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Golgi Apparatus Activity and Membrane Flow During Scale Biogenesis in the Green Flagellate *Scherffelia dubia* (*Prasinophyceae*). II: Cell Wall Secretion and Assembly

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Summary

Secretion of the cell wall (theca) in the scaly green flagellate *Scherffelia dubia (Prasinophyceae)* has been examined by electron microscopy during cytokinesis. The bi-laminate wall forms by the extracellular amalgamation of two layers of scales produced in the Golgi apparatus (GA). Each mature GA cisterna contains ca. 12,000 scales of two distinct varieties arranged in two layers on the cisternal membrane. GA cisternae undergo turnover and one scale containing cisterna matures from the *trans*-face of each dictyosome every 3–4 minutes. Cisternae then fuse with the plasma membrane at the anterior end of the cell releasing the scales onto the cell surface. The two layers of wall scales integrate on the cell surface in a time-dependent self-assembly process. The first scales deposited commence assembly at the cell posterior and the wall develops anteriorly by edge growth. The daughter cell wall is composed of ca. 1.2 million scales deposited in about 3 hours. Calculations of net membrane flow strongly indicate extensive endocytosis during wall deposition.

Keywords: Cell wall; Golgi apparatus; Green Algae; Membrane flow; Scales; *Scherffelia*; Self-assembly; Theca.

1. Introduction

Investigations of the mechanism of the ordered deposition and assembly of biological structures such as cell walls are fundamental to our understanding of morphogenesis. Amongst the various groups of algae are observed a diverse spectrum of cell wall forms and materials, perhaps the best known of which is the crystalline glycoprotein wall of chlorophycean algae (ROBERTS *et al.* 1982), and in particular *Chlamydomonas* (*e.g.*, O'NEILL and ROBERTS 1981, ROBERTS *et al.* 1972, 1980, ROBERTS 1974, CATT *et al.* 1976, 1978).

While the cell wall of *Chlamydomonas* is relatively well characterized biochemically (see above), only preliminary data are available on the composition of the cell wall (theca) of the scaly green flagellate *Tetraselmis* (LEWIN 1958, GOODAY 1971, MANTON *et al.* 1973). The cell wall of *Tetraselmis* is pectic in nature and yields mainly galactose, galacturonic acid, and arabinose after hydrolysis (LEWIN 1958, GOODAY 1971). High levels of calcium, probably complexed to galacturonic acid, are present in the cell wall of *Tetraselmis* (MANTON *et al.* 1973). In *Chlamydomonas* deposition of the multilaminate wall occurs by extracellular crystallization of soluble precursors derived from the Golgi apparatus (GA) (CATT *et al.* 1976, 1978). The bi- or trilaminate wall of *Tetraselmis* also forms by extracellular assembly of GA derived material (MANTON and PARKE 1965, DOMOZYCH *et al.* 1981, GOODAY 1971), but the wall precursors are non-soluble moieties (scales). Developing wall scales can be observed in the GA cisternae of *Tetraselmis* and the sequential progression of maturation stages from the *cis*- to the *trans*-face (MANTON and PARKE 1965, DOMOZYCH *et al.* 1981) suggests that the scales may be transported through the GA by cisternal turnover. However, recent re-evaluations of the membrane flow hypothesis (FARQUHAR 1978, FARQUHAR and PALADE 1981, ROTHMAN and LENARD 1984, ROTHMAN 1985) make it necessary to re-examine scale secretion

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more carefully to determine the GA *modus operandi* (for further discussion see McFadden and Melkonian 1986).

Wall scales of *Tetraselmis*, released to the cell surface by exocytosis, coalesce to form an electron dense wall (Manton and Parke 1965, Domozych *et al.* 1981) but the detailed structure of the scales and the manner in which they coalesce has not yet been resolved.

In this paper we investigate wall secretion and assembly during cytokinesis in *Scherffelia dubia*; a close relative of *Tetraselmis* (Melkonian and Preisig 1986). Developing scales are transported through the GA by cisternal turnover, thereby supporting the original hypothesis of endomembrane flow (Morré *et al.* 1971). Wall formation in *Scherffelia* occurs by amalgamation of two layers of structurally different scales by a self-assembly mechanism. Comparison of the total number of scales deposited in a wall with the number of scales in the GA at any one time allows extrapolation of scale production rates and membrane turnover. Calculations indicate that extensive reclamation of membrane occurs at the cell surface during wall assembly, and a membrane flow model including endocytosis is presented.

2. Materials and Methods

Culture, fixation (simultaneous type) and thin section electron microscopy (EM) of *Scherffelia dubia* (Perty) Pascher emend. Melkonian and Preisig was performed as described by Melkonian and Preisig (1986). Deep-etch analysis was done as described by McFadden and Wetherbee (1985).

3. Results

The general interphase morphology of *Scherffelia dubia* is described in detail elsewhere (Melkonian and Preisig 1986). Immediately prior to division, the flagellate cell is obovate in lateral view and ellipsoidal in cross-section (see Figs. 1–7 and 11–16 in Melkonian and Preisig 1986) and averages 15 μm long, 10 μm wide and 6 μm broad (average of 17 cells measured). By dividing the cell into segments as shown in Fig. 1, the cell surface area prior to division is calculated as 400 μm^2.

3.1. The Cell Cycle

Cell division in *Scherffelia dubia* is described in Melkonian and Preisig (1986), and is similar to what has been described for species of the closely related *Tetraselmis* (*cf.*, Ricketts and Davey 1980, Domozych *et al.* 1981, Manton and Parke 1965, Stewart *et al.* 1974). Two oppositely oriented daughter cells form within the distended parent wall and deposit new walls commencing at the respective posterior ends (Fig. 2). During the very latter stages of wall formation, the flagella begin to regenerate (not illustrated). The entire process of wall formation, from cytokinesis to completion of the daughter walls, takes about 3 hours. The daughter cells (15 cells of which averaged 12 μm × 7.3 μm × 4.8 μm, surface area = 260 μm^2) are released through a rupture of the parent wall and the abandoned wall promptly rolls in upon itself with the distal side outward (see Fig. 33 in Melkonian and Preisig 1986).

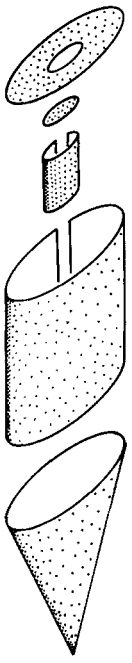


Fig. 1. Exploded diagram showing the surface of the cell of *Scherffelia dubia* broken into pieces to calculate the area

3.2. GA Morphology During Wall Formation

The general characteristics of the GA are similar to those described by McFadden and Melkonian (1986). The number of cisternae during cytokinesis (average of 16 ± 1 from 17 cells) is less than during interphase (average of 19 ± 2 from 11 cells). Cisternal diameter increases from 1.3 μm at the *cis*-face to ca. 1.8 μm at the *trans*-face (Figs. 2 and 3). The cisternae, particularly those at the *trans*-pole, are dilated to accomodate the wall scales (Fig. 3). Dilation of the cisternae has the effects of reducing the diameter and increasing the height of the stack of cisternae, the latter increasing from an average of 0.6 μm during interphase (eleven

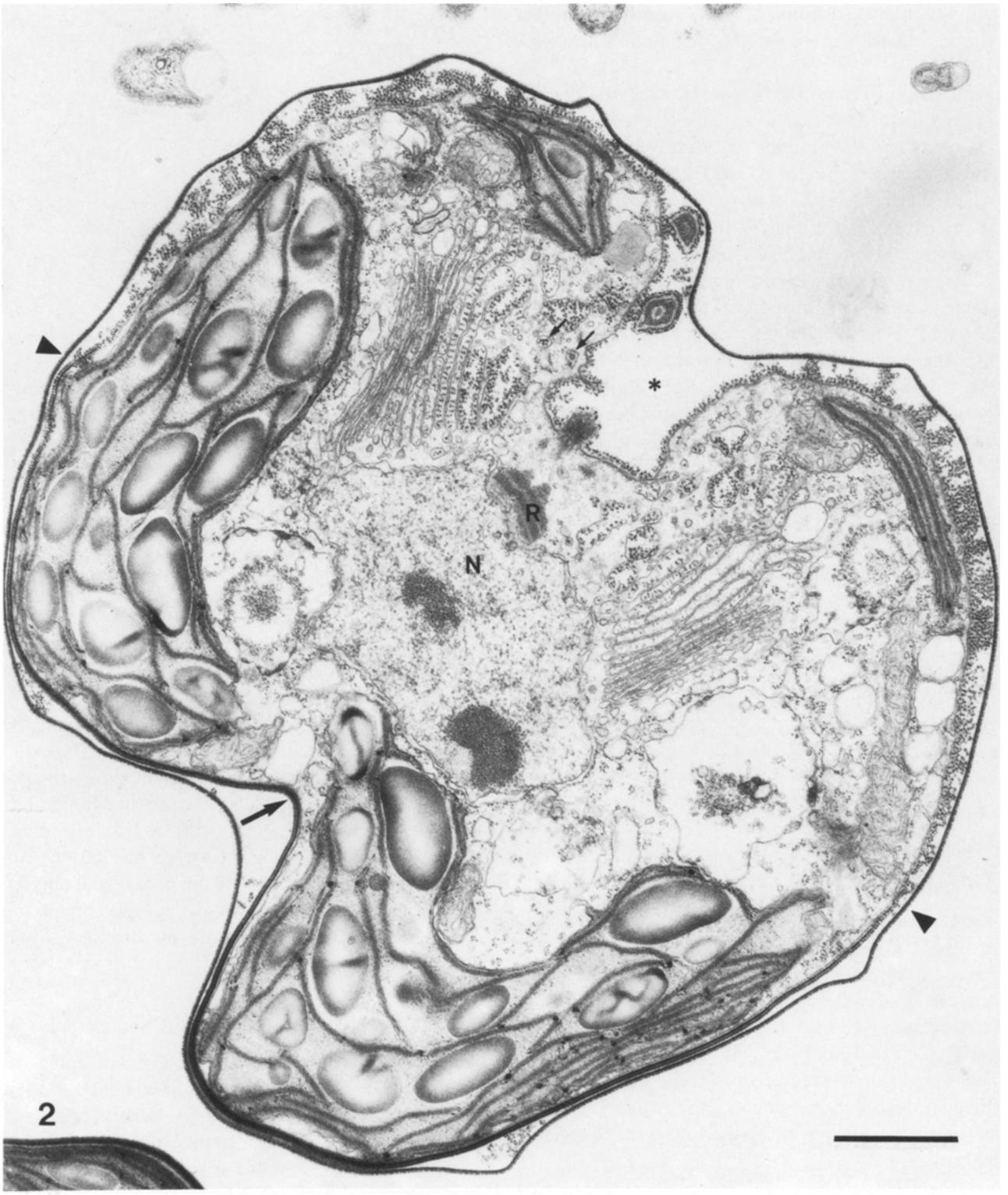


Fig. 2. Oblique longitudinal section of a daughter cell within the parent cell wall of *Scherffelia dubia*. The two dictyosomes (subtended by rough ER) lie on either side of the forming flagellar groove (asterisk). Wall scales are visible in the *trans*-most GA cisternae. The surface of the daughter cell bears exocytosed wall scales that have coalesced at the posterior end to form a new daughter wall (arrow). Assembly of the two scale layers proceeds toward the anterior end (arrowheads). Cross-sections of two flagella (possibly belonging to another daughter cell) are visible within the parent wall. One striated flagellar root (*R*) is shown in oblique section by the nucleus (*N*). Portions of the reduced flagellar scale reticulum are visible between the GA and the flagellar groove (small arrows) (scale bar = 1 μm)

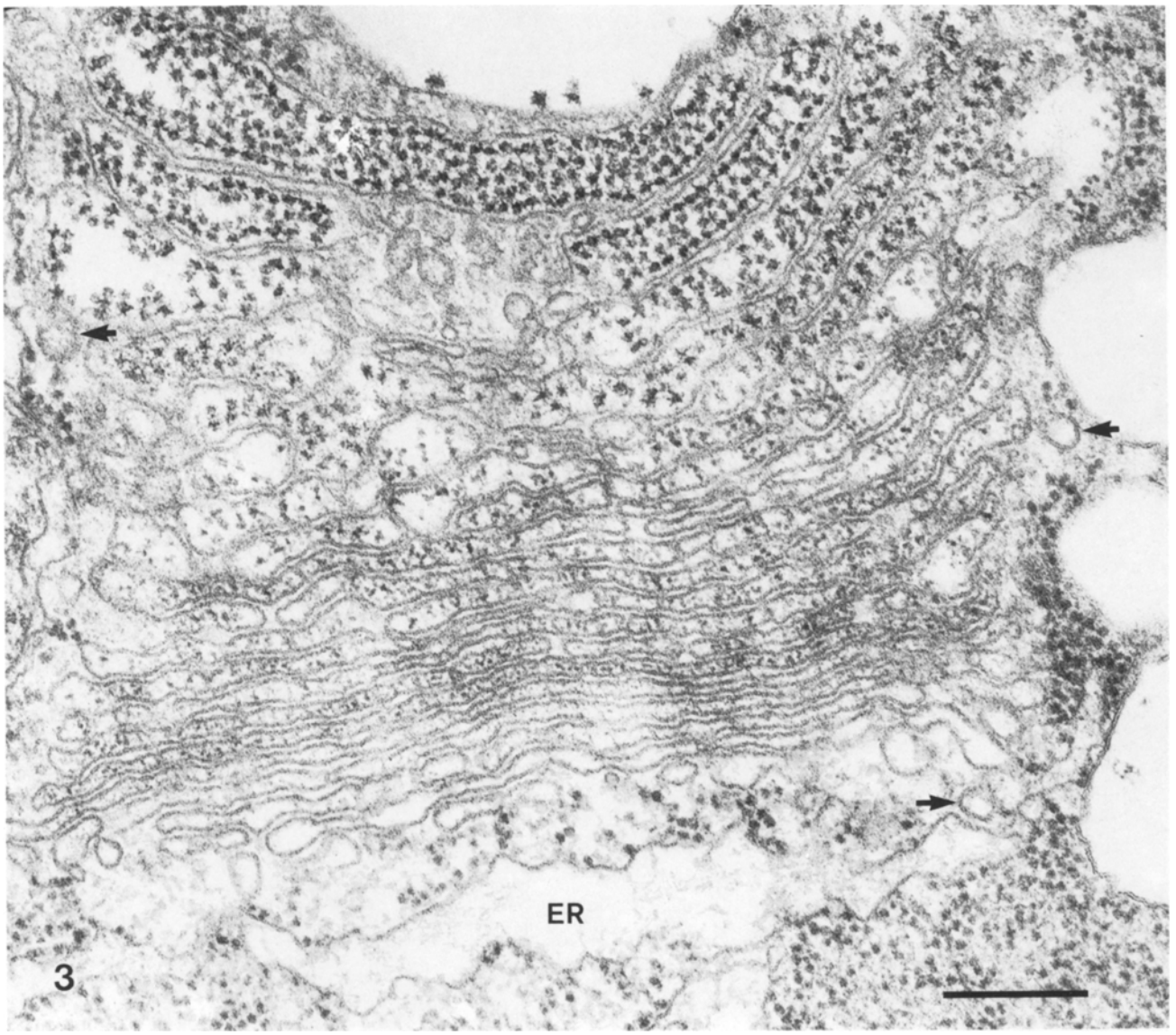
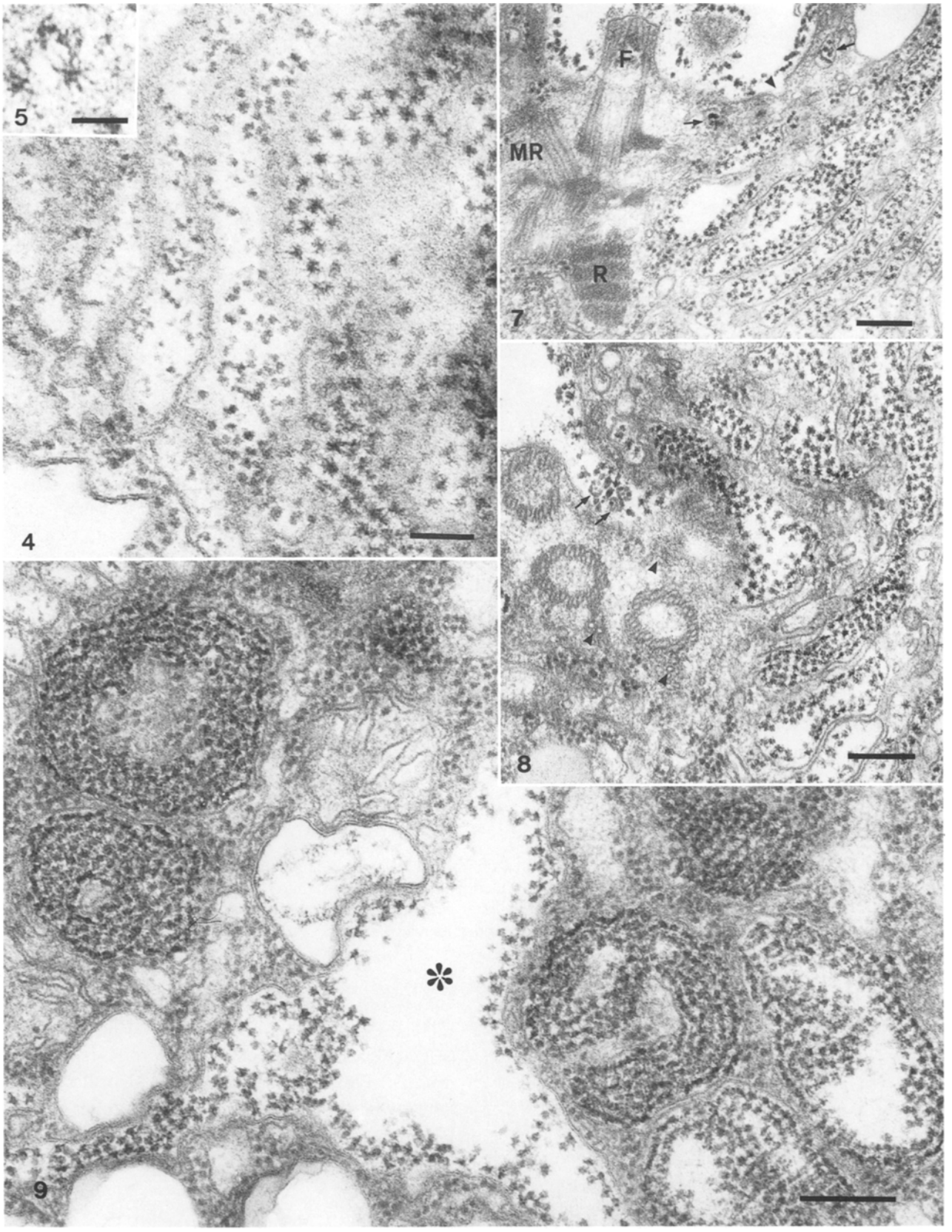


Fig. 3. Cross-section of a dictyosome during wall formation showing the stack of 17 cisternae. The *cis*-face is subtended by dilated rough ER (*ER*) that contains fibrillar tufts. Several transition vesicles are visible at the dictyosome peripheries (arrows). Dark granules, visible within the fourth cisterna from the *cis*-face, are elaborated into wall scales in cisternae at the *trans*-pole. The mature wall scales are of two types arranged in two layers on the dictyosomes membrane resulting in four layers within the cisternal lumen (scale bar = 200 nm)

cells evaluated) to ca. 1.1 µm during wall production (*e.g.*, Fig. 3; 4 cells evaluated). During cytokinesis no flagellar scales are observed in the GA cisternae (Figs. 3 and 4). Polygonal vesicles, which occur at the *trans*-face of the GA during flagellar regeneration (*cf.*, McFadden and Melkonian 1986), are absent. However, the "scale reticulum" containing pentagonal flagellar scales (*cf.*, Melkonian and Preisig 1986, McFadden and Melkonian 1986) is present during cytokinesis (Figs. 2 and 7). Since the distended GA cisternae protrude into the zone normally occupied by the scale reticulum (Figs. 2, 3, and 7), the latter is reduced during division. The *cis*-face of the GA is subtended by dilated rough endoplasmic reticulum (ER) (Fig. 2) that contains faint tufts of fibrillar material (Fig. 3). Numerous vesicles (diameter 60 nm) are present at the cisternal rims, between the ER and *cis*-cisterna, and between nuclear envelope and cisternal rims (Figs. 2, 3, and 7).

3.3. Scale Production in the GA

In the fourth cisterna from the *cis*-face numerous electron-dense granules are visible and these granules



Figs. 4–9

are progressively more elaborate toward the *trans*-face where they are distinguishable as scales (Figs. 2 and 3). The scales are of two varieties that are organized into two distinct layers on the cisternal membrane (Fig. 3). Closest to the cisternal membrane is a layer of disk-shaped scales having indistinct morphology (diameter = 22 nm, height = 13 nm, Fig. 3). These inner scales are positioned on the membrane of the cisternae in a planar grid, interspaced in each dimension by 29 nm (Fig. 3). The inner type scales are distinctly more electron-dense (Fig. 3). The second or outer scale variety has a distinctive morphology (Figs. 4–6) and resembles a human match-stick figure in lateral section view (Fig. 11). The outer scales (diameter = 35 nm, height = 30 nm) are positioned with the "head" toward the inner scale beneath, and the three "feet" projecting into the lumen of the cisterna. The three feet (diameter of each foot = 4 nm) project straight out from an equilateral triangular base that has 9 nm sides (Figs. 6 and 13). Each outer scale also has ca. five lateral radiating arms (Figs. 5 and 6), but we are unable to determine the exact number. The two scale types are present in the cisterna in a 1 : 1 numerical ratio, each inner scale having an outer scale above it (Fig. 3). The two scale layers are present on both sides of the cisternal membrane, thereby giving four scale layers in each cisterna (Fig. 3).

3.4. Scale Release

Both dictyosomes secrete similar numbers of scales (Fig. 2). Mature cisternae leave the dictyosome stack from the *trans*-face (Figs. 2 and 3). In early stages of division the cisternae appear to fuse within the cell to form a cleavage zone (Fig. 9). The cleavage plane and zones of wall assembly are subtended by secondary (*sensu* PICKETT-HEAPS 1975) cytoskeletal microtubules (Fig. 10, arrowheads). Cisternae fuse with the plasma membrane of each daughter cell near the basal bodies at the anterior end (Figs. 7 and 8). Matured cisternae are sometimes folded or perhaps fragmented prior to release (Fig. 9). In a very few flagellated cells we observed production of wall scales without cell division (Fig. 14). In these aberrant cells the scales are not released but accumulate in vacuoles, wherein they coalesce in the same manner as scales released to the cell surface (*cf.*, Figs. 15 and 10), eventually forming small spheres of inverted wall within the cell (Fig. 14). Membrane pits are visible both on the plasma membrane in the flagellar groove (Fig. 7, arrowhead) and on the limiting membrane of scale containing vacuoles in aberrant cells (arrows in Fig. 15).

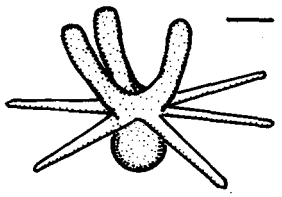


Fig. 6. Drawing illustrating the presumed structure of the outer wall scale with a proximal "head", five lateral "arms", and three distal "feet" (scale bar = 5 nm)

3.5. Wall Assembly

The outer membrane surface of the cleavage zone and the newly formed daughter cell membrane bear a loose agglomeration of scales (Figs. 2, 9, and 10). The scales amalgamate initially at the posterior end of each daughter cell (Figs. 2 and 10); early thecal formation stages typically being observed at opposite ends within the parent cell. The scales on the surface of the daughter cells show a similar arrangement to that observed within the GA cisternae (*cf.*, Figs. 3 and 10). The underlayer is closely affiliated with the cell membrane, and the outer layer of scales associates on a one-to-one basis with those of the underlayer (Figs. 10 and 11). Wall assembly occurs in two distinguishable steps. Firstly, the amorphous inner scales become more

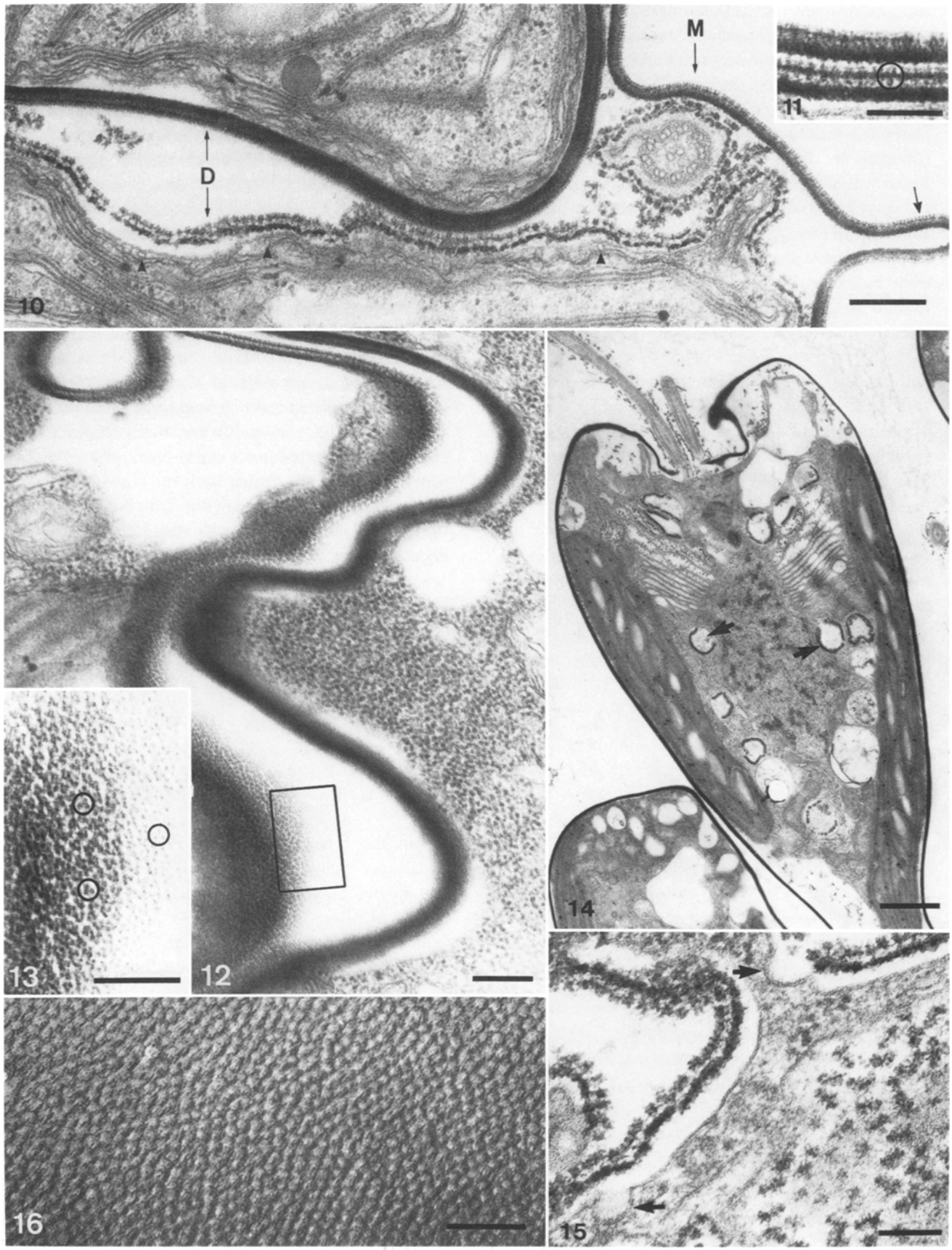
Fig. 4. Oblique/*en face* view of GA cisternae showing the outer layer scales arranged in a planar grid within the cisternae. A maximum of eight appendages decorate the scales. Nascent scales are visible in the cisternae toward the *cis*-face (left side of micrograph) (scale bar = 100 nm)

Fig. 5. High magnification of an outer layer scale showing eight appendages (scale bar = 30 nm)

Fig. 7. Longitudinal section of the flagellar groove region showing a flagellar stump (*F*), striated flagellar root (*R*), microtubular flagellar root (*MR*), scale reticulum (arrows), a pit in the plasma membrane (arrowhead), and GA cisternae (scale bar = 200 nm)

Fig. 8. Cross-section through the flagellar groove region showing three basal bodies with associated microtubular roots (arrowheads). Presumptive exocytosis of wall scales into the flagellar groove is visible. Several pentagonal flagellar scales (arrows) are also present on the flagellar groove membrane (scale bar = 200 nm)

Fig. 9. Cleavage zone (asterisk) with wall scales on the external surface of the membrane. Several folded GA cisternae are visible in the vicinity of the forming cleavage zone (scale bar = 200 nm)



Figs. 10–16

closely associated until, at center to center spacing of ca. 21 nm, they are no longer resolvable as separate entities (Figs. 10–13). At this stage, the overlying outer scales are contiguous by the interlocking "arms", but still distinguishable and have the appearance of a row of cut-out paper dolls (Figs. 10 and 11). The second phase involves the compression of the two layers from a thickness of ca. 55 nm in the early stages of aggregation, down to ca. 44 nm in the mature wall (Fig. 10). This compression results in (from?) the "heads" of the outer scales becoming embedded into the subtending inner layer (Fig. 11). The "feet" of the outer scales remain distinct in thin-section material, and are spaced ca. 9 nm apart in either dimension in mature walls (Fig. 10). However, when mature walls are examined in deep-etch preparations for EM, the "feet" are indistinct; only individual scales (diameter = 20 nm) representing the outer scales can be seen in off-set rows (Fig. 16). It is possible that the distinctive appearance of the "feet" in embedded material is artifactual.

The last portion of the wall to form is the anterior flagellar groove (Fig. 2) finishing with the specialized zone forming the wall slit, but we have not observed details. In embedded material the parent wall was often convoluted (Fig. 10), but when examined by light microscopy the wall is always smooth (not illustrated).

4. Discussion

4.1. Wall Scale Morphology

Wall assembly in *Scherffelia* clearly involves distinctive scales with defined morphology that aggregate in a precise manner, and appears homologous to wall formation in species of *Tetraselmis* (*cf.*, MANTON and PARKE 1965, DOMOZYCH *et al.* 1981, RICKETTS and DAVEY 1980, DOMOZYCH 1984). The scales that comprise the outer portion of the cell wall in *Scherffelia* bear a striking morphological resemblance to those that form the outer layer on cells of *Nephroselmis pyriformis* (*cf.*, Figs. 23–25 in MOESTRUP 1983), and not to the square shaped underlayer scales of prasinophytes as suggested by DOMOZYCH *et al.* (1981). Affinities of the scales comprising the inner portion of the cell wall of *Scherffelia* remain uncertain.

4.2. Cell Wall Assembly and Structure

The mechanism controlling the aggregation of wall scales into a precisely constructed wall is not known, but it seems likely that it is another example of self-assembly of biological precursors producing a predetermined structure. MANTON and PARKE (1965) suggested that since assembly commences near the pyrenoid, perhaps that organelles releases an assembly moderating enzyme. *Scherffelia* lacks a pyrenoid (MELKONIAN and PREISIG 1986), but assembly still commences at the posterior end where the pyrenoid is positioned in *Tetraselmis*. DOMOZYCH *et al.* (1981) also pointed out the possibility of sequential release of an assembly enzyme or effector molecule, such as an ion or sugar. Since Ca^{2+} will precipitate dissolved walls (MANTON *et al.* 1973, McFADDEN and MELKONIAN, unpublished) it may be an important component in wall assembly. We have also found that removal of Ca^{2+} renders isolated walls of *Scherffelia* and *Tetraselmis* soluble

Fig. 10. Section showing two daughter walls (*D*) within a convoluted parent wall (*M*). The wall of the lower daughter cell is at an early stage of assembly showing the inner and outer scale layers. Secondary cytoskeletal microtubules subtend the plasma membrane (arrowheads) during scale aggregation. The wall on the upper daughter cell is almost complete. One flagellum is also seen in cross-section inside the parent wall. In the parent wall the "feet" of the outer scales are distinct (arrow) (scale bar = 200 nm)

Fig. 11. High magnification of a daughter wall assembling beneath a parent wall. The inner scales have coalesced to form a congruent layer but the outer scales (one scale circled) are still distinguishable. The "heads" are oriented toward the inner scales beneath, and the "feet" will eventually become the outermost portion of the wall (scale bar = 100 nm)

Fig. 12. Section of two daughter walls. The left hand wall is still in the process of assembly and curves from a vertical section at the top of the micrograph to a tangential section lower down (scale bar = 200 nm). An enlargement of the marked area is given in Fig. 13

Fig. 13. Enlargement of tangentially sectioned daughter wall in Fig. 12. Rows of outer scales having a triangular core can be distinguished. On the outer-most area (right hand side) the three "feet" of each scale (3 trios circled) that project outward can be seen (scale bar = 100 nm)

Fig. 14. Longitudinal section of a flagellated (interphase) cell producing wall scales in the GA. The scales are not secreted but accumulated in vacuoles (arrows) within which the scales coalesce as seen in normal wall assembly (*cf.*, Fig. 10) (scale bar = 1 μm)

Figs. 15. High magnification of scale accumulation vacuoles in an aberrant cell similar to that depicted in Fig. 14. The scales are coalescing, and the vacuole membranes exhibit pits (arrows) (scale bar = 100 nm)

Fig. 16. Rotary-shadowed deep-etch of cell wall of *Scherffelia dubia*. Off-set, curving rows of scales that bear fine lateral protrusions are visible (scale bar = 100 nm).

(McFadden and Melkonian, unpublished), and Melkonian *et al.* (1981) and Melkonian (1982a) showed Ca^{2+} to be essential in maintenance of flagellar scale disposition in *Tetraselmis*. However, we do not believe that sequential release of an effector substance (if existent) mediates wall assembly in *Scherffelia*. Rather, the self-assembly proceeds automatically, aggregation being most complete in the earliest deposited precursors—those at the posterior end. In this way, edge assembly of arriving precursors proceeds anteriorly from the posterior; eventually closing in the anterior flagellar groove. The observation of vacuoles containing assembled wall scales (retained in aberrant cells producing wall scales during interphase) corroborates this hypothesis. The observation of pits, reminiscent of clathrin coated pits, in the plasma membrane and limiting membrane of the scale containing vacuoles may indicate that reduction of the membrane area by endocytosis occurs concomitantly with scale coalescence (see also below).

Several interesting questions concerning the cell wall in *Scherffelia* (and *Tetraselmis*) remain unanswered: 1. what induces the separation of the parent wall from the protoplast and the subsequent loss of the original wall conformation; 2. what causes the parent wall to rupture, releasing the daughter cells; 3. what is the mechanism of interphase expansion of the wall; and 4. why has the wall an inherent tensibility making it roll up when abandoned? In answer to the latter it seems that the tensibility would act in maintaining the conformation of the flagellar groove and also in the closure of the wall slit upon flagellar autotomy. If the assembling wall is bent against the inherent curling force created by the wall substructure (a possible role for the subtending cytoskeletal elements that appear during wall formation), this might also explain the loss of the original conformation prior to division, and the convolutions induced by embedding procedures. Cytoskeletal elements probably define the protoplast shape while wall scales self-assemble independently on the surface. Domozych *et al.* (1981) illustrated that the microtubular flagellar roots probably mould the formation of the anterior invagination during wall deposition, later retracting to attach to the cell wall via the rhizankyrae (*sensu* Salisbury *et al.* 1981). We believe that the rhizankyral attachments would thereby hold the free ends of the completed wall against the curling force, maintaining the conformation automatically. This system appears to be a further instance of morphogenetic role for the flagellar roots (*cf.*, Melkonian 1982b, 1984, Melkonian and Robenek 1984).

One final query concerns the force by which the secreted scales are transported from the anterior release site to the wall coalescence zone. Transport would be most easily explained by simple insertion displacement wherein newly exocytosed scales force preceding units away from the point of release (anterior end) toward the wall coalescence zone (initially the posterior end). Scales would then be immobilized as a two-dimensional aggregation in the plane of the membrane.

4.3. Scale Production and Membrane Flow Rates

Since the assembly of the scale units into a wall is clearly visible in *Scherffelia*, we are able to estimate the number of scales comprising an entire wall. Taking the last discernible center-to-center distance between the scales as 21 nm, it is reckoned that at least 2,300 scales compose each μm^2 of mature wall. The total surface area of wall around each daughter cell is ca. 260 μm^2 meaning that ca. 600,000 scales are in each layer. Since scales within GA cisternae have a constant center-to-center spacing (29 nm), the number of scales per cisterna can be estimated. Each μm^2 surface area of GA cisternal membrane bears ca. 1,200 amorphous inner scales, plus an equivalent number of outer scales. The total area of a GA cisterna equals 5 μm^2 ($\pi r^2 \times 2$ sides $= 3.14 \times 0.9 \times 0.9 \times 2\,\mu m^2$), giving a total of ca. 12,000 scales per GA cisterna. Secretion of enough scales for a new wall must therefore involve the production of of ca. 100 GA cisternae. Since wall secretion takes ca. 3 hours, some 6,700 scales are produced per minute. This scale production rate indicates maturation of one cisterna from each dictyosome every three to four minutes. Total turnover of the stack of 16 cisternae would occur every hour. These rates are comparable to those calculated for various granulocrine secretory processes (for review see Robinson and Kristen 1982).

Interestingly, the amount of membrane involved in the secretion of the wall is far more than is necessary for the formation of the plasma membrane. The plasma membrane surface area of the parent protoplast is ca. 400 μm^2 and the total for the two daughter cells is ca. 520 μm^2. In secreting enough scales for two walls, ca. 1,000 μm^2 (5 μm^2/cisterna $\times$ 100 cisternae $\times$ 2) of GA cisternal membrane is released. We must assume that the original plasma membrane from the parent protoplast is either incorporated into the daughter cells with new wall scales being attached somehow, or that the 400 μm^2 are resorbed. Assuming the latter, there is still some 480 μm^2 excess membrane secreted to the cell's surfaces during wall deposition. Although we have no

direct evidence, these calculations strongly suggest that endocytosis plays a major role during cell wall coalescence in *Scherffelia dubia*.

The system of cell wall secretion in *Scherffelia* involves the exocytosis of entire GA cisternae without any extensive internal sorting of the membrane prior to release, as seen during flagellar regeneration (McFADDEN and MELKONIAN 1986). The secretory mode observed during wall formation is more commensurate with the coincidental requirement of both bulk product deposition and plasma membrane expansion. Thus, the actual cisternal production during wall secretion is much slower in the absence of internal membrane cycling, but the scale production rate is greatly enhanced.

Scale biogenesis in *Scherffelia dubia* involves two modes of membrane flow (see also McFADDEN and MELKONIAN 1986) each adapted to the sorting, packaging, and deposition requirements of the two scale systems produced (flagellar scales or wall scales). Both modes of secretion involve cisternal progression to transport the secretory product from the *cis*- to the *trans*-face of the GA.

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