HELGOLÄNDER MEERESUNTERSUCHUNGEN
Helgoländer Meeresunters. 42, 601–612 (1988)

Amphiesmal ultrastructure in *Noctiluca miliaris* Suriray (Dinophyceae)*

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ABSTRACT: The ultrastructure of the cell covering (amphiesma) of vegetative cells of *Noctiluca miliaris* (Dinophyceae) was studied in detail using thin sections. The amphiesma is typically amphidinoid and contains the following components (starting from the outside): (a) a continuous outer membrane (plasmamembrane) surrounding the cell; (b) a layer of contiguous vesicles (amphiesmal vesicles) that contain a thin "honeycomb-patterned" layer of material appressed mainly to the outer portion of the vesicle membrane; (c) a finely granular pellicular layer that lies beneath the amphiesmal vesicles and (d) groups of cortical microtubules (only present in certain regions of the cell). The pellicular layer is always present but its thickness is highly variable (20–800 nm) depending on regional specializations of the amphiesma. Trichocysts and mucocysts project through the pellicular layer and amphiesmal vesicles, the apical portion of their limiting membrane docks at the plasmamembrane. Small vesicles that presumably contain material for the "honeycomb-patterned" layer traverse the pellicular layer through discontinuities and presumably fuse with the amphiesmal vesicles. We conclude that *Noctiluca* has a typical dinophycean (i.e. amphidinoid) cell covering, and that the most recent proposal for the developmental origin of the dinoflagellate pellicle should be revised.

INTRODUCTION

Although *Noctiluca miliaris* had been recognized and first described in the 18th century by Henry Baker (Baker, 1753) and would thus qualify as the first known dinoflagellate, its systematic affiliation to the Dinophyceae remained controversial during the following two hundred years (Haeckel, 1873; Jollos, 1910; Doflein, 1916; Kofoid, 1920). Kofoid (1920) seemed to have settled this controversy by homologizing structures of *Noctiluca* with the sulcus, girdle, longitudinal and transverse flagellum of tentaculate Dinophyceae. The apparent diplontic life history of *Noctiluca* and the absence of a true "dinokaryotic" nucleus from the vegetative cells (Zingmark, 1970a, 1970b) have, however, led some investigators to conclude that "the question of its systematic position remains unsolved" (Pfiester, 1984).

One of the most important structural characteristics of the Dinophyceae is the cell covering or amphiesma. Traditionally it has served as one of the bases for the classification of the Dinophyceae (Dodge, 1984). The ultrastructure of the amphiesma has recently

* Dedicated to Dr. Dr. h. c. P. Kornmann on the occasion of his eightieth birthday.

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been reviewed by Morrill & Loeblich (1983) and Netzel & Dürr (1984). Morrill & Loeblich (1983) identified several structural types of amphiesmae in the Dinophyceae, thus extending earlier studies by Dodge & Crawford (1970). They were, however, unable to assign *Noctiluca* to one of their structural types and concluded that "with the possible exceptions of . . . *Noctiluca scintillans*, with some reinterpretation the amphiesmae of all dinoflagellates discussed fit into the scheme . . ." Although *N. miliaris* (syn. *N. scintillans* Macartney) has been the subject of a number of ultrastructural studies over the last 25 years, none has dealt with the cell surface in detail, and micrographs depicting the cell surface are rare (Afzelius & Halyarson, 1964; Soyer, 1968a, 1968b, 1969, 1970a; Sweeney, 1978; Lucas, 1982). Often fixations and/or magnification of micrographs in these publications appear to be inadequate to resolve the finer details. We therefore decided to study the ultrastructure of the cell surface of *N. miliaris* in detail using an improved fixation procedure. We demonstrate that the cell surface of *Noctiluca* represents a true dinoflagellate (amphidinoid) amphiesma whose most prominent constituent is a pellicular layer underlying the amphiesmal vesicles.

MATERIAL AND METHODS

Noctiluca miliaris Suriray was kindly provided by Dr. G. Uhlig (Biologische Anstalt Helgoland) and cultured in petri dishes in a synthetic seawater medium (ASP-H; McFadden & Melkonian, 1986) at 15 °C in a 14/10 h light/dark cycle. The cells were fed every 3–4 days with *Dunaliella bioculata* (strain 19–4; Sammlung von Algenkulturen, Göttingen; Schlösser, 1982). Cells were fixed for thin section electron microscopy using a simultaneous fixation according to Melkonian (1982). The final concentrations of the fixatives in full-strength culture medium were 1.25 % glutaraldehyde and 0.4 % osmic acid. Fixation was performed for 15 min at 4 °C. Individual cells were then washed twice in ASP-H, dehydrated in ethanol and prepared for electron microscopy as usual (Melkonian, 1975). Sections were cut with a diamond knive and observed in a Siemens Elmiskop 102. A total of 12 different cells were sectioned.

RESULTS

In its vegetative state *Noctiluca miliaris* is a fragile, almost spherical cell which may reach nearly 1 mm in diameter. The cell surface exhibits a number of grooves and other specializations (e.g. Lucas, 1982 for an SEM analysis). Consequently, we have found a number of variations in the ultrastructure of the cell surface within a single cell, but have made no attempt to correlate these with the known surface features. We describe first the most common arrangement of the components of the cell surface of vegetative non-dividing cells and then indicate some of the structural variations that were encountered.

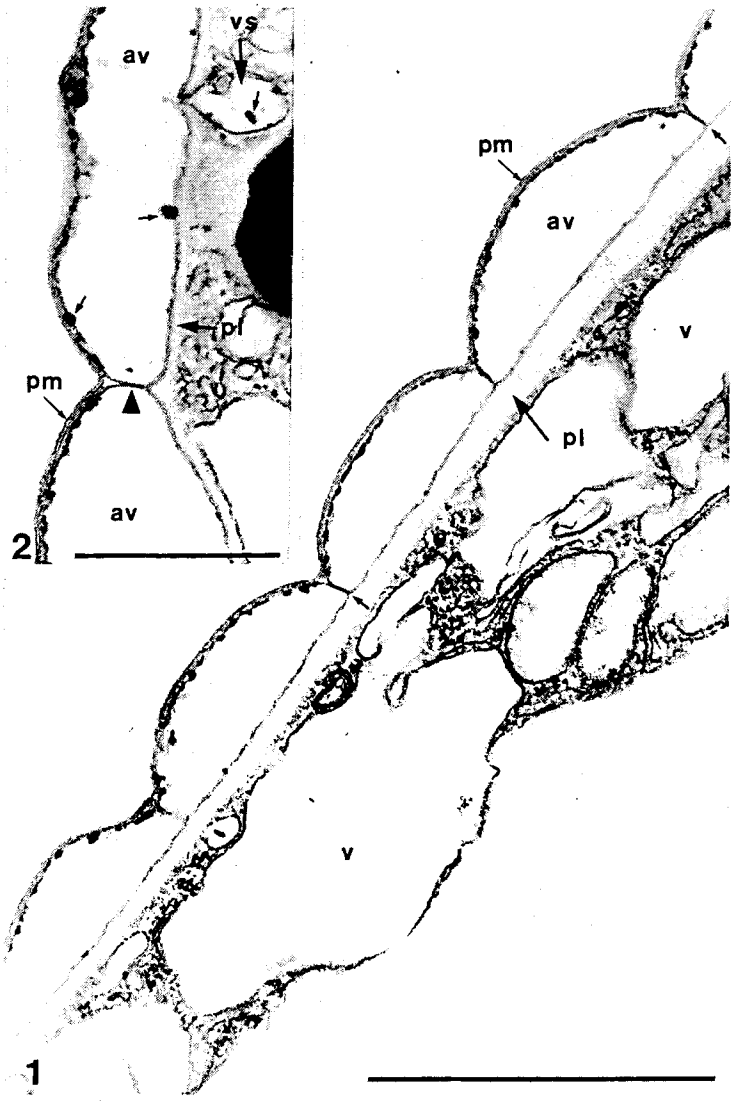
The cell surface typically consists of the following components:

(1) A continuous membrane enclosing the cell is the outermost component and thus represents the plasmamembrane (Figs 1, 2, 9). It takes an undulatory path because it is closely appressed to underlying vesicles (see below).

(2) Beneath the plasmamembrane lies a series of contiguous vesicles that in our fixation have a peculiar shape when cross-sectioned (Figs 1, 2, 9): the vesicles (termed amphiesmal vesicles) are flattened parallel to the cell surface and measure about 1 μm in

diameter. Their outer membrane (i.e. the vesicle membrane attached to the plasmamembrane) always bulges towards the cell surface (convex shape; Figs 1, 9). Most often the curvature of the outer membrane is very regular describing a circular segment, sometimes the curvature is more irregular (Figs 4, 5). The inner membrane of the flattened vesicles has a variable appearance depending on the elaboration of the underlying pellicular layer (see below). If the pellicular layer is very thin (Figs 2, 3), the inner vesicle membrane also bulges towards the cell interior resulting in an ellipsoid shape of the vesicle (Fig. 2). More often, however, the underlying pellicular layer is thicker (Figs 1, 9, 10). In this case, the inner vesicle membrane takes a straight path (Figs 1, 9, 10) suggesting a considerable mechanical strength of the pellicular layer. The contiguous lateral membranes of the vesicles are closely appressed to each other (Figs 1, 2, 9) and it requires favourable sections and high resolution to resolve them as two separate membranes. The best resolution of the lateral vesicle membranes is obtained when the pellicular layer is thin (Fig. 2, triangle); however, even when a thick pellicular layer is present, the individuality of the vesicles is maintained (Fig. 9). The appressed portions of the lateral vesicle membranes are a natural shear point for shedding of the outer membranes (Fig. 14, see below). A consequence of the presence of laterally appressed vesicles is that the space between the plasmamembrane and the outer vesicle membrane is not filled with cytoplasm and the two membranes are closely associated. Although the lumen of the vesicles appears empty in the electron microscope (Figs 1–6, 9–12), close evaluation shows that a small amount of material is invariably present, attached to the inner surface of the vesicle membranes (Figs 1–3, 5, 9, 11, 14). In cross sections through vesicles, this material consists of irregularly distributed electron-dense spots and patches primarily attached to the inner surface of the outer vesicle membrane (e.g. Figs 1, 2). Occasionally some material may also be seen associated with the inner surface of the inner vesicle membrane (Figs 2, 4, 6, 14). If the outer vesicle membrane is tangentially sectioned (Figs 3, 5) it may be seen that the patches and spots are interconnected in a honeycomb-like network. Fine filaments interconnect the patches that occur at the junctions of the polygons of the network (Figs 3, 5). Except for this honeycomb-patterned material attached to the inner surface of the outer vesicle membrane, no other electron dense structures were observed in the lumen of vesicles.

(3) Beneath the layer of vesicles we have consistently found a continuous layer of amorphous, granular material of only moderate electron density (Figs 1–3, 5, 6, 9–14). We call this layer the pellicular layer. The pellicular layer varies highly in thickness depending on regional specializations of the cell surface of vegetative cells. Even when the pellicular layer is extremely thin (20 nm, Figs 2, 6), it is still recognizeable because it excludes cytosolic components like ribosomes or microtubules. In certain areas of the cell, the pellicular layer continuously increases in thickness (e.g. Fig. 1, from left to right) and along ridges or grooves of the cell surface it may reach up to 800 nm in thickness (Figs 9, 10). When such a thick pellicular layer is favourably sectioned it may be seen to consist of sublayers of granular material (Fig. 9). The outer surface of the pellicular layer is always in close contact to the inner amphiesmal vesicle membrane (Figs 1, 9, 10), the inner surface of the pellicular layer borders the cytosol. Usually there is no membrane separating the cytosol from the inner surface of the pellicular layer (Figs 1, 2, 5, 9). Often peripheral large vacuoles lie close to the pellicular layer (Figs 3, 10, 11, 13, 14). In these cases, the outer vacuolar membrane underlies the pellicular layer.



Figs 1–2. *Noctiluca miliaris.* 1 Thin section through the cell surface of *N. miliaris.* Arrows = appressed lateral membranes of two amphiesmal vesicles. Magnification bar = 1 μm. 2 Higher magnification of amphiesmal layers. Subpellicular vesicles (vs) with electron dense patches resembling similar contents of amphiesmal vesicles (small arrows). Arrowhead = two lateral membranes of contiguous amphiesmal vesicles are clearly resolved. Magnification bar = 0.5 μm

List of abbreviations: av = amphiesmal vesicle, m = mitochondrial profile, mb = mucocyst, ol = outer layer, pl = pellicular layer, pm = plasmamembrane, tr = trichocyst, v = peripheral vacuoles, vs = subpellicular vesicle

(4) Cortical microtubules were sometimes observed. They lie immediately beneath the pellicular layer in the peripheral cytosol often in groups of 2–4 microtubules (Fig. 4). They do not occur regularly but seem to be confined to certain regions of the cell more often underlying a thin pellicular layer rather than a thick pellicular layer.

The above description refers to the general ultrastructure of the cell surface of *Noctiluca miliaris*. Before considering some variations that were found and which are presumably related to certain stages of the cell cycle, we would like to describe a few additional vesicular structures that are an integral part of the cell surface.

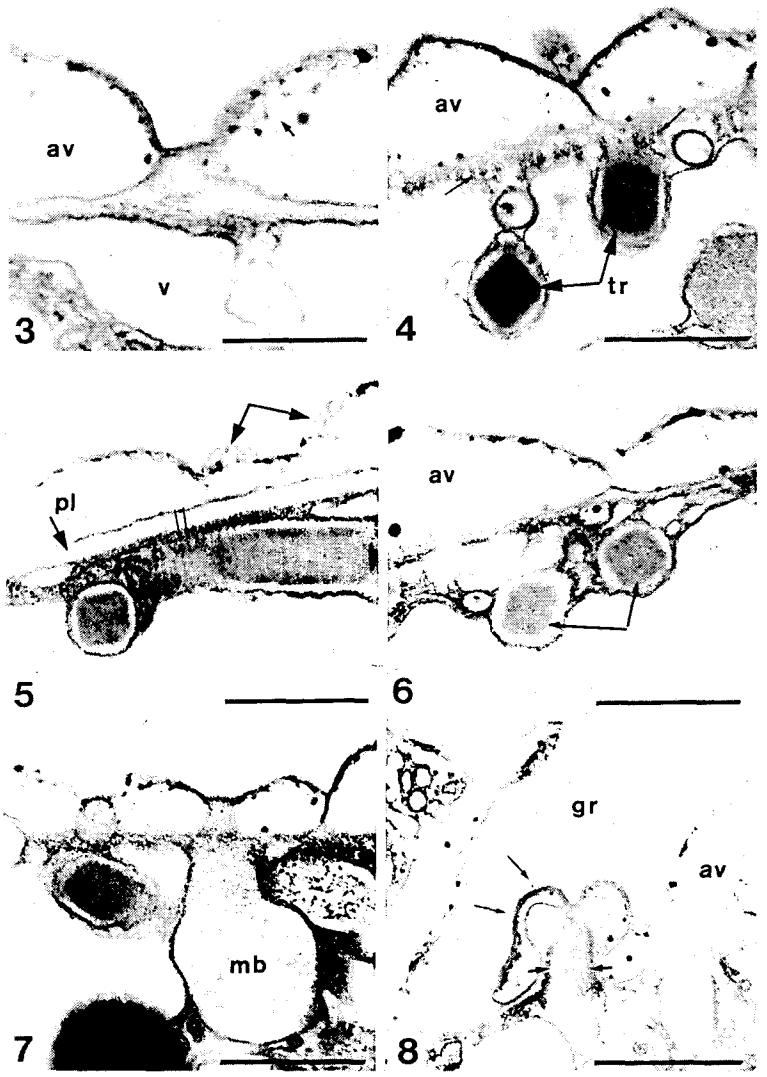
Three types of vesicular structures traverse part of the amphiesmal layers in *N. miliaris*:

(a) A small type of vesicle (about 200 nm in diameter) has often been found located immediately beneath the pellicular layer (Figs 2, 14). These vesicles project through the pellicular layer with a small nose-like extension. Sometimes, continuites between the vesicle membrane and the inner amphiesmal vesicle membrane at the tips of the nose-like projections have been observed (not illustrated). The vesicles contain patches of electron dense material that appear to be identical to the "honeycomb"-patterned material which is present inside the amphiesmal vesicles (Figs 2, 14). This type of vesicle was primarily found in areas where the pellicular layer is relatively thin (20–50 nm). In areas where the pellicular layer is thick, similar but more irregularly shaped vesicles are present beneath the pellicular layer (Fig. 9). The pellicular layer overlying these vesicles is discontinuous and these discontinuities are channel-like (Fig. 10). Inside those channels we regularly observed a number of small spherical vesicles (70 nm diameter) filling the lumen of the channel (Fig. 10). These smaller vesicles also contain electron dense material similar to the "honeycomb"-patterned material of the amphiesmal vesicles (not illustrated).

(b) Whereas the above mentioned vesicles only traverse the pellicular layer, the trichocysts and mucocysts project through the amphiesmal vesicles also. The former are rod-spaped vesicles with a paracrystalline central body that is square in cross-section (Figs 4–6, 13, 14), and reveals a fine longitudinal striation (Fig. 5). Most trichocysts in our material appeared to be in an immature state since the rods were mainly located parallel to the cell surface and not perpendicular to it (Fig. 5). The trichocyst membrane is ruffled and in tangential longitudinal sections thin filaments can be seen to run perpendicular to the rod surrounding it (Fig. 5). The mature trichocyst consists of a shaft and an elongated tip (Fig. 13). The tip contains a group of twisted fibres (Fig. 13) and traverses the pellicular layer and the amphiesmal vesicles. The tip membrane is appressed to the plasmamembrane and during discharge fuses with the latter (Fig. 13). We have found trichocysts mainly in areas where the pellicular layer is thin.

Mucocysts are less frequent than trichocysts. They consist of a sac-like vacuole with fluffy, granular contents (Fig. 7). They also possess a neck region that traverses both the pellicular layer and the amphiesmal vesicles to dock at the plasmamembrane (not shown).

A description follows of some ultrastructural specializations of the cell surface of *N. miliaris* that we found in some cells or in some particular areas of a cell. *N. miliaris* contains several prominent grooves, the short straight section of the major groove near the site of the origin of the single flagellum contains the so-called cytostome, where food particles are presumably ingested (Soyer, 1968b; Lucas, 1982). A section through the



Figs 3–8. *Noctiluca miliaris*. 3 Tangential section through outer membranes of the cell surface revealing "honeycomb"-patterned electron dense patches (small arrow). Magnification bars in Figs 3–8 = 0.5 μm. 4 A slightly oblique section through the cell surface. Groups of subpellicular microtubules can be seen (arrows). 5 Section through the cell surface. One trichocyst is cross-sectioned, another longitudinally sectioned. The latter reveals a ruffled trichocyst membrane and parallel oriented filaments running around the central paracrystalline core of the trichocyst (small arrows). Notice also "honeycomb"-patterned material (prominent arrows). 6 Another section through the cell surface with immature trichocysts in cross section (arrows). 7 A section through the cell surface depicting a mucocyst. 8 Section through the cytostome region at the bottom of the major groove (gr). Short arrows = dome-like fibrillar material attaching to the plasmamembrane, long arrows = fuzzy surface material associated with the exterior of the plasmamembrane in the cytostome region

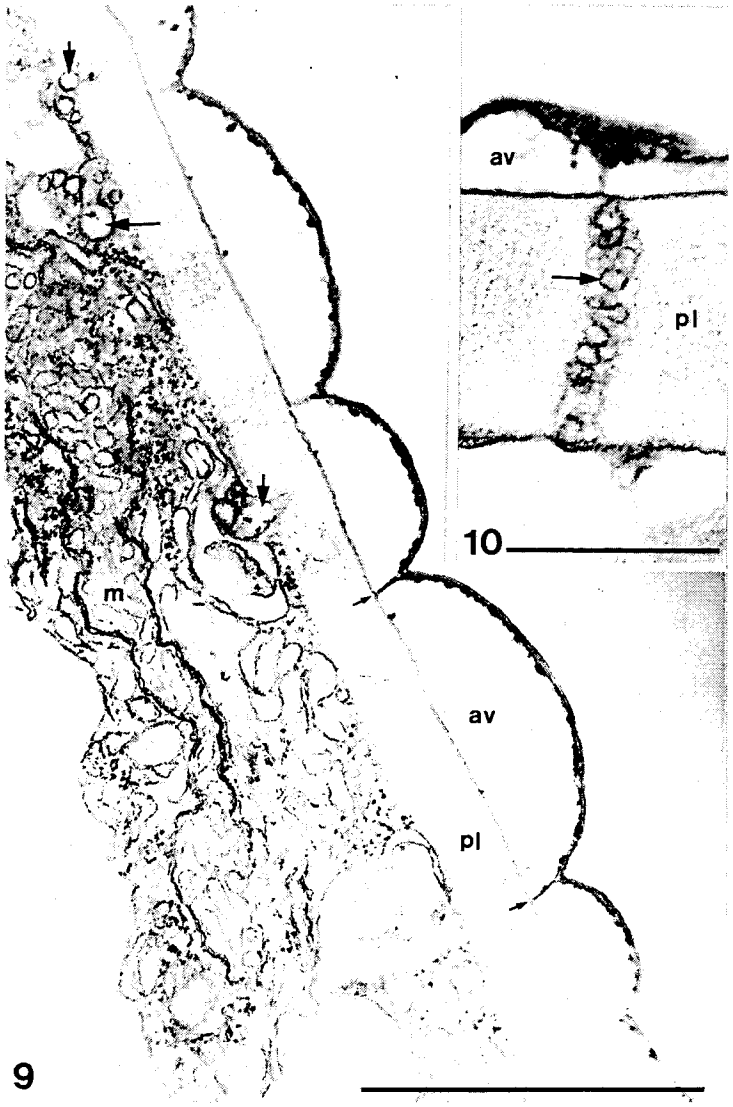
bottom of the groove in this area depicts the cytostome which represents a specialized portion of the cell surface (Fig. 8). The cytostome is about 500 nm in diameter and in principal consists of specialized amphiesmal vesicles (or perhaps a single vesicle with a central hole; no serial sections were taken) that are arranged nearly perpendicular to the cell surface but bent towards the cell interior (Fig. 8). They are not laterally appressed but are separated by a distance of about 80 nm. In this central area, the cell is covered only by the plasmamembrane and an underlying electron dense layer of 10 nm thickness. Attached to this layer is amorphous electron dense material which, towards the cell interior, splits into what appears to be a dome-like structure with a central fibrillar region (Fig. 8, prominent arrows). The electron dense material then curves around the pellicular layer and tapers. The pellicular layer appears to be discontinuous and presumably terminates on both sides of the dome-like structure (Fig. 8). Distal from the termination point of the pellicle the area beneath the specialized amphiesmal vesicle(s) is externally associated with a fuzzy coat (Fig. 8, small arrows).

A prominent specialization of the cell surface of *N. miliaris* that we observed in certain regions of almost all cells sectioned is an additional layer of granular material located outside the plasmamembrane (Figs 11, 12). The layer, that we term outer granular layer, is similar in appearance to the pellicular layer but is more electron dense (Fig. 11). It also has a variable thickness. It originates attached to the outside of the plasmamembrane as a thin layer but increases continuously in thickness to a maximum of about 700 nm before it again decreases in thickness and terminates (Figs 11, 12). Below the outer granular layer lie the normal cell surface layers, i.e. the plasmamembrane, the amphiesmal vesicles with "honeycomb"-patterned material, a usually thin pellicular layer and often large vacuoles (Figs 11, 12).

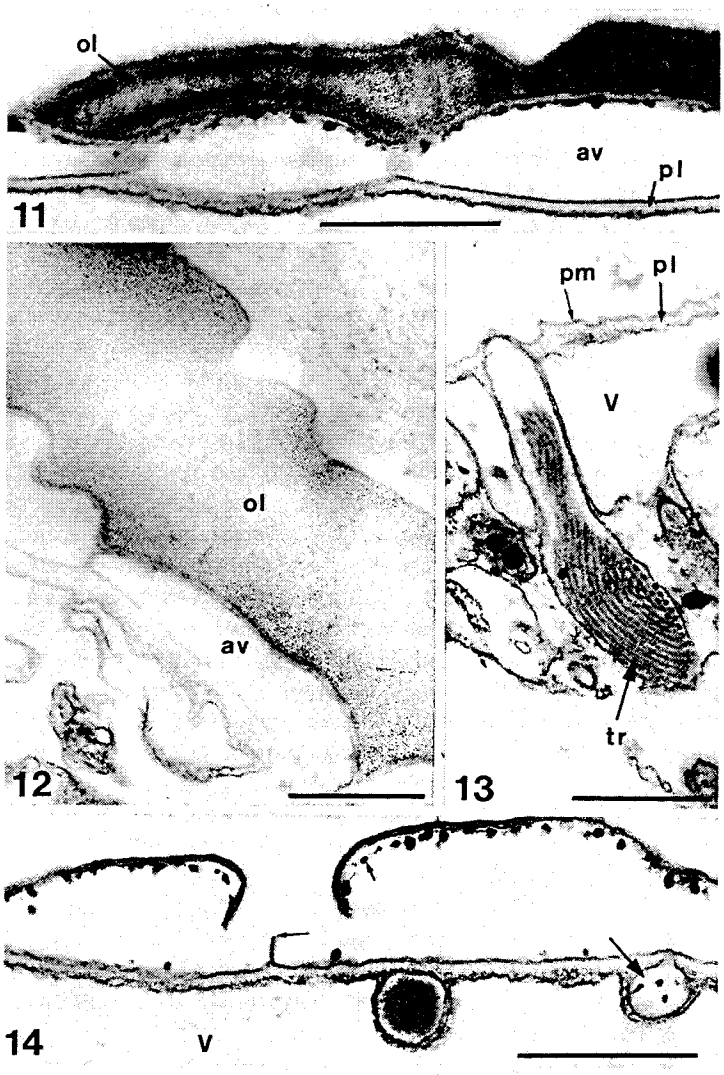
Occasionally we observed two further variations of the general ultrastructural pattern of the cell surface:

(a) An absence of the amphiesmal vesicles (Fig. 13). This occurred only in some regions of two cells that we sectioned, whereas other regions of the same cells appeared normal. The cells in question did not seem to be damaged in a recognizeable way. In the specialized area, the cell surface consists only of the plasmamembrane plus an underlying thin pellicular layer which occasionally is traversed by trichocysts (Fig. 13) and mucocysts (not shown). Often, large empty looking vacuoles are located adjacent to the pellicular layer.

(b) Finally, in some cells, parts of the outer cell surface layers were in the process of dissociation from the rest of the cell (Fig. 14). Again this was seen only in certain areas of a cell, whereas other regions had a normal complement of cell surface layers. "Shedding" apparently occurs by disruption of the lateral amphiesmal vesicle membranes in the appressed regions (Fig. 14). The shed structures are the plasmamembrane, which often disrupts also at the vesicle junctions (Fig. 14), the outer amphiesmal vesicle membrane and its associated "honeycomb-patterned" material. The latter partially dissociates from the amphiesmal vesicle membrane and thus becomes more prominent than in intact amphiesmal vesicles (Fig. 14, small arrow). The portions of the cell surface layers remaining with the cell proper consist of the inner amphiesmal vesicle membrane and the thin pellicular layer (Fig. 14). The inner amphiesmal vesicle membrane becomes a continuous membrane because portions of the lateral vesicle membranes remain closely appressed and apparently seal the individual inner vesicle membranes (Fig. 14). Often,



Figs 9–10. *Noctiluca miliaris*. 9 Section through the cell surface in a region where the pellicular layer is relatively thick. Prominent arrows = subpellicular vesicles partially projecting into the pellicular layer. Small arrows = appressed lateral membranes of amphiesmal vesicles. Magnification bar = 1 μm. 10 Higher magnification of section through a thick pellicular layer. Arrow = channel in the pellicular layer filled with small vesicles. Magnification bar = 0.5 μm



Figs 11–14. *Noctiluca miliaris*. 11 Section through the cell surface revealing a granular electron dense outer layer located outside the plasmamembrane. Magnification bars Figs 11–14 = 0.5 μm. 12 Another section through a very thick outer layer that is also located outside the plasmamembrane. 13 Section through a region of the cell surface that lacks amphiesmal vesicles. The amphiesmal layers consist of only the plasmamembrane and a thin pellicular layer. A mature trichocyst projects through the pellicular layer and is docked with its tip membrane to the plasmamembrane. The trichocyst is apparently in the process of exocytosis. 14 Section through a region of the cell surface where the outer membranes are apparently in the process of shedding. Small short arrow = "honeycomb"-patterned layer of patches partially retracted from the outer amphiesmal vesicle membrane. Small long arrow = point of breakage of the appressed lateral membranes of amphiesmal vesicles. Large arrow = subpellicular vesicle presumably exocytosing "honeycomb"-patterned material into amphiesmal vesicles of their remnants

large vacuoles are associated with the inner surface of the pellicular layer in these regions (Fig. 14).

DISCUSSION

The present study on the ultrastructure of the cell surface of *Notiluca miliaris* Suriray extends earlier studies on this species (Afzelius & Halyarson, 1964; Soyer, 1968a, 1969, 1970a, 1970b; Sweeney, 1978) and demonstrates that the cell surface of *N. miliaris* represents a typical dinoflagellate amphiesma of a type common e.g. in species of *Amphidinium* (Dodge & Crawford, 1970; Wilcox et al., 1982). Soyer (1968a, 1969, 1970a) first distinguished three "layers" of the cell surface of *N. miliaris* which she termed C1, C2 und C3. These "layers" apparently correspond to the plasmamembrane plus outer amphiesmal vesicle membrane (C3), inner amphiesmal vesicle membrane plus pellicular layer (C2), and layer of microtubules (C1). The existence of amphiesmal vesicles was not recognized, possibly because the lateral amphiesmal membranes were not preserved during fixation or sectioning. Later, Sweeney (1978), studying the ultrastructure of a *Noctiluca* with *Pedinomonas* endosymbionts, recognized the presence of amphiesmal vesicles. Because of poor fixation (OsO_4 without osmotic buffering) these vesicles were, however, collapsed and details (presence of a plasmamembrane, a pellicular layer, material inside the amphiesmal vesicles, etc.) could not be resolved. Sweeney (1978) concluded that a "pellicle" underlying the amphiesmal vesicles is present only in the region of the "gullet". Because of these insufficient analyses Morrill & Loeblich (1983) were unable to assign the cell surface of *N. miliaris* to one of their newly erected types of dinoflagellate amphiesmae. They (as well as Sweeney, 1978) noted that in principal the cell surface of *N. miliaris* is similar to a basic arrangement found in *Amphidinium* (a group 2 arrangement according to Dodge & Crawford, 1970), i.e. a continuous outer plasmamembrane and contiguous empty amphiesmal vesicles are present, but that in addition a finely granular layer lies beneath the amphiesmal vesicles. Since Morrill & Loeblich (1983; see also Morrill, 1984) assume that the pellicle of dinoflagellates originates exclusively within amphiesmal vesicles, they reinterpreted the ultrastructure of the cell surface of *N. miliaris* to present a type (their Fig. 6d) in which fused amphiesmal vesicles and fused discontinuous layers form an amphiesma of two outer membranes, a pellicle and an inner "cytoplasmic" membrane. We have never found such a type of amphiesma in *N. miliaris*. In contrast, we found that individual amphiesmal vesicles are always present together with a continuous pellicular layer that always lies beneath the amphiesmal vesicles. Beneath this pellicular layer we have never found a continuous membrane, and it is thus clear that the pellicular layer of *N. miliaris* does not originate within amphiesmal vesicles. Since Morrill & Loeblich (1983) define pellicles as "continuous, nonmembranous layers which form a part or the major component of the amphiesma, . . ." we can faithfully call the pellicular layer of *N. miliaris* a pellicle. To what extent is the presence of a pellicle beneath amphiesmal vesicles a unique feature of *Noctiluca?* The amphiesma of *Kofoidinium* Pavillard, a dinoflagellate closely related to *Noctiluca*, apparently consists also of a layered pellicle beneath amphiesmal vesicles (Cachon & Cachon, 1974). Even in species of *Amphidinium* (Wilcox et al., 1982) and *Gymnodinium* (Wilcox & Wedemayer, 1984) there is a thin layer of material present, separating amphiesmal vesicles from the cytosol. This material corresponds in thickness and structure to the thin portion of the pellicular layer of *N. miliaris*. Our own unpub-

lished observations also demonstrate that in *Gymnodinium splendens* and *Amphidinium rhynchocephalum* a thin pellicle underlies the amphiesmal vesicles. This layer thickens during ecdysis in *A. rhynchocephalum* and represents the major portion of the pellicle of this taxon which is also, therefore, not formed within amphiesmal vesicles (Melkonian & Höhfeld, unpubl.). A reinterpretation of the formation mechanism of the dinoflagellate pellicle is in view of the above observations overdue (Melkonian & Höhfeld, unpubl.). In conclusion, the pellicle of *N. miliaris* both in its structure and location is widespread among the non-thecate Dinophyceae. The only unusual feature of the pellicle of *N. miliaris* is its variable thickness which is presumably related to different cell surface specializations (grooves, elevations, etc.). The similarity between an *Amphidinium*-type amphiesma and that of *N. miliaris* is further emphasized by observations of Wilcox et al. (1982) who showed that in *Amphidinium cryophilum* the lumen of amphiesmal vesicles is not empty but contains a very thin layer of material that in tangential sections exhibits a "honeycomb"-pattern, exactly like the pattern observed in *N. miliaris*. The "honeycomb"-patterned layer in *A. cryophilum* does not seem to be associated with either of the two vesicle membranes, while in *N. miliaris* it is appressed to the outer vesicle membrane, but this aspect may depend on the conditions of fixation (osmolarity, etc.) used. Since the structure and location of trichocysts and mucocysts are also characteristic, there can be no doubt that *Noctiluca* exhibits a typical *Amphidinium*-type amphiesma and, based on this character, is therefore a genuine dinoflagellate.

There are a number of additional new observations which may shed light on some functional and developmental aspects of the amphiesma of dinoflagellates. It appears that vesicles containing material that resembles the "honeycomb"-patterned material fuse with amphiesmal vesicles, which thereby presumably increase in size. The opposite process, endocytosis of vesicles from the amphiesmal vesicles, is less likely. If the pellicle is thick, channels in the pellicle exist, through which small vesicles may be fed into amphiesmal vesicles. How the plasmamembrane grows during the possible enlargement of amphiesmal vesicles also remains unclear, although recruitment of membranes from trichocysts or mucocysts is a possibility.

The extensive outer granular layer that was observed in certain regions of almost all cells of *N. miliaris* has to our knowledge not been described before in *Noctiluca* or any other dinoflagellate. It could represent remnants of the pellicle of the previous cell generation. Since *Noctiluca* simply divides into two progeny cells during cell division and some rearrangements of ridges and grooves must take place during cell division, it is possible that in certain areas of the dividing cell part of the amphiesma is shed in which case the thickened portion of the pellicle presumably remains associated with the progeny cells. A new amphiesma would then be formed beneath these thickened areas of the old pellicle. During the next cell division the outer granular layer is also shed together with the underlying amphiesmal vesicles. This hypothesis should be tested by investigating the ultrastructure of the cell surface of *N. miliaris* during cell division.

Acknowledgements. This study was supported by the Deutsche Forschungsgemeinschaft (Me 658/5-1) and by NSF Grant BSR 85-06413 (to K. R. Roberts and M. Melkonian). Part of this study was performed at the Biologische Anstalt Helgoland (BAH) and I. Höhfeld wants to thank the staff of the BAH for help during various stages of the work. We would also like to thank B. Berns and W. Krüger for excellent technical assistance. M. Melkonian is supported as a Heisenberg fellow by the Deutsche Forschungsgemeinschaft.

LITERATURE CITED

Afzelius, B. A. & Halyarson, M., 1964. The fine structure of the photogenic granules in dinoflagellates. – Proc. Eur. reg. Conf. Electron Microsc. *3* (B), 175–176.

Baker, M., 1753. Employment for the microscope. Dodsley, London, 403 pp.

Cachon, J. & Cachon, M., 1974. Le système stomopharyngien de *Kofoidinium* Pavillard. Comparisons avec celui de divers peridiniens libres et parasites. – Protistologica *10*, 217–222.

Dodge, J. D., 1984. Dinoflagellate taxonomy. In: Dinoflagellates. Ed. by D. L. Spector. Acad. Press, London, 17–42.

Dodge, J. D. & Crawford, R. M., 1970. A survey of thecal fine structure in the Dinophyceae. – Bot. J. Linn. Soc. *63*, 53–67.

Doflein, F., 1916. Lehrbuch der Protozoenkunde. Fischer, Jena, 1190 pp.

Haeckel, E., 1873. Natürliche Schöpfungsgeschichte. Reimer, Berlin, 688 pp.

Jollos, V., 1910. Dinoflagellatenstudien. – Arch. Protistenk. *19*, 178–206.

Kofoid, C. A. 1920. A new morphological interpretation of the structure of *Noctiluca*, and its bearing on the status of the Cystoflagellata (Haeckel). – Univ. Calif. Publs Zool. *19*, 317–334.

Lucas, I. A. N., 1982. Observations on *Noctiluca scintillans* Macartney (Ehrenb.) (Dinophyceae) with notes on an intracellular bacterium. – J. Plankt. Res. *4*, 401–409.

McFadden, G. I. & Melkonian, M., 1986. Use of Hepes buffer for microalgal culture media and fixation for electron microscopy. – Phycologia *25*, 551–557.

Melkonian, M., 1975. The fine structure of the zoospores of *Fritschiella tuberosa* Iyeng. (Chaetophorineae, Chlorophyceae) with special reference to the flagellar apparatus. – Protoplasma *86*, 391–404.

Melkonian, M., 1982. Effect of divalent cations on flagellar scales in the green flagellate *Tetraselmis cordiformis*. – Protoplasma *111*, 221–233.

Morrill, L. C., 1984. Ecdysis and location of the plasma membrane in the dinoflagellate *Heterocapsa niei*. – Protoplasma *119*, 8–20.

Morrill, L. C. & Loeblich, A. R., 1983. Ultrastructure of the dinoflagellate amphiesma. – Int. Rev. Cytol. *82*, 151–180.

Netzel, H. & Dürr, G., 1984. Dinoflagellate cell cortex. In: Dinoflagellates. Ed. by D. L. Spector. Acad. Press, London, 43–105.

Pfiester, L. A., 1984. Sexual reproduction. In: Dinoflagellates. Ed. by D. L. Spector. Acad. Press, London, 181–199.

Schlösser, U. G., 1982. Sammlung von Algenkulturen. – Ber. dt. bot. Ges. *95*, 181–276.

Soyer, M.-O., 1968a. Étude cytologique ultrastructurale d'un dinoflagellé libre, *Noctiluca miliaris* Suriray: trichocystes et inclusions paracristallines. – Vie Milieu *19*, 305–314.

Soyer, M.-O., 1968b. Présence de formations fibrillaires complexes chez *Noctiluca miliaris* Suriray et discussion de leur rôle dans la motilité de ce Dinoflagellé. – C. r. hebd. Séanc. Acad. Sci. Paris (D) *266*, 2428–2430.

Soyer, M.-O., 1969. Étude ultrastructurale des inclusions paracristallines intra-mitochondriales et intra-vacuolaires chez *Noctiluca miliaris* S. Dinoflagellé, Noctilucidae et observations concernant leur rôle dans la genèse des trichocystes fibreux et muqueux. – Prostistologica *5*, 327–334.

Soyer, M.-O., 1970a. Les ultrastructures liées aux fonctions de relation chez *Noctiluca miliaris* S. (Dinoflagellata). – Z. Zellforsch. mikrosk. Anat. *104*, 29–55.

Soyer, M.-O., 1970b. Etude ultrastructurale de l'endoplasme et des vacuoles chez deux types de Dinoflagellés appartenant aux genres *Noctiluca* (Suriray) et *Blastodinium* (Chatton). – Z. Zellforsch. mikrosk. Anat. *105*, 350–388.

Sweeney, B. M., 1978. Ultrastructure of *Noctiluca miliaris* (Pyrrophyta) with green flagellate symbionts. – J. Phycol. *14*, 116–120.

Wilcox, L. W. & Wedemayer, G. J., 1984. *Gymnodinium acidotum* Nygaard (Pyrrophyta), a dinoflagellate with an endosymbiotic cryptomonad. – J. Phycol. *20*, 236–242.

Wilcox, L. W., Wedemayer, G. J. & Graham, L. E., 1982. *Amphidinium cryophilum* sp. nov. (Dinophyceae) a new freshwater dinoflagellate. II. Ultrastructure. – J. Phycol. *18*, 18–30.

Zingmark, R. G., 1970a. Ultrastructural studies on two kinds of mesocaryotic nuclei. – Am. J. Bot. *57*, 586–592.

Zingmark, R. G., 1970b. Sexual reproduction in the dinoflagellate *Noctiluca miliaris* Suriray. – J. Phycol. *6*, 122–126.