BIOCHEMICAL AND CYTOCHEMICAL EVIDENCE FOR ATPase ACTIVITY IN BASAL BODIES ISOLATED FROM OVIDUCT

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ABSTRACT

Biochemical and cytochemical techniques were used to determine whether oviduct basal bodies have ATPase activity. All studies were carried out on basal bodies isolated and purified from the chicken oviduct. These preparations contained structurally intact basal bodies with basal feet, rootlet, and alar sheet accessory structures. Whereas the specific activity of the basal body ATPase in 2 mM Ca^{++} or 2 mM Mg^{++}, 1 mM ATP, pH 8.0, averaged 0.04 μmol P_i/min per mg protein, higher concentrations of either cation inhibited the enzyme activity. Furthermore, the pH optimum for this reaction was pH 8.5. In comparison, the ATPase activity in cilia purified and measured under conditions identical to those for determining the basal body ATPase activity averaged 0.07 μmol P_i/min per mg protein. However, the activity increased at higher concentrations of divalent cation, and the pH optimum was pH 10.0. By cytochemical procedures for localizing ATPase activity, ATP-dependent reaction product in isolated basal bodies was found to be confined to: (*a*) the cross-striations of the rootlet; (*b*) the outer portion of the basal foot; (*c*) the alar sheets; and (*d*) the triplet microtubules. It is concluded that basal bodies have an intrinsic ATPase activity that, by a variety of criteria, can be distinguished from the ATPase activity found in cilia.

The basal body and its homologue, the centriole, have attracted the interest of cell biologists and cytologists through the years. To date, there is a good understanding of the structure of this organelle in a variety of organisms (2, 12, 15, 24, 30, 35, 41), and the morphological events of replication have been extensively studied (4, 8, 19, 33, 34, 38). However, it has been difficult to experimentally probe the functional potential of this organelle. Presently, the two most accepted theories for explaining how the basal body functions are: (*a*) it acts as the initiation site for cilium formation during ciliogenesis (11), and (*b*) it is an anchoring device that holds the cilium in the cell (10). These theories are based on the observations that: (*a*) all organisms studied so far form their cilium or flagellum from the distal end of a basal body, and (*b*) in those cells that produce multiple cilia, the basal body is retained, and it contains accessory structures, e.g., rootlets, that appear to be adapted for anchoring the basal body-cilium complex within the cell.

There is no definitive proof that basal bodies do function as initiating sites or as anchors for cilia. Furthermore, this organelle may have other functions. It is possible that the basal body can act as a type of pacemaker that regulates the initiation of the ciliary beat (16, 18) and/or coordinate the beat cycle of the cilia within each cell. Another possibility is that this organelle may control the direction of the ciliary beat (9, 12).

An important question is whether any of the

postulated functions of the basal body are active or passive in nature. That is, do basal bodies carry out any energy-requiring activities or do they function simply by virtue of their shape and juxtaposition to other cellular elements, e.g., microfilaments, in the cell? As a first step towards answering this question, it is important to determine whether this organelle has the machinery to utilize ATP. If basal bodies contain enzymes that are specific for the hydrolysis of ATP, then this would constitute presumptive evidence that regardless of the function of this organelle, it involves some type of active process.

Several investigators have examined basal bodies and centrioles for cytochemical evidence of ATPase activity (1, 22, 27, 28). Abel et al. (1) have reported the presence of an ATPase associated with centrioles in several cell lines. Markwald[1] has obtained similar results. Two investigators have reported ATPase activity in basal bodies. Matsusaka (22) found activity over the cross-striations of the basal body rootlet in the inner rod segment of human retinal rods. Reaction product was also present over the basal body. Nayak (27) and Nayak and Wu (28) found ATPase activity associated with the basal bodies of porcine, bovine, and rabbit oviducts but did not find any activity over the cross-striations of the basal body rootlets.

In the present report, we present additional evidence that basal bodies have ATPase activity. These studies combine both biochemical and cytochemical techniques to investigate the activity of this enzyme in preparations of purified basal bodies isolated from the chicken oviduct. We conclude that the basal body ATPase has ionic requirements distinct from those for the ATPase of oviduct cilia, and that the enzyme activity is located in at least four different portions of the organelle.

MATERIALS AND METHODS

Isolation of Basal Bodies and Cilia

Adult laying hens were obtained from a local supplier and sacrificed by intracardial injection of Nembutal (Abbott Laboratories, South Pasadena, Calif.). Each oviduct was immediately removed, cut into small pieces, and placed in a 1-liter trypsinizing flask (Grand Island Biological Co., Grand Island, New York) with 200 ml of Hanks' balanced salt solution (HBSS). A magnetic stirring bar was placed in the flask and the mixture was stirred on a magnetic stirrer at high speed for 1 min. The HBSS was removed and fresh HBSS was added. The pieces of oviduct were washed four times with this procedure (which is presented diagrammatically in Fig. 1).

ISOLATION OF CILIA: Cilia were isolated by the previously described techniques (3). After the last wash in HBSS, the medium in each flask was replaced with 300 ml of a solution that contained 0.25 M sucrose, 0.02 M piperazine-*N*-N'-bis[2-ethane sulfonic acid] (PIPES), pH 5.5, 0.01 M $CaCl_2$, 0.01 M KCl, and 0.05% Triton X-100 (Sigma Chemical Co., St. Louis, Mo.). The pieces of oviduct were stirred vigorously in this solution for 5 min. The supernate, which contained the cilia, was removed and centrifuged at 600 *g* for 10 min in an HS-4 rotor (Dupont Instruments, Sorvall Operations, Newtown, Conn.). The pellet was discarded and the supernate was centrifuged at 9,000 *g* to pellet the cilia. Most of the supernate was discarded, and the pellet in each tube was then resuspended in the small amount of remaining supernate and layered over a PIPES-buffered 1.5, 2.0-M sucrose step gradient, pH 6.5, in a 50-ml tube. This was centrifuged at 10,000 *g* for 30 min in an HB-4 rotor, and the purified cilia were collected from the 1.5–2.0-M sucrose interface. Cilia were stored frozen in the sucrose-containing buffer.

ISOLATION OF CORTICES: The pieces of oviduct remaining after the removal of the deciliation solution still contained intact epithelial cells with the basal bodies in their normal cortical arrangement (3). These pieces of unciliated oviduct were then stirred vigorously for 5 min in 300 ml of a solution that contained 0.25 M sucrose, 0.02 M *N*-2-hydroxyethylpiperazine-*N'*-2-ethane sulfonic acid (HEPES), pH 7.5, 0.002 M EDTA, 0.01 M KCl, and 0.025% Triton X-100 (see Fig. 1). The pieces of oviduct were then discarded, and the supernate was centrifuged at 600 *g* for 5 min to pellet the basal body-containing cortices and the nuclei. This pellet was washed once by resuspension in a solution of 1 M sucrose, 0.002 M EDTA, 0.02 M HEPES, 0.01 M KCl, 0.025% Triton X-100, and 1 mg/ml bovine serum albumin, pH 7.5, and centrifuged at 4,000 *g* for 15 min. The pellet was resuspended in PIPES-buffered 2.5 M sucrose, pH 6.5, over which was layered equal volumes of PIPES-buffered 2.2 M and 1.5 M sucrose, pH 6.5, and the gradient was centrifuged in an SW27 swinging bucket rotor (Beckman Instruments, Spinco Div., Palo Alto, Calif.) at 40,000 *g* for 2 h. The partially purified cortices were collected from the 2.5–2.2-M interface and frozen in the sucrose buffer.

ISOLATION OF BASAL BODIES: The frozen cortices could be stored for several weeks at −10°C. When purified basal bodies were desired, the cortices were thawed and the molarity of the sucrose was lowered by adding excess 0.02 M PIPES buffer, pH 6.5. This mixture was centrifuged at 4,000 *g* for 15 min and the supernate was discarded. The pellet was resuspended in a buffer that contained 0.02 M HEPES, 0.01 M KCl, pH 7.0, and 0.05% Triton X-100 (HKT), and sonicated for

[1] R. R. Markwald, personal communication.

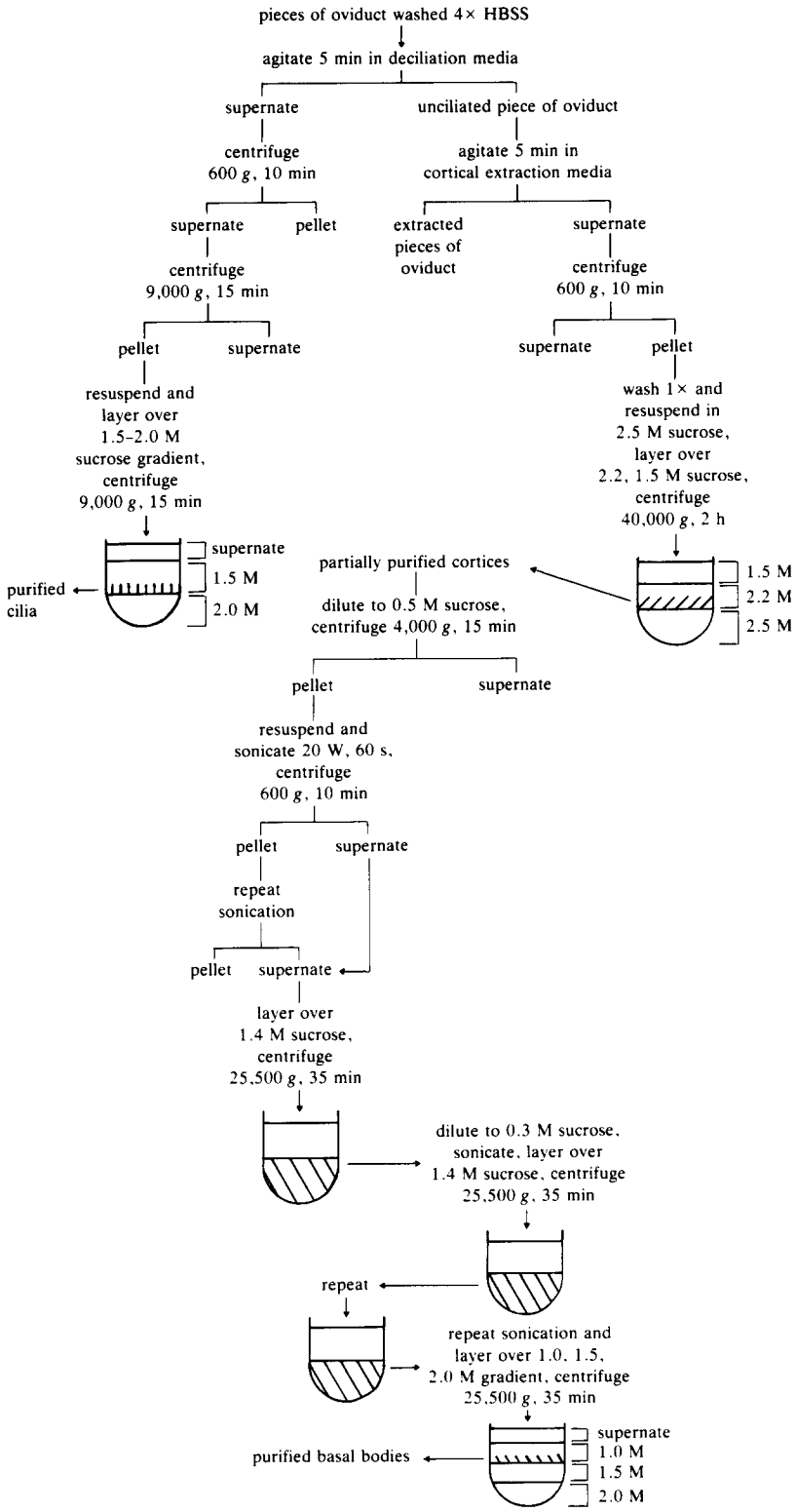


FIGURE 1 A diagram that shows the various steps in the purification of chicken oviduct cilia and basal bodies.

1 min at 20 watts of power in a 30-ml tube with a Heat Systems-Ultrasonics, Inc. (Plainview, N. Y.) sonicator that contained a microtip. The sonicate was centrifuged at 600 *g* for 10 min to pellet any undisrupted cortices. The supernate was saved; the pellet was resuspended in HKT buffer and resonicated. This sonicate was centrifuged at 600 *g* for 10 min and the pellet was discarded.

The two supernates, which contained disrupted cortices, were pooled and layered over 1.4 M sucrose buffered with 0.02 M HEPES, pH 7.0, and centrifuged at 25,500 *g* in an HB-4 rotor for 35 min. All of the basal bodies as well as some contaminating material entered the 1.4-M sucrose. The sucrose and any pellet were resuspended by adding enough of the HKT buffer to adjust the concentration of sucrose to 0.3 M. This mixture was sonicated at 20 watts of power for 30 s, layered over 1.4 M sucrose, and centrifuged at 25,500 *g* for 35 min. This whole procedure was repeated once more. After the third 1.4-M sucrose layer and pellet was diluted and sonicated, it was layered over a step gradient of 1.0, 1.5, and 2.0 M sucrose buffered with 0.02 M HEPES, pH 7.0, and centrifuged at 25,500 *g* for 35 min. After this final centrifugation, the purified basal bodies appeared as a pink band just below the 1.0–1.5-M interface. The basal bodies were recovered from the gradient and either used immediately for biochemical measurements or frozen overnight for use in cytochemical experiments.

Biochemical ATPase Measurements

All ATPase determinations on basal bodies utilized organelles freshly isolated as described above. However, the ATPase activity of cilia was measured in preparations that had been sonicated to break the cilia into smaller pieces. To prepare these ciliary fragments, frozen, purified cilia were thawed and diluted with 0.02 M PIPES buffer, pH 6.5, before centrifuging at 9,000 *g* for 15 min. The pellet was resuspended in HKT buffer and sonicated at 20 W for 1 min. This was then layered over 1.4 M sucrose and centrifuged at 25,500 *g* for 30 min. All of the broken cilia entered the 1.4-M sucrose layer. This fraction was diluted with HKT buffer, resonicated, layered over 1.4 M sucrose, and centrifuged into the 1.4-M sucrose once again. The ciliary fragments from the 1.4-M sucrose were used for measurements of ciliary ATPase.

To measure ATPase activity, 0.2-ml aliquots of either basal bodies or ciliary fragments (containing ~100 μg of protein) suspended in 0.2 M Tris-HCl, pH 8.0, were added to 1.8 ml of reactivation media. The basic medium contained 0.15 M KCl, 5×10^{-4} M EDTA (Sigma Chemical Co.), 10^{-4} M dithiothreitol (Sigma Chemical Co.), 0.2 M Tris-HCl (Sigma Chemical Co.), pH 8.0, 0.001 M substrate (either ATP, ITP, GTP, ADP, AMP, pyrophosphate, or *p*-nitrophenyl phosphate [all from Sigma Chemical Co.]) and either $MgCl_2$ or $CaCl_2$. The mixture was incubated for 15 min at 25°C, cooled to 4°C, and centrifuged at 25,500 *g* for 30 min at 4°C. The supernate was removed and the concentration of phosphate was determined by the Gomori technique (17). The protein concentration in the pellets was determined by the Lowry procedure (20).

Cytochemical Detection of ATPase Activity

A modification (21, 23) of the Wachstein-Meisel technique (39) was used to localize ATPase activity in isolated basal bodies. Purified basal bodies were suspended in 20 ml of a solution containing 0.002 M $Pb(NO_3)_2$, 0.004 M ATP, 0.004 M $MgSO_4$, 0.075 M Tris-maleate, pH 7.3, 5% sucrose, 0.1 M NaCl, 5×10^{-4} M EDTA, and 0.05 M KCl. The mixture was incubated for 20 min at 25°C. The reaction was stopped by adding 2 ml of 2% glutaraldehyde buffered with 1% Na cacodylate, pH 7.3. The suspension was centrifuged at 25,500 *g* for 35 min and the supernate was discarded. The pellet was resuspended in 1 ml of fresh 2% glutaraldehyde and pelleted in a microfuge tube.

In some experiments, basal bodies were pelleted, fixed with 2% glutaraldehyde buffered with 1% Na cacodylate, pH 7.3, and then exposed to 20 ml of the cytochemical reactivation mixture for 20 min at room temperature. The reactivation mixture was removed; the pellet was resuspended in 2% glutaraldehyde and pelleted in a microfuge tube.

Electron Microscopy

Microfuge pellets of basal bodies were washed with 5% sucrose, 1% Na cacodylate, pH 7.3, before postfixation in 1% OsO_4 buffered with 1% Na cacodylate, pH 7.3. Fixed pellets were then embedded in Araldite, sectioned on a Porter-Blum MT-2B ultramicrotome (DuPont Instruments, Sorvall Operations) and examined with either a Philips 200 or Philips 300 electron microscope.

The purity of the basal body preparations was checked by placing a drop of a basal body suspension on a parlodion-covered grid, drying the sample and then staining with 1% uranyl acetate. These preparations were examined directly in the electron microscope (as shown in Fig. 2).

RESULTS

Evaluation of Basal Body Isolation Procedure

The first step in isolating chick oviduct basal bodies was to obtain partially purified unciliated cortices. This procedure, which was adapted from a technique developed in this laboratory for isolating cortices from the rabbit oviduct (3), gave cortices that contained basal bodies, some cytoplasmic contamination, an occasional cilium or group of cilia, and some nuclei. It was found

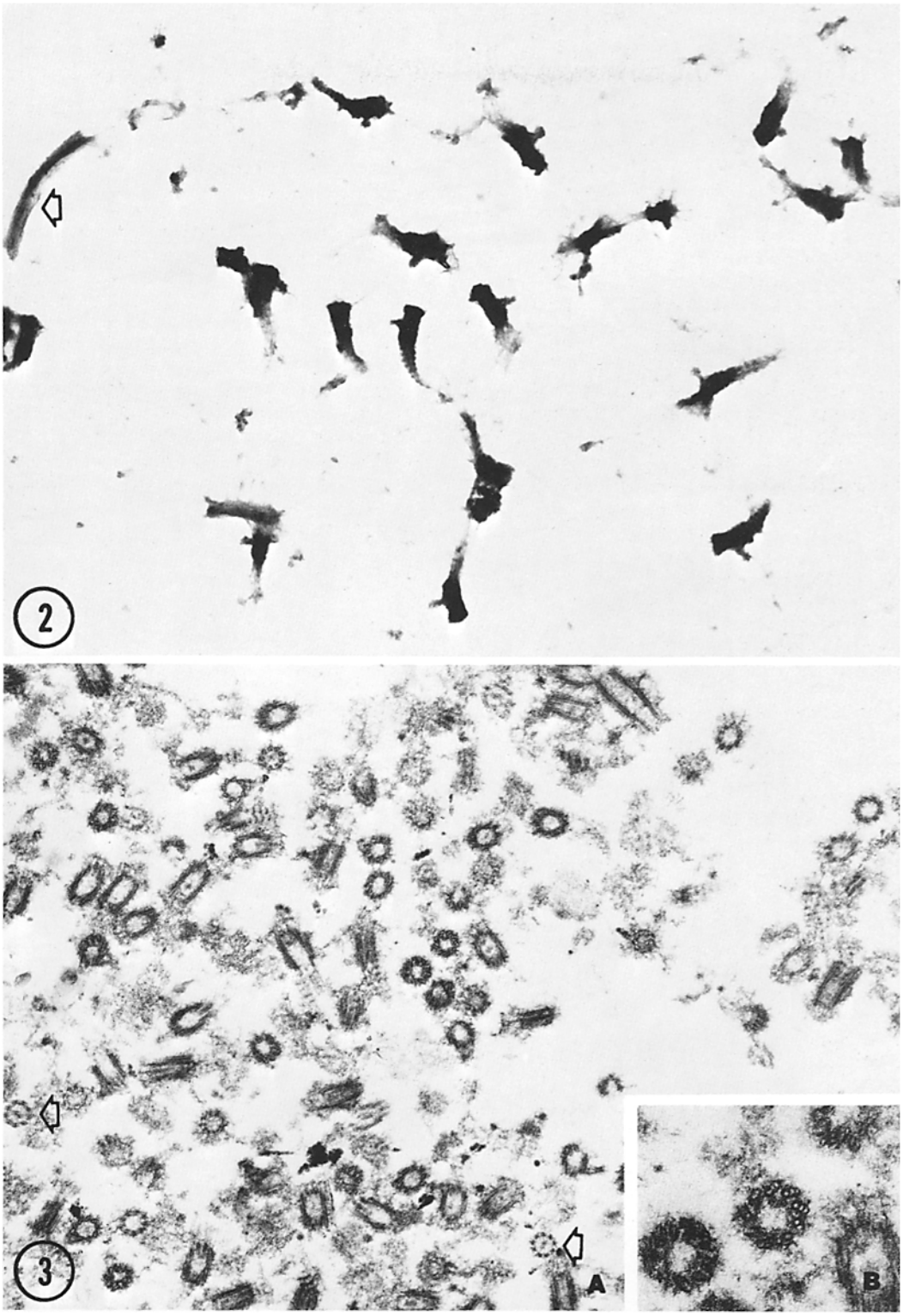


FIGURE 2 A sample of a typical preparation of purified basal bodies that was dried on a parlodion-coated grid and stained with uranyl acetate. These preparations contained a few ciliary fragments (open arrow) derived from the sonication of those whole cilia that contaminated the initial cortical preparation. × 17,500.

FIGURE 3 A micrograph of a pellet of purified basal bodies. (*A*) Low-magnification view that demonstrates the purity of these preparations. Occasional pieces of cilia are found (arrows). × 24,000. (*B*) A high-magnification view of a cross section through the proximal end of an isolated basal body. The characteristic organization of the triplet microtubules is preserved in these preparations. × 60,000.

that the gentle sonication disrupted the cortices and the nuclei and produced a suspension of free basal bodies with some adhering cytoplasmic matrix. Repeated sonication followed by centrifugation into 1.4 M sucrose removed most of the cytoplasmic contamination, and the final centrifugation into a sucrose step gradient separated basal bodies from most of the contaminating cilia. On the average, 80–100 μg of basal body protein was obtained from each laying-hen oviduct.

Fig. 3 is an electron micrograph of a pellet of purified basal bodies. These organelles retained all of their normal morphological characteristics during the isolation procedure. Rootlets, basal feet, and alar sheet (2) accessory structures were present, and the nine sets of triplet microtubules were preserved (Fig. 3 *B*). In addition to a small amount of cytoplasmic contamination, some fragments of broken cilia were also present. The number of contaminating cilia varied among preparations; however, we estimate from negatively stained preparations of whole basal bodies (Fig. 2) that, on the average, 10–15% of the fraction was ciliary fragments with no more than 25% contamination in any one preparation.

To date, all attempts to remove the contaminating cilia have failed. The cilia came from those cortices that were initially incompletely deciliated. The sonication broke these cilia into pieces about the size of a basal body, and, therefore, the two organelles could not be separated by density gradient centrifugation. Because of this contamination, the ATPase activity in isolated basal body preparations had to be compared with the activity in preparations of fragmented cilia (prepared as described in Materials and Methods).

Basal Body ATPase Activity

When purified basal bodies were incubated in reactivation mixture without divalent cations, negligible amounts of inorganic phosphate were released. However, in the presence of either 2 mM Ca^{++} or 2 mM Mg^{++}, ATPase activity ranged from 0.03 to 0.06 μmol P_i/min per mg of protein (average 0.04 μmol P_i/min per mg of protein). Both ions were equally effective in activating the ATPase. For comparison, under exactly the same conditions, purified ciliary fragments had an ATPase activity that ranged from 0.04 to 0.16 μmol P_i/min per mg of protein with an average of 0.07 μmol P_i/min per mg of protein. Therefore, on the average, basal bodies had about half the specific activity of ciliary fragments.

Basal body and ciliary ATPase activity behaved quite differently in response to increasing concentration of divalent cation (Figs. 4, 5). Optimal activity for basal bodies was at 2 mM Ca^{++} or 2 mM Mg^{++}, with the activity decreasing at higher concentrations; however, with ciliary fragments, ATPase activity continued to increase at concentrations of Mg^{++} or Ca^{++} higher than 2 mM.

The basal body ATPase also responded differently than the ciliary ATPase to pH (Fig. 6). The basal body ATPase had a pH optimum of pH 8.5 and was inhibited at higher pH. However, the ciliary ATPase increased in activity to pH 10.0 and then decreased. This pH optimum for oviduct cilia is in agreement with that reported for bound dynein in sea urchin flagella using 0.15 M KCl in the assay mixture (14).

To determine the substrate specificity for the basal body enzyme, the ability of this organelle to hydrolyze various nucleotides was examined (Table I). With ATP, the specific activity was 0.04

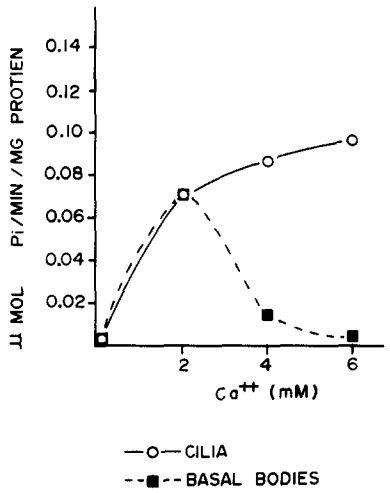


FIGURE 4 Comparison of ATPase activity in basal bodies and in ciliary fragments at various concentrations of calcium. 0.2 ml of the purified preparations were mixed with 1.8 ml of reactivation mixture, pH 8.0, and incubated for 15 min as described in Materials and Methods. Whereas basal body ATPase activity was inhibited at concentrations of calcium above 2 mM, ciliary ATPase activity continued to increase. The dramatic, reproducible inhibition of basal body ATPase activity at 4 and 6 mM calcium indicates that this enzyme activity is not coming from any cilia present in the basal body preparations.

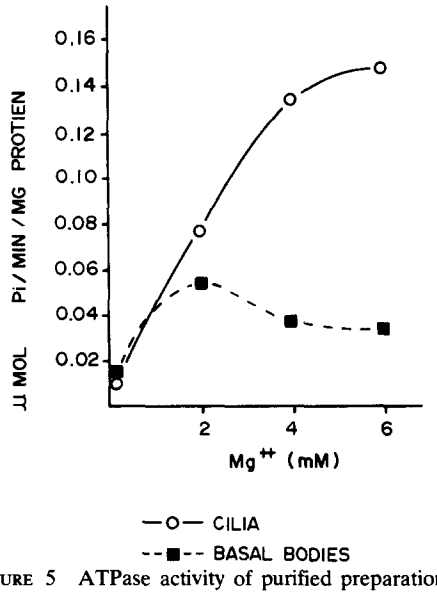


FIGURE 5 ATPase activity of purified preparations of basal bodies and of ciliary fragments in response to different concentrations of magnesium. Assays were performed at pH 8.0 for 15 min as described in Materials and Methods. Notice that even though the activity for the ciliary ATPase increased dramatically above 2 mM Mg^{++}, the basal body ATPase activity decreased. These results show that 4 mM Mg^{++} was a better activator of the ciliary ATPase than 4 mM Ca^{++}, and that higher concentrations of Mg^{++} did not inhibit the basal body ATPase nearly as well as did higher concentrations of Ca^{++} (Fig. 4).

μmol P_i/min per mg protein. However, with ITP as the substrate, only 0.009 μmol P_i/min per mg protein was liberated, and with GTP only 0.002 μmol P_i/min per mg protein was released. ADP was also a poor substrate (0.003 μmol P_i/min per mg protein), and no inorganic phosphate was released when AMP was the substrate. Furthermore, no phosphate was released when either pyrophosphate or *p*-nitrophenyl phosphate was the substrate.

Cytochemical Localization of ATPase Activity

To further establish that basal bodies have ATPase activity, cytochemical experiments were carried out to see whether any ATP-dependent lead phosphate precipitate could be localized in this organelle. In these experiments, the reaction was carried out on both fixed and unfixed basal bodies. As a control, we examined preparations that had been reacted in the absence of ATP and in the absence of divalent cations.

Reaction product was localized over basal bodies in all incubations that contained both ATP and Mg^{++} (Fig. 7*B,C,D*, and *F-L*); however, precipitate was not present when either ATP (Fig. 7*A* and *E*) or divalent cation (Fig. 8) was omitted. Prefixation of basal body preparations did not eliminate the reaction, although there seemed to be a little less precipitate (Fig. 7*C*). In some of the experiments on unfixed basal bodies with and without ATP, some nonspecific lead precipitate was present (Fig. 7*A* and *B*). However, this precipitate was never localized over basal bodies. As further evidence of the specificity of the reaction, ATP- and cation-dependent precipitate was associated with those ciliary frag-

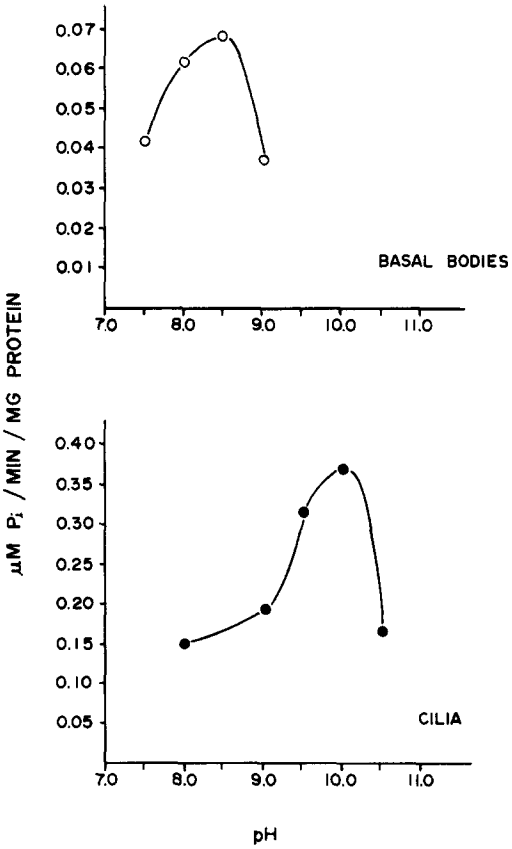


FIGURE 6 ATPase activity of purified preparations of basal bodies and ciliary fragments in response to pH. The assay was done as described in Materials and Methods, using 2 mM Mg^{++} as the divalent cation. These curves show that there is a dramatic difference between the pH optimum for basal bodies and the pH optimum for cilia. The high pH optimum for the cilia apparently is related to the dissociation of the dynein from the cilia at high pH and a subsequent change in the enzyme activity (14).

TABLE I

Enzyme Activity in Purified Basal Bodies

Substrate	μmol P_i/min/mg protein
ATP	0.04
ADP	0.003
AMP	0
ITP	0.009
GTP	0.002
p-Nitrophenyl phosphate	0
Pyrophosphate	0

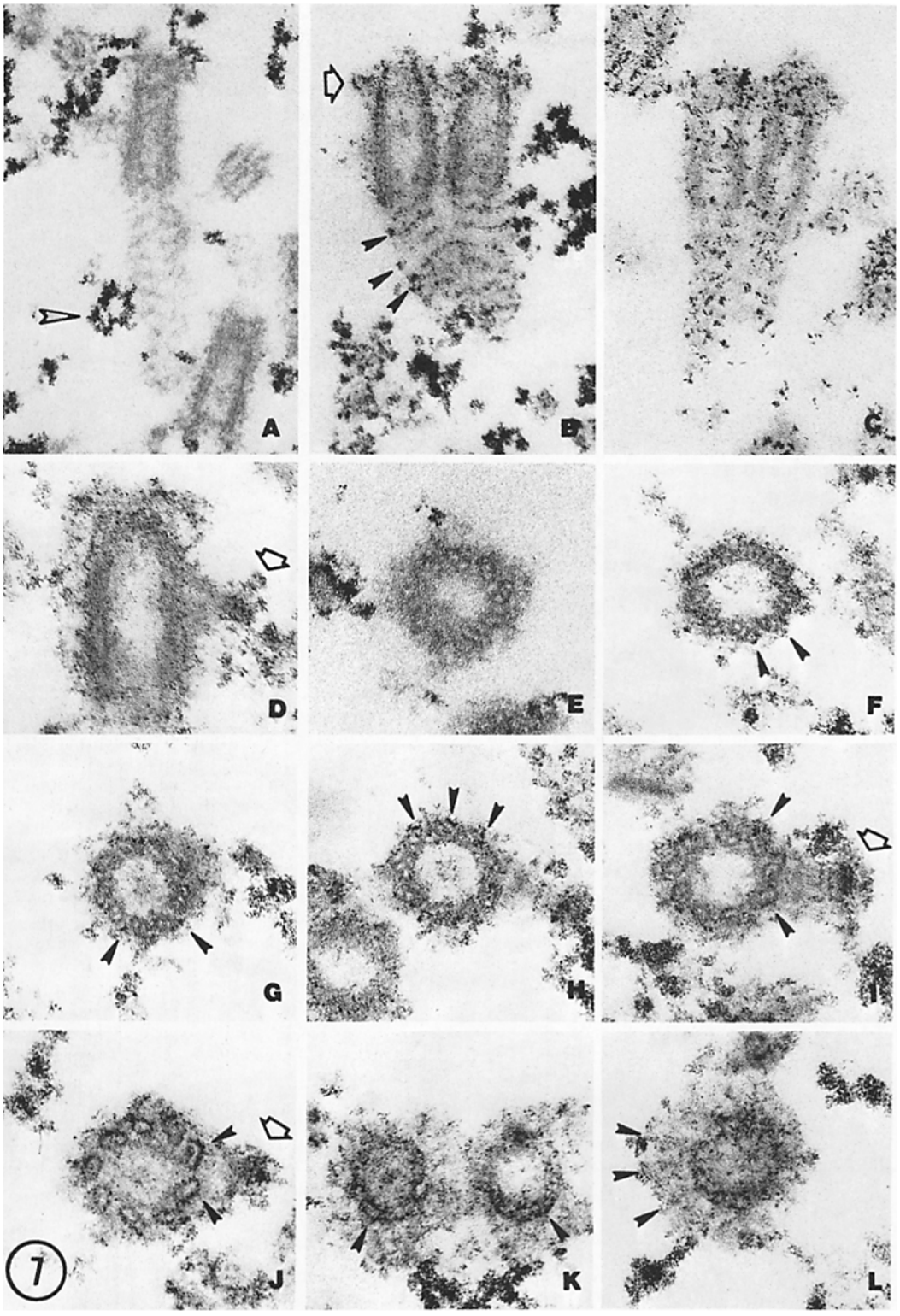
For each measurement, 0.2 ml of purified basal bodies was incubated with 1.8 ml of reaction medium that contained 0.15 M KCl, 0.002 M $MgCl_2$, 5×10^{-4} M EDTA, 10^{-4} M dithiothreitol, 0.001 M substrate, 0.2 M Tris-HCl, pH 8.0. Incubations were carried out for 15 min at 25°C followed by cooling to 4°C and centrifugation to remove the basal bodies. Phosphate determinations were done on the supernate as described in Materials and Methods.

ments that contaminated our preparations of basal bodies.

Although the presence of basal body ATPase activity could easily be detected in these experiments, we also wanted to determine which parts of the organelle had enzyme activity. In some cases, this was impossible because the lead phosphate precipitate was too large to give adequate resolution (Fig. 7*C*). However, we were fortunate that, in some experiments, a very fine precipitate was produced that remained associated with specific regions of the organelle. Fig. 7 is a series of electron micrographs that show this specificity. From these micrographs, we have determined that the reaction product is associated with four regions of the basal body: (*a*) the rootlet (Fig. 7*B*); (*b*) the nine sets of triplet microtubules (Fig. 7*F,G,* and *H*); (*C*) the outermost globular portion of the basal foot (Fig. 7*D*); and (*d*) the nine sets of alar sheets (Fig. 7*B*; see reference 2 for structure terminology).

The rootlet, which is composed of longitudinally arranged filaments that are interrupted at 57-nm intervals by perpendicularly oriented densities 19 nm thick (the cross-striations), had enzyme activity only over the cross-striations

FIGURE 7 A series of electron micrographs showing the cytochemical localization of ATPase activity in purified preparations of basal bodies. All reactions were carried out as described in Materials and Methods. (*A*) Unstained longitudinal section of a basal body reacted in the absence of ATP. Even though no reaction product is located over the basal body, some nonspecific lead precipitate was present (arrow). × 47,500. (*B*) Unstained, longitudinal section of two basal bodies reacted in the presence of ATP. Notice that reaction product is located over the whole alar sheet (open arrow) as well as over each cross-striation on the rootlet (solid arrows). In addition, there is a proximal to distal gradient in the amount of reaction product associated with the microtubular component of the basal body. × 53,300. (*C*) Unstained, longitudinal section of basal bodies that were reacted in the presence of ATP after fixation with 2% glutaraldehyde. Although enzyme activity could be demonstrated in these preparations, neither the amount of activity nor the localization of activity was as good as in unfixed basal bodies. × 51,500. (*D*) Longitudinal view of a basal body reacted in the presence of ATP that shows localization of activity on outer, globular portion of the basal foot (open arrow). Strained for 5 min with uranyl acetate and for 1 s with lead citrate. × 81,000. (*E*) Unstained cross section through the proximal end of a basal body that was reacted in the absence of ATP. No reaction product was associated with the microtubules in these preparations. × 88,000. (*F, G,* and *H*) Several cross sections through the proximal part of basal bodies that were reacted in the presence of ATP. The solid arrows point to the outer region of the *C* tubule where reaction product appears to be preferentially located at this level of the basal body. Triplets facing counterclockwise in (*F*) and (*G*) but clockwise in (*H*). Stained as in 7*D*.× 91,000. (*I*) Cross section through the mid-region of a basal body reacted in the presence of ATP. More reaction product is deposited over the triplet microtubules (solid arrows) at this level of the basal body; however, the precipitate is still preferentially located on the outside of the triplet. This view also shows reaction product over the outer, globular part of the basal foot (open arrow). Stained as in 7*D*. × 91,000. (*J*) A view similar to (*I*), but through a slightly more distal region. Here, the whole outer portion of each triplet is outlined by reaction product (solid arrows). The basal foot is also labeled (open arrow). Stained as in 7*D*. × 91,000. (*K*) A cross section through the distal region of a basal body reacted in the presence of ATP. The triplet microtubules are completely outlined by reaction product at this level of the basal body (arrows). Stained as in 7*D*. × 80,000. (*L*) In this cross-sectional view through the distal end of the basal body, one can see reaction product associated with the cross-sectional profiles of the alar sheets (arrows). Stained as in 7*D*. × 87,500.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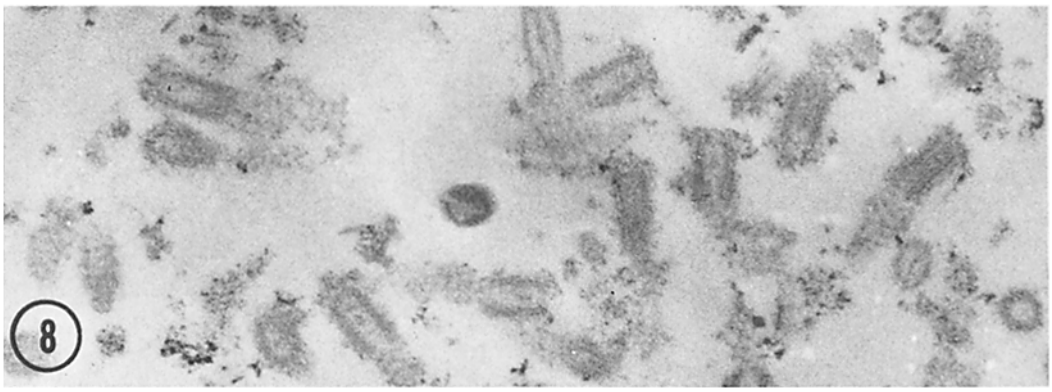

FIGURE 8 Section through the pellet of basal bodies that were cytochemically stained for ATPase in the presence of ATP but without Mg^{++} added. No reaction product was seen associated with either basal bodies or cilia in these preparations. This result agrees with the low biochemical activity in these preparations in the absence of divalent cations. Unstained. × 27,000.

(Fig. 7*B*). The length of the rootlet was variable, and it decreased in diameter as it extended from the proximal end of the basal body; however, in all cases, each of the cross-striations had equivalent amounts of enzyme activity. The localization was so specific that even when these striations were distorted or bent, the reaction product followed the contour of this portion of the rootlet (Fig. 7*B*).

A proximal to distal increase in the amount of ATP-dependent reaction product was associated with the nine sets of triplet microtubules (Fig. 7*B*). The small amount of reaction product in the base region was seen in cross section to be associated with the outer region of the triplets, primarily at the tip of the *C* (outer) microtubule (Fig. 7*F,G,* and *H*). Cross sections through the upper mid-region (Fig. 7*I* and *J*) showed some reaction product around the periphery of the whole triplet, with most of the activity on the outer portion of each set of microtubules. At the distal end, each set of tubules was completely outlined by reaction product (Fig. 7*K* and *L*). The association of reaction product with the microtubule components could also be detected in oblique sections of the organelle.

Some reaction product was located in the amorphous wall material of the basal body (Fig. 7*F* and *H*). However, it could not be determined with accuracy that this finding indicated activity in this portion of the organelle because of the possibility that phosphate may have diffused away from the triplets and formed a lead phosphate precipitate that was trapped in the amorphous wall coat.

The basal foot is a conically shaped structure, the base of which is attached to two or three of the triplet sets within the wall of the basal body (2). It is composed of fibers embedded in an amorphous matrix that periodically varies in density. This variation in density sometimes gives the basal foot a striated appearance. The apex of this accessory structure is rounded, and, within the cell, it is often the site of attachment of cytoplasmic microtubules (3). It is this rounded apical portion that had most of the ATPase activity associated with this accessory structure (Fig. 7 *D*, *I*, and *J*). Occasionally, there was reaction product scattered in the matrix material; however, it did not seem to be associated with any specific structural component of the foot, e.g., the striations.

The alar sheets, which are triangular-shaped sheets of material that are attached to each triplet set at the distal end of the organelle, were not preserved well in isolated basal bodies. However, when they could be identified in the cytochemical preparations, they were heavily stained with reaction product (Fig. 7*B* and *L*). In both longitudinal (Fig. 7*B*) and transverse views (Fig. 7*L*), the whole accessory structure was covered with lead phosphate precipitate.

For comparison, we also determined the location of the ATP-dependent reaction product associated with the ciliary fragments that contaminated the basal body preparations. ATPase activity was localized over the nine outer doublet microtubules as well as the central pair of microtubules (Fig. 9). Furthermore, the reaction product associated with transverse sections of the

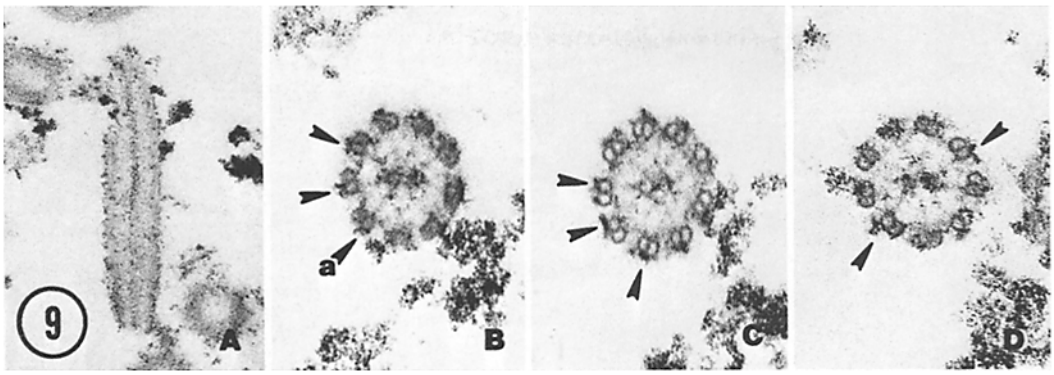

FIGURE 9 A series of electron micrographs that show the distribution of ATPase activity in those ciliary fragments present in the purified basal body preparations. All cytochemical procedures were carried out as described in Materials and Methods, in the presence of ATP and divalent cation. (*A*) An unstained, longitudinal section showing enzyme activity over the outer doublet microtubules as well as over the central pair of microtubules. × 39,000. (*B*, *C*, and *D*) Cross-sectional views of cytochemically reacted cilia stained for 5 min with uranyl acetate and for 1 s with lead citrate. Reaction product is almost exclusively located very close to the outer dynein arms on the outer doublets (arrows) as well as over the central pair of microtubules. Some lead phosphate is located over the radial linkers (e.g., arrow *a*, Fig. 9*B*) close to where they join the central pair. The location of reaction product so close to the known site of the ciliary ATPase on the outer doublet (13) attests to the specificity of the cytochemical procedure for determining the location of ATPase activity in these preparations. × 97,000.

outer doublets was located primarily over the dynein arms (Fig. 9*B*,*C*, and *D*). Not every dynein arm was labeled, and the outer arms were more often labeled than the inner arms. Sometimes the precipitate was on the outside of the inner *A* tubule close to the outer dynein arms. Some activity was also associated with the radial links (Fig. 9*B*) near their junction with the central tubules (probably where the link heads are located [40]).

DISCUSSION

Evidence for Basal Body ATPase

Although several reports have been published on the cytochemical localization of ATPase activity in basal bodies (22, 27, 28) and centrioles (1), there has been no corroborative biochemical evidence for the existence of such an enzyme. The data presented in this paper are the first demonstration of basal body ATPase activity by both cytochemical and biochemical techniques. At least three lines of evidence indicate that these basal bodies do contain an enzyme(s) that is specific for the hydrolysis of ATP: (*a*) the specific activity and substrate specificity of the ATPase in isolated basal bodies; (*b*) the characteristic behavior of the ATPase in response to divalent cations and pH; and (*c*) the specificity of the cytochemical localization of ATPase activity in the isolated organelle.

The biochemical measurements of basal body ATPase activity cannot be accounted for by assuming that the enzyme activity is due to the fragments of cilia in these preparations. For instance, a comparison of the activity in purified basal bodies with the activity in fragments of cilia prepared under nearly identical conditions indicates that in incubations that contain 2 mM Mg^{++} at pH 8.0, basal bodies had almost 60% of the activity of cilia. Thus, even if we assume a 25% contamination of the basal body preparations with cilia, not all of the activity could have come from the cilia. Moreover, the basal body ATPase and ciliary ATPase responded quite differently to Mg^{++}, Ca^{++}, and pH. Like ciliary ATPase, the basal body ATPase was activated by either cation; however, at ion concentrations above 2 mM, ciliary ATPase activity increased, but basal body ATPase activity decreased. Increasing the Ca^{++} concentration was more effective in inhibiting the basal body ATPase than was increasing the Mg^{++} concentration. Finally, the pH optimum for the basal body ATPase was pH 8.5, but for the purified ciliary fragments it was pH 10.0.

There seems to be little doubt that this basal enzyme is a nucleotide phosphatase that is specific for ATP. No inorganic phosphate was re-

leased when basal bodies were incubated with pyrophosphate or *p*-nitrophenyl phosphate. Further, other nucleotides were not hydrolyzed nearly so well as ATP. The specific activities for ITP and GTP were one-fourth and one-twentieth, respectively, of that for ATP. ADP also was a poor substrate, whereas AMP was not hydrolyzed.

The results of the biochemical experiments were confirmed and extended by the cytochemical experiments. Not only was there an ATP-dependent lead phosphate precipitate associated with isolated basal bodies, but also the reaction product was localized over four specific regions of the organelle: (*a*) the rootlet; (*b*) the basal foot; (*c*) the alar sheets; and (*d*) the triplet microtubules. The internal control for the localization of reaction product was the contaminating ciliary fragments. We found that, in this organelle, lead phosphate was located over or close to the dynein arms on the outer doublet microtubules (a known ATPase [13]). Therefore, the ion and nucleotide dependence of the cytochemical reaction, together with the specific localization of reaction product, strongly suggests that the basal body-associated lead phosphate precipitate was due to the presence of ATPase activity in this organelle.

Interpretations of ATPase Activity

The presence of ATPase activity in isolated basal bodies suggests that this organelle has an energy-requiring function within the cell. Because the basal body-cilium complex is specialized for motility, it seems reasonable that the basal body ATPase may be involved in some aspect of this motility. In fact, since four different parts of the organelle have enzyme activity, each part may have a different, energy-requiring role to play during the beat cycle of the cilium.

Although it has been proposed that centrioles (and, therefore, basal bodies) might be capable of some type of movement (35), there is no evidence to support this hypothesis. If this organelle could move, by analogy to cilia, it might involve some type of sliding microtubule mechanism (31, 36). Our finding that ATPase activity is associated with basal body microtubules at least makes this hypothesis tenable. However, the presence of the microtubule-associated enzyme on the outer portions of the triplet primarily over the *C* tubule, and the relationship of this activity to the angular disposition of the tubules in the basal body wall, make it difficult to envision how microtubule-microtubule interaction would take place. Furthermore, unlike that in cilia, the amount of ATPase activity in basal bodies, as determined by the quantity and distribution of lead phosphate precipitate, varies over the length of the triplet tubules, with most of the activity in the distal portion of the organelle. Therefore, if the triplet microtubule ATPase can generate basal body movements, most likely the form of this movement is quite different from that generated by the sliding of microtubules in the cilium.

The finding of ATPase activity in the cross-striations of the rootlet agrees with the results of studies reported by Matsusaka (22), who found a similar distribution of activity over retinal rod basal bodies. In addition, the reports that mitochondria (5, 29) and the endoplasmic reticulum (7) are associated with the rootlet can now be related to the presence of an ATP-utilizing system in this part of the basal body. However, even though one might imagine that this ATPase may be involved in the generation of filament sliding and, therefore, movement of the rootlet, it must be kept in mind that this accessory structure is present in various modified, nonmotile cilia (22) and not present in some motile cilia (3).

Whereas it is difficult to understand how the basal foot might move, the structure and organization of the alar sheets (2) immediately suggest how this accessory structure might function. Each of these nine structures attaches the basal body to the cell membrane (2). During the beat cycle of the cilium, the sliding of microtubules must exert some force on the basal body. This force may tend to shift the basal body from its normal perpendicular orientation to the membrane. The alar sheets, then, may actively function to maintain the basal body in its proper upright position. The ATPase would be involved in providing the energy required for this function.

The presence of ATPase activity in basal bodies does not necessarily mean that this organelle is motile. Other energy-requiring functions that do not depend on an intrinsic motility are quite possible. As suggested before, basal bodies may be important for providing the signal to initiate the ciliary beat cycle. This might be coupled with some type of inter-basal body communication system that functions to coordinate ciliary activity within one cell. The argument against this idea is the observation of several investigators that sperm flagella can beat both in vitro (6) and in vivo without a basal body (11). However, even

though cilia may be able to beat without this organelle, in many cells it may be required to modulate ciliary activity. This would explain, for example, how *Chlamydomonas* flagella that are attached to basal bodies can beat coordinately in opposite directions (18) even when the membrane is removed. Similarly, the cilia of both *Paramecium* (26) and *Tetrahymena* (25) can beat coordinately as well as reverse their direction of beat in response to calcium (25, 26) after the membrane has been removed. As further evidence, Torres and Renaud (37) have noted that the requirements for the reactivation of oviduct cilia are quite different for free cilia than for cilia that are attached to a basal body.

An interesting finding from these studies is the narrow range of divalent cation concentrations at which basal body ATPase activity is maximal. This suggests that if the basal body can influence ciliary activity, ciliary function might be impaired at concentrations of calcium above 2 mM. In this regard, Satir (32) has reported that when clam gills are exposed to 6 mM Ca^{++} in the presence of a calcium ionophore, cilia stop beating. Our studies have shown that in the chick oviduct, these levels of Ca^{++} almost completely inhibit basal body ATPase activity but increase ciliary ATPase activity.

Taken together, the results of this study establish that basal bodies have ATPase activity and, therefore, that this organelle probably has an active rather than passive function within the ciliated cell. Precisely how the basal body utilizes the energy released upon the dephosphorylation of ATP will be the subject of further investigations. But, for the moment, it seems reasonable to think that basal bodies may have an important function in ciliary motility.

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