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Molecular mechanisms of Cl^- transport in fishes: New insights and their evolutionary context

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Abstract

With remarkably few exceptions, aquatic vertebrates maintain internal Cl^- homeostasis despite strong and sometimes fluctuating Cl^- concentration gradients between extracellular fluids and external environments. In this "Perspective," we discuss recent advances in the understanding of epithelial Cl^- transport at the molecular level within key osmoregulatory organs in fishes. New insights into mechanisms for epithelial Cl^- transport in basal lineages are highlighted to provide an evolutionary context. We describe Cl^- transport processes that employ: cystic fibrosis transmembrane conductance regulator, cation-chloride cotransporters, voltage-gated chloride channels, and chloride-anion exchangers. As the collective understanding of Cl^- transport processes continues to expand, investigators are equipped to more precisely characterize how endocrine factors promote hydromineral balance. We, therefore, conclude our discussion by paying special attention to recently defined roles for prolactin and corticosteroids in the regulation of Cl^- transport in basal and derived clades.

KEYWORDS

gill, hormones, ionocytes, lamprey, sturgeon, teleost

1 | INTRODUCTION

Fishes are grouped into three classes: Agnatha (jawless fishes), Chondrichthyes (cartilaginous fishes), and Osteichthyes (bony fishes) (Figure 1). Teleosts (class Osteichthyes; subclass Actinopterygii; infraclass Neopterygii; division Teleostei) and lampreys (members of Class Agnatha) typically maintain extracellular fluids between 270 and 400 mOsm/kg, with the major dissolved ions, Na^+ and Cl^-, maintained between approximately 130–180 and 100–150 mmol/l, respectively (Ferreira-Martins et al., 2016; Marshall & Grosell, 2006). Freshwater (FW) and marine environments offer formidable challenges to the maintenance of ionic and osmotic homeostasis. Teleosts and lampreys residing in FW are hyperosmotic to the dilute external environment; therefore, they are dually threatened by diffusive ion losses and excessive hydration. To maintain hydromineral balance, the gills absorb Na^+ and Cl^- against strong concentration gradients while the kidney reabsorbs ions and exhibits high glomerular filtration rates to produce large volumes of dilute urine (Marshall & Grosell, 2006). Teleosts and lampreys residing in seawater (SW), on the other hand, are hyposmotic to the external environment and face the passive gain of ions and dehydration. In turn, they must drink ambient SW that is initially desalinated by the esophagus to enable subsequent solute-linked water absorption by the intestine (Barany et al., 2020; Grosell, 2006; Takei et al., 2017). The gills are specialized for Na^+ and Cl^- secretion while the kidney exhibits low glomerular filtration rates and produces small volumes of isosmotic urine (Marshall & Grosell, 2006). The branchial, gastrointestinal, and renal epithelia of both FW- and SW-acclimated fishes must leverage various pathways to actively transport Cl^-, the major anion in extracellular fluids, to sustain hydromineral balance of the organism.

In the past two decades, a rapid increase in the genomic resources available for the study of teleost fishes has illuminated the diversity of molecular strategies for osmoregulation. Recent sequencing efforts for pirarucu (*Arapaima gigas*; Vialle et al., 2018), spotted gar (*Lepisosteus oculatus*; Braasch et al., 2016), sterlet sturgeon (*Acipenser ruthenus*; Cheng et al., 2019; Du et al., 2020), bichir (*Polypterus senegalus*;

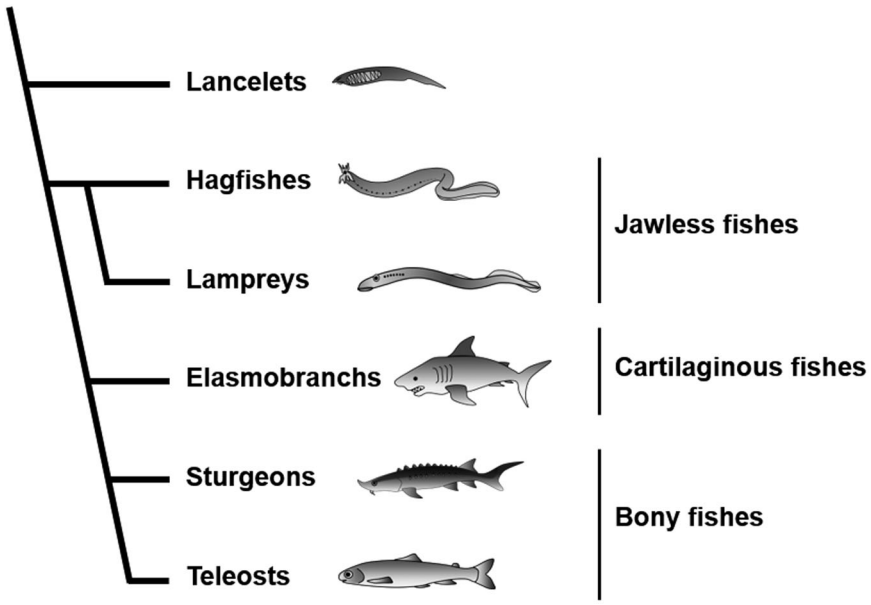

FIGURE 1 General phylogeny of major fish taxa

Chiu et al., 2004), African coelacanth (*Latimeria chalumna*; Amemiya et al., 2013), inshore hagfish (*Eptatretus burgeri*; Yamaguchi et al., 2020), and sea lamprey (*Petromyzon marinus*; Smith et al., 2013, 2018) will now support future investigations into the mechanisms underlying Cl^- transport in basal actinopterygian and pre-actinopterygian lineages. Here, we first present recent insights into the molecular mechanisms underlying Cl^- transport in fishes with special reference to their operation in basal lineages. We then discuss how these Cl^- transport pathways are functionally controlled by the pituitary hormone, prolactin, and corticosteroids.

2 | MOLECULAR MECHANISMS OF Cl^- TRANSPORT IN FISHES

2.1 | Cystic fibrosis transmembrane conductance regulator (Cftr)

For decades it has been known that marine/SW-acclimated teleosts express Cftr on the apical surface of branchial ionocytes where it serves as the conduit for active Cl^- secretion (Marshall & Singer, 2002). In these same ion-secreting cells, termed "SW-type" ionocytes, Na^+/K^+-ATPase (Nka) and $Na^+/K^+/2Cl^-$ cotransporter 1 (Nkcc1) are coexpressed in the basolateral membrane to energize and facilitate the Na^+- and K^+-coupled passage of Cl^- from blood plasma into ionocytes (Figure 2). The coordinated operation of Cftr, Nkcc1, and Nka is represented in multiple Cl^--secreting vertebrate epithelia, including shark rectal gland, teleost gill, avian/reptilian salt glands, and the mammalian intestinal crypt and airway. Recent characterizations of functionally divergent Cftr isoforms in fishes have provided new insight into the structural and functional evolution of Cftr in vertebrates.

Given its rich population of ionocytes and structural advantages for electrophysiological analyses, the opercular membrane of mummichog (*Fundulus heteroclitus*) has a long-standing record as an in vitro model for the study of Cl^- transport (Karnaky et al., 1977; Marshall & Bellamy, 2010; Marshall & Singer, 2002). Fittingly, the first teleost Cftr was described in the opercular epithelium, gill, and intestine of SW-acclimated mummichog (Singer et al., 1998). Two genes encoding distinct Cftr isoforms, denoted *cftr1* and *cftr2*, were subsequently discovered in Atlantic salmon (*Salmo salar*; Chen et al., 2001). With the SW-adaptive role for Cftr1 in teleost gill firmly established, recent investigations focused on discerning the function(s) of Cftr2 in the intestine. Salmon *cftr2* decreased in the intestine during smoltification and SW exposure, patterns indicative of a FW-adaptive role (or a deleterious activity in SW) for this particular isoform (Sundh et al., 2014). In euryhaline sea bream (*Sparus aurata*), intestinal *cftr* expression was similarly diminished by high salinities (Gregório et al., 2013). In Japanese eel (*Anguilla japonica*) where duplicate *cftr* isoforms were also identified, branchial *cftrb* (*cftr1*) was upregulated in SW, whereas intestinal *cftra* (*cftr2*) was diminished in SW (Wong et al., 2016). Given the apical localization of Cftr in mummichog enterocytes, Marshall et al. (2002) proposed that intestinal Cftr supports salt and fluid secretion into the lumen to facilitate the clearance of toxic bacterial flora. This hypothesized role for intestinal Cftr makes intuitive sense; whereas intestinal Cftr activity may be necessary for FW-acclimated teleosts that are not ingesting fluids, it would be unnecessary in the intestine of SW-acclimated teleosts where imbibed fluid is present to facilitate solute-linked water absorption. Functional studies are required to more fully resolve the significance of intestinal Cftr2 expression in FW-acclimated fishes.

In basal fishes, the molecular pathways of branchial Cl^- secretion are far less resolved than in teleosts. Our preliminary analysis using BLAST (Altschul et al., 1990) revealed that *cftr* orthologues are present in the genomes of sturgeon, bichir, coelacanth, and hagfish, yet none of these orthologues have been functionally characterized.

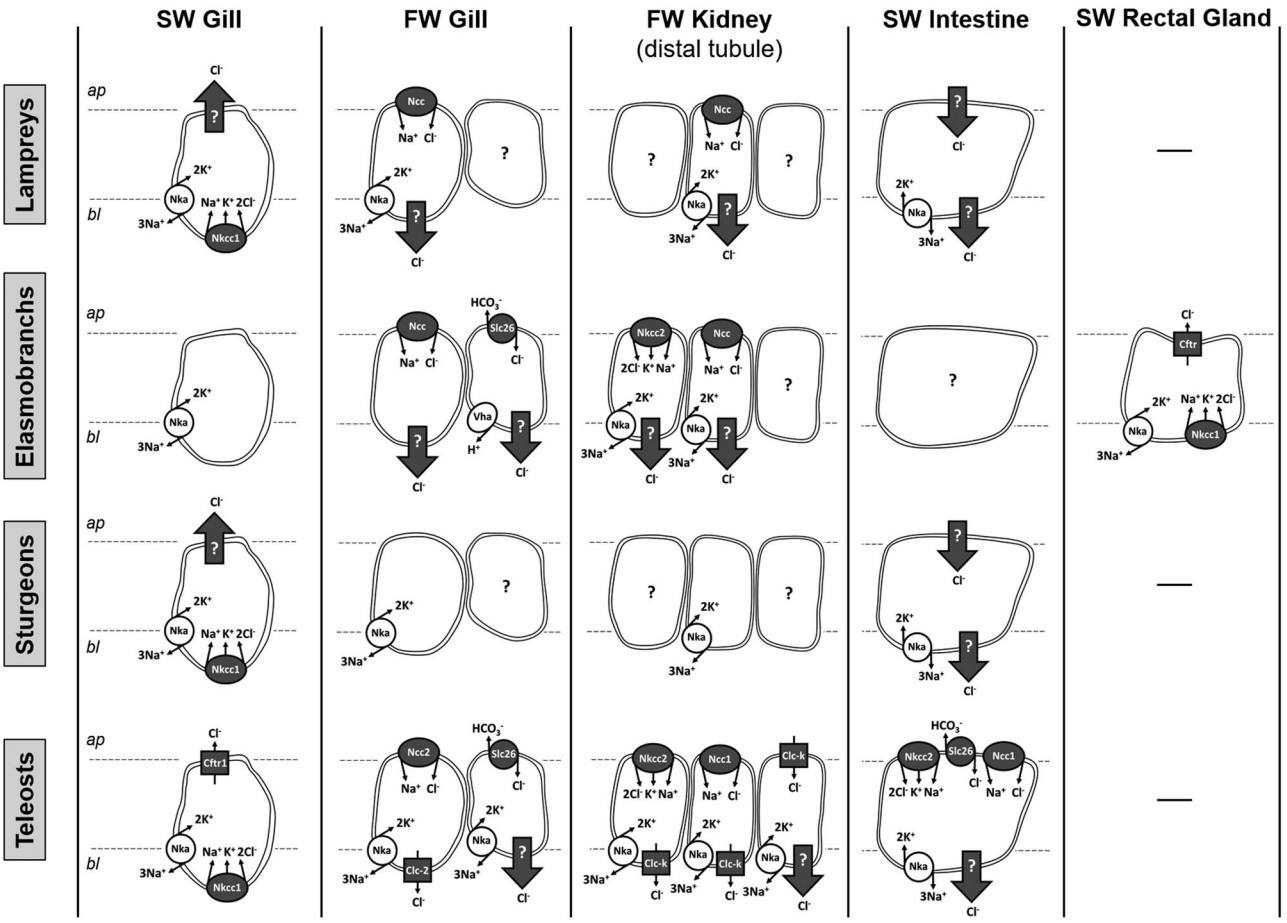

FIGURE 2 Cellular and molecular mechanisms of Cl^-transport in key ionoregulatory organs in fishes. Schematic diagrams present molecular mechanisms for Cl^-transport that operate in SW and FW gill, FW renal distal tubule, and SW intestine of lampreys, elasmobranchs, sturgeons, and teleosts. Apical and basolateral sides are presented at the top and bottom of cells, respectively. Cell models were derived from protein and/or gene transcript expression patterns depending on data availability. The figure provides a summary of the recently characterized Cl^-transport pathways discussed in this "Perspective" and does not intend to serve as an exhaustive depiction of all established or putative pathways. Where Cl^-transport is indicated with an arrow and question mark, the presence of a Cl^-transport pathway is presumed to exist based on functional observations, but the particular molecular pathway has not been identified. See text for citations. ap, apical; bl, basolateral; Cftr, cystic fibrosis transmembrane conductance regulator; Clc, voltage-gated Cl^-channel; FW, freshwater; Ncc, Na^+/Cl^-cotransporter; Nka, Na^+/K^+-ATPase; Nkcc, $Na^+/K^+/2Cl^-$cotransporter; Slc26, anion exchanger; SW, seawater; Vha, V-H^+-ATPase

A single *cftr* orthologue was previously identified in a genome-wide scan of the sea lamprey, the most basal extant osmoregulating vertebrate (Ren et al., 2015). Ensuing RNAseq analyses in sea lamprey detected low levels of *cftr* expression in all larval, juvenile, and adult tissues except for the intestine of FW-acclimated larvae (Ren et al., 2015). A more recent study confirmed high Cftr expression in the distal intestine of FW-acclimated larvae, and importantly, reported major functional differences between the lamprey Cftr and orthologues from jawed vertebrates. For example, lamprey Cftr exhibits limited Cl^- conductance and reduced activation by cAMP versus human Cftr (Cui et al., 2019). Moreover, eel Cftrb and lamprey Cftr exhibit structural divergence in their regulatory domains compared to mammalian Cftrs (Cui et al., 2019; Wong et al., 2016). Intestinal Cftr isoforms of salmon and eel, and the Cftr orthologue of sea lamprey, all possess a reduced number of phosphorylation sites and reduced responsiveness to protein kinase A (PKA). Thus, PKA-activated Cl^- secretion via Cftr does not appear to be a common trait among all vertebrate Cftr isoforms.

Although Cftr orthologues appear to be present throughout the vertebrate phylogeny, it is plausible that its functionality as a Cl^- channel has changed throughout vertebrate evolution. Given the limited Cl^- conductance of lamprey Cftr and the lack of a *cftr* transcriptional response to SW exposure in the gill (Shaughnessy et al., unpublished), it stands entirely unresolved whether Cftr mediates branchial Cl^- secretion at the molecular level in lamprey (Figure 2). Given that SW-acclimated lampreys do in fact utilize Nka- and Nkcc1-expressing SW-type branchial ionocytes (see below), the possibility that an alternate Cl^- channel operates as the primary molecular route for branchial Cl^- secretion is an exciting avenue for exploration.

2.2 | Cation-chloride cotransporters (Slc12 family)

The Slc12 family of electroneutral cation-chloride cotransporters includes $Na^+/K^+/2Cl^-$ cotransporters (Nkcc1/Slc12a2 and Nkcc2/Slc12a1), Na^+/Cl^- cotransporters (Ncc1/Slc12a3 and Ncc2/Slc12a10), and K^+/Cl^- cotransporters (Kcc1-3/Slc12a4-6). All Slc12 family members are responsible for transporting Cl^-, with Na^+ and/or K^+, into a given cell, although they may differ in their transport stoichiometry and cellular location (apical vs. basolateral). The participation of a basolateral Nkcc1 in Cl^--secreting ionocytes of the teleost gill and elasmobranch rectal gland is firmly established (Gagnon et al., 2002; Marshall & Grosell, 2006; Pelis et al., 2001). Nkcc2 and Ncc are expressed within the apical membrane of Cl^--absorptive cells within the gill (Ncc2) and kidney/urinary bladder (Ncc1 and Nkcc2) of FW-acclimated teleosts and within the intestine (Nkcc2) of SW-acclimated teleosts (Figure 2; Kato et al., 2011; Li et al., 2014).

The involvement of Nkcc1 in branchial Cl^- secretion by basal fishes has only recently been investigated. Nkcc1 and Nka were increased in sturgeons exposed to salinities ≥25‰ (McCormick et al., 2020; Sardella & Kültz, 2009; Shaughnessy et al., 2015). In sea lamprey, gill *nkcc1* increased during metamorphosis (the preparatory phase preceding SW migration) and following exposure to SW. Sea lamprey Nkcc1 is colocalized with Nka in presumptive Cl^--secreting branchial ionocytes and functionally critical to maintaining Cl^- homeostasis in SW (Shaughnessy & McCormick, 2020).

In SW-acclimated/marine teleosts, the intestine mediates solute-linked water uptake via a suite of ion transporters, channels, and pumps (Grosell, 2006; Sundell & Sundh, 2012). Apically located Nkcc2 mediates the entry of Na^+ and Cl^- into the interior of enterocytes before their subsequent transport across the basolateral membrane (Grosell, 2014; Whittamore, 2012). In Mozambique tilapia (*Oreochromis mossambicus*), Nkcc2 is highly expressed on the luminal surface of the intestine in animals inhabiting SW (Li et al., 2014). In Atlantic salmon, intestinal *nkcc2* increases during smolting and SW acclimation (Sundh et al., 2014). Functional studies exploiting the bumetanide-sensitivity of Nkcc2 have associated *nkcc2* expression with bumetanide-inhibitable current across the intestine of SW-acclimated Japanese eel (Ando & Takei, 2015). In green sturgeon (*A. medirostris*), imbibed Cl^- is progressively removed from the gastrointestinal lumen, but the molecular mechanisms for desalinization in the sturgeon gut remain unknown (Allen et al., 2009). It was suggested that sea lamprey intestine lacks bumetanide sensitivity (Shaughnessy & McCormick, 2020), and that Nkcc2 might not be a major pathway for intestinal Cl^- transport in lampreys. Nonetheless, it was shown that Cl^- transport does support SW-adaptive processes operating within the sea lamprey intestine (Barany et al., 2020). Therefore, it stands unresolved whether an Slc12 family member underlies luminal Cl^- removal and supports SW adaptation in this basal vertebrate species (Figure 2).

In contrast to the well-established paradigm for ion secretion by "SW-type" ionocytes, various models seek to explain how "FW-type" ionocytes (within the gill and skin) absorb ions from dilute environments (Evans, 2008; Takei et al., 2014). These varying models reflect, in part, the evolution of multiple strategies for ion uptake in FW (Dymowska et al., 2012; Takei et al., 2014; Yan & Hwang, 2019). Hiroi et al. (2008) discovered that FW-acclimated Mozambique tilapia express Ncc in the apical membrane of ionocytes. This Ncc is denoted Ncc2 (Slc12a10) and is not a member of the "conventional" Ncc1 (Slc12a3) clade (Takei et al., 2014). Ionocytes that express Ncc2 in tilapia (coined "Type-II" ionocytes) leverage Ncc2 for the transport of Na^+ and Cl^- from FW into the ionocyte interior. Zebrafish (*Danio rerio*) also exhibit Ncc2-mediated Cl^- uptake by a subset of ionocytes termed "Ncc-cells" (Wang et al., 2009). Convincing evidence established that Ncc2-expressing ionocytes are in fact critical to Cl^- homeostasis (Hiroi et al., 2008; Inokuchi et al., 2008; Kwong & Perry, 2016; Wang et al., 2009). Ncc2-expressing ionocytes operate in species spanning across teleost clades but are seemingly absent in salmonids (Hiroi & McCormick, 2012; Hsu et al., 2014; Inokuchi et al., 2017; Lema et al., 2018). Recently, mummichogs were found to express Ncc2 in the apical region of FW-type ionocytes (Breves et al., 2020). This was a curious finding given that branchial epithelium was previously considered a minor contributor to Cl^- uptake by FW-acclimated mummichogs (Marshall et al., 1997; Wood & Laurent, 2003). Rather, the diet may provide Cl^- for intestinal absorption with the branchial epithelium operating to minimize Cl^- loss via the coordinated expression of tight-junction proteins (Chasiotis et al., 2012; Patrick et al., 1997).

Roles for Ncc in the osmoregulatory organs of basal fishes stand largely undefined. A recent study, however, implicated Ncc in branchial ion absorption by cartilaginous fishes. In Japanese-banded houndshark (*Triakis scyllium*), a "conventional" *ncc1* (*slc12a3*) is expressed in a subpopulation of gill ionocytes (type-B cells) where its expression was increased upon transfer from full SW to 30% SW (Takabe et al., 2016). In turn, Takabe et al. (2016) proposed that for elasmobranchs in which Ncc2 is absent, Ncc1 mediates branchial Na^+ and Cl^- absorption (Figure 2). Similarly, sea lamprey *ncc* is highly expressed in the gill and kidney and is downregulated during acclimation to high-salinity (>50% SW) conditions (Ferreira-Martins et al., 2016). These two studies further indicate that the FW gill Ncc2, which is not expressed in the FW kidney, ought to be regarded as a "ray-finned fish-specific" or "bony fish-specific" Ncc. It follows then that an Ncc2 should be expressed in the gill of FW-acclimated sturgeon. Our preliminary analysis of the sterlet sturgeon genome indicates that two distinct *ncc*-like paralogues exist that have yet to be characterized.

Renal Ncc1 supports Na^+ and Cl^- reabsorption and the production of dilute urine by teleosts inhabiting FW (Kato et al., 2011; Teranishi et al., 2013; Wingert et al., 2007; Figure 2). By localizing Ncc1 to the distal tubules and collecting ducts of FW-acclimated bullshark (*Carcharhinus leucas*), Imaseki et al. (2019) provided the first evidence that Ncc1 contributes to tubular Na^+ and Cl^- reabsorption in elasmobranchs. Finally, hydrochlorothiazide-sensitive transporters such as Ncc1 and Ncc2 were recently proposed as candidate transporters of Na^+ and Cl^- in the desalination of SW by the esophagus of Japanese eel (Takei et al., 2017).

2.3 | Anion exchangers (Slc26 family)

Emerging evidence associates Slc26 family anion exchangers with the uptake of environmental Cl^- in exchange for HCO_3^- by ionocytes (Figure 2). In euryhaline Atlantic stingray (*Dasyatis sabina*), anti-human Slc26a4 (pendrin) immunoreactivity was localized within the branchial epithelium of FW-acclimated animals (Piermarini et al., 2002). In zebrafish, three distinct *slc26a3*, *-4*, and *-6c* genes are expressed by ionocytes. Slc26a4 and -6c, however, assume greater roles in Cl^- uptake when environmental Cl^- is severely depleted (Bayaa et al., 2009; Perry et al., 2009). It remains unclear whether Slc26a3, -4, and -6c operate within a type of ionocyte that is distinct from the four types already described in zebrafish (HR-, NaR-, Ncc-, or KS-cells; Guh et al., 2015). More recently, it was reported that branchial *slc26a6* expression was higher in FW- versus SW-acclimated rainbow trout (*Oncorhynchus mykiss*), thereby adding additional support for the operation of Slc26-dependent pathways for Cl^-/HCO_3^- exchange in FW-acclimated fishes (Boyle et al., 2015; Leguen et al., 2015). The presence and function of Slc26 family anion exchangers in lampreys and sturgeons remain unresolved and should be the focus of future investigations.

2.4 | Voltage-gated Cl^- channels (Clc Cl^- channel family members)

Among members of the Clc Cl^- channel family, Clc-2, Clc-3, and Clc-K support Cl^- balance in fishes (Figure 2). Ion-absorptive ionocytes that express Ncc2 in the apical membrane must transport Cl^- across the basolateral membrane. In zebrafish, Clc-2c mediates the movement of Cl^- from the interior of Ncc-cells into blood plasma (Pérez-Rius et al., 2015; Wang et al., 2015). Accordingly, knockdown of *clc-2c* perturbed whole-body Cl^- balance (Wang et al., 2015). In tilapia, branchial *clc-2c* expression is elevated in parallel with *ncc2* during FW acclimation (Breves et al., 2017). Recall that salmonids lack Ncc2-cells (Hiroi & McCormick, 2012); thus, Clc-2 is not restricted to Ncc2-expressing ionocytes given its gene expression in FW-type rainbow trout ionocytes (Leguen et al., 2015). In pufferfish (*Tetraodon nigroviridis*), European sea bass (*Dicentrarchus labrax*), and Mozambique tilapia, Clc-3 is expressed within branchial ionocytes and may contribute to Cl^- uptake from FW (Bossus et al., 2013; Miyazaki et al., 1999; Tang & Lee, 2011; Tang et al., 2010). Within esophageal epithelium, Clc-2 supports the desalination of imbibed SW that enables intestinal water absorption by Japanese eel (Takei et al., 2017). The in vitro effects of serosally applied diphenylamine-2-carboxylic acid (DPC) indicated that Clc-2 mediates the basolateral transport of Cl^- by columnar cells within the esophagus (Takei et al., 2017). Finally, Clc-K underlies the capability of renal tubules to reabsorb Cl^- in teleosts residing in FW (Kato et al., 2011; Miyazaki et al., 2002; Pérez-Rius et al., 2019; Wingert et al., 2007). Barttin, an accessory subunit that stabilizes Clc-K, is coexpressed with Clc-K in the apical region of distal nephrons of zebrafish (Pérez-Rius et al., 2019). Clc-K is indispensible to Cl^- balance given that knockout of *clc-k* is lethal to embryonic zebrafish undergoing mesonephrogenesis (Pérez-Rius et al., 2019). It currently stands unresolved how zebrafish Clc-K-expressing cells mediate basolateral Cl^- transport (Figure 2).

3 | ENDOCRINE REGULATION OF Cl^- TRANSPORT IN FISHES

Perturbations to hydromineral balance elicit the release of hormones that orchestrate the expression, localization, and function of solute transporters/channels. While a broad collection of hormones undoubtedly contributes to Cl^- balance in teleosts, prolactin and cortisol have widely accepted roles as "FW- and SW-adapting" hormones, respectively (Takei et al., 2014). We conclude our discussion by highlighting new insights into the roles of prolactin and cortisol (and other corticosteroids) in regulating mechanisms of Cl^- transport.

3.1 | Prolactin

In agreement with prolactin being recognized as a "FW-adapting" hormone (Pickford & Phillips, 1959), prolactin binding and *prolactin receptor* gene expression occur in key ionoregulatory organs including the gill, kidney, gut, and skin (Breves et al., 2014, 2020). The prolactin receptor was further localized to ionocytes in the gill (Santos et al., 2001; Weng et al., 1997) and to the proximal segment of pronephric tubules (Liu et al., 2006). Only recently have discrete Cl^--transporting processes been linked with prolactin. The failure of hypophysectomized tilapia to adequately recruit Ncc2-expressing ionocytes during FW acclimation was rescued by prolactin replacement (Breves et al., 2010). Prolactin acts directly on the gill and does not require a systemic intermediary to promote Ncc2 expression within ionocytes (Inokuchi et al., 2015). A prolactin-Ncc2 regulatory link also operates in zebrafish and Japanese medaka (*Oryzias latipes*) gill (Bollinger et al., 2018; Breves et al., 2013). In medaka, prolactin stimulated *ncc2* expression via Stat5 activation and not through ERK- or AKT-dependent signal transduction pathways (Bollinger et al., 2018). Moreover, *prolactin*-deficient zebrafish embryos do not fully express *ncc1* and *-2* in the pronephros and epidermis, respectively (Breves et al., 2014; Shu et al., 2016). Given that Ncc2 and Clc-2c are coexpressed within branchial and epidermal ionocytes to facilitate transcellular Cl^- transport (Wang et al., 2015), it is logical that prolactin also promotes *clc-2c* expression (Breves et al., 2017; Breves, 2019). To date, any links between prolactin and teleost Slc26s remain entirely uncharacterized.

A distinct *prolactin* gene arose in a vertebrate ancestor that preceded Agnathans (Ocampo Daza & Larhammar, 2018). While only growth hormone has been identified in sea lamprey (Kawauchi et al., 2002), seemingly due to loss of the *prolactin* gene (Ocampo Daza & Larhammar, 2018), sea lamprey express distinct *prolactin* and *growth hormone receptor* genes (Gong et al., 2020). Thus, lampreys are now poised to offer unique insights into how prolactin receptor signaling

(perhaps with growth hormone as the cognate ligand) connects with Cl^- balance in a basal vertebrate. Perhaps prolactin receptor-mediated signaling underlies the branchial expression of *ncc* in lampreys residing in FW (Ferreira-Martins et al., 2016).

3.2 | Corticosteroids

Cortisol is the primary mineralocorticoid and glucocorticoid in teleosts (Prunet et al., 2006), with both functions mediated primarily via the glucocorticoid receptor. Given these dual roles for cortisol in teleosts, glucocorticoid receptors are widely distributed throughout the body with robust expression in osmoregulatory (gill, kidney, intestine) and gluconeogenic (liver) organs (Greenwood et al., 2003). As an established osmoregulatory hormone in teleosts, cortisol stimulates the abundance and/or activity of ion transporters and pumps that underlie branchial Cl^- secretion (e.g., Nka, Nkcc1, and Cftr; McCormick, 2001; Takei et al., 2014). The realization that cortisol can promote branchial ion uptake in some FW-acclimated teleosts arrived long after its SW-adaptive role was firmly established. In the gill and yolk sac of particular teleosts, Na^+/H^+ exchanger 3 (Nhe3) and Ncc2 are expressed in distinct ionocytes where they mediate ion absorption (Guh et al., 2015; Hiroi & McCormick, 2012). In larval zebrafish, cortisol stimulated Na^+ uptake in a fashion dependent upon the presence of Nhe3b-expressing cells (Kumai et al., 2012) and promoted the differentiation of Ncc-cells from a progenitor population (Cruz et al., 2013; Kumai et al., 2012). Moreover, Lin et al. (2016) showed that cortisol treatment increased *ncc2* expression and Ncc2-dependent ion absorption. Cortisol similarly increased *ncc2b* expression in ex vivo Japanese medaka gill (Bossus et al., 2017).

Only recently have roles for corticosteroid signaling towards Cl^- balance in basal fishes become clearer. Cortisol is firmly established as a glucocorticoid in sturgeons (Webb et al., 2007). A recent study in Atlantic sturgeon (*Acipenser oxyrhynchus oxyrhynchus*) reported that plasma cortisol is elevated after SW exposure and promotes branchial Nka and Nkcc1 expression, and, thereby, provided evidence that cortisol is in fact an osmoregulatory hormone in sturgeons (McCormick et al., 2020). Steroidogenic pathways in elasmobranchs result in a novel circulating corticosteroid, 1α-hydroxycorticosterone, which exerts some mineralocorticoid-like activity (Anderson, 2012). Lampreys cannot produce cortisol because they lack 11β-hydroxylase; alternatively, 11-deoxycorticosterone (DOC) and the biochemical precursor to cortisol, 11-deoxycortisol (S), are the primary circulating corticosteroids (Close et al., 2010; Rai et al., 2015; Roberts et al., 2014). In vivo administration of S, but not DOC, promoted Cl^- homeostasis during a SW challenge by stimulating branchial Nka and Nkcc1, suggesting that discriminative corticosteroid control of Cl^- homeostasis appeared early in vertebrate evolution (Shaughnessy et al., 2020). A functional explanation for the substantial levels of circulating DOC in lamprey is lacking. Although DOC does not appear to control active Cl^- transport by the gill (Shaughnessy et al., 2020), it could be that DOC controls tight-junction permeability and barrier functions in the gill and/or other osmoregulatory organs such as the intestine.

4 | CONCLUSION

New perspectives on how epithelial Cl^- transport is achieved have fostered the discovery of regulatory connections between the endocrine system and the molecular mechanisms that allow teleost fishes to inhabit diverse aquatic habitats. Prospective studies on basal fishes, which are now enabled by newly available genomic resources, will offer valuable insight into how processes underlying Cl^- transport evolved within the vertebrate lineage. As the most basal extant osmoregulating vertebrate, and with its well-developed genome assembly, the sea lamprey will serve as an important model organism in this effort. It will be particularly exciting to unravel how the evolution of vertebrate endocrine systems paralleled the evolution of ionoregulatory strategies.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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