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The Unusual, Lepidosome-coated Resting Cyst of *Meseres corlissi* (Ciliophora: Oligotrichea): Encystment and Genesis and Release of the Lepidosomes

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Summary. *Meseres corlissi* Petz and Foissner (1992) is an oligotrichine ciliate covering the resting cyst with epicortical scales called lepidosomes. We studied in detail encystment as well as the genesis and release of the lepidosomes, using live observation, morphometry, and transmission electron microscopy. Encystment is remarkable in changing body shape distinctly and showing two phases of intense rotation. When encysting, the conical body becomes globular and forms a discoidal "head" *via* a fibrous ligament. Then, the cell rotates rapidly about its main axis for a minute. We speculate that this rotation transports the lepidosomes and cyst wall precursors to the cell's cortex. When rotation stops, the lepidosomes are released within about 5 - 20 s. Then occurs a second, slow rotation phase lasting several minutes and possibly distributing the material released by the cyst wall precursors. The lepidosomes develop in Golgi vesicles. Maturation thus occurs in a membrane-bound vesicle and is a complex process with seven distinct stages. The central cavity of the lepidosome develops asymmetrically, and the large meshes of the lepidosome wall develop earlier than the small ones. Growth of the lepidosomes does not occur by vesicular transport processes. The lepidosomes are released by classical exocytosis. The lepidosome (vesicle) membrane closes the port and becomes part of the newly forming cortex. Detailed data on encystment and lepidosome genesis are rare. However, it turned out that both, encystment and lepidosome genesis are more complex in *M. corlissi* than in most other ciliates.

Key words: cystic ciliates, exocytosis, oligotrichine ciliates, *Strombidium oculatum*, transmission electron microscopy, vegetative ciliates, volume relationship.

INTRODUCTION

Lepidosomes are epicortical, organic structures of definite shape produced intracellularly by trophic and/or cystic ciliates (Foissner *et al*. 2005). They occur in a variety of ciliates and often have nice shapes and structures. Lepidosomes were recognized only recently as a specific structure of ciliates (Foissner *et al*. 2005), likely because they have been described under a bewildering variety of names, e.g., Schleim, curieux éléments, gelatinous covering, foam, external scale layer, epicortical scales, "scales", chalice-like structures, and yellow or brownish globules. In *Meseres corlissi*, lepidosomes occur only in the cystic stage, where they are part of the pericyst (Foissner 2005, Foissner *et al*. 2005).

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Except of *Colpoda cucullus* (Kawakami and Yagiu 1963a, b), the genesis and release of the lepidosomes remained unknown. This contrasts other protist groups, especially algae, where external scale genesis and release have been studied in great detail (e.g. Hibberd 1980, Romanovicz 1981, Pienaar 1994). Thus, the lepidosome-coated resting cyst of *Meseres corlissi* provided an excellent opportunity to study scale genesis and release in a ciliate.

Our investigations showed that lepidosome and cyst wall genesis are complex processes connected with the production of four types of highly organized cyst wall precursors which produce, inter alia, the slime adhering the lepidosomes to the resting cyst. These data and cyst wall genesis will be described in a forthcoming paper (Foissner and Pichler 2006). Here, we concentrate on lepidosome genesis and release as well as on encystment which shows several peculiarities likely related to the lepidosomes and slime precursors.

We studied encystment previously (Foissner *et al.* 2005), but missed several important processes because the light of the microscope and the microaquaria disturbed encystment whose conditions can be only partially reproduced. Now, we used another method which provided epidemic encystment, and we could study the process in great detail in the light and electron microscope.

MATERIALS, METHODS AND TERMINOLOGY

Material and cultivation. The population studied was isolated from a meadow soil of Upper Austria, i.e., in the surroundings of the town of Kefermarkt, using the non-flooded Petri dish method (Foissner *et al.* 2002).

Cultures of *M. corlissi* were established with about 20 cells on Eau de Volvic (French table water) enriched with some squashed wheat grains and *Cryptomonas lucens* (UK Culture Collection of Algae and Protozoa, CCAP, Windermere).

Induction of encystment. The various encystment stages were obtained by transferring about 2000 specimens from an exponentially growing culture (about 10% dividers) into a Petri dish 5 cm across, together with 7 ml culture medium and the food contained therein. This isolated part of the culture was then controlled hourly with a dissecting microscope. Encysted specimens were recognized already after 5 h, but epidemic encystment occurred after 20 h when food was visibly reduced.

Encysting and non-encysting specimens were isolated with a fine pipette and transferred into micro-aquaria made of vaseline and a coverslip on a microscope slide. Microscopic observation was performed under dimmed light because ordinary light intensity greatly disturbs the encystment process; full light was used only for the micrographs.

Morphological and cytological methods. The methods described in Foissner (2005) and Foissner *et al.* (2005) were used.

Morphometry. We provide detailed morphometric data for most of the structures described. This is uncommon in cyst research and was criticized by both reviewers. The first reviewer suggested that it "would make much more sense to give round figures", and the second one even believes that the "tabulated values should be deleted". Thus, some comment is necessary.

In our opinion, a basic, descriptive statistics greatly improves the value and interpretation of the observations because of showing the variability of the data and the number of specimens and/or observations they are based. As concerns the present and a forthcoming study, morphometry was indispensable for discriminating between the about 35 developmental stages of five types of cyst wall precursors. Certainly, the measurements are influenced by, e.g., shrinkage or inflation during fixation and embedding, the magnetic hysteresis of the lenses of the electron microscope, and the uncertainty to know whether the section passes through the widest part of the structure (when globular, this can be checked by the membranes whose tripartite structure can be seen clearly only if the section is in or near to the mid). Accordingly, the data are less exact than they appear in Table 2. However, this applies to most measurements, and the more they are distorted by external factors the more statistics is required (Sachs 1984)! In any case, data with statistics are much more reliable than the frequently used "about". Today, international journals would not accept ciliate descriptions without appropriate statistics, though the data are influenced by the preparation procedures, the calibration error of the microscope, and other shortcomings. However, we know of these problems and thus can estimate their influence and, if appropriate, can test for differences with comparative statistics. Thus, more exact data should become the rule in cystology, too.

Terminology. General ciliate terminology follows Corliss (1979), cytological terminology is according to Alberts *et al.* (1994), and cyst terminology is according to Gutiérrez *et al.* (2003), Foissner (2005), and Foissner *et al.* (2005).

RESULTS

Trophic cell

The specimens from Upper Austria are highly similar to those from the Salzburg type locality (Petz and Foissner 1992) and the Dominican Republic (Foissner *et al.* 2005). Usually, cells are obconical or pot-shaped and have a size of about 70×50 µm *in vivo* (Figs 2, 4). The somatic cortex is covered by an average of eight rows of about 15 µm long bristles. The anterior body end is occupied by about 16 collar and 15 ventral adoral membranelles. The whole cell, including somatic cilia and adoral membranelles, is covered by the so-called perilemma and a thin layer of mucus (Foissner *et al.* 2005). The perilemma is typical for some groups of spirotrich ciliates and consists of one to several tripartite

membranes lying upon the cell membrane of the ciliate (Foissner 2005; Fig. 57).

Encystment

Protargol impregnation and transmission electron microscopy showed that about one third of the specimens of exponentially growing cultures have lepidosomes and cyst wall precursors in various stages of development; 10% even contain few or many fully differentiated lepidosomes. Thus, encysting cells are found throughout the life cycle. We consider this as a specific survival strategy, details of which will be reported in a separate paper.

The above mentioned specimens look like ordinary cells, even if they contain many mature lepidosomes, while "truly" encysting specimens show a series of morphological and physiological changes described in the following paragraphs (Figs 1, 4-20). Although encystment is a continuous process, six distinct stages can be distinguished, each associated with certain morphological and/or physiological changes.

Stage (a): The first morphological changes recognizable are a considerable reduction of the cell volume and a slight elevation of the peristomial bottom, likely caused by some constriction of the peristominal collar (Figs 1a, 4, 5, 16, Table 1). The basic cell shape is maintained, and many developing and some mature lepidosomes are recognizable in the light- and electron microscope. The somatic cilia become shorter gradually and swimming speed decreases.

Stage (b): Next, the cell becomes globular and develops a very distinct constriction anteriorly, i.e., reaches the "head stage" which is significantly shorter than the theront stage, while the volume remains the same (Figs 1b, 6, 7, 17, Table 1). The formation of the head is achieved by an about 200 nm thick ring of fibres (Fig. 18). Many mature or almost mature lepidosomes are now scattered throughout the cell and length reduction of the somatic cilia continues (Fig. 13).

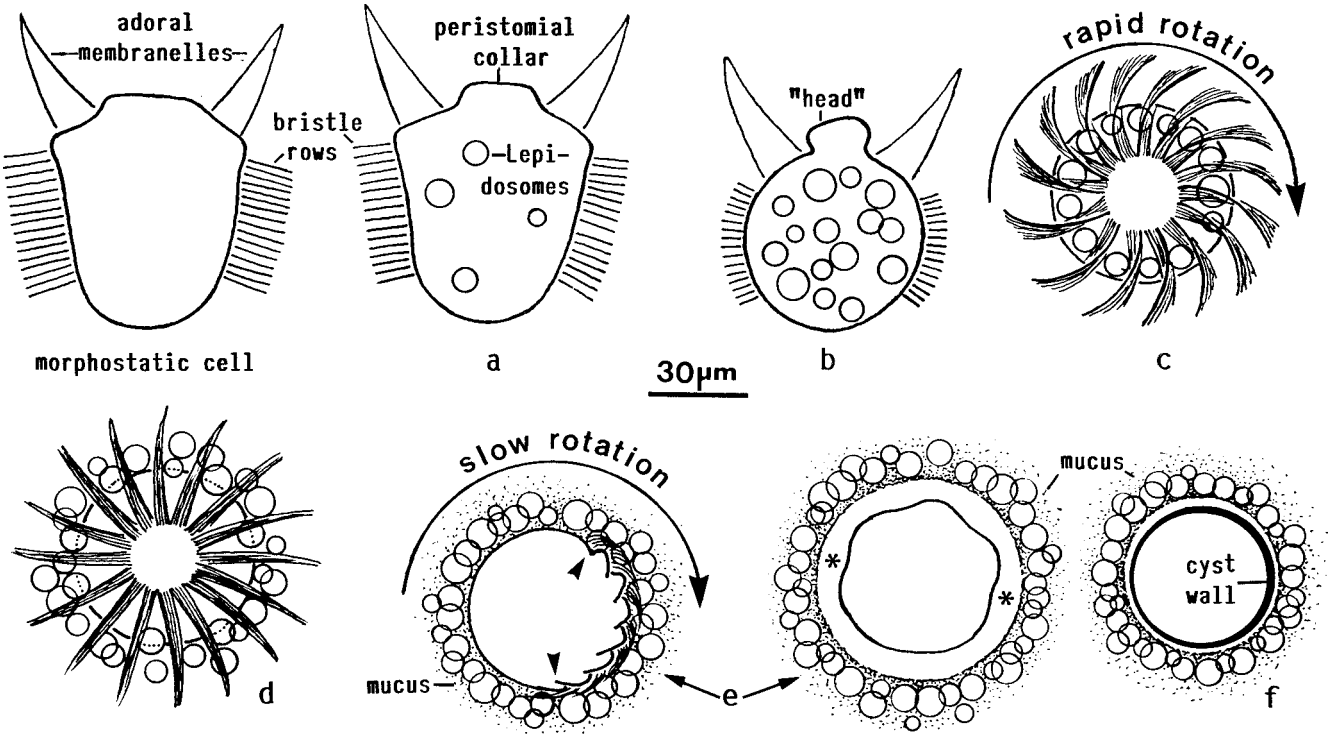
Stage (c): When stage (b) is fully developed, a conspicuous process commences, that is, the cell rotates rapidly about the main body axis for a minute or so (Figs 1c, 8). Obviously, this rotation is caused by the adoral membranelles, which are almost unshortened (Figs 9, 17). During rotation, the lepidosomes and cyst wall precursors accumulate in the periphery of the cell (Figs 8, 9, 17). When specimens are disturbed, they perform a few short, rapid jumps and then continue to rotate.

Stage (d): Then, the cell splays the adoral membranelles and the rotation stops abruptly (Fig. 9). The lepidosomes are now tightly underneath the cortex and are released immediately. The release of the lepidosomes occurs within 5 - 20 s, rarely within 60 s, as observed in 10 specimens (Figs 1d, 10, 19). Lepidosome release is spectacular, that is, they appear to glide through the

Table 1. Size and volume changes of *Meseres corlissi* during encystment. Data based on fixed, Epon-embedded specimens as used for transmission electron microscopy, except of trophont measurements which are from life. Measurements in µm. CV - coefficient of variation in %, M - median, Max - maximum, Min - minimum, n - number of specimens investigated, SD - standard deviation, SE - standard error of mean, $\overline{x}$ - arithmetic mean.

Characteristics	$\overline{x}$	M	SD	SE	CV	Min	Max	n
Trophonts								
Length	72.7	76	15.2	3.5	21.0	60	88	19
Width	59.4	64	7.9	1.8	13.3	48	42	19
Volume (truncated cone)[a]	118 488 µm³							19
Theronts ready to encyst								
Length	62.9	64	6.1	1.4	9.7	52	76	19
Width	48.4	48	2.8	0.6	5.8	44	55	19
Volume (truncated cone)[a]	66 367 µm³							19
Encysting cell in "head" stage								
Length (with head)	55.9	56	5.3	1.2	9.4	44	64	19
Width	48.0	48	3.2	0.7	6.7	40	52	19
Volume (ellipsoid)[a]	67 523 µm³							19
Early resting cysts								
Length	41.4	40	3.6	0.7	8.7	36	48	19
Width	38.8	40	4.4	1.0	11.4	28	44	19
Volume (sphere)[a]	33 493 µm³							19

[a] Geometric figure applied for volume calculation



Figs 1a-f. *Meseres corlissi*, encystment according to live observations and micrographs (Figs 4-15). **a** - early stage of encystment, where the peristomial collar narrows and some almost mature lepidosomes occur; **b** - "head" stage, where the cell becomes globular and has many mature lepidosomes; **c** - frontal view during the rapid rotation phase which lasts for about 1 min and possibly slings the lepidosomes to the periphery of the cell; **d** - then, the cell stops rotation and all lepidosomes are released within about 20 s; **e** - next, the cell rotates slowly for about 5 min, reducing the adoral membranelles and secreting mucous material. Two important processes occur during the slow rotation phase: cell size is reduced causing the wrinkled shape, and cyst wall material is secreted eventually appearing as an up to 10 µm wide, bright zone (asterisks); **f** - young resting cyst with lepidosome layer 0 - 3 µm distant from cyst wall.

cortex, whereby the cell becomes slightly but distinctly inflated, just like it would take a deep breath. For cytological details, see electron microscopy below.

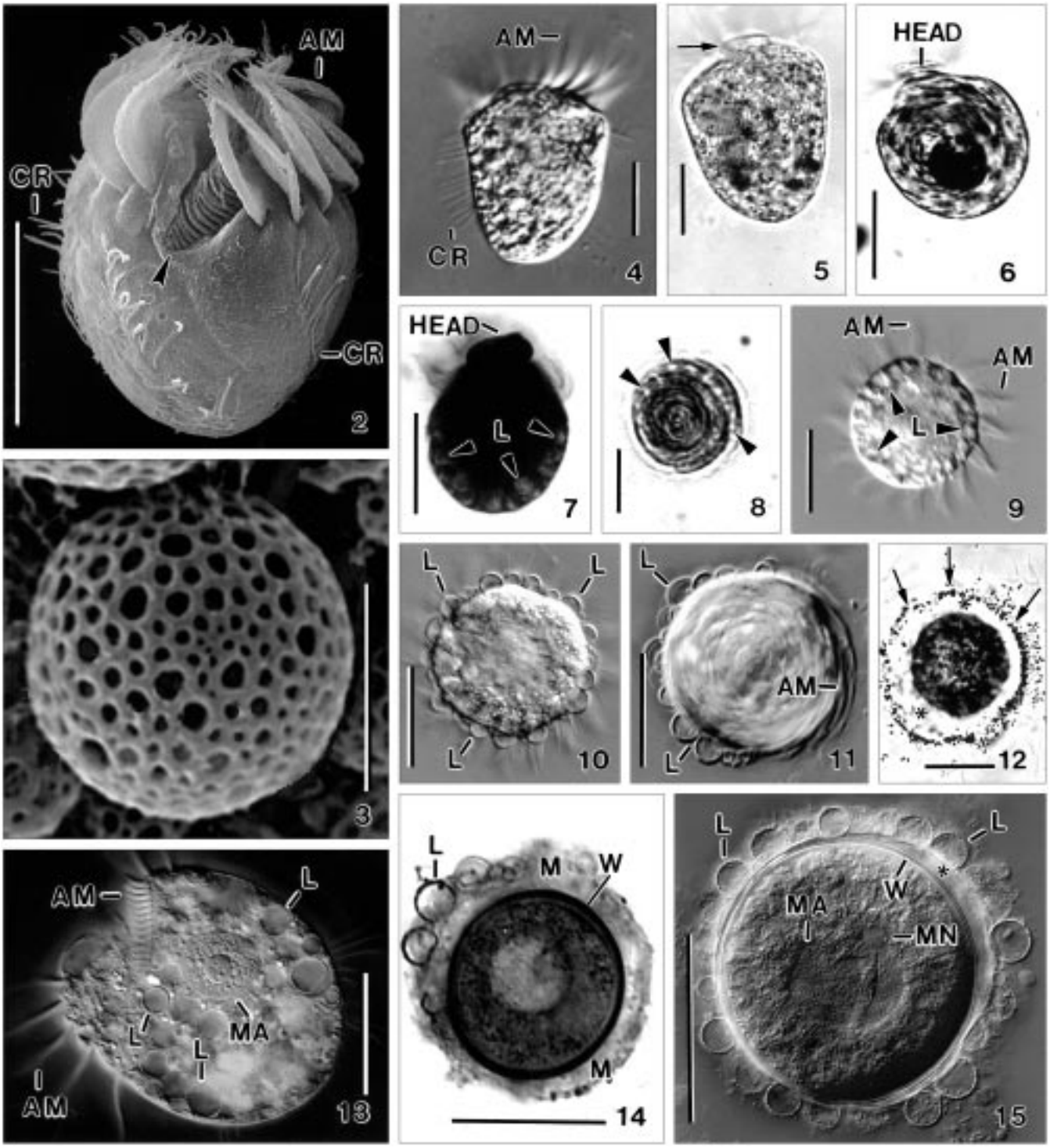
Stage (e): Within the next two minutes, the shortening adoral membranelles attach to the cell which then commences to rotate slowly for about five minutes; likely, the rotation is caused by the shortened adoral membranelles, though no distinct movements of their cilia are recognizable (Figs 1e, 11). During rotation, the cell gets a slightly irregular outline and becomes smaller, that is, reaches a diameter of about 40 µm, likely due to the high activity of the contractile vacuole which expels water every 10 s. Further, the rotation is associated with the secretion of an up to 10 µm thick slime layer which pushes away the lepidosomes. The slime is very hyaline and recognizable mainly due to the adhering bacteria; it does not stain with uranyl acetate and lead citrate, and thus a wide, clear zone becomes recognizable between cell and lepidosome coat in the light- and electron microscope (Figs 12, 20).

Stage (f): When rotation ceases, the cyst wall *s. str.* is assambled and separated from the mucous coat by 1-3 µm (Figs 1f, 15). When such cysts are treated with alcian blue, the newly produced cyst wall and the mucous coat stain heavily (Fig. 14), showing the presence of acid mucopolysaccharides (Foissner *et al*. 2005). In mature cysts, the wall does not stain with alcian blue (see Foissner *et al*. 2005 for more detailed cytochemical data).

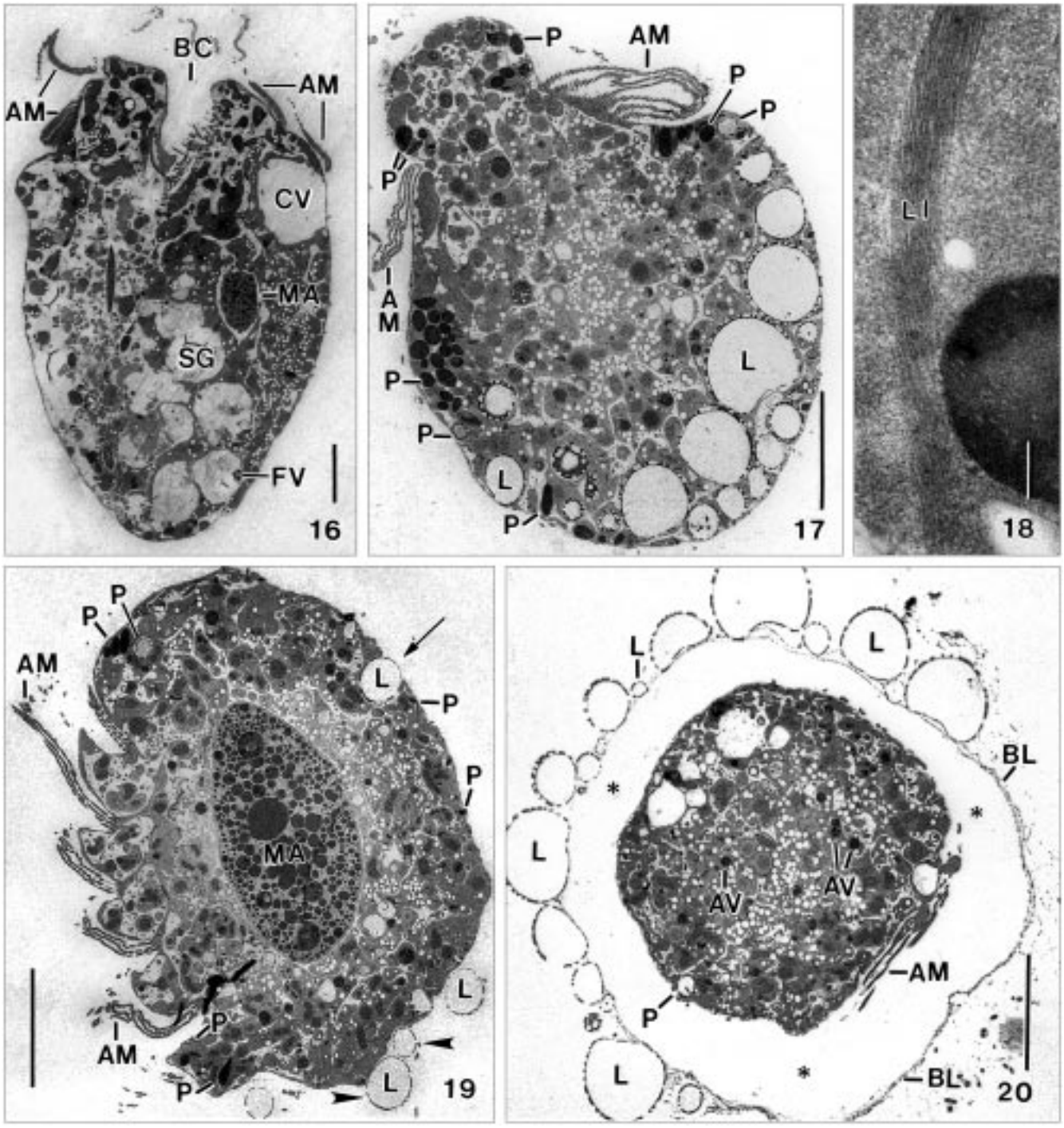
Mature resting cyst

The mature resting cyst of *M. corlissi* has been investigated by Foissner (2005) and Foissner *et al*. (2005). Thus, we provide only a very brief description needed for understanding the present paper.

Mature cysts of *M. corlissi* are globular and about 45 µm across (Fig. 15). They belong to the kinetosome-



Figs 2-15. *Meseres corlissi*, vegetative and encysting specimens in the scanning electron microscope (2, 3), *in vivo* in the bright field (5, 6, 8, 12) and interference contrast (4, 9-11, 13, 15) microscope, after fixation as used for transmission electron microscopy (7), and stained with alcian blue (14). **2, 4** - ventral views showing general organization and buccal vertex (arrowhead); **3** - a lepidosome; **5** - early encystment stage with narrowed peristomial collar (arrow); **6, 7, 13** - "head" stage with many lepidosomes (L). **8** - rapid rotation stage where the lepidosomes (arrowheads) accumulate in the cell's periphery; **9, 10** - after rapid rotation (8), the cell stops (9) and releases the lepidosomes (10) within about 20s; **11, 12, 14** - then the cell begins to rotate slowly, reduces the adoral membranelles (11), and releases cyst wall material which forms a thick, bright coat (12, asterisks) deeply staining with alcian blue (14). Arrows in figure (12) mark coat margin quickly colonized by bacteria; **15** - young cyst with narrow space (asterisk) between cyst wall and lepidosome coat. AM - adoral membranelles, CR - ciliary rows, L - lepidosomes, M - mucous layer, MA - macronucleus, MN - micronucleus, W - cyst wall. Scale bars: 5 µm (3); 30 µm (2, 4-15).



Figs 16-20. *Meseres corlissi*, overviews of a vegetative cell (16) and of encysting specimens (17-20) in the transmission electron microscope. **16** - (corresponds to specimens like those shown in Figures 2, 4) - longitudinal section showing the overall organization of a vegetative specimen; **17, 18** - (correspond to specimens like those shown in Figures 6, 7) - a "head stage" specimen at the end of the first rotation phase, as recognizable by the peripheral location of the lepidosomes (L) and cyst wall precursors (P). The specimen rounds up and constricts the oral area by a fibrous ligament (18), producing a highly characteristic knob, the "head"; **19** - (corresponds to specimens like that shown in Figure 10) - a globular specimen just extruding the lepidosomes (arrow); those marked with arrowheads have just left the cell and are shown at higher magnification in Figure 53. The adoral membranelles (AM) are still intact, and many cyst wall precursors (P) are recognizable; **20** - (corresponds to specimens like that shown in Figure 12) - the cell now commences to rotate slowly, reduces the adoral membranelles, and releases the cyst wall precursors which push away the lepidosomes, producing a thick, slimy, stainless zone around the cell (asterisks). AM - adoral membranelles, AV - autophagous vacuoles, BC - buccal cavity, BL - basal layer of lepidosome coat, CV - contractile vacuole, FV - food vacuole, L - lepidosomes, LI - ligament, MA - macronucleus, P - three types of cyst wall precursors described in a forthcoming paper, SG - starch grain. Scale bars: 200 nm (18); 10 µm (16, 17, 19, 20).

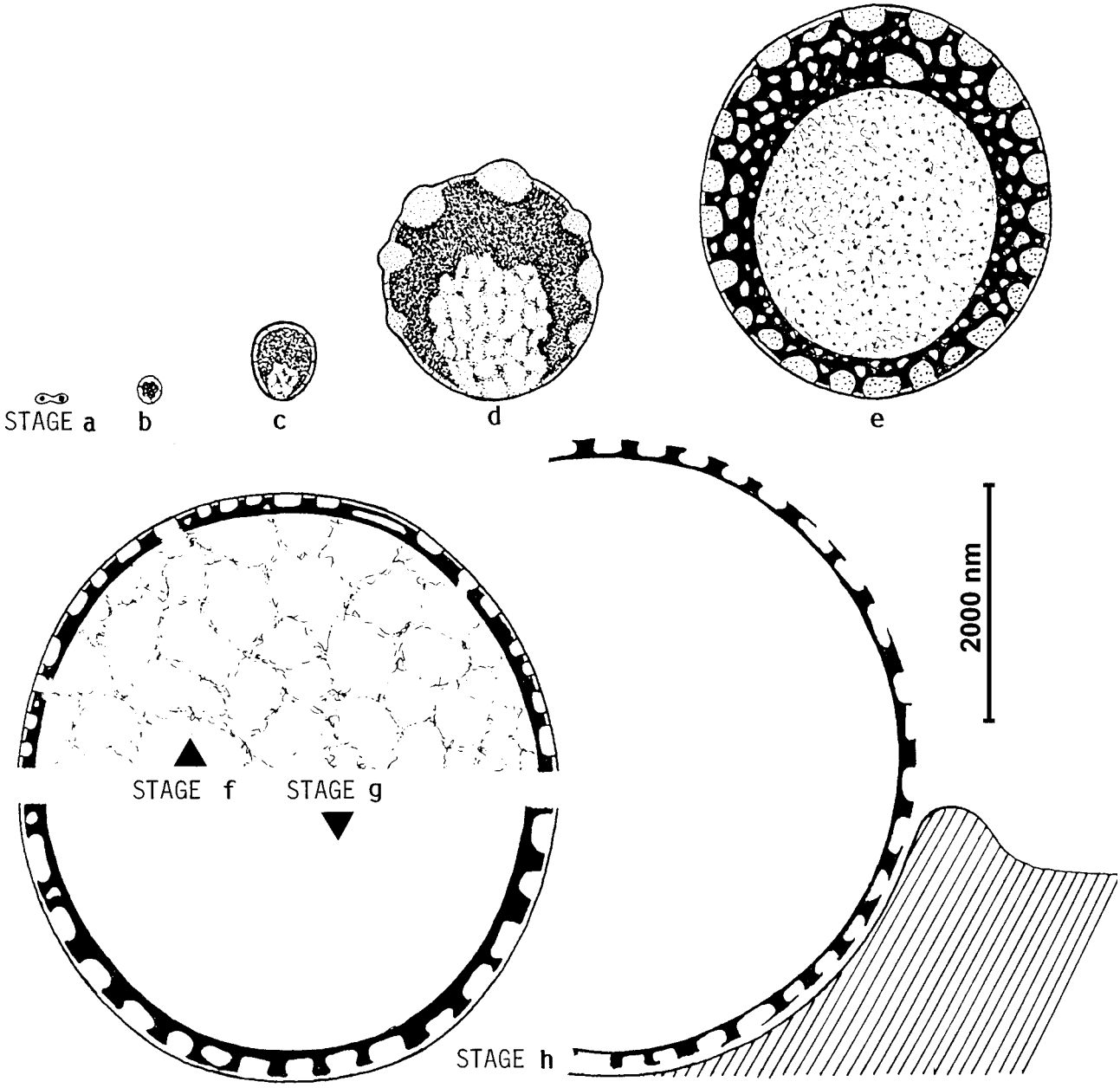
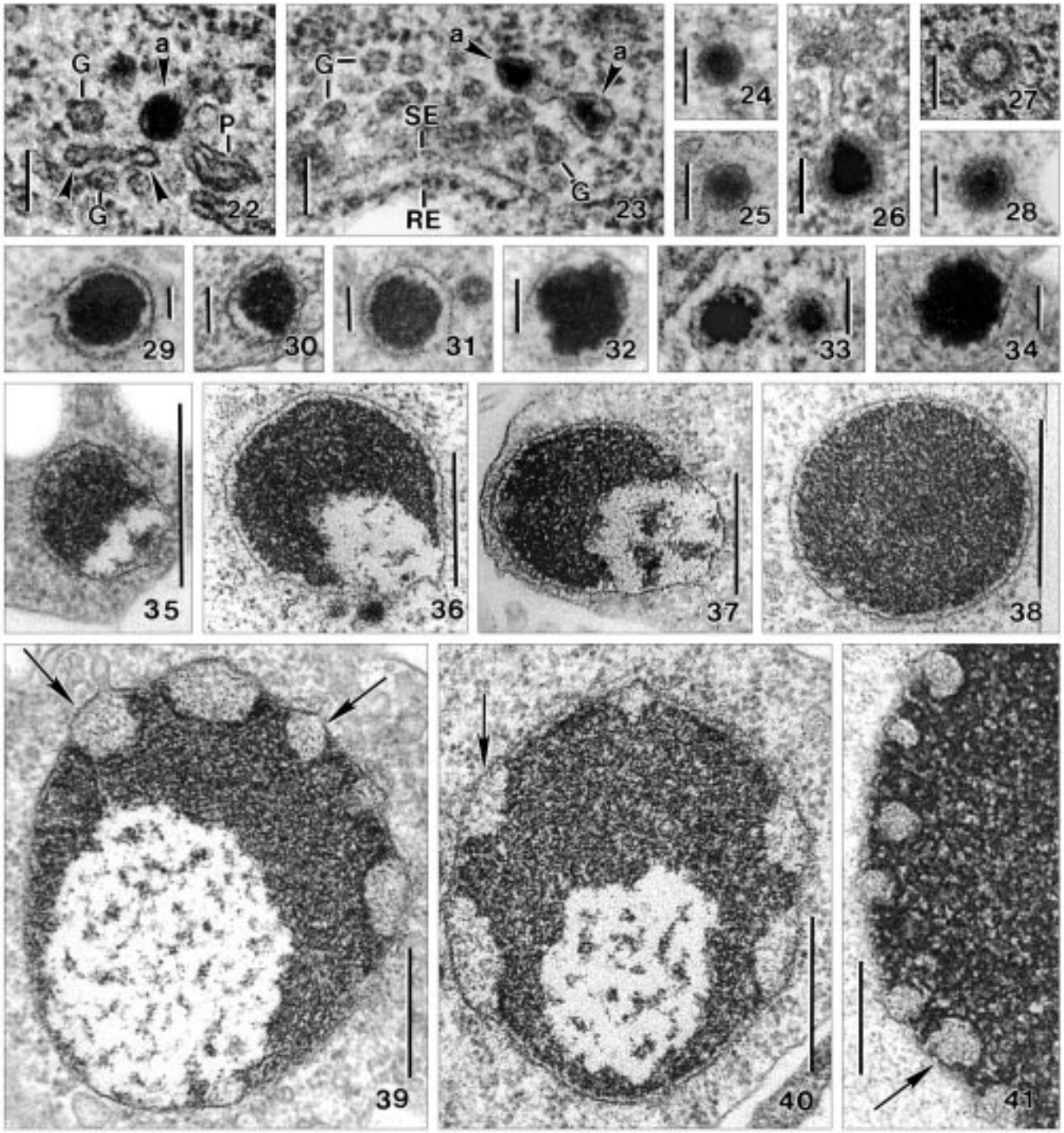


Fig. 21. *Meseres corlissi*, schematic figures drawn to scale of lepidosome genesis (a-g) and release (h). See text and Figures 22-58 for more detailed explanation.

resorbing type and have a conspicuous coat of extracellular organic scales, termed lepidosomes, embedded in a thick layer of mucus mainly composed of acid mucopolysaccharides. The lepidosomes, which likely consist of glycoproteins, are finely faceted, hollow spheres with a diameter of 2 - 14 µm (Fig. 3). The cyst wall is about 1.5 µm thick, smooth, and consists of five complex layers.

Genesis of the lepidosomes

Although lepidosome genesis is a continuous process, we have distinguished seven stages roughly correlating with the six encystment stages described above (Fig. 21). However, the sometimes high coefficients of variation indicate that several distinct stages have been



Figs 22-41. *Meseres corlissi*, transmission electron micrographs of stages (a-d) of lepidosome genesis. **22-25** - stage (a) shows that the lepidosomes are generated pairwise in dumbbell-shaped vesicles (arrowheads) of the medial Golgi cisternae. The vesicles, which have an average size of 85 × 78 nm, become filled with strongly osmiophilic material [arrowheads with (a)]. Figure 23 shows that the Golgi apparatus and vesicle production of *M. corlissi* correspond to textbook knowledge: transition vesicles arriving from the smooth side of the endoplasmic reticulum (SE) fuse with membranes of the cis-Golgi network, while lepidosome precursors bud off from the trans-Golgi network; **26-28** - coated transport vesicles; **29-34** - stage (b) precursors have a size of about 200 nm and show a cogged dense core when mature; **35-38** - stage (c) is very distinctive because a bright area develops at the margin of the vesicle. The bright area is not recognizable, if the section goes trough the electron-dense portion, which is composed of finely reticular material; **39-41** - stage (d) is characterized by the occurrence of mesh precursors (arrows) in the periphery of the dense core. The lepidosome precursors have now light-microscopical dimension, i.e., a size of about 2 µm. G - Golgi vesicles, P - cyst wall precursor, RE - rough endoplasmic reticulum, SE - smooth endoplasmic reticulum. Scale bars: 100 nm (22-34); 400 nm (35-41).

lumped or the process runs very fast (Table 2). The general fine structure of the cytoplasm of *M. corlissi* matches that of other ciliates and textbook knowledge, except of the endoplasmic reticulum and the Golgi apparatus which are comparatively distinct, likely because lepidosomes and cyst wall precursors are produced throughout the cell cycle (see above).

Stage (a): Lepidosome genesis commences with the appearance of dense core vesicles in the dilated rims of the medial and trans-Golgi cisternae. Thus, the lepidosomes originate in pairs, producing highly characteristic, dumbbell-shaped vesicles with an average size of 251 × 74 nm (Figs 21a, 23, Table 2). When the vesicles have pinched off, they become globular and have an average size of 85 × 78 nm (Figs 22, 24, 25, Table 2). Both the dense core and the surrounding membrane are more or less wrinkled. Usually, there is a minute space, often traversed by fibrogranular bridges, between membrane and core. The dense core is composed of strongly osmiophilic, very fine-grained material.

Two other vesicle types are also rather frequent and have the same size as the lepidosome precursors. They are coated by an about 30 nm thick, finely granular material, and one is filled with heavily osmiophilic material (Figs 26, 28), while the other appears bright (Fig. 27). Likely, these are transport vesicles.

Stage (b): The vesicles are globular to broadly ellipsoidal and doubled the average size to 206 × 182 nm (Table 2). Usually, the vesicle membrane and the dense core are less wrinkled than in stage 1, but the core surface often shows a cogged pattern (Figs 32-34). The dense core is very narrowly reticular and slightly smaller than the vesicle, leaving a narrow, bright space bridged by fine strands of fibrogranular material (Figs 21b, 29-31).

Stage (c): The lepidosome precursors are now broadly ellipsoidal and have a size of 615 × 525 nm on average (Table 2). Stage (c) is characterized by the appearance of a bright area at the margin of the dense core, which is connected to this area by many fine strands of fibrogranular material. The bright area, which has an average size of 465 × 312 nm, contains a loose reticulum of fibrogranular core material and, frequently, some patches of condensed core material (Figs 35, 37). Rarely, two or more bright areas occur, and sometimes the area distinctly projects from the vesicle proper, producing a rather irregular outline of the vesicle. The dense core has a finely spotted appearance, indicating a reticular arrangement of the material. Still, the core is separated from the surrounding vesicle membrane by an about 15 nm wide space bridged by many fine strands of fibrogranular material (Figs 21c, 35-38, Table 2).

Stage (d): The next stage distinguishable has already light microscopical dimension, that is, has an average size of 2020 × 1827 nm (Table 2). Frequently, the precursors are broadly ovate with the bright portion being narrower and more or less protruding. The fine structure of the bright area and the dense core are as described in stage (c), but the plane of the former decreased from 45% to 26%. However, the main feature of this stage is the appearance of mesh precursors opposite to the bright area (Figs 21d, 39-41). The mesh precursors are pot-shaped blisters or concavities in the periphery of the dense core and contain fluffy, weakly stained material hardly distinguishable from the background; they are not bounded by a membrane, except distally, where the precursor membrane covers the blisters. The blisters have an average size of 228 × 174 nm and are comparatively rare, indicating that they will form the large meshes of the lepidosomes.

Stage (e): By further growth, the lepidosome precursors reached an average size of 3350 × 2962 nm, that is, are broadly ellipsoidal and have a tubercular outline due to the more or less distinctly protruding mesh blisters (Figs 42-44, Table 2). Morphologically, two main changes occur and can be followed *via* several transition stages (Figs 21e, 42-44). First, the dense core material, which has a finely granular structure (Fig. 46), becomes distinctly reticulate (Fig. 45), showing that it forms the lepidosome wall. Second, the bright area, which contains very fine-meshed, fibrogranular material, commences to move centripetally and grows distinctly, now occupying an average of 49% of the lepidosome plane (Figs 42-44). Obviously, the bright area forms the lepidosome cavity.

Stage (f): The lepidosome precursors have almost mature dimension, that is, show an average size of 4571 × 3831 nm (Table 2). Most precursors are globular or broadly ellipsoidal and very near to the ciliate´s cortex (Figs 47, 49); rarely, they are impressed on one side and thus hemispherical or slightly wrinkled. Morphologically, the precursors are already highly similar to mature lepidosomes, that is, they consist of a thin, darkly stained wall and a large, bright cavity (Figs 21f, 47). However, details are still in work. The precursor wall material is still fibrogranular (Fig. 46), while the wall becomes distinctly reticular. However, the basal layer, which separates the wall from the central cavity, is still thin and partially lacking (Fig. 48); thus, cytoplasmic intrusions

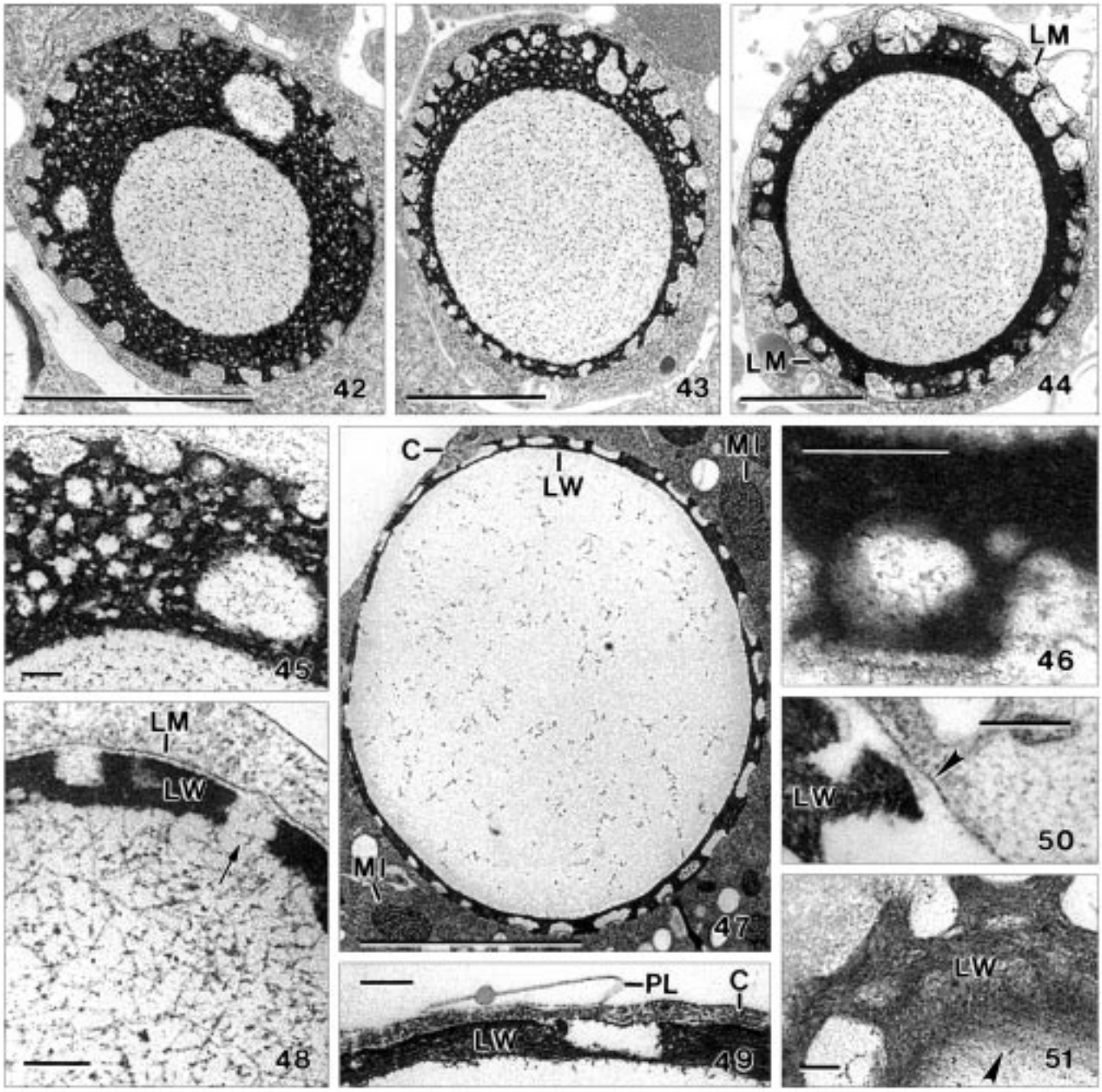
Table 2. Morphometric data on lepidosome precursors. All measurements in nm and from transmission electron micrographs. CV - coefficient of variation in %, I - number of cysts investigated, M - median, Max - maximum, Min - minimum, n - number of precursors measured, SD - standard deviation, $\overline{\overline{X}}$ - arithmetic mean.

Characteristics[1]	$\overline{\overline{X}}$	M	SD	CV	Min	Max	n	I
Stage (a), length[a]	251	225	72	28.8	183	413	11	2
width	74	75	16	22.2	47	100	11	2
Stage (a), length[b]	85	90	20	23.5	35	109	20	3
width	78	83	21	26.5	30	100	20	3
Stage (b), length	206	200	27	13.0	167	250	14	3
width	182	174	29	16.1	150	227	14	3
Stage (c), length	615	636	114	18.5	413	767	11	4
width	525	527	108	20.6	387	767	11	4
bright area, length	465	400	163	35.0	300	767	9	2
width	312	283	132	42.4	120	600	9	2
Stage (d), length	2020	1875	90	44.4	884	3947	26	6
width	1827	1675	84	46.2	714	3684	26	6
bright area, length	1081	1050	327	30.3	507	1682	13	5
width	894	975	262	29.3	453	1318	13	5
mesh precursors, length	228	250	69	30.4	120	320	13	5
width	174	150	68	39.3	107	319	13	5
Stage (e), length	3350	3107	1536	45.9	1679	6250	11	5
width	2962	2643	1356	45.8	1607	5833	11	5
bright area, length	2368	1884	1228	51.8	950	4750	10	5
width	2043	1590	1081	52.9	825	4250	10	5
Stage (f), length	4571	3737	2336	51.1	2250	9300	14	7
width	3831	3079	1911	49.9	1875	7895	14	7
wall, thickness	169	158	70	39.2	90	281	13	7
Stage (g), length	5842	5600	2227	38.1	3500	9700	12	7
width	4904	4500	2289	46.7	2500	9300	12	7
wall, thickness	250	211	103	41.1	158	500	10	7
Stage (h), length	5578	4500	2959	53.0	2400	10300	13	2
width	4754	4000	2622	55.2	1800	9000	13	2
wall, thickness	155	135	62	40.0	68	267	18	2
Just extruded lepidosomes, length	4490	4600	1625	36.2	2300	7600	12	2
width	3952	4035	1389	35.1	1895	6667	12	2

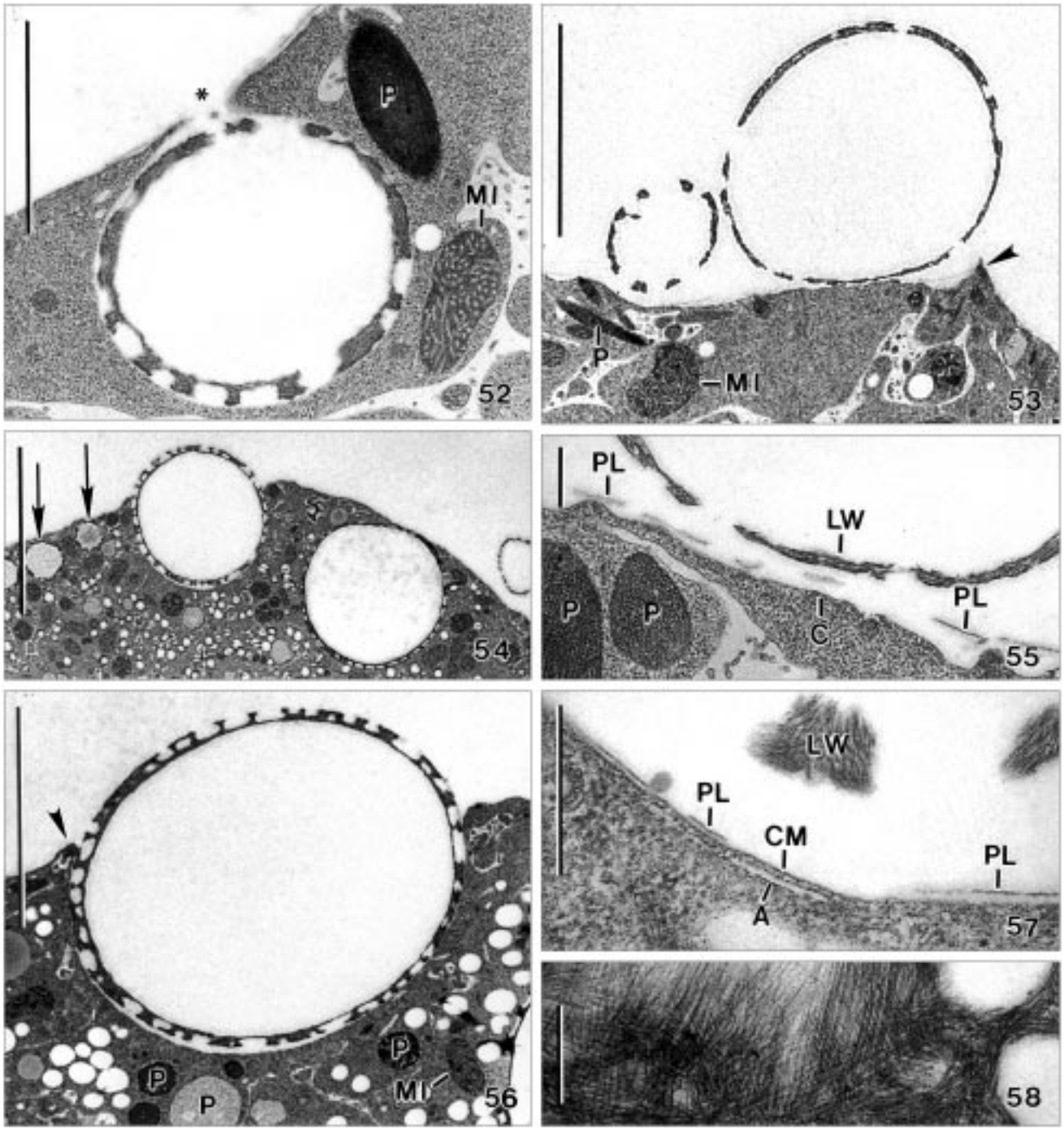
[a]dumbbell-shaped vesicles, [b]single vesicles.

are sometimes found. The large, bright central cavity of the precursor contains a loose, sometimes very distinct (Fig. 48) fibrogranular reticulum (Fig. 47), which completes the precursor wall in the next stage.

Stage (g): By further growth, the precursors reached their mature size, that is, 5842 × 4904 nm on average (Table 2). Most precursors are globular or slightly ellipsoidal and very near to the cortex of the cell; rarely, they are impressed on one side and thus hemispherical or are near to the cell centre. Morphologically, two features change: (i) the wall material organizes to sheets, possibly via a microfibrillar transition stage, which are still finer and less distinct (Figs 50, 51) than in extruded lepidosomes (Fig. 58), and (ii) the central cavity becomes clear, likely



Figs 42-51. *Meseres corlissi*, transmission electron micrographs of stages (e-g) of lepidosome genesis. **42-46** - stage (e) precursors have a size of about 3 µm and show the rising lepidosome structure, viz., a thick, strongly osmiophilic wall surrounding a large, bright centre with fibrogranular contents (Figs 42-44). The wall, which evolved from the dense core described in the previous stages, still consists of finely granular material (Figs 45, 46) which, however, now forms a coarse reticulum with meshes increasing in size from proximal to distal (Figs 42-44); **47-49** - stage (f) precursors, which have a diameter of about 4 µm and are thus much larger than the mitochondria (Fig. 47), already resemble mature lepidosomes because the wall is much thinner than the central cavity (Fig. 47). Figure 49 is a few sections away from that shown in Figure 47 and demonstrates that the lepidosome is near to the ciliate's cortex and the wall is getting a microfibrillar structure. The central cavity is filled with fibrogranular material (Fig. 47), which is sometimes very distinct (Fig. 48). The lepidosome wall is still in work, often partially lacking the basal layer (Fig. 48, arrow); **50, 51** - stage (g) precursors are very similar to stage (f) precursors, but the microfibrillar structure of the wall changes to more distinct, sheet-like structures (Fig. 51, arrowhead), as typical for the mature lepidosome (Fig. 58). The arrowhead in Figure 50 marks the membrane surrounding the developing lepidosome. The lepidosome cavity becomes clear because the fibrogranular material (Figs 47, 48) attaches to the wall which thus becomes thicker (Table 2). C - ciliate cortex, LM - lepidosome membrane, LW - lepidosome wall, MI - mitochondria, PL - ciliate perilemma. Scale bars: 4000 nm (47); 2000 nm (42-44); 200 nm (45, 46, 48-51).



Figs 52-58. *Meseres corlissi*, transmission electron micrographs of lepidosome release. **52** - the cortex opens (asterisk) to release a lepidosome; **53** - (for an overview, see Figure 19) - two lepidosomes which just left the cell, as recognizable by the collar (arrowhead) surrounding leaving lepidosomes (cp. Fig. 56); **54, 56** - two sections of a lepidosome leaving the cell. Likely, the cell's turgor transports the lepidosome out of the cell. The lepidosome membrane closes the opening caused by the lepidosome and transforms into a new cell membrane (see also Figures 55, 57). The arrowhead marks the minute collar forming around the leaving lepidosome. The arrows denote some cyst wall precursors; **55, 57** - some fortunate sections show that the lepidosome membrane becomes part of the new ciliate cortex closing the lepidosome opening (see also Fig. 56). This process runs very fast, i.e., the new cortex becomes visible immediately after the lepidosome has left the cell. The cortex consists of a perilemma membrane (PL), the cell membrane (CM), and the membrane-bounded alveoli (A); **58** - the wall of the mature lepidosome consists of interwoven, very thin sheets, which form from the fibrogranular material composing the wall of the developing lepidosome (Figs 46, 49-51). A - cortical alveolus, C - cortex, CM - cell membrane, LW - lepidosome wall, MI - mitochondria, P - cyst wall precursors, PL - perilemma. Scale bars: 2 µm (52); 4 µm (53, 56); 10 µm (54); 400 nm (55, 57, 58).

Table 3. Resting cyst volume as percentage of the vegetative cell volume. All calculations were done or redone by us, using simple geometric figures (cone, sphere etc.) and average size values as given by the authors cited. Pericyst excluded!

Species	Cyst volume %	Literature
Oligotrichs		
Meseres corlissi	28	This paper; see Table 1
Halteria grandinella	38	Foissner (unpubl.)
Pelagostrombidium spp.	58	Müller *et al.* (2002)
Strombidium oculatum	161	Jonsson (1994)
Stichotrichs		
Oxytricha bifaria	20	Ricci *et al*. (1985)
Kahliella simplex	20	Foissner and Foissner (1987)
Engelmanniella mobilis	33	Wirnsberger-Aescht *et al*. (1990)
Parakahliella halophila	26	Foissner *et al.* (2002)
Heterotrichs		
Condylostomides etoschensis	3	Foissner *et al.* (2002)
Blepharisma japonicum	~100	Giese (1973)
Blepharisma americanum	92	Foissner (unpubl.)
Peritrichs		
Vorticella echini	51	Foissner *et al.* (2002)
Opisthonecta henneguyi	24	Rosenberg (1938)
Opisthonecta henneguyi	5	Walker *et al.* (1989)
Colpodids		
Colpoda cucullus	69	Foissner (1993)
Maryna umbrellata	52	Foissner *et al.* (2002)
Kuehneltiella namibiensis	84	Foissner *et al.* (2002)
Platyophrya spumacola	71	Foissner (1993)
Haptorids		
Enchelydium blattereri	66	Foissner *et al.* (2002)
Spathidium turgitorum	~100	Foissner *et al.* (2002)
Arcuospathidium cultriforme	97	Xu and Foissner (2005)

due to the accumulation of the fibrogranular reticulum to the precursor's wall, whose thickness increases from 169 nm in stage (f) to 250 nm in the (g) stage (Figs 21g, 52, Table 2).

Stage (h): The precursor size is similar to that in the previous stage (Table 2), while the thickness of the precursor wall decreases from 250 nm in stage (g) to 155 nm in stage (h) and the wall sheets become distinct and thicker (Fig. 58), as typical for the mature state (Foissner 2005 and discussion below). The membrane surrounding the precursor is very near to the lepidosome wall (Figs 21g, 48, 50). The central cavity is clear and structureless (Figs 52, 56).

Release of the lepidosomes

When the mature lepidosome leaves the encysting cell, usually after the head stage (Fig. 1d), the cortex opens forming a low wall around the port (Figs 21h, 54, 56). Concomitantly, the precursor membrane is incorporated into the newly forming cortex consisting of perilemma, cell membrane and alveoli (Figs 21h, 53, 55, 57). Thus, the cytoplasm is membrane-covered during the whole extrusion process. We studied the release in nine lepidosomes and are thus sure that the lepidosome membrane is incorporated into the newly forming cortex.

DISCUSSION

Encystment

Encystment of *M. corlissi* shows two peculiarities, viz., body shape changes early and distinctly (Figs 4-7) and two phases of intense rotation occur (Figs 8, 11). The mature cyst has also two extraordinary features, viz., a coat of lepidosomes (Figs 3, 15) and a chitinous layer in the cyst wall (Foissner 2005, Foissner *et al*. 2005).

Distinct body changes during encystment occur in a variety of ciliates, for instance, in *Bursaria truncatella*, a colpodid ciliate (Foissner 1993). However, usually the shape becomes simpler, while the encysting *Meseres*

looks like another species (Figs 6, 7). Preliminary observations on *Halteria grandinella*, a close relative of *Meseres corlissi* (Katz *et al*. 2005), showed the same, that is, the globular vegetative cell becomes cylindroidal, and this stage has been described, indeed, as a distinct species (Foissner, unpubl.). *Strombidium oculatum*, the sole other oligotrich where detailed data are available on encystment, simply rounds up, as do many other ciliates (Montagnes *et al*. 2002).

The two rotation phases of the encysting *Meseres* are another extraordinary feature. In the literature, we found only one other ciliate doing the same, viz., *Strombidium oculatum*, suggesting that this phenomenon is characteristic for oligotrichs. Montagnes *et al*. (2002) described it as follows: "Cysts form in minutes on the bottom of tissue plates. A ciliate will swim to a surface, repeatedly bump into the surface, and begin to rotate around its long axis. The ciliate then presses against the surface and splays its adoral membranelles. After ~30 s, the ciliate begins to rotate, forms a ball, and within ~1min it is covered by a cyst wall". Possible functions of these rotations will be discussed below. The peculiarities discussed above, motivated us to compare the cyst volume of *M. corlissi* with that of other ciliates (Table 3). However, *M. corlissi* is not special in this respect, while the cyst volume of *Strombidium oculatum* is considerably larger than that of the vegetative cell, an extraordinary feature not found in other ciliates. The cyst volume of colpodids and haptorids is between 50% and 100% and is thus usually larger than that of oligotrichs and stichotrichs (20%-60%). The wide volume ratios of the heterotrichs are also remarkable. Generally, the highly different ratios shown in Table 3 cry for more detailed investigations and a functional explanation. The data from *S. oculatum* suggest that the ratios could be related to the organism's ecology/biology (Jonsson 1994, Montagnes *et al*. 2002).

Lepidosome genesis

Many organelles of protists are produced in cisterns of the endoplasmic reticulum or in vesicles of the Golgi apparatus, for instance, trichocysts (Hausmann 1978, Peck *et al*. 1993) and the organic scales of various amoebae, flagellates and algae (Hibberd 1980, Romanovicz 1981, Pienaar 1994). In ciliates, the genesis of the cyst wall precursors is insufficiently known. Calvo *et al*. (1986) proposed that the wall precursors of a stichotrich ciliate, *Histriculus similis*, arise from the endoplasmic reticulum and the Golgi apparatus. This has been confirmed by Walker *et al*. (1989), who documented that the cyst wall precursor of a peritrich ciliate, *Telotrochidium henneguyi*, develops pairwise in dilated Golgi cisterns, very similar to what we found in *M. corlissi* (Figs 21a, 23).

As yet, lepidosome genesis and development has been investigated only in *Colpoda cucullus*. Kawakami and Yagiu (1963 a, b) showed that they develop from minute, dense vesicles which grow, *via* a granular stage, to about 1 µm-sized "network structures" composed of honey-combed units. The extrusion of these structures has been not documented, but the micrographs show that the extruded networks are globular and lack a surrounding membrane (see also Chessa *et al*. 2002), just as do the lepidosomes of *Meseres*. Obviously, lepidosome genesis is simpler in *Colpoda* than in *Meseres*. Seen from a more general aspect, the processes of pinching off vesicles from the smooth side of the endoplasmic reticulum and incorporating them into the dictyosome are as described in textbooks (Figs 21a, 23).

A few peculiarities in the genesis of the lepidosomes of *M. corlissi* should be mentioned, although we do not understand them. Why develops the central cavity asymmetrically? Why do the large meshes of the lepidosome wall develop earlier than the small ones? How do the lepidosomes grow? We never saw vesicles entering developing lepidosomes, although we studied hundreds of them. Thus, one may speculate that growth is carrier-mediated (Alberts *et al*. 1994). Accordingly, growth of the lepidosomes is different from that of trichocysts which grow by vesicle fusion (Peck *et al*. 1993).

Figure 21 shows a semi-schematic summary of lepidosome genesis and release in *M. corlissi*.

Lepidosome release

The lepidosomes are released rather early, viz., when the ciliary structures are still functioning (Figs 1d, 9, 10, 19). The release of the lepidosomes is a spectacular event because they are numerous and have an average size of 6 µm. Functionally, it is an exocytotic process par excellence, i.e., as described in textbooks (Alberts *et al*. 1994, Plattner and Hentschel 2002): the membrane surrounding the lepidosome fuses with the cortex membranes and becomes a new cell membrane; concomitantly, a new perilemma and cortical alveoli develop (Figs 52-57). This is different from trichocyst release, where the surrounding membrane remains in the cytoplasm and is recycled (Hausmann 1978). Likely, the huge number and the large size of the lepidosomes require that the lepidosomal membrane replaces the cortical membranes. Otherwise, the plasm would be

exposed to the environment because closing of an opening of up to 15 µm needs some time. Further, the about 200 lepidosomes are released almost concomitantly. It is difficult to imagine that the cell would not burst if the new cell membrane had to be generated *de novo*. Certainly, details of the process need to be studied with refined methods, e.g., marking the old cell membrane with ruthenium red.

The extrusion of the lepidosomes occurs within about 20 s and is followed by the release of four types of cyst wall precursors (Foissner and Pichler, submitted). Considering that there are about 200 lepidosomes with an average size of 6 µm, it can be calculated that two thirds of the cortex must be restored; and if the many wall precursors are added, this percentage increases to near 100%. Thus, production and release of the lepidosomes are extremely energy-demanding, suggesting that they have an important function which, however, is not known (Foissner *et al*. 2005). This situation resembles *Paramecium* which invests 40% of its total protein contents in the trichocysts, now known to have a protective function (Plattner 2002).

We did not find any structures, for instance, microtubules transporting the newly formed lepidosomes and cyst wall precursors to the cell periphery, while Walker *et al*. (1980) observed microtubules associated with the cyst wall precursors of *Gastrostyla steinii*, a stichotrich ciliate. Thus, we hypothesize that the intense first rotation phase, which occurs just before lepidosome release, slings the lepidosomes and wall precursors to the cell's periphery. The curious, breath-like inflation of the cell associated with the lepidosome release is possibly caused by a sudden influx of water ejecting the lepidosomes. The second, slower rotation phase might distribute the precursor material. At first glance, these hypotheses appear reasonable. However, many other ciliates produce the resting cyst without specific rotation phases. Thus, our speculations must be rigorously tested whether they are applicable at the low Reynold's numbers operating in the microscopic world.

Lepidosomes: composed of fibres or of thin sheets?

Foissner (2005) described the lepidosomes to be composed of "about 20 nm thick fibres likely longer than 1 µm". However, when looking at the micrographs, it becomes obvious that the "fibres" must be very thin sheets because roundish or ring-like transverse sections, as typical for fibres and tubules, are not recognizable. This was confirmed by a reinvestigation of Foissner's unpublished materials and the present study. Both show that the lepidosomes consist of very irregularly arranged, thin sheets (Fig. 58) which originate from a fribrogranular mass (Fig. 46). We could not determine the size of the sheets, but likely they are narrow (~20 nm ?), long bands or broad sheets folded like the bellows of a camera.

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