Acta Zoologica
KUNGL. VETENSKAPS AKADEMIEN
Published for The Royal Swedish Academy of Sciences and The Royal Danish Academy of Sciences and Letters

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Journal:	*Acta Zoologica*
Manuscript ID:	AZ--02-2014-0011.R2
Manuscript Type:	Original Manuscript
Date Submitted by the Author:	n/a
Complete List of Authors:	Alvarez Nogal, Rafael; Universidad de León, Molecular Biology Molist, Pilar; University of Vigo, Functional Biology and Health Science
Keywords:	Haliotis, ultrastructure, lectins, shell, mantle

The outer mantle epithelium of *Haliotis tuberculata* (Gastropoda Haliotidae): an ultrastructural and histochemical study using lectins

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"Mantle epithelium of the adult abalone"

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Abstract

The mantle of mollusks has been the subject of many studies since it is the organ that forms the shell. Microscopic studies in particular focus on the outer mantle epithelium, but few studies address this epithelium in a histochemical way. In the present paper the outer mantle epithelium in adult specimens of *Haliotis tuberculata* is studied, that is, in specimens involved in maintaining and repairing the shell rather than in generating it. The epithelial cells are studied by scanning (SEM) and transmission electron microscopy (TEM), and by histochemical techniques, including the use of lectins for their biochemical characterization. The epithelium is composed of ~~posseses~~ pigmented epidermal cells with small microvilli and junctional complexes. It furthermore contains~~'s also composes by~~ a few ciliated cells, as well as two types of secretory cells which differ in the ultrastructural appearance of their secretory granules and their glycoconjugate content. Histochemical study shows secretory cells containing sulfated glycoconjugates such as glycosaminoglycans or mucins rich in N-acetylgalactosamine, and N-glycoproteins rich in fucose. Furthermore, the apical regions of the epidermal cells are positive for lectins that label fucose, mannose and N-acetylglucosamine. The role of epithelial cells in the synthesis of structural components of the shell is discussed.

Keywords: mantle, shell, lectins, ultrastructure, *Haliotis*

Introduction

The external soft body of the abalone *Haliotis tuberculata* Linnaeus 1758 consists of three different parts: the foot (lateral and ventral part), the epipodium (formed by papillae and tentacles) and the mantle (Fig. 1A). According to Sud et al. (2002) the mantle is composed of an external epithelium facing the shell and an internal epithelium facing the body cavity. Between these two epithelia there is a folded area called the periostracal groove. The nomenclature used to refer to parts of the mantle varies between authors, even within the same species. Thus, in *H. tuberculata* the "inner epithelium" of the mantle described by Sud et al. (2002) corresponds to the "inner circumpedal epithelium" described by Jolly et al. (2004) and to the "side foot epithelium" described by Bravo et al. (2012). The present study focuses on the outer mantle epithelium.

Within the outer mantle epithelium of *H. tuberculata*, Sud et al. (2002) distinguish two areas: the "tubular area" near the periostracal groove, and the rest of the area that is generically referred to as "outer epithelium". These structures correspond to the “distal portion of the outer fold” and the “proximal portion of the outer fold” of *Haliotis asinine* Linnaeus, 1758, respectively (McDougall et al. (2011). Histological differences between these areas were observed, which correspond to different regions with respect to the secretion of various components involved in the formation of the shell (Jolly et al. 2004; McDougall et al. 2011). It should be kept in mind that the "tubular area" presents different stages of development depending on the age of the specimen (Jolly et al. 2004; McDougall et al. 2011); it is well-developed in young individuals whereas in adults it is poorly developed or absent altogether (Sud et al. 2002; Jolly et al. 2004; McDougall et al. 2011). In the present work adult specimens are studied, and therefore the tubular area is not studied.

The mantle of mollusks has been the subject of many studies as it is the organ that forms the shell (Wilbur 1964, 1972). The mineralization of the shell is controlled by the epithelial cells of the external mantle: they regulate both the secretion of the organic matrix and the movement of inorganic ions into the

mantle space (Saleudin 1976; Simkiss and Wilbur 1989; Jolly et al. 2004). The components of the organic matrix (consisting of a complex mixture of proteins, glycoproteins, polysaccharides and lipids) and the mineral precursors are secreted by the mantle cells (Addadi et al. 2005).

While most of the work in gastropods focuses on the biochemical study of the organic matrix of the shell or on the ultrastructural study of the mantle cells, few studies ~~had been~~ address~~ed to consider~~ the histochemistry of the outer epithelium of the mantle (Bielefeld et al. 1993b; Yi Yan et al. 2004; Di et al. 2012).

The aim of the present paper is to provide an ultrastructural and histochemical description of the outer epithelium of the mantle of *H. tuberculata*. It aims to characterize –by using lectins– the chemical nature of the secretory products of the different epithelial cells involved in the generation of the shell. Lectins are useful tools because of their ability to bind to specific sugars, sugar linkages or oligomers in complex carbohydrates (Spicer and Schulte, 1992).

Most of the work done in the genus *Haliotis* ~~have been~~ focuse~~d~~ on young specimens, which present much activity in the outer mantle epithelium associated with formation of the shell (Jolly et al. 2004; McDougall et al. 2011). In the present study, done on adult specimens, we shed~~s~~ light on the activity of mantle cells in relation to maintenance and repair of the shell.

Material and Methods

Four adult specimens of *Haliotis tuberculata* of about 12 cm long were collected in Vigo (Spain) in March and October of 2009. They were transported in sea water with aeration to the laboratory, where they were anesthetized with 5% $MgCl_2$. They were dissected to obtain mantle samples for both optical microscopy and electron microscopy. All procedures for animal experiments were in accordance with the rules of the animal care committee of the Autonomous Government of Galicia and according to the guidelines of the European Community.

Light microscopy

Samples were fixed in formaldehyde for 24-48 hours at room temperature, embedded in paraffin, and 8 µm sections were cut. Sections were stained with: 1: alcian blue (AB) at different pH values (2.5, 1.0 and 0.5) to demonstrate the presence of acidic glycoconjugates; 2: high iron diamine combined with alcian blue (HID/AB) to distinguish between acidic, sulphated and carboxylated glycoconjugates; 3: periodic acid Schiff

reagent (PAS) for detecting glycoconjugates with neutral monosaccharides and sialic acid; and 4/ to see if the PAS-positive components correspond to glycogen, the amylase test was done using rat liver sections as controls.

Lectins. Lectins were labeled with digoxigenin (DIG) or with biotin. For DIG-labeled lectins, sections were incubated for 2 hours at room temperature with: L1/ GNA (*Galanthus nivalis,* L. 1753), specific for mannose; L2/ SNA (*Sambucus nigra,* L. 1753) and MAA (*Maackia amurensis,* Rupr. 1856), both specific for sialic acid; L3/ PNA (*Arachis hypogaea,* L. 1753) to recognize the sequence galactose (beta 1-3) N-acetylgalactosamine; or L4/ AAA (*Aleuria aurantia,* (Fries) Fuckel 1870), specific for fucose. The following lectins were labeled with biotin: L5/ WGA (*Triticum vulgare,* Vill. 1786), specific for N-acetylglucosamine; L6/ DBA (*Dolichos biflorus,* L. 1753), specific for N-acetylgalactosamine; L7/ UEA I (*Ulex europaeus,* L. 1753) and LTA (*Lotus tetragonolobus,* L. 1753), both specific for fucose; and L8/ ConA (*Canavalia ensiformis,* (L.) DC. 1825), specific for mannose. In this case sections were incubated overnight at 4 ºC.

All lectins were used at a concentration of 10 µg/mL except for MAA and SNA, which were used at three concentrations (10, 25, and 50 µg/mL). The specificity of the lectins was assessed by two controls: a: replacement of the lectin by buffer; and b: preincubation of the lectins with their specific mono/oligosaccharide. In addition, a desulphation treatment was done before incubation with lectins to remove sulphate ester groups. All staining protocols were carried out according to Kiernan (2008) and Molist et al. (2011) and are described in more detail in Bravo et al. (2012).

Electron microscopy.

Scanning electron microscopy (SEM). Pieces of mantle of 3-4 mm^2 were fixed for four hours in 2.5% glutaraldehyde in filtered seawater. They were then washed in cacodylate buffer, dehydrated in ethanol and isoamyl acetate and critical point-dried with CO_2. Finally, they were coated with gold for observation under a Philips XL 30 SEM.

Transmission electron microscopy (TEM). Mantle samples were fixed in 2.5% paraformaldehyde / 2% glutaraldehyde in cacodylate buffer for 3 hours. They were postfixed in osmium tetroxide, dehydrated in acetone, and embedded in Spurr resin. Semithin (1 µm) sections stained with toluidine blue were studied under a bright-field light microscope to locate the area for ultrastructural study. Ultrathin (70 nm) sections were contrasted with uranyl acetate and lead citrate and examined with a Jeol 1010 TEM.

Results

General description and Light microscopy

Dorsal to the epipodium there is a space covered by the shell that is called the pallial, or mantle, cavity (Fig. 1A). The epithelium lining the cavity extends rostrocaudally around the animal's body and has a pale yellow color. The most part of the mantle epithelium is a simple columnar epithelium with microvilli. It also contains very few ciliated cells and two types (B and G types) of proposed~~ly~~ secretory cells (Fig. 1B). Their proposed secretory function is ~~d interprete~~based on two main criteria, the histochemical reaction of the cells (Fig. 2) ~~together with~~and their ultra~~e~~structural morphology (Fig 3). ~~**As they have been observed by SEM, TEM and histochemical methods.**~~

The epithelial cells contain different types of glycoconjugates (Fig. 2). Staining with AB at pH 2.5 shows a moderate distribution of secretory cells (Fig. 2A) showing the presence of acidic glycoconjugates; staining decreases when using AB pH 0.5. These cells are no longer observed when the sections are subjected to desulphation treatment. Staining with HID/AB (Fig. 2B) shows secretory cells stained black or dark brown, which indicates the presence of glycoconjugates containing sulfated sugars. Both types of acidic glycoconjugates (carboxylated and sulphated) were not observed within the same cell. Taken together, these results indicate that the secretory cells contain mostly glycoconjugates with O-sulphated groups, but that they are not heavily sulphated.

A moderate amount of secretory cells were stained intensely with PAS (Fig. 2C), which –after doing the amylase test to exclude the possibility that it involved glycogen – shows that they contain glycoconjugates with neutral monosaccharide residues and/or sialic acid.

Double AB pH 2.5 / PAS staining shows 2 types of secretory cells (stained blue and red), which present glycoconjugates containing sulphated acidic and neutral sugars, respectively (Fig. 2D). With the combined AB pH 0.5 / PAS staining, AB-positive cells are no longer observed, indicating that the sulphated glycoconjugates do not contain strongly sulphated groups (Fig. 2E).

Lectins. The results with biotin or digoxigenin labeled lectins were classified into six groups according to the monosaccharide for which they are specific: fucose, mannose, N-acetylglucosamine, galactose, N-acetylgalactosamine and sialic acid.

For the detection of fucose, AAA, LTA and UEA-I were used. AAA binds strongly to secretory cells after desulphation (Fig. 2F). LTA labels the apical surface of the microvillous epidermal cells and spreads with less intensity into their apical cytoplasm (Fig. 2G). Very few secretory cells are labeled with UEA-I and the intensity of staining is very low (Fig. 2H). For the detection of mannose, GNA and Con A were used. The staining pattern was similar with both lectins, and they uniformly labeled the apical portions of the microvillous epidermal cells with strong intensity (Fig. 2I, J). Furthermore, GNA staining intensity increased after desulphation.

The presence in the secretory cells of galactose linked to N-acetylgalactosamine could be detected with PNA only after desulphation (Fig. 2K). N-acetylglucosamine was detected with WGA, and it was observed in the apical regions of the microvillous epidermal cells (Fig. 2L). However, the terminal N-acetylgalactosamine that is recognized by DBA is only seen in a small number of secretory cells (Fig. 2M), although this number increases slightly after desulphation (Fig. 2N). SNA and MAA were used for the detection of sialic acid, and no labeling of any cell type was seen.

In conclusion, the epithelial cells of the outer mantle have specificity with respect to the glycoconjugate they contain. In this respect, a variety of saccharides such as L-fucose, mannose and N-acetylglucosamine form part of the luminal surface and the apical cytoplasm of microvillous epidermal cells. Nevertheless, secretory cells contain specific glycoconjugates, i.e. L-fucose and N-acetylgalactosamine. L-fucose is a neutral monosaccharide that may be detected by PAS staining, and it is therefore an integral part of glycoproteins. N-acetylgalactosamine is a sulphated monosaccharide that may be detected with alcian blue staining, and it is therefore a constituent of proteoglycans. Thus, the specificity or affinity of the secretory cells of the mantle for certain sugars is evidenced by both conventional histochemical techniques and by the use of lectins.

Electron microscopy.

SEM observation shows that the surface of the mantle is slightly folded (Fig. 3A) and covered with microvilli. On the outer surface of the cells different sized secretory vesicles can be distinguished that are

fusing with each other to form a more uniform layer corresponding to the mucus layer. Between the microvilli openings of the secretory cells are observed (Fig~~.~~s 3B, C).

With the TEM, three types of cells can be distinguished in the epithelium: 1: microvillous epidermal cells, 2: ciliated cells, and 3: secretory cells (Figs. 1B, 3D).

The microvillous epidermal cells are columnar (Fig. 3D). They have very short microvilli (about 0.5 µm long) with poorly developed actin filaments (Fig. 3E). They also show junctional complexes between them: two apical adherens junctions followed by one unusual occluding junction seems to be crossed by thin ladder-like septa. Below the junctional complex and down to the basal lamina, the membranes of neighboring cells are slightly folded and the intercellular space is relatively wide (Fig. 3E)~~.~~. These cells contain variable amounts of varied content ~~pigments~~ (Fig. 3F), which accumulate as small grains or as tubular (membrane-like) structures that sometimes seem to roll up into a spiral (Fig. 3G). Grains and membranes intertwine and form electron-dense areas in the apical portion of the cytoplasm. They also have Golgi complexes in the cytoplasm (clustered mainly in the apical region) and numerous mitochondria (many of them close to the nucleus) (Fig. 3F). As the mantle has a pale yellow color, we ~~can~~ hypothes~~t~~ize that these cells contain some kind of pigment ~~that could~~ cause~~e~~ing this color ~~the mantleto~~ .

Ciliated cells are not abundant, and when present (Fig. 3H) contain ~~they have cilia with~~ cilia with the typical 9 +2 microtubule pattern characteristic of ciliated cells involved in the movement of particles.

Two types of proposed~~ly~~ secretory cells were observed (Fig. 3D): type B cells and type G cells. Type B cells contain medium-sized secretory vesicles with a granular material of homogeneous electron density. Type G cells also contain electron-dense vesicles. They also present a very well developed Golgi complex and an extensive RER in the ventrolateral portion of the cell. Two types of vesicles are observed: small, electron-dense vesicles associated with the trans face of the Golgi complex (fusion between these vesicles was not observed), and larger, less electron-dense vesicles located at the apex of the cell (Fig. 3I). Vesicles of the first type show a dense central area whereas the other vesicles show two regions with different electron densities, with denser material in one half than in the other half (Fig. 3I).

Discussion

In the present study conducted in adult specimens of *H. tuberculata*, the cellular composition of the outer mantle is described. Epidermal pigmented cells and two types of secretory cells are described: type B

and G cells, continuing the nomenclature of Bravo et al. (2012). There is also a fourth –very scarce– type of ciliated cell.

The presence of secretory cells in *H. tuberculata* is in disagreement with the observations of Sud et al. (2002), as they do not describe any type of secretory cell in the outer mantle of this species. On the other hand, McDougall et al. (2011) do establish the existence of two types of glandular cell –by ultrastructural studies– in *H. asinina*.

The so-called B type cell observed in the present study is a typical mucocyte. It is widely distributed in the foot epithelium of *H. tuberculata* (Bravo et al. 2012) and corresponds to what was described by McDougall et al. (2011) as A type cells in *H. asinina*. Furthermore, Sud et al. (2002) place this cell type in the inner mantle epithelium along with other secretory cells described previously in the side foot of *H. tuberculata*. The G type cell observed in the present study, however, is not equivalent to any previously described secretory cell in *H. tuberculata*.

Histochemical tests done in the present study show that each type of secretory cell contains a specific glycoconjugate. One type ~~–type B, most likely,~~ AB positive– is rich in acidic sulphated glycoconjugates. Labeling with lectins (PNA and DBA) shows the presence of N-acetyl-galactosamine and of the terminal sequence galactose-N-acetylgalactosamine as a part of the glycoconjugates. These monosaccharides are constituents of glycosamino-glycans and sulphated mucins. It has been shown that the glycosaminoglycans of proteoglycans (consisting of acetylhexosamines, sulphated or not) are not PAS-positive (Hooghwinkel and Smits, 1957, as cited by Kiernan, ~~(~~2008~~)~~). ~~– and sulphated mucins. glycosaminoglycansThese monosaccharides are constituents of~~These data are consistent with most biochemical analyses of mollusk shells, which indicate that sulphated carbohydrates are the major component of the matrix glycoconjugates (Marxen and Becker 1997; Marxen et al. 2003; Marie et al. 2007; Pavat et al. 2012). Given their ~~By its~~ content and ultrastructural morphology ~~–~~similar to mucocytes, type B ~~could~~cells may correspond to AB positive cells~~-~~.

The other type of secretory cell ~~–ype G, most likely, t~~PAS ~~–~~positive– contains neutral glycoproteins. Neutral sugars like L-fucose may be detected by PAS staining, and ~~it is~~they are therefore an integral part of glycoproteins (Leblond et al., 1957, as cited by Kiernan, 2008). Labeling with lectin AAA indicates the presence of a terminal fucose and of fucose attached to N-acetylglucosamine residues of N-glycoproteins

(Osawa and Tsuji 1987). This kind of fucosylation has been described in different aquatic species of gastropods (Guternigg et al. 2007) only in the case of AAA lectin. It has been known since the studies of Crenshaw (1972) in the marine bivalve *Mercenaria mercenaria* (Linnaeus 1758) that glycoproteins involved in crystallization processes (Falini et al. 1996) present in the organic matrix of the shell have a high affinity for calcium ions. Dense vesicles in type G secretory cells could may correspond to glycoproteins, which are PAS positive.

The microvillous epidermal cells of the outer mantle of *H. tuberculata* –studied in the present work– have very short microvilli and poorly developed actin microfilament bundles. They display an inconspicuous brush border. In the side foot and the inner mantle epithelium of the same species, however, epidermal cells have a prominent brush border (Sud et al. 2002; Bravo et al. 2012). Perhaps the brush border observed in the present study is particularly specialized in increasing flexibility, somehow facilitating the movement of substances into the mantle cavity.

The occluding junctions transversed by thin ladder-like septa are reminiscent of the so-called septate junctions characteristic of invertebrates (Fawcett 1981). In the freshwater snail *Biomphalaria glabrata* (Say, 1818) it has been demonstrated that the septate junctions of the outer mantle epithelium do not allow the passage of proteins into the mantle space, but they do allow calcium ions in low concentrations to diffuse freely (Bielefeld et al. 1993a). Histological data of Fleury et al. (2008) also point to a paracellular calcium transport. The occluding junctions between epithelial cells in vertebrates close passage through the intercellular space of the epithelium (Fawcett 1981). On the other hand, the adherens junctions observed in this study and others described in other prosobranchs and bivalves (Bielefeld et al. 1992), leave large intercellular spaces that would allow the transport of calcium ions (Simkiss and Wilbur 1989) from the connective tissue into the extrapallial space. Taken together, the observed junction complexes may contribute to the maintenance of high metabolic activity as they facilitate the interaction between the internal and the external environment.

The epidermal cells of the mantle are associated with pigmentation of the shell and soft tissues of the prosobranchs (Fox 1983). Thus, melanin was histologically characterized in the epithelium of the side foot of *Patella vulgata* (Linnaeus 1758) (Grenon and Walker 1978) and of *H. tuberculata* (Bravo et al. 2012). In both cases the pigment is in the form of membrane-bound vesicles or melanosomes. In the present study another

probablеy form of pigment storage is observed, in small grains interspersed with structures that are arranged as spiral membranes. Similar structures have been described in the epithelium of the cephalic tentacles of *Diodora* sp. (Kunz and Haszprunar 2001), although in that study they were described as secretory granules belonging to mucous cells. However, in the present study they were not observed in any secretory cell.

The lectins used in the present study revealed that the microvillous epidermal cells contain fucose, mannose and N-acetylglucosamine in areas rich in pigments. Because of its relationship with chitin, the presence of N-acetylglucosamine is of special interest. Chitin is formed by N-acetylglucosamine monosaccharide units linked by β 1-4 bonds, which is precisely the preferential binding site of the WGA lectin used (Spicer and Schulte 1992). Sulfated N-acetylglucosamine residues have also been described in the mantle of other gastropods such as *B. glabatra* (Bielefeld et al. 1993b). Biochemical analyses of some bivalve shells revealed the presence of chitin (Marie et al. 2007; de Paula and Silveira 2009) and it was proposed that chitin plays a structural role in the matrix of the shell (Levi-Kalisman et al. 2001).

It can be hypothesized that the pigment granules of the epidermal cells of the outer mantle also contribute to the formation and structure of the shell through chitin. The presence of a large number of mitochondria in the apical cytoplasm of these epidermal cells favors this hypothesis (Istin and Masoni 1973).
In view of all the results observed in this study, we propose a hypothesis that can be tested by future research. For one thing, it must be established whether there is a correspondence between juvenile / adult specimens and their different types of glands. At the same time it must be established whether juvenile / adult specimens correspond with specimens involved in shell formation / specimens involved in maintaining and restoring the shell. In addition, the role of epidermal cells of the outer mantle must be specified: they are either only involved in the pigmentation of the shell, or they are additionally involved in the synthesis of its structural components. It must also be determined whether epidermal cells similar to the ones observed here are also located in other epithelia of *H. tuberculata* or in other species.

Our study sheds new light on the molecular components contained and secreted by mantle cells to form the shell. However, despite the large number of studies on the mantle, the mechanisms and cell types involved in shell formation are still not very well defined.

ACKNOWLEDGMENTS

The authors thank Inés Pazos and Suso Méndez from the Electron Microscopy Unit of CACTI (University of Vigo) for technical assistance and SEM and TEM facilities. They are also grateful to Ron Hartong for critical reading, correction and translation of -the manuscript.

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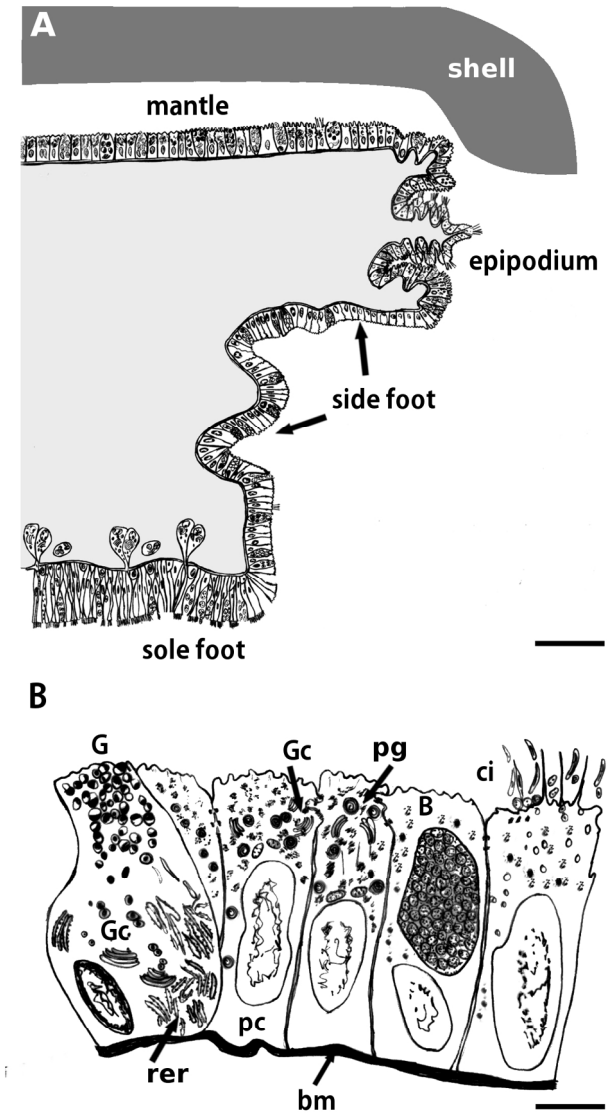
FIGURE LEGENDS

Figure 1. A. Squematic representation ~~drawings~~ showing the different parts of the external body *Haliotis tuberculata*. *B.* ~~*(1)*~~ Squematic drawing ~~and~~ of the outer mantle epithelium based on figure 3 ~~(B)~~ which consists ~~in~~ of pigmented cells (pc), ciliated cells (ci) and type B and G secretory cells (B, G) lined by the basal membrane (bm). Golgi complex (Gc), rough endoplasmic reticulum (rer), pigmented granules (pg). Bars: A~~a~~. 5 µm, B~~b~~. 40 µm

Figure 2. Outer mantle epithelium of *Haliotis tuberculata*. Histochemistry. **A**. Secretory cells positive for AB at pH 2.5, containing acidic glycoconjugates. **B**. Combining the above staining technique with HID staining, the same cells are stained black indicating the presence of acidic sulfated glycoconjugates. **C**. PAS-positive secretory cells showing the presence of neutral glycoconjugates. **D**. Combined PAS / AB pH 2.5 stain contrasted with hematoxylin. The two types of secretory cells are stained red and blue, respectively; no mixed colors were observed. **E**. Combined PAS / AB pH 0.5 stain. Only one of the secretory cell types is stained, suggesting the absence of heavily sulfated acidic glycoconjugates in the other cell type. **F**. AAA lectins label secretory cells after desulfation, indicating that they contain fucose. **G**. Fucose recognized by LTA lectins labels the apical surface of the epidermal cells. **H**. Few secretory cells show fucose labeled with UEAI. **I-J**. Mannose is visualized in the apical areas of the epidermal cells with the lectins Con A (**I**) and GNA after desulfation (**J**). **K**. PNA positive secretory cells after desulfation (PNA recognizes galactose). **L**. Apical regions of epidermal cells labeled with WGA (WGA recognizes N-acetylglucosamine). **M-N**. Secretory cells labeled with DBA (DBA recognizes N-acetylgalactosamine). The number of stained secretory cells increases after desulfation (**N**). **F, J, K**. Digoxigenin-labeled lectins. **G, M**. Biotin-labeled lectins counterstained with hematoxylin. **H, I, L, N.** Biotin-labeled lectins. Bars: 200 µm.

Figure 3. Outer mantle epithelium of *Haliotis tuberculata*. Ultrastructure. **A**. General SEM view showing slightly folded outer surface (arrows). **B**. Detail of the surface epithelium coated with microvilli (mv). Note the different sized secretory vesicles (sv) from secretory cells (arrow). **C**. The vesicles fuse with each other to form the mucus layer (mu). **D**. General TEM view. Note the two types of secretory cells (types B and G) and the epidermal cells showing small microvilli (mv) and apical pigment granules (pg). Basal membrane (bm). **E**. Detail of the apical region of two adjacent epidermal cells. The junctional complex has two adherens junctions (aj) and a septate junction (sj). The microvilli (mv) are short and the cytoskeleton is poorly developed. **F**. The pigment of the epidermal cells is concentrated in the apical region where also several Golgi complexes (Gc) and some mitochondria (m) are observed close to the nucleus (n). **G**. Detail of the organization of the pigment in groups formed by small granules (arrow) or in the form of spiral membranes (arrowheads). **H**. Apical region of a ciliated cell (ci). The cilia have a basal body from which typical 9+2 microtubules originate. Cross sections of microvilli (arrows) on the surface of the epithelium confirm the poor development of the actin microfilaments **I**. Secretory cell type G. Secretory vesicles (arrows) associated with the Golgi complex (Gc) are changing size and electron density as they move toward the apical region (white

arrowheads). **A-C**. SEM. **D-I**. TEM. Bars: **A**. 50µm. **B**. 4µm. **C**. 5µm. **D**. 1 µm. **E**. 0,25µm. **F-H**. 1µm. **G** 100nm. **I**. 1µm.



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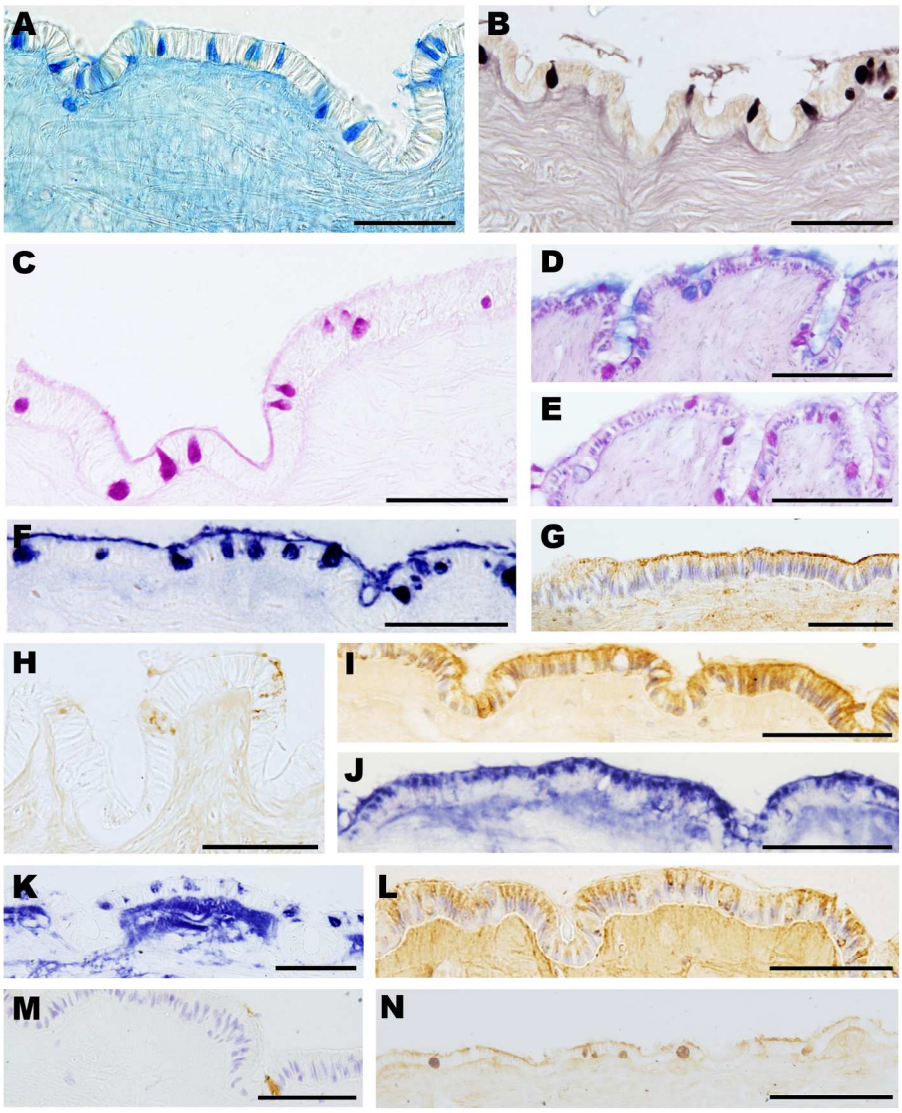

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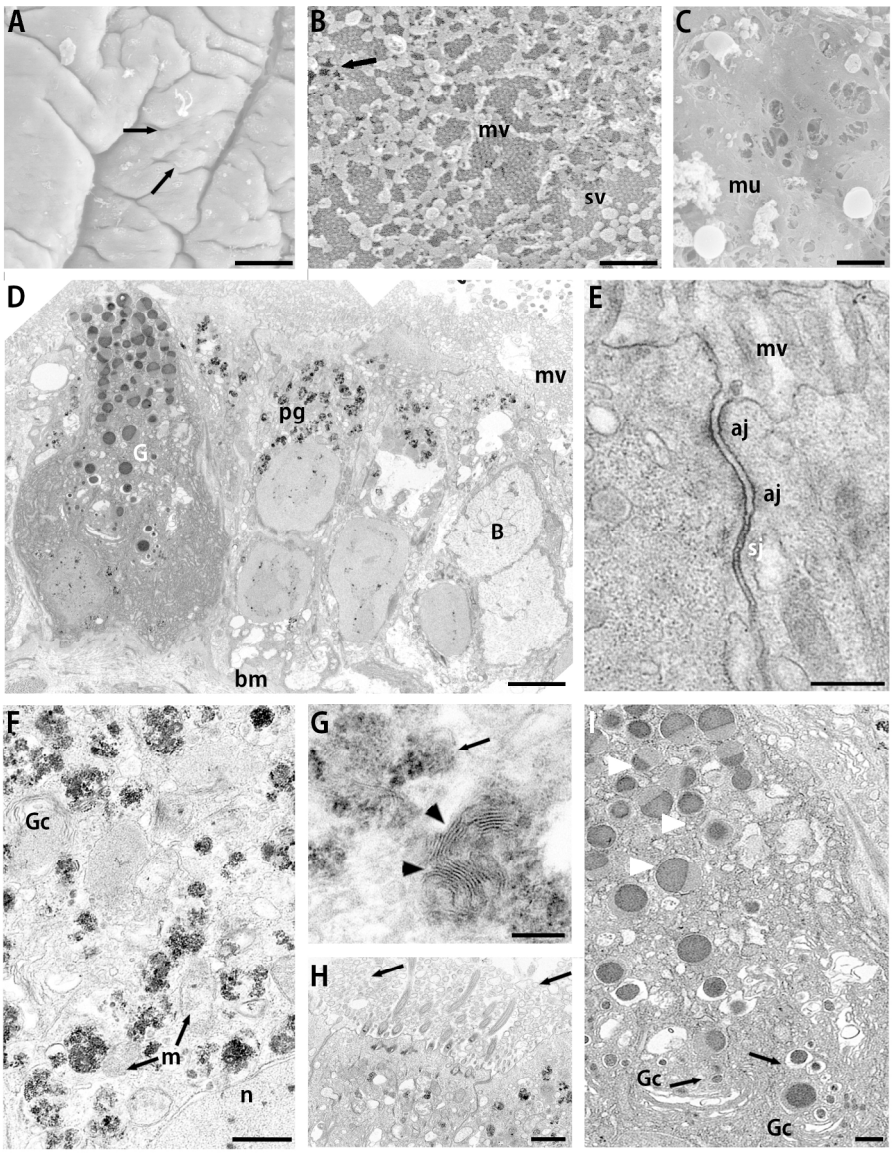

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