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The flagellar apparatus and temporary centriole-associated microtubule systems at the interphase–mitosis transition in the green alga *Gloeomonas kupfferi*: an example of the spatio-temporal flexibility of microtubule-organizing centres

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SUMMARY

The interphase–mitosis transition in the green flagellate *Gloeomonas kupfferi* (Chlamydomonadales) was analysed using indirect immunofluorescence microscopy and transmission electron microscopy of serial sections, with emphasis on the organization of the microtubular cytoskeleton. At interphase, cortical microtubules originate from different parts of the flagellar apparatus, including a connecting fibre, two basal bodies and flagellar roots. The interphase–mitosis transition is characterized by: (1) loss of motility and reduction of the flagellar apparatus, resulting in two remaining, widely separated, plasma membrane-associated basal body pairs; (2) migration of the nucleus towards the anterior (basal body-containing) side of the cell, presumably resulting from contraction of a cytoskeletal system recently discovered in related flagellates; (3) depolymerization of the cortical cytoskeleton; (4) transformation of the basal body pairs and associated pericentricular material into centrosomes that are involved in spindle formation; (5) dissociation of the centrosomes from the spindle microtubules at metaphase. The present study strongly suggests that microtubule-organizing centres in green flagellates are flexible entities that may be associated with diverse components of the flagellar apparatus at interphase. At the interphase–mitosis transition, centrosomes are activated to form astral microtubule systems. The dissociation of centrosomes from the metaphase spindle suggests that microtubule initiation and microtubule (re)organization are two different processes. These findings are discussed with respect to current ideas on the control of microtubule synthesis and organization in animal cells.

Key-words: astral microtubules, basal body, centriole, flagellar apparatus, *Gloeomonas*, interphase–mitosis transition, MTOC.

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INTRODUCTION

The interphase–mitosis transition, one of the most dynamic stages in the plant division cycle, is characterized by considerable changes in the spatial organization of the microtubular cytoskeleton (Clayton 1985). At this stage, the interphase microtubule (MT) system becomes disassembled and the mitotic spindle is being formed. In most acentric plant cells, a transient plasma membrane-associated MT system, the (pre)prophase band, signals the onset of cell division (Gunning 1982; Lloyd 1987). However, in centric plant cells, such as the majority of green algae, the interphase–mitosis transition is marked by the occurrence of astral MT systems. In the filamentous green alga *Aphanochaete magna* Godward, the presence of astral MT systems at the interphase–mitosis transition during vegetative cell division (*sensu* Ettl 1988) has been extensively documented (Segaar 1989). However, the behaviour of MT systems at the interphase–mitosis transition in sporulating green flagellates (*sensu* Ettl 1988) has remained poorly understood, particularly with respect to the identity and behaviour of the MT-organizing centres (MTOCs; *sensu* Pickett-Heaps 1969) and the relation to other structural changes in the premitotic cell.

Following extensive studies on the endomembrane system and the cytokinetic apparatus in the walled green flagellate *Gloeomonas kupfferi* (Skuja) Gerloff (Domozych 1989a,b; Segaar *et al.* 1989), the present study highlights the dynamic changes that occur in the structure of the flagellar apparatus, the behaviour of the nucleus, and the simultaneous changes in the organization of MTs at the interphase–mitosis transition in this alga. The results strongly suggest that MTOCs in green flagellates are flexible entities and that basal bodies play an important role in the spatial organization of MTs during the interphase–mitosis transition in these organisms.

MATERIALS AND METHODS

Preparation of cells for indirect immunofluorescence microscopy and transmission electron microscopy was done following protocols described earlier (Segaar *et al.* 1989).

Terminology

The basal body is a cylindrical MT structure supporting a flagellum. As pointed out elsewhere (Pickett-Heaps 1971; Coss 1974; Heywood 1987), a centriole is the structural equivalent (analogue) of the basal body once it loses its flagellum prior to mitosis. However, following general practice in the literature on green flagellates, the term basal body will be used also when the flagellum becomes detached, while the term 'probasal body' will be used to designate the basal body precursor (a newly formed template for a basal body) that is oriented perpendicular to the flagellum-bearing basal body at interphase (Gould 1975; Gaffal 1988).

The flagellar apparatus (interphase) is the complex of flagella and its appendages. The flagellar apparatus of *Gloeomonas* consists of two widely separated, possibly non-identical 'halves', each consisting of one flagellum-bearing basal body, one probasal body, two flagellar roots and fibrous connectives (Schnepf *et al.* 1976; Segaar *et al.* 1989; the present study; Berry & Floyd 1989; B.A. Berry *et al.* manuscript in preparation). The two flagella-bearing basal bodies are interconnected via a long connecting fibre (Fig. 1a, small arrows).

The cortical cytoskeleton is the plasma membrane-associated MT system, sometimes referred to as the 'secondary' MT cytoskeleton in non-dividing flagellated cells to distinguish it from flagellar roots (Melkonian *et al.* 1980).

The aster is the star-shaped MT system that is focused onto basal bodies/centrioles during or at specific stages of mitosis.

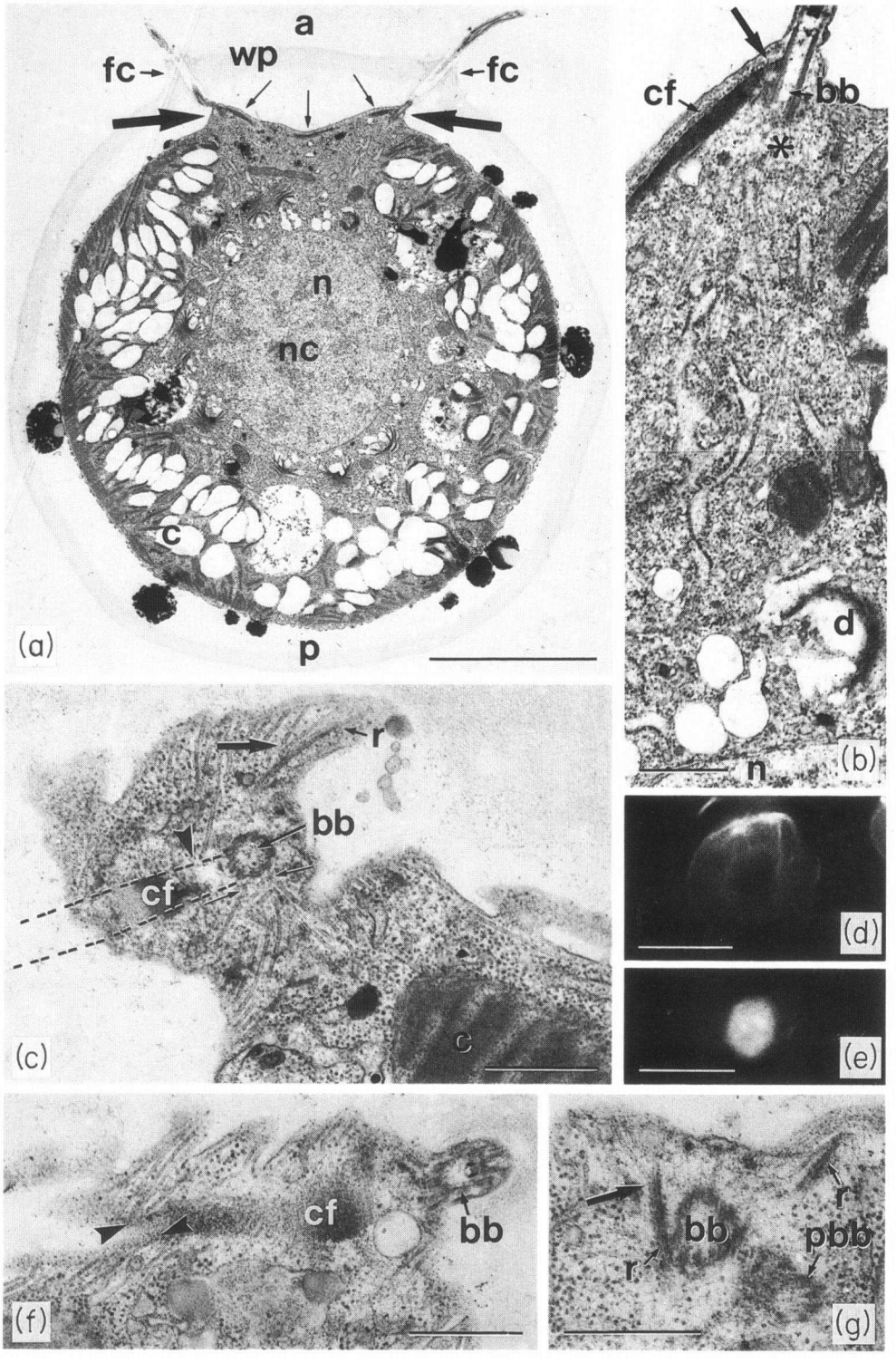
RESULTS

The flagellar apparatus and MT organization at the interphase–mitosis transition

Following cytokinesis, interphase morphology of biflagellate daughter cells is established within the sporangial wall (Segaar *et al.* 1989). In released daughter cells with a centrally positioned nucleus, MTs are found exclusively in the flagellar apparatus [(pro)basal bodies and flagellar roots] and are associated with the plasma membrane (cortical cytoskeleton). In mature interphase cells, cortical MTs appear to originate at different components of the flagellar apparatus, e.g. short flagellar roots (large arrow in Fig. 1c and arrows in Fig. 1g), a large electron-opaque fibre that interconnects the two widely separated 'halves' of the flagellar apparatus (arrowheads in Fig. 1c,f), and basal bodies (small arrows in Fig. 1c).

The onset of cellular division is marked by the gradual disappearance of the nucleolus and the appearance of two new MT systems (Fig. 1d), which seem to radiate from a differentiated cytoplasmic region associated with each of the basal bodies (asterisk in Fig. 1b) towards the still centrally located nucleus (Fig. 1a,e). The following events mark the progression of the cell into the mitotic cycle: (i) the nucleus migrates to the anterior side of the cell (compare nuclear position in Fig. 1a with that in Figs 4 and 5a); (ii) chromosomes start to condense (Fig. 2b,e); (iii) flagella are either shed or resorbed, resulting in the cells becoming immotile; (iv) most components of the flagellar apparatus, except for (pro)basal bodies, start to disintegrate; (v) former probasal bodies mature and reorientate such that one end becomes associated with the plasma membrane, thereby establishing a 'V-shape' configuration with the former basal body (angle approximately 17 degrees), a configuration that is maintained throughout mitosis (Fig. 5b); (vi) the two new MT systems described above develop into discrete asters, the MTs of which are focused onto granular material that is associated with each basal body pair (Fig. 2); (vii) at the same time the cortical cytoskeleton disassembles completely (compare Fig. 2a–d). It is emphasized that basal bodies do not become associated with the nuclear envelope, but that, throughout the division cycle, they remain associated with the plasma membrane. Astral MTs are oriented either perpendicular to the nuclear envelope (small arrow in Fig. 2e), run tangentially over the nuclear poles (Fig. 2c,e and f), or run into channels in the reticulate chloroplast (not shown).

As chromosomes become progressively condensed (Fig. 3b), the two MT asters start to penetrate the nucleus, thereby establishing the conspicuously focused bipolar spindle (Fig. 3a). However, as soon as the metaphase spindle is formed, spindle MTs become detached from the basal bodies (Figs 3c–f, 4 and 5b–e), and spindle poles become much less focused and may even appear as multiple 'minipoles' (Figs 3e and 4b). Serial section analysis clearly reveals that any structural relationship between basal bodies and the spindle MTs is lost once the intranuclear spindle has been formed (Fig. 5b–e). The spindle can be considered to be closed, although many perforations exist in the nuclear envelope (Figs 4b and 5d). In addition to the original two basal bodies and a presumptive remnant of the connecting fibre per half of the flagellar apparatus (Fig. 5b,d, arrow), two new probasal bodies were found orientated perpendicular to the V-shaped (original) basal body pair (Fig. 5c,e), indicating that replication of these organelles occurs at this stage. Thus, at late mitosis, eight basal bodies are present in two groups, each of which will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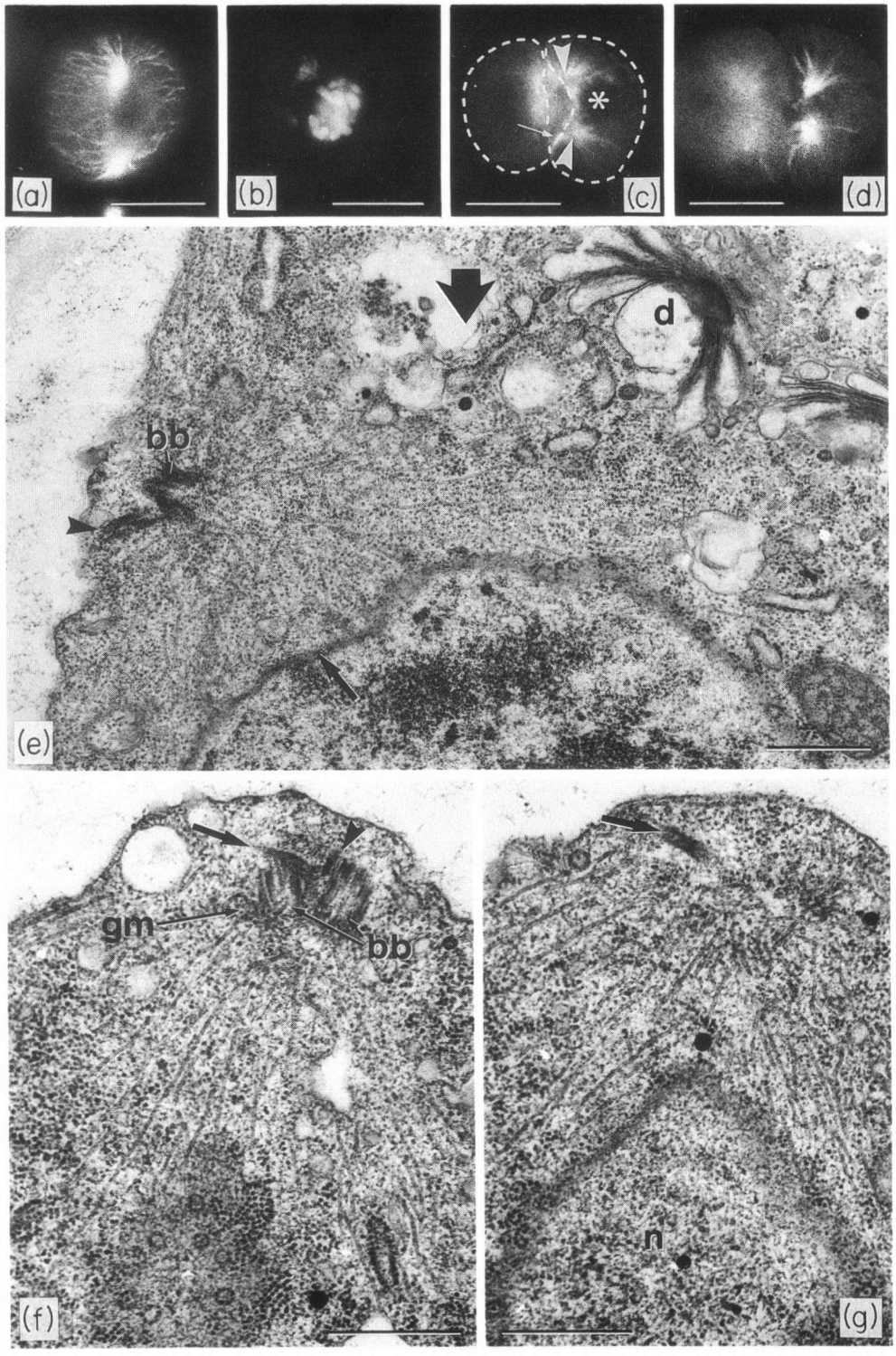
develop into the flagellar apparatus of the daughter cells (Segaar *et al.* 1989). Apart from basal bodies and the spindle, cells in metaphase–anaphase are completely devoid of MTs, as seen in IIF preparations (Fig. 3). A 'metaphase band' of MTs (Johnson & Porter 1968; Doonan & Grief 1987) is not present in this alga. However, a new cytoplasmic MT system is formed at the anaphase–telophase transition. The developmental fate and the significance of this new MT system, a flagellar root-based phycoplast, have been reported in a previous paper (Segaar *et al.* 1989).

DISCUSSION

Organization of the interphase cortical cytoskeleton in green flagellates and zoospores

Flagellated green algal cells have a distinct cortical cytoskeleton that appears to be involved in determining cell shape (Marchant 1979; Melkonian *et al.* 1980). Interphase cortical MTs in *G. kupfferi* appear to originate at flagellar roots, basal bodies and at the connecting fibre between the basal bodies (the present study). Among green algae, interphase cortical MTs have been reported to originate at a connecting fibre in *G. simulans* (Schnepf *et al.* 1976; Melkonian 1980), at flagellar roots in walled (Katz & McLean 1979) and naked flagellates (Brown *et al.* 1976, 1982), in walled zoospores of filamentous or thallose genera (Melkonian 1979; Sluiman *et al.* 1980), and at osmiophilic material associated with basal bodies in walled (Greuel & Floyd 1985) and in naked (Marano *et al.* 1985) flagellates. Cytoplasmic MTs are able to grow from flagellar roots (Stearns & Brown 1981) as well as from basal bodies (Snell *et al.* 1974) *in vitro*, even in the spatial configuration that is characteristic of the intact interphase cells (Stearns & Brown 1981). Thus, flagellar roots as well as basal bodies can be considered to represent MTOCs. In contrast, in naked zoospores of the cladophoralean alga *Chaetomorpha spiralis* Okamura, cortical MTs have been seen to originate at an electron-dense 'crown' at the anterior side of the flagellar apparatus (Hirayama & Hori 1984), while they appear to be more diffusely organized by plasma membrane-associated electron-dense granules that are not connected to the flagellar apparatus in the naked flagellate *Spermatozopsis exsultans* Korsch. (Melkonian *et al.* 1987a) and in the scaly prasinophyte *Pyramimonas gelidicola* McFadden *et al.* (McFadden & Wetherbee 1984). In walled *Pediastrum* zoospores, two separate cortical MT systems coexist that appear to be organized by two different MTOCs, one associated

Fig. 1. *Gloeomonas kupfferi*: (late) interphase; IIFM (d), DAPI (e) and TEM micrographs. (a) Late interphase cell revealing almost centrally positioned nucleus, reticulate cup-shaped chloroplast filled with starch, parts of the large connecting fibre (small arrows) interconnecting the two halves of the flagellar apparatus (large arrows), and the broad wall papilla at the anterior side of the cell with flagellar collars at its edges. Notice perinuclear organization of dictyosomes and vacuoles. Bar represents 5 μm. (b) Detail of serial section adjacent to that shown in (a) revealing MTs apparently arising from a differentiated cytoplasmic region (asterisk) associated with the basal body of one of the halves of the flagellar apparatus. Notice attachment site of the connecting fibre at the basal body (arrow). Bar represents 0·5 μm. (c) Interphase. Section almost perpendicular to one of the basal bodies showing cortical MTs that originate at or near the basal body (small arrows), flagellar root (arrow) or connecting fibre (arrowhead), as confirmed from serial sections. Position of connecting fibre indicated with dashed lines. Bar represents 0·5 μm. (d) and (e) Optical section of late interphase cell showing two MT foci at the anterior side, from which MTs radiate (d) towards the centrally positioned, DAPI-stained nucleus (e). Flagella have been excised or resorbed. Bar represents 10 μm. (f) and (g) Interphase. Two serial sections (sequence numbers 57 and 62) of another interphase cell showing cortical MTs originating at the connecting fibre (arrowheads in f) and at a flagellar root (arrow in g). Notice probasal body in (g). Bar represents 0·5 μm. Abbreviations used in figures: a, anterior side; bb, basal body; c, chloroplast; cf, connecting fibre; d, dictyosome; fc, flagellar collar; gm, granular material; k, kinetochore; n, nucleus; nc, nucleolus; p, posterior side; pbb, probasal body; r, flagellar root; sp, spindle; wp, wall papilla.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with the flagellar apparatus and one associated with the tips of developing horns that are characteristic of these zoospores (Marchant 1979).

From this summary it appears that the presence of basal body MTOCs or flagellar root MTOCs is not related to the presence or absence of a cell wall. In addition, the site of organization of the interphase cortical MT cytoskeleton in flagellated green algal cells is rather flexible. In *Pediastrum* zoospores (Marchant 1979) and in cells of *G. kupfferi* (the present study), flexibility in the localization of the MTOCs for (the) cortical MT system(s) is even shown within the same cell. The presence of multiple sites of origin for cortical MTs in *G. kupfferi* may be the result of the considerable size of the cells of this alga (25–50 μm), which might prevent the organization of all cortical MTs exclusively at small focal points, such as the basal bodies or the relatively short flagellar roots. The large connecting fibre appears to be most suitable for the organization of a considerable number of the cortical MTs present in the interphase cell.

The interphase–mitosis transition in green flagellates

The interphase–mitosis transition in walled flagellates such as *G. kupfferi* is characterized by a series of complex subsequent structural changes in the cell. The preparation for mitosis in these organisms is complicated by the fact that the cell becomes temporarily immotile at the time of division, which results in flagellar apparatus transformation. This transformation is accompanied by nuclear migration, followed directly by centrosome and spindle formation, as discussed in the following paragraphs.

1. Transformation and replication of the flagellar apparatus. In *G. kupfferi*, the connecting fibre and flagellar roots disintegrate at the interphase–mitosis transition, which is presumably related to the temporary loss of flagella and, thus, to the loss of motility. As a result of this reduction in the flagellar apparatus, two widely separated, V-shaped basal body pairs, each consisting of the former basal body and the maturated probasal body, remain. Splitting of these basal body pairs does not occur prior to mitosis (late G_2 phase of the cell cycle), as in most centriole-containing eukaryotes, but instead occurs directly after cytokinesis of the previous division cycle (early G_1 phase) (Segaar *et al.* 1989). This unusual timing of basal body segregation explains the characteristic structure of the interphase cell of *Gloeomonas*, showing two widely separated flagella (Fig. 1a). In this respect, *Gloeomonas* species are different from most other unicellular flagellates in which flagella are implanted close to each other at interphase and splitting of basal bodies occurs prior to mitosis.

Fig. 2. *Gloeomonas kupfferi*: interphase–mitosis transition; IIFM (a, c, d), DAPI (b) and TEM micrographs. (a) and (b) Cell in early prophase, as seen on top of the anterior side. Chromosomes condensing in the nucleus (b) while two MT foci proliferate (a). The cortical cytoskeleton at the anterior side is still intact. Bar represents 10 μm. (c) Lateral view of same stage in a dyad (prophase of second round of division). Astral MTs originating at two foci (arrowheads) radiate into the cytoplasm, thereby partially ensheathing the future spindle poles of the anteriorly positioned nucleus (asterisk). An anterior cortical MT bundle is indicated by an arrow; daughter cell peripheries with dashed lines. Bar represents 10 μm. (d) Later stage revealing the lack of the cortical cytoskeleton and two remaining MT asters. Bar represents 10 μm. (e) Lateral view of early prophase (chromosomes condensing but intranuclear MTs still absent) revealing one of the two MT asters. Astral MTs focused onto a basal body (second one not grazed in this section) radiate towards and partly ensheath the nucleus. Remaining cortical MTs and a remnant of a flagellar root (arrowhead) are also present. Bar represents 0·5 μm. (f) and (g) Two serial sections [sequence numbers 18 (f) and 16 (g)] of similar stage as shown in (e); nucleus seen from above as indicated with the large arrow in (e). Astral MTs originate from granular material that is associated with the basal body pair. The nuclear envelope at the pole, showing many nuclear pores (f, bottom), is ensheathed by astral MTs. Remnants of a striated connective (arrowhead) and of a flagellar root (arrows) are also visible; other components have disappeared. Bar represents 0·5 μm. Abbreviations as for Fig. 1.

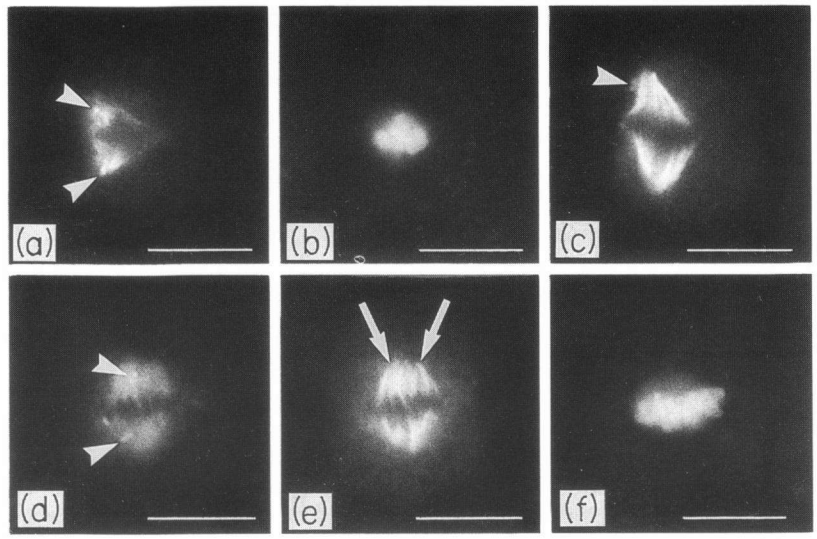

Fig. 3. *Gloeomonas kupfferi*: prometaphase–metaphase; DAPI (b, f) and IIF micrographs. (a) and (b) Nucleus unilaterally penetrated by two half spindles that are focused onto two fluorescent spots (arrowheads in a); DAPI-stained chromosomes, lying in between the half spindles, are conspicuously condensed (b). Bar represents 10 μm. (c) Metaphase spindle consisting of two well-developed half spindles. The two fluorescent spots at the anterior side of one of the spindle poles (arrowhead) mark the position of a basal body pair (remnant of one half of the flagellar apparatus); the other pair is out of the plane of focus. Bar represents 10 μm. (d–f) Metaphase. Spindle seen on the anterior side revealing, in two consecutive foci and starting at the level of the plasma membrane at the anterior side, two plasma membrane-associated basal body pairs (arrowheads) well below the position of the spindle poles (d) and an optical section of the spindle at the level of the relatively broad spindle poles revealing multiple foci (arrows in e). DAPI-stained chromosome plate (f) photographed at the same level. Bar represents 10 μm.

While splitting of basal body pairs can be demonstrated relatively easily, the timing of replication of these organelles is much more difficult to detect, as it has become clear that basal bodies in flagellates show distinctly separated replication and maturation cycles (Gould 1975; Gaffal 1988), the latter presumably requiring more than one cell cycle (Melkonian *et al.* 1987b). With respect to *Chlamydomonas reinhardtii* Dangeard, Gaffal (1988) concluded that basal bodies duplicate at identical stages during each mitotic round and that mature duplicated basal body pairs segregate in a semi-conservative manner (each daughter cell receiving equal numbers of 'old' and 'new' basal bodies) at the onset of the next division cycle. Semi-conservative replication during mitosis has also been shown to occur for flagellar roots (Aitchison & Brown 1986; Gaffal 1988). This could not be demonstrated in *G. kupfferi*; it is possible that original roots become completely disassembled in this alga (e.g. Fig. 5b–e) and that four new roots per daughter flagellar apparatus are formed at the end of mitosis (Segaar *et al.* 1989).

2. Premitotic nuclear migration. In walled flagellates like *G. kupfferi*, premitotic migration of the nucleus towards the anterior side of the cell appears to be mediated by (Ca^{2+}-induced?) contraction of a delicate system of fibres that interconnects the basal bodies with the nucleus (Wright *et al.* 1985; Schulze *et al.* 1987; Salisbury *et al.* 1987, 1988). In addition, the formation of MT asters prior to mitosis (Doonan & Grief 1987; the present study) coincides with a change in the immunolocalization pattern of these fibres (Salisbury *et al.* 1988), but it is not yet known how these two cytoskeletal systems interact with each other.

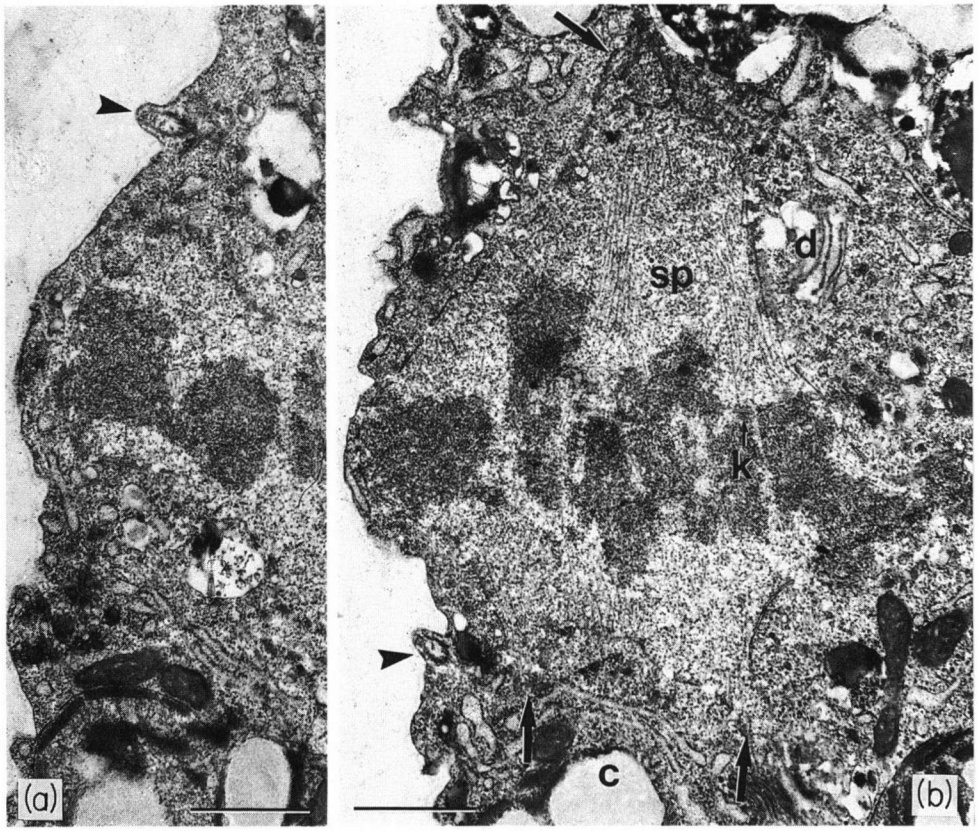

Fig. 4. *Gloeomonas kupfferi*: metaphase; TEM micrographs. Two serial sections [sequence numbers 45 (a) and 56 (b)] of a cell in early metaphase revealing spindle 'minipoles' (arrows) and plasma membrane-associated remnants of the flagellar apparatus (arrowheads). Serial section analysis revealed that these remnants each consist of two basal bodies and two short roots. Notice conspicuous kinetochore in (b). Bar represents 1 μm. Abbreviations as for Fig. 1.

3. Centrosomes, asters and spindle formation. At the time of nuclear migration in *G. kupfferi* cells, astral MT systems develop from granular material that is associated with the remaining basal body pairs (Fig. 2f). Thus, each basal body pair and its associated granular material can be considered to represent a centrosome, which is one of the most common MTOCs in centric cells (Peterson & Berns 1980; Wheatley 1982; Mazia 1984, 1987; Brinkley 1985; Karsenti & Maro 1986; Vorobjev & Nadezhdina 1987). The present results are in line with numerous other observations of premitotic, centric (green) algal cells (e.g. Segaar 1989, and references therein) and with findings that the eukaryotic centrosome and the green algal flagellar apparatus share immunologically related components (Salisbury *et al.* 1986). However, in contrast to most other green algae, centrosomes in flagellates like *G. kupfferi* remain associated with the plasma membrane and are not intimately associated with the nuclear poles. Because of the unusual lateral position of the centrosomes in *G. kupfferi* and in related flagellates [interpretation of data shown by Triemer & Brown (1974) and Doonan & Grief (1987)], the nuclear poles become ensheathed by MTs in a way similar to the MT 'baskets' found in the syncytial blastoderm

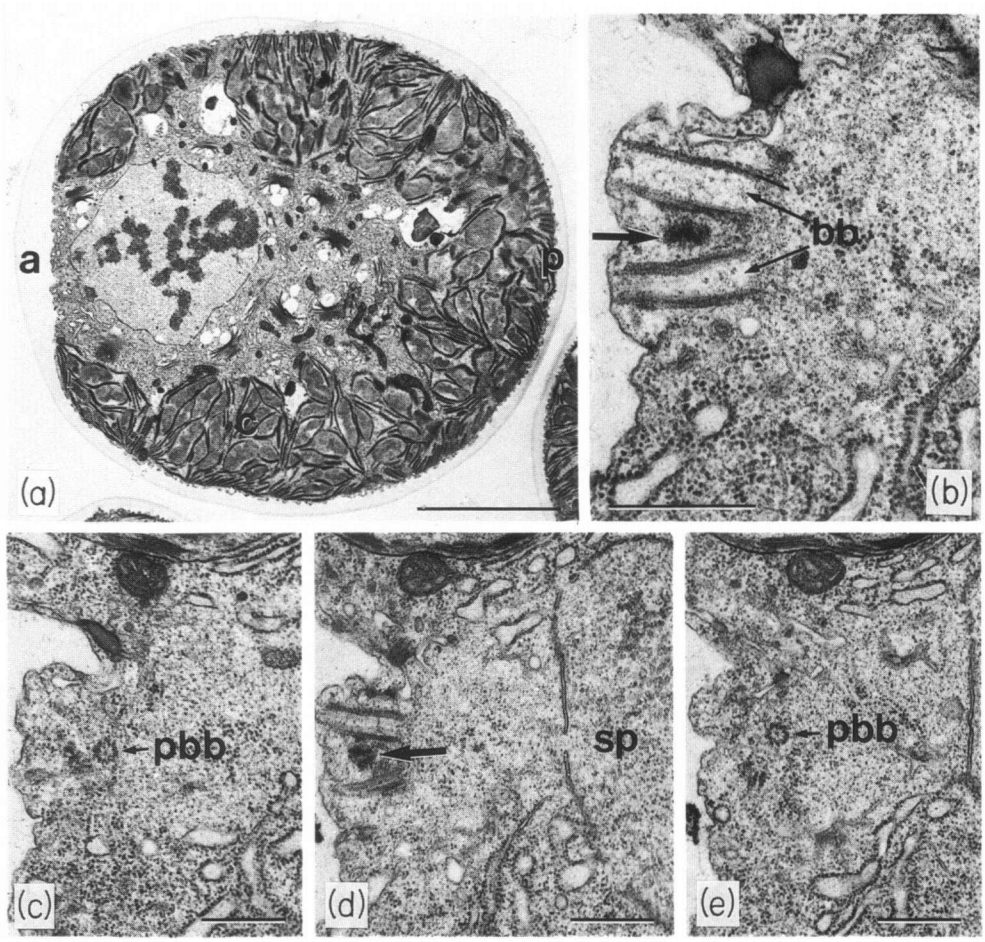

Fig. 5. *Gloeomonas kupfferi*: metaphase; TEM micrographs. Serial sections [sequence numbers 8 (a), 45 (c), 47 (d), 48 (b), and 51 (e)] cut approximately parallel to the division plane of a cell containing an anteriorly positioned metaphase spindle. (a) Median section through the chromosome plate. Bar represents 5 μm. (b–e) Details of the remnants of one of the halves of the flagellar apparatus, showing the two original basal bodies in a V-shaped configuration (b, d), two newly formed probasal bodies oriented perpendicular to the original ones (c, d) and a remnant of the connecting fibre (arrows in b, d). Notice absence of connections between the basal bodies and the spindle. Bar represents 0·5 μm. Abbreviations as for Fig. 1.

of *Drosophila* embryos (Callaini & Anselmi 1988). Presumably as a result of unilateral penetration of the nucleus, the bipolar mitotic spindle that develops from the two asters has a curved appearance (Fig. 3a) that persists until the end of mitosis (Segaar *et al.* 1989). A similar type of spindle formation seems to occur in the prymnesiophycean flagellate *Pavlova*, but in this alga the unilaterally curved spindle appears to be organized by fibrous flagellar roots (system II fibres; *sensu* Melkonian 1980) throughout mitosis (Green & Hori 1988). This is fundamentally different from the situation in *G. kupfferi* in which centrosomal MTOCs appear to be involved in spindle initiation only. In addition, fibrous roots have not been seen in mitotic cells of *G. kupfferi*.

Since there is no indication in *G. kupfferi* of a reorientation of intact cortical MTs into the new directions shown by astral MTs, it appears that asters are formed by

repolymerization of MTs from the tubulin pool that results from the disintegration of the cortical cytoskeleton. Thus, in this alga the conversion of the interphase cortical MT system into the mitotic astral MT systems appears to occur via depolymerization/repolymerization events, as in the filamentous green alga *Aphanochaete magna* (Segaar 1989).

From these data, it may be concluded that centrosomes in *G. kupfferi* function, albeit only during a relatively short stage of the mitotic cycle, to provide the spindle precursor consisting of two half spindles. However, the dissociation of the centrosomes from the spindle MTs at metaphase suggests that events such as chromosome movements and spindle elongation are controlled by different organelles or processes. This supports the general idea that MT initiation and subsequent (re)organization are two separate events in the MT cycle (Lloyd 1987). On the other hand, the spatio-temporal behaviour of MTs in the spindle, and the mechanism of mitosis *sensu stricto*, is subject to considerable debate in the literature on cell biology (reviewed in Hyams & Brinkley 1989).

In addition to being involved in spindle formation, centrosomal asters in *G. kupfferi* may also be involved in the polarization of the cytoplasm and thus impose directionality onto premitotic nuclear migration. Once the nucleus has been positioned in the correct location, the asters are easily transformed into a functional bipolar spindle. This hypothesis is supported by reports on cone- or cage-like MT systems unilaterally ensheathing nuclei that appear to be involved in nuclear migration in various green algae (Meindl 1985; Menzel 1986; Pickett-Heaps & Wetherbee 1987).

MTOCs as flexible entities in the green algal division cycle

Detailed studies of the MT cycle in *G. kupfferi* have revealed that MTs appear to be organized by basal body-, connecting fibre- and flagellar root-associated MTOCs at interphase, by centrosomal MTOCs at the interphase–mitosis transition (the present study) and by flagellar root MTOCs at cytokinesis (Segaar *et al.* 1989). On the other hand, in the filamentous green alga *Aphanochaeta magna*, MTs appear to be organized by diffusely dispersed plasma membrane-associated MTOCs at interphase (P. J. Segaar 1989, unpublished results), by centrosomal MTOCs at the interphase–mitosis transition (P.J. Segaar 1989, unpublished results), and by a cytoplasmic region associated with the centre of the interzonal spindle at the time of phycoplast formation (Segaar & Lokhorst 1988, unpublished results). In dividing higher plant cells, different 'MT assembly sites' have been demonstrated using a MT drug recovery technique (Falconer *et al.* 1988). Thus it appears that, during the division cycle, centric as well as acentric plant cells are capable of 'switching' the organization of their MT systems to different cellular loci, in particular organelles and membranes.

With respect to animal cells, it is increasingly accepted that the controlling machinery for MT synthesis resides in the pericentriolar material (PCM) instead of the centriole (Peterson & Berns 1980; Karsenti & Maro 1986). Antibodies against PCM antigens have been found to stain acentriolar spindle poles in animal (Calarco-Gillam *et al.* 1983) and higher plant cells (Lloyd 1987) and, in addition, antigenic sites closely associated with the nuclear envelope in higher plant cells, a site that appears to be actively involved in MT polymerization (Lloyd 1987). It is expected that in (flagellated) green algae, material similar to PCM will be found to be associated with diverse structures such as basal bodies/centrioles, flagellar roots, etc.

Evidence from recent studies points to two alternative (but not necessarily mutually exclusive) pathways of the spatio-temporal control of MT organization in eukaryotic

cells. (i) Spatially fixed MTOCs are activated (and deactivated) to synthesize MTs at specific moments in the cell cycle. An example is the phosphorylation of the centrosome, which has been suggested to increase its MT nucleating capacity and which might represent an activation event in the interphase–mitosis transition in mammalian cells (Vandre & Borisy 1985). (ii) MT nucleating material, either dispersed in the cytoplasm or confined to large surfaces such as the nuclear envelope, becomes reorganized at small organelles such as basal bodies/centrioles, resulting in polarization of the MT system formed and, thus, polarization of the surrounding cytoplasm. This implies that MT nucleating material is transported through the cell (Calarco-Gillam *et al.* 1983; Tassin *et al.* 1985). In his recent review on the plant cytoskeleton, Kristen (1986) concluded, in what appears to be a universal hypothesis to explain MT dynamics in eucaryotic cells: 'The dynamics of MT arrays during the cell cycle and during cell differentiation implicates the existence of either migrating or fixed, temporarily inactivated MTOCs'. In conclusion, the temporary activation of centrosome-associated MT nucleation sites in *G. kupfferi* (the present study), and the migratory behaviour often displayed by basal bodies/centrioles during cytokinesis in green algae (e.g. Segaar *et al.* 1989), enable these organisms to control the formation of focused MT systems and, thus, polarize the cytoplasm at specific times in the cell cycle and at defined loci in the cell.

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