# Tubulins in *C. elegans*

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Last revised: Augst, 2017
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WormBook Early Online, published on January 30, 2018 as doi: 10.1895/wormbook.1.182.1.

**Abstract**

**The *C.elegans* tubulin family is composed of nine α-tubulins, six β-tubulins, and one γ-tubulin. Tubulins are highly conserved, functioning as α-β heterodimers that assemble into microtubules. These cylindrical and ubiquitous components of the cytoskeleton are critical for nearly all cellular and developmental processes. *C. elegans* has provided a model for the study of microtubules in multiple settings including separation of chromosomes, cellular polarity, and neuronal sensation. Tubulins and microtubules interact with a long list of other cellular proteins that regulate tubulin homeostasis, modify microtubule dynamics, and control incorporation into or disassociation of higher-order cellular structures such as spindles or ciliary axonemes. A collection of enzymes modifies tubulins, often at the variable carboxyl-terminal tail, adding another layer of regulation to microtubule structure and function. Genetic and cytological studies in *C. elegans* have revealed roles for tubulin and its associated proteins in numerous contexts from embryogenesis to adult behavior.**

## 1. Tubulin, microtubules, and tubulin mutations in *C. elegans*

Tubulins are ~450 amino acid globular proteins of the cytoplasm that comprise microtubules (Figure 1). The sequences of both α- and β-tubulins (Figure 2), which form heterodimers, indicate that they are part of a large family of proteins that bind to and hydrolyze guanosine triphosphate (GTP). Tubulin-like proteins appear early in evolution, and they have remained highly conserved in the eukaryotic lineage. Tubulins are related to the FtsZ proteins of eubacteria. Like tubulins, FtsZ proteins polymerize and depolymerize with a GTPase cycle and play a role in cell division (Ingerson-Mahar and Gitai, 2012: PMID 22092065). Genes for FtsZ homologs are widely distributed in prokaryotic lineages, but closer relatives to eukaryotic tubulins are found in a few archaeal species (Yutin and Koonin, 2012: PMID 22458654). Amongst eukaryotes, the α- and β-tubulins are nearly identical to each other within their families, as most of the sequence divergence is located in the carboxyl-terminal tails which emanate from the outer surface of the microtubule and can impart specific functions to tubulins, perhaps through interactions with motor proteins and/or other microtubule-associated proteins (Hsu et al., 2014: PMID 25392990; Nogales et al., 1999: PMID 9989499; Popodi et al., 2008: PMID 18157906).

Microtubules are semi-rigid, cylindrical cytoskeletal polymers composed of repeating units of the α- and β-tubulin heterodimer. Heterodimers, which are formed soon after synthesis of monomers, associate head to tail into protofilaments. Kinetic and structural data suggest that the further assembly of heterodimers and short protofilaments into microtubules is explained by a lattice model. This model suggests that incorporation into a microtubule straightens soluble, curved heterodimers, while nucleotide status tunes the strength of contacts within the lattice of dimers. Hydrolysis of GTP to GDP in the exchangeable site (E-site) of β-tubulin within a microtubule causes compaction and other conformational changes in both subunits of the dimer that weaken the contacts holding the lattice together, making the microtubule unstable (Rice et al. 2008: PMID 18388201; Alushin et al., 2014: PMID 24855948). The structure and nucleotide hydrolysis capacity of tubulin imparts microtubules with dynamic instability, an intrinsic property of self-assembly and disassembly (Mitchison and Kirschner, 1984: PMID 6504138).

*In vivo*, microtubules are commonly nucleated by the γ-tubulin ring complex and other components of centrosomes (Teixido-Travesa et al., 2012: PMID 23132930), which pattern protofilaments into the helical pitch of a microtubule. Nematode microtubules are 11 protofilaments in most cells, while typical eukaryotic microtubules are 13 protofilaments. Touch receptor cells in nematodes have 15 protofilament microtubules (Chalfie and Thomson, 1982: PMID 7068753).

Microtubules are polar polymers with assembly and disassembly occurring preferentially at the plus end. Similar activities take place at the minus end, although at a slower rate. Polymer polarity is a hallmark of microtubules (and actin), and it often establishes and/or maintains cellular polarity (e.g., see Dubey et al. 2015: PMID 26441521).

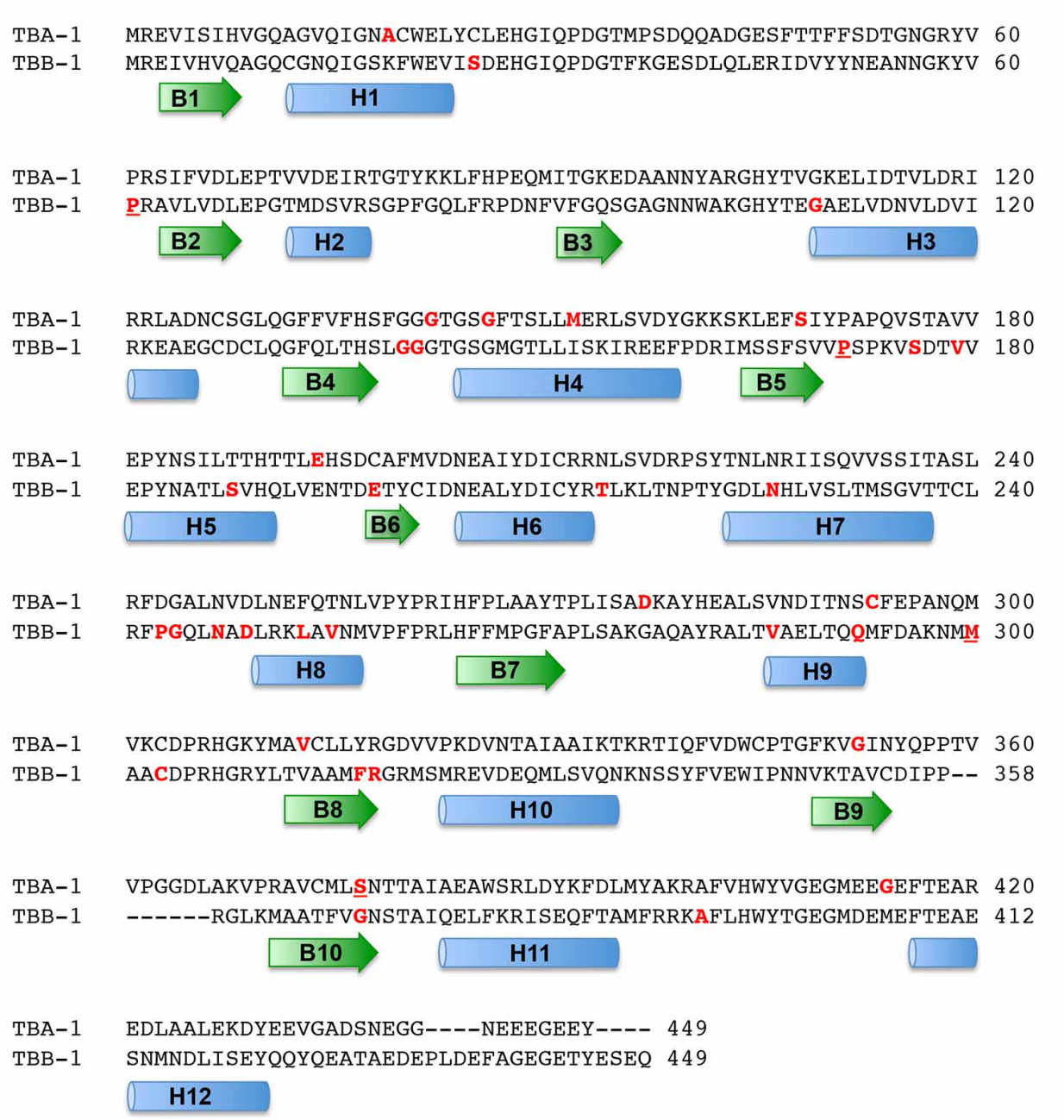


**Figure 1. A pairwise alignment of TBA-1 and TBB-1 with dominant alleles and secondary structure.** Genetically defined dominant, semi-dominant, and/or gain of function alleles are shown in red and underlined if they have been independently discovered more than once (Table 1). α-helices (blue cylinders) and β-sheets (green arrows) are shown below the primary structure.


**Figure 2. Multiple alignment of α-tubulin and β-tubulin C-termini.** An alignment of the C-terminal tails (CTTs) of the nine α-tubulins and the six β-tubulins. Tubulins within each family are nearly identical to each other throughout the rest of their structure (not shown). The axonemal motif in TBB-4 is shown in bold. For an alignment of complete sequences, see Gogonea et al., 1999 (PMID 10680114).

Mutations in genes encoding tubulins in *C. elegans* have been discovered in screens for a variety of phenotypes. The forward genetic approach has produced a large collection of alleles for which there is a rich and growing collection of phenotypic information. This allows for understanding the cellular role of tubulin in many different contexts (see Section 2). In addition, because the *C. elegans* genome has a fairly large family of tubulin genes (with many being non-essential), dominant mutations in tubulin have been isolated and propagated. Mapping of these alleles to the structure has provided insight into the function of tubulin, especially in the case of genetically explored and molecularly identified point mutations (Figure 2 and Table 1, Section 6).

Tubulin can be divided into three domains. Domain one spans the first 200 amino acids, and it includes the Rossman fold and nucleotide-binding portion that faces the lumen of the microtubule. Dominant mutations have been found in the glycine-rich stretch (GGGTG[T/S]G, sometimes called the tubulin signature motif) in between B4 and H4 of both α and β-tubulin (Figure 1 and Figure 2). Dominant mutations of the first of a conserved set of prolines (aa170) that reside in the pocket around the purine base of GTP have been found twice, both in β-tubulin. Other dominant mutations are scattered in the amino-terminal half of both tubulins, often in between or near the ends of α helices or β ribbons. Two intragenic revertants of a dominant mutation in the conserved S169 have also been identified.

The second domain (sometimes called the activation domain) spans the remaining conserved amino acids up to the divergent C-terminal tails (CTTs, Figure 2). As noted by Erickson, (1998 PMID 9695825), dominant mutations near or in the highly conserved G244, N247, and D249 of the 'synergy loop' have only been found in β-tubulin, typically in *mec-7*, although the nearby L253 in H8 is altered in

an allele of *tbb-4*. This collection of alleles is curious as the amino acids they change in β-tubulin form part of the binding pocket for the non-exchangeable GTP nucleotide in the dimer-dimer interface that is mostly bound by α-tubulin (Figure 1 and Figure 2). More exploration will reveal whether this is specific to these worm tubulins or telling something broader about dimer formation and the non-exchangeable GTP. A mutation of the conserved methionine at position 300 has been independently isolated twice (both in *mec-7*), and the serine at position 377 is mutated to phenylalanine in two independent alleles of *tba-1*. Negative charges around a glycine (aa416 in *mec-12*, aa414 in Figure 2) near H12 modulate the interaction between microtubules and motor proteins such as dynein (Hsu et al., 2014: PMID 25392990). As is the case in the amino terminal portion of tubulin, many of the dominant mutations are in between or near the ends of the α-helices and/or β-ribbons. None have yet been found in a CTT.

**2. The multi-tubulin hypothesis and cellular and developmental roles for microtubules**

The *C. elegans* tubulin superfamily consists of nine α-tubulins, six β-tubulins, and a single γ-tubulin (Table 2). The presence of multiple, closely related α- and β-tubulin paralogs (also referred to as isoforms or isotypes) in eukaryotic genomes has historically suggested three hypotheses, which can also be applied to other families of paralogous proteins. First, multiple genes may be present to ensure a stable and redundant pool of tubulin; subtle sequence changes are simply due to post-duplication genetic drift. Second, particular tubulins may be required for specific environmental conditions and are expressed in response to these conditions. Third, the subtle sequence changes of the monomers impart functional specificity to tubulin dimers, which can optimize the function of the microtubule cytoskeleton for its role in a particular cell type or developmental context (Cleveland, 1987: PMID 3546332; Wade, 2007: PMID 18085218; Wilson and Borisy, 1997: PMID 9204761). These explanations are not mutually exclusive as genetic and cytological evidence support redundancy and specificity among the members of the tubulin superfamily in *C. elegans* and other metazoans.

Worm tubulins have been studied for thirty years in multiple cellular and developmental contexts. *C. elegans* research made early contributions to establishing a linkage between mechanosensation and microtubule structure/function (Chalfie and Thomson, 1982: PMID 7068753; Fukushige et al., 1999: PMID 9885292). These pioneering genetic studies mostly supported the notion that certain tubulins play specific biological roles. Further support for this came from the early observation that certain monoclonal tubulin antibodies labeled specific subsets of neuronal cells (Siddiqui et al., 1989: PMID 2475594). More recently, non-motile primary cilia at the tip of the dendrite of many sensory neurons have provided a model to study intraflagellar transport processes (see WormBook chapter The sensory cilia of *Caenorhabditis elegans*; Müller et al., 2011: PMID 21537177), and they give another example of the use of specific tubulins to optimize behavioral responses (Hao et al., 2011: PMID 21642982; Hurd et al., 2010: PMID 20421600).

As a core component of the metazoan cytoskeletal system, microtubules composed of the less diverged, more broadly expressed, and likely more redundant tubulins play numerous and essential roles at all phases of the cell and life cycle. The early *C. elegans* embryo has become a model to study particular processes such as the assembly and function of the spindle (see Figure 3 and WormBook chapter Cell division; Müller-Reichert et al., 2010: PMID 20339898; Lu and Mains, 2005: PMID 15781712; Lu et al., 2004: PMID 14565976; Cheeranbathur et al., 2013: PMID 24231804), microtubule dynamics (Bajaj and Srayko, 2013: PMID 23936530), or the accumulation of spindle proteins after nuclear envelope breakdown (Hayashi et al., 2012: PMID 22398724). In addition, the early embryo is also a venue to study the role of microtubules in the developmental processes of establishing polarity through asymmetric cell divisions and axis formation, although it is disputed whether centrosomes or the microtubules that emanate from them are the initial symmetry breaking entities (for a history of the

evidence both ways, see Wallenfang and Seydoux, 2000: PMID 11081513; Cowan and Hyman, 2004: PMID 15343338; Tsai and Ahringer, 2007: PMID 17967950; Motegi et al. 2011: PMID 21983565; Bienkowska and Cowan, 2012: PMID 22425158).

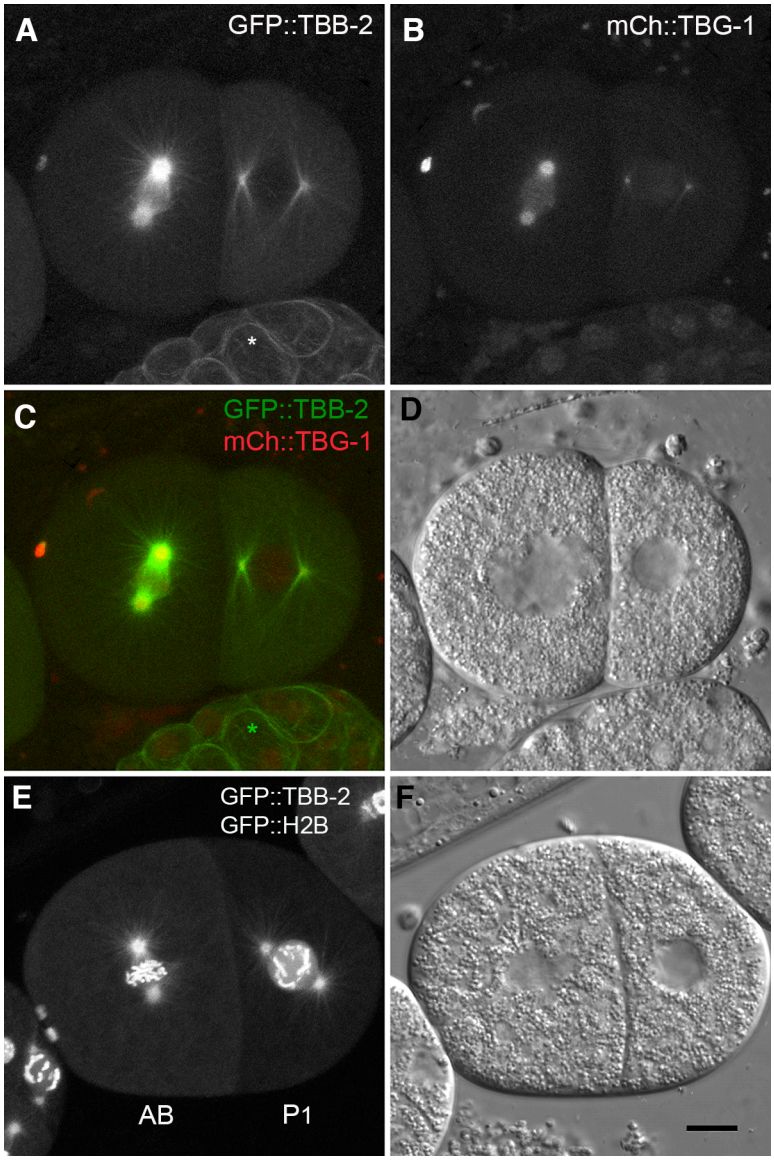


**Figure 3. Translational fusions to the promoter of *pie-1* allow for live visualization of tubulins and other components of mitosis in embryonic blastomeres**. Strain SA250 contains GFP::TBB-2 as shown in panel A and mCh::TBG-1 in panel B, along with mCh::HIS-48 (histone 48), which appears more strongly in later blastomeres (Toya et al., 2010: PMID 20719280). A merge of A and B is shown in panel C, and a DIC image is shown in panel D. Strain TY3558 contains GFP::TBB-2 and GFP::H2B (histone 2B) as shown in panel E, with corresponding DIC image in panel F (this strain is not referenced). In all panels, anterior is to the left. Note the asymmetric distribution of one or both of these components at the two-cell stage as seen by a brighter cytoplasm in AB relative to P1 (panel E) and that interphase blastomeres in older embryos show a cortical tubulin network (asterisks in panels A and C). Both strains are available at the *Caenorhabditis* Genetics Center (CGC). Scale bar is 10 µm.

Evidence for both redundancy and specificity among *C. elegans* tubulins has come from studies of the tubulins that function in somatic tissues in processes such as neuronal polarity, synaptogenesis, and plasticity (Baran et al., 2010: PMID 20300184; Ou and Shen, 2011: PMID 21557505; Driscoll et al., 1989: PMID 2592410; Kurup et al., 2015: PMID 26051896). The broadly expressed tubulins, perhaps with some contribution from the more specialized family members, likely function as the predominant microtubule tracks for the movement, positioning, and anchoring of intracellular membranous organelles such as yolk granules (McNally et al., 2010: PMID 20036653) and nuclei (Tapley and Starr, 2013: PMID 23149102; see Section 4.3.15 for description of NOCA-1). In a process involving the polarity protein PAR-3 microtubules and components of centrosomes relocalize during establishment of intestinal epithelial polarity (Feldman and Priess, 2012: PMID 22425160). Under control of the formin EXC-6 (excretory cell abnormal) microtubules help organize tubulogenesis (Shaye and Greenwald, 2015: PMID 25771894). In the absence of centrosomes, microtubules can polymerize from and help remodel epidermal junctions, where they function alongside the LET-502 (Rho kinase) pathway during embryonic elongation (Quintin et al., 2016: PMID 26586219; Wang et al. 2015: PMID 26371552). Microtubules also form the tracks for dynein-based movement of chromosomes during oocyte cell division (Muscat et al., 2015: PMID 26026148). During differentiation of reproductive muscles microtubules undergo regulated changes in their behavior that are controlled by regulatory proteins and/or compartmentation of soluble monomer (Lacroix et al., 2014: PMID 24780738; Lacroix et al., 2016: PMID 26985017).

### 2.1. The mitotic and neuronal tubulins: TBA-1, TBA-2, TBB-1, TBB-2, and BEN-1

Two α- (TBA-1 and TBA-2) and two β- (TBB-1 and TBB-2) tubulins show some degree of redundancy and are major components of the mitotic spindle microtubules and of the axonal microtubules in motor neurons. A third β-tubulin, BEN-1, functions redundantly in neurons.

#### 2.1.1. TBA-1 and TBA-2

TBA-1 is expressed in the germline and is involved in meiotic and mitotic spindle assembly in the early embryo. It is partially redundant with TBA-2 as RNAi depletion of both results in embryonic lethality while RNAi or deletion of either causes little effect on embryonic viability (Lu and Mains, 2005: PMID 15781712; Phillips et al., 2004: PMID 15083533; Honda et al. 2017: PMID 28302908). TBA-1 is also broadly expressed in excitatory motor neurons (Table 2), where it functions with DLK-1 (dual leucine zipper kinase), a mitogen-activated protein kinase kinase kinase, in synaptogenesis and remodeling events (Kurup et al., 2015: PMID 26051896). Dominant or semi-dominant missense alleles, but not deletion alleles, cause defects in cell division events and neuronal function. These molecular and cellular defects underlie embryonic lethality (O'Rourke et al. 2011: PMID 21390299) and uncoordination, respectively (Baran et al., 2010: PMID 20300184). Suppressors of the neuronal phenotypes caused by the combination of a dominant allele of TBA-1 and a loss of DLK-1 include a neomorphic allele of TBB-2, and novel missense alleles of kinesin, dynein/dynactin, and the casein kinase TTBK-3 (tau tubulin kinase) (Kurup et al. 2017: PMID 28636662).

The primary structure of TBA-2 is very closely related to that of TBA-1 (98.5% identical, 99.5% similar), which likely provides the basis for redundancy. Sequence similarity extends through their CTTs, which are also the most similar to α-CTTs of other organisms. TBA-2 is expressed in the germline and in certain neurons (Table 2), overlapping with TBA-1 in the VB and DB motor neurons and the PLM sensory neurons (Fukushige et al., 1993: PMID 8263934). Like TBA-1, TBA-2 functions in the mitotic spindle of the early embryo as dominant missense alleles cause embryonic lethality (Lu and Mains, 2005: PMID 15781712; Phillips et al., 2004: PMID 15083533). Both TBA-1 and TBA-2 are also expressed in touch receptor neurons, but they do not seem to be required for the structure or

function of those cells (Lockhead et al. 2016: PMID 27654945). However, minor sequence differences between TBA-1 and TBA-2, which are mostly at the amino-terminus, result in differential sensitivity to the actions of the microtubule severing protein, katanin (MEI-1/MEI-2) and in differences in microtubule dynamics in the early embryo (Lu and Mains, 2005: PMID 15781712; Honda et al. 2017: PMID 28302908).

### 2.1.2. TBB-1 and TBB-2

Like the broadly expressed α-tubulins, TBB-1 and TBB-2 (97.6% identical and 98.9% similar) both function in a partially redundant way in the mitotic spindle in embryonic blastomeres, and they interact differently with MEI-1/MEI-2 katanin (Lu et al., 2004: PMID 14565976; Wright and Hunter, 2003: PMID 12937270). Similarly, they are partially redundant in motor neurons as loss of either causes mild axonal outgrowth and synaptic defects similar to the dominant mutation of *tba-1* (loss of both is lethal). However, loss of either enhances the defects caused by a dominant allele of *tba-1* (Baran et al., 2010: PMID 20300184). Both are expressed in many unidentified neurons and other tissues, but rarely does loss of function of one cause any significant defect (Lockhead et al. 2016: PMID 27654945).

TBB-2 functions during microtubule-dependent processes in the early embryo such as spindle placement and rotation, where dominant missense alleles cause stronger defects than do null alleles (Ellis et al., 2004: PMID 14702387; Wright and Hunter, 2003: PMID 12937270, O'Rourke et al., 2011: PMID 21390299). Loss of TBB-2 causes an up-regulation of TBB-1 in the early embryo, providing a genetic mechanism for redundancy (Ellis et al., 2004: PMID 14702387). Quantitative expression analysis in the early embryo indicates that TBB-2 is more prevalent than TBB-1, and substitution experiments suggest that the particular tubulins present and their concentration contribute to diverse microtubule behaviors (Honda et al. 2017: PMID 28302908).

Large-scale RNAi screens indicate that TBB-2 is also required for P-granule localization (Updike and Strome, 2009: PMID 19805813). Along with TBA-2, TBB-2 is required for correct sexual specification of the male gonad (Kalis et al., 2010: PMID 20308279). It is broadly expressed in many somatic tissues and the germline (Hunt-Newbury et al., 2007: PMID 17850180; Lu et al., 2004: PMID 14565976; McKay et al., 2003: PMID 15338614), and regulated by the formin EXC-6, it also plays a role in excretory cell morphogenesis (Shaye and Greenwald, 2015: PMID 25771894).

### 2.1.3. BEN-1

Benomyl is an anti-mitotic benzimidazole compound that acts to inhibit dynamic instability of microtubules (Gupta et al., 2004: PMID 15157098; Singh et al., 2008: PMID 18384115). When wild-type *C. elegans* are reared in its presence, growth is slowed, movement is uncoordinated, and fewer neuronal processes emanate from ventral cord motor neurons. The *ben-1* locus was identified in a screen for mutations that offer resistance to benomyl (Driscoll et al., 1989: PMID 2592410). Deletion of *ben-1* and certain missense mutations confer resistance while causing no other gross abnormalities. Mutations that cause either resistance or hypersensitivity to benomyl have been linked to a β-tubulin in both nematodes and fungi (Nakaune and Nakano, 2007: PMID 17507270). *ben-1* is broadly expressed in the nervous system (Figure 4), which supports the idea that it is involved in the neuronal control of locomotion. However, no cytological phenotypes have been reported. The structure of the CTT of BEN-1 is similar to TBB-4 (Figure 2), although it lacks a recognizable axonemal motif (see Section 2.2.4).

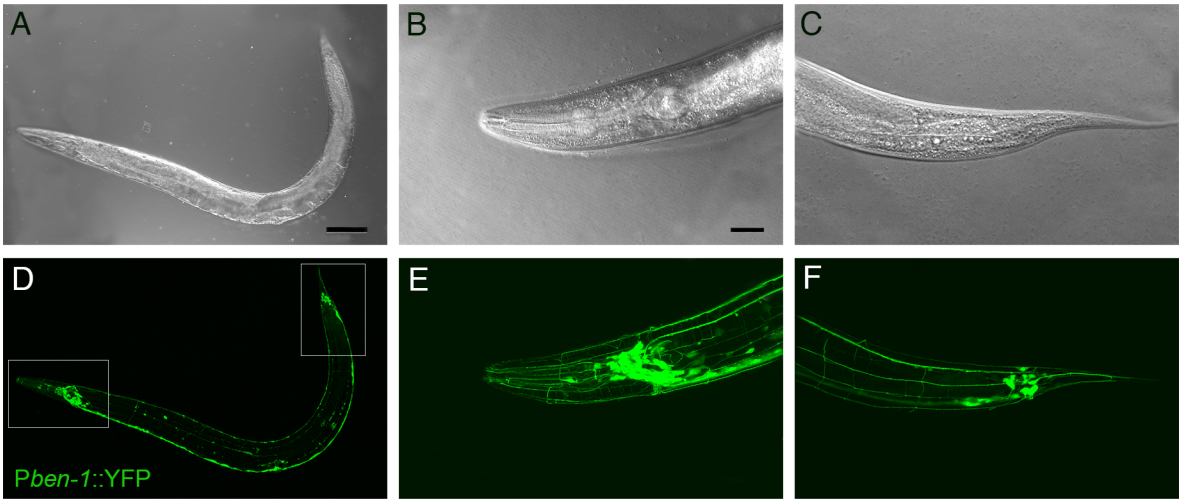


**Figure 4. *ben-1* is expressed in multiple neurons.** A transcriptional fusion of the *ben-1* promoter to yellow fluorescent protein (YFP, from pPD136.64/L4817 in the Fire Lab vector kit 1999) reveals broad expression in sensory, motor, and likely interneurons. Scale bars = 100 µm in panels A and D and 10 µm in B, C, E and F. Boxes in D indicate views shown in E and F. Strain to be available at the CGC.

### 2.2. The sensory neuron axonemal tubulins: TBA-5, TBA-6, TBA-9, and TBB-4

*C. elegans* sense their environment with an array of chemo- and mechanosensory neurons such as the anterior amphid neurons and posterior phasmid neurons. The non-motile cilia at the tips of the dendrites in these neurons are built on a 9+0 axoneme structure, and they have become a model in which to study homologs of the ciliopathy genes in humans (see WormBook chapter The sensory cilia of *Caenorhabditis elegans*;)

#### 2.2.1. TBA-5

TBA-5 is an axonemal α-tubulin expressed in amphid and phasmid sensory neurons where it is localized to cilia. It was isolated in a screen to identify mutants in ciliary dye-filling. A missense allele causes a Dyf (dye-filling defective) phenotype and shortened cilia while loss-of-function (deletion) mutations do not show defects in dye-filling (Hao et al., 2011: PMID 21642982). TBA-5, TBB-4, and perhaps additional axonemal tubulins are transported by intraflagellar transport (IFT) mechanisms involving a number of microtubule motor proteins (Hao et al., 2011: PMID 21642982). No behavioral defects have been associated with the perturbation of *tba-5*.

#### 2.2.2. TBA-6

TBA-6 was identified as a ciliary tubulin by combining serial analysis of gene expression (SAGE) and microarray datasets. It is co-expressed with PKD-2 (a polycystin) in ciliated, male-specific sensory neurons (CEMs and RnBs) in addition to inner labial neurons found in both sexes. Mutant analysis indicated that it is required, along with TBB-4, for the normal structure of CEM cilia and the normal localization of PKD-2 to the distal tips of RnB cilia (Hurd et al., 2010: PMID 20421600). It is also required for multiple aspects of the specialized structure and function of male specific CEM neurons including axoneme ultrastructure, motor protein velocity, and extracellular vesicle function (Silva et al., 2017: PMID 28318980). The combination of these abnormalities likely underlies the male mating behavior defects shown by mutants (Hurd et al., 2010: PMID 20421600). TBA-6 mRNA is presumably

expressed in the germline as it is found in the one-cell embryo. It is also expressed in other somatic tissues (Baleanu-Gogonea and Siddiqui, 2000: PMID 12167287), but deletion mutants are fertile and without obvious morphological abnormalities. Structurally, TBA-6 lacks a recognizable α-tubulin axonemal CTT found in other ciliary α-tubulins.

### 2.2.3. TBA-9

TBA-9 was also identified as a candidate ciliary α-tubulin by combining SAGE and microarray datasets (Hurd et al., 2010: PMID 20421600). It is expressed in many ciliated sensory neurons in the head of both sexes, often with TBB-4 (Table 2). It is required for many normal behaviors including male mating, retraction after nose touch, locomotory rate/posture, exploratory behavior, and the basal slowing response upon contact with food. Deletion causes only subtle defects in the localization of signaling proteins (D. D. Hurd, unpublished results), although it is required for the normal distribution of TBB-4. Structurally, it lacks a recognizable axonemal CTT found in other ciliary α-tubulins (Hurd et al., 2010: PMID 20421600).

### 2.2.4. TBB-4

TBB-4 is a broadly expressed ciliary β-tubulin orthologous to ciliary β-tubulins in other species (Hao et al., 2011: PMID 21642982). It has a recognizable axonemal motif in its CTT, and it is expressed in many, but not all ciliated sensory neurons, including certain amphid, certain labial, and both classes of male tail ray neurons (Table 2) where it is required for the normal localization of signaling proteins, such as PKD-2. TBB-4 is required for normal male mating and nose-touch behaviors (Hurd et al., 2010: PMID 20421600). It is enriched in the ciliary axoneme, and is transported by intraflagellar transport. Some missense mutations are more severe than null (deletion) mutations (Hao et al., 2011: PMID 21642982).

## 2.3. The mechanosensory tubulins: MEC-12 and MEC-7

The MEC-12 α-tubulin and the MEC-7 β-tubulin were originally discovered in screens for mutants that did not respond to mechanical stimulation along the body (Chalfie and Sulston, 1981: PMID 7227647). They are both expressed in the six body wall touch receptor neurons (TRNs) sometimes referred to as the microtubule cells (ALML/R, PLML/R, AVM, and PVM). These sensory neurons contain cross-linked 15-protofilament microtubules that terminate near the plasma membrane (Chalfie and Thomson, 1982: PMID 7068753). MEC-12 is the only *C. elegans* α-tubulin with a lysine as aa40, a position that is frequently modified via post-translational acetylation (see Section 4.2.1). Antibodies to acetylated tubulin strongly label the touch receptor cells (Akella et al., 2010: PMID 20829795; Shida et al., 2010: PMID 21068373; Siddiqui et al., 1989: PMID 2475594).

MEC-12 and MEC-7 are required for the 15 protofilament microtubules in the TRNs, for mechanoreceptor currents, and for touch sensitivity (Bounoutas et al., 2009: PMID 19615905; O'Hagan et al., 2005: PMID 15580270). Mutations often cause the appearance of 11 protofilament microtubules, which can alter the distribution and/or expression of the components of the mechanosensation signal transduction pathway (Bounoutas et al., 2011: PMID 21368137; Bounoutas et al., 2009: PMID 19615905; Huang et al., 1995: PMID 7477350). Mutations in these two tubulins that cause the most severe defects are not null, but rather dominant or semi-dominant missense (gain-of-function) alleles (Hsu et al., 2014: PMID 25392990; Savage et al., 1994: PMID 7983175; Kirszenblat et al., 2013: PMID 23223572). Loss of PTL-1 (see Section 4.3.1) causes an enhancement of touch insensitivity in double mutant combinations with certain alleles of *mec-12* or *mec-7* (Gordon et al., 2008: PMID 18807071).

## 2.4. α- and β- tubulins with undiscovered roles

### 2.4.1.TBA-4

TBA-4 has not been studied in detail. Data from RNAi experiments have suggested that TBA-4 may play a role in embryonic development or larval growth (Simmer et al., 2003; PMID 14551910). Its anatomic expression resembles that of *tba-7* (see Table 2 and Figure 5).

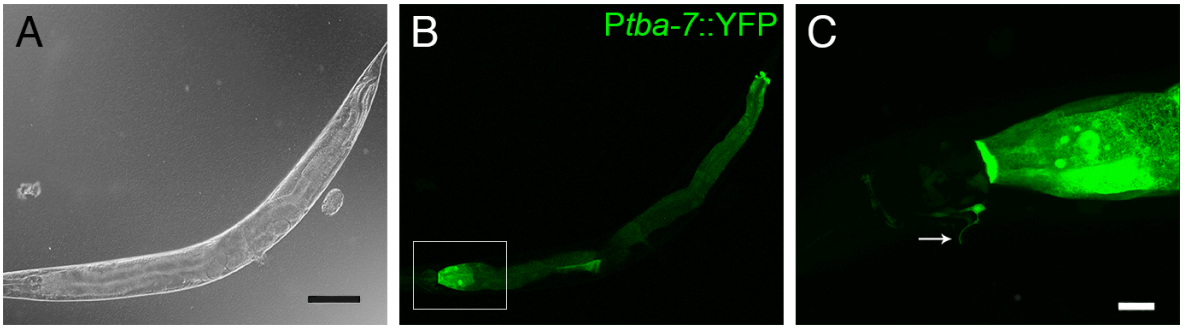


**Figure 5. *tba-7* is primarily expressed in the intestine.** A transcriptional fusion of the *tba-7* promoter to YFP reveals expression predominantly in the gut (see Table 2). It is also expressed in the pore cell of the excretory system (arrow in panel C, which is a high magnification view of the junction of the posterior pharynx and anterior intestine). Scale bars = 100 µm in panel A and 10 µm in B and C. Box in panel B indicates view in panel C. Strain to be available at the CGC.

### 2.4.2. TBA-7

A transcriptional reporter indicates that *tba-7* is primarily expressed in the intestine (Figure 5). However, neither a deletion mutation nor RNAi causes an overt phenotype (D. D. Hurd, unpublished results).

### 2.4.3. TBA-8

The primary structure of TBA-8 is notable for its diverged CTT that has a phenylalanine as the last amino acid (Figure 2). Nearly all other α-tubulins have a tyrosine that is often removed after translation. Transcriptional reporters indicate that it is expressed in seam cells and unidentified neurons (D. D. Hurd and D. S. Portman, unpublished results). No loss of function analysis has been undertaken.

### 2.4.4. TBB-6

TBB-6 is the most structurally diverged β-tubulin as it lacks most of what is considered to be a CTT (typically acidic amino acids, see Figure 2). It appears to have arisen recently in the *Caenhorhabditis* lineage (Saunders et al. 2013: PMID 23416426). Its anatomic expression closely resembles that of *tba-7* (Table 2). Neither a deletion mutation nor RNAi depletion causes an overt phenotype (D. D. Hurd, unpublished results).

## 3. Beyond α- and β-tubulin

### 3.1. TBG-1

γ-tubulin functions in the nucleation and anchoring of microtubules. Antibody staining and translational GFP fusions show that γ-tubulin is a component of centrosomes (Bobinnec et al., 2000:

PMID 11034903; Strome et al., 2001: PMID 11408582). It is also found at the base of cilia in amphid and phasmid sensory neurons and the apical cortex in the intestinal epithelium (Bobinnec et al., 2000: PMID 11034903). RNAi depletion of γ-tubulin causes polyploid germline cells and early blastomeres that contain a disorganized, ineffective mitotic spindle (Bobinnec et al., 2000: PMID 11034903; Strome et al., 2001: PMID 11408582). Too much γ-tubulin at the centrosome is caused by loss of the RNA-binding protein ATX-2 (ataxin-2), and this also brings about multiple defects in cell division (Stubenvoll et al., 2016: PMID 27689799). γ-tubulin is not absolutely required for microtubule nucleation, but instead is required for proper placement of microtubule minus ends within centrosomes and normal centriole structure (O'Toole et al., 2012: PMID 22253783).

### 3.2. Other tubulins

The *C. elegans* genome does not contain orthologs of other tubulins (δ-, ε- or η-tubulin), which are found in some single-celled eukaryotes and vertebrates. These tubulins are perhaps involved in basal body and centriole function (Dutcher, 2003: PMID 14662361; Oakley, 2000: PMID 11121746).

## 4. Cellular control of microtubules

Microtubules underlie a variety of essential cellular processes and are regulated by diverse mechanisms at many levels including biogenesis, heterodimer formation, nucleation, polymerization, depolymerization, post-translational modification, and association with other proteins (see Table 3).

### 4.1. Tubulin biogenesis and homeostasis

Newly synthesized tubulin (and actin) monomers pass through a series of chaperone complexes prior to being incorporated into dimers and eventually microtubules. The first of these is the prefoldin complex, which accepts nascent tubulin monomers from the ribosome and delivers them to the CCT (chaperonin-containing TCP-1) complex. The CCT complex finalizes folding of monomers and presents them to the tubulin folding cofactors A-E for dimerization. Tubulin degradation is thought to be mediated by the ubiquitin-proteasome pathway and other regulators/sensors of tubulin homeostasis, such as parkin or cofactor E-like proteins (Lundin et al., 2010: PMID 20116259).

PFD-1 through PFD-6 are subunits of the prefoldin complex, and CCT-1 through CCT-8 compose the CCT complex. Both transgenic fusion proteins and antibody staining indicate that they are expressed in nearly all cells with the most intense expression (of transgene) in bodywall muscle, pharyngeal muscle, vulval muscle, hypodermal cells, and the somatic gonad. Inhibition by RNAi in the early embryo indicates that prefoldin, CCT, and TXDC-9 function are required for multiple events in the early embryo that are dependent on microtubule function in addition to distal tip cell migration (Leroux and Candido, 1997: PMID 9434769; Lundin et al., 2008: PMID 18062952; Ogawa et al., 2004: PMID 15009089; Srayko et al., 2005: PMID 16054029)). Post-embryonic RNAi depletion of *cct-5* has shown that it is required for proper levels of tubulin and normal organization of intestinal actin and microvilli (Saegusa et al., 2014: PMID 25143409). The tubulin folding cofactors have not been studied in detail. Parkin has been implicated in the ubiquitination of tubulin in other systems, but the role for the broadly expressed *C. elegans* ortholog (PRD-1) in tubulin homeostasis has not been investigated (Springer et al., 2005: PMID 16204351).

### 4.2. Post-translational modification

Eukaryotic tubulins can be covalently modified after translation in a number of ways. These include tubulin-specific modifications such as removal/replacement of a terminal tyrosine (α-tubulin), addition of an acetyl group to an amino-terminal lysine (α-), addition of glycines or glutamates to residues in the CTT (both α- and β-). Tubulins also undergo more general modifications such as phosphorylation/dephosphorylation (β-) and palmitylation (α-). The most intensely studied modifications are those that are unique to tubulins (Hammond et al., 2008: PMID 18226514; Janke and Bulinski, 2011: PMID 22086369).

#### 4.2.1. Acetylation

MEC-12 is the only α-tubulin with a lysine at position 40, suggesting that it is the only acetylated α-tubulin in *C. elegans*. Supporting this idea, antibodies that recognize acetylated tubulin intensely stain the bodywall TRNs, and neurons implicated in nose mechanosensation that express MEC-12 (Shida et al., 2010: PMID 21068373; Siddiqui et al., 1989: PMID 2475594).

Acetylation is accomplished by two α-tubulin acetyltransferases (aTATs), MEC-17 and ATAT-2. They are expressed in TRNs and are required for acetylation of tubulin on the lumenal side of the microtubule wall in these cells. Acetylated α-tubulin is required for optimal touch sensation (Akella et al., 2010: PMID 20829795; Shida et al., 2010: PMID 21068373) and the structure and mechanical properties of the specialized 15 protofilament microtubules found in touch receptor neurons, although MEC-17 might play an additional role in microtubule structure (Cueva et al., 2012: PMID 22658592; Topalidou et al., 2012: PMID 22658602, Davenport et al., 2014: PMID 24846647).

#### 4.2.2. Glutamylation/deglutamylation

Tubulins are glutamylated by tubulin tyrosine ligating enzymes (TTLLs). Some members of this family initiate while some elongate chains of glutamate. These chains are added to a glutamate residue in the primary structure of tubulin CTTs. TTLL-4 glutamylates microtubules in the amphid channel cilia and certain labial cilia (See Table 3). Individual TTLL mutations cause little overt phenotypic effect, but a triple mutant of TTLL-4, TTLL-5 and TTLL-11 eliminates glutamylation and causes reduced male mating efficiency (Chawla et al. 2016: PMID 27635036).

Certain members of the cytosolic carboxypeptidase (CCPP) family remove glutamate chains from tubulin. Both CCPP-1 and CCPP-6 have been shown to suppress poly- or hyper-glutamylation (Kimura et al., 2010: PMID 20519502; O'Hagan et al., 2011: PMID 21982591). Proper levels of glutamylation are required for axonemal microtubule ultrastructure, normal velocity of motor proteins, localization of signaling proteins, and certain behaviors that require input from ciliated neurons (O'Hagan et al., 2011: PMID 21982591).

### 4.3. Microtubule-associated proteins (MAPs)

Microtubules are found in their numerous cellular contexts in either highly dynamic and transient structures, such as the spindle during mitosis, more stable structures, such as the axoneme supporting a cilia, or in variably stable structures, such as neuronal microtubules during development or after injury. These higher order structures and/or changes in dynamic behavior are regulated by a collection of microtubule-associated proteins that can promote growth/stabilize, promote shrinkage/destabilize, sever, bundle, bind to plus or minus ends, or perform some combination of these functions.

#### 4.3.1. PTL-1

*tau* and other similar MAPs bind to and stabilize microtubules. In vertebrates, dysfunction of *tau* causes disassociation from microtubules and self-aggregation, which underlies a broad class of neuronal pathologies known collectively as tauopathies (Sabbagh and Dickey, 2016: PMID 26834532). *C. elegans* has a single identified protein that is similar to mammalian *tau* (Goedert et al., 1996: PMID 8937984; McDermott et al., 1996: PMID 8755720). PTL-1 (protein with *tau*-like repeats) is most highly conserved in both structure and function to *tau* and other MAPs in the carboxyl-terminal microtubule binding repeats (Hashi et al., 2016: PMID 26906882; Gordon et al., 2008: PMID 18807071). It is most prominently expressed in mechanoreceptor cells. Loss of PTL-1 causes no overt phenotypic effect, but *ptl-1* mutations dominantly enhance the loss of touch sensation caused by lack of either MEC-7 or MEC-12 (Goedert et al., 1996: PMID 8937984; Gordon et al., 2008: PMID 18807071). Loss of both PTL-1 and spectrin (UNC-70) also causes a rearrangement of microtubule bundles, which sensitizes TRN neurites to mechanical damage (Krieg et al. 2017: PMID 28098556). PTL-1 is required for the normal behavior of microtubule motor proteins and the distribution of synaptic components in touch receptor cells (Tien et al., 2010: PMID 21569846).

### 4.3.2. ELP-1

EMAP (echinoderm microtubule-associated protein) family members bind to and can either stabilize (Houtman et al., 2007: PMID 17196341) or destabilize microtubules (Eichenmüller et al., 2002: PMID 11694528). Consensus domains across family members include ~6 WD40 repeats and another domain specific to the EMAP-like proteins, the ELP domain. *C. elegans* has a single member of the family, ELP-6 (Suprenant et al., 2000: PMID 10603080), which has a predicted amino terminal coiled domain found in some of the vertebrate orthologs (Hueston et al., 2008: PMID 19014691). It has been shown to bind to microtubules and is expressed in a number of cell types including bodywall muscle, sensory neurons (IL1, male ray neurons, TRNs), vulval muscles, the spermethecal valve cell, intestinal cells, and seam cells (Hueston et al., 2008: PMID 19014691). Loss of ELP-1 through mutation or RNAi causes defects in touch reception, and in combination with loss of the dystrophin homolog DYS-1, causes paralysis and death (Hueston and Suprenant, 2009: PMID 19582871).

### 4.3.3. TAC-1

The transforming acidic coiled-coil (TACC-1) family of proteins functions in the regulation of microtubule length by promoting growth (Mortuza et al, 2014: PMID 25246530). The *C. elegans* protein TAC-1 consists solely of the TACC-1 domain, while orthologous proteins have more complex and larger structures (Bellanger and Gönczy, 2003: PMID 12956950; Le Bot et al., 2003: PMID 12956951). TAC-1 accumulates in the centrosomes of dividing blastomeres (dependent upon ZYG-9) where it physically associates and functions with ZYG-9 and ZYG-8 to regulate microtubule length, pronuclear migration, and spindle positioning as a polymerizing factor (Bellanger et al., 2007: PMID 17666432; Bellanger and Gönczy, 2003: PMID 12956950; Srayko et al., 2003: PMID 12956952).

### 4.3.4. ZYG-8

Doublecortin kinases bind to microtubules to stabilize and/or straighten them (Jean et al., 2012: PMID 23001563). ZYG-8 is the ortholog of the human protein DCLK (doublecortin-like kinase), as it contains a doublecortin domain and a kinase domain similar to calcium/calmodulin-regulated kinases. ZYG-8 localizes to microtubules in the mitotic spindle in early blastomeres (and in cultured mammalian cells), where it functions to promote microtubule growth required for proper spindle placement (Gönczy et al., 2001; PMID 11702948). It is also expressed in certain neurons where it is required for process outgrowth, locomotion, and touch sensitivity (Bellanger et al., 2012: PMID 22956537). ZYG-8

physically associates with TAC-1 through its doublecortin domain (Bellanger et al., 2007: PMID 17666432) and with EFA-6 during regeneration after axonal injury (see section 4.3.8).

### 4.3.5. ZYG-9

The XMAP215 (*Xenopus* microtubule-associated protein) family of microtubule binding proteins is composed of three TOG (tumor overexpressed gene) domains that interact with tubulin dimers and promote assembly (Al-Bassam and Chang, 2011: PMID 21782439). ZYG-9 is the *C. elegans* member, originally discovered in screens for mutations that alter polarity in the early embryo (Kemphues et al., 1986; PMID 3949074). It was subsequently shown to be required for spindle function (Matthews et al., 1998: PMID 9606208). ZYG-9 localizes to the centrosome in dividing blastomeres (dependent upon TAC-1), where it functions with ZYG-8 and TAC-1 to regulate the length of microtubules (Bellanger et al., 2007: PMID 17666432; Bellanger and Gönczy, 2003: PMID 12956950; Srayko et al., 2003: PMID 12956952).

### 4.3.6. CLS-1, CLS-2, and CLS-3

The CLASP (cytoplasmic linker associated protein) family of microtubule regulators also contains TOG domains. They function in microtubule stabilization by promoting rescue of shrinking microtubules (Al-Bassam and Chang, 2011: PMID 21782439). CLS-1, CLS-2, and CLS-3 are the members of the CLASP family in *C. elegans*. RNAi depletion causes defects in nuclear rotation, maintenance of spindle length, and spindle placement in the one-cell embryo, likely due to fewer astral microtubules. CLS-2 is found on the mitotic spindle, centrosomes, and kinetochores in dividing cells in early embryogenesis and in the cytoplasm of interphase cells (Espiritu et al, 2012: PMID 22613359).

### 4.3.7. CHE-12

Crescerins (*crescere*: to grow) are another family of TOG domain proteins that bind to and regulate microtubules in cilia. This family is represented by CHE-12 in *C. elegans*, and is part of a larger family that includes the CLASPs (CLS-1, -2 and -3) and ch-TOG/XMAP215 (ZYG-9) proteins. CHE-12 localizes to the cilia of the amphid channel neurons and the phasmid neurons. Loss of function through mutation causes altered cilia structure, inability to take up lipophilic dyes, and failure in chemotaxis (Bacaj et al., 2008: PMID 18245347; Das et al., 2015: PMID 26378256).

### 4.3.8. EFA-6

Members of the EFA-6 (exchange factor for ARF6 family GTPases) protein family function to limit microtubule growth. These proteins are composed of a Sec7 guanine nucleotide exchange factor domain, a pleckstrin homology (PH) domain, and regions of coiled-coil. *C. elegans* EFA-6 is found in the cortex of blastomeres where loss of function through mutation or RNAi treatment causes an increase in the total number of microtubules, an increase in the fraction of the population that is growing, and an increase in microtubule length (Chen et al., 2011: PMID 21943602; O'Rourke et al., 2010: PMID 21076413). EFA-6 is also found dynamically rearranging from a smooth distribution to punctae of microtubule minus ends during axotomy-induced regeneration in PLMs. This is controlled by an 18 amino acid N-terminal motif through which it also makes physical association with TAC-1 and ZYG-8. Loss-of-function mutations cause neurite overgrowth after injury (Chen et al., 2015: PMID 26339988).

### 4.3.9. RMD-1, RMD-2, RMD-3, RMD-4, RMD-5, and RMD-6

RMD-1 through -6 are a family of proteins that might function in the regulation of microtubule dynamics. They all contain predicted coiled-coil domains and have orthologs in vertebrates, although little is known about the vertebrate homologs. Only RMD-1 has been studied in detail; it appears to function in the attachment of microtubules to kinetochores as depletion through RNAi causes delayed anaphase and incorrectly segregated DNA during mitosis. RMD-1 localizes to the spindle of the one-cell embryo and binds to microtubules *in vitro* (Oishi et al., 2007: PMID 18070910).

#### 4.3.10. MEI-1 and MEI-2

Katanins are a family of microtubule severing proteins (Roll-Mecak and McNally, 2010: PMID 19963362). MEI-1 is the p60 catalytic subunit (AAA ATPase) of *C. elegans* katanin, and MEI-2 is the p80 activator/regulator of MEI-1. They were originally identified in screens for defects in meiosis and mitosis (Clark-Maguire and Mains, 1994: PMID 8150281; Mains et al., 1990: PMID 2249759). They are maternally expressed and dependently colocalize in the meiotic spindle of oocytes (Srayko et al., 2000: PMID 10809666). It was originally thought that microtubule severing activity was required for functional meiotic spindles (Srayko et al., 2006: PMID 17027492), but recent analysis of specific point mutations suggests that MEI-1 might also bind/bundle microtubules (McNally and McNally, 2011: PMID 21372175; Connolly et al., 2014: PMID 24554763; McNally et al., 2014: PMID 24501424). Levels of MEI-1 decrease after meiosis due to the activity of the MEL-26 E3 ubiquitin ligase (Johnson et al., 2009: PMID 19361490; Pintard et al., 2003: PMID 13679921).

#### 4.3.11. SPAS-1

Like katanins, spastins are microtubule severing proteins in the AAA ATPase family of enzymes (Roll-Mecak and McNally, 2010: PMID 19963362). SPAS-1 was identified via homology in a search for proteins related to spastin, the human protein that is defective in a form of spastic paraplegia. SPAS-1 is reportedly expressed in a broad variety of tissues in worms. A precise *in vivo* function is not evident from studies of a deletion mutation, which causes pleitropic defects in growth, vulval development, and reproduction (Matsushita-Ishiodori et al., 2007: PMID 17531954). Biochemically, SPAS-1 forms hexamers, and its ATPase activity is stimulated by microtubules. Conserved basic amino acids interact with acidic CTTs of tubulin, and a conserved tryptophan in SPAS-1 is required for microtubule severing (Matsushita-Ishiodori et al., 2009: PMID 19619244).

#### 4.3.12. KLP-7

KLP-7 is a kinesin-like protein of the MCAK (mitotic centromere-associated kinesin) family, which functions to depolymerize microtubules in many systems (Sanhaji et al., 2011: PMID 22249213). It is found at the kinetochore in the blastomeres of the early embryo (Oegema et al., 2001: PMID 11402065). KLP-7 is phosphorylated by Aurora kinases, and it regulates nucleation rate; loss of *klp-7* expression through RNAi or mutation increases the number of astral and midzone microtubules while decreasing growth rate (Srayko et al., 2005: PMID 16054029; Han et al., 2015: PMID 26168236). KLP-7 also functions to establish a bipolar meiotic spindle in the absence of centrosomes (Connolly et al., 2015: PMID 26370499) In adult touch receptor neurons, KLP-7 functions to maintain a dynamic population of microtubules in normal cells and to inhibit microtubule upregulation in axotomized cells (Ghosh-Roy et al. 2012: PMID 23000142).

#### 4.3.13. EBP-1, EBP-2, and EBP-3

Microtubule end-binding proteins (also known tip-interacting proteins or +TIPs) recognize and bind to a structural feature at the plus ends of microtubules (Jiang and Akhmanova, 2011: PMID

20817499). EBP-1 and EBP-2 fusion proteins (driven by the *pie-1* promoter) decorate the growing plus ends of microtubules in the spindle of early embryos (Motegi et al., 2006: PMID 16580995; Srayko et al., 2005: PMID 16054029). EBP-1 is required for efficient axon regrowth in PLM neurons after laser axotomy (Chen et al., 2011: PMID 21943602). Perturbation by mutation or RNAi does not cause severe phenotypes, perhaps due to redundancy, and analysis of anatomic expression from native promoters for these three paralogs has not been reported.

#### 4.3.14. PTRN-1

Members of the CAMSAP (calmodulin-regulated spectrin-associated protein)/Nezha/patronin family decorate and stabilize minus ends of microtubules (Akhmanova and Hoogenraad, 2015: PMID 25689915). The proteins share a conserved structure, which includes a calponin homology domain, a coiled-coil domain(s), the namesake CAMSAP (also called CKK) domain, and a carboxyl-terminal microtubule binding domain. PTRN-1 in *C. elegans* is broadly expressed in neurons and has been shown to be associated with microtubules in puncta in nerve and muscle cells (Marcette et al. 2014: PMID 24569480; Richardson et al., 2014: PMID 24569477; Chuang et al. 2014: PMID 25437544). It is not required in general for normal movement, sensation, or reproduction. The effects of loss-of-function mutations, such as overgrowth or ectopic neurite formation, are more readily observed in microtubule-sensitized situations such as exposure to the depolymerizing drug colchicine, or in specific developmental contexts such as neuronal remodeling in touch receptor neurons or after injury. Loss of DLK-1 (dual leucine zipper kinase), a mitogen-activated protein kinase kinase kinase, suppresses the subtle defects caused by loss of PTRN-1, suggesting they may work in the same pathway (Marcette et al. 2014: PMID 24569480; Richardson et al., 2014: PMID 24569477; Chuang et al. 2014: PMID 25437544).

#### 4.3.15. NOCA-1

Ninein proteins function to anchor and perhaps stabilize and/or nucleate microtubules through association with the minus end (Srivatsa et al. 2015: PMID 25741725). The *C. elegans* homolog of vertebrate ninein-like proteins is NOCA-1, encoded by a complex locus that produces several variants. It was originally identified as a gene that phenocopied γ-tubulin defects. A deletion causes abnormal positioning of germline nuclei and associated sterility, with no other obvious phenotypes. NOCA-1 works with γ-tubulin and PTRN-1 to establish non-centrosomal microtubule arrays that position nuclei and/or provide structural integrity (along with LET-502) to the hypodermis (Quintin et al., 2016: PMID 26586219). NOCA-1 and γ-tubulin colocalize, and NOCA-1 and PTRN-1 bind to microtubules in extracts (Wang et al. 2015: PMID 26371552).

#### 4.3.16. CYLC-1 and CYLC-2

Cylicin was originally discovered in the cytoskeletal calyx that surrounds part of the nucleus in the head of mammalian sperm; structurally it is characterized by numerous small repeats of the charged amino acids KKD/E (Hess et al. 1993: PMID 8354692). *C. elegans* has two highly conserved proteins, CYLC-1 and CYLC-2, that are related to mammalian cylicin and potentially to stathmin. Inhibition by RNAi causes altered tubulin concentrations and misregulated growth of microtubules in a region of uterine muscle cells, suggesting that these proteins function in the intracellular compartmentation of tubulin levels (Lacroix et al., 2016: PMID 26985017). An anatomic expression pattern has not been reported.

## 5. Concluding remarks and future directions

As a genetic and cytological model in which to study a family of closely related structural proteins, *C. elegans* provides evidence for both redundancy and specificity in the use of α- and β-tubulins. In support of redundancy, mutagenesis screening has produced a collection of missense alleles that change conserved amino acids in tubulin (e.g., in the *tba-1*, *tba-5*, *tbb-2*, *mec-12*, *mec-7,* and *tbb-4* genes) that often cause more severe defects than deletions/nulls. This means that it is often more deleterious to incorporate an altered tubulin into a microtubule than it is to incorporate a wild-type version of another tubulin. In addition, genetic and/or RNAi elimination of multiple mitotic tubulins (*tba-1*, *tba-2*, *tbb-1,* and *tbb-2*) is required to disrupt embryogenesis, further supporting redundancy among tubulins. Nevertheless, specific expression patterns (Figure 6), the presence of variant CTTs, and the quantifiable cytological and behavioral defects caused by the loss of single tubulins (e.g., *tba-5*, *tba-6*, *tba-9*, *mec-12*, *tbb-4,* or *mec-7*) all support the idea that tubulins can be specialized for certain developmental and physiological contexts. Whether tubulins in *C. elegans* are regulated in response to environmental change has not been explored.

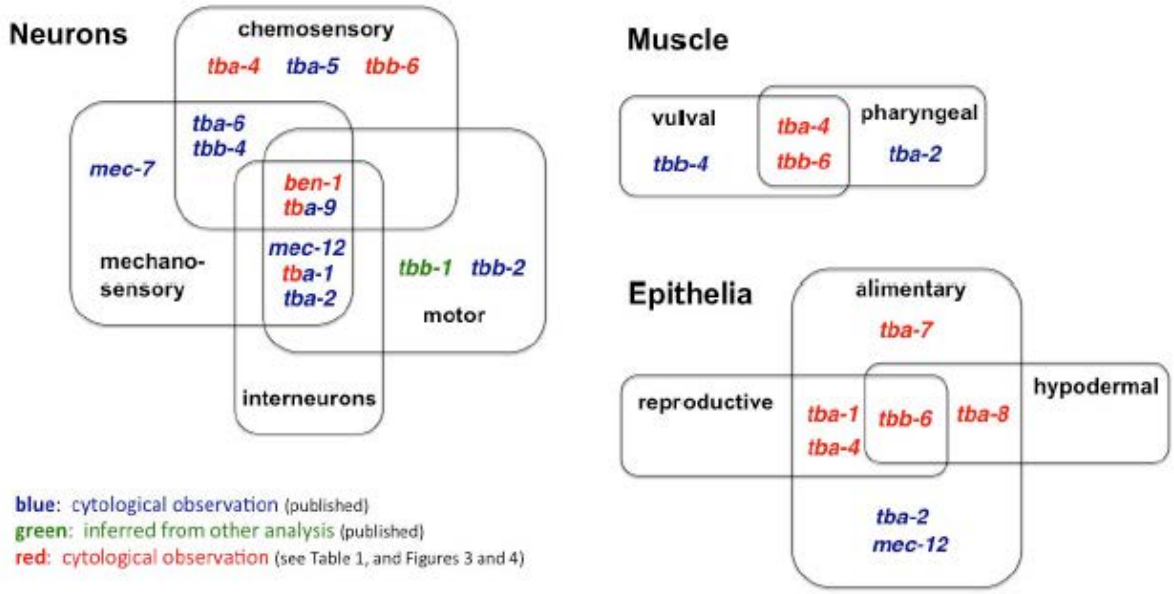


**Figure 6. Summary of the somatic expression of the α- and β-tubulins.** Venn diagrams depict the anatomic expression of the tubulin family members in non-germline tissues/organs. The type of data used to place a particular tubulin in a particular area is depicted using color. See text for references.

*C. elegans* provides the ability to understand tubulin biology from the molecular to the organismal level. Worm tubulins will continue to be discovered playing roles in diverse and unforeseen biological processes, whether through *de novo* mutagenesis screening, systems level analysis, or novel approaches. At the molecular level, new alleles can continue to shed insight into the structure and function of the tubulin dimer and the myriad other proteins that interact with tubulin, either soluble or assembled. Both forward and reverse genetic screens and searching for modifiers of existing phenotypes, especially among paralogs expressed in the same tissues and/or with regulatory factors, allows understanding of tubulin usage and regulation within an organism. Technological advances (e.g., studying transport velocities of cellular components in neurons or other tissues), a strength of the nematode model system, should provide a higher resolution and more precise quantitative description of the role of tubulin in metazoan biology.

## 6. Tables

**Table 1. WormBase (WS248) alleles that have been classified genetically, have an identified molecular change, or are represented by a strain at the CGC.**

| Gene | Allele | Protein change | Strain | Dominant, Semi-Dominant, Recessive, Gain-of-function, Loss-of-function | Reference/ Source |
|---|---|---|---|---|---|
| *tba-1* | *ju89* | G414R | | Dominant | Baran et al., 2010: PMID 20300184 |
| *tba-1* | *or346* | S377F | EU1135 | Dominant | Philips et al., 2004: PMID: 15083533; O'Rourke et al., 2011: PMID 21390299 |
| *tba-1* | *or594* | S377F | EU1161 | Semi-dominant | O'Rourke et al., 2011: PMID 21390299 |
| *tba-2* | *sb25* | E277K | HR899 | Dominant | Lu, C. et al., 2005: PMID 15781712 |
| *tba-2* | *sb27* | E194K | HR973 | Dominant | Lu, C. et al., 2005: PMID 15781712 |
| *tba-2* | *sb51* | S168Y | HR505 | Dominant | Lu, C. et al., 2005: PMID 15781712 |
| *tba-2* | *sb116* | E22K | | Intragenic revertant of sb51 | Lu, C. et al., 2005: PMID 15781712 |
| *tba-2* | *sb117* | E69K | | Intragenic revertant of sb51 | Lu, C. et al., 2005: PMID 15781712 |
| *mec-12* | *e1605* | H192Y | CB3284 | Recessive | Bounoutas et al., 2009: PMID 19615905; Bounoutas et al., 2011: PMID 21368137 |
| *mec-12* | *e1607* | G144S | | Semi-Dominant (haploinsufficient), Loss of function, Recessive | Hsu et al., 2014: PMID 25392990; Bounoutas et al. 2011: PMID 21368137; Bounoutas et al., 2009: PMID 19615905 |
| *mec-12* | *gm379* | G416E | | Semi-dominant, Neomorphic | Hsu et al., 2014: PMID 25392990 |
| *mec-12* | *u159* | C295Y | | Semi-dominant | Gu et al., 1996: PMID 8692859; Bounoutas et al., 2009: PMID 19615905 |
| *mec-12* | *u204* | S178F | | | Chalfie and Au 1989: PMID 2646709; Bounoutas et al., 2009: PMID 19615905 |
| *mec-12* | *u241* | G354E | | Dominant, Gain of function | Fukushige et al, 1999: PMID 9885292; Bounoutas et al., 2011: PMID 21368137; |

| | | | | | |
|---|---|---|---|---|---|
| | | | | | Bounoutas et al., 2009: PMID 19615905 |
| *mec-12* | *u279* | T179I | | | Gu et al., 1996: PMID 8692859 |
| *mec-12* | *u50* | H192Y | | Recessive | Bounoutas et al., 2009: PMID 19615905; Chalfie and Au, 1989: PMID 2646709 |
| *mec-12* | *u63* | E415K | | Recessive, Gain of function | Fukushige et al., 1999: PMID 9885292; Bounoutas et al., 2011: PMID 21368137 |
| *mec-12* | *u67* | M154I | | Temperature-sensitive dominant | Gordon et al., 2008: PMID 18807071; Bounoutas et al., 2009: PMID 19615905 |
| *mec-12* | *u76* | D69N | | | Fukushige et al., 1999: PMID 9885292 |
| *mec-12* | *u94* | G148S | | Semi-Dominant | Gu et al., 1996: PMID 8692859; Bounoutas et al., 2009: PMID 19615905 |
| *tba-5* | *qj14* | A19V | | Gain-of-Function | Hao et al., 2011: PMID 21642982 |
| *tbb-2* | *or362* | G141E | EU858 | Semi-Dominant, Dominant | Ellis et al, 2004: PMID 14702387; Lu et al., 2004: PMID 14565976 |
| *tbb-2* | *or600* | G140E | EU1588 | Semi-Dominant | O'Rourke et al., 2011: PMID 21390299 |
| *tbb-2* | *qt1* | E198K | HC48 | Gain-of-Function | Wright et al., 2003: PMID 12937270 |
| *tbb-2* | *sb26* | E439K | HR1060 | Semi-Dominant, Gain of function | Lu, et.al., 2004: PMID 14565976; Shaye et al., 2015: PMID 25771894 |
| *tbb-2* | *t1623* | V313M | GE2255 | Semi-Dominant | Wright et al., 2003: PMID 12937270 |
| *mec-7* | *e1343* | P171L | CB1477; CB2217 | Semi-Dominant | Savage et al., 1994: PMID 7983175 |
| *mec-7* | *e1505* | | | Semi-Dominant | Savage et al., 1994: PMID 7983175 |
| *mec-7* | *e1506* | M1I | CB3270 | Recessive | Savage et al., 1994: PMID 7983175 |
| *mec-7* | *e1522* | F317I | | Semi-Dominant | Savage et al., 1994: PMID 7983175 |
| *mec-7* | *e1527* | V286D | CB3276 | Dominant | Savage et al., 1994: PMID 7983175 |
| *mec-7* | *ky852* | P220S | | | Kirszenblat et.al. 2013: PMID 23223572 |
| *mec-7* | *n434* | N247I | TU300 | Dominant | Savage et al., 1994: PMID 7983175 |
| *mec-7* | *u10* | S188F | | Semi-Dominant | Savage et al., 1994: PMID 7983175 |
| *mec-7* | *u127* | P171L | | Semi-Dominant | Savage et al., 1994: PMID 7983175 |

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| *mec-7* | *u129* | G244S | | Dominant | Savage et al., 1994: PMID 7983175 |
| *mec-7* | *u136* | | | Semi-Dominant | Savage et al., 1994: PMID 7983175 |
| *mec-7* | *u142* | Q279stop | | Recessive | O'Hagan et.al 2005: PMID 15580270 |
| *mec-7* | *u143* | G369R | | Semi-Dominant | Savage et al., 1994: PMID 7983175 |
| *mec-7* | *u156* | W344X | | Semi-Dominant | Savage et al., 1994: PMID 7983175 |
| *mec-7* | *u162* | D249N | | Dominant | Savage et al., 1994: PMID 7983175 |
| *mec-7* | *u170* | E405K | | Recessive | Savage et al., 1994: PMID 7983175 |
| *mec-7* | *u173* | G402R | | Recessive | Savage et al., 1994: PMID 7983175 |
| *mec-7* | *u178* | Q280stop | | Recessive | Savage et al., 1994: PMID 7983175 |
| *mec-7* | *u18* | A393T | | Dominant | Savage et al., 1994: PMID 7983175 |
| *mec-7* | *u222* | G109E | | Semi-Dominant | Savage et al., 1994: PMID 7983175 |
| *mec-7* | *u223* | P61L | | Semi-Dominant | Savage et al., 1994: PMID 7983175 |
| *mec-7* | *u225* | T214P | | Semi-Dominant | Savage et al., 1994: PMID 7983175 |
| *mec-7* | *u234* | R318Q | | Semi-Dominant | Savage et al., 1994: PMID 7983175 |
| *mec-7* | *u249* | P61S | | Semi-Dominant | Savage et al., 1994: PMID 7983175 |
| *mec-7* | *u262* | N226Y | | Semi-Dominant | Savage et al., 1994: PMID 7983175 |
| *mec-7* | *u275* | G148R | | Recessive | Savage et al., 1994: PMID 7983175 |
| *mec-7* | *u278* | C303Y | | Semi-Dominant | Savage et al., 1994: PMID 7983175 |
| *mec-7* | *u283* | P243L | | Dominant, Gain of function | Savage et al., 1994: PMID 7983175; Bounoutas et al., 2011: 21368137 |
| *mec-7* | *u305* | G34S | | Recessive | Savage et al., 1994: PMID 7983175; Sze et al., 1997: PMID 9102303 |
| *mec-7* | *u319* | S25F | | Semi-Dominant | Savage et al., 1994: PMID 7983175 |
| *mec-7* | *u382* | E410:Tc5 | | Recessive | Savage et al., 1994: PMID 7983175 |
| *mec-7* | *u388* | E410:Tc5 | | Recessive | Savage et al., 1994: PMID 7983175 |
| *mec-7* | *u427* | V255M | | Semi-Dominant | Savage et al., 1994: PMID 7983175 |
| *mec-7* | *u428* | G369E | | Recessive | Gu et al., 1996: PMID 8692859 |

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| *mec-7* | *u429* | G141E | | Recessive | Savage et al., 1994: PMID 7983175 |
| *mec-7* | *u430* | A97V | | Recessive | Savage et al., 1994: PMID 7983175 |
| *mec-7* | *u431* | Q280stop | | Recessive | Sze et al., 1997: PMID 9102303 |
| *mec-7* | *u433* | G141E | | Recessive | Savage et al., 1994: PMID 7983175 |
| *mec-7* | *u440* | W101X | | Recessive | Savage et al., 1994: PMID 7983175 |
| *mec-7* | *u443* | | | Recessive | Savage et al., 1994: PMID 7983175 |
| *mec-7* | *u445* | M300V | | Semi-Dominant | Savage et al., 1994: PMID 7983175 |
| *mec-7* | *u448* | | | Recessive | Savage et al., 1994: PMID 7983175 |
| *mec-7* | *u449* | V179A | | Semi-Dominant | Savage et al., 1994: PMID 7983175 |
| *mec-7* | *u451* | Q292P | | Semi-Dominant | Savage et al., 1994: PMID 7983175 |
| *mec-7* | *u453* | | | Recessive | Savage et al., 1994: PMID 7983175 |
| *mec-7* | *u48* | S176F | | Semi-Dominant | Savage et al., 1994: PMID 7983175 |
| *mec-7* | *u58* | P61L | | Semi-Dominant | Savage et al., 1994: PMID 7983175 |
| *mec-7* | *u80* | | | Recessive | Savage et al., 1994: PMID 7983175 |
| *mec-7* | *u88* | R318stop | | Recessive | Sze et al., 1997: PMID 9102303 |
| *mec-7* | *u9* | W101stop | | Recessive | Savage et al., 1994: PMID 7983175 |
| *mec-7* | *u98* | M300T | | Semi-Dominant | Savage et al., 1994: PMID 7983175 |
| *tbb-4* | *sa127* | L253F | SP1742 | Gain of function | Hao et al., 2011: PMID 21642982 |
| *ben-1* | *e1880* | | CB3474 | Semi-Dominant | Driscoll et al., 1989: PMID 2592410 |
| *ben-1* | *e1910* | | | Semi-Dominant | Driscoll et al., 1989: PMID 2592410 |
| *ben-1* | *e1911* | | | Semi-Dominant | Driscoll et al., 1989: PMID 2592410 |
| *ben-1* | *u102* | | | Semi-Dominant | Driscoll et al., 1989: PMID 2592410 |
| *ben-1* | *u116* | | | Semi-Dominant | Driscoll et al., 1989: PMID 2592410 |
| *ben-1* | *u134* | | | Semi-Dominant | Driscoll et al., 1989: PMID 2592410 |
| *ben-1* | *u462* | | | Semi-Dominant | Driscoll et al., 1989: PMID 2592410 |
| *ben-1* | *u463* | | | Semi-Dominant | Driscoll et al., 1989: PMID 2592410 |
| *tbb-6* | *oxTi207* | | EG7952 | | |

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| *tbg-1* | *t1465* | A401V | GE2959 | | Hannack et al., 2002: PMID 12011109 |

Mutant alleles of all tubulin genes were classified using the terms dominant, semi-dominant, gain of function, recessive and/or loss of function according author's descriptions found by Textspresso full-text searching of published articles. In many cases these were dependent upon the type of screen employed or the phenotype observed and/or the particular combinations of alleles that were tested. See the text of the particular papers for details. The majority of these alleles have an identified molecular change in the primary structure of tubulin (mapped in Figure 1), and there are strains carrying some these alleles available at the CGC. The vast majority of alleles generated in the large-scale mutation projects have no phenotypic data yet, and they were excluded from this analysis.

## Table 2. Summary of the expression and function of worm tubulins.

| Locus | Gene Model | Expression | Cellular, developmental, behavioral roles | Selected references |
|---|---|---|---|---|
| *tba-1* | F26E4.8 | ALM, PLM, AVM, PVM, VA/VB/DA/DB motor neurons, multiple other neurons, *germline*, early embryo | cellular architecture, meiosis/mitosis, neuronal pathfinding, synaptogenesis | Lu and Mains, 2005: PMID 15781712; Phillips et al., 2004: PMID 15083533; Baran et al., 2010: PMID 20300184 |
| *tba-2* | C47B2.3 | pharynx, intestine, VB/DB motor neurons, PLM, ALA, other anterior neurons, *germline, early embryo* | cellular architecture, meiosis/mitosis | Lu and Mains, 2005: PMID 15781712; Phillips et al., 2004: PMID 15083533 |
| *mec-12* | C44B11.3 | ALM, PLM, AVM, PVM, other neurons | sensory neuron dendritic microtubules, mechanosensation | Chalfie and Sulston, 1981: PMID 7227647; Bounoutas et al., 2009: PMID 19615905; O'Hagan et al., 2005: PMID 15580270; Bounoutas et al., 2011: PMID 21368137 |
| *tba-4* | F44F4.11 | intestine and other tissues | unknown/redundant | D. D. Hurd, unpublished (expression) |
| *tba-5* | F16D3.1 | PHA, PHB, amphid neurons, perhaps other sensory neurons | sensory neuron axonemal microtubules, *chemosensation and/or mechanosensation* | Hao et al., 2011: PMID 21642982 |
| *tba-6* | F32H2.9 | IL2, CEM, HSN, RnB, HOB | sensory neuron axonemal/dendritic microtubules, chemosensation and/or mechanosensation | Hurd et al., 2010: PMID 20421600 |

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|---|---|---|---|---|
| *tba-7* | T28D6.2 | intestine and excretory pore cell (Figure 5) | unknown/redundant | |
| *tba-8* | ZK899.4 | seam cells, neurons | unknown/redundant | D. D. Hurd and D. S. Portman, unpublished (expression) |
| *tba-9* | F40F4.5 | CEP, OLQ, ASI, ADF, AFD, AWA, AWC, ASE, ADE, URX, RIG, PDE, PQR, RnA, HOA, various motor neurons, other neurons and neuronal support cells | axonal/axonemal/ dendritic microtubules, chemosensation and/or mechanosensation | Hurd et al., 2010: PMID 20421600 |
| *tbb-1* | K01G5.7 | *germline, early embryo* | cellular architecture, meiosis/mitosis | Lu et al., 2004: PMID 14565976; Wright and Hunter, 2003: PMID 12937270 |
| *tbb-2* | C36E8.5 | germline, early embryo, many neurons, excretory cell | cellular architecture, meiosis/mitosis, neuronal pathfinding, synaptogenesis, excretory cell tubulogenesis | Shaye and Greenwald, 2015: PMID 25771894; Lu et al., 2004: PMID 14565976; Wright and Hunter, 2003: PMID 12937270 |
| *mec-7* | ZK154.3 | ALM, PLM, AVM, PVM, FLP, PVD, BDU | sensory neuron dendritic microtubules, mechanosensation | Chalfie and Sulston, 1981: PMID 7227647; Bounoutas et al., 2009: PMID 19615905; O'Hagan et al., 2005: PMID 15580270; Bounoutas et al., 2011: PMID 21368137 |
| *tbb-4* | B0272.1 | CEP, OLQ, CEM, ASK, ADL, ASI, ASJ, ASH, AWB, AWA, AWC, ASE, ASG, FLP, HSN, PDE, PQR, PHA, PHB, RnA, RnB, HOA, vulval muscles | axonemal/dendritic microtubules, chemosensation and/or mechanosensation | Hurd et al., 2010: PMID 20421600; Hao et al., 2010: PMID 21642982 |
| *ben-1* | C54C6.2 | multiple motor, sensory, and interneurons (Figure 4) | axonal pathfinding, sensitivity to benzimidazole | Driscoll et al., 1989: PMID 2592410 |
| *tbb-6* | T04H1.9 | intestine and other tissues | unknown/redundant | D. D. Hurd, unpublished (expression) |
| *tbg-1* | F58A4.8 | early embryo, sensory neurons, other tissues | spindle organization | Bobinnec et al., 2000: PMID 11034903; Strome et al., 2001: |

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|---|---|---|---|---|
| | | | | PMID 11408582; O'Toole et al., 2012: PMID 22253783 |

In the expression column, both cells express in all examined cases if a neuron is part of a left/right bilateral pair. In both the expression and function column, *italics* are used when expression is inferred from mutant analysis or *vice versa*

## 7. Acknowledgements

I thank Douglas Portman (University of Rochester) for initiating my interest in the *C. elegans* tubulins and the continued use of equipment in his laboratory. I also thank Noveera Ahmed (St. John Fisher College) for critically reading versions of the manuscript, Nicholas Hoffend and Katelyn Giardino (St. John Fisher College undergraduates) for making the DNA constructs that led to Figure 4 and Figure 5, and Michelle Price (St. John Fisher College Lavery Library) for the reference collecting and TextPresso searching that led to Table 1. Finally, I acknowledge the community of *C. elegans* researchers for the production of seminal and important data in the field of tubulin biology.

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