ISJ 6: S77-S86, 2009 ISSN 1824-307X

RESEARCH REPORT

Ultrastructural comparative analysis on the adhesive papillae of the swimming larvae of three ascidian species

G Dolcemascolo, R Pennati[1], F De Bernardi[1], F Damiani, M Gianguzza

Dipartimento di Biopatologia e Metodologie Biomediche, Sez. di Biologia e Genetica, Università di Palermo, Palermo, Italy
[1]Dipartimento di Biologia, Università di Milano, Milano, Italy

Accepted March 13, 2009

Abstract

This paper presents a preliminary report on the papillae of the swimming larvae of three ascidian species: *Ascidia malaca, Phallusia mammillata* and *Ciona intestinalis*. The investigations, carried out at ultrastructural level and at confocal laser microscope, have evidenced, in the adhesive papillae of the three studied species, three different cell-types: axial columnar cells, collocytes, sensory cells respectively. The adhesive papillae of *A. malaca* and *P. mammillata* show central axial columnar cells with long microvilli emerging from the apical edge and extending throughout the hyaline cap. Collocytes are elongated secreting cells, lying in middle-lateral side. Sensory cells have a cilium at the apical side and an axon proceeding from the basal side. The adhesive papillae of *C. intestinalis* present some differences in the ultrastructure of the axial columnar cells, which bear a big digitiform protrusion, extending throughout the hyaline cap and a lot of microtubules along the cell axis. The investigations, carried out at confocal microscopy, have evidentiated a clear fluorescence in the papillae of the three studied species and a network of nervous fibers projecting from the papillar base up to cerebral vesicle of the cephalenteron. The characteristic of simple and coniforme type and the adhesive and sensorial functions of adhesive papillae of three ascidian species examined are confirmed.

Key words: swimming larvae ascidiae; papillae; ultrastructure; comparative analysis

Introduction

Ascidians are marine invertebrates commonly known as sea squirts. At the adult stage, they are sessile and live in the shoreline, often fixed to the walls of harbours, to the keels and sides of boats and ships. The adult stage is preceded by a larval stage, during which the larvae are free swimming in the marine environment (swimming larvae). This stage lasts from 10 to 12 h, after which the ascidian larvae adhere to a substratum by means of adhesive material. The adherence process is due to the functional activity of adhesive papillae, organs of ectodermic origin, located in the anterior region of the cephalenteron (Grave, 1926; Millar, 1971; Cloney, 1977, 1979; Turon, 1991; Gianguzza and Dolcemascolo, 1994, 1997; Gianguzza *et al.,* 1999). In *Ascidia malaca* the three papillae of swimming larvae, like those of *Phallusia mammillata* (Sotgia *et al.*, 1998) and *Ciona intestinalis* (Burighel and Cloney, 1997) are simple, coniform and located at the vertices of a triangular field. Rarely, as in the larvae of *Eurherdmania claviformis* (Trason, 1957) and in numerous species of the *Didemnum* genus (Lafargue, 1976) papillae are two, located along a sagittal plane.

As widely reported in several morphological, ultrastructural and cytochemical investigations, main function of papillae of ascidian larvae is to secrete an adhesive substance which enables larvae to adhere to a substratum and start metamorphosis (Cloney, 1961, 1977, 1978, 1979; Millar, 1971; Turon 1991; Gianguzza and Dolcemascolo, 1994, 1997; Sotgia *et al*., 1998; Gianguzza *et al.,* 1999; Groppelli *et al*., 2003; Zega *et al*., 2005). According to classification scheme of Burighel and Cloney (1997), two types of adhesive papillae can be found in ascidian larvae: a) eversible papillae whose shape is rapidly changed after secretion of the adhesive material; b) non eversible papillae whose structure is maintained on secretion of the adhesive material. To the former type belong adhesive papillae with a very complex ultrastructural and

Corresponding author:
Giuseppe Dolcemascolo
Dipartimento di Biopatologia e Metodologie Biomediche
Sez. di Biologia e Genetica, Università di Palermo
via Divisi 83, 90133 Palermo, Italy
E-mail: dolcemas@unipa.it

functional organization [*Distaplia occidentalis* (Cloney, 1977), *Diplosoma listerianum* (Lane, 1973), *Diplosoma macdonaldi* (Cavey and Cloney, 1976), *E. claviformis* (Trason, 1957), *Eudistoma ritteri* (Levine, 1962)]. On the other hand, larvae belonging to *Ciona*, *Ascidia*, *Styela*, *Pyura* genera present papillae of the latter type characterized by a very simple ultrastructural and morpho-functional organization (Millar, 1971; Anderson *et al.*, 1976; Cloney and Cavey, 1982; Torrence, 1983; Cloney and Torrence, 1984; Turon, 1991; Gianguzza and Dolcemascolo, 1994, 1997; Gianguzza *et al.,* 1999). The adhesive function of papillae is ensured by secretory activity of peculiar cells known as collocytes. Larvae of some Molgulidae which are devoid of papillae can adhere to a substratum by means of the external layer, entirely adhesive, of the cephalenteron test (Berrill, 1950; Grave, 1926; Cloney, 1961). The secretion process of the adhesive material from the collocytes and the identification of chemical composition of secrete has been examined in the larvae of *A. malaca* by means ultrastructural and cytochemical investigations (Gianguzza and Dolcemascolo, 1997). This study has shown that adhesive secreted substance is of glycoproteic nature. Besides secretion, also a sensory function is attributed to the papillae of ascidian larvae. The sensory function is ascertained in eversible adhesive papillae with a very complex ultrastructural and morpho-functional organization. In *D. occidentalis*, for instance, Cloney (1977, 1978, 1979) reported that the sensory function is played by anchor cells, basal cells and, probably, by axial columnar cells. The presence of microvilli and cilia in the above cells enables them to exercise the sensory function through recognition of chemical and/or mechanical stimuli from the substratum. Eversible papillae with a sensory function have been found in larvae of *D. macdonaldi* (Torrence and Cloney, 1983) *Polysyncraton lacazei, Diplosoma spongiforme* and *Ecteinascidia turbinata* (Turon, 1991). Primary sensory neurons have been recently described by light microscopy and by histochemical and immunochemical techniques also in non eversible papillae with a simple coniform organization as those of Ascidiidae (Sotgia *et al.*, 1998; Groppelli *et al*. 2001, 2003), Cionidae (Takamura, 1998; Imai and Meinertzhagen, 2007a, b) and Styelidae (*Botrylloides leachi,* Pennati *et al.*, 2007*)*. Different neurotransmitters, like acetylcholine, dopamine and serotonin were also localized by immunohistochemistry in primary neurons of the papillae of some species and a mechanism was proposed by which these neurotransmitters may modulate the timing of metamorphosis (Groppelli *et al*., 2003; Zega *et al*., 2005; Pennati *et al*., 2007).

However, few papers reported the ultrastructure of the adhesive papillae with a simple, coniform and non-eversible organization (Gianguzza and Dolcemascolo, 1994, 1997; Gianguzza *et al.*, 1999). In order to fully describe their structure a comparative investigation has been carried out, in this paper, on the ultrastructural and morpho-functional organization of adhesive papillae of swimming larvae of three ascidian species: *A. malaca, P. mammillata* and *C. intestinalis* belonging to the order of Phlebobranchiata. These investigations have been carried out at scanning electronic microscopy (SEM), transmission electronic microscopy (TEM) and confocal microscopy.

Materials and Methods

Adult specimens of *Ascidia malaca*, *Phallusia mammillata* and *Ciona intestinalis* were collected in the Gulf of Palermo and in the harbours of Termini and Sciacca in July, September and October. They were transferred in an aquarium and maintained at 18-20 °C. Following gametes removal, fertilization occurred in Syracuse dishes containing pasteurized and filtered sea water. Hatching started 18 h after fertilization, at 18 °C. The swimming larvae (6-8 h after hatching) were withdrawn with a pipette and utilized for optical and electron microscopy investigations.

Scanning electron microscopy (SEM)

A. malaca swimming larvae were fixed in PAF (picric acid-formaldehyde) 1200 mOsm pH 7.5. The fixed larvae were dehydrated in a graded ethanol series, critical point dried and gold sputtered. They were than observed and photographed in a Cambridge Stereoscan S 250 Mk2 scanning electron microscope.

Transmission electron microscopy (TEM)

Swimming larvae of *A. malaca*, *P. mammillata* and *C. intestinalis* (6-8 h after hatching) were fixed in 2.5 % glutaraldehyde in 0.2 M phosphate buffer pH 7.5 and postfixed in 1 % osmium tetroxide in the same buffer. After fixation the larvae were dehydrated in a graded ethanol series and embedded in Epon 812 (Luft, 1961). The sections obtained with the Ultracut E (Reichert-Jung) microtome were contrasted with uranyl acetate and lead citrate (Reynolds, 1963) and photographed with a Philips EM 410 at 80 kV accelerating voltage using Kodak electron microscope film (Estar thick base 4489).

Cytochemical investigations: Tannic acid reaction

C. intestinalis swimming larvae were fixed in a mixture containing 2.5 % glutaraldehyde and 4 % tannic acid (Hayat, 1993) in 0.2 M phosphate buffer pH 7.5 and postfixed in 1 % osmium tetroxide in the same buffer. The fixed material was dehydrated in a graded ethanol series and embedded in Epon 812 (Luft, 1961). The sections obtained with the Ultracut E (Reichert-Jung) microtome were contrasted with uranyl acetate and lead citrate (Reynolds, 1963) and photographed with a Philips EM 410 at 80 kV accelerating voltage using Kodak electron microscope film (Estar thick base 4489).

Confocal laser scanning microscopy

Swimming larvae of *A. malaca*, *P. mammillata* and *C. intestinalis* (6-8 h after hatching) were fixed at -20 °C in methanol containing 1 % formaldehyde (from a 37 % formaldehyde solution) for 2 h or longer. They were gradually rehydrated to PBS (phosphate-buffered saline), extracted for 20 min with 0.25 % Triton X-100 in PBS, rinsed in PBS and

then incubated overnight at 4 °C in anti-β-tubulin (mouse) monoclonal N357 (Amersham) antibody diluted 1:50. After repeat rinsing in 0.1 % Tween-PBS, larvae were incubated with FITC-conjugated anti-mouse IgG diluted 1:50. They were then rinsed in PBS and muted in Citifluor. The larvae were observed under a Leica TCS 4D confocal laser scanning microscope equipped with an argon/krypton laser. 40x and 10x objectivies were used, and images were obtained from eight scans of laser beam.

Results

Papillae of Ascidia malaca

Adhesive papillae of the swimming larvae of *A. malaca*, three in number, are located at the vertices of a triangular field in the anterior region of cephalenteron (Figs 1a, b). In according to previous data (Gianguzza and Dolcemascolo, 1994, 1997; Gianguzza *et al.,* 1999), our ultrastructural observations showed that each adhesive papilla presents a sort of hyaline cap covering the papillar body. The hyaline cap is dome-shaped expansion of the test located at the apex of each papilla. Along its whole length it contains numerous microvilli that originate from the axial columnar cells (Fig. 1c) and in his apex can be noted the presence of an electrondense substance that corrisponde to adhesive

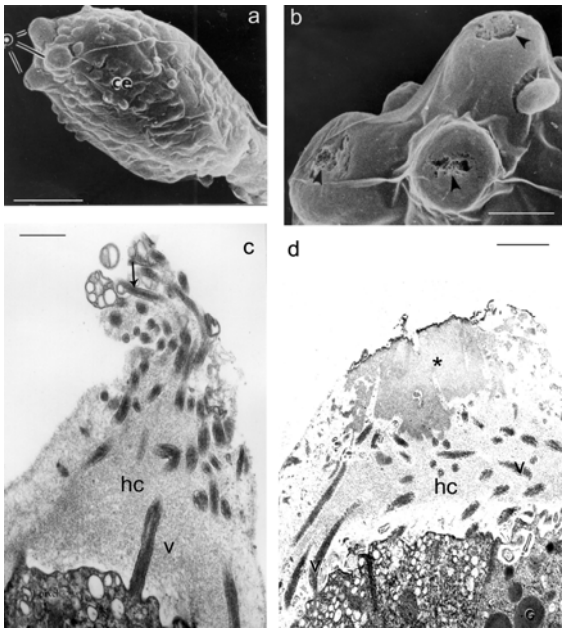

Fig. 1 *A. malaca* papillae. a) b) SEM images showing the disposition of three adhesive papillae (p) in the anterior end of the cephalenteron (ce) at the vertices of a triangular field. b) Papillae at a higher magnification, showing fenestrations in the apical part (arrows). c) Above the papillary body is present the hyalin cap (hc), dome-shaped, that bears long microvilli (v) extending for the whole cap length and protruding outside hyalin cap fenestrations (arrows). d) In the apex of hyalin cap can be noted a mass of electrondense substance (*). Bars = a) 40 μm, b) 10 μm, c) 0,5 μm, d) 1 μm.

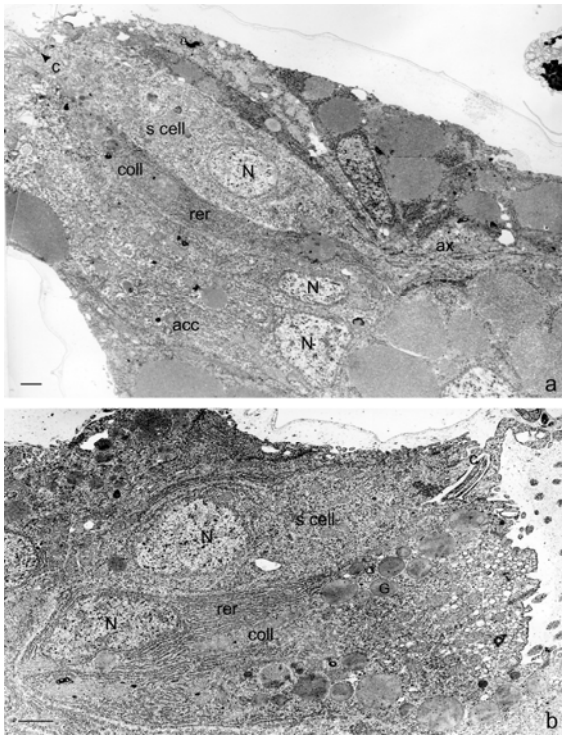

Fig. 2 *A. malaca* papillae. a) Ultrastructure of three types of cells costituting the papillar body. At the centre of papilla collocytes (coll) with numerous rough endoplasmic reticulum vesicles (rer) can be noted. In the marginal position are present sensory cells (s cell) that bear a single cilium (c) at their apex and extend at the base in a long axon (ax). The axial columnar cells (acc), lied in semilateral position, are caracterized by presence of a long microvilli that extend inside the hyalin cap. b) Sensory cells and collocytes of papillae observed at high magnification. G, granule. Bars = a), b) 1 μm.

secretion (Fig. 1d). The papillar body essentially consists of three types of cells: axial columnar cells, collocytes and sensory cells (Fig. 2a).

The axial columnar cells, elongated in shape, lie in the middle-papillar region. A peculiar characteristic of these cells is the presence, in their apical part, of long microvilli extending for the whole hyaline cap length (Fig. 1c). Some observations have highlighted microvillar ends protruding out of the hyaline cap via fenestrations (Fig. 1c). In their cytoplasm are present poor very elongated rough endoplasmic reticulum (RER) cisternae, and a few Golgi profiles (Fig. 2a).

The ultrastructure of the second type of cells, known as collocytes, is typical of cells with a secretory activity. These cells, certainly deputed to form adhesive secretion, lie in the central or middle-lateral side of the papillar body. Their cytoplasm is characterized by the presence of numerous RER vesicles and of a Golgi complex apparently engaged in synthesis process (Figs 2a, b).

The third type of cells, present in the papillary body, is represented by the sensory cells. These cells occupy a very marginal position in the papilla and show an ovoidal and elongated shape (Figs 2a,

b). In their cytoplasm mitochondria and rare RER elements can be noted. The nucleus lies at the cell base. The apical part of these cells is caracterized by the presence of a single cilium originating from a sort of pocket of the membrane (Figs 2a, b). A further peculiarity of these cells is their basal part prolonged into a long axon running under ectodermic layer of the cephalenteron (Fig. 2a). In order to highlight the run of nervous fibers that arise from the papillar base, investigations were carried out at laser confocal microscopy after treatment with anti-β-tubulin antibody. The observations made in the cephalenteron of *A. malaca* have shown a clear fluorescence in the central part of the adhesive papillae and in a small network of nervous fibers projecting from the papillar base and converging into one nerve extending from each papilla up to cerebral vesicle (Figs 6a, b).

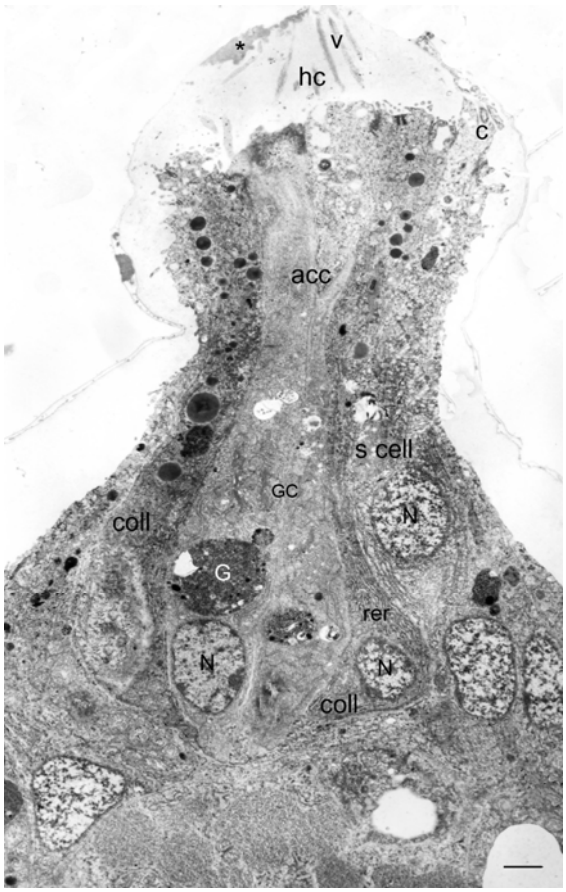

Fig. 3 *P. mammillata* papillae. The papillar body, covered in the apex by hyalin cap (hc), consists of three types of cells: sensory cells (s cell) axial columnar cells (acc) and collocytes (coll). The sensory cells, lied in the marginal position of papillae, bear at their apex a single cilium (c) while their basal part reduce as a funnel. The axial columnar cells, presented in the central position, bear in their apical part long microvilli that extend inside the hyalin cap. The ultrastructure of collocytes is typical of secretory cells with a cytoplasm rich of rough endoplasmic reticulum vesicles (rer). GC, Golgi complex, N, nucleus. Bar = 2 μm.

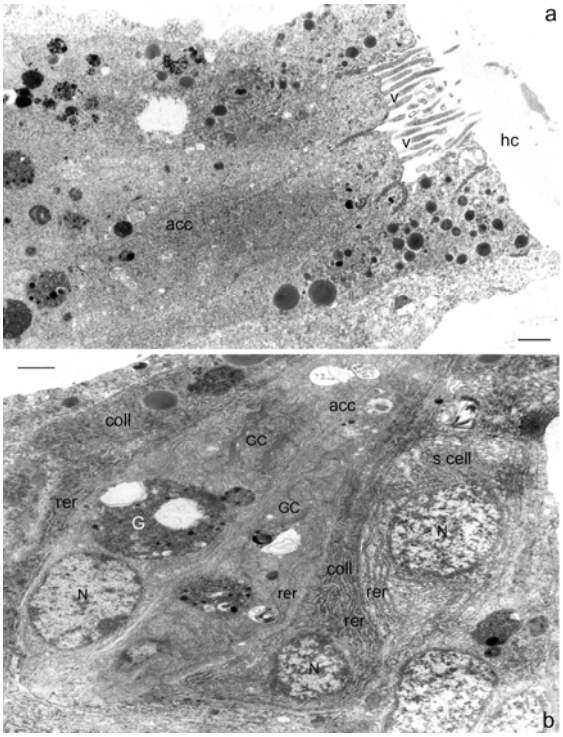

Fig. 4 *P. mammillata* papillae. a) The hyalin cap (hc) is crossed, for the whole length, microvilli (v) originating from axial columnar cells (acc). b) Cells of papillar body observed at high magnification. In the basal part of the sensory cells (s cell) can be noted the nucleus (N) and some vesicles of rough endoplasmic reticulum (rer). In the cytoplasm of axial columnar cells (acc) are present a Golgi complex (GC), large granules (G) and rare profile of r.e.r. In the cytoplasm of collocytes the rough endoplasmic reticulum vesicles. are very numerous. Bars = a), b) 1 μm.

Papillae of Phallusia mammillata

The ultrastructural observations carried out on adhesive papillae of *P. mammillata* have evidenced clear similarity with those of *A. malaca*. The papillae are three, located in the anterior region of cephalenteron, at the vertices of a triangular fied. Each papilla present a hyaline cap arranged above the papillary body. The cells that make the body of papillae are of three types and show an ultrastructure very similar those of cells present in the papillae of *Ascidia malaca*: axial columnar cells, collocytes and sensory cells (Fig. 3).

The investigations carried out by TEM have shown that the axial columnar cells, elongates in shape, occupy in the papillar body a central position. These cells are caracterized by the presence of long microvilli that, originating from their apical part, extend for the whole hyaline cap length. In their cytoplasm a Golgi complex, RER vesicles, mitochondria and large granules can be noted. The nucleus is located in the basal side of cell (Figs 4a, b).

The collocytes, elongated in shape, occupy the semi-lateral region of the papillar body. Their ultrastructure is typical of cells with secretory activity: in their cytoplasm numerous elongated vesicles of RER and a Golgi complex in active phase

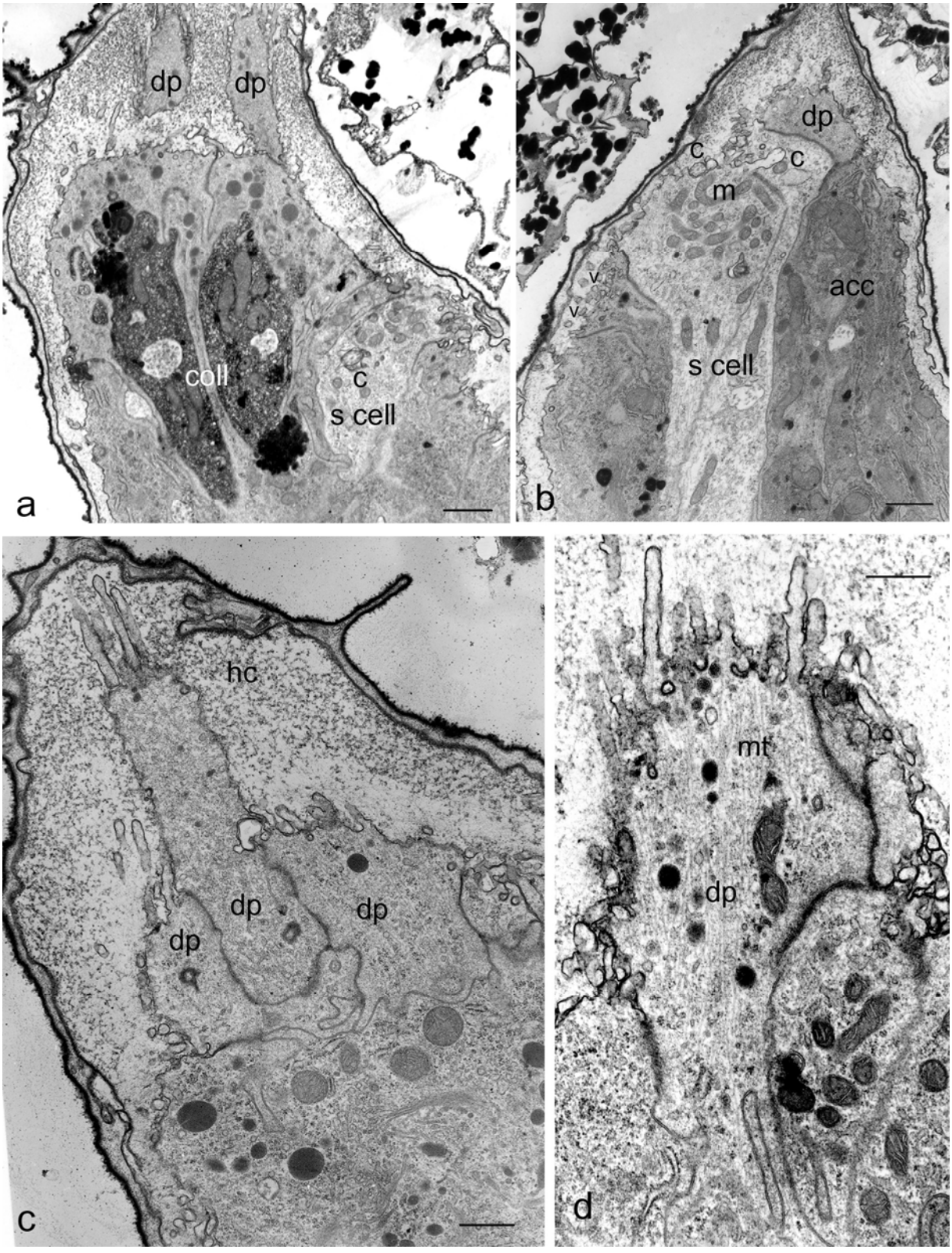

Fig. 5 *C. intestinalis* papillae fixed in glutaraldehyde plus tannic acid. a) b) Ultrastructural caracteristics of collocytes (coll), axial columnar cells (acc) and sensory cells (s cell). The collocytes show the typical ultrastructure of secretory cells and result positive to tanic acid reaction. The sensory cells, elongated in shape, bear their apical part flared as sort of platform. In this platform can be noted the presence of a cilium (c) and of microvilli (v). c) d) The ultrastructure of axial columnar cells evidence the presence of big digitiform protrutions (dp) that extend in side the hyalin cap. The tannic acid reaction evidence the presence of bundles of microtubules (mt) in side of these protrusion and in the cell cytoplasm. Bars = a), b), c), d) 1 μm.

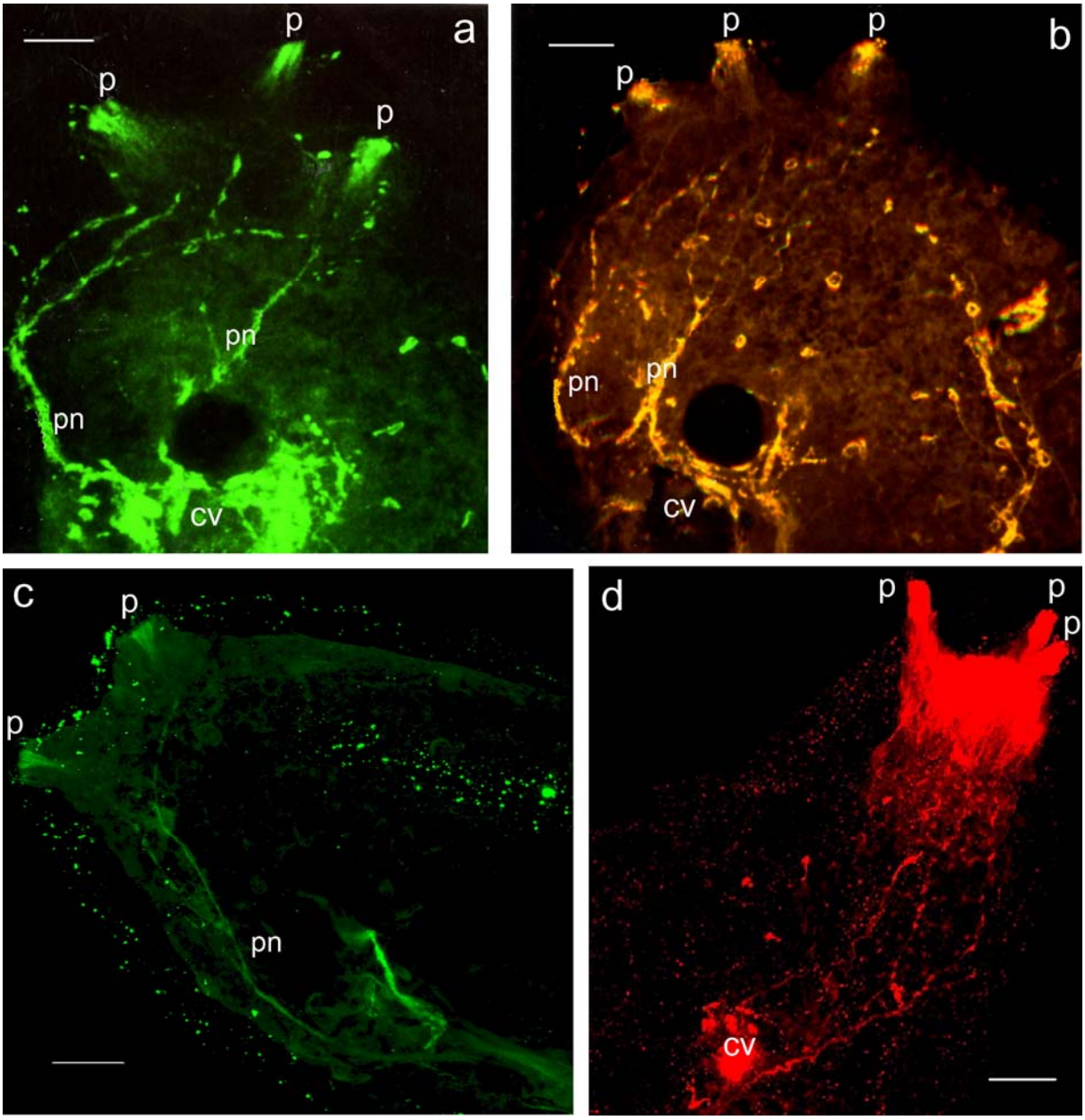

Fig. 6 Swimming larvae stained by FITC-conjugated anti-β-tubulin antibody observed by laser confocal scanning microscope. a) b) Larvae of *A. malaca*. In the cephalenteron can be evidenced a small nervous network of nervous fibers proiecting from the base of papillae (p), converge into only one papillar nerve (pn) extending to cerebral vesicle (cv). c) Larvae of *P. mammillata.* Image shows axons emerging from central cells of papilla where fluorescence is very clear. d) Larvae of *C. intestinalis*: The papillae are more positive to reaction than those of *A. malaca* and *P. mammillata*. The reaction has also evidentiated a nervous netwoork in the cephalenteron of these larvae even if the fluorescence signal is less strong respect to that evidenced in *A. malaca* and *P. mammillata*. Bars = a) 10 μm, b) 10 μm, c) 20 μm d) 20 μm.

of synthesis can be noted (Figs 4a, b). On the basis of their ultrastructure the function of synthesis and secretion of adhesive substances can be assigned to these cells.

The sensory cells, present in a lateral/marginal position of the papillar body, show an ovoidal elongated shape and in their basal part became funnel like (Fig. 4b). The apical part of these cells, like those of *A. malaca*, is characterized by the presence of a single cilium. Our observations have not evidenced axonal process originate from the base of these cells. The confocal microscopy investigations, made by use of anti-β-tubulin antibodies, have shown a clear fluorescence in the cells of central part of the papilla and in a network of nervous fibers projecting from the base of papilla up to cerebral vesicle of the cephalenteron. The confocal image have also evidenced axons emerging from central cells of papilla (Fig. 6c).

Papillae of Ciona intestinalis

Ultrastructural investigations carried out on the adhesive papillae of *C. intestinalis* larvae evidenced, like in other two species, the presence of three types of cells: axial columnar cells, collocytes and sensory cells (Figs 5a, b). The investigations have been performed on sections of larvae fixed in glutaraldehyde plus tannic acid, a technique that highlight, at the ultrastructural level, the presence of proteins or glycoproteins.

The ultrastructure of the axial columnar cells of *C. intestinalis,* underlines some differences with those of *A. malaca* and *P. mammillata*. In the apical part of these cells are present big digitiform protrusion that extend along the whole hyaline cap length (Fig. 5c). Observations reported in this paper did not find evidence for the presence of microvilli in the apical part of these cells. The acid tannic reaction have evidenced in the cytoplasm of these cells and inside the apical protrusion the presence of numerous microtubules running paralleling along longitudinal cell axis (Fig. 5d). Moreover, immunofluorescence with anti-β-tubulin antibodies showed a clear fluorescence in these cells of central part of the papilla (Fig. 6d).

The collocytes, present in semi-lateral position in the body of papilla, show the typical ultrastructure of cells with a secretory activity. Some of these cells are evidently positive to the reaction with tannic acid showning a strong activity of synthesis (Fig. 5a).

The sensory cells, elongated in shape, occupy the lateral/marginal position in the body of papilla. Their apical part appear flared, as a sort of "platform", and go into contact with the inner cuticular layer of test (Fig. 5b). In the apical part of the platform can be noted a cilium and numerous microvilli that proliferate in the hypocuticular space of test (Fig. 5b). Even if the ultrastructural investigations have not evidenced at the base of these cells axonic process, the investigations carried out at confocal microscopy in the larval cephalenteron, have shown a clear fluorescence in a nervous network extending from the base of papilla up to cerebral vesicle, in addition to a strong fluorescence in the body of papillae (Fig. 6d). The reaction with tannic acid have also facilitate the identification of neuronal cells present in a cephalenteron region included between the base of papillae and cerebral vesicles. These neurons are formed by ovoidal cellular body extending in a long axon surrounded by a sheath positive to acid tannic reaction (Fig. 7a). The observations, carried out at high magnification, have shown, in the cytoplasm of cellular body, a large nucleus with an evident nucleolus, numerous mitochondria rounds in shape, a Golgi complex and poor very elongated r.e.r. cisternae (Fig. 7b). Inside the axon bundles of microtubules, running paralleling along longitudinal axis, can be noted (Fig. 7c).

Discussion

Ultrastructural comparative investigations carried out on the adhesive papillae of *A. malaca* and *P. mammillata* larvae have shown several similarities. The adhesive papillae of these two species are coniform and show in their apex a hyalin cap, dome shaped, formed by an espansion of the test. In the apical part of hyalin cap there is an electron dense substance of proteoglycan nature (Gianguzza and Dolcemascolo, 1997) useful to adhere to the substratum. The papillar body is essentially constituted of three types of cells: axial columnar cells, collocytes and sensory cells respectively. The collocytes are cells with an ultrastructure typical of secretory cells and lie in a semi-lateral position of papillar body. Ultrastructurally and functionally this cell type matches the collocytes described by Cloney (1977) in *D. occidentalis* and secretorial cells by Turon (1991) in *E. turbinata*. The second cell type, the axial columnar cells, lies in the middle-central papillar region and extend for the whole papillar length. These cells could have a supporting role, as suggested by Cloney (1977) for cells with a similar ultrastructure found in papillae of *D. occidentalis.* The ultrastructural investigations reported in this paper have evidenced the presence, in the apical part of the cells, of long microvilli extending through the whole length of the hyaline cap and beyond it with their far ends, through fenestrations of the tunic. The presence of microvilli in the apycal part of papillae undoubtedly facilitates the perception of stimuli coming from the substratum. Therefore a possible sensorial function for these cells, strongly β-tubulin positive, cannot be ruled out, as already hypothesized by Sotgia *et al.* (1998) and for the analogous central cells of the *B. leachi* papillae (Pennati *et al.*, 2007). In agreement with this hypothesis the confocal investigations, carried out on the *P. mammillata* larvae, have shown axons emerging from central cells of papillae where the signal of fluorescence is very clear. The third cell type, in *A. malaca* and *P. mammillata,* is represented by sensory cells that are located in a definitely marginal area of papillae. The apical part of these cells bears a cilium that extends to touch the internal cuticle of the hyaline cap. In *A. malaca* the main feature of these cells is their basal part prolonging into a long axon. The presence of a cilium in the apical region and of an axonal process at their base indicate these cells as primary sensory neurons. In *P. mammillata* the axonal process have not been found but this can be due to the observations of unfavourable sections. However the confocal microscopy observations have evidenced in both species a small network of several nervous fibers projecting from the papillar base and converging into one nerve extending from each papilla up to cerebral vesicle.

Ultrastructural investigations carried out to papillae of *C. intestinalis* have confirmed the presence, in the papillary body, of the three types of cells characterizing their function: collocytes, axial columnar cells and sensory cells. The ultrastructure of collocytes of *C. intestinalis* is that of cells with secretory activity. The sensory cells of the *C. intestinalis* are very similar to the "anchor cells" present in the papillae of 11 different ascidians species (Cloney, 1979). These cells present, in fact, the same spindle-shaped somata flared in their apical part, a sort of "platform" whose ultrastructure is very similar to the pedestal of anchor cells. The sensory function can be assigned to these cells on

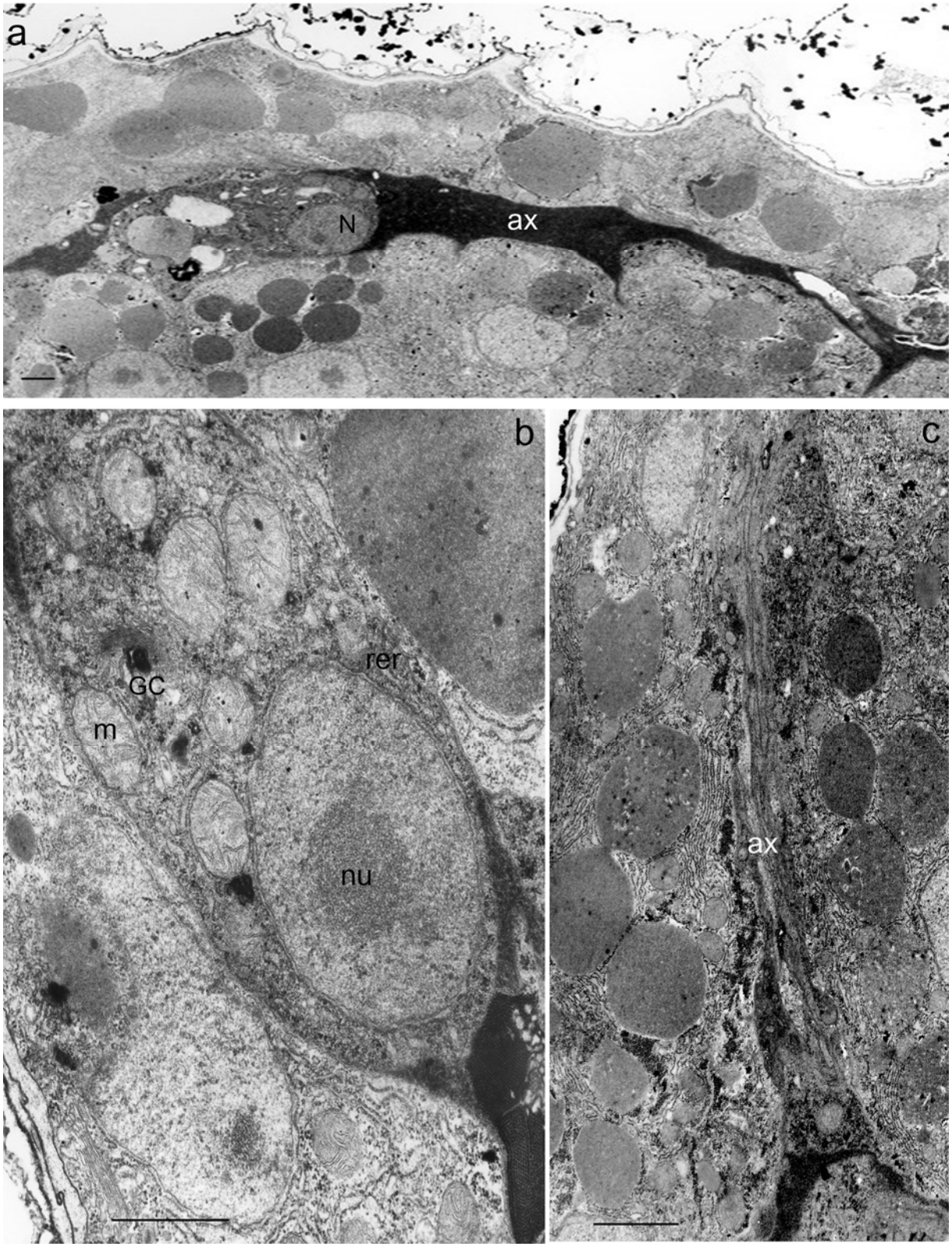

Fig. 7 *C. intestinalis* larvae. a) Ultrastructure of neuron present in the cephalenteron area between the papillar body and the cerebral vesicle. The neuron, elongated in shape, is formed by an ovoidal cellular body and a long axon (ax). b) Cellular body of neuron at high magnification. In the nucleus is present an evident nucleulus (nu). In the cytoplasm can ben noted numerous mitochondria (m), a Golgi complex (GC) and rare profile of rough endoplasmic reticulum vesicles. c) Inside the axon are present bundles of microtubules running paralleling longitudinal axis. Bars = a), b), c) 1 μm.

the basis of presence, in the apical platform, of a cilium and microvilli extending in the hypocuticular space of test. The lack of evidence of axonal process at the base of these cells is certainly due to observation of unfavourable sections. However the axons have been, recently, described in the *Ciona* larvae by Imai and Meinertzhagen (2007a, b) and confirmed by confocal microscopy observations reported in this paper. The ultrastructure of axial columnar cells differ from those of *A. malaca* and *P. mammillata*. The apical part of these cells shows big digitiform protrusion of membrane extending along the whole of hyaline cap length. Even if the ultrastructural observations reported in this paper have not evidenced microvilli in the apical part of axial cells, their presence do not be excluded. The presence of numerous bundles of microtubules in the cytoplasm and in the protrusion of these cells indicate a possible sensory function during the phase preceding the adhesion. In many species of ascidians the axial columnar cells bear, in their apical part, microvilli and/or digitiform protrusions, [*D. occidentalis*, (Cloney, 1977), *A. malaca* (Gianguzza *et al*., 1999), *B. leachi* (Pennati *et al.*, 2007)] structures that undoubtedly facilitate the registration of mechanical and/or chemical stimuli. The above reported data suggest the hypothesis that these cells, beside a support function, could also exercise a sensory function. Moreover, Imai and Meinertzhagen (2007b) suggest that in *Ciona* two subpopulations of papillar neurons may exist as two nerve bundles project from each papilla. The ultrastructural investigations have also evidenced in *C. intestinalis* cephalenteron, between the base of papilla and the cerebral vesicle, the presence of neuronal cells. These neurons, for position, correspond to those described by Takamura (1998) as Ia, Ib, Ic and by Imai and Meinertzhagen (2007b) as RTEN (Rostral Trunk Epidermal Neuron). However, the ultrastructural observations of this paper have not evidenced dendritic process that originate from neuronal body extending inside the larval test. Very probably these neurons take part in the extensive nervous network present in the cephalenteron area.

In conclusion the ultrastructural and immunohistochemical investigations reported in the present paper confirmed the characteristic of simple and coniforme type and the adhesive and sensorial functions of adhesive papillae of the three ascidian species studied. Moreover, close similarities were observed for the papillae of *A. malaca* and *P. mammillata.* The different structure of the apical region of the axial columnar cells of *C. intestinalis* might be related to the different extension of the papillae and of the hyaline cap in this species.

References

Anderson DT, White BM, Egan EA. The larval development and metamorphosis of the ascidians *Pyura praeputialis* (Heller) and *Pyura pachydermatina* (Herdman) (Pleurogona, family Pyuridae). Proc. Linn. Soc. N.S.W. 100: 205-217, 1976.

Berrill NJ. The Tunicata. with an account of the British species. The Royal Society, London, 1950.

Burigel P, Cloney RA. Urochordata: Ascidiacea. In: Microscopic Anatomy of Invertebrates. vol.15: Hemichordata, Chaetognatha and the Invertebrates Chordates. Wiley-Liss Inc., pp 221-347, 1997.

Cavey MJ, Cloney RA. Ultrastructure and differentiation of ascidian muscle. I. Caudal musculature of the larva of *Diplosoma macdonaldi*. Cell Tissue Res. 174: 289-313, 1976.

Cloney RA. Observations on the mechanism of tail resorption in ascidians. Amer. Zool. 1: 67-87, 1961.

Cloney RA. Larval adhesive organs and metamorphosis in ascidians. I. Fine structure of the everting papillae of *Distaplia occidentalis*. Cell Tissue Res. 183: 423-444, 1977.

Cloney RA. Ascidian metamorphosis: review and analysis. In: Chia FS, Rice M (eds), Settlement and metamorphosis of marine invertebrate larvae, Elsevier/North-Holland Biomedical Press, New York, pp 255-282, 1978.

Cloney, RA. Larval adhesive organs and metamorphosis in ascidians. II.The mechanism of eversion of the papillae of *Distaplia occidentalis*. Cell Tissue Res. 200: 453-473, 1979.

Cloney RA, Cavey MJ. Ascidian larval tunic: extraembryonic structures influence morphogenesis. Cell Tissue Res. 222: 547-562, 1982.

Cloney RA, Torrence SA. Ascidian larvae: structure and settlement. In: Costlow JD, Tipper C (eds), Marine biodeterioration: an interdisciplinary study, Annapolis Naval Institute Press, pp141-148, 1984.

Gianguzza M, Dolcemascolo G. Adhesive papillae in the swimming larva of *Ascidia malaca*: an ultrastructural investigation. Eur. Arch. Biol. 105: 51-62, 1994.

Gianguzza M, Dolcemascolo G. Adhesive organs in the swimming larvae of *Ascidia malaca*: an ultrastructural and cytochemical study. Anim. Biol. 6: 53-60, 1997.

Gianguzza M, Dolcemascolo G, Fascio U, De Bernardi F. Adhesive papillae of *Ascidia malaca* swimming larvae: investigations on their sensory function. Inv. Reprod. Dev. 35: 239-250, 1999.

Grave C. *Molgula citrina* (Alder and Hancock). Activities and structure of the free-swimming larva. J. Morph. 42: 453-467, 1926.

Groppelli S, Pennati R, Sotgia C, De Bernardi F. AChE localization in adesive papillae of ascidian larva: effects of citral, a retinoic acid synthesis inhibitor. Inv. Reprod. Dev. 40: 95-102, 2001.

Groppelli S, Pennati R, Scarì G, Sotgia C, De Bernardi F. Observations on the settlement of *Phallusia mammillata* larvae: effects of different lithological substrata. Ital. J. Zool. 70: 321-326, 2003.

Hayat MA. Fixation for electron microscopy, Academic Press, New York, 1981.

Imai JH, Meinertzhagen IA. Neurons of the ascidian larval nervous system in *Ciona intestinalis*: I. Central nervous system. J. Comp. Neurol. 501: 316-334, 2007a.

Imai JH, Meinertzhagen IA. Neurons of the ascidian larval nervous system in *Ciona intestinalis*: II. Peripheral nervous system. J. Comp. Neurol. 501: 335-352, 2007b.

Lafargue F. Révision taxonomique des Didemnidae des côtes de France (ascidies composées). Les espèces de Banyuls-sur-mer. Genre Didemnum. 2^{e} partic: larves à deux ventouses. Ann. Inst. Océanogr. (Paris) 52: 259-281, 1976.

Lane DJW. Attachment of the larva of the ascidian *Diplosoma listerianum*. Mar. Biol. 21: 47-58, 1973.

Levine EP. Studies on the structure, reproduction, development and accumulation of metals in the colonial ascidian *Eudistoma ritteri* Van Name, 1945. J. Morph. 111: 105-138,1962.

Luft JH. Improvements in epoxy resin embedding methods. J. Bioph. Biochem. Cytol. 9: 409-427, 1961.

Millar RH. The biology of ascidians. Adv. Mar. Biol. 9: 1-100, 1971.

Pennati R, Zega G, Groppelli S, De Bernardi F. Immunohistochemical analysis of the adesive papillae of *Botrylloides leachi* (Cordata: Tunicata Ascidiacea): implications for their sensory function. Ital. J. Zool. 74: 325-329, 2007.

Reynolds ES. The use of lead citrate at high pH as an electron opaque stain in electron microscopy. J. Cell Biol. 17: 208-212, 1963.

Sotgia C, Fascio U, Melone G, De Bernardi F. Adhesive papillae of *Phallusia mamillata* larvae: morphology and innervation. Zool. Sci. 15: 363-370, 1998.

Takamura K. Nervous network in larvae of the ascidian *Ciona intestinalis*. Dev. Genes Evol. 208: 1-8, 1998.

Torrence SA. Ascidian larval nervous system: anatomy, ultrastructure and metamorphosis. Doctoral Thesis, University of Washington, pp 1-177, 1983.

Torrence SA, Cloney RA. Ascidian larval nervous system: primary sensory neurons in adhesive papillae. Zoomorphology 102: 111-123, 1983.

Trason WB. Larval structure and development of the oozooid in the ascidian *Euherdmania claviformis*. J. Morph. 100: 509-545, 1957.

Turon X. Morphology of the adhesive papillae of some ascidian larvae. Cah. Biol. Mar. 32: 295-309, 1991.

Zega G, Pennati R, Groppelli S, Sotgia C, De Bernardi F. Dopamine and serotonin modulate the onset of metamorphosis in the ascidian *Phallusia mammillata*. Dev. Biol. 282: 246-256, 2005.