Purification and Characterization of Native Conventional Kinesin, HSET, and CENP-E from Mitotic HeLa Cells*

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We have developed a strategy for the purification of native microtubule motor proteins from mitotic HeLa cells and describe here the purification and characterization of human conventional kinesin and two human kinesin-related proteins, HSET and CENP-E. We found that the 120-kDa HeLa cell conventional kinesin is an active motor that induces microtubule gliding at ~30 μm/min at room temperature. This active form of HeLa cell kinesin does not contain light chains, although light chains were detected in other fractions. HSET, a member of the C-terminal kinesin subfamily, was also purified in native form for the first time, and the protein migrates as a single band at ~75 kDa. The purified HSET is an active motor that induces microtubule gliding at a rate of ~5 μm/min, and microtubules glide for an average of 3 μm before ceasing movement. Finally, we purified native CENP-E, a kinesin-related protein that has been implicated in chromosome congression during mitosis, and we found that this form of CENP-E does not induce microtubule gliding but is able to bind to microtubules.

Conventional kinesin and kinesin-related proteins (KRPs) constitute a rapidly expanding superfamily of microtubule-associated motor proteins that perform a variety of cellular functions (1, 2). Conventional kinesin was originally identified as a fast moving (~30 μm/min) microtubule-based motor in squid giant axons (3), and it is believed to function as a heterotetramer consisting of two heavy chains and two light chains (reviewed in Ref. 4). It has since been identified in virtually all cell types and has multiple roles in vesicle trafficking (5–9); however, it is not yet clear if conventional kinesin has a role in animal cell mitosis. Microinjection of antibodies specific to kinesin heavy chain had no effect on mitotic progression of early sea urchin embryos (10), and no mitotic defects in kinesin heavy chain mutants have been detected in Drosophila melanogaster (11). However, severe defects in early mitoses were found in C. elegans kinesin heavy chain mutants (12). In contrast to conventional kinesin, many KRPs are known to have mitotic functions, including roles in spindle formation, spindle maintenance, and chromosome movement. These mitotic KRPs include members of the BimC subfamily, the C-terminal subfamily, the MKLP1 subfamily, chromokinesins, and others (reviewed in Refs. 13 and 14).

More than 200 kinesins and KRPs have been identified and catalogued. Whereas most of the biochemical characterization performed on these motors has been on recombinantly expressed protein or protein fragments, the characterizations of the biochemical properties of kinesins and KRPs in their native forms from natural host cells have been limited. In the case of conventional kinesin, which has been the most extensively studied kinesin in native form, the cell types from which it has been isolated and thoroughly studied are restricted for the most part to brain tissue and early embryonic cells (reviewed in Ref. 4). Furthermore, only two KRPs have been isolated from natural host cells. These are KRP85/95 and KRP130, both of which were purified from embryonic cells and found to exist in multimeric complexes (16, 17).

In order to study human kinesins and KRPs in their native forms, we have developed a method for the purification of microtubule motor proteins from HeLa cells. We were especially interested in mitotic kinesins; thus, we used cells that were blocked in the mitotic stage of the cell cycle. In this study, we describe a method for the large scale purification of native kinesin and KRPs and focus on the purification of three motors: conventional kinesin, HSET (human spleen, embryo, testes; Ref. 18), and CENP-E (centromere protein-E). We found that the active form of purified HeLa cell kinesin lacks light chains and moves microtubules at ~30 μm/min. In addition, we purified the KRP HSET, a 75-kDa protein that moves microtubules at a rate of ~5 μm/min. Finally, we purified the native form of the mitotic KRP, CENP-E, and found that although it binds to microtubules, it apparently lacks microtubule gliding activity.

EXPERIMENTAL PROCEDURES

Large Scale Culturing and Harvesting of HeLa Cells—HeLa S3 cells were grown in suspension in 20-liter carboys maintained on large spinner plates at 37 °C in half Ham’s F-12 medium and half Dulbecco’s modified Eagle’s medium supplemented with modified Eagle’s medium-nonessential amino acids, penicillin (5000 units/ml), streptomycin (5 mg/ml), 10 mM Hepes, 1.5 g/liter glucose, and 2% iron-rich calf serum. Asynchronously dividing cells were collected from exponentially growing cultures after reaching a density of ~106 cells/ml (~4% of the cells were in mitosis, and 96% were in interphase). A highly enriched mitotic

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1 The abbreviations used are: KRP, kinesin-related protein; CENP-E, centromere protein-E; HSET, human spleen, embryo, and testes expressed (also previously referred to as Tctex7 and Knsl2); AMPPPNP, 5′-adenylimidodiphosphate; Pipes, 1,4-piperazinediethanesulfonic acid; NEM, N-ethylmaleimide; HSS, high speed supernatant; ATPγS, adenosine 5′-O-(thiotriphosphate).

2 E. A. Greene and S. Henikoff, The Kinesin Home Page at www.blocks.fhcrc.org/∼kinesin.
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细胞总数（90%的细胞处于分裂期）通过与12 mM vinblastine硫酸盐2 h（每10个细胞的完整细胞周期）进行培养而获得。

Purification of Motors—Approximately 70 liters of cells from several carboys were harvested using a continuous flow rotator adapter system (Sorvall model KSB-R, Kendro Laboratories, New York, NY). The cells were resuspended in the release buffer, centrifuged for 10 min at 600 × g, and gently mixed on a vortex mixer before sonication with a Sono-Tek Sonicator (Monticello, NY). An 8-ml gelatin cushion (1.5% gelatin in PEM100 buffer) was used to facilitate the release of the motors. The supernatants were clarified by centrifugation at 2000 × g for 1 h and then filtered through 250-μm filters. The filtered samples were loaded onto a 1.5-ml column of a release buffer consisting of 10 mM Pipes, 1 mM EGTA, 1 mM MgSO4, and 0.05% sodium azide at pH 6.9 (PEM 50) and sedimented to a pellet in a tabletop centrifuge. The pellet was resuspended in 150 ml of PEM50 buffer containing 1 mM dithiothreitol (Dtt), 1 mM microcystin (PEG50-P), and 10% glycerol at 37 °C and sheared through a 25-gauge needle five times. Sheared seeds were mixed with 100,000 x g for 1 h (4 °C) to obtain a clarified high speed supernatant (HSS). The HSS, which contained between 1 and 2 g of protein in 50–100 ml, was batch-adsorbed to 50–100 ml of hydrated DEAE-cellulose (DE52, Whatman, Maidstone, England) (equal volumes of HSS to hydrated DE52) by incubation on a rocking platform (4 °C for 30 min). The slurry was then poured into a 2.5 × 50-cm column and allowed to pack (DE-FT), with the cells containing the motors, was collected. The column was then washed with two column volumes of PEM50-P, which was added to the DE-FT. The remaining adsorbed proteins were eluted with ~1 column volumes of 150 mM NaCl in PEM50-P, and the eluate, which contained additional motors, was collected but not further pursued here. The proteins present in the DE-FT fraction were precipitated by the gradual addition of ammonium sulfate to 60% saturation (w/v) and incubation (24 h, 4 °C). Precipitated proteins were separated by centrifugation in a Sorvall centrifuge (SS-34 rotor, 20,000 rpm, 20 min, 4 °C) and resuspended in PEM50-P (identical to PEM50-P but containing 100 mM Pipes). Resuspended samples were clarified by centrifugation for 20 min at 20,000 rpm (SS-34 rotor, 4 °C), and depending upon the motor of interest, the resulting supernatant (~8–10 ml) was loaded onto a 2.5 × 120-cm column containing either 500 ml of a Biogel A-1.5-m or 500 ml of a BioGel A-15-m gel filtration matrix. Protein filters were filtered through the columns with PEM50-P, and 7-ml fractions were collected. HSET and CENP-E were identified in specific column fractions by SDS-polyacrylamide gel electrophoresis and immunoblotting with specific antibodies, and conventional kinesin was identified by immunoblotting and analysis of motor activity (see “Results”). HSET was collected as a peak from the column with PEM100-P, and 7-ml fractions were collected. HSET antibodies were generously provided by Dr. Duane Compton (Dartmouth Medical School, Hanover, NH) and used for immunoblots at a dilution of 1:6000. Antibodies to CENP-E were generously provided by Dr. T. E. Yen (Fox Chase Cancer Center, Philadelphia, PA) and used for immunoblots at a dilution of 1:6000.

RESULTS

Our primary goal was to characterize motors from mitotic cells; thus, a highly enriched mitotic cell population was obtained by incubation of a 16° cells/ml suspension with 12 mM vinblastine sulfate for one cell cycle (see “Experimental Procedures”). This concentration was chosen to produce a cell popu-
were again sedimented and resuspended in PEM100-P plus 10 mM MgATP and 300 mM NaCl to release the proteins that were bound in an ATP-sensitive manner (Fig. 4, lane 4). The released proteins were then fractionated on a 5–50% sucrose gradient, and two-drop fractions were collected (see "Experimental Procedures"). The fast motor fractionated as a single peak in fractions 26–29 of the gradient (data not shown). The pooled fractions contained a single polypeptide that migrated at ~120 kDa (Fig. 4, lane 5, upper arrow) and a trace amount of residual bovine brain tubulin (Fig. 4, lane 5, lower arrow). No other proteins could be detected on heavily overloaded Coomasie Blue-stained gels or on silver-stained gels (data not shown); thus, we conclude that the motor had been purified to homogeneity. The purified protein had a sedimentation coefficient of ~7 S as determined by comparison with proteins of known S values (data not shown).

The 120-kDa protein was identified as conventional kinesin heavy chain by mass spectrometry. The band was excised from a Coomasie Blue-stained SDS gel, and the protein was sequenced by microcapillary reverse-phase high pressure liquid chromatography nanoelectrospray tandem mass spectrometry (Harvard Microchemistry Facility). The sequences of multiple individual tryptic fragments of the protein corresponded exactly with sequences present in human ubiquitously expressed kinesin heavy chain (data not shown). In addition, the kinesin heavy chain antibody, SUK-4, recognized the 120-kDa polypeptide on immunoblots (Fig. 4, lane 5'), further indicating that the protein was conventional kinesin.

The active purified kinesin motor did not appear to contain light chains, since no additional protein bands could be detected either by Coomasie Blue stain (Fig. 4, lane 5) or by silver stain (data not shown). To determine if kinesin light chains were present in the active kinesin motor fractions, we performed Western blots using antibodies against the conserved region of kinesin light chain. No cross-reactivity to the antibodies was detected in the active motor fractions. The antibodies did, however, recognize light chains in other gradient fractions and so clearly were able to detect light chains. Therefore, the active human 7 S conventional kinesin that we isolated is composed solely of the heavy chain(s).

**Motility Characteristics of Human Kinesin**—The purified 7 S kinesin moved microtubules at a rate of 32.2 ± 1.5 μm/min at 25 °C (n = 240), a rate similar to that of the activity detected in the clarified lysate. When assayed at physiological temperature (37 °C), the gliding rate increased ~2-fold to 61.5 ± 2.0 μm/min (n = 50). The directionality of the kinesin was tested using polarity-marked fluorescent microtubules prepared with NEM-labeled tubulin (19). The microtubules glided with their brightly labeled minus-ends leading (Fig. 5), indicating that the kinesin is a plus-end-directed motor. However, when directionality was tested using polarity-marked microtubules in the absence of NEM, the microtubules glided in both directions (data not shown).

To determine whether the HeLa cell kinesin was similar to other conventional kinesins, we examined the nucleotide specificity of the gliding activity and inhibition by known kinesin inhibitors. As shown in Table I, HeLa cell kinesin could utilize GTP to produce microtubule gliding but at only 10% of the efficiency of ATP. Neither 1 mM ATP-γS, 1 mM etheno-ATP, nor 8-bromo-ATP supported microtubule gliding. Microtubule gliding was supported by 1 mM 2'-deoxy-ATP, 3'-deoxy-ATP, and 2',3'-dideoxy-ATP, but the rate was reduced to 84, 63, and 18%, respectively, of the gliding rate in the presence of 1 mM ATP. As reported in Table II, the HeLa cell kinesin gliding was inhibited with 1 mM AMPNNP, whereas gliding was not affected by

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3 DeLuca and Wilson, unpublished observation.
Panels

2 mM NEM. Finally, vanadate inhibited the gliding of kinesin at 100 μM but not at 50 μM.

HeLa cells blocked in mitosis by vinblastine were used as the source material for purified kinesin, since we were primarily interested in isolating mitotic motor proteins. However, we also purified kinesin from asynchronous HeLa cells, which consisted primarily of cells in interphase (~96%). The biochemical properties of kinesin purified from the interphase cells were not distinguishable from those of the kinesin purified from mitotic cells. Specifically, the active form of the motor consisted only of heavy chains and induced plus-end-directed microtubule gliding at a rate of ~30 μm/min (data not shown).

Purification of HSET—We found, using specific antibodies, that the DE-PT fraction also contained HSET, the human homolog of Drosophila Ned (non-claret disjunction). HSET belongs to the C-terminal kinesin motor subfamily, since its motor domain resides in the C terminus of the polypeptide. The basic purification scheme (Fig. 1) used to purify kinesin from mitotic HeLa cells was modified to purify HSET. The proteins in the DE-PT fraction were concentrated and applied to a BioGel-A 1.5-m gel filtration column, from which HSET eluted in the void volume (data not shown). The void volume fractions were pooled and subjected to a microtubule affinity purification step similar to that described previously for kinesin. In Fig. 6, lanes 1–5 show the supernatant and pellet fractions from the microtubule-binding and release steps. Proteins released from the microtubules with ATP (Fig. 6, lane 5) were fractionated on a 5–50% sucrose gradient by ultracentrifugation. SDS-polyacrylamide gel electrophoresis followed by immunoblot analysis revealed that HSET migrated as a single peak in fractions 31–33 of the gradient (data not shown). The peak obtained from the pooled sucrose fractions is shown in Fig. 6, lane 6, as a Coomassie Blue-stained SDS gel, and lane 5 is from an immunoblot probed with the kinesin heavy chain antibody, SUK4. Lanes 1 (pre MT), pooled, concentrated 1.5-m gel filtration column fractions containing the rapid gliding activity; lane 2 (binding sup), supernatant following initial centrifugation of microtubules and bound proteins; lane 3 (wash sup), supernatant following 300 mM NaCl microtubule wash and centrifugation; lane 4 (release sup), supernatant following 10 mM ATP, 300 mM NaCl wash and centrifugation; lanes 5 and 6 (purified kinesin), pooled 5–50% sucrose gradient fractions 26–29 containing purified kinesin. The upper arrow indicates 120-kDa kinesin, and the lower arrow indicates bovine brain tubulin from the microtubule affinity purification procedure. Molecular mass standards (M) are indicated in kilodaltons.

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CENP-E was detected in the DE-FT, although some CENP-E was detected in the 150 mM eluate (data not shown). We found that a critical step for the purification of CENP-E was the use of a BioGel-A 15-m gel filtration column rather than the Bio-Gel-A 1.5-m column used for kinesin and HSET. Thus, the proteins in the DE-FT were concentrated as described previously for kinesin and applied to a BioGel-A 15-m gel filtration column and eluted with PEM100-P buffer. The fractions containing CENP-E were pooled and subjected to a microtubule affinity purification step as described for kinesin (Fig. 8, lanes 1–4). Proteins released from microtubules with ATP (Fig. 8, lane 5) were fractionated on a 5–50% sucrose gradient containing initial centrifugation of microtubules and bound proteins; lane 3 (wash sup), supernatant following 300 mM NaCl microtubule wash and centrifugation; lane 4 (release pel), microtubule pellet following 10 mM ATP, 300 mM NaCl wash and centrifugation; lane 5 (release sup), supernatant following 10 mM ATP/300 mM NaCl wash and centrifugation; lanes 6 and 6′ (purified HSET), pooled 5–50% sucrose gradient fractions 31–33 containing purified HSET. The upper arrow indicates 75-kDa HSET, and the lower arrow indicates bovine brain tubulin from the microtubule affinity purification. Molecular mass standards are indicated in kilodaltons.

from the 15-m column in some of the preparations, there may have been contaminating Eg5, which then co-purified along with CENP-E throughout the microtubule affinity purification step and the sucrose gradient fractionation step.

Initial attempts to demonstrate microtubule binding or gliding by purified CENP-E using the protocol described previously for kinesin (see "Experimental Procedures") were negative. It is conceivable that the CENP-E did not bind to the glass slide under those conditions. To facilitate CENP-E binding to the slide, antibodies to the C-terminal tail domain of CENP-E were

### Table I

**Nucleotide specificity of HeLa cell kinesin**

Control gliding is the gliding rate of purified kinesin at 25 °C in the presence of 1 mM ATP (32.7 ± 1.9 µm/min, n = 92). All nucleotides and analogs were used in the assays at 1 mM, and gliding was tested at 25 °C. The results are an average of 3 experiments per nucleotide or analog, and in each experiment, an average of 30 microtubules was scored.

| Nucleotide       | Rate of gliding (percentage of control)* |
|------------------|------------------------------------------|
| ATP              | 100                                      |
| GTP              | 10 ± 4                                   |
| ATPγS            | 0                                        |
| 1, N'-ethenoATP  | 0                                        |
| 8-Bromo-ATP      | 0                                        |
| 3'-Deoxy-ATP     | 84 ± 6                                   |
| 3'-Deoxy-ATP     | 63 ± 8                                   |
| 2'3'-Deoxy-ATP   | 18 ± 2                                   |

* Gliding was tested for each condition at 25 °C in the presence of 1 mM ATP plus the inhibitor at the indicated concentration. A plus indicates that microtubule gliding was observed; a minus indicates that no microtubule gliding was observed.

### Table II

**Inhibitor profile of HeLa cell kinesin**

Inhibitor profile of HeLa cell kinesin (Fig. 6). Purified mitotic HeLa cell kinesin was adsorbed to glass slides and assayed for motor directionality by the addition of polarity-marked microtubules polymerized with NEM-treated tubulin (see "Experimental Procedures"). The microtubules glided only with the brightly labeled fluorescent seeds leading, indicating plus-end-directed activity. Numbers 1–3 mark examples of gliding microtubules. Time lapse images were taken every 5 s, and the time, in seconds, is indicated. Scale bar, 10 µm.

| Inhibitor | Concentration | Microtubule gliding* |
|-----------|---------------|------------------------|
| AMPNPN    | 1 mM          | –                      |
| NEM       | 2 mM          | +                      |
| Vanadate  | 50 µM         | –                      |
| Vanadate  | 100 µM        | –                      |

* Control gliding is the gliding rate of purified kinesin at 25 °C in the presence of 1 mM ATP plus the inhibitor at the indicated concentration. A plus indicates that microtubule gliding was observed; a minus indicates that no microtubule gliding was observed.

![Image 152x666 to 453x729]
first adsorbed to the slide by adding a solution of antibody (100 μg/ml) onto the slide. After a 10-min incubation, unbound antibodies were washed off, and the purified CENP-E was adsorbed to the antibody-coated glass surface. When assayed under these conditions, CENP-E did support microtubule binding (Fig. 9). Binding was specific for CENP-E; glass slides coated with antibody alone or sucrose gradient fractions not containing CENP-E did not support microtubule binding.

Although microtubules did bind to CENP-E, they did not glide upon the addition of ATP at concentrations as high as 10 mM. Many assay conditions were tested, but none supported gliding. Specifically, the pH was varied between pH 5.5 and 8.3, and the NaCl concentration was varied between 0 and 200 mM. In addition, we tried several buffers known to support gliding, including Pipes buffers (10–100 mM), Tris buffers (10–50 mM), and Hepes buffers (10–50 mM). No gliding occurred under any of these conditions. While the CENP-E did not exhibit microtubule motor activity, it did support a pivoting behavior, since microtubules bound to CENP-E-coated slides pivoted about fixed points on the microtubules. Interestingly, pivoting was not dependent on added ATP, since microtubules bound to the CENP-E on slides still pivoted after extensive washing with buffer containing no ATP. No other motor or protein fraction that we tested produced the pivoting movement; thus, this behavior was specific to CENP-E.

DISCUSSION

A major goal of this work was to develop a strategy for large scale purification of kinesin and KRP s in their native forms from mitotic human cells. While there are advantages to studying recombinantly expressed motors and motor fragments, including large yields and relative ease of purification (24), there are also important advantages to studying native motors. Namely, native motors may retain modifications acquired in vivo, they may retain their natural folded state, and they may remain complexed with important associated proteins.

We were interested primarily in purifying motors from mitotic cells. Therefore, HeLa cells were an ideal source, since they exhibit a high mitotic index (~90%) when treated with low concentrations of vinblastine (22). Under these conditions, the cells block in late prometaphase with an intact, relatively normal mitotic spindle (21, 22). This was important, because we had hypothesized that many mitotic motors might be bound to the spindle microtubules. These mitotically blocked cells differ slightly from naturally occurring mitotic cells in that the blocked cells are incapable of exiting mitosis in a normal fashion. The cells are in a “checkpoint-active” state, in which the cell cycle checkpoint machinery that monitors the progression into anaphase is “on,” preventing further cell cycle advancement.

Mitotic HeLa cells proved to be valuable for purification of native mitotic human microtubule motor proteins. In this report, we describe the purification and partial characterization of three motors: conventional kinesin, the C-terminal mitotic KRP HSET, and the mitotic KRP CENP-E. In addition, using this method, we identified and partially purified additional native microtubule motor proteins including the KRP s MKLP1, KLP2, and Eg5 and also a large cytoplasmic dynein complex (data not shown). The purification scheme described in this study for HeLa cell kinesin and KRP s is similar to previously described purification schemes in that a critical enrichment step is the binding of motors to microtubules in the presence of AMPNP and the release of motors with ATP and NaCl (3, 25–28). The major difference in our purification is that we developed a strategy for large scale HeLa cell culture growth and harvesting; thus, we were able to purify human kinesins in their native form in sufficient quantities for biochemical study.

The large majority of previous studies on purified native kinesin or KRP s has involved the use of either neuronal tissue (3, 28) or embryonic cells (16, 17, 25, 27), since these systems can also yield ample protein for biochemical characterization.

Human Conventional Kinesin—We found that mitotic HeLa cell extracts contained an active form of conventional kinesin. The microtubule gliding rate (~30 μm/min at 25 °C) did not change throughout purification, suggesting that the motor was not altered during the purification procedure. The gliding rate of the HeLa cell kinesin is similar to most, but not all, conventional kinesins previously studied (3, 25, 26). For example,
conventional kinesins purified from sea urchin embryos and from bovine brain tissue glide at ~30 μm/min (27, 28), whereas Drosophila kinesin glides along microtubules at ~54 μm/min (25), and kinesin from Neurospora glides at ~177 μm/min (26).

The nucleotide requirements and inhibition patterns of HeLa cell kinesin were similar to those of other previously purified native kinesins (26, 27, 29–31). Conventional kinesins promote microtubule gliding in the presence of the deoxy-ATP analogs in the order 2′ > 3′ > 2′3′ and do not promote efficient gliding in the presence of ATPyS (26, 27, 31), consistent with the results we obtained (Table I). However, the activities we observed with GTP and 1,N-etheno-ATP do differ from those reported in the literature. In the case of GTP, the HeLa cell kinesin produced gliding at only 10% of the rate it produced with ATP, while both Neurospora and sea urchin egg kinesins use GTP more efficiently, at 50–100% of control gliding in ATP (26, 27). These results may suggest that the nucleotide requirement for HeLa kinesin is more specific than that of other kinesins. Alternatively, the differences may simply reflect contamination of earlier kinesin preparations with a nucleotide diphosphokinase. Also, in contrast with bovine brain and Neurospora (26, 31), HeLa cell kinesin did not produce gliding in the presence of 1,N-etheno-ATP. While this may suggest that the kinesins vary in their abilities to hydrolyze 1,N-etheno-ATP, it could be that the various motors have different affinities for nucleotide analogs.

The inhibition pattern of HeLa cell kinesin (Table II) was found to be similar to those patterns of previously characterized conventional kinesins purified from bovine brain tissue, sea urchin eggs, and Neurospora (26, 27, 30). The gliding activity of the HeLa cell kinesin was insensitive to low concentrations of vanadate, but at concentrations above ~50 μM, gliding was inhibited, consistent with previous data (26, 27, 30). In addition, AMPNP has been shown to reduce or inhibit the gliding activity of conventional kinesins at ~1 mM (26, 27), while the alkylating agent NEM at ~2 mM has no effect on gliding activity (26, 27, 30). These findings are consistent with our data regarding the HeLa cell kinesin (Table II).

We examined the directionality of the purified human kinesin on microtubules in which tubulin was grown onto stable seeds in the presence of NEM-modified tubulin (Fig. 6; see “Experimental Procedures”). This procedure results in microtubule constructs with microtubules grown only at the plus-ends of the seeds, making designation of direction unambiguous. The microtubules glided with the plus-ends trailing the seeds, indicating that the kinesin produced plus-end-directed gliding (Fig. 5). These results are consistent with previous studies on the motility of conventional kinesin (3, 25, 27). It is interesting, however, that when we tested the direction of movement with polarity-marked microtubules polymerized without NEM-modified tubulin (32), the HeLa cell kinesin produced both plus- and minus-end-directed microtubule gliding (data not shown). We are currently investigating further the directionality of the purified HeLa kinesin.

A major difference between the active HeLa cell kinesin and previously characterized conventional kinesins is the lack of association of the HeLa cell kinesin with light chains. The HeLa cell kinesin had a sedimentation coefficient of ~7 S and did not appear to be associated with any other proteins. Also, light chain antibodies did not react with any proteins in the active purified kinesin preparation, although the antibodies did recognize light chains in other fractions (not shown).

Although the majority of conventional kinesins purified to date are heterotetrameric (reviewed in Ref. 4), containing both heavy and light chains, this is not the first example of an active native kinesin composed solely of heavy chains. Hackney et al. (33) purified two active bovine brain kinesins, a 7 S form consisting only of heavy chains and a 9 S form containing both heavy and light chains. In addition, purified conventional kinesin from Neurospora lacks light chains (26). Finally, we found that HeLa cells do contain a form of kinesin that contains both heavy and light chains, but this form lacks microtubule gliding activity.3

HSET—HSET belongs to the C-terminal family of KRP; members of this family have their motor domains in the C terminus as opposed to conventional kinesin, whose motor domain resides in the N terminus. The recombinant forms of the family members Kar3, CHO2, and Ncd exhibit minus-end-directed gliding activity at a rate of ~1–8 μm/min (34–36). These motors have been proposed to provide “inward” directed forces on the mitotic spindle to oppose the “outward” directed forces provided by the BimC subfamily (reviewed in Ref. 37). In vitro experiments have suggested that the C-terminal family motors may cross-link spindle microtubules and exert force by motoring toward the MT minus-ends (38). Interestingly, the Xenopus C-terminal family member, XCTK2, does not exhibit microtubule gliding activity in vitro and cannot cross-link microtubules (39). Therefore, the known members of this family may not be functionally equivalent.

We identified HSET in mitotic HeLa cell lysates using an HSET antibody and purified it by modifying the basic purification scheme (Fig. 1) for kinesin. HSET migrated as a single band at ~75 kDa on SDS gels and did not appear to purify with any associated proteins. The native HSET is an active microtubule motor that moves microtubules at ~5 μm/min (Fig. 7). This is consistent with previous results demonstrating microtubule motor activity for several other C-terminal family homologs including Ncd, CHO2, and Kar3 (34–36). However, this is the first demonstration that a native C-terminal KRP supports microtubule gliding activity and, moreover, that human HSET possesses gliding activity. Many additional experiments probing the biochemical nature of active native HSET are now feasible, including detailed characterization of its motor activity and determination of its microtubule cross-linking ability and ultrastructure.

CENP-E—CENP-E is a cell cycle-regulated, 312-kDa KRP that associates transiently with the kinetochores of mitotic chromosomes (23, 40). Disruption of CENP-E function by microinjection of antibodies to CENP-E or by antisense oligonucleotide transfection results in a failure of complete chromosome alignment at metaphase (41, 42), suggesting that CENP-E is involved in mitotic chromosome movements. This idea is supported by experiments of Lombillo et al. (43) in which antibodies to CENP-E blocked microtubule depolymerization-dependent minus-end-directed chromosome movements in vitro.

The nature of CENP-E’s motor activity and thus its function in mitosis remains poorly understood. In initial attempts to purify CENP-E from HeLa cells, it was found to be tightly associated with a fast, minus-end-directed microtubule motor activity (44). The form of CENP-E purified here does not appear to possess such activity. One simple explanation is that in the previous work, the partially purified CENP-E was contaminated with another motor. Another possibility is that the robust motor activity was associated with a different form of CENP-E. More recently, the recombinant motor domain of the Xenopus homolog, XCENP-E, exhibited slow, plus-end-directed microtubule gliding activity (45). Thus, it is clear that at least certain CENP-E segments can glide along microtubules.

The form of CENP-E we purified from the mitotic HeLa cells does not possess intrinsic microtubule gliding activity but rather may function to tether microtubules, perhaps to the...
kinetochores. Using a wide range of motility assay conditions, we were unable to detect any microtubule gliding, only microtubule binding. Many of the CENP-E-tethered microtubules pivoted around a fixed point, a behavior that occurred in similar fashion in the presence or absence of ATP. Because pivoting occurred in the absence of ATP, we suspect that the pivoting motion may be driven by diffusion rather than by a genuine motor activity.

It is possible that other forms of CENP-E might function as motors. For example, we found that a portion of the HeLa cell CENP-E remained bound to the DE-52 column and did not emerge in the DE-FT. This CENP-E form, which could be eluted from the column by 150 mM NaCl, has not yet been characterized, but it could have motor activity. In support of this idea, the CENP-E-containing 150 mM NaCl eluate did contain a slow microtubule gliding activity that moved microtubules when assayed in PEM50-P buffer but not in PEM100-P buffer. A second possibility is that the native CENP-E we purified may be modified (e.g. by phosphorylation) so that its gliding activity is inhibited. Consistent with this possibility, phosphorylation of CENP-E during mitosis has been implicated in its microtubule binding ability (46).

Another possibility is that the quantity of native CENP-E in our purified preparation may be too low for detectable gliding. In support of this possibility, it has been previously shown that when very low concentrations of conventional kinesin were examined in motility assays, the kinesin supported a similar behavior to that which we observed with CENP-E (microtubule binding and pivoting about a fixed point) (47, 48). However, in those experiments, some microtubules did exhibit gliding, whereas we never observed gliding with the HeLa cell CENP-E.

An interesting possibility is that the role in mitosis of the form of CENP-E we purified involves tethering of spindle microtubules to the kinetochores. In support of this idea, Lombillo et al. (43) found that antibodies to CENP-E caused chromosomes to lose their tethering to microtubules in vitro. Active microtubule gliding activity was not required in their assay, since ATP was not needed for the chromosome movement. This would not be the first example of an essential mitotic KRP that is incapable of producing microtubule gliding, since XKCM1, a KIN I kinesin that is essential for mitosis, does not support microtubule gliding but does induce microtubule disassembly in an ATP-dependent manner (15).

In summary, our experiments with CENP-E are consistent with a model in which the CENP-E binds to mitotic kinetochores and serves to tether the kinetochores microtubules. As suggested by Lombillo et al. (43), CENP-E may be essential for proper poleward chromosome movements by tethering them to actively depolymerizing microtubules. With such a mechanism, gliding activity would not be required for CENP-E’s function. Further characterization of the properties of native CENP-E will undoubtedly shed further light on its function.

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