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

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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), Xklp1 (Vernos et al., 1995), and Drosophila Ncd (Afsar 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; Mattheis 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). How- ever, 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 mei-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,
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 iso-
type-specific RNA interference (RNAi) experiments demon-
strate 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 micro-
tubules containing the TBB-2-β-tubulin isoform 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), gl129, gl130, tbb-1(gk207). 
tbb-2 deletion alleles gl129 and gl130 and tbb-1 deletion allele gk207 were 
purchased from 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 1, including the start ATG 
without generating any downstream in-frame ATG. Both alleles were out-
crossed 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 
myl-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 BamHI site of pGEX-3 × (Amersham 
Pharmacia, Uppsala, Sweden) to create a GST-fusion. Bacterially expressed 
protein was purified with a glutathione-Sepharose 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 
the synthetic peptide (Alberta Peptide Institute, Edmonton, AB, Canada) corre-
spending to the amino acids 432–442 of TBB-2 (see Figure 3A). Western 
analysis was performed as described in 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 Kempfues et al. (1986). Affinity-purified anti-TBB-2 
antiser was 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 antibody (Piperno and 
and Name:/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 de-
fects caused by ectopic MEI-1/MEI-2 serving 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 effec-
tively suppresses (allelizes) the lethality caused by ectopic 
MEI-1/MEI-2 activity resulting from the mei-1(ct46gf) muta-
tion (Table 1, lines 1–5). In mei-1(ct46gf) embryos, the first 
mitotic spindle is smaller than wild-type and is often mis-
positioned orthogonally to the anterior-posterior axis (Figure 
IC). In contrast, when mei-1(ct46gf) is combined with the 
tbb-2(sb26) mutation, embryos have wild-type spindle mor-
phology 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-1and mei-2. On their own, mei-1 and 
mei-2If mutations result in defects in meiotic spindle forma-
tion, most likely due to decreased meiotic microtubule sev-
ering, and these defects were enhanced when combined with 
tbb-2(sb26) (Table 1, lines 10–11). Whereas the meiotic 
defect of a weak mei-2lf allele, c98, results in 86% hatching 
at the nonpermissive temperature of 25°C, lethality increased 
dramatically when combined with tbb-2(sb26), resulting in a 
37% hatching under the same conditions. In C. elegans, 
nondisjunction of the X chromosome results in a normal XO 
males, and thus the frequency of males among the survivors is 
a measure of meiotic failure. Normally, ~1/500 zygotes from a selfed hermaphro-
dite is a male (Hodgkin and Bren-
ner, 1977), but among the mei-2(ct98); tbb-2(sb26) escapers at 
25°C, 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, 
sDfJ30, failed to dominantly suppress the maternal-effect 
lethality of mei-1(ct46gf), although tbb-2(sb26)+/+ did so (Ta-
ble 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 METH-
ODS). These produced no detectable protein on immuno-
blots with TBB-2–specific antibodies (see below). In contrast to 
tbb-2(sb26), heterozygosity for a tbb-2 null allele decreased
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 mis-sense 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.

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

<table>
<thead>
<tr>
<th>Maternal genotype</th>
<th>% hatching (%) male*</th>
</tr>
</thead>
<tbody>
<tr>
<td>mei-1(ct46gf)*</td>
<td>15 20 25</td>
</tr>
<tr>
<td>tbb-2(sb26)</td>
<td>23 0 1.5 0</td>
</tr>
<tr>
<td>mei-1(ct46gf); tbb-2(sb26)</td>
<td>96 92 0 67 1.4</td>
</tr>
<tr>
<td>mei-1(ct46gf)/+</td>
<td>— 38 1.2</td>
</tr>
<tr>
<td>mei-1(ct46gf)/+; tbb-2(sb26)/+</td>
<td>— 84 37 74</td>
</tr>
<tr>
<td>tbb-2(t1623)/+</td>
<td>96 74</td>
</tr>
<tr>
<td>mei-1(ct46gf)/+; tbb-2(t1623)/+</td>
<td>16 0.6 95 25 0</td>
</tr>
<tr>
<td>mei-1(ct46gf)/+; tbb-2(or362)/+</td>
<td>— 98 95 25 0</td>
</tr>
<tr>
<td>mei-1(ct46gf)/+; tbb-2(or362)/+</td>
<td>— 25 0 74 2.6</td>
</tr>
<tr>
<td>mei-1(ct98)*</td>
<td>96 97 0.2 74 2.6</td>
</tr>
<tr>
<td>mei-2(ct98); tbb-2(sb26)</td>
<td>89 64 4.7 3.7 (17)</td>
</tr>
</tbody>
</table>

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

* 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.

* Not determined.

* 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

<table>
<thead>
<tr>
<th>Maternal genotype</th>
<th>% hatching (20°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>mei-1(ct46gf)/+</td>
<td>39</td>
</tr>
<tr>
<td>mei-1(ct46gf)/+; tbb-2(sb26)/+</td>
<td>75 7.6</td>
</tr>
<tr>
<td>mei-1(ct46gf)/+; sDf130/</td>
<td>12</td>
</tr>
<tr>
<td>mei-1(ct46gf)/+; tbb-2(gk129)/+</td>
<td>99 89</td>
</tr>
</tbody>
</table>

* Corrected for the 43% zygotic lethality resulting from the deficiency.

* tbb-2(gk129) is a predicted molecular null allele. Another null allele, gk130, behaved similarly.
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 ethylmethanesulfonate-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°C (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.
nads were dissected from gravid hermaphrodites, fixed, and stained with TBB-2–specific antisera and contained 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 gfp-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-tubulins that is expressed at high levels in the germ line, TBB-1, share $\geq 85\%$ 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 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 if 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 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 if mei-2 allele (Clandinin and Mains, 1993) 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 microtubule-severing protein complex katanin (Srayko et al., 2003), and Ellis et al. (2004) showed that TBB-2 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).
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 microtubules at different time points of cold-induced depolymerization (our unpublished data). Furthermore, tbb-2(sb26) does not genetically interact with the if 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.

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 benomyl (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 isoforms 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 If mei-2(ct198) were enhanced by depleting tbb-2(+)/tbb-1/ by either RNAi or a deletion allele (Table 4). However, removal of the other β-tubulin

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

<table>
<thead>
<tr>
<th>Maternal genotype</th>
<th>% hatching at 25°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>tbb-2(RNAi)</td>
<td>86*</td>
</tr>
<tr>
<td>tbb-2(gk129)</td>
<td>75</td>
</tr>
<tr>
<td>tbb-1(RNAi)</td>
<td>92*</td>
</tr>
<tr>
<td>tbb-1(gk207)</td>
<td>88</td>
</tr>
<tr>
<td>tbb-2(RNAi); tbb-1(RNAi)</td>
<td>0*</td>
</tr>
<tr>
<td>tbb-2(gk129): tbb-1(RNAi)</td>
<td>0.5</td>
</tr>
<tr>
<td>tbb-2(sb26): tbb-1(RNAi)</td>
<td>85</td>
</tr>
<tr>
<td>mei-2(ct98)</td>
<td>74</td>
</tr>
<tr>
<td>mei-2(ct98); tbb-2(RNAi)</td>
<td>1.7</td>
</tr>
<tr>
<td>mei-2(ct98): tbb-2(gk129)</td>
<td>5.8</td>
</tr>
<tr>
<td>mei-2(ct98): tbb-1(RNAi)</td>
<td>71</td>
</tr>
<tr>
<td>mei-2(ct98); tbb-1(gk207)</td>
<td>60</td>
</tr>
</tbody>
</table>

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

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