Inhibition of Kinesin-driven Microtubule Motility by Monoclonal Antibodies to Kinesin Heavy Chains

Amie L. Ingold,* Stanley A. Cohn,* and Jonathan M. Scholey*‡

* Department of Molecular and Cellular Biology, National Jewish Center for Immunology and Respiratory Medicine, Denver, Colorado 80302; and ‡ Department of Cellular and Structural Biology, and the Department of Biochemistry, Biophysics and Genetics, University of Colorado Health Science Center, Denver, Colorado 80206

Abstract. We have prepared and characterized seven mouse monoclonal antibodies (SUK 1-7) to the 130-kD heavy chain of sea urchin egg kinesin. On immunoblots, SUK 3 and SUK 4 cross-reacted with *Drosophila* embryo 116-kD heavy chains, and SUK 4, SUK 5, SUK 6, and SUK 7 bound to the 120-kD heavy chains of bovine brain kinesin. Three out of seven monoclonal antikinesins (SUK 4, SUK 6, and SUK 7) caused a dose-dependent inhibition of sea urchin egg kinesin-induced microtubule translocation, whereas the other four monoclonal antibodies had no detectable effect on this motility. The inhibitory monoclonal antibodies (SUK 4, SUK 6, and SUK 7) appear to bind to spatially related sites on an ATP-sensitive microtubule binding 45-kD chymotryptic fragment of the 130-kD heavy chain, whereas SUK 2 binds to a spatially distinct site. None of the monoclonal antikinesins inhibited the microtubule activated MgATPase activity of kinesin, suggesting that SUK 4, SUK 6, and SUK 7 uncouple this MgATPase activity from motility.

VARIOUS forms of intracellular transport are thought to depend upon microtubule-associated mechanochemical ATPases (Vale, 1987). One such enzyme, kinesin (Vale et al., 1985*a*), binds microtubules in a nucleotide sensitive fashion, and displays a microtubule-activated ATPase activity that drives movement of kinesin-coated objects towards the plus end of microtubules (MTs)[1] in vitro (Vale et al., 1985*a*, *b*; Scholey et al., 1985; Brady, 1985; Kuznetsov and Gelfand, 1986; Porter et al., 1987; Cohn et al., 1987; Saxton et al., 1988; Gelles et al., 1988). Kinesin from a variety of animals contains a major polypeptide with an apparent molecular mass of 110–140 kD, encoded by a single gene in *Drosophila* (Yang et al., 1988), plus copurifying light chains of M_r = 40–80 kD (Vale et al., 1985*a*,*b*; Amos, 1987; Kuznetsov and Gelfand, 1986; Saxton et al., 1988). Bovine brain kinesin is an $\alpha_2\beta_2$ heterotetramer consisting of two 120-kD heavy chains plus two 60-kD light chains, assembled into an elongated molecule (Amos, 1987; Kuznetsov et al., 1988; Bloom et al., 1988). Kinesin is proposed to participate in organelle/vesicle transport, in organizing the endomembrane system, and in mitosis (Vale et al., 1986; Vale, 1987; Leslie et al., 1987; Schroer and Sheetz, 1988), but direct evidence concerning the biological functions of kinesin is currently lacking.

Antibodies that inhibit mechanochemical activity have been extremely useful in probing the functions of myosin and dynein. For example, an antiserum that inhibits the ATPase activity of sea urchin sperm dynein caused a corresponding decrease in the beat frequency of reactivated sea urchin sperm, supporting the hypothesis that the dynein ATPase drives axonemal motility (Ogawa and Mohri, 1975; Okuno et al., 1976; Gibbons et al., 1976). In addition, polyclonal and monoclonal myosin antibodies have been used to inhibit the actin activated ATPase activity of myosin, (Mabuchi and Okuno, 1977; Kiehart and Pollard, 1984*b*; Peltz et al., 1985) to block myosin-driven motility in vitro (Kiehart and Pollard, 1984*b*; Flicker et al., 1985) to examine the relationship between myosin polymerization and ATPase activity (Kiehart and Pollard, 1984*a*) as well as to probe the conformation and polymerization state of myosin (Citi and Kendrick-Jones, 1987). Inhibitor myosin antibodies have provided evidence for the role of myosin in actin-based organelle transport (Adams and Pollard, 1986) and in cytokinesis (Mabuchi and Okuno, 1977; Kiehart et al., 1982).

Kinesin antibodies have been used to establish immunological relatedness between kinesin from various cell types, as well as to identify, purify, and localize their corresponding antigens (Scholey et al., 1985; Vale et al., 1985*b*; Porter et al., 1987; Leslie et al., 1987; Saxton et al., 1988; Kuznetsov et al., 1988; Neighbors et al., 1988). However, none of these antikinesins was reported to inhibit kinesin activity. For example, antibodies raised against the 130-kD subunit of sea urchin egg kinesin prepared by preparative SDS gel electrophoresis and thereby denatured (Scholey et al., 1985), had no detectable effect on kinesin-driven microtubule motility in vitro (Scholey, J. M., and M. E. Porter, unpublished observations).

1. *Abbreviations used in this paper*: MT(s), microtubule(s); PMEG, 0.9 M glycerol, 0.1 M Pipes, pH 6.9, 5 mM EGTA, 2.5 mM $MgSO_4$, 0.5 mM EDTA, 1 mM DTT, 100 μg/ml soybean trypsin inhibitor, 1 mg/ml *p*-tosyl-L-arginine methyl ester hydrochloride, 10 μg/ml leupeptin, pepstatin, and aprotinin; RT, room temperature.

We report here the preparation and characterization of monoclonal antibodies that were raised in animals immunized using native kinesin as antigen. Amongst these are antibodies that serve as potent inhibitors of the kinesin driven translocation of microtubules over glass surfaces in vitro.

Materials and Methods

Materials and General Methods

Sea urchins were obtained from Marinus (Long Beach, CA) and handled as described previously (Scholey et al., 1984, 1985; Porter et al., 1987, 1988). Taxol was a gift of Dr. M. Suffness at the National Products Branch, National Cancer Institute. GTP was obtained from Boehringer Mannheim Biochemicals, Indianapolis, IN. Other chemicals were purchased from Sigma Chemical Co., St. Louis, MO. Chromatography resins and antibody conjugates were usually from Bio-Rad Laboratories, Richmond, CA. Cellulose phosphate (P11) and DEAE cellulose (DE 52) resins were from Whatman Inc., Hillsboro, OR.

General biochemical procedures were carried out as described previously (Scholey et al., 1984, 1985, 1988; Porter et al., 1987, 1988; Cohn et al., 1987). Immunological procedures were carried out following the advice of colleagues, in particular Drs. John Kappler and Philippa Marrack, National Jewish Center, Denver, CO, and methods cited in the following literature: Hudson and Hay, 1980; Kiehart et al., 1984*a*, 1986; Peltz et al., 1985; Citi and Kendrick-Jones, 1987.

Purification of Sea Urchin Egg Kinesin Antigen

Purification of kinesin was carried out essentially as described previously (Scholey et al., 1985; Cohn et al., 1987). Sea urchin eggs were homogenized in PMEG extraction buffer (0.9 M glycerol, 0.1 M Pipes, pH 6.9, 5 mM EGTA, 2.5 mM $MgSO_4$, 0.5 mM EDTA, 1 mM DTT, 1 mg/ml *p*-tosyl-L-arginine methyl ester hydrochloride, 100 μg/ml soybean trypsin inhibitor, 10 μg/ml leupeptin, pepstatin, and aprotinin), and centrifuged to yield the "high speed extract supernatant" (HSS), which was treated with hexokinase, glucose, taxol/GTP, plus AMPPNP and the resulting microtubules were pelleted. The MTs were washed in extraction buffer supplemented with 20 mM EDTA plus 2 mM Mg-free ATP, then centrifuged again at 55,000 *g* for 20 min, 4°C. The pellet was resuspended in PMEG containing 10 mM ATP, 100 μM GTP, 100 mM KCl, 10 μM taxol plus 10 mM $MgSO_4$, incubated overnight on ice, supplemented with 5 mM MgATP, then spun to pellet the MTs. Microtubule-associated proteins in the supernatant were loaded onto a 1.6 × 17.5 cm gel filtration column (Biogel A5M; Bio-Rad Laboratories). The kinesin fractions were further purified by addition of 1.5 mg/ml phosphocellulose-purified bovine brain tubulin (polymerized with 20 μM taxol and 0.5 mM GTP for 15 min at 22–25°C), 20 mM EDTA, and 2 mM ATP, and incubated for 20 min before centrifugation at 45,000 *g*, 20 min, 20°C. The kinesin-microtubule pellet was resuspended in PMEG plus 10 mM ATP, 12.5 mM $MgSO_4$, 30 μM taxol, and 0.1 mM GTP for 30 min, room temperature (RT), then centrifuged again at 45,000 *g*, 20 min, 20°C to yield purified kinesin in the supernatant. This recycled kinesin was used as antigen.

Monoclonal Antibody Production

Four BALB/c mice were immunized subcutaneously in four spots (hips and shoulders) with 50 μg ATP/EDTA recycled kinesin emulsified 1:1 (vol/vol) with complete Freund's adjuvant. 2 wk later the mice were boosted intraperitoneally with 20 μg kinesin emulsified 1:1 (vol/vol) with incomplete Freund's adjuvant. 2 wk after the first boost, the mice were bled from the tail and their serum was tested using an ELISA and Western blotting for antibodies against the 130-kD subunit of kinesin. Each mouse was boosted and bled until its antikinesin titer was a minimum of 1:1,000. The mice were rested for at least 3 wk after the last boost, before a final immunization of 50 μg kinesin in PMEG buffer was given intraperitoneally 3 d before they were killed.

B-cell hybridomas were produced by standard technique using polyethylene glycol fusion of splenic B cells to the variant myeloma line P3x63S-68.653 and selection in HAT medium. ELISA was used for primary screening of the hybridoma supernatants. Briefly, 1 μg of Biogel fractionated kinesin was coated to microtiter plate wells and incubated overnight at 4°C. Wells were washed with PBS, incubated with 1% BSA in PBS-0.05% Tween for 1–2 h, 20–25°C, washed again with PBS, incubated with hybridoma supernatants for 1–2 h, 20–25°C, then washed again with PBS. Goat anti-mouse IgG (H + L) horseradish peroxidase secondary was added (1:300 dilution in PBS) for 1–2 h, 20–25°C, after which the wells were washed with PBS. A substrate mixture (170 mM citric acid, 650 mM phosphate buffer, pH 6.3) was added to each well and treated with orthophenylene-diamine and H_2O_2 to develop the color. The reaction was quenched with 4.5 M H_2SO_4. Nonspecific binding was determined by reacting hybridoma supernatants to wells coated with tubulin. Positives were rescreened using ELISA assay and Western blotting before being cloned at limiting dilution. Ascitic fluid was prepared for each.

Solid Phase RIA

Assays were performed using polyvinyl chloride microtiter plates (Dynatech Corp., Alexandria, VA). 50 μl (20 μg/ml) of kinesin in PMEG was placed in the microtiter plate wells overnight at 4°C. The wells were washed with PBS after which 200 μl of blocking solution (blotto) containing 5% nonfat dry milk and 0.05% sodum azide in PBS was added for 1–2 h at 23°C. The wells were again washed with PBS, incubated with 50 μl monoclonal antibody solution (diluted in blotto) for 1–2 h, at 23°C, washed again with PBS, then incubated with 50 μl 20 μg/ml ^{125}I-goat anti-mouse Ig at a specific activity of ~25,000 cpm/μg for 1–2 h. The wells were washed again with PBS before separation of the wells with scissors. Gamma counting for bound radioactivity was performed (gamma 300; Beckman Instruments Inc., Palo Alto, CA). The level of nonspecific binding was determined by counting wells coated with BSA (20 μg/ml) or tubulin (20 μg/ml), and wells coated with kinesin incubated with a nonspecific control antibody KJ23 (Kappler et al., 1987).

Competitive Binding Radioimmunoassay (RIA)

Purified antibodies were iodinated using an iodination Reagent (IODOGEN, Pierce Chemical Co., Rockford, IL), 1,3,4,6-tetrachloro 3α 6α-diphenyl glycoluril from Pierce Chemical Co. (Markwell and Fox, 1978). Competitive RIAs were done by modification of the methods of Kiehart et al. (1986) and Peltz et al. (1985). Briefly, 50 μl of 20 μg/ml kinesin was placed in microtiter plate wells (Dynatech Corp.) for 2 h at room temperature. The wells were washed in PBS after which 200 μl blocking solution (blotto) was added for 1 h at room temperature. The wells were again washed with PBS, then incubated with 50 μl 400 μg/ml "blocking" antibody, 30 min, room temperature. 50 μl of 20 μg/ml ^{125}I-labeled challenge antibody at 1–2,000 cpm/μl was added. After 1 h the wells were washed with PBS before separation of the wells with scissors and gamma counting. Nonspecific binding was determined by counting microtiter plate wells coated with BSA (20 μg/ml) and nonspecific blocking levels were determined with 400 μg/ml mouse IgG (Jackson Immunoresearch Lab. Inc., West Grove, PA).

Chymotryptic Digestion of Kinesin and Analysis of Fragments by Immunoblotting

Kinesin–microtubule complexes were prepared from cytosolic extracts in AMPPNP, then washed in PMEG buffer. 2 mg/ml microtubule–kinesin complexes were digested with 1 μg/ml α-chymotrypsin (type I-S from bovine pancreas; Sigma Chemical Co.) for 20 min, RT, in order to protect the microtubule binding domain on kinesin during digestion. Then the reaction was quenched with 3 mM PMSF. The resulting fragments were subjected to SDS-PAGE and immunoblotting with rabbit polyclonal antikinesin, and SUK 1-7. To analyze ATP-sensitive MT binding by the 45-kD fragment, Biogel fractionated kinesin (100 μg/ml) in PMEG + 5 mM MgATP was incubated with 2 μg/ml chymotrypsin for 20 min, RT in total volume ≅ 3 ml. PMSF (1 mM) and Triton X-100 (0.1%) were added, then 3 × 1 ml aliquots were supplemented with taxol (30 μM) and GTP (0.1 mM) plus; (*a*) 1 mg/ml tubulin/10 mM MgATP; (*b*) 1 mg/ml tubulin/10 mM AMPPNP; or (*c*) no microtubules with or without ATP or AMPPNP. Each aliquot was centrifuged to pellet microtubules; supernatants were concentrated to 50 μl in centricons, then supernatants and pellets were mixed with 100 μl SDS gel sample buffer and analysed on Coomassie Blue–stained SDS gels and SUK 4 immunoblots to determine if the 45-kD fragment had bound and cosedimented with MTs.

Antibody Isotyping

Monoclonal antibodies were initially isotyped using Ouchterlony double diffusion analysis (1% agarose in PBS) against rabbit Ig typing sera (a gift of Dr. Ralph Kubo, National Jewish Center) for mouse IgG1, IgG2A, IgG2B, IgM, and IgA. Cloned hybridoma supernatants were concentrated

~50-fold by precipitation against 50% ammonium sulphate followed by resuspension and dialysis into PBS. Isotyping and determination of protein A binding was also performed by immunoprecipitations using anti-mouse Ig or protein A coupled to agarose beads. Briefly, 1 ml of 3 mg/ml ammonium sulphate cut hybridoma culture supernatants were mixed with 25 μl beads in PBS for 2 h at room temperature. The beads were washed four times in PBS, once in distilled water, boiled in 50 μl Laemmelli gel sample buffer, clarified, and run on SDS-PAGE to detect immunoglobulin polypeptides that bind and cosediment with the beads. Confirmation of antibody isotype was done by analysis of purified preparations on SDS gels, where the IgM (μ = 70 kD) and IgG (γ = 50 kD) heavy chains could be distinguished.

Antibody Purification

SUK 1, 3, 6, and 7 do not bind efficiently to protein A under our conditions. These Igs were prepared from ascitic fluid as follows: 5 ml ascitic fluid was strained through glass wool, adjusted to pH 5 by addition of 1 M sodium acetate pH 5, then incubated with 1/20 vol octanoic acid for 30 min at room temperature, clarified 10,000 *g*, 15 min, then an equal volume of saturated ammonium sulphate was added. The crude Ig was pelleted at 10,000 *g*, 15 min, and resuspended in appropriate buffer. SUK 1 and SUK 3 (IgMs) were further purified by dialysis against low ionic strength buffer, and SUK 6 and SUK 7 by DE 52 ion exchange chromatography, essentially as described by Kiehart et al. (1986). The octanoic acid treatment at pH 5 was omitted for SUK 6, since the yield was greatly decreased by this step.

Those monoclonal antibodies (SUK 2, 4, 5) which bound to protein A under our conditions were purified on a large scale basis via Affi-gel protein A MAPS II (Monoclonal Antibody Purification System; Bio-Rad Laboratories) kit from ascitic fluid. Absorbance (A280) was used to calculate the concentration of the purified antibody using an extinction coefficient of 1.4 for IgG at A280. Antibodies were stored at 4°C (short term) and at −20° or −70°C (long term). Proteolytic fragments [Fab and $F(ab)_2$] of SUK 2 and SUK 4 were prepared and screened using PAGE under reducing and nonreducing conditions, as described by Parham, 1986.

Electrophoresis and Blotting

Samples on SDS gels were transferred onto nitrocellulose (Schleicher & Schuell, Inc., Keene, NH), stained with Ponceau S (Sigma Chemical Co.), destained, then incubated in solutions of primary antibody diluted into 5% nonfat dry milk in PBS-0.05% Tween overnight at 4°C or for 1 h at 37°C, then washed with several changes of PBS-0.05% Tween. The secondary antibody (either affinity-purified goat anti-mouse IgG [H + L] or goat anti-rabbit IgG [H + L] horseradish peroxidase conjugate [Bio-Rad Laboratories]) was then applied at a 1:1,000 dilution in 5% nonfat dry milk in PBS-0.05% Tween. Incubation was the same as primary antibody. The blots were washed again with PBS-0.05% Tween followed by Tris-saline, and developed by addition of 4 chloro-1-naphthol with H_2O_2.

Motility Assays

The ability of test antibodies to inhibit MT motility was assayed using video-enhanced microscopy and computer-assisted velocity analysis of kinesin/MT samples as described previously (Cohn et al., 1987). 14 μl of 100–300 μg/ml kinesin solutions were adsorbed onto coverslips for 10–20 min at room temperature in a humidified chamber before addition of the test antibodies. Antibody solutions were then added onto the coverslip for an additional 10–20 min. Phosphocellulose-purified bovine brain MTs (to 20 μg/ml) and MgATP (to 10 mM) were added, and the sample sealed to a slide using VALAP (Vaseline/lanoline/paraffin; 1:1:1; wt/wt/wt) and observed with the video-enhanced microscope. The velocity of 10–20 microtubules randomly selected from several microscopic fields was measured for each data point. The velocity of each MT was determined over an interval of at least 10 s. Each set of data points and standard deviations on Figs. 7 and 8 are from single typical experiments that have been reproduced many times. Tests using culture supernatants required using relatively large volumes of antibody solution in the assay (>5 μl) and for these samples the kinesin/antibody solution was removed after incubation and replaced with 14 μl of PMEG buffer before MT and MgATP addition. Controls using such large volumes of PMEG buffer alone were performed to determine that motility inhibition was not due to dilution effects. Solutions of monoclonal antibodies purified from ascitic fluid were at sufficiently high concentrations that only small (1-2 μl) volumes were added, and removal of the kinesin/antibody mixture was not required.

Results

Preparation of Mouse Monoclonal Antikinesins

Native sea urchin egg kinesin was prepared by microtubule affinity binding and Biogel A5M chromatography as described previously (Scholey et al., 1985) followed by an additional cycle of microtubule binding in EDTA/ATP and release in MgATP. This material contained contaminating tubulin, and was partially depleted of the putative 75-kD light chains (1 mol 130 kD: 0.2 mol 75 kD: Fig. 1, lane 2), but retained microtubule-translocating activity.

We prepared monoclonal antibodies from mice immunized using such native sea urchin egg kinesin (Fig. 1, lane 2), and seven hybridomas (SUK 1–7) which secrete antibodies to the antigen were produced. The properties of the SUK 1–7 antibodies are summarized in Table I. The hybridomas were cloned, and their antibodies isotyped (SUK 1, 3 = IgM; SUK 5 = IgG2b; SUK 2, 4, 6, 7 = IgG1), and assayed for binding to protein A (SUK 2, 4, 5 bind efficiently under conditions used, others weakly [SUK 7] or not detectably). Hybridoma clones were grown as ascitic tumors, and the immunoglobulin was purified from the ascitic fluid by successive steps of octanoic acid clarification, ammonium sulphate precipitation, and chromatography on protein A agarose or DEAE cellulose (Kiehart et al., 1986). Purity of the antibodies was confirmed on SDS-polyacrylamide gels (data not shown).

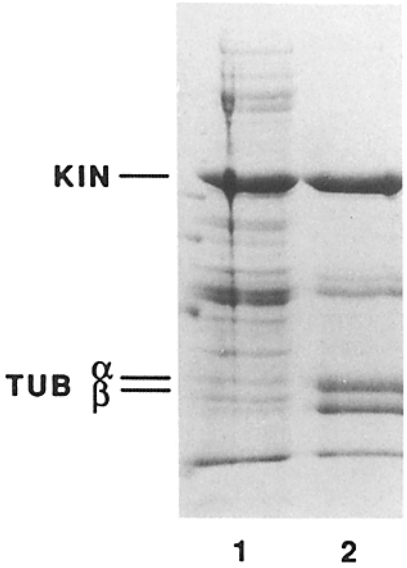

Figure 1. Purification of sea urchin egg kinesin antigen. Pooled Biogel A5M-fractionated kinesin samples (lane *1*) were prepared by AMPPNP-MT affinity binding, "EDTA/ATP washing" of the MT-kinesin complexes, MgATP extraction, and Biogel A5M chromatography. This material was further purified by incubation with phosphocellulose-purified taxol-stabilized MTs and 1 mM ATP + 10 mM EDTA. The MT/kinesin mix was centrifuged, and the MT pellets extracted in 10 mM MgATP. The extract supernatant (lane 2) was significantly purified, containing predominantly 130-kD kinesin heavy chain (*KIN*), unpolymerized tubulin (*TUB*), and minor polypeptides in the 75–80-kD range.

Table I. Kinesin 130-kD Monoclonal Antibodies

Antibody	Isotype	Bind protein A	Cross-reaction on immunoblots				Inhibits motility
			U	B	S	D	
SUK 1	IgM	–	+	–	–	–	–
SUK 2	IgG1	+	+	–	–	–	–
SUK 3	IgM	–	+	–	–	+	–
SUK 4	IgG1	+	+	+	–	+	+
SUK 5	IgG2b	+	+	+	–	–	–
SUK 6	IgG1	–	+	+	–	–	+
SUK 7	IgG1	Weak	+	+	–	–	+

Summary of the properties of SUK 1-7. *U*, urchin; *B*, bovine; *S*, squid; *D*, *Drosophila*.

Characterization of Binding of SUK 1-7 to Sea Urchin Egg Kinesin

We investigated binding of SUK 1-7 to kinesin using a number of procedures. Solid phase radioimmunoassays showed that SUK 1-7 bind to purified sea urchin egg kinesin under conditions where a control antibody (KJ23 monoclonal anti-T-cell receptor) exhibits no detectable binding (Fig. 2). The monoclonals differed in their apparent affinity for kinesin, however. SUK 2 and SUK 4 appeared to bind most efficiently, exhibiting a plateau of binding extending down to 0.1 and 1 μg/ml, respectively; with SUK 6 and SUK 7, a plateau was observed above 100 μg/ml and with SUK 1, 3, and 5 which appeared to have lower affinity for kinesin in RIAs, no plateau was seen under the conditions used. We also examined the ability of SUK 1-7 to bind to native sea urchin egg kinesin in immunoprecipitation experiments; immune complexes were precipitated with protein A or goat anti-mouse Ig coupled to agarose beads. SUK 2, SUK 4, and SUK 5 (which binds weakly in RIAs) bound most efficiently to kinesin, forming immune complexes that could be precipitated by protein A or anti-mouse Ig agarose. The other SUK antibodies appeared to bind more weakly in assays using either protein A or antimouse Ig beads to precipitate the immune complexes (data not shown).

Immunoblotting was used to further analyze the specificity of binding of SUK 1-7 to sea urchin egg kinesin. Routinely, blots of sea urchin egg cytosolic extracts, microtubules prepared in the presence or absence of AMPPNP, or purified kinesin were probed with 1-10 μg/ml SUK 1-7, and typical results are shown in Fig. 3. All antibodies exhibit strong staining of the 130-kD kinesin heavy chain in these fractions, although they differ in their degree of specificity (Fig. 3). SUK 2-7 typically bind only to the 130-kD subunit but they sometimes also bind to low molecular polypeptides in the AMPPNP-microtubule preparations. The variable occurrence of these smaller species leads us to believe that they are proteolytic fragments of the kinesin heavy chain. In contrast, SUK 1 (an IgM) consistently binds to a variety of polypeptides in addition to the 130-kD subunit (Fig. 3), but the significance of this observation is not known. We did not obtain antibodies to the 75-kD polypeptides which were present

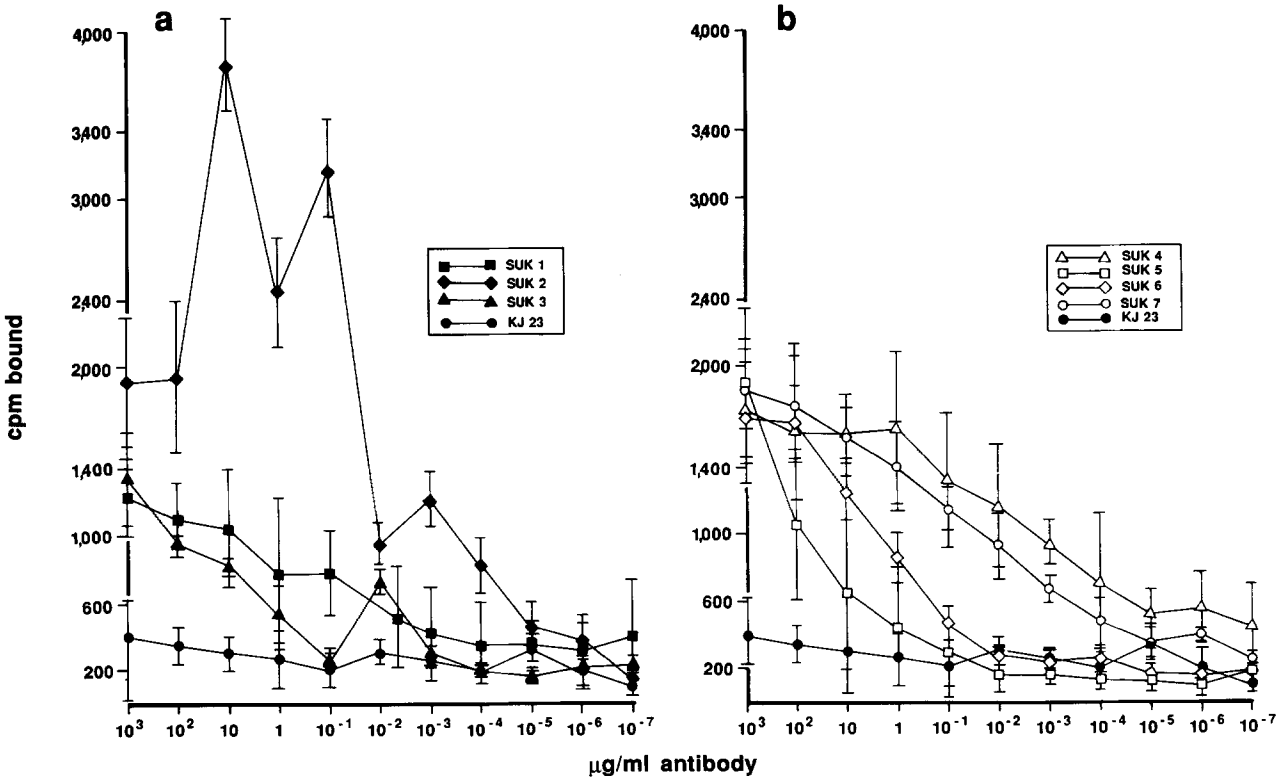

Figure 2. Titration of SUK 1-7 binding to sea urchin egg kinesin using solid phase radioimmunoassays (RIAs). Assays were done as described by Peltz et al., 1985. SUK 1-3 are shown on the left (*a*) and SUK 4-7 are shown on the right (*b*). The KJ23 anti-T-cell receptor antibody (Kappler et al., 1987) was used as a control and gave no significant signal above background. In RIAs with bovine serum albumin or bovine brain tubulin adsorbed to the microtiter plates instead of kinesin, the SUK 1-7 antibodies gave no signal above background (range = 100-300 cpm; see Fig. 4).

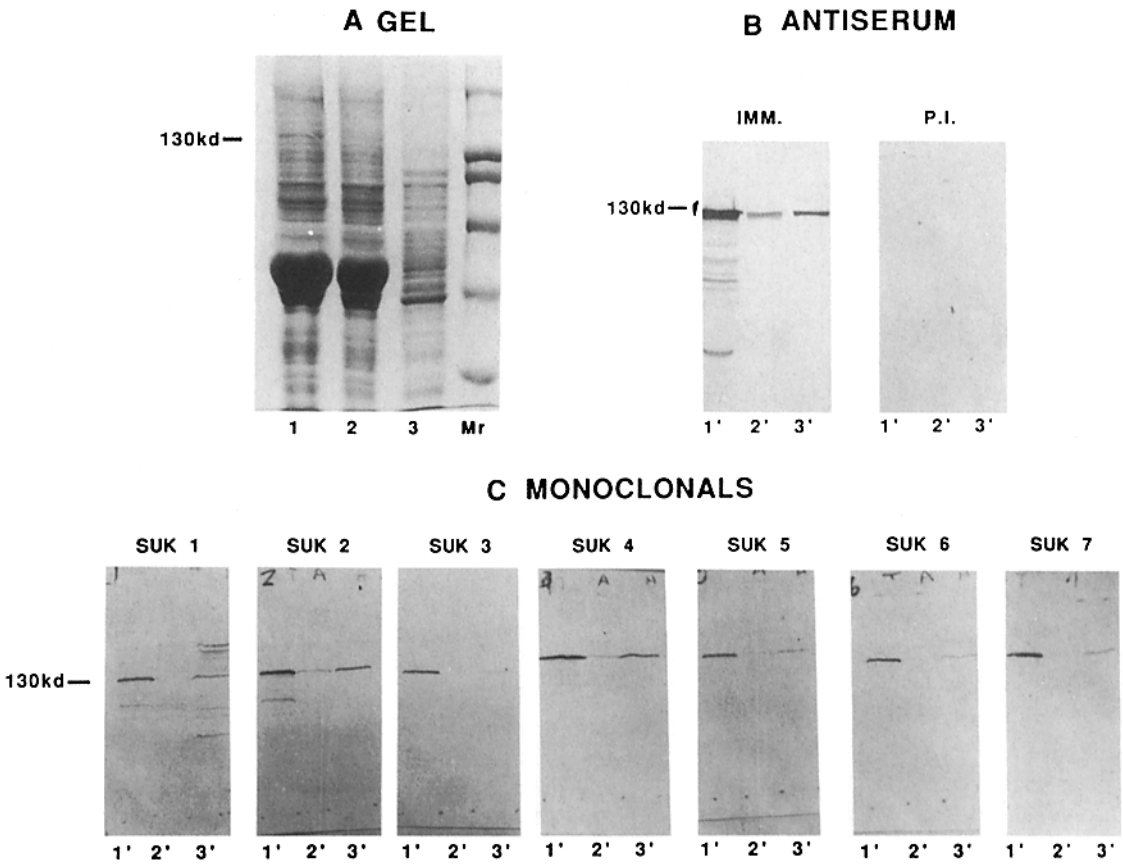

Figure 3. Immunoblots demonstrating the binding of polyclonal antikinesin and monoclonals SUK 1-7 to the 130-kD heavy chain of sea urchin egg kinesin. The *A GEL* shows a Coomassie Blue-stained SDS gel of relative molecular mass standards (*Mr*), *S. purpuratus* microtubules prepared in the presence (*1*) and absence (*2*) of AMPPNP, and a sea urchin egg high speed cytosolic extract supernatant (*3*). Proteins in lanes *1*, *2*, and *3* from parallel gels were transferred to nitrocellulose (*1'*,*2'*,*3'*, respectively). The blots in *B* were probed with a 1:3,000 dilution of serum from rabbits immunized with native sea urchin kinesin (*IMM.*), or a 1:1,000 dilution of corresponding preimmune serum (*P.I.*). The blots in *C* were probed with 1 μg/ml SUK 1-7 as indicated. The following control antibodies displayed no reaction with the 130-kD kinesin heavy chain; 5 μg/ml mouse monoclonal anti-T-cell receptor (KJ 23) (Kappler et al., 1987), 1 μg/ml mouse gamma globulin, and 1 μg/ml each of mouse IgM, mouse IgG1, and mouse IgG2B fractions.

in small quantities in the antigen (Fig. 1, lane *2*) and are candidates for sea urchin egg kinesin light chains.

SUK 4, SUK 6, and SUK 7 Bind to Spatially Related Sites on the 130-kD Heavy Chain

Competitive binding RIAs (Peltz et al., 1985; Kiehart et al., 1986) were used to identify SUK antibodies that bind to topographically related sites on the sea urchin egg kinesin heavy chain (Fig. 4). We compared SUK 4, SUK 6, and SUK 7 (which inhibit motility; see below) with SUK 2, but we did not analyze SUK 1, 3, and 5 which bind relatively poorly to kinesin in RIAs (Fig. 2). Radioiodinated SUK 2, 4, 6, and 7 bound to kinesin but not to BSA coated onto microtiter plates (Fig. 4). ^{125}I-SUK antibody binding to kinesin was not blocked by an unlabeled control antibody, but in all cases binding was reduced in the presence of corresponding unlabeled antibody (Fig. 4). Binding of ^{125}I-SUK 2 to kinesin was not inhibited by SUK 4, 6, or 7, and vice versa, indicating that SUK 2 binds to a site on the 130-kD heavy chain that is spatially distinct from the binding sites of SUK 4, 6, and 7. In contrast, SUK 4, 6, and 7 all inhibited each other's binding to the kinesin-coated microtiter plates (Fig. 4) indicating that these three antibodies bind to spatially related sites on the 130-kD heavy chain.

Limited chymotryptic digestion of sea urchin egg kinesin-microtubule complexes produced 130-kD fragments of 76 and 45 kD which were both stained using rabbit polyclonal antikinesin on immunoblots (Fig. 5). SUK 4, SUK 6, and SUK 7 all bound to the 45-kD chymotryptic fragment on immunoblots, whereas the other monoclonal antikinesins recognized neither fragment. The results suggest that SUK 4, SUK 6, and SUK 7, which inhibit kinesin-driven motility, bind to spatially related sites located on the 45-kD kinesin subfragment.

Cross-reactivity of SUK 1-7

Immunoblotting was used to examine the binding of SUK 1-7 to the heavy chains of kinesins isolated from sea urchin eggs, squid brain, *Drosophila* embryos and vertebrate (chick and bovine) brain (Fig. 6). The antibodies varied in their degree of cross-reactivity towards the kinesins used; SUK 1 and 2 appear to bind an epitope that is sea urchin specific; SUK 3 binds urchin and *Drosophila* kinesin heavy chains; SUK 5, 6, 7 bind to both urchin and bovine kinesin heavy chains;

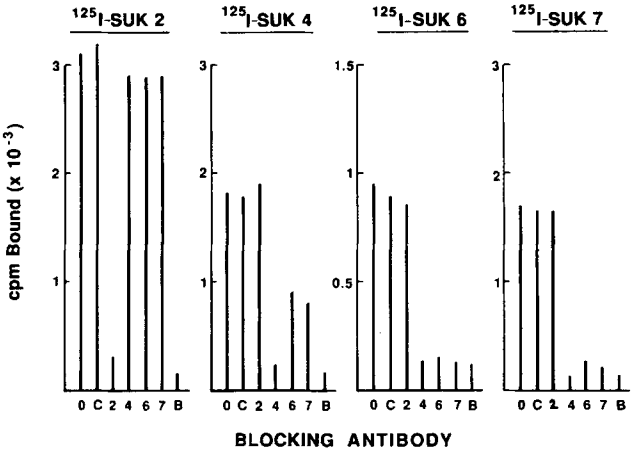

Figure 4. Solid-phase competitive binding RIAs to show that SUK 4, SUK 6, and SUK 7 bind to topographically related sites on the 130-kD kinesin heavy chain. Purified sea urchin egg kinesin (1 μg, Fig. 1, lane 2) was adsorbed onto wells of microtiter plates, and incubated with 1 μg ^{125}I-SUK antibody plus 20 μg unlabeled "blocking" antibody. The blocking antibodies were: *0*, none; *C*, control IgG; *2*, SUK 2; *4*, SUK 4; *6*, SUK 6; and *7*, SUK 7. *B* is a control that shows binding of ^{125}I-SUK antibody to a well coated with BSA instead of kinesin (no blocking antibody).

finally SUK 4 is the most cross-reactive member of our panel of antibodies, and it binds to related epitopes on the heavy chain of sea urchin egg, bovine brain, and *Drosophila* embryo kinesins. Interestingly, none of our monoclonal antibodies cross-react with squid brain kinesin.

Effects of SUK 1–7 on Kinesin-induced Microtubule Translocation

Purified SUK 1–7 were screened for their ability to inhibit kinesin-driven motility by analyzing their effects on the velocity of microtubule translocation over kinesin-coated glass surfaces using video-enhanced differential interference contrast light microscopy. When tested at concentrations of 100 μg/ml, SUK 4, 6, and 7 caused a complete inhibition of microtubule movement (Table II). In contrast, SUK 1, 2, 3, and 5 had negligible effects on the velocity of microtubule gliding.

We have compared the effects of protein A–agarose–purified SUK 2 and SUK 4 on motility in some detail (Fig. 7) because they bind efficiently to the sea urchin egg kinesin heavy chain in all assays used but they appear to bind to spatially distinct sites. In addition, on two-dimensional gels, the heavy chain of purified sea urchin egg kinesin formed an asymmetric spot which was stained identically with SUK 2 and SUK 4 on immunoblots (Willy, P. J., unpublished observation. We think that any differences in the effects of these antibodies on kinesin activity do not reflect an inability of either antibody to bind to the sea urchin egg kinesin being assayed.

SUK 4 caused a dose-dependent inhibition in the velocity of sea urchin egg kinesin-induced microtubule gliding, causing a 50% reduction in rate of gliding at ~5 μg/ml, whereas SUK 2 did not reduce the velocity of movement at concentrations as high as 330 μg/ml (Fig. 7).

To further analyze these effects of SUK 2 and SUK 4 on motility, we prepared univalent and divalent proteolytic fragments from purified IgGs, demonstrated that they displayed

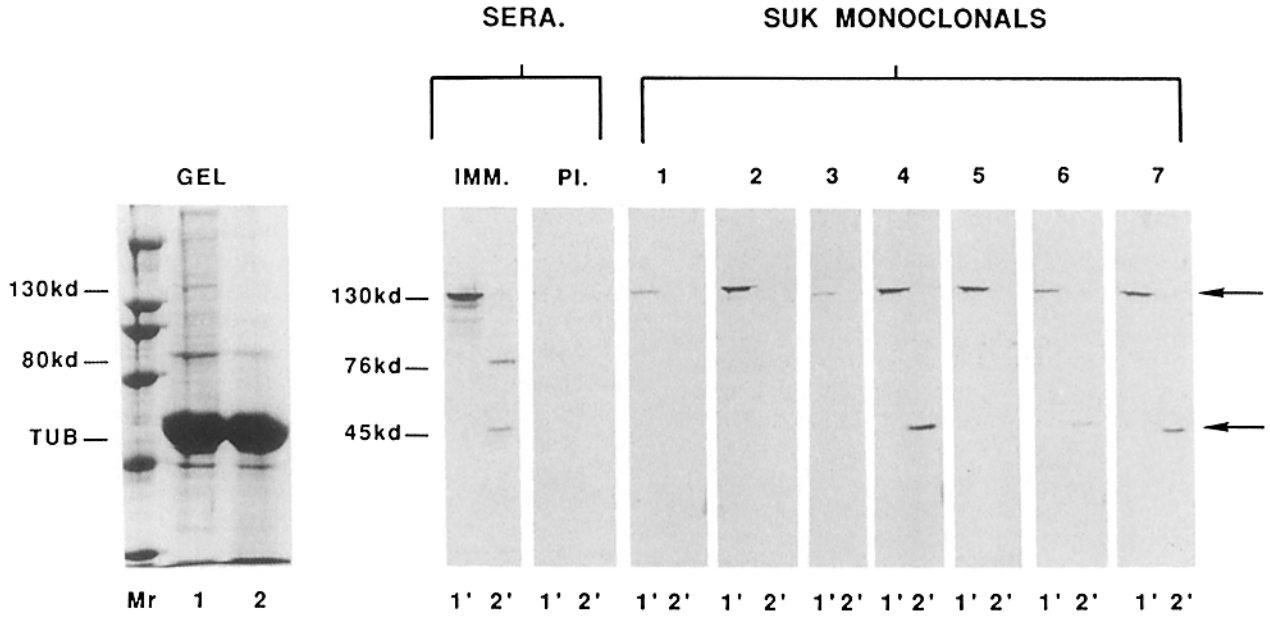

Figure 5. Immunoblotting experiment to show that SUK 4, SUK 6, and SUK 7 bind to the same chymotryptic fragment of the 130-kD heavy chain. Kinesin bound to MTs in AMPPNP was digested with α-chymotrypsin (0.05% enzyme: protein wt/wt) for 20 min, RT, or incubated in buffer alone. Control microtubule-kinesin complexes (lanes *1*,*1'*) and chymotryptic digest (*2*,*2'*) were run on SDS-PAGE (*GEL*), transferred to nitrocellulose, and probed with kinesin antibodies: *IMM.*, 1:1,000 dilution of rabbit polyclonal antikinesin; *PI.*, 1:1,000 dilution of corresponding preimmune serum; monoclonal antikinesins SUK 1–7 are as indicated. The polyclonal antikinesin binds to the 130-kD heavy chain, and chymotryptic fragments of $M_r \cong 76$ and $\cong 45$ kD. SUK 4, 6, and 7 react with the 45-kD fragment, but no reaction with either fragment could be seen using the other monoclonals.

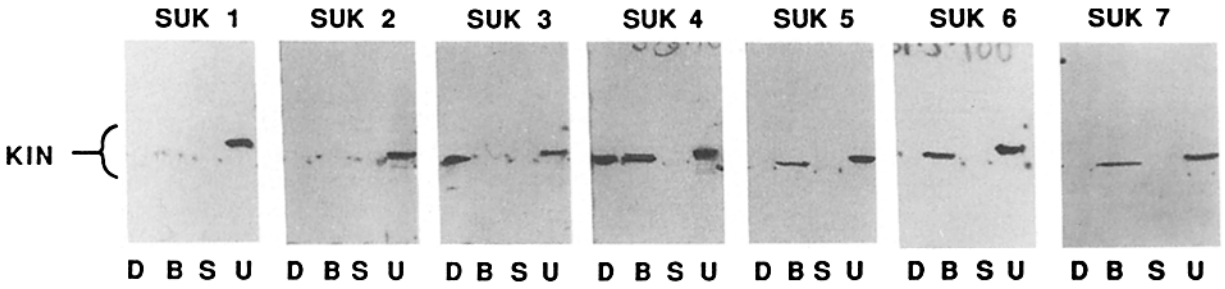

Figure 6. Immunoblots demonstrating the binding of SUK 1-7 to the heavy chains of kinesins isolated from phylogenetically diverse animal types. Preparations of sea urchin egg (*U*), squid brain (*S*), bovine brain (*B*), and *Drosophila* embryo (*D*) kinesins were subjected to SDS-PAGE and immunoblotting with SUK 1-7. The antikinesins were tested at a range of concentrations between 50 ng/ml and 50 μg/ml. SUK 1-7 all reacted with the 130-kD subunit of sea urchin egg kinesin at the lowest concentrations used (50 ng/ml). In some cases, the cross-reaction shown here was observed at higher antibody concentrations, as follows: 2.5 μg/ml for SUK 5 vs. *B*; >20 μg/ml for SUK 6 vs. *B*; >10 μg/ml for SUK 7 vs. *B*. The minimum concentration of antibody required was not determined, however. No staining was observed using the anti-T-cell receptor, KJ23 (Kappler et al., 1987) as a control at 50 ng/ml and 50 μg/ml.

characteristic mobilities on SDS-PAGE under reducing and nonreducing conditions (Parham, 1986) and that they retained binding to the 130-kD polypeptide on immunoblots at concentrations of 1 μg/ml, before investigating their effects on kinesin-driven motility (Fig. 7). SUK 2 Fab and $F(ab)_2$, like the intact IgG, had no effect on the velocity of MT translocation. SUK 4 univalent Fab reduced the velocity of MT gliding routinely to levels of ~50% control values at saturating concentrations, whereas divalent SUK 4 $F(ab)_2$, like the intact IgG, was a potent inhibitor of motility. However, whereas saturating concentrations of SUK 4 IgG completely stopped MT gliding, in excess levels of SUK 4 $F(ab)_2$, MTs continued to glide, but at an almost imperceptibly slow rate (<0.02 μm/s). We think that SUK 4 Fabs reduce the rate of gliding by binding close to a functional domain on kinesin, and this effect is potentiated in SUK 4 IgG or $F(ab)_2$ by steric blocking or epitope cross-linking.

In motility assays performed in 10–20 μg/ml SUK 4 IgG, microtubules are attached immotile to the glass coverslip, but as the concentration of SUK 4 is increased above 50 μg/ml we observe increasing release of MTs from the glass surface. A possible explanation for these results is that SUK 4 IgG blocks motility by inducing dissociation of kinesin from the coverslip to a level below the critical concentration for MT movement (Cohn et al., 1987) or that SUK 2 has no effect on motility because of its inability to bind due to steric hindrance of the glass coverslip. However, several observations suggest that these explanations are unlikely. (*a*) We found that the inhibition of motility by SUK 4 and lack of inhibition by SUK 2 were independent of whether the antibody was incubated with the kinesin before or after the kinesin was adsorbed to the glass coverslip. (*b*) In modifications of competitive binding assays (Fig. 2) using glass coverslips as the substrate for adsorption of kinesin, ^{125}I-SUK 2 bound to the kinesin coated coverslips to a similar extent whether or not the coverslip was preincubated with 20-fold excess unlabeled SUK 4 for 1 h and rinsed three times with PBS to remove unbound protein. (*c*) We observed that intermediate concentrations of SUK 4 (<10 μg/ml) caused reduced velocities of MT translocation with unimodal distributions. This is in contrast to the effect seen at limiting concentrations of kinesin in dilution experiments, where intermediate velocities are not observed and individual MTs are either immotile or moving at control velocities. (*d*) When coverslips were incubated with apyrase to deplete ATP, we observed no effect of SUK 4 on the density of immotile MTs bound to the kinesin-coated glass. (*e*) In high concentrations of SUK 4 $F(ab)_2$, no release of MTs from the kinesin-coated coverslip was observed, yet MT motility was strongly inhibited.

The inhibitory effect of SUK 4 was abolished by boiling the antibody solution for 10 min and the activity coeluted with the antibody heavy and light chains through Biogel A 1.5 M chromatography (not shown) strongly suggesting that inhibition is not due to the presence of a contaminant in our antibody preparations. Preincubation of kinesin-coated coverslips with SUK 2 had no effect on the ability of SUK 4 to inhibit kinesin-induced microtubule motility, supporting the hypothesis that SUK 4 may bind to an epitope on the kinesin molecule that is important for kinesin-induced microtubule translocation and that SUK 2 binds to a different epitope.

We compared the effects of SUK 2, SUK 4, and SUK 7 on microtubule gliding over glass induced by sea urchin egg, *Drosophila* embryo, and squid brain kinesins (Fig. 8). Sea urchin egg kinesin activity was inhibited in a similar dose-dependent manner by SUK 4 and SUK 7 but not by SUK 2. None of the antibodies bind to squid kinesin and, as ex-

Table II. Monoclonal Antikinesin Effects on ATPase and Motility

Antibody	Motility (% maximal velocity)	Microtubule-activated ATPase (% maximal activity)
None	0.77 ± 0.04 μm/s 100	70 nanomol/min per mg 100
SUK 1	100	124 (n = 2)
SUK 2	97	125 (n = 13)
SUK 3	97	112 (n = 2)
SUK 4	<2	222 (n = 19)
SUK 5	97	92 (n = 6)
SUK 6	<2	255 (n = 7)
SUK 7	<2	413 (n = 9)

Summary of effects of SUK 1-7 on the microtubule translocating and microtubule-activated ATPase activities of sea urchin egg kinesin. The kinesin was preincubated for 15 min with 100 μg/ml SUK 1-7, before addition of microtubules for assaying motility or ATPase activity. Assays were performed as described by Cohn et al. (1987).

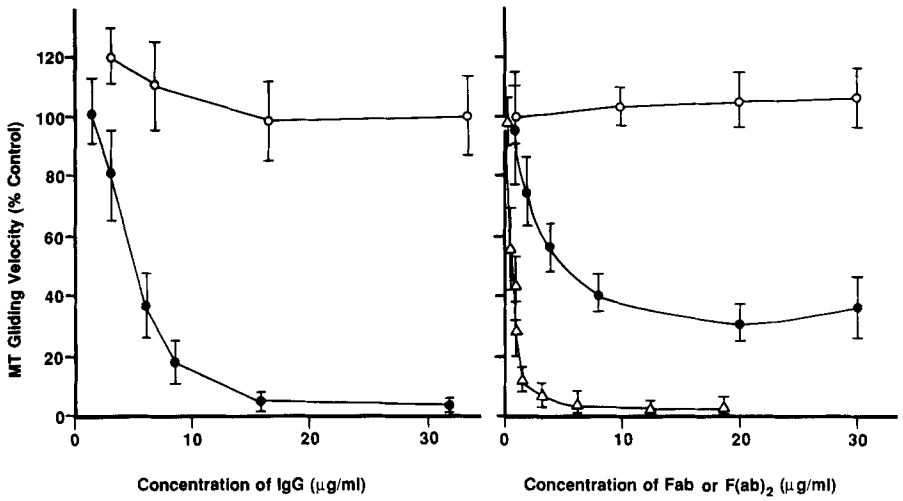

Figure 7. Effect of SUK 2 and SUK 4 on the velocity of microtubule translocation over glass driven by sea urchin egg kinesin. The effects of purified IgGs, SUK 2 (○), and SUK 4 (●) are shown on the left. Proteolytic fragments were used in the experiments on the right, namely SUK 2 Fab (○), SUK 4 Fab (●), and SUK 4 $F(ab)_2$ (△). (SUK 2 $F[ab]_2$ had no effect; data not shown.) Velocities of microtubule gliding were determined as described in Cohn et al., 1987. Control MT velocity in the experiment on the left was 0.54 ± 0.11 μm/s and on the right was 0.69 ± 0.07 μm/s (Fabs), and 0.8 ± 0.04 μm/s [$F(ab)_2$].

pected, they have no effect on the corresponding motility. Similarly, SUK 2 and SUK 7 do not bind or inhibit the activity of *Drosophila* kinesin. Interestingly, SUK 4 cross-reacts with *Drosophila* kinesin (Fig. 6), but it causes only a partial inhibition of motility (40% inhibition with no further decrease in gliding velocity with doses increased above 40 μg/ml). In experiments done in collaboration with Dr. Moshe Rozdzial, University of Colorado at Boulder, we observed no inhibition of bovine brain kinesin-driven motility by SUK 4.

Analysis of Mechanism of Inhibitory Activity of SUK 4, 6, and 7

To investigate the mechanism by which SUK 4, 6, and 7 inhibit sea urchin egg kinesin activity, we initially tested the antibody preparations for proteolytic activity directed against the kinesin heavy chain (data not shown). Kinesin was incubated with purified antibody under conditions similar to those used in the motility assays, then analyzed by immunoblotting with the polyclonal antibody raised against native kinesin. No degradation of the kinesin heavy chain could be detected, suggesting that the inhibitory activity of SUK 4, 6, and 7 is not due to the presence of contaminating proteases.

We used sedimentation assays to compare the effects of SUK 2 and SUK 4 on the nucleotide-sensitive binding of Biogel A5M chromatographed kinesin to microtubules assembled from phosphocellulose-purified bovine brain tubulin. In the absence of antibody, kinesin bound and cosedimented with microtubules in the absence but not in the presence of MgATP. Addition of SUK 2 or SUK 4 did not change the amount of kinesin heavy chain that bound and cosedimented with the microtubules under these conditions. We used gel densitometry of the kinesin heavy chain and the immunoglobulin light chains in microtubule/kinesin/SUK antibody pellets to estimate the molar stoichiometry of binding of SUK 2/kinesin and SUK 4/kinesin (Fig. 9). In both cases we observed that incubation of the kinesin-microtubule complexes with excess antibody resulted in a maximum level of 1 mol Ig heavy chain per 1 mol kinesin heavy chain in the kinesin–microtubule pellet.

We compared the effects of SUK 1–7 on the motility and the microtubule activated ATPase activities of sea urchin egg kinesin (Table II). SUK 1, 2, 3, and 5 had little effect either on the velocity of microtubule translocation or on the rate of ATP hydrolysis. Surprisingly, SUK 4, 6, and 7, which completely blocked kinesin-induced microtubule motility, did

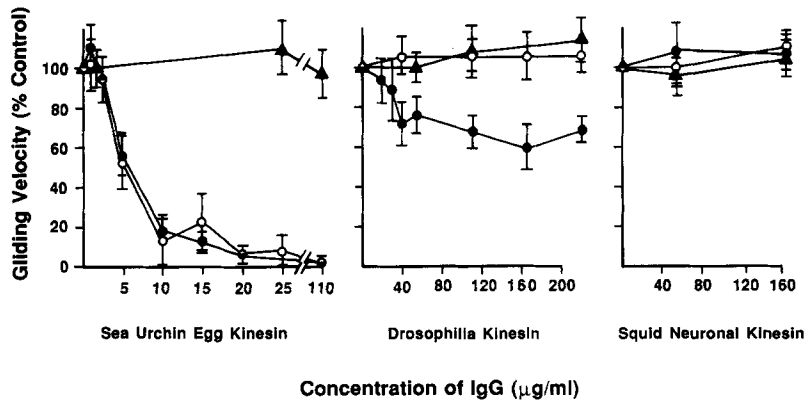

Figure 8. Effect of mAbs (SUK 2, 4, and 7) on MT motility driven by kinesin from three phylogenetically diverse animal sources. Three monoclonal antibodies raised against sea urchin egg kinesin ([▲] SUK 2; [●] SUK 4; [○] SUK 7;) were tested for their effect on MT motility using kinesin from three separate sources (sea urchin egg, *Drosophila* embryo, squid neuron). SUK 2 had no effect on egg kinesin-driven MT motility although it binds tightly to sea urchin egg kinesin. SUK 7 inhibited only sea urchin egg kinesin, while SUK 4 inhibited MT motility driven by either sea urchin egg or, to a lesser extent, *Drosophila* kinesin. None of the antibodies inhibited motility driven by squid neuronal kinesin. Note that the inhibition of *Drosophila* kinesin produced by SUK 4 does not increase at IgG concentrations above 40 μg/ml. Control velocities were 0.65 ± 0.05 μm/s (sea urchin), 0.62 ± 0.05 μm/s (*Drosophila*), and 0.70 ± 0.05 μm/s (squid).

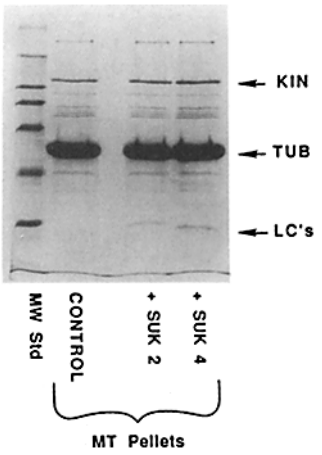

Figure 9. SDS-PAGE showing SUK 2 and SUK 4 that cosedimented with kinesin–microtubule complexes. Microtubule binding assays were performed as described previously using kinesin desalted of ATP by gel filtration. Monoclonal antibodies were incubated with Biogel A5M chromatographed sea urchin egg kinesin at a stoichiometry of at least 10 mol IgG per 1 mol kinesin for 20 min at room temperature, before addition of 1 mg/ml taxol-assembled phosphocellulose-purified bovine brain tubulin in the absence of ATP. The mixture was incubated for an additional 20 min before centrifugation at 42,000 *g* for 20 min to pellet kinesin–microtubule–SUK antibody complexes. Pellets and supernatants were analyzed on SDS-PAGE and laser densitometry to determine the molar stoichiometry. In the absence of microtubules or in the presence of MgATP, no SUK antibody polypeptides could be detected in the pellets (not shown). The gel here shows relative molecular mass standards, kinesin–MT complexes pelleted in the absence of SUK antibody (*CONTROL*), and the SUK–microtubule–kinesin complexes that pelleted in the absence of ATP and were analyzed by densitometry (*TUB*, tubulin; *KIN*, 130-kD heavy chain; and *LC*'s indicate the SUK 2 and SUK 4 IgG light chains). The excess SUK antibody remained in the supernatant.

not inhibit MT-activated ATPase activity but instead they caused a two- to fourfold increase in the microtubule activated ATPase activity of sea urchin egg kinesin (Table II). In preliminary experiments, SUK 4 Fab caused a similar stimulation of MT-activated ATPase activity. We could not detect any effect of SUK 1–7 on the ATPase activity in the absence of microtubules.

Finally, sedimentation assays were used to analyze nucleotide-sensitive MT binding by the 45-kD chymotryptic fragment to which SUK 4, 6, and 7 bind (Fig. 10). The 45-kD peptide pelleted in the presence of MTs plus AMPPNP, but not in the presence of MTs plus ATP or the absence of MTs. This nucleotide-sensitive MT binding suggests that this fragment may contain both MT and nucleotide-binding sites, and that binding of SUK 4, 6, and 7 to the region of kinesin represented by this fragment may disrupt the mechanochemical cycle of kinesin, resulting in an inhibition of motility.

Discussion

In this report we have described the preparation and characterization of antibodies that inhibit kinesin-induced microtubule motility. Our aim was to obtain antibodies that might serve as probes for the function of kinesin in blastomeres of early sea urchin embryos, where kinesin may participate in various aspects of cell motility, cell division, and intracellular transport (Scholey et al., 1985, 1988; Leslie et al., 1987; Porter et al., 1987; Cohn et al., 1987). To this end, the three monoclonal antibodies, SUK 4, 6, and 7 that disrupt kinesin activity in vitro might prove useful for disrupting kinesin function in vivo after their microinjection into living cells (Mabuchi and Okuno, 1977; Kiehart et al., 1982).

These inhibitory antibodies were raised in animals immunized using native, active kinesin as antigen, whereas polyclonal antibodies prepared against 130-kD gel bands (Scholey et al., 1985) were previously found to have no effect on kinesin-driven motility. It remains to be seen whether native kinesin antigen will generally yield a higher fraction of inhibitory antibodies than denatured kinesin. The observation that antibodies to the 130-kD polypeptide block activity supports the hypothesis that kinesin heavy chains are important for mechanochemical function. Further, our results suggest that the different SUK antibodies can differentiate between epitopes on the 130-kD polypeptide that are important for function and those that are not. The inhibitory monoclonal antibodies SUK 4, 6, and 7 inhibit each other's binding in competitive RIAs and they all bind to a 45-kD chymotryp-

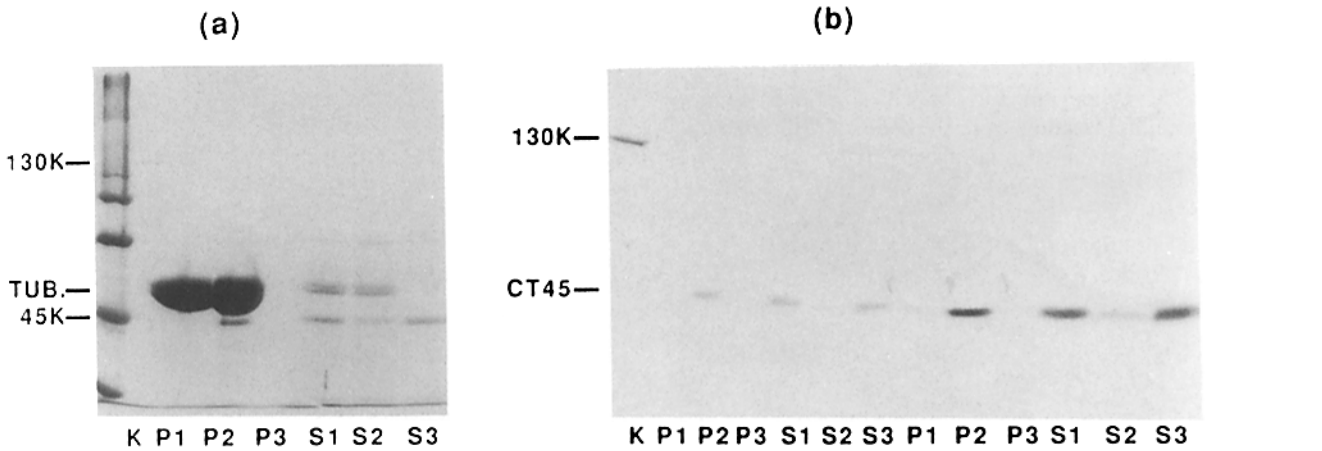

Figure 10. Inhibitory antibody, SUK 4, binds to a 45-kD chymotryptic fragment of kinesin that binds MTs in a nucleotide-sensitive fashion. Kinesin was digested with chymotrypsin and the resulting 45-kD fragment assayed for MT binding using sedimentation assays (see Materials and Methods). (*a*) Coomassie Blue–stained SDS gel; (*b*) SUK 4-probed immunoblot (duplicate samples were loaded on the blot at 2 and 20 μl). *K*, kinesin; *P1–P3*, pellets; *S1–S3*, supernatants. *P1*, *S1* = MTs + ATP; *P2*, *S2* = + MTs + AMPPNP; *P3*, *S3* = no MTs. *TUB.*, tubulin; the 130-kD heavy chain, and the 45-kD fragment of kinesin are indicated. Note that the SUK 4-reactive 45-kD fragment pellets only in the presence of MTs + AMPPNP (P2 and S2).

tic peptide fragment of the kinesin heavy chain, suggesting that they bind to spatially related sites.

The SUK monoclonal antibodies exhibit a range of cross-reactivity; SUK 2, for example, was found to be relatively specific for sea urchin egg kinesin, whereas SUK 4, the most cross-reactive of this panel of antibodies, bound to vertebrate, *Drosophila*, and sea urchin egg kinesins, but not to squid kinesin on immunoblots. The lack of cross-reactivity of any of the SUK monoclonals with squid kinesin is notable.

We characterized the effects of SUK 2 and SUK 4 on sea urchin egg kinesin activity in some detail. Both antibodies bind efficiently to the 130-kD subunit of sea urchin egg kinesin but SUK 2 has no detectable effect on motility whereas SUK 4 is a potent inhibitor of activity. The observation that SUK 2 does not reduce the inhibition of motility caused by SUK 4 supports the idea that these two antibodies bind to distinct epitopes on the 130-kD polypeptide. While SUK 4 completely inhibits sea urchin egg kinesin–induced motility, this antibody serves as a partial inhibitor of *Drosophila* kinesin activity, presumably because it partially inhibits the mechanochemical cycle of *Drosophila* kinesin.

At the present time, we do not know the mechanism by which SUK 4 inhibits kinesin-induced microtubule motility. Results with SUK 4 proteolytic fragments (Fig. 7) lead us to propose that SUK 4 Fab causes a partial inhibition of motility as a result of binding close to a functionally important domain on kinesin and its effects are potentiated by the bulkier, divalent $F(ab)_2$ and IgG as a result of cross-linking or steric blocking. The observation that SUK 4 binds and cosediments with rigor microtubule–kinesin complexes suggests that microtubules and SUK 4 bind to distinct sites on the kinesin molecule in the absence of ATP. However, SUK 4 (plus SUK 6 and SUK 7) binds to a 45-kD chymotryptic kinesin peptide that binds to microtubules in a nucleotide-sensitive fashion, suggesting that this fragment contains the microtubule and ATP-binding sites of kinesin (Fig. 10). This observation is of interest because Goldstein and colleagues have studied the nucleotide-sensitive MT-binding properties of *Drosophila* kinesin molecules containing varying sized deletions, and have concluded that the ATP- and MT-binding sites lie in a region spanning 40% of the heavy chain starting from the amino terminal (Yang et al., 1988; Goldstein, 1988). The 45-kD chymotryptic fragment that binds SUK 4, 6, and 7 may correspond to this amino-terminal region and could represent a mechanochemical domain whose activity is disrupted as a result of binding these antibodies. Further analysis of the 45-kD fragment binding site will be of obvious interest.[2]

In motility assays, at high concentrations of SUK 4 we observe increasing release of microtubules from the kinesin-coated glass surface. Our observations suggest that SUK 4 does not dissociate kinesin from glass coverslips, and the results are most consistent with the idea that SUK 4 interferes with microtubule binding in the presence of ATP, perhaps blocking the transition of attached kinesin cross-bridges from one state to another, but further work will be needed to probe this possibility.

SUK 4 (and also the other inhibitory antibodies, SUK 6 and SUK 7) does not block the microtubule-activated ATPase activity of kinesin; surprisingly, it causes a twofold activation of the rate of ATP hydrolysis. While we do not understand this effect, we believe that it does support the notion that SUK 4 exerts an effect on mechanochemical coupling. While some monoclonal antimyosins have been reported to block both motility and enzymatic activity, others have been reported to block motility without blocking the actin-activated ATPase activity. For example, antibodies M2.2, M2.15, and M2.17 to *Acanthamoeba* myosin 2 block actomyosin mediated gel contraction but not actomyosin ATPase activity (Kiehart and Pollard, 1984*b*) and antibodies My1, My5, My8, and My10 to *Dictyostelium* myosin block the translocation of myosin-coated beads along actin filaments, but not the actin activated ATPase activity of myosin (Flicker et al., 1985; Peltz et al., 1985). How all these antibodies can apparently uncouple motility from actin or microtubule activated ATPase activity is not understood.

This is the first report of antibodies that inhibit kinesin activity. While our data support the view that specific domains on kinesin heavy chains are important for mechanochemical function, we believe that another important aspect of our work is to produce reagents for investigating kinesin function in cells. Thus we are using the inhibitory SUK antibodies to analyze the role of kinesin in organelle/vesicle transport along microtubules in cell extracts (Pryer et al., 1986; Adams and Pollard, 1986) to localize important functional domains on the molecule in conjunction with electron microscopy (Kiehart et al., 1984*b*; Flicker et al., 1985) as well as to disrupt kinesin function in vivo (Mabuchi and Okuno, 1977; Kiehart et al., 1982). We anticipate that the antibodies described in this report will be useful for many cell biologists who are interested in kinesin-driven MT-based motility.

We are particularly grateful to Drs. John Kappler and Philippa Marrack for providing advice, tissue culture/animal facilities, and most importantly, enthusiastic support for our monoclonal antibody work. We also thank Janice White, Eleonora Kushnir, Ralph Kubo, Dave Hancock, John Spencer, and Michelle Pigeon for immunological help. Taxol was a gift of Dr. Matthew Suffness (Natural Products Branch, National Cancer Institute) and Drs. Bruce Schnapp, Ron Vale, Mike Sheetz, Moshe Rozdzial, and Bill Saxton provided kinesin-containing samples for analysis of antibody cross-reactivity. We are grateful to Barry Silverstein, Leigh Landskroner, and Nadia deStackelburg for their artwork, and to Shirley Downs for efficiently preparing the manuscript. A thank you also goes to Dr. Cynthia Troxell for helpful comments on the manuscript.

This work was generously supported by a grant from the American Cancer Society to J. M. Scholey (ACS #BC-530) and was aided by a March of Dimes Birth Defects Foundation Basil O'Connor starter Scholar Research award (#5), also to J. M. Scholey. S. A. Cohn was supported by an American Cancer Society postdoctoral fellowship (#PF-2925).

Received for publication 31 May 1988, and in revised form 12 August 1988.

2. After acceptance of this manuscript, we learned that Kuznetsov and coworkers (Kuznetsov, S. A., Y. A. Vaisberg, S. W. Rothwell, D. B. Murphy, and V. I. Gelfand. 1989. *J. Biol. Chem.* In press.) have found that bovine brain kinesin can be cleaved to a 45-kD fragment which displays an elevated microtubule-activated Mg-ATPase but it does not drive microtubule motion. This result complements our observation that antibodies that bind to the 45-kD domain on intact kinesin block microtubule motion and also stimulate the microtubule-activated Mg-ATPase activity. Thus the stimulation of ATP hydrolysis may be a general consequence of uncoupling ATPase activity from kinesin-driven motility.

References

Adams, R. J., and T. D. Pollard. 1986. Propulsion of organelles isolated from *Acanthamoeba* along actin filaments by myosin-1. *Nature (Lond.).* 322:754–

756.
Amos, L. A. 1987. Kinesin from pig brain studied by electron microscopy. *J. Cell Sci.* 87:105–111.
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.
Brady, S. T. 1985. A novel brain ATPase with the properties expected for the fast axonal transport motor. *Nature (Lond.).* 317:73–75.
Citi, S., and J. Kendrick-Jones. 1987. Studies on the structure and conformation of Brush Border myosin using monoclonal antibodies. *Eur. J. Biochem.* 165: 315–325.
Cohn, S. A., A. L. Ingold, and J. M. Scholey. 1987. Correlation between the ATPase and microtubule translocating activities of sea urchin egg kinesin. *Nature (Lond.).* 328:160–163.
Flicker, P. F., G. Peltz, M. P. Sheetz, P. Parham, and J. A. Spudich. 1985. Site-specific inhibition of myosin-mediated motility in vitro by monoclonal antibodies. *J. Cell Biol.* 100:1024–1030.
Gelles, J., B. J. Schnapp, and M. P. Sheetz. 1988. Tracking kinesin-driven movements with nanometer-scale precision. *Nature (Lond.).* 331:450–453.
Gibbons, B. H., K. Ogawa, and I. R. Gibbons. 1976. The effect of antidynein 1 serum on the movement of reactivated sea urchin sperm. *J. Cell Biol.* 71: 823–831.
Goldstein, L. S. B. 1988. What can molecular biology and genetics tell us about MAPs and motors? *In* Cell Movement. Vol. 2. Kinesin, Dynein and Microtubule Dynamics. F. D. Warner and J. R. McIntosh, editors. Alan R. Liss, Inc. NY. In press.
Hudson, L., and F. C. Hay. 1980. Practical Immunology. 2nd ed. Blackwell Scientific Publications Inc., Boston. 359 pp.
Kappler, J. W., T. Wade, J. White, E. Kushnir, M. Blackman, J. Bill, N. Roehm, and P. Marrack. 1987. A T-cell receptor V_β segment that imparts reactivity to a class II major histocompatibility complex product. *Cell.* 49: 263–271.
Kiehart, D. P., and T. D. Pollard. 1984*a*. Stimulation of Acanthamoeba actomyosin ATPase activity by myosin-II polymerization. *Nature (Lond.).* 308:864–866.
Kiehart, D. P., and T. D. Pollard. 1984*b*. Inhibition of *Acanthamoeba* actomyosin II ATPase activity and mechanochemical function by specific monoclonal antibodies. *J. Cell Biol.* 99:1024–1033.
Kiehart, D. P., D. A. Kaiser, and T. D. Pollard. 1984*a*. Monoclonal antibodies demonstrate limited structural homology between myosin isozymes from *Acanthamoeba. J. Cell Biol.* 99:1002–1014.
Kiehart, D. P., D. A. Kaiser, and T. D. Pollard. 1984*b*. Direct localization of antibody-binding sites on *Acanthamoeba* myosin-II and inhibition of filament formation by antibodies that bind to specific sites on the myosin II tail. *J. Cell Biol.* 99:1015–1023.
Kiehart, D. P., D. A. Kaiser, and T. D. Pollard. 1986. Antibody inhibitors of nonmuscle myosin function and assembly. *Methods Enzymol.* 134:423–453.
Kiehart, D. P., I. Mabuchi, and S. Inoue. 1982. Evidence that myosin does not contribute to force production for chromosome movement. *J. Cell Biol.* 94: 165–178.
Kuznetsov, S. A., and V. I. Gelfand. 1986. Bovine brain kinesin is a microtubule-activated ATPase. *Proc. Natl. Acad. Sci. USA.* 83:8530–8534.
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.
Leslie, R. J., R. B. Hird, L. Wilson, J. R. McIntosh, and J. M. Scholey. 1987. Kinesin is associated with a nonmicrotubule component of sea urchin mitotic spindles. *Proc. Natl. Acad. Sci. USA.* 84:2771–2775.
Mabuchi, I., and M. Okuno. 1977. The effect of myosin antibody on the division of starfish blastomeres. *J. Cell Biol.* 74:251–263.
Markwell, M. A. K., and C. F. Fox. 1978. Surface-specific iodination of membrane proteins of viruses and eukaryotic cells using 1,3,4,6-tetrachloro-3α, 6α-diphenylglycoluril. *Biochemistry.* 17:4807–4817.
Neighbors, B. W., R. C. Williams, Jr., and J. R. McIntosh. 1988. Localization of kinesin in cultured cells. *J. Cell Biol.* 106:1193–1204.
Ogawa, K., and H. Mohri. 1975. Preparation of antiserum against a tryptic fragment (fragment A) of dynein and an immunological approach to the subunit composition of dynein. *J. Biol. Chem.* 250:6476–6483.
Okuno, M., K. Ogawa, and H. Mohri. 1976. Inhibition of movement and ATPase activity of demembranated spermatazoa by antidynein antiserum. *Biochem. Biophys. Res. Commun.* 68:901–906.
Parham, P. 1986. Preparation and purification of active fragments from mouse monoclonal antibodies. *In* Handbook of Immunology. Vol. 1. Immunochemistry. 4th ed. D. M. Weir, editor. Blackwell Scientific Publishers Inc., Boston. 14.1–14.23.
Peltz, G., J. A. Spudich, and P. Parham. 1985. Monoclonal antibodies against seven sites on the head and taiil of *Dictyostelium myosin. J. Cell Biol.* 100:1016–1023.
Porter, M. E., P. M. Grissom, J. M. Scholey, E. D. Salmon, and J. R. McIntosh. 1988. Dynein isoforms in sea urchin eggs. *J. Biol. Chem.* 263:6759–6771.
Porter, M. E., J. M. Scholey, D. L. Stemple, G. P. A. Vigers, R. D. Vale, M. P. Sheetz, and J. R. McIntosh. 1987. Characterization of the microtubule movement produced by sea urchin egg kinesin. *J. Biol. Chem.* 262:2794–2802.
Pryer, N. K., P. Wadsworth, and E. D. Salmon. 1986. Polarized microtubule gliding and particle saltations produced by soluble factors from sea urchin eggs and embryos. *Cell Motil. Cytoskeleton.* 6:537–548.
Saxton, W. M., M. E. Porter, S. A. Cohn, J. M. Scholey, E. C. Raff, and J.R. McIntosh. 1988. *Drosophila* kinesin: characterization of microtubule motility and ATPase. *Proc. Natl. Acad. Sci. USA.* 85:1109–1113.
Scholey, J. M., S. A. Cohn, and A. L. Ingold. 1988. Biochemical and motile properties of sea urchin egg kinesin. *In* Cell Movement. Vol. 2. Kinesin, Dynein and Microtubule Dynamics. F. D. Warner and J. R. McIntosh, editors. Alan R. Liss, Inc., NY. In press.
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 egg. *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.
Schroer, T. A., and M. P. Sheetz. 1988. The role of kinesin and kinesin-associated proteins in organelle transport. *In* Cell Movement Vol. 2. Kinesin, Dynein and Microtubule Dynamics. F. D. Warner and J. R. McIntosh, editors. Alan R. Liss, Inc., NY. In press.
Vale, R. D. 1987. Intracellular transport using microtubule-based motors. *Annu. Rev. Cell Biol.* 3:347–378.
Vale, R. D., T. S. Reese, and M. P. Sheetz. 1985*a*. Identification of a novel force generating protein, kinesin, involved in microtubule-based motility. *Cell.* 42:39–50.
Vale, R. D., B. J. Schnapp, T. S. Mitchison, E. Steuer, T. S. Reese, and M. P. Sheetz. 1985*b*. Different axoplasmic proteins generate movement in different directions along microtubules *in vitro. Cell.* 43:623–632.
Vale, R. D., J. M. Scholey, and M. P. Sheetz. 1986. Kinesin: possible biological roles of a new microtubule motor. *Trends Biochem. Sci.* 11:464–468.
Yang, J. T., W. M. Saxton, and L. S. B. Goldstein. 1988. Isolation and characterization of the gene encoding the heavy chain of *Drosophila* kinesin. *Proc. Natl. Acad. Sci. USA.* 85:1864–1868.