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Fine Structure of the Chloride Cell in the Gill Epithelium of *Brachydanio rerio* (Cyprinidae, Teleostei)

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Abstract: The main ultrastructural properties of the gill epithelium of the zebrafish, *Brachydanio rerio* are investigated. The pavement cells are distinguished with their microvilli, infolding protrusions and construction of an intercellular channel system, while the chloride cells sited in filamental and especially respiratory lamellar epithelia are characterized by a high number of well developed, large mitochondria and an expansive, membranous tubular system. The close relationship observed between these cell types indicates that, at least ultrastructurally, chloride cells are communicated with the external milieu via the channel system of the pavement cells.

Key Words: Osmoregulation, gill filaments, epithelia, pavement cells. chloride cells, *Brachydanio rerio* (Teleostei).

Brachydanio rerio'nun (Cyprinidae, Teleostei) Solungaç Epitelindeki Klorid Hücrelerin İnce Yapısı

Özet: *Brachydanio rerio'da* (Zebra Balığı) solungaç epitelinin temel ultrastrüktürel özellikleri araştırılmıştır. Döşeyici hücreler mikrovillusları, katlanmış uzantıları ve hücrelerarası bir kanal sistemi oluşturmaları ile ayırt edilmişlerdir. Filament epitelinde ve özellikle solungaç lamellerinde yer alan klorid hücreler çok sayıda iri ve iyi gelişmiş mitokondriumların yanısıra zarsı yapıda, yaygın tübüler sistemleriyle karakteristiktir. Bu hücre tipleri arasında gözlenen sıkı ilişki, en azından ultrastrüktürel olarak, klorid hicrelerin dış ortamla iletişimlerinin döşeyici hücrelerin kanal sistemi aracılığı ile sağlandığını işaret etmektedir.

Anahtar Sözcükler: Su-tuz dengesi, solungaç filamenti, epitel, döşeyici hücreler, klorid hücreler, *Brachydanio rerio* (Teleostei).

Introduction

As a surface for the respiratory gas diffusion, ionoregulation and waste excretion, opercular and gill epithelium of teleosts had been widely investigated (1, 2). The gill is composed of the filaments and lamellae covered by a special epithelium (3,4). This epitelium consists of three cell types: the most abundant pavement cells arranged in a cobblestone fashion (2), mucous cells and typically differentiated chloride cells.

It is clear that ionic composition of an aquatic environment is effected by so many factors. In order to adaptate to the new conditions, gill epitelia should have the ability to excrete and/or absorb salts (5). The chloride cells had been first identified in some seawater and freshwater teleosts during 1930's, and are resembled the acid-secreting cells of the stomach (2). Since that time, many investigations on the structure and function of the chloride cells have been performed of various species including *Fundulus sp.* (2,6,7), *Oreochromis mossambicus* (5), *Mugil capito* (8), *Anguilla japonica* (9) and *Rivulus marmoratus* (10).

Surely, morphological researches are the main route for providing the basic data which can be indicative for physiological investigations. From this viewpoint, the purpose of this study is to identify the fine structure of the chloride cells of zebrafish, *Brachydanio rerio,* and to discuss their function.

Materials and Methods

Zebrafishes were obtained from commercial dealers and acclimated to filtered and well aerated tap water in 100 lt. aquaria at 23 ± 14 °C for two weeeks. The fishes were exposed to a photoperiod of 14L: 10D. Commercial flake food was utilized for rearing.

After sacrification, gill filaments of gill arches of each fish were dissected out and fixed in freshly prepared cacodylate buffered glutaraldehyde and osmium tetraoxide. Thin sections of epon-embedded material were stained in uranyl acetate-lead citrate and examined under JEOL 100 C transmission electron microscope.

Results

On each side of their head, teleosts have four gill arches. Each of them is made up of hundreds of filaments. An individual gill filament is consisted of an eccentrically placed cartilaginous rod and supporting connective tissue which are covered by epithelium. Flattened respiratory lamellae (secondary lamellae) extend in two rows from the sides of the filaments. Between the layers of flattened pavement cells, chloride cells can be seen in either filamental or lamellar epithelia, however, most of them is observed in lamellae (Fig. 1).

Even at a relatively low magnification, it is easy to distinguish the pavement cells with their conspicuous microvilli and microridges, and largely infolding protrusions (Fig.2). These surfaces are covered with a thin coat of finely filamentous, mucoid material. The pavement cells contain a small amount of smooth endoplasmic reticulum in the form of flattened cisternae and a lot of free ribosomes (Fig. 3,4). Their Golgi apparatus consist mainly of lamellar and vesicular components. Randomly distributed mitochondria are numerous, although they are smaller when compared to the mitochondria of chloride cells. The nuclei of the pavement cells are large and irregular, sometimes lobular in shape (Fig. 2,3).

The presence of some widened channels is found to be the most distinctive feature of the pavement cells. These channels which are originated from the plasma membrane, appeared to be in continuity with the largely infolding protrusions and the enlarged intercellular area (Fig. 3,4,5). Thus, they form an intercellular network. It is known that the microvilli, microridges and protrusions serve to widen the surface placed between the external and internal media. Such an organization is especially necessary for the diffusion of the respiratory gases. In addition to that organization, a construction of an intercellular channel system is found to be very interesting.

The chloride cells which are surrounded by the pavement cells can be seen on either filamental or lamellar epithelia (Fig.1). High number of well developed, large mitochondria and an extensive, membranous tubular system are the main characteristic features of the chloride cells. The nuclei are round or ovoid in shape (Fig.5,6).

During the lifetime of the chloride cells, three progressive stages can be observed. At the initial stage, a clear cytoplasm which contains a few mitochondria is typical (Fig. 6a). Later, mitochondria increase in number and the cytoplasm is getting darker (Fig. 6b). The cells in the last stage are full of mitochondria (Fig. 6c). The tubular system is seen to have a less organized network initially, however, it becomes more anastomosing and is greatly elaborated furtherly. Widening of the perinuclear area and the occurence of small clusters of the multivesicular bodies are the common features for all of these stages.

Cytoplasm of a chloride cell exhibits small amounts of smooth endoplasmic reticulum, a few free ribosomes, multivesicular bodies and numerous, large mitochondria. The anastomosing tubular system is widely interspersed in the cytoplasm and the tubules are often in close association with the plasma membranes (Fig. 7).

Discussion

In *Brachydanio rerio,* it is observed that the chloride cells localize both in the filamental and lamellar epithelia. This finding is consistent with the previous studied performed on *Oreochromis mossambicus* (5), *Solea solea* (11) and *Onchorhynchus mykiss* (12).

Figure 1. Light micrograph of the cross section of gill filaments. Cartilaginous rod (CR), respiratory lamellae (RL), connective tissue (CT) and chloride cells (→) sited on both lamellar and filamental epithelia. x500.

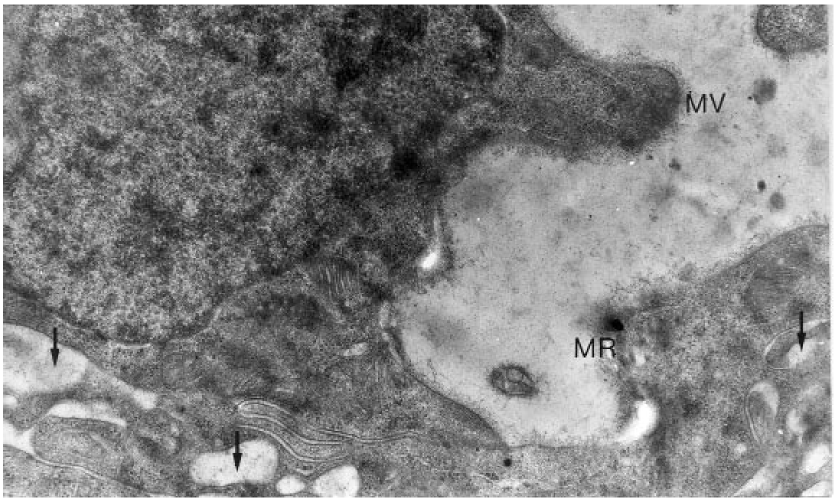

Figure 2. Pavement cell with microvillus (MV), microridge (MR) and infolding protrusions (→) covered by finely filamentous, mucoid material. x19.900.

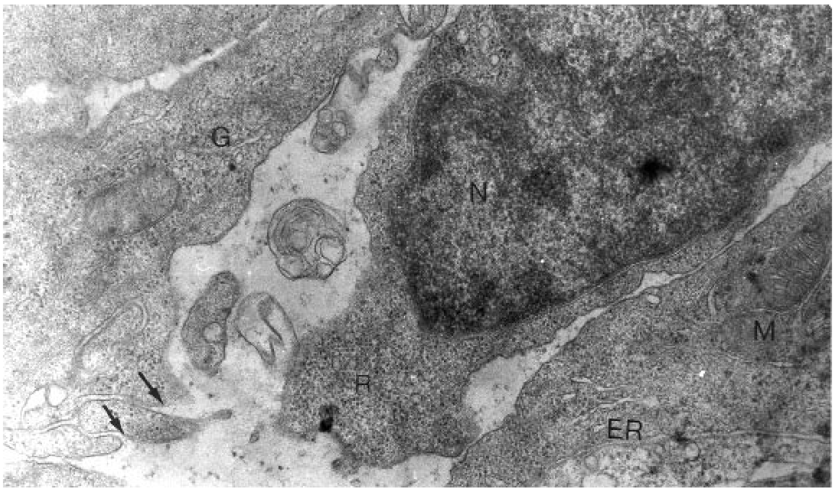

Figure 3. The cell body and infolding protrusions of the pavement cells contained rough endoplasmic reticulum (ER), numerous free ribosomes (R), Golgi area (G), mitochondria (M) and irregularly forming nucleus (N). The channels of the pavement cells may also be seen with their opengings (→) to the widened intercellular area. x27.500.

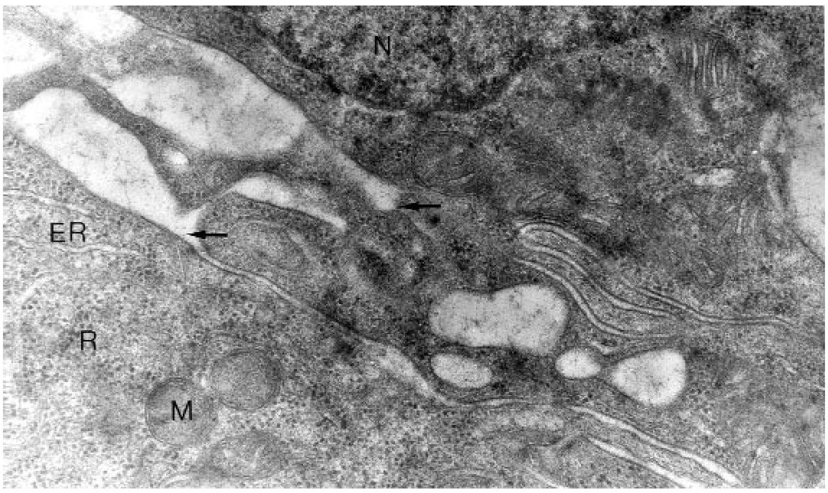

Figure 4. The channels forming an intercellular channel system. Note that the ending of the chanels (→) with the widened intercellular area. Rough endoplasmic reticulum (ER), ribosomes (R), mitochondria (M) and nucleus (N). x29.900.

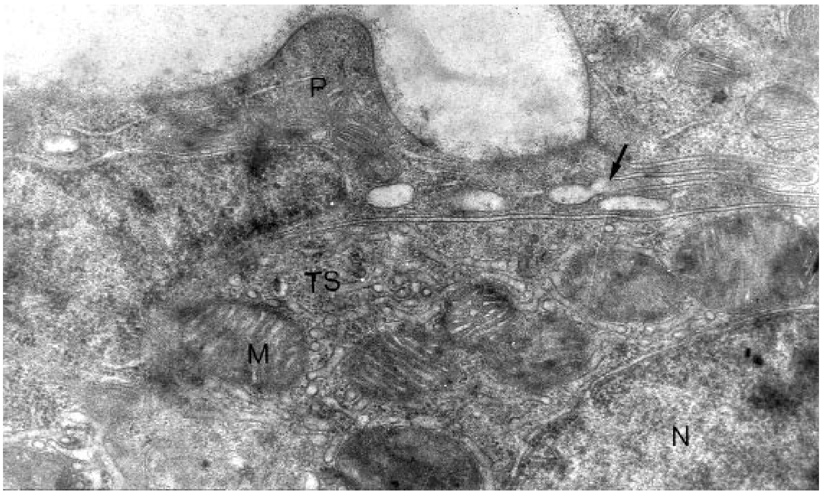

Figure 5. Chloride cells surrounded by the pavement cells (P). Comparatively large, well-developed, abundant mitochondria (M), tubular system (TS) and nucleus (N). Note that the channels of the pavement cells ended with the infoldings. x23.000.

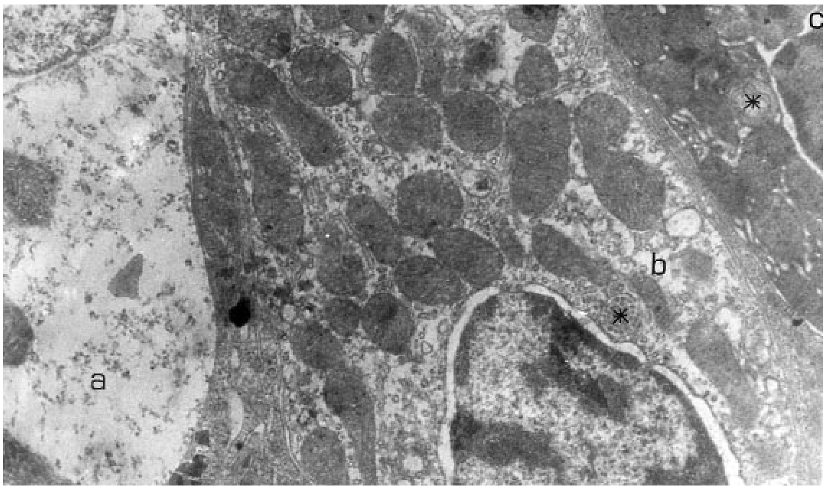

Figure 6. Chloride cells in different stages of their lifespent period. At the initial stage cytoplasm is clear (a); mitochondria increase in number at second stage (b) and cytoplasm is in the last stage (c). Widening perinuclear area, multivesicular bodies (*) and developing of the tubular system are also seen in all stages. x15.000.

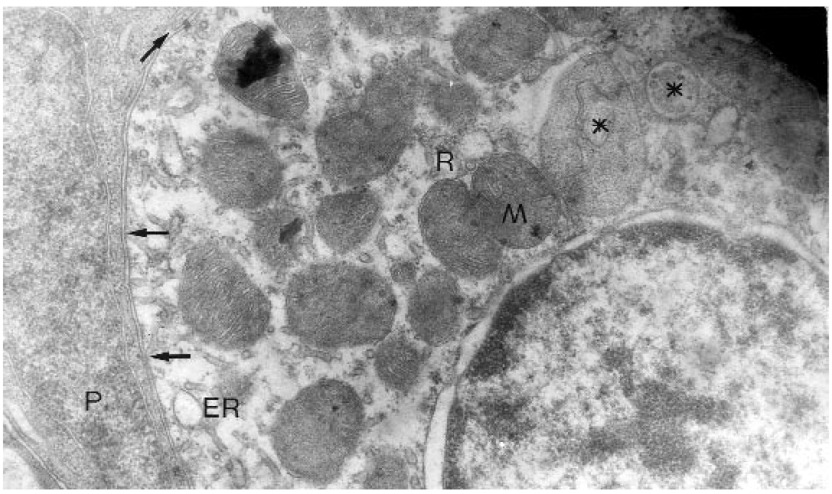

Figure 7. Mature chloride cell exhibiting numerous mitochondria (M) endoplasmic reticulum (ER), a few free ribosomes (R) and multivesicular bodies (*). Note the interspersing of the elaborated, anastomosing tubular system and its close association with the plasma membranes (→). x17.000

Although generalizations for all of the teleosts have to be made with caution, it is possible to conclude that the main ultrastructural aspects of the chloride cells are similar in species investigated. From this viewpoint, the presences of the numerous, well developed, large mitochondria and an expansive tubular system in the chloride cells of *Brachydanio rerio* are found to be similar to those reported previously (2, 5, 9, 10). However, such a general similarity does not indicate a functional equality. There is no speculation about the function of the chloride cells of the seawater fishes; salt excretion of the specialized epithelial cells has been postulated to be vital in seawater, however, it is difficult to answer the critical question concerning their role in freshwater.

Whether they live in freshwater or seawater, teleosts should have to solve severe osmotic problems. In contrast to the conditions in seawater, the internal fluids of a freshwater fish contain more salts than external milieu. In order to survive, fish drinks little water and absorbs ions from the external medium via the gills. Reabsorbtion of Na^+ and Cl^- ions from the large amounts of diluted urine is also of great significance. By all of these mechanisms, both Na^+ and Cl^- are actively transported from the milieu to the blood, but, this conclusion is based upon only *in vitro* experiments. Moreover, it has been presented that all of the Na^+ and Cl^- influx is through the lamellar epithelium in the trout gill (13). Because of the chloride cells are located not only in the lamellar epithelium, but also in the filamental epithelium, this finding is supposed to be an evidence that, ionic absorbtion does not occure in the chloride cells of freshwater fishes. Additionally, a notable difference between the opercular and filamental epithelia of hypo-osmoregulating tilapia was reported recently (5), this study suggests that the opercular epithelium may only be involved in NaCl secretion, and, the filamental epithelium is the site of NaCl absorbtion and secretion.

The locatization of the most chloride cells on the secondary lamellae of the intact zebrafish may bring a different viewpoint for these speculations. At least ultrastructurally, it is possible to state that lamellar epithelium is not involved only with the gas exchange, but also with the ionic exchange.

An ultrastructural hallmark of the seawater chloride cells is an invagination called apical crypt (8). This structure is correlated with adaptation to high salinities (2, 9). Although it has been noticed that there are just a few reports pointing out the presence of apical crypts in freshwater teleosts, we failed to observe the single or multicellular formation of the crypts in the chloride cells of *Brachydanio rerio.*

The multicellular complexity of apical crypts is defined in some seawater species including *Mugil capito* and *Anguilla anguilla* (8); *Solea solea* (11) and *Blennius pholis* (14). In some freshwater teleosts *(Oreochromis mossambicus, Plectoglossus altivelis* and *Cyprinus carpio)* the multicellular structures are also reported (15, 16). The observation of three progressive stages of the chloride cells of *Brachydanio rerio* can not be suggested as an evidence for such a separated from each other by the protrusions of the pavement cells.

It is known that the microvilli and infolding protrusions of the pavement cells serve to form an expansive respiratory surface. The presence of an intercellular channel system constructed by these cells is probably raise a question whether this system is involved with another function.

Although it can be supported only ultrastructurally, we suggest that the intercellular channel system of the pavement cells is closely related with the tubular system of the chloride cells; this relationship may play a mediatory role in the regulation of internal ionic composition. In other words, although always placed interiorly, and never found to be exposed to the external milieu, chloride cells of *Brachydanio rerio* communicate with the external milieu via the intercellular channel system of the pavement cells.

Then the critical question arises: do the chloride cells communicate directly with the external milieu and form an apical crypt during the adaptation period to the high salinities? In *Oreochromis mossambicus,* it is reported that the secondary epitelium was not modified by salinity (5). Although *Brachydanio rerio* is found to be a hardly resisting species to the high rate of ambient salinities (17), this question remains to be answered with precise transfer experimerts.

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