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Regulation of gill claudin paralogs by salinity, cortisol and prolactin in Mozambique tilapia (*Oreochromis mossambicus*)

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ABSTRACT

In euryhaline teleosts, reorganization of gill tight junctions during salinity acclimation involves dynamic expression of specific claudin (Cldn) paralogs. We identified four transcripts encoding Cldn tight junction proteins in the tilapia gill transcriptome: *cldn10c*, *cldn10e*, *cldn28a* and *cldn30*. A tissue distribution experiment found *cldn10c* and *cldn10e* expression levels in the gill to be 100-fold higher than any other tissues examined. *cldn28a* and *cldn30* levels in the gill were 10-fold greater than levels in other tissues. Expression of these genes in Mozambique tilapia was examined during acclimation to fresh water (FW), seawater (SW), and in response to hormone treatments. Transfer of tilapia from FW to SW elevated *cldn10c* and *cldn10e*, while *cldn28a* and *cldn30* were stimulated following transfer from SW to FW. In hypophysectomized tilapia transferred to FW, pituitary extirpation induced reduced expression of *cldn10c*, *cldn10e* and *cldn28a*; these effects were mitigated equally by either prolactin or cortisol replacement. *In vitro* experiments with gill filaments showed that cortisol stimulated expression of all four *cldns* examined, suggesting a direct action of cortisol *in situ*. Our data indicate that elevated *cldn10c* and *cldn10e* expression is important during acclimation of tilapia to SW possibly by conferring ion specific paracellular permeability. On the other hand, expression of *cldn28a* and *cldn30* appears to contribute to reorganization of branchial epithelium during FW acclimation. Hormone treatment experiments showed that particular FW- and SW-induced *cldns* are controlled by cortisol and prolactin.

1. Introduction

Euryhaline teleosts like Mozambique tilapia (*Oreochromis mossambicus*) must regulate the permeability of their surface epithelia when they acclimate to changes in environmental osmolality and ion conditions. As the main interface with the surrounding water, the gill epithelium mediates not only essential iono- and acid/base-regulatory functions but is also the main site of external respiration in the adult fish (Evans et al., 2005). In turn, passive transepithelial fluxes of solutes and water are continuously counteracted to maintain internal plasma osmolality at about one-third that of full-strength seawater (SW) in both concentrated marine and dilute freshwater (FW) environments.

Tight junction strands regulate paracellular solute and water movement by making up the apical-lateral barriers, or gates, between epithelial cells (Hou et al., 2013). In the gill of fish acclimated to FW, tight junctions between adjacent pavement cells and ionocytes, and in between individual pavement cells, are deep with multi-stranded connections (Sardet et al., 1979). In SW, shallow tight junctions connect accessory cells with adjacent ionocytes permitting "leaky" paracellular pathways between these cells (Sardet et al., 1979; Cozzi et al., 2015). In SW-type ionocytes, NaCl secretion involves transcellular chloride transport *via* a basolateral Na^+,K^+,$2Cl^-$ cotransporter (Nkcc) and an apical cystic fibrosis transmembrane conductance regulator anion channel (Evans et al., 2005). Chloride transport is secondarily coupled to the activity of Na^+,K^+-ATPase (Nka) found in the extensive tubular network which extends from the basolateral membrane (Karnaky et al., 1976). The active Cl^- secretion is thought to generate a serosal positive transepithelial potential driving Na^+ extrusion *via* a paracellular pathway (Degnan and Zadunaisky, 1980; Evans et al., 2005). In contrast with hyposmoregulating marine fishes, FW teleosts are subject to passive ion losses and osmotic water gain. The gill epithelium does not readily exhibit complexes of ionocytes and accessory cells with shallow tight junctions as seen in marine teleosts, and is considered very "tight" with respect to electrical resistance (Marshall, 1977; Sardet et al., 1979; Wood et al., 2002; Zhou et al., 2003; Chasiotis et al., 2012). FW-type

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ionocytes actively absorb NaCl from the dilute environment *via* transcellular routes that exchange mucosal Na^+ and Cl^- with serosal H^+ and HCO_3^- (see Dymowska et al., 2012; Kwong and Perry, 2013) or by the action of an apical Na^+ and Cl^- cotransporter (Ncc; Hiroi and McCormick, 2012).

Transmembrane tight junction proteins include claudins (Cldns), occludin and tricellulin, all of which have four trans-membrane spanning domains, two extracellular loops, and a cytosolic carboxy-terminal linked to the cytoskeleton through adaptor molecules (Hou et al., 2013). Members of the Cldn superfamily, with 27 mammalian isoforms, appear to determine the permeability and ion selectivity of tight junctions (Furuse et al., 1998; Hou et al., 2013). Site-directed mutagenesis and overexpression studies have shown that paracellular permeability of specific ions is controlled by charged amino acids in the first extracellular loop (Colegio et al., 2003; Hou et al., 2013). Therefore, the expression of specific Cldn isoforms controls the overall permeability and selective barrier functions critical to a specific tissue. Accordingly, the expression levels of various Cldns often change during development in tissue- and cell-specific manners (Loh et al., 2004; Tipsmark et al., 2008a; Baltzegar et al., 2013; Madsen et al., 2015).

In Atlantic salmon (*Salmo salar*), spotted green puffer fish (*Tetraodon nigroviridis*) and Japanese medaka (*Oryzias latipes*), SW-acclimation entails the elevated expression of branchial *cldn10c*, *cldn10d* and *cldn10e* (Tipsmark et al., 2008a; Bui and Kelly, 2014; Bossus et al., 2015). Based on the amino acid sequences of their first extracellular loops, these paralogs could be involved in forming the cation-selective pathway associated with Na^+ extrusion (Günzel et al., 2009; Bossus et al., 2015). Mammalian Cldn4 shares a common ancestor with 13 teleost Cldns (Baltzegar et al., 2013), some of which (Cldn28a and Cldn30) have salinity-dependent expression in the gill of Atlantic salmon and Mozambique tilapia. In both species, *cldn28a* and *cldn30* levels are elevated in FW-acclimated animals (Tipsmark et al., 2008a, 2008b). Cldn30 is directly involved in the tightening of fish epithelia (Engelund et al., 2012; Kwong and Perry, 2013), while the sequence of Cldn28a suggests a similar capacity as its barrier-forming mammalian homologs (Hou et al., 2013; Bossus et al., 2015). Cortisol and prolactin directly decrease the permeability of primary gill cultures of trout epithelia (Kelly and Wood, 2002; Zhou et al., 2003). Accordingly, blood plasma levels of both hormones are elevated during the acute phase of FW acclimation in surveyed teleosts (McCormick, 2001), including tilapia (Kajimura et al., 2004). Furthermore, both cortisol (Atlantic salmon: Tipsmark et al., 2009; goldfish, puffer fish: Bui et al., 2010; Chasiotis and Kelly, 2011) and prolactin (Atlantic salmon: Tipsmark et al., 2009) upregulate expression of specific gill *cldn* paralogs suggesting close endocrine control of epithelial permeability.

In the present study, we first identified *cldn* transcripts in the gill transcriptome of the congeneric Nile tilapia (*Oreochromis niloticus*) in order to probe how tight junctions are regulated by environmental salinity and hormones in Mozambique tilapia. To date, only one previous study has addressed the molecular physiology of tight-junctions in a cichlid (Tipsmark et al., 2008b). Our approach was to examine changes in gene expression of identified *cldns* following transfer from FW to SW, and *vice versa*. Then, hypophysectomy and subsequent replacement therapy trials with prolactin (Prl), growth hormone (Gh) and/or cortisol were conducted to assess the contribution of endocrine signaling to the expression of identified *cldns*. Lastly, we tested the *in vitro* effects of cortisol on *cldn* expression in incubated gill filaments.

2. Materials and methods

2.1. Fish

For tissue distribution analyses, salinity-challenge experiments, and *in vivo* hormone replacement experiments, male Mozambique tilapia (*O. mossambicus*) were selected from a population maintained at the Hawai'i Institute of Marine Biology (HIMB; University of Hawai'i). Fish were maintained outdoors at 24–26 °C with a continuous flow of FW (municipal water: 0.98 mM Na^+, 0.25 mM Ca^{2+}, 0.01 mM Mg^{2+}, 0.04 mM K^+) under natural photoperiod and fed daily (Silver Cup Trout Chow, Nelson & Sons, Murray, UT). Some fish were acclimated to SW (Kāne'ohe Bay, HI; 34‰) for 4 weeks before the start of the FW-transfer experiment. The SW-transfer experiment was conducted with SW-naïve animals. The Institutional Animal Care and Use Committee of the University of Hawai'i approved all experimental protocols conducted at HIMB. For *in vitro* experiments, gill filaments were collected from tilapia maintained within the Department of Biological Sciences (University of Arkansas, Fayetteville). These fish were maintained indoors with recirculated FW at 22–24 °C (dechlorinated municipal water: 0.34 mM Na^+, 0.64 mM Ca^{2+}, 0.09 mM Mg^{2+}, 0.03 mM K^+) under artificial photoperiod (14 L:10D) and fed daily (Aquamax Starter Fingerling 300; PMI Nutrition International, Brentwood, MO). The Institutional Animal Care and Use Committee of the University of Arkansas approved all experiments at the University of Arkansas.

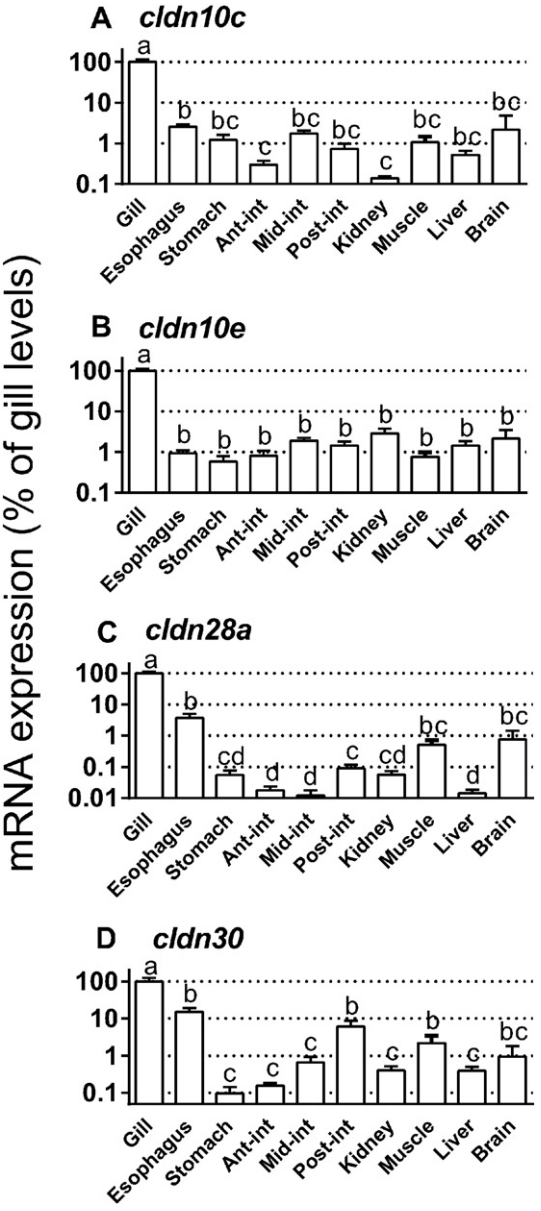

Fig. 1. Relative expression of *cldn10c* (A), *cldn10e* (B), *cldn28a* (C) and *cldn30* (D) mRNA in gill, esophagus, stomach, anterior intestine (Ant-int), middle intestine (Mid-int), posterior intestine (Post-int), kidney, skeletal muscle (Muscle), liver, and brain in FW-acclimated tilapia. Groups that do not share letters are significantly different ($P < 0.05$). Means + S.E.M. ($n = 4–5$) are presented as a percent of gill expression levels.