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INSERM U836, Grenoble institut des neurosciences ; iRTSV, Groupe de Physiopathologie du Cytosquelette ; CEA Grenoble, 17 avenue des martyrs 38054 Grenoble

Corresponding author: Cécile Boscheron, Groupe de Physiopathologie du cytosquelette, CEA; INSERM U836; UJF; 17 rue des martyrs 38054 Grenoble France. Tel : +33 438 78 43 25 E-Mail : cecile.boscheron@cea.fr

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Abbreviations : microtubule (MT), Spindle Pole Body (SPB), Green Fluorescent Protein (GFP), microtubule + ends tracking protein (+TIP)

Abstract

Bik1p is the budding yeast counterpart of the CLIP-170 family of microtubule + end tracking proteins, which are required for dynein localization at + ends and dynein dependent spindle positioning. CLIP-170 proteins comprise a CAP-Gly microtubule binding domain, which sustains their microtubule + end tracking behaviour. However, in yeast, where Bik1p is transported towards + ends in a complex with the kinesin Kip2p, current models postulate that Bik1p tracks + ends principally as a cargo of Kip2p. Here, we have tested this possibility by examining Bik1p localization in yeast strains expressing mutant, Glu, tubulin, whose interaction with CAP-Gly domains is severely impaired. In Glu tubulin strains, despite presence of robust Kip2p comets at microtubule + ends, Bik1p failed to track + ends. Interestingly, despite Bik1p depletion, dynein positioning at the same + ends was unperturbed. Videomicroscopy and genetic evidence indicated that dynein was transported at microtubule + ends in a complex with Kip2p and Bik1p, and was then capable to track Bik1p depleted + ends. We conclude that, whereas Bik1p interaction with Kip2p cannot mediate Bik1p + end tracking as previously suggested, it sustains a new pathway of dynein transport and positioning at microtubule ends.

Introduction

Microtubules (MTs) are fibrous structures in the cytoplasm of eukaryotic cells and play a vital role in cell organization, motility, and division. MTs are intrinsically polar polymers with a fast growing + end and a slow-growing - end (Carvalho et al., 2003; Desai and Mitchison, 1997). In fungi and animals, the - ends of MTs are usually at or adjacent to the microtubule organizing center and the + ends are oriented peripherally. A group of proteins called + end tracking proteins or +TIPs specifically associates with the + ends of MTs (Galjart and Perez, 2003). In live cell experiments, GFP-labelled + TIPs appear as comet- or dot-like structures that remain on the MT + ends (Perez et al., 1999). + TIPs form structural links between microtubule + ends and polarized membrane sites or kinetochores (Akhmanova et al., 2001; Carvalho et al., 2003; Coquelle et al., 2002; Dujardin and Vallee, 2002; Fukata et al., 2002; Howard and Hyman, 2003; Lin et al., 2001; Pierre et al., 1992; Tai et al., 2002) and are important for the regulation of microtubule dynamics (Han et al., 2001; Maiato et al., 2003; Rogers et al., 2002; Tirnauer et al., 1999).

The first discovered + TIP, CLIP-170, contains specific microtubule binding domains (CAP-Gly domains), which are shared by related proteins such as CLIP-115, $P150^{Glued}$, in mammalian cells, or its orthologs Bik1p and Tip1p in budding and fission yeasts. The CAP-Gly-containing proteins are central for astral microtubule interactions with cortical dynein-dynactin patches during mitosis and for the delivery of motors such as dynein to their site of action (Coquelle et al., 2002; Lansbergen et al., 2004; Niccoli et al., 2004; Tai et al., 2002; Vaughan et al., 2002; Xiang et al., 2000). In budding yeast, dynein is delivered to the cortex on the + ends of polymerizing MTs. Furthermore, dynein recruitment at MT tip is thought to involve dynein interactions with Bik1p at microtubule + ends (Sheeman et al., 2003).

How CLIP-170 and related proteins 'recognize' microtubule + ends is not completely clear. In mammalian cells, there is evidence that CLIP-170 accumulates at microtubule ends both by copolymerizing with tubulin (treadmilling mechanism) and by associating with another + TIP, EB1 (hitch hiking mechanism). An additional mechanism has been proposed in yeast where Bik1p and Tip1p are thought to be principally localized at microtubule + ends by kinesin motor proteins (Kip2p in budding yeast, Tea2p in fission yeast).

We have previously demonstrated that in budding yeast removal of the C-terminal phenylalanine residue of alpha tubulin dramatically impairs the recruitment of Bik1p at microtubule + ends (Badin-Larcon et al., 2004; Erck et al., 2005; Peris et al., 2006). We have recently reported related observations in mammalian cells, in which CLIP-170 and other CAP-Gly + TIPs association with microtubule + ends is severely impaired in cells expressing

detyrosinated, Glu, tubulin. Several studies have provided a structural basis for our observations, by demonstrating a crucial requirement of the C-terminal aromatic residue of alpha-tubulin for tubulin interaction with CAP-Gly domains (Weisbrich et al., 2007)(+PNAS). In budding yeast; models in which Bik1p is principally localized as a Kip2p cargo do not require direct interactions of Bik1p with tubulin. There is an apparent conflict between such models and the massive mis-localization of Bik1p caused by the Glu mutation in budding yeast.

Here we demonstrate that, actually, whereas Kip2p can mediate the transport of Bik1p towards microtubule + ends, it cannot support subsequent Bik1p + end tracking. Additionally, our data indicate that dynein is transported at microtubule + ends in a complex with Kip2p and Bik1p. Apparently, dynein can subsequently track microtubule + ends even when Bik1p is severely depleted at those ends. Thus, although Kip2p cannot mediate Bik1p + end tracking as previously proposed, it seems to be central in a new pathway for dynein localization at + ends, which can support apparently normal dynein function even when the interaction of Bik1p with tubulin is disrupted.

Results

Bim1p is required for remaining Bik1p accumulation at microtubule + ends in Glu tubulin mutant

We have previously shown that Bik1p accumulation at microtubule + ends is decreased about three folds in yeast strain expressing phenylalanine deleted, Glu tubulin (*tub1-Glu*) compared to strain expressing *wt*, Phe tubulin (*TUB1*) (Badin-Larcon et al., 2004). However, these results were observed using over expressed Bik1p. In such conditions, Kip2p may be saturated, and we could have looked principally at the localization of excess free Bik1p. To examine the localization of Bik1p expressed at physiological levels we imaged a functional fusion between Bik1p and a 3GFP cassette (Bik1p-3GFP) in cells in which SBP were also labelled with Spc42p-RedStar. Fluorescence analysis in *TUB1* strain showed a Bik1p-3GFP signal as dots at microtubule +ends (Figure 1A) as previously described (Lin et al., 2001). Bik1p dots were still visible in *tub1-Glu* strain, but, quantitative analysis indicated a three fold decrease of the Bik1p signal at + ends compared to *TUB1* strain, as previously observed with plasmid over-expressed Bik1p (Fig. 1B). Thus the Glu mutation affects Bik1p localization, even when expressed at physiological levels.

We then searched for factors responsible for persistent localization of some Bik1p at microtubule + ends in *tub1-Glu* strain. We tested a role of Bim1p. Bim1p orthologs can localize CAP-Gly proteins at +ends in many systems (Lansbergen and Akhmanova, 2006). In previous studies, budding yeast looked as an exception, since Bik1p localization was insensitive to BIM1 deletion. However, we thought that the situation may be different in *tub1-Glu* strains. We examined the consequences of *BIM1*deletion on Bik1p-3GFP localization, in our strains. Both strains *TUB1 bim1Δ* and *tub1-Glu bim1Δ* were viable and grew normally (data not shown). Microtubule length was somewhat decreased in both *TUB1 bim1*Δ and *tub1-Glu bim1*Δ strains (Table 1). As expected, *BIM1* deletion had no detectable effect on Bik1p-3GFP localization in *TUB1 bim1*Δ strain (Fig. 1A and for quantitative analysis Fig. 1B). In contrast, in *tub1-Glu bim1*Δ strain, Bik1p-3GFP signal was reduced about 5 fold compared to *tub1-Glu* strain, to reach barely detectable levels (Fig. 1A, B). Bik1p localization at spindle pole bodies (SPB) was also reduced (Fig. 1A, B). Blot analysis showed similar levels of Bik1p-3GFP expression in *TUB1* and *tub1-Glu* strains deleted or not for *BIM1* (Fig. 1C), indicating that Bik1p depletion at microtubule + ends in Glu tubulin strains did not reflect variations in protein expression levels. Thus Bim1p deletion in *tub1-Glu* strain suppressed almost completely + end tracking by Bik1p. Whatever the exact mechanism through which Bim1p affected Bik1p localization in *tub1-Glu* strains, for practical purposes,

BIM1 deleted strains yielded a cell system in which Bik1p + end tracking was fully dependent of the presence or absence of the Glu tubulin mutation, which simplified further analysis.

Kip2p in Glu tubulin mutant

Why should the Glu tubulin mutation suppress Bik1p tracking of + end? In the context of current models, in which Bik1p end tracks +ends principally as a cargo of Kip2p, Bik1p + end tracking could be affected due to abnormal interactions of Kip2p with Glu microtubules. To test this possibility, we examined the behaviour of a Kip2p-3YFP fusion protein expressed at physiological levels. In both *TUB1* and *tub1-Glu* strains, Kip2p-3YFP was visible as fluorescent dots at microtubule ends (Fig. 2A). In a quantitative analysis, Kip2p-3YFP signals at microtubules tips were not affected by the Glu tubulin mutation (Fig. 2B). Kip2p has been shown to end-track both growing and shrinking microtubules. Accordingly, in video-microscopy experiments, Kip2p-3YFP tracking of growing and shrinking microtubules + ends was visible, in both *TUB1* and *tub1-Glu* strains (data not shown and Fig. 2C, D, video S1, S2). The number of Kip2p decorated microtubule ends as well as microtubule + end dynamics were similar in *TUB1* and *tub1-Glu* strains (Table 2). In previous studies, Bim1p removal enhanced Kip2p microtubule + end labelling (Carvalho et al., 2004). Accordingly, in both *TUB1 bim1Δ* and *tub1-Glu bim1Δ* strains the Kip2p-3YFP signal at microtubule + ends increase (Fig. 2A, B). *BIM1* deletion induced modifications of microtubule + end dynamics, as previously observed (Tirnauer et al., 1999) but the microtubule + end dynamics were not significantly affected by the Glu tubulin mutation (Tables 1, 2). Thus Kip2p localization and dynamic behaviour at microtubule + ends was not detectably affected by the Glu tubulin mutation.

In wild type strain, the motion of fluorescent Kip2p along astral microtubules towards microtubule + ends can be visualized as low-intensity speckles moving along microtubules toward the + end at a rate that exceed that of MT polymerization (Carvalho et al., 2004). Such speckles could be detected in *tub1-Glu* strain (Fig. 2E, video S3). In wild type strains, Bik1p is transported towards microtubule + ends as a cargo Kip2p. Accordingly, we observed Bik1p-3GFP low intensity speckles sliding along Glu microtubules in a manner suggesting motor-dependent transport (Fig. 2F, video S4). Thus, apparently, Kip2p + end directed motion along astral microtubules, as well as Bik1p transport on astral microtubules, were present in Glu tubulin expressing strain.

Our results demonstrate that a Bik1p comet are suppressed at + ends in Glu tubulin strains despite persistence of normal Kip2p comets. Such results indicate that microtubule + end

tracking by Bik1p and Kip2p are distinct processes and that Bik1p cannot track microtubule ends as a cargo of Kip2p.

A new Kip2p dependent pathway for dynein localization at microtubule + ends

In current models, dynein is recruited at microtubule + ends by Bik1p. One would then expect dynein to be depleted at Glu microtubule + ends. However, when we analyzed the localization of Dyn1p-3GFP fusion proteins expressed at physiological levels, Dyn1p-3GFP was visible as fluorescent dots at the + ends of growing or shrinking microtubules in both *TUB1* and *tub1-Glu* strains (Fig. 3 and Video S5, S6). Furthermore, *BIM1* deletion produced no detectable difference in Dyn1p-3GFP dot signals and intensity even in *tub1-Glu bim1Δ* strain where Bik1p-3GFP is not detectable at microtubules + ends (Fig. 3A, B). It could be that whereas Bik1p is required for dynein localization at microtubule + ends in wild type strains, Bik1p is no more needed for dynein localization at microtubule + ends in the Glu tubulin mutant. We tested this possibility by examining dynein localization in *TUB1 bik1Δ* and *tub1-Glu bik1Δ* strains. In both cases, dynein became undetectable at microtubule + ends (Fig. 3B). Thus, Bik1p is strictly required for dynein positioning at microtubule + ends even in the Glu tubulin mutant.

A possibility was that in *tub1-Glu* and *tub1-Glu bim1Δ* strains, dynein could be delivered at microtubule ends as a partner of Bik1p, in a complex with Kip2p, and could subsequently end-track microtubules independently of Bik1p. To test for the existence of dynein transport along astral microtubules, we searched for dynein fluorescent speckles moving along microtubules in *tub1-Glu* strain. We could indeed observe such speckles (Fig. 3E, and video S7) strongly indicative of motor dependent dynein transport toward microtubules + ends.

To test whether Kip2p was indeed involved in dynein transport, we constructed double deleted *tub1-Glu bim1*Δ *kip2*Δ strain. In this strain, cell viability was compromised, with 56% spore lethality. However, viable cells grew normally (not shown) and astral microtubules were readily observable (Fig. 4A). Interestingly, dynein was no more detectable at microtubule +ends (Fig. 3B). Correlatively, in video microscopy experiments, spindle positioning was conspicuously perturbed with spindle elongation occurring in the mother cell and a marked delay of spindle penetration in the bud (Fig. 4B, C, D). There are obviously interesting questions concerning the mechanisms which finally position the spindle in *tub1-Glu bim1*Δ *kip2*Δ strains. Within the scope of our study, we principally conclude that, both

Bik1p and Kip2p are essential for dynein localization in *tub1-Glu bim1*Δ strain, compatible with dynein transport to + ends in a complex with Bik1p and Kip2p.

Apart from this main conclusion we note that astral microtubules of sizeable length persisted in *KIP2* deleted strain, which contrasted with previous observations of drastic microtubule length reduction following *KIP2* deletion. We believe that this phenotypic variations, which we observed in *TUB1* and *tub1-Glu* strains (Cottingham and Hoyt, 1997), may result from the absence of *TUB3* in our strains (Table 1 and Table S1).

Alternative pathways for dynein localization at microtubule + ends

The data shown above demonstrate the existence of a pathway for dynein positioning in which Bik1p is only needed as a linker between Kip2p an dynein, being otherwise dispensable at microtubule + ends. However, such results did not exclude the possibility of dynein recruitment at + ends by Bik1p, as in previous models. To test for such recruitment in our strains, we examined strains lacking Kip2p, but still displaying variable amounts of Bik1p at microtubule + ends. As stated above, we note that in our *KIP2* deleted strains, microtubules length is not affected as dramatically as previously described on different genetic backgrounds (Table 1).

We examined the effect of *KIP2* deletion in *tub1-Glu* strain. Bik1p was detectable at microtubule + ends in such strains, compatible with Bim1p mediated localization (see above). Dynein was also detectable at *tub1-Glu kip2Δ* microtubule ends.

We similarly examined the effect of *KIP2* deletion in *TUB1* strain. *KIP2* deletion induced proportional depletion of Bik1p and dynein at microtubule + ends (Fig. 3B). Additional deletion of *BIM1* in these wild type tubulin strains did not induce any detectable changes in Bik1p or dynein signals at microtubule ends (Table 1).

Collectively, these results indicate that dynein can be directly recruited at + ends by Bik1p, when the latter protein is present at microtubule ends.

Discussion

In this study we provide strong evidence that Bik1p cannot end track microtubules as a cargo of Kip2p, as proposed in previous models. Our data indicate that although Bik1p is brought to microtubule + ends by Kip2p, microtubule end tracking by the two proteins are distinct processes. In yeast as in other systems, Bik1p seems to track + ends based on its intrinsic interaction with tubulin.

A key factor for deciphering the molecular interactions involved in Bik1p + end tracking has been the use of the Glu tubulin mutation, which, in recent studies, has been shown to specifically inhibit the interaction of CAP-Gly microtubule binding domains with the C-terminus of alpha tubulin. In our previous study, the Glu mutation only induced a three fold decrease of the Bik1p signal at microtubule ends. The existence of a sizeable remaining Bik1p signal could suggest incomplete inhibition of Bik1p interaction with tubulin by the Glu mutation in budding yeast. However, in this study, we demonstrate that in *tub1-Glu bim1*Δ strain the Bik1p signal at microtubule ends decreases to reach barely detectable levels. Such an effect of *BIM1* deletion is compatible with the possibility that in Glu tubulin strains Bik1p localizes at + ends mainly by hitch-hiking Bim1p, not by interacting with Glu tubulin. Actually, in many cell types, Bik1p orthologs can be localize by hitch-hicking EB1 orthologs (Lansbergen and Akhmanova, 2006). Budding yeast looked as an exception, Bik1p localization being insensitive to *BIM1* deletion in *wt* strains (Lin et al., 2001) (Fig. 1). Apparently the contribution of Bim1p hitch-hicking to Bik1p localization is minimal in *wt* strains, but becomes readily apparent in Glu tubulin mutant strains.

Our data indicate that Bik1p is transported to microtubule + ends in a complex with Kip2p which dissociates upon arrival at + ends with further microtubule + end tracking by Bik1p depending on Bik1p interaction with tubulin. What could trigger the dissociation the Bik1p/Kip2p complex at + ends? Possibly, Kip2p conformation and interactions with tubulin are different when the motor walks on microtubules and when it end-tracks microtubules. The two processes actually occur with profoundly different velocities, Kip2p motion on microtubules being 3 to 5 fold faster than microtubules growth (Carminati and Stearns, 1997; Carvalho et al., 2004; Shaw et al., 1997). In the case of another + end directed +TIP kinesin MCAK, + end tracking involves domains distinct from the motor domain, which is apparently inactive during end tracking (Moore et al., 2005). Kip2p may similarly be inactive as a motor during + end tracking, with a conformation which does not allow interaction with Bik1p. How could Bik1p end track microtubules, once dissociated from Kip2p? Possibly, Bik1p binds to free tubulin and co-polymerizes with tubulin exactly as suggested in the case of CLIP-170 in

mammalian cells. However, such a model would only accounts for Bik1p tracking of growing + ends, whereas Bik1p also tracks shrinking + ends in budding yeast, suggesting the possibility of a direct association of Bik1p with + ends whether growing or shrinking; May be, the structures of growing and shrinking microtubule + ends, which are conspicuously different in the case of polymers made of mammalian tubulin ( ), are less different in polymers made from yeast tubulin.

Collectively, our data suggest that in wild type strains Bik1p tracks microtubule + ends principally based its intrinsic tubulin binding capacity, in a manner very much similar to that proposed for mammalian cells, and then functions to recruit dynein at microtubules + ends. Kip2p transport of Bik1p apparently facilitates Bik1p concentration close to + ends, without being strictly required. However, Kip2p is apparently central in a new functionally redundant pathway for Bik1p dependent dynein localization and transport. In this pathway, dynein is apparently brought to + ends in a ternary complex with Bik1p and Kip2p, and following dissociation of this complex, can end track microtubules even in the absence of Bik1p. Previous studies have indicated that dynein may normally end track microtubules in a ternary complex with Pac1p and Bik1p. May be, when Bik1p is lacking, dynein interaction with Pac1p is sufficient to sustain dynein tracking of + ends. One could then wonder why dynein could not be recruited directly by Pac1p. May be, dynein interaction with Bik1p releases an inhibition that otherwise precludes dynein interaction with Pac1p. A schematic summary of these models and conclusion is shown Figure 5.

Finally, it will be of obvious interest to know whether a kinesin dependent pathway for CLIP-170 mediated localization of dynein at + ends exists in mammalian cells. One way to know could be to test for the existence of dynein comets in tubulin tyrosine ligase deficient cells (Glu tubulin cells). The observation of such comets would strongly favour the possibility of kinesin mediated transport of both CLIP-170 and dynein in mammalian cells, as in yeast.

Materials and methods

Yeast strains and plasmids

The plasmids and strains used in this study were isogenic to S288c background and are listed in Table S1. Deletion mutants were obtained by one-step gene replacement, and verified by PCR. pFC1 is the integrating Bik1p-3GFP plasmid, the hphMX4 cassette from pMJ696 (pAG32 in (Goldstein and McCusker, 1999)) was cloned *Acc*I/*Not*I in place of *TRP1* in pB1587 (Lin et al., 2001). pFC2 is the 3'region of *DYN1* fused to 3*GFP* of pB1761 (Sheeman et al., 2003) cloned *Eco*RI/*Not*I in pRS406. pFC3 is the 3'region of *KIP2* fused to 3*YFP* of pMG108 (Carvalho et al., 2004) cloned *Sal*I/*Not*I to pRS406. pAFS125-Glu is *tub1-Glu* C-terminal mutation cloned *Sac*I/*BstE*II from pUC18-*tub1-Glu* (Badin-Larcon et al., 2004) in pAF125.

Antibodies

Western blots were performed on whole cell extract from the indicated strain with anti-GFP antibody (Molecular Probes). Correction for loading equality was achieved with whole alpha tubulin signal quantification using mAb YOL1/34 (Sera-Lab, Crawley Down, Sussex, U.K.).

Microscopy and image analysis

Cell imaging was performed on a Zeiss Axiovert microscope equipped with a Cool Snap ES CCD camera (Ropper scientific). All images were captured using 2x2 binning and 17 sequential z-axis collected in 0.3-µm exposure time varying between constructions**.** Time lapse video microscopy for Kip2p-3YFP and Dyn1p-GFP were collected with 5 sequential z-axis 0.5µm steps and an exposure time of 800ms every 40s. For microtubule length measurements: images of maximal intensity projection of cells bearing *TUB1*-GFP or *tub1-Glu*-GFP fusions integrated at the *URA3* locus are used to visualize microtubules. For microtubule dynamic measurements Kip2p-3YFP is the reporter and images were collected with 5 sequential z-axis 0.5µm steps every 10s. Speckles were observed using a single plane exposed 1s every 5s for Dyn1p-3GFP and every 2.5s for Bik1p-3GFP and Kip2p-3YFP. All image manipulations and fluorescence intensity measurements were performed using METAMORPH software (universal imaging). For technical consideration and fluorescent intensity those experiment are done with only one protein tagged. SPB and +ends are distinguish based on their movement during the whole time lapse analysis (SPB share very few movement, +ends share intensive movements). Fluorescent-counting was performed with maximal intensity projections computed from the original datasets and corrected for cytoplasmic

background fluorescence. Bik1p-3GFP quantifications were done on pre anaphase cells. Plus ends fluorescent quantification are an average microtubule's + end signal over cell number. We proceeded to statistical analysis (Student t-test) and determined that all obvious differences were significant $p<0.001$.

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Figure legends

Figure 1: Bik1p localization is Bim1p dependent in Glu tubulin strains.

(A) Images of representative cells from different backgrounds expressing Bik1p-3GFP from the native locus. From left to right: Bik1p-3GFP fluorescence (green); Spc42p-RedStar fluorescence (red); GFP and RedStar merged imaged. Arrows indicate +end of astral microtubules. (Bar 3μm). (B) Shows the quantification of fluorescent intensity at the +ends of astral microtubules. 25 to 35 cells were scored for each strain. The bars indicate the s.e.m. (C) Left: western blotting of whole cell extracts with anti-GFP against Bik1p-3GFP and YoL1/34 against alpha tubulin. Right: ratio between the anti-GFP signal and the anti-alpha tubulin signal obtained for four independent western blotting experiments. The bars indicate the s.e.m. Strains are (1) *TUB1*, (2) *TUB1 bim1Δ*, (3) *tub1-Glu*, (4) *tub1-Glu bim1Δ.*

Figure 2: Kip2p in Glu tubulin strains

(A) Merged images of representative cells of the indicated diploid strain, expressing Kip2p-3YFP (green) and Spc42p-RedStar (red). Arrows indicates +end of astral microtubules. (Bar 3μm). (B) Shows the quantification of Kip2p-3YFP fluorescent intensity at the +ends of astral microtubules in the indicated strain. 50 cells were scored for each strain. The bars indicate the s.e.m. Kip2p-3YFP localizes to + ends of both growing (C) and shrinking MTs (D) in Glu tubulin cells. (E) Illustration of Kip2p-3YFP speckle movement on Glu microtubules. (F) Illustration of Bik1p-3GFP speckle movement on Glu microtubules. Diploid cells for (C) to (F) arrows indicate +end of astral microtubules arrowheads SPB and double arrowheads highlight speckles moving towards the + ends. Time is in seconds; bars are 3μm.

Figure 3: Dynein in Glu tubulin strains

Merged images of representative cells expressing Dyn1p-3GFP (green) and Spc42p-RedStar (red) of the indicated diploid strain. Arrows indicate +end of astral microtubules. (Bar 3μm). (B) Shows the quantification of Dyn1p-3GFP fluorescent intensity at the +ends of astral microtubules in the indicated strain.). 50 cells were scored for each strain. No signal was detectable in Bik1 deleted cells (N.D. The bars indicate the s.e.m. Dyn1p-3GFP localizes to the plus ends of both growing (C) and shrinking (D) MTs in Glu tubulin cells. (E) Illustration of Dyn1p-3GFP speckle movement on Glu microtubules For (C) to (E) diploid cells, arrows

indicate +end of astral microtubules arrowheads SPB and double arrowheads highlight speckles moving towards the + ends. Time is in seconds; bars are 3μm. (F) Shows the quantification of Bik1p-3GFP fluorescent intensity at the +ends of astral microtubules in the indicated strain. 50 cells were scored for each strain. Bars are s.e.m.

Figure 4: Mitosis in the *tub1-Glu bim1Δ kip2Δ* strain.

We monitored mitosis in the *tub1-Glu bim1Δ kip2Δ* strain using tub1-Glu-GFP fusion integrated at the *URA3* locus. (A) Illustration of microtubules behaviour. Contrast phase of cells are in the left and fluorescent image of tub1-Glu-GFP are on the right. Astral microtubules are visible at different cell stage (arrowhead). (B) Selected frames from representative time-lapse series analyzed mitosis. Astral microtubules are not detectable, we imaged spindle which elongate in the mother cell. Finding the neck lumen was apparently problematic for the spindle and this delayed the penetration of the elongating spindle into the bud. (C) Selected frames from representative time-lapse series of spindle bending in the mother cell. For (A) to (C) time is in hours minutes; bars are 3μm. (D) Quantitative analysis of the percentage of spindles in contact with the cell cortex prior to passage of the spindle through the neck. Bars are s.e.m.

Figure 5: A new role for kinesin directed transport of Bik1p. This figure presents a model interpreting results presented in this paper. (A) Present the current model of Kip2ptransport of Bik1p toward microtubules +ends where dynein is recruited. (B) Our result show that dynein was transported at microtubule + ends with Kip2p and Bik1p, and was then capable to track + ends. The Glu tubulin strain provides evidence that Kip2p cannot mediate Bik1p + end tracking. Finally, analysis of *KIP2* deleted strains confirmed a direct recruitment of dynein through Bik1p. It appears that dynein could be recruited at microtubules + ends through redundant mechanism both Bik1p dependent.