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ULTRASTRUCTURE OF THE FLAGELLAR ROOTS IN *CHLAMYDOMONAS* GAMETES

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SUMMARY

The cytoskeleton of *Chlamydomonas reinhardtii* gametes has been studied by electron microscopy. The microtubular system, consisting of four flagellar roots inserted into the basal apparatus, is shown to include two daughter basal bodies and two striated fibres, newly described in this report. One new fibre associates with the 3-over-1 root and is similar to its counterpart, the striated fibre of the 2-member root. These similar root fibres connect each daughter basal body to the V-shaped microtubular root pair. The other new striated fibre joins the daughter basal body to both flagellar roots and is similar to the proximal striated fibre. In mt^+ gametes, the conventional root microtubules make direct contact with the doublet zone of the non-activated mating structure. During activation, doublet zone microfilaments associate with the daughter basal body and the finely striated fibre of the 3-over-1 root. These observations suggest that the cytoskeleton acts as a scaffolding for membrane extension by the mt^+ mating structure microfilaments.

INTRODUCTION

Our understanding of the organization of the cytoskeleton of *Chlamydomonas reinhardtii* is based on the classical description by Ringo (1967) and several subsequent reports (Cavalier-Smith, 1974; Goodenough & Weiss, 1978; Gould, 1975; Hyams & Borisy, 1975). These investigations present the flagellar root system as an X-shaped arrangement, stabilized at the centre by the basal apparatus and along one root, the compound root, by a finely striated fibre. Because the only reported connection of the cytoskeleton is limited to the centre (Ringo, 1967; Goodenough & Weiss, 1978) and because current models do not accurately reflect how the microtubule sets intersect in this zone, a major question that arises is how the cytoskeleton with four independent roots is organized to absorb effectively the stress of agglutination and at the same time provide the necessary support or scaffolding for mating structure activation. This study of *Chlamydomonas* gametes provides new details of the intersection of the flagellar roots and their attachment to daughter basal bodies and the mating structure.

MATERIALS AND METHODS

Gametes of wild-type strains, RC3 mt^+ and NO mt^- were prepared from 7-day-old plates as described (Martin & Goodenough, 1975). Low-temperature cell fusion was performed at 11 °C for 25 min. Fixation methods for electron microscopy have already been described (Millikin & Weiss, 1984). Tannic acid fixation was similar to a procedure recently reported (Goodenough, Detmers & Hwang, 1982). Micrographs were taken with a Philips 300 electron microscope.

RESULTS

Striated fibres of the cytoskeleton

The cytoskeleton consists of the flagellar apparatus and four sets of microtubules. Two of the sets are 3-over-1 roots and the other two are 2-member roots with a striated fibre (Goodenough & Weiss, 1978). My serial sectioning has revealed two new striated fibres associated with the cytoskeleton. The arrangement of these two fibres is presented diagrammatically in Fig. 1. The first fibre is a finely striated 3-over-1 root fibre, which together with the 2-member root fibre joins each daughter basal body to the cytoskeleton. The second new fibre, the lateral striated fibre, connects the daughter basal body to the 2-member root. Striated fibres appear to associate with microtubules or basal bodies (Melkonian, 1980), joining together the various parts of the cytoskeleton. Each fibre contributing to the cytoskeleton will be considered below.

Flagellar root intersection

The 2-member root microtubules terminate in the basal body zone, but the root fibre is continuous and passes between the basal bodies (Goodenough & Weiss, 1978). Fig. 2 illustrates the first detectable intersection of the two roots. Of interest here is

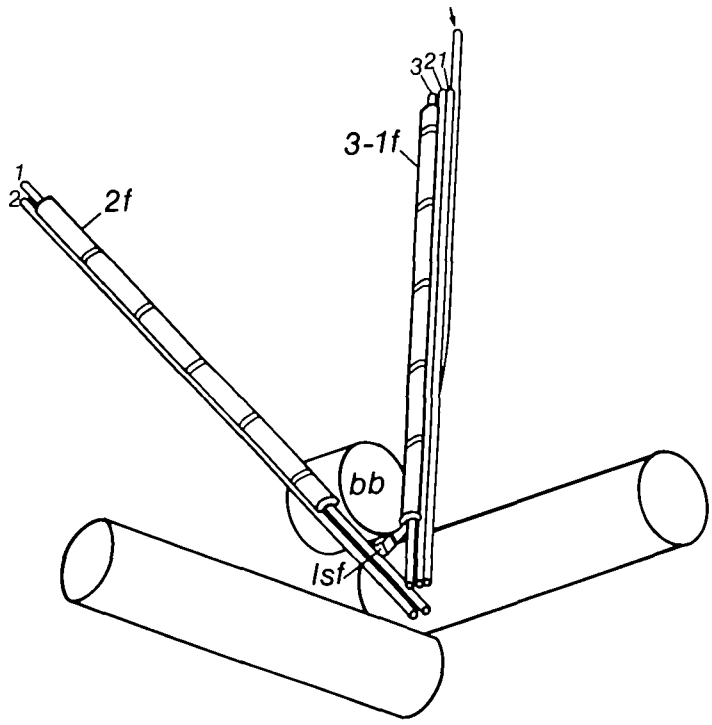


Fig. 1. Diagrammatic illustration of the arrangement of the flagellar apparatus. The 3-over-1 root (*3-1f*) enters the basal body (*bb*) from the right and intersects the 2-member root. The flagella appear as long cylinders; microtubules are labelled *1–3*; arrow marks 'over-1' microtubule; *2f*, 2-member root fibre; *lsf*, lateral striated fibre.

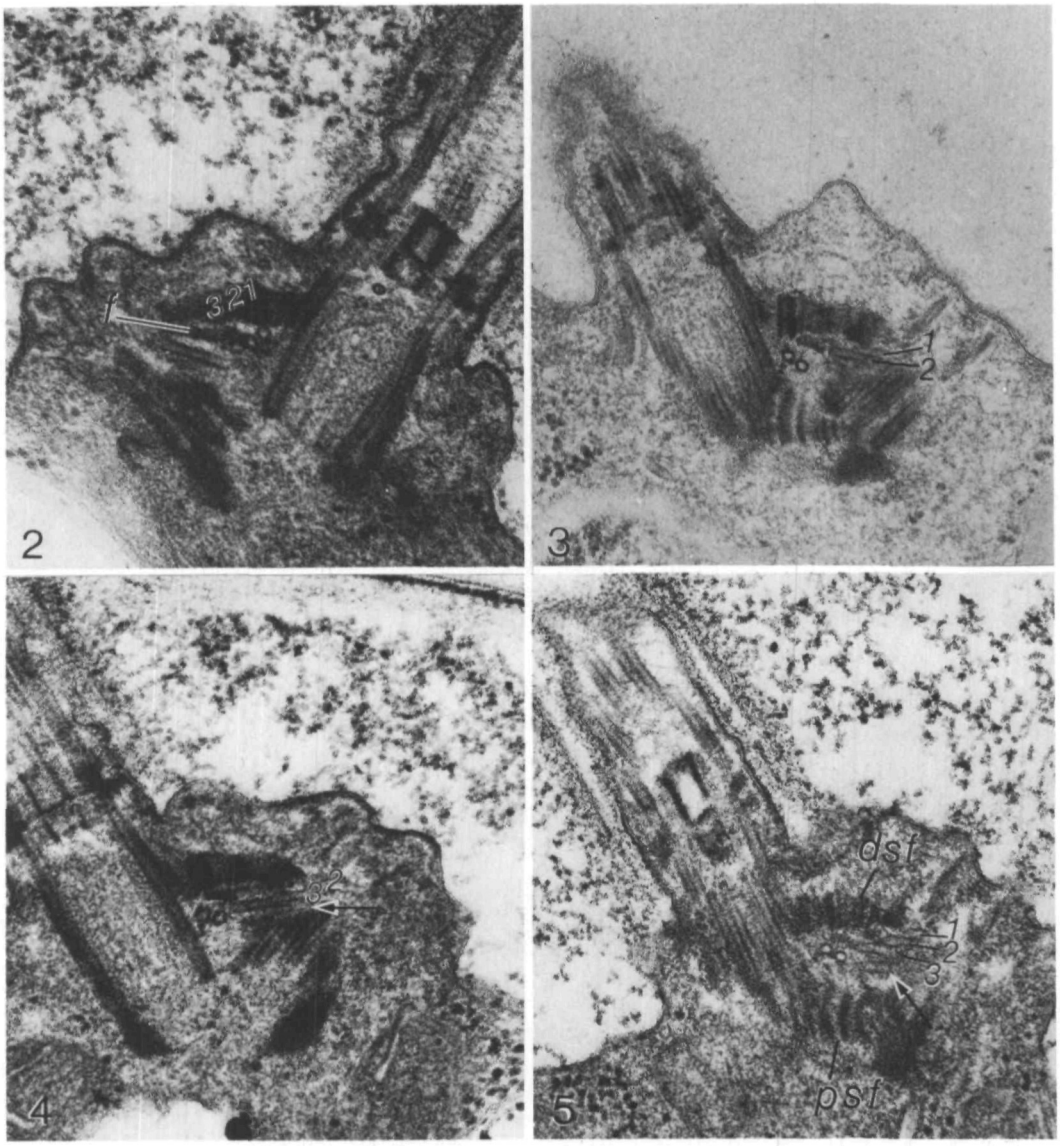


Fig. 2. Transverse section through 3-over-1 root near the basal body. The three tubules, labelled *1–3*, are tilted at an angle relative to the finely striated fibre (*f*) of the 2-member root. The fibre, shown in oblique longitudinal section, intersects the central microtubule (no. *2*) in the row of three. ×82 000.

Fig. 3. Intersection of the 3-over-1 root microtubule no. *2* and 2-member root striated fibre. Longitudinal section of microtubule no. *2* as it contacts the 2-member root at the edge of the striated fibre and terminates. A separation of the fibre from the 2-member root microtubule pair is shown. ×82 000. (Compare with Fig. 2.)

Fig. 4. Insertion of 3-over-1 root microtubule no. *3* into the 2-member root. Microtubule no. *3* is inserted between the finely striated fibre and the underlying 2-member root microtubule. 3-over-1 root microtubule no. *2* and an extra microtubule (arrow) can also be seen. ×82 000.

Fig. 5. Extra 3-over-1 root microtubule. Three flagellar root microtubules (*1–3*) appear above an extra microtubule (arrow), which terminates beneath the 2-member root; *psf*, proximal striated fibre; *dsf*, distal striated fibre. ×82 000.

the location of the three transversely sectioned 3-over-1 root microtubules with respect to the 2-member root striated fibre. The point of intersection with the fibre (*f*), occurs in the centre of the three microtubules. This is more obvious in longitudinal section in Fig. 3, which shows the central microtubule (no. *2*) in contact with the edge of the striated fibre. As seen here and suggested in Fig. 2, 3-over-1 root microtubule no. *1* lies distal to the striated fibre, but the insertion site remains to be determined. The remaining microtubule (no. *3*) ends in the compound root.

Fig. 4 illustrates the insertion of microtubule no. *3* into the 2-member root. The tubule appears in the space between the striated fibre and the underlying root microtubule. This interpretation predicts that in transverse section this microtubule (no. *3*) would appear very close to or below the striated fibre. This is exactly what is seen in Fig. 2, in which the tubule appears to approach the 2-member root below the striated fibre. It is also logical to expect some overlap of the fibre and the microtubule (no. *3*), since the fibre in Fig. 2 appears in oblique section, but Fig. 4 suggests that the fibre and the microtubule are closely associated. This association was observed in two sets of serial sections and in several other micrographs, which presented different views of the insertion of microtubule no. *3*. Here also, even the arrangement was similar. For example, Fig. 5 shows microtubule no. *3* as it approaches the insertion site in the 2-member root and other micrographs illustrated the insertion of the microtubule viewed from a distal location. Finally, in Fig. 4, 3-over-1 root microtubule no. *2* is vaguely apparent in longitudinal section at the level of the striated fibre, as is an extra microtubule (arrow) seen below the 3-over-1 root.

The number of 3-over-1 root microtubules in the root intersection site is variable. Fig. 2 shows only three of the 3-over-1 root microtubules, but Fig. 5 illustrates these three tubules and shows an extra microtubule (arrow) beneath the root. This interpretation is further supported by the microtubule in Fig. 4 (arrow), which lies below the 3-over-1 root.

Association of daughter basal bodies with the cytoskeleton

An important feature of the basal apparatus is that the daughter basal bodies lie nearby in the cytoplasm. When the basal apparatus is isolated, the daughter basal bodies usually remain with the basal body complex (Gould, 1975; Cavalier-Smith, 1974), indicating that they might be physically attached. Serial sectioning suggests that attachment includes three striated fibres, i.e. two striated flagellar root fibres and a third fibre resembling the proximal striated fibre (Ringo, 1967).

Fig. 6 illustrates a longitudinal section through the conventional root in an mt^- gamete. Finely striated fibres (*sf*) are seen in association with a narrow zone overlying microtubule no. *3* on each 3-over-1 root. The repeating unit of the fibre consists of a pair of light striations separated by a dark line, an arrangement that is similar to the finely striated 2-member root fibre, but not nearly as obvious. We found, using the 2-member root striations as an internal standard, that the repeat distance was 24 nm, which is exactly the same as the repeat distance of the striations of the 2-member root (Goodenough & Weiss, 1978). Serial sections show that the striated fibre extends from the basal apparatus to the mating structure, but regions beyond this have not been

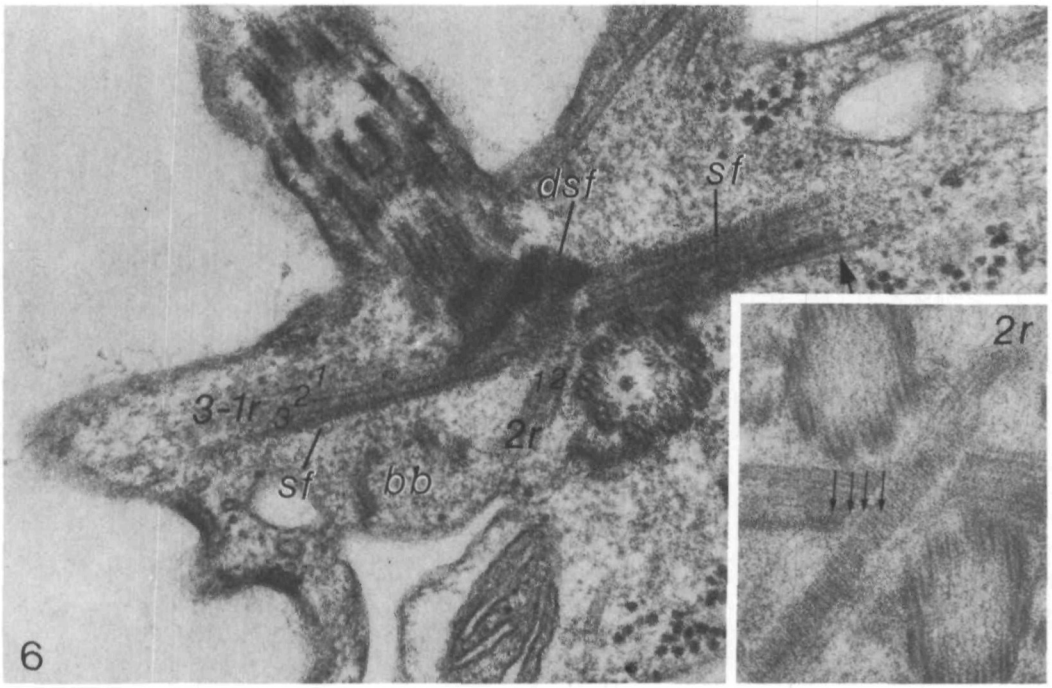
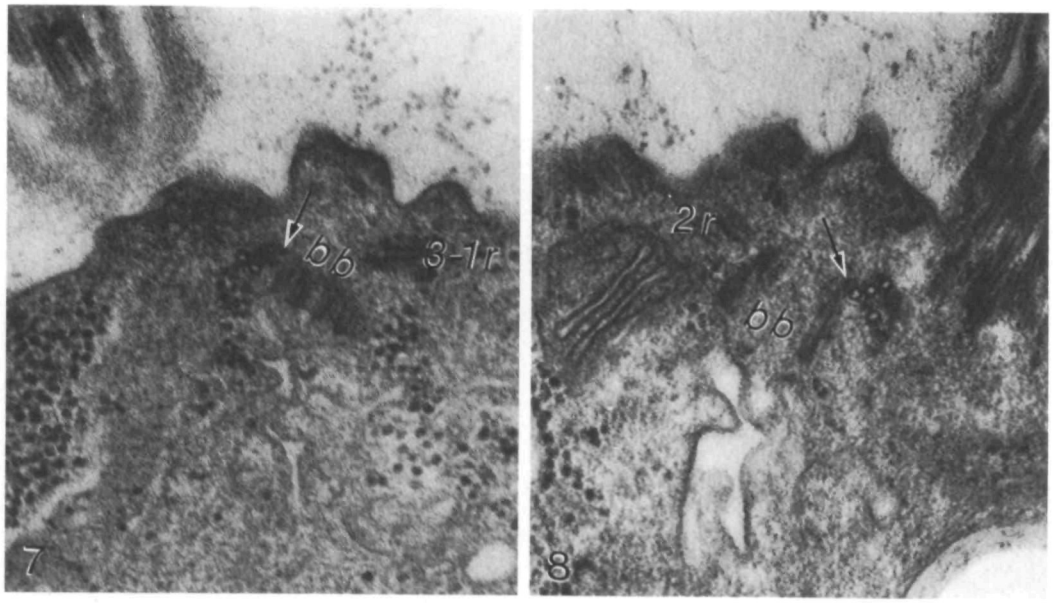


Fig. 6. Finely striated fibre of the 3-over-1 root (*3-1r*). The fine striations of the root preferentially associate with microtubule no. *3*. The striated fibre (*sf*) passes beneath the distal striated fibre (*dsf*) in the region of the basal body. One daughter basal body (*bb*) appears between the 3-over-1 and 2-member (*2r*) roots. The arrow marks an extra root microtubule. Inset: shows intersection of two root fibres in basal body area; the arrows mark striations. ×82 000; inset ×100 000.

examined. Comparison of the two fibres in the inter-basal body region (Fig. 6, inset) suggests that the 2-member fibre lies above and very close to the 3-over-1 fibre. This suggestion is consistent with Figs 2 and 4, which show the 3-over-1 root microtubule no. *3* fibre close to the 2-member root fibre. One additional similarity of particular importance to this study is that both root fibres associate with the medial microtubule and therefore lie very close to the daughter basal body.

The arrangement of both flagellar roots is difficult to see in a transverse section through a daughter basal body because the angle of the V-shaped roots precludes viewing them simultaneously. When viewed separately, however, the finely striated fibres appear to serve as attachment sites, joining the daughter basal body to the cytoskeleton. This is shown in Fig. 7 for the 2-member root where attachment to the daughter basal body apparently occurs at the edge of the fibre and by means of an overlying tubule link (arrow). The observation that the root fibre, the tubule link and the daughter basal body are closely associated and lie in parallel planes, distinct from the horizontal cell surface, supports the suggestion of a firm attachment.

When the 3-over-1 root appears in transverse section, as in Fig. 8, the comparable view of its association with the daughter basal body is seen. The angle of the 3-over-1 root, relative to the daughter basal body, suggests that attachment of the root fibre



Figs 7, 8. Attachment of the daughter basal body to the cytoskeleton.

Fig. 7. Transverse section of the 2-member root showing the finely striated fibre–daughter basal body (*bb*) association. Distal to the fibre is a linking tubule (arrow). The 3-over-1 root (*3-1r*) is shown.

Fig. 8. Transverse section through the 3-over-1 root. The striated fibre overlying microtubule no. *3* and marked by an arrow is attached to the daughter basal body (*bb*). The 2-member root (*2r*) is seen to the left. Figs 7, 8, ×82 000.

(arrow) occurs at the distal surface of the daughter basal body. Other micrographs suggest that the fibre–daughter basal body association may be broad. For example, in Fig. 6 – an oblique section through the daughter basal body – more than one tubule subfibre appears to associate with the striated fibre.

In order to describe the arrangement of the second new fibre, the lateral striated fibre, the orientation of the basal body complex must first be presented. Fig. 9 illustrates the parent and daughter basal bodies in an mt^- gamete. Each daughter basal body (*bb*) is oriented with its longitudinal axis parallel to that of a flagellar basal body (Ringo, 1967). Lying between the parallel basal body and the daughter basal body on the right is a faintly defined lateral striated fibre (*lsf*). It appears in vertical longitudinal section as a series of alternating bands seen close to the daughter basal body. These same bands are shown with slightly more contrast in the left inset; they appear similar to the proximal striated fibre (*psf*). The appearance of the lateral fibre in 'horizontal' oblique section is shown in Fig. 9 on the left side of the basal body. Several characteristic features should be noted. First, the fibre makes direct contact with the daughter basal body, as suggested by the image (Fig. 9) on the right side of the basal body. Second, the cross-striations (*c*) are not as dense as in the left inset (Fig. 9). Third, filamentous connections (arrows) extend from the single striation on the basal body to the paired striations and on towards the compound root. Finally, only the middle zone along the fibre length is seen, so that interpretation of the image is difficult. In

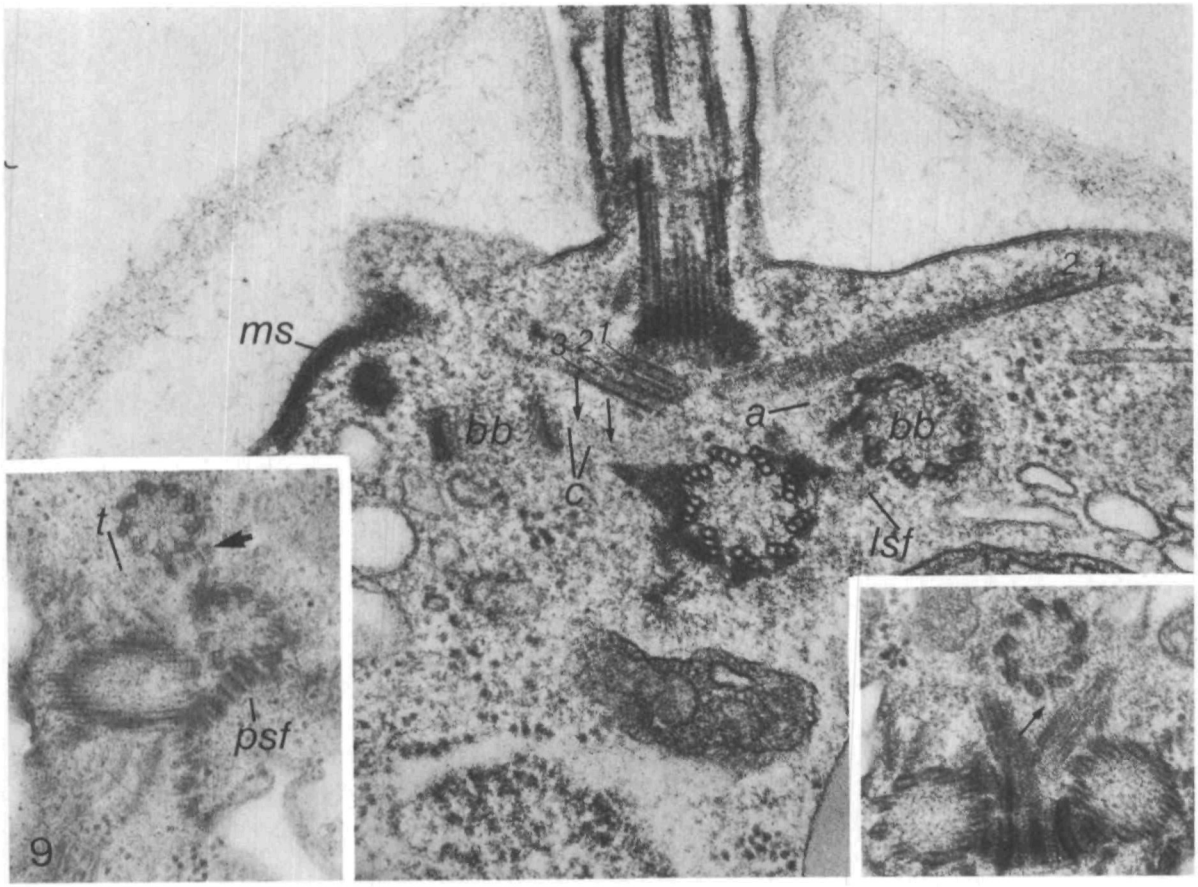
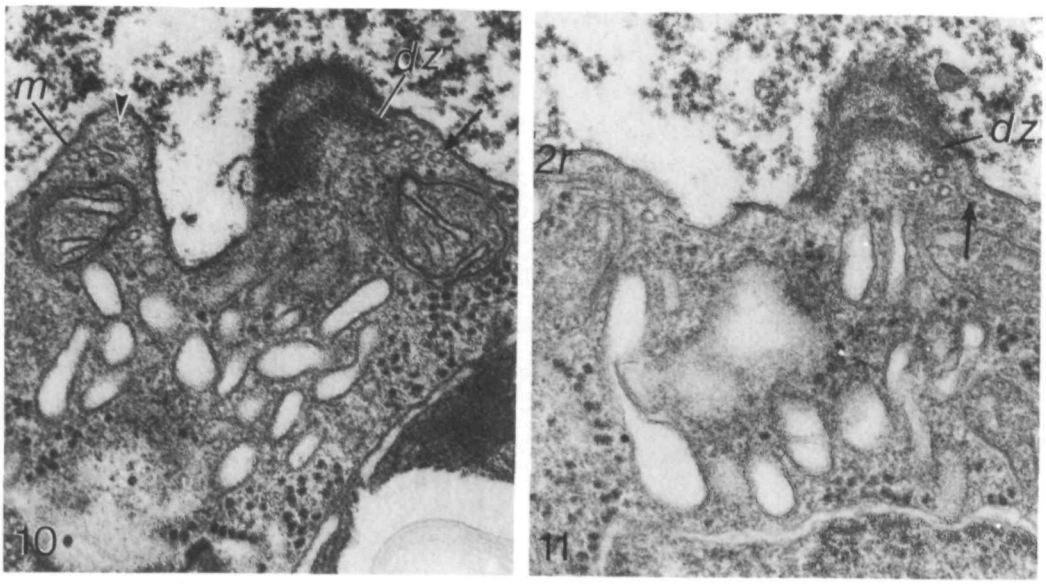


Fig. 9. Lateral striated fibre (*lsf*) in an unactivated mt^- gamete. The fibre appears on the right between the daughter (*bb*) and parent basal bodies and presumably associates with the 2-member root at site *a*. On the left, cross-striations (*c*) extend from the daughter basal body towards the compound root and are connected by filaments (arrows). The 2-member root microtubules on the right are labelled *1* and *2*. The 3-over-1 root microtubules, labelled *1–3*, and the mating structure (*ms*) appear to the left. The left inset shows a lateral striated fibre (arrow) between daughter and parent basal bodies; short tubules (*t*) and proximal striated fibre (*psf*). The right inset shows the connection of a fibre cross-striation (arrow) to the 3-over-1 root. ×82 000; left inset, ×66 000; right inset, ×66 000.

most micrographs the lateral fibre was usually obliquely sectioned, so that again only a part of the fibre length was seen in a single section. The daughter basal body usually appeared in oblique section as well, so that the lateral striated fibre appeared to be unrelated to any distinct structure and resembled section 'noise' that would normally be ignored (e.g. see fig. 3 of Goodenough & Weiss, 1978). Although it is difficult to interpret the small rod-like parts in a single section, serial reconstructions suggest that it extends between the two microtubule roots and joins and daughter basal body as shown in Fig. 1.

Fig. 9 (right inset) shows a rod-like structure, interpreted to be a cross-striation (arrow) of the lateral fibre. The close association of the striation with the microtubule



Figs 10, 11. Non-activated mt^+ mating structures.

Fig. 10. The base of the doublet zone (*dz*) is associated with microtubules 2 and 3 of the 3-over-1 root. On the left are the 3-over-1 root striated fibre and two extra microtubules. One is associated with the distal surface of the fibre (arrowhead) and the other (*m*) with the microtubule root.

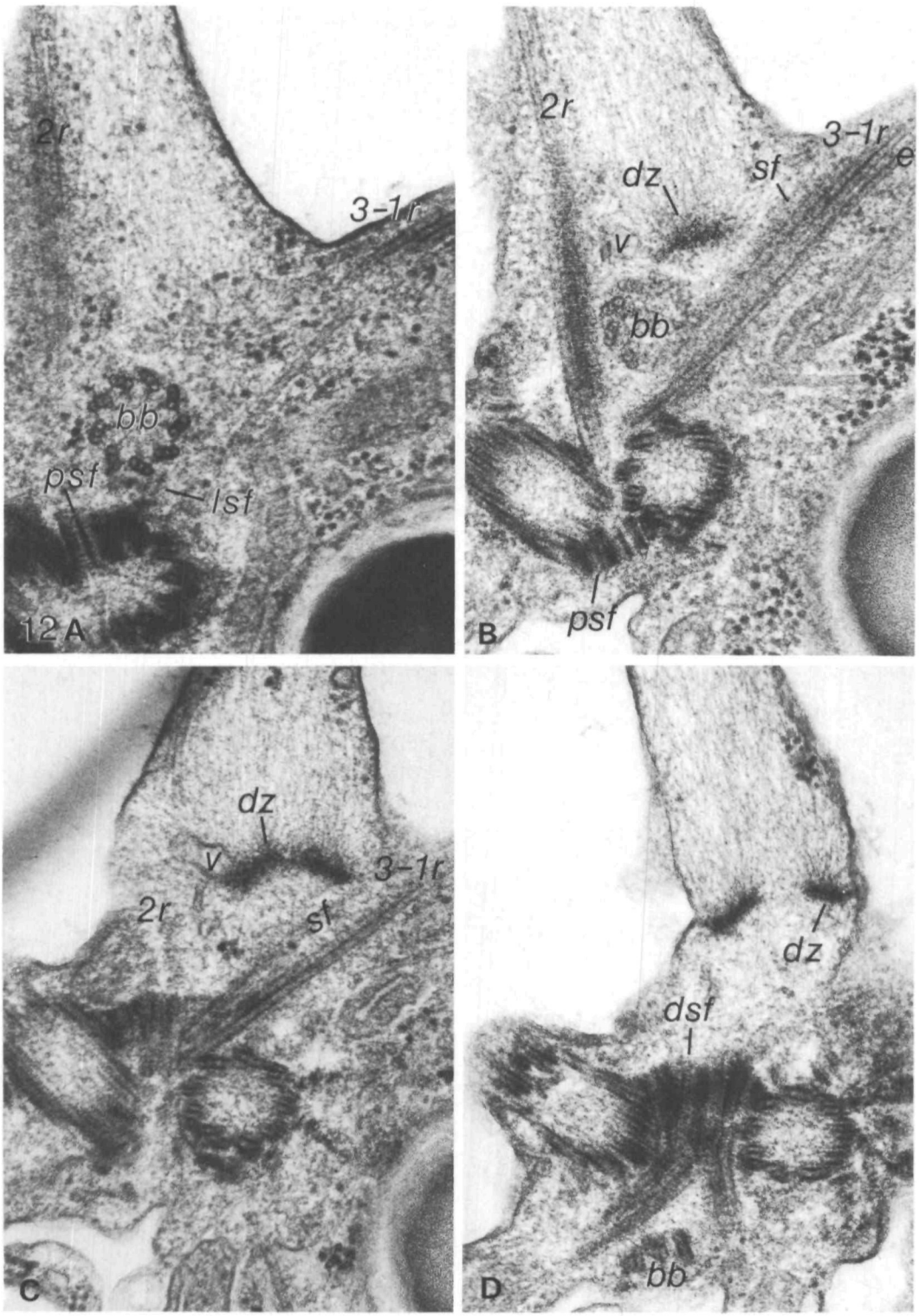
Fig. 11. 3-over-1 root microtubule no. 1 contacts the inner surface of the doublet zone (*dz*). The 3-over-1 root is tilted at a slight angle relative to the cell surface. The 2-member root (*2r*) appears on the left. In Figs 10 and 11, the arrow marks the extra 3-over-1 root microtubule. Tannic acid fixation; ×82 000.

suggests that the end of the lateral fibre is connected to the 3-over-1 root. The comparable association with the compound root is indicated in Fig. 9 at site *a*. Here, no distinct structural connection was observed, but a pair of short tubular structures (Fig. 9, left inset, *t*) appears in the presumed contact zone (Fig. 9, *a*). Several micrographs suggest that these short tubules make direct contact with the 2-member root microtubules, indicating that the fibre may be continuous across the V formed by the two microtubule root sets.

Association of the 3-over-1 root with the mt^+ *mating structure*

The microtubules of the 3-over-1 root lie directly beneath the doublet zone of the mt^+ mating structure. This is readily seen when both the fibre and the doublet zone are cut transversely, as in Figs 10 and 11. In Fig. 10 there is a slight separation of the

Fig. 12. Consecutive sections showing attachment of the activated mt^+ mating structure to the cytoskeleton. In A, the lateral striated fibre (*lsf*) lies between the parent and daughter basal bodies (*bb*); the proximal striated fibre (*psf*) also appears. In B, the activated doublet zone (*dz*) is associated with the daughter basal body and a vesicle (*v*) resembling a coated vesicle; in C, it is associated with the 3-over-1 root striated fibre (*sf*) and the same vesicle; and in D, with the plasma membrane. The 2-member (*2r*) and 3-over-1 (*3-1r*) roots and the distal striated fibre (*dsf*) can also be seen; *e*, single microtubule (B). ×82 000.


2r
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C
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Fig. 12

doublet zone and the underlying microtubules; material that appears to surround the microtubules fills the separation zone. In the case of Fig. 11, one root microtubule makes direct contact with the doublet zone. In each micrograph, the mating structure doublet zone appears to be oriented in essentially the same plane as that formed by the three microtubules of the 3-over-1 root, so that the mating structure lies at an angle, relative to the cell surface.

Each flagellar root is seen to associate with extra microtubules. One extra microtubule (Figs 10, 11, arrows) is associated with the 3-over-1 root and may terminate beneath the 2-member root in the basal body region (Fig. 5). The 2-member root is seen in Fig. 10 as an association of four microtubules, two root microtubules and two extra microtubules. One extra microtubule associates with the striated fibre at its distal surface (arrowhead) and the other (*m*) is associated directly with a root microtubule. The extra microtubules may correspond to the tubules occasionally seen overlying the basal apparatus (Ringo, 1967; Weiss, 1982), but their course through the anterior cytoplasm has not been established.

Contact between the 3-over-1 root and the doublet zone is altered by mating structure activation. As shown in Fig. 12 in consecutive sections, the activated doublet zone makes direct contact with the striated fibre of the 3-over-1 root and the daughter basal body. The contact sites also include short microtubules and membrane vesicles. As shown in Fig. 12B, C, these microtubules lie near the 2-member root and attach to a small vesicle (*v*) associated with the activated doublet zone. The vesicle seems similar to a coated vesicle (Weiss, 1984).

Another feature revealed here by serial sectioning (Fig. 12A, B) is the extra 3-over-1 root microtubule of Figs 10 and 11. This single microtubule (*e*) travels alongside the root in longitudinal section (Fig. 12B), until it approaches the basal apparatus. The tubule then is seen at an angle below the root (Fig. 12B) towards the basal apparatus, and it terminates proximal to microtubule no. *3* (Fig. 12C). The proximal termination is also supported by Fig. 5, which illustrates this extra microtubule beneath the 3-over-1 root.

DISCUSSION

Functional interpretations of striated fibres

Several reports have considered the function of finely striated fibres. Brown, Massalski & Patenaude (1976) suggested that striated microtubule roots in *Polytomella* may serve as microtubule-organizing centres. In another study, Goodenough & Weiss (1978) examined the 2-member root striated fibre, which makes contact with the mating structure. They suggested that the association may have a morphogenetic function or the fibre may function in signal transmission during activation for cell fusion. Several other studies proposed that striated roots might function in absorbing the stress of flagellar motility or in the co-ordination of flagellar motility (Hyams & Borisy, 1975; Ringo, 1967; Salisbury & Floyd, 1978; Stephens, 1975). Stress absorption seems the most relevant function to consider in the present study on *Chlamydomonas* mating, because my results show that the finely striated flagellar

root fibres and the lateral fibre join the cytoskeletal components together in regions that resist the motions of gametic agglutination and at the same time provide support for membrane extension during mating structure activation.

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REFERENCES

BROWN, D. L., MASSALSKI, A. & PATENAUDE, R. (1976). Organization of the flagellar apparatus and associated cytoplasmic microtubules in the quadriflagellate alga *Polytomella agilis*. *J. Cell Biol.* **69**, 106–125.

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.

GOODENOUGH, U. W., DETMERS, P. A. & HWANG, C. (1982). Activation for cell fusion in *Chlamydomonas*: Analysis of wild-type gametes and nonfusing mutants. *J. Cell Biol.* **92**, 378–386.

GOODENOUGH, U. W. & WEISS, R. L. (1978). Interrelationships between microtubules, a striated fiber, and the gametic mating structure of *Chlamydomonas reinhardi*. *J. Cell Biol.* **76**, 430–438.

GOULD, R. R. (1975). The basal bodies of *Chlamydomonas reinhardtii*. Formation from probasal bodies, isolation, and partial characterization. *J. Cell Biol.* **65**, 65–74.

HYAMS, J. S. & BORISY, G. G. (1975). Flagellar coordination in *Chlamydomonas reinhardtii*: Isolation and reactivation of the flagellar apparatus. *Science, N.Y.* **189**, 891–893.

MARTIN, N. C. & GOODENOUGH, U. W. (1975). Gametic differentiation in *Chlamydomonas reinhardtii*. I. Production of gametes and their fine structure. *J. Cell Biol.* **67**, 587–605.

MELKONIAN, M. (1980). Ultrastructural aspects of basal body associated fibrous structures in green algae: A critical review. *BioSystems* **12**, 85–104.

MILLIKIN, B. E. & WEISS, R. L. (1984). Distribution of concanavalin A binding carbohydrates during mating in *Chlamydomonas*. *J. Cell Sci.* **66**, 223–279.

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

SALISBURY, J. L. & FLOYD, G. L. (1978). Calcium-induced contraction of the rhizoplast of a quadriflagellate green alga. *Science, N.Y.* **202**, 975–977.

STEPHENS, R. E. (1975). The basal apparatus. Mass isolation from the molluscan ciliated gill epithelium and a preliminary characterization of striated rootlets. *J. Cell Biol.* **64**, 408–420.

WEISS, R. L. (1982). The flagellar apparatus and fusing membranes in *Chlamydomonas*. *J. Cell Biol.* **95**, 258a (abstr.).

WEISS, R. L. (1984). Coated vesicles in the contractile vacuole/mating structure region of *Chlamydomonas*. *J. Ultrastruct. Res.* (in press).

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