

A Nucleus-Basal Body Connector in *Chlamydomonas reinhardtii* That May Function in Basal Body Localization or Segregation

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ABSTRACT We have isolated a nucleus-basal body complex from *Chlamydomonas reinhardtii*. The complex is strongly immunoreactive to an antibody generated against a major protein constituent of isolated *Tetraselmis striata* flagellar roots (Salisbury, J. L., A. Baron, B. Surek, and M. Melkonian, *J. Cell Biol.*, 99:962–970). Electrophoretic and immunoelectrophoretic analysis indicates that, like the *Tetraselmis* protein, the *Chlamydomonas* antigen consists of two acidic isoforms of ~20 kD. Indirect immunofluorescent staining of nucleus-basal body complexes reveals two major fibers in the connector region, one between each basal body and the nucleus. The nucleus is also strongly immunoreactive, with staining radiating around much of the nucleus from a region of greatest concentration at the connector pole. Calcium treatment causes shortening of the connector fibers and also movement of nuclear DNA towards the connector pole. Electron microscopic observation of negatively stained nucleus-basal body complexes reveals a cluster of ~6-nm filaments, suspected to represent the connector, between the basal bodies and nuclei.

A mutant with a variable number of flagella, *vfl-2-220*, is defective with respect to the nucleus-basal body association. This observation encourages us to speculate that the nucleus-basal body union is important for accurate basal body localization within the cell and/or for accurate segregation of parental and daughter basal bodies at cell division.

A physical association between nuclei and basal bodies or centrioles has been observed in a variety of algal, protozoan, and metazoan cells, although the nature of the association, in terms of both structure and function, has been obscure. We believe it likely that fibrous connectors homologous to those described here for *Chlamydomonas* are general features of centriole-bearing eucaryotic cells.

The basal body cycle of *Chlamydomonas* resembles the classical centriole cycle in most, if not all, essential respects, and so an analysis of the *Chlamydomonas* basal body cycle may provide information about centriole cycles in general. The cytological behavior of centrioles during the cell cycle is well known (43). Landmark events in the centriole cycle include duplication during interphase, migration to the nuclear poles before mitosis, presence at the mitotic spindle poles, and equal segregation to daughter cells at division. Despite a very large body of literature on centriole structure and cytology, the cellular function of centrioles remains obscure, and most

of the questions raised when centrioles were observed more than one hundred years ago remain unanswered. Why are centrioles maintained in such a wide variety of organisms? How do centriole duplication and segregation occur? Is proper subcellular localization of centrioles important to their function?

Protozoan centrioles are generally referred to as basal bodies, and we will follow this convention throughout this paper. But the reader should bear in mind that in many cell types, both metazoan and protozoan, a basal body or centriole may carry an axoneme during one interval in the cell cycle and

behave like a centriole during another. Thus, the fact that a basal body is flagellated does not exclude its behaving like a centriole.

Throughout interphase the *Chlamydomonas* cell carries two anterior flagella whose basal bodies are oriented at $\sim 90^\circ$ to one another and are connected by one distal and two proximal striated fibers (32). Early in G1 two probasal bodies develop adjacent to the two flagellated basal bodies. These mature into ultrastructurally complete basal bodies later in G1, so that four basal bodies are available for segregation at cell division (8, 15). As mitosis approaches, the two flagella resorb and the basal bodies migrate in pairs to the mitotic poles of the nucleus (9). The mitotic nucleus is bent in such a way that each spindle pole is positioned very close to a basal body pair even though the basal bodies remain near the cell surface. At metaphase, numerous spindle microtubules radiate from near each basal body, passing into the nucleus through large fenestrae in the persistent nuclear membrane (18). After karyokinesis numerous microtubules appear along an anterior/posterior plane that passes between the two nuclei and between the two basal body pairs. The cleavage furrow forms along this plane, moving from anterior to posterior as it divides the cell in two. As a consequence of these events basal bodies are segregated equally to daughter cells. Thus, the landmark events of the centriole cycle—duplication, migration, and segregation—have analogous counterparts in the *Chlamydomonas* basal body cycle.

The behavior of centrioles during cell division has led to the hypothesis that centrioles have essential roles in organizing the mitotic spindle and that they thereby govern nuclear division in a fundamental way. Recent evidence that cells which normally possess centrioles can undergo apparently normal mitotic events in their absence has made this view untenable (5, 11, 43), although it may be that the centrosomes, with or without associated centrioles, play the roles formerly assigned to centrioles (25, 26, 30). Likewise, results of several studies support the notion that basal bodies per se are not essential components of division in *Chlamydomonas*. The mutant *bald-2* (14), which has only very rudimentary basal bodies, shows no apparent cell cycle defect. *vfl-2*, which has variable flagellar (and basal body) number, also shows no cell cycle defect (21). Thus, having too many or too few basal bodies has no obvious effect on mitosis or cell division in *Chlamydomonas*. This is consistent with published observations in metazoan cell culture systems, e.g., a *Drosophila* cell line without centrioles divides normally (11), as do neuroblastoma cells with excess centrioles (31).

If centrioles are not needed for mitosis, what are we to make of the striking and nearly universal temporal and spatial coordination that exists between the mitotic and centriole cycles? One simple possibility is that the highly orchestrated phenomena observed exist to ensure accurate centriole segregation at cell division; that is, cells may use such a complex and evolutionarily conserved procedure for centriole segregation precisely because accurate segregation is very important. What remains mysterious is the centriole's function—with the notable exception of its role as the nucleation organelle for axonemes in ciliated or flagellated cells. In this context it should be remembered that centrioles may well have critical cellular functions other than roles in ciliogenesis or cell division. In fact, a number of recent speculations about centriole function have centered on possible roles in generating or maintaining cell asymmetry or polarity (2, 3, 20).

In this report we document the existence of a connector between the basal body pair and the nucleus in interphase *C. reinhardtii* cells. We show that the connector contains a 20-kD protein homologous to that isolated from the flagellar roots of *Tetraselmis*. Observations of wild-type and mutant cells support the view that the connector plays a central role in ensuring accurate basal body placement in the cell and/or accurate basal body segregation at cell division.

MATERIALS AND METHODS

Strains and Culture Conditions: Wild-type strains were the 137c derivatives NOmt⁺ and NOmt⁻ provided by Dr. U. Goodenough (Washington University, St. Louis, MO). Cell wall defective mutants, *cw-15* (10) and *cw-92* (17), were obtained from the *Chlamydomonas* Culture Collection, Duke University. *vfl-1* was isolated after mutagenesis with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (1). *vfl-2-220* (21) and *vfl-3-207* (45) were derived from NOmt⁺ after ultraviolet light mutagenesis. Strains with a cell wall were grown at 25°C in medium I of Sager and Granick (35); strains without cell walls were grown in the same medium, supplemented with 0.02 M sodium acetate, 0.4% yeast extract, and 0.5% proteose peptone. Cultures were bubbled continuously with filtered air and illuminated with white light (General Electric F28T12-CW-HO fluorescent tubes, General Electric Co., Wilmington, MA). Experiments were performed on log-phase cultures at densities of $\sim 10^6$ cells/ml.

Preparation of Nucleo-flagellar Complexes: Log-phase liquid cultures were harvested by centrifugation (250 g for 5 min), resuspended in MT buffer (30 mM Tris-acetate, 5 mM MgSO₄, 5 mM EGTA, 25 mM KCl, 1 mM dithiothreitol, pH 7.3), recentrifuged as above through a layer of 30% Percoll (Pharmacia Fine Chemicals, Piscataway, NJ) in MT buffer, resuspended in MT buffer, and placed on ice. An equal volume of lysis solution (MT buffer with 2% Nonidet P-40 [NP-40],¹ 0.01% aprotinin, 0.005% phenylmethylsulfonyl fluoride) was added, and the suspension was mixed vigorously. Cell lysis was observed to be rapid and complete. (When cells had been deflagellated before addition of lysis solution, they were much less susceptible to detergent lysis and required stirring on ice for ~ 15 min before sufficient lysis had occurred to continue with the protocol.) An equal volume of 50% Percoll in MT buffer was added to the lysate and the suspension was centrifuged in 40-ml aliquots at 10,000 rpm in a Sorvall SA-600 rotor (E. I. duPont de Nemours & Co., Inc., Sorvall Instruments Div., Newtown, CT) for 30 min. The nucleo-flagellar apparatuses banded at the top of the gradient, just below the interface between the aqueous and Percoll phases. The interface was removed with a syringe and diluted 50 times in MT buffer. The apparatuses were pelleted at 10,000 rpm for 15 min, resuspended in MT buffer, and repelleted as above. The purified apparatuses were suspended in sample buffer and boiled for 1 min (for SDS gel electrophoresis) or were lyophilized for subsequent two-dimensional electrophoresis. For all biochemical analysis, cells were deflagellated before isolation of nucleo-flagellar apparatuses. The proteins present, therefore, represent those of the basal bodies, striated fibers, connectors, and nucleus, as well as residual portions of rootlet microtubules.

Electron Microscopy: *cw-15* or *cw-92* cells were allowed to adhere (via their flagella) to carbon-Formvar-coated grids. The grids were sequentially floated on MT buffer, lysis solution, and fixative (1% glutaraldehyde in MT) before staining with 1% uranyl acetate or 1% phosphotungstic acid. Grids were observed in a Philips 300 electron microscope at 60 kV. A 1–3-s treatment with lysis solution yielded preparations with unlysed nuclei, whereas treatments longer than 30 s yielded preparations with all nuclei lysed.

Fluorescence Microscopy: Slides (10-well, Carlson Scientific, Peotone, IL) were washed, dried, treated with 0.1% aqueous polyethylenamine for several seconds, rinsed thoroughly in distilled water, and air dried. *cw-92* cells were harvested and washed several times by pelleting and resuspension in MT buffer, then applied to the wells and allowed to adhere. The slides were washed twice in MT buffer and then directly fixed (10% formaldehyde in MT buffer for 15 min at room temperature) or extracted with 0.5% NP-40 in MT buffer before fixation. The slides were washed twice in MT buffer then plunged into methanol and acetone (1 min each, -20°C). Slides were air dried, and blocker (10% calf serum, 2.5% bovine serum albumin in phosphate-buffered saline [PBS]) was applied to the well and incubated for 15 min. Diluted antiserum was added directly to the blocker in the wells and incubated for an additional 1 h. The slides were washed three times in 0.05% Tween-20 in PBS, then four times in PBS only. Blocker was applied as above and the fluorescein-conjugated secondary antibody was added directly. After incubation for 1 h, the slides were

¹ Abbreviations used in this paper: DAPI, 4,6-diamidino-2-phenylindole; NP-40, Nonidet P-40.

washed as above and then incubated for 15 min in a solution of 1 $\mu\text{g/ml}$ 4,6-diamidino-2-phenylindole (DAPI) in MT buffer. The slides were rinsed once in PBS, and coverslips were mounted with FA Mounting Medium (Difco Laboratories Inc., Detroit, MI) and sealed with nail varnish. The samples were observed with a Zeiss Epi-fluorescence microscope at 1,000 \times . Pictures were taken on Tri-X black and white film at exposures of 1 min for DAPI and 2 min for fluorescein.

Immunoblot Analysis: Proteins were separated by SDS gel electrophoresis (23) through a 5–15% polyacrylamide gradient. Two-dimensional separations followed O'Farrell (28). Protein transfer to nitrocellulose and immunostaining followed Towbin et al. (42). Antisera were produced in rabbits and directed against the 20-kD flagellar root protein of *Tetraselmis striata* (37).

RESULTS

Detergent Lysis Releases Nucleus-Basal Body Complexes from *C. reinhardtii*

Treatment with the nonionic detergent NP-40 released nucleus-basal body-axoneme complexes from the cell wall-defective mutants *cw-15* and *cw-92* and from wild type cells whose walls had been first digested by treatment with *Chlamydomonas* autolysin (38). We call the complex the nucleo-flagellar apparatus (44). In freshly released complexes observed by light microscopy (Fig. 1) the distance between the nucleus and the basal bodies was $\sim 1 \mu\text{m}$, approximately the distance between nucleus and basal bodies seen by electron microscopy in thin-sectioned cells. The complexes were morphologically stable for several days at 4°C and for at least 24 h at room temperature. It is important to emphasize the absolute one-to-one correlation between nucleus and basal body pairs in the complexes. Neither free nuclei, unattached basal body/axoneme pairs, nor nuclei with multiple basal body pairs were observed indicating that the connection between basal bodies and nucleus does not occur by random association after cell lysis. Complexes could also be released from gametic cells, as well as from dikaryon and diploid zygotes present 3 h after *mt+* and *mt-* gametes were mixed. Axonemes typically remained attached to the basal bodies in the complexes because free calcium levels in our standard lysis buffer were low. However, complexes without axonemes were efficiently released from cells that had first been deflagellated by pH shock or dibucaine treatment.

Immunological Identification of a Major Protein Component of the Nucleo-flagellar Apparatus

Recently Salisbury et al. (37) described the isolation of striated flagellar roots from the quadriflagellate green alga *Tetraselmis striata*. A 20-kD protein consisting of two acidic isoforms was identified as the major constituent of the isolated roots, and an antibody to the protein was raised. We used this antibody for immunoblot analysis of protein from whole cells and from deflagellated nucleo-flagellar apparatuses. The results, presented in Figs. 2 and 3, demonstrate that the *Chlamydomonas* antigen is also a 20-kD protein that resolves into two acidic components, α and β , by two-dimensional electrophoresis.

Immunofluorescent Localization of the 20-kD Antigen in the Nucleo-flagellar Apparatus

Examination of *C. reinhardtii* cells by indirect immunofluorescence using the anti-20-kD antibody revealed intense staining in two fiberlike strands between the nucleus and the basal body pair. Additional strands of immunofluorescence extended from the connector pole in a basketlike array around the nuclear perimeter (Fig. 4). In many cases, a pair of bright spots of fluorescence were seen on the nucleus at the points where the strands from the basal bodies met the nuclear surface. (Note that the nucleus is unambiguously defined in our preparations by staining with the DNA-specific fluorescent dye DAPI.) The proximal portion of the basal bodies also reacted with the antibody, producing a "diamond ring" pattern within each cell. Bright but diffuse staining over much of the nucleus was also present; staining was brightest in the region of the connector pole and extended around much of the nucleus.

In addition to the pattern described above, the anti-20-kD antiserum occasionally stained axonemes. This immunofluorescence was variable, however, and sometimes cells with both stained and unstained axonemes could be observed in a single field. The variability in immunofluorescence and the absence of a prominent band in immunoblots of flagellar proteins make us unsure at the present time whether the 20-kD antigen is regularly present in flagella.

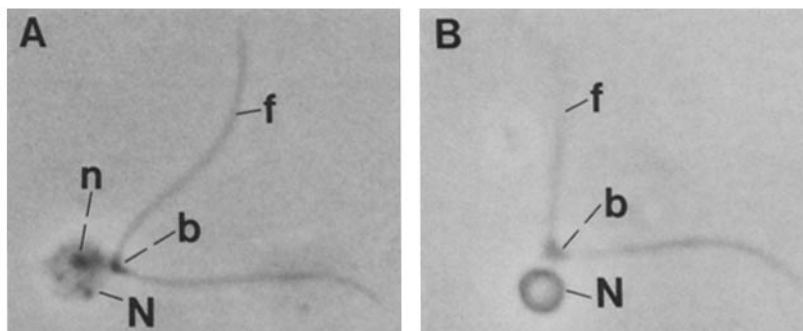


FIGURE 1 Nucleo-flagellar apparatuses are released in detergent lysates of *cw-92* vegetative cells. Cells were lysed in 1% NP-40 in MT buffer without calcium and photographed under phase-contrast optics. Two morphological classes are typically observed, based on nuclear appearance. (A) Nucleo-flagellar apparatus with a presumed lysed nucleus. In these cases, the nuclei appear slightly irregular in shape, are nonuniform in phase density, and have clearly visible nucleoli. (B) Apparatus with a presumed unlysed nucleus. Here the nuclei are very smoothly rounded, with a uniform and highly refractile appearance under phase optics. Nucleoli are not visible. "Lysed" (class A) nuclei are similar in size to nuclei in vivo (see Fig. 4 and 5). "Unlysed" (class B) nuclei are smaller than nuclei visualized in whole cells, as though the nucleus initially contracts after release from the cell. The proportion of class A nuclei increases with longer incubation in detergent or when excessive pressure is exerted on the coverslip. *b*, basal bodies; *f*, flagella; *n*, nucleolus; *N*, nucleus. $\times 2,100$.

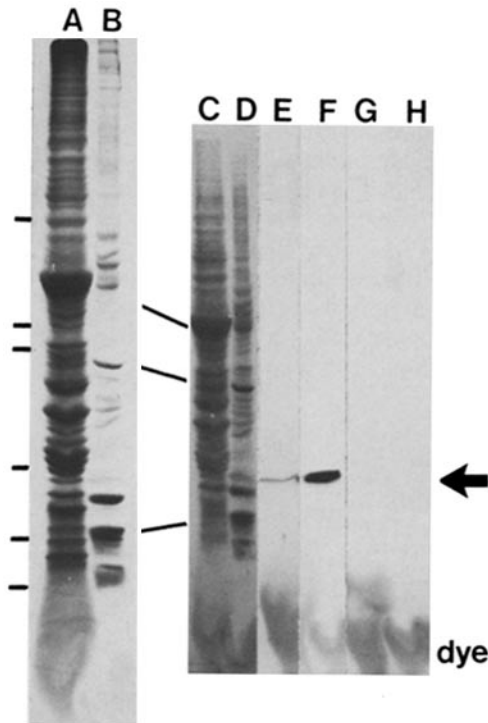


FIGURE 2 The *C. reinhardtii* nucleus-basal body complex contains a protein homologous to a major flagellar root protein from *Tetraselmis*. Lanes A and B, SDS polyacrylamide gels of whole cells (lane A) and nucleus-basal body complexes (lane B) stained with Coomassie Brilliant Blue. Lanes C–F, proteins from whole cells and nucleus-basal body complexes transferred to nitrocellulose and stained with naphthol blue black (lanes C and D); immune anti-20-kD serum (lanes E and F); and preimmune serum (lanes G and H). Molecular weight markers indicated at left are from top to bottom, (bovine serum albumin, 68,000; ovalbumin, 45,000; pepsin, 34,700; trypsinogen, 24,000; β -lactoglobulin, 18,400; and lysozyme, 14,300. Tracking dye (Pyronin Y) was transferred to the nitrocellulose near the bottom of lanes E–H.

Isolated nucleo-flagellar apparatuses showed essentially the same antibody-staining patterns as were seen in whole cells (Fig. 5). “Lysed” and “unlysed” nuclei (see Fig. 1) stained with similar intensity, indicating that the nuclear antigen is not peripherally bound to the nuclear surface.

Calcium Promotes the Shortening of the Nucleus-Basal Body Connector and the Concomitant Movement of DNA to the Nuclear Pole

The striated flagellar roots of *Tetraselmis* show calcium-mediated contraction (37), and, since the 20-kD component of the *Chlamydomonas* nucleo-flagellar apparatus appeared to be homologous to the 20-kD *Tetraselmis* root protein, we thought it probable that the *Chlamydomonas* connector would show calcium-mediated contraction as well. Therefore, *Chlamydomonas* nucleus-basal body complexes were prepared in media with or without millimolar free calcium. In complexes released in the presence of calcium the connector appeared distinctly shorter and wider, and the strands of immunofluorescent staining that had surrounded much of the nucleus were now seen only at the connector pole, also shorter and wider (Fig. 6). A simple interpretation of these

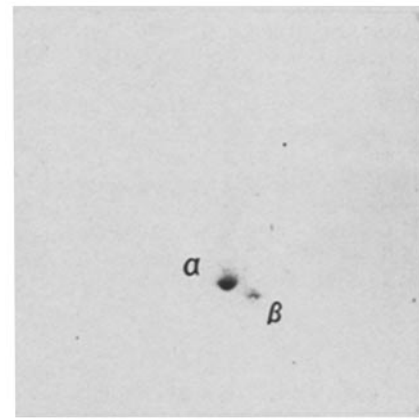


FIGURE 3 The *C. reinhardtii* 20-kD antigen consists of α - and β -subunits. Proteins in nucleo-flagellar apparatuses were separated by two-dimensional electrophoresis, transferred to nitrocellulose, and probed with anti-20-kD antiserum. The immunoblot is printed with the low molecular weight region at the bottom and the acidic region at the right. Only the relevant section of the immunoblot is shown.

results is that calcium promotes contraction by the connector. It is interesting that the nuclear DNA also seemed to have moved toward the connector pole (compare Figs. 4 and 6). This leads us to speculate, though without further evidence at this time, that connector fibers could be attached directly or indirectly to the chromosomes.

The reduction in distance between the basal bodies and nucleus was also observed in nucleo-flagellar apparatuses released in the absence of free calcium then incubated in the presence of 1 mM calcium. In this case, the average distance was initially 1.2 μ m and decreased to 0.034 μ m after incubation in 1 mM calcium for 30 min. After the calcium incubation, the basal body pair was often observed directly on the nuclear surface, a condition not observed in the absence of calcium.

The Nucleo-flagellar Apparatus in Mutant Cells: *vfl-2-220* Has a Connector Defect

Instead of being uniformly biflagellate, certain mutants of *C. reinhardtii* have variable numbers of flagella per cell—the so-called *vfl* phenotype. We examined lysates of three such mutants: *vfl-1-1* (1), *vfl-2-220* (21), and *vfl-3-207* (45) to see if they had readily discernible nucleus-basal body connector defects. The *vfl-1* and *vfl-3* mutants yielded nucleo-flagellar apparatuses of normal stability, but *vfl-2* behaved very differently. Instead of all flagellar apparatuses being attached to nuclei, ~40% of the flagellar apparatuses in fresh lysates were free of connections to nuclei, and, after overnight incubation at 4°C, none at all could be observed. This was in marked contrast to wild type, *vfl-1* or *vfl-3* in which all apparatuses survived for 12 h or longer. Thus, by the operational criterion available to us, *vfl-2-220* is defective with respect to its nucleo-flagellar connectors. We emphasize that the connector need not be absent in *vfl-2-220*; it could be present but incomplete or functionally defective.

In the *vfl-1* and *vfl-3* lysates, nucleo-flagellar apparatuses of normal stability were observed, and the distribution of flagellar apparatuses on the nuclei reflected the distributions of flagella on the cells before lysis. Basal bodies were often widely separated on individual nuclei, and this too reflected the

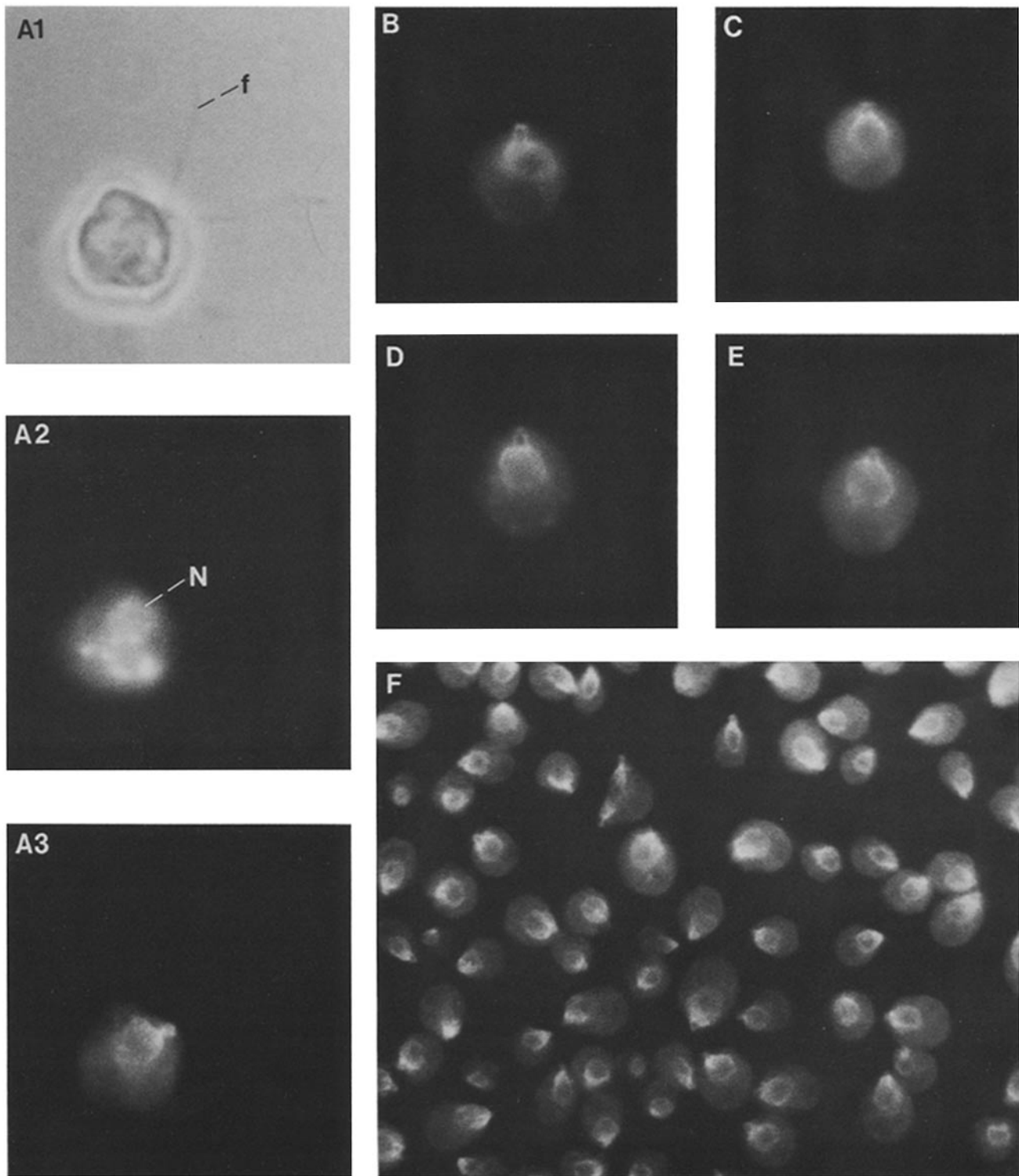


FIGURE 4 The connector region in whole *C. reinhardtii* cells is immunoreactive to anti-20-kD antiserum. Shown are phase (A1), DAPI fluorescence (A2), and anti-20-kD fluorescence (A3) images of the same cell. The smaller, more intense spots in A2 are chloroplast nucleoids. (B–F) Anti-20-kD staining of different cells. The pattern of staining in these cells is similar: the proximal portion of the basal bodies, two strands from the basal bodies to the nucleus, and a basketlike array around the nucleus itself are evident. Background staining is due to the autofluorescence of residual chlorophyll. *f*, flagella; *N*, nucleus. (A–E) $\times 1,700$. (F) $\times 650$.

variable location of flagella on *vfl-1* and *vfl-3* cells before lysis. As in wild-type lysates, no free flagellar apparatuses were observed; every one was attached to a nucleus. In contrast to wild type, some free nuclei were observed, and these were in the frequencies expected based on the proportions of nonfla-

gellated cells present in the original cultures. Fig. 7 compares the distribution of flagella on *vfl-3* cells with the distribution of axonemes on isolated *vfl-3* nuclei. The distributions are identical. These results reinforce our conclusion that the associations between basal bodies and nuclei do not derive

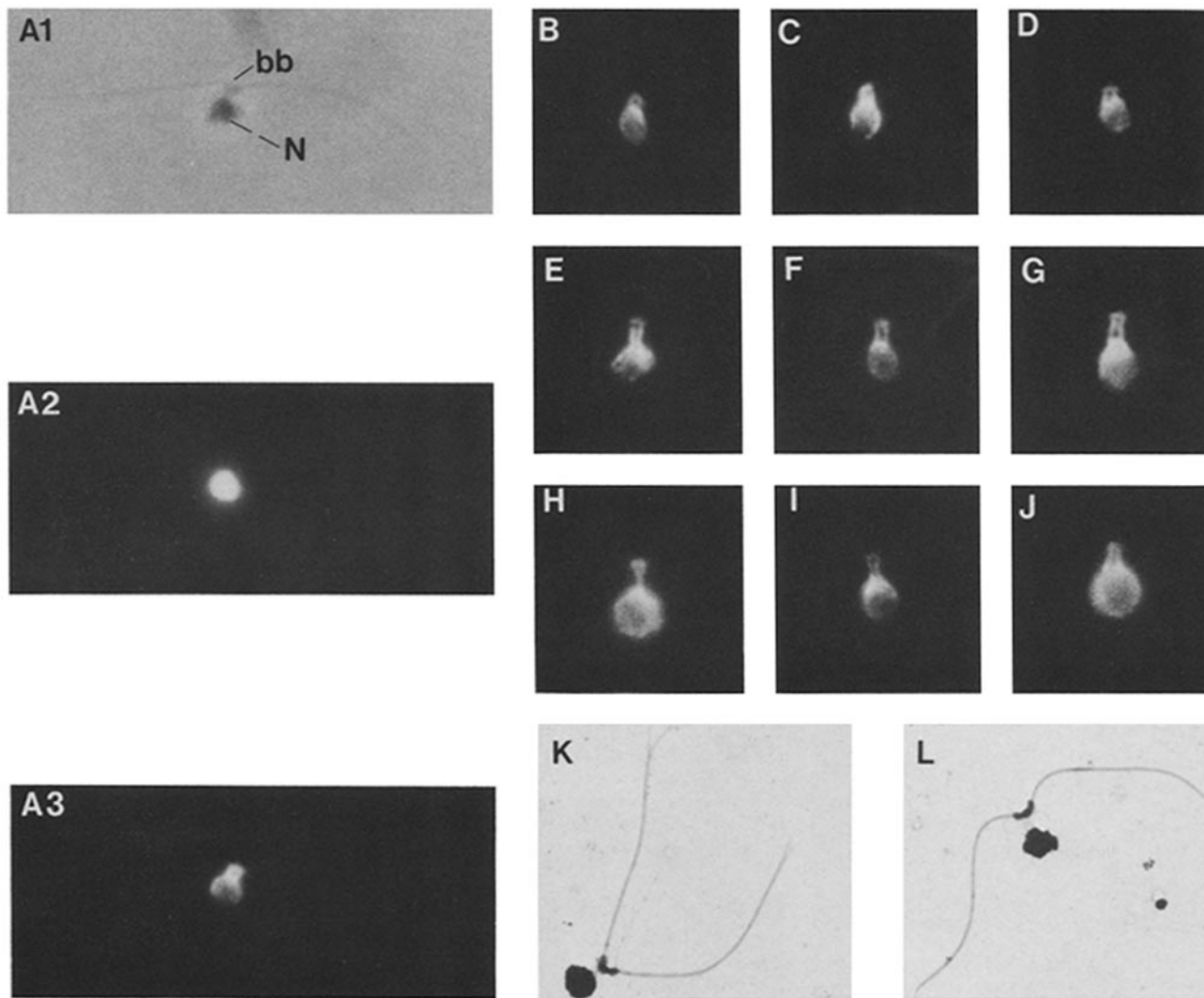


FIGURE 5 The anti-20-kD immunofluorescence pattern of isolated nucleoflagellar apparatuses is similar to that of whole cells. Shown are phase (A1), DAPI fluorescence (A2), and anti-20-kD fluorescence (A3) of the same apparatus. (B–J) Anti-20-kD staining of different apparatuses. Apparatuses in A–C have “unlysed” nuclei; those in H–J have “lysed” nuclei (see Fig. 1). (K–L) Electron micrographs of positively stained nucleoflagellar apparatuses with “unlysed” nuclei. bb, basal bodies; N, nucleus. $\times 1,700$.

from random postlysis events. Furthermore, they indicate that the basal body–nucleus connection does not depend on basal body–associated distal or proximal striated fibers, since these are defective in the *vfl-1* and *vfl-3* mutants (1, 45), nor does it depend on normally positioned basal body–associated rootlet microtubules, since these too are defective in the mutants (1, 45).

Electron Microscopy

Negative stain electron microscopic images of the nucleoflagellar apparatus are shown in Fig. 8. In all these images the nuclear membranes are lysed (see Fig. 5 for electron microscopic images with the nucleus intact). Fig. 8A shows a typical grid square; note the attachment of every flagellar apparatus to a nucleus. In most images, chromatin from the lysed nuclei fills the connector region, but in favorable cases two presumed connectors, one from each basal body, are present, and in some cases they can be seen to contain a bundle of fine filaments whose diameter is ~ 6 nm (Fig. 8D).

We have attempted to visualize the connector in thin sections of fixed and embedded nucleoflagellar apparatuses.

These efforts have not met with success, although they have confirmed that the lysed nuclei in the NP-40-derived complexes are substantially membrane free.

DISCUSSION

The Nucleus-Basal Body Connector

Nucleus-basal body complexes have been isolated from lysed *C. reinhardtii* cells, and a connecting strand between each basal body and the nucleus has been visualized by indirect immunofluorescence using an antibody generated against the major component of isolated *Tetraselmis striata* flagellar roots. Treatment with 1 mM Ca^{++} leads to pronounced shortening of the connector in vitro. The protein recognized by the antibody is ~ 20 kD and consists of two acidic isoforms. In these respects, the protein is just like that of *Tetraselmis*. It has been demonstrated that one of the *Tetraselmis* isoforms labels with short pulses of ^{32}P and one does not (37), and we expect that the *Chlamydomonas* isoforms differ in an equivalent way. The functional significance of the isoforms is not clear at this time, although it has been

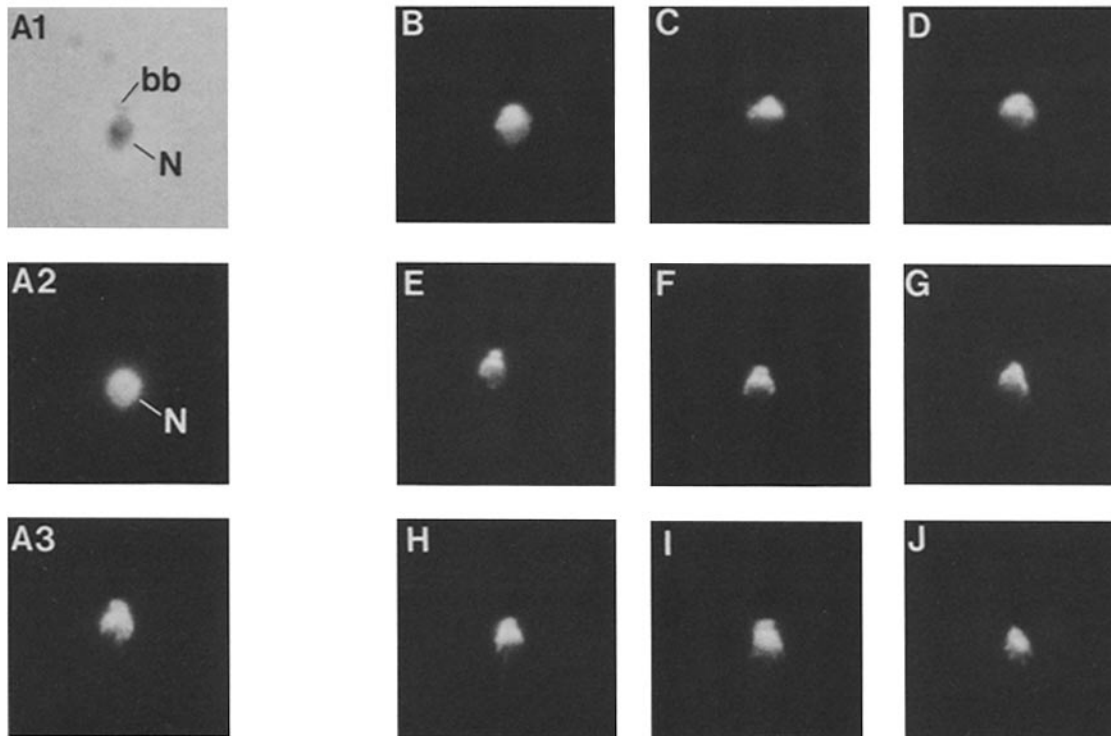


FIGURE 6 Nucleo-flagellar apparatuses prepared in the presence of 1 mM Ca^{++} show an altered pattern of anti-20-kD immunofluorescence. Shown are phase (A1), DAPI fluorescence (A2), and anti-20-kD fluorescence (A3) of the same apparatus. Note the absence of flagella (lysis in the presence of Ca^{++} deflagellates the cell), the shift in DAPI staining toward the basal bodies (compare Fig. 4 A2), and the altered anti-20-kD staining pattern. In the presence of calcium, basal bodies are typically found directly against the nucleus, making it quite difficult to clearly observe them. (B–J) Anti-20-kD immunofluorescence of different nucleo-flagellar apparatuses. All nuclei shown had the “unlysed” morphology (see Fig. 1). *bb*, basal bodies; *N*, nucleus. $\times 1,700$.

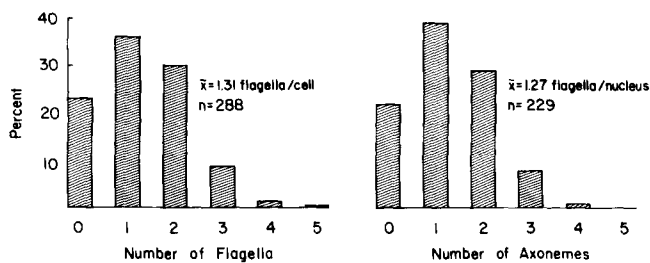


FIGURE 7 Left, prelysis number of flagella per cell. Right, postlysis, number of axonemes per nucleus. The distribution of flagella on *vfl-3* cells is identical to the distribution of axonemes on isolated nucleo-flagellar apparatuses, indicating that all basal bodies remain attached to the nuclei after lysis.

speculated that phosphorylation of the protein may be important in regulating calcium-mediated contraction *in vivo* (37). Numerous possible biological functions for connector contraction can be imagined, such as roles in coordination of flagellar beating (34), movement of the basal bodies before mitosis (9, 37a), or transduction of signals for flagellar resorption during the cell cycle or for the turning on of flagellar genes after deflagellation (24). However, we stress that elaboration of such speculations is premature, since at present we do not even know whether connector contraction occurs *in vivo*.

Connector antigen is present in considerable quantity in the nucleus, where it appears to be localized at or near the nuclear surface. Antigen is most concentrated at the connector pole, but a gradient of diffuse staining, as well as some distinct fluorescent strands, extends around much of the nucleus.

Because nuclear antigen is not extracted by detergent treatments that extract much or all of the nuclear membrane, we suspect that the protein is associated with the nuclear matrix, or perhaps with the chromosomes. As for possible roles for the nuclear antigen, we can say little beyond the obvious role of anchoring the basal body connector fibers to the nucleus. We suspect that there is more to the story than this, since the antigen is present in the nucleus in such abundance.

Electron microscopic observation of connector ultrastructure has been difficult. We have been unable to visualize the connector in thin sections of either whole cells or isolated nucleo-flagellar apparatuses. We are not particularly disturbed by these negative results; after all, if the structure were readily apparent it would have been described years ago, not only in *C. reinhardtii* but also, if we are right about the biological generality of the connector, in numerous other eucaryotic cell types. Indeed, it may be the case that a small and/or loosely organized connector is sufficient to do the biological job in most cell types and that it has been elaborated and/or enlarged by evolution only in cases where it plays a special role, such as anchoring beating flagella to the nucleus in cells that lack a rigid force-absorbing wall (33, 39).

The Nucleo-flagellar Apparatus Is Not an Artifact

The ready interpretation of the results presented here, and the one we strongly favor, is that there exists a structure *in vivo* that holds basal bodies and nuclei together. Could it be, however, that the nucleo-flagellar apparatus forms artifactually during or after lysis? We are convinced that it does not, for the following reasons: (a) Nucleo-flagellar apparatuses do

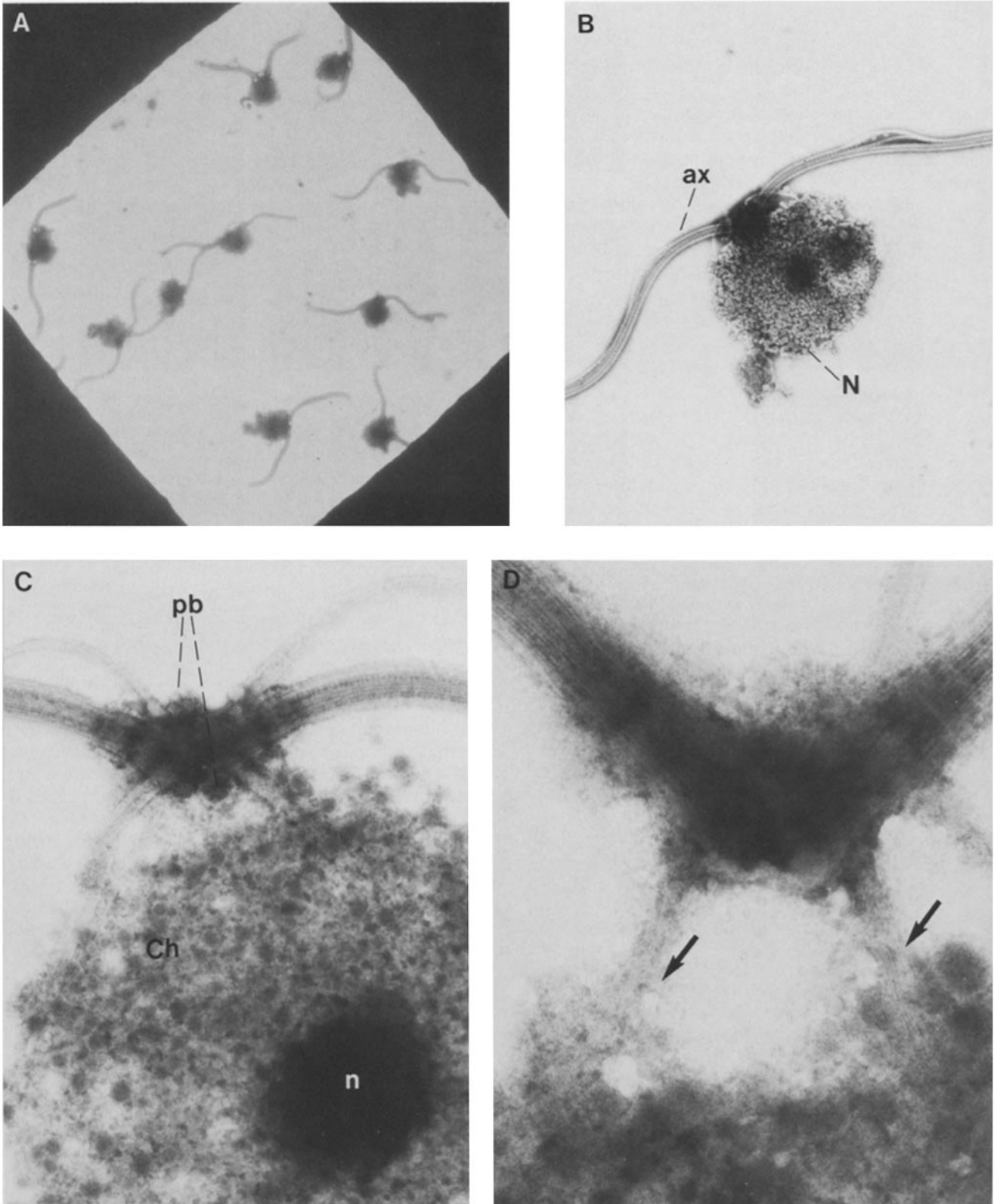


FIGURE 8 Electron microscopy of negatively stained nucleo-flagellar apparatuses. Preparations in which the nuclei had lysed (shown here) gave much better images in the connector region than those in which nuclei were intact, apparently because excessive stain in the connector region otherwise obscures ultrastructural detail. (A) An entire grid square of apparatus preparations demonstrates the one-to-one correlation of nucleus to flagellar apparatus. Often the nucleus overlies the flagellar apparatus, making visualization of the connectors impossible. (B) A single, negatively stained apparatus. Note the absence of the nuclear membrane (compare Fig. 3, K-L for apparatuses with intact nuclear membranes). $\times 7,500$. (C) At higher magnifications, the basal bodies, probasal bodies, and portions of the microtubule rootlet system are evident. $\times 27,000$. (D) In favorable instances a cluster of ~ 6 -nm filaments is evident within the connector. ax, axoneme; Ch, chromatin; N, nucleus; n, nucleolus; pb, probasal bodies. $\times 69,000$.

not arise from random association of basal bodies and nuclei following lysis, since the distribution of axonemes on nuclei directly reflects that of intact cells. This is true both for wild-type and for the variable flagellar number mutants, *vfl-1* and *vfl-3* (Fig. 7). (b) Nucleo-flagellar apparatuses do not arise because material, such as chromatin, is released from the lysed nuclei and attaches to the basal bodies. We know this to be true because apparatuses with intact nuclei can be released from cells by homogenization in the absence of detergents. Furthermore, the association between the nucleus and basal body pair is not disrupted by treatment with DNase (unpublished results). (c) Nucleo-flagellar apparatuses do not arise from the collapse of the rootlet microtubules around the nucleus. This is indicated by several observations. First, mutants with gross rootlet defects (*vfl-1* and *vfl-3*) yield apparatuses of normal stability. Second, in vivo the rootlet microtubules are far from the nucleus; they radiate around the outside of a cup-shaped chloroplast and the nucleus is inside the cup. Because of this geometry, the chloroplast represents a physical barrier to the collapse of the microtubules onto the nucleus. Third, portions of all four rootlets can often be observed on nucleus-basal body apparatuses, both at the light and electron microscopic levels. In these instances, it is clear that there are additional and distinct structures bridging the space between the basal bodies and nuclear surface. (d) Finally, there is the very fact that the nucleus-basal body connector is visualized by indirect immunofluorescence in whole cells. This does not by itself prove that the antigen is in an integral connecting structure, but the fact that the cellular pattern of immunofluorescence is the same as that of isolated apparatuses suggests strongly that it is.

Genetic Evidence for a Possible Role for the Connector in Basal Body Localization and/or Segregation

Our results with the *vfl* mutants are particularly suggestive about possible connector function. Nucleo-flagellar apparatuses isolated from *vfl-2* cells, but not from *vfl-1* or *vfl-3* cells, are either extremely unstable or absent entirely. *vfl-2* has a variable number of basal bodies per cell, yet cell division in the mutant appears to be normal (21). This is in contrast to *vfl-1* and *vfl-3*, in which both basal body number and cell division are dramatically aberrant (1, 45). The fact that cell division is normal in *vfl-2* may mean that normal attachment of the basal bodies to the nucleus is not essential for normal mitosis and cell division, a view consistent with other evidence (see Introduction) that the cell cycle is not governed by the basal body cycle.

The variable basal body-number phenotype of *vfl-2* invites the speculation that the nucleus-basal body connector plays an essential role in basal body localization and segregation. Specifically, we hypothesize that due to its connector defect the *vfl-2* cell fails to localize its basal bodies precisely enough to guarantee equal segregation at cytokinesis. We anticipate that the analysis of additional mutants with connector defects will provide more definitive evidence about connector function.

Nucleus-Centriole and Nucleus-Basal Body Connectors Homologous to Those in Chlamydomonas May Be General Features of Eucaryotic Cells

A close relationship between basal bodies or centrioles and the nucleus has been reported in a variety of algal, protozoan, and metazoan cells (3, 6, 22, 27, 29, 33, 40, 44). We believe it likely that connectors homologous to those we describe here for *Chlamydomonas* mediate nucleus-centriole associations in general. Indeed, the antibody that we used here to visualize the connector in *Chlamydomonas* cross-reacts with material near the centrosomes and mitotic spindle poles of mammalian cells (36). What might the functions of such connectors be? We find several possibilities attractive. Connectors might serve to physically couple the basal body or centriole cycle to the mitotic cycle; when centrioles or basal bodies migrate to the nascent mitotic poles, their movements might be guided by the connectors. In this way the connector would indirectly determine the location of the mitotic spindle. The connector might also be directly involved in organizing the spindle; material recognized by the anti-20-kD antibody is present in mammalian spindles (36), and electron microscopic images exist showing that system II striated fibers (see below) can act as nucleating sites for spindle microtubules (7, 41). Connectors might serve to properly localize centrioles or basal bodies with respect to the nucleus, and therefore with respect to the cell as a whole. Correct basal body location is obviously important for flagella-bearing cells, since flagella would otherwise be improperly localized on their surfaces. More generally, if centrioles and basal bodies play important roles in establishing or maintaining cell polarity, as some investigators suspect they do (see the introduction), then the connectors could be vital to a wide variety of functions in many different cell types.

There exist a number of distinct classes of striated fibers associated with centrioles and basal bodies (12, 27, 43). In the classification scheme of Melkonian (27) the *Tetraselmis* root is a system II fiber. System II fibers constitute a very heterogeneous class, being large and easily visualized by thin section electron microscopy in some cases and barely discernable in others. We can now add that *C. reinhardtii* has system II fibers that are not discernible by standard procedures of fixation and thin-section electron microscopy. In fact, though, a pair of small type II fibers attached to the basal bodies and pointing toward the nucleus has been described in another *Chlamydomonas* species, *C. moewusii* (19), and Hoops and Witman have recently observed what appear to be system II fibers in thin sections of detergent-extracted, calcium-treated *C. reinhardtii* (personal communication).

As a final speculation, we note that in *C. reinhardtii* there exists a nuclear linkage group that shows unusually high second division segregation at meiosis (16). If the nucleo-flagellar connectors directly or indirectly attach to a particular chromosome (4), if this chromosome represents the "uni linkage group," and if the basal bodies, connectors, and chromosome segregate in the first meiotic division in the way that basal bodies appear to segregate in mitosis, i.e., then semiconservatively (13, 16), then high second division segregation is expected. This would be highly unorthodox, as it

would require sister chromatid segregation in the first meiotic division, but it is formally consistent with the observed segregation patterns.

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Note added in proof: We have examined the immunofluorescence patterns of *vfl-2* and *vfl-3*, utilizing the anti-20-kD antiserum. Connectors and nuclei were visualized in *vfl-3*, both in whole cells and nucleo-flagellar apparatuses. However, neither connectors nor nuclei were stained in *vfl-2* preparations.

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