

INTERRELATIONSHIPS BETWEEN MICROTUBULES, A STRIATED FIBER, AND THE GAMETIC MATING STRUCTURE OF *CHLAMYDOMONAS REINHARDI*

URSULA W. GOODENOUGH and RICHARD L. WEISS

From Cell and Developmental Biology, The Biological Laboratories, Harvard University, Cambridge, Massachusetts 02138. Dr. Weiss' present address is Department of Botany, San Diego State University, San Diego, California 92182.

ABSTRACT

The microtubule system associated with the *Chlamydomonas reinhardtii* flagellar apparatus is shown to differ from previous descriptions; two of the four flagellar "roots" possess only two microtubules and are associated with a finely striated fiber. In gametic cells this fiber underlies the gametic mating structure and makes contact with it. Functional interpretations are offered.

KEY WORDS microtubules · flagellum · striated fiber · mechanoreceptors · gametogenesis · *Chlamydomonas*

The single-celled eukaryote *Chlamydomonas reinhardtii* is presently under intensive investigation by students of flagellar motility (7, 12, 14, 15, 21, 30) and gametic agglutination and cell fusion (2, 9, 25, 26, 31). Of major importance to such studies is the fact that the ultrastructure of the *C. reinhardtii* flagellar apparatus (5, 10, 16, 23) and mating apparatus (6, 8, 11, 29, 31) has been carefully detailed. The present paper describes structural features of these systems that have not been previously reported, and considers their evolutionary and functional significance.

MATERIALS AND METHODS

Gametes of wild-type strain 137c were prepared from week-old plate cultures as previously described (19). All micrographs except Fig. 7 were prepared from cells that were chilled and fixed in cold 0.008% glutaraldehyde in 10 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethane sulfonic acid (HEPES) buffer, pH 7 for 15 min; an equal volume of cold 1% OsO₄ was then added for 30 min, after which the cells were dehydrated and

embedded in Spurr's low viscosity resin. Fig. 7 is a section serial to a previously published micrograph (Fig. 1 of reference 31); fixation methods are described in that paper.

Micrographs were taken with a Philips 300 electron microscope. Direct measurements of fiber periodicities were made with a Nikon Shadowgraph (Nikon Inc., Instrument Div., EPOI, Garden City, N. Y.). Five images were also subjected to optical diffraction by Dr. D. A. Goodenough, using a helium neon laser. The camera length of the optical diffractometer was calibrated with a lattice having a unit cell size of 0.255 mm. Measurements of the diffracted images were made with a Nikon Shadowgraph (Nikon Inc.).

RESULTS

Arrangement of Flagellar Roots

Our studies of vegetative and gametic cells of *C. reinhardtii* in general confirm the classical analysis of the flagellar apparatus offered by Ringo 10 years ago (23). Extensive serial sectioning of the flagellar region has revealed, however, that the four sets of cortical microtubules ("flagellar roots" *in sensu* Manton) that radiate out from the basal apparatus are not, as Ringo believed, iden-

tical. Instead, as diagrammed in Fig. 1, only two of the sets are of the 3-over-1 variety described by Ringo. The other two qualify as compound roots (4, 13); each set contains two microtubules associated with a finely striated fiber.

Figs. 2-4 show serial sections through the compound roots of a gametic cell. Conspicuous is the finely striated fiber which extends from one side of the basal apparatus to the other, passing just under the wide distal fiber that interconnects the two mature basal bodies. At higher magnification (Fig. 5), the basic repeating unit of the fiber is seen to be a triplet of three equidistant cross-striations, the outer two (arrows) being slightly more electron dense than the central. Between such units is a space lacking a central line.

An optical diffraction image of a finely striated fiber is shown in Fig. 6, where the strong periodicity of the fiber is reinforced. The repeat distance

in such diffracted images is found to be precisely 12 nm for five independent fibers, including fibers fixed in 0.008 and 3% glutaraldehyde. Direct measurements of real images reveal the distance between any two sets of heavy lines to be 12 nm, meaning that the fiber has an overall center-to-center repeat of 24 nm. No variability in fiber periodicity is evident in the small sample studied (cf. reference 24).

The finely striated fiber is continuous from one side of the basal apparatus to the other (Figs. 2-4). Lying beneath the fiber on either side of the basal apparatus is a set of two microtubules (labeled 1 and 2). Each microtubule pair ends in the interbasal body region (Fig. 4, large arrowheads) and makes no apparent insertion into the fiber. The finely striated fiber associates preferentially with the microtubule designated no. 1 in Figs. 2-4. This asymmetry is more readily visualized when the fiber and the microtubules are sectioned transversely, as in Figs. 7 and 8. In each case, the fiber appears as a dense line (arrow), and in both cases it overlies the cross-sectioned microtubule labeled no. 1. This asymmetric relationship can be seen to persist through at least nine consecutive serial sections (our unpublished micrographs).

The microtubules of the compound roots make a right-angle approach to the plane of the basal apparatus; a longitudinal section through the distal fiber cuts these microtubules transversely (cf. Fig. 2 of reference 32 and several Figures in reference 23). Each 3-over-1 root, on the other hand, approaches the basal apparatus from one side such that it makes a precise 50° angle with one of the compound roots (the apparent 90° angles between the root systems in Fig. 1 are the result of artistic license). Within each "V" formed by the intersecting root systems lies a short daughter basal body (Figs. 2-4, bb). There is no evidence that the 3-over-1 roots insert into the fiber, nor do other microtubules associate with it along its length (cf. reference 4).

The fact that the compound roots have not previously been reported in such an extensively studied organism as *C. reinhardi* merits comment. Ringo (23) interpreted the fiber to be a short dense plate into which four-membered microtubular roots insert (cf. Fig. 27 of reference 23). The striations are in fact easy to miss. They are most obvious when the fiber is cut in sagittal section but, because the fiber is only about 22 nm thick at its midpoint and tapers at either end, such sections are relatively rare. Moreover, in a

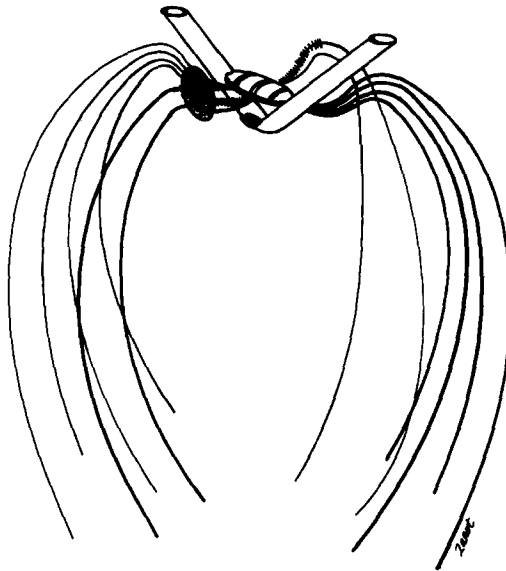
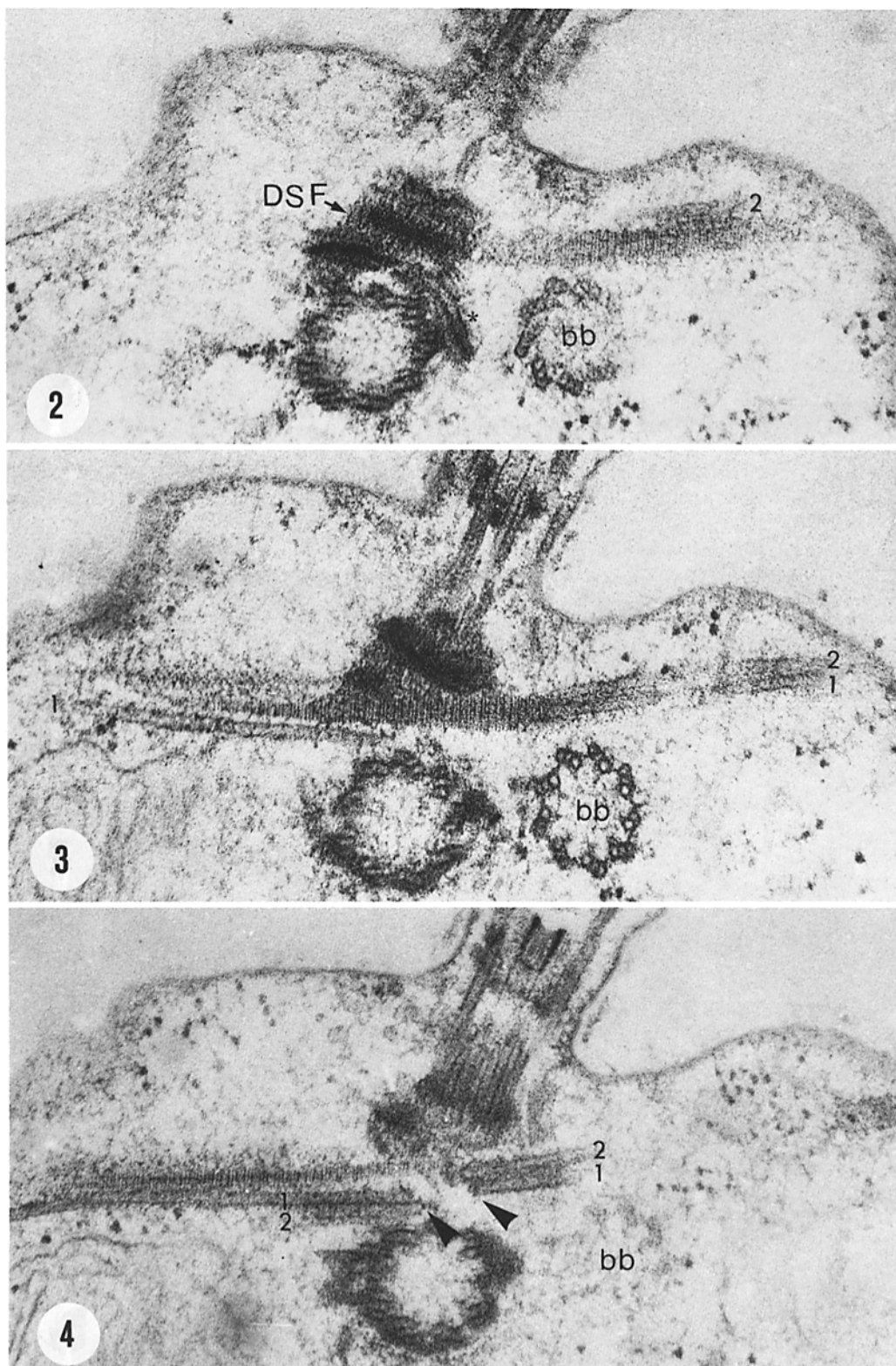


FIGURE 1 A schematic drawing of the general form of the cellular microtubule system, based on Fig. 28 of Ringo's article (23). The emerging flagella are represented by truncated cylinders, and the relative sizes of the microtubules and the basal apparatus are not to scale. Details of how the microtubules end in the posterior region of the cell are not known. Shown is the wide distal striated fiber interconnecting the two basal bodies and one of the two proximal striated fibers. Each of the two roots emerging at right angles to the distal fiber contains two microtubules, one of which is overlain by a flat, finely striated fiber. The position of the gametic mating structure is indicated as a saucer-shaped stippled area.



FIGURES 2-4 Consecutive serial sections through the anterior of a gametic cell in an mt^+/mt^- mixture. The large distal striated fiber (DSF) connects the two mature basal bodies; a short daughter basal body is indicated at *bb*. The two compound-root microtubules are labeled 1 and 2; their sites of termination are indicated by the arrowheads in Fig. 3. The finely striated fiber is included to the right in Fig. 2, central in Fig. 3, and to the left in Fig. 4. One 3-over-1 root is marked by asterisk in Fig. 2 (*). $\times 86,000$.

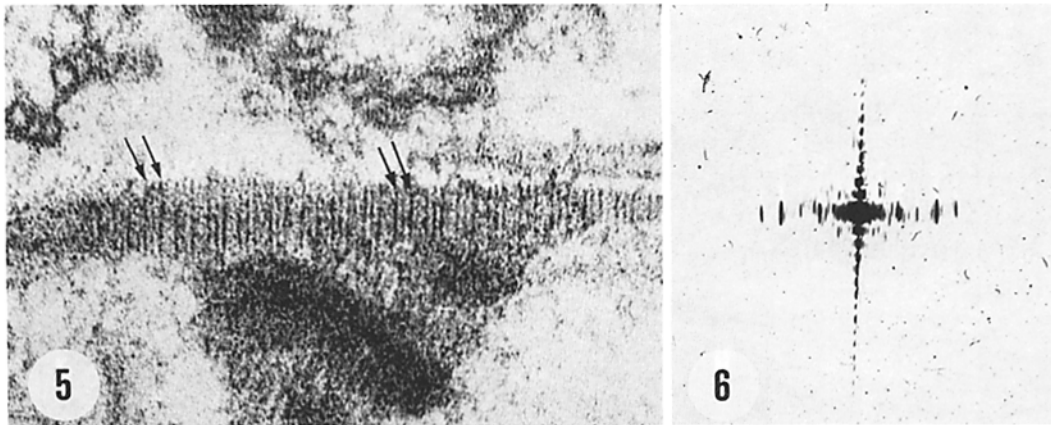
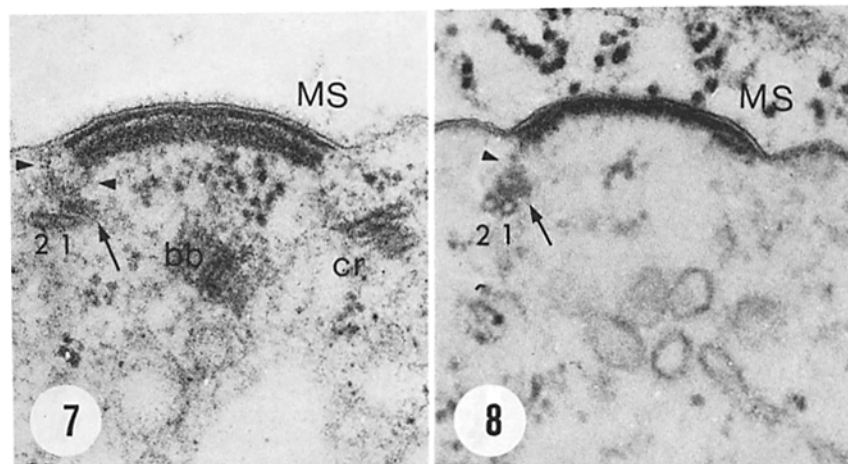


FIGURE 5 Enlargement of the striated fiber shown in Fig. 3. $\times 163,000$.

FIGURE 6 Optical diffraction image of a finely striated fiber. Distance between diffraction spots is 12 nm. The alternation between dense and light spot intensities is presumed to reflect the contribution made in alternate unit cells by the central line. The image is enlarged such that 1 mm corresponds to 30 nm in real space.



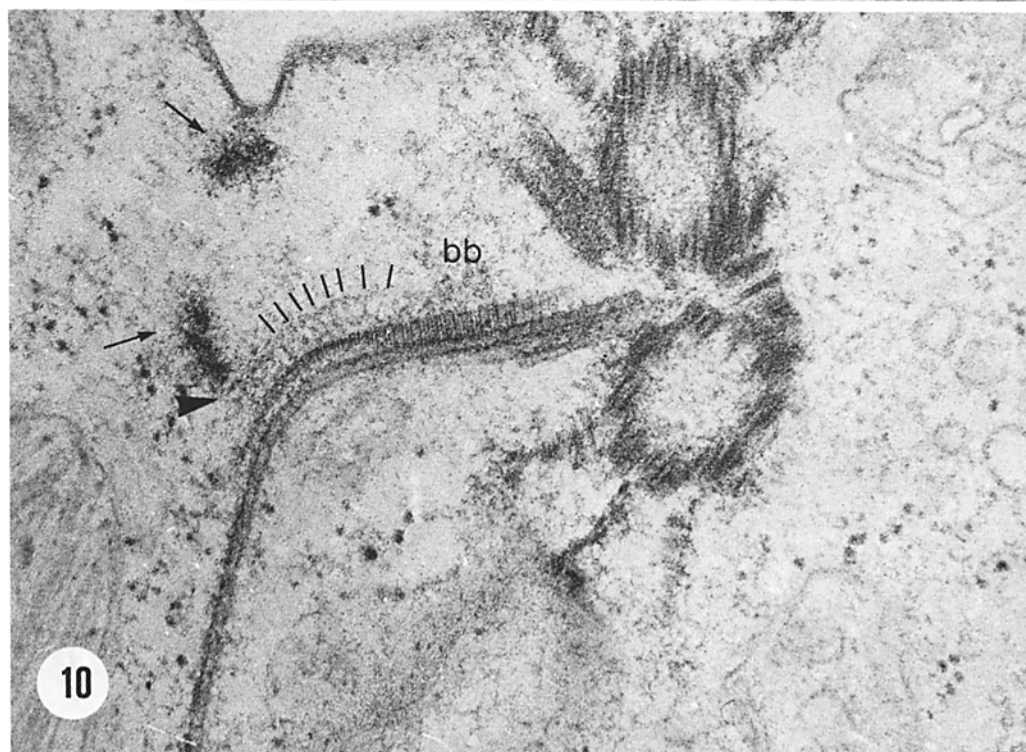
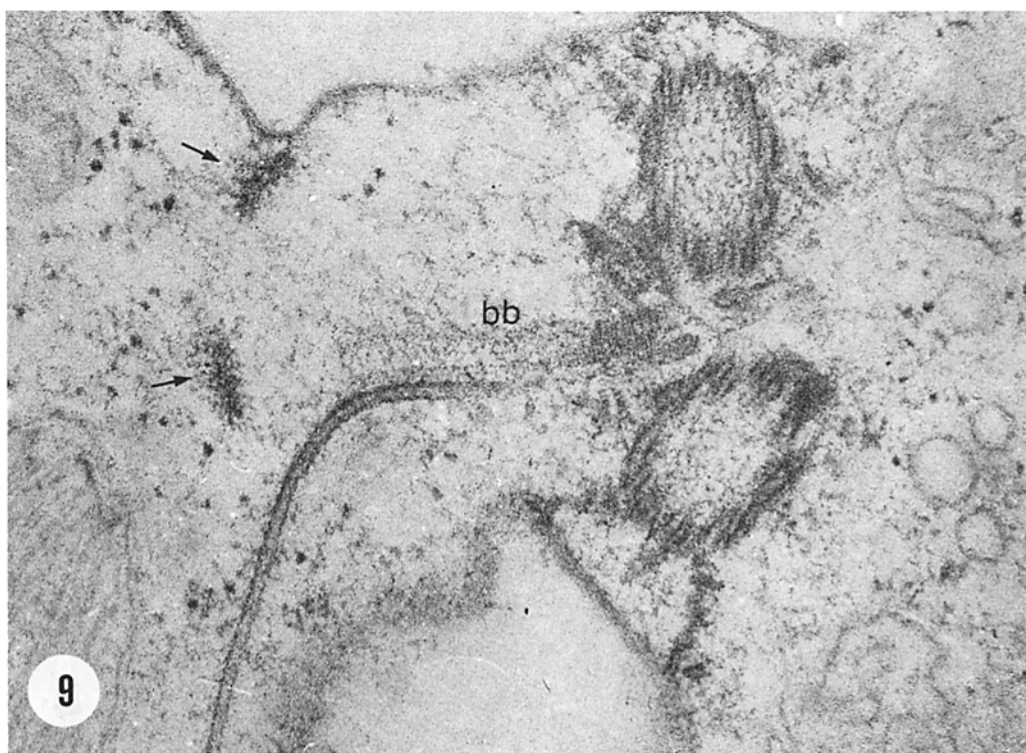
FIGURES 7 and 8 Mating structures (*MS*) of an *mt*⁺ (Fig. 7) and an *mt*⁻ (Fig. 8) gamete. The two compound root microtubules are labeled 1 and 2. The striated fiber overlying microtubule no. 1 is indicated by an arrow, and the connective-lattice material between the fiber and the membrane is indicated by arrowheads. A "conventional" (3-over-1) root is at *cr* in Fig. 7. (Fig. 7 is a section serial to a previously published micrograph, Fig. 1. of reference 31.)

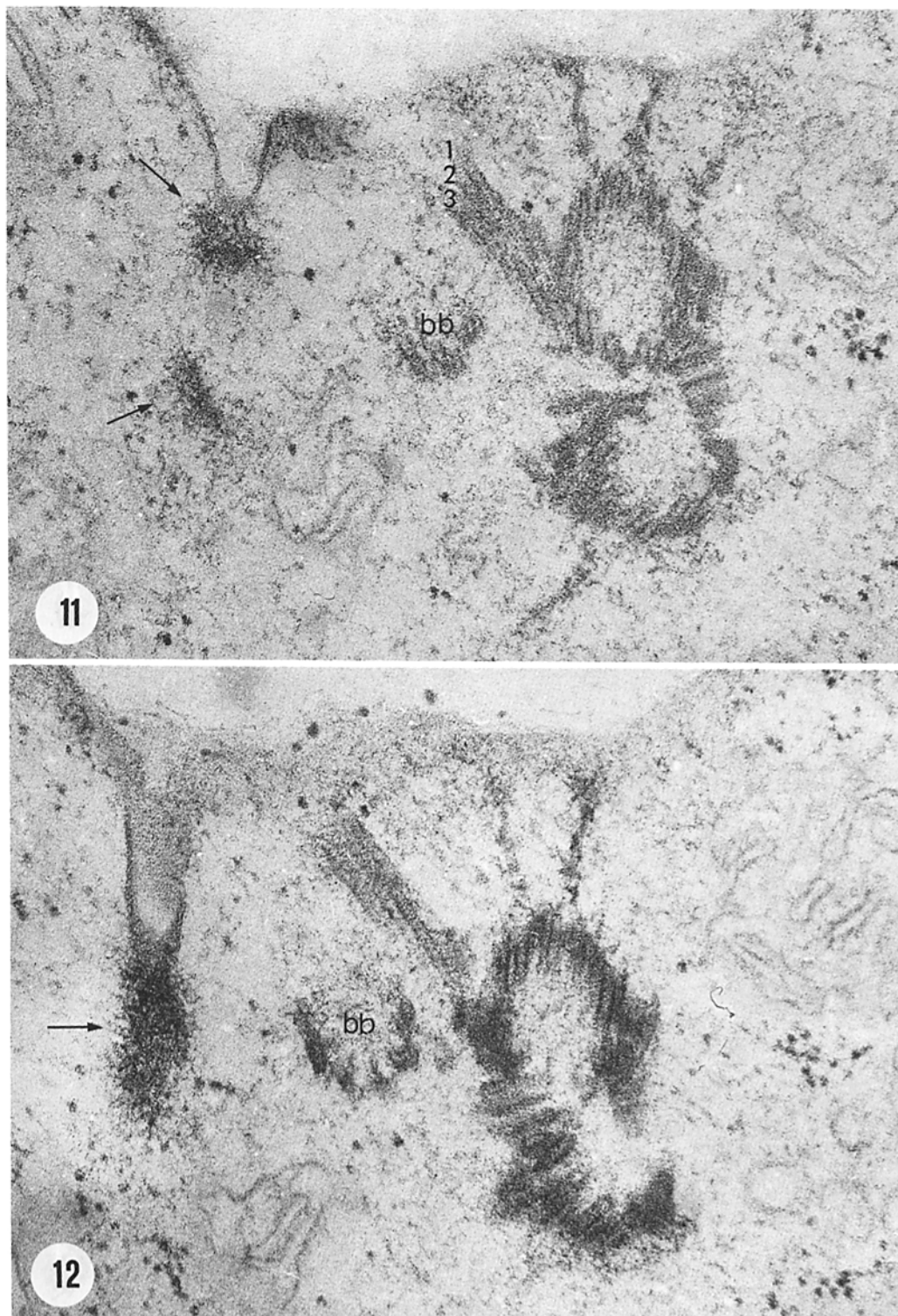
typical silver section 70 nm thick, the 12-nm striations are difficult to resolve. Very thin sections and/or tissue from which much cytoplasmic material has been extracted (obtained when dilute glutaraldehyde concentrations are employed) are therefore necessary to visualize the fiber clearly.

Association of the Compound Root with the Mating Structure

The compound root of Fig. 7 lies below and to

one side of an *mt*⁺ mating structure (*MS*); a comparable arrangement is shown in Fig. 8 for an *mt*⁻ mating structure. In both cases, strands of material (arrowheads) extend from the finely striated fiber to the edge of the mating structure and, in Fig. 7, to the membrane adjacent to the mating structure as well. The nature of this material is more clearly visualized in Figs. 9–12. Shown are two gametes in the act of zygotic cell fusion, with the mating structure (arrows), the basal apparatus, and the compound root all deriving





FIGURES 9-12 Consecutive serial sections through a newly formed zygote. The mu^+ mating structure is indicated by arrows. The periodicity of the connective lattice associated with the compound root is demarcated in Fig. 10 by a series of short lines on its upper surface. The interconnection between the connective lattice and the mu^+ mating structure is shown at the large arrowhead in Fig. 10. A daughter basal body or its putative location are indicated at *bb*, and the three microtubules of a conventional root are labeled 1-3 in Fig. 11. The connection between one edge of the mating structure and the anterior cell membrane of the zygote is evident in all four sections. $\times 96,000$.

from the mt^+ partner. As the end of the finely striated fiber passes by one edge of the mating structure in Fig. 10, a zone of fibrous material connects the two. In such a lateral view the material is seen to take the form of a thin sheet, less than one thin-section thick and 50–60 nm across, whose lattice-like substructure has an irregular periodicity (parallel lines) that bears no apparent relationship to the periodicity of the finely striated fiber. We propose the name connective lattice for this structure.

Serial sections through a number of mt^+ mating structures reveal that direct contact between the connective lattice and the mating structure is made towards the basal-apparatus-distal edge of the mating structure. The contact persists during the activation stage (31) of the mating reaction, as well as in the early zygote (Figs. 9–12). In the early zygote, this is the edge seen to have detached from the cytoplasmic bridge membrane (31) at the time of cell fusion (Fig. 10).

A connective lattice does not appear to be associated with the striated fiber of vegetative cells (our unpublished micrographs). It is difficult to make this statement categorically, however, since vegetative cells have no mating structure and, without a mating structure for orientation, detection of connective-lattice material may be difficult.

DISCUSSION

Evolutionary Considerations

Students of evolution have regarded the radial symmetry of the *C. reinhardi* flagellar root system to be a major dilemma in relating *Chlamydomonas* to its taxonomic neighbors (28). This report establishes the *C. reinhardi* root system to be asymmetric, with two four-membered roots and two two-membered compound roots in a cruciate pattern. Several quadriflagellated relatives of *Chlamydomonas* (3, 4) possess four of each type of root system, confirming Ringo's speculation that such quadriflagellates might possess "doublings" of the basic biflagellate unit of *Chlamydomonas*. Indeed, it is intriguing to speculate further that some of these organisms may have arisen as zygotic *Chlamydomonads* that lost, by mutation, their ability to undergo meiosis.

The finely striated fiber of *C. reinhardi* appears to have taxonomic significance in itself, for fibers of identical appearance and similar periodicity have been found in the zoospores produced by

several filamentous green algae (3, 13, 17). Particularly interesting is the recent study by Brown et al. (4) on the structure of *Polytomella*, a colorless quadriflagellated unicell. Present are two finely striated fibers with an identical morphology, although a slightly greater reported periodicity (30 nm center-to-center) compared to that of *C. reinhardi*, which pass between the basal bodies. The apparent conservation of this structure bears testimony to Manton's original insight (18) as to the potential phyletic importance of flagellar structures.

Functional Significance

The function of striated fibers and microtubule roots is not, to our knowledge, known for any organism. These structures are usually thought to function either in absorbing the stress of the ciliary/flagellar beat or in ciliary/flagellar coordination (15, 22–24, 27). Of the two, a stress-absorbing function appears the more applicable to the compound root system described here. Because our particular interest lies in the *Chlamydomonas* mating reaction, we shall consider briefly the possible significance of the association between the compound root and the mating structure.

First, the association may have morphogenetic significance. The mating structure appears to differentiate during the mitosis that marks the completion of gametogenesis (19), and the finely striated fiber and its connective lattice may serve as a yardstick (or, more aptly, as a micronstick) to position the mating structure in its fixed location with respect to the basal apparatus (31). More generally, it seems attractive to us to consider that organelle positioning might in many cases be effected by fibrous molecules of specific lengths.

A second possibility would endow the fiber with a more active function. Several investigators (9, 26) have recognized that during the mating reaction some signaling mechanism must couple the occurrence of sexual agglutination at the flagellar tip with cell-wall lysis and mating-structure activation at the cell body. A reciprocal signal must then be generated at the time of cell fusion to cause agglutinating flagella to lose their sexual adhesiveness. The most obvious medium for transmitting such signals is, of course, the flagellar/plasma membrane. The observations reported here, however, demonstrate that flagellar axonemes, basal bodies, compound roots, lattice con-

nectives, and mating structures are all intimately associated with one another. Therefore, there exists at least a structural basis for proposing that this network of cytoplasmic fibrous elements may play a role in the signaling process. Specifically, one could imagine that sexual-agglutination perturbations at the flagellar membrane surface might be transduced down the axoneme and out to the mating structure via this network; ionic changes might then be elicited such that microfilament polymerization can proceed. One could also imagine that the loss of contact between the lattice connectives and the cell membrane that occurs at the time of cell fusion (Figs. 9–12) might in some way initiate the loss of adhesiveness at the flagellar tips; we observe this loss of adhesiveness to occur precisely at the time of cytoplasmic confluence. Our speculations are necessarily phrased very generally because we have no notion of what signal-transmission-along-fibers might mean at a molecular level.

Solter and Gibor (26) were the first to make the analogy between gametic flagella of *Chlamydomonas* and the cilia of such sensory cells as mechanoreceptors; in both cases a stimulus at the flagellar/ciliary tip sends a signal to the cell body which leads to a very rapid response. The way that mechanoreceptor signals are transduced is not at all understood, except that the ciliary tip is clearly implicated as the initial receptor (20) and the basal body region appears intimately involved in the process (reviewed in references 1, 33). In perusing the literature on the fine structure of mechanoreceptors, we have been struck by the prominence of striated fibers and microtubule roots in these cells. Because the cilia of mechanoreceptors are reportedly immotile, it seems unlikely that the cytoplasmic fibrous elements function in these cells to absorb stress or coordinate motion. Therefore, in mechanoreceptors as well as in *Chlamydomonas*, it is conceivable that microtubules and fibers may somehow be sensitive to, and capable of transmitting, stimuli generated by perturbations of the membrane/axonemal complex of cilia and flagella.

We thank Dan Goodenough for the optical diffraction images.

This research was supported by grants GM 23058, GM 06637, and HD 00415 from the National Institutes of Health, and by a grant from the Maria Moors Cabot Foundation for Botanical Research, Harvard University.

Received for publication 3 August 1977, and in revised form 7 October 1977.

REFERENCES

1. BARBER, V. C. 1974. Cilia in sense organs. In *Cilia and Flagella*. M. A. Sleight, editor. Academic Press Inc. Ltd., London. 403–433.
2. BERGMAN, K., U. W. GOODENOUGH, D. A. GOODENOUGH, J. JAWITZ, and H. MARTIN. 1975. Gametic differentiation in *Chlamydomonas reinhardtii*. II. Flagellar membranes and the agglutination reaction. *J. Cell Biol.* **67**:606–622.
3. BIRKBECK, T. E., K. D. STEWART, and K. R. MATTOX. 1974. The cytology and classification of *Schizomeris leibleinii* (Chlorophyceae). II. The structure of quadriflagellate zoospores. *Phycologia*. **13**:71–79.
4. BROWN, D. L., A. MASSALSKI, and R. PATENAUE. 1976. Organization of the flagellar apparatus and associated cytoplasmic microtubules in the quadriflagellate alga *Polytomella agilis*. *J. Cell Biol.* **69**:106–125.
5. CAVALIER-SMITH, T. 1974. Basal body and flagellar development during the vegetative cell cycle and the sexual cycle of *Chlamydomonas reinhardtii*. *J. Cell Sci.* **16**:529–556.
6. CAVALIER-SMITH, T. 1975. Electron and light microscopy of gametogenesis and gamete fusion in *Chlamydomonas reinhardtii*. *Protoplasma*. **86**:1–18.
7. DENTLER, W. L., and J. L. ROSENBAUM. 1977. Flagellar elongation and shortening in *Chlamydomonas* III. Structures attached to the tips of flagellar microtubules and their relationship to the directionality of flagellar microtubule assembly. *J. Cell Biol.* **74**:747–759.
8. FRIEDMANN, I., A. L. COLWIN, and L. H. COLWIN. 1968. Fine structural aspects of fertilization in *Chlamydomonas reinhardtii*. *J. Cell Sci.* **3**:115–128.
9. GOODENOUGH, U. W. 1977. Mating interactions in *Chlamydomonas*. In *Microbial Interactions. Receptors and Recognition*, Series B. J. Reissig, editor. Chapman & Hall Ltd., London. 3:322–350.
10. GOODENOUGH, U. W., and H. S. ST. CLAIR. 1975. *Bald-2*: a mutation affecting the formation of doublet and triplet sets of microtubules in *Chlamydomonas reinhardtii*. *J. Cell Biol.* **66**:480–491.
11. GOODENOUGH, U. W., and R. L. WEISS. 1975. Gametic differentiation in *Chlamydomonas reinhardtii*. III. Cell wall lysis and microfilament-associated mating structure activation in wild-type and mutant strains. *J. Cell Biol.* **67**:623–637.
12. HEIDEMANN, D. R., G. SANDER, and M. W. KIRSCHNER. 1977. Evidence for a functional role of RNA in centrioles. *Cell*. **10**:337–350.
13. HOFFMAN, L. R. 1970. Observations on the fine structure of *Oedogonium*. VI. The striated component of the compound flagellar “roots” of *O. cardiacum*. *Can. J. Bot.* **48**:189–196.
14. HUANG, B., M. R. RIFKIN, and D. J. L. LUCK. 1977. Temperature-sensitive mutations affecting

- flagellar assembly and function in *Chlamydomonas reinhardtii*. *J. Cell Biol.* **72**:67-85.
15. HYAMS, J. S., and G. G. BORISY. 1975. Flagellar coordination in *Chlamydomonas reinhardtii*: isolation and reactivation of the flagellar apparatus. *Science (Wash. D. C.)*. **189**:891-893.
 16. JOHNSON, U. G., and K. R. PORTER. 1968. Fine structure of cell division in *Chlamydomonas reinhardtii*. Basal bodies and microtubules. *J. Cell Biol.* **38**:403-425.
 17. MANTON, I. 1964. Observations on the fine structure of the zoospore and young germling of *Stigeoclonium*. *J. Exp. Bot.* **15**:399-411.
 18. MANTON, I. 1965. Some phyletic implications of flagellar structure in plants. In *Advances in Botanical Research*. R. D. Preston, editor. Academic Press Inc. Ltd., London. **2**:1-34.
 19. MARTIN, N. C., and U. W. GOODENOUGH. 1975. Gametic differentiation in *Chlamydomonas reinhardtii*. I. Production of gametes and their fine structure. *J. Cell Biol.* **67**:587-605.
 20. MORAN, D. T., J. C. ROWLEY, S. N. ZILL, and F. G. VARELA. 1976. The mechanism of sensory transduction in a mechanoreceptor. *J. Cell Biol.* **71**:832-847.
 21. PIPERNO, G., B. HUANG, and D. J. L. LUCK. 1977. Two-dimensional analysis of flagellar proteins from wild-type and paralyzed mutants of *Chlamydomonas reinhardtii*. *Proc. Natl. Acad. Sci. U. S. A.* **74**:1600-1604.
 22. PITELKA, D. R. 1974. Basal bodies and root structures. In *Cilia and Flagella*. M. A. Sleight, editor. Academic Press Inc. Ltd., London. 437-469.
 23. RINGO, D. L. 1967. Flagellar motion and fine structure of the flagellar apparatus in *Chlamydomonas*. *J. Cell Biol.* **33**:543-571.
 24. SIMPSON, P. A., and A. D. DINGLE. 1971. Variable periodicity of the rhizoplast of *Naegleria flagellates*. *J. Cell Biol.* **51**:323-328.
 25. SNELL, W. J. 1976. Mating in *Chlamydomonas*: a system for the study of specific cell adhesion. I. Ultrastructural and electrophoretic analysis of flagellar surface components involved in adhesion. *J. Cell Biol.* **68**:48-69.
 26. SOLTER, K. M., and A. GIBOR. 1977. Evidence for role of flagella as sensory transducers in mating of *Chlamydomonas reinhardtii*. *Nature (Lond.)*. **265**:444-445.
 27. 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.
 28. STEWART, K. D., and K. R. MATTOX. 1975. Comparative cytology, evolution and classification of the green algae with some consideration of the origin of other organisms with chlorophylls A and B. *Bot. Rev.* **41**:104-135.
 29. TRIEMER, R. E., and R. M. BROWN. 1975. Fertilization in *Chlamydomonas reinhardtii*, with special reference to the structure, development, and fate of the choanoid body. *Protoplasma*. **85**:99-107.
 30. WEEKS, D. P., and P. S. COLLIS. 1976. Induction of microtubule protein-synthesis in *Chlamydomonas reinhardtii* during flagellar regeneration. *Cell*. **9**:15-27.
 31. WEISS, R. L., D. A. GOODENOUGH, and U. W. GOODENOUGH. 1977. Membrane differentiations at sites specialized for cell fusion. *J. Cell Biol.* **72**:144-160.
 32. WEISS, R. L., D. A. GOODENOUGH, and U. W. GOODENOUGH. 1977. Membrane particle arrays associated with the basal body and with contractile vacuole secretion in *Chlamydomonas*. *J. Cell Biol.* **72**:133-143.
 33. WIEDERHOLD, M. L. 1976. Mechanosensory transduction in "sensory" and "motile" cilia. *Annu. Rev. Biophys. Bioeng.* **5**:39-62.