

**ACTIVATION AND INACTIVATION
OF MITOCHONDRIA-RICH CELLS
IN TILAPIA LARVAE
ACCLIMATED TO AMBIENT CHLORIDE CHANGES**

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Introduction

Mitochondria-rich (MR) cells in branchial epithelium of freshwater teleost are responsible for absorbing Cl^- and Ca^{2+} actively from external environment. Resembling to other ion-transporting epithelial cells, MR cells contact with both internal and external milieus by basolateral and apical membrane domains, respectively, and pump the ions transcellularly from environment into blood circulation. Ambient Cl^- is suggested to be transported against gradient by Cl^- transporters distributed on the apical opening (apical membrane) of MR cells. It is well known that the apical openings of MR cells recessed to form apical crypts in seawater-acclimated fishes, but flush or slightly raised above the adjacent pavement cells in most freshwater-acclimated fishes (reviewed by Perry and Laurent, 1993). However, in the special case of tilapia, *Oreochromis mossambicus*, recessed apical surface of MR cells were also founded in freshwater-acclimated individuals. In our previous study on freshwater tilapia, we categorized these MR cells with different apical surface as wavy-convex, shallow-basin, and deep-hole subtypes (Lee et al., 1996). We found the structure of these apical openings varied with ambient Cl^- levels and suggested that the three subtypes of cells representing MR cells equipped with distinct capabilities

of Cl⁻ uptake (Lin and Hwang, 2001). Moreover, our evidences implicated that MR cells modulate their apical structure quickly (in hours) to compensate for ambient Cl⁻ disturbance. In the present study, we provide further evidences to support our model that MR cells in tilapia can be activated or inactivated to modulate their Cl⁻ uptake capabilities through modifying their exposed apical openings.

Experimental designs and results

Yolk-sac MR cells in tilapia larvae were examined with immunocytochemistry and vital staining during acclimation to high or low ambient Cl⁻ levels (High Cl⁻, 10 mM; Low Cl⁻, 0.005 mM; Normal Cl⁻, 0.5 mM). By using Concanavalin-A (Con-A) and Na pump double-staining, the MR cell on yolk-sac were labeled and discriminated to active (Con-A positive) or inactive (Con-A negative) groups. Therefore, the ratios of active MR cells in total labeled MR cells were counted and compared between the 3 Cl⁻ acclimating groups. Results showed that ratio of active cells increased gradually during 48 h acclimation to low Cl⁻ medium, but declined during acclimation to high Cl⁻ medium (fig. 1). However, the total numbers of MR

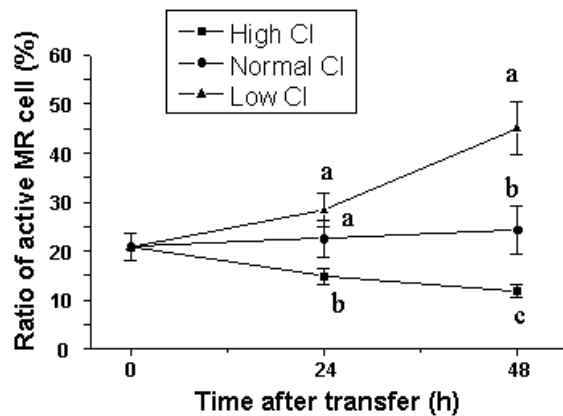


Figure 1. Changes of active MR cell in tilapia larvae acclimated to high, normal, and low Cl⁻ (10, 0.5, 0.05 mM) for 48 h. Mean±SD (N=5) is indicated. Different letters indicate significant difference between different groups.

cells were not different between different Cl⁻ acclimation groups. Moreover, we also labeled the yolk-sac MR cells with vital staining (DESPEI) and traced the marked MR cells in living larvae. After transfer the labeled larvae from normal water to high or low Cl⁻ water, we seriously observed the changes of DESPEI- stained cells every 6 h for 24 h. Under confocal scanning, we found that the cell numbers were maintained

constantly during acclimation, and the newly generated or degenerated cells were less than 10 %. These evidences consistently indicated that no significant turnover of MR cells occurred in larvae during acclimation to high or low ambient Cl⁻. Thus, the dramatic changes of apical openings subtypes within 24 h of acclimation (Lin and Hwang 2001) can be consider as morphological and functional modification of terminal differentiated MR cells. Furthermore, we suggested that MR cells can be activated from a inactive cells that are not contact with external environment and gradually expend their openings to upregulate Cl⁻ uptake capability, and vice versa, inactivated through constricting their opening to totally being covered by apical adjacent pavement cells. In addition, we also labeled MR cells with Con-A in living animal to examine the structural changes of apical opening during MR cell activation or inactivation. Interestingly, Con-A labeled vesicles were observed inside MR cells during the acclimation, indicating internalization of apical membrane might be involved in the process of activation or inactivation. In the aspect of physiological significance, our finding reveals that through activation or inactivation of MR cells in short-term regulation, tilapia larvae are capable of maintaining internal Cl⁻ constant and resisting to ambient Cl⁻ fluctuation.

References

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