The *mec-3* gene contains *cis*-acting elements mediating positive and negative regulation in cells produced by asymmetric cell division in *Caenorhabditis elegans*

Jeffrey C. Way¹, Lili Wang, Jin-Quan Run, and Alice Wang

Department of Biology, Busch Campus, Rutgers University, Piscataway, New Jersey 08855-1059 USA

The homeo box-containing genes *mec-3* and *unc-86* are necessary to specify the fate of a defined set of mechanoreceptors in *Caenorhabditis elegans*. Previous experiments have shown that *mec-3* expression can be divided into two phases: initial synthesis mediated in part by *unc-86*, and continued synthesis that requires *mec-3* itself. We now identify sequences that have been conserved during *Caenorhabditis* evolution and are necessary for establishment, maintenance, and repression of *mec-3* expression. Upstream of the start codon for the *mec-3*-coding sequence are four segments (regions I–IV) of 71, 29, 28, and 24 bp, which are almost identical between *C. elegans* and *Caenorhabditis vulgarensis*. Region I is the only conserved sequence that effects establishment of *mec-3* synthesis. Maintenance of *mec-3* expression is mediated primarily by region II. Repression appears to be controlled by several segments: Mutation of region III, region IV, and parts of region I in a *mec-3-lacZ* fusion results in β -galactosidase expression in some non-*mec-3*-expressing sisters of *mec-3*-positive cells. These results indicate that the *mec-3* 5' region contains target sequences that mediate a genetic switch between alternative fates expressed by sister cells in a stereotyped cell lineage.

[Key Words: *mec-3*; *C. elegans*; autoregulation; asymmetric cell division; *unc-86*; lineage]

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Mechanisms for choosing between two differentiated states are found throughout development. A λ -infected *Escherichia coli* cell can develop along a lytic or lysogenic pathway. Each fate is controlled by a distinct DNA-binding protein, λ repressor or Cro, whose expression is mutually exclusive (Ptashne 1987). The choice between the two sexual states in *Drosophila* is controlled at the level of RNA splicing, rather than transcription. The initial synthesis of *Sex-lethal* protein is controlled by the X-to-autosome ratio. Once this protein is synthesized, it modifies the pattern of its own splicing so that a coding form of mRNA is generated (Bell et al. 1988). In both of these examples, there is a protein whose expression represents commitment to a particular fate: λ repressor or *Sex-lethal*. Each protein has a distinct establishment phase of synthesis and is then necessary for the maintenance of its own expression. If synthesis of these regulatory genes is repressed or never established, an alternative fate is chosen.

Development of *Caenorhabditis elegans* is characterized by repeated cell divisions in which two sister cells

acquire different fates. To understand this process, we are studying *mec-3*, a homeo box-containing gene that is expressed in three sets of mechanoreceptors and appears to control their identity, thus playing a role similar to λ repressor or *Sex-lethal*. *mec-3* also has establishment and maintenance modes of synthesis. In a wild-type *C. elegans* strain carrying a *mec-3-lacZ* fusion, the fusion is expressed throughout development after each *mec-3*-synthesizing cell arises (Fig. 1A), whereas in a *mec-3* mutant, a *mec-3-lacZ* fusion is expressed in the correct set of cells but only transiently after each cell is produced. Thus, the *mec-3* product is proposed to be necessary for its own continued expression (Way and Chalfie 1988, 1989).

The *unc-86* gene is necessary for the production of *mec-3*-expressing cells and for the production and differentiation of many other neurons in *C. elegans* (Chalfie et al. 1981; Way and Chalfie 1989; Finney and Ruvkun 1990). In *unc-86* mutants, patterns of cell division and differentiation are altered so that in place of *mec-3*-expressing cells and their sisters, other cell types or incorrectly differentiating cells are produced. The *unc-86* protein is expressed in each cell that is a parent of a *mec-3*-expressing cell; and after this cell divides, *unc-86* is

¹Corresponding author.

Figure 1. Cells that can express *mec-3-lacZ* in *C. elegans*. (A) Schematic diagram of *C. elegans* showing cells that normally express *mec-3* (solid ovals; Way and Chalfie 1989) and additional cells (open ovals) that express *mec-3-lacZ* from plasmids with alterations in the *mec-3* promoter (see Tables 1 and 2). (B) A young L2 animal carrying pR4delA-Z (*jeEx8*), seen from the dorsal aspect. This animal shows expression in the SDQR and SDQL cells in the middle of the animal and in the ALNR cell in the tail, as well as having β -galactosidase activity in most of the normal *mec-3*-expressing cells. (The PVD neurons have not arisen by this stage of development. This particular animal appears to be a genetic mosaic that has lost the *jeEx8* element from PLML and ALNL.) (C) The tail of an L2 animal carrying pR4delA-Z, viewed from the left side, showing β -galactosidase activity in the PLM cells and their sisters, the ALNs.

detectable in both the *mec-3*-expressing cell and its non-*mec-3*-expressing sister (Fig. 2). In an *unc-86* mutant, *mec-3* is never expressed, which may be the primary reason why subsequent mechanoreceptor differentiation does not occur. *unc-86* is thus necessary for establishment of *mec-3* expression.

The role of *mec-3* in terminal differentiation is best understood for the six touch receptor neurons, which are a subset of the *mec-3*-expressing cells (Chalfie and Au 1989; Way 1990; Wolinsky and Way 1990). Mutations in *mec* (mechanosensory) genes cause defects in the touch receptors, so that mutant animals are insensitive to a gentle touch with an eyebrow hair. *mec-1*, *-2*, and *mec-*

4–17 are necessary for the function of the touch receptors, whereas *mec-3* is necessary for the overall differentiation of these cells. Two genes in the former class have also been cloned: *mec-7* is a touch receptor-specific β -tubulin (Savage et al. 1989; M. Hamelin, I. Scott, J. Way and J. Culotti, in prep.), whereas *mec-4* encodes an apparent membrane protein (Driscoll et al. 1991). *mec-3* may directly turn on genes such as *mec-4* and *mec-7*. In the other cells in which *mec-3* is expressed, analogous “downstream” genes have not been identified. The sisters of the 10 *mec-3*-expressing cells do not synthesize *mec-3* (except perhaps transiently after they are produced; Fig. 1B; see below) and are unaffected in *mec-3* mutants (Way and Chalfie 1989). *mec-3* is therefore a plausible candidate “switch” gene whose expression represents commitment of a cell to a particular developmental program.

To understand how *mec-3* is expressed in only one cell after an asymmetric cell division and only upon termination of cell proliferation, a detailed analysis of control sequences in the *mec-3* promoter has been initiated. The results that follow indicate that the *mec-3* 5' region includes discrete sequences for establishment and maintenance of *mec-3* synthesis, as well as regions for repression of *mec-3* in the sisters of cells that normally express this gene.

Figure 2. Relationship between *mec-3* and *unc-86* expression in *C. elegans*. Some *unc-86*-expressing cells (hatched regions) divide several times to produce sets of related cells that contain this protein. At most, one daughter of such a cell will express *mec-3*: This cell is always an anterior daughter. Other cells express *unc-86* after the final cell division: These cells never express *mec-3* (Way and Chalfie 1989; Finney and Ruvkun 1990). These results suggest that a cell must go through a division cycle while expressing *unc-86* to express *mec-3*. Some alterations of the *mec-3* 5' region cause expression in the posterior sisters of a subset of *mec-3*-expressing cells. These changes may disrupt the control of asymmetric expression of *mec-3*. (The cases of ectopic expression all occur in lineages in which the *unc-86*-expressing cell divides twice before *mec-3* is expressed, the significance of which is unclear.)

Results

Isolation of a *mec-3* homolog and identification of *mec-3* sequences conserved during evolution

Candidate sequences in the *mec-3* gene that could regulate its expression were initially identified by a comparison of the *mec-3* gene from two species. This revealed several conserved noncoding sequences. Such sequences are presumed to be conserved because they are positively selected during evolution.

Using low-stringency cross-hybridization, an apparent *mec-3* homolog from *Caenorhabditis vulgarensis* (C. Link, pers. comm.) was identified. This species is similar to *C. elegans* in size, appearance, and behavior. *C. vul-*

garensis also has a touch response like *C. elegans*, suggesting that it would have touch receptor neurons and a *mec-3* homolog to control their development. The plasmid pJW91-1 (Fig. 3A), which carries a 3.0-kb DNA fragment from *C. vulgarensis* that cross-hybridizes with *C. elegans mec-3*, can rescue *C. elegans* strains carrying the *mec-3* alleles *u6* or *u298* upon microinjection (see Materials and methods), indicating that this DNA corresponds to *mec-3* itself and not a functionally distinct gene.

The 3.0-kb fragment from *C. vulgarensis* contains sequences corresponding to all of the exons of *mec-3* in *C. elegans* (Fig. 3A). Comparison of the two sequences indicates that the first two *mec-3* exons proposed by Way and Chalfie (1988) are incorrect and that the correct protein sequence of *C. elegans mec-3* corresponds to that shown in Figure 3B. This interpretation of the coding sequence is consistent with that proposed by Freyd et al. (1990) because of the similarity of *mec-3* to the *lin-11* protein sequence. The amino acid sequence of the *mec-3* protein from the two species is 91% identical (Fig. 3B).

Intron sequences appear to be completely unrelated between the two species. For example, the first intron in *C. elegans mec-3* is 496 bp in length, whereas in *C. vulgarensis* this intron is only 73 bp long. The lengths of the other introns are also not conserved, except for the short intron within the homeo box, which is 44 bp in *C. vulgarensis* and 43 bp in *C. elegans*. Within this intron, 11 of 31 bp between the donor and acceptor sites can be aligned.

Upstream of the coding sequence are four long stretches of conserved bases (regions I–IV), as well as several shorter regions of similarity (Fig. 4A). Regions I–IV are 71, 29, 28, and 24 bp long, respectively. These regions may include binding sites for the *unc-86* and *mec-3* proteins which, on the basis of genetic evidence, are known to regulate *mec-3* (Fig. 4B; Way and Chalfie 1989). *unc-86* is a member of the POU family of homeo domain proteins (Ruvkun and Finney 1991). From the known binding sites of other members of this family, the consensus CAT[–]T/A^TAATNCAT has been derived, which is similar to the sequence CATNNGAAATGCAT that occurs at the 5' side of both regions I and II (Fig. 4B). The *mec-3* protein sequence is related to that of the pancreatic islet transcription factor Isl-1, which binds to the sequence TTAATAATCTAA in the insulin gene (Karls-son et al. 1990). A closely related sequence, TTAATAATCGAT, is found in region III, and a somewhat less related sequence, TCTATTATCGTC, is found in region II. However, because only one binding site for any member of the LIM family of proteins has been identified, the significance of the latter sequence relationships is unclear.

In the last intron of *C. vulgarensis mec-3*, there is a small region (base pairs 2442–2532), immediately 5' to the last exon, that is similar to the corresponding region in *mec-3* of *C. elegans*. In this region, 63 of 91 bases are identical (with two gaps in the alignment), and it includes one stretch of 18 identical base pairs. This sequence might be important for RNA processing or some

other function that determines the absolute level of *mec-3* synthesis but does not appear to be involved in cell-specific regulation of *mec-3* (see below; data not shown). This conserved region has not been studied further.

Function of regions I–IV in *mec-3* regulation

To determine the function of conserved noncoding regions, various parts of *mec-3* from a *mec-3-lacZ* fusion were mutated or deleted, and effects on expression of β -galactosidase in *C. elegans* were examined (Table 1). To confirm some assignments of function to certain sequences, the same mutations were introduced into an otherwise intact *mec-3* gene to examine the effect on its ability to rescue a *mec-3* mutant (Fig. 4). With a wild-type *mec-3-lacZ* fusion (pTU28/*uEx4*, where pTU28 is a *mec-3-lacZ* fusion plasmid and *uEx4* is the extrachromosomal array derived from it; Table 1; Way and Chalfie 1989), β -galactosidase synthesis is seen in only 10 cells in *C. elegans*. Expression is first seen after the final division that produces these cells (Fig. 2), and can be observed for the remainder of development. Of the constructs described below, some give a staining pattern identical to the wild-type pattern, some show no expression or expression that is uniformly reduced across all stages in each cell type, and some show regulatory defects in establishment, maintenance, or repression (Table 1; Figs. 1 and 4). For all of these constructs, the 10 *mec-3*-expressing cells appear to be affected equally: There are no constructs that cause a significant reduction in a subset of cells. None of the plasmids cause expression in any other cell type besides the normal cells or their sisters.

If sequences outside the conserved upstream regions are deleted (upstream to base pair 1494 and downstream from base pair 1968) there is no observable effect on expression of a *mec-3-lacZ* fusion (pUpsA-Z in Table 1; data not shown). In addition, sequences up to base pair 1494 can be removed without affecting the ability of an intact *mec-3* gene to rescue the *mec-3* mutants *u298* or *u6* (Way and Chalfie 1989; data not shown).

Regions I, II, and III are sufficient to mediate expression in the 10 *mec-3*-expressing cells when coupled to heterologous promoter elements. To test the conserved regions in the *mec-3* promoter for enhancer activity, restriction fragments containing parts of the *mec-3* upstream region were fused to a *C. elegans* HSP16-41 heat shock promoter (Fig. 5; Jones et al. 1986). pR123-HS contains regions I, II, and III (base pairs 1418–1870) fused to a single heat shock enhancer element, the heat shock TATA box, followed by *lacZ* and a 3'-end region from the *C. elegans* myosin heavy-chain gene *unc-54*.

C. elegans that carries pR123-HS does not express detectable β -galactosidase activity unless it is heat-shocked, whereupon *lacZ* expression can be seen only in the 10 *mec-3*-expressing cells. Apparently the activity of either the *mec-3* or heat shock enhancer alone is insufficient to stimulate detectable *lacZ* expression, but the two sets of enhancers must be active in the same cell at

Figure 3. (A) The sequence of the *mec-3* gene from *C. vulgarens* (the insert from pJW91.1). This region contains an intact *mec-3* gene, as defined by its ability to rescue the *C. elegans* *mec-3* mutants *u6* and *u298*. The coding sequences shown are inferred from comparison with the *C. elegans* sequence. (B) Alignment of the *mec-3* protein sequences from *C. vulgarens* (top) and *C. elegans* (bottom). The *mec-3* protein sequence can be divided into three regions: a cysteine- and histidine-rich LIM region consisting of two LIM repeats (Freyd et al. 1990), a homeodomain, and an acidic region encoded by the final exon (Way and Chalfie 1988). The sequence alignment shown here suggests that the first two *C. elegans* *mec-3* exons are at base pairs 2021–2098 and 2494–2544 and are shown incorrectly in Way and Chalfie (1988).

the same time and can “cooperate” to mediate *lacZ* transcription. This type of cooperativity is a common property of enhancers (Kakidani and Ptashne 1988; Webster et al. 1988) and does not suggest anything unusual about the *mec-3* or HSP-16 regulatory sequences. This result indicates that a restriction fragment carrying conserved regions I, II, and III can cause cell-specific transcriptional activation in *mec-3*-expressing cells.

Establishment of *mec-3* expression

Sequences including region I, but not regions II, III, or IV, are necessary and sufficient for establishment expression of *mec-3*. A *mec-3-lacZ* fusion that lacks region I and upstream sequences does not express β -galactosidase activity in a *mec-3*-independent manner (pR1delA-Z, Table 1; Fig. 4D). However, this region I (–) *mec-3-lacZ* fusion is still expressed in a *mec-3*(+) strain, indicating that region I is not required for maintenance of *mec-3* expres-

Figure 4. Regions of similarity between the *mec-3* 5' regions from *C. elegans* and *C. vulgarens* and their functions. (A) Computer alignment of *mec-3* 5' regions from *C. vulgarens* (top) and *C. elegans* (bottom; Way and Chalfie 1988). Vertical lines show identical base pairs. In addition, a 13-nucleotide region of identity found between region IV and the start codon is underlined (base pairs 546–568 in *C. vulgarens* and 1958–1970 in *C. elegans*), as is the start codon. Alterations introduced into the *C. elegans* intact *mec-3* gene or *mec-3-lacZ* fusion are also shown. (B) Relationship of conserved *mec-3* 5' sequences to target sites for POU proteins (Ruvkun and Finney 1991). Because *unc-86* is a member of this family, it may bind to these sequences. (C) Computer alignment of conserved regions within the final intron of *mec-3* from *C. vulgarens* and *C. elegans*. (D) Effects of mutations and deletions of conserved upstream regions on expression of a *mec-3-lacZ* fusion or on the ability of an intact *mec-3* gene to rescue the *mec-3(u6)* mutant. Transient rescue by intact *mec-3* shows the ability of each construct, when injected into *mec-3(u6)* with the Roller plasmid pRF4, to rescue touch receptor function: Shown is the number of touch-sensitive animals over the total number of Rollers identified among progeny of injected animals. The parent of the intact *mec-3* plasmids described here is pTU48 (Table 2; for construction, see Materials and methods). For the deletions shown, the lines represent DNA that remains in a construct. (*) Only plasmid pR1B-I was tested.

sion. Compared with a nonmutant *mec-3-lacZ* fusion, *lacZ* from pR1delA-Z is expressed at a reduced level. Sequences removed by this deletion may play a role in determining the overall level of *mec-3* transcription.

A DNA fragment containing only region I can mediate transient gene expression in *mec-3*-expressing cells after these cells arise, suggesting that region I is sufficient to direct establishment but not maintenance synthesis of *mec-3*. The plasmid pR1-HS contains a *mec-3* restriction fragment with only conserved region I fused to the *C. elegans* HSP16-41 heat shock promoter (Fig. 5). This construct is similar to pR123-HS except that it lacks regions II and III. Like pR123-HS, the *mec-3* and heat shock promoter elements are insufficient by themselves to induce transcription but appear to cooperate when both are active.

When *C. elegans* carrying pR1-HS are heat-shocked, β -galactosidase activity can be seen in the postembryonic AVM, PVM, and PVD neurons shortly after these cells arise but not at later times. In a mixed population of L1-L4 larval animals carrying pR1-HS, 8 of 60 showed expression in either AVM, PVM, or one or both PVDs. Only animals at ~11–14 hr of postembryonic development show expression in AVM or PVM. Because the heat shock is for 1 hr, followed by a 2-hr recovery, this result suggests that the regulatory elements that mediate *mec-3* establishment are active at ~8–11 hr of development, which is about the time that these cells arise (Sulston and Horvitz 1977).

We interpret these results by assuming that the conserved regions can function as enhancers. Other models are formally possible, but all would require that region I respond to signals present only in the *mec-3*-expressing cells around the time they are generated.

Mutation of the POU consensus site within region I does not affect establishment of *mec-3* expression (pR1B-Z, Table 1). It is possible that other nonconserved sequences upstream of region I can also mediate establishment, that mutation 1B does not abolish function of this site, or that *unc-86* does not act through this site.

Maintenance of mec-3 expression is mediated primarily by region II

A mutation in region II causes a *mec-3-lacZ* fusion to be expressed in a wild-type *C. elegans* strain in the correct set of cells for only a short period after they arise, after which all expression is lost (pR2-Z, Table 1). This pattern is the same as that seen for an intact *mec-3-lacZ* fusion in a *mec-3* mutant background (Table 1; Way and Chalfie 1989). In addition, an otherwise intact *mec-3* gene with a region II mutation cannot rescue a *mec-3* mutant (Fig. 4). A deletion that removes both region I and part of region II (pR1+2-Z, Table 1) prevents all expression, suggesting that both establishment and maintenance expression have been abolished.

A region II mutation in the chromosomal *mec-3* gene is defective in maintenance of touch receptor function. The *mec-3* mutation *u298* has a transposon Tc1 insertion in region II of *mec-3*. Most *u298* young larvae are

detectably touch sensitive, whereas animals carrying the strong *mec-3* allele *u6* newly hatched larvae are touch insensitive throughout development. In one experiment in which animals <4 hr old were tested for touch sensitivity in the tail, 48 of 70 *u298* larvae and 4 of 61 *u6* larvae responded to this stimulus. In later stages, touch sensitivity is completely absent in both *mec-3* mutants (J. Way, unpubl.; Chalfie and Au 1989). It thus appears that in *u298* animals, *mec-3* is expressed transiently in the PLM neurons and mediates their differentiation into touch receptors. However, *mec-3* synthesis is not maintained, so synthesis of touch receptor-specific structural genes is discontinued; and as the animal and the touch receptor grow larger, the cell can no longer function.

Together, these results indicate that alteration of region II causes a defect in *mec-3* maintenance, which is mediated by *mec-3* itself. If such a mutation alters *mec-3* action upon itself, then the level of *lacZ* expression in a *mec-3* (–); *mec-3-lacZ* (region II –) strain should be the same as in a strain that is mutant only in *mec-3* or region II. Alternatively, if the mutation in region II somehow simply causes an overall reduction in expression of *mec-3-lacZ* fusion, or an array carrying such a construct is somehow defective, then a chromosomal *mec-3* mutation should further reduce expression of the fusion. The first possibility appears to hold: When the *mec-3-lacZ* fusion carrying a region II mutation is crossed into a *mec-3(u6)* background, its expression level is not reduced and is no less than that of a nonmutant fusion [Table 1, cf. pTU28; *mec-3(u6)*, pR2-Z, and pR2-Z; *mec-3(u6)*]. This implies that the alteration in region II interferes with the action of *mec-3* on its own promoter. [In fact, expression from the *mec-3-lacZ* (region II –) fusion is somewhat higher in *mec-3(u6)* than in *mec-3(+)*; we have no explanation for this result.]

Region II contains a POU consensus site, suggesting that *unc-86* might bind to this region, but thus far genetic data failed to show a role for *unc-86* in the continued expression of *mec-3*. The temperature-shifting of the *unc-86-ts* mutant, *n848*, after the establishment phase of *mec-3* expression has no detectable effect on either touch sensitivity or expression of the wild-type *mec-3-lacZ* fusion. (M. Finney, pers. comm.; J. Way, unpubl.).

A mutation in region III may have a weak effect on maintenance of *mec-3-lacZ* fusion expression (pR3-Z in Table 1). Mutations in region I or region IV have no effect on maintained expression (Table 1).

Sequences for repression of mec-3

Mutations in region III, region IV, and some sites in region I all cause expression of *mec-3-lacZ* fusion in some sisters of the normal *mec-3*-expressing cells. Staining of these cells is seen most dramatically after the cells arise and gradually decreases during development. In L1 and L2 larvae, β -galactosidase expression can be seen in the ALN, SDQ, and AIZ neurons, which are sisters of the PLM, AVM/PVM, and FLP cells, respectively (Fig. 1; Sulston et al. 1983; data not shown). AIZ and SDQ can be identified by their position in DAPI-stained animals,

Table 1. Expression of *mec-3-lacZ* fusions in the embryonic ALM, PLM, FLP, and PLM sister (ALN) neurons at different stages of *C. elegans* development

Plasmid	Host genotype	Stage of development					
		L1 larvae	L2 larvae	L3 larvae	L4 larvae	young adult	gravid adult
Nonmutant elements							
pTU28(<i>uEx4</i>)	<i>mec-3(+)</i>						
ALM, PLM, and FLPs		63% (96)	83% (54)	69% (54)	67% (30)	72% (18)	71% (24)
Putative PLM sisters		0% (32)	0% (18)	0% (18)	0% (10)	0% (6)	0% (8)
pTU28(<i>jeIn2</i>)	<i>mec-3(+)</i>			Experiment A			
ALM, PLM, and FLPs		74% (114)	61% (36)	60% (42)	61% (18)	60% (30)	69% (36)
Putative PLM sisters		0% (38)	0% (12)	0% (14)	0% (6)	0% (10)	0% (12)
pTU28(<i>jeIn2</i>)	<i>mec-3(+)</i>			Experiment B			
ALM, PLM, and FLPs		76% (42)	75% (102)	74% (54)	73% (30)	56% (18)	—
Putative PLM sisters		0% (14)	0% (34)	0% (18)	0% (10)	0% (6)	—
pTU28(<i>uEx4</i>)	<i>mec-3(u6)</i>			Experiment A			
ALM, PLM, and FLPs		43% (108)	0% (18)	—	—	—	0% (42)
Putative PLM sisters		6% (36)	0% (6)	—	—	—	0% (14)
pTU28(<i>uEx4</i>)	<i>mec-3(u6)</i>			Experiment B			
ALM, PLM, and FLPs		56% (30)	22% (18)	10% (42)	3% (30)	0% (36)	0% (42)
Putative PLM sisters		20% (10)	0% (6)	0% (14)	0% (10)	0% (12)	0% (14)
pTU28(<i>uEx4</i>)	<i>mec-3(u298)</i>						
ALM, PLM, and FLPs		30% (30)	33% (30)	2% (42)	0% (18)	0% (30)	0% (48)
Putative PLM sisters		0% (10)	0% (10)	0% (14)	0% (6)	0% (10)	0% (16)
pUpsA-Z	<i>mec-3(+)</i>						
ALM, PLM, and FLPs		72% (36)	38% (24)	50% (6)	30% (30)	33% (6)	40% (90)
Putative PLM sisters		0% (12)	0% (8)	0% (2)	0% (10)	0% (2)	0% (30)
pR123-HS	<i>mec-3(+)</i>						
ALM, PLM, and FLPs		59% (54)	77% (84)	42% (12)	53% (30)	54% (24)	40% (48)
Putative PLM sisters		0% (18)	0% (28)	0% (4)	0% (10)	0% (8)	0% (16)
Region I alterations							
pR1delA-Z	<i>mec-3(+)</i>						
ALM, PLM, and FLPs		13% (30)	21% (24)	17% (24)	22% (18)	17% (36)	8% (12)
Putative PLM sisters		0% (10)	0% (6)	0% (8)	0% (6)	0% (12)	0% (4)
pR1delA-Z	<i>mec-3(u6)</i>						
ALM, PLM, and FLPs		0% (96)	0% (48)	0% (72)	0% (18)	—	0% (24)
Putative PLM sisters		0% (32)	0% (16)	0% (24)	0% (6)	—	0% (8)
pR1A-Z	<i>mec-3(+)</i>						
ALM, PLM, and FLPs		78% (42)	70% (90)	81% (54)	83% (30)	78% (36)	42% (12)
Putative PLM sisters		0% (14)	0% (30)	0% (18)	0% (10)	0% (12)	0% (4)
pR1B-Z	<i>mec-3(+)</i>						
ALM, PLM, and FLPs		89% (36)	91% (66)	67% (48)	75% (12)	78% (18)	72% (36)
Putative PLM sisters		0% (12)	0% (22)	0% (16)	0% (4)	0% (6)	0% (12)
pR1B-Z	<i>mec-3(u6)</i>						
ALM, PLM, and FLPs		56% (18)	23% (30)	14% (42)	13% (48)	—	—
Putative PLM sisters		0% (6)	0% (10)	0% (14)	0% (16)	—	—
pR1C-Z	<i>mec-3(+)</i>						
ALM, PLM, and FLPs		42% (42)	38% (42)	50% (12)	17% (24)	30% (30)	22% (18)
Putative PLM sisters		43% (14)	21% (14)	25% (4)	0% (8)	0% (10)	0% (6)
pR1D-Z	<i>mec-3(+)</i>						
ALM, PLM, and FLPs		83% (30)	100% (6)	75% (12)	—	58% (24)	29% (54)
Putative PLM sisters		80% (10)	100% (2)	25% (4)	—	0% (8)	6% (18)
pR1E-Z	<i>mec-3(+)</i>						
ALM, PLM, and FLPs		63% (24)	94% (48)	61% (18)	100% (6)	25% (12)	83% (48)
Putative PLM sisters		25% (8)	6% (16)	0% (6)	0% (2)	0% (4)	0% (16)
Region II alterations							
pR2-Z	<i>mec-3(+)</i>						
ALM, PLM, and FLPs		27% (60)	2% (42)	1% (102)	0% (36)	0% (42)	0% (54)
Putative PLM sisters		18% (20)	7% (14)	3% (34)	0% (12)	0% (14)	0% (18)
pR2-Z	<i>mec-3(u6)</i>			Experiment A			
ALM, PLM, and FLPs		52% (96)	19% (36)	—	—	—	3% (36)
Putative PLM sisters		32% (16)	8% (12)	—	—	—	0% (12)

(Table 1 continued on next page)

Table 1. (Continued)

Plasmid	Host genotype	Stage of development					
		L1 larvae	L2 larvae	L3 larvae	L4 larvae	young adult	gravid adult
pR2-Z	<i>mec-3(u6)</i>	Experiment B					
ALM, PLM, and FLPs		50% (66)	36% (30)	6% (18)	13% (24)	13% (30)	10% (48)
Putative PLM sisters		23% (22)	40% (10)	33% (6)	0% (8)	0% (10)	6% (16)
Region I and II alterations							
pR1 + 2-Z	<i>mec-3(+)</i>						
ALM, PLM, and FLPs		0% (18)	0% (42)	—	0% (36)	0% (18)	0% (54)
Putative PLM sisters		0% (6)	0% (14)	—	0% (12)	0% (6)	0% (18)
Region III alterations							
pR3-Z (<i>jeEx5</i>)	<i>mec-3(+)</i>	Experiment A					
ALM, PLM, and FLPs		67% (66)	—	67% (6)	28% (18)	28% (42)	10% (66)
Putative PLM sisters		58% (22)	—	0% (2)	0% (6)	7% (14)	5% (22)
pR3-Z (<i>jeEx5</i>)	<i>mec-3(+)</i>	Experiment B					
ALM, PLM, and FLPs		88% (24)	92% (24)	55% (60)	83% (18)	83% (6)	6% (48)
Putative PLM sisters		50% (8)	63% (8)	25% (20)	16% (6)	0% (2)	0% (8)
pR3-Z (<i>jeIn5</i>)	<i>mec-3(+)</i>						
ALM, PLM, and FLPs		90% (72)	79% (42)	—	72% (18)	33% (12)	—
Putative PLM sisters		25% (24)	35% (14)	—	0% (6)	25% (4)	—
pR3-Z (<i>jeIn5</i>)/+	<i>mec-3(+)</i>						
ALM, PLM, and FLPs		73% (90)	43% (96)	35% (60)	30% (30)	0% (6)	28% (18)
Putative PLM sisters		53% (30)	22% (32)	0% (20)	0% (10)	0% (2)	0% (6)
Region IV alterations							
pR4delA-Z (<i>jeEx8</i>)	<i>mec-3(+)</i>	Experiment A					
ALM, PLM, and FLPs		42% (36)	27% (54)	25% (36)	17% (18)	25% (12)	26% (42)
Putative PLM sisters		75% (12)	67% (18)	17% (12)	17% (6)	0% (4)	14% (14)
pR4delA-Z (<i>jeEx8</i>)	<i>mec-3(+)</i>	Experiment B					
ALM, PLM, and FLPs		50% (108)	60% (36)	26% (42)	100% (12)	—	38% (48)
Putative PLM sisters		69% (36)	50% (12)	0% (14)	50% (4)	—	13% (16)

To determine the importance of conserved sequences in the *mec-3* 5' region, deletions and point mutations were introduced into a wild-type *mec-3-lacZ* fusion plasmid and tested for their effect on expression of β -galactosidase. These data show that for a wild-type fusion, the frequency of staining cells is approximately constant during development and that the expression of the fusion in extra cells in the tail usually can not be detected. Certain mutant constructions cause a decrease in the frequency of staining cells during development, indicating a defect in maintenance of expression of the fusion. Other constructs give a high frequency of staining of extra cells in the tail, suggesting a defect in repression. *mec-3-lacZ* fusion plasmids were injected into *C. elegans* with the Twitcher plasmid pPD10.41 to generate inherited extrachromosomal arrays of these two plasmids. Some extrachromosomal elements were integrated by gamma-ray treatment (see Materials and methods) (*jeIn*-elements), *jeEx* elements are extrachromosomal. All tested plasmids are part of extrachromosomal elements unless otherwise noted. Transgenic animals were stained with X-gal and scored as described in Materials and methods. Percentages shown represent the sum of the ALM, PLM, and FLP neurons, the *mec-3*-expressing cells that arise during embryogenesis, divided by the number of such cells that were present (shown in parentheses). When one or two cells in the tail were stained, these were scored as PLM cells. Putative PLM sisters indicates more than two cells and are presumably ALNs (for identification, see Materials and Methods). Certain elements were crossed into the *mec-3* mutants *u6* or *u298* to examine how a lack of *mec-3* would affect the expression from a given construct. (—) No animals of this stage were sampled. All of the data shown are from representative observations. Data are shown from two experiments (A and B) to illustrate the degree of variation from one set of observations to the next. *jeIn5*/+ worms were examined to determine whether reduction of plasmid copy number affected expression in PLM sisters. These animals are heterozygous for the integrated *jeIn5* element and were generated by crossing a marked *jeIn5* strain with N2 males (see Materials and methods). Southern blot analysis indicates that *jeIn5*/+ animals have about one-half as many copies of the *mec-3-lacZ* fusion as *uEx4* animals (see Materials and methods).

whereas the ALNs are in a cluster of neurons in the tail. The additional staining cells in the tail (Fig. 1C) appear to be the ALNs because they can be seen immediately after hatching (data not shown), and because, with the PLMs, these are the only paired cells in the tail that express *unc-86* at this time (Finney and Ruvkun 1990). Moreover, staining of more than four cells was never seen, suggesting that the products of the *unc-86*-requir-

ing T lineage do not express *mec-3-lacZ* from these constructs (Sulston and Horvitz 1977; Chalfie et al. 1981). Staining of the BDUs, sisters of the ALMs, was not seen in hatched animals. Occasionally, developing embryos with 12 staining cells were observed, but the identities of these cells have not been determined. Staining of the PVD sisters, which undergo programmed cell death, was seen very rarely. For all of the *mec-3-lacZ* constructs

Figure 5. Plasmids carrying *mec-3*-heat shock hybrid promoters. Plasmids pR123-HS and pR1-HS contain regions I–III and region I, respectively, fused to elements of the heat-inducible *C. elegans* HSP16-41 promoter (Jones et al. 1986). The fusion junction is within the heat shock element. *lacZ* is fused in-frame to the HSP16-41-coding sequence (E. Stringham and P. Candido, pers. comm.)

that cause expression in extra cells, the ALNs are seen most often, whereas the AIZ and SDQ cells are seen (stained) less often. For example, for animals with pR3-Z, which carries the region III mutant fusion, the population of L1s and L2s shown in Table 1 had staining in 21 of 38 ALNs, 5 of 14 SDQs, and 7 of 38 AIZs.

Staining of presumptive ALN cells in the tail can also be seen in L1 stage larvae carrying *uEx4*, other extra-chromosomal elements carrying pTU28 (the nonmutant *mec-3-lacZ* fusion), or other constructs with mutations outside of conserved noncoding sequences, although the staining of additional cells is seen at a lower frequency and does not persist as long (Table 1). Expression of β -galactosidase from wild-type constructs in other sisters of the normal cells is seen in at most 1–10% of such cells. [Expression of the wild-type fusion in extra cells in *mec-3* mutant backgrounds (Table 1) is generally within this range and does not suggest that *mec-3* plays any role in these cells.] The additional *mec-3*-expressing cells in the tail probably correspond to the occasional extra *mec-4(d)*-expressing cells in the tail noted by Way and Chalfie (1988). Expression of *mec-3-lacZ* in additional cells is not the result of increased plasmid copy number in animals carrying mutant *mec-3-lacZ* fusions (Table 1; Materials and methods).

The expression in extra cells is transient, suggesting that expression of the *mec-3-lacZ* fusion cannot be maintained because *mec-3* itself is not expressed in these cells. None of the constructs tested caused ectopic *mec-3-lacZ* expression in cells besides sisters of the usual *mec-3*-expressing cells.

These results indicate that several different sequences must be intact for *mec-3* to be repressed in the sisters of

the cells that normally express *mec-3*. Because there is no apparent relationship between these sequences, it may be that distinct proteins must act at various sites in the *mec-3* 5' region to prevent *mec-3* expression in sister cells.

Discussion

The data presented here indicate that the *mec-3* gene contains a large region involved in the initial establishment of *mec-3* synthesis, a distinct site necessary for *mec-3*-dependent maintenance expression, and additional sequences for repression of *mec-3* in sisters of cells that normally express this gene (Fig. 4D). These results suggest that the 5' region of the *mec-3* gene controls certain asymmetric cell divisions by allowing *mec-3* expression in one of two sister cells and repressing this expression in the other. The lines of evidence supporting these conclusions are as follows:

1. A comparison of the *mec-3* sequences from *C. elegans* and *C. vulgarensis* reveals four blocks of conserved noncoding sequence (regions I–IV) upstream from the apparent start codon for the *mec-3*-coding sequences (Fig. 3A). Because they have been conserved in the course of evolution, these sequences may be important regulatory sites.
2. A large upstream sequence that includes region I and not regions II–IV is necessary for establishment, but not maintenance, of *mec-3* expression. When this upstream sequence is coupled to heterologous promoter elements, it can stimulate transcription in *mec-3*-expressing cells as they arise but not at later times.
3. Alteration of region II causes a defect in maintenance expression of *mec-3*. Alterations in region I or IV cause other defects but still allow maintenance synthesis of *mec-3* while alteration of region III has a slight effect on continued synthesis of a *mec-3-lacZ* fusion (Table 1).
4. Mutations in regions III, IV, and parts of region I can cause transient expression of a *mec-3-lacZ* fusion in some sisters of the normal *mec-3*-expressing cells.

Pattern of *mec-3* expression

mec-3 is expressed in 10 neurons in *C. elegans*. These neurons share several features: All appear to be mechanoreceptors, and each one is the anterior daughter of an asymmetric cell division. In addition, these cells express the *unc-86* protein, their parent cells express *unc-86*, and both the *mec-3*-expressing cells and their sisters are absent in *unc-86* mutants (Fig. 2; Chalfie et al. 1981; Way and Chalfie 1989; Finney and Ruvkun 1990). These common features suggest that *mec-3* expression may be regulated in the same way in each of the 10 cells that synthesize it.

One way to identify candidate regulatory sequences in a gene is to compare sequences of the gene from two related species. For example, several important regulatory sequences in the *Drosophila* DOPA decarboxylase

gene were first identified by comparison of noncoding sequences from *Drosophila melanogaster* and *D. virilis* (Bray et al. 1988). The nematode strain *C. vulgarensis* is physically similar to *C. elegans* and has a *mec-3* homolog capable of substituting for the *mec-3* gene of *C. elegans*. The DNA sequences of *mec-3* from each species are similar only in the coding sequence, a region within the 350 bp 5' of the *mec-3* start codon, and a small region in the last intron of the gene. The sequences show no other similarity: The introns are different in both length and sequence, suggesting that these two genes have diverged in the course of evolution to the point where only functionally important DNA sequences are conserved.

Establishment and maintenance of mec-3 synthesis

Previous work defined two modes of *mec-3* expression (Way and Chalfie 1989). The initial establishment of *mec-3* synthesis is independent of the *mec-3* product but requires *unc-86*. Subsequent expression for the remainder of development is maintained by the *mec-3* product. *unc-86* may act directly on *mec-3*: In the lineages that produce *mec-3*-expressing cells, *unc-86* is only expressed a few hours before *mec-3* (in Fig. 2, the time between cell divisions is ~2 hr), and in a large screen for mutants affecting the *C. elegans* touch receptors, no genes were identified that act between *unc-86* and *mec-3* during touch receptor differentiation (Chalfie and Au 1989).

The analysis of the *mec-3* promoter presented here has identified *cis* regions that mediate establishment and maintenance of *mec-3*. Region I and surrounding sequences mediate establishment of *mec-3*, so *unc-86* may bind to this region. (One candidate site for *unc-86* binding would be the POU consensus site in region I, but mutation of this site alone has no effect on *mec-3* regulation.) By the same reasoning, *mec-3* would act through region II. Regions I and II are large enough that several different proteins may bind to these sites, so there may be additional unidentified regulatory proteins necessary for *mec-3* establishment and maintenance. Region I may be particularly complex, because it is 71 bp, and mutation of some sites within this region leads to expression in the sisters of the normal *mec-3*-expressing cells.

None of the deletions or mutations examined cause expression of *mec-3-lacZ* only in a subset of the normal *mec-3*-expressing cells (legend to Table 1). This would be predicted if *mec-3* were sufficient for its own maintenance and there were no subtype-specific factors required for maintenance expression.

Repression of mec-3 synthesis

Mutational analysis of the *mec-3* 5' region revealed an additional mode of *mec-3* regulation: repression in sisters of cells that normally express *mec-3* (Table 1; Figs. 1 and 2). Mutations in region I, region III, and deletion of region IV and surrounding sequences can all cause ectopic expression of a *mec-3-lacZ* fusion in some sisters of the normal *mec-3*-expressing cells (Table 1; Results). This expression is transient, presumably because these

additional cells do not express *mec-3* itself, and so expression of the fusion is not maintained. Because alteration of several sites can cause this phenotype, the repression process may be complex and could require the action of several proteins at various sites to be complete.

One feature of *C. elegans* development is that asymmetric cell divisions always occur in a given orientation with respect to the rest of the animal (Sulston and Horvitz 1977; Sulston et al. 1983). For example, all *mec-3*-expressing cells are anterior daughters. Several genes necessary for this process have been identified in *C. elegans*, such as *lin-17* (Sternberg and Horvitz 1988), but their molecular function is unknown. The mechanism of such a process cannot be easily extrapolated from an understanding of single-celled organisms such as yeast and *E. coli*.

Because the *mec-3*-independent establishment expression of *mec-3* is brief, there must be some transiently active regulatory factor present during or immediately after the cell division that produces the *mec-3*-expressing cells. It is possible that a transient signal such as a cell-cycle regulator could act in combination with cell-specific regulators to actually turn on *mec-3*. Several groups have found that passage through a particular part of the cell cycle may be necessary for expression of differentiated phenotypes but that cell division itself need not occur (Albertson et al. 1978; White et al. 1982; Edgar and McGhee 1988).

The expression patterns of *unc-86* and *mec-3* indicate that *unc-86* is always present in a parent cell if one of its descendants expresses *mec-3*: If a cell expresses *unc-86* but never divides, it does not express *mec-3* (Fig. 2; Way and Chalfie 1989; Finney and Ruvkun 1990). It may be that in addition to requiring *unc-86*, establishment synthesis of *mec-3* only occurs during a certain part of the cell cycle and cannot take place in a nondividing cell. In this context, it is interesting to note that the sequence CGAAA, which constitutes the common core of the yeast histone and HO gene cell-cycle regulatory sequences, is found three times in the 150 bp that is conserved in the upstream region of *mec-3* [base pairs 1679–1683, 1707–1711 (region I), and 1959–1963 (3' to region IV) in Fig. 4A; Nasmyth 1985; Osley et al. 1986].

Materials and methods

Isolation of mec-3 from C. vulgarensis

A genomic λ library of DNA from *C. vulgarensis* was obtained from Chris Link (pers. comm.). Several hundred thousand plaques were screened for cross-hybridization to *mec-3* by using nick-translated 2.7-kb *Bam*HI fragment from pTU24 as a probe (this fragment contains the last four exons of *mec-3*). After washing at 37°C in 2 $\times$ SSC, several positive plaques were identified at a frequency of ~1/50,000. Each positive plaque had an apparently identical 3.0-kb *Eco*RI fragment that cross-hybridized with the *C. elegans mec-3* probe. From one such phage, this fragment was subcloned into Bluescript pKS(–) to yield pJW91-1 and sequenced by standard Sanger/M13 techniques (Fig. 3A; Sanger et al. 1977; Yanisch-Perron et al. 1985).

To verify that the cloned DNA contained a *mec-3* homolog

and not a closely related but functionally different gene, pJW91-1 was coinjected with the "Roller" plasmid pRF4 (Kramer et al. 1990; C. Mello, unpubl., as cited in Liu and Ambros 1991) into *mec-3(u6)*—after which one of five Roller transformants was touch sensitive—and into *mec-3(u298)*—from which one of one Roller transformant was touch sensitive and gave rise to a line of touch-sensitive Roller progeny.

Similarities between the *C. elegans* and *C. vulgarensis* sequences were identified by inspection and by computer.

Plasmid constructions

Most plasmids used in this study are derived from pTU28, a *mec-3-lacZ* fusion plasmid, or pTU48, which contains intact *mec-3* (Way and Chalfie 1989). pJW89-138 was generated by deletion of the internal *EcoRI* fragments of pTU48; pUpsA-Z is pJW89-138 with the 6-kb *XhoI-SalI lacZ* fragment from PS118 (Silver et al. 1988) in the unique *XhoI* site in the *mec-3* homeo box. pR4delA-Z and pR1+2-Z were derived from pTU28 by deletion of the internal *XbaI* and *NsiI* sites, respectively (Table 2). pR1delA-I is identical to pTU48 with base pairs –1237 to +1734 removed; pR1D-I (Fig. 4; see below), which has an additional *Clal*-digestible site at base pair 1735, was deleted by partial digestion with *Clal* and complete digestion with *EcoRV*, followed by filling in and ligation. pR1delA-Z was created from pR1delA-I by insertion of the *XhoI-SalI lacZ* fragment from PS118 (Silver et al. 1988).

pHS16.25 (E. Stringham and P. Candido, pers. comm.) contains the HSP16-41 promoter region (Jones et al. 1986) in pPD16.51 (Fire et al. 1990), so that the *lacZ* gene is heat inducible. pR123-HS has the *HindIII-XbaI mec-3* fragment containing conserved regions I, II, and III (base pairs 1418–1874) inserted into *HindIII*- and *XbaI*-cut pHS16.25: The *mec-3* region is immediately upstream of base pair 1266 of HSP16-41, so that pR123-HS includes part of one heat shock element and the heat shock TATA box. pR1-HS is similar and was constructed as follows: the *HindIII-Sau3a* fragment containing region I of *mec-3* (base pairs 1418–1735) was inserted into *HindIII* and *BamHI*-digested Bluescript pKS(+), which places an *XbaI* site adjacent to the *Sau3a* site; the resulting *HindIII-XbaI* fragment was inserted into *HindIII/XbaI*-cut pHS16.25 to generate pR1-HS.

Site-directed mutagenesis

Oligonucleotides for site-directed mutagenesis were designed according to the general principles outlined in Sambrook et al. (1989). As a substrate for mutagenesis, either pTU28 (the intact *mec-3-lacZ* fusion) or pJW90-19 [pTU24 (Way and Chalfie 1988) with a deletion of 2.7-kb *BamHI* fragment (base pairs 3121–5661)] was used. Because these plasmids have a pIB130 backbone, they contain an M13/f1 origin of replication/packaging: Each was converted to single-stranded DNA by coinfection with a helper phage. Standard mutagenesis procedures were followed (Sambrook et al. 1989). Mutations generated in pJW90-19 were subsequently transferred into plasmids containing an intact *mec-3* gene or *mec-3-lacZ* fusion by substituting the *KpnI-BamHI* fragment (base pairs 130–3154) for the corresponding fragment of either pJW89-139 (pTU48 with a deletion between *XbaI* sites at base pairs 1870 and 2489) or pR4delA-Z. Several attempts at oligonucleotide mutagenesis of region II all failed, perhaps because this region is very AT-rich and the mutagenic oligonucleotides failed to hybridize with sufficient specificity. Therefore, to mutate region II, a 38-bp *TcI* insertion remnant in this site was used (see pTU1 in Way and Chalfie 1988). pR2-I was constructed by insertion of the 2.7-kb *BamHI-XbaI mec-3*

Table 2. Plasmids used to generate transgenic *C. elegans* strains

Plasmid	Array	
A. <i>mec-3-lacZ</i> fusion plasmids used to generate extrachromosomal arrays		
pTU28	<i>uEx4</i>	wild-type <i>mec-3-lacZ</i> fusion
PTU28	<i>jeln2</i>	gamma ray-induced integration of <i>uEx4</i> into a chromosome
pUpsA-Z	<i>jeEx9</i>	pTU28 with deletion from –213 to 1494 (upstream nonessential regions)
pR123-HS	<i>jeEx19</i>	1418–1874 bp (regions I–III) fused to heat shock promoter (Fig. 5)
pR1delA-Z	<i>jeEx20</i>	pTU28 with deletion from –1237 to 1734 (removes region I; Fig. 4)
pR1A-Z	<i>jeEx11</i>	pTU28 with mutation 1A (region I; Fig. 4)
pR1B-Z	<i>jeEx21</i>	pTU28 with mutation 1B (region I; Fig. 4)
pR1C-Z	<i>jeEx14</i>	pTU28 with mutation 1C (region I; Fig. 4)
pR1D-Z	<i>jeEx13</i>	pTU28 with mutation 1D (region I; Fig. 4)
pR1E-Z	<i>jeEx12</i>	pTU28 with mutation 1E (region I; Fig. 4)
pR2-Z	<i>jeEx6</i>	pTU28 with <i>TcI</i> remnant (region II; Fig. 4)
pR1 + 2-Z	<i>jeEx15</i>	pTU28 with deletion from –1129 to 1810
pR3-Z	<i>jeEx5</i>	pTU28 with mutation 3 (region III; Fig. 4)
pR3-Z	<i>jeln5-1</i>	gamma ray-induced integration of <i>jeEx5</i> into a chromosome
pR4delA-Z	<i>jeEx8</i>	pTU28 with deletion from 1874 to 2489 (region IV and adjacent sequences)
pR1-HS	<i>jeEx22</i>	1418–1735 bp (region I) fused to heat shock promoter in pHS16.25 (Fig. 5)
B. Intact <i>mec-3</i> plasmids used to generate extrachromosomal arrays		
pJW91-1	<i>jeEx51</i>	<i>mec-3</i> homolog from <i>C. vulgarensis</i> in pKS(–)
pTU23	—	intact <i>mec-3</i> plasmid (Way and Chalfie 1989)
pTU48	—	pTU23 with ~100-bp deletion in 3' noncoding sequences (Way and Chalfie 1989)
pR1B-I	<i>jeEx58</i>	mutation 1B in pTU48 (region I; Fig. 4)
pR1E-I	<i>jeEx57</i>	mutation 1E in pTU48 (region I; Fig. 4)
pR2-I	<i>jeEx62</i>	Contains 125–5661 bp with <i>TcI</i> remnant in region II (Fig. 4)
pR3-I	<i>jeEx56</i>	mutation 3 in pTU48 (region III; Fig. 4)

Plasmids and corresponding extrachromosomal elements. *mec-3-lacZ* fusion plasmids were injected into wild-type *C. elegans* (N2) with the antisense *unc-22* plasmid pPD10.41, which confers a dominant Twitcher phenotype (Fire et al. 1990). Intact *mec-3* plasmids were injected into *mec-3(u6)* with the plasmid pRF4, which confers a dominant Roller phenotype. Some of these extrachromosomal arrays were derived from injections described in Fig. 4D. Extrachromosomal arrays are named according to standard *C. elegans* nomenclature: (*jeEx*) Elements are extrachromosomal arrays generated by microinjection of DNA and behave as unstable free duplications; (*jeln*) elements are integrated into a chromosome and are therefore stable. Sequence numbers refer to the *mec-3* sequence of Way and Chalfie (1988). From the comparison of the *C. elegans* and *C. vulgarensis mec-3* sequences (Fig. 4A), the apparent start of *C. elegans mec-3* translation is at 2021 bp.

homeo box-containing fragment from pTU25 into *BamHI*- and *XbaI*-digested pTU1 (Way and Chalfie 1988); this plasmid is analogous to other intact *mec-3* plasmids except that it lacks base pairs –1237 to +125. pR2-Z was constructed in the same manner as site-directed mutant *mec-3-lacZ* fusion plasmids.

C. elegans strains, constructions, and injections and staining

C. elegans strains were handled as described by Wood (1988). General properties of *mec* mutants are described in Chalfie and

Au (1989). The *mec-3* mutant *U298* contains a transposon Tc1 insertion in region II of the *mec-3* 5' region (Way and Chalfie 1988; this study). *u6* is one of several ethylmethane sulfonate (EMS)-induced mutations in *mec-3* (Chalfie and Au 1989). All *mec-3* mutations tested cause a reduction, but not elimination, of expression of a *mec-3-lacZ* fusion. *u298* causes the mildest reduction, whereas *u6* causes the most severe reduction (M. Hamelin, I. Scott, J. Way, and J. Culotti, in prep.). It is not known whether any existing alleles are null. The *unc-86* alleles *n846* (null) and *n848* (ts) are described by Finney and Ruvkun (1990) and Finney (1987).

jeln2 is an insertion of the *uEx4* element into the *unc-22* region of chromosome IV. This insertion was generated by gamma ray treatment (C. Kari, A. Fire, and R. Herman, pers. comm.). Twenty *uEx4* L4-stage animals were treated with 3800 rads from a ¹³⁷Cs source and placed on separate plates. From the 8 animals that had normal broods, a total of 107 F₁ *uEx4* progeny were picked to individual plates. From each of the 56 F₁ animals with normal-brood sizes, 3 F₂ animals were picked to separate plates and examined for the failure to segregate non-*uEx4* animals. Two such independent isolates were identified, *jeln2* and *jeln3*. *jeln2* was outcrossed once to eliminate an unlinked temperature-sensitive sterility. *jeln5* and *jeln8* were constructed in a similar manner from *jeEx5* and *jeEx8*, respectively. Their chromosomal locations are unknown.

C. elegans strains were injected according to Fire (1986). Sometimes animals were injected in the gonad syncytium, which was convenient for generating large numbers of animals that were transformed for one generation. *mec-3-lacZ* fusions were coinjected with the "Twitcher" plasmid pPD10.41 (Fire et al. 1990) into the N2 strain, whereas intact *mec-3* plasmids were coinjected with the Roller plasmid that carries the *rol-6* gene with the dominant mutation *su1006* (Kramer et al. 1990; C. Mello and V. Ambros, unpubl. as cited in Liu and Ambros 1991) into *u6* or *u298*.

Interpretation of X-gal staining

C. elegans strains carrying *lacZ* fusions were stained for 24 hr at 37°C according to Way and Chalfie (1989) and Fire et al. (1990). As described in Way and Chalfie (1989), in adult *uEx4* animals two neurons in the head (probably the AIZ cells), several motor neurons in the posterior ventral cord, and occasional intestinal cells can express β-galactosidase activity. This expression may be artifactual, as none of these cells is known to be affected by *mec-3* mutations. Because β-galactosidase activity is seen long after these cells are produced, the control of *lacZ* expression must be different in these cells than it is in the 10 canonical *mec-3*-expressing cells. Most of the constructs do not affect this expression, and it has not been investigated in detail.

Not all of the canonical *mec-3*-expressing cells express the *mec-3-lacZ* fusion in animals carrying *jeln2*, as detected in our standard assay. This is probably because the level of β-galactosidase activity in these cells is near the threshold of detectability with this assay and not because these cells are differentiating incorrectly. When animals carrying *uEx4* or *jeln2* are grown at 25°C instead of 20°C, almost 100% of the *mec-3*-expressing cells show β-galactosidase activity, and growth at 15°C causes β-galactosidase activity to be low (Way and Chalfie 1989; unpubl.). Because these temperature conditions are not known to affect touch receptor differentiation, it would appear that synthesis or stability of β-galactosidase is somewhat temperature sensitive in *C. elegans* and that expression of *lacZ* from the *mec-3* promoter at 20°C gives levels of β-galactosidase activity near the threshold of detection. The apparent variation from cell to cell in activity levels may be the result of variations in fixation. No

attempt is made to quantify differences in staining intensity: Each cell is scored as staining or not staining. To be considered stained, a cell must be distinctly blue.

Transgenic *C. elegans* strains were examined by Southern blot to determine whether different staining patterns could be explained by variations in plasmid copy number within different arrays. The *uEx4* element contains ~40 copies per cell of both pTU28 and pPD10.41 (Way and Chalfie 1989). Compared with a *uEx4* strain, strains carrying pR2-Z, pR3-Z, or pR4delA-Z did not have fewer copies of the *mec-3-lacZ* fusion plasmid, whereas the copy number of pUpsA-Z in *jeEx9* is ~10-fold lower than that of pTU28 in *uEx4* (data not shown). Southern analysis indicates that *uEx4* and *jeln5*, respectively, contain about five and four times as many copies of the *mec-3-lacZ* fusion as does *jeln2*. In spite of these differences, *jeln2* and *uEx4* strains have the same overall staining pattern, as do *jeEx5*, *jeln5*, and *jeln5/+* strains. Also, *jeln5/+* animals carry fewer copies of the *mec-3-lacZ* fusion than animals carrying *uEx4*; however, in spite of this, *jeln5/+* animals show β-galactosidase activity in sisters of the normal *mec-3*-expressing cells, and *uEx4* animals do not. It is thus unlikely that this ectopic expression is the result of variations in copy number of the *mec-3-lacZ* fusion in different transgenic lines. *jeln5/+* animals were generated by constructing a *unc-24(e138) mec-17(u265) mec-3(u467) dpy-20(e1282); jeln5* strain, then mating with N2 males, and picking non-Unc, non-Dpy animals to be stained.

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Note added in proof

Sequence data described in Fig. 3 have been submitted to the EMBL/GenBank data libraries.

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