Morphology and Function of Gill Mitochondria-Rich Cells in Fish Acclimated to Different Environments

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ABSTRACT

The objective of this study is to test the hypothesis that morphologically different mitochondria-rich (MR) cells may be responsible for the uptake of different ions in freshwater-adapted fish. Tilapia (*Oreochromis mossambicus*) were acclimated to high-Ca, mid-Ca, low-Ca, and low-NaCl artificial freshwater, respectively, for 2 wk. Cell densities of wavy-convex, shallow-basin, and deep-hole types of gill MR cells as well as whole-body Ca^{2+} , Na^+ , and Cl^- influxes were measured. Low-Ca fish developed more shallow-basin MR cells in the gills and a higher Ca^{2+} influx than those acclimated to other media. However, fish acclimated to low-NaCl artificial freshwater predominantly developed wavy-convex cells, and this was accompanied by the highest Na^+ and Cl^- influxes. Relative abundance of shallow-basin and wavy-convex MR cells appear to be associated with changes in Ca^{2+} and Na^+/Cl^- influxes, suggesting that shallow-basin and wavy-convex MR cells are mainly responsible for the uptake of Ca^{2+} and Na^+/Cl^- , respectively.

Introduction

Mammalian collecting duct, amphibian skin, and fish gill are useful models to study the mechanisms of transepithelial trans-

port of ions. Intercalated cells in mammalian collecting duct and mitochondria-rich (MR) cells in amphibian skin and fish gills share similar characteristics in morphology and function and, thus, are the target cells for studies on ion transporting cells.

For a long time, studying how these cells perform the transport functions of different ions has been an important and interesting topic. On the basis of the differential expressions of band 3 and vacuolar H^+ -ATPase, two types, α (or A) and β (or B), of intercalated cells in the mammalian collecting duct were identified (Alper et al. 1989). Moreover, the two types of cells were demonstrated to be responsible for the transport of bicarbonates and protons, respectively (Madsen and Tischer 1986; Schuster and Stokes 1987). In amphibian skin, different subtypes or subpopulations of MR cells have also been identified on the basis of functional performances (Larsen 1991; Rick 1992) or cytochemical patterns (Katz et al. 1997). However, evident linkages between morphology and function of different types of MR cells are not yet well established.

The branchial MR cell in fish is another good model system to elucidate the relations of morphologies and functions of transepithelial cells. Teleosts inhabit environments ranging from hypotonic to hypertonic water. Gills are the most important extrarenal organs responsible for ion regulation in teleosts, and the gill MR cells have been demonstrated to play major roles in ionic exchanges by actively excreting or absorbing salts in hyper- or hypotonic milieus, respectively (Epstein et al. 1980; Foskett and Scheffey 1982; Perry 1997). Evident changes in morphology in gill MR cells and in ion regulation have been well documented in teleosts on their acclimation to environments with different osmolarities or ion compositions (Hwang 1987, 1988). Using the vibrating probe technique, Foskett and Scheffey (1982) provided definitive evidence for the location of active Cl^- secretion. Moreover, many morphological, biochemical, and physiological studies support the current model (Silva et al. 1977) for salt secretion of MR cells (Epstein et al. 1980; Hwang 1987; Hwang et al. 1988, 1989; Laurent and Perry 1991; Zadunaisky 1984). However, in hypotonic environments, the ion-uptake function ascribed to MR cells is still a matter of discussion (Perry 1997). Uptake of diverse ions, that is, Na^+ , Cl^- , and Ca^{2+} , was shown to correlate significantly with the fractional area of exposed MR cells on the gill filament epithelium of some teleosts, which were treated with hormones or acid/base disturbances (Perry et al. 1992a, 1992b; Bindon et al. 1994). These phenotypic changes were suggested to be a crucial mechanism for ionic (Pisam et al. 1995) or acid-base

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Table 1: Ionic compositions (mM) in the artificial freshwater

Medium	[Na ⁺]	[Cl ⁻]	[Ca ²⁺]	[K ⁺]	[Mg ²⁺]
Low Ca	.529 ± .108	.303 ± .067	.004 ± .001	.198 ± .027	.135 ± .035
Mid-Ca	.547 ± .104	.308 ± .074	.148 ± .027	.194 ± .030	.166 ± .016
High Ca	.807 ± .199	.321 ± .077	9.493 ± 1.96	.199 ± .029	.161 ± .022
Low NaCl	.017 ± .008	.023 ± .02	.151 ± .021	.189 ± .028	.167 ± .016

Note. Mean ± SD (N = 7) is indicated.

regulation (Goss et al. 1992). However, the relationships between uptake of diverse ions and phenotypic changes of MR cells are still uncertain up to now. In the past, Wendelaar Bonga and van der Meij (1989) described MR cells as pleomorphic. Pisam et al. (1987) also showed that at least two types of MR cells, α cells and β cells, existed in the gill epithelium of freshwater teleosts. Thus, based on this information, we proposed that subpopulations of MR cells with their own function and morphology may exist in the gill epithelium of the teleosts to deal with diverse ionic changes in varied hypotonic milieus. Our previous studies (Lee et al. 1996a, 1996b) have indicated that several types of MR cells with different morphologies in apical crypts exist in fish gills; the densities of these cells are associated with the ionic compositions of environments to which fish were acclimated. However, there is no physiological data to demonstrate that these morphologically different MR cells are responsible for the transport of different ions.

In this study, tilapia (*Oreochromis mossambicus*) were acclimated to artificial freshwater containing different ionic compositions, and the densities of different types of gill MR cells and ion influxes of Ca²⁺, Na⁺, and Cl⁻ then were measured. The results indicate that the relative densities of different types of MR cells are closely associated with the changes in influxes of Ca²⁺, Na⁺, and Cl⁻, suggesting that these cells with different morphologies are responsible for the uptake of different ions.

Material and Methods

Animals

Male or female tilapia (*Oreochromis mossambicus*) of 0.3–0.7 g (for ion flux measurements) and 4.4–12.3 g (for gill morphology) body weights were obtained from laboratory stocks. The group of small-size fish was used in the ion-influx experiments to decrease the volume of waste isotopic media. The large-size group was used for morphological observations because of convenience in sample preparation. Preliminary studies have confirmed that no significant differences in both morphology and ion influxes were found between these two groups of tilapia. All individuals were reared in aerated local tap water at 26°–28°C with a photoperiod of 12L : 12D before the acclimation experiments.

Acclimation Experiments

Four kinds of artificial freshwater, high Ca, mid-Ca, low Ca, and low NaCl, were prepared by adding appropriate amounts of NaCl, MgSO₄, K₂HPO₄, KH₂PO₄, and CaSO₄ to double-deionized water (Milli-RO60, Millipore). The ion concentrations of mid-Ca were near the ranges of local freshwater. The ionic compositions (Table 1) of the four media were confirmed by measuring the Na⁺, K⁺, Ca²⁺, and Mg²⁺ concentrations with an atomic absorption spectrophotometer (Hitachi Z-8000, Tokyo) and measuring Cl⁻ with a spectrophotometer (Hitachi U-2000, Tokyo). The pH of the media was kept between 6.2 and 6.7, and the water temperature was 26°–28°C. Tilapia were acclimated to the low-Ca, mid-Ca, high-Ca, and low-NaCl media for 2 wk and then were sampled for morphological observation (five individuals for each test) and ion influx measurements (four to eight individuals for each test). The acclimation experiment was repeated two to three times. To maintain the water quality, the acclimation media were aerated with a filtered air pump, and the artificial freshwater was prepared and changed every 2 d. The fish were fed with commercial pellets 1 h before the water change every 2 d, but feeding was stopped 3 d before the sampling.

Morphology of Gill MR Cells

After anesthetization with MS222, five fish from each medium were killed to excise the gills. The first gill arch from each side was fixed at 4°C in fixative consisting of 5% glutaraldehyde and 4% paraformaldehyde in 0.2 M phosphate buffer (PB; pH 7.2) for 12 h. After rinsing with 0.1 M PB, the specimens were postfixed with 1% osmium tetroxide in 0.2 M PB for another 1 h. After rinsing with PB and dehydration with ethanol and acetone, the specimens were infiltrated and embedded in LR-white resin. Ultrathin sections were cut with glass or diamond knives in an ultramicrotome (Ultracut E, Reichert-Jung, Vienna). Sections were mounted on formvar-coated copper grids, double stained in uranyl acetate and lead citrate, and examined with a transmission electron microscope (TEM; Hitachi, H-7000, Tokyo) at 75 kV. For scanning electron microscopy, dehydrated specimens were critical-point dried using liquid CO₂ in a critical-point drier (Hitachi HCP-2, Tokyo) and sputter coated for 3 min with a gold-palladium complex in a vacuum evaporator (Eiko 1B-2, Tokyo). The coated specimens were

examined in a scanning electron microscope (SEM; Hitachi S-2500, Tokyo) at an accelerating voltage of 15 kV.

It has been confirmed previously that in tilapia gill, MR cells were concentrated on the afferent and interlamellar regions of the filament, but no MR cell was found on the efferent filamental surface and the lamellae (Lee et al. 1996a, 1996b). Densities of different types of MR cells were measured according to Lee et al. (1996a, 1996b). The numbers of different types of MR cells among different acclimation media were analyzed by one-way ANOVA. Areas on the afferent side of the filament near the lamellae were chosen at random for counting at 1250 $\times$ magnification to measure the densities of different types of MR cells. Three areas ($48 \times 84 \mu\text{m}^2 = 4,032 \mu\text{m}^2$ each) were counted on each of six gill filaments from a fish. An average of these 18 areas was obtained. Five individuals per group were examined.

Ion Influxes

Whole-body Na^+ , Ca^{2+} , and Cl^- influxes were measured following Wood (1992) with some modifications. Tracer media were prepared by adding appropriate amounts of $^{24}\text{NaHCO}_3$ (prepared by 1-mW Tsing Hwa Open-Pool Reactor, Nuclear Science and Technology Development Center, National Tsing Hua University, Hsinchu, Taiwan), $^{45}\text{Ca}^{2+}$ (Amersham, Piscataway, N.J.), or $^{36}\text{Cl}^-$ (Amersham) to mid-Ca medium to give a final working specific activity: $^{24}\text{Na}^+$, 20,000–36,000 cpm μmol^{-1} ; $^{45}\text{Ca}^{2+}$, 510,000–540,000 cpm μmol^{-1} ; $^{36}\text{Cl}^-$, 210,000–260,000 cpm μmol^{-1} . After rinsing briefly with mid-Ca water, four (for $^{36}\text{Cl}^-$ or $^{24}\text{Na}^+$) or eight (for $^{45}\text{Ca}^{2+}$) fish from each acclimation media were transferred to plastic flux chambers with 20 mL tracer media for 2.5 h. The tracer media in flux chambers were gently aerated, and the water qualities were confirmed to show no significant change during the period of incubation. The plot of accumulated radioisotope against time was linear within the first 6 h ($R = 0.974$; $N = 6$; sampling time: 1, 2, 3, 4, 5, and 6 h), and the calculated influxes at each period of the first 6 h were constant. Moreover, preliminary experiments with fish overanaesthetized on ice indicated that radioisotope sticking to the surfaces of flux chamber and fish was <2% of the total radioisotope in the chamber and that the sticking saturated within the first 10 min of incubation ($N = 5$; sampling time: 0, 10, 20, 30, 60, and 150 min). Therefore, water samples (100 μL ; three samples for each determination) were collected 0.5 and 2.5 h after incubation to exclude the effects of sticking. Water samples from the $^{24}\text{Na}^+$ media were counted for radioactivities with an auto- γ counter (B5002, Packard, Meriden, Conn.). Counting solution (Fluoran-safe Scintran, BDH, Poole) was added to water samples from the $^{45}\text{Ca}^{2+}$ or $^{36}\text{Cl}^-$ media, and then radioactivities were counted with a β counter (LS6500, Beckman, Fullerton, Calif.). Volumes of the tracer media were measured at 0.5 and 2.5 h after incubation. The ion compositions of the tracer media followed

those of mid-Ca medium ($[\text{Ca}^{2+}] = 0.148$, $[\text{Na}^+] = 0.547$, and $[\text{Cl}^-] = 0.308$ mM; see above). The ion influxes in fish from different acclimation media were measured in the same levels of ions to compare the ion uptake capacities, although these data did not reflect the actual ion influxes that would occur in the acclimation media.

Ca^{2+} , Na^+ , or Cl^- influx was calculated by the following formula:

$$J_i = \frac{(Q_i V_i - Q_0 V_0)}{[1/2(SA_i + SA_0)tW]},$$

where Q_i and Q_0 (cpm mL^{-1}) refer to initial (0.5 h) and final (2.5 h) radioactivities in the tracer media, V_i and V_0 (mL) refer to initial and final volumes of the tracer media, SA_i and SA_0 are initial and final specific activities (cpm μmol^{-1}), t (2 h) is incubation time, and W (g) is body weight of fish. The decay of ^{24}Na during influx experiments was corrected. As all water samples were in the same condition, the effects of quenching on data were excluded through calculation.

Statistical Methods

All values are presented as means $\pm$ SE (N). The significance of difference between treatments was assessed by one-way ANOVA (Tukey's pairwise comparison).

Results

Gill MR cells

MR cells with different morphologies of apical surfaces—deep hole, shallow basin, and wavy convex—were observed on the afferent side of gill filaments (Fig. 1). As Lee et al. (1996a, 1996b) reported, the morphologies of apical membranes of different types of MR cells, which were examined with the TEM (Fig. 1), reflected what was observed under the SEM (Fig. 2). With larger size of dimension, the apical membranes of the wavy-convex MR cells gave a convex surface appearance. The ovoid apertures of shallow-basin MR cells with medium size in dimension were occasionally ornamented with short microvilli. The deep-holed MR cells with smaller size in apical dimension were narrow, deep, round-to-oval pores in which little or no internal structure was visible.

Low-NaCl groups developed more MR cells (total number = 5.62 ± 0.60 cells per unit area of filament) than other groups (low Ca, 3.34 ± 0.55 ; mid-Ca, 3.26 ± 0.40 ; high Ca, 2.96 ± 0.56). However, dramatic changes were found in the compositions of the three types of gill MR cells among the different groups of tilapia (Figs. 2, 3, 4). Both high-Ca and low-NaCl groups dominantly developed wavy-convex MR cells, while shallow-basin MR cells dominated in the mid-Ca and low-Ca groups. Among the four groups, low-NaCl tilapia was the highest in both cell density and relative density of wavy-

convex cells, while low-Ca fish showed the highest value in both parameters of shallow-basin cells. With regard to deep-hole type of MR cells, no significant differences were found in cell density among the four groups of tilapia. These results indicate that declining environmental Ca^{2+} concentrations stimulate the development of shallow-basin-type MR cells; in contrast, high environmental Ca^{2+} or low environmental NaCl levels enhance the development of wavy-convex-type MR cells in tilapia gills.

Ca^{2+} Influx

Ca^{2+} influxes in tilapia acclimated to different artificial media; low Ca, mid-Ca, high Ca, and low NaCl were compared, and the results indicate significant differences among the four groups (Fig. 5A; one-way ANOVA, Tukey's pairwise comparison). Low-Ca tilapia showed a significantly higher Ca^{2+} influx that was about 1.3–1.9-fold higher than that of other groups (Fig. 5A). Moreover, environmental Ca^{2+} levels induced a concentration-dependent effect on Ca^{2+} influx in tilapia based on the data of low-Ca, mid-Ca, and high-Ca groups (Fig. 5A). No significant difference was found between high-Ca and low-NaCl groups (Fig. 5A). These results indicate that declining environmental Ca^{2+} levels stimulate Ca^{2+} influx in tilapia.

Na^+ Influx

Significant differences in the Na^+ influxes were also found among the four groups (Fig. 5B; one-way ANOVA, Tukey's pairwise comparison). Na^+ influxes of low-NaCl groups were significantly higher than those of high-Ca, low-Ca, and mid-Ca groups (Fig. 5B). Low-NaCl tilapia revealed the highest Na^+ influx, about 3.4, 3.3, and 1.8 times those of low-Ca, mid-Ca, and high-Ca tilapia, respectively.

Cl^- Influx

Similar to the data of Ca^{2+} and Na^+ influxes as described above, significant differences in Cl^- influxes were found among the four groups (Fig. 5C; one-way ANOVA, Tukey's pairwise comparison). Mid-Ca tilapia showed a significantly lower level of Cl^- influx than those of the low-Ca, high-Ca, and low-NaCl groups. Low-NaCl tilapia revealed a Cl^- influx about 1.7 times higher than those of mid-Ca tilapia, indicating the effect of environmental Na^+/Cl^- levels on Cl^- influx in tilapia (Fig. 5C).

Figure 1. TEM images of branchial MR cells with different apical morphologies in freshwater tilapia. A, Wavy-convex MR cell; B, shallow-basin MR cell; C, deep-hole MR cell. CC = MR cell; PC = pavement cell. Magnification, $7,000\times$.

Discussion

According to cell shape, ultrastructure, and localization in gills under TEM, two distinguishable MR cell types, α and β , have been identified (Pisam et al. 1987). In this study, three MR cell types, shallow basin, deep hole, and wavy convex (Lee et al. 1996b), were used to describe modification in apical surface of MR cell under SEM. According to amounts of apical carbohydrates or cell density in low-Ca artificial freshwater, Tsai and Hwang (1998b) proposed that WGA-positive (WGA = wheat germ agglutinin, one kind of lectin that can bind with carbohydrates) MR cells (apical carbohydrates rich) are considered to be similar to β MR cells whose apical region is rich in carbohydrates (Pisam et al. 1995) or to shallow-basin-type MR cells that are the dominant MR cell type in low- Ca^{2+} artificial freshwater (Lee et al. 1996b). Thus, β MR cells in Pisam's papers look like shallow-basin MR cells. Moreover, in seawater-adapted tilapia, there is only one type of MR cells, which were named α MR cells (Pisam et al. 1987) or deep-hole MR cells (Lee et al. 1996b, 2000). Hence, Pisam's α MR cells should be deep-hole MR cells in our study. Meanwhile, there is no morphological description in Pisam's studies similar to the wavy-convex-type MR cells in our previous study (Lee et al. 1996b). However, no direct evidence is available to elucidate this possible linkage in these two MR cell classifications. Methods (SEM and TEM) or media for acclimation are perhaps the causes for the difference in these two MR cell classifications.

There is no direct evidence to confirm the different function of α and β MR cells. On the basis of extensive studies examining the effects of environmental salinities or ion levels and exogenous hormones on the appearance of α and β MR cells (Pisam et al. 1993), it was suggested that β MR cells may be associated with Ca^{2+} uptake, while α MR cells exert dual functions: Cl^- secretion in salt water and Na^+ uptake in freshwater (Pisam et al. 1993, 1995). However, no influx data are available to support their inference, and the functions of α and β MR cells in freshwater are still a matter of discussion (Perry 1997). Perry (1997) suggested that physiological significance of the different MR cell types needed to be proved. According to variability in the surface area of exposed MR cells on the gill epithelia in cortisol-treated fish, it was suggested that MR cells are involved in Ca^{2+} and Na^+ or Cl^- uptake (Perry et al. 1992a, 1992b). Conversely, Goss et al. (1992) suggested that Cl^- influx and Na^+ influx changed with exposed area of MR cells and pavement cells, respectively, during hypercapnic acidosis. These studies indicated that MR cells are the sites for Ca^{2+} and Cl^- uptake, while

Figure 2. SEM images of the apical morphologies of branchial MR cell in tilapia acclimated to different media. Shallow-basin MR cells (S) were predominant in low Ca (A), while wavy-convex MR cells (W) were predominant in high Ca (B) and low NaCl (C). D = deep-hole MR cells. Magnification, $2,200\times$.

Figure 3. Densities of different types of gill MR cells in tilapia acclimated to different media. Means $\pm$ SE ($N = 5$) is shown. One-way ANOVA was conducted among different media for each type of MR cell, and different letters indicate significant differences between different media (Tukey's pairwise comparison). Unit area of filament was described in "Material and Methods."

pavement cells are responsible for Na^+ uptake. However, these authors never distinguished gill MR cells with different morphologies, nor did they provide any correlated data between different MR cell types and the uptake of various ions. In this study, artificial freshwater media with various ionic compositions were used to stimulate the influxes of various ions and the development of different types of gill MR cells, and the correlated results suggested that the morphological alternation of gill MR cells reflect their ionic demand.

Previously, we identified wavy-convex, shallow-basin, and deep-hole MR cells based on the apical morphology under SEM observations and found a close association between the relative densities of these cells and environmental ion (Na^+ , Cl^- , and Ca^{2+}) compositions (Lee et al. 1996a, 1996b). Therefore, we proposed the functions of NaCl uptake and Ca^{2+} uptake for wavy-convex and shallow-basin MR cells, respectively. These results provide direct influx data to support our hypothesis. These artificial freshwater media (low Ca, mid-Ca, high Ca, and low NaCl) differ in concentrations of Na^+ , Cl^- , and Ca^{2+} , and thus, this experimental protocol enabled us to identify more clearly the relation between ion fluxes and the dominant types of MR cells. Decreasing environmental Ca^{2+} appears to stimulate the Ca^{2+} influx and the occurrence of shallow-basin type of MR cells, while decreasing environmental NaCl enhances Na^+ and Cl^- influxes as well as the occurrence of wavy-convex-type MR cells.

Previous studies have suggested the effects of environmental $[\text{Ca}^{2+}]$ on the permeability of other ions such as Na^+ and Cl^- (Avella et al. 1987). When we carefully compared the ion compositions of the artificial media, densities of MR cells, and

influxes of ions, complicated interactions between ions were also found. The low NaCl has the same environmental $[\text{Ca}^{2+}]$ as the mid-Ca group, yet there were significantly fewer shallow-basin cells and lower Ca^{2+} influx in the former groups (Table 1; Figs. 2–5), suggesting a possible effect of environmental $[\text{Na}^+/\text{Cl}^-]$ on Ca^{2+} balance. The high-Ca, mid-Ca, and low-Ca groups, which have similar environmental $[\text{Na}^+/\text{Cl}^-]$, were different in influxes of Na^+ and Cl^- and in densities of wavy-convex cells (Table 1; Figs. 2–5), implying that the effects of environmental $[\text{Ca}^{2+}]$ on Na^+ and on Cl^- fluxes were different. It is difficult, and not the major objective of this study, to distinguish the interactions among different ions. In this study, artificial freshwater media with various ionic compositions were used as a tool to stimulate the influxes of various ions (Ca^{2+} , Na^+ , and Cl^-) and the development of morphologically different MR cells to elucidate the correlation between functions and morphologies of MR cells. Despite the complicated interactions among different ions, these results evidently indicated that positive correlation occurred only between density of wavy-convex cells and influxes of $[\text{Na}^+]$ and $[\text{Cl}^-]$ (comparison between low-NaCl and mid-Ca groups) and between shallow-basin cells and Ca^{2+} influxes (comparison among high-Ca, mid-Ca, and low-Ca groups).

In tilapia (*Oreochromis mossambicus*) gill, Tsai and Hwang (1998b) identified WGA-positive MR cells based on amounts of apical carbohydrates and suggested that these cells are similar to shallow-basin-type MR cells. When acclimated to low-Ca freshwater ($[\text{Ca}^{2+}] = 0.015 \text{ mM}$) for 4 d, the number of WGA-positive MR cells increased in tilapia (Tsai and Hwang 1998b). In a subsequent study, Tsai and Hwang (1998a) demonstrated

Figure 4. Relative abundance of different types of gill MR cells in tilapia acclimated to different media. Means $\pm$ SE ($N = 5$) is shown. One-way ANOVA was conducted among different media for each type of MR cell, and different letters indicate significant differences between different media (Tukey's pairwise comparison).

Figure 5. Ca^{2+} (A), Na^{+} (B), and Cl^{-} (C) influxes in tilapia acclimated to different media. Means $\pm$ SE ($N = 8, 4$, and 4 for Ca^{2+} , Na^{+} , and Cl^{-} , respectively) are shown. One-way ANOVA was conducted among different media for each ion influx, and different letters indicate significant differences between different media (Tukey's pairwise comparison).

the association between WGA-binding sites in MR cells and Ca^{2+} uptake in gills of tilapia, which was suggested to be involved in adjusting the microtubule network. Treatment with WGA concurrently enhanced Ca^{2+} influx and fluorescence intensity of the microtubules in gill MR cells in tilapia kept in extremely low Ca freshwater ($[\text{Ca}^{2+}] = 0.002 \text{ mM}$; Tsai and Hwang 1998a). These findings, while similar to this study, also support the hypothesis about the major function of shallow-basin-type (or WGA-positive) MR cells: that they are associated with Ca^{2+} uptake in freshwater fish.

MR cells are believed to be the principle site for Cl^{-} uptake, while the role of MR cells in Na^{+} uptake is still being debated. MR cells are suggested to be the major site for Cl^{-} uptake via apical $\text{Cl}^{-}/\text{HCO}_3^{-}$ exchanger in the model of amphibian skin (Harvey 1992; Klein et al. 1997) and probably also in the model for freshwater-adapted fish gills (Perry 1997). In amphibian

skin, the H^{+} -ATPase in MR cells hyperpolarizes the apical membrane and, consequently, facilitates passive electrodiffusion of Na^{+} through Na^{+} channels present in both MR cells and principal cells (another type of epithelial cell in skin; Harvey 1992; Klein et al. 1997). The Na^{+} channels in MR cells provide the major route for Na^{+} absorption when an acid load closes the Na^{+} channels in principal cells (Harvey 1992; Klein et al. 1997). However, some studies have indicated that amiloride-inhibitable uptake of Rb^{+} was restricted to principal cells irrespective of the experimental conditions, and Na^{+} uptake across MR cells appears to be negligible (Nagel and Dorge 1996). In gills of freshwater fish, a similar Na^{+} absorption mechanism via H^{+} -ATPase and Na^{+} channels was suggested to be restricted mainly to gill pavement cells and not to MR cells (Perry 1997). No correlation was found between MR cell surface area and Na^{+} uptake/acid excretion on acid-base disturbance, and environmental Na^{+} concentrations affected the intracellular Na^{+} levels in pavement cells only (Morgan et al. 1995). However, the data about the localization of H^{+} -ATPase in MR cells or pavement cells are still controversial (Perry 1997). In this study, the correlated data between wavy-convex MR cell density and influxes of Na^{+} and Cl^{-} could not answer whether the key factor stimulating the occurrence of wavy-convex MR cells is Na^{+} , Cl^{-} , or both. This is because the environmental Na^{+} and Cl^{-} levels were designed to change in parallel following our previous study (Lee et al. 1996a, 1996b). In a further study, experiments dealing respectively with Na^{+} or Cl^{-} should be designed to certify whether wavy-convex MR cells are mainly responsible for the uptake of Na^{+} , Cl^{-} , or both in freshwater-adapted fish.

The function of deep-hole MR cells is not yet clear from this study, as the changes in the densities of deep-hole cells did not show evident positive relation either with the stimulation of Ca^{2+} uptake or with those of Na^{+} and Cl^{-} . It was suggested that the function of deep-hole MR cells may be associated with the secretion of Na^{+} and/or Cl^{-} because only deep-hole MR cells were observed in $>5\%$ seawater treatment (Lee et al. 1996b, 2000). Recently, we found that deep-hole MR cells increased in cell number and the size of apical opening following the increase of salinity to which fish were acclimated (Lee et al. 2000). This implies that deep-hole MR cells appearing in hypotonic freshwater might be in a resting state and preparing to conduct the excretion of Na^{+} and/or Cl^{-} on higher salinity challenges. This needs further study to confirm.

In conclusion, this study provides, for the first time, direct linkage between ion influxes and relative densities of morphologically different MR cells. These data demonstrate multiple functions of morphologically different MR cells; that is, the wavy-convex and shallow-basin types (or subtypes) are responsible mainly for the uptake of $\text{Na}^{+}/\text{Cl}^{-}$ and Ca^{2+} , respectively, in freshwater-adapted fish. Much remains to be done in the future, such as the quantification and the distribution of the ion transporters.

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