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# BASAL BODY AND FLAGELLAR DEVELOPMENT DURING THE VEGETATIVE CELL CYCLE AND THE SEXUAL CYCLE OF *CHLAMYDOMONAS REINHARDII*

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## SUMMARY

Basal body development and flagellar regression and growth in the unicellular green alga *Chlamydomonas reinhardii* were studied by light and electron microscopy during the vegetative cell cycle in synchronous cultures and during the sexual life cycle.

Flagella regress by gradual shortening prior to vegetative cell division and also a few hours after cell fusion in the sexual cycle. In vegetative cells basal bodies remain attached to the plasma membrane by their transitional fibres and do not act as centrioles at the spindle poles during division. In zygotes the basal bodies and associated microtubular roots and cross-striated connexions all dissolve, and by 6·5 h after mating all traces of flagellar apparatus and associated structures have disappeared. They remain absent for 6 days throughout zygospore maturation and then are reassembled during zygospore germination, after meiosis has begun.

Basal body assembly in developing zygospores occurs close to the plasma membrane (in the absence of pre-existing basal bodies) via an intermediate stage consisting of nine single A-tubules surrounding a central 'cartwheel'. Assembly is similar in vegetative cells (and occurs prior to cell division), except that new basal bodies are physically attached to old ones by amorphous material. In vegetative cells, amorphous disks, which may possibly be still earlier stages in basal-body development occur in the same location as 9-singlet developing basal bodies. After the 9-singlet structure is formed, B and C fibres are added and the basal body elongates to its mature length. Microtubular roots, striated connexions and flagella are then assembled. Both flagellar regression and growth are gradual and sequential, the transitional region at the base of the flagellum being formed first and broken down last. The presence of amorphous material at the tip of the axoneme of growing and regressing flagella suggests that the axoneme grows or shortens by the sequential assembly or disassembly at its tip.

In homogenized cells basal bodies remain firmly attached to each other by their striated connexions. The flagellar transitional region, and parts of the membrane and of the 4 microtubular roots, also remain attached; so also do new developing basal bodies, if present. These structures are well preserved in homogenates and new fine-structural details can be seen.

These results are discussed, and lend no support to the idea that basal bodies have genetic continuity. It is suggested that basal body development can be best understood if a distinction is made between the information needed to specify the structure of a basal body and that needed to specify its location and orientation.

## INTRODUCTION

The unicellular biflagellate green alga *Chlamydomonas reinhardii* is the most suitable eukaryote for combined genetical, electron-microscopic, physiological and biochemical studies of flagella and basal bodies (Randall, Cavalier-Smith, McVittie, Warr &

Hopkins, 1967; Rosenbaum, Moulder & Ringo, 1969). The study reported in this paper was carried out in the belief that it is essential to have as complete an understanding as possible of the ultrastructural changes which occur in wild type cells during the development of flagella and basal bodies, as a basis for hypotheses about the mechanisms and control of flagellar and basal body development.

The fine structure of the mature flagellar apparatus of *C. reinhardii* has been described in detail by Ringo (1967*a*, *b*) and by me (Cavalier-Smith, 1967). This paper describes the developmental cycle of flagella and basal bodies in synchronous cultures of *C. reinhardii* and during the sexual cycle. Further details of the structure of basal bodies and associated structures as seen in cell homogenates are described. My observations on synchronous cultures and the sexual cycle were summarized briefly in the review by Randall *et al.* (1967). Since their completion a paper on cell division in 'logarithmically' grown cells has appeared (Johnson & Porter, 1968). Johnson & Porter's findings agree with those reported here in some, but not all, respects; in particular their interpretation of flagellar regression is substantially different.

## MATERIALS AND METHODS

### *Synchronous culture method*

Wild type strain 32D (minus mating type) of *Chlamydomonas reinhardii* from the Culture Collection, Botany School, Cambridge, England, was synchronized by a method based on that used by Bernstein (1960, 1964) for *Chlamydomonas moewusii*, the major difference being that aeration was by filtered air instead of 5 % carbon dioxide in air. Cells were grown at 25 °C in alternating 12-h periods of light and darkness, the light being provided by 'cool white' and 'daylight' fluorescent lamps at an intensity of about 6500 lux (measured by a Gossen Lunasix CdS exposure meter). Cells were grown in 2-l. Erlenmeyer flasks in 1 l. of a liquid medium based on Medium I of Sager & Granick (1953), but with ferric chloride replaced by 0·01 g/l. ferric citrate and 0·01 g/l. citric acid.

To assess the degree of synchrony cells were counted in a haemocytometer after fixation in glutaraldehyde or formaldehyde, and growth curves obtained by plotting cell numbers/ml against time (Fig. 1). All observations were made after at least one (and usually two or more) full light-dark cycles (each of 24 h) had elapsed since inoculation of the culture.

### *Mating and zygospore maturation and germination*

Plus and minus wild type strains (32D and 32C) of *C. reinhardii* were grown in synchronous culture, and gametes were prepared by transferring cells, after 6 h in the light period, into sterile $5 \times 10^{-4}$ M $CaCl_2$ solution and incubating in continuous light for 18–24 h. Equal quantities of plus and minus cells were mixed and incubated in the light to allow mating to occur. After zygote formation, the cells were either suspended in fresh liquid medium or plated out on 2 % agar plates and then incubated in the light for 1 day and in the dark for 5 days. The resulting zygospores were placed in the light on fresh agar plates for germination to occur. Because zygospores 'matured' in liquid were very inhomogeneous and usually failed to germinate (Cavalier-Smith, 1967), only agar-matured zygospores were used for studies of zygospore germination. Zygospores matured on agar by a method known to lead subsequently to very synchronous germination (Lawrence, 1965) were kindly provided by Dr Lawrence, and then germinated at 25 °C and 5000 lux by transferring them on to fresh 1·5 % agar in a medium with the following composition, per litre: $K_2HPO_4$, 1·44 g; $KH_2PO_4$, 0·72 g; $NH_4NO_3$, 0·4 g; $CaCl_2.6H_2O$, 0·07 g; $MgSO_4.7H_2O$, 0·1 g; ferric citrate, 0·01 g; citric acid, 0·01 g; sodium citrate, 0·05 g; and trace element solution (Sager & Granick, 1953), 10 ml.

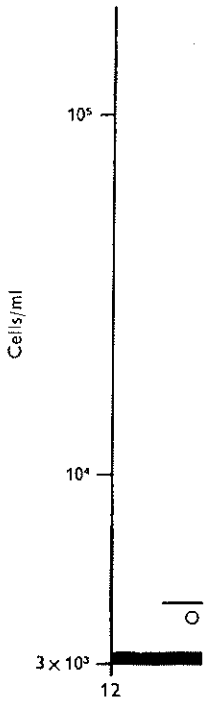


Fig. 1. Growth curve of
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### *Microscopy*

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### *Cell disruption*

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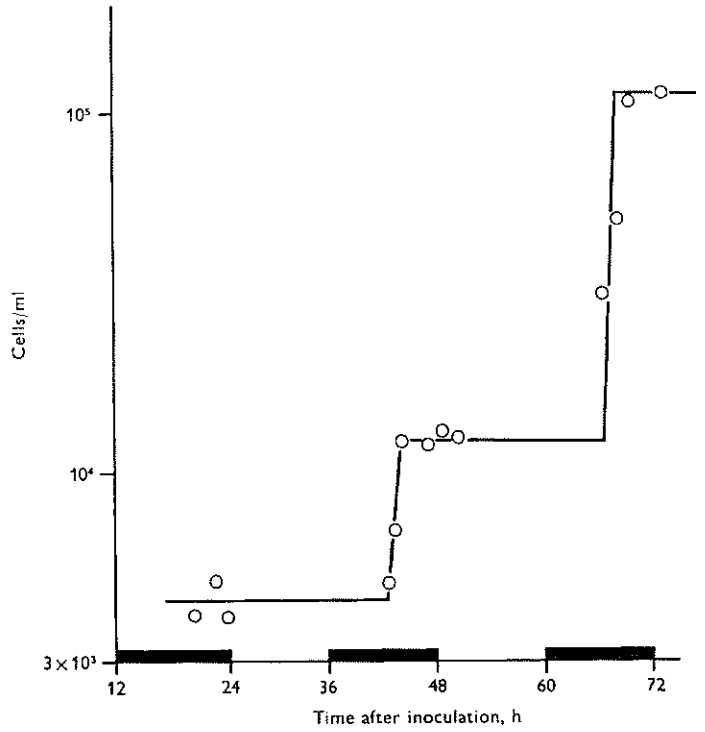


Fig. 1. Growth curve of synchronously cultured vegetative cells plotted on a $\log_{10}$ scale. The vertical steps show that daughter cell liberation occurs during the 12-h dark periods indicated by the horizontal black bars.

## *Microscopy*

Living cells were examined at all stages by phase-contrast and bright-field microscopy, and cells were sampled and fixed for electron microscopy in the middle of the light period and at 0, 1, 3, 5, 6, and 8 h after the beginning of the dark period and at various intervals during the sexual cycle. Cells were either centrifuged into a pellet to which fixative was added, or an equal volume of fixative was added directly to the suspension and the cells subsequently centrifuged and fresh fixative added to the resulting pellet. Fixation was for 1 h with 1 % glutaraldehyde in 0·02 M phosphate buffer pH 7·4 (total osmolarity 140 mosmol, measured on an Advanced osmometer). Higher osmolarities caused cell shrinkage. Cells were washed overnight in 0·05 M phosphate buffer (102 mosmol), postfixed in 1 % osmium tetroxide in 0·02 M phosphate pH 7·4 and embedded in Araldite (Luft, 1961). Sections were stained in 2 % uranyl acetate or uranyl magnesium acetate (in distilled water or 50 % ethanol) for from 15 min to several hours and post-stained in lead citrate (Reynolds, 1963) for 5–30 min. A Siemens Elmiskop I was used at 80 kV with 50-$\mu$m objective apertures.

## *Cell disruption*

Cells of strain $89^+$ from the Indiana culture collection were suspended at 4 °C in a 15 % (w/v) sucrose solution containing 1 mM ethylenediaminetetra-acetic acid (EDTA), pH 8, and passed through a French press at a pressure of $1{\cdot}38\times10^4$ kN m$^{-2}$. The resulting homogenate was either negatively stained with 2 % potassium phosphotungstate (pH 7·0) after 5 min

fixation in $OsO_4$ vapour, or, alternatively, centrifuged to give a pellet, which was fixed in glutaraldehyde and $OsO_4$, embedded in Epon, sectioned and stained as described for whole cells.

## RESULTS

### *General features of the vegetative cell cycle*

The overall behaviour of flagella and basal bodies during the vegetative cell cycle is shown in Fig. 2. The flagella regress before cell division begins, by gradually becoming shorter over a period of approximately 30 min, just as in *C. moewusii* (Lewin, 1952). No flagellar fragments sufficiently large to be seen under phase contrast are

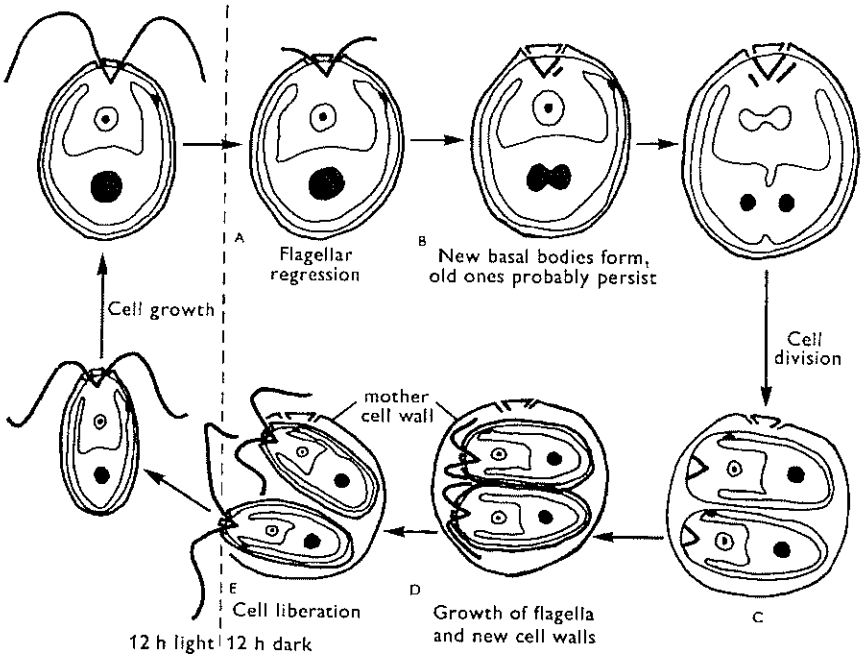


Fig. 2. Diagram summarizing the times during the vegetative cell cycle when the flagella and basal bodies are formed or regress. For simplicity only 2 daughter cells are shown, though 4 or 8 cells are normally produced under the growth conditions and cell densities used for this study. This results from a second, and often a third, cell division occurring between stages C and D, directly after the completion of the first division.

broken off and cast into the external medium; moreover, during regression the remaining part of the flagellum may beat apparently normally, which suggests that it is fairly intact.

The cell apex remains pointed until the 2 flagellar stumps have completely regressed; it then becomes rounded and about 15 min later a cleavage furrow begins to form in the colourless apical cytoplasm at the point where the flagella had been. At

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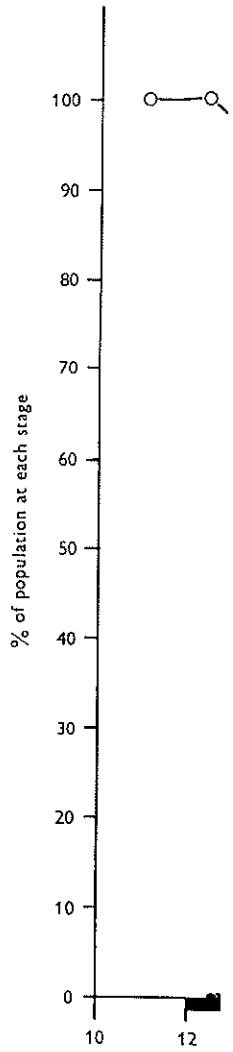


Fig. 3. Graph show
division during the
division cycle. The d
○, parent 1-cell; ■,

some stage during cytokinesis (and not 'just prior to nuclear division' as reported by Buffaloe, 1958) the protoplast rotates through 90° relative to the cell wall, so that by the end of cytokinesis (which takes about 7 min) the cleavage furrow lies at right angles to the former long axis of the cell.

Under the growth conditions used here a second cleavage (and often later a third) begins about 12 min later; thus 4 or 8 daughter cells are formed and remain enclosed by the mother cell wall until daughter cell liberation occurs. Each daughter cell grows

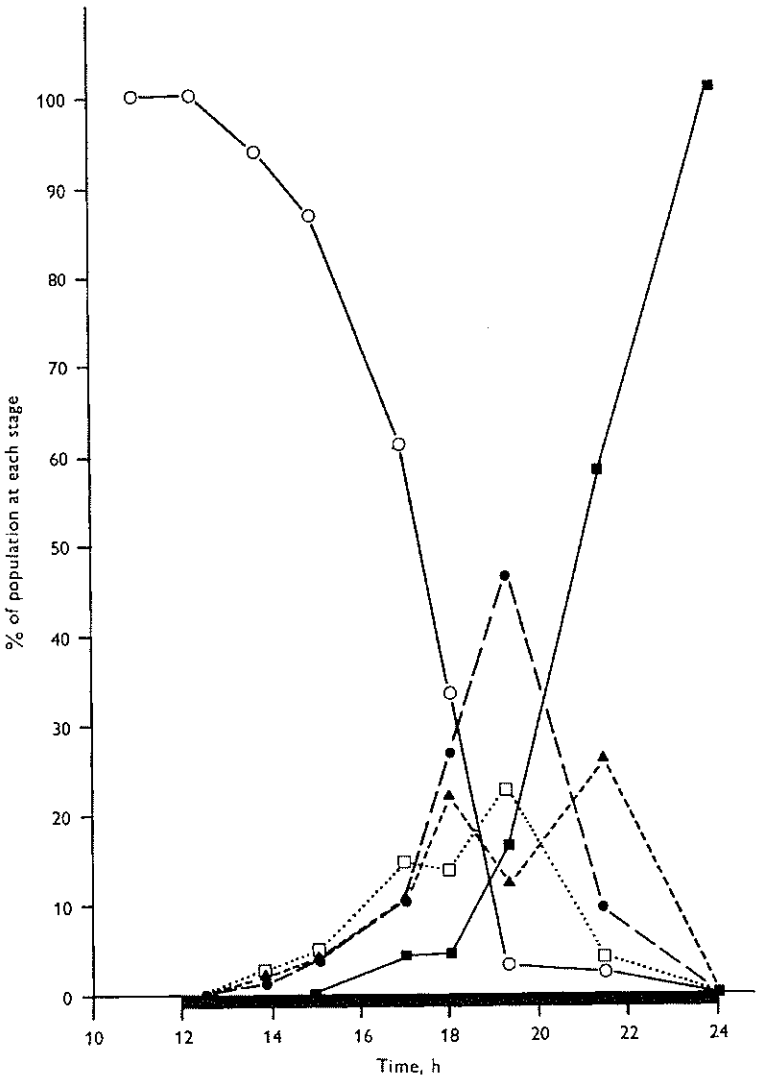


Fig. 3. Graph showing the percentages of the population at various stages of cell division during the 12-h dark period (black horizontal bar) of a single cell growth-division cycle. The details vary from cycle to cycle and from experiment to experiment. ○, parent 1-cell; ■, daughter 1-cell; □, 2-cell; ▲, 4-cell; ●, 8-cell.

2 flagella, which become motile before the mother cell wall breaks down; indeed their 'writhing' inside the swollen wall possibly helps to break it open. Rupture occurs at any point on the cell surface, and the flagellar tunnels (see below) are visible as 2 dark spots on the cell wall, which later disintegrates.

Although all the cells divide during each dark period, the fact that the successive cell divisions are spread throughout several hours of the dark period (Fig. 3), coupled with the short duration of each cell division, means that only a small proportion of the population is in the same stage of cell division at any one time. Flagellar regression, likewise, takes place during a period of about 5 h, so that at any one time not more than 10% of the population are regressing. Daughter cell liberation (Figs. 1, 3) is somewhat better synchronized, usually occurring in a 1–3 h period.

*General features of the sexual cycle*

Fig. 4 summarizes the overall behaviour of the flagellar apparatus during the sexual cycle. Flagellar regression begins a few hours after the biflagellate + and − gametes have fused to form a quadriflagellate zygote. As in vegetative cells, regression occurs

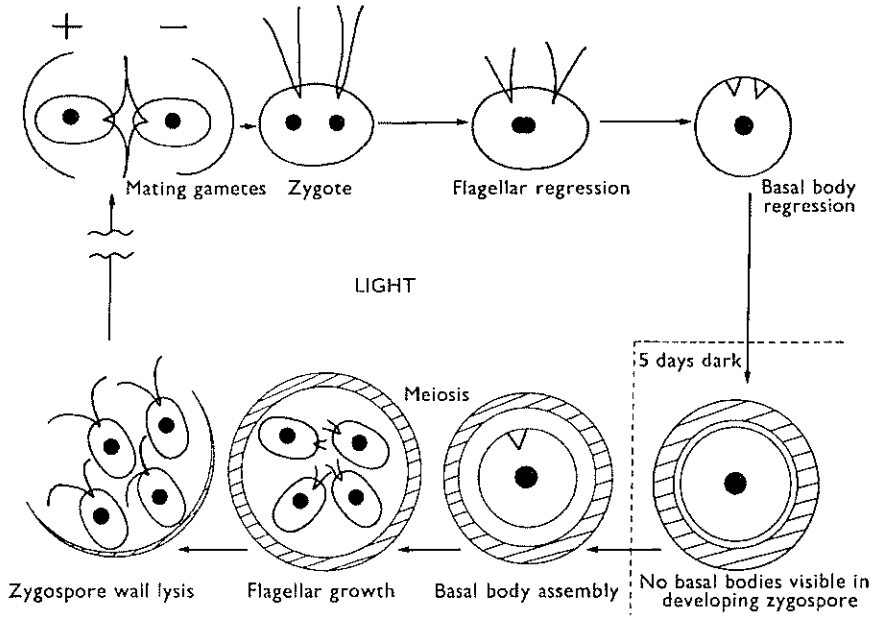


Fig. 4. Summary of the sequence of events during the regression and the development of the flagellar apparatus in maturing and germinating zygospores.

by the gradual shortening of the flagella and not by breakage. Shortening is easier to observe than in vegetative cells, because in a good mating it can occur more or less simultaneously in many cells. Shortening takes about 0·5 h and all 4 flagella shorten at the same rate. Electron microscopy (see below) shows that after the disappearance of the flagella, the basal bodies, striated connexions and microtubular roots also dis-

appear from the cell. By 6·5 h after mating all trace of flagellar apparatus and associated structures have disappeared. Nuclear fusion has occurred and chloroplast fusion (Cavalier-Smith, 1970) is nearly complete; the diploid zygote is growing rapidly in size and the sticky outer layer of the zygospore wall is already laid down (Cavalier-Smith, 1967). Throughout the remaining 18 h in the light, and the 5 days in the dark, needed for the maturation of the zygospore, basal bodies and associated structures are completely absent. During this period the complex multilayered zygospore wall is formed and the cytoplasm of the zygospore (including the chloroplast) undergoes drastic dedifferentiation.

After 5 days in the dark the now mature zygospores are transferred to fresh agar and placed in the light. They germinate synchronously and undergo meiosis, which begins about 6 h, and is complete about 9·5–10 h after the transfer to fresh medium in the light (Lawrence & Davies, 1967). During germination the cytoplasm undergoes a dramatic redifferentiation which restores its structure to that characteristic of vegetative cells (Cavalier-Smith, 1967). The zygospore wall is gradually broken down during meiosis, and the 4 or 8 daughter cells (zoospores) acquire flagella and cell walls before the zygospore wall finally bursts (at about 12–18 h after the beginning of germination) and liberates them to the exterior. The first traces of new basal bodies (see below) are visible at 6 h.

Thus during the sexual cycle of *Chlamydomonas* basal bodies and flagella regress completely in the early zygotes and only reappear 6 days later, during zygospore germination. This clearcut separation in time between regression and assembly (and also the relative synchrony of each process throughout the population) makes electron-microscopic study of the events much simpler and interpretation less liable to error than in the case of the vegetative cycle.

*Flagellar and basal body regression: electron microscopy*

Fig. 5 summarizes the structure of a mature *Chlamydomonas* flagellum and basal body and the names of the parts. Further details are given by Ringo (1967*a*, *b*), Cavalier-Smith (1967), and Hopkins (1970).

Electron microscopy shows that during mitosis the basal bodies remain in the cell, perpendicular to the plasma membrane, and still apparently attached to it, presumably by their transitional fibres. The flagellar axoneme and transitional region, however, have disappeared completely. Electron micrographs of cells from cultures where regression is occurring show no axonemes free in the cytoplasm. Therefore the flagella do not regress by the withdrawal of their axonemes into the cytoplasm. Since no visible fragments were liberated into the medium the mechanism is probably one of gradual breakdown. In vegetative cells at 3 h after onset of darkness, longitudinally sectioned flagella include some (Fig. 6) which may reasonably be interpreted as regressing. The membrane at their tips is sectioned perpendicularly, so it is unlikely that such flagella appear short simply because they curve out of the plane of the section. The 9+2 axoneme is apparently intact right to the tip of the flagellum, though the membrane 'balloons' away from it more frequently than at other stages, possibly because its connexions to the axoneme are already broken. Nor is it likely that these

are broken flagella, since accidental breaks (e.g. during specimen preparation) usually occur (as in artificial deflagellation, Figs. 10 and 11; and see Randall, 1969) at the junction between the axoneme proper and the transitional region, just *below* where the central pair stops: this leaves a stump consisting only of the transitional region and basal body (Figs. 10, 11). Such stumps occur frequently in regressing populations;

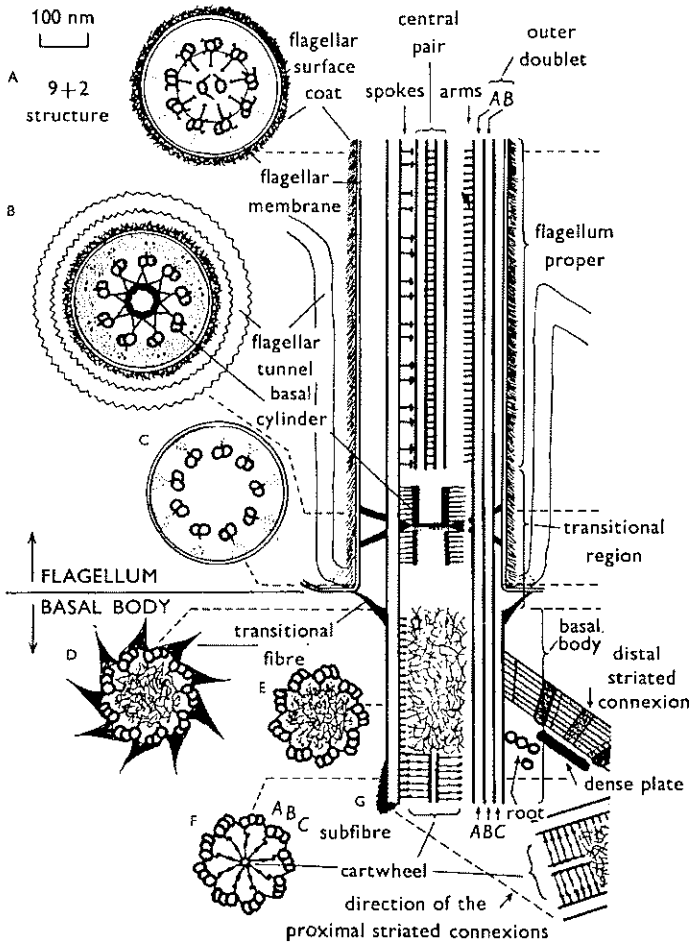


Fig. 5. Diagrammatic reconstruction of flagellum, transitional region and basal body. A to F show transverse sections at the levels indicated by the dotted lines (A, of flagellum, B, C, of transitional region, D, E, F, basal body). G shows a median longitudinal section in the plane of the central pair; however, the outer fibre on the right is turned sideways (as it would appear in a tangential longitudinal section) to show it more clearly; also one of the 4-member microtubular roots is included to show the level at which it is attached to the basal body, even though it would not be visible in a 50- to 80-nm-thick median section passing through both basal bodies, since all 4 roots terminate at about 40–50 nm to the side of the median plane. The dimensions are those for the fixative used in this paper. With a cacodylate-buffered fixative containing 3 mM $CaCl_2$ most dimensions would be about 20% smaller (Cavalier-Smith, 1967). ×68000 approx.

though many of them probably do represent an intermediate stage of lysis, at least some must be stumps left after breakage, since they are also found to a small extent when regression is not taking place.

In zygotes the much greater synchrony of flagellar regression results in a very much greater frequency of accurately longitudinal sections through short, presumably regressing, flagella. These reveal that the axoneme is always complete, except at its tip where amorphous material often occurs (Figs. 24, 27). This material is probably protein, derived from the disassembly of the axonemal tubules, which has not yet passed into the body of the cell. The intactness of the axonemes and the fact that the flagella can beat normally during shortening indicate that disassembly probably occurs only at the tip of the axoneme, and proceeds sequentially to the base of the flagellum. As in vegetative cells, no axonemes were ever seen free in the cytoplasm.

It seems that in zygotes the transitional fibres connecting the basal body to the plasma membrane, and the annular connexion between the transitional region and the flagellar membrane are both broken before the transitional region itself is dissolved, since the transitional region is often partly withdrawn into the cell (Fig. 25). However, these connexions are not broken until the 9+2 axoneme is completely dissolved. The transitional region, the striated connexions and the microtubular roots are then broken down and the basal body is left free in the cytoplasm some distance from the plasma membrane. Such basal bodies are often much shorter than their mature length (Fig. 28). Sometimes one basal body appears longer than its partner (Fig. 26). By 5·5 h after mixing the gametes only traces of basal bodies and/or roots remain. At 6·5, 7·5, 9·5, 12, and 24 h, I have been unable to detect any trace of basal bodies or related structures despite careful examination of many sections for each time point. At these times the cytoplasm is not markedly dense and one would have expected to see very short 'probasal bodies', like those reported by Renaud & Swift (1964) in *Allomyces*, if any were present.

*Basal body continuity and development in zygospores*

The cytoplasm of mature zygospores (matured on agar) is so dense that it would be possible to overlook the presence of very short probasal bodies. However, any full-sized basal bodies should have been visible, but none was seen. By 3 h after the beginning of germination, the cytoplasm has become noticeably less dense (Cavalier-Smith, 1967) but still no traces of basal bodies can be seen. By 6 h the cytoplasm has become much less dense and structures stand out with much better contrast; most cells still appear to lack basal bodies. I have seen an indistinct basal body in only 2 cells. At 7 h, although prophase of meiosis has begun (Cavalier-Smith, 1967), basal bodies are very rare. Even at 8 h (when some cells are in metaphase or anaphase and a few have completed the first meiotic nuclear division) basal bodies are rare, and usually much shorter than their mature length. They are commoner at 9 h, particularly at the newly formed surfaces of daughter cells. By 10·5 h, when most zygospores contain 4 highly granular daughter cells, most daughter cells have basal bodies.

At 9 h and 10 h (during the peak of basal body assembly), 9-singlet structures, consisting of 9 single microtubules surrounding a central 'cartwheel', occur

frequently just below the cell surface. They occur in zygospores prior to cytokinesis (Fig. 30), in dividing cells (Fig. 32) and in daughter cells which have already grown flagella (Fig. 31). There can be no doubt that these structures are developing basal bodies and not some stage in mature or regressing ones, since they occur at a time of extensive basal body assembly following a period of 6 days when basal bodies were entirely absent. Similar 9-singlet structures are found in vegetative cells only at stages when new basal bodies are being formed. They cannot be confused with structures other than basal bodies since they have such a very well defined geometry, which corresponds closely with *part* of the structure of the proximal end of a mature basal body. Their overall diameter is about 0·16 $\mu$m, i.e. the same as that of the A-fibres alone, and not that of a complete basal body (0·22 $\mu$m).

The 9 tubules are connected to the 9 spokes in the same way as are the A-subfibres of the mature basal body (see below), which shows that the 9 singlets actually are A-subfibres. Their diameter and the subunit structure of their 5-nm-thick dense walls is also the same as those of the A-subfibres. Dense material is usually present between the 9 singlets but it usually does not take the form of discrete links. The structure of the hub and inner parts of the spokes more nearly resembles the diagrammatic interpretation given in Fig. 5 (i.e. a central ring and 9 radiating lines) than does that of mature basal bodies, because the hub and spokes appear intrinsically denser and have less obscuring background material.

Longitudinal sections of basal bodies are much less easy to interpret, but it is clear that at 9 and 10·5 h, many basal bodies are shorter than mature ones. It is often not possible to tell whether they consist of singlets or triplets, though sometimes basal bodies are seen which appear to consist of singlets throughout their length (Fig. 29). The 'singlet' basal body in Fig. 29 appears to be full length and to have a cartwheel hub and spokes throughout at least three quarters of its length, in marked contrast to mature basal bodies.

*Basal body development during the vegetative cell cycle*

Most mid-light period cells contain only the 2 flagella-bearing basal bodies, but a few already have a third basal body. More cells have extra basal bodies at the beginning of the dark period, and 3 h later (when most cells are preparing to divide and about 13% have partly done so) a high proportion have one or two extra basal bodies. Since the 8 daughter cells derived from each parent contain altogether at least 16 basal bodies, and since I have seldom seen more than four (never more than five) basal bodies in one cell, many basal bodies must be made after the onset of the first division, i.e. between, during or after subsequent divisions.

At 3 and 5 h after the beginning of the dark period one observes transverse sections of basal bodies (in daughter cells) which consist of 9 *singlet* tubules surrounding a central cartwheel (Fig. 8), like the developing basal bodies seen in germinating zygospores, rather than the 9 triplets (Fig. 18) characteristic of the proximal cartwheel-containing region of mature basal bodies. Occasionally, amorphous 'disks' similar in diameter to a basal body are seen (Figs. 9–11); although some may be grazing sections across the ends of mature basal bodies it is more likely that most are transverse

sections at some level through developing ones, especially those that have the smaller diameter (0·16 $\mu$m) characteristic of 9-singlet basal bodies.

*The development of flagella and accessory structures*

In vegetative cells flagellar development does not begin until cell division is over and basal bodies of mature length and apparently mature structure are formed (Fig. 7). Developing flagella are distinguishable from regressing ones since they are attached to daughter cells still inside the mother cell wall. At the mid-dark period many daughter cells have flagella, some of which are shorter than usual (Fig. 9). Some of these show an only partially complete transitional region and no (9 + 2) axoneme, while others have a complete transitional region but a poorly organized (9 + 2) axoneme and dense amorphous material may be present inside the often-irregular membrane. If longer flagella are assumed to represent later stages in development, these observations indicate that the transitional region is formed before the (9 + 2) axoneme, which suggests that the flagellum is assembled sequentially, beginning at the base and growing by adding material to the tip. The existence of a disorganized region at the tip of developing flagella and not throughout their length may also be taken as evidence for growth at the tip rather than intercalary growth. Daughter cells at this stage frequently have more than two basal bodies, though the extra ones may be incomplete.

In germinating zygospores, as in vegetative cells, flagellar development does not begin until the basal bodies are fully mature, and are joined together by striated connexions and associated with microtubular roots. Development is sequential, the transitional region being formed before the 9 + 2 axoneme proper. A few daughter cells already have flagella at 9 h and many do so at 10·5 h. From the beginning, new basal bodies are found close to the plasma membrane, but microtubular roots and striated connexions are generally not visible at the earliest stages when basal bodies appear. However, microtubular roots, at least, are sometimes seen before the zygospore undergoes cytokinesis. As in vegetative cell division, pairs of basal bodies, not yet joined by striated connexions, are frequent. After basal bodies have become joined in pairs, new ones form nearby, linked to them by amorphous material, so that groups of up to four basal bodies can occur as in vegetative cells (Figs. 29, 32).

*Observations on basal bodies and associated structures in cell homogenates*

The homogenization procedure breaks the flagellar axoneme away from the transitional region, confirming the evidence from artificial deflagellation (Figs. 10, 11; Randall, 1969; Rosenbaum *et al.* 1969), that the axoneme-transitional region junction is the weakest part of the flagellar apparatus. The 9 + 2 part of the flagellum is grossly disrupted, presumably partly by the homogenization itself and partly by the EDTA which solubilizes certain components (Jacobs & McVittie, 1970). The transitional region and basal bodies, on the other hand, are remarkably well preserved and more details of their fine structure are visible than in intact cells (Figs. 13–23).

The transitional region normally remains firmly attached to the basal bodies, but very occasionally becomes detached and after negative staining lies flat on the grid (Fig. 13).

The 4·5-nm globular subunits comprising the outer doublets are then clearly seen (the circular A-tubule has about 13 subunits, and the C-shaped B-tubule about 9). In addition, it can be seen that the V-shaped projections from the basal cylinder, which form the 'star' pattern, and the link attaching the apex of the V to the A fibres, are also composed of globular subunits, somewhat smaller than those of the tubules themselves. The link from the A-tubule to the star points probably consists of 3 subunits, the third of which forms the apex of the 'star'. Each arm of the V consists of 5 subunits in addition to this common apical one. Positively stained sections of transitional region (Figs. 14, 15) show that at least some B-tubules have a hitherto unnoticed dense 'knob' (comparable in size to one, or at most two, microtubular subunits), which projects into their lumen from the wall diametrically opposite to the A-tubule. Jacobs & McVittie (1970 and personal communication) have seen identical 'B-tubule knobs' in isolated flagella of *C. reinhardii*. In their pictures only 3 out of the 9 B-tubules appear to have knobs; a pair of adjacent knob-bearing doublets is separated from the third one by two groups of 3, apparently knob-free, doublets. It is unlikely that these B-tubule knobs are merely an artifact of isolation in EDTA since 3 such knobs with the typical arrangement can clearly be seen in Ringo's (1967*b*) fig. 7 (and fig. 9), though usually they are not clearly visible in intact cells. In the transitional region there sometimes appear to be more than 3 B-tubule knobs (e.g. about 5 in Fig. 15). I have seen at least 2 B-tubule knobs in triplet basal bodies, so they are present throughout the basal-body-flagellar axoneme.

Further details can also be seen in the dense 'annular connexion' which joins the transitional region doublets to the flagellar membrane. Each of the 9 doublets bears a dense 10-nm 'doublet outer projection', which projects radially outwards from the junction point of the A and B tubules (Figs. 4, 13, 15). In longitudinal sections (Fig. 17) densities visible at 25-nm intervals along the length of the outer doublets are probably best explained as end-on views of doublet outer projections. The dense material of the annular connexion itself is not homogeneous, but contains densities placed in pairs opposite each doublet outer projection (Figs. 14, 15).

Sections through the apex of isolated basal bodies (Fig. 19) show that each transitional fibre attaches at its broader end not simply to the B-tubule of each triplet (Ringo, 1967*a*), but also to the A-fibre, and to the C-fibre of the adjacent triplet. The tapering ends often remain securely attached to fragments of plasma membrane, so that there can be little doubt that the function of the transitional fibres is to anchor the basal body firmly perpendicularly to the plasma membrane.

The lumen of the basal bodies often contains the cartwheel structure at its proximal end (Figs. 18, 20) and amorphous material of medium density at its distal end, as in intact cells. Frequently, however, this material is lost (Fig. 21), and because the lumen is empty a series of projections spaced at 15-nm intervals on the inner side of the A-tubule stand out clearly (Fig. 21). These projections are essentially comparable to the dense 'feet' seen in mammalian centrioles (Stubblefield & Brinkley, 1967) and also in *Paramecium* (Dippel, 1968), so I shall call them 'A-tubule feet'. In their orientation and attachment to the A-tubule they resemble the radial 'spokes' of the outer doublets of flagella (Hopkins, 1970), but are only 12 nm long instead of 33 nm. Like

the flagellar spokes, they consist of a filament terminated by a knob. Ringo (1967*a*) interpreted these knobs as A–C connexions. This cannot be the case, because A–C connexions would not project into the lumen in median longitudinal sections such as Fig. 22, and can only be seen in tangential longitudinal sections which pass through 2 outer fibres (Fig. 17). In transverse sections through the distal end of the basal body the 'feet' tend to be obscured by the dense material in the lumen, but are normally visible in the cartwheel region. In monkey oviduct centrioles Anderson & Brenner (1971) have interpreted the links between the A-tubules and terminal blob of the cartwheel spokes as sections through an 'A-tubule attachment sheet' instead of separate filaments. However, the very close resemblance in detailed structure between the basal bodies of *Chlamydomonas* and *Paramecium* (Dippell, 1968) and Chinese hamster centrioles (Stubblefield & Brinkley, 1967) and Anderson & Brenner's actual micrographs encourages the expectation that in mammals, too, these links will eventually turn out to be filaments rather than sheets.

Thus there are 3 quite distinct kinds of inwardly pointing radial projections from the A-tubule: (1) the A-tubule feet; (2) the A-tubule-star point links in the transitional region; and (3) the spokes in the 9 + 2 region of the flagellum. It is conceivable, however, that the proximal part of all 3 kinds of projections (corresponding to the 3 globular subunits of the A-tubule-star point links) is homologous or even identical.

The structure of the cartwheel (Figs. 4, 16, 18) is the same as in mammals (Stubblefield & Brinkley, 1967) and in *Paramecium* (Dippell, 1968). Each spoke is terminated by a knob which is distinct from, but attached to, the knob of an A-tubule foot; each spoke is somewhat thicker at the hub end and has another thickening near its mid-point.

Basal bodies in homogenates usually remain attached together in pairs by the distal and 2 proximal striated connexions; this supports the idea that these connexions have a structural role. The dense plate underlying the distal striated connexion is also present (Figs. 21, 22) but is often (like parts of the distal connexion) at least partially dissolved. The 4 microtubular roots are always broken at some point, but part of each remains connected to the basal bodies by amorphous material (Fig. 23). Daughter basal bodies are also physically attached to the old basal body complex by similar amorphous material of medium density (Fig. 16). A very dense material is associated with the proximal end of the basal bodies. This material is much more pronounced on the side of each basal body opposite to that where the distal connexion and microtubular roots attach (Figs. 21, 22); the triplets on this side are longer than on the other, but are often indistinct because they are embedded in the dense material.

## DISCUSSION

### *Flagellar regression*

Johnson & Porter (1968), by using the phrase 'dissociation of basal bodies from flagella', imply that *Chlamydomonas reinhardii* flagella are lost from the cell by breakage or detachment prior to cell division. My observations by light microscopy show that this is not the case, but that the flagella regress gradually by shortening

(as Lewin (1952) observed in *C. moewusii*), at least until the flagella are too short to project from the flagellar tunnels, and therefore disappear from view. I suggest that this shortening is brought about by the solubilization of the axoneme proteins (and possibly also the membrane) by a sequential process starting at the tip of the flagellum and ending at the transitional region.

What happens to the transitional region itself seems to be more variable. In the synchronous cultures studied what usually happens is that the sequential dissolution of the axoneme continues through the transitional region and dissolves it. The connexion between the base of the flagellar tunnel and the base of the flagellar membrane is then broken (this connexion must be fairly strong since it resists homogenization in some cells at least). Fragments of flagellar membrane remain stuck in the flagellar tunnel, and may persist even after the mother wall is cast, but usually the tunnel is empty. In Johnson & Porter's (1968) 'logarithmic' cultures, however, it seems that the entire transitional region (and sometimes some of the 9 + 2 axoneme also) remains trapped in the tunnels. I have also seen an entire (or more usually a partly degraded) transitional region (but never also a 9+2 axoneme) trapped in the flagellar tunnels of cells recently washed off agar into liquid medium.

The reason for this variability may lie in the variable timing of the 90° rotation of the protoplast with respect to the cell wall. This rotation probably breaks the connexion between the cell membrane and the base of the flagellar tunnel. If the rotation does not begin until regression is complete, the flagellar tunnels in the mother cell wall will be empty, or have only membrane fragments, as in my synchronous cultures. If rotation begins before regression is complete, short lengths of flagella will be broken away from the basal bodies and remain in the tunnels (Johnson & Porter, 1968).

Regression in developing zygospores (which possess no cell wall or flagellar tunnels to complicate matters) very clearly occurs by gradual shortening, first of the axoneme and then the transitional region. The withdrawal of the transitional region into the cytoplasm before it is lysed, frequently observed in developing zygospores, was not observed in vegetative cells. I suggest that this is because the transitional fibres joining the basal body to the plasma membrane remain unbroken in vegetative cells (the basal body remains attached to the plasma membrane throughout cell division), but are broken down in zygospores.

Flagellar regression before cell division, and in developing zygospores, takes about 0·5 h, like regression following unilateral deflagellation (Rosenbaum *et al.* 1969). It is likely that the same mechanism is involved in all three cases. The evidence that flagellar protein is conserved by the cell during regression following unilateral deflagellation (Coyne & Rosenbaum, 1970) is consistent with the light- and electron-microscope evidence reported here for regression by shortening and not by breakage.

*Basal body continuity*

Basal bodies appear to be absent for 6 days in *Chlamydomonas* zygospores yet new basal bodies appear during germination. Thus *Chlamydomonas* can be added to the growing list of organisms in which basal bodies appear to be entirely absent for some part of the life cycle (Fulton, 1971). It is clear that basal bodies can be formed in the

complete absence of existing mature basal bodies. Therefore, the widespread current notion (e.g. see Pitelka, 1969; Gibor & Granick, 1967; Jinks, 1964; Wilkie, 1964) that basal bodies are 'self-replicating' is, in its simplest and most obvious sense, simply untrue for many cells.

Since, as Fulton (1971) and I (Cavalier-Smith, 1967) have argued, there is no good evidence either for the presence of DNA in basal bodies or for the self-replication or genetic continuity of basal bodies, it would be better if authors ceased to use these terms with respect to basal bodies and centrioles; Beisson & Sonneborn (1965) and Sonneborn (1970), point out that even if DNA were present in basal bodies this would not explain their results. Indeed their results, and comparable ones for *Tetrahymena* (Nanney, 1968), and the electron-microscopic observations on basal body development in numerous organisms can be explained without postulating self-replication. All these results are compatible with the coding of all basal body proteins by nuclear genes and their synthesis on ordinary cytoplasmic ribosomes. Moreover, there is no reason to postulate that old basal bodies contribute any information about the pattern of assembly of new ones into the 9-triplet plus cartwheel structure. This can be explained by a sequential assembly process (see below) with no necessity for any kind of template.

All that the electron-microscopic and experimental observations require is that there is a mechanism for ensuring that the assembly of new basal bodies begins only at a definite location and with a definite orientation of the new basal body. Such mechanisms must exist for many different organelles (cf. Tucker, 1971) in most eukaryotic cells, but their molecular basis is a mystery. Where 'old' basal bodies are present (e.g. in *Chlamydomonas* vegetative cells) amorphous material close to existing basal bodies appears to serve as a nucleating centre for the first steps of assembly (Dippell, 1968). In *Chlamydomonas* my observations on homogenates show that this material is actually attached to both new and old basal bodies. In some cases where new basal bodies are not formed adjacent to old ones fibrous or granular material seems to act as a nucleation centre (e.g. Anderson & Brenner, 1971), but in others no obvious material is present (Pickett-Heaps, 1971). It is clear that in germinating *Chlamydomonas* zygospores new basal bodies are always formed just below the plasma membrane. Clearly something must ensure this precise location and orientation. Even though basal bodies themselves are absent, it is conceivable that the amorphous material seen in vegetative cells remains, perhaps attached to the plasma membrane throughout the sexual cycle.

Whatever the mechanisms ensuring the precise location and orientation of new basal bodies and other organelles (referred to by Sonneborn as 'cytotaxis'), they alone would be sufficient to explain the inheritance of cortical patterns in ciliates. The simplest type of mechanism is the sequential assembly of molecules, starting with the attachment of the first components to a site with a specific location and orientation on a relatively rigid pre-existing structure. In this way the location and orientation of the new organelle would depend on 2 quite separate factors. One is the geometry and chemical specificity of the component molecules of both the new and of the old structure; this structure is probably coded by normal DNA genes. The second factor

is simply the orientation that the nucleation site happens to have with respect to the other cell structures at the time of assembly. If this orientation is experimentally altered (Beisson & Sonneborn, 1965) then such alteration will automatically be inherited, because of the inherent specificity of the molecules and the assembly process.

Thus, in the case of cortical inheritance it is as correct to speak of the cortical pattern, or even individual kineties, as 'showing genetic continuity' or 'self-replication' as it is for a DNA molecule. But there is no more justification for saying that elements of the pattern such as individual basal bodies, kinetodesmata and so on, are 'self-replicating' than there is for saying that deoxyribonucleotides are self-replicating. Of course, some elements of a cortical pattern may indeed, in some sense, be 'self-replicating', as mitochondria are, but this requires independent evidence (which is lacking for basal bodies) and is neither necessary nor sufficient for the inheritance of the overall pattern.

*Basal body development*

The observations on homogenates show that new basal bodies are firmly attached to the old pair by 'amorphous' material. I suggest that this material serves as a nucleus for the initial stages of assembly and determines the site and orientation of new basal bodies through its geometry and chemical specificity.

It is clear that the 9-singlet structure represents a definite stage in the development of basal bodies. In strain 32 D all such structures contained a well defined central cartwheel, which led me to postulate (Cavalier-Smith, 1967) a sequential mechanism for the assembly of basal bodies: (1) the tubular hub of the cartwheel might be assembled first and (2) each of its 9 subunits visible in transverse sections (Gibbons & Grimstone, 1960) could serve as a site for the assembly of a single spoke; (3) one A-tubule is assembled at the end of each spoke to produce the observed 9-singlet structure; (4) the B and C tubules are added, and connexions made between A and C tubules; and (5) the basal body elongates, transitional fibres form and attach it to the plasma membrane. It is clear that this sequence occurs only in part in *Paramecium*, since Dippel (1968) found that the A-tubules are assembled *before* the cartwheel. In *C. reinhardii* strain $89^+$ I have once seen a 9-singlet structure lacking a cartwheel; this may have been a section through a developing basal body whose A fibres had already lengthened beyond a cartwheel region not included in the section, but it could equally well mean that in *Chlamydomonas* also the cartwheel is not formed first but is added after the formation of a ring of A-tubules.

Some of the dense disk-like objects seen (Figs. 9–11) in the same position as developing basal bodies may be still earlier stages in basal body assembly, corresponding to the morphologically similar 'generative disks' which are the first basal body precursor to appear in *Paramecium* (Dippell, 1968). Similar structures have also been observed during basal body formation in monkey oviduct (Anderson & Brenner, 1971) and in chick trachea (Kalnins & Porter, 1969).

I have not seen developing basal body outer fibres consisting of doublets, though developing basal bodies consisting partly of singlets and partly of triplets do occur. This suggests that the C-tubule is added to the B-tubule immediately the B-tubule is

itself added to the A-tubule. Johnson & Porter (1968, fig. 19) show a 'basal body' consisting of 8 doublets and 1 triplet; because it lacked transitional fibres they considered it to be a developing basal body and not simply a section through the junction of the basal body with the transitional region. However, the absence in their micrograph of transitional fibres is to be expected (apart from the fact that they sometimes fix poorly), since they attach to the extreme upper end of the *triplet* region of the basal body and *not* to the doublet fibres of the transitional region. The closeness of the flagellar membrane except on the side facing the triplet, is also characteristic of sections through the extreme base of the transitional region.

Although my observations are less complete than those of Dippell (1968) for *Paramecium*, they are sufficient to show that the sequence of assembly differs in at least one respect in *Chlamydomonas*: the cartwheel is assembled in *Chlamydomonas* before any B-fibres are complete, whereas in *Paramecium* B-fibres and at least some C-fibres are formed before the cartwheel appears. This means that one should not expect to find an identical sequence of assembly in all organisms, and implies that the detailed mechanisms may differ. Developing basal bodies in *Tetrahymena* (Allen, 1969) and *Tetraspora* (Pickett-Heaps, 1973) also go through a 9-singlet stage.

*Flagellar growth*

It is clear that in *Chlamydomonas* as in many other organisms (e.g. Dippell, 1968; Dingle & Fulton, 1966; Allen, 1969), flagellar growth does not begin until the basal bodies are completely assembled and attached to the plasma membrane of daughter cells by their transitional fibres. Furthermore, flagellar growth does not begin until the 2 basal bodies are at their characteristic angle to each other. Presumably at this stage the distal striated connexion, dense plates and 2 proximal striated connexions are also completely assembled, though one cannot be sure of this without complete serial sections of several daughter cells with stubby flagella. The 4 microtubular roots are assembled (at least in the immediate vicinity of the basal bodies) before flagellar growth begins.

My observations indicate that the transitional region is assembled first, before the axoneme proper. The presence of amorphous material at the tip of short, presumably growing, flagella suggests that the axoneme itself grows sequentially at the tip, which is supported by autoradiographic evidence (Rosenbaum *et al.* 1969). Indeed, my observations are consistent with the view that the whole flagellar-basal body axoneme complex is assembled in a base-to-tip sequence, beginning at the proximal end of the basal body and ending at the tip of the flagellar axoneme. Flagellar growth after artificial deflagellation (Randall, 1969; Rosenbaum *et al.* 1969) appears to be essentially the same as in daughter cells after cell division, except that after deflagellation the transitional region remains and does not have to be formed anew.

The manner of association between the flagellum and the cell wall is of especial interest in *Chlamydomonas*. The proximal regions of mature flagella lie in tunnels in the cell wall and their membranes are fairly firmly attached to the base of the tunnels at the level of the proximal end of the basal cylinder in the transitional region. It appears that the flagella grow a certain length before the cell wall and tunnels are

formed. It may well be that the tunnel is laid down around the flagellum. Unfortunately, mutants completely lacking a flagellar axoneme and transitional region have not yet been found (Randall *et al.* 1967; McVittie, 1972); such mutants would be very interesting since they could show whether the flagella serve in any way as a template or mould for the formation of the flagellar tunnels or whether their structure is an intrinsic property of the cell walls. Even if the latter is true, the flagella must presumably play a role in determining *where* in the cell wall tunnels are formed (unless one postulates that the cell rotates within its new wall, and the flagella somehow 'find' the tunnels and wriggle into them).

*Basal body behaviour and association with other organelles*

Basal bodies are not found directly at the spindle poles in *C. reinhardii*, though sometimes one or more is not far from one of the poles (Fig. 12; and Cavalier-Smith, 1967; Johnson & Porter, 1968). Indeed, they remain attached to the plasma membrane throughout mitosis and cytokinesis, whereas the spindle poles are usually 1 $\mu$m or more from the cell surface. Basal bodies should therefore not be called 'centrioles' in *Chlamydomonas*.

It is unclear whether the 2 daughter cells each receive one old and one new basal body, or whether the old basal bodies remain joined together and both pass to the same daughter cell. Nor is it clear whether every daughter cell necessarily receives a basal body in cases where 2 or more successive cell divisions occur. However, in some cases, the first cell cleavage clearly passes exactly between 2 pairs of basal bodies, so that 2 do go to each daughter cell.

Since in predivision cells (as shown above) the 2 new basal bodies and the 2 old ones all appear to be attached together, it follows that at least some of these links must be broken prior to cytokinesis to allow separation, whatever the manner of distribution of new and old basal bodies to daughter cells. Moreover, since the old basal bodies are linked to each other and to the 4 microtubular roots which underlie an extensive area of the cell surface, at least some of the links involving only 'old' organelles must be broken to allow the cleavage furrow to pass. Thus the 'basal body cycle' in vegetative cells involves, not merely the assembly of new organelles and the movement of both new and old organelles, but also at least a partial alteration of existing structures; it would be very difficult to establish the extent of such an alteration; it might be as little as the temporary breakage of links between old basal bodies and old roots, and between old and new basal bodies, or as great as the complete lysis of all existing roots, striated connexions and the dense plate.

Daughter cells often have 4 basal bodies but grow only 2 flagella. Such cells never have more than one distal striated connexion or 4 microtubular roots. This indicates that the number of these structures is controlled much more rigidly than that of basal bodies. This control of flagella number seldom breaks down in diploid cells, where I have only very rarely observed 3 or 4 flagella at the anterior end; but Starling (1969) has shown that 2–5 % of diploid cells have 3 flagella (and occasionally more). In the 'twin' *C. reinhardii* mutant (Warr, 1968) the number of nuclei and of *pairs* of flagella are closely correlated. Interphase cells have a fibrous area of cytoplasm (the 'fibrous

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REFERENCES

ALLEN, R. D. (1969
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BEISSON, J. & SON
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BERNSTEIN, E. (196
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DINGLE, A. D. & F
*J. Cell Biol.* **31**, 4
DIPPELL, R. V. (196
*U.S.A.* **61**, 461–4
FULTON, C. (1971).
H. Urspring), pp
GIBBONS, I. R. &
*J. biophys. biochem*
GIBOR, S. & GRAN
*Prog. Nucleic Aci*
HOPKINS, J. M. (19
*J. Cell Sci.* **7**, 82
JACOBS, M. & MCV
*reinhardii. Expl C*
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band' (Cavalier-Smith, 1967)), which is free of ribosomes and links the ribosome-free fibrous matrix surrounding the basal bodies and associated structures to the nuclear envelope. Warr (1968) has suggested that the fibrous band could be the physical basis for the correlation between nuclear and flagellar number. The fibrous band is often visible during mitosis joining the nuclear envelope in the region of one of the spindle poles to the basal bodies which remain at the cell surface.

The observations on synchronous cultures and sexual stages were included in a thesis submitted in part fulfilment of the requirements for the Ph.D. degree of London University (Cavalier-Smith, 1967); the observations on homogenates were made during the tenure of a Damon Runyon Cancer Research Fellowship at the Rockefeller University, New York. I thank Professors Sir John Randall and David Luck for encouragement and support.

REFERENCES

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**, 716–733.

ANDERSON, R. G. W. & BRENNER, R. M. (1971). The formation of basal bodies (centrioles) in the Rhesus monkey oviduct. *J. Cell Biol.* **50**, 10–34.

BEISSON, J. & SONNEBORN, T. M. (1965). Cytoplasmic inheritance of the organisation of the cell cortex in *Paramecium aurelia*. *Proc. natn. Acad. Sci. U.S.A.* **53**, 275–282.

BERNSTEIN, E. (1960). Synchronous division in *Chlamydomonas moewusii*. *Science, N.Y.* **131**, 1528–1529.

BERNSTEIN, E. (1964). Physiology of an obligate photoautotroph, *Chlamydomonas moewusii*. I. Characteristics of synchronously and randomly reproducing cells and an hypothesis to explain their population curves. *J. Protozool.* **11**, 56–74.

BUFFALOE, N. D. (1958). A comparative cytological study of four species of *Chlamydomonas*. *Bull. Torrey bot. Club* **85**, 157–178.

CAVALIER-SMITH, T. (1967). *Organelle Development in Chlamydomonas reinhardii*. Ph.D. Thesis, University of London.

CAVALIER-SMITH, T. (1970). Electron microscopic evidence for chloroplast fusion in zygotes of *Chlamydomonas reinhardii*. *Nature, Lond.* **228**, 333–335.

COYNE, B. & ROSENBAUM, J. L. (1970). Flagellar elongation and shortening in *Chlamydomonas*. Reutilization of flagellar proteins. *J. Cell Biol.* **47**, 42a–43a.

DINGLE, A. D. & FULTON, C. (1966). Development of the flagellar apparatus of *Naegleria*. *J. Cell Biol.* **31**, 43–54.

DIPPELL, R. V. (1968). The development of basal bodies in *Paramecium*. *Proc. natn. Acad. Sci. U.S.A.* **61**, 461–468.

FULTON, C. (1971). Centrioles. In *Origin and Continuity of Cell Organelles* (ed. J. Reinert & H. Urspring), pp. 170–221. Berlin: Springer-Verlag.

GIBBONS, I. R. & GRIMSTONE, A. V. (1960). On flagellar structure in certain flagellates. *J. biophys. biochem. Cytol.* **7**, 697–716.

GIBOR, S. & GRANICK, A. (1967). The DNA of chloroplasts, mitochondria and centrioles. *Prog. Nucleic Acid Res. & molec. Biol.* **6**, 103–186.

HOPKINS, J. M. (1970). Subsidiary components of the flagella of *Chlamydomonas reinhardii*. *J. Cell Sci.* **7**, 823–839.

JACOBS, M. & MCVITTIE, A. (1970). Identification of the flagellar proteins of *Chlamydomonas reinhardii*. *Expl Cell Res.* **63**, 53–61.

JINKS, J. L. (1964). *Extrachromosomal Inheritance*. Englewood Cliffs, New Jersey: Prentice Hall.

JOHNSON, U. & PORTER, K. (1968). Fine structure of cell division in *Chlamydomonas reinhardii*: basal bodies and microtubules. *J. Cell Biol.* **38**, 403–425.

KALNINS, V. I. & PORTER, K. R. (1969). Centriole replication during ciliogenesis in the chick tracheal epithelium. *Z. Zellforsch. mikrosk. Anat.* **100**, 1–30.

LAWRENCE, C. W. (1965). The effect of dose duration in the influence of irradiation on recombination in *Chlamydomonas*. *Mutation Res.* **2**, 487–493.

LAWRENCE, C. W. & DAVIES, D. R. (1967). The mechanism of recombination in *Chlamydomonas reinhardii*. I. The influence of inhibitors of protein synthesis on intergenic recombination. *Mutation Res.* **4**, 137–146.

LEWIN, R. A. (1952). Studies on the flagella of algae. I. General observations on *Chlamydomonas moewusii* Gerloff. *Biol. Bull. mar. biol. Lab., Woods Hole* **103**, 74–79.

LUFT, J. H. (1961). Improvements in epoxy resin embedding methods. *J. biophys. biochem. Cytol.* **9**, 409–414.

MCVITTIE, A. (1972). Flagellum mutants of *Chlamydomonas reinhardii*. *J. gen. Microbiol.* **71**, 525–540.

NANNEY, D. L. (1968). Cortical patterns in cellular morphogenesis. *Science, N.Y.* **160**, 496–502.

PICKETT-HEAPS, J. D. (1971). The autonomy of the centriole: fact or fallacy? *Cytobios* **3**, 205–214.

PICKETT-HEAPS, J. D. (1973). Cell division in *Tetraspora*. *Ann. Bot.* **37**, 1017–1025

PITELKA, D. R. (1969). Centriole replication. In *Handbook of Molecular Cytology* (ed. A. Lima-de-Faria), chapter 44. Amsterdam: North-Holland Publishing.

RANDALL, J. T. (1969). The flagellar apparatus as a model organelle for the study of growth and morphopoiesis. *Proc. R. Soc.* B **173**, 31–58.

RANDALL, J. T., CAVALIER-SMITH, T., MCVITTIE, A., WARR, J. R. & HOPKINS, J. M. (1967). Developmental and control processes in the basal bodies and flagella of *Chlamydomonas reinhardii*. *Devl Biol.*, Suppl. **1**, 43–83.

RENAUD, F. L. & SWIFT, H. (1964). The development of basal bodies and flagella in *Allomyces arbusculus*. *J. Cell Biol.* **23**, 339–354.

REYNOLDS, E. S. (1963). The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J. Cell Biol.* **17**, 208–212.

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

RINGO, D. L. (1967*b*). The arrangement of subunits in flagellar fibres. *J. Ultrastruct. Res.* **17**, 266–277.

ROBERTSON, J. D., BODENHEIMER, T. S. & STAGE, D. E. (1963). The ultrastructure of Mauthner cell synapses and nodes in goldfish brains. *J. Cell Biol.* **19**, 159–199.

ROSENBAUM, J. L., MOULDER, J. E. & RINGO, D. L. (1969). Flagellar elongation and shortening in *Chlamydomonas*. The use of cycloheximide and colchicine to study the synthesis and assembly of flagellar proteins. *J. Cell Biol.* **41**, 600–619.

SAGER, R. & GRANICK, S. (1953). Nutritional studies with *Chlamydomonas reinhardii*. *Ann. N.Y. Acad. Sci.* **56**, 831–838.

SONNEBORN, T. M. (1970). Gene action in development. *Proc. R. Soc.* B **176**, 347–366.

STARLING, D. (1969). Complementation tests on closely linked flagellar genes in *Chlamydomonas reinhardii*. *Genet. Res.* **14**, 343–347.

STUBBLEFIELD, E. & BRINKLEY, B. R. (1967). Architecture and function of the mammalian centriole. In *Formation and Fate of Cell Organelles*, Symp. int. Soc. Cell Biol. No. **6** (ed. K. B. Warren), pp. 175–218. New York and London: Academic Press.

TUCKER, J. B. (1971). Spatial discrimination in the cytoplasm during microtubule morphogenesis. *Nature, Lond.* **232**, 387–389.

WARR, J. R. (1968). A mutant of *Chlamydomonas reinhardii* with abnormal cell division. *J. gen. Microbiol.* **52**, 243–251.

WATSON, M. R. & HYNES, R. D. (1966). Starch gel electrophoresis of the fibrillar proteins of the cilia of *Tetrahymena pyriformis*. *Expl Cell Res.* **42**, 348–356.

WILKIE, D. (1964). *The Cytoplasm in Heredity*. London: Methuen.

(*Received* 5 *February* 1973 – *Revised* 21 *May* 1974)



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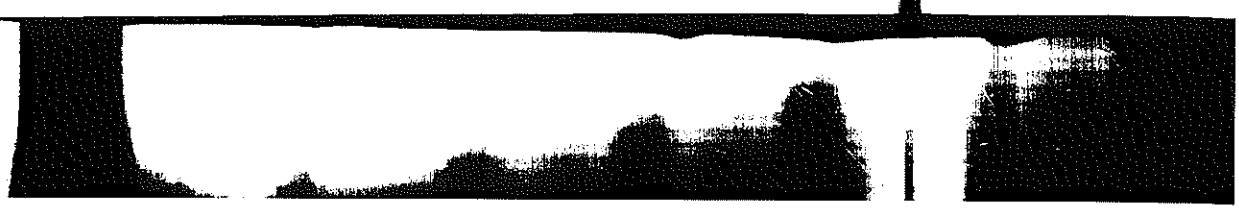


Fig. 6. Median longitudinal section of short flagellum 3 h after the beginning of the dark period. This is probably a regressing flagellum since the membrane at its tip is sectioned approximately perpendicularly, and there are traces of the bottom end of the central pair just distal to the transitional region (arrows), which do not occur in stumps left after a flagellum has broken off (cf. Figs. 10 and 11). Note the ribosome-free 'fibrous band' (*f*) (Cavalier-Smith, 1967) which joins the basal bodies to the nucleus, and the associated smooth-surfaced vesicles (*v*). × 36000.

Fig. 7. Accurately longitudinal tangential section of 2 basal bodies before the outgrowth of flagella (in daughter cell 5 h after the beginning of the dark period). Note the triplets present in each, and their orientation roughly perpendicular to 2 protuberances of the plasma membrane. The basal bodies are not yet perpendicular to each other and only a small amount of dense amorphous material (arrow) is present in the region where the distal striated connexion would probably later be formed. Daughter cell walls and flagellar tunnels are not yet formed, but a layer of fibrous material is present on the outer surface of the plasma membrane. × 80000.

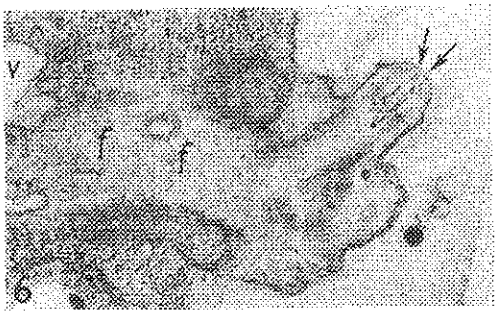
Fig. 8. Transverse section of 9-singlet structure with a central cartwheel; interpreted as a stage in the development of basal bodies. In a daughter cell 3 h after the beginning of the dark period. × 55000.

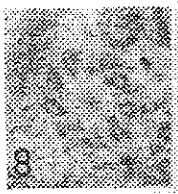
Fig. 9. Slightly oblique longitudinal section through basal body and flagellum of daughter cell 6 h after the beginning of the dark period. This is probably a growing flagellum; note the patch of amorphous material at the tip of the axoneme inside the membrane. A flagellar surface coat, but no cell wall, is already present. Two roots and a second triplet basal body are shown in oblique section. The 2 vague disk-shaped objects (arrows) have the characteristic diameter and location of newly formed basal bodies. They could either be developing basal bodies or (less likely) glancing sections across the ends of mature basal bodies. × 60000.

Fig. 10. Two mature and 2 young basal bodies in a cell artificially deflagellated by the ethanol-calcium method (Watson & Hynes, 1966). Note the 4 microtubular roots (*r*), in one of which ($r_1$), only the dense, cross-striated, material which is characteristically associated with the microtubules (Cavalier-Smith, 1967) can be seen. × 80000.

Fig. 11. Artificially deflagellated cell showing roots (*r*) distal striated connexion (*sc*), and an amorphous disk (which may be a very early stage in basal body development). A single A-tubule (arrow) can just be seen in the edge of the amorphous disk. Figs. 10 and 11 both show the characteristic site of flagellar breaking (and efficient re-sealing of the flagellar membrane) at the distal end of the transitional region. × 105000.

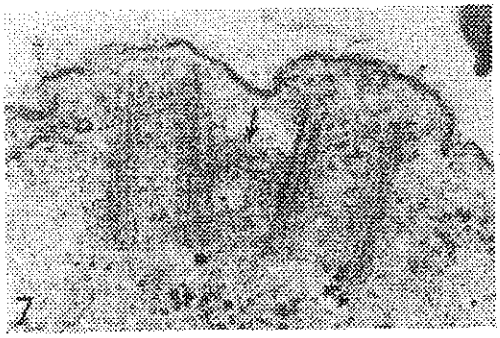
Fig. 12. An exceptional mitosis in which the nuclear envelope is broken down far more than is usual and where the spindle pole (arrow) is much nearer the cell surface and therefore also to the basal body, which remains attached to the plasma membrane (and not to the spindle pole) throughout division (Cavalier-Smith, 1967). Note the intact Golgi apparatus (*g*) the spindle microtubules (*m*), and chromosomes (*c*). × 45000.

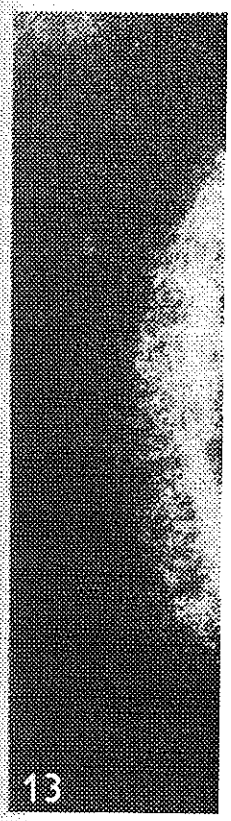


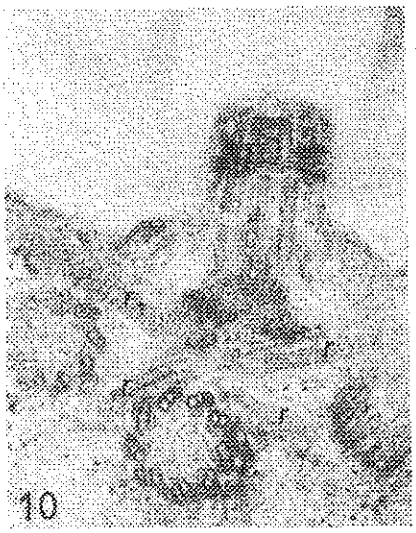



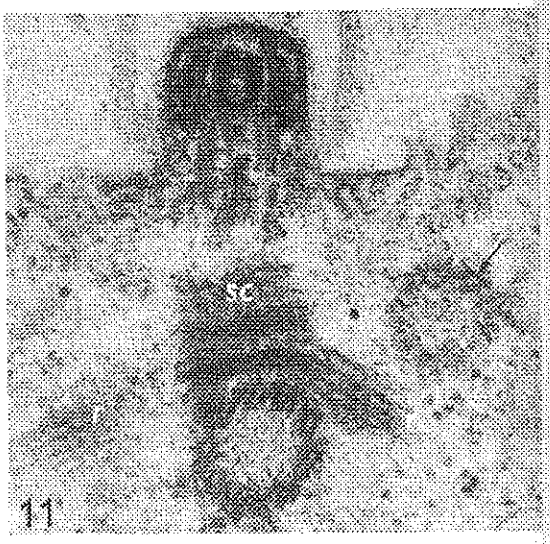




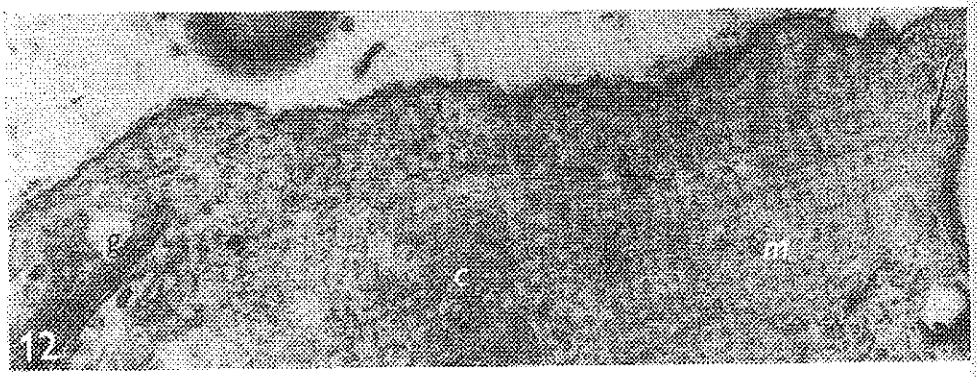


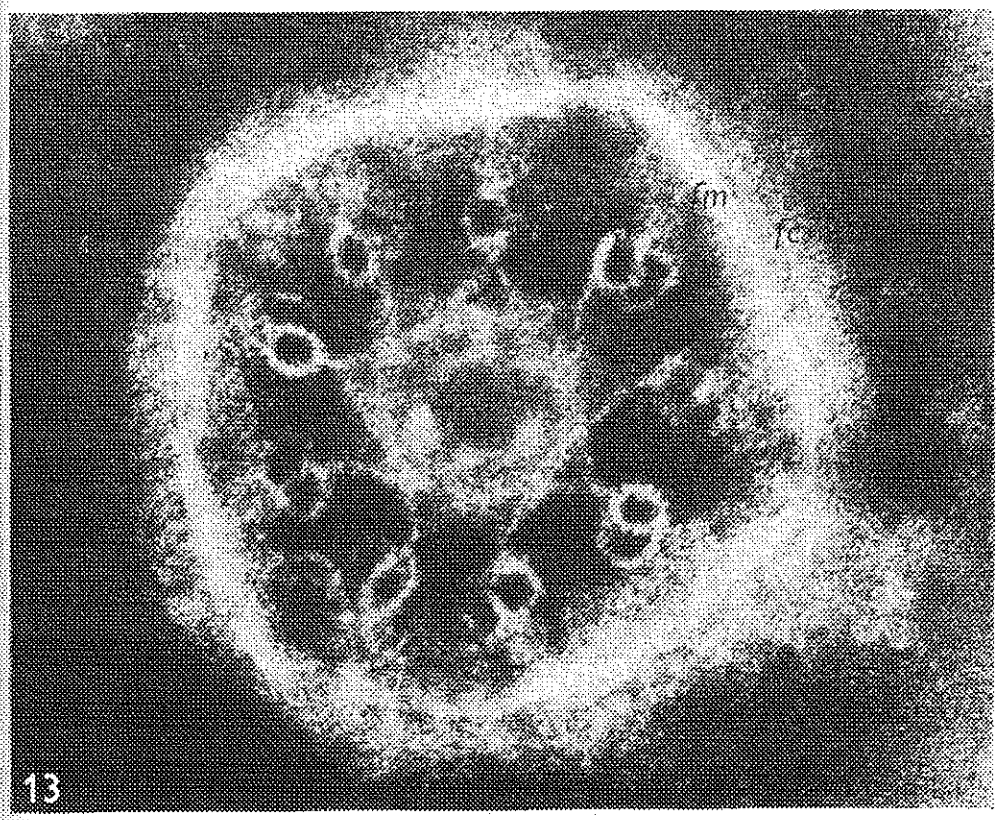



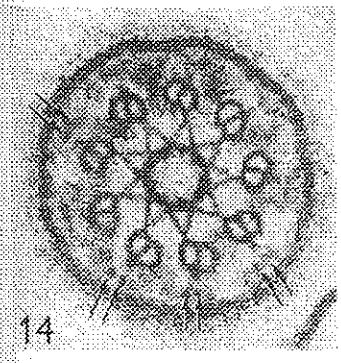


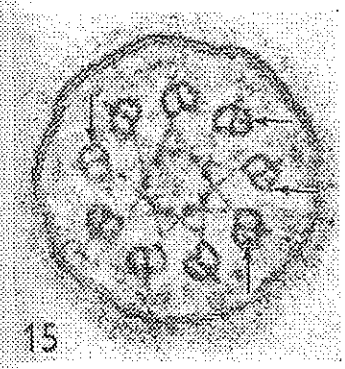


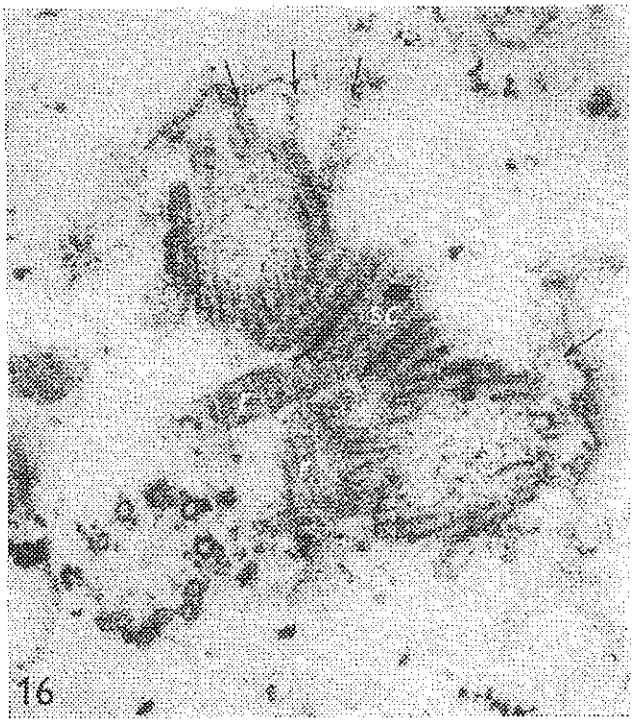


Figs. 6–12. For legend see previous page.




Figs. 13–16. For legend see p. 552.

Figs. 13–16. *Chlamydomonas* transitional regions and basal bodies from cell homogenates.

Fig. 13. Negatively stained transitional region lying flat on the grid, showing flagellar surface coat (*fc*), flagellar membrane (*fm*) and the globular substructure of the outer doublets, and of the star pattern surrounding the basal cylinder. × 320000.

Fig. 14. Transverse section through the distal part of the transitional region. That part of the annular connexion lying between the outer doublets and the membrane is not homogeneous but contains densities placed in pairs (arrows) opposite each doublet outer projection. × 120000.

Fig. 15. Transverse section through the proximal part of the transitional region's basal cylinder. Doublet outer projections and paired densities are present, and B-tubules knobs can be seen clearly in places (arrows). The flagellar surface coat is very well preserved. × 120000.

Fig. 16. Two mature triplet basal bodies still joined to each other by the distal striated connexion (*sc*) and to the plasma membrane fragments by transitional fibres (arrows). A developing basal body consisting of a cartwheel plus 6 singlets and 3 triplets is connected to the mature basal body pair by amorphous material that appears to be attached to fragments of the microtubular roots (*r*). × 120000.

Fig. 17. Tangential longitudinal section through isolated basal body and transitional region. The double annular connexion (*ac*) joining the outer doublets to the flagellar membrane is very clear. The dense blobs (arrows) are probably end-on views of doublet outer projections. The upper end of the C-fibres (*c*), the A–C connexions (arrow-heads), a microtubular root (*r*), and the dense material (*d*) attached to the proximal end of the basal body, are all visible. × 120000.

Fig. 18. Section through an isolated basal body pair retaining both the cartwheel (lower basal body) and the amorphous material in the lumen of the distal region (upper basal body, which is attached to a membrane fragment by poorly preserved transitional fibres (arrows)). × 120000.

Fig. 19. Transverse section of extreme distal end of basal body showing transitional fibres, 5 of which connect the triplets to a membrane fragment. × 60000.

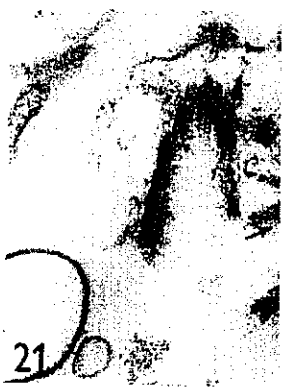
Fig. 20. Median longitudinal section through isolated basal body. Even though the cartwheel and amorphous material in the lumen are well preserved, the A-tubule feet show as 2 rows of dense spots (between 2 pairs of arrows). *r*, root. × 50000.

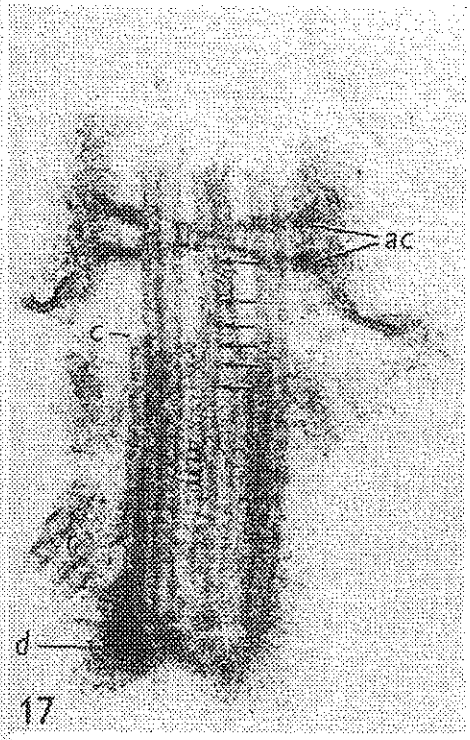
Fig. 21. Basal body pair where the cartwheel and amorphous material is dissolved, showing the A-tubule feet more clearly (between arrows). Note also that the distal striated connexion and dense plate have dissolved more than in Fig. 23. × 50000.

Fig. 22. Isolated basal body pair showing excellent preservation of all structures, except for a slight dissolution of the distal striated connexion (*sc*) and dense plate (*d*). × 60000.

Fig. 23. Isolated basal body pair showing excellent preservation of microtubule roots. × 35000.







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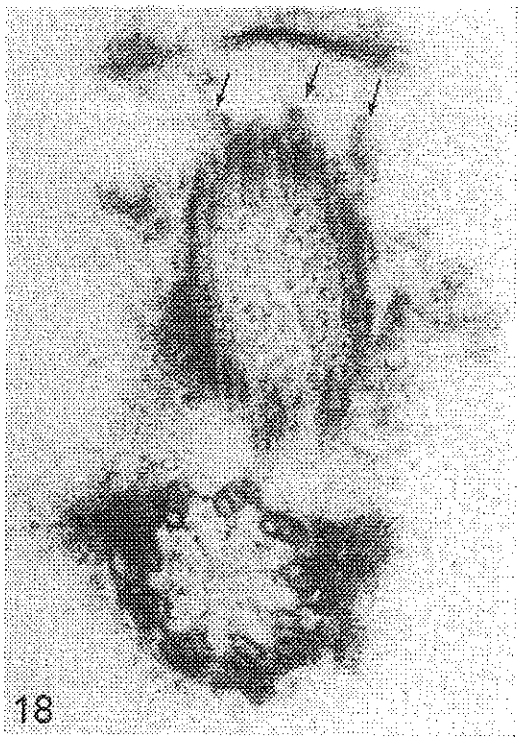
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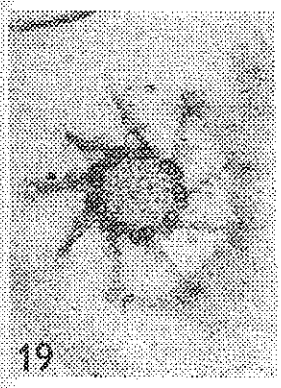
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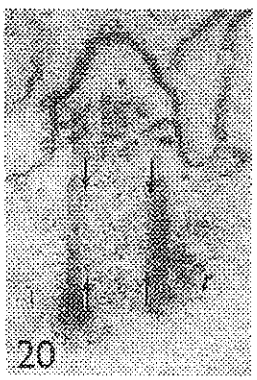
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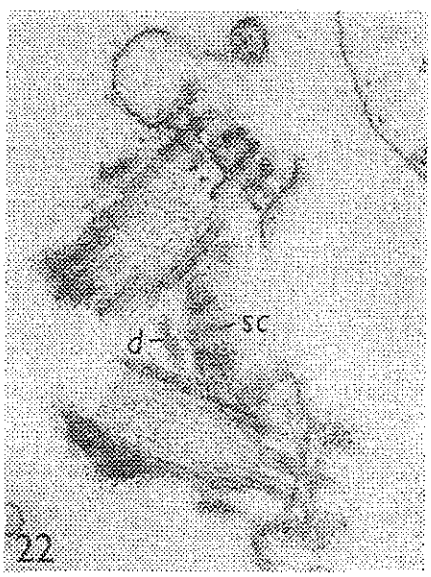

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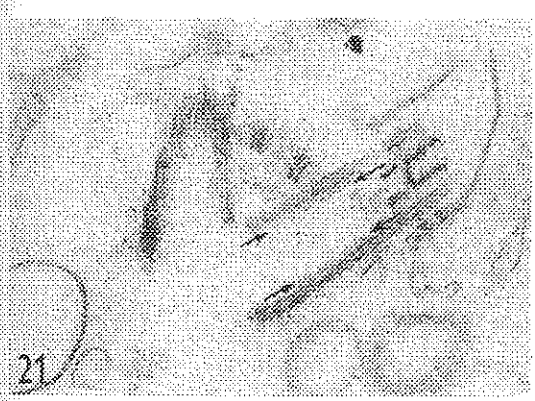
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Figs. 24–28. Stages in the regression of flagella and basal bodies in zygotes fixed 4·75 h after mixing the gametes.

Fig. 24. Median longitudinal section of short regressing flagellum. The basal body and transitional region are still complete, but the central pair is absent and the outer doublets are (unequally) cut short. Note the amorphous material just below the perpendicularly sectioned membrane at the flagellum tip, some membrane fragments (*mf*), and the transverse fibres which connect the basal cylinder to the A-subfibres of the outer doublet (arrow). × 70000.

Fig. 25. A later stage in flagellar regression. The 9 + 2 axoneme is completely gone, but the basal body and transitional region are intact. The transitional region is slightly withdrawn into the cell; this probably indicates that the annular connexion that joins it to the flagellar membrane, and the transitional fibres that join the basal body to the membrane, are both broken or dissolved by this stage. The dense mass attached to the base of the basal body is well shown here (arrow). × 80000.

Fig. 26. Tangential longitudinal section of a regressing flagellum before the annular connexions (small arrows) between the transitional region axoneme and the flagellar membrane are broken. The structure shown by a large arrow is possibly the short proximal end of a basal body which has regressed faster than the one still attached to the transitional region; if so, the irregular structures between it and the larger basal body will be remains of the striated connexion. The outer fibrous layer of the zygospore wall (Cavalier-Smith, 1967) is already partly formed and covers even the flagellar stub. × 45000.

Fig. 27. Oblique longitudinal section of regressing flagellum at a stage intermediate between those shown in Figs. 24 and 25. Note that the outer fibrous layer (*f*) of the zygospore wall is already partly formed. × 60000.

Fig. 28. The 2 dense bodies (*d*) are interpreted as the outer fibres of a very short (0·1 $\mu$m) basal body seen in longitudinal section about 0·75 $\mu$m below the cell surface. It is located close to the obliquely sectioned nuclear envelope and separated from the cell surface by an area of amorphous/fibrous cytoplasm, largely free of ribosomes. The outer fibrous layer (*f*) of the developing zygospore wall is well developed. × 60000.

Figs. 29–32. Stages in the development of new basal bodies in germinating zygospores, and in the resulting daughter cells (zoospores).

Fig. 29. Three basal bodies (*1*, *2* and *3*) in a daughter cell (still within the zygospore wall) 9 h after the beginning of germination. Basal body *1* appears to be a complete 0·4-$\mu$m-long triplet basal body (in slightly oblique tangential longitudinal section), above which the proximal 0·1-$\mu$m part of the transitional region (brackets, *tr*) is being assembled within a protuberance of the plasma membrane. The other 2 basal bodies are probably incomplete. Basal body *2* is sectioned accurately in the longitudinal median plane. It appears to be full length (0·4 $\mu$m) but to consist only of singlet outer fibres (A-tubules), and has a well developed cartwheel throughout at least three quarters of its length. Like transversely sectioned 9-singlet basal bodies, it is 0·165 $\mu$m in diameter instead of the 0·22 $\mu$m characteristic of mature 9-triplet basal bodies. The cartwheel hub appears as 2 staggered rows of dense dots (between large arrows). The knobs on the A-tubule feet can be seen as a dense line just on the luminal side of the upper A-tubule (between small arrows). The third basal body (*3*) is probably a short developing one, but it is too indistinct for one to be sure of this. The microtubule (*m*) is probably part of a developing microtubular root. × 60000.

Legends continued on p. 556.

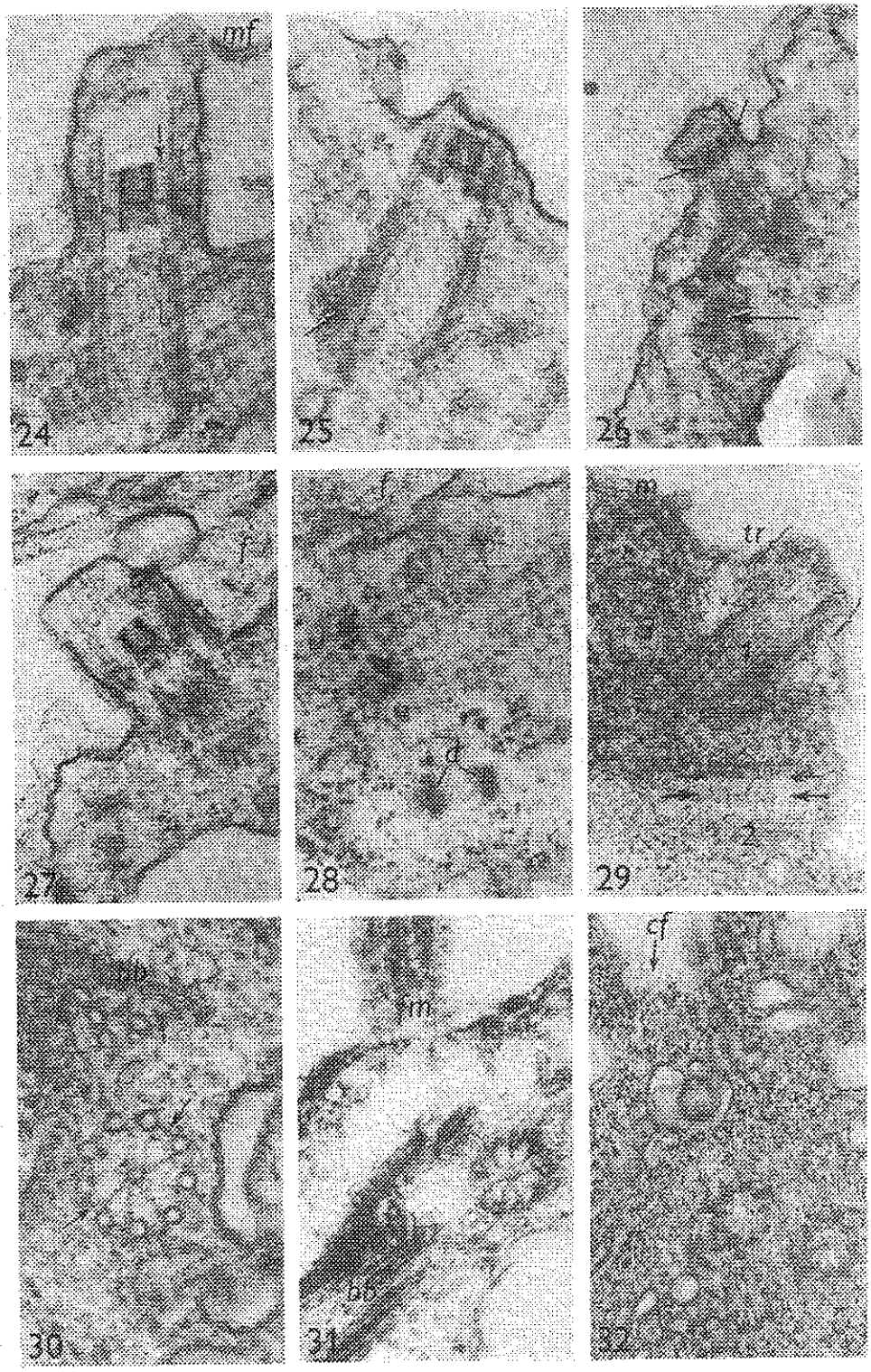


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Fig. 30. Transverse section through 9-singlet developing basal body, and the adjacent 4-member microtubular root (above), which is attached to one side of the neighbouring, 9-triplet body (*bb*) of a 9-h germinating zygospore. The central cartwheel, the A-tubule feet and the globular substructure of the 9 A-tubules themselves are clearly visible. Apparent cross-links (with a central blob, arrows) are visible between a few of the A-tubules. × 120 000.

Fig. 31. Nine-singlet basal body in an already (at least partially) flagellate daughter cell (not yet released from zygospore wall) 10·5 h after beginning of germination. Some of the A-tubules appear incomplete. One of the 2 flagella-bearing basal bodies (*bb*) is obliquely sectioned; the other is out of the plane of the section, though a grazing section of its flagellum's membrane (*fm*) is seen; this flagellum (not shown) had well developed doublets. Very thin section (grey-to-black interference colour), stained with potassium permanganate (Robertson, Bodenheimer & Stage, 1963) in between the uranyl and lead stains. × 80 000.

Fig. 32. Nine-hour germinating zygospore undergoing cytokinesis. The 4 basal bodies are apparently in the middle of the cell. Three have cartwheels and two have triplet fibres. Two have singlet fibres on part of their circumference, but apparently triplet fibres elsewhere. Note the numerous smooth-surfaced vesicles bounded by an 8–9 nm trilaminar membrane indistinguishable from the plasma membrane. These and the 4 basal bodies lie to one side of the cleavage furrow (*cf*) whose plane is shown by the arrow. × 40 000.

*J. Cell Sci.* 16, 557–577 (
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## I. THREE-T
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SARAH P. G
*Department of I*

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# THE CHLO
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## I. THREE-DI
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SARAH P. GIB
*Department of Biol*

SUMMARY

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