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

Cathy Savage,^{1,5} Michel Hamelin,² Joseph G. Culotti,² Alan Coulson,³ Donna G. Albertson,^{3,4} and Martin Chalfie¹

¹Department of Biological Sciences, Columbia University, New York, New York, 10027 USA; ²Division of Molecular Immunology and Neurobiology, Mt. Sinai Hospital Research Institute, Toronto, Ontario, Canada M5G 1X5; ³Laboratory of Molecular Biology, Medical Research Council Centre, Cambridge CB2 2QH, UK

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 neu-

rons 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 cell 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.

⁴Present address: Molecular Genetics Unit, Medical Research Council Centre, Cambridge CB2 2QH, U.K.

⁵Corresponding author.

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 (*uDf1/+*) 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 was 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*)/*uDf1* 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(r)* and *u156(sd)*, with *uDf1* 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 (PLML and PLMR; 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 cosmid 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

Table 1. Touch-insensitive phenotype of *mec-7* mutants

Class	Allele	<i>m/m</i> (25°C) ^a		<i>m/+</i> (25°C) ^a		<i>m/+</i> (15°C) ^a	
		head	tail	head	tail	head	tail
Strong recessive	<i>e1506</i>	–	–	+	+	+	+
	<i>u142</i>	–	–	+	+	+	+
	<i>u176</i>	–	–	+	+	+	+
	<i>u178</i>	–	–	+	+	+	+
	<i>u275</i>	–	–	+	+	+	+
	<i>u388</i>	–	–	+	+	+	+
	<i>u428</i>	–	–	+	+	ND	ND
	<i>u429</i>	–	–	+	+	ND	ND
	<i>u432</i>	–	–	+	+	ND	ND
	<i>u433</i>	–	–	+	+	ND	ND
	<i>u434</i>	–	–	+	+	ND	ND
	<i>u440</i>	–	–	+	+	ND	ND
	<i>u443</i>	–	–	+	+	ND	ND
	<i>u448</i>	–	–	+	+	ND	ND
	<i>u453</i>	–	–	+	+	ND	ND
Weak recessive ^b	<i>u80</i>	–	–	+	+	+	+
	<i>u88</i>	–	–	+	+	+	+
	<i>u170</i>	±	±	+	+	+	+
	<i>u173</i>	+	±	+	+	+	+
	<i>u305</i>	±	±	+	+	+	+
	<i>u382</i>	–	–	+	+	+	+
	<i>u430</i>	±	±	+	+	ND	ND
	<i>u431</i>	–	–	+	+	ND	ND
Weak semi-dominant ^b	<i>e1522</i>	–	–	–	±	+	+
	<i>u48</i>	–	–	–	±	±	+
	<i>u58</i>	–	–	±	+	+	+
	<i>u127</i>	–	–	±	+	+	+
	<i>u136</i>	–	–	±	+	+	+
	<i>u145</i>	–	±	±	+	+	+
	<i>u156</i>	–	±	±	+	+	+
	<i>u225</i>	–	–	–	+	+	+
	<i>u262</i>	–	±	±	+	+	+
	<i>u278</i>	–	±	±	+	+	+
	<i>u319</i>	–	±	±	+	+	+
	<i>u427</i>	–	±	±	+	ND	ND
Strong semi-dominant	<i>e1343</i>	–	–	–	+	+	+
	<i>e1505</i>	–	–	±	+	+	+
	<i>u9</i>	–	–	±	+	+	+
	<i>u10</i>	–	–	±	+	+	+
	<i>u98</i>	–	–	–	+	+	+
	<i>u143</i>	–	–	±	+	+	+
	<i>u222</i>	–	–	±	+	+	+
	<i>u223</i>	–	–	–	±	+	+
	<i>u234</i>	–	–	–	±	+	+
	<i>u249</i>	–	–	±	±	+	+
	<i>u445</i>	–	–	–	±	ND	ND
	<i>u449</i>	–	–	±	+	ND	ND
	<i>u451</i>	–	–	±	+	ND	ND
Dominant	<i>e1527</i>	–	–	–	–	±	+
	<i>n434</i>	–	–	–	–	–	–
	<i>u18</i>	–	–	–	–	–	±
	<i>u129</i>	–	–	–	–	–	+
	<i>u162</i>	–	–	–	–	±	+
	<i>u283</i>	–	–	–	–	±	+

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).

^a (+) 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).

mec-7. 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 non*Mec* 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

Sensitivity at:

Allele	A	B	C	D
wild type	+	+	+	+
<i>e1506</i>	-	-	-	-
<i>e1522</i>	+/-	-	-	-
<i>u48</i>	+/-	-	-	-
<i>u58</i>	+/-	-	-	-
<i>u80</i>	+/-	-	-	-
<i>u88</i>	+/-	-	-	-
<i>u127</i>	+/-	-	-	-
<i>u136</i>	+/-	-	-	-
<i>u145</i>	+/-	-	+/-	+/-
<i>u156</i>	+/-	-	-	+/-
<i>u170</i>	+	+/-	-	+/-
<i>u173</i>	+	+	+/-	+/-
<i>u225</i>	+/-	-	-	-
<i>u262</i>	-	-	-	+/-
<i>u275</i>	-	-	-	+/-
<i>u278</i>	+/-	-	+/-	+/-
<i>u305</i>	+	+/-	+/-	+/-
<i>u319</i>	-	-	-	+/-
<i>u382</i>	+/-	-	-	-
<i>u427</i>	+/-	-	-	+/-
<i>u430</i>	+	+/-	-	+/-
<i>u431</i>	+/-	-	-	-

Figure 2. Position dependence of touch sensitivity. (+) Touch sensitive; ($\pm$) 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 *Bgl*III fragment isolated from cosmid R02A8 that contains the β -tubulin sequence. The coding sequence is contained within the 3.5- and 4.4-kb *Eco*RI 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.

(Fig. 3). Five mutants, two from EMS mutagenesis (*e1505* and *u156*) and three from γ -ray mutagenesis (*u443*, *u448*, and *u453*) contain deletions. The EMS mutations delete ~ 600 and 300 bp, respectively, of DNA from the 4.4-kb wild-type *Eco*RI fragment, which contains most of the tubulin-coding sequence and ~ 3 kb of upstream sequences. Two of the γ -ray mutations (*u443* and *u448*) delete ~ 28 and 18 kb, respectively, of DNA, which includes the region that hybridizes with a chicken β -tubulin cDNA probe (Fig. 3B). Animals homozygous for these mutations are completely touch insensitive but show no other behavioral defects. The third γ -ray-induced mutation (*u453*) deletes >40 kb of DNA (Fig. 3B) and results in additional defects (partial lethality at 25°C and poor male mating). These additional phenotypes are probably associated with defects in one or more adjacent genes. DNAs from two other mutants [*u382* and *u388*, both derived from a mutator strain, TR679 (Collins et al. 1987)] contain insertions of ~ 3.5 kb in the 3.5-kb wild-type *Eco*RI fragment (Fig. 3A). The insertions are not seen in wild-type revertants derived from these animals (Fig. 3A and data not shown). The insertions are located between the *Eco*RI site at position 1830 in the coding sequence and a *Bgl*III site ~ 600 bp downstream of the coding sequence. Taken together, these data provide strong evidence that *mec-7* is the *tub-2* β -tubulin gene.

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).

1 TTCAAAAATGGCTTTTGTGTTGGATCTTGTATCTCGTCTCTTTCTTCTTCTTCTCGGACCACTCATTATTCGAATGCCATGCATTGTTGA
 101 TGATGCGCAGATGGTCGCACACTACAACAGATATGATGGTGGCTGTGCGTGGTGCAGAGCTCATGAATAAGTGATGGGACCGAGATGTGACTCCT
 201 CCCATTATTCGTCTTGAGTCGCATCTTTTGTCTTGGTCCGTTGTGAGTCGAGCCTCAAGGCAGCCGTTCCGCCAGTGATCGCTGCTAAGAAGTTGTAGG
 MetArgGluIleValHisIleGlnAlaGlyGlnCysGlyAsnGlnIleGlySerLys
 301 TGTAGAGGAAGAAACGTCGGGTCCAATTTCAAGCAACATGCGCGAGATCGTTCATATTCAAGCCGACAATGCGGTAAATCAATCGGTTCAAGGTTAG
 PheTrpGluValIleSerAspGluHisGlyIleAspProSerGlyGlnTyr
 401 TATTTTCAGTTATAATGTTCTAGTTGTGCTTTATGCCAAGCAATTTCAAGTCTGGGAGGTAATATCCGATGAACACGGCATTGACCCATCTGCCAGTA
 ValGlyAspSerAspLeuGlnLeuGluArgIleAsnValTyrTyrAsnGluAlaGlySerAsnLysTyrValProArg
 501 TGTGGGAGACTCTGATCTTCAACTCGAAAGAATCAACGTCTACTATAATGAGGCCGAAGCAACAATATGTTCAAGGTATAGATATAGTTTGT
 601 CATAATTTTTCATGAAATATTTTTTGTATCTAGACGTTTTCATGATTTTGTACATATACCGATTTTGTCCACCGTTTACCTAAGTGTATGTTTTCAGA
 AlaValLeuValAspLeuGluProGlyThrMetAspSerValArgSerGlyProPheGlyGlnLeuPheArgProAspAsnTyrValPheGlyGlnSerGly
 701 GCTGTCTTGTGACTTGGAGCCAGGAACCATGGATTCTGTCCGTTCTGGACCATTCGGACAACCTTTCCGTCCAGACAATTACGTATTTGGTCAAAGCG
 AlaGlyAsnAsnTrpAlaLysGlyHisTyrThrGluGlyAlaGluLeuValAspAsnValLeuAspValValArgLysGluAlaGluSerThrAspCys
 801 GTGCTGGAACAATTTGGGCCAAGGACACTACACCGAGGAGCAGAACTGTAGACAATGTTCTTGACGTTGTGAGAAAGAGCAGAAAGCACAGATTG
 LeuGlnGlyPheGlnLeuThrHisSerLeuGlyGlyGlyThrGlySerGlyMetGlyThrLeuLeuIleSerLysIleArgGluGluTyrProAspArg
 901 CCTTCAAGGATTTCAACTTACTCACTCACTTGGAGGAGGTACTGGATCTGGAATGGGAACCCCTTCTCATTTCAAAATTCGGAGGAATATCCTGACCGG
 IleMetAsnThrPheSerValValProSerProLysValSerAspThrValValGluProTyrAsnAlaThrLeuSerValHisGlnLeuValGluAsnThr
 1001 ATAATGAACACCTTCTCGGTAGTTCCAAAGTCCAAAGTATCCGACACTGTAGTCGAACCTACAATGCCACCTCTCTGTCCACCGCTGTGTCGAGAACA
 AspSerThrPheCysIleAspAsnGluAlaLeuTyrAspIleCysPheArgThrLeuLysLeuThrThrProThrTyrGlyAspLeuAsnHisLeuVal
 1101 CCGACTCGACGTTCTGCATTGATAATGAGGCCTTGTATGACATCTGTTTCCGCACTCTGAAACTGACGACACCGACATATGGAGACCTTAACCATCTGGT
 SerAlaThrMetSerGlyValThrThrCysLeuArgPheProGlyGlnLeuAsnAlaAspLeuArgLysLeuAlaValAsnMetValProPheProArg
 1201 CAGTGCCACAATGAGCGCGTCACTACTTGCCCTCCGCTTCCCTGGTCAACTCAATGCGGATCTACGAAAGTTGGCAGTGAACATGGTTCATTCCCACGT
 LeuHisPhePheMetProGlyPheAlaProLeuThrSerArgSerAsnGlnGln
 1301 CTTCACTTCTTCATGCCAGGATTCGCACCACTGACTAGCAGAAGCAATCAGCAGGTTTGAATAATGAAAAAACAACCTTTGTGATACTTAGTTTTTA
 TyrArgAlaIleThrValProGluLeuThrGlnGlnCysPheAspAlaLysAsnMetMetAlaAlaCysAspProArgHisGlyArgTyrLeuThrAla
 1401 GTATCGTGCCATTACTGTCCCTGAGCTGACCAACAATGTTTCGACGCAAGAACATGATGGCTGCATGCGATCCAAGACATGGACGTTATCTCACCCT
 AlaAlaIlePheArgGlyArgMetSerMetLysGluValAspGluGlnMetLeuAsnIleGlnAsnLysAsnSerSerTyrPheValAspTrpIleProAsn
 1501 GCTGCCATTTTCCGAGGAAGAATGAGCATGAAGGAGTTGACGAGCAGATGCTCAACATCCAGAACAAGAACTCTTCTACTTCGTGGACTGGATTCCAA
 AsnValLysThrAlaValCysAspIleProProArgGlyLeuLysMetSerAlaThrPheIleGlyAsnSerThrAlaIleGlnGluLeuPheLysArg
 1601 ACAATGTCAAGACCGCAGTTTGTGACATTCCACCAAGAGGATTAAAGATGTCCGCAACATTCATCGGAACTCCACCGCCATTCAAGAGCTCTTTAAGAG
 IleSerGluGlnPheThr AlaMetPheArgArgLysAlaPheLeuHisTrpTyr
 1701 AATCTCAGAGCAGTTTCACAGTAAAAATTTTACGATTGTATTTTGACTAATTTTCATATTTTTCAGCTATGTTCCGCGCAAGCTTTCTTCATTGGT
 ThrGlyGluGlyMetAspGluMetGluPheThrGluAlaGluSerAsnMetAsnAspLeuValSerGluTyrGlnGlnTyrGlnGluAlaAlaAlaAsp
 1801 ACATGGCGAGGGAATGGACGAGATGGAATTCAGTGAAGCGGAGAGCAACATGAACGATCTTGTTCGGAATACCAACAATACCAGGAGGCAGCTGCTGA
 GluAspAlaAlaGluAlaPheAspGlyGluOC
 1901 TGAAGATGCCGCCGAAGCGTTCGACGGAGAGTAAATCATATTTCTAACCAAGTTTTATTGATCGATTGTTATATCATTTTATTCAGCCTTTTGTCTTG
 2001 CTTTTTTTTTTCATGGGATTCACAATTGATAAATTTTGATTAGCTAAACAACGTGTTAAACACGGAATTTAAGGCTGTTTTTTTTTAAATCAAAAAGCT

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 *Xba*I site at position 629, which was not detected by restriction mapping analysis.

(Fig. 5). Three of these substitutions represent significant alterations in hydrophobicity (residues 35, 278, and 293), and another results in a charge difference (residue 198). Two of these changes are in amino acids that have hitherto been invariant [Cys (127) and Glu (343)].

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/uDf1* 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.

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mec-7 MREIVHIQAGQCGNQIGSKFWEVISDEHGDIDPSGQYVGSDILQLERINVYNEAGSNKYVPRAVLVDLEP 70
      O
β2t      G      C AT T Y      TGA      I
mec-7 GTMDSVRSGPFGQLFRPDNYVFGQSGAGNNWAKGHYTEGAELVDNVLDVVRKEAESTDCLQGFQLTHSLG 140
      R I F      S      S GC
      ●
mec-7 GGTGSGMGTLISKIREEYPDRIMNTFSVVPSPKVS DTVVEPYNATLSVHQLVENTDSTFCIDNEALYDI 210
      O
β2t      E Y
mec-7 CFRTLKLTPTTYGDLNHLVSATMSGVTTCLRFPGQLNADLRKLAVNMVFPRLHFFMPGFAPLTSRSNQ 280
      O
β2t      GS
mec-7 YRAITVPELTQQCFDAKNMAACDPRHGRYLTA AAI FRGRMSMKEVDEQMLNIQKNSSYFVDWIPNNVK 350
      O
β2t      L      M      V      F E C
      ●
mec-7 TAVCDIPPRGLKMSATFIGNSTAIQELFKRISEQFTAMFRRKAFLHWYTGE GDEMEFTEAESNMNDLVS 420
β2t      V
mec-7 EYQQYQEA ADEDAEAEAFDGE
      O O O O
β2t      T EGEFDE E GGGDE

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Figure 5. Comparison of amino acid sequences from *mec-7* and *Drosophila* β 2-tubulin. The entire predicted amino acid sequence of the *mec-7* β -tubulin is shown, but only those differences in the *Drosophila* sequence (β 2t) are indicated. (○) 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; Kempfues 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
<i>e1527/+</i>	49	94	6	0
<i>e1527/+/+</i>	45	0	0	100
<i>n434/+</i>	37	97	3	0
<i>n434/+/+</i>	32	3	97	0
<i>u18/+</i>	15	67	33	0
<i>u18/+/+</i>	16	0	75	25
<i>u129/+</i>	9	44	56	0
<i>u129/+/+</i>	40	5	60	35
<i>u162/+</i>	28	50	50	0
<i>u162/+/+</i>	41	0	71	29
<i>n434/+ (15°)</i>	10	100	0	0
<i>n434/+/+ (15°)</i>	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

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

β -Tubulin	Percent identity	Reference
<i>Physarum</i> β 1	86 (398 aa)	Singhofer-Wowra et al. (1986)
<i>Physarum</i> β 2	79	Burland et al. (1988)
<i>Chlamydomonas reinhardtii</i>	85	Youngbloom et al. (1984)
Trypanosome	81	Kimmel et al. (1985)
<i>Neurospora crassa</i>	82	Orbach et al. (1986)
<i>Aspergillus</i> β 1/2	81	May et al. (1987)
<i>Aspergillus</i> β 3	76	May et al. (1987)
<i>S. pombe</i>	75	Hiraoka et al. (1984)
<i>S. cerevisiae</i>	74	Neff et al. (1983)
<i>Drosophila</i> β 2	92	Rudolph et al. (1987)
<i>Drosophila</i> β 3	88	Rudolph et al. (1987)
Chicken β 1	92	Sullivan et al. (1985)
Chicken β 2	92	Sullivan et al. (1985)
Chicken β 3	92	Sullivan and Cleveland (1986)
Chicken β 4	90	Sullivan and Cleveland (1984)
Chicken β 5	90	Sullivan and Cleveland (1986)
Human M40	91	Lewis et al. (1985)
Human β 2	93	Lewis et al. (1985)
Human β 4	90 (379 aa)	Sullivan and Cleveland (1986)
Human β 5	91	Lewis et al. (1985)
Pig β 1	92	Kraus et al. (1981)
Mouse β 2	91 (204 aa)	Sullivan and Cleveland (1986); Lewis et al. (1985)
Mouse β 4	92 (375 aa)	Lewis et al. (1985)
Mouse β 4'	93 (371 aa)	Sullivan and Cleveland (1986)
Mouse β 5	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).

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

(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. Chalfie, 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 (Chalfie and Thomson 1982). Because process outgrowth in these mutants is inhibited by benomyl (Chalfie 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 (Chalfie 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.

The testis-specific β -tubulin gene ($\beta 2t$) in *Drosophila* also functions cell specifically (Kemphues et al. 1983). The absence of all microtubule types in the testes of $\beta 2t$ mutant males contrasts with our findings, where the loss of *mec-7* gene activity prevents only the assembly of

15-protofilament microtubules. Although the $\beta 2t$ 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 $\beta 2t$ 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(e78)*, *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 (Chalfie and Sulston 1981; Chalfie and Thomson 1982; Chalfie 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 mutator strain TR679 [*mut-2(r459)*] (Collins et al. 1987) that generates spontaneous mutations by increasing the rate of transposition.

Touch sensitivity

Touch sensitivity was assessed as described previously (Chalfie and Sulston 1981). Touch-sensitive (+) animals move away when a fine hair is drawn across their bodies. Completely touch-insensitive (−) animals respond to < 30% of the touches. Some animals show a partial ($\pm$) response in that they respond to between 30 and 70% of the touches. Finally, some animals show a position dependence of touch sensitivity in that they respond to touch only at certain positions along their lengths.

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 progeny 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 ^{137}Cs gammator, mated them to *mec-7(e1506) dpy-6* hermaphrodites, and screened for touch-insensitive cross progeny. Of 2700 F_1 progeny, one such mutation, *uDf1*, was identified. This deficiency is lethal as a homozygote; *uDf1/mec-7 dpy-6* heterozygotes segregate only heterozygotes and *mec-7 dpy-6* homozygotes. *uDf1* fails to complement *xol-1* (L. Miller, pers. comm.), *unc-6*, *dpy-7*, *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 8800 and 1700 F_1 progeny, respectively, were tested for touch sensitivity. Two wild-type revertants (*u382u473* and *u388u474*) 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.

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/ $\times$;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 OsO_4 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 a *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 ^{32}P 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 *XbaI* fragment was subcloned from the *tub-2* clone (Gremke 1986) as two *XbaI–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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References

- Aamodt, E.J. and J.G. Culotti. 1986. Microtubules and microtubule-associated proteins from the nematode *Caenorhabditis elegans*: Periodic cross-links connect microtubules in vitro. *J. Cell Biol.* **103**: 23–31.
- Adachi, Y., T. Toda, O. Niwa, and M. Yanagida. 1986. Differential expressions of essential and nonessential α -tubulin genes in *Schizosaccharomyces pombe*. *Mol. Cell. Biol.* **6**: 2168–2178.
- Albertson, D.G. 1985. Mapping muscle protein genes by in situ hybridization using biotin-labeled probes. *EMBO J.* **4**: 2493–2498.
- Brenner, S. 1974. The Genetics of *Caenorhabditis elegans*. *Genetics* **77**: 71–94.
- Burchill, B.R., J.M. Oliver, C. B. Pearson, E.D. Leinbach, and R. D. Berlin. 1978. Microtubule dynamics and glutathione metabolism in phagocytizing human polymorphonuclear leukocytes. *J. Cell Biol.* **76**: 439–447.
- Burland, T.G., E.C.A. Paul, M. Oetliker, and W.F. Dove. 1988. A gene encoding the major β -tubulin of the mitotic spindle in *Physarum polycephalum* plasmodia. *Mol. Cell Biol.* **8**: 1275–1281.
- Burland, T.G., T. Schedl, K. Gull, and W.F. Dove. 1984. Genetic analysis of resistance to benzimidazoles in *Physarum*: Differential expression of β -tubulin genes. *Genetics* **108**: 123–141.
- Burton, P.R., R.E. Hinkley, and G.B. Pierson. 1975. Tannic acid-stained microtubules with 12, 13, and 15 protofilaments. *J. Cell Biol.* **65**: 227–233.
- Chalfie, M. and M. Au. 1988. Genetic control of differentiation of the *C. elegans* touch receptor neurons. *Science* **243**: 1027–1033.
- Chalfie, M. and J. Sulston. 1981. Developmental genetics of the mechanosensory neurons of *Caenorhabditis elegans*. *Dev. Biol.* **82**: 358–370.
- Chalfie, M. and J.N. Thomson. 1982. Structural and functional diversity in the neuronal microtubules of *Caenorhabditis elegans*. *J. Cell Biol.* **93**: 15–23.
- Chalfie, M., E. Dean, E. Reilly, K. Buck, and J.N. Thomson. 1986. Mutations affecting microtubule structure in *Caenorhabditis elegans*. *J. Cell Sci. Suppl.* **5**: 257–271.
- Chalfie, M., J.E. Sulston, J.G. White, E. Southgate, J.N. Thomson, and S. Brenner. 1985. The neural circuit for touch sensitivity in *Caenorhabditis elegans*. *J. Neurosci.* **5**: 956–964.
- Cleveland, D.W. 1987. The multi-tubulin hypothesis revisited: What have we learned? *J. Cell Biol.* **104**: 381–383.
- Cleveland, D.W. and K. F. Sullivan. 1985. Molecular biology and genetics of tubulin. *Annu. Rev. Biochem.* **54**: 331–365.
- Cleveland, D.W., M.A. Lopata, R.J. Macdonald, N.J. Cowan, W.J. Rutter, and M.W. Kirschner. 1980. Number and evolutionary conservation of α - and β -tubulin and cytoplasmic β - and λ -actin genes using specific cloned cDNA probes. *Cell* **20**: 95–105.
- Collins, J., B. Saari, and P. Anderson. 1987. Activation of a transposable element in the germ line but not the soma of *Caenorhabditis elegans*. *Nature* **328**: 726–728.
- Coulson, A., J. Sulston, S. Brenner, and J. Karn. 1986. Toward a physical map of the genome of the nematode *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci.* **83**: 7821–7825.
- Dustin, P. 1984. *Microtubules*, 2nd ed. Springer-Verlag, Berlin, New York.
- Edgley, M.L. and D.L. Riddle. 1987. The nematode *Caenorhabditis elegans*. *Genet. Maps* **4**: 351–365.
- Eichenlaub-Ritter, U. and J.B. Tucker. 1984. Microtubules with more than 13 protofilaments in the dividing nuclei of ciliates. *Nature* **307**: 60–62.
- Emmons, S.C. 1988. The genome. In *The nematode Caenorhabditis elegans* (ed. W.B. Wood), pp. 47–79. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
- Emmons, S.C., M.R. Klass, and D. Hirsch. 1979. Analysis of the constancy of DNA sequences during development and evolution of the nematode *Caenorhabditis elegans*. *Proc. Nat. Acad. Sci.* **76**: 1333–1337.
- Evans, L., T. Mitchison, and M. Kirschner. 1985. Influence of the centrosome on the structure of nucleated microtubules. *J. Cell Biol.* **100**: 1185–1191.
- Fuller, M.T., 1986. Genetic analysis of spermatogenesis in *Drosophila*: The role of the testis-specific β -tubulin and interacting genes in morphogenesis. In *Gametogenesis and the early embryo* (ed. J. Gall), pp. 19–41. Forty-fourth Symposium of the Society for Developmental Biology, Alan R. Liss, New York.
- Fulton, C. and P.A. Simpson. 1976. Selective synthesis and utilization of flagellar tubulin. The multitubulin hypothesis. In *Cell motility* (ed. R. Goldman, T. Pollard, and J. Rosenbaum), pp. 987–1005. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
- Gremke, L. 1986. "Cloning and molecular characterization of the tubulin genes of *Caenorhabditis elegans*: Nucleotide sequence analysis of a tubulin gene." Ph.D. thesis, Northwestern University, Evanston, Illinois.
- Heine, U. and T. Blumenthal. 1986. Characterization of regions of the *Caenorhabditis elegans* X chromosome containing vitellogenin genes. *J. Mol. Biol.* **188**: 301–312.
- Henikoff, S. 1984. Unidirectional digestion with exonuclease III creates targeted breakpoints for DNA sequencing. *Gene* **28**: 351–359.
- Hiraoka, T., T. Toda, and M. Yanagida. 1984. The NDA3 gene of fission yeast encodes β -tubulin: A cold sensitive *nda3* mutation reversibly blocks spindle formation and chromosome movement in mitosis. *Cell* **39**: 349–358.
- Kemphues, K.J., E.C. Raff, and T.C. Kaufman. 1983. Genetic analysis of $\beta 2t$, the structural gene for a testis-specific β -tubulin subunit in *Drosophila melanogaster*. *Genetics* **105**: 345–356.
- Kemphues, K.J., R.A. Raff, T.C. Kaufman, and E.C. Raff. 1979. Mutation in a structural gene for a β -tubulin specific to testis in *Drosophila melanogaster*. *Proc. Natl. Acad. Sci.* **76**: 3991–3995.
- Kimmel, B.E., S. Samson, J. Wu, R. Hirschberg, and L.R. Yarbrough. 1985. Tubulin genes of the African trypanosome *Trypanosoma brucei rhodesiense*: Nucleotide sequence of a 3.7 kb fragment containing genes for alpha and beta tubulins. *Gene* **35**: 237–248.
- Kraus, E., M. Little, T. Kempfl, R. Hofer-Warbinek, W. Ade, and H. Ponstingl. 1981. Complete amino acid sequence of β -tubulin from porcine brain. *Proc. Natl. Acad. Sci.* **78**: 4156–4160.
- Lewis, S.A., M.E. Gilmartin, J.L. Hall, and N.J. Cowan. 1985.

- Three expressed sequences within the human β -tubulin multigene family each define a distinct isotype. *J. Mol. Biol.* **182**: 11–20.
- L'Hernault, S.W. and J.L. Rosenbaum. 1985. Reversal of the posttranslational modification on *Chlamydomonas* flagellar α -tubulin occurs during flagellar resorption. *J. Cell Biol.* **100**: 457–462.
- Maniatis, T., E.F. Fritsch, and J. Sambrook. 1982. *Molecular cloning: A laboratory manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
- Matthews, K.A. and T.C. Kaufman. 1987. Developmental consequences of mutations in the 84B α -tubulin gene of *Drosophila melanogaster*. *Dev. Biol.* **119**: 100–112.
- May, G.S., M.L.-S. Tsang, H. Smith, S. Fidel, and N.R. Morris. 1987. *Aspergillus* beta-tubulin genes are unusually divergent. *Gene* **55**: 231–243.
- Mogensen, M.M. and J.B. Tucker. 1987. Evidence for microtubule nucleation at plasma membrane-associated sites in *Drosophila*. *J. Cell Sci.* **88**: 95–107.
- Morris, N.R., M.H. Lai, and C.E. Oakley. 1979. Identification of a gene for α -tubulin in *Aspergillus nidulans*. *Cell* **16**: 437–442.
- Nagano, T. and F. Suzuki. 1975. Microtubules with 15 subunits in cockroach epidermal cells. *J. Cell Biol.* **64**: 242–245.
- Neff, N.F., J.H. Thomas, P. Grisafi, and D. Botstein. 1983. Isolation of the β -tubulin gene from yeast and demonstration of its essential function in vivo. *Cell* **33**: 211–219.
- Nishida, E. and T. Kobayashi. 1977. Relationship between tubulin SH groups and bound guanine nucleotides. *J. Biochem. (Tokyo)* **81**: 343–347.
- Orbach, M.J., E.B. Porro, and C. Yanofsky. 1986. Cloning and characterization of the gene for the β -tubulin from a benomyl-resistant mutant of *Neurospora crassa* and its use as a dominant selectable marker. *Mol. Cell Biol.* **6**: 2452–2461.
- Raff, E.C. 1984. Genetics of microtubule systems. *J. Cell Biol.* **99**: 1–10.
- Rudolph, J.E., M. Kimble, H.D. Hoyle, M.A. Subler, and E.C. Raff. 1987. Three *Drosophila* β -tubulin sequences: A developmentally regulated isoform (β 3), the testis-specific isoform (β 2), and an assembly-defective mutation of the testis-specific isoform (β 2^t) reveal both an ancient divergence in metazoan isotypes and structural constraints for β -tubulin function. *Mol. Cell Biol.* **7**: 2231–2242.
- Saito, K. and K. Hama. 1982. Structural diversity of microtubules in the supporting cells of the sensory epithelium of guinea pig organ of Corti. *J. Electron Microsc.* **31**: 278–281.
- Sanger, F., S. Nicklen, and A. R. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci.* **74**: 5463–5467.
- Schatz, P.J., F. Solomon, and D. Botstein. 1986. Genetically essential and nonessential α -tubulin genes specify functionally interchangeable proteins. *Mol. Cell Biol.* **6**: 3722–3733.
- Scheele, R.B., L.G. Bergen, and G. B. Borisy. 1982. Control of the structural fidelity of microtubules by initiation sites. *J. Mol. Biol.* **154**: 485–500.
- Sheir-Neiss, G., M.H. Lai, and N.R. Morris. 1978. Identification of a gene for β -tubulin in *Aspergillus nidulans*. *Cell* **15**: 639–647.
- Singhofer-Wowra, M., L. Clayton, P. Dawson, K. Gull, and M. Little. 1986. Amino-acid sequence data of β -tubulin from *Physarum polycephalum myxamoebae*. *Eur. J. Biochem.* **161**: 669–679.
- Stretton, A.O.W., R.E. Davis, J.D. Angstadt, J. E. Donmoyer, and C.D. Johnson. 1985. Neural control of behavior in *Ascaris*. *Trends Neuro. Sci.* **8**: 294–300.
- Sullivan, K.F. and D.W. Cleveland. 1984. Sequence of a highly divergent tubulin reveals regional heterogeneity in the β -tubulin polypeptide. *J. Cell Biol.* **99**: 1754–1760.
- . 1986. Identification of conserved isotype-defining variable region sequences for four vertebrate β -tubulin polypeptide classes. *Proc. Nat. Acad. Sci.* **83**: 4327–4331.
- Sullivan, K.F., J.T.Y. Lau, and D.W. Cleveland. 1985. Apparent gene conversion between β -tubulin genes yield multiple regulatory pathways for a single β -tubulin polypeptide isotype. *Mol. Cell Biol.* **5**: 2454–2465.
- Thomas, J.H., N.F. Neff, and D. Botstein. 1985. Isolation and characterization of mutations in the β -tubulin gene of *Saccharomyces cerevisiae*. *Genetics* **112**: 715–734.
- Tilney, L.G., J. Bryan, D.J. Bush, K. Fujiwara, M.S. Mooseker, D.R. Murphy, and D. H. Snyder. 1973. Microtubules: Evidence for 13 protofilaments. *J. Cell Biol.* **59**: 267–275.
- Tucker, J.B. 1979. Spatial organization of microtubules. In *Microtubules* (ed. K. Roberts and J.S. Hyams), pp. 315–357. Academic Press, London.
- Tucker, J.B., S.A. Matthews, K.A.K. Hendry, J.B. Mackie, and D.L.J. Roche. 1985. Spindle microtubule differentiation and deployment during micronuclear mitosis in *Paramecium*. *J. Cell Biol.* **101**: 1966–1976.
- Waterston, R.H. and S. Brenner. 1978. A suppressor mutation in the nematode acting on specific alleles of many genes. *Nature* **275**: 715–719.
- Weatherbee, J.A., G.S. May, J. Gambino, and N.R. Morris. 1985. Involvement of a particular species of beta-tubulin (β 3) in conidial development of *Aspergillus nidulans*. *J. Cell Biol.* **101**: 705–711.
- Wills, N., R.F. Gesteland, J. Karn, L. Barnett, S. Bolten, and R.H. Waterston. 1983. The genes *sup-7 X* and *sup-5 III* of *C. elegans* suppress amber nonsense mutations via altered transfer RNA. *Cell* **33**: 575–583.
- Winston, F. and D. Botstein. 1981. Control of lysogenization by phage P22: 2. Mutations (*clvA*) in the *cl* gene that cause increased lysogenization. *J. Mol. Biol.* **152**: 233–246.
- Yamamoto, M. 1980. Genetic analysis of resistant mutants to antimetabolic benzimidazole compounds in *Schizosaccharomyces pombe*. *Mol. Gen. Genet.* **180**: 231–234.
- Youngbloom, T., J.A. Schloss, and C.D. Silflow. 1984. The two β -tubulin genes of *Chlamydomonas reinhardtii* code for identical proteins. *Mol. Cell Biol.* **4**: 2686–2696.

Note

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

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

C Savage, M Hamelin, J G Culotti, et al.

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