Proc. Natl. Acad. Sci. USA
Vol. 94, pp. 322–327, January 1997
Plant Biology

In vitro motility of AtKCBP, a calmodulin-binding kinesin protein of *Arabidopsis*

(microtubule motor/myosin/calcium regulation)

HEBOK SONG*, MAXIM GOLOVKIN†, A. S. N. REDDY†, AND SHARYN A. ENDOW*‡

*Department of Microbiology, Duke University Medical Center, Durham, NC 27710; and †Department of Biology, Colorado State University, Fort Collins, CO 80523

Communicated by James A. Spudich, Stanford University, Stanford, CA, November 5, 1996 (received for review July 30, 1996)

ABSTRACT **AtKCBP is a calcium-dependent calmodulin-binding protein from *Arabidopsis* that contains a conserved kinesin microtubule motor domain. Calmodulin has been shown previously to bind to heavy chains of the unconventional myosins, where it is required for *in vitro* motility of brush border myosin I, but AtKCBP is the first kinesin-related heavy chain reported to be capable of binding specifically to calmodulin. Other kinesin proteins have been identified in *Arabidopsis*, but none of these binds to calmodulin, and none has been demonstrated to be a microtubule motor. We have tested bacterially expressed AtKCBP for the ability to bind microtubules to a glass surface and induce gliding of microtubules across the glass surface. We find that AtKCBP is a microtubule motor protein that moves on microtubules toward the minus ends, with the opposite polarity as kinesin. In the presence of calcium and calmodulin, AtKCBP no longer binds microtubules to the coverslip surface. This contrasts strikingly with the requirement of calmodulin for *in vitro* motility of brush border myosin I. Calmodulin could regulate AtKCBP binding to microtubules in the cell by inhibiting the binding of the motor to microtubules. The ability to bind to calmodulin provides an evolutionary link between the kinesin and myosin motor proteins, but our results indicate that the mechanisms of interaction and regulation of kinesin and myosin heavy chains by calmodulin are likely to differ significantly.**

The *Arabidopsis* protein AtKCBP is a recently identified calmodulin target protein that binds to calmodulin in the presence of micromolar concentrations of calcium (1). AtKCBP contains a region homologous to the motor domain of the kinesin microtubule (MT) motor proteins, but the predicted tail and stalk regions of AtKCBP do not show significant sequence similarity to any of the known kinesin proteins. AtKCBP is the first kinesin-related heavy chain to be reported that is capable of binding specifically to calmodulin. The protein is highly expressed in developing flowers and cultured cells. Homologues that share extensive sequence similarity with the head, stalk, and tail regions of AtKCBP have been isolated from potato and tobacco by screening expression libraries using labeled calmodulin as a probe (2, 3).

Calmodulin has been known for many years to bind to the heavy chains of the unconventional myosins and is thought to regulate myosin function. The effect of calcium and calmodulin on myosin function has been best described for brush border myosin I, a vertebrate myosin present in intestinal epithelia. Brush border myosin I can bind to three or four calmodulins, but only two of these remain bound in the presence of calcium (4). Calcium causes partial dissociation of calmodulin from the motor and can completely inhibit motility in coverslip assays (5, 6). The motility can be restored by adding back purified calmodulin, indicating that brush border myosin I motility in *in vitro* assays is dependent on bound calmodulin. Calcium and calmodulin have been shown to have complex effects on the ATPase activities of brush border myosin I and other unconventional myosins (7).

The calmodulin binding sites in the myosins consist of highly basic ≈23-residue repeats with a core consensus sequence of IQXXXRGXXXR, known as the IQ motif (8), present near the motor–stalk junction or motor–tail junction. Unlike AtKCBP, many of the unconventional myosins can bind calmodulin with high affinity in the absence of calcium (7). The region of AtKCBP that binds calmodulin has been mapped to a 23-residue peptide located near the C terminus of the protein (1). This peptide does not contain an IQ motif and bears little sequence similarity to the calmodulin-binding IQ repeats of the myosins. The calmodulin-binding region of AtKCBP is highly conserved in potato and tobacco KCBP but is not present in any of the other known kinesin proteins.

The function of AtKCBP is not known, nor is the relationship of its function to the ability to bind calmodulin. Calmodulin, a multifunctional and ubiquitous calcium-binding protein in eukaryotes, is thought to control diverse cellular functions in plants by modulating the activity of its target proteins (9, 10). Among other processes, calcium and calmodulin are implicated in MT organization and dynamics during the cell cycle (11–14), and cytokinesis (12). During cell division, calmodulin is found associated with MT arrays of the spindle apparatus and phragmoplast (11, 12).

The finding of a kinesin protein that binds to calmodulin raises many questions. Foremost among them are the questions of whether the protein functions as an MT motor protein and, if so, what effect calmodulin has on motor activity and function in the cell. Although several kinesin proteins have previously been reported to exist in *Arabidopsis* (15, 16), none of these has yet been demonstrated to be capable of moving on MTs. Furthermore, none of the previously reported kinesin proteins from *Arabidopsis* or any other organism contains a calmodulin-binding sequence like that of KCBP, implying differences in regulation among the proteins. To begin to address the questions of function and regulation of this novel calmodulin-binding kinesin from a higher plant, we have analyzed the motility properties of AtKCBP. We report here the finding that AtKCBP induces gliding of MTs in *in vitro* motility assays and is therefore an MT motor protein. We have further determined the polarity of AtKCBP movement on MTs and the effect of calmodulin on AtKCBP motility.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

0027-8424/97/94322-6$2.00/0
PNAS is available online at **http://www.pnas.org**.

Abbreviations: MT, microtubule; GST, glutathione *S*-transferase; NEM, *N*-ethylmaleimide.

‡To whom reprint requests should be addressed. e-mail: endow@galactose.mc.duke.edu.

MATERIALS AND METHODS

Plasmid Construction. pGEX/AtKCBP was constructed by ligating a 1.5-kb *Eco*RI fragment of AtKCBP to *Eco*RI-digested pGEX-3X (17). The orientation of the insertion was determined by digesting the recombinant plasmids from bacterial transformants with *Bam*HI and *Xho*I restriction endonucleases, which cut at unique sites in the vector and insertion, respectively. A plasmid with the insertion in the correct orientation was selected and transformed into BL21(DE3)pLysS host cells (18) for expression of GST/AtKCBP. Expression of pGEX/AtKCBP was monitored by probing Western blots of uninduced and induced cultures with biotinylated calmodulin or anti-HIPYR antibody (19).

Bacterial Expression and Preparation of Motors. Bacterial cultures containing pGEX/AtKCBP or pGEX/MC1 (20) in BL21(DE3)pLysS host cells were grown at 37°C to an OD_{550} of 1.2 or 0.8, respectively, then induced by addition of 0.4 mM isopropyl β-D-thiogalactopyranoside followed by shaking at 22°C for 3.5–5 hr. Cells were harvested, washed in PB (10 mM sodium phosphate, pH 7.2/1 mM EGTA/1 mM $MgCl_2$) or HEM (10 mM Hepes, pH 7.2/1 mM EGTA/1 mM $MgCl_2$), frozen in liquid N_2, and stored at −80°C until use. Clarified cell lysates were prepared for motility assays as described (21). Briefly, cells were thawed in PB containing protease inhibitors and lysed by freezing in liquid N_2 followed by thawing at 37°C. Bacterial DNA was digested by addition of 5 mM $MgCl_2$, 1 mM DTT, and 100 μg/ml DNAse I and incubation on ice for 20 min. The lysate was clarified by centrifugation at 27,000 × *g* in a Sorvall SS-34 rotor for 20 min at 4°C and then at 155,000 × *g* in a Beckman TLA 100.3 rotor for 20 min at 2°C. The final supernatant was used for motility assays. Purified kinesin from bovine brain was obtained from Cytoskeleton (Denver, CO).

Axoneme–MT Complexes. *Chlamydomonas* axonemes were prepared as described (22) with modifications. *Chlamydomonas reinhardtii ida 1 mt*$^+$ cells (strain CC-2664), cultured for 3 days under fluorescent light, were obtained from the *Chlamydomonas* stock center at Duke University. Cells (600 ml) were harvested by centrifugation at 230 × *g* for 7 min at 22°C in a Sorvall GSA rotor, washed in 150 ml of distilled water and pelleted, and then resuspended in 150 ml of 10 mM Hepes (pH 7.2) and pelleted again. The loosely packed cells were resuspended in 10 ml of cold HMDS [10 mM Hepes, pH 7.2/5 mM $MgCl_2$/1 mM DTT/4% (wt/vol) sucrose] and deflagellated by addition of Nonidet P-40 to 0.02% followed by gentle mixing and inversion on ice for 4 min. An equal volume of ice-cold HMDS + 0.5 mM EGTA was added, and cell bodies were removed by centrifugation for 10 min at 1600 × *g* in a Sorvall SS-34 rotor. The resulting supernatant was gently layered onto the top of a 5-ml cushion of HMDS-25 [HMDS + 25% (wt/vol) sucrose] and centrifuged at 20,000 × *g* for 30 min at 4°C. The flagellar pellet was resuspended in 1 ml of salt extraction buffer (10 mM Hepes, pH 7.2/0.6 M NaCl/5 mM $MgCl_2$/1 mM EGTA/7 mM 2-mercaptoethanol/1 mM DTT) and incubated for 30 min at 22°C with occasional swirling to remove axonemal membranes and dyneins. The extraction mix was centrifuged for 15 min at 12,000 rpm in a microfuge at room temperature, and the resulting pellet was resuspended in 300 μl of axoneme storage buffer [10 mM Hepes, pH 7.2/0.5 mM EGTA/1 mM $MgCl_2$/3.5 mM 2-mercaptoethanol/1 mM DTT/1 mM Mg·GTP/50% (wt/vol) glycerol] and stored at −80°C after freezing in liquid N_2.

Tubulin was isolated from porcine brains as described (23). Three mg of phosphocellulose-purified tubulin (6 mg/ml) was modified by treatment with *N*-ethylmaleimide (NEM; ref. 24). Both 0.1 mM Mg·GTP and 1 mM NEM were added to the tubulin, and the reaction mix was incubated for 10 min on ice. The NEM was quenched by adding 8 mM 2-mercaptoethanol, and the mix was incubated an additional 10 min on ice. Excess NEM and 2-mercaptoethanol were removed by passing the reaction mix over a Sephadex G25 column equilibrated with PEM (100 mM Pipes, pH 6.9/1 mM EGTA/1 mM $MgCl_2$). The peak fraction was divided into aliquots, frozen in liquid N_2, and stored at −80°C.

For preparation of axoneme–MT complexes, axonemes (20 μl) were thawed, diluted with 200 μl of PEM, and centrifuged at 12,000 rpm for 10 min at 22°C in a microfuge. The pellet was gently resuspended in 15 μl of PEM containing 1 mM Mg·GTP + 20 μg of NEM–tubulin + 20 μg of phosphocellulose–purified tubulin and was incubated for 5 min at 37°C. Seven microliters of PEM + 40 μM taxol at 37°C was added, and the assembly mix was transferred to room temperature. Further dilution (1:5 or 1:3) of the axoneme–MT complexes with PEM containing taxol was usually necessary before use to produce an appropriate density.

Motility Assays. MT gliding assays were performed using methods described previously (21). Briefly, thoroughly cleaned coverslips were coated with 6–7 μl of anti-glutathione *S*-transferase (GST) antibody ($OD_{280} \approx 0.5$). After rinsing off excess antibody with PB (two times, 30 μl), the coverslips were drained and gently coated with clarified bacterial cell lysates containing expressed motor protein, followed by addition of Mg·ATP and MTs or axoneme–MT complexes. Coverslips were then sealed to a microscope slide with VALAP (1:1:1 mix of Vaseline/lanolin/paraffin). For kinesin, assays were prepared by adding the motor (2.5 units) directly to the clean coverslips, followed by addition of Mg·ATP and MTs or axoneme–MT complexes, and PB to 8 μl.

Gliding of MTs or axoneme–MT complexes was observed using video-enhanced differential interference contrast microscopy (25) and recorded onto tape. MT or axoneme–MT gliding velocity was determined by manually tracking MT ends using a mouse-controlled cursor overlaid on the video image and a tracking program written for this purpose (a gift of N. Gliksman and T. Salmon, University of North Carolina Chapel Hill, NC). Taped images of gliding axoneme–MT complexes were converted to digital images using the S-video capture capability of a Power Macintosh 8500AV/120 and Apple Video Player software. Images were converted to stacks and cropped using the stack macros of the public domain program NIH IMAGE version 1.59. Montages were made and lettered, and image contrast was adjusted with Adobe PHOTOSHOP version 3.0.4.

Motility Assays with Calmodulin. Bacterial lysates to determine the effect of calmodulin on AtKCBP motility were prepared as described above except that buffers without EGTA were used. Lysates containing induced GST/AtKCBP or GST/MC1 motor proteins were preincubated on ice with calmodulin and $CaCl_2$ for 2–5 min before preparing the coverslip assay. Preincubation mixes contained 8 μl of clarified lysate, 1 μl of 4.32 mg/ml purified bovine brain calmodulin in 25 mM Tris (pH 7.5) + 5 mM $CaCl_2$ (a gift of A. Means, Duke University, Durham, NC) and 1 μl of $CaCl_2$ (0.5, 5, or 10 mM) to give final preincubation mix concentrations of 0.55, 1, or 1.5 mM $CaCl_2$. After coverslips were coated with anti-GST antibody and washed with PB without EGTA, coverslips were drained and 6 μl of the lysate + calmodulin was added, followed by 0.8 μl of 100 mM Mg·ATP and 1 μl of MTs. The MTs were prepared by incubating 5.8 mg/ml tubulin containing 2 mM EGTA and 1 mM Mg·GTP for 25–30 min at 37°C. Taxol was added to 40 μM, and the incubation was continued for 5 min at 37°C. The MTs were divided into aliquots, flash-frozen, and stored at −80°C until use. For use in motility assays, MTs were thawed at 37°C and diluted 1:5 with 100 mM Hepes (pH 7.2) containing taxol (final EGTA concentration = 0.4 mM; final taxol concentration = 40–80 μM).

RESULTS

AtKCBP Is an MT Motor Protein. A plasmid for expression of AtKCBP as a fusion protein with GST was constructed and

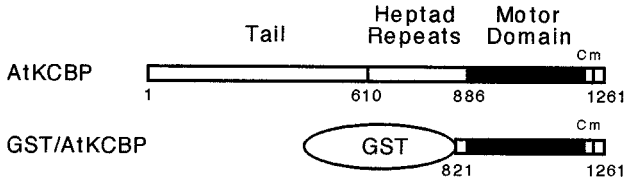

FIG. 1. AtKCBP and GST/AtKCBP. The predicted AtKCBP kinesin-related protein is 1261 aa long, and the conserved motor domain (residues 886–1217) is present at the C terminus of the protein. A region of heptad repeats of hydrophobic amino acids (residues 610–890) with a high probability of coiled-coil formation is present in the center of the molecule. The 23-aa calmodulin-binding peptide (residues 1218–1240) lies immediately adjacent to the conserved motor domain, near the C terminus of the protein. The GST/AtKCBP fusion protein consists of GST fused to the N terminus of the protein at residue 821 and contains ≈70 residues of the predicted coiled-coil together with the conserved motor domain including the calmodulin-binding peptide. Cm, calmodulin-binding peptide.

transformed into bacterial host cells. The pGEX/AtKCBP plasmid encodes a fusion protein consisting of an N-terminal ≈26 kDa GST moiety, followed by residues 821-1261 of AtKCBP (Fig. 1). The AtKCBP sequences correspond to ≈70 residues of the central predicted α-helical coiled-coil and the entire C-terminal motor domain including the calmodulin-binding residues that lie to the 3′ side of the conserved motor domain. AtKCBP, expressed in bacteria as a fusion protein with GST, binds MTs to coverslips and translocates the bound MTs across the glass surface. MTs gliding on GST/AtKCBP bound to coverslips were tracked at leading and lagging ends to determine motor velocity. The leading and lagging ends of the MTs moved with the same velocity, and the MT lengths did not change significantly during the periods of observation. The leading and lagging end velocities were 10.1 ± 0.5 μm/min (x ± SE, n = 20) and 9.9 ± 0.4 μm/min (x ± SE, n = 20), respectively (Fig. 2).

AtKCBP Is a Minus End-Directed MT Motor. The polarity of the AtKCBP motor was determined by assaying gliding of axoneme–MT complexes on GST/AtKCBP bound to coverslips. The axoneme–MT complexes consisted of MTs grown from the plus ends of *Chlamydomonas* flagellar axonemes or axoneme fragments. NEM-modified tubulin (24) was added to the assembly mix to suppress growth of MTs from the minus ends of the axonemes. The axoneme–MT complexes bound to the GST/AtKCBP motor on the coverslip and glided with the MT (plus) ends of the complexes leading. A time-lapse sequence of an axoneme–MT complex gliding on GST/AtKCBP is shown in Fig. 3. Because the motor was attached to the coverslip, movement of the complexes with the plus ends leading indicates that the GST/AtKCBP motor moves on the MTs toward the MT minus ends. A complex gliding on kinesin bound to a coverslip is shown in Fig. 3 for comparison. The axoneme–MT is moving on kinesin with the axoneme (minus) end of the complex leading, consistent with the plus-end polarity of kinesin translocation on MTs.

The polarity and velocity of AtKCBP translocation on MTs were analyzed by observing the gliding movement of 39 axoneme–MT complexes bound to GST/AtKCBP (Table 1). All 39 axoneme–MT complexes glided on GST/AtKCBP with the MT (plus) end leading, with a velocity of 8.1 ± 0.6 μm/min (x ± SE), confirming the minus-end polarity of AtKCBP movement on MTs. As a control, polarity of movement and velocity were determined for 37 axoneme–MT complexes bound to purified bovine brain kinesin attached to a coverslip. All of the 37 axoneme–MT complexes scored (Table 1) glided on kinesin with the minus end of the complex leading, with a velocity of 48.3 ± 2.2 μm/min (x ± SE), consistent with the plus-end polarity and velocity of kinesin. As a further control, gliding of axoneme–MT complexes on GST/Ncd (GST/MC1)

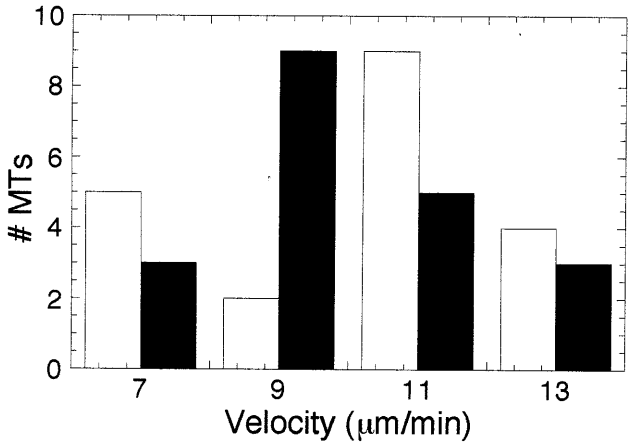

FIG. 2. Velocity of MT gliding on AtKCBP. MTs gliding on GST/AtKCBP bound to a coverslip were tracked at leading and lagging ends using a mouse-controlled cursor overlaid on the video images and specially written software. Velocity data are shown above. The leading end velocity was 10.1 ± 0.5 μm/min (x ± SE, n = 20), and the lagging end velocity was 9.9 ± 0.4 μm/min (x ± SE, n = 20). Open bar, leading end; filled bar, lagging end.

(20) was determined. Ncd has been demonstrated previously to be a minus-end MT motor (26, 27). All 64 of the complexes scored (Table 1) glided with the MT end of the complex leading, with a velocity of 10.0 ± 0.4 μm/min (x ± SE), consistent with the previously reported minus-end polarity and velocity of Ncd.

Calmodulin Inhibits Binding of AtKCBP to MTs. AtKCBP was originally isolated as a calcium-dependent calmodulin-binding protein (1). The region of AtKCBP that binds calmodulin has been mapped to 23 residues that lie to the 3′ side of the conserved motor domain and are present near the C terminus of the GST/AtKCBP fusion protein. The bacterially expressed GST/AtKCBP is unlikely to contain bound calmodulin, because calmodulin is thought to be absent from bacterial cells. Motility assays were carried out to determine the effect of calmodulin on AtKCBP motility.

A bacterial lysate containing induced GST/AtKCBP was preincubated on ice for 2–5 min with purified bovine brain calmodulin in the presence of 550 μM $CaCl_2$, as described in *Materials and Methods*. The total soluble protein in the clarified lysate was ≈20–30 mg/ml using the Bio-Rad protein concentration reagent. The amount of soluble GST/AtKCBP in clarified lysates was estimated to be <1% of the total soluble protein, based on Coomassie blue staining of SDS/polyacrylamide gels. This gives a rough estimate of <3–4 μM GST/AtKCBP in the lysate, assuming an M_r for GST/AtKCBP of 76,300. Based on this estimate, the concentration of GST/AtKCBP in the preincubation mix was <2.4–3.2 μM. The concentration of purified calmodulin in the preincubation mix was 26 μM, assuming an M_r of 16,680 for bovine brain calmodulin (28). The molar concentration of calmodulin in the preincubation mix was therefore in great excess of the molar concentration of GST/AtKCBP.

Coverslips were prepared using taxol-stabilized MTs, Mg·ATP, and GST/AtKCBP preincubated with calcium and calmodulin. The final concentrations of reagents in the coverslip motility assays were approximately 0.24 mM $CaCl_2$, 0.03 μM EGTA, and 11 μM calmodulin, assuming an assay volume of ≈14 μl (6 μl of lysate + calmodulin, 0.8 μl of Mg·ATP, 1 μl of MTs, and ≈6 μl of residual PB from the washes after anti-GST antibody coating of the coverslip).

Assays carried out with motor protein preincubated with calmodulin showed no binding of MTs to the GST/AtKCBP on the coverslip, but numerous MTs could be observed floating in solution. The concentration of $CaCl_2$ in the preincubation

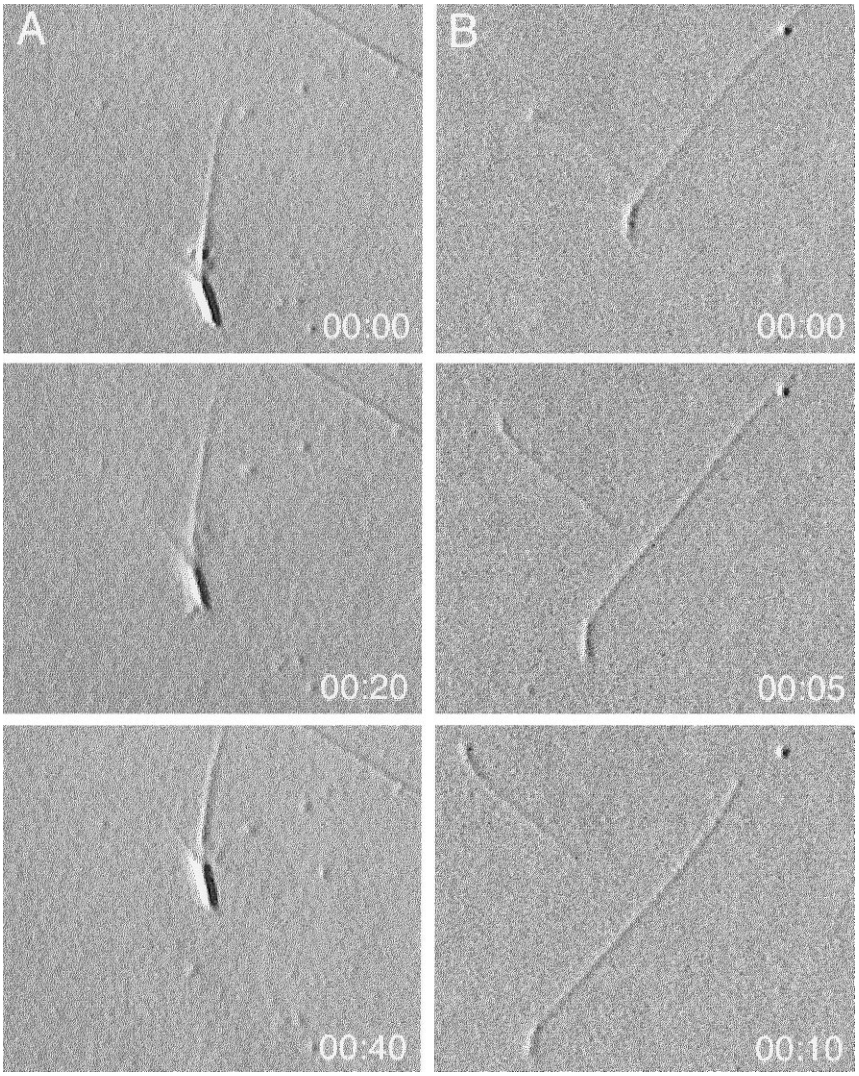

FIG. 3. Axoneme—MT complexes gliding on AtKCBP or kinesin. Shown are time-lapse sequences of axoneme—MT complexes gliding on GST/AtKCBP (*A*) or kinesin (*B*) bound to a coverslip. The time (in minutes and seconds) is indicated at the bottom right of each image. (*A*) The axoneme—MT is gliding on GST/AtKCBP with the MT (plus) end of the complex leading, relative to fixed points on the coverslip. The plus-end movement of the complex indicates that the GST/AtKCBP motor is moving toward the minus end of the MT. (*B*) The axoneme—MT is moving on kinesin with the axoneme (minus) end of the complex leading, consistent with the plus-end translocation of kinesin on MTs. A second axoneme—MT complex is binding to the coverslip at time 00:00 and also glides with the axoneme end leading.

mix was increased to 1 mM or 1.5 mM to ensure binding of calmodulin to GST/AtKCBP, giving final concentrations of ≈0.43 mM and ≈0.64 mM $CaCl_2$ in the assays. The assays containing higher concentrations of $CaCl_2$ again showed no binding of MTs to the coverslip, although many MTs were observed floating in solution. To control for a possible effect of $CaCl_2$ on the binding of MTs to GST/AtKCBP, lysate containing induced GST/AtKCBP was preincubated with 1 mM $CaCl_2$ without calmodulin. The assay showed binding of MTs to the motor on the coverslip and MT gliding with leading and lagging end velocities of 8.0 ± 0.5 μm/min ($x \pm$ SE, n = 8) and 7.6 ± 0.4 μm/min ($x \pm$ SE, n = 8), respectively, comparable to the leading and lagging end velocities of 8.7 ± 0.8 μm/min ($x \pm$ SE, n = 7) and 7.8 ± 0.5 μm/min ($x \pm$ SE, n = 8), respectively, determined in assays without $CaCl_2$ using the same preparation of GST/AtKCBP.

The effect of calmodulin on GST/Ncd (GST/MC1) (20), another minus-end kinesin motor, was tested to determine whether calmodulin affects the ability of GST/Ncd to bind MTs to the coverslip under the same conditions as those for GST/AtKCBP. GST/MC1 was preincubated with calmodulin in the presence of 1 mM $CaCl_2$ and assayed for motility. MTs bound to the GST/MC1 on the coverslip and glided with leading and lagging end velocities of 12.1 ± 0.3 μm/min ($x \pm$ SE, n = 8) and 12.4 ± 0.4 μm/min ($x \pm$ SE, n = 8), respectively, similar to the leading and lagging end velocities of 12.0 ± 0.3 μm/min ($x \pm$ SE, n = 13) and 12.1 ± 0.5 μm/min ($x \pm$ SE, n = 10), respectively, determined in assays without calmodulin or $CaCl_2$, using the same preparation of GST/MC1.

DISCUSSION

AtKCBP, a kinesin-related protein from *Arabidopsis*, was expressed in bacteria as a fusion protein with GST. The GST/AtKCBP fusion protein binds MTs to a coverslip surface and induces gliding of MTs across the glass surface with a velocity of 8–10 μm/min. The polarity of AtKCBP motor movement on MTs was determined to be minus end-directed, in contrast to the plus-end polarity of kinesin movement on MTs. The polarity of AtKCBP is the same as that of Ncd, another kinesin protein that, like AtKCBP, has been classified as a member of the C-terminal motor kinesin subfamily. The velocity of AtKCBP movement on MTs is similar to the 8- to 12-μm/min velocity of Ncd (refs. 20 and 29 and this report).

Table 1. Polarity of AtKCBP translocation on MTs

Motor protein	Movement		
	Plus-end	Minus-end	Velocity, μm/min
Kinesin	37	0	48.3 ± 2.2
GST/Ncd	0	64	10.0 ± 0.4
GST/AtKCBP	0	39	8.1 ± 0.6

Axoneme–MT complexes bound to motor protein attached to glass coverslips were observed using video-enhanced differential interference contrast microscopy. Asymmetrical complexes were scored for polarity of gliding. Plus-end movement refers to axonemes (minus ends) leading; minus-end movement, to MTs (plus ends) leading. The velocity of axoneme–MT gliding was determined by tracking gliding movement of complexes (n = 15 for each motor protein) from videotapes. Assays were carried out as described in *Materials and Methods*. GST/Ncd = GST/MC1.

Unlike Kar3, another member of the C-terminal motor subfamily that is also a minus-end kinesin motor (30), AtKCBP did not show MT shortening in our assays. The ability of Kar3 to destabilize MTs has been demonstrated to be nucleotide-dependent and to occur preferentially at MT minus ends (30), and is consistent with the abnormally long MTs observed in *kar3* mutant cells (31). AtKCBP differs from Kar3 in its ability to destabilize MTs in *in vitro* assays and probably lacks this function *in vivo*.

AtKCBP was originally isolated as a protein that binds calmodulin in the presence of calcium. The region of AtKCBP that binds to calmodulin has been mapped to a 23-residue peptide near the C terminus of the protein, to the 3′ side of the conserved motor domain. Tests of calmodulin for its effect on motility showed that the AtKCBP motor does not bind MTs to the coverslip surface in the presence of calmodulin, and that this effect depends on calmodulin and not on the presence of $CaCl_2$, which is required for binding of calmodulin to AtKCBP. The presence of $CaCl_2$ but not calmodulin in the assay had little or no effect on the ability of the AtKCBP motor to bind MTs to the coverslip surface and support MT gliding across the glass surface.

In contrast to AtKCBP, tests of GST/MC1 showed that the ability of Ncd to bind MTs to the coverslip and translocate MTs across the glass surface was not affected by preincubation of the motor with calmodulin. Calmodulin also does not show specific binding to Ncd in Western blot assays in the presence of Ca^{2+} (data not shown). These observations support the conclusion that Ncd and AtKCBP differ in their ability to bind specifically to calmodulin. The presence of calmodulin plus $CaCl_2$ in the assay did not produce any significant effect on the ability of the Ncd motor to bind to and translocate MTs across the coverslip surface.

Differences in the ability of the AtKCBP motor to destabilize MTs and bind to calmodulin indicate significant differences in the motility properties of AtKCBP compared with Kar3 and Ncd, despite the same polarity of movement on MTs of AtKCBP as Kar3 and Ncd, and the classification of all three motors as members of the C-terminal motor kinesin subfamily. The C-terminal motor subfamily of kinesin proteins is more divergent than the other groups of kinesin proteins by phylogenetic analysis (32), an indication that the *in vivo* functions of the proteins are divergent. Moreover, the AtKCBP motor does not group with the other C-terminal motor *Arabidopsis* kinesin proteins (AtKATA, AtKATB, and AtKATC) within the subfamily (unpublished data). The differences in motility of AtKCBP compared with the other C-terminal motor kinesin proteins that have been characterized, and the sequence differences among the *Arabidopsis* C-terminal motor kinesins, imply that AtKCBP represents a divergent group within the C-terminal motor subfamily.

In addition to Ncd, AtKCBP is likely to differ from other members of the C-terminal motor subfamily in the ability to bind to calmodulin (Fig. 4). Kar3 lacks sequences beyond the conserved motor domain, including the 23-residue peptide corresponding to the AtKCBP calmodulin-binding site. This peptide in AtKCBP lies within a 45-residue region that extends beyond the conserved motor domain, at the C terminus of the protein, and is highly basic, containing seven Arg + Lys residues. Ncd contains 24 residues at the C terminus beyond the conserved motor domain. This region of Ncd differs in sequence from the C terminus of AtKCBP but contains a stretch of highly basic residues. Deletion of the C-terminal 27 residues of Ncd causes the motor to bind weakly to MTs (unpublished data), indicating that the basic amino acids may stabilize interactions of the motor with MTs. If the C terminus of AtKCBP is also involved in motor interactions with MTs, binding of calmodulin might sterically inhibit binding of AtKCBP to MTs. This would explain the inhibition of AtKCBP binding of MTs to the coverslip surface in the presence of calmodulin.

The effect of calmodulin on AtKCBP motility differs from its effect on brush border myosin I, where calmodulin has been demonstrated to be required for motility in *in vitro* assays (5, 6). This difference indicates that calmodulin regulation of AtKCBP motility differs from that of myosin. The binding of calmodulin both to a kinesin heavy chain and to heavy chains of unconventional myosins suggests an evolutionary link between the kinesin and myosin motors. Calmodulin has been previously demonstrated to show Ca^{2+}-dependent binding to the light chains of bovine brain kinesin and to inhibit the ATPase of kinesin upon binding (33), suggesting regulation of kinesin mediated by binding to light chains. However, AtKCBP is the first kinesin-related heavy chain shown to bind specifically to calmodulin.

What is the role of calmodulin in AtKCBP function? Calmodulin has been localized to mitotic spindles in plant cells (11, 12), where it could bind to kinesin spindle motors like AtKCBP. The cellular localization of AtKCBP has not yet been reported, but the ability of calmodulin to inhibit binding of the AtKCBP motor to MTs could be important in regulation of motor activity *in vivo*, acting to prevent binding of the motor to MTs in the cell. The Ca^{2+}-dependence of calmodulin binding to AtKCBP provides a potentially important link to

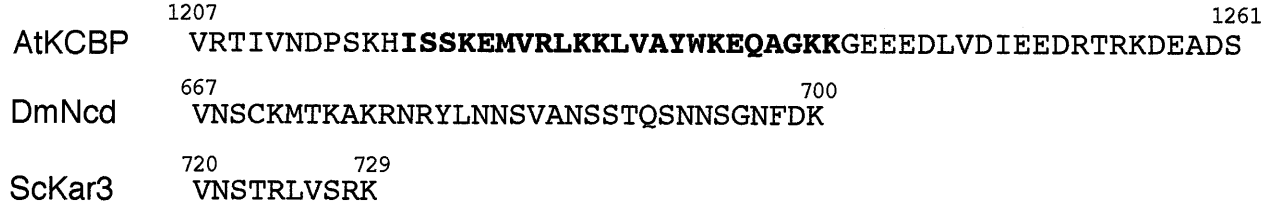

FIG. 4. C-terminal sequences of three minus-end kinesin proteins. The amino acid sequences at the C terminus of the minus-end kinesin motor proteins, *Arabidopsis* KCBP (AtKCBP), *Drosophila* Ncd (DmNcd), and *Saccharomyces* Kar3 (ScKar3) are shown. The 23-aa calmodulin-binding peptide of AtKCBP is shown in boldface type. Ncd contains 24 amino acids beyond the end of the conserved motor domain, but this region differs in sequence from the C terminus of AtKCBP. Kar3 ends at residue 729, at the end of the conserved motor domain.

Ca^{2+}-activated signal transduction pathways. Finally, MT-associated, calmodulin-binding proteins have been proposed to stabilize MTs in living cells (34, 35). The binding of calmodulin to the AtKCBP motor could serve to inhibit binding of the motor to MTs and, at the same time, cause a decrease in MT stability by removing calmodulin from MTs, resulting in MT depolymerization. The net effect would be comparable to the destabilization of MTs by the Kar3 motor, although the mechanisms of MT destabilization by the AtKCBP and Kar3 motors would differ significantly.

AtKCBP is the first plant kinesin for which motility has been demonstrated, although a plus end-directed microtubule gliding activity has been observed in tobacco phragmoplast extracts and attributed to an MT motor (36). The finding that higher plant kinesin proteins such as AtKCBP are MT motors is significant and indicates that the kinesin proteins in plants function in a manner similar to that of animal and fungal species. The similarity in motor function among widely divergent organisms that include plants, animals, and fungi implies a common origin and related functions of the motors in basic cellular processes.

We thank E. Harris for *Chlamydomonas* cell cultures, provided through the Duke University *Chlamydomonas* Stock Center, and A. Means for a generous gift of purified bovine brain calmodulin. The MT tracking and velocity determination program was generously provided by T. Salmon and N. Gliksman. We also thank J. Sellers and K. Collins for clarification of previous work on brush border myosin I. Taxol was obtained from N. R. Lomax (National Cancer Institute). This work was supported by grants from the National Institutes of Health and American Cancer Society to S.A.E. and a grant from the National Science Foundation to A.S.N.R.

1. Reddy, A. S. N., Safadi, F., Narasimhulu, S. B., Golovkin, M. & Hu, X. (1996) *J. Biol. Chem.* **271,** 7052–7060.
2. Reddy, A. S. N., Narasimhulu, S. B., Safadi, F. & Golovkin, M. (1996) *Plant J.* **10,** 9–21.
3. Wang, W., Takezawa, D., Narasimhulu, S. B., Reddy, A. S. N. & Poovaiah, B. W. (1996) *Plant Mol. Biol.* **31,** 87–100.
4. Collins, K. & Matsudaira, P. (1991) *J. Cell Sci. Suppl.* **14,** 11–16.
5. Collins, K., Sellers, J. R. & Matsudaira, P. (1990) *J. Cell Biol.* **110,** 1137–1147.
6. Wolenski, J. S., Hayden, S. M., Forscher, P. & Mooseker, M. S. (1993) *J. Cell Biol.* **122,** 613–621.
7. Wolenski, J. S. (1995) *Trends Cell Biol.* **5,** 310–316.
8. Cheney, R. E. & Mooseker, M. S. (1992) *Curr. Opin. Cell Biol.* **4,** 27–35.
9. Roberts, D. M. & Harmon, A. (1992) *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **43,** 375–414.
10. Poovaiah, B. W. & Reddy, A. S. N. (1987) *CRC Crit. Rev. Plant Sci.* **6,** 47–103.
11. Vantard, M., Lambert, A. M., Mey, J. D., Picquot, P. & Van Eldik, L. J. (1985) *J. Cell Biol.* **101,** 488–499.
12. Wick, S. M., Muto, S. & Duniec, J. (1985) *Protoplasma* **126,** 198–206.
13. Cyr, R. J. (1991) *J. Cell Sci.* **100,** 311–317.
14. Durso, N. A. & Cyr, R. J. (1994) *Plant Cell* **6,** 893–905.
15. Mitsui, H., Yamaguchi-Shinozaki, K., Shinozaki, K., Nishikawa, K. & Takahashi, H. (1993) *Mol. Gen. Genet.* **238,** 362–368.
16. Mitsui, H., Nakatani, K., Yamaguchi-Shinozaki, K., Shinozaki, K., Nishikawa, K. & Takahashi, H. (1994) *Plant Mol. Biol.* **25,** 865–876.
17. Smith, D. B. & Johnson, K. S. (1988) *Gene* **67,** 31–40.
18. Studier, F. W., Rosenberg, A. H., Dunn, J. J. & Dubendorff, J. W. (1990) *Methods Enzymol.* **185,** 60–89.
19. Sawin, K. E., Mitchison, T. J. & Wordeman, L. G. (1992) *J. Cell Sci.* **101,** 303–313.
20. Chandra, R., Salmon, E. D., Erickson, H. P., Lockhart, A. & Endow, S. A. (1993) *J. Biol. Chem.* **268,** 9005–9013.
21. Chandra, R. & Endow, S. A. (1993) *Methods Cell Biol.* **39,** 115–127.
22. Allen, C. & Borisy, G. G. (1974) *J. Mol. Biol.* **90,** 381–402.
23. Williams, R. C., Jr. & Lee, J. C. (1982) *Methods Enzymol.* **85,** 376–385.
24. Hyman, A., Drechsel, D., Kellogg, D., Salser, S., Sawin, K., Steffen, P., Wordeman, L. & Mitchison, T. (1991) *Methods Enzymol.* **196,** 478–485.
25. Walker, R. A., Gliksman, N. R. & Salmon, E. D. (1990) in *Optical Microscopy for Biology*, eds. Herman, B. & Jacobson, K. (Wiley–Liss, New York), pp. 395–407.
26. Walker, R. A., Salmon, E. D. & Endow, S. A. (1990) *Nature (London)* **347,** 780–782.
27. McDonald, H. B., Stewart, R. J. & Goldstein, L. S. B. (1990) *Cell* **63,** 1159–1165.
28. Watterson, M., Sharief, F. & Vanaman, T. (1980) *J. Biol. Chem.* **255,** 962–975.
29. Moore, J. D., Song, H. & Endow, S. A. (1996) *EMBO J.* **15,** 3306–3314.
30. Endow, S. A., Kang, S. J., Satterwhite, L. L., Rose, M. D., Skeen, V. P. & Salmon, E. D. (1994) *EMBO J.* **13,** 2708–2713.
31. Meluh, P. B. & Rose, M. D. (1990) *Cell* **60,** 1029–1041.
32. Moore, J. D. & Endow, S. A. (1996) *BioEssays* **18,** 207–219.
33. Matthies, H. J. G., Miller, R. J. & Palfrey, H. C. (1993) *J. Biol. Chem.* **268,** 11176–11187.
34. Pirollet, F., Rauch, C. T., Job, D. & Margolis, R. L. (1989) *Biochemistry* **28,** 835–842.
35. Pirollet, F., Derancourt, J., Haiech, J., Job, D. & Margolis, R. L. (1992) *Biochemistry* **31,** 8849–8855.
36. Asada, T. & Shibaoka, H. (1994) *J. Cell Sci.* **107,** 2249–2257.