THE FINE STRUCTURE OF GONYAULAX POLYEDRA, A BIOLUMINESCENT MARINE DINOFLAGELLATE

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SUMMARY

An electron-microscope study of Gonyaulax polyedra, a holophytic marine dinoflagellate which is bioluminescent, has revealed 2 structures not previously described in free-living dinoflagellates: a polyvesicular body occurring at intervals along the cell’s periphery, and a large spherical body which may function in intracellular digestion. Coated vesicles were found in the nucleus-Golgi region. Chloroplast lamellae were widely and regularly spaced in portions of the chloroplasts at the interior of cells harvested during the light period of the culture cycle. The lamellae were closer together and not so regularly arrayed in peripherally located chloroplast branches, or in chloroplasts harvested during the dark period. Other aspects of the ultrastructure of G. polyedra are described and discussed.

INTRODUCTION

Gonyaulax polyedra is a holophytic dinoflagellate about 35 × 45 μm in size. Of ecological interest because of its role in oxygen production, as a red tide organism, and as part of a link in the food chain, its bioluminescence has prompted a considerable amount of biochemical investigation (Hastings, 1968).

Light-microscopic observations have yielded information about the elaborate cell wall, the unusual nucleus, and other cytological aspects of Gonyaulax (Kofoid, 1911; Dodge, 1964b).

Published electron-microscope studies have dealt with specific aspects of this organism’s ultrastructure but have not described its morphology in detail (Bouck & Sweeney, 1966; Sweeney & Bouck, 1966). A more thorough ultrastructure study is of special interest because of the isolation from extracts of G. polyedra of subcellular particles, termed scintillons, which gave bioluminescence in an in vitro assay (DeSa, Hastings & Vatter, 1963; DeSa & Hastings, 1968). In addition, G. polyedra and other dinoflagellates have been found to exhibit persistent daily (circadian) rhythms in several of their physiological processes, including bioluminescence, photosynthetic capacity, and cell division. The understanding of the mechanism involved in such rhythms might be aided by an ultrastructural knowledge of the cell as related to the time of day and/or time in its circadian cycle.
MATERIALS AND METHODS

Growth of cells

*Gonyaulax polyedra* was grown at 21 °C in a supplemented sea-water medium described by Fogel & Hastings (1970). Fluorescent lighting was programmed to provide an alternating cycle of 12 h light–12 h dark.

Electron microscopy

Cells were collected by gentle centrifugation (lowest setting on an IEC model CL centrifuge) after addition of a small amount of glutaraldehyde fixative (see below) to each tube. Cells were fixed for 1 h at 22 °C in 6% glutaraldehyde in 0.15 M Sorensen's phosphate buffer, pH 7.2, and 0.2 M sucrose. Several washes with 0.1 M phosphate buffer containing 0.2 M sucrose followed fixation. Post-fixation was carried out at room temperature in 1% OsO4 in 0.05 M phosphate buffer. After one buffer wash, the cells were dehydrated rapidly in an ethanol series. Alternatively, uranyl acetate or uranyl nitrate was dissolved in all ethanol solutions except the first, and cells were left in each solution for 15-30 min. Two changes of propylene oxide preceded Epon/Araldite embedding. Beem capsules were used; blocks were polymerized at 60 °C for 48 h. Sections were cut with a diamond knife using a Porter–Blum MT-2 ultramicrotome, stained with lead citrate (Reynolds, 1963) or lead tartrate (Millonig, 1961), and viewed at 50 kV with the Hitachi HU-11 C electron microscope.

Cytochemistry

Staining reactions were carried out on 1-2 μm sections of Epon/Araldite-embedded material fixed as above or on cells fixed in glutaraldehyde and embedded in glycol methacrylate (Feder & O'Brien, 1968).

Munger's (1961) method for the periodic acid/Schiff (PAS) reaction was used, and starch was stained using the iodine-potassium iodide method of Johansen (1940). Tests for cellulose employed the method of Rawlins & Takahashi (1952).

RESULTS

Nucleus

The nucleus of *Gonyaulax polyedra* is C-shaped and lies near the apical end of the cell (see Fig 1). The nucleolus is characteristically found along the inner curvature of the C, within the nucleus. Ribosome-like particles occur in the nucleolar region and along adjacent portions of the nuclear envelope. The nuclear envelope possesses pores (Figs. 2, 3), which appear to be annulate when viewed in tangential section (Fig. 3). The endoplasmic reticulum is continuous with the nuclear envelope at many points (Figs. 2, 4).

The nuclear envelope does not break down during division in dinoflagellates (Leadbeater & Dodge, 1967b; Kubai & Ris, 1969). Nuclear separation is somehow effected by cytoplasmic channels which push through the nucleus. These channels contain numerous microtubules, and Kubai & Ris have suggested that the tubules are responsible for elongation of the channels. Fig. 4 shows a portion of a prophase nucleus containing such channels. Microtubules 19 nm in diameter are clearly visible in the inset. Each channel is formed by invagination of the nuclear envelope and therefore contains cytoplasmic material.

The chromosomes (k, Fig. 4) of *G. polyedra* appear less electron-dense than does the nucleoplasm, unless specimens are stabilized with uranyl acetate or uranyl nitrate.
Fig. 1. A 3-dimensional diagram of *Gonyaulax polyedra* harvested during the light period. A portion of the C-shaped nucleus is seen at left centre. Within it, coiled chromosomes (k) and the nucleolus can be seen. In the Golgi region at the centre of the diagram are 2 pre-trichocyst bodies, r'. A charged trichocyst (t) is at the upper left. The general locations of the 'compact' (co) and 'expanded' (e) forms of the chloroplasts are indicated. Starch granules are drawn as stippled rings. The PAS-body is located at lower centre. Vesicles of the pusule system (s) are indicated by the heavy outlines at right centre. Accessory microtubules of the flagellar apparatus are marked by the arrow. Polyvesicular bodies (pv) occur at intervals around the periphery of the cell. Several mitochondria (m) are indicated. The elaborate cell wall is outlined at the upper left. (Approximate magnification, × 7500.)
before embedding. A core region can be seen in the chromosomes in Fig. 4, an unstabilized preparation. One might expect the core material to be proteinaceous, although it has been reported that dinoflagellate chromosomes are not associated with histones (Dodge, 1964a; Kubai & Ris, 1969). After en bloc treatment with uranyl acetate, chromosomes appear as dense aggregates of fibrils arranged in waves (Figs. 2, 3). An electron-transparent halo may surround the chromatin.

**Nuclear-Golgi region**

Golgi dictyosomes are localized in a shell around the inner curvature of the nucleus (see Fig. 1). They are composed of numerous stacked cisternae and attendant vesicles. The association of Golgi-packaged material with formation of the crystalline shaft of dinoflagellate trichocysts has been described in detail by Bouck & Sweeney (1966).

Coated vesicles (Fig. 5, v) are reported for the first time in a dinoflagellate. They are about 90 nm in diameter and occur near the nuclear-Golgi region of the cell. Tubules of smooth endoplasmic reticulum or Golgi cisternae (arrows) are interconnected with the coated vesicles.

**Endoplasmic reticulum**

Dilated cisternae of rough or rough/smooth endoplasmic reticulum containing fibrous material (f) occur frequently in the cytoplasm (Figs. 4, 8). Some of these fibrous inclusions are within cisternae that are continuous with the nuclear envelope (Fig. 4). No function is presently ascribable to the fibrous areas, although several possibilities are evident and will be discussed.

**Chloroplasts and starch**

The number of chloroplasts present within a single *Gonyaulax* is uncertain, but it is clear from serial sections that the chloroplasts are reticular in form. Profiles branch and merge with others; portions of chloroplasts at the cell's periphery connect with profiles at the centre of the cell. Serial sectioning of the entire organism has yet to be achieved, and a statement of the number of chloroplasts per cell must await completion of that study.

The chloroplast envelope of *Gonyaulax polyedra* is composed of 3 membrane layers (Fig. 2, arrows). As in other dinoflagellates the chloroplasts are not bounded by endoplasmic reticulum, and a chloroplast girdle lamella is lacking (Dodge, 1968). Chloroplast lamellae are formed by the apposition, usually, of 2 or 3 disks. The disks are often staggered, rather than running the entire length of a lamella (Figs. 2, 4), and branched lamellae occur. Chloroplast ribosomes (r) and regions of chloroplast DNA (d) are common features of the stroma (Fig. 2).

Pronounced differences were observed between chloroplast profiles of cells fixed during the light and dark periods. Plastid profiles seen at the periphery of cells fixed during the light period contain 10–20 lamellae of 2 or 3 disks each. The lamellae are closely spaced, and not regularly so (Figs. 4, 12). Those portions of the plastid at the interior of the same cells possess the strikingly different morphology depicted in Fig. 6. The 2-disk lamellae are regularly arrayed, with an interlamellar spacing of 130–140 nm.
when cut in cross-section. These interior 'expanded' chloroplast regions contain fewer lamellae per profile than do the peripheral, closely lamellated regions. Lamellae, rather than bands of stroma, occur adjacent to the chloroplast envelope. Plastid profiles of cells fixed during the later hours of the dark period are all of the 'compact' type found at the periphery of light-harvested cells. They contain 10-20 closely spaced lamellae. Ribosomes are found in both plastid conformations. It is emphasized that the regularly arrayed 'expanded' conformation occurs only at the interior of cells harvested during or just after the light period and is not found in cells sampled after mid-dark period. The exact time course of the disappearance of the 'expanded' plastid configuration has not been determined.

Starch granules (Fig. 8, st) as indicated by PAS and iodine-potassium iodide staining of thick (1-2 μm) sections are present during the light period and absent after about 4 h into the dark period. The granules are not membrane-bounded and are not structurally associated in any obvious way with the chloroplasts or other organelle.

PAS-body

A large spherical body from 1 to 4 μm in diameter and bounded by a single membrane is found at the subapical end of the cell (Figs. 1, 8). Portions of this body are stained by the PAS reaction, and for convenience the term PAS-body will be used. These bodies contain aggregates of electron-dense material, fibrous areas, and membranous vesicles. PAS-bodies are morphologically similar to the digestive granules found by Slatterback (1967) in absorptive cells of _Hydra_ and the food vacuoles of _Ceratium hirundinella_ (Dodge & Crawford, 1970a), and may be related to the 'accumulation bodies' described by Taylor (1968) in the symbiotic dinoflagellate, _Symbiodinium microadriaticum_.

Mitochondria

The mitochondria of _Gonyaulax polyedra_ (m in Figs. 4, 8 and 12) are bounded by double membranes and have the tubular cristae characteristic of protozoa. They occur throughout the cell, but appear less numerous in the Golgi region.

Guanine crystals

A prominent feature of the cytoplasm is a branching collection of dilated membranes containing birhombohedral crystals (labelled c in Figs. 4, 9 and 10). The extent of interconnexion among the dilated membranes is difficult to determine. The crystals, when stained with lead, either dissolve away or tend to disintegrate in the electron beam, accounting for the holes seen in Figs. 8, 12 and 13. Crystals isolated from _Gonyaulax polyedra_ have been identified as guanine and were hypothesized to be an integral part of a subcellular luminescent particle, the scintillon (DeSa et al. 1963; DeSa & Hastings, 1968).

The guanine crystals in preparations stained _en bloc_ with uranyl salts retain a dark outline (arrows, Fig. 10), even when the typical electron-dense appearance of the unstained crystal (Fig. 9) has been destroyed by subsequent staining of the section with basic lead stains. This observation suggests the presence of a single membrane, a lipid layer, or some other substance closely applied to the surface of the crystal.
Polyvesicular bodies

Previously undescribed polyvesicular bodies occur regularly at the periphery of the cell (Figs. 11 and 12, pv). These consist of a number of small single-membrane vesicles of various diameters contained within a closely adhering double membrane. At some points within a polyvesicular body (Fig. 11, arrows) the vesicles seem to arise from invaginations of the inner membrane.

Flagellar pusule

A ramifying system of double-membranous vesicles containing amorphous material and an assortment of single and double-membrane-bounded vesicles comprises the flagellar pusule system (Fig. 12). The large double-membrane pusule vesicles (s) converge on a central, single-membrane-bounded region (z). There is an apparent concentration of mitochondria in the pusule region. It is uncertain whether their presence is related to pusule function or to the proximity of the flagellar apparatus. (The flagellar apparatus will not be dealt with here; see Leadbeater & Dodge, 1967a, for a description of the flagellar apparatus in the dinoflagellates Woloszyńska and Gymnodinium.)

Two alternative functions have been suggested for the pusule system: by means of its connexion to the outside through the flagellar pores it may be involved in the discharge of fluid from the dinoflagellate cell (F. Schütz, interpreted by Kofoid, 1909); or it may function in the uptake of material from the surrounding medium (Kofoid, 1909).

Cell wall

The cell wall of Gonyaulax polyedra is a complex structure composed of membranes, an armour layer and a darkly staining layer only partially membranous (Fig. 13). It can easily be seen that there are two membranes (labelled 1 and 2) outside the armour layer (a). Directly beneath the armour is a darkly staining layer (arrow), with a membrane beneath.

Various interpretations of these layers are possible: in classical terminology (Kofoid, 1911) the darkly staining layer would be termed the pellicle, and the membrane just beneath it the plasma membrane. The armour layer outside the pellicle would then be the theca. Kofoid reported that G. polyedra can shed and regenerate its theca, and he suggested that a new theca differentiates from the pellicle.

Dodge & Crawford (1970b) have derived another interpretation of this complex wall from a comparison of the thecae of many dinoflagellate genera. In their interpretation the outermost membrane (labelled 1) is the plasma membrane. Armour material is found within a membranous sac, the outer portion of which is marked 2 in Fig. 13. A part of the darkly stained layer appears to comprise the inner portion of the sac. Thus, the armour plates are within the plasma membrane, rather than outside it as in the Haptophyceae and Chrysophyceae (Manton, 1966; Manton & Harris, 1966). This interpretation is similar to the results of studies on the cuticular structure of such ciliates as Coleps. Fauré-Fremiet, André & Ganier (1968) described the formation of
Fine structure of Gonyaulax

the calcium phosphocarbonate-containing armour within membrane sacs inside the plasma membrane.

The armour of *G. polyedra* consists largely of polysaccharide which yields glucose upon hydrolysis (J. W. Hastings, cited in Salton, 1960). A thorough analysis was not carried out to determine what linkages are involved. The wall is stained by the zinc-chlor-iodide reaction for cellulose, however.

**DISCUSSION**

*Gonyaulax polyedra* possesses many interesting structural features which either have not been reported in a dinoflagellate before or which have not been discussed. These include coated vesicles, PAS-bodies, polyvesicular bodies, guanine crystals, and the unusual chloroplasts.

**Coated vesicles**

Coated vesicles have been found either as specialized invaginations of the plasma-lemma (see references in Friend & Farquhar, 1967, and Slautterback, 1967) or associated with, and perhaps derived from, Golgi elements (Bruni & Porter, 1965). Two general functions have been postulated for coated vesicles in animal tissues: cellular uptake of protein (Roth & Porter, 1964); and transport, either of enzymes (Bruni & Porter, 1965) or of soluble products of intracellular digestion (Novikoff, Roheim & Quintana, 1966). As pointed out by Friend & Farquhar (1967), coated vesicles may have multiple transport functions. These might include transporting enzymes or membrane material to the cell surface as well as intracellular transport duties.

Coated vesicles have been found in association with the contractile vacuole of the green alga *Stigeoclonium* ('hairy vesicle', Manton, 1964) and in *Euglena spirogyra* ('alveolate vesicles', Leedale, Meuse & Pringsheim, 1965). A role in osmoregulation has been suggested for coated vesicles in algal flagellates (Leedale *et al.* 1965). Branches of the pusule system do occur adjacent to regions containing coated vesicles in *G. polyedra*, but no contractile vacuole *per se* exists in this organism.

Bearing in mind that in a compact single cell, proximity does not necessarily imply a functional relationship, the vesicles also occur adjacent to whorled membrane configurations which could suggest a role in intracellular digestion. An association of small, coated vesicles with Golgi cisternae and hydrolytic enzyme-containing multivesicular bodies has been studied cytochemically in the epithelium of the rat vas deferens (Friend & Farquhar, 1967). That study indicates that the small, coated vesicles are involved in the transport of hydrolytic enzymes from the Golgi complex to the multivesicular bodies.

The possible occurrence of coated vesicles in the vicinity of the PAS-body, which may be a site of intracellular digestion, has been investigated, thus far with negative results. Coated vesicles have been found only in the nuclear-Golgi region of cells harvested during the early hours of the light period. Additional study will establish whether they occur only at that time. For example, cell division takes place within a few hours of 'dawn' when cells are grown on a 12 h dark–12 h light cycle.
It may be that the microtubules involved in nuclear channel formation are formed initially within a membrane system, the membranes being digested away prior to channel formation.

**Fibrous areas**

Several possible functions can be suggested for the fibrous areas found in *G. polyedra*. The fibres could comprise unpolymerized microtubule protein for use in nuclear channel formation or the formation of microtubules occurring beneath the theca. Or, they might represent an early form of the fibrous portion of charged trichocysts, the ontogeny of which has not been described. Finally, it has recently been suggested (Leedale, Leadbeater & Massalski, 1970) that the ‘fibrous body’ described by Leadbeater & Dodge (1966) in another dinoflagellate comprises precursor material for flagellar hair formation. Aggregates of fibrils were found by Hepler & Newcomb (1964) in *Coleus* cells, and they suggested that the presence of the fibrils within cisternae of the endoplasmic reticulum might be related to the accumulation of products in the rough endoplasmic reticulum of animal cells prior to secretion.

**Chloroplasts**

The ‘expanded form’ chloroplasts described in this study present a problem in terminology. Structurally, these chloroplast regions are nearly identical with the pyrenoids described in detail by Kowallik (1969) in *Prorocentrum micans*. Dodge & Crawford (1969) described chloroplasts at the centre of *Gymnodinium fuscum* as containing much stroma and few lamellae compared with those at the periphery, but the highly ordered structure described here and by Kowallik (1969) was not depicted. Dodge & Crawford (1970a) reported the absence of pyrenoids in *Ceratium hirundinella* and noted that chloroplasts can be found at the centre of the cell, which contains few lamellae. They suggested that ‘these chloroplasts take the place of pyrenoids’.

‘Pyrenoid’ is an ambiguous term, and as such its utility here is in question. One can consider alternative schemes in which the ‘pyrenoid’ could be involved, all of which are related to its being a physiologically differentiated region of the chloroplast proper, not a separate entity.

These expanded-form regions might represent chloroplast growth. This is an attractive idea for 2 reasons. First, incompletely assembled chloroplasts might be expected not to function efficiently in photosynthesis. By extending the chloroplast toward the interior of the cell, only functional ‘old’ chloroplast regions would occupy the prime light-harvesting regions at the cell’s periphery. Secondly, the highly ordered arrangement of the lamellae could provide guidelines for the assembly of new disks, possibly from protein or lipoprotein subunits contained in the ordered, granular stroma. The stroma regions were not stained by the bromophenol blue method for protein of Mazia, Brewer & Alfert (1953) however.

The chloroplasts of some algae and higher plants in experimental systems go through morphological stages not unlike this one. For example, in *Chlorella vulgaris*, of which the wild type produces chlorophyll and develops a chloroplast in the presence or absence of light, a mutant form requires light to produce a photosynthetically active
Fine structure of Gonyaulax

chloroplast (Bryan, Zadylak & Ehret, 1967). In the presence of light, a tubule system develops into parallel primary disks. Blebs from the primary disks extend to form adjacent secondary disks. Ultimately, the 3-disk lamellae typical of this organism’s chloroplast are formed. During greening in etiolated shoots of Nicotiana tabacum (Stetler & Laetsch, 1969), widely spaced 2-disk lamellae are present in the chloroplasts after 8 h in the light. (After 10 min in the light, N. tabacum chloroplasts contain a few lamellae and a well developed prolamellar body. G. polyedra plastids do not contain prolamellar bodies, and only rarely are proplastid-like structures seen in cells from the dark period.) In a mature N. tabacum chloroplast a granum contains 4–9 disks. It is interesting to note that many chloroplasts appear to divide during the 2-disk/lamella stage of plastid development in Nicotiana (Stetler & Laetsch, 1969).

Clearly, a quantitative study of the amount of expanded-form chloroplast present at different points during the light period should be made, to determine whether its amount increases progressively with longer exposure to light. Further, an intensive study of time points at the beginning of the dark period should reveal whether or not a transition to the compact form chloroplast proceeds by the formation of new disks. If so, one might expect to see partially formed lamellae between lamellae of the expanded form at early times in the dark period.

It is possible that the variation in chloroplast morphology might be correlated with photophosphorylation. Zurzycki (1967) found that chloroplasts in Mnium undulatum increase in area after illumination with light of various wavelengths and intensities. He suggested that these light-induced changes were coupled with photophosphorylation.

Since G. polyedra is known to possess rhythms (Hastings & Sweeney, 1958; Sweeney, 1960; Sweeney & Hastings, 1957, 1958) it is of interest to know whether the morphological changes in the chloroplasts at different times in the light-dark cycle are the result of light induction or are a rhythmic phenomenon. Dodge (1968) reported that the lamellae of Woloszynskia chloroplasts were spaced farther apart if grown under 500 ft-c (1715 lx) illumination than if grown at 100 ft-c (343 lx). In studies on Euglena, König (1965) found that lamellated pyrenoids were present at mid-light period and not at mid-dark period.

Guanine crystals

Recent biochemical studies indicate that the guanine crystals in G. polyedra are not directly involved in the particulate bioluminescence (Fogel, 1970; Fogel & Hastings, 1971; M. Fogel, R. E. Schmitter & J. W. Hastings, in preparation). Crystals like those found in G. polyedra occur in a number of other dinoflagellates, both luminescent and non-luminescent, although these have not been isolated and identified as guanine (Sweeney & Bouck, 1966; Schmitter, unpublished observations). The occurrence of large numbers of crystals in these cells is not unreasonable, since plant cells are known to sequester substances as crystals (Robbins, Weier & Stocking, 1965; Esau, 1965). If the guanine is an unwanted by-product of metabolism, then such a segregation mechanism would be desirable. Calcium oxalate crystals occur the most commonly in plant cells (Esau, 1965), and crystals identified as calcium oxalate have been found in the symbiotic dinoflagellate Symbiodinium microadriaticum (Taylor, 1968).
The cells of yeasts, such as *Candida utilis*, can actively take up guanine, adenine, and other purines added to the culture medium (Roush, Questiaux & Domnas, 1959; Cowie & Bolton, 1957). Crystals of the purines form within the yeast's vacuole, and these have been studied by light (Roush et al. 1959) and ultraviolet microscopy (Svihla, Dainko & Schlenk, 1963). Many of the purines studied can be utilized as a nitrogen source by this yeast, regardless of whether they constitute the sole nitrogen source (Roush et al. 1959) or are present in conjunction with another nitrogen source (Cowie & Bolton, 1957). It has been suggested (Bertha Livingstone, cited in Syrett, 1962) that *Chlamydomonas moewusii* can use nitrogen from guanine for growth. Thus, it is possible that the guanine of *G. polyedra* serves as a nitrogen store.

The guanine crystals could function as reflectors. Ordered arrays of guanine crystals are found in the photophores of the luminous organs of certain fishes (Bassot, 1966) and in the guanophores and iridophores of the skin of amphibians and fishes (Kawaguti, Kamishima & Sato, 1965; Kawaguti & Kamishima, 1966; Setoguti, 1967). In such instances the crystals apparently do function as reflectors. However, it is unlikely that the guanine crystals in *G. polyedra* serve as reflectors, since they appear to be randomly oriented.

**PAS-body**

Taylor (1968) described an 'accumulation body' in the symbiotic dinoflagellate *Symbiodinium microadriaticum*. Composed of a dense outer covering and electron-dense regions, this body did not contain membranous material. Taylor suggested that the body is an accumulation of waste materials, based on cytochemical studies and the observation that the body increases in size during ageing or starvation of the cell.

Apart from considering the PAS-body an accumulation body of the type described by Taylor, it is possible that this structure is involved in active digestive processes. The fact that membrane profiles are at times present within the PAS-body is suggestive of a digestive function. PAS-bodies are found in cells fixed during both dark and light portions of the dark cycle; their occurrence is probably related to some factor such as age of the cell or growth conditions, rather than to the time of day.

The PAS-bodies of *G. polyedra* differ from the food vacuoles described by Dodge & Crawford (1970a) in the dinoflagellate *Ceratium hirundinella* in several respects. The former occur singly, in the subapical region of the cell, while several food vacuoles can be found in *C. hirundinella* in different parts of the cell. Bacterial or algal contents have not been identified within PAS-bodies, but pieces of membrane or fibrous material are seen consistently. Axenically grown cells of *G. polyedra* do possess PAS-bodies, so it is probable that any digestive function of PAS-bodies in this organism involves such processes as autophagy or utilization of stored metabolites.

**Polyvesicular bodies**

It is possible that the polyvesicular bodies are transverse sections of trichocyst sacs remaining after discharge of the shaft, but I do not favour this interpretation. If it were so, one would expect to see elongated longitudinal profiles of the collapsed sac frequently. This is not the case.
Polyvesicular bodies of the type described here do occur in purified scintillon preparations from extracts of *G. polyedra* (Fogel & Schmitter, unpublished observations), but any possible relationship between these bodies and the particulate bioluminescence remains to be elucidated.

The similarity of the polyvesicular bodies to mitochondria cannot be overlooked. Both possess a basic double-membrane structure, with elaborate infoldings of the inner membrane. However, the polyvesicular bodies do not usually exhibit what could be described as a matrix. If they are related ontogenetically to mitochondria, their location only at the cell’s periphery would suggest a specialized function. The proximity of well fixed mitochondria and other organelles seems to preclude that such bodies result from inadequate fixation.

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Fine structure of Gonyaulax


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Fig. 2. Portion of an interphase nucleus. The nuclear envelope (ne) is formed by a double membrane, which is interrupted at intervals by pores (p). Some elements of the rough endoplasmic reticulum (rer) are continuous with the nuclear envelope. The chromosomes (k) consist of fibrils arranged in waves. Chloroplast DNA (d) and chloroplast ribosomes (r) are evident. The chloroplast envelope consists of 3 membranes (arrows). (m, mitochondrion.)

Fig. 3. Tangential section of the nuclear envelope. The nuclear pores (arrows) appear annulate when viewed in tangential section. (k, chromosome.)
Fine structure of Gonyaulax
Fig. 4. Portion of a prophase cell. Arrows indicate endoplasmic reticulum which is continuous with the nuclear envelope. Chromosomes (k) are less electron-dense than the nucleoplasm in this preparation, which was not stained en bloc with uranyl salts. The nuclear channels (nc) associated with division are bounded by the nuclear envelope and contain cytoplasmic material. The inset shows microtubules (arrows) within one of the channels. (c, guanine crystal; ch, chloroplast; er, endoplasmic reticulum; f, fibrous area; g, Golgi dictyosome; m, mitochondrion; t, trichocyst; t', forming trichocyst.)
Fig. 5. Detail of the nuclear-Golgi region. Coated vesicles (v) occur in this region. They frequently connect with tubular profiles presumed to be elements of the smooth endoplasmic reticulum or Golgi cisternae (arrows).

Fig. 6. Chloroplast profiles at the interior of a ‘day’ cell. The 2-disk lamellae are parallel to each other and are regularly spaced at 130–140 nm. The granular stroma appears organized, especially in the regions marked by arrows. Several clusters of chloroplast ribosomes are also seen (r). The area outlined is shown at higher magnification in Fig. 7. (c, guanine crystal; m, mitochondrion; ′, forming trichocyst.)
Fine structure of Gonyaulax
Fig. 7. Portion of chloroplast in the outlined region in Fig. 6 seen at higher magnification. The particulate nature of the stroma is evident; some of the particles appear linearly arrayed. The limiting membrane of a single disk is circled, and one lamella is indicated by the vertical bar.

Fig. 8. A single-membrane-bounded body found at the subapical end of the cell. Portions of the body stain with the PAS reaction. Fibrous (x) and electron-dense (y) materials are typically present. (c, guanine crystal; ch, chloroplast; f, fibrous area; m, mitochondrion; st, starch.)
Fine structure of Gonyaulax
Fig. 9. Guanine crystals (c) in an unstained section.

Fig. 10. Guanine crystals (c) in a lead-stained section. Although the crystals no longer appear electron-dense, they are outlined by a layer of dense material (arrows). (m, mitochondrion; t, trichocyst.)

Fig. 11. Section near the periphery of *G. polyedra*. Polyvesicular bodies (pv) of undetermined function occur at the periphery of the cell. Arrows indicate regions where the doubleness of the closely appressed membranes is evident. Note that these bodies are distinct from typical mitochondria (m), which can be seen nearby. (c, guanine crystal; rer, rough endoplasmic reticulum; t, trichocyst.) Inset: polyvesicular body at higher magnification.
Fine structure of Gonyaulax
Fig. 12. Flagellar pusule system. Membranous vesicles of the pusule system are marked s. These double-membrane-bounded vesicles appear associated with a central, single-membrane-bounded region (z). Arrows indicate accessory microtubules of the flagellar root system. (c, guanine crystal; ch, chloroplast; m, mitochondrion; pv, polyvesicular body; rer, rough endoplasmic reticulum; t, trichocyst.)
Fig. 13. Portion of *G. polyedra* illustrating the cell wall. The armour layer (a) is contained within a membranous sac. The sac consists of membrane 2 and part of the darkly staining layer (arrow). The plasma membrane is labelled 3. (e, guanine crystal; ch, chloroplast; m, mitochondrion; t, trichocyst.)