Tubulin nucleotide status controls Sas-4-dependent pericentriolar material recruitment

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Regulated centrosome biogenesis is required for accurate cell division and for maintaining genome integrity1. Centrosomes consist of a centriole pair surrounded by a protein network known as pericentriolar material1 (PCM). PCM assembly is a tightly regulated, critical step that determines the size and capability of centrosomes2–4. Here, we report a role for tubulin in regulating PCM recruitment through the conserved centrosomal protein Sas-4. Tubulin directly binds to Sas-4; together they are components of cytoplasmic complexes of centrosomal proteins5,6. A Sas-4 mutant, which cannot bind tubulin, enhances centrosomal protein complex formation and has abnormally large centrosomes with excessive activity. These results suggest that tubulin negatively regulates PCM recruitment. Whereas tubulin–GTP prevents Sas-4 from forming protein complexes, tubulin–GDP promotes it. Thus, the regulation of PCM recruitment by tubulin depends on its GTP/GDP-bound state. These results identify a role for tubulin in regulating PCM recruitment independent of its well-known role as a building block of microtubules7. On the basis of its guanine-bound state, tubulin can act as a molecular switch in PCM recruitment.

Centrosome biogenesis is a multi-step process that begins with centriole formation followed by PCM recruitment to form a functional organelle4. PCM recruitment begins with the formation of cytoplasmic protein complexes and requires3,5,8,9 Sas-4 (CPAP in humans). Recently, we reported that Sas-4, a protein known to have a role in centriole and PCM formation10,11, scaffolds centrosomal protein complexes (S-CAP complexes) that include Cnn, Asl, D-PLP, CP-190 and tubulin (αβ-tubulin dimer), and tethers the S-CAP complexes to centrosomes8. Sas-4 also exists in complexes with γ-tubulin3 and γ-tubulin ring proteins (S-γ-tubulin complexes), suggesting that Sas-4 may also be associated with the assembly intermediates of γ-tubulin ring complexes (Fig. 1). S-γ-tubulin complexes are recruited to developing centrosomes in a Sas-4-dependent manner (Fig. 2). Together, these results suggest that Sas-4 regulates PCM recruitment through several protein complex types. Interestingly, it seems that tubulin exists in the multiple Sas-4 complex types (Fig. 1b–d). As tubulin is significantly more abundant than other centrosomal proteins, Sas-4 probably interacts with free tubulin before the formation of the centrosomal protein complexes. If the Sas-4–tubulin interaction is a first step, then tubulin may regulate centrosomal complex formation and PCM recruitment.

We began testing this hypothesis by comparing the abilities of Sas-4, which can bind tubulin, to a mutated version of Sas-4, which cannot bind tubulin. For this, we used an amino-terminal fragment of Sas-4 (Sas-4-N) that includes its tubulin-binding site12,13; we also used a mutated version of Sas-4-N (Sas-4-NΔT), which lacks the two amino acids essential for tubulin binding12,13. As expected, Sas-4-NΔT failed to pull down tubulin from embryonic high-speed lysates (HSLs). Surprisingly, Sas-4-NΔT pulled down significantly more Cnn, Asl, D-PLP, γ-tubulin and Grip128 than was pulled down by Sas-4-N (Fig. 3a). We then tested the effects of tubulin on the ability of Sas-4-N or Sas-4-NΔT to bind centrosomal proteins. Increasing amounts of tubulin progressively inhibited the binding of Sas-4-N to centrosomal proteins but did not inhibit the binding of Sas-4-NΔT (Fig. 3b,c). These results suggest that tubulin can negatively regulate the formation of centrosomal protein complexes.

To test this hypothesis in vivo, we generated transgenic Drosophila that express full-length Sas-4ΔT in the sas-492214 null (sas-4-92214) phenotype of uncoordination14; sas-4-92214 flies stood but could barely walk (Supplementary Movie). Furthermore, sas-4-92214 sperm axonemes were abnormal (Supplementary Fig. S1a). These phenotypes suggest defects in centrosome biogenesis14,15 and that tubulin binding to Sas-4 is essential for centrosome function and proper cilia formation. However, sas-4-92214 flies had correct numbers of centrosomes, indicating that Sas-4ΔT rescued this aspect of...
Figure 1 Tubulin is present in each Sas-4 complex type. (a) Immunopurification of Sas-4 complexes from HSL of Drosophila embryonic extracts using anti-Sas-4 antibody revealed associations between Sas-4 with Grip proteins that are components of γ-tubulin ring complexes (γ-TuRCs): Grip91, Grip84, Grip163, Grip128 and Grip75, and γ-tubulin small complexes (γ-TuSCs): Grip91 and Grip84 (ref. 36). Embryonic extracts were used as a positive control; mouse IgG beads were used as a negative control. The use of extract depleted of centrosomes and the absence of the centriole-core protein Sas-6 indicate that the purified complexes were not centrosomes. (b) Immunopurified Sas-4 complexes fractionate at distinct densities in a 5-40% sucrose gradient. Individual fractions are analysed by western blot. Tubulin co-fractionates with Sas-4 across the gradient. Co-fractionation patterns probably represent different complex types: S-CAP (Cnn, Asl and D-PLP proteins, solid rectangle; S-γ-TuSC and S-γ-TuRC, dashed rectangles. The fractionation pattern of S-CAP complexes in a narrow range of low-density fractions, and γ-tubulin and Grip protein fractionation at intermediate and high densities, were consistent with previous reports5,36. However, the fractionation patterns of γ-TuSC and γ-TuRC protein complexes do not exhibit clear peaks as previously demonstrated, suggesting that Sas-4 interacts with assembly intermediates of γ-tubulin ring proteins. (c, d) The immunopurified Sas-4 complexes are unlikely to be part of an unstable large complex that destabilizes during immunopurification. HSL (high-speed lysate) of Drosophila embryonic extract was first fractionated in a 5–40% sucrose gradient (c) and the immunopurifications of the distinct Sas-4 complex types were performed on distinct fractions (marked by dashed rectangles and named S-CAP, S-γ-TuSCs and S-γ-TuRCs; d). Note that Sas-4 and tubulin were detected in all of the complex types. Embryonic extract was used as a positive control; mouse IgG beads were used as a negative control. In a–d, HSLs were diluted such that the tubulin concentration was below 0.2 μM to prevent tubulin polymerization; furthermore, Sas-4 complexes were purified at 4 °C in the presence of nocodazole (330 nM). In b and c, the arrows mark the corresponding peaks of the sedimentation coefficient standards.
**Figure 2** Sas-4 is essential for recruiting S-γ-tubulin complexes to centrosomes. (a–e) Centriolar structures labelled by Ana-1-GFP in control testes, but not in sas-4<sup>S2214</sup> null mutant testes, recruit components of S-γ-tubulin complexes as tested using antibodies specific to γ-tubulin, Grip75, Grip84, Grip91 and Grip163. The dashed squares mark the enlarged areas shown in the lower panels. The histograms on the right show the fraction of Ana-1-positive centriolar structures (CS) that are also positive for the respective proteins tested in the control (grey) and in sas-4<sup>S2214</sup> (white). As described previously<sup>5</sup>, Ana-1-GFP-labelled centriolar structures from each testis were counted within a 20 μm<sup>2</sup> area that is ~25 μm away from the tip of a testis (dotted lines). The mean ± s.e.m. of three independent testes are shown. (a–e) Scale bars, 10 μm (main images) and 1 μm (insets). ***P < 0.001.
Figure 3 Tubulin negatively regulates PCM recruitment. (a) Comparison of the ability of Sas-4-NAT and Sas-4-N to interact with centrosomal proteins in embryonic extracts. Increasing loading amounts of Sas-4-N (one-to-fourfold) pull down Sas-4-interacting proteins from embryonic HSL. Sas-4-NAT does not pull down tubulin, but pulls down approximately three times more Cnn, Asl, γ-tubulin and GPIP128 than Sas-4-N. Purified recombinant proteins are shown in Coomassie-stained gels. (b,c) The addition of increasing amounts of free tubulin to Sas-4-N pulldown experiments from HSL proportionally inhibits Sas-4-N binding to its interacting partners (b) with a half maximal inhibitory concentration (IC50) of 0.1–0.3 μM (c). There is no significant change in the ability of Sas-4-NAT to bind Sas-4-interacting proteins in the presence of tubulin. CP-190 binding to Sas-4-N did not change significantly, suggesting that tubulin specifically interferes with the binding of Cnn, Asl and γ-tubulin. The purified recombinant proteins used are shown in Coomassie-stained gels. (d) In Sas-4::sas-4ΔT and Sas-4::sas-4ΔT mutant spermatocytes (magenta) is not detected in interphase centrosomes. In contrast, Sas-4ΔT::sas-4ΔT interphase centrosomes contain Cnn. The dotted lines mark a cell boundary. The histogram shows the percentage of centrosomes positive for Cnn. The mean ± s.e.m. of six independent testes is shown. P = 0.001. Scale bar, 2 μm. (e) Unlike Sas-4::sas-4ΔT and Sas-4ΔT::sas-4ΔT, interphase spermatocyte centrosomes contain Cnn. Centrosomes are marked by Ana-1–tdT (red) and Sas-4–GFP (green). The squares mark the magnified areas. The histogram shows the percentage of centrosomes positive for Cnn. The mean ± s.e.m. of six independent testes is shown, P < 0.001. Scale bars, 2 μm. (f) Sas-4ΔT::sas-4ΔT mitotic centrosomes have increased Cnn immunoreactivity. The histogram shows centrosome size for Sas-4::sas-4ΔT and Sas-4ΔT::sas-4ΔT, as measured by Cnn immunolabelling. The mean ± s.e.m. of six independent testes is shown. P < 0.001. Scale bar, 2 μm. (g) Sas-4ΔT::sas-4ΔT interphase spermatocyte centrosomes emanate microtubule asters. Microtubules are stained by α-tubulin (magenta). The squares mark the magnified areas. The histogram shows the percentage of centrosomes emanating microtubule asters in Sas-4::sas-4ΔT (grey) and Sas-4ΔT::sas-4ΔT (white). The mean ± s.e.m. of six independent testes is shown. P < 0.001. Scale bar, 2 μm.

the sas-4ΔT phenotype14 (Supplementary Fig. S1b). Thus, the Sas-4–tubulin interaction is not essential for maintaining the centrosome number. Yet, sas-4ΔT::sas-4ΔT spermatocyte centrosomes were slightly shorter (Supplementary Fig. S1c). This is consistent with reports that Sas-4, and in particular, the tubulin–Sas-4 interaction, is required for centriole elongation13,16–18.
In addition to its well-known role in centriole formation, Sas-4 plays an important role in PCM formation and in regulating centrosome size. Achieving proper centrosome size and capability requires Sas-4 and Cnn (refs 2,3). As Sas-4 scaffolds centrosomal complexes that include Cnn, regulation of Sas-4 complex formation may indirectly control centrosome size. Indeed, although Cnn is normally detected only in mitotic or meiotic centrosomes, interphase spermatogonium and spermatocyte centrosomes of *sas-4 ΔT::sas-4* 2214 contained Cnn (Fig. 3d,e; ref. 19). Moreover, mitotic and meiotic centrosomes of *sas-4 ΔT::sas-4* 2214 contained twice the Cnn immunolabelling as control centrosomes (Fig. 3f and Supplementary Fig. S2a). Thus, tubulin can negatively regulate the timing, distribution and quantity of protein recruitment to centrosomes, through Sas-4.

In Drosophila, interphase centrosomes do not nucleate microtubules. As *sas-4 ΔT::sas-4* 2214 centrosomes prematurely contain Cnn and the human orthologue of Cnn stimulates microtubule nucleation, we investigated whether the *sas-4 ΔT* mutation affects microtubule nucleation. Interphase *sas-4 ΔT::sas-4* 2214 centrosomes had premature microtubule nucleation (Fig. 3g) and their meiotic centrosomes had massive microtubule asters, which could fill a significant fraction of a cell (Supplementary Fig. S2b,c). Similarly, in cultured cells, *sas-4 ΔT* produced massive asters (Supplementary Fig. S2d,e). These results suggest that the tubulin present in wild-type Sas-4 complexes is not a building block of microtubule asters, but instead seems to be essential in the regulation of PCM recruitment.

To gain insight into how disruption of the Sas-4–tubulin interaction affects meiosis and mitosis, we analysed spermatids and larval brain cells. We found that over 95% of *sas-4 ΔT::sas-4* 2214 round spermatids exhibit normal morphology, suggesting that meiotic cell division can conclude normally (Supplementary Fig. S2f). In larval brain cells, unlike control cells, which recruit significant amounts of Cnn and form robust asters only during mitosis, *sas-4 ΔT::sas-4* 2214 cells recruited Cnn and formed asters before entry into mitosis (Supplementary Fig. S3a–c). During mitosis, control larval brain cells have Cnn enrichment in only one centrosome, but in *sas-4 ΔT::sas-4* 2214 cells, Cnn was distributed more evenly to both centrosomes (Supplementary Fig. S3d). Finally, spindle orientation relative to Bazooka’s crescent (a polarity establishment marker), were abnormal in *sas-4 ΔT::sas-4* 2214, suggesting that these centrosomes have difficulty properly aligning their spindles (Supplementary Fig. S3e,f). Taken together, these results suggest that the interaction of tubulin with Sas-4 is essential for normal PCM recruitment and centrosome function in larval brain cells.

To better understand how tubulin operates in the regulation of PCM recruitment, we focused on the biochemical properties of the Sas-4–tubulin interaction. Tubulin is a guanine binding protein having GTase activity, which hydrolyses tubulin–GTP into tubulin–GDP (ref. 7). Tubulin has a different conformation when present as tubulin–GTP versus tubulin–GDP and its conformation acts as a molecular switch that regulates microtubule dynamics. Therefore, we speculated that the conformation of tubulin might also regulate the formation of Sas-4 complexes. For this, we analysed the binding of tubulin to Sas-4-N in the presence of GDP or GMPCPP (a non-hydrolysable GTP analogue) 24. Sas-4-N, which includes the tubulin-binding site of Sas-4, prevents microtubule polymerization when present in excess. Tubulin–GMPCPP at 0.5 μM (which is below the concentration necessary for microtubule polymerization) had fourfold less binding to Sas-4-N than tubulin–GDP at the same concentration (Fig. 4a). Similar results were obtained by isothermal titration calorimetry experiments, indicating that tubulin–GDP has a higher affinity for Sas-4 than tubulin–GMPCPP has (Supplementary Fig. S4). However, the affinity of tubulin to Sas-4 seems to be high (Fig. 3b,c and Supplementary Fig. S4) relative to the cytoplasmic concentration of free tubulin (~10 μM; ref. 27), it is likely that cytoplasmic Sas-4 is bound to either tubulin–GDP or tubulin–GTP. Therefore, it is possible that conformation of this bound tubulin (depending on which guanine is present) regulates the formation of Sas-4-containing complexes.

To investigate this, we purified and analysed Sas-4 complexes from HSLs exposed to GDP or GMPCPP. Although the quantity of Sas-4 present in the purified complexes was unaffected by GDP or GMPCPP exposure, the amounts of other centrosomal proteins in the Sas-4 complex were affected. More specifically, HSLs exposed to GDP had 6–12-fold increases in the amounts of particular centrosomal proteins relative to HSLs exposed to GMPCPP (Fig. 4b,c). Therefore, when bound to tubulin–GDP, Sas-4 acts similarly to Sas-4-ΔT in that it accumulates excess centrosomal proteins in its complexes. Perhaps, the binding of tubulin–GTP to Sas-4 sterically hinders the binding of Sas-4 to other centrosomal proteins and tubulin–GDP reverses the steric hindrance, allowing Sas-4 to bind the centrosomal proteins. Together, it seems that the binding of Sas-4 to tubulin–GDP (but not tubulin–GTP) favours the formation of centrosomal protein complexes.

To confirm this, we first investigated whether Sas-4 complexes preferentially contain GDP. We immunopurified Sas-4 complexes from embryonic HSLs treated with [α-32P]GTP and analysed the complexes using thin-layer chromatography. Tubulin that was not bound to Sas-4 contained [α-32P]GTP, whereas purified Sas-4 complexes instead contained mostly [α-32P]GDP, which is the hydrolysed product of [α-32P]GTP (Fig. 4d). Sas-4-N, but not Sas-4-NΔT, was able to pull down GDP (Fig. 4e). Accordingly, when in a Sas-4 complex, tubulin binds GDP.

Second, we investigated the effects of treatments with griseofulvin, a compound that changes the conformation of tubulin and induces hydrolysis of tubulin-bound GTP into GDP (ref. 28), on the composition of Sas-4 complexes. Griseofulvin increased the quantity of centrosomal proteins in purified Sas-4 complexes (Fig. 4f); this is consistent with our data for HSLs exposed to GDP (Fig. 4b,c). Together, these results suggest that the conformation of tubulin can regulate the formation of cytoplasmic Sas-4 complexes.

We then studied how tubulin modulates PCM recruitment. Typical GTP-binding proteins (G proteins), that is, heterotrimeric G proteins and the small GTPases belonging to the Ras superfamily, act as molecular switches whose function depends on their GTP- or GDP-bound state. G proteins have both low intrinsic GTase and guanine exchange activities and require GTase-activating proteins (GAPs) and guanine exchange factors (GEFs) as catalysts. Accordingly, we investigated whether tubulin can exhibit the characteristics of a typical G protein during PCM recruitment by acting as a molecular switch.

It is known that free tubulin has low intrinsic GTase activity and exists as tubulin–GTP (ref. 7). Therefore, if a tubulin switch is involved in PCM recruitment, it is expected that a GAP exists that induces tubulin to hydrolyse its bound GTP into GDP. We investigated whether Sas-4 functions as a tubulin GAP and found that Sas-4-N

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enhanced the intrinsic GTPase activity of tubulin, as measured by the release of inorganic phosphate (Fig. 5a). This suggests that Sas-4 can function as a tubulin GAP.

Tubulin is known to have high guanine exchange activity and readily exchanges its GDP with GTP (ref. 31). On the other hand, although tubulin–GTP disfavors the formation of centrosomal protein complexes (Fig. 4b), Sas-4 complexes are quite stable regardless of whether they are exposed to GDP or GMPCPP (Supplementary Fig. S5a–c). Therefore, for a Sas-4 complex to remain stable, the guanine exchange activity of tubulin must remain low. To assay the effects of Sas-4 on the guanine exchange activity of tubulin, we added \([\alpha-\text{32P}]\text{GTP}\) to tubulin bound to Sas-4 (Sas-4–N) or not bound to Sas-4 (Sas-4–N\(\Delta T\)). The amount of exchanged \([\alpha-\text{32P}]\text{GTP}\) was then determined by scintillation counting and thin-layer chromatography (Fig. 5b and Supplementary Fig. S5d). Consistent with previous reports, tubulin had a high rate of guanine exchange in the absence of bound \(^{32}\text{P}\text{GTP} (n = 3)\).}

\(\frac{\text{Relative intensity (fold)}}{\text{control}}\) (Fig. 5b and Supplementary Fig. S5d). This indicates that centrosomes seem to undo the inhibition of the guanine exchange when tubulin is unbound to Sas-4.

In contrast, GTP exchange was not observed in the presence of Sas-4. These results suggest that Sas-4 inhibits the guanine exchange activity of tubulin and can stabilize the Sas-4–tubulin complex.

Eventually, Sas-4 complexes are recruited to centrosomes. So, we investigated how centrosomes affect guanine exchange and the stability of Sas-4 complexes. In the presence of centrosomes, tubulin did not have an increase in guanine exchange when tubulin is unbound to Sas-4 (Fig. 5b and Supplementary Fig. S5d). This indicates that centrosomes cannot increase the intrinsic guanine exchange activity of tubulin. In contrast, in the presence of centrosomes, the guanine exchange activity of tubulin was significantly increased when it was bound to Sas-4.

Therefore, centrosomes seem to undo the inhibition of the guanine exchange activity of tubulin by Sas-4. Consistently, centrosomes also destabilize the Sas-4–tubulin interaction (Fig. 5c). In the absence of centrosomes or in the presence of Sas-4 complexes, Sas-4–tubulin complexes remained stable; however, in the presence of Sas-4 complex, Sas-4 complexes were recruited to centrosomes.
Figure 5 GAP and guanine exchange activities in PCM recruitment. (a) Sas-4-N functions as a tubulin GAP. Specific activity of tubulin GTPase as determined by [$\text{P}]$ release (micromoles per minute per micromole of tubulin). As the GTPase activity is greater at low tubulin concentrations, the observed increase in GTPase activity is unlikely to be mediated by tubulin–tubulin interactions occurring during microtubule polymerization.\textsuperscript{6,12,25} (b) Centrosomes induce guanine exchange of the tubulin–Sas-4 complex. [$\alpha$-32P]GTP was added to biotinylated tubulin bound to Sas-4 (Sas-4-N) or not bound to Sas-4 (Sas-4-NΔT). Scintillation counting shows that the inclusion of centrosomes increases the GTP exchange of the Sas-4–tubulin complex but not free tubulin. (a,b) The mean ± s.e.m. of three independent experiments is shown. (c) Centrosomes disrupt the Sas-4–tubulin–GDP interaction. When GMPCPP or GDP is added to the Sas-4–biotinylated–tubulin–GDP complex immobilized to resin through Sas-4-N, biotinylated tubulin remains bound to tubulin–GDP (upper row); likewise, when GMPCPP or GDP is added to the Sas-4–biotinylated–tubulin–GDP complex immobilized to resin through Sas-4-N, biotinylated tubulin remains bound to Sas-4-N. However, when centrosomes (+Cen) and GMPCPP are added together, the interaction between tubulin and Sas-4 is weakened, releasing the partner that is not immobilized to the resin; this is not observed when centrosomes and GDP are added together. (d) Centrosomes can induce Sas-4 complex disassembly allowing Sas-4–interacting proteins to remain in the centrosome and Sas-4 to be released into the cytoplasm. Isolated centrosomes (Cen) were mixed with Sas-4 complexes (Com) in the presence of GMPCPP or GDP, and subjected to velocity sedimentation. Proteins bound to the centrosome are found in the pellet (P), whereas proteins not bound to the centrosome are found in the supernatant (S). (e) Taxol-treated (1 μm) mitotic centrosomes of S2 cells have a reduced amount of Cnn (magenta). S2 cells transfected with Sas-4 but not with Sas-4 are less sensitive to taxol treatment. Scale bar, 2 μm. (f) Griseofulvin-treated (250 μM) mitotic centrosomes of S2 cells have an increased amount of Cnn. Scale bar, 2 μm. (e,f) Signal intensity with mean ± s.e.m. of ten cells is shown. (g) Model for tubulin in regulating PCM recruitment. Uncropped images of blots/gels are shown in Supplementary Fig. S6.
of centrosomes exposed to GMPCPP, Sas-4–tubulin complexes were destabilized and dissociated, potentially allowing Sas-4 to be released into the cytoplasm (Fig. 5c).

To further investigate this, we mixed isolated centrosomes, purified Sas-4 complexes and either GMPCPP or GDP. The reaction mixture was subjected to velocity sedimentation, which pelleted centrosomes along with their bound proteins. When exposed to GDP, Sas-4–complex proteins, Sas-4 and tubulin were in the pellet (Fig. 5d), indicating that the Sas-4 complexes were bound to centrosomes. However, when exposed to GMPCPP, the Sas-4–complex proteins were in the pellet, yet some Sas-4 and tubulin were released into the supernatant. This indicates that centrosomes have guanine exchange activity that releases Sas-4 and tubulin from Sas-4 complexes, whereas other complex proteins remain in the centrosome (Fig. 5d). This is consistent with the observation that Sas-4 traffics between centrosomes and cytoplasm.

PCM recruitment is tightly coupled to the cell cycle32. Mathematical models and analyses of global cytoskeleton remodelling predict that microtubule breakdown releases tubulin–GDP, causing its concentration to increase when cells enter mitosis. Given our above observations that tubulin–GDP promotes complex formation, this increase in its concentration may promote PCM recruitment. At present, there are no tools to determine this directly. Therefore, we analysed centrosomes of cells treated with taxol, a compound that stabilizes microtubules and reduces tubulin–GDP release into the cytoplasm33. As expected, taxol-treated mitotic centrosomes of Sas-4–GFP-transfected cells had significantly less Cnn, whereas, mitotic centrosomes of Sas-4 AT-transfected cells were less sensitive to taxol (Fig. 5e). Although taxol may affect centrosomes through multiple mechanisms, our data suggest that cytoskeleton remodelling regulates recruitment of Sas-4 complexes to centrosomes. Furthermore, treating cells with griseofulvin, which enhances Sas-4 complex formation, recruitment of Sas-4 complexes to the centrosome (Fig. 5f). Together, the taxol and griseofulvin experiments show that modulating tubulin in cells affects PCM formation.

Our findings reveal a previously unknown function of tubulin. We show that tubulin can negatively control Sas-4 complex formation and, thereby, regulate PCM recruitment. Tubulin is a molecular switch that can regulate the formation of Sas-4 complexes and the recruitment of centrosomal proteins to a developing centrosome. The data described above were used to formulate a model whereby tubulin coordinates normal PCM recruitment (Fig. 5g). In the cytoplasm, tubulin–GTP binds Sas-4, which prevents Sas-4 from forming complexes with centrosomal proteins (Fig. 5gi). When Sas-4 activates the GTPase of tubulin, hydrolysis of tubulin–GTP into tubulin–GDP takes place; tubulin–GDP can initiate Sas-4 complex formation (Fig. 5gii). In addition, Sas-4 complex formation may be enhanced when the GTPase activity of tubulin is increased owing to microtubule depolymerization (Fig. 5gii). Sas-4 binding to tubulin–GDP stabilizes the Sas-4–tubulin complex by blocking the exchange of GDP with GTP. Sas-4–tubulin–GDP then interacts with other centrosomal proteins to form one of the various types of Sas-4-containing complex (Fig. 5gvi). When a Sas-4 complex tethers to a centrosome, the guanine exchange activity of tubulin is induced by the centrosome, causing the release of tubulin and Sas-4 and allowing the recruitment of centrosomal proteins to the centrosome (Fig. 5gv).

Typical G proteins act as molecular switches whose function depends on their GDP- or GTP-bound state. Here, during PCM recruitment, we show that tubulin acts as a molecular switch whose function depends on its GDP- or GTP-bound state. Therefore, in PCM recruitment, tubulin acts like a typical G protein. By manipulating this switch, it may be possible to target cancerous cells, which are known to have abnormal centrosomes and PCM (ref. 1).

METHODS

Methods and any associated references are available in the online version of the paper.

Note: Supplementary Information is available in the online version of the paper.

ACKNOWLEDGEMENTS

We would like to thank J. Iwasa for scientific illustrations; T. Mitchison, A. Johnson, I. Cheeseman and J. Malicki for scientific discussions; T. Kaufman, J. Raff, R. Raynaud-Messina and T. K. Tang (Institute of Biomedical Sciences, Taipei, Taiwan) for reagents; R. Reed laboratory (Harvard Medical School, USA), F. Eric, A. Hari, R. Rodriguez for technical help with biophysical experiments; E. Koundakjian for scientific editing and discussions; and electron microscopy facility staff at HMS for help with electron microscopy analyses. This work was supported by a grant (RO1GM098394) from the National Institute of General Medical Sciences.

AUTHOR CONTRIBUTIONS

J.G. and T.A.R. conceived the project. J.G performed most of the experiments described herein. T.A.R. supervised the project. Y.-F.C. performed phase and electron microscopy analyses. A.H. performed biochemical complex analyses. M.L.B. generated constructs and took part in the biochemical purification of recombinant proteins. D.A.L. and N.M.R. advised on and discussed larval brain analyses.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

Published online at www.nature.com/doifinder/10.1038/ncel2527

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1. Nigg, E. A. & Raff, J. W. Centrioles, centrosomes, and cilia in health and disease. Cell 139, 663–678 (2009).
2. Conduit, P. T. et al. Centrosomes regulate centrosome size by controlling the rate of Cnn incorporation into the PCM. Curr. Biol. 20, 2178–2186 (2010).
3. Kirkham, M., Muller-Reichert, T., Oegema, K., Grill, S. & Hyman, A. A. SAS-4 is a C. elegans centriolar protein that controls centrosome size. Cell 112, 575–587 (2003).
4. Pielh, M., Tulu, U. S., Wadsworth, P. & Cassimeris, L. Centrosome maturation: measurement of microtubule nucleation throughout the cell cycle by using GFP-tagged EB1. Proc. Natl Acad. Sci. USA 101, 1584–1588 (2004).
5. Gopalakrishnan, J. et al. Sas-4 provides a scaffold for cytoplasmic complexes and tethers them in a centrosome. Nat. Commun. 2, 359 (2011).
6. Hung, L. Y., Chen, H. L., Chang, C. W., Li, B. R. & Tang, T. K. Identification of a novel microtubule-destabilizing motif in CPAP that binds to tubulin heterodimers and inhibits microtubule assembly. Mol. Biol. Cell 15, 2797–2806 (2004).
7. Desai, A. & Mitchison, T. J. Microtubule polymerization dynamics. Annu. Rev. Cell Dev. Biol. 13, 83–117 (1997).
8. Dammermann, A., Maddox, P. S., Desai, A. & Oegema, K. SAS-4 is recruited to dynamic structures in newly forming centrioles that is stabilized by the γ-tubulin–mediated addition of centriolar microtubules. J. Cell Biol. 180, 771–785 (2008).
9. Hung, L. Y., Tang, C. J. & Tang, T. K. Protein 4.1 R-135 interacts with a novel centrosomal protein (CPAP) which is associated with the γ-tubulin complex. Mol. Cell Biol. 20, 7813–7825 (2000).
10. Pielhier, L., O’toole, E., Schwager, A., Hyman, A. A. & Muller-Reichert, T. Centriole assembly in Caenorhabditis elegans. Nature 444, 619–623 (2006).
11. Leidel, S. & Gonczy, P. SAS-4 is essential for centrosome duplication in C elegans and is recruited to daughter centrioles once per cell cycle. Dev. Cell 4, 431–439 (2003).
12. Hsu, W. B. et al. Functional characterization of the microtubule-binding and -destabilizing domains of CPAP and d-SAS-4. Exp. Cell Res. 314, 2591–2602 (2008).
13. Tang, C. J., Fu, R. H., Wu, K. S., Hsu, W. B. & Tang, T. K. CPAP is a cell-cycle regulated protein that controls centriole length. Nat. Cell Biol. 11, 825–831 (2009).
14. Basta, R. et al. Flies without Centrioles. Celf 125, 1375–1386 (2006).
15. Blachon, S. et al. Drosophila asterless and vertebrate Cep152 Are orthologs essential for centriole duplication. Genetics 180, 2081–2094 (2008).
16. Blachon, S. et al. A proximal centriole-like structure is present in Drosophila spermatids and can serve as a model to study centriole duplication. Genetics 182, 133–144 (2009).
17. Kohlmaier, G. et al. Overly long centrioles and defective cell division upon excess of the SAS-4-related protein CPAP. *Curr. Biol.* **19**, 1012–1018 (2009).
18. Schmidt, T. I. et al. Control of centriole length by CPAP and CP110. *Curr. Biol.* **19**, 1005–1011 (2009).
19. Li, K. et al. *Drosophila* centrosomin protein is required for male meiosis and assembly of the flagellar axoneme. *J. Cell Biol.* **141**, 455–467 (1998).
20. Rogers, G. C., Rusan, N. M., Peifer, M. & Rogers, S. L. A multicomponent assembly pathway contributes to the formation of acentrosomal microtubule arrays in interphase *Drosophila* cells. *Mol. Biol. Cell* **19**, 3163–3178 (2008).
21. Choi, Y. K., Liu, P., Sze, S. K., Dai, C. & Qi, R. Z. CDK5RAP2 stimulates microtubule nucleation by the γ-tubulin ring complex. *J. Cell Biol.* **191**, 1089–1095 (2010).
22. Rusan, N. & Peifer, M. A role for a novel centrosome cycle in asymmetric cell division. *J. Cell Biol.* **177**, 13–33 (2007).
23. Giansanti, M., Gatti, M. & Bonaccorsi, S. The role of centrosomes and astral microtubules during asymmetric division of *Drosophila* neuroblasts. *Development* **128**, 1137–1182 (2001).
24. Sandoval, I. V., Jameson, J. L., Niedel, J., MacDonald, E. & Cuatrecasas, P. Role of nucleotides in tubulin polymerization: effect of guanosine 5′-methylene diphosphonate. *Proc. Natl Acad. Sci. USA* **75**, 3178–3182 (1978).
25. Cormier, A. et al. The PN2-3 domain of centrosomal P4.1-associated protein implements a novel mechanism for tubulin sequestration. *J. Biol. Chem.* **284**, 9899–9917 (2009).
26. Mozzoneacci, J., Sandblad, L., Wachsmuth, M., Brunner, D. & Karsenti, E. Tubulin dimers oligomerize before their incorporation into microtubules. *PLoS One* **3**, e3821 (2008).
27. Hiller, G. & Weber, K. Radioimmunoassay for tubulin: a quantitative comparison of the tubulin content of different established tissue culture cells and tissues. *Cell* **14**, 795–804 (1978).
28. David-Pfeuty, T., Simon, C. & Pantaloni, D. Effect of antimotic drugs on tubulin GTPase activity and self-assembly. *J. Biol. Chem.* **254**, 11696–11702 (1979).
29. Cabrera-Vera, T. M. et al. Insights into G protein structure, function, and regulation. *Endocr. Rev.* **24**, 765–781 (2003).
30. Takai, Y., Sasaki, T. & Matozaki, T. Small GTP-binding proteins. *Physiol. Rev.* **81**, 153–208 (2001).
31. Melki, R., Carlier, M. F., Pantaloni, D. & Timasheff, S. N. Cold depolymerization of microtubules to double rings: geometric stabilization of assemblies. *Biochemistry* **28**, 9143–9152 (1989).
32. Kobayashi, T. & Dynlacht, B. D. Regulating the transition from centriole to basal body. *J. Cell Biol.* **193**, 435–444 (2011).
33. Janulevicius, A., van Pelt, J. & van Ooyen, A. Compartment volume influences microtubule dynamic instability: a model study. *Biophys. J.* **90**, 788–798 (2006).
34. Zhai, Y., Kronebusch, P. J., Simon, P. M. & Borisy, G. G. Microtubule dynamics at the G2/M transition: abrupt breakdown of cytoplasmic microtubules at nuclear envelope breakdown and implications for spindle morphogenesis. *J. Cell Biol.* **135**, 201–214 (1996).
35. Schift, P. D. & Horwitz, S. B. Taxol stabilizes microtubules in mouse fibroblast cells. *Proc. Natl Acad. Sci. USA* **77**, 1561–1565 (1980).
36. Oegema, K. et al. Characterization of two related *Drosophila*-tubulin complexes that differ in their ability to nucleate microtubules. *J. Cell Biol.* **144**, 721–733 (1999).
METHODS

Plasmids, cells and flies. Transgenic flies and sas-4 constructs were prepared as described previously13,15. P[lacW][3]S2214 (sas-4E224) and sas-4 complementary DNA (GM21734) were obtained from the Bloomington Stock Center and Berkeley Drosophila Genome Project, respectively. sas-4-N and sas-4-X7 (Arg 114 and 115 of Sas-4 were replaced with glutamic acid) constructs in pGEX-2T were a gift from the Tang laboratory. sas-4-N was subcloned into a modified pET24(+)-vector, which contained both a Bgl II and His tag at the N terminus, to generate a fusion protein37. To generate Drosophila expressing full-length Sas-4:AT in the sas-4E224 null background, the sas-4 cDNA amino acids Arg 114 and 115 of Sas-4 were replaced with glutamic acids. For in vivo expression of wild-type or mutant Sas-4, constructs that include the sas-4 promoter were subcloned into a PUA vector with an in-frame carboxy-terminal GFP such that Sas-4 was expressed at near physiological levels and was not overexpressed. Stable Drosophila S2 cell lines expressing variants of Sas-4 were prepared as previously described13,15. For experiments with taox, Drosophila S2 cells were incubated for 10 h with 50 nM taox.

Drosophila embryonic extracts. Drosophila embryonic extracts of low-speed lysates or HSLS were prepared as described previously38. Low-speed lysates was used to isolate centrosomes. HSL was used for the immunopurification of Sas-4 complexes and pulldown assays. All HSLS used in this study contained 330 nM nocodazole and all manipulations were performed at 4 °C to avoid any spontaneous microtubule nucleation.

Western blot. Samples were resolved in 8% polyacrylamide gels and transferred into nitrocellulose membranes. The blots were incubated with primary antibodies overnight at 4 °C followed by peroxidase-conjugated secondary antibodies at room temperature for 1 h. Super Signal West Pico or Femto Chemiluminescent substrate (Pierce) was used to detect peroxidase activity. Molecular masses were determined by comparison to Precision Plus Protein Standard (Bio-Rad).

Antibodies. Antibody concentrations were as follows. Western blots: mouse anti-Sas-4 (1:500; ref. 5), rabbit anti-Cnn (1:5,000, courtesy of C. Kaufman, Indiana University, Bloomington, Indiana, USA), rabbit anti-Asl (1:5,000; ref. 15), rabbit anti-D-PLP (1:1,000, courtesy of M. Pfeiffer, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA), mouse anti-γ-tubulin (1:5,000, Sigma-Aldrich, T6557, clone GTU-88), rabbit anti-β-tubulin (1:5,000, Sigma, T9392), rabbit anti-Sas-6 (1:5,000; ref. 39), rabbit anti-D-PLP (1:5,000; ref. 20), rabbit anti-Grip antibodies (1:5,000, courtesy of B. Raymond-Messina (Centre National de la Recherche Scientifique—Pierre Fabre, Toulouse, France) and D. Agard (University of California—San Francisco, San Francisco, California, USA) and rabbit anti-CP-190 (1:5,000, courtesy of D. Agard); peroxidase-conjugated secondary antibodies were used at 1:5,000 (Vector Labs). Immunofluorescence analysis: rat anti-α-tubulin (1:200, Chemicon, MAB1864, Lot 0601019632), mouse anti-γ-tubulin (1:200, Sigma-Aldrich, T6557, clone GTU-88), anti-Sas-4 (1:100), rabbit anti-Cnn (1:200, courtesy of C. Kaufman), rabbit anti-Asl (1:200, ref. 15), rabbit anti-phospho H3 (1:200, Sigma-Aldrich, H0412), rabbit anti-bazooka (1:1,000, courtesy of A. Wodarz38, Institut für Genetik, Heinrich-Heine-Universität Düsseldorf, Universitätstrasse, Düsseldorf, Germany) and rabbit anti-D-PLP (1:1,000, courtesy of J. Raff, The Gurdon Institute, Cambridge, UK); secondary antibodies Cy5 goat anti-mouse and rhodamine donkey anti-rat were used at 1:200 (Jackson ImmunoResearch, 115-175-146 and 115-295-146), DAPI (1 μg ml–1, Sigma) stained DNA.

Recombinant and purified proteins. Sas-4-N and Sas-4-NAT were expressed in Esherichia coli strain BL21(DE3)pLysE by IPTG induction. For tubulin binding to GST-Sas-4-N, isotothermal titration calorimetry (ITC) and GTP experiments, buffer containing recombinant proteins was exchanged with sucrose-free embryonic extract buffer. Recombinant proteins were then subjected to size-exclusion chromatography using a Superdex 200 column and the purified peak fractions were collected. Bovine tubulin (TL258) and biotinylated porcine tubulin (T333P) were obtained from Cytoskeleton.

Immunofluorescence and electron microscopy. Pupal tests were dissected in PBS and permeabilized with 0.1% Triton X-100 in PBS for 10 min. Blocking was done with 1% BSA and 0.1% Triton X-100 in PBS for 45 min. Antibody labelling was performed for 1 h at room temperature or overnight at 4 °C followed by three washes in PBS. Confocal images were collected using a Leica TCS SP5 scanning confocal microscope. Images were processed using Adobe Photoshop. Electron microscopy was performed as previously described15,19.

Immunopurification of native Sas-4 complexes and pulldown assays. Embryonic HSL was added to the anti-Sas-4-coated protein G beads and incubated at 4 °C for 4 h. The beads were extensively washed with sucrose-free embryonic extract buffer with 0.1% Triton X-100, and then washed twice with embryonic extract buffer. Sas-4 complexes were eluted from the beads using 500 μg ml–1 of a synthetic peptide that encompassed the epitope sequence of Sas-4 (5′-GLKQRQFKNIDPLQRELHYKANHPQ-3′).

For GST pulldowns, the bait was a recombinant GST-tagged Sas-4-N or Sas-4-NAT immobilized to glutathione–agarose resin. The bait-bound resins were mixed with HSL for 3 h at 4 °C. Glutathione (20 mM) was used to elute the complexes from resins. An anti-tubulin antibody (Sigma, T3952) was used for tubulin immunopurification in the co-immunoprecipitation experiments.

Sucrose gradient velocity sedimentation. Immunopurified Sas-4 complexes or centrosomes were fractionated using sucrose gradient velocity sedimentation of 15–60% or 5–40%. Centrifugation at 243,000g for 13 h at 4 °C was performed using an SW-40 rotor (Beckman Coulter). For sedimentation coefficient standards, we used BSA (4.35), alcohol dehydrogenase (7.4S), beta-amylose (8.9S) and bovine globulin (12S) that ran on an identical gradient.

Biochemical assays. To test for [α-32P]GDP incorporation in Sas-4 complexes, [α-32P]GTP (3,000 Ci mmol–1; PerkinElmer) was spiked into HSL. Sas-4 complexes were immunopurified and eluted using 8 M urea solution. Denatured proteins were then spun in a 3 kD cut-off centrifuge filter (Millipore) and the filtrate was spotted onto a thin-layer chromatography (TLC) sheet (cellulose PEI, Sigma). The air-dried sheet was developed in 1 M NaH2PO4, pH 4 until the front reached 9 cm beyond the sample application line. The TLC sheets were exposed to a Molecular Dynamics phosphorimager screen (Bio-Rad). Standards of GDP and GTP were run in parallel and their relative positions were marked after using ultraviolet illumination. A similar procedure was followed for measuring [α-32P]GTP uptake by the Sas-4-N–tubulin complex. In addition to TLC, scintillation counting was performed. In these experiments, biotinylated tubulin was bound with streptavidin resins to avoid analysing endogenous tubulins.

GAP activity of Sas-4 was estimated according to the manufacturer’s instruction using a malachite green reagent (GTPase assay kit, Innova Biosciences). The colour change due to the release of inorganic phosphate was measured photometrically at 650 nm.

To investigate the effect of centrosomes on the stability of Sas-4–tubulin–GDP, a biotinylated-tubulin was used. A Sas-4–tubulin–GDP complex was initially made by mixing Sas-4-N and biotinylated-tubulin in the presence of GDP. The resulting mixture was bound to either streptavidin (through biotin) or IgG (through the GBI tag of Sas-4–tubulin) resins. Step gradient velocity sedimentation was used as previously described to investigate the effect of the guanine exchange activity of centrosomes on the stability of native Sas-4–complexes39.

ITC. Protein samples were prepared in sucrose-free embryonic extract buffer with 0.5 mM TCEP. ITC (ITC200 system, Microcal) was run at an equilibrium temperature of 25 °C. The concentration of the protein in the well (tubulin) was 20 mM and the concentration of the protein (Sas-4–N) in the syringe was 200 mM. The results were integrated using Origin (OriginLab) and fitted with the nonlinear least-squares curve fitter provided by the software package.

Statistical methods. Statistical analyses were done with GraphPad Prism 5. A two-tailed, unpaired Student t-test (with samples that do not have equal variances) was used to test the difference between Sas-4–sas-4E224 and Sas-4–T immobilized to glutathione–agarose resin. The bait-bound resins were mixed with HSL for 3 h at 4 °C. Glutathione (20 mM) was used to elute the complexes from resins. An anti-tubulin antibody (Sigma, T3952) was used for tubulin immunopurification in the co-immunoprecipitation experiments.

13. Zhou, P., Lugovskoy, A. A. & Wagner, G. A solubility-enhancement tag (SET) for NMR studies of poorly behaving proteins. J. Biomol. NMR. 20, 11–14 (2001).
14. Moritz, M. et al. Three-dimensional structural characterization of centrosomes from early Drosophila embryos. J. Cell Biol. 130, 1149–1159 (1995).
15. Gopalkrishnan, J. et al. Self-assembling SAS-6 multimer is a core centriole building block. J. Biol. Chem. 285, 8759–8770 (2010).
16. Wemmer, E., Ramrath, A., Kinkela, U. & Knust, E. Bazooka provides an apical cue for Inscurtate localization in Drosophila neuroblasts. Nature 402, 544–547 (1999).
Figure S1 Sas-4ΔT does not affect centrosome numbers but affects centriole size and axoneme structure. (a) The tubulin-Sas-4 interaction is essential for normal cilium structure. The sperm tail normally contains two mitochondrial derivatives (yellow arrows) and an axoneme with distinct structure (red arrow). sas-4ΔT::sas-4Δs2214 sperm contain the two mitochondrial derivatives but lack a normal axoneme. Scale bar, 500 nm; and 100 nm for lower and higher magnification, respectively. (b) The tubulin-Sas-4 interaction is dispensable for normal centrosome number. Spermatogonium cells of sas-4::sas-4Δs2214 (control) and sas-4ΔT::sas-4Δs2214 exhibit the same numbers of centrosomes, indicating that Sas-4ΔT rescued this aspect of the sas-4Δs2214 phenotype. Dotted lines mark testes boundaries. The mean ± SD of six independent tests are shown. Significance p=0.61. Scale bar, 10 μm; and 1 μm for lower and higher magnification, respectively. (c) Based on Ana-1-tdT labeling3, sas-4ΔT::sas-4Δs2214 exhibits a slight reduction (~20-%) in centriole length compared to sas-4::sas-4Δs2214 (control). Centriole lengths were measured at Stages S3-S13 of spermatocyte development. *** marks the significant difference (P<0.001) in centriole length between Sas-4 and Sas4-ΔT.
**Figure S2** sas-4ΔT::sas-4Δ2214 exhibits an increased amount of PCM and microtubules. (a) Immunostaining with α-tubulin antibody and Cnn confirm the increased amount of spindle microtubules and Cnn recruitment during meiosis in sas-4ΔT::sas-4Δ2214 compared to sas-4::sas-4Δ2214 (control). Analysis by phase microscopy finds that mature spermatocytes (b) and early spermatids (c) of sas-4ΔT::sas-4Δ2214 exhibit massive microtubule asters (b, yellow lines) emanating from the centrosomes, which is surrounded by an enlarged volume of PCM (red dotted line). Microtubule asters in meiotic spermatocytes of sas-4ΔT::sas-4Δ2214 are stained with α-tubulin antibody (purple in c). White solid line, cell membrane. White dotted line, nucleus. n=61. Scale bar, 2 μm. (d-e) Overexpression of Sas-4ΔT in Drosophila cells induces the formation of centrosomes that emanate excessive microtubule asters. Sas-4-GFP (control) and Sas-4ΔT-GFP overexpression in S2 cells label the centrosomes and induce additional foci containing Sas-4 and Asl (a, magenta) or D-PLP (b, magenta). One focus in Sas-4ΔT-GFP overexpressed S2 cells emanates excessive microtubule asters and is presumed to be the centrosome. Microtubules are marked by anti α-tubulin antibody (red). DNA is stained by DAPI (Blue). Scale bar, 1 μm. (f) Spermatids of sas-4::sas-4Δ2214 and sas-4ΔT::sas-4Δ2214 exhibit normal morphology. Round spermatids contain a phase-light nucleus and a phase-dark mitochondrial derivative of similar size, suggesting that meiotic cell division can conclude normally. Scale bar, 10 μm.
Figure S3  

**Figure S3**  

**sas-4ΔT::sas-4^2214** brain cells exhibit premature Cnn recruitment microtubule aster formation and exhibit abnormal mitosis.  

(a) Interphase cells, which are characterized by uncondensed chromosomes and attached centrosomes, contain significant amounts of Cnn (magenta) in sas-4ΔT::sas-4^2214 but not in sas-4::sas-4^2214. N=65, Scale bar, 2 μm.  

(b) Interphase cells of sas-4ΔT::sas-4^2214 but not control cells nucleate robust microtubule asters (Interphase), whereas mitotic cells of both sas-4ΔT::sas-4^2214 and control nucleate microtubules (Mitosis).  

Phosphohistone H3 (red) was used to differentiate mitotic or interphase cells. Microtubules are marked by anti α-tubulin antibody (magenta). n=60, Scale bar, 2 μm.  

(c) Neuroblast centrosomes sas-4ΔT::sas-4^2214 recruit enhanced levels of Cnn and nucleate greater microtubule asters, as shown by immunostaining with Cnn antibody (purple) and α-tubulin antibody (red). White dotted line, cell boundary. Graphs show distribution and mean±SEM of the relative intensity between the two centrosomes in a cell. Scale bar, 2 μm. n=12  

(d) In control mitotic cells, Cnn (magenta) was enriched asymmetrically in one of the centrosomes, whereas sas-4ΔT::Sas-4^2214 cells exhibited more similar levels of Cnn in both of centrosomes. Graph shows distribution and mean±SEM of the relative intensity between the two centrosomes in a cell. Scale bar, 2 μm. n=45.  

(e) sas-4ΔT::sas-4^2214 cells (ii) exhibited an abnormal distribution of the asymmetric cell division marker Bazooka (magenta) as compared to control (i). Microtubules are marked by anti α-tubulin antibody (red). DNA is stained by DAPI (Blue). n>35. Scale bar, 2 μm. The estimated position of apical centrosomes relative to the Bazooka crest is given in the graph. About 15% of monopolar cells with an abnormal distribution of Bazooka (magenta) were also observed (iii).  

(f) sas-4ΔT::sas-4^2214 cells exhibited an abnormal configuration of mitotic spindles. Control cells consistently aligned mitotic spindles parallel to the metaphase plate (Normal), whereas, many sas-4ΔT::sas-4^2214 cells exhibited abnormal mitotic spindles (abnormal) to the metaphase plate. This may reflect that sas-4ΔT::sas-4^2214 cells nucleate microtubule asters prematurely. Scale bar, 2 μm. n=63.
**Figure S4** ITC of the recombinant Sas-4-N-tubulin interaction. 

**a)** Purified recombinant Sas-4-N used in Figure 3b-d and in isothermal titration calorimetry (ITC). Coomassie stained Sas-4-N tagged with HIS-GB1. 

**b-e)** ITC of the Sas-4-N-tubulin interaction in the presence of GMPCPP or GDP. Raw data representing the response to injections of Sas-4-N into tubulin in the presence of GMPCPP (b) or GDP (c). Integrated heat change (closed squares) and associated curve fit (red line) in the presence of GMPCPP (d) or GDP (e). Sas-4-N had a calculated $K_d$ of ~2 μM for tubulin-GMPCPP and 0.3 μM for tubulin-GDP.
Figure S5  Affinity purified Sas-4 complexes are stable in GTP, GDP or GMPCPP and Centrosomes can mediate GTP exchange within the Sas-4N-tubulin complex. Immuno-purified Sas-4 complexes fractionate similarly in a 5-40% sucrose gradient in the presence of 2 mM GTP (a), GDP (b) or GMPCPP (c). Individual fractions were analyzed by Western blot. (d) TLC analysis reveals that centrosomes mediate tubulin GTP exchange. GTP exchange was observed with tubulin when the reaction mixture contained Sas-4-N-ΔT, but was not observed with Sas-4-N. Adding centrosomes induces GTP exchange of Sas-4N-tubulin. Standards of GDP and GTP were run in parallel and their position is indicated.
Figure S6 Full scans
Supplementary Movie

The movement of sas-4ΔT::sas-4Δ2214 flies. Note that the flies are severely uncoordinated, indicating that the Sas-4ΔT transgene cannot rescue the sas-4 null mutant phenotype.