DOCTORAL THESIS

Functional studies of gill epithelial cells isolated from Japanese eels (anguilla japonica)

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Date of Award:
2008

Functional Studies of Gill Epithelial Cells Isolated from Japanese Eels (*Anguilla japonica*)

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A thesis submitted in partial fulfillment of the requirements

for the degree of

Doctor of Philosophy

Principal Supervisor: Dr. WONG Kong Chu

Hong Kong Baptist University

January 2008

Abstract

Eel (*Anguilla japonica*) is a euryhaline fish that resides both in freshwater and seawater. The fish, however, encounters totally opposite osmotic challenges in the two media. In freshwater, the fish deals with osmotic water uptake and loss of salts and vice versa in seawater. To tolerate the wide range of salinities during migration, they develop special structures and mechanisms for osmoregulation, of which are the chloride cells (CCs) and pavement cells (PVCs) in the gill epithelium. Over the years, studies on the ion regulation in gills were mainly based on the electrophysiological, biochemical, and immunocytochemical staining methods on whole gill preparation. Although a great deal of information has been gathered, the direct interaction of the hormones and osmotic effects with individual type of gill cells in mediating cellular mechanisms in ion transport is still not fully understood. The progress of the study is basically hampered by the anatomical complexity of the gill tissues and the limited nucleotide databases as well as the commercially available antibodies for fish protein.

In the present study, we developed a model that is able to extract purified isolated chloride cells (CCs) and pavement cells (PVCs) from the gill of acclimating fish as well as to establish the primary gill cell culture to study the effect of osmotic stress on the expression profiles of ion transporters and ion channels. To increase the availability of molecular tools in studying fish osmoregulation, in chapter 2 we have cloned a number of ion transporters/channels and osmotic-stress induced transcription factor from the Japanese eel model. Partially cDNAs of sodium-hydrogen exchanger 3 (NHE3), sodium-bicarbonate co-transporters (NBC1), vacuolar H^+-ATPase and osmotic stress transcription factor 1 (Ostf1) were cloned. Real-time PCR primers were designed for mRNA quantification. For the NHE3, NBC1 and vacuolar H^+-ATPase, their expression levels were in general higher in freshwater gill cells. Our data indicated that the expression level of Ostf1 was significant higher in freshwater CCs than that of the PVCs. Upon seawater transfer, significant inductions of Ostf1 transcripts were observed in both CCs and PVCs. Interestingly in seawater to freshwater transfer experiments, there was no significant change in Ostf1 expression levels. Our data also indicated that the activation of Ostf1 expression was sensitive to water Na^+ and Cl^- levels.

In chapters 3 & 4, we aimed to determine the expression profiles of ion transporters and ion channels in CCs and PVCs of freshwater- and seawater-acclimating eels. Upon freshwater to seawater transfer (Chapter 3), significant increase of the mRNA and/or protein expressions of Na^+/K^+-ATPase α\ and β£] subunits, cystic fibrosis transmembrane conductance regulator (CFTR), sodium potassium chloride co-transporter (NKCC), and inwardly rectifying potassium channel (eKir) were found in gill CCs. However, reduction of aquaporin 1 and 3 (AQP1, and 3) transcript levels

were detected. While in seawater to freshwater transfer (Chapter 4), increased phosphorylation of some signaling proteins like mitogen-activated protein kinases (MAPK), myristoylated alanine-rich C-kinase substrate (MARCKs), and cAMP response element-binding (p-CREB) were observed. The expression levels of the transporters like anion exchanger 1 (AE1), NHE3, NBC1, and V-H^+-ATPase were significantly increased in freshwater CCs. The results suggested that the rapid response of signaling molecules in freshwater acclimation modulated the expression levels of different ion transporters for osmoregulation. In these two chapters, we provide the first evidence to demonstrate the expression profiles of different ion transporters and ion channels in individual cell types in fish gills. Our data demonstrated that the hyper- or hypo-osmotic adaptation remodeled the tissue distribution of CCs as well as the differential gene expression in the cells. The study support the notion that gill CCs are important in both freshwater and seawater adaptation.

To elucidate the importance of freshwater CCs in Na^+ and Cl^- uptake, in chapter 5 we attempted to establish an experimental model to study the reactivation mechanism in branchial ion uptake. Deionized water adapted eels were transferred to artificial freshwater (ion-supplement deionized water, ISDI) with or without Na^+ and/or Cl^-. Upon the transfer, our data demonstrated that the fish acclimating in ISDI without Na^+, showed significant inductions in the expression of the ion transporters (i.e. NHE1, NHE3, NBC1, and V-H^+-ATPase) in gill CCs. In the acclimation at ISDI without Cl^-, significant inductions of AE and NKCC in gill CCs were detected. Comparatively, the changes in the transporter expression levels were trifling in gill PVCs. Our study demonstrated that the gill CCs were the major targets in the reactivation of ion absorption. The data further suggest the importance of freshwater CCs in ion uptake.

In the last chapter (Chapter 6), we have developed a primary gill cell culture model and studied the direct effect of osmotic stress on cell volume disturbance and the expression of ion transporters in the cells. Our data demonstrated that the hypertonic (500 mOsm) treatment of PVCs induced cell shrinkage, followed by regulatory volume increase (RVI). The cell volume regulation was found to be modulated by the transporters – Na^+/K^+-ATPase, NKCC, and NHE1. The stimulators, dexamethasone (DEX) and dibutyryl cAMP treatments significantly induced the expression levels of the three ion transporters. Collectively, this thesis represents the first to combine *in-vivo* and *in-vitro* studies to obtain a multi-facet understanding on the gill cell functions.

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