The role of the flagellar transition region: inferences from the analysis of a *Chlamydomonas* mutant with defective transition region structures

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Summary

Thin-section electron microscopy of the *Chlamydomonas reinhardtii* mutant *vfl-2* revealed striking defects in the transition region between basal body and flagellum. In place of the highly organized transition cylinders and stellate fibers characteristic of wild type, variable quantities of poorly organized electron-dense material were present. In many cases the transition region was penetrated by central pair microtubules that passed from the axoneme into the basal body. On the basis of these observations we propose that an important function of the structures present in the normal transition region is to physically exclude the central pair microtubules from the basal body.

The transition region is the site of flagellar autotomy – the process by which doublet microtubules are severed and flagella are released from the cell. It has been claimed that autotomy is caused by contraction of the centrin-containing stellate fibers, resulting in the mechanical severing of the doublet microtubules and a concomitant reduction of the diameter of the axoneme adjacent to the abscission point. Our observations do not support this claim in that *vfl-2* cells, which lack organized stellate fibers, display effective autotomy unaccompanied by detectable narrowing of the axoneme.

Key words: autotomy, flagella, *Chlamydomonas*, transition region, microtubules, centrin.

Introduction

The specialized region between the eucaryotic cilium or flagellum and its basal body is known as the *transition region* or *transition zone*. The transition region can be said to begin where the C-tubules of the basal body end (i.e. where triplet microtubules give way to doublet microtubules) and to end where the central pair microtubules begin (Pitelka, 1974), or, in a variation on this definition, to end where the axoneme first assumes its standard ultrastructure (Gibbons, 1961). Most transition regions are 200–400 nm in length, although longer examples exist.

The transition region can be conveniently divided into an *inner zone* and an *outer zone*, with the doublet microtubules forming the boundary between the two. The ultrastructure of the outer zone shows great evolutionary conservation, with all transition regions possessing Y-shaped elements linking the doublets to the membrane as well as two or more rings of distinctive membrane-associated particles known collectively as the ciliary necklace (Ringo, 1967; Gilula and Satir, 1972; Rohlich, 1975). In contrast, inner zone structures vary greatly among organisms, and, accordingly, they go by a variety of names such as axosomes, transverse septa or basal plates. In *Chlamydomonas* the transition region inner zone contains two prominent *transition cylinders* and a set of *stellate fibers* that link the cylinders to the doublet microtubules.

The transition region is responsible for a number of critical functions, including partitioning the flagellar membrane from the plasma membrane (Musgrave *et al.* 1986; Kaneshiro, 1989), partitioning the flagellar interior from the cytosol (Besharse and Horst, 1990), and autotomy, or flagellar shedding (Blum, 1971). In addition, the transition region may play a major role in the control of flagellar assembly (Dentler, 1987).

The biflagellate green alga *Chlamydomonas reinhardtii* provides an excellent model system for studying organelle structure and function using genetic, molecular and ultrastructural approaches (Harris, 1989). As a means of studying basal body structure and localization, we have found it useful to analyze a class of mutants that, instead of being biflagellate, have a variable number of flagella per cell. These mutants are designated *vfl*, for *v*ariable *fl*agella number. *vfl* mutants are typically defective in basal body placement and/or segregation at cell division, and, in a number of cases, their functional defects have been shown to be associated with particular ultrastructural abnormalities (Wright *et al.* 1983; Hoops *et al.* 1984; Adams *et al.* 1985; Wright *et al.* 1985; Wright *et al.* 1989).

Previously published results from our laboratory (Wright *et al.* 1989) showed that the mutant *vfl-2* has defects with respect to the quantity and localization of the protein centrin (also known as caltractin) – a small acidic calcium-binding protein related to calmodulin. In wild type, centrin is found in the contractile *nucleus–basal body connectors* that link the basal bodies to the nucleus, in the *distal striated fibers* between the members of each basal body pair, and in the inner zone of the transition region (Wright *et al.* 1985; Huang *et al.* 1988; Melkonian *et al.* 1988; Salisbury *et al.* 1988; Sanders and Salisbury,

1989; Wright *et al.* 1989). In this report we describe the ultrastructure of the *vfl-2* basal body region. Not only are NBBCs and distal striated fibers absent, but dramatic defects are present in the transition region – defects whose nature has interesting implications with regard to normal transition region function.

Materials and methods

Strains and culture conditions

Wild-type *C. reinhardtii* were either *137c mt*$^+$ or the *137c* derivitive *NO mt*$^+$ obtained from the Chlamydomonas Culture Collection, Duke University, Durham, NC. Cell wall-defective strains *cw15 mt*$^+$ and *cw92 mt*$^+$ (Hyams and Davies, 1972) and the *Chlamydomonas* natural isolates *C. smithii* (Hoshaw and Ettl, 1966) and *S1D2* (Gross *et al.* 1988) were obtained from the same source. The mutant *vfl-2* was isolated in our laboratory (Kuchka and Jarvik, 1982), as were the *C. reinhardtii* natural isolates *6*, *224* and *356* (Spanier, Graham and Jarvik, unpublished). Cells were grown under constant light in M-medium (Medium I of Sager and Granick, 1953) supplemented with 0.1 % yeast extract, 0.1 % proteose peptone and 0.01 M sodium acetate.

Cell ghosts

Cell ghosts were prepared by pelleting 40 ml samples of log phase cells at approx. 10^6 cells ml^{-1} for 3 min at 2500 revs min^{-1} in a Beckman TJ6 centrifuge, resuspending in 8 ml nucleus buffer without calcium (NB minus Ca^{2+}: 25 mM KCl, 10 mM $MgCl_2$, 3.7 mM EGTA, 0.67 mM Tris–HCl, pH 7.3), repelleting as before, and resuspending in NB minus Ca^{2+} plus 1 % Nonidet P-40 (NP-40). After extraction for 30 min at room temperature, the cell ghosts were fixed for electron microscopy in 1 % glutaraldehyde in NB minus Ca^{2+}.

Nucleo-flagellar apparatuses

Nucleo-flagellar apparatuses (Wright *et al.* 1985) were prepared by a procedure identical to that described above, except that the cells carried the *cw15* or *cw92* mutation.

Deflagellated cells

Deflagellated cells were prepared by centrifuging 20 ml samples of log phase cells at approx. 10^6 cells ml^{-1} for 3 min at 2500 revs min^{-1} in a Beckman TJ6 centrifuge; resuspending in 1 ml M medium; adding 1 ml of 10 mM dibucaine, 5 % sucrose; incubating for 30 s; and fixing for electron microscopy by adding 2 ml of 2 % glutaraldehyde in M medium.

Electron microscopy

Log-phase cells were harvested by centrifugation (5 min at 2500 revs min^{-1} in a Beckman TJ6 centrifuge) and resuspended in 2 % glutaraldehyde in growth medium or NB minus Ca^{2+} as appropriate. After 30 to 60 min they were washed several times in fresh medium with 2 % glutaraldehyde, and the pellets were postfixed for 30 min. with 1 % osmium tetroxide and rinsed twice with water. Samples were *en bloc* stained in 1 % uranyl acetate for 30 min, dehydrated in a graded ethanol series, rinsed twice in propylene oxide, and infiltrated with resin (Polybed 812). Infiltration was facilitated by use of a vacuum oven (50 °C, 10 psi;) and the resin was polymerized at the same setting for 12–24 h. Silver to gold sections were cut using a diamond knife on a Sorvall MT-2B microtome. Sections were stained with 1 % aqueous uranyl acetate and Reynold's lead citrate and examined using a Philips 300 electron microscope at 60 kV.

Results

The wild-type transition region

A number of wild-type strains of *C. reinhardtii*, including the standard laboratory strain *137c* and five other natural isolates (see Materials and methods) were observed by thin-section transmission electron microscopy. The transition regions in all strains appeared identical and showed the constellation of structural features typical of *C. reinhardtii* (Ringo, 1967; Cavalier-Smith, 1974). These features (Fig. 1) include: (1) two prominent electron-dense *transition cylinders*, one *distal* and one *proximal*; (2) a set of *stellate fibers* linking the transition cylinders to the surrounding outer doublet microtubules. In longitudinal sections the stellate fibers appear as projections between the transition cylinders and the outer doublet microtubules, and in transverse sections they are seen as a set of fibers that appear to originate at the A-tubules of the outer doublets and to contact the transition cylinders tangentially; collectively the stellate fibers produce the image of a nine-pointed star; (3) a specialized segment of the flagellar membrane that maintains a constant distance of about 50 nm from the axoneme and is refractory to solubilization with nonionic detergents (Goodenough, 1983; Kamaya and Witman, 1984). We refer to this as *the transitional membrane*; (4) a set of Y-shaped *membrane–microtubule connectors*, apparent in transverse sections, connecting the outer doublet microtubules to the transitional membrane (see Fig. 1C and D); (5) a set of fuzzy V-shaped *annular connections* projecting from the transitional membrane towards and between the doublet microtubules (Fig. 1E–G). We suspect that in transverse sections the annular connections are represented by the clouds of electron density that surround the doublet microtubules and extend outwards to the transitional membrane (e.g. Fig. 1C); (6) a *clear zone* of reduced electron density about 100 nm deep between the proximal transition cylinder and the internal matrix of the basal body; (7) a *transition cylinder fiber* extending from the proximal transition cylinder through the clear zone and into the internal matrix of the basal body (e.g. Fig. 1F). In our micrographs, the central pair microtubules typically end above the distal transition cylinder (Fig. 1E and F), but it is not at all uncommon for them to reach right to it (Fig. 1G). Note also the prominent *transitional fibers* associated with the triplet microtubules of the basal body just below the transition region (e.g. Fig. 1B). These serve to link the basal body to the plasma membrane; despite their name, they are not strictly part of the transition region.

vfl-2 *has abnormal transition regions that are often penetrated by central pair microtubules*

Thin-section transmission electron microscopy of cells and cell lysates of *vfl-2* revealed grossly abnormal transition regions, with no normal case seen in many hundreds of transition regions examined. Examples are shown in Fig. 2. Electron-dense material reminiscent of the transition cylinders was typically observed inside the transition region, but its abundance and morphology were highly variable and it was never organized into a true cylinder as in wild type. Membrane–microtubule connectors were present, as were transitional membranes maintaining a standard distance from the axoneme. Stellate fibers were not observed nor were annular connections. A clear zone was sometimes present but transition cylinder fibers were absent. Finally, in about one third of the cases, the central pair microtubules were seen to penetrate into or through the transition region, and it was not at all unusual to observe them extending through the basal body and into the cell. In a number of cases we also observed what appeared to be additional

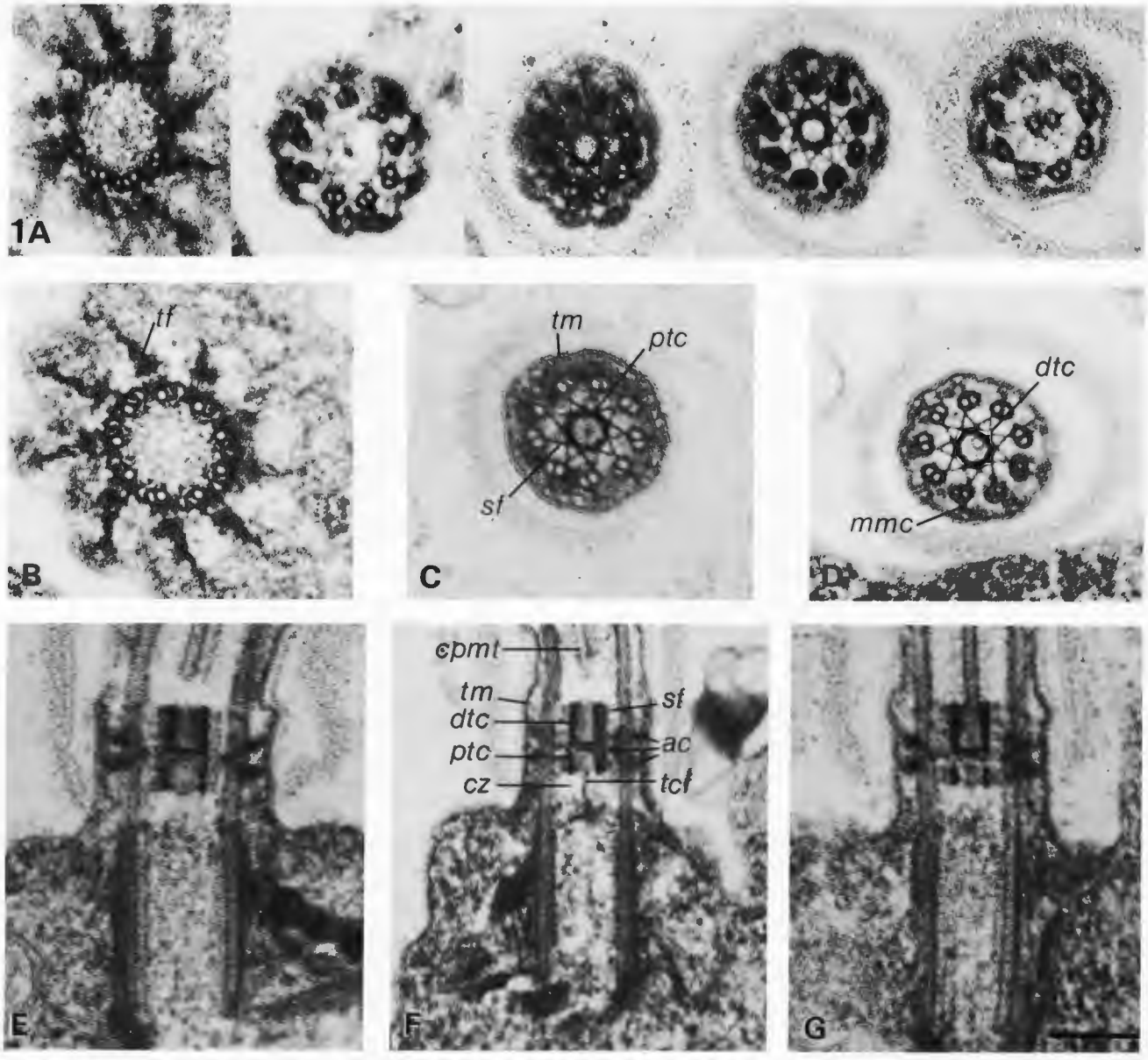

Fig. 1. Wild-type transition region. (A) Consecutive transverse serial sections. (B–D) Transverse sections at three consecutive levels. Note dot in center of transition cylinder in C that probably represents the transition cylinder fiber, and the ring inside the transition cylinder in D that probably represents the end of a central pair microtubule. (E–G) Longitudinal sections. A,B,D, strain *137c*. C,E, strain *6*. F,G, strain *356*. *ac*, annular connection; *cpmt*, central pair microtubules; *cz*, clear zone; *dtc*, distal transition cylinder; *mmc*, membrane–microtubule connector; *ptc*, proximal transition cylinder; *sf*, stellate fiber; *tcf*, transition cylinder fiber; *tf*, transitional fiber; *tm*, transitional membrane. Bar, 200 nm.

short microtubules of unknown significance inside the *vfl-2* transition region. Examples can be seen in Fig. 2B and C (see also Fig. 3D).

Flagellar autotomy in vfl-2

Many treatments are known to induce *Chlamydomonas* to shed its flagella by an active calcium-dependent process known as flagellar autotomy (Lewin *et al.* 1982; Huber *et al.* 1986). This process involves the severing of the outer doublet microtubules and a pinching-off of the flagellar membrane just distal to the transition region, so that the basal body remains with the cell and the flagellum is released into the medium. It has been shown that the morphology of the transition region changes during autotomy, with a distinct reduction in the diameter of the axoneme just below the point of severing, accompanied by a narrowing of the open end of the distal transition cylinder and an apparent contraction of the stellate fibers (Lewin and Lee, 1985; Sanders and Salisbury, 1989).

Wild-type and *vfl-2* cells were treated with the deflagellating agent dibucaine for 30 s and then fixed and prepared for electron microscopy (Fig. 3). Both strains were efficiently deflagellated by the procedure. Although the outer doublet microtubules of *vfl-2* were severed, there was little or no narrowing of the axoneme below the abscission point as in wild type. In a number of cases central pair microtubules were observed to pass out of the

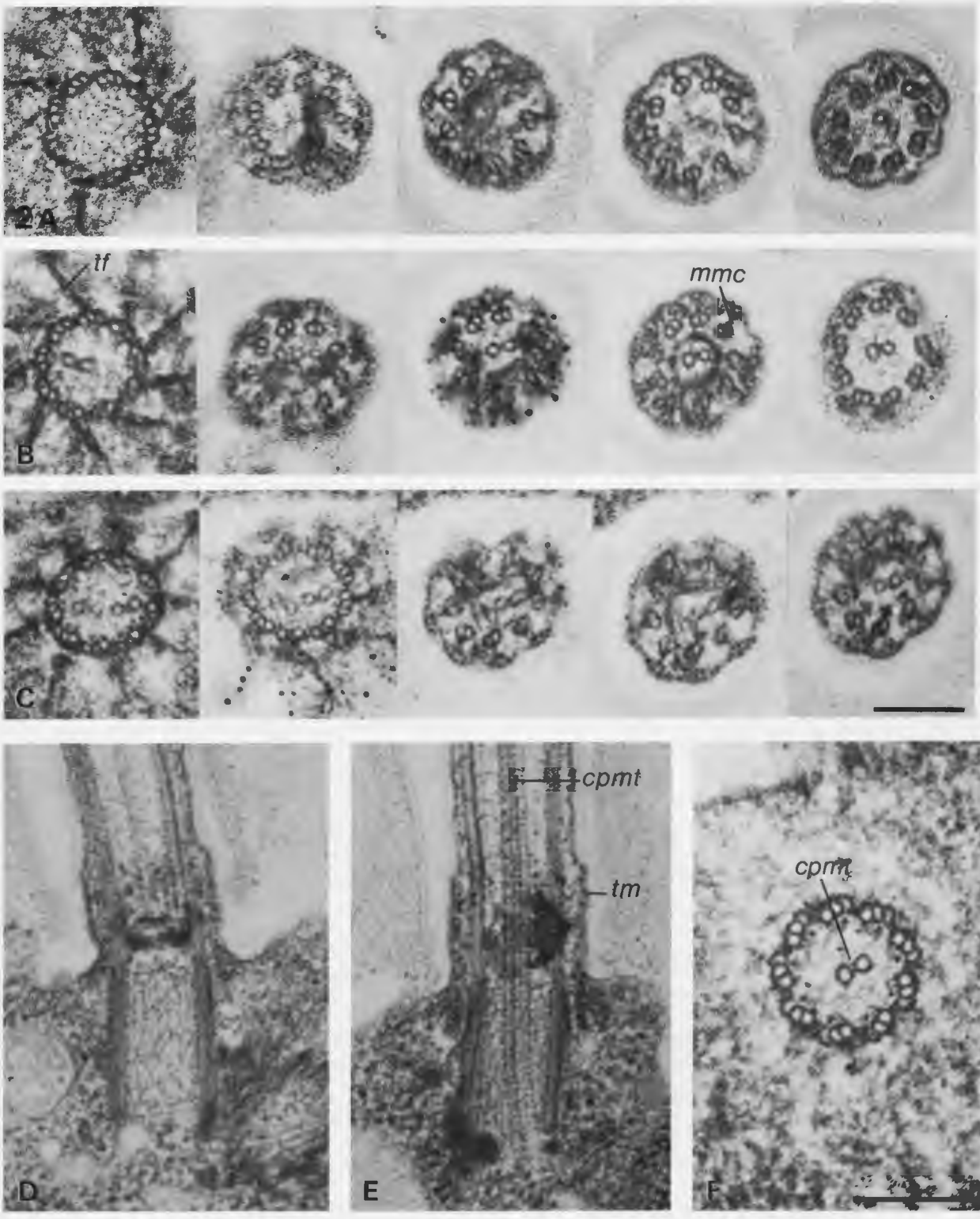

Fig. 2. Defective transition region in *vfl-2*. (A–C) Three sets of consecutive transverse serial sections. (D,E) Longitudinal sections, one showing the penetration of the transition region by central pair microtubules. (F) Transverse section at the level of the basal body (note triplet microtubules) showing central pair microtubules. *mmc*, membrane–microtubule connector; *tf*, transitional fiber; *tm*, transitional membrane; *cpmt*, central pair microtubules. Bars, 200 nm; all micrographs except F are at the same scale.

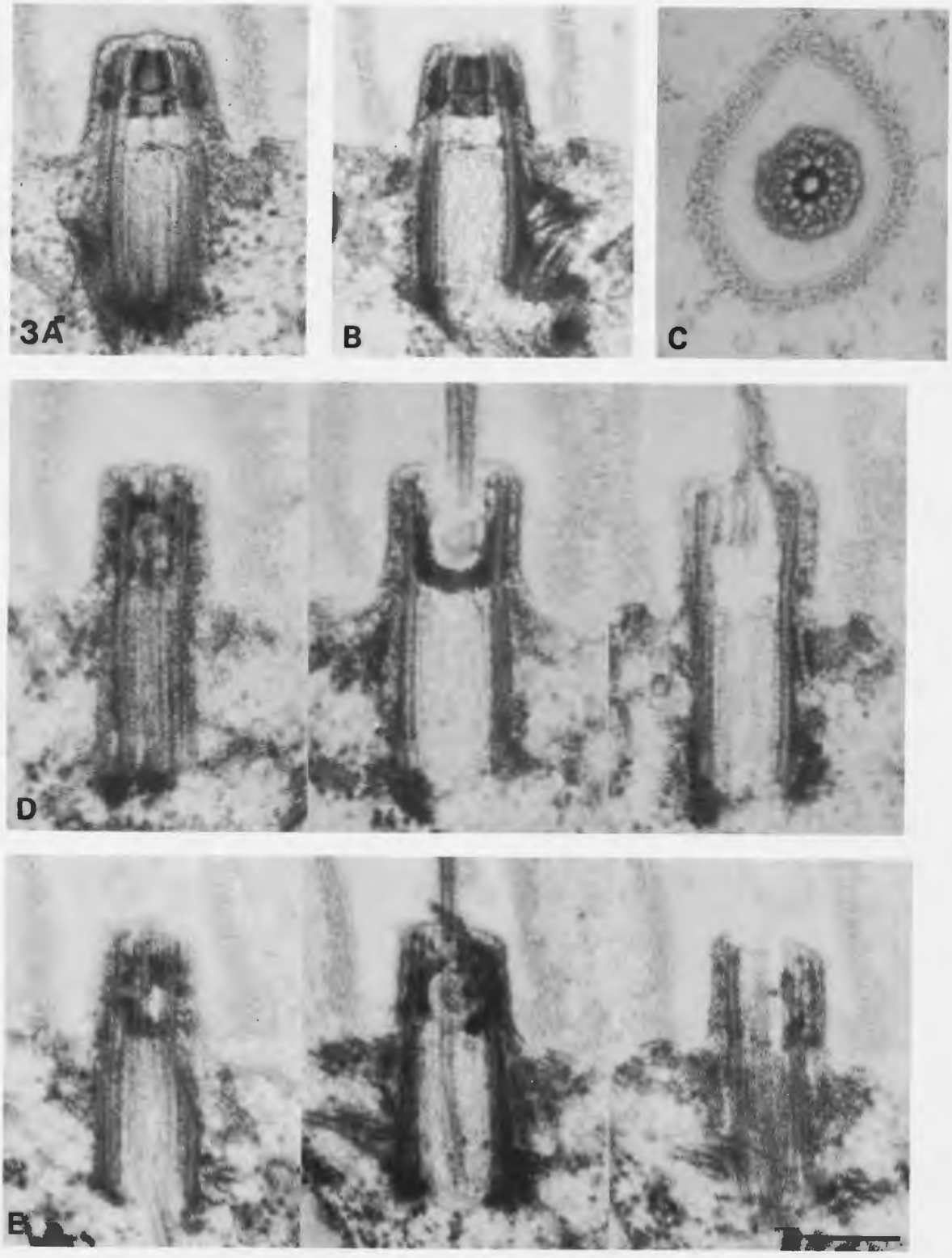

Fig. 3. Transition region in deflagellated wild-type and *vfl-2* cells. (A–C) Wild-type (strain NO^+). Note distal narrowing of axoneme and transition cylinder. (D,E) Two sets of consecutive serial sections of *vfl-2*. Note absence of narrowing at distal end and penetration by central pair microtubules. Bar, 200 nm.

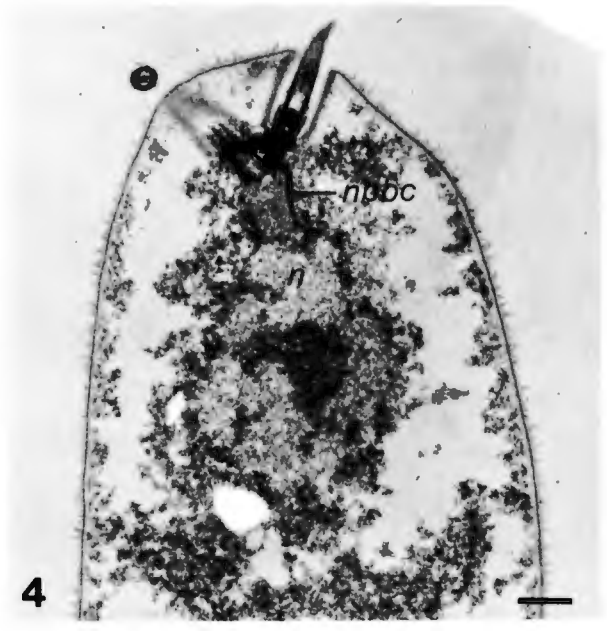

Fig. 4. Wild-type cell ghost (strain *137c*) showing nucleus–basal body connector. *nbbc*, nucleus–basal body connector; *n*, remnant of nucleus. Bar, 500 nm.

severed axoneme and through the tip of the flagellar stump. Two sets of consecutive serial sections showing this are included in Fig. 3. The appearance of the electron-dense amorphous material in the *vfl-2* transition regions did not seem to change as a consequence of deflagellation, although, because of the variability from case to case, we cannot be certain that there was no change at all.

In both wild type and *vfl-2* the point of severing corresponded closely to the most cell-distal end of the transitional membrane. Thus the pre-deflagellation lengths from the base of the basal body to the end of the transitional membrane were 582+47 nm (n=10) for wild type and 603+33 nm (n=10) for *vfl-2*, and the post-deflagellation lengths from the base of the basal body to the end of the flagellar stump were 556+24 nm (n=8) for wild type and 553+33 nm (n=22) for *vfl-2*.

vfl-2 *cells lack nucleus–basal body connectors*

We knew from previous analysis that *vfl-2* cells are defective for the centrin-containing fibers that link the basal bodies to the nucleus (Wright *et al.* 1989). Electron microscopy extended this result to the ultrastructural level. To make visualization of the NBBC easier, we examined cell ghosts that had been extracted with the nonionic detergent NP-40 prior to fixation (Kimaya and Witman, 1984); extractions were performed in calcium-free medium to prevent contraction of the NBBCs (Wright *et al.* 1985). A wild-type example is shown in Fig. 4. The observation of NBBCs in wild-type cell ghosts was common, with one or two connectors apparent in about 40% of all sections that contained the nucleus and both basal bodies. In identically prepared *vfl-2* cells, however, NBBCs were never observed, despite examination of many dozens of appropriate sections.

vfl-2 *lacks distal striated fibers*

The *vfl-2* basal body apparatus was completely devoid of the prominent centrin-containing distal striated fibers that link the members of each wild-type basal body pair together (Fig. 5). Observations of numerous *vfl-2* basal bodies in a variety of preparations – including whole cells, cell ghosts, and free nucleo-flagellar apparatuses – never revealed a complete or partial distal striated fiber, even in the not-infrequent cases in which the basal bodies were found in pairs. *vfl-2* is thus more defective with respect to the distal striated fiber than the previously described mutants *vfl-1* and *vfl-3*, in which many basal bodies carry partially assembled distal striated fibers (Wright *et al.* 1983; Adams *et al.* 1985).

Discussion

vfl-2 *and the protein centrin*

The ultrastructural results reported here demonstrate that *vfl-2* is defective for all cellular structures known to contain the protein centrin: the nucleus–basal body connectors (NBBCs), the distal striated fibers, and the stellate fibers. These observations are fully consistent with our previously reported light-microscopic results showing that centrin immunostaining in *vfl-2* was absent in the region of the NBBC and unusually weak at or near the basal bodies (Wright *et al.* 1989). The *vfl-2* phenotype is unique; we have examined approximately 20 other *vfl* mutants by electron microscopy and none has shown this phenotype. Whether the *vfl-2* mutation is in the structural gene for centrin or in a gene involved in regulating centrin gene expression or centrin function is unknown.

The *vfl-2* transition region is defective with respect to four distinct structures: the stellate fibers, the transition cylinders, the annular connections, and the transition cylinder fibers. Since *vfl-2* has a general defect with respect to centrin, and since evidence exists to show that centrin is localized in the stellate fibers (Melkonian *et al.* 1988; Sanders and Salisbury, 1989), we think it likely that the mutant's primary transition region defect is in the stellate fibers, with the defects in the other three components arising as secondary consequences.

The transition region as a physical barrier to the penetration of the basal body by the central pair microtubules

As documented in this paper, the mutant *vfl-2* is grossly defective in the transition region and it frequently suffers a penetration of the central pair microtubules into the basal body. On the basis of these facts, as well as other considerations discussed below, we propose that an important function of the structures in the inner zone of the normal transition region is to act as a physical barrier to prevent the central pair microtubules from entering the basal body.

The immediate barrier to central pair penetration appears to be the distal transition cylinder, with the stellate fibers appearing to hold the cylinder in place in the center of the transition region. Perhaps the stellate fibers serve as rigid struts that keep the transition cylinder in position. Alternatively, they could serve as tensile elements, all pulling on the transition cylinder to keep it centered. Given what is known about the physical properties of centrin and centrin-containing fibers (Salisbury *et al.* 1984), the latter possibility seems the more likely. Indeed, if the stellate fibers are continuously in tension, then the observation of transverse contraction of the axoneme in association with flagellar autotomy makes immediate sense, since, once the microtubules have been

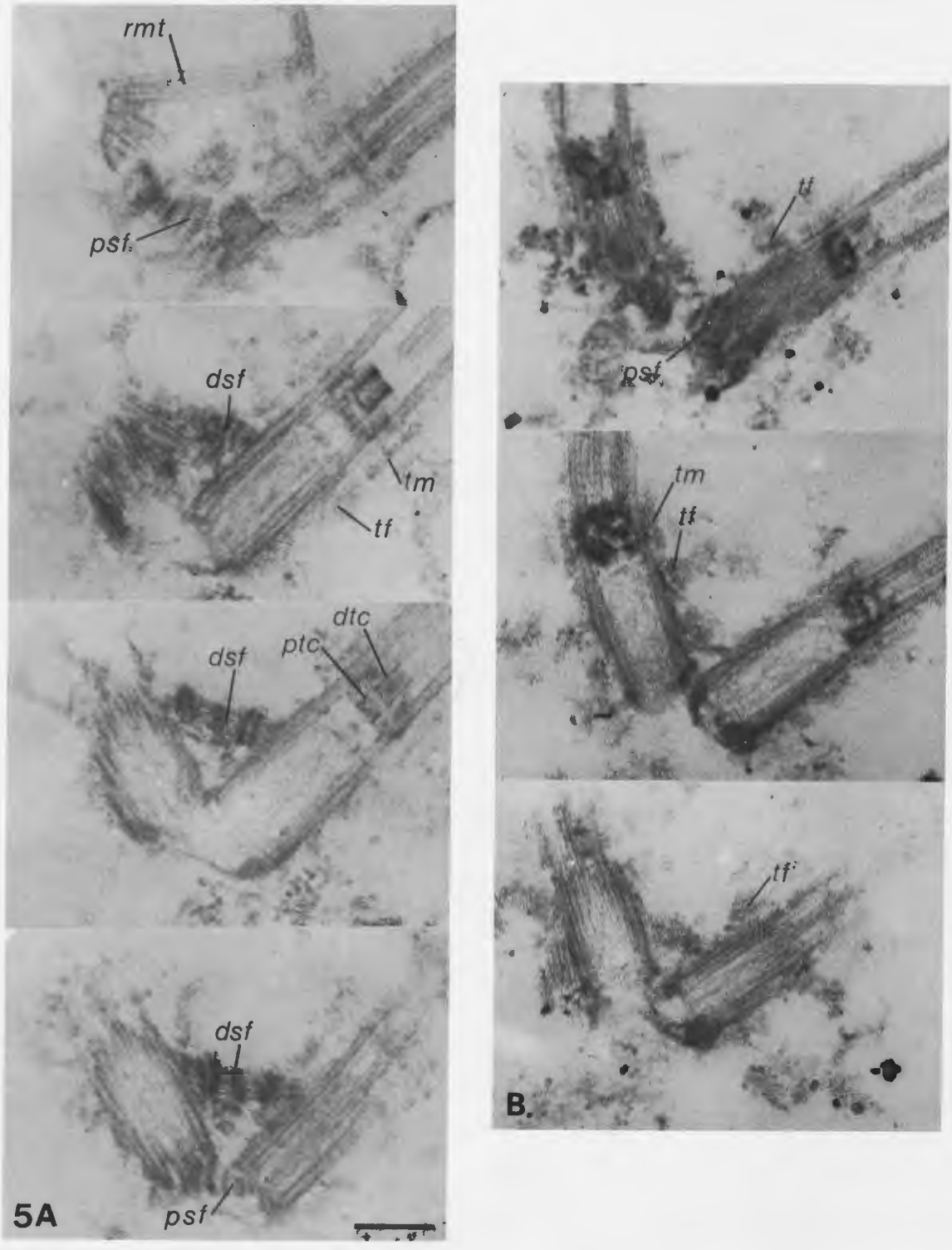

Fig. 5. Distal striated fibers in wild type but not *vfl-2*. (A) Consecutive serial sections through a basal body pair in a lysate containing wild-type nucleo-flagellar apparatuses (strain *cw92*). (B) Consecutive serial sections through a basal body pair in a *vfl-2* lysate (*cw15*, *vfl-2*). Note that the features in the *vfl-2* sections that superficially resemble partially assembled distal striated fibers are in fact transitional fibers. *dsf*, distal striated fiber; *dtc*, distal transition cylinder; *psf*, proximal striated fiber; *ptc*, proximal transition cylinder; *rmt*, rootlet microtubules; *tf*, transitional fiber; *tm*, transitional membrane. Bar, 200 nm.

severed, they ought to bend at their ends in response to transverse force.

There exists a reasonable, and rather obvious, reason for keeping the central pair microtubules out of the basal body. As the flagellum beats, any central pair microtubules that pass into the basal body would move about inside it and quite probably compromise its structure. To our knowledge, penetration of the central pair microtubules into the basal body is not a standard feature of basal body ultrastructure in any organism. This generalization supports our expectation that such penetration would be deleterious, and that it would have been selected against in evolution. Indeed, we know of only one report of central pair microtubules inside basal bodies (Melkonian and Peveling, 1987), and this was in zoospores rather than in vegetative cells.

The transition region and the control of flagellar microtubule assembly

The hypothesis that the transition region represents a barrier to the central pair microtubules may have significance with respect to the question of how the lengths of the outer doublet and central pair microtubules are coordinately controlled. In particular, we propose that the transition region regulates central pair assembly by physically limiting the lengths to which the central pair microtubules can grow. We reason as follows. The outer doublet microtubules are continuous with the A and B microtubules of the basal body, whereas the central pair microtubules are not continuous with microtubules in the basal body and are suspended, as it were, within the axoneme. During flagellar growth, elongation of the outer doublet microtubules occurs at their cell-distal ends (Witman, 1975), and growth of the central pair seems to occur there as well (Dentler, 1987). Growth of the central pair microtubules, if it were even slightly more rapid than growth of the doublets, would tend to push the central pair into the basal body, whereas if it were even slightly slower it would produce a substantial gap between the transition region and the end of the central pair. Such a gap is never seen in wild type, which is consistent with a model in which the intrinsic assembly rate of the central pair microtubules is greater than that of the outer doublets with transition region structures preventing the central pair microtubules from entering the basal body. An alternate, but formally similar, possibility is that the transition region normally promotes depolymerization of the central pair microtubules.

The presence of central pair microtubules inside the basal bodies was frequent but not universal in *vfl-2*. This heterogeneity could reflect the fact that the relative growth rates of central pair and outer doublet microtubules are very similar, so that random fluctuations determine that some basal bodies are penetrated and some are not. Alternatively, the heterogeneity in basal body penetration could reflect heterogeneity in the transition regions themselves, with some representing effective barriers to central pair penetration and some not.

Comparative ultrastructure of the transition region inner zone

For the green algae and higher plants, the literature on transition region ultrastructure has been critically reviewed (Moestrup, 1982; Melkonian, 1984). On the basis of these reviews we can state with considerable confidence that in these organisms inner zone structures that could represent barriers to the central pair microtubules are generally present in some form or another. Even a known counter-example can be viewed as an 'exception that proves the rule': in certain euglenoids the transition region is free of internal structures, but here the axoneme also lacks central pair microtubules in its cell-proximal portion (Dynesius and Walne, 1975), making an inner zone barrier unnecessary.

It is tempting to speculate that barriers to central pair microtubule penetration are general features of transition region inner zones in all taxa. Indeed, our hypothesis may help to explain the great natural diversity of transition region inner zone ultrastructure, since the structural constraints on an element that serves as a barrier ought to be relatively relaxed. Numerous examples can be cited of transition region structures that might act as barriers to the central pair microtubules (e.g. see Pitelka, 1974; Holley, 1982; Hard and Reider, 1983; Arima *et al.* 1984; Sandoz *et al.* 1988), but the critical question is whether there also exist cases *without* such structures. To our knowledge, all relevant transition regions that have been examined with appropriate care have shown evidence of possible barrier structures, but we must also state that the literature on basal body ultrastructure is immense and that we have not attempted to survey it comprehensively.

Flagellar autotomy does not depend upon lateral contraction in the transition region

Sanders and Salisbury (1989) recently confirmed earlier results showing that the calcium-binding protein centrin is generally present in green algal transition regions (Melkonian *et al.* 1988) and that autotomy involves a narrowing of the diameter of the distal transition cylinder just below the abscission point (Lewin and Lee, 1985). They presented ultrastructural evidence for contraction of the stellate fibers at or near the abscission point, and they concluded from these observations that flagellar autotomy is *caused* by mechanical shearing of the microtubules as the result of centrin-based contraction of the stellate fibers. Our results are not supportive of this conclusion for three reasons. (1) *vfl-2* cells are capable of effective flagellar autotomy, indicating that the phenomenon does not depend on properly assembled transition cylinders or organized stellate fibers. (2) The abscission point in *vfl-2* is close to the cell-distal end of the transitional membrane where, in many cases, no misassembled presumptive transition cylinder or stellate fiber material is present. (3) Autotomy in *vfl-2* occurs without detectable narrowing of the axoneme.

Even though outer doublet severing can occur without lateral contraction of the flagellum, it is clear that in wild type a considerable narrowing of axoneme does occur just below the abscission point, and it is reasonable to attribute this to contraction of the stellate fibers. On the basis of our present knowledge, however, the case can be made that the phenomenon has no functional significance with respect to autotomy itself, but rather, as discussed earlier in this paper, reflects the fact that the fibers are normally in tension in order to hold the transition cylinders in place. In this context, it is worth noting that in a wide variety of organisms flagellar autotomy occurs at the transition region without apparent transverse contraction (Kennedy and Brittingham, 1968; Blum, 1971; Satir *et al.* 1976; Boisville-Ulrich, 1980; Lewin *et al.* 1982; Verra *et al.* 1990)

What might be the mechanism of outer doublet scission?

If centrin-based lateral contraction of the flagellum in the transition region is not the cause of flagellar abscission,

then what is? One intriguing possibility is that there exists a microtubule-severing activity in the transition region that is activated by the deflagellation signal (Lewin and Lee, 1985). The substrate for such an activity – the outer doublet microtubules at the abscission point – might contain a distinct form of tubulin (Oakley *et al.* 1990) or tektin (Linck and Langevin, 1982), or they could be differentiated by the presence (or absence, as the case might be) of one or more microtubule-associated proteins. In this context it should be noted that evidence has recently been reported for the presence in *Xenopus* oocytes of a microtubule severing activity whose substrate is thought to be cytoskeletal microtubules (Vale, 1991).

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References

Adams, G. M. W., Wright, R. L. and Jarvik, J. W. (1985). Defective temporal and spatial control of flagellar assembly in a variable flagellar number mutant of *Chlamydomonas reinhardtii*. *J. Cell Biol.* **100**, 955–964.

Arima, T., Shibata, Y. and Yamamoto, T. (1984). A deep-etching study of guinea pig tracheal cilium with special reference to the ciliary transitional region. *J. Ultrastruct. Res.* **89**, 34–41.

Besharse, J. C. and Horst, C. J. (1990). The photoreceptor connecting cilium: a model for the transition zone. In *Ciliary and Flagellar Membranes* (ed. R. Bloodgood), pp. 389–417. Plenum Publishing, New York.

Blum, J. J. (1971). Existence of a breaking point in cilia and flagella. *J. theor. Biol.* **33**, 257–263.

Boisville-Ulrich, E., Sandoz, D. and Chailley, B. (1980). A thin section and freeze-fracture study of deciliation in bird oviduct. *Biol. Cell* **37**, 261–268.

Cavalier-Smith, T. (1974). Basal body and flagellar development during the vegetative cell cycle and the sexual cycle of *Chlamydomonas reinhardii*. *J. Cell Sci.* **16**, 529–556.

Dentler, W. (1987). Cilia and flagella. *Int. Rev. Cytol. Suppl. 17*, 391–456.

Dynesius, R. A. and Walne, P. L. (1975). Ultrastructure of the reservoir and flagella in *Phacus pleuronectes* (Euglenophyceae). *J. Phycol.* **11**, 125–130.

Gibbons, I. R. (1961). The relation between the fine structure and direction of beat in gill cilia of a lamellibranch mollosc. *J. biophys. biochem. Cytol.* **11**, 179–205.

Gilula, N. B. and Satir, P. (1972). The ciliary necklace. A ciliary membrane specialization. *J. Cell Biol.* **53**, 494–509.

Goodenough, U. (1983). Motile detergent-extracted cells of *Tetrahymena* and *Chlamydomonas*. *J. Cell Biol.* **96**, 1610–1621.

Gross, C. H., Ranum, L. P. W. and Lefebvre, P. A. (1988). Extensive restriction fragment length polymorphisms in a new isolate of *Chlamydomonas reinhardtii*. *Curr. Genet.* **13**, 503–508.

Hard R. and Reider, C. L. (1983). Mucociliary transport in newt lungs: the ultrastructure of the ciliary apparatus in isolated epithelial sheets and in functional triton-extracted models. *Tissue & Cell* **15**, 227–243.

Harris, E. (1989). *The* Chlamydomonas *Sourcebook: A Comprehensive Guide to Biology and Laboratory Use*. Academic Press, San Diego, CA.

Holley, M. C. (1982). The control of anthozoan cilia by the basal apparatus. *Tissue and Cell* **14**, 607–620.

Hoops, H. J., Wright, R. L., Jarvik, J. W. and Witman, G. B. (1984). Flagellar waveforms and rotational orientation in a *Chlamydomonas* mutant lacking normal striated fibers. *J. Cell Biol.* **98**, 818–824.

Hoshaw, R. W. and Ettl, H. (1966). *Chlamydomonas smithii* sp. nov. – a Chlamydomonad interfertile with *Chlamydomonas reinhardtii*. *J. Phycol.* **2**, 93–96.

Huang, B., Watterson, D. M., Lee, V. D. and Schibler, M. J. (1988). Purification and characterization of a basal body-associated Ca^{2+}-binding protein. *J. Cell Biol.* **107**, 121–131.

Huber, M. E., Wright, W. G. and Lewin, R. A. (1986). Divalent cations and flagellar autotomy in *Chlamydomonas reinhardtii* (Volvocales, Chlorophyta). *Phycologia* **25**, 408–411.

Hyams, J. S. and Davies, D. R. (1972). The induction and characterization of cell wall mutants of *Chlamydomonas reinhardtii*. *Mutat. Res.* **14**, 381–389.

Kamiya, R. and Witman, G. B. (1984). Subbicromolar levels of calcium control the balance between the beating of the two flagella in demembranated models of *Chlamydomonas reinhardtii*. *J. Cell Biol.* **98**, 97–107.

Kaneshiro, E. (1989). Lipids of ciliary and flagellar membranes. In *Ciliary and Flagellar Membranes* (ed. R. Bloodgood), pp. 241–263. Plenum Publishing, New York.

Kennedy, J. R. and Brittingham, E. (1968). Fine structure changes during chloral hydrate deciliation of *Paramecium caudatum*. *J. Ultrastruct. Res.* **22**, 530–545.

Kuchka, M. and Jarvik, J. (1982). Analysis of flagellar size control using a mutant of *Chlamydomonas reinhardtii* with a variable number of flagella. *J. Cell Biol.* **92**, 170–175.

Lewin, R. A. and Lee, K. W. (1985). Autotomy of algal flagella: electron microscope studies of *Chlamydomonas* (Chlorophyceae) and *Tetraselmis* (Prasinophyceae). *Phycologia* **24**, 311–316.

Lewin, R. A., Lee, T.-H. and Fang, L.-S. (1982). Effects of various agents on flagellar activity, flagellar autotomy and cell viability in four species of *Chlamydomonas* (Chlorophyta:Volvocales). In *Prokaryotic and Eukaryotic Flagella* (ed. W. B. Amos and J. G. Duckett), *Soc. Exp. Biol. Symp.* vol. 35, pp. 421–437. Cambridge University Press, Cambridge.

Linck, R. W. and Langevin, G. L. (1982). Structure and chemical composition of insoluble filamentous components of sperm flagellar microtubules. *J. Cell Sci.* **58**, 1–22.

Melkonian, M. (1984). Flagellar apparatus ultrastructure in relation to green algal classification. In *Systematics of the Green Algae* (ed. D. E. G. Irvine and D. M. John), pp. 73–120. Academic Press, London and Orlando.

Melkonian, M. and Peveling, E. (1987). Zoospore ultrastructure in species of *Trebouxia* and *Pseudotrebouxia* (*Chlorophyta*). *Pl. Syst. Evol.* **158**, 183–210.

Melkonian, M., Schulze, D., McFadden, G. I. and Robenek, H. (1988). A polyclonal antibody (anticentrin) distinguishes between two types of fibrous flagellar roots in green algae. *Protoplasma* **144**, 56–61.

Moestrup, O. (1982). Flagellar structure in algae: a review, with new observations, particularly on the Chrysophyceae, Phaeophyceae (Fucophyceae), Euglenophyceae and *Reckertia*. *Phycologia* **21**, 427–528.

Musgrave, A., de Wildt, P., van Etton, I., Scholma, C., Kooyman, R., Homan, W. and van den Ende, H. (1986). Evidence for a functional membrane barrier in the transition zone between the flagellum and cell body of *Chlamydomonas eugamatos* gametes. *Planta* **167**, 544–553.

Oakley, B. R., Oakley, C. E., Yoon, Y. and Jung, M. K. (1990). γ-tubulin is a component of the spindle pole body that is essential for microtubule function in *Aspergillus nidulans*. *Cell* **61**, 1289–1301.

Pitelka, D. R. (1974). Basal bodies and root structures. In *Cilia and Flagella* (ed. M. A. Sleigh), pp. 437–469. Academic Press, New York.

Ringo, D. L. (1967). Flagellar motion and fine structure of the flagellar apparatus in Chlamydomonas. *J. Cell Biol.* **33**, 543–571.

Rohlich, P. (1975). The sensory cilium of retinal rods is analogous to the transitional zone of motile cilia. *Cell Tiss. Res.* **161**, 421–430.

Sager, R. and Granick, S. (1953). Nutritional studies with *Chlamydomonas reinhardtii*. *Ann. N.Y. Acad. Sci.* **56**, 831–838.

Salisbury, J. L., Bacon, A. T. and Sanders, M. A. (1988). The centrin-based cytoskeleton of *Chlamydomonas reinhardtii*: distribution in interphase and mitotic cells. *J. Cell Biol.* **107**, 635–642.

Salisbury, J. L., Baron, A. T., Surek, B. and Melkonian, M. (1984). Striated flagellar roots: Isolation and partial characterization of a calcium-modulated contractile organelle. *J. Cell Biol.* **99**, 962–970.

Sanders, M. A. and Salisbury, J. L. (1989). Centrin-mediated microtubule severing during flagellar excision in *Chlamydomonas reinhardtii*. *J. Cell Biol.* **108**, 1751–1760.

Sandoz, D., Chailley, B., Boisvieux-Ulrich, E., Lemullois, M., Laine, M.-C. and Bautista-Harris. G. (1988). Organization and functions of cytoskeleton in metazoan ciliated cells. *Biol. Cell* **63**, 183–193.

Satir. B., Sale, W. S. and Satir, P. (1976). Membrane renewal after dibucaine deciliation of *Tetrahymena*. *Expl Cell Res.* **97**, 83–91.

Snell, W. J., Dentler, W. L., Haimo, L. T., Binder, L. I. and Rosenbaum, J. L. (1974). Assembly of chick brain tubulin onto isolated basal bodies of *Chlamydomonas reinhardtii*. *Science* **185**, 357–360.

Vale, R. D. (1991). Severing of stable microtubules by a mitotically activated protein in *Xenopus* egg extracts. *Cell* **64**, 827–839.

Verra, F., Escudier, E., Pinchon, M.-C., Fleury, J., Bignon, J. and Bernaudin, J.-F. (1990). Effects of local anaesthetics on the structure and function of ciliated respiratory epithelial cells. *Biol. Cell* **69**, 99–105.

Witman, G. B. (1975). The site of *in vitro* assembly of microtubules. *Ann. N.Y. Acad. Sci.* **253**, 178–191.

Wright, R. W. Adler, S. A., Spanier, J. G. and Jarvik, J. W. (1989). The nucleus-basal body connector of *C. reinhardtii*: Evidence for a role

in basal body segregation and against essential roles in mitosis or in determining cell polarity. *Cell Motil. Cytoskel.* **14**, 516–526.

WRIGHT, R. L., CHOJNACKI, B. AND JARVIK, J. W. (1983). Abnormal basal-body number, location, and orientation in a striated fiber-defective mutant of *Chlamydomonas reinhardtii*. *J. Cell Biol.* **96**, 1697–1707.

WRIGHT, R. L., SALISBURY, J. AND JARVIK, J. (1985). A nucleus–basal body connector in *Chlamydomonas reinhardtii* which may function in basal body localization or segregation. *J. Cell Biol.* **101**, 1903–1912.

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