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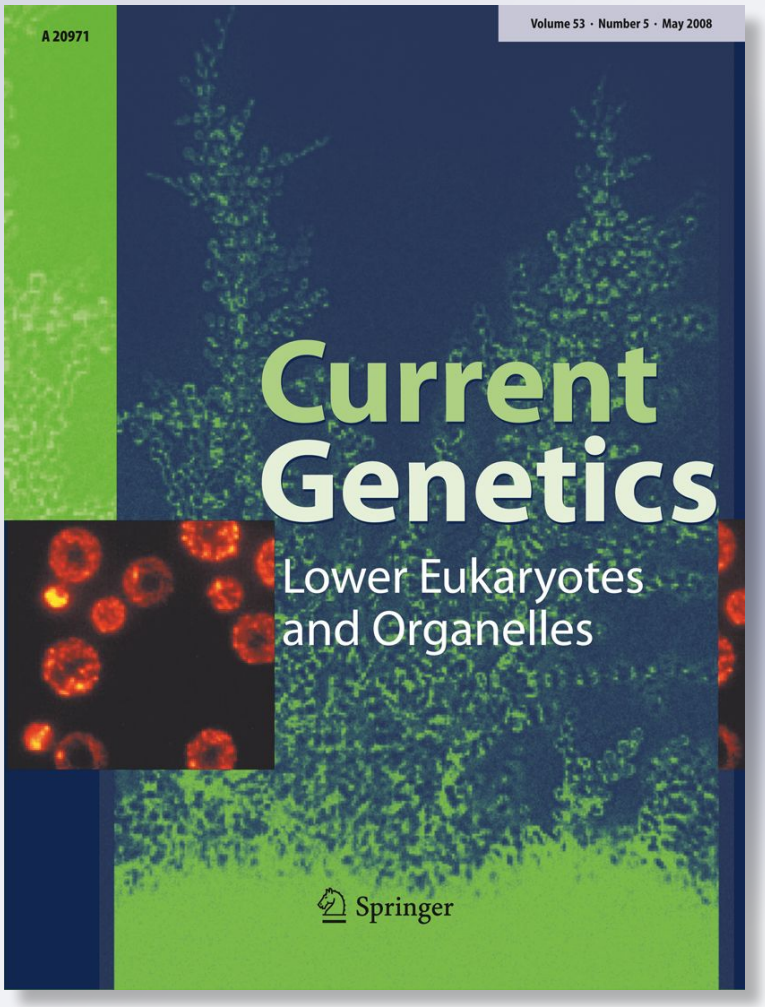

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RESEARCH ARTICLE

The *Aspergillus nidulans* CENP-E kinesin KipA is able to dimerize and to move processively along microtubules

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Abstract Kinesin molecular motors serve a variety of cellular functions usually in dynamic processes. One characteristic feature of many kinesins is their ATP-dependent processive movement along polymerized microtubules. However, many kinesins work as stationary polymerases or depolymerases. Therefore, it needs to be determined for each motor, whether it moves processively along microtubules or not. The *Schizosaccharomyces pombe* kinesin-7, Tea2, has been shown to be involved in cell end marker transportation towards the cortex to organize the actin cytoskeleton. In human, kinesin 7 promotes microtubule polymerization. In *Aspergillus nidulans*, the machinery for determining growth directionality is conserved, but there is no evidence yet that kinesin 7, KipA is potentially involved in the transportation of the cell end marker proteins, TeaA or TeaR or newly identified proteins such as KatA. We expressed KipA in *Escherichia coli* and determined the catalytic properties of this kinesin. Here we show that KipA is an active ATPase, which is able to dimerize and move processively along microtubules with a speed of 9.48 μm/min.

Keywords *Aspergillus* · Kinesin · Tea2 · KipA

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Introduction

Molecular motors are fascinating mechanochemical enzymes, many of which couple the hydrolysis of ATP with the generation of force and processive movement along microtubules. They are involved in many different cellular and developmental processes such as organelle movement, localization of polarity marker proteins, mitosis or meiosis (Hirokawa et al. 2009b; Schliwa and Woehlke 2003). The transport machinery normally consists of polar cytoskeletal tracks, motors as engines, ATP as fuel, accessory factors as regulators and specific cargos (Hirokawa et al. 2009a, Marx et al. 2009). Molecular motors are classified into microtubule-dependent kinesins and dynein and actin-dependent myosins. All eukaryotes contain motors of all three classes, although the number of motors of each class varies in different organisms. For instance *Saccharomyces cerevisiae* harbors six kinesins, whereas the related filamentous fungus *Aspergillus nidulans* contains 11, and mammalian genomes encode about 50 putative kinesins (Hirokawa 1998; Rischitor et al. 2004). Kinesins are classified into 17 different families, which used to be named after e.g. the founding members. However, a novel nomenclature has been introduced and the families are distinguished by numbers (Lawrence et al. 2004). The family of conventional kinesin is therefore now called kinesin-1 family, and the Kip2 family was re-named to kinesin-7. Conventional kinesin is probably one of the best-studied motors. Although discovered and purified from squid axons because of its ATP hydrolysis activity, already early on an invitro assay was developed to show that kinesin-1 is a processive motor moving along polymerized and stabilized microtubules (Vale et al. 1985). Meanwhile the processive movement of kinesin-1 has even been followed at the single-molecule level (Verbrugge

et al. 2009). However, not all kinesin motors are able to move processively along microtubules. Many kinesins work as stationary microtubule depolymerases or polymerases (Howard and Hyman 2007).

Kinesin-7 is a motor protein, which is found in lower and higher eukaryotes. In mammals, it is called CENP-E. It is undetectable in non-dividing tissues and before late G2 in cycling cells. CENP-E accumulates at the end of G2 and is degraded after mitosis (Brown et al. 1994). Depletion of this kinesin causes chromosome segregation errors due to a weaker spindle assembly checkpoint and impaired interaction between the centromeres and the microtubules of the mitotic spindle (Putkey et al. 2002; Weaver et al. 2003). CENP-E is responsible for the localization and the activity of BubR1 at the kinetochore. BubR1 phosphorylates the checkpoint component Mad2 resulting in an arrest of mitosis in metaphase. This mitotic delay is necessary for correct attachment of the sister kinetochores to the spindle. Without CENP-E, the checkpoint is inactive, resulting in missegregation of sister chromatids (Putkey et al. 2002). After alignment of chromosomes, CENP-E is dephosphorylated by PP1 resulting in stable attachment to microtubules (Kim et al. 2010). In addition, there is an evidence that CENP-E promotes microtubule elongation at the plus end (Sardar et al. 2010). In this study, it was also shown that CENP-E is able to move in vitro processively along microtubules with velocities of 0.66 μm/min. In other systems, the speed of a CENP-E motor has been determined to 20.5 μm/min, which is still well below the speed for kinesin-1 (up to several μm/s) (Röhlk et al. 2008; Yardimci et al. 2008). The poleward force to segregate sister chromatids is generated by a microtubule minus-end directed dynein (Sharp et al. 2000).

In *S. cerevisiae* kinesin-7 (Kip2) is required for microtubule plus-end accumulation of Bik1 (CLIP-170 homologue) (Carvalho et al. 2004). In *S. pombe* kinesin-7 (Tea2) performs the same function as Kip2 in *S. cerevisiae*, the transport of a CLIP-170 homologue, Tip1, to the microtubule plus end. These results suggested a processive motor activity. The transportation system could indeed be reconstituted in vitro, clearly showing that no other components such as membranous vesicles are required for the transport of the proteins (Bieling et al. 2007). In addition, Tea2 transports the cell end marker protein Tea1 (Browning et al. 2003). The protein is anchored at the membrane of the growing tip through the interaction with membrane associated Mod5, scaffolds a protein complex and recruit formin, which in turn catalyzes the formation of actin cables. The latter serve as tracks for myosin-dependent vesicle secretion, necessary for membrane and cell wall extension (Snaith and Sawin 2003).

In *A. nidulans* kinesin-7 (KipA) is—like in *S. pombe*—involved in polarity determination (Konzack et al. 2005). In addition to Tea2, other cell end factors from *S. pombe* are also conserved in this filamentous fungus. Tea1 (*A. nidulans* TeaA), Mod5 (TeaR), Tea4 (TeaC), and For3 (SepA) have been characterized and a role in determining the zone for polarized growth has been shown (Fischer et al. 2008; Higashitsuji et al. 2009; Sharpless and Harris 2002; Takeshita et al. 2008; Yardimci, et al. 2008). However, TeaA and TeaC are apparently transported to the cortex independently of KipA (Higashitsuji et al. 2009; Konzack, et al. 2005) although TeaA (and probably TeaC) accumulates at microtubule plus ends (Takeshita and Fischer 2011). Another example is KatA, a KipA-interacting protein (Herrero et al. 2011). KatA is related to CENP-H and is involved in microtubule–kinetochore interaction. Again, the localization of KatA was independent of KipA. Taken together, it was not clear whether KipA is a processive motor or acts only as a linker protein and thus serves different functions than the transportation of proteins. In addition, sequence similarity between Tea2 and KipA outside the highly conserved motor domain (47% identity) is only low with an overall identity of 29%.

In order to study the catalytic properties of KipA, we expressed the protein in *Escherichia coli* and determined ATPase and the microtubule gliding activity.

Materials and methods

Expression and purification of KipA

The *A. nidulans kip*A gene was amplified by PCR using cDNA and the primers KipAfor (5′-gatcatatgTCTACACTGCCGCAGCC-3′) and KipArev (5′-cgaagcttTGCACTTGGACGACTGTTTAG-3′) for the full length region (pTS13). The amplificate was cloned into a pET21b vector (Novagen) and transformed into BL21 (DE3) codon plus RIL (BL21 RIL) strain, which carries extra copies of the *argU*, *ileY*, and *leuW* tRNA genes (Stratagene). Cells were incubated in TPM medium with 50 μM ampicillin at 37°C. Expression was induced by adding 0.1 mM IPTG at a cell density corresponding to an OD of 0.6–0.8. Cells were centrifuged and resuspended in lysis buffer containing protease inhibitors, DNAse, and lysozyme, followed by disintegration in a French Press (Aminco). After clarification by centrifugation, the supernatant was incubated with Ni–NTA fast flow slurry (GE Healthcare), washed and eluted with imidazole according to the instruction manual. Eluted fractions containing KipA were incubated with taxol-stabilized microtubules in the presence of 2 mM AMP-PNP to induce strong kinesin-microtubule binding. After washing, active kinesins were released by buffer containing 10 mM ATP. The release step was repeated

twice. The released fraction was used in the ATPase assays described below.

Motility assays were performed using a KipAhktail construct (pTS37). To this end, the C-terminal part of KipA (aa 640–889) was deleted by PCR using phosphorylated primers taildelfor (5′-gtgaattcAAGCTTGCGGCCGCACT CGAGC-3′) and taildelrev (5′-cggatccTCCTTGTGAGTT GACGCCTGTGG-3′) (pTS36). In the inserted restriction sites (lower case letters, *Eco*RI and *Bam*HI, respectively), the amplified coiled-coil region part of human Kif5B (aa 432–546) was ligated (pTS37), which is known to attach unspecifically to glass surfaces (Copppin et al. 1996; Jaud et al. 2006). After expression in BL21RIL strain, the protein was purified using Ni–NTA Fast Flow material and subsequently the eluted fractions were loaded onto a HiTrap SP HP column. After washing, the KipAhktail protein eluted between 250 and 300 mM NaCl. The eluted fractions were used in motility assays and the mantADP release experiments.

Microtubule stimulated ATPase assay

The MT-stimulated ATP hydrolysis of kinesin was measured for full-length KipA (pTS13) with a coupled enzyme assay (Huang et al. 1994). All reactions were performed at 20°C in BRB80 containing additional 1 mM MgATP and 20 μM taxol. The ATPase reaction of KipA was measured at different MT concentrations (0–7 μM). Tubulin was purified from porcine brain as described (Mandelkow et al. 1985). The reactions were started by adding ATP and measured for 2 min in a Spectrophotometer (Uvicon 930, Lohhof). The data points were plotted according to Michaelis–Menten.

Yeast two-hybrid analysis

The yeast two-hybrid analysis was performed using the Matchmaker™ Library Construction & Screening system (BD Clontech). For bait generation, a *kipA* cDNA fragment corresponding to the C-terminal half of KipA (505–889 amino acids) with primers KipA-F(NcoI) (5′-CCATG-GATTCGCTGGTCAGTATTCTTTG) and KipA_SR (5′-GCGCGTCGACTCATGCACTTGGACGACTG-3′) was amplified and cloned in the pGBKT7 vector, which contains the GAL4 DNA-BD and the *TRP1* marker (BD Clontech). The same kipA cDNA fragment was subcloned into the pGADT7-Rec vector, which contains the GAL4 DNA-AD and the *LEU2* marker (BD Clontech).

For the truncated versions of the KipA bait, primers with phosphorylated 5′-ends were used to amplify the entire bait vector except the deleted region. The primers used for the KW2 mutant were KW2F (P-5′-GATGGAAAGGCGA GTGCTCAA) and KW1R (P-5′-AAGCTCTTTCTCGG CCTGCGC; for the KW3 KW3F (P-5′-AACCAATCGT TACCAAAAGAG) and KW2R (P-5′-GTGGAATTCTTG GCTATGGAC) and for KW4 KW4F (P-5′-TAAGTCG ACCTGCAGCGGCCG) and KW3R (P-5′-GGAGCGAA GAGCTGTCAGCAT). pGBK7 associated plasmids were transformed in yeast AH109 (mating type *MAT*a) and pGADT7 associated plasmids were transformed in yeast Y187 (mating type *MAT*α). The system utilizes two reporter genes (*HIS3* and *LACZ*) under the control of the *GAL4*-responsive UAS.

MantADP release experiment

The mantADP release can be determined directly by following the ADP release because each stable binding of kinesin to microtubules releases one ADP from the motor. Therefore, KipAhktail (full length, pTS37) was incubated with a fluorescent ATP species, *N*-methylanthranoyl-ATP (mantATP), which yields a kinesin–mantADP complex. Addition of microtubules to the reaction releases mantADP first from one head. Addition of ATP causes activation of the motor and release of mantADP from the second motor head. This causes a step-wise decrease of the fluorescence and indicates dimerization of the kinesin.

Motility assay

Motility assays were performed on coverslips with KipAhktail motors (Paschal and Vallee 1993). Since the ATPase activities of truncated kinesins attached to the coiled-coil region of human Kif5B were indistinguishable from those of the corresponding untagged versions, the tag is unlikely to alter the motile behavior (Jaud et al. 2006). The KipAhktail motor protein adhered to the glass surface and was used for gliding assays under multiple motor conditions (motor concentration 0.1–0.5 mg/ml). Dilutions below 0.1 mg/ml caused a lack of microtubule binding. For imaging an AxioVision system from Zeiss was used.

Results

Kinesin-7, KipA is an active ATPase

To address whether the fungal kinesin KipA reaches the plus end of microtubules using its own motor activity, we expressed several kinesin constructs in *E. coli*. (Fig. 1). KipA was purified using Ni–NTA affinity purification, followed by affinity purification with polymerized microtubules in the presence of the non-hydrolyzable ATP analog AMP-PNP. This step increased the homogeneity of the protein fraction and increased the percentage of active motor protein, because inactive KipA cannot bind to

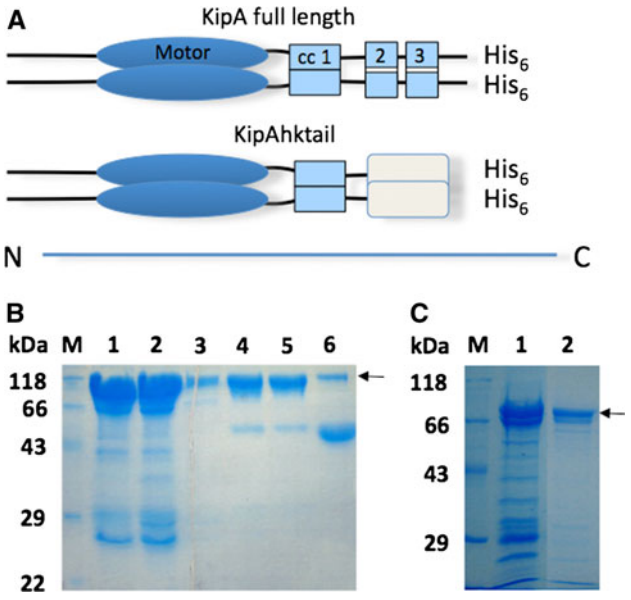

Fig. 1 Purification of KipA constructs. **a** Domain model of full length KipA and KipAhktail. The three coiled coil regions are indicated as cc1, 2 and 3. Only one coiled coil region is *left* in the hybrid molecule. **b** Purification of full length KipA protein (*arrows*) through Ni-affinity (lanes *1–3*) and activity purification [release fractions (lanes *4*, *5*); pellet fraction (lane *6*)]. In the activity purification fractions, tubulin is visible at 55 kDa. 10–60 µg protein were loaded per lane. **c** Purification of KipAhktail (*arrows*) through Ni-affinity (lane *1*) and ion exchange chromatography (lane *2*) as used in the motility assay. 10–50 µg protein were loaded per lane. For further experimental details see "Materials and methods"

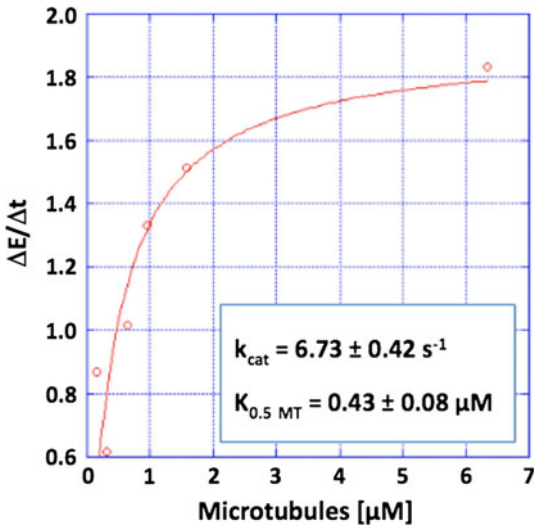

Fig. 2 Microtubule-stimulated ATPase activity of KipA. Microtubule-stimulated ATPase assay as a function of microtubule concentration. Final concentrations: 0.1 µM KipA, 0–7 µM tubulin, 20 µM paclitaxel, 1 mM MgATP, enzyme mixture (LDH/PK 0.2 U each), 0.2 mg/ml PEP, 0.2 mM NADH. Inset: calculation data of k_{cat} and $K_{0.5,MT}$

microtubules. The purified kinesin protein was used for activity measurements (Fig. 2). In this assay, phosphoenol pyruvate is converted by pyruvate kinase to pyruvate. ADP, required in this reaction, is provided if the kinesin has ATPase activity. The produced pyruvate is further reduced to lactate, where NADH/H+ serves as electron donor. The kinetics of NADH to NAD can be monitored spectrophotometrically and is directly proportional to the hydrolyzed ATP [modified after (Huang et al. 1994)]. The initial enzyme activities were plotted against the microtubule concentration. The dependence of the kinesin ATPase activity followed a Michaelis–Menten kinetics and k_{cat} was calculated to $6.73 \pm 0.42\ s^{-1}$ and the $KM_{0.5MT}$ to 0.43 ± 0.08 µM. This is comparable with previous findings for CENP-E of *Xenopus laevis* (Rosenfeld et al. 2009; Wood et al. 1997). This vertebrate Kinesin-7 has similar biochemical properties with a k_{cat} of $13.2\ s^{-1}$. The KipA $KM_{0.5MT}$ is also in agreement with the ones of other dimeric kinesins, whereas this value is around 1 µM for monomeric proteins (Moyer et al. 1998). In comparison to KipA, conventional kinesin of *A. nidulans* KinA has a higher ATP turnover rate [$k_{cat} = 55.4\ s^{-1}$, (Requena et al. 2001)].

KipA forms homodimers and moves along microtubules

Next, we asked whether KipA would not only be active as ATPase but also move along microtubules. Because most kinesins move along microtubules as dimers, with always being attached to the microtubule through at least one kinesin protein, we first asked whether KipA is able to dimerize. The presence of coiled coil regions in the tail of KipA suggested already self-interaction. This question was addressed in two ways, the yeast-two-hybrid assay and a direct measurement of the ATP consumption in a mantADP release experiment [modified after (Ma and Taylor 1995)].

To confirm the potential to dimerize, we tested the interaction of KipA with itself using the yeast two-hybrid system (Fig. 3a). The tail of KipA contains three coiled-coil regions (I, II, III), which all together or individually could mediate protein–protein interaction. Recently, we observed that the interaction of KipA with certain cargo proteins depends on a C-terminal cargo-binding domain, but coil-coiled region 2 and 3 (KW2) appeared also to contribute to binding of KatA (Herrero et al. 2011). In order to test which regions were necessary for dimerization of KipA, the same deletion versions of KipA as used previously have been tested (Fig. 3a). Surprisingly, removal of any single coiled-coil region had no effect on dimerization. Even KipA with only coiled-coil region I (KW2) showed interaction. A slight reduction was only obvious with the construct lacking the cargo-binding domain (KW4).

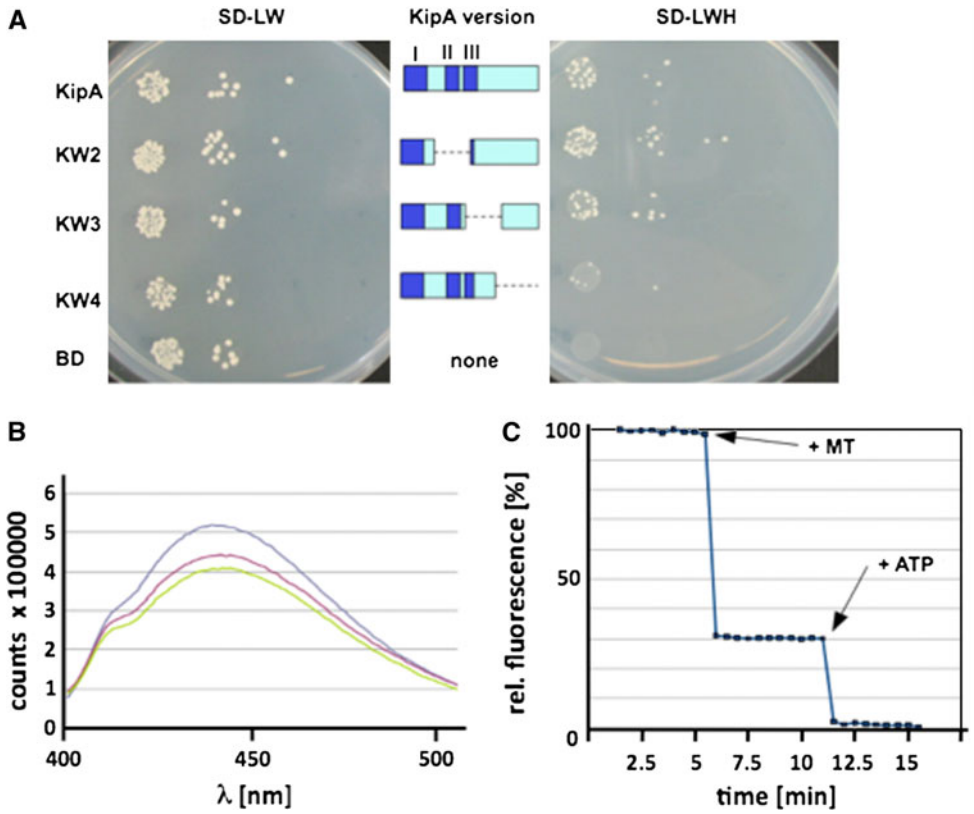

Fig. 3 Evidence for dimerization of KipA. **a** Yeast-two-hybrid analysis. As bait, different deleted versions of the C-terminal half of KipA, which includes the three coiled-coils (depicted in *dark grey*) and the cargo-binding regions, were used. As prey the KipA clone without deletions was taken. As negative control the Gal4 binding domain alone (BD) was chosen. KW means KipA window. SD-LW and SD-LWH means synthetic dropout medium without leucine and tryptophan and without leucine, tryptophan and histidine, respectively. **b** MantADP release experiment. Fluorescence of 100–250 μM KipA*MantADP complexes were measured under starting conditions (*upper line*), after addition of equimolar amounts of MTs (0.1–1 μM; *middle line*) and after ATP addition (1 mM; *lower line*). Stepwise decrease of the relative fluorescence by mantADP release stimulated by addition of microtubules (MT) and ATP. **c** After addition of equimolar amounts of microtubules, relative fluorescence decreased immediately to 30%. After ATP addition, relative fluorescence decreased to the background level

As a second proof for dimerization, a mantADP release experiment was conducted (Fig. 3b). Basal ADP release of kinesin is very slow but can be strongly activated by microtubule binding (Ma and Taylor 1995). Since the mant-fluorophore displays stronger fluorescence when bound to a protein than when free in solution, the release of mantADP can be followed in a fluorimeter (Ma and Taylor 1997). All assays were performed in a FluoroMax2 spectrofluorimeter (Jobin–Yvon) at 23°C in 1 ml cuvettes and using the ATPase buffer. The excitation wavelength was 365 nm, the emitted fluorescence was monitored at 445 nm. 100–250 nM of kinesin*mantADP complexes were first mixed with at least equimolar amounts of microtubules (0.1–1 μM final concentration). This causes binding of one motor head to the microtubule, release of the bound mantADP and a drop of the fluorescence to 30%. After 5 min, ATP was added for the activation of the bound motor and the release of the second mantADP visible as a further decrease of the fluorescence to a basal level (Fig. 3b). This step-by-step reduction of the fluorescence indicates the mantADP release from dimeric kinesin motor domains. The fact that the initial reduction of the fluorescence was more than the expected 50% could be explained by monomeric KipA molecules, which also could bind to microtubules and release mantADP. A further reduction of the fluorescence of these species after addition of ATP would be impossible.

A standard assay to measure the activity of kinesin motor proteins and to determine the speed of a motor is the motility assay. In this assay motor proteins are attached to a cover slip and fluorescently labeled microtubules are added. Upon addition of ATP, microtubules are transported on the kinesin layer, which is visible in the fluorescence microscope (Paschal and Vallee 1993). This assay relies on the binding of kinesin to the glass surface. In the case of human conventional kinesin this is a spontaneous reaction, because the C-terminal part sticks unspecifically to several surfaces (Jaud et al. 2006). However, purified KipA did not

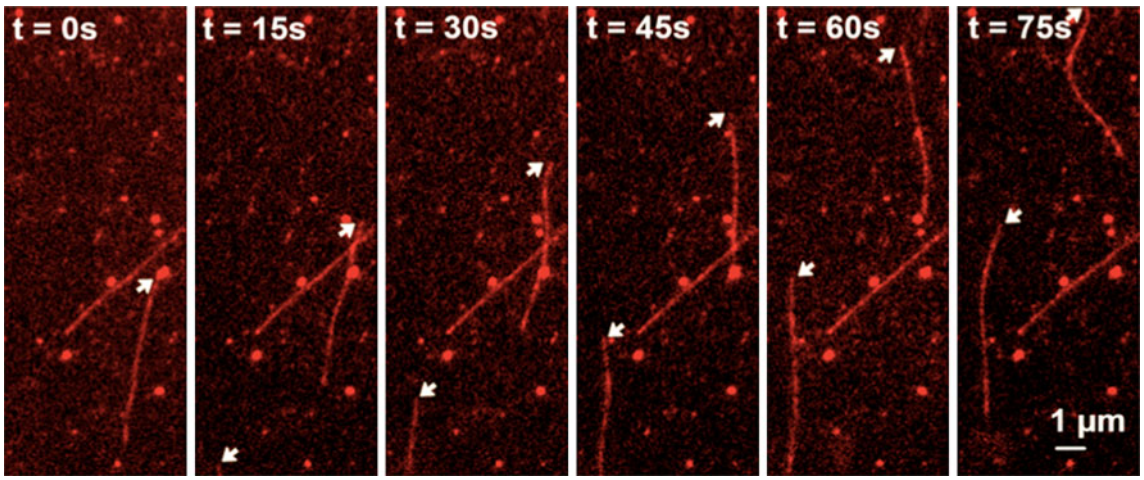

Fig. 4 Microtubule gliding assay for KipA. Rhodamine-labeled microtubules are transported by KipAhktail constructs in a multimotor gliding assay. *Panels* show fluorescence microscopy images every 15 s during the motility assay. *Arrows* representing the minus end of moving microtubules. The speed was calculated from moving microtubules (9.48 ± 1.8 µm/min; $n = 114$). The corresponding movie is available as material

attach to the surface. In order to reach this goal, the KipA N-terminal domain including the motor domain plus the first coiled-coil region for KipA–KipA interaction [amino acids 1–639 (out of 889 aa)] was fused to a coiled–coiled region (114 aa) of human Kif5B as done in other studies (Copppin et al. 1996; Ebbing et al. 2008; Jaud et al. 2006). The protein was expressed like full-length KipA and Ni-affinity purification was followed by cation-exchanger chromatography to obtain a homogeneous protein fraction (Fig. 1b). Using this modified KipA protein to perform motility assays, velocities of 9.48 ± 1.8 µm/min were calculated ($n = 114$) (Fig. 4).

Discussion

In this paper, we show that *A. nidulans* kinesin 7 is an active ATPase, is able to dimerize and moves along microtubules with velocities of ca. 10 µm/min. The value for KipA is in good agreement with single molecule measurements with the *S. pombe* Tea2 protein, where velocities of ca. 5 µm/min were determined (Bieling et al. 2007). The fungal kinesin-7 motor is thus 10–25 times slower than the velocity of *A. nidulans* conventional kinesin, which was determined to 120 µm/min (Requena et al. 2001). KinA is responsible for long distance transport in fungal hyphae and thus high kinesin velocities could be an advantage for fast growth. In contrast, KipA acts in chromosome alignment and segregation during mitosis or in short distance transportation of cell end markers towards the microtubule plus ends (in *S. pombe*). In these processes, moderate speeds are apparently sufficient.

KipA has been shown to be a microtubule plus end tracking protein. This localization could be due to loading and attachment of KipA to the plus ends during the initiation of the polymerization at the microtubule-organizing centers. Alternatively, the endogenous motor activity could be responsible for the accumulation of KipA at the microtubule ends. The maximum speed of microtubule polymerization in living *A. nidulans* hyphae was measured with 8.93 ± 1.1 µm/min (Han et al. 2001). The fact that the velocity of KipA was determined in vitro to ca. 10 µm/min allows the possibility that KipA moves behind the growing microtubule plus end and reaches it after some time. Hence, KipA movement could also explain the transportation and accumulation of cell end markers at the microtubule plus ends. However, TeaA and TeaR were delivered at the tip independently of KipA (Konzack et al. 2005). Nevertheless, deletion of *kipA* phenocopied the *teaR* deletion and affected the positioning of TeaA and TeaR (Takeshita et al. 2008). This strongly suggests that KipA is required for the correct transportation and positioning of as yet unidentified cell end marker proteins. It will be one of the challenges for further research to discover these proteins.

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References

Bieling P, Laan L, Schek H, Munteanu EL, Sandblad L, Dogterom M, Brunner D, Surrey T (2007) Reconstitution of a microtubule plus-end tracking system in vitro. Nature 450:1100–1105

Brown KD, Coulson RM, Yen TJ, Cleveland DW (1994) Cyclin-like accumulation and loss of the putative kinetochore motor CENP-E. Dev Cell 3:351–365

Browning H, Hackney DD, Nurse P (2003) Targeted movement of cell end factors in fission yeast. Nat Cell Biol 5:812–818

Carvalho P, Gupta MLJ, Hoyt MA, Pellman D (2004) Cell cycle control of kinesin-mediated transport of Bik1 (CLIP-170) regulates microtubule stability and dynein activation. Dev Cell 6:815–829

Copppin CM, Finer JT, Spudich JL, Vale RD (1996) Detection of sub-8-nm movements of kinesin by high-resolution optical trap microscopy. Proc Natl Acad Sci USA 93:1913–1917

Ebbing B, Mann K, Starosta A, Jaud J, Schöls L, Schüle R, Woehlke G (2008) Effect of spastic paraplegia mutations in KIF5A kinesin on transport activity. Hum Mol Genet 17:1245–1252

Fischer R, Zekert N, Takeshita N (2008) Polarized growth in fungi—interplay between the cytosekelton, positional markers and membrane domains. Mol Microbiol 68:813–826

Han G, Liu B, Zhang J, Zuo W, Morris NR, Xiang X (2001) The Aspergillus cytoplasmic dynein heavy chain and NUDF localize to microtubule ends and affect microtubule dynamics. Curr Biol 11:19–24

Herrero S, Takeshita N, Fischer R (2011) The *Aspergillus nidulans* CENP-E kinesin motor KipA interacts with the fungal homologue of the centromere-associated protein CENP-H at the kinetochore. Mol Microbiol 80:981–994

Higashitsuji Y, Herrero S, Takeshita N, Fischer R (2009) The cell end marker protein TeaC is involved in both growth directionality and septation in *Aspergillus nidulans*. Eukaryot Cell 8:957–967

Hirokawa N (1998) Kinesin and dynein superfamily proteins and the mechanism of organelle transport. Science 279:519–526

Hirokawa N, Nitta R, Okada Y (2009a) The mechanisms of kinesin motor motility: lessons from the monomeric motor KIF1A. Nat Rev Mol Cell Biol 10:877–884

Hirokawa N, Noda Y, Tanaka Y, Niwa Sq (2009b) Kinesin superfamily motor proteins and intracellular transport. Nat Rev Mol Cell Biol 10:682–696

Howard J, Hyman AA (2007) Microtubule polymerases and depolymerases. Curr Opin Cell Biol 19:31–35

Huang TG, Suhan J, Hackney DD (1994) Drosophila kinesin motor domain extending to amino acid position 392 is dimeric when expressed in *Escherichia coli*. J Biol Chem 269:16502–16507

Jaud J, Bathe F, Schliwa M, Rief M, Woehlke G (2006) Flexibility of the neck domain enhances kinesin-1 motility under load. Biophys J 91:1407–1412

Kim Y, Holland AJ, Lan W, Cleveland DW (2010) Aurora kinases and protein phosphatase 1 mediate chromosome congression through regulation of CENP-E. Cell 142:444–455

Konzack S, Rischitor P, Enke C, Fischer R (2005) The role of the kinesin motor KipA in microtubule organization and polarized growth of *Aspergillus nidulans*. Mol Biol Cell 16:497–506

Lawrence CJ, Dawe RK, Christie KR, Cleveland DW et al (2004) A standardized kinesin nomenclature. J Cell Biol 167:19–22

Ma YZ, Taylor EW (1995) Kinetic mechanism of kinesin motor domain. Biochemistry 34:13233–13241

Ma YZ, Taylor EW (1997) Interacting head mechanism of microtubule-kinesin ATPase. J Biol Chem 272:724–730

Mandelkow EM, Herrmann M, Rühl U (1985) Tubulin domains probed by limited proteolysis and subunit-specific antibodies. J Mol Biol 185:311–327

Marx A, Hoenger A, Mandelkow E (2009) Structures of kinesin motor proteins. Cell Mot Cytoskel 66:958–966

Moyer ML, Gilbert SP, Johnson KA (1998) Pathway of ATP hydrolysis by monomeric and dimeric kinesin. Biochemistry 37:800–813

Paschal BM, Vallee RB (1993) Microtubule and axoneme gliding assays for force production by microtubule motor proteins. Meth Cell Biol 39:65–74

Putkey FR, Cramer T, Morphew MK, Silk AD, Johnson RS, McIntosh JR, Cleveland DW (2002) Unstable kinetochore-microtubule capture and chromosomal instability following deletion of CENP-E. Dev Cell 3:351–365

Requena N, Alberti-Segui C, Winzenburg E, Horn C, Schliwa M, Philippsen P, Liese R, Fischer R (2001) Genetic evidence for a microtubule-destabilizing effect of conventional kinesin and analysis of its consequences for the control of nuclear distribution in *Aspergillus nidulans*. Mol Microbiol 42:121–132

Rischitor P, Konzack S, Fischer R (2004) The Kip3-like kinesin KipB moves along microtubules and determines spindle position during synchronized mitoses in *Aspergillus nidulans* hyphae. Eukaryot Cell 3:632–645

Röhlk C, Rohlfs M, Leier S, Schliwa M, Liu X, Parsch J, Woehlke G (2008) Properties of the kinesin-1 motor DdKif3 from *Dictyostelium discoideum*. Eur J Cell Biol 87:237–249

Rosenfeld SS, van Duffelen M, Behnke-Parks WM, Beadle C, Correia J, Xing J (2009) The ATPase cycle of the mitotic motor CENP-E. J Biol Chem 284:32858–32868

Sardar HS, Luczak VG, Lopez MM, Lister BC, Gilbert SP (2010) Mitotic kinesin CENP-E promotes microtubule plus-end elongation. Curr Biol 20:1648–1653

Schliwa M, Woehlke G (2003) Mol Motors. Nature 422:759–765

Sharp DJ, Rogers GD, Scholey JM (2000) Cytoplasmic dynein is required for poleward chromosome movement during mitosis in Drosophila embryos. Nat Cell Biol 2:922–930

Sharpless KE, Harris SD (2002) Functional characterization and localization of the *Aspergillus nidulans* formin SEPA. Mol Biol Cell 13:469–479

Snaith HA, Sawin KE (2003) Fission yeast mod5p regulates polarized growth through anchoring of tea1p at cell tips. Nature 423:647–651

Takeshita N, Fischer R (2011) On the role of microtubules, cell end markers, and septal microtubule organizing centers on site selection for polar growth in *Aspergillus nidulans*. Fungal Biol 115:506–517

Takeshita N, Higashitsuji Y, Konzack S, Fischer R (2008) Apical sterol-rich membranes are essential for localizing cell end markers that determine growth directionality in the filamentous fungus *Aspergillus nidulans*. Mol Biol Cell 19:339–351

Vale RD, Reese TS, Sheetz MP (1985) Identification of a novel force-generating protein, kinesin, involved in microtubule-based motility. Cell 42:39–50

Verbrugge S, Lansky Z, Peterman EJ (2009) Kinesin's step dissected with single-motor FRET. Proc Natl Acad Sci USA 106:17741–17746

Weaver BAA, Bonday ZQ, Putkey FR, Kops GJPL, Silk AD, Cleveland DW (2003) Centromere-associated protein-E is essential for the mammalian mitotic checkpoint to prevent aneuploidy due to single chromosome loss. J Cell Biol 162:551–563

Wood KW, Sakowicz R, Goldstein LS, Cleveland DW (1997) CENP-E is a plus end-directed kinetochore motor required for metaphase chromosome alignment. Cell 91:357–366

Yardimci H, van Duffelen M, Mao Y, Rosenfeld SS, Selvin PR (2008) The mitotic kinesin CENP-E is a processive transport motor. Proc Natl Acad Sci USA 105:6016–6021