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Research paper

Insights into molecular and cellular mechanisms of hormonal actions on fish ion regulation derived from the zebrafish model

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ABSTRACT

Fish have sophisticated mechanisms of ionic and acid-base regulation for maintaining body fluid homeostasis. Many hormones have been proposed to control the ionic and acid-base regulation mechanisms in fishes; however, lots of the proposed actions lack convincing cellular/molecular evidence. With the advantages of available genetic databases and molecular manipulation techniques, zebrafish has become an emerging model for research into ion transport physiology and functional regulation. Different types of ionocytes were found to transport ions through various sets of ion transporters, and the molecular mechanisms of ionocyte proliferation and differentiation have also been dissected, providing a competent platform with which to precisely study the ion transport pathways and ionocytes targeted by hormones, including isotocin, prolactin, cortisol, stanniocalcin-1, calcitonin, endothelin-1, vitamin D, parathyroid hormone 1, catecholamines, the renin-angiotensin-system, estrogen-related receptor α, and calcitonin gene-related peptide, which have been demonstrated to positively or negatively regulate ion transport through specific receptors at different molecular levels (transcriptional, translational, or posttranslational) or at different developmental stages of ionocytes (proliferation or differentiation). The knowledge obtained in zebrafish not only enhances our understanding of the hormonal control of fish ion regulation, but also informs studies on other animal species, thereby providing insights into related fields.

1. Introduction

Teleost fish, like mammals, have developed sophisticated mechanisms of ionic and acid-base regulation, which maintains body fluid homeostasis to enable cell activity and physiological processes to proceed normally. In fish, the gills (or the skin during embryonic stages before the gills are functionally developed) are the major organs responsible for ion transport, functionally equivalent to the kidney in mammals; ionocytes within the gills mediate the active transport of ions (Hwang and Lee, 2007). Hormones are known to tightly control ionic and acid-base regulation mechanisms to achieve body fluid homeostasis in mammals. Lots of these hormones have been proposed to exert similar actions on fish ionic and acid-base regulation; however, convincing cellular/molecular evidence of many proposed actions in fish still largely lack (Evans et al., 2005; Hwang and Chou, 2013). Possible reasons which hindered the related studies include the following: (1) lack of the exact understanding of the ion transport pathway (related ion transporter isoforms or ionocyte subtypes), increasing the difficulty to determine the target cells or effectors of a hormone action, (2) limited approaches to manipulate the homologous/endogenous level of a hormone or the receptor(s), and to distinguish the actions mediated by different receptor isoforms and (3) difficulty in precisely determining the action of a hormone on the developmental stage of ionocytes or the molecular pathway of ionocyte number control. These problems or constraints highlight the potential of zebrafish as a model for studies in hormonal control of ion regulation in fish. Given the advantages of having comprehensive genetic databases and well-developed molecular physiological approaches available, zebrafish has become an emerging model for research into ion transport physiology and functional regulation. Different types of ionocytes were identified to carry out ion transport functions through respective sets of ion transporters, and the molecular mechanisms of ionocyte proliferation and differentiation were also dissected, providing a competent platform with which to precisely study the ion transport pathways and ionocytes targeted by a given hormone. Moreover, the ready identification of receptor isoforms in zebrafish and the suitability for gene overexpression and knockdown provide powerful approaches to alter homologous/endogenous levels

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of hormone or receptor isoforms. It is now feasible to look at the actions of a hormone on an ion transport pathway through specific receptors at different molecular levels (transcriptional, translational, or posttranslational) or at the different developmental stages of ionocytes (proliferation or differentiation). Although there are several comprehensive and comparative reviews on the hormonal control of fish iono- and osmoregulation (Evans et al., 2005; Guh et al., 2015; McCormick and Bradshaw, 2006; Takei et al., 2014), efforts have been made continuously in addressing this issue. Several new findings, including the regulation of ionocyte differentiation, acid secretion and Cl^- uptake by hormones, have been reported recently. The present review focuses on recent progress in the related studies on zebrafish with emphasis on the underlying cellular and molecular mechanism to provide an updated overview of endocrine control of ion regulation in zebrafish.

2. Ion regulation in zebrafish

Zebrafish, a stenohaline freshwater (FW) teleost, can survive well in extremely soft ($[Na^+] = 0.03–0.1$ mM, $[Cl^-] = 0.03–0.04$ mM, $[Ca^{2+}] = 0.02–0.05$ mM), acidic (pH 4), or alkaline (pH 10) FW, as well as in water containing high ammonia (5 mM NH_4^+), similar to many other teleost species that exhibit marked habitat diversity (Hwang and Perry, 2010). Zebrafish, much like other FW teleost species, have to actively absorb ions (mainly, Na^+, Cl^- and Ca^{2+}), excrete waste ammonia, and secrete excess acid (or alkaline) equivalents, to achieve body fluid ionic and acid-base homeostasis (Hwang, 2009; Hwang and Chou, 2013; Hwang and Perry, 2010).

2.1. Ion transport and ionocytes

Various ionocyte subtypes have been identified and proposed to be associated with the transport of different ions in several FW species (Dymowska et al., 2012; Hwang et al., 2011; Hwang and Lin, 2014). Functional analyses of various ionocyte subtypes in zebrafish were performed by multiple labelling of the transporters and certain convincing molecular physiological approaches (e.g. loss- or gain-of-function of genes, ion fluxes, or electrophysiology). In zebrafish, there are at least 5 subtypes of ionocytes in zebrafish gills and skin: H^+-ATPase-rich (HR), Na^+-K^+-ATPase-rich (NaR), Na^+-Cl^- cotransporter (NCC), solute carrier 26-expressing (SLC26), and K^+ secreting (KS)-cells (Fig. 1). In HR cells, (i) apical H^+-ATPase (HA), (ii) Na^+/H^+ exchanger 3b (NHE3b) and ammonia transporter (Rhcg1), and (iii) basolateral anion exchanger 1b (AE1b) and Na^+-K^+-ATPase (atp1a1a.5), combined with membrane and cytosolic forms of carbonate anhydrases (CA15a and CA2-like a, respectively), mediate Na^+ uptake, H^+ secretion (i.e., HCO_3^- uptake), and ammonia excretion, respectively (Horng et al., 2007; Lee et al., 2011; Liao et al., 2009; Lin et al., 2006, 2008; Shih et al., 2008, 2013, 2012; Yan et al., 2007). NaR cells employ apical epithelial Ca^{2+} channel (ECaC) and basolateral plasma membrane Ca^{2+}-ATPase 2 (PMCA2), Na^+/Ca^{2+} exchanger 1b (NCX1b), and Na^+-K^+-ATPase (atp1a1a.1) for Ca^{2+} uptake (Liao et al., 2009; Pan et al., 2005). NCC cells express apical NCC2b (formerly, NCC like 2, SLC12A10.2), basolateral Cl^- channel 2c (CLC2c), Na^+-HCO_3^- cotransporter 1b (NBCe1b), and Na^+-K^+-ATPase (atp1a1a.2) to absorb Na^+ and Cl^- (Lee et al., 2011; Liao et al., 2009; Wang et al., 2009, 2015). In SLC26 cells, apical SLC26A3 (-4 and/or -) and basolateral Na^+-K^+-ATPase (atp1a1a.1) are co-expressed and responsible for HCO_3^- secretion and Cl^- uptake (Bayaa et al., 2009; Perry et al., 2009b). KS cells apically express K^+ channel (Kir1.1) and may excrete K^+ (Abbas et al., 2011). There are still some unresolved issues regarding transporter function and expression in the identified ionocytes. The functional role of NBCe1 (an HCO_3^- transporter) in acid base regulation is unclear. The identities and related pathways of the transporters other than SLC26s in SLC26 cells await further exploration. In KS cells, there is no information about any transporter other than Kir1.1, and the physiological significance of K^+ secretion in zebrafish (in which plasma K^+ concentration is higher than that of FW) is unknown.

2.2. Differentiation of ionocytes

Increasing the number of ionocytes (and thus the expression levels of ion transporters) is a strategy by which fish regulate ion transport in the gills to cope with environmental challenges (Hwang et al., 2011; Hwang and Lin, 2014; Takei and Hwang, in press). Therefore, it is an important issue to know how ionocytes proliferate, differentiate, and mature. The zebrafish is the most studied of all fish models in terms of the molecular pathways of

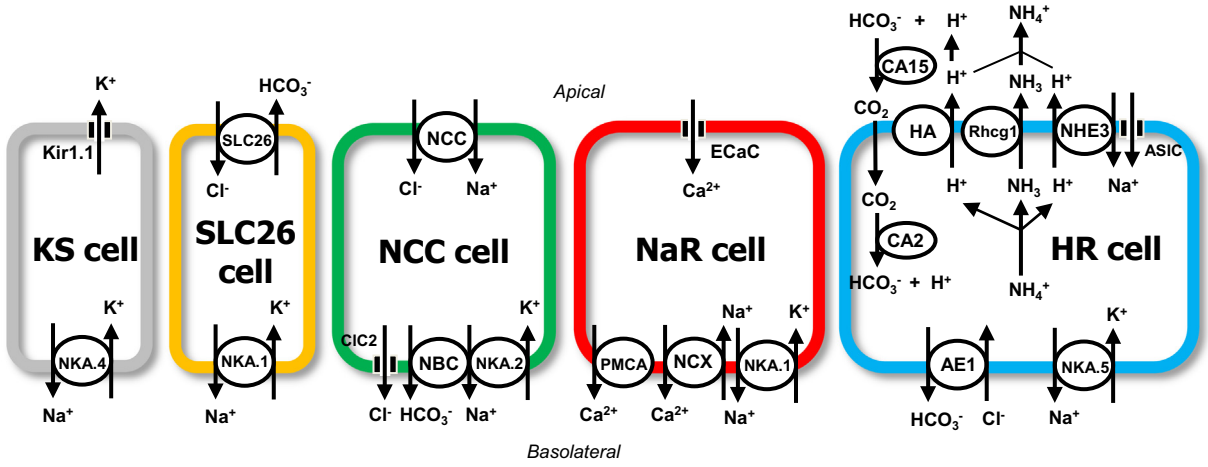

Fig. 1. Model of ionocytes and ion transport pathways in zebrafish gills and skin (modified from Guh et al. (2015)). Five types of ionocytes were proposed: H^+-ATPase-rich (HR), Na^+-K^+-ATPase-rich (NaR), Na^+-Cl^- cotransporter (NCC), solute carrier 26-expressing (SLC26), and K^+ secreting (KS)-cells. For details of the transport pathways, please refer to the text. AE1, anion exchanger 1b; ASIC, acid-sensing ion channels 2; CA, carbonic anhydrase 2-like a; CA2 (-15), carbonic anhydrase 2-like a (-15a); ClC2, Cl^- channel 2c; ECaC, epithelial Ca^{2+} channel; HA, H^+-ATPase; ROMK, an ortholog of the mammalian renal outer medullary K^+ channel (Kir1.1); NBC, electrogenic Na^+-HO_3^- cotransporter 1b; NCC, Na^+-Cl^- cotransporter 2b; NCX, Na^+/Ca^{2+} exchanger 1b; NHE3, Na^+/H^+ exchanger 3b; NAK.1 ~ 5, Na^+-K^+-ATPase α1 subunit subtypes (atp1a1a.1 ~ 5); PMCA, plasma membrane Ca^{2+}-ATPase 2; Rhcg1, Rhesus glycoprotein; SLC26, SLC26A3, -4 and -6.

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