Dynein-like Mg^{2+}-ATPase in Mitotic Spindles Isolated from Sea Urchin Embryos (*Strongylocentrotus droebachiensis*)

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ABSTRACT Two distinctly different ATPases have been reported to be endogenous to the mitotic apparatus: a Mg^{2+}-ATPase resembling axonemal dynein, and a Ca^{2+}-ATPase postulated to be bound in membranes. To examine the nature of the Mg^{2+}-ATPase, we isolated membrane-free mitotic spindles from *Strongylocentrotus droebachiensis* embryos by rapidly lysing them in a calcium-chelating, low-ionic-strength buffer (5 mM EGTA, 0.5 mM $MgCl_2$, 10 mM PIPES, pH 6.8) that contained 1% Nonidet P-40. The fibrous isolated mitotic spindles closely resembled spindles in living cells, both in general morphology and in birefringence. In electron micrographs, the spindles were composed primarily of microtubules, free from membranes and highly extracted of intermicrotubular cytoplasmic ground substance. As analyzed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE), the pelleted spindles contain 18% tubulin, variable amounts of actin (2–8%), and an unidentified protein of 55 kdaltons in a constant weight ratio to tubulin (1:2.5). The isolated spindles also contained two polypeptides, larger than 300 kdaltons, that comigrated with egg dynein polypeptides, and ATPase activity (0.02 μmol P_i/mg·min) that closely resembled both flagellar and egg dynein. The spindle Mg^{2+}-ATPase showed a ratio of Ca^{2+}-/Mg^{2+}-ATPase = 0.85, had minimal activity in KCl and EDTA, and cleaved GTP at 35% of the rate of ATP. The Mg^{2+}-ATPase was insensitive to ouabain or oligomycin. The spindle Mg^{2+}-ATPase was inhibited by sodium vanadate but, like egg dynein, was less sensitive to vanadate than flagellar dynein. The spindle Mg^{2+}-ATPase does not resemble the mitotic Ca^{2+}-ATPase described by others. We propose that the spindle Mg^{2+}-ATPase is egg dynein. Bound carbohydrate on the two high-molecular-weight polypeptides of both egg dynein and the spindle enzyme suggest that these proteins may normally associate with membranes in the living cell.

Dynein is the mechanochemical cross-bridge protein that reversibly interconnects the outer doublet microtubules of ciliary and flagellar axonemes to produce motility (11, 26, 60). Recent physiological evidence implicates a dynein-like ATPase as the motive force in chromosome movement (4, 26, 47–49). Biochemical evidence for ATPase activity within the mitotic spindle has come mainly from analysis of isolated mitotic apparatus from sea urchin eggs (see references 31, 40, 48 for reviews). The structure, protein composition, and amount and characteristics of ATPase activity reported for the isolated mitotic apparatus have been as varied as the isolation methods themselves (31, 35, 40, 48).

Two distinctly different ATPases have been reported to be constituents of the mitotic apparatus: a Mg^{2+}-ATPase, also activated by Ca^{2+} (29, 33, 61), and a Ca^{2+}-ATPase, not significantly activated by Mg^{2+} (30, 37, 39–42). The Mg^{2+}-ATPase resembled axonemal dynein (61). The mitotic Ca^{2+}-ATPase, characterized by Petzelt, Mazia, and co-workers (30, 40), is now postulated to be associated with the smooth endoplasmic reticulum that is integral to the mitotic apparatus, and to be involved in actively sequestering calcium ions (40–42).

Mg^{2+}-ATPase activity was initially identified in the isolated mitotic apparatus by Mazia et al. (29) and Miki (33) in the early 1960s. In 1968, Weisenberg and Taylor (61) purified from isolated mitotic apparatus a soluble Mg^{2+}-ATPase that had a sedimentation coefficient and nucleotide and ionic specificities similar to those of ciliary axonemal dynein. The specific activity of the mitotic apparatus was three times the Mg^{2+}-ATPase specific activity of the whole egg cytoplasm (61; see also reference 33). Weisenberg and Taylor suggested that the cyto-

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plasmic dynein-like ATPase was a ciliary precursor and thus might be a cytoplasmic component trapped within the isolated mitotic apparatus. Subsequently, Mohri et al. (34) showed that both the sea urchin mitotic apparatus and the egg cortex appeared brightly fluorescent when stained by a preparation of antibody to the dynein 1 component of sea urchin flagellar dynein, using indirect immunofluorescence techniques. A Mg^{2+}-ATPase has been purified from isolated cortices of sea urchin eggs. This ATPase is soluble in 0.6 M KCl and has a sedimentation coefficient and enzymatic activity similar to those of axonemal dynein (20, 24).

Recently, Pratt (44, 45) has shown that the soluble dynein-like Mg^{2+}-ATPase purified from sea urchin eggs during early development is probably not a ciliary precursor as proposed earlier (61), but a cytoplasmic component of the egg. This 14S enzyme shares with axonemal dynein a typical sedimentation coefficient, high molecular weight polypeptides (320–385 kdaltons), ionic and nucleotide specificities (Mg^{2+}-ATPase/Ca^{2+}-ATPase ratio near 1.0, no activity in KCl-EDTA, and low Mg^{2+}-GTPase activity), and inhibition by low concentrations of sodium vanadate (44, 45). Ciliary axonemal dynein is composed of at least four major high molecular weight polypeptides, but the cytoplasmic dynein termed "egg dynein" by Pratt (45) has two predominant high molecular weight polypeptides. Sea urchin embryos contain a large amount of egg dynein, but its function in the egg is not known.

This study was designed to determine whether isolated mitotic apparatus that have had membranous and most cytoplasmic components removed contain either the egg dynein Mg^{2+}-ATPase or the mitotic Ca^{2+}-ATPase. Salmon and Jenkins (52) recently reported a procedure for isolating relatively stable spindles from the developing eggs of *Strongylocentrotus droebachiensis* by use of a calcium-chelating, detergent lysis buffer. This procedure isolated the fibrous skeleton of the mitotic apparatus plus attached chromosomes and centrosomes (termed here the "mitotic spindle") with little entrapped cytoplasm and no membranes detectable in electron micrographs.

Gibbons et al. (14) have proposed that the term "dynein" be used to refer to a class of enzymes that have ATPase activity, high molecular weight subunits (>300 kdaltons), and are associated with microtubules. Enzymatically, dynein ATPases exhibit characteristic ionic and nucleotide specificities (13, 45) and inhibition by sodium vanadate (4, 12, 19, 36, 51). We have compared the enzymatic characteristics of the ATPase activity of the isolated spindles with those of both egg and flagellar dynein, and the polypeptide components of the spindle with whole egg and ciliary proteins.

We found that the spindles isolated by the procedure of Salmon and Jenkin (52) contained the high molecular weight polypeptides of egg dynein, and an Mg^{2+}-ATPase activity that resembles egg dynein in both ionic and nucleotide specificity and in response to various inhibitors, including sodium vanadate. We found no evidence that other cytoplasmic ATPases, including the mitotic Ca^{2+}-ATPase or myosin ATPase, were retained in the isolated spindles.

A preliminary account of this work has appeared in abstract form (38).

MATERIALS AND METHODS

Organisms and Collection of Gametes

Sea urchins (*Strongylocentrotus droebachiensis*), collected from the coast of Maine, were kept at Woods Hole, Massachusetts, in running natural seawater (10°C) or at Chapel Hill, North Carolina, in Instant Ocean Artificial Seawater (Aquarium Systems Inc., Eastlake, Ohio) (5°C). To collect gametes, 2–5 ml of 0.56 M KCl was injected into the sea urchin's body cavity. Semen was collected from the surface of the test as it was shed. Eggs were shed into 50–100 ml of cold seawater (4°C). Before fertilization, eggs were washed twice in 150 ml of fresh seawater by allowing them to settle (57).

Isolation of the Mitotic Spindles

Procedures for isolating spindles were adapted from Salmon and Jenkins (52), Rebhun et al. (46), and Sakai et al. (50). We suspended 20–30 ml of eggs in an equal volume of seawater at 8°C, then fertilized them by adding ~10 drops of diluted sperm (one drop of semen/12 ml seawater). 2.5 min after the addition of sperm, we quickly reduced the pH of the seawater to 5.25 by adding 0.1 N HCl, which removed the egg jelly. The eggs were then pelleted by gentle hand centrifugation and washed twice in 1 M glycerol, 5 mM Tris-HCl, pH 8.0, to remove the fertilization membranes. Finally, the eggs were resuspended in seawater at 8°C and plated out into large bowls kept in an 8°C incubator.

We monitored the stage of development with a polarization microscope. 5 min before the onset of anaphase, eggs were collected and washed twice in an isotonic solution, 19 parts NaCl:1 part KCl, to remove divalent cations and to weaken the hyaline layer. The pelleted eggs were resuspended in 35–50 vol of 5-mM EGTA lysis buffer containing 5 mM EGTA, 0.5 mM $MgCl_2$, 10 mM PIPES, pH 6.8, and 1% Nonidet P-40 at 19°–22°C and shaken vigorously to lyse the eggs quickly. The detergent concentration was twice that used previously by Salmon and Jenkins (52) to lyse rapidly large numbers of eggs for biochemical analysis. After 30 min, whole eggs and fertilization membranes were removed by filtering through 50-μm-pore Nitex screen. Spindles were pelleted by centrifugation for 10 min at 500 *g*. The pellet was washed once in 1.5 ml of lysis buffer. Washed pellets were solubilized in Tris-glycine sample buffer (see below) for gel electrophoresis or in 10 mM Tris-HCl, pH 8.0, for ATPase assays that were performed immediately. In two experiments, the protein composition of the spindles isolated at 8° and 15°C was compared to that of spindles isolated at 19°–22°C.

Preparation of Cilia, Flagella, and Axonemal and Egg Dynein

Ciliary axonemes were prepared from embryos as described by Stephens (59). Sperm flagellar axonemes were isolated according to the method of Stephens (56).

Flagellar dynein was prepared by dialysis of the washed axonemes against 1 mM Tris-HCl, pH 8.0, 0.1 mM EDTA, 0.1% β-mercaptoethanol at 2°–4°C for 12–24 h, according to the method of Gibbons and Fronk (13). The dialysate was centrifuged at 25,000 *g* for 15 min. The supernate, which without further purification was ~20% dynein, was used in the flagellar dynein assays.

Egg dynein was prepared according to the method of Pratt (45), a modification of the original method of Weisenberg and Taylor (61). Sea urchin eggs were dejellied by acid treatment and washed in Millipore-filtered seawater. The eggs were homogenized in an isotonic buffer (1 M sucrose, 5 mM $MgSO_4$, 50 mM Tris-HCl, pH 8.0), and the homogenate was centrifuged for 2 h at 100,000 *g*. The supernate was dialyzed to reduce the sucrose concentration and loaded onto a 5–20% linear sucrose gradient containing 50 mM Tris-HCl, pH 8.0, 0.1 M KCl, and centrifuged at 100,000 *g* for 16 h at 2°C. Gradient fractions were collected and assayed for ATPase activity, and the three fractions which constituted the 14S ATPase peak were pooled. After dialysis against 10 mM Tris-HCl, pH 8.0, to remove excess sucrose, the partially purified egg dynein pool was used for ATPase assays and gel electrophoresis.

Gel Electrophoresis and Densitometry

All samples were prepared for SDS-polyacrylamide gel electrophoresis (SDS-PAGE) in a sample buffer containing 2.5 mM Tris-glycine, pH 8.3, 1% SDS, 1% β-mercaptoethanol, 5% sucrose, 0.05% bromophenol blue, and 50 μg/ml phenylmethyl sulfonyl fluoride (PMSF) (1). 5% polyacrylamide gels were run in a Tris-glycine buffer system (1, 58), and 3% gels were run in a phosphate buffer system (54). All gels were stained according to the method of Fairbanks et al. (8), using Coomassie Blue. Carbohydrate was detected by the periodic acid-Schiff reagent (PAS) method of Fairbanks et al. (8). Coomassie Blue–stained gels were scanned in an Ortec 4310 densitometer (Ortec Inc., EG&G, Inc., Oak Ridge, Tenn.) equipped with a green filter. Planimetry was used to measure the area under the curve as described by Pratt (45). All overlapping peaks were assumed to be Gaussian.

Determination of Protein Content and ATPase Activity

Protein content was determined by the method of Lowry et al. (23), using bovine serum albumin as a standard.

ATPase activity was measured at 23°–25°C. Standard buffer conditions were 50 mM Tris-HCl, pH 8.0, 5 mM $MgSO_4$, 0.1 M KCl, and 1 mM ATP. Deionized water was used throughout. Modifications were made by equimolar substitution of $CaCl_2$ or EDTA for $MgSO_4$, or GTP for ATP, or by addition of inhibitors to the standard buffer. For ATPase assay, the spindle pellets were dispersed in 10 mM Tris-HCl, pH 8.0, which solubilized the microtubule protein. Typically, an aliquot of the solubilized spindles or of flagellar or egg dynein was added to the appropriate assay buffer and the reaction was started by the addition of ATP.

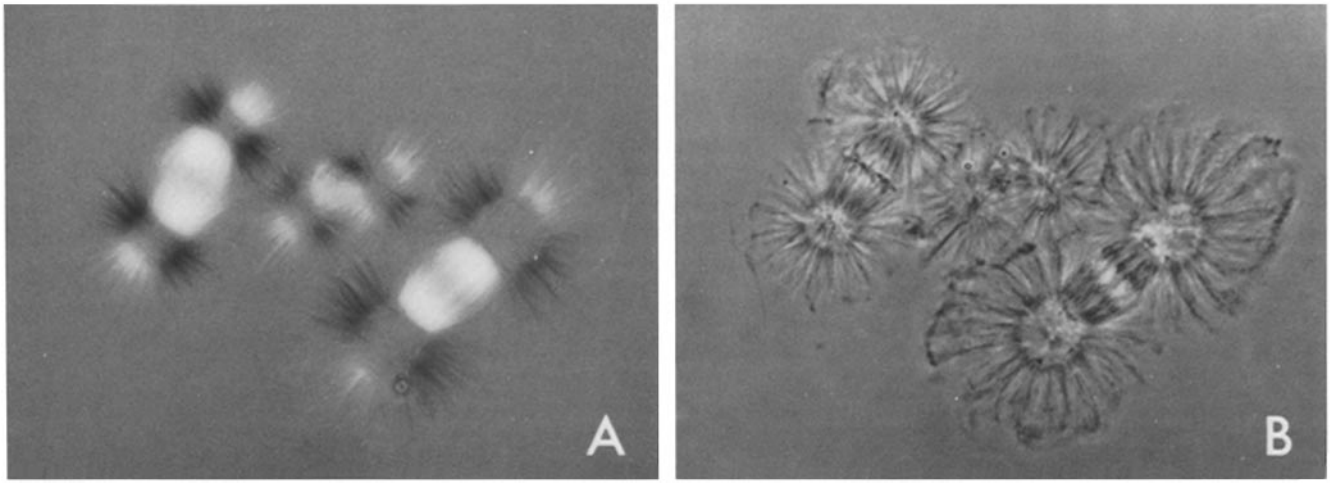

FIGURE 1 Mitotic spindles isolated in 5 mM EGTA lysis buffer containing 1% Nonidet P-40 at 19°C seen by polarization (*A*) and phase-contrast (*B*) microscopy. Differences in the distribution of birefringence are preserved for metaphase (left), prometaphase (middle), and anaphase (right) spindles (*A*). In phase contrast, the spindles are distinctly fibrous in appearance, with little adhering material. × 450.

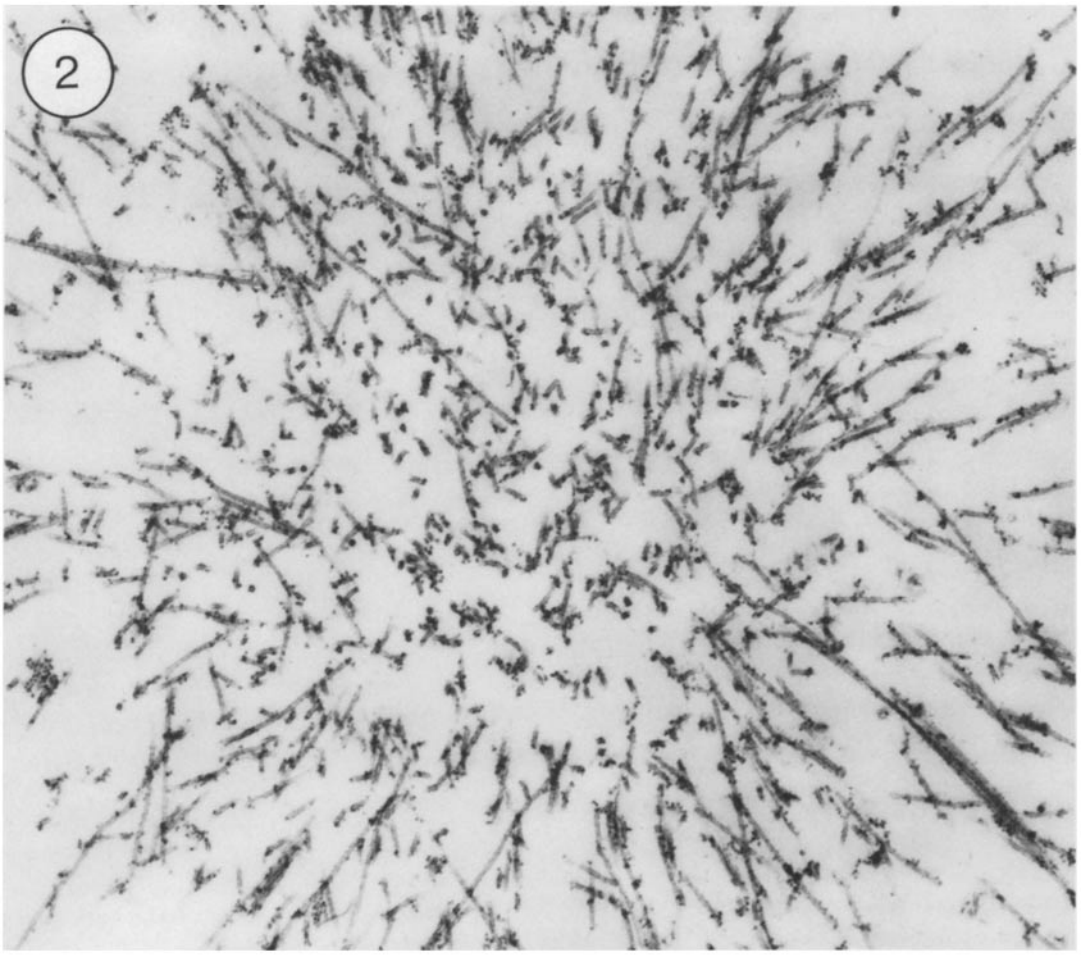

FIGURE 2 Thin section through astral region of isolated prometaphase spindle. Microtubules are the major fibrous components. Spindles appear highly extracted of membranes and intermicrotubule ground cytoplasm. A relatively high density of ribosomelike particles, clusters of particles, and fine filaments remains associated with the microtubules. Chromosomes are above the field of view. × 22,200.

The reaction was stopped and inorganic phosphate release was measured by the method of Pollard and Korn (43), a modification of the original method of Martin and Doty (27). An identical assay was prepared and stopped immediately after the addition of ATP (time 0) to correct for inorganic phosphate present in the enzyme mixture and the ATP stock.

Microscopy

As described by Salmon and Segall (53), specimens were observed and spindle birefringence retardation (BR) was measured in a Zeiss Photomicroscope I using either a Zeiss × 25 Neofluor phase-contrast objective, or × 10 or × 20 Nikon rectified polarization objectives with the Zeiss differential interference contrast condenser lens. The light source was an HBO 200 W mercury arc lamp equipped with heat-cut and 540-nm interference filters.

Photographs were taken on Kodak Plus X 35-mm film. Polarization photographs were taken at 4–5 nm of BR compensation.

Electron Microscopy

For fixation, the isolated spindles were pelleted and resuspended for 1 h at room temperature in the 5-mM EGTA buffer (no detergent) to which had been added 3% glutaraldehyde (Electron Microscopy Science, Fort Washington, Pa., no. 80127, 25% ampules). The fixed spindles were washed three times by centrifugation in EGTA buffer, postfixed for 30 min in 1% OsO_4 at 0°C, rinsed, stained with 1% uranyl acetate for 2 h at 22°C, then dehydrated in an ethanol series, embedded in Mollenhauer no. 2 Epon-Araldite, sectioned with a diamond knife, stained in lead citrate, and viewed in a Hitachi HU11B electron microscope at 75 kV.

RESULTS

Structure

The isolated spindles retained the morphology and distribution of BR typical of the spindle fibers seen in living cells at the same stage (Fig. 1). There was no loss of birefringence over a period of several hours when the isolated spindles were in 5 mM EGTA lysis buffer at 19°–22°C. As seen in Fig. 1 *B*, little globular cytoplasmic material adhered to the spindles, which were fibrous in appearance when viewed with phase contrast optics. Because of slight asynchrony in development, prometaphase, metaphase, and early anaphase spindles are all present in a single preparation. Stage-specific differences (including distribution of BR, size of the centrospheres, and positioning of the chromosomes) were preserved in the isolated spindles. Ultrastructural analysis will be reported in detail elsewhere, but Fig. 2 illustrates that the spindle fibers are structured primarily by microtubules and microtubule-associated material. No membranes are seen and the spindles are highly extracted of intermicrotubular ground substance.

Protein Composition

SDS-PAGE analysis of the isolated spindles showed a large amount of tubulin (Fig. 3). On 5% Tris-glycine gels, two equimolar proteins from the isolated spindles comigrated with the α and β subunits of tubulin from *S. droebachiensis* blastula cilia (Fig. 4). As determined by densitometry and planimetry of Coomassie Blue–stained gels, 17.8 ± 0.6% (n = 13) of the spindle protein was contained in these two tubulin polypeptides. The polypeptides of the 22S and 2.5S yolk proteins are not detectable on stained gels of our spindle pellets (Fig. 3). The 22S and 2.5S yolk proteins are major constituents of the egg cytoplasm and frequent contaminants of isolated mitotic apparatus (9, 55, 57). The absence of these proteins implies that these isolated spindles are relatively free of cytoplasmic contaminants.

One unidentified polypeptide from the isolated spindles migrated consistently on the gels at 55 kdaltons between the α- and β-tubulin bands. This 55-kdalton polypeptide was present in a constant weight ratio to tubulin of 1:2.5 ± 0.15 (n = 13). Although the electrophoretic mobility of the 55-kdalton band on Tris-glycine buffered gels was similar to that of ciliary component 20 (22, 59) (see Fig. 3), densitometry of these gels and analysis of Tris-HCl buffered gels (21) showed that the 55-kdalton polypeptide did not comigrate with ciliary component 20 (data not shown).

The isolated spindles also contain a 43–45-kdalton polypeptide that comigrates on SDS-PAGE with muscle actin. Although present in nearly every preparation of spindles, this polypeptide varies considerably in amount (2–8% of the total protein), and it is not present in a constant ratio to any other major component.

Two high molecular weight polypeptides in the isolated spindles comigrated on 3% polyacrylamide gels with the two high molecular weight components of egg dynein (Fig. 5). Densitometric tracings of 3% Tris-glycine gels of spindle samples showed that ~4% of the spindle protein is contained in the two high molecular weight bands. The weight ratio of these polypeptides to tubulin in the mitotic spindle is 1:5.2 ± 0.7 (n

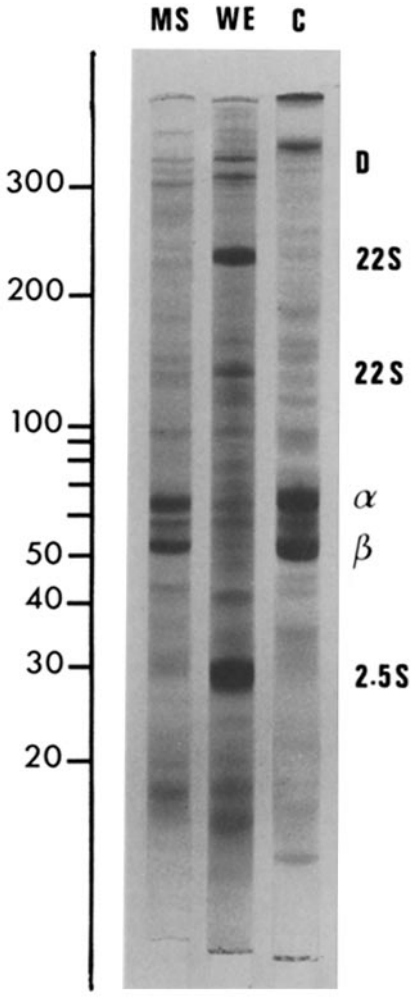

FIGURE 3 5% Tris-glycine-buffered SDS-polyacrylamide gels of mitotic spindles (*MS*), whole eggs (*WE*), and blastula cilia (*C*). The spindle contains proteins identical in electrophoretic mobility to the α- and β-subunits from cilia, and several bands in the dynein region (*D*). No 22S nor 2.5S yolk protein can be detected in the spindle sample. An approximately molecular weight scale is at the left (× 10^{-3}).

= 13). Occasionally, more than two bands were seen in the dynein molecular weight range on the gels (52), but usually only the two egg dynein-like proteins were observed.

These two high molecular weight spindle polypeptides stain positively by the PAS staining method for carbohydrates as do the two high molecular weight cytoplasmic egg dynein polypeptides (Fig. 6 and reference 45). PAS-stained gels of ciliary or flagellar axoneme samples, however, do not show carbohydrate-containing material in the dynein region. Of the proteins typically seen on gels of whole sea-urchin embryos, only the egg dynein bands and those comprising the 22S yolk protein (55) are PAS-positive.

The composition of the isolated *S. droebachiensis* spindles did not vary considerably with the temperature of isolation. Mitotic spindles isolated at 8°, 15°, or 20°C all had similar protein components as determined by SDS-PAGE. The total amount of protein, however, was an increasing function of isolation temperature. An isolation temperature of 20°C was chosen to maximize the incorporation of birefringent material into the spindle, as shown earlier by Stephens (57). At 20°C, lysis of 60 mg (0.6 ml) of embryos yielded 0.30 mg of spindle protein, or ~0.5% of total embryo protein.

Enzymatic Activity

As shown in Table I, the washed spindle pellets contained ATPase activity which, at 23°–25°C and pH 8.0, was activated by both Mg^{2+} and Ca^{2+} (Ca^{2+}-/Mg^{2+}-ATPase = 0.84). The spindle enzyme hydrolyzed ATP three times faster than it hydrolyzed GTP. The ATPase of the isolated spindles had little activity in KCl plus EDTA. The specific activity of the pelleted spindle Mg^{2+}-ATPase is ~0.02 μmol P_i/mg·min.

To examine further the dynein-like nature of the spindle ATPase, we assayed its activity in the presence of sodium vanadate in the +5 oxidation state ($NaVO_3$ and Na_3VO_4), a

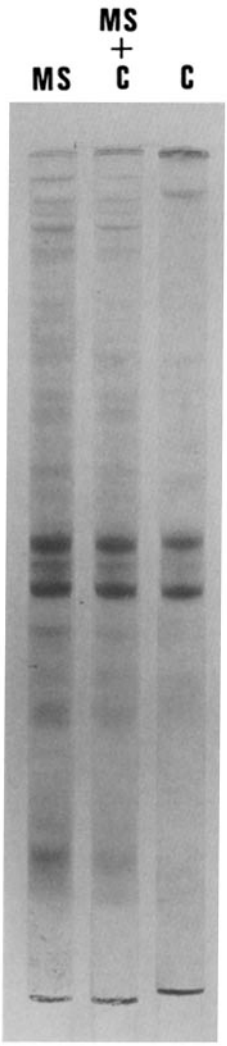

FIGURE 4 Comigration of mitotic spindle (*MS*) proteins and blastula ciliary axonemes (*C*) on 5% Tris-glycine-buffered gels. The α- and β-tubulin subunits of the two samples have identical electrophoretic mobilities, but the 55-kdalton proteins that run between the tubulin bands are not coincident. The center gel was produced by mixing the ciliary and spindle samples 1:1, and loading a volume that contained one half the protein in gel *MS* and one half the protein in gel *C*.

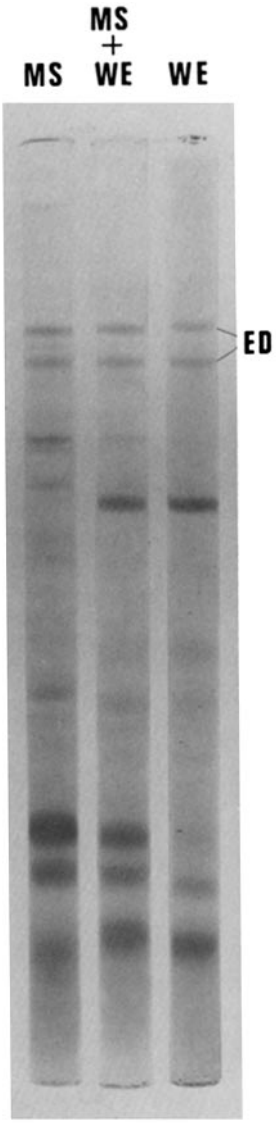

FIGURE 5 3% phosphate-buffered gels of spindles (*MS*), whole egg (*WE*), and a comigration (*MS* + *WE*). The center gel was produced by mixing the whole egg sample and the spindle sample 1:1 before loading. The upper and lower bands of egg dynein (*ED*) comigrate with two major spindle proteins. A third minor band is visible in this preparation of spindles between the two dyneinlike spindle proteins.

potent inhibitor of flagellar dynein (4, 12, 19, 36, 51). The Mg^{2+}-ATPase activity of the isolated spindles was inhibited by sodium vanadate at concentrations between 2 μM and 1 mM. However, at a given concentration of vanadate, the spindle ATPase was inhibited less than was flagellar dynein isolated from *S. droebachiensis* sperm tails (Fig. 7): 50% inhibition of flagellar dynein occurred at 2 μM sodium vanadate, whereas 50% inhibition of spindle ATPase required 75 μM sodium vanadate.

The spindle Mg^{2+}-ATPase was not inhibited by ouabain or oligomycin (Table I) in concentrations known to inhibit Na^+,K^+-ATPase and proton-activated mitochondrial ATPase, respectively (10). The spindle enzyme was slightly stimulated by 10^{-4} M ouabain as has been previously reported for egg and flagellar dynein (45).

We compared the ATPase activity of our spindle enzyme to that of the mitotic Ca^{2+}-ATPase described by Petzelt (39). Elevated calcium ion concentrations, substitution of magnesium for calcium, substitution of Tris-HCl for *N*-2-hydroxyethylpiperazine-*N'*-3-propanesulfonic acid (EPPS), or elevated EDTA levels decrease significantly the activity of the mitotic Ca^{2+}-ATPase. In contrast, the spindle ATPase activity changed only slightly when assayed under these various buffer conditions (Table II).

DISCUSSION

The ultrastructure and protein composition of the isolated spindles reflect the fibrous morphology. Microtubules are the predominant structures visible in the electron micrographs of spindle fibers, and ~18% of the total spindle protein is tubulin. This value lies within the range of other estimates of 8% (48), 25% (5), and 30–45% tubulin (31) reported for other types of isolated mitotic apparatus. The 55-kdalton protein, which can be seen in our electrophoretic analysis to be distinct from α- and β-tubulin, is ~8% of the total spindle protein. Because its

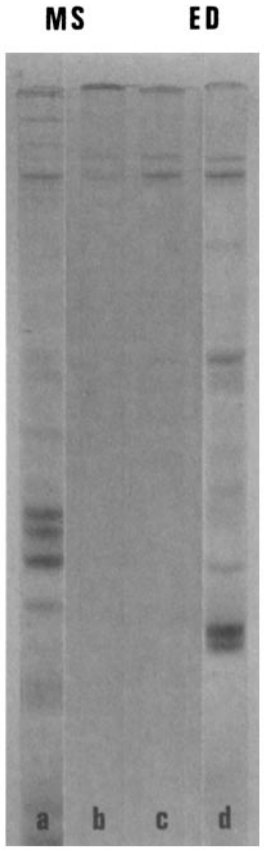

FIGURE 6 Comparison of egg dynein (*ED*, gels *c* and *d*) purified by sucrose gradient centrifugation with spindle pellets (*MS*, gels *a* and *b*), on 5% Tris-glycine-buffered gels. Gels *a* and *d* were stained for protein with Coomassie Brilliant Blue, and gels *b* and *c* were stained to detect carbohydrate by the PAS method. Both the partially purified egg dynein and the high molecular weight bands in the spindle stain positively for carbohydrate.

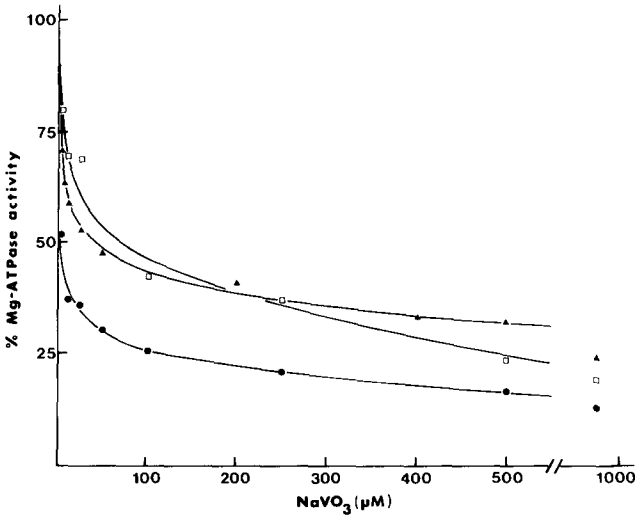

FIGURE 7 Inhibition of Mg^{2+}-ATPase activity by sodium vanadate for dialysis-extracted flagellar dynein (●), egg dynein (▲), and the spindle Mg^{2+}-ATPase (□). 50% inhibition of the spindle enzyme required ~25 μM sodium vanadate and ~2 μM vanadate inhibited the flagellar enzyme by 50%. Points plotted are the mean of three determinations for egg dynein and two determinations for flagellar dynein and the spindle Mg^{2+}-ATPase.

TABLE I

Specific Activities of Flagellar Dynein, Egg Dynein, and Spindle Mg^{2+}-ATPase

	Mg^{2+}-ATPase	Ca^{2+}-ATPase	K^+-EDTA ATPase	Mg^{2+}-GTPase	Mg^{2+}-ATPase + oligomycin	Mg^{2+}-ATPase + ouabain
Flagellar dynein	.211 ± .004	.169 ± .027	.003 ± 0	.003 ± 0	.180	.180
Egg dynein	.019 ± .001	.007 ± .001	0	.002 ± 0	.017	.016
Spindle Mg^{2+}-ATPase	.019 ± .003	.016 ± .007	.001 ± 0	.007 ± .002	.019	.021

Dialysis-extracted flagellar dynein, 14S egg dynein, and whole mitotic spindle pellets were assayed for enzymatic activity under the conditions shown. The assay medium was 50 mM Tris-HCl, pH 8.0, 0.1 M KCl, 5 mM cation or EDTA, 1 mM nucleotide. Inhibitor concentrations were 10 μg/ml oligomycin, and 10^{-4} M ouabain. Specific activities are expressed as mean μmol P_i/mg total protein · min ± standard error of the mean (measurements of three to five different preparations of each enzyme). Only one measurement was made in each case of oligomycin and ouabain sensitivity.

TABLE II

Comparison of the Spindle Mg^{2+}-ATPase and Mitotic Ca^{2+}-ATPase

	Standard conditions	5 mM $CaCl_2$	5 mM $MgCl_2$	1 mM EDTA	10 mM Tris-HCl
	%				
Spindle Mg^{2+}-ATPase	100	90	80	100	80
Mitotic Ca^{2+}-ATPase*	100	60	13	50	50

Comparison of spindle Mg^{2+}-ATPase with the mitotic Ca^{2+}-ATPase. Standard conditions were 50 mM EPPS, pH 8.5, 2.5 mM $CaCl_2$, 1.25 mM ATP, 0.5 mM EDTA. Substitutions included 5.0 mM $CaCl_2$ or 5.0 mM $MgCl_2$ for 2.5 mM $CaCl_2$; 1.0 mM EDTA for 0.5 mM EDTA; 10.0 mM Tris-HCl, pH 8.0, for 50 mM EPPS, pH 8.5. In each case, the column heading lists the substitution made. Values are expressed as percent of activity in standard conditions.

* Data calculated from Pelzelt (39).

molecular weight is nearly identical to that of tubulin, this unidentified protein may have been included in previous estimates of spindle tubulin content by electrophoretic analysis (5, 52). All of the pellets of isolated spindles contained some actin. Actin makes up at least 2% of the protein of the pelleted spindles and may be a bonafide fibrous component of the spindle fibers as proposed by others (9, 40, 48). However, the amount of actin is highly variable with respect to tubulin concentration, which indicates that actin could be a contaminant, probably from pelleted fragments of the egg cortex (2).

Cosedimenting with the isolated spindle is a Mg^{2+}-ATPase. There are enzymatic similarities between the spindle Mg^{2+}-ATPase and egg dynein, including activation by both Mg^{2+} and Ca^{2+} at a ratio Ca^{2+}-/Mg^{2+}-ATPase = 0.5–1.0, inactivity in the presence of KCl and EDTA, specificity for ATP over GTP, inhibition by vanadate, and slight stimulation by 10^{-4} M ouabain (Table I and reference 45).

The spindle ATPase described here is distinctly different from the mitotic Ca^{2+}-ATPase reported by Petzelt, Mazia, and co-workers (30, 39–42). The spindle ATPase is activated by both Mg^{2+} and Ca^{2+} (Ca^{2+}-/Mg^{2+}-ATPase = 0.85), whereas the mitotic Ca^{2+} ATPase is not significantly activated by Mg^{2+} (Ca^{2+}-/Mg^{2+}-ATPase = 5.0) (39). As shown in Table II, our spindle ATPase activity does not show the sensitivities to EDTA or Tris-HCl in the presence of calcium that are characteristic of the mitotic Ca^{2+}-ATPase (39). Petzelt (40) has recently reported that the mitotic Ca^{2+}-ATPase activity is insensitive to sodium vanadate. In contrast, the spindle Mg^{2+}-ATPase is significantly inhibited by sodium vanadate.

The mitotic Ca^{2+}-ATPase is now postulated to be membrane bound and active in sequestering calcium within the microenvironment of the mitotic apparatus (40–42). Membrane vesicles, lamellae, and cisternae are prominent components of the mitotic apparatus in the living cell (15–18). However, these membranes have been removed by our cell lysis procedure, leaving a highly extracted spindle (Fig. 2). If the Ca^{2+}-ATPase is either membrane bound as postulated, or a soluble cytoplasmic enzyme, then it is not surprising to find that it is absent from the spindle's fibrous structure analyzed here.

In addition, we found no evidence that other membrane-bound or cytoplasmic ATPases were retained in the pelleted spindles. The spindle Mg^{2+}-ATPase was not inhibited by concentrations of ouabain that block Na^+,K^+-ATPase or of oligomycin that block proton-activated mitochondrial ATPase (10). Unlike myosin ATPase, the ATPase of the isolated spindles was not activated by KCl plus EDTA (Table I). Although starfish egg myosin seems less sensitive to KCl and EDTA than other cytoplasmic myosins, the starfish myosin ATPase is activated by Ca^{2+} and strongly inhibited by Mg^{2+} (25). In contrast, the spindle ATPase is activated by both Mg^{2+} and Ca^{2+}.

From the above evidence, we conclude that a dynein-like Mg^{2+}-ATPase is associated with the spindle fibers in these isolates. The spindle Mg^{2+}-ATPase is probably egg dynein (45), and several features distinguish it from axonemal dynein. The spindle ATPase, like egg dynein, is less sensitive to inhibition by sodium vanadate than is axonemal dynein. This difference in vanadate sensitivity may explain the unexpectedly high concentrations of vanadate required to significantly slow chromosome movements in mitotic model systems (4, 47). In addition, two prominent high molecular weight polypeptides in the isolated spindle comigrate in SDS-PAGE with two egg dynein polypeptides. PAS staining shows carbohydrate bound to the two high molecular weight peptides of both the spindle Mg^{2+}-ATPase and egg dynein (45). Axonemal dynein consists of at least four major high molecular weight polypeptides that are not PAS positive.

Absolute identification of the spindle Mg^{2+}-ATPase as egg dynein must await copurification and characterization of the high molecular weight polypeptides and the Mg^{2+}-ATPase activity from isolated spindles. Although the spindle ATPase is dynein-like in enzymatic properties, it is possible that the ATPase activity is not associated with the high molecular weight, PAS-positive egg dynein polypeptides. The enzymatic activity may reside in a lower molecular weight component, and thus, may not strictly be characterized as a dynein according to the definition of Gibbons et al. (14).

Coisolation of a dynein-like Mg^{2+}-ATPase with spindle fibers implies that this enzyme associates with microtubules. Further evidence of such an association could be shown by an in vitro microtubule binding assay (if the enzyme could be purified in sufficient quantity), or by copurification of the Mg^{2+}-ATPase and spindle tubulin. It is also possible that, in vivo, the ATPase is bound to some other mitotic structure. If the high molecular weight, PAS-positive egg dynein peptides are components of the spindle Mg^{2+}-ATPase, then the presence of carbohydrate suggests that an unknown fraction of the enzyme normally is membrane associated (8). Thus, in the living cell, the Mg^{2+}-ATPase may link microtubules and membranes. Because the mitotic membranes are dispersed by detergent during isolation, the isolated mitotic spindle may contain only that fraction of the in vivo ATPase that is tightly bound to the microtubules. Although dynein is typically associated with microtubules (14, 26), membrane-associated dynein has been identified recently in the ciliary membranes of *Tetrahymena* and of scallop gill cilia, where it appears to link the ciliary membrane with the axonemal outer doublet microtubules (6, 7). Membrane-microtubule links have been seen in ultrastructural studies of the mitotic apparatus (18).

The functional significance of this spindle ATPase is still open to question. A dynein cross-bridge mechanism between microtubules has been postulated to generate forces that move chromosomes to the spindle poles and that elongate the central, or interpolar, spindle during mitosis (3, 4, 26, 28, 31, 32, 47–49). Pratt (44) has shown that the egg dynein present in sea urchin embryos is not a ciliary dynein precursor, as once was postulated (61). One possible cytoplasmic function for this

enzyme is in force generation for chromosome movement. The data presented here are consistent with the presence of egg dynein in the mitotic apparatus.

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