manipulations. These two procedures enabled us to mate relatively easily all but three mutant strains. To mate hermaphrodites carrying the Vul mutation n300, we examined 3×10^5 hermaphrodites before a single egg-laying competent hermaphrodite was obtained. To mate strains carrying either the Vul mutation n676 or the Muv mutation n177, we mutagenized these strains and obtained extragenic mutations that permitted mating.

A new mutation was first tested for complementation against alleles of known genes of the same phenotypic class. As detailed below, Muv mutations in different genes result in different and often

distinctive phenotypes, so that in most cases it was necessary to test a new Muv mutation for complementation against Muv alleles of only one or a few genes. However, most Vul mutations result in similar phenotypes, and, thus it was necessary to test most new Vul mutations for complementation against Vul alleles of all genes. If a new mutation complemented alleles of those genes with mutations resulting in a similar phenotype, the mutation was mapped to one of the six linkage groups by testing for linkage to a standard marker on each chromosome using the protocol described in TRENT, TSUNG and HORVITZ (1983). Three-factor crosses were then performed to position the gene on the linkage group (Table 2-2). We have assigned new complementation groups defined by the Muv and Vul mutants lin (lineage abnormal) gene names. These genes are distributed fairly uniformly throughout the genome (Figures 2-1 and 2-2). Some details concerning these mapping and complementation experiments are presented below.

Mutant phenotypes and genetic characterization: This section, which is summarized in Table 2-3, describes characteristics of mutants defective in the genes affecting the vulval cell lineages. Phenotypes as visualized with a dissecting microscope are presented first, followed by data detailing genetic tests or strain manipulations. We constructed double mutants between all alleles of genes that were not already known to have amber alleles and one of the amber suppressors, sup-5 or sup-7 (see Materials and Methods). Only data from those suppression experiments in which we observed partial or complete suppression are presented below (or in Materials and Methods).

Table 2-2. Three-factor crosses were performed as described by BRENNER (1974). From heterozygotes of genotype ab/c recombinants A non-B and B non-A were picked. The progeny of each recombinant hermaphrodite were examined for the expression of the trans marker, c. In occasional crosses either C or AB hermaphrodites were picked and scored for the segregation of the trans marker(s). lin-8(n111); lin-9(n112) is a synthetic Muv strain; n111 and n112 each results in a wild-type phenotype when isolated, and, thus, each requires the presence of the other for scoring in mapping experiments. sup-9 and sup-10 are recessive suppressors of unc-93(e1500) (GREENWALD and HORVITZ 1980). The mapping of lin genes relative to these two genes was performed in the presence of unc-93(e1500).

Table 2-2

Three- and four-factor crosses

Gene	Genotype of heterozygote	Phenotype of selected hermaphrodites	Genotype of selected hermaphrodites (with respect to unselected marker)
<u>lin-25</u>	<u>+ unc-42 +/dpy-11 + lin-25</u>	Dpy	3/5 <u>unc-42/+</u>
	<u>+ him-5 +/lin-25 + unc-76</u>	Lin	3/10 <u>unc-42/+</u>
	<u>+ sma-1 +/unc-42 + lin-25</u>	Lin	2/9 <u>him-5/+</u>
		Unc	4/9 <u>him-5/+</u>
		Lin	4/6 <u>sma-1/+</u>
		Unc	11/14 <u>sma-1/+</u>
<u>lin-26</u>	<u>+ lin-26 +/dpy-10 + unc-4</u>	Unc	3/11 <u>lin-26/+</u>
	<u>lin-26 + +/+ vab-9 unc-4</u>	Unc	11/11 <u>lin-26/+</u>
	<u>+ vab-9 +/lin-26 + unc-4</u>	Vab	0/3 <u>lin-26/+</u>
	<u>+ lin-26 +/tra-2 + unc-4</u>	Unc	15/21 <u>vab-9/+</u>
	<u>+ rol-6 +/lin-26 + unc-4</u>	Unc	12/17 <u>lin-26/+</u>
		Unc	10/13 <u>rol-6/+</u>
	<u>+ lin-5 +/lin-26 + unc-4</u>	Vul	0/2 <u>rol-6/+</u>
		Unc	46/49 <u>lin-5/+</u>
<u>lin-31</u>	<u>+ unc-85 +/lin-31 + bli-2</u>	Lin	3/4 <u>unc-85/+</u>
	<u>lin-31 + +/+ unc-85 bli-2</u>	Lin	1/49 <u>unc-85 bli-2/+ +</u>
	<u>sup-9 + +/+ lin-31 unc-85; unc-93</u>	Sup	17/125 <u>lin-31 unc-85/+ +</u>
<u>lin-33</u> (n1043)	<u>lin-33 + +/+ unc-22 unc-30</u>	Unc-30	13/13 <u>lin-33/+</u>
	<u>(lin-33 +) +/ (+ unc-8) dpy-20</u>	Unc	0/13 <u>lin-33/+</u>
	<u>+ + lin-33/unc-17 dpy-13 +</u>	Unc	3/3 <u>lin-33/+</u>
		Dpy	0/3 <u>lin-33/+</u>
		non-Lin, non-Unc, non-Dpy	1/20 <u>lin-33/+ + +</u>
			1/20 <u>unc-17 dpy-13 lin-33/+ + lin-33</u>
<u>lin-33</u> (n1044)	<u>lin-33 + +/+ unc-22 unc-30</u>	Unc-30	44/44 <u>lin-33/+</u>
	<u>(lin-33 +) +/ (+ unc-8) dpy-20</u>	Unc	0/10 <u>lin-33/+</u>
	<u>+ (+ lin-33)/unc-17 (dpy-13 +)</u>	Unc	15/16 <u>lin-33/+</u>
		Dpy	0/19 <u>lin-33/+</u>
<u>lin-34</u>	<u>+ (lin-34 +)/unc-8 (+ dpy-20)</u>	Unc	4/4 <u>lin-34/+</u>
	<u>(lin-34 +) +/ (+ dpy-20) unc-22</u>	Unc	7/7 <u>lin-34/+</u>
	<u>+ (lin-34 +)/lin-3 (+ dpy-20)</u>	Dpy	0/16 <u>lin-34/+</u>
<u>let-23</u> (n1045)	<u>+ + let-23/dpy-10 lin-26 +</u>	Dpy	4/4 <u>let-23/+</u>
	<u>+ let-23 +/lin-5 + unc-4</u>	Unc	10/11 <u>let-23/+</u>
	<u>+ let-23 +/vab-9 + unc-4</u>	Unc	4/5 <u>let-23/+</u>

Table 2-2
Three- and four-factor crosses

Gene	Genotype of heterozygote	Phenotype of selected hermaphrodites	Genotype of selected hermaphrodites (with respect to unselected marker)
<u>lin-13</u>	<u>+ lin-13 +/daf-4 + unc-32</u> <u>+ lin-13 unc-36 +/lin-16 + + unc-86</u> <u>+ dpy-19 +/lin-13 + unc-32</u>	Unc Unc-86 Unc	11/25 <u>lin-13/+</u> 7/13 <u>lin-13 +/+</u> 1/13 <u>lin-13 unc-36/+</u> 3/21 <u>dpy-19/+</u>
<u>lin-15</u>	<u>unc-93; + lin-15 +/unc-3 + sup-10</u>	Sup	5/21 <u>lin-15/+</u>
<u>lin-17</u>	<u>lin-17 + +/+ unc-11 dpy-5</u> <u>bli-3 + +/+ lin-17 dpy-5</u> <u>+ + unc-13/bli-3 lin-17 +</u> <u>+ lin-6 +/bli-3 + lin-17</u>	Dpy Lin Lin Lin-17	4/4 <u>lin-17/+</u> 1/21 <u>bli-3/+</u> 1/17 <u>unc-13/+</u> 4/14 <u>lin-6/+</u>
<u>lin-18</u>	<u>unc-78 + +/+ lin-18 lon-2</u> <u>unc-78 lin-18 lon-2/+ + +</u> <u>+ dpy-23 +/lin-18 + lon-2</u>	Lin Lon Lon non-Unc Lin	1/41 <u>unc-78/+</u> 6/11 <u>lin-18/lin-18</u> 5/11 <u>lin-18/+</u> 4/4 <u>dpy-23/+</u>
<u>lin-24</u> (n432)	<u>+ + lin-24/unc-8 dpy-20 +</u> <u>+ lin-24 +/dpy-20 + unc-31</u> <u>+ + lin-24/dpy-20 unc-22 +</u> <u>+ unc-22 +/dpy-20 + lin-24</u> <u>+ lin-24 +/dpy-20 + unc-30</u> <u>+ lin-24 +/unc-22 + dpy-26</u>	Dpy Unc Dpy Unc Unc Unc Lin Dpy Unc Unc	0/24 <u>lin-24/+</u> 25/25 <u>lin-24/+</u> 6/11 <u>lin-24/+</u> 4/6 <u>lin-24/+</u> 0/3 <u>lin-24/+</u> 2/2 <u>lin-24/+</u> 2/9 <u>lin-24/+</u> 9/10 <u>lin-24/+</u> 3/10 <u>lin-24/+</u>
<u>lin-24</u> (n1057)	<u>+ + lin-24/dpy-20 unc-22 +</u> <u>+ lin-24 +/unc-22 + unc-30</u> <u>+ lin-24 +/unc-22 + dpy-26</u>	Unc Unc-22 Unc-30 Unc	0/5 <u>lin-24/+</u> 1/7 <u>lin-24/+</u> 17/18 <u>lin-24/+</u> 1/5 <u>lin-24/+</u>
<u>lin-25</u>	<u>+ + lin-25/dpy-11 unc-42 +</u> <u>+ lin-25 +/dpy-11 + unc-76</u>	Dpy Unc Dpy Unc	7/7 <u>lin-25/+</u> 0/3 <u>lin-25/+</u> 3/6 <u>lin-25/+</u> 6/9 <u>lin-25/+</u>

Table 2-2

Three- and four-factor crosses

Gene	Genotype of heterozygote	Phenotype of selected hermaphrodites	Genotype of selected hermaphrodites (with respect to unselected marker)
<u>lin-3</u>	<u>+ unc-8 +/unc-5 + lin-3</u> <u>+ + unc-31/unc-5 lin-3 +</u> <u>+ dpy-20 +/lin-3 + unc-31</u>	Lin Lin Lin	24/30 <u>unc-8/+</u> 0/12 <u>unc-31/+</u> 8/27 <u>dpy-20/+</u>
<u>lin-8</u>	<u>sup-9 + + +/+ lin-8 unc-85 dpy-10; unc-93 lin-9</u> <u>+ lin-8 + +/sup-9 + lin-31 unc-85; unc-93 lin-9</u>	Sup Sup	2/40 <u>lin-8 unc-85 dpy-10/+ + +</u> 11/17 <u>lin-8/+</u>
<u>lin-9</u>	<u>lin-8; + lin-9 +/dpy-19 + unc-32</u> <u>lin-8; + + unc-32/dpy-19 lin-9 +</u> <u>+ lin-9(n942) +/dpy-19 + unc-32</u> <u>+ lin-9(n942) +/sma-2 + unc-32</u>	Dpy Unc Dpy Lin Dpy Unc Sma Unc	16/16 <u>lin-9/+</u> 0/15 <u>lin-9/+</u> 15/15 <u>unc-32/+</u> 0/16 <u>unc-32/+</u> 5/5 <u>lin-9/+</u> 1/10 <u>lin-9/+</u> 3/4 <u>lin-9/+</u> 1/13 <u>lin-9/+</u>
<u>lin-10</u>	<u>+ + lin-10/dpy-5 unc-13 +</u> <u>+ lin-10 +/dpy-5 + unc-56</u> <u>+ unc-13 +/dpy-5 + lin-10</u>	Dpy Unc Dpy Unc Lin	10/10 <u>lin-10/+</u> 0/7 <u>lin-10/+</u> 13/15 <u>lin-10/+</u> 2/9 <u>lin-10/+</u> 3/19 <u>unc-13/+</u>
<u>lin-11</u>	<u>+ lin-11 +/dpy-5 + unc-75</u> <u>+ + lin-11/dpy-5 unc-29 +</u> <u>+ unc-29 +/dpy-5 + lin-11</u>	Dpy Unc Dpy Unc Dpy Lin	11/21 <u>lin-11/+</u> 3/7 <u>lin-11/+</u> 15/15 <u>lin-11/+</u> 0/15 <u>lin-11/+</u> 8/10 <u>unc-29/+</u> 4/21 <u>unc-29/+</u>
<u>lin-13</u>	<u>lon-1 + +/+ lin-13 unc-32</u> <u>+ lin-13 +/lon-1 + unc-32</u> <u>+ lin-13 +/lon-1 + unc-36</u>	Unc Unc Lon Lon Unc	12/12 <u>lon-1/+</u> 4/7 <u>lin-13/+</u> 5/12 <u>lin-13/+</u> 30/34 <u>lin-13/+</u> 1/16 <u>lin-13/+</u>

Figure 2-1. Partial genetic map of C. elegans indicating the markers used in this study. Genes that affect the vulval cell lineages are drawn above the lines representing the C. elegans linkage groups. The extents of deficiencies (Dfs) and duplications (Dps) are indicated below the lines. mnDf88 fails to complement lin-5 and lin-26. mnDf68 fails to complement vab-9, rol-6 and let-23. nDf27 fails to complement the genes in the interval between dpy-20 and ced-3.

Figure 2-2. Expanded genetic map showing the region of LGIII extending from lon-1 through lin-12. The precise map positions of lin-16, unc-86, sup-5 and sma-2 were determined by M. FINNEY (personal communication).

Figure 2-2

lin-1(e1026,e1275,e1777,n176,n303,n304,n383,n430,n431,n746,n753,n757,n1047,n1054,n1140,n1141) IV: Multivulva: The pattern of ventral protrusions in lin-1 hermaphrodites ranges from four large fairly evenly spaced protrusions to a single vast ventral protrusion at the vulva (Figure 2-4a). Many of the most severely affected hermaphrodites rupture at the vulva after the L4 molt. Males do not have ventral protrusions. One allele, e1275, is weaker and slightly heat-sensitive. e1275 males mate if grown at low temperatures. We have maintained some alleles as balanced heterozygotes, lin-1/nT1(IV); +/nT1(V). lin-1 was mapped by HORVITZ and SULSTON (1980).

As detailed in Table 2-1, two of nine lin-1 alleles tested, e1777 and n431, were suppressed by the amber suppressor sup-7 and thus probably eliminate lin-1 gene activity. Both were suppressed by a single copy of the suppressor, i.e., hermaphrodites of genotype lin-1(amber)/lin-1(amber); sup-7/+ were phenotypically wild-type. To further reduce the amount of suppressed lin-1 product by a factor of two, hermaphrodites of genotype lin-1(amber)/lin-1(null, nonamber); sup-7/+ were constructed. Of 36 hermaphrodites of genotype lin-1(e1777)/lin-1(e1026); sup-7/+, 35 had small protrusions at or near the vulva; however, the remainder of the ventral hypodermis appeared normal. The lin-1 alleles e1026, e1275, n176, n303, n304, n383 and n430 are not suppressed by sup-7.

lin-2(e1309,e1424,e1437,e1453,n105,n167,n305,n380,n397,n670,n674,n768,n1052) X: Vulvaless: The penetrance of the Vul defect in lin-2

Table 2-3. Reference alleles are those alleles used in mapping studies and/or studies of gene interactions (E. FERGUSON and P. STERNBERG, unpublished observations). Hermaphrodite and male phenotypes are described as viewed with a dissecting microscope. Data concerning the penetrances and expressivities of the Vul mutations are presented in Table 2-4. An "*" following "Vul" indicates that one or more alleles of the gene result in a phenotype described in the legend of Table 2-4. Data concerning male mating ability are presented in Table 2-13. Some genes have alleles that were not identified in our screen for Muv and Vul mutants. These alleles result in lethal or sterile phenotypes. The number of such alleles, if any, is noted following the number of Vul or Muv alleles of that gene, e.g., let-23, which has one allele that results in a Vul phenotype and three alleles that result in a larval lethal phenotype, has "1+3 non-Vul" alleles. am, amber; cs, cold-sensitive; hs, heat-sensitive; Egl, Egg-laying defective; WT, wild-type.

a--n300 has not been assigned to a gene (see Results).

Table 2-3

Genes that affect the vulval cell lineages

Genes	No. of alleles	Types of alleles	Reference allele(s)	Hermaphrodite phenotype	Male phenotype	Nature of alleles (evidence)
<u>lin-24 IV</u>	2	<u>n1057am</u> (non-null)	<u>n432</u>	Vul [*] : <u>n432</u> - incompletely penetrant, partially dominant. <u>n1057</u> - wild-type as homozygote; incompletely penetrant as heterozygote.	Wild-type morphology. Efficient mating.	Partially dominant, novel function?
<u>lin-25 V</u>	2	<u>n545hs</u>	<u>e1446</u> , <u>n545hs</u>	Vul: highly penetrant, some sterility.	Wild-type morphology. Mating abolished.	Recessive, unknown
<u>lin-26 II</u>	1		<u>n156</u>	Vul: highly penetrant. Slightly dumpy.	Very abnormal tail. Mating abolished.	Recessive, partially decreased function (phenotype enhanced by Df)
<u>lin-31 II</u>	11		<u>n301</u>	Muv: C-4 small ventral protrusions.	Wild-type morphology. Mating abolished.	Recessive, null (no. of alleles, phenotype not enhanced by Df)
<u>lin-33 IV</u>	2		<u>n1043</u>	Vul [*] : incompletely penetrant.	Wild-type morphology. Efficient mating.	Partially dominant, novel function?
<u>lin-34 IV</u>	1	<u>n1045am</u> (non-null)	<u>n1045am</u>	Muv: variable numbers of large protrusions.	Wild-type morphology. Mating greatly reduced.	Partially dominant, unknown
<u>let-23 II</u>	1 + 3 non-Vul	<u>n1045cs am</u> (non-null)	<u>n1045cs am</u>	Vul [*] : incompletely penetrant. Some <u>n1045</u> animals arrest during larval development. Non-Vul alleles result in fully penetrant larval arrest.	Wild-type morphology. Mating greatly reduced.	Recessive, partially reduced function (phenotype enhanced by Df, other alleles)
^a	1		<u>n300</u>	Vul: highly penetrant.	Slightly abnormal tail. Mating abolished.	Recessive, unknown, associated with translocation <u>nI(IV;V)</u>

Table 2-3

Genes that affect the vulval cell lineages

Genes	No. of alleles	Types of alleles	Reference allele(s)	Hermaphrodite phenotype	Male phenotype	Nature of alleles (evidence)
<u>lin-10 I</u>	3		<u>e1439</u>	Vul: incompletely penetrant.	Wild-type morphology. Efficient mating.	Recessive, null (phenotype not enhanced by Df)
<u>lin-11 I</u>	4		<u>n389</u>	Vul: 3 alleles highly penetrant. (n566 100% Egl but can mate.) Slightly Unc.	Wild-type morphology. Inefficient mating.	Recessive (n672 partially dominant), unknown
<u>lin-12 III</u>	7		<u>n137</u> (Muv) <u>n302</u> (Vul)	Muv: 5 small ventral protrusions. No vulva. Vul: highly penetrant as homozygotes, incompletely penetrant as heterozygotes.	Muv: homozygous: 5 ventral protrusions. Mating abolished. Heterozygous: 1-2 ventral protrusions. Efficient mating. Vul: wild-type morphology. Efficient mating.	Partially dominant, increased activity (dosage studies)
<u>lin-13 III</u>	2	<u>n387hs</u> , <u>n388hs</u>	<u>n387hs</u>	Muv: sterile, 0-4 ventral protrusions. Maternal effects.	Sterile, occasional ventral protrusions. Mating abolished.	Recessive, partially decreased function (phenotype enhanced by Df)
<u>lin-15 X</u>	5	<u>n765hs</u>	<u>n309</u> , <u>n765hs</u>	Muv: variable numbers of large protrusions. <u>n765</u> can display maternal effect.	Ventral protrusions. Mating abolished.	Recessive, partially decreased function? (phenotype possibly enhanced by Df)
<u>lin-17 I</u>	5		<u>n671</u>	Muv: small protrusion posterior to vulva.	Very abnormal tail. Mating abolished.	Recessive, unknown
<u>lin-18 X</u>	2	<u>e620hs</u> , <u>n1051hs am</u>	<u>e620hs</u> , <u>n1051hs am</u>	Muv: small protrusion posterior to vulva. Maternal effects.	Wild-type morphology. Efficient mating.	Recessive, null? (amber allele results in heat-sensitive phenotype)

Table 2-3

Genes that affect the vulval cell lineages

Genes	No. of alleles	Types of alleles	Reference allele(s)	Hermaphrodite phenotype	Male phenotype	Nature of alleles (evidence)
<u>lin-1 IV</u>	16	<u>e1777am</u> , <u>n431am</u> , <u>e1275hs</u>	<u>e1777am</u> , <u>e1275hs</u>	Muv: 1 large to 4 small protrusions.	Wild-type morphology. Mating abolished.	Recessive, null (no. of alleles, 2 amber alleles)
<u>lin-2 X</u>	13	<u>e1453am</u> (non-null), <u>n105hs</u>	<u>e1309</u> , <u>e1453am</u>	Vul ⁸ : incompletely penetrant.	Wild-type morphology. Efficient mating.	Recessive, null? (no. of alleles)
<u>lin-3 IV</u>	2 + 2 non-Vul		<u>e1417</u>	Vul: incompletely penetrant. Non-Vul alleles result in larval arrest or sterility.	Wild-type morphology. Efficient mating.	Recessive, partially decreased function (phenotype enhanced by non-Vul alleles)
<u>lin-4 II</u>	1		<u>e912</u>	Vul: highly penetrant. Thin and slightly long.	Very abnormal. Mating abolished.	Recessive, unknown
<u>lin-7 II</u>	13	<u>e974am</u> , <u>e1413am</u> , <u>e1449am</u> , <u>n308cs</u> , <u>n701cs</u>	<u>e1413am</u>	Vul ⁸ : incompletely penetrant.	Wild-type morphology. Efficient mating.	Recessive, null (no. of alleles, 3 amber alleles)
<u>lin-3 II</u>	1		<u>n111</u>	WT: Muv with <u>lin-9</u> (strain CB1322).	Wild-type morphology. Efficient mating.	Recessive, non-null? (phenotype possibly enhanced by Df)
<u>lin-9 III</u>	1		<u>n112</u>	WT: Muv with <u>lin-8</u> (strain CB1322).	Wild-type morphology. Efficient mating.	Recessive, partially decreased function (phenotype enhanced by sterile alleles)
CB1322				Muv: variable numbers of large protrusions. Slightly heat sensitive.	Ventral protrusions. Efficient mating.	

mutants ranges from a high of 90-95%, in e1309 and n1052 animals, to a low of about 21%, in n768 animals (Table 2-4). One allele, n105, is somewhat heat-sensitive: at 15^o, 24% of n105 hermaphrodites are Vul, whereas at 25^o, 90% of n105 hermaphrodites are Vul. One or rarely two ventral protrusions are evident in 15% of the Vul hermaphrodites of genotype lin-2(e1309). Males are phenotypically wild-type as viewed with a dissecting microscope. lin-2 was mapped by HORVITZ and SULSTON (1980).

Weak alleles of lin-2 (n768, n167, n105 at 15^o) result in two distinct phenotypes not seen in hermaphrodites carrying stronger alleles of lin-2 (e1309, n1052). First, some of these hermaphrodites are severely egg-laying defective (Egl) but not Vul; these hermaphrodites do not lack a vulva but rather have an abnormal vulva and are able to release some eggs or larvae. Second, some egg-laying competent hermaphrodites are Muv; these animals have a functional vulva and one or two ectopic supernumerary vulva-like structures (Table 2-4 and Figure 2-3g).

The penetrance of the Vul defect in e1309 hermaphrodites that pass through a dauer larval stage (CASSADA and RUSSELL 1975; RIDDLE, SWANSON and ALBERT 1981) is equivalent to that of unstarved animals (95% Vul, n = 227); however, the penetrance of the Vul phenotype markedly decreases in e1309 hermaphrodites that do not pass through a dauer larval stage but have been starved before reaching adulthood (36% Vul, n = 416).

Figure 2-3. Brightfield photomicrographs of a wild-type hermaphrodite, representative Vul hermaphrodites, and of non-Vul hermaphrodites carrying mutations in genes with Vul alleles. a, Wild-type hermaphrodite, the arrow indicates the vulva (bar, 200 μ m). b, The midsection of the same wild-type hermaphrodite as in "a"; the arrow indicates the vulva (bar, 100 μ m). c, A Vul hermaphrodite of genotype lin-24(n432) in which the progeny have hatched internally (bar, 200 μ m); this phenotype of a "bag of worms" is common to all Vul mutants and allows the scoring of the Vul phenotype in the dissecting microscope. d, The midsection of a Vul hermaphrodite of genotype lin-12(n302), the arrow indicates where the vulva is located in wild-type hermaphrodites (bar, 100 μ m). e, A hermaphrodite of genotype let-23(n1045) that has arrested during larval development (bar, 200 μ m); let-23(n1045) hermaphrodites that do not arrest during larval development display a cold-sensitive Vul phenotype. f, A hermaphrodite of genotype lin-3(n1059) that has arrested during larval development (bar, 100 μ m); two other alleles of lin-3, e1417 and n378, result in a Vul phenotype. g, The midsection of an egg-laying competent lin-7(n701) hermaphrodite with a functional vulva, indicated by an arrow, and an additional ventral protrusion, indicated by a line (bar, 100 μ m); although most lin-7(n701) hermaphrodites are Vul in phenotype, about 45% are not.

Figure 2-4. Brightfield photographs of representative Muv hermaphrodites. All photographs are to the same scale (bar, 200um).

a, A lin-1(e1777) hermaphrodite with two small ventral protrusions and a single large ventral protrusion, indicated by lines. The phenotype of lin-1(e1777) hermaphrodites ranges from one very large protrusion at the vulva to four evenly spaced smaller protrusions. The majority of lin-1(e1777) hermaphrodites do not have functional vulvae. b, A lin-8(n111); lin-9(n112) hermaphrodite with a functional vulva, indicated by an arrow, and two ventral protrusions indicated by lines. lin-8(n111); lin-9(n112) hermaphrodites usually have a normal vulva and between one and four ventral protrusions. c, A lin-12(n137) hermaphrodite that lacks a functional vulva and has five small ventral protrusions, indicated by lines. The middle protrusion is slightly larger than the other four protrusions. The phenotypes of lin-12(n137) hermaphrodites do not vary extensively. d, A lin-13(n387) hermaphrodite grown at 25° that is sterile and has four ventral protrusions, indicated by lines. lin-13 hermaphrodites that are grown at 25° are slightly smaller than the wild type, are sterile and usually have between two and four ventral protrusions. e-h, Four hermaphrodites carrying lin-15(n309) showing the variability of the Muv phenotype. e, A lin-15(n309) hermaphrodite with two ventral protrusions, indicated by lines, and a functional vulva, indicated by an arrow. f, A lin-15(n309) hermaphrodite with three ventral protrusions, indicated by lines, and a functional vulva, indicated by an arrow. g, A lin-15(n309) hermaphrodite with three ventral protrusions, indicated by lines. h, A lin-15(n309) hermaphrodite with six ventral protrusions, indicated by lines. i, A lin-17(n671)

hermaphrodite with a single ventral protrusion, indicated by a line, posterior to a functional vulva, indicated by an arrow. lin-17(n671) hermaphrodites are slightly longer than the wild type and have a single protrusion immediately posterior to the vulva; ventral protrusions are never observed in other locations. j, A lin-18(n1051) hermaphrodite with a single ventral protrusion, indicated by a line, posterior to a functional vulva, indicated by an arrow. lin-18 hermaphrodites have a single ventral protrusion immediately posterior to a vulva; ventral protrusions are never observed in other locations. k, A lin-31(n301) hermaphrodite with four ventral protrusions, indicated by lines. lin-31(n301) hermaphrodites have between zero and four small ventral protrusions and some lin-31 hermaphrodites lack a functional vulva. l, A lin-34(n1046) hermaphrodite with two ventral protrusions, indicated by lines, and a functional vulva, indicated by an arrow. lin-34(n1046) hermaphrodites usually have between zero and three variably-sized ventral protrusions either anterior or posterior to a functional vulva.

Figure 2-5. Brightfield photomicrographs of males carrying mutations that affect the vulval cell lineages. Only visibly mutant males are shown. All photographs are to the same scale (bar, 200um). a, Wild type. b, A lin-4(e912) male with a deformed tail. c, A lin-8(n111); lin-9(n112) male that has a single ventral protrusion, indicated by a line, and an ectopic hook-like structure in the tail, indicated by an arrowhead. lin-8(n111); lin-9(n112) males have between zero and two ventral protrusions. The ectopic hook is not usually visible in the dissecting microscope. ?rNomarski optics were used to determine that these tail structures have hook-like morphology and are generated by a lineage pattern similar to that that generates the hook (SULSTON and HORVITZ, 1981; P. STERNBERG, personal communication). d, A lin-12(n137) male with four small ventral protrusions, indicated by lines, and two ectopic hooks, indicated by arrowheads. The phenotype of lin-12(n137) males does not vary extensively. Only one ectopic hook is usually visible in the dissecting microscope. Nomarski optics were used to determine that these tail structures have hook-like morphology and are generated by a lineage pattern equivalent to that that generates the hook (GREENWALD, STERNBERG and HORVITZ, 1983). e, A lin-15(n309) male with two ventral protrusions, indicated by lines, and an ectopic hook-like structure, indicated by an arrowhead. lin-15(n309) males have between one and three ventral protrusions. The ectopic hook is not usually visible in the dissecting microscope. Nomarski optics were used to determine that these tail structures have hook-like morphology and are generated by a lineage pattern similar to that that generates the hook (P. STERNBERG, personal communication). f, A lin-17(n671) male with a deformed tail.

The allele e1453 does not result in the strongest lin-2 phenotype (Table 2-4) and, thus, probably does not totally eliminate lin-2 gene activity. Nonetheless, e1453 is an amber mutation. e1453 is suppressed in a sup-5 heterozygote, *i.e.*, hermaphrodites of genotype sup-5/+; lin-2(e1453)/lin-2(e1453) are wild-type (HORVITZ and SULSTON 1980). To reduce the amount of suppressed lin-2 gene product, hermaphrodites of genotype sup-5/+; lin-2(e1453)/lin-2(nonamber) were constructed. The nonamber allele used was e1309, the lin-2 allele of highest penetrance. Of 23 hermaphrodites of genotype sup-5/+; lin-2(e1453)/lin-2(e1309), two did not have functional vulvae. Although hermaphrodites of genotype sup-5/+ can have a vulval defect (three of 257 such hermaphrodites had abnormal vulvae), the penetrance of the vulval defect in hermaphrodites of genotype sup-5/+; lin-2(e1453)/lin-2(e1309) is probably greater, suggesting that the vulval defect of these animals results from the incomplete suppression of the Lin-2 phenotype.

lin-3(e1417,n378,n1058,n1059) IV: Vulvaless: The penetrance of the Vul defect of e1417 hermaphrodites is 89%, whereas that of n378 hermaphrodites is 97% (Table 2-4). In both cases 15% of the Vul hermaphrodites have a single ventral protrusion. Males are phenotypically wild-type as viewed with a dissecting microscope. lin-3 was mapped between unc-5 and dpy-20 by HORVITZ and SULSTON (1980).

The penetrance of the Vul phenotype in n378 hermaphrodites that pass through a dauer larval stage decreases from 97 to 70% (n = 389).

Table 2-4. To determine the penetrance and expressivity of the phenotype of each allele of the Vul genes, about ten plates each containing 25 L2 hermaphrodites were placed at 20° or at the indicated temperature. The percentage of hermaphrodites that turned into bags of worms was then determined. An "*" following the percentage of Vulvaless hermaphrodites indicates that some hermaphrodites carrying this Vul allele have an abnormal vulva and are severely Egl but are able to release some progeny. To determine the penetrance of these mutations, we measured the percentage of animals that were severely egg-laying defective without regard to their abilities to release progeny, and we report these data here as "% Vul." Most mutations in most Vul genes do not completely prevent vulval cell divisions (P. STERNBERG, personal communication). The vulval cells that are produced can form one or more ventral protrusions. For at least one allele of each gene, the percentage of Vul hermaphrodites with given numbers of ventral protrusions was determined. For weak alleles of three genes, lin-2, lin-7 and let-23, some egg-laying competent hermaphrodites also have ventral protrusions. The frequency of ventral protrusions in such hermaphrodites is prefaced by the phrase "non-Vul." For let-23(n1045), the phrase "non-Vul" refers only to adult hermaphrodites and not to let-23(n1045) hermaphrodites that arrest during larval development.

^a --n300 has not been assigned to a gene (see Results).

Table 2-4

Penetrance and expressivity of Vul alleles

			% Vul hermaphrodites with given No. of ventral protrusions				
Gene	Allele	%Vul		0	1	2	3
<u>lin-2</u>							
	<u>e1309</u> (n=286)	93		85	13	2	0
	<u>e1424</u> (n=358)	69		46	40	13	1
	<u>e1437</u> (n=221)	54					
	<u>e1453</u> (n=348)	50		38	53	8	1
15°	<u>n105</u> (n=261)	24*					
25°	<u>n105</u> (n=175)	90					
	<u>n167</u> (n=278)	41*					
	<u>n305</u> (n=251)	85					
	<u>n380</u> (n=267)	75					
	<u>n397</u> (n=308)	77					
	<u>n670</u> (n=339)	71					
	<u>n674</u> (n=295)	79					
	<u>n768</u> (n=358)	21*					
			Vul	12	46	34	8
			non-Vul	68	27	5	0
	<u>n1052</u> (n=190)	89					
<u>lin-3</u>							
	<u>e1417</u> (n=280)	89		84	16	0	0
	<u>n378</u> (n=266)	97		86	13	1	0
<u>lin-4</u>							
	<u>e912</u> (n=204)	100		96	4	0	0
<u>lin-7</u>							
	<u>e974</u> (n=272)	94		75	23	2	0
	<u>e1413</u> (n=341)	98		73	21	6	1
	<u>e1449</u> (n=214)	95					
	<u>n106</u> (n=362)	33*					
			Vul	50	36	12	2
			non-Vul	79	19	2	0
15°	<u>n308</u> (n=253)	91					
20°	<u>n308</u> (n=276)	65*					
			Vul	37	40	20	3
			non-Vul	67	16	16	1
25°	<u>n308</u> (n=179)	28*					
	<u>n385</u> (n=272)	91					
	<u>n673</u> (n=312)	94		80	17	3	0

Table 2-4

Penetrance and expressivity of Vul alleles

			% Vul hermaphrodites with given No. of ventral protrusions			
Gene	Allele	%Vul	0	1	2	3
<u>lin-7</u>						
	<u>n699</u> (n=387)	91	70	21	9	0
15°	<u>n701</u> (n=196)	96				
20°	<u>n701</u> (n=345)	62*				
25°	<u>n701</u> (n=236)	56*				
	<u>n759</u> (n=240)	91				
	<u>n760</u> (n=229)	94				
	<u>n763</u> (n=176)	93				
	<u>n764</u> (n=208)	94				
<u>lin-10</u>						
	<u>e1438</u> (n=220)	93	55	34	11	0
	<u>e1439</u> (n=282)	95	76	20	4	0
	<u>n299</u> (n=211)	97	84	13	3	0
<u>lin-11</u>						
	<u>n382</u> (n=182)	100	2	98	0	0
	<u>n389</u> (n=115)	100	3	97	0	0
	<u>n566</u> (n=233)	100	0	100	0	0
	<u>n672</u> (n=122)	100	3	97	0	0
<u>lin-24</u>						
	<u>n432</u> (n=232)	97*	92	8	0	0
	<u>n1057</u> (n=203)	0				
	<u>n1057/+</u> (n=314)	33*				
<u>lin-25</u>						
	<u>e1446</u> (n=279)	100	19	81	0	0
	11% are sterile					
15°	<u>n545</u> (n=305)	8				
25°	<u>n545</u> (n=234)	100	9	91	0	0
	18% are sterile					
<u>lin-26</u>						
	<u>n156</u> (n=268)	100	88	12	0	0
<u>lin-33</u>						
	<u>n1043</u> (n=221)	96*	95	5	0	0
	<u>n1044</u> (n=253)	95*	93	7	0	0

Table 2-4

Penetrance and expressivity of Vul alleles

Gene	Allele	%Vul	% Vul hermaphrodites with given No. of ventral protrusions			
			0	1	2	3
<u>let-23</u>	15°	<u>n1045</u> (n=219) 50				
		49% undergo larval arrest				
	20°	<u>n1045</u> (n=763) 14*				
		Vul 14 64 22 0				
		non-Vul 74 25 1 0				
25°	<u>n1045</u>	51% undergo larval arrest				
		(n=310) 2*				
		Vul 14 72 14 0				
		non-Vul 62 36 2 0				
a		28% undergo larval arrest				
	<u>n300</u>	(n=202) 100	100	0	0	0

In addition, the percentage of Vul hermaphrodites that have one or more ventral protrusions increases from 14 to 42%, suggesting that in these animals a greater number of vulval precursor cells have divided. However, the penetrance of the Vul defect in n378 hermaphrodites that do not pass through a dauer larva stage but have been starved before reaching adulthood is equivalent to that of unstarved animals (99% Vul, n = 180).

In an effort to determine the null phenotype of lin-3, a complementation screen to obtain new lin-3 alleles was performed against lin-3(e1417), as described in Materials and Methods. Two additional alleles of lin-3, n1058 and n1059, were obtained. n1059 animals arrest during early larval development with a rigid, rod-like phenotype (Figure 2-3f). Although most n1058 hermaphrodites are sterile adults, some arrest with a phenotype similar to that caused by n1059. To determine whether it is the lin-3 mutation in the lin-3(n1058) strain that results in the observed sterility, 30 Unc non-Dpy recombinants were picked from among the progeny of hermaphrodites of genotype unc-8(e49) + dpy-20/+ lin-3(n1058) +. These recombinant hermaphrodites were allowed to produce self progeny for 1 day and then were mated with lin-3(e1417) males. The self progeny these hermaphrodites were scored for the presence of sterile Unc animals, and the cross progeny were scored for the presence of Vul animals. In all cases the sterility cosegregated with lin-3(n1058). unc-8 and dpy-20 are approximately 2 map units apart. Thus, the sterility is linked to within 0.07 map units of lin-3(n1058) and probably results from the same mutation.

The phenotypes of hermaphrodites heterozygous for various lin-3 alleles are described in Table 2-5. n1059 increases the severity of the other lin-3 alleles in trans and, thus, probably results in a phenotype more close, resembling the null phenotype of lin-3. However, although n1058 results in a more severe phenotype than e1417, i.e., some n1058 animals undergo larval arrest, as do all animals of genotype n1058/n1059, n1058 reduces the penetrance of the Vul phenotype of e1417 in trans. These results are consistent with the hypothesis that these three mutations -- e1417, n1058, n1059 -- result in successively greater reductions of a single lin-3 activity and that e1417 and n1058 display partial intragenic complementation. However, these results are also consistent with an alternate hypothesis that lin-3 has two activities, an early function essential during larval development, which may also be necessary for fertility, and a later function needed during vulval development.

lin-4(e912) II: Vulvaless: Greater than 99% of lin-4 hermaphrodites form bags of worms and about 95% do not have ventral protrusions (Table 2-4). lin-4 hermaphrodites are thinner and slightly longer than the wild type. Some lin-4 males die before adulthood; others have incompletely formed tail structures (Figure 2-5b). lin-4 was mapped by HODGKIN (1974). The phenotype of lin-4 animals has been described by SULSTON and HORVITZ (1981) and CHALFIE, HORVITZ and SULSTON (1981) and interpreted as reflecting temporal transformations in the fates of certain cells by AMBROS and HORVITZ (1984). For ease of genetic manipulation lin-4 is maintained in two balanced heterozygote strains, lin-4(e912)/C1 dpy-10 unc-52 and

Table 2-5. Interactions among lin-3 alleles. Either the penetrance of the Vul phenotype (expressed as "% Vul") or a statement of the phenotype ("sterile" or "arrested larvae") is presented. Animals heteroallelic for different lin-3 mutations were obtained in the following ways. n378/e1417: non-Unc progeny from the mating of lin-3(n378); him-5 males with unc-32; lin-3(e1417) hermaphrodites. n378/n1058: non-Unc, non-Dpy progeny from hermaphrodites of genotype + lin-3(n378) dpy-20/unc-8(e49) lin-3(n1058) +. n378/n1059: non-Dpy progeny from hermaphrodites of genotype lin-3(n378) dpy-20/lin-3(n1059) +. e1417/n1058: non-Unc, non-Dpy progeny from hermaphrodites of genotype + lin-3(e1417) dpy-20/unc-8(e49) lin-3(n1058) +. e1417/n1059: non-Dpy progeny from hermaphrodites of genotype lin-3(e1417) dpy-20/lin-3(n1059) +. The phenotype of hermaphrodites of genotype lin-3(n1058)/lin-3(n1059) was determined by mating males of genotype + lin-3(n1059) +/unc-8(e49) + dpy-20 with hermaphrodites of genotype unc-8(e49) lin-3(n1058) +/unc-8(e49) + dpy-20. Many arrested larvae were seen among the progeny of the mating. The genotypes of 44 non-Unc progeny were determined; all were of genotype + lin-3(n1059) +/unc-8(e49) + dpy-20.

Table 2-5
Phenotypes of lin-3 homozygous
and heteroallelic strains

<u>lin-3</u> allele	<u>n378</u>	<u>e1417</u>	<u>n1058</u>	<u>n1059</u>
<u>n378</u>	97% Vul (n=266)	88% Vul (n=226)	78% Vul (n=364)	100% Vul (n=665)
<u>e1417</u>		89% Vul (n=351)	59% Vul (n=414)	99.8% Vul (n=471)
<u>n1058</u>			Sterile; occasional arrested larvae	Arrested larvae
<u>n1059</u>				Arrested larvae

lin-4(e912)/C1 dpy-10 unc-52; him-5.

e912 results in a slightly heat-sensitive phenotype. Although no egg-laying competent e912 hermaphrodites were observed at any temperature, at 15° 4% (n = 326) of e912 hermaphrodites have recognizable vulval structures as viewed with a dissecting microscope. Although 4% of e912 hermaphrodites grown at 20° have a ventral protrusion (Table 2-4), no such vulval structure is observed in these hermaphrodites.

lin-7(e974,e1413,e1449,n106,n308,n385,n673,n699,n701,n759,n760,n763,n764) II: Vulvaless: The penetrance of the Vul phenotype of all but one of the lin-7 mutants is about 95% (Table 2-4). The Vul phenotype of n106 hermaphrodites is much less penetrant (33%, n = 362). In addition, two alleles, n701 and n308, are cold-sensitive: at 15° both result in a Vul phenotype of about 95% penetrance, whereas at 25° the Vul phenotype resulting from both mutations is much less penetrant (62%, n701; 28%, n308). In general 20-30% of lin-7 hermaphrodites have one, or rarely two, ventral protrusions. Males are phenotypically wild-type as viewed with a dissecting microscope. lin-7 was mapped by HORVITZ and SULSTON (1980).

Weak alleles of lin-7 (n106, n308 at 25°, n701 at 25°) result in two distinct phenotypes not seen in hermaphrodites carrying a stronger allele of lin-7 (e.g., e1413). First, some of these hermaphrodites are severely Egl but not Vul; these hermaphrodites do not lack a vulva but rather have an abnormal vulva and are able to release some eggs or

larvae. Second, some egg-laying competent hermaphrodites are Muv; these animals have a functional vulva and one or two ectopic supernumerary vulva-like structures (Table 2-4).

The penetrance of the Vul phenotype in e1413 hermaphrodites that pass through a dauer larval stage is probably equivalent to that of unstarved animals (87% Vul, n = 171); however, the penetrance of the Vul phenotype markedly decreases in e1413 hermaphrodites that do not pass through a dauer larva stage but have been starved before reaching adulthood (22% Vul, n = 273).

e1413 is an amber mutation (HORVITZ and SULSTON 1980) and, thus, probably eliminates lin-7 gene activity. It is suppressed by a single copy of sup-7, i.e., hermaphrodites of genotype lin-7(amber)/lin-7(amber); sup-7/+ are phenotypically wild-type (HORVITZ and SULSTON 1980). As detailed in Table 2-1, two of the eight other lin-7 alleles tested, e974 and e1449, were also suppressed by a single copy of sup-7. To further reduce the amount of suppressed lin-7 product by a factor of two, hermaphrodites of genotype lin-7(amber)/lin-7(null, nonamber); sup-7/+ were constructed. Of 55 hermaphrodites of genotype lin-7(e1413)/lin-7(n385); sup-7/+, six were either egg-laying defective or lacked a functional vulva. Although hermaphrodites of genotype sup-7/+ can have a vulval defect (two of 226 such hermaphrodites had abnormal vulvae), the penetrance of the vulval defect in hermaphrodites of genotype lin-7(amber)/lin-7(null, nonamber); sup-7/+ is much greater, suggesting that the vulval defect of these animals results from the incomplete suppression of the lin-7

phenotype. The alleles n308, n385, n673, n701, n699 were not suppressed by sup-7.

CB1322: Multivulva: The Muv strain CB1322 carries two unlinked mutations, lin-8(n111) II and lin-9(n112) III. Each of these mutations alone results in a wild-type phenotype (HORVITZ and SULSTON 1980). Many CB1322 hermaphrodites have two ventral protrusions, one anterior and one posterior to the vulva (Figure 2-4b). Some hermaphrodites have an abnormal vulva and are egg-laying defective. The males of this strain have from zero to two ventral protrusions (Figure 2-5c).

lin-8(n111) II: Wild-type: When separated from lin-9(n112), lin-8(n111) results in a wild-type phenotype in both hermaphrodites and males. lin-8 was mapped between cat-2 and unc-85 by HORVITZ and SULSTON (1980).

We have established that nDf3 fails to complement lin-8. Males of genotype lin-8(n111) dpy-10/+ +; lin-9(n112)/+ were mated with hermaphrodites of genotype nDf3 +/lin-31 bli-2. [nDf3 fails to complement lin-31 (see below) but complements bli-2 and unc-85 (GREENWALD and HORVITZ 1980).] From F1 progeny hermaphrodites of genotype lin-8(n111) dpy-10/nDf3 +; lin-9(n112)/+, Muv non-Dpy hermaphrodites of putative genotype lin-8(n111) dpy-10/nDf3 +; lin-9(n112) were picked. These hermaphrodites segregated approximately 1/4 dead eggs, confirming the presence of nDf3.

The phenotype of hermaphrodites of genotype lin-8(n111)/nDf3; lin-9(n112) suggests that n111 may not eliminate lin-8 gene activity. The phenotype of these hermaphrodites is similar to the phenotype of lin-8(n111); lin-9(n112) hermaphrodites except that, relative to lin-8(n111); lin-9(n112) hermaphrodites, hermaphrodites of genotype lin-8(n111)/nDf3; lin-9(n112) have a higher incidence of sterility and general sickness. This sterility and sickness is not seen in either hermaphrodites of genotype lin-8(n111)/nDf3, which have a wild-type phenotype, or in hermaphrodites of genotype nDf3/+; lin-9(n112). [Hermaphrodites of the former genotype were constructed by picking progeny of hermaphrodites of genotype lin-8(n111) dpy-10/nDf3 +; lin-9(n112)/+ that segregated approximately 1/4 dead eggs and did not segregate any Muv progeny. Hermaphrodites of the latter genotype were constructed by mating males of genotype unc-85/+; dpy-17 lin-9(n112)/+ with hermaphrodites of genotype nDf3 +/lin-31 bli-2, and, from F1 progeny of genotype nDf3 +/unc-85; dpy-17 lin-9/+ +, picking F2 Dpy non-Unc animals of genotype nDf3 +/unc-85; dpy-17 lin-9.]

lin-9(n112) III: Wild-type: When separated from lin-8(n111), lin-9(n112) results in a wild-type phenotype in both hermaphrodites and males. lin-9 was shown to map near unc-32 by HORVITZ and SULSTON (1980). A second allele of lin-9, n942, that results in a sterile phenotype and will be described elsewhere was used to map lin-9 between sma-2 and unc-32.

n112 results in a temperature-dependent maternal effect, which is evident at 15° but not at 20° or 25°. At 15°, from parental

hermaphrodites of genotype lin-8(n111); dpy-17 lin-9(n112)/+ +, only 2% (n = 150) of the progeny hermaphrodites of genotype lin-8(n111); dpy-17 lin-9(n112) were Muv.

lin-10(e1438,e1439,n299) I: Vulvaless: The penetrance of the Vul phenotype of all three alleles of lin-10 is about 95% (Table 2-4). Sixteen percent (n = 211) of n299 hermaphrodites that are Vul have one, or occasionally two, ventral protrusions. However, even though 93% (n = 220) of e1438 hermaphrodites are Vul, 45% of the Vul hermaphrodites have one or more ventral protrusions. Males are phenotypically wild-type as viewed with a dissecting microscope.

Unlike the six other genes with incompletely penetrant Vul mutations (lin-2, lin-3, lin-7, lin-24, lin-33 and let-23), the penetrance of the Vul phenotype of lin-10(n299) hermaphrodites is not decreased either by starvation or by passage through the dauer larval stage (E. FERGUSON, unpublished results).

A series of deficiencies in the unc-13 region were used to further map lin-10. The deficiencies sDf5 and nDf23 complement lin-10, whereas the deficiencies nDf24 and nDf25 fail to complement lin-10. [sDf5 complements both nDf24 and nDf25; nDf23, nDf24 and nDf25 all fail to complement lin-28 (E. FERGUSON, unpublished results).] The penetrance of the Vul phenotype in hermaphrodites of genotype lin-10(e1439)/Df is not enhanced relative to the penetrance of the Vul phenotype in e1439 hermaphrodites: 92% (n = 177) of hermaphrodites of genotype dpy-5 lin-10(e1439)/+ nDf25 were Vul, and 88% (n = 139) of

hermaphrodites of genotype dpy-5 lin-10(e1439)/+ nDf24 were Vul. These results are consistent with the hypothesis that e1439 reduces or eliminates lin-10 activity and that lack of lin-10 gene activity results in a Vul phenotype.

lin-11(n382,n389,n566,n672) I: Vulvaless: The egg-laying defect of all four mutants defective in this gene is 100% penetrant; greater than 95% of all hermaphrodites have a single ventral protrusion and all form bags of worms. No hermaphrodites of genotypes n382, n389 or n672 have been observed to form a functional vulva. However, n566 hermaphrodites can form a functional vulva, as some animals are able to mate. Hermaphrodites and males carrying any of the four alleles are slightly uncoordinated. Males have an otherwise wild-type phenotype as viewed in a dissecting microscope.

Males of genotypes n382, n389 or n682 were originally obtained by heat shock and mated to unc-32; him-5 hermaphrodites to generate lin-11; him-5 strains, which were used for subsequent genetic manipulations. Because lin-11 males mate inefficiently (Table 2-13), about 20 L4 lin-11; him-5 males were generally used in each mating.

n382, n389 and n566 are recessive mutations. n672 is slightly semidominant, as approximately 5% of heterozygous hermaphrodites are Vul. n672 was assigned to lin-11 (i.e., interpreted as allelic with the three recessive mutations) based on its map position and on complementation data. n672; him-5 males were mated to hermaphrodites of genotype dpy-5 + lin-11(n389)/dpy-5 unc-29 +. Eleven F1 Lin

non-Dpy hermaphrodites were picked. All of their progeny were Vul, indicating that they were of genotype dpy-5 lin-11(n389)/+ lin-11(n672). Sixteen F1 non-Dpy egg-laying-competent hermaphrodites were also picked. The progeny of these hermaphrodites indicated that their genotype was + + lin-11(n672)/dpy-5 unc-29 +. From one of these hermaphrodites three Unc non-Dpy and eight Dpy non-Unc animals were picked. Zero of three Unc animals and eight of eight Dpy animals segregated n672, confirming that n672 is close to or right of unc-29, as is lin-11(n389).

lin-12(n137,n177,n427,n302,n379,n676,n769) III: Multivulva and Vulvaless: Seven partially dominant mutations affecting the vulval cell lineages map to the same region of LGIII: all seven of these mutations are closely linked to unc-32 III, and most have been shown to map slightly to the right of unc-32 (Table 2-6). These mutations result in three different phenotypes: n137, n177 and n427 are semidominant Muv mutations; n302, n379 and n676 are semidominant Vul mutations; n769 is a dominant Vul and a recessive Muv mutation. The three semidominant Muv mutations are slightly cold-sensitive (Table 2-7).

Homozygous Muv hermaphrodites lack a functional vulva, have a small brood size and generally have five ventral protrusions (Figure 2-4c). Heterozygous Muv hermaphrodites are similar but more fertile (Table 2-8). The penetrance of the Vul defect is greater than 95% in all three homozygous Vul strains but is lower in heterozygous strains (Table 2-9).

Homozygous Muv males (Figure 2-5d) have four midbody ventral protrusions. In addition, Muv males have two ventral protrusions just anterior to the tail; however, only the anterior these two protrusions is usually visible as viewed with a dissecting microscope. A single protrusion just anterior to the tail is observed in about 70% of the heterozygous Muv males at 20°. About 25% of these males have either one or two protrusions midbody. Vul males have a wild-type phenotype as viewed with a dissecting microscope.

The lack of a functional vulva is a highly penetrant defect in all of the homozygous Vul and Muv lin-12 strains. To permit genetic manipulations, these mutations are maintained in a number of different genetic backgrounds. Because the homozygous Vul males mate efficiently, the three Vul strains are maintained as lin-12(Vul); him-5 strains. However, because neither homozygous males nor heterozygous hermaphrodites of the other four lin-12 alleles are able to mate, heterozygous males of two different genotypes [+ lin-12/unc-32 +; him-5 or lin-12/eT1(III); him-5/eT1(V) him-5] are used to transfer these mutations. [n886, a recessive lethal mutation linked to eT1(III;V) (M. FINNEY, personal communication) is also present in the latter strain. him-5 was placed on eT1(V) by recombination (V. AMBROS, personal communication).]

Heteroallelic lin-12 strains were constructed to examine the interactions between the different mutations. Three classes of heteroallelic strains were constructed: lin-12(Muv)/lin-12(Muv), lin-12(Muv)/lin-12(Vul) and lin-12(Vul)/lin-12(Vul). The

Table 2-6. Three-factor crosses that indicate the map position of most lin-12 alleles. In these crosses n137 was treated as a strictly dominant mutation, and n769 was treated as a dominant mutation with different phenotypes as a heterozygote and as a homozygote. Because of the incompletely penetrant dominant phenotypes of the lin-12 Vul alleles n379, n302 and n676, the progeny of recombinant hermaphrodites were examined for the segregation of these markers. WT, wild-type.

Table 2-6

Map data for lin-12 alleles: three- and four-factor crosses

Allele	Genotype of heterozygote	Phenotype of recombinant	Genotype of selected recombinants
<u>lin-12(n137)</u>	<u>+ + lin-12/dpy-19 unc-32 +</u>	Unc	10/10 <u>+ unc-32 +/dpy-19 unc-32 +</u>
		Dpy	11/11 <u>dpy-19 + lin-12/dpy-19 unc-32 +</u>
		WT	8/8 <u>+ + +/dpy-19 unc-32 +</u>
	<u>unc-36 + +/+ unc-32 lin-12</u>	WT	3/19 <u>+ unc-32 +/unc-36 + +</u>
			16/19 <u>+ + +/unc-36 + +</u>
	<u>+ dpy-19 + +/unc-36 + unc-32 lin-12</u>	WT	1/1 <u>unc-36 + + +/+ dpy-19 + +</u>
		Unc-36 Lin	2/3 <u>unc-36 + + +/unc-36 + unc-32 lin-12</u>
			1/3 <u>unc-36 dpy-19 + +/unc-36 + unc-32 lin-12</u>
		Unc-32 Lin	2/4 <u>+ dpy-19 unc-32 lin-12/unc-36 + unc-32 lin-12</u>
			2/4 <u>+ + unc-32 lin-12/unc-36 + unc-32 lin-12</u>
	<u>unc-36 + +/+ dpy-19 lin-12</u>	WT	3/3 <u>+ dpy-19 +/unc-36 + +</u>
<u>lin-12(n302)</u>	<u>+ (+ lin-12)/dpy-19 (unc-32 +)</u>	Unc	9/9 <u>+ + +/dpy-19 (unc-32 +)</u>
		Dpy	2/2 <u>dpy-19 (+ lin-12)/dpy-19 (unc-32 +)</u>
<u>lin-12(n379)</u>	<u>+ + lin-12/dpy-19 unc-32 +</u>	Unc	7/7 <u>+ + +/dpy-19 unc-32 +</u>
		Dpy	5/5 <u>dpy-19 + lin-12/dpy-19 + unc-32</u>
	<u>+ unc-32 + +/dpy-19 + lin-12 unc-69</u>	Dpy	5/9 <u>dpy-19 unc-32 + +/dpy-19 + lin-12 unc-69</u>
			4/9 <u>dpy-19 + + +/dpy-19 + lin-12 unc-69</u>
<u>lin-12(n676)</u>	<u>+ + lin-12/dpy-19 unc-32 +</u>	Dpy	1/1 <u>dpy-19 + lin-12/dpy-19 unc-32 +</u>
		Unc	3/3 <u>+ unc-32 +/dpy-19 unc-32 +</u>
	<u>+ lin-12 +/dpy-19 + unc-69</u>	Unc	6/11 <u>+ lin-12 unc-69/dpy-19 + unc-69</u>
			5/11 <u>+ + unc-69/dpy-19 + unc-69</u>
	<u>dpy-19 + +/+ unc-32 lin-12</u>	Unc	3/3 <u>+ unc-32 +/+ unc-32 lin-12</u>
<u>lin-12(n759)</u>	<u>+ lin-12 +/dpy-19 + unc-69</u>	Dpy	2/7 <u>dpy-19 lin-12 +/dpy-19 + unc-69</u>
		Unc	5/7 <u>dpy-19 + +/dpy-19 + unc-69</u>
			10/11 <u>+ lin-12 unc-69/dpy-19 + unc-69</u>
			1/11 <u>+ + unc-69/dpy-19 + unc-69</u>
	<u>+ unc-32 +/dpy-19 + lin-12</u>	WT	2/2 <u>+ unc-32 +/dpy-19 + +</u>
		Unc Lin/+ (Vul)	1/1 <u>+ unc-32 +/+ unc-32 lin-12</u>
		Dpy Lin/+ (Vul)	1/1 <u>dpy-19 unc-32 +/dpy-19 + lin-12</u>
	<u>+ unc-32 +/dpy-17 + lin-12</u>	WT	1/1 <u>+ unc-32 +/dpy-17 + +</u>

Table 2-7. The numbers of ventral protrusions of males were counted using a dissecting microscope. Males homozygous for the Muv alleles of lin-12 -- n137 and n427 -- or for lin-12(n769) have one or more midventral protrusions. Males homozygous for the Vul alleles of lin-12 -- n379, n302, and n676 -- or heterozygous for lin-12(n769) are phenotypically wild-type. When numbers of protrusions are specified, one protrusion is directly anterior to the tail; the remaining protrusions are located in the midsection of the animal. These numbers do not include a second protrusion just anterior to the tail that is usually not visible as viewed with a dissecting microscope. The "Other" category contains animals with unusual numbers or positions of protrusions. These data are presented in increasing order of severity of phenotypic effect upon the male hypodermis. Except for lin-12(n769), the alleles used in this study have also been ranked according to the severity of their effect upon the gonadal anchor cell by GREENWALD, STERNBERG and HORVITZ (1983). The relative orders of the two rankings are equivalent, except that lin-12(n379) appears to result in a higher level of lin-12 activity in the hypodermis than does lin-12(n302), opposite to their relative order of elevation of lin-12 activity in the gonad. The following strains were used. n427/+: non-Unc male cross progeny from the mating of + lin-12(n427)/unc-32 +; him-5 males with unc-32 hermaphrodites. n137/+: non-Unc males from the strain + unc-32 lin-12(n137)/unc-36 +; him-5. n769/n769: males from the strain dpy-19 lin-12(n769); him-5. n137/n769: Dpy male cross progeny from the mating of dpy-17 + lin-12(n769)/+ unc-32 +; him-5 males with dpy-17 + lin-12(n137)/+ unc-32 lin-12(n137 n720) hermaphrodites. lin-12(n137 n720) is a null

allele of lin-12 (GREENWALD, STERNBERG and HORVITZ 1983);
approximately 10% of the hermaphrodites of genotype
lin-12(n137)/lin-12(n137 n720) form an anchor cell and are able to
mate. n137/n302: non-Unc males from the strain + unc-32
lin-12(n137)/unc-86(e1416) + lin-12(n302); him-5. n137/n379: non-Unc
males from the strain + unc-32 lin-12(n137)/unc-86(e1416) +
lin-12(n379); him-5. n137/n676: Unc male cross progeny from the
mating of + unc-32 lin-12(n137)/dpy-19 + +; him-5 males with + unc-32
lin-12(n676)/dpy-19 + + hermaphrodites. n137/n137: Unc-32 males
from the strain + unc-32 lin-12(n137)/unc-36 + +; him-5.

Table 2-7

Interactions among lin-12 mutations: male ventral hypodermis

Genotype	Temperature	% males with given no. of protrusions						
		0	1	2	3	4	5	Other
<u>n427/+</u>	15° (n=128)	26	51	14	2	1		6
	25° (n=132)	75	20	2				3
<u>n137/+</u>	15° (n=182)	20	47	16	10	2		5
	20° (n=159)	29	52	11	4	1		3
	25° (n=160)	63	34					3
<u>n769/n769</u>	15° (n=170)	39	28	14	3	3	1	12
<u>n137/n769</u>	15° (n=84)		3	7	6	25	43	16
	25° (n=85)	13	18	8	13	24	13	11
<u>n137/n302</u>	15° (n=65)			1	11	28	53	7
	20° (n=71)			13	17	24	35	11
	25° (n=65)	1	9	20	29	29	3	7
<u>n137/n379</u>	15° (n=87)				3	6	78	13
	20° (n=71)		1	8	15	37	37	2
	25° (n=54)	4	9	11	21	24	9	22
<u>n137/n676</u>	20° (n=86)			1	7	27	58	7
<u>n137/n137</u>	15° (n=70)					9	86	5
	20° (n=68)					9	85	6
	25° (n=83)				4	10	82	4

heteroallelic interactions of the Muv alleles with each other and with the Vul alleles could not be differentiated in hermaphrodites of different genotypes because all such heteroallelic hermaphrodites have a Muv phenotype. However, these interactions could be quantified in males by counting the number of ventral protrusions. The interactions between the Vul mutations were quantified by measuring the penetrance of the Vul defect in the heteroallelic strain. [n769, which results in a totally penetrant Vul phenotype as a heterozygote, was examined in trans only with a lin-12(Muv) allele.] To generate males carrying different pairs of Muv alleles in trans, heterozygous Muv males (lin-12(n177)/+ or lin-12(n427)/+) were mated with sup-17(n316); lin-12(n137) hermaphrodites. [sup-17(n316) I is an extragenic suppressor of partially dominant lin-12 alleles isolated by phenotypically reverting lin-12(n177); E. FERGUSON, unpublished results.] In both cases approximately one-half of the progeny males were phenotypically indistinguishable from homozygous n137 males, indicating that n177 and n427 interact with n137. Similarly, the penetrance of the Vul defect in hermaphrodites bearing two different Vul mutations in trans is similar to the penetrance of the homozygous Vul strains (Table 2-7). In addition, the Vul mutations enhance the phenotype of the Muv mutation n137 in trans: lin-12(Muv)/lin-12(Vul) is more mutant than lin-12(Muv)/+ but less mutant than lin-12(Muv)/lin-12(Muv) (Tables 2-7 and 2-8). This pattern can be seen both in the fertility of hermaphrodites and in the number of ventral protrusions of males.

A detailed genetic analysis of these and other partially dominant

Table 2-8. Progeny produced at 25° were counted. In the majority of lin-12 strains, all hermaphrodites lack functional vulvae and turn into bags of worms. However, in a few lin-12 strains, some hermaphrodites are egg-laying competent. These hermaphrodites have a much larger brood size than the egg-laying defective individuals of the same strain. Thus, to compare the brood sizes of differing lin-12 strains, only progeny from hermaphrodites unable to lay eggs were counted. The following strains were used. n379/+: non-Unc Vul cross progeny from the mating of lin-12(n379); him-5 males and unc-32 hermaphrodites. n302/+: non-Dpy Vul cross progeny from the mating of wild-type males and hermaphrodites of genotype dpy-19 + lin-12(n302)/dpy-19 unc-32 +. n137/+: non-Unc, non-Dpy hermaphrodites from the strain + unc-32 lin-12(n137)/dpy-19 + +. n137/n379: non-Unc hermaphrodites from the strain + unc-32 lin-12(n137)/unc-86(e1416) + lin-12(n302). n137/n302: non-Unc, non-Dpy hermaphrodites from the strain + unc-32 lin-12(n137)/dpy-19 + lin-12(n302).

Table 2-8

Interactions among lin-12 mutations:

brood sizes of hermaphrodites

Genotype	Average no. of progeny
<u>n379/+</u>	53 (n=23)
<u>n302/+</u>	58 (n=34)
<u>n137/+</u>	57 (n=47)
<u>n137/n379</u>	38 (n=54)
<u>n137/n302</u>	20 (n=49)
<u>n137/n137</u>	11 (n=54)

Table 2-9. L3 hermaphrodites of each genotype were picked and later scored for their ability to lay eggs. The following strains were used. n379/+: non-Unc hermaphrodites from the strain unc-86(e1416) + + lin-12(n379)/+ dpy-19 unc-32 +. n302/+: non-Unc hermaphrodites from the strain unc-86(e1416) + + lin-12(n302)/+ dpy-19 unc-32 +. n676/+: non-Unc hermaphrodites from the strain + + unc-32 lin-12(n676)/unc-36 dpy-19 + +. n769/+: non-Unc cross progeny from the mating of + lin-12(n769)/unc-32 + males and unc-32 hermaphrodites. n302/n379: non-Dpy, non-Unc hermaphrodites from the strain + dpy-19 lin-12(n302)/unc-86(e1416) + lin-12(n379). n676/n379: non-Dpy non-Unc hermaphrodites from the strain dpy-19 + lin-12(n379)/+ unc-32 lin-12(n676). n676/n302: non-Dpy non-Unc hermaphrodites from the strain dpy-17 + lin-12(n302)/+ unc-32 lin-12(n676).

Table 2-9

Interactions among lin-12 mutations: percentages
of Vulvaless hermaphrodites

	<u>+</u>	<u>n379</u>	<u>n302</u>	<u>n676</u>
<u>n379</u>	9 (n=172)	97.6 (n=427)		
<u>n302</u>	66 (n=221)	99.1 (n=426)	99.8 (n=419)	
<u>n676</u>	75 (n=387)	99.1 (n=451)	100 (n=488)	100 (n=482)
<u>n769</u>	100 (n=425)			

lin-12 alleles has been reported (GREENWALD, STERNBERG, and HORVITZ 1983). These studies have (1) led to the identification of amber alleles of lin-12 and shown that loss of lin-12 function does not result in a Vul or Muv phenotype and (2) demonstrated that the Muv and Vul mutations we are describing in this paper are alleles of lin-12 and result in increases in lin-12 gene activity. The differing phenotypes (Muv and Vul) caused by these alleles appear to reflect differences in the magnitude of the increase in lin-12 activity. The elevated level of lin-12 activity resulting from all seven partially dominant lin-12 alleles is sufficient to prevent the formation of the gonadal anchor cell, which is necessary for the generation of a functional vulva. Thus, all seven mutants fail to form a functional vulva. However, only in the three Muv strains is the increase in the level of lin-12 activity apparently sufficient to alter directly the lineages of the cells of the ventral hypodermis and to thereby result in a Muv phenotype.

lin-13(n387,n388) III: Multivulva: Both alleles of lin-13 result in a heat-sensitive sterile Muv phenotype and a temperature-dependent maternal effect. The protrusions of the Muv hermaphrodites are variably sized and evenly spaced along the ventral side. The phenotype of homozygous lin-13 hermaphrodites segregating from a heterozygous strain depends on the temperature at which the strain was grown. At 25⁰, n387 hermaphrodites segregating from a heterozygote are both Muv and sterile (Figure 2-4d). At 20⁰, about half of the n387 hermaphrodites segregating from a heterozygote are sterile, but only a few of the animals are Muv. At 15⁰, n387 hermaphrodites

segregating from a heterozygote are almost wild-type in appearance and fertility. However, if the progeny of these 15° animals are grown at 15°, all are sterile and some are Muv. If the progeny of these 15° animals are grown at 25°, some animals arrest during larval growth, and the rest are both sterile and Muv. The male phenotype similarly is heat-sensitive; only males that are the progeny of lin-13 hermaphrodites and are grown at 20° or 25° have ventral protrusions. The lin-13 alleles are maintained in the balanced strains + lin-13 +/dpy-17 + unc-86, lin-13 +/+ unc-32; him-5 or lin-13/eT1(III); him-5/eT1(V) him-5.

lin-13 acts both maternally and zygotically. The maternal activity of lin-13 can be observed at 15°: lin-13 homozygous hermaphrodites that are the progeny of a lin-13/+ parent are phenotypically wild-type, whereas their progeny, which lack the maternal component of lin-13 activity, are sterile and sometimes Muv. However, the zygotic activity of lin-13 is sufficient to generate a wild-type phenotype: if fertile lin-13 hermaphrodites are mated with wild-type males, cross progeny hermaphrodites of genotype lin-13/+ lack the maternal component of lin-13 activity but are phenotypically wild-type.

Both lin-13 alleles were obtained by discovering sterile Muv animals among the F2 progeny of mutagenized hermaphrodites and picking many phenotypically wild-type hermaphrodites from the same plate. A few such hermaphrodites proved to be heterozygous for the Muv mutation. To map (and balance) these mutations, hermaphrodites

heterozygous for the Muv mutations were mated with wild-type males and the male progeny (one-half of which were heterozygous for the Muv mutation) were mated with hermaphrodites of the two mapping strains described in Materials and Methods. Both mutations were balanced by unc-32.

The deficiency nDf16 (V. AMBROS and M. FINNEY, personal communication) fails to complement lin-13. The phenotype of hermaphrodites of genotype lin-13(n387)/nDf16 was determined by mating lin-13(n387) + unc-32/+ unc-86(n848) + males with hermaphrodites of genotype nDf16 +/unc-86(e1416) unc-32. The non-Unc-32 non-Unc-86 progeny were of genotype lin-13(n387) unc-32/nDf16 +. (nDf16 fails to complement unc-86 but complements unc-32.) The phenotype of lin-13(n387)/nDf16 hermaphrodites depended upon the temperature at which the cross was performed. At 15° these hermaphrodites were sterile and had a single protrusion at the vulva. At 25° these hermaphrodites arrested during what appeared by size to be the L2 stage. Because nDf16 in trans to a lin-13 allele increased the severity of the lin-13 phenotype at all temperatures tested, it is likely that the two Muv alleles of lin-13 do not result in the null phenotype of the locus.

lin-15(e1763,n309,n377,n765,n1139) X: Multivulva: All alleles of lin-15 result in a similar Muv phenotype (Figure 2-4e-h): some hermaphrodites have a normal vulva and two or three large protrusions (one or two anterior to the vulva and one posterior), and other hermaphrodites have similar patterns of protrusions but lack a

functional vulva. A number of hermaphrodites rupture at the vulva shortly after the L4 molt. Males have between one and three ventral protrusions (Figure 2-5e). One allele, n765, is heat-sensitive and has a temperature-dependent maternal effect. n765 animals are phenotypically wild-type at 15° but are mutant at 20° and 25°. At 20°, but not at 25°, n765 hermaphrodites that are the progeny of parents of genotype lin-15(n765)/+ have fewer and generally smaller ventral protrusions than do n765 hermaphrodites that are the progeny of n765 animals. Hemizygous n765 males mate at 15° and 20° but do not mate at 25°.

The presence of F1 Muv males and non-Muv hermaphrodites from a cross between wild-type males and n309 hermaphrodites suggested that the mutation was X-linked. Because these males do not mate, males of genotype tra-1(e1099); lin-15(n309)/+ were used to perform complementation tests; tra-1 transforms genotypically XX hermaphrodites into phenotypic males that are able to mate, although inefficiently (HODGKIN and BRENNER 1977).

A three-factor cross (Table 2-2) localized lin-15 to the right end of LGX. A series of deficiencies for this region (MENEELY and HERMAN 1979; 1981) was used to map lin-15 further. These deficiencies are maintained in balanced strains, mnDp1/+; mnDf. (mnDp1 is a duplication of the right end of the X chromosome attached to LGV; mnDp1 is homozygous sterile or larval lethal.) Wild-type males were crossed with these balanced strains, and F1 males (genotype mnDp1/+; mnDf/0) were crossed with unc-17; lin-15(n309) hermaphrodites. The presence

of Muv non-Unc hermaphrodite progeny (genotype unc-17/+; lin-15/mnDf) established that the deficiency failed to complement lin-15. mnDf1, mnDf4 and mnDf11 failed to complement lin-15; mnDf43 and mnDf19 complemented lin-15 (Figure 2-1).

The phenotype of lin-15 in trans to a deficiency of the locus suggests that the known alleles of lin-15 may not result in a total lack of gene activity. Specifically, when unc-84 lin-15(n765) males were mated with hermaphrodites of genotype mnDp1/+; mnDf4 at 25[°], the great majority (95%, n = 79) of F1 Muv hermaphrodites of genotype unc-84 lin-15(n765)/+ mnDf4 were the size of L3 larvae and were sterile. (At 25[°], n765 results in a phenotype equivalent to that of n309.) However, at 20[°], the deficiency did not appear to grossly enhance the lin-15 phenotype.

Four genes (let-15, let-18, let-38 and let-40) with sterile or lethal alleles have been mapped to this region (MENEELY and HERMAN 1979, 1981). To test whether any of these let mutations failed to complement lin-15 for the Muv phenotype, males of genotype mnDp1/+; unc-3 let/0 were mated with unc-17; lin-15(n309) hermaphrodites. No Muv hermaphrodites were observed among the non-Unc progeny. To confirm the results of the above experiments and to test whether lin-15/let was Let, mnDp1/+; unc-3 let/0 males were mated with unc-3 lin-15(n309) hermaphrodites. For all four let genes, Unc non-Muv hermaphrodite progeny (genotype unc-3 + lin-15(n309)/unc-3 let +) were observed. Thus, lin-15 appears to complement all known lethal mutations in the region.

lin-17(e1456,n669,n671,n677,n698) I: Multivulva: About half of n671 and n677 hermaphrodites have a single protrusion posterior to the vulva (Figure 2-4i). The other three lin-17 mutations may be of somewhat lower penetrance for this phenotype. Hermaphrodites are slightly uncoordinated and have a long irregularly shaped tail. In some lin-17 hermaphrodites, one of the two arms of the gonad does not develop. A few lin-17 hermaphrodites are sterile. lin-17 males have undeveloped tails and some rupture at the anus after the L2 molt (Figure 2-5f).

lin-18(e620,n1051) X: Multivulva: Fewer than half of lin-18 hermaphrodites have a single protrusion posterior to the vulva [30% (n = 157), e620 at 25^o] (Figure 2-4j). Both lin-18 alleles are slightly heat-sensitive and result in a slight maternal effect. lin-18 males are phenotypically wild-type as viewed with a dissecting microscope.

n1051 is an amber mutation (Table 2-1) and, hence, is likely to eliminate lin-18 gene activity. However, n1051 results in a heat-sensitive phenotype, which suggests that an altered but potentially functional lin-18 gene product may be synthesized in lin-18(n1051) animals. Alternatively, the heat-sensitive phenotypes resulting from both lin-18 alleles may reflect a temperature-sensitive process revealed or induced by eliminating lin-18 activity (other C. elegans temperature-sensitive mutants of this type have been identified; SULSTON and HORIVITZ 1981; GOLDEN and RIDDLE 1984; W. FIXSEN, personal communication).

n1051 was suppressed by a single copy of the amber suppressor sup-5, i.e., hermaphrodites of genotype sup-5/+; lin-18(n1051)/lin-18(n1051) were phenotypically wild-type. To reduce the amount of suppressed lin-18 product, hermaphrodites of genotype sup-5/+; lin-18(n1051)/lin-18(nonamber) were constructed. The nonamber allele used, e620, results in a phenotype similar to that of n1051. Of 148 hermaphrodites of genotype sup-5/+; lin-18(n1051)/lin-18(e620) at 25^o, two were either egg-laying defective or had an abnormal vulva. However, the severity of the vulval defect in these hermaphrodites was not very different from that observed in hermaphrodites of genotype sup-5/+ (three of 257 such hermaphrodites had abnormal vulvae), suggesting that the vulval defect of hermaphrodites of genotype sup-5/+; lin-18(n1051)/lin-18(e620) is completely suppressed.

lin-24(n432,n1057) IV: Vulvaless: Most lin-24 hermaphrodites that turn into bags of worms are Vulvaless. However, a minority of the egg-laying defective (Egl) hermaphrodites that become bags of worms are nonetheless able to release some progeny. This observation suggests that in these animals at least some vulval cells are generated. In determining the penetrance of the Vul phenotype that results from these two mutations (and the penetrance of the two alleles of lin-33; see below), we measured the percentage of hermaphrodites that were severely Egl without regard to their ability to release progeny.

n432 results in a partially dominant Vul phenotype: 95% (n = 458)

of n432 homozygous hermaphrodites are Vul, whereas 57% (n = 396) of n432/+ heterozygotes are Vul. n1057 results in a wild-type phenotype when homozygous (0/203 were Vul), but results in an Vul phenotype when heterozygous with a wild-type allele (33% Vul, n = 314). The males of both strains are phenotypically wild-type as viewed with a dissecting microscope. The penetrance of the Vul defect markedly decreases in n432 hermaphrodites that have passed through a dauer larval stage (29% Vul, n = 455).

nDf27 is a deletion that fails to complement unc-22 and unc-31, visible markers flanking lin-24 (H. ELLIS, personal communication). Hermaphrodites heterozygous for nDf27 have no obvious vulval abnormalities in that 0/105 hermaphrodites of genotype unc-8(e49)/+ nDf27 were Vul. (nDf27 complements unc-8.) Thus, it is unlikely that the Vul phenotype of the lin-24 alleles results from a loss of lin-24 activity. The phenotype of lin-24(n432)/nDf27 hermaphrodites was determined by examining the non-Unc progeny from hermaphrodites of genotype unc-8(e49) lin-24(n432)/+ nDf27. Fifty-four percent (n = 452) of these hermaphrodites were Vul. Thus, the presence or absence of a wild-type lin-24 allele does not alter the penetrance of the Vul defect in hermaphrodites heterozygous for n432. The phenotype of lin-24(n1057)/nDf27 was established by mating n1057 males with hermaphrodites of genotype nDf27 +/unc-30 dpy-4. (nDf27 fails to complement unc-30 but complements dpy-4.) Non-Unc cross progeny were picked and scored for their ability to lay eggs, and their progeny were tested to determine their genotypes. One hundred and three of 103 hermaphrodites of genotype lin-24(n1057)/nDf27 were phenotypically

wild-type, suggesting strongly that the mutant phenotype of lin-24(n1057)/+ animals results from an interaction between the mutant and wild-type products of the lin-24 gene.

The vulval precursor cells of the hermaphrodite, P(5-7).p, are a subset of the ventral hypodermal cells P(1-12).p present in both males and hermaphrodites (SULSTON and HORVITZ 1977). Observations using Nomarski optics have shown that in n432 animals some of the P(1-12).p ventral hypodermal cells die during the L1 and early L2 stages (P. STERNBERG, personal communication). These cell deaths are not suppressed in the double mutant lin-24(n432) ced-3(n717).

[ced-3(n717) blocks the onset of the normal program of cell death in C. elegans (HORVITZ et al. 1983; H. ELLIS, personal communication).] In addition the P(1-12).p cells that die in n432 animals do not have the appearance of cells in which the normal program of cell death has been activated (H. ELLIS, personal communication). Thus, it is possible that the aberrant cell deaths in n432 hermaphrodites may be the result of the action of an altered, cytotoxic form of the lin-24 gene product. lin-24 may encode a product utilized in the cells P(1-12).p, and the mutation n432 may produce an altered lin-24 product that kills these cells. A similar phenomenon has been observed for the dominant mutation mec-4(e1611), which causes the death of the six microtubule cells that mediate touch sensitivity in C. elegans (CHALFIE and SULSTON 1981; M. CHALFIE, personal communication).

We have tentatively made an assignment of allelism between the two partially dominant Vul mutations n432 and n1057 based on three

criteria. First, both mutations map to the interval between unc-22 and dpy-26 on LGIV (Table 2-2). Second, both mutations result in similar defects in the vulval cell lineages and in the presence of ectopic cell deaths in the ventral hypodermal cells during the L1 stage (P. STERNBERG, personal communication). Third, 92% (n = 350) of hermaphrodites of genotype unc-22 lin-24(n1057)/+ lin-24(n432) were Vul. The penetrance of the vulval defect of this strain is equivalent to the penetrance of the vulval defect of homozygous n432 hermaphrodites, suggesting that the two mutations may be allelic. However, we have also observed a similar increase in the penetrance of the Vul phenotype between two partially dominant Vul mutations in different genes: the penetrance of the Vul defect in hermaphrodites of genotype lin-33(n1043) +/+ lin-24(n432) is 99% (n = 442). Thus, the possibility remains that n432 and n1057 are mutations of separate, closely linked genes.

Although n1057 does not result in the loss of lin-24 gene function, this allele is an amber mutation as it is suppressed by the amber suppressor sup-5 (see Materials and Methods). Suppression requires two copies of the suppressor. Specifically, the penetrance of the Vul phenotype of hermaphrodites of genotype unc-22 lin-24(n1057)/+ + (22%, n = 301) was much higher than the penetrance of the Vul phenotype of hermaphrodites of genotype dpy-19 sup-5; unc-22 lin-24(n1057)/+ + (3%, n = 279). n1057 is not suppressed by a single copy of sup-5, as 13 of 21 Vul hermaphrodites that were the progeny of hermaphrodites of genotype dpy-19 sup-5/+ +; unc-22 lin-24(n1057)/+ + segregated Dpy animals.

lin-25(e1446,n545) V: Vulvaless: n545 is heat-sensitive. The phenotype of n545 at 25° is similar to that of e1446. n545 hermaphrodites at 25° and e1446 hermaphrodites have never been observed to lay eggs: approximately 85% form bags of worms, whereas the rest are sterile [18% (n = 234), n545 at 25°; 11% (n = 279), e1446]. Nonetheless, a few lin-25 hermaphrodites are able to mate, indicating that an occasional hermaphrodite forms an abnormal but functional vulva. Most [91% (n = 234), n545 at 25°; 81% (n = 279), e1446] lin-25 hermaphrodites have a single protrusion at the vulva. At the permissive temperature of 15°, all n545 hermaphrodites are fertile and only 8% (n = 305) are Vul. lin-25 males are phenotypically wild-type as viewed with a dissecting microscope. e1446 is maintained in the balanced strain +/nT1(IV); lin-25(e1446)/unc(n754) nT1(V). [unc(n754) is a dominant mutation resulting in uncoordinated locomotion linked to nT1(IV;V) (E. FERGUSON, unpublished results).]

lin-26(n156) II: Vulvaless: The Vul phenotype of n156 is highly penetrant, as greater than 99% (n = 268) of the hermaphrodites form bags of worms. A single ventral protrusion is seen in 14% (n = 268) of the hermaphrodites. However, a small percentage (1-2%) of the hermaphrodites can mate, indicating that an occasional hermaphrodite forms an abnormal but functional vulva. lin-26 hermaphrodites have a smaller, slightly fatter body than does the wild type. Males are very small and scrawny with a rounded tail. For genetic manipulations, the mutation is maintained in two balanced strains, lin-26(n156)/C1 dpy-10 unc-52; him-5 and + lin-5 unc-4/lin-26(n156) + unc-4.

Hermaphrodites of genotype lin-26(n156)/mnDf88 arrest during larval development (K. EDWARDS, personal communication), suggesting that n156 does not result in the total loss of lin-26 activity and that the null phenotype of lin-26 is probably lethal. SIGURDSON, SPANIER and HERMAN (1984) have mapped two let genes, let-253 and let-236, to the same region of LGII to which n156 has been localized. n156 complements mutations in both of these let genes (K. EDWARDS, personal communication).

lin-31(e1750,n301,n376,n428,n429,n435,n762,n1048,n1049,n1050,n1053) II: Multivulva: Hermaphrodites have between zero and four small, widely spaced ventral protrusions (Figure 2-4k). In some hermaphrodites the vulva is nonfunctional, causing these animals to become bags of worms. Males do not have ventral protrusions.

GREENWALD and HORVITZ (1980) isolated four deletions of lin-31, nDf2 through nDf5, and showed that lin-31(n301)/Df animals are of the same gross phenotype as lin-31(n301) animals. This fact and the large number of alleles isolated suggest that some, if not all, of the known alleles of lin-31 eliminate lin-31 gene activity. [At the time of the above paper, we had erroneously assigned n301 to the gene lin-8. However, lin-31(n301) is not allelic to lin-8(n111), as these mutations complement and are separated by approximately 1 map unit (Table 2-2).]

lin-33(n1043,n1044) IV: Vulvaless: Most lin-33 hermaphrodites that turn into bags of worms are Vulvaless. However, a minority of

the Egl hermaphrodites that become bags of worms are nonetheless able to release some progeny. This observation suggests that in these animals at least some vulval cells are generated. In determining the penetrance of the Vul phenotype resulting from these two mutations (as in determining the penetrance of the two alleles of lin-24; see above), we measured the percentage of hermaphrodites that were severely Egl without regard to their ability to release progeny.

n1043 results in a partially dominant Vul phenotype: 95% (n = 502) of n1043 homozygous hermaphrodites are Vul, whereas 77% (n = 447) of n1043/+ heterozygotes are Vul. n1044 also results in a partially dominant Vulvaless phenotype: 96% (n = 425) of n1044 homozygous hermaphrodites are Vul, whereas 79% (n = 411) of n1044/+ heterozygotes are Vul. Males are phenotypically wild-type as viewed with a dissecting microscope.

The penetrance of the Vul defect of n1043, like that of lin-24(n432), is lower in animals that have recovered from the dauer larval stage. Fifty-three percent (n = 235) of n1043 hermaphrodites that had recovered from dauer larvae were Vul. In n1043 hermaphrodites, as in lin-24 hermaphrodites, some of the cells P(1-12).p die, suggesting that n1043 also may result in the production of a toxic product (P. STERNBERG, personal communication).

n1044 maps to a position similar to that of n1043 (Table 2-2). The penetrance of the Vul defect in hermaphrodites of genotype lin-33(n1043)/lin-33(n1044) is 98% (n = 248), equivalent to the

penetrance of the Vul defect in homozygous n1043 hermaphrodites, which suggests that these mutations may be allelic. However, the penetrance of the Vul defect in hermaphrodites of genotype lin-33(n1043) +/+ lin-24(n432) is 99% (n = 442), even though n1043 and n432 are mutations in different genes. Nonetheless, we have decided on the basis of the similarity of the map positions and phenotypes of n1043 and n1044 to consider them provisionally to be alleles of the same gene. However, the possibility remains that they are mutations of separate, closely linked genes.

lin-34(n1046) IV: Multivulva: n1046 results in an incompletely penetrant but partially dominant Muv phenotype; 57% (n = 426) of n1046 homozygous hermaphrodites were Muv, whereas 17% (n = 194) of lin-34(n1046)/+ heterozygotes were Muv. Many n1046 hermaphrodites have two protrusions, one anterior to the vulva, and one posterior (Figure 2-41). Both heterozygous and homozygous n1046 males are wild-type in phenotype as viewed with a dissecting microscope.

n1046 was mapped relative to lin-3 using the lin-3 allele n1059, which results in a larval lethal phenotype (see above). The phenotype of animals of genotype lin-34(n1046)/nDf27 is equivalent to the phenotype of lin-34(n1046)/+ animals; thus, it can not be determined whether nDf27 fails to complement n1046.

As detailed in Materials and Methods, n1046 is an amber mutation as it is suppressed by sup-7. The suppression of n1046 by sup-7 is recessive in that a single copy of the suppressor does not markedly

reduce the penetrance of the mutation as a homozygote. Fifty-seven percent ($n = 426$) of hermaphrodites of genotype lin-34(n1046) unc-22 were Muv, whereas 68% ($n = 104$) of hermaphrodites of genotype lin-34(n1046) unc-22; sup-7 dpy-7/+ + were Muv and 13% ($n = 182$) hermaphrodites of genotype lin-34(n1046) unc-22; sup-7 dpy-7 were Muv. However, sup-7 may be a partially dominant suppressor of lin-34(n1046)/+, because, at 25°, 17% ($n = 339$) of hermaphrodites of genotype lin-34(n1046) unc-22/+ + were Muv, whereas 8% ($n = 199$) of hermaphrodites of genotype lin-34(n1046) unc-22/+ +; sup-7 dpy-7/+ + were Muv.

let-23(mn23,mn216,mn224,n1045) II: Vulvaless: n1045 results in a cold-sensitive Vul phenotype with an associated cold-sensitive larval lethality. At 15°, 49% of n1045 hermaphrodites arrest during larval development, 50% are Vul and 1% have a functional vulva ($n = 219$). At 20°, 51% of n1045 hermaphrodites arrest during larval development, 14% are Vul or severely egg-laying defective (Egl) and 35% have a functional vulva ($n = 763$). At 25°, 28% of n1045 hermaphrodites arrest during larval development, 2% are Vul or severely Egl, and 70% have a functional vulva ($n = 310$). The n1045 hermaphrodites that arrest during larval development do so with a rigid rod-like phenotype during what appears by size to be the L2 stage (Figure 2-3e). Males are phenotypically wild-type as viewed with a dissecting microscope.

At 20° and 25° n1045 hermaphrodites have two distinct phenotypes not seen in n1045 hermaphrodites grown at 15°. First, some of these hermaphrodites are Egl but not Vul; these hermaphrodites do not lack a

Table 2-10. The penetrance of the larval lethal phenotype is presented as a percentage of the hermaphrodites of differing genotypes at different temperatures that arrested during larval development. Animals of differing genotypes and at different temperatures were obtained in the following ways. n1045; sup-7/+, 15°: 15 animals of genotype let-23(n1045) unc-4; sup-7 dpy-7/+ + were allowed to lay eggs for 1 day and the number of progeny that arrested as larvae was counted. n1045; sup-7, 20°: ten fertile animals of genotype let-23(n1045) unc-4; sup-7 dpy-7 were allowed to lay eggs and the number of hermaphrodites that arrested as larvae was counted. n1045; sup-7, 22.5°: the degree of larval lethality was measured as described at 20°. n1045/Df, 25°: males of genotype mnDf68 unc-4/C1 dpy-10 unc-52 were mated with hermaphrodites of genotype let-23(n1045) unc-4; dpy-7. The number of Unc non-Dpy hermaphrodites of genotype let-23(n1045) unc-4/mnDf68 unc-4; dpy-7/+ and the number of wild-type hermaphrodites of genotype let-23(n1045) unc-4/C1 dpy-10 unc-52; dpy-7/+ were counted. The number of wild-type hermaphrodites was much greater than the number of Unc non-Dpy hermaphrodites, and the ratio of the number of Unc non-Dpy hermaphrodites to the number of wild-type hermaphrodites was taken as the percentage of Unc hermaphrodites that did not arrest during larval development. n1045/Df; sup-7/+, 25°: males of genotype mnDf68 unc-4/C1 dpy-10 unc-52 were mated with hermaphrodites of genotype let-23(n1045) unc-4; sup-7 dpy-7. The above protocol was followed to determine the degree of larval lethality. ND, not determined.

Table 2-10

Suppression by sup-7 of the larval lethal defect of let-23

<u>let-23</u> genotype	Temper- ature	% hermaphrodites of given genotype that arrest during larval development		
		<u>sup-7(+)/sup-7(+)</u>	<u>sup-7/sup-7(+)</u>	<u>sup-7/sup-7</u>
<u>n1045</u>	15°	49 (n=219)	2 (n=919)	ND
	20°	51 (n=763)	ND	0.5 (n=206)
	22.5°	41 (n=673)	ND	5 (n=95)
<u>n1045/Df</u>	25°	78 (n=85)	33 (n=35)	ND

Table 2-11. The penetrance of the Vul phenotype is presented as a percentage of adult hermaphrodites of differing genotypes at different temperatures that were Vulvaless. Animals of differing genotypes and at different temperatures were obtained in the following ways. n1045; sup-7/+, 15[°]: 15 animals of genotype let-23(n1045) unc-4; sup-7 dpy-7/+ + were allowed to lay eggs for 1 day. n1045; sup-7/+, 20[°]: let-23(n1045); him-5 males were mated with hermaphrodites of genotype let-23(n1045) unc-4; sup-7 dpy-7 and the fertile non-Unc progeny were scored for their ability to lay eggs. n1045; sup-7, 20[°]: 141 L4 hermaphrodites of genotype let-23(n1045) unc-4; sup-7 dpy-7 were picked separately; of those that were fertile, the percentage that were egg-laying competent was measured. The remaining hermaphrodites were sterile as a consequence of the presence of sup-7; however, a vulval structure was also observed in these hermaphrodites with the dissecting microscope. n1045/Df, 25[°]: the penetrance of the Vulvaless defect in hermaphrodites of genotype dpy-10 let-23(n1045)/mnDf68 unc-4 (P. STERNBERG, personal communication). n1045/Df; sup-7/+, 25[°]: males of genotype mnDf68 unc-4/C1 dpy-10 unc-52 were mated with hermaphrodites of genotype let-23(n1045) unc-4; sup-7 dpy-7. If the Unc non-Dpy hermaphrodites of genotype let-23(n1045) unc-4/mnDf68 unc-4; sup-7 dpy-7/+ + were fertile, they were scored for the ability to lay eggs; if not, the presence of a vulval structure was ascertained either using Nomarski optics or with a dissecting microscope. ND, not determined.

^a -- many fertile, egg-laying competent hermaphrodites of putative genotype let-23(n1045) unc-4; sup-7 dpy-7/+ + were observed

among the progeny of hermaphrodites of genotype let-23(n1045) unc-4;
sup-7 dpy-7/+ + grown at 15^o, but their number was not ascertained.

Table 2-11

Suppression by sup-7 of the Vulvaless defect of let-23

<u>let-23</u> genotype Genotype	Temper- ature	% adult hermaphrodites of given genotype that have a Vul phenotype		
		<u>sup-7(+)/sup-7(+)</u>	<u>sup-7/sup-7(+)</u>	<u>sup-7/sup-7</u>
<u>n1045</u>	15°	98 (n=111)	a	ND
	20°	28 (n=373)	8 (n=97)	3 (n=40)
<u>n1045/Df</u>	25°	100 (n=20)	4 (n=23)	ND

vulva but rather have an abnormal vulva and are able to release some eggs or larvae. Second, some egg-laying competent hermaphrodites are Muv; these animals have a functional vulva and one or two ectopic supernumerary vulva-like structures (Table 2-4).

At 15^o, the penetrance of the Vul defect in n1045 hermaphrodites that pass through a dauer larval stage is equivalent to that of unstarved animals: 95% (n = 79) of adult hermaphrodites that have passed through a dauer larval stage are Vul, equivalent to the 98% (n = 111) of unstarved adult hermaphrodites that are Vul. However, the penetrance of the Vul phenotype markedly decreases in n1045 hermaphrodites that do not pass through a dauer larval stage but have been starved before reaching adulthood; 47% (n = 287) of n1045 hermaphrodites that have been starved before reaching adulthood are Vul.

n1045 was mapped to LGII between vab-9 and unc-4 by three-factor crosses (Table 2-2). Recently, a detailed genetic analysis of this region of LGII has identified many essential genes (SIGURDSON, SPANIER and HERMAN 1984). Using a series of deficiencies, C. SIGURDSON (personal communication) mapped n1045 to the same region of LGII as let-23 and subsequently determined that n1045 failed to complement an allele of let-23. Hermaphrodites bearing any of the other three let-23 alleles, mn23, mn216 and mn224 (HERMAN 1978; SIGURDSON, SPANIER and HERMAN 1984), arrest during larval development with the same phenotype that is seen among n1045 hermaphrodites and fail to complement n1045 for the Vul activity. Thus, let-23 appears to have

two activities, one that functions during early larval development and another that functions later during vulval development.

The phenotype caused by n1045 is enhanced when n1045 is in trans to other let-23 alleles or to a deficiency of the locus (P. STERNBERG, personal communication). Thus, n1045 probably does not totally eliminate let-23 activity. Nonetheless, n1045 is an amber mutation as it is suppressed by the amber suppressor sup-7 (see Materials and Methods). Based upon the phenotype observed using a dissecting microscope, n1045 appears to be suppressed completely by two copies of sup-7 but only partially by one copy (Tables 2-10 and 2-11).

MT300, n300 and nT1(IV;V): MT300 hermaphrodites never form ventral protrusions or vulvae. Males have slightly rounded tails.

The isolation of the strain MT300 is described in Materials and Methods. The single egg-laying competent MT300 hermaphrodite that was identified was mated with wild-type males. The resulting F1 males were mated with the two mapping strains. Fewer than 1/4 Vul animals were seen among the F2 progeny from these crosses. In addition unc-5 IV and dpy-11 V appeared to be genetically linked. These two observations suggested that there might be a chromosome abnormality in this strain, possibly a translocation between LGIV and LGV. The results of several experiments, detailed in Table 2-12, support this hypothesis. First, the translocation heterozygote segregates approximately 10/16 dead eggs. This is the fraction of inviable progeny that would segregate from a hermaphrodite heterozygous for a

Table 2-12. ^a Progeny counts from hermaphrodites of genotype nT1(IV)/unc-5; nT1(V)/dpy-11. Single hermaphrodites were allowed to lay eggs for 24 hr and then removed. Eggs that had not hatched after 24 hr were counted as "dead eggs."

b Progeny counts from hermaphrodites of genotype nT1(IV)/unc-17 dpy-4; nT1(V)/+.

c Progeny counts from hermaphrodites of genotype nT1(IV)/+; nT1(V)/unc-60 dpy-11.

Table 2-12

Characterization of nT1(IV;V)Linkage of LGIV and LGV^a

<u>Dead eggs</u>	<u>Wild type</u>	<u>Dpy-11</u>	<u>Unc-5</u>	<u>Dpy-11 Unc-5</u>	<u>Vul</u>
1049 66.6%	400 25.4%	0 0%	0 0%	75 4.8%	50 3.2%

Crossover suppression of the right arm of LGIV^b

<u>Dead eggs</u>	<u>Wild type</u>	<u>Dpy-4</u>	<u>Unc-17</u>	<u>Dpy-4 Unc-17</u>	<u>Vul</u>
1181 64.4%	469 25.6%	0 0%	0 0%	120 6.6%	62 3.4%

Crossover suppression of the left arm of LGV^c

<u>Dead eggs</u>	<u>Wild type</u>	<u>Dpy-11</u>	<u>Unc-60</u>	<u>Dpy-11 Unc-60</u>	<u>Vul</u>
1175 64.3%	463 25.4%	1 0.05%	0 0%	108 5.9%	79 4.1%

reciprocal translocation if all aneuploid progeny do not survive. Second, mutations on LGIV and LGV appear to be tightly linked. Third, the translocation suppresses crossing over on the right arm of LGIV and on the left arm of LGV. Crossover suppression is not observed on the left arm of LGIV (between the markers dpy-9 and unc-17) and on the right arm of LGV (between the markers dpy-11 and unc-51) (E. FERGUSON, unpublished results). This translocation was named nT1(IV;V) and is maintained in the balanced strains nT1(IV)/unc-5; nT1(V)/dpy-11 or nT1(IV)/unc-8(n491); nT1(V)/unc-60. Vul hermaphrodites segregating from a homozygous nT1(IV;V) parent are much healthier than those segregating from a balanced heterozygote.

We have named the mutation in the strain MT300 that is responsible for the Vul phenotype n300. n300 and the translocation have always cosegregated. All hermaphrodites heterozygous for nT1(IV;V), i.e., those hermaphrodites that segregate 10/16 dead eggs and that exhibit pseudolinkage between LGIV and LGV, have in addition always segregated n300. We do not know whether the Vul phenotype is caused by the translocation, possibly by one or both of its breakpoints, or whether the Vul mutation is simply a secondary mutation present on the translocation. Complementation screening experiments (GREENWALD and HORVITZ 1980) to induce a point mutation failing to complement the Vul phenotype of n300 have not succeeded (E. FERGUSON, unpublished results), and, thus, we do not know whether n300 defines a single gene that can mutate to result in a Vul phenotype.

Efficiency of male mating: Vulval cell lineages occur only in

hermaphrodites. Since cells homologous to the vulval precursor cells generate male-specific structures required for mating, it was of interest to determine whether mutations that affect vulval development also have effects in males. The phenotypes of mutant males as viewed with a dissecting microscope are described above and in Table 2-3. Males also were tested for their abilities to mate, as described in Materials and Methods. For each gene an allele of high penetrance was chosen. If a mutant was severely deficient in male mating ability, a mutant carrying a second allele of that gene (if available) was tested to decrease the likelihood that the effect was caused by a secondary mutation.

The results of these tests are presented in Table 2-13. The numbers of male progeny vary considerably among experiments and provide only an approximate indication of mating ability. Even so, one generalization is apparent: male mating ability is eliminated by most Muv mutations. Specifically, lin-1, lin-13, lin-15, lin-17 and lin-31 males do not mate, lin-12(Muv) homozygous males do not mate and lin-34 males mate very poorly. However, lin-8; lin-9 and lin-18 males mate. Males carrying Vul mutations in any of six genes -- lin-4, lin-11, lin-25, lin-26, let-23 and n300 -- either do not mate or mate very poorly. Males carrying Vul mutations in any of seven other genes -- lin-2, lin-3, lin-7, lin-10, lin-12(Vul), lin-24, lin-33 -- mate with an efficiency approximately equal to that of the wild type.

Table 2-13

Male mating ability of Lin mutants

Genotype	No. of cross progeny	% control
N2	1789	
<u>him-5(e1467)</u>	1894	
<u>him-9(e1487)</u>	1500	
Vul		
<u>lin-2(e1309); him-5</u>	1383	73
<u>lin-3(e1417); him-5</u>	1299	69
<u>lin-4(e912); him-5</u>	0	0
<u>lin-7(e1413); him-5</u>	2089	110
<u>lin-10(1439); him-5</u>	1181	62
<u>lin-11(n382); him-5</u>	0	0
<u>lin-11(n389); him-5</u>	2	0.1
<u>lin-12(n302); him-5</u>	845	45
<u>lin-12(n379); him-5</u>	872	46
<u>lin-24(n432)</u>	2586	146
<u>lin-24(n1057)/+</u>	1907	106
<u>lin-25(e1446) him-5</u>	0	0
<u>lin-25(n545) him-5</u>	0	0
<u>lin-26(n156); him-5</u>	0	0
<u>lin-33(n1043)</u>	1593	89
<u>let-23(n1045); him-5</u>	352	19
<u>n300; him-9</u>	0	0
Muv		
<u>lin-1(e1777); him-5</u>	0	0
<u>lin-8(n111)</u>	1016	57
<u>lin-9(n112); him-5</u>	1477	78
<u>lin-8(n111); lin-9(n112); him-5</u>	569	30
<u>+ lin-12(n137)/unc-32 +; him-5</u>	1899	100
<u>lin-12(n137); him-5</u>	0	0
<u>lin-12(n427); him-5</u>	0	0
<u>lin-13(n387); him-5</u>	0	0
<u>him-5; lin-15(n309)</u>	0	0
<u>lin-15(n377)</u>	2	0.1
<u>lin-17(n671); him-5</u>	0	0
<u>him-5; lin-18(e620)</u>	734	38
<u>lin-31(n301); him-5</u>	0	0
<u>lin-31(e1750); him-5</u>	0	0
<u>lin-34(n1046); him-5</u>	95	5

DISCUSSION

We have identified and characterized 95 mutants of the C. elegans hermaphrodite in which the vulval cell lineages are altered. These mutants have been isolated based on their displaying one of two phenotypic abnormalities in vulval anatomy: Vulvaless (Vul) mutants lack a vulva, and Multivulva (Muv) mutants have one or more protrusions along the ventral midline. These mutants define 22 complementation groups, 15 of which are represented by multiple alleles. The phenotypes of most, but not all, of these mutants result from single gene recessive mutations. The Vulvaless phenotype of one strain, MT300, is associated with a reciprocal translocation involving linkage groups IV and V. Thirteen mutations that result in partially dominant phenotypes have been assigned to four complementation groups, lin-12 III, lin-24 IV, lin-33 IV and lin-34 IV. One allele of lin-24 results in a Vulvaless phenotype when heterozygous with a wild-type allele but results in a wild-type phenotype when homozygous. Certain mutations in each of the genes lin-9, lin-13 and lin-15 result in maternal effects at low temperatures. The penetrance of the Vul phenotype resulting from mutations in three genes is reduced in hermaphrodites that have passed through a dauer larval stage. [A dauer larva is an alternate developmental stage that is entered as a consequence of starvation during the first larval stage (CASSADA and RUSSELL 1975).] The penetrance of the Vul phenotype resulting from mutations in three other genes is reduced in hermaphrodites that did not pass through a dauer larval stage but that have been starved

before reaching adulthood. Ten mutations in seven genes have been shown to be amber mutations; however, as discussed below, four of these amber mutations may not result in the complete loss of function of their respective loci.

To understand how these 22 genes function in the development of the vulva, it is necessary to determine how the mutations that define these genes affect gene function, to observe the effects of these mutations on the vulval cell lineages and to examine the patterns of interaction among mutations in different genes. In this manuscript, we have analyzed how these mutations affect gene function. In addition, for some of the mutations that do not eliminate gene function, we have determined the phenotype that probably results from the complete absence of gene function.

We have used four criteria in attempting to determine the nature of these mutations and, in particular, to determine which of these mutations result in a loss of gene function: (1) the number of alleles of a given gene (the isolation of a large number of alleles suggests that the observed phenotype may be the result of the elimination of gene function); (2) the nature of the phenotype (dominant phenotypes usually do not result from either the loss or partial reduction of gene function; recessive phenotypes that are not the most extreme observed phenotype are likely to result from a reduction rather than an elimination of gene function); (3) the phenotype that results when a mutation is in trans to a deficiency of the locus (an enhancement of the phenotype of a recessive mutation in

trans to a deficiency of the locus strongly suggests that the mutation does not completely eliminate gene function); (4) the existence of and phenotypes caused by amber mutations (amber mutations usually, but not always, result in the total lack of gene function; see below).

These four criteria suggest that existing mutations in six of the 22 genes result in the absence of gene function. Four genes, the Vul genes lin-2 and lin-7 and the Muv genes lin-1 and lin-31, have 11 or more recessive alleles. We have identified amber alleles of three of these genes, lin-1, lin-2 and lin-7 (although the single amber allele of lin-2 may not eliminate lin-2 gene function; see below), and have determined that the severity of the effect of lin-31 alleles is not enhanced by a deficiency of this locus. The phenotype resulting from mutations in the Vul gene lin-10, which has three alleles, also is not enhanced by a deficiency of the locus, and, thus, mutations in this gene may also result in the absence of gene activity. In addition, we have identified an amber allele of the Muv gene lin-18. Although only two alleles of lin-18 have been isolated, these mutations result in a subtle phenotype; thus, other alleles may have been missed in the screening process. Thus, the Vul phenotypes of lin-2, lin-7 and lin-10 and the Muv phenotypes of lin-1, lin-18 and lin-31 may be null phenotypes.

In contrast, it is likely that mutations in seven other genes (lin-3, let-23, lin-26, lin-13, lin-15, lin-8, lin-9) reflect a partial decrease in, but not a complete loss of, gene function. In particular, lethal alleles of the Vul genes lin-3 and let-23 have been

identified. For both let-23 (P. STERNBERG, personal communication) and lin-3, lethal alleles increase the penetrance of the Vul defect in trans to a Vul mutation, suggesting that these lethal alleles result in a further decrease, and possibly a total absence, of gene activity. In addition, the severity of the phenotypes resulting from alleles of three genes, the Vul gene lin-26 and the Muv genes lin-13 and lin-15, is greater when any of these alleles is in trans to an appropriate deficiency. In these cases, hermaphrodites of genotype lin/Df either arrest during larval development or have an increased incidence of sterility, suggesting that the null phenotypes of these three loci are lethality or sterility. The mutations lin-8(n111) and lin-9(n112), which were isolated because together they result in a Muv phenotype, also may not be null alleles. Two other alleles of lin-9 that will be described elsewhere result in a sterile phenotype and appear to be stronger alleles of the gene, suggesting that lin-9(n112) may only partially decrease lin-9 gene activity. Although hermaphrodites of genotype lin-8/Df are phenotypically wild-type, hermaphrodites of genotype lin-8/Df; lin-9(n112) have a much higher incidence of sterility than do hermaphrodites of genotype lin-8; lin-9(n112), suggesting that the single allele of lin-8 may not result in the null phenotype of this locus.

Although the null phenotypes of the other five genes with recessive mutations (the Vul genes lin-4, lin-11, lin-25 and n300 and the Muv gene lin-17) are not known, mutations in these genes seem likely to either decrease or eliminate gene activity. However, it is unlikely that the Muv and Vul mutations in four genes (lin-12, lin-24,

lin-33 and lin-34) that are represented only by dominant alleles decrease or eliminate the function of these genes. Dominant mutations usually result in the acquisition of a novel function by the gene product, in an increase in gene activity and/or in ectopic gene expression. The dominant Muv and Vul alleles of lin-12 described in this paper already have been demonstrated to result in an increased activity and, possibly, in the ectopic expression of the lin-12 gene; in addition, amber alleles of lin-12 have been isolated and shown not to result in a Muv or Vul phenotype (GREENWALD, STERNBERG and HORVITZ 1983). The partially dominant Vul alleles of lin-24 and lin-33 cause vulval precursor cells to die (P. STERNBERG, personal communication). Hermaphrodites heterozygous for a deficiency of the lin-24 locus are not Vul, suggesting that the phenotype observed in lin-24 hermaphrodites does not result from a reduction in function of the lin-24 locus but rather may result from either an increase in the amount of normal lin-24 gene product or from the production of an altered lin-24 gene product with a novel, cytotoxic function. A second putative allele of lin-24, n1057, results in a similar Vul defect when heterozygous to a wild-type allele but results in a wild-type phenotype as a homozygote or in trans to a deficiency of the region. These observations suggest that the mutant phenotype of n1057 is the result of an interaction between the wild-type and mutant lin-24 gene products to generate a novel, cytotoxic activity.

In addition to lin-12, seven of the other 21 genes described in this study are represented by one or more amber alleles. As discussed above, amber mutations in the genes lin-1, lin-7, lin-12 and lin-18

probably result in the null phenotypes of these genes. However, amber mutations in the other four genes most likely do not result in null phenotypes. Specifically, the amber mutations lin-2(e1453) and let-23(n1045) appear to reduce, but not completely eliminate, gene function: many alleles of lin-2 are of higher penetrance than lin-2(e1453), and the severity of the Vul phenotype of let-23(n1045) is enhanced in trans both to other alleles of the gene and to a deficiency of the locus. Presumably, the amber fragments in these mutants retain some gene activity. Also, lin-24(n1057) is an amber allele, and, thus, the Vul phenotype of hermaphrodites of genotype lin-24(n1057)/+ probably results from the interaction between the amber fragment of lin-24(n1057) and the wild-type lin-24 gene product. Since the amber mutation lin-34(n1046) results in a partially dominant phenotype, it also probably does not cause a reduction or loss of gene function. Other amber mutations that do not result in total loss of gene function have been observed in both prokaryotes, e.g., the first identified mutation in E. coli DNA polymerase (DE LUCIA and CAIRNS 1969; GROSS and GROSS 1969), and in C. elegans, i.e., null mutations in the gene tra-1 of C. elegans result in the transformation of XX animals to phenotypic males (HODGKIN and BRENNER 1977; J. HODGKIN, personal communication), and one of the eight amber alleles of this gene results in incomplete transformation and thus is likely to retain some gene activity (J. HODGKIN, personal communication).

The amber alleles of lin-1, lin-2, lin-7 and lin-18 were suppressed well by a single copy of one of the amber suppressors sup-5 or sup-7, i.e., lin(amber)/lin(amber); sup/+ animals were

phenotypically wild-type, suggesting that relatively little activity is needed from these genes to produce a wild-type phenotype. For three of these genes, lin-1, lin-2 and lin-7, a further two-fold reduction in gene activity resulted in a partially mutant phenotype, as some hermaphrodites of genotype lin(amber)/lin(null, nonamber); sup/+ were visibly abnormal. lin-18 hermaphrodites of similar genotype were phenotypically wild-type. We can estimate the relative amount of lin gene product needed to result in a wild-type phenotype. In hermaphrodites homozygous for both sup-7 and unc-15(e1214), an amber allele of a gene that probably encodes paramyosin, approximately 40% of the wild-type level of paramyosin was restored (WATERSTON 1981). If the suppressed polypeptide products of lin-1, lin-2 and lin-7 function with an efficiency equal to that of the corresponding wild-type products, approximately 10% of the wild-type gene activity of these three genes is needed for the production of a wild-type phenotype. Similarly, less than 10% of the wild-type lin-18 gene activity is needed for the production of a wild-type phenotype. [These estimates assume that both the amber and nonamber alleles used in these experiments eliminate gene activity. However, the lin-2 and lin-18 alleles used may not totally eliminate gene activity (see Results) and, hence, the estimates for these two genes of the amount of gene activity that is necessary for the production of a wild-type phenotype may be low.] Similar or higher thresholds have been found for a number of different gene-enzyme systems in Drosophila melanogaster (reviewed by O'BRIEN and MACINTYRE 1978), suggesting that lin-1, lin-2, lin-7 and lin-18 may encode products that function catalytically (as opposed to stoichiometrically; e.g., see SNUSTAD

1967).

The suppression by amber suppressors of the mutations lin-24(n1057) and lin-34(n1046) is recessive; i.e., the mutant phenotypes are suppressed only in hermaphrodites homozygous for the suppressor. If these mutations result in abnormally functioning gene products (as suggested above), then the dose-dependent suppression of these mutations is caused by either the necessity of the restoration of a stoichiometric amount of wild-type gene product or by the necessity of a reduction in the amount of abnormally functioning gene product.

One of our goals has been to identify all genes that affect the vulval cell lineages. So far we have identified 25 genes that can mutate to generate a Muv or Vul phenotype (Table 2-14). Twenty-two of these genes are described in this manuscript and three genes (unc-83, unc-84 and lin-14) have been described elsewhere (HORVITZ and SULSTON 1980; SULSTON and HORVITZ 1981; AMBROS and HORVITZ 1984). The alleles of lin-14 that result in a Vul phenotype are dominant and result in the overproduction and/or ectopic expression of lin-14 gene activity (AMBROS and HORVITZ 1984). Some recessive mutations in unc-83 and unc-84 probably result in loss of gene function (W. FIXSEN, personal communication). The class of genes for which we were most likely to saturate is the set with null alleles that result in a Muv or Vul phenotype without also causing lethality or sterility. The eight genes (lin-1, lin-2, lin-7, lin-10, lin-18, lin-31, unc-83, unc-84) believed to be of this class are all represented by multiple alleles,

Table 2-14. The extent of saturation for genes that can mutate to a fertile Muv or Vul phenotype. The numbers of unc-83 and unc-84 alleles were obtained from W. FIXSEN (personal communication), and the number of dominant lin-14 alleles was obtained from AMBROS and HORVITZ (1984). If a mutation in each gene can occur with equal likelihood (i.e., if the probability of a gene's having a given number of alleles follows a binomial distribution), the Poisson function can be used to estimate the number of genes not yet identified. However, it is evident that some genes in each class have a greater number of alleles than would be expected if a mutation in every gene of that class could be recovered with equal frequency, prohibiting a formal application of the Poisson function to these data. Nonetheless, to obtain a very approximate estimate of the number of genes not yet identified, we have applied the Poisson function to these data. In an effort to reduce an overemphasis on genes with large numbers of alleles, for the last three classes of genes we calculated m, the average number of alleles per gene, according to MENEELY and HERMAN (1981), using the formula $f = (1 - e^{-m} - me^{-m}) / (1 - e^{-m})$, where f is the fraction of identified genes represented by more than one allele.

Table 2-14

The extent of saturation for genes that can mutate to a fertile Muv or Vul phenotype

No. of alleles of genes in each class

(1) Genes that were identified by recessive mutations and that have a viable null phenotype		(2) Genes that were identified by recessive mutations and that have a lethal or sterile null phenotype		(3) Genes that were identified by recessive mutations and that have an unknown null phenotype		(4) Genes that were identified by dominant mutations	
<u>lin-1</u>	16	<u>lin-3</u>	2	<u>lin-4</u>	1	<u>lin-12</u>	7
<u>lin-2</u>	13	<u>lin-8</u>	1	<u>lin-11</u>	4	<u>lin-24</u>	2
<u>lin-7</u>	13	<u>lin-9</u>	1	<u>lin-17</u>	5	<u>lin-33</u>	2
<u>lin-10</u>	3	<u>lin-13</u>	2	<u>lin-25</u>	2	<u>lin-34</u>	1
<u>lin-18</u>	2	<u>lin-15</u>	5	<u>n300</u>	1	<u>lin-14</u>	2
<u>lin-31</u>	11	<u>lin-26</u>	1				
<u>unc-83</u>	10	<u>let-23</u>	1				
<u>unc-84</u>	16						
Poisson estimate of the number of unidentified genes	0		2.5		1.0		0.3

six of them by ten or more alleles. It seems likely that we have identified most or all such genes; if so, there are very few genes of this class (Table 2-14). However, 12 other genes are defined by recessive mutations, which presumably result in loss or reduction of function. Seven of these genes (lin-3, lin-8, lin-9, lin-13, lin-15, lin-26, let-23) probably have lethal or sterile null phenotypes, and five other genes (lin-4, lin-11, lin-17, lin-25, n300) have unknown null phenotypes. We may also have identified most genes that by reduction of gene function can mutate to give a Muv or Vul phenotype (Table 2-14). The Muv or Vul alleles of these genes may be relatively infrequent mutations either that generally reduce (but not eliminate) gene function or that selectively reduce gene function in the vulval cells. The frequency with which we might expect to identify in a given gene mutations that result in a dominant phenotype -- because of altered, increased or ectopic gene activity -- is difficult to estimate. Nonetheless, we may have identified most genes that are able to mutate to a dominant Muv or Vul phenotype with frequencies comparable to the frequencies of dominant mutations in the five genes listed in Table 2-14.

Other classes of genes in addition to those we have identified may be involved in the control of the vulval cell lineages. We would not necessarily have identified genes that can mutate to generate sterile Muv or Vul animals, nor would we have identified genes with Muv or Vul mutations that display maternal effects. More generally, genes with redundant functions may be able to be identified only as a result of rare multiple mutations [or, in some cases, as a result of relatively

rare dominant mutations, e.g., see GREENWALD and HORVITZ (1980)].

Other genes involved in the control of the vulval lineages may not be able to mutate to give a Muv or Vul phenotype. Some such genes might be identified by mutations that act either to suppress or to enhance phenotypes caused by mutations known to affect vulval lineages.

Extragenic suppressors of mutations in lin-12 (E. FERGUSON, unpublished results) and of mutations in lin-1 (K. EDWARDS, personal communication) and extragenic enhancers of mutations in lin-8 or lin-9 (E. FERGUSON, unpublished results) have been identified that define additional genes that may be involved in vulval development. We hope that the identification and characterization of many of the genes responsible for vulval development will reveal aspects of the genetic and, ultimately, of the molecular specification of cell lineage in C. elegans.

Chapter III

Genetic analysis of the pathway of vulval development in Caenorhabditis elegans

This chapter is a draft of a paper: Ferguson, E. L., P. W. Sternberg
and H. R. Horvitz. Genetic analysis of the pathway of vulval
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Developmental Biology.

ABSTRACT

During the development of the vulva of the nematode Caenorhabditis elegans, six equipotential cells of the ventral hypodermis express one of three specific fates in a precise spatial pattern in response to a graded inductive signal from the gonadal anchor cell (Sulston and Horvitz, 1977; Sulston and White, 1980; Kimble, 1981; Sternberg and Horvitz, 1985a). Two classes of mutants abnormal in vulval morphology were previously identified: Vulvaless (Vul) and Multivulva (Muv) (Horvitz and Sulston, 1980; Ferguson and Horvitz, 1985). In this paper, we have characterized the action of the 22 genes defined by these mutants in four ways: (1) observation of the vulval cell lineage defects resulting from a mutation in each of these 22 genes, (2) localization of the site of action of each of these genes to one of the two tissues involved in vulval development, (3) for some genes, determination of the time of gene action, and (4) observation of the interactions among mutations in different genes. These data, in conjunction with the analysis of the effects of these mutations upon gene function (Greenwald et al., 1983; Ferguson and Horvitz, 1985), have allowed us to assign 17 of these 22 genes to one of five steps in the pathway of vulval development (Sternberg and Horvitz, 1985a). Specifically, three genes are necessary for the production of cells competent to undergo vulval cell lineages, one gene affects the formation of the anchor cell of the gonad, eleven genes are necessary for the determination of the fates of the vulval precursor cells in response to the anchor cell signal, and three genes are necessary for the execution of the fates of the vulval precursor cells.

INTRODUCTION

The development of a multicellular organism involves complex patterns of cell division and cell fate, known as cell lineages. We have begun to study the genetic specification of cell lineage in the small nematode Caenorhabditis elegans. C. elegans consists of relatively few cells (959 somatic nuclei in the adult hermaphrodite) of many classes of differentiated cell types (Sulston and Horvitz, 1977, Kimble and Hirsh, 1979, Sulston et al., 1980; 1983). The cellular anatomy and patterns of cell division and cell fate in C. elegans are essentially invariant throughout development (Sulston and Horvitz, 1977; Deppe et al., 1978; Kimble and Hirsh, 1979; Sulston et al., 1980; 1983). The results of cell ablation experiments using a laser microbeam suggest that the fates of a majority of cells are determined by their ancestry (Sulston et al., 1983). However, the fates of a small number of cells or groups of cells have been shown to be determined both by their ancestry and their position; these cells or groups of cells have the potential to express one of a number of alternate sublineages -- intrinsically determined lineages that generate a specific set of progeny -- dependent upon their position in the animal. One group of cells whose fates depend upon position are the cells that generate the vulva of the adult hermaphrodite. During the development of the vulva, six equipotential cells of the ventral hypodermis, P(3-8).p, respond to a graded inductive signal from the gonadal anchor cell by expressing one of three sublineages in a precise spatial pattern.

Vulval development in the wild type has been modelled as a branched pathway of five temporally separate steps (Sulston and Horvitz, 1977; Sulston and White, 1980; Kimble, 1981; Sternberg and Horvitz, 1985a) (summarized in Figure 1-1): (1) generation of cells competent to undergo vulval cell lineages, (2) generation of the anchor cell and production of the inductive signal, (3) determination of the fates of the vulval precursor cells in response to the inductive signal, (4) execution of the vulval sublineages, and (5) morphogenesis of the vulva.

To determine the genetic specification of the vulval cell lineages, we have attempted to identify all genes that affect these lineages. To date, we have isolated 98 mutants, defining 22 genes, that have alterations in vulval anatomy. These mutants display one of two general phenotypes as viewed with a dissecting microscope (Horvitz and Sulston, 1980; Sulston and Horvitz, 1981; Greenwald et al., 1983; Ferguson and Horvitz, 1985). A "Vulvaless" (Vul) hermaphrodite lacks a vulva and can not release eggs. (Some Vul hermaphrodites have a single protrusion at the site of the vulva.) Consequently, the progeny hatch inside the body of the parent and devour it. A Vul hermaphrodite can be recognized with a dissecting microscope by the presence of a "bag of worms", in which the parental cuticle encloses its progeny larvae. The progeny later escape from the parental cuticle. A "Multivulva" (Muv) hermaphrodite has one or more supernumerary protrusions or "pseudovulvae" along its ventral side. (Some Muv hermaphrodites also lack a functional vulva.)

In this paper, we report the alterations in the vulval cell lineages caused by mutations in each of the 22 identified genes. In addition, we characterize the wild-type function of these genes by determining the site, and for some genes, the time of gene action. These data, together with our observations of the patterns of interaction among mutations in different genes, allow us to assign 17 of the 22 genes to specific steps in the pathway of vulval development.

MATERIALS AND METHODS

Strains and genetic nomenclature: Caenorhabditis elegans var. Bristol strain N2 and most of the mutant strains used for strain construction were obtained from Brenner (1974) or from the Caenorhabditis Genetics Center, which is supported by contract number NO1-AG-9-2113 between the National Institutes of Health and the Curators of the University of Missouri. Except where noted, these genes are described by Brenner (1974) and/or by Swanson et al. (1984). The alleles used are either the reference alleles listed in the above publications or alleles that result in similar phenotypes. The map positions of all genes are indicated in Figure 3-1. N2 is the wild-type parent of all nematode strains used in this work.

LGI: unc-29(e1072).

LGII: dpy-10(e128), unc-4(e120), him-9(e1487), unc-52(e444), mnDf61, mnDf67, mnDf68 (Sigurdson, et al., 1984), C1 dpy-10(e128) unc-52(e444). C1 dpy-10 unc-52 is an aberration of LGII that balances the right half of the chromosome (Herman, 1978).

LGIII: unc-36(e251), sup-5(e1464), lin-16(e1743), unc-86(e1507), unc-32(e189), tra-1(e1488) (J. Hodgkin, personal communication), dpy-18(e364).

LGIV: dpy-13(e184), dpy-20(e1362), unc-22(e66), unc-31(e169).

Figure 3-1 Partial genetic map of *C. elegans* indicating the markers used in this study. Genes that affect the vulval cell lineages are drawn above the lines representing the *C. elegans* linkage groups. The extents of deficiencies (Dfs) are indicated below the lines.

0 5 10
map units

Figure 3-1

LGV: him-5(e1467,e1490). Unless otherwise specified, him-5(e1467) was used in all genetic manipulations.

LGX: sup-7(st5), unc-58(e665)

This paper follows the standardized C. elegans genetic nomenclature (Horvitz et al., 1979). All vulval cell lineage mutants except n300 and n1045 were assigned lin, for lineage abnormal, gene names. It is not known if n300, the mutation responsible for the Vulvaless phenotype associated with nT1(IV;V), is a mutation present on the translocation or is a result of the translocation itself (Ferguson and Horvitz, 1985). For this reason we have not assigned a gene name to n300 although it defines a separate complementation group. Because n1045 was shown to be an allele of the previously defined gene let-23 (Sigurdson et al., 1984; Ferguson and Horvitz, 1985), it was not assigned a lin gene name.

General techniques: Methods for the culturing, handling and genetic manipulation of C. elegans have been described (Brenner, 1974). Most experiments were done at 20°, except those involving lin-13, sup-7, or lin-18, which were done either at 20° or 25°. Experiments involving let-23(n1045) were performed at 15°, 20° or 25°. Cell lineages and anatomy were determined using the methods of Sulston and Horvitz (1977); in general, only vulval cell lineages have been determined. Males carrying vulval cell lineage mutations were obtained either from a male stock or from strains containing him-5 or him-9, which result in a high incidence of males (Hodgkin et al., 1979).

Sources of vulval cell lineage mutants: The sources of the vulval cell lineage mutants characterized in this manuscript were described by Ferguson and Horvitz (1985).

Construction of double mutant strains: We have constructed double mutants carrying multiple vulval cell lineage mutations. Table 3-1 summarizes the double or multiple mutants that were constructed; Table 3-2 lists the strain names and exact genotypes of the double or multiple mutant strains. The phenotypes of some of the strains listed in Table 3-2 are described in Sternberg and Horvitz (1985b). The methods used in the construction of these strains are detailed below.

Muv-Vul double mutants: In general, Multivulva ("Muv") - Vulvaless ("Vul") double mutants were constructed by picking about ten Muv and ten Vul progeny from animals of genotype muv/+; vul/+. The segregants of these animals that had a phenotype different from that of their parents (e.g., Vul animals derived from Muv parents) were the desired double mutants. (To construct hermaphrodites of genotype muv/+; vul/+, males homozygous or heterozygous for one vulval cell lineage mutation were mated with hermaphrodites homozygous for the second vulval cell lineage mutation. As some vulval cell lineage mutations render hermaphrodites unable to mate, such mutations were passed through males. Most such males were generated from strains containing him-5; lin-4 +/+ dpy-10; him-5, lin-11; him-5, lin-12(n302); him-5, + lin-12(n137)/unc-32 +; him-5, lin-13 +/+ unc-32; him-5, lin-26/C1 dpy-10 unc-52; him-5. Males heterozygous for n300 were generated by mating wild-type males with hermaphrodites of genotype nT1(IV)/unc-5;

Table 3-1 Double or multiple mutant strains containing different vulval cell lineage mutations. The exact genotype and strain name of each of these mutant strains is listed in Table 3-2. *, a double or multiple mutant strain exists; L, the double or multiple mutant strain was constructed but was subsequently lost; h, a double or multiple mutant strain containing a him mutation exists. [him mutations increase the frequency of male self progeny, (Hodgkin et al., 1979).] All him strains were constructed with him-5, except MT1475 him-9; nT1(IV); nT1(V).

Table 3-1

Double and multiple mutant strains

Production of P(3-8).p Cells					Vulvaless					Determination of P(3-8).p Cell Fate Multivulva					lin-12			Execution of 2° Sublineages			Other		
lin-26	n300	lin-4	lin-24	lin-33	lin-3	let-23	lin-2	lin-7	lin-10	lin-15	lin-8	lin-9	lin-13	lin-34	lin-12 (Muv)	lin-12 (Vul)	lin-12 (O)	lin-11	lin-17	lin-18	lin-1	lin-25	lin-31
lin-26	*h									L	*	L			L	*					*		*
n300		*h	*				*	*	*	L	*	*	*		L	*					*		*
lin-4			*h				*h	*	*	L	*	*	*		L	*					*		*
lin-24				*			*	*	*	*	*	*	*h		*h	*h					*		*
lin-33				*			*	*	*	*	*	*	*		*	*					*		*
=====																							
			lin-3		*h	*	*h	*	*h	*h	*	*h	*		*	*h	*h		*h	*		*	*h
			let-23			*h	*	*	*	*h	*	*	*		*h	*h	*h		*h	*		*h	*
			lin-2				*h	*h	*	*h	*	*	*h		*h	*h	*		*h	*		*	*
			lin-7					*h	*h	*h	*h	*h	*h		*h	*	*		*h	*		*	*
			lin-10						*h	*h	*	*h	*		*h	*h	*h		*h	*		*	*h
=====																							
			lin-15							*h					*	*h	*h		*h	*		*	*
			lin-8; lin-9								*h								*h	*		*	*
			lin-13									*h							*h	*		*	*
			lin-34										*h						*h	*		*	*
=====																							
			lin-12 (Muv)												*h				*h	*		*	*
			lin-12 (Vul)													*h			*h	*		*	*
			lin-12 (O)														*h		*	*		*	*
=====																							
			lin-11															*h	*	*		*	*
			lin-17																*h	*		*	*
			lin-18																	*h		*	*
=====																							
			lin-1																		*h	*	*
			lin-25																			*h	*
			lin-31																				*h
=====																							

Table 3-2 The single, double or multiple mutant strains described in this manuscript. Strains containing mutations in two or more lin genes are listed under the lin gene with the lowest gene number; e.g., MT1321 lin-10(e1439); lin-2(e1453) is listed under lin-2. See Materials and Methods for details of strain construction.

Table 3-2

Strain list

lin-1

MT876	<u>lin-1(e1275)</u>
MT1001	<u>lin-1(e1777)</u>
MT521	<u>lin-1(e1275); him-5(e1467)</u>
MT1468	<u>lin-1(e1777); him-5(e1467)</u>
CB2275	<u>lin-1(e1275); lin-2(e1309)</u>
MT519	<u>lin-1(e1275) lin-3(e1417)</u>
CB2228	<u>lin-4(e912); lin-1(e1275)</u>
MT520	<u>lin-7(e1413); lin-1(e1275)</u>
MT544	<u>lin-10(e1438); lin-1(e1275)</u>
MT1324	<u>lin-11(n389); lin-1(e1275)</u>
MT1323	<u>lin-11(n389); lin-1(e1275); him-5(e1467)</u>
MT2385	<u>dpy-19(e1259) lin-12(n137); lin-1(e1777)</u>
MT965	<u>lin-12(n302); lin-1(e1275)</u>
MT966	<u>lin-12(n302); lin-1(e1275); him-5(e1467)</u>
MT2345	<u>+ unc-32(e189) lin-12(n137 n720)/unc-36(e251) + +;</u> <u>lin-1(e1777)</u>
MT1763	<u>lin-17(n671); lin-1(e1275)</u>
MT1768	<u>lin-1(e1275) lin-24(n432)</u>
MT1777	<u>lin-1(e1275); lin-25(e1446)</u>
MT1756	<u>lin-26(n156); lin-1(e1275)</u>
MT2393	<u>let-23(n1045); lin-1(e1777)</u>
MT2471	<u>let-23(n1045); lin-1(e1777); him-5(e1467)</u>

lin-2

CB1309	<u>lin-2(e1309)</u>
MT887	<u>lin-2(e1453)</u>
MT491	<u>him-5(e1467); lin-2(e1309)</u>
MT640	<u>him-5(e1467); lin-2(e1453)</u>
MT969	<u>lin-3(e1417); lin-2(e1453)</u>
MT970	<u>lin-3(e1417); him-5(e1467); lin-2(e1453)</u>

MT959 lin-4(e912); lin-2(e1453)
 MT960 lin-4(e912); him-5(e1467); lin-2(e1453)
 MT620 lin-7(e1413); lin-2(e1453)
 MT625 lin-7(e1449); him-5(e1467); lin-2(e1453)
 MT525 lin-8(n111); lin-9(n112); lin-2(e1309)
 MT1321 lin-10(e1439); lin-2(e1453)
 MT1479 lin-11(n389); lin-2(e1453)
 MT1322 lin-11(n389); him-5(e1467); lin-2(e1453)
 MT1330 lin-12(n137); lin-2(e1453)
 MT2387 lin-12(n137); him-5(e1467); lin-2(e1309)
 MT1481 lin-12(n302); lin-2(e1309)
 MT1482 lin-12(n302); him-5(e1467); lin-2(e1309)
 MT2346 + unc-32(e189) lin-12(n137 n720)/unc-36(e251) + +;
lin-2(e1309)
 MT1375 lin-13(n387) +/- unc-32(e189); lin-2(e1309)
 MT1376 lin-13(n387) +/- unc-32(e189); him-5(e1467);
lin-2(e1309)
 MT1313 lin-2(e1453) lin-15(n309)
 MT1779 lin-24(n432); lin-2(e1309)
 MT1476 lin-31(n301); lin-2(e1309)
 MT2392 let-23(n1045); lin-2(e1309)
 MT2479 let-23(n1045); lin-2(n768)
 MT1342 nT1(IV); nT1(V); lin-2(e1453)
 MT2184 tra-1(e1488); lin-2(e1309)

lin-3

CB1417 lin-3(e1417)
 MT2202 lin-3(n1059) +/- lin-3(e1417) dpy-20(e1362)
 MT490 lin-3(e1417); him-5(e1467)
 MT1317 lin-4(e912); lin-3(e1417)
 MT589 lin-7(e1413); lin-3(e1417)
 MT524 lin-8(n111); lin-9(n112); lin-3(e1417)
 MT626 lin-10(e1439); lin-3(e1417)
 MT627 lin-10(e1439); lin-3(e1417); him-5(e1467)
 MT1326 lin-11(n389); lin-3(e1417)
 MT1327 lin-11(n389); lin-3(e1417); him-5(e1467)

MT547 lin-12(n137); lin-3(e1417)
 MT973 lin-12(n302); lin-3(e1417)
 MT974 lin-12(n302); lin-3(e1417); him-5(e1467)
 MT2347 + unc-32(e189) lin-12(n137 n720)/unc-36(e251) + +;
lin-3(e1417)
 MT2348 + unc-32(e189) lin-12(n137 n720)/dpy-19(e1259) +
lin-12(n137); lin-3(e1417); him-5(e1467)
 MT1377 lin-13(n387) +/+ unc-32(e189); lin-3(e1417)
 MT1363 lin-13(n387) +/+ unc-32(e189); lin-3(e1417);
him-5(e1467)
 MT636 lin-3(e1417); lin-15(n309)
 MT2730 lin-3(n378) + unc-22(e66)/lin-3(n1059) dpy-20(e1362) +;
him-5(e1467); lin-15(n765)
 MT1776 lin-24(n432) lin-3(e1417)
 MT975 lin-31(n301); lin-3(e1417)
 MT976 lin-31(n301); lin-3(e1417); him-5(e1467)
 MT2391 let-23(n1045); lin-3(e1417)
 MT2185 tra-1(e1488); lin-3(e1417)

lin-4

MT873 lin-4(e912)
 MT1155 lin-4(e912)/ C1 dpy-10(e128) unc-52(e444); him-5(e1467)
 MT624 lin-4(e912) lin-7(e1413)
 MT523 lin-8(n111) lin-4(e912); lin-9(n112)
 MT623 lin-10(e1439); lin-4(e912)
 MT986 lin-4(e912); lin-12(n302); him-5(e1467)
 MT1380 lin-4(e912); lin-13(n387) +/+ unc-32(e189)
 MT1315 lin-31(n301) lin-4(e912)
 MT1367 lin-4(e912) +/+ dpy-10(e128); nT1(IV); nT1(V)

lin-7

MT877 lin-7(e1413)
 MT495 lin-7(e1413); him-5(e1467)
 MT526 lin-8(n111) lin-7(e1413); lin-9(n112)
 MT596 lin-8(n111) lin-7(e1449); lin-9(n112); him-5(e1467)
 MT622 lin-10(e1439); lin-7(e1413)

MT621 lin-10(e1439); lin-7(e1413); him-5(e1467)
 MT1478 lin-11(n389); lin-7(e1413)
 MT1314 lin-11(n389); lin-7(e1413); him-5(e1467)
 MT1332 lin-7(e1413); lin-12(n137)
 MT1333 lin-7(e1413); lin-12(n137); him-5(e1467)
 MT985 lin-7(e1413); lin-12(n302)
 MT2349 lin-7(e1413); + unc-32(e189) lin-12(n137 n720)/
unc-36(e251) + +
 MT1378 lin-7(e1413); lin-13(n387) +/- unc-32(e189)
 MT1379 lin-7(e1413); lin-13(n387) +/- unc-32(e189);
him-5(e1467)
 MT637 lin-7(e1413); lin-15(n309)
 MT638 lin-7(e1413); him-5(e1467); lin-15(n309)
 MT2380 lin-17(n671); lin-7(e1413)
 MT1773 lin-7(e1413); lin-24(n432)
 MT987 lin-31(n301) lin-7(e1413)
 MT2389 let-23(n1045) lin-7(e1413)
 MT1339 lin-7(e1413); nT1(IV); nT1(V)
 MT2188 lin-7(e1413); tra-1(e1488)

lin-8; lin-9

CB1322 lin-8(n111); lin-9(n112)
 MT522 lin-8(n111); lin-9(n112); him-5(e1467)
 MT545 lin-10(e1438); lin-8(n111); lin-9(n112)
 MT983 lin-11(n389); lin-8(n111); lin-9(n112)
 MT1331 lin-11(n389); lin-8(n111); lin-9(n112); him-5(e1467)
 MT1758 lin-17(n671); lin-8(n111); lin-9(n112)
 MT1767 lin-8(n111); lin-9(n112); lin-24(n432)
 MT1765 lin-8(n111); lin-9(n112); lin-25(n545)
 MT1762 lin-8(n111) lin-26(n156); lin-9(n112)
 MT2494 lin-8(n111) let-23(n1045); lin-9(n112)
 MT1480 lin-8(n111); lin-9(n112); nT1(IV); nT1(V)

lin-10

CB1439 lin-10(e1439)
 MT993 lin-10(e1439); him-5(e1467)

MT1318 lin-10(e1439); lin-12(n137)
 MT1319 lin-10(e1439); lin-12(n137); him-5(e1467)
 MT979 lin-10(e1439); lin-12(n302)
 MT980 lin-10(e1439); lin-12(n302); him-5(e1467)
 MT2351 lin-10(e1439); + unc-32(e189) lin-12(n137 n720)/
dpy-19(e1259) + lin-12(n137)
 MT2352 lin-10(e1439); + unc-32(e189) lin-12(n137 n720)/
dpy-19(e1259) + lin-12(n137); him-5(e1467)
 MT1365 lin-10(e1439); lin-13(n387) +/+ unc-32(e189)
 MT1364 lin-10(e1439); lin-13(n387) +/+ unc-32(e189);
him-5(e1467)
 MT981 lin-10(e1439); lin-15(n309)
 MT982 lin-10(e1439); him-5(e1467); lin-15(n309)
 MT1772 lin-10(e1439); lin-24(n432)
 MT1320 lin-10(e1439); lin-31(n301)
 MT1477 lin-10(e1439); lin-31(n301); him-5(e1467)
 MT2390 lin-10(e1439); let-23(n1045)
 MT1336 lin-10(e1439); nT1(IV); nT1(V)
 MT2186 lin-10(n299); tra-1(e1488)

lin-11

MT389 lin-11(n389)
 MT633 lin-11(n389); him-5(e1467)
 MT963 lin-11(n389); lin-12(n137)
 MT964 lin-11(n389); lin-12(n137); him-5(e1467)
 MT967 lin-11(n389); lin-12(n302)
 MT968 lin-11(n389); lin-12(n302); him-5(e1467)
 MT2353 lin-11(n389); + unc-32(e189) lin-12(n137 n720)/+ +
unc-36(e251)
 MT1381 lin-11(n389); lin-13(n387) +/+ unc-32(e189)
 MT1382 lin-11(n389); lin-13(n387) +/+ unc-32(e189);
him-5(e1467)
 MT1325 lin-11(n389); lin-15(n309)
 MT984 lin-11(n389); him-5(e1467); lin-15(n309)
 MT2379 lin-17(n671) lin-11(n382)
 MT2381 lin-11(n382); him-5(e1467); lin-18(e620)

MT977 lin-11(n389); lin-31(n301)
 MT978 lin-11(n389); lin-31(n301); him-5(e1467)
 MT1355 lin-11(n389); nT1(IV); nT1(V)

lin-12(Muv)

MT137 lin-12(n137)
 MT686 + lin-12(n137)/unc-32 +; him-5(e1467)
 MT2384 lin-12(n137); lin-15(n309)
 MT2383 lin-17(n671); lin-12(n137); him-5(e1467)
 MT2386 lin-12(n137); him-5(e1467); lin-18(e620)
 MT1770 lin-12(n137); lin-24(n432)
 MT1771 lin-12(n137); lin-24(n432); him-5(e1467)
 MT1822 + lin-12(n137)/unc-32(e189) +; lin-25(e1446)
 MT2366 let-23(n1045); dpy-19(e1259) + lin-12(n137)/+
 unc-32(e189) lin-12(n137 n720)
 MT2367 let-23(n1045); dpy-19(e1259) + lin-12(n137)/+
 unc-32(e189) lin-12(n137 n720); him-5(e1467)
 MT1337 lin-12(n137); nT1(IV); nT1(V)

lin-12(Vul)

MT302 lin-12(n302)
 MT1329 lin-12(n302); him-5(e1467)
 MT971 lin-12(n302); lin-15(n309)
 MT972 lin-12(n302); him-5(e1467); lin-15(n309)
 MT1774 lin-12(n302); lin-24(n432)
 MT1775 lin-12(n302); lin-24(n432); him-5(e1467)
 MT961 lin-31(n301); lin-12(n302)
 MT962 lin-31(n301); lin-12(n302); him-5(e1467)
 MT1341 lin-12(n302); nT1(IV); nT1(V)

lin-12(O)

MT1527 + unc-32(e189) lin-12(n137 n720)/unc-36(e251) + +
 MT2344 + unc-32(e189) lin-12(n137 n720)/dpy-19(e1259) +
 lin-12(n137); him-5(e1467)
 MT2354 + unc-32(e189) lin-12(n137 n720)/unc-36(e251) + +;
 lin-15(n309)

MT2355 unc-32(e189) + lin-12(n137 n720)/dpy-19(e1259) +
lin-12(n137); him-5(e1467); lin-15(n309)
 MT2356 lin-17(n671); + unc-32(e189) lin-12(n137 n720)/
unc-36(e251) + +
 MT2358 + unc-32(e189) lin-12(n137 n720)/unc-36(e251) + +;
lin-18(e620)
 MT2359 + unc-32(e189) lin-12(n137 n720)/unc-36(e251) + +;
him-5(e1467); lin-18(e620)
 MT2368 + unc-32(e189) lin-12(n137 n720)/unc-36(e251) + +;
lin-25(e1446)
 MT2362 lin-31(n301); + unc-32(e189) lin-12(n137 n720)/
unc-36(e251) + +
 MT2364 lin-31(n301); + unc-32(e189) lin-12(n137 n720)/
dpy-19(e1259) + lin-12(n137); him-5(e1467)
 MT2365 let-23(n1045); + unc-32(e189) lin-12(n137 n720)/
unc-36(e251) + +

lin-13

MT1982 + lin-13(n387) unc-36(e251) +/lin-16(e1743) + +
unc-86(e1507)
 MT1373 lin-13(n387) +/+ unc-32(e189); him-5(e1467)
 MT1825 lin-13(n387) +/+ unc-32(e189); lin-24(n432)
 MT1826 lin-13(n387) +/+ unc-32(e189); lin-24(n432);
him-5(e1467)
 MT1824 lin-13(n387) +/+ unc-32(e189); lin-25(e1446)
 MT1366 lin-13(n387) +/+ unc-32(e189); nT1(IV); nT1(V)

lin-15

MT309 lin-15(n309)
 MT1470 him-5(e1467); lin-15(n309)
 MT2382 lin-17(n671); lin-15(n309)
 MT1769 lin-24(n432); lin-15(n309)
 MT1764 lin-25(n545); lin-15(n309)
 MT2464 let-23(n1045); lin-15(n309)
 MT2465 let-23(n1045); him-5(e1467); lin-15(n309)
 MT1340 nT1(IV); nT1(V); lin-15(n309)

lin-17

MT1306 lin-17(n671)
MT1464 lin-17(n671); him-5(e1467)
MT1760 lin-17(n671); lin-31(n301)
MT2394 lin-17(n671); let-23(n1045)

lin-18

CB620 lin-18(e620)
MT1469 him-5(e1467); lin-18(e620)

lin-24

MT1007 lin-24(n432)
MT1766 lin-31(n301); lin-24(n432)

lin-25

CB1446 lin-25(e1446)
MT1175 lin-25(n545)
MT1757 lin-25(e1446) him-5(e1467)
MT1778 lin-31(n301); lin-25(e1446)
MT2193 tra-1(e1488); lin-25(n545)

lin-26

MT156 lin-26(n156)
MT1816 lin-26(n156)/ C1 dpy-10(e128) unc-52(e444);
him-5(e1467)

lin-31

MT301 lin-31(n301)
MT376 lin-31(n376)
MT1639 lin-31(n762)
MT1466 lin-31(n301); him-5(e1467)
MT2400 lin-31(n301) let-23(n1045)
MT1338 lin-31(n301); nT1(IV); nT1(V)

lin-34

MT2124 lin-34(n1046)

MT2481 lin-34(n1046); him-5(e1467)

let-23

MT2123 let-23(n1045)

MT2483 let-23(n1045); him-5(e1467)

n300

MT300 nT1(IV); nT1(V)

MT1475 him-9(e1487); nT1(IV); nT1(V)

nT1(V)/dpy-11.)

To facilitate the construction of double mutants between the partially dominant Muv mutation lin-12(n137) and different Vul mutations, the closely linked marker unc-32 was used in trans to lin-12(n137). Specifically, males of genotype + lin-12(n137)/unc-32 +; him-5 were mated with hermaphrodites of genotype unc-32; vul. Cross-progeny Muv hermaphrodites of genotype + lin-12(n137)/unc-32 +; vul/+; him-5/+ were picked and the phenotypes of their progeny were examined. If lin-12(n137) was epistatic to the Vul mutation, no non-Unc Vul progeny were observed. In such cases, Muv hermaphrodites were picked. Some Muv hermaphrodites were of genotype + lin-12(n137)/unc-32 +; vul, as they segregated some Unc progeny, all of which were Vul. From such Muv hermaphrodites, the lin-12(n137); vul strain was established by picking Muv hermaphrodites that failed to segregate Unc progeny. If the Vul mutation was epistatic to lin-12(n137), non-Unc Vul hermaphrodites were observed among the progeny of a + lin-12(n137)/unc-32 +; vul/+ hermaphrodite. In such cases, the lin-12(n137); vul strain was established by picking Vul animals that failed to segregate any Unc progeny.

Similarly, to construct double mutants carrying either of the two partially dominant Vul mutations -- lin-12(n302) and lin-24(n432) -- and different Muv mutations, a closely linked marker was used in trans to each of the Vul mutations. (unc-32 was used in trans to lin-12(n302); unc-31 was used in trans to lin-24.)

To facilitate the construction of the double mutant between the Vul mutation let-23(n1045) and the partially dominant Muv mutation lin-34(n1046), unc-22 was used in cis to lin-34. To construct a strain of genotype lin-8 let-23; lin-9, Muv non-Dpy recombinants were picked from the progeny of hermaphrodites of genotype lin-8 dpy-10 +/+ + let-23; lin-9 (lin-8; lin-9 hermaphrodites are Muv). The Vul animals that segregated from these hermaphrodites were of the desired genotype.

As some of the lin-25(e1446); muv double mutants were sterile, the lin-25; muv double mutant strains were constructed using the heat-sensitive allele of lin-25, n545. At 25⁰, from the progeny of hermaphrodites of genotype muv/+; lin-25(n545)/+, Vul animals were picked and placed at 15⁰. Some of these animals segregated Muv progeny, which were of the desired genotype. The phenotype of these double mutant strains were then examined at the restrictive temperature of 25⁰.

Because Muv mutations in lin-13 result in a heat-sensitive phenotype and display a maternal effect, the epistatic interactions between lin-13 and different Vul mutations were determined using the following protocol. From the progeny of hermaphrodites of genotype lin-13 +/+ unc-32; vul that were grown at 15⁰, fertile hermaphrodites of genotype lin-13; vul were picked and allowed to produce progeny at 25⁰, which were examined.

Vul-Vul double mutants: To construct double mutants between pairs of

Vul mutations in the genes lin-2, lin-3, lin-7, lin-10, lin-11 and let-23(n1045), a recessive mutation, r, was used in trans to one of the Vul mutations. Specifically, if vul-a and vul-b are two Vul mutations and r is a recessive mutation that is closely linked to vul-a, the double mutant strain vul-a; vul-b was constructed by mating vul-a; him-5 males with r; vul-b hermaphrodites. From cross progeny hermaphrodites of genotype vul-a +/+ r; vul-b/+, Vul non-R animals were picked. The Vul hermaphrodites that segregated R animals were presumably of genotype vul-a +/+ r; vul-b. Progeny of these Vul hermaphrodites that did not segregate R animals were presumably of genotype vul-a; vul-b. The genotypes of Vul-Vul double mutants that were of incomplete penetrance were subsequently confirmed by complementation experiments. (dpy-13 was used in trans to lin-3, unc-52 was used in trans to lin-7, unc-29 was used in trans to lin-11, and dpy-10 was used in trans to let-23. The dominant mutation unc-58 was used in trans to lin-2.) The n300; vul double mutant strains were constructed by mating males of genotype vul/+; b/+ (where b was any of various balancers for nT1(IV;V)) with hermaphrodites of genotype unc-32; nT1(IV)/dpy-13; nT1(V)/+ (unc-32 was used to distinguish self progeny from cross progeny). Because lin-4 hermaphrodites have a distinctive body shape, the lin-4; vul double mutant strains were constructed by mating vul; him-5 males with lin-4 +/+ dpy-10 hermaphrodites. In the F2, vul hermaphrodites were picked; those that segregated lin-4 animals were of the desired genotypes.

Gonad ablation studies: Young L1 hermaphrodites with four cells in the gonad primordium were anesthetized in 0.5% (v/v) 1-phenoxy

2-propanol in M9 buffer until motionless (about 1 minute) (Sulston and Horvitz, 1977). Animals were irradiated using the laser microbeam system described by Sternberg and Horvitz (1981). Each of the four cells of the gonad primordium was irradiated 4-10 times (until visible damage to each cell was observed). The animal was then allowed to recover. The irradiated animals were reexamined using Nomarski optics before adulthood to ascertain that the gonad was destroyed and that there was no apparent damage to the hypodermal cells. Animals that did not meet these criteria were discarded.

After reaching adulthood, irradiated animals of Muv strains that normally generate more than one supernumerary ventral protrusion -- lin-1(e1275), lin-8(n111); lin-9(n112), lin-12(n137), lin-13(n387), lin-15(n309), lin-31(n301) -- were examined either with a dissecting microscope or with Nomarski optics to ascertain the presence or absence of such protrusions.

Irradiated animals of the other three strains -- lin-11(n389), lin-17(n671), let-23(n1045) grown at 25° -- were examined with Nomarski optics during the late L3 or early L4 stages for the presence or absence of vulval cells. Since the Muv phenotype of let-23(n1045) hermaphrodites is only evident at 25°, the let-23 hermaphrodites that were to be irradiated were grown at 25° until the early L1. At this time, which is prior to the temperature-sensitive period of let-23(n1045) (see Results), the let-23 hermaphrodites were removed from 25° and their gonads were ablated. The irradiated let-23(n1045) hermaphrodites were then returned to 25° after an absence of less than

one hour.

Temperature-shift experiments: Temperature-shift experiments were performed on hermaphrodites carrying a heat-sensitive allele of any of three genes -- lin-13, lin-15, lin-25 -- or a cold-sensitive allele of a fourth gene -- let-23. Because lin-13(n387) results in sterility, the temperature-sensitive period of lin-13 was determined by examining the phenotype of unc-36 lin-13 hermaphrodites segregating from parental hermaphrodites of genotype + lin-13 unc-36 +/ lin-16 + + unc-86. Because the Muv phenotype of lin-15 is expressed in males as well as in hermaphrodites, the temperature-sensitive period of lin-15 was determined in both sexes by performing the temperature-shift experiments on male and hermaphrodite progeny of him-5; lin-15 hermaphrodites.

For all strains except let-23, the temperature-shift experiments were performed according to the following protocol. Stocks of the strain to be shifted were grown at 15°. A synchronous population of worms was obtained by allowing three or four adult hermaphrodites to lay eggs for two hours on each of 36 Petri dishes at 15°. Each Petri dish contained 30-60 eggs. Immediately after the removal of the parental hermaphrodites, 18 of the Petri dishes were shifted to 25° while the other 18 were kept at 15°. At various times during the development of the progeny, a pair of Petri dishes was shifted either from 15° to 25° or from 25° to 15°. In most cases, three or four animals were staged at the time of transfer using Nomarski optics; otherwise, the stage of the animals at the time of transfer was

inferred from animals observed at other time points during the same experiment. One pair of Petri dishes at each temperature was not shifted. The phenotypes of the shifted animals were then examined after they reached adulthood. lin-15 animals (both males and hermaphrodites) were scored for the presence of ventral protrusions. lin-25 hermaphrodites were scored for their ability to lay eggs. lin-13 hermaphrodites were scored both for fertility and for the presence of ventral protrusions. If the lin-13 hermaphrodites were fertile, the phenotypes of their progeny were also examined.

Temperature-shift experiments with let-23(n1045) hermaphrodites were performed by growing let-23 hermaphrodites at both permissive (25°) and restrictive (15°) temperatures. At various times during development, let-23 hermaphrodites were removed from one temperature and the stage of their development was ascertained with Nomarski optics using cell lineage criteria. These hermaphrodites were then placed on Petri dishes at the other temperature. The patterns of divisions of P(3-8).p in these hermaphrodites were later observed in L3 and/or L4 larvae using Nomarski optics.

Lineage nomenclature: The cell lineage nomenclature is as described by Sulston and Horvitz (1977). In Figure 1-1f, which contains a diagrammatic representation of the vulval cell lineages, the anterior daughter of each cell is drawn on the left, the posterior daughter of each cell is drawn on the right. The progeny of transverse divisions are indicated by "l" (left) and "r" (right). "P(3-8).p" refers collectively to the six cells P3.p, P4.p, P5.p, P6.p, P7.p and P8.p.

"Pn.p" refers to the cells P1.p through P11.p. "x" refers to both sister cells; e.g., P7.px refers collectively to P7.pa and P7.pp.

RESULTS

To determine how the 22 genes defined by the Muv and Vul mutants function during the development of the vulva, we have characterized the action of these genes in four ways. (1) Mutant Phenotype: we have examined the alterations in the vulval cell lineages resulting from a mutation in each of the genes. (2) Site of Gene Action: we have localized the action of each of the genes to one of the two tissues involved in vulval development. (3) Time of Gene Action: for those genes with temperature-sensitive alleles, we have determined the time of gene action. (4) Interactions between Mutations: we have investigated the patterns of interactions between mutations in different genes. We summarize our results in Table 3-3.

(1) Mutant Phenotypes

We have determined that the morphological defects that cause the Muv and Vul phenotypes of these mutant strains result from alterations in the vulval cell lineages. In this section, we describe the vulval cell lineage defects that result from a canonical allele of each of these 22 genes. The canonical allele of each gene was chosen using two criteria: (1) as viewed with a dissecting microscope, the canonical allele had as strong an effect upon the anatomy of the vulva as any other allele of the gene; and (2) the canonical allele of a gene was genetically well characterized (i.e., the allele had been used for mapping studies and/or for studies of mutant interactions). However, for some genes we have determined the vulval cell lineage

Table 3-3 Canonical alleles are those alleles used for studies of vulval cell lineages, for mapping studies and/or for studies of gene interactions (Ferguson and Horvitz, 1985; also see Results). A complete listing of the alleles of these genes and the effects of these mutations upon wild-type gene activity is reported by Ferguson and Horvitz (1985).

Table 3-3

Step in pathway of vulval development	Genes that affect the vulval cell lineages		
	Gene; canonical allele(s)	Hermaphrodite phenotype	Type of mutation
Production of vulval precursor cells	<u>lin-26</u> <u>n156</u>	Vul: P(1-11).p become neuronal-like.	Recessive, reduction of function
	<u>n300</u>	Vul: P(3-8).p join hypodermal syncytium without dividing.	Recessive, unknown (associated with reciprocal translocation nT1(IV;V))
	<u>lin-4</u> <u>e912</u>	Vul: P(3-8).p divide. Expression of L1-specific divisions?	Recessive, unknown
	<u>lin-24</u> ^a <u>n432</u>	Vul: P(1-11).p can die in L1, P(3-8).p undergo abnormal L3 lineages.	Partially dominant, probably gain of function
	<u>lin-33</u> ^a <u>n1043</u>	Vul: P(1-11).p can die in the L1, P(3-8).p undergo abnormal L3 lineages.	Partially dominant, probably gain of function
Determination of the fates of P(3-8).p	<u>lin-2</u> <u>e1309</u> <u>e1453</u>	Vul: P ₀ (5-7).p express 3 fates, incompletely penetrant.	Recessive, probably null
	<u>lin-7</u> <u>e1413</u>	Vul: P ₀ (5-7).p express 3 fates, incompletely penetrant.	Recessive, null
	<u>lin-10</u> <u>e1439</u>	Vul: P ₀ (5-7).p express 3 fates, incompletely penetrant.	Recessive, null

Table 3-3

Step in pathway of vulval development	Gene; canonical allele(s)	Hermaphrodite phenotype	Type of mutation
	<u>let-23</u> <u>n1045cs</u>	Vul: 15 ⁰ -- P(5-7).p express 3 ⁰ fates, incompletely penetrant. 25 ⁰ -- P(3-8).p can express non-3 ⁰ fates.	Recessive, reduction of function
	<u>lin-3</u> <u>e1417</u>	Vul: P(5-7).p express 3 ⁰ fates, incompletely penetrant.	Recessive, reduction of function
	CB1322 <u>lin-8(n111);</u> <u>lin-9(n112)</u>	Muv: P(5-7).p express normal fates. P(3,4,8).p undergo 1 ⁰ , 2 ⁰ or novel sublineages.	--
	<u>lin-8</u> <u>n111</u>	WT	Recessive, unknown
	<u>lin-9</u> <u>n112</u>	WT	Recessive, reduction of function
	<u>lin-15</u> <u>n309</u>	Muv: P(5-7).p express normal fates; P(3,4,8).p undergo 1 ⁰ , 2 ⁰ or novel sublineages.	Recessive, reduction of function
	<u>lin-13</u> <u>n387hs</u>	Muv: 20 ⁰ -- P(5-7).p express normal fates. P(3,4,8).p undergo 1 ⁰ , 2 ⁰ or novel sublineages. 25 ⁰ -- P(3-8).p divide in the L2; additional L3 divisions generate neuronal-like cells.	Recessive, reduction of function

Table 3-3

Step in pathway of vulval development	Gene; canonical allele(s)	Hermaphrodite phenotype	Type of mutation
	<u>lin-34</u> <u>n1046</u>	Muv: P(5-7).p express normal fates; P(3,4,8).p can undergo either 1 ^o , 2 ^o or novel sublineages.	Partially dominant, unknown
	<u>lin-12(d)</u> <u>n137</u>	Muv: P(3-8).p express 2 ^o sublineages.	Partially dominant, increase in function or ectopic expression
	<u>lin-12(0)</u> <u>n137 n720</u>	st: P(3-8).p undergo non-2 ^o sublineages, <u>i.e.</u> 1 ^o , 3 ^o or novel	Recessive, null
Execution of vulval sublineages	<u>lin-11</u> <u>n389</u> <u>n382</u>	Vul: P5.p and P7.p undergo novel sublineages; planes of division of P6.p are altered.	Recessive, unknown
	<u>lin-17</u> <u>n671</u> <u>n677</u>	Muv: P7.p and sometimes P5.p undergo novel sublineages.	Recessive, unknown
	<u>lin-18</u> <u>e620</u> <u>n1051</u>	Muv: 25 ^o -- P5.p and P7.p express novel sublineages.	Recessive, null
Other	<u>lin-1</u> <u>e1777</u> <u>e1275</u>	Muv: P(3-8).p undergo non-3 ^o sublineages.	Recessive, null
	<u>lin-31</u> <u>n301</u>	Muv: P(3-8).p divide in L2, additional L3 divisions generate neuronal-like cells.	Recessive, null

Table 3-3

Step in pathway of vulval development	Gene; canonical allele(s)	Hermaphrodite phenotype	Type of mutation
	<u>lin-25</u> <u>e1446</u> <u>n545</u>	Vul: P(1-11).p can divide in the L1. P(5-7).p undergo abnormal divisions in L3.	Recessive, unknown

defects resulting from other alleles, or allelic states, of the gene. If multiple vulval cell lineage defects were observed in a strain carrying the canonical allele of a gene, the vulval cell lineages of hermaphrodites carrying a second allele of the same gene were also examined. The presence of similar defects in both strains strongly suggested that the multiple defects were a consequence of a single mutation. Additionally, if other alleles, or allelic states, of a gene resulted in a phenotype different from that caused by the canonical allele (e.g., if the penetrance and/or expressivity of the canonical allele was enhanced in trans to a deficiency of the locus), the vulval cell lineages of such mutant hermaphrodites were also examined.

In the majority of the mutant strains, the timing of the vulval cell divisions is similar to that observed in the wild type and the descendent cells have vulval-like morphologies. For these mutant strains, we detail the vulval cell lineages that we observed in individual mutant hermaphrodites using the nomenclature that is used to describe the vulval cell divisions in the wild type (see Figure 1-1 and below). However, in some mutant strains the vulval cell lineages differ from the wild type in either the timing of the vulval cell divisions or the morphologies of the descendent cells. For these strains, we present a general description of the mutant phenotype.

During vulval development in the wild type, each of the cells P(3-8).p expresses one of three fates (i.e., undergoes one of the three vulval sublineages -- 1⁰, 2⁰, or 3⁰). As described in Sternberg

and Horvitz (1985a) and summarized in Figure 1-1, each of the three vulval sublineages are distinguishable by the numbers and types of progeny produced. If, as described below, a P(3-8).p cell in a mutant underwent a lineage that had specific characteristics of one of the three vulval sublineages, we inferred that the P(3-8).p cell expressed that particular fate. In P(3-8).p cells that underwent lineages similar to the 3⁰ sublineage expressed by the cells P(3,4,8).p in the wild type, the P(3-8).p cell divided once and its progeny joined the hypodermal syncytium, represented by a lineage pattern of "[S S]." (P3.p, one of the cells that normally expresses a 3⁰ sublineage, can join the hypodermal syncytium without dividing, represented by a lineage pattern of "[S].")

In some mutants, some (or all) of the P(3-8).p cells underwent lineages similar to the 1⁰ or 2⁰ sublineages expressed by P(5-7).p in the wild type and generated vulval-like cells. The 1⁰ and 2⁰ sublineages can be distinguished from each other by differences in the individual P(3-8).pxx cells, specifically in their ability to divide, in the plane of cell division, and in the subsequent morphogenesis of the descendent cells. In P(3-8).p cells that underwent a lineage similar to 1⁰ sublineage expressed by P6.p in the wild type, the four P(3-8).pxx cells divided transversely, represented as "[TTTT]" (each of the four letters refers to the plane of division of one of the P(3-8).pxx cells). At the L3 molt the eight descendent cells moved dorsally and separated from the ventral cuticle. Thus, the main criteria for the expression of a 1⁰ sublineage were the transverse divisions of the P(3-8).pxx cells, and the lack of adhesion of the

eight descendent cells to the ventral cuticle.

P(3-8).px cells that underwent lineages similar to the 2⁰ sublineages expressed by P5.p and P7.p in the wild type displayed a polarity in both the lineage and morphogenesis of the descendent cells. Such cells underwent one of two lineage patterns -- "[LLTN]" or "[NTLL]." If a P(3-8).p cell underwent an [LLTN] lineage, the two P(3-8).pax cells divided longitudinally, represented by "[LL]", the P(3-8).ppa cell divided transversely, represented by "[T]", and the P(3-8).ppp cell did not divide, represented by "[N]." The four cells derived from the two longitudinal divisions adhered to the ventral cuticle at the L3 molt. Thus, the main criteria for the expression of a 2⁰ sublineage were the appearance of longitudinal planes of division in both daughters of one of the P(3-8).px cells and the subsequent adherence of the four cells derived from these divisions to the ventral cuticle.

There are three main classes of vulval cell lineage defects in these mutant strains. (1) In mutants defective in any of five genes, the lineages of P(3-8).p are altered prior to the time of normal vulval cell divisions in the early L3, suggesting that these genes may be necessary for the production of P(3-8).p cells competent to undergo vulval cell divisions. (2) In mutants defective in any of eleven genes, some of the cells P(3-8).p adopt fates normally expressed by other members of this set of cells, suggesting that these genes may be involved in either the production of the inductive signal by the gonadal anchor cell, or the response to the inductive signal by the

vulval precursor cells of the hypodermis. (3) In mutants defective in any of three genes, P5.p and/or P7.p undergo aberrant vulval cell lineages, suggesting that these three genes may be necessary for the proper execution of the vulval sublineages. As the genes defined by each group of mutants are likely to affect a similar step (or steps) in the pathway of vulval development, we describe the lineage defects of each set of mutants in a single section. Mutants defective in any of the remaining three genes share characteristics of two or more of the above classes of mutants and are described separately.

(1.a) Mutations that Result in Lineage Defects Prior to the Early L3

In wild-type hermaphrodites, the Pn cells divide during the mid-L1 to generate the Pn.p cells. The six potential vulval precursor cells, P(3-8).p, divide further during the early L3 and generate the cells of the vulva. In this section, we describe the lineage defects of five mutant strains -- lin-26, n300, lin-4, lin-24, and lin-33 -- in which the lineages and/or fates of P(3-8).p are altered prior to the early L3. Although mutants defective in any of three other genes -- lin-13, lin-25 and lin-31 -- may have vulval cell lineage defects prior to the early L3, we describe these mutants in other sections (see below).

lin-26(n156): The highly penetrant Vul phenotype of n156 hermaphrodites appears to result from the transformation of the Pn.p cells to neuronal cells or neuroblasts during the L1. In n156 hermaphrodites, the Pn.p cells either become neuronal-like or divide

shortly after their formation during the L1 to produce two or occasionally four neuronal-like progeny (H. Ellis, personal communication). We have observed a similar transformation of the fates of the Pn.p cells in n156 males.

n300: The completely penetrant Vul phenotype of n300 hermaphrodites results from the failure of P(3-8).p to divide. Rather, these cells appear to join the hypodermal syncytium as P(1,2,9,10,11).p do normally (Table 3-4a). However, in a small percentage of animals, one or two P(3-8).p cells do not join the syncytium; these cells divide ([LLLL] and [LLLT] lineages have been observed) to generate eight progeny that neither organize into a vulva nor connect with the anchor cell.

lin-4(e912): The highly penetrant Vul phenotype of e912 hermaphrodites appears to result from the inability of P(3-8).p to express L3-specific vulval cell lineages. The phenotype of lin-4 mutants has been described by Sulston and Horvitz (1981) and Chalfie et al. (1981). The cell lineages of P(3-8).p are complex; the number of descendants of each of the cells is variable, and the cells that are generated do not undergo vulval morphogenesis. On the basis of similarity in phenotype between lin-4 hermaphrodites and hermaphrodites carrying the dominant mutation lin-14(n536), Ambros and Horvitz (1984) have proposed that the lateral hypodermal cells of lin-4 hermaphrodites reiterate an early (L1-L2) larval program during the L3 stage. If such a defect is present in the ventral hypodermal cells of lin-4 hermaphrodites, the cells P(3-8).p may not be able to

execute the normal L3-specific vulval cell divisions.

lin-24(n432): The partially dominant Vul phenotype of n432 hermaphrodites appears to result from a combination of two defects: the deaths of some of the Pn.p cells during the L1, and the generation of abnormal cell lineages by the remaining vulval precursor cells during the L3. In lin-24 hermaphrodites, the Pn.p cells generally become refractile shortly after their formation in the mid-L1; between zero and six of the the Pn.p cells die during the late L1 or early L2. These deaths appear similar to, but not identical with, the programmed cell deaths that occur during the normal development of wild-type animals (Sulston and Horvitz, 1977). The mutation ced-3(n717) IV, which blocks the onset of the normal program of cell death and allows cells that normally would die to survive and differentiate (Horvitz et al., 1983; H. Ellis and R. Horvitz, manuscript in preparation), does not suppress the deaths of the Pn.p cells in a lin-24 ced-3 double mutant (Ferguson and Horvitz, 1985), suggesting that the deaths of the Pn.p cells in lin-24 animals do not result from the inappropriate activation of the normal program for cell death but rather result from the production of a cytotoxic lin-24 product. As not all of the Pn.p cells in n432 hermaphrodites die during the L1 and L2 stages, the Vul phenotype of these animals results, at least in part, from the fact that the surviving P(3-8).p cells often undergo abnormal cell lineages during the L3. Specifically, some of the remaining P(3-8).p cells move dorsally within the ventral cord and generate up to eight compact nuclei; these cells appear to be more neuronal-like than vulval- or hypodermal-like in character.

Table 3-4 Vulval cell lineages in wild-type and mutant hermaphrodites. Each letter, with the exception of "S", indicates the plane of division of one of the four granddaughters of a P(3-8).p cell. The plane of division of a P(3-8).pxx cell was an accurate indication of the morphologies of the descendent cells (see Results for details). "L", a P(3-8).pxx cell divided longitudinally; "T", a P(3-8).pxx cell divided transversely; "N", a P(3-8).pxx cell did not divide but moved dorsally and had a morphology characteristic of P5.ppp or P7.aaa; "U", undivided, a P(3-8).pxx cell did not divide, but lacked the characteristic morphology of an "N" nucleus; "O", a P(3-8).pxx cell divided with an oblique plane of division; "?", the pattern of division of a P(3-8).pxx cell was unclear; "D", a P(3-8).pxx cell divided, but the plane of division was not observed. The symbol "S" indicates that a P(3-8).p, P(3-8).px or P(3-8).pxx cell joined the hypodermal syncytium without dividing. All animals were grown at 20° unless otherwise indicated. The lineages of lin-18 hermaphrodites grown at 25° were determined by growing lin-18 hermaphrodites at 25° until the P(5-7).pxx cells had divided and then shifting these animals to 20° for observation. The cell lineages of some other mutant strains are reported in Sulston and Horvitz (1981), or in Sternberg and Horvitz (1985b). The vulval cell lineages of those mutants in which the P(3-8).p cells were affected prior to the early L3, or of those mutants in which either the timing of the vulval cell divisions in the L3 differs from that of the wild type or the descendent cells are not vulval-like in morphology, are not presented here but rather are summarized in Results. a, Vulval cell lineages of mutants in which P(3-8).p fail to form competent vulval precursor

cells. b, Vulval cell lineages of mutants in which some of the cells P(3-8).p adopt fates characteristic of other members of this set of cells. c, Vulval cell lineages of mutants in which the execution of the vulval sublineages are altered. d, Vulval cell lineages of lin-1 mutants.

Table 3-4

Vulval cell lineages of mutant hermaphrodites

	<u>#ac</u>	<u>P3.p</u>	<u>P4.p</u>	<u>P5.p</u>	<u>P6.p</u>	<u>P7.p</u>	<u>P8.p</u>	<u>No.</u>
wild type	1	S S	S S	<u>LLTN</u>	TTTT	<u>NTLL</u>	S S	
A)								
<u>n300</u>		S S	S S	S S	S LLLL	S S	S S	rare
B)								
<u>let-23(n1045)</u>	1	S S	S S	S S	S S	S S	S S	29
<u>15^o</u>		S						
	1	S S	S S	S DD	S S	S S	S S	1
	1	S	SSSS	S TN	S S	S S	S S	1
	1	S S	S S	S S	TT S	S S	S S	1
	1	S	S S	LLD?	S S	?DLL	S S	1
	1	S S	S S	LLTN	S S	NTLL	S S	1
	1	S	S S	SSTN	TT S	NTLL	S S	1
	1	S S	S S	LLTN	S S	S OO	S S	1
	1	S	DDDD	DDDD	S S	NTLL	S S	1
	1	S	S DD	LLOO	S S	OOLL	S S	1
	1	S S	S TT	S TT	TTTT	S S	S S	1
	1	S S	S S	LLTN	TTTT	NTLL	S S	2
<u>lin-8 (n111);</u>								
<u>lin-9 (n112)</u>	1	LLOO	TTTO	LLLN	TTTO	NOLL	OOTO	1
	1	LOLT	LTTO	LLTN	TTTT	NLLL	LOTO	1
	1	LLOO	OOLL	LLLN	TTTT	NLLL	LLTO	1
	1	TTOO	LLLN	TTTT	NLLL	LLOO	LLO?	1
<u>lin-13(n387)</u>								
<u>20^o</u>	1	LLOO	LLOO	LLON	T??T	NOLL	S S	1
	1	S S	S S	LLTN	TTTT	NLLL	S OO	1
	1	S	OODL	LLTN	O??T	NTLL	S S	1
	2	OLOT	S OO	LLTN	TTTT	NTLL	S S	1
	2	S S	S LL	LLON	TTTT	NTLL	TTLS	1
C)								
<u>lin-11(n382)</u>	1	S	S S	LLLL	TOOT	LLOO	S S	1
	1	?	S S	LLLO	TTOT	OLOL	S S	1
	1	?	S S	LLLL	OTTO	OOLL	S S	1
	1	S	S S	LLL?	TTTT	?LLO	S S	1

Table 3-4

Vulval cell lineages of mutant hermaphrodites

	#ac	P3.p	P4.p	P5.p	P6.p	P7.p	P8.p	No.
<u>lin-11(n389)</u>	1	S	S S	LLLO	TTOT	OOLL	S S	1
	1	S	S S	LOLO	TTOT	OLLL	S S	1
	1	S	S S	OLLO	TTTT	LLOL	S S	1
<u>lin-17(n671)</u>	1	S S	S S	LLTN	TTTT	LLTN	S S	1
	1	S S	S S	LLTN	TTTT	LLLL	S S	4
	1	S S	SSSS	LLTT	T?TT	LLLL	S S	1
	1	S	S S	LLLT	TTTT	LLTT	S S	1
	1	?	S S	LLTN	TTTT	TTLL	S S	1
	1	?	S S	LLTN	TTTT	LLOO	S S	1
<u>lin-17(n677)</u>	1	?	S S	LLTT	TTTT	LLLL	S S	1
	1	S	S S	LLTT	TTTT	LLLO	S S	1
	1	S S	S S	LLTT	TTTT	TOLL	S S	1
<u>lin-18(e620)</u> 20	1	S S	S S	LLTN	TTTT	NTLL	S S	3
	1	S S	S S	LLTN	TTTT	TTLL	S S	1
	1	S	S S	LLTN	TTTT	LLTN	S S	1
	1	S S	S S	LLTN	TTTT	LLNT	S S	2
	1	S S	S S	LLTN	TTTT	LLNO	S S	1
<u>lin-18(e620)</u> 25	1	?	S S	LLTN	TTTT	LLLL	S S	1
	1	S	S S	LLTN	TTTT	TOLL	S S	1
	1	S S	S S	LLLL	TDTT	LLLL	S S	1
	1	?	S S	LLTO	TTTT	LLLL	S S	1
	1	?	S S	LLTT	TTTT	NTLL	S S	1
D)								
<u>lin-1(e1777)</u>	1	LOTT	LOTT	TLTN	TTTT	NTLL	OOLL	1
	1	S	LLTT	OOTT	NTTO	OTOL	LLTT	1
	1	S TT	OLOT	LLTT	OTTT	TTLL	TO S	1
	1	S TT	LLON	OTTT	LLTN	TTTT	TTTL	1
	1	S S	LLOO	OOTT	TTTT	NTLL	LL S	1
	1	L000	LLOO	LDDT	LOTT	NTTL	OO S	1

A second putative lin-24 allele, n1057, (Ferguson and Horvitz, 1985) results in a wild-type phenotype when homozygous but results in a Vulvaless phenotype when heterozygous. Hermaphrodites of genotype of lin-24(n1057)/+ also have Pn.p cell deaths in the L1 and have abnormal vulval cell lineages in the L3, suggesting that both classes of lineage defects observed in n432 hermaphrodites result from a single mutation.

lin-33(n1043): Hermaphrodites carrying the partially dominant Vul mutation n1043 display the two classes of lineage defects observed in lin-24 hermaphrodites; Pn.p cell death in the L1 or early L2, and abnormal vulval cell lineages during the L3.

(1.b) Mutations that Alter the Fates of P(3-8).p

The fates of the equipotential cells P(3-8).p appear to be determined in the early L3 by a signal from the gonadal anchor cell. As a result of these interactions, P6.p, the cell closest to the anchor cell, is determined to express a 1^o fate; P5.p and P7.p, the cells adjacent to P6.p, are determined to express 2^o fates, and P(3,4,8).p are determined to express 3^o fates. Mutations in eleven genes alter the determination of the fates of P(3-8).p so that one or more of these cells adopt a fate characteristic of another member or members of this set of cells. Based on the phenotypes that result from these mutations, we have divided these mutants into three groups: Vulvaless mutants, Multivulva mutants, and lin-12 mutants.

(1.b1) Vulvaless mutations

Vul mutations in five genes cause P(3-8).p to adopt 3⁰ fates. The fact that P(3-8).p also adopt 3⁰ fates in wild-type hermaphrodites in which the inductive signal has been eliminated by ablation of the anchor cell suggests that these mutations might reduce or eliminate the activity of genes involved in the inductive process, either in the production of the inductive signal by the anchor cell or in the response to the signal by P(3-8).p.

Mutations in lin-2, lin-7 and lin-10 result in similar incompletely penetrant Vul phenotypes. Because some mutant alleles of each of these genes probably eliminate gene activity (Ferguson and Horvitz, 1985), it is likely that their wild-type functions are not absolutely required for vulval induction.

lin-2(e1309,e1453,n768): The penetrance of the Vul defect in hermaphrodites carrying any of the thirteen alleles of lin-2 ranges from a low of 27% (n768) to a high of 97% (e1309) (Ferguson and Horvitz, 1985). The vulval cell lineage defects resulting from two lin-2 alleles, e1453 and e1309, have been reported by Sulston and Horvitz (1981) and Sternberg and Horvitz (1985b). In three of the ten e1309 animals that were examined, P(5-7).p expressed 3⁰ fates, in one animal P(5-7).p adopted wild-type fates, and in the remaining six animals from one to three of the P(5-7).px cells divided.

lin-7(e1413,n106): All of the thirteen lin-7 alleles, except lin-7(n106) (see below), result in a Vul phenotype of 95% penetrance

(Ferguson and Horvitz, 1985). The vulval cell lineages of e1413 hermaphrodites are reported in Sulston and Horvitz (1981) and Sternberg and Horvitz (1985b). In two of the 14 e1413 animals that were examined, P(5-7).p expressed 3⁰ fates, while in the remaining 12 animals from one to six of the P(5-7).px cells divided.

lin-10(e1438,e1439,n299): The three alleles of lin-10 result in a Vul phenotype of approximately 95% penetrance (Ferguson and Horvitz, 1985). The vulval cell lineages of e1439 hermaphrodites are reported in Sternberg and Horvitz (1985b). In eight of the 15 e1439 hermaphrodites examined, P(5-7).p expressed 3⁰ fates; in the remaining seven animals, one or more of the six P(5-7).px cells divided and underwent its normal lineage.

As viewed with a dissecting microscope, some egg-laying competent hermaphrodites carrying non-null alleles of lin-2 or lin-7 are Muv having one, or sometimes two supernumerary pseudovulvae. We examined the vulval cell lineages of hermaphrodites of genotypes lin-2(n768) and lin-7(n106); in some animals, more than three P(3-8).p cells express 1⁰ or 2⁰ fates (Table 3-6). This weak Muv phenotype is discussed in a separate section (see below).

Mutations in two other genes -- lin-3 and let-23 -- also result in an incompletely penetrant Vul phenotype. However, the Vul mutations in each of these genes reduce but do not eliminate gene activity (Ferguson and Horvitz, 1985), and, as demonstrated below, the activity of each of these genes is absolutely required for vulval induction.

lin-3(e1417,n378,n1059): Ninety percent of e1417 hermaphrodites are Vul, while 97% of n378 hermaphrodites are Vul (Ferguson and Horvitz, 1985). The vulval cell lineages of e1417 hermaphrodites have been described by Sulston and Horvitz (1981) and Sternberg and Horvitz (1985b). In 9 of the 22 e1417 hermaphrodites examined, P(5-7).p adopted 3° fates, in another 11 animals only P6.p adopted a non- 3° fate, and in the remaining two animals P6.p and either P5.p or P7.p adopted non- 3° fates.

lin-3(e1417) does not result in complete loss of lin-3 gene function, since lin-3(n1059), which results in larval lethality, increases the penetrance of the Vul phenotype in trans to lin-3(e1417) (Ferguson and Horvitz, 1985). In 31 of 39 hermaphrodites of genotype lin-3(e1417) dpy-20/lin-3(n1059) +, P(3-8).p expressed 3° fates, in seven other hermaphrodites P5.p and P7.p, but not P6.p, expressed 3° fates, and in one hermaphrodite P(5-7).p expressed wild-type fates. No egg-laying competent hermaphrodites were observed among 665 hermaphrodites of genotype lin-3(n378)/lin-3(n1059) (Ferguson and Horvitz, 1985), so it is likely that lin-3 activity is absolutely required for vulval induction.

let-23(mn23,mn216,mn224,n1045): n1045 results in a cold-sensitive Vul phenotype with an associated larval lethality (Ferguson and Horvitz, 1985). At 15° , in 29 of the 50 n1045 hermaphrodites examined, P(5-7).p adopted 3° fates, in two hermaphrodites P(5-7).p adopted wild-type fates, and in the remaining 19 hermaphrodites from one to six P(5-7).px cells divided. (The vulval cell lineages of 42 of these

animals are described in Table 3-4a.) In seven of the 19 n1045 animals in which some P(5-7).px cells divided, P5.p or P7.p expressed a 2⁰ sublineage while P6.p expressed a 3⁰ sublineage. Thus, in let-23(n1045) hermaphrodites P5.p and P7.p preferentially express their normal lineages, while in lin-3(e1417) hermaphrodites, P6.p preferentially expresses its normal lineage.

At 25⁰, n1045 hermaphrodites are not Vul. Viewed with a dissecting microscope, most n1045 hermaphrodites appear phenotypically wild-type; however, some egg-laying competent n1045 hermaphrodites are Muv, having one or sometimes two supernumerary pseudovulvae. We have examined the vulval cell lineages of n1045 hermaphrodites grown at 25⁰; in many of these hermaphrodites, more than three of the P(3-8).p cells expressed 1⁰ or 2⁰ fates (Table 3-6). This weak Muv phenotype is discussed in a separate section (see below).

let-23(n1045) does not result in complete loss of let-23 gene activity: the penetrance of the Vul defect of n1045 is enhanced in trans to either a deficiency of the locus, or to other let-23 alleles that result in larval lethality (Table 3-5). Since all the P(3-8).p cells in hermaphrodites of genotype let-23(n1045)/mnDf67 that are grown at 15⁰ adopt 3⁰ fates (Table L23), it is likely that let-23 activity (like lin-3 activity) is absolutely required for vulval induction.

Two of the three lethal alleles of let-23, mn23 and mn216, result in an enhancement of the Vul phenotype in trans to let-23(n1045), as

Table 3-5 Interaction among let-23 alleles. The number of P(3-8).px cells that underwent vulval cell lineages in hermaphrodites of different let-23 genotypes is expressed as a percentage of the number (6) of P(3-8).px cells that divide in the wild type. Animals of each genotype were raised at the indicated temperatures until the P(3-8).px cells had divided; these animals were subsequently shifted to 20° for observation. Animals heteroallelic for different let-23 mutations were obtained in the following ways. n1045/+: non-Dpy progeny from the mating of wild-type males with dpy-10 let-23(n1045) hermaphrodites. Hermaphrodites carrying n1045 in trans to any of the three lethal alleles of let-23 -- mn23, mn216, mn224 -- were obtained as the non-Dpy progeny from the mating of males of genotype let-23 unc-4/C1 dpy-10 unc-52 with hermaphrodites of genotype dpy-10 let-23(n1045). Hermaphrodites carrying n1045 in trans to any of the three deficiencies of the let-23 locus -- mnDf61, mnDf67, mnDf68 -- were obtained as the non-Dpy progeny from the mating of males of genotype Df unc-4/C1 dpy-10 unc-52 with hermaphrodites of genotype dpy-10 let-23(n1045).

Table 3-5

let-23 heteroallelic interactions

Hermaphrodites carrying let-23(n1045) in trans
to other alleles of let-23

% division of P(3-8).px cells

Temper- ature	+	<u>n1045</u>	<u>mn224</u>	<u>mn216</u>	<u>mn23</u>	<u>mnDf68</u>	<u>mnDf67</u>	<u>mnDf61</u>
25°	101 n=138	134	109 n=21	50 n=4	26 n=12	5 n=10	25 n=10	65 n=13
22.5°	ND	>100	ND	25 n=6	ND	ND	ND	ND
20°	ND	>100	ND	12 n=4	0 n=2	ND	0 n=2	0 n=5
15°	100 n=51	25 n=60	92 n=13	2 n=18	0 n=14	ND	0 n=11	0 n=1

does a deficiency of the let-23 locus, suggesting that these two alleles may substantially reduce let-23 gene activity (Table 3-5); however, the third lethal allele of let-23, let-23(mn224), does not enhance the Vul phenotype of let-23(n1045) in trans (Table 3-5). These interactions are similar to the pattern of interactions among different lin-3 alleles (Ferguson and Horvitz, 1985) and are consistent with either of two interpretations of let-23 activity. let-23 could have a single activity and n1045 and mn224 display partial intragenic complementation. Alternatively, let-23 could have two activities, an early function essential during larval development and a later function needed during vulval development.

(1.b2) An analysis of the weak Muv phenotype that results from non-null alleles of let-23, lin-2 and lin-7

Most hermaphrodites carrying non-null alleles of either let-23, lin-2, or lin-7 are not Vul; instead some of the egg-laying competent hermaphrodites of each of these genotypes display a weak Muv phenotype, with one or rarely two small protrusions anterior or posterior to a functional vulva. We have examined hermaphrodites of three genotypes -- let-23(n1045) grown at 25^o, lin-2(n768) and lin-7(n106) -- and have observed two similarities in the vulval cell lineage defects in these mutant strains (Table 3-6): (1) in a single animal, more than three P(3-8).p cells can express 1^o and/or 2^o fates; and (2) a normal vulva can be formed from two alternative sets of Pn.p cells -- P(4-6).p or P(6-8).p -- with the anchor cell centered over the cell that undergoes the 1^o sublineage.

Table 3-6 Vulval cell lineages of hermaphrodites of genotypes lin-2(n768), lin-7(n106), or let-23(n1045) grown at 25°. In hermaphrodites of all three genotypes, more than the wild-type number of P(3-8).px cells divide, resulting in a weak Muv phenotype. In a majority of animals, only the fact that a P(3-8).px cell had divided was ascertained; the lineages of the descendent cells were not followed.

Table 3-6

Vulval cell divisions in hermaphrodites carrying non-null alleles of lin-2, lin-7, or let-23

<u>Geno- type</u>	<u>Temper- ature</u>	<u>P3.p</u>	<u>P4.p</u>	<u>P5.p</u>	<u>P6.p</u>	<u>P7.p</u>	<u>P8.p</u>	<u>No.</u>
<u>let-23</u> <u>n1045</u>	25°	S	LLON	OTTT	TTTT	NTLL	S S	1
		S	S00	LLTN	TTTT	NTLL	S S	1
		S	LLON	OOTT	TTTT	NTOL	NOLL	1
		3°	2°	1°	2°	3°	3°	2
		3°	2°	2°	1°	2°	3°	7
		3°	3°	2°	1°	2°	2°	2
		3°	2°	1°	1°	2°	3°	7
		3°	2°	1°	1°	1°	2°	1
		3°	3°	2°	1°	2°	3°	6
<u>lin-2</u> <u>n768</u>	20°	S	S --	----	S --	S S	S S	1
		S	S --	----	----	S --	S S	1
		S S	S S	S S	S S	TTLL	-- S	1
		S S	S S	S TT	TT S	S S	S S	1
		S	----	----	----	S S	S S	4
		S	LLTT	TTTT	NTLL	S S	S S	1
		S S	----	----	----	----	S S	1
		S S	S TT	LLTT	S S	S S	S S	1
		S S	S TT	----	----	----	S S	1
		S S	S TN	TTTT	----	----	S S	1
		S S	S S	LLTT	TTTT	TTLL	S S	1
		S S	S S	LLTN	TTTT	NTLL	S S	1
		S	? --	----	----	S S	S S	1
<u>lin-7</u> <u>n106</u>	20°	S	S TT	LLTN	TTTT	NT S	S S	1
		S S	S --	----	----	----	S S	1

Because lin-2, lin-7 and let-23 act in the hypodermis (see below), and because the Muv phenotype of at least let-23(n1045) hermaphrodites depends on the presence of the inductive signal of the anchor cell (see below), the Muv phenotypes observed in these mutants appear to result from an increased sensitivity in the hypodermis to the inductive signal of the gonad. As the loss of activity of any of these three genes causes P(3-8).p to adopt 3⁰ fates and results in a Vul phenotype, the Muv phenotypes of these strains could result from: (1) the acquisition of a novel function by these mutant gene products, or (2) the response of P(3-8).p to a reduction in, but not elimination of, normal gene activity. Three observations favor the latter hypothesis. (1) n1045 hermaphrodites can undergo a transition from Muv to Vul by decreasing the dose of the n1045 gene product. Specifically, at 25⁰, 130% of the wild-type number of P(3-8).px cells divide in let-23(n1045) hermaphrodites, while at this same temperature only 25% of the wild-type number of P(3-8).px cells divide in hermaphrodites of genotype let-23(n1045)/mnDf67. Because a similar reduction in the number of P(3-8).px cells that divide is observed in let-23(n1045) hermaphrodites grown at successively lower temperatures (P. Sternberg, unpublished observations), is it likely that the cold-sensitive phenotype of let-23(n1045) results from a reduction in the activity of the let-23(n1045) gene product. (2) A similar phenotypic transition from wild-type to Muv to Vul is observed when the dose of an essentially wild-type lin-2 or lin-7 gene product is reduced. Specifically, both of the genes lin-2 and lin-7 have amber alleles, which are suppressible by either of the amber suppressors sup-5 or sup-7, as well as non-amber alleles that probably eliminate

gene activity. Hermaphrodites of genotype lin(amber)/lin(amber); sup/+ have a wild-type phenotype; hermaphrodites of genotype lin(amber)/lin(null, non-amber); sup/+, which have one-half of the dose of the suppressed gene product, can have a Muv phenotype; and hermaphrodites of genotype lin(amber)/lin(amber) are Vul. If the suppressed lin-2 and lin-7 gene products, which presumably differ from the wild-type gene products by a single amino acid substitution, have wild-type activities, it is likely that the Muv phenotypes of hermaphrodites of genotypes lin(amber)/lin(nonamber); sup/+, and by analogy the Muv phenotypes of hermaphrodites of genotypes lin-2(n768) and lin-7(n106), result from a response by P(3-8).p to a decreased level of lin-2 or lin-7 gene activity. (3) Although at 25° both let-23(n1045) and lin-2(n768) result in Muv phenotypes, a double mutant strain of genotype let-23(n1045); lin-2(n768) has a Vul phenotype. If these two genes affect independent steps in a pathway, the penetrance of the phenotype of a double mutant carrying both of these mutations should be enhanced. Thus, it is likely that the Vul phenotype of the double mutant is indicative of the phenotype that results from a greater reduction in the activity of either of the mutant gene products.

Thus, the Muv phenotypes of these strains appears to result from a response in the hypodermal cells to a reduction in, but not elimination of, gene activity. One possible explanation of this phenomenon consistent with a model involving the reception of a graded inductive signal by the vulval precursor cells (Sternberg and Horvitz, 1985a) is that the gene products that are involved in the reception of

a diffusible signal also act as a barrier to its further diffusion, perhaps by internalization or modification of the signal. A specific reduction in the activity of any one of such genes might allow the reception of enough signal to permit induction of the cells P(5-7).p but result in the inability to prevent the diffusion of the signal to other P(3-8).p cells. A further reduction in activity of such a gene product would then lead to the failure of the reception of the anchor cell signal and the production of a Vul phenotype.

(1.b3) Multivulva mutations

In Muv mutants defective in any of five genes, P(5-7).p usually express their normal fates and form a functional vulva, while P(3,4,8).p adopt 1⁰ and 2⁰ fates and form one or more pseudovulvae.

CB1322, lin-8(n111); lin-9(n112): The Muv phenotype of CB1322 results from the interaction of lin-8(n111) and lin-9(n112) (Horvitz and Sulston, 1980). As described below, each of these two mutations results in a wild-type phenotype. Many CB1322 hermaphrodites have two ectopic pseudovulvae, one anterior and one posterior to a normal vulva. In the CB1322 animals that were examined, P(5-7).p usually underwent normal lineages to form a functional vulva; however, P(3,4,8).p usually expressed 1⁰ or 2⁰ fates or underwent novel lineages (Table 3-4b).

lin-8(n111): We examined 22 n111 hermaphrodites in different stages of vulval development using Nomarski optics. All were normal.

lin-9(n112): We examined 19 n112 hermaphrodites in different stages of vulval development using Nomarski optics. All were normal.

lin-15(e1763,n309,n377,n765): The Muv phenotype of n309 hermaphrodites is similar to, but more severe than, the Muv phenotype of CB1322 hermaphrodites. n309 hermaphrodites can have between two and six ventral protrusions; some n309 hermaphrodites lack a functional vulva. In the n309 hermaphrodites that were examined (Sternberg and Horvitz, 1985b), P(5-7).p underwent their normal lineages [2° , 1° , 2°] and formed a vulva, which was sometimes abnormal; however, P(3,4,8).p also expressed 1° or 2° fates or underwent novel lineages.

lin-13(n387): lin-13(n387) displays a maternal effect and results in a heat-sensitive sterile Muv phenotype: at 25° , n387 hermaphrodites that are the progeny of heterozygous parents are both sterile and Muv; at 15° , n387 hermaphrodites that are the progeny of heterozygous parents have a wild-type phenotype but generate all sterile progeny. The vulval cell lineage defects in the sterile lin-13 progeny were dependent on the temperature at which these animals were raised. If such lin-13 hermaphrodites were grown at 20° , P(5-7).p usually underwent their normal lineages [2° , 1° , 2°], and P(3,4,8).p underwent non- 3° lineages about 50% of the time (Table 3-4b). However, if such lin-13 animals were grown at 25° , some of the cells P(3-8).p divided once or twice during the late L2; subsequently during the early L3, many of the vulval precursor cells (i.e., P(3-8).p, or their descendants, P(3-8).px or P(3-8).pxx) divided to generate eight

hypodermal-like progeny, some generated two syncytial cells, and others generated several small nuclei, which were neuron-like in morphology.

lin-34(n1046): The partially dominant Muv phenotype of n1046 hermaphrodites is similar to the Muv phenotype of CB1322 hermaphrodites; many lin-34 animals have two ectopic pseudovulvae, one anterior and one posterior to a normal vulva. In most n1046 hermaphrodites that were examined, P(5-7).p underwent normal lineages [2^0 , 1^0 , 2^0] and formed a normal vulva; however, P(3,4,8).p usually underwent non- 3^0 lineages. Specifically, when we examined the vulval anatomy of 12 lin-34 animals, we found that P3.p expressed non- 3^0 fates in nine animals, P4.p expressed non- 3^0 fates in ten animals, and P8.p expressed non- 3^0 fates in seven animals.

(1.b4) lin-12 mutations

The phenotypes that result from mutations in lin-12 have been described in detail by Greenwald et al. (1983) and Sternberg and Horvitz (1985b); we summarize their observations here. There are two major classes of alleles of lin-12: dominant alleles ("lin-12(d)"), exemplified by lin-12(n137), that result in an increased level of lin-12 activity, and null alleles ("lin-12(0)"), exemplified by lin-12(n137 n720), that eliminate lin-12 activity. These two classes of alleles cause discrete and reciprocal transformations in the fates of several sets of cells, among them the two cells of the gonadal equivalence group, Z1.ppp and Z4.aaa, which generate the anchor cell and a ventral uterine precursor cell, and the six potential vulval

precursor cells, P(3-8).p. Thus, lin-12 mutations affect both tissues involved in vulva development, the gonad and the ventral hypodermis.

lin-12(n137) hermaphrodites are Muv and lack an anchor cell; in these hermaphrodites P(3-8).p all undergo 2⁰ sublineages (Sternberg and Horvitz, 1985b). Each of the four cells P(3,4,7,8).p generally generates one pseudovulva. In hermaphrodites in which the sublineages of P5.p and P6.p are of opposite polarity (i.e., P5.p undergoes an [LLTN] lineage and P6.p undergoes a [NTLL] lineage), the descendants of these two cells organize into one larger pseudovulva. In n137 hermaphrodites in which P5.p and P6.p have the same polarity, the descendants of these two cells organize separately to generate two pseudovulvae. In the absence of the anchor cell, the vulval cells do not connect to the uterus.

A second class of partially dominant alleles of lin-12, represented by n302, result in a less extreme elevation of the lin-12 activity. n302 hermaphrodites lack an anchor cell and consequently do not form a functional vulva. However, in n302 hermaphrodites, P(3-8).p usually adopt 3⁰ fates as a result of the absence of an anchor cell. Consequently, n302 hermaphrodites are Vul.

Hermaphrodites of genotype lin-12(n137 n720) lack lin-12 activity; these hermaphrodites are sterile, have two or more anchor cells, and have a single large protrusion at the site of the vulva. In these hermaphrodites P(3-8).p undergo novel lineages. In general, there are 1⁰ lineages, 3⁰ lineages, or lineages with both 1⁰ characteristics and

3⁰ characteristics [S TT], but no 2⁰ lineages (Sternberg and Horvitz, 1985b).

(1.c) Mutations that Affect the Execution of the Vulval Sublineages

During the L3, each of the cells P(3-8).p undergo one of the three vulval sublineages -- 1⁰, 2⁰, or 3⁰. In mutants defective in any of three genes, P5.p and P7.p undergo novel vulval-like divisions.

lin-11(n382,n389): The completely penetrant Vul phenotype of lin-11(n382) or lin-11(n389) hermaphrodites results from alterations in the lineages of P(5-7).p. In the lin-11 hermaphrodites that were examined, the [LL] portion of the 2⁰ sublineage appeared to be duplicated, i.e., many P5.p and P7.p cells underwent [LLLL] lineages instead of the normal 2⁰ lineages of [LLTN] or [NTLL] (Table 3-4c). The 24 progeny generated by P(5-7).p failed to form a functional vulva but rather formed a non-functional vulva-like protrusion at the site of the vulva. As the divisions of the P6.px daughter cells were usually transverse, mutations in lin-11 may not affect the execution of 1⁰ sublineages.

lin-17(n671,n677): The single ventral protrusion posterior to the vulva that is characteristic of lin-17 hermaphrodites results from a reversal in the polarity of the P7.p lineage, i.e., in many lin-17 hermaphrodites, P7.pa instead of (or in addition to) P7.pp underwent the [LL] portion of the 2⁰ sublineage (Table 3-4c). In such animals, the descendants of P7.p organized independently of the descendants of

P5.p and P6.p and formed a pseudovulva posterior to a vulva. The lineages of P5.p were also affected in about one-half of the lin-17 animals examined, while the lineages of P6.p were usually normal, suggesting that lin-17 may specifically affect the execution of the 2⁰ sublineages.

lin-18(e620,n1051): The single ventral protrusion posterior to the vulva that is characteristic of lin-18 hermaphrodites results from a reversal in the polarity of the P7.p lineage, i.e. in some lin-18 hermaphrodites P7.pa instead of (or in addition to) P7.pp underwent the [LL] portion of the 2⁰ sublineage (Table 3-4c). In such animals, the descendants of P7.p organized independently of the descendants of P5.p and P6.p and formed a pseudovulva posterior to the vulva.

lin-18(e620) is slightly heat-sensitive. At 20⁰, the vulval cell lineages were normal in three of seven e620 hermaphrodites examined, and in the other four, only P7.p appeared to be affected. Of five e620 hermaphrodites that were grown at 25⁰ until completion of the second round of P(5-7).p divisions, three had alterations in both the P5.p and the P7.p lineages; the other two had alterations in only the P7.p lineage. In all lin-18 animals, the lineages of P6.p were usually normal, suggesting that lin-18 may specifically affect the execution of the 2⁰ sublineages.

(1.d) Other Mutations

In this section, we summarize the vulval cell lineage defects resulting from mutations in the remaining three genes -- lin-1, lin-25

and lin-31.

lin-1(e1275,e1777): The Muv phenotype of lin-1 hermaphrodites ranges from four large evenly-spaced protrusions to a single vast ventral protrusion at the vulva. The lineage defects of hermaphrodites carrying lin-1(e1777), an amber allele (Ferguson and Horvitz, 1985), are similar to the lineage defects of lin-1(e1026) and lin-1(e1275) described by Sulston and Horvitz (1981) (Table 3-4d). Generally, in lin-1 hermaphrodites P(3-8).p undergo non-3⁰ lineages; some P(3-8).p cells undergo 1⁰-like lineages and other P(3-8).p cells undergo 2⁰-like lineages.

lin-25(e1446,n545): lin-25 hermaphrodites have a highly penetrant Vul phenotype. Two classes of vulval cell lineage defects were observed in lin-25(e1446) hermaphrodites. In the L1, one or two of the Pn.p cells divided and generated two hypodermal-like daughters, in essence producing an extra "Pn.p-like" cell. These ectopic divisions were not restricted to the cells P(3-8).p; e.g. we observed P(2,5,9).p divide in one animal and inferred (from anatomy) that P(3,4,6,10).p had divided during the L1 in a second animal. In the early L3 many of the vulval cells (P(3-8).p and the P(3-8).px cells generated during the L1) divided once, and their progeny joined the hypodermal syncytium. Occasionally one or two of the vulval cells underwent a non-3⁰ lineage, usually [TTTT] or [LOTN], and formed one small ventral protrusion. At 25⁰, lin-25(n545), a heat-sensitive allele of lin-25, also results in ectopic Pn.p divisions during the L1, suggesting that both classes of lineage defects observed in lin-25 hermaphrodites are

caused by a single mutation.

lin-31(n301,n376,n762): The Muv phenotype of lin-31 hermaphrodites is variable; individual hermaphrodites have from zero to four small ventral pseudovulvae. In the n301 hermaphrodites that were examined, some P(3-8).p cells divided precociously during the late L2 producing two to four descendants. During the L3, some of the vulval precursor cells (P(3-8).p and their L2-derived descendants, P(3-8).px and P(3-8).pxx) underwent three additional rounds of division, generating either vulval-like cells or compact neuron-like cells; other vulval precursor cells did not divide and became compact. Precocious divisions have also been observed in n376 and n762 hermaphrodites, suggesting that both classes of lineage defects observed in lin-31 hermaphrodites result from a single mutation.

(2) Site of action

Since the vulva is formed as a result of an interaction between the anchor cell of the gonad and the cells P(3-8).p of the ventral hypodermis, the lesions of the Muv and Vul mutants could be in either (or both) of these tissues. In this section we demonstrate that all 22 genes have a site of action in the hypodermis, and that one gene, lin-12, acts in both tissues.

In the majority of Muv mutants -- lin-1, lin-8; lin-9, lin-12(n137), lin-13, lin-15, lin-31, lin-34, let-23(n1045) at 25° -- P(3,4,8).p, as well as P(5-7).p, express non-3° fates. The ectopic

cell divisions of P(3,4,8).p could be caused by: (1) an abnormality in the gonad resulting in an increase in the strength of the gonadal inductive signal; (2) an abnormality in the hypodermis resulting in an increased response to the gonadal signal; or (3) an abnormality in the hypodermis resulting in an inductive response independent of the gonadal signal. For each of the Muv strains, we can distinguish between these alternatives by using a laser microbeam to ablate the gonads of L1 larvae carrying the Muv mutation (Sulston and White 1980, Sternberg and Horvitz, 1981). If such adult hermaphrodites, which lack a gonad, are still Muv, then the Muv phenotype is independent of the gonadal signal and the site of action of the Muv mutation is localized to the hypodermis. However, if the ablation of the gonad in hermaphrodites carrying a Muv mutation causes P(3-8).p to express 3⁰ fates, then the expression of the Muv phenotype is dependent upon the gonadal signal, and the site of action of the Muv mutation could be in either the gonad or the hypodermis.

For each of the Muv strains lin-1(e1275), lin-8(n111); lin-9(n112), lin-12(n137), lin-13(n387), lin-15(n309), lin-31(n301), and let-23(n1045), we ablated the gonads of five hermaphrodites during the L1. Upon reaching adulthood, all of the irradiated hermaphrodites, except the let-23(n1045) hermaphrodites, became Muv, indicating that the Muv mutations in the strains lin-1(e1275), lin-8(n111); lin-9(n112), lin-12(n137), lin-13(n387), lin-15(n309), and lin-31(n301) cause an inductive response in the hypodermal cells which is independent of the gonad. In all five let-23(n1045) hermaphrodites in which the gonad was ablated during the L1, the six

cells P(3-8).p adopted 3^o fates. Thus, the weak Muv phenotype of let-23(n1045) hermaphrodites grown at 25^o could result from either an abnormal gonadal signal, or an abnormal hypodermal response to the gonadal signal. Since let-23(n1045) acts in the hypodermis (see below), it is likely that the weak Muv phenotype observed in let-23(n1045) hermaphrodites grown at 25^o results from an abnormal hypodermal response to the gonadal signal. [The gonads of hermaphrodites carrying the Muv mutation lin-34(n1046) were not ablated. However, in lin-34(n1046) males, P(3-6).p occasionally divide (P. Sternberg and E. Ferguson, unpublished observations). The occurrence of divisions of P(3-6).p in lin-34 males, which lack the inductive signal of the hermaphrodite gonad, suggests that the divisions of P(3-8).p in lin-34(n1046) hermaphrodites are also probably independent of the gonad.]

Conversely, the lack of vulval cell divisions by P(5-7).p in a Vul strain could result from: (1) a reduction or elimination of the action of the inducing signal of the gonad, either by a reduction of the amount of the signal itself or by an alteration of some property of the signal such as its timing; or (2) a reduction in the ability of the vulval precursor cells of the hypodermis to respond to the inducing signal. For each of the Vul mutations, we can distinguish between these alternatives by examining the phenotypes of a double mutant carrying the Vul mutation and a known hypodermal Muv mutation. If a Vul mutation is expressed in a double mutant with a hypodermal Muv, the Vul mutation must have a site of action in the hypodermis, as the elimination of the gonadal signal does not change the phenotype

resulting from a hypodermal Muv mutation. However, if all hypodermal Muv mutations are epistatic to a given Vul mutation, the Vul mutation could act either in the gonad or in an early step in the pathway of hypodermal cell response.

We constructed double mutants among Vul alleles of the genes lin-2, lin-3, lin-4, lin-7, lin-10, lin-24, lin-25, lin-26 and n300 and different hypodermal Muv mutations (see Materials and Methods for the protocols used and Tables 3-1 and 3-2 for a list of the strains constructed). All of the Vul mutations, with the exception of lin-3, were epistatic to, or coexpressed with, at least one hypodermal Muv mutation (Table 3-7), indicating that the genes lin-2, lin-4, lin-7, lin-10, lin-24, lin-25, lin-26 and n300 have a site of action in the hypodermis. [Although no double mutants were constructed between the Vul mutation in lin-33 and any of the hypodermal Muv mutations, lin-33, like the hypodermal Vul mutation lin-24, causes some of the Pn.p cells to die in the L1.]

The Vul phenotype of lin-3 was not expressed in any of the double mutants containing a hypodermal Muv mutation, but lin-3(e1417) results in only a partial loss of gene function (see above). To determine if a greater reduction in lin-3 activity could allow the Vul phenotype of lin-3 to be expressed in a multiply mutant strain containing a hypodermal Muv mutation, we constructed a strain of genotype lin-3(n378) + unc-22/lin-3(n1059) dpy-20 +; him-5; lin-15(n765). [lin-3(n1059), which results in larval lethality, increases the penetrance of the Vul phenotype in trans to lin-3(e1417), suggesting

Table 3-7 The epistatic relationship between a Muv and a Vul mutation was determined by examining the vulval anatomy of a double mutant hermaphrodite with a dissecting microscope. See Results for details of the phenotype of the lin-12(d); lin-25 double mutant. Muv/Vul, both mutations were expressed in the double mutant strain (see Results for details); ND, not determined. a -- The double mutant between lin-1 and n300 could not be constructed.

Table 3-7

Muv-Vul double mutants

V u l	M u v					
	<u>lin-12</u> <u>n137</u>	<u>lin-1</u>	<u>lin-15</u>	<u>lin-31</u>	<u>lin-8;</u> <u>lin-9</u>	<u>lin-13</u>
<u>lin-26</u> <u>n300</u> <u>lin-4</u> <u>lin-24</u>	Vul	Vul ^a	Vul	Vul	Vul	Vul
<u>lin-25</u>	Muv	Vul	Vul	Vul	Vul	Vul
<u>let-23</u>	Muv	Muv	Muv/Vul	Muv/Vul	Vul	ND
<u>lin-10</u> <u>lin-7</u> <u>lin-2</u>	Muv	Muv	Muv	Muv/Vul	Muv/Vul	Vul
<u>lin-3</u>	Muv	Muv	Muv	Muv	Muv	Muv
<u>lin-12</u> <u>n302</u>	ND	Muv	Muv	Muv	ND	ND

that this mutation results in a greater loss of lin-3 gene activity than does lin-3(e1417) (Ferguson and Horvitz, 1985). lin-15(n765) results in a heat-sensitive Muv phenotype: at 15[°], lin-15(n765) hermaphrodites are wild-type, and at 20[°] and 25[°] lin-15(n765) hermaphrodites are Muv. However, n765 results in a more severe Muv phenotype at 25[°] than at 20[°] (Ferguson and Horvitz, 1985).] At 20[°], not all hermaphrodites of genotype lin-3(n378) + unc-22/lin-3(n1059) dpy-20 +; him-5; lin-15(n765) were Muv; some hermaphrodites were wild-type, and occasional hermaphrodites were Vul. We also examined the males of this strain. Unlike wild-type males, lin-15(n765) males have ventral protrusions resulting from the vulval-like divisions of the cells P(3-6).p (P. Sternberg, unpublished observations). However, at 20[°], none of the males of genotype lin-3(n378) + unc-22/lin-3(n1059) dpy-20 +; him-5; lin-15(n765) had ventral protrusions. These observations indicate that lin-3 has a site of action in the hypodermis and is involved in an early step in the pathway of hypodermal response to the inductive signal.

Mutations in lin-17 and lin-11 cause alterations in the lineages of P(5-7).p, but do not affect the lineages of P(3,4,8).p. To determine if the vulval cell lineage defects resulting from these mutations were independent of the gonadal signal, the gonads of four lin-11 hermaphrodites and five lin-17 hermaphrodites were ablated during the L1. In irradiated hermaphrodites of both genotypes, P(5-7).p adopted 3[°] fates, indicating that the expression of the lin-11 and lin-17 defects are not independent of the gonad. The lineage defects of these two mutants are also expressed in double

mutant strains containing the hypodermal Muv mutation lin-12(n137) (see below and Table 3-10a), indicating that these genes are likely to act in the hypodermal cells after the fates of P(3-8).p have been determined.

lin-12 has a site of action in the gonad as well as in the hypodermis. Dominant mutations in lin-12 result in the transformation of the anchor cell to a ventral uterine precursor cell and eliminate the gonadal signal (Greenwald et al., 1983). Recessive mutations in lin-12 result in the production of two or more anchor cells. In hermaphrodites homozygous for the partially dominant Vul mutation lin-12(n302), no anchor cell is present. lin-12(n302) acts as a gonad-defective Vul mutation; all double mutants carrying lin-12(n302) and a hypodermal Muv mutation have a Muv phenotype. Thus, all of these 22 genes have a site of action in the hypodermis and only one gene, lin-12, has been shown to act in the gonad.

(3) Time of Action

Temperature-shift experiments performed on animals carrying a temperature-sensitive (ts) allele of a gene can determine the interval (the temperature-sensitive period or TSP) during which the temperature sensitive activity of a mutation, either ts synthesis or ts function, is required for normal development to occur (Suzuki, 1970; Swanson and Riddle, 1981). The TSP does not necessarily correspond to the time at which the phenotype of a mutation in the gene is manifest (Suzuki, 1970, Hartwell et al., 1974). The beginning of the TSP is defined as the earliest point at which a shift from restrictive to permissive

temperatures results in a mutant phenotype; conversely, the end of the TSP is defined as the earliest point at which a shift from permissive to restrictive temperatures results in a non-mutant phenotype (Suzuki, 1970). In this section, we determine the TSPs of four genes involved in vulval development -- lin-13, lin-15, lin-25, and let-23.

lin-15(n765): The TSP of lin-15 begins at hatching, as some lin-15 hermaphrodites that were shifted from restrictive to permissive temperatures any time after hatching became Muv (Figure 3-2a). However, both the penetrance and expressivity of the Muv phenotype was lower in hermaphrodites that were shifted to the permissive temperature earlier in development. The TSP of lin-15 ends at the L2 molt, since individual hermaphrodites that were shifted from permissive to restrictive temperatures during the L2 molt became Muv, while hermaphrodites that were shifted from permissive to restrictive temperatures in the early L3 before the divisions of P(3-8).p became wild-type (Figure 3-2a). The TSP of lin-15 in males is equivalent to the TSP of lin-15 in hermaphrodites (E. Ferguson, unpublished observations).

These data suggest that the temperature-sensitive activity of lin-15 begins at hatching (or before) and ends prior to the divisions of P(3-8).p in the early L3. If lin-15(n765) is heat-sensitive for both synthesis and function (Jarvik and Botstein, 1973), the lin-15 product may be synthesized continuously from hatching and act during the early L3.

Figure 3-2 Temperature-shift experiments performed on hermaphrodites carrying temperature-sensitive alleles of genes that affect the vulval cell lineages. The solid line represents the phenotypes of animals shifted from the permissive to the restrictive temperature (P → R). The dashed line represents the phenotypes of animals shifted from the restrictive to the permissive temperature (R → P). The open circles and squares indicate the phenotypes of mutant hermaphrodites that were grown at that temperature but were not shifted. a, lin-15:

Hermaphrodites of genotype him-5; lin-15(n765) were shifted at various times during development. Times of shift represent hours after hatching at the permissive temperature (15°) before shift to the non-permissive temperature (25°) or hours after hatching at 25° before shift to 15°. P → R: 0 hr (n=36); 4 hr (n=53); 15 hr (n=49); 25 hr (n=46); 35 hr (n=64); 43 hr (n=48); 50 hr (n=57); 58 hr (n=37); not shifted (n=51). R → P: 0 hr (n=51); 7 hr (n=33); 12 hr (n=86); 18 hr (n=67); 22 hr (n=62); 25 hr (n=45); 29 hr (n=26); 34 hr (n=23); 38 hr (n=31); not shifted (n=36). b, lin-13: The lin-13(n387) unc-36

progeny of hermaphrodites of genotype + lin-13 unc-36 +/ lin-16 + + unc-86 were shifted at various times during development. P → R: 4 hr (n=19); 15 hr (n=17); 25 hr (n=24); 35 hr (n=26); 43 hr (n=22); 50 hr (n=18); 58 hr (n=15); not shifted (n=27). R → P: 0 hr (n=27); 8 hr (n=17); 13 hr (n=19); 20 hr (n=14); 26 hr (n=16); 29 hr (n=13); 34 hr (n=10); 38 hr (n=23); not shifted (n=19). c, let-23:

Hermaphrodites carrying the cold-sensitive mutation let-23(n1045) were shifted between two different temperatures at various times during development. The number in parentheses following the time of shift indicates the number of hermaphrodites shifted at that time. The time

of shift was determined by using cell lineage criteria to stage the development of individual hermaphrodites in Nomarski optics. P → R: L1 (n=15); L1 molt (n=4); early L2 (n=1); mid-L2 (n=6); late L2 (n=4); L2 molt (n=7); very early L3 (n=6); early L3 (n=4); L3 second round of Pn.p divisions (n=3). R → P: L1 (n=11); L1 molt (n=1); early L2 (n=2); mid-L2 (n=3); late L2 (n=11); L2 molt (n=8); very early L3 (n=2); early L3 (n=2); L3 second round Pn.p (n=6). d, lin-25: Hermaphrodites of genotype lin-25(n545) were shifted at various times during development. P → R: 0 hr (n=25); 4 hr (n=37); 15 hr (n=26); 25 hr (n=35); 34 hr (n=45); 42 hr (n=24); 50 hr (n=45); 58 hr (n=33); not shifted (n=58). R → P: 0 hr (n=58); 7 hr (n=18); 12 hr (n=65); 18 hr (n=52); 22 hr (n=11); 25 hr (n=47); 29 hr (n=31); 34 hr (n=30); 38 hr (n=32); not shifted (n=25).

lin-13(n387): Because lin-13(n387) results in a sterile Muv phenotype, the temperature shift experiments were performed on lin-13 hermaphrodites that were the progeny of heterozygous parents. The results of these experiments (Figure 3-2b) indicate that if a lin-13 hermaphrodite is grown at the permissive temperature any time between the late L1 and the early L3, it will become wild-type. lin-13 hermaphrodites that were shifted from permissive to restrictive temperatures during the mid-L1 became sterile, but were not Muv (E. Ferguson, unpublished observations).

The results of the temperature shift experiments are consistent with either of two models of lin-13 action. In both models the temperature-sensitive activity of lin-13 is completed prior to the divisions of P(3-8).p in the early L3. In the first model, lin-13 has two separate TSPs, and wild-type lin-13 activity during either of the TSPs is sufficient to generate a wild-type phenotype. As the genetic analysis of lin-13 indicates that lin-13 has both a maternal and a zygotic component (Ferguson and Horvitz, 1985), it is possible that the earlier TSP, which ends during the mid-L1, reflects the maternal lin-13 activity and the later TSP, which ends at the L2 molt, reflects the zygotic activity of lin-13. The alternative model, which is similar to a model proposed by Hirsh and Vanderslice (1976) during their studies of temperature-sensitive lethal mutations in C. elegans, is that lin-13 has a single TSP, is temperature-sensitive for synthesis, and is made in excess continuously from the early L1 until the L2 molt, after which time it functions. If so, the exposure of a lin-13 hermaphrodite to permissive temperatures any time between the

early L1 and L2 molt would result in a wild-type phenotype, provided that enough functional lin-13 product could be produced during a short period of exposure to a permissive temperature.

let-23(n1045): let-23(n1045) results in a cold-sensitive Vul phenotype: in let-23(n1045) hermaphrodites that are grown at 15°, P(5-7).p undergo 3° fates; conversely, in let-23(n1045) hermaphrodites that are grown at 25°, more than 3 P(3-8).p cells express non-3° fates. This weak Muv phenotype most likely results from a decrease in let-23 gene activity (see above). The TSP of let-23(n1045) is during the mid L2 (Figure 3-2c). Hermaphrodites shifted from restrictive to permissive temperatures after the mid L2 had a Vul phenotype. Conversely, hermaphrodites shifted from permissive to restrictive temperatures after the mid L2 had a weak Muv phenotype.

If the let-23 product is cold-sensitive for synthesis, then it must be synthesized during the mid-L2 and function at that time or later. If the let-23 product is cold-sensitive for function, then it must function during the mid-L2.

lin-25(n545): The TSP of the Vul phenotype of lin-25 begins during the early L2, since hermaphrodites shifted from the restrictive to permissive temperatures before the early L2 did not display a Vul phenotype (Figure 3-2d). Thus, the lack of lin-25 activity during the ectopic division of the Pn.p cells in the L1 does not result in a Vul phenotype. However, some n545 hermaphrodites that were shifted from permissive to restrictive temperatures during the L1 were egg-laying

competent, suggesting that wild-type lin-25 activity during the L1 can be sufficient to generate an egg-laying competent phenotype.

The TSP of the Vul phenotype of lin-25 ends in the mid-L3 as all hermaphrodites that were shifted from permissive to restrictive temperatures after the mid-L3 were egg-laying competent. However, some n545 hermaphrodites that were shifted from restrictive to permissive temperatures after P(5-7).p had divided were egg-laying competent, suggesting that lin-25 can act after the divisions of P(5-7).p to generate an egg-laying-competent phenotype.

These results demonstrate that the Vul phenotype of lin-25 hermaphrodites, which is caused by the aberrant cell divisions of the P(3-8).p cells (and the L1-derived daughter cells of P(3-8).p) during the L3, does not result from the lack of lin-25 activity during the L1, but rather results from the necessity of lin-25 activity in one or more of the later steps in the pathway of vulval development.

Comparison of the TSPs of lin-13(n387), lin-15(n765), lin-12(n137 n460), let-23(n1045) and lin-25(n545)

The TSPs of lin-13 and lin-15 end during the L2 molt, prior to the divisions of P(3-8).p in the early L3. If lin-13 and lin-15 are heat-sensitive for function, the activities of these genes precede, or are concurrent with, the time of the determination of the fates of P(3-8).p in the wild type (Sternberg and Horvitz, 1985a). Thus, the TSPs of both genes are consistent with their involvement in the

determination of P(3-8).p cell fates. However, because the TSPs of both genes encompass periods of earlier larval development, the lin-13 and lin-15 gene products are either continuously synthesized until the early L3, at which time they act, or these products function continuously from early larval development until the early L3.

The TSP of let-23 is during the mid-L2. If the let-23 product is cold-sensitive for function, the action of this gene appears to be complete by the L2 molt. Kimble (1981) and Sternberg and Horvitz (1985a) have shown that the period of anchor cell induction is from L2 ecdysis to sometime before the divisions of P(3-8).p in the early L3, suggesting that let-23 may act in the hypodermal cells prior to the time of anchor cell induction. Alternatively, let-23(n1045) could be cold-sensitive for synthesis. If this is the case, then let-23 is synthesized during the mid-L2 and functions after that time.

The TSP of lin-12 in the ventral hypodermis was determined by performing temperature shift experiments on hermaphrodites carrying the temperature-sensitive allele lin-12(n137 n460), which results in a wild-type phenotype at 25° and a Muv phenotype at 15° (Greenwald et al., 1983). In the hypodermis, the TSP of lin-12 is very narrow, extending from just after the L2 molt until just prior to the divisions of P(3-8).p in the early L3. Thus, in contrast with lin-13 and lin-15, lin-12 acts only during the time of the determination of the fates of P(3-8).p.

The TSPs of mutations in the genes lin-12, lin-13, lin-15 and

let-23, are consistent with their action in the determination of the fates of P(3-8).p. However, lin-25 appears to act during at least three of the steps of vulval development. The lack of lin-25 action during the L1 causes some of the Pn.p cells to divide, indicating the involvement of lin-25 in the formation of the vulval precursor cells. In addition, since the TSP for the Vul phenotype of lin-25 extends from the early L2 through the mid-L3, lin-25 appears to act during the time of both the determination of P(3-8).p cell fates and the execution of the vulval sublineages.

(4) Interactions among Mutations

To determine the patterns of interactions among mutations in the 22 genes, we constructed double mutant strains containing pairs of vulval cell lineage mutations. (See Tables 3-1 and 3-2 for the strains constructed and Materials and Methods for the protocols employed.) An examination of the phenotypes of the double mutant strains has allowed us, for some genes, to deduce the order of gene function. For other genes, the phenotypes of the double mutant strains have permitted us to determine the number of independent functions affected by these genes.

The epistatic relationship between two mutations that result in different phenotypes (e.g., Muv and Vul) is dependent upon the interaction between the two genes. Specifically, if two genes affect the same pathway and the completion of the activity of one gene is required for the initiation of the activity of a second gene (e.g., if

two genes specify sequential enzymatic steps in a biochemical pathway), a mutation that eliminates the activity of the first gene will be epistatic to a mutation that eliminates the activity of the second gene. However, if two genes affect the same pathway but the latter gene is negatively regulated by the former gene (e.g., the repression by the lacI gene product of the activities of the lacZ, lacY and lacA genes), a mutation that eliminates the activity of the second gene will be epistatic to a mutation that eliminates the activity of the first gene. Thus, the epistatic relationship between two mutations can only be used to determine the order of gene function if the interrelationship between the two genes can be determined.

The majority of the steps in the pathway of vulval development occur independently of one another, as the preceding step must be completed before the succeeding step can be initiated (e.g., the formation of the vulval precursor cells is a prerequisite for their ability to respond to the inductive signal of the anchor cell). Thus, genes that are postulated to affect different steps in the pathway of vulval development should act independently of one another, and the epistatic relationship between mutations in such genes should be similar to the order of gene function. However, since the interrelationships among the activities of genes that affect a single step in the pathway of vulval development are not presently known, the order of gene action cannot be deduced from the epistatic relationships among mutations in such genes.

The patterns of interactions among mutations in a set of genes can

also specify the minimal number of functions encoded by the set of genes. Specifically, if two genes identified by mutations that result in a similar phenotype have a different epistatic relationship with a third mutation that confers an opposing phenotype, the two genes most likely encode different functions. Similarly, the patterns of interaction between incompletely penetrant mutations that result in the same phenotype can specify the number of different functions affected by a set of genes. If two genes encode different functions, the phenotype of a double mutant carrying mutations in both genes should display higher penetrance than that of either single mutant. Alternatively, if two genes affect a single function, the double mutant phenotype could show the same or higher penetrance than that of either single mutant. Thus, if the penetrance of a double mutant is not higher than the penetrance of either single mutant, the two genes most likely affect a single function.

We describe the interactions among the vulval cell lineage mutations in four sections: (1) interactions between the early-acting Vul mutations and other vulval cell lineage mutations; (2) interactions among the mutations that alter the fates of P(3-8).p; (3) interactions between mutations in lin-12 and mutations that alter the execution of the 2⁰ sublineage; (4) interactions between mutations in lin-1, lin-25, and lin-31 and other vulval cell lineage mutations.

(4.a) Interactions Among Early-Acting Vul Mutations and Other lin Mutations

Four of the Vul mutations that result in vulval cell lineage alterations prior to the early L3 -- lin-4, lin-24, lin-26, and n300 -- are epistatic to all Muv mutations tested (Table 3-7). As the majority of Muv mutations cause P(3-8).p to express 1^o and/or 2^o sublineages, it is likely that these four Vul mutations render P(3-8).p unable to express vulval cell lineages.

We also constructed a number of double mutants carrying an early-acting Vul mutation and a Vul mutation that causes P(5-7).p to express 3^o fates (Table 3-1). In all of the double mutant strains, the penetrance of the Vul phenotype was equivalent to the penetrance of the Vul phenotype of the early-acting Vul mutation, suggesting that the early-acting Vul mutant phenotypes are expressed in these strains. In addition, we observed the defects in the vulval cell lineages in four of these double mutant strains -- n300; lin-2, lin-7; n300, lin-10; n300, and lin-24; lin-2 -- with Nomarski optics; in all four strains, the vulval cell lineage defects resulting from the early-acting Vul mutation were present, suggesting that the early-acting Vul mutations also block the ability of P(3-8).p to express 3^o fates.

These results strongly suggest that at least three of these genes -- lin-4, lin-26, and n300 -- act prior to all genes that are involved in the determination of P(3-8).p cell fate and that the activities of these three genes are probably necessary for the production of vulval precursor cells competent to undergo vulval cell lineages. lin-24, however, is represented only by partially dominant alleles that

probably result in a gain of function (Ferguson and Horvitz, 1985). Thus, it is not known whether the wild-type activity of this gene is necessary for, or active in, the production of competent vulval precursor cells.

(4.b) Interactions Among Mutations That Alter the Fates of P(3-8).p

To determine the patterns of interactions among the mutations that alter the fates of P(3-8).p, we constructed three classes of double mutants: (1) double mutants carrying a Muv and a Vul mutation, (2) double mutants carrying two Vul mutations, and (3) double mutants carrying a Vul mutation and a lin-12(d) mutation.

(4.b1) Interactions between Vul and Muv mutations

The epistatic relationships (Table 3-7) among the Vul mutations in the five genes -- lin-2, lin-3, lin-7, lin-10, let-23 --, and the Muv strains of genotypes lin-8; lin-9, lin-13, and lin-15, suggest that the five genes identified by Vul mutations affect at least three independent functions.

let-23 affects a function different from the functions affected by the other four genes; unlike Vul mutations in the other four genes, let-23(n1045) is coexpressed with the Muv phenotype of lin-15 and is epistatic to the Muv phenotypes of both lin-8; lin-9 and lin-13. The coexpression of let-23(n1045) and lin-15 is dependent upon the residual gene activity of let-23(n1045); at 15°, hermaphrodites of genotype dpy-10 let-23(n1045) +/- let-23(mn23) unc-4; lin-15(n309)

have a Vul phenotype. [let-23(mn23) results in larval lethality and may eliminate let-23 gene activity.]

The Vul mutations in lin-2, lin-7 and lin-10 have similar epistatic relationships with the aforementioned Muv strains (Table 3-7) and, thus, might affect a similar function. lin-15 is epistatic to these Vul mutations, while these Vul mutations are epistatic to the Muv phenotype of lin-13. Vul mutations in these three genes are coexpressed with the Muv phenotype of lin-8; lin-9. We have observed hermaphrodites in which some of the cells P(3,4,8).p expressed 1⁰ and 2⁰ fates (expression of the Muv phenotype), and some of the cells P(5-7).p expressed 3⁰ fates (expression of the Vul phenotype), indicating that the coexpression of the Muv and Vul phenotypes can occur in individual hermaphrodites. In such strains, the probability of each of the cells P(3-8).p expressing a non-3⁰ fate is greater than the probability of each of the cells P(5-7).p expressing a non-3⁰ fate in the Vul mutant, suggesting that this coexpression of the Muv and Vul phenotypes in these strains is not due to the incomplete penetrances of the Vul mutations.

The Muv mutations in lin-15, lin-8; lin-9 and lin-13 are all epistatic to the Vul mutation lin-3(e1417), which reduces but does not eliminate lin-3 activity. A greater reduction in lin-3 activity does not allow expression of the lin-3 Vul phenotype in a highly penetrant Muv background; at 25⁰ hermaphrodites of genotype + lin-3(n378) unc-22/dpy-20 lin-3(n1059) +; lin-15(n765) are Muv. [lin-3(n1059) results in larval lethality and may eliminate lin-3 gene activity.]

lin-15(n765) is a heat-sensitive allele of lin-15.] Thus, lin-3 most likely affects a separate function from the other four Vul genes.

(4.b2) Interactions among Vul mutations in lin-2, lin-3, lin-7, lin-10, and let-23

As the Vul mutations in the genes lin-2, lin-3, lin-7, lin-10 and let-23 result in incompletely penetrant phenotypes, the penetrances of the Vul phenotypes of the double mutant strains carrying two of these mutations can also allow us to determine the number of functions affected by these five genes. We constructed all of the ten possible double mutant strains among this set of genes.

The epistatic interactions between these Vul mutations and the Muv mutations suggested that these five genes specified at least three different functions. However, since lin-2, lin-7 and lin-10 displayed similar epistatic relationships with the Muv mutations, the number of different functions specified by the three genes could not be determined. The penetrance of the Vul phenotypes of the three double mutants each carrying alleles of two of these genes was not higher than the penetrance of the Vul phenotypes of the respective single mutants (Table 3-8), suggesting that lin-2, lin-7 and lin-10 specify a single function.

The remaining seven double mutant strains contained two Vul mutations postulated to specify different functions. The penetrance of the Vul phenotypes of each of these double mutants was higher than

Table 3-8 The penetrances of the Vul phenotype of double mutants carrying a pair of Vul mutations in the genes lin-2, lin-7, lin-10 and lin-3 was determined by placing Petri dishes containing between 25-50 L2 hermaphrodites at 20° and measuring the percentage of hermaphrodites that turned into bags of worms. The penetrance of the Vul phenotype of the single mutant strains is from Ferguson and Horvitz (1985).

Table 3-8

Penetrance of the Vul phenotype in double mutants
carrying two Vul mutations

<u>Genotype</u>	<u>% Vul hermaphrodites</u>
<u>lin-2</u>	75 (n=275)
<u>lin-3</u>	87 (n=470)
<u>lin-7</u>	91 (n=478)
<u>lin-10</u>	92 (n=451)
<u>lin-2; lin-7</u>	84 (n=474)
<u>lin-2; lin-10</u>	84 (n=445)
<u>lin-7; lin-10</u>	96 (n=543)
<u>lin-3; lin-2</u>	96 (n=460)
<u>lin-3; lin-7</u>	99.3 (n=897)
<u>lin-3; lin-10</u>	99.3 (n=755)

Table 3-9 The number of P(3-8).px cells that divided in hermaphrodites carrying let-23(n1045) and/or a Vul mutation in one of the genes lin-2, lin-3, lin-7, or lin-10 are expressed as a percentage of the number (6) of the P(3-8).px cells that divide in the wild type. "% all 3⁰" is the percentage of hermaphrodites of a given genotype in which none of the cells P(3-8).px divided.

Table 3-9

No. of P(3-8).px cells that divide in strains carrying
one or two Vul mutations

<u>let-23</u> genotype	Vul geno- type	Temper- ature	No. P(3-8).px cells participating in vulval development								N	% induct- ion	% all 3 ^o
			0	1	2	3	4	5	6	>6			
<u>n1045</u>	<u>+</u>	15 ^o	29	3	2	4	5	4	3	0	50	25	58
		20 ^o	0	0	1	0	0	0	16	12	29	>100	0
		25 ^o	0	0	0	0	0	0	7	18	25	>100	0
<u>+</u>	<u>lin-3</u>	15 ^o	5	5	2	2	3	2	1	0	20	37	25
		20 ^o	5	3	3	2	1	0	0	0	13	25	38
		25 ^o	4	3	6	2	0	1	0	0	16	27	25
<u>n1045</u>	<u>lin-3</u>	15 ^o	39	1	0	0	0	0	0	0	40	0.4	98
		20 ^o	17	5	1	1	1	0	0	0	25	9	68
		25 ^o	14	3	2	3	1	0	0	0	23	15	61
<u>+</u>	<u>lin-10</u>	15 ^o	27	2	1	1	0	2	0	0	33	9	82
		20 ^o	9	2	4	2	3	1	3	0	24	35	38
		25 ^o	19	2	5	1	2	0	3	0	32	22	59
<u>n1045</u>	<u>lin-10</u>	15 ^o	23	0	0	0	0	0	0	0	23	0	100
		20 ^o											
		25 ^o	36	1	1	0	0	0	1	0	39	7	92
<u>+</u>	<u>lin-7</u>	15 ^o	10	2	2	1	0	2	1	0	18	22	56
		20 ^o	8	2	1	1	4	1	2	1	20	40	40
		25 ^o	6	1	3	1	5	2	5	0	23	50	17
<u>n1045</u>	<u>lin-7</u>	15 ^o	27	0	0	0	0	0	0	0	27	0	100
		22.5 ^o	29	0	0	0	0	0	0	0	29	0	100
		25 ^o	31	0	0	0	0	0	0	0	31	0	100
<u>+</u>	<u>lin-2</u>	15 ^o	7	0	0	0	1	0	1	0	9	19	78
		25 ^o	16	1	1	0	1	0	1	0	20	11	80
<u>n1045</u>	<u>lin-2</u>	15 ^o	13	0	0	0	0	0	0	0	13	0	100
		20 ^o	15	0	0	0	0	0	0	0	15	0	100
		25 ^o	26	0	0	0	0	0	0	0	26	0	100

the penetrance of the phenotypes of the respective single mutants (Tables 3-8 and 3-9), supporting the hypothesis that the two mutations in each of the double mutants specify different functions.

(4.b3) Interactions between Vul and lin-12(d) mutations

The lin-12(d) mutation, n137, is epistatic to Vul mutations in the genes lin-2, lin-3, lin-7, lin-10 and let-23 (Table 3-7). However, these Vul mutations are epistatic to the hypodermal defects resulting from a lin-12(0) mutation (Sternberg and Horvitz, 1985b).

(4.c) Interactions Between Sublineage-Specific Mutations and lin-12 Mutations

Mutations in the genes lin-11, lin-17 and lin-18 affect the lineages of P5.p and P7.p. To determine if these genes are active in all cells that adopt a 2⁰ fate, or if the action of these genes is specific to P5.p and P7.p, we examined the phenotypes of double mutants that were constructed between these mutations and the two classes of lin-12 mutations, lin-12(d) and lin-12(0).

lin-11 mutations affect all 2⁰ sublineages in a lin-12(d) hermaphrodite. In lin-12(d) hermaphrodites, P(3-8).p all undergo [LLTN] or [NTLL] lineages and generate five small ventral protrusions. In hermaphrodites of genotype lin-11; lin-12(d), P(3-8).p undergo [LLLL] lineages, just as P5.p and P7.p do in lin-11 hermaphrodites, and generate five or six smaller pseudovulvae. Similarly, lin-17 and lin-18 mutations affect the polarity of the 2⁰ sublineages in

Table 3-10 Vulval cell lineages in double mutant hermaphrodites. The nomenclature used to indicate the planes of division of the P(3-8).pxx cells is as described in Table 3-4. a, Vulval cell lineages of double mutant hermaphrodites carrying a lin-12(d) mutation and a mutation in lin-11, lin-17 or lin-18. b, Vulval cell lineages of double mutant hermaphrodites carrying a lin-1 mutation and either of the two classes of lin-12 mutations, lin-12(d) or lin-12(0).

Table 3-10

Vulval cell lineages of double mutant hermaphrodites

	#ac	<u>P3.p</u>	<u>P4.p</u>	<u>P5.p</u>	<u>P6.p</u>	<u>P7.p</u>	<u>P8.p</u>	No.
A)								
<u>lin-11;</u>	0	LLLO	LLLO	LLLN	LLLL	LLLL	LLOL	1
<u>lin-12(d)</u>	0	LLLO	LLLO	LLL?	LLLL	LLLL	LLLL	1
	0	<u>LOLL</u>	<u>LOOL</u>	<u>LLLL</u>	<u>LLOL</u>	<u>LLLL</u>	<u>LOLO</u>	1
	0	<u>LOLO</u>	<u>LLLL</u>	<u>LLLL</u>	<u>LLLL</u>	<u>LLLL</u>	<u>LLLO</u>	1
	0	<u>S</u>	<u>LLLO</u>	<u>OLLL</u>	<u>LOLL</u>	<u>OOLL</u>	<u>LLOL</u>	1
	0	LOOL	<u>LLOL</u>	<u>LLLL</u>	<u>LOLL</u>	<u>OOLL</u>	<u>LLOL</u>	1
<u>lin-17;</u>	0	LLOT	LLTO	LLTT	LLOO	LLTT	LLTT	1
<u>lin-12(d)</u>	0	OLTT	LLOT	LLTT	LLTT	LLOO	LLTN	1
	0	LLOL	LLTT	LLLN	LLLL	LLTO	LLTT	1
	0	OLTT	LLLL	LLLO	LLTO	LLTT	LLTT	1
	0	LLOO	LLLO	LLTL	LLLL	LOLO	LT00	1
<u>lin-17;</u>	2	S	S S	SSTT	TTTT	TTTO	S S	1
<u>lin-12(0)</u>	2	S S	S LT	OTTT	LTTT	S S	S S	1
	2	S S	S S	TTTT	TTTT	TT S	S S	1
	2	S S	S S	LOTT	TTTT	TTOL	S S	1
	2	S S	S S	TTTT	TTTT	TO S	S S	1
	2	S	S DD	LOT?	TTTT	TTTO	DL S	1
	2	S	S S	TTTT	TTTT	TTTT	TO S	1
<u>lin-12(d);</u>	0	TOTO	LLTO	LLON	LLOT	LLOO	OLTT	1
<u>lin-18</u>	20° 0	LLOT	LLOO	LLOO	LLOT	LLTO	LLTT	1
<u>lin-12(0);</u>	3	S S	S ??	OTDD	DDTT	S S	S S	1
<u>lin-18</u>	20° 2	S	S UU	TTTT	TTTT	TTSO	S S	1
	2	S S	S S	O?TT	TTTT	TT S	S S	1
	1	S	S S	S DD	TTTT	?? S	S S	1

Table 3-10

Vulval cell lineages of double mutant hermaphrodites

B)	#ac	<u>P3.p</u>	<u>P4.p</u>	<u>P5.p</u>	<u>P6.p</u>	<u>P7.p</u>	<u>P8.p</u>	No.
<u>dpy-19</u>								
<u>lin-12(d);</u>	0	OOTT	LLTN	OLTT	TOTT	NTLL	TTOT	1
<u>lin-1(e1777)</u>	0	LLTT	LLTN	OLTN	TTNT	NTLL	OOTN	1
	0	LLTN	LLON	LLON	TTTT	NOLL	NTOL	1
	0	LLTT	OOTN	OTTN	TTTT	NTTO	NTLL	1
<u>lin-12(0);</u>	2	S	S S	S TT	TTTT	S S	S S	1
<u>lin-1(e1777)</u>	2	S	S S	TN S	OT S	TTTT	S S	1
	2	LDDL	??OT	??OO	UUTT	TT S	S S	1
	2	S OT	S TT	S TT	SSTO	OT S	OOSS	1
	2	S	S OT	S LT	S TT	OT S	S S	1
	2	S SS	S T?	S TT	SSTT	TT S	TT S	1
	2	S TT	S TT	S TT	S TT	TT S	TT S	1
	?	LTTT	S TT	S TT	S TT	TT S	S S	1
	?	S S	S S	TT S	TTTT	S S	S S	1
	?	S TT	TT S	S TT	S TT	TT S	S S	1
	?	S TT	S TT	TTTT	TT S	S S	S S	1
	2	S	S TT	S TT	TTTT	TT S	S S	1

lin-12(d) hermaphrodites. In most lin-12(d) hermaphrodites, P(3-5).p undergo 2⁰ lineages that have the polarity of a P5.p lineage in the wild type, and P7.p and P8.p express 2⁰ lineages that have the polarity of a P7.p lineage in the wild type. In lin-17; lin-12(d) hermaphrodites and in lin-12(d); lin-18 hermaphrodites all six cells P(3-8).p express 2⁰ lineages that have the polarity of the P5.p lineage in the wild type, resulting in the appearance of six pseudovulvae of equal size. Conversely, mutations in lin-11, lin-17 and lin-18 do not appear to affect the vulval cell lineages of lin-12(0) hermaphrodites, suggesting that these three genes are not expressed in cells undergoing 1⁰ or 3⁰ sublineages.

(4.d) Interactions Between Mutations in the Genes lin-1, lin-25 and lin-31 and Other Vulval Cell Lineage Mutations

lin-1: The patterns of interactions between lin-1 and the other vulval cell lineage mutations do not resolve whether lin-1 affects the the execution of the three vulval sublineages or whether lin-1 is involved in the determination of the fates of P(3-8).p. lin-1 is epistatic to or coexpressed with all mutations that alter the fates of P(3-8).p, suggesting that lin-1 may be involved in the execution of all three vulval sublineages. Specifically, the Muv phenotype of lin-1 is epistatic to Vul mutations in lin-2, lin-3, lin-7, lin-10 and let-23 (Table 3-7), demonstrating that the lack of lin-1 activity can alter the lineages of cells that are determined to express 3⁰ fates. The phenotype of lin-1 is also expressed in hermaphrodites of

genotypes lin-12(d); lin-1 and lin-12(0); lin-1 (Table 3-10b). Specifically, in lin-12(d); lin-1 hermaphrodites, P(3-8).p can undergo non-2^o lineages (including some with 1^o character), demonstrating that the lack of lin-1 activity can alter the lineages of P(3-8).p cells that are determined to undergo 2^o fates. lin-1 has two effects upon the vulval cell lineages of lin-12(0) hermaphrodites. In a lin-12(0); lin-1 double mutant, more of the cells P(3-8).p express non-3^o patterns of division (indicating the expression of a lin-1 Muv phenotype) than in lin-12(0) hermaphrodites. Second, many of the P(3-8).p cells that divide express a novel lineage pattern of [S TT] or [TT S], demonstrating that the lack of lin-1 activity can affect the lineages of cells that are determined to undergo 1^o fates.

However, there is also indirect evidence that lin-1 could affect the determination of the fates of P(3-8).p. lin-1 probably acts in the same pathway as let-23 in at least some non-vulval cells. Specifically, lin-1(e1777) suppresses the larval lethality that results from any of the four let-23 alleles -- the Vul allele, n1045, and the larval lethal alleles, mn23, mn216, and mn224. The suppressed let-23(lethal); lin-1 hermaphrodites are not wild-type, rather these hermaphrodites grow slowly and become sterile Muv hermaphrodites. This result suggests that the larval lethality of let-23 could result from an elevation of lin-1 activity, i.e., let-23 could be a negative regulator of lin-1 activity. If let-23 and lin-1 interact in a similar fashion in the vulval cells, the Vul phenotype of let-23 could result from an elevation of lin-1 activity in these cells. If so, one necessary component of vulval induction could be the lowering of lin-1

activity in all cells undergoing non-3⁰ fates.

lin-31: The Muv phenotype of lin-31 is coexpressed with four Vul mutations -- lin-2, lin-7, lin-10, and let-23 -- that cause P(3-8).p to adopt 3⁰ fates (Table 3-7). In these double mutant strains fewer pseudovulvae are observed than in lin-31 hermaphrodites. However, as we have not examined the lineages of these double mutant strains, we do not know whether these Vul mutations suppress any of the L2 divisions of P(3-8).p that are observed in lin-31 hermaphrodites. lin-31 is epistatic to the Vul mutation lin-3(e1417).

lin-25: The Vul phenotype of lin-25 is epistatic to all Muv mutations except the lin-12(d) mutation, n137 (Table 3-7). In lin-12(d); lin-25 hermaphrodites, the ectopic L1 divisions of the Pn.p cells characteristic of lin-25 hermaphrodites generate vulval precursor cells (the P(3-8).p cells and their L1-derived daughters) that express 2⁰ lineages during the L3, resulting in the appearance of five to eight pseudovulvae. These results suggest that the vulval cells derived from the L1 Pn.p divisions in lin-25 hermaphrodites are able to undergo at least 2⁰ sublineages. The phenotype of the lin-12(0); lin-25 double mutant may be equivalent to the phenotype of lin-12(0) hermaphrodites. In both mutant strains, vulval precursor cells typically can produce [TTTT] lineages. The lineages of the vulval precursor cells in the lin-12(0); lin-25 double mutant are qualitatively similar to the P(3-8).p lineages in lin-12(0) hermaphrodites. In the double mutant possibly more cells undergo 3⁰ fates. Thus, lin-25 may not alter the L3 divisions in either

lin-12(d) or lin-12(0) mutants.

These results suggest that the wild-type product of lin-25 may function during the L3 to elevate the activity of lin-12 in the ventral hypodermis, since lin-12(n137), a mutation that results in increased or ectopic lin-12 activity, is epistatic to the Vul phenotype of a mutation in lin-25, while a mutation in lin-25 may not affect the phenotype resulting from a lin-12(0) mutation.

DISCUSSION

Genetic Pathway of Vulval Development

We have constructed a genetic pathway of vulval development in the nematode Caenorhabditis elegans by characterizing the action of each of 22 genes defined by Multivulva and Vulvaless mutants. First, we examined the phenotype resulting from a mutation in each of these genes. The observation that the anatomical defects in these mutant strains are due to alterations in the vulval cell lineages, coupled with an analysis of the effects of each of the mutations upon gene activity (Greenwald et al., 1983; Ferguson and Horvitz, 1985), has enabled us to infer the wild-type functions of many of the genes. Second, we determined the site (i.e., tissue) and, for some genes, the time of gene action. These results have permitted us to correlate the activity of each of these genes with the time and site of each of the steps of vulval development. Third, we examined the patterns of interactions among mutations in different genes. For some genes, these results have allowed us to determine the order of gene function. For other genes, these results have allowed us to identify the number of different functions affected by a set of genes. We describe below (and summarize in Figure 3-3) the assignment of 17 of these 22 genes to specific steps in the pathway of vulval development.

(1) Genes necessary for the production of cells competent to undergo the vulval cell lineages.

Figure 3-3 The genetic pathway of vulval development. See text for details.

Figure 3-3

We have identified seven genes that are necessary for the production of P(3-8).p cells that are competent to undergo vulval cell lineages. Three of these genes -- lin-26, n300, and lin-4 -- are described in this manuscript; the other four genes -- unc-83, unc-84, lin-14, lin-28 -- are described elsewhere (Sulston and Horvitz, 1980; Horvitz and Sulston, 1981; Ambros and Horvitz, 1984; W. Fixsen and R. Horvitz, manuscript in preparation). We have used two criteria to make these assignments. (1) Recessive loss or reduction of function mutations in these genes cause four classes of vulval cell lineage defects prior to the early L3, suggesting that these genes are necessary for the production of cells competent to undergo vulval cell lineages. (2) The Vul mutations in lin-26, lin-4 and n300 are epistatic to all Muv mutations. As most of the Muv mutations cause P(3-8).p to express vulval cell lineages (albeit incorrectly), it is likely that the three genes defined by the Vul mutations act prior to the genes defined by the Muv mutations and that these Vul mutations preclude the expression of vulval cell lineages.

The lineage defects resulting from mutations in these seven genes suggest that these genes are involved in four independent, temporally ordered functions. Although no double mutants have been constructed among mutations in this set of genes, it is likely that the order of gene function reflects the temporal order of these functions during development. (1) The activities of unc-83 and unc-84 are necessary for the migration of the P cell nuclei into the ventral nerve cord (Sulston and Horvitz, 1980; Horvitz and Sulston, 1981; W. Fixsen and R. Horvitz, manuscript in preparation). In hermaphrodites lacking the

activity of either gene, the P cell nuclei do not migrate into the nerve cord, and the vulval precursor cells, P(3-8).p are not generated. (2) The activity of lin-26 is necessary for the Pn.p cells to adopt a hypodermal fate; if the activity of this gene is reduced, the Pn.p cells express neuronal fates. (3) In n300 hermaphrodites, P(3-8).p join the hypodermal syncytium without dividing, suggesting that this mutation prevents P(3-8).p from expressing any of the three vulval sublineages. Since n300 is associated with the reciprocal translocation nT1(IV;V) (Ferguson and Horvitz, 1985), it is not known whether the Vul phenotype of this strain results from an alteration in the activity of a single gene. However, K. Edwards (personal communication) has isolated a recessive mutation which complements n300 but results in a similar phenotype, supporting the notion that the activities of at least one gene, and probably more, are necessary for P(3-8).p to undergo vulval sublineages. (4) Three genes, lin-4, lin-14, and lin-28, are involved in the temporal specification of the lineages of the lateral and ventral hypodermal cells. Recessive loss of function mutations in both lin-14 and lin-28 cause P(3-8).p to undergo vulval cell divisions prior to the early L3. Conversely, recessive mutations in lin-4 and dominant mutations in lin-14 cause the cells of the lateral hypodermis to reiterate early larval division during later larval stages. The vulval cell lineage defects caused by these two classes of mutations are complex; however, it is likely that these mutations also cause P(3-8).p to reiterate early larval divisions during the L3.

(2) Genes necessary for the formation of the gonadal anchor cell and

the production of its inductive signal

We have identified one gene -- lin-12 -- that, in addition in its action in the cells P(3-8).p (see below), is also involved in the formation of the anchor cell of the gonad. In the wild type, the anchor cell is formed during the L2 as the result of an interaction between the two cells Z1.ppp and Z4.aaa. One of these cells becomes the anchor cell, while the other cell becomes a ventral uterine precursor cell (VU). In hermaphrodites carrying lin-12(d) alleles that elevate lin-12 gene activity, both Z1.ppp and Z4.aaa become VU cells; conversely, in hermaphrodites carrying lin-12(0) alleles that eliminate lin-12 activity, both cells become anchor cells (Greenwald et al., 1983).

Mutations that alter the vulval cell lineages during the L3 could affect either the production of the inductive signal by the anchor cell or the response to the inductive signal by P(3-8).p. However, as described in Results, all of the 22 genes studied have a site of action in the cells P(3-8).p. Thus, although it is likely that we have identified most or all of the genes that can mutate to a fertile Muv or Vul phenotype (Ferguson and Horvitz, 1985), we did not identify any genes that specifically affect the production of the inductive signal of the anchor cell. One possible explanation of these results is that the gene, or genes, that encode the anchor cell signal could also act in the hypodermis, masking their action in the anchor cell. A second possibility is that if the gene, or genes, that encode the anchor cell signal have other essential functions, such genes may

identifiable only by rare mutations that reduce but not eliminate gene activity. Alternatively, it may not be possible to eliminate the anchor cell signal by a mutation in a single gene. If the anchor cell signal has more than one component (e.g., Sternberg and Horvitz (1985a) have proposed that the inductive signal could have two components separated in time), or if the inductive signal is encoded by members of a gene family, the elimination of the activity of one of the genes encoding the inductive signal would not necessarily result in a visible phenotype.

(3) Genes that affect the determination of the fates of P(3-8).p

By two criteria, we have identified eleven genes -- lin-2, lin-3, lin-7, lin-8, lin-9, lin-10, lin-12, lin-13, lin-15, lin-34, let-23 -- that are involved in the determination of the fates of P(3-8).p. (1) Recessive, reduction or loss of function mutations in these genes cause some of the P(3-8).p cells to adopt the fates of other members of this set of cells. (2) The site of gene action, and, for those genes for which it is known, the time of gene action, suggest that these genes are involved in the hypodermal response to the anchor cell signal. All of these genes act in the cells P(3-8).p, and the temperature-sensitive periods (TSPs) of four genes -- lin-12 (Greenwald et al., 1983), lin-15, lin-13, and let-23 -- suggest that these genes act prior to, or during the time of P(3-8).p determination in the early L3.

In Vul strains defective in any of five genes -- lin-2, lin-3,

lin-7, lin-10, let-23 --, P(3-8).p all adopt 3⁰ fates, indicating that the activities of these five genes are necessary for P(3-8).p to express 1⁰ and 2⁰ fates. The patterns of interactions among these Vul mutations and between these Vul mutations and different Muv mutations suggest that these five genes specify three different functions. let-23 and lin-3 specify separate functions, both of which are absolutely required for the production of 1⁰ and 2⁰ fates. lin-2, lin-7 and lin-10, however, most likely specify a single function, which is necessary but not absolutely required for the production of 1⁰ and 2⁰ fates.

In Muv strains defective in five genes -- lin-8, lin-9, lin-13, lin-15, lin-34 --, P(3-8).p express 1⁰ or 2⁰ fates. The analysis of these genes suggests that they specify a common function during the determination of P(3-8).p cell fate (Chapter 4, this thesis). In the wild type, this function might be required for determination of P(3-8).p cell to express a 3⁰ fate; i.e., in these Muv mutants, all of the P(3-8).p cells express 1⁰ or 2⁰ fates because none of the P(3-8).p cells can express a 3⁰ fate. Alternatively, during wild-type vulva development, the inductive signal of the anchor cell may result in the reduction or elimination of a function, specified by these genes, which would otherwise inhibit the expression of 1⁰ and 2⁰ fates, i.e., in these Muv mutants, all of the cells P(3-8).p express 1⁰ or 2⁰ fates because these mutations simulate the response to the anchor cell signal.

The activity of lin-12 is required for the production of 2⁰ fates,

since in mutants lacking lin-12 activity, none of the cells P(3-8).p express 2⁰ fates. In mutants carrying a lin-12(d) allele which elevates lin-12 activity, all the cells P(3-8).p express 2⁰ fates, demonstrating that lin-12 activity is not only necessary, but can be sufficient to generate a 2⁰ fate.

Combinations of mutations in these three classes of genes -- vul, muv and lin-12 -- can constrain P(3-8).p to express any one of the three vulval sublineages [1⁰, 2⁰ or 3⁰] independent of the anchor cell (Sternberg and Horvitz, 1985b). Specifically, in muv; lin-12(0) hermaphrodites, P(3-8).p adopt 1⁰ fates, in lin-12(d); vul hermaphrodites P(3-8).p adopt 2⁰ fates and in lin-12(0); vul hermaphrodites P(3-8).p adopt 3⁰ fates. These results suggest that we have identified the major classes of genes that act to interpret the anchor cell signal and determine the fates of P(3-8).p.

Although the epistatic interactions among these mutations do not permit an unambiguous ordering of gene activity during the determination of P(3-8).p cell fate, it is possible, with some assumptions, to construct a model of the interactions among these genes. If the function specified by the muv genes must be lowered to enable cells to express 1⁰ or 2⁰ fates, it is likely that, in response to an anchor cell signal, one or more genes acts to lower the activities of the muv genes. lin-3 may encode such a function, since the Muv mutants are epistatic to the Vul phenotype of lin-3, as this model would predict. Thus, lin-3 could be involved in a very early step in the pathway of hypodermal cell response to the anchor cell,

even possibly in the reception of the anchor cell signal. Vul mutations in let-23 are epistatic to the Muv mutations, which can be interpreted in either of two ways. let-23 could encode an activity essential for the muv genes and act prior to, or concurrently with, the muv genes. Alternatively, the activity of let-23 could be controlled by the muv genes. The patterns of interactions between lin-12 and the genes identified by the Muv and Vul mutations are described more fully by Sternberg and Horvitz (1985b). Their results suggest that, in response to the anchor cell signal, the end product or products of the interaction between the muv and vul genes specify the determination of a 1^o fate in P6.p and serve to elevate the level of lin-12 activity in at least P5.p and P7.p, resulting in the expression of 2^o fates by these two cells.

(4) Genes that are necessary for execution of the three vulval sublineages.

By three criteria, three genes -- lin-11, lin-17, and lin-18 -- are involved in the execution of 2^o sublineages. (1) Mutations in these three genes result in the production of novel vulval cell lineages by P5.p and P7.p. (2) The mutations in these three genes most likely reduce or eliminate gene activity. All mutations in lin-17 and lin-18 are recessive. Three of the four lin-11 alleles are recessive, while the fourth lin-11 allele is partially dominant. If these alleles reduce or eliminate lin-11 activity, then the activity of this gene is also required for the proper execution of 2^o fates.

(3) As described in Results, the phenotypes that result from mutations in these three genes are expressed in all cells that are determined to undergo 2⁰ sublineages, but are not expressed in cells that are determined to undergo 1⁰ or 3⁰ sublineages.

The observation that, after the initiation of vulval cell divisions, the vulval cells lose their ability to alter their fates in response to the ablation of neighboring cells, suggested that the inductive response by the cells P(3-8).p to the anchor cell signal might have two independent components, the determination of cell fate and the execution of the vulval sublineages. The identification of genes that are specific for the execution of the 2⁰ sublineages confirms that the inductive response of the P(3-8).p cells is comprised of two genetically independent functions. These results also suggest that the activity of the genes required for the execution of the vulval sublineages are controlled by the genes that determine the fates of the P(3-8).p cells. Thus, the activities of the three genes -- lin-11, lin-17, lin-18 -- that are specific for the execution of 2⁰ sublineages are probably controlled by lin-12, the gene responsible for the generation of 2⁰ fates.

The Genetic Control of Cell Lineage

Mutations in any of the 17 genes that affect a single step in the pathway of vulval development result in the homeotic transformation of the fates of some or all of the vulval precursor cells or their descendants. Mutations in genes that are necessary for P(3-8).p to

express vulval fates cause P(3-8).p to adopt the fates of non-vulval cells; mutations in genes that are involved in the determination of the fates of P(3-8).p cause some of these cells to express the fates of other members of the set; mutations in the genes involved in the execution of the 2⁰ sublineages cause some of the P5.px and P7.px cells to express the fates of other P5.px and P7.px cells. Many of the mutations that affect the vulval cell lineages also affect some non-vulval cell lineages (P. Sternberg and E. Ferguson, unpublished observations). The effects of the vulval cell lineage mutations upon non-vulval cells are similar to the effects of these mutations upon vulval cells. For example, in the male P(9-11).p are a set of equipotential cells that generate some of the structures used in mating. Like P(3-8).p in hermaphrodites, each of these cells adopts one of three fates [1⁰, 2⁰, or 3⁰] dependent upon position. Many of the Muv mutations that cause P(3-8).p to express 1⁰ or 2⁰ fates result in a similar transformation of the fates of P(9-11).p in the male. Additionally, mutations in three other genes -- lin-12, lin-17 and lin-4 -- result in transformations in the fates of many groups of non-vulval cells. These transformations are analogous to the observed transformations in vulval cell fate. Specifically, in lin-12 mutants, certain homologous cells that normally adopt different fates express similar fates (Greenwald et al., 1983); in lin-17 mutants, certain cells that normally produce asymmetric progeny that express different fates instead produce similar progeny that adopt identical fates (P. Sternberg and R. Horvitz, manuscript in preparation); in lin-4 mutants, the temporal pattern of divisions of the cells of the ventral hypodermis is altered (V. Ambros and R. Horvitz, manuscript in

preparation). As other mutations that alter the cell lineages of C. elegans also appear to transform the fates of certain groups of cells [e.g., ced-3, ced-4 (H. Ellis, personal communication), lin-22 (W. Fixsen, personal communication), mab-5 (C. Keynon, personal communication), unc-86 (Chalfie et al., 1981)], it is possible that many of the genes that are involved in the control of cell lineage in C. elegans may function by regulating the expression of a set of programs, each of which encodes the specification of a particular cell fate.

Chapter IV

Synthetic Multivulva mutants of the nematode

Caenorhabditis elegans

ABSTRACT

The Multivulva (Muv) phenotype of CB1322, one of the mutants of Caenorhabditis elegans defective in the vulval cell lineages of the hermaphrodite, had previously been shown to result from an interaction between two mutations, lin-8(n111) II and lin-9(n112) III, each of which resulted in a wild-type phenotype (HORVITZ and SULSTON, 1980). By mutagenizing hermaphrodites homozygous for either lin-8 or lin-9, we obtained 12 Muv strains, each of which contained a newly-identified mutation that interacted with either lin-8 or lin-9 to generate a Muv phenotype. The new synthetic mutations in seven of these strains defined four additional genes involved in vulval development -- lin-35, lin-36, lin-37, lin-38. The new synthetic mutations in the remaining five strains were alleles of lin-15, a gene previously defined by Muv mutations. --To determine the patterns of interaction among these synthetic mutations, we examined the phenotypes of double mutant strains carrying pairs of these mutations. The phenotypes of these double mutants strains suggest that the Muv phenotypes of these strains result from the necessity of alterations in the functions of two independent processes; an alteration in the function of each process alone is not sufficient to generate a Muv phenotype.

INTRODUCTION

The cellular anatomy, and the patterns of cell division and cell fate of the nematode *Caenorhabditis elegans* are essentially invariant during development (SULSTON and HORVITZ, 1977; DEPPE et al., 1978; KIMBLE and HIRSH, 1979; SULSTON, ALBERTSON and THOMSON, 1980; SULSTON et al., 1983). We have begun an attempt to understand the genetic specification of a particular set of cell lineages in *C. elegans*, those that are involved in the development of the vulva of the hermaphrodite. The vulva is formed during the third larval (L3) stage by the descendants of three of six equipotential cells of the ventral hypodermis in response to a graded inductive signal from the gonadal anchor cell (SULSTON and HORVITZ, 1977; SULSTON and WHITE, 1980; KIMBLE 1981; STERNBERG and HORVITZ, 1985a).

In previous papers (HORVITZ and SULSTON, 1980; SULSTON and HORVITZ, 1981, GREENWALD, STERNBERG and HORVITZ, 1983, FERGUSON and HORVITZ, 1985, FERGUSON, STERNBERG and HORVITZ, 1985) we have described the isolation and characterization of 98 mutants, defective in any of 22 genes, that were abnormal in the vulval cell lineages. Eleven of these 22 genes are postulated to be involved in the determination of the fates of the six potential vulval precursor cells in response to the anchor cell signal (FERGUSON, STERNBERG and HORVITZ, 1985). Mutations in any of these eleven genes result in the homeotic transformation of the fates of some of the six vulval precursor cells to fates normally expressed by other members of this set of cells. In one class of such mutants, "Multivulva" (Muv)

mutants, the three equipotential cells that normally do not adopt vulval fates undergo vulval-like lineages and generate multiple vulva-like protrusions along the ventral side of the animal. The Muv phenotypes of all but one of these strains results from a mutation in any of three genes -- lin-13, lin-15 or lin-34. The Muv phenotype of the remaining strain CB1322 is synthetic, resulting from an interaction between two unlinked mutations, lin-8(n111) II and lin-9(n112) III (HORVITZ and SULSTON, 1980). A hermaphrodite homozygous for either lin-8(n111) or lin-9(n112) has a wild-type phenotype (HORVITZ and SULSTON, 1980; FERGUSON, STERNBERG and HORVITZ, 1985).

In this paper, we further characterize this set of genes. Specifically, we have identified five additional genes, including lin-15, that can mutate to interact with either lin-8(n111) or lin-9(n112) to generate a Muv phenotype. The pattern of interactions among these synthetic mutations suggest that the homeotic transformations in cell fate in these strains result from the necessity of alterations in the functions of two independent processes and that an alteration in the function of either process alone is not sufficient to cause a Muv phenotype.

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 Institute 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.

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

LG II: bli-2(e768); dpy-10(e128); rol-1(e91); unc-52(e444); C1 dpy-10(e128) unc-52(e444). C1 dpy-10 unc-52 is an LG II chromosomal abnormality that balances the right half of the chromosome (HERMAN, 1978).

LG III: dpy-17(e164); lon-1(e185); daf-4(e1364); unc-36(e251); unc-86(e1507); dpy-19(e1259); sup-5(e1464); sma-2(e502); unc-32(e189); lin-12(n137, n137 n720), nDf16 (V. AMBROS and M. FINNEY, personal communication).

LG IV: unc-5(e53); lin-3(e1417); unc-22(e66).

LGV: dpy-11(e224); him-5(e1467, e1490); dpy-21(e428).

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 synthetic Muv mutations were assigned lin, for lineage abnormal, gene names.

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

Identification of hermaphrodites of genotypes lin-8(n111) and lin-9(n112): To obtain hermaphrodites homozygous for lin-8(n111), wild-type males were mated with hermaphrodites of genotype lin-8(n111); dpy-17 lin-9(n112). From F1 hermaphrodites of genotype lin-8(n111)/+; dpy-17 lin-9(n112)/+ +, wild-type progeny were picked. Some of these hermaphrodites were of genotype lin-8(n111); dpy-17 lin-9(n112)/+ +, as they segregated some Dpy progeny, all of which were Muv. The progeny of these animals that did not segregate any Dpy hermaphrodites were of putative genotype lin-8(n111). This genotype was confirmed by mating males of genotype dpy-17 lin-9(n112)/+ + with hermaphrodites of putative genotype lin-8(n111) and picking wild-type progeny. Some of the wild-type progeny segregated Dpy animals. On all such plates, some of the Dpy animals were Muv, confirming that the strain was homozygous for lin-8(n111).

To obtain hermaphrodites homozygous for lin-9(n112), males of genotype unc-32/+ were mated with hermaphrodites of genotype lin-8(n111) dpy-10; lin-9(n112). From F1 hermaphrodites of genotype lin-8(n111) dpy-10/+ +: lin-9(n112) +/+ unc-32, wild-type progeny were picked. Some of these animals were of genotype lin-9(n112) +/+ unc-32, as they segregated Unc hermaphrodites but did not segregate any Dpy Muv or Muv hermaphrodites. The progeny of these hermaphrodites that did not segregate any Unc animals were of putative genotype lin-9(n112). This genotype was confirmed by mating males of genotype lin-8(n111) dpy-10/+ + with hermaphrodites of putative genotype lin-9(n112) and picking wild-type progeny. Some of the wild-type progeny segregated Dpy animals. On all but one such plate, some of the Dpy animals were Muv, confirming that the strain was homozygous for lin-9(n112). (The exceptional hermaphrodite that segregated Dpy animals but no Muv animals was presumably of genotype dpy-10/+ lin-9/+.)

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) (BRENNER, 1974). Individual mutagenized hermaphrodites were put on separate 100mm NGM plates (BRENNER, 1974) and allowed to produce 20-40 progeny. The progeny of these F1 hermaphrodites 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. (The progeny of 2900 F1 animals were examined during the mutagenesis of lin-8(n111))

hermaphrodites; similarly, the progeny of 4100 F1 animals were examined during the mutagenesis of lin-9(n112) hermaphrodites.) Muv hermaphrodites were picked onto separate plates and allowed to self-fertilize. If a candidate appeared to be sterile, it was mated with wild-type males and, if progeny were produced, Muv hermaphrodites were reisolated from the F2 progeny. Only one Muv strain from each parental plate was characterized to ensure that each isolate carried an independently derived mutation.

Isolation and characterization of Muv strains after mutagenesis of the strain MT1312: The strain MT1312 is a highly penetrant Vul strain. 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 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 of both the mutation responsible for the Vul phenotype of MT1312, lin-12(n676), and the mutations that resulted in the Muv phenotypes of these

strains. Studies of lin-12(n676) are described elsewhere (GREENWALD, STERNBERG and HORVITZ, 1983; FERGUSON and HORVITZ, 1985).

For two reasons, it was likely that the Muv phenotypes of these strains resulted from interactions between two mutations: (1) the Muv phenotypes of these strains were similar to the Muv phenotype of the lin-8(n111); lin-9(n112) strain CB1322, and (2) less than 1/4 of the segregants from the cross progeny of the matings described above had a Muv phenotype. Subsequently, the two mutations -- lin-36(n766) III and lin-15(n767) X -- responsible for the Muv phenotype of one of these strains, MT1643, displayed linkage to unc-32 III and unc-3 X respectively (Table 4-1), confirming this hypothesis. The frequency of isolation of these Muv strains (1.25×10^{-3} per mutagenized F1 chromosome) was similar to the frequencies with which Muv strains were isolated after mutagenesis of lin-8 or lin-9 hermaphrodites (see Results), suggesting that a synthetic Muv mutation was present in the strain MT1312 prior to these reversion experiments. The mutations responsible for the expression of the Muv phenotypes of three of the remaining four Muv isolates also displayed linkage to two linkage groups (Table 4-1). One of the mutations in each of the four Muv strains was linked to LGX, while the other mutation in each strain displayed linkage to one of three chromosomes (Table ISO). Thus, it is likely that the synthetic Muv mutation lin-15(n767) X was present in the strain MT1312 and interacted with the newly derived mutations to generate a Muv phenotype. (The mutations that are responsible for the expression of the Muv phenotype of the remaining strain MT1646, are allelic to the mutations present in MT1643.)

Complementation: Complementation tests between two synthetic mutations, a and b, that mapped to the same linkage group were performed in the background of c, a third synthetic Muv mutation with which both a and b interacted to give a Muv phenotype. Wild-type males or males carrying c were mated with Muv hermaphrodites of genotype c; a. Progeny males of genotypes c; a/+ or c/+; a/+ were mated with hermaphrodites of genotype c; b that also carried a recessive marker, r, used to distinguish self progeny from cross progeny. If any of the cross progeny hermaphrodites were Muv, some of the Muv animals were picked onto separate plates and allowed to self-fertilize. If all the progeny of these Muv hermaphrodites were also Muv, the mutations a and b failed to complement and were considered to be allelic.

Two alleles of lin-36, n747 and n750, were isolated after a mutagenesis of lin-8(n111) hermaphrodites. The other two alleles of lin-36, n766 and n772, were isolated after a mutagenesis of hermaphrodites carrying lin-15(n767). To demonstrate that n747 and n766 were allelic, n766 was separated from lin-15(n767) (see below) and a Muv strain of genotype lin-8(n111); lin-36(n766) unc-32 was constructed. The availability of this Muv strain allowed the complementation test between these two mutations to be performed using the protocol described above.

Construction of strains homozygous for a single synthetic Muv mutation: Three protocols were used to construct strains homozygous for a single synthetic Muv mutation. (1) For most mutations, a

balancer chromosome was used in trans to one of the synthetic Muv mutations. From the progeny of hermaphrodites of genotype b/+; a/r (a is the synthetic Muv mutation to be separated from the synthetic Muv mutation b, and r is a chromosome carrying mutations in one or more genes linked to a), wild-type hermaphrodites were picked. Some of these animals were heterozygous for r, but did not segregate any Muv progeny and were of putative genotype +/+; a +/+ r. Progeny from these animals that did not segregate hermaphrodites of the R phenotype were of putative genotype a. This genotype was confirmed by mating b males with hermaphrodites of putative genotype a and observing the presence of Muv hermaphrodites among the F2 progeny. (dpy-5 unc-13 was used in trans to lin-35(n745); unc-52 was used in trans to lin-38(n751); lon-1 unc-86 dpy-19 was used in trans to lin-36(n766), and unc-3 was used in trans to lin-15(n767)). (2) A strain homozygous for lin-37(n758) was constructed by using a linked marker, lon-1, in cis to lin-37(n758). From hermaphrodites of genotype lin-8(n111)/+; lon-1 lin-37(n758)/+ +, Lon non-Muv progeny were picked. Those hermaphrodites that failed to segregate any Muv progeny in two generations were of putative genotype lon-1 lin-37(n758). The genotype of this strain was confirmed by mating Lon hermaphrodites with lin-8(n111) males and noting that all cross progeny hermaphrodites segregated Lon Muv animals. (3) Strains homozygous for either of two alleles of lin-15, n433 and n744, were constructed by mating wild-type males with Muv hermaphrodites of genotypes lin-8(n111); lin-15(n744) and lin-9(n112); lin-15(n433). The progeny males were mated with Muv hermaphrodites of the parental strain. All non-Muv cross progeny were of genotypes lin-8(n111)/+; lin-15(n744) or

lin-9(n112)/+; lin-15(n433). The progeny of these hermaphrodites that did not segregate any Muv animals were of the desired genotype.

Construction of double mutants containing two synthetic Muv mutations: As described in Results, these synthetic Muv mutations were of two classes, "class-8" and "class-9", after lin-8 and lin-9. A double mutant containing a member of each class was Muv; a double mutant containing two members of the same class was not Muv. To construct a double mutant between a class-8 and a class-9 mutation, males heterozygous or homozygous for one synthetic Muv mutation were mated with hermaphrodites homozygous for the second synthetic Muv mutation and a linked cis marker. The Muv animals that were picked from among the progeny of the F1 hermaphrodites were of the desired genotype. If, as a result of a maternal effect, no Muv hermaphrodites were observed in the F2, hermaphrodites homozygous for the cis marker were picked. Approximately 1/4 of such hermaphrodites segregated all Muv progeny.

Because double mutants between two class-8 mutations or two class-9 mutations are not Muv, strains containing two mutations of the same class were constructed by using linked markers in cis or in trans to identify each of the chromosomes carrying a synthetic Muv mutation. However, at 25° one class-9 mutation lin-15(n744) results in a visible phenotype. At this temperature, lin-15(n744) hermaphrodites are thinner and less fertile than the wild type. The visible phenotype resulting from lin-15(n744) enabled doubly mutant strains containing lin-15(n744) and another class-9 mutation to be constructed without

using a linked marker to identify lin-15(n744) animals.

Construction of class-8 - class-9 double mutants: lin-8(n111);
lin-36(n766): males of genotype lin-8(n111) were mated with
lin-36(n766) unc-32 hermaphrodites. Of 20 Unc non-Muv hermaphrodites
picked in the F2, five produced all Muv progeny and were of genotype
lin-8(n111); lin-36(n766) unc-32. lin-35(n745); lin-38(n751): males
of genotype lin-35(n745) unc-13/+ + were mated with lin-38(n751)
hermaphrodites and Unc Muv hermaphrodites were picked in the F2.
lin-38(n751); lin-36(n766): males of genotype lin-38(n751) were mated
with lin-36(n766) unc-32 hermaphrodites and Unc Muv hermaphrodites
were picked in the F2. lin-38(n751); lin-37(n758): males of genotype
lon-1 lin-37(n758)/+ + were mated with lin-38(n751) hermaphrodites.
Of 24 Lon non-Muv progeny picked in the F2, six produced all Muv
progeny and were of genotype lin-38(n751); lon-1 lin-37(n758).
lin-38(n751); lin-15(n744): males of genotype rol-1 lin-38(n751)
unc-52/+ + + were mated with hermaphrodites of genotype lin-15(n744)
and Rol Muv Unc hermaphrodites were picked in the F2. lin-35(n745);
lin-15(n767): males of genotype lin-15(n767)/0 were mated with
lin-35(n745) unc-13 hermaphrodites and Unc Muv hermaphrodites were
picked in the F2. lin-37(n758); lin-15(n767): males of genotype
lin-15(n767)/0 were mated with lon-1 lin-37(n758) hermaphrodites and
Lon Muv hermaphrodites were picked in the F2. lin-9(n112);
lin-15(n767): males of genotype dpy-17 lin-9(n112)/+ + were mated
with lin-15(n767) hermaphrodites and Dpy Muv hermaphrodites were
picked in the F2.

Construction of class-8 - class-8 double mutants: lin-8(n111)
lin-38(n751): males of genotype rol-1 lin-38(n751) unc-52/+ + +;
lin-9(n112)/+ were mated with hermaphrodites of genotype dpy-10
lin-8(n111). From F1 hermaphrodites of genotype dpy-10 lin-8(n111) +
+ +/+ + rol-1 lin-38(n751) unc-52; lin-9(n112)/+, 39 Dpy
hermaphrodites were picked. Three of these hermaphrodites were of
putative genotype dpy-10 lin-8(n111) + + +/dpy-10 lin-8(n111) rol-1
lin-38(n751) unc-52; lin-9(n112)/+. Progeny hermaphrodites of these
three animals were mated with lin-8(n111) males. Nine of 57 F1
hermaphrodites were of putative genotype dpy-10 lin-8(n111) rol-1
lin-38(n751) unc-52/+ lin-8(n111) + + +. The non-Dpy Rol Unc progeny
were of putative genotype lin-8(n111) rol-1 lin-38(n751) unc-52 and
were not Muv. lin-8(n111); lin-15(n767): males of genotype dpy-10
lin-8(n111)/+ + were mated with lin-15(n767) hermaphrodites. The
progeny males were primarily of two genotypes lin-15(n767)/0 and
dpy-10 lin-8(n111)/+ +; lin-15(n767)/0 and were mated with
lin-15(n767) hermaphrodites. Ten of 48 progeny hermaphrodites
segregated Dpy progeny; on no plates were the Dpy hermaphrodites Muv.
Seven of these Dpy isolates of putative genotype dpy-10 lin-8(n111);
lin-15(n767) were tested by mating the Dpy hermaphrodites with
lin-35(n745) unc-13/+ + males and confirming that the great majority
of Dpy Unc hermaphrodites in the F2 were Muv. Two of the seven
isolates satisfied this criterion and were presumed to be of genotype
dpy-10 lin-8(n111); lin-15(n767). lin-38(n751); lin-15(n767): males
of genotype rol-1 lin-38(n751) unc-52/+ + +; lin-9(n112)/+ were mated
with lin-15(n767) hermaphrodites. The progeny males were mated with
lin-15(n767) hermaphrodites. Two of 40 F1 hermaphrodites segregated

Rol Unc hermaphrodites and were of putative genotype rol-1
lin-38(n751) unc-52/+ +; lin-15(n767). The Rol Unc progeny of these
two animals were of putative genotype rol-1 lin-38(n751) unc-52;
lin-15(n767) and did not have a Muv phenotype.

Construction of class-9 - class-9 double mutants: lin-35(n745);
lin-37(n758): males of genotype lon-1 lin-37(n758)/+ + were mated
with lin-35(n745) unc-13 hermaphrodites. Lon Unc hermaphrodites of
putative genotype lin-35(n745) unc-13; lon-1 lin-37(n758) were picked
in the F2. No Lon Unc hermaphrodites were Muv, nor did they segregate
any Muv progeny. lin-35(n745); lin-36(n766): males of genotype
dpy-19 sma-2/+ + were mated with hermaphrodites of genotype
lin-35(n745) unc-13. Progeny males were mated with hermaphrodites of
genotype lin-36(n766); lin-15(n767). Five of 46 F1 hermaphrodites
picked were of genotype lin-35(n745) unc-13/+ +; + dpy-19 sma-2/
lin-36(n766) + +; lin-15(n767)/+. Of 24 Unc progeny, 11 were of
putative genotype lin-35(n745) unc-13; + dpy-19 sma-2/lin-36(n766) + +
and segregated no Muv progeny. The lin-35(n745) unc-13; lin-36(n766)
strain was established by picking hermaphrodites that segregated no
Dpy Sma animals. lin-35(n745); lin-9(n112): males of genotype
lin-35(n745) unc-13/+ +; lin-8(n111)/+ were mated with hermaphrodites
of genotype dpy-17 lin-9(n112). Of 25 F1 animals, six were of
genotype lin-35(n745) unc-13/+ +; dpy-17 lin-9(n112)/+ +. At 20^o,
almost all Dpy Unc segregants from these F1 parents were not Muv, but
were sterile with a single protrusion at the vulva. lin-35(n745);
lin-15(n744): males of genotype lin-35(n745) unc-13/+ + were mated
with lin-15(n744) hermaphrodites. Twelve of 16 lin-15(n744)

hermaphrodites that were picked in the F2 segregated non-Muv Unc progeny of putative genotype lin-35(n745) unc-13; lin-15(n744). lin-36(n766); lin-15(n744): males of genotype lin-36(n766) unc-32/+ + were mated with hermaphrodites of genotype lin-15(n744). Five of eight lin-15(n744) hermaphrodites that were picked in the F2 segregated non-Muv Unc progeny of putative genotype lin-36(n766) unc-32; lin-15(n744). lin-37(n758); lin-15(n744): males of genotype lon-1 lin-37(n758)/+ + were mated with lin-15(n744) hermaphrodites. Seven of nine lin-15(n744) hermaphrodites that were picked in the F2 segregated non-Muv Lon progeny of putative genotype lon-1 lin-37(n758); lin-15(n744). lin-9(n112); lin-15(n744): males of genotype dpy-17 lin-9(n112)/+ + were mated with lin-15(n744) hermaphrodites. Three of four lin-15(n744) hermaphrodites that were picked in the F2 segregated non-Muv Dpy progeny of putative genotype dpy-17 lin-9(n112); lin-15(n744). Some of the Dpy animals were sterile at 25°.

lin-9 complementation screen: To obtain mutations that failed to complement the synthetic Muv mutation lin-9(n112), lin-8(n111) L4 males 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 (six hermaphrodites and six males) were transferred to a new plate every day and the F1 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. Such hermaphrodites could

carry a mutation that resulted in a dominant Muv phenotype. In addition, as hermaphrodites of genotype lin-8(n111); lin-9(n112)/+; a/+ (where a is a recessive class-9 synthetic Muv mutation in another gene) can sometimes be Muv, such hermaphrodites could also carry a class-9 synthetic mutation in a second gene. Approximately 10,000 cross progeny were examined and five Muv hermaphrodites were isolated. Two of these hermaphrodites subsequently were shown to carry synthetic Muv mutations in other genes, n1137 and n1138. One of these mutations, n1138, is linked to unc-3 X. The other three Muv hermaphrodites carried additional alleles of lin-9. One 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 alleles, n942 and n943, result in sterility and are maintained in balanced strains of genotypes lin-8; lin-9/unc and lin-9/unc (unc-32 was used to balance lin-9(n942), and unc-36 was used to balance lin-9(n943)).

Mapping of lin-15 synthetic alleles with deficiencies: To map lin-15(n744), lin-8(n111) males were mated with hermaphrodites homozygous for one of two deficiencies of the X chromosome, mnDf43 or mnDf4, and heterozygous for mnDp1, i.e. of genotype mnDp1/+; mnDf. The progeny males of genotype lin-8(n111)/+; mnDp1/+; mnDf/0 were mated with hermaphrodites of genotype lin-8(n111); unc-32; lin-15(n744). When the cross was performed with hermaphrodites carrying mnDf4, non-Unc Muv progeny of genotype lin-8(n111); unc-32/+; lin-15(n744)/mnDf4 were observed. Sixteen such animals were picked and gave rise to all Muv progeny. When the cross was performed with

hermaphrodites carrying mnDf43, no non-Unc Muv hermaphrodites were observed among approximately 200 cross progeny. An analogous series of crosses established the map position of lin-15(n767). lin-36(n766) males were mated with hermaphrodites of genotype mnDp1/+; mnDf. The progeny males of genotype lin-36(n766)/+; mnDp1/+; mnDf/0 were mated with hermaphrodites of genotype lin-36(n766) dpy-19; lin-15(n767). When the cross was performed with hermaphrodites carrying mnDf4, non-Dpy Muv progeny of genotype lin-36(n766) dpy-19/lin-36(n766) +; lin-15(n767)/mnDf4 were observed. Fourteen such animals were picked and gave rise to all Muv progeny. When the cross was performed with hermaphrodites carrying mnDf43, no non-Dpy Muv hermaphrodites were observed among approximately 250 cross progeny.

Construction of hermaphrodites of different lin-15 genotypes:

lin-8(n111); lin-15(n309): males of genotype mnDp1/+; unc-3 lin-15(n309)/0 were mated with hermaphrodites of genotype lin-8(n111) dpy-10. From cross progeny of genotype lin-8(n111) dpy-10/+ +; unc-3 lin-15(n309)/+ +, five Dpy Unc Muv hermaphrodites of putative genotype lin-8(n111) dpy-10; unc-3 lin-15(n309) were picked. The genotypes of these hermaphrodites were confirmed by mating lin-15(n765) males with hermaphrodites of all five isolates and placing eight cross progeny from each mating at 15⁰. (At this temperature lin-15(n765) results in a wild-type phenotype, but it interacts with lin-8(n111) to generate a Muv phenotype.) If the majority of Dpy segregants of all F1 cross progeny of a single mating were Muv, an isolate was considered to be homozygous for lin-8(n111). Four of the five isolates were of the desired genotype. lin-8(n111); lin-15(n309)/+; lin-8(n111) males

were mated at 25° with hermaphrodites of genotype dpy-10 lin-8(n111); unc-3 lin-15(n309). Of 154 cross-progeny, 129 were wild-type and 25 had a single ventral protrusion. lin-8(n111); lin-15(n309)/lin-15(n744): lin-8(n111) males were mated with hermaphrodites of genotype lin-8(n111); lin-15(n744). Cross progeny 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) at 25°. All of the 108 cross progeny hermaphrodites were Muv.

lin-8(n111); lin-15(n744)/+: lin-8(n111) males were mated at 25° with hermaphrodites of genotype lin-8(n111); unc-32; lin-15(n744). No Muv hermaphrodites were observed among 164 cross progeny. lin-8(n111); lin-15(n767)/lin-15(n744): lin-8(n111) males were mated with hermaphrodites of genotype lin-8(n111) dpy-10; lin-15(n767). Progeny 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). No Muv hermaphrodites were observed among more than 200 cross progeny. lin-36(n766); lin-15(n309): males of genotype mnDp1/+ unc-3 lin-15(n309)/0 were mated with hermaphrodites of genotype lon-1 lin-36(n766) unc-32. From cross-progeny hermaphrodites of genotype lon-1 lin-36(n766) unc-32/+ + +; unc-3 lin-15(n309)/+ +, Lon Unc-32 hermaphrodites were picked. The Muv segregants of these hermaphrodites were of genotype lon-1 lin-36(n766) unc-32; unc-3 lin-15(n309). lin-36(n766); lin-15(n309)/+: lin-36(n766); him-5 males were mated at 20° with hermaphrodites of genotype lon-1 lin-36(n766) unc-32; unc-3 lin-15(n309). No Muv hermaphrodites were observed among 195 cross progeny. lin-36(n766); lin-15(n309)/lin-15(n767): lin-36(n766); him-5 males were mated with

hermaphrodites of genotype lin-36(n766); lin-15(n767). Progeny 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). All of the 325 cross progeny hermaphrodites were Muv. 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) at 25°. No Muv hermaphrodites were observed among the 105 cross progeny. lin-36(n766); lin-15(n767)/lin-15(n744): males of genotype lin-36(n766); him-5 were mated with hermaphrodites of genotype lin-36(n766); lin-15(n767). Progeny 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). No Muv hermaphrodites were observed among more than 460 cross progeny. lin-15(n767)/lin-15(n309): males of genotype lin-15(n767)/0 were mated at 25° with hermaphrodites of genotype unc-3 lin-15(n309). Of 247 cross progeny, 154 had a Muv phenotype of decreased expressivity. lin-15(n744)/lin-15(n309): males of genotype lin-15(n744)/0 were mated at 20° with hermaphrodites of genotype unc-3 lin-15(n309). No Muv hermaphrodites were observed among the 230 cross progeny.

Construction of double mutants between lin-15(n765) and different synthetic Muv mutations: At 15° lin-15(n765) results in a wild-type phenotype. To determine if at this temperature lin-15(n765) interacts with any synthetic Muv mutations to generate a Muv phenotype, double mutants were constructed between lin-15(n765) and synthetic class-8 and class-9 mutations. lin-15(n765) males were mated with hermaphrodites homozygous for a synthetic Muv mutation and a linked

cis marker. The cross progeny of the mating were grown at 25^o, and F2 Muv hermaphrodites homozygous for lin-15(n765) were picked and shifted to 15^o. The progeny of these animals that were homozygous for the linked cis marker were of the desired genotype. [unc-13 was used in cis to lin-35(n745); dpy-10 was used in cis to lin-8(n111); unc-52 was used in cis to lin-38(n751); unc-32 was used in cis to lin-36(n766); and dpy-17 was used in cis to lin-9(n112).]

Construction of hermaphrodites of genotype lin-13(n387);
lin-15(n767): At 15^o, hermaphrodites of genotype lin-13(n387) that are the progeny of hermaphrodites of genotype lin-13(n387)/+ are phenotypically wild-type. To determine if at 15^o lin-13(n387) can interact with a class-8 mutation to generate a Muv phenotype, a double mutant was constructed between lin-13(n387) and lin-15(n767). unc-32/+ males were mated with lin-15(n767) hermaphrodites. Cross progeny males were mated at 15^o with unc-36 lin-13(n387) hermaphrodites. From F1 progeny of genotype lin-13(n387) unc-36 +/+ + unc-32; lin-15(n767)/+, non-Unc animals were picked. Four of 25 such animals were of putative genotype lin-13(n387) unc-36 +/+ + unc-32; lin-15(n767) as all Unc-36 progeny were both fertile and Muv. The genotype of these hermaphrodites was confirmed by mating fertile Unc-36 hermaphrodites with wild-type males. Progeny males of genotype lin-13(n387) unc-36/+ + were mated with unc-32; lin-15(n767) hermaphrodites. F1 non-Unc hermaphrodites of putative genotype lin-13(n387) unc-36 +/+ + unc-32; lin-15(n767) were placed at 15^o. Fifteen of these F1 hermaphrodites segregated Unc-36 animals; in all cases the Unc-36 hermaphrodites were Muv, confirming that the original

strains were homozygous for lin-15(n767).

Construction of hermaphrodites of genotypes lin-8; lin-34; sup-7 and lin-9; lin-34; sup-7: lin-34(n1046) results in a partially dominant Muv phenotype similar to the Muv phenotype of lin-8; lin-9 hermaphrodites. lin-34(n1046) is also an amber mutation as the Muv phenotype resulting from this mutation is suppressed in double mutant strains of genotypes lin-34; sup-7 or sup-5; lin-34. To determine if the suppression of lin-34 by sup-7 restores wild-type lin-34 gene activity or results in either a class-8 or class-9 synthetic Muv phenotype, the penetrance of the Muv phenotype in hermaphrodites of genotypes lin-8(n111); lin-34(n1046) unc-22; sup-7 dpy-7 and lin-9(n942) unc-32; lin-34(n1046) unc-22; sup-7 dpy-7 were determined.

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. Of 45 F1 hermaphrodites, nine segregated lin-9(n942) unc-32 progeny. One of the nine 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 single hermaphrodite, 55 hermaphrodites were picked. Two of these hermaphrodites were of the desired genotype lin-8(n111); lin-9(n942) unc-32/+ +; lin-34(n1046) unc-22/+ +; sup-7 dpy-7/+ + as all lin-9(n942) unc-32 progeny were Muv. From the progeny of these two animals, 44 hermaphrodites were

picked. Seven of these hermaphrodites were of the desired 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 construct a strain of genotype lin-9(n942) unc-32; unc-34(n1046) unc-22; sup-7 dpy-7, from the progeny of 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 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.

The phenotypes of hermaphrodites of genotypes lin-15(n767)/mnDf4 and lin-15(n765)/mnDf4: At 22.5°, lin-15(n767) results in a wild-type phenotype, but can result in a Muv phenotype in trans to a deficiency of the locus. To determine the penetrance of the Muv phenotype in hermaphrodites of genotype lin-15(n767)/mnDf4 at 22.5°, males of genotype lin-15(n767)/0 were mated with hermaphrodites of genotype mnDp1/+; mnDf4. A Muv hermaphrodite of genotype lin-15(n767)/mnDf4 was picked and transferred to a fresh plate daily. The phenotypes of all of the surviving progeny were determined. As 1/3 of the surviving progeny were of genotype lin-15(n767) and had a wild-type phenotype, the penetrance of the Muv phenotype in the remaining 2/3 of the progeny, which were of genotype lin-15(n767)/mnDf4, could then be estimated. Similarly, at 15°, lin-15(n765) results in a wild-type

phenotype, but can result in a Muv phenotype in trans to a deficiency of the locus. A similar protocol to that described above was used to estimate the penetrance of the Muv phenotype in hermaphrodites of genotype lin-15(n765)/mnDf4.

RESULTS

Identification of additional synthetic Muv strains: The phenotype of the Muv strain CB1322 is synthetic, resulting from an interaction between two mutations lin-8(n111) II and lin-9(n112) III; however hermaphrodites homozygous for either of these two mutations alone have a wild-type phenotype (HORVITZ and SULSTON, 1980; FERGUSON, STERNBERG and HORVITZ, 1985). To identify other mutations that in combination with either lin-8(n111) or lin-9(n112) would generate a Muv phenotype, we mutagenized phenotypically wild-type hermaphrodites homozygous for either lin-8(n111) or lin-9(n112). We isolated twelve Muv strains from the mutagenesis of lin-8(n111) hermaphrodites. Similarly, we isolated six Muv strains from the mutagenesis of lin-9(n112) hermaphrodites. To determine if the Muv phenotype of each of these strains required the presence of lin-8(n111) or lin-9(n112), the mutation(s) responsible for the Muv phenotype was (were) tested for linkage to a standard marker on each chromosome (Table 4-1). The mutations responsible for the Muv phenotypes of 12 of these strains displayed linkage to two linkage groups (one of which was the linkage group of the synthetic mutation present in the parental strain). Since one of the mutations responsible for the Muv phenotype of a thirteenth strain, MT1627, which was isolated after mutagenesis of lin-9(n112) hermaphrodites, displayed linkage to LGIII, it is possible that that the Muv phenotype of this strain results from an interaction between lin-9 and a newly identified synthetic mutation.

Table 4-1 To determine if the phenotypes of the Muv strains isolated after mutagenesis of hermaphrodites homozygous for either lin-8(n111), lin-9(n112) or lin-15(n767) were dependent on two mutations (the mutation present in the parental strain and a newly isolated mutation), the mutation(s) that were responsible for the Muv phenotypes of each of these strains was (were) mapped to one or more of the six linkage groups. Males heterozygous for the Muv mutation(s) were mated with hermaphrodites of genotypes dpy-5 I; bli-2 II; unc-32 III and unc-5 IV; dpy-11 V; lon-2 X (TRENT, TSUNG and HORVITZ, 1983). Muv animals were picked in the F2 and the frequency of segregation of each of the markers was noted. Mutations in two genes -- lin-38 II and lin-15 X -- did not display linkage to any of the above six markers. Using the protocol described above, mutations in lin-15 were mapped to LGX by establishing linkage to unc-3 X. Similarly, mutations in lin-38 were mapped to LGII by establishing linkage to C1 dpy-10 unc-52.

Table 4-1

Synthetic Muv strains: origin and chromosomal linkage

Origin	Strain	Genotype	Chromosomal linkage	
			Parental mutation	Second mutation
	CB1322	<u>lin-8(n111) II; lin-9(n112) III</u>		
mutagenesis of <u>lin-8(n111)</u>	MT1624	<u>lin-35(n745) I; lin-8(n111) II</u>	2/12 <u>bli-2 II/+</u>	0/12 <u>dpy-5 I/+</u>
	MT663	<u>lin-35(n373) I; lin-8(n111) II</u>	0/7 <u>bli-2 II/+</u>	1/7 <u>dpy-5 I/+</u>
	MT1635	<u>lin-8(n111) II; lin-37(n758) III</u>	1/10 <u>bli-2 II/+</u>	0/10 <u>unc-32 III/+</u>
	MT1629	<u>lin-8(n111) II; lin-36(n750) III</u>	1/12 <u>bli-2 II/+</u>	0/14 <u>unc-32 III/+</u>
	MT1626	<u>lin-8(n111) II; lin-36(n747) III</u>	5/11 <u>bli-2 II/+</u>	0/11 <u>unc-32 III/+</u>
	MT1623	<u>lin-8(n111) II; lin-15(n744) X</u>	1/10 <u>bli-2 II/+</u>	0/19 <u>unc-3 X/+</u>
	MT664	<u>lin-8(n111) II; lin-15(n374) X</u>	4/22 <u>bli-2 II/+</u>	5/17 <u>lon-2 X/+</u>
	MT1622	<u>lin-8(n111) II; lin-15(n743) X</u>	1/11 <u>bli-2 II/+</u>	0/13 <u>unc-3 X/+</u>
mutagenesis of <u>lin-15(n767)</u>	MT1643	<u>lin-36(n766) III; lin-15(n767) X</u>	1/35 <u>unc-3 X/+</u>	1/14 <u>unc-32 III/+</u>
	MT1646	<u>lin-36(n772) III; lin-15(n767) X</u>		
	MT1648	<u>lin(n770) II; lin-15(n767) X</u>	2/22 <u>unc-3 X/+</u>	0/7 <u>C1 II/+</u>
	MT1645	<u>lin(n771) III; lin-15(n767) X</u>	3/16 <u>lon-2 X/+</u>	0/13 <u>unc-32 III/+</u>
	MT1753	<u>lin(n833) I; lin-15(n767) X</u>	0/8 <u>unc-3 X/+</u>	2/10 <u>dpy-5 I/+</u>
mutagenesis of <u>lin-9(n112)</u>	MT1630	<u>lin-38(n751) II; lin-9(n112) III</u>	0/15 <u>unc-32 III/+</u>	0/11 <u>C1 II/+</u>
	MT1638	<u>lin-38(n761) II; lin-9(n112) III</u>	0/24 <u>unc-32 III/+</u>	0/11 <u>C1 II/+</u>
	MT990	<u>lin-9(n112) III; lin-15(n433) X</u>	0/14 <u>unc-32 III/+</u>	6/17 <u>lon-2 X/+</u>
	MT1628	<u>lin-9(n112) III; lin-15(n749) X</u>	0/10 <u>unc-32 III/+</u>	7/17 <u>lon-2 X/+</u>
	MT1627	<u>lin-9(n112) III; lin(n748)</u>	0/10 <u>unc-32 III/+</u>	

However, the new mutation in the strain, lin(n748), did not display linkage to any of the markers used for mapping, and the strain has not been characterized further. Thus, 12 (or possibly 13) of these 18 Muv strains carried new mutations that interacted with either lin-8 or lin-9 to generate a Muv phenotype.

Four of the other five strains carried new alleles of genes already represented by mutations that result in a Muv phenotype. 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. The mutation responsible for the Muv phenotype of the fifth strain, MT665, which was isolated after mutagenesis of lin-8(n111) hermaphrodites, did not display linkage to LGII but displayed linkage to unc-3 X.

As described in Materials and Methods, five additional Muv strains were isolated during the mutagenesis of a strain carrying the synthetic Muv mutation lin-15(n767). The Muv phenotypes of these strains required mutations linked both to LGX and to another chromosome (Table 4-1).

Complementation and mapping: If two synthetic mutations mapped to the same linkage group, a complementation test was performed to determine if the two mutations were alleles of the same gene (see Materials and Methods). Two synthetic Muv mutations, a and b, were considered to be allelic if, and only if, hermaphrodites of genotype a/b; c were Muv and produced all Muv progeny (c is a third synthetic

Muv mutation with which both a and b interact to give a Muv phenotype). It was necessary to examine the phenotypes of the progeny of hermaphrodites of genotype a/b; c for two reasons. (1) Hermaphrodites heterozygous for two synthetic Muv mutations in different genes (i.e., of genotype a +/+ b; c) could have a Muv phenotype (e.g., when males of genotype lin-8(n111); lin-9(n112); him-5 were mated with hermaphrodites of genotype dpv-5; lin-8(n111); lin-37(n758), two of the 23 cross progeny of genotype dpv-5/+; lin-8(n111); lin-37(n758) +/+ lin-9(n112) had a Muv phenotype of reduced expressivity). However, all such Muv hermaphrodites segregated many wild-type progeny. (2) Some synthetic Muv mutations resulted in a partially dominant phenotype (e.g., when males of genotype lin-15(n767)/0 were mated with hermaphrodites of genotype lin-36(n766) dpv-19; lin-15(n767), 12% (n=237) of the cross-progeny hermaphrodites of genotype lin-36(n766) dpv-19/+ +; lin-15(n767) had a Muv phenotype of reduced expressivity).

As described below, six synthetic Muv mutations were shown to be alleles of lin-15, a gene previously defined by five Muv mutations. The other nine synthetic Muv mutations defined four additional genes involved in vulval development -- lin-35, lin-36, lin-37, lin-38. Three-factor crosses (Table 4-2) were performed to position these four genes on their respective linkage groups (Figures 4-1 and 4-2).

General characteristics of the synthetic Muv strains: The synthetic Muv strains share three phenotypic and genotypic characteristics. (1) The expressivity of the Muv phenotype in every

Table 4-2 Three- and four-factor crosses were performed essentially as described by BRENNER (1974). Because the synthetic Muv mutations result in a wild-type phenotype, these mutations were scored in the presence of a second synthetic Muv mutation. From heterozygotes of genotype a + b/+ c +, recombinant A non-B and B non-A hermaphrodites were picked. The progeny of each recombinant hermaphrodite were examined for the expression of the trans marker. In one cross, wild-type hermaphrodites were picked and scored for the segregation of AB and C progeny.

Table 4-2

Three- and four-factor crosses

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

Figure 4-1. Partial genetic map of C. elegans indicating the markers used in this study. Genes with synthetic Muv mutations are drawn above the lines representing the C. elegans linkage groups. The extents of duplications (Dps) and deficiencies (Dfs) are indicated below the line.

Figure 4-1

Figure 4-2. Expanded genetic map showing the region of LGIII extending from lon-1 through lin-12. The precise map positions of unc-86, sup-5 and sma-2 were determined by M. FINNEY (personal communication).

Figure 4-2

strain is variable and, in some strains, the penetrance of the Muv phenotype is incomplete. The penetrance of the Muv phenotype of a strain and the expressivity of the Muv phenotype in individual hermaphrodites of the strain are correlated (i.e., the expressivity of the Muv phenotype in individual hermaphrodites of an incompletely penetrant Muv strain is reduced compared to the expressivity of the Muv phenotype in individual hermaphrodites of a completely penetrant Muv strain). In incompletely penetrant Muv strains, some Muv hermaphrodites have a single small protrusion anterior or posterior to a normal vulva; other Muv hermaphrodites have two large protrusions, one anterior and one posterior to a normal vulva. In completely penetrant Muv strains, some Muv hermaphrodites have a functional vulva and two large protrusions; other Muv hermaphrodites lack a functional vulva and have between three to five irregularly sized ventral protrusions. The most severely affected Muv hermaphrodites are usually egg-laying defective and many become "bags of worms", in which the progeny larvae hatch inside the parental cuticle. (2) The phenotypes of many of the synthetic Muv strains are heat-sensitive, as at high temperatures the penetrance and/or expressivity of the Muv phenotype increases. Additionally, if some of the Muv strains are grown at 25^o, many hermaphrodites become thinner and less fertile than the wild type. It is likely that the heat-sensitive phenotypes of these strains reflect a temperature-sensitive process revealed or induced by these mutations. [Other C. elegans temperature-sensitive mutants of this type have been identified (HORVITZ and SULSTON, 1980; GOLDEN and RIDDLE, 1984; W. FIXSEN, personal communication).] (3) Many of the synthetic mutations responsible for the Muv phenotypes of

these strains display maternal effects; i.e., the penetrance and expressivity of the Muv phenotype in hermaphrodites of genotype a; b that are the progeny of hermaphrodites of genotype a/+; b/+ is often reduced compared to the penetrance and expressivity of the Muv phenotype in the strain of genotype a; b.

The phenotypes of hermaphrodites homozygous for a single synthetic Muv mutation: Hermaphrodites homozygous for a synthetic Muv mutation in each of the genes -- lin-15, lin-35, lin-36, lin-37 and lin-38 -- had a wild-type phenotype at 20°. However, at 25° hermaphrodites of genotypes lin-9(n112), lin-15(n744), lin-35(n745) and lin-37(n758) could display any of a number of phenotypic abnormalities, including a thinner body shape than the wild type, reduced fertility compared to the wild type, and a defect in their ability to lay eggs. However, even at 25° no hermaphrodites of any of these genotypes were Muv.

Interactions between synthetic Muv alleles: To determine the pattern of interactions among the synthetic Muv mutations, we constructed a series of double mutant strains, each carrying the canonical synthetic Muv allele of two genes (see Materials and Methods). The phenotypes of these double mutant strains (Table 4-3) indicate that the synthetic Muv mutations are of two classes. A double mutant strain carrying a mutation of each class was Muv, while a double mutant strain carrying two mutations of the same class was not Muv. We have named these "class-8" after lin-8(n111) and "class-9" after lin-9(n112). With the exception of the synthetic mutations in lin-15 (see below), the pattern of interactions among

Table 4-3 The protocols used in the construction of the double mutants are described in Materials and Methods. On the basis of the phenotypes of the double mutant strains, these mutations are of two classes, class-8, after lin-8(n111), and class-9, after lin-9(n112). M, Multivulva; nM, not Multivulva; st, sterile; ND, not determined.

Table 4-3

Phenotypes of double mutants carrying two synthetic mutations

		Class 8		Class 9				
		<u>lin-38</u> (n751)	<u>lin-15</u> (n767)	<u>lin-9</u> (n112)	<u>lin-35</u> (n745)	<u>lin-36</u> (n766)	<u>lin-37</u> (n758)	<u>lin-15</u> (n744)
Class 8	<u>lin-8(n111)</u>	nM	nM	M	M	M	M	M
	<u>lin-38(n751)</u>		nM	M	M	M	M	M
	<u>lin-15(n767)</u>			M	M	M	M	ND
Class 9	<u>lin-9(n112)</u>			st nM	ND	ND		nM
	<u>lin-35(n745)</u>					nM	nM	nM
	<u>lin-36(n766)</u>						ND	nM
	<u>lin-37(n758)</u>							nM

this set of mutations was gene specific, allele non-specific. Table 4-4 contains a listing, by class of mutation, of the alleles of each of these genes.

lin-15 is a complex locus with three classes of alleles: Six synthetic Muv mutations displayed linkage to LGX. Complementation tests established that the three X-linked class-9 mutations that were isolated after mutagenesis of lin-8(n111) hermaphrodites, n744, n374 and n743, were allelic. Similarly, complementation tests established that n767, the synthetic class-8 Muv mutation present in MT1312, was allelic with two X-linked class-8 synthetic mutations, n433 and n749, that were 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 further map n767 and n744. As described in Materials and Methods, mnDf4, but not mnDf43, failed to complement both mutations. Thus, n767 and n744 both mapped to the same region of LGX as lin-15, a gene previously defined by mutations that result in a Muv phenotype.

To demonstrate that these synthetic mutations were alleles of lin-15, complementation tests were performed involving these three classes of mutations (Table 4-5). Each of the three classes of mutations was first shown to result in a recessive phenotype; hermaphrodites of genotypes lin-8(n111); lin-15/+ and lin-36(n766); lin-15/+ had wild-type phenotypes (where "lin-15" refers to any of the three classes of lin-15 alleles). lin-15(n309), which confers a Muv

Table 4-4 The synthetic Muv mutations result in wild-type phenotypes and are of two classes, class-8 and class-9. See Results for details of the experiments concerning lin-13(n387) and lin-34(n1046). ts, temperature-sensitive.

phenotype, failed to complement both classes of synthetic alleles; hermaphrodites of genotypes lin-8(n111); lin-15(n744)/lin-15(n309) and lin-36(n766); lin-15(n767)/lin-15(n309) were Muv, indicating that these two synthetic mutations were alleles of lin-15. However, lin-15(n744) complemented lin-15(n767), as hermaphrodites of genotypes lin-8(n111); lin-15(n767)/lin-15(n744) and lin-36(n766); lin-15(n767)/lin-15(n744) were phenotypically wild-type (Table 4-5).

These experiments indicate that lin-15 is a complex locus with two independently mutable activities, a class-8 activity and a class-9 activity. Mutations in the lin-15 locus can alter each activity independently, or a single mutation can coordinately affect both activities. Mutations affecting one lin-15 activity totally complement mutations affecting the other lin-15 activity.

One of the Muv alleles of lin-15, n765, results in a heat-sensitive Muv phenotype. At 15°, more than 95% of lin-15(n765) hermaphrodites are phenotypically wild-type, while at 25° all lin-15(n765) hermaphrodites are Muv. To determine if at the permissive temperature of 15°, lin-15(n765) results in wild-type lin-15 activity or in a synthetic phenotype of either class, a series of double mutants, detailed in Materials and Methods, were constructed between lin-15(n765) and class-8 and class-9 synthetic mutations. At 15°, lin-15(n765) interacted with the class-8 alleles -- lin-8(n111) and lin-38(n751) -- to generate a Muv phenotype, but did not interact with any of the class-9 alleles tested -- lin-9(n112), lin-35(n745) and lin-36(n766) --- to generate a Muv phenotype. Thus at 15°,

Table 4-5 8, the class-8 synthetic mutation lin-8(n111); 9, the class-9 synthetic mutation lin-36(n766); lin-15(Muv), lin-15(n309); lin-15(8), lin-15(n767); lin-15(9), lin-15(n744). Animals of the different genotypes were obtained as described in Materials and Methods. WT, wild-type.

Table 4-5

Interactions among *lin-15* alleles

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

Genotype	Phenotype	Genotype	Phenotype
<u>8; <i>lin-15</i>(9)/+</u>	WT	<u>9; <i>lin-15</i>(8)/+</u>	WT
<u>8; <i>lin-15</i>(Muv)/+</u>	WT	<u>9; <i>lin-15</i>(Muv)/+</u>	WT
<u>8; <i>lin-15</i>(9)/<i>lin-15</i>(9)</u>	Muv	<u>9; <i>lin-15</i>(8)/<i>lin-15</i>(8)</u>	Muv
<u>8; <i>lin-15</i>(9)/<i>lin-15</i>(Muv)</u>	Muv	<u>9; <i>lin-15</i>(8)/<i>lin-15</i>(Muv)</u>	Muv
<u>9; <i>lin-15</i>(9)/<i>lin-15</i>(8)</u>	WT	<u>9; <i>lin-15</i>(8)/<i>lin-15</i>(9)</u>	WT

lin-15(n765) does not restore wild-type lin-15 activity but rather results in a class-9 synthetic Muv phenotype.

lin-13 and lin-34 have both class-8 and class-9 activities: Hermaphrodites carrying either lin-13(n387) or lin-34(n1046) have a Muv phenotype similar to the Muv phenotypes of many of the synthetic Muv strains. However, under certain conditions, both lin-13(n387) and lin-34(n1046) can result in wild-type phenotypes (FERGUSON and HORVITZ, 1985). As demonstrated below, under these conditions both mutations interacted with a class-8 synthetic Muv mutation to generate a Muv phenotype, indicating that the wild-type activities of these two genes are not restored. Thus, it is likely that lin-13 and lin-34 have both class-8 and class-9 activities.

Mutations in lin-13 are heat-sensitive and display a heat-sensitive maternal effect (FERGUSON and HORVITZ, 1985). At 25°, lin-13 hermaphrodites are sterile and Muv; however at 15°, lin-13 hermaphrodites from a heterozygous parent are phenotypically wild-type, but their progeny are sterile and not Muv. Because, as described below, the activity of at least one class-9 genes is required for fertility, it was possible that the sterility of lin-13(n387) hermaphrodites could result from the elimination of the activity of a class-9 gene. To test the hypothesis, we constructed a double mutant (described in Materials and Methods) between lin-15(n767), a class-8 synthetic lin-15 allele, and lin-13(n387). At 15°, 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 both fertile and Muv. Thus, because lin-13(n387) results in a class-9 synthetic phenotype at 15°, it is likely that the Muv phenotype of lin-13(n387) hermaphrodites at 25° results from a combination of a class-9 synthetic phenotype and a heat-sensitive class-8 synthetic phenotype.

The single allele of lin-34, n1046, results in a partially dominant incompletely penetrant Muv phenotype. lin-34(n1046) is an amber allele (FERGUSON and HORVITZ, 1985); the penetrance of the Muv phenotype of lin-34 is reduced in strains of genotypes sup-5 III; lin-34 and lin-34; sup-7 X. [sup-5 III and sup-7 X are amber suppressor t-RNA mutations (WATERSTON and BRENNER, 1978; WATERSTON, 1981; WILLS *et al.*, 1983).] Specifically, the penetrance of the Muv phenotype of hermaphrodites of genotype lin-34(n1046); sup-7 is reduced from 56% to 13% (FERGUSON and HORVITZ, 1985). To determine if the action of sup-7 restores wild-type lin-34 gene activity, or whether the action of sup-7 on lin-34(n1046) results in the generation of either a class-8 or class-9 synthetic Muv phenotype, hermaphrodites of genotypes lin-8(n111); lin-34(n1046) unc-22; sup-7 dpy-7 and lin-9(n942) unc-32; lin-34(n1046) unc-22; sup-7 dpy-7 were obtained as described in Materials and Methods. As the penetrance of the Muv phenotype in hermaphrodites of the former genotype (59%, n=224), but not in hermaphrodites of the latter genotype (16%, n=25), was greater than the penetrance of the Muv phenotype in lin-34; sup-7 hermaphrodites, it is likely that the activity of sup-7 does not completely restore wild-type lin-34 gene activity but rather generates a class-9 synthetic Muv phenotype.

lin-9(n942) probably results in a greater decrease in lin-9 activity than does lin-9(n112); at 15[°], lin-9(n112), but not lin-9(n942), results in a maternal effect. Specifically, from parental hermaphrodites of genotype lin-8(n111); dpy-17 lin-9(n112)/+, only 2% (n=150) of the progeny hermaphrodites of genotype lin-8(n111); dpy-17 lin-9(n112) were Muv. lin-9(n942) greatly reduces or eliminates this maternal effect, as from parental hermaphrodites of genotype lin-8(n111); + lin-9(n942) unc-32/sma-2 + +, 72% (n=109) of the segregants of genotype lin-8(n111); lin-9(n942) unc-32 were Muv. These observations suggest that lin-9(n942) results in a smaller maternal dowry of lin-9 activity than does lin-9(n112), and thus probably reduces lin-9 activity to a greater extent than does lin-9(n112).

Two observations suggest, albeit indirectly, that the activities of two other genes identified by class-9 mutations may also be necessary for fertility. (1) At 25[°], hermaphrodites carrying lin-15(n744), a class-9 allele of lin-15, grow much more slowly and are thinner than the wild type. Additionally, at 25[°] a few lin-15(n744) hermaphrodites are sterile. Thus, it is likely that at least the class-9 activity of lin-15 is necessary for fertility. (2) Although lin-35(n745) hermaphrodites are fertile at 20[°], hermaphrodites of genotype lin-35(n745); lin-9(n112) are sterile. As the phenotype of the double mutant is similar to the phenotype resulting from lack of lin-9 gene activity, it is possible that lin-35 activity may also be necessary for fertility.

The severity of the Muv phenotype resulting from certain lin-15 alleles is correlated with the dose of the mutant lin-15 gene: Some alleles of lin-15 result in an incompletely penetrant Muv phenotype. In an effort to determine the sensitivity of the Muv phenotype to a change in the amount of lin-15 gene product, we varied the dose of the mutant lin-15 gene. The data from these experiments suggest, and we demonstrate, that the severity of the Muv phenotypes resulting from lin-15 alleles can be used as a genetic assay to measure the level of expression of X-linked genes.

The penetrance of the Muv phenotypes resulting from two alleles of lin-15 -- n767, a class-8 synthetic allele, and n765, a heat-sensitive Muv allele -- are increased when these mutations are in trans to a deficiency of the lin-15 locus. Specifically, at 15⁰, 6% (n=127) of lin-15(n765) hermaphrodites are Muv, while 52% (n=202) of hermaphrodites of genotype lin-15(n765)/mnDf4 are Muv. Similarly, at 22.5⁰, all lin-15(n767) hermaphrodites are wild-type (n=367), while 58% (n=165) of hermaphrodites of genotype lin-15(n767)/mnDf4 are Muv. Conversely, the penetrance of the Muv phenotype resulting from an interaction between lin-15(n767) and an autosomal synthetic mutation is decreased in hermaphrodites that carry three copies of the mutant lin-15 gene, i.e., in triplo-X hermaphrodites. Specifically, at 20⁰, the penetrance of the Muv phenotype in hermaphrodites of genotype lin(n833); him-5; lin-15(n767) is 96% (n=213), while the penetrance of the Muv phenotype of hermaphrodites of genotype lin(n833); him-5; lin-15(n767)/lin-15(n767)/lin-15(n767) is 53% (n=32). [him-5 hermaphrodites produce both male and triplo-X self-progeny, which have

a slightly Dpy phenotype, as a result of meiotic nondisjunction (HODGKIN, HORVITZ and BRENNER, 1970).]

The quantifiable sensitivity of the Muv phenotype to at most a two-fold change in the dose of a mutant lin-15 gene suggests that the severity of the Muv phenotype resulting from mutations in lin-15 can be used as a genetic assay for the level of X-chromosome expression. In C. elegans, the level of X chromosome gene expression is coordinately regulated to equalize the level of transcription of X-linked genes between males, which have a single X chromosome, and hermaphrodites, which have two X chromosomes. Mutations in genes involved in this process, known as dosage compensation, are likely to result in the alteration of the level of transcription of X-linked genes in one or both of the sexes. One gene that is likely to be involved in dosage compensation is dpy-21 V (HODGKIN, 1984; MENEELY and WOOD, 1984), as mutations in this gene result in a hermaphrodite-specific Dpy phenotype, similar to that seen in triplo-X hermaphrodites. In addition, triplo-X dpy-21 hermaphrodites are dead, suggesting that the level of transcription of X-linked genes in a dpy-21 hermaphrodite may be elevated. To determine if mutations in dpy-21 could reduce the penetrance of the Muv phenotype in hermaphrodites of genotype lin(n833); lin-15(n767), a strain of genotype lin(n833); dpy-21 him-5; lin-15(n767) was constructed. At 20°, 32% (n=242) of the hermaphrodites of this genotype were Muv, similar to the penetrance of the Muv phenotype in hermaphrodites of genotype lin(n833); lin-15(n767)/lin-15(n767)/ lin-15(n767), and less than the penetrance of the Muv phenotype in hermaphrodites of genotype

lin(n833); lin-15(n767), supporting the involvement of dpy-21 in the process of dosage compensation. Subsequently, mutations in dpy-21 have been shown to suppress the phenotypes resulting from other lin-15 alleles (P. MENEELY, personal communication) as well as the phenotypes resulting from reduction-of-function mutations in other X-linked genes (P. MENEELY and L. DE LONG, personal communication). Recently, B. MEYER and W. CHAMPNESS (personal communication) have obtained biochemical evidence that relative to the level of transcription of autosomal genes, dpy-21 hermaphrodites have a higher level of transcription of X-linked genes than do wild-type hermaphrodites.

DISCUSSION

The Multivulva (Muv) phenotype of CB1322, one of the mutants of the nematode C. elegans altered in the cell lineages of the vulva, results from an interaction between two unlinked mutations, lin-8(n111) II and lin-9(n112) III, each of which results in a wild-type phenotype (HORVITZ and SULSTON, 1980; FERGUSON, STERNBERG and HORVITZ, 1985). We have identified 12 additional synthetic Muv mutations that can interact with either lin-8(n111) or lin-9(n112) to generate a Muv phenotype. Seven of these synthetic Muv mutations define four additional genes involved in vulva development -- lin-35, lin-36, lin-37 and lin-38. The remaining five synthetic Muv mutations were shown to be alleles of lin-15, a gene previously defined by Muv mutations. Five additional synthetic Muv strains were isolated during a reversion of the highly penetrant Vul mutation in the strain MT1312. One of the mutations in each of these five strains displayed linkage to unc-3 X, suggesting that this mutation, lin-15(n767), was present in the strain MT1312 prior to the reversion experiments. Like lin-8 or lin-9 hermaphrodites, hermaphrodites carrying a canonical synthetic Muv allele of each of the genes -- lin-15, lin-35, lin-36, lin-37 and lin-38 -- had a wild-type phenotype.

To understand how these genes function in the development of the vulva, it is first necessary to ascertain the number of genes that could mutate to result in a synthetic Muv phenotype, to determine the cause of the synthetic phenotypes of these strains, and to

characterize the effects of these mutations on the activities of their respective genes. Although we have not identified all the genes that can mutate to a synthetic Muv phenotype, it is possible that we have identified many of them. Four of the six genes defined by the synthetic mutations are represented by more than one allele, and one, lin-15, is represented by six alleles. In addition, if the activity of each member of this set of genes can be reduced or eliminated by EMS at a frequency comparable to the standard frequency of elimination of gene activity, 5×10^{-4} per mutagenized F1 chromosome (BRENNER, 1974; GREENWALD and HORVITZ, 1980), the frequencies with which we isolated synthetic mutations that interacted with either lin-8(n111), 1.4×10^{-3} per mutagenized F1 chromosome, or lin-9(n112), 1.7×10^{-4} per mutagenized F1 chromosome, to generate a Muv phenotype suggests that there may not be a large number of genes, possibly less than ten, that are able to mutate to interact with either lin-8(n111) or lin-9(n112) to generate a Muv phenotype.

The synthetic nature of the Muv phenotype of these strains could result from any of three classes of interactions among these mutations. (1) If these genes encode products with a single function (i.e., if these genes are members of a gene family), the Muv phenotypes of these strains could result from the cumulative reduction in the activity of the product below a certain threshold. Such a reduction could necessitate the reduction or elimination of the activities of two or more of the members of the gene family, resulting in a synthetic phenotype. (2) The Muv phenotypes of these strains could result from specific interactions between two mutant gene

products. (3) The Muv phenotype of these strains could result from the necessity of alterations in the activity of genes involved in two independent processes. Each of these three models predicts that the synthetic Muv mutations would interact in a different but specific pattern. When we constructed double mutants carrying two synthetic Muv mutations in different genes, we observed that the synthetic mutations were of two classes: a double mutant homozygous for a synthetic mutation of each class was Muv, while a double mutant homozygous for two synthetic mutations of the same class was not Muv. The two classes of mutations were named "class-8" after lin-8(n111) II and "class-9" after lin-9(n112) III.

The patterns of interaction among these mutations are inconsistent with those predicted by the first two models, but are consistent with the pattern of interaction predicted by the third model. Specifically, it is not likely that the Muv phenotype of these strains results from the cumulative reduction of a single activity below a threshold. One specific prediction of such a model is that if any two strains of genotypes a; b and c; d are Muv (where a, b, c, and d are synthetic Muv mutations in differing genes), then at least one of the strains of genotypes a; c or b; d must also have a Muv phenotype. However, the interactions among the synthetic mutations is not consistent with this prediction. If a and c are any two class-8 mutations and b and d are any two class 9 mutations, double mutants of genotypes a; b and c; d are Muv, while neither of the double mutants of genotypes a; c or b; d has a Muv phenotype.

For three reasons, it is also not likely that the Muv phenotypes of these strains result from a specific interaction between two mutant gene products. (1) We obtained many of these synthetic mutations after mutagenesis of hermaphrodites of genotypes lin-8(n111) or lin-9(n112). If the newly identified mutations resulted in specific alterations to their respective gene products, the frequency of isolation of these mutations should be lower than the frequency of isolation of mutations that disrupt gene activity. However, the frequencies with which we obtained these mutations were similar to the frequency of isolation of mutations that eliminate gene function. Thus, it is likely that the newly-identified synthetic mutations do not result in specific alterations to the structure of their respective gene products. (2) These mutations displayed a gene-specific, allele non-specific pattern of interaction; any class-8 and any class-9 mutation interacted to generate a Muv phenotype. (3) Mutations that most likely eliminated the activity of lin-9 interacted with lin-8(n111) to generate a Muv phenotype, suggesting that the loss of gene activity does not prohibit the generation of a synthetic Muv phenotype.

The patterns of interactions among the synthetic Muv mutations suggest that the Muv phenotype of these strains results from the necessity of alterations in the functions of two independent pathways and that an alteration in the function of either pathway is not sufficient to result in a Muv phenotype. The seven genes defined by these synthetic mutations are of three classes: two genes -- lin-8 and lin-38 -- are represented by class-8 alleles; four genes -- lin-9,

lin-35, lin-36 and lin-37 -- are represented by class-9 alleles, and one gene -- lin-15 -- is represented by Muv mutations and by synthetic mutations of both classes. It is likely that the activity of lin-15 is required in both pathways, since both class-8 and class-9 alleles of lin-15 have been identified. However, the activity of each of the remaining six genes could be specific to one pathway, or each of the genes could be active in both pathways. To determine the wild-type activity of each of these genes, it is necessary to determine the phenotype that results from the elimination of gene activity. We have isolated mutations that probably eliminate the activity of lin-9. Because these mutations do not result in a Muv phenotype, but rather interact with the class-8 synthetic mutation lin-8(n111) to generate a Muv phenotype, this suggests that the action of lin-9 is specific to one of the two pathways defined by these genes.

As lin-15 has Muv alleles and synthetic alleles of both classes, the lin-15 locus could encode a single protein with two separately mutable functions, or the lin-15 locus could comprise two separate closely-linked genes, each of which functions in one of the two pathways. To ascertain if lin-15 encodes one or two polypeptide products, and ultimately to determine lin-15 gene function, it may be necessary to isolate and characterize the DNA of the lin-15 locus. Recently, a transposable element, Tc1, has been identified in C. elegans (EMMONS et al., 1983; LIAO, ROSENZWEIG and HIRSH, 1983; EIDE and ANDERSON, 1985). The Bristol strain of C. elegans has about 30 copies of this element, while a related strain, C. elegans var. Bergerac, has about 600 copies of Tc1. In a Bergerac background Tc1

has been shown to transpose into additional chromosomal locations with low frequency. Such transpositions can eliminate gene function and result in a visible phenotype. If however, the gene of interest has a recessive lethal phenotype, as lin-15 does (FERGUSON and HORVITZ, 1985), the majority of transpositions of Tc1 into such a gene may not be able to be identified, as the transpositional event would eliminate gene activity and result in recessive lethality. However, for lin-15, it is possible to construct a strain that allows the identification and subsequent recovery of such a transpositional event. Specifically, while at 22.5° hermaphrodites carrying a class-8 allele of lin-15, n767, are wild-type, hermaphrodites of genotype lin-15(n767)/mnDf4 are fertile and can be Muv. Thus, in a lin-15(n767) background, a transposition of Tc1 into lin-15 would result in the generation of a dominant a Muv phenotype, allowing the recovery and subsequent analysis of the transpositional event. (Such transpositional events may also allow identification and characterization of the DNA of other genes with class-9 alleles, as at 25° class-9 synthetic mutations can be haplo-insufficient; i.e., hermaphrodites of genotypes muv-9/+; lin-15(n767) can have a Muv phenotype (E. FERGUSON, unpublished observations).)

Because the two other genes -- lin-13 and lin-34 -- that can mutate to a similar Muv phenotype also have class-8 and class-9 activities, it is likely that this set of genes is involved in a common process (or processes) in the determination of the fates of the vulval precursor cells. The wild-type function (or functions) of these genes could be elucidated by observing the interactions between

the synthetic Muv mutations and mutations in other genes involved in the determination of vulval precursor cell fate. Additionally, if the two classes of synthetic Muv mutations affect separate processes, each of the two classes of synthetic Muv mutations could interact differently with mutations in other genes. As described below, we have obtained preliminary evidence that class-8 and class-9 mutations differentially affect the phenotypes resulting from mutations in two genes -- lin-3 and lin-12 -- that are involved in the determination of the fates of the vulval precursor cells.

To describe these interactions, we first present a brief summary of the effects of these mutations upon the cell lineages of the vulva. In the wild type, the reception of a graded signal from the anchor cell causes the vulval precursor cells, P(3-8).p, to adopt one of three fates dependent upon their position. The anchor cell-proximal cells P(5-7).p are induced and undergo vulval lineages; P6.p expresses a 1^o fate, and P5.p and P7.p express 2^o fates. The anchor cell-distal cells P(3,4,8).p are not induced and do not generate vulval cells; these three cells express 3^o fates. The expression of each of the three vulval fates results in the generation of distinct numbers and types of progeny.

The activity of lin-3 is necessary for the expression of 1^o and 2^o fates; in lin-3 hermaphrodites, P(5-7).p express 3^o fates characteristic of P(3,4,8).p and do not form a vulva. Because lin-3 hermaphrodites lack a vulva, they cannot lay eggs and their progeny hatch internally. The progeny subsequently devour the body of the

parent resulting in a phenotype known as a "bag of worms." The activity of lin-12 is necessary, and can be sufficient, for the expression of 2° fates. In hermaphrodites carrying a mutation that eliminates lin-12 activity, P(3-8).p fail to express 2° fates; conversely, in hermaphrodites carrying a mutation that elevates the level of lin-12, lin-12(n137), all of the cells P(3-8).p express 2° fates.

In the synthetic Muv strains, P(3,4,8).p, as well as P(5-7).p, express 1° or 2° fates. However, in a double mutant carrying lin-15 and a mutation that eliminates lin-12 activity, none of the cells P(3-8).p express 2° fates, rather all cells express 1° fates (STERNBERG and HORVITZ, 1985b). These results indicate that the ectopic expression of the 2° fates observed in the synthetic Muv strains results from an elevation of lin-12 activity in some or all of the cells P(3-8).p.

To determine whether a class-8 synthetic Muv mutation or a class-9 synthetic Muv mutation alone could elevate lin-12 activity, we observed whether a class-8 or a class-9 synthetic Muv mutation could enhance an incompletely penetrant defect resulting from a partially dominant lin-12 allele. [As the partially dominant phenotype of these lin-12 alleles appears to result from an increase in normal gene activity (GREENWALD, STERNBERG and HORVITZ, 1983), a elevation of lin-12 activity by a synthetic Muv mutation would enhance the phenotype caused by the lin-12 mutation.] In wild-type males P(3-8).p do not divide; however, in lin-12(n137)/+ males, some of the these

cells undergo vulval-like divisions, resulting in the appearance of one or more midbody ventral protrusions. We examined the phenotypes of males of genotypes + lin-12(n137)/unc-32 +; him-5(e1467); lin-15(n744) and lin-8(n111); + lin-12(n137)/unc-32 +; him-5(e1467). At 20⁰, lin-15(n744), but not lin-8(n111), enhanced both the penetrance and expressivity of the lin-12(n137)/+ defect. Specifically, while 19% (n=88) of lin-12(d)/+ males and 28% (n=86) of lin-8(n111); lin-12(n137)/+ males had ventral protrusions, 61% (n=390) of lin-12(n137)/+; lin-15(n744) males had ventral protrusions. In addition, lin-15(n744) increased the expressivity of the lin-12(n137)/+ defect; while only 11% of lin-12(n137)/+ males and 8% of lin-8(n111); lin-12(n137)/+ males had more than one ventral protrusion, 42% of lin-12(n137)/+; lin-15(n744) males had more than one ventral protrusion. If other class-8 and class-9 mutations display the same pattern of interactions with lin-12, a reduction of the activity of the genes defined by class-9 synthetic mutation in the vulval precursor cells could cause an elevation of lin-12 activity, and thereby result in a predisposition to express a 2⁰ fate.

Class-8 and class-9 mutations also interacted differently with a Vul mutation in lin-3. The Muv phenotype resulting from lin-8(n111); lin-9(n112) is epistatic to the Vul phenotype resulting from lin-3(e1417) (FERGUSON, STERNBERG and HORVITZ, 1985). Hermaphrodites of genotype lin-8(n111); lin-9(n112); lin-3(e1417) have an egg-laying competent Muv phenotype. To determine if either lin-8(n111) or lin-9(n112) alone could suppress the Vul phenotype of lin-3(e1417) (*i.e.*, cause P(5-7).p to express their normal fates), hermaphrodites

of genotypes lin-8(n111); lin-3(e1417) and lin-9(n112); lin-3(e1417) were constructed. Neither double mutant had a wild-type phenotype, suggesting that in a lin-3 hermaphrodites neither lin-8(n111) nor lin-9(n112) alone can cause P(5-7).p to adopt their normal fates. However, many lin-8(n111); lin-3(e1417) hermaphrodites were able to release some eggs or larvae before becoming bags of worms, indicating that many of these hermaphrodites were not Vulvaless but rather had an abnormal vulva. Such abnormal vulvae were not present in either lin-3 hermaphrodites or in lin-9; lin-3 hermaphrodites. As the cells derived from the expression of a 1^o fate are necessary for (and can be sufficient to) generate a vulval structure that allows egg-laying, while the cells derived from the expression of a 2^o fate are neither necessary for or sufficient to generate a functional vulva (STERNBERG and HORVITZ, 1985a), it is likely that in lin-8; lin-3 hermaphrodites, P6.p expresses a 1^o fate with a higher frequency than in lin-3 or lin-9(n112); lin-3 hermaphrodites. Thus, a reduction in the activities of the genes identified by class-8 mutations in the vulval precursor cells could result in a predisposition to express a 1^o fate.

Prior observations (GREENWALD, STERNBERG and HORVITZ, 1983; STERNBERG and HORVITZ, 1985b) suggested that a high level of lin-12 activity in the cells P(3-8).p did not prohibit the expression of a 1^o fate (e.g., in lin-12(n137); lin-15(n309) hermaphrodites, some P(3-8).p cells express 1^o fates). However, as described below, the expression of a 1^o fate can be dependent on the level of lin-12 activity. Specifically, hermaphrodites of genotype lin-8(n111); + lin-12(n137)/unc-32 +; lin-15(n744) have a Muv phenotype similar to

that of hermaphrodites of genotype lin-8(n111); lin-15(n744), suggesting that some of the cells P(3-8).p express 1⁰ fates. However, hermaphrodites of genotype lin-8(n111); lin-12(n137); lin-15(n744) have a phenotype identical to hermaphrodites of genotype lin-12(n137), suggesting that in these hermaphrodites all of the cells P(3-8).p express 2⁰ fates. Thus, in hermaphrodites of the latter genotype an approximately two-fold increase in the level of lin-12 activity eliminated the expression of 2⁰ fates by the cells P(3-8).p, indicating that the decision of a P(3-8).p cell to express a 1⁰ or a 2⁰ fate may be dependent on the level of lin-12 activity.

If these observations, albeit limited, are indicative of the action of the synthetic Muv genes in the wild type, differences in the levels of the two functions specified by this set of genes could conceivably cause the vulval precursor cells to express each of the three vulval fates [1⁰, 2⁰ and 3⁰]. Specifically, if the level of either, or both, of the class-8 and class-9 functions in a particular P(3-8).p cell is high, the P(3-8).p cell would be determined to express a 3⁰ fate. If, however, both class-8 and class-9 functions are lowered, a P(3-8).p cell could express either a 1⁰ or a 2⁰ fate. The decision of which of these two fates to express could be specified by the relative level of reduction of each of the two functions. If the class-9 function is reduced to a greater extent than the class-8 function, the P(3-8).p cell would be determined to express a 2⁰ fate. Conversely, if the class-8 function is reduced to a greater extent than the class-9 function, the P(3-8).p cell would be determined to express a 1⁰ fate.

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