The XMAP215 homologue Stu2 at yeast spindle pole bodies regulates microtubule dynamics and anchorage

Takeo Usui^{1,2}, Hiromi Maekawa¹,
Gislene Pereira¹ and Elmar Schiebel^{1,3}

¹The Paterson Institute for Cancer Research, Christie Hospital
NHS Trust, Wilmslow Road, Manchester M20 4BX, UK and

²Antibiotics Laboratory, RIKEN Institute for Discovery Research,
Hirosawa 2-1, Wako-shi, Saitama 351-0198, Japan

³Corresponding author

e-mail: eschiebel@picr.man.ac.uk

T.Usui and H.Maekawa contributed equally to this work

The yeast protein Stu2 belongs to the XMAP215 family of conserved microtubule-binding proteins which regulate microtubule plus end dynamics. XMAP215-related proteins also bind to centrosomes and spindle pole bodies (SPBs) through proteins like the mammalian transforming acidic coiled coil protein TACC or the yeast Spc72. We show that yeast Spc72 has two distinct domains involved in microtubule organization. The essential 100 N-terminal amino acids of Spc72 interact directly with the γ -tubulin complex, and an adjacent non-essential domain of Spc72 mediates binding to Stu2. Through these domains, Spc72 brings Stu2 and the γ -tubulin complex together into a single complex. Manipulation of Spc72–Stu2 interaction at SPBs compromises the anchorage of astral microtubules at the SPB and surprisingly also influences the dynamics of microtubule plus ends. Permanently tethering Stu2 to SPBs by fusing it to a version of Spc72 that lacks the Stu2-binding site in part complements these defects in a manner which is dependent upon the microtubule-binding domain of Stu2. Thus, the SPB-associated Spc72–Stu2 complex plays a key role in regulating microtubule properties.

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Introduction

Microtubules (MTs) are hollow cylinders that assemble from a heterodimer of α - and β -tubulin. MTs are inherently polar structures, so that the two ends of an MT are different. Only the MT minus ends are attached to MT organizing centres such as the centrosome of vertebrate cells and the spindle pole body (SPB) of yeast. MT plus ends are directed away from the centrosome towards the cell periphery. MTs are highly dynamic structures and the ends switch from states of growing to shrinking (called catastrophe) and from shrinking to growing (rescue). The plus ends of MTs are more dynamic than the minus ends (Howard and Hyman, 2003).

When measured *in vivo*, MTs are more dynamic than MTs assembled *in vitro* from pure tubulin, due to the

presence of regulatory factors. Members of the conserved family of MT-binding proteins named Stu2 in budding yeast, XMAP215 in *Xenopus*, mini-spindles (Msps) in *Drosophila*, Dis1 and Alp14 in *Schizosaccharomyces pombe*, DdCP224 of *Dictyostellium* and the colonic-hepatic tumour-overexpressed gene (ch-TOG) in mammalian cells represent proteins that regulate MT dynamics (Nabeshima *et al.*, 1995; Wang and Huffaker, 1997; Gräf *et al.*, 2000).

XMAP215-like proteins regulate MT function by a number of mechanisms. One place of action is the MT plus end. Work in *Xenopus* extract has shown that XMAP215 stabilizes MT plus ends by opposing the destabilizing activity of the kinesin XKCM1 (Tournéize *et al.*, 2000). In contrast, studies using stabilized MTs identified XMAP215 as an MT-destabilizing factor (Shirasu-Hiza *et al.*, 2003). XMAP215-like proteins also bind to centrosomes and SPBs independently of MTs. In *Drosophila*, the centrosomal protein D-TACC, named after the human counterpart of the transforming acidic coiled coil (TACC) protein, mediates binding of Msps to centrosomes (Gergely *et al.*, 2000; Lee *et al.*, 2001). If D-TACC levels are reduced, Msps does not efficiently concentrate at centrosomes and the centrosomal MTs are destabilized (Lee *et al.*, 2001). The interpretation of this result is complicated by the fact that Msps and D-TACC are also present at MT plus ends. The three human TACC proteins target ch-TOG to centrosomes and TACC-3 has a role in stabilizing centrosomal MTs (Gergely *et al.*, 2003). A number of studies suggest that defects in ch-TOG and the three TACC proteins may lead to cancer; however, the function of these proteins in mammalian cells is poorly understood (Raff, 2002).

The budding yeast Stu2 is associated with SPBs, kinetochores, nuclear and astral MTs, whereas Stu2 probably fulfils diverse functions (Wang and Huffaker, 1997; Chen *et al.*, 1998; He *et al.*, 2001). It is not surprising, therefore, that total depletion of Stu2 leads to a mixture of phenotypes including fewer and less dynamic astral MTs, cell cycle arrest and a failure to elongate the mitotic spindle (Kosco *et al.*, 2001; Severin *et al.*, 2001). Although recent evidence suggests that Stu2 is a plus end-binding MT destabilizer (Kosco *et al.*, 2001; van Breugel *et al.*, 2003), the function of Stu2 at SPBs is not understood. It is only known that Stu2 interacts with the SPB component Spc72 (Chen *et al.*, 1998).

The SPB is a large multi-layered structure that is embedded in the nuclear envelope throughout the cell cycle. The cytoplasmic side of the SPB organizes the astral MTs involved in nuclear positioning, whereas the SPB nuclear side assembles the nuclear MTs that have a role in chromosome segregation. The protein Spc72 is only associated with the cytoplasmic side of the SPB. The C-terminus of Spc72 targets the protein to this side of the

SPB through binding to the SPB components Nud1 and Kar1 (Pereira *et al.*, 1999; Gruneberg *et al.*, 2000; Schaerer *et al.*, 2001). Besides Nud1, Kar1 and Stu2, Spc72 also interact with the yeast γ -tubulin complex, named the Tub4 complex. Spc72 recruits the Tub4 complex to the cytoplasmic face of the SPB, thereby mediating nucleation of astral MTs (Knop and Schiebel, 1998).

The little knowledge we have of the function played by XMAP215-like proteins at centrosomes and SPBs is due in large part to the lack of mutants that specifically affect the centrosome/SPB-associated function while leaving the other functions unaffected. Here, we show that the Tub4 complex and Stu2 bind to adjacent but distinct domains in Spc72. Stu2 and the Tub4 complex are tethered together through their independent associations with Spc72, and they cooperate in organizing astral MTs. Analysis of a *SPC72* mutant that fails to bind Stu2 demonstrates that SPB-associated Stu2 is required for astral MTs anchorage and regulation of MT dynamics.

Results

The 35 most C-terminal amino acids of Stu2 confer binding to Spc72 and astral MT plus ends

In order to understand how Stu2 binds to SPBs, we investigated which domain of Stu2 confers binding to the SPB component Spc72. In the yeast two-hybrid system, the 35 most C-terminal amino acids of Stu2 (Stu2^{853–888}) were sufficient to mediate interaction with Spc72 (Figure 1A, lane 3). Consistently, deletion of C-terminal amino acids of Stu2 (Stu2^{1–855}) completely abolished interaction with Spc72 (Figure 1A, lane 2). The Stu2^{1–855} two-hybrid construct was functional, since it interacted strongly with *STU2*^{648–888} (Figure 1A). The two-hybrid data were confirmed by an *in vitro* approach. The C-terminal truncated Stu2^{1–855} from insect cells had only a weak interaction with recombinant *Escherichia coli*-expressed N-Spc72 (Figure 1B, lane 6), whereas Stu2 showed strong interaction (lane 4). Thus, amino acids 853–888 of Stu2 are essential for Spc72 binding.

We then asked whether the Spc72-interaction domain of Stu2 is essential for viability and whether *STU2*^{1–855} cells are specifically defective in Stu2 binding to SPBs. Using a PCR approach, the chromosomal *STU2* was terminated after codon 855. Although *STU2*^{1–855} cells were viable when grown between 14–37°C (data not shown), cells showed nuclear and astral MT defects (see Supplementary figure 1 available at *The EMBO Journal* Online). Moreover, localization studies showed that the Stu2^{1–855} protein not only failed to bind to SPBs but also associated with strongly reduced efficiency (79.6% in wild-type and 16.7% in *STU2*^{1–855} cells) with MT plus ends (see Supplementary figure 2C). Thus, the astral MT defect of *STU2*^{1–855} cells is likely to arise from the failure of Stu2 to function at both ends of astral MTs.

Distinct domains of Spc72 facilitate interaction with the fully assembled Tub4 complex and with Stu2

The requirement for the C-terminal 35 amino acids of Stu2 for its function at both the plus and minus ends of astral MTs probably explains why we failed to obtain *STU2* mutants with defects in only one of the two activities.

Fig. 1. The 35 C-terminal amino acids of Stu2 mediate binding to Spc72. **(A)** The 35 C-terminal amino acids of Stu2 interact with Spc72 in the yeast two-hybrid system. The domain structure of Stu2 is shown. The MT-binding domain is from amino acids 558–657 (Wang and Huffaker, 1997), the coiled coil domain was predicted from amino acids 697–758 and the low complexity region (LC) from amino acids 766–781. Abbreviations: –, +, ++ symbolize no, good or strong interaction in the two-hybrid system, respectively. **(B)** Stu2^{1–855} failed to bind to N-Spc72 *in vitro*. Sf9 cell extracts with Stu2 or Stu2^{1–855} (lanes 1 and 2) were incubated with *E. coli*-expressed purified GST (lanes 3 and 5) or GST-N-Spc72 (lanes 4 and 6) bound to glutathione-Sepharose beads. After washing, the bound proteins were eluted and analysed by immunoblotting.

Based on two-hybrid and co-immunoprecipitation experiments, it was suggested that Spc72 interacts with the Tub4 complex and Stu2 (Chen *et al.*, 1998; Knop and Schiebel, 1998; Souès and Adams, 1998). If Stu2 and the Tub4 complex bind to distinct regions of Spc72, it should be possible to construct *SPC72* mutants in which the association with Stu2 is defective yet the interaction with the Tub4 complex is unaffected. We therefore mapped the binding sites of Spc72 for the Tub4 complex subunit Spc98 (Geissler *et al.*, 1996) and Stu2.

As reported previously (Chen *et al.*, 1998; Knop and Schiebel, 1998), full-length Spc72 interacted with Spc98 and Stu2 in the two-hybrid system (Figure 2A, lane 1). Interaction of Stu2 with Spc72 was relatively weak, which is explained by the recruitment of the Stu2 construct to MTs. Analysis of Spc72 subfragments then showed that the C-terminal Spc72^{231–622} fragment (Spc72^{SPB}), which interacts with the SPB components Nud1 and Kar1 by two-hybrid (Pereira *et al.*, 1999; Gruneberg *et al.*, 2000), failed to bind to Spc98 and Stu2 (lane 7). This suggests that the N-terminus of Spc72 mediates interaction with Spc98 and Stu2. Indeed, the 267 N-terminal amino acids of Spc72 (N-Spc72) showed two-hybrid interactions with Spc98 and Stu2 (lane 4). N-terminal deletion constructs of Spc72 were then used to address whether Spc98 and Stu2 bind to distinct or overlapping regions of Spc72. Spc72 constructs lacking amino acids 116–230 (Spc72^{ΔStu2*}, line 2) or 176–230 (Spc72^{ΔStu2}, line 3) interacted with Spc98 but not Stu2. Similarly, Spc72^{1–116} (N-Spc72^{ΔStu2}, line 6) bound to Spc98 but not Stu2. In contrast, Spc72^{99–267} (N-Spc72^{ΔTub4}, line 5) only interacted with Stu2 but not Spc98. These data suggest that the N-terminus of

Fig. 2. The N-terminus of Spc72 has distinct binding sites for Stu2 and the Tub4 complex. (A) Two-hybrid interactions of *STU2* and *SPC98* with indicated fragments of *SPC72*. Abbreviations are as in Figure 1A. (B) Anti-HA immunoprecipitations from *SPC72* (lanes 1 and 5), *SPC72-3HA* (lanes 2 and 6), *SPC72 Δ Stu2⁺* (lanes 3 and 7), *SPC72 Δ Stu2⁺-3HA* (lanes 4 and 8), *SPC72 Δ Tub4* (lanes 9 and 11) and *SPC72 Δ Tub4-3HA* cells (lanes 10 and 12). For *SPC72 Δ Tub4* and *SPC72 Δ Tub4-3HA* cells the rare, slow growing survivors of Figure 3A were used. Cell extracts (lanes 1–4 and 9–10) were incubated with the anti-HA antibodies followed by protein G Sepharose. The washed precipitations (lanes 5–8 and 11–12) were then analysed by immunoblotting with affinity-purified anti-Spc72, anti-Tub4, anti-Spc97 and anti-Spc98 antibodies (lanes 5–8) or only with anti-Spc72 and anti-Tub4 antibodies (lanes 11–12). The Spc72 doublets seen in lanes 8, 10 and 12 are due to partial degradation of Spc72. The asterisks in lanes 1–4 indicate the position of the Spc72 proteins. (C) Yeast total extracts of cells expressing *STU2* (–) (pRS426-*STU2*; pSM564) or *STU2-3HA* (+) (pRS426-*STU2-3HA*; pSM669) and chromosomally integrated *SPC72-9Myc* (lanes 1 and 2) or *SPC72 Δ Stu2⁺-9Myc* (lanes 3 and 4) were incubated with anti-HA antibodies. The immunoprecipitations (IP) were probed with anti-HA and anti-Myc antibodies (lanes 5–8). (D) Only the complete Tub4 complex bound strongly to Spc72. Tub4 complex subunits expressed in the indicated combination in Sf9 insect cells were incubated with recombinant and purified GST or GST-N-Spc72 from *E.coli*. After washing, the eluted proteins were analysed by immunoblotting with the indicated affinity-purified antibodies. (E) *In vitro* binding of recombinant and purified Tub4 complex and Stu2 (lane 1) from Sf9 cells to *E.coli* expressed and purified GST (lane 2), GST-N-Spc72 (lane 3), GST-Spc72 Δ Stu2 (lane 4) and GST-Spc72 Δ Tub4 (lane 5). Proteins were detected by immunoblotting.

Spc72 carries distinct binding sites for the Tub4 complex and Stu2.

Immunoprecipitation experiments were performed to confirm the two-hybrid data. Co-immunoprecipitation of Tub4 complex subunits with Spc72 (Figure 2B, lane 6), Spc72 Δ Stu2⁺ (lane 8) and Spc72 Δ Stu2 (data not shown) but not with Spc72^{99–622} (Spc72 Δ Tub4, lane 12) was consistent with the notion that the Tub4 complex binding site of Spc72 resided in the 100 N-terminal amino acids and that deletion of the Stu2-binding domain did not affect Tub4 complex binding to Spc72. Moreover, confirming that

Spc72 Δ Stu2 fails to associate with Stu2 *in vivo*, Stu2-3HA co-immunoprecipitated Spc72-9Myc (Figure 2C, lane 6) but not Spc72 Δ Stu2-9Myc (lane 8).

Although the two-hybrid and immunoprecipitation experiments clearly demonstrated that the Tub4 complex and Stu2 interacted with Spc72, it remained unclear whether these interactions were direct or indirect. *In vitro* binding experiments were performed to show direct binding of the Tub4 complex and Stu2 to Spc72. Reconstituted Tub4 complex from insect cells (Vinh *et al.*, 2002) interacted strongly with recombinant N-Spc72

Fig. 3. Only the Tub4 complex binding domain but not the Stu2-binding site of Spc72 is essential for viability. (A) *Δspc72* pRS316-*SPC72* cells were transformed with the indicated *LEU2*-based plasmids. Transformants were grown on YPD and 5-FOA plates for 3 days at 30°C. After 7 days at 30°C, a few 5-FOA resistant *SPC72^{ΔTub4}* and *SPC72¹⁷⁶⁻⁶²²* colonies were obtained (data not shown). (B) 5-FOA resistant colonies of (A) were analysed by immunoblotting for Spc72 expression using anti-C-terminus Spc72 antibodies.

(Figure 2D, lane 6). In contrast, Spc72 association with individual subunits (Spc97 and Spc98) and heterodimers (Spc97-Tub4 and Spc98-Tub4) was much weaker (lanes 7–12). Thus, only the fully assembled Tub4 complex binds with high efficiency to Spc72. When N-Spc72 subfragments were tested, Stu2 but not the Tub4 complex interacted with N-Spc72^{ΔTub4} (Figure 2E, lane 5). In contrast, only the Tub4 complex but not Stu2 bound to N-Spc72^{ΔStu2} (lane 4). These binding data suggest that the N-terminal 100 amino acids of Spc72 interact directly with the Tub4 complex and amino acids 99–267 of Spc72 with Stu2. The Stu2-binding site is not required for Tub4 complex binding and vice versa.

The Tub4 complex but not the Stu2-binding region of Spc72 is essential

In our S288c strain background *SPC72* is an essential gene (Knop and Schiebel, 1998). This allowed us to address whether the Stu2 or Tub4 complex binding domains of Spc72 were required for viability. Cells from which the chromosomal *SPC72* was deleted and which were kept alive by *SPC72* on a *URA3*-based plasmid were transformed with *LEU2*-based plasmids containing *SPC72* derivatives. Cells were then tested for growth on 5-FOA. As the 5-FOA selects against the *URA3*-based *SPC72* plasmid, the *LEU2*-based *SPC72* construct was the only source of *SPC72* activity in the 5-FOA resistant colonies. Despite its expression, *SPC72^{ΔTub4}* failed to support growth on 5-FOA plates (Figure 3A, sector 3). Only after long incubation did a few, poor-growing colonies expressing *SPC72^{ΔTub4}* but not *SPC72* appear (Figure 3B, lane 2). Similarly *SPC72¹⁷⁶⁻⁶²²* cells lacking the Tub4

complex and part of the Stu2-binding regions of Spc72 were unable to grow on 5-FOA (sector 4). Thus, the Tub4 complex binding region of Spc72 is essential for viability. However, the Tub4 complex binding domain by itself was not sufficient to support viability (sectors 7 and 8). Therefore, this domain has to be attached to the C-terminal SPB targeting region of Spc72 (Knop and Schiebel, 1998) in order to fulfil its essential function. Finally, both *SPC72^{ΔStu2*}* and *SPC72^{ΔStu2}* constructs allowed growth on 5-FOA (Figure 3A, sectors 5 and 6, 5-FOA), indicating that the Stu2-binding domain of Spc72 is non-essential. Together, the Tub4 but not the Stu2-binding domain of Spc72 is required for cell viability.

Spc72^{ΔStu2} is specifically defective in Stu2 interaction at SPBs

Spc72^{ΔStu2} was characterized to ensure that it only affected the interaction between Spc72 and Stu2 and not the association of Stu2 with the plus ends of astral MTs. The functional Stu2–4GFP localized in *SPC72* wild-type (Figure 4A, arrow head) and *SPC72^{ΔStu2}* cells (Figure 4B, arrow) to astral MT plus ends. Analysis of the Stu2–4GFP signal intensity then revealed that Stu2 binding to astral MT plus ends was not altered in *SPC72^{ΔStu2}* cells compared with *SPC72* wild-type cells (Figure 4C). Thus, the binding of Stu2 to astral MT plus ends is not affected by the *SPC72^{ΔStu2}* mutation.

D-TACC, the functional homologue of Spc72, and Msp5 interact not only at centrosomes but also at MT plus ends (Lee *et al.*, 2001). A similar interaction of Spc72 and Stu2 at astral MT plus ends would complicate the interpretation of the *SPC72^{ΔStu2}* phenotype. Previous localization studies using a *SPC72-GFP* gene fusion may have missed a weak Spc72 signal at astral MT plus ends (Chen *et al.*, 1998; Knop and Schiebel, 1998). We therefore re-investigated the localization of the fully functional Spc72–4GFP in *CFP-TUB1* cells. Under conditions that allowed detection of Stu2–4GFP protein at astral MT plus ends (Figure 4A), it was only detected at SPBs but not along astral MTs or MT plus ends (Figure 4D). *Spc72^{ΔStu2}* behaved as Spc72 (data not shown). Thus, Stu2 and Spc72 only co-localize at SPBs. It is likely, therefore, that the *SPC72^{ΔStu2}* mutation only affects Stu2 at SPBs but not astral MT plus ends.

Astral MTs detach from the SPB of *SPC72^{ΔStu2}* cells

The phenotype of *SPC72^{ΔStu2}* cells was determined to address the function of Stu2 at SPBs. Because astral MT behaviour is cell cycle dependent, we analysed cells in different phases of the cell cycle (Carminati and Stearns, 1997; Vogel *et al.*, 2001). Wild-type *SPC72* and *SPC72^{ΔStu2}* cells were synchronized in the G₁ phase of the cell cycle by α -factor block and release. In G₁, SPBs of *SPC72^{ΔStu2}* and *SPC72* cells had similar numbers of MTs (Figure 5A). A more detailed analysis by time-lapse video microscopy, however, showed that astral MTs detached from the SPB of *SPC72^{ΔStu2}* (Figure 5E, arrows) but not *SPC72* cells (data not shown). Thus, binding of Stu2 to Spc72 is important for the anchoring of astral MTs to SPBs.

Astral MT defects were also observed in pre-anaphase cells. Twenty-three per cent of pre-anaphase *SPC72^{ΔStu2}* cells showed astral MTs that were disconnected from the

Fig. 4. Stu2 binds to the plus ends of astral MTs of *SPC72 Δ Stu2* cells. Wild-type (A) and *SPC72 Δ Stu2* cells (B) with *STU2-4GFP CFP-TUB1* were analysed for Stu2 localization by fluorescence microscopy. The arrowhead in (A) indicates Stu2 associated with astral MT plus ends. The asterisks in (B) highlight the position of the spindle pole, and the arrows point towards the astral MT plus ends containing Stu2. (C) The relative fluorescence intensity of the Stu2-4GFP signal at astral MT plus ends of cells in (A) and (B) was determined. $n > 50$. (D) Spc72 is not associated with astral MT plus ends. Using *SPC72-4GFP CFP-TUB1* cells, Spc72-4GFP localization was determined by fluorescence microscopy. Scale bars: 5 μ m.

SPB (Figure 5B, arrow). Time-lapse analysis revealed that these MTs were initially organized from the SPB but subsequently detached (see Supplementary figure 3A). Moreover, 60% of *SPC72 Δ Stu2* cells either lacked (Figure 5B, arrow head) or had fewer astral MTs than *SPC72* cells (Figure 5B). These astral MT defects resulted in spindles that were no longer oriented along the mother-bud axis in around 80% of *SPC72 Δ Stu2* cells (Figure 5B, arrow head).

SPC72 Δ Stu2 cells in anaphase did not show detached astral MTs. This either means that the detachment of astral MT is restricted to pre-anaphase cells or that detached astral MTs of anaphase cells are highly unstable. Despite the lack of MT detachment, astral MTs of anaphase *SPC72 Δ Stu2* cells were far from normal. Instead, the *SPC72 Δ Stu2* mutation affected the two astral MT bundles organized of the two SPBs in a different and distinct manner. There was only a slight reduction in the number of astral MTs associated with the SPB, which was located within the bud (Figure 5C). However, in 40–50% of these cells, the MTs were about 10 times longer and curved around the bud tip back into the mother cell (Figure 5C, arrows). Time-lapse video microscopy analysis of these *SPC72 Δ Stu2* cells showed that the astral MTs extended

normally into the bud at the beginning of anaphase (Figure 5F, 0–285 s, arrows). Instead of the normal depolymerization that is seen in wild-type cells (Carminati and Stearns, 1997), these bud proximal astral MTs of *SPC72 Δ Stu2* cells did not depolymerize, but slid along the bud cortex as anaphase progressed. In contrast, the mother ward directed SPBs of *SPC72 Δ Stu2* cells were not associated with elongated astral MTs. In fact, these SPBs were more often devoid of detectable astral MTs than the bud-ward directed SPB (Figure 5D; 20% versus 45%). In summary, the Stu2-binding domain of Spc72 fulfils multiple functions during the cell cycle. It is required for the anchorage of astral MTs to SPBs in pre-anaphase cells, for formation of sufficient astral MTs throughout the cell cycle, and for depolymerization of astral MTs in late anaphase.

Altered astral MTs dynamics of *SPC72 Δ Stu2* cells

The dynamic properties of astral MTs of pre-anaphase *SPC72* and *SPC72 Δ Stu2* cells were determined from time-lapse data (see Supplementary figure 3B). Although the astral MTs of pre-anaphase *SPC72 Δ Stu2* cells depolymerized at the same rate as seen in wild-type *SPC72* cells (Table I), their growth rate was moderately increased by a

factor of 1.3. The most significant change was the 1.5- and 2.3-fold reduction in catastrophe and rescue frequencies, respectively (Table I). These changes resulted in more continuous growth and shrinkage of astral MTs of *SPC72 Δ Stu2* cells (see Supplementary figure 3B). Thus, Stu2 binding to Spc72 at the SPB has a significant and important impact upon the dynamics of astral MTs.

Stu2, the Tub4 complex and Spc72 assemble into a complex and cooperate

Both the Tub4 complex and Stu2 are recruited to the cytoplasmic side of the SPB by binding to Spc72 (Chen *et al.*, 1998; Knop and Schiebel, 1998), where they have important roles in astral MT organization (Figures 3 and 5). The close spatial link of the two binding sites in Spc72 (Figure 2) suggests that the Tub4 complex and Stu2 may, in a large complex, cooperate in astral MT organization. To address this possibility, we first asked whether the Tub4 complex and Stu2 are present in common complexes. In such a case, the Tub4 complex subunit Spc97 would be expected to co-immunoprecipitate with Stu2. An anti-HA immunoprecipitate from cells expressing HA-tagged Spc97 contained not only Spc97-3HA but also the Tub4 complex components Spc98 and Tub4, Spc72 and Stu2-3Myc independently of whether logarithmically growing or G₁ arrested (α -factor) cells were used

(Figure 6A). Thus, the Tub4 complex, Spc72 and Stu2 are part of a larger complex.

In vitro binding studies were performed to address how the Tub4 complex, Spc72 and Stu2 interact. Recombinant Tub4 complex and Stu2-3HA were incubated with GST or GST-N-Spc72, followed by the precipitation of Stu2-3HA with anti-HA antibodies. In the presence of GST, the Tub4 complex did not co-precipitate with Stu2-3HA (Figure 6B, lane 8). Addition of N-Spc72 enabled co-immunoprecipitation of the Tub4 complex and Stu2 (Figure 6B, lane 9). Similar results were obtained with N-Spc72-6His (data not shown). This result has two important implications. First, it suggests that the Tub4 complex and Stu2 do not interact directly. Secondly, the Tub4 complex and Stu2 assemble into a larger complex via their association with Spc72. However, since in the yeast two-hybrid assay N-Spc72 still interacted with itself (data not shown), our data do not enable us to determine whether Stu2 and the Tub4 complex bind to the same Spc72 molecule or to different Spc72 molecules, which subsequently form dimers (Chen *et al.*, 1998).

The above experiments show Spc72-mediated complex formation between the Tub4 complex and Stu2. This interaction and the common function in astral MT organization suggest that the Tub4 complex and Stu2 cooperate at SPBs. Functional cooperation of Stu2 and the

Tub4 complex should result in genetic interaction between *SPC72^{ΔStu2}* and alleles of components of the Tub4 complex. *spc98-2 SPC72^{ΔStu2}* cells were less viable at 23°C and 30°C compared with *spc98-2* or *SPC72^{ΔStu2}* cells (Figure 6C, top). The *tub4-1* allele also weakly interacted with *SPC72^{ΔStu2}* (Figure 6C, 35°C). A genetic interaction was not observed when the conditional lethal *spc97-1* and *spc97-3* alleles (Knop *et al.*, 1997) were combined with *SPC72^{ΔStu2}* (data not shown). This allele specific interaction suggests functional cooperation between the Tub4 complex and Stu2 in astral MT organization.

Intramolecular complementation of *SPC72* mutants

Our data indicate that the Tub4 complex and Stu2 cooperate in astral MT organization. For this cooperation, the Tub4 complex and Stu2 have to bind to either the same Spc72 molecule or to distinct Spc72 molecules, which are then brought together by Spc72 dimerization (Chen *et al.*, 1998). In the latter case, two Spc72 derivatives, one able to bind only the Tub4 complex and the other only Stu2, should show trans-complementation when coexpressed in cells lacking any other form of Spc72.

Fig. 5. The Stu2-binding site of Spc72 fulfils distinct functions during the cell cycle. (A–D) Astral MT number and morphology of synchronized *SPC72* wild-type and *SPC72^{ΔStu2}* cells with *GFP-TUB1* were analysed throughout the cell cycle. $n > 100$. (A) Astral MTs appear to be normal in *SPC72^{ΔStu2} GFP-TUB1* cells in G₁. (B) Pre-anaphase budded *SPC72^{ΔStu2} GFP-TUB1* cells have fewer and detached astral MTs. The arrow highlights a detached astral MT and the arrowhead a short mispositioned spindle without astral MTs. (C and D) *SPC72^{ΔStu2} GFP-TUB1* cells in anaphase. The number of astral MTs associated with the SPB in the bud (C) or in the mother cell body (D) is indicated. The arrows indicate largely extended astral MTs in *SPC72^{ΔStu2} GFP-TUB1* cells. (E) Time-lapse analysis of a *SPC72^{ΔStu2} GFP-TUB1* cell in G₁. A time-lapse series of the same cell was taken every 15 s. The arrows highlight a detaching astral MT. (F) Time-lapse analysis of a *SPC72^{ΔStu2} GFP-TUB1* cell in anaphase. A time-lapse series of the same cell was taken every 15 s. The arrows indicate astral MTs that contact the bud cell cortex and then fail to depolymerize. Scale bars: 5 μm.

Table I. Astral microtubule dynamics

	<i>SPC72</i> (<i>n</i> = 12)	<i>SPC72^{ΔStu2}</i> (<i>n</i> = 12)	<i>SPC72^{ΔStu2}</i> <i>STU2¹⁻⁸⁸⁸-SPC72^{SPB*}</i> (<i>n</i> = 8)	a	b
Rates (μm/min)					
Growing	1.19 ± 0.59	1.55 ± 0.52	1.23 ± 0.50	<i>P</i> < 0.001	<i>P</i> < 0.001
Shrinking	1.72 ± 0.76	1.76 ± 0.61	1.78 ± 0.70	<i>P</i> = 0.72	<i>P</i> = 0.83
Frequencies (event/s)					
Catastrophe	0.0147	0.0098	0.0119		
Rescue	0.0206	0.0089	0.0150		
Event duration (min)					
Growing	0.74 ± 0.45	0.82 ± 0.46	0.96 ± 0.55	<i>P</i> = 0.43	<i>P</i> = 0.27
Shrinking	0.55 ± 0.25	1.01 ± 0.43	0.77 ± 0.31	<i>P</i> < 0.001	<i>P</i> = 0.017
Pause	0.61 ± 0.16	0.85 ± 0.46	0.67 ± 0.29	<i>P</i> = 0.073	<i>P</i> = 0.24
Event length changes (μm)					
Growing	0.88	0.96	1.18		
Shrinking	1.27	1.74	1.37		
Total times (%)					
Growing	49.6	41.1	52.4		
Shrinking	35.3	41.8	35.1		
Pausing	15.2	17.1	12.4		

a: *t*-test between *SPC72* and *SPC72^{ΔStu2}*.b: *t*-test between *SPC72^{ΔStu2}* and *SPC72^{ΔStu2} STU2¹⁻⁸⁸⁸-SPC72^{SPB*}*.

The position of the nucleus is dependent on astral MTs (Palmer *et al.*, 1992). To test for trans-complementation, we investigated whether *SPC72^{ΔTub4}* suppressed the nuclear position defect of pre-anaphase *SPC72^{ΔStu2}* cells. In 45% of *SPC72^{ΔStu2}* cells, the nucleus was not seen close to the bud neck (Figure 7A, lane 1). This defect was reduced to the same extent by either *SPC72* (lane 2) or *SPC72^{ΔTub4}* (lane 4), but was not significantly reduced by *SPC72¹⁷⁶⁻⁶²²* (lane 3), *SPC72^{ΔStu2*}* (lane 5) or *SPC72^{ΔStu2}* (lane 6), all lacking at least part of the Stu2-binding domain. Thus, *SPC72^{ΔTub4}* suppresses the nuclear position defect of *SPC72^{ΔStu2}* cells.

The number of astral MTs per cell was determined to confirm that the *SPC72^{ΔTub4}* construct rescued the astral MT defect of *SPC72^{ΔStu2}* cells. *SPC72^{ΔTub4}* increased the number of astral MTs per *SPC72^{ΔStu2}* cell by a factor of 1.5. Wild-type *SPC72* was with a two-fold increase, slightly more efficient (Figure 7B). However, full complementation of the astral MT defect of *SPC72^{ΔStu2}* cells by *SPC72^{ΔTub4}* was hardly expected, considering that the functional entity of Spc72 is a homodimer (Chen *et al.*, 1998). The active Spc72^{ΔStu2}-Spc72^{ΔTub4} dimer would therefore only be expected to occur in 50% of Spc72-Spc72 dimer pairings. Together, the correction of the nuclear position defect and the increase in astral MT number of *SPC72^{ΔStu2}* cells by *SPC72^{ΔTub4}* show that the Tub4 complex and Stu2 do not have to bind to the same Spc72 molecule in order to effectively organize astral MTs.

SPB-associated Stu2 requires the MT-binding domain

Given that the Stu2 and Tub4 complex binding domains do not have to be encoded by the same Spc72 molecule, a fusion between *STU2* and the SPB targeting domain of *SPC72* might be expected to complement astral MT defects of *SPC72^{ΔStu2}* cells if the main defect in these cells lies in the recruitment of Stu2 to SPBs. If is the case,

deletion analysis will reveal the domains of Stu2 that are essential for its function at the SPB.

We fused the entire *STU2* gene (codons 1–888) to the *SPC72²⁷⁷⁻⁶²²* subfragment, which lacked the Stu2 and Tub4 complex binding sites but encoded the SPB-binding domain (Knop and Schiebel, 1998). The fusion protein was named Stu2¹⁻⁸⁸⁸-Spc72^{SPB*}. To ensure that Stu2¹⁻⁸⁸⁸-Spc72^{SPB*} functions at SPBs, we first studied the subcellular localization of the GFP-tagged protein. In 96% of cells Stu2-Spc72^{SPB*}-GFP only associated with SPBs (Spc42-RFP) but not with astral MTs or the central spindle (Figure 8A). A weak astral MT-like staining was only apparent in ~4% of cells. The *STU2-SPC72^{SPB*}* gene fusion did not complement a deletion of *STU2* (Figure 8B), indicating that it fulfilled only a subset of Stu2 functions. Thus, Stu2-Spc72^{SPB*} is predominantly associated with SPBs.

The nuclear position defect of *SPC72^{ΔStu2}* cells (Figure 8C, lane 1) was reduced by *STU2¹⁻⁸⁸⁸-SPC72^{SPB*}* (lane 8) as strongly as by *SPC72* (lane 2). This suppression was a direct consequence of altered astral MTs. First, in the presence of *STU2¹⁻⁸⁸⁸-SPC72^{SPB*}*, more astral MTs were associated with SPBs of *SPC72^{ΔStu2}* cells (Figure 8D) but not wild-type cells (data not shown). Secondly, the strong elongation of bud-ward directed astral MTs in anaphase *SPC72^{ΔStu2}* cells was suppressed by *STU2¹⁻⁸⁸⁸-SPC72^{SPB*}* (Figure 8E). Thirdly, *STU2¹⁻⁸⁸⁸-SPC72^{SPB*}* restored, in part, the dynamic behaviour of astral MTs of *SPC72^{ΔStu2}* cells (Table I).

Complementation of *SPC72^{ΔStu2}* defects by *STU2¹⁻⁸⁸⁸-SPC72^{SPB*}* allowed us to investigate the region of Stu2 that was important for Stu2 function at SPBs. The C-terminal 33 amino acids of Stu2 were required for MT plus end binding (see Supplementary figure 2C). However, deletion of these same 33 amino acids still enabled a 70–80% complementation of the fusion protein (Figure 8C, lane 7). Deletion of the N-terminal HEAT repeats reduced complementation by 30–40% (lane 4). Hardly any

Fig. 6. The Tub4 complex, Stu2 and Spc72 assemble into one complex and act in a cooperative manner. (A) Tub4 complex, Stu2 and Spc72 are part of common complexes. Lysates of logarithmically growing or α -factor arrested *SPC97* and *SPC97-3HA* cells, both with *STU2-3Myc*, were subjected to anti-HA immunoprecipitation. Precipitated proteins were detected by immunoblotting with the indicated antibodies. (B) Tub4 complex (lanes 1–3 and 7–9) and Stu2-3HA (lanes 4–9), both from Sf9 cells, were incubated with recombinant and purified GST or GST-N-Spc72. After an initial incubation step, anti-HA antibodies were added followed by protein G Sepharose beads. The anti-HA precipitates were analysed by immunoblotting with the indicated antibodies. (C) Genetic interaction between *SPC98* and *SPC72 Δ Stu2*. Serial dilutions of the indicated yeast cells were grown at the indicated temperatures on YPD plates for 3 days.

complementation was observed when the MT-binding domain of Stu2 was removed (lanes 5, 6 and 9). All Stu2–Spc72^{SPB*} derivatives were expressed similarly (data not shown). The MT-binding domain is therefore the part that is most important for the function of Stu2 at SPBs.

Discussion

In this report, we investigated the binding and function of Stu2 at SPBs and its interaction with the Tub4 complex. A model of how Stu2 and the Tub4 complex bind to SPBs via Spc72 is given in Figure 9. Consistent with previous results (Chen *et al.*, 1998; Knop and Schiebel, 1998), Stu2 and the Tub4 complex bind directly to the SPB component Spc72. The first 100 amino acids of Spc72 are essential

and interact with the Tub4 complex. Only the Tub4 complex but not individual subunits or subcomplexes bind with high efficiency to Spc72. This ensures that only the fully assembled Tub4 complex that is competent to promote MT nucleation is targeted to SPBs. The second, Stu2-binding domain of Spc72, encoded by amino acids 100–230, is non-essential. Interaction of Stu2 and the Tub4 complex via Spc72 (Figure 6) suggests that Spc72 joins Stu2 and the Tub4 complex together in a single, larger complex. This can be achieved in two ways. The Tub4 complex and Stu2 could interact through the spatially distinct binding sites with the same Spc72 molecule. Alternatively, considering that Spc72 forms homodimers (Chen *et al.*, 1998), the Tub4 complex could bind to one Spc72 molecule while Stu2 binds to the other. In either case, complementation of the *SPC72 Δ Stu2* phenotype by *SPC72 Δ Tub4* (Figure 7) indicates that Stu2 and the Tub4 complex do not have to interact with the same Spc72 molecule in order to be functional (as illustrated in Figure 9).

The functional analysis of Stu2 at SPBs was complicated by the fact that Stu2 is associated not only with SPBs but also with astral MT plus ends, kinetochores, the spindle midzone and along MTs (Wang and Huffaker, 1997; He *et al.*, 2001; Kosco *et al.*, 2001). Truncation of Stu2 at the C-terminus abolished not only Stu2 binding to SPBs but also localization to astral MT plus ends (see Supplementary figure 2) where Stu2 probably destabilizes MTs (van Breugel *et al.*, 2003). An alternative approach was to study *SPC72* mutants that specifically fail to interact with Stu2. For the interpretation of the phenotype of such mutants, it was important to know whether the Spc72 protein is only important at SPBs or, as is the case of D-TACC (Lee *et al.*, 2001), also associates with MTs and MT plus ends. The fully functional Spc72 protein tagged with four copies of GFP was only detected at SPBs but not along MTs or at MT plus ends (Figure 4D). The conditions used were such that they allowed the detection of Stu2–4GFP at astral MT plus ends (Figure 4A, B and C). In addition, the Tub4 complex and Stu2-binding domains of Spc72 fulfil their function in astral MT organization when fused to the integral SPB components Kar1 and Cnm67 (Pereira *et al.*, 1999; Gruneberg *et al.*, 2000). When taken together, these results support the view that Spc72 only functions at SPBs.

To impair the binding of Stu2 to Spc72 at SPBs, we deleted the non-essential Stu2-binding domain of Spc72. *Spc72 Δ Stu2* is specifically defective in Stu2 interaction at SPBs, but is normal with regards to the interaction with the Tub4 complex (Figure 2), Stu2 localization to MT plus ends (Figure 4A, B and C) and other functions of Spc72 in astral MT organization. This notion is further supported by the finding that Stu2 permanently tethered to SPBs strongly reduced the astral MT defect of *SPC72 Δ Stu2* cells (Figure 8).

The dynamic properties of astral MTs were altered in *SPC72 Δ Stu2* cells. There was a marked reduction in catastrophe and rescue frequencies. Similarly, defects in the budding and fission yeast γ -tubulin complexes (which are like the Spc72–Stu2 complex, associated with MT minus ends) also affected astral MT dynamics (Spang *et al.*, 1996; Paluh *et al.*, 2000; Vogel *et al.*, 2001; Fujita *et al.*, 2002). Since astral MTs in budding yeast are only

Fig. 7. Intramolecular complementation of Spc72 molecules. (A) The nuclear position defect of *SPC72* ^{Δ Stu2} cells was rescued by *SPC72* ^{Δ Tub4}. Logarithmically growing *SPC72* ^{Δ Stu2} cells with chromosomally integrated pRS306 control plasmid (lane 1), pRS306-*SPC72* (lane 2), pRS306-*SPC72*¹⁷⁶⁻⁶²² (lane 3), pRS306-*SPC72* ^{Δ Tub4} (lane 4), pRS306-*SPC72* ^{Δ Stu2} (lane 5) or pRS306-*SPC72* ^{Δ Stu2} (lane 6) were fixed, the DNA was stained with DAPI and the position of the nucleus was determined as outlined in the figure. $n > 100$. (B) *SPC72* ^{Δ Tub4} increases the number of astral MTs of *SPC72* ^{Δ Stu2} cells. The number of astral MTs of *SPC72* ^{Δ Stu2} *GFP-TUB1* cells carrying *SPC72* ^{Δ Tub4} or *SPC72* was determined by fluorescence microscopy. $n > 100$.

dynamic at their plus ends (Maddox *et al.*, 2000), these observations suggest that proteins associated with the MT minus ends influence MT plus end dynamics. Two models are consistent with this phenotype. Stu2 and Tub4 complex binding to the MT minus ends could impose an altered conformation onto interacting tubulin subunits, which then would be transmitted from the minus ends onward toward the dynamic plus ends of MTs through tubulin–tubulin interactions. Alternatively, MT plus end regulators could be loaded onto SPBs before travelling along MTs to their plus ends. Recent work has provided evidence that a complex of Kar9, the MT-binding protein Bim1 and yeast Cdk1, Cdc28, are transported from SPBs to MT plus ends by the kinesin motor Kip2 (Liakopoulos *et al.*, 2003; Maekawa *et al.*, 2003). Since Kar9 and Stu2 are present in common complexes (Miller *et al.*, 2000), Kar9 loading onto the SPB could require complex formation between Spc72 and Stu2. To this end, known MT regulators such as Bik1 (Berlin *et al.*, 1990), Kip2 (Huyett *et al.*, 1998), Kip3 (Miller *et al.*, 1998), Cdk1/Cdc28 and Kar9 still associate with MT plus ends in *SPC72* ^{Δ Stu2} cells (see Supplementary figure 4; data not shown). However, our data do not exclude the possibility that interaction of an MT regulator with Stu2 at SPBs is required for them to obtain activity.

We also observed that the two SPBs of anaphase *SPC72* ^{Δ Stu2} cells were affected differently. It is possible that the selective loading of the Bim1–Kar9 complex onto the bud-ward directed pre-existing old SPB, accompanied by the transport of the Bim1–Kar9 complex along astral MTs to the MT plus ends (Pereira *et al.*, 2001;

Liakopoulos *et al.*, 2003; Maekawa *et al.*, 2003), stabilizes the astral MTs organized by the old SPB. In contrast, the astral MTs generated in the mother cell body by the new SPB do not carry the Bim1–Kar9 complex and remain unstable.

Our study indicates that the full function of SPB-associated Stu2 is dependent upon its MT-binding site (Figure 8) (Wang and Huffaker, 1997). Moreover, Stu2 at SPBs promotes MT formation and prevents the detachment of astral MTs from SPBs (Figure 5). To explain these phenotypes, we propose that Spc72 binds the Tub4 complex that initiates MT formation from tubulin subunits. Stu2 associated via its C-terminus (Figure 1) either to the same Spc72 or to a second Spc72 molecule within the Spc72–Spc72 homodimer (Figure 9) (Chen *et al.*, 1998). The MT-binding domain of Stu2 first stabilizes short MT seeds nucleated by the Tub4 complex. When the MT cylinder is extended, the MT-binding site of Stu2 cooperates with the Tub4 complex to anchor astral MTs to SPBs. The data presented here also indicate the astral MT promoting function of Stu2 at SPBs is not essential for the viability of yeast cells. The essential function of Stu2 is therefore either at kinetochores, the formation of anaphase spindles or both (He *et al.*, 2001; Severin *et al.*, 2001).

In human cells, ch-TOG and the three TACC proteins have been implicated in cancer, but the transforming activity of these proteins is unclear (Raff, 2002). Our finding now suggests that many astral MT defects observed in Stu2 depleted cells (Kosco *et al.*, 2001) are actually caused by the failure of Stu2 to function at SPBs.

Fig. 8. The MT-binding domain of Stu2 is important for its function at SPBs. (A) A Stu2¹⁻⁸⁸⁸-Spc72^{SPB*}-GFP fusion protein is predominantly associated with SPBs. Cells with the SPB marker *SPC42-RFP* and *STU2*¹⁻⁸⁸⁸-*SPC72*^{SPB*}-GFP were analysed by fluorescence microscopy. Scale bar: 5 μ m. (B) *STU2*¹⁻⁸⁸⁸-*SPC72*^{SPB*} did not fulfil the essential functions of *SPC72* and *STU2*. *Δspc72* pRS316-*SPC72* and *Δstu2* pRS316-*STU2* cells were transformed with just the *LEU2*-based plasmid pRS315 or pRS315 with *SPC72*, *STU2*, or *STU2*¹⁻⁸⁸⁸-*SPC72*^{SPB*}. Transformants were grown on 5-FOA plates at 30°C for 3 days. (C) The nuclear position defect of *SPC72*^{Δstu2} cells was rescued by *STU2*¹⁻⁸⁸⁸-*SPC72*^{SPB*}. The experiment was performed as described for Figure 7A. *SPC72*^{Δstu2} cells were transformed with the integration plasmid pRS306 without an insert (lane 1), with pRS306-*SPC72* (lane 2), or the pRS306-*STU2*-*SPC72*^{SPB*} constructs (lanes 3–9). The correction of the nuclear position defect of *SPC72*^{Δstu2} cells by *SPC72* was set as 100% complementation. The domain structure of Stu2 is indicated. Note that *STU2* was fused to *SPC72*²⁷⁷⁻⁶²² (*SPC72*^{SPB*}) only encoding the SPB-binding domain (highlighted in lane 2 as light blue bar; the dark blue bar indicates the Tub4 complex and Stu2-binding regions of Spc72), which targets fusion proteins to SPBs (Knop and Schiebel, 1998). (D) The number of astral MTs of pre-anaphase *SPC72*^{Δstu2} *GFP-TUB1* cells with the integrated control vector pRS402, the pRS402-*SPC72* or pRS402-*STU2*¹⁻⁸⁸⁸-*SPC72*^{SPB*} was determined. $n > 100$. (E) *STU2*¹⁻⁸⁸⁸-*SPC72*^{SPB*} suppresses the elongated anaphase astral MT defect of *SPC72*^{Δstu2} cells. *SPC72* and *SPC72*^{Δstu2} cells with the integrated control vector pRS402 ('vector') and *SPC72*^{Δstu2} cells with the integrated pRS402-*STU2*¹⁻⁸⁸⁸-*SPC72*^{SPB*}, all in anaphase, were scored when the angle of the astral MTs in the bud exceeded 60° (as shown in the figure). $n > 100$.

In particular, we present the first evidence that the Spc72–Stu2 complex at SPBs contributes to the regulation of MT plus end dynamics. This finding makes XMAP215/Stu2-like proteins and their centrosomal anchors, TACC proteins and Spc72, important regulators of astral MT properties. OP18/stathmin, another protein with a role in cancer development, also regulates MT dynamics (Cassimeris, 2002). It will therefore be interesting to investigate whether a change in the MT regulatory activity of the TACC/ch-TOG complex at centrosomes is the basis of the transforming activity.

Materials and methods

Plasmids and yeast strains

Strains and plasmids are listed in Table II. Yeast strains were derivatives of YPH499 (Sikorski and Hieter, 1989). Yeast strains were constructed by PCR-based methods (Knop *et al.*, 1999; Maekawa *et al.*, 2003). Two-hybrid interactions were determined as described previously (Maekawa *et al.*, 2003).

Cell cycle analysis and growth conditions

Yeast strains were grown in yeast extract, peptone, dextrose medium containing 100 μ g/l adenine (YPAD medium) (Sherman, 1991). Cells were blocked in G₁ by the addition of 10 μ g ml⁻¹ mating pheromone,

Fig. 9. Model for the cooperation of Stu2 and the Tub4 complex at the cytoplasmic side of the SPB. See text for details.

α -factor. After 2.5 h at 23°C or 2 h at 30°C, cells were released from the block by washing and re-suspension in YPAD medium.

Expression of recombinant proteins

Recombinant *TUB4*, *SPC98*, *SPC97*, *STU2*, *STU2¹⁻⁸⁵⁵* and *STU2-3HA* were expressed in Sf9 cells by baculovirus expression system. The Tub4 complex was purified as described previously (Vinh *et al.*, 2002). Fragments of Spc72 were expressed in *E. coli* BL21 (DE3) as GST fusion proteins following induction by the addition of 0.5 mM IPTG for 4 h at 30°C.

In vitro binding experiments

For assessment of the *in vitro* binding between Spc72 and the Tub4 complex or Stu2, GST-fused Spc72 proteins bound to glutathione–Sepharose (GSH–Sepharose, AmershamPharmacia Biotech) were mixed with Sf9 cell extracts that expressed Tub4 complex components or Stu2.

Table II. Yeast strains and plasmids

Name	Genotype-construction	Source of reference
Yeast strains		
ATU39	MATa <i>ura3-52 trp1Δ63 his3Δ200 leu2Δ1 GFP-TUB1-URA3 STU2¹⁻⁸⁵⁵::klTRP1</i>	This study
ATU74	MATa <i>ura3-52 trp1Δ63 his3Δ200 leu2Δ1 SPC42-RFP-NatNT2 STU2-GFP-klTRP1</i>	This study
ATU86	MATa <i>ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 Δspc72::kanMX6 SPC72-LEU2</i>	This study
ATU99	MATa <i>ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 Δspc72::kanMX6 SPC72-9Myc-klTRP1-LEU2</i>	This study
ATU100	MATa <i>ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 Δspc72::kanMX6 SPC72^{ΔStu2}-9Myc-klTRP1-LEU2</i>	This study
ATU102	MATa <i>ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ2 Δspc72::kanMX6 SPC72-3HA-klTRP1-LEU2</i>	This study
ATU105	MATa <i>ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 Δspc72::kanMX6 SPC72^{ΔTub4}-LEU2</i>	This study
ATU106	MATa <i>ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 Δspc72::kanMX6 SPC72^{ΔTub4}-3HA-kanMX6-LEU2</i>	This study
ATU171	MATa <i>ura3-52 trp1Δ63 his3Δ200 leu2Δ1 Δspc72::kanMX6 SPC72^{ΔStu2*}-LEU2</i>	This study
ATU177	MATa <i>ura3-52 trp1Δ63 his3Δ200 leu2Δ1 Δspc72::kanMX6 SPC72^{ΔStu2*}-3HA-klTRP1-LEU2</i>	This study
ATU256	MATa <i>ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 Δspc72::kanMX6 SPC72^{ΔStu2}-LEU2 ADE2 GFP-TUB1-URA3</i>	This study
ATU257	MATa <i>ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 Δspc72::kanMX6 SPC72^{ΔStu2}-LEU2 SPC72-ADE2 GFP-TUB1-URA3</i>	This study
ATU263	MATa <i>ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 Δspc72::kanMX6 SPC72^{ΔStu2}-LEU2 pSPC72-STU2¹⁻⁸⁸⁸-SPC72^{SPB*}-ADE2 GFP-TUB1-URA3</i>	This study
ATU264	MATa <i>ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 Δspc72::kanMX6 SPC72-LEU2 ADE2 GFP-TUB1-URA3</i>	This study
ATU300	MATa <i>ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 Δspc72::kanMX6 SPC72^{ΔStu2}-LEU2 pSPC72-STU2¹⁻⁸⁸⁸-SPC72^{SPB*}-GFP-klTRP1-ADE2 SPC42-RFP-NatNT2</i>	This study
ESM278	MATa <i>ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 spc98-2</i>	Knop <i>et al.</i> (1997)
ESM356-1	MATa <i>ura3-52 trp1Δ63 his3Δ200 leu2Δ1</i>	This study
ESM448	MATa <i>ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 Δspc72::kanMX6 pSM447</i>	Knop and Schiebel (1998)
ESM489	MATa <i>ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 STU2-3Myc-kanMX6 Δsst1::URA3</i>	This study
ESM549	MATa <i>ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 STU2-3Myc-kanMX6 SPC97-3HA-His3MX6 Δsst1::URA3</i>	This study
ESM605	MATa <i>ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 Δstu2::kanMX6 pSM599</i>	This study
ESM709	MATa <i>ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 Δspc72::kanMX6 SPC72^{ΔStu2}-LEU2</i>	This study
ESM710	MATa <i>ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 Δspc72::kanMX6 SPC72^{ΔStu2*}-LEU2</i>	This study
SGY37	MATa <i>ura3-52::URA3-lexA-op-LacZ trp1Δ63 his3Δ200 leu2Δ1</i>	Geissler <i>et al.</i> (1996)
SHM277	MATa <i>Δspc72::kanMX6 STU2-GFP-kanMX6 spc25-7-LEU2 Δspc25::klTRP1</i>	This study
SHM295	MATa <i>ura3-52 trp1Δ63 his3Δ200 leu2Δ1 GFP-TUB1-URA3</i>	This study
SHM480	MATa <i>ura3-52 trp1Δ63 his3Δ200 leu2Δ1 STU2-4GFP-kanMX6 SPC42-RFP-NatNT2 CFP-TUB1-URA3</i>	This study
SHM534	MATa <i>ura3-52 trp1Δ63 his3Δ200 leu2Δ1 SPC72^{ΔStu2}-LEU2 Δspc72::hphMX4 STU2-4GFP-kanMX6 CFP-TUB1-URA3</i>	This study
SHM536	MATa <i>ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 GFP-TUB1-URA3</i>	This study
SHM537	MATa <i>ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 Δspc72::kanMX6 SPC72^{ΔStu2}-LEU2 GFP-TUB1-URA3</i>	This study
SHM622	MATa <i>ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 SPC72-4GFP-kanMX6 CFP-TUB1-URA3</i>	This study
SHM631	MATa <i>ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 Δspc72::kanMX6 SPC72^{ΔStu2}-LEU2 tub4-1 GFP-TUB1-URA3</i>	This study
SHM632	MATa <i>ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 tub4-1 GFP-TUB1-URA3</i>	This study
SHM633	MATa <i>ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 GFP-TUB1-URA3</i>	This study

After 60 min incubation, GSH–Sepharose was washed with extraction buffer and proteins were eluted with SDS sample buffer. The samples were analysed by immunoblotting with affinity-purified rabbit anti-Tub4, anti-Spc98, anti-Spc97, anti-Stu2, and monoclonal mouse anti-HA (12C5)

antibodies. For *in vitro* binding of Tub4 complex, Stu2 and Spc72, purified GST or GST–N-Spc72 was incubated for 60 min in Sf9 cell extract in which the Tub4 complex, Stu2-3HA or both were expressed. Stu2-3HA was immunoprecipitated with anti-HA antibody (12CA5).

Table II. *Continued*

Name	Genotype-construction	Source of reference
SHM634	MATa <i>ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 Aspc72::kanMX6 SPC72^{ΔStu2} LEU2 spc98-2</i>	This study
SHM662	MATa <i>ura3-52 leu2Δ1 his3Δ200 trp1Δ63 SPC72-4GFP-kanMX6 CFP-TUB1-URA3</i>	This study
YPH499	MATa <i>ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1</i>	Sikorski and Hieter (1989)
YPH500	MATα <i>ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1</i>	Sikorski and Hieter (1989)
Plasmids		
pAcSG2	Baculovirus expression vector	Pharmingen
pACT2	2 μm, <i>LEU2</i> -based vector carrying the <i>GAL4</i> activator domain	Durfee <i>et al.</i> (1993)
pCU6-3	pAcSG2 carrying <i>SPC97</i>	This study
pCU8-2	pAcSG2 carrying <i>SPC98</i>	This study
pEG202	2 μm, <i>HIS3</i> -based vector carrying the <i>LexA</i> DNA-binding domain	Gyuris <i>et al.</i> (1993)
pGEX-5X-1	<i>E.coli</i> expression vector containing GST under control of the <i>lacZ</i> promoter	Amersham pharmacia
pGP62	pACT2 carrying <i>SPC72</i>	Knop and Schiebel (1998)
pGP63	pACT2 carrying <i>N-SPC72</i>	Knop and Schiebel (1998)
pGP64	pACT2 carrying <i>SPC72^{SPB}</i>	Knop and Schiebel (1998)
pGP68	pGEX-5X-1 carrying <i>N-SPC72</i>	Knop and Schiebel (1998)
pMK232	pRS315 carrying <i>SPC72^{176–622}</i>	Knop and Schiebel (1998)
pMK267	pACT2 carrying <i>N-SPC72^{ΔStu2}</i>	This study
pMK269	pACT2 carrying <i>N-SPC72^{ΔTub4}</i>	This study
pRS305	<i>URA3</i> -based integration plasmid	Sikorski and Hieter (1989)
pRS315	<i>LEU2</i> -based CEN plasmid	Sikorski and Hieter (1989)
pRS316	<i>URA3</i> -based CEN plasmid	Sikorski and Hieter (1989)
pRS402	<i>ADE2</i> -based integration plasmid	Sikorski and Hieter (1989)
pSG56	pEG202 carrying <i>SPC98</i>	Geissler <i>et al.</i> (1996)
pSM412	pRS315 carrying <i>SPC72</i>	Knop and Schiebel (1998)
pSM447	pRS316 carrying <i>SPC72</i>	Knop and Schiebel (1998)
pSM564	pRS426 carrying <i>STU2</i>	This study
pSM576	pEG202 carrying <i>STU2</i>	This study
pSM588	pACT2 carrying <i>STU2^{648–888}</i>	This study
pSM599	pRS316 carrying <i>STU2</i>	This study
pSM662	pRS315 carrying <i>SPC72^{ΔStu2*}</i>	This study
pSM661	pRS315 carrying <i>SPC72^{ΔStu2}</i>	This study
pSM669	pRS426 carrying <i>STU2-3HA-kanMX6</i>	This study
pSM681	pRS315 carrying <i>SPC72^{ΔTub4}</i>	This study
pSM713	pRS315 carrying <i>SPC72^{ΔStu2}</i>	This study
pSM714	pRS315 carrying <i>N-SPC72</i>	This study
pTU1	pGEX-5X-1 carrying <i>N-SPC72^{ΔStu2}</i>	This study
pTU2	pGEX-5X-1 carrying <i>N-SPC72^{ΔTub4}</i>	This study
pTU4	pAcSG2 carrying <i>STU2</i>	This study
pTU5	pAcSG2 carrying <i>STU2-3HA</i>	This study
pTU17	pEG202 carrying <i>STU2^{1–855}</i>	This study
pTU22	pAcSG2 carrying <i>TUB4</i>	This study
pTU42	pAcSG2 carrying <i>STU2^{1–855}</i>	This study
pTU94	pEG202 carrying <i>STU2^{853–888}</i>	This study
pTU119	pRS306 carrying <i>SPC72^{176–622}</i>	This study
pTU125	pRS306 carrying <i>SPC72^{ΔTub4}</i>	This study
pTU141	pRS306 carrying <i>pSPC72-ATG-NotI-SPC72^{SPB*}</i>	This study
pTU146	pRS306 carrying <i>pSPC72-ATG-NotI-STU2^{501–855}-SPC72^{SPB*}</i>	This study
pTU147	pRS306 carrying <i>pSPC72-ATG-NotI-STU2^{501–888}-SPC72^{SPB*}</i>	This study
pTU148	pRS306 carrying <i>pSPC72-ATG-NotI-STU2^{648–855}-SPC72^{SPB*}</i>	This study
pTU149	pRS306 carrying <i>pSPC72-ATG-NotI-STU2^{648–888}-SPC72^{SPB*}</i>	This study
pTU152	pRS306 carrying <i>SPC72^{ΔStu2}</i>	This study
pTU153	pRS306 carrying <i>SPC72^{ΔStu2*}</i>	This study
pTU166	pRS306 carrying <i>pSPC72-ATG-NotI-STU2^{1–855}-SPC72^{SPB*}</i>	This study
pTU167	pRS306 carrying <i>pSPC72-ATG-NotI-STU2^{1–888}-SPC72^{SPB*}</i>	This study
pTU173	pRS305 carrying <i>pSPC72-ATG-NotI-STU2^{1–888}-SPC72^{SPB*}</i>	This study
pTU191	pRS306 carrying <i>pSPC72-ATG-NotI-STU2^{Δ558–657}-SPC72^{SPB*}</i>	This study
pTU192	pRS402 carrying <i>SPC72</i>	This study
pTU203	pRS402 carrying <i>STU2^{1–888}-SPC72^{SPB*}</i>	This study

SPC72^{Δ116–230}; *SPC72^{ΔStu2*}*; *SPC72^{Δ176–230}*; *SPC72^{ΔStu2}*; *SPC72^{99–622}*; *SPC72^{ΔTub4}*; *SPC72^{231–622}*; *SPC72^{SPB*}*; *SPC72^{277–622}*; *SPC72^{SPB*}*; *SPC72^{1–267}*; *N-SPC72*; *SPC72^{1–116}*; *N-SPC72^{ΔStu2}*; *SPC72^{99–267}*; *N-SPC72^{ΔTub4}*.

NatNT2 encodes the *Streptomyces noursei* *nat1* gene.

klTRP1 encodes the *Kluyveromyces lactis* *TRP1* gene.

hphMX4 encodes the *E.coli* *hph* gene.

Antibodies and immunoprecipitation

Antibodies specific for Stu2 were produced and affinity purified using recombinant 6His-Stu2^{2504–888}. Spc72 antibodies were raised against TrpE-Spc72p^{1–428}. Rabbit anti-C-Spc72 antibodies were affinity purified using 6His-Spc72^{231–622} purified from *E.coli*. Rabbit anti-Tub4, anti-Spc72, anti-Spc98 and anti-Spc97 antibodies have been described previously (Knop and Schiebel, 1998). Immunoprecipitation of Spc72-3HA was performed using a standard protocol (Knop and Schiebel, 1998).

Image analysis

GFP or RFP-labelled cells were analysed by fluorescence microscopy after fixation with 3.7% formaldehyde. DNA was stained with 4',6-diamino-2-phenylindole (DAPI) (Pereira *et al.*, 2000). Time-lapse analysis of living cells was as described previously (Pereira *et al.*, 2001). Time-lapse series were acquired in Z-series 11 planes 0.4 µm apart. At each time point, the length of MT was measured. Growth, shrinkage and pause were defined as described by Kosco *et al.* (2001).

Supplementary data

Supplementary data are available at *The EMBO Journal* Online.

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References

- Berlin, V., Styles, C.A. and Fink, G.R. (1990) BIK1, a protein required for microtubule function during mating and mitosis in *Saccharomyces cerevisiae*, colocalizes with tubulin. *J. Cell Biol.*, **111**, 2573–2586.
- Carminati, J.L. and Stearns, T. (1997) Microtubules orient the mitotic spindle in yeast through dynein-dependent interactions with the cell cortex. *J. Cell Biol.*, **138**, 629–641.
- Cassimeris, L. (2002) The oncoprotein 18/stathmin family of microtubule destabilizers. *Curr. Opin. Cell Biol.*, **14**, 18–24.
- Chen, X.P., Yin, H. and Huffaker, T.C. (1998) The yeast spindle pole body component Spc72p interacts with Stu2p and is required for proper microtubule assembly. *J. Cell Biol.*, **141**, 1169–1179.
- Durfee, T., Becherer, K., Chen, P.L., Yeh, S.H., Yang, Y., Kilburn, A.E., Lee, W.H. and Elledge, S.J. (1993) The retinoblastoma protein associates with the protein phosphatase type 1 catalytic subunit. *Genes Dev.*, **7**, 555–569.
- Fujita, A., Vardy, L., Garcia, M.A. and Toda, T. (2002) A fourth component of the fission yeast γ -tubulin complex, Alp16, is required for cytoplasmic microtubule integrity and becomes indispensable when γ -tubulin function is compromised. *Mol. Biol. Cell*, **13**, 2360–2373.
- Geissler, S., Pereira, G., Spang, A., Knop, M., Souès, S., Kilmartin, J. and Schiebel, E. (1996) The spindle pole body component Spc98p interacts with the γ -tubulin-like Tub4p of *Saccharomyces cerevisiae* at the sites of microtubule attachment. *EMBO J.*, **15**, 3899–3911.
- Gergely, F., Kidd, D., Jeffers, K., Wakefield, J.G. and Raff, J.W. (2000) D-TACC: a novel centrosomal protein required for normal spindle function in the early *Drosophila* embryo. *EMBO J.*, **19**, 241–252.
- Gergely, F., Draviam, V.M. and Raff, J.W. (2003) The ch-TOG/XMAP215 protein is essential for spindle pole organization in human somatic cells. *Genes Dev.*, **17**, 336–341.
- Gräf, R., Daumberer, C. and Schliwa, M. (2000) *Dictyostelium* DdCP224 is a microtubule-associated protein and a permanent centrosomal resident involved in centrosome duplication. *J. Cell Sci.*, **113**, 1747–1758.
- Gruneberg, U., Campbell, K., Simpson, C., Grindlay, J. and Schiebel, E. (2000) Nud1p links astral microtubule organization and the control of exit from mitosis. *EMBO J.*, **19**, 6475–6488.
- Gyuris, J., Golemis, E., Chertkov, H. and Brent, R. (1993) Cdi1, a human G1 and S phase protein phosphatase that associates with Cdk2. *Cell*, **75**, 791–803.
- He, X., Rines, D.R., Espelin, C.W. and Sorger, P.K. (2001) Molecular analysis of kinetochore-microtubule attachment in budding yeast. *Cell*, **106**, 195–206.
- Howard, J. and Hyman, A.A. (2003) Dynamics and mechanics of the microtubule plus end. *Nature*, **422**, 753–758.
- Huyett, A., Kahana, J., Silver, P., Zeng, X. and Saunders, W.S. (1998) The Kar3p and Kip2p motors function antagonistically at the spindle poles to influence cytoplasmic microtubule numbers. *J. Cell Sci.*, **111**, 295–301.
- Knop, M. and Schiebel, E. (1998) Receptors determine the cellular localization of a γ -tubulin complex and thereby the site of microtubule formation. *EMBO J.*, **17**, 3952–3967.
- Knop, M., Pereira, G., Geissler, S., Grein, K. and Schiebel, E. (1997) The spindle pole body component Spc97p interacts with the γ -tubulin of *Saccharomyces cerevisiae* and functions in microtubule organization and spindle pole body duplication. *EMBO J.*, **16**, 1550–1564.
- Knop, M., Siegers, K., Pereira, G., Zachariae, W., Winsor, B., Nasmyth, K. and Schiebel, E. (1999) Epitope tagging of yeast genes using a PCR-based strategy: more tags and improved practical routines. *Yeast*, **15**, 963–972.
- Kosco, K.A., Pearson, C.G., Maddox, P.S., Wang, P.J., Adams, I.R., Salmon, E.D., Bloom, K. and Huffaker, T.C. (2001) Control of microtubule dynamics by Stu2p is essential for spindle orientation and metaphase chromosome alignment in yeast. *Mol. Biol. Cell*, **12**, 2870–2880.
- Lee, M.J., Gergely, F., Jeffers, K., Peak-Chew, S.Y. and Raff, J.W. (2001) Msp/XMAP215 interacts with the centrosomal protein D-TACC to regulate microtubule behaviour. *Nat. Cell Biol.*, **3**, 643–649.
- Liakopoulos, D., Kusch, J., Grava, S., Vogel, J. and Barral, Y. (2003) Asymmetric loading of Kar9 onto spindle poles and microtubules ensures proper spindle alignment. *Cell*, **112**, 561–574.
- Maddox, P.S., Bloom, K.S. and Salmon, E.D. (2000) The polarity and dynamics of microtubule assembly in the budding yeast *Saccharomyces cerevisiae*. *Nat. Cell Biol.*, **2**, 36–41.
- Maekawa, H., Usui, T., Knop, M. and Schiebel, E. (2003) Yeast Cdk1 translocates to the plus end of cytoplasmic microtubules to regulate bud cortex interactions. *EMBO J.*, **22**, 438–449.
- Miller, R.K., Heller, K.K. and Rose, M.D. (1998) The kinesin-related proteins, Kip2p and Kip3p, function differently in nuclear migration in yeast. *Mol. Biol. Cell*, **9**, 2051–2068.
- Miller, R.K., Cheng, S.-C. and Rose, M.D. (2000) Bim1p/Yeb1p mediates the Kar9p-dependent cortical attachment of cytoplasmic microtubules. *Mol. Biol. Cell*, **11**, 2949–2959.
- Nabeshima, K., Kurooka, H., Takeuchi, M., Kinoshita, K., Nakaseko, Y. and Yanagida, M. (1995) p93dis1, which is required for sister-chromatid separation, is a novel microtubule and spindle pole body-associated protein phosphorylated at the Cdc2 target sites. *Genes Dev.*, **9**, 1572–1585.
- Palmer, R.E., Sullivan, D.S., Huffaker, T. and Koshland, D. (1992) Role of astral microtubules and actin in spindle orientation and migration in the budding yeast *Saccharomyces cerevisiae*. *J. Cell Biol.*, **119**, 583–593.
- Paluh, J.L., Nogales, E., Oakley, B.R., McDonald, K., Pidoux, A.L. and Cande, W.Z. (2000) A mutation in γ -tubulin alters microtubule dynamics and organization and is synthetically lethal with the kinesin-like protein pkl1p. *Mol. Biol. Cell*, **11**, 1225–1239.
- Pereira, G., Grueneberg, U., Knop, M. and Schiebel, E. (1999) Interaction of the yeast γ -tubulin complex binding protein Spc72p with Kar1p is essential for microtubule function during karyogamy. *EMBO J.*, **18**, 4180–4196.
- Pereira, G., Höfken, T., Grindlay, J., Manson, C. and Schiebel, E. (2000) The Bub2p spindle checkpoint links nuclear migration with mitotic exit. *Mol. Cell*, **6**, 1–10.
- Pereira, G., Tanaka, T.U., Nasmyth, K. and Schiebel, E. (2001) Modes of spindle pole body inheritance and segregation of the Bfa1p/Bub2p checkpoint protein complex. *EMBO J.*, **20**, 6359–6370.
- Raff, J.W. (2002) Centrosomes and cancer: lessons from a TACC. *Trends Cell Biol.*, **12**, 222–225.
- Schaerer, F., Morgan, G., Winey, M. and Philippsen, P. (2001) Cnm67p is a spacer protein of the *Saccharomyces cerevisiae* spindle pole body outer plaque. *Mol. Biol. Cell*, **12**, 2519–2533.
- Severin, F., Habermann, B., Huffaker, T. and Hyman, T. (2001) Stu2 promotes mitotic spindle elongation in anaphase. *J. Cell Biol.*, **153**, 435–442.
- Sherman, F. (1991) Getting started with yeast. *Methods Enzymol.*, **194**, 3–21.
- Shirasu-Hiza, M., Coughlin, P. and Mitchison, T. (2003) Identification of XMAP215 as a microtubule-destabilizing factor in *Xenopus* egg extract by biochemical purification. *J. Cell Biol.*, **161**, 349–358.
- Sikorski, R.S. and Hieter, P. (1989) A system of shuttle vectors and yeast

- host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. *Genetics*, **122**, 19–27.
- Souès,S. and Adams,R. (1998) *SPC72*: a spindle pole body component required for spindle orientation in the yeast *Saccharomyces cerevisiae*. *J. Cell Sci.*, **111**, 2809–2818.
- Spang,A., Geissler,S., Grein,K. and Schiebel,E. (1996) γ -Tubulin-like Tub4p of *Saccharomyces cerevisiae* is associated with the spindle pole body substructures that organize microtubules and is required for mitotic spindle formation. *J. Cell Biol.*, **134**, 429–441.
- Tournebise,R.A. *et al.* (2000) Control of microtubule dynamics by the antagonistic activities of XMAP215 and XKCM1 in *Xenopus* egg extracts. *Nat. Cell Biol.*, **2**, 13–19.
- van Breugel,M., Drechsel,D. and Hyman,H. (2003) Stu2, the budding yeast member of the conserved Dis/XMAP215 family of microtubule-associated proteins is a plus end-binding microtubule destabilizer. *J. Cell Biol.*, **161**, 359–369.
- Vinh,D.B.N., Kern,J.W., Hancock,W.O., Howard,J. and Davis,T.N. (2002) Reconstitution and characterization of the budding yeast γ -tubulin complex. *Mol. Biol. Cell*, **13**, 1144–1157.
- Vogel,J., Drapkin,B., Oomen,J., Beach,D., Bloom,K. and Snyder,M. (2001) Phosphorylation of γ -tubulin regulates microtubule organization in budding yeast. *Dev. Cell*, **1**, 621–631.
- Wang,P.J. and Huffaker,T.C. (1997) Stu2p: a microtubule-binding protein that is an essential component of the yeast spindle pole body. *J. Cell Biol.*, **139**, 1271–1280.

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