

Sequence and Submolecular Localization of the 115-kD Accessory Subunit of the Heterotrimeric Kinesin-II (KRP_{85/95}) Complex

Karen P. Wedaman, David W. Meyer, Dana J. Rashid, Douglas G. Cole, and Jonathan M. Scholey

Section of Molecular and Cellular Biology, University of California, Davis, California 95616

Abstract. The heterotrimeric kinesin-II holoenzyme purified from sea urchin (*Strongylocentrotus purpuratus*) eggs is assembled from two heterodimerized kinesin-related motor subunits of known sequence, together with a third, previously uncharacterized 115-kD subunit, SpKAP115. Using monospecific anti-SpKAP115 antibodies we have accomplished the molecular cloning and sequencing of the SpKAP115 subunit. The deduced sequence predicts a globular 95-kD non-motor "accessory" polypeptide rich in alpha-helical segments that are generally not predicted to form coiled coils. Electron microscopy of individual rotary shadowed kinesin-II holoenzymes also suggests that SpKAP115 is globular, with a somewhat asymmetric morphology. Moreover, the SpKAP115 subunit lies at one end of the

51-nm-long kinesin-II complex, being separated from the two presumptive motor domains by a ~26-nm-long rod, in a manner similar to the light chains (KLCs) of kinesin itself. This indicates that SpKAP115 and the KLCs may have analogous functions, yet SpKAP115 does not display significant sequence similarity with the KLCs. The results show that kinesin and kinesin-II are assembled from highly divergent accessory polypeptides together with kinesin related motor subunits (KRPs) containing conserved motor domains linked to divergent tails. Despite the lack of sequence conservation outside the motor domains, there is striking conservation of the ultrastructure of the kinesin and kinesin-II holoenzymes.

THE kinesins constitute a family of microtubule-based motor proteins that play critical roles in various intracellular transport events (Vale and Goldstein, 1990; Vale, 1992; Skoufias and Scholey, 1993; Walker and Sheetz, 1993; Bloom and Endow, 1994; Cole and Scholey, 1995a). Kinesin holoenzymes are assembled from kinesin-related polypeptides consisting of conserved motor domains that hydrolyze ATP and actually move along microtubules. The motor domains are linked to non-conserved "tail" domains that serve as oligomerization and cargo-binding domains, and allow different kinesin-related polypeptides to assemble, with or without accessory polypeptides, into a variety of oligomeric states (Cole and Scholey, 1995a) and to bind to a variety of different subcellular cargoes (Vale and Goldstein, 1990).

Kinesin itself (Brady, 1985; Scholey et al., 1985; Vale et al., 1985) is a $\alpha_2\beta_2$ heterotetramer consisting of two apparently identical heavy chains (KHC)¹ together with two

light chains (KLC) (Bloom et al., 1988; Kuznetsov et al., 1988). Both types of subunit have been cloned and sequenced from a variety of organisms (Yang et al., 1989; Cyr et al., 1991; Wright et al., 1991; Gauger and Goldstein, 1993; Wedaman et al., 1993). The amino-terminal ends of the two heavy chains fold into globular motor domains (Hirokawa et al., 1989; Scholey et al., 1989) that are linked to a central "stalk" domain that is the site of dimerization of the two heavy chains via coiled-coil formation (deCuevas et al., 1992). There is a second globular domain at the carboxyl-terminal end of the heavy chain that displays membrane binding activity (Skoufias et al., 1994) and is juxtaposed to the associated light chains (Hirokawa et al., 1989).

It is now known that the KHC is the founder member of a large family of kinesin-related polypeptides (KRPs), many of which have been sequenced and characterized using molecular genetic approaches (Goldstein, 1993). To identify KRPs in cells and cell extracts, peptide antibodies that react with highly conserved sequences in the kinesin family motor domain were generated and shown to react with multiple members of the kinesin family on immunoblots (Cole et al., 1992; Sawin et al., 1992). We used these antibodies in a biochemical screen to identify and purify kinesin-related holoenzymes via their AMPPNP-enhanced, ATP-sensitive microtubule binding (Cole et al., 1992, 1993, 1994; Cole and Scholey, 1995b). The first kinesin-

Address all correspondence to Jonathan M. Scholey, Section of Molecular and Cellular Biology, University of California, Davis, CA 95616. Tel.: (916) 752-2271. Fax: (916) 752-1449. E-mail: jmscholey@ucdavis.edu

1. *Abbreviations used in this paper:* SpKAP115, *S. purpuratus* kinesin accessory polypeptide with 115-kD M_r ; SpKRP85 and SpKRP95, *S. purpuratus* kinesin-related polypeptides with 85- and 95-kD M_r , respectively; KHC, kinesin heavy chain; KLC, kinesin light chain; RT, room temperature; MT, microtubule.

related holoenzyme to be identified and purified this way is the heterotrimeric kinesin, previously named KRP_{85/95} from sea urchin (*S. purpuratus*) eggs (Cole et al., 1992, 1993; Rashid et al., 1995).

The heterotrimeric kinesin is an $\alpha\beta\gamma$ protein consisting of 1 mol 85 kD:1 mol 95 kD:1 mol 115 kD polypeptides, with a Stokes radius of 7.9 nm, a sedimentation coefficient of 9.8S and an estimated molecular mass of 300 kD (Cole et al., 1992). It binds to microtubules in an AMPPNP-enhanced, ATP-sensitive fashion and moves towards the plus ends of microtubules at $\sim 0.4 \mu\text{m/s}$ (Cole et al., 1992, 1993). The 85- and 95-kD subunits are kinesin-related polypeptides that are hypothesized to bind together by the formation of a heterodimeric coiled coil, homodimerization of these polypeptides being destabilized by unfavorable electrostatic interactions between residues of like charge (Rashid et al., 1995). The heterotrimeric kinesin appears to be associated with vesicle-like structures in the metaphase half spindles and anaphase interzone of dividing sea urchin embryonic cells, leading us to hypothesize that it moves vesicles towards the plus ends of microtubules during mitosis in this system, possibly delivering new membrane to the developing cleavage furrow during cytokinesis (Henson et al., 1995).

An intriguing question regarding the heterotrimeric kinesin concerns the identity and function of the 115-kD subunit (SpKAP115), which does not react with pan-kinesin peptide antibodies and is therefore proposed to be an accessory subunit possibly analogous to the kinesin light chains (Cole et al., 1992). We have speculated that this subunit may play a regulatory role or could function as an adaptor for cargo attachment (Cole et al., 1993). As a first step towards elucidating the role of SpKAP115 we have determined its localization within the kinesin-II holoenzyme and completed the cloning and sequencing of cDNAs encoding SpKAP115. We find that the deduced amino acid sequence predicts that SpKAP115 is distinct from other motor protein-associated polypeptides including the kinesin light chains (KLCs), but like the KLCs it is associated with the "tail" end of the corresponding kinesin holoenzyme. All three of the subunits of the heterotrimeric kinesin have therefore been sequenced (Cole et al., 1993; Rashid et al., 1995; this report). As this heterotrimeric kinesin is the first kinesin-related holoenzyme to be purified and characterized, we refer to it as kinesin-II.

Materials and Methods

Purification of Sea Urchin Egg Kinesin II

We developed an improved procedure for purifying kinesin-II. Sea urchins, *Strongylocentrotus purpuratus*, were collected from tide pools on the Pacific coast, north of Bodega Bay, and transported to the Bodega Bay marine station where they were maintained in a refrigerated, thousand-gallon holding tank. The collection of sea urchin eggs and preparation of egg cytosol were performed as described previously (Scholey et al., 1984; Buster and Scholey, 1991). Cytosol was frozen rapidly in liquid nitrogen and stored at -80°C . Starting with 200 ml frozen sea urchin egg cytosol, we depleted ATP by addition of hexokinase (10 U/ml; Sigma Chem. Co., St. Louis, MO) and glucose (50 mM), stirred slowly at room temperature (RT). The resulting actomyosin precipitate was removed by centrifugation at 17,000 rpm for 25 min at 10°C (Sorvall SS34 rotor). The assembly of endogenous microtubule proteins into microtubules was induced in the clarified extract by addition of GTP to 1 mM and taxol to 20 μM at RT

(Scholey et al., 1984, 1985; Buster and Scholey, 1991). Binding of kinesin-related holoenzymes to the assembled microtubules was induced by addition of AMPPNP to 1 mM with rocking at RT 20 min. MT-motor complexes were pelleted through a 15% sucrose cushion in PMEG (100 mM Pipes, pH 6.9, 2.5 mM MgSO_4 , 0.5 mM EDTA, 5.0 mM EGTA, 900 mM glycerol, 1 mM DTT, containing a protease inhibitor cocktail) plus 100 μM GTP, 100 μM AMPPNP, and 5 μM taxol by centrifugation at 13,000 rpm for 1 h at 10°C (Sorvall SS34 rotor). MTs were resuspended and washed in PEG, (100 mM Pipes, pH 6.9, 15 mM EDTA, 5 mM EGTA, 0.9 M glycerol, 1 mM DTT, plus a protease inhibitor cocktail consisting of 1 $\mu\text{g/ml}$ pepstatin, 2 $\mu\text{g/ml}$ aprotinin, 1 $\mu\text{g/ml}$ leupeptin, 100 $\mu\text{g/ml}$ soy bean trypsin inhibitor, and 0.1 mM phenylmethylsulfonyl fluoride) 0.1 mM GTP, 0.01 mM taxol, and 0.1 mM AMPPNP by five passes with a pestle in a glass tissue grinder. Washed MTs were pelleted at 25,000 rpm for 20 min at 10°C (Beckman 50.2Ti rotor; Beckman Instrs., Carlsbad, CA). Elution of bound kinesin holoenzymes from MTs was performed by resuspending MTs in PME (100 mM Pipes, pH 6.9, 2.5 mM MgSO_4 , 5 mM EDTA, 0.5 mM EDTA) containing 10 mM ATP, 10 mM MgSO_4 , 150 mM KCl, 1 mM GTP, and 10 μM taxol homogenized by five to eight passes with a pestle in a glass tissue grinder, and incubated at 4°C for a minimum of 4 h. Eluted motors were separated from MTs by centrifugation at 40,000 rpm for 20 min at 4°C , (Beckman 70.1Ti rotor) and further fractionated by gel filtration through a Biogel A1.5M gel filtration chromatography column, equilibrated with PME + 0.1 mM ATP. Peak kinesin-II fractions were pooled and dialyzed into TEMD buffer (20 mM Tris, pH 8.1, 0.5 mM EDTA, 2.5 mM MgSO_4 , 1 mM DTT) + 0.1 mM ATP. The dialyzed pool was filtered through a 0.22- μm filter and bound to a Pharmacia FPLC Mono Q anion exchange column equilibrated with TEMD + 0.1 mM ATP. The large volume of the sample, usually 15–20 ml, was injected via a 50-ml Super Loop (Pharmacia LKB Biotechnology Inc., Piscataway, NJ). Two major protein peaks, each ~ 1.5 ml in volume, were eluted from the Mono Q column using a 0–0.75 M NaCl gradient to desorb bound proteins, and these were identified as kinesin and kinesin-II (see Fig. 1 B). Kinesin-II was separated from all other remaining proteins by running 200 μl of peak FPLC fraction through a 5 ml 5–20% sucrose gradient in PMEG containing 100 μM ATP at 55,000 rpm in a Beckman SW55Ti rotor, for 9 h at 4°C . This method yields highly purified kinesin-II (Fig. 1) in a yield of $\sim 1 \mu\text{g}$ per ml of starting cytosol and is now used routinely to prepare active kinesin-II.

Electron Microscopy and Determination of Sedimentation Coefficient

Rotary shadow electron microscopy of single kinesin-II molecules was performed using highly purified kinesin-II (Fig. 1) or sometimes kinesin-II bound to microtubules polymerized from purified sea urchin egg tubulin (Cole et al., 1992) and pelleted through a 20% sucrose cushion (identical kinesin-II molecules were observed irrespective of the microtubule binding step, indicating that kinesin-II-microtubule complexes dissociate during processing for EM). The samples were mixed with an equal volume of 80% glycerol containing 1 M ammonium acetate and sprayed onto freshly cleaved mica plates. The plates were then vacuum-dried, rotary shadowed with platinum at a 6° angle using a Balzers BAF 400T freeze fracture device and processed according to Tyler and Branton, 1980. The replicas were visualized on a Philips 410LS electron microscope at 80 kV (Philips Electronic Instrs. Co., Mahwah, NJ). The sedimentation coefficient was determined as a function of the ionic strength using methods and marker proteins as described by Cole et al. (1992).

Preparation of the SpKAP115 Antiserum

Antisera to the three subunits of the heterotrimeric kinesin-II complex were raised in rabbits using routine procedures. Briefly, the kinesin-II holoenzyme was purified as described by Cole et al. (1992, 1993) and the three subunits were separated by preparative SDS-PAGE and visualized using 0.05% Coomassie brilliant blue. Rabbits were immunized by serial injection of the excised gel bands at the UCD Animal Resource Service, one rabbit per polypeptide (no. 7115 with the 115-kD band, no. 7116 with the 95-kD band and no. 7118 with the 85-kD band). Antisera collected from these rabbits were screened for immunoreactivity against subunits of kinesin-II by immunoblotting. The resulting 115-kD antiserum was blot affinity purified (Olmsted, 1991) against kinesin-II prepared by the aforementioned purification protocol (Fig. 1). The affinity-purified antibody was used to clone bona fide SpKAP115 by immunoscreening an unfertilized sea urchin egg λ ZAP cDNA library (Wright et al., 1991).

Antibody Purification against the SpKAP115 Subunit of Native Kinesin-II

Purified kinesin-II was run on a 7.5% SDS-PAGE curtain gel, transferred to nitrocellulose, washed in H₂O 10', stained with Ponceau S, and destained with H₂O to identify the 85-, 95-, and 115-kD subunits. The band at 115 kD was cut from the nitrocellulose and washed with PBS-Tween for 10' with shaking to elute the Ponceau S. The anti-115-kD rabbit polyclonal antibody was diluted 1:10 in PBS and the 115-kD band/nitrocellulose strip was rocked in this diluted antibody in a sealed tube overnight at 4°C. The strip with antibody bound to antigen was washed with rocking at 4°C in the following: PBS-Tween (3 × 10'), PBS (3 × 10') and wash buffer (50 mM Tris, pH 7.5, 5 mM EDTA, 150 mM NaCl) (3 × 20'). The antibody/antigen-bound nitrocellulose was cut into 2.5-mm squares and nonspecific antibody was removed by several buffer washes through two connected Luer-Lok (Becton Dickinson, San Jose, Ca.) syringes. The antibody was eluted with 3 ml of 3 M KSCN (in 20 mM Tris, pH 7.5) incubated 5' on ice with emulsification every 1'. To dilute and filter out the KSCN as well as concentrate the antibody, the solution was added to 9 ml of ice-cold PBS and concentrated to ~0.5 ml with centrifugation at 4°C in a Centriprep 30. This procedure was repeated two additional times until the final concentration of ~0.0012 M KSCN was achieved. To stabilize the antibody 1 mg/ml of BSA was added and it was stored at 4°C.

Library Screening

A λ ZAP (Stratagene Inc., La Jolla, CA) library prepared from poly(A)⁺ RNA from unfertilized eggs of *Strongylocentrotus purpuratus* (Wright et al., 1991) was used to screen ~1 × 10⁶ clones with a 1:100 dilution of blot affinity-purified polyclonal antibodies to the 115-kD subunit of kinesin II. Clones that reacted with the polyclonal anti-SpKAP115 were plaque-purified and the five positive clones that reacted most strongly with the antibody were in vivo excised and transformed into *E. coli* SOLR cells (Stratagene Inc.). cDNA inserts from the positive clones were characterized by restriction mapping (Sambrook et al., 1989) and standard Sanger dideoxy sequencing methods. The data obtained showed that the five positives were divided into three groups with no overlapping sequence between the three groups—two clones of identical sequence containing a 5' end start sequence followed by an ORF (SpKAP115N), one clone containing an ORF followed by a stop and noncoding sequence (SpKAP115C), and two clones that were false positives.

To obtain a full-length SpKAP115 cDNA or a clone containing overlapping sequence for SpKAP115N and SpKAP115C, subsequent library screens were performed using ³²P-labeled DNA probes prepared from regions within SpKAP115N and SpKAP115C. Numerous positive clones were obtained which matched the previous clones, however none produced a full-length cDNA or overlapping sequence. It was hypothesized that inefficient methylation during library construction resulted in the cleavage of a full-length SpKAP115 cDNA by EcoRI at an internal site. This hypothesis was confirmed by PCR amplification and sequencing of cDNAs that span the SpKAP115N-SpKAP115C junction.

PCR Procedures

First-strand cDNA was synthesized from reverse transcribed poly(A)⁺ RNA (from *S. purpuratus*) using random primers (a generous gift from F. McNally, University of California, Davis, CA). The PCR procedure was used under standard conditions to amplify regions of DNA between specific primers from SpKAP115N and SpKAP115C to show that the two clones represent one full-length cDNA encoding the SpKAP115 subunit, separated only by an EcoRI site. This was done using a 100-μl reaction mixture prepared using a Pfu polymerase protocol (Stratagene Inc.). To sequence through the region of the amplified product corresponding to the junction between clones SpKAP115N and SpKAP115C, Cyclist Exo(-) Pfu DNA Sequencing (Stratagene Inc.) was performed under standard conditions.

Sequencing Methods and Computer-assisted Sequence Analysis Procedures

DNA sequencing was performed by the Sanger dideoxy procedure (Sambrook et al., 1989). Sequence analysis was done using standard procedures (Doolittle, 1987; Gribskov and Devereux, 1991) and the GCG sequence analysis software package (Devereux et al., 1984).

Expression and Purification of SpKAP115N and SpKAP115C

cDNAs expressing SpKAP115N, SpKAP115C and a false positive negative control (Sp108) in pBluescript were excised and subcloned into the pRSET expression vectors (Invitrogen, San Diego, CA) in all three alternative translational reading frames. The resulting recombinant plasmids were transformed into DH5α competent cells and the cells grown up for plasmid preparations. To test protein expression, BL21(DE3) host cells (Novagen, Inc., Madison, WI) were transformed with the recombinant plasmids, and recombinant protein expression was induced using 0.4 mM IPTG at 37°C. Expressed fusion protein was detected using the T7-Tag monoclonal antibody (Novagen, Inc.) that revealed that the pRSETB vector expressed SpKAP115N, SpKAP115C and Sp108 fused to the T7-Tag in the correct translational reading frame. For large scale preparation of SpKAP115N, SpKAP115C and Sp108 fusion proteins, BL21(DE3) host cells transformed with the recombinant pRSETB expression vectors were grown at 37°C in 250–300 ml LB carbenicillin liquid cultures to an optical density of ~A₆₀₀ = 0.6. Protein expression was induced overnight by addition of IPTG to a final concentration of 0.4 mM. The induced cells were pelleted and the cell pellets frozen at -80°C. Recombinant protein was extracted from the transformed cells by resuspension in 6 M guanidine hydrochloride 1 h, RT and centrifugation to pellet cell debris. Solubilized protein was applied to a 1–2-ml Ni-NTA agarose affinity column (Qiagen, Inc., Chatsworth, CA) preequilibrated in guanidine hydrochloride buffer. The column was washed using 8 M urea-containing buffer, and bound recombinant proteins were eluted with 8 M urea buffer containing 0.5 M imidazole according to the manufacturer's recommendations. SDS-PAGE and immunoblotting with the T7-Tag antibody revealed the recovery of relatively pure recombinant proteins that were used for blot affinity purification of monospecific anti-SpKAP115 antibodies.

Affinity Purification of Anti-SpKAP115 Antibody against Recombinant SpKAP115N and SpKAP115C

Purified SpKAP115N and SpKAP115C were subjected to 10% SDS-PAGE, electrophoretically transferred to nitrocellulose and their migration position determined by probing a strip cut from the end of the filter with T7-Tag antibody and staining the remainder of the blot with Ponceau-S. The stained SpKAP115N and SpKAP115C bands were excised and used as affinity matrices for purifying monospecific anti-SpKAP115 antibody from the crude antiserum using the procedure described above (Olmsted, 1981). As a negative control, the recombinant false positive, Sp108 was processed identically.

Protein Microsequencing

To confirm that the polypeptide encoded by the cDNA clones was the 115-kD SpKAP115 subunit of the heterotrimeric kinesin-II complex, partial protein microsequencing of chymotryptic fragments of electrophoretically separated 115-kD subunit was performed as previously described (Cole et al., 1993).

Results

Molecular Structure of Kinesin-II Holoenzymes

We used electron microscopy to investigate the structure of kinesin-II, and to determine the position of SpKAP115 within this heterotrimeric complex. Sea urchin egg kinesin-II was highly purified (Fig. 1), sprayed onto mica, rotary shadowed and examined in the EM (Fig. 2). Many individual kinesin-II holoenzymes were visible as elongated structures 50.7 ± 2.4 nm in length (*n* = 100). At one end of the molecule, two small globular heads were visible, 10.3 ± 0.9 nm in diameter (*n* = 100). As these structures are the same size as the kinesin motor domains (Hirokawa et al., 1989; Scholey et al., 1989) and because the number of amino acids in the deduced sequences of the motor domains of SpKRP85, SpKRP95, and KHC are very similar (Wright et al., 1991; Cole et al., 1993; Rashid et al., 1995),

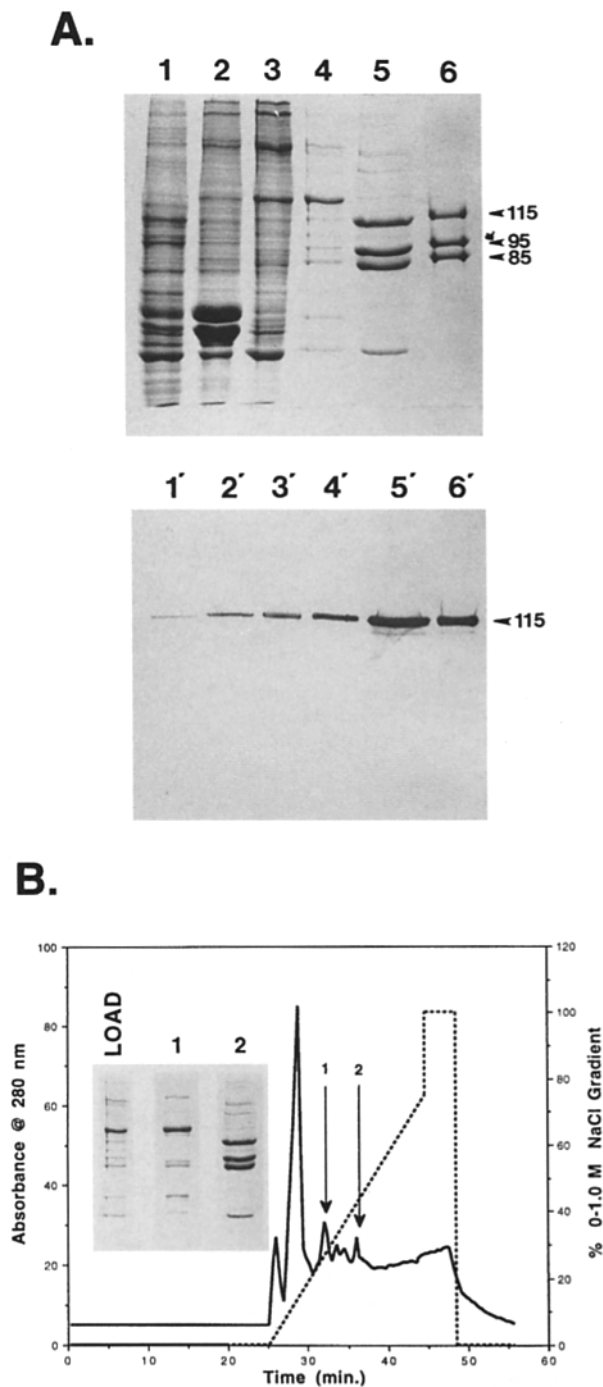


Figure 1. Purification of kinesin-II holoenzyme from sea urchin eggs and characterization of the 115-kD antibody. (A) Coomassie stained gel (top) and corresponding anti-SpKAP115-probed immunoblot (bottom). Lanes 1 and 1', 168 μ g sea urchin egg cytosol. Lanes 2 and 2', 151 μ g AMPPNP-microtubule protein. Lanes 3 and 3', 46 μ g ATP-eluted microtubule-associated proteins. Lanes 4 and 4', 5 μ g of the peak fractions of kinesin-II from the Biogel A1.5M column. Lanes 5 and 5', 12 μ g of the peak kinesin-II fractions eluted from an FPLC Mono Q column (Pharmacia) using a linear 0–0.75 M NaCl gradient. Lanes 6 and 6', 9 μ g purified kinesin-II from a 5–20% sucrose gradient. (B) Shows the separation of kinesin from kinesin-II as a result of binding the two proteins to a Mono Q FPLC anion exchange resin at low ionic strength then desorbing them with a linear gradient of NaCl (full line is absorbance at 280 nm, broken line indicates 0–1.0 M NaCl gradi-

these globular heads are likely to correspond to the amino-terminal motor domains of kinesin-II. The two globular domains are linked to a rod with a length of 26.3 ± 1.5 nm ($n = 98$), corresponding to the heterodimeric coiled-coil that we predicted to be 35 nm long based on sequence analysis (Rashid et al., 1995). At the end of the rod opposite the two globular heads is an asymmetric globular structure whose longest dimension is 23.6 ± 1.8 nm ($n = 100$). By comparing electron microscopic images of native kinesin-II (Fig. 2) with corresponding images of heterodimerized recombinant mouse homologues of SpKRP85 and SpKRP95 lacking their accessory subunit (Yamazaki et al., 1995) we conclude that this asymmetric globular structure corresponds to SpKAP115 (see discussion). Thus in the kinesin-II holoenzyme, SpKAP115 appears to be localized at the COOH-terminal ends of the heterodimerized motor subunits, much the same as the KLCs in the kinesin holoenzyme.

In addition to the extended kinesin-II molecules, we observed many globular particles. Some of these were clearly identified as being folded kinesin-II molecules, but others had a less distinct morphology, making it difficult to determine if they were also folded molecules or something else such as broken parts of intact kinesin-II holoenzymes, for example. The uncertainty in identification of some of the globular particles makes it impossible to estimate the ratio of folded to extended kinesin-II molecules. Kinesin and nonmuscle myosin also exist in folded (10S) and extended (6S) conformations, and the 10S conformer can be converted to the 6S conformer by elevating the ionic strength (Craig et al., 1983; Hisanaga et al., 1989; Hackney et al., 1992). We observed that kinesin-II molecules undergo similar ionic strength-dependent shifts in their sedimentation coefficients (Fig. 3). In our standard PMEG buffer, kinesin-II displays a sedimentation coefficient of $\approx 9.8S$, but this value decreases as the ionic strength increases, reaching a minimum value of 8S when the buffer is supplemented with ~ 200 mM KCl (Fig. 3).

Isolation of SpKAP115-encoding cDNA Clones and Confirmation of their Identities

Blot affinity-purified anti-SpKAP115 antibody reacted specifically with the 115-kD polypeptide that copurified with the 85- and 95-kD subunits of kinesin-II in fractions obtained during the purification of kinesin-II (Fig. 1 A). By using this antibody to immunoscreen a sea urchin unfertilized egg λ ZAP cDNA library (Wright et al., 1991) we obtained five strongly reactive putative positive SpKAP115 clones. Two of these positive clones encoded protein with identical deduced sequences. This protein, SpKAP115N (see map in Fig. 4 A), corresponds to the amino terminal portion of the SpKAP115 subunit and it has the ability to affinity purify, from the crude 115-kD antiserum, monospecific antibody that reacted strongly with the 115-kD polypeptide present in purified kinesin-II preparations and with a 115-kD polypeptide which dis-

ent). Arrows point to the peak of sea urchin kinesin (1) and kinesin-II (2). The corresponding Coomassie blue-stained gels of these fractions together with the load are shown on the left.

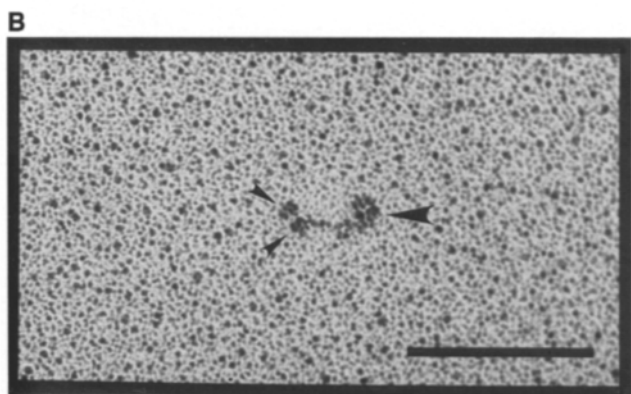
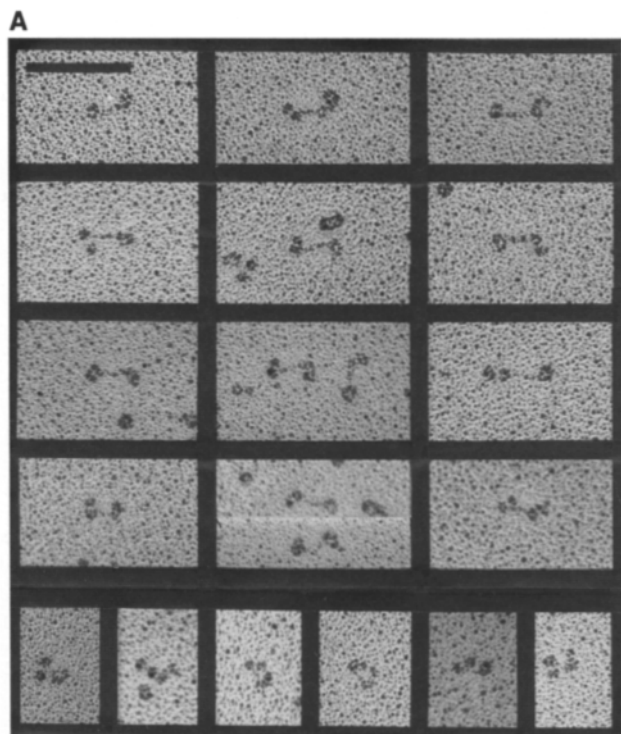


Figure 2. Rotary shadow electron micrographs of the kinesin-II holoenzyme. (A) Gallery of representative kinesin-II molecules, oriented with the 10-nm motor domains to the left and SpKAP115 to the right, linked by the central coiled-coil rod. The first four rows show extended molecules, whereas the lower row shows folded molecules. (B) Higher magnification representation of a single extended kinesin-II molecule. Small arrowheads indicate the motor domain "heads" and large arrowhead indicates the globular, asymmetric SpKAP115 subunit. Bars, 100 nm.

played AMPPNP-enhanced, ATP-sensitive microtubule binding (data not shown). This affinity purified antibody behaved in a similar fashion to antibody that was affinity purified against the native 115-kD subunit of kinesin-II. An additional positive clone encoded SpKAP115C (Fig. 4 A), the carboxyl-terminal portion of SpKAP115; the product of this cDNA was also able to affinity purify anti-SpKAP115 antibody with properties similar to that purified by the native 115-kD subunit of kinesin-II. Moreover, it contained two deduced stretches of sequence that were identical to partial peptide sequences determined by Ed-

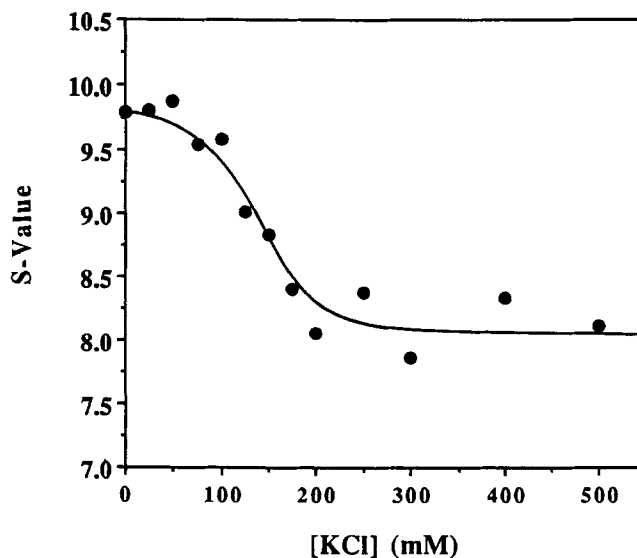
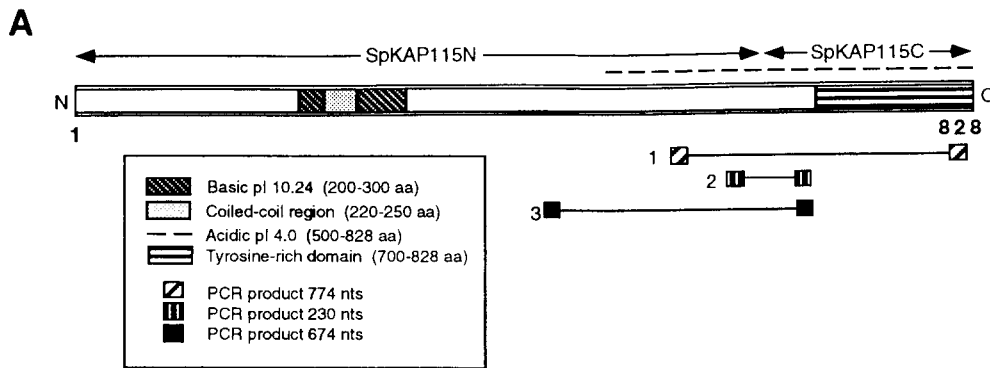


Figure 3. Ionic strength-dependent shift in the sedimentation coefficient of kinesin-II. On 5–20% sucrose density gradients containing PMEG with no additional salt, kinesin-II sediments as a monodisperse peak with a sedimentation coefficient of 9.8S. However, as the buffer is supplemented with incremental increases in KCl the S-value drops to ~8S.

man degradation and protein microsequencing (Fig. 4 B). Finally two clones encoding proteins of identical sequence were false positives that were unable to affinity purify anti-SpKAP115 antibody and whose deduced sequence did not match the SpKAP115 peptide sequences. Examination of the nucleotide sequence of the cDNAs encoding SpKAP115N and SpKAP115C suggested that the two cDNAs correspond to a single contiguous open reading frame as indicated in the map in Fig. 4 A, beginning with an initiator methionine preceded by three in-frame stop codons within the first 100 upstream nts, and ending in a TGA termination codon. The two cDNA fragments appeared to be linked by an EcoRI site that was only partially methylated leading to EcoRI cleavage of the intact cDNA during library construction. The hypothesis that the SpKAP115N and SpKAP115C cDNAs do indeed correspond to a single full-length contiguous open reading frame was tested by PCR amplification of cDNA (from unfertilized egg poly(A)⁺RNA) using primers designed to anneal on opposite sides of the predicted junction (Fig. 4 A). Fragments of the predicted size were obtained; 774 nts for PCR product no. 1, 230 nts for PCR product no. 2 and 674 nts for PCR product no. 3. To confirm that the PCR products were cDNAs representative of a SpKAP115N/SpKAP115C containing a junction-forming EcoRI restriction site, two cycle sequencing reactions were performed using PCR product no. 3. Each reaction used one specific primer ~80 nts on either side of the junction. Analysis of the resulting sequences confirmed that SpKAP115N and SpKAP115C adjoin one another at the predicted EcoRI site and form one full-length cDNA which encodes the SpKAP115 subunit of the kinesin-II holoenzyme.

Sequence Analysis of the 115-kD Subunit of Kinesin-II

The deduced sequence of the 115-kD subunit of kinesin-II



B

1 MQAEDARYLK RKVKGGSIDV HPTEKALVVN YELEATILGE MGDPLMGERK
 51 ECQKIIRLRS LNSTTDIAAL AREVVEKCKL IHPSKLPVEVE QLLYYLQNRK
 101 ETTQKAGAKK GELTGKLDKDP PPYEGTELNE VANINDIEDY IELLYEDNPE
 151 KVRGTALILQ LARNPDNLEE LQSNETVLGA LARVLREEWK HSVELATNIV
 201 YIFFCFSSFS QFHSIVAHFK IGALVMGVVE HELKKHLSWN EELLKKKKTA
 251 EENPASKRDF EKSTKKYHGL LKKQEQLLRV SFYLLLNLSSE DPKVELKMKR
 301 KNIIRLLIKT LERDNAELLI LVVSFLKKLK IYVENKNEMA EQQIIERLAK
 351 LVPCDHEDLL NITLRLLLNL SFDTDLRGKM IKLGMLPKFV ELLANDNHRL
 401 VVLCVLYHVS QDDKAKSMFT YTDCIPMIMK MMLESPTERL EIELVALGIN
 451 LACNKHNAQL ICEGNGLRLL MRRALKFRDP LLLKMIRNIS QHDGPSKAQF
 501 IEYISDVARI IKESNDEELV VEALGIMANL TIPDLDYEMI INEFLGLVPWI
 551 KEKLEPGAAE DDLVLEVVMV VGTVATDDSC AAMLAKSGII QSLIELLNAK
 601 QEDDEIVCQI VYVFYQMVFH EATREVIKIL TQAPAYLIDL MHDKNSEIRK
 651 VCDNTLDIIS EFDEEWARKI KLEKFRWHNS QWLEMVESQQ IDDGEGGLM**X**
 701 GDES**Y**SP**Y**IQ ESDILDRPDL F**Y**QGT**Y**EDG EM**Y**DGQVTP**E** **Y**VDE**Y**GNVQ**N**
 751 GE**Y**LGRPAS**A** M**Y**GNR**Y**QSE**Y** ESLRPGSRVA **Y**LTE**D**GQPLD **E****Y**GNPIEV**Y****A**
 801 **E**SV**D**Q**Y**GRPV **T**P**Y**Q**Y**GSQA Q**Q**GP**Y**P**Y**

Figure 4. Map and deduced amino acid sequence of SpKAP115, the 115kD subunit of the heterotrimeric kinesin-II holoenzyme. (A) The map shows the location of the two fragments of SpKAP115, namely SpKAP115N and SpKAP115C in relation to the full length, 828 residue SpKAP115 polypeptide, with potentially interesting features indicated. The positions of the PCR products, 1, 2, and 3 that span the junction between SpKAP115N and SpKAP115C, together with the primers that were used to amplify them, are also indicated. (B) The deduced amino acid sequence of SpKAP115 is presented using the single letter code for amino acids. Tyrosines in the tyrosine-rich COOH-terminal region are bold. The bracketed residues indicate stretches of deduced amino acid sequence that match partial peptide sequence obtained from chymotryptic fragments of the electrophoretically separated 115-kD subunit of kinesin-II. The deduced sequence and the corresponding nucleotide sequence are available from Genbank under accession number U38655.

(SpKAP115) predicts an 828-amino acid residue chain with a molecular weight of 94,646 Daltons (Fig. 4). The predicted isoelectric point of SpKAP115 is 4.9; the carboxyl-terminal region from residue 500–828 is very acidic (pI ~ 3.9) whereas the first 500 residues are more basic (pI ~ 8.1) with a highly basic region between residues 200–300 (pI ~ 10.2; Fig. 4 A). The segment lying between residues 700–828 is rich in tyrosine residues (16% of the amino acids are Y), many of which are potential targets for tyrosine protein kinases which phosphorylate tyrosines that have acidic residues (usually glu) at one to five amino acids upstream (Aitken, 1990).

Secondary structure predictions suggest that SpKAP115 is a globular protein rich in alpha helical conformation (at least 54% alpha helix overall, with residues 1–690 displaying at least 65% alpha helix; see map of SpKAP115 in Fig. 5 B). The predicted alpha helical segments are joined by short stretches of β -sheet and turns. The tyrosine rich region at the carboxyl terminus is predicted to contain abundant turns, consistent with the appearance of multiple pro-

line residues (9% of residues), and is predicted to be hydrophilic. The algorithm of Lupas et al. (1991) suggests that most of the alpha helical segments of SpKAP115 do not participate in coiled-coil formation; there is a region between residues 220 and 250 with a high probability of coiled-coil formation (Fig. 5 A) but this is much shorter than the regions of the two motor subunits of kinesin-II that are predicted to heterodimerize to form a coiled-coil stalk 35 nm in length (Fig. 5 A, and Rashid et al., 1995).

We compared the deduced sequence of SpKAP115 to those of other proteins in the sequence databases using the BLAST program. No matches to any proteins of known function were observed. In particular we observed no significant sequence similarity with kinesin light chains, kinectin, or other motor proteins or motor protein-associated polypeptides. However, the protein whose deduced amino acid sequence produced the highest scoring segment pairs with SPKAP115 was the product of a *C. elegans* gene, F08F8.3. This gene was cloned and sequenced during the *C. elegans* genome sequencing project (Wilson et al.,

1994). It is located on chromosome III between genes *mec14* and *lin39* and encodes a protein whose function is currently unknown. SpKAP115 and *C. elegans* F08F8.3 display 34% sequence identity and 57% similarity throughout their length.

Discussion

Here we have reported the molecular ultrastructure of the heterotrimeric sea urchin egg kinesin, together with the sequence, predicted structural organization, and submolecular localization of the 115-kD accessory subunit, SpKAP115. As predicted from the lack of reactivity with pan-kinesin peptide antibodies (Cole et al., 1992), SpKAP115 is not a kinesin-related polypeptide, but rather is an accessory polypeptide like the conventional kinesin light chains (Cyr et al., 1992; Wedaman et al., 1993). Previously we reported the sequences of the heterodimerized 85- and 95-kD kinesin-related motor subunits of the heterotrimeric kinesin (Cole et al., 1993; Rashid et al., 1995). Thus all three of the subunits of this sea urchin egg protein, previously named KRP_{85/95}, have now been sequenced. For this reason, and because it is the first kinesin-related holoenzyme to be purified and characterized, we feel justified in referring to this heterotrimeric motor protein as kinesin-II.

Immunological and primary structural evidence confirm that cDNAs SpKAP115N and SpKAP115C encode the entire 115-kD subunit (SpKAP115) of kinesin-II. Sequencing of the SpKAP115N and SpKAP115C cDNAs themselves, as well as a PCR product that spans the junction between these two cDNAs, confirms that SpKAP115N and SpKAP115C correspond, respectively, to the amino and carboxyl-terminal portions of an entire 95-kD polypeptide. The cDNA fragments that encode SpKAP115N and SpKAP115C form a single open reading frame and are linked by an EcoRI restriction site that was presumably cut during library preparation. The full length SpKAP115 contains two stretches of deduced sequence identical to partial peptide sequences derived from chymotryptic fragments of the 115-kD subunit of kinesin-II. Both SpKAP115N and SpKAP115C are capable of immunoadsorbing monospecific antibody that reacts with a 115-kD polypeptide that copurifies extensively with kinesin-II, and displays AMP-PNP-enhanced, ATP-sensitive microtubule binding. The latter result is significant as only kinesin and kinesin-related polypeptides are known to display AMPPNP-enhanced, ATP-sensitive microtubule binding (Cole and Scholey, 1995b). The sequence of SpKAP115 reveals that it is not a kinesin-related polypeptide, so the most plausible explanation is that its association with the 85- and 95-kD kinesin-related subunits of kinesin-II is responsible for the observed pattern of nucleotide-sensitive microtubule binding displayed by SpKAP115. Together these data provide strong evidence in support of the hypothesis that we have cloned SpKAP115.

Rotary shadow electron microscopy of individual kinesin-II molecules reveals that they have a remarkably similar ultrastructure to molecules of kinesin, with the accessory subunits associating with the divergent tails (Fig. 5 C). At one end of each kinesin-II molecule, there are two presumptive motor domains, ~10 nm in diameter, which are

likely to represent the amino-terminal domains predicted from the sequence of SpKRP85 and SpKRP95 (Cole et al., 1993; Rashid et al., 1995). The motor domains are linked to a 26.3-nm-long rod (shorter than that of kinesin) corresponding to the heterodimeric SpKRP85-SpKRP95 coiled-coil (Rashid et al., 1995). Based on sequence analysis we predicted a 35-nm-long rod (Rashid et al., 1995) so the slightly smaller length measured using EM might reflect the occlusion of a portion of the coiled-coil by SpKAP115 which appears to be an asymmetric structure associated with the end of the molecule opposite the motor domains. As with kinesin, the motor domains of kinesin-II can be released from the rod by limited proteolysis, suggesting that they are bound to the rod by a flexible, protease-sensitive segment (Scholey et al., 1989; Cole et al., 1993).

Kinesin-II undergoes an ionic strength-dependent change in S-value, much like kinesin, nonmuscle myosin-II and smooth muscle myosin-II (Craig et al., 1983; Hisanaga, 1989; Hackney et al., 1992). The 10S forms of kinesin and myosin-II are folded and elevating the ionic strength converts them to the 6S extended form. Kinesin-II also appears to exist in both folded and extended forms, suggesting that the ionic strength-dependent change from the 9.8S to the 8S form of kinesin-II may correlate with the extension of a folded conformer, although for technical reasons (Results), we were unable to confirm this directly by measuring the ratio of folded to extended molecules under different ionic strength conditions in the EM. The regulatory light chains of myosin-II play important roles in controlling myosin-II conformation, suggesting that the accessory SpKAP115 subunit of kinesin-II and the KLCs of kinesin may also play a role in regulating the conformation of the corresponding holoenzymes.

Our rotary shadow EM results are consistent with the EM observations of Yamazaki et al. (1995) who examined the molecular structure of murine Kif3a/b heterodimers which are homologues of SpKRP85/SpKRP95 heterodimers, and lack any accessory structures; the heterodimerized motor polypeptides were ~50 nm long consisting of two 10-nm heads attached to a short rod with a small globular structure at the end, possibly corresponding to the tails of the two motor subunits (Yamazaki et al., 1995). Molecules of the heterotrimeric kinesin-II complex are identical to Kif3a/b heterodimers, except for the large asymmetric globular structure at the end opposite the motor domains, which must correspond to SpKAP115 (Fig. 2).

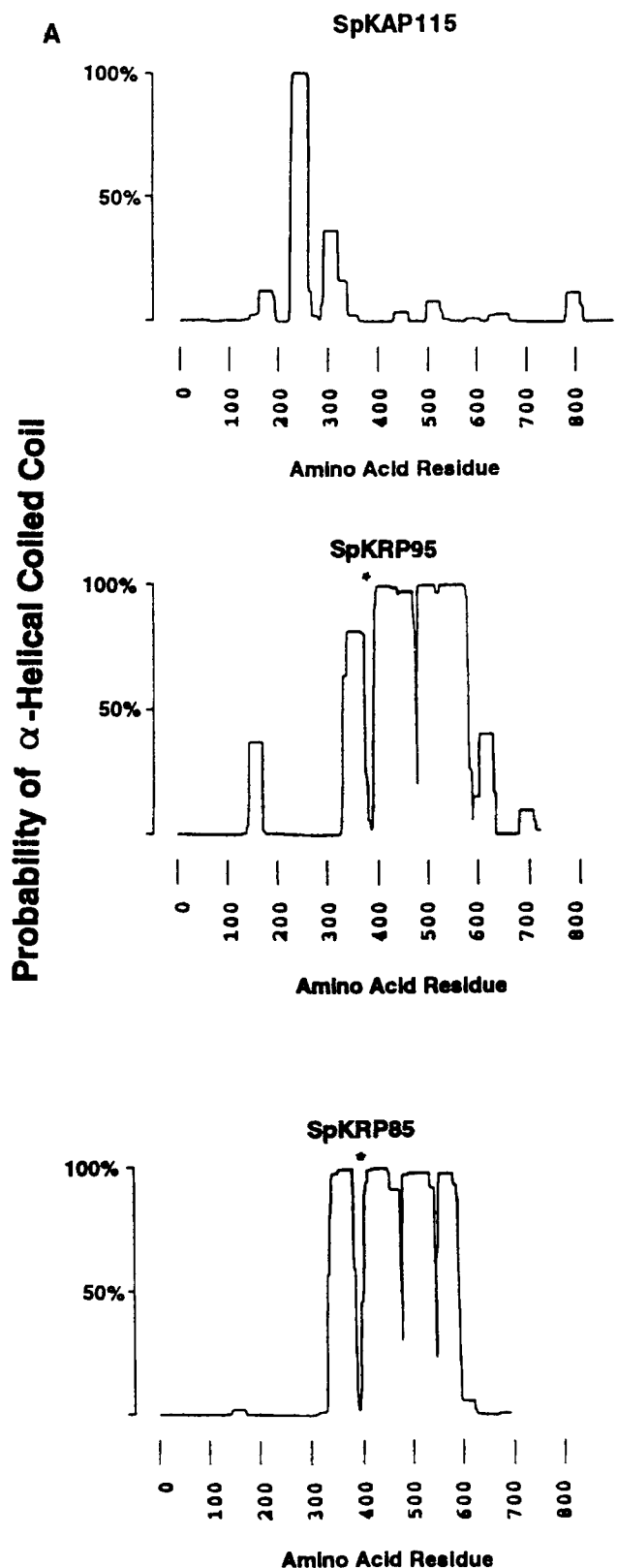
Based on our electron microscopic observations, we have hypothesized that a logical site of interaction of SpKAP115 with the heterodimerized SpKRP85 and SpKRP95 subunits is through their carboxyl-terminal tail regions. These tails are predicted to be extremely basic (SpKRP85 pI = 10.8, SpKRP95 pI = 9.6; Cole et al., 1993; Rashid et al., 1995), and might bind via ionic bonds to the acidic carboxyl-terminal region of SpKAP115, for example. We hypothesize that ionic bonds also stabilize SpKRP85/SpKRP95 heterodimeric coiled-coils relative to homodimers (Rashid et al., 1995). As noted above, the predicted topology of the tail regions of SpKRP85/SpKRP95/SpKAP115 heterotrimers resembles that of the conventional KHC-KLC complex (Hirokawa et al., 1989) suggesting that the KLCs and SpKAP115 may play analogous roles, for example in regulating motor protein conformation or modulat-

ing cargo-binding. However, in this context it is striking to note that we can observe no primary structural relatedness between SpKAP115 and the KLCs, even those from the same organism (Wedaman et al., 1993). Similarly, we have noted no significant similarity between SpKAP115 and any other known motor protein-associated polypeptides whose sequences are deposited in the sequence databases.

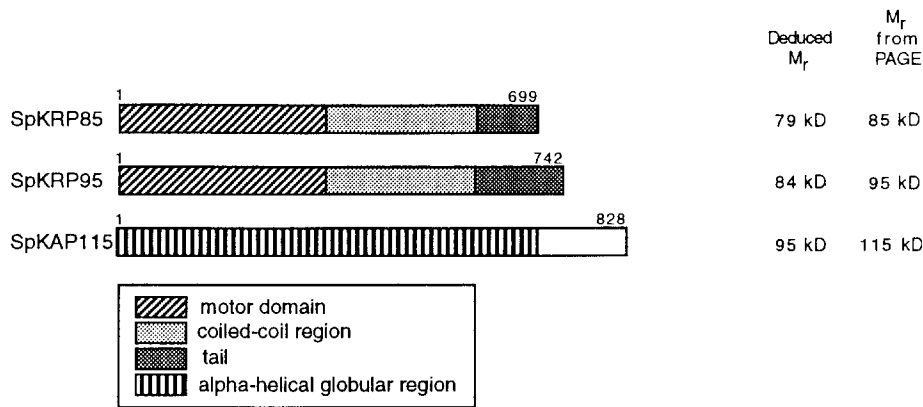
Homologues of the two motor subunits of kinesin-II, SpKRP85 and SpKRP95, have been found in a variety of organisms, including mouse (Aizawa et al., 1992; Kondo et al., 1994; Yamazaki et al., 1995), *Chlamydomonas* (Walther et al., 1994), *Caenorhabditis elegans* (Shakir et al., 1993; Tabish et al., 1995) and *Drosophila melanogaster* (Pesavento et al., 1994) leading us to hypothesize that these homologues might also be components of heterotrimeric complexes containing accessory polypeptides related to SpKAP115 (Cole et al., 1993; Cole and Scholey, 1995; Rashid et al., 1995). In support of our hypothesis, Yamazaki et al. (1995) have recently reported that the murine homologues of SpKRP85 and SpKRP95, namely Kif3a and Kif3b, respectively, form a heterotrimeric complex with a third polypeptide, KAP3, that has not yet been characterized. Indeed, it is remarkable that the data of Yamazaki et al., 1995 replicate our results so exactly (Cole et al., 1992). It seems likely, therefore, that KAP3 will turn out to be a homologue of SpKAP115.

It is also possible that a close relative of SpKRP85 and SpKRP95 in *C. elegans*, OSM-3, may interact with an accessory subunit encoded by the F08F8.3 gene that is related by sequence to SpKAP115. The product of the *C. elegans* F08F8.3 gene is the only protein in the sequence databases that displayed significant relatedness with SpKAP115. The deduced sequence of this protein was determined in the *C. elegans* genome project, but so far as we know, the function of this protein has not been determined. Interestingly, however, this gene is located between *mec14* and *lin39* on the genetic map of chromosome III, where the *osm-10* mutation is also located. This may be significant because mutations in both *osm-10* and *osm-3* cause defects in osmotic avoidance (Shakir et al., 1993; Tabish et al., 1995) and, in the case of *osm-3*, this phenotype is thought to be due to defects in motor-driven axonal transport in chemosensory neurons open to the external environment (Tabish et al., 1995). Based on the results presented here, therefore, we speculate that the *osm-10* gene corresponds to F08F8.3 and encodes a close relative of SpKAP115, with the *osm-3* and *osm-10* gene products being two subunits of a *C. elegans* heterotrimeric kinesin closely related to the kinesin-II complex. This would mean that the accessory subunit of kinesin-II equivalent to SpKAP115 in *C. elegans* has an essential function in axonal transport. To test this hypothesis it will be important to sequence the OSM-10 protein, to determine if F08F8.3 can complement the *osm-10* mutation, and to purify the presumptive OSM-3/OSM-10 complex from *C. elegans*. The purification protocol for kinesin-II described here may be a useful guide in this endeavor.

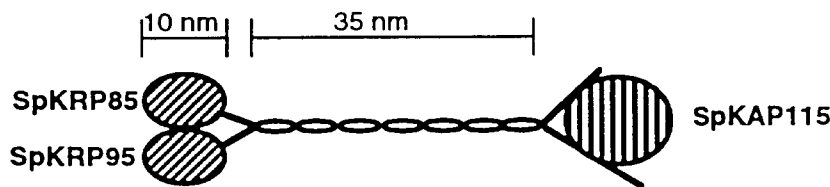
We have now purified two kinesin holoenzymes, kinesin and kinesin-II, from sea urchin eggs and embryos, we have characterized their molecular structures and we have determined the sequence of each of their subunits. Kinesin and kinesin-II have generally similar structures, consisting



B



C



consists of two different kinesin related polypeptides, SpKRP85 and SpKRP95, with amino-terminal motor domains, a central alpha helical domain that forms a heterodimeric coiled-coil, and a globular, presumptive cargo-binding domain at the carboxyl-terminal end (Cole et al., 1992, 1993; Rashid et al., 1995). The 115-kD kinesin-II-associated polypeptide, SpKAP115 is predicted to be a highly alpha-helical, globular protein with a hydrophilic, tyrosine-rich carboxyl-terminal tail domain. (C) Model of the quaternary structure of kinesin-II based on sequence analysis and electron microscopy. The motor domains of the heterodimerized SpKRP85 and SpKRP95 motor subunits appear as globular, 10-nm "heads". The heterodimeric coiled-coil regions form a central rod that is predicted to be 35 nm long based on the Lupas et al. (1991) algorithm, but the association of SpKAP115 with the carboxyl-terminal ends of SpKRP85 and SpKRP95 appears to occlude a portion of the rod and reduces its length to ~26 nm as observed by electron microscopy.

of dimerized motor polypeptides with amino terminal motor domains linked to coiled-coil rods, terminating in a second globular domain at the COOH-terminal end, where the accessory KLC or SpKAP115 polypeptides are localized. The coiled-coil rod of kinesin-II is heterodimeric and somewhat shorter than that of the homodimeric kinesin rod, for reasons that are unclear. Both types of kinesin move towards the plus ends of microtubule tracks at speeds of approximately half a micron per second (Cohn et al., 1989; Cole et al., 1993), and both these kinesin holoenzymes are thought to function as vesicle motors in the spindle. However, kinesin affiliates with exocytic vesicles in the spindle asters (Wright et al., 1991, 1993; Steinhardt et al., 1994) whereas kinesin-II is associated with vesicles in the metaphase half spindles and anaphase interzone (Henson et al., 1995). An intriguing question concerns the mechanism by which kinesin and kinesin-II are targeted to vesicles located in different regions of the same spindles. Given the fact that the KLCs and SpKAP115 are highly divergent accessory polypeptides localized at the tail end of kinesin and kinesin-II respectively, we hypothesize that the accessory polypeptides play a role in targeting kinesin and kinesin-II to different classes of spindle vesicles. As all the subunits of sea urchin egg kinesin and kinesin-II have

been cloned and sequenced, it may now be possible to test the role of the accessory polypeptides in targeting these two motors to different classes of spindle vesicles by switching their accessory subunits to create different types of motor-accessory subunit hybrids.

We are particularly indebted to Dr. R. Baskin for teaching the rotary shadow electron microscopy procedures to D. Rashid. We thank members of the Scholey laboratory plus Drs. K. McKim and J. Lye for helpful discussion and Drs. R. Yount, C. Cremo and G. Munske for help with peptide sequencing. We are very grateful to Dr. F. McNally for providing unfertilized sea urchin egg RNA for PCR. We thank K. Chiem for excellent technical assistance.

This work was supported by National Institutes of Health grant no. GM50718.

Received for publication 9 October 1995 and in revised form 10 November 1995.

References

- Aitken, A. 1990. Identification of Protein Consensus Sequences. Ellis Horwood publishers, NY. 151-160.
- Aizawa, H., Y. Sekine, R. Tekamura, Z. Zhang, M. Nangaku, and N. Hirokawa. 1992. Kinesin family in murine central nervous system. *J. Cell Biol.* 119: 1287-1296.
- Biou, V., J. F. Gibrat, J. M. Levin, B. Robson, and J. Garnier. 1988. Secondary structure prediction: combination of three different methods. *Protein Eng.* 2:

Figure 5. Summary of the structural organization of the three subunits of the heterotrimeric kinesin-II holoenzyme. (A) Probability of coiled-coil formation of the three subunits of kinesin-II, as predicted by the Lupas et al. (1991) algorithm. The 85- and 95-kD motor subunits are predicted to form similar sized coiled-coils that are the basis for heterodimerization of these two polypeptides (Rashid et al., 1995). In contrast, only a short stretch of SpKAP115, lying between residues 220 and 250, is predicted to participate in coiled-coil formation. This analysis is not consistent with the idea that the three subunits of kinesin-II form a heterotrimeric coiled-coil extending throughout the length of the region of SpKRP85-SpKRP95 heterodimerization. (B) Maps showing the domain organization of the three subunits of kinesin-II derived from sequence analysis. The heterotrimeric kinesin-II motor

- 185–191.
- Bloom, G. S., M. C. Wagner, K. K. Pfister, and S. T. Brady. 1988. Native structure and physical properties of bovine brain kinesin and identification of the ATP binding subunit polypeptide. *Biochemistry*. 27:3409–3416.
- Bloom, G. S., and S. Endow. 1994. Kinesins. *Protein Profile*. 1:1059–1116.
- Brady, S. T. 1985. A novel brain ATPase with properties expected for the fast axonal transport motor. *Nature (Lond.)*. 317:73–75.
- Buster, D., and J. M. Scholey. 1991. Purification and assay of kinesin from sea urchin eggs and early embryos. *J. Cell Sci.* 14s:109–115.
- Cohn, S. A., A. L. Ingold, and J. M. Scholey. 1989. Quantitative analysis of sea urchin egg kinesin-driven microtubule motility. *J. Biol. Chem.* 264:4290–4297.
- Cole, D. G., W. Z. Cande, R. J. Baskin, D. A. Skoufias, C. J. Hogan, and J. M. Scholey. 1992. Isolation of a sea urchin egg kinesin-related protein using peptide antibodies. *J. Cell Sci.* 101:291–301.
- Cole, D. G., S. W. Chinn, K. P. Wedaman, K. Hall, T. Vuong, and J. M. Scholey. 1993. Novel heterotrimeric kinesin-related protein purified from sea urchin eggs. *Nature (Lond.)*. 366:268–270.
- Cole, D. G., W. M. Saxton, K. B. Sheehan, and J. M. Scholey. 1994. A “slow” homotetrameric kinesin related motor protein purified from *Drosophila* embryos. *J. Biol. Chem.* 269:22913–22916.
- Cole, D. G., and J. M. Scholey. 1995a. Structural variations among the kinesins. *Trends Cell Biol.* 5:259–262.
- Cole, D. G., and J. M. Scholey. 1995b. Purification of kinesin-related protein complexes from eggs and embryos. *Biophys. J.* 68:158s–162s.
- Craig, R., R. Smith, and J. Kendrick-Jones. 1983. Light chain phosphorylation controls the conformation of smooth muscle and nonmuscle myosin. *Nature (Lond.)*. 302:436–439.
- Cyr, J. L., K. K. Pfister, G. S. Bloom, C. A. Slaughter, and S. T. Brady. 1991. Molecular genetics of kinesin light chains: generation of isoforms by alternative splicing. *Proc. Natl. Acad. Sci. USA*. 88:10114–10118.
- deCuevas, M., T. Tao, and L. S. Goldstein. 1992. Evidence that the stalk of *Drosophila* kinesin heavy chain is an alpha-helical coiled coil. *J. Cell Biol.* 116:957–965.
- Devereux, J., P. Haeberli, and O. Smithies. 1984. A comprehensive set of sequence analysis programs for the VAX. *Nucleic Acids Res.* 12:387–395.
- Doolittle, R. F. 1987. Of ORFs and URFs. University Science Books, Mill Valley, CA, 103 pp.
- Gauger, A. K., and L. S. B. Goldstein. 1993. The *Drosophila* kinesin light chain. Primary structure and interaction with kinesin heavy chain. *J. Biol. Chem.* 268:13657–13666.
- Goldstein, L. S. B. 1993. With apologies to Sheherazade: tails of 1001 kinesin motors. *Annu. Rev. Genet.* 27:319–351.
- Gribskov, M., and J. Devereux. 1991. Sequence Analysis Primer. Stockton Press, London. 1–279.
- Hackney, D. D., J. D. Levitt, and D. D. Wagner. 1991. Characterization of $\alpha 2\beta 2$ and $\alpha 2$ forms of kinesin. *Biochem. Biophys. Res. Commun.* 174:810–815.
- Hackney, D. D., J. D. Levitt, and J. Suhan. 1992. Kinesin undergoes a 9 S to 6 S conformational transition. *J. Biol. Chem.* 267:8696–8701.
- Henson, J. H., D. G. Cole, M. Terasaki, D. Rashid, and J. M. Scholey. 1995. Immunolocalization of the heterotrimeric kinesin related protein KRP_{85,95} in the mitotic apparatus of sea urchin embryos. *Dev. Biol.* 171:182–194.
- Hirokawa, N., K. K. Pfister, H. Yorifuji, M. C. Wagner, S. T. Brady, and G. S. Bloom. 1989. Submolecular domain of brain kinesin identified by electron microscopy and monoclonal antibody decoration. *Cell*. 56:867–878.
- Hisanaga, S., H. Murofushi, K. Okuhara, R. Sato, Y. Masuda, H. Sakai, and N. Hirokawa. 1989. The molecular structure of adrenal medulla kinesin. *Cell Motil. Cytoskeleton*. 12:264–272.
- Johnson, C. S., D. Buster, and J. M. Scholey. 1990. Light chains of sea urchin kinesin identified by immunoabsorption. *Cell Motil. Cytoskeleton*. 16:204–213.
- Kondo, S., R. Sato-Yoshitake, Y. Noda, H. Aizawa, T. Nakata, Y. Matsuura, and N. Hirokawa. 1994. Kif3A is a new microtubule-based motor in the nerve axon. *J. Cell Biol.* 125:1095–1107.
- Kuznetsov, S. A., E. A. Vaisberg, N. A. Shanina, N. N. Magretova, V. Y. Chernyak, and V. I. Gelfand. 1988. The quaternary structure of bovine brain kinesin. *EMBO (Eur. Mol. Biol. Organ.) J.* 7:353–356.
- Kyte, J., and R. F. Doolittle. 1982. A simple method for displaying the hydrophobic character of a protein. *J. Mol. Biol.* 157:105–132.
- Lupas, A., M. Van Dyke, and J. Stock. 1991. Predicting coiled coils from protein sequences. *Science (Wash. DC)*. 252:1162–1164.
- Olmsted, J. B. 1981. Affinity-purification of antibodies from diazotized paper blots of heterogeneous protein samples. *J. Biol. Chem.* 256:11955–11957.
- Pesavento, P. A., R. J. Stewart, and L. S. Goldstein. 1994. Characterization of the Klp68D kinesin-like protein in *Drosophila*: possible roles in axonal transport. *J. Cell Biol.* 127:1041–1048.
- Rashid, D. J., K. P. Wedaman, and J. M. Scholey. 1995. Heterodimerization of the two motor subunits of the heterotrimeric kinesin, KRP_{85,95}. *J. Mol. Biol.* 252:157–162.
- Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
- Sawin, K. E., T. J. Mitchison, and L. G. Wordeman. 1992. Evidence for kinesin-related proteins in the mitotic apparatus using peptide antibodies. *J. Cell Sci.* 101:303–313.
- Scholey, J. M., B. Neighbors, J. R. McIntosh, and E. D. Salmon. 1984. Isolation of microtubules and a dynein-like MgATPase from unfertilized sea urchin eggs. *J. Biol. Chem.* 259:6516–6525.
- Scholey, J. M., M. E. Porter, P. M. Grissom, and J. R. McIntosh. 1985. Identification of kinesin in sea urchin eggs and evidence for its localization in the mitotic spindle. *Nature (Lond.)*. 318:483–486.
- Scholey, J. M., J. Heuser, J. T. Yang, and L. S. B. Goldstein. 1989. Identification of globular mechanochemical heads of kinesin. *Nature (Lond.)*. 338:355–357.
- Shakir, M., T. Fukushige, H. Yasuda, J. Miwa, and S. S. Siddiqui. 1993. *C. elegans osm-3* gene mediating osmotic avoidance behavior encodes a kinesin-like protein. *Neuroreport*. 4:891–894.
- Skoufias, D. A., and J. M. Scholey. 1993. Cytoplasmic microtubule-based motor proteins. *Curr. Opin. Cell Biol.* 5:95–104.
- Skoufias, D., D. G. Cole, K. P. Wedaman, and J. M. Scholey. 1994. The carboxyl-terminal domain of kinesin heavy chain is important for membrane binding. *J. Biol. Chem.* 269:1477–1485.
- Steinhardt, R. A., G. Bi, and J. M. Alderton. 1994. Cell membrane resealing by a vesicular mechanism similar to neurotransmitter release. *Science (Wash. DC)*. 263:390–393.
- Tabish, M., Z. K. Siddiqui, K. Nishikawa, and S. S. Siddiqui. 1995. Exclusive expression of *C. elegans osm-3* kinesin gene in chemosensory neurons open to the external environment. *J. Mol. Biol.* 247:377–389.
- Tyler, J. M., and D. Branton. 1980. Rotary shadowing of extended molecules dried from glycerol. *J. Ultrastr. Res.* 71:95–102.
- Vale, R. D., T. S. Reese, and M. P. Sheetz. 1985. Identification of a novel force-generating protein, kinesin, involved in microtubule-based motility. *Cell*. 42:39–50.
- Vale, R. D., and L. S. B. Goldstein. 1990. One motor many tails; an expanding repertoire of force-generating molecules. *Cell*. 60:883–885.
- Vale, R. D. 1992. Microtubule motors: many new models off the assembly line. *Trends Biochem. Sci.* 17:300–304.
- Walker, R. A., and M. P. Sheetz. 1993. Cytoplasmic microtubule-associated motors. *Annu. Rev. Biochem.* 62:429–451.
- Walther, Z., M. Vashishtha, and J. L. Hall. 1994. The *Chlamydomonas fla10* gene encodes a novel kinesin-homologous protein. *J. Cell Biol.* 126:175–188.
- Wedaman, K. P., A. E. Knight, J. Kendrick-Jones, and J. M. Scholey. 1993. Sequences of sea urchin kinesin light chain isoforms. *J. Mol. Biol.* 231:155–158.
- Wilson, R., R. Ainscough, K. Anderson, C. Baynes, M. Berks, J. Bonfield, J. Burton, M. Connell, T. Copsy, J. Cooper, et al. 1994. 2.2 Mb of contiguous nucleotide sequence from chromosome III of *C. elegans*. *Nature (Lond.)*. 368:32–38.
- Wright, B. D., J. H. Henson, K. P. Wedaman, P. J. Willy, J. N. Morand, and J. M. Scholey. 1991. Subcellular localization and sequence of sea urchin kinesin heavy chain: evidence for its association with membranes in the mitotic apparatus and interphase cytoplasm. *J. Cell Biol.* 113:817–833.
- Wright, B. D., M. Terasaki, and J. M. Scholey. 1993. Roles of kinesin and kinesin-like proteins in sea urchin embryonic cell division: evaluation using antibody microinjection. *J. Cell Biol.* 123:681–689.
- Yamazaki, H., T. Nakata, Y. Okada, and N. Hirokawa. 1995. KIF3A/B: a heterodimeric kinesin superfamily protein that works as a microtubule plus end-directed motor for membrane organelle transport. *J. Cell Biol.* 130:1387–1399.
- Yang, J. T., R. A. Laymon, and L. S. B. Goldstein. 1989. A three-domain structure of kinesin heavy chain revealed by DNA sequence and microtubule binding analyses. *Cell*. 56:879–889.