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Comparative Morphology of the Stolonic Vessel in a Didemnid Ascidian and Some Related Tissues in Colonial Ascidians

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ABSTRACT—The stolonic vessel is a tubular projection of the epidermis from the anterior part of the abdomen in the didemnid ascidians, and the vessel has been supposed to be closely related to the stolons, vascular appendages, and the posterior ends of the abdomen in other aplousobranch ascidians. We compared the morphology of the stolonic vessels of *Diplosoma virens* with similar or related tissue in other colonial ascidians, e.g. stolons of *Clavelina,* vascular appendages of *Distaplia* and *Eudistoma,* tunic vesicle of *Aplidium,* and vascular ampullae of *Botrylloides*. The epidermis of the stolonic vessel is composed of cuboidal cells in lateral wall and columnar cells at the distal tip of the vessel. The cuboidal cells have microvilli that probably anchor the stolonic vessel to the tunic. The columnar cells contain round granules that may concern with the secretion of some tunic components. The secretion of the granules, however, could not observed in this study. The stolonic vessel of *D. virens* is similar in morphology to the vascular ampullae of *Botrylloides* and the tunic vesicle of *Aplidium* rather than the other tissue examined here. Since the cell morphology is supposed to reflect its function but not the phylogenetic relationship, the present study could not provide conclusive evidences to prove the homology and the phylogenetic relationship among the tubular, epidermal projections in the colonial ascidians.

Key words: ultrastructure, phylogeny, colonial ascidian, epidermis, Tunicata

INTRODUCTION

The colonial ascidians of the family Didemnidae usually possess tubular projections of epidermis extending from the abdominal region of zooids, and they are referred to as stolonic vessels. In some species, the stolonic vessels are so short and inconspicuous, but they are often very long in other species (Cf. Kott, 2001). The vessels are sometimes branched and their distal ends form terminal ampullae. Tubular, epidermal appendages are also found in many ascidian species, and homology among these structures has been discussed (Cf. Kott, 2001): the stolonic vessels of didemnids may be homologous with non-vegetative stolon of holozoinids, vascular appendages projecting from posterior end of zooids in aplousobranchs, or tunic vessels of stolidobranch and phlebobranch ascidians. For instance, Millar (1951) supposed that the Didemnidae are derived from the clavelinid stock (most primitive group) through the *Distaplia*-like forms. Moreover, the stolonic vessels, budding area, and muscular appendix are all derived from the posterior end of the zooid of an ancestral ascidian.

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The terminal ampullae of the stolonic vessels consist of columnar epidermis containing spherical granules, and Millar (1951) indicate that the epidermal cells produce tunic substance based on the histological observation. As for cellulose fibrils of the tunic, cellulose synthase gene is contained in the genome of *Ciona intestinalis* (Dehal *et al*., 2002). Since the cellulose synthase embedded in cell membrane elongate cellulose microfibrils to the outside of cell surface (see Read and Bacic, 2002), cellulose synthesis would involve neither exocytosis nor apocrine secretion. Moreover, Kimura and Itoh (1996) showed that the cellulose fibrils are synthesized by the terminal complexes in the plasma membrane of epidermal cells in ascidians. On the other hand, some other components of tunic are probably produced by some other sites of epidermal cells and/or tunic cells. Besides the stolonic vessels of didemnids, possible secretory functions of glandular, epidermal cells has been reported in relation to tunic formation in some ascidians, such as tunic vesicles of a polycitorid (Oka and Usui, 1944), pad cells of a botryllid (Katow and Watanabe, 1978), glandular tip cells of metamorphosing molgulids (Torrence and Cloney, 1981; Bates, 1991). These glandular, epidermal cells may share homologous functions and similar cell structures.

The present study compared the morphology of the stolonic vessels of a didemnid ascidian with some possible related structures of non-didemnid ascidians in reference to their possible functions and phylogeny. By means of light and electron microscopy, we examined the stolonic vessels of *Diplosoma virens* (Didemnidae, Aplousobranchia), the stolon in *Clavelina miniata* (Clavelininae, Polycitoridae, Aplousobranchia), the vascular appendages in *Distaplia* sp. (Holozoinae, Polycitoridae, Aplousobranchia), the epidermis of posterior part of zooids and the vascular appendages in *Eudistoma glaucus* (Polycitorinae, Polycitoridae, Aplousobranchia), the epidermis of posterior part of zooids and the tunic vesicle in *Aplidium yamazii* (Polyclinidae, Aplousobranchia), and the pad cells in the terminal ampullae of tunic vessels in *Botrylloides simodensis* (Botryllidae, Stolidobranchia).

MATERIALS AND METHODS

Animals

Colonies of *Diplosoma virens* (Hartmeyer, 1909) were collected in the vicinity of Maeda Point (Yomitan, Okinawa, Japan) and those of *Eudistoma glaucus* (Sluiter, 1909) were in Teniya (Nago, Okinawa, Japan). Colonies of *Aplidium yamazii* (Tokioka, 1949), *Botrylloides simodensis* Saito et Watanabe, 1981, and *Clavelina miniata* Watanabe et Tokioka, 1973 were collected in Nabeta (Shimoda, Shizuoka, Japan). Some colonies of *Distaplia* sp. from Nabeta were kindly provided by Mis. M. Atsumi (Shimoda Marine Research Center).

Light Microscopy

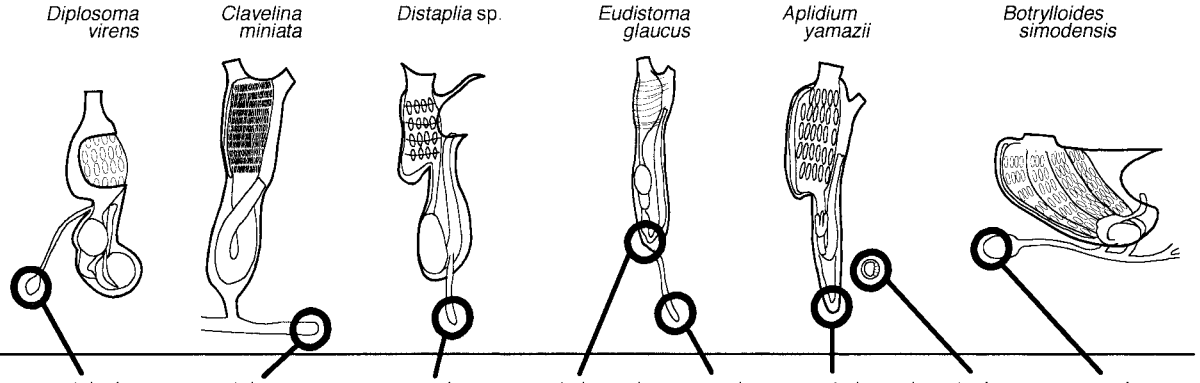
The colonies of *D. virens* were observed under a stereomicroscope equipped with a video camera IK-900N (Nakamura, Tokyo, Japan), and the expansion and contraction of the terminal ampullae was recorded by a videotape recorder.

Colonies of *D. virens* were fixed in 10% formaline-seawater or 2.5% glutaraldehyde-0.1M sodium cacodylate-0.45M sucrose (pH 7.6). The colonies of the other species were fixed in 2.5% glutaraldehyde-0.1M sodium cacodylate-0.45M sucrose (pH 7.6). The formaline-fixed specimens were dehydrated through an ethanol-xylen series, embedded in paraffin, and serially sectioned at 8 μm. The sections were stained with hematoxylin and eosin. The glutar-fixed specimens were rinsed with 0.1M sodium cacodylate-0.45M sucrose, post-fixed in 1% osmium tetroxide-0.1M sodium cacodylate, dehydrated through an ethanol series. The specimens were cleared with *n*-butyl glycidyl ether and embedded in low viscosity epoxy resin. Some of the specimens of *D. virens* were also embedded in styrene resin. These resin-embedded specimens were sectioned at 0.5 to 1 μm thick, and stained with toluidine blue.

Electron microscopy

Thin sections of the epon-embedded specimens were stained with uranyl acetate and lead citrate, and observed in a transmission electron microscope (TEM; Hitachi HS-9, JEOL JEM-1010). The styren-embedded specimens of *D. virens* were sectioned until the desired structures were exposed, and then the resin was removed from the specimens in acetone (1 hr, 2 times). The specimens were immersed hexamethyldisilazane (30 min, 2 times), and then, they were air-dried and sputter coated with gold-palladium, and observed in a Hitachi S-570 scanning electron microscope (SEM).

Table 1. Morphological features of the stolonic vessel and related tissues.



Tissue	stolonic vessel	stolon	vascular appendage	posterior end of zooid	vascular appendage	posterior end of zooid	tunic vesicle	vascular ampulla
Interconnection among zooids	no	yes	no	—	no	—	—	yes
Swelling of distal end	yes	no	no	—	no	—	—	yes
Lumen	hemocyte	hemocyte	hemocyte	—	mesenchymal cell[#]	—	none	hemocyte
Epidermal cell								
Lateral wall (Inclusions)	cuboidal*	columnar* (heterogeneous materials in vesicles)	cuboidal (dense materials in ER, granules)	squamous or cuboidal (vacuoles)	columnar*	columnar (vesicles)	squamous	squamous
Distal tip (Inclusions)	columnar (round granules)	columnar* (heterogeneous materials in vesicles)	cuboidal (dense materials in ER, granules)	squamous or cuboidal (vacuoles)	columnar*	columnar (vesicles)	cuboidal (round granule)	columnar (round granules)

*, Microvilli are present in the apical side. #, Cell are loosely packed in the lumen.

RESULTS

The present study described the morphology of eight tissues (five tubular projection of the epidermis, two posterior end of zooid, and tunic vesicle) in six colonial species of four families. Schematic drawings and morphological features of these tissues are listed in Table 1.

Diplosoma virens: Stolonic vessel

The colonies of *D. virens* grow on the branches of dead coral in the coral lagoon through the year in our collection site. Stolonic vessels are extend from each zooid, and the ampular termini of the stolonic vessels can be found as the small, white spots scattered in the tunic (Fig. 1). Some of them extend toward the colonial margin, and the thickened epidermis of the ampular tip is observed as dark caps with transmission light (Fig. 2). In live specimens, the ampullae periodically expand and contract in a cycle of about 200 sec (Fig. 3 and 4).

The stolonic vessels extend from the apical part of the abdomen (Fig. 5). Two or more stolonic vessels often arise from the same part of the abdomen, but the stolonic vessel

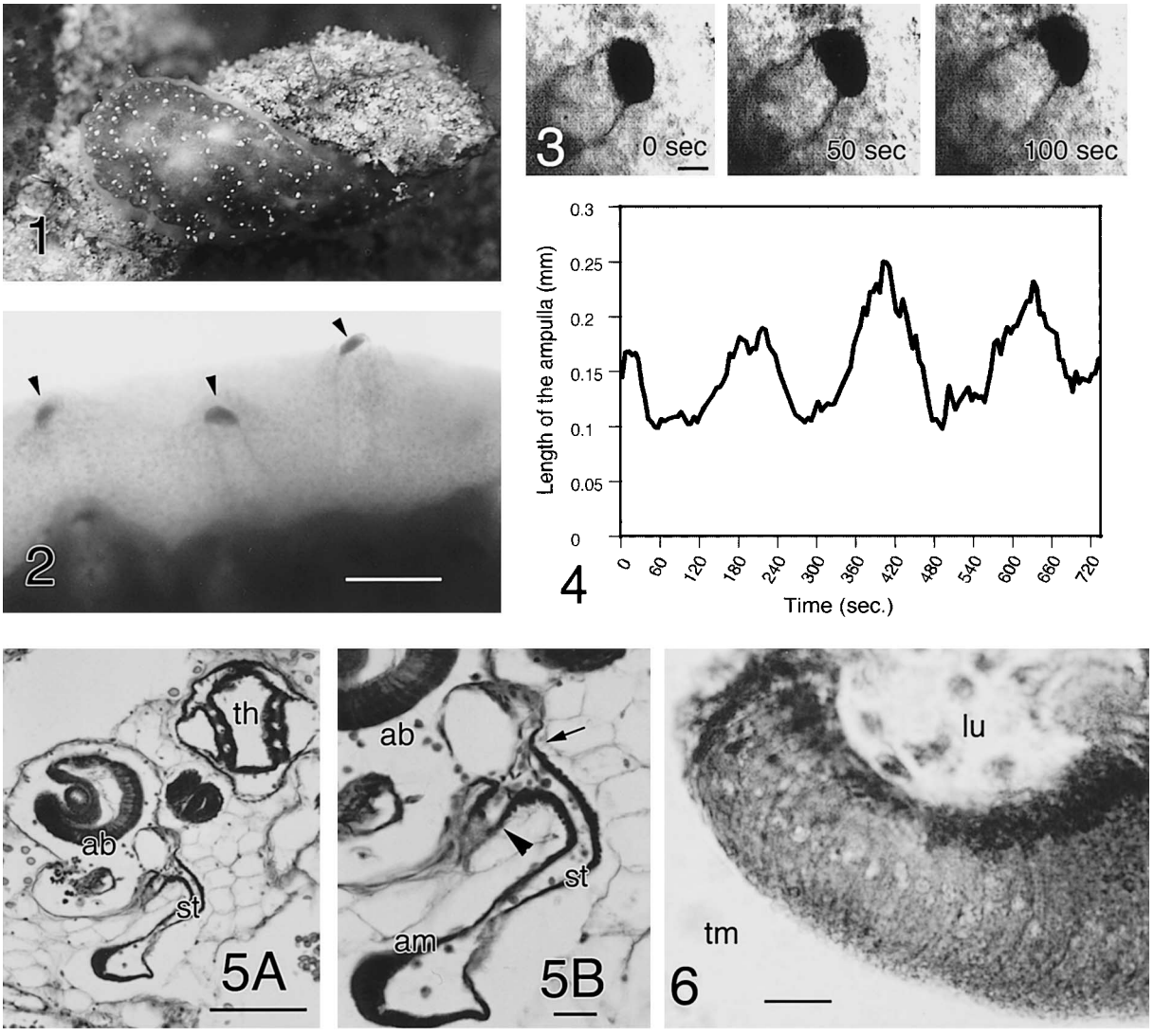


Fig. 1. A colony of *Diplosoma virens* growing on the dead coral branch. White spots scattered in the colony are ampular termini of the stolonic vessels.
Fig. 2. Colony periphery of *Diplosoma virens* showing three ampular termini of the stolonic vessels. Dark cap of the ampular tip consists of the columnar epidermis (arrowheads). Scale bar, 0.2 mm.
Fig. 3. Expansion of the ampular terminus of the stolonic vessel. Scale bar, 0.1 mm.
Fig. 4. Time course of the expansion and contraction of the ampulla.
Fig. 5. Histological section of the zooid of *Diplosoma virens* (A), and the enlargement of the stolonic vessel (B) (paraffin section stained with hematoxylin and eosin). Arrow indicates the point of the origin of the stolonic vessel. Arrowhead indicates another stolonic vessel. ab, abdomen; am, ampular terminus; st, stolonic vessel; th, thorax. Scale bar; 0.1 mm for A, 20 μm for B.
Fig. 6. Columnar cells of the ampular epidermis (paraffin section stained with hematoxylin and eosin). lu, lumen of the stolonic vessel; tm, tunic matrix. Scale bar, 10 μm.

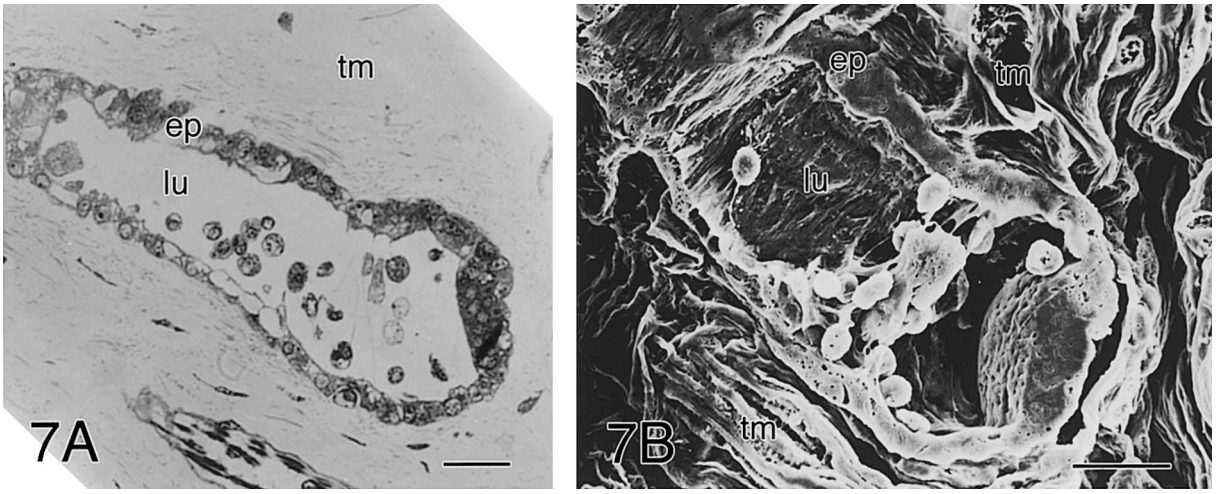


Fig. 7. A pair image of resin section (A) and SEM (B) of the ampular terminus of the stolonic vessel. ep, epidermis of the vessel wall; lu, lumen of the vessel; tm, tunic matrix. Scale bars, 20 μm.

never connects with another one. The vessel ends in a sausage shaped swelling, ampulla. The vessel wall is consists of cuboidal epidermal cells, and the distal tip of the vessel consists of columnar cells (Fig. 6–8). The columnar cells are stained with eosin (Fig. 6) and the cytoplasm is filled with round granules that are well stained with toluidine blue (Fig. 8). Hemocytes are distributed in the lumen of the stolonic vessel.

The cuboidal cell of ampular wall has numerous microvilli in the apical side (Fig. 9A), while its cell membrane of basal side is lined with fibrous basal lamina and highly enfolded (Fig. 9B). There are no granular components in the cytoplasm of the cuboidal cells. On the contrary, the columnar cells in distal tip of the ampullae contain numerous round granules (Fig. 10–11). We could not observe the secretion of these granules so far examined. Granule-free bulge of the cytoplasm is often found in the apical end of the columnar cells (asterisk in Fig. 10A). This may indicate the occurrence of apocrine secretion. The cell membrane of basal side is smooth and lined with thin basal lamina (Fig. 10B). The round granules are membrane-bound and have heterologous substructure; inner part of high electron-density and peripheral part of moderate electron-density (Fig. 12). Moreover, the inner part has striated patterns (Fig. 13). The presence of rough ER suggests the active biosynthesis of the columnar cells.

Clavelina miniata: distal tip of the stolon

The stolon connects zooids with one another and terminates in a blunt end. The distal tip and lateral wall in the distal end of the stolon consists of columnar cells (Fig. 14). Many hemocytes are found in the stolonal lumen, and some are attached on the epidermal wall. The epidermal cells form a smooth line and their basal side lined with basal lamina is usually flat (Fig. 15). The cytoplasm of the epidermal cells is full of rough ER, and vesicles containing heterogeneous materials are often found in the apical half of the cytoplasm (Fig. 15 and 16). Many microvilli protrude into the tunic from the apical membrane of the epidermal cells (arrowheads, Fig. 16).

Distaplia sp.: vascular appendage

The vascular appendages extend from zooids and terminate at the colonial margin. The distal end of the appendage is closed and does not swell. There are no interconnections in the appendages. The epidermal wall consists of cuboidal cells, and some hemocytes are distributed in the lumen (Fig. 17A). The apical side of the epidermis is corrugated, because the apical halves of the epidermal cells are not in contact with the adjacent cells. At the distal tip, many epidermal cells bulge into the tunic, and some are loosely attached with neighbor cells (Fig. 17B). Rough ER occupies the large part of the cytoplasm in the epidermal cell, and the lumen of the ER is often filled with moderately electron-dense materials (Fig. 18). The ER sometimes swells and becomes vesicles (asterisk, Fig. 18). Some electron-dense granules are occasionally found in the cytoplasm (arrows, Fig. 18).

Eudistoma glaucus: epidermis and vascular appendage

The vascular appendages extend from the posterior end of the abdomen (Fig. 19). The appendage often forked but never connects with another vascular appendages. The distal ends of the appendages do not swell to form ampullae. The epidermis of the posterior part of abdomen is squamous or cuboidal (Fig. 20A). Free cells and muscle are distributed in the mesenchymal space between the epidermis and the intestinal wall. The epidermal cells have several vacuoles that are homogeneously filled with moderately electron-dense materials (Fig. 20B). In the distal part of the vascular appendage, the epidermal wall consists of columnar cells, and mesenchymal cells are loosely packed in the lumen of the appendage (Fig. 21). The mesenchymal cells are most packed at the distal end of the appendage and the

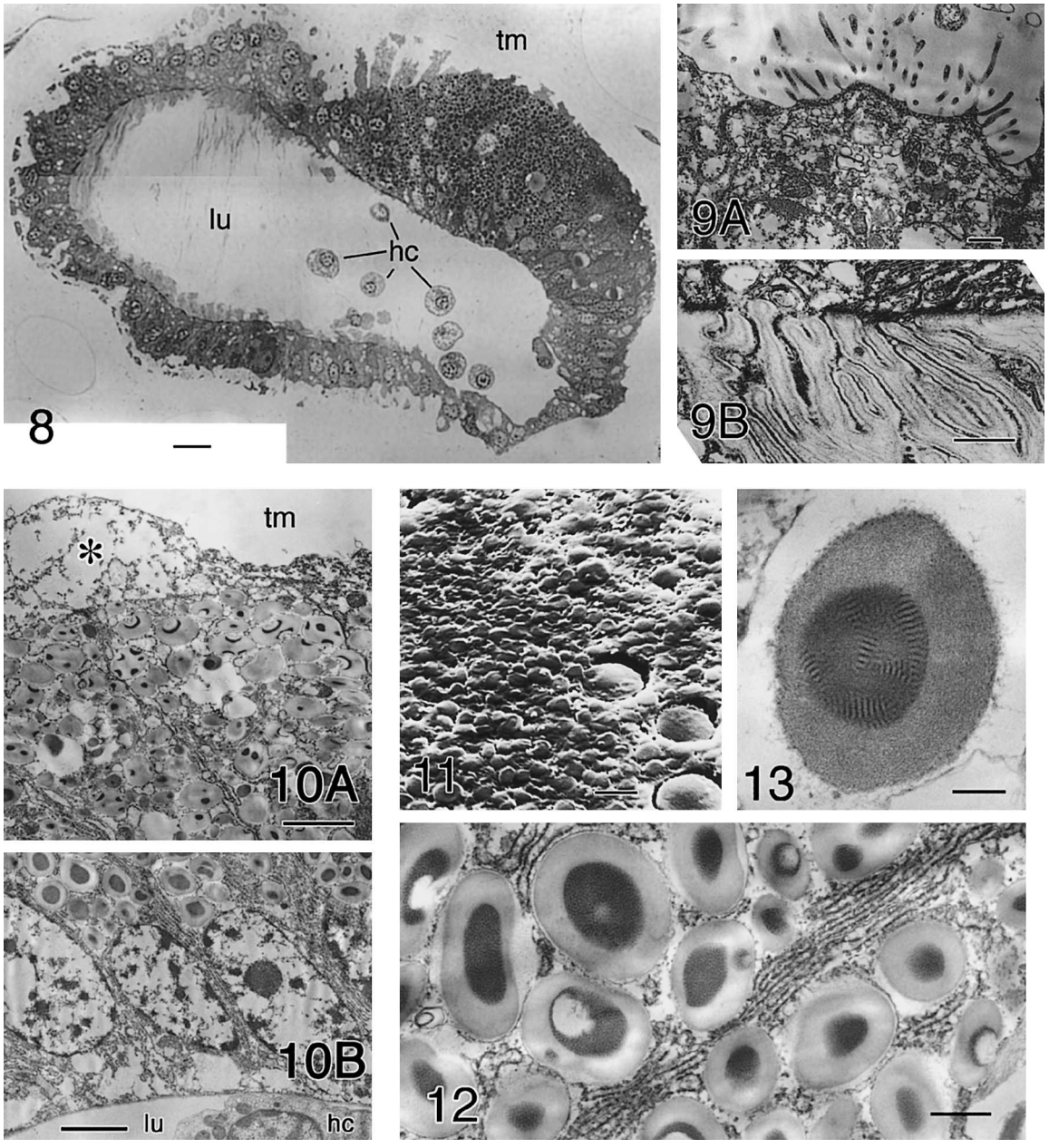


Fig. 8. An ampular terminus of the stolonic vessel in *Diplosoma virens* (resin section stained with toluidine blue). hc, hemocytes; lu, lumen of the vessel; tm, tunic matrix. Scale bar, 10 μm.
Fig. 9. Apical (A) and basal (B) parts of the cuboidal cells in the ampular epidermis. Scale bars, 1 μm.
Fig. 10. Apical (A) and basal (B) parts of the columnar cells in the ampular epidermis. asterisk, granule-free bulge of the cytoplasm; hc, hemocyte; lu, lumen of the vessel; tm, tunic matrix. Scale bars, 2 μm.
Fig. 11. Cytoplasm of the columnar cells is full of round granules (SEM in section). Scale bar, 2 μm.
Fig. 12. Round granules and rough ER in the cytoplasm of the columnar cells. Scale bar, 0.5 μm.
Fig. 13. Enlargement of the round granule. The inner-dark part has striated patterns. Scale bar, 0.2 μm.

cell density tends to be gradually decreased toward the proximal part of the appendage. There are many microvilli in the apical side of the epidermal cells, and the basal side of the epidermis is lined with a thick basal lamina (Fig. 22). Granular inclusions are rarely found in the cytoplasm of the epidermis.

***Aplidium yamazii:* epidermis and tunic vesicle**

In the posterior part of the zooid, the epidermis consists columnar cells (Fig. 23). Since the tunic matrix around the zooids is metachromatically stained with toluidine blue, the epidermis supposed to secrete some materials to the tunic. In TEM observation, irregularly shaped vesicles are found in the epidermal cell, and the vesicles contain moderately electron-dense material (arrowheads in Fig. 23B). The apical

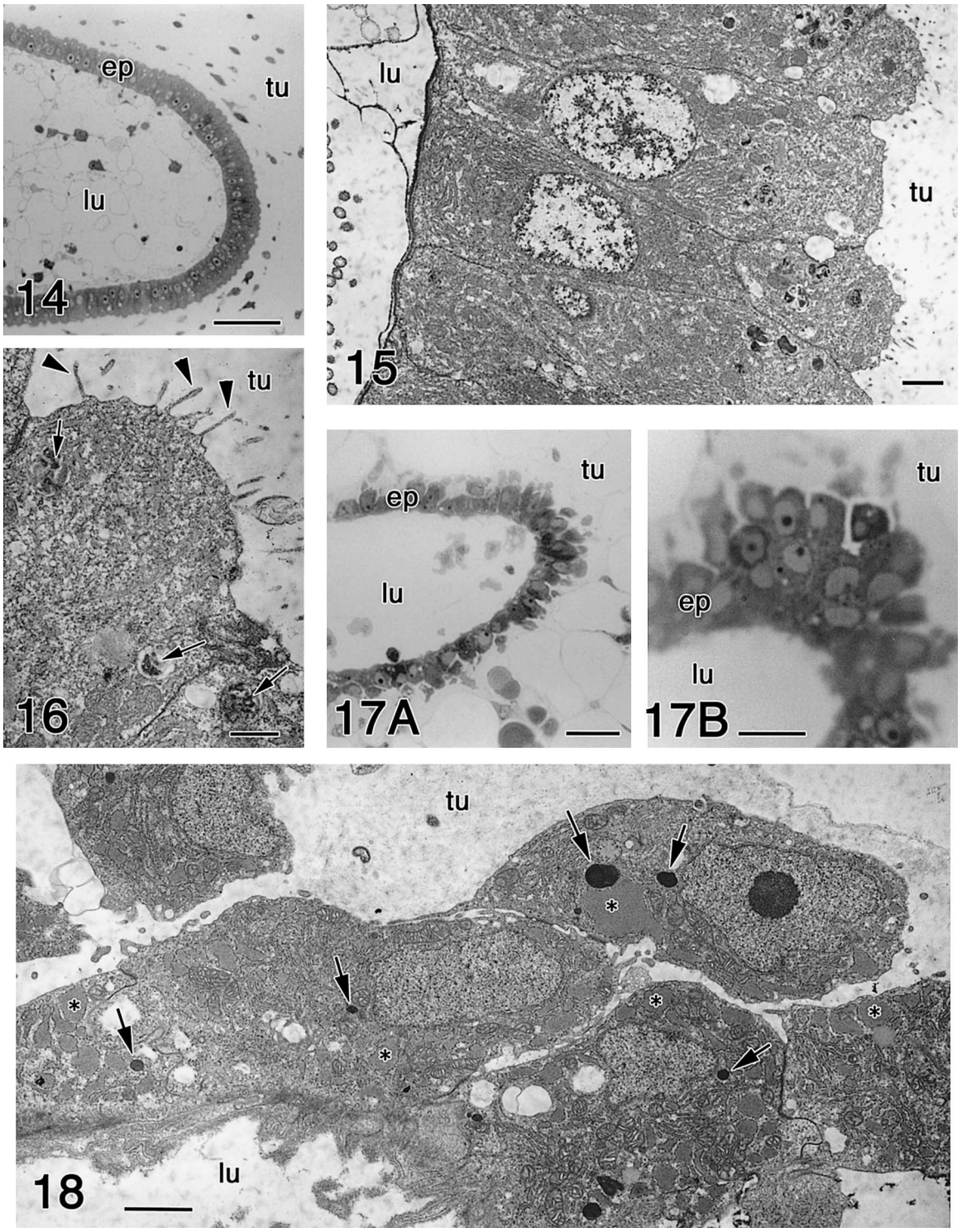


Fig. 14. Distal tip of the stolon in *Clavelina miniata* (resin section stained with toluidine blue). ep, epidermis; lu, lumen; tu, tunic. Scale bar, 50 μm.
Fig. 15. The columnar epidermis of the stolon in *Clavelina miniata.* lu, lumen; tu, tunic. Scale bar, 2 μm.
Fig. 16. Apical side of the epidermal cell of *Clavelina miniata.* Arrows, vesicles containing heterogeneous materials; arrowheads, microvilli; tu, tunic. Scale bar, 1 μm.
Fig. 17. The vascular appendage in *Distaplia* sp. (A) and the enlargement of the distal tip (B) (resin section stained with toluidine blue). ep, epidermis; lu, lumen; tu, tunic. Scale bars, 20 μm for A, 10 μm for B.
Fig. 18. The epidermal cells of the vascular appendage in *Distaplia* sp. Arrows, electron-dense granules; asterisks, vesicles containing moderately electron-dense materials; lu, lumen; tu, tunic. Scale bar, 2 μm.

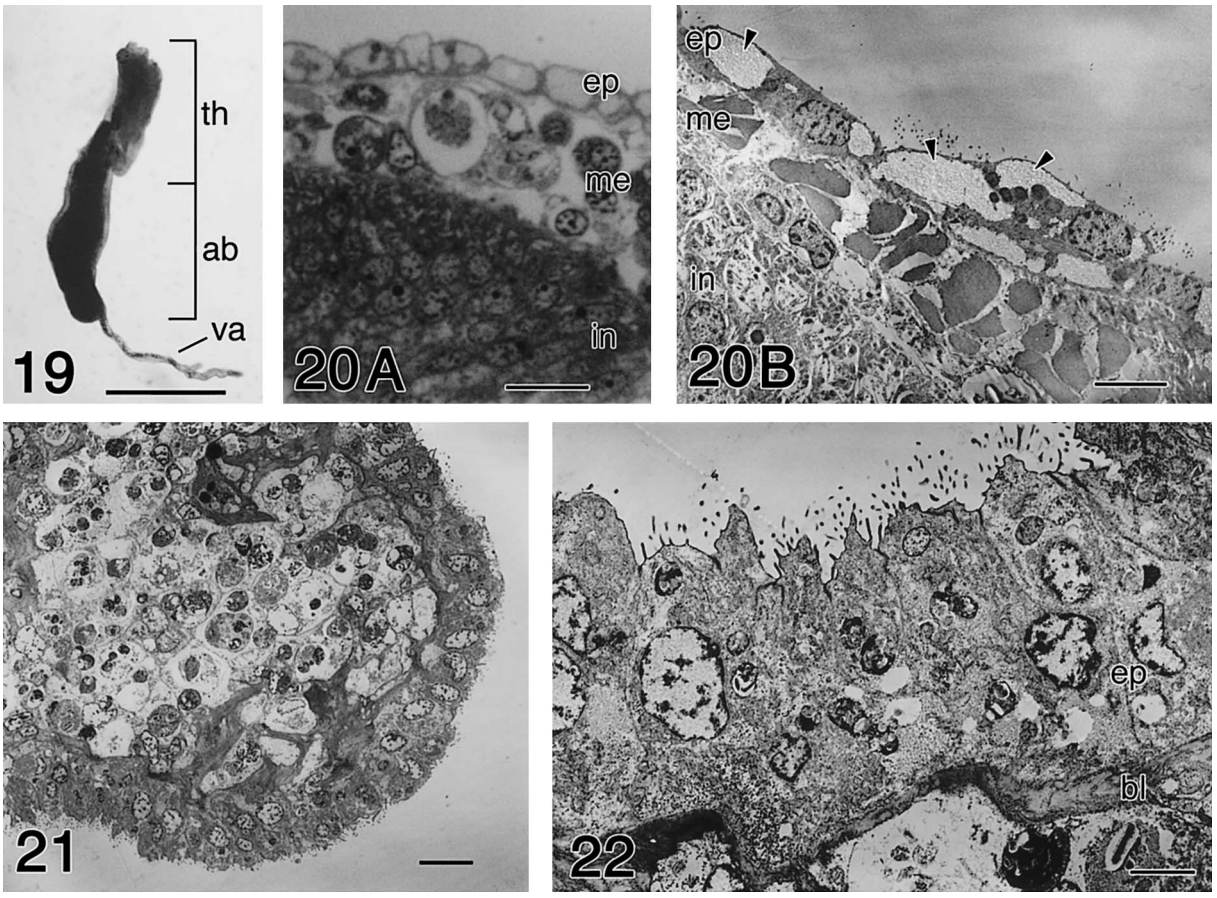


Fig. 19. A zooid of *Eudistoma galaucus*. A vascular appendage (va) extending from the posterior end of the abdomen (ab). th, thorax. Scale bar, 1 mm.
Fig. 20. Epidermis (ep) of the posterior part of abdomen in *Eudistoma galaucus* (A, resin section stained with toluidine blue; B. TEM). Squamous cells have several vacuoles containing moderately electron-dense materials (arrowheads). in, intestinal wall; me, mesenchymal space. Scale bars: 10 μm for A, 5 μm for B.
Fig. 21. A tip of the vascular appendage in *Eudistoma galaucus*. The epidermis consists of columnar cells, and mesenchymal cells are loosely packed in the lumen. Scale bar, 10 μm.
Fig. 22. Enlargement of the epidermis (ep) of the vascular appendage in *Eudistoma galaucus*. The epidermal cells have many microvilli in the apical side, and the basal side is lined with a thick basal lamina (bl). Scale bar, 2 μm.

part of the cytoplasm often forms bulge toward the tunic (asterisks in Fig. 23B).

Tunic vesicle is a hollow multicellular vesicle that is distributed in the tunic without any cellular connections with zooids (Fig. 24). The wall of tunic vesicle is a simple epithelium that consists of squamous and cuboidal cells. Any hemocytes are not distributed in the lumen. The cuboidal cells of the epidermis are abundant in round granules and rough ER, suggesting active biosynthesis (Fig. 25). The round granules are membrane-bound and homogeneous in electron-density. Secretion of these granules has never found so far examined.

***Botrylloides simodensis:* pad cells in terminal ampullae**

In the colony of botryllid ascidians, zooids are interconnected with blood vessels in which hemocytes circulate throughout the colony. The blood vessels are anastomosed and terminate in a sausage shaped ampullae in the colonial margin (Fig. 26). The vessel wall mostly consists of simple squamous cells, and columnar cells (pad cells) compose the ampular wall at the distal tip (Fig. 27). In the pad cells, the nucleus is situated in the basal half of the cell, the cytoplasm is filled with rough ER and round granules, and a thin layer of basal lamina lines the basal side of the cell membrane (Fig. 28A). The round granules bound in membrane are well stained with toluidine blue (Fig. 27) and have heterogeneous substructures (Fig. 28B). Some cytoplasmic protrusions are occasionally found in the apical side.

DISCUSSION

The stolonic vessel of the didemnid ascidians is a tubular projection of the epidermis extending from the anterior part of the abdomen. It is often branched and each of the distal ends is expanded to form terminal ampulla. These structures are essentially similar to those of the inter-zooidal blood vessels in polyzoinid and botryllid species, although the stolonic vessels are never interconnected but the blood

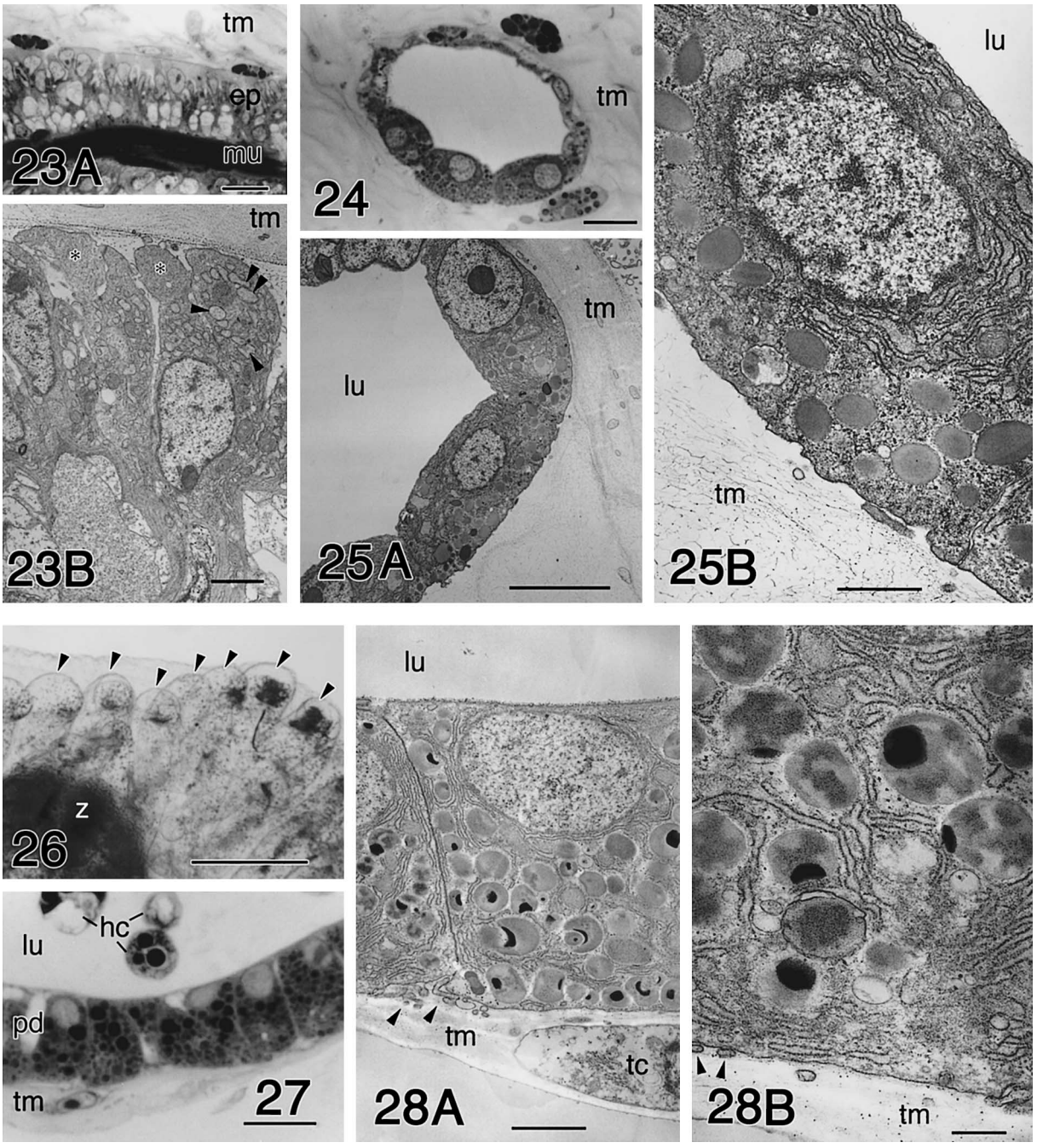


Fig. 23. Epidermis (ep) of the posterior end of a young zooid in *Aplidium yamazii* (A, resin section stained with toluidine blue; B. TEM).The apical part of the cytoplasm is often rich in irregularly shaped vesicles (arrowheads). Asterisks, apical bulge of the cytoplasm; ep, epidermis; mu, muscle; tm, tunic matrix. Scale bars: 10 μm for A, 2 μm for B.

Fig. 24. A tunic vesicle of *Aplidium yamazii* (resin section stained with toluidine blue). The vesicle consists of cuboidal and squamous cells. tm, tunic matrix. Scale bar, 10 μm.

Fig. 25. The cuboidal cells of the tunic vesicle in *Aplidium yamazii* (A), and the enlargement (B). The cytoplasm is rich in round granules and rough ER. lu, lumen of the tunic vesicle; tm, tunic matrix. Scale bars: 5 μm for A, 1 μm for B.

Fig. 26. Colony periphery of *Botrylloides simodensis.* Blood vessels terminate as sausage-shaped ampullae (arrowheads) in the colonial margin. z, zooid. Scale bar, 0.5 mm.

Fig. 27. The ampular epithelium consists of pad cells (pd) at the tip of the vascular ampullae of *Botrylloides simodensis* (resin section stained with toluidine blue). hc, hemocyte; lu, ampular lumen; pd, pad cells; tm, tunic matrix. Scale bar, 10 μm.

Fig. 28. Pad cells of *Botrylloides simodensis* (A) and the enlargement (B). Arrowheads, protrusions of apical cytoplasm; lu, lumen; tc, tunic cells; tm, tunic matrix. Scale bars: 2 μm for A, 0.5 μm for B.

vessels are always anastomosed. Besides them, the didemnid stolonic vessels are supposed to have morphological similarities and/or phylogenetic relationships with vascular appendages of other aplousobranchs and posterior part of the abdomen/post-abdomen of aplousobranchs. According to the ascidian phylogeny proposed by Millar (1966), the members of the subfamily Clavelininae have the most primitive characteristics among the existing aplousobranch ascidians, and clavelinids, didemnids, and polyclinids have a close origin based on their larval forms. Millar (1951) supposed that didemnids were derived from the clavelinid stock through *Distaplia*-like forms, and the posterior end of the body of ancestral form were involved in the budding and it moved forward through the evolutionary course of the Didemnidae. This hypothesis indicates that the didemnid stolonic vessels are closely related with posterior ends of the abdomen and vascular appendages in polycitorid ascidians. On the other hand, the suborder Aplousobranchia have been claimed to be doubtful (Kott, 1969), and Kott (1990) originally established some families in this suborder although these taxa have not been accepted widely yet. The fine structures of the tunic cuticle and the presence of the particular types of tunic cells also suggest that Aplousobranchia is a polyphyletic group (Hirose *et al*., 1997; Hirose, 2001). Here, we wish to discuss structural similarity, possible functions, and phylogenetic relationship of the didemnid stolonic vessel, based on the morphological comparison with some other similar or related tissue.

Hemocytes are sparsely distributed in the lumen of the stolonic vessel. Since the lumen of the stolonic vessel is directly connected with the mesenchymal space between epidermis and intestinal wall, hemocytes may freely migrate between the lumen and the mesenchymal space. As mentioned above, the stolonic vessels are not connected with one another, and thus, the hemocytes in the lumen are not exchanged among the zooid. The function of these hemocytes is uncertain. Hemocytes are also found in the lumen of the stolon of *Clavelina miniata* and the vascular appendage of *Distaplia* sp. Because the stolon of *C. miniata* connects zooids, the hemocytes circulate throughout the colony. In *Eudistoma glaucus,* although free cells are distributed in the lumen, they are loosely packed and unlikely circulate. On the contrary, the lumen is empty in the tunic vesicle of *A. yamazii.* The blood vessels of botryllids are interconnected among the zooids, and hemocytes in the lumen circulate throughout the colony. These circulating hemocytes in botryllid ascidians are involved in budding (Oka and Watanabe, 1957), oogenesis (Mukai and Watanabe, 1976) and colonial allorecognition (Cf.Hirose *et al*., 2003).

The distal tip of the stolonic vessel swells and ends in an ampulla that periodically expands and contracts. The cell membrane of the basal side is highly enfolded in the cuboidal cells of ampular wall. These folds are probably caused by the contraction of the epidermal cells. The periodic movement of the ampullae may be involved in the locomotion of the colonies reported in some didemnid species (Carlisle, 1961; Birkeland *et al*., 1981). In polycitorid stolon and vascular appendage, the distal tip does not swell to form ampular ends. In botryllid ascidians, vascular ampullae also show the periodical movement of expansion and contraction, and there is a thin layer of microfilaments at the basal region of the cell, suggesting the contractility of the cell (De Santo and Dudley, 1969; Katow and Watanabe, 1978). In metamorphosing juvenile of molgulids, ampular epidermis shows peristaltic movement, and the contractions are thought to be mediated by a thin layer of microfilaments in the base of the ampular epidermis (Torrence and Cloney, 1981). These periodic movements of the ampular structure may contribute to expansion of the tunic spreading on the substratum and circulation of the fluid in the lumen.

The epidermal wall of the stolonic vessel consists of cuboidal cells in which granules and vesicles are rarely found, and the ampular tip consists of columnar cells containing many round granules. In the distal tip of the stolons of *C. miniata,* the columnar cells contain some vesicles containing heterogeneous materials, but the amount of vesicle is much less than the granules in the columnar cells of the stolonic vessel. In the cuboidal cells of the vascular appendage in *Distaplia* sp., the lumen of ER is usually filled with moderately electron-dense materials, while there are only a few electron-dense granules in the cytoplasm. In *E. glaucus,* the epidermal cells of the posterior pat of the abdomen are squamous and highly vacuolated, whereas the columnar cells of the vascular appendage contain few vacuoles and granules. As described above, the cytoplasmic inclusions, e.g., round granules, are much different in morphology among the epidermal cells of the didemnid stolonic vessel and the polycitorid stolon/vascular appendage. In *Aplidium yamazii,* the epidermis of the posterior part of the zooid consists of columnar cell containing vesicular components in the apical cytoplasmic bulge, and it is much different in morphology from the epidermal cells comprising the stolonic vessel. On the contrary, the tunic vesicle of *A. yamazii* has similar cell composition to the stolonic vessel: squamous cells with few granules and cuboidal cells with full of rough ER and round granules. The vascular ampulla of *Botrylloides simodensis* also has similar cell composition to the stolonic vessel. On the other hand, there are many microvilli in the apical side of the cuboidal cells of the stolonic vessel in *D. virens*, the columnar cells of the stolon in *C. miniata,* and the cuboidal cells of the vascular appendage in *E. glaucus*. They are not found in the columnar cells of the stolonic vessel in *D. virens,* the vascular appendage in *Distaplia* sp., the abdomen and tunic vesicle in *A. yamazii,* and the vascular ampulla in *B. simodensis*. These microvilli are supposed to anchor the zooid or stolon/appendage to the tunic.

In the columnar cells of the stolonic vessel, round granules are heterogeneous in electron-density and the inner electron-dense parts have striated patterns. Secretion of the granules were not observed, while the occurrence of apocrine secretion might be suggested by the granule-free

bulge of the cytoplasm in the apical side of the columnar cells. These characteristics are well consistent with the histological observation on the stolonic vessels described by Millar (1951). In *A. yamazii,* metachromatic stainability of the tunic around the zooid indicates that the epidermis of the zooid probably secrete some materials to the tunic. The apical cytoplasmic bulge and vesicles containing moderately electron-dense material suggest the occurrence of apocrine secretion and exocytosis of vesicular contents, respectively. The round granules in the tunic vesicles are homogeneously electron-dense and not similar in ultrastructure to those of the didemnid stolonic vessels. Degranulation of these granules of the tunic vesicle could not observed so far examined. The pad cells of the vascular ampullae in *B. simodensis* are full of rough ER and round granules. The granules have heterogeneous substructure in electron-density and are similar in morphology to the round granules of the didemnid stolonic vessels. We could not observe the secretion of the granules. Katow and Watanabe (1978) described more detailed observation on the ampular epidermis of *Botryllus primigenus:* the pad cells have cytoplasmic bulge in the apical side and contain round granules that are heterogeneous in electron-density, and these granules are referred as to adhesive vesicle. According to their description, the adhesion vesicles fuse with apical cell membrane and discharge their contents into the tunic. Moreover, exocytosis of coated vesicles is observed in some epidermal cells consisting lateral wall of the ampullae. Epidermal cells having similar to the botryllid pad cells have been reported in some other stolidobranch ascidians. In the ampular epidermis of metamorphosing larvae of *Molgula occidentalis* (Molgulidae), Torrence and Cloney (1981) reported the presence of numerous secretory vesicles in the glandular cells and the exocytosis of coated vesicles in parietal cells. Similarly, in *Polyandrocarpa misakiensis* (Styelidae), the epidermal cells have round granules having vesicular substructures and apical cytoplasmic bulges suggesting apocrine secretion (Hirose and Mukai, 1992).

It has been thought that the granular inclusions are involved in secretion of adhesive material to attach the substrata in didemnid stolonic vessel (Millar, 1951), tunic vesicle (Oka and Usui, 1944), botryllid pad cells (Katow and Watanabe, 1978), and glandular tip cells of molgulids (Torrence and Cloney, 1981). This is not contradictory to the observations described above, but secretion of the granules has never observed in this study. It may be possible that the degranulation occurs responding to a particular event, although we have never met such a situation so far. Alternatively, the granules are merely temporal storage of the tunic components that are discharged into the tunic through the apocrine secretion of apical cytoplasmic bulges. In either case, the exact function of the granules is still uncertain.

The present observations showed that the stolonic vessel of *Diplosoma* is similar in morphology to the vascular ampullae of *Botrylloides* and the tunic vesicle of *Aplidium* rather than the stolons of *Clavelina,* the vascular appendages of *Distaplia* and *Eudistoma,* and the epithelia of the posterior ends of *Eudistoma* and *Aplidium*. Many ascidians possess tubular extensions of epidermis. Some of them have granular epidermal cells at the distal tip, whereas these ascidians are not always related closely with one another. The cell morphology is supposed to reflect its function but not the phylogenetic relationship, and thus the histological and ultrastructural investigations could not provide conclusive evidences to prove the phylogenetic relationship among the stolonic vessels and the other tissues examined here. It is necessary to reveal their functions to discuss the homology and phylogenetic relationship among the tubular projections of epidermis in ascidians.

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