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Transformation and development of the flagellar apparatus of *Cryptomonas ovata* (Cryptophyceae) during cell division

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Summary. In *Cryptomonas ovata*, long, dorsal flagella are produced which transform during the following cell division into short, ventral flagella. At division there is a reorientation in cell polarity, and the parental basal apparatus, which comprises the basal bodies and associated roots, is distributed to the daughter cells via a complex sequence of events. Flagellar apparatus development includes the transformation of a four-stranded microtubular root into a mature root of different structure and function. Each newly formed basal body nucleates new microtubular roots, but receives a striated fibrous root from a parental basal body. The striated roots are originally produced on the transforming basal body and are "transferred" to the new basal bodies at each successive division. The development of the asymmetric flagellar apparatus throughout the cell cycle is described.

Keywords: Basal bodies; Cell symmetry; *Cryptomonas*; Cryptophyceae; Flagellar apparatus; Flagellar transformation.

Introduction

Flagellar transformation is the maturation process of a basal body/flagellum over two or more cell cycles. It follows that all basal bodies in a cell develop to become identical. First demonstrated in the green alga *Nephroselmis olivacea* (Melkonian et al. 1987), flagellar transformation has now been shown to occur in many algae (for review, see Beech et al. 1991), and is described here for the Cryptophyceae. As a similar developmental sequence can be deduced from published observations on the centrioles of mammalian PtK2 cells (Rieder and Borisy 1982) and amoebae of the myxomycete *Physarum polycephalum* (Wright et al. 1985), it has been proposed that the extension of basal body or centriolar development over two or more generations is a ubiquitous, intrinsic feature of all eukaryotic cells (Beech et al. 1988).

The transformation of a basal body may be accompanied by a reorientation and reorganization of the basal apparatus (= basal body and attached flagellar roots). This reorganization may have major implications for the development of the cytoskeleton, and hence cell symmetry. Flagellar transformation can be observed directly but direct observations of flagellar root development are not possible, and therefore our knowledge of these processes is less complete. Observations on basal apparatus development throughout the cell cycle have been made on a few algal species (for review, see Beech et al. 1991). In the organisms studied thus far, first generation basal bodies appear to assemble their flagellar roots de novo, while the flagellar roots of the second and subsequent generation basal bodies disassemble or are maintained (at least in a reduced form) for subsequent cell cycles.

The unicellular, asymmetric cells of *Cryptomonas ovata* (Cryptophyceae, Cryptophyta) possess two distinctly heteromorphic and heterodynamic flagella. Direct observations of living cells, in conjunction with ultrastructural studies, show that the longer, dorsal flagellum transforms during cell division into a short, ventral flagellum. Coinciding with flagellar transformation, a microtubular root associated with the dorsal basal body, transforms during cell division into a microtubular root with different associations. A striated fibrous root, which forms on the dorsal basal body while it is

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transforming, ends up with a direct connection to a newly formed basal body. These fibrous roots are retained in subsequent generations, but at each division they become associated with a newly formed basal body. This is the first description of flagellar apparatus development in a cryptomonad and includes yet undescribed mechanisms of basal apparatus ontogeny.

Materials and methods

Cultures of *Cryptomonas ovata* Ehrenberg were maintained in an enriched soil water medium and kept at 16 °C under a 15:9 h or 14:10 h photoperiod. Cells were harvested for observation and fixation 4 h before the light period.

Light microscopy

For light microscopy, live cells were observed with a Zeiss Photomicroscope III equipped with a Micro Flash II apparatus and Nomarski optics. Cells were also observed with a Nikon Optiphot-Pol microscope coupled to an Interactive Video System (IVS) Image I processor, which allowed recording in real time on video for repeated observation.

Transmission electron microscopy

For transmission electron microscopy, cells were initially fixed for 1 h in an equal volume of 4% glutaraldehyde buffered to pH 7.3 with 0.05 M sodium cacodylate then washed twice in growth medium at room temperature. The suspension was then put on ice and post-fixed for 1 h in 1% OsO_4 in buffer, rinsed three times in buffer, dehydrated in acetone and embedded in Spurr's resin (Spurr 1969). The resin preparation was placed into blocks; or "thin-embedded" between two Teflon-coated, polished microscope slides prior to polymerization. Thin-embedded cells were selected and photographed on the Zeiss Photomicroscope III, individually cut out and mounted onto a resin block for serial sectioning (for technique, see Reymond and Pickett-Heaps 1982). All sections were stained with uranyl acetate and lead citrate and viewed on a Siemens 102 transmission electron microscope. All electron micrographs and drawings are presented looking down the flagellar axis towards the proximal end of the basal bodies, unless stated otherwise.

Results

Nomenclature

In interphase cells, the oldest (mature) basal body/flagellum (BB/F) is designated 1, while the next oldest basal body/flagellum is designated 2 (nomenclature according to Heimann et al. 1989, Moestrup and Hori 1989). During flagellar duplication, the newly formed basal body/flagellum that associates with the mature parental flagellum (F1) is designated 2^1, while the newly formed basal body/flagellum that pairs with the transforming parental flagellum (F2) is designated 2^2. Prior to cytokinesis when basal body/flagellum 2 already occupies its new mature position, it is designated 1_2.

Stages in basal body development

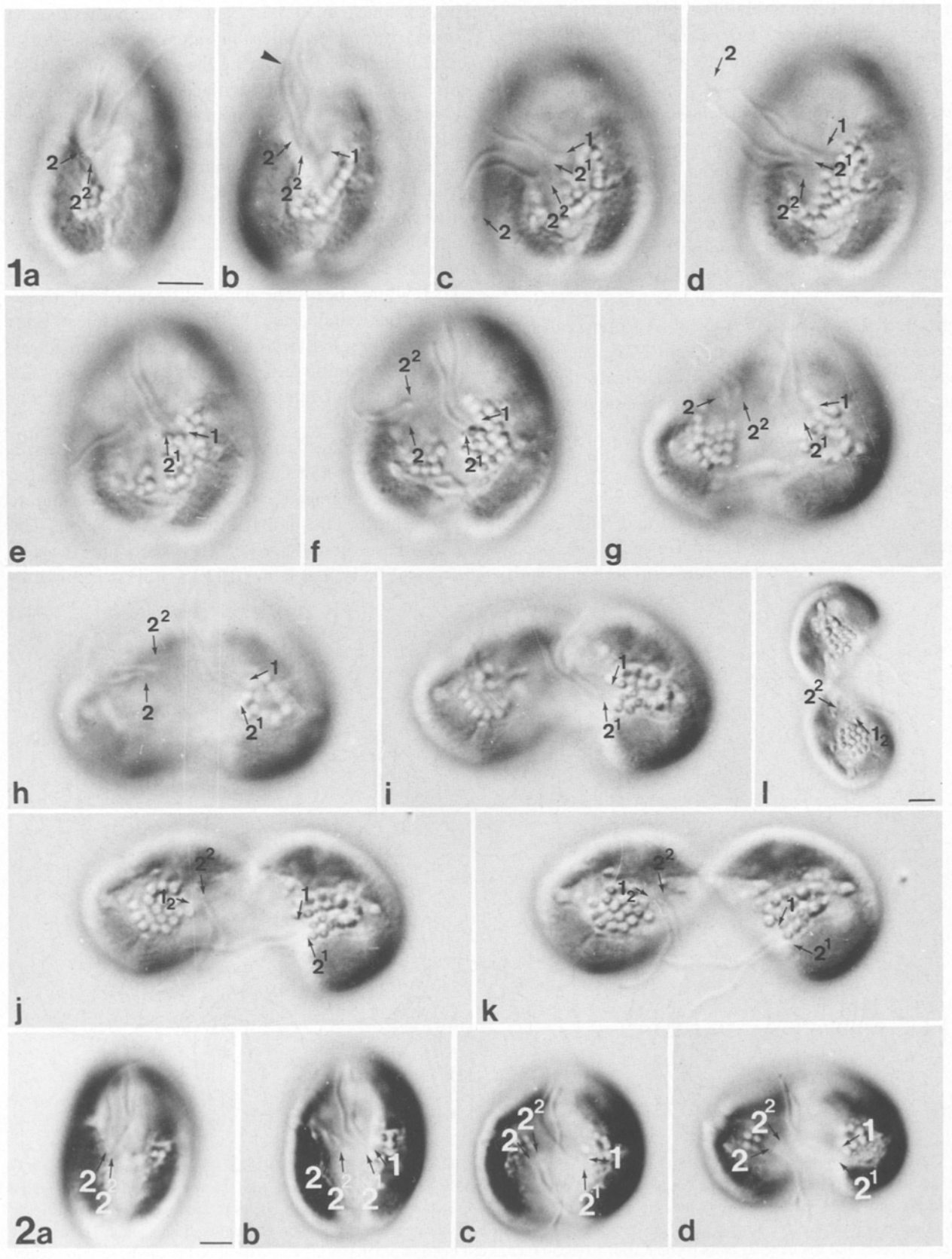
Basal body development in *Cryptomonas ovata* (and probably all biflagellate algae that do not possess probasal bodies) occurs over three developmental cycles. Stage one: During cell division two new basal bodies arise and develop flagella and flagellar roots of the same type. Each new basal body segregates with one of the parental basal bodies, and is termed basal body 2. Stage two: During the next cell division, basal body 2 transforms into basal body 1, with accompanying flagellar and flagellar root alterations. The transforming flagellum pairs, reorientates and segregates with the new basal body 2^2. Stage three: During its third division cycle, a basal body (now a basal body 1) reestablished its stage two flagellum and root associations, pairing and segregating with the new basal body 2^1. We consider this third stage an additional developmental cycle, as the basal body pairs and segregates with a different new basal body, and reestablishes the same flagellar and root structures rather than trans-

Nomenclature in figures and legends: *1* ventral (mature) basal body/flagellum (BB/F); *2* dorsal (transforming) BB/F; 2^1 new BB/F associating with BB/F 1; 2^2 new BB/F associating with BB/F 2; 1_2 transformed BB/F prior to cytokinesis. *ML* mitochondrion associated lamella root; *Rhs* rhizostyle, *SR* striated fibrous root; *SRm* striated root associated microtubules, *4r* four-stranded microtubular root; *AF* anchoring fibre; after Roberts 1984. *Cr* two-stranded root; after Mignot et al. 1968. *F* fibrous fan-like structure, *I* idiosome. *NB.* Left refers to cell's left, i.e., viewer's right

Figs. 1 and **2.** *C. ovata* during division. Bars: 2 μm

Fig. 1. a–d The new $F2^2$ precedes the dorsal (transforming) F2 as this pair migrates posteriorly, and the ventral (mature) F1 precedes the new $F2^1$ as this pair migrates posteriorly and to the cell's left. A membranous bleb on the transforming flagellum is indicated in **b** by arrowhead, in **c** and **d** by the position of the arrow. **e–g** The four flagella align at the mid-plane of the cell with parental flagella occupying the outer positions. **h** and **i** The new flagella move to the right side of each developing cell with the parental flagella trailing slightly to their left. **j** and **k** The new flagella now occupy a more right and dorsal position in relation to the parental basal bodies as the daughter anteriors form at the parental mid-plane. **l** Sideways view of the stage shown at **k**, indicating that the new flagella (which are now longer than the parental flagella) occupy the position of dorsal flagella

Fig. 2. a and **b** All four flagella are aligned along the cell's midplane with the longer (parental) flagella to the outer. **c** and **d** Cell division progresses with the new flagella still central but to the right side of each developing daughter cell


1a
b
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forming them. Presumably basal body 1 continues to develop in this manner, segregating with basal body 2^1 at the beginning of subsequent cell cycles.

Flagellar structure and insertion in an asymmetric cell

Cells of *C. ovata*, like all cryptomonad flagellates, are asymmetric in morphology, and possess two heteromorphic and heterodynamic flagella arising from a subapical vestibulum. The shorter, ventral flagellum emerges slightly to the left and posterior of the longer, dorsal flagellum (Fig. 3 a). Note that "left" refers to the left side of the parental cell which is the viewer's right. Both flagella have the typical cryptophycean surface ornamentation (Hibberd et al. 1971), the dorsal flagellum possessing two rows of elongate bipartite hairs while the ventral flagellum has a single row of shorter bipartite hairs.

Flagellar/basal body transformation

Direct observations of cell division and flagellar development were made on many live cells of *C. ovata* using light microscopy and were corroborated with thin-sectioned material. New basal bodies/flagella formed during cell division initially become dorsal basal bodies/flagella. During the subsequent cell division the dorsal basal body/flagellum (BB/F2) is observed to transform into a ventral basal body/flagellum (BB/F1) that presumably remains as such throughout successive generations (summarized in Figs. 1 and 3). Prior to cytokinesis, two new basal bodies form, one on each side of the parental pair (Fig. 3 a). The new basal bodies produce flagella, and from the earliest stages of their growth the new flagella maintain a rapid and rhythmic beat. The parental flagella beat irregularly during cell division. In one series of thin sections through a pre-division (prophase) cell, the new flagellum ($BB2^1$), can be seen to bear two opposing rows of tubular hairs, identifying it as a developing dorsal flagellum (Fig. 8 a).

When the new flagella are approximately half the length of the parental flagella, the cell's furrow widens, the basal bodies segregate into pairs, and rapidly migrate (ca. 1–2 min) to the mid-ventral region of the cell. The ventral basal body/flagellum (BB1) precedes the new left basal body/flagellum ($BB2^1$) as this pair migrates

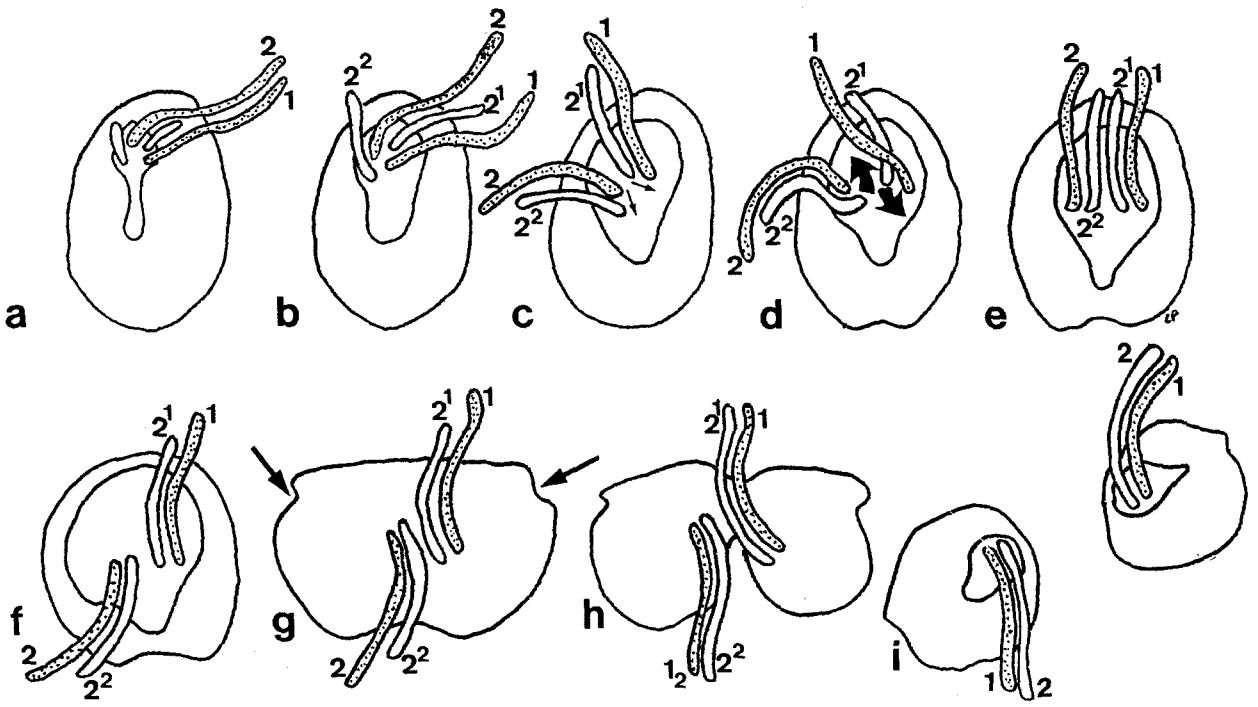


Fig. 3. Diagrammatic representation of flagellar development during division in *C. ovata*. Parental flagella are stippled. **a–c** The new $F2^2$ precedes the dorsal (transforming) F2 as this pair migrates posteriorly, and the ventral (mature) F1 precedes the new $F2^1$ as this pair migrates posteriorly and to the cell's left (arrows indicate direction of migration of each pair). **d–f** The basal bodies of each pair migrate relative to each other so that all four are aligned along the mid-plane (curved arrows indicate relative migration of basal bodies). **g** and **h** The parental gullet disappears and the flagellar pairs become skew to each other as cell division progresses. The new basal bodies can be seen to occupy the position of the dorsal, right flagellum in each developing daughter cell (arrows indicate posteriors of the developing daughter cells). **i** Ventral view of the daughter cells, after cytokinesis

to the left side of the future division plane (Figs. 1 b–d and 3 c, d). The new right basal body/flagellum ($BB2^2$) precedes the dorsal basal body/flagellum (BB2) as they migrate almost directly posteriorly (Figs. 1 a–d and 3 c). Electron micrographs of cells sectioned at stages of basal body pairing show a connection forming between basal bodies 2 and 2^2 confirming their association as a new pair (Fig. 10 b).

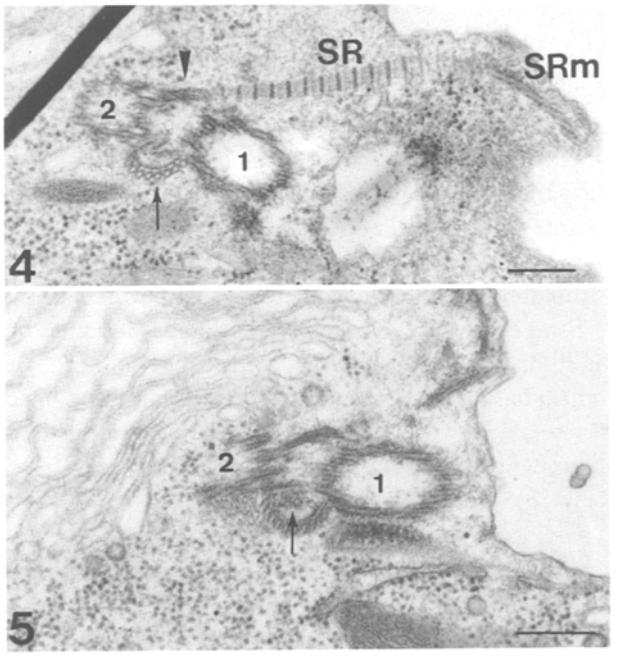
The basal bodies of the right pair, 2 and 2^2, then migrate relative to each other, with basal body 2^2 moving anteriorly and to its partner's left (Figs. 1 d–g and 3 d). The basal bodies of the left pair undergo a similar, but opposite, relative migration with basal body 2^1 moving posteriorly and to the right of its partner (BB1) (Figs. 1 d–f and 3 d). Thus, all four basal bodies/flagella are aligned along the transverse midplane of the parental cell, with the two new basal bodies/flagella occupying the central positions (Figs. 1 g, 2 a–c, and 3 e). When longitudinal cleavage commences, the right basal body pair migrates slightly anteriorly, and the left pair posteriorly (that is towards the right sides of the developing daughter cells). The new basal body of each pair is directed slightly more to the right of each developing cell. In the case of the right side pair, the new basal body (BB^2) will be more anterior (relative to the parental cell) than its partner (BB2). In the case of the left side pair, the new basal body ($BB2^1$) will be more posterior than its partner (BB1). The two basal body pairs now point in opposite directions (Figs. 1 h, 2 d, and 3 g). The parental flagella are still longer than the new flagella, although they shorten somewhat (ca. 20% of their original length) while the new flagella elongate. During cell division, the anterior of each daughter cell forms at the mid-plane of the parental cell, thereby effecting the change in cell polarity described for dividing cryptomonads (Perasso et al. in prep.). This cell reorientation places the new basal bodies/flagella, which have remained nearest this mid-region, in the more dorsal position in each developing cell (Figs. 1 j, k, 2 d, and 3 h). Thus, both new basal bodies/flagella have taken up the position of the dorsal basal body/flagellum in each future daughter cell. When dividing cells are viewed side on (Fig. 11), the orientation of the forming daughter cells is seen more clearly, with each new basal body/flagellum right and dorsal to its associated parental basal body/flagellum. At late stages of cell division, the new flagella become marginally longer than the parental flagella, while the transforming flagellum becomes distinctly motionless for 1–2 min.

Structure of the basal apparatus

A detailed description of the interphase basal apparatus of *C. ovata*, including flagellar root nomenclature, has been published by Roberts (1984), and is summarized here along with our own observations (refer to Fig. 6). The basal apparatus, which consists of two basal bodies and associated fibrous and microtubular roots, is asymmetrical.

The rhizostyle (Rhs), emerges from the right side of the basal body pair (Figs. 4 and 5) and descends posteriorly into the cell. We have observed an additional two microtubules at the proximal end of the rhizostyle, which appear freely suspended within its concave surface, and might extend for most of its length (Fig. 5). A finely striated, mitochondrion associated lamella root (ML) emerges from between the two basal bodies and stretches to their right. A sparsely striated anchoring fibre (AF) links the ventral basal body (BB1) to the furrow. The idiosome (I, an amorphous body), surrounds the ventral and left surfaces of the ventral basal body (BB1) below the AF.

A striated fibrous root (SR), emerging from a dense



Figs. 4 and **5.** Flagellar apparatus of *C. ovata* at interphase. Bars: 0.2 μm

Fig. 4. Arrow indicates Rhs. Arrowhead indicates the fibrous attachment band connecting the SR to the dorsal BB2. The distal end of the SRm is observed where the root commences to traverse the path of the SR

Fig. 5. Arrow indicates an additional two microtubules suspended within the concave surface of the Rhs

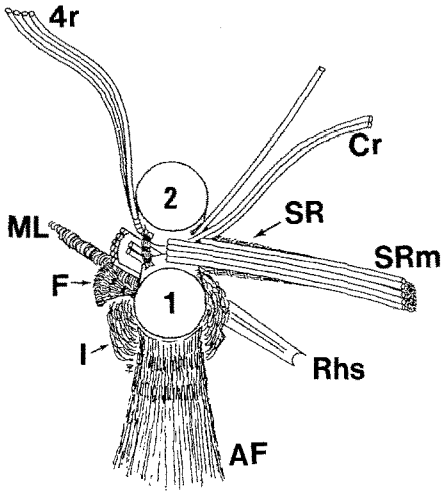


Fig. 6. Three dimensional representation of the flagellar apparatus of *C. ovata* during interphase

fibrous attachment band on the dorsal basal body (BB2), passes by the ventral basal body (BB1) and then progresses centrally through the anterior of the cell (Fig. 4). Traversing the path of the SR, just above it, are the striated root associated microtubules (SRm) (Fig. 4). The five-stranded SRm is adpressed to the dorsal surface of the ventral basal body at its origin. A two-stranded microtubular root (Cr) emerges at an approximate angle of 45° to the SR and ascends dorsally. A four-stranded microtubular root (4r) emerges from the right side of the dorsal basal body (BB2) in an anterio-dorsal direction at near right angles to the SRm. (A single microtubule is observed adjacent to the CR in dividing cells and sometimes in interphase cells.) A distal connecting band connects the basal bodies on the right side of the pair. The triplet that connects the ventral basal body (BB1) to the distal connecting band is associated with a fibrous fan-like structure, itself adjacent to the AF.

Summary of basal apparatus development and transformation in Cryptomonas ovata

Flagellar root transformation occurs when the 4r becomes the SRm during division, the parental SRm being maintained as a mature root. The SR is formed on the transforming basal body (BB2), prior to a fibrous attachment band forming between this new SR and the new basal body ($BB2^2$). The parental SR is retained beneath the SRm but its interphase attachment to the dorsal basal body (BB2) breaks down and later a new fibrous attachment band forms between it and the new basal body ($BB2^1$). First generation 4r and Cr roots are formed de novo on the two new basal bodies. The AF is formed on the transforming (dorsal) basal body (BB2) as it matures to become a ventral basal body ($BB1_2$), and remains with this basal body in subsequent generations. These processes are described in detail below.

Duplication of the basal apparatus

During the initial stages of basal apparatus duplication (refer to Fig. 7), the parental distal connecting band breaks down (Fig. 10 a); however, the parental basal bodies are still closely associated at their proximal ends enabling their identification (Figs. 8 c, 9 b, 10 d, and 11 d, e). In some cells, a new distal connecting band is seen forming between basal bodies 2 and 2^2 (Fig. 10 a). Even at this early stage each new basal body pair with its associated flagellar apparatus can be identified.

The parental AF (Figs. 8 c, 10 a, and 11 a), the fibrous fan (Figs. 10 a and 11 a) and the idiosome (Figs. 8 c, 10 c, d, and 11 b, c) maintain their association with the mature ventral basal body (BB1) and enable its identification throughout division. The SRm maintains its interphase association with the dorsal surface of the mature ventral basal body (BB1) (Fig. 11 a). The attachment of the parental SR to the dorsal basal body (BB2) appears to break down but this SR remains

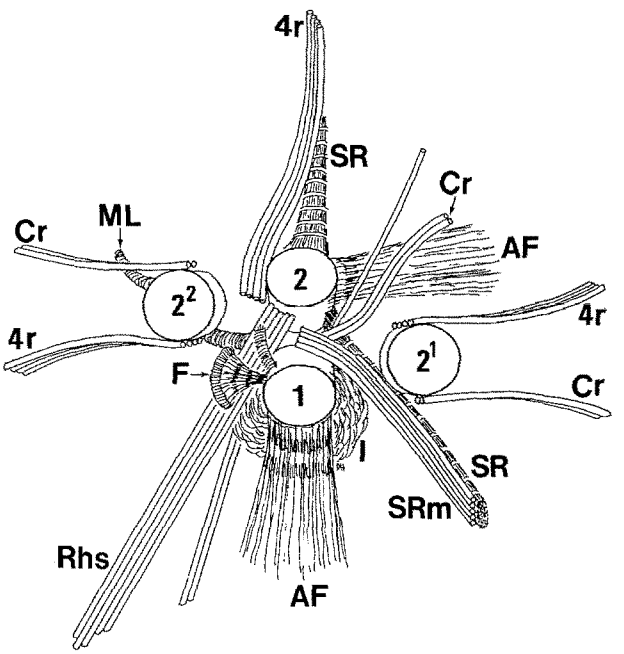
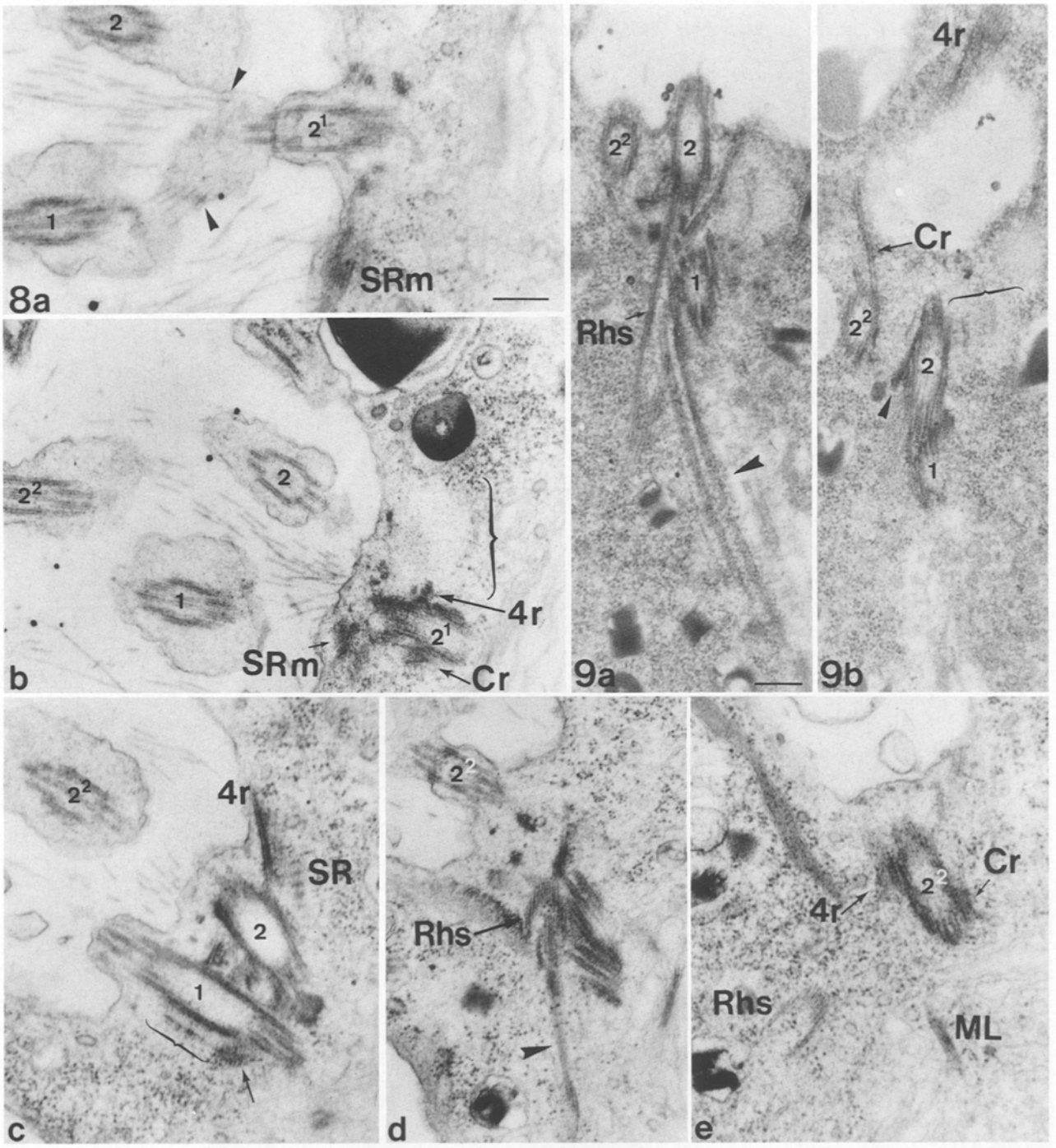


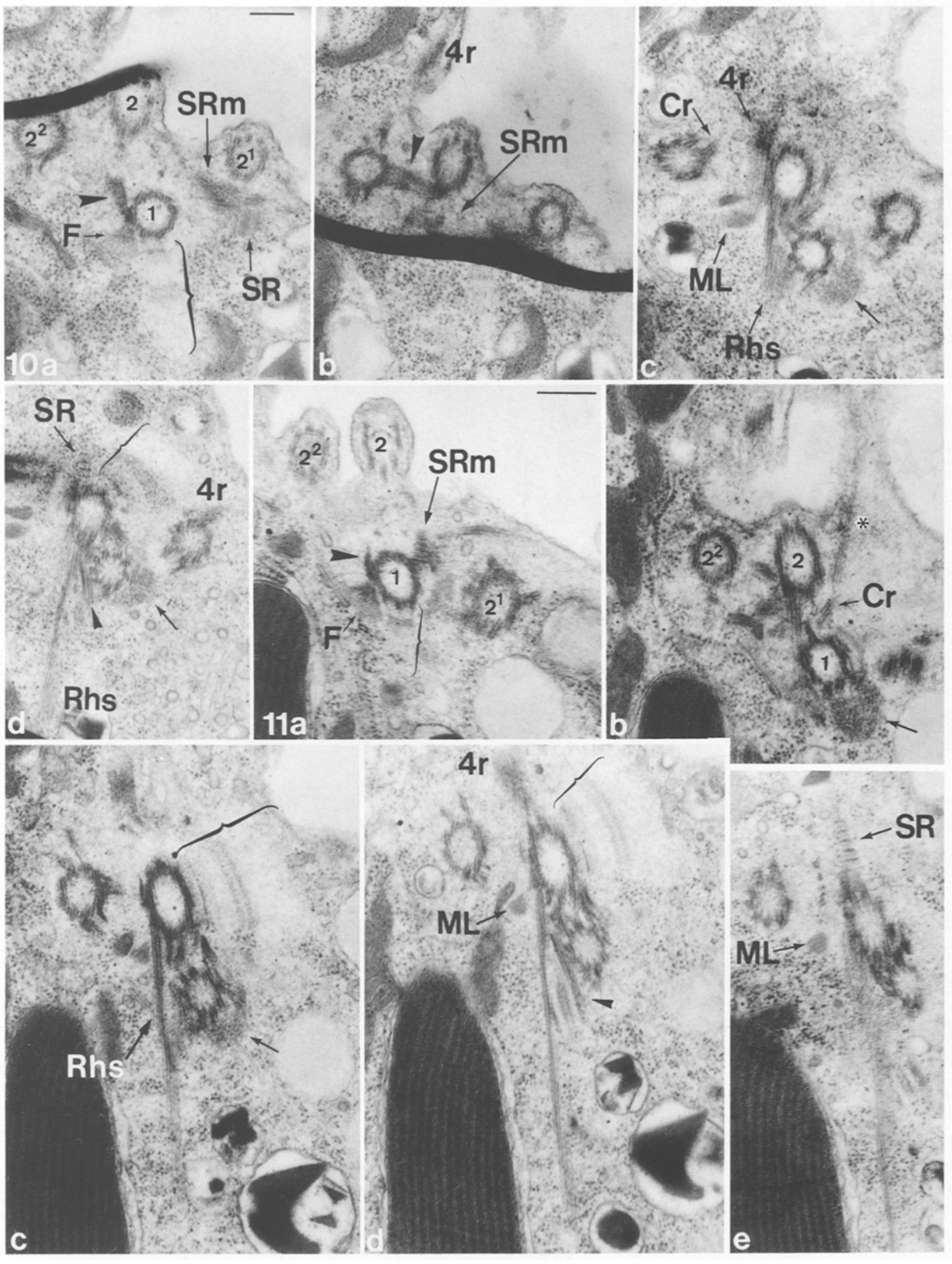
Fig. 7. Three dimensional representation of the flagellar apparatus of *C. ovata* during its duplication but prior to segregation of basal bodies



Figs. 8 and **9.** Nonsequential serial sections through two cells of *C. ovata* during duplication of the flagellar apparatus. Bars (in Fig. 8 a and Fig. 9 a for Fig. 8 a–e and Fig. 9 a and b, respectively): 0.2 μm

Fig. 8. a Arrowheads indicate two rows of flagellar hairs on the new flagellum 2^1, evidence that it will become a dorsal flagellum. **b** A new Cr and new 4r nucleate from $BB2^1$, and the SRm passes between it and the ventral (mature) BB1. A new AF (bracket) forms to the cell's left of the dorsal (transforming) BB2. **c** The parental AF (bracket) remains associated with BB1, as does the idiosome (arrow). The parental 4r, which passes BB2 on its right surface, is subtended by the developing SR. **d** The Rhs is visible on the right side of the parental basal body pair with two central microtubules (arrowhead) central within its arc. **e** More posteriorly the arc of the Rhs is still visible, as is the parental ML. A new Cr nucleates from the new $BB2^2$, on the side adjacent BB2, while a new 4r nucleates from its opposite side

Fig. 9. a The Rhs has attached to BB2, while a number of microtubules (arrowhead) remain behind, skew to the arc. **b** A new AF (bracket) nucleates from BB2. The parental 4r curves in sinusoidal fashion from the anterior of the cell, passing by the right surface of BB2 (in glancing section; arrowhead), between BB2 and $BB2^2$. A new Cr nucleates from $BB2^2$ on the side adjacent BB2


10a
SRm
2
2²
2¹
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SR
b
4r
SRm
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4r
Cr
ML
Rhs
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Rhs
11a
SRm
2²
2
1
2¹
F
b
*
2²
2
Cr
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subtending the SRm (Fig. 10 a). The new left basal body ($BB2^1$) develops dorsal to the parental SR and SRm (Figs. 8 a, b, and 10 a), and thus these two roots run between basal bodies 1 and 2^1, as they did previously between 1 and 2. (In micrographs from Roberts et al. (1981) of *Chilomonas* these roots are also observed running between the parental and new basal body. Note that these micrographs view the basal bodies from proximal to distal, contrary to ours.) A new two-stranded Cr nucleates adjacent to basal body 2^1 and extends between it and 1, emerging from between this pair on the same side as the SRm (Fig. 8 b). Three to four microtubules (a new 4r) also nucleate from basal body 2^1 on the opposite surface to the Cr (Figs. 8 b and 10 d).

In viewing the other new pair, the transformation of the 4r into the SRm can be observed. The parental 4r remains on the right surface of the dorsal basal body (BB2) (Figs. 8 c, 10 c, and 11 d) while the new basal body ($BB2^2$) develops to its right (Figs. 10 c and 11 d). Hence the 4r is running between the transforming basal body (BB2) and the new right basal body ($BB2^2$), in the fashion of an SRm. It maintains this association to become the SRm in the right daughter cell, as the dorsal basal body (BB2) moves to become a ventral basal body ($BB1_2$). This transformation requires the parental 4r to gain another microtubule as the SRm is five-stranded. A new SR nucleates directly from the transforming basal body (BB2) (Figs. 8 c, 10 d, and 11 e); there is no fibrous attachment band connecting it to the basal body as in interphase cells (cf. Fig. 4). This SR subtends the path of the transforming 4r, reflecting the relationship of the SR subtending the SRm in interphase cells. A new AF emanates from the left surface of the (transforming) dorsal basal body (BB2) at right angles to the parental 4r (Figs. 8 b, 9 b, 10 d, and 11 c, d). A new two-stranded Cr nucleates adjacent to the new right basal body ($BB2^2$) between it and basal body 2 (Figs. 8 e, 9 b, and 10 c), while from this same new basal body a new 4r nucleates on the opposite surface (Fig. 8 e).

At these early stages, the parental Cr lies in its interphase position (Figs. 10 c and 11 b), but in some cells it appears to divide in half (not illustrated). A single microtubule is visible adjacent to the Cr (Figs. 10 c and 11 b). The parental ML is also in its interphase position (Figs. 8 e, 10 c, and 11 d, e). The rhizostyle becomes attached to the ventral surface of the dorsal basal body (BB2) and lies between the parental basal bodies (Figs. 9 a, 10 c, d, and 11 b–d). A number of microtubules of the rhizostyle appear to remain in their normal position, and, hence, are skew to its arc (Figs. 9 a, 10 d, and 11 d).

Segregation of the basal bodies

The pairing of the basal bodies and their migration to the mid-plane of the cell where they become aligned occurs rapidly (ca. 1–2 min). Once aligned, the major roots are already associated with their basal body pairs as they appear in the interphase basal apparatus (refer to Fig. 13, cf. Fig. 6). The distal connecting band attaches opposing triplets along the side of each pair that will face the right side of each daughter cell (Figs. 12 f and 14 a). New 4rs extend from this side of each new basal body to the centre of the parental cell (Figs. 12 c,

Figs. 10 and **11.** Nonsequential serial sections through *C. ovata* during duplication of the flagellar apparatus. Bars (in Fig. 10 a and Fig. 11 a for Fig. 10 a–d and Fig. 11 a–e, respectively): 0.2 μm

Fig. 10. a The parental AF (bracket) emanates from the ventral (mature) BB1, with the fan (*F*) and distal connecting fibre (arrowhead) to its right. The SRm passes between BB1 and the new $BB2^1$ above the parental SR. **b** A new distal connecting band (arrowhead) has already formed between the dorsal (transforming) BB2 and the new $BB2^2$. The SRm ends below BB2. Distal regions of the parental 4r are observed. **c** The parental 4r meets BB2 at its right side, running between BB2 and $BB2^2$, in the fashion of an SRm, indicating its forthcoming role. The Rhs runs posteriorly from the ventral surface of BB2, to which it has become associated. The idiosome (arrow) remains associated with the ventral BB1. A new Cr emanates from $BB2^2$. **d** A new 4r emanates from $BB2^1$. A new SR nucleates from BB2 subtending the parental 4r, with a new AF (bracket) just beginning to form to its left. The Rhs extends posteriorly, with a few microtubules (arrowheads) now skew to its arc

Fig. 11. a The parental AF (bracket) emanates from BB1, with the fan (*F*) and distal connecting fibre (arrowhead) to its right. The SRm lies on the dorsal surface of BB1, between BB1 and BB2. **b** The idiosome (arrow) remains associated with the ventral and left surfaces of BB1. A single microtubule (asterisk) emerges from between the basal bodies near the parental Cr. **c** The Rhs has attached to BB2 (note also in **b** and **d**), and a new AF (bracket) nucleates from this same basal body. The idiosome is indicated by the arrow. **d** The parental 4r meets BB2 at its right side (see also in **c**), at right angles to the new AF and runs between BB2 and $BB2^2$, mimicking the SRm of interphase cells. The ML lies in its interphase position, as do a few microtubules of the Rhs (arrowhead), now skew to the arc of the Rhs. **e** A new SR emanates from BB2, subtending the parental 4r in the fashion of the SR to SRm in interphase cells

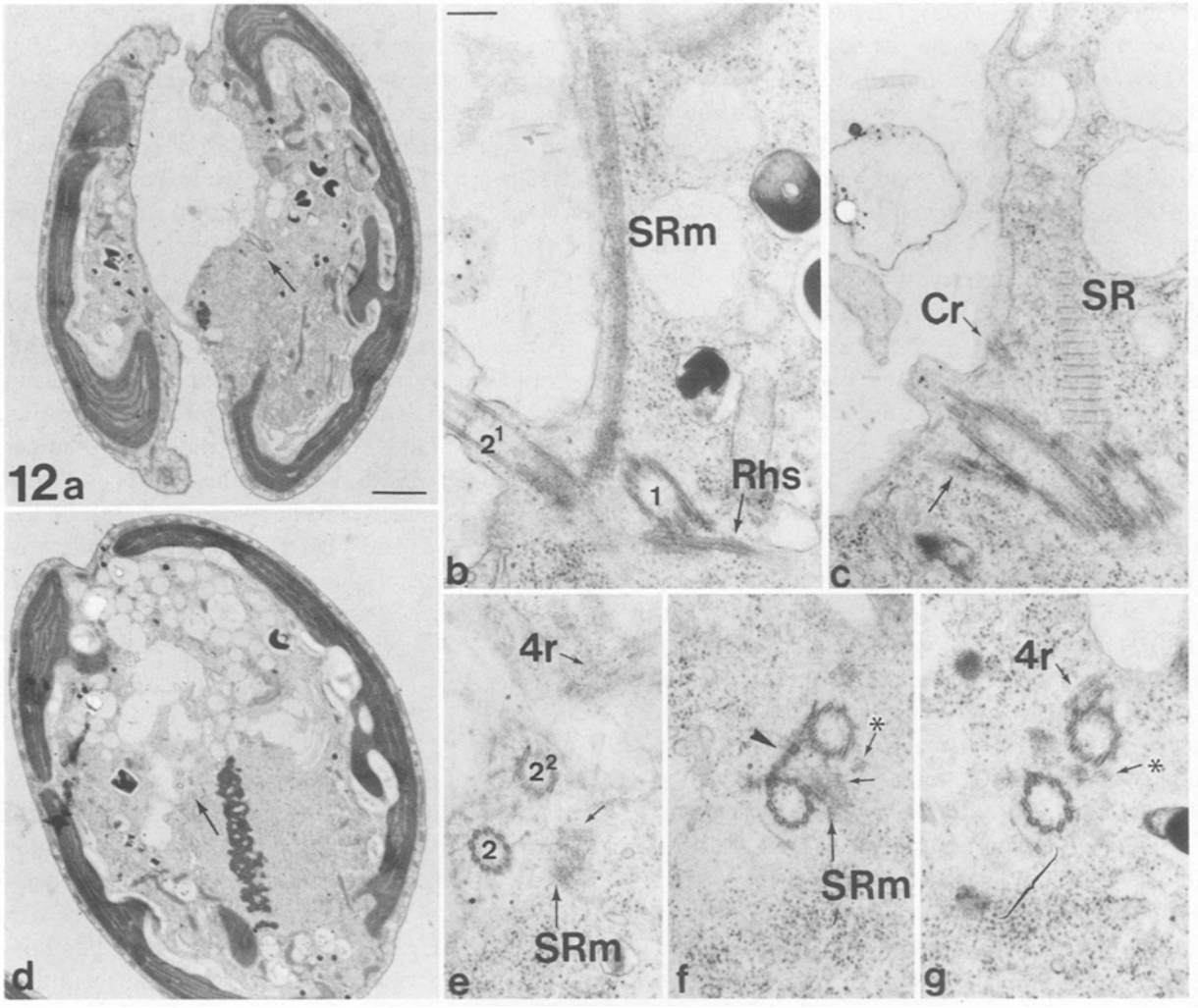

Fig. 12. Nonsequential serial sections through a *C. ovata* cell following segregation of basal bodies and their alignment at the cell's mid-plane. Bars: for a and d, 2 μm; for b, c, and e–g, 0.2 μm. **a** and **d** Whole cell showing position of basal body pairs within the cell (arrows), section shown in **a** bears the cell's left (viewer's right) basal body pair and is more ventral than the section shown in **d**. **b** and **c** Sections through the cell's left basal body pair showing the parental SRm passing between the ventral (mature) BB1 and its new partner $BB2^1$. A new Cr emerges at ca 45° C to the SRm, a new 4r (arrow) originates on the opposite side of $BB2^1$ to the new Cr. The new SR subtends the parental SRm with its origin not clearly on either basal body. **e–g** Sections through the cell's right basal body pair. The former 4r (now an SRm) passes between the pair along with a new Cr (small arrow), which is below and at ca 45° C to it. A single microtubule (asterisked small arrow) emerges from $BB2^2$ on the opposite surface to the Cr. A new distal connecting band (arrowhead) couples opposing basal body triplets on what will be the right side of the pair, with the new AF (bracket) emanating from the transformed $BB1_2$

e–g, and 14 b, c, h). The SRms (the parental SRm and transformed 4r) extend from the basal body pair at approximately 90° to the new 4rs (Figs. 12 b, e, f, and 14 b, g).

SRs subtend the SRms; the parental SR subtends the parental SRm on the left basal body pair, while the new SR subtends the new SRm (former 4r) on the right pair (Figs. 12 c and 14 b, c, h). At this stage, the origin of each SR is difficult to determine. In some cells, the new SR still appears to be attached to the transformed basal body ($BB1_2$), its nucleating site (Fig. 14 c). The parental SR (Figs. 12 c and 14 h) remains directly beneath the mature SRm and hence its origin is closer to the ventral basal body (BB1); however, it is not clearly connected to either basal body and there is no fibrous attachment band present (cf. Fig. 4).

An AF emanates from each parental basal body, the newly formed AF associating with the transformed basal body ($BB1_2$), and the parental AF with the parental mature basal body (BB1) (Figs. 12 g and 14 a, f).

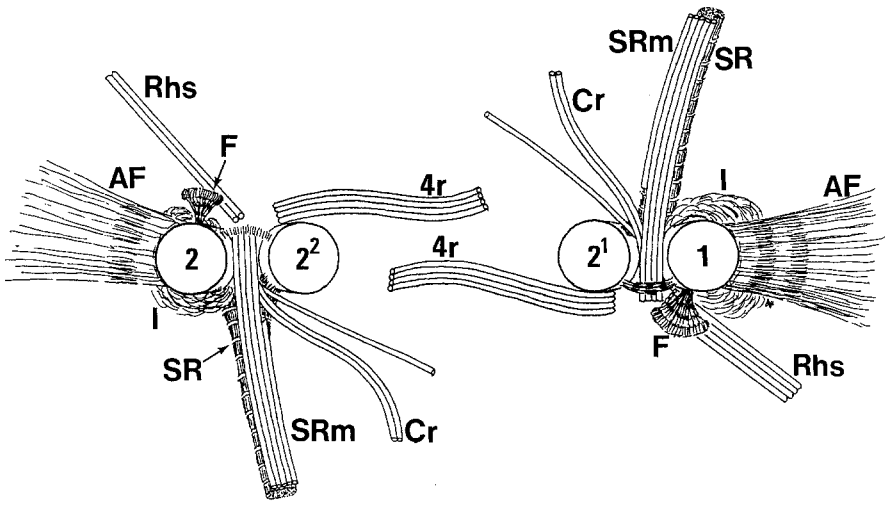

Fig. 13. Three dimensional representation of the developing flagellar apparatus of the forming daughter cells, following pairing and segregation of the basal bodies, and their migration to the parental cell's mid-plane

The new Crs emerge from between the basal bodies below the SRms (Figs. 12 c, e, f, and 14 b, h). A single microtubule is often seen adjacent to the new Crs (Figs. 12 f, g, and 14 c).

A few microtubules of each rhizostyle run towards the parental cell's lateral regions (the two regions that become the daughter cell posteriors) (Figs. 12 b and 14 b, h).

Further migration of the basal bodies

Further development of the basal apparatus occurs during the completion of cytokinesis, when the basal body pairs and associated roots migrate to occupy an interphase position in the forming daughter cell. The transforming 4r, which lay on the right surface of the dorsal basal body (BB2) during the previous cell cycle (Fig. 15 a), is now the SRm on the dorsal surface of the ventral (transformed) basal body ($BB1_2$) (Figs. 14 b and 15 c, d). The new AF, which nucleated from the left surface of the (transforming) dorsal basal body (almost opposite the parental 4r), is now in its interphase position on the ventral surface of basal body 1_2 (Fig. 14 a). The point of association of these two roots to this basal body illustrates the sum rotation that the dorsal basal body undergoes during cell division (for summary, see Fig. 15).

The origin of both SRs is still undefined during late stages of division (Fig. 14 c and h). Even in these later stages, both basal body pairs bear an apparently reduced form of rhizostyle (Fig. 14 b and h). We have not observed an ML after segregation of the basal bodies, hence cannot determine its behaviour during division. It is possible that the ML breaks down and two new ones are formed.

Discussion

Flagellar transformation and basal body reorientation during cell division

During cell division in *Cryptomonas ovata*, a major reorientation in cell polarity occurs as the posterior of each forming daughter cell develops in an anterio-lateral region of the parental cell (Perasso et al. in prep.). With this reorientation, basal body/flagellar transformation is effected. The new basal body pairs migrate approximately 90° from their origin to either side of the division plane of the parental cell (where the anterior of each daughter cell will develop). Each basal body also reorients relative to its partner as the pairs migrate. As a result of the reorientation, the parental basal bodies take up the position of the ventral basal body in each daughter cell.

As flagellar roots often nucleate cytoskeletal microtubules, either directly or indirectly, they mediate a spatial arrangement of organelles within the cell and play a role in determining cell shape. In *C. ovata*, cell and basal body reorientation provides for maintenance of cell and flagellar apparatus asymmetry, and maintenance of a functional orientation of basal bodies (to their respective partners) as they reassociate during cell division.

The exact degree of basal body reorientation in *C. ovata* is difficult to determine due to the migration and changes in cell shape occurring at division (see summaries in Figs. 3 and 15). Basal body reorientation during division was reported in a number of algae prior to the first description of flagellar transformation (for review see O'Kelly and Floyd 1984). These earlier reports focused attention on the necessity of maintaining the absolute orientation of the flagellar apparatus dur-

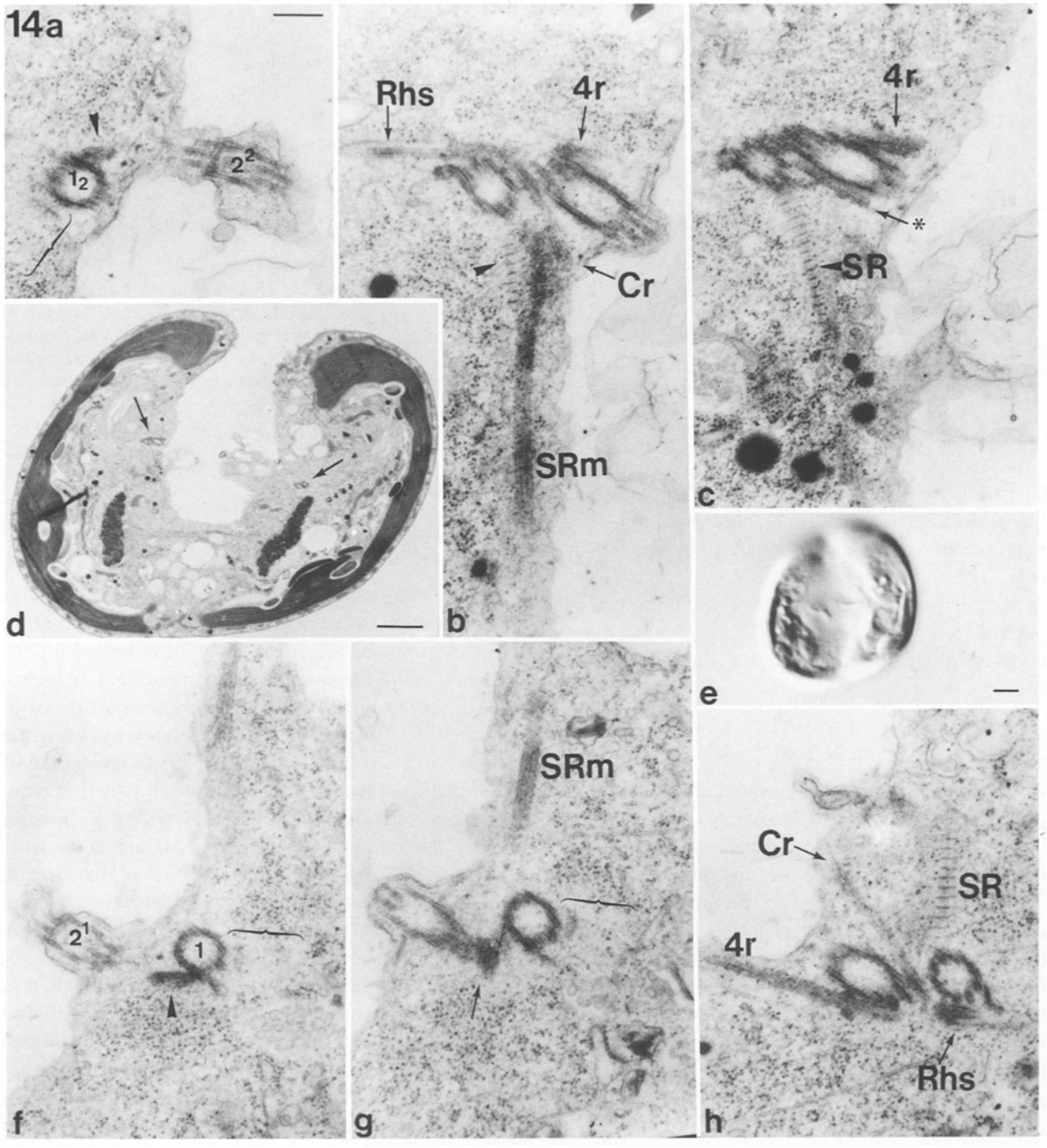

Fig. 14. Nonsequential serial sections through a *C. ovata* cell during basal body pair migration to interphase positions. Bars: for a–c and f–h, 0.2 µm; for d and e, 2 µm. **a–c** Sections through the cell's right (viewer's left) basal body pair. **a** The transformed $BB1_2$ bears the new AF (bracket) and the new distal connecting band (arrowhead). **b** and **c**. The SRm (formerly 4r) passes between the pair and progresses posteriorly. The new SR lies beneath the SRm, its origin still at the basal body from which it was nucleated. A new Cr passes between the pair at ca 45° to the SRm. A single microtubule (asterisked arrow) emerges from between the basal bodies near the Cr. A new 4r emanates from the new dorsal $BB2_2$ at right angles to the SRm. A portion of the Rhs points laterally towards what will be the posterior region of the daughter cell. **d** Whole cell of **c**, showing both left and right basal body pairs (arrows). **e** Light micrograph of the same cell prior to sectioning. **f–h** Sections through the cell's left basal body pair. **f** The parental ventral BB1 has maintained the parental AF (bracket, see also in **g**), the distal band (arrowhead) connects it to its new partner. **g** and **h** The parental SRm passes between the basal body pair (arrow) below the distal connecting band, with the parental SR beneath. A new CR also passes between the basal bodies at ca 45° to the SRm. A new 4r emanates from $BB2^1$ at right angles of the SRm. A portion of the Rhs points laterally towards what will become the posterior region of the daughter cell

ing its division and segregation into daughter cells. Although basal body/flagellar transformation could have been inferred from these observations, it was not directly discussed.

Flagellar root development and transformation

During cell division in *C. ovata*, at least one root (4r) is transformed (along with its associated basal body) into a mature root (SRm). The majority of the parental flagellar roots are maintained, while new roots are synthesized at each cell cycle from the transforming basal body as well as the new basal bodies. Flagellar root transformation has not previously been demonstrated, though such a mechanism has been anticipated (Melkonian et al. 1987, Moestrup and Hori 1989).

One of the few reports to describe flagellar root behaviour during basal body transformation concerns the synurophyte *Mallomonas splendens* (Beech and Wetherbee 1990 b). *M. splendens* achieves flagellar root reorganization through the disassembly of the parental microtubular root and rhizoplast, and the simultaneous formation of new structures by the new basal bodies. Likewise, in *Pleurochrysis carterae*, it is probable that the parental flagellar roots break down and all roots are formed de novo (Beech et al. 1988). In the green algae *Polytomella* (Aitchison and Brown 1986), *Chlamydomonas* (Gaffal 1988), *Brachiomonas* (Segaar and Gerritsen 1989), *Chlorosarcina* (Sluiman and Blommers 1990), as well as the euglenoid *Ploeotia* (Farmer and

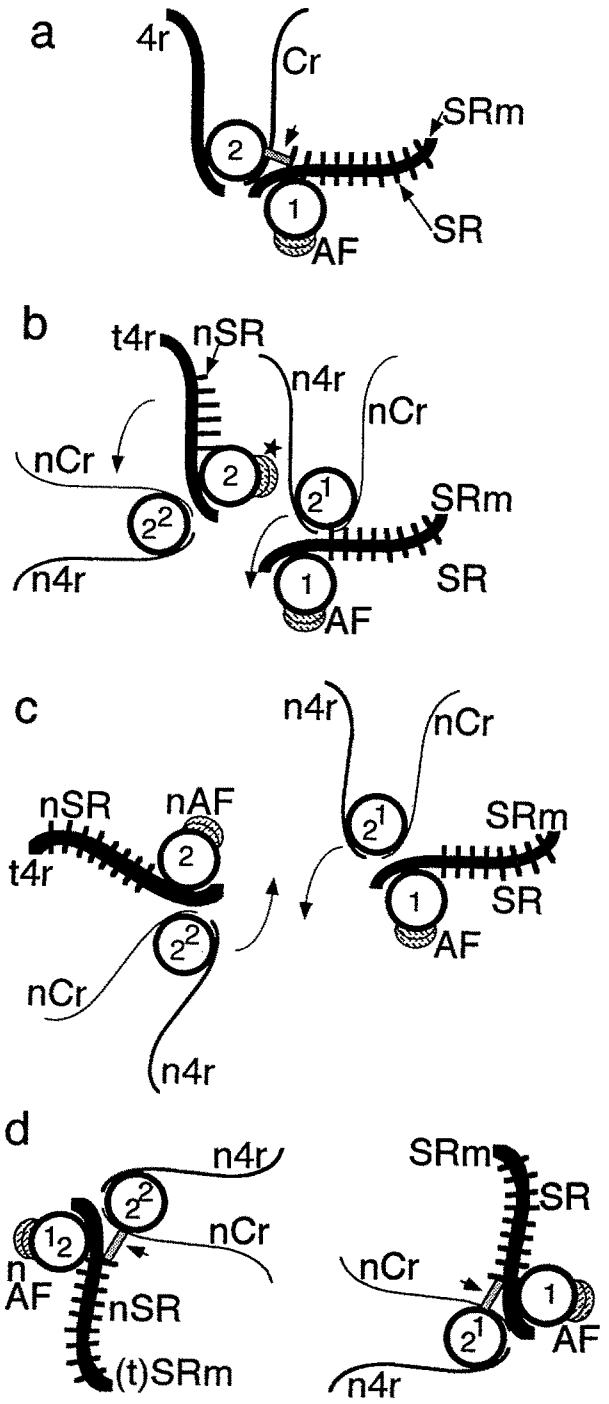

Fig. 15. Two dimensional representation of part of the basal apparatus of *C. ovata*, illustrating relative migration of basal bodies, and transformation of the 4r. Not all roots illustrated. *nCr* new Cr, *n4r* new 4r, *nAF* new AF, *nSR* new SR, *t4r* transforming 4r, *(t)SRm* new SRm, having transformed from the 4r; the SRm is represented by the solid curved line only, the SR is represented by the cross striations and lies beneath the SRm. **a** Interphase flagellar apparatus. Arrow indicates the fibrous attachment band linking the SR to the dorsal BB2. **b** Predivision flagellar apparatus: the new basal bodies (2^1, 2^2) have formed on either side of the pair. The fibrous attachment band has broken down. The parental SR remains beneath the parental SRm, and these roots now pass between BB1 and $BB2^1$ as they previously did between BB1 and BB2. The new SR nucleates from the dorsal (transforming) BB2 beneath the parental 4r. These pass between BB2 and $BB2^2$ as the SR and SRm pass between BB1 and $BB2^1$. The new AF also nucleates from BB2, at right angles to the 4r, mimicking the positional association of the parental AF and SRm. Both new basal bodies nucleate a Cr from the side adjacent their partner basal body, and a 4r from their opposite side. The curved arrows indicate direction of movement of each basal body pair as they segregate and migrate to the mid-phase of the dividing cell. **c** Arrows indicate relative migration of the new basal bodies to the parental basal bodies, within each pair, to effect alignment along the mid-plane. **d** The basal body pairs continue to migrate throughout division until they lie skew to each other at the completion of cytokinesis. The new basal bodies now occupy the position reserved for the dorsal flagellum within each forming daughter cell. The flagellar roots, which have migrated with the basal bodies, now exhibit normal interphase associations, with the parental 4r occupying the position of an SRm. The two SRs lie beneath the SRms. Arrows indicate where the fibrous attachment bands will form to mediate a connection between the SRs and the new dorsal basal bodies

Triemer 1988), the roots of new basal bodies are formed de novo, while the parental roots are conserved, for the most part, and distributed semi-conservatively to the daughter cells. Both basal bodies of the pair in *Polytomella, Chlamydomonas, Brachiomonas,* and *Chlorosarcina* bear roots similar in morphology. Nevertheless, *Chlamydomonas* cells have been shown to be asymmetrical due to the position of the single eyespot associated with one flagellar root of the immature basal body (Moestrup 1978, Melkonian and Robenek 1984, Holmes and Dutcher 1989). Therefore, as the flagellar roots are initially associated with the immature basal body, and then the mature basal body in the following cell cycle, root transformation can be said to be occurring in *Chlamydomonas*.

In *C. ovata*, the rhizostyle may be distributed semi-conservatively to the daughter cells, as each new basal apparatus bears an apparently reduced form of rhizostyle in late stages of division. We have not observed an ML after segregation of the basal bodies, and hence assume it breaks down and two new ones form. The Cr appears to divide in half during cell division, which may account for the origin of the extra microtubule that the 4r gains when transforming into the SRm. The origin and fate of the single microtubule observed adjacent to the Cr in dividing cells is unknown. It may persist through interphase, and may also be derived from the Cr of the previous generation.

During division in *C. ovata*, the SR is nucleated on the transforming basal body (BB2), but becomes attached to a newly formed basal body ($BB2^2$) via a fibrous attachment band. During the next cell division, this SR is transferred to another new basal body ($BB2^1$), which necessitates the attachment band breaking down and a new one forming. This is the first report of flagellar roots being formed on one basal body and "transferring" to another. However, the main body of the SR does not appear to undergo any significant movement relative to the basal bodies. It is in fact the formation of the fibrous attachment band between the SR and respective new basal body that mediates their connection. This attachment band is somewhat similar in appearance to the distal connecting band in *C. ovata*. Perhaps the attachment band behaves similarly to the distal connecting band, breaking down (at least partially) at the start of division and reforming when new basal body associations have been established. However, we have not observed this fibrous attachment band in all interphase cells, and it is possible that the connection does not form until some stage after cytokinesis.

Flagellar apparatus dynamics

Based on the ability of the distal connecting fibre of the basal bodies in *Spermatozopsis similis* (Chlorophyta) to contract and effect basal body reorientation during swimming reversal (McFadden et al. 1987), it has been postulated that a similar fibre may be the cause of basal body reorientation during division in *Pleurochrysis carterae* (Beech et al. 1988) and in *Brachiomonas submarina* (Segaar and Gerritsen 1989). It has also been hypothesized that flagellar root mediated sliding may (partly) effect the segregation of basal bodies and flagellar roots among daughter cells during division in *Brachiomonas* (Segaar and Gerritsen 1989) and *Chlorosarcina* (Sluiman and Blommers 1990).

Whether components of the flagellar apparatus are responsible for basal body movement during division in *C. ovata* cannot be determined in this type of study. It is apparent, however, that the majority of the flagellar apparatus components form prior to basal body segregation and remain associated with their respective basal body pairs during their migration.

Development and transformation of the flagellar surface

The surface ornamentation of the dorsal and ventral flagella in *C. ovata* is quite different (Hibberd et al. 1971). As parental flagella only retract marginally during division, surface modifications accompanying flagellar transformation must occur on a flagellum persisting from a previous cell cycle. In reported cases of transformation involving flagella with flagellar hairs (*Epipyxis pulchra*, Wetherbee et al. 1988; *Mallomonas splendens*, Beech and Wetherbee 1990 a), the parental flagella are retracted for most of their length. Thus surface modifications on an existing flagellum are not required, as the cells essentially regenerate all flagella anew during each division cycle. We are presently investigating the process by which this transfer of surface ornamentation occurs in *C. ovata*.

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