Ultrastructure of the Mid-gut Epithelium of *Tigriopus japonicus*\textsuperscript{*1}

Kazuma Yoshikoshi*\textsuperscript{2}

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The mid-gut epithelium in the metasome consists of two kinds of epithelial cells, strongly vacuolated cells and non-vacuolated cuboidal or columnar cells. The vacuolated cells are characterized by prominent endocytosis, suggesting both absorptive and digestive functions. The non-vacuolated cells possess well developed microvilli and plentiful cytoplasmic organelles, particularly the conspicuous granular endoplasmic reticulum, Golgi complexes, and numerous lysosomal vacuoles, suggesting both absorptive and secretory functions. The mid-gut epithelium in the uroscope consists of rather flattened non-vacuolated cells, the primary function of which is probably absorption. The mid-gut epithelial cells exhibit various ultrastructural features depending largely on their maturation stages and functional conditions.

In previous papers dealing with histological structure of the alimentary canal and cell renewal in the mid-gut epithelium of *Tigriopus japonicus*,\textsuperscript{1,2} I reported the existence of two kinds of epithelial cells in the mid-gut, which were referred to as the glandular cells and the absorptive cells, and some ultrastructural findings on cell renewal. It seemed impossible, however, to infer the primary function of each cell type from these findings, since the ultrastructural examination was not thorough enough. It is from this aspect that a further ultrastructural examination was made using improved methods and an excellent electron microscope.

This paper describes new and supplementary findings on the ultrastructure of the mid-gut epithelium of *Tigriopus*.

Materials and Methods

Adult females of *T. japonicus* were obtained from a laboratory culture.

The specimens were fixed in a cold formaldehyde-glutaraldehyde fixative containing 2% formaldehyde (freshly prepared from paraformaldehyde) and 3% glutaraldehyde buffered with 0.1 M cacodylate, pH 7.3, for 3 hours. Immediately after immersing the specimens in the fixative, both anterior and posterior portions of the body were cut off using a razor blade. The fixed specimens were rinsed in a 1:9 mixture of the cacodylate buffer and a saline solution for marine crabs containing 2.5% NaCl, 0.26% KCl, 0.23% CaCl\(_2\), and 0.16% MgCl\(_2\). After that, the specimens were post-fixed in 1% OsO\(_4\) in the buffered saline solution mentioned above, dehydrated in ethanol, and embedded in Spurr's resin. Ultrathin sections were cut using a Porter-Blum MT-1 ultramicrotome, stained with uranyl acetate and lead citrate or with lead citrate alone, and observed using a JEOL JEM 100S electron microscope.

Results

The mid-gut epithelium in the metasome consists of two kinds of epithelial cells.

The vacuolated cells which were referred to as the glandular cells in earlier papers\textsuperscript{1,2} are characterized by prominent endocytosis (Fig. 1). The cells possess numerous microvilli of ca. 0.7 μm in length and ca. 0.07 μm in diameter, in which fine filaments, probably actin filaments, are found to be arranged longitudinally in a bundle. The plasma membrane of the microvilli is ca. 8 nm in thickness. In the cytoplasm beneath the free surface numerous endocytotic vacuoles are present. They coalesce into larger heterophagic vacuoles, each containing an electron dense spherical body which is probably an indigestible residue (Figs. 1 and 2). Beneath the heterophagic vacuoles there is an enormous basal vacuole occupying a great portion of the cells. This basal vacuole contains a material similar to that of the smaller hetero-
phagic vacuoles except that the occurrence of the residual bodies is less frequent. Occasionally, the vacuole increases in size to such an extent that the nucleus is compressed and displaced to one side and the cytoplasm becomes extremely thinned out. Mitochondria, the granular endoplasmic reticulum, and Golgi complexes are present in the narrow cytoplasm surrounding the heterophagic and basal vacuoles, but they are not so plentiful as in the cells of another type. A few mitochondria are also present between the endocytotic vacuoles in the apical region of the cells. Secretory vacuoles and lipid droplets are not found in the cells. On the lateral surface immediately beneath the free surface of the cells the septate junction is present, where the opposing plasma membranes measure ca. 8 nm in thickness and the space between the membranes ca. 9.2 nm (Fig. 1). From the septate junction to the base of the cells there are 3 to 4 gap or tight junctions, but desmosomes and other junctional apparatus are not found. The inter-digitation of the lateral surface is not seen. Occasionally, the strongly condensed vacuolated cells are found between the ordinary cells (Fig. 3).

The epithelial cells of another type which were referred to as the absorptive cells in earlier papers, cuboidal or columnar in shape, possess plentiful cytoplasmic organelles (Fig. 4). Numerous microvilli of ca. 1.0 µm in length and ca. 0.08 µm in diameter are seen in the free surface of the cells, the internal structure of which is the same as in the vacuolated cells mentioned above. The length of microvilli varies to some extent from cell to cell and tends to increase as aging of the cells progresses, measuring 1.3 µm in an aged cell. The number of microvilli is ca. 60/µm². Immediately beneath the free surface there is a filament-rich layer where the core filaments of microvilli enter and the granular endoplasmic reticulum and many lysosomal vacuoles are present. This layer differs in the presence of these cytoplasmic organelles from the terminal web of the mammalian intestinal absorptive cells excluding cytoplasmic organelles. The granular endoplasmic reticulum is very plentiful throughout the cells and tends to lie parallel to the lateral surface of the cells. Golgi complexes are also plentiful in the supranuclear region, where numerous lysosomal vacuoles containing a homogeneous and moderately dense material are formed. Mitochondria, varying in shape and size and possessing densely packed cristae, are present throughout the cells. Lipid droplets are always present, though their number and size considerably vary from specimen to specimen. The nucleus, spherical or ovoid in shape, is situated in the basal half of the cells. The infoldings of the basal plasma membrane are less conspicuous. The ultrastructure of the lateral surface of the cells is the same as in the vacuolated cells.

The mid-gut epithelium in the urosome consists of rather flattened non-vacuolated cells which develop from relatively undifferentiated cells lying in the basal region of the epithelium (Fig. 5), as has been described in the anterior mid-gut epithelium. The mature epithelial cells possess numerous microvilli of ca. 0.6 µm in length and ca. 0.08 µm in diameter and their number is ca. 60/µm². The internal structure of the microvilli is the same as in the cells in the metasome. Cytoplasmic organelles of the mature cells are much less plentiful than in the non-vacuolated cells in the metasome. The structure of the cell junction is the same as in the cells in the metasome, but the lateral surface is somewhat complicated by the overlapping of the cells. The developing cells are situated beneath the mature cells and possess conspicuous Golgi complexes, numerous vacuoles, and a few mitochondria (Figs. 5 and 6). Immature cells are abundant in free ribosomes (Fig. 5). Occasionally, a colliquative break down is found to occur in the mature cells and these cells are then renewed by underlying developing cells (Fig. 6). The numerous vacuoles found in the developing cells supply the membranous material in the formation of microvilli (Fig. 7).

Discussion

It is doubtless that fixation is the most important first step for better tissue preservation. In this respect, double fixation with formaldehyde-glutaraldehyde and OsO₄ fixatives combined with cutting some body portions off at the primary fixation provided fruitful results.

It was revealed by improved tissues preservation that the vacuolated mid-gut epithelial cells of Tigriopus which had been regarded as the glandular cells probably function as the absorptive cells. Similar vacuolated cells have been found in the mid-gut of a number of free-living copepods including calanoid, cyclopoid, and harpacticoid copepods and regarded as the glandular cells producing digestive enzymes or a peritrophic membrane. Recently, Arnaud et al. described that the B cells of Centropages...
typicus, the vacuolated cells, are characterized by prominent pinocytosis and subsequent intracellular digestion of nutritive substances, and that the B cells are extruded into the gut lumen after maturation and the cell content is absorbed by the R cells, being the absorptive cells. More recently, they\(^9\) suggested that the B cells of calanoid copepods are not only digestive but also excretory in function. The ultrastructure of the vacuolated cells of Tigriopus resembles in many respects that of Centropages and the other calanoid copepods, but there is a remarkable difference between the two: the luminal extrusion of the vacuolated cells in Tigriopus probably takes place as senile decay accompanying a marked condensation of the cells. Briggs\(^14\) reported in Paranthessius anemoniae, a cyclopoid associate of the snakelocks anemone, that the ameboid cells, a type of the mid-gut epithelial cells, engulf the gut content by phagocytosis. Similar cells were found in some cyclopoid associates of marine bivalves and fish parasites (in preparation). It is likely that intracellular digestion of the gut content is an essential process in the digestive physiology of copepods. Its biological significance in copepods seems to be in compensating relatively insufficient luminal digestion of the gut content at a rather low phylogenetic stage. Intracellular digestion of the gut content has also been reported in the mid-gut gland cells of malacostracans:\(^15,16\) these cells digest nutritive substances and use nutrients at least in part for the synthesis of digestive enzymes.\(^14\)

It is evident in Tigriopus that the non-vacuolated cells in the metasome function as the absorptive cells in the possession of well developed microvilli. Of particular interest is the secretory function of the cells. The ultrastructural features such as the abundant granular endoplasmic reticulum, Golgi complexes, and numerous lysosomal vacuoles and their occurrence just beneath the free surface of the cells (Fig. 4), strongly suggest that the cells also function as the secretory cells of digestive enzymes.\(^14\)

Numerous microvilli and few lysosomal vacuoles in the non-vacuolated cells in the urosome suggest that the primary function of the cells is absorption. Less abundant cytoplasmic organelles also suggest rather reduced functions in the cells.

The present study reconfirmed the existence of strongly condensed mid-gut epithelial cells in the metasome and adduced new evidences on cell renewal in the mid-gut epithelium in the urosome. It is likely that subtle or remarkable differences in the structure of the mid-gut epithelial cells are closely associated with their maturation stages and functional conditions as well as with cell types.

References
Explanation of Figures

Fig. 1. Vacuolated mid-gut epithelial cell in the metasome. Arrow indicates the septate junction. N: nucleus, V: basal vacuole, ×9,000. Inset: partial enlargement of the septate junction, ×130,000.

Fig. 2. Partial enlargement of Fig. 1, showing the coalescence of endocytic vacuoles into larger heterophagic vacuoles (arrow). ×25,000.

Fig. 3. Mid-gut epithelial cells in the metasome. ×6,000.

Fig. 4. Non-vacuolated mid-gut epithelial cell in the metasome. Arrows indicate lysosomal vacuoles situated just beneath the free surface of the cell. G: Golgi complex, Mv: microvilli, ×25,000. Inset: apical region of the cell shown in Fig. 4.

Fig. 5. Mid-gut epithelial cells in the urosome. Bl: basal lamina, Cm: circular muscle, a-c: developing cells, d: mature cell, ×21,000.

Fig. 6. Mid-gut epithelial cells in the urosome. e: cell under colliquative break down, ×21,000.

Fig. 7. Mid-gut epithelial cell in the urosome. Pm: peritrophic membrane, ×30,000.
Mid-gut Epithelium of Tigriopus