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**LA THÈSE A ÉTÉ
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BIOCHEMICAL AND CYTOCHEMICAL STUDIES OF ADENOSINE
TRIPHOSPHATASE ACTIVITIES ASSOCIATED WITH THE
FLAGELLAR APPARATUS OF POLYTOMELLA.

A Thesis Submitted
to the School of Graduate Studies
of
The University of Ottawa
in partial fulfillment of requirements
for the degree of
Masters of Science in Biology

by

R. Bruce White

1980

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UNIVERSITÉ D'OTTAWA
UNIVERSITY OF OTTAWA

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ABSTRACT

Biochemical and cytochemical methods were used to examine ATPase activities in the flagellar apparatus of the quadriflagellate green alga Polytomella. In vivo, the basal bodies of the four flagella are linked in opposite pairs by interconnecting fibers, with flagellar rootlets of two types terminating between adjacent basal bodies. Several of the interconnecting fibers and one type of rootlet fiber are striated and composed of fine filaments (Brown, Massalski and Patenaude, 1976). An isolation and purification procedure is used which preserves the structural organization of the basal apparatus. Under conditions of optimal pH and divalent cation requirements, the basal apparatus and isolated flagellar axonemes demonstrate the highest specific activities with ATP as substrate. The basal apparatus ATPases were different from those of the axoneme on the basis of pH activity profiles, optimal divalent cation concentration and activity profiles, K_m values for ATP and in the sensitivity of the ATPases to urea and thiourea. In addition, the basal apparatus ATPases were much more insensitive to vanadate than axoneme ATPases. The results indicate the basal apparatus contains a characteristic set of ATPase enzymes unlike those of the flagellar axoneme.

Conditions established for optimal ATPase activities were utilized in the cytochemical studies which used an

adaptation of the Wachstein and Meisel (1957) technique for cytochemical localization of ATPase activity. ATP-dependent reaction product was localized on the rootlet microtubules and in a cross-banding pattern on the rootlet striated fiber and striated interconnecting fibers. A heavy deposition of reaction product was localized in the central core of the basal body-transition region and on the connections to the basal body microtubules. ATPase activity is also associated with the outer surfaces of the basal body microtubules. Several structures of the basal apparatus of many different cell types have been suggested to play direct or indirect roles in the control of flagellar movement. These proposed roles are discussed in terms of the localization of ATPase activities, which I have demonstrated in the basal apparatus of Polytomella.

RESUME

Les méthodes biochimiques et cytochimiques étaient utilisées pour examiner les activités ATPasiques dans l'algue vert comprenant quatre flagelles Polytomella. In vivo, les corpuscules basaux des quatre flagelles sont reliés en pair opposé par des fibres conjugués, avec deux types de radicules flagellaires se terminant entre des corpuscules basaux adjacents. Plusieurs des fibres conjugués et un type de radicule sont striés et composés de fins filaments (Brown, Massalski et Patenaude, 1976). Un procédé de purification et d'isolement qui préserve l'organisation structurale d'appareil basal est utilisé. Sous conditions de l'optimum pH et des exigences des cations divalent, les appareils basaux et des axonèmes de flagelles isolées démontrent les activités spécifiques les plus élevées avec l'ATP comme substrat. L'ATPasiques de l'appareil basal sont différents de ceux de l'axonème basés sur les profils des activités du pH, l'optimum concentré des cations divalent et leur profils de l'activité, les valeurs K_m pour l'ATP et dans les sensibilités de l'ATPasique aux urea et thiourea. Comme supplément, l'appareil basal ATPasique est plus insensible au vanadate que l'axonème ATPasique. Les résultats indiquent que l'appareil basal contient un ensemble caractéristique d'enzymes ATPasiques différent de ceux de l'axonème des flagelles. Des conditions établies pour les activités optimales ATPasiques ont été utilisées pour les études cytochimiques dont une adaptation des techniques de Wachstein et Meisel (1957) localisent les activités cytochimiques de l'ATPasique. Les produits d'opération enclenchés de l'ATP ont été localisés sur les radicules microtubules et dans un paterne striation transverse

v

régulière sur les fibreux striés des radicelles et sur les fibres striés conjugués. Une forte déposition des produits réactifs a été localisée dans le noyau central de la région transitoire des corpuscules basaux. Les activités de l'ATPasique sont aussi associées avec les surfaces externes des microtubules des corpuscules basaux. Certaines structures des appareils basaux de plusieurs différents types de cellules ont été suggérées de jouer des rôles directs ou indirects⁴⁰ pour le control des mouvements des flagelles. Ces rôles proposés sont discutés sous termes de localisation des activités de l'ATP, dont j'ai démontré dans l'appareil basal de Polytomella.

SOURCE OF CHEMICALS

bovine serum albumin - Sigma Chemical Co.
DMSO - Fisher Chemicals Ltd.
DTT - Sigma Chemical Co.
EDTA - Fisher Chemicals Ltd.
[γ - 32 P]ATP - New England Nuclear
 β -glycerophosphate - Sigma Chemical Co.
lead citrate - Fisher Chemicals Ltd.
lead nitrate - Fisher Chemicals Ltd.
p-nitrophenylphosphate - Sigma Chemical Co.
Nonidet P-40 - Shell Chemicals
nucleoside phosphates - Sigma Chemical Co.
ATP - Boehringer Mannheim Corporation
PCMB - Sigma Chemical Co.
pyrophosphate - Sigma Chemical Co.
Tris - Sigma Chemical Co.
Triton X-100 - Harleco.
Uni/verse LSC Cocktail - J.T. Baker Chemical Co.
uranyl acetate - Polysciences Inc.

LIST OF ABBREVIATIONS

ADP	adenosine 5'-diphosphate
ADPase	adenosine 5'-diphosphatase
AMP	adenosine 5'-monophosphoric acid
ATP	adenosine 5'-triphosphate
ATPase	adenosine 5'-triphosphatase
CTP	cytidine 5'-triphosphate
DMSO	dimethyl sulfoxide
DTT	dithiothreitol
EDTA	disodium ethylenediamine-tetraacetate
GTP	guanosine 5'-triphosphate
GTPase	guanosine 5'-triphosphatase
IMP	inosine 5'-monophosphoric acid
ITP	inosine 5'-triphosphate
PCMB	p-chloromercuribenzoate
Tris	2-amino-2-hydroxymethyl 1-1, 3-propanediol
UDP	uridine 5'-diphosphate
UMP	uridine 5'-monophosphoric acid
UTP	uridine 5'-triphosphate

INTRODUCTION

The beating of a flagellum results in a cell being propelled through an aqueous medium or the medium being propelled over the cell surface. The basal body and associated structures, collectively termed the basal apparatus, in conjunction with the flagellum forms a complex specialized for motility.

In addition to a function of anchoring the flagellum in the cytoplasm, it has been suggested that components of the basal apparatus actively participate in the initiation of flagellar beat, the coordination of flagella in multiflagellated cells and in control of the direction of flagellar beat. These suggestions have been supported by cytochemical studies which have localized nucleoside triphosphatase activities (particularly ATPase) on structures of the basal apparatus in a variety of cell types.

The long term goal of these studies is to establish the relationship between ATPase activity in structures of the basal apparatus and control of flagellar or ciliary beat. This will require precise cytochemical localization and the biochemical characterization of the enzyme systems in the isolated organelle.

Components of the basal apparatus

The flagella of cells arise from and are anchored by basal bodies in the cytoplasm. Fibers which interconnect basal bodies, and root structures are sometimes present. This complex of basal bodies, interconnecting fibers and root structures is the basal apparatus (Wolfe, 1972;

Pitelka, 1974).

a. The basal body

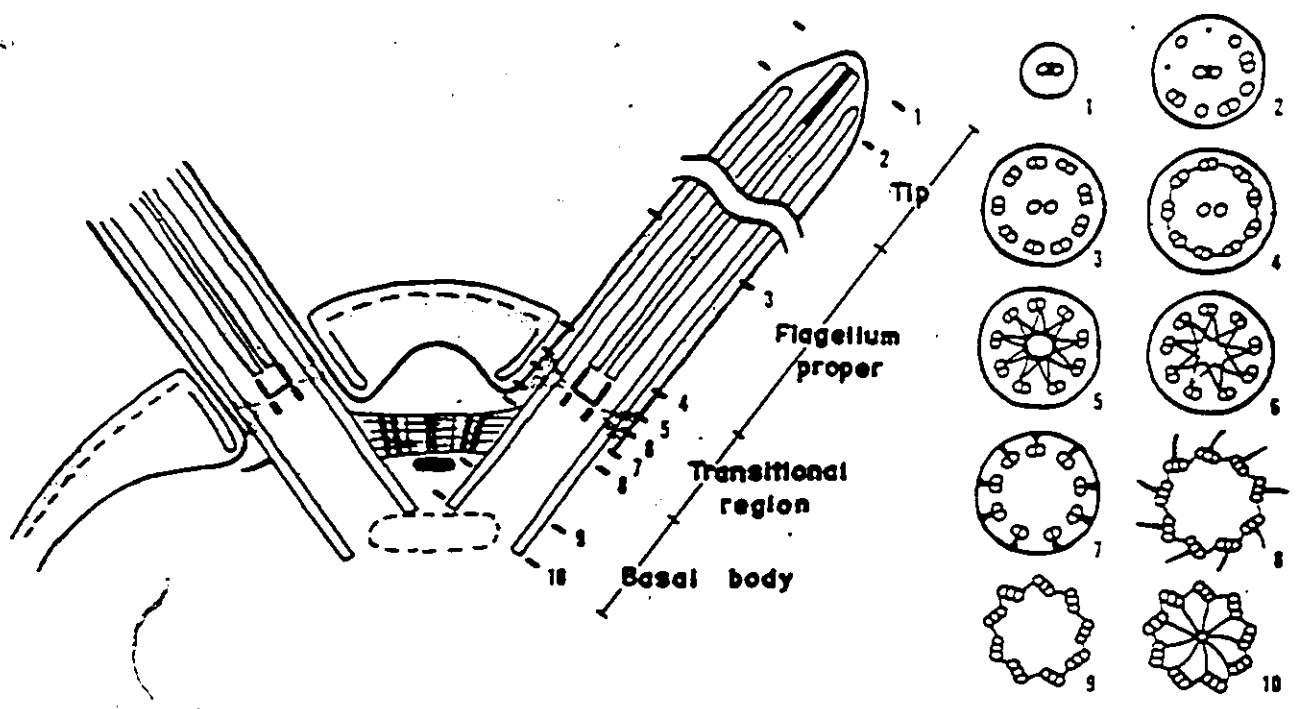
A basal body is a cylindrical structure measuring approximately $0.2\mu\text{m} \times 0.5\mu\text{m}$. The walls of the cylinder are composed of nine sets of triplet microtubules. The triplet microtubules consist of A, B and C subfibers. The A subfiber is tubular and contains 13 protofilaments; the B and C subfibers are 'cup-shaped' and share protofilaments with their adjacent subfibers to complete the tubule. The triplet sets of microtubules are connected to one another by a series of linkages forming a continuous ring around the periphery of the triplets (Turner, 1968; Perkins, 1970).

The A and B subfibers are continuous with the A and B subfibers of the flagellar axoneme. The C subfiber is not continuous into the flagellum. Although the function of the C subfiber is unknown, in some basal bodies the C subfiber gives rise to fibers that appear to attach the end of the basal body to the cell surface (Gibbons, 1961).

The basal body is typically capped at the distal end by a basal plate. In some organisms such as the green algae the basal plate is not present, but a stellate structure (Fig. 1) (Manton, 1964; Ringo, 1967; Turner, 1968) or an electron-dense helix (Hibberd, 1979) occurs in the transitional region of the basal body. The proximal portion is characterized by a cartwheel structure consisting of a cylindrical hub and one or more tiers of nine radiating spokes (Fig. 1) attached to the A subfibers (Allen, 1967; Ringo, 1967).

Figure 1.

A schematic drawing of an idealized section through the basal bodies of Chlamydomonas. Four regions of the basal body and flagellum are designated, and 10 typical cross sections are shown for the numbered points marked along the length of one flagellum (from Ringo, 1967).



The lumen of the basal body may appear empty in some cell types, but others show granules of various sizes and densities (Dingle and Fulton, 1966; Doolin and Birge, 1966; Sakai and Shigenaga, 1967). Enzymatic digestion studies suggest an RNA-protein complex may be present in the lumen of Paramecium basal bodies (Dippell, 1976). The functional significance of this complex is unknown.

b. Root structures

Root structures are found in all flagellated or ciliated cells and are made up of amorphous or finely filamentous materials, bundles of packed filaments that may or may not show periodic cross-banding, or microtubules (Piteka, 1974). There is great variation in pattern, structure and number of root structures in ciliated or flagellated cells.

Large striated roots (rhizoplasts) originate as branched structures in dense material near the proximal portion of the basal body. As the rhizoplasts extend down into the cytoplasm the branches combine forming a structure up to 250 nm wide and 5 μ m in length which tapers as it extends towards or around the nucleus (Anderson, 1962; Dingle and Fulton, 1966; Outka and Kluss, 1967).

Smaller striated root(let)s are found associated with basal bodies of ciliated epithelial cells. Rootlets of chicken oviduct originate from the proximal portion of the basal body and taper as they extend down into the cytoplasm (Anderson, 1977). Striated roots have a complex striation pattern and probably always have a substructure of longitudinally arranged filaments (Pitelka, 1974).

Rootlets found in flagellated cells of algae, protozoa and fungi consist of striated, filamentous and/or microtubular structures. Many algal cells have been examined and there is a great diversity in the number and structure of rootlets, although a cruciate arrangement is a common feature. The rootlets and other structural features of the basal apparatus can serve as a phylogenetic indicator, and aid in analyzing evolutionary trends and the assigning of algae to various groupings (Melkonian, 1980). The rootlet structures are far too varied and complex for an adequate summary here, only a few examples will be mentioned.

Chlamydomonas has four rootlets, two consisting of four microtubules in a 3 over 1 pattern and the other two of a striated fiber over two tubules (Goodenough and Weiss, 1978). The green flagellate Schizomeris has four rootlets in a cruciate arrangement, each consisting of four tubules (Birbeck, Stewart and Mattox, 1974). More complex patterns of rootlets also exist. Urospora has four rootlets consisting of nine tubules and four striated rootlets (Kristiansen, 1974).

The basal foot is a short, dense, banded cone projecting laterally from dense material near the mid-region of the basal body in ciliated cells. The basal feet are usually uniformly oriented (Pitekla, 1974) and in some cell types extend in the direction of the effective stroke of their cilia (Gibbons, 1961).

Other microtubule structures are sometimes associated with the basal bodies of ciliated protozoa (Pitelka and

Child, 1964; Pitelka, 1965). The microtubule bundles or sheets run in close proximity to the proximal ends of basal bodies and have been speculated to function as a communication line to coordinate ciliary beat (Allen, 1967).

c. Interconnecting fibers

Another class of fibers in multi-flagellated cells are those which interconnect basal bodies. These fibers, striated or non-striated, consist of 30 - 70 A filaments (Wolfe, 1972).

The arrangement of the interconnecting fibers differs among cell types. The biflagellate alga Chlamydomonas has two basal bodies joined at their mid-region by a broad fiber with three electron dense cross-bands and proximally by two smaller striated fibers (Ringo, 1967; Johnson and Porter, 1968). Other biflagellate cells characteristically show a broad striated fiber linking the two basal bodies (Anderson, 1962; Lang 1963).

Quadriflagellate cells have a more complicated system of interconnecting fibers. These fibers usually interconnect opposite pairs of basal bodies (Moestrup and Thomsen, 1974; Brown, Massalski and Patenaude, 1976); however Schizomeris has adjacent pairs of basal bodies interconnected by distal and proximal fibers (Birbeck et al., 1974).

ATPase activities of the basal apparatus

ATPase activities have been cytochemically localized on the basal body microtubules in a number of cell types (Abel, 1969; Abel, Jokelainen and Verhage, 1972; Nayak,

1972; Nayak and Wu, 1975).

Recently, more precise cytochemical localization of ATPases have been demonstrated. ATPase activities have been shown to be associated with the outer portions (primarily) of the nine sets of basal body microtubule triplets (Anderson, 1977; Dentler, 1977). ATPase activity is also associated with the basal foot (Anderson, 1977). Studies of Tetrahymena demonstrate ATPase activity on the basal plate and in the central core of the basal body (Dentler, 1977) as well as on the microtubule bundles associated with the basal bodies (Sharp, Fitzsimons and Kerkut, 1979).

It is believed the basal body plays a role in the development of the axoneme and anchors it in the cell since the A and B subfibers are continuous with the peripheral tubules of the axoneme. Anchoring of the flagellum may require active functioning of structures in the basal body. It has been speculated that basal body microtubules slide (Anderson, 1972; Dentler, 1977) but the localization of ATPases make it difficult to envisage how sliding would occur (Anderson, 1977).

The observation that several types of root structures have a striated appearance has prompted speculation that these structures may have muscular or contractile properties (Simpson and Dingle, 1971). Salisbury and Floyd (1978) have induced the striated root (rhizoplast) of the green alga Platymonas to contract on the addition of Ca^{++} and ATP to the intact cell. The extended rhizoplast consisted

of 10 to 12 fibrous zones and the contracted organelle was shorter and wider with amorphous dense bands. They suggest the rhizoplast may be acting as a mechanical aid in flagellar coordination.

ATPase activity has been localized in a cross-banded pattern on rootlets of human retinal rods (Matsusaka, 1967). The only study to date using isolated basal apparatus shows ATPase activity at positions corresponding to striations on the basal body rootlets of ciliated epithelial cells (Anderson, 1977). The localization of ATPase activity on striated rootlets indicates contraction may occur.

It is not known if striated roots are constructed of muscle type protein (actin-myosin) or other proteins. The only attempt to study the protein composition of striated rootlets was done by Stephens (1975). He found that solubilized rootlets of gill epithelial cells consistently showed the presence of two proteins with molecular weights of 230,000 and 250,000 that are distinct from muscle components. Studies on the protein composition of other root structures is necessary.

Interconnecting fibers link the basal bodies of the basal apparatus. It has been suggested that the large striated interconnecting fibers that are present in cells such as Chlamydomonas and Polytomella might act in the control of flagellar beat by either initiating or conveying the initiation of the flagellar beat (Ringo, 1967; Brown et al., 1976). Hyams and Borisy (1975) have demonstrated

that when the two basal bodies with their flagella are isolated intact from Chlamydomonas, flagellar beat can be reactivated in the presence of Mg^{++} and ATP. The movement of the flagella on the isolated structure was largely synchronous and indistinguishable from the movement of flagella in living cells. They suggested that the distal striated fiber interconnecting the two basal bodies may be controlling flagellar beat. There has been no cytochemical localizations of ATPases on the basal apparatus of cells containing interconnecting fibers.

The flagellum

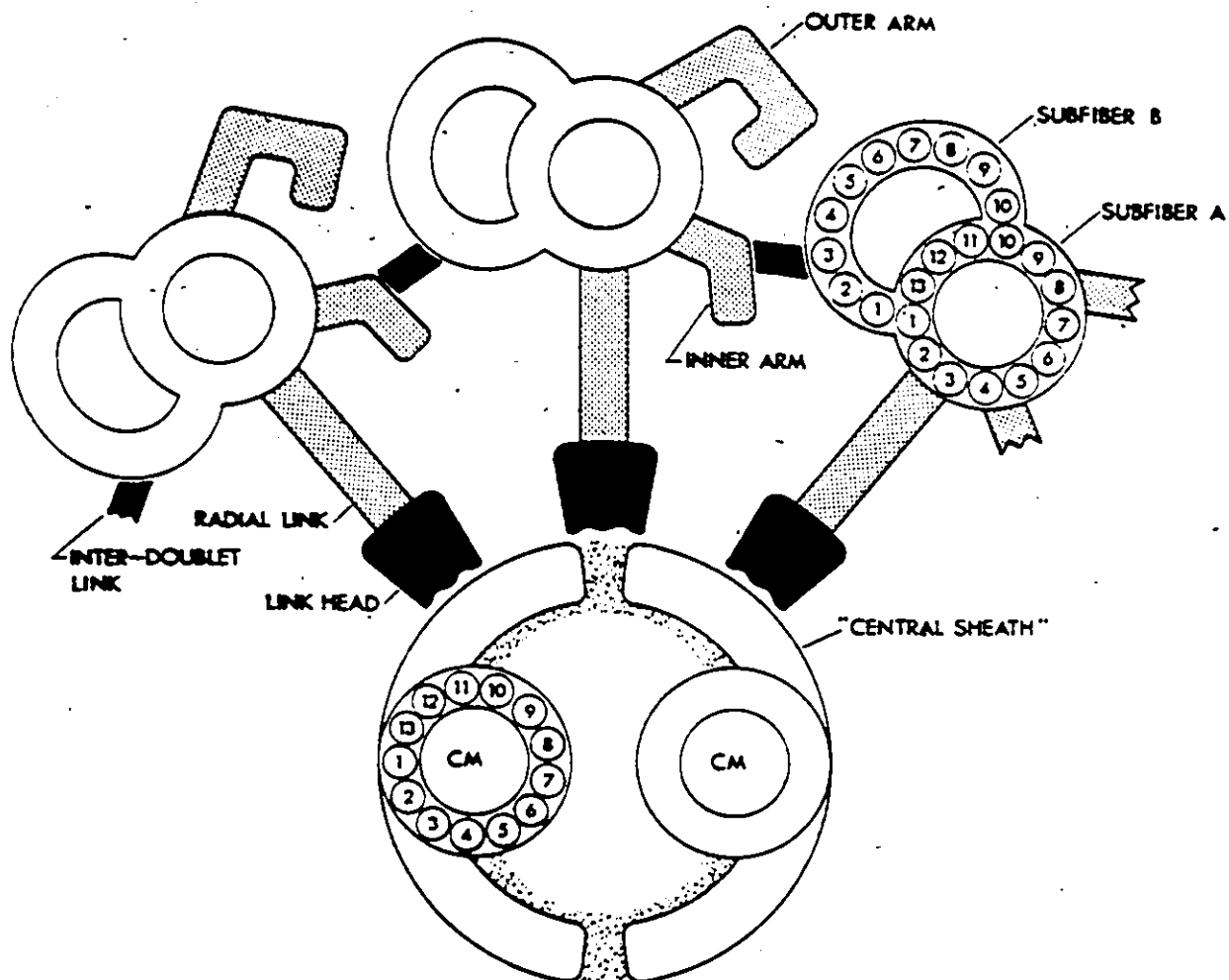
The flagellar axoneme is constructed of nine interconnected doublet microtubules equidistantly positioned around and joined by radial links to the central sheath surrounding the two central tubules (Fig. 2). Each doublet consists of an A subfiber of 13 protofilaments and a cup-shaped B subfiber sharing some protofilaments of the A subfiber.

Between the adjacent doublets are inner and outer dynein ATPase arms attached to the A subfiber. An interdoublet link connects the terminal portion of the inner dynein arms to the adjacent B subfiber (Allen, 1968; Kifer, 1970). Fibers also extend between the doublet microtubules and the adjacent flagellar membrane (Ringo, 1967; Sattler and Staehlin, 1974).

The ATPase activities of flagella and axonemes isolated from a number of cell types have been

Figure 2.

An interpretive diagram of a portion of an axoneme. The outer doublet microtubules are joined to the central sheath attached to the two central microtubules (from Warner, 1974).



determined (Gibbons, 1966; Gibbons, 1968; Stephens and Levine, 1970; Gibbons and Fronk, 1972; Ogawa and Mohri, 1972; Watanabe and Flavin, 1976; Gibbons et al., 1978). Cytochemical studies have shown ATPase activities on the outer doublet microtubules (Nelson, 1958; Lansing and Lamy, 1961; Daems, Persijn and Tates, 1963; Gordon and Barrnett, 1967; Anderson, 1977; Dentler, 1977; Sharp et al., 1979), the central sheath or radial linkers (Anderson, Personne and André, 1968; Burton, 1973; Anderson 1977; Dentler, 1977; Sharp et al., 1979), and at positions corresponding to the location of the dynein arms (Gordon and Barrnett, 1967; Anderson, 1977).

The basal apparatus of *Polytomella*

Polytomella agilis is a single celled alga (c. Chlorophyceae) that exists as a free swimming form in exponentially growing cultures. These cells are elongate being approximately 12 μm long and 7 μm in width. The cells are quadri-flagellates with the flagella emerging from depressions at the base of the anterior papilla. The external appearance of the cells, the structural organization of the cells and their microtubule systems have been described by Brown et al., (1976) (Fig. 3-5).

In this cell the four basal bodies are linked in opposite pairs. The A pair of the basal bodies is interconnected distally by a large striated fiber (40-50 \AA filaments) with seven equally spaced cross-bands, and proximally by two smaller striated fibers (Fig. 4). A single nonstriated fiber (80-90 \AA filaments) interconnects

Figure 3.

A diagrammatic view of Polytomella showing the cell shape, the A and B pairs of flagella and their corresponding basal bodies at the anterior end of the cell, the microtubular rootlets (MR), the compound rootlets (CR) and the cytoplasmic microtubule system (CM).

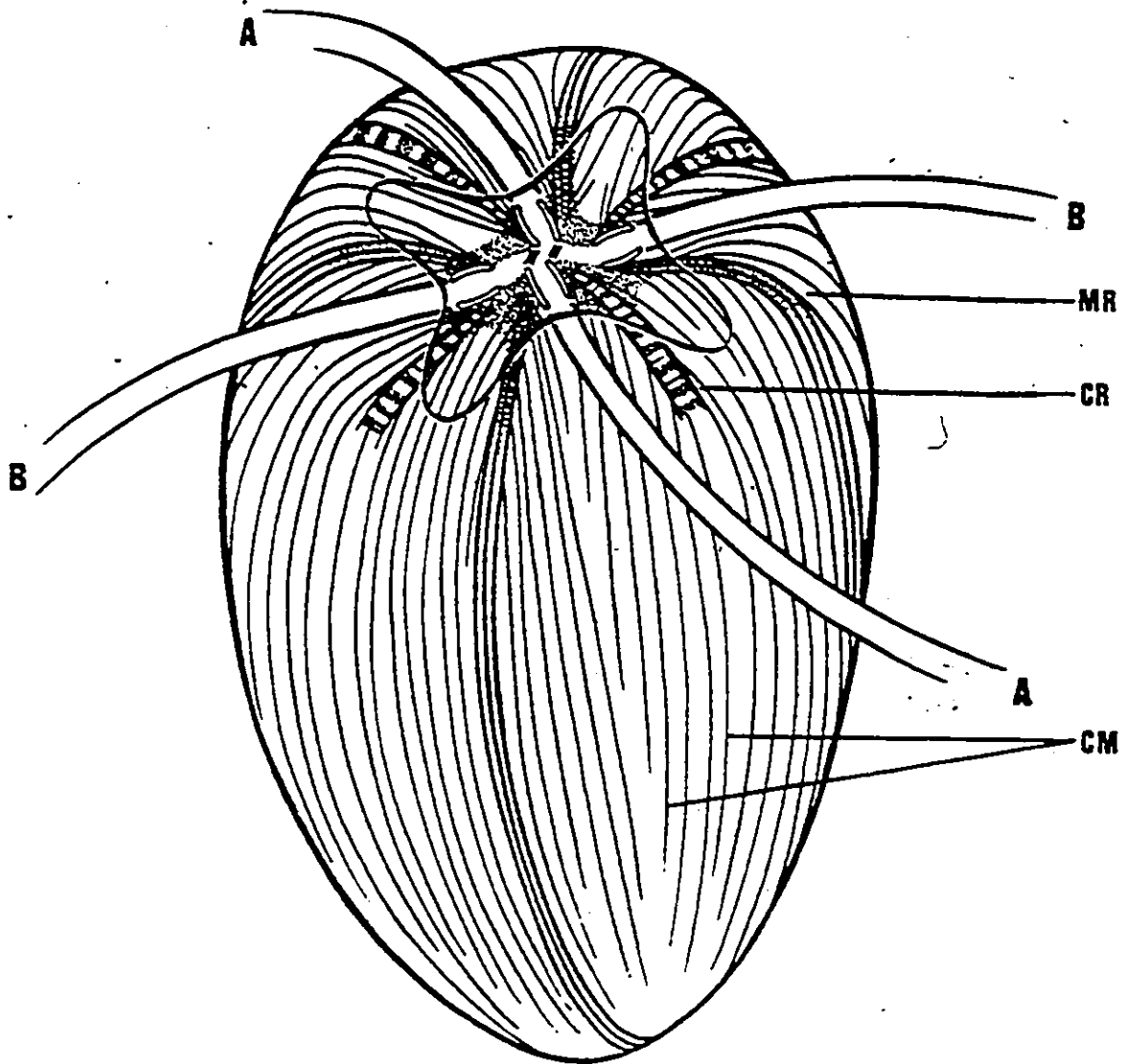


Figure 4.

Schematic diagram of the arrangement of the four basal bodies and the interconnecting fibers of Polytomella. A - basal bodies of the A pair of flagella; B - basal bodies of the B pair of flagella; D - distal striated fiber; P - proximal striated fiber of the A pair; P_n - proximal nonstriated fiber of the B pair (from Brown et al., 1976).

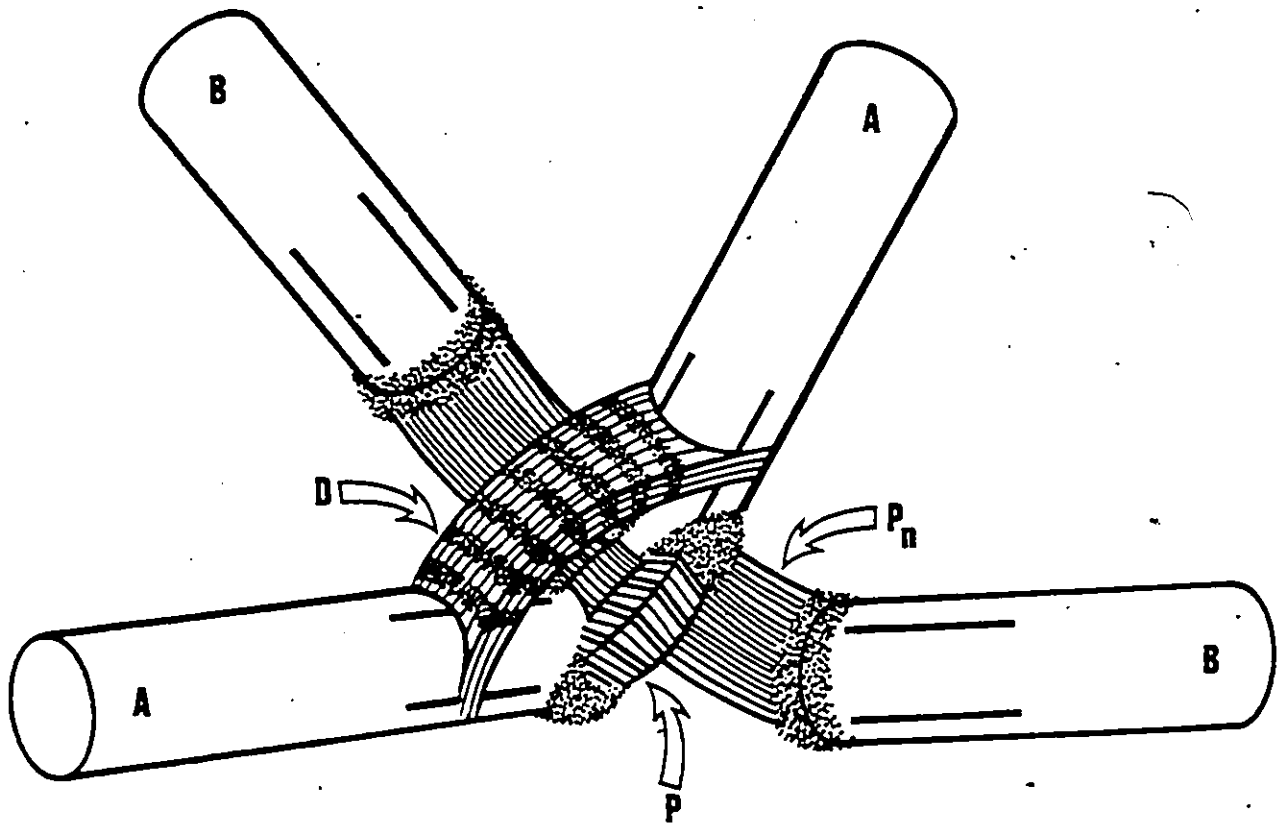
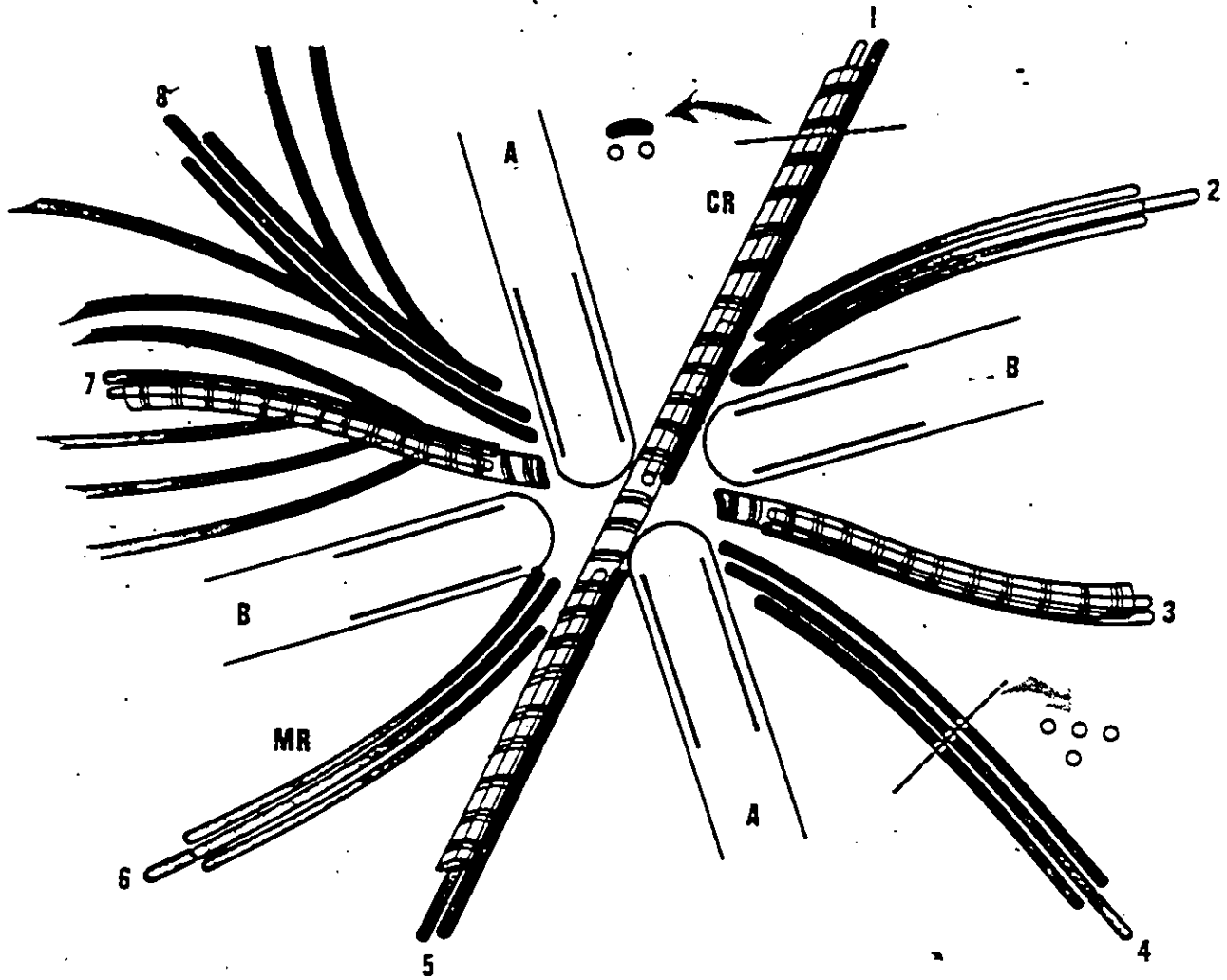


Figure 5.

Diagrammatic representation of the structure and arrangement of the eight flagellar rootlets. The rootlets are labeled 1 to 8. A - A pair of basal bodies; B - B pair of basal bodies; CR - compound rootlet; MR - microtubular rootlet. Cytoplasmic microtubules are shown attached to rootlets 7 and 8 (from Brown *et al.*, 1976).



the B pair of basal bodies at their proximal ends. There are no fibers directly connecting A basal bodies to B basal bodies, however the four basal bodies are held together by connections to and between rootlets.

Eight flagellar rootlets of two types terminate between adjacent basal bodies (Fig. 5). Four of the rootlets termed microtubular rootlets, consist of four microtubules in a 3 over 1 pattern. The remaining rootlets termed compound rootlets, consist of a striated fiber running parallel to and above two microtubules. The rootlets appear to be firmly attached to the basal bodies, and by lateral association link the adjacent basal bodies.

The flagellar beat pattern has been analyzed in detail by Gittleson and Jahn (1968). The flagella beat back alongside the cell in a breast stroke-like motion. There is good coordination between the two pairs of flagella, but they are not synchronous as the pairs beat with different frequencies. The pair of flagella in Chlamydomonas also exhibit a breast stroke-like beat pattern (Ringo, 1967). Ringo has suggested the distal striated fiber may control flagellar coordination. The striated fibers interconnecting the A pair of basal bodies and the fiber interconnecting the B pair of basal bodies in Polytomella may function in a similar manner (Brown et al., 1976).

It has been suggested that structures such as those in the basal apparatus of Polytomella are so situated that they could play a role in the control of flagellar beat. This might require a process requiring energy. The chemical energy of ATP can be released by transferring the terminal phosphate group (by way of ATPase enzymes) to certain specific acceptor mol-

ecules, which become energized and can do work. This energy transfer occurs in motile processes such as flagellar bending (Satir, 1974) and muscle contraction (Rüegg, Straub and Twarog, 1963). For this reason it was of interest to determine if the basal apparatus of Polytomella had associated ATPase activities that could function in the hydrolysis of ATP, with the result being a motile process acting in control of flagellar beat. I also wished to determine if this ATPase activity was similar to the ATPase found in flagella.

Methods are available to isolate the basal apparatus (Stearns, Connolly and Brown, 1976) and flagellar axonemes (Connolly, 1974) intact and free of other cellular components. Using the optimal conditions of basal apparatus and axoneme ATPases it is possible to differentiate between the ATPase activities in these two organelles, an initial step in the characterization of the enzymes. The ATPase activities of the basal apparatus and axonemes were examined over a range of conditions including varying the divalent cations and substrates used and varying their concentrations. Agents that affect ATPase activity were also examined.

The optimal levels of divalent cation and pH required for ATPase activity were determined and then the sites of enzyme activity were localized by cytochemical procedures. The use of the isolated basal apparatus permits a more precise cytochemical localization of ATPase activities than can be carried out in the intact cell. The results show that ATPase activity is associated with structures that have been proposed to play a role in the control of flagellar beat.

MATERIALS AND METHODS

Cell cultures

Cultures of Polytomella agilis were grown in 4-liter erlenmeyer flasks in a complex medium containing 0.1% tryptone, 0.2% yeast extract and 0.2% sodium acetate. Cultures were inoculated to give an initial population density of approximately 10^4 cells/ml. The cultures were grown at 25°C in a dark constant temperature incubator and were harvested in middle exponential growth phase, approximately 40 hr after inoculation, at a cell density of $2-5 \times 10^6$ cells/ml.

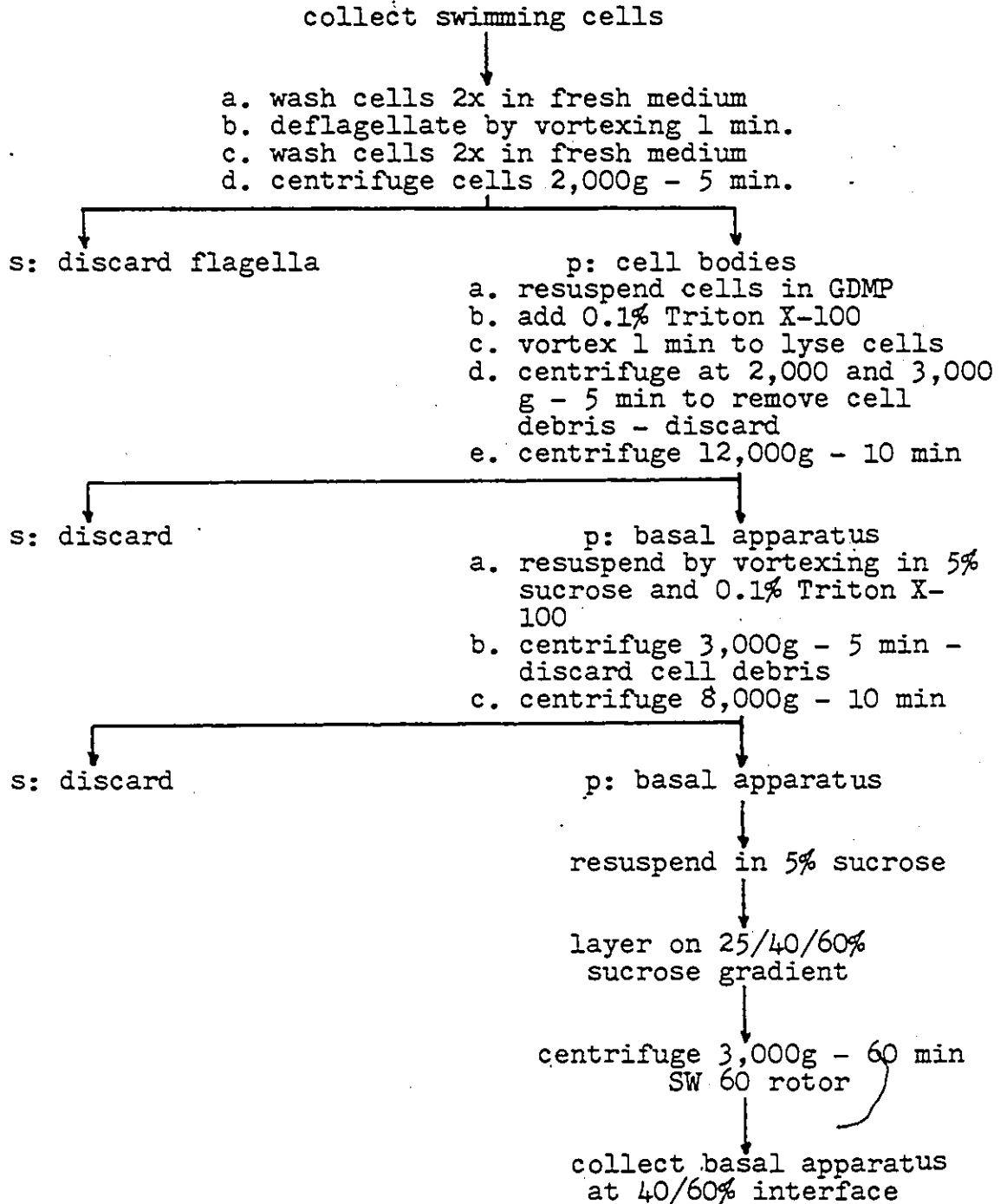
Isolation of basal apparatus

The procedure for isolating the basal apparatus outlined in figure 6, is that of Stearns et al. (1976).

Swimming cells were deflagellated by vortex agitation in a fluted tube for 1 min. The cell bodies were then centrifuged and the supernatant containing detached flagella discarded. To purify large numbers of basal apparatus the structures must be maintained intact and stable. The basal apparatus are stabilized by lysis of the cell into a medium containing 50% (vol/vol) glycerol, 10% (vol/vol) dimethylsulfoxide, 5 mM MgCl₂, 5 mM NaH₂PO₄, pH 6.8 (GDMP). The addition of 0.1% Triton X-100 causes little or no lysis itself, but the cells are fragile and easily lysed by mechanical agitation. A gentle vortex agitation at this stage releases the basal apparatus intact. This treatment

Figure 6.

Flow chart of procedure for isolating the basal apparatus of Polytomella.



leaves much of the cell debris as large fragments which can be removed by low speed centrifugation. The basal apparatus in the GDMP medium can subsequently be pelleted at a higher centrifugation speed. The basal apparatus must be pelleted at a centrifugal force which will allow complete dispersal of these structures during resuspension. Following resuspension in 5% sucrose, 0.1% Triton X-100 is added and the suspension is vortex agitated to remove membrane material still associated with the basal apparatus. The basal apparatus are then centrifuged, resuspended and layered on a sucrose gradient. These are later collected at the 40/60% sucrose interface. The basal apparatus can be monitored throughout the procedure by light microscopy and counted using a Spencer hemacytometer. Yields of 1.8×10^8 ($\pm 9\%$) basal apparatus are obtained from 10 liters of cells harvested at a concentration of $2-5 \times 10^6$ cells/ml.

The basal apparatus were very resistant to the alkaline solutions commonly used in the determination of protein concentration. For this reason the basal apparatus were solubilized in a solution containing 20 mM Tris-HCl, 20 mM EDTA, 2% SDS and 0.1% β -mercaptoethanol, pH 6.8. The protein concentration using the Hartree (1972) procedure and bovine serum albumin as the standard, was determined to be 6.90 μg per 10^6 basal apparatus. In experiments measuring ATPase activities, the number of basal apparatus were determined and converted to μg of protein using this value.

In some experiments the basal apparatus were dialyzed

against 5% sucrose for 1.5 hr and then extracted by dialysis at 4°C against an excess volume of 1 mM Tris, 0.1 mM EDTA, 0.01% β -mercaptoethanol, pH 8.0 for 20 min according to the procedures of Bloodgood and Rosenbaum (1976) and Stearns and Brown (1979). The extracted basal apparatus were separated from the extracted proteins by centrifugation at 12,000g for 10 min, and resuspended in phosphatase reactivation solution. The supernatant containing the extracted proteins was centrifuged at 100,000g for 30 min to pellet remaining basal apparatus and then dialyzed against ATPase assay solution.

Isolation of axonemes

Flagella were removed from cells by a modification of the procedure of Witman et al. (1972). Five liters of cells were harvested by centrifugation, washed once and resuspended in 80 ml of 10 mM Tris, pH 7.8. This cell suspension was cooled to 10°C, 300 ml of cold (0 - 2°C) 15 mM Tris, 2.5 mM EDTA, 11% ethanol, 30 mM KCl, pH 7.8 (TEEP) was added and the suspension was stirred vigorously. CaCl_2 was added immediately to bring the final calcium concentration to 15 mM. Deflagellation was greater than 95% within 60 sec with very little cell breakage. The suspension was centrifuged at 3,000g for 10 min. The supernatant which contained flagella was centrifuged at 25,000g for 30 min. The pellet was resuspended in 5% sucrose and centrifuged twice at 200g for 5 min to pellet cell debris. Flagella were pelleted by centrifugation at 25,000g for 30 min, and

resuspended at 4°C for 6 min in 1 mM Tris and 0.02% of the detergent Non-Idet P-40, pH 8.3 to remove the flagellar membranes. Demembrated flagella (axonemes) were pelleted by centrifugation at 25,000g for 30 min, resuspended in 5% sucrose and layered over 40% sucrose. Centrifugation at 16,000g for 60 min in a SW-60 swinging bucket rotor pelleted the axonemes through the 40% sucrose and left detached membranes as a whitish band at the interface. Five liters of cells yielded approximately 400 µg of purified axonemes.

To isolate dynein ATPases, axonemes were resuspended in and dialyzed at 4°C against 1 mM Tris, 0.1 mM EDTA, 0.01% β-mercaptoethanol, pH 8.0 (Bloodgood and Rosenbaum, 1976) for 18 hr. The suspension was centrifuged at 70,000g for 60 min, and the supernatant containing dynein was then dialyzed against ATPase assay solution.

Determination of ATPase specific activities

The basal apparatus or axonemes were resuspended in ATPase assay solution containing 0.2 M Tris-HCl, 0.25 M KCl, 5×10^{-4} M EDTA, 10^{-4} M DTT, 1.5 mM phosphate-containing substrate and divalent cation or 1 mM EDTA, pH 8.5. Agents affecting ATPase activity were added to the assay solution in some experiments.

Samples containing basal apparatus or axonemes were incubated for 20 min at 25°C, cooled to 4°C and centrifuged at 25,000g for 30 min at 4°C. The supernatant was removed and the concentration of inorganic phosphate determined by the colorimetric method of Gomori (1942). This procedure

utilizes the addition of a molybdate-sulfuric reagent which is subsequently reduced with methyl-p-aminophenol sulfate and the absorbance read at 650 nm. Termination of the reaction with molybdate-sulfuric reagent prior to centrifugation did not give reproducible color development. Alternatively, the method of Pollard and Korn (1973) was used to verify results from the Gomori method. In this method the reaction was terminated by the addition of butanol-benzene which produced an upper organic phase and a lower phase. Ammonium molybdate was added which extracted the inorganic phosphate forming phosphomolybdic acid in the upper phase. The lower phase contained coagulated proteins, ATP and ADP. Following this 0.15 ml of the upper phase containing radioactive and non-radioactive phosphomolybdic acid was added to 10 ml of Uni/verse LSC Cocktail and counted in a Beckman LS-233 liquid scintillation spectrometer. Total amount of inorganic phosphate released was determined by reducing 1.0 ml of the upper phase with stannous chloride and reading the absorbance at 720 nm.

Cytochemical localization of ATPase activity

Localization of ATPase activity employed a modification of the Wachstein-Meisel (1957) technique. Lead forms a precipitate with inorganic phosphate at the sites of ATPase activity. This precipitate is referred to as ATP-dependent reaction product. The purified samples were resuspended in 10 ml of solution containing 75 mM Tris-maleate, 100 mM NaCl, 50 mM KCl, 0.5 mM EDTA, 5% sucrose, 2 mM $\text{Pb}(\text{NO}_3)_2$,

2.5 mM ATP and divalent cation or 1 mM EDTA, pH 8.5. Controls omitting either divalent cation or ATP or using p-nitrophenylphosphate (a non-hydrolyzable substrate) were included. The mixture was incubated 45 min at 25°C and the reaction stopped by adding 2% glutaraldehyde buffered with 0.038 M Na cacodylate, pH 8.5. The mixture was centrifuged at 25,000g for 30 min and the pellet was prepared for thin-section electron microscopy.

Electron microscopy

The basal apparatus or axonemes were pelleted and fixed at 25°C for 1 hr in 2% glutaraldehyde in 0.038 M Na cacodylate pH 8.5, post-fixed at 4°C for 1 hr in 1% osmium tetroxide in the same buffer and dehydrated by a series of 10, 20, 50, 70, 90 and 100% acetone washes. Pellets were infiltrated and embedded in Spurr's plastic (Spurr, 1969). Thin-sections were cut with a diamond knife on a Porter-Blum MT2-B ultramicrotome. When necessary sections were stained with 2% uranyl acetate and 1% lead citrate. Sections were then examined in an AEI-EM 6B or Philips 201C electron microscope.

For negative staining, samples were first fixed by the addition of an equal volume of 4% glutaraldehyde in 0.023 M Na cacodylate, pH 8.5. A drop of sample was deposited on a 200 mesh, formvar-carbon coated grid followed by washing with glutaraldehyde and 0.4% photo-flo. Samples were stained with 1% uranyl acetate containing 0.1% cytochrome c and washed with 95% ethanol.

RESULTS

Structure of the isolated basal apparatus of *Polytomella*

In a negatively stained preparation of isolated basal apparatus (Fig. 7), the long rootlets can be seen extending away from the four basal bodies. Note that no flagella remain attached to the basal bodies and that the cytoplasmic tubules which usually terminate on the rootlets are also absent. Thin-section electron microscopy shows the structural organization of the basal apparatus is preserved during the isolation procedure (Fig. 8). In situ, the A pair of basal bodies are inserted at an angle of 120° to one another, and are interconnected by a distal striated fiber showing seven equally spaced major cross striations and by two smaller proximal striated fibers. The B pair of basal bodies insert at an angle of about 150° and are interconnected at their proximal ends by a filamentous non-striated fiber. Two types of rootlets, microtubular and compound rootlets, terminate between adjacent basal bodies. The compound rootlets have a striated fiber showing a cross-banded pattern with a center-to-center spacing of 300A (Brown et al., 1976). In the isolated basal apparatus the angle between the basal bodies is preserved (Fig. 8 B, C). The interconnecting fibers of the basal bodies are present. The distal and proximal fibers interconnecting the A pair of basal bodies show their characteristic

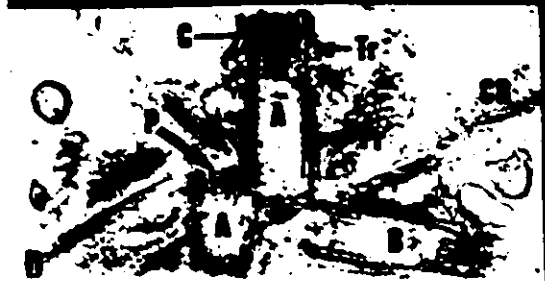
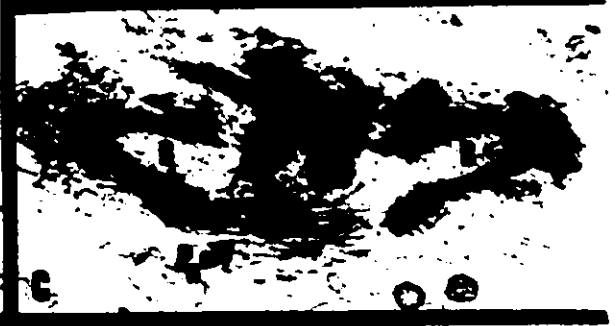
Figure 7.

Negative stain of isolated basal apparatus. Rootlets extend from the densely stained basal bodies. x 28,650.



Figure 8.

Structures of the isolated basal apparatus. A) Face view showing the A and B pair of basal bodies, the proximal striated interconnecting fibers (P) and one of the microtubular rootlets (MR). B) and C) are sections at right angles to the view shown in A). B) The A pair of basal bodies interconnected by the distal striated fiber (D). The proximal non-striated fiber (P_n) is seen in cross-section. C) The proximal non-striated fiber interconnecting the B pair of basal bodies is seen in longitudinal view. D) A median longitudinal section through the A pair of basal bodies showing the central core (C), transition region (Tr) and proximal region (Pr) of the basal body. Striations are evident on the proximal striated fibers and the compound rootlet (CR). E) Cross-section through the transition region of a basal body showing the microtubule doublets and their attachments to the central core. F) Cross-section through the proximal region of a basal body showing the nine sets of triplet microtubules. Magnifications: A)-D), x45,875; E),F), x125,000.



striated appearance (Fig. 8 B,D), and the proximal non-striated fiber which interconnects the B pair of basal bodies is filamentous (Fig. 8 C). Rootlets of two types terminate between the adjacent basal bodies. The fiber which runs parallel to and above the two microtubules of the compound rootlet has a striated appearance (Fig. 8 D). Cross-sections of the basal body show it to have nine sets of triplet microtubules in the proximal portion (Fig. 8 F), and nine sets of doublets connected to the central core in the transition region (Fig. 8 E).

Flagellar axonemes isolated from Polytomella have the normal structure of nine doublets surrounding a central pair of microtubules. One or both microtubules of the central pair may be lost during the isolation procedure. (Fig. 9).

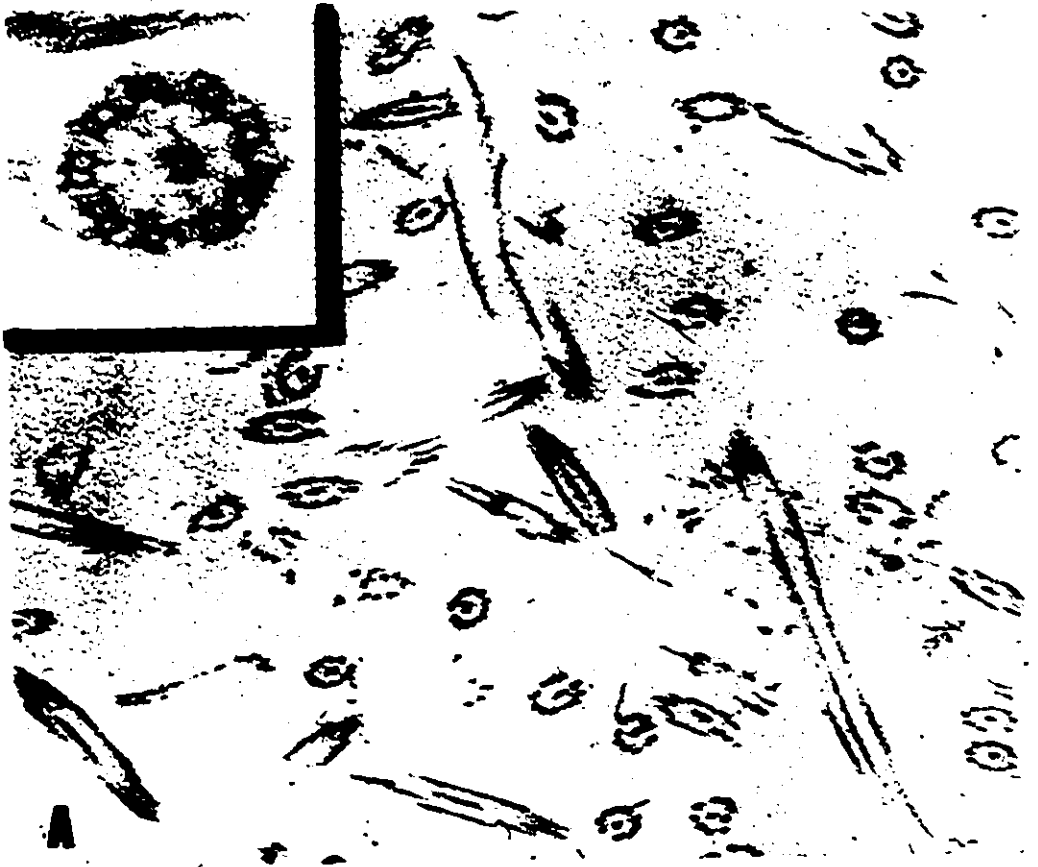
Optimal conditions for basal apparatus and axoneme ATPase activity

Prior to cytochemical localization of ATPase activity it was necessary to determine the pH optima and the concentrations of divalent cation required for maximal basal apparatus and axoneme ATPase activity. Two physiological activators of ATPase activities, Mg^{++} and Ca^{++} were chosen for these studies.

The basal apparatus ATPase activities show a pH optimum of 8.5 in the presence of either Mg^{++} or Ca^{++} (Fig. 10). These activities are maximal at divalent cation

Figure 9.

A preparation of isolated axonemes stained with uranyl acetate and lead citrate. No membranous material is present. x28,500. Inset: cross-section showing the nine outer doublets and one of the central pair of microtubules. x172,000.



concentrations of 1 mM Mg^{++} or 2 mM Ca^{++} with specific activities (expressed as μ mole inorganic phosphate released/min/mg protein) of 7.10 and 1.43 respectively (Fig. 11). ATPase activity is not detected in the absence of divalent cations or substrate. All further experiments were conducted at optimal pH and divalent cation requirements.

The pH optimum of axoneme ATPases is 8.5 (Fig. 12). These ATPases require 2 mM Mg^{++} or 6 mM Ca^{++} for maximal activity and show specific activities of 25.0 and 7.9 respectively (Fig. 13). A second peak of ATPase activity occurs at a concentration close to the optimum of basal apparatus ATPases. No ATPase activity is detected in the absence of divalent cations or substrate. All cytochemical studies using axonemes were conducted under conditions optimal for ATPase activity.

Cytochemical localization of ATPase and GTPase activities

To determine if ATPase activities in basal apparatus and axonemes are associated with a specific site or structure that could be involved in the coordination of flagellar beat, a cytochemical study was undertaken. Figures 14, 16, 17, and 18 show the localization of ATPase activity in views comparable to those in Figure 8.

Control experiments incubating the basal apparatus in the absence of divalent cation (Fig. 14, A, B) or ATP (Fig. 14 C-F) were conducted. No ATP-dependent reaction

Figure 10.

ATPase activity of the basal apparatus in response to change in pH at optimal divalent cation concentrations.

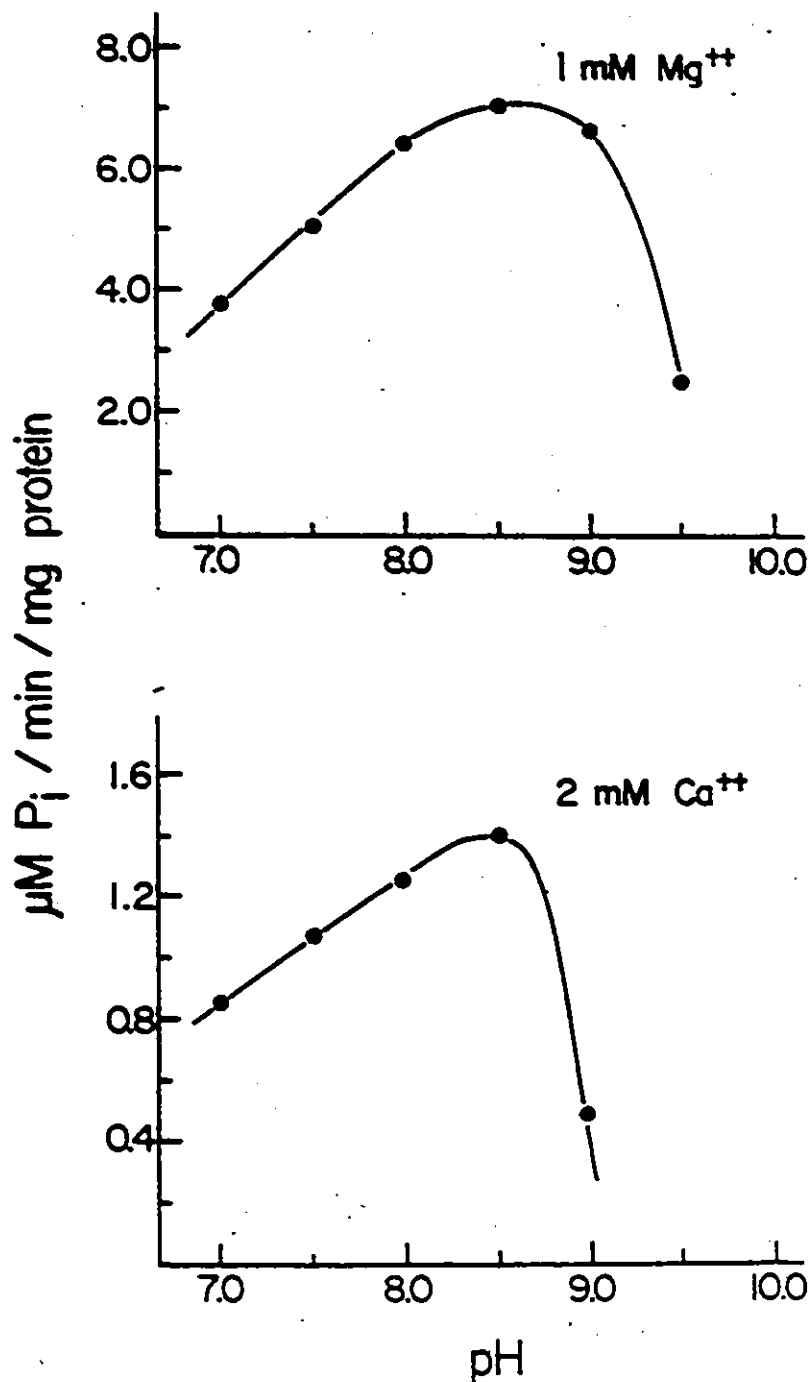


Figure 11.

Comparison of ATPase activity in the basal apparatus at varying concentrations of Mg^{++} or Ca^{++} .

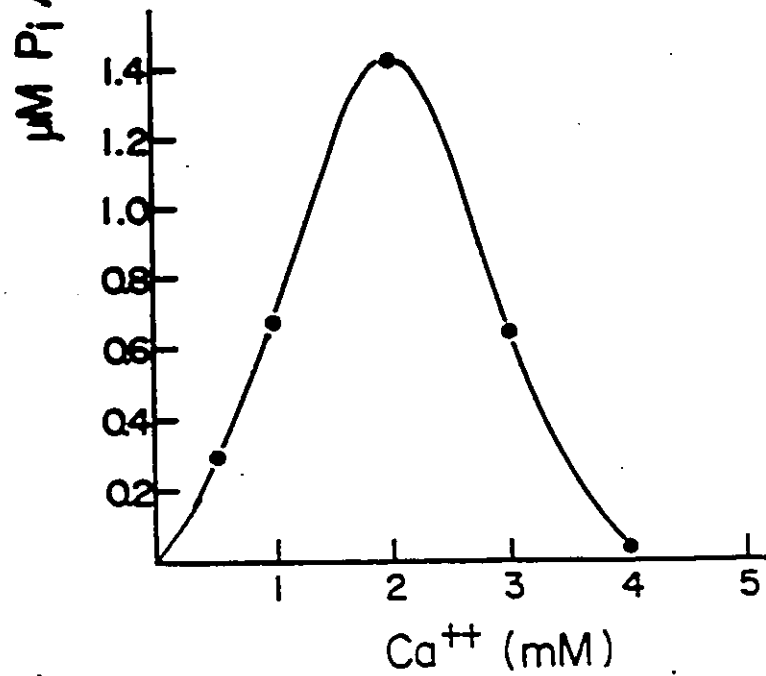
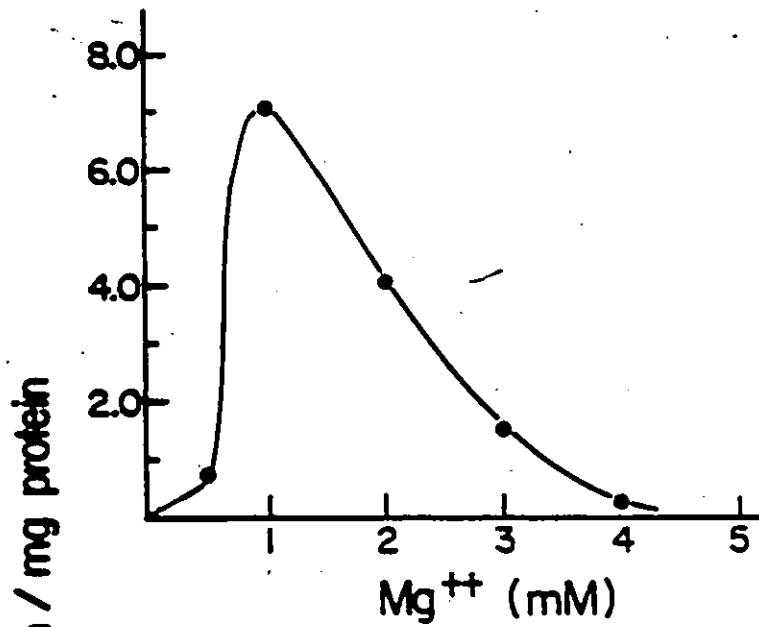


Figure 12.

ATPase activity of axonemes in response to change in pH at divalent cation concentrations of 1 mM Mg^{++} or 2 mM Ca^{++} .

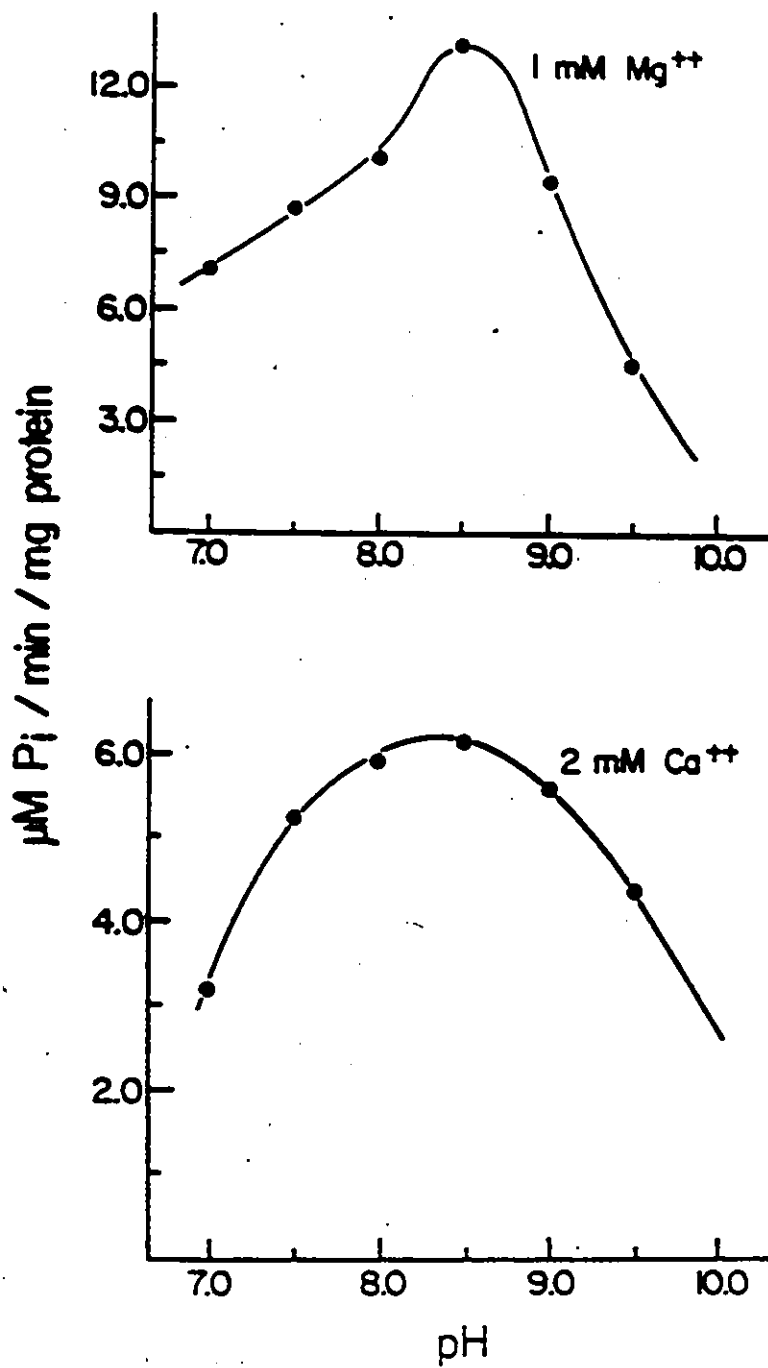
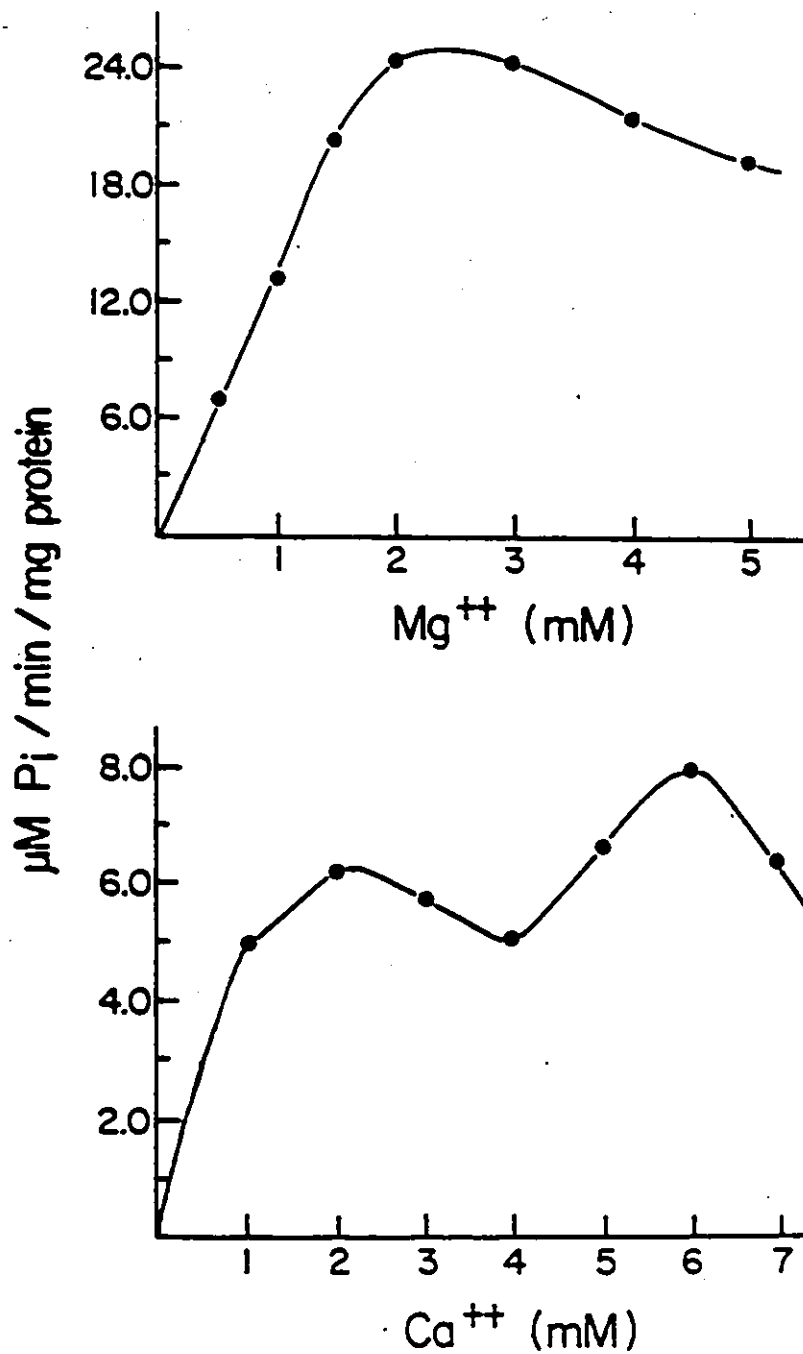


Figure 13.

Comparison of ATPase activity in the axonemes at varying concentrations of Mg^{++} or Ca^{++} .



product was detected in the unstained thin sections. Some non-specific lead phosphate precipitate is noted in the sections, but there is no specific localization and no regular pattern of deposition. The interconnecting fibers are present (Fig. 14 B, C, D) but the cross-banded pattern normally present on the distal and proximal striated fibers can not be detected. Rootlets are present (Fig. 14 A), but it can not be determined whether they are compound or microtubular rootlets. The individual microtubules in the transition and proximal regions of the basal body (Fig. 14 E, F) can not be discerned.

Control experiments were also conducted using axonemes (Fig. 15). ATP-dependent reaction product is ~~not~~ found associated with the microtubules of the axonemes in the absence of divalent cation (Fig. 15 C) or ATP (fig. 15 A, C). When p-nitrophenylphosphate is used as a control, no reaction product is associated with the microtubules of the axoneme (Fig. 15 B, E).

ATP-dependent reaction product is deposited on several structures in the basal apparatus. The pattern of deposition is the same whether Mg^{++} or Ca^{++} is used as divalent cation, except that in the presence of Ca^{++} reaction product is more heavily deposited on the striated fibers.

Both types of rootlets show the presence of ATP-dependent reaction product. The striated fibers of the compound rootlet show ATPase activity in a cross-banded

Figure 14.

Unstained sections of the basal apparatus incubated in the absence of divalent cations A) and B); or the absence of ATP C) - F). No deposition of ATP-dependent reaction product can be detected. A) shows a face view of the basal apparatus with rootlets (R) present. B) shows the A pair of basal bodies and the distal striated fiber (D). C) The B pair of basal bodies and proximal non-striated fiber (P_n) are present. D) The proximal striated fibers (P) and transition region of the basal body (Tr) are indicated by arrows. E) and F) cross-sections through the transition region and proximal region of the basal body respectively. Magnifications: A)-D), x46,785; E),F), x125,000.

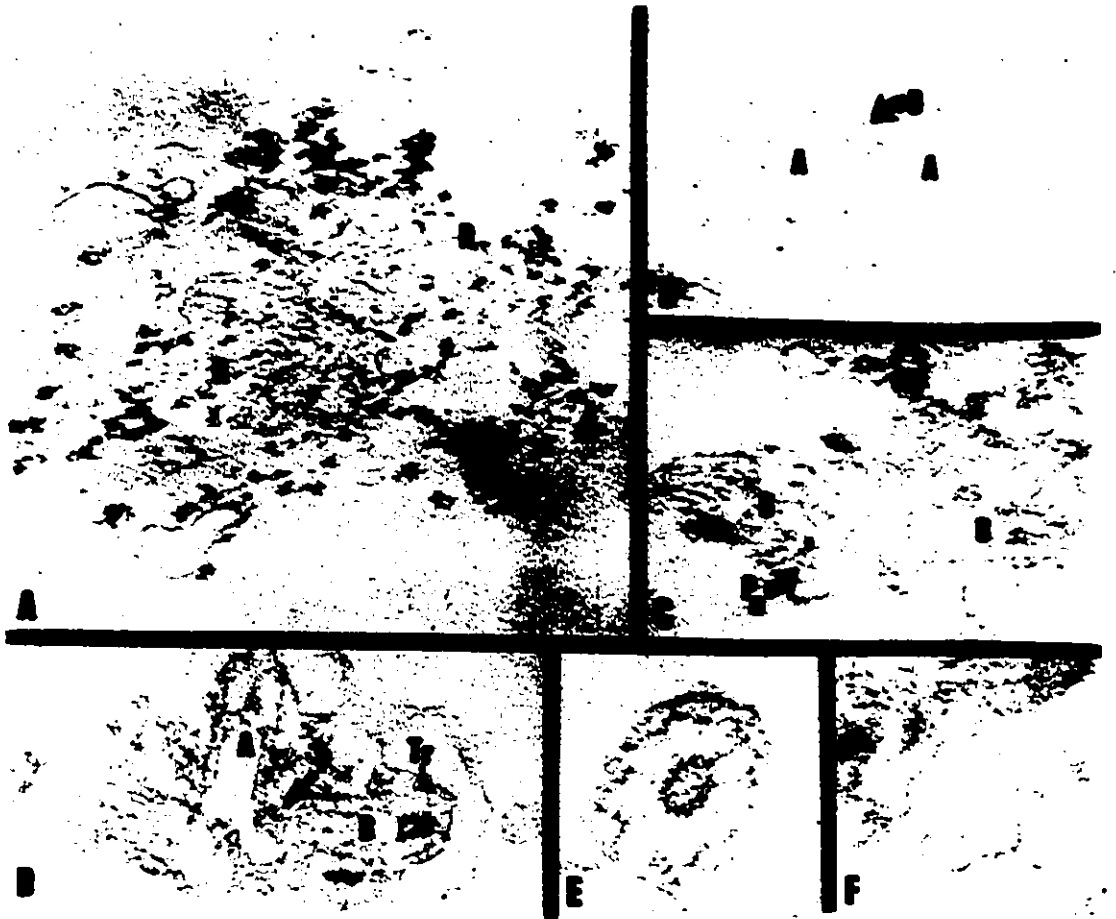
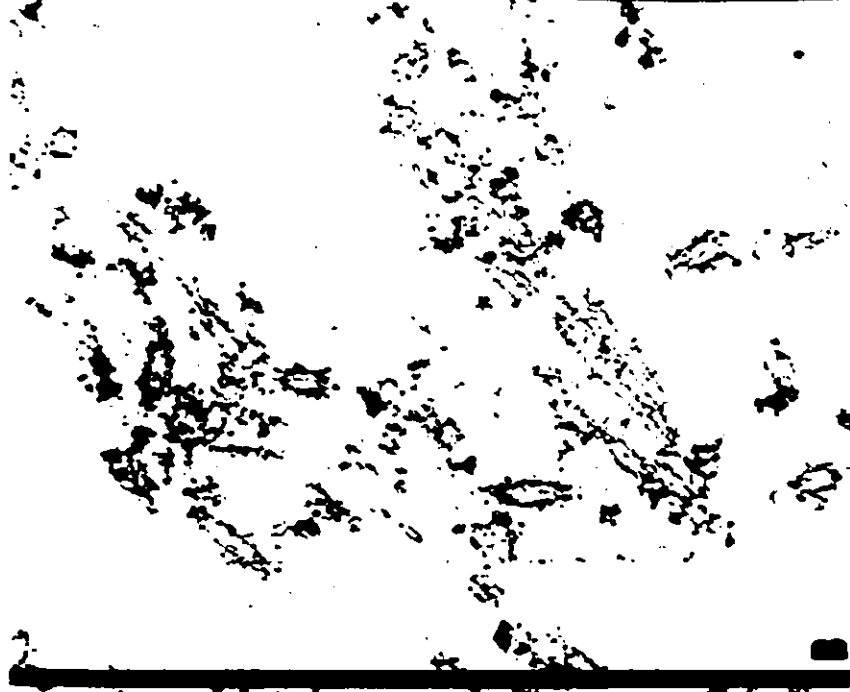


Figure 15.

Sections of axonemes incubated in the absence of divalent cation C), ATP A) and D), or using p-nitrophenylphosphate as substrate B) and E). The cross-sections were stained with uranyl acetate 5 min so that the microtubules of the axoneme could be visualized. Magnifications: A),B), x16,500; C),D),E), x125,000.



pattern that corresponds to the major striations with a 300 Å center-to-center spacing (Fig. 16 A; Fig. 17 A). ATP-dependent reaction product coats the microtubular rootlets. No regular pattern of deposition is seen (Fig. 16 A).

In the presence of Ca^{++} , ATP-dependent reaction product is found on the filamentous, proximal non-striated fiber which interconnects the B pair of basal bodies in longitudinal (Fig. 17 C) and cross-sectional views (Fig. 17 B). ATP-dependent product was not detected on this fiber in the presence of Mg^{++} (Fig. 16 C). The proximal striated fibers show ATPase in a cross-banded pattern (Fig. 16 A, B; Fig. 17 D), and on the periphery of the fibers in longitudinal sections (Fig. 16 B; Fig. 17 B). The distal striated fiber shows a heavy deposit of ATP-dependent reaction product in a pattern that may correspond to the seven equally spaced major cross-bands (Fig. 16 B; Fig. 17 B). This fiber also shows a heavy deposition of product when seen in cross-section (Fig. 16 C, Fig. 17 C).

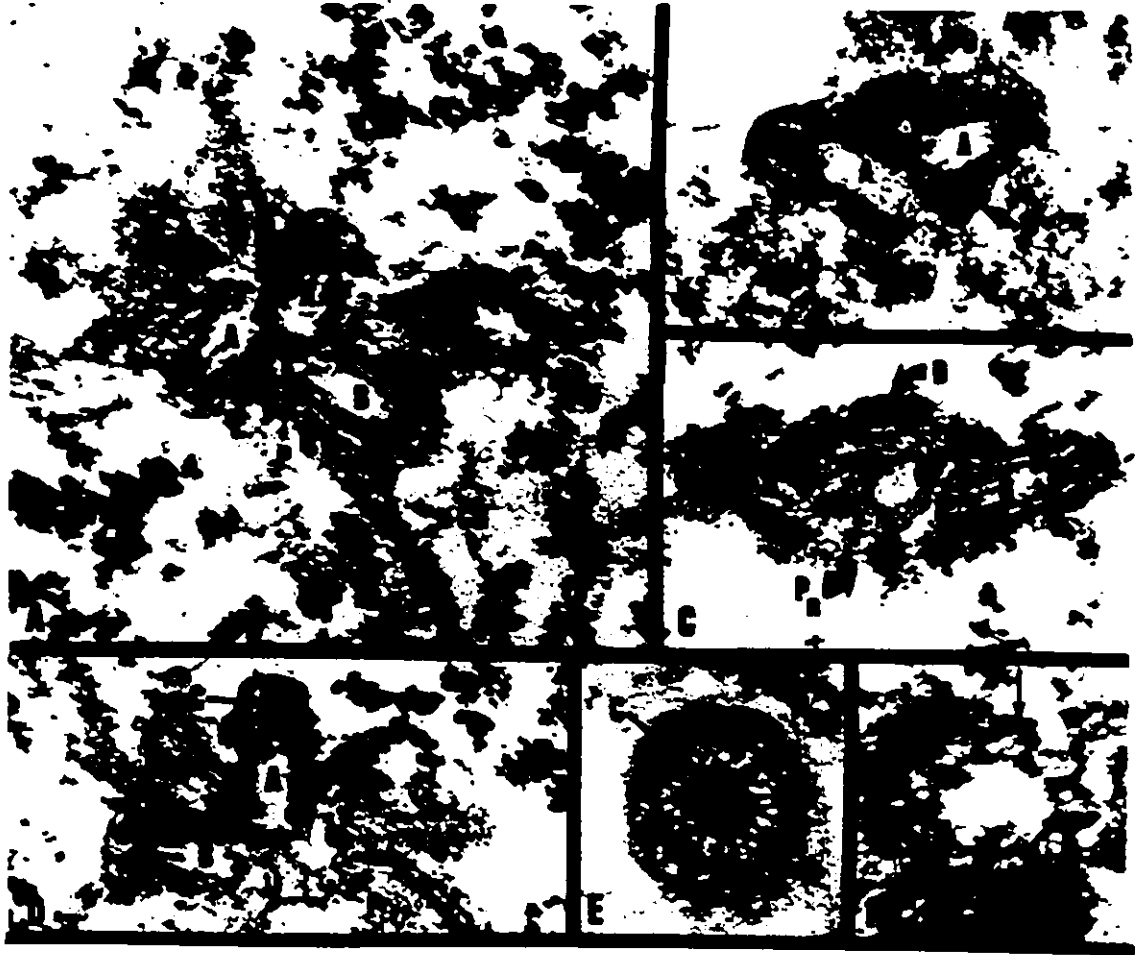
The basal bodies also have an associated ATPase activity. In the proximal region, ATPase activity is found primarily on the outside of the A, B and C triplet microtubules and on the inside of the A tubule (Fig. 16 F, Fig. 17 F). In the transition region of the basal body, ATP-dependent reaction product is deposited as a single 'spot' on the outside edge of each of the nine doublet microtubules (Fig. 16 E; Fig. 17 E). A heavy deposit of ATP-dependent

reaction product is found in the central core of the basal body transition region (Fig. 16 B, D, E; Fig. 17 A, D, E). In longitudinal view the pattern resembles little spokes projecting laterally from around a hollow core (Fig. 16 D). This ATPase may be localized on the attachments which connect the central core to the nine sets of outer doublet microtubules.

An examination of substrate specificity (see following section) showed a relatively high level of GTPase activity. In cytochemical studies using GTP as substrate, reaction product was found associated with several structures in the basal apparatus. The localization of GTP-dependent reaction product is almost identical to that of ATP-dependent reaction product. GTP-dependent reaction product is found on the striated fiber of the compound rootlet in a cross-banded pattern (Fig. 18 A). The distal striated fiber, as when ATP is used as substrate, shows a cross-banded pattern (Fig. 18 A, B) as well as showing a heavy deposition of GTP-dependent reaction product when seen in cross-section (Fig. 18 D). The proximal non-striated fiber shows GTPase activity as seen in longitudinal (Fig. 18 D) and cross-sectional (Fig. 18 B) views. The GTP-dependent reaction product deposited on the basal body microtubules and in the central core of the transition region is identical to the deposition of ATP-dependent reaction product (Fig. 18 E, F). The only difference in the cytochemical localization of nucleoside triphosphatase activity is that a

Figure 16.

Sections through the basal apparatus incubated in the presence of 1 mM Mg^{++} and ATP showing the deposition of ATP-dependent reaction product. The sections are comparable to those in Figure 8. A) - D) are unstained. E) and F) have been stained 5 min in uranyl acetate so the microtubules of the basal body could be visualized. ATP-dependent reaction is found on the microtubular (MR) and compound rootlet (CR), A). The proximal interconnecting fibers (P) show ATP-dependent reaction product deposited in a cross-banded pattern, A), D). The distal striated fiber (D) also shows ATPase activity in a cross-banded pattern, B). No ATP-dependent reaction product could be detected on the proximal non-striated fiber (P_n), C). A heavy deposition of ATP-dependent reaction product is present in the central core (C) of the basal body transition region B), D), E). ATPase activity is associated with the doublet and triplet microtubules in the transition and proximal regions of the basal body, E), F). ATPase activity is also associated with the connections between the doublet microtubules and central core, E), see also Fig. 8E. Magnifications: A)-D), x46,785; E), F), x 125,000.



2

3

Figure 17.

Sections through the basal apparatus incubated in the presence of 2 mM Ca^{++} and ATP showing the deposition of ATP-dependent reaction product. A) - D) are unstained. E) and F) have been stained 5 min in uranyl acetate. ATP-dependent reaction product is deposited in a striated pattern on the compound rootlet (CR), A). Both the proximal (P) and distal (D) striated interconnecting fibers show ATP-dependent reaction product in a cross-banded pattern, B), D). The proximal non-striated fiber (P_n) as seen in cross-section, B) and longitudinal view, C) shows ATP-dependent reaction product associated with the filaments. ATP-dependent reaction is deposited in the central core (C), A), D), E). ATP-dependent reaction product is also found on the doublet and triplet microtubules of the basal body, E), F). Magnifications: A)-D), x46,875, E),F), x125,000.

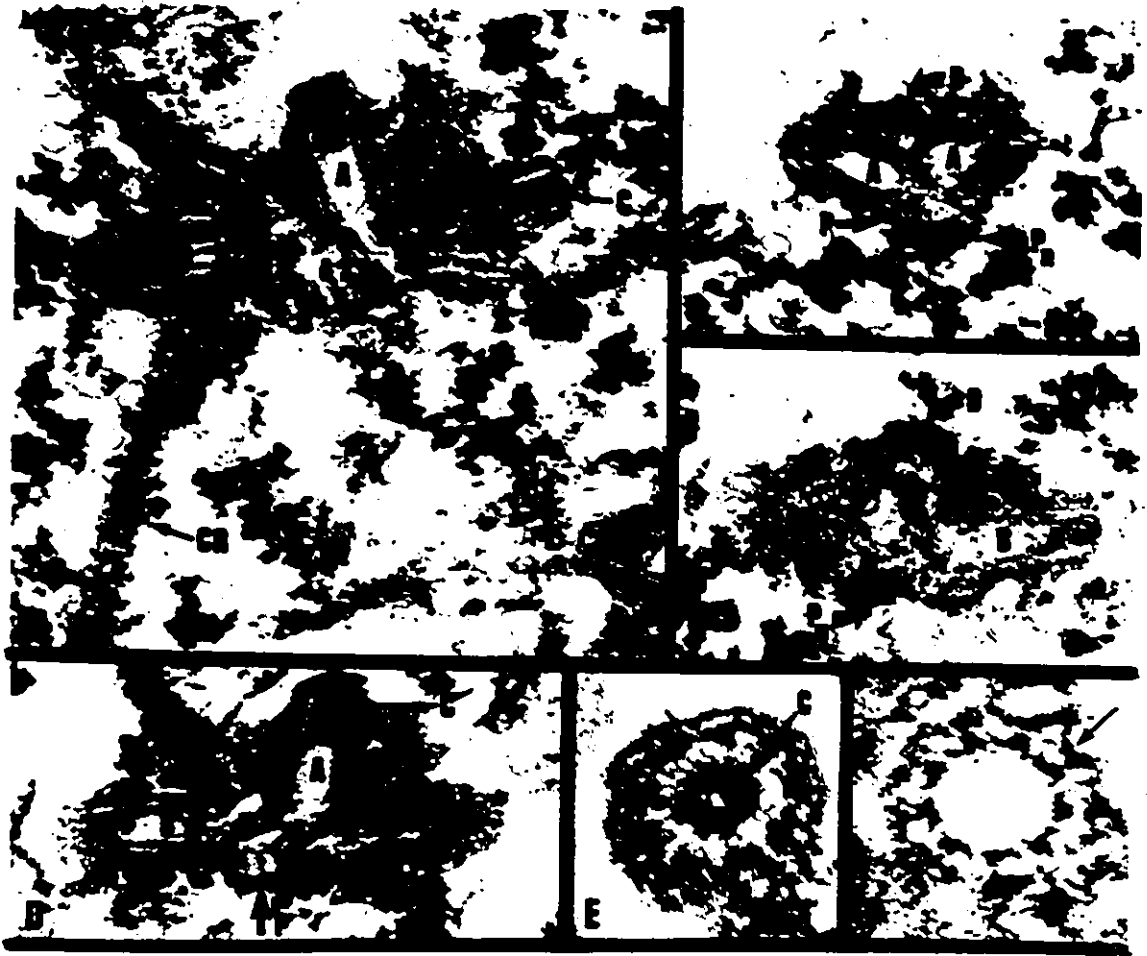


Figure 18.

Sections through the basal apparatus incubated in the presence of 2 mM Ca^{++} and GTP showing the deposition of nucleoside triphosphate-dependent reaction product. A) - D) are unstained. E) and F) have been stained 5 min in uranyl acetate. GTP-dependent reaction product is deposited on the compound rootlet (CR) in a striated pattern, A). Reaction product is deposited on the distal striated fiber (D) in a cross-banded pattern, A). The proximal striated fiber (P) only has reaction product on the outside edges, B), C). GTPase activity is associated with the filaments of the proximal non-striated fiber (P_n) as seen in cross-section, B) and longitudinal view D). Reaction product is also heavily deposited in the central core (C), C), E). GTPase activity is found on the outer doublet and triplet microtubules in the transition, E) and proximal, F) regions on the basal body. Magnifications: A)-D), x46,875; E),F), x125,000.

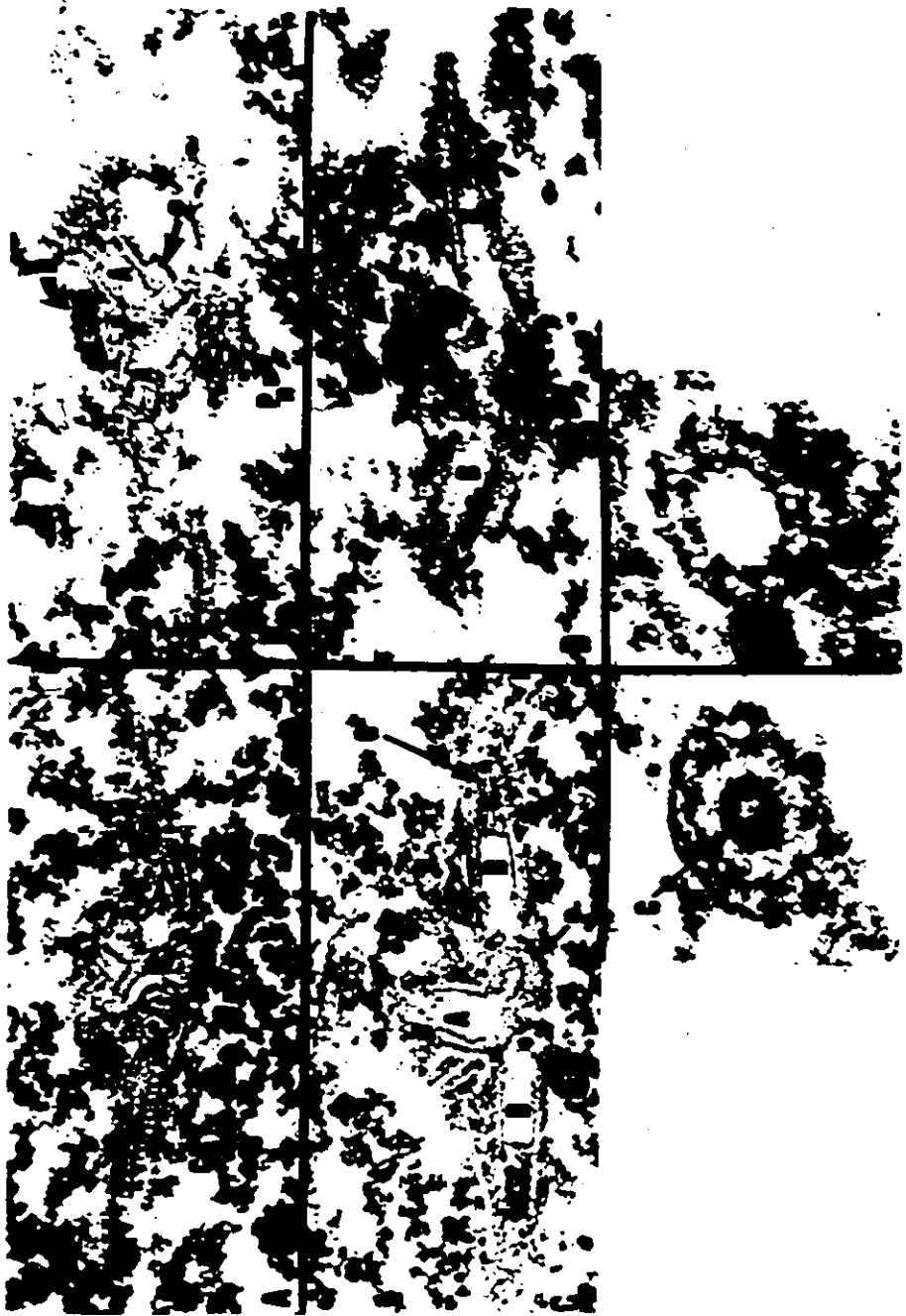


Figure 19.

Sections through the axoneme incubated in the presence of 2 mM Mg^{++} and ATP showing the deposition of ATP-dependent reaction product. The cross-sections have been stained 5 min in uranyl acetate. A) longitudinal section of axonemes. x16,500. B), C) and D) cross-sections through the axonemes. x125,000. CS - central sheath, RL - radial links, D - dynein ATPase arms.



NS

cross-banded pattern of reaction product was not detected on the proximal striated fiber (Fig. 18 C).

Localization of ATPase activity on axonemes isolated from Polytomella was also examined. ATP-dependent reaction product is found on the outer edges of the A and B microtubules (Fig. 19 B), on the central sheath and where the radial links attach to the A microtubules of the outer doublets (Fig. 19 B, C). ATPase activity is also found at a position corresponding to the location of the dynein ATPase arms (Fig. 19 C, D). The localization of ATP-dependent reaction product is the same in the presence of Mg^{++} or Ca^{++} . The localization of reaction product in the presence of Ca^{++} is not shown.

Properties of basal apparatus and axoneme ATPase activities

a. Divalent cation activation

The ATPase in the basal apparatus is activated by a number of divalent cations (Table I). The experiments were conducted at a pH of 8.5, and a divalent cation concentration of 1 mM or 2 mM. The highest specific activity recorded is when Mn^{++} is present. With the exception of Ba^{++} and Ca^{++} , the specific activities decrease as the concentration of divalent cations increase.

b. Substrate specificity

The basal apparatus hydrolyze ATP, some other nucleoside triphosphates and ADP (Table II). The highest specific activity is found with ATP as substrate. In the presence of 1 mM EDTA (Ca^{++} and Mg^{++} absent) ATP, ITP and UTP are

Table I

ATPase activity in the basal apparatus in response to different divalent cations. *

Divalent cation	Specific activities	
	1 mM	2 mM
Mn ⁺⁺	9.08	4.78
Mg ⁺⁺	7.12	4.13
Co ⁺⁺	3.51	1.87
Fe ⁺⁺	3.41	1.71
Cd ⁺⁺	2.46	1.42
Zn ⁺⁺	2.05	0.90
Cu ⁺⁺	1.84	1.67
Sr ⁺⁺	1.71	1.60
Ni ⁺⁺	1.60	0.77
Ba ⁺⁺	0.77	0.83
Ca ⁺⁺	0.71	1.46

* Values are the average of two trials. In the absence of divalent cation, ATP hydrolysis can not be detected.

Table II

Substrate specificity of the basal apparatus. *

Incubation conditions	Substrate	Specific activities	
1 mM Mg ⁺⁺	ATP	7.10	(6.74 - 7.58)
	ITP	2.16	(1.95 - 2.37)
	ADP	1.56	(0.84 - 2.20)
	GTP	1.17	(0.84 - 1.42)
	UTP	1.12	(0.92 - 1.34)
	CTP	0.82	(0.54 - 0.98)
2 mM Ca ⁺⁺	ATP	1.43	(0.88 - 1.83)
	GTP	0.48	(0.33 - 0.67)
1 mM EDTA	ATP	0.22	(0.10 - 0.35)
	ITP	0.09	(0. - 0.19)
	UTP	0.04	(0. - 0.07)

* Specific activities are average values. The range of values over at least three trials are in brackets. In the absence of divalent cation or EDTA, ATP hydrolysis can not be detected.

hydrolyzed at much lower rates.

The basal apparatus ATPase activities in the presence of EDTA are low, possibly indicating that only certain enzymes on some structures are active. Cytochemical localization of ATPase activity (Fig. 20) shows the deposition of ATP-dependent reaction product on three structures only. The distal striated fiber shows reaction product deposited in a cross-banded pattern (Fig. 20 A), this heavy deposition can also be seen in cross-section (Fig. 20 B). ATPase activity is also found in the central core of the basal body transition region (Fig. 20 A, B, C) and associated with the outer doublet microtubules of the transition region (Fig. 20 C).

In the presence of both 1 mM Mg^{++} and 2 mM Ca^{++} with ATP as substrate, the basal apparatus ATPase specific activity is 8.23. ATPase activities can not be detected in the basal apparatus when either nucleoside monophosphates, nucleoside diphosphates (other than ADP), pyrophosphate, p-nitrophenylphosphate or β -glycerophosphate are used as controls.

Stearns and Brown (1979) have shown that it is possible to isolate a protein fraction from the basal apparatus that will stimulate in vitro microtubule assembly. This is done by dialyzing the isolated basal apparatus in a Tris-EDTA solution for 20 min and centrifuging the preparation. The supernatant containing the extracted proteins was examined for the presence of ATPase activity.

Figure 20.

Sections through the basal apparatus incubated in the presence of 1 mM EDTA and ATP showing the deposition of ATP-dependent reaction product. A) and B) are unstained. C) has been stained 5 min in uranyl acetate. ATP-dependent reaction product is deposited in a cross-banded pattern on the distal striated fiber (D), A). ATPase activity is also associated with the central core (C), A) B) and C) and the outer doublets, C) of the basal body transition region. ATP-dependent reaction product is not detected on any other structures. Magnifications: A),B), x46,875; C), x125,000.



Inorganic phosphate is liberated from all nucleoside phosphate substrates, pyrophosphate and β -glycerophosphate indicating non-specific hydrolysis by the extracted proteins. The level of inorganic phosphate released varies greatly from one trial to another. In the extracted basal apparatus no hydrolysis of ITP, UTP or CTP is detected. The extracted basal apparatus still retains the capacity to hydrolyze ATP, GTP and ADP (Table III). Hydrolysis of GTP and ADP are not greatly affected by the extraction procedure (compare with Table II), but the ATPase activity in the presence of Mg^{++} is only about one-half of the unextracted control. This could be due to inactivation of the enzymes during the extraction procedure. The extracted basal apparatus appears intact. (Fig. 21).

Axoneme ATPases hydrolyze ATP, ITP, UTP, GTP and ADP (Table IV). The specific activity of the axonemes when ATP is used as substrate is much higher than with any other substrate. ATP is probably the natural substrate in axonemes. No hydrolysis of other nucleoside phosphates, pyrophosphate, p-nitrophenylphosphate or β -glycerophosphate is detected.

The specific activities of dynein ATPases isolated by Tris-EDTA extraction were also determined in the presence of 1 mM Mg^{++} or 2 mM Ca^{++} and are 13.08 and 10.92 respectively. Neither axonemes nor dynein demonstrated ATPase activity in the presence of EDTA.

Table III

Substrate specificity of the extracted basal apparatus. *

Incubation conditions	Substrate	Specific activities	
1 mM Mg ⁺⁺	ATP	3.76	(3.67 - 4.25)
	GTP	1.61	(1.31 - 2.19)
	ADP	1.53	(0.87 - 1.75)
2 mM Ca ⁺⁺	ATP	1.48	(1.17 - 1.78)
	GTP	0.91	(0.53 - 1.28)

*Specific activities are average values. The range of values over at least three trials are in brackets.

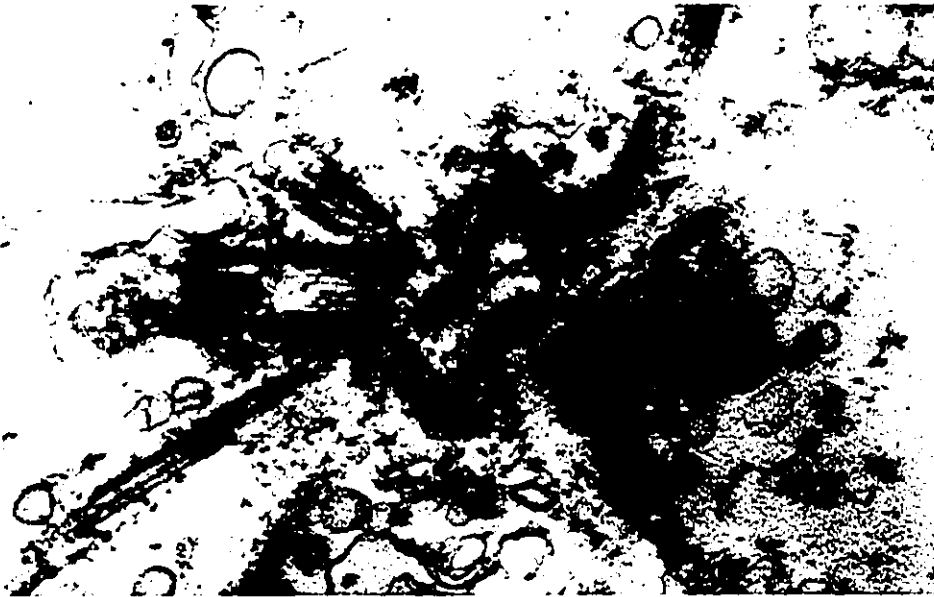


Fig. 21 Section of the isolated basal apparatus after extraction in Tris-EDTA at 4° for 20 min. The basal bodies and rootlets appear intact.

Table IV

Substrate specificity of axonemes. *

Incubation conditions	substrate	specific activities	
1 mM Mg ⁺⁺	ATP	13.22	(11.42 - 14.86)
	ITP	0.19	(0.11 - 0.26)
	UTP	0.97	(0.85 - 1.05)
	ADP	2.13	(1.87 - 2.42)
2 mM Ca ⁺⁺	ATP	6.25	(5.31 - 7.91)
	GTP	0.10	(0.09 - 0.12)
	ITP	0.02	(0.02 - 0.03)

* Specific activities are average values. The range of values over at least three trials are in brackets.

c. Michaelis constants

The basal apparatus and axoneme ATPase activities were compared by determining the values of the Michaelis constants (K_m) for ATP from Lineweaver-Burk plots (Fig. 22 and Fig. 23). The K_m values of the basal apparatus ATPases are 1.67×10^{-4} M in the presence of 1 mM Mg^{++} and 4.65×10^{-5} M in the presence of 2 mM Ca^{++} (Fig. 22). The K_m values of the axoneme ATPases are 2.63×10^{-5} M and 2.27×10^{-5} M respectively in the presence of Mg^{++} and Ca^{++} (Fig. 23). In these experiments the KCl concentration of the ATPase assay solution was lowered to 25 mM, since Lineweaver-Burk plots of axoneme ATPases in the presence of Mg^{++} at KCl concentrations greater than approximately 100 mM have been shown to be non-linear (Gibbons and Fronk, 1972).

d. Effects of various agents on ATPase activities

Various agents have been reported to affect ATPase activity and can be used to distinguish between the different ATPase activities of various cellular components. Some of these agents inhibit specific ATPases, others are less specific inhibitors.

The inhibitors oligomycin and ouabain at concentrations of 10^{-3} M have no effect on ATPase activities of the basal apparatus or axoneme preparations, indicating no significant mitochondrial or membrane ATPase contamination.

Figure 22.

Lineweaver-Burk plots (least squares fit) of the ATPase activity of basal apparatus as a function of ATP concentration. ■ 2 mM Ca^{++} ; ● 1 mM Mg^{++} .

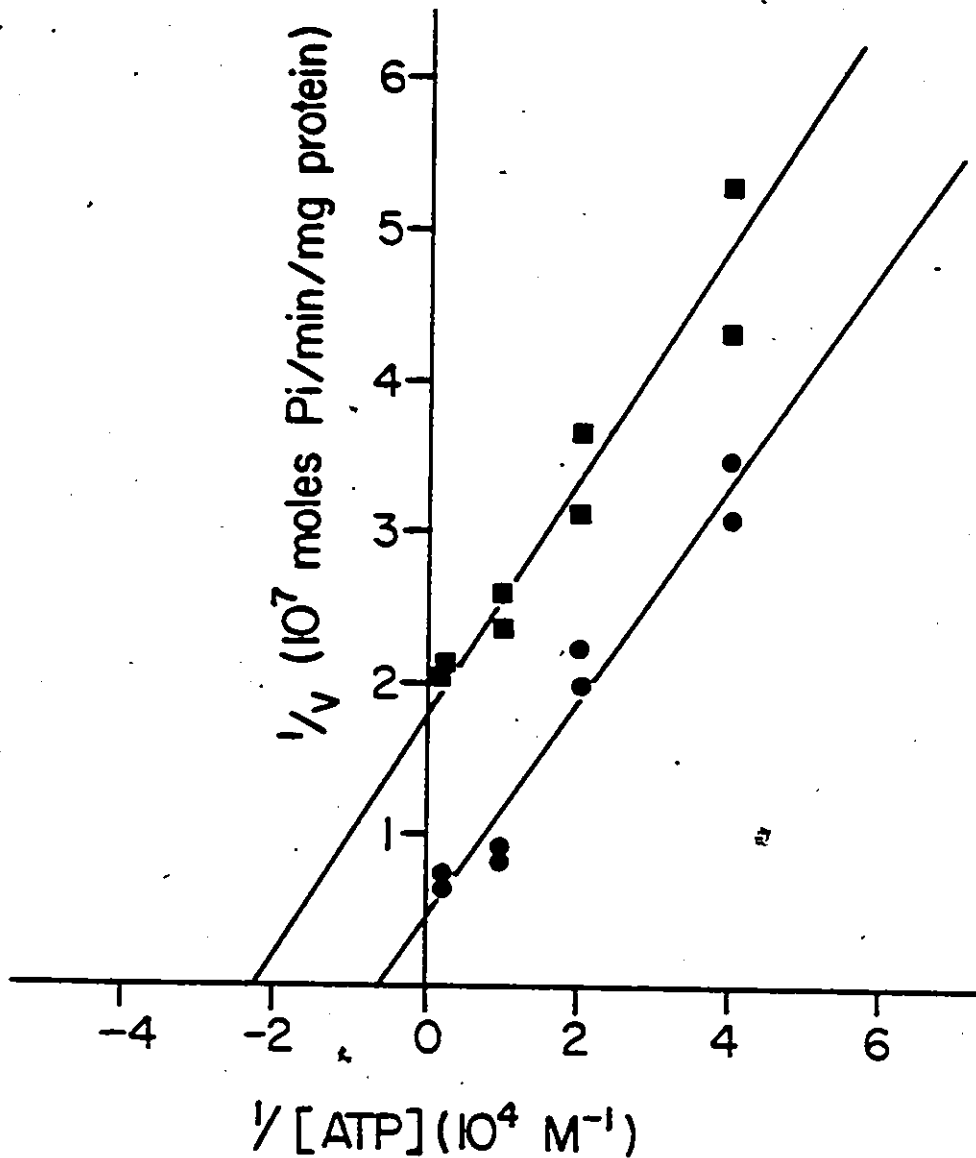
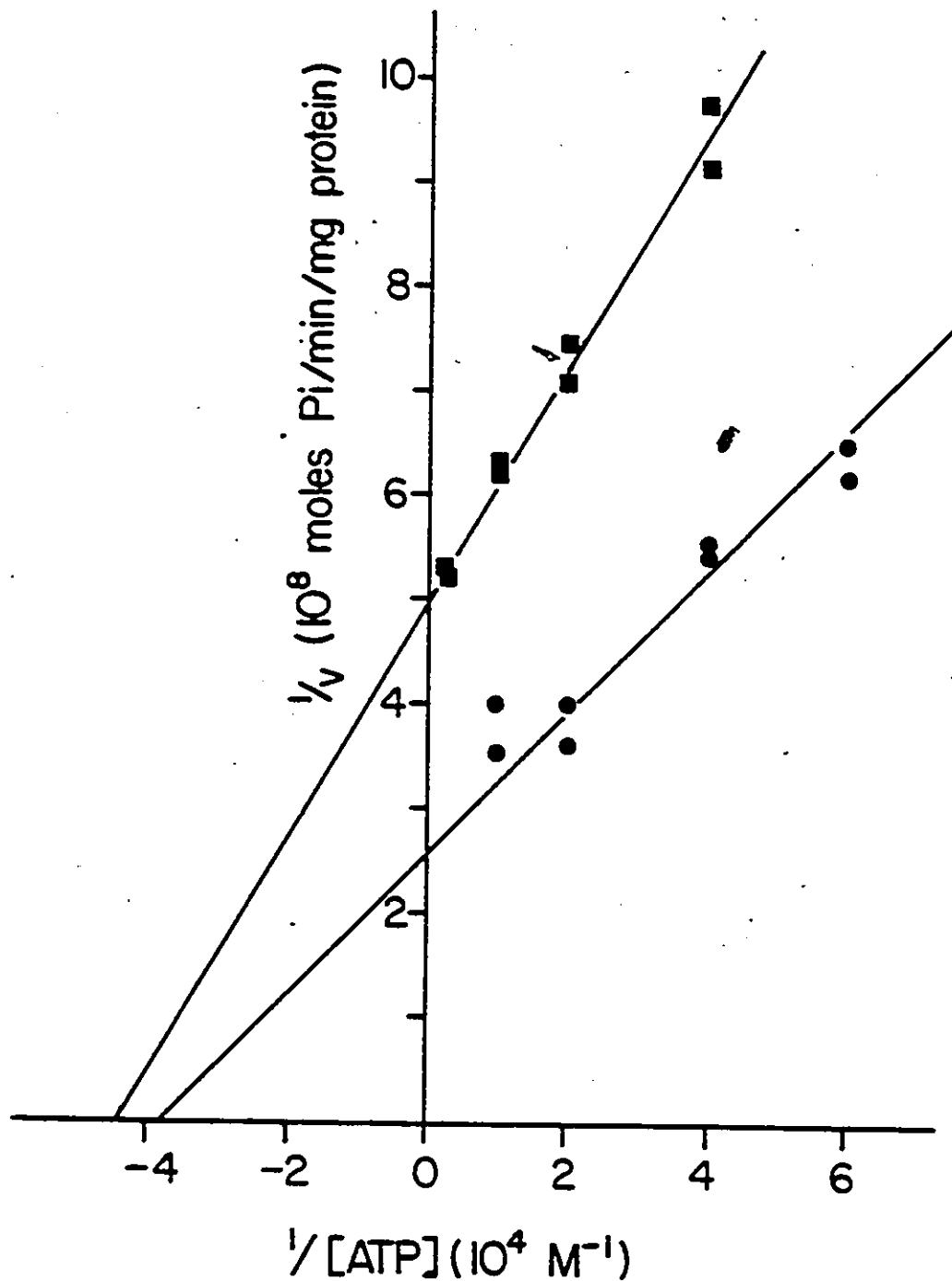


Figure 23...

Lineweaver-Burk plots (least squares fit) of the ATPase activity of axonemes as a function of ATP concentration. ■ 2 mM Ca^{++} ; ● 1 mM Mg^{++} .



Under conditions favourable for membrane (Na-K)-ATPase activity (Cantley, Cantley and Josephson, 1978), ouabain sensitive ATPases in the basal apparatus preparations represented only 4.5% of the total specific activity.

Many recent studies have used vanadate to distinguish between ATPase activities (see Simons, 1979 for a review). Table V shows the effect of varying concentrations of vanadate on the specific activities of the basal apparatus axoneme and dynein ATPases of Polytomella. Kobayashi et al., (1978) has found that 250 mM KCl interferes with the action of vanadate on axoneme and dynein ATPase activity. For this reason vanadate inhibition studies were carried out at both 25 mM and 250 mM KCl. The ATPases in the axoneme are about ten-fold less sensitive to vanadate than dynein ATPases. Axoneme ATPases are more highly inhibited when the salt concentration (KCl) is lower, this is especially evident in the presence of Mg^{++} at a vanadate concentration greater than 10^{-4} M. When axonemes were incubated with 10^{-3} M vanadate in the presence of Mg^{++} and ATP, no ATP-dependent reaction product was detected cytochemically. Axoneme and dynein ATPases are less sensitive to vanadate when in the presence of Ca^{++} .

The vanadate inhibition of basal apparatus ATPase activity is markedly different. At vanadate concentrations as high as 10^{-3} M, basal apparatus ATPase activity is at

Table V.

The effect of varying concentrations of vanadate on ATPase activities in the basal apparatus, axonemes and dynein. *

The control values of the specific activities are below, the range of values over at least three trials are in brackets:

	25 mM KCl		250 mM KCl	
<i>a</i>	13.10	(10.93 - 13.82)	7.10	(6.74 - 7.58)
<i>b</i>	4.81	(4.59 - 4.93)	1.43	(0.88 - 1.83)
<i>c</i>	0.45	(0.37 - 0.52)	0.22	(0.10 - 0.35)
<i>d</i>	21.61	(18.72 - 25.02)	13.22	(11.42 - 14.86)
<i>e</i>	19.33	(17.29 - 20.21)	6.25	(5.31 - 7.91)
<i>f</i>	13.08	(12.52 - 13.65)		n.d.
<i>g</i>	10.92	(9.67 - 12.34)		n.d.

n.d. not determined

*Vanadate was incubated with the basal apparatus, axonemes or dynein in ATPase assay solution for 20 min before addition of ATP. Each value is the average of three trials.

Table V

	Incubation conditions	vanadate concentration	ATPase activity (% control)	
			25 mM KCl	250 mM KCl
basal apparatus	1 mM Mg ⁺⁺ <i>a</i>	10 ⁻⁵ M	84.4	82.5
	"	10 ⁻⁴ M	73.4	64.5
	"	10 ⁻³ M	54.4	43.0
	2 mM Ca ⁺⁺ <i>b</i>	10 ⁻⁵ M	96.7	83.0
	"	10 ⁻⁴ M	81.5	67.5
	"	10 ⁻³ M	31.7	46.2
	1 mM EDTA <i>c</i>	10 ⁻⁴ M	137.4	n.d.
	"	5 x 10 ⁻⁴ M	137.4	n.d.
	"	10 ⁻³ M	137.4	n.d.
axonemes	1 mM Mg ⁺⁺ <i>d</i>	10 ⁻⁵ M	63.3	68.4
	"	10 ⁻⁴ M	17.7	47.2
	"	10 ⁻³ M	4.5	13.0
	2 mM Ca ⁺⁺ <i>e</i>	10 ⁻⁵ M	80.0	92.5
	"	10 ⁻⁴ M	52.3	49.3
	"	10 ⁻³ M	24.7	19.0
dynein	1 mM Mg ⁺⁺ <i>f</i>	10 ⁻⁵ M	18.0	n.d.
	"	10 ⁻⁴ M	4.3	n.d.
	"	10 ⁻³ M	0	n.d.
	2 mM Ca ⁺⁺ <i>g</i>	10 ⁻⁵ M	76.2	n.d.
	"	10 ⁻⁴ M	48.5	n.d.
	"	10 ⁻³ M	29.5	n.d.

least 31.7% of control levels. Unlike axoneme ATPase inhibition, the inhibition of basal apparatus ATPase activity by vanadate does not show great differences dependent on salt concentration. In the presence of EDTA, the basal apparatus ATPases are stimulated by all vanadate concentrations examined, to levels 37.4% over control specific activities.

The sensitivity of basal apparatus and axoneme ATPases to other agents affecting ATPase activity were examined (Table VI). PCMB (p-chloromercuribenzoate) at 10^{-3} M totally inhibits axoneme ATPases. The basal apparatus ATPases also show very high inhibition (19.6% of control levels). NaF (sodium fluoride) at a concentration of 10^{-2} M is a relatively poor inhibitor of axoneme and basal apparatus ATPase activities. The basal apparatus ATPases in the presence of Mg^{++} showed the least sensitivity. Thiourea is an effective inhibitor of ATPase activities at a concentration of 0.5 M. The basal apparatus ATPases are only about 5% of control levels, and the axonemes are also strongly inhibited, especially in the presence of Ca^{++} . Basal apparatus ATPase activity decrease from about 60% of control levels at 0.5 M urea to 31.8% and 19.2% respectively in the presence of Mg^{++} and Ca^{++} at 1.0 M urea. Axoneme ATPases show less sensitivity to urea.

Basal apparatus ATPase activity in the presence of EDTA and ATP was most sensitive to the agents examined,

Table VI

The effect of various agents on ATPase activity in the basal apparatus and axonemes. *

	ATPase Activity (% control)			
	PCMB 10 ⁻³ M	NaF 10 ⁻² M	thiourea 0.1 M	urea 0.5 M
Basal apparatus				
1 mM Mg ⁺⁺	19.6	90.5	87.8	5.7
2 mM Ca ⁺⁺	15.8	70.6	84.5	4.0
1 mM EDTA	0	66.7	33.3	0
Axonemes				
1 mM Mg ⁺⁺	0	76.7	63.3	12.7
2 mM Ca ⁺⁺	0	70.1	62.4	2:0
			76.8	53.2
			61.2	45.9

* The agent was incubated with the basal apparatus or axoneme in ATPase assay solution for 20 min before addition of ATP. Each value is the average of three trials.

but, the control specific activity (shown in Table II) is very low and only represents a small fraction of the total basal apparatus ATPase activity.

DISCUSSION

The role of flagella and cilia in cell motility is now well understood at the molecular level. An ATP-splitting property of bull spermatazoa was first demonstrated in 1945 (Lardy, Hansen and Phillips) and in 1954 this ATPase activity was shown to be associated with the tail region (flagellum) of the spermatazoa (Nelson). Subsequently, the ATPase activity was cytochemically localized on outer doublet microtubules of the flagellar axoneme (Nelson, 1958). A component from the flagella with a high level of ATPase activity (dynein) was isolated (Gibbons and Rowe, 1965), purified (Gibbons, 1966) and shown to be associated with a specific structure. These dynein ATPase arms are involved in axonemal microtubule sliding resulting in flagellar movement (Gibbons and Gibbons, 1972; Satir, 1974). Recent cytochemical studies have localized ATPase activity in intact axonemes at positions corresponding to the location of the dynein arms (Anderson, 1977). The enzymatic properties of axonemes and dynein have been analyzed in detail (see review, Gibbons et al., 1976).

The general approach in determining the role of ATPase activities in flagellar motility was initially to localize at finer and finer levels the structures involved in this motility. I have used a similar approach to begin the analysis of the role of

the basal apparatus in the control of flagellar beat patterns. High levels of ATPase were detected in the isolated basal apparatus, optimal conditions for this activity were established and the ATPase activity was cytochemically localized. The basal apparatus ATPases were then compared to the better understood ATPases of the axoneme.

Studies of flagellar axoneme or dynein ATPase activities have been carried out under a variety of conditions. In most previous studies, particularly those of the basal apparatus, no attempt was made to establish the optimal conditions. Flagellar studies generally use pH levels between 8.0 and 8.5 with divalent cation concentrations ranging from 1 to 20 mM (Stephens and Levine, 1970; Gibbons and Fronk, 1972; Ogawa and Mohri, 1972). Similarly, cytochemical studies of basal apparatus ATPases have been conducted at a variety of pHs and divalent cation concentrations (Matsusaka, 1967; Abel et al., 1972; Anderson, 1977; Dentler, 1977; Sharp et al., 1979).

Only one study to date has combined biochemical and cytochemical methods to compare the ATPase activities of basal apparatus and cilia isolated from the same cell type (Anderson, 1977). The pH optimum for the chicken oviduct basal apparatus ATPases was 8.5, that of the ciliary ATPases was 10.0. The optimal divalent cation requirements for basal apparatus ATPases were 2 mM Mg^{++} or 2 mM Ca^{++} , whereas, the ATPase activities of the cilia continued to increase at levels exceeding 6 mM Ca^{++} or Mg^{++} . His results indicated that basal bodies have an intrinsic ATPase activity that

can be distinguished from the ATPase activity found in cilia.

I have carried out similar, but more comprehensive, comparative studies in order to distinguish between the ATPases in basal apparatus and axonemes isolated from Polytomella. A major reason for carrying out these studies was to establish the optimal conditions for basal apparatus ATPases for use in the cytochemical studies.

The basal apparatus ATPases can be activated by a great number of divalent cations indicating that they are necessary for ATPase activity, but it does not appear the size of the cation has any effect. Similarly, axoneme (Gibbons, 1966) and dynein (Ogawa and Mohri, 1972) ATPases can be activated by numerous divalent cations.

Both the basal apparatus and axoneme ATPases of Polytomella have a pH optimum of 8.5 in the presence of either Mg^{++} or Ca^{++} . The ATPase activity of the basal apparatus declines rapidly above this pH while the axoneme ATPases are capable of activity over a wider pH range. Concentrations of 1 mM Mg^{++} or 2 mM Ca^{++} are required for maximal basal apparatus ATPase activity and the specific activities decline rapidly above and below these optimal levels. The axoneme ATPases have optimal divalent cation requirements of 2 mM Mg^{++} or 6 mM Ca^{++} , but function well over a wider range of concentrations than the basal apparatus ATPases.

The basal apparatus and axoneme ATPases can hydrolyze

a variety of nucleoside triphosphates and ADP. Polytomella axonemes show the highest specific activity when ATP is substrate, other nucleoside triphosphates and ADP are hydrolyzed at a much lower rate. Flagella isolated from other sources also show the highest specific activity in the presence of ATP. The apparent hydrolysis of ADP in flagella has been shown to be due to phosphorylation of ADP to ATP by adenylate kinase (Brokaw, 1961). The hydrolysis of nucleoside triphosphates other than ATP may be the result of transphosphorylases (Brokaw, 1961).

The basal apparatus in the presence of Mg^{++} hydrolyzes ATP most rapidly; significant levels of other nucleoside triphosphate activities were also detected. Only the hydrolysis of ATP and GTP was detected in the presence of Ca^{++} . Anderson (1977) showed that chicken oviduct basal body ATPases have the highest specific activity and that very low levels of ADP, GTP and ITP hydrolysis were detected. Whether the hydrolysis of substrates other than ATP is due to adenylate kinase or transphosphorylases is not known. These results suggest that ATP is probably the natural substrate for the majority of basal apparatus phosphatases.

The K_m values of ATP have been determined for the phosphatases of both the basal apparatus and axonemes of Polytomella. The basal apparatus ATPases in the presence of Mg^{++} have a K_m of 1.67×10^{-4} M, about three times higher than the K_m in the presence of Ca^{++} (4.65×10^{-5} M). The axoneme ATPases have K_m values of 2.65×10^{-5} M and $2.27 \times$

10^{-5} M in the presence of Mg^{++} and Ca^{++} respectively, which are considerably lower than those of the basal apparatus. It is difficult to compare these results with the literature axonemal ATPase K_m values because the experiments were carried out under different conditions. For example, the K_m for sea urchin axonemes in the presence of Mg^{++} (no KCl present) is 4.1×10^{-5} M; but in the presence of Ca^{++} and 0.15 M KCl is 1.1×10^{-4} M (Gibbons and Fronk, 1972).

Vanadate in the +5 oxidation state has been shown to inhibit a number of ATPase activities including: axonemal ATPases (Kobayashi et al., 1978); dynein ATPases (Gibbons et al., 1978; Sale and Gibbons, 1979); the dynein-like ATPases found associated with microtubules in mitotic spindles (Cande and Wolniak, 1979); the (Na-K)-ATPase associated with cation transport (Josephson and Cantley, 1977; Cantley et al., 1978); several other plasma membrane ATPases (O, Neal, Rhoads and Racker, 1979) and acid (Lindquist, Lynn and Lienhard, 1974) and alkaline (Lopez, Stevens and Lindquist, 1976) phosphatases. Based on studies of vanadate inhibition of myosin ATPases, Goodno (1979) has suggested vanadate (V_i) acts as an analog of inorganic phosphate (P_i) leading to inhibition of ATPase activities.

The differential inhibition of these enzyme systems by vanadate has been used to distinguish between various ATPases. My results show that the axoneme ATPases are much more sensitive to vanadate than the basal apparatus ATPases. The basal apparatus ATPases are at least 43% and 31% of

control levels, at 10^{-3} M vanadate in the presence of Mg^{++} and Ca^{++} respectively. The vanadate stimulation of the low ATPase activity in the presence of EDTA resembles the activation of actomyosin or myosin ATPases in the presence of 5×10^{-4} M vanadate (Gibbons et al., 1978). In agreement with previous reports (Kobayashi et al., 1978), axoneme ATPases are about ten times less sensitive to vanadate than dynein ATPases and are almost totally inhibited in the presence of Mg^{++} at a vanadate concentration of 10^{-3} M. Both dynein and axoneme ATPases are more sensitive to vanadate inhibition in the presence of Mg^{++} than Ca^{++} (see also Gibbons et al., 1978).

A variety of agents, some specific (oubain, oligomycin) and others non-specific (PCMB, NaF, thiourea and urea) have been shown to affect ATPase activities. The effects of these agents on flagellar motility (Abé, 1963; Brokaw, 1966; Tash and Mann, 1973), ATPases in flagella or axonemes (Brokaw and Benedict, 1971; Ogawa and Mohri, 1972) and actin-myosin interactions (Kielley and Bradley, 1956; Bárány, Bárány and Trautwein, 1960; Rüegg, Straub and Twarog, 1963) have been studied. Bloodgood (1975) has used a variety of agents to study ATPases and motility in isolated axostyles. In Polytomella, the basal apparatus and axoneme ATPases show similar sensitivities to PCMB and NaF, and show increasing inhibition of ATPases with increasing levels of thiourea or urea. Axoneme ATPases are more sensitive to 0.1 M thiourea, and the basal apparatus ATPases show greater

sensitivity to urea.

ATPases of the basal apparatus and axonemes can be distinguished by differences in pH activity profiles, optimal divalent cation concentration and activity profiles, K_m values for ATP and the sensitivity of the ATPases to vanadate, thiourea and urea. This preliminary characterization indicates the basal apparatus and axonemes contain their own set of ATPases enzymes.

Under conditions of Mg^{++} and Ca^{++} activation, the specific activities of the basal apparatus and axoneme ATPases of Polytomella are greater than those previously reported in studies of other cell types. This may be due to differences between organisms used or differences in isolation and assay conditions. Gibbons et al., (1976) have shown that nonmotile axonemal fragments isolated under some conditions have a relatively low activity, but have a higher activity when isolated under other conditions. They have suggested this increase in activity results from the uncoupling of activity from motility. They also found that dynein ATPase activity could be increased up to 10 fold by preincubating in 1% Triton X-100. The exposure to detergent in the procedures I have used to isolate basal apparatus and axonemes may account for the high activities.

Neither axonemes nor dynein show ATPase activity in the presence of EDTA (see also Gibbons, 1966; Stephens and Levine, 1970). In contrast, basal apparatus ATPase activities are activated by 1 mM EDTA if K^+ is present. I .

have found in the presence of K^+ /EDTA that basal apparatus ATPases are stimulated by vanadate and strongly inhibited by thiourea and urea. The myosin ATPases have been shown to be activated by K^+ /EDTA (Offer, 1964) and vanadate (Gibbons et al., 1978), and strongly inhibited by thiourea and urea (Bárány et al., 1960; Rüegg et al., 1963). This may indicate the presence of a myosin-like ATPase in the basal apparatus. The K^+ /EDTA activated basal apparatus ATPases only accounts for a small proportion of the total phosphatase activity; however cytochemical studies indicate this ATPase activity is localized only on the distal striated fiber and in the central core of the basal body transition region.

For the flagella to beat in their characteristic manner there must be mechanisms controlling initiation, coordination and direction of flagellar beat. Several structures of the basal apparatus have been suggested to play a role in the control of flagellar beat in a variety of cell types. Such suggestions have usually been based on observations that specific basal apparatus structures are in the correct positions to influence flagellar movement.

Root structures have been suggested to serve as a structural support for the basal bodies to dissipate the forces created by the flagellum as it beats back and forth (Allen, 1967). A structural or anchoring role would require the rootlets be attached to the basal body and interact with some other structure(s). The rootlets of Polytomella

are certainly attached to the basal bodies since they remain attached throughout the isolation procedure. In situ the rootlets have been shown (Brown et al., 1976) to extend from the basal body just underneath the plasma membrane for the length of the cell. They appear to be firmly attached to the plasma membrane since they are still observed associated with this membrane in cells which have been exposed to hydrostatic pressure to disassemble cytoskeletal microtubules. The cytoskeletal microtubules terminating on the rootlets do not appear necessary for anchoring the basal body since cells lacking the cytoskeletal microtubules are still motile (Brown, personal communication). The observation of ATP-dependent reaction product in a non-patterned distribution on the microtubular rootlets and the microtubules of the compound rootlet, and deposited on the major cross-bands of the compound rootlet striated fiber may indicate interaction with the plasma membrane in vivo, the other rootlet microtubules, or both. The association of root structures with basal bodies of non-motile cells (Nagano, 1962; Sakaguchi, 1965; Scalzi, 1967) indicates anchoring may not be their sole function. The rootlet microtubules may transmit signals or impulses (Matsusaka, 1967) similar to that proposed for the microtubule sheets or bundles in ciliates (Pitelka, 1965; Allen, 1967).

Rootlets of the type found in Polytomella do not appear to be directly involved in the coordination of flagellar

beat. Hyams and Borisy (1975) have demonstrated that flagellar coordination in the isolated flagellar apparatus of Chlamydomonas, in which only root remnants are present, is indistinguishable from that seen in living cells.

The demonstration of ATP-dependent reaction product on cross-striations of rootlets (Matsusaka, 1967; Anderson, 1977) has suggested that the rootlet filaments may function in contraction. Direct evidence of contraction of a striated root (rhizoplast) has been shown in Platymonas (Salisbury and Floyd, 1978). They have suggested that the rhizoplast may act in the initiation of flagellar power and recovery strokes, coordination of the stroke cycle and directional control of flagellar beat.

It has been suggested that the striated fiber interconnecting the basal bodies in cells such as Chlamydomonas and Polytomella could play an important role in the control of flagellar beat (Ringo, 1967; Hyams and Borisy, 1975; Brown et al., 1976), perhaps functioning like a "miniature" rhizoplast (Salisbury and Floyd, 1978). The positioning, cross-banding and filamentous structure of the distal striated fiber indicates a contractile function may be possible. The distal striated fiber interconnecting the A pair of basal bodies in Polytomella may be involved in a structural role maintaining the angle of insertion of the basal bodies with respect to one another. Ringo (1967) has suggested a similar role for the distal striated fiber in Chlamydomonas. Perhaps as the flagellar beat back alongside

the cell, the pair of basal bodies begin to be pulled apart and this is counteracted by a contraction of the distal striated fiber. Alternatively, the contraction of the distal striated fiber could initiate and thus coordinate flagellar beat. Hyams and Borisy (1975), who demonstrated that flagellar coordination in the isolated flagellar apparatus is identical to that seen in living cells, have suggested coordination of flagella is coupled to structural elements of the basal apparatus, and that the distal striated fiber interconnecting the basal bodies is responsible for this coupling. There is no direct evidence for contraction of the distal striated fiber in Polytomella, however the heavy deposition of ATP-dependent reaction that I have found is consistent with a contractile function.

The two pairs of flagella in Polytomella beat with different frequencies, but the flagella of each pair are coordinated. The proximal striated fibers of the A pair, and the proximal non-striated fiber of the B pair of basal bodies may also be involved in initiating the coordinated beat of the flagellar pairs. An initiating signal to commence flagellar beat would then have to be transmitted to the flagellar axoneme.

The basal body microtubules are the only obvious link between the interconnecting fibers and flagella. The axoneme microtubules are continuous with those of the basal bodies and the interconnecting fibers terminate on the basal body. The basal body microtubules may transit signals

or impulses from the interconnecting fibers to the axonemes. A similar role has been suggested for the microtubule sheets or bundles of ciliates (Pitelka, 1965; Allen, 1967).

The basal body triplet microtubules have ATP-dependent reaction product localized primarily on the outer surfaces. Similar observations have been made on the basal bodies of other cell types (Anderson, 1977; Dentler, 1977). The presence of ATPase activity on the basal body microtubules has led to speculation that sliding occurs (Dentler, 1977). In Polytomella, ATP-dependent reaction product is localized on the outer surface of the doublets in the transition region of the basal body and on the outer surface of the triplets in the remainder of the basal body. These ATPase localizations are at the attachment sites of the linkages which interconnect the sets of basal body microtubules. There is a heavy deposition of ATP-dependent reaction product in the central core region of the basal body and on the connections between the basal body microtubules and the central core (compare Figs. 8 E and 16 E). These linkages and interconnections would probably be involved in any basal body microtubule sliding.

In Opalina (Tamm and Horridge, 1970) and Paramecium (Omato and Kung, 1979) the central pair of microtubules have been shown to twist to remain perpendicular to the plane of flagellar beat. Tamm and Horridge (1970) suggest the angle of the central tubules could control flagellar direction or be the consequence of the flagellar beat. The

high level of ATPase activity found in the basal body central core region of Polytomella, may be involved in twisting of the central pair resulting in directional control of flagellar beat.

A structure that probably does not play a direct role in the control of flagellar beat is the flagellar membrane. The inclusion of detergents which demembrate flagella in the reactivation solution of isolated, swimming, flagellar apparatus does not affect either the form of flagellar beat or the timing of the beat cycle (Hyams and Borisy, 1978).

The cytochemical localization of basal apparatus ATP-dependent reaction product is almost identical in the presence of Mg^{++} or Ca^{++} . It is not clear whether this represents the presence of two (or more) ATPases or a single ATPase with broad divalent cation activation. Evidence for more than one enzyme activity comes from the cytochemical localization of ATP-dependent reaction product in the presence of EDTA. ATPase activity is only associated with a few structures, although these sites also show ATPase activity in the presence of Mg^{++} and Ca^{++} , suggesting that some enzymes may be preferentially activated by EDTA. Some additional evidence for the presence of more than one ATPase comes from the Tris-EDTA extraction studies. The Mg^{++} -ATPase specific activity of the extracted basal apparatus decreased while the Ca^{++} -ATPase activity had not been significantly altered. This may indicate selective extraction of a specific ATPase activity, but it could also be due to

alteration of the binding site of only the Mg^{++} -ATPase enzymes. The extracted proteins demonstrated hydrolysis of any phosphate-containing substrate added. This could have been the result of exposing enzymes normally surrounded by other proteins and therefore inaccessible by substrate, or the activation of other enzymes such as phosphatases. Additional work will be required to establish how many different phosphate-bond hydrolyzing enzymes are present in Polytomella.

The localization of GTP-dependent reaction product is almost identical to that when ATP is substrate. Sharp et al., (1979) have found that Tetrahymena shows lead phosphate precipitate in the presence of ATP, UTP, GTP or CTP. It is possible the reaction product found with substrates other than ATP is the result of transphosphorylases (Brokaw, 1961).

This study is the first clear demonstration that ATPase activities are associated with a number of structures in the basal apparatus that have been suggested to play a role in the control of flagellar beat. The preliminary biochemical characterization strongly suggests the presence of ATPases specific to the basal apparatus which can be distinguished on the basis of several criteria from those in the flagellar axoneme.

Further studies are needed to determine how many nucleoside triphosphate hydrolyzing enzymes are present in the basal apparatus of Polytomella, and whether these enzymes are localized on specific structures. This might be accomplished by the straight-forward approach of selective

solubilization of components of the basal apparatus. This approach would also facilitate studies aimed at establishing the presence or absence of known contractile proteins such as actin and myosin in basal apparatus structures. To directly demonstrate that specific structures are involved in control of flagellar beat will require developing an in vitro functional assay such as that described by Hyams and Borisy (1975). This would then permit analysis of the function of specific structures in the basal apparatus by the methods of selective removal and reconstitution, used in identifying the functional proteins of the flagellar axoneme.

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