ular structural goals. As implied by Fig. 1, different applications require different precursors. Acid-catalyzed alkoxides form dense films that sinter at relatively low temperature (*29*). If porous films, on the other hand, are desired for index-matched coatings, then base-catalyzed materials (even colloidal particles) provide the required rigidity necessary to achieve porous films with tailorable refractive index (*30*).

For the preparation of bulk monoliths, other considerations apply. Although acid catalysis leads to relatively dense solids, these do not sinter well because closed porosity precludes the escape of organics. The local rigidity afforded by base-catalyzed systems, however, allows sufficient open porosity to effectively sinter bulk monoliths. These examples show that kinetic models are useful, not only for explaining existing data on the structure and processibility of sol-gel–derived materials, but also as a framework for the control variables that can be manipulated to achieve particular structural and processing goals.

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Genetic Control of Differentiation of the *Caenorhabditis elegans* Touch Receptor Neurons

MARTIN CHALFIE AND MACY AU*

The genetic control of neuronal differentiation has been studied by examining mutations that affect the development and function of the six touch receptor neurons of the nematode *Caenorhabditis elegans*. By screening for touch-insensitive mutants, it has been possible to identify 18 genes (represented by 417 mutations) that are required at various stages in the developmental program for touch cell differentiation. Two of the genes are needed for the generation of precursors in the touch cell lineages; without the precursors, touch cells are not made. A third gene, *mec-3*, specifies the differentiation of the touch cells, probably by acting as a transcription factor. The remaining 15 genes are likely targets of *mec-3* action; mutants defective in these genes have nonfunctioning, yet differentiated, touch cells. Some of these latter genes are needed for the formation of cell-specific components of the touch cells, such as a set of microtubules that are only found in these cells. The study of the touch genes should help us understand how touch cell fate is determined, how microtubule form is specified, and, perhaps, how mechanical stimuli are transduced.

NERVOUS SYSTEMS CONSIST OF MANY TYPES OF NEURONS that differ from each other structurally and biochemically in such features as shape, patterns of connectivity, neurotransmitters, receptors, and channels. Considerable diversity is seen even in organisms with relatively few neurons. For example, the 302 nerve cells of the nematode *Caenorhabditis elegans* have been classified into 118 groups (*1*). The mechanism by which cells attain their individual, differentiated features is not known; cell-cell interactions, growth factors, and intrinsic determinants are all thought to influence the expression of cell-specific characteristics.

One method of studying the control of the differentiation of

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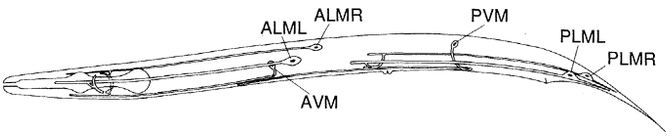

Fig. 1. The position of the touch cells. AVM and PVM arise postembryonically (*4*); the other touch cells arise embryonically (*5*). ALML, ALMR, and AVM contribute to the touch reflex in the head and PLML and PLMR contribute to a similar circuit in the tail. PVM shares features with the other cells, including making a similar set of synapses (*2, 5*), but does not contribute in a major way to touch-mediated movement unless inappropriately positioned, as in *mab-5* mutants, where it contributes to the anterior touch circuit (*32*). Loss of the PVM touch cell has no detectable effect in wild-type animals (*2*).

neurons (and other cells) is to identify and characterize mutations that disrupt the activity of single classes of cells. This approach is particularly useful when the loss of a single cell type results in a distinguishable and viable phenotype, such as a specific behavioral abnormality. Because the mutations disrupt the in vivo activity of the cells, this method has the advantage of identifying functionally important components that are needed for cell differentiation.

We have used this approach to study the genes required for the development and function of a set of six touch receptor neurons in *C. elegans* (*2*) (Fig. 1). The touch cells are well characterized: the lineages giving rise to the cells (*3, 4*) and the shape and synaptic connectivity of the mature cells (*1, 5*) have been described and are invariant from animal to animal. Like many *C. elegans* neurons, these cells have a simple shape with very few branches. However, the touch cells can be unambiguously identified because their cell processes contain large diameter (15-protofilament) microtubules and are attached near the cuticle by an associated extracellular material, the mantle (*2, 6*).

The touch cells mediate a readily assayed, yet nonessential, behavior; they are the first step in a simple reflex circuit (touch cell, interneuron, motorneuron, and muscle) for touch sensitivity (*5*). Animals normally move backward or forward when a fine hair is drawn across their body at the head or tail, respectively (*2*). The animals no longer respond at the head when the anterior touch cells (ALML, ALMR, and AVM; Fig. 1) have been killed by laser ablation or at the tail when the posterior touch cells (PLML and PLMR) have been killed. Such laser-treated animals, however, will move in response to the prodding of a harder stimulus, such as a platinum wire, and are viable. This selective touch insensitivity is characteristic of the loss of the touch cells; ablation of the other cells in the touch circuit results in additional behavioral defects (*5*).

Characterization of Touch-Insensitivity Mutations

We have screened for mutants that have this selective touch insensitivity. Since this defect is found only when the touch receptors are killed, mutations producing this phenotype must be in genes that either act intrinsically in the touch cells or their precursors or are required for cellular interactions associated with touch cell development. The genes identified in such a screen represent an important subset of genes required for touch cell development: genes that are necessary for touch cell differentiation and function and are potentially expressed in a highly specific fashion within the animal. Because touch sensitivity is not essential for viability and, therefore, mutations in touch cell–specific genes should not be

Table 1. Characterization of touch sensitivity mutations. Columns indicate the number of alleles arising at 25°C after EMS mutagenesis (EMS25), at 15°C after EMS mutagenesis (EMS15), spontaneously in the TR679 mutator strain, or at 25°C after γ-ray mutagenesis (7). Complementation and mapping of mutations followed standard procedures (*11*). Also listed are alleles discussed elsewhere by ourselves and others (*2, 7, 13, 14, 16, 17, 21*). One apparent difference among the different screens, the relatively small number of *mec-5, mec-12*, and *mec-15* mutations obtained in the EMS15 screen, is explained by the finding that most of the alleles of these genes from the EMS25 screen produce temperature-sensitive phenotypes. Amber (am) mutations or mutations that are expressed in a temperature-sensitive (ts), dominant or semidominant (d), or partial (p) fashion from the EMS25, EMS15, TR679, and γ-ray screens or from our previous studies (*2, 6*) are listed. Some mutations are listed in two categories: one, two, and four of the *mec-2, mec-5*, and *mec-15* mutations, respectively, are both p and ts; five *mec-12* mutations are ts and d, and three *mec-7* mutations are p and d. In addition, all of the dominant and semidominant mutations of *mec-7* (*6, 14*) and four of those of *mec-12* are expressed in a ts manner in heterozygotes. "Same" indicates that the phenotype of *mec/deletion* heterozygotes was not detectably different from that of *mec/mec* homozygotes. The *mec-1, mec-15*, and *mec-17* mutations are uncovered by deletions *sDf20, mnDf29* and *mDf7*, respectively (*38*). The unmapped genes are mutations [*mec(u211)I, mec(u420)IV, mec(u192)X*, and *mec(u264)X*] that produce a variable or weak touch-insensitive phenotype. They complement representative alleles of mapped *mec* genes, but may possibly be unusual alleles of known genes. Chromosome numbers follow the gene name.

Gene	Mutation source					Total	Notable alleles				*mec/del*
	EMS25	EMS15	TR679	γ-ray	Other		am	ts	d	p	
mec-1 V	28	15	3	6	11	63	1				Same
mec-2 X	28	15	2	2	7	54	1	2	6	5	
mec-3 IV	3	2	3		7	15				1	Same (*13*)
mec-4 X	27	17	3	3	9	59		2	3	6	Same (*2*)
mec-5 X	22	6	1	3	5	37		16		3	Same (*2*)
mec-6 I	5	1		1	2	9		1			
mec-7 X	17	14	2	7	14	54			31	5	Same (*14*)
mec-8 I	2	2		2	1	7	1	1			
mec-9 V	14	8	3	3	5	33	3				Same (*2*)
mec-10 X	1	2		1	2	6					
mec-12 III	9	4		2		15		7	11		
mec-14 III	3	4	2			9					
mec-15 II	5					5		4		5	Same
mec-17 IV	1					1				1	Same
mec-18 X	2	2		2		6				6	
egl-5 III	1				5	6				1	
lin-32 X	1				1	2				2	
unc-86 III	9	3	1	3	20	36					Same (*21*)
Unmapped	2	1	1			4				4	
Total	180	96	21	35	89	421					

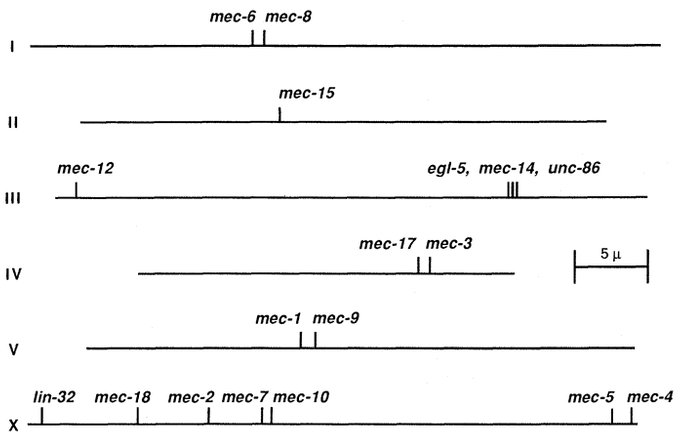

Fig. 2. Genetic map of *C. elegans*, indicating the positions of genes that affect touch sensitivity (*41*).

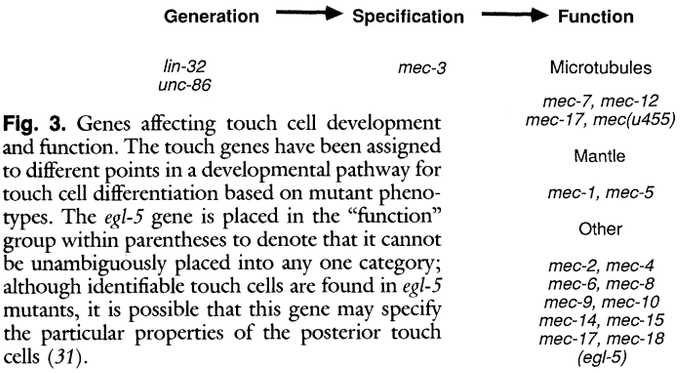

Fig. 3. Genes affecting touch cell development and function. The touch genes have been assigned to different points in a developmental pathway for touch cell differentiation based on mutant phenotypes. The *egl-5* gene is placed in the "function" group within parentheses to denote that it cannot be unambiguously placed into any one category; although identifiable touch cells are found in *egl-5* mutants, it is possible that this gene may specify the particular properties of the posterior touch cells (*31*).

lethal, our mutant screens should provide an estimate of the number of such genes. We would not expect to identify all genes required for touch cell development; genes, which when mutated heighten touch sensitivity or produce more severe defects (for example, uncoordination or lethality), would not, for the most part, be detected.

In our initial study (*2*) we identified 42 mutations in 12 genes that resulted in touch insensitivity (most of these genes are named *mec* for *mec*hanosensory abnormality). We and others have now generated a total of 421 touch-insensitive mutants by means of a variety of mutagenic protocols (7) (Table 1). Of the mutations, 417 have been assigned to 18 complementation groups: 12 previously identified touch genes (*mec-1* to *mec-10*, *mec-12*, and *unc-86*), 4 new genes (*mec-14*, *mec-15*, *mec-17*, and *mec-18*), and 2 previously known genes (*egl-5* and *lin-32*) for which touch-insensitive phenotypes had not been noted. These 18 genes are distributed randomly on all six *C. elegans* chromosomes (Fig. 2). Similar sets of mutations, most of which are recessive, arose with different mutagenic protocols. Many mutations with unusual properties, including nonsense (*8*), temperature-sensitive, and dominant alleles were also recovered (Table 1).

It is likely that we have identified most, if not all, genes that when mutant result in animals that are completely touch-insensitive yet have few or no other abnormalities. There are 13 genes of this type (*mec-1* to *mec-10*, *mec-12*, *mec-14*, and *unc-86*), and each is represented by several mutant alleles. Two other genes (*lin-32* and *egl-5*) result in a loss of touch insensitivity only at the tail. These 15 genes appeared to be required for touch insensitivity. Mutations in the three remaining genes (*mec-15*, *mec-17*, and *mec-18*) and the four unmapped mutations (Table 1) result in a partial loss of touch sensitivity (*9*). Because mutations resulting in a marginal defect are more difficult to identify, it is possible that other genes of this type remain to be identified.

Specificity of Gene Action

The phenotype of touch insensitivity alone cannot tell us whether the genes are restricted in their expression to just the touch cells. The cellular pattern of touch gene expression must await the availability of molecular probes for these genes and their products, especially if any of these genes are required in cells whose loss does not result in a readily detectable phenotype. Nonetheless, a number of observations suggest that many of the genes act in a very specific fashion (Table 1). (i) Many of the touch genes are mutated at relatively high frequencies (a result that is consistent with the loss of gene function) (*10, 11*) and some are represented by amber alleles. Most of the putative null mutations do not detectably affect other behaviors, such as movement, chemotaxis (*2*), or osmotic avoidance (*12*). (ii) For many of the genes, animals carrying a single copy of a mutation over a deletion of the region have the same phenotypes as homozygous mutants. This is even true for mutations of *mec-15* and *mec-17*, which produce partial touch insensitivity. (iii) Mutant alleles of *mec-3* (*13*) or *mec-7* (*14*) that were identified because they failed to complement existing mutations of these genes produce no other behavioral phenotype than touch insensitivity. These data suggest that the loss-of-function phenotype for many of these genes is touch insensitivity.

Although the touch genes do not appear to be generally required in *C. elegans* development, many of the genes do function in other cells. Mutations in *egl-5*, *lin-32*, and *unc-86* are quite pleiotropic and result in additional behavioral phenotypes (*15–17*). Mutations in *mec-1*, *mec-6*, and *mec-8* affect other neurons (*2, 18, 19*), but do not produce any detectable behavioral abnormalities except touch insensitivity.

Thus, the number of genes that may act in a touch cell–specific fashion is quite small. Only 12 genes may act in this way. As the expression patterns of these genes are examined in greater detail, this number may be reduced further.

Stages of Touch Cell Development

We have classified the touch genes into three groups according to whether their mutations affect the differentiation of the touch cells or their precursors (*20*) (Fig. 3). Mutants defective in genes in the first two categories lack identifiable touch cells. Mutations of genes in the first category (generation) prevent the production of the touch cells and other cells from the appropriate cell lineages by changing the apparent fate of the precursor cells. Mutations of the single gene in the second category (specification) appear to change the fate of the touch cells but not that of any other cells in the touch cell lineages. Mutations in any of the genes in the third category (function) result in touch-insensitive animals with differentiated touch cells. The phenotypes of the touch mutants have, thus, allowed us to identify genes that are required for various aspects of the developmental program for the touch cells.

Two genes, *lin-32* and *unc-86*, affect the generation of the touch cells (*2, 15, 17*). As seen in the postembryonic lineages that give rise to the AVM and PVM touch cells (Fig. 4), mutations in these genes appear to affect the fates of specific precursors so that lineages that should give rise to the touch cells do not occur. Both genes affect the production of the embryonic touch cells as well (*lin-32* mutants lack PLM cells and *unc-86* mutants lack both the ALM and PLM cells), but the nature of the lineage defects are not known. Consistent with the hypothesis that these genes act early in touch cell development is

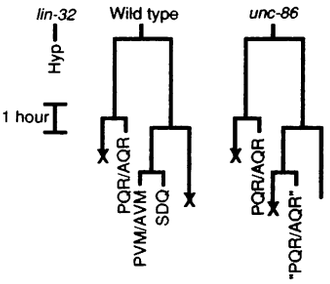

Fig. 4. Effects of *unc-86* and *lin-32* mutations on the AVM/PVM lineages. The AVM and PVM cells arise from similar postembryonic lineages on the right and left sides of the animal, respectively (*3*). The lineages each produce two other neurons and two cells that undergo programmed cell death (X). In *lin-32* mutants the initial precursor cell does not divide and appears to remain a hypodermal cell (Hyp). In the *unc-86* lineages (*15*) the first posterior daughter (the cell to the right of the diagram) appears to repeat the lineage of its parent. As a consequence, multiple copies of the PQR/AQR neurons arise, but no touch cells are seen.

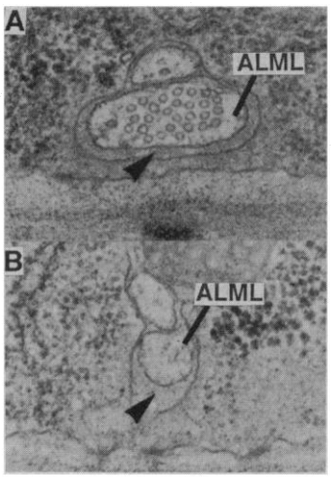

Fig. 5. Electron micrographs of the ALML processes of (**A**) *mec-12(u50)* and (**B**) *mec-12(u76)* mutants. Touch cell processes of both mutants have the mantle (arrowheads), but only the *u50* cell has the characteristic bundle of microtubules seen in wild-type cells. Similar phenotypic variation is seen in other *mec-12* mutants: the touch cell processes in *el605* and *u63* mutants have normal numbers of large diameter microtubules and those of *el607*, *u67*, *u76*, *u94*, *u241*, and *u204* mutants have few or none. Magnification is ×60,000.

the finding of an embryonic temperature-sensitive period for *unc-86* mutants (*21, 22*).

The *lin-32* and *unc-86* mutations affect the fates of two different precursors in the touch cell lineages. Mutations affecting other precursors in the lineages have not been identified, either because they were more pleiotropic or because they resulted in the overproduction, rather than the loss, of the touch cells (just as certain neurons are overproduced in *unc-86* mutants; Fig. 4) (*15*).

The developmental fates of a number of cells in other lineages are affected (*15, 17*) by *lin-32* and *unc-86* mutations. This pleiotropy suggests that although the production of the appropriate precursors are needed to generate the touch cells, neither gene is the sole or final determinative event in touch cell development.

The specification of cell fate, which is the second stage of touch cell development, is affected by mutations in a single gene in our collection, *mec-3*. The *mec-3* mutants have lineages that appear normal, but the cells that should differentiate into the touch cells have none of the characteristic features of the touch cells: they lack the 15-protofilament microtubules and the mantle (*2*). The cells are not blocked in their development, but appear to differentiate as other types of neurons. The most striking examples are the cells that would normally become the ALM touch cells; in *mec-3* mutants they are more laterally positioned and grow both anteriorly and posteriorly directed processes (the wild-type ALM cell has only an anteriorly directed process) (*13, 23*). Indirect evidence suggests that the cells may be transformed into cells that are like their lineal sisters (*13*), and, thus, *mec-3* may be important in specifying the differences between the sister cells.

The *mec-3* gene codes for a product with a homeodomain [a likely DNA-binding domain (*24*)] and an acid-rich domain, features that have been associated with regulators of transcription (*13*). Thus, *mec-3* could specify touch cell differentiation either as a positively acting transcription factor or as an antirepressor.

Possible targets of *mec-3* activity are the genes of the third group, those needed for touch cell function and terminal differentiation. Mutations in five genes affect individual features of the differentiated cells, the 15-protofilament microtubules [*mec-7* (*5*), *mec-12* (Fig. 5), and *mec-17* (*9*)] or the mantle [*mec-1* (*2*) and *mec-5* (*25*)], suggesting that both features are required for sensory transduction. Consistent with the hypothesis that these genes encode end products of cell differentiation is the finding that the *mec-7* gene encodes a β-tubulin (*14*).

The ten remaining genes in this class can be mutated to give touch insensitivity without any detectable alterations in touch cell morphology (although dominant alleles of *mec-4* result in the degeneration of the touch cells) (*26*). The structure of the AVM touch cell has been examined in more detail in *mec-5*, *mec-6*, and *mec-8* mutants, but no obvious defects were seen; all had branches with gap junctions and apparent chemical synapses. It is, perhaps, not surprising that a majority of the genes do not grossly affect the structure of the touch cells; mutations in genes for receptors, channels, or enzymes needed by the touch cells, for example, might not produce obvious morphological abnormalities.

The temperature-sensitive periods for mutants of many genes in this class, unlike that of the *unc-86* mutants, last through most of larval development (Fig. 6). Because wild-type larvae are touch-sensitive soon after hatching, these temperature-sensitive periods occur after the time in which the touch cells have grown to their targets and established synaptic connections. Thus, it is unlikely that genes of this class are required for these early steps in touch cell development. However, the touch cells grow considerably during larval development: process length increases 2.4-fold and the total length of microtubules in the process increases nearly 50-fold (*6*). Many of the genes in the function class appear to be needed in the larva to accommodate this growth after the establishment of synaptic connections. The phenotype of *mec-17(u265)* mutants is intriguing in this context; the animals are touch-sensitive as newly hatched larvae, but become insensitive as they mature (Fig. 7).

The touch mutations identify three steps in the developmental program for the touch cells that can be disrupted in a selective fashion. We do not know how directly the genes of one stage affect the expression of those of the next, since genes acting in a less specific manner may also be required. Thus, other intervening stages are possible.

Other Aspects of Touch Cell Differentiation

Analysis of the existing touch genes should help us investigate such problems as the molecular control of touch cell differentiation, the regulation of microtubule structure, and, perhaps, the molecular mechanism of mechanosensation. There are other aspects of touch cell differentiation, however, for which our mutants are not as informative. As indicated above, *mec-3* activity is required for the ALM cell to be positioned correctly and to grow appropriately directed processes. However, none of the mutants defective in the genes in the function class affect these features of the touch cells. It is likely that such touch cell characteristics are regulated by genes that act in a more general fashion in *C. elegans* development. In support of this hypothesis is the finding that *C. elegans* mutants that have abnormal neuronal outgrowth are severely uncoordinated (*27, 28*). Some of these mutants have touch cell processes in incorrect positions (*29*). In addition, mutants with displaced ALM cell bodies have other types of neurons in inappropriate positions (*27, 28*).

Other features of touch cell differentiation may also require genes

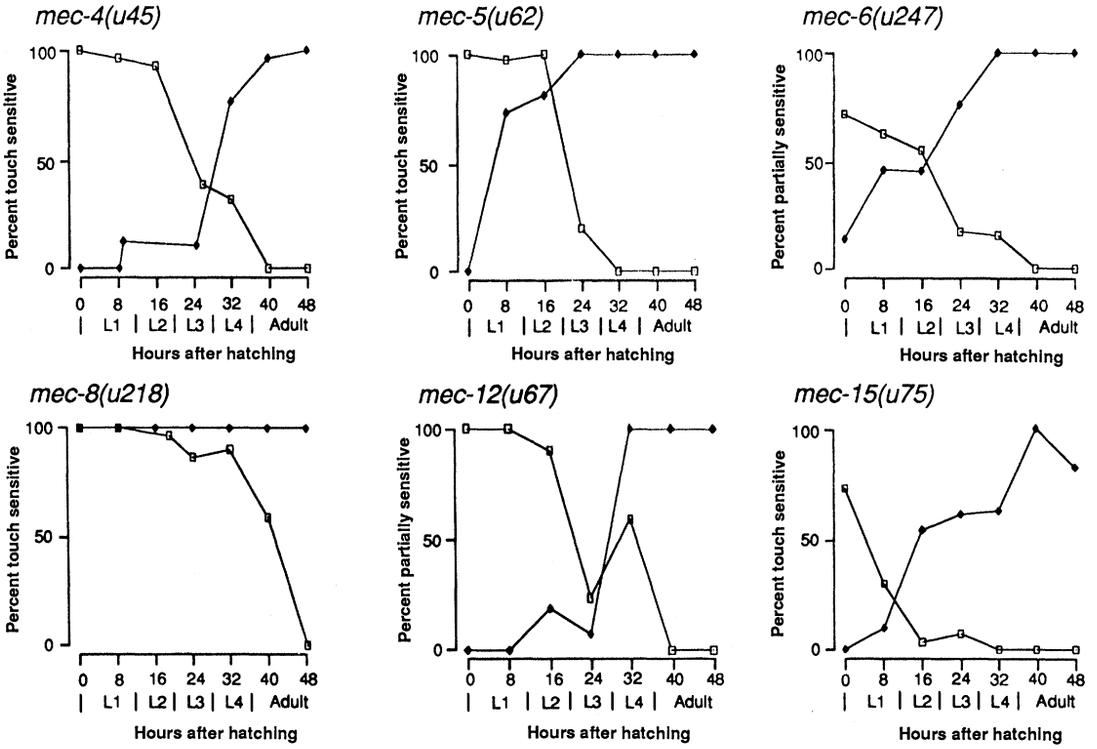

Fig. 6. Temperature-sensitivity of *mec* mutants. Temperature shifts were conducted as in (*2*): animals from stocks that had grown at the appropriate temperature for at least two generations were synchronized at hatching, shifted from 15° to 25°C (shift up, ♦) or 25° to 15°C (shift down, □) at the indicated times, and tested for touch sensitivity as egg-laying adults (at approximately 48 hours or at least 12 hours after the shift, whichever was greater). Animals were scored as touch-sensitive if they responded at only one end. The *mec-6* mutants were only partially sensitive at 15°C, and many of the *mec-12* mutants were partially sensitive at 25°C. For these mutants "percent partially sensitive" refers to the proportion of animals that were partially or completely touch-sensitive. Times are given in 25°C equivalents. Similar results were found with one, eleven, two, and three other strains of *mec-4*, *mec-5* (*2*), *mec-12*, and *mec-15*, respectively. The extensive temperature-sensitive periods (the region of crossover of the upshift and downshift curves) of *mec-4*, *mec-6*, *mec-12*, and *mec-15* mutants (also *mec-7* mutants) (*8*) suggest that gene activity is needed throughout larval development. Activity of *mec-5* is also required during this period, but perhaps for a shorter time. Not shown are two *mec-2* mutants (one heat-sensitive, the other cold-sensitive) that are reversibly touch-sensitive: switching them between the permissive and restrictive temperature at any time during larval growth or early adulthood changes their phenotype. These observations suggest that *mec-2* function must be maintained during this period. In contrast, growth of *mec-8(u218)* animals at 15°C during embryogenesis or any time during larval development is sufficient to rescue the touch-insensitive phenotype. This observation suggests that only a small amount of the *mec-8* product is needed for touch cell function.

that are needed for the development of other types of neurons. The collection of *unc* (*unc*oordinated) genes (*11*) may contain genes of this type. Recently we identified a temperature-sensitive mutation from γ-ray mutagenesis, *mec(u455)X*, that produces uncoordinated and touch-insensitive animals whose touch cells lack the large diameter microtubules. We have also tested representative mutants of the majority of the *unc* genes and find that they are either touch-sensitive or so severely paralyzed as to make them impossible to test (*30*). These latter mutants have identifiable touch cells by Nomarski optics, so the nature of a touch cell defect, if any, is unknown.

Another aspect of touch cell differentiation for which we have little information is the production of subsets of touch cells. Touch cells in different parts of the animal are not the same; for example, different synapses are made by the touch cells in the head and in the tail (*5*). Although we have identified mutations that result in touch insensitivity only at the head or the tail, most of these are in genes (*mec-4* and *mec-7*) for which other alleles result in complete touch insensitivity. Mutations in only two genes (*lin-32* and *egl-5*) affect a subset of cells, causing the animals to be touch-insensitive only at the tail. Either or both of these genes may be important for the anterior-posterior differences seen among the touch cells, but it is difficult to interpret the function of these genes because mutations in them act pleiotropically and may not be complete loss-of-function alleles (*31*).

It is possible that the six touch cells constitute a single cell type whose differences result from local cellular interactions. We have previously shown that the AVM cell (Fig. 1) must be in the correct cellular environment to branch (*32*) and that this branch requires the presence of a pair of cells, the BDU cells, to be directed to its synaptic targets (*22*). White *et al.* (*33*) have also suggested that the formation of appropriate synapses is position dependent in *C. elegans*.

How Characteristic Is Touch Cell Differentiation?

The question arises whether the differentiation of other neurons, and perhaps of other cell types, is programmed similarly to that of the touch cells. From our analysis the key component of touch cell differentiation is *mec-3*, a gene that may specify touch cell fate by regulating transcription. Transcriptional regulation could be either through the de novo synthesis of a transcription factor or through the modification of the existing transcription machinery. The differ-

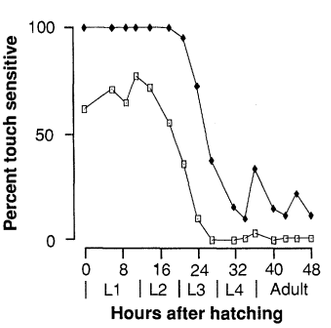

Fig. 7. Development of touch insensitivity in *mec-17(u265)* mutants at 25°C. Animals were touched once at the head and tail at various times after hatching. If they responded to both touches, they were scored as wild type for touch sensitivity; if they only responded to one touch, they were scored as partially touch-insensitive. Different batches of animals were examined at different times (each with at least 80 animals). Thus, the variability in the response is caused partly by the uncertainty in the detection and partly by differences among sets of animals. ⊡, wild type; ♦, wild type or partial.

entiation of the touch cells appears to use the first mechanism. This may also be the way that homeo box genes such as *fushi tarazu*, *even-skipped*, and *cut* in *Drosophila* (*34*) and *unc-86* in *C. elegans* (*35*) regulate the expression of specific cellular characteristics of terminally differentiated cells or their precursors. It is possible that the genes of the *achaete-scute* complex, which are needed for neuronal differentiation in *Drosophila*, and the MyoD1 gene, which induces myogenesis in mouse fibroblasts in culture (*36*), may act similarly. Genes such as *Notch* and *sevenless* in *Drosophila* and *lin-12* in *C. elegans* (*37, 38*) may act through the second mechanism. These genes are thought to act at the cell surface and be involved in cell-cell interactions that lead to the specification of particular cellular fates, so one must assume that their effects on transcription are indirect. The specification of cell differentiation, thus, may utilize a variety of strategies. However, these distinctions of mechanism may be misleading; although we can identify important regulatory elements, we cannot say that they are the only factors acting. It is possible that a combination of regulatory elements is involved; we can only identify those that can be disrupted genetically. Nonetheless, the *mec-3* gene provides us with a candidate that is the most proximate regulator of gene expression.

Although we do not know the pattern of expression of the *mec-3* gene, one striking property of the gene is that it appears to be required for the differentiation of a very restricted set of cells. Such selectivity has been seen for very few other genes. In *Drosophila* mutations in the *sevenless* gene appear to affect only one class of photoreceptor (*38*), and in *C. elegans* mutations in the *unc-55* gene appear to affect one class of motorneurons (*39*). Most regulatory genes, however, do not appear to be this selective. For example, the other homeo box genes discussed above affect a number of cell types. In addition, although genes regulating the differentiation of the HSN cells, a pair of motorneurons needed for egg-laying in *C. elegans*, have been found, all of them also affect the development of other cells (*16*). These results argue that in many cases the specification of cell fate is not an example of one regulatory gene per cell type.

We have identified two genes that appear to act before *mec-3* to specify the cell fate of precursors in the touch cell lineages. The phenotypes of the *lin-32* and *unc-86* mutants suggest a hierarchy of gene action in the production of the cell lineages. The question remains whether these genes, and perhaps others that may affect the production of precursors, directly regulate the expression of *mec-3*. The finding that *unc-86* is a homeo box gene (*35*) raises the possibility that there may be an interacting set of regulatory factors that corresponds to the lineage hierarchy. A somewhat analogous cascade of regulatory factors has been invoked in the development of *Drosophila* (*40*). If this hierarchy does occur in the development of the *C. elegans* touch cells, it would represent a molecular mechanism for intrinsic control of cell fate. However, although many of the features of the touch cells require the expression of *mec-3*, at least one characteristic, the branching pattern of the AVM cell, requires cell-cell interactions. Thus, the touch cells appear to be cells whose differentiation, although regulated primarily by intrinsic factors, can be modified by interactions in their cellular environment. The identification of the touch genes provides a means to begin to study the molecular mechanisms involved in the differentiation of these cells. In particular, the analysis of the recently cloned genes *unc-86* (*35*), *mec-3* (*13*), and *mec-7* (*14*) should elucidate the nature of the molecular interactions required for touch cell differentiation.

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8. Nonsense alleles derived from EMS and γ-ray mutagenesis were identified by suppression by the amber transfer RNA (tRNA) suppressors *sup-5(el464)* (for X-linked mutations) or *sup-7(st5)* (for autosomal mutations) [R. H. Waterston and S. Brenner, *Nature* **275**, 715 (1978); R. H. Waterston, *Genetics* **97**, 307 (1981); N. Wills *et al.*, *Cell* **33**, 575 (1983)]. Dpy or Lon progeny from *lon-1(el85) sup-5*/++; *mec*/+ or *mec*/+; *dpy-7 (el324) sup-7*/++ hermaphrodites were examined for touch insensitivity at 20°C (or 25°C if the *mec* mutation was heat sensitive). If no LonMec or DpyMec animals were seen (an indication of suppression), individual Mec animals were screened for the production of Lon or Dpy animals (the absence of which indicates dominant suppression). The amber mutations are *mec-1(u39)*, *mec-2(u8)*, and *mec-9(u27, u164,* and *u258)*. Only *mec-9(u164)* and the previously identified amber mutation *mec-8(e398)* (*2*) are suppressed in a dominant fashion.
9. Animals with *mec-15*, *mec-17*, or *mec-18* mutations are often, but not always, touch-insensitive just at the head, and the insensitivity is often incomplete in that the animals respond to some touches (such partial insensitivity is seen with some alleles of *mec-2*, *mec-4*, and *mec-7*; Table 1).
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17. We have not seen the PLM touch cells in *lin-32(u282)* animals, a finding consistent with their posterior touch insensitivity. The ALM cells of these mutants are situated closer to the head than usual, but are functional since the animals are touch-sensitive at the head. Since only two *lin-32* mutations have been identified (with the *u282* mutation producing the more severe phenotype), it is possible that *lin-32* mutations affecting the function of all the touch cells will be found. Additional phenotypes are seen in *lin-32(u282)* mutants; a number of neurons are in unusual positions (immediately posterior to the pharynx and anterior to the anus) and males cannot mate. Similar observations have been made by C. Kenyon and E. Hedgecock (personal communication) on the first isolate of this gene (*el926*).
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20. Representative mutants of newly identified genes have been examined by Normarski differential interference contrast microscopy (*3*) and electron microscopy (except *lin-32* mutants in the latter case). Animals were fixed in glutaraldehyde and acrolein or OsO_4 and processed for electron microscopy as before [M. Chalfie and J. N. Thomson, *J. Cell Biol.* **82**, 278 (1979)], except that some animals were embedded in epon or araldite and epon.
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23. It is likely that the change in position is a consequence and not a cause of the altered differentiation of the ALM cells because (i) the other touch cells in these mutants do not have the microtubules or mantle, but are not displaced (*2*); (ii) functional, yet displaced, ALM cells are found in *lin-32* mutants (*17*); and (iii) other mutations that displace these cells (*27*) do not result in touch insensitivity.
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25. The touch cells stained with fluoresceinated peanut lectin in a pattern that suggests that binding is to the mantle; *mec-5* mutants lack this staining (E. M. Hedgecock and M. Chalfie, unpublished data).
26. The dominant *mec-4(el611)* mutation [originally called *mec-13* in (*2*)] was assigned to *mec-4* because no wild-type recombinants were found among 3379 progeny from +*mec-4(el611)/lon-2 mec-4(el497)* ($P < 0.06\%$) and EMS reversion yielded a recessive *mec-4* mutation (*el879*) that does not produce degenerating touch cells. Two other dominant *mec-4* alleles (*u214* and *u231*), but none of the remaining *mec-4* mutations, all of which are recessive, result in touch cell degeneration.
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31. There are only two *lin-32* mutations. The more severe of these affects all touch cells, but does not eliminate the anterior touch response (*17*). Since the touch cells arise from similar, but not identical, lineages (*3, 4*), it is possible that the *lin-32* mutations reveal a lineage component necessary for the differentiation of the various touch cells. Alternatively, the posterior touch insensitivity of these mutants may result from a partial loss of gene activity. The *egl-5* mutations affect a number of cells in the tail (*16*). The PLM touch cells in these mutants appear normal, that is, they have both the large diameter microtubules and the mantle. Thus, we cannot determine whether the effect on touch sensitivity is direct (for example, causing an alteration in touch cell synapses) or indirect (for example, causing a displacement of the interneuronal processes onto which the touch cells must synapse).
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42. Genes have been positioned based on published data [(*2*); (M. L. Edgley and I Riddle, *Genet. Maps* **4**, 351 (1987)] as well as our own unpublished data depo with the Caenorhabditis Genetics Center (University of Missouri).
43. We are indebted to E. Bergholz, K. Buck, N. Hom, C. Masuoka, anc McDonald for technical assistance; to P. Brickman, P. Josephson, R. Goldstei Mindich, S. Shaham, and J. Srinivasan for the isolation and characterization o TR679-derived mutants; to M. Driscoll, E. Ferguson, L. Fischer, C. Savage Walthall, and J. Way for mapping data and helpful discussions; and to M. Le and S. Mount for suggestions on the manuscript. We are also grateful tc Caenorhabditis Genetics Center (University of Missouri) and our fellow *C. el* researchers for providing both Mec mutants and mapping strains. Supporte U.S. Public Health Service grant GM30997 to M.C.

Research Articles

Reverse Transcriptase in a Clinical Strain of *Escherichia coli*: Production of Branched RNA-Linked msDNA

BERT C. LAMPSON, JING SUN, MEI-YIN HSU, JORGE VALLEJO-RAMIREZ, SUMIKO INOUYE, MASAYORI INOUYE

Branched RNA-linked multicopy single-stranded DNA (msDNA) originally detected in myxobacteria has now been found in a clinical isolate of *Escherichia coli*. Although lacking homology in the primary structure, the *E. coli* msDNA is similar in secondary structure to the myxobacterial msDNA's, including the 2′,5′-phosphodiester linkage between RNA and DNA. A chromosomal DNA fragment responsible for the production of msDNA was cloned in an *E. coli* K12 strain; its DNA sequence revealed an open reading frame (ORF) of 586 amino acid residues. The ORF shows sequence similarity with retroviral reverse transcriptases and ribonuclease H. Disruption of the ORF blocked msDNA production, indicating that this gene is essential for msDNA synthesis.

AN UNUSUAL SATELLITE DNA CALLED MSDNA (MULTICOPY single-stranded DNA) was originally found in *Myxococcus xanthus*, a Gram-negative bacterium living in soil (*1*). The satellite consists of a 162-base single-stranded DNA, the 5′ end of which is linked to a branched RNA (msdRNA) of 77 bases by a 2′,5′-phosphodiester linkage at the 2′ position of the 20th rG residue (*2*). There are approximately 700 copies of msDNA per genome. msDNA is widely distributed among various myxobact including the closely related *Stigmatella aurantiaca*, which has msDNA—msDNA-Sa163 (*3*), highly homologous to msDI Mx162 from *M. xanthus* (*4, 5*). Several *M. xanthus* strains, indep dently isolated from different sites, contain msDNA (*6*). We fo that *M. xanthus* contains another smaller species of msDNA form called mrDNA and now termed msDNA-Mx65 (*7*). In contras the close homology between msDNA-Mx162 and msDNA-Sa1 there is no primary sequence homology between msDNA-Mx and the small molecule, msDNA-Mx65. However, msDNA-M does share key secondary structures such as a branched rG residu DNA-RNA hybrid at the 3′ ends of the msDNA and the msdRI and stem-loop structures in RNA and DNA strands.

We have shown that msdRNA is derived from a much lor precursor RNA (pre-msdRNA), which can form a very stable st and-loop structure (*2*). A novel mechanism for msDNA synth was proposed, in which the stem-and-loop structure of msdRNA serves as a primer for initiating msDNA synthesis as as a template to form the branched RNA-linked msDNA, predicted that a reverse transcriptase (RT) is required for reaction (*2*). We now report that msDNA also exists in *Escher coli* and that a gene with sequence similarity to retroviral RT's

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