Isolation of Microtubules and a Dynein-like MgATPase from Unfertilized Sea Urchin Eggs*

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Taxol was used to prepare microtubules from unfertilized eggs of sea urchins Lytechinus pictus, Strongylocentrotus droebachiensis, and Strongylocentrotus purpuratus. By electron microscopy, these microtubules possessed normal morphology and were decorated with projections. The polypeptides present were tubulin plus microtubule-associated proteins (MAPs) which included various high molecular weight polypeptides, and a Mᵦ = 80,000 polypeptide. These MAPs were extracted from the microtubules by differential centrifugation in high ionic strength buffers, yielding a pellet of microtubules which were not decorated with projections. The Mᵦ = 80,000 and high molecular weight MAPs were separated using Bio-Gel A-1.5m chromatography, and shown to bind taxol-stabilized microtubules assembled from purified bovine brain tubulin.

A dynein-like MgATPase activity is present in sea urchin egg extracts. 10-20% of this MgATPase copelleted with the taxol-assembled microtubules, under conditions where >80% of the tubulin pelleted. During subsequent fractionation of the microtubules, by (i) high salt extraction followed by gel filtration or sucrose density gradient fractionation or (ii) ATP extraction, the MgATPase co-purified with high Mᵦ MAPs. The MgATPase which remained in the microtubule-depleted egg extract was partially purified by (NH₄)₂SO₄ fractionation, followed by Bio-Gel A-5m and hydroxyapatite chromatography. The high Mᵦ MAP MgATPase and the hydroxyapatite MgATPase both contained a prominent polypeptide (Mᵦ ~ 350,000), which co-migrated on sodium dodecyl sulfate gels with the major heavy chain of dynein extracted from sperm axonemes. Our data suggest that this Mᵦ ~ 350,000 polypeptide is cytoplasmic dynein.

Microtubules are major structural and functional elements of mitotic spindles (1-4). Spindle MAPs¹ are therefore likely to play significant roles in mitosis, for example, in regulating the state of spindle microtubule assembly or stability, and in generating forces for microtubule sliding and chromosome movement.

Sea urchins are potentially an excellent source of spindle-specific MAPs, because the major function of microtubules in early sea urchin embryos is in mitosis. Cell division in such embryos is synchronous, allowing the preparation of relatively large amounts of isolated mitotic spindles (5-8). Such spindle preparations are reported to contain “cytoplasmic” or “egg” dynein, a 12-14 S vanadate-sensitive MgATPase present in unfertilized sea urchin egg extracts (9-14), and also a Mᵦ = 80,000 tubulin-binding polypeptide (15). These proteins are therefore candidates for spindle MAPs.

In order to obtain sufficient quantities of spindle MAPs for biochemical and immunological analysis, we have sought methods for preparing MAP-containing microtubules from unfertilized sea urchin eggs. These cells contain large pools of soluble tubulin (16, 17) (and conceivably MAPs), which assemble into the mitotic spindle subsequent to fertilization. However, the assembly of microtubules from this soluble protein pool does not occur in extracts of unfertilized sea urchin eggs incubated under a wide range of conditions (18-22). Thus, the preparation of microtubules from these extracts via cycles of temperature-dependent assembly and disassembly (23, 24) is impossible. The chromatographic procedures which have been employed by various groups to purified sea urchin egg tubulin (25, 26) yield preparations containing low concentrations of MAPs.

As an alternative approach, we have investigated the use of taxol for inducing the assembly of the pools of microtubule protein present in cell extracts into microtubules. This approach is based on the work of Vallee (27) who has shown that taxol can be used to prepare microtubule protein from extracts of mammalian cells, and that the polypeptide composition of this taxol-assembled microtubule protein is essentially the same as that of microtubules prepared via cycles of temperature-dependent assembly and disassembly. In this report, we describe (i) the morphology and polypeptide composition of MAP-containing microtubules prepared using taxol from extracts of unfertilized eggs of three species of sea urchins; (ii) our investigation of the presence of a dynein-like MgATPase activity in these microtubule preparations; and (iii) the partial purification of a dynein-like MgATPase activity from the microtubule-depleted egg extracts.

mm MgCl₂, 0.1 mm EDTA, 1 mm EGTA, pH 6.9; PMSF, phenylmethylsulfonyl fluoride; TAME HCl, F-tosyl-L-arginine methyl ester hydrochloride; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; 80K, 200K, and 150K, proteins of Mᵦ = 80,000, 200,000, and 150,000.

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RESULTS

Preparation of Taxol-microtubule Protein and MAPs—Homogenates of unfertilized eggs of Lytechinus pictus, Strongylocentrotus purpuratus, and S. droebachiensis contained soluble tubulin which did not pellet during centrifugation at 180,000 × g for 90 min at 4 °C. Incubation of the resulting supernatant, however, with taxol plus GTP at 22 °C resulted in the assembly of microtubules, which were pelleted by centrifugation at approximately 30,000 × g through a 15% (w/v) sucrose cushion (yield approximately 2–5 mg/10 ml of eggs) and then analyzed for protein composition on SDS gels (Fig. 1) and by electron microscopy (Fig. 2).

The microtubules from all three species contained α- and β-tubulins together with high molecular weight polypeptides (M, > 300,000) and a prominent polypeptide with M, ~ 80,000. These components were all greatly enriched in the microtubule pellets, relative to the extract supernatant. Densitometry and planimetry of SDS gels of L. pictus taxol-MTP prepara-

![Fig. 1. SDS-polyacrylamide gel analysis of fractions obtained during preparation of taxol-assembled microtubules from extracts of unfertilized (a) L. pictus and (b) S. droebachiensis eggs.](image)

![Fig. 2. Electron microscopic analysis of taxol-assembled MTP.](image)

2 Portions of this paper (including "Materials and Methods," Table II, and Fig. 9) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. 83M-3270, cite authors, and include a check or money order for $3.20 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.
tions (Fig. 1) revealed a weight ratio of HMr:80K:tubulin of 1:3:30, which we estimate represents 1 mol of HMr plus 15 mol of 80K/130 mol of tubulin dimers.

Various precautions were routinely used to minimize proteolysis during the preparation of the microtubules. Nevertheless, the microtubule preparations occasionally contained other major polypeptides, in addition to the HMr, 80K, and tubulin components. For example, the *S. droebachiensis* microtubules shown in Fig. 1 contained prominent polypeptides with *M*ₚ ~ 220,000 and 150,000.

The HMr and 80K polypeptides (and also the 220K and 150K species, when present) appear to be specifically associated with the taxol-assembled microtubules, by the following criteria: 1) these polypeptides were not removed from the microtubules by washing in PME buffer containing GTP plus taxol. 2) When taxol and GTP were not added to the extract supernatant, these polypeptides did not pellet, but remained in the supernatant with the disassembled tubulin (Fig. 1, lanes 66 and 76). 3) Similar, when taxol-dependent microtubule assembly was inhibited by preincubating the extract in 300 μM NaCl, none of these putative MAP polypeptides pelleted, and neither did tubulin (Fig. 1, lanes 6b and 7b). 4) When taxol microtubules were assembled in the presence of 0.5 M NaCl to dissociate ionic bonds between MAPs and tubulin (27), the microtubule pellet was found to contain pure tubulin, essentially free of any associated polypeptides (Fig. 1, lanes 4b and 5b).

Therefore, the pelleting of the HMr and 80K polypeptides (plus the 220K and 150K polypeptides of *S. droebachiensis*) depends upon their binding and co-sedimenting with microtubules. By these criteria, they are specific MAPs.

*Morphology of the Taxol-assembled Microtubules*—When preparations of microtubules (Fig. 1, lanes 3a and 2b) were examined in the electron microscope, only morphologically normal microtubules (and no free protofilaments or protofilament sheets) were visible (Fig. 2). The diameter of these microtubules was 24.4 ± 0.8 nm (n = 25). In sections of microtubule pellets, “fuzzy” filamentous material was often visible between the microtubules. Numerous projections were visible on microtubules negatively stained using the procedure of Langford (41) (Fig. 2, a, c, and d). These projections displayed an obvious axial periodicity in some regions along the microtubule surface. For example, the projections in Fig. 2d display a periodicity of 38.6 ± 1.2 nm. Projections are not present on microtubules sedimented in high ionic strength buffers (Fig. 2e), conditions which dissociate MAPs from microtubules.

*Further Fractionation of the HMr and 80K MAPs*—The HMr and 80K MAPs appear to bind to microtubules via ionic bonds which are dissociated at high ionic strength (Fig. 1, lanes 4b and 5b; see also Refs. 27 and 29) and, thus, they could be extracted from microtubules by differential centrifugation in approximately 0.6 M NaCl buffers which contained taxol and GTP to maintain microtubule assembly, yielding preparations greatly enriched in the HMr and 80K MAPs (Fig. 3).

In addition, HMr 2 and HMr 3 (see the following section), but not HMr 1 or the 80K MAP, could be extracted from microtubules by differential centrifugation in ATP (Fig. 3, lanes 4–6). In the absence of high salt or ATP, negligible quantities of the HMr or 80K MAPs were extracted from the microtubules (Fig. 3, lane 7). Gel filtration chromatography of the high salt-extracted MAPs on Bio-Gel A-1.5m (Fig. 4) yielded two major protein peaks. The leading peak, which eluted with a partition coefficient (Kₚ) of <0.10, contained the HMr MAPs (yield approximately 0.1 mg/10 ml of eggs), whereas the main component of the retarded peak was the 80K protein (yield approximately 0.1–0.5 mg of 80K MAP/10 ml of eggs). The latter species eluted with a Stokes radius of 35 Å, suggesting it is a rather compact molecule.

Following gel filtration chromatography, the 80K MAP and all three HMr MAPs (HMr 1, HMr 2, and HMr 3) of *S. droebachiensis* (see below) were competent to bind and co-sediment with taxol-assembled microtubules prepared from purified bovine brain tubulin (data not shown).

*SDS Gel Analysis of the HMr MAPs Associated with Taxol Microtubules and Isolated Mitotic Spindles*—We consistently observed three HMr MAP polypeptides in preparations of *S. droebachiensis* microtubules which we refer to as HMr 1, HMr 2, and HMr 3 (in order of decreasing *M*ₚ) (Fig. 5). Densitometry and planimetry of SDS gels suggested that these polypeptides were present in the MTP preparations at weight ratios of HMr 1:HMr 2:HMr 3 = 3:2:2. The compositions of the HMr MAPs present in *L. pictus* and *S. purpuratus* microtubules were different; *S. purpuratus* MTP contained large amounts of polypeptides which co-migrated with HMr 2 and HMr 3 of *S. droebachiensis*, whereas a polypeptide corresponding to HMr 1 was present in much lower quantities (Fig. 5b). The major HMr polypeptide of *L. pictus* MTP co-migrated with HMr 3, and little HMr 1 or HMr 2 was present (Fig. 5d). HMr polypeptides present in *S. droebachiensis* mitotic spindle preparations co-migrated with HMr 1 and HMr 3 of the MTP preparations, but a peptide corresponding to HMr 2 was not seen (Fig. 5c). Thus, these different preparations vary with respect to HMr 1 and HMr 2, but they all contained an HMr polypeptide (*M*ₚ ~ 350,000) which co-migrated with HMr 3 of *S. droebachiensis*. This component co-migrated on SDS gels with the major heavy chain of 0.6 M NaCl-extracted *S. droebachiensis* sperm axonemal dynein.
Sea Urchin Microtubule Protein

FIG. 4. Bio-Gel A-1.5m gel filtration chromatography of high salt-extracted S. droebachiensis MAPs. Between 0.5 and 1.0 ml of high salt-extracted MAPs at 0.5-1.0 mg/ml concentration (Fig. 3) were routinely chromatographed on a 1 x 11 cm column of Bio-Gel A-1.5 m, preequilibrated, and eluted in PME buffer, containing protease inhibitors plus 0.1 mM GTP; flow rate, 35 ml/h; temperature, 4°C; 0.5-ml fractions were collected. Fractions were analyzed for protein concentration (33) and MgATPase activity (O--O). SDS gels of the fractions indicated are shown. Lanes marked 80K and HMr show pooled 80K and HMr MAP preparations, respectively. Tub, tubulin.

(Fig. 5, a and b), as does purified sea urchin egg cytoplasmic dynein (13).

The MgATPase Activity of Taxol-Microtubules—The extract supernatants obtained from unfertilized eggs of S. droebachiensis, S. purpuratus, and L. pictus contain MgATPase activity which appears to be 30-50% vanadate sensitive (measured in 50 μM Na₃VO₄; Table I). The ATPase activity present in L. pictus egg extracts was observed to elute as a single peak from columns of Bio-Gel A-5m (Kᵥ ≈ 0.15). It was estimated that 14-18% of the MgATPase activity pelleted from egg extracts with the taxol-assembled microtubules, which exhibited specific activity of 10-20 nmol/min/mg (Table I). The corresponding level of depletion of tubulin from the extract supernatant was, however, much greater. Using anti-tubulin immunoblotting, we estimate that at least 90-95% of the tubulin present in the extract pelleted as taxol-microtubule protein (Fig. 9, Miniprint). In the absence of microtubule assembly, negligible quantities of MgATPase were pelleted from egg extracts. For example, when one aliquot of an S. purpuratus egg extract was incubated in taxol plus GTP to induce microtubule assembly, whereas taxol and GTP were omitted from a control aliquot, we observed that the microtubule pellet obtained by centrifuging the former aliquot hydrolyzed 11.9 nmol of MgATP/min whereas the control pellet hydrolyzed only 0.4 nmol of ATP/min.

Extraction of the MgATPase from Microtubules—Two procedures for extracting the ATPase from the MTs have been attempted, namely high salt extraction and ATP extraction (Table II, Miniprint; Fig. 3), since these protocols are known to extract dynein from sperm axonemal microtubules (43). Differential centrifugation of the taxol-MTP in high salt usually extracted about one-quarter of the MgATPase activity of the microtubules (Table II, Miniprint) (specific activity approximately 30 nmol/min/mg), together with the high salt-extracted MAPs.

Incubation of taxol-microtubules from S. droebachiensis in
FIG. 5. SDS-PAGE analysis of the HMr MAPs present in sea urchin egg taxol-MTP and isolated mitotic spindle preparations. a, b, and c show lanes from 5% SDS gels, which were stained with Coomassie brilliant blue, whereas d shows 4–11% polyacrylamide gradient gels, stained with silver (38). In gel a, S refers to isolated spindles from *S. droebachiensis* eggs, and D refers to purified *S. droebachiensis* sperm axonemal dynein. In gel b, M refers to MAPs extracted from *S. purpuratus* taxol-microtubules by differential centrifugation in 0.6 M NaCl and D refers to *S. droebachiensis* sperm axonemal dynein. In c, M refers to taxol-stabilized microtubules from *S. droebachiensis* and S refers to isolated spindles from the same species. In d, 1 shows taxol microtubule protein from *S. droebachiensis* and 2 shows taxol-microtubule protein from *L. pictus*. Note that *S. droebachiensis* contains three HMr polypeptides; HMr1, HMr2, and HMr3. The major *S. purpuratus* HMr MAPs correspond to HMr2 and HMr3, whereas the prominent HMr polypeptide in *L. pictus* MAPs is HMr3. Isolated spindles contain polypeptides which co-migrate with HMr1 and HMr3. HMr3, which appears in all the preparations examined, co-migrates with A subunits of *S. droebachiensis* sperm axonemal dynein. TUB, tubulin.

**Table I**

Precipitation of Na<sub>3</sub>VO<sub>4</sub>-sensitive MgATPase activity together with taxol-assembled microtubules from extracts of unfertilized sea urchin eggs

Taxol-assembled microtubules were prepared by incubating extracts of unfertilized eggs with taxol and GTP as usual, followed by centrifugation to pellet the microtubules. ATPase activities of the extract supernatant, the pelleted microtubules, and the residual supernatant were measured in the standard assay buffer, in the presence or absence of 50 μM Na<sub>3</sub>VO<sub>4</sub>. Specific activities are expressed in nanomoles of ATP hydrolyzed/min/mg of protein, at 22°C. Total activity is specific activity × mg of protein. Vanadate sensitivity = (1 – (ATPase + 50 μM Na<sub>3</sub>VO<sub>4</sub>)) × 100%

<table>
<thead>
<tr>
<th>Fraction</th>
<th>L. pictus MgATPase</th>
<th>S. purpuratus MgATPase</th>
<th>S. droebachiensis MgATPase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Specific activity</td>
<td>Vanadate sensitivity</td>
<td>Total activity ATPase</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>Extract supernatant</td>
<td>1.45</td>
<td>52</td>
<td>176</td>
</tr>
<tr>
<td>Taxol-assembled microtubules</td>
<td>17.5</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>Residual supernatant</td>
<td>1.9</td>
<td>33</td>
<td>158</td>
</tr>
</tbody>
</table>

In contrast, when the taxol-MTP was incubated in PME buffer containing no ATP, very little HMr 2 or 3 and correspondingly less MgATPase activity were extracted.

Co-purification of the MgATPase with HMr MAPs—When
high salt-extracted MAPs were chromatographed on Bio-Gel A-1.5m, the MgATPase activity co-eluted with the HMr MAPs (Fig. 4). The pooled HMr MAP fractions routinely possessed specific activity between 40 and 70 nmol of ATP split/min/mg. On 5-20% sucrose density gradients (Fig. 6), the MgATPase activity co-sedimented with HMr 3 and HMr 1 as an approximately 12 S peak. There was a sharp correspondence between the MgATPase activity and HMr 3, whereas a number of fractions containing HMr 1 did not possess MgATPase activity. This result, together with the observed absence of HMr 1 in the ATP-extracted MAP fraction is consistent with the hypothesis that HMr 3 is the MgATPase polypeptide. The egg HMr MAP MgATPase activity demonstrated sedimentation behavior similar to S. droebachiensis dynein extracted from sperm axonemes using high salt in the presence of taxol (Fig. 6).

Dynein-like Characteristics of the HMr MAP MgATPase Activity—The specific activity of the Bio-Gel A-1.5m-chromatographed HMr MAPs (40–70 nmol/min/mg) was considerably lower than that of sperm axonomal dynein prepared by a similar procedure (1.0–2.5 μmol/min/mg). In other respects, however, the two enzymes were rather similar. For example, both enzymes showed similar divalent cation requirements; the HMr MAP MgATPase exhibited 100% activity in presence of Mg2+, 63% maximal activity in Ca2+, and 0% activity in EDTA, the corresponding values for axonomal dynein being 100% activity in Mg2+, 57% in Ca2+, and 0% in EDTA. Furthermore, the MgATPase activities of axonomal dynein and the HMr MAPs showed strikingly similar patterns of inhibition by Na3VO4 (50% inhibition at 50 μM Na3VO4; Fig. 7). A MgATPase activity associated with isolated mitotic spindles exhibited similar vanadate sensitivity, whereas a bovine brain microtubule-associated MgATPase activity was not inhibited by Na3VO4 concentrations below 0.1 mM. The HMr MAP and spindle MgATPase activities were inhibited by EHNA (Fig. 7), which also inhibits axonomal dynein MgATPase activity (44).

**Isolation of the Dynein-like MgATPase Activity from the**

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**FIG. 6. Sucrose density gradient centrifugation of high salt-extracted MAPS (upper trace) and sperm axonomal dynein (lower trace) from S. droebachiensis.** Approximately 1 ml of high salt-extracted MAPS and crude sperm axonomal dynein (prepared by extracting axonemes with PME buffer containing 0.2 mM GTP, 10 μM taxol, 0.1 mM ATP plus 0.5 M NaCl) were layered onto 5-20% (w/v) sucrose gradients containing PME buffer plus 0.6 M NaCl. The gradients were centrifuged at 26,000 rpm for 20 h in a Beckman SW 27 swinging bucket rotor at 4 °C. 1.4-ml fractions were collected from both tubes, and were assayed for protein concentration (33) and for MgATPase activity (O--O). Top marks the top of the sucrose gradients. Refractive index measurements showed that the egg MAP MgATPase and the axonomal dynein MgATPase sedimented to positions of similar sucrose concentration in their respective gradients. The insets show SDS gels of fractions indicated by the numbers shown and also the pooled MgATPase preparation. Note that the fractions enriched in MgATPase activity are also enriched in polypeptides corresponding to the egg MAPs HMr 1 and HMr 3.
to specifically co-pellet with microtubules from all three species of sea urchin studied, and they were capable of binding to bovine brain microtubules. However, these proteins were dissociated from microtubules in high ionic strength buffers. We therefore conclude that the HMr and 80K polypeptides are MAPs which associate with microtubules in vitro through the formation of ionic bonds. Vallee (27) has similarly noted a likely role of ionic bonds in the interaction between MAPs and microtubules from mammalian cells.

In addition to the 80K and HMr polypeptides, there are also polypeptides (e.g. the 220K and 150K components of some S. droebachiensis preparations; Fig. 1) which appear in variable quantities in different preparations. The significance of the variability is difficult to evaluate; it may depend upon proteolysis, despite the use of protease inhibitors, or upon subtle changes in the physiology of the organisms themselves.

Electron microscopy of negatively stained taxol-assembled microtubules (41) comprising tubulin, 80K, and HMr MAPs (e.g. Fig. 1, lane 3a), revealed prominent projections from the microtubule surface. These projections were "extracted," together with the 80K and HMr MAPs, by differential centrifugation in high ionic strength buffers, giving rise to smooth walled microtubules in the pellet. These results suggest that the projections represent either the 80K MAP, the HMr MAPs, or both. Brain high Mr, MAPs (45) and axonemal dynein (49, 50) are examples of high Mr polypeptides which appear as projections on microtubules. The 80K MAP which is associated with the taxol-assembled microtubules may be related to the 80K tubulin-binding MAP identified by Keller and Rebhun (15) in isolated sea urchin mitotic spindles, which is proposed to function as an intermicrotubule cross-bridge (47). Whatever the identity of the projections present on the taxol-assembled microtubules (Fig. 2), it is tempting to speculate that they are related to the arms and bridges which are visible by electron microscopy in mitotic spindles and other cellular arrays of microtubules (6, 46).

One approach to the isolation of sea urchin spindle MAP-containing microtubules, described here, was based on work by Vallee (27) who used taxol to prepare microtubules from mammalian cells. Recently, Vallee's group (28, 29) reported similar studies using the sea urchins Lytechinus variegatus and S. purpuratus. Their microtubule preparations also contained tubulin, a 77K polypeptide, plus various high Mr components, and in the electron microscope, projections and cross-bridges were seen to be associated with the microtubules. Furthermore, monoclonal antibodies raised against various components of their preparations were shown to be components of the mitotic spindle of early sea urchin embryos (29), demonstrating the usefulness of sea urchin taxol-assembled microtubules as "affinity ligands" for preparing spindle MAPs.

One component of unfertilized sea urchin eggs which might function as a spindle MAP (14) is cytoplasmic dynein (9–13). We therefore investigated the association of the dynein-like MgATPase activity present in sea urchin egg extracts with our taxol-microtubule preparations, in the hope that microtubules might serve as a useful intermediate step in the purification of cytoplasmic dynein (analogous to the purification of actomyosin as an intermediate step in the purification of cytoplasmic myosin (e.g. Ref. 35)). In the conditions used, however, the MgATPase apparently bound weakly to microtubules in the extract, so that less than 20% of the activity pelleted, (even though >80% tubulin pelleted with the microtubules), resulting in a low recovery of the MgATPase. Indeed, the residual supernatant, depleted of microtubule protein using taxol, proved to be an excellent source.

**DISCUSSION**

We estimate that at least 90–95% tubulin can be pelleted from egg extracts using taxol (Fig. 9, Miniprint). Our yields of taxol-microtubules are usually approximately 2–5 mg/10 ml of eggs, which is adequate for biochemical analysis.

High molecular weight and 80K polypeptides were observed to specifically co-pellet with microtubules from all three species of sea urchin studied, and they were capable of binding to bovine brain microtubules. However, these proteins were dissociated from microtubules in high ionic strength buffers. We therefore conclude that the HMr and 80K polypeptides are MAPs which associate with microtubules in vitro through the formation of ionic bonds. Vallee (27) has similarly noted a likely role of ionic bonds in the interaction between MAPs and microtubules from mammalian cells.

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High molecular weight and 80K polypeptides were observed
FIG. 8. Isolation of the dynein-like MgATPase activity from the residual supernatant. A 0-55% saturated (NH₄)₂SO₄ fraction from the residual supernatant which remained after pelleting taxol-stabilized microtubules from an extract of *L. pictus* eggs (Fig. 1, lane 4a) was chromatographed on a column of Bio-Gel A-5m, and fractions were analyzed for protein concentration (C) and MgATPase activity (O) and on 6% SDS gels (A). Fractions 16-20, designated by the shaded area, which possessed specific activity of approximately 50 nmol of ATP split/min/mg were pooled for subsequent hydroxylapatite chromatography (B). The pooled ATPase fractions in 50 mM sodium phosphate buffer were loaded onto a hydroxylapatite column preequilibrated in the same buffer. The ATPase was eluted by increasing the phosphate concentration. The main peak of ATPase (shaded area) eluted at about 0.15 M sodium phosphate (ATPase specific activity approximately 150 nmol of ATP split/mg/min). The silver-stained 4-8% SDS gradient gel (B, insets) show lanes of (1) axonemal dynein and (2) the ATPase prepared by hydroxylapatite chromatography. The arrow indicates the dynein heavy chains.

of the dynein-like MgATPase (Fig. 8) for further biochemical studies. The specific activity of the MgATPase associated with the microtubules was consistently approximately 10 times higher than that of the whole cell extract, which compares with a 3-fold (or less) increase in specific activity of isolated mitotic spindles over cytoplasmic extracts (10).

Our data support the idea that HMr 3 is the MgATPase. (i) The MgATPase co-purified with the HMr MAPs during high salt extraction of the microtubules and subsequent gel filtration chromatography. (ii) The MgATPase consistently co-sedimented with HMr 1 and HMr 3 of *S. droebachiensis* on sucrose density gradients. (iii) ATP extracted mainly HMr 2 and HMr 3 from the microtubules, plus a corresponding amount of the MgATPase activity. (iv) The HMr 3 polypeptide, like the MgATPase activity (but unlike HMr 1 or HMr 2), is common to microtubules prepared from all three species of sea urchin examined. (v) A polypeptide which co-migrates with HMr 3 was present in isolated *S. droebachiensis* mitotic spindles, which also contain dynein-like MgATPase activity (Ref. 14; Figs. 5 and 7). (vi) A polypeptide which co-migrates with HMr 3 co-purified with the dynein-like MgATPase activity from the residual supernatant (Fig. 8), and was the major component of the hydroxylapatite MgATPase preparations.

The data suggest that HMr 3 may be cytoplasmic dynein. For example, (i) gel filtration chromatography and sucrose density gradient sedimentation suggest that the MgATPase possesses high molecular weight. (ii) On SDS gels, HMr 3 co-
migrates with the major heavy chain of dynein extracted from sperm axonemes under conditions which are known to extract predominantly the A subunits (43). The major polypeptide of purified cytoplasmic dynein similarly co-migrates with an A subunit of sperm axononal dynein (13). (iii) The HMr MAP MgATPase exhibits divalent cation requirements and sensitivity to vanadate and EHNA similar to those described for axonemal and cytoplasmic dyneins (9–14, 44, 48). (iv) Axonemal dyneins bind to microtubules by a salt-solubilable structural site and an ATP-sensitive site (43, 49, 50). Similarly, some of the HMr MAP MgATPase was extracted from microtubules in high salt buffers or in ATP, together with HMr 3 (Fig. 3; Table II). Other workers have also observed ATP-sensitive binding of cytoplasmic dynein to microtubules. The formation of ATP-sensitive bonds between cytoplasmic dynein and microtubules is consistent with the idea that a mechanochemical interaction occurs between them.

We do not know why 80% of the dynein-like MgATPase does not co-sediment with the taxol-assembled microtubules. It is possible that the solution conditions (e.g. ionic strength, nucleotide concentrations, presence or absence of required “co-factors,” etc.) in the extract may be unsuitable for optimal binding, or that other MAPs compete with the MgATPase for microtubule-binding sites. It would be interesting to know whether the residual dynein-like MgATPase is competent to bind microtubules. Our observations using taxol-assembled microtubules are comparable to those of Hisanaga and Sakai (13) who found that approximately 30% of their cytoplasmic dynein bound to outer doublet microtubules of sperm axonemes.

Numerous workers have described an association between MgATPase activity and mammalian brain microtubules (51–60). Thus, a \( M_0 = 33,000 \) ATPase dependent upon tubulin and Ca\(^{2+} \) for activity (57) and an ATPase associated with membrane vesicles (57, 59, 60) have been shown to co-purify with neuronal microtubules. These brain enzymes appear to be different from the sea urchin egg dynein-like MgATPase described in this and other reports (9–14). However, a rigorous evaluation of the relationship between these MgATPases must await a detailed comparison of the brain and sea urchin enzymes.

Our results lend support to the view that unfertilized sea urchin eggs contain a pool of cytoplasmic dynein (9–14), a fraction of which co-pellets with taxol-assembled microtubules from egg extracts. Microtubules are known to function as components of the early sea urchin embryo mitotic spindle. Whether the dynein-like MgATPase functions as a mechanochemical enzyme during mitosis remains to be established.

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3 P. Hollenbeck and W. Z. Cande, personal communication; D. J. Asai and L. Wilson, personal communication.
Supplementary Material

Isolation of microtubules and a dynin-like multifunction from unperturbed sea urchin embryos

By J. W. Schelby, H. Niwa, D. J. McConnohy, and T. D. Salmon

Organisms and collection of samples

Auricles from the species Echinus esculentus and Strongylocentrotus purpuratus were obtained from Pacific Biological Station, New Zealand, California, during the months of July and August, respectively. Strongylocentrotus drookeri were collected from the coast of Maine during July and August. The animals were shipped by air from New Zealand and Maine, respectively, and were placed in artificial seawater as described previously (1). Eggs and sperm were collected, following intraperitoneal injection of 0.5 ml of a 2 mmol/L HCl, in ice-cold seawater (7).

Preparation of taxol-stabilized microtubules

Fresh microtubules were prepared using modifications of the method of Vallee (27). Mature eggs were dejellied in 0.2 M NaOH and suspended in 0.5 M tricine, pH 7.4 (32). The suspension was stirred at 20°C, allowing the gelatinous matrix to slowly sediment, and the supernatant was collected at 25°C. The supernatant was filtered through a 0.45 filter and used as extract supernatant. An aliquot of extract supernatant was used to test for tubulin concentration using a dye exclusion assay (33). The supernatant was dialyzed against 0.1 M tricine, pH 7.4, containing 0.1 mmol/L dithiothreitol (dithiothreitol in the presence of small amounts of ATP and GTP at 20°C) (32) for 24 h and used for ATPase assays.

Preparation of ATP hydrolyzing activity from microtubule-depleted egg extract

A 1.4 A natural unfertilized egg extract was prepared in 100 mM NaCl and 100 mmol/L HEPES (pH 7.4) containing 0.1 mmol/L dithiothreitol (dithiothreitol in the presence of small amounts of ATP and GTP at 20°C). The extract was then subjected to microtubule depletion at 0°C for 30 min in the presence of 20 μg of taxol/mg of extract supernatant. The supernatant was collected and dialyzed against 0.1 M tricine, pH 7.4, containing 0.1 mmol/L dithiothreitol (dithiothreitol in the presence of small amounts of ATP and GTP at 20°C) for 24 h. The supernatant was dialyzed against 0.1 M tricine, pH 7.4, containing 0.1 mmol/L dithiothreitol (dithiothreitol in the presence of small amounts of ATP and GTP at 20°C) for 24 h and used for ATPase assays.

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