Molecular Biology of the Cell
Vol. 15, 142–150, January 2004

The *Caenorhabditis elegans* Microtubule-severing Complex MEI-1/MEI-2 Katanin Interacts Differently with Two Superficially Redundant β-Tubulin Isotypes

Chenggang Lu,* Martin Srayko,*† and Paul E. Mains*‡§

*Genes and Development Research Group and ‡Department of Biochemistry and Molecular Biology, University of Calgary, Calgary, Alberta, T2N 4N1 Canada

Submitted June 18, 2003; Revised August 25, 2003; Accepted September 8, 2003
Monitoring Editor: Susan Strome

The microtubule-severing protein complex katanin is required for a variety of important microtubule-base morphological changes in both animals and plants. *Caenorhabditis elegans* katanin is encoded by the *mei-1* and *mei-2* genes and is required for oocyte meiotic spindle formation and must be inactivated before the first mitotic cleavage. We identified a mutation, *sb26*, in the *tbb-2* β-tubulin gene that partially inhibits MEI-1/MEI-2 activity: *sb26* rescues lethality caused by ectopic MEI-1/MEI-2 expression during mitosis, and *sb26* increases meiotic defects in a genetic background where MEI-1/MEI-2 activity is lower than normal. *sb26* does not interfere with MEI-1/MEI-2 microtubule localization, suggesting that this mutation likely interferes with severing. Tubulin deletion alleles and RNA-mediated interference revealed that TBB-2 and the other germline enriched β-tubulin isotype, TBB-1, are redundant for embryonic viability. However, limiting MEI-1/MEI-2 activity in these experiments revealed that MEI-1/MEI-2 preferentially interacts with TBB-2–containing microtubules. Our results demonstrate that these two superficially redundant β-tubulin isotypes have functionally distinct roles in vivo.

INTRODUCTION

During cell division, precise segregation of chromosomes into the daughter cells relies on the dynamic organization of a bipolar spindle. With spindle microtubules nucleating from the centrosomes, a bipolar mitotic spindle forms as microtubules grow out and capture the chromosomes. Compared with mitosis of animal cells, female meiosis in most animals has a distinct mechanism to achieve the spindle bipolarity (reviewed in Schatten, 1994; Merdes and Cleveland, 1997). The oocyte lacks centrosomes, so spindle microtubules are nucleated around the meiotic chromatin and are then bundled into antiparallel arrays by multimeric plus end-directed motors such as Eg5 (Sharp *et al*., 1999). These microtubule arrays are driven with their minus ends out by plus end-directed microtubule motors, such as chromokinesin (Wang and Adler, 1995), Xklp 1 (Vernos *et al*., 1995), and *Drosophila* Nod (Afshar *et al*., 1995). Minus end-directed motors, such as cytoplasmic dynein or *Drosophila* Ncd then tether the spindle ends into focused poles (Gaglio *et al*., 1996; Matthies *et al*., 1996). Based on the morphological differences between the two spindle structures, microtubule dynamics in meiosis are likely also differentially regulated in accordance to the forces generated by motor proteins.

C. elegans female meiosis takes place in the fertilized zygote. Therefore, the zygote cytoplasm must support the formation of both the meiotic and the first mitotic spindle, which form within 20 min of one another (Kemphues *et al*., 1986). This along with other convenient genetic and molecular tools makes *C. elegans* an ideal system (Brenner, 1974) to study how these different types of spindles form. Previously, we showed that *mei-1* and *mei-2* encode two meiotic spindle-specific components. MEI-1 and MEI-2 are the *C. elegans* homologs of the p60 (catalytic) and p80 (localization) subunits of the sea urchin microtubule-severing complex katanin (Clark-Maguire and Mains, 1994a,b; Hartman *et al*., 1998; Srayko *et al*., 2000). Because MEI-1 and MEI-2, like sea urchin p60 and p80, disassembled interphase microtubules when coexpressed in HeLa cells, we proposed that they specifically regulate spindle microtubule dynamics and/or restrict microtubule length in meiosis, and thus are required for the formation of a bipolar spindle in the absence of centrosomes (Srayko *et al*., 2000). In *mei-1* and *mei-2* loss-of-function (*lf*) mutants, meiotic spindles fail to form properly but subsequent mitotic spindles are not affected (Mains *et al*., 1990a; Clandinin and Mains, 1993; Srayko *et al*., 2000). However, ectopic MEI-1/MEI-2 activity in mitosis, resulting either from the *mei-1(ct46)* gain-of-function (*gf*) mutation, or loss of the *mei-1*/*mei-2* postmeiotic inhibitor *mel-26*, instead disrupt mitotic spindle structure (Mains *et al*., 1990a; Clark-Maguire and Mains, 1994a,b; Dow and Mains, 1998; Kurz *et al*., 2002). This ectopic microtubule-severing activity results in smaller, mispositioned mitotic spindles. Consistent with reduced microtubule length caused by ectopic microtubule-severing activity, *mei-1(ct46gf)* is phenocopied by low doses of the microtubule-destabilizing drug nocodazole (Strome and Wood, 1983; Hyman and White, 1987).

To better understand the role of MEI-1/MEI-2 katanin in spindle formation, we performed a screen for suppressors that rescue the lethality of ectopic katanin activity caused by the *mei-1(ct46gf)* mutation (Clandinin and Mains, 1993). Here, we describe the analysis of an extragenic suppressor,

Article published online ahead of print. Mol. Biol. Cell 10.1091/mbc.E03–06–0418. Article and publication date are available at www.molbiolcell.org/cgi/doi/10.1091/mbc.E03–06–0418.
† Present address: Max-Planck-Institute of Molecular Cell Biology and Genetics, Pfotenhauerstrasse 108, D-01307 Dresden, Germany.
§ Corresponding author. E-mail address: mains@ucalgary.ca.
Abbreviations used: *gf*, gain-of-function; *lf*, loss-of-function.

 © 2003 by The American Society for Cell Biology

sb26, which is a missense allele of the β-tubulin gene *tbb-2*. *tbb-2(sb26)* genetically behaves as if it produces meiotic and mitotic microtubules that are resistant to katanin severing. Immunofluorescence with TBB-2–specific antibodies shows ubiquitous TBB-2 expression in microtubule structures throughout worm development. Furthermore, tubulin isotype-specific RNA interference (RNAi) experiments demonstrate a redundant role for *tbb-2* with the closely related gene *tbb-1* during the early cleavage divisions. Finally, using a sensitized genetic background, we demonstrate that microtubules containing the TBB-2 β-tubulin isotype are preferred for MEI-1/MEI-2 activity.

MATERIALS AND METHODS

Nematode Strains and Culture Conditions

C. elegans was cultured under standard conditions (Brenner, 1974) and brood analysis was done as described by Mains *et al.* (1990b). Hatching rates were scored among 500-2000 embryos. The following genes and alleles were used: *mei-2(ct98)*, *mei-1(ct46)*, *unc-29(e1072)*, *tbb-2(sb26*, *gk129*, *gk130)*, *tbb-1(gk207)*. *tbb-2* deletion alleles *gk129* and *gk130* and *tbb-1* deletion allele *gk207* were provided by the *C. elegans* Gene Knockout Consortium (elegans.bcgsc.bc.ca/knockout.shtml). Both *tbb-2* deletion alleles are predicted molecular nulls, removing some promoter region and most of exon I, including the start ATG without generating any downstream in-frame ATG. Both alleles were outcrossed at least three times by selecting for the healthiest strains that did not stain with anti-TBB-2 antibody. The *tbb-1* allele *gk207* is also a predicted molecular null, removing most of gene's promoter and part of its first exon. The strain was outcrossed six times and genotype was verified by polymerase chain reaction (PCR).

Genetic Mapping and Cloning of tbb-2(sb26)

Three-factor crosses placed *sb26* between two cloned markers, *pat-3* and *mpk-1* on LGIII (data submitted to WormBase, www.wormbase.org), a region covered by 10 overlapping cosmids. The genomic sequence of *tbb-2(sb26)* was PCR amplified from *sb26* homozygous mutants and sequenced from two independent PCR products as described by Srayko *et al.* (2000).

Antisera Production and Immunoblotting

PCR-amplified sequence corresponding to the last 20 amino acids of TBB-2 (residues 431–450) was inserted into the *Bam*HI site of pGEX-3 × (Amersham Pharmacia, Uppsala, Sweden) to create a GST-fusion. Bacterially expressed protein was purified with a glutathione-Sephadex column (Pharmacia) and run on an SDS-PAGE gel. Immunization of rabbits was performed following Srayko *et al.* (2000). Crude sera were affinity purified against a column of a synthetic peptide (Alberta Peptide Institute, Edmonton, AB, Canada) corresponding to the amino acids 432–442 of TBB-2 (see Figure 3A). Western analyses was performed as described by Srayko *et al.* (2000), by using affinity purified TBB-2 antisera at 1/1000 dilution.

Microscopy and Immunofluorescence

Embryos and dissected gonads were freeze-cracked and fixed with methanol-acetone as described by Kemphues *et al.* (1986). Affinity-purified anti-TBB-2 antisera were used at 1/100 dilution for 1 h at 37°C. α-Tubulin localization was determined with either a mouse monoclonal antibody (Piperno and Fuller, 1985) at 1/100 dilution or the mouse DM 1A α-tubulin monoclonal antibody (Sigma-Aldrich, St. Louis, MO) at 1/200 dilution. Secondary antibodies (Jackson ImmunoResearch Laboratories, West Grove, PA) were fluorescein isothiocyanate-conjugated goat anti-rabbit, tetramethylrhodamine B isothiocyanate-conjugated goat anti-mouse, or Texas Red-conjugated goat anti-mouse. DNA was visualized with 4,6-diamidino-2-phenylindole (DAPI) as described in Srayko *et al.* (2000). Photographs were either taken with an Axioplan fluorescence microscope camera (Carl Zeiss, Thornwood, NY) with Techpan film (Eastman Kodak, Rochester, NY) developed at ASA100, or with a Hamamatsu ORCA-ER digital camera and deconvolved using Zeiss AxioVision software.

RNAi

tbb-1 and *tbb-2* cDNA clones yk51f5 and yk84c10, respectively, were obtained from Dr. Yuji Kohara (University of Tokyo, Tokyo, Japan). Primers were designed to amplify the divergent C-terminal coding region and 3′-untranslated regions (UTRs) from each cDNA. Primers used for *tbb-2* were forward: 5′-AAGCTTCACTTCACTCAGAT-3′ and reverse: 5′-GAAGCAACTGCCGAAGACGAC-3′. Primers used for *tbb-1* were forward: 5′-AAGGTACCGCCGAAGACGAGCC-3′ and reverse: 5′-AATACGACTCACTATAG-3′ (T7). PCR fragments were cloned into pBluescript SK(+) (Stratagene, La Jolla, CA). RNA was transcribed using the Megascript system (Ambion, Austin, TX), and DNA templates were digested with DNase I. The resulting RNAs were purified and annealed as described by Fire (ftp.ciwemb.edu/PNF:byName:/FireLabWeb/) and 150 μg/ml was used for injection. The resulting embryos were dissected for fixation 24 h after injection.

RESULTS

tbb-2(sb26) Prevents Lethality Caused by Ectopic MEI-1/MEI-2 Expression

The *mei-1(ct46gf)* mutant shows a dominant, temperature-sensitive maternal-effect lethality due to mitotic spindle defects caused by ectopic MEI-1/MEI-2 severing activity (Mains *et al*., 1990a; Srayko *et al*., 2000). In a previous screen to identify interacting genes (Clandinin and Mains, 1993), we isolated an extragenic *mei-1(ct46gf)* suppressor, *sb26*. As shown below, *sb26* is an allele of the β-tubulin gene *tbb-2*. Homozygous *tbb-2(sb26)* single mutant embryos develop the same as wild type, giving rise to fertile adults with wild-type brood sizes and hatching. However, although *tbb-2(sb26)* has no obvious mitotic phenotypes (Figure 1, A and B), it effectively suppresses (alleviates) the lethality caused by ectopic MEI-1/MEI-2 activity resulting from the *mei-1(ct46gf)* mutation (Table 1, lines 1–5). In *mei-1(ct46gf)* embryos, the first mitotic spindle is smaller than wild-type and is often mispositioned orthogonally to the anterior-posterior axis (Figure 1C). In contrast, when *mei-1(ct46gf)* is combined with the *tbb-2(sb26)* mutation, embryos have wild-type spindle morphology and orientation (Figure 1D). This result suggests that *tbb-2(sb26)* somehow interferes with the ectopic MEI-1/MEI-2 activity associated with the *mei-1(ct46gf)* mutation. To test whether *tbb-2(sb26)* also interferes with normal MEI-1/MEI-2 activity during meiosis, we looked at mutations that cause partial loss of *mei-1*and *mei-2.* On their own, *mei-1* and *mei-2 lf* mutations result in defects in meiotic spindle formation, most likely due to decreased meiotic microtubule severing, and these defects were enhanced when combined with *tbb-2(sb26)* (Table 1, lines 10–11). Whereas the meiotic defect of a weak *mei-2(lf)* allele, *ct98*, results in 86% hatching at the nonpermissive temperature of 25°, lethality increased dramatically when combined with *tbb-2(sb26)*, resulting in a 3.7% hatching under the same conditions. In *C. elegans*, nondisjunction of the X chromosome results in a normal XO male, and thus the frequency of males among the survivors is a measure of meiotic failure. Normally, ~1/500 zygotes from a selfed hermaphrodite is a male (Hodgkin and Brenner, 1977), but among the *mei-2(ct98); tbb-2(sb26)* escapers at 25°, 17% were male (Table 1, line 11), likely indicating a failure in meiotic spindle function. Therefore, in either wild-type meiosis or abnormal mitosis as a result of ectopic MEI-1/MEI-2 expression, we find that *tbb-2(+)* is required for *mei-1* and *mei-2* function, suggesting that *tbb-2(sb26)* lowers the overall sensitivity of microtubules to MEI-1/MEI-2 activity.

The sb26 Mutation Alters Rather than Eliminates Gene Function

Several lines of evidence indicate that the *tbb-2* allele *sb26* represents an altered rather than a loss of gene function. A chromosomal deficiency that removes the *tbb-2* locus, *sDf130*, failed to dominantly suppress the maternal-effect lethality of *mei-1(ct46gf)*, although *tbb-2(sb26)/+* did so (Table 2, lines 1–3). We also acquired two *tbb-2* deletion alleles, both predicted to be molecular nulls, from the *C. elegans* Gene Knockout Consortium (see MATERIALS AND METHODS). These produced no detectable protein on immunoblots with TBB-2–specific antibodies (see below). In contrast to *tbb-2(sb26)*, heterozygosity for a *tbb-2* null allele decreased

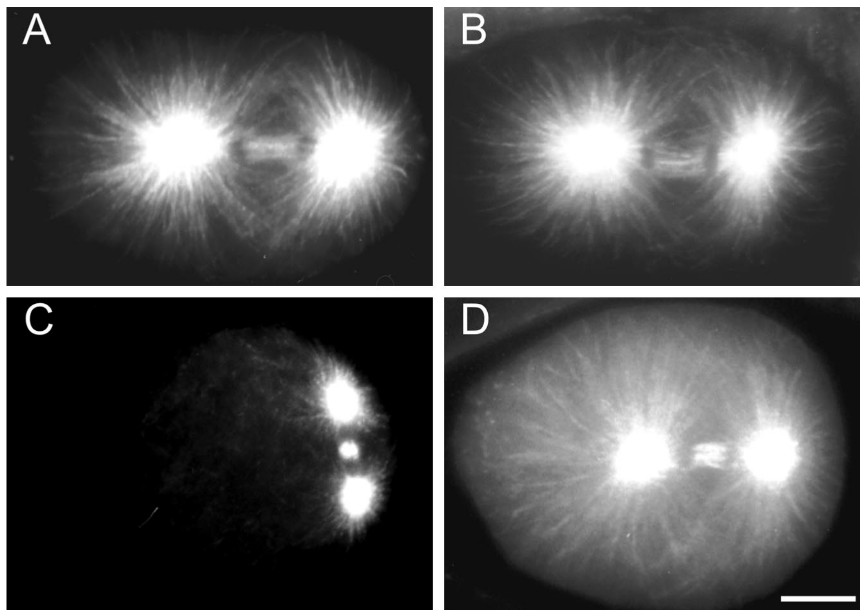

Figure 1. sb26 prevents the mitotic spindle defect caused by ectopic MEI-1/MEI-2 expression. Shown are indirect immunofluorescence images of anti-α-tubulin staining of the first mitotic spindles of wild-type (A), *sb26* (B), *mei-1(ct46)*, which causes ectopic MEI-1/MEI-2 expression (C) and *mei-1(ct46gf); tbb-2(sb26)* double mutants (D). Embryos here and in all other figures are shown with anterior to the left. Bar, 10 μm.

hatching when ectopic MEI-1/MEI-2 was present in mitosis (Table 2, line 4). Likewise, when the wild-type *tbb-2* allele in *mei-1(ct46gf)/+; tbb-2(sb26)/+* was removed and replaced with the molecular null *gk129*, suppression of the *mei-1(ct46gf)* embryonic lethality improved, although not to the extent of the complete suppression seen with *sb26/sb26* (Table 2, compare lines 2, 5, and 6). This indicates that the wild-type *tbb-2* allele interferes with the suppression by the *sb26* allele. Finally, *mei-1(ct46)* lethality caused by the ectopic MEI-1/MEI-2 expression was rescued by transformation with *tbb-2(sb26)* but not with *tbb-2(+)* (see below). Together, these genetic studies clearly indicate that *tbb-2(sb26)* is a *gf* suppressor of *mei-1(ct46gf)*.

Other *tbb-2* alleles that behave as gain-of-function mutations with mitotic spindle defects similar to *mei-1(gf)* are described by other groups (Ellis *et al*., 2004; Wright and Hunter, 2003). Neither *tbb-2(t1623)* nor *tbb-2(or362)*, two missense *tbb-2* alleles of such kind, suppresses *mei-1(ct46gf)*, but rather acted as dominant enhancers (Table 1, lines 6–9). This likely results from the fact that these *tbb-2* alleles and *mei-1(ct46gf)* both destabilize mitotic microtubules. Thus, *tbb-2* interacts with *mei-1* and *mei-2* in an allele-specific manner.

tbb-2(sb26) Does Not Prevent Ectopic MEI-1/MEI-2 Localization

MEI-1 and MEI-2 localize exclusively to wild-type female meiotic spindles; both proteins are found throughout the spindle but are more concentrated at the poles and on chromatin (Clark-Maguire and Mains, 1994a; Srayko *et al*., 2000). In mutants resulting in ectopic MEI-1 and MEI-2 expression, both MEI-1 and MEI-2 localize to the analogous regions during mitosis (Figure 2, A and C), disrupting mitotic spindle function (Clark-Maguire and Mains, 1994a; Srayko *et al*., 2000). We asked whether the suppression of mitotic spindle defects by *tbb-2(sb26)* was due to absent (or reduced) MEI-1 and/or MEI-2 in the mitotic apparatus. Immunolocalization using anti-MEI-1 or MEI-2 showed that the gene products still persisted in the double mutants (Figure 2, B and D).

Table 1. Interaction of *tbb-2* with *mei-1* and *mei-2* alleles

	% hatching (% male[a])		
Maternal genotype	15°	20°	25°
mei-1(ct46gf)[b]	23 (0)	1.5	0
tbb-2(sb26)	100 (0)	—[c]	97 (0.1)
mei-1(ct46gf); tbb-2(sb26)	96 (0.1)	92 (0.9)	67 (1.4)
mei-1(ct46gf)/+	—	38	1.2
mei-1(ct46gf)/+; tbb-2(sb26)/+	—	84	37
tbb-2(t1623)/+	—	96	74
mei-1(ct46gf)/+; tbb-2(t1623)/+	—	16	0.6
tbb-2(or362)/+	—	98	95
mei-1(ct46gf)/+; tbb-2(or362)/+	—	25	0
mei-2(ct98)[d]	96 (0)	97 (0.2)	74 (2.6)
mei-2(ct98); tbb-2(sb26)	89 (0.4)	64 (4.7)	3.7 (17)

[a] Percentage of males among hatched embryos that survived to late larval stages, which is a measure of meiotic failure.

[b] *mei-1(ct46gf)* is a semidominant temperature-sensitive maternal-effect lethal mutation that results in relatively normal meiotic MEI-1 function, but ectopic activity persists into mitosis.

[c] Not determined.

[d] *mei-2(ct98)* decreases but does not eliminate *mei-2* activity.

Table 2. Suppression of *mei-1(ct46gf)* by *tbb-2(sb26)* results from a gain-of-function mutation

Maternal genotype	% hatching (20°)
mei-1(ct46gf)/+	39
mei-1(ct46gf)/+; tbb-2(sb26)/+	75
mei-1(ct46gf)/+; sDf130/+	7.6[a]
mei-1(ct46gf)/+; tbb-2(gk129)[b]*/+*	12
mei-1(ct46gf)/+; tbb-2(sb26)	99
mei-1(ct46gf)/+; tbb-2(sb26)/tbb-2(gk129)	89

[a] Corrected for the 43% zygotic lethality resulting from the deficiency.

[b] *tbb-2(gk129)* is a predicted molecular null allele. Another null allele, *gk130*, behaved similarly.

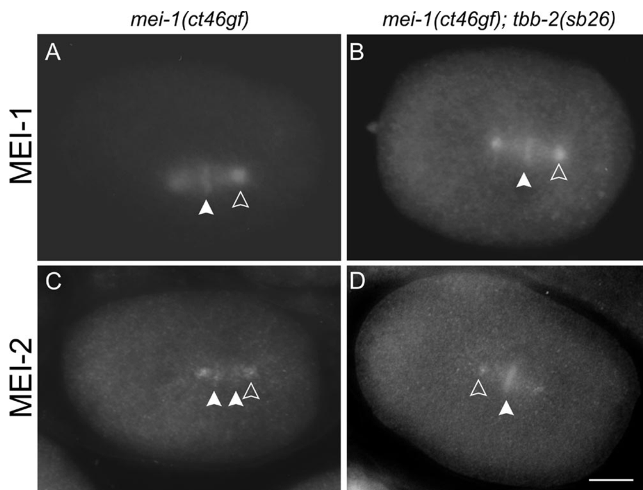

Figure 2. Mislocalization of MEI-1 and MEI-2 to mitotic centrosomes and chromosomes in *mei-1(ct46); tbb-2(sb26)* double mutant embryos. Indirect immunofluorescence images of anti-MEI-1 staining in *mei-1(ct46)* shows ectopic MEI-1 expression (A) and *mei-1(ct46); tbb-2(sb26)*, which continues to show ectopic MEI-1 staining. Similar results were seen with anti-MEI-2 staining in *mei-1(ct46)* (C) and *mei-1(ct46); tbb-2(sb26)* (D). Both MEI-1 and MEI-2 localize to mitotic spindle microtubules, centrosomes (open arrowheads), and chromosomes (white arrowheads). The photograph in D was digitally deconvolved. Bar, 10 μm.

Therefore, *sb26* suppresses *mei-1(ct46gf)* despite the presence of ectopic MEI-1/MEI-2 protein. This suggests that *sb26* interferes with MEI-1/MEI-2 function via a mechanism other than blocking localization or causing degradation of the complex and instead likely acts by inhibiting microtubule severing (see DISCUSSION).

sb26 Has a Missense Mutation in the β-Tubulin Gene tbb-2

sb26 was genetically mapped (see MATERIALS AND METHODS) to a region containing the β-tubulin gene *tbb-2* (C36E8.5) (Gremke, 1986). Sequencing of *tbb-2* in *sb26* mutants revealed a single G-to-A transition at the 3′ end of the coding region, resulting in a Glu to a Lys change (Figure 3A). Reverse transcription-PCR showed that a SL1 leader sequence (Krause and Hirsh, 1987) was *trans*-spliced onto the 5′ end of the *tbb-2* transcript; sequencing confirmed the predicted gene structure in WormBase (www.wormbase-.org). To eliminate the possibility that the mutation we found in *tbb-2* was an unrelated ethylmethansulfonate-induced lesion very tightly linked to *sb26*, we transformed worms with *tbb-2(+)* and *tbb-2(sb26)* genomic constructs. One transgenic line of *tbb-2(+)* and three independent lines of *tbb-2(sb26)* were obtained. Wild-type worms carrying extrachromosomal arrays of either *tbb-2(+)* (*sbEx156)* or the mutant *tbb-2(sb26)* (e.g., *sbEx145*) showed essentially wild-type hatching rates (Table 3, lines 2 and 3), although both showed an incompletely penetrant protruding vulva phenotype. As expected from the genetic nature of *tbb-2(sb26)*, only transgenic arrays made from *sb26* mutant genomic DNA rescued the embryonic lethality of *mei-1(ct46gf)*, with hatching rates increasing nearly 10-fold for *tbb-2(sb26)* compared with the control at 20° (Table 3, lines 1, 4, and 5). In addition, two independent TBB-2::GFP expressing lines failed to rescue *mei-1(ct46gf)* (our unpublished data). These results confirm that *sb26* alters rather than eliminates gene function and that the *tbb-2* mutation corresponds to *sb26*.

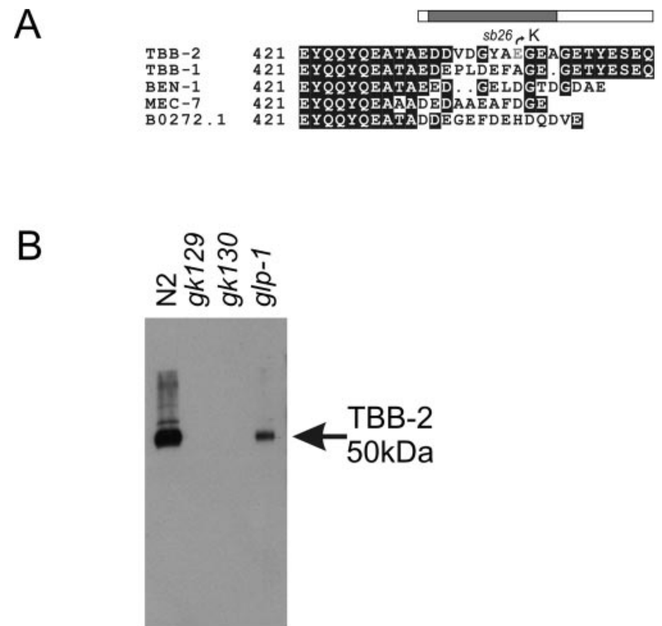

Figure 3. TBB-2 specific antisera production. (A) Alignment of the C termini of TBB-2 and four other *C. elegans* β-tubulins. Black shading indicates identity to TBB-2. The box above shows the region used to generate antisera, whereas the shaded region of that box indicates the peptide used for antisera affinity purification. The *sb26* mutation is indicated. (B) Immunoblots of wild-type, *tbb-2* deletion alleles and germline absent (*glp-1)* worms with affinity purified anti-TBB-2 antibodies, demonstrating antibody specificity. Twenty adult hermaphrodites of each genotype were lysed and loaded per lane. Lane 1, wild type; lanes 2 and 3: *tbb-2* deletion alleles *gk129* and *gk130*, respectively; lane 4, *glp-1*. The arrow indicates the TBB-2 band.

TBB-2 Is Widely Expressed throughout the Life Cycle

Because *tbb-2(sb26)* suppresses ectopic *mei-1* activity during early embryogenesis, we expected *tbb-2* to be a component of the meiotic and early mitotic spindles, where it perhaps facilitates MEI-1/MEI-2 microtubule severing. To explore the expression pattern of TBB-2, we generated specific polyclonal antisera directed to a nonconserved region of 11 amino acids in the C terminus (Figure 3A; see MATERIALS AND METHODS). Affinity-purified antisera recognized a single band at the expected size of 50 kDa on Western blots, and this band disappeared when blotting either of the two *tbb-2* deletion strains (Figure 3B, lanes 1–3), demonstrating antisera specificity.

We predicted that TBB-2 is maternally provided and should be present during early embryogenesis. Female go-

Table 3. Suppression of *mei-1(ct46gf)* by *tbb-2* extrachromosomal transgenic arrays

	% hatching	
Maternal genotype	15°	20°
mei-1(ct46gf)	16	1.3
+; *sbEx156*[*tbb-2(+)*]	92	98
+; *sbEx145*[*tbb-2(sb26)*]	100	99
mei-1(ct46gf); sbEx156[*tbb-2(+)*]	16	2.2
mei-1(ct46gf); sbEx145[*tbb-2(sb26)*]	29	11

nads were dissected from gravid hermaphrodites, fixed, and stained with TBB-2–specific antisera and costained with a generic monoclonal anti-α-tubulin antibody to visualize microtubule structures. TBB-2 was expressed throughout the female gonad, from the distal syncytial arm to maturing oocytes (Figure 4, A–C). We next examined TBB-2 expression in meiotic and early mitotic spindles, the developmental stage at which the genetic interactions take place. Figure 4, D–F, shows TBB-2 localization in a meiosis metaphase I spindle in a pattern identical to that of the α-tubulin staining. Digital deconvolution microscopy was used to examine in detail TBB-2 expression in mitotic spindles. As shown in Figure 4, G–I, TBB-2 was expressed in every subset of spindle microtubules recognized by the generic anti-α-tubulin antibody, including spindle midzone and astral microtubules.

TBB-2 expression persisted in mitotic spindles and asters throughout embryogenesis (our unpublished data), beyond the end of the temperature-sensitive period of *mei-1(ct46gf)* (Mains *et al.*, 1990a). Indeed, TBB-2 expression was not germline and embryo specific, because the protein was also expressed (albeit at lower levels) in a *glp-1* mutant, which lack germline and fertilized embryos (Figure 3B, lane 4). Finally, TBB-2 was detected postembryonically in larval and adult neuronal tissues (Figure 4K). TBB-2 is therefore widely expressed throughout worm development. TBB-2(*sb26*) showed the same expression pattern as wild-type TBB-2.

tbb-2 and tbb-1 Are Partially Redundant during Early Development

Because *sb26* results in an altered rather than a loss of gene activity, we used RNAi (Fire *et al.*, 1998) to deplete its expression during embryonic development to better determine the gene's normal function. However, TBB-2 and the other β-tubulin that is expressed at high levels in the germline, TBB-1, share >85% DNA sequence identity within their coding regions. Therefore, full-length RNAi to either gene results in simultaneous inhibition of both gene's function (our unpublished data). Because sequence similarities among the 3′ coding and 3′-UTRs of these worm β-tubulins are <50%, we examined the effect of silencing *tbb-2* by using dsRNA targeted to the last 210 base pairs of the transcript. This reduced TBB-2 expression to nearly undetectable levels by immunofluorescence (Figure 5F). However, 86% of *tbb-2*(RNAi) embryos hatched and grew to fertile adults (Table 4, line 1), similar to the level of lethality seen with either of the *tbb-2* deletion strains (Table 4, line 2). These data suggest that other β-tubulins can compensate for loss of *tbb-2*.

Because *tbb-1* is the only other β-tubulin expressed in early embryos at a significant level (Reinke *et al.*, 2000; Baugh *et al.*, 2003), we used RNAi to determine whether *tbb-1* functions redundantly with *tbb-2*. Like *tbb-2*(RNAi), injection of dsRNA directed to the divergent 3′ coding and 3′-UTR of *tbb-1* showed little embryonic lethality (Table 4, line 3), also similar to the level of lethality associated with a *tbb-1* deletion strain, *gk207* (Table 4, line 4). However, RNAi to both *tbb-1* and *tbb-2* resulted in 100% embryonic lethality and completely abolished spindle formation, and all embryos arrested without division (Table 4, line 5; and Figure 5, G–I). Oocyte meiosis also failed because no polar bodies were seen. Similar results were obtained by doing *tbb-1*(RNAi) in animals carrying a *tbb-2* deletion (Table 4, line 6). Therefore, *tbb-2* and *tbb-1* function redundantly during embryonic development.

TBB-1– and TBB-2–containing Microtubules Interact Differently with MEI-1/MEI-2

During meiosis, microtubules fully resistant to MEI-1/MEI-2 katanin activity should result in meiotic defects similar to the complete loss of either *mei-1* or *mei-2*. However, *tbb-2(sb26)* alone does not have any meiotic defects. It is possible that the MEI-1/MEI-2–interfering property of TBB-2(*sb26)* is simply masked by the presence of the functionally redundant TBB-1(+). Alternatively, TBB-2(*sb26)* may also permit some MEI-1/MEI-2 activity even in the absence of TBB-1(+). If the former is true, *mei-1* and *mei-2 lf* phenotypes might be expected when TBB-1 is depleted by RNAi in the *tbb-2(sb26)* mutant. However, this only slightly increased the embryonic lethality (Table 4, line 7). Because no other β-tubulins are expressed at significant levels at this stage (Reinke *et al.*, 2000; Baugh *et al.*, 2003), microtubules containing TBB-2(*sb26)* as the only β-tubulin component are still partially sensitive to katanin activity, thus favoring the latter model described above. However, the high survival rate associated with removal of TBB-2 by RNAi or the *tbb-2* null allele demonstrates that microtubules containing TBB-1 as the only β-tubulin isotype are also sensitive to MEI-1/MEI-2 (Table 4, lines 1–2).

In the absence of the *sb26* mutation, it seems that microtubules are effective katanin substrates whether they contain only TBB-1 or only TBB-2 as the β-tubulin component. However, because *mei-1* activity is present in excess during meiosis (Clandinin and Mains, 1993), microtubules containing either of the two β-tubulin isotypes could nonetheless differ in terms of their effectiveness of being MEI-1/MEI-2 katanin substrates. We took advantage of the partial *lf mei-2* allele *ct98*, because in this sensitized background, differences in the genetic interactions between the different tubulin isotypes and katanin can be monitored more easily. As shown in Table 4 (lines 8–10), removal of TBB-2 by either a null mutation or RNAi in *mei-2(ct98)* animals substantially decreased hatching. In contrast, removal of TBB-1 by RNAi or the null mutation had very little effect (Table 4, lines 11–12). Therefore, MEI-1/MEI-2 interacts with microtubules more efficiently when TBB-2 is present, revealing a functional difference between the two β-tubulin isotypes. It is unlikely that this effect stems from TBB-2 being the more prevalent embryonic isotype because both *tbb-1* and *tbb-2* are expressed at similar levels at this stage (Reinke *et al.*, 2000; Baugh *et al.*, 2003), and Ellis *et al.* (2004) showed that TBB-1 expression is up-regulated in *tbb-2* null mutants. Therefore, although TBB-1 and TBB-2 isotypes can each support embryogenesis on their own, these data demonstrate that MEI-1/MEI-2 katanin interacts with the two tubulins differently in vivo.

DISCUSSION

mei-1 and *mei-2* encode subunits of the *C. elegans* homolog of microtubule-severing protein complex katanin (Srayko *et al.*, 2000). Although MEI-1 and MEI-2 had been shown to disassemble interphase microtubules when coexpressed in HeLa cells, interactions of *tbb-2(sb26)* with *mei-1* and *mei-2* alleles provide the first genetic evidence that MEI-1 and MEI-2 indeed directly regulate meiotic spindle microtubule function, likely their length or dynamics, in *C. elegans* embryos. TBB-2 does not represent a tubulin specialized only for interactions with *mei-1* and *mei-2* because it is widely expressed throughout development, but TBB-2 does represent the β-tubulin that results in microtubules that are most susceptible to MEI-1/MEI-2 activity.

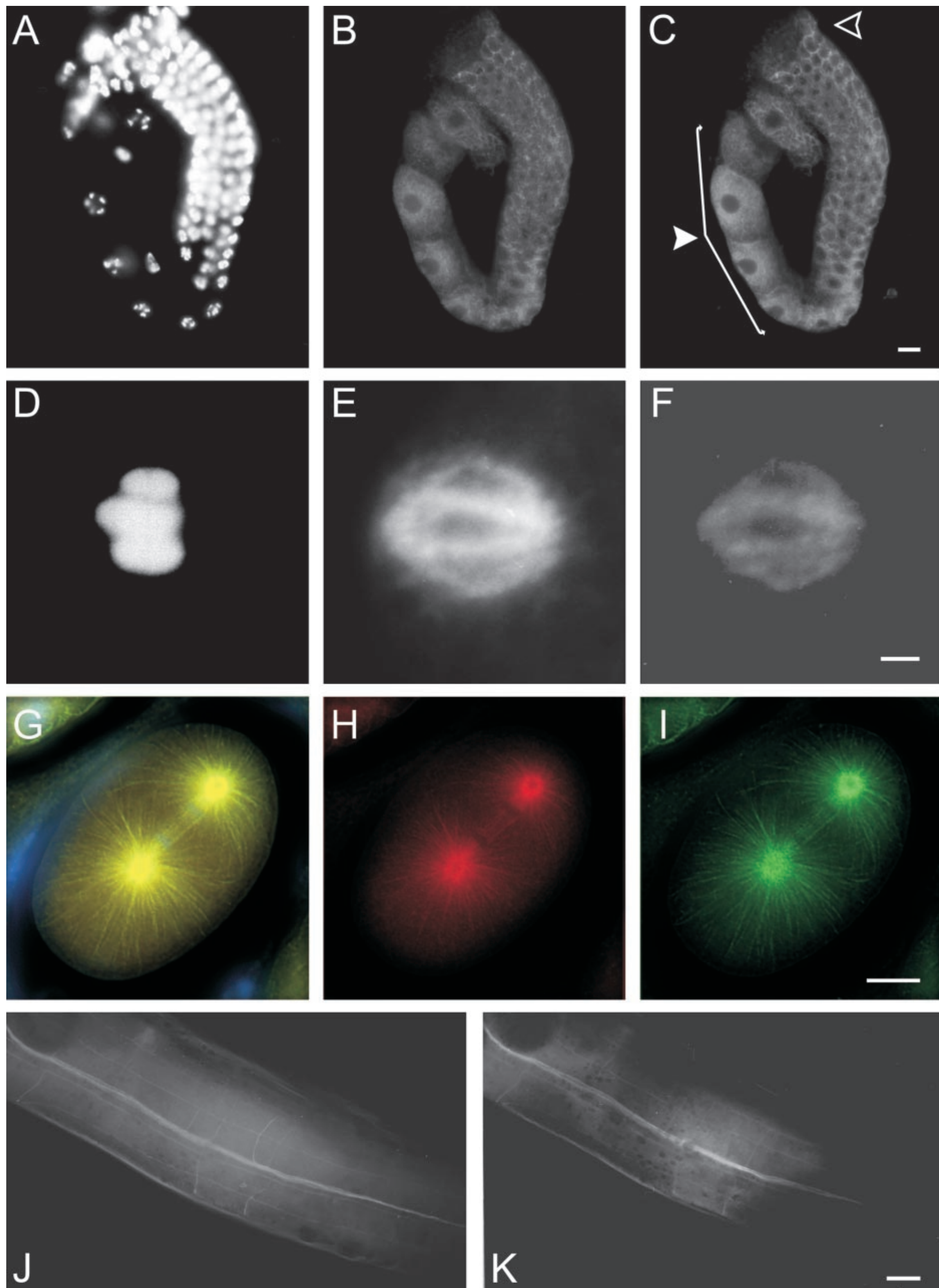

Figure 4. TBB-2 expression pattern. Dissected gonads and embryos were visualized by indirect immunofluorescence after staining DNA with DAPI (A and D), anti-α-tubulin (B, E, and H) and anti-TBB-2 (C, F, and I). TBB-2 was strongly expressed in the germline, starting from distal syncytial gonad arms (open arrowhead in C) and was also seen in maturing oocytes (white arrowhead) and the meiotic spindle (F). TBB-2 is present in all mitotic spindle microtubules recognized by the α-tubulin in the first cleavage spindle, as demonstrated by the digitally deconvolved merged image (G; blue: DAPI). Postembryonic TBB-2 expression was examined by anti-TBB-2 staining (K) and compared with anti-α-tubulin staining (J). TBB-2 is strongly expressed in adult neurons (K). Bars, 10 μm (C, I and K); 2 μm (F).

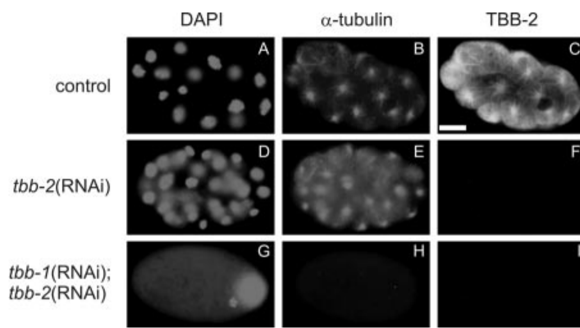

Figure 5. *tbb-1* and *tbb-2* are redundant during embryogenesis. Hermaphrodites were injected with gene-specific double-stranded RNA and were stained with DAPI (A, D, and G), anti-α-tubulin (B, E, and H) and anti-TBB-2 (C, F, and I) and visualized by indirect immunofluorescence. TBB-2 expression was reduced to near undetectable levels with *tbb-2*(RNAi) (F). However, microtubules as visualized with anti-α-tubulin staining were unaffected (E) compared with uninjected control (B). In embryos simultaneously treated with *tbb-1* and *tbb-2* dsRNA (G–I), no microtubules were detected by anti-α-tubulin antibody (H). Embryos arrested as one cell, with massive amounts of DNA (G). Bar, 10 μm.

Our genetic evidence shows that the *gf* mutation *tbb-2(sb26)* inhibits MEI-1/MEI-2 katanin's activity on microtubules. This was demonstrated by the ability of *tbb-2(sb26)* to suppress mutations that cause ectopic mitotic MEI-1/MEI-2 expression and its enhancement of mutations that limit meiotic MEI-1/MEI-2 activity. However, *sb26*-containing microtubules are clearly not fully resistant to MEI-1/MEI-2 activity; otherwise *tbb-2(sb26)*, which is wild type on its own, would be lethal as seen for null alleles of *mei-1* or *mei-2*.

MEI-1 and MEI-2 still ectopically localize to mitotic spindles in *mei-1(ct46gf); tbb-2(sb26)* double mutants (Figure 2). These data, together with the known biochemical activity of MEI-1/MEI-2 katanin and the similarity of the *mei-1(ct46gf)* phenotype to that of embryos treated with microtubule-depolymerizing drugs (Strome and Wood, 1983; Hyman and White, 1987), strongly suggests that the *sb26* tubulin mutation reduces the sensitivity of microtubules to the severing activity of katanin without interfering with other intrinsic properties of the MEI-1/MEI-2 complex, such as its intracellular localization. However, taxol-stabilized microtubules from *sb26* and wild-type worms were at least qualitatively equally susceptible to human katanin severing in vitro (McNally, personal communication). This result was not surprising because completely resistant microtubules would be lethal, similar to *mei-1* or *mei-2* null mutations, and the in vitro assay may not be sensitive enough to detect subtle differences. An alternative way that *tbb-2(sb26)* could suppress ectopic MEI-1/MEI-2 severing is that it could result in intrinsically more stable microtubules. According to this model, *sb26* microtubules might depolymerize more slowly in a cold environment (Hannak *et al*., 2002). However, wild-type and *sb26* embryos showed similar length and organization of their mitotic spindles after different time periods of cold-induced depolymerization (our unpublished data). Furthermore, *tbb-2(sb26)* does not genetically interact with the *lf zyg-9* allele *b244* (our unpublished data), a mutation that results in a *mei-1(ct46gf)*-like mitotic phenotype, albeit due to loss of a microtubule-stabilizing protein (Kemphues *et al*., 1986; Matthews *et al*., 1998). This again suggests that *sb26* specifically inhibits activity of the MEI-1/MEI-2 katanin complex rather than altering general microtubule dynamics.

Table 4. TBB-1 and TBB-2 are redundant for embryonic development but are not equally preferred for katanin activity

Maternal genotype	% hatching at 25°
*tbb-2(*RNAi*)*	86[a]
tbb-2(gk129)[b]	75
*tbb-1(*RNAi*)*	92[a]
tbb-1(gk207)[c]	88
*tbb-2(*RNAi*); tbb-1(*RNAi*)*	0[a]
*tbb-2(gk129); tbb-1(*RNAi*)*	0.5
*tbb-2(sb26); tbb-1(*RNAi*)*	85
mei-2(ct98)[d]	74
*mei-2(ct98); tbb-2(*RNAi*)*	1.7
mei-2(ct98); tbb-2(gk129)	5.8
*mei-2(ct98); tbb-1(*RNAi*)*	71
mei-2(ct98); tbb-1(gk207)	60

[a] Percentage of hatching at 20°.
[b] *gk129* is a predicted molecular null allele of *tbb-2.*
[c] *gk207* is a predicted molecular null allele of *tbb-1.*
[d] *mei-2(ct98)* decreases but does not eliminate *mei-2* activity.

The *sb26* lesion is at the extreme C terminus of the β-tubulin protein, a region that is not essential for in vitro polymerization of microtubules (Lu and Ludueña, 1994). Interestingly, the small C-terminal fragments of both α- and β-tubulins are required for microtubule severing by katanin (McNally and Vale, 1993): when the C-terminal regions of the tubulins were removed by subtilisin digestion, katanin was able to bind the microtubules and hydrolyze ATP but could not disassemble the microtubules. This suggests that *sb26* specifically disrupts a site required for katanin-mediated microtubule disassembly.

C. elegans has six β-tubulins. Among them, *mec-7* is expressed exclusively in six touch neurons and two other neurons and is required only for touch sensitivity (Savage *et al*., 1989, 1994; Hamelin *et al*., 1992). Null alleles of the β-tubulin *ben-1*, which is also nonessential for development, confer resistance to the microtubule-depolymerizing drug benzimidazole (Driscoll *et al*., 1989). Among all the β-tubulins, microarray data indicates that only *tbb-1* and *tbb-2* mRNAs are expressed at a significant (and also at an equivalent) level in embryos (Reinke *et al*., 2000; Baugh *et al*., 2003). *tbb-2*–specific RNAi using the 3′ region resulted in only slight lethality (Table 4), consistent with the phenotype of *tbb-2* deletion alleles. Similarly, depleting TBB-1 by either RNAi or a deletion allele also results in slight lethality (Table 4). However, simultaneous RNAi to both *tbb-2* and *tbb-1* resulted in 100% lethality and eliminated all microtubule structures (Figure 5), indicating that these tubulin isotypes function redundantly during embryonic development. Similar findings on developmental redundancies of α- and β-tubulins are also reported by Phillips *et al.* (personal communication) and Wright and Hunter (2003).

Even though katanin is involved in different microtubule-mediated developmental processes in a variety of species (Ahmad *et al*., 1999; Lohret *et al*., 1999; Webb *et al*., 2002; Bouquin *et al*., 2003), little is known about whether katanin prefers specific types of microtubules. Interestingly, although both *tbb-1* and *tbb-2* suffice for early *C. elegans* development and viability, MEI-1/MEI-2 katanin complex seems to prefer TBB-2 over TBB-1 for its activity (Table 4). The meiotic defects resulting from the weak *lf mei-2(ct98)* were enhanced by depleting *tbb-2(+)* by either RNAi or a deletion allele (Table 4). However, removal of the other β-tubulin

isotype, TBB-1, by RNAi or the null mutation had no effect. Therefore, MEI-1/MEI-2 katanin severs spindle microtubules more efficiently when the TBB-2 β-tubulin isotype is present. Because the *tbb-2* deletion allele alone does not give complete embryonic lethality, MEI-1/MEI-2 likely also uses other tubulin sites, β or α, in the absence of TBB-2, albeit less efficiently. The relatively high viability of animals lacking *tbb-2* likely reflects the excess *mei-1(+)* activity normally present during meiosis (Clandinin and Mains, 1993), which can compensate for the decreased sensitivity of microtubules when TBB-2 is removed by mutations or RNAi.

Eukaryotic cells often express multiple α- and β-tubulin isotypes simultaneously, and these may also differ in their posttranslational modifications (Ludueña, 1998). Although different tubulin isotypes seem to be largely redundant, evidences for functional differences among tubulin isotypes do exist. For example, the budding and fission yeasts both express a pair of α-tubulin genes that are interchangeable for viability but the budding yeast isotypes differ in their effects on in vitro microtubule dynamics (Bode *et al*., 2003). Likewise, different mammalian β-tubulin isoforms have varying effects on in vitro microtubule dynamics and drug sensitivities (Panda *et al*., 1994; Derry *et al*., 1997). Our results demonstrate that different tubulin isotypes play different roles during katanin severing. Although at a superficial level tubulin isotypes may seem to be completely interchangeable, closer examination reveals that they indeed have specializations that may be important in nature.

ACKNOWLEDGMENTS

We thank B. Bowerman, C. Hunter, G. Ellis, J. Phillips, and A. Wright for communicating unpublished results; T. Clandinin and H. Fu for early work on this project; W. Brook, J. McGhee, F. McNally, T. Fukushige, and the Mains laboratory for discussion and technical advice. We thank the *C. elegans* gene Knockout Consortium for *tbb-2* and *tbb-1* deletion strains, Y. Kohara for cDNA clones and T. Stiernagle at the *Caenorhabditis elegans* Genetics Center (funded by the National Institutes of Health Center for Research Resources) for providing several strains used in this study. This work was supported by grants from the Canadian Institute of Health Research and the Alberta Heritage Foundation for Medical Research to P.E.M. and a Canadian Institute of Health Research graduate student voucher to C.L.

REFERENCES

Afshar, K., Scholey, J., and Hawley, R.S. (1995). Identification of the chromosome localization domain of the *Drosophila* nod kinesin-like protein. J. Cell Biol. *131*, 833–843.

Ahmad, F.J., Yu, W., McNally, F.J., and Baas, P.W. (1999). An essential role for katanin in severing microtubules in the neuron. J. Cell Biol. *145*, 305–315.

Baugh, L.R., Hill, A.A., Slonim, D.K., Brown, E.L., and Hunter, C.P. (2003). Composition and dynamics of the *Caenorhabditis elegans* early embryonic transcriptome. Development *130*, 889–900.

Bode, C.J., Gupta, M.L., Suprenant, K.A., and Himes, R.H. (2003). The two alpha-tubulin isotypes in budding yeast have opposing effects on microtubule dynamics in vitro. EMBO Rep. *4*, 94–99.

Bouquin, T., Mattsson, O., Naested, H., Foster, R., and Mundy, J. (2003). The *Arabidopsis lue1* mutant defines a katanin p60 ortholog involved in hormonal control of microtubule orientation during cell growth. J. Cell Sci. *116*, 791–801.

Brenner, S. (1974). The genetics of *Caenorhabditis elegans.* Genetics *77*, 71–94.

Clandinin, T.R., and Mains, P.E. (1993). Genetic studies of *mei-1* gene activity during the transition from meiosis to mitosis in *Caenorhabditis elegans*. Genetics *134*, 199–210.

Clark-Maguire, S., and Mains, P.E. (1994a). Localization of the *mei-1* gene product of *Caenorhabditis elegans*, a meiotic-specific spindle component. J. Cell Biol. *126*, 199–209.

Clark-Maguire, S., and Mains, P.E. (1994b). mei-1, a gene required for meiotic spindle formation in *Caenorhabditis elegans*, is a member of a family of ATPases. Genetics *136*, 533–546.

Derry, W.B., Wilson, L., Khan, I.A., Ludueña, R.F., and Jordan, M.A. (1997). Taxol differentially modulates the dynamics of microtubules assembled from unfractionated and purified beta-tubulin isotypes. Biochemistry *36*, 3554–3562.

Dow, M.R., and Mains, P.E. (1998). Genetic and molecular characterization of the *Caenorhabditis elegans* gene, mel-26, a postmeiotic negative regulator of mei-1, a meiotic-specific spindle component. Genetics *150*, 119–128.

Driscoll, M., Dean, E., Reilly, E., Bergholz, E., and Chalfie, M. (1989). Genetic and molecular analysis of a *Caenorhabditis elegans* beta-tubulin that conveys benzimidazole sensitivity. J. Cell Biol. *109*, 2993–3003.

Ellis, G.C., Phillips, J.A., O'Rourke, S., Lyczak, R., and Bowerman, B. (2004). Maternally expressed and partially redundant β-tubulins in *Caenorhabditis elegans* exhibit autoregulation. J. Cell Sci. (*in press*).

Fire, A., Xu, S., Montgomery, M.K., Kostas, S.A., Driver, S.E., and Mello, C.C. (1998). Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans.* Nature *391*, 806–811.

Gaglio, T., Saredi, A., Bingham, J.B., Hasbani, M.J., Gill, S.R., Schroer, T.A., and Compton, D.A. (1996). Opposing motor activities are required for the organization of the mammalian mitotic spindle pole. J. Cell Biol. *135*, 399–414.

Gremke, L. (1986). Cloning and Molecular Characterization of the Tubulin Genes of *Caenorhabditis elegans*: Nucleotide Sequence Analysis of a Tubulin Gene. Ph.D. Thesis. Evanston, IL: Northwestern University.

Hamelin, M., Scott, I.M., Way, J.C., and Culotti, J.G. (1992). The *mec-7* beta-tubulin gene of *Caenorhabditis elegans* is expressed primarily in the touch receptor neurons. EMBO J. *11*, 2885–2893.

Hannak, E., Oegema, K., Kirkham, M., Gonczy, P., Habermann, B., and Hyman, A.A. (2002). The kinetically dominant assembly pathway for centrosomal asters in *Caenorhabditis elegans* is gamma-tubulin dependent. J. Cell Biol. *157*, 591–602.

Hartman, J.J., Mahr, J., McNally, K., Okawa, K., Iwamatsu, A., Thomas, S., Cheesman, S., Heuser, J., Vale, R.D., and McNally, F.J. (1998). Katanin, a microtubule-severing protein, is a novel AAA ATPase that targets to the centrosome using a WD40-containing subunit. Cell *93*, 277–287.

Hodgkin, J.A., and Brenner, S. (1977). Mutations causing transformation of sexual phenotype mutations causing transformation of sexual phenotype in the nematode *Caenorhabditis elegans.* Genetics *86*, 275–287.

Hyman, A.A., and White, J.G. (1987). Determination of cell division axes in the early embryogenesis of *Caenorhabditis elegans.* J. Cell Biol. *105*, 2123–2135.

Kemphues, K.J., Wolf, N., Wood, W.B., and Hirsh, D. (1986). Two loci required for cytoplasmic organization in early embryos of *Caenorhabditis elegans.* Dev. Biol. *113*, 449–460.

Krause, M., and Hirsh, D. (1987). A trans-spliced leader sequence on actin mRNA in *C. elegans.* Cell *49*, 753–761.

Kurz, T., Pintard, L., Willis, J.H., Hamill, D.R., Gonczy, P., Peter, M., and Bowerman, B. (2002). Cytoskeletal regulation by the Nedd8 ubiquitin-like protein modification pathway. Science *295*, 1294–1298.

Lohret, T.A., Zhao, L., and Quarmby, L.M. (1999). Cloning of *Chlamydomonas* p60 katanin and localization to the site of outer doublet severing during deflagellation. Cell Motil. Cytoskeleton *43*, 221–231.

Lu, Q., and Ludueña, R.F. (1994). In vitro analysis of microtubule assembly of isotypically pure tubulin dimers. Intrinsic differences in the assembly properties of alpha beta II, alpha beta III, and alpha beta IV tubulin dimers in the absence of microtubule-associated proteins. J. Biol. Chem. *269*, 2041–2047.

Ludueña, R.F. (1998). Multiple forms of tubulin: different gene products and covalent modifications. Int. Rev. Cytol. *178*, 207–275.

Mains, P.E., Kemphues, K.J., Sprunger, S.A., Sulston, I.A., and Wood, W.B. (1990a). Mutations affecting the meiotic and mitotic divisions of the early *Caenorhabditis elegans* embryo. Genetics *126*, 593–605.

Mains, P.E., Sulston, I.A., and Wood, W.B. (1990b). Dominant maternal-effect mutations causing embryonic lethality in *Caenorhabditis elegans.* Genetics *125*, 351–369.

Matthews, L.R., Carter, P., Thierry-Mieg, D., and Kemphues, K. (1998). ZYG-9, a *Caenorhabditis elegans* protein required for microtubule organization and function, is a component of meiotic and mitotic spindle poles. J. Cell Biol. *141*, 1159–1168.

Matthies, H.J., McDonald, H.B., Goldstein, L.S., and Theurkauf, W.E. (1996). Anastral meiotic spindle morphogenesis: role of the non-claret disjunctional kinesin-like protein. J. Cell Biol. *134*, 455–464.

McNally, F.J., and Vale, R.D. (1993). Identification of katanin, an ATPase that severs and disassembles stable microtubules. Cell *75*, 419–429.

Merdes, A., and Cleveland, D.W. (1997). Pathways of spindle pole formation: different mechanisms; conserved components. J. Cell Biol. *138*, 953–956.

Panda, D., Miller, H.P., Banerjee, A., Ludueña, R.F., and Wilson, L. (1994). Microtubule dynamics in vitro are regulated by the tubulin isotype composition. Proc. Natl. Acad. Sci. USA *91*, 11358–11362.

Piperno, G., and Fuller, M.T. (1985). Monoclonal antibodies specific for an acetylated form of alpha-tubulin recognize the antigen in cilia and flagella from a variety of organisms. J. Cell Biol. *101*, 2085–2094.

Reinke, V., *et al*. (2000). A global profile of germline gene expression in *C. elegans.* Mol. Cell *6*, 605–616.

Savage, C., Hamelin, M., Culotti, J.G., Coulson, A., Albertson, D.G., and Chalfie, M. (1989). mec-7 is a beta-tubulin gene required for the production of 15-protofilament microtubules in *Caenorhabditis elegans.* Genes Dev. *3*, 870–881.

Savage, C., Xue, Y., Mitani, S., Hall, D., Zakhary, R., and Chalfie, M. (1994). Mutations in the *Caenorhabditis elegans* beta-tubulin gene *mec-7*: effects on microtubule assembly and stability and on tubulin autoregulation. J. Cell Sci. *107*, 2165–2175.

Schatten, G. (1994). The centrosome and its mode of inheritance: the reduction of the centrosome during gametogenesis and its restoration during fertilization. Dev. Biol. *165*, 299–335.

Sharp, D.J., McDonald, K.L., Brown, H.M., Matthies, H.J., Walczak, C., Vale, R.D., Mitchison, T.J., and Scholey, J.M. (1999). The bipolar kinesin, KLP61F, cross-links microtubules within interpolar microtubule bundles of *Drosophila* embryonic mitotic spindles. J. Cell Biol. *144*, 125–138.

Srayko, M., Buster, D.W., Bazirgan, O.A., McNally, F.J., and Mains, P.E. (2000). MEI-1/MEI-2 katanin-like microtubule severing activity is required for *Caenorhabditis elegans* meiosis. Genes Dev. *14*, 1072–1084.

Strome, S., and Wood, W.B. (1983). Generation of asymmetry and segregation of germ-line granules in early *C. elegans* embryos. Cell *35*, 15–25.

Vernos, I., Raats, J., Hirano, T., Heasman, J., Karsenti, E., and Wylie, C. (1995). Xklp1, a chromosomal *Xenopus* kinesin-like protein essential for spindle organization and chromosome positioning. Cell *81*, 117–127.

Wang, S.Z., and Adler, R. (1995). Chromokinesin: a DNA-binding, kinesin-like nuclear protein. J. Cell Biol. *128*, 761–768.

Webb, M., Jouannic, S., Foreman, J., Linstead, P., and Dolan, L. (2002). Cell specification in the *Arabidopsis* root epidermis requires the activity of ECTOPIC ROOT HAIR 3– a katanin-p60 protein. Development *129*, 123–131.

Wright, A.J., and Hunter, C.P. (2003). Mutations in a β-tubulin disrupt spindle orientation and microtubule dynamics in the early *C. elegans* embryo. Mol. Biol. Cell *14*, 4512–4525.