THE BASAL BODIES OF *CHLAMYDOMONAS REINHARDTII*

Formation from Probasal Bodies, Isolation, and Partial Characterization

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ABSTRACT

The assembly and composition of basal bodies was investigated in the single-celled, biflagellate green alga, *Chlamydomonas reinhardtii*, using the cell wall-less strain, *cw*15. In the presence of EDTA, both flagellar axonemes remained attached to their basal bodies while the entire basal body-axoneme complex was separated from the cell body, without cell lysis, by treatment with polyethylene glycol-400. The axonemes were then removed from the basal bodies in the absence of EDTA, leaving intact basal body pairs, free from particulate contamination from other regions of the cell. The isolated organelles produced several bands on sodium dodecyl sulfate-urea polyacrylamide gels, including two tubulin bands which co-electrophoresed with flagellar tubulin.

The formation of probasal bodies was observed by electron microscopy of whole mount preparations. Synchronous cells were lysed, centrifuged onto carbon-coated grids, and either negatively stained or shadowed with platinum. The two probasal bodies of each cell appeared shortly after mitosis as thin "annuli," not visible in thin sections, each consisting of nine rudimentary triplet microtubules. Each annulus remained attached to one of the mature basal bodies by several filaments about 60 Å in diameter, and persisted throughout interphase until just before the next cell division. It then elongated into a mature organelle. The results revive the possibility of the nucleated assembly of basal bodies.

Basal bodies are the cell structures composed of nine triplet microtubules which nucleate the formation of ciliary and flagellar microtubules. The most puzzling feature of the basal body is that it is usually assembled adjacent and perpendicular to a pre-existing basal body. This has led to suggestions that the mature organelle in some way influences the formation of its successor. Yet in many plants and in some exceptional animal species, basal bodies appear to assemble *de novo*, in the absence of any pre-existing basal bodies (see Pickett-Heaps, 1971; Fulton, 1971; and Wolfe, 1972 for reviews). A resolution of this dilemma should bear on the general problem of how microtubules are initiated and oriented within the cell.

Our knowledge of basal body assembly has been limited by two factors: the difficulty of observing the smallest basal body precursors ("probasal bodies") in thin sections, and the difficulty of isolating basal bodies for biochemical analysis and for comparison with other microtubule systems.

This report first describes the isolation of basal

body pairs from the single-celled, biflagellate green alga, *Chlamydomonas reinhardtii*, and presents some preliminary findings on their composition. The basal body pairs, with both flagella attached, were separated from their cell bodies, thus minimizing contamination from other cell structures, and were then separated from their flagella and further purified. It was found that *Chlamydomonas* basal bodies are composed predominantly of microtubule protein that co-electrophoreses with flagellar tubulin on sodium dodecyl sulfate (SDS)-urea polyacrylamide gels.

This report also describes the structure and maturation of probasal bodies from *Chlamydomonas*, as seen in negatively stained whole mount preparations of the entire basal body system, obtained from an easily lysed mutant lacking a cell wall. The two probasal bodies of each cell appeared shortly after cell division as thin rings, or "annuli," which remained attached to the two mature basal bodies by several filaments. Each annulus remained dormant throughout interphase and then, just before the ensuing cell division, rapidly elongated into a mature organelle. The results of this work clarify several structural and temporal aspects of basal body formation.

MATERIALS AND METHODS

Cultures

The cell wall-less strain *cw*15 (Davies and Plaskitt, 1971) of *C. reinhardtii* was used in all experiments. Cells destined for basal body isolation were grown on Tris-acetate-phosphate medium (Gorman and Levine, 1965) supplemented with nicotinamide (1 mg/ml) and thiamine (1 mg/ml), in 4-liter flasks under continuous light at 25°C. Cultures were harvested in the late log phase of growth (6×10^6 cells/ml).

Cells destined for electron microscopy were grown as above, but in 500-ml flasks on rotatory shakers. Synchronous cultures were grown in 4-liter flasks on high salt liquid medium (Sueoka, 1960), aerated with 5% CO_2, using a 14-10-h light-dark cycle.

Basal Body Isolation

HARVESTING: 6 liters of cells were gently pelleted at 500 *g* for 5 min at room temperature, washed once with unbuffered, 5% aqueous sucrose, pelleted again, and gently resuspended in an equal volume (8 ml) of 5% aqueous sucrose.

PARTIAL REMOVAL OF THE CELL MEMBRANE: The suspension was slowly pipetted into 100 ml of polyethylene glycol-400 (Fisher Scientific Co., Pittsburgh, Pa.) and slowly stirred for 5 s, crenating the cells. It is important that all the above steps be performed with a minimum of turbulence, since the flagella of this mutant are easily sheared from the cell bodies and may be inadvertently lost. The resulting suspension was in turn slowly diluted with 250 ml of a cold (0°C) solution containing 0.5 M sucrose, 0.01 M sodium-EDTA, pH 7.5, and 0.015 M Tris-HCl, pH 7.8. Upon rehydration, much of the cell membrane is solubilized but the chloroplast and nucleus remain intact. All succeeding steps were carried out at 0°-4°C.

CONCENTRATION OF THE CELLS: The suspension was divided between eight 50-ml polycarbonate tubes and was underlaid with 1-ml cushions of 1.5 M sucrose, 5% (vol/vol) polyethylene glycol-400 (PEG), 0.01 M EDTA, pH 7.5, and 0.015 M Tris-HCl, pH 7.8. After centrifugation at 600 *g* (Sorvall swinging bucket rotor, HB-4 [DuPont Instruments, Sorvall Operations, Newtown, Conn.], 2,000 rpm) for 10 min, the cushions were pooled and slowly diluted with 10% PEG, 0.01 M EDTA, pH 7.5., 0.015 M Tris-HCl, pH 7.8 (PET buffer) to a final volume of 50 ml.

SEVERING THE BASAL BODY-AXONEME COMPLEX: The resulting suspension was magnetically stirred in a 100-ml beaker with increasing vigor for 5 min or until all the basal body-axoneme complexes were detached from the cell bodies.

REMOVAL OF THE CELL BODIES: 25-ml portions of the suspension were then layered over 25-ml aliquots of 25% sucrose in PET buffer and were centrifuged in a Sorvall HB-4 rotor at 2,500 rpm for 1 h, pelleting most of the cell bodies. The basal body-axoneme complexes remained in the supernate.

FURTHER PURIFICATION: The supernate was centrifuged at 23,000 *g* (Beckman Model L, SW25.1 rotor [Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.], 15,000 rpm) for 15 min into 0.1-ml cushions of 1.75 M sucrose in PET buffer. The cushions were diluted with PET buffer, layered over 15 ml of 10% sucrose in PET buffer, and centrifuged at 1,000 *g* for 25 min in a Sorvall HB-4 rotor, pelleting any remaining cell bodies.

REMOVAL OF MEMBRANE CONTAMINATION: The supernate was then made 0.5% in Triton X-100, layered over a discontinuous gradient consisting of 1.75 M, 2.0 M, 2.25 M, and 2.5 M sucrose in PET buffer, and centrifuged for 4 h at 63,000 *g* (Beckman SW25.1 rotor, 25,000 rpm). The 2.0/2.25 M interface, containing the basal body-axoneme complexes, was withdrawn and diluted with 15 mM Tris-HCl, pH 7.8, to a volume of 7 ml.

DETACHMENT OF THE AXONEMES: The basal body-axoneme complexes, resuspended in the absence of EDTA, were layered over 24 ml of 1.65 M sucrose in 15 mM Tris-HCl, pH 7.8, 0.1% Triton X-100, and centrifuged for at least 4 h at 63,000 *g* (Beckman SW25.1 rotor, 25,000 rpm). The supernate was discarded and the pellet, containing axonemes and free basal body pairs, was resuspended in 20 ml of 0.1 mM EDTA, 1 mM

Tris-HCl, pH 7.8, and 0.75% Kodak Photo-flo-200 (Eastman Kodak Co., Rochester, N. Y.).

REMOVAL OF THE AXONEMES: The suspension was then passed three times through a Yeda pressure cell (Yeda Instruments, Rehovot, Israel) under 300 pounds per square inch of nitrogen, dissociating the axonemes but leaving the basal body pairs undamaged. 5-ml aliquots of the resulting mixture were layered over 40 ml of 10% hexylene glycol (Eastman Kodak Co.), 15 mM Tris-HCl, pH 7.8, 0.1 mM EDTA, and centrifuged at 7,500 rpm for 45 min in a Sorvall HB-4 rotor. The supernate, containing fragmented axonemes, was discarded. The very scant pellet contained pure, intact basal body pairs.

Electrophoresis

The proteins of basal body pairs were analyzed by electrophoresis on SDS-urea polyacrylamide gels using a modification of the system described by Laemmli (1970). The proteins were solubilized in 2% SDS, 2% 2-mercaptoethanol, and 0.0625 M Tris-HCl, pH 6.8, at 100°C. for 2 min. After cooling, the solution was made 8 M in urea. The 7.5% polyacrylamide gels were 6 mm × 80 mm, and contained 0.1% SDS in 8 M urea. All gels were stained with Coomassie Brilliant Blue (I. C. I. America Inc., Stamford, Conn.) and destained in 7.5% acetic acid, 40% methanol.

Electron Microscopy

WHOLE MOUNTS: Cells were prepared for electron microscopy by a modification of the elegant method of Miller et al. (1970), originally used for studies of DNA transcription. 10 ml of cultures were pelleted, washed once with 5% aqueous sucrose, pelleted again, and resuspended in a minimum volume of distilled water. A 10-μl aliquot of cells was pipetted into 1 ml of cold (0°C) lysis medium containing 0.1 M sucrose and 0.4% Kodak Photo-flo, pH 7.8. A drop of the lysate was sedimented at 3,200 *g* for 5 min through a cushion of 5% sucrose and 10% formalin, pH 7.8, directly onto 400-mesh carbon-coated grids. The pH of all buffers was adjusted with pH 10, Mallinckrodt "buffAR" standard pH-meter buffer. Some grids were negatively stained with 2% aqueous uranyl acetate or 2% phosphotungstic acid. Other grids were rinsed in 0.4% Kodak Photo-flo, pH 7.0, dried, and either observed directly by dark-field microscopy or shadowed with platinum.

Whole mounts of isolated basal bodies were prepared by direct centrifugation onto carbon-coated grids as above. The grids were rinsed in 0.4% Kodak Photo-flo, dried, and stained with 2% uranyl acetate.

THIN SECTIONS: Isolated basal bodies were also pelleted, fixed, embedded in Epon and thin sectioned for microscopy as described by Witman et al. (1972). Grids were stained with 2% uranyl acetate and 2% lead citrate, and were observed in a Philips 300 electron microscope.

RESULTS

Basal Body Isolation

When the cell wall-less strain *cw15* of *Chlamydomonas* was disrupted in the presence of at least 1 mM EDTA its flagellar axonemes remained firmly attached to their basal bodies forming a V-shaped structure that was easily visible in the phase microscope. The axonemes were then used as a convenient handle for further purification of the basal body pairs. Outright lysis of the cell with detergents generated too many contaminating fragments for an effective isolation procedure, so a method was devised for removing the basal body-axoneme "complex" while leaving the cell body virtually intact.

The complex was severed from the rest of the cell by first weakening the cell membrane with polyethylene glycol-400 (Fig. 1), and then stirring vigorously until all the complexes were removed (Fig. 2). Once separated from the cell body, the complex remained intact even when resuspended in the absence of EDTA.

The complexes were separated by differential centrifugation from the cell bodies and from any cell fragments that may have been generated. The remaining flagellar membranes and other residual contaminants were removed by brief treatment with the nonionic detergents Triton X-100 or Kodak Photo-flo. Both of these reagents apparently had no adverse effect on the structure of the basal body at the concentrations used. Polyethylene glycol-400 and EDTA appeared to slightly stabilize the basal body, as determined by electron microscopy, and were therefore retained in the isolation buffers.

A typical basal body-axoneme complex is shown in Fig. 3. The two basal bodies have maintained their normal perpendicular orientation, and each one bears an axoneme. Emanating from the basal body pair are the remains of four bands of cortical microtubules which in vivo extend under the cell membrane, anchoring the basal bodies (Ringo, 1967). Each basal body is also attached at its proximal end to the probasal body (discussed below) destined to become its new partner after cell division.

The axonemes, cortical microtubules, and probasal bodies were easily detached from the basal body pair by sedimenting the entire complex through a viscous sucrose solution at high speed in the absence of EDTA. The torques generated by this procedure were evidently sufficient to detach

the axonemes just distal to the transition region (Ringo, 1967).

Despite the disparity in their sizes, the free axonemes and basal body pairs had similar sedimentation coefficients and were not readily fractionated by differential centrifugation. Therefore the mixture was rapidly sheared at low pressure in a Yeda pressure cell, causing the axonemes to splay apart into their component outer doublet microtubules. The very stable basal body pairs were not disrupted by this procedure and were easily pelleted. Up to 200 μg of basal body pairs were obtained from 6 liters of cells.

Purity of the Preparation

The isolated basal body pairs were intact and completely free of particulate contamination from other regions of the cell (Fig. 4 *a* and *b*). However, each basal body was firmly associated with several accessory structures. These included the striated fiber that connects the two basal bodies at their midpoints (Ringo, 1967), and some fibrous material (Fig. 4 *a*) that may be a remnant of the specialized cell wall "collar" into which the basal bodies are inserted (Roberts et al., 1972). A portion of this collar is apparently present even in the cell wall-less strain *cw*15 (R. R. Gould and C. L. F. Woodcock, unpublished observations). Although these structures complicated the task of biochemical analysis, they could not be removed without damage to the basal body, and they were considered an integral part of the basal body pair itself.

The possibility of even low levels of adsorbed contamination posed a serious problem, since many interesting components of the basal body, such as the molecules that comprise the cartwheel or the "stellate structures" of the transition region (Ringo, 1967), are present in very few copies per organelle. The most prevalent adsorbed contaminant was probably DNA, released from the few nuclei that were observed to lyse during the isolation procedure.

The basal bodies occasionally suffered slight damage during isolation, such as loss of some of the outermost C tubules (Fig. 4 *c*). In addition, the attachment fibers that bind both basal bodies at their proximal ends were sometimes broken, apparently allowing them to pivot from their normal orientation. The final stages of sample preparation are currently being modified to reduce these problems.

Gel Electrophoresis

The proteins of the basal body pair were consistently separated into several components by electrophoresis on SDS-urea polyacrylamide gels. The two most prominent bands were a closely spaced doublet, corresponding to molecular weights of approximately 52,000 and 55,000, which contained more than 80% of the total protein on the gel, as determined from gel scans. These two bands represented the proteins of the nine triplet microtubules of the basal body. They had the same electrophoretic mobility as flagellar tubulin (Fig. 5), and are typical of all known tubulins studied to date (Olmsted and Borisy, 1973).

The minor bands visible on the SDS-urea gels probably corresponded to the nonmicrotubular structures of the basal body pair, such as the intertriplet linkers, the transition region structures, and the distal striated connecting fiber (Ringo, 1967). Rigorous assignment of these bands must await further fractionation of the basal body pairs.

Basal Body Assembly

STRUCTURE OF THE PROBASAL BODY: - Whole mount preparations of *Chlamydomonas* basal bodies were rapidly obtained for electron microscopy by centrifuging cell lysates directly onto carbon-coated grids. Fig. 6 *a* shows an unstained preparation, made about 1 h after cell division, that was typical of the several hundred basal body pairs that were observed. The basal bodies have been distorted from their normal perpendicular orientation while settling on the grid, and the flagellar axonemes have fallen off, since the cells were lysed in the absence of EDTA. The probasal bodies are symmetrically disposed on either side of the basal body pair and are not necessarily seen in their native orientation. Each probasal body is a ring, or "annulus," whose nine components are shown below to be rudimentary triplet microtubules. Each annulus is attached by two filaments (arrows) which run from two of the triplets to two different sites on the proximal end of the partner basal body.

Fig. 7 shows a basal body pair prepared in identical fashion, except that it was negatively stained with 2% uranyl acetate, which affords optimum preservation of the fine structure. Four triplets of the probasal body annulus are clearly visible. The remaining five triplets, as well as the two attachment filaments, have been obscured by

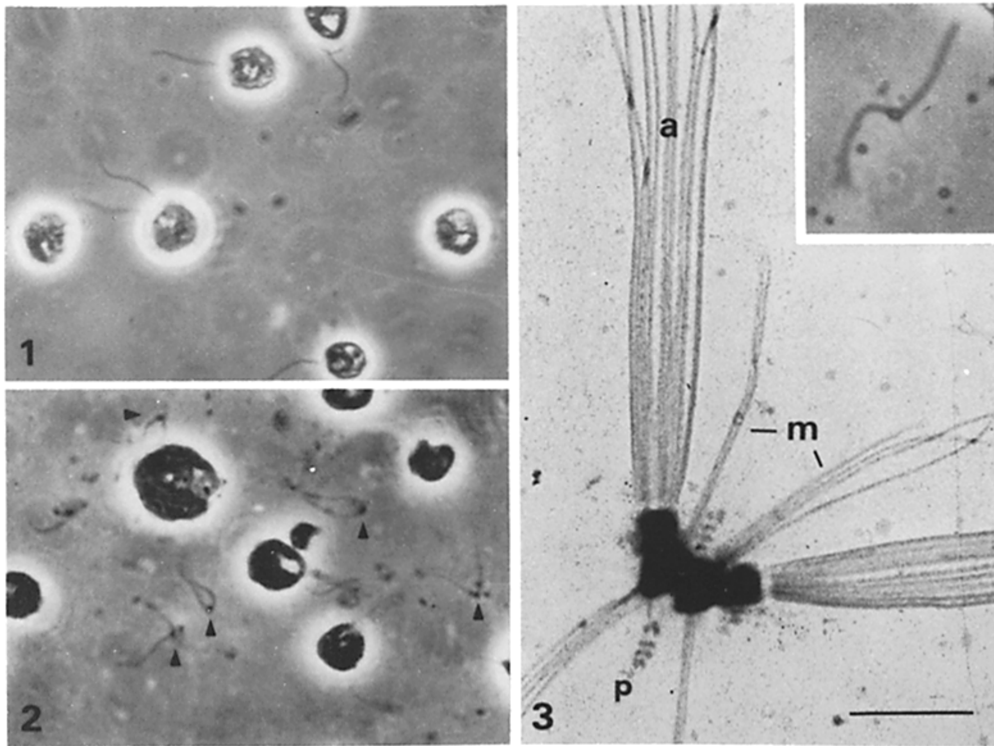

FIGURE 1 Phase micrograph of cells treated briefly with PEG-400 and then rehydrated. The cell membrane and subjacent cytoplasm have been partially solubilized, leaving the cup-shaped chloroplast and nucleus intact. The flagella are still attached. × 800.

FIGURE 2 After stirring, the basal body pairs (arrowheads), with both flagella still attached, are severed from the cell bodies. Phase micrograph. × 800.

FIGURE 3 Electron micrograph of a basal body-axoneme complex after brief treatment with detergent. Each axoneme (*a*) has begun to splay into nine outer doublet microtubules. Two probasal body "annuli" (*p*) and four bands of cortical microtubules (*m*) remain attached to the pair of basal bodies. Lightly shadowed with platinum. The bar is 1 μm. × 16,000. Inset, phase micrograph of a basal body-axoneme complex that has been separated from its cell body. × 2,000.

stain, although in some preparations all nine triplets can be discerned.

GROWTH OF THE PROBASAL BODY: The progress of the probasal body was followed throughout the cell cycle using synchronously grown cells. The annulus formed within an hour after cell division and persisted throughout the cell cycle, without apparent elongation, until shortly before the next cell division. It then elongated to form a mature basal body.

Several stages of this process are shown in Fig. 6. The two elongating probasal bodies in Fig. 6 *d* have fallen lengthwise onto the grid and their triplet microtubules have been pulled apart during drying. In Fig. 6 *e* the new basal bodies have fully matured, and in Fig. 6 *f*, at the onset of cell division, even the cortical microtubules have been duplicated, although not all of them have survived the sample preparation.

The basal bodies in Fig. 6 *a–c* include their transition regions (Ringo, 1967), while those in Fig. 6 *d–f* do not. This apparently reflects the fact that just before mitosis in *Chlamydomonas*, the flagellar axonemes become detached from their basal bodies in vivo, proximal to the transition region (see Fig. 5 of Johnson and Porter, 1968). Thus there are two zones of discontinuity between the basal body and its axoneme, one on either side of the transition region.

Due to the difficulty of obtaining precise syn-

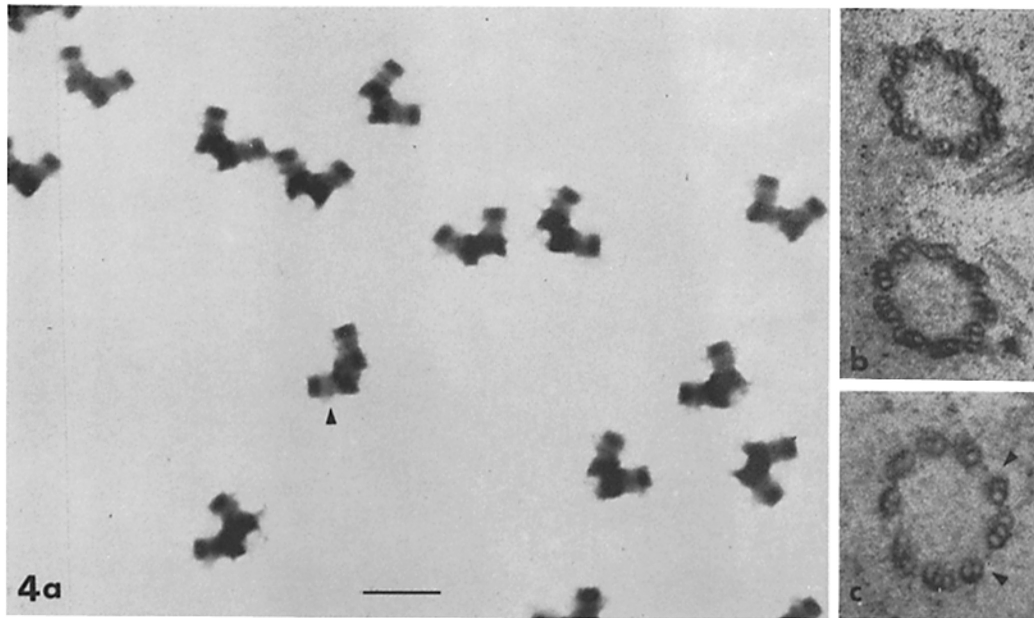

FIGURE 4 Electron micrographs of isolated basal body pairs. (*a*) Whole mount preparation stained with 2% uranyl acetate. Fibrous material (arrowhead) may be part of the cell wall "collar" into which the basal body is inserted in vivo. The bar is 1 μm. × 9,800. (*b*) Thin section of a basal body pair showing the nine intact triplets of each organelle. × 60,000. (*c*) Thin section of a damaged basal body. Several C tubules are missing (arrowheads) but the links between triplets remain. × 86,000.

chrony with the cell wall-less strain, the exact timing of probasal body elongation was not determined. In studies using rapidly growing, nonsynchronous cultures, the vast majority of probasal bodies were observed to be in the initial "annulus" stage of development, while only very few probasal bodies were seen in some stage of elongation. This suggests that, once initiated, elongation is completed in a relatively short time.

ATTACHMENT OF THE PROBASAL BODY: On probasal bodies that had begun to elongate, several more attachment fibers were occasionally observed by negative staining. Fig. 8 shows a preparation made shortly before cell division, similar to that of Fig. 6 *d*. The probasal body has fallen well beyond the distal end of the basal body, and its nine immature triplet microtubules have been pulled apart during drying. At least four of the triplets are each still attached by one or two filaments, about 60 Å in diameter (arrowheads), which run towards the proximal end of the basal body. The attachment fibers apparently persist throughout cell division, when the new basal body pairs are distributed semi-conservatively to the daughter cells. They thus help bind each new basal body pair at its proximal end until the new distal and proximal striated connecting fibers (Ringo, 1967) can be added.

DISCUSSION

Basal Body Isolation

Since it has long been known that cilia may be detached from their basal bodies by treatment with calcium ions (Watson et al., 1961), it is not surprising that the chelating agent EDTA is required to remove the basal body-axoneme complex intact from *Chlamydomonas*. Recently Anderson (1974) has reported a similar procedure using EDTA for isolating ciliated basal bodies from the rabbit oviduct.

Once the basal body-axoneme complex is free of the cell body, however, it remains intact even in the presence of calcium ions. This suggests that strong torques about the points of attachment are also necessary to sever the axoneme from its basal body. These torques are normally present when the basal body is anchored to the cell by the four bands of cortical microtubules, but must be greatly reduced as soon as the basal body is detached.

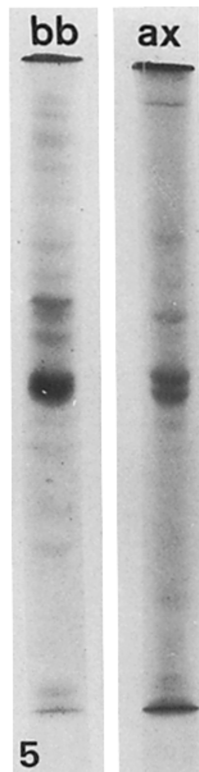

FIGURE 5 SDS-urea gels of isolated basal body pairs (left, *bb*) compared with fragmented flagellar axonemes (right, *ax*). The two prominent bands on each gel are microtubule protein. The basal body gel has been slightly overloaded to bring out the minor bands, obscuring the doublet nature of basal body tubulin.

There are two potential drawbacks to the basal body isolation procedure described here. First, the flagella of the cell wall-less strain are quite fragile and are apt to be severed prematurely from the cell, without their basal bodies, when the cells are initially harvested and resuspended at high cell density. Therefore, care must be taken to avoid excessive turbulence until after the treatment with polyethylene glycol. Second, the method is sensitive to how the cells are cultured: cells that have remained either in the stationary phase of growth or above 25°C yield contaminating fragments when the complexes are severed from the cell bodies, making the subsequent isolation more difficult.

A major advantage of the method is that it avoids the use of Ca^{++}, ethanol, low pH, or extended treatment with detergent, all of which might interfere with subsequent biochemical analysis. Furthermore, since complete cell lysis is avoided, preparations of great purity can be obtained.

Gel Electrophoresis

Basal body microtubules differ from their flagellar counterparts in several respects. They are short, triplet microtubules of precisely determined length, and they are modified to accommodate both a cartwheel structure at one end and a complex stellate array at the other end. In *Chlamydomonas*, basal body and flagellar microtubules also differ in their state of aggregation at various times during the cell cycle. During mitosis, for example, the flagellar axonemes are normally detached from their basal bodies and then depolymerized (Johnson and Porter, 1968). During interphase, the axoneme of one flagellum may be made to shorten by removing the other flagellum (Rosenbaum et al., 1969). The basal bodies, on the other hand, do not depolymerize under these conditions, and have not yet been induced to shorten.

These observations suggest that basal body and flagellar tubulin may differ slightly either in their primary structure or in some post-translational modification. This report has shown that the triplet microtubules of the basal body are composed of proteins that are indistinguishable from flagellar tubulin by SDS-urea gel electrophoresis. It remains to determine whether or not the corresponding tubulins from the two organelles are actually identical and also whether the C tubule of the basal body contains a distinct species of tubulin.

Basal Body Assembly

Basal body formation in *C. reinhardtii* has been shown to proceed in at least two discrete stages: first, the assembly of the thin probasal body annulus very early in the cell cycle, and second, the sudden elongation of the annulus into a mature basal body just before cell division. The annulus is thus a stable and complex structure, an organelle in itself, whose apparent function is to nucleate the formation of the basal body much as the basal body in turn nucleates the formation of the flagellar axoneme.

Probasal bodies have been seen only rarely in thin section studies of *Chlamydomonas* (Johnson and Porter, 1968), generally at the end of interphase (Ringo, 1967), despite the fact that both

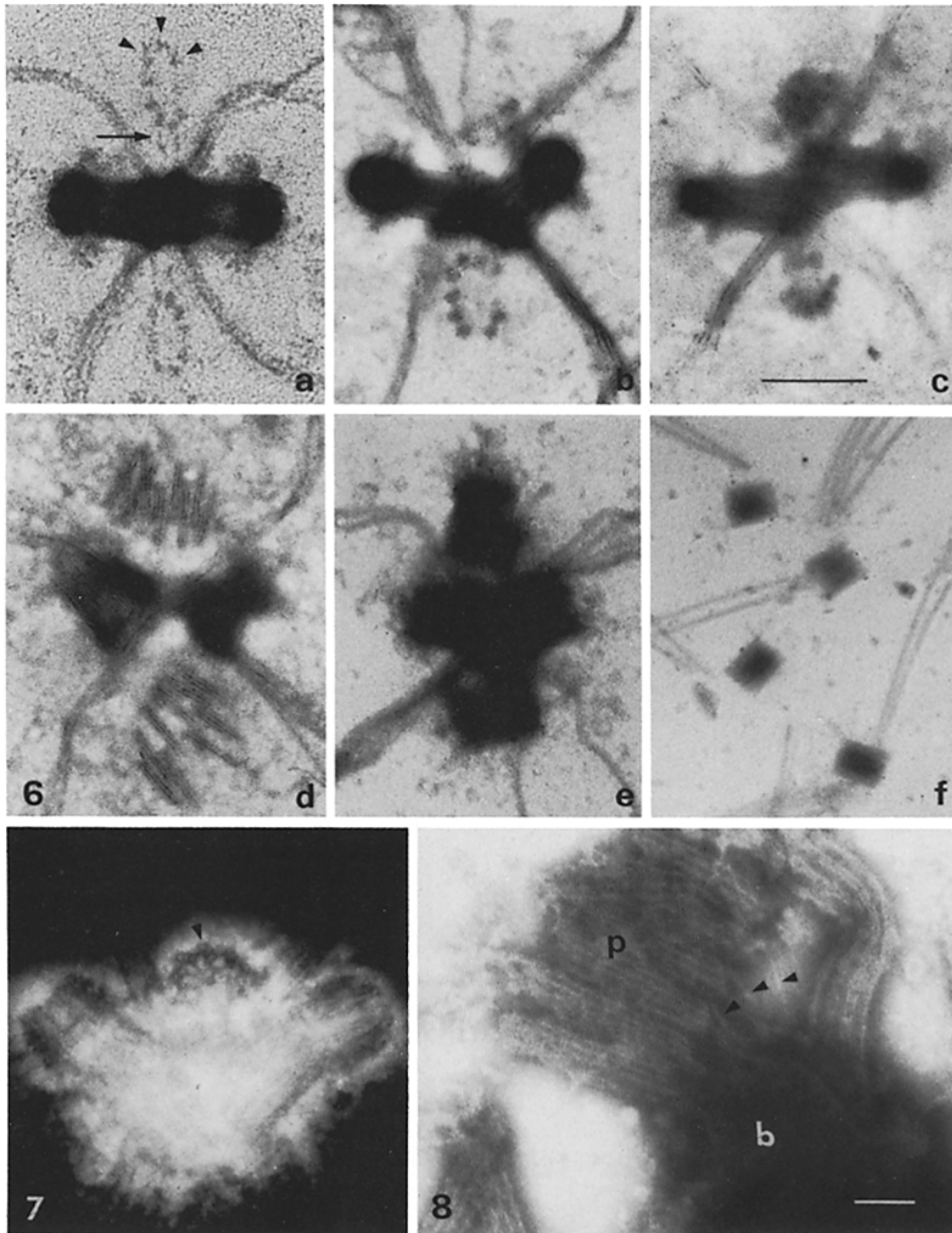

FIGURE 6 Several stages of probasal body maturation. (*a*) A basal body pair, shortly after cell division. Each probasal body is an annulus of nine components (arrowheads), two of which are connected to the proximal end of the partner basal body by fibers (long arrow). Whole mount, unstained. (*b-d*) Shortly before cell division, the probasal bodies elongate. By the onset of mitosis, elongation is completed (*e*) and the new cortical microtubules are assembled (*f*). Negatively stained with 2% phosphotungstic acid. The bar is 0.5 μm. *a-e*, × 30,000. *f*, × 19,000.

FIGURE 7 Reversal print of an interphase basal body pair, negatively stained with 2% uranyl acetate. Part of a probasal body annulus is visible between the basal bodies, showing four triplets (arrowhead), a hub, and several spokes of the cartwheel. The second annulus has not been preserved. × 51,000.

FIGURE 8 An elongating probasal body (*p*) that has fallen past the distal end of its partner basal body (*b*), stretching its attachment fibers. Several of the triplets bear fibers (arrowheads) which run towards the proximal end of the basal body, out of the field of view. The bar is 0.1 μm. × 78,900.

annuli are present throughout most of interphase. This suggests that the annuli escape detection in thin sections, perhaps because they may not survive the embedding process, or because they are very thin and may not be constrained to lie in a plane. Only after they have begun to elongate do the probasal bodies seem to be detectable in thin sections.

When negatively stained with 2% uranyl acetate, a probasal body annulus was always observed as a ring of triplet microtubules, even very early in the cell cycle. One never observed the rings of nine singlet microtubules, or of mixed singlets, doublets, and triplets, that have been reported in thin section studies of newly forming basal bodies in *Chlamydomonas* (Johnson and Porter, 1968) and *Paramecium* (Dippell, 1967). This suggests that the probasal body annulus is indeed not visible in thin sections, and also suggests that the rings of singlet and doublet microtubules observed in thin sections correspond to sections of *elongating* probasal bodies in which the A tubules elongate fastest and the C tubules slowest. Sections through the growing end of the structure would then show nine singlets, while slightly oblique and proximal sections would show a combination of singlets, doublets, and triplets. Preliminary observations on whole mounts of elongating probasal bodies suggest that the A tubules actually do elongate fastest.

This report has also shown that the probasal body is attached to the proximal end of its "parent" basal body by at least two fibers. This explains why the new basal body matures adjacent to its partner: simply because the nucleating center for the new basal body is tethered to its partner.

Although the probasal body attachment fibers have not been previously reported in other organisms, the region between the two basal bodies or centrioles of a pair has often been described in thin section studies as "fibrous" or "filamentous" (Fulton, 1971). With hindsight, some possible candidates for these attachment fibers may be discerned in previously published micrographs (e.g., Fig. 2 of Kalnins and Porter, 1969).

IMPLICATIONS FOR A THEORY OF BASAL BODY FORMATION: The central question that remains is whether the probasal body annulus assembles independently ("*de novo*" formation, "spontaneous self-assembly") and is then attached to its partner, or whether the pre-existing basal body plays a role in the actual assembly of the annulus ("nucleated assembly"). The findings of this investigation are equally compatible with either alternative.

A nucleated assembly would allow the cell to precisely control the number and location of new basal bodies. Since the only known *essential* function of the basal body is to serve as a nucleating site for axoneme formation, it is quite plausible that a basal body (or probasal body) might be required to nucleate the assembly of new basal bodies. Furthermore, it has been recently postulated that microtubule assembly in vitro may be nucleated by disk-shaped structures (Borisy and Olmsted, 1972).

On the other hand, there is suggestive evidence that centrioles, which are structurally identical to basal bodies, can form *de novo*, particularly in the case of artificially activated sea urchin eggs (Dirksen, 1961; Kato and Sugiyama, 1971). It has also been clearly shown that basal bodies can form in the absence of other mature basal bodies (Mizukami and Gall, 1966; Fulton and Dingle, 1971; Grimes, 1973). However, none of these studies unequivocally ruled out the presence of very thin pre-existing probasal bodies or other nucleating structures. Indeed, the results of the present work emphasize that it is insufficient to rely on thin sections to rule out the presence of probasal bodies in a given cell.

New approaches will be needed to explore the key stages of basal body assembly.

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Note added in proof: Chlamydomonas basal bodies have recently been isolated by a different procedure (Snell, W. J., W. L. Dentler, L. T. Haimo, L. I. Binder, and J. L. Rosenbaum. 1974. *Science* (*Wash. D. C.*). **185:** 357). A thin-section study of the formation of *Chlamydomonas* basal bodies also has appeared recently (Cavalier-Smith, T. 1974. *J. Cell Sci.* **16:**529).

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