mec-7 is a β-tubulin gene required for the production of 15-protofilament microtubules in Caenorhabditis elegans

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In the nematode Caenorhabditis elegans, microtubules with 15 protofilaments are a specialized feature of six touch-receptor neurons; microtubules found in other C. elegans neurons have 11 protofilaments. Mutations in the gene mec-7 result in touch-insensitive animals whose touch cells lack the 15-protofilament microtubules. We have characterized 54 mutations in the mec-7 gene. The absence of mec-7 activity results selectively in the recessive loss of touch sensitivity. Partial loss-of-function alleles result in a partial loss of touch sensitivity. Dominant mutations, which are isolated at an unusually high proportion, may encode abnormal products. We have cloned the mec-7 gene; it encodes a β-tubulin which is 90–93% identical to vertebrate β-tubulin. Our results are consistent with the hypothesis that tubulin heterogeneity contributes to the formation of structurally and functionally distinct sets of microtubules.

[Key Words: Caenorhabditis elegans; β-tubulin, tubulin mutants, microtubule structure, protofilament number]

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Although ubiquitous in eukaryotes, microtubules display considerable structural diversity. They can exist as singlets or doublets and in specialized arrays (Tucker 1979; Dustin 1984) and can vary in the number of protofilaments (Tilney et al. 1973; Burton et al. 1975; Nagano and Suzuki 1975; Chalfie and Thomson 1982; Saito and Hama 1982; Eichenlaub-Ritter and Tucker 1984; Tucker et al. 1985; Mogensen and Tucker 1987). How this structural diversity is produced is not known. Fulton and Simpson (1976) and, more recently, Cleveland (1987) have suggested that diversity in tubulin protein structure, encoded genetically, may be partially responsible for the formation of structurally and functionally distinct sets of microtubules. Post-translational modifications of tubulin (L'Hernault and Rosenbaum 1985) and/or different microtubule-associated proteins may also contribute to the production of specialized microtubules. Experiments by Scheele et al. (1982) and Evans et al. (1985), for example, have suggested that protofilament number is determined, at least in part, by microtubule initiation.

We have studied the specification of microtubule structure and function in Caenorhabditis elegans because this organism possesses an unusual, cell-specific microtubule that can be altered by mutation. Most neurons in C. elegans have microtubules with 11 protofilaments, the six touch-receptor neurons contain ~450 15-protofilament microtubules and, at most, 1 or 2 11-protofilament microtubules (Chalfie and Thomson 1982). Mutations in the gene mec-7 result in animals that are insensitive to gentle touch and whose touch cells processes lack the 15-protofilament microtubules. Touch cells in the two mec-7 mutants examined in serial sections [e1506 and e1343] contain ~100 11-protofilament microtubules that do not associate into structured bundles as do the 15-protofilament microtubules (Chalfie and Thomson 1982). The 11-protofilament microtubules allow the outgrowth of touch cell processes but are insufficient for sensory transduction. Microtubule structure in other cells does not appear to be affected in mec-7 mutants.

In this paper we describe a genetic and molecular analysis of the mec-7 gene. We have characterized 54 mec-7 mutations. Some mutations, including two complete deletions of the gene, result in the recessive loss of touch sensitivity. Other mutations are expressed as hypomorphs or antimorphs yet still appear to affect only touch sensitivity. The sequence of the cloned mec-7 gene reveals that it encodes a β-tubulin that is similar to vertebrate β-tubulins. Our data suggest that the formation of structurally and, perhaps, functionally unique microtubules can, at least in part, be mediated by the use of a specialized β-tubulin.
Results

mec-7 mutations produce a range of touch insensitive phenotypes

We have classified the 54 mec-7 mutations into five phenotypic groups (Table 1) [1] strong recessive [15 alleles]; [2] weak recessive [8 alleles]; [3] weak semidominant (i.e., both homozygotes and heterozygotes show a weak phenotype; 12 alleles); [4] strong semidominant (i.e., the homozygotes have a strong phenotype, and the heterozygotes, a weak one; 13 alleles); [5] strong dominant (6 alleles). Mutants showing a strong phenotype are completely touch insensitive; those showing a weak phenotype are partially touch sensitive and display a position dependence in their response (see below).

Dominant mec-7 mutations are antimorphic

More than half of the mec-7 alleles produce a dominant or semidominant phenotype. These alleles probably act as antimorphs, generating products that interfere with normal mec-7 function. As described in Methods, we constructed animals containing a chromosomal deficiency of the mec-7 region [Fig. 1] in trans to a copy of the wild-type mec-7 gene [uDfl/+] to determine whether a loss of gene activity produces a dominant phenotype. Because these animals are phenotypically wild type, the complete loss of gene activity is expressed recessively. The dominant phenotypes are therefore not the result of haploinsufficiency. We increased mec-7(+)/+ dosage (as described in Methods) to determine whether the dominant phenotypes are due to the overexpression of normal gene product or to the expression of an aberrant product. mec-7/+/+ strains constructed with the translocated duplication stDp2, which contains a copy of the wild-type mec-7 gene, and the dominant alleles e1527, n434, u18, u129, and u162 showed partial rescue of touch sensitivity by the extra dose of mec-7(+) at 20°C (Table 2). In addition, the mec-7(+)/+ construct with the semidominant allele e1505 was completely wild type at 25°C.

Additional support for the hypothesis that these alleles encode aberrant products comes from an examination of temperature effects. Chalfie and Thomson [1982] found that both the expressivity and penetrance of the touch-insensitive phenotype were higher at 25°C than at 15°C for heterozygotes containing the e1343, e1505, e1522, and e1527 alleles. We have found this temperature sensitivity to be a general property: Heterozygotes carrying a single copy of any of the dominant or semidominant mec-7 alleles (except n434) are more completely touch insensitive at 25°C than at 15°C [Table 1]. In addition, n434/+/+ animals are more completely touch insensitive at 20°C than at 15°C [Table 2].

Loss of mec-7 function causes only touch insensitivity

The recessive expression of complete touch insensitivity (i.e., the strong, recessive phenotype) is likely to be the result of the complete loss of mec-7 function. Two γ-ray-induced mutations, u443 and u448, which produce this phenotype, are deletions of ~28 and 18 kb, respectively, which include the mec-7 gene (see below). Furthermore, a screen for noncomplementing mutations (described in Methods) yielded only mec-7 alleles that result in touch insensitivity. Because mec-7 [e1506]/

uDfl animals are touch insensitive, mutations causing more severe defects, such as uncoordination or lethality, could in theory be isolated in a screen for mutations that fail to complement e1506 for touch sensitivity. None of the eight mutations identified in this way from 9700 ethyl methanesulfonate (EMS)-mutagenized chromosomes caused more severe defects: Six produced a strong recessive phenotype, one a weak recessive phenotype, and one a weak semidominant phenotype. All eight are X linked and thus likely to be mec-7 defects.

Heterozygotes of two of the weak alleles, u305[s] and u156[sd], with uDfl are more strongly touch insensitive than the corresponding homozygotes. Thus, the weak alleles are not due to the complete loss of mec-7 gene activity but are likely to be hypomorphic, partial loss-of-function mutations.

Position dependence of touch sensitivity in weak alleles

Chalfie and Sulston [1981] showed that touch in the anterior of the worm is sensed by three anterior touch cells (ALML, ALMR, and AVM, Fig. 2), whereas touch in the posterior of the worm is sensed by two posterior touch cells (PLMR and PLML, Fig. 2). Mutants with different mec-7 mutations differ in their response to touch at various positions along their bodies (Table 1; Fig. 2). Wild-type worms sense touch along their entire lengths, whereas homozygotes for strong mec-7 alleles, for example, e1506, do not respond to touch at any position [except at the very tips of the head and tail, where even animals lacking touch cells respond [Chalfie and Sulston 1981]]. Many mutants, however, display a partial phenotype. These animals respond more readily when touched at the pharynx (position A in Fig. 2) than at a position more posterior to the pharynx (position B). Similarly, many mutants respond more readily when touched at the tail at a position near the anus (position D) than at a more anterior location (position C). The spatial differences in touch sensitivity do not correlate with the distance from the touch cell body. Rather, the degree of touch sensitivity correlates with the distance from the touch cell synapses, which are made near position A in the head and near position D in the tail [Chalfie et al. 1985].

mec-7 is a β-tubulin gene

The mec-7 gene appears to encode a β-tubulin. A number of C. elegans tubulin genes have been cloned and characterized [Gremke 1986]. One of these (a β-tubulin gene originally called tub-2) is contained in a series of contiguous cosmids clones [Coulson et al. 1986]. One of the cosmids in this series mapped by in situ hybridization to the region of the X chromosome near
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Table 1. *Touch-insensitive phenotype of mec-7 mutants*

| Class          | Allele | m/m [25°C]* | m/ + [25°C]* | m/ + [15°C]* |
|----------------|--------|-------------|--------------|--------------|
|                |        | head | tail | head | tail | head | tail |
| Strong recessive | e1506  | -    | -    | +    | +    | +    | +    |
|                 | u142   | -    | -    | +    | +    | +    | +    |
|                 | u176   | -    | -    | +    | +    | +    | +    |
|                 | u178   | -    | -    | +    | +    | +    | +    |
|                 | u275   | -    | -    | +    | +    | +    | +    |
|                 | u388   | -    | -    | +    | +    | +    | +    |
|                 | u428   | -    | -    | +    | +    | ND   | ND   |
|                 | u429   | -    | -    | +    | +    | ND   | ND   |
|                 | u432   | -    | -    | +    | +    | ND   | ND   |
|                 | u433   | -    | -    | +    | +    | ND   | ND   |
|                 | u434   | -    | -    | +    | +    | ND   | ND   |
|                 | u440   | -    | -    | +    | +    | ND   | ND   |
|                 | u443   | -    | -    | +    | +    | ND   | ND   |
|                 | u448   | -    | -    | +    | +    | ND   | ND   |
|                 | u453   | -    | -    | +    | +    | ND   | ND   |
| Weak recessive | u80    | -    | -    | +    | +    | +    | +    |
|                 | u88    | -    | -    | +    | +    | +    | +    |
|                 | u170   | ±    | ±    | +    | +    | +    | +    |
|                 | u173   | ±    | ±    | +    | +    | +    | +    |
|                 | u305   | ±    | ±    | +    | +    | +    | +    |
|                 | u382   | ±    | ±    | +    | +    | +    | +    |
|                 | u430   | ±    | ±    | +    | +    | ND   | ND   |
|                 | u431   | ±    | ±    | +    | +    | ND   | ND   |
| Weak semi-dominant | e1522 | -    | -    | -    | ±    | ±    | +    |
|                  | u48    | -    | -    | -    | ±    | ±    | +    |
|                  | u58    | -    | -    | -    | ±    | ±    | +    |
|                  | u127   | -    | -    | -    | ±    | ±    | +    |
|                  | u136   | -    | -    | -    | ±    | ±    | +    |
|                  | u145   | -    | -    | -    | ±    | ±    | +    |
|                  | u156   | -    | -    | -    | ±    | ±    | +    |
|                  | u225   | -    | -    | -    | ±    | ±    | +    |
|                  | u262   | -    | -    | -    | ±    | ±    | +    |
|                  | u278   | -    | -    | -    | ±    | ±    | +    |
|                  | u319   | -    | -    | -    | ±    | ±    | +    |
|                  | u427   | -    | -    | -    | ±    | ±    | +    |
| Strong semi-dominant | e1343 | -    | -    | -    | +    | +    | +    |
|                  | e1505  | -    | -    | ±    | +    | +    | +    |
|                  | u9     | -    | -    | ±    | +    | +    | +    |
|                  | u10    | -    | -    | ±    | +    | +    | +    |
|                  | u98    | -    | -    | ±    | +    | +    | +    |
|                  | u143   | -    | -    | ±    | +    | +    | +    |
|                  | u222   | -    | -    | ±    | +    | +    | +    |
|                  | u223   | -    | -    | +    | ±    | +    | +    |
|                  | u234   | -    | -    | +    | ±    | +    | +    |
|                  | u249   | -    | -    | +    | ±    | +    | +    |
|                  | u445   | -    | -    | -    | ±    | ND   | ND   |
|                  | u449   | -    | -    | ±    | ND   | ND   | ND   |
|                  | u451   | -    | -    | ±    | ND   | ND   | ND   |
| Dominant        | e1527  | -    | -    | -    | ±    | +    | +    |
|                  | u434   | -    | -    | -    | -    | -    | -    |
|                  | u18    | -    | -    | -    | -    | -    | -    |
|                  | u129   | -    | -    | -    | -    | -    | -    |
|                  | u162   | -    | -    | -    | -    | -    | -    |
|                  | u283   | -    | -    | -    | -    | -    | -    |

Animals were touched at position B (Fig. 2) in the head and at position D (Fig. 2) in the tail, as described in Chalfie and Sulston (1981).

* ( + ) Touch sensitive; ( ± ) partially touch sensitive; ( - ) touch insensitive, as defined in Methods; (ND) not determined.

b Weak alleles are those that produce partial phenotypes at any of the positions indicated in Fig. 2.
Figure 1. Genetic map surrounding the mec-7(X) locus. The open bar shows the extent of the chromosomal deficiency uDf1; the solid bar shows the extent of the duplication stDp2. The gene xol-1 has been mapped to the interval between mec-7 and dpy-6 (L. Miller, pers. comm.). All other map positions are from data presented in the text or from Edgley and Riddle (1987).

Genetic analysis of mec-7 β-tubulin

Probes containing this β-tubulin gene identified DNA fragment length differences in several mec-7 mutant strains. One of these differences, a deletion in the e1505 strain (Fig. 3), was used to map the β-tubulin gene relative to mec-7. None of 9 Unc and 17 of 18 Dpy recombinants from the heterozygote unc-18 + dpy-6/+ mec-7(e1505) + showed the Mec phenotype. Genomic DNA from all of the Mec animals, but from none of the nonMec animals, contained the deletion (data not shown). Thus, the deletion and the mec-7 mutation are within 0.02 map units.

We have used the cosmid R02A8 containing the β-tubulin gene and a subfragment of this cosmid to probe genomic DNA from wild type and from mec-7 mutants. DNAs from seven mec-7 mutants exhibit restriction fragment length differences when probed with R02A8.

Figure 2. Position dependence of touch sensitivity. (+) Touch sensitive; (-) partially touch sensitive; (-) touch insensitive.
Figure 3. Southern blots of wild-type and mec-7 mutants. (A) Genomic DNAs from wild-type (N2), two EMS-derived mec-7 mutants (e1505 and u156), two TR679-derived mec-7 mutants (u382 and u388), and a wild-type revertant of the TR679 mutant u382 (u382u473) were probed with a 10-kb BglII fragment isolated from cosmid R02A8 that contains the β-tubulin sequence. The coding sequence is contained within the 3.5- and 4.4-kb EcoRI fragments. Open arrows identify deletions in the 4.4-kb fragment; solid arrows identify insertions in the 3.5-kb fragment. (B) Genomic DNAs from wild-type and three γ-ray-induced mutants (u443, u448, and u453) were probed with the entire R02A8 cosmid.

Sequence of the mec-7 gene

We have determined the nucleotide sequence of 2100 bp of DNA that appears to include the entire coding region of mec-7 and 338 bp of upstream sequence (Fig. 4). The putative tubulin-coding sequence is interrupted by four small introns. Each putative intron is bounded by consensus C. elegans donor and acceptor splice sites (Emmons 1988), and each contains stop codons in all three reading frames. The tubulin predicted from the putative exon sequences has 441 amino acids. No known regulatory sequences were found in the upstream region, nor does it contain consensus splice acceptor or splice donor sites. Therefore, this region is unlikely to be an intron.

The coding sequence is 90–93% identical to that of vertebrate β-tubulins and the testis-specific β-tubulin of Drosophila (Table 3, Fig. 5). Sequence identity is lower in comparison with other β-tubulins. The carboxyterminal region beyond amino acid 430 is highly divergent in mec-7, as it is in other β-tubulins and is the shortest of any reported β-tubulin (Sullivan and Cleveland 1986; Rudolph et al. 1987; Burland et al. 1988). In addition to these differences at the carboxyl terminus, only seven residues in the mec-7 peptide sequence are not shared by other reported β-tubulins: residues 35 (Gln), 127 (Thr), 198 (Ser), 278 (Asn), 293 (Cys), 343 (Asp), and 429 (Ala).
Genetic analysis of mec-7 β-tubulin

The nucleotide sequence of mec-7 is shown below, with predicted translational start and stop sites indicated. Predicted splice acceptor and donor sites are underlined. One anomaly in the sequence is the predicted XbaI site at position 629, which was not detected by restriction mapping analysis.

Figure 4. Nucleic acid and predicted amino acid sequence of mec-7. Predicted translational start and stop sites are at positions 339 and 1932, respectively. Predicted splice acceptor and splice donor sequences are underlined. One anomaly in the sequence is the predicted XbaI site at position 629, which was not detected by restriction mapping analysis.

Discussion

Nature of mec-7 activity

mec-7 is likely to encode a β-tubulin that is selectively required in the C. elegans touch cells; mutations in mec-7 do not detectably affect other cells. Various data suggest that loss-of-function mutations are recessive and result in the complete loss of touch sensitivity: (1) Two deletions of the mec-7 locus produce the strong recessive phenotype; (2) the mec-7 locus is not haploinsufficient; (3) e1506/ud[1] animals have no stronger phenotype than e1506 homozygotes; and (4) a screen for alleles that fail to complement mec-7(e1506) identified only mutations resulting in touch insensitivity. Similarly, null mutations in β-tubulin genes from Saccharomyces cerevisiae [Neff et al. 1983] and Drosophila melanogaster [Kemphues et al. 1983] are expressed recessively.
Figure 5. Comparison of amino acid sequences from mec-7 and Drosophila β2-tubulin. The entire predicted amino acid sequence of the mec-7 β-tubulin is shown, but only those differences in the Drosophila sequence (B2t) are indicated. (o) Novel amino acid substitution; (●) substitution in a previously invariant amino acid.

Dominant and semidominant mec-7 alleles act as antimorphs. Fifteen protofilament microtubules are not found in the touch-insensitive heterozygotes of dominant alleles [Chalfie and Thomson 1982]. Abnormal gene products in these animals are likely to interfere with wild-type proteins to prevent the formation and/or stability of these microtubules. The sequences of α- and β-tubulins are highly conserved among eukaryotes, and the homologies extend throughout the proteins [Cleveland and Sullivan 1985]. This degree of conservation suggests that tubulins cannot tolerate structural change without loss or change of function. Thus, one would expect a large proportion of tubulin mutations to encode aberrant products that fail to interact normally with other microtubule proteins. These mutations are likely to be expressed dominantly, as we have found for the mec-7 β-tubulin gene. Consistent with this hypothesis, dominant alleles constitute a considerable proportion of the β-tubulin mutations in Schizosaccharomyces pombe [9 of 9; Yamamoto 1980], S. cerevisiae [15 of 65; Thomas et al. 1985], and D. melanogaster [2 of 5; Kemphues et al. 1979, 1983].

Position dependence of touch sensitivity

The hypomorphic mutants show a position dependence of touch sensitivity in the head and in the tail. The degree of touch sensitivity does not correlate with the dis-

Table 2. Effects of increased gene dosage on the expression of dominant mec-7 phenotypes

| Genotype     | Number of animals | Percent touch insensitive | Percent partially touch sensitive | Percent touch sensitive |
|--------------|-------------------|---------------------------|----------------------------------|-------------------------|
| e1527/+      | 49                | 94                        | 6                                | 0                       |
| e1527/++     | 45                | 0                         | 0                                | 100                     |
| n434/+       | 37                | 97                        | 3                                | 0                       |
| n434/++      | 32                | 3                         | 97                               | 0                       |
| u18/+        | 15                | 67                        | 33                               | 0                       |
| u18/++       | 16                | 0                         | 75                               | 25                      |
| u129/+       | 9                 | 44                        | 56                               | 0                       |
| u129/++      | 40                | 5                         | 60                               | 35                      |
| u162/+       | 28                | 50                        | 50                               | 0                       |
| u162/++      | 41                | 0                         | 71                               | 29                      |
| n434/+[15°]  | 10                | 100                       | 0                                | 0                       |
| n434/++ [15°]| 13                | 0                         | 62                               | 38                      |
tance from the cell bodies of the touch cells but with the distance from the touch cell synapses. Because these mutants can respond to touch at some positions, the touch cells appear to make proper connections. Other observations, using Nomarski optics and immunofluorescence microscopy, confirm that the touch cell processes appear normal (C. Savage and M. Chalfie, unpubl.).

We do not yet know whether touch cells in these mutants contain 15-protofilament microtubules. The partial touch sensitivity suggests that the mec-7 β-tubulin plays a role in sensory transduction. The 15-protofilament microtubules may function, actively or passively, in the generation of signals (Chalfie and Thomson 1982). Motor neurons in the large nematode Ascaris do not produce action potentials, only graded potentials (Stretton et al. 1985). If the touch cells in C. elegans act similarly, the magnitude of the signal may be so reduced in hypomorphic mec-7 mutants that it cannot reach the synapses unless generated very near to them, or these mutants may contain less stable microtubules that can be stabilized by proteins found preferentially near the synapses. Alternatively, the microtubules may be necessary for structural integrity; in the absence of these microtubules, a signal cannot propagate along the touch cell process. We believe that this last possibility is unlikely, however, because other neurons in the nematode can propagate signals without 15-protofilament microtubules.

Tubulin protein structure and function

When polymerized in vitro, tubulins vary greatly in protofilament number unless grown from axonemal seeds (Scheele et al. 1982) or centrosomes (Evans et al. 1985). These experiments have led to the conclusion that nucleation of microtubules is a determinant of protofilament number. Our finding that mec-7 is a β-tubulin gene suggests that the constituent tubulins of the microtubule may also affect the number of protofilaments. Still, the mec-7 product could reside at the nucleation sites of touch cell microtubules or could affect protofilament number via specific binding to a microtubule-associated protein. Moreover, mec-7 is not sufficient for the formation of 15-protofilament microtubules because mutations in three other genes also result in the loss of these microtubules (Chalfie and Au 1988); nor do we know whether mec-7 is expressed in other cell types that do not contain 15-protofilament microtubules.

The mec-7 β-tubulin may be required for the formation of 15-protofilament microtubules because of structural features inherent in the protein. Alternatively, the mec-7 gene may be necessary because it maintains a particular level of tubulin expression. Either of these possibilities represents an unusual method of influencing microtubule structure. We believe, however, that the second possibility, control of β-tubulin levels, is unlikely because (1) sufficient tubulin [from other genes] is produced in mec-7 null mutants to form ~100 11-protofilament microtubules (Chalfie and Thomson 1982); (2) altering wild-type gene dosage has no effect on touch sensitivity; and (3) Scheele et al. (1982) found no significant effect of tubulin concentration of protofilament number of microtubules polymerized in vitro. Moreover, experiments by Aamodt and Culotti (1986) support a role for microtubule proteins in the determination of protofilament numbers: Tubulins and microtubule-associated proteins isolated from C. elegans predominantly formed microtubules with 9–11 protofilaments in vitro, whereas those from bovine brain predominantly formed microtubules with 13 protofilaments.

The mec-7 tubulin is very similar to other β-tubulins, so that only a few amino acid changes may be needed to permit the assembly of 15-protofilament microtubules. Figure 5 compares the mec-7 sequence with that of the testis-specific β-tubulin from Drosophila (Rudolph et al. 1987). This latter β-tubulin is required for the formation of all cytoplasmic, spindle, and axonemal microtubules.

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Table 3. Percentage identity between the amino acid sequence of mec-7 and that of other β-tubulins

| β-Tubulin      | Percent identity | Reference                        |
|----------------|------------------|----------------------------------|
| Physarum β1    | 86 (398 aa)      | Singhofer-Wowra et al. (1986)    |
| Physarum β2    | 79               | Burland et al. (1988)            |
| Chlamydomonas reinhardtii | 85 | Youngblom et al. (1984) |
| Trypanosome    | 81               | Kimmel et al. (1985)             |
| Neurospora crassa | 82  | Orthbach et al. (1986)          |
| Aspergillus β1/2 | 81 | May et al. (1987)          |
| Aspergillus β3  | 76               | May et al. (1987)                |
| S. pombe       | 75               | Hiraoka et al. (1984)            |
| S. cerevisiae  | 74               | Neff et al. (1983)               |
| Drosophila β2  | 92               | Rudolph et al. (1987)            |
| Drosophila β3  | 88               | Rudolph et al. (1987)            |
| Chicken β1     | 92               | Sullivan et al. (1985)           |
| Chicken β2     | 92               | Sullivan et al. (1985)           |
| Chicken β3     | 92               | Sullivan and Cleveland (1986)    |
| Chicken β4     | 90               | Sullivan and Cleveland (1984)    |
| Chicken β5     | 90               | Sullivan and Cleveland (1986)    |
| Human M40      | 91               | Lewis et al. (1985)              |
| Human β2       | 93               | Lewis et al. (1985)              |
| Human β4       | 90 (379 aa)      | Lewis et al. (1986); Lewis et al. |
| Human β5       | 91               | Lewis et al. (1985)              |
| Pig β1         | 92               | Krauhs et al. (1981)             |
| Mouse β2       | 91 (204 aa)      | Sullivan and Cleveland (1987); Lewis et al. |
| Mouse β4       | 92 (375 aa)      | Lewis et al. (1985)              |
| Mouse β4'      | 93 (371 aa)      | Sullivan and Cleveland (1986)    |
| Mouse β5       | 91               | Sullivan and Cleveland (1986)    |

β-Tubulin amino acid sequences were compared to mec-7 along its 441-amino-acid length, except where partial sequences were available, as indicated by the amino acid numbers in parentheses (aa).

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(none of which have 15-protofilaments) during spermatogenesis, beginning with meiosis [Raff 1984]. Of the first 430 residues in the mec-7 peptide, only 7 are not shared by the Drosophila β-tubulin or by any other known β-tubulins [Fig. 5]. Possibly of most significance to the secondary structure of mec-7, because sulfhydryl groups may play a role in the regulation of tubulin assembly [Nishida and Kobayashi 1977; Burchill et al. 1978], are the substitutions of a threonine for a cysteine at residue 127 and a cysteine for a methionine at residue 293. The cysteine at position 127 has been conserved in every other sequenced β-tubulin [Sullivan and Cleveland 1986; Rudolph et al. 1987; Burland et al. 1988], including two other C. elegans β-tubulins [Gremke 1986; M. Driscoll, E. Dean, E. Reilly, E. Bergholz, and M. Challie, unpubl.]. Residue 293 lies in the 'hinge' region between the amino- and carboxyterminal domains. A single amino acid change at position 288 in the hinge region in the Drosophila β-tubulin prevents polymerization of the tubulin into functional microtubules without preventing heterodimer or protofilament formation [Rudolph et al. 1987]. This region may thus be important for interactions between protofilaments.

Effects of tubulin mutations on microtubule structure and function

Although the mec-7 β-tubulin is required specifically in the touch cells in C. elegans, it is not the only β-tubulin that can be expressed in this cell type. In the touch cell process of mec-7(e1343) and mec-7(e1506) mutants, the 15-protofilament microtubules have been replaced by 11-protofilament microtubules [Challie and Thomson 1982]. Because process outgrowth in these mutants is inhibited by benomyl [Challie and Thomson 1982], the microtubules in these processes are likely to contain a benomyl-sensitive component. Studies of benomyl sensitivity in C. elegans have shown that only one gene product, the β-tubulin encoded by ben-1, confers benomyl sensitivity to microtubules [Challie et al. 1986]. Therefore, the mec-7 mutant touch cells are likely to be expressing the β-tubulin gene ben-1. We have now examined a deletion null mutant [mec-7(u448)] and found that the touch cell processes in these animals also contain small-diameter microtubules like those found in other neurons [data not shown]. We do not yet know whether the ben-1 gene or other β-tubulin genes are expressed in wild-type touch cells. β-Tubulin expression in these mutants may be similar to that in Aspergillus nidulans, where the benA22 benomyl-resistant β-tubulin can substitute in conidiation for the β-3 tubulin in fungi lacking β-3 activity [Weatherbee et al. 1985]. In mec-7 mutants, however, the ben-1 β-tubulin is not sufficient for the assembly of 15-protofilament microtubules. Although the β2t tubulin is multifunctional, it may be specifically required for the formation of one or more of the microtubule types in which it is expressed. Consistent with this hypothesis, some partial loss-of-function mutations in β2t have effects either on the meiotic spindle or on the flagellar axoneme, but not both [Fuller 1986]. Similarly, we do not yet know whether the mec-7 tubulin is incorporated into 11-protofilament microtubules in other cell types.

Other tubulin mutations have been identified by resistance or supersensitivity to benzimidazole compounds in A. nidulans [Sheir-Neiss et al. 1978; Morris et al. 1979] Physarum [Burland et al. 1984], S. pombe [Yamamoto 1980], and S. cerevisiae [Thomas et al. 1985]. These mutations have been useful in identifying tubulin genes and in determining when and where these genes are expressed. Because such alleles represent a specific class of tubulin mutations, however, they cannot directly elucidate the complete loss-of-function phenotype. Mutations in essential tubulin genes have been identified in the yeasts S. cerevisiae [Hiraoka et al. 1984; Adachi et al. 1986] and S. pombe [Thomas et al. 1985; Schatz et al. 1986] and in Drosophila [Matthews and Kaufman 1987]. Because of the lethality of these mutations, the effects of the mutant tubulins on microtubule structure are difficult to determine. Because the mec-7 β-tubulin is not essential for the worm or for touch cell growth, we have been able to isolate a large, heterogeneous set of mutations in the gene. It should be possible to identify protein domains required for incorporation into the 15-protofilament microtubules by mapping and sequencing the mutations in these alleles.

Methods

Growth and maintenance of nematode strains

Animals were grown as described by Brenner [1974]. Unless otherwise noted, animals were kept at 25°C because they grow most rapidly at this temperature. Most strains derive from the wild-type C. elegans var. Bristol [strain N2]. In addition to the mec-7 alleles, we used the following mutations on linkage group X: lon-2(e678), mec-2(e1084), unc-6(e76), dpy-7(e88), unc-18(e81), dpy-6(e14), uDF1, and stDp2(X;II); and the following mutations on linkage group III: lon-1(e185) and sup-5(e1464). Except for uDF1, stDp2, and sup-5, all of these mutations were characterized by Brenner [1974]. The deficiency uDF1 was isolated as described below. stDp2 is a duplication of the region of the X chromosome that includes mec-7, which has been translocated to chromosome II {R. Waterston, pers. comm.}. Two copies of stDp2 are lethal. The amber suppressor mutation sup-5(e1464) is described by Waterston and Brenner [1978] and Wills et al. [1983]. Except for the mec-7 alleles identified by failure to complement mec-7(e1506), as described below, all mec-7 alleles were isolated in previous screens for touch-insensitive mutants [Challie and Sulston 1981; Challie and Thomson 1982; Challie and Au 1988], 37 were identified following EMS mutagenesis, 7 [u440, u443, u445, u448, u449, u451, u453] were identified following γ-ray mutagenesis, and 2 [u382 and u388] derived from the mutant strain TR679 [mut-2(r459)] [Collins et al. 1987] that generates spontaneous mutations by increasing the rate of transposition.
Mutagenesis
We screened for noncomplementing mec-7 alleles by mating lon-2 mec-7(e1506) males to dpy-6 hermaphrodites that had been mutagenized with EMS [Brenner 1974] and examining their progeny for touch sensitivity. Touch-insensitive (+) animals were isolated and allowed to segregate animals homozygous for new mec-7 alleles linked to dpy-6. These mutations have allele numbers u427–u434.

To screen for deficiencies, we irradiated wild-type males for 4.25 min (total radiation = 3000 rads) with a lSFCs gammator, and screened for touch-insensitive cross progeny. Of 2700 F1 progeny, one such mutation, uDF1, was identified. This deficiency is lethal as a homozygote, uDF1/mec-7 dpy-6 hermaphrodites (L. Miller, pers. comm.), unc-6, dpy-6, unc-18, and mec-7, complements lon-2 and dpy-6, and is completely covered by stDp2. Because unc-18 and xol-1 flank mec-7 on opposite sides on the genetic map, it is likely that the deficiency uDF1 completely deletes the mec-7 gene.

Identification of revertants of TR679-derived mutations
To revert the TR679-derived alleles u382 and u388, we first reintroduced the mut-2(r459) mutation [Collins et al. 1987] from the strain HH*10 (a gift from M. Finney, Massachusetts General Hospital, Boston). Individual mut-2, mec-7(u382) and mut-2; mec-7(u388) animals were plated at 20°C, and 8000 and 1700 F1 progeny, respectively, were tested for touch sensitivity. Two wild-type revertants (u382tu473 and u388tu474) that segregated only heterozygotes and mec-7(d) homozygotes were identified and separated from mut-2 by repeated crosses with mec-7(e1506).

Genetic mapping
The position of mec-7 on the genetic map [Fig. 1] was refined by the following three-factor cross using standard procedures [Brenner 1974]: 2 of 35 Unc and 36 of 38 Dpy recombinants from unc-18 + dpy-6/+ mec-7 + animals segregated mec-7 progeny. These data place mec-7 –0.02 map units to the right of unc-18 on the X chromosome.

Genetic analysis of mec-7 β-tubulin

Genetic analysis of mec-7 β-tubulin

Suppression tests
To identify nonsense alleles, we looked for effects of the amber suppressor mutation sup-5(e1464) on mec-7 expression. For each allele of mec-7, a hermaphrodite of genotype lon-1 sup-5/+ mec-7(+)/+ was constructed at 20°C and its Lon progeny were examined for touch sensitivity. None of the mutations were detectably suppressed. Because many of the mec-7 alleles produce a semidominant or dominant phenotype, the insertion of a novel amino acid into the protein by a suppressor tRNA might enhance, rather than suppress, an amber mutation [Winston and Botstein 1981]. The phenotypes of the recessive alleles were not, however, detectably enhanced in the sup-5 background.

Construction of mec-7(d)/+/+ strains
We increased wild-type mec-7 dosage in animals with dominant mec-7 mutations [designated as mec-7(d)] by constructing strains containing stDp2. stDp2/+; uDF1 males were mated to dpy-7 mec-7(d) hermaphrodites. Their non-Dpy male cross progeny (stDp2/+; dpy-7 mec-7) were mated to dpy-7 unc-18 hermaphrodites. Non-Dpy hermaphrodites from this mating [genotype stDp2 × dpy-7 unc-18 + dpy-7 + mec-7(d)] have two copies of the wild-type gene and one copy of a dominant allele of mec-7. These animals are designated as mec-7(d)/+/+.

Construction of uDF1/+ animals
We determined the effect on touch sensitivity of decreased wild-type mec-7 dosage by mating wild-type males to uDF1/mec-7(e1506)dpy-6(e14) hermaphrodites. Non-Dpy hermaphrodites, which are either uDF1/+, mec-7 dpy-6/+, or uDF1/mec-7 dpy-6, were picked and scored for touch sensitivity. Those animals that segregated dead eggs and no Dpy progeny were designated uDF1/+.

Electron microscopy
Animals were fixed in glutaraldehyde, acrolein, and OsO4 and processed for electron microscopy as before (Chalfie and Thomson 1979), except that the animals were stained en bloc in 0.5% uranyl acetate and embedded in epon.

Recombinant DNA techniques.
DNA was extracted from C. elegans, as described by Emmons et al. [1979]. Genomic DNA was digested with EcoRI from New England Biolabs in accordance with their instructions, except that 5 mM spermidine was added to all reactions. Southern blots of genomic DNA were probed with the λ clone NW #BT4, the cosmid R02A8, or the BglII subfragment of ~10 kb isolated from R02A8. The R02A8 cosmid was identified as containing the tub-2 β-tubulin gene [Gremke 1986] by comparing its fingerprint [Coulson et al. 1986] to that of the λ clone NW #BT4 that contains this β-tubulin. The BglII fragment contains all of the DNA sequences of R02A8 that hybridize to a chicken β-tubulin cDNA clone [Cleveland et al. 1980], a gift of N. Cowan [New York University Medical Center]. Probes were labeled with 32P using an oligolabeling kit from Pharmacia or by nick translation. Other techniques were performed according to Maniatis et al. [1982].

The R02A8 cosmid is part of a contiguous set of cosmids [contig] that also contains the vit-1 gene [Heine and Blumenthal 1986]. An adjacent cosmid in this set, C27C10, was used to localize the contig to the central portion of the X chromosome, the same region as mec-7, by in situ hybridization as described previously [Albertson 1985].

DNA sequencing was performed according to the method of Sanger et al. [1977]. A 5.0-kb Xbal fragment was subcloned from the tub-2 clone [Gremke 1986] as two Xbal–HindIII fragments into Stratagene's pKS vector. Subclones in both orientations were treated according to the method of Heinkoff [1984] to create a series of deletions. Single-stranded DNA from superinfected cultures was then subjected to sequence analysis. The sequence shown in Figure 4 was confirmed on both strands.
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**Note**

The nucleotide sequence data reported will appear in the EMBL, GenBank, and DDBJ nucleotide sequence databases under the accession number X15242 mec-7.

**Genetic analysis of mec-7 β-tubulin**
mec-7 is a beta-tubulin gene required for the production of 15-protofilament microtubules in Caenorhabditis elegans.

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