General and Comparative Endocrinology 203 (2014) 21–28

Contents lists available at ScienceDirect

General and Comparative Endocrinology

journal homepage: www.elsevier.com/locate/ygcen

Review

Prolactin and teleost ionocytes: New insights into cellular and molecular targets of prolactin in vertebrate epithelia

Jason P. Breves [a,*], Stephen D. McCormick [a,b], Rolf O. Karlstrom [a]

[a] Department of Biology & Center for Neuroendocrine Studies, University of Massachusetts, Amherst, MA 01003, USA
[b] USGS, Conte Anadromous Fish Research Center, Turners Falls, MA 01376, USA

ARTICLE INFO

Article history:
Available online 13 January 2014

Keywords:
Osmoregulation
Gill
Differentiation
Pituitary
NCC
Zebrafish

ABSTRACT

The peptide hormone prolactin is a functionally versatile hormone produced by the vertebrate pituitary. Comparative studies over the last six decades have revealed that a conserved function for prolactin across vertebrates is the regulation of ion and water transport in a variety of tissues including those responsible for whole-organism ion homeostasis. In teleost fishes, prolactin was identified as the "freshwater-adapting hormone", promoting ion-conserving and water-secreting processes by acting on the gill, kidney, gut and urinary bladder. In mammals, prolactin is known to regulate renal, intestinal, mammary and amniotic epithelia, with dysfunction linked to hypogonadism, infertility, and metabolic disorders. Until recently, our understanding of the cellular mechanisms of prolactin action in fishes has been hampered by a paucity of molecular tools to define and study ionocytes, specialized cells that control active ion transport across branchial and epidermal epithelia. Here we review work in teleost models indicating that prolactin regulates ion balance through action on ion transporters, tight-junction proteins, and water channels in ionocytes, and discuss recent advances in our understanding of ionocyte function in the genetically and embryonically accessible zebrafish (*Danio rerio*). Given the high degree of evolutionary conservation in endocrine and osmoregulatory systems, these studies in teleost models are contributing novel mechanistic insight into how prolactin participates in the development, function, and dysfunction of osmoregulatory systems across the vertebrate lineage.

1. Introduction

The pituitary gland has long been viewed as a central player in the homeostatic regulation of salt and water balance in vertebrates (McCormick and Bradshaw, 2006). The pituitary hormone prolactin is secreted from lactotrophs of the rostral pars distalis and plays a role in ion and water transport in many tissues throughout the vertebrate lineage. In 1959, prolactin was identified as a "freshwater-adapting hormone" in the teleost fish, *Fundulus heteroclitus* (Pickford and Phillips, 1959). Prolactin was later shown in a series of euryhaline species to promote ion conserving and water secreting processes of the whole animal by acting on the gill, kidney, gut and urinary bladder (Hirano, 1986). Subsequently, prolactin was shown to influence solute and water transport across renal, intestinal, mammary and amniotic epithelial membranes in mammals (Bole-Feysot et al., 1998; Freeman et al., 2000). Despite decades of focused and sustained research on prolactin action in both fishes and mammals, a detailed picture of the mechanisms underlying prolactin action has remained largely undeveloped due to limitations in our understanding of ion and water transport across osmoregulatory epithelia. Recent work has begun to identify the molecular mechanisms of epithelial ion transport, opening the door to a new understanding of the mechanisms by which prolactin regulates a variety of cellular responses in target tissues, including cell proliferation, differentiation, and gene expression. Insight into the mechanisms of prolactin action is key to an understanding of diseases linked with prolactin dysfunction such as breast cancer, diabetes, infertility, and atherosclerosis (Neville et al., 2002; McHale et al., 2008; Georgiopoulus et al., 2009; Bernichtein et al., 2010; Balbach et al., 2013). Prolactin is in fact a candidate therapeutic factor for diseases such as diabetic retinopathy (Arnold et al., 2010) and prolactin may be critical for neurogenic events needed for effective stem-cell related therapies (Walker et al., 2012).

The first insights into prolactin function in teleosts came in the middle of the last century when early studies identified prolactin as a key osmoregulatory hormone. Pituitary removal (hypophysectomy) was used to show that pituitary function was essential for survival of euryhaline species in freshwater environments. In a landmark study, Pickford and Phillips (1959) demonstrated that prolactin replacement therapy promoted survival of hypophysectomized killifish (*F. heteroclitus*) in fresh water. Ball and Ensor (1965) then showed in sailfin molly (*Poecilia latipinna*) that prolactin

* Corresponding author. Fax: +1 413 545 3243.
E-mail address: breves@bio.umass.edu (J.P. Breves).

0016-6480/$ - see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.ygcen.2013.12.014

supports freshwater survival by preventing the fall in plasma electrolytes that occurs following hypophysectomy. Subsequently it was established that prolactin mediates freshwater acclimation by acting on osmoregulatory tissues (e.g., gill, kidney, intestine, and urinary bladder) to regulate ion-conserving and water-secreting processes (Hirano, 1986). *Prolactin* gene expression and/or plasma prolactin levels rise in response to reductions in environmental salinity (Yada et al., 1994; Shepherd et al., 1999; Lee et al., 2006; Liu et al., 2006; Hoshijima and Hirose, 2007; Fuentes et al., 2010); in some cases these responses are driven by direct sensing of extracellular osmolality by lactotrophs (Sage, 1968; Ingleton et al., 1973; Kwong et al., 2009; Seale et al., 2012). Based on the broad range of tissues known to respond to prolactin across teleosts, it is widely believed that prolactin is a conserved regulator of physiological responses to low salinity environments (reviewed by Loretz and Bern, 1982; Bern, 1983; Hirano, 1986; Manzon, 2002; Sakamoto and McCormick, 2006).

Teleost models have been useful in uncovering the osmoregulatory functions of prolactin in part because teleosts have evolved remarkable capacities for Na^+ and Cl^- transport through the activities of specialized 'ionocytes' (also termed 'chloride cells' and 'mitochondrion-rich cells') of the branchial epithelia and epidermis. Ionocytes play an essential role in maintaining systemic salt and water balance, and in this regard, are functionally analogous to ion and water transporting cells of tetrapod renal tubules (Evans et al., 2005; Chang and Hwang, 2011). In this review we describe how recent advances in our understanding of teleost ionocyte function at the sub-cellular level have paved a path to characterizing the osmoregulatory actions of prolactin in a more mechanistic fashion than was previously possible. We discuss how research in three key areas is needed to reveal the tissue-level actions of prolactin in osmoregulation: (a) the expression patterns and signaling characteristics of prolactin receptors, (b) the transcriptional targets of prolactin signaling within ionocytes, and (c) the mechanisms by which prolactin regulates ionocyte and/or ionocyte precursor populations. Given the conserved characteristics of endocrine systems and ion transporting epithelia across vertebrates, these studies of prolactin function in teleosts promise to contribute insight into how prolactin participates in the development, function, and disease of osmoregulatory systems across the vertebrate lineage.

2. The initiation of cellular responses: signaling via prolactin receptors

The first prolactin receptor was cloned in rat by Boutin et al. (1988), and the first teleost prolactin receptor was cloned in Nile tilapia (*Oreochromis niloticus*) (Sandra et al., 1995). Ensuing molecular comparisons between teleost and mammalian prolactin receptors revealed the presence of highly conserved functional domains including an extracellular ligand-binding domain, a single-pass transmembrane region, and a Box 1 region (Prunet and Auperin, 1994; Bole-Feysot et al., 1998; Prunet et al., 2000; Huang et al., 2007; Pierce et al., 2007; Fiol et al., 2009). Mammals possess a single prolactin receptor gene with long and short splice variants (Bole-Feysot et al., 1998; Freeman et al., 2000) while teleosts possess multiple prolactin receptor gene loci. Ligand binding leads to dimerization and cross phosphorylation events that activate JAK/STAT signaling within the cytoplasm, with JAK2 and STAT5 identified as the key mediators of prolactin signal transduction (Han et al., 1997; Bole-Feysot et al., 1998; Freeman et al., 2000). Phosphorylated STAT proteins translocate to the nucleus and bind target regulatory DNA elements, thereby regulating the transcription of prolactin responsive genes. In addition to JAK/STAT signaling, MAPK, PI3K, and Src kinase pathways are also potentially activated in prolactin-responding cells (Bole-Feysot et al., 1998; Freeman et al., 2000), complicating analyses of the cellular responses to prolactin. Due to the involvement of these signaling cascades in an array of cell signaling pathways there is currently no specific transcriptional or cellular reporter for prolactin signaling. In the absence of specific gene read-outs for prolactin signaling, labeling with anti-phosphorylated STAT5 antibodies has been widely used as an indicator of active prolactin signaling within responding cells (Furth et al., 2011).

An obvious but important first step in identifying potential target tissues for prolactin action has been to determine which tissues express prolactin receptors. In one survey of prolactin receptor expression across vertebrate tissue types, key osmoregulatory epithelia emerged as hot spots of expression (Bole-Feysot et al., 1998). In teleosts, prolactin binding was first characterized in gill, kidney, intestine, liver and gonad preparations of Mozambique tilapia (*Oreochromis mossambicus*) (Fryer, 1979; Edery et al., 1984; Dauder et al., 1990; Prunet and Auperin, 1994). Consistent with prolactin directing ionoregulatory processes, *prolactin receptor* transcripts are expressed in the gill of all teleosts examined to date (Sandra et al., 1995; Prunet et al., 2000; Tse et al., 2000; Higashimoto et al., 2001; Santos et al., 2001; Lee et al., 2006; Huang et al., 2007; Pierce et al., 2007; Fiol et al., 2009; Breves et al., 2013). In tilapia and sea bream (*Sparus aurata*), prolactin receptors have been further localized to ionocytes of the gill (Weng et al., 1997; Santos et al., 2001).

A key advance in our understanding of prolactin signaling in teleosts came with a description of two separate *prolactin receptor* genes in black porgy (*Acanthopagrus schlegeli*) (Huang et al., 2007). Fiol et al. (2009) and Chen et al. (2011) subsequently established the existence of two distinct *prolactin receptor* genes in tilapia and zebrafish (*Danio rerio*), respectively. Expression of the two *prolactin receptors* in the gill is highly plastic and differentially impacted by both osmoregulatory challenges and hormone treatments (Huang et al., 2007; Pierce et al., 2007; Fiol et al., 2009; Tomy et al., 2009; Breves et al., 2010b; Rhee et al., 2010; Breves et al., 2011; Flores and Shrimpton, 2012; Breves et al., 2013; Jeong et al., 2013). For example, *in vivo* levels of branchial *prolactin receptor a*, but not *prolactin receptor b*, are stimulated by transfer to ion-poor water and prolactin injection in zebrafish (Breves et al., 2013). Dynamic *prolactin receptor* expression may provide a mechanism to modulate target-tissue sensitivity to circulating hormone. Because the two prolactin receptors initiate the activation of distinct target genes upon ligand binding, at least *in vitro* (Huang et al., 2007; Fiol et al., 2009; Chen et al., 2011), the two receptors likely mediate both distinct and overlapping physiological responses to circulating prolactin. An important next step is to define the *in vivo* transcriptional targets of the two prolactin receptors during physiological challenges such as changes in environmental salinity. Morpholino based gene-silencing approaches have been successful in resolving the functions of hormone receptor gene families in the zebrafish model system (Lin et al., 2011; Kumai et al., 2012; Griffin et al., 2013) and can improve our understanding of the functional consequences of growth hormone/prolactin-family receptor gene duplications that occurred during teleost evolution (Fukada et al., 2005; Fukamachi and Meyer, 2007).

3. Does prolactin control the expression of ionoregulatory genes in the gill?

In freshwater fishes, ionocytes of the gill and epidermis are the site of active ion uptake that counteract diffusive losses to the external environment (see reviews by Evans et al. (2005), Kaneko et al. (2008), Hwang and Lee (2007) and Dymowska et al. (2012)). To date, several models have been proposed to explain how ionocytes facilitate ion uptake against strong electrochemical

gradients (reviewed by Evans (2011)) and uncertainty regarding the cellular mechanisms of ion uptake has impeded progress towards understanding the specific actions of prolactin. Nonetheless, exogenous prolactin has been shown to stimulate ion uptake by cultured branchial epithelia (Zhou et al., 2003), suggesting that genes regulating freshwater-type ionocyte function might be good candidates as targets of prolactin. The recent discovery of specific genes/proteins involved in ion uptake by ionocytes (Table 1) suggests that teleost ionocytes can provide a tractable model for understanding how prolactin regulates ion transport at the molecular level in other osmoregulatory epithelia such as renal tubules, mammary glands, and the gastrointestinal tract.

3.1. Na^+/Cl^- cotransporter (NCC)

Employing Mozambique tilapia as a model, Hiroi et al. (2008) were the first to localize an electroneutral Na^+/Cl^- cotransporter (NCC) to the apical membrane of teleost ionocytes. Convincing biochemical, morphological and pharmacological evidence indicates that NCC-expressing ionocytes (NCC-cells) are key effectors of Cl^- uptake (Hiroi et al., 2008; Inokuchi et al., 2008; Horng et al., 2009; Wang et al., 2009). Branchial *ncc* gene and protein expression are induced by exposure to low Cl^- conditions (Hiroi et al., 2008; Inokuchi et al., 2008; Wang et al., 2009), pointing to the essential role of NCC-cells in maintaining Cl^- homeostasis. Employing a classic endocrine paradigm modified for use in tilapia (Nishioka, 1994), Breves et al. (2010b) found that hypophysectomy blocked the increase in *ncc* expression that accompanies freshwater acclimation in euryhaline tilapia and severely reduced the number of NCC-cells on gill filaments. Replacement therapy with ovine prolactin restored both *ncc* expression and NCC-cell numbers in hypophysectomized animals, suggesting that prolactin may affect *ncc* expression by promoting the differentiation of NCC-cells from a currently unknown stem/progenitor cell population (see Section 4). The establishment of a link between prolactin and NCC in tilapia ionocytes provided the first evidence that prolactin directly regulates an ion-uptake pathway in target tissue.

The zebrafish has recently emerged as a powerful genetic and experimental system to study the hormonal control of ion uptake in teleosts (Tseng et al., 2009; Chou et al., 2011; Lin et al., 2011; Kumai et al., 2012). Three distinct ionocyte sub-types have now been characterized based on the expression of specific integral membrane ion transporters/exchangers. In addition to NCC-cells that mediate Cl^- uptake, HR-cells (H^+-ATPase-rich) and NaR-cells (Na^+/K^+-ATPase-rich) function in the uptake of Na^+ and Ca^{2+}, respectively (Pan et al., 2005; Esaki et al., 2007; Liao et al., 2007; Wang et al., 2009). HR-cells specifically express a Na^+/H^+ exchanger (NHE3b) while NaR-cells express an epithelial Ca^{2+} channel (ECaC) (see Fig. 1). Characterization of these functionally distinct zebrafish ionocyte sub-types now allows a detailed analysis of how prolactin mediates ionocyte lineages, and thus ion uptake capacities, during embryonic development.

Our lab took advantage of these ionocyte markers to examine the specificity by which prolactin influences the genes affecting ion uptake. We showed that prolactin positively regulates *ncc* expression in the zebrafish gill both *in vivo* and in culture (Breves et al., 2013), suggesting direct action of prolactin on ionocytes or ionocyte precursors as in tilapia (Breves et al., 2010b). The use of a specific prolactin antagonist generated by modifying the human prolactin peptide (Δ1–9-G129R-hPRL; Bernichtein et al., 2003) confirmed that prolactin acts gill-autonomously through transmembrane receptors to affect *ncc* expression. In light of the demonstrated actions of prolactin on internal Na^+, Cl^- and Ca^{2+} levels in other teleosts (Hirano, 1986), we were surprised to find that the actions of prolactin were remarkably specific, with no apparent effects on *nhe3b* or *ecac* expression in the zebrafish gill (Breves et al., 2013). Collectively, these studies suggest that prolactin targets NCC-dependent ion-uptake pathways in a subset of both euryhaline and stenohaline species. Hiroi and McCormick (2012) recently described the prevalence of NCC-expressing ionocytes across teleost groups and suggest NCC ion-uptake pathways operate in specific clades of *Ostariophysi* and *Acanthopterygii*. A more nuanced understanding of this seemingly conserved prolactin-NCC link will emerge from probing whether prolactin is linked with NCC in representatives of these taxa.

In addition to NCC-mediated Cl^- uptake, ionocytes in zebrafish may also regulate Cl^-/HCO_3 exchange via members of the SLC26 family of anion exchangers. Three distinct genes have been isolated in zebrafish (*slc26a3*, *slc26a4* and *slc26a6c*). The *slc26a4* and *slc26a6c* isoforms appear to assume greater roles in Cl^- uptake when environmental Cl^- is severely depleted (Bayaa et al., 2009; Perry et al., 2009). While these exchangers are localized to ionocytes (Perry et al., 2009), it remains to be determined whether *slc26*-expressing ionocytes are distinct from NCC-, HR- or NaR-cells. Cl^-/HCO_3 exchangers are proposed to mediate prolactin-dependent HCO_3^- transport across mammalian endometrial epithelium (Deachapunya et al., 2008), suggesting prolactin regulates the expression of *slc26* genes or the kinetics of Cl^-/HCO_3 exchange. Teleost ionocytes now provide a new model for understanding prolactin regulation of HCO_3^- transport, which appears to play a key role in supporting implantation and embryonic development in mammals (Deachapunya et al., 2008).

3.2. Na^+/K^+-ATPase α1 subunits

The Na^+/K^+-ATPase (NKA) enzyme is a ubiquitously expressed ion pump consisting of three subunits (α, ß, and γ) that is responsible for the maintenance of the Na^+ and K^+ gradients across all cell membranes. In the basolateral membrane of freshwater- and seawater-type ionocytes, the NKA enzyme plays a critical role in energizing branchial ion transport (McCormick, 1995). The catalytic α-subunit of the NKA enzyme possesses binding sites for ATP, Na^+, and K^+ (Geering, 2008). Reciprocal expression of NKA-α1-subunit genes (*nka-α1a* and *nka-α1b*) was first described in salmonids that were transitioning between freshwater and seawater environments (Richards et al., 2003; Mackie et al., 2005; Bystriansky et al., 2006; Madsen et al., 2009; McCormick et al., 2009), a transition that is highly dependent on endocrine signaling. More recently, Tipsmark et al. (2011) showed that Mozambique tilapia also undergo *nka-α1a* and *nka-α1b* switching upon salinity changes, the first observation of this gene expression pattern in a

Table 1
Recently identified prolactin target genes associated with freshwater-type ionocyte function.

Gene	Function	Species	References
aqp3	Water/small solute channel	*O. mossambicus*	Breves et al. (unpublished)
claudin-28a	Tight-junction protein	*S. salar*	Tipsmark et al. (2009)
fxyd-11	Na^+/K^+-ATPase regulatory subunit	*O. mossambicus*	Tipsmark et al. (2011)
ncc	Na^+/Cl^- cotransport	*D. rerio*	Breves et al. (2013)
		O. mossambicus	Breves et al. (2010)
		O. niloticus	Breves et al. (unpublished)
nka-α1a	Na^+/K^+-ATPase catalytic subunit	*O. mossambicus*	Tipsmark et al. (2011)
		O. niloticus	Breves et al. (unpublished)
prolactin receptor a	Prolactin receptor isoform	*D. rerio*	Breves et al. (2013)

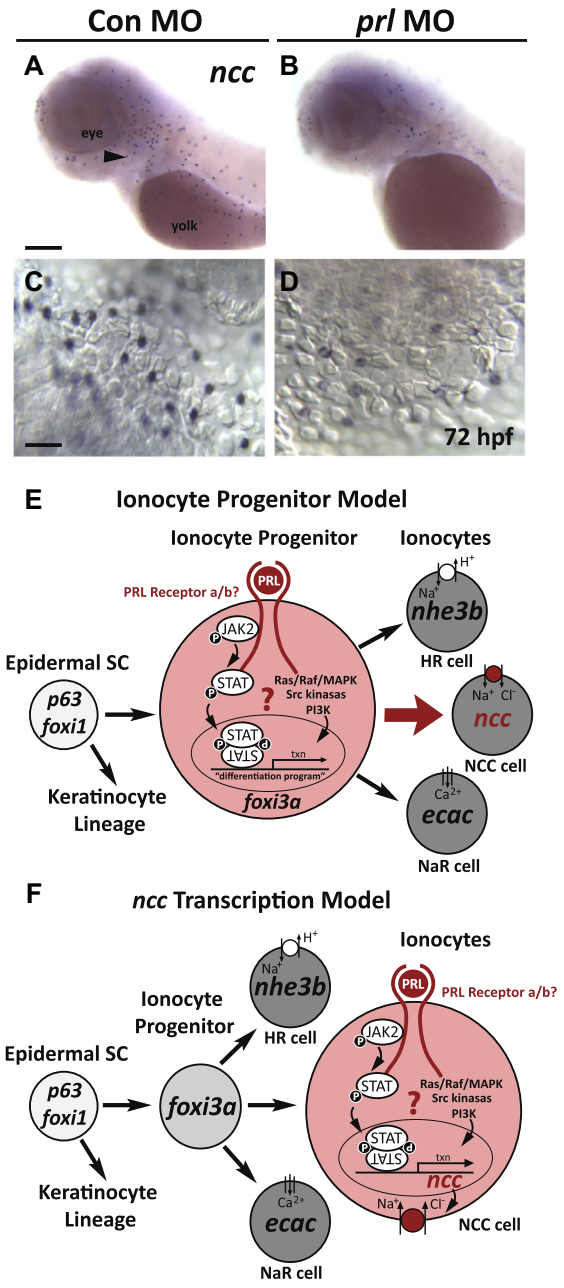

Fig. 1. Models for Prolactin (PRL) Regulation of Ionocytes. (A–D) 72 h post fertilization (hpf) zebrafish embryos labeled to reveal *ncc*-expressing ionocytes in the epidermis. (A and C) In control embryos, approximately 300 ionocytes in the epidermis express *ncc* by 72 hpf. Ionocytes are concentrated over the yolk sac and epidermis of the head, including the developing branchial arches (arrow head). Scale bar = 200 μm. Panel C shows a higher magnification view of *ncc*-expressing ionocytes dispersed in the skin posterior to the eye. Scale bar = 40 μm. (B and D) Morpholino (MO) knockdown of *prl* function results in a diminished number of *ncc*-expressing ionocytes in the epidermis, while *ecac*- and *nhe3b*-expressing ionocytes are unaffected (data not shown). Panel D shows a higher magnification view and the approximately 70% reduction in *ncc*-expressing cells. Ionocyte Progenitor Model for Prolactin Action. (E) In zebrafish embryos, epidermal stem cells (SCs) expressing *p63* and *foxi1* give rise to skin keratinocytes and *foxi3a*-expressing ionocyte progenitors. These progenitors subsequently differentiate into ionocytes (NaR-cells, HR-cells or NCC-cells). Prolactin may act on ionocyte progenitors to drive differentiation of *ncc*-expressing ionocytes (NCC-cells), or to increase proliferation of NCC precursor cells. In this diagram, signaling initiated by the binding of prolactin to one of the two prolactin receptors activates JAK/STAT, Ras/Raf/MAPK, Src kinases, or PI3K second messengers, activating genes that drive NCC-cell differentiation. *ncc* Transcription Model for Prolactin Action. (F) Alternatively (or in addition), prolactin could act directly and specifically on differentiated NCC-cells to modulate expression from the *ncc* locus. Ionocyte lineage diagram adapted from Hwang and Chou (2013).

non-salmonid. Hypophysectomy and hormone replacement studies showed that prolactin stimulates *nka-α1a* expression in Mozambique tilapia (Tipsmark et al., 2011), while Nile tilapia (*O. niloticus*) also exhibited prolactin-dependent *nka-α1a* expression in fresh water (Breves et al., unpublished). This action of prolactin on *nka-α1* expression appears to be specific to tilapia, as prolactin, alone or in combination with cortisol, failed to stimulate the "freshwater inducible" *nka-α1a* isoform in Atlantic salmon (*Salmo salar*) (Tipsmark and Madsen, 2009). Similarly, prolactin had no effect on NKA activity in brown trout (*Salmo trutta*) (Madsen et al., 1995). In zebrafish, three NKA-α1-subunit paralogues, *nka-α1a1a.5*, *nka-α1a1a.2*, *nka-α1a1a.1*, are specifically expressed in ionocyte subtypes that are responsible for Na^+, Cl^- and Ca^{2+} uptake, respectively (Liao et al., 2009), allowing a more detailed analysis of prolactin action on *nka-α1a* expression and ionocyte function.

The γ-subunit of the NKA enzyme, or FXYD, participates in the regulation of enzymatic activity by associating with the Na^+/K^+ pump complex (Geering, 2008; Pavlovic et al., 2013). In Mozambique tilapia, Tipsmark et al. (2011) showed synergy between prolactin and cortisol to promote *fxyd-11* gene expression in hypophysectomized animals transferred to fresh water. However, *fxyd-11* levels in Atlantic salmon ionocytes were not responsive to prolactin treatment (Tipsmark et al., 2010a). The disparate sensitivity of *fxyd-11* to prolactin may reflect fundamental differences between tilapia and salmonids regarding the necessity of pituitary hormones for freshwater acclimation. For example, pituitary hormones are essential for freshwater survival of Mozambique tilapia (Dharmamba and Maetz, 1972), while coho salmon (*Oncorhynchus kisutch*) and rainbow trout (*Salmo gairdneri*) can maintain osmotic balance in fresh water following hypophysectomy (Björnsson and Hansson, 1983; Björnsson et al., 1987). The functions of FXYD proteins in teleosts are becoming better resolved (Tipsmark, 2008; Wang et al., 2008; Saito et al., 2010; Yang et al., 2013), and based on how NKA enzyme activity is regulated during salinity challenges (McCormick, 1995), it is likely that prolactin will emerge as a key modulator of NKA kinetics upon freshwater transfer via FXYD-11. Links between prolactin and FXYD proteins clearly warrant further comparative study in euryhaline models such as spotted green pufferfish (*Tetraodon nigroviridis*) and medaka (*Oryzias dancena* and *O. latipes*) (Wang et al., 2008; Yang et al., 2013).

3.3. Tight-junction and aquaporin proteins

For fish to survive in freshwater environments, both ion loss and water gain across the large surface area of the branchial epithelium must be minimized. While the majority of experimental evidence supporting a role for prolactin as a freshwater-adapting hormone relates to effects on ion exchange, there is also evidence for prolactin control of osmotic permeability (reviewed by Hirano (1986); Brown and Brown (1987); Manzon et al., 2002). This is an area of keen interest for health-related research, as prolactin can also act on epithelial membrane permeability in the mammalian mammary gland (Linzell et al., 1975; Stelwagen et al., 1999), blood–brain barrier (Rosas-Hernandez et al., 2013), and amnion (Raabe and McCoshen, 1986).

Paracellular solute movements across epithelia are governed in large part by the barrier properties of tight junction complexes composed of occludin and claudin family proteins (Chasiotis and Kelly, 2008; Tipsmark et al., 2008a,b). In the teleost gill, changes in salinity and pH lead to a general "tightening" of the branchial epithelium. In tilapia, freshwater exposure leads to up-regulation of *claudin-28a* transcription (Tipsmark et al., 2008a), and *claudin-28a* expression in the gill of Atlantic salmon has now been linked with prolactin (Tipsmark et al., 2009), establishing a possible role for prolactin in regulating tight junction properties. Surprisingly,

claudin-28a was not induced during freshwater acclimation of salmon (Tipsmark et al., 2009). *Occludin* expression is strongly regulated by salinity and pH (Chasiotis et al., 2009; Kumai et al., 2011; Whitehead et al., 2011), making it a likely player in branchial epithelial tightening and a good candidate for regulation by prolactin, although this has yet to be examined. Rosas-Hernandez et al. (2013) recently showed that prolactin stimulates the expression of both *claudin-5* and *occludin* (in parallel with decreasing epithelial permeability) in a bovine *in vitro* model of the blood–brain barrier. With the large suite of occludin and claudin genes/proteins that have now been identified in both euryhaline and stenohaline teleosts (reviewed by Chasiotis et al. (2012)), these models are poised to contribute to a better understanding of the regulation of vertebrate tight junction proteins by prolactin and other systemic signals.

Aquaporins (AQPs) constitute a superfamily of integral membrane proteins that facilitate passive movements of water and small non-ionic compounds across cell membranes (Cerdà and Finn, 2010). Teleosts are equipped with an especially large suite of AQPs that are expressed in a wide array of tissues (see reviews by Cutler et al. (2007a) and Tingaud-Sequeira et al. (2010)). A subset of *aqps* are expressed in the gill, and expression is affected by salinity and/or pH (Hirata et al., 2003; Tse et al., 2006; Tingaud-Sequeira et al., 2010; Tipsmark et al., 2010b). For example, in European eel (*Anguilla anguilla*), Japanese eel (*A. japonica*) and Mozambique tilapia, *aqp3* expression is markedly reduced following transfer from fresh water to seawater (Cutler and Cramb, 2002; Lignot et al., 2002; Watanabe et al., 2005; Breves et al., 2010a). Prolactin is known to decrease the osmotic permeability of the gill (reviewed by Brown and Brown (1987)), an action that could be mediated by controlling *aqp3* expression or function. One study reported endocrine (cortisol) regulation of *aqp3* expression in the gill of the European eel (Cutler et al., 2007b) and our unpublished results suggest that *aqp3* expression is greatly diminished in freshwater tilapia following hypophysectomy, with expression subsequently restored by prolactin replacement. Future work is now required to determine whether and how prolactin acts to regulate gill permeability via AQP3. In addition to contributing to branchial permeability, Watanabe et al. (2005) proposed that AQP3 mediates autonomous osmosensing capacities of ionocytes; therefore, prolactin may also fine-tune aspects of environmental sensing.

4. Working models for prolactin action on ionocyte populations

Many actions of prolactin across vertebrates have been ascribed to the promotion of cell proliferation and/or differentiation events (Bole-Feysot et al., 1998; Freeman et al., 2000; Sakamoto and McCormick, 2006). For example, one of the best-studied actions of prolactin is the initiation of mammary gland development through the differentiation and proliferation of alveolar epithelia (Henninghausen and Robinson, 2001). Based on the observation that changes in prolactin affect the number of cells expressing different osmoregulatory proteins and/or genes (Herndon et al., 1991; Pisam et al., 1993; Kelly et al., 1999; Breves et al., 2010b), it has been hypothesized that prolactin directly stimulates freshwater-type ionocyte differentiation from a progenitor/stem cell pool (Fig. 1E). It is also possible that prolactin may influence ionocyte numbers by regulating the proliferation of already differentiated yet proliferative ionocytes and/or by pushing the transdifferentiation of seawater-type ionocytes into freshwater-type ionocytes (McCormick, 2001; Hiroi and McCormick, 2012). Finally, a model in which prolactin directly regulates transcription of specific ionoregulatory genes within differentiated ionocytes is also compatible with the current data (Fig. 1F).

The recent characterization of epidermal stem cells and the ionocyte lineage (Pellegrini et al., 2001; Reis-Filho and Schmitt, 2002; Hwang and Chou, 2013), in combination with the identification of the prolactin-responsive osmoregulatory genes described above (*ncc*, *nka-α1a*, *fxyd-11*, *claudin-28a*, *aqp3*), now makes it possible to distinguish between these models in the developing zebrafish embryo. Ionocytes appear in the zebrafish embryonic epidermis (Fig. 1A) early in development over the yolk sac (Chang and Hwang, 2011) and become concentrated in the branchial arches as the gills begin to form (Wang et al., 2009). Epidermal stem cells express the proliferation marker p63 and give rise to both keratinocytes and *foxi3a*-expressing ionocyte progenitor cells, the latter first appearing around 12 h post fertilization (hpf) (Hsiao et al., 2007; Jänicke et al., 2007; Esaki et al., 2009; Hwang and Chou, 2013). These ionocyte progenitor cells then give rise to the three functionally mature ionocytes discussed above (NaR-cells, HR-cells or NCC-cells) (Chang and Hwang, 2011; Chang et al., 2013). As in the gill, NaR-cells specifically express *ecac*, NCC-cells express *ncc*, while HR-cells express *nhe3b* (Pan et al., 2005; Yan et al., 2007; Hwang, 2009; Lin et al., 2011).

Embryonic *prolactin* gene expression is modulated in response to osmoregulatory challenges, suggesting prolactin could play a role in coordinating adaptive responses to challenges to homeostasis even during early embryonic stages (Liu et al., 2006; Hoshijima and Hirose, 2007). Prolactin signaling may also be involved with the first appearance of ionocytes in the epidermis independent of changes in external ion concentrations. In a preliminary set of experiments to test whether prolactin levels could affect formation of embryonic ionocytes, we injected 2-cell embryos with a translation-blocking *prolactin* morpholino (Zhu et al., 2007) and assayed *ncc*, *nhe3b* and *ecac* gene expression at 72 hpf. Loss of prolactin signaling led to a clear reduction in the number of *ncc*-expressing cells in the epidermis (Fig. 1 A–D), suggesting prolactin is required for the normal complement of NCC-cells. Prolactin expression begins at approximately 18 hpf as the pituitary gland forms at the anterior margin of the neural plate (Sbrogna et al., 2003; Liu et al., 2006), consistent with a role in directing early *ncc* expression. It now remains to be determined whether prolactin acts directly on ionocyte precursors (Fig. 1E) or on ionocytes themselves (Fig. 1F) to modulate ionoregulatory functions in the early embryo. Previous work has revealed that cortisol and isotocin help regulate ionocyte differentiation at various levels in the ionocyte lineage (Chou et al., 2011; Cruz et al., 2013), suggesting complex hormonal control of ionocyte differentiation and function. Given the remarkable similarity in the differentiation programs between ionocytes and intercalated cells of mammalian kidney (Hwang and Chou, 2013), the study of hormonal action on zebrafish ionocyte differentiation may provide insight into whether and how prolactin and other pituitary hormones direct cell differentiation in incipient renal tubules.

5. Concluding remarks

Comparative approaches to study prolactin action in teleosts have successfully identified prolactin as a key hormonal regulator of ion and water transport in osmoregulatory tissues. More recently, the zebrafish has emerged as a powerful model system that is helping reveal the cellular mechanisms by which prolactin exerts its osmoregulatory effects. By combining new tools for manipulating gene function with recently established ionocyte lineage markers it is now possible to begin a detailed analysis of the effects of prolactin on specific ionocyte precursors and/or differentiated ionocytes. Recent work in tilapia and zebrafish suggests that prolactin is a key regulator of NCC, an ion cotransporter that is also an important player in the vertebrate kidney. Indeed, a renal

isoform of NCC that is expressed in mammalian distal convoluted tubules is responsible for the re-absorption of ~10% of the total filtered Na^+ and Cl^- (Obermuller et al., 1995). Loss of NCC function causes Gitelmann's syndrome, a disease associated with low blood pressure and NaCl wasting. Thus, besides uncovering the basic cellular and molecular mechanisms that allow teleosts to meet the challenges associated with life in aquatic environments, these studies may contribute to future diagnoses and treatments of diseases causing hydromineral imbalances in human patients.

Acknowledgments

This work was supported by training grants from the National Institute of Mental Health (T32-MH020051) and the National Institute of Diabetes and Digestive and Kidney Diseases (F32-DK095575) to J.P.B and NIH NS039994 to R.O.K. We appreciate the invaluable laboratory support and encouragement of Ms. Meng-Chieh Shen.

References

Arnold, E., Rivera, J.C., Thebault, S., Moreno-Paramo, D., Quiroz-Mercado, H., Quintanar-Stephano, A., Binart, N., Martinez de la Escalera, G., Clapp, C., 2010. High levels of serum prolactin protect against diabetic retinopathy by increasing ocular vasoinhibins. Diabetes 59, 3192–3197.

Balbach, L., Wallaschofski, H., Völzke, H., Nauck, M., Dörr, M., Haring, R., 2013. Serum prolactin concentrations as risk factor of metabolic syndrome or type 2 diabetes? BMC Endocrinol. Disord. 13, 12.

Ball, J.N., Ensor, D.M., 1965. Effect of prolactin on plasma sodium in the teleost, *Poecilia latipinna*. J. Endocrinol. 32, 269–270.

Bayaa, M., Vulesevic, B., Esbaugh, A., Braun, M., Ekker, M.E., Grosell, M., Perry, S.F., 2009. The involvement of SLC26 anion transporters in chloride uptake in zebrafish (*Danio rerio*) larvae. J. Exp. Biol. 212, 3283–3295.

Bern, H.A., 1983. Functional evolution of prolactin and growth hormone in lower vertebrates. Am. Zool. 23, 663–671.

Bernichtein, S., Kayser, C., Dillner, K., Moulin, S., Kopchick, J.J., Martial, J.A., Norstedt, G., Isaksson, O., Kelly, P.A., Goffin, V., 2003. Development of pure prolactin receptor antagonists. J. Biol. Chem. 278, 35988–35999.

Bernichtein, S., Touraine, P., Goffin, V., 2010. New concepts in prolactin biology. J. Endocrinol. 206, 1–11.

Björnsson, B.T., Hansson, T., 1983. Effects of hypophysectomy on the plasma ionic and osmotic balance in rainbow trout, *Salmo gairdneri*. Gen. Comp. Endocrinol. 49, 240–247.

Björnsson, B.T., Yamauchi, K., Nishioka, R.S., Deftos, L.J., Bern, H.A., 1987. Effects of hypophysectomy and subsequent hormonal replacement therapy on hormonal and osmoregulatory status of coho salmon, *Oncorhynchus kisutch*. Gen. Comp. Endocrinol. 68, 421–430.

Bole-Feysot, C., Goffin, V., Edery, M., Binart, N., Kelly, P.A., 1998. Prolactin (PRL) and its receptor: actions, signal transduction pathways and phenotypes observed in PRL receptor knockout mice. Endocr. Rev. 19, 225–268.

Boutin, J.M., Jolicoeur, C., Okamura, H., Gagnon, J., Edery, M., Shirota, M., Banville, D., Dusanter-Fourt, I., Djiane, J., Kelly, P.A., 1988. Cloning and expression of the rat prolactin receptor, a member of the growth hormone/prolactin receptor gene family. Cell 53, 69–77.

Breves, J.P., Fox, B.K., Pierce, A.L., Hirano, T., Grau, E.G., 2010a. Gene expression of growth hormone family and glucocorticoid receptors, osmosensors, and ion transporters in the gill during seawater acclimation of Mozambique tilapia, *Oreochromis mossambicus*. J. Exp. Zoolog. A 313, 432–441.

Breves, J.P., Seale, A.P., Helms, R.E., Tipsmark, C.K., Hirano, T., Grau, E.G., 2011. Dynamic gene expression of GH/PRL-family hormone receptors in gill and kidney during freshwater-acclimation of Mozambique tilapia. Comp. Biochem. Physiol. A 158, 194–200.

Breves, J.P., Watanabe, S., Kaneko, T., Hirano, T., Grau, E.G., 2010b. Prolactin restores branchial mitochondrion-rich cells expressing Na^+/Cl^- cotransporter in hypophysectomized Mozambique tilapia. Am. J. Physiol. Regul. Integr. Comp. Physiol. 299, R702–R710.

Breves, J.P., Serizier, S.B., Goffin, V., McCormick, S.D., Karlstrom, R.O., 2013. Prolactin regulates transcription of the ion uptake Na^+/Cl^- cotransporter (*ncc*) gene in zebrafish gill. Mol. Cell. Endocrinol. 369, 98–106.

Brown, P.S., Brown, S.C., 1987. Osmoregulatory actions of prolactin and other adenohypophysial hormones. In: Pang, P.K.T., Schreibman, M.P., Sawyer, W.H. (Eds.), Vertebrate Endocrinology: Fundamentals and Biomedical Implications. Academic Press, London, pp. 45–84.

Bystriansky, J.S., Richards, J.G., Schulte, P.M., Ballantyne, J.S., 2006. Reciprocal expression of gill Na^+/K^+-ATPase-subunit isoforms 1a and 1b during seawater acclimation of three salmonid fishes that vary in their salinity tolerance. J. Exp. Biol. 209, 1848–1858.

Cerdà, J., Finn, R.N., 2010. Piscine aquaporins: an overview of recent advances. J. Exp. Zoolog. A 313, 623–650.

Chang, W.J., Hwang, P.P., 2011. Development of zebrafish epidermis. Birth Defects Res. C 93, 205–214.

Chang, W.J., Wang, Y.F., Hu, H.J., Wang, J.H., Lee, T.H., Hwang, P.P., 2013. Compensatory regulation of Na^+ absorption by Na^+/H^+ exchanger and Na^+–Cl^- cotransporter in zebrafish (*Danio rerio*). Front. Zoolog. 10, 46.

Chasiotis, H., Effendi, J.C., Kelly, S.P., 2009. Occludin expression in goldfish held in ion-poor water. J. Comp. Physiol. B. 179, 145–154.

Chasiotis, H., Kelly, S.P., 2008. Occludin immunolocalization and protein expression in goldfish. J. Exp. Biol. 211, 1524–1534.

Chasiotis, H., Kolosov, D., Bui, P., Kelly, S.P., 2012. Tight junctions, tight junction proteins and paracellular permeability across the gill epithelium of fishes: a review. Respir. Physiol. Neurobiol. 184, 269–281.

Chen, M., Huang, X., Yuen, D.S.H., Cheng, C.H.K., 2011. A study on the functional interaction between the GH/PRL family of polypeptides with their receptors in zebrafish: evidence against GHR1 being the receptor for somatolactin. Mol. Cell. Endocrinol. 337, 114–121.

Chou, M.Y., Hung, J.C., Wu, L.C., Hwang, S.P.L., Hwang, P.P., 2011. Isotocin controls ion regulation through regulating ionocyte progenitor differentiation and proliferation. Cell. Mol. Life Sci. 68, 2797–2809.

Cruz, A.A., Chao, P.L., Hwang, P.P., 2013. Cortisol promotes differentiation of epidermal ionocytes through Foxi3 transcription factors in zebrafish (*Danio rerio*). Comp. Biochem. Physiol. A 164, 249–257.

Cutler, C.P., Cramb, G., 2002. Branchial expression of an aquaporin 3 (AQP-3) homologue is downregulated in the European eel *Anguilla anguilla* following seawater acclimation. J. Exp. Biol. 205, 2643–2651.

Cutler, C.P., Martinez, A.S., Cramb, G., 2007a. The role of aquaporin 3 in teleost fish. Comp. Biochem. Physiol. A 148, 82–91.

Cutler, C.P., Phillips, C., Hazon, N., Cramb, G., 2007b. Cortisol regulates eel (*Anguilla anguilla*) aquaporin 3 (AQP3) mRNA expression levels in gill. Gen. Comp. Endocrinol. 152, 310–313.

Dauder, S., Young, G., Hass, L., Bern, H.A., 1990. Prolactin receptors in liver, kidney, and gill of the tilapia (*Oreochromis mossambicus*): characterization and effect of salinity on specific binding of iodinated ovine prolactin. Gen. Comp. Endocrinol. 77, 368–377.

Deachapunya, C., Poonyachoti, S., Krishnamra, N., 2008. Regulation of electrolyte transport across cultured endometrial epithelial cells by prolactin. J. Endocrinol. 197, 575–582.

Dharmamba, M., Maetz, J., 1972. Effects of hypophysectomy and prolactin on the sodium balance of *Tilapia mossambica* in fresh water. Gen. Comp. Endocrinol. 19, 175–183.

Dymowska, A.K., Hwang, P.P., Goss, G.G., 2012. Structure and function of ionocytes in the freshwater fish gill. Respir. Physiol. Neurobiol. 184, 282–292.

Edery, M., Young, G., Bern, H.A., Steiny, S., 1984. Prolactin receptors in tilapia (*Sarotherodon mossambicus*) tissues: binding studies using I-125 labeled ovine prolactin. Gen. Comp. Endocrinol. 56, 19–23.

Esaki, M., Hoshijima, K., Kobayashi, S., Fukuda, H., Kawakami, K., Hirose, S., 2007. Visualization in zebrafish larvae of Na^+ uptake in mitochondria-rich cells whose differentiation is dependent on foxi3a. Am. J. Physiol. Regul. Integr. Comp. Physiol. 292, R470–R480.

Esaki, M., Hoshijima, K., Nakamura, N., Munakata, K., Tanaka, M., Ookata, K., Asakawa, K., Kawakami, K., Wang, W., Weinberg, E.S., Hirose, S., 2009. Mechanism of development of ionocytes rich in vacuolar-type H(+)-ATPase in the skin of zebrafish larvae. Dev. Biol. 329, 116–129.

Evans, D.H., 2011. Freshwater fish gill ion transport: August Krogh to morpholinos and microprobes. Acta Physiol. 202, 349–359.

Evans, D.H., Piermarini, P.M., Choe, K.P., 2005. The multifunctional fish gill: dominant site of gas exchange, osmoregulation, acid–base regulation, and excretion of nitrogenous waste. Physiol. Rev. 85, 97–177.

Fiol, D.F., Sanmarti, E., Sacchi, R., Kültz, D., 2009. A novel tilapia prolactin receptor is functionally distinct from its paralog. J. Exp. Biol. 212, 2007–2015.

Flores, A.M., Shrimpton, M.J., 2012. Differential physiological and endocrine responses of rainbow trout, *Oncorhynchus mykiss*, transferred from fresh water to ion-poor or salt water. Gen. Comp. Endocrinol. 175, 244–250.

Freeman, M.E., Kanyicska, B., Lerant, A., Nagy, G., 2000. Prolactin: structure, function and regulation of secretion. Physiol. Rev. 80, 1523–1631.

Fryer, J.N., 1979. Prolactin-binding sites in tilapia (*Sarotharadon mossambicus*) kidney. Gen. Comp. Endocrinol. 39, 397–403.

Fuentes, J., Brinca, L., Guerreiro, P.M., Power, D.M., 2010. PRL and GH synthesis and release from the sea bream (*Sparus auratus* L.) pituitary gland *in vitro* in response to osmotic challenge. Gen. Comp. Endocrinol. 168, 95–102.

Fukamachi, S., Meyer, A., 2007. Evolution of receptors for growth hormone and somatolactin in fish and land vertebrates: lessons from the lungfish and sturgeon orthologues. J. Mol. Evol. 65, 359–372.

Fukada, H., Ozaki, Y., Pierce, A.L., Adachi, S., Yamauchi, K., Hara, A., Swanson, P., Dickhoff, W.W., 2005. Identification of the salmon somatolactin receptor, a new member of the cytokine receptor family. Endocrinology 146, 2354–2361.

Furth, P.A., Nakles, R.E., Millman, S., Diaz-Cruz, E.S., Cabrera, M.C., 2011. Signal transducer and activator of transcription 5 as a key signaling pathway in normal mammary gland developmental biology and breast cancer. Breast Cancer Res. 13, 220.

Geering, K., 2008. Functional roles of Na, K-ATPase subunits. Curr. Opin. Nephrol. Hypertens. 17, 526–532.

Georgiopoulos, G.A., Stamatelopoulos, K.S., Lambrinoudaki, I., Lykka, M., Kyrkou, K., Rizos, D., Creatsa, M., Christodoulakos, G., Alevizaki, M., Sfikakis, P.P., Papamichael, C., 2009. Prolactin and preclinical atherosclerosis in menopausal women with cardiovascular risk factors. Hypertension 54, 98–105.

Griffin, L.B., January, K.E., Ho, K.W., Cotter, K.A., Callard, G.V., 2013. Morpholino-mediated knockdown of ERα, ERβa, and ERβb mRNAs in zebrafish (*Danio rerio*) embryos reveals differential regulation of estrogen-inducible genes. Endocrinology 154, 4158–4169.

Han, Y., Watling, D., Rogers, N.C., Stark, G.R., 1997. JAK2 and STAT5, but not JAK1 and STAT1, are required for prolactin-induced beta-lactoglobulin transcription. Mol. Endocrinol. 11, 1180–1188.

Hennighausen, L., Robinson, G.W., 2001. Signaling pathways in mammary gland development. Dev. Cell 1, 467–475.

Herndon, T.M., McCormick, S.D., Bern, H.A., 1991. Effects of prolactin on chloride cells in opercular membrane of seawater-adapted tilapia. Gen. Comp. Endocrinol. 83, 283–289.

Higashimoto, Y., Nakao, N., Ohkubo, T., Tanaka, M., Nakashima, K., 2001. Structure and tissue distribution of prolactin receptor mRNA in Japanese flounder (*Paralichtys olivaceus*): conserved and preferential expression in osmoregulatory organs. Gen. Comp. Endocrinol. 123, 170–179.

Hirano, T., 1986. The spectrum of prolactin action in teleosts. Prog. Clin. Biol. Res. 205, 53–74.

Hirata, T., Kaneko, T., Ono, T., Nakazato, T., Furukawa, N., Hasegawa, S., Wakabayashi, S., Shigekawa, M., Chang, M.H., Romero, M.F., Hirose, S., 2003. Mechanism of acid adaptation of a fish living in a pH 3.5 lake. Am. J. Physiol. Regul. Integr. Comp. Physiol. 284, R1199–R1212.

Hiroi, J., McCormick, S.D., 2012. New insights into gill ionocyte and ion transporter function in euryhaline and diadromous fish. Respir. Physiol. Neurobiol. 184, 257–268.

Hiroi, J., Yasumasu, S., McCormick, S.D., Hwang, P.P., Kaneko, T., 2008. Evidence for an apical Na–Cl cotransporter involved in ion uptake in a teleost fish. J. Exp. Biol. 211, 2584–2599.

Horng, J.L., Hwang, P.P., Shih, T.H., Wen, Z.H., Lin, C.S., Lin, L.Y., 2009. Chloride transport in mitochondrion-rich cells of euryhaline tilapia (*Oreochromis mossambicus*) larvae. Am. J. Physiol. Cell Physiol. 297, C845–854.

Hoshijima, K., Hirose, S., 2007. Expression of endocrine genes in zebrafish larvae in response to environmental salinity. J. Endocrinol. 193, 481–491.

Hsiao, C.D., You, M.S., Guh, Y.J., Ma, M., Jiang, Y.J., Hwang, P.P., 2007. A positive regulatory loop between foxi3a and foxi3b is essential for specification and differentiation of zebrafish epidermal ionocytes. PLoS One 2, e302.

Huang, X., Jiao, B., Fung, C.K., Zhang, Y., Ho, W.K., Chan, C.B., Lin, H., Wang, D., Cheng, C.H.K., 2007. The presence of two distinct prolactin receptors in seabream with different tissue distribution patterns, signal transduction pathways and regulation of gene expression by steroid hormones. J. Endocrinol. 194, 373–392.

Hwang, P.P., 2009. Ion uptake and acid secretion in zebrafish (*Danio rerio*). J. Exp. Biol. 212, 1745–1752.

Hwang, P.P., Chou, M.Y., 2013. Zebrafish as an animal model to study ion homeostasis. Pflugers Arch. 465, 1233–1247.

Hwang, P.P., Lee, T.H., 2007. New insights into fish ion regulation and mitochondrion-rich cells. Comp. Biochem. Physiol. A 148, 479–497.

Ingleton, P.M., Baker, B.I., Ball, J.N., 1973. Secretion of prolactin and growth hormone by teleost pituitaries *in vitro*. I. Effect of sodium concentration and osmotic pressure during short-term incubations. J. Comp. Physiol. 87, 317–328.

Inokuchi, M., Hiroi, J., Watanabe, S., Lee, K.M., Kaneko, T., 2008. Gene expression and morphological localization of NHE3, NCC and NKCC1a in branchial mitochondria-rich cells of Mozambique tilapia (*Oreochromis mossambicus*) acclimated to a wide range of salinities. Comp. Biochem. Physiol. A 151, 151–158.

Jänicke, M., Carney, T.J., Hammerschmidt, M., 2007. Foxi3 transcription factors and Notch signaling control the formation of skin ionocytes from epidermal precursors of the zebrafish embryo. Dev. Biol. 307, 258–271.

Jeong, S.Y., Kim, J.H., Lee, W.O., Dahms, H.U., Han, K.N., 2013. Salinity changes in the anadromous river pufferfish, *Takifugu obscurus*, mediate gene regulation. Fish Physiol. Biochem. 23, 24–56. http://dx.doi.org/10.1007/s10695-013-9837-z.

Kaneko, T., Watanabe, S., Lee, K.M., 2008. Functional morphology of mitochondrion-rich cells in euryhaline and stenohaline teleosts. Aqua-BioSci. Monogr. 1, 1–62.

Kelly, S.P., Chow, I.N., Woo, N.Y., 1999. Effects of prolactin and growth hormone on strategies of hypoosmotic adaptation in a marine teleost, *Sparus sarba*. Gen. Comp. Endocrinol. 113, 9–22.

Kumai, Y., Bahubeshi, A., Steele, S., Perry, S.F., 2011. Strategies for maintaining Na^+ balance in zebrafish (*Danio rerio*) during prolonged exposure to acidic water. Comp. Biochem. Physiol. A 160, 52–62.

Kumai, Y., Nesan, D., Vijayan, M.M., Perry, S.F., 2012. Cortisol regulates Na^+ uptake in zebrafish, *Danio rerio*, larvae via the glucocorticoid receptor. Mol. Cell. Endocrinol. 364, 113–125.

Kwong, A.K., Ng, A.H., Leung, L.Y., Man, A.K., Woo, N.Y., 2009. Effect of extracellular osmolality and ionic levels on pituitary prolactin release in euryhaline silver sea bream (*Sparus sarba*). Gen. Comp. Endocrinol. 160, 67–75.

Lee, K.M., Kaneko, T., Aida, K., 2006. Prolactin and prolactin receptor expression in a marine teleost, pufferfish *Takifugu rubripes*. Gen. Comp. Endocrinol. 146, 318–328.

Liao, B.K., Chen, R.D., Hwang, P.P., 2009. Expression regulation of Na^+–K^+-ATPase alpha1-subunit subtypes in zebrafish gill ionocytes. Am. J. Physiol. Regul. Integr. Comp. Physiol. 296, R1897–R1906.

Liao, B.K., Deng, A.N., Chen, S.C., Chou, M.Y., Hwang, P.P., 2007. Expression and water calcium dependence on calcium transporter isoforms in zebrafish gill mitochondrion-rich cells. BMC Genomics 8, 354.

Lignot, J.H., Cutler, C.P., Hazon, N., Cramb, G., 2002. Immunolocalisation of aquaporin 3 in the gill and the gastrointestinal tract of the European eel *Anguilla anguilla* (L.). J. Exp. Biol. 205, 2653–2663.

Lin, C.H., Tsai, I.L., Su, C.H., Tseng, D.Y., Hwang, P.P., 2011. Reverse effect of mammalian hypocalcemic cortisol in fish: cortisol stimulates Ca^{2+} uptake via glucocorticoid receptor-mediated vitamin D_3 metabolism. PLoS One 6 (8), e23689.

Linzell, J.L., Peaker, M., Taylor, J.C., 1975. The effects of prolactin and oxytocin on milk secretion and on the permeability of the mammary epithelium in the rabbit. J. Physiol. 253, 547–563.

Liu, N.A., Liu, Q., Wawrowsky, K., Yang, Z., Lin, S., Melmed, S., 2006. Prolactin receptor signaling mediates the osmotic response of embryonic zebrafish lactotrophs. Mol. Endocrinol. 20, 871–880.

Loretz, C.A., Bern, H.A., 1982. Prolactin and osmoregulation in vertebrates. Neuroendocrinology 35, 292–304.

Mackie, P., Wright, P.A., Glebe, B.D., Ballantyne, J.S., 2005. Osmoregulation and gene expression of Na^+/K^+ ATPase in families of Atlantic salmon (*Salmo salar*) smolts. Can. J. Fish. Aquat. Sci. 62, 2661–2672.

Madsen, S.S., Jensen, M.K., Nhr, J., Kristiansen, K., 1995. Expression of Na(+)–K(+)-ATPase in the brown trout, *Salmo trutta*: *in vivo* modulation by hormones and seawater. Am. J. Physiol. 269, R1339–R1345.

Madsen, S.S., Kiilerich, P., Tipsmark, C.K., 2009. Multiplicity of expression of Na^+, K^+-ATPase α-subunit isoforms in the gill of Atlantic salmon (*Salmo salar*): cellular localisation and absolute quantification in response to salinity change. J. Exp. Biol. 212, 78–88.

Manzon, L.A., 2002. The role of prolactin in fish osmoregulation: a review. Gen. Comp. Endocrinol. 125, 291–310.

McCormick, S.D., 1995. Hormonal control of gill Na^+, K^+-ATPase and chloride cell function. In: Wood, C.M., Shuttleworth, T.J. (Eds.), Fish Physiology. Academic Press, New York, pp. 285–315.

McCormick, S.D., 2001. Endocrine control of osmoregulation in teleost fish. Am. Zoolog. 41, 781–794.

McCormick, S.D., Bradshaw, D., 2006. Hormonal control of salt and water balance in vertebrates. Gen. Comp. Endocrinol. 147, 3–8.

McCormick, S.D., Regish, A.M., Christensen, A.K., 2009. Distinct freshwater and seawater isoforms of Na^+/K^+-ATPase in gill chloride cells of Atlantic salmon. J. Exp. Biol. 212, 3994–4001.

McHale, K., Tomaszewski, J.E., Puthiyaveettil, R., Livolsi, V.A., Clevenger, C.V., 2008. Altered expression of prolactin receptor-associated signaling proteins in human breast carcinoma. Mod. Pathol. 21, 565–571.

Neville, M.C., McFadden, T.B., Forsyth, I., 2002. Hormonal regulation of mammary differentiation and milk secretion. J. Mammary Gland Biol. Neoplasia 7, 49–66.

Nishioka, R.S., 1994. Hypophysectomy of fish. In: Hochachka, P.W., Mommsen, T.P. (Eds.), Biochemistry and Molecular Biology of Fishes: Analytical Techniques. Elsevier, New York, pp. 49–58.

Obermuller, N., Bernstein, P., Velazquez, H., Reilly, R., Moser, D., Ellison, D.H., Bachmann, S., 1995. Expression of the thiazide-sensitive Na–Cl cotransporter in rat and human kidney. Am. J. Physiol. Ren. Physiol. 269, F900–F910.

Pan, T.C., Liao, B.K., Huang, C.J., Lin, L.Y., Hwang, P.P., 2005. Epithelial Ca^{2+} channel expression and Ca^{2+} uptake in developing zebrafish. Am. J. Physiol. Regul. Integr. Comp. Physiol. 289, R1202–R1211.

Pavlovic, D., Fuller, W., Shattock, M.J., 2013. Novel regulation of cardiac Na pump via phospholemman. J. Mol. Cell. Cardiol. 61, 83–93.

Pellegrini, G., Dellambra, E., Golisano, O., Martinelli, E., Fantozzi, I., Bondanza, S., Ponzin, D., McKeon, F., De Luca, M., 2001. P63 identifies keratinocyte stem cells. Proc. Natl. Acad. Sci. USA 98, 3156–3161.

Perry, S.F., Vulesevic, B., Grosell, M., Bayaa, M., 2009. Evidence that SLC26 anion transporters mediate branchial chloride uptake in adult zebrafish (*Danio rerio*). Am. J. Physiol. Regul. Integr. Comp. Physiol. 297, R988–R997.

Pickford, G.E., Phillips, J.G., 1959. Prolactin, a factor in promoting survival of hypophysectomized killifish in fresh water. Science 130, 454–455.

Pierce, A.L., Fox, B.K., Davis, L.K., Visitacion, N., Kitashashi, T., Hirano, T., Grau, E.G., 2007. Prolactin receptor, growth hormone receptor, and putative somatolactin receptor in Mozambique tilapia: tissue specific expression and differential regulation by salinity and fasting. Gen. Comp. Endocrinol. 154, 31–40.

Pisam, M., Auperin, B., Prunet, P., Rentier-Delrue, F., Martial, J., Rambourg, A., 1993. Effects of prolactin on alpha and beta chloride cells in the gill epithelium of the saltwater adapted tilapia "*Oreochromis niloticus*". Anat. Rec. 235, 275–284.

Prunet, P., Auperin, B., 1994. Prolactin receptors. In: Sherwood, N.M., Hew, C.L. (Eds.), Fish Physiology: Molecular Endocrinology of Fish, vol. 13. Academic Press, New York, pp. 367–391.

Prunet, P., Sandra, O., Le Rouzic, P., Marchand, O., Laudet, V., 2000. Molecular characterization of the prolactin receptor in two fish species, tilapia *Oreochromis niloticus* and rainbow trout, *Oncorhynchus mykiss*: a comparative approach. Can. J. Physiol. Pharmacol. 78, 1086–1096.

Raabe, M.A., McCoshen, J.A., 1986. Epithelial regulation of prolactin effect on amniotic permeability. Am. J. Obstet. Gynecol. 154, 130–134.

Reis-Filho, J.S., Schmitt, F.C., 2002. Taking advantage of basic research: p63 is a reliable myoepithelial and stem cell marker. Adv. Anat. Pathol. 5, 280–289.

Rhee, J.S., Kim, R.O., Seo, J.S., Lee, J., Lee, Y.M., Lee, J.S., 2010. Effects of salinity and endocrine-disrupting chemicals on expression of prolactin and prolactin receptor genes in the euryhaline hermaphroditic fish, *Kryptolebias marmoratus*. Comp. Biochem. Physiol. C 152, 413–423.

Richards, J.G., Semple, J.W., Bystriansky, J.S., Schulte, P.M., 2003. Na^+/K^+-ATPase-isoform switching in gills of rainbow trout (*Oncorhynchus mykiss*) during salinity transfer. J. Exp. Biol. 206, 4475–4486.

Rosas-Hernandez, H., Cuevas, E., Lantz, S.M., Hamilton, W.R., Ramirez-Lee, M.A., Ali, S.F., Gonzalez, C., 2013. Prolactin and blood–brain barrier permeability. Curr. Neurovasc. Res. 10, 278–286.

Sage, M., 1968. Responses to osmotic stimuli of *Xiphophorus* prolactin cells in organ culture. Gen. Comp. Endocrinol. 10, 70–74.
Saito, K., Nakamura, N., Ito, Y., Hoshijima, K., Esaki, M., Zhao, B., Hirose, S., 2010. Identification of zebrafish Fxyd11a protein that is highly expressed in ion-transporting epithelium of the gill and skin and its possible role in ion homeostasis. Front. Physiol. 1, 129.
Sakamoto, T., McCormick, S.D., 2006. Prolactin and growth hormone in fish osmoregulation. Gen. Comp. Endocrinol. 147, 24–30.
Sandra, O., Sohm, F., de Luze, A., Prunet, P., Edery, M., Kelly, P.A., 1995. Expression cloning of a cDNA encoding a fish prolactin receptor. Proc. Natl. Acad. Sci. USA 92, 6037–6041.
Santos, C.R.A., Ingleton, P.M., Cavaco, J.E.B., Kelly, P.A., Edery, M., Power, D.M., 2001. Cloning, characterization, and tissue distribution of prolactin receptor in the sea bream (*Sparus aurata*). Gen. Comp. Endocrinol. 121, 32–47.
Sbrogna, J.L., Barresi, M.J., Karlstrom, R.O., 2003. Multiple roles for Hedgehog signaling in zebrafish pituitary development. Dev. Biol. 254, 19–35.
Seale, A.P., Watanabe, S., Grau, E.G., 2012. Osmoreception: perspectives on signal transduction and environmental modulation. Gen. Comp. Endocrinol. 176, 354–360.
Shepherd, B.S., Sakamoto, T., Hyodo, S., Nishioka, R.S., Ball, C., Bern, H.A., Grau, E.G., 1999. Is the primitive regulation of pituitary prolactin ($tPRL_{177}$ and $tPRL_{188}$) secretion and gene expression in the euryhaline tilapia (*Oreochromis mossambicus*) hypothalamic or environmental? J. Endocrinol. 161, 121–129.
Stelwagen, K., McFadden, H.A., Demmer, J., 1999. Prolactin, alone or in combination with glucocorticoids, enhances tight junction formation and expression of the tight junction protein occludin in mammary cells. Mol. Cell. Endocrinol. 156, 55–61.
Tingaud-Sequeira, A., Calusinska, M., Finn, R.N., Chauvigné, F., Lozano, J., Cerdà, J., 2010. The zebrafish genome encodes the largest vertebrate repertoire of functional aquaporins with dual paralogy and substrate specificities similar to mammals. BMC Evol. Biol. 10, 38.
Tipsmark, C.K., 2008. Identification of FXYD protein genes in a teleost: tissue-specific expression and response to salinity change. Am. J. Physiol. Regul. Integr. Comp. Physiol. 294, R1367–R1378.
Tipsmark, C.K., Baltzegar, D.A., Ozden, O., Grubb, B.J., Borski, R.J., 2008a. Salinity regulates claudin mRNA and protein expression in the teleost gill. Am. J. Physiol. Regul. Integr. Comp. Physiol. 294, R1004–R1014.
Tipsmark, C.K., Breves, J.P., Seale, A.P., Lerner, D.T., Hirano, T., Grau, E.G., 2011. Switching of Na^+, K^+-ATPase isoforms by salinity and prolactin in the gill of a cichlid fish. J. Endocrinol. 209, 237–244.
Tipsmark, C.K., Jørgensen, C., Brande-Lavridsen, N., Engelund, M., Olesen, J.H., Madsen, S.S., 2009. Effects of cortisol, growth hormone and prolactin on gill claudin expression in Atlantic salmon. Gen. Comp. Endocrinol. 163, 270–277.
Tipsmark, C.K., Kiilerich, P., Nilsen, T.O., Ebbesson, L.O., Stefansson, S.O., Madsen, S.S., 2008b. Branchial expression patterns of claudin isoforms in Atlantic salmon during seawater acclimation and smoltification. Am. J. Physiol. Regul. Integr. Comp. Physiol. 294, R1563–R1574.
Tipsmark, C.K., Madsen, S.S., 2009. Distinct hormonal regulation of Na^+, K^+-atpase genes in the gill of Atlantic salmon (*Salmo salar* L.). J. Endocrinol. 203, 301–310.
Tipsmark, C.K., Mahmmoud, Y.A., Borski, R.J., Madsen, S.S., 2010a. FXYD-11 associates with Na^+–K^+-ATPase in the gill of Atlantic salmon: regulation and localization in relation to changed ion-regulatory status. Am. J. Physiol. Regul. Integr. Comp. Physiol. 299, R1212–R1223.
Tipsmark, C.K., Sørensen, K.J., Madsen, S.S., 2010b. Aquaporin expression dynamics in osmoregulatory tissues of Atlantic salmon during smoltification and seawater acclimation. J. Exp. Biol. 213, 368–379.
Tomy, S., Chang, Y.M., Chen, Y.H., Cao, J.C., Wang, T.P., Chang, C.F., 2009. Salinity effects on the expression of osmoregulatory genes in the euryhaline black porgy *Acanthopagrus schlegeli*. Gen. Comp. Endocrinol. 161, 123–132.
Tse, W.K., Au, D.W., Wong, C.K., 2006. Characterization of ion channel and transporter mRNA expressions in isolated gill chloride and pavement cells of seawater acclimating eels. Biochem. Biophys. Res. Commun. 346, 1181–1190.
Tse, D.L.Y., Chow, B.K.C., Chan, C.B., Lee, L.T.O., Cheng, C.H.K., 2000. Molecular cloning and expression studies of a prolactin receptor in goldfish (*Carassius auratus*). Life Sci. 66, 593–605.
Tseng, D.Y., Chou, M.Y., Tseng, Y.C., Hsiao, C.J., Kaneko, T., Hwang, P.P., 2009. Effects of stanniocalcin 1 on calcium uptake in zebrafish (*Danio rerio*) embryo. Am. J. Physiol. Regul. Integr. Comp. Physiol. 296, R549–557.
Walker, T.L., Vukovic, J., Koudijs, M.M., Blackmore, D.G., Mackay, E.W., Sykes, A.M., Overall, R.W., Hamlin, A.S., Bartlett, P.F., 2012. Prolactin stimulates precursor cells in the adult mouse hippocampus. PLoS One 9, e44371.
Wang, P.J., Lin, C.H., Hwang, H.H., Lee, T.H., 2008. Branchial FXYD protein expression in response to salinity change and its interaction with Na^+/K^+-ATPase of the euryhaline teleost *Tetraodon nigroviridis*. J. Exp. Biol. 211, 3750–3758.
Wang, Y.F., Tseng, Y.C., Yan, J.J., Hiroi, J., Hwang, P.P., 2009. Role of SLC12A10.2, a Na–Cl cotransporter-like protein, in a Cl uptake mechanism in zebrafish (*Danio rerio*). Am. J. Physiol. Regul. Integr. Comp. Physiol. 296, R1650–R1660.
Watanabe, S., Kaneko, T., Aida, K., 2005. Aquaporin-3 expressed in the basolateral membrane of gill chloride cells in Mozambique tilapia *Oreochromis mossambicus* adapted to freshwater and seawater. J. Exp. Biol. 208, 2673–2682.
Weng, C.F., Lee, T.H., Hwang, P.P., 1997. Immune localization of prolactin receptor in the mitochondria-rich cells of the euryhaline teleost (*Oreochromis mossambicus*) gill. FEBS Lett. 405, 91–94.
Whitehead, A., Roach, J.L., Zhang, S., Galvez, F., 2011. Genomic mechanisms of evolved physiological plasticity in killifish distributed along an environmental salinity gradient. Proc. Natl. Acad. Sci. USA 108, 6193–6198.
Yada, T., Hirano, T., Grau, E.G., 1994. Changes in plasma levels of the two prolactins and growth hormone during adaptation to different salinities in the euryhaline tilapia (*Oreochromis mossambicus*). Gen. Comp. Endocrinol. 93, 214–223.
Yan, J.J., Chou, M.Y., Kaneko, T., Hwang, P.P., 2007. Gene expression of Na^+/H^+ exchanger in zebrafish H^+-ATPase-rich cells during acclimation to low-Na^+ and acidic environments. Am. J. Physiol. Cell Physiol. 293, C1814–C1823.
Yang, W.K., Kang, C.K., Chang, C.H., Hsu, A.D., Lee, T.H., Hwang, P.P., 2013. Expression profiles of branchial FXYD proteins in the brackish medaka *Oryzias dancena*: a potential saltwater fish model for studies of osmoregulation. PLoS One 8, e55470.
Zhou, B., Kelly, S.P., Ianowski, J.P., Wood, C.M., 2003. Effects of cortisol and prolactin on Na^+ and Cl^- transport in cultured branchial epithelia from FW rainbow trout. Am. J. Physiol. Regul. Integr. Comp. Physiol. 285, R1305–R1316.
Zhu, Y., Song, D., Tran, N.T., Nguyen, N., 2007. The effects of the members of growth hormone family knockdown in zebrafish development. Gen. Comp. Endocrinol. 150, 395–404.