BASAL BODY REPLICATION AND CILIOGENESIS IN A SUCTORIAN, *TOKOPHRYA INFUSIONUM*

LYNDELL L. MILLECCHIA and MARIA A. RUDZINSKA

The Rockefeller University, New York 10021

ABSTRACT

Basal body replication and ciliogenesis in *Tokophrya infusionum* were studied in synchronized cultures. Basal body replication occurs during the 1st hr of reproduction, which in *Tokophrya* is by internal budding. The number of basal bodies increases from about 20 to over 300 within this period. New basal bodies develop in association with mature basal bodies; they are formed at right angles to the mature basal body as short "probasal" bodies, which elongate, slant upward, become parallel to the mature basal body, and elongate to the mature size. Ciliogenesis occurs only during reproduction; the nonreproducing adult is not ciliated, and has only 18–25 barren basal bodies. Cilia first appear as short bulges above the basal body. The axonemal structure is incomplete at first, with one or both central microtubules absent, and occasionally the B fibers of the outer doublets are missing. Several accessory fibers are associated with the basal bodies, both in the adult and during reproduction. One of the fibers appears only after the cilia have sprouted. The scheme of basal body replication and ciliogenesis in *Tokophrya* is compared to that reported in other organisms, and the role of the accessory fibers is discussed.

INTRODUCTION

Suctoria belong to the class Ciliatea (15), and they are most unusual ciliates because they do not have cilia during the adult life. They are sedentary and are attached to the substrate by a disc (14, 38) throughout their adult life, which may last for 10 days or more (33, 34). Cilia appear in Suctoria during reproduction and are retained in the embryo, a motile form that swims freely in the medium for a comparatively short time lasting only several minutes to several hours. The embryo then stops moving, attaches itself to the substrate, and undergoes a complete metamorphosis; it loses its cilia and acquires all the characteristics of the adult form (36). The two stages, the sessile adult and the swimming embryo, characteristic for the life cycle of Suctoria, result from the complex way of reproduction by exogenous or endogenous budding (6, 13). The latter process was studied by means of electron microscopy in *Tokophrya infusionum* (20, 21, 37). Endogenous budding in *Tokophrya* starts with an invagination of the pellicle and plasma membrane. The progressively growing invagination cuts off a part of the parent body to form an ovoid body, the embryo, which is covered by several hundred cilia. From previous electron microscope studies, it is known that the adult *Tokophrya* possesses about 20 barren basal bodies, all assembled in the vicinity of the contractile vacuole pore (35). The fact that a ciliated offspring equipped with hundreds of cilia can arise from a nonciliated parent having only a few basal bodies offered an excellent opportunity to study the replication of basal bodies and the problem of ciliogenesis (23). The results of such studies are presented in this paper.

MATERIALS AND METHODS

Bacteria-free cultures of *Tokophrya infusionum* were grown in 125-mm screw-capped pyrex tubes in a yeast extract medium as described in previous papers (22, 35, 39). In the study of basal body replication and ciliogenesis, it was important to use organisms that were reproducing synchronously. Several methods were tried (24), and in this work, organisms in plateau cultures were stimulated to reproduce by pouring off the old yeast medium and pipetting in fresh yeast medium. 1 and 2 hr after exchanging the medium, *Tokophrya* was fixed for 25 min in 1.0% glutaraldehyde in 0.1 M phosphate buffer (pH 7.3), washed in 0.1 M phosphate buffer, pelleted, and post-fixed in phosphate-buffered 1.0% OsO_4 for 1 hr. After dehydration in alcohols and propylene oxide, the pellets were embedded in Epon (18) and polymerized at 55°C for 3 days. Sections were cut with a diamond knife on a Porter-Blum MT-2 ultramicrotome, and were picked up on naked mesh grids (Ernest F. Fullam Co., P.O. Box 444, Schenectady, N.Y., 200 mesh, No. 2213). The sections were stained by floating the grids on drops of uranyl acetate (7.5% aqueous uranyl acetate + 100% ethanol, 2:1 for 5–10 min, washed in distilled water, and counterstained with lead citrate (0.4%) for 1 min (42). The grids were then washed, dried, and examined with an RCA microscope, EMU 3F (50 kv). At the 1-hr time point, 50–60% of the organisms viewed were in early stages of reproduction, and steps in formation of basal bodies and cilia could be found after careful search. Most of the organisms at the 2-hr time point were in middle-to-late stages of reproduction, and the structure of the mature cilia could be elucidated.

RESULTS

Basal Bodies in the Adult

There are only 18–25 basal bodies in the adult *Tokophrya*, and these are always adjacent to the contractile vacuole pore (35). In the nonreproducing adult, the basal bodies are devoid of cilia, and are arranged in six short parallel rows extending away from the contractile vacuole pore (Fig. 1). The basal bodies are 0.2 μ in diameter and 0.5 μ long, and have the conventional structure of nine triplet fibers (12). The fiber in each triplet that is closest to the center of the basal body is a microtubule, about 240 A in diameter, which has been designated by Gibbons and Grimstone (12) as fiber A. Adjacent to fiber A and joining to it is an incomplete tubule, fiber B, and a third tubule, C, which is also incomplete, adjoins fiber B. When the basal body is viewed from the proximal end, the fibers A–C run counterclockwise (Fig. 5); this is the same orientation as that found so far in cells of all other organisms (12). Near the distal tip of the basal body the C fibers end, leaving nine doublets (Fig. 3).

A single tubule appears in the center of the proximal third of the basal body (Fig. 5). Nine fibers radiate from the central tubule to the A microtubules of each triplet (Fig. 5). This is the cartwheel structure found in most basal bodies and centrioles (12). In the middle and distal portions of the basal body, no distinct structures appear (Figs. 3, 4); there is no dense core like that found in many other ciliates (27).

Accessory Fibers in the Adult

It was believed that the basal body system in Suctoria was different from that in other ciliates because of the lack of accessory fibers. However, early electron microscope studies revealed the presence of such fibers in *Tokophrya infusionum* (35). Several accessory fibers are associated with the basal bodies in this species. Beneath each row of basal bodies are two ribbons of microtubules, each composed of five to seven microtubules (Figs. 2, 6). They appear to run continuously beneath each row (Figs. 8, 11, 12). Perpendicular to the microtubular ribbons are two fibers. The first, to the right[1] of the row and toward the contractile vacuole pore, is composed of three microtubules (Figs. 1–5, 7). It originates near the base of the basal body (Fig. 5), rises parallel to the basal body (Fig. 4), then bends slightly to the right and ends near the plasma membrane a short distance from the distal tip of the basal body (Fig. 3). The second fiber, which is striated in some sections (Fig. 18), arises about a third of the way up the basal body (Fig. 4). It runs parallel to the microtubular ribbons along the right side of the row and *away* from the contractile vacuole pore (Figs. 1–3, 7), gradually slants up toward the plasma membrane, and terminates near the adjacent basal body.

Proliferation of Basal Bodies during Reproduction

The first sign of reproduction in *Tokophrya* is the invagination of the pellicle and plasma membrane,

[1]Right and left sides of basal bodies will be given as though the organism were viewed from *inside*, and along the row looking *away* from the contractile vacuole pore. All basal bodies are viewed from the proximal (innermost) end.

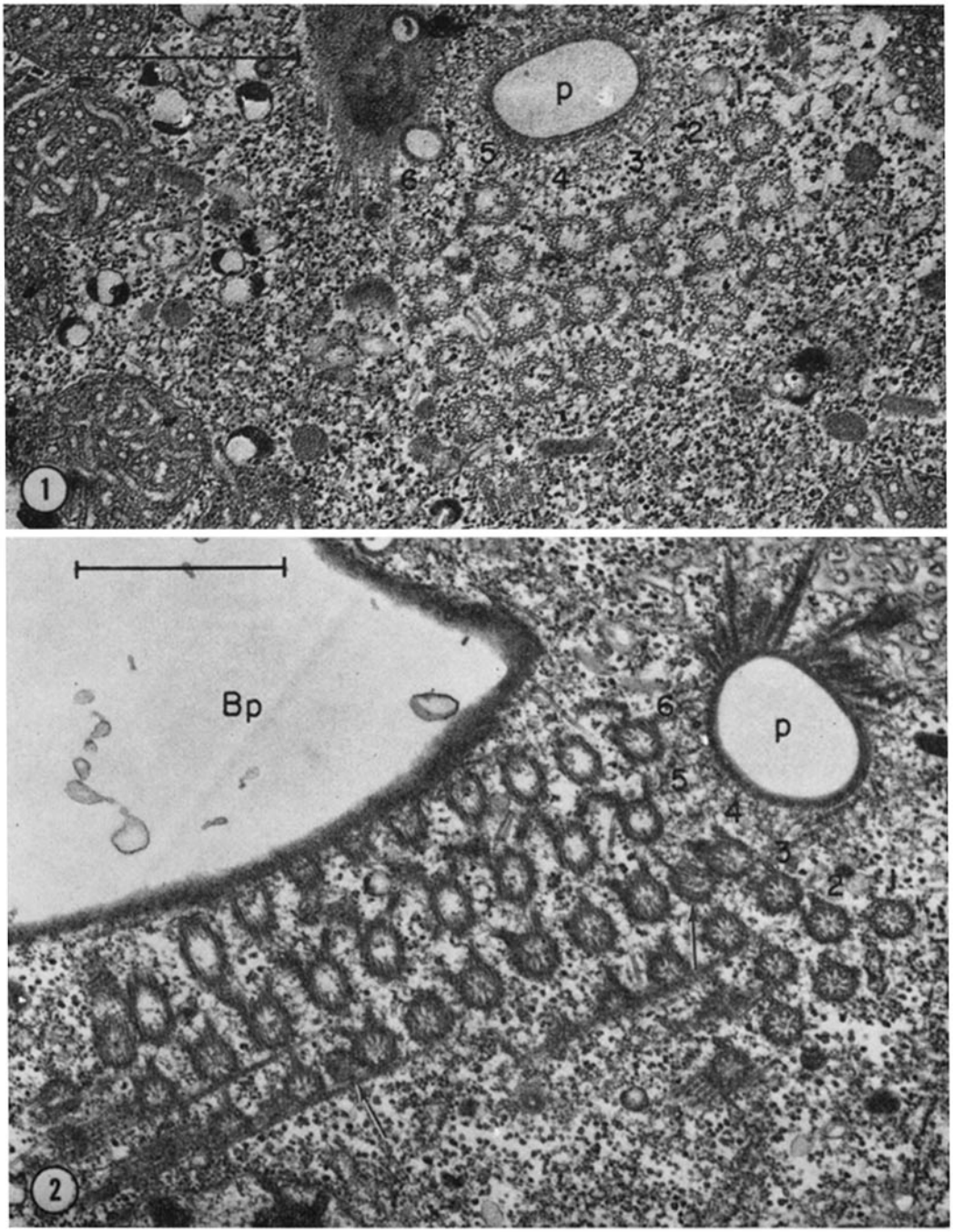
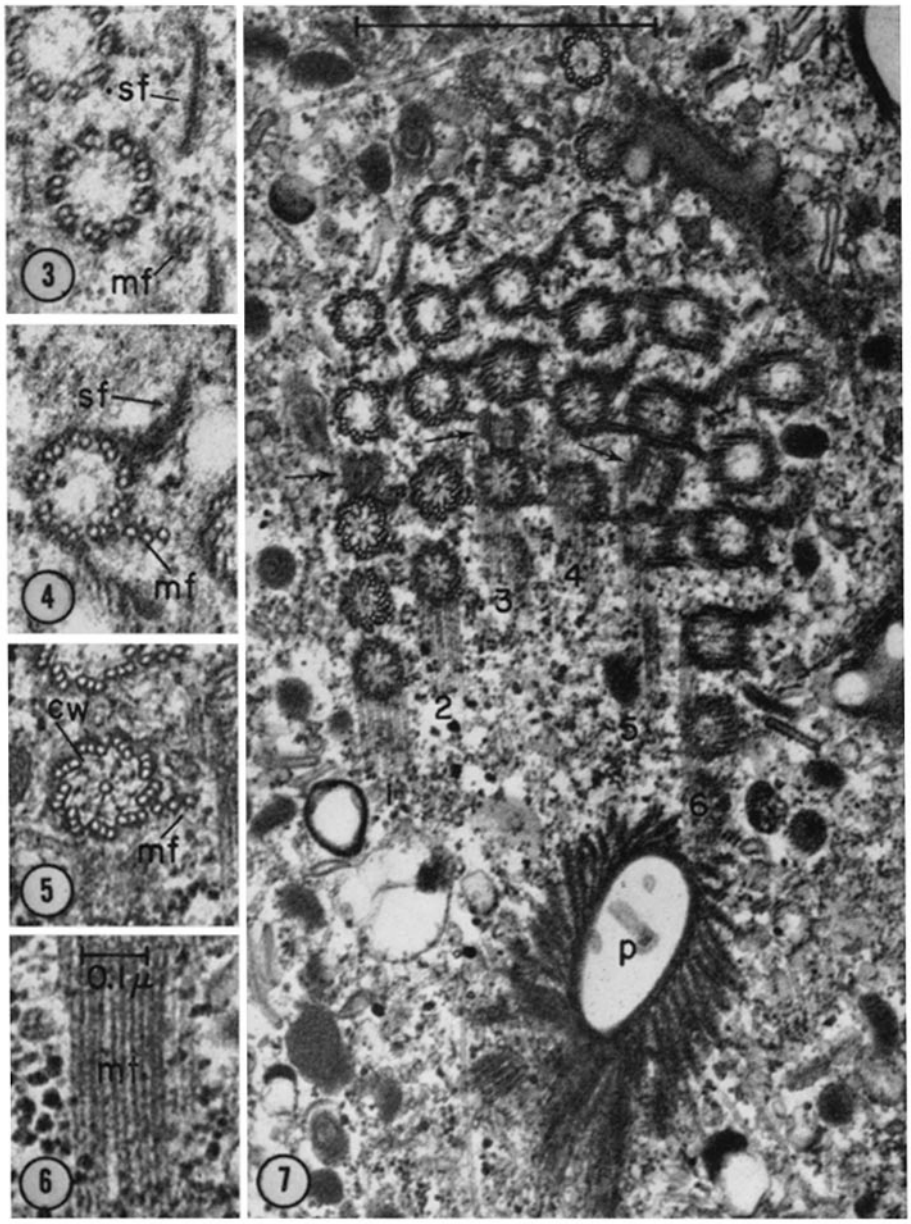


FIGURE 1 Cross-section through the contractile vacuole pore (*p*) and six rows of nonciliated basal bodies (*1–6*) in a nonreproducing adult *Tokophrya infusionum*. × 34,000.

FIGURE 2 Basal body proliferation in a reproducing *T. infusionum*. Six rows of basal bodies (*1–6*) extend away from the contractile vacuole pore (*p*) along the membranes of the brood pouch (*Bp*). In the fourth row, two probasal bodies (arrows) can be seen. × 30,000.



FIGURES 3–6 The structure of the basal body and accessory fibers in the adult *T. infusionum*. × 76,500.

FIGURE 3 Distal end of basal body. The C fiber is missing in some of the triplets. The striated fiber (*sf*) and the microtubular fiber (*mf*) are seen just to the right of the basal body.

FIGURE 4 Middle of basal body, showing the origin of the striated fiber (*sf*) and the three microtubules of the microtubular fiber (*mf*).

FIGURE 5 Proximal end of basal body, with central cartwheel (*cw*) and microtubular fiber (*mf*).

FIGURE 6 One of the two ribbons of microtubules (*mt*) that run beneath each row of basal bodies.

FIGURE 7 Arrangement of basal bodies in the adult *T. infusionum*, and the relationship of the basal bodies and accessory fibers to the contractile vacuole pore. The six rows of basal bodies (*1–6*) extend away from the pore (*p*). The microtubular fiber is on the right side of each basal body *toward* the pore, and the striated fiber is on the right side of the row running *away* from the pore. Probasal bodies are present in rows *1, 3, 5* (arrows); they arise within the row, and *away* from the contractile vacuole pore. Reproduction has just been completed in this adult, and the presence of probasal bodies at this time may indicate either that the adult is replacing basal bodies it had contributed to the embryo, or that formation of a second embryo has already begun. × 40,000.

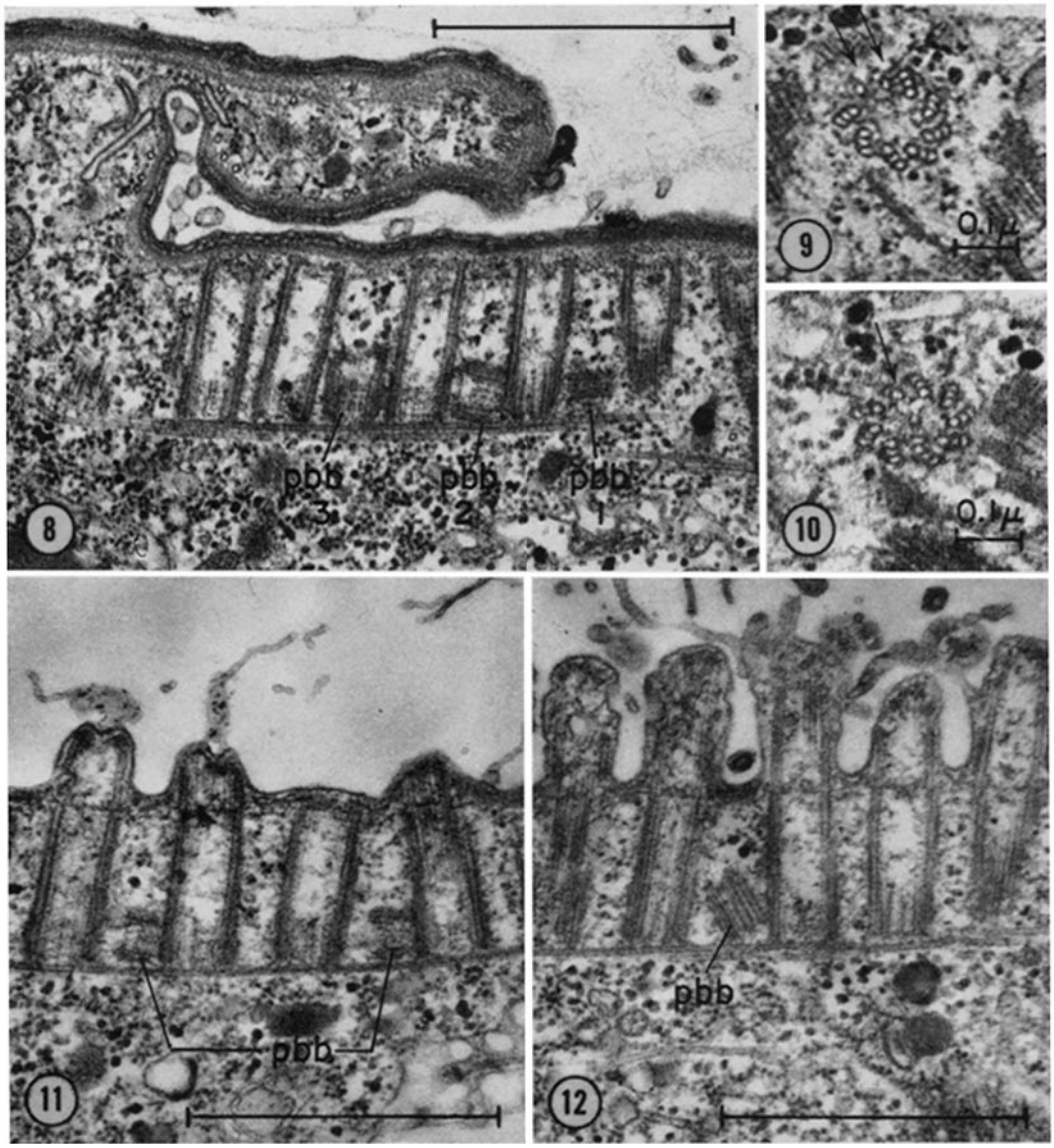


FIGURE 8 Invagination of parental membranes at the start of embryogenesis in *T. infusionum*, and the formation of new basal bodies. Three different stages in basal body replication appear. In the first stage (*pbb 1*), the probasal body is perpendicular to the adjacent mature basal body; in the second (*pbb 2*), it is just beginning to slant upward; and in the third stage (*pbb 3*), the probasal body is parallel to the mature basal bodies, but is still not fully grown. × 40,000.

FIGURES 9 and 10 Serial sections through an elongating basal body. × 80,000.

FIGURE 9 Distal portion of probasal body. Two of the fibers are incomplete (arrows); only the A fiber is present.

FIGURE 10 Proximal portion of probasal body. The C fiber is missing in one of the triplets (arrow), but the other eight triplets are complete.

FIGURES 11 and 12 Basal body replication and ciliogenesis in *T. infusionum*. × 40,000.

FIGURE 11 Two probasal bodies are located just above the microtubular ribbon and perpendicular to the adjacent mature basal bodies. At the same time, cilia are starting to grow. The fibers of the basal body appear to extend up into the bulge of cytoplasm above the basal body, which is covered by a single membrane.

FIGURE 12 The probasal body has elongated, and it slants upward. The cilia are elongating, with the basal body microtubules extending into the ciliary bulges. The ends of the bulges appear to be composed of vesicles.

which always takes place in the region of the contractile vacuole pore and the neighboring basal bodies. Both structures, the pore and the six short rows of basal bodies, are carried inward together with the invaginating membranes. As the invagination grows, it encircles and finally cuts off a part of the parent cell to form an ovoid body, the embryo, located within a space, the brood pouch. The whole process of reproduction takes about 2 hr, but the period of basal body proliferation, with the number of basal bodies increasing from about 25 to over 300, occurs within the 1st hr.

An early stage of proliferation of basal bodies in the forming embryo can be seen in Fig. 2. Some of the six rows already contain 10 to 11 basal bodies instead of the usual three to five found in a row in the adult organism (Fig. 1). The formation of new basal bodies starts at the very beginning of reproduction, simultaneously with the onset of the invagination of the pellicle and plasma membrane, as depicted in Fig. 8. In the first stage of development, a short probasal body appears which is perpendicular to the mature basal body and is closely adjacent to it (Figs. 8, 11). It is parallel to and just above the basal microtubules (Figs 8, 11). When the probasal body is about 0.17 μ long, it begins to slant upward toward the adjacent mature basal body (Figs. 8, 12). In the next stage, the probasal body is found parallel to the mature basal body, but it is only about 0.2 μ long (Fig. 8). At this stage of development, there is a small distance of 25 mμ between the probasal body and the mature basal body. The probasal body then elongates until it reaches the maximum length, and moves farther away from the mature basal body, probably as a result of growth of the pellicle and plasma membranes between the two basal bodies (17).

So far, we have not found the dense plaques or single microtubules of the very early probasal bodies reported by Dippell (8). We did find one probasal body that had two single and seven triplet fibers (Fig. 9). This probably represents a fairly late stage in probasal body formation since a section serial to that in Fig. 9 reveals one doublet and eight triplet fibers (Fig. 10). Elongation of the A fibers, therefore, apparently precedes that of the B and C fibers during basal body formation.

Probasal bodies arise only within a row and appear only on the side of the basal body away from the contractile vacuole pore (Figs. 2, 7). Since the striated fiber is at this side of the basal body, it could be assumed that some kind of association exists between probasal bodies and striated fibers (Fig. 7).

FIGURE 13 Cross-section through early cilia. The central microtubules are absent in one cilium (far left), and only one central microtubule is seen in the middle three. In the second cilium from the right, several of the outer doublets are incomplete. The fibrous structure is complete in the cilium to the far right, but arms are not clearly visible on the outer doublets. × 76,500.

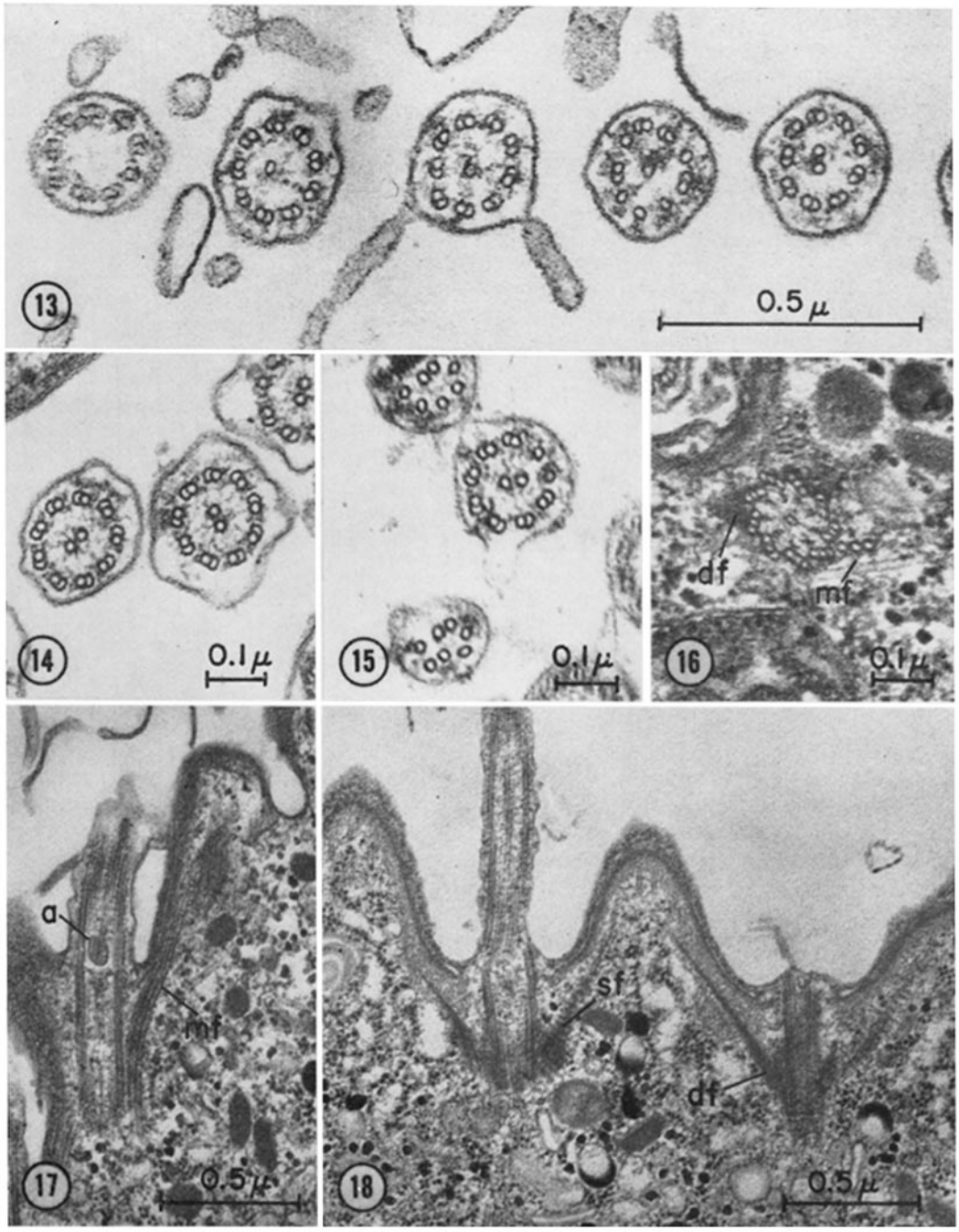
FIGURE 14 Cross-section through mature cilia, showing typical 9+2 axonemal structure. Arms, which point in a clockwise direction when the cilium is viewed from base to tip, are found on the A fiber of each doublet. × 76,500.

FIGURE 15 Tips of mature cilia. The B fibers end first, then the A fibers, and finally the central microtubules. × 76,500.

FIGURE 16 Cross-section through a basal body in the embryo. A new accessory fiber, the dense fiber (*df*), originates close to the proximal end of the basal body, on the opposite side from the microtubular fiber (*mf*). × 76,500.

FIGURE 17 Basal body and cilium in the embryo. The central microtubules originate in the axosome (*a*), a dense granule just distal to the basal body. The microtubular fiber (*mf*) extends from the basal body up into a ridge adjacent to the cilium. × 40,000.

FIGURE 18 Two of the accessory fibers in the embryo: the new dense fiber (*df*) on the anterior side of the basal body, and the striated fiber (*sf*) on the posterior side. × 40,000.


13
0.5 μ
14
0.1 μ
15
0.1 μ
df
mf
16
0.1 μ
a
mf
17
0.5 μ
sf
df
18
0.5 μ

The Appearance of Cilia during Reproduction

Ciliogenesis occurs early in reproduction, and at the same time as basal body proliferation. It begins with the elongation of the A and B fibers of the basal body. A single membrane, continuous with the outer pellicular membrane, covers the growing microtubular part of the cilium (Figs. 11, 12). The cilia are at first short bulges (Fig. 11), and their distal tips sometimes seem to be composed of vesicles (Fig. 12). Early cilia do not have the complete axonemal structure; one or both of the central microtubules are absent, and some of the doublets are incomplete (Fig. 13). The completed cilium has the typical 9 + 2 fibrous structure (12), and arms are present on the A fiber of each doublet (Fig. 14); the arms are not distinct in the early cilia (Fig. 13). Near the tip of the cilium the B fibers end first, then the A fibers, and finally the central microtubules (Fig. 15). Both central microtubules of the cilium originate in the axosome, a dense granule just distal to the basal body (Fig. 17).

Basal Bodies, Cilia, and Accessory Fibers in the Embryo

The basal bodies and cilia of the completed embryo are arranged in five long rows, which encircle the body of the embryo near its anterior end. One short row is located at the posterior end. Ridges, formed by outpocketings of the pellicle and plasma membrane, border the rows of basal bodies (Fig. 18). The microtubular fiber (Fig. 17) and the striated fiber (Fig. 18) extend up into the ridges, and the microtubular ribbons encircle the embryo at the base of each row. It is of great interest that a new fiber arises in the embryo after the cilium is formed. This is a dense fiber, neither striated nor microtubular, which originates on the left side of the basal body (Fig. 16) and extends from the base of the basal body up into the ridge (Fig. 18). In the embryo, the striated fiber and the microtubular fiber lie on the posterior side of the row, while the dense fiber is on the anterior side.

DISCUSSION

Basal Body Replication

Lwoff (19) thought of the replication of basal bodies as an actual splitting or division process. Electron microscope studies, however, have shown that basal bodies do not divide, but are formed in either of two different ways: in association with a preexisting basal body or centriole, or in the absence of this organelle. Gall (11) found that during spermiogenesis in the snail, *Viviparus*, a procentriole is formed at right angles to the mature centriole. This procentriole is a cylinder that has the same triplet structure as the centriole, but is much shorter. The procentriole lengthens during spermiogenesis and moves to the cell periphery, where the flagellum is formed. This type of basal body formation in association with a centriole is also seen during differentiation of mammalian ciliated epithelia. In the trachea and lungs of fetal rats (9, 41) and in the monkey oviduct (5), some of the basal bodies arise in association with centrioles, but others appear in the vicinity of amorphous dense regions (proliferative elements) in the cytoplasm, and are not associated with centrioles.

Both types of basal body formation have been found in protozoa. In the ameboid form of the ameboflagellates, *Naegleria* (7, 40) and *Tetramitus* (25), no basal body or centriole is present, but most of the amebas acquire basal bodies and flagella after they have been suspended in a dilute buffer. No precursor material was identified in *Naegleria*, but in *Tetramitus* plaques of dense amorphous material were seen during the transformation of the amoeboid to the flagellated stage (25). The water mold, *Allomyces*, also sprouts a flagellum when placed in a dilute aqueous medium, but in this case the basal body is formed from a procentriole, which arises at right angles to the centriole in the nonflagellated stage (31). During some stages in the life cycle of the phytoflagellate, *Chlamydomonas*, new basal bodies are formed in association with a preexisting basal body, but in other stages new basal bodies appear in the absence of a preexisting basal body (30).

Only in the past year has basal body replication been seen in the ciliates. The most detailed report was given by Dippell (8) for the gymnostome ciliate, *Paramecium*. During division of *Paramecium*, thousands of basal bodies appear in the vicinity of the new mouth. Dippell selected single organisms in early division stages, and was able to trace a whole sequence of steps in basal body formation. She found that a dense plaque of amorphous material seemed to be the first stage of basal body replication. Within the dense material, singlet microtubules appeared one at a time; once the pattern of nine was complete, doublets began to appear, and finally the typical triplet pattern

emerged. The short probasal body is formed at right angles to the mature basal body, but later it turns toward the surface, elongates, and becomes aligned parallel to the mature basal body. A similar scheme for basal body replication was proposed for another gymnostome ciliate, *Tetrahymena* (2).

Stages in basal body replication in the suctorian, *Tokophrya infusionum*, were found through the use of synchronized cultures (24). Basal body replication in *Tokophrya* seems to follow, in general, the pattern seen in the gymnostome ciliates. The short probasal body is formed perpendicular to the mature basal body. It slants upward, becomes aligned parallel to the mature basal body, and completes elongation to the size of the mature basal body, as in *Paramecium* (8) and *Tetrahymena* (2). In *Tokophrya*, the A fibers of the probasal body seem to elongate ahead of the B and C fibers; this would be expected if the A fibers are formed first, as in *Paramecium* (8). There is every reason to believe that the dense plaque and singlets precede the appearance of probasal bodies in *Tokophrya*, and, in fact, with the recent finding of Kalnins and Porter (16) that singlets precede the formation of centrioles in chick epithelia, it could be predicted that most organisms follow the same basic scheme in the development of this ubiquitous organelle.

Ciliogenesis

Ciliogenesis in *Tokophrya* appears to be the result of elongation of the A and B fibers of the basal body with concomitant membrane growth above the basal body, similar to that found in the rumen ciliates (32) and the ameboflagellate, *Tetramitus* (25). In *Tetramitus*, however, the central pair of microtubules is formed early, always before the peripheral cylinder of doublets is complete, while in *Tokophrya* the formation of the central microtubules lags behind the outer doublets, at least very early in ciliogenesis. Also, the early cilia in *Tokophrya* do not display the "ballooning" found in *Tetramitus* flagella, and the fibrils never appear disorganized. As in the probasal body, elongation of the A fibers precedes that of the B fibers. In the early cilium, one or both central microtubules are absent; perhaps the axosome must be formed before the central microtubules appear. Growth of the central microtubules overtakes that of the outer fibers later in ciliogenesis, however, since in the mature cilium the central microtubules are longer than the doublets.

Accessory Fibers

Accessory fibers have been reported in two other suctorians, *Acineta* (3), which is closely related to *Tokophrya*, and *Discophrya* (4), a more distant relative. In both of these organisms basal microtubules, a microtubular fiber, and a striated fiber, all of which bear a very close resemblance to the fibers in *Tokophrya*, are present.

There are several similarities between the accessory fibers of suctorians and those of the gymnostome ciliates. The microtubular fiber of *Tokophrya* resembles the postciliary fibers of *Tetrahymena* (1, 26) and *Paramecium* (28), and there seems to be a striking similarity between the striated fiber in *Tokophrya* and the kinetodesmos (27) of *Tetrahymena* (2). The striated fiber of *Tokophrya* is shorter and less complex than the kinetodesmos but may very well be homologous. Since the gymnostomes and suctorians are thought to have a common ancestor in the rhabdophorine ciliates (13), perhaps it is not too surprising that they share similar traits, but in view of the long evolutionary history of the two orders, it would seem that the accessory fibers have exceptionally stable characteristics.

The role of accessory fibers is not really known. Many workers (2, 10, 29) tend to favor the idea that accessory fibers are structural elements, but others (1) believe that some fibers may serve as communication links between basal bodies. The basal microtubular ribbons in *Tokophrya* certainly would be a prime candidate for coordinating ciliary beat in the embryo. But in the adult the ribbons, as well as the striated fiber and the microtubular fiber, must have a role other than that of ciliary coordination. Perhaps, they serve to "anchor" the basal bodies, not to prevent lashing cilia from displacing them (29) but to prevent cytoplasmic movements from carrying the basal bodies away from their position near the contractile vacuole pore. Or, as Allen suggested (2), the arrangement of the basal bodies may be determined by interrelationships among the cortex, the basal bodies, and the accessory fibers.

A structural role for the accessory fibers is suggested by the fact that ridges appear in the embryo. The microtubular fiber, the striated fiber, and the new dense fiber all extend into the ridges, and it is possible that they help to sculpture the cortex of the embryo.

The very well-developed microtubular ribbons in *Tokophrya* may be homologous to the basal microtubules recently reported in *Tetrahymena* by

Allen (2). Allen felt that the basal microtubules and kinetodesmos in *Tetrahymena* may play a role in orienting new basal bodies. The association of the basal microtubular ribbons and the striated fiber with probasal bodies in *Tokophrya* suggests that these fibers play a similar role in Suctoria.

This study is supported by a Graduate Fellowship at The Rockefeller University, awarded to Dr. Millecchia, and also by Grant A1-01407-12 from the United States Public Health Service awarded to Dr. Rudzinska.

Received for publication 21 January 1970.

REFERENCES

1. ALLEN, R. D. 1967. Fine structure, reconstruction, and possible functions of components of the cortex of *Tetrahymena pyriformis*. *J. Protozool.* **14**:553.
2. ALLEN, R. D. 1969. The morphogenesis of basal bodies and accessory structures of the cortex of the ciliated protozoan, *Tetrahymena pyriformis*. *J. Cell Biol.* **40**:716.
3. BARDELE, C. F. 1968. *Acineta tuberosa*. I. Der Feinbau des adulten Suktors. *Arch. Protistenk.* **110**:403.
4. BATISSE, A. 1968. Quelque donnees sur la permanence des cinetosomes durant la phase adult des Acinetiens. *C. R. Acad. Sci. (Paris).* **266**:130.
5. BRENNER, R. M. 1969. Renewal of oviduct cilia during the menstrual cycle of the Rhesus monkey. *Fert. Steril.* **20**:599.
6. COLLIN, B. 1912. Etude monographique sur les Acinetiens. II. Morphologie, physiologie, systematique. *Arch. Zool. Exp. Gen.* **51**:1.
7. DINGLE, A. D., and C. FULTON. 1966. Development of the flagellar apparatus of *Naegleria*. *J. Cell Biol.* **31**:43.
8. DIPPELL, R. V. 1968. The development of basal bodies in *Paramecium*. *Proc. Nat. Acad. Sci. U.S.A.* **61**:461.
9. DIRKSON, E. R., and T. T. CROCKER. 1966. Centriole replication in differentiating ciliated cells of mammalian respiratory epithelium. *J. Microsc.* **5**:629.
10. FAWCETT, D. 1961. Cilia and flagella. *In* The Cell. J. Brachet and A. E. Mirsky, editors. **2**:217.
11. GALL, J. G. 1961. Centriole replication. A study of spermatogenesis in the snail, *Viviparus*. *J. Biophys. Biochem. Cytol.* **10**:163.
12. GIBBONS, I. R., and A. V. GRIMSTONE. 1960. On flagellar structure in certain flagellates. *J. Biophys. Biochem. Cytol.* **7**:697.
13. GUILCHER, Y. 1951. Contribution à l'étude des cilies gemmipares, chonotriches, et tentaculiferes. *Ann Sci. Natur. Zool.* Series 11. **8**:33.
14. HASCALL, G., and M. A. RUDZINSKA. 1968. Formation of the stalk and disk during metamorphosis in *Tokophrya infusionum*; an electron microscope study. *J. Protozool.* **15**(Suppl.):11.
15. HONIGBERG, B. M., W. BALAMUTH, E. C. BOVEE, J. O. CORLISS, M. GOJDICS, R. P. HALL, R. R. KUDO, N. D. LEVINE, A. R. LOEBLICH, JR., J. WEISER, and D. H. WENRICH. 1964. A revised classification of the phylum Protozoa. *J. Protozool.* **11**:7.
16. KALNINS, V. I., and K. R. PORTER. 1969. Centriole replication during ciliogenesis in the chick tracheal epithelium. *Z. Zellforsch.* **100**:1.
17. KORMOS, J. 1958. Experimentelle Untersuchung des Knospungsgradienten. *Acta Biol. (Szeged).* **9**:105.
18. LUFT, J. H. 1961. Improvements in epoxy resin embedding methods. *J. Biophys. Biochem. Cytol.* **9**:409.
19. LWOFF, A. 1950. Problems of Morphogenesis in Ciliates. John Wiley & Sons, New York.
20. MILLECCHIA, L. 1969. Embryogenesis in the Suctorian, *Tokophrya infusionum*. A light and electron microscope study. Ph.D thesis. The Rockefeller University, New York.
21. MILLECCHIA, L., and M. A. RUDZINSKA. 1968 *a*. The fine structure of embryogenesis in *Tokophrya infusionum*. *J. Protozool.* 15(Suppl.):12.
22. MILLECCHIA, L., and M. A. RUDZINSKA. 1968 *b*. An abnormality in the life cycle of *Tokophrya infusionum*. *J. Protozool.* **15**:665.
23. MILLECCHIA, L., and M. A. RUDZINSKA. 1969 *a*. Replication of basal bodies in a Suctorian, *Tokophrya infusionum*. *Prog. Protozool., Proc. 3rd Int. Congr. Protozool. Acad. Sci. U.S.S.R.*, Leningrad. 100.
24. MILLECCHIA, L., and M. A. RUDZINSKA. 1969 *b*. Synchronized reproduction in *Tokophrya infusionum*. *J. Protozool.* **16**(Suppl.): 20.
25. OUTKA, D. E., and B. KLUSS. 1967. Ameba-to-flagellate transformation in *Tetramitus*. II. Microtubule morphogenesis. *J. Cell Biol.* **35**: 323.
26. PITELKA, D. R. 1961. Fine structure of the silverline and fibrillar system of three tetrahymenid ciliates. *J. Protozool.* **8**:75.
27. PITELKA, D. R. 1963. Electron Microscopic Structure of Protozoa. Pergamon Press, MacMillan Co., New York.
28. PITELKA, D. R. 1965. New observations on cortical ultrastructure in *Paramecium*. *J. Microsc.* 4:373.

29. PITELKA, D. R., and F. M. CHILD. 1964. The locomotor apparatus of ciliates and flagellates: Relations between structure and function. *In* Biochemistry and Physiology of Protozoa. S. H. Hutner, editor. Academic Press Inc., New York. **3**:131.
30. RANDALL, J., T. CAVALIER-SMITH, A. MCVITTIE, J. R. WARR, and J. M. HOPKINS. 1967. Developmental and control processes in the basal bodies and flagella of *Chlamydomonas reinhardii*. *Develop. Biol. Suppl.* **1**:43.
31. RENAUD, F. L., and H. SWIFT. 1964. The development of basal bodies and flagella in *Allomyces arbusculus*. *J. Cell Biol.* **23**:339.
32. ROTH, L. E., and Y. SHIGENAKA. 1964. The structure and formation of cilia and filaments in rumen protozoa. *J. Cell Biol.* **20**:249.
33. RUDZINSKA, M. A. 1951. The influence of amount of food on the reproduction rate and longevity of a suctorian (*Tokophrya infusionum*). *Science (Washington)*. **113**:10.
34. RUDZINSKA, M. A. 1952. Overfeeding and life span in *Tokophrya infusionum*. *J. Gerontol.* **7**:544.
35. RUDZINSKA, M. A. 1958. An electron microscope study of the contractile vacuole in *Tokophrya infusionum*. *J. Biophys. Biochem. Cytol.* **4**:195.
36. RUDZINSKA, M. A. 1961. The use of a protozoan for studies on aging. I. Differences between young and old organisms of *Tokophrya infusionum* as revealed by light and electron microscopy. *J. Gerontol.* **16**:213.
37. RUDZINSKA, M. A., and K. R. PORTER. 1954 *a*. The origin and fine structure of the brood pouch in *Tokophrya infusionum*. *J. Protozool.* **1**:7.
38. RUDZINSKA, M. A., and K. R. PORTER. 1954 *b*. Electron microscope study of intact tentacles and disc in *Tokophrya infusionum*. *Experientia*. **10**:460.
39. RUDZINSKA, M. A., and K. R. PORTER. 1955. Observations on the fine structure of the macronucleus of *Tokophrya infusionum*. *J. Biophys. Biochem. Cytol.* **1**:421.
40. SCHUSTER, F. 1963. An electron microscope study of the amoebo-flagellate *Naegleria gruberi*. I. The amoeba and flagellate stages. *J. Protozool.* **10**:297.
41. SOROKIN, S. P. 1968. Reconstruction of centriole formation and ciliogenesis in mammalian lungs. *J. Cell Sci.* **3**:207.
42. VENABLE, J. H., and R. COGGESHALL. 1965. A simplified lead citrate stain for use in electron microscopy. *J. Cell Biol.* **25**:407.