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Combinatorial control of touch receptor neuron expression in *Caenorhabditis elegans*

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SUMMARY

Six touch receptor neurons with distinctive morphological features sense gentle touch in *Caenorhabditis elegans*. Previous studies have identified three genes (*lin-32, unc-86 and mec-3*) that regulate touch cell development. However, since other cell types also require these genes, we suspected that other genes help restrict the expression of touch cell characteristics to the six neurons seen in the wild type. To identify such genes, we have examined mutants defective in genes required for the development of other *C. elegans* cells for changes in the pattern of touch cell-specific features. Mutations in seven genes either reduce (*lin-14*) or increase (*lin-4, egl-44, egl-46, sem-4, ced-3* and *ced-4*) the number of touch receptor-like cells. The combinatorial action of these genes, all of which are required for the production of many cell types, restrict the number of cells expressing touch receptor characteristics in wild-type animals by acting as positive and negative regulators and by removing cells by programmed cell death.

Key words: *Caenorhabditis elegans*, combinatorial regulation, specification of cell fate

INTRODUCTION

Combinatorial specification has often been proposed as a means of generating the large variety of cell types seen in animals and plants, but the combinatorial factors specifying cell fate are largely unknown (e.g., Gierer, 1974; Yamamoto, 1985; Johnson and McKnight, 1989; Simmons et al., 1990; Benfey et al., 1990). Genetic experiments on the specification of nerve cell fate in the nematode *Caenorhabditis elegans* also support the view of development as a combinatorial process. In particular, studies of two cell types, a set of six sensory receptors for gentle touch (Chalfie and Au, 1989; Way and Chalfie, 1989) and a pair of motor neurons needed for egg-laying (Desai et al., 1988), suggest that genes regulating cell fate are not expressed in a cell-specific fashion. The question that we address here is how cell fate, in this case the ability to become a *C. elegans* touch receptor neuron, is restricted to specific cells, i.e. what combinatorial factors result in cell-specific differentiation and how is the final number of these cells determined?

The six touch receptors (ALML, ALMR, PLML, PLMR, AVM and PVM; Fig. 1) can be distinguished from other *C. elegans* cells by several identifying features, including their position, structure and possession of large-diameter (15-protofilament) microtubules and an associated extracellular material called the mantle (Chalfie and Sulston, 1981; Chalfie and Thomson, 1982). Screens for touch-insensitive mutants (Chalfie and Sulston, 1981; Chalfie and Au, 1989) identified several genes that are needed for touch cell function. These include *mec-7*, a gene encoding a β-tubulin that is required for the production of the 15-protofilament microtubules (Savage et al., 1989), and *mec-4*, a gene encoding a putative membrane protein that can be mutated to cause the specific death of the touch cells (Driscoll and Chalfie, 1991). As we show below, these genes are expressed predominantly in the six touch receptor neurons and, thus, are excellent markers for touch cell differentiation.

Previous genetic studies also identified three genes that were needed for the production of the touch receptors. Mutations in two genes, *lin-32* and *unc-86*, result in abnormal precursor cells such that touch receptors are not generated (Chalfie et al., 1981; Sulston et al., 1983; Chalfie and Au, 1989; E. Hedgecock and C. Kenyon, personal communication). The third gene, *mec-3*, is not needed for the production of precursors but for the differentiation of the cells as touch receptors; the cells develop as neurons, but these cells do not have touch receptor features (Chalfie and Sulston, 1981; Way and Chalfie, 1988; Chalfie and Au, 1989). All three genes are needed for the development of additional cells (Chalfie et al., 1981; Chalfie and Au, 1989; Way and Chalfie, 1989; E. Hedgecock and C. Kenyon, pers.

comm.). In particular, *mec-3*, the most specific of the genes, is expressed and required in two other pairs of neurons (the FLP and PVD cells; Way and Chalfie, 1989; J. Kaplan and H.R. Horvitz, personal communication; see below). *unc-86* is also expressed in these cells (and others) in wild-type animals (Finney and Ruvkun, 1990) and is needed for their development (Hamelin et al., 1992; Xue et al., 1992).

Multiple alleles have been identified for almost all of the genes that mutate to a touch-insensitive phenotype (Chalfie and Au, 1989). Thus, it is unlikely that any nonredundant genes specifically controlling touch cell development will be found. Genes having more general patterns of expression, however, could help specify touch cell fate. Previously, we had speculated that *lin-14*, based on its mutant phenotype, might be one of these genes (Way and Chalfie, 1989). In this paper we show that at least five genes (*lin-4*, *lin-14*, *egl-44*, *egl-46* and *sem-4*) are needed in addition to *lin-32*, *unc-86* and *mec-3* to direct the correct cellular expression of touch cell characteristics. Together these genes provide a combinatorial basis for the specification of this single cell type. The number of cells expressing the touch receptor fate is further restricted by programmed cell death.

MATERIALS AND METHODS

Strains

Wild-type *C. elegans* (var. Bristol, N2) and mutant stocks were grown at 25˚C as before (Brenner, 1974; Way and Chalfie, 1988). Animals with temperature-sensitive phenotypes were grown at both 25˚C and 15˚C for at least two generations before testing.

Strains with the following mutations were used:

L.G. I: *lin-17(n671), lin-44(n1792), lin-35(n745), sem-4(n1378, n1971, n2087), lin-10(e1439), lin-28(n719), lin-11(n389), unc-59(e261)*

L.G. II: *lin-8(n111), lin-31(n301), egl-44(n998, n1080, n1087), lin-23(e1883), lin-4(e912), lin-26(n156), lin-5(e1348), unc-4(e120), lin-29(n333, n836, n1440), lin-7(e1413)*

L.G. III: *ced-4(n1162), lin-16(e1743), lin-37(n758), lin-13(n387), mab-5(e1239), egl-5(u202, n945), lin-36(n766), unc-86(e1416), lin-9(n112), lin-12(n941, n302), lin-19(e1756), lin-30(e1908), lin-39(n1792)*

L.G. IV: *lin-1(e1275), lin-3(e1417), lin-22(n372), lin-33(n1043), mec-17(u265), mec-3(e1338, u6), lin-34(n1041), lin-24(n432), ced-3(n717)*

L.G. V: *dpy-11(e224), egl-46(n1075, n1076, n1127), lin-25(e1446), him-5(e1490), unc-61(e228)*

L.G. X: *lin-32(u282), lin-18(e620), mec-7(u443, u448), vab-3(e1796)* (a.k.a *lin-20*), *lin-14(n179, n536, n355, n355n531, n536n540), lin-2(e1309), lin-15(n309,n767), mec-4(e1611)*

References for the mutations are: *ced* mutations: Ellis et al. (1986); *egl* and *sem-4* mutations: Trent et al. (1983) and Desai et al. (1988); *him-5* mutation: Hodgkin et al. (1979); *mab-5*: Hodgkin (1983); *mec*, *lin-32 and unc-86* mutations: Chalfie and Sulston (1981) and Chalfie and Au (1989); other *lin* mutations: Horvitz and Sulston (1980), Ambros and Horvitz (1984), Ferguson and Horvitz (1985), and M. Chalfie (unpublished data) and E. Hedgecock (personal communication); and other *unc* and the *dpy-11* mutations: Brenner (1974). Strains containing these mutation were either in our collection or generously provided by the Caenorhabditis Genetics Center, Victor Ambros, Michael Basson, Gian Garriga, Ed Hedgecock and Bob Horvitz. Some of the double mutant strains were provided by these same individuals: *lin-8(n111); lin-9(n112), lin-36(n766); lin-15(767), lin-37(n758); lin-15(n767)* and *egl-44(n1080); unc-4(e120) egl-46(n1075)*. The remaining multiple mutant strains were constructed by standard procedures (Brenner, 1974).

In situ hybridization

Digoxigenin in situ hybridization using an antisense oligonucleotide to the 3′ end of the *mec-7* sequence (5′ GAACGCTTCG-GCGGCATCTT 3′) followed the procedure in the Boehringer Mannheim Genius Kit and of Tautz and Pfeifle (1989) except that after a short fixation animals were treated with β-mercaptoethanol (Cox et al., 1981) and proteinase K to permeabilize the cuticle. To stain nuclei, 0.5 μg/ml diamidinophenylindole (DAPI) was included in the final wash.

The hybridization is *mec-7* dependent; it is absent in animals with mutations that delete the gene (*u443* and *u448*; Savage et al., 1989). *mec-7* mRNA is first detected in L2 larvae. The signal increases until the early L4 stage, when it begins to decline. Only the PLM cells stain in egg-laying adults. Usually L2-L4 larvae grown at 25˚C (Way and Chalfie, 1989) were examined.

Immunofluorescence

Animals were prepared for immunofluorescence microscopy according to the method of Finney and Ruvkun (1990) as modified by these authors (personal communication). Basically animals are fixed in 2% formaldehyde on ice for 30 minutes in the presence of EGTA, spermidine, 25% methanol. Fixation is followed by reduction first with β-mercaptoethanol and then with dithiothreitol. The animals are subsequently oxidized with H_2O_2.

Samples were incubated with shaking for 24 hours at room temperature with a 1:450 dilution of a rabbit anti-*mec-7* antibody (C. Savage and M. Chalfie, unpublished data), washed 7 times with buffer AbA (Finney and Ruvkun, 1990), incubated with rhodamine isothiocyanate-conjugated goat anti-rabbit IgG antibody (Cooper Biochemical) and washed an additional 8 times with buffer AbB (Finney and Ruvkun, 1990). The primary antibody was preabsorbed with acetone powders prepared by the method of Johnson (1989) from whole *mec-7(u443)* worms (the *u443* mutation deletes the *mec-7* gene; Savage et al., 1989). The secondary antibody was preabsorbed with acetone powders prepared from wild-type animals. Animals were observed with a Bio-Rad MRC-600 Confocal imaging system.

Only the touch receptor cells (ALML, ALMR, AVM, PVM, PLML and PLMR) stain intensely in wild-type animals; other cells stain less strongly (no cells stain in *u443* mutants). To quantitate the differences in intensity, we measured fluorescence output of individual cell bodies using the histogram software provided with the confocal microscope (the mean of three readings taken from cells in composite images of optically sectioned animals was used). The relative intensity of staining of the AVM cells compared to the staining of the ALM cells is 0.87±0.16 (7) [mean±s.e.m. (number of animals)]. Non-touch receptor cells have 5-9% of the staining of the touch cells in wild-type animals [FLP: 0.07±0.01 (4); PVD: 0.09±0.01 (4); BDU: 0.05±0.01 (4)]. This low-level staining is referred to as ‘weak.’ Other weakly staining cells include one to four cells in the tail and two cells in the ventral ganglion that are also seen in *mec-3* and *unc-86* mutants. Given their position, number and bipolar shape, these latter neurons are likely to be the AVF cells (White et al., 1986). In contrast, the transformed FLP cells in *egl-46(n1076)* mutants (see RESULTS) display fluorescence comparable to that of the touch receptor neurons [0.71±0.17 (5); AVM cells in these mutants have a relative intensity of 0.86±0.09 (4)].

mec-4lacZ fusion expression

The *mec-4lacZ* fusion vector (TU#44) was constructed using the *mec-4* genomic DNA sequence of TU#12 (Driscoll and Chalfie, 1991) and the *lacZ*-containing plasmid pPD22.04 (Fire et al.,

1990). We introduced an *Sph*I site by site-directed mutagenesis (Kunkel, 1985) after position 4639 in TU#12 using the oligonucleotide 5′ AAGGCATGCAAAAAT 3′. Introduction of this site results a 4.6 kb *Hin*dIII-*Sph*I restriction fragment that contains the *mec-4* 5′ regulatory sequences and genomic DNA encoding all but the last seven amino acids of the protein [as well as a substitution of Cys for Lys at position 490 (Driscoll and Chalfie, 1991]. This fragment was ligated to pPD22.84 that had also been digested with *Hin*dIII and *Sph*I.

TU#44 and pRF4, a plasmid that includes the dominant *rol-6* allele *su1006* [the *rol-6* mutations causes animals to roll and serves as a marker for transformation (Kramer et al., 1990; Mello et al., 1991)], were coinjected at 50 μg/ml into wild-type animals. A strain that segregated approximately 80% rollers was obtained from the progeny of the injected animals. The concatemerized array of *rol-6(su1006)* and *mec-4lacZ* DNA in this strain (TU1422) was designated as *uEx86*.

To generate a strain in which *rol-6(su1006)* and *mec-4lacZ* were stably integrated into one of the *C. elegans* chromosomes, 40 rollers of strain TU1422 were irradiated at 330 rads/minute from a ^{137}Cs source for 12 minutes. Irradiated parents were allowed to lay eggs and 125 F_1 progeny were picked onto individual plates. Six F_2 rollers from each plate were put onto individual plates and three animals (from different F_1 parents) that produced only roller progeny were identified. BZ2 is one of the strains that contains the integrated DNA *bzIs1*.

Standard genetic procedures (Brenner, 1974) were used to place either *uEx86* or *bzIs1* into various genetic backgrounds. Animals containing either fusion were stained for β-galactosidase activity by the procedure of A. Fire (personal communication, see Xue et al., 1992).

Electron microscopy

Animals were fixed in buffered 2.5% glutaraldehyde, stained in 1% osmium tetroxide, positioned within a small agarose block, dehydrated and embedded in Medcast resin (cf. Sulston et al., 1983). Intermittent transverse thin sections and thick sections were cut through either the head or the tail, so that ganglia and nerve cords could be examined at 5-10 μm intervals, using a Philips CM10 electron microscope. Thin sections were poststained with uranyl acetate and lead citrate before microscopy.

The sampling procedure was generally thorough enough to confirm that extra processes were localized longitudinally in the same region as the expected touch neurons, as judged from the *mec-7* antibody staining experiments. However, no attempt was made to preselect animals with extra touch receptors, nor to trace processes in serial sections back to their origin. These extra touch processes did not extend very far into the midbody region and often had fewer microtubules and more patchy mantle than their wild-type counterparts.

Laser ablations

Both ALM cells and the AVM precursor, QR, were killed in one hour old larvae with a Laserscience laser (Chalfie and Sulston, 1981; Seydoux and Greenwald, 1989). Touch sensitivity (Chalfie and Sulston, 1981) of the resulting animals was tested in double blind tests over the next few days.

RESULTS

Markers of touch cell differentiation

In order to follow cell fate rather than the expression of a particular gene, we have employed several methods to characterize touch cell differentiation. Initially we used in situ hybridization to *mec-7* mRNA (Table 1). This mRNA is detected only in the six touch receptor neurons in wild-type animals. To examine cell morphology, we have used a serum antibody specific to the *mec-7* β-tubulin (Table 2; Fig. 1). This is a more sensitive method: intense staining is seen in wild-type animals only in the six touch receptor neurons, while weak staining is seen in the PVD cells, the FLP cells, the BDU cells (the sister cells to the ALM touch cells) and a few other cells. We have also examined *mec-4* expression using a *mec-4lacZ* fusion (Table 3; Fig. 2). This fusion is expressed in wild-type animals primarily in the six touch receptor neurons, although the FLP, PVD and BDU cells stain infrequently. In some cases, we also determined whether the degeneration-causing mutation *mec-4(e1611)*, which causes the death of the touch receptor neurons (Chalfie and Sulston, 1981; Driscoll and Chalfie, 1991), caused the deaths of ectopic touch receptor-like cells. Finally, we examined cells by electron microscopy (Fig. 3). This allows us to determine whether the large diameter microtubules and mantle are present and how the processes are positioned relative to other neuronal processes. Examination of mutants known to be disrupted in touch cell development with these methods gave results that were consistent with (or enlarged upon) the previously noted phenotypes of the mutants (Tables 1-3; Figs 1, 2). For the mutants described in the following sections, all of these methods gave similar results, suggesting that the ectopic touch receptor-like cells that we find are cells whose fates have been changed.

Several genes affect touch cell production

To identify genes that regulate touch cell fate, we used these methods to examine mutants defective in 48 genes that had previously been shown to be needed for cell development (Tables 1-4; Figs 1-3). Because several genes are discussed in the following sections, we will invert the normal order of presenting information by providing a model that describes our results (Fig. 4) and then presenting the data on which the model is based.

In this model, the *unc-86*, *mec-3* and *lin-14* genes are needed to activate the expression of touch cell characteristics. The number of touch-receptor neurons is restricted to six in wild-type animals because of negative regulation of these positively acting genes or their effects. Specifically, the other *mec-3*- and *unc-86*-expressing cells do not develop as touch cells because either *lin-4* represses *lin-14* activity (PVD neurons) or the effects of these three genes (not their expression) are repressed by the *egl-44* and *egl-46* genes (FLP neurons). Another pair of neurons, tentatively identified as the PHC cells, also fails to develop as touch receptors (and do not express *mec-3*) because *sem-4* represses *mec-3* activity. Four additional cells that could express touch cell features die by programmed cell death, a process involving the genes *ced-3* and *ced-4*.

Positive action of *lin-14*

Gain-of-function mutations in the heterochronic gene *lin-14* cause the repetition of several first larval stage (L1) lineages, while loss-of-function mutations result in the premature appearance of later stage lineages (Ambros and Horvitz, 1984). The *lin-14* gene appears to be negatively regulated by the *lin-4* gene (Ambros, 1989; Arusa et al., 1991); a *lin-*

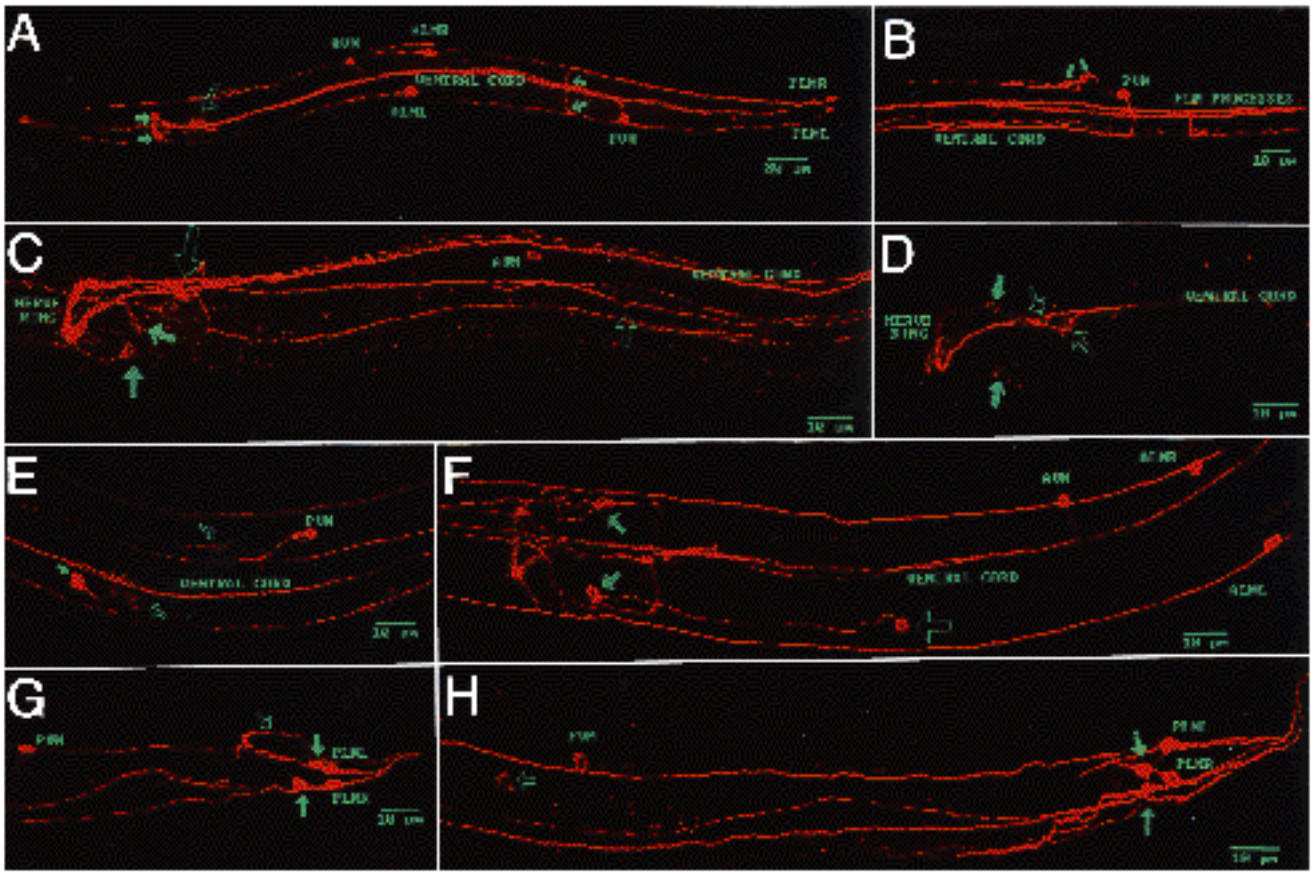

Fig. 1. *mec-7* immunofluorescence. Each figure is a composite of optical sections of an L4 larva obtained by confocal imaging. (A) Wild-type. Filled arrows indicate the positions of the PLM branches into the ventral nerve cord or the ALM and AVM branches into the nerve ring. Open arrows indicate the positions of the putative AVF cells. (B) Wild-type. The arrows indicate the cell bodies of the PVD cells. (C) *mec-3(u6)*. The small open arrow points to an ALM cell with the altered morphology expected in this mutant (Chalfie and Sulston, 1981; Way and Chalfie, 1988). Modified FLP cells (lacking the posteriorly directed processes of the wild-type cells; filled arrows) and putative AVF cells (large open arrow) are indicated. Approximately 10% of the *mec-3* animals had PLM cells with anteriorly directed processes that looped back toward the tail. (D) *unc-86(e1416)*. Only the putative FLP cells (filled arrows) and AVF cells (open arrows) stain in these mutants. (E) *lin-14(n355)* (gain-of-function). The extra strongly staining cell in the PVM region (filled arrow) does not look like a PVD cell. The open arrows indicate weakly staining cells. (F) *egl-46(n1076)*. Transformed FLP cells (filled arrows) often bifurcate anteriorly to the nerve ring and sometimes send processes that project into the position of the ALM processes (not shown) unlike the wild-type cells. The open arrow indicates an extra AVM-like cell that, like 10% of the AVM cells in this mutant, does not project into the ventral cord near its cell body (AVM and PVM show a similar outgrowth defect in *egl-44* animals). (G) *sem-4(n1971)*. The additional stained cells in the tail (filled arrows) often have bent processes (open arrow). (H) *ced-4(n1162)*. Additional strongly staining cells in the tail (filled arrows) and near PVM (open arrow) are indicated.

4 mutation causes similar lineage defects to those produce by gain-of-function *lin-14* mutations (Chalfie et al., 1981; Ambros and Horvitz, 1984). *lin-14* protein is normally found in cells of the embryo and L1 larva, but not the L2 larva (Ruvkun and Giusto, 1989); its production is presumably turned off by *lin-4* at this latter time.

We find that *lin-14* activity is needed for the choice of whether the two postembryonic touch cells (AVM and PVM) or the two PVD cells are produced. These two pairs of cells are generated from different precursors during the L1 and L2 stages, respectively (Sulston and Horvitz, 1977). *lin-14* loss-of-function mutants lack the AVM and PVM cells (Tables 1, 2); the cells that should give rise to the AVM and PVM cells produce a PVD-type cell lineage instead (Ambros and Horvitz, 1984). In contrast, both a gain-of-function *lin-14* mutation, *n355*, which results in the continued production of *lin-14* protein (Ruvkun and Giusto, 1989), and a *lin-4* mutation produce an effect opposite to that of the *lin-14* loss-of-function mutations: two (or more) extra cells express *mec-7* and *mec-4* in the PVD region in 4-40% of the animals (Tables 1-3). It seems likely that the PVD lineage has been altered so as to produce AVM-/PVM-like cells. Precursors giving rise to migrating cells (normally found in the L1 AVM/PVM lineages) are sometimes found in second stage larvae with *lin-14* gain-of-function mutations (V. Ambros, pers. comm.). The PVD lineage is also transformed in *lin-4* animals (Chalfie et al., 1981). The infrequent appearance of the extra touch-like cells may be due to the absence of necessary precursors because of earlier lineage defects in the mutants. Consistent with the model

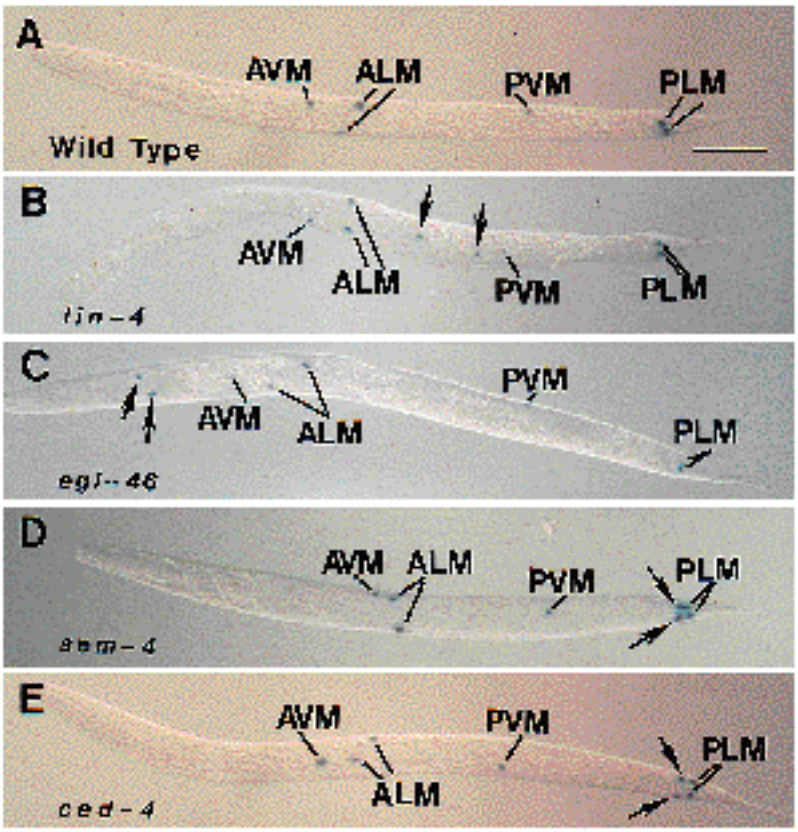

Fig. 2. Expression of a *mec-4lacZ* fusion in wild-type and mutant animals. Cells that express the fusion in mutants, but not in wild-type animals, are indicated by arrows. All animals are L4 larvae and have the *bzIs1* construct except for the *egl-46* strain which has *uEx86*. Scale bar (100 μm) applies to all animals. (A) Wild type. (B) *lin-4(e912)* animal with two extra expressing cells near PVM. (C) *egl-46(n1076)* animal with two extra expressing cells where the FLP cells are normally found. (D) *sem-4(n1971)* animal with two extra expressing cells in the tail. (E) *ced-4(n1162)* animals with two extra expressing cells in the tail.

that *lin-4* negatively controls *lin-14* expression, double mutants containing the *lin-4* mutation and *lin-14* loss-of-function mutations detectably expresses *mec-7* only in the ALM and PLM cells (Tables 1, 2).

These experiments suggest that *lin-14*, controlled by *lin-4*, acts as a genetic switch controlling the choice between the AVM/PVM and PVD lineages [two other heterochronic genes, *lin-28* and *lin-29* (Ambros and Horvitz, 1984) do not appear to be required; Table 1). We do not know whether *lin-14* affects only the precursors of the AVM, PVM and PVD cells or also acts on the cells directly, but the results with the *egl-44* and *egl-46* mutants suggest that this latter role is possible (see below).

One paradox concerning the role of *lin-14* as a coactivator in touch cell development is that *lin-14* mutations do not apparently affect the function or development of the ALM and PLM cells (*lin-14* mutants are touch sensitive; Ruvkun and Giusto, 1989; M. Chalfie, unpublished data). Since *lin-14* is needed for the development of ectopic touch receptor-like cells (see below), its activity may be redundant in the ALM and PLM cells.

Negative regulation by *egl-44* and *egl-46*

The *egl-44* and *egl-46* genes, which are needed for the differentiation of the HSN cells [a pair of neurons needed for egg-laying (Desai et al., 1988; Desai and Horvitz, 1989)], appear to encode negatively acting regulators that prevent the expression of touch cell features. Mutations in either gene result in the appearance of a pair of touch receptor-like cells in the region of the second pharyngeal bulb (Tables 1-3; Figs 1-3). In addition, mutants containing the dominant, degeneration-causing mutation *mec-4(e1611)* and a mutation in either *egl-44* and *egl-46* have one or two additional dying cells in the position of the FLP cells in newly hatched larvae (7/13 *egl-44* animals and 11/17 *egl-46* animals). It is likely that the ectopic cells are transformed FLP neurons, since they are in the normal FLP position and are the only cells in this position in *egl-46* mutants to express a *mec-3-lacZ* fusion (D. Xue and M. Chalfie, unpublished data). The *egl-44(n998); egl-46(n1076)* double mutant did not display a greater transformation.

This transformation requires the *lin-32*, *unc-86*, *mec-3* and *lin-14* genes (Table 1). The requirement for *lin-14* is somewhat surprising, even though *lin-14* protein is found in wild-type FLP cells (Ruvkun and Giusto, 1989; G. Ruvkun, personal communication), since the FLP cells are non-dividing, embryonically derived cells (Sulston et al., 1983) and *lin-14* defects had previously been described only for cells that divide in larvae (Ambros and Horvitz, 1984). These results suggest that the involvement of *lin-14* in touch cell differentiation may be more direct than simply acting to determine the fate of precursor cells. In this way, *lin-14* may act similarly to *unc-86*, which is needed at several stages in touch cell development.

We tested the extent of the FLP transformation by ablating the ALM and AVM cells (to abolish anterior touch sensitivity; Chalfie and Sulston, 1981) in *egl-44(n998)* (n=12) and *egl-46(n1127)* (n=5) mutants. The resulting animals were not detectably touch sensitive, perhaps because the ectopic touch receptor-like cells are unable to make appropriate synapses (these mutations also cause errors in neuronal outgrowth; Desai et al., 1988; Fig. 2).

Table 1. *mec-7* mRNA expression

Relevant genotype	*n*	Temp	Percent animals stained[1]					
			Touch receptor neurons			Extra stained cells		
			ALM	PLM	AVM/PVM	PVD	FLP	tail
A. Touch cell development[2]								
Wild type	>100	25	100	100	100	0	0	0
lin-32(u282)X	24	25	100d	0	0	0	0	0
unc-86(e1416)III	>20	25	0	0	0	0	0	0
mec-3(e1338)IV	>20	25	0	0	0	0	0	0
mec-17(u265)IV	22	25	0	0	0	0	0	0
mab-5(e1239)III[3]	14	25	100	100	100d	0	0	0
B. *lin-14* and *lin-4*								
lin-14(n179)X lf	18	25	100	100	33	0	0	0
	17	15	100	100	88	0	0	0
lin-14(n355n531)X lf	23	25	100	100	0	0	0	0
lin-14(n536n539)X lf	20	25	100	100	0	0	0	0
	20	15	100	100	10	0	0	0
lin-14(n355)X gf	23	25	100	100	100	9	0	0
lin-14(n536)X gf	>20	25	100	100	100	0	0	0
lin-4(e912)II	44	25	100	100	100	9	0	0
lin-4(e912); lin-14(n179)	26	25	100	100	38	4	0	0
	24	15	100	100	100	8	0	0
lin-4(e912); lin-14(n536n540)	4	25	100	100	0	0	0	0
lin-28(n719)I	>20	25	100	100	100	0	0	0
lin-29(n333)II[4]	20	25	100	100	100	10	0	15
lin-29(n836)II	>20	25	100	100	100	0	0	0
lin-29(n1440)II	>20	25	100	100	100	0	0	0
C. *egl-44* and *egl-46*								
egl-44(n998)II	21	25	100	100	100	0	67(100)	0
egl-44(n1080)II	31	25	100	100	100	0	23(52)	0
egl-44(n1087)II	21	25	100	100	100	0	57(90)	0
egl-44(n998); mec-3(e1338)	>20	25	0	0	0	0	0	0
egl-44(n998); unc-86(e1416)	>20	25	0	0	0	0	0	0
egl-44(n998); lin-32(u282)	>20	25	100d	0	0	0	0	0
egl-44(n998); lin-14(n179)	16	25	100	100	100	0	0(38)	0
	15	15	100	100	100	0	27(94)	0
egl-46(n1075)V	23	25	100	100	100	0	13(22)	0
egl-46(n1076)V	25	25	100	100	100	0	60(88)	0
egl-46(n1127)V	23	25	100	100	100	0	57(96)	0
mec-3(e1338); egl-46(n1127)	>20	25	0	0	0	0	0	0
unc-86(e1416); egl-46(n1127)	>20	25	0	0	0	0	0	0
egl-46(n1127); lin-32(u282)	>20	25	100d	0	0	0	0	0
egl-46(n1127); lin-14(n179)	21	25	100	100	100	0	5(43)	0
	21	15	100	100	100	0	24(72)	0
egl-44(n1080); egl-46(n1075)[5]	22	25	100	100	100	0	45(86)	0
D. *sem-4*								
sem-4(n1378)I[6]	22	25	100	100	100	0	0	(55)
sem-4(n1971)I[6]	25	25	100	100	100	0	0	(100)
sem-4(n2087)I[6]	23	25	100	100	100	0	0	(91)
sem-4(n1971); mec-3(e1338)	>20	25	0	0	0	0	0	0
sem-4(n1971); unc-86(e1416)	>20	25	0	0	0	0	0	0
sem-4(n1971); lin-14(n179)	24	15	100	100	ND	0	0	38(59)
	22	25	100	100	ND	0	0	86(100)
E. *ced-3* and *ced-4*								
ced-3(n717)IV[7]	30	25	100	100	100	0	0	37(87)
ced-3(n717); lin-32(u282)	>20	25	100d	0	0	0	0	0
unc-86(e1416); ced-3(n717)	>20	25	0	0	0	0	0	0
ced-4(n1162)III[7]	30	25	100	100	100	0	0	37(84)
ced-4(n1162); mec-3(e1338)	>20	25	0	0	0	0	0	0

[1]Values indicate the percentage of L2-L4 (except *mec-17)* animals expressing *mec-7* mRNA in both cells of an indicated pair; numbers in parentheses indicate the percentage of animals with expression in at least one member of the pair. The extra cells are labeled by their presumed source (PVD or FLP) or by their position (tail; presumably the PHC cells in *sem-4* mutants or the undying cells in the *ced* mutants). Abbreviations: *n,* number of animals; ND, not determined; Temp, growth temperature in °C (some strains are temperature sensitive); d, displaced cell body; gf, gain-of-function mutation; lf, loss-of-function mutation. No defects were seen in strains with representative alleles of the following genes: *egl-5, lin-1 - lin-3, lin-5, lin-7 - lin-13, lin-15 - lin-19, lin-22 - lin-26, lin-30, lin-31, lin-33 -lin-37, lin-39, lin-44, unc-59, unc-61* and *vab-3*.

[2]These results are consistent with touch cell defects previously noted for these mutants (Chalfie et al., 1983; Chalfie and Au, 1989). These include the loss of expression of all cells in *unc-86* and *mec-3* animals; the displacement anteriorly of the ALM cells and the loss of other cells in *lin-32* animals; and the anterior displacement of the PVM cells in the 86% of *mab-5* animals. In the *mec-17* experiment egg-bearing adults and cocultured *dpy-11(e224)V* adults were co-processed for *in situ* hybridization. In contrast to the *mec-17* animals, virtually all (24/27) of the *dpy-11* animals had PLM staining (this is the only staining left at this time). This loss is consistent with the suggested role of *mec-17* in the maintenance of touch cell differentiation (Chalfie and Au, 1989; Way and Chalfie, 1989).

[3]The strain also contained a *him-5(e1490)V* mutation.

[4]Only this *lin-29* strain (MT333) had infrequent, ectopic touch receptor-like cells. Because the other strains contained putative null mutations (V. Ambros personal comm.), this staining is presumably due to a second site mutation.

[5]The strain also contained an *unc-4(e120)II* mutation.

[6]In these experiments, records were not kept on whether one or two additional cells stained. In a separate experiment with *n1971,* all animals had two extra expressing cells in the tail.

[7]In larger fields of these animals, extra AVM- or PVM-like cells were seen rarely (1-2%).

Table 2. *mec-7* immunocytochemistry

Genotype	*n*	Percent animals stained[1]								
		ALM	AVM	+	PVM	+	PLM	FLP	PVD	Tail
Wild type[2]	74	100	100	0	100	0	100	0(58)	0(81)	0(80)
mec-3(e1338)[3]	62	0(55)	0(13)	0	0(23)	0	52(29)	0(29)	0	0(21)
mec-3(u6)[3]	62	0(90)	0(48)	0	0(53)	0	65(26)	0(85)	0	0(29)
unc-86(e1416)[3]	74	0	0	0	0	0	0	0(54)	0	0
lin-4(e912)[4]	74	100	100	0	100	40	100	ND	?	0(82)
lin-14(n355)[4]gf	103	100	100	0	100	4	100	ND	?(19)	0(35)
lin-14(n536)[4]gf	55	100	100	0	98	0	100	0(9)	0(85)	0(11)
lin-14(n536n540)[5]lf	50	100	0	0	0	0	100	0	0	0
lin-4(e912); lin-14(n179)[6]	72	100	79	0	87	11	100	0(22)	0(17)	0
egl-44(n998)[7]	54	100	81	4	83	4	100	67(30)	0(39)	0(13)
egl-46(n1076)[7]	100	100	90	0	100	9	100	48(18)	ND	0(31)
egl-44(n998); egl-46(n1076)[7]	53	100	80	2	90	0	100	60(11)	0(2)	0
sem-4(n1971)[8]	80	100	100	0	100	0	100	ND	ND	100(11)
ced-4(n1162)[9]	66	100	100	18	100	38	100	ND	ND	78(30)

[1]Values give the percentage of animals with at least one of the indicated cell types staining (all are cell pairs except AVM and PVM); numbers in parentheses indicate the percentage of animals with weakly staining cells of the indicated type. Animals sometimes varied in the number of additional, strongly staining cells they contained. The number of weakly staining cells in the tail varied, from one to four. The abbreviations used are gf, gain-of-function; lf, loss-of-function; WT, wild-type staining pattern and intensity; +, additional cells of the indicated type; N, number of animals examined; ND, not determined.

[2]All animals, even *unc-86* mutants, also had two cells that weakly stained in the ventral ganglion (probably the AVF cells). Wild-type animals, but not *mec-3* or *unc-86*, had 58% of the BDU cells that also stained weakly.

[3]The "FLP" cells in *mec-3* and *unc-86* mutants lacked the normal FLP morphology; the posteriorly directed process was usually absent. In addition, eight percent of *mec-3(e1338)* and eleven percent of *mec-3(u6)* animals had PLM cells with anteriorly directed processes that looped back posteriorly. Hamelin et al. (1992) also saw low levels of *mec-7* expression in the touch cells of *mec-3* mutants and suggested that this expression was activated by *unc-86*. Although likely, we also find low level expression in a few cells (the AVF cells) in *unc-86* animals. Unlike these workers, we find that the *mec-3(e1338)* mutation was a more severe allele than the *mec-3(u6)* mutation.

[4]In *lin-4* animals one (20%), two (8%), three (5%) or more than three (7%) extra strongly staining cells were seen in the PVD/PVM region (the maximum number seen was five). In the *lin-14(n355)* animals one (1%) or two cells (3%) were seen. Although the structure of these extra cells varied, because they do not look like PVD cells and do have the strong *mec-7* staining, we have listed them as extra PVM cells and list ? under PVD.

[5]This loss-of-function mutation had no detectable expression in the usual weakly expressing cells in the indicated experiment. In a second experiment (n=25), no AVM and PVM staining was seen, but the weak staining was present.

[6]The AVM cells were displaced posteriorly to positions near the ALM cells in 36% of the animals.

[7]The FLP cells have an unusual morphology, *e.g.*, they often bifurcate anteriorly to the nerve ring. Sometimes processes are seen that project posteriorly toward the positions of the ALM cell bodies, and infrequent separations ("bubbles") are seen along the ALM process suggesting that multiple processes (one from an ALM cell and one from a transformed FLP cell) may run together. The AVM process projected laterally rather than ventrally in 15%, 10% and 20% of the *egl-44*, *egl-46* and *egl-44; egl-46* animals, respectively, as did the PVM process in 15%, 0% and 10% of the animals.

[8]One (8%), two (66%), or three or more (26%) additional cells were seen in the tail. 60% of the animals had tail cell processes that exhibited large bends as they projected anteriorly (see Fig. 1).

[9]One (59%), two (17%), or three or more (2%) additional cells were seen in the tail. Only single additional AVM-like cells were seen; all were on the same side of the animal as AVM. One (36%) or two (2%) additional PVM-like cells were seen; these were also on the same side of the animals as PVM.

Table 3. *mec-4lacZ* expression

Genotype	*n*	Percent animals stained[1]								
		ALM	AVM	+	PVM	+	PLM	FLP	PVD	Tail
Wild type	44	100	100	0	100	0	100	0(14)	7	0
mec-3(e1338)	82	0	0	0	0	0	0	0	0	0
mec-3(u6)	75	0	0	0	0	0	0	0	0	0
unc-86(e1416)	100	0	0	0	0	0	0	0	0	0
lin-4(e912)[2]	65	100	100	5	99	31	100	0(26)	?	0
egl-46(n1076)[3]	65	100	68	0	91	9	97	95	0	0
sem-4(n1971)[4]	75	100	100	4	99	16	100	0(8)	0	96
ced-3(n717)[5]	61	100	100	16	99	25	100	0(20)	0	58
ced-4(n1162)[5]	60	100	98	10	95	30	100	0(8)	0	67

[1]The abbreviations used are the same as in Table 2.

[2]In *lin-4* animals one (20%), two (8%), or three or more (3%) strongly staining cells were seen in the PVD/PVM region (the maximum number seen was four). We have listed these cells as extra PVM-like cells.

[3]Unlike the other strains, the *egl-46* strain did not have an integrated *mec-4lacZ* fusion gene. This may account for the lower level of AVM staining.

[4]One (20%), two (67%), or three or more (9%) additional cells were seen in the tail.

[5]One (52% and 48%) or two (15% and 10%) additional cells were seen in the tails of the *ced-3* and *ced-4* animals, respectively. Only single additional AVM-like cells were seen; all were the same side of the animal as AVM. Usually one (13% and 25%) additional PVM-like cell was seen, and this was also on the same side as PVM.

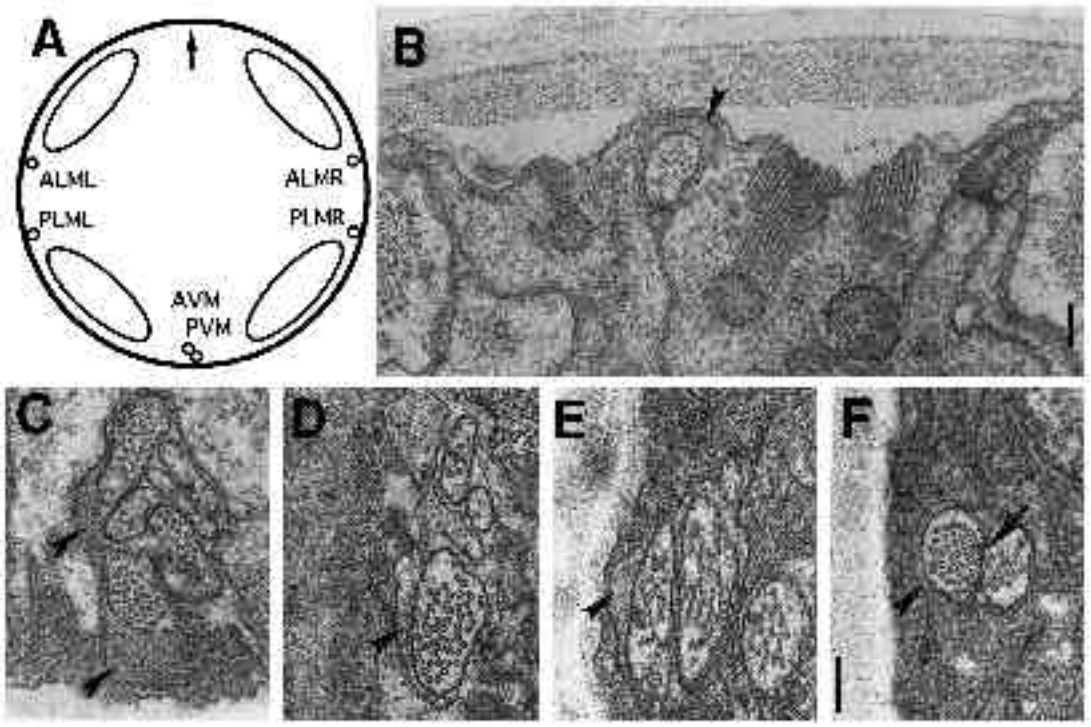

Fig. 3. Electron microscopy of touch receptor processes. (A) Schematic drawing indicating the positions in transverse section of normal touch receptor processes (small circles) closely apposed to the cuticle at the margins of longitudinal body muscles (ovals). Extra processes arise in the mutants at all of these positions and in the dorsal cord (arrow). (B) *egl-46* head, anterior to nerve ring. This dorsally located process has both the large-diameter microtubules and mantle (arrowhead). (C) *egl-44* head, anterior to nerve ring. The anterior end of AVM receptor process in the ventral nerve cord (the ventralmost process) is accompanied by two additional processes containing large-diameter microtubules (one has detectable mantle). Nearby axons have smaller-diameter microtubules. (D) *sem-4* tail. A touch receptor-like process with a small amount of mantle occupies a dorsal-lateral position. (E) *sem-4* tail. The PLML receptor process (on the left) is accompanied by two additional touch receptor-like processes with no mantle. (F) *ced-4* tail. The PLML receptor process (on the left) is accompanied by an additional receptor process (with no mantle) that appears to form a gap junction (arrow) with it. Scale bars: 0.2 μm bar in (B); 0.2 μm bar in (F) applies to panels C-F.

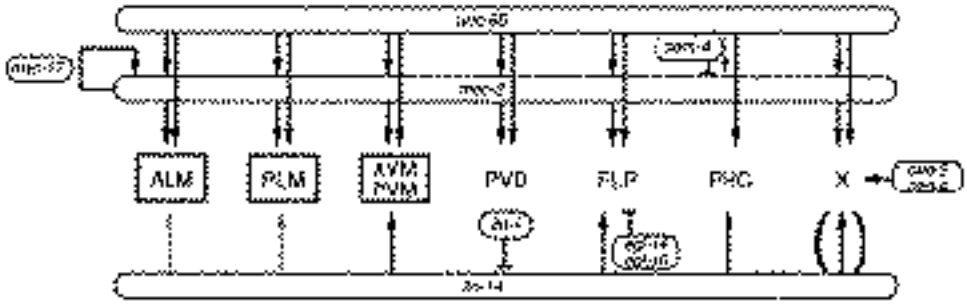

Fig. 4. Genetic interactions in the regulation of touch receptor characteristics. Wild-type animals contain a pair of each of the indicated cell types and four relevant dying cells (X). Only the boxed cells expressed touch receptor characteristics in wild-type animals. j , positive regulatory effects; ¾|, negative regulatory effects. The *lin-32* gene (not indicated on the figure) is presumed to act before *unc-86*. The double arrow from *unc-86* denotes that this gene may act both in regulating *mec-3* and, subsequently, with *mec-3*, on target genes such as *mec-7* and *mec-4* (A. Duggan and M. Chalfie, unpublished data). The arrow from *mec-3* onto itself signifies the role of this gene in maintaining its own expression; maintenance of touch receptor differentiation (but perhaps not of other cells) also requires the *mec-17* gene (Way and Chalfie, 1989). The arrows from *lin-14* to the ALM and PLM cells are shaded to indicate that its function with regard to these cells is not known. The effect of *lin-14* on the expression of the cells that do not die in *ced-3* and *ced-4* animals is hypothesized; it has not been tested.

Negative regulation by *sem-4*

The *sem-4* gene, which is needed for the development of the HSN egg-laying neurons (Desai et al., 1988), also acts as a negative regulator in touch cell development. *sem-4* mutants have two additional cells in the tail with touch cell-like features, including the large-diameter microtubules and mantle (Tables 1-3; Figs 1-3). This ectopic touch cell expression requires *lin-14*, *lin-32*, *unc-86* and *mec-3* (Table 1). Similar electron microscopy observations to ours have been made by M. Basson and H. R. Horvitz (personal communication), who have suggested that these extra cells arise from altered lineages that normally generate the PHC neurons. Since *unc-86* (Finney and Ruvkun, 1990), but not *mec-3* (Way and Chalfie, 1989), is expressed in the PHC cells in wild-type animals, we suggest that the wild-type product of the *sem-4* gene may negatively regulate *mec-3* expression in these cells.

Table 4. Presence of extra touch receptor-like processes in *C. elegans* mutants

			Animals with extra			Extra processes/animal[4]						Extra process position[5]				
Genotype	Area[1]	*n*[2]	Processes	Mantle	GJ[3]	1	2	3	4	5	6	ALM	PLM	VC	DC	other
egl-44(n998)	head	4	4	3	1	1			1	2		8		6	1	
egl-46(n1127)	head	10	7	5	2	3	2	1	1			8		4	2	
sem-4(n1971)	tail	8	8	4			3	1	2	1	1	3	18	4		3
ced-3(n717)	tail	10	4	2	1	3	1						5			
ced-4(n1162)	tail	12	8	3	2	5	1		2			2	8	4		1
ced-4(n1162)	head	6	2	1		1	1					1		2		

[1]Sections were examined from the head and anterior midbody (head) or the tail and posterior midbody (tail). We have also examined the central midbody region of *ced-4(n1162)* animals and have seen extra processes that are likely to arise from extra PVM-like cells, but since we could not exclude the possibility that these were from extra tail cells, we have not included them in this table.

[2]*n*, number of animals examined.

[3]The extra processes usually had patchy mantle, but sometimes lacked it completely. The numbers given indicate animals in which at least one process had associated mantle. Occasionally gap junctions (GJ) were seen between adjacent touch receptor-like processes. The ALM touch cells form gap junctions with the AVM cell in wild-type animals (Chalfie et al., 1985; White et al., 1986).

[4]Several animals had more processes than expected from the *mec-7* antibody staining. Since we did not follow cells to their cell bodies, we do not know how many cells produce the extra processes. In addition, some animals, particularly in *sem-4* mutants, had extra touch cell processes only in a few cross-sections (over a distance of 5-10 μm), which were otherwise not found. Although this could signify that these processes are branches of nearby processes, it seems more likely that they represent glancing sections of processes directed circumferentially along the body wall (as suggested from the *mec-7* antibody staining; Fig. 1).

[5]The extra processes (i.e. those in addition to the ALM, PLM, AVM and PVM cells) were primarily seen either in the dorsal lateral position that is the normal location of the ALM cells, the ventral lateral position that is the normal location of the PLM cells, the ventral cord (VC, the normal position of the AVM and PVM processes), or the dorsal cord (DC). Exceptions (other) are one mediolaterial process in one *sem-4* and one *ced-4* animal (these could be processes in transit from lateral touch receptor cell bodies) and two processes associated with the anal ridge in the lumbar region (this is the position of the PVR process, in which large-diameter microtubules are infrequently seen in wild-type animals; Hall and Russell, 1991).

Cryptic touch cells

Prevention of programmed cell death by *ced-3* and *ced-4* mutations leads to the appearance of additional differentiated cells (Ellis and Horvitz, 1986; Avery and Horvitz, 1987; Chalfie and Wolinsky, 1990; White et al., 1991). We find that these mutations result in the appearance of four additional touch receptor-like cells (Tables 1-3; Figs 1-3) in regions where several cells normally die (Sulston and Horvitz, 1977; Sulston et al., 1983). Two cells are found in the tail near the PLM cells. These cells also degenerate in *ced-4(n1162); mec-4(e1611)* double mutants. The ectopic *mec-7* mRNA expression in these cells requires *lin-32*, *unc-86* and *mec-3* (Table 1; the effect of *lin-14* mutations has not been tested). The two other cells, which look like the AVM and PVM cells and are situated near them, arise less frequently than the cells in the tail (Tables 1-3; Fig. 1). Their relative rarity may indicate that the transformation is less complete in these cells (incomplete function for cells prevented from dying has been seen previously by Avery and Horvitz, 1987).

Because the four extra touch receptor-like cells in these mutants are located near the PLMR, PLML, AVM and PVM cells, it seems likely that the new cells arise from the same lineages as the wild-type touch receptors. Two cells die in each of these four lineages (Sulston and Horvitz, 1977; Sulston et al., 1983), but only one (Q(R/L).pp and AB.p(r/l)apapppppp) expresses detectable *unc-86* protein (Finney and Ruvkun, 1990) and is, thus, likely to be the extra touch receptor-like cell.

DISCUSSION

Our results suggest that *C. elegans* has solved the problem of making four different types of neurons by using combinations of positively and negatively acting factors (Fig. 4). A further restriction in cell number is caused by programmed cell death. None of the genes that specify or restrict touch cell expression acts in a cell-specific fashion (Desai et al., 1988; Chalfie and Au, 1989; Desai and Horvitz, 1989; Way and Chalfie, 1989; Finney and Ruvkun, 1990).

There are at least two stages in the specification of the touch cells: the expression of the regulatory gene *mec-3* (controlled by *unc-86* and *sem-4*) and the expression of touch cell features [controlled by *mec-3*, *lin-14*, *egl-44*, *egl-46* and *unc-86* (Hamelin et al., 1992; present results ; A. Duggan and M. Chalfie, unpublished data)]. The specification of cell fate, however, does not appear to be a strictly linear process; the cumulative effect of successively acting regulatory factors is also important. The low level expression of *mec-7* protein in *unc-86* and *mec-3* mutants (RESULTS and Hamelin et al., 1992) and the requirement for *lin-14* in the touch cell-like differentiation of FLP cells in *egl-44* and *egl-46* mutants suggest that *unc-86*, *lin-14* and, perhaps, other genes act at more than one regulatory stage.

We do not know whether the genetic interactions diagrammed in Fig. 4 are direct or indirect. *unc-86* and *mec-3* encode DNA-binding proteins (Xue et al., 1992) found in the touch cells (Way and Chalfie, 1989; Finney and Ruvkun, 1990) and other genes may regulate the expression of genes within these cells. As argued above, the *lin-14* gene, which encodes a nuclearly localized protein of unknown molecular function (Ruvkun and Giusto, 1989), may be required in the FLP cells for them to acquire touch receptor characteristics in *egl-44* and *egl-46* mutants. Alternatively, some genes, e.g. *lin-14*, may (or may also) regulate the production of touch cell features indirectly by affecting the fate of the immediate (parental and grandparental) precursor cells. The roles of genes such as *egl-44*, *egl-46* and *sem-4* should become better

understood when these gens are cloned and their expression patterns examined.

Because mutations in *lin-14*, *egl-44*, *egl-46* and *sem-4* affect expression of *mec-4* and *mec-7* and the ultrastructure of the affected cells, these genes specify cell fate. However, the changes in the cell fate produced by these mutations do not appear to be complete: many of the extra cells in *lin-4* or *lin-14* (gain-of-function) mutants do not migrate to the AVM position, the extra cells in the *egl-44* and *egl-46* mutants cannot mediate a touch response, and the extra cells in several mutants sometimes have fewer microtubules and less mantle than wild-type cells. We do not know whether these defects result from incomplete transformation because necessary factors are lacking, inappropriate transformation because other factors are present (discussed in Dickinson, 1988), pleiotropic effects of the mutations, or inappropriate cell interactions or timing (the PVD cells arise later and the FLP cells are positioned more anterior than any of the touch receptor neurons). Pleiotropy may explain why *egl-44* and *egl-46* are needed both to repress touch receptor features in the FLP cells and to allow proper development of the AVM touch cells: the effects on nerve outgrowth, for AVM and perhaps the transformed FLP cells and other cells (Desai et al., 1988), may be indirect. In addition, cell interactions are necessary for the proper outgrowth of the AVM touch cell (Chalfie et al., 1983; Walthall and Chalfie, 1988).

We have identified several of the components needed for the combinatorial specification of touch cell differentiation. However, since we only examined the effects of known cell differentiation mutations, we would be surprised if we had identified all the genes needed for this process. At least one other factor may make *lin-14* redundant in the ALM and PLM touch cells (see above), and other genes may be needed to produce the different synapses made by the touch receptor neurons (Chalfie et al., 1985). Other candidate genes include *lin-32*, which appears from the existing three alleles to be minimally needed for the development of the ALM touch cells (Chalfie and Au, 1989), and *ceh-18*, a recently identified POU-type homeobox gene of unknown function that is expressed in the ALM and AVM cells but not in other neurons (D. Greenstein, S. Hird and G. Ruvkun, personal communication).

Although future experiments will undoubtedly refine our picture of touch cell differentiation (e.g., screens for mutations that alter the pattern of *mec-7* and *mec-4* expression may reveal other genes in this developmental pathway), it is clear that combinatorial control is needed to direct cell differentiation. The genetic interactions that we have found in touch receptor differentiation are similar to the genetic circuits deduced for embryonic development in *Drosophila* (reviewed e.g., in Akam, 1987; Ingram, 1988). Our results show that similar developmental pathways also direct the terminal specification of cell fate.

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REFERENCES

Akam, M. (1987). The molecular basis for metameric pattern in the *Drosophila* embryo. *Development* **101**, 1-22.

Ambros, V. (1989). A hierarchy of regulatory genes controls a larva-to-adult developmental switch in C. elegans. *Cell* **57**, 49-57.

Ambros, V. and Horvitz, H. R. (1984). Heterochronic mutants of the nematode *Caenorhabditis elegans*. *Science* **226**, 409-416.

Arasu, P., Wightman, B. and Ruvkun, G. (1991). Temporal regulation of *lin-14* by the antagonistic action of two other heterochronic genes, *lin-4* and *lin-28*. *Genes Dev.* **5**, 1825-1833.

Avery, L. and Horvitz, H. R. (1987). A cell that dies during wild-type C. elegans development can function as a neuron in a *ced-3* mutant. *Cell* **51**, 1071-1078.

Benfey, P. N., Ren, L. and Chua, N.-H. (1990). Combinatorial and synergistic properties of CaMV 35S enhancer subdomains. *EMBO J.* **9, 1685-1696.**

Brenner, S. (1974). The genetics of *Caenorhabditis elegans*. *Genetics* **77**, 71-94.

Chalfie, M. and Au, M. (1989). Genetic control of differentiation of the *C. elegans* touch receptor neurons. *Science* **243**, 1027-1033.

Chalfie, M., Horvitz, H. R. and Sulston, J. E. (1981). Mutations that lead to reiterations in the cell lineages of Caenorhabditis elegans. *Cell* **24**, 59-69.

Chalfie, M. and Sulston, J. (1981). Developmental genetics of the mechanosensory neurons of *Caenorhabditis elegans*. *Dev. Biol.* **82**, 358-370.

Chalfie, M., Sulston, J. E., White, J. G., Southgate, E., Thomson, J. N. and Brenner, S. (1985). The neural circuit for touch sensitivity in *Caenorhabditis elegans*. *J. Neurosci.* **5**, 956-964.

Chalfie, M. and Thomson, J. N. (1982). Structural and functional diversity in the neuronal microtubules of *Caenorhabditis elegans*. *J. Cell Biol.* **93,** 15-23.

Chalfie, M., Thomson, J. N. and Sulston, J. E. (1983). Induction of neuronal branching in *Caenorhabditis elegans*. *Science* **221**, 61-63.

Chalfie, M and Wolinsky, E. (1990). The identification and suppression of inherited neurodegeneration in *Caenorhabditis elegans*. *Nature* **345**, 410-416.

Cox, G. N., Kusch, M. and Edgar, R. S. (1981). Cuticle of *Caenorhabditis elegans*: its isolation and partial characterization. *J. Cell Biol.* **90**, 7-17.

Desai, C., Garriga, G., McIntire, S. L. and Horvitz, H. R. (1988). A genetic pathway for the development of the *Caenorhabditis elegans* HSN motor neurons. *Nature* **336**, 638-646.

Desai, C. and Horvitz, H. R. (1989). *Caenorhabditis elegans* mutants defective in the functioning of the motor neurons responsible for egg-laying. *Genetics* **121**, 703-721.

Dickinson, W. J. (1988). On the architecture of regulatory systems: Evolutionary insights and implications. *BioEssays* **8**, 204-208.

Driscoll, M. and Chalfie, M. (1991). The *mec-4* gene is a member of a family of *Caenorhabditis elegans* genes that can mutate to induce neuronal degeneration. *Nature* **349**, 588-593.

Ellis, H. M. and Horvitz, H. R. (1986). Genetic control of programmed cell death in the nematode C. elegans. *Cell* **44**, 817-829.

Ferguson, E. L. and Horvitz, H. R. (1985). Identification and characterization of 22 genes that affect the vulval cell lineages of the nematode *Caenorhabditis elegans*. *Genetics* **110**, 17-72.

Finney, M. and Ruvkun, G. (1990). The *unc-86* gene product couples cell lineage and cell identity in C. elegans. *Cell* **63**, 895-905.

Fire, A., Harrison, S. W. and Dixon, D. (1990). A modular set of *lacZ* fusion vectors for studying gene expression in *Caenorhabditis elegans*. *Gene* **93**, 189-198.

Gierer, A. (1974). Molecular models and combinatorial principles in cell differentiation and morphogenesis. *Cold Spr. Harb. Symp. Quant. Biol.* **38**, 951-961.

Hall, D. H. and Russell, R. L. (1991). The posterior nervous system of the nematode *Caenorhabditis elegans*: Serial reconstruction of identified neurons and complete pattern of synaptic interactions. *J. Neurosci.* **11**, 1-22.

Hamelin, M., Scott, I. M., Way, J. C. and Culotti, J. G. (1992). The *mec-7* β-tubulin gene of *C. elegans* is expressed primarily in the touch receptor neurons. *EMBO J.* **11**, 2885-2893.

Hodgkin, J. (1983). Male phenotypes and mating efficiency in *Caenorhabditis elegans*. *Genetics* **103**, 43-64.

Hodgkin, J., Horvitz, H. R. and Brenner, S. (1979). Nondisjunction mutations of the nematode *Caenorhabditis elegans*. *Genetics* **91**, 67-94.

Horvitz, H. R. and Sulston, J. E. (1980). Isolation and genetic characterization of cell-lineage mutants of the nematode *Caenorhabditis elegans*. *Genetics* **96**, 435-454.

Ingham, P. W. (1988). The molecular genetics of embryonic pattern formation in *Drosophila*. *Nature* **335**, 25-34.

Johnson, G. D. (1989). Immunofluorescence. In *Antibodies, A Practical Approach* vol. 2. (ed. D. Catty), pp. 179-200. Oxford: IRL Press.

Johnson, P. F. and McKnight, S. L. (1989). Eukaryotic transcriptional regulatory proteins. *Ann. Rev. Biochem.* **58**, 799-839.

Kramer, J. M., French, R. P., Park, E.-U., Johnson, J. J. (1990). The *Caenorhabditis elegans rol-6* gene, which interacts with the *sqt-1* collagen gene to determine organismal morphology, encodes a collagen. *Molec. Cell. Biol.* **10**, 2081-2089.

Kunkel, T. A. (1985). Rapid efficient site-specific mutagenesis without phenotypic selection. *Proc. Natl. Acad. Sci. USA* **82**, 488-492.

Mello, C. C., Kramer, J. M., Stinchcomb, D. and Ambros, V. (1991). Efficient gene transfer in *C. elegans*: Extrachromosomal maintenance and integration of transforming sequences. *EMBO J.* **10**, 3959-3970.

Ruvkun, G. and Giusto, J. (1989). The *Caenorhabditis elegans* heterochronic gene *lin-14* encodes a nuclear protein that forms a temporal developmental switch. *Nature* **338**, 313-319.

Savage, C., Hamelin, M., Culotti, J. G., Coulson, A. Albertson, D. G. and Chalfie, M. (1989). *mec-7* is a β-tubulin gene required for the production of 15-protofilament microtubules in *Caenorhabditis elegans*. *Genes Dev.* **3**, 870-881.

Seydoux, G. and Greenwald, I. (1989). Cell autonomy of *lin-12* function in a cell fate decision in C. elegans. *Cell* **57**, 1237-1245.

Simmons, D. M., Voss, J. W., Ingraham, H. A., Holloway, J. M., Broide, R. S., Rosenfeld, M. G. and Swanson, L. W. (1990). Pituitary cell phenotypes involve cell-specific Pit-1 mRNA translation and synergistic interactions with other classes of transcription factors. *Genes Dev.* **4**, 695-711.

Sulston, J. E. and Horvitz, H. R. (1977). Post-embryonic cell lineages of the nematode, *Caenorhabditis elegans. Dev. Biol.* **56**, 110-156.

Sulston, J. E., Schierenberg, E. White, J. G. and Thomson, J. N. (1983). The embryonic cell lineage of the nematode *Caenorhabditis elegans*. *Dev. Biol.* **100**, 64-119.

Tautz, D. and Pfeifle, C. (1989). A non-radioactive in situ hybridization method for the localization of specific RNAs in *Drosophila* embryos reveals translational control of the segmentation gene *hunchback*. *Chromosoma* **98**, 81-85.

Trent, C., Tsung, N. and Horvitz, H. R. (1983). Egg-laying defective mutants of the nematode *Caenorhabditis elegans*. *Genetics* **104**, 619-647.

Walthall, W. W. and Chalfie, M. (1988). Cell-cell interaction in the guidance of late-developing neurons in *Caenorhabditis elegans*. *Science* **239**, 643-645.

Way, J. C. and Chalfie, M. (1988). *mec-3*, a homeobox-containing gene that specifies differentiation of the touch receptor neurons in C. elegans. *Cell* **54**, 5-16.

Way, J. C. and Chalfie, M. (1989). The *mec-3* gene of *Caenorhabditis elegans* requires its own product for maintained expression and is expressed in three neuronal cell types. *Genes Dev.* **3**, 1823-1833.

White, J. G., Southgate, E., Thomson, J. N. and Brenner, S. (1986). The structure of the nervous system of *Caenorhabditis elegans. Philos. Trans. R. Soc. Lond. B Biol. Sci.* **314**, 1-340.

White, J. G., Southgate, E. and Thomson, J. N. (1991). On the nature of undead cells in the nematode *Caenorhabditis elegans*. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **331**, 263-271.

Xue, D., Finney, M., Ruvkun, G and Chalfie, M. (1992) Regulation of the *mec-3* gene by the *C. elegans* homeoproteins UNC-86 and MEC-3. *EMBO J.* **11**, 4969-4979.

Xue, D., Tu, Y. and Chalfie, M. (1993) Cooperative interaction between the *C. elegans* homeoproteins UNC-86 and MEC-3. *Science* **261**, 1324-1328.

Yamamoto, K. R. (1985). Steroid receptor regulated transcription of specific genes and gene networks. *Ann. Rev. Genet.* **19**, 209-252.

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