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ORIGINAL ARTICLE

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Gill ultrastructure and symbiotic bacteria in *Codakia orbicularis* (Bivalvia, Lucinidae)

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Summary The cellular organization of the gill, which harbors symbiotic bacteria, is described in juveniles and adults of *Codakia orbicularis,* a large tropical Lucinidae. The ciliary zone is similar in every species of Lucinidae described and includes the large clear cell which has been previously described as an intermediary cell. The intermediary zone is composed of a few narrow unciliated cells, which bind adjacent filaments together and constitute channels through which sea water circulates along the abfrontal part of the filaments. The lateral zone is more complex in *C. orbicularis* than in other Lucinidae, being composed of four cell types and differentiated into two distinct regions. The bacteriocytes and intercalary cells occupy the outermost bacteriocyte zone, while mucocytes and numerous cells crowded with proteinic, cystine-rich granules constitute the innermost secretory zone which has not been described in other species. The newly described granule cells are considered to be a key factor in the storage and metabolic conversion of sulfur compounds.

A. Introduction

Symbioses between Lamellibranchia and intracellular chemoautotrophic bacteria (Felbeck et al. 1981) were first described in deep-sea hydrothermal vent fauna. The discovery of endosymbiotic sulfur-oxidizing bacteria in the gill of *Calyptogena magnifica* Boss and Turner, 1980, was rapidly followed up by the identification of a similar symbiosis in coastal bivalve species which inhabit areas with a considerable degree of ecological stress where oxygen and reduced sulfur compounds are simultaneously available, as in *Solemya reidi* (Bernard, 1980) inhabiting reduced sediment (Felbeck 1983) and *Codakia orbicularis* (Linné, 1758) which inhabits sea-grass beds (Berg and Alatalo 1982, 1984). A similar type of symbiosis is now known in every species of the family Lucinidae so far examined, as well as in some species of the related family Thyasiridae (for review, see Somero et al. 1989; Fiala-Medioni and Felbeck 1990; Fisher 1990; Reid 1990). Most of the Lucinidae are small species with the exception of a few tropical ones such as *C. orbicularis* which grows up to 90 mm in length (Frenkiel and Mouëza 1988). The association of endocellular gram-negative bacteria within the gill cells of *C. orbicularis* was demonstrated by Berg and Alatalo (1984) through enzymatic activities related to sulfur oxidation and chemolithotrophic syntheses by endocellular bacteria; but this study provided no information about the structure of the gill. In view of the thickness and complexity of a large adult's gill structure, we decided to study the organization of the gill in juveniles (2 mm shell length) and in small individuals (7–15 mm shell length). For juveniles, metamorphosis is completed notwithstanding the fact that the filaments are not fused as they are in an adult's gill. Such a simple organization allows a better appreciation of the basic structure and intercellular relationships than in adults. As a clear understanding of the organization of the gill tissue is considered basic to a future investigation of the transmission of bacteria to juveniles, this paper is designed to describe the organisation of the gill filament in recently differentiated gills as well as in the adult stage.

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B. Material and methods

Juveniles and small adults of *C. orbicularis* were collected by sieving and sorting sediment from sea-grass beds off the island of Guadeloupe. Juveniles were fixed whole for SEM and for TEM observations. Small pieces, dissected from different locations in the gills of adults, were fixed for TEM. All the pieces were prefixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer adjusted to pH 7.2 and 1100 mOsM with a saline solution designed for marine invertebrates (Hernandez-Nicaise and Amsellem 1980). After a short rinse, they were fixed in 1% osmium tetroxide in the same cacodylate buffer for one hour at 4° C. An alternative, one-step fixation procedure using a mixture of 0.8% glutaraldehyde and 1%

osmium tetroxide in cacodylate buffer at 4° C, adapted to marine invertebrates (Mouëza 1982) from Hirsh and Fedorko's method (1968) was also used. After either procedure, the pieces were rinsed in distilled water and postfixed in 2% aqueous uranyl acetate solution at room temperature for one hour.

The shell of the juveniles was dissolved, thereafter, in 1% ascorbic acid, according to Dietrich and Fontaine's technique (1975). Whole juveniles were observed in a Hitachi 2500 SEM after dehydration by acetone, critical-point drying in carbon dioxide, and sputter-coating with gold. Other juveniles and the pieces of gills were dehydrated in a graded ethanol series followed by propylene oxide prior to embedding in Epon-Araldite according to Mollenhauer (in Glauert 1975) and sectioning on a Leica Ultracut E ultramicrotome. Semithin sections, 1 µm thick, were stained with toluidine blue in 1% borax buffer and observed on a Leica Vario-Orthomat microscope. Fine sections, 60 nm thick, were contrasted for 10 min in lead citrate prior to observation in a Hitachi H8000 TEM.

Some histochemical information was obtained from paraffin sections of adults. Whole individuals fixed in 3% formaldehyde in sea water or in Bouin's fluid were embedded in paraplast and 7-µm-thick sections were cut. Histological and histochemical techniques (in Gabe 1968) were performed to obtain information on the content of secretory cells: trichrome staining according to Goldner, using two different acidic dyes; Mann-Dominici; alcian blue at varying pH; fuchsin-paraldehyde with and without previous permanganic oxidation; and PAS reaction were used to identify mucosubstances. The tetrazoreaction according to Danielli (in Gabe 1968) was used to ascertain the presence of proteinic inclusions and the DDD reaction according to Barrnett and Seligman (in Gabe 1968) to detect sulfur amino acids with and without reduction of disulfur bonds and adequate controls to ascertain the presence of cysteine or cystine.

C. Results

I. Morphology

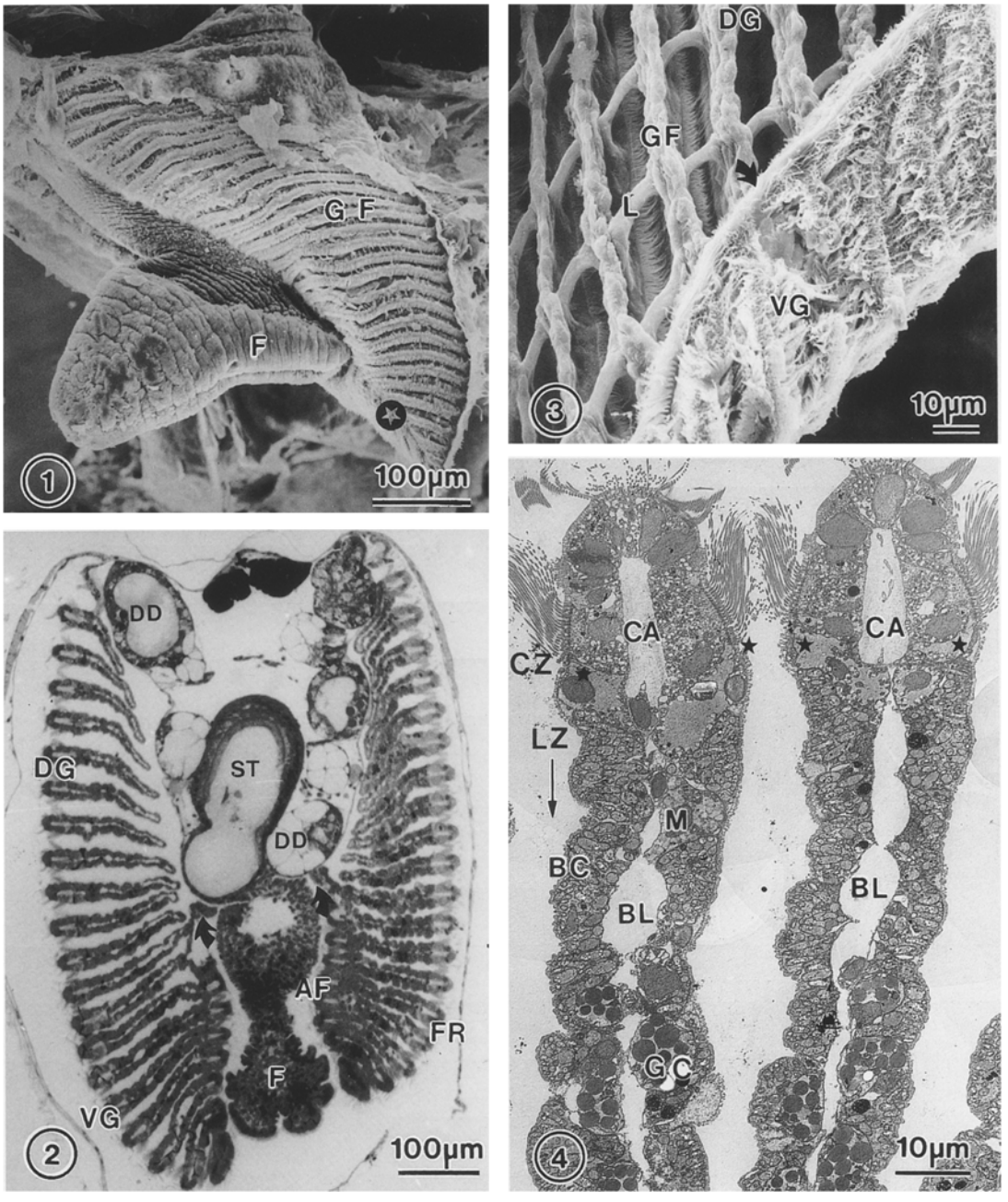
The creamy colored, thick gills of *C. orbicularis* consist of a single demibranch covering the visceral mass on either side and extending posteriorly below the heel of the foot. In 2- to 3-mm-long juveniles, they consist of a number of gill filaments ranging from 20 to 30 (Figs. 1, 2). These gill filaments are linked together, being connected by transverse connective tissue (Fig. 3) which will develop into the cancellate structure of the adult's gill. The gill filament anlagen are posteroventral on either side of the siphonal septum. In the posterior area, between the foot and the siphonal septum, the two gills are in close contact with each other (Fig. 1). A transverse section (Fig. 2) at the level of the stomach shows that, in the suprabranchial area, the gill filaments are composed of a frontal ciliated zone and a lateral zone devoid of cilia, whereas in the infrabranchial area two ciliated zones, frontal and abfrontal, are connected by a lateral zone devoid of cilia.

II. Gill filament structure

Each gill filament consists of a simple epithelium which, at the level of the ciliated zone, is in contact with a connective axis and which encloses a blood lacunar space at the level of the lateral zone (Fig. 4). This lacunar space is partitioned, at irregular intervals, by apposition of the basal laminae which are highly convoluted at these spots. All the cells which are in contact with sea water develop microvilli at their apical surface.

The ciliated zone is short (Fig. 5) with differentiated cell types as described following the terminology defined by Atkins (1938) with modifications introduced by Owen and McCrae (1976). Frontal cells bear short cilia without a precise orientation and possess basal bodies without ciliary rootlets. Narrow prolaterofrontal cells bear two rows of long orientated cilia. The large apical surface of eulaterofrontal cells bears a large curved cirrus composed of some 30 cilia fused together, and which possess long ciliary rootlets each being shared between adjacent basal bodies. Prolateral cells are devoid of cilia and have long regular microvilli. The eulateral cells, which bear some 30 rows of long independent cilia, constitute a functional group composed of three cell types (Figs. 5–7). The first one, located near the prolateral cells, has an electron translucent cytoplasm and mitochondria scattered at the periphery. Its nucleus is in the apical part and it possesses cilia only on a small part of its apical area. The second one consists of two cells which exhibit cilia all along their apical surface and the apical region of their cytoplasm is occupied by striated rootlets and many mitochondria. The third cell type is much like the first one but, being larger, it is even more conspicuous, with clear cytoplasm, many peripheral mitochondria, an apical or lateral nucleus, and only some 4–5 rows of cilia on a narrow apical pole. This cell is partly covered by two elongated, non-ciliated cells which are intermediary cells located between the ciliated zone and the lateral zone (Figs. 6, 7).

The lateral zone constitutes the main part of the filament and accounts for the thickness and color of the gill. It is composed of four cell types. The bacteriocytes, which are the most prevalent cells in the gill filament, in juveniles, have a basal nucleus of a standard length of 5 µm (Fig. 8). The main part of the cell volume is occupied by vacuoles with a single bacterium visible in most sections (Figs. 8, 10, 13) or, less frequently, a dividing one (Fig. 16). In 2-mm-long juveniles, the bacteriocytes are much wider than they are high. In the suprabranchial area, their height is no more than 5 µm in the abfrontal region (Fig. 8), but 10 µm in the outer region near the ciliated zone (Fig. 10). The bacteriocyte height is related to the number of bacteria. It may reach 20 µm in the gills of a 7-mm-long *C. orbicularis* and at least 35 µm in the gill of a large adult. The apical surface differentiates microvilli linked by a fibrous glycocalyx and develops a broad contact area with the pallial sea water. The basal membranes are convoluted at intervals (Fig. 10) when they come into contact with each other or with hemocytes (defined here as any cell type seen in the hemolymph lacunae). The organelles are scarce (Fig. 11) with most of the cell space being occupied by bacteria. Sometimes, partly destroyed bacteria are associated with complex lysosomal structures (Fig. 12). More frequently, round, heterogeneous inclusions as large as the nucleus are located in the basal part of bacteriocytes (Fig. 19).



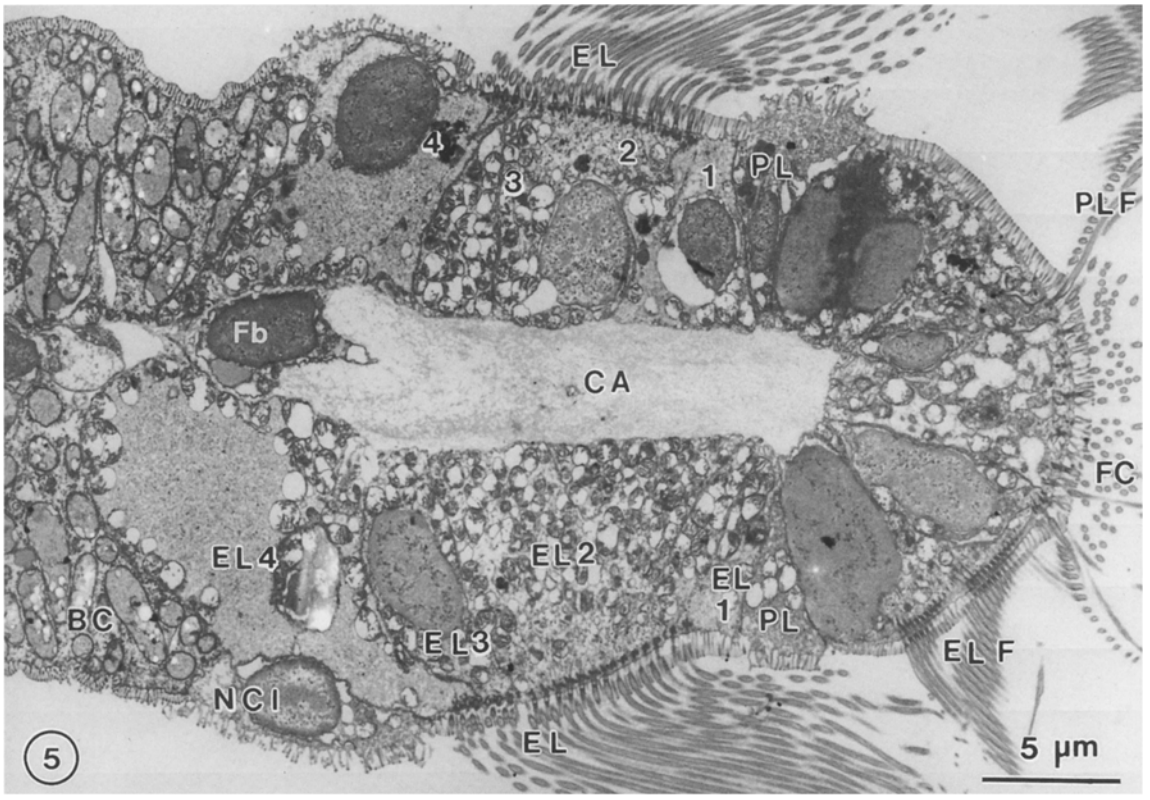
Figs. 1–4 *Codakia orbicularis,* gill structure in juveniles

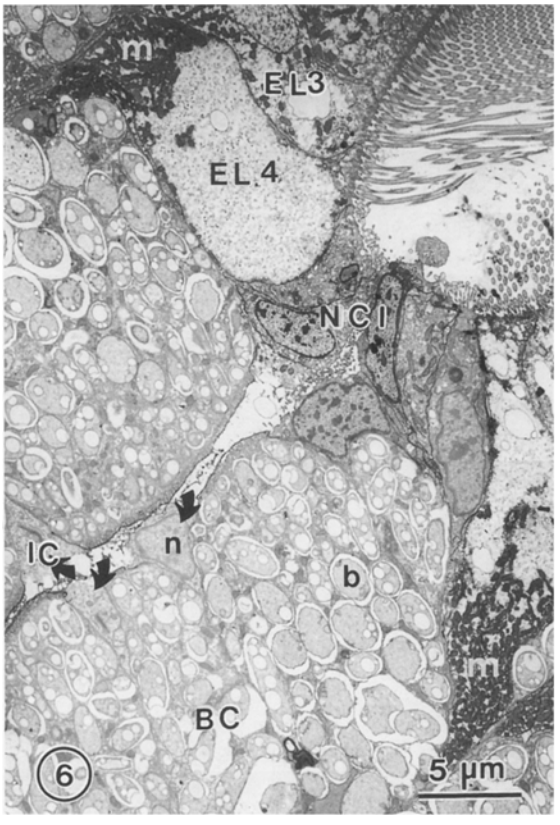
Fig. 1 SEM of a juvenile (3 mm shell length) with approximately 30 gill filaments. The gill filaments (*GF*) are affixed to each other *(star)* at the back of the foot (*F*) and with the lateral wall of the foot

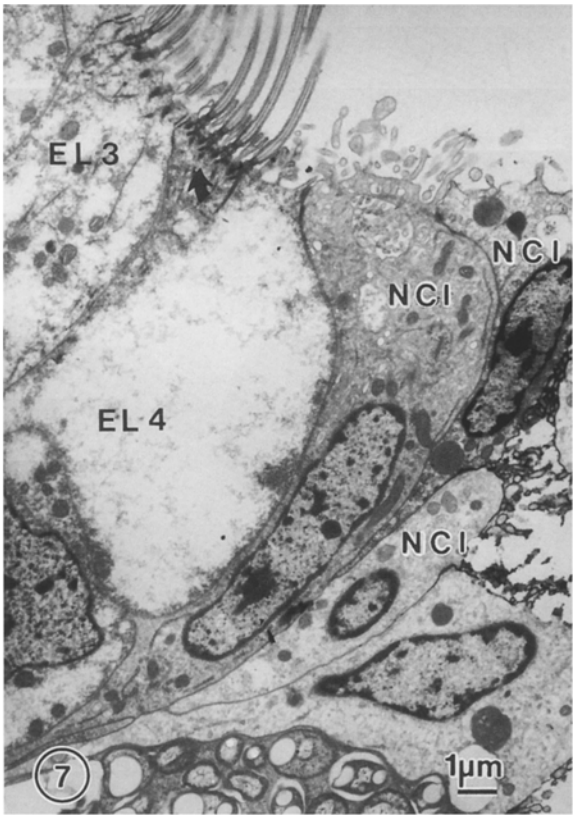
Fig. 2 Light micrograph (LM) of a semithin transverse section at the level of the stomach (*ST*) and digestive diverticula (*DD*) of a juvenile (2 mm shell length). The dorsal part of the gill filaments (*DG*) has a single frontal ciliated zone (*FR*) while the ventral part (*VG*) has a frontal (*FR*) and an abfrontal ctenidial zone (*AF*) separated by the lateral zone. The gills are affixed on each side of the foot (*F*) (*curved arrow* on Figs. 2, 3)

Fig. 3 SEM of inner side of the gill of a juvenile (2 mm shell length). The connective links (*L*) between the gill filaments (*GF*) are observed in the dorsal part (*DG*) facing the visceral mass in the suprabranchial region. They will develop into the cancellate design observed on adult gills. The ventral part of the gill (*VG*) is still short at this stage but will constitute most of the gill in adults. Its upper limit, marked by a *curved arrow,* is affixed along a line which delimits the foot and the visceral mass

Fig. 4 TEM of two adjacent gill filaments with a short ciliated zone (*CZ*) limited by a large clear cell which is the last ciliated eulateral cell (marked by a *star*). In the lateral zone (*LZ*), the bacteriocytes (*BC*) are intermingled with "granule cells" (*GC*) and some mucocytes (*M*). The ciliated part of the filament is stiffened by a collagen axis (*CA*) while the axis of the lateral zone is occupied by a blood lacunar space (*BL*)







Figs. 5–7 *C. orbicularis,* ciliated and intermediary zone

Fig. 5 TEM of the ciliated zone in a juvenile gill filament. Collagen axis (*CA*), fibroblast (*Fb*), frontal cilia (*FC*), prolaterofrontal cilia (*PLF*), eulaterofrontal cilia (*ELF*), prolateral cells (*PL*), and eulateral cells (*EL 1–4*). The apical pole of the ciliated clear cell (*EL4*) is partly covered by non-ciliated intermediary cells (*NCI*). Bacteriocytes (*BC*) are part of the lateral zone

Fig. 6 TEM of the intermediary zone of united adjacent filaments, in a small adult (7 mm shell length). Notice the close apposition of the non-ciliated intermediary cells (*NCI*). The clear ciliated eulateral cell (*EL 4*) has many mitochondria (*m*), at its base. Bacteriocytes (*BC*) are crowded with bacteria (*b*) while the intercalary cells (*IC*) *(arrows)* are narrow and characterized by an apical nucleus (*n*)

Fig. 7 TEM of the intermediary zone at a higher magnification. The large clear cell (*EL4*) possesses four rows of cilia *(arrow)* and belongs to the functional group of lateral ciliated cells. It is partly covered by narrow non-ciliated intermediary cells (*NCI*)

The most conspicuous inclusions of the bacteriocytes are the bacteria. They are ovoid or rod-shaped, with the characteristic outer membrane of gram-negative bacteria (Fig. 9). Bacterial sizes vary from 3 µm to 5 µm in length and 0.8 µm to 2 µm in width. Periplasmic inclusions, up to 0.75 µm, are very frequent and appear generally electron lucent (Fig. 13) but may occasionally retain a granular matrix (Fig. 14). The bacterial cytoplasm displays variable aspects in different individuals and even in different bacteria of the same individual. Most frequently, it contains numerous non-membrane-bound irregular inclusions of a size varying from 50 nm to 80 nm (Fig. 13). The nuclear area is generally identified as a well-defined network which appears to be less conspicuous in juveniles than in older individuals. It is frequently associated with a lamellar annular structure which reaches 0.3 µm in diameter and 40 nm in thickness (Figs. 13–15). This annular structure is composed of clear and dark laminae (Fig. 15), the number of which varies from 4 to 15, with a mean thickness of 1.5–1.7 nm each. This structure has been identified in bacteria observed in adult individuals of various sizes but not in juveniles.

Inconspicuous intercalary cells are interspersed between bacteriocytes. Their main feature is an apical nucleus (Fig. 6) and long microvilli (Figs. 19, 20) are frequently the only hint of their presence in sections, as their basal, narrow part is almost devoid of any organelle.

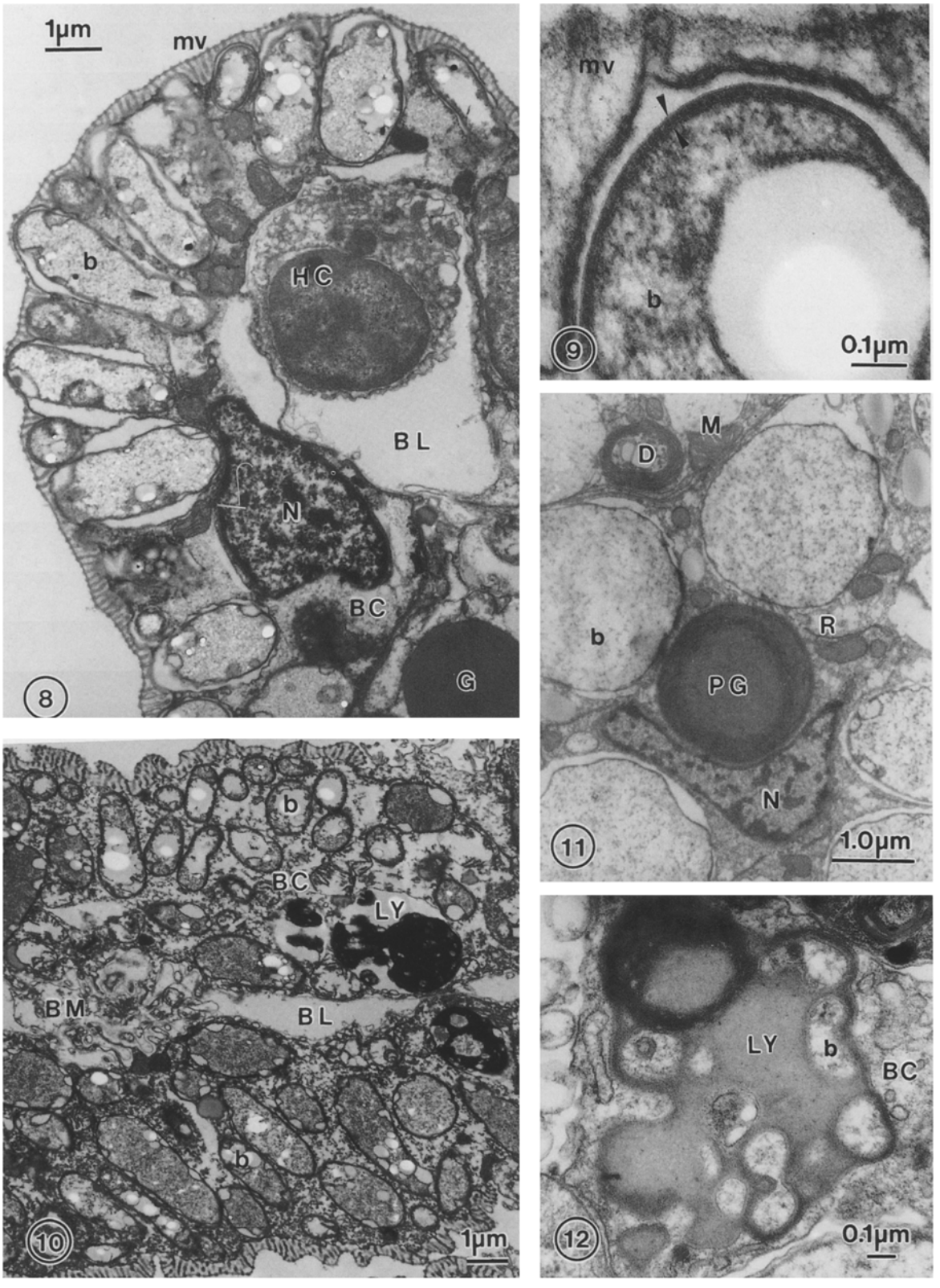
In juveniles, mucocytes were identified at various levels of the filament (Fig. 4). Their nucleus is basal and numerous Golgi stacks are their most conspicuous cytoplasmic organelles (Fig. 20). The apical pole is occupied by some short microvilli. The mucous granules are electron lucent and show a red (γ) metachromasia with toluidine blue on semithin sections. Histological techniques as well as histochemical staining with alcian blue at pH 0.5 but not at pH 3, and the absence of a PAS reaction, indicate that their main product is a sulfated proteoglycan.

In gill sections of both adults and juveniles, large globular cells with a narrow neck were identified and termed "granule cells" (Figs. 4, 19). They are often partly covered by the widened apical pole of the bacteriocytes. In juveniles, their apical pole has short microvilli which are no longer in evidence in older individuals. The basal nucleus of these granule cells is approximately 5 µm in length and mitochondria and a well-developed Golgi apparatus are not uncommon in sections of their cytoplasm. Their large, membrane-bound, osmiophilic inclusions, 3–5 µm in diameter, are homogeneous granules which stain orthochromatically with toluidine blue on semithin sections. By using histological techniques, such as Goldner triple staining or Mann-Dominici, these inclusions appear to be strongly acidophilic; they are PAS negative and alcian blue negative at pH 3 as well as at pH 0.5. These results exclude the possibility of their content being either a proteoglycan or a mixture of glycoprotein and proteoglycan, typical of mucous droplets. Their staining by tetrazoreaction is conclusive of a proteinic content; moreover the DDD reaction shows that their proteinic content is rich in cystine residues with disulfur bonds. The most conspicuous components of the gill filament, other than the bacteriocytes, are these "granule cells". On a juvenile filament section (Figs. 4, 17), they are more numerous than mucocytes but less so than bacteriocytes and are encountered more frequently in the innermost zone. With growth and the increase in thickness of the gill, the proportion of "granule cells" and the heterogeneity of their distribution increases (Fig. 18). In adult gill filaments, the "granule cells" become more prevalent in the deeper part of the filament, whereas the bacteriocytes occupy the part of the filament immediately adjacent to the intermediary zone. These granule cells, being intermingled with mucocytes and a few bacteriocytes, make up a distinct part of the lateral zone as this becomes progressively separated into a superficial bacteriocyte zone and a deep "granule cells" zone. In adults, this last zone becomes twice as thick as the bacteriocyte zone.

D. Discussion

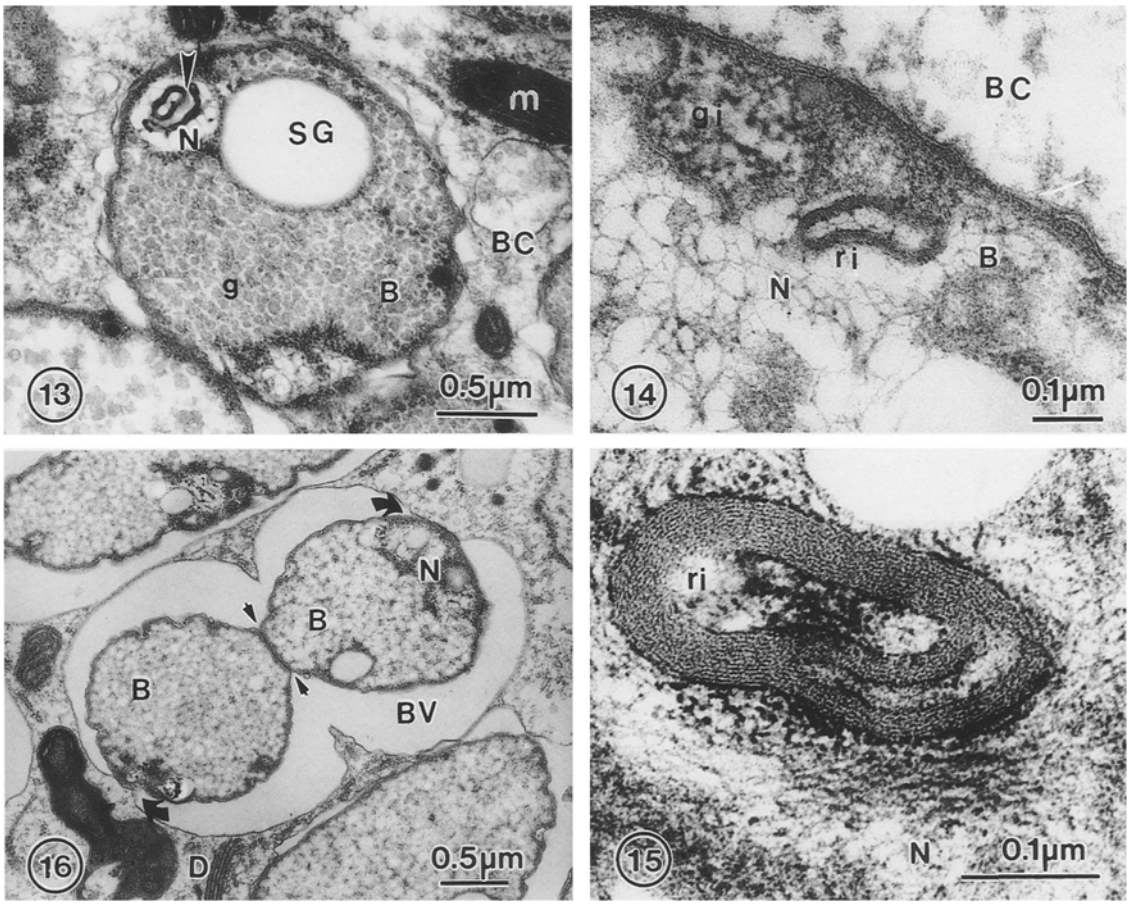
I. Ciliated and intermediary zone

In juvenile *C. orbicularis,* the overall structure of the ciliated part of the gill is very similar to that described by Ansell (1962) in *Venus striatula* (Da Costa, 1778) during the first part of its ctenidial development. In Veneridae as well as in most other Lamellibranchia, this development is followed by the development of an outer demibranch whereas in *C. orbicularis* and in other Lucinidae (Allen 1958), only the inner demibranch is to be found. The gill structure of the various species so far examined shows similar patterns of organization; they have a reduced ciliated zone with few specific variations and an important lateral zone with significant variations. The frontal, prolaterofrontal, eulaterofrontal, and lateral ciliary complements are functional in *C. orbicularis* as well as in other Lucinidae. Contradictory interpretations have been advanced for the organization of the intermediary zone. The large electron-lucent cell which borders the ciliated zone was classified as a "storage cell" by Reid and Brand (1986) and as an "intermediary cell" by Herry et al. (1989), but its ciliary complement has not been mentioned as yet. The fact that it is found to be PAS positive and alcian blue positive in *Parvilucina tenuisculpta* (Carpenter, 1864) by Reid and Brand (1986) but PAS negative and alcian blue negative in *C. orbicularis,* implies a different content and possibly a different function. The function of the storage cell supposed by Reid and Brand (1986) was contested by Herry et al. (1989) who considered this cell to be the main part of the intermediary zone. The fact that this cell is obviously ciliated, not only in juvenile and adult *C. orbicularis* but also in adult *Loripes lucinalis* (Lamarck, 1818) (Fig. 1D in Herry et al. 1989), is not consistent with a transitory ciliation. The abundance of mitochondria and the aspect of the cell,



which closely resembles the first ciliated lateral cell, indicate that this large clear cell is, in fact, part of the functional group of lateral ciliated cells.

The non-ciliated intermediary cells have been considered to play a role in the formation of new bacteriocytes by Herry et al. (1989). However, no formal proof of this hypothesis has yet been offered. The apposition of the gill filaments that we observed during the first stage of their fusion in *C. orbicularis* (Fig. 6) indicates that these intermediary cells play a role in binding the adjacent fil-



Figs. 13–16 *C. orbicularis,* bacteria in the bacteriocytes

Fig. 13 TEM of an endocellular bacterium (*B*), tightly enclosed inside the bacteriocyte vacuole. The bacterial cytoplasm contains numerous non-membrane-bound inclusions, probably glycogenic storage (*g*). The nuclear area (*N*) contains an unidentified ribbon-like inclusion *(arrowhead)* with a ring or spiral shape. The large electron-lucent vacuole is a periplasmic sulphur globule (*SG*) frequent in sulphur bacteria. The bacteriocyte (*BC*) contains various organelles such as mitochondria (*m*)

Fig. 14 Detail of a bacterium showing a smaller periplasmic granular inclusion (*gi*). The nuclear area (*N*) contains a ribbon-like inclusion (*ri*)

Fig. 15 Higher magnification of a ribbon-like inclusion (*ri*) which appears to be constituted of a variable number of alternately dark and clear laminae

Fig. 16 Dividing bacterium (*B*) with a hyaloplasm devoid of storage inclusions. The large vacuolar space around the bacterium does not seem to be an artifact of fixation as tight or large vacuolar spaces (*BV*) may be observed in the same host cell. Some special contacts keep the bacteria affixed to the vacuolar membrane *(curved arrows)* near the nuclear areas. Small arrowheads show the dividing plane between bacteria

Figs. 8–12 *C. orbicularis,* bacteria in the bacteriocytes

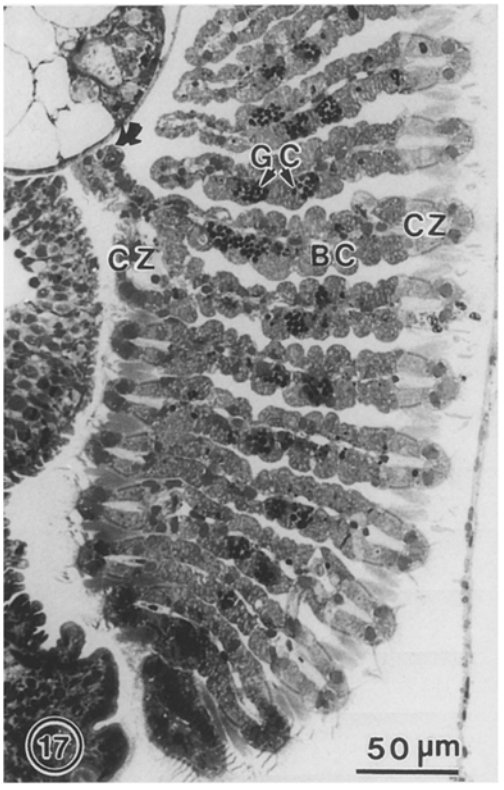
Fig. 8 TEM of the abfrontal part of a filament in the suprabranchial area of a juvenile. Notice that only a single bacterium (*b*) can fit in the height of bacteriocyte (*BC*); bacteria are salient in the microvilli border (*mv*). The basal nucleus (*N*) is as large in such small cells as it is in much larger bacteriocytes of older individuals. A hemocyte (*HC*) is in close contact with the basal membrane inside the blood lacuna (*BL*). A dark homogeneous granule (*G*) is part of a "granule cell"

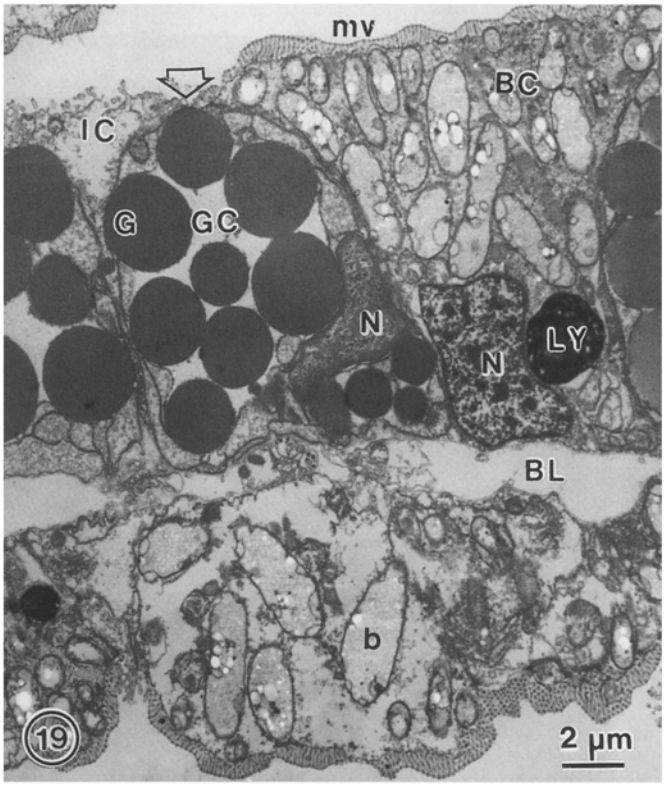
Fig. 9 TEM of a bacterium (*b*) located inside the microvilli border (*mv*). Notice the two membranes typical of gram-negative bacteria (between *arrowheads*)

Fig. 10 At the medium level of the filament, the bacteriocyte height is larger and the bacteriocyte (*BC*) accomodates many more bacteria (*b*) than in the abfrontal part shown on Fig. 8. The basal membranes (*BM*) are convoluted in their contact areas which partition the blood lacuna (*BL*); dark heterogeneous granules are lysosomal residual bodies (*LY*) located at the bases of bacteriocytes

Fig. 11 Bacteria (*b*) occupy most of the volume in the bacteriocyte of a small adult. However, there is some space left for hyaloplasm and normal organelles, such as mitochondria (*M*), dictyosomes (*D*), and scarce flat reticulum cisterns (*R*); large pigment granules display a more or less heterogeneous aspect (*PG*)

Fig. 12 In bacteriocytes (BC) of small adults, bacteria (*b*) are engulfed and destroyed in lysosomal structures (*LY*)


GC
CZ
CZ
BC
17
50 μm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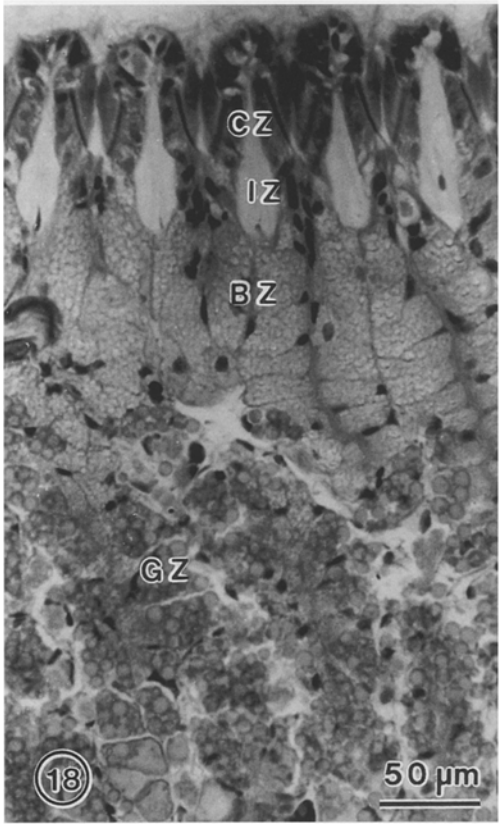
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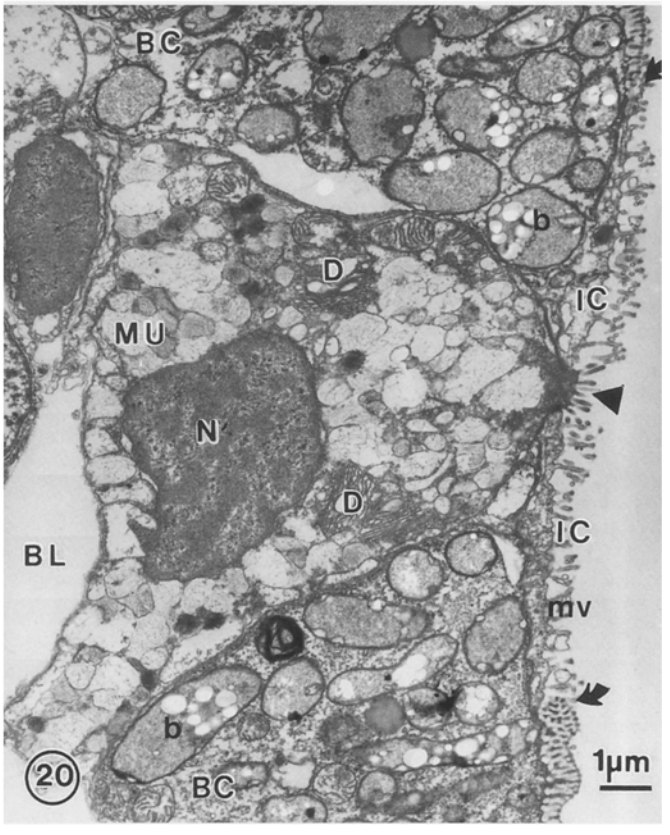
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aments together and thus form channels similar to those described in *Lucinoma aequizonata* (Stearns, 1890) by Distel and Felbeck (1987).

II. Lateral zone

The different cell types found in the lateral zone within various species have also led to contradictory interpretations. In *C. orbicularis,* most of the volume of the bacteriocytes is occupied by bacteria except for a relatively small basal nucleus and a large basal inclusion which is characterized by its heterogenous structure. Such large inclusions, which appear golden-brown when observed in light microscopy without staining, have been identified in the gill tissue of Lucinidae by Allen (1958). They were considered by Fisher and Hand (1984), to be pigment granules, containing hemoglobin, in *Lucina floridana* Conrad, 1833, which is consistent with Read's diagnosis (1962) in *Phacoides pectinatus* (Gmelin, 1791) where histochemical staining of iron was supposed to indicate the presence of hemoglobin. However, the histochemical technique, used by Read (1962) and by Fisher and Hand (1984) is more likely to detect a breakdown product of hemoproteins, which may be abundant in lysosomes, than to detect hemoglobin. Giere (1985) and Distel and Felbeck (1987) interpreted similar inclusions as being lysosomal residual bodies characterized by myelin figures, dark concretions and dense aggregates of membranes. A cytoenzymological study of adult gill cells, which is beyond the scope of the present paper, will be necessary to identify the real location of hemoproteins in the gill cells of Lucinidae.

The size of the bacteriocytes is correlated with the abundance of bacteria, whereas the size of the nucleus is not modified by the increase in cell size which ranges from a few µm to more than 35 µm in large adults. This increase may be related either to the multiplication of the bacteria inside the bacteriocytes, as some dividing stages have been identified, or to the endocytosis of an increasing number of bacteria, as the relationships of bacteria with the microvillar apical surface of the bacteriocytes suggest. However, no bacteria of the same type have been identified in the extracellular space and the two hypotheses remain equally probable.

The endocellular bacteria contain various inclusions. Large periplasmic vacuoles, considered as being the location of sulfur globules secreted by the bacteria (Shively 1974; Vetter 1985; Distel and Fellbeck 1987) are typical of sulfur-oxidizing bacteria. Their apparent content is correlated with the degree of dissolution of sulfur by chemicals during fixation and dehydration. Some conspicuous hyaloplasmic non-membrane-bound inclusions of a diameter ranging from 50 to 80 nm in mean diameter and with a polyhedral irregular shape, are similar to those interpreted by Giere (1985) as being carboxysomes according to Shively's study (1974), but carboxysomes described by Shively (1974) are given as being much larger, 90–500 nm, membrane-bound inclusions, located in the nucleoplasmic region of the cell. The much smaller, non-membrane-bound inclusions observed in the symbiotic bacteria of *C. orbicularis* are more likely to be glycogen inclusions more or less abundant according to the metabolic status of bacteria. The annular structure located in the nuclear area of the bacteria has not been described previously. It is neither similar to a mesosomal structure, such as described in gram-positive bacteria, nor to the extensive membrane system characteristic of methylotrophic bacteria and its nature and function are unknown at the moment.

The intercalary cells, having almost no characteristic organelles, have been supposed by Reid and Brand (1986) to replace old bacteriocytes. However the location of their nucleus, in the apical part of the cell, is not consistent with that hypothesis. In *C. orbicularis,* the trumpet-shaped intercalary cells encroach slightly upon the apical part of the bacteriocytes which are covered by microvilli being exposed to sea water through their large apical surface. The intercalary cells do not limit, in that case, the direct contact of the bacteriocytes with sea water as it was considered by Distel and Felbeck (1987) to be the case in all the Lucinidae.

Unusual "granule cells" have been identified in *C. orbicularis.* Their granules look quite similar to the spherical globules which have a homogeneous content as described in the intercalary cells of *Lucina costata* d'Orbigny, 1842 [= *Codakia costata* (d'Orbigny, 1842)] by Giere (1985) and supposed to be mucous droplets or

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Figs. 17–20 *C. orbicularis,* cellular components and organization of the gill filament

Fig. 17 LM of the ventral part of the gill in a 2-mm-long juvenile. The line of fixation of the gill to the body wall is marked by a *curved arrow.* The two ciliated zones (*CZ*) are linked by a continuous lateral zone in which bacteriocytes (*BC*) are intermingled with granule cells (*GC*)

Fig. 18 LM of the gill of a 15-mm-long adult individual. The lateral zone is divided into a discrete granular zone (*GZ*) and a bacteriocyte zone (*BZ*), separated from the ciliated zone (*CZ*) by a more elongated intermediate zone (*IZ*) than in the gill filaments of juveniles

Fig. 19 TEM of the gill filament of a 2-mm-long juvenile with typical "granule cells" (*GC*) adjacent to a bacteriocyte (*BC*). The bacteriocyte has a large apical pole, being covered by microvilli (*mv*) of uniform aspect enmeshed in a fibrous glycocalyx, a basal nucleus (*N*) and a lysosomal inclusion (*LY*). Bacteria (*b*) occupy most of the cell volume. The granule cell (*GC*) has a narrow apical pole *(white arrow),* a basal nucleus (*N*) but no lysosomal inclusion; most of its cell volume is occupied by large homogeneous proteinic granules (*G*). Between the two granule cells, an intercalary cell (*IC*) is characterized by a narrow base and a large apical region with uneven microvilli without a glycocalyx; its nucleus is not in this plane of section. The blood lacuna (*BL*) of the filament is irregularly partitioned

Fig. 20 TEM of a gill filament of a 2-mm-long juvenile. A typical mucocyte has a narrow neck (marked by a *triangular arrow*) between the enlarged apical poles of intercalary cells (*IC*) and bacteriocytes (*BC*). Except for the basal nucleus (*N*), the most conspicuous structures of the mucocyte are numerous dictyosomes (*D*) and clear mucous granules (*MU*); *curved arrows* show the limits between uneven microvilli (*mv*) of the intercalary cells (*IC*) and those of the bacteriocytes (*BC*) enmeshed in the glycocalyx

pigment granules. A similar type of cell has been described as "storage cells" in *Myrtea spinifera* Montagu, 1803, by Southward (1986). Southward also describes some heterogenous granules in *Lucinoma borealis* (Linné, 1767) and assumes that both inclusion types, which have been supposed to be proteinic, have a similar function. This type of "storage cell" is present in only a few species of Lucinidae. Some very similar inclusion bodies, interpreted by Fiala-Medioni et al. (1986) in *Bathymodiolus* sp. as being dense mucous droplets, have not been described in other deep-sea bivalves. Herry and Le Pennec (1987), who have not, apparently, had the opportunity to observe such cells, conclude that the storage cells described by Southward were "modified bacteriocytes" with a high level of reserves originating from the lysosomal destruction of the bacteria. However, the micrographs provided by these authors show typical lysosomal residual bodies, frequent in bacteriocytes, which are quite different from the granules identified in other cells in *Lucina costata* by Giere (1985), in *Myrtea spinifera* by Southward (1986), or in *Bathymodiolus* sp. by Fiala-Medioni et al. (1986) and in *C. orbicularis*. The extreme case of *C. orbicularis,* where these "granule cells" are particularly abundant, as well as the use of juveniles to clarify the spatial relationships between gill cells, leaves no doubt as to the existence of a cell type distinct from bacteriocytes as well as from intercalary cells. In view of their homogeneous osmiophilic granules and of the histochemical information obtained, it is possible to rule out the idea that the granule cells may be mucocytes as supposed by Giere (1985) as well as by Fiala-Medioni et al. (1986). However, not having, as yet, enough precise information, either on the nature and origin of their proteinic content, or on their function, we prefer to consider them as "granule cells" rather than "storage cells" as they were denominated by Southward (1986). Whatever their role, the existence of this cell type, more or less abundant, in a limited number of species, would suggest an evolution in the relationship between the bacteria and their host. Their high sulfur protein content and the abundant proteinaceous fluid which occupies the interlamellar space and is expelled through the exhalent siphon in adult's gills, are indicative of their implication in the turnover of sulfur between the bacteria and the host cells either in a detoxification or in a nutritional pathway.

Another remarkable feature of the gill structure of *C. orbicularis* is the abundance of mucocytes. In the various species of Lucinidae studied, the mucocytes are described as scarce in *Lucinoma borealis* (see Southward 1986), very scarce in *Loripes lucinalis* (see Herry et al. 1989), and even non-existent in *Myrtea spinifera* (see Southward 1986). This is not the case with *C. orbicularis* where the mucocytes are to be found here and there along the gill filaments in juveniles and intermingled with the granule cells in the internal zone in older individuals. Their distribution becomes uneven with growth and their abundance reaches its maximum in the posterodorsal region of the gill. Such an unusual abundance of mucocytes may be either related to the large size of the species or indicative of a response to its habitat.

In conclusion, the ultrastructural study of the gill filaments of *C. orbicularis* allows the emphasis of a more diversified cellular structure of the lateral zone than described in other symbiont-bearing bivalves. Bacteriocytes are prevalent in the structure of the gill filaments in juveniles. However, they are progressively restricted to the outermost part of the lateral zone while the innermost zone is occupied by two secretory cell types which become prevalent in the adult's gill. Both are considered to play a role in the sulfur detoxification process; moreover the cystine-rich protein content of the granule cells may be considered as a metabolic storage of sulfur compounds more extensive than in other species. However, the fact that similar granule cells have been identified in the deep-sea bivalve *Bathymodiolus* sp. supports the idea that such a storage is not restricted to some species of Lucinidae but may have a larger functional significance.

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