Msps/XMAP215 interacts with the centrosomal protein D-TACC to regulate microtubule behaviour

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The XMAP215/ch-TOG/Msps family of microtubule-associated proteins (MAPs) promote microtubule growth *in vitro* and are concentrated at centrosomes *in vivo*. We show here that Msps (mini-spindles protein) interacts with the centrosomal protein D-TACC, and that this interaction strongly influences microtubule behaviour in *Drosophila* embryos. If D-TACC levels are reduced, Msps does not concentrate at the centrosomes efficiently and the centrosomal microtubules appear to be destabilized. If D-TACC levels are increased, both D-TACC and Msps accumulate around the centrosomes/spindle poles, and the centrosomal microtubules appear to be stabilized. We show that the interaction between D-TACC and Msps is evolutionarily conserved. We propose that D-TACC and Msps normally cooperate to stabilize centrosomal microtubules by binding to their minus ends and binding to their plus ends as they grow out from the centrosome.

Centrosomes are the main microtubule-organizing centres (MTOCs) in animal cells[1]. Centrosomes have an important role in organizing many cellular processes, but surprisingly little is known about how they function at the molecular level[1–3].

In recent years, great progress has been made in understanding how centrosomes nucleate microtubules. The protein γ-tubulin is concentrated at the MTOCs, and is essential for MTOC function[4–8]. In the cell, γ-tubulin usually exists in the form of a large ring complex, and this complex has many of the properties expected of a microtubule-nucleating complex[9]. Ring-like structures that contain γ-tubulin have been visualized in the peri-centriolar material of centrosomes[10], and it is now widely accepted that γ-TuRCs are directly involved in the nucleation of centrosomal microtubules[11,12].

It is clear, however, that the interaction between centrosomes and microtubules is more complex than just a simple nucleation event. In mitosis, for example, many centrosomal microtubules are released from their nucleating sites, but are then 'captured' by complexes of microtubule-motor proteins, such as dynein/dynactin/NuMA that function to keep these microtubules focused around the centrosomes[13–15]. Furthermore, there are several other proteins that are concentrated at centrosomes and have been shown to interact with microtubules[16–21]. Particularly intriguing among these proteins are the XMAP215/ch-TOG family of MAPs. These proteins bind directly to microtubules and seem to stabilize them by modulating their dynamics[22–25]. Surprisingly, although these proteins seem to influence mainly microtubule plus-end dynamics, they all seem to be highly concentrated at centrosomes in cells[16,24,26–29]. The function of these proteins at centrosomes, if any, is therefore unclear.

We recently identified a novel *Drosophila* centrosomal protein, called D-TACC, that is essential for mitotic spindle function in the *Drosophila* embryo: when D-TACC function is perturbed by mutation or antibody injection, centrosomal microtubules are abnormally short, and the embryos die owing to an accumulation of mitotic defects[30]. The ~200 amino acids at the carboxy terminus of D-TACC are predicted to form a coiled-coil, and this region is related to a family of mammalian proteins called the transforming acidic coiled-coil-containing (TACC) proteins. These proteins have all been implicated in cancer[31–33], but their normal functions are unknown. We have shown that all the human TACC proteins associate with centrosomes and microtubules, at least during mitosis, indicating that the TACC domain is a conserved microtubule/centrosome-interacting domain[34].

Although D-TACC, or a glutathione *S*–transferase (GST)- or maltose-binding protein (MBP)-fusion protein that contains the conserved TACC domain, strongly interacts with microtubules in embryo extracts, these proteins do not strongly interact with purified microtubules[30]. We suspected, therefore, that the TACC domain interacts with microtubules indirectly through another protein. We show here that D-TACC interacts with Msps, the *Drosophila* homologue of XMAP215, and that this interaction seems to strongly influence the stability of centrosomal microtubules. We show that the interaction between these families of proteins is highly conserved in evolution. Moreover, we show that the TACC domain of D-TACC can markedly stabilize microtubules in *Drosophila* embryos, and this process seems to require the Msps protein. We propose that D-TACC and Msps normally cooperate to regulate the stability of centrosomal microtubules.

Results

D-TACC interacts with Msps. We previously showed that an MBP- or GST-fusion protein that contains the conserved C-terminal TACC domain of D-TACC was concentrated at centrosomes in embryos and associated with microtubules in embryo extracts[30]. These same fusion proteins, however, did not interact significantly with purified microtubules *in vitro*[30] (Fig. 1a). To identify other factors present in the extract that might mediate the interaction between D-TACC and microtubules, we added a purified MBP–TACC-domain fusion protein (MBP–TD, or MBP–CT in ref. 30) to embryo extracts, and then re-isolated the fusion protein on an amylose column. On Coomassie-blue-stained gels, the re-isolated fusion protein co-purified with two proteins of a higher relative molecular mass of ~220,000 (M_r ~220K) and ~180K (Fig. 1b). This re-purified MBP–TD fusion protein complex now strongly associated with purified microtubules in spin-down experiments (Fig. 1a, lower panel).

We used mass spectroscopic methods to identify the ~220K and ~180K proteins. In two separate experiments, the ~220K protein

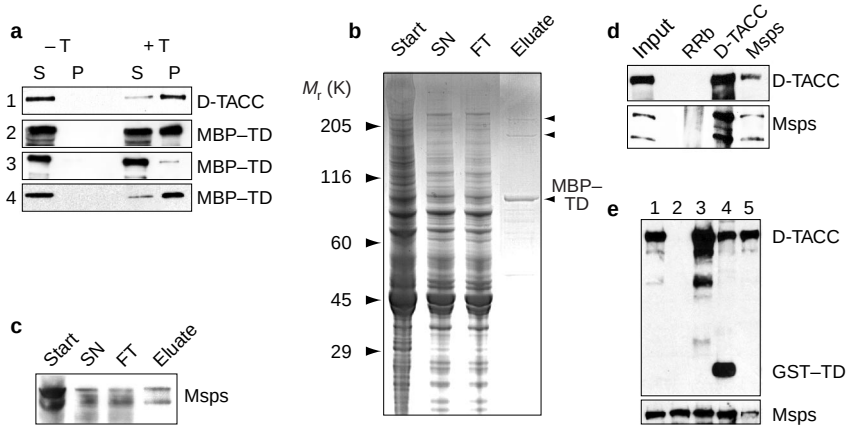

Figure 1 **Interaction between D-TACC and Msps. a**, Microtubule spin-down experiments were performed either in embryo extracts (panels 1, 2) or with purified tubulin (panels 3, 4) in the absence (–T) or presence (+T) of taxol. S, supernatant; P, pellet. In embryo extracts, the endogenous D-TACC (panel 1) or an exogenously added MBP–TD fusion protein (panel 2) pelleted together with microtubules. MBP–TD does not bind strongly to purified microtubules (panel 3), unless it is mixed with embryo extracts and re-purified (panel 4). **b**, A Coomassie-blue stained gel of the proteins that bind to MBP–TD when it is mixed with embryo extracts. Start, crude embryo extract; SN, high speed supernatant with added MBP–TD; FT, proteins that passed through the amylose column; Eluate, proteins that bind to the amylose column. All of the proteins smaller than MBP–TD are breakdown products (they were all present in the MBP–TD originally purified from bacteria; data not shown). The ~220K and ~180K proteins are highlighted with arrowheads. **c**, A western blot of the protein fractions shown in **b**, probed with anti-Msps antibodies. **d**, A western blot of an immunoprecipitation experiment performed with random rabbit IgG, anti-D-TACC or anti-Msps antibodies, and probed with anti-D-TACC antibodies (upper panel) or anti-Msps antibodies (lower panel). **e**, The amounts of D-TACC (upper panel) and Msps (lower panel) in embryos of different genotypes: wild type (lane 1); *d-tacc*^{stella592} (lane 2); 4 × D-TACC (lane 3); GST–TD (lane 4); *msps*^{MJ15} (lane 5).

was identified as the product of the *msps* gene, the *Drosophila* homologue of the well-characterized *Xenopus* MAP-XMAP215 (ref. 16), whereas the ~180K band seemed to contain a mixture of Msps and D-TACC. The presence of Msps in these bands was confirmed by western blots with affinity-purified anti-Msps antibodies that recognized the full-length Msps as a ~220K protein and a prominent breakdown product of ~180K in embryo extracts and in the re-purified MBP-TD protein fraction (Fig. 1c). To test whether the full length D-TACC could interact with Msps, we carried out immunoprecipitation experiments on wild-type embryo extracts using affinity-purified anti-D-TACC or anti-Msps antibodies. Each protein specifically co-immunoprecipitated with the other (Fig. 1d). Quantification of these experiments, and an analysis of the behaviour of the D-TACC and Msps proteins in embryo extracts by gel-filtration chromatography, indicated that not all of the D-TACC or Msps in embryo extracts existed together in a complex (data not shown). The two proteins, however, seemed to co-localize extensively in *Drosophila* embryos (Fig. 2a).

The D-TACC/Msps interaction influences centrosomal microtubules. It has been shown that both D-TACC and Msps are integral centrosomal proteins that remain concentrated at the centrosomes even in the presence of microtubule-destabilizing agents[16,30]. We therefore tested whether either protein was required for the centrosomal localization of the other. We have shown that the majority of *d-tacc* mutant embryos fail in pronuclear fusion and do not develop significantly[30]. In the embryos that do develop, the microtubules that are associated with the centrosomes are abnormally short at all stages of the cell cycle, and the embryos eventually die owing to an accumulation of mitotic defects. We found that in developing *d-tacc* mutant embryos (which expressed <1% of the normal amounts of D-TACC; Fig. 1e, lane 2), Msps was essentially absent from the centrosome in ~50% of spindles (Fig. 2b), was severely reduced at the centrosome in ~25% of spindles, and was normally localized at the centrosome in ~25% of them (72 spindles were scored from 10 different embryos). This indicates that D-TACC is involved in, but is not essential for, the efficient localization of Msps to centrosomes. Interestingly, even in embryos where Msps was no longer detectably concentrated at the centrosomes, it seemed to be associated normally with the short spindles present in these embryos (Fig. 2b), which indicates that D-TACC is not required for Msps to interact with spindle microtubules.

As with *d-tacc* mutant embryos, most *msps* mutant embryos that expressed ~10–20% of the normal amount of Msps (Fig. 1e, lane 5) failed in pronuclear fusion and did not develop significantly. In the few embryos that did develop, the spindles were often highly disorganized but D-TACC was still detectable on the centrosomes and spindles. As some Msps protein was still present in these embryos, this result is difficult to interpret.

To test whether D-TACC could recruit Msps to the centrosomes, we overexpressed D-TACC in early embryos. In embryos that overexpressed D-TACC by about fourfold (Fig. 1e, lane 3; hereafter referred to as 4 × DT embryos), extra D-TACC was recruited to the area around the centrosomes/spindle poles, where it was often clustered into large aggregates (Fig. 2c, d). Msps strongly co-localized with the clusters of extra D-TACC at the spindle pole (Fig. 2c). Two lines of evidence indicate that this recruitment of Msps to the centrosome/spindle pole area is not simply a secondary consequence of the stabilization by D-TACC of centrosomal microtubules. First, we examined the distribution of several other centrosomal proteins that interact with microtubules. In contrast to Msps, γ-tubulin (Fig. 2d), CP60 and CP190 (data not shown) did not seem to co-localize with the extra D-TACC at the centrosome/spindle pole. Second, we treated wild-type embryos with the microtubule-stabilizing drug taxol. In these embryos, both D-TACC and Msps associated with the extra microtubules, but neither protein was specifically recruited to the centrosomal region (see Supplementary Information, Fig. 1).

The amount of D-TACC that interacts with Msps at centrosomes/spindle poles was correlated with the number and/or stability of centrosomal microtubules. In *d-tacc* mutant embryos, the number and length of the astral and spindle microtubules seemed to be reduced[30] (Fig. 2f), whereas in embryos that overexpressed D-TACC, the number and length of astral and spindle microtubules seemed to be increased (Fig. 2g). Thus, the amount of D-TACC in the embryo influences both the amount of Msps associated with

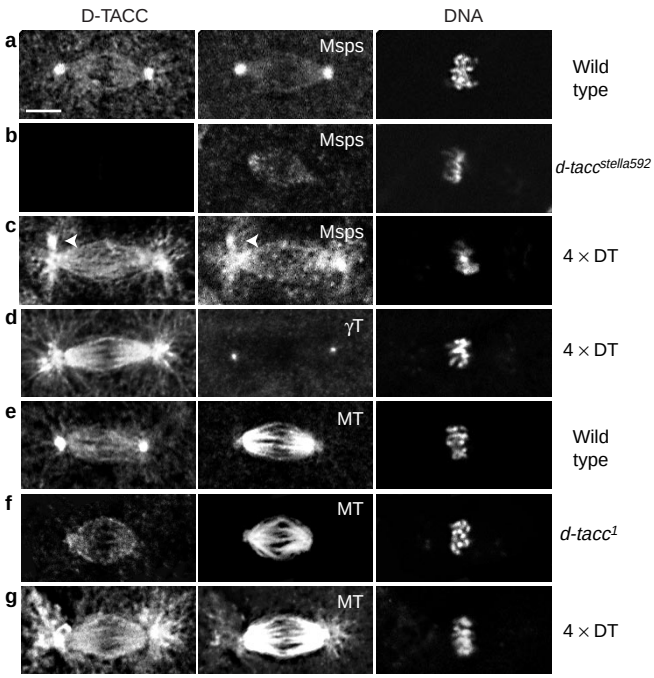

Figure 2 **Localization of D-TACC and Msps in embryos containing different amounts of D-TACC.** The localization of D-TACC (left hand panels), DNA (right hand panels) and Msps (**a–c**), γ-tubulin (**d**) or microtubules (**e–g**) (middle panels) in metaphase spindles in embryos that contained different amounts of D-TACC. The spindles appeared shorter and weaker in embryos with less D-TACC, and larger and more robust in embryos with extra D-TACC. Quantification of the average pixel intensities of these spindles (see Methods) revealed that the 4 × DT spindles were about twofold brighter on average than the *d-tacc*^{1} mutant spindles (data not shown). In **a–d** and **e–g**, the images were acquired using identical settings on the confocal microscope. The arrows in **c** highlight the co-localization of D-TACC and Msps in the large aggregates of D-TACC that often concentrate around the centrosome/spindle pole in 4 × DT embryos. Scale bar, 5 μm.

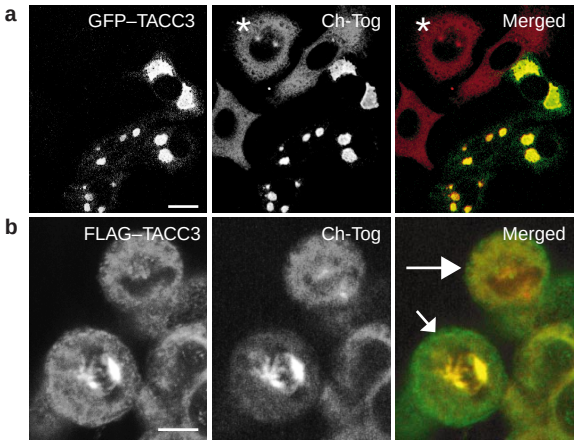

Figure 3 **The human TACC proteins can bind to ch-TOG. a**, Interphase cells transfected with GFP–TACC3 (detected here by the fluorescence of GFP, left hand panels, green in merged image) and with ch-TOG (middle panels, red in merged image). ch-TOG associates strongly with TACC3–GFP polymers and is largely depleted from the cytoplasm. In non-transfected cells, ch-TOG is mainly cytoplasmic, and it associates with the centrosomes as cells enter mitosis (cell marked with an asterisk). Similar results were obtained when any of the TACC proteins or their TACC domains were overexpressed in HeLa cells. **b**, In mitotic cells transiently transfected with FLAG–TACC3, the fusion protein (detected here with anti-FLAG antibodies, left hand panels, green in merged image) binds to centrosomes where it recruits extra ch-TOG (middle panels, red in merged image). Compare the levels of ch-TOG at the centrosome in the transfected cell (marked with a small arrow) and non-transfected cell (marked with a large arrow). Scale bar, 10 μm (**a**), 5 μm (**b**).

centrosomes/spindle poles and the number and/or stability of centrosomal microtubules. The overall amount of Msps in the D-TACC mutant and 4 × DT embryos did not seem to be altered (Fig. 1e, lanes 1–3), which indicates that D-TACC was not influencing centrosomal microtubules by simply regulating the total concentration of Msps protein in the embryo.

The interaction between D-TACC and Msps is highly conserved. As Msps seems to interact with D-TACC through the conserved C-terminal TACC domain, we tested whether the human TACC proteins could also interact with the human homologue of Msps, ch-TOG. When overexpressed in HeLa cells, all the TACC proteins (or the TACC domains from any of the TACC proteins) can form large polymers that can interact with microtubules and/or tubulin in the cytoplasm under certain conditions[34]. We found that ch-TOG was highly concentrated in the polymers that were formed by any of the full-length human TACC proteins or their TACC domains (Fig. 3). We observed the same effect when the TACC domain from *Drosophila* TACC was overexpressed in human cells (data not shown), which showed that the interaction between the TACC domain and Msps/ch-TOG has been highly conserved in evolution. In contrast, the centrosomal protein γ-tubulin and the spindle-pole component NuMA were not concentrated in any of the TACC/TACC domain polymers (data not shown).

We have shown that, like D-TACC in *Drosophila*, overexpressed TACC3 becomes concentrated around centrosomes in human cells in mitosis, and this leads to an apparent increase in the number and/or stability of centrosomal microtubules[34]. Extra ch-TOG was also recruited to the centrosomes/spindle poles in these mitotic cells (Fig. 3b). Thus, in both *Drosophila* and human cells, by overexpressing a TACC protein, extra Msps/ch-TOG can be recruited to the centrosome/spindle pole area, and there seems to be a concomitant increase in the number and/or stability of centrosomal microtubules.

The TACC domain may modulate the microtubule-stabilizing activity of Msps. We have shown that the ability of the TACC domain to interact with microtubules is modulated by its context within the full-length TACC protein[34]. To test whether this was also the case in *Drosophila*, we overexpressed a *Drosophila* GST–TACC-domain-fusion protein (GST–TD) in embryos by about fivefold (compared with the endogenous protein; Fig. 1e, lane 4). To our surprise, virtually none of these embryos developed at all. Instead, they were often filled with microtubules that were usually organized into asters which centred around aggregates of the GST–TD protein (Fig. 4a). Msps was also concentrated at the centre of these asters (Fig. 4b), but γ-tubulin (Fig. 4c), CP190 and CP60 (data not shown) were not. As a control, we also generated transgenic embryos that expressed the GST protein alone at comparable levels to the GST–TD protein in GST–TD embryos. We did not detect any asters of microtubules in these embryos, and they developed and hatched at normal rates (data not shown).

To test whether this marked effect of the GST–TD fusion protein on microtubules was dependent on Msps, we expressed GST–TD in *msps* mutant embryos that expressed only ~10–20% of the normal amount of Msps (Fig. 1e, lane 5). Although the asters of microtubules were easily detectable in >80% of wild-type embryos that expressed GST–TD ($n = 78$), we did not detect any asters of microtubules in *msps* mutant embryos that expressed GST–TD ($n = 85$; Fig. 4d). Although none of these embryos developed at all, the male and female pronuclei in both the wild-type and *msps*

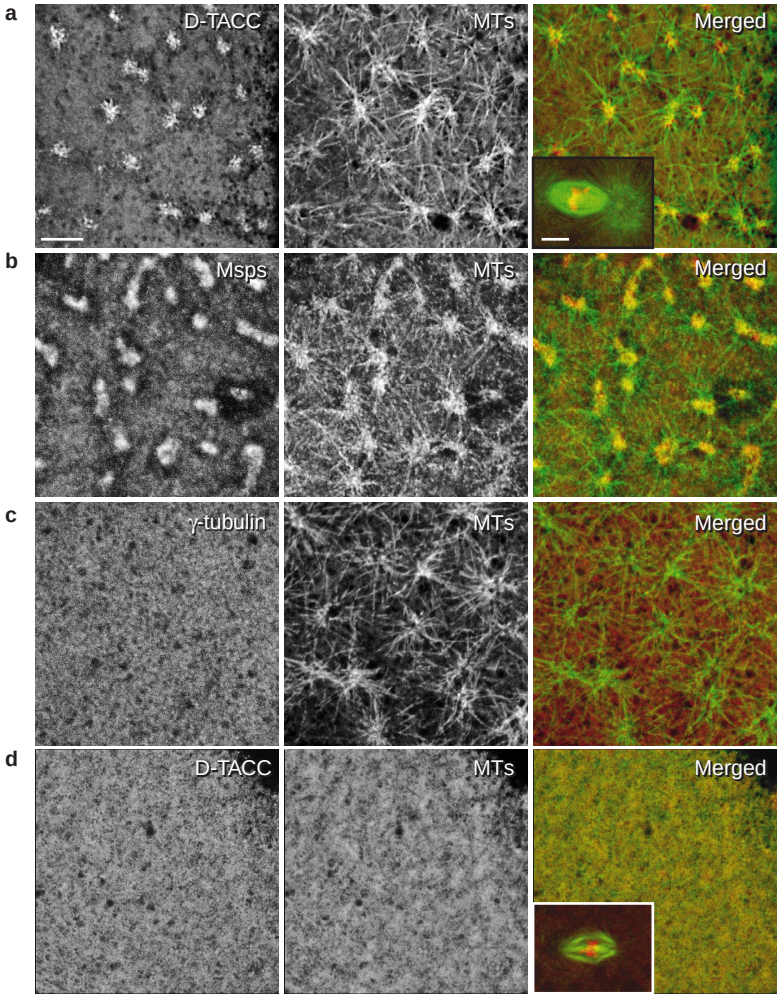

Figure 4 **The GST–TD fusion protein seems to stabilize microtubules in *Drosophila* embryos in an Msps-dependent manner.** The distribution of microtubules (MTs, green in merged images) and D-TACC (**a**, **d**), Msps (**b**) or γ-tubulin (**c**) (red in merged images), in embryos that overexpressed the GST–TD fusion protein. In **a–c**, GST–TD has been overexpressed in otherwise wild-type embryos. In **d**, GST–TD has been overexpressed in an *msps*^{MJ15} mutant embryo. The insets in **a** and **d** show close-up views of typical pronuclei observed in embryos that expressed GST–TD. In wild-type embryos (**a**), robust spindle-like arrays of microtubules (green) surround the condensed chromosomes (red), whereas in *msps* mutant embryos, the chromosomes are condensed but the microtubule arrays are much weaker. Scale bar, 10 μm (inset, 5 μm).

GST–TD embryos always seemed to be in a mitotic state, with spindle-like structures organized around the condensed chromosomes (insets in Fig. 4a, d). The difference in behaviour of the microtubules in the wild-type and *msps* GST–TD embryos is, therefore, unlikely to be simply a consequence of the embryos being arrested at different stages of the cell cycle. Thus, the GST–TD fusion protein seems to require Msps to stabilize the microtubules in these embryos. As the amounts of Msps are not altered in GST–TD embryos (Fig. 1e, lane 4), it seems that the GST–TD fusion protein can somehow activate the microtubule-stabilizing function of Msps.

Both Msps and D-TACC may interact with the plus ends of centrosomal microtubules. The results described here indicate that the interaction between D-TACC and Msps is involved in regulating the stability and/or nucleation of microtubules. Although both of these proteins are strongly concentrated at the centrosomes, Msps family members mainly influence microtubule plus-end dynamics[22–25]. We therefore wondered whether these proteins might localize to centrosomes as an efficient way of binding to the plus ends of the microtubules that grow out from the centrosome. To test this possibility, we monitored the behaviour of a D-TACC–GFP (green fluorescent protein) fusion protein and an Msps–GFP fusion protein in living embryos using time-lapse confocal microscopy. Both fusion proteins showed a very similar localization and were strongly concentrated at the centrosomes and more weakly associated with the microtubules throughout the cell cycle (data not shown). However, many small dots of both fusion proteins could be seen moving to and from the centrosome as though they were attached to the plus ends of the microtubules (Fig. 5). This result is consistent with the possibility that D-TACC and Msps might bind to the plus ends of the microtubules that grow out from the centrosome.

Discussion

We have shown that D-TACC is an integral centrosomal protein that associates with microtubules and is essential for mitotic spindle function in *Drosophila* embryos[30]. D-TACC seems to interact with microtubules indirectly[30], and we show here that D-TACC interacts with Msps, a member of the well-characterized XMAP215/ch-TOG family of microtubule-binding proteins. We

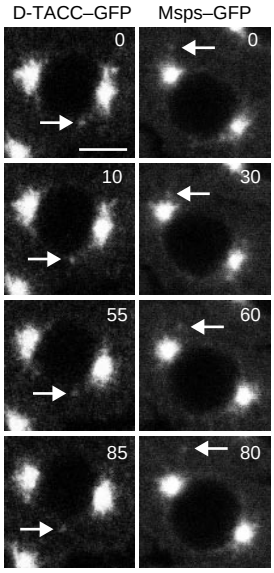

Figure 5 **The behaviour of D-TACC–GFP or Msps–GFP in living embryos.** Both fusion proteins are strongly concentrated around the centrosomes throughout the cell cycle, but many small dots were oscillating to and from the centrosome, as though the proteins were bound to the plus ends of the centrosomal microtubules. (This phenomenon is clearer in movies of these embryos; see Supplementary Information). The behaviour of one of these dots is highlighted with arrows. Time is shown in seconds. Scale bar, 5 μm.

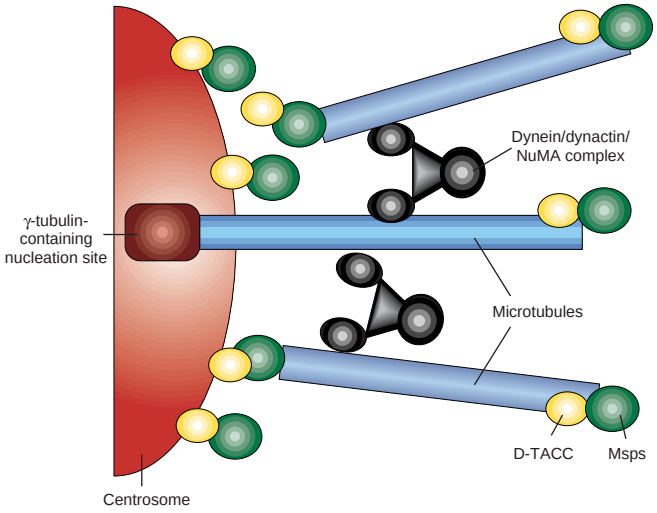

Figure 6 **A model of how D-TACC and Msps might cooperate to stabilize centrosomal microtubules.** D-TACC (yellow) and Msps (green) co-localize at the periphery of the centrosome (red). These proteins cooperate to stabilize centrosomal microtubules (blue) in two ways. First, they bind to and stabilize the minus ends of microtubules that have been released from their γ-tubulin-containing nucleation sites (brown). Second, the concentration of D-TACC and Msps at the centrosome ensures that they bind to the plus ends of the centrosomal microtubules as they grow out from the centrosome. In this way, centrosomal microtubules are stabilized by D-TACC and Msps. The centrosomal microtubules also interact with motor-protein complexes, such as dynein/dynactin/NuMA (black), which function to keep the released microtubules focused at the poles.

speculate, therefore, that D-TACC normally interacts with microtubules through its interaction with Msps.

The interaction between D-TACC and Msps seems to be important in efficiently localizing Msps to the centrosomes. In strong *d-tacc* mutant embryos, Msps can still associate with spindle microtubules but it is no longer efficiently concentrated at the centrosomes. Moreover, in embryos that overexpress D-TACC, extra Msps is recruited to the area around the centrosomes/spindle poles by the extra D-TACC. Thus, D-TACC seems to be involved in recruiting Msps to, or in maintaining its localization at, the centrosomes/spindle poles. In this issue, Cullen and Ohkura have shown that D-TACC and the microtubule motor Ncd are involved in localizing Msps to the polar regions of the acentrosomal meiosis I spindle in *Drosophila* females[35], which indicates that D-TACC is involved in localizing Msps to spindle poles even in the absence of centrosomes.

The interaction between D-TACC and Msps seems to influence the number and/or length of centrosomal microtubules. When the concentration of D-TACC at the centrosome/spindle pole is reduced, the amount of Msps at the centrosome/spindle pole is also often reduced too, as are the number and/or length of the centrosomal microtubules[30]. When the concentration of D-TACC at the centrosome/spindle pole is increased, the level of Msps at the centrosome/spindle pole also increases, as does the number and/or length of the centrosomal microtubules. The TACC3 protein seems to function in a similar manner in human cells. We previously showed that overexpressing TACC3 leads to an accumulation of TACC3 at the centrosomes/spindle poles and to an increase in the number and/or length of centrosomal microtubules[34]. We have now shown that this extra TACC3 also recruits ch-TOG to the centrosome/spindle pole. Thus, in both human cells and *Drosophila* embryos, overexpressing a TACC protein seems to recruit extra Msps/ch-TOG to the centrosome/spindle pole area, and there is a concomitant increase in the number and/or length of centrosomal microtubules.

As the Msps/XMAP215 family of proteins are known to promote microtubule polymerization, perhaps the simplest interpretation of our data is that D-TACC influences microtubule stability through its interaction with Msps. In support to this possibility, overexpressing the GST–TD-fusion protein in embryos led to a complete failure of their development, and these embryos were often filled with long microtubules that were organized into astral arrays around aggregates of the GST–TD and Msps proteins. This marked effect of GST–TD on microtubules seemed to be Msps dependent, as we did not detect any microtubule asters in *msps* mutant embryos that overexpressed the GST–TD fusion protein. This indicates that the TACC domain of D-TACC can stabilize microtubules in a Msps-dependent manner. However, we cannot rule out the possibility that D-TACC and Msps normally function to nucleate microtubules (rather than to stabilize them), nor that D-TACC may influence microtubule behaviour through a mechanism that does not involve Msps.

Both the GST–TD protein and Msps are strongly concentrated in the middle of the microtubule asters that form in the GST–TD embryos. This indicates that the GST–TD–Msps complex has a preference for binding to one (or both) end(s) of microtubules. D-TACC seems to be concentrated mainly at the minus ends of the spindle microtubules[30], and both D-TACC and Msps are concentrated at the poles of the acentrosomal female meiosis I spindle[35]. Our observation of D-TACC–GFP and Msps–GFP fusion proteins moving to and from the centrosome in living embryos also raises the possibility that these proteins can interact preferentially with microtubule plus ends (although we cannot rule out that these proteins are simply moving backwards and forwards along microtubules by alternately associating with plus- and minus-end-directed motors). Moreover, there is biochemical evidence that ch-TOG has a preference for binding to microtubule ends[25]. Taken together, these results indicate that D-TACC and Msps interact

preferentially with microtubule ends.

We envisage at least two ways in which D-TACC and Msps might cooperate to influence centrosomal microtubules (Fig. 6). First, D-TACC and Msps may bind to and stabilize the minus ends of the microtubules that are normally released from their centrosomal nucleation sites and maintained in the vicinity of the spindle poles by the activity of microtubule-motor complexes such as dynein/dynactin/NuMA[13–15]. In support of this possibility, XMAP215 has been shown to increase the growth rate of microtubule minus ends by about two- to threefold *in vitro*[22]. Second, concentrating D-TACC and Msps at the centrosome could serve to 'load' D-TACC and Msps onto the plus ends of microtubules as they initially grow out from the centrosome. In support of this possibility, we have observed small dots of the D-TACC–GFP or Msps–GFP fusion proteins that oscillate to and from the centrosome, as though binding to the growing and shrinking plus ends of the centrosomal microtubules. This model is particularly attractive as it would explain the apparent paradox of why the XMAP215 family of MAPs are all highly concentrated at centrosomes but seem mainly to affect microtubule plus-end dynamics. Moreover, in this model, the concentration of D-TACC and Msps at the centrosome would help to ensure that centrosomal microtubules are more likely to be stabilized by these proteins than non-centrosomal microtubules, which might form spontaneously in the cytoplasm, for example. □

Methods

Purification and identification of MBP–TD-associated proteins.

The MBP–TD and GST–TD fusion proteins used in these experiments were described in ref. 30, in which they were referred to as MBP–CT and GST–CT, respectively. Fusion proteins were purified according to the manufacturer's instructions. The proteins were desalted on a P6 (Bio-Rad) column into C-buffer (50 mM HEPES pH 7.6, 50 mM KCl, 1 mM $MgCl_2$, 1 mM EGTA) using a Bio-Logic FPLC system (Bio-Rad). Glycerol was added to 50% of the total volume, and the proteins were stored at –20 °C. High speed, 0–4-h-old embryo extracts were prepared in C-buffer as described[30], and 5–10 µg of either MBP or MBP–TD protein was added to 2 ml of embryo extract. The mixture was incubated at room temperature for 20 min, at 4 °C for 20 min, and then loaded onto a 2 ml amylose–resin column at a flow rate of 0.1 ml min^{-1} using a Bio-Logic FPLC system. The column was washed with 20 volumes of C-buffer plus 0.1% Tween-20, and then eluted with 5 volumes of C-buffer plus 10 mM maltose. The peak protein fractions eluting from the column were pooled, precipitated with 10% trichloroacetic acid and resuspended in protein-sample buffer[36]. The mixture was neutralized with ammonium hydroxide and loaded onto 8% or 10% polyacrylamide gels or 4–12% gradient gels (Novex). Gels were either stained with Coomassie blue, or blotted onto nitrocellulose as described[30].

To identify the proteins that specifically co-purified with MBP–TD, the protein bands of interest were excised from a Coomassie-blue-stained gel, washed, in-gel digested with trypsin and subjected to MALDI-TOF mass spectroscopic analysis as described[37]. The MS-FIT programme was used for the interpretation of MS data and identification of proteins.

Microtubule spin-down experiments.

Microtubule spin-down experiments in embryo extracts or with purified tubulin and purified fusion proteins expressed in bacteria were performed as described[30].

Antibodies.

The following antibodies have been used in this study. The affinity-purified rabbit anti-D-TACC antibodies and mouse anti-D-TACC sera have been described[30], as have affinity-purified rabbit anti-γ-tubulin[38], anti-CP190, anti-CP60[20] and anti-ch-TOG[24] antibodies. Our own affinity-purified anti-Msps antibodies were raised and purified against an MBP–Msps fusion protein (containing amino acids 1200–1612 of the Msps protein) as described[39]. These affinity-purified antibodies were used in all the experiments reported here. The anti-FLAG M2 monoclonal antibody was purchased from Sigma.

Fly stocks.

The following fly stocks were used in this study: *d-tacc*^{1}, an EMS (ethylmethane sulphonate)-induced allele of *d-tacc* that produces ~10% of the normal wild-type amount of D-TACC[30]; *d-tacc*^{stella592}, a P-element allele of D-TACC that produces essentially no detectable D-TACC protein (kindly provided by B. Williams and M. Goldberg); *msps*^{MJ15}, a female-sterile allele of Msps that produced ~10–20% of the wild-type amounts of Msps[16]. The transgenic lines that overexpress full-length D-TACC were constructed using standard genetic methods to generate two independent stocks that each contained six copies of a transgene that expressed the full length D-TACC protein driven by the polyubiquitin promoter. One copy of this transgene completely rescues the *d-tacc*^{1} mutation[30]. Both lines behaved similarly, and the results described here are pooled from both lines. Transgenic lines that overexpressed the GST–TD fusion protein were constructed by creating transformed flies that carried a construct in which GST–TD was subcloned into the pUASp vector for GAL4-driven expression of the transgene in the germline[40]. Several independent lines were crossed to several transgenic lines in which the GAL4 protein was driven from the maternal 67C tubulin promoter (D. St Johnston, personal communication). All of these lines expressed the GST–TD at about the same levels and were essentially completely female sterile. As a control, GST alone was subcloned into the pUASp vector and the transgenic flies generated with this construct were crossed to the same GAL4 driver lines.

Immunoprecipitation.

Immunoprecipitation experiments were carried out on 0–4-h-old embryo extracts using random rabbit IgG, affinity-purified anti-D-TACC, or anti-Msps antibodies, as described[30].

SDS–PAGE and western blotting.

SDS–PAGE and western blotting experiments were performed as described[36,41]. As the enhanced chemiluminescent methods used to probe western blots were non-linear, blots were quantified by parallel control blots that contained serial dilutions of extracts or the appropriate fusion protein.

Fixation and antibody staining.

Embryos were fixed and processed for immunofluorescence as described[30]. The transient transfection, fixation, staining and observation of HeLa cells were performed as described[34].

Quantification of microtubule density.

To quantify the density of microtubules in *d-tacc* mutant, wild-type and 4 × DT spindles, four to five embryos of each genotype were identified on the basis of their chromatin staining as being in metaphase. Images of the spindles in each embryo were then acquired using identical (non-saturating) settings on the confocal microscope. Five spindles from each embryo were randomly selected for quantification using NIH Image. The area of each spindle (excluding the astral microtubules) was defined manually and the average pixel intensity per unit area of spindle was calculated.

Live analysis of embryos.

Transgenic embryos that expressed either the full-length D-TACC-GFP[30] or the full length Msps–GFP (full cloning details available upon request) were observed using time-lapse confocal microscopy as described[30].

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Supplementary Information is available on *Nature Cell Biology*'s website (http://cellbio.nature.com).

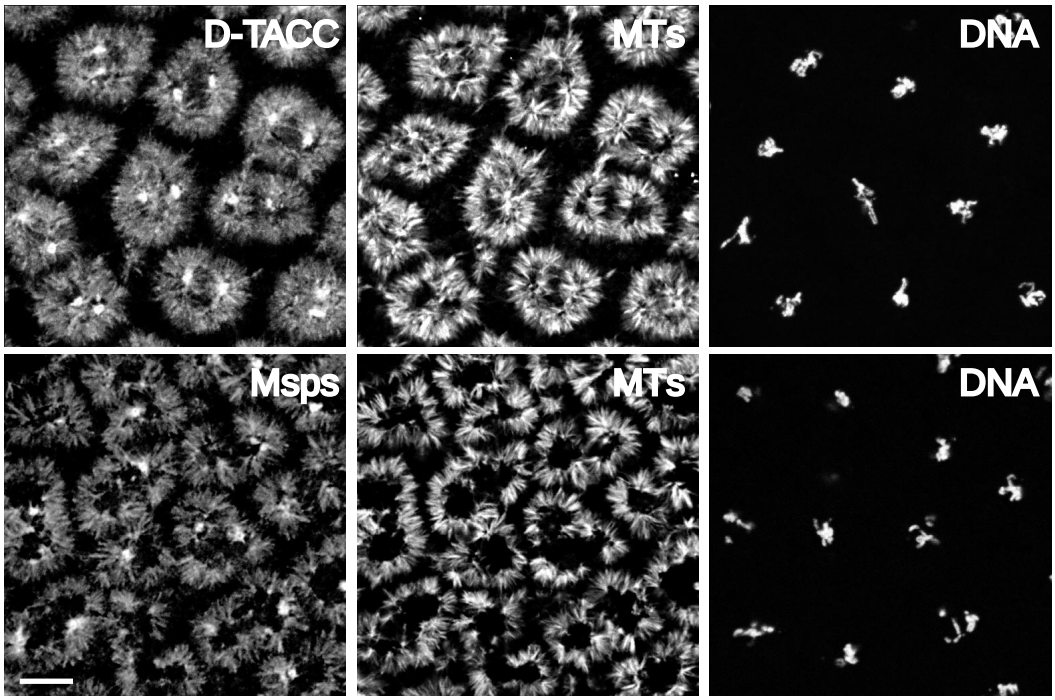

Figure S1. **The behaviour of D-TACC (top left panel), Msps (bottom left panel), microtubules (middle panel) and DNA (right panel) in embryos treated with taxol before fixation.** Note how both proteins bind to the extra microtubules polymerised in the presence of taxol, but both proteins are still somewhat concentrated around the centrosomes. Scale bar, 10 mm.

Movie 1. **The behaviour of a D-TACC–GFP fusion protein in a living embryo.** Note how small dots of the fusion protein can be observed oscillating to and fro from the centrosomal region, as though binding to the plus ends of the centrosomal microtubules.

Movie 2. **The behaviour of a Msps–GFP fusion protein in a living embryo.** Note how small dots of the fusion protein can be observed oscillating to and fro from the centrosomal region, as though binding to the plus ends of the centrosomal microtubules.