The Role of $\gamma$-Tubulin in Centrosomal Microtubule Organization

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Abstract

As part of a multi-subunit ring complex, $\gamma$-tubulin has been shown to promote microtubule nucleation both in vitro and in vivo, and the structural properties of the complex suggest that it also seals the minus ends of the polymers with a conical cap. Cells depleted of $\gamma$-tubulin, however, still display many microtubules that participate in mitotic spindle assembly, suggesting that $\gamma$-tubulin is not absolutely required for microtubule nucleation in vivo, and raising questions about the function of the minus end cap. Here, we assessed the role of $\gamma$-tubulin in centrosomal microtubule organisation using three-dimensional reconstructions of $\gamma$-tubulin-depleted C. elegans embryos. We found that microtubule minus-end capping and the PCM component SPD-5 are both essential for the proper placement of microtubules in the centrosome. Our results further suggest that $\gamma$-tubulin and SPD-5 limit microtubule polymerization within the centrosome core, and we propose a model for how abnormal microtubule organization at the centrosome could indirectly affect centriole structure and daughter centriole replication.

Introduction

In most animal cells, the centrosome is the major microtubule-organizing center (MTOC). It consists of a pair of centrioles surrounded by a complex, three-dimensional matrix of electron-dense, pericentriolar material (PCM) [1]. Observed over 100 years ago, centrosomes are typically 1.5-2 μm in diameter, but can vary depending on tissue type and cell size [1,2]. Although many centrosomal components are now known [3,4,5], the composition and precise organization of the individual structural components within this complex organelle and the nucleation and outgrowth of microtubules from the centrosome are still not fully understood.

The PCM of the centrosome provides a kinetically favorable site for cellular microtubule nucleation as well as a structural hub for anchoring the microtubule minus ends [6]. Microtubule outgrowth results in a polarized microtubule astral array, with the majority of microtubules plus ends polymerizing away from centrosomes [7]. This arrangement of microtubules around centrosomes is exploited for a variety of functions including intracellular trafficking, cellular polarity, mitotic spindle assembly, and cytokinesis. The ability of a centrosome to promote the robust initiation of microtubule growth is due, in part, to $\gamma$-tubulin [8]. Two molecules of $\gamma$-tubulin and one copy each of the accessory proteins Spc97 and Spc98 compose the 300-kDa $\gamma$-tubulin small complex ($\gamma$-TuSC) [9]. In metazoans, multiple $\gamma$-TuSCs associate with additional proteins to form open $\gamma$-tubulin ring complexes (γTuRCs) [10,11], which have been shown to serve as templates for polymerization of 13-protofilament microtubules [12,13,14,15].

Despite the information on the mechanism of microtubule nucleation from $\gamma$-tubulin and associated proteins, the in vivo consequence of $\gamma$-tubulin depletion on the structure of the centrosomes and microtubules has not been addressed [16]. RNAi directed against the sole C. elegans $\gamma$-tubulin gene, tbg-1, results in a significant reduction but not complete loss of centrosomal microtubules [7,17,18,19]. Defects also include aberrant spindle microtubule organization [18]. Studies using EB1::GFP to measure growing microtubule plus ends have shown that, although tbg-1(RNAi) embryos have ~60% of wild-type centrosomal microtubule levels, the existing microtubules emanate from centrosomes with normal growth rate and polarity [7]. Therefore, the polarity of centrosomal microtubules in tbg-1(RNAi) embryos appear largely unaffected, which raises questions as to the function of $\gamma$-tubulin in microtubule arrangement and centrosome function. Although a decrease in centrosomal microtubule nucleation alone could interfere directly with mitotic microtubule-based processes,
our data suggest that γ-tubulin plays an important role in organizing microtubule minus ends at the centrosome to create a polarized, radial microtubule array [20].

Results

γ-tubulin organizes capped microtubule ends at the centrosome periphery

To investigate the role of γ-tubulin on mitotic centrosome organization, we first visualized the distribution of α-tubulin and γ-tubulin on immunostained wild-type and *tbg-1* (RNAi) embryos (Figure 1). A linescan through a wild-type centrosome revealed a clear reduction in α-tubulin levels within the core region. This core region coincided with a peak of fluorescence intensity of γ-tubulin (Figure 1A). In contrast, a linescan through the most concentrated mass of microtubules within a single confocal plane in the *tbg-1* (RNAi) embryos indicated that α-tubulin was not excluded in a similar pattern (Figure 1B–C). These results suggested that γ-tubulin provides a microtubule-organizing function for the centrosome that includes the formation of a microtubule-free zone within the central core, consistent with previous reports [18].

In order to elucidate the precise arrangement and structure of the microtubules nearest the centrosome, we performed electron tomography in combination with 3-D modeling on wild-type

![Figure 1. Distribution of α- and γ-tubulin in the C. elegans centrosome.](#)

A. Immunofluorescence images (projections of confocal stacks) of a wild-type embryo (left column) and a *tbg-1* (RNAi) embryo (right column) showing α-tubulin (red), γ-tubulin (green), and DNA (blue). B. Line scans from single confocal planes over centrosome regions as indicated in (A) used for measurements of pixel intensity. C. Linescan intensity plots of fluorescence intensity for α-tubulin (red) and γ-tubulin (green) along the marked lines presented in (B). In contrast to the *tbg-1* (RNAi) embryo, the concentration of α-tubulin is locally reduced in the wild-type centrosome. Bar: 10 μm in A.

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(Figure 2A) and tbg-1(RNAi) C. elegans embryos (Figure 2B). The centrosomes of wild-type, one-cell metaphase embryos appeared homogenous with a density characteristic of the PCM and an average diameter of ∼1500 nm (Figure 2A, dashed circle). Using tomography of 300 nm-thick serial slices, we modeled centrioles and all nearby microtubules to determine the position of their pole-proximal ends in 3-D (Figure 2A; Movie S1; Figure S1A for other examples). In agreement with fluorescence images and previous results [18], wild-type metaphase centrosomes displayed a “central core”, i.e. a zone of exclusion of pole-proximal microtubule ends around the centriole pair.

In contrast, the metaphase tbg-1(RNAi) embryo displayed vesicles within the centrosome core, suggesting a loss of centrosome integrity (Figure 2A). Furthermore, 3-D modeling revealed that numerous microtubules encircled or traversed the central region of the tbg-1(RNAi) centrosome, many coming within 100 nm of a centriole (Figure 2B; Movie S2; Figure S1B–C for other examples). In addition, fewer microtubule ends were observed in the centrosome of the tbg-1(RNAi) embryo compared to wild type (average of 497 vs. 970 from three tbg-1(RNAi) and two wild-type embryos, respectively; Table 1; Figure S1B–C for other examples).

Next, we examined the microtubule end morphology in the wild-type and tbg-1(RNAi) embryos. To image these ends in detail, we extracted a slice from the 3-D volume that contained the axis of each microtubule (Figure 3A). We identified closed (i.e. conical) and open (i.e. blunted or slightly flared) pole-proximal ends [21], and marked them with purple and yellow spheres, respectively (Figure 3B). In wild-type centrosomes, 79% (1537/1942; Table 1) of the ends nearest the centrioles were closed and 21% (405/1942) were open (Figure 3B). In contrast, only 30% (456/1494) of the pole-proximal ends were closed in tbg-1(RNAi) embryos and the rest (1030/1494) were open (Table 1 and Figure S1 for other examples). These results suggest that γ-tubulin contributes to the closed-end morphology of microtubules at the centrosome. We suspect that the residual capped ends in tbg-1(RNAi) result from incomplete depletion of γ-tubulin, however, the existence of a redundant microtubule end-capping mechanism cannot be ruled out. The presence of a few capped ends in tbg-1(RNAi) embryos allowed us to determine whether or not capped microtubules specifically maintain a proper position within the centrosome. The closed pole-proximal ends in tbg-1(RNAi) embryos occupied positions at an average of 695±243 nm from the center of the centrosome (Figure 3D; Table 1). These positions overlapped with their wild-type counterparts (average of 719±192 nm; Figure 1D; Table 1). This suggests that the end capping is necessary for proper microtubule positioning within the PCM.

Depletion of γ-tubulin causes overly long centriolar microtubules

We next examined the ultrastructure of centrioles in tbg-1(RNAi) embryos. Wild-type cells contain two centrioles oriented at approximate right angles to one another. C. elegans centriole biogenesis initiates with the formation of a central tube that is subsequently surrounded by nine singlet microtubules. The singlet microtubules are normally not longer than the central tube at any stage during the first embryonic cell division [22]. Tomograms of tbg-1(RNAi) embryos revealed a consistent centriole defect whereby varying numbers of centriolar microtubules extended beyond the distal end of the central tube (Figure 4A–C; Movie S3; Figure S1 for more examples). This defect was observed on both the mother and the newly-assembled daughter centrioles. Most central tubes (10/11) had a mean length that was similar to wild type (Figure 3D); however, 1/11 centrioles had a central tube that was twice as long as wild type (Figure 4C). In some centrosomes (5/8), only one centriole was detected, or the daughter was incomplete. This suggests a role for TBG-1 in centriole formation, consistent with previous light microscopy experiments [23].

Microtubule end placement in the centrosome periphery requires SPD-5

We further tested whether the centriolar microtubule defects were due directly to the loss of γ-tubulin or an indirect consequence of PCM dysfunction and subsequent disorganization of the microtubule array. SPD-5 is a major PCM component in C. elegans; it is required for centrosome maturation via the recruitment of γ-tubulin and other core components [24,25,26] and is essential for robust microtubule outgrowth (Figure 5A; Movie S4) [24]. We used spd-5(RNAi) to compromise centrosome structure/function and interfere with the intracellular distribution of TBG-1 (Figure 5B–C). We applied electron tomography to visualize the distribution and end morphology of the microtubules (Figure 5D). Similar to tbg-1(RNAi) embryos, microtubules in spd-5(RNAi) traversed the centriole vicinity (Figure 5E–F; Movie S5; and Figure S2 for other examples). We determined the ratio of closed-to-open pole-proximal ends within this 3-D model. The majority of the microtubule pole-proximal ends were open (Figure 5G; Table 1). Microtubules in the spd-5(RNAi) embryos might originate from the chromatin rather than centriolar regions, therefore it is possible that many of these open ends could represent growing plus tips [7,24,27]. Interestingly, unlike the tbg-1(RNAi) embryos,
where the few capped ends that remained were situated at near-wild-type distances from the centrosome center, the capped ends in \( spd-5(\text{RNAi}) \) embryos were very close to the centrosome center (405±206 nm; Table 1). Therefore both SPD-5 and TBG-1 are important for the proper positioning of microtubule ends around the centrosomes [35]. In wild-type nematode embryos, the closed, cone-shaped ends are reminiscent of microtubule minus ends observed near the centrioles [31] and at isolated Drosophila centrosomes [35]. In wild-type nematode embryos, the closed, centrosome-proximal microtubule ends outnumber the open ends 4:1, and the majority of the open ends are on kinetochore microtubules [21]. Our electron tomography results verify that \( \gamma \)-tubulin contributes a structural role to microtubule ends \textit{in vivo}. The frequency of the conical cap was also greatly diminished in \( spd-5(\text{RNAi}) \) and we suggest two likely explanations for this result. First, because of the severe reduction in centrosomal microtubule nucleation in \( spd-5(\text{RNAi}) \) [23,24], many of the open ends we observed near the centrioles could be plus-ends originating from chromatin-initiated microtubules as suggested by others [7,24,27]. Second, the PCM scaffold in wild-type cells might facilitate microtubule minus-end capping by recruiting \( \gamma \)-tubulin and providing a docking site for the \( \gamma \)-tubulin ring complex [6].

**Table 1.** Comparison of wild type embryos with TBG-1 and SPD-5 depletion phenotypes.

| Strain   | # MT ends | MT ends from centrosome centre | # closed pole-proximal MT ends | # open pole-proximal MT ends | Mean distance closed ends centrosome centre | Mean distance open ends centrosome centre |
|----------|-----------|--------------------------------|-------------------------------|-----------------------------|---------------------------------------------|-------------------------------------------|
| WT       | 866       | 766±192 nm                     | 81% (702/866)                | 19% (164/866)              | 743±184 nm                                  | 867±195 nm                                |
| (Fig. 1, 2) | 1076   | 725±201 nm                     | 78% (835/1076)               | 22% (241/1076)            | 698±196 nm                                  | 818±191 nm                                |
| All WT   | 1942      | 743±255 nm                     | 79% (1537/1942)              | 21% (405/1942)            | 719±192 nm                                  | 837±194 nm                                |
| \( tbg-1(\text{RNAi}) \) | 650      | 829±248 nm                     | 30% (207/650)                | 64% (443/650)             | 786±241 nm                                  | 849±249 nm                                |
| (Fig. 1, 2) | 415     | 661±235 nm                     | 33% (138/415)                | 67% (277/415)             | 630±217 nm                                  | 676±242 nm                                |
| (Fig. S1C) | 429     | 661±236 nm                     | 26% (111/429)                | 74% (318/429)             | 608±219 nm                                  | 679±238 nm                                |
| All \( tbg-1(\text{RNAi}) \) | 1494     | 734±255 nm                     | 31% (456/1494)               | 69% (1038/1494)          | 695±243 nm                                  | 751±258 nm                                |
| \( spd-5(\text{RNAi}) \) | 205      | 405±208 nm                     | 46% (95/205)                 | 54% (110/205)            | 332±200 nm                                  | 468±194 nm                                |
| (Fig. 4E, F) | 284     | 395±200 nm                     | 34% (98/284)                 | 66% (186/284)            | 341±195 nm                                  | 424±198 nm                                |
| (Fig. S2B) | 261     | 509±197 nm                     | 36% (94/261)                 | 64% (167/261)            | 414±147 nm                                  | 563±201 nm                                |
| (Fig. S2C) | 652     | 558±235 nm                     | 23% (147/652)                | 77% (505/652)            | 491±218 nm                                  | 578±237 nm                                |
| All \( spd-5(\text{RNAi}) \) | 1402     | 494±229 nm                     | 31% (434/1402)               | 69% (968/1402)           | 405±206 nm                                  | 533±228 nm                                |

**Discussion**

Taken together, our observations suggest that \( \gamma \)-tubulin is important for the proper microtubule end-morphology and end-placement in the periphery of the centrosome (Figure 6E). In other systems, pericentrin, a large coiled-coil-containing protein, has been implicated in anchoring microtubule minus ends to the PCM [28]. In C. elegans, SPD-5 is required for centrosome structure and recruitment of TBG-1 and other factors [24]. Our results further suggest that microtubule-end placement in the centrosome periphery also requires SPD-5. The placement of microtubules into the PCM could also have consequences for microtubule polymer dynamics, as many regulators of microtubules localize to centrosomes in a \( \gamma \)-tubulin-dependent manner [6,29,30]. In the absence of functional \( \gamma \)-tubulin, microtubules in Drosophila S2 cells assemble near the centrosome, but they become unusually long and curved [31], consistent with our findings in C. elegans \( tbg-1(\text{RNAi}) \) embryos.

When nucleated from \( \gamma \)-tubulin \textit{in vitro}, microtubules minus ends display a closed, cone-shaped morphology [14,32,33]. Such cone-shaped ends are reminiscent of microtubule minus ends observed at yeast spindle pole bodies [34] and at isolated Drosophila centrosomes [35]. In wild-type nematode embryos, the closed, centrosome-proximal microtubule ends outnumber the open ends 4:1, and the majority of the open ends are on kinetochore microtubules [21]. Our electron tomography results verify that \( \gamma \)-tubulin contributes a structural role to microtubule ends \textit{in vivo}. The frequency of the conical cap was also greatly diminished in \( spd-5(\text{RNAi}) \), and we suggest two likely explanations for this result. First, because of the severe reduction in centrosomal microtubule nucleation in \( spd-5(\text{RNAi}) \) [23,24], many of the open ends we observed near the centrioles could be plus-ends originating from chromatin-initiated microtubules as suggested by others [7,24,27]. Second, the PCM scaffold in wild-type cells might facilitate microtubule minus-end capping by recruiting \( \gamma \)-tubulin and providing a docking site for the \( \gamma \)-tubulin ring complex [6].

The over-extension of centriolar microtubules in \( tbg-1(\text{RNAi}) \) embryos seems inconsistent with the established role for \( \gamma \)-tubulin in promoting microtubule polymerization \textit{in vitro}. However, centriolar microtubule extension has also been observed in \( \gamma \)-tubulin-depleted Drosophila S2 cells [31] and in cells depleted of the distal centriole microtubule-capping protein CP110 [36,37,38]. This
Figure 3. Distribution and morphology of pole-proximal microtubule ends in mitotic centrosomes. A. Gallery of pole-proximal ends (wild-type: left column; tg-1(RNAi): right column). Electron tomography reveals closed- and open-end morphologies. B. 3-D modeling of closed (purple spheres) and open (yellow spheres) microtubule ends. C. The ratio of closed/open pole-proximal ends is reversed in γ-tubulin compromised embryos. D. Distribution of closed microtubule pole-proximal ends. Although reduced in number, the closed pole-proximal ends in tg-1(RNAi) embryos occupy a similar distance from the centrosome center as wild type. Bars: 50 nm in A; 200 nm in B.
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Figure 4. Effect of γ-tubulin-RNAi on centriole ultrastructure. A. Tomographic slice showing centriolar microtubules extending beyond the distal ends of the two centrioles (arrows). B–C. 3-D models of centrioles showing that both mother and daughter centrioles can contain elongated centriolar microtubules (B), however some tomograms contained only single centrioles (C). D. Length of central tubes from tbg-1(RNAi) centrosomes showing double or single centrioles. Bars: 200 nm A–C.

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**Figure 5. Effect of spd-5(RNAi) on centrosome structure and microtubule end morphology.** A. Live-cell imaging of a wild-type and spd-5(RNAi) embryo expressing β-tubulin::GFP. B. Imaging of TBG-1::GFP in spd-5(RNAi) embryos. C. Western blot. γ-tubulin is present in the SPD-5-compromised embryo. A non-specific band from the anti-SPD-5 antiserum served as a loading control (l.c.). D. Gallery of closed (left images) and open
raises the possibility that γ-tubulin could play additional roles in recruiting and/or activating this type of protein [6], although a CP110p equivalent is not apparent in *C. elegans*. To date, SAS-4 is the only centriolar component required specifically for the formation of the centriolar microtubules and not the central tube [22,39]. Furthermore, mammalian tissue culture cells over-expressing the SAS-4 homologue, CPAP, have over-extended centriolar microtubules. Interestingly, in a previous *C. elegans* study using light microscopy and photobleaching techniques, γ-tubulin was shown to be important for the

Figure 6. Model explaining the relationship between centrosomal microtubule organization and centriole morphology. A–B. Centriole pairs in *spd-5(RNAi)* embryos with variable lengths of the central tube of daughter centrioles. C. Centrosome region with three centrioles at one pole. Although it is unclear how one extra centriole could arise within a single cell cycle, the loss of SPD-5 may cause excessive movement of mother and daughter centrioles during the duplication process. If a daughter is dislodged at an early stage of this process, perhaps the same mother centriole can support the assembly of a second daughter. D. Quantification of central tube length in wild-type versus *spd-5(RNAi)* embryos. E. Model explaining the effect of TBG-1 and SPD-5 depletion on the structural organization of the centrosome. In wild type, the PCM component SPD-5 and γ-tubulin might contribute to a zone of exclusion visible as a ring-shaped area of pole-proximal microtubule ends around centrioles that limits microtubule polymerization and physically prevents movement of microtubule polymers into the centrosome core. Centriolar microtubule assembly occurs normally during late prophase, in a SAS-4 dependent manner. The zone of exclusion might trap SAS-4 within this central core, which could specifically promote the assembly of centriolar microtubules in an environment expected to be otherwise non-conducive to microtubule polymerization. In *tbg-1(RNAi)* or *spd-5(RNAi)* embryos, microtubule polymerization occurs near the centrioles, exchange with cytoplasmic SAS-4 becomes possible, and centriolar microtubules over-extend. Microtubule numbers are greatly reduced in *spd-5(RNAi)* but less affected in *tbg-1(RNAi)*, where the majority of microtubule ends near the centrosome exhibit an open, rather than a closed morphology. Bars: 200 nm A–C.

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stabilization of a pool of SAS-4 protein at the centrioles during later stages of centrosome maturation [40]. It was speculated that loss of SAS-4 stability at the centrosome in tbg-1(RNAi) embryos might directly interfere with centriolar microtubule assembly. We observed single centrioles in over half of the tbg-1(RNAi) embryos, indicating a role for γ-tubulin in daughter centriole formation. However, the over-extension of centriolar microtubules in tbg-1(RNAi) embryos also suggests that γ-tubulin is not required for centriolar microtubule production but may be important in regulating the extent of the process.

In tbg-1(RNAi) embryos, mis-positioning of microtubule ends at the centrosomes could also contribute to a deregulation of tubulin polymer dynamics within the centrosome core. Normally, the arrangement of all capped microtubule minus ends facing inward would be expected to limit microtubule polymerization in the central region due to low free tubulin concentration. Observations of a centrosomal core region have been reported as early as 1897 by E. B. Wilson [41]. In our study, we found a clear reduction in the level of γ-tubulin within the wild-type centrosome core by immunofluorescence and an absence of microtubules in this area via EM tomography. Our results further suggest that both γ-tubulin and SPD-5 contribute to the formation of the microtubule-free zone around the centrioles. We therefore speculate that γ-tubulin could facilitate the formation of a microtubule-free zone in at least two ways. First, a high concentration of γ-tubulin in the centrosome could physically restrict the movement of free α/β tubulin subunits into the core, thus limiting polymerization of microtubules. Second, γ-tubulin could limit microtubule polymerization within the core because of its own polarized placement within the centrosome, such that any free α/β tubulin would be expected to polymerize away from the center exclusively.

An inappropriate increase in overall microtubule polymerization within the centrosome core would also explain why the centriolar extensions in tbg-1(RNAi) embryos are not limited to the newly forming centriole but are also apparent on the sperm-derived mother centrioles. Furthermore, pushing forces resulting from polymerization of microtubules near to and between centrioles could also explain why the centrioles are often separated from one another in tbg-1(RNAi) and spd-5(RNAi) embryos.

It has been reported that γ-tubulin is required to immobilize SAS-4 within mature centrosomes [40]. Early SAS-4 recruitment to centrioles could occur prior to the formation of this zone of exclusion, but once established as the centrosome matures, a certain fraction of SAS-4 could become trapped within the central core. The SAS-4 homologue, CPAP, promotes centriolar microtubule assembly through binding of tubulin heterodimers to promote procentriole elongation [36,37,38,42]. We suggest that C. elegans SAS-4 might be necessary for controlled centriolar microtubule growth within a centrosomal core that is otherwise non-conducive to microtubule polymerization. A centrosomal core environment that limits microtubule polymerization could be important to maintain the structural integrity of centrosomes, ensuring that mother and daughter centrioles do not separate prematurely, due to physical forces arising from inappropriate microtubule assembly.

Materials and Methods

RNAi Methods, Light Microscopy and Quantification of Fluorescence

Wild-type or TH27 [β-tubulin::GFP; Histone::GFP] worms were injected with dsRNA as in Sommichini et al. [4] and cultured at 25°C for 22–28 hr prior to dissection. Loss-of-function phenotypes were first confirmed by observing the RNAi embryos via fluorescence or light microscopy prior to freezing. Confocal fluorescence images of fixed and living embryos were obtained with a Hamamatsu Orca R2 camera on an inverted Olympus IX51 (60×, NA 1.42 oil objective) microscope with a Yokogawa CSU-10 spinning disc confocal head (Quorum Technologies) controlled by MetaMorph software. Embryos were fixed for immunofluorescence as previously described [43]. Wild-type and tbg-1(RNAi) embryos were combined and fixed on the same slide and treated with primary antibodies DM1A (10 μg/mL, mouse monoclonal, Sigma) and rabbit-anti-TBG-1 (35 μg/mL) [19] and fluorescently-labeled secondary antibodies and Alexa 488 anti-mouse and Alexa 546 anti-rabbit secondary antibodies (Invitrogen). DAPI (1 μg/mL) was used to visualize the chromatin. Image stacks were deconvolved using Huygens Essential software and maximum projections of image stacks were generated for the whole embryo using false colors for display. Line-scans (50 pixels long) were performed on a single deconvolved image plane exhibiting the highest intensity of γ-tubulin within a circle (10 μm diameter) using MetaMorph software. Orientation of the line was arbitrary and did not alter the overall conclusions.

Immunoblotting

L4 larvae were injected with spd-5(RNAi). After 27–30 h at 20°C, 50 wild-type and spd-5(RNAi) worms were transferred to 1 mL of M9 buffer and embryos were isolated as previously described [19]. Samples were run on a 10% SDS-PAGE gel and immunoblots were blotted at 70 kDa. Immunoblots were then probed using rabbit anti-SPD-5 or rabbit anti-γ-tubulin. Binding of SPD-5 (155 kDa) and TBG-1 (50 kDa) were detected with an HRP-conjugated secondary antibody (1:5000; Bio-Rad Laboratories).

Specimen Preparation for Electron Microscopy

C. elegans hermaphrodites were dissected in M9 buffer containing 20% BSA (Sigma). Single embryos were collected into capillary tubing to observe the development of the one-celled embryos as described [44]. Embryos at specific stages were transferred to 100 μL deep membrane carriers (Leica) and frozen using the EM-PACT2+RTS high-pressure freezer (Leica). Specimens were then freeze-substituted in anhydrous acetone containing 1% osmium tetroxide and 0.1% uranyl acetate over 24–48 h. Epon/Araldite infiltrated specimens were flat embedded in a thin layer of resin and polymerized for 2–3 d at 60°C. Serial semi-thick sections (300 nm) were cut using a Leica UCT ultramicrotome. Sections were collected on Formvar-coated copper slot grids and post-stained with 2% uranyl acetate in 70% methanol and Reynolds’ lead citrate.

Electron Tomography

Dual-axis electron tomography was performed as described [44]. Briefly, 15-nm colloidal gold particles (Sigma-Aldrich) were attached to each surface of the semi-thick sections to serve as fiducial markers for subsequent image alignment. Tilt series datasets were imaged using a TECNAI F30 intermediate-voltage electron microscope (FEI) operated at 300 kV. The SerialEM program [45], was used to automatically acquire images every 1° over a ±60° range using a Gatan 2K×2K CCD camera at a pixel size of 1.2 nm. Tomograms from 3-4 serial sections were calculated using the IMOD software package and joined to produce a final volume containing most of the centrosome (2.4 μm×2.4 μm×0.9 μm) [46,47]. We recorded 4 wild-type centrosomes, 8 tbg-1(RNAi) centrosomes, and 7 centrosomes of spd-5(RNAi) embryos. By electron tomography we checked that GFP-tubulin labeling had no effect on the morphology of the pole-proximal microtubule ends.
Modeling and Analysis of Tomographic Data

Tomograms were displayed and analyzed using the IMOD program, 3dmod [46]. The centrioles, including the central tube (orange) and the centriolar microtubules (light blue) were modeled in 8 wild-type, 11 tbg-1(RNAi), and 15 spd-5(RNAi) centrioles. The centrosomal microtubules (red) as well as closed (purple spheres) and open (yellow spheres) microtubule pole-proximal ends were modeled and tracked throughout the volume of 2 wild-type, 3 tbg-1(RNAi) and 4 spd-5(RNAi) centrosomes. In addition, we modeled closed in the centrosome. The projections of the 3-D models were displayed and rotated to study their 3-D geometry.

Supporting Information

Figure S1 Three-dimensional modeling of centrosome regions in early C. elegans embryos. A. Wild-type embryo. B–C. γ-tubulin compromised embryos. The distribution of closed (purple) and open (yellow) microtubule ends and centrioles is shown on the right. Bars: 200 nm in A–C. (TIFF)

Figure S2 Three-dimensional modeling of spd-5(RNAi) embryos. The distribution of closed (purple) and open (yellow) microtubule ends and centrioles is shown on the right. Bars: 200 nm in A–C. (TIFF)

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Author Contributions

Conceived and designed the experiments: EOT MS TMR. Performed the experiments: EOT GG KIL MS TMR. Analyzed the data: EOT MS TMR. Contributed reagents/materials/analysis tools: EOT MS TMR. Wrote the paper: EOT MS TMR.
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