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Morphological Modification of Mitochondria-Rich Cells of the Opercular Epithelium of Freshwater Tilapia, *Oreochromis mossambicus*, Acclimated to Low Chloride Levels

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Yi-Er Shieh, Ru-Shiow Tsai and Pung-Pung Hwang (2003) Morphological modification of mitochondria-rich cells of the opercular epithelium of freshwater tilapia, *Oreochromis mossambicus*, acclimated to low chloride levels. *Zoological Studies* **42**(4): 522-528. The present study attempted to elucidate the effect of Cl^- concentration on the morphology of the opercular epithelium of freshwater tilapia, *Oreochromis mossambicus*. Similar to the situation in gills, 3 types of mitochondria-rich (MR) cells with different morphologies of apical crypts (wavy-convex, shallow-basin, and deep-hole) were identified in the opercular epithelium of freshwater tilapia. Most of the MR cells were located in the upper central area of the opercular epithelium, and the shallow-basin type dominated. When freshwater tilapia were acclimated to 3 artificial fresh waters, low-Na^+ low-Cl^- (L-Na-L-Cl), high-Na^+ low-Cl^- (H-Na-L-Cl), and high-Na^+ high-Cl^- (H-Na-H-Cl), for 1 wk, cell densities of MR cells were higher in L-Na-L-Cl and H-Na-L-Cl media than in the H-Na-H-Cl medium. The wavy-convex MR cells appeared in the low-Cl^- media only and were never observed in the H-Na-H-Cl medium. In an acute-exposure experiment, tilapia preacclimated to H-Na-H-Cl were transferred to a low-Cl^- environment, and the numbers of MR cells dramatically increased within 6 h (significantly different from that at 0 h, $p < 0.05$, *t*-test), and increased linearly for 96 h. The wavy-convex MR cells were first seen at 24 h and increased until 96 h. On the contrary, deep-hole MR cells began to decrease after 6 h of exposure to the low-Cl^- environment. The increased cell densities and morphological changes in MR cells suggest that the expanded apical surfaces of MR cells are associated with the stimulated Cl^- uptake by tilapia in a low-Cl^- environment.
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Key words: Acclimation, Apical crypts, Wavy-convex type, Shallow-basin type, Deep-hole type.

Physiological conditions of fish are tremendously influenced by environmental factors such as ion concentrations, salinity, temperature, oxygen, and pH value in the water. Marine teleosts living in hypertonic environments drink large volumes of seawater to compensate for water loss from their bodies, and extrude excess ions via gills, the opercular epithelium, and feces (Foskett and Scheffey 1982, Marshall 1985, Flik et al. 2002). Freshwater teleosts living in hypotonic environments can excrete excess water through the kidneys, and ion loss by passive diffusion is balanced by salt intake from food and active salt uptake via gills and the opercular epithelium (Jobling 1995).

Gills are important for ion regulation not only in marine but also in freshwater teleosts. Although the structure and functions of gills have been well studied (Laurent and Dunel 1980, Laurent and Perry 1991, Goss et al. 1998, Evans et al. 1999, Chang et al. 2001), the complexity and fragility of intact gills are difficult to overcome when studying the mechanism of ion movement in the gill epithelium. Burns and Copeland (1950) reported that the opercular epithelium lining inside the operculum contains pavement cells, chloride cells, mucus cells, and undifferentiated cells. The opercular epithelium of seawater teleosts actively transports

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chloride ions from the blood side to the seawater side, being involved in osmoregulation as are gills, when isolated, mounted as a membrane in an Ussing chamber, and short-circuited (Karnaky and Degnan 1977, Marshall 1977). From that time on, the opercular epithelium has been widely used as a model tissue for analyzing ion transport processes in fish gills (Girard and Payan 1980, McCormick 1990, Marshall et al. 1995). "Chloride cells", also called mitochondria-rich (MR) cells, were suggested to be the site of salt secretion (Keys and Willmer 1932). Involvement of cortisol and prolactin in the differentiation of chloride cells and the stimulation of Na^+, K^+-ATPase activity in the opercular epithelium was also demonstrated (McCormick 1990, Herndon et al. 1991). Using a vibrating probe, MR cells were directly proven to actively secrete Cl^- and to show high ionic permeability in the opercular epithelium of *Sarotherodon mossambicus* (syn. *Oreochromis mossambicus* Trewevas, 1983), (Foskett and Scheffey 1982). Burgess et al (1998) studied freshwater-adapted teleosts by clamping the opercular epithelia in Ussing chambers to record the transepithelial potential and the current in open-circuit or short-circuit conditions, and by applying isotopic ions across the epithelium. They found that non-diffusive uptake of Cl^- and Na^+ as well as Ca^{2+} from the mucosal FW occurred in *O. niloticus*, but only non-diffusive uptake of Cl^- and passive movement of Na^+ occurred in killifish, *Fundulus heteroclitus*. Also the uptake of Ca^{2+}, Cl^-, and Na^+ was demonstrated in the opercular epithelium of tilapia (*O. mossambicus* and *O. niloticus*) and killifish (*F. heteroclitus*) (McCormick et al. 1992, Marshall et al. 1995, Burgess et al. 1998). However, the mechanisms for NaCl uptake in MR cells of freshwater-adapted teleosts are still being debated (Perry 1997, Marshall 2002). One possible reason is that there have only been a very few studies conducted on the opercular epithelium that are suitable for such kind of experiments.

Two subtypes of MR cells, α and β cells were postulated by Pisam and colleagues (Pisam and Rambourg 1991, Pisam et al. 1995). Our previous studies (Lee et al. 1996a b) demonstrated that there are 3 subtypes of MR cells in tilapia (*O. mossambicus*) gills: wavy-convex, shallow-basin, and deep-hole MR cells according to the different morphologies of their apical surfaces, and the cell density of each subtype was associated with various ion concentrations in the water. In recent studies, wavy-convex MR cells, characterized by an expanded apical surface, were related to the stimulated capacity for Cl^- but not Na^+ uptake (Chang et al. 2002). However, little is known about the relationship between the morphologies and functions of opercular MR cells in freshwater-adapted fish, which is important basic information for further studies on ion uptake mechanisms.

Therefore, the purpose of the present study was to examine the effects of environmental ion (particularly Na^+ and Cl^-) concentrations on the morphologies of opercular MR cells in freshwater-adapted tilapia and to compare these results with those of gills.

MATERIALS AND METHODS

Animals

Tilapia (*O. mossambicus*) of both genders obtained from the Tainan Branch of the Taiwan Fisheries Research Institute were reared in aerated and circulated local fresh water under a photoperiod of 12 L: 12 D at 26 ± 2°C. Fish weighing 7-10 g were used in the following acclimation experiments under controlled conditions as described above.

Three kinds of artificial fresh water, low-Na^+ low-Cl^- (L-Na-L-Cl), high-Na^+ low-Cl^- (H-Na-L-Cl), and high-Na^+ high-Cl^- (H-Na-H-Cl), were prepared by adding appropriate amounts of NaCl, Na_2SO_4, $CaSO_4$, $MgSO_4$, KH_2PO_4, and K_2HPO_4 to double-deionized water (dd-H_2O) (Milli-RO60, Millipore, Mass., USA) (Table 1). The ion concentrations of K^+, Ca^{2+}, and Mg^{2+} in the artificial fresh water were near the ranges in local fresh water. The ion compositions of the artificial fresh water were confirmed by measuring the Na^+, K^+, Ca^{2+}, and Mg^{2+} concentrations with an atomic absorption spectrophotometer (Hitachi Z-8000, Tokyo, Japan) and

Table 1. Ionic compositions (mM) of 3 artificial media of fresh water (Mean ± SE)

Medium	Na^+	Cl^-	Ca^{2+}	K^+	Mg^{2+}
L-Na-L-Cl	0.39 ± 0.01	< 0.001	0.19 ± 0.01	0.19 ± 0.02	0.19 ± 0.01
H-Na-L-Cl	9.8 ± 0.290	< 0.001	0.2 ± 0.003	0.18 ± 0.02	0.19 ± 0.003
H-Na-H-Cl	9.7 ± 0.01	9.5 ± 0.45	0.28 ± 0.01	0.18 ± 0.02	0.19 ± 0.003

Cl^- with a spectrophotometer (Hitachi U-2000; Zall et al. 1956). The pH of the media was maintained at between 6.2 and 6.7 during the experiments.

Acclimation experiments

I. *Long-term acclimation experiment*

In order to examine the effects of low environmental Na^+ and Cl^- on the morphology of opercular MR cells, freshwater fish were acclimated to H-Na-H-Cl, H-Na-L-Cl, and L-Na-L-Cl media respectively for 1 wk, and then were sampled for morphological observations (6 individuals for each test). In the case of H-Na-L-Cl, Na_2SO_4 was used to replace the NaCl, and a previous study confirmed that environmental SO_4^{2-} levels are not correlated with the appearance of MR cells (Lee et al. 1996). The artificial media were renewed every 2 d. After anesthetization with MS-222 (*m*-aminobenzoic acid ethyl ester methansulfonate, 200 μg/ml, pH 6.7, Alpharma, USA), opercula were dissected for scanning electron microscopic (SEM) observation. In order to maintain the water quality, the acclimation media were aerated with a filtered air pump, and changed every 2 d. Fish were fed with commercial pellets 1 h immediately prior to the water changes every 2 d, but feeding was stopped 3 d before sampling.

II. *Time-course exposure experiment*

Based on results of the long-term acclimation experiment, tilapia were preacclimated to H-Na-H-Cl medium for 1 wk and then were transferred directly to H-Na-L-Cl medium. Sampling (6 individuals per sampling time) was conducted at 0, 3, 6, 12, 24, and 96 h after transfer. Samples were treated as described above.

In order to diminish the effect of handling stress, medium was transferred by exchanging it using a siphon, and fish were not handled at all until the time immediately before sampling.

SEM observation and quantification

After being anesthetized with MS222, fish were sacrificed in order to excise the operculum. Dissected bilateral opercula were prefixed in a fixative containing 5% glutaraldehyde and 4% paraformadehyde in 0.2 M phosphate buffer (PB, pH 7.2) at 4°C for 12 h, then rinsed with PB and postfixed with 1% osmium tetraoxide in 0.2 M PB for 1.5 h at room temperature. After rinsing with PB and dd-H_2O, tissues were dehydrated in ascending concentrations of ethanol from 50% to 100%, then specimens were critical-point-dried using liquid CO_2 in a critical-point drier (Hitachi HCP-2), and sputter-coated with a gold-palladium complex for 4 min in a vacuum evaporator (Eiko 1B-2, Tokyo, Japan). The coated specimens were examined in an SEM (Hitachi S-2500) at an accelerating voltage of 15 kV.

The distribution of MR cells in the opercular epithelium was investigated. Criteria for the cell type classification of MR cells followed Lee et al. (1996). Most MR cells were distributed in a certain area as shown in figure 1A. From this area, 6 images (4250 μm^2 for each, 1200x magnification) were randomly obtained for counting the number of MR cells and measuring the longest diameter of MR cell openings. Six individuals in each group were measured.

Statistical analysis

Values are presented as the mean ± SE and were assessed by one-way analysis of variance (ANOVA) and Tukey's pair-wise comparison. The percentage (proportional) data (obtained by dividing the number of a subtype with the total number of MR cells) were subjected to arcsine transformation prior to analysis.

RESULTS

Distribution and morphology of MR cells in the opercular epithelium

Similar to that in other species, tilapia opercular epithelium, an area of membrane structure lining the inside of the opercular bone, exposes its apical side to the aqueous environment of the gill chamber and connects to an underlying layer of loose connective tissue on its basal side (Fig. 1A).

MR cells in the opercular epithelium of freshwater tilapia, *O. mossambicus*, are not evenly distributed. Most MR cells are located in the upper central area of the opercular epithelium with cell densities of about 2.0-9.0 cells/4250 μm^2 (about 3.4%-13.4% of the total cells) (Fig. 1A), and only very few MR cells appeared in the remaining areas of the membrane. Three types of apical openings of MR cells were observed in the opercular epithelium (Fig. 1B-D). According to previous descriptions by Lee et al. (1996), they were identified as wavy-convex, shallow-basin, and deep-hole types of MR cells. The diameters of the apical openings of wavy-convex, shallow-basin, and deep-hole

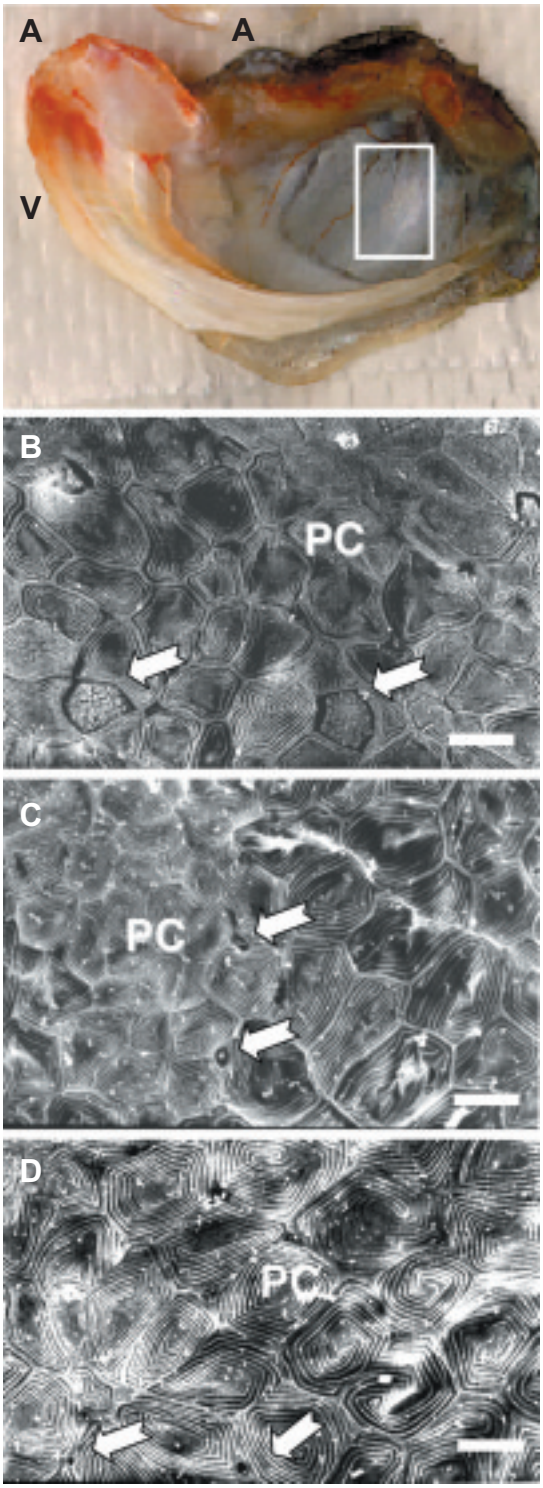

Fig. 1. Image of the operculum of tilapia, *Oreochromis mossambicus*. (A) Square area with a higher density of mitochondria-rich cells on the opercular epithelium; (B) wavy-convex type of mitochondria-rich cell (arrow); (C) shallow-basin type of mitochondria-rich cell (arrow); (D) deep-hole type of mitochondria-rich cell (arrow). A, anterior position; PC, pavement cells; V, ventral side. Scale bar = 10 μm.

types were 6.5 ± 0.7, 3.2 ± 0.2, and 0.8 ± 0.1 μm, respectively.

MR cells in the opercular epithelium after long-term acclimation to different media

After 7-d acclimation, cell densities of opercular MR cells in both low-Cl^- groups, L-Na-L-Cl and H-Na-L-Cl, were significantly higher (7.8 ± 0.5 and 9.0 ± 1.6 cells/unit area) than that in the H-Na-H-Cl group (2.0 ± 0.5 cells/unit area) (Fig. 2). All 3 subtypes of MR cells were observed in tilapia acclimated to L-Na-L-Cl and H-Na-L-Cl media, but no wavy-convex type was observed in H-Na-H-Cl-acclimated fish (Fig. 2). The cell density of shallow-basin MR cells was higher in H-Na-L-Cl-acclimated tilapia than in L-Na-L-Cl- or H-Na-H-Cl-acclimated fish (Fig. 2). Cell densities of the deep-hole type remained similar among the 3 groups (Fig. 2).

Percentage changes of different cell types of MR cells among total MR cells (Table 2) indicated that changes in the concentration of environmental Cl^-, but not Na^+, stimulated the appearance of wavy-convex MR cells. The shallow-basin type was the dominant type in all 3 media. The percentages of wavy-convex and deep-hole MR cells did not change significantly when the fish were acclimated to L-Na-L-Cl or H-Na-L-Cl media. However, percentages of wavy-convex and deep-hole MR cells in H-Na-H-Cl-acclimated fish significantly differed from those in the other 2 groups of fish.

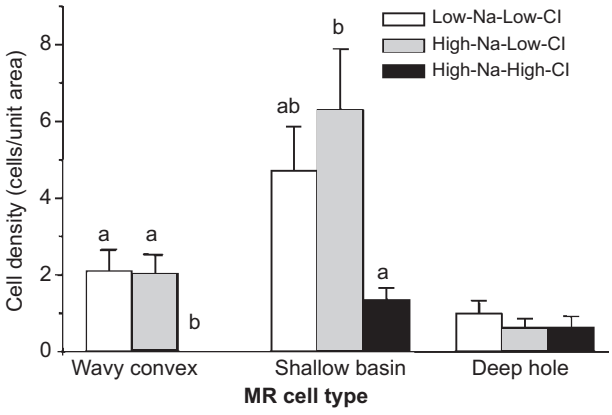

Fig. 2. Densities of different subtypes of mitochondria-rich cells on the opercular epithelium of tilapia acclimated to different media. No wavy-convex type was observed in high-Cl^- medium (H-Na-H-Cl). Values with different letters significantly differ (by one-way ANOVA).

Modification of MR cells in the opercular epithelium upon acute exposure to a low-Cl⁻ environment

With acute transfer from H-Na-H-Cl to low Cl⁻ (H-Na-L-Cl), the cell density of MR cells in tilapia opercular epithelium almost doubled within 6 h (significantly different from that at 0 h, *t*-test), and continued to increase slowly over 96 h (Fig. 3).

Wavy-convex MR cells were not observed until 24 h after transfer to low-Cl⁻ medium (Fig. 4A), and the relative percentage of the wavy-convex type increased to 16.5% ± 8.5% within 96 h of acclimation (Fig. 4B). Shallow-basin MR cells began to increase within 24 h (from 54.5% ± 8.2% to 94.1% ± 2.8%) and thereafter maintained a higher relative density (Fig. 4). The relative percentage of deep-hole MR cells decreased from 45.5% ± 8.2% to 28.7% ± 5.0% within the first 12 h and finally decreased to 1.4% ± 0.8% between 24 and 96 h (Fig. 4B). The data for cell densities showed similar patterns of changes (Fig. 4A).

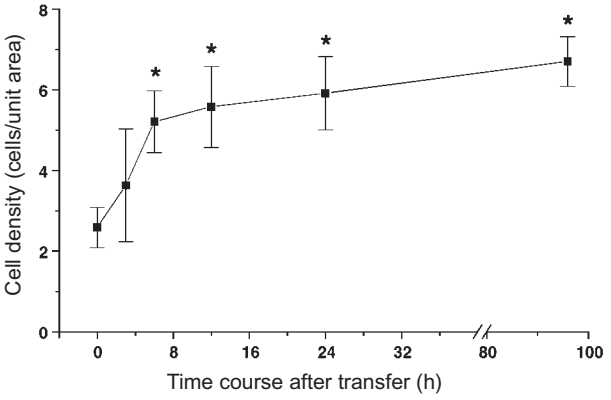

Fig. 3. Changes in mitochondria-rich (MR) cell densities on the opercular epithelium of tilapia after being transferred from high-Cl⁻ (H-Na-H-Cl) to low-Cl⁻ (H-Na-L-Cl) media. An asterisk (*) indicates significant differences from the time 0 h for each subtype of mitochondria-rich cell (n = 4; p < 0.05, by Student's t-test).

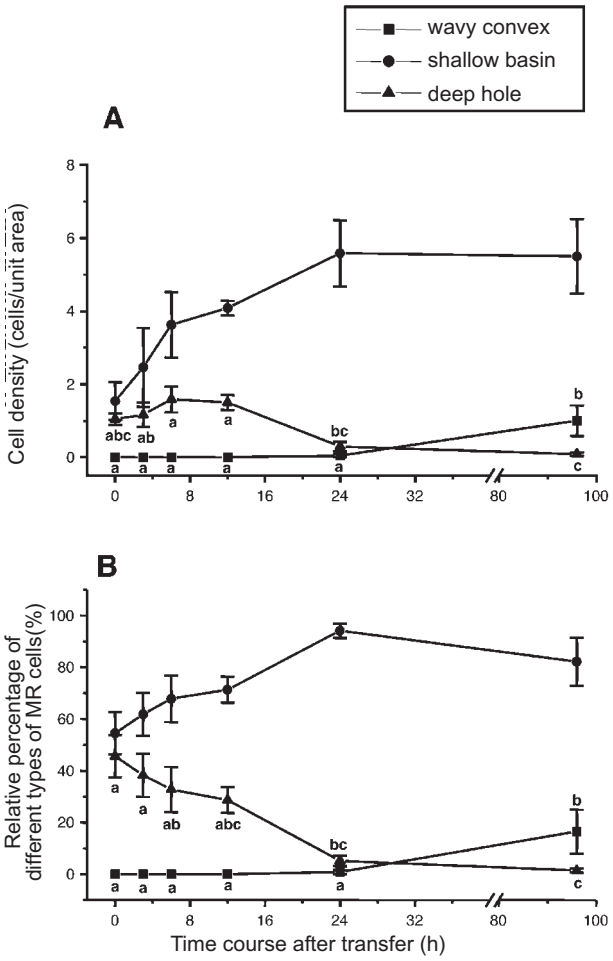

Fig. 4. Changes in different subtypes of mitochondria-rich (MR) cells on the opercular epithelium of tilapia after being transferred from high-Cl⁻ (H-Na-H-Cl) to low-Cl⁻ (H-Na-L-Cl) media. (A) Changes in cell density; (B) changes in the relative percentages. Values with different letters significantly differ among different times for each subtype of mitochondria-rich cell. Percentage data were subjected to arcsine transformation prior to one-way ANOVA.

Table 2. *Relative cell percentage of the opercular MR cells in tilapia acclimated to different artificial freshwaters (Mean ± SE, n = 6)

	Medium		
	Low-Na-Low-Cl	High-Na-Low-Cl	High-Na-High-Cl
Wavy convex	28.28 ± 8.0[a]	27.12 ± 8.1[a]	0[b]
Shallow basin	58.35 ± 11.8	65.5 ± 8.1	72.08 ± 6.3
Deep hole	13.38 ± 4.5[ab]	7.38 ± 2.0[a]	27.92 ± 6.3[b]

Different letters indicated statistical significance among artificial freshwater groups. Data were subjected to arcsine transformation prior to one-way ANOVA.

* Relative cell percentage = subtype MR cells/ total MR cells × 100%

DISCUSSION

Abundant early studies focused on ion regulation by the opercular epithelium of fish adapted to seawater (Degnan et al. 1977, Marshall and Nishioka 1980, Foskett et al. 1981, Marshall 1995), yet very few studies were conducted on ion transport in the opercular epithelium of fish adapted to fresh water (McCormick et al. 1992, Burgess et al. 1998). The major findings of this study were that (1) 3 subtypes of MR cells were observed in opercular epithelium, i.e., wavy-convex, shallow-basin, and deep-hole types; (2) MR cells were concentrated in a certain area of the opercular epithelium; (3) wavy-convex MR cells appeared in the opercular epithelium in low-Cl^- environments only, and more opercular MR cells appeared in low- than in high-Cl^- environments; and (4) upon acute exposure to a low-Cl^- environment, the relative percentage of deep-hole MR cells decreased within 24 h, while that of wavy-convex MR cells began to increase 24 h after transfer.

Compared with the MR cell density (34.58 ± 2.61 cells/4250 μm^2) of tilapia gills (Chang et al. 2001 2002), tilapia opercular epithelium developed a much lower cell density of MR cells (9.0 ± 1.6 cells/4250 μm^2). The difference in MR cell number between the 2 tissues is more apparent, if one considers the much larger surface area of gills compared to the opercular membrane on which MR cells appear in only a small area. However, the morphologies and apical diameters of different types of MR cells in the opercular epithelium did not differ from those in gills of freshwater tilapia. Enlargement of the apical surface of MR cells is related to ion uptake of fish in low-ion environments (Laurent et al. 1985, Perry and Laurent 1989, Greco et al. 1996). Low environmental Cl^- levels cause an increase in the density of wavy-convex type MR cells with enlarged apical surfaces and a stimulation of Cl^- uptake in the whole body or larval skin of freshwater tilapia, indicating the involvement of MR cells in the Cl^- uptake mechanism of freshwater fish (Lin and Hwang 2001, Chang et al. 2002). Wavy-convex MR cells appeared in the opercular epithelium of tilapia acclimated to low-Cl^- medium only. Taken together, opercular MR cells probably also perform similar functions of ion uptake as do gill MR cells, although the total number of cells in the opercular epithelium is much lower.

Cl^- absorption is thought to occur by active transport through the apical membrane of MR cells by Cl^-/HCO_3^- exchangers (Perry 1997). Thus the increase in apical membrane area might allow more Cl^-/HCO_3^- exchangers to be inserted, which would consequently produce an increase in the transport of Cl^-. In contrast, when the Cl^- level in the water is raised, the apical membrane and the exchangers on it may be internalized to reserve energy and maintain the Cl^- balance. Gill epithelial cells are renewed in about 4 d (Mackinnon and Enesco 1980, Tsai and Hwang 1998). Therefore, reconstruction of the apical surface seems to be critical for modulating Cl^- uptake activities, and transformations among different subtypes of MR cells upon acclimation to low Cl^- might reflect acute modifications in the apical membranes of MR cells prior to renewal of the cells, as proposed by Lin and Hwang (2001). Modulation of apical surface structures might be achieved through membrane turnover, cytoskeleton reorganization, and other intracellular modifications. Actin around MR cells was found to regulate its apical crypts, and cytochalasin D, which disrupts actin polymerization, was found to block the conductance to hypotonic shock and the reduction in apical crypt density of MR cells (Daborn et al. 2001). Effects of a low-Cl^- environment on modifications in apical crypts of opercular MR cells may be associated with the cytoskeletal activity of cells and interactions with pavement cells. These cellular events and their regulation may be similar in both gill and opercular MR cells. However, the sensitivity to environmental Cl^- and the speed with which the modification and regulation occur are somewhat lower in opercular MR cells than in gill cells. The increase in wavy-convex MR cells in the opercular membrane occurred 24 h after the low-Cl^- challenge, while it occurs within 6 h in gills (Chang et al. 2002).

In comparison with gills, the opercular epithelium has fewer MR cells and reveals a lower sensitivity and slower regulation upon environmental challenges, indicating that it plays a minor or accessory role in the ion- and osmoregulation of the whole fish. However, the mechanisms for Cl^- uptake may be similar in both gill and opercular MR cells. Therefore, opercular epithelium could be a suitable model for studying ion uptake mechanisms.

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REFERENCES

Burgess DW, WS Marshall, CM Wood. 1998. Ionic transport by the opercular epithelia of freshwater acclimated tilapia (*Oreochromis mossambicus*) and killifish (*Fundulus hete-*

roclitus). Comp. Biochem. Phys. A **121:** 155-164.
Burns J, DE Copeland. 1950. Chloride excretion in the head region of *Fundulus heteroclitus*. Biol. Bull. **99:** 381-385.
Chang IC, PP Hwang. 2002. Cl^- uptake mechanism in freshwater-adapted tilapia (*Oreochromis mossambicus*). PhD dissertation, National Taiwan Univ.
Chang IC, TH Lee, CH Yang, YY Wei, FI Chou, PP Hwang. 2001. Morphology and function of gill mitochondria-rich cells in fish acclimated to different environments. Physiol. Biochem. Zool. **74:** 111-119.
Daborn K, RRF Cozzi, WS Marshall. 2001. Dynamics of pavement cell-chloride cell interactions during abrupt salinity change in *Fundulus heteroclitus*. J. Exp. Biol. **204:** 1889-1899.
Degnan KJ, KJ Karnaky Jr, JA Zadunaisky. 1977. Active chloride transport in the in vitro opercular skin of a teleost (*Fundulus heteroclitus*), a gill-like epithelium rich in chloride cells. J. Physiol. London **271:** 155-191.
Evans DH, PM Poermarini, WTW Potts. 1999. Ionic transport in the fish gill epithelium. J. Exp. Zool. **283:** 641-652.
Foskett JK, CD Logsdon, T Turner, TE Machen, HA Bern. 1981. Differentiation of the chloride extrusion mechanism during sea water adaptation of a teleost fish, the cichlid *Sarotherodon mossambicus*. J. Exp. Biol. **93:** 209-224.
Foskett JK, C Scheffey. 1982. The chloride cell: definitive identification as the salt-secretory cell in teleost. Science **215:** 164-166.
Girard JP, P Payan. 1980. Ion exchanges through respiratory and chloride cells in freshwater- and seawater-adapted teleosteans. Am. J. Physiol. **238:** R260-R268.
Goss GG, JN Perry, P Laurent. 1998. Gill morphology and acid-base regulation in freshwater fishes. Comp. Biochem. Phys. A **119:** 107-115.
Greco AM, JC Fenwick, SF Perry. 1996. The effects of soft-water acclimation on gill structure in the rainbow trout *Oncorhynchus mykiss.* Cell Tissue Res. **285:** 75-82.
Herndon TM, SD McCormick, HA Bern. 1991. Effects of prolactin on chloride cells in opercular membrane of seawater-adapted tilapia. Gen. Comp. Endocr. **83:** 283-289.
Hwang PP. 1987. Tolerance and ultrastructural responses of branchial chloride cells to salinity changes in the euryhaline teleost *Oreochromis mossambicus*. Mar. Biol. **94:** 643-649.
Jobling M. 1995. Osmotic and ionic regulation-water and salt balance. *In* M Jobling, ed. Environmental biology of fishes. Lodon: Chapman & Hall Press, pp. 211-249.
Karnaky KJ Jr, KJ Degnan, JA Zadunaisky. 1977. Chloride transport across isolated opercular epithelium of killifish: a membrane rich in chloride cells. Science **195:** 203-205.
Keys AB, EN Willmer. 1932. "Chloride secreting cells" in the gills of fishes, with special reference to the common eel. J. Physiol. London **76:** 368-378.
Laurent P, S Dunel. 1980. Gill morphology of gill epithelia in fish. Am. J. Physiol. **238:** R147-R159.
Laurent P, H Hobe, S Dunel-Erb. 1985. The role of environmental sodium chloride relarive to calcium in gill morphology of freshwater salmonid fish. Cell Tissue Res. **240:** 675-692.
Laurent P, SE Perry. 1991. Environmental effects on fish gill morphology. Physiol. Zool. **64:** 4-25.
Lee TH, PP Hwang, SH Feng. 1996a. 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 TH, PP Hwang, HC Lin, FL Hwang. 1996b. Mitochondria-rich cells in the branchial epithelium of the teleost, *Oreochromis mossambicus*, acclimated to various hypotonic environments. Fish Physiol. Biochem. **15:** 513-523.
Lin LY, PP 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, HE Enesco. 1980. Cell renewal in the gills of the fish *Barbus conchonius*. Can. J. Zool. **58:** 650-653
Marshall WS. 1977. Transepithelial potential and short-circuit current across the isolated skin of *Gillicthys mirabilis* (Teleostei: Gobiidae), acclimated to 5% and 100% seawater. J. Comp. Physiol. B **114:** 157-165.
Marshall WS. 1995. Transport processes in isolated teleost epithelia: opercular epithelium and urinary bladder. *In* CM Woods, TJ Shuttleworth, eds. Cellular and molecular approaches to fish ionic regulation. New York: Academic Press, pp. 1-24.
Marshall WS. 2002. Na^+, Cl^-, Ca^{2+} and Zn^{2+} transport by fish gills retrospective review and prospective synthesis. J. Exp. Zool. **293:** 264-283.
Marshall WS, SE Bryson, JS Burghardt, PM Verbost. 1995. Ca^{2+} transport by opercular epithelia of the fresh water adapted euryhaline teleost, *Fundulus heteroclitus*. J. Comp. Physiol. B **165:** 268-277.
Marshall WS, RS Nishioka. 1980. Relation of mitochondria-rich chloride cells to active chloride transport in the skin of marine teleost. J. Exp. Zool. **214:** 147-188.
McCormick SD. 1990. Cortisol directly stimulates differentiation of chloride cells in tilapia opercular membrane. Am. J. Physiol. **259:** R857-R863.
McCormick SD, S Hasegawa, T Hirano. 1992. Calcium uptake in the skin of a freshwater teleost. Proc. Natl. Acad. Sci. USA **89:** 3635-3638.
Perry SF. 1997. The chloride cell: structure and function in the gills of freshwater fishes. Ann. Rev. Physiol. **59:** 325-347.
Perry SF, P Laurent. 1989. Adaptational responses of rainbow trout to lowered external NaCl concentration: contribution of the branchial chloride cells. J. Exp. Biol. **147:** 147-168.
Pisam M, LEC Moal, B Auperin, P Prunet, A Rambourg. 1995. Apical structures of "mitochondria-rich" α and β cells in euryhaline fish gill: their behavior in various living conditions. Anat. Rec. **241:** 13-24.
Pisam M, A Rambourg. 1991. Mitochondria-rich cells in the gill epithelium of teleost fishes: an ultrastructural approach. Int. Rev. Cytol. **130:** 191-232.
Trewevas E. 1983. Tilapiine fishes of the genera *Sarotherodon*, *Oreochromis* and *Danakilia*. British Museum of Natural History, Publ. no. 878. Ithaca, NY: Comstock Publishing Assoc, 583 pp.
Tsai JC, PP Hwang. 1998. 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.
Zall DM, D Fisher, MQ Garner. 1956. Photometric determination of chlorides in water. Anal. Chem. **28:** 1665-1668.