Characterization of Seven Genes Affecting *Caenorhabditis elegans* Hindgut Development

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ABSTRACT

We have identified and characterized 12 mutations in seven genes that affect the development of the *Caenorhabditis elegans* hindgut. We find that the mutations can disrupt the postembryonic development of the male-specific blast cells within the hindgut, the hindgut morphology in both males and hermaphrodites, and in some cases, the expression of a hindgut marker in hermaphrodite animals. Mutations in several of the genes also affect viability. On the basis of their mutant phenotypes, we propose that the genes fall into four distinct classes: (1) *egl-5* is required for regional identity of the tail; (2) *sem-4* is required for a variety of ectodermal and mesodermal cell types, including cells in the hindgut; (3) two genes, *lin-49* and *lin-59*, affect development of many cells, including hindgut; and (4) three genes, *mab-9*, *egl-38*, and *lin-48*, are required for patterning fates within the hindgut, making certain hindgut cells different from others. We also describe a new allele of the *Pax* gene *egl-38* that is temperature sensitive and affects the conserved β-hairpin of the EGL-38 paired domain. Our results suggest that a combination of different factors contribute to normal *C. elegans* hindgut development.

THE development of a digestive system is critical to animal viability and may include processes of organogenesis established early in animal evolution. Recent molecular investigations have identified similarities in the genes involved in development of the digestive system among vertebrate and invertebrate animals (reviewed in Simon and Gordon 1995). Notably, there is a conservation of gene expression and function in hindgut development, despite classical distinctions in the embryonic source of hindgut tissues in different organisms (*e.g.*, endoderm *vs.* ectoderm; Hoch and Pankratz 1996; Wu and Lengyel 1998). Thus, genetic studies of hindgut development in invertebrates may identify genes with a common role in development as well as investigate factors important to organogenesis.

The *Caenorhabditis elegans* digestive system includes a foregut (pharynx), midgut (intestine), and hindgut (rectum; Sulston *et al.* 1983). The cells of each structure derive from distinct embryonic precursors and have different developmental properties. All cells of the intestine are clonally derived embryonically from the endodermal founder cell, E. In contrast, pharyngeal cells are not born clonally and derive both from the mesodermal founder cell, MS, and an ectodermal precursor, ABa. The cells of the hindgut derive from another ectodermal precursor, ABp. Most of the hindgut cells are not closely related to each other by lineage, and cell ablation results suggest the proper specification of some hindgut cell types requires cell interactions during both early and late embryogenesis (Bowerman *et al.* 1992). To better understand the development and organogenesis of the *C. elegans* hindgut, we have identified and characterized mutations that disrupt this process.

The *C. elegans* hindgut is composed of 11 cells and eight distinct cell types (Sulston *et al.* 1983; Figure 1). Together, these cells form an opening to the intestine, yet maintain the structural integrity of the animal. The eight hindgut cell types can be distinguished on the basis of cellular morphology, the expression of specific markers, and their behavior during postembryonic development. Although development of the *C. elegans* digestive system and the differentiation of hindgut cell types occurs during embryogenesis, the hindgut cells also contribute to larval development as one (in hermaphrodite animals) or five (in male animals) of the cells undergo further postembryonic cell divisions (Sulston and Horvitz 1977; Sulston *et al.* 1980). Each of the dividing cells generally produces at least one progeny cell that continues as part of the hindgut, as well as other distinct cell types, such as neurons.

The four most posterior hindgut cells (F, U, B, Y) are termed male-specific blast cells. These four cells represent four distinct cell types in both males and hermaphrodites and can be distinguished on the basis of cellular morphology, behavior, and the expression of molecular markers. In addition, part of male sexual specialization includes the subsequent postembryonic

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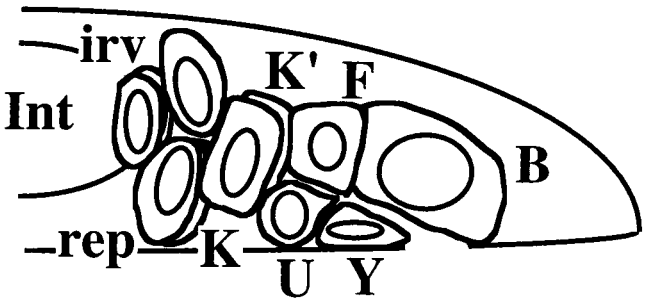

Figure 1.—Diagram of hindgut cells in early L1 larval stage (after Sulston *et al.* 1983). The *C. elegans* hindgut cells form five tiers (or rings) of cells connecting the intestine to the anal pore. At hatching, the Y cell is part of the hindgut, but during larval development it is replaced by P12.pa. In hermaphrodites, Y moves anterior into the preanal ganglion to become PDA. In males, U, F, B, and Y cells divide during postembryonic development. K divides postembryonically in both males and hermaphrodites. Int, intestine; irv, intestinal rectal valve cells (also called vir); rep, rectal epithelial cells. Anterior left, dorsal up.

division of these cells. In males, each cell divides with a stereotypic pattern and produces a different set of differentiated progeny. For example, the B cell divides to produce 47 progeny, including all of the cells of the spicules, which are sensory structures important for male mating (Liu and Sternberg 1995). The different cell division patterns and progeny produced in the male reflect the distinct cell types of the four precursor cells, and thus the male cell lineage can be used as an indicator of precursor cell type. In general, mutations that affect specification of cell type for these hindgut cells in both sexes can be identified and characterized because of the effects on the postembryonic male development (Chisholm and Hodgkin 1989; Chisholm 1991; Chamberlin *et al.* 1997). Consequently, we have identified 12 mutations in seven genes that affect hindgut development in both males and hermaphrodites among a collection of mutants with abnormal male tail development. Using cell morphology, cell lineage, tissue differentiation, and gene expression criteria, we have subdivided the genes into several classes that suggest contributions from a combination of regional, local, and tissue-type factors in hindgut development.

MATERIALS AND METHODS

Strains: Nematode strains were cultured according to standard techniques (Brenner 1974; Sulston and Hodgkin 1988). Mutations used are described by Hodgkin (1997).

Linkage group (LG) I: *egl-30(n686), mek-2(q425), fog-1(e2121), unc-11(e47), unc-74(e883), dpy-5(e61), sem-4(n1378), unc-13(e51). qDf3.*
LG II: *mab-9(e1245), rol-6(e187), unc-4(e120).*
LG III: *unc-93(e1500), unc-103(n500), dpy-17(e164), egl-5(n945), unc-32(e189). yDf10, sDf121, sDf130.*
LG IV: *dpy-13(e184), unc-5(e53), unc-24(e138), mes-6(bn66), lin-49(s1198), fem-3(e1996), elt-1(zu180), egl-20(n585), egl-38(s1775), egl-38(n578), daf-14(m77), unc-43(e408), mec-3(e1338), let-312(s1234), lin-3(n378), dpy-20(e1282), dpy-20(e1362), unc-22(s7), unc-31(e169). eDf19, eDf18, mDf7.*
LG V: *dpy-11(e224), him-5(e1490).*
LG X: *lon-2(e678).*
Extrachromosomal array: *pkEx246* (Pettitt *et al.* 1996).

Isolation of mutations: The mutations described in this article were isolated in a screen for mutants with abnormal male tail morphology. We used the strain CB1490 *him-5(e1490)* as a convenient source of phenotypically wild-type males, since *him-5* mutant hermaphrodites (XX) segregate about 40% male (XO) self-progeny (Hodgkin *et al.* 1979). *him-5(e1490)* hermaphrodites (P0) were mutagenized with 50 mm EMS for 4 hr (Sulston and Hodgkin 1988), placed individually on agar plates, and allowed to produce self-progeny. After 4 days, four to five F_1 L4 hermaphrodite progeny were removed from each parental plate and placed individually on fresh plates. After another 4–5 days, each F_2 brood was screened for presence of 1/4 or more morphologically abnormal individuals among the male self-progeny. Sibling hermaphrodites were selected from plates that yielded abnormal males to recover a homozygous mutant strain. In screens of over 25,000 mutagenized gametes, we identified 86 mutations that result in a *m*ale *ab*normal (Mab) phenotype. These mutants were then observed using Nomarski optics and placed into different phenotypic classes based on the morphological defects of larval and adult males.

Genetic mapping and complementation tests: All mutations were backcrossed at least twice to N2 (wild-type) stocks, usually during the process of mapping. Assignment to specific chromosomes was performed as described by Brenner (1974), generally using the following markers: *dpy-5* I, *rol-6* II, *unc-32* III, *unc-5* IV, *dpy-11* V, and *lon-2* X. Once a mutation was assigned to a specific chromosome, it was either tested for complementation with known hindgut genes (in the case of *mab-9* and *egl-5* alleles) or mapped further using multipoint and deficiency mapping (Table 1). Linkage and complementation tests with two mutations, *sa417* and *sa423*, showed that they were alleles of *mab-9*. These two alleles were subsequently lost. They are included in this study for completeness and to correctly represent the frequency of allele recovery.

Cell lineage analysis and laser ablation: Divisions of nuclei in the male F, U, and B lineages were observed directly in living animals using Nomarski differential interference contrast optics as described by Sulston and Horvitz (1977). Nomenclature follows the standard of Sulston and Horvitz (1977), with modifications of Chamberlin and Sternberg (1993). For F and U cell lineages in Figure 4, the initial cell division was inferred on the basis of observations from other mutant animals and the position of the presumptive F.l/r and U.l/r cells in the animals. All lineages were followed from early L3 through the L3 molt. For B cell lineages in Table 3, all lineages were followed from the first divisions of the B.a(l/r)xx cells (early to mid-L3 larval stage; x represents both progeny of a division) through the L3 molt.

Laser killing of cells was performed by the method of Sulston and White (1980) using the laser microbeam system and procedure of Avery and Horvitz (1987). Animals were anesthetized on pads of 5% agar in water containing 5 mm sodium azide. F, U, and B or B.a were killed in late L1 or early L2 larval stage males. Animals were recovered, checked several hours later for successful surgery, and then raised to adulthood and scored for differentiation of spicule socket cells. Socket cell differentiation was scored on the basis of the production of refractile spicule cuticle (Jiang and Sternberg 1999). In wild type, this material is expressed by both spicule cells (derived from B.a) and proctodeal cells (derived from

TABLE 1

Genetic mapping and complementation tests of mutations

Gene	Heterozygote[a]	Recombinants	Results
egl-5	*egl-5(sy279)/egl-5(n945); unc-24/+; him-5*		*sy279* fails to complement *n945* (Egl, Mab)[b]
sem-4	*sem-4(sa416)/unc-74 dpy-5; him-5*	Unc non-Dpy	*unc-74* (9) *sem-4* (0) *dpy-5*
		Dpy non-Unc	*unc-74* (6) *sem-4* (0) *dpy-5*
	sem-4(sa416)/dpy-5 unc-13; him-5/+	Unc non-Dpy	*dpy-5* (9) *sem-4* (0) *unc-13*
		Dpy non-Unc	*dpy-5* (10) *sem-4* (1) *unc-13*
	sem-4(sa416)/dpy-5 sem-4(n1378); him-5/+		*sa416* fails to complement *n1378* (Egl, Mab)
lin-49	*lin-49(sy238)/unc-24 mec-3 dpy-20; him-5*	Unc non-Dpy	*unc-24* (15) *lin-49* (6) *mec-3* (3) *dpy-20*
		Dpy non-Unc	*unc-24* (4) *lin-49* (3) *mec-3* (4) *dpy-20*
	lin-49(sy238)/unc-24 fem-3	Unc non-Fem	*unc-24* (19) *lin-49* (10) *fem-3*
	lin-49(sy238) unc-22/unc-24 mes-6 dpy-20	Unc-24 non-Dpy	*unc-24* (6) *mes-6* (0) *lin-49* (18) *dpy-20*
		Dpy non-Unc-24	*unc-24* (13) *mes-6* (2) *lin-49* (51) *dpy-20*
	unc-24 lin-49(sy238)/eDf18; him-5/+		*eDf18* deletes *lin-49* (Let)
	unc-24 lin-49(sy238)/eDf19; him-5/+		*eDf19* deletes *lin-49* (Let)
lin-59	*lin-59(sa489)/unc-11 dpy-5; him-5*	Unc non-Dpy	*unc-11* (0) *lin-59* (10) *dpy-5*
		Dpy non-Unc	*unc-11* (0) *lin-59* (5) *dpy-5*
	lin-59(sa489)/egl-30 unc-11; him-5/+	Egl non-Unc	*egl-30* (8) *lin-59* (2) *unc-11*
	lin-59(sa489)/mek-2 unc-11; him-5/+	Unc non-Mek	*mek-2* (7) *lin-59* (0) *unc-11*
	lin-59(sa489)/fog-1 unc-11; him-5/+	Unc non-Fog	*fog-1* (8) *lin-59* (1) *unc-11*
	lin-59(sa489) unc-11/qDf3; him-5/+		*qDf3 deletes lin-59* (Let)
mab-9	*mab-9(sa473) unc-4/mab-9(e1245); him-5*		*sa473* fails to complement *e1245* (Mab)
egl-38	*egl-38(sy294)/unc-24 dpy-20; him-5/+*	Unc non-Dpy	*unc-24* (6) *egl-38* (6) *dpy-20*
		Dpy non-Unc	*unc-24* (7) *egl-38* (4) *dpy-20*
	egl-38(sy294)/elt-1 dpy-20; him-5/+	Dpy non-Elt	*elt-1* (7) *egl-38* (35) *dpy-20*
	egl-38(sy294)/unc-24 daf-14; him-5/+	Unc non-Daf	*unc-24* (50) *egl-38* (6) *daf-14*
	egl-38(sy287)/let-312 lin-3; him-5	Lin non-Let	*let-312* (0) *egl-38* (5) *lin-3*
	egl-38(sy294) unc-22 unc-31/elt-1 unc-43	non-Elt non-Unc-22(d)	*elt-1* (14) *egl-38* (4) *unc-43* (88) *unc-22*
	egl-38(sy294) unc-22 unc-31/unc-24 egl-20 dpy-20	Unc-24 non-Dpy	*unc-24* (23) *egl-20* (3) *egl-38* (21) *dpy-20*
		Dpy non-Unc-24	*unc-24* (15) *egl-20* (5) *egl-38* (19) *dpy-20*
	dpy-13 egl-38(sy294)/eDf18 unc-22; him-5/+		*eDf18* does not delete *egl-38*
	egl-38(sy294) unc-22 unc-31/eDf19		*eDf19* deletes *egl-38* (Let)
	egl-38(sy294) unc-22 unc-31/dpy-13 mDf7		*mDf7* deletes *egl-38* (Let)
lin-48	*lin-48(sa469) unc-32/unc-93 dpy-17; him-5/+*	Unc-93 non-Dpy	*unc-93* (6) *lin-48* (2) *dpy-17*
		Dpy non-Unc-93	*unc-93* (1) *lin-48* (3) *dpy-17*
	lin-48(sa469)unc-32/unc-103 dpy-17; him-5/+	Dpy non-Unc-103	*unc-103* (0) *lin-48* (6) *dpy-17*
		non-Unc-32 non-Unc-103(d)	*unc-103* (0) *lin-48* (7) *dpy-17* (18) *unc-32*
	lin-48(sa469) unc-32/yDf10 unc-32; him-5/+		*yDf10* deletes *lin-48* (Mab)
	lin-48(sa469) unc-32/sDf130 unc-32; him-5/+		*sDf130* deletes *lin-48* (Mab)
	lin-48(sa469) unc-32/sDf121 unc-32; him-5/+		*sDf121* does not delete *lin-48*

[a] Alleles are listed in materials and methods.
[b] The phenotypes observed in *trans*-heterozygotes are listed in parentheses.

B.p). Although both types of cells must be removed to completely eliminate refractile cuticle expression, killing the B cell is not tolerated well by the animals, and many do not survive to adulthood. To enhance viability of the operated animals, B.a, rather than B, was killed in many animals, and B.p was allowed to develop normally. These animals produce proctodeal cells, but these are distinct from both the normal and the ectopic spicule cells. In all cases, production of spicule socket cells in B.a-killed and B-killed animals was the same.

Analysis of *cdh-3::gfp* expression: The strain NL1008 *dpy-20(e1362); pkEx246* (Pettitt *et al.* 1996) was used as a parent strain for all crosses and as the wild-type control. *pkEx246* was crossed into strains and maintained on the basis of its ability to rescue *dpy-20* mutations. To score expression, L1 and L2 larvae from non-Dpy parents were anesthetized on pads of 5% agar in water containing 5 mm sodium azide and scored for larval stage and green fluorescent protein (GFP) expression at ×1000 magnification. Transgenic animals were verified by confirming expression of *cdh-3::gfp* in nonhindgut cells prior to scoring. Cells were scored positive for expression if any GFP fluorescence was detected above background.

Tests for strain viability: The viability of different strains was tested by counting the full brood of three to four homozygous mutant hermaphrodites. All strains contained the mutation *him-5(e1490)*, which causes a background of ~5% X-aneuploid progeny that fail to hatch (Hodgkin *et al.* 1979). CB1490 *him-5(e1490)* was used as the wild-type control for comparison. To score viability at 20°, L4 hermaphrodites were placed singly

TABLE 2

Summary of hindgut genes recovered in Mab screen

Gene	LG	Alleles isolated	No. other alleles	References
egl-5	III	*sy279*	>10	Chisholm (1991); Wang *et al.* (1993)
sem-4	I	*sa416*	6	Basson and Horvitz (1996)
lin-49	IV	*sy238*, *sa470*	1	Clark (1990)
lin-59	I	*sa489*	0	
mab-9	II	*sa417*, *sa423*, *sa473*	3	Chisholm and Hodgkin (1989)
egl-38	IV	*sy287*, *sy294*	2	Trent *et al.* (1983); Chamberlin *et al.* (1997)
lin-48	III	*sy234*, *sa469*	1	Jiang and Sternberg (1999)

on plates and then transferred to fresh plates every 24 hr until they stopped producing self-progeny. Twenty-four hours after transferring the parent, unhatched eggs and dead L1 stage larvae were counted and removed from the plate. Forty-eight or more hours after removing the dead eggs, the remaining progeny were counted and assessed for stage of development. These times were modified for tests at 15° and 25° to compensate for altered growth rates. In all cases, however, animals were scored when the wild-type control animals were all hatched and again when they had reached adulthood. Because many dying larvae disintegrate rapidly, dead larvae, especially L1s, may be underrepresented when counted by this method.

Temperature-sensitive tests with *lin-49*, *lin-59*, and *egl-38* mutants: *lin-49(sy238)*, *lin-49(sa470)*, and *lin-59(sa489)* are all temperature sensitive, and homozygous mutant strains cannot be maintained at 25°. To test *cdh-3::gfp* expression and animal viability at 25° and 15°, parents were shifted from 20°, and then their progeny were analyzed. *egl-38(sy287)* is temperature sensitive, but viable. Homozygous strains were maintained at least two generations at the experimental temperature prior to scoring phenotypes.

Scoring hindgut, tail, and egg-laying phenotypes in *egl-38* mutants: Hindgut (Scar, Con) and male tail (Mab) morphology were scored in adult animals at ×1000 magnification using Nomarski optics. Adult hermaphrodites were scored as egg-laying defective (Egl) if they retained more than the normal single row of eggs in the uterus and the retained embryos had developed past the gastrulation stage.

DNA sequencing: We used PCR to amplify DNA including the exons coding for the paired domain (exons 2–5; Chamberlin *et al.* 1997) of *egl-38* from *egl-38(sy287)* mutants according to the single worm PCR method of Barstead *et al.* (1991). PCR products were directly sequenced by the California Institute of Technology sequencing facility using an ABI 373 DNA sequencer (Perkin-Elmer, Norwalk, CT). Both strands of DNA were sequenced from three independent reactions to confirm the mutations.

RESULTS

Male tail-defective mutants identify genes affecting hindgut development: In a genetic screen for mutations that disrupt male tail development, we identified several distinct classes of mutants (H. M. Chamberlin, unpublished results). One class consisting of 12 mutations shared several features that suggested a common effect on hindgut development (Table 2). These mutants display profoundly abnormal male tail development and morphology (Figure 2) and less penetrant phenotypes of abnormal hindgut morphology apparent at hatching and in hermaphrodites as well as males at all stages (Figure 3). These morphological features can result in constipation of larvae and adults; animals can be sufficiently deformed or damaged to result in lethality. Since the defects are present at hatching and are in both males and hermaphrodites, we infer that these genes function in the embryonic development of the hindgut in both males and hermaphrodites. The defects in male tail development and morphology reflect the effects on hindgut development, but they may also reflect additional male-specific functions of these genes.

Genetic mapping and complementation tests placed these 12 mutations into seven complementation groups. Three genes (*egl-5*, *sem-4*, and *mab-9*) had previously been identified by other researchers as affecting hindgut development (Chisholm and Hodgkin 1989; Chisholm 1991; Basson and Horvitz 1996). One gene (*egl-38*) had been identified for other functions but not hindgut development (Trent *et al.* 1983). Three genes (*lin-48*, *lin-49*, and *lin-59*) were novel and were not previously identified in screens for developmental mutants in *C. elegans*.

Altered male cell lineage in hindgut mutants: To investigate the cause of the male tail defects we observed the development of the four hindgut cells that divide in males (F, U, B, and Y). Initial observations indicated that all the mutants had abnormal divisions of some or all of these cells. We carried out cell lineage analysis of F, U, and B in *lin-48* and *lin-49* mutants (Figure 4; Table 3). Cell lineage analysis for *egl-5*, *mab-9*, and *egl-38* has been reported previously (Chisholm and Hodgkin 1989; Chisholm 1991; Chamberlin *et al.* 1997).

In *lin-48* mutants, the presumptive F and U cells produce more progeny than in wild type, and both cells produce a cell division pattern similar to each other (Figure 4B). Since the cell division pattern in *lin-48* mutants is abnormal and not similar to a cell lineage normally found in wild type, it alone does not suggest a specific role for *lin-48* in the development of F and U cells. Cell ablation experiments (see below) were used to further investigate the role of *lin-48* in F and U development.

We also followed the development of the B cell in *lin-48* mutants. Normally the B cell develops in three steps: (1) An initial set of divisions produces 10 progeny. (2) Eight of these progeny (the progeny from B.a) then

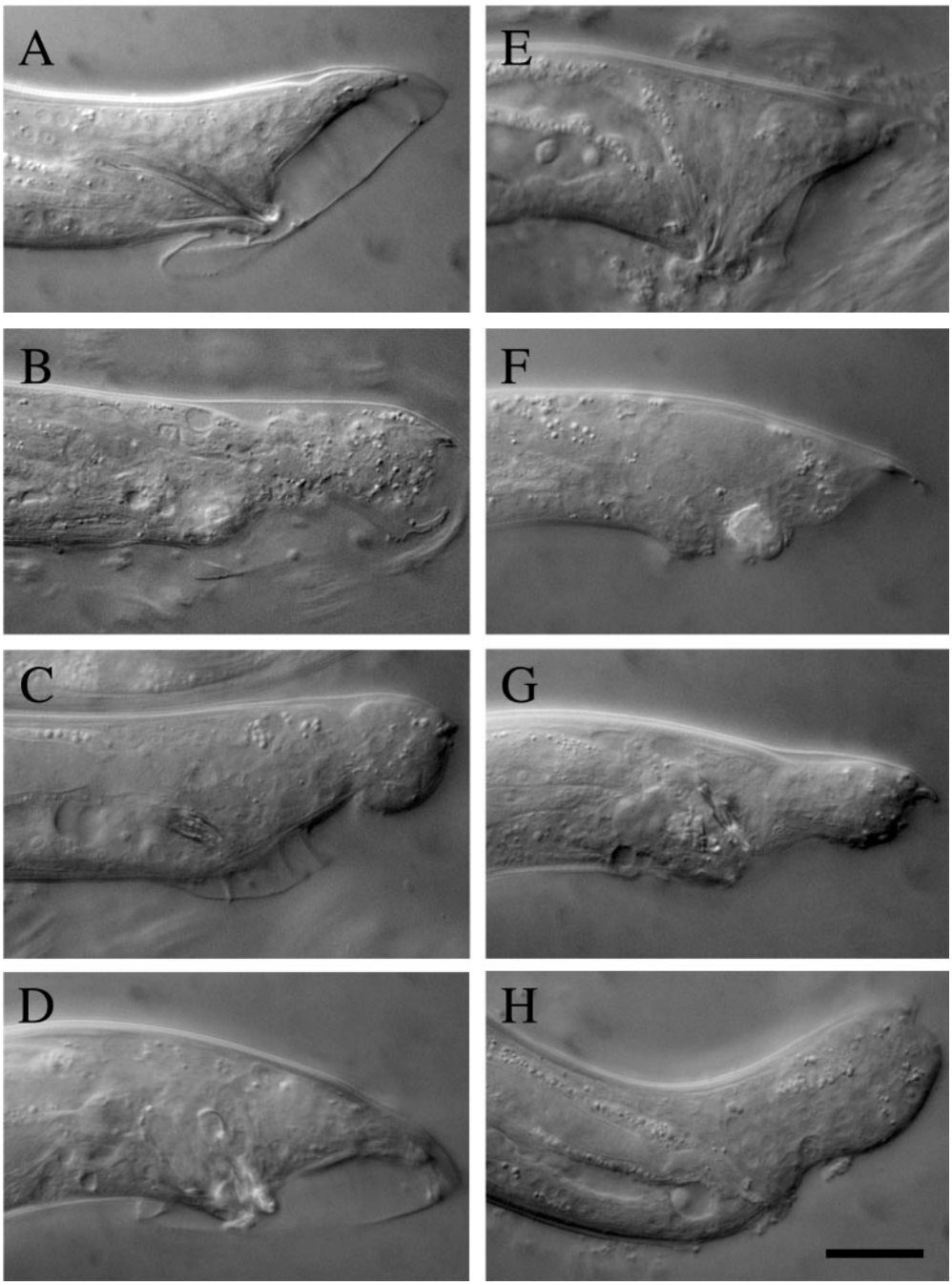

Figure 2.—Nomarski photomicrographs comparing the wild-type *C. elegans* adult male tail and those of mutants recovered in the genetic screen. (A) Wild type; (B) *mab-9(sa473)*; (C) *egl-38(sy294)*; (D) *lin-48(sa469)*; (E) *egl-5(sy279)*; (F) *sem-4(sa416)*; (G) *lin-49(sy238)*; (H) *lin-59(sa489)*. Anterior left, dorsal up. Bar, 20 μm.

participate in short-range migrations. (3) All 10 progeny then undergo a second set of cell divisions (Sulston *et al.* 1980). The third phase of B cell development reflects response of the eight migrating cells to a collection of cell interactions. In particular, the eight cells form pairs of cells in which one cell is more anterior and the other more posterior. These cell pairs are subject to competing signals: signals that promote the anterior fate and signals that inhibit it (or promote posterior fate; Figure 5; Chamberlin and Sternberg 1993). The F and U cells (or their progeny) are a source of the anterior-promoting signal. Normally, of the two cells in the pair, the physically anterior cell is closer to the progeny of F and U, and it adopts the anterior fate. However, experimental conditions that result in ectopic anterior-promoting signal or eliminate the inhibiting signal can result in physically posterior cells behaving like their more anterior neighbors (Chamberlin and Sternberg 1993, 1994). We find that in *lin-48* mutants the first set of B cell divisions and the cell migrations are generally normal. However, the subsequent development of some B progeny is abnormal. For example, the posterior cell of the B.a(l/r)aa (**aa**) cell pair normally produces a cell lineage (β) that results in 6 progeny, while the anterior **aa** cell produces a cell lineage (α) that results in 4 progeny. In *lin-48* mutants the posterior cell often produces 5 or 4 progeny rather than the normal 6, suggesting it is developing in a manner more similar to its

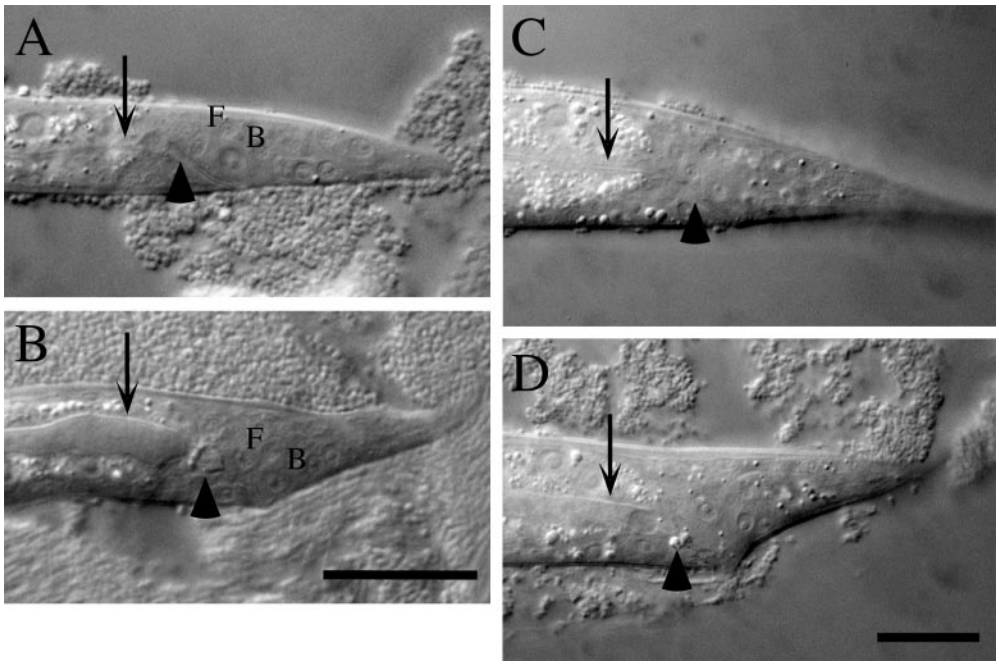

Figure 3.—Nomarski photomicrographs comparing larval tail morphology in wild type and representative mutants. (A) Wild-type L1 larval stage male animal. Although there are some cellular differences, L1 larval males and hermaphrodites are morphologically similar. (B) *lin-49(sy238)* L1 male. (C) Wild-type L4 larval stage hermaphrodite. (D) *egl-38(sy294)* L4 hermaphrodite. Arrows indicate lumen of the intestine, which is distended in mutants, indicating constipation. Arrowheads indicate the hindgut, which is morphologically abnormal and sometimes blocked in mutants. Anterior left, dorsal up. Bars, 20 μm.

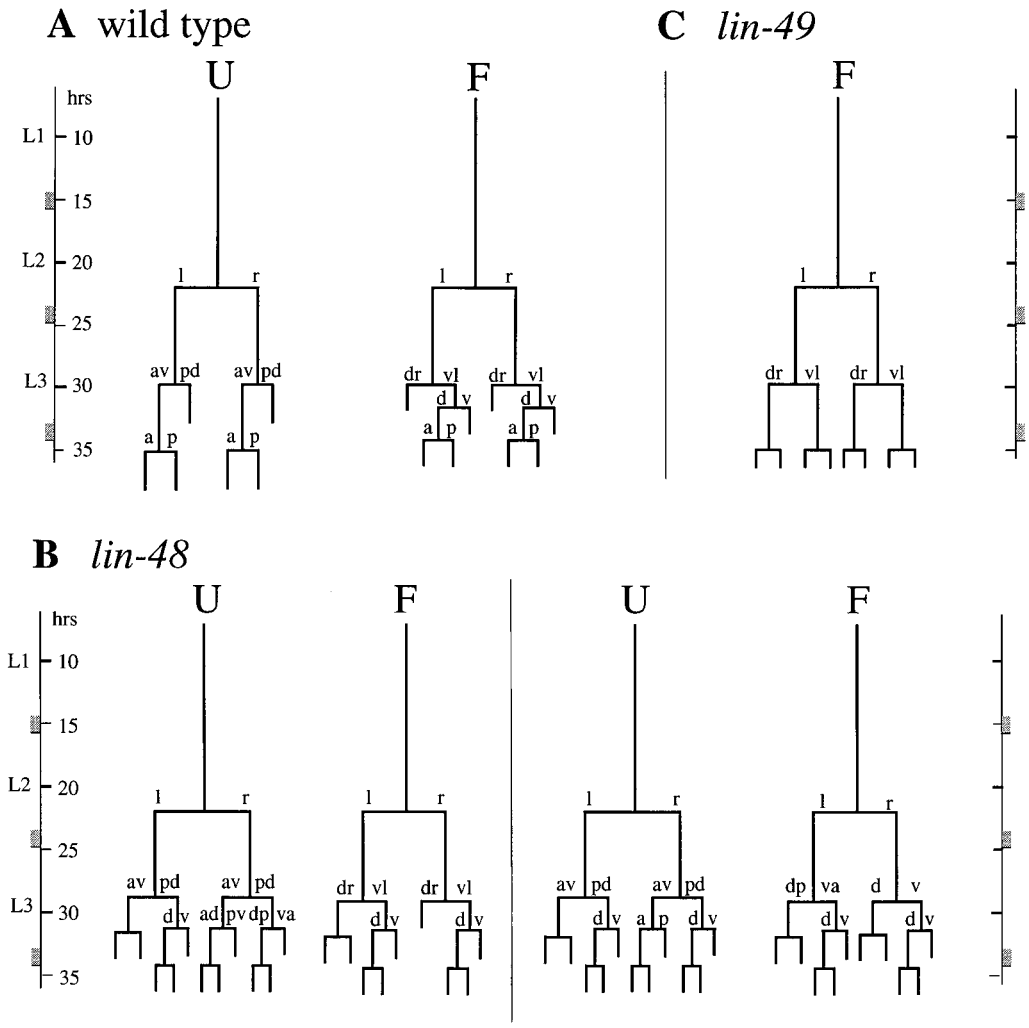

Figure 4.—Abnormal F and U cell lineage in hindgut mutants. (A) Wild-type male F and U cell lineages (after Sulston *et al.* 1980). The U.(l/r)aa cells do not divide in all animals. (B) The F and U cell lineages from two *lin-48(sy234)* mutant males. (C) The F cell lineage from one *lin-49(sy238)* male. The U cell in this animal did not divide. Side bars indicate approximate developmental time from hatching (in hours), and larval stage. Shading indicates the lethargus period prior to molting.

TABLE 3

Abnormal male B cell lineage in hindgut mutants

Genotype	**aa** ant.	**aa** post.	**pp** ant.	**pp** post.
Wild type	α (4)	β (6)	γ (6)	δ (2)
lin-48[a]	α	abn-6[b]	abn-7	δτ
	ατ[c]	α	γτ	δτ
	abn-5(l)[d]	abn-5(r)	γτ	δτ
	ατ(r)	abn-5(l)	γτ	δτ
	α	abn-5	abn-7	δτ
lin-49[e]	α(l)	β(r)	γ*[f]	δ

Each line represents the observed cell lineage for B.a(l/r)aa (**aa**) and B.a(l/r)pp (**pp**) from one male animal. Other B cell progeny developed normally in all animals. For wild type, the number of progeny from each cell type is indicated in parentheses. Abnormal cell lineages are indicated by underscoring.

[a] Genotype: *lin-48(sy234); him-5(e1490).*

[b] abn-*n* indicates that the cell lineage was abnormal, but produced *n* progeny.

[c] τ indicates the cell produced the normal pattern of progeny, but cell division axes were abnormal.

[d] (l) and (r) indicate that the cells failed to migrate to their normal anterior/posterior positions. One cell (l) remained on the left, and one cell (r) remained on the right.

[e] Genotype: *lin-49(sy238); him-5(e1490).*

[f] γ* is a commonly observed abnormal lineage (Chamberlin and Sternberg 1993). It results in four progeny instead of the normal six.

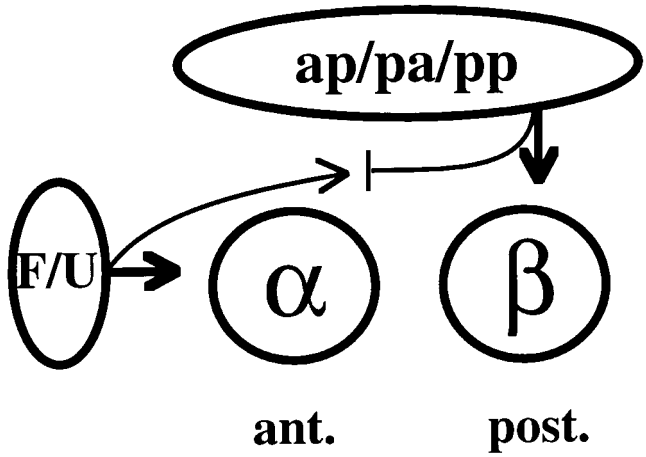

Figure 5.—A model for some cell interactions that affect fate choice of B.a(l/r)aa (**aa**) cells (figure after Chamberlin and Sternberg 1993). Normally, the two **aa** cells adopt distinct fates that correspond to their relative anterior/posterior position. The anterior cell adopts the fate and produces the cell lineage termed α. The posterior cell adopts the fate and produces the cell lineage termed β. Killing F and U cells results in both cells adopting the posterior fate (β). Killing the cousin and sibling cells (**ap**, **pa**, and **pp**) or ectopic expression of an anterior-promoting signal (*lin-3*; Chamberlin and Sternberg 1994) results in both cells adopting the anterior fate (α).

anterior neighbor. The abnormal cell lineage observed in the anterior **pp** cell is also consistent with ectopic signal. Even though F and U cells develop abnormally in *lin-48* mutants, the B cell progeny do not behave as if the F and U cells have been removed. Instead, the B cell progeny in *lin-48* mutants behave like cells exposed to either increased anterior-promoting signal or removal of signal that inhibits anterior fate.

lin-49 mutant males also have cell lineage defects in F, U, and B cells. We find that the presumptive U cell (or sometimes the presumptive F cell) often fails to divide, and the other cell divides without asymmetry and produces fewer progeny than normal (Figure 4C). We observed similar effects in *lin-59* mutants (data not shown). We followed the B cell lineage in one *lin-49* mutant (Table 3). The B cell lineage defect in this animal is consistent with a reduction of the signal that promotes anterior fate (see above). Lineage defects of this sort are observed in wild-type animals in which F and U cells have been killed (Chamberlin and Sternberg 1993).

***lin-48* mutants produce ectopic spicule cells from U:** In addition to the cell lineage defects (see above), we observed that some adult *lin-48* mutant males produced ectopic refractile spicule cuticle (Figure 6). The spicule cuticle is made by spicule socket cells (Jiang and Sternberg 1999) and thus serves as a marker for the differentiation of these spicule cells. In wild type, all of the cells of the spicules derive from a single precursor, the B cell (Sulston *et al.* 1980). To test whether the ectopic socket cells derive from some cell other than B, we killed the B cell (or the B.a daughter; see materials and methods) in *lin-48* mutants and found that 14 of 21 mutants still produced ectopic spicule socket cells (Table 4). Since U and F cells produce abnormal lineages in *lin-48* mutants, we killed these cells each in combination with the B cell and found that killing the U cell essentially eliminated the production of ectopic spicule socket cells. We infer that U is producing the ectopic spicule socket cells. Since spicule socket cells are a cell type normally produced only by the B cell, we conclude that *lin-48* plays a role in making the presumptive U cell different from B.

Altered *cdh-3::gfp* expression in some hindgut mutants: To further investigate the role of these genes in hindgut development, we tested whether expression of *cdh-3::gfp* could serve as a marker for specific hindgut cells. *cdh-3* is a *C. elegans* cadherin gene described by Pettitt *et al.* (1996), who found that *cdh-3::gfp* is expressed in the F and U cells of the hindgut in both males and hermaphrodites. We confirmed the expression of this construct in F and U, but detected expression at lower frequency and level in other hindgut cells in hermaphrodites grown at 20° (Table 5). We also found that detectable expression is greatly increased in animals grown at 25°. For example, we detected expression of *cdh-3::gfp* in K or K′ (or both) in 19% of wild-type hermaphrodites grown at 20°, but in 84% of those grown

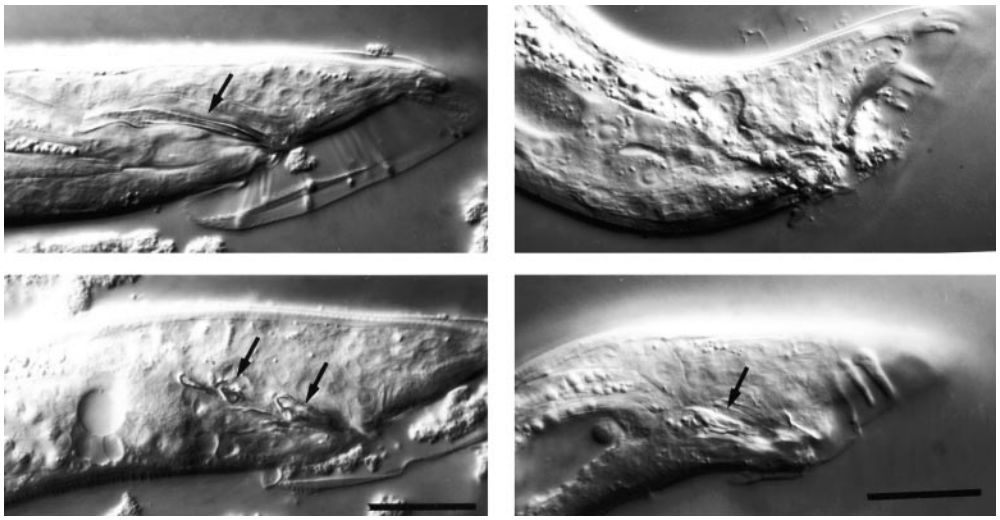

Figure 6.—Ectopic production of refractile spicule cuticle in *lin-48* mutants. Arrows indicate cells expressing spicule cuticle. Intact adult males: (Top left) wild type; (bottom left) *lin-48(sa469)*. Note the second, ectopic clump of cells in the *lin-48* mutant. Adult males in which the B cell was killed in early larval development: (Top right) wild type; (bottom right) *lin-48(sa469)*. The *lin-48* male still produces spicule cuticle. Bars, 20 μm.

at 25°. Detectable expression also increased in F and U, as well as B and repD, at the higher temperature. These observations suggest that *cdh-3::gfp* expression in the hindgut is not restricted to F and U, but that it is expressed at different levels or with different stability in different hindgut cells.

We tested expression of *cdh-3::gfp* in different hindgut mutants (Table 5). We found that mutations in *sem-4* and *egl-5* did not affect expression, despite the fact that morphological and cell lineage results indicate that these genes profoundly affect the fates of hindgut cells (Chisholm 1991; Basson and Horvitz 1996). Our observations suggest that these genes act independently (or possibly downstream) of the processes that affect hindgut *cdh-3::gfp* expression.

We found that only about half of *egl-38(sy294)* mutants that express *cdh-3::gfp* in the hindgut express the marker in both presumptive U and F (Table 5). Of animals expressing in only one cell, F was the expressing cell 87% of the time. This is consistent with our conclusion that in *egl-38* mutants the U and F cells adopt fates more similar to their posterior neighbors, Y and B, respectively (Chamberlin *et al.* 1997). In wild type, *cdh-3::gfp* expression is never observed in Y; so a U to Y cell fate transformation should result in failure to express (or reduced expression) in the presumptive U cell, as observed. In contrast, the B cell expresses *cdh-3::gfp* at low levels in wild type; so a transformation of F to B cell fate might affect expression in the presumptive F cell to a lesser extent. It is also possible that the *sy294* mutation affects the development of U to a greater extent than F (*sy294* reduces *egl-38* function, but is not a null allele; Chamberlin *et al.* 1997). *egl-38* mutants were the only tested mutants that exhibit a marked asymmetry in expression between F and U.

Mutations in *lin-49* and *lin-59* modestly affect *cdh-3::gfp* expression in cells expressing threshold levels of detectable GFP. Expression in U and F cells is affected at 20°, and expression in K and K′ cells is affected at 25°. This suggests that *lin-49* and *lin-59* may play either a role in establishing the fates of several hindgut cells, or a more general role in gene expression.

Hindgut mutants can have reduced viability: We observed that the hindgut morphological defects reduced the health of affected animals and in some cases appeared to be a cause of lethality due to blockage or damage to the hindgut and surrounding tissue. Previously, we have shown that *egl-38* is an essential gene, with strong mutations resulting in lethality at hatching (Chamberlin *et al.* 1997). We tested whether the genes identified in our screen similarly affect the animals' viability. Both mutations in *lin-48* confer only a slight decrease in viability compared to wild-type controls (Table 6). Animals bearing either allele *in trans* to deficiencies that delete *lin-48* are also viable (Table 1). Thus, existing *lin-48* alleles affect viability to a modest extent. Similar modest effects on viability are seen in *mab-9* mutants (Table 6; Chisholm and Hodgkin 1989).

In contrast, we found that the alleles of *lin-49* and *lin-59* recovered in our screen cause sharply reduced viability and are temperature sensitive. In particular, *lin-49* mutants are inviable at 25° (Table 6 and data not shown). *lin-49* and *lin-59* alleles are also lethal *in trans* to deficiencies that delete the gene (Table 1). We identi-

TABLE 4

Production of ectopic spicule cuticle in *lin-48* mutants

Genotype	Cell(s) killed	Percentage with spicule cuticle	*N*
Wild type	B[a]	0	10
lin-48[b]	B	67	21
lin-48	F, B	57	14
lin-48	U, B	5	20

[a] B includes animals in which B or B.a was killed (see materials and methods).

[b] Includes both *lin-48(sy234)* and *lin-48(sa469)* animals.

TABLE 5

Altered expression of *cdh-3::gfp* in some hindgut mutants

		Percentage of animals								
		U, F expression[a]			K, K′ expression[a]			Other cells		
Temp.	Genotype	2	1	0	2	1	0	B	repD	*N*
20°	Wild type[b]	72	15	13	11	8	81	8	13	109
	egl-5	85	9	6	23	24	52	3	18	103
	sem-4	79	12	9	12	13	75	3	6	107
	lin-49(sy238)	50	25	25	4	9	87	4	5	109
	lin-49(sa470)	62	23	15	10	10	79	3	7	106
	lin-59	79	13	8	17	19	64	2	13	107
	egl-38	47	43	10	6	0	94	16	25	108
	lin-48	70	19	11	5	14	81	6	13	109
25°	Wild type	89	7	4	65	19	15	42	32	113
	egl-5	87	7	6	61	21	17	14	21	103
	lin-49(sy238)	86	3	10	26	24	50	22	34	58
	lin-49(sa470)	86	9	5	10	29	58	18	36	112
	lin-59	86	7	8	39	25	37	14	22	106

[a] 2, 1, and 0 indicate expression in two of the cells, only one, or none, respectively.

[b] All genotypes include *pkEx246* and a *dpy-20* allele (*e1282* or *e1362*) for maintenance of the transgene. Alleles tested: *egl-5(sy279)*, *sem-4(sa416)*, *egl-38(sy294*), *lin-48(sa469)*, *lin-59(sa489)*. *egl-5*, *lin-59*, and *lin-48* strains included *him-5(e1490)* in the background. In these strains, both male and hermaphrodite L1 and L2 larvae were scored for expression.

fied a nonconditional early larval lethal allele of *lin-49*, *s1198*, among lethal mutations linked to *unc-22* and left of *sDf2* recovered by Clark (1990). We conclude that *lin-49* and, possibly, *lin-59* are essential genes, and the alleles of these genes isolated in our screen are non-null.

TABLE 6

Lethality associated with hindgut mutations

		Developmental stage[a] (percentage of animals)			
Temp.	Genotype	e/L1	L2/L3	L4/Ad	*N*
15°	Wild type[b]	6	1	93	1407
	lin-49	29	21	50	272
	lin-59	11	9	80	263
20°	Wild type	3	1	96	1334
	lin-49	35	35	30	601
	lin-59	13	23	65	559
	mab-9	6	12	82	955
	lin-48(sy234)	14	6	81	1094
	lin-48(sa469)	14	5	81	775
25°	Wild type	14	3	84	642
	lin-49	59	41	0	175
	lin-59	38	29	33	173

[a] Developmental stage at which animals arrest or the stage they had reached when counted. e/L1 indicates animals arrest as embryos or L1 larvae. L2/L3 indicates animals arrest as L2 or L3 larvae (also includes slow-growing animals). L4/Ad indicates animals had reached L4 or adulthood.

[b] All genotypes include *him-5(e1490)*. Alleles tested: *lin-49(sy238)*, *lin-59(sa489)*, *mab-9(sa473)*.

***egl-38(sy287)* is a temperature-sensitive allele:** The *Pax* gene *egl-38* is required for both the development of the hindgut and the development of the hermaphrodite egg-laying system. Previously we have shown that two viable mutations in *egl-38*, *n578* and *sy294*, preferentially disrupt different functions of the gene and represent distinct amino acid substitutions within the DNA-binding paired domain of EGL-38 (Chamberlin *et al.* 1997). To investigate whether *egl-38(sy287)* showed similar defects we tested hermaphrodite egg laying, male and hermaphrodite hindgut morphology, and adult male tail morphology in mutants at different temperatures (Table 7). We find that *egl-38(sy287)* is temperature sensitive for these functions. However, in general, *egl-38(sy287)* preferentially affects male tail development compared to egg laying. For example, at 20° 100% of males have abnormal spicule morphology, whereas only 4% of hermaphrodites are egg-laying defective. We sequenced genomic DNA from *egl-38(sy287)* mutants and found that it has a missense mutation affecting the β-hairpin portion of the EGL-38 paired domain (Figure 7). *In vitro* studies have identified this domain as important for protein interactions between Pax and Ets transcription factors (Wheat *et al.* 1999).

DISCUSSION

Several classes of genes affect hindgut development: In a genetic screen for mutations that affect male tail development, we identified mutations in seven genes that affect the hindgut in both males and hermaphro-

TABLE 7

***egl-38(sy287)* is a temperature-sensitive allele**

		Hermaphrodite			Male				
Temp.	Genotype	Egl	Scar	*N*	Scar	Con	Mab (sp)	Mab (hook)	*N*
	Wild type	0	0	Many	0	0	0	0	Many
15°	*sy287*[a]	0	0	17	0	0	50	0	10
20°	*sy287*	4	32	25	0	50	100	0	10
25°	*sy287*	69	31	26	100	100	100	100	11
20°	*sy287/s1775*[b]	56	ND	16	97	97	97	97	40
	sy287/n578[c]	0	ND	10	ND	ND	20	ND	10

Numbers represent percentage of animals with indicated phenotypes. Phenotypes: Egl, hermaphrodite egg-laying defective; Scar, damage, blockage, or scarring of the hindgut; Con, constipated; Mab (sp), male spicules morphologically abnormal; Mab (hook), male hook morphologically abnormal; ND, not determined.

[a] Full genotype: *egl-38(sy287); him-5(e1490).*

[b] Full genotype: *unc-24(e138) egl-38(sy287)/unc-24(e138) egl-38(s1775) unc-22(s7) unc-31(e169); him-5(e1490)/+.*

[c] Full genotype: *egl-38(sy287) dpy-20(e1282)/egl-38(n578); him-5(e1490).*

dites. We have combined our analysis of these genes and that of other investigators to suggest that these seven genes represent four distinct classes. (1) *egl-5* encodes a homeodomain protein and is part of a *C. elegans* HOX gene cluster (Wang *et al.* 1993). Phenotypic analysis suggests *egl-5* is required for regional identity in the tail (Chisholm 1991). (2) *sem-4* encodes a zinc finger protein and is required for the normal development of several mesodermal and ectodermal cells throughout the animal (Basson and Horvitz 1996). *sem-4* may contribute to the tissue-type identity of hindgut cells. (3) Mutations in the two genes *lin-49* and *lin-59* affect the development of hindgut cells, but they are highly pleiotropic. We have observed that mutant animals are variably Unc, Sma, and Egl, and some cell lineages in both males and hermaphrodites can be disrupted (data not shown). Thus, although we initially identified these genes as affecting hindgut development, they may function in the development of many cells of *C. elegans.* (4) The three genes *mab-9*, *egl-38*, and *lin-48* function to make certain hindgut cells different from each other and thus contribute to the patterning of cell fates within the hindgut. In particular, the activity of these three genes reflects the three spatial axes involved in distinguishing the four posterior-most hindgut cells. *mab-9* is required to make the two dorsal cells (F and B) different from their ventral neighbors (U and Y; Chisholm and Hodgkin 1989). *egl-38* is required to make the two anterior cells (F and U) different from their posterior neighbors (B and Y; Chamberlin *et al.* 1997). *lin-48* plays a role in distinguishing the presumptive U cell from B (this work). Embryonically, U and B are lineal homologs, with each cell being born from a similar pattern of cell divisions, but one born from a precursor on the right of the animal, and one from a precursor on the left (Sulston *et al.* 1983; U is ABplpppapa, B is ABprpppapa). Thus *lin-48* functions to make left/right symmetrical cells different from each other.

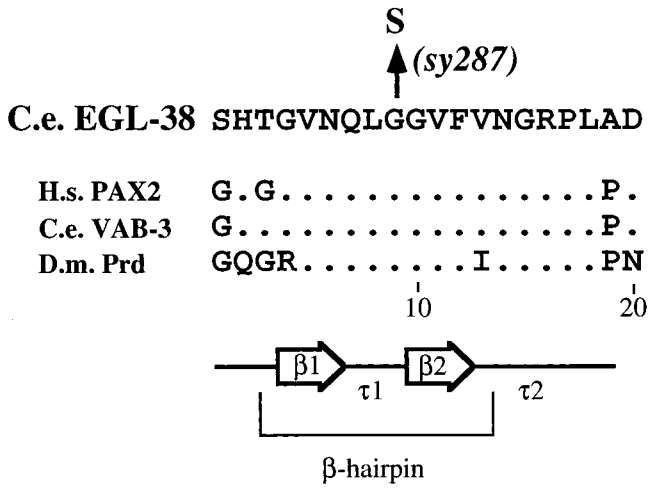

Figure 7.—Missense mutation in *egl-38(sy287).* The paired domain is composed of an amino-terminal β-hairpin followed by two helix-turn-helix motifs (Xu *et al.* 1995). The figure includes the first 20 amino acids of the paired domain from EGL-38 and other Pax proteins (from Chamberlin *et al.* 1997) and indicates the β-hairpin domain. *egl-38(sy287)* is a temperature-sensitive missense mutation of Ggc to Agc. This results in a serine substitution at a normally invariant glycine within the β-hairpin.

Developmental consequences of *lin-48* mutations on male tail development: We have observed cell lineage defects in the F, U, and B cells in *lin-48* mutant males. The F and U cells produce more progeny than in wild type and develop in a manner similar to each other. However, the consequences for each are not identical, as the presumptive U cell can produce differentiated spicule socket cells whereas the presumptive F cell cannot. Since spicule socket cells normally derive only from the B cell, one function of *lin-48* is to prevent the presumptive U cell from developing like B. Although the presumptive U cell can produce differentiated B progeny, it did not develop with a cell lineage similar to that

of B in the two animals we followed. In addition, the presumptive U cell in *lin-48* mutants does not produce all types of B cell progeny (Jiang and Sternberg 1999). Thus, we find that certain differentiated cell types can be produced in the absence of a complete transformation of the developmental potential of the precursor cell.

In *lin-48* mutants development of the B cell in males is affected in a manner consistent with overproduction of an anterior-promoting signal. In wild type, the F and U cells (or their daughters) are required for this signal, and B progeny act antagonistically to it (Chamberlin and Sternberg 1993). Although we have not directly tested whether the B cell lineage effect results from the F and U defects in *lin-48* mutants, our observations are not consistent with a reduction of F and U signaling in *lin-48* mutants, since removing these cells causes B cell lineage defects opposite to those we observed. Thus the B cell lineage defect is consistent with F and U retaining some of their wild-type developmental features in spite of abnormal cell lineage and abnormal differentiated progeny. Alternatively, the *lin-48* mutant effect on B cell development may reflect a function independent of its effect on the development of F and U.

***cdh-3::gfp* expression and hindgut cell fates:** We have extended the observations of Pettitt *et al.* (1996) and found that *cdh-3::gfp* is expressed in many hindgut cells, with highest levels in the F and U cells, and that the expression of the transgene or stability of the product is sensitive to temperature. *cdh-3::gfp* expression is reduced in the presumptive U cell in *egl-38* mutants, consistent with cell lineage analysis that suggests *egl-38* is required to make U different from its posterior neighbor Y (Chamberlin *et al.* 1997). *cdh-3::gfp* expression is also moderately affected in *lin-49* and *lin-59* mutants. In contrast, expression is not affected in *egl-5* or *sem-4* mutants, even though mutations in these genes affect the morphology and the development of hindgut cells. This suggests that these two genes function independently (or downstream) of the processes that regulate *cdh-3::gfp* expression in the hindgut. These genes may affect certain aspects of hindgut development, but not others. Alternatively, *cdh-3::gfp* expression may coincide with hindgut cell fate, but not actually reflect specific cell fate choices.

Hindgut development and viability: Mutations in genes affecting hindgut development can significantly affect viability (Chisholm and Hodgkin 1989; Basson and Horvitz 1996; Chamberlin *et al.* 1997; this work). In this work we have recovered non-null alleles of essential genes, and other essential genes that affect hindgut development have been identified by other researchers (*lag-1* and *lag-2*, Lambie and Kimble 1991; Bowerman *et al.* 1992; *pha-4*, Mango *et al.* 1994; Horner *et al.* 1998; Kalb *et al.* 1998). Although multiple developmental defects may contribute to the inviability of the mutant animals, our observations suggest that common defects associated with abnormal development of the hindgut, including blocking of the intestine and compromising the structural integrity of the tail, are sufficient to cause lethality. In our genetic screen we have identified viable mutations in seven genes that play a role in hindgut development, representing potential regional, local, and tissue-type factors. These genes provide the genetic framework for beginning the study of different factors that contribute to hindgut development. However, further screens to directly identify essential genes that contribute to hindgut development will be required to understand the full repertoire of factors involved in coordinating the development of the 11 distinct hindgut cells into a functional organ.

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