Phosphotransferases Associated with the Regulation of Kinesin Motor Activity*

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Lisa Lindesmith‡, James M. McIlvain, Jr.§, Yair Argon§, and Michael P. Sheetz¶

From the ¶Department of Cell Biology, Duke University Medical Center, Durham, North Carolina 27710 and §Department of Pathology, University of Chicago, Chicago, Illinois 60637

Kinesin, a plus-end-directed microtubule motor protein, functions in concert with accessory factors that have been shown to regulate enzyme activity and may also provide cargo specificity. This report identifies the 79-kDa kinesin-associated phosphoprotein as a phosphoisoform of kinesin light chain. Increased phosphorylation of this light chain isoform is sufficient to account for the increase in kinesin-mediated microtubule-gliding activity. Additionally, it was found that the degree of phosphorylation of this isoform is regulated by a 100-kDa kinase and 150-kDa type 1 phosphatase. Both the kinesin light chain kinase and phosphatase co-purify with the kinesin heavy chain, suggesting that kinesin exists in a large complex capable of self-regulation.

The microtubule (MT) motor protein kinesin plays an important role in intracellular motility because it moves membranous organelles and vesicles toward the plus end of MTs in many cell types (for reviews see Refs. 1–3). Kinesin is a tetramer composed of two heavy chains and two light chains (4). Each heavy chain can be divided into three regions (5). The N-terminal head domain is globular and contains the ATPase and MT-binding activities characteristic of kinesin. The central region forms an extended coiled coil with a second heavy chain molecule, forming a stalk. The C terminus again forms globular domains, around which the two light chains coil producing the fully functional molecule. Since both the ATPase activity and MT-binding activity are located within the heavy chain, a kinesin heavy chain dimer has in vitro motor activity (6). The association of the two light chains with the heavy chains may confer cargo specificity to kinesin in vivo.

Although the function and structure of kinesin are well characterized, little is known about its regulation. Since phosphorylation is important in the regulation of the myosin motor proteins (7), it is also expected to play a role in the regulation of the MT motor proteins. Indeed, a growing body of evidence suggests that this is the case. Kinesin heavy and light chains can be phosphorylated in vitro by purified protein kinase C and protein kinase A (8, 9), but these kinases are not likely to phosphorylate kinesin in vivo (10). Cytoplasmic dynein and kinesin have both been shown to exist as phosphoisoforms in vitro (11, 12), with both kinesin heavy chain (KHC) and kinesin light chain (KLC) shown to be phosphorylated on multiple sites (12). Increased phosphorylation of cytoplasmic dynein correlates with dissociation from lysosomal vesicles (11). In contrast, the membrane-bound form of kinesin has a higher state of phosphorylation than soluble kinesin (12). In vivo studies by Hamm-Alvarez et al. (13) have shown that treatment of serum-starved CV-1 cells in culture with the phosphatase (PPase) inhibitor okadaic acid (OA) resulted in an increase in the number of vesicle movements within the cells. McIlvain et al. (14) found that treatment of the MT-depleted cytosol with OA before purification of the motor proteins resulted in a 2-fold increase in kinesin motor activity in an in vitro MT-gliding assay. Further, they were able to show that this treatment resulted in increased phosphorylation of three kinesin-associated proteins, a 150-, 79-, and 73-kDa kinesin-associated phosphoprotein (KAPP). These data provided the first direct correlation between phosphorylation of particular proteins and kinesin motor activity.

Here we demonstrate that the 79-kDa KAPP is a kinesin light chain isoform (KLC79) and that phosphorylation of KLC alone is sufficient to reproduce the increased activity described previously. We find that the phosphorylation state of KLC is mediated by a 100-kDa kinase and a 150-kDa type 1 PPase, both of which co-purify with KHC. Together with the other data, the present results suggest that kinesin exists as a complex consisting of at least KHC, KLC, KLC kinase, and KLC PPase.

EXPERIMENTAL PROCEDURES

Cell Culture—The murine T hybridoma cell line 2B23.18 was grown in Dulbecco’s modified Eagle’s medium with high glucose and 5% fetal calf serum. The human myelocytic leukemia cell line HL-60 was cultured in RPMI 1640 and 10% fetal calf serum. Chick embryo brains were collected from 12-day-old chick embryos. All media were supplemented with 50 units/ml penicillin and 50 μg/ml streptomycin. All cell culture reagents were purchased from Life Technologies, Inc.

Preparation of Cytosol—Cytosolic fractions were prepared as described previously for cell lines (14) and chick embryo brains (15), except endogenous MTs were resuspended in 1 μM taxol, diluted to 0.5 μM KCl with PMEE/20 μM taxol and pelleted at 100,000 × g, 15 min, 21 °C before being used for MT affinity.

Immunoprecipitation—The MT-depleted cytosol was incubated with protein A-agarose beads (Pierce) for 1 h before addition of antibody. After removing the preclearing beads, antibody was added and the samples left on ice for 1 h. Fresh protein A-agarose beads were added, and the samples left on a rotator at 4 °C overnight. The beads were then washed repeatedly in PMEE before being used in an assay. The bead-coupled immune complexes were then treated with either buffer or
inhibitors before the addition of [γ-32P]ATP. After the phosphorylation reactions were complete, the beads were washed, and the immune complexes released by boiling in SDS-PAGE sample buffer. The kinase-associated proteins were separated by SDS-PAGE, and the phosphorylated proteins were visualized by autoradiography.

Treatment with Ovidac Acid, Inhibitor-2, Staurosporine, and [γ-32P]ATP—Samples were treated with either of the PPase inhibitors, OA (LC Services, Woburn, MA) or inhibitor-2 (I-2) (generously provided by David Brautigan, University of Virginia, Charlottesville), or the kinase inhibitor staurosporine (LC Services) for at least 15 min before the phosphorylation reactions were initiated by the addition of [γ-32P]ATP. The reactions were incubated at 37 °C with 3,000 Ci/ml [γ-32P]ATP (Amersham Corp.) in the presence or absence of inhibitors. Motor fraction phosphotubulbin was at a concentration of 200 μCl/ml. Bead-bound KHC immunoprecipitates were adjusted to a volume equal to the preimmunoprecipitation cytosol with PMEE and incubated with 10 μCl/ml [γ-32P]ATP.

**Purification of Microtubule Motor Proteins**—MT motors were purified by a MT affinity/ATP elution protocol as described before (14) with an additional step. Actin and myosin were removed by the addition of 9 mg/ml glucose and 10 units/ml hexokinase (Boehringer Mannheim) to the MT-depleted cytosol. The samples were incubated at room temperature until a white flocculate precipitate developed and then centrifuged at 100,000 × g, for 30 min at 21 °C to pellet the complex before processing with the MT affinity protocol.

**MT-gliding Assay**—MT-gliding assays were performed as described previously (14). Briefly, microtubules were polymerized by diluting 4 volumes of 3–3.5 mM/ml purified porcine brain tubulin with 1 volume of PMEE containing 5 mM GTP and 100 μm taxol and incubated at 37 °C for 15 min. A flow chamber was constructed using acid-washed coverslips overlaid on two strips of double stick tape on a standard microscope slide. Tape strips were spaced 2–3 mm apart, forming a chamber of 5–10 μl. The chamber was filled with 1 volume of motor fraction, incubated for 5 min at 24 °C, and washed with 3 volumes of 150 μg/ml casein (60% α, 40% β, from bovine milk (Sigma)) in PMEE. Where indicated, the motor fraction was preincubated with buffer or 125 mM OA before addition to the slide chamber, as described previously (14). Two volumes of microtubule/nucleotide mixture containing 20 μg/ml of polymerized microtubule stock (sheared by trituration to give an average microtubule length of 3–5 μm), 10 mM ATP, 150 μg/ml casein, 10 mM creatine phosphate (Boehringer Mannheim), and 80 μg/ml creatine kinase (Boehringer Mannheim) in PMEE were then flowed into the chamber and incubated for 5 min before visualization by video-enhanced differential interference microscopy. The gliding assays were done in excess limiting dilutions of the motor fraction in the presence of excess microtubules and analyzed as described previously (14).

**Embedded Kinase Renaturation Assay**—The kinase was identified by its ability to autophsorylate under renaturing conditions while embedded within an SDS-PAGE gel matrix. MT affinity-purified motors were separated by SDS-PAGE, and the gels were washed in 20% isopropanol in 0.05 M Tris, pH 8.0, 4 mM dithiothreitol (buffer A) before addition to the slide chamber, as described previously (14). Two volumes of microtubule/nucleotide mixture containing 20 μg/ml of polymerized microtubule stock (sheared by trituration to give an average microtubule length of 3–5 μm), 10 mM ATP, 150 μg/ml casein, 10 mM creatine phosphate (Boehringer Mannheim), and 80 μg/ml creatine kinase (Boehringer Mannheim) in PMEE were then flowed into the chamber and incubated for 5 min before visualization by video-enhanced differential interference microscopy. The gliding assays were done in excess limiting dilutions of the motor fraction in the presence of excess microtubules and analyzed as described previously (14).

**Phosphorylation of Kinesin Light Chain**—Using CKLC.2.7F8 (15) were previously produced in our laboratory. The mono- and polyclonal antibodys were then used to identify the components of the immune complex. The monoclonal anti-KHC antibodies CKLC.3.9C1 (12, 17) and CKLC.2.7F8 (15) were previously produced in our laboratory. Using CKLC.3.9C1 only the 70-kDa predominant isofrom of KHC was seen by Coomassie staining of the gel, and [32P]ATP was added to the bead-coupled immune complex incubated with [γ-32P]ATP. As expected, the major components of the SUK4 immune complex were KHC and KLC (Fig. 1). Immunoblotting with a series of antibodies was then used to identify the components of the immune complex. The monoclonal anti-KHC antibodies CKLC.3.9C1 (12, 17) and CKLC.2.7F8 (15) were previously produced in our laboratory. Using CKLC.3.9C1 only the 70-kDa predominant isofrom of KLC, as seen by Coomassie staining of the gel, was detected. However, CKLC.2.7F8 cross-reacted with the predominant 70-kDa KLC and an additional isoform of KLC with an apparent molecular mass of 79 kDa. Autoradiography of the blots showed that this 79-kDa KLC was the only phosphoprotein within the KHC immune complex. This conclusion is supported by studies that used bovine anti-KLC antibodys.2 These results identify the 79-kDa KAPP as a phosphoisoform of KLC.

**Hyperphosphorylation of KLC Increases Kinesin Motor Activity**—Several KAPPs are present in the purified motor fraction from 2B23.18 cells (14). To determine the effect of phosphorylation of KLC79 on kinesin activity, motor proteins were pre- pared from 2B23.18 cells by a MT affinity protocol and incubated with [γ-32P]ATP in the presence or absence of OA. The 79-kDa KLC was the only protein found to be consistently phosphorylated under these conditions (Fig. 2A). The 150- and 73-kDa KAPPs were not observed. To determine if increased

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**RESULTS**

The 79-kDa KAPP Is Kinesin Light Chain—Our previously reported correlation between increased phosphorylation of the KAPPs and increased kinesin motor activity suggested that phosphorylation of these proteins plays an important regulatory role. Therefore, the identification of these proteins would lead to a better understanding of kinesin function. Since the 79-kDa KAPP falls within the range of predicted molecular weights for chick KLC, we began by evaluating the isoforms of KLC found in chick embryo brains. The MT-depleted cytosol was immunoprecipitated with the anti-KHC monoclonal antibody SUK4 (16) and the bead-coupled immune complex incubated with [γ-32P]ATP. As expected, the major components of the SUK4 immune complex were KHC and KLC (Fig. 1). Immunoblotting with a series of antibodies was then used to identify the components of the immune complex. The monoclonal anti-KHC antibodies CKLC.3.9C1 (12, 17) and CKLC.2.7F8 (15) were previously produced in our laboratory. Using CKLC.3.9C1 only the 70-kDa predominant isofrom of KLC, as seen by Coomassie staining of the gel, was detected. However, CKLC.2.7F8 cross-reacted with the predominant 70-kDa KLC and an additional isoform of KLC with an apparent molecular mass of 79 kDa. Autoradiography of the blots showed that this 79-kDa KLC was the only phosphoprotein within the KHC immune complex. This conclusion is supported by studies that used bovine anti-KLC antibodys.2 These results identify the 79-kDa KAPP as a phosphoisoform of KLC.

**Sizing of the PPase**—To determine the size of the PPase, MT affinity-purified motor proteins were applied to a Superose 6 gel filtration column (Pharmacia Biotech Inc.). The column was developed at 0.5 ml/min with 30 ml PMEE, 0.1% 2-mercaptoethanol, 5% glycerol, and 0.5 ml fractions collected. An elution volume standard curve was constructed using proteins of known molecular weight, and the molecular weight of the PPase determined by comparison of the elution volume of the peak of PPase activity with this curve.

**Analysis of Phosphorimages—Phosphotubulbin was analyzed using a Fuji Fujix BAS1000 phosphorimaging system (Fuji, Stamford, CT). A Fuji imaging plate was exposed to a dried radiolabeled gel inside an Amersham film cassette. After exposure, the imaging plate was scanned by the BAS1000 and converted into digital form. Analysis of the image was done using Fuji Fujix MacBas software for the BAS1000.**

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2 J. M. McIlvain, Jr., unpublished results.
Fig. 2. Hyperphosphorylation of KLC increases kinesin motor activity. A, MT affinity-purified motors were treated with a buffer or 125 nM OA before the addition of [γ-32P]ATP to initiate the phosphorylation reaction. The proteins were then separated by SDS-PAGE, and the phosphorylated proteins were visualized by autoradiography. B, motor fractions were treated with buffer or 125 mM okadaic acid before being absorbed onto acid-washed coverslips. After absorption, taxol-stabilized microtubules and 10 mM ATP were flowed into the slide chamber. The number of microtubules bound and gliding was visualized by video-enhanced differential interference microscopy. The error bars represent the S.E.

phosphorylation of KLC alone was sufficient to stimulate kinesin motor activity, the purified and phosphorylated motor fraction from 2B23.18 cells was assayed for the ability to support MT gliding in vitro. Compared with untreated samples, twice as many microtubules were observed to be bound or gliding in the samples treated with OA (Fig. 2B). Since the same relation between enhanced phosphorylation of KLC79 and kinesin motor activity was seen as when the 150- and 79-kDa KAPPs were hyperphosphorylated (14), we conclude that phosphorylation of KLC79 alone is sufficient for regulating kinesin motor activity as measured in the MT-gliding assay.

A 100-kDa Kinase Activity Associates with Purified Kinesin—The response of KLC79 in the purified motor fraction (Fig. 2A) suggested that the KLC kinase and PPase remain associated with the motors during MT affinity purification. To further investigate the KLC kinase activity, proteins of purified motor fractions were separated by SDS-PAGE and assayed for their ability to renature and autophosphorylate within an acrylamide gel matrix. Under these conditions, one phosphoprotein was seen at 100 kDa. To verify that this 100-kDa phosphoprotein represented an autophosphorylating kinase and not an ATP-binding protein, the renatured gel was pretreated with the kinase inhibitor staurosporine at 100 nM before the addition of [γ-32P]ATP. The observed inhibition of phosphorylation confirms that the protein is a kinase (Fig. 3) and makes it a likely candidate for the KLC kinase. Using this technique, a similar 100-kDa putative kinase was found in different species and cell types, including the mouse hybridoma 2B23.18 and the human myelocytic leukemia line HL-60 (data not shown) as well as chick embryo brains (Fig. 3).

A 150-kDa PPase Associates with Purified Kinesin—The enhanced phosphorylation of KLC79 upon OA treatment of the MT-affinity-purified motors (Fig. 2A) indicates that either a type 1 or type 2A Ser/Thr PPase dephosphorylates the protein.

Fig. 2. A 100-kDa kinase co-purifies with the motor proteins. Kinesin purified by MT affinity was further isolated by sucrose gradient density sedimentation, and the proteins were separated by SDS-PAGE. The kinesin-associated proteins were assayed for ability to autophosphorylate under renaturing conditions while embedded within an acrylamide gel matrix in the presence or absence of the kinase inhibitor staurosporine (STR).

PP1 and PP2A activities can be distinguished from each other based on their sensitivity to OA. The PP2A family is inhibited at 1–10 nanomolar concentrations of OA, whereas the PP1 family remains active at concentrations 100-fold higher (18). To determine if a PP1 or PP2A was responsible for dephosphorylation of KLC79, the MT affinity-purified motors from 2B23.18 and HL-60 cells were used to dephosphorylate [32P]phosphorylase α, a well-characterized substrate for PP1 and PP2A PPases. When compared with the buffer controls, little change in the amount of PPase activity was observed upon addition of 5 mM OA to the reaction mixture, whereas the PPase activity was significantly inhibited in the presence of 500 mM OA. This result indicates that a PP1 phosphatase was present in the motor fraction (Fig. 4A).

To further characterize the PPase, the MT affinity-purified motors were fractionated by a Superose 6 gel filtration column (Fig. 4B), and the resulting fractions were assayed for the ability to dephosphorylate [32P]phosphorylase α. Two peaks of PPase activity were identified, corresponding to molecular masses of approximately 150- and 80-kDa. The first peak is the PPase holoenzyme, while the second peak co-eluted with a commercial PP1 catalytic subunit, representing a dimer of the PPase catalytic subunit freed during enzyme purification (19). Similar peaks of activity were also identified from gel filtration analysis of motor proteins purified from HL-60 cells (data not shown).

To confirm that the single PPase identified in the bulk motor fraction was a PP1 enzyme, biochemical classification studies were done on the PPase holoenzyme and catalytic subunit isolated from the 2B23.18 cells. MT affinity-purified motors were separated by velocity sedimentation through a 5–20% linear sucrose gradient, and each fraction was assayed for its ability to dephosphorylate [32P]phosphorylase α (Fig. 5A). The fractions corresponding to the holoenzyme (which co-sedimented with kinesin) and the catalytic subunit were incubated with OA (Fig. 5B) or I-2 (Fig. 5C) before being analyzed for PPase activity. I-2 is a PP1-specific inhibitor. It does not inhibit PP2A even at micromolar concentrations. The PPase holoenzyme was not affected by 5 mM OA, showed little activity after incubation with 125 mM OA, and was inhibited by I-2 at nanomolar concentrations. The PPase catalytic subunit was slightly more sensitive to the inhibitors, but exhibited the same overall characteristics as the holoenzyme. In contrast, a commercial PP2A catalytic subunit was completely inhibited at 5 mM OA but was not effected by 200 mM I-2 (data not shown), which confirms that the 150-kDa motor-associated PPase is a member of the PP1 family.

Kinesin Exists in a Large Complex Containing KHC, KLC,
KLC kinase, and KLC PPase—The antibody SUK4 binds kinesin heavy chain near the motor domain (16) and immunoprecipitates a complex of proteins including KHC, KLC, and other accessory factors needed for kinesin motility in vitro (20). To determine if the KLC kinase and PPase are among these accessory proteins, the SUK4 immune complex from 2B23.18 cells was incubated with $^32$P-ATP in the presence or absence of phosphotransferase inhibitors. Without treatment with inhibitors, the two major proteins that were consistently phosphorylated within the kinesin heavy chain immune complex of these cells were the 150-kDa KAPP and KLC79 (Fig. 6) (as previously reported (14)), which confirms that the KLC kinase is kinesin associated.

To evaluate the effect of kinase inhibition on the phosphorylation of KLC79, the SUK4 immune complex was pretreated with 100 nM staurosporine before incubation with $^32$P-ATP. Under these conditions phosphorylation of KLC79 was eliminated (Fig. 6A). These data are in agreement with the autophosphorylation inhibition of the kinase shown in Fig. 3 and further suggest that the 100-kDa kinase present in the MT affinity-purified motor fraction phosphorylates KLC within the kinesin complex.

In addition, pretreatment of the kinesin heavy chain immune complex with 500 nM OA before incubation with $^32$P-ATP increased phosphate incorporation into KLC (Fig. 6B) and demonstrated that the KLC PPase is active within the kinesin complex.

DISCUSSION

Identification of the kinesin-associated phosphoproteins and the phosphotransferases that regulate them may elucidate the control of vesicle motility mediated by the motor proteins. Our original work describing the KAPPs (14) and later work on

![Fig. 4. A 150-kDa PPase co-purifies with the motor proteins. A, MT affinity-purified motors from 2B23.18 (■) and HL-60 cells (□) were incubated with $^32$P-phosphorylase a in the presence of OA. Trichloroacetic acid was added to stop the reactions and precipitate the proteins. PPase activity was equated to the amount of $^32$P released from the substrate by PPase activity found in the trichloroacetic acid-soluble phase. B, MT affinity-purified motor proteins from 2B23.18 cells (●) and a commercial PP1 catalytic subunit (○) were analyzed by gel filtration through a Superose 6 sizing column. The resulting fractions were assayed for PPase activity. The arrow denotes the elution position of alcohol dehydrogenase (apparent molecular mass, 150 kDa).](image)

![Fig. 5. The motor-associated PPase is a type 1 enzyme. MT affinity-purified motor proteins from 2B23.18 cells (●) and a commercial PP1 catalytic subunit (○) were analyzed by velocity sedimentation through 5% (last fraction) to 20% (first fraction) linear sucrose gradients (panel A). Fractions were assayed for PPase activity. The peak fractions corresponding to the motor-associated PPase holoenzyme and the motor-associated PPase catalytic subunit were then assayed for PPase activity in the presence of increasing concentrations of OA (panel B) or the PP1-specific inhibitor I-2 (panel C).](image)

![Fig. 6. Kinesin exists in a large complex consisting of at least KHC, KLC, KLC kinase, and KLC PPase. The MT-depleted cytosol from 2B23.18 cells was immunoprecipitated with the anti-kinesin heavy chain antibody SUK4, and the immune complex was left coupled to the Protein A beads during phosphorylation reactions in the presence of the broad-spectrum kinase inhibitor 100 nM staurosporine (STR) (panel A) or 500 nM OA (panel B). KLC (12) suggested that the 79-kDa KAPP was not KLC. This conclusion was based primarily on results in both laboratories from Western blots using the CKLC.3.9C1 antibody. However,](image)
our current work characterizes CKLC.3.9C1 as cross-reactive with only the predominant isoform of KLC and explains why Lee and Hollenbeck (12) did not observe cross-reactivity of the 70–80-kDa KAPPs with CKLC.3.9C1 in their studies. An alternative anti-KLC monoclonal antibody (CKLC.2.7F8) made this clarification possible because it cross-reacted with multiple isoforms, including the 79-kDa KAPP of KLC in multiple species, making it a particularly useful tool in our studies on phosphoisoforms of KLC.

KLCs are suspected to be involved in cargo selection. They are associated with the KHC fan-shaped tail and therefore are located at the proposed vesicle docking site (5). This strongly suggests that the multiple isoforms of KLC provide cargo specificity. An OA-induced increased phosphorylation state of the KLC, represented by KLC79, may resemble the membrane-bound form of KLC and thus activate kinesin motor activity. This idea is consistent with data showing that the more highly phosphorylated forms of kinesin are vesicle-bound, whereas less phosphorylated forms are soluble (12).

The experiments that led to the identification of the KAPPs and the correlation between KAPP phosphorylation and kinesin activity (14) relied on cytosolic phosphorylation of the activating proteins. Here we demonstrate that further purification did not result in a loss of hyperphosphorylation activity. In fact, we were still able to obtain the same level of activation as observed in the previous report under conditions where only KLC was consistently phosphorylated.

The level of phosphorylation of KLC is controlled by two phosphotransferases that can be found in association with the kinesin molecule. The kinase is at present uncharacterized, but its size and sensitivity to specific kinase inhibitors indicates that it may be a novel enzyme. The phosphatase has been characterized to be a type 1 Ser/Thr PPase. Other type 1 PPases have been implicated in the regulation of both cytoplasmic dynein (21) and the nonmuscle myosin motors (7). Although the apparent molecular weight of the motor-associated PPI is similar to the 150-kDa KAPP, they are different proteins, since the PPI size was estimated for the native holoenzyme, whereas the KAPP size was derived from SDS-PAGE analysis. Work is underway to define which of the three subsets of PPI is the relevant KLC phosphatase.

The present data show that phosphorylation of the 150-kDa KAPP is not essential for the enhancement of kinesin activity in the MT-gliding assay. However, it is associated with kinesin through various purification steps in several cell lines that we have examined and thus is likely to play a regulatory role that is not measured in the in vitro MT-gliding assay. For example, the 150-kDa KAPP may be important in maintaining the kinesin motor complex together in membrane association of the kinesin complex or in another step of in vivo vesicle motility, since highly purified kinesin is unable to transport vesicles in vitro (22). We are currently investigating the parameters that define the association of the 150-kDa KAPP with kinesin as well as regulation of its phosphorylation state in different kinesin preparations.

The 100-kDa kinase was the only kinase activity detected in the MT affinity-purified motor preparation, and the KLC was the only phosphoprotein detected when the MT affinity-purified motors were incubated with \(^{32}\)P-ATP. This suggests that the 100-kDa kinase is a KLC kinase. This hypothesis is supported by similar results obtained when a different method, immunoprecipitation, was used in the purification. These data suggest that autophosphorylation activates the 100-kDa kinase, which then results in phosphorylation of the KLC and subsequent activation of kinesin motor activity. To unequivocally identify the 100-kDa kinase and 150-kDa PPase as KLC-specific, all of the components will have to be purified to homogeneity. Nonetheless, the work presented here and in a previous paper (14) suggests that the active kinesin motor is an assembly of a number of proteins, including kinesin heavy and light chains, a kinase, and a phosphatase.

We consider it likely that phosphorylation of kinesin accessory factors is one step in a cascade of signal transduction that regulates kinesin activity and anterograde organelle movement. Our data support this by demonstrating that the phosphorylation level of KLC is modulated by a kinesin-associated kinase and PPase, and the level of KLC phosphorylation directly correlates with activation of kinesin motor activity. Future studies will address how the activities of the KLC kinase and PPase are controlled in turn.

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