Stimulation of Cl^- Uptake and Morphological Changes in Gill Mitochondria-Rich Cells in Freshwater Tilapia (*Oreochromis mossambicus*)

Il-Chi Chang[1]
Yuan-Yaw Wei[2]
Fong-In Chou[2]
Pung-Pung Hwang[3,*]
[1]Institute of Fisheries Science, National Taiwan University, Taipei, Taiwan, Republic of China; [2]Nuclear Science and Technology Development Center, National Tsing Hua University, Hsinchu, Taiwan, Republic of China; [3]Institute of Zoology, Academia Sinica, Taipei, Taiwan 11529, Republic of China

Accepted 1/24/03

ABSTRACT

The purpose of the present article is to examine the relationships between ion uptakes and morphologies of gill mitochondria-rich (MR) cells in freshwater tilapia. Tilapia were acclimated to three different artificial freshwaters (high Na [10 mM], high Cl [7.5 mM]; high Na, low Cl [0.02–0.07 mM], and low Na [0.5 mM], low Cl) for 1 wk, and then morphological measurements of gill MR cells were made and ion influxes were determined. The number and the apical size of wavy-convex MR cells positively associated with the level of Cl^- influx. Conversely, Na^+ influx showed no positive correlation with the morphologies of MR cells. The dominant MR cell type in tilapia gills changed from deep-hole to wavy-convex within 6 h after acute transfer from a high-Cl^- to a low-Cl^- environment. Deep-hole MR cells became dominant 24–96 h after acute transfer from a low-Cl^- to a high-Cl^- environment. We conclude that wavy-convex MR cells associate with Cl^- uptake but not Na^+ uptake, and the rapid formation of wavy-convex MR cells reflects the timely stimulation of Cl^- uptake to recover the homeostasis of internal Cl^- levels on acute challenge with low environmental Cl^-.

* Corresponding author; e-mail zophwang@ccvax.sinica.edu.tw.

Physiological and Biochemical Zoology 76(4):544–552. 2003. 1522-2152/2003/7604-2105$15.00

Introduction

The gill is an important extrarenal organ responsible for ion regulation in teleosts. In seawater, gill mitochondria-rich (MR) cells were demonstrated to actively secrete Cl^- (Foskett and Scheffey 1982). Conversely, freshwater teleosts' gill MR cells were thought to play a crucial role in the uptake of diverse ions, Na^+, Cl^-, and Ca^{2+}, and in acid-base regulation (Avella et al. 1987; Laurent and Perry 1990; Perry et al. 1992; Bindon et al. 1994). More than one type of MR cell has been observed in the gill epithelia of freshwater teleosts (Pisam et al. 1987, 1993; Hwang 1988; Lee et al. 1996*a*, 1996*b*; Chang et al. 2002). However, the relationships between ion uptake and different MR cell types are still unclear, and further studies are required to address them (Perry 1997).

By manipulation in pH of the acclimation media (Goss et al. 1992*a*), gill MR cells with an expanded apical surface were associated with Cl^- uptake in brown bullhead (*Ictalurus nebulosus*). Using an X-ray microanalysis technique, it was demonstrated that the intracellular Cl^- concentration of MR cells in brown trout (*Salmo trutta*) changed with external Cl^- levels (Morgan et al. 1994; Morgan and Potts 1995). Although the cell type associated with the function of Cl^- uptake was not discussed in the above studies, relationships between Cl^- uptake and MR cells were established in salmonids. However, the model for salmonids may not be completely applicable to other species, for example, freshwater mummichog (*Fundulus heteroclitus*), in which Cl^-/base exchange was unimportant or absent, and Na^+ uptake was mainly via Na^+/H^+ exchanger (Patrick and Wood 1999). Moreover, remarkable interspecies morphological differences in MR cells were documented among four freshwater teleosts: brown bullhead, American eel, rainbow trout, and tilapia (Perry 1997). Therefore, it is necessary and important to examine the relationships between Cl^- uptake and MR cells in species other than salmonids.

In our previous study (Lee et al. 1996*b*), three MR cell types (deep hole, wavy convex, and shallow basin) were identified in gills of freshwater tilapia (*Oreochromis mossambicus*). Moreover, it was implied that they were associated with uptake of different ions (Lee et al. 1996*b*). Recently, it was shown that densities of wavy-convex MR cells and shallow-basin MR cells increased with enhanced NaCl and Ca^{2+} uptake, respectively (Chang et al. 2001). In tilapia larvae, skin wavy-convex MR cells were also demonstrated to be associated with Cl^- uptake (Lin and

Hwang 2001). However, no direct data were provided to exclude the relationships between Na^+ influx and wavy-convex MR cells.

The object of this study was to examine whether wavy-convex MR cells are responsible for Cl^- uptake but not for Na^+ uptake in freshwater tilapia. Artificial media with different Na^+ and Cl^- compositions were used to stimulate the influxes of Na^+ and Cl^- as well as the development of different types of gill MR cells in tilapia. The results indicate that the increased density and apical size of wavy-convex MR cells coincided with the stimulation in Cl^- influx, suggesting the involvement of these cells in Cl^- uptake in tilapia gills.

Material and Methods

Animals

Tilapia (*Oreochromis mossambicus*) at 0.3–0.7 g were obtained from laboratory stocks. All individuals were reared in 26°–28°C aerated local tap water with a photoperiod of 12L : 12D before the acclimation experiments.

Preparation of Artificial Freshwater

Three kinds of artificial freshwater (low Na, low Cl [L-Na-L-Cl]; high Na, low Cl [H-Na-L-Cl]; high Na, high Cl [H-Na-H-Cl]) were prepared by adding appropriate amounts of NaCl, Na_2SO_4, $MgSO_4$, K_2HPO_4, KH_2PO_4, and $CaSO_4$ to double-deionized water (Milli-RO60, Millipore, Billerica, Mass.). The ion concentrations of K^+, Ca^{2+}, and Mg^{2+} in the artificial freshwater were near the ranges of local freshwater. The ionic compositions (Table 1) of the three media were confirmed by measuring the Na^+, K^+, Ca^{2+}, and Mg^{2+} concentrations with an atomic absorption spectrophotometer (Hitachi Z-8000, Tokyo), and the Cl^- concentration was determined by the ferricyanide method (Franson 1985) with a spectrophotometer (Hitachi U-2000, Tokyo). The pH of the media was adjusted with K_2HPO_4 and KH_2PO_4 and kept between 6.2 and 6.7, and the water temperature was 26°–28°C.

Morphology of Gill MR Cells

After anesthetization with MS222 (100–200 mg L^{-1}), fish were killed and their gills excised. 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, 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, specimens were critical-point dried using liquid CO_2 in a critical-point dryer (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 (Hitachi S-2500, Tokyo) at an accelerating voltage of 15 kV.

It was previously confirmed that in tilapia gills, MR cells are 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. 1996*a*, 1996*b*). Densities of different types of MR cells were measured according to Lee et al. (1996*a*, 1996*b*). Briefly, areas on the afferent side of the filament near the lamellae were chosen at random for measuring at × 1,200 magnification the densities of different types of MR cells. One area (4,250 μm^2 each) was counted on each of two branchial filaments from a fish. The greatest linear diameters of MR cells were measured as the indicator of the apical size according to Franklin (1990). Filamental surfaces associated with the afferent artery were considerably broad compared with those with the efferent artery (Lee et al. 1996*a*, 1996*b*) so that it was quite easy to find surfaces with minimum curves for morphological measurements in order to diminish errors from a curved surface. An average of each morphological parameter of the MR cell in these two areas was obtained.

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 $^{24}NaHCO_3$ (prepared with a 1-mW Tsing Hwa Open-Pool Reactor, Nuclear Science and Technology Development Center, National Tsing Hua University, Hsinchu, Taiwan) and $^{45}Ca^{2+}$ or $^{36}Cl^-$ (Amersham, Piscataway, N.J.) to an artificial freshwater ($[Ca^{2+}]$ = 0.148 mM, $[Na^+]$ = 0.547 mM, and $[Cl^-]$ = 0.308 mM) to give the final working specific activity: $^{24}Na^+$, 20,000–36,000 cpm × μmol^{-1}; $^{45}Ca^{2+}$, 510,000–540,000 cpm × μmol^{-1}; $^{36}Cl^-$, 210,000–260,000 cpm × μmol^{-1}. After rinsing briefly with the same artifical freshwater, fish from each acclimation medium were transferred to plastic flux chambers with 20 mL of tracer media for 2.5 h. The tracer medium in the flux chambers was gently aerated, and the water quality was

Table 1: Ionic compositions (mM) in artificial freshwater

Medium	$[Na^+]$	$[Cl^-]$	$[Ca^{+2}]$	$[K^+]$	$[Mg^{2+}]$	pH
L-Na-L-Cl	.55 ± .01	.002 ± .001	.182 ± .006	.16 ± .01	.18 ± .01	6.79 ± .09
H-Na-L-Cl	10.47 ± .31	.007 ± .005	.171 ± .004	.16 ± .01	.20 ± .01	6.81 ± .06
H-Na-H-Cl	9.65 ± .38	7.597 ± .283	.188 ± .002	.16 ± .01	.18 ± .01	6.74 ± .04

Note. Means ± SE (N = 7) is indicated.

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 times: 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 over anesthetized on ice indicated that radioisotope sticking to the surfaces of flux chamber and fish accounted for less than 2% of the total radioisotope in the chamber, and the amount sticking reached saturation within the first 10 min of incubation ($N = 5$; sampling times: 0, 10, 20, 30, 60, and 150 min). Therefore, water samples (100 μL; three samples for each determination) were collected at 0.5 and 2.5 h after incubation in order to exclude the effects of sticking. Radioactivities of water samples from the $^{24}Na^+$ media were counted with an auto-gamma counter (B5002, Packard, Meriden, Conn.). Counting solution (Fluoran-Safe Scintran, BDH, Poole) was added to water samples from the $^{45}Ca^{2+}$ or $^{36}Cl^-$ media, and then radioactivities were counted with a beta counter (LS6500, Beckman, Fullerton, Calif.). Volumes of the tracer media were measured at 0.5 and 2.5 h after incubation. The ion influxes in fish from different acclimation media were measured with the same levels of ions in order to compare the ion uptake capacities.

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

$$J_i = \frac{(Q_f \times V_f) - (Q_i \times V_i)}{1/2 \times (SA_i + SA_f) \times t \times W},$$

where Q_i and Q_f (cpm $\times$ mL^{-1}) refer to the initial (0.5 h) and final (2.5 h) radioactivities in the tracer media; V_i and V_f (mL) refer to the initial and final volumes of the tracer media; SA_i and SA_f are the initial and final specific activities (cpm $\times$ μmol^{-1}); t (2 h) is incubation time; and W (g) is the body weight of the fish. The decay of radioisotopes during the influx experiments was corrected for. Because 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 the mean $\pm$ SE (N). The significance of the difference between treatments was assessed by one-way ANOVA (Tukey's pairwise comparison).

Experimental Design

Acclimation Experiment 1. Effects of low environmental Na^+ or Cl^- on MR cell morphology and capacity of ionic regulation were examined in this experiment. Tilapia (5 to 6 individuals for each test) were acclimated to the H-Na-H-Cl, H-Na-L-Cl, and L-Na-L-Cl media for 1 wk and then were sampled for morphological observations and ion influx measurements. In our preliminary experiments, tilapia acclimated to local freshwater with presence or absence of additional 20 mM mannitol and indicated that osmolality difference, which resulted from mannitol, did not result in morphological alteration in apical surface of gill MR cells. Moreover, effects of SO_4^{2-} were shown previously that did not correlate with the appearance of MR cells (Lee 1996). Thus, morphological modification in gill MR cells of tilapia that acclimated to different artificial freshwater may result from different Na^+ and Cl^- composition in artificial freshwater. The acclimation experiment was repeated three times. In order to maintain the water quality, the acclimation media were aerated with a filtered air pump and changed every 2 d, and the water samples for ion measurements were collected at the beginning of acclimation and before and after the water renewal. Fish were fed commercial pellets 1 h immediately prior to the water change every 2 d, but feeding was stopped 3 d before the sampling.

Acclimation Experiment 2. In order to examine the time-course changes of the apical modifications in branchial MR cells during Cl^- acclimation, tilapia acclimated to H-Na-H-Cl and H-Na-L-Cl were directly transferred to H-Na-L-Cl and H-Na-H-Cl media, respectively. After the transfer, gills of six individuals for each sampling time were sampled for morphological observations at 0, 1.5, 3, 6, 12, 24, 96, and 168 h.

Results

Gill MR Cells in Tilapia Acclimated to Different Artificial Freshwaters

Low-Cl groups developed more MR cells than did H-Cl groups (Table 2). Wavy-convex MR cells predominantly developed in gills of both H-Na-L-Cl and L-Na-L-Cl groups while deep-hole MR cells dominated over the other two types of MR cells in the H-Na-H-Cl group (Fig. 1). Data of relative percentages of different MR cell types indicate that declining environmental Cl^- but not Na^+ concentration stimulated the development of wavy-convex MR cells (Table 2). Frequency distributions of the apical diameters of each type of MR cell varied among the three groups (Fig. 2), and the average apical diameter of wavy-convex MR cells in H-Na-L-Cl tilapia was significantly larger than that in the L-Na-L-Cl and H-Na-H-Cl groups (Table 2). It was also noted that decreased environmental Na^+ resulted in a reduction in the apical diameter of wavy-convex MR cells in the low-Cl groups, while decreased environmental Cl^- caused a dramatic increase in the apical diameter of the same type of MR cells in high-Na tilapia gills (Table 2). With regard to shallow-basin MR cells, no significant differences were found in the cell density among the three groups of tilapia (Fig. 2).

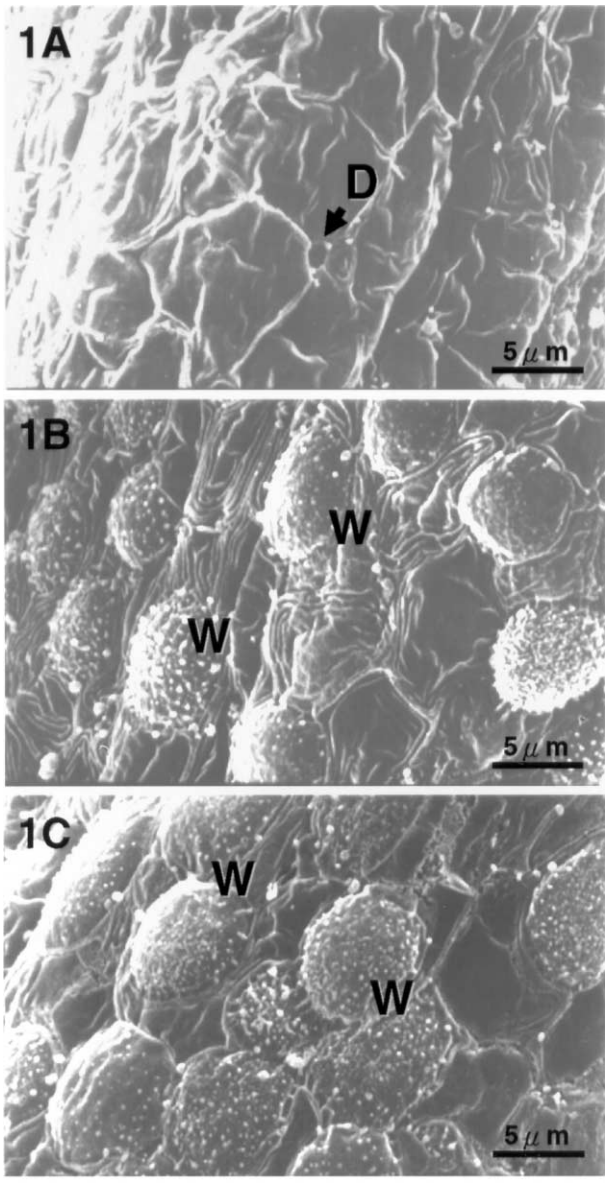

Figure 1. Scanning electron microscopic images of branchial mitochondria-rich (MR) cells in tilapia acclimated to different artificial freshwater mediums. *A*, high Na, high Cl; *B*, high Na, low Cl; *C*, low Na, low Cl. *D*, deep-hole MR cell; *W*, wavy-convex MR cell. Magnification × 2,400.

Modification of Gill MR Cells in Tilapia on Acute Acclimation to High Environmental Cl^-

During acclimation to high Cl^-, the total number of MR cells was maintained within 24 h (34.1 ± 1.3 cells/unit area of filament) and then dramatically decreased to 33%–40% (13.0 ± 1.6 to 15.5 ± 0.5 cells/unit area of filament) at 96–168 h compared with that at 0 h (38.7 ± 6.8 cells/unit area of filament; Fig. 3*A*). On high-Cl acclimation, predominant MR cells in gills changed gradually from the wavy-convex to the deep-hole type. Initially, the relative percentage of wavy-convex MR cells gradually decreased from 86% (33.2 ± 5.5 cells/unit area of filament) to 53%–52% (20.2 ± 3.9 to 17.5 ± 4.0 cells/unit area of filament) between 12 h and 24 h and rapidly down to 0% at 96 h. On the contrary, the relative percentage of deep-hole MR cells increased rapidly from 10% (4.0 ± 1.6 cells/unit area of filament) to 40% (12.5 ± 1.0 cells/unit area of filament) by 24 h and subsequently up to 94%–98% (12.3 ± 1.6 to 15.3 ± 0.6 cells/unit area of filament) by the end of acclimation at 168 h. Shallow-basin MR cells showed no significant change in relative percentage during high-Cl acclimation.

Modification of Gill MR Cells in Tilapia on Acute Acclimation to Low Environmental Cl^-

The total number of MR cells obviously increased within 3 h from 15.7 ± 0.6 to 25.5 ± 3.6 cells/unit area of filament and then remained constant (36.2 ± 2.6 cells/unit area of filament). At the end of low-Cl acclimation (168 h), MR cells had increased threefold in number (45.1 ± 5.9 cells/unit area of filament), and the dominant type had rapidly changed from deep-hole to wavy-convex (Fig. 3*B*), in contrast to what was observed during acclimation to high Cl. The relative percentage of deep-hole MR cells decreased rapidly from 100% (15.7 ± 0.6 cells/unit area of filament) to 30% (25.8 ± 3.6 cells/unit area of filament) within the first 6 h of acclimation and finally down to 15% (6.8 ± 1.5 cells/unit area of filament) between 96 and 186 h. On the other hand, wavy-convex MR cells increased from 0% to 67% (25.8 ± 3.6 cells/unit area of filament) during the first 6 h of acclimation and remained between 60% and 80% (22.0 ± 5.5 to 37.0 ± 5.7 cells/unit area of filament). Shallow-basin MR cells increased to 28%–32% at 1–3 h but returned to a constant level, about 2%–8%, thereafter.

Ion Influxes in Tilapia Acclimated to Different Artificial Freshwaters

Na^+ influx in the L-Na-L-Cl group was significantly higher than those in the H-Na-L-Cl and H-Na-H-Cl groups at about 2.6 and 3.0 times, respectively (Fig. 4*A*). There was no significant difference in the Na^+ influx between the H-Na-L-Cl and H-Na-H-Cl groups. These data suggest that a low environmental Na^+ level resulted in enhancement of Na^+ uptake in tilapia.

Cl^- influx in H-Na-L-Cl and H-Na-H-Cl tilapia was significantly higher and lower than that in L-Na-L-Cl tilapia at about 1.1 and 0.2 times, respectively (Fig. 4*B*). These results indicate that declining environmental Cl^- levels stimulated Cl^- influx in tilapia, while low environmental Na^+ levels showed inhibitory effects on Cl^- uptake. On the other hand, no significant differences were found in the Ca^{2+} influxes among the three groups of tilapia (Fig. 4*C*).

Table 2: Comparisons of morphological parameters of mitochondria-rich (MR) cells in tilapia acclimated to different artificial freshwater mediums

Artificial Freshwater Medium	Relative Percentage (%) of MR Cell Types			Total Number of MR Cells (Cells/Unit Area)	Apical Diameter of Wavy Convex (μm)
	Deep Hole	Shallow Basin	Wavy Convex		
L-Na-L-Cl	3.5 ± .8^{A}	1.1 ± .9^{A}	95.2 ± .8^{A}	34.8 ± 4.9^{A}	7.78 ± .11^{A}
H-Na-L-Cl	2.3 ± 1.4^{A}	.7 ± .5^{A}	96.8 ± 1.6^{A}	34.6 ± 6.4^{A}	8.55 ± .10^{B}
H-Na-H-Cl	83.8 ± 3.5^{B}	7.2 ± 2.3^{B}	8.8 ± 1.8^{B}	14.8 ± 2.6^{B}	3.87 ± .31^{C}

Note. Means ± SE (N = 5 to 6) is indicated. One-way ANOVA was conducted among different mediums for each type of MR cell, the total number of MR cells, and the apical diameter of wavy convex cells, respectively; different letters indicate significant differences between different media (Tukey's pairwise comparison).

Discussion

The major findings of the present study are that (1) relationships between Cl^- uptake and gill MR cells are established in tilapia (*Oreochromis mossambicus*), a species other than salmonids; (2) the appearance of wavy-convex MR cells characterized by expanded apical surfaces is associated with the stimulated capacity for Cl^- uptake but not with those for Na^+ uptake or for Ca^{2+} uptake; and (3) the dominant MR cell type in tilapia gills changes from deep-hole to wavy-convex within 6 h of acclimation to a low-Cl^- environment, while the deep-hole type becomes dominant much more slowly, at about 24–96 h, on acclimation to high Cl^- medium.

Polymorphism of MR cells in fish gills is well documented and is believed to play crucial roles in the uptake of diverse ions as well as in acid-base regulation (Perry 1997). However, until recently there were no comprehensive data to illustrate the relationships between morphological differences of gill MR cells and the uptake of diverse ions in freshwater fish. Based on the ultrastructure and location of the cells, Pisam et al. (1987) identified α and β MR cells in gills of several teleosts. In subsequent studies using salinity or exogenous hormone treatments (Pisam et al. 1993, 1995), it was suggested that α MR cells were associated with Ca^{2+} uptake while α MR cells displayed dual functions: Cl^- secretion in seawater and Na^+ uptake in freshwater. However, there were no ion flux data or other substantial data to support their inferences. Previously, we identified wavy-convex, shallow-basin, and deep-hole MR cells, based on the apical morphology using scanning electron microscope observations and found a close association between the relative densities of these cells and environmental Na^+, Cl^-, and Ca^{2+} compositions (Lee et al. 1996*b*). In our subsequent studies, manipulation of the ion compositions of the acclimation media, an approach that is simple, repeatable, and lacking pH disturbances that were reported to affect contributions from principal cells and MR cells to Na^+ uptake in amphibian skin (Harvey 1992), was used to examine the morphology-function relationships of gill MR cells (Chang et al. 2001; Lin and Hwang 2001; Chang et al. 2002; this article). Based on data of ionic influx and relative densities of the above three different MR cell types (Chang et al. 2001), it was found that MR cells with different apical surfaces display different capacities for differential ion uptake; for example, wavy-convex MR cells are associated with NaCl uptake. However, the particular relations between MR cells and Na^+ or Cl^- could not be distinguished in Chang et al. (2001) because the concentrations of Na^+ and Cl^- in artificial diluted media were changed in parallel in that experimental design. In the present study, various combinations of Na^+ and Cl^-, which could induce differential changes in the morphologies of gill MR cells and ion influxes, were designed to examine further the correlations among these parameters. Total number of MR cells and apical size and relative density of wavy-convex cells were highly positively correlated with Cl^- influx, while relative density of deep-hole cells was negatively correlated (Table 3), indicating that appearance of wavy-convex MR cells with larger apical surfaces was associated with a stimulation of Cl^- uptake. Similar results were reported in our previous article on tilapia larvae (Lin and Hwang 2001) and stenohaline goldfish (Chang et al. 2002). Moreover, the present study also provides data for examination of the relations between MR cells and Na^+ uptake. All the morphological parameters of MR cells did not show statistically significant correlations with the changes in Na^+ influx (Table 3), suggesting that MR cells may not be the sites for Na^+uptake, as proposed previously (Goss et al. 1995; Perry 1997). On the other hand, it is worthwhile to note that in the low-Na^+ environment, Na^+uptake caused a significant decrease of wavy-convex MR cells on the apical surface and consequently an inhibition of Cl^- uptake. This indicates that wavy-convex MR cells and pavement cells compete with each other on the gill surface for Cl^- and Na^+uptake, respectively. As proposed by Goss et al. (1992*a*), manipulation of the alkalinity and acidity resulted in competition between MR cells and pavement cells on the gill epithelial surface and hence led to changes in Cl^- and Na^+ influxes.

MR cells have been suggested to be the site for Ca^{2+} uptake (Perry and Wood 1985; Ishihara and Muyiya 1987). Our previous study demonstrated that Ca^{2+} influx was positively correlated with the number of shallow-basin MR cells only (Chang et al. 2001). In the present study there was no significant difference in Ca^{2+} uptake among the three groups of tilapia, which

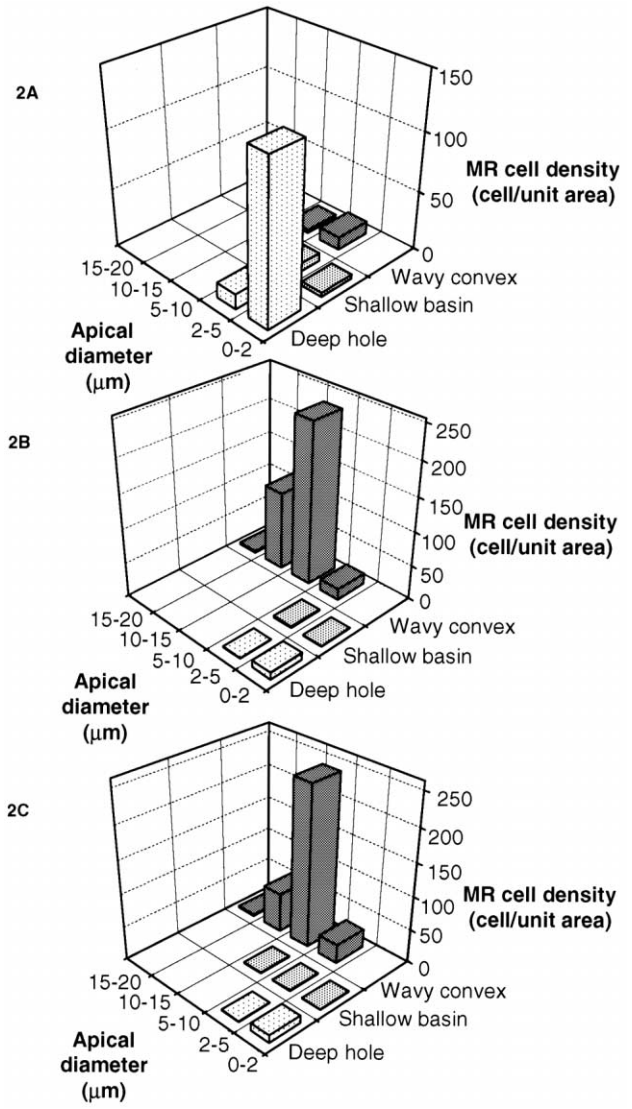

Figure 2. Longest diameter and number of the apical opening of different types of branchial mitochondria-rich cells in tilapia acclimated to different media. *A*, High Na, high Cl; *B*, high Na, low Cl; *C*, low Na, low Cl.

developed different numbers of wavy-convex and deep-hole MR cells but which had the similar numbers of shallow-basin MR cells. The appearance of wavy-convex or deep-hole MR cells caused no change in Ca^{2+} influx. The roles of wavy-convex and deep-hole MR cells in the mechanism of Ca^{2+} uptake remain to be studied. In the current Ca^{2+} uptake model (Flik et al. 1993), Ca^{2+}enters an MR cell via apical Ca^{2+} channels down an electrochemical gradient, and then Ca^{2+} exits the cell through basolateral Ca^{2+}-ATPase. Modification of the apical membrane of MR cells may not be necessary to reflect the activity of apical Ca^{2+} channels, while the activity of the basolateral Ca^{2+}-ATPase may play a more crucial role in the regulation of Ca^{2+} uptake.

In the present study, the dynamic cellular profile of the three types of MR cells was also examined during acute acclimation to different levels of environmental Cl^-. The relative density of the three types of MR cells began to change 3 to 12 h after acclimation to low or high Cl^-. In previous studies (Mackinnon and Enesco 1980; Tsai and Hwang 1998*b*), it was demonstrated that the time needed for the renewal of gill epithelial cells is about 4 d. This implies that direct morphological conversion on the apical surface of MR cells occurs prior to renewal of the cells. Several studies (Shikano and Fujio 1998; Tsai and Hwang 1998*b*; Hiroi et al. 1999) have also indicated that direct morphological conversion between different MR cell types occurs to deal with environmental challenges such as Ca^{2+} level or salinity. Recently, Lin and Hwang (2001) pointed out that remodeling of MR cells (conversion between wavy-convex and deep-hole types) in tilapia larvae skin without a change in the total number of MR cells occurred in response to low ambient Cl^- medium. In mammals, cytoskeleton reorganization has been demonstrated to be associated with osmoregulatory properties such as regulatory volume decreases or increases (Al-Habori 1994). Accumulative evidence also indicates that the

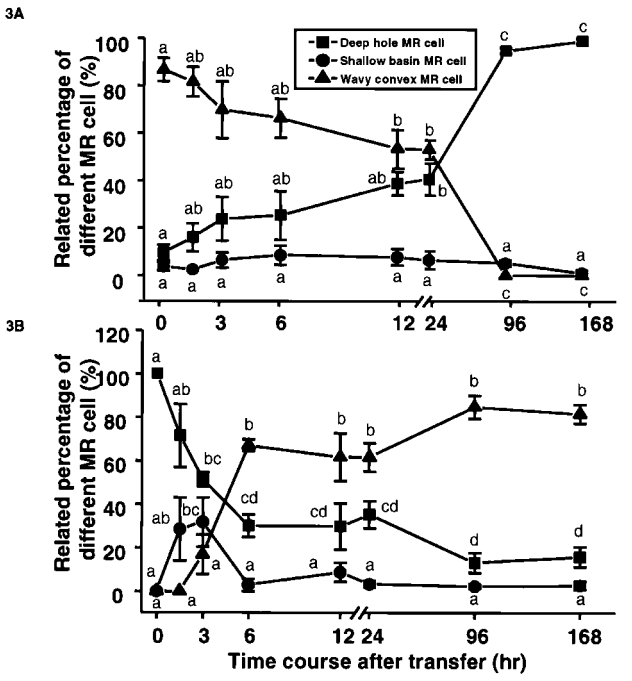

Figure 3. Relative cellular profile of branchial mitochondria-rich cells in tilapia acclimated from low to high environmental Cl^-media (*A*) and from high to low environmental Cl^-media (*B*). Means ± SE ($N = 6$) is shown. A one-way ANOVA was conducted among different sampling time points, and different letters indicate significant differences among different sampling times (Tukey's pairwise comparison).

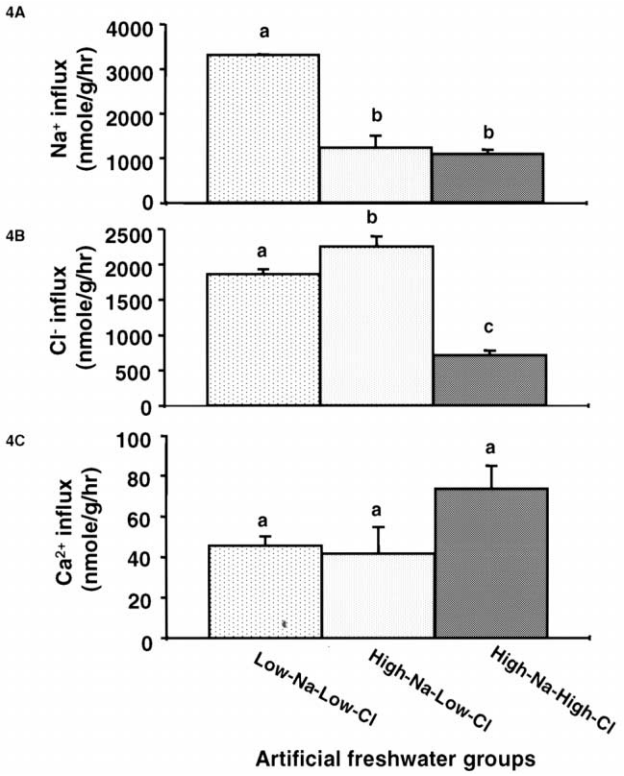

Figure 4. Na^+ (*A*), Cl^- (*B*), and Ca^{2+} (*C*) influxes in tilapia acclimated to different media. Means ± SE ($N = 5$ to 6) is shown. A one-way ANOVA was conducted among different media for each ion influx, and different letters indicate significant differences between different media (Tukey's pairwise comparison).

cytoskeleton system is involved in the stabilization, delivery, and activity of membrane proteins that mediate transport events (Mills and Mandel 1994). Several studies have also proposed that the cytoskeleton may participate in the modification of the apical surface of MR cells to deal with the transport of various ions in fish. Modification of the microtubule network in MR cells was found to play a critical role in Ca^{2+} uptake and NaCl secretion in tilapia (*O. mossambicus*) and mullet (*Mugil capito*; Maetz and Pic 1977; Tsai and Hwang 1998*a*). Waterborne Cd^{2+} was also reported to cause morphological alternation in MR cells via actin reorganization in salmon (*Salmo salar*; Devos et al. 1998), and more recently a strong actin ring at the apex of MR cells was found to be involved in the active adjustment of the opening/closing of the apical crypt of cells in killifish (*Fundulus heteroclitus*; Daborn et al. 2001). It will be important and interesting to see whether remodeling of MR cells, which results in the changes in Cl^- uptake in tilapia gills, is also achieved by reorganization of such components of the cytoskeleton as actin.

On acute acclimation, morphological transformations of MR cells began more rapidly in the case of low-Cl^- acclimation (3–6 h) than in that of high-Cl^- acclimation (12–24 h). This implies that tilapia are more sensitive and thus respond more rapidly to low ambient Cl^- than to high ambient Cl^-. Goss et al. (1992*a*, 1992*b*) suggested that the number and exchange rate of teleost branchial Cl^-/HCO_3^- exchangers can be modulated and result in adjustment of Cl^- uptake and modification of the MR cell apical surface. Wilson et al. (2000) demonstrated that the Cl^-/HCO_3^- exchanger was localized in tilapia MR cell apical membranes. Wavy-convex MR cells with an expanded apical surface may contain a much higher number of Cl^-/HCO_3^- exchangers for Cl^- uptake than do the other two types of cells. Taken together, prompt transformation to wavy-convex from the other two types may reflect the timely and efficient stimulation of Cl^- uptake to recover the homeostasis of internal Cl^- levels when challenged with low environmental Cl^-.

Table 3: Correlation between ion influx and morphology of gill mitochondria-rich (MR) cells

Morphological Parameter	Na^+ Influx		Cl^- Influx	
	r	*P*	*r*	*P*
Total number of MR cells	.419	.262	.923	<.001
Apical size of wavy convex	.437	.239	.943	<.001
Relative percentage of wavy convex	.536	.137	.962	<.001
Relative percentage of shallow basin	.159	.680	−.624	.013
Relative percentage of deep hole	.544	.130	−.953	<.001

Note. Data from the three groups, H-Na-H-Cl, H-Na-L-Cl, and L-Na-L-Cl, were combined for correlation analysis (Pearson correlation, Minitab, 2000).

Acknowledgments

This study was supported by grants to P.-P.H. from the National Science Council (NSC 91-2313-B001-032) and Academia Sinica (Major Group-Research Project), Taiwan, Republic of China. Appreciation is expressed to Y. E. Shieh and M. Y. Chou for their assistance in micrograph preparation and statistical analysis.

Literature Cited

Al-Habori M. 1994. Cell volume and ion transport regulation. Int J Biochem 26:319–334.

Avella M., A. Masoni, M. Bornancin, and N. Mayer-Gostan. 1987. Gill morphology and sodium influx in the rainbow trout (*Salmo gairdneri*) acclimated to artificial freshwater environments. J Exp Zool 241:159–169.

Bindon S.D., K.M. Gilmour, J.C. Fenwick, and S.F. Perry. 1994. The effects of branchial chloride cell proliferation on respiratory function in the rainbow trout, *Oncorhynchus mykiss*. J Exp Biol 197:47–63.

Chang I.C., T.H. Lee, H.C. Wu, and P.P. Hwang. 2002. Effects of environmental Cl^- levels on Cl^- uptake and mitochondria-rich cell morphology in gills of the stenohaline goldfish, *Carassius auratus*. Zool Stud 41:236–243.

Chang I.C., T.H. Lee, C.H. Yang, Y.Y. Wei, F.I. Chou, and P.P. Hwang. 2001. Morphology and function of gill mitochondria-rich cells in fish acclimated to different environments. Physiol Biochem Zool 74:111–119.

Daborn K., R.R.F. Cozzi, and W.S. Marshall. 2001. Dynamics of pavement cell–chloride cell interactions during abrupt salinity change in *Fundulus heteroclitus*. J Exp Biol 204:1889–1899.

Devos E., P. Devos, and M. Cornet. 1998. Effects of cadmium on the cytoskeleton and morphology of gill chloride cells in parr and smolt Atlantic salmon(*Salmo salar*). Fish Physiol Biochem 18:15–27.

Flik G., J.A. Van Der Velden, K.J. Dechering, P.M. Verbost, T.J.M. Schoenmakers, Z.I. Kolar, and S.E. Wendelaar Bonga. 1993. Ca^{2+} and Mg^{2+} transport in gills and gut of tilapia, *Oreochromis mossambicus*: a review. J Exp Zool 265:356–365.

Foskett J.K. and C. Scheffey. 1982. The chloride cell: definitive identification as the salt-secretory cell in teleosts. Science 215: 164–166.

Franklin C.E. 1990. Surface ultrastructural changes in the gills of sockeye salmon (Teleostei: *Oncorhynchus nerka*) during seawater transfer: comparison of successful and unsuccessful seawater adaptation. J Morphol 206:13–23.

Franson M.A.H. 1985. Standard Methods for the Examination of Water and Waste Water. 16th ed. American Public Health Association, Washington D.C.

Goss G.G., P. Laurent, and S. F. Perry. 1992*a*. Evidence for a morphological component in acid-base regulation during environmental hypercapnia in the brown bullhead (*Ictalurus nebulosus*). Cell Tissue Res 268:539–552.

Goss G.G., S.F. Perry, P. Laurent. 1995. Gill morphology and acid-base regulation. Pp. 257–284 in C.M. Wood and T.J. Shuttleworth, eds. Fish Physiology. Vol. 14. Academic Press, New York.

Goss G.G., S.F. Perry, C.M. Wood, and P. Laurent. 1992*b*. Mechanisms of ion and acid-base regulation at the gills of freshwater fish. J Exp Zool 263:143–159.

Harvey B.J. 1992. Energization of sodium absorption by the H^+-ATPase pump in mitochondria-rich cells of frog skin. J Exp Biol 172:289–309.

Hiroi J., T. Kaneko, and M. Tanaka. 1999. In vivo sequential changes in chloride cell morphology in the yolk-sac membrane of Mozambique tilapia (*Oreochromis mossambicus*) embryos and larvae during seawater adaptation. J Exp Biol 202:3485–3495.

Hwang P.P. 1988. Multicellular complex of chloride cells in the gills of freshwater-adapted teleosts. J Morphol 196:15–22.

Ishihara A. and Y. Muyiya. 1987. Ultrastructural evidence of calcium uptake by chloride cells in the gills of goldfish, *Carassius auratus*. J Exp Zool 242:121–129.

Laurent P. and S.F. Perry. 1990. The effects of cortisol on gill chloride cell morphology and ionic uptake on the freshwater trout, *Salmo gairdneri*. Cell Tissue Res 259:429–442.

Lee T.H. 1996. Morphological and functional studies on mitochondria-rich cells in the gills epithelium of the tilapia, *Oreochromis mossambicus*. PhD diss. Taiwan University, Taipei.

Lee T.H., P.P. Hwang, and S.H. Feng. 1996*a*. Morphological studies on gill and mitochondria-rich cells in the stenohaline cyprinid teleosts, *Cyprinus carpio* and *Carassius auratus*, adapted to various hypotonic environments. Zool Stud 35: 272–278.

Lee T.H., P.P. Hwang, H.C. Lin, and F.L. Huang. 1996*b*. Mitochondria-rich cells in the branchial epithelium of the teleost, *Oreochromis mossambicus*, acclimated to various hypotonic environments. Fish Physiol Biochem 15:513–523.

Lin L.Y. and P.P. Hwang. 2001. Modification of morphology and function of integument mitochondria-rich cells in tilapia larvae (*Oreochromis mossambicus*) acclimated to ambient chloride levels. Physiol Biochem Zool 74:469–476.

Mackinnon M. and H.E. Enesco. 1980. Cell renewal in the gills of the fish *Barbus conchonius*. Can J Zool 58:650–653.

Maetz J. and P. Pic. 1977. Microtubules in the chloride cell of the gill and disruptive effects of colchicine on the salt balance of the sea water adapted *Mugil capito*. J Exp Zool 199:325–338.

Mills J.W. and L.J. Mandel. 1994. Cytoskeletal regulation of membrane transport events. Fed Am Soc Exp Biol 8:1161–1165.

Morgan I.I. and W. Potts. 1995. The effects of the adrenoreceptor agonist phenylephrine and isoproterenol on the intracellular ion concentrations of branchial epithelial cells of brown trout (*Salmo trutta* L.). J Comp Physiol B 165:458–463.

Morgan I.I., W. Potts, and K. Oates. 1994. Intracellular ion concentrations in branchial epithelial cells of brown trout (*Salmo trutta* L) determined by X-ray microanalysis. J Exp Biol 194:139–151.

Patrick M.L. and C.M. Wood. 1999. Ion and acid-base regulation in the freshwater mummichog (*Fundulus heteroclitus*): a departure from the standard model for freshwater teleosts. Comp Biochem Physiol A 122:445–456.

Perry S.F. 1997. The chloride cell: structure and function in the gills of freshwater fishes. Annu Rev Physiol 59:325–347.

Perry S.F., G.G. Goss, and P. Laurent. 1992. The interrelationships between gill chloride cell morphology and ionic uptake in four freshwater teleosts. Can J Zool 70:1775–1786.

Perry S.F. and C.M. Wood. 1985. Kinetics of branchial calcium

uptake in the rainbow trout: effects of acclimation to various external calcium levels. J Exp Biol 116:411–433.

Pisam M., B. Auperin, P. Prunet, and A. Rambourg. 1993. Effects of prolactin on α and β chloride cells in the gill epithelium of the saltwater adapted tilapia *Oreochromis niloticus.* Anat Rec 235:275–284.

Pisam M., A. Caroff, and A. Rambourg. 1987. Two types of chloride cells in the gill epithelium of a freshwater-adapted euryhaline fish, *Lebistes reticulatus*: their modification during adaptation to salt water. Am J Anat 179:40–50.

Pisam M., C. LeMoal, B. Auperin, P. Prunet, and A. Rambourg. 1995. Apical structures of "mitochondria-rich" alpha and beta cells in euryhaline fish gill: their behavior in various living conditions. Anat Rec 241:13–24.

Shikano T. and Y. Fujio. 1998. Changes in salinity tolerance and branchial chloride cells of newborn guppy during freshwater and seawater adaptation. J Exp Zool 284:137–146.

Tsai J.C. and P.P. Hwang. 1998*a*. Effects of wheat germ agglutinin and colchicine on microtubules of the mitochondria-rich cells and Ca^{2+} uptake in tilapia (*Oreochromis mossambicus*) larvae. J Exp Biol 201:2263–2271.

———. 1998*b*. The wheat germ agglutinin binding sites and development of the mitochondria-rich cells in gills of tilapia (*Oreochromis mossambicus*). Fish Physiol Biochem 19:95–102.

Wilson J.M., P. Laurent, B.L. Tufts, D.J. Benos, M. Donowitz, A.W. Vogl, and D.J. Randall. 2000. NaCl uptake by the branchial epithelium in freshwater teleost fish: an immunological approach to ion-transport protein localization. J Exp Biol 203:2279–2296.

Wood C.M. 1992. Flux measurements as indices of H^+ and metal effects on freshwater fish. Aquat Toxicol 22:239–264.