ROLE OF ION CHANNELS IN SALT SECRETION BY ATLANTIC SALMON GILLS DURING ACCLIMATION TO SEAWATER

Francisco J. Morera[1,*], David Baez-Nieto[2], Yenisleidy Lorenzo[2], Karen Castillo[2], Amaury Pupo[2], Luis Vargas-Chacoff[3] and Carlos Gonzalez[2,*]

[1]Institute of Pharmacology and Morphophysiology, Faculty of Veterinary Sciences, Universidad Austral de Chile, Valdivia, Chile
[2]Interdisciplinary Center for Neuroscience of Valparaiso, Faculty of Sciences, Universidad de Valparaiso, Valparaiso, Chile
[3]Institute of Marine Sciences and Limnology, Faculty of Sciences, Universidad Austral de Chile, Valdivia, Chile

***Correspondence to:**
Dr. González C. (carlos.gonzalezl@uv.cl)
Dr. Morera FJ. (fjmorera@uach.cl)

ABSTRACT

Smoltification, also called parr-smolt transformation, is a complex developmental process that consists of a number of independent, but coordinated changes, in the biochemistry, physiology, morphology and behavior of juvenile salmon in their transition from freshwater to seawater life. A key component of smoltification is represented by the physiological adaptations that enable smolts to thrive in hyperosmotic environments. Instrumental to this process is the ability of smolt gills to gradually become capable of actively secreting salt through specialized cells known as mitochondria-rich (MR) cells, ionocytes or chloride cells. NaCl secretion by teleost gills is therefore accomplished via the secondary active transport of Cl^- and the passive transport of Na^+. The driving force for active transport is provided by Na^+/K^+ ATPase, which maintains low intracellular Na^+ and high intracellular K^+ concentrations. However, this NaCl secretion mechanism needs at least two different ion channels: A CFTR type chloride channel for the passive exit of Cl^- and a potassium channel to recycle extracellular K^+, which is a thermodynamic prerequisite to work under conditions imposed by high extracellular salinity in seawater. The characteristics of K^+ channels required for NaCl secretion from MR cells into seawater are still unknown for *Salmo salar* and only recently have begun to be studied in other teleosts.

Keywords: smoltification, salt secretion, ion channels, atlantic salmon

Original received, March 27, 2015; revision received, April 30, 2015; accepted, May 4, 2015

1. GENERAL BACKGROUND

1.1. General summary of the salmon life cycle

Salmonids begin life in freshwater. After adult salmons spawn in freshwater, eggs hatch into alevins and begin their development into fry and parr. At this stage, environmental cues initiate the smoltification process, thus preparing the fish for downstream migration and entrance into seawater, where they will grow to become a marine, predatory species [1, 2]. This anadromous strategy confers reproductive and developmental advantages to salmon, because it enables them to utilize a relatively safe environment provided by freshwater for reproduction, whereas juvenile migration towards the ocean allows them to feed on a rich supply of fish and other marine organisms [3]. Hence, smoltification represents the key turning point in the anadromous life cycle of *Salmo salar* [2, 4].

1.2. Physiological changes during smoltification

Smoltification, is a complex process driven by the endocrine system which consists of a series of changes, from cellular physiology to morphology in the juvenile salmon, as an adaptive response in order to survive in the new seawater environment. All of these changes prepare the fish for downstream migration and transition to the marine life stage [2, 3]. Key elements of the parr-smolt transformation are: i) environmental cues, such as primarily photoperiod and temperature [1]; ii) endocrine control of smoltification [2]; and iii) the physiological changes in osmoregulation that allow the smolt to thrive in high-salt environments [5, 6].

1.3. Hormonal and environmental regulation of smoltification

It is well established that the photoperiod and seasonal temperature fluctuations are two important environmental signals that work together in the transformation of Atlantic salmon parrs into smolts [5]. Smoltification is complete by spring, when a rising temperature by around 8-10^{o}C initiates the migration of wild smolts into seawater [5, 7]. The mechanism by which photoperiodic information is translated into a neuroendocrine response in teleosts has not been elucidated. Even so, the melatonin secretion by the pineal gland of salmonids can be directly entrained by a specific photoperiod [8] but the relationship between melatonin rhythm and the photoperiod in fish is unclear [9].
The Parr-smolt transformation involves several endocrine signaling systems. Some examples of well-studied endocrine mediators of smoltification are the growth hormone/insulin-like growth factor I system (GH-IGF-I), cortisol and thyroid hormones, all of them promote the smoltification process [10]. On the other hand, there is other hormones such as prolactin, which is considered to play an inhibitory function in the smoltification process [10, 11]. The light–brain–pituitary axis is stimulated by a size- or growth-related threshold, resulting in higher levels of GH, cortisol and thyroid hormones [8, 12]. GH, modulates the fish intermediary metabolism and osmoregulatory mechanisms by stimulating the activity of somatomedins, such as insulin-like growth factors IGF-1 and IGF-2 [13]. GH and cortisol interact to control hyperosmoregulatory mechanisms in gills, gut, and kidneys, promoting increased salinity tolerance as well as changes in growth and metabolism. In gills, cortisol and the GH/IGF-I axis promote the

differentiation of salt-secreting ionocytes (see section 3.2), which is a process that requires the up regulation of three major osmoregulatory membrane transporters: sodium-potassium ATPase (NKA), sodium-potassium-2 chloride cotransporter 1 (NKCC1), and the cystic fibrosis transmembrane conductance regulator (CFTR) [13]. Lastly, thyroid hormones regulate imprinting, metabolism, morphological changes such as silvering, and possibly behavior [8].

1.4. Osmoregulatory changes during smoltification

As was mentioned above, salmonids begin their life cycle in freshwater, where they present a hyperosmotic intracellular equilibrium respect to the external medium. In this situation, osmotic pressure favors the entry of water into the body and the loss of salt by diffusion across the gill. To compensate for this passive flow of water and ions, the fish eliminate excess water as diluted urine and obtain salts from food and by active uptake through the gill's epithelium [14]. However, when they move into seawater, the osmotic gradient is reversed and they become hyposmotic relative to the external medium (the internal fluids of salmonids are approximately one-third the osmolarity of seawater). Therefore, salmon lose water and gain salts by passive diffusion. As compensatory mechanisms, they drink seawater, reduce their urine production and actively secrete salts across the gill's epithelium through specialized type cell, the MR cells [5] (See Fig. 1).

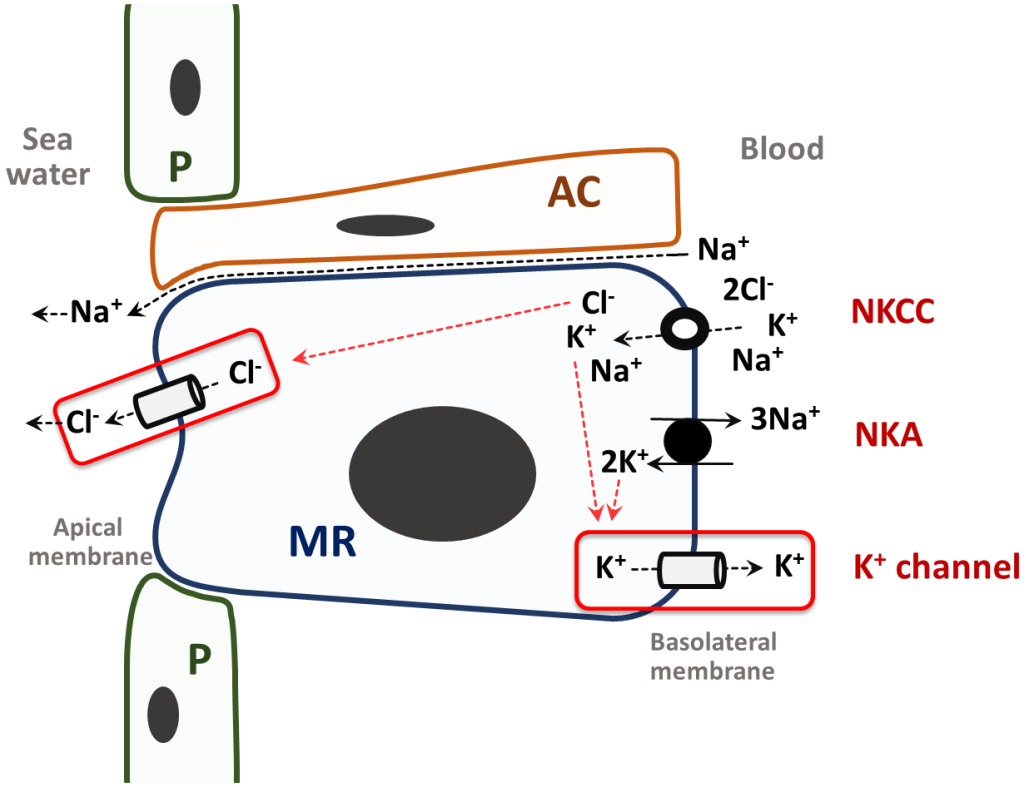

Fig. 1 Model of NaCl secretion by mitochondria-rich (MR) cells in gill epithelium. Cl^- enters MR cells via a $Na^+/K^+/2Cl^-$ co-transporter driven by the Na^+ gradient, which is maintained by Na^+/K^+ ATPase (NKA). Chloride ions accumulates intracellularly and exits at the apical membrane through CFTR-type anion channels. Na^+ is secreted paracellularly. The red rectangle highlights the potassium channels required in this process. AC: accessory cell (Marshall and Grosell, 2006).

One of the key events in osmoregulatory changes during parr-smolt transformation involves the fine-tuning of the ion-transporting machinery in the gill epithelium [14]. Ion transport is primarily carried out by MR cells [3], and requires their rearrangement as well as changes in the expression of NKA, NKCC1 and other critical ion transport proteins at the cell surface [13]. A strong upregulation of gill NKA and NKCC1 is associated with seawater acclimation in most euryhaline teleosts [13, 15, 16].

1.5. Molecular mechanism of salt secretion by MR cells.

The NaCl secretion mechanism by MR cells in gills of teleost fish living in seawater has been studied extensively [for further reading see Evans et al., 2005 and Hiroi and McCormick, 2012], leading to a consensus model of NaCl transport (Fig. 1). This working model for NaCl secretion includes ouabain-sensitive NKA and bumetanide-sensitive NKCC1, both located in the basolateral membrane, as well as the anion channel CFTR in the apical membrane. NKCC1 mediates the entry of Na^+ and Cl^- into the cellular compartment down the electrochemical gradient provided by NKA, followed by the passive exit of Cl^- and Na^+ through the apical channel CFTR and the paracellular tight-junction pathway, respectively [8, 14, 16]. NaCl secretion by teleost gills is accomplished via secondary active transport of Cl^- and passive transport of Na^+ (Fig. 1). The driving force for active transport is provided by NKA, which maintains intracellular Na^+ at low levels and high intracellular K^+ concentration [15]. However, this NaCl secretion mechanism needs an additional condition to work under seawater, namely a thermodynamic requirement to recycle K^+ out, via conductive pathways mechanism (potassium channels, shown in Figure 1).

1.6. General principles of physiological osmoregulatory mechanisms in teleost fish

Ion and water homeostasis is an important challenge for fish, especially because they have to maintain their internal milieu in despite of the amount of salt present in the aqueous environment. In some cases, the water that fish are in contact with could vary in a range from freshwater (FW) with very few osmolytes (~0 mOsmol kg^{-1}) to approximately 1000 mOsmol kg^{-1} in seawater (SW).

All FW fishes maintain their internal salt concentration at approximately 250–320 mOsmol kg^{-1}. They have to counter a diffusive ion loss and osmotic gain of water across the gill epithelium and other external surfaces. They solved this problem through two different process: a) to obtain ions via active transport of both Na^+ and Cl^- across the gill epithelium from water and via the intestine from ingested food and b) the production of large volumes of dilute urine [17]. On the other hand, SW teleosts maintain their extracellular fluids at approximately one-third the osmotic concentration of SW. They suffer an osmotic loss of water across their gills and other epithelial surfaces. To counter this, they drink SW to maintain osmotic balance, absorbing water and salts but excreting the excess salts through ionocytes in the epithelium of the gills and skin. The transepithelial potential of the gill in SW is positive and allow the paracellular transport of Na^+, whereas the transport of Cl^- is carried out by an active transcellular process. Any excess uptake of divalent ions is excreted by the kidney [17].

In general, different fishes have evolved and adapted to deal with both dehydrating and highly dilute environments. Most fishes are unable to move between freshwater and seawater (stenohaline), but around of 3–5% of fishes are capable of surviving in a wide range of salinities (euryhaline). Euryhaline fishes represent important model species for elucidating the physiological mechanisms of salt and water balance among vertebrates [18] and it is important to mention that some euryhaline fishes such as salmon, eels, and tilapia are economically important in worldwide fisheries and aquaculture.

2. STRUCTURAL INSIGHTS OF ION CHANNELS INVOLVED IN NaCl SECRETION IN THE GILL

2.1 CFTR.

Cystic fibrosis transmembrane conductance regulator (CFTR) belongs to the ATP-binding cassette (ABC) transporter family. The members of this family is spread through the different branches among of life kingdoms (Higgins, 1992). The canonical architecture of ABC transporters consist of 2 transmembrane domains (TMDs).

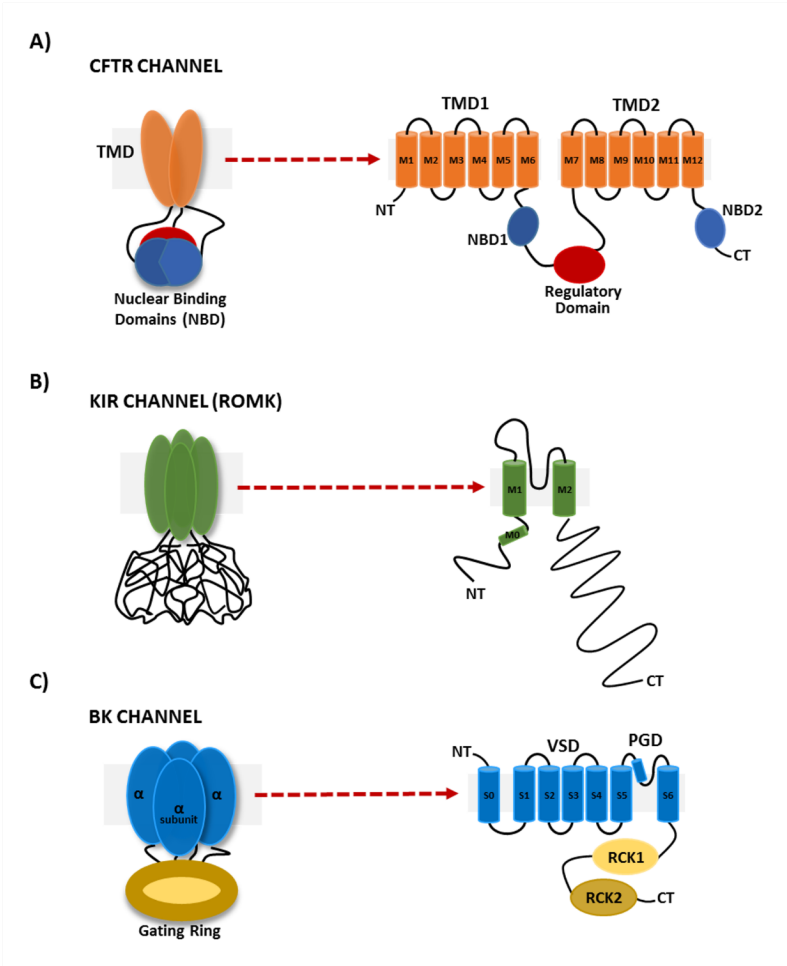

Fig. 2 Architecture of different ion channels involved in smoltification process. **A.** The CFTR chloride channel is divided in two transmembrane structural domains (TMD), each one contains six transmembrane segments (TM1-TM6 / TM7-TM12). It possess two intracellular nucleotides binding domains and a regulatory domain, which controls the activity of the channel. **B.** Kir channels present a tetrameric structure, where each monomer consist of two transmembrane domains (M1-M2) and intracellular helix (M0) in the N-terminal domain. The intracellular C-terminus harbors the pH regulation domain, and important structural determinants associated to gating and rectification. **C**. BK channel is a Ca^{2+} activated K^{+} channel, in contrast to Kv channel it has an extra transmembrane segment domain (S0), and the N-terminus facing the extracellular environment. BK channel activity is tightly regulated by intracellular Ca^{2+} concentrations, the molecular machinery that confers the Ca^{2+} sensitivity to BK is located in the C-terminus intracellular domain. This channel present two RCK domains, which conform a macro domain called gating-ring, which controls the BK channel activity to the local increases of intracellular Ca^{2+}.

Each one is formed by 6 transmembrane segments, S1-S6 harbored in the TMD1 and S7-S12 in the TMD2. In addition this protein presents two intracellular nucleotide-binding domains (NBDs) and a regulatory domain (Fig. 2A). This domains expressed as fusion protein do not prefer any order (TM1D/TMD2 or TMD2/TMD1) to produce fully functional channels [19]. Interestingly most of the member of ABC family function as classical transporters, CFTR is an anionic non-selective channel, with increased permeability to chloride and bicarbonate ions. Structural details and transport mechanism insights has been recently reviewed by Cant et al. (2014) [20].

2.2 Potassium channels

The hypothesis that K^+ ions entering the cell through NKA and NKCC1 are recycled via K^+ channels has been supported by experiments measuring epithelial short-circuit currents in the opercular epithelium from killifish. The different conductances present, whether in the apical or basolateral side of the cell, were revealing with an ussing-style membrane chamber, which works as a voltage-clamp for the whole epithelium. The addition of 2 mM Ba^{2+} (a non-specific blocker for potassium channels**)** to the basolateral side is able to inhibit short-circuit currents by 77%, suggesting that the function of K^+ channels in the basolateral membrane is to recycle K^+ ions, pumped into the cell by NKA (reviewed by Evans et al., 1999 and 2005). The molecular identity of this K^+ conductance is still unknown in salmonids, but there are several K^+ channels involved in MR cell function in other teleosts fish [15]. One plausible candidate for the K+ recycling is the inward-rectifying K^+ channel (eKir), which is highly expressed in gills of the seawater-acclimated Japanese eel [21]. More recently, an inward-rectifying K^+ channel (Kir1.1 or ROMK for **R**enal **O**uter **M**edullary **K**$^+$ channel) and a BK channel have been detected in MR cells from the *Mozambique tilapia* [22]. Another candidate is a large-conductance calcium-activated K^+ channel (called BK for "**B**ig **K**$^+$" given its large unitary conductance compared to other K^+ channels), whose expression was recently detected in gills from the teleost fish *Porichthys notatus* [23] and recently in *Salmo salar* [24].

2.1.1. Kir and ROMK channels

Inward rectifying K^+ (Kir) channels are tetrameric complexes, assemble around its symmetry axis, which is the K^+ conductive pathway. Interestingly, Kir channel lacks the classical voltage sensor domain (VSD) present in other voltage-gated K^+ channel, each subunit is composed just by two transmembrane segments (M1 and M2) [25]. These transmembrane segments resemble the S5-S6 domain, present in voltage-gated K^+ channel, which confer the selectivity to K^+ ions over others [25]. In addition, each monomer presents short intracellular alpha-helix (M0), which precedes M1 and M2. This K^+ conductance is highly regulated by intracellular domains N and C-terminus [26] (Fig. 2B). Inward rectification refers to the ability of an ion channel to permeate ions in the inward direction rather than outward direction. The outcome is that Kir channel present robust inward currents at hyperpolarized potential, and there is no ionic current at positive voltages. There are several rectification mechanisms, being the particular one for Kir channel that given by a fast blockage of outward current by intracellular Mg^{2+} or polyamines [27]. In the case of smoltification, the Kir channel type involved in the process is Kir1.1 or ROMK, which among others Kir channels shows a relative weak

rectification. Kir1.1 presents a tight regulation by internal pH, at physiological range, which could be important for the smoltification process [28].

2.1.2. BK channels

The high conductance voltage- and Ca^{2+}-activated K^+ channel is one of the most broadly expressed channels in metazoans. The name 'Big K' stems from its single-channel conductance that can be as large as 250 pS under symmetrical 100 mM K^+ condition [29, 30]. BK channels are homotetramers formed by its α-subunit, encoded by the *slo-1* gene (KNCMA1), it is member of the voltage-gated potassium (Kv) channel superfamily [31]. BK channel architecture is different from typical Kv channel, it has an extra transmembrane segment (S0), thus each monomer presents seven transmembrane segments instead of six [32].
This channel presents an exquisite regulation by intracellular Ca^{2+}, achieving its maximum activity (Po ~ 1) at 100 μM [33]. The structural motif that confers to BK Ca^{2+} sensitivity is the **R**egulator of **C**onductance of **K**+ (RCK) domain, which is part of the intracellular regulatory domain called gating ring conformed by the N and C-terminus (see part 2.1.3) (Fig. 2C) [34, 35]. BK channels have been implicated in a variety of physiological processes, from the regulation of smooth muscle tone [36] to the modulation of hormone and neurotransmitter release [37]. Interestingly, BK channels are also involved in modulating K^+ transport in the mammalian kidney [38, 39], pulmonary epithelium [40] and colon epithelium [41]. These channels have been characterized in different species, from *Drosophila* to humans, nonetheless detailed studies about their properties and functions in teleosts fish are lacking, with the exception of earlier work from Rohmann and colleagues, which identified BK currents as one of the major outward currents in teleost fish hair cells [23]. The genomic organization of *slo-1* has only recently been reported in the zebrafish (*Danio rerio*) [42]. BK channel transcripts have also been detected in the intestinal epithelium of the European eel (*Anguilla Anguilla*) [43], in the nervous system of different teleost fish and in gills of the midshipman fish (*Porichthys notatus*) [23] and the Mozambique tilapia (*Oreochromis mossambicus*) [22].

3. Coda

The smoltification process is the consequence of an adaptive response of the fish in order to survive the change of its environment, from freshwater to seawater. This tightly regulated process encompasses from huge morphological to subtle physiological changes in the fish. Within this changes, one of the most important is the change in the expression pattern of different set of membrane proteins and ion channels in specialized cells in the gills, called MR cells. The different biophysical properties of the channels mentioned above, help the fish to achieve the osmoregulation in different osmotic conditions (freshwater and seawater). Understanding the functioning of these channels is key to understand the smoltification process in salmonids, which is important for different productive processes, depending on the smoltification, like fish farming.

Acknowledgments: This work was supported by FONDECYT Grants 11130308 (F.J.M); 1120802 (C.G), and ANILLO Grant ACT 1104 (C.G.); (Y.L.) and (A.P.) are funded by PhD fellowship CONICYT. The Centro Interdisciplinario de Neurociencia de

Valparaíso (CINV) is a Millennium Institute supported by the Millennium Scientific Initiative of the Chilean Ministry of Economy, Development and Tourism.

References

[1] **Bjornsson BT & Bradley TM.** Epilogue: Past successes, present misconceptions and future milestones in salmon smoltification research. *Aquaculture* 2007; 273 (2-3):384-391.

[2] **Bjornsson BT, Stefansson SO, & McCormick SD.** Environmental endocrinology of salmon smoltification. *General and comparative endocrinology* 2011; 170(2):290-298.

[3] **Stefansson SO, Björnsson BT, Ebbesson LO, & McCormick SD.** 2008 Smoltification. In: Fish Larval Physiology (Finn, R.N. and Kapoor, B.G., Eds). Science Publishers, Inc. Enfield (NH) & IBH Publishing Co. Pvt. Ltd., New Delhi ISBN 978-1-57808-388-6. (*Science Publishers, Inc.).*

[4] **McCormick SD.** Evolution of the hormonal control of animal performance: Insights from the seaward migration of salmon. *Integrative and comparative biology* 2009; 49(4):408-422.

[5] **Clarke WC, Saunders RL, & McCormick SD.** Smolt Production In: Principles of Salmonid Culture. Eds:W. Pennel and B.A. Barton. *CRC Press, Elsevier. pp. 517-567.* (*Elsevier*).

[6] **Breves JP, Serizier SB, Goffin V, McCormick SD, & Karlstrom RO.** Prolactin regulates transcription of the ion uptake Na+/Cl- cotransporter (ncc) gene in zebrafish gill. *Mol Cell Endocrinol 2013;* 369(1-2):98-106.

[7] **Jonsson B & Ruud-Hansen J.** Water temperature as the primary influence on timing of seaward migrations of Atlantic salmon (Salmo salar) smolts. *Can J Fish Aquat Sci 1985;* 42(3):593-595.

[8] **McCormick SD, Regish AM, Christensen AK, & Bjornsson BT.** Differential regulation of sodium-potassium pump isoforms during smolt development and seawater exposure of Atlantic salmon. *J Exp Biol* 2013; 216(7):1142-1151.

[9] **Falcon J, Besseau L, Sauzet S, & Boeuf G.** Melatonin effects on the hypothalamo-pituitary axis in fish. *Trends in endocrinology and metabolism: TEM* 2007; 18(2):81-88.

[10] **Sakamoto T & McCormick SD.** Prolactin and growth hormone in fish osmoregulation. *General and comparative endocrinology* 2006; 147(1):24-30.

[11] **Manzon LA.** The role of prolactin in fish osmoregulation: a review. *General and comparative endocrinology* 2002; 125(2):291-310.

[12] **McCormick SD.** Endocrine control of osmoregulation in teleost fish. *American Zoologist* 2001; 41(4):781-794.

[13] **McCormick SD*, et al.*** Physiological and endocrine changes in Atlantic salmon smolts during hatchery rearing, downstream migration, and ocean entry. *Can J Fish Aquat Sci* 2013; 70(1):105-118.

[14] **Evans DH, Piermarini PM, & Choe KP.** The multifunctional fish gill: dominant site of gas exchange, osmoregulation, acid-base regulation, and excretion of nitrogenous waste. *Physiological reviews* 2005; 85(1):97-177.

[15] **Marshall W & Grosell M.** Ion transport, osmoregulation, and acid-base balance. *The physiology of fishes* 2006; 3:177-230.
[16] **Hiroi J & McCormick SD.** New insights into gill ionocyte and ion transporter function in euryhaline and diadromous fish. *Respiratory physiology & neurobiology* 2012; 184(3):257-268.
[17] **Edwards SL & Marshall WS.** Principles and patterns of osmoregulation and euryhalinity in fishes. *Fish Physiology* 2013; 32:1-44.
[18] **McCormick SD.** Smolt physiology and endocrinology In: Fish Physiology: Euryhaline Fishes: Fish Physiology Vol 32 Eds by McCormick, SD., Farrel, AP and Brauner, CJ. Academic Press; First edition. pag. 199-251.
[19] **Higgins CF.** ABC transporters: from microorganisms to man. *Annual review of cell biology* 1992; 8:67-113.
[20] **Cant N, Pollock N, & Ford RC.** CFTR structure and cystic fibrosis. *The international journal of biochemistry & cell biology* 52:15-25.
[21] **Suzuki Y, et al.** Identification by differential display of a hypertonicity-inducible inward rectifier potassium channel highly expressed in chloride cells. *The Journal of biological chemistry* 1999; 274(16):11376-11382.
[22] **Furukawa F, Watanabe S, Kimura S, & Kaneko T.** Potassium excretion through ROMK potassium channel expressed in gill mitochondrion-rich cells of Mozambique tilapia. *American journal of physiology. Regulatory, integrative and comparative physiology* 2012; 302(5):R568-576.
[23] **Rohmann KN, Deitcher DL, & Bass AH.** Calcium-activated potassium (BK) channels are encoded by duplicate slo1 genes in teleost fishes. *Molecular biology and evolution* 2009; 26(7):1509-1521.
[24] **Loncoman CA, et al.** Application of a real-time PCR assay to detect BK potassium channels expression in samples from Atlantic salmon (Salmo salar) and Rainbow trout (Oncorhynchus mykiss) acclimated to freshwater. *Archivos de medicina veterinaria* 2015 (In press).
[25] **Kuo A, et al.** Crystal structure of the potassium channel KirBac1.1 in the closed state. *Science* 2003; 300(5627):1922-1926.
[26] **Pegan S, et al.** Cytoplasmic domain structures of Kir2.1 and Kir3.1 show sites for modulating gating and rectification. *Nature neuroscience* 2005; 8(3):279-287.
[27] **Nishida M & MacKinnon R.** Structural basis of inward rectification: cytoplasmic pore of the G protein-gated inward rectifier GIRK1 at 1.8 A resolution. *Cell* 2002; 111(7):957-965.
[28] **Rapedius M, et al.** Structural and functional analysis of the putative pH sensor in the Kir1.1 (ROMK) potassium channel. *EMBO reports* 2006; 7(6):611-616.
[29] **Pallotta BS, Magleby KL, & Barrett JN.** Single channel recordings of Ca^{2+}-activated K+ currents in rat muscle cell culture. *Nature* 1981; 293(5832):471-474.
[30] **Latorre R, Vergara C, & Hidalgo C**. Reconstitution in planar lipid bilayers of a Ca^{2+}-dependent K+ channel from transverse tubule membranes isolated from rabbit skeletal muscle. *Proceedings of the National Academy of Sciences of the United States of America* 1982; 79(3):805-809.
[31] **Butler A, Tsunoda S, McCobb DP, Wei A, & Salkoff L.** mSlo, a complex mouse gene encoding "maxi" calcium-activated potassium channels. *Science* 1993; 261(5118):221-224.
[32] **Meera P, Wallner M, Song M & Toro L.** Large conductance voltage- and calcium-dependent K+ channel, a distinct member of voltage-dependent ion channels

with seven N-terminal transmembrane segments (S0-S6), an extracellular N terminus, and an intracellular (S9-S10) C terminus. *Proc Natl Acad Sci USA*. 1997; 94(25):14066-71.

[33] **Stefani E, et al.** Voltage-controlled gating in a large conductance Ca^{2+}-sensitive K^{+}channel (hslo). *Proceedings of the National Academy of Sciences of the United States of America* 1997 ; 94(10):5427-5431.

[34] **Cui J, Yang H, & Lee US**. Molecular mechanisms of BK channel activation. *Cellular and molecular life sciences:CMLS* 2009; 66(5):852-875.

[35] **Latorre R, Morera FJ, & Zaelzer C.** Allosteric interactions and the modular nature of the voltage- and Ca^{2+}-activated (BK) channel. *The Journal of physiology* 2010; 588(Pt 17):3141-3148.

[36] **Ledoux J, Werner ME, Brayden JE, & Nelson MT.** Calcium-activated potassium channels and the regulation of vascular tone. *Physiology* 2006; 21:69-78.

[37] **Nassar-Gentina V, Pollard HB, & Rojas E.** Electrical activity in chromaffin cells of intact mouse adrenal gland. *The American journal of physiology* 1988; 254(5 Pt 1):C675-683.

[38] **Grimm PR, Foutz RM, Brenner R, & Sansom SC**. Identification and localization of BK-beta subunits in the distal nephron of the mouse kidney. *American journal of physiology. Renal physiology* 2007; 293(1):F350-359.

[39] **Grimm PR & Sansom SC.** BK channels and a new form of hypertension. *Kidney international* 2010; 78(10):956-962.

[40] **Manzanares D, et al.** Functional apical large conductance, Ca^{2+}-activated, and voltage-dependent K+ channels are required for maintenance of airway surface liquid volume. *The Journal of biological chemistry* 2011; 286(22):19830-19839.

[41] **Sorensen MV, Strandsby AB, Larsen CK, Praetorius HA, & Leipziger J**. The secretory KCa1.1 channel localises to crypts of distal mouse colon: functional and molecular evidence. *Pflugers Archiv: European journal of physiology* 2011; 462(5):745-752.

[42] **Rohmann KN, Tripp JA, Genova RM, & Bass AH.** Manipulation of BK channel expression is sufficient to alter auditory hair cell thresholds in larval zebrafish. *J Exp Biol* 2014; 217(Pt 14):2531-2539.

[43] **Lionetto MG, et al.** Molecular and functional expression of high conductance Ca^{2+} activated K^{+} channels in the eel intestinal epithelium. *Cellular physiology and biochemistry : international journal of experimental cellular physiology, biochemistry, and pharmacology* 2008; 21(5-6):373-384.

About the authors

Dr. Carlos Gonzalez is full Professor at Interdisciplinary Center for Neuroscience (CINV) at Universidad de Valparaíso. He did his bachelor and Master in Science in Biophysics at State University of Moscow. He got his PhD in Molecular Cell Biology and Neurosciences (Biophysics) from Universidad de Chile. He has a vast experience in ion channel biophysics and function-structure studies, with three postdoctoral trainings in NINDS-NIH, University of Virginia and University of Miami, respectively.

Dr. Francisco J. Morera is Assistant Professor at Institute of Pharmacology, Faculty of Veterinary Sciences, Universidad Austral de Chile. He is a biochemist from P. Universidad Católica de Chile and he did his PhD under supervision of Dr. Ramon Latorre in ion channels biophysics. Currently, he is interested in study of the K^+ channels involved in seawater adaptation in salmonids.
Dr. Luis Vargas-Chacoff is Associate Professor at Institute of Marine Sciences and Limnology, Faculty of Sciences, Universidad Austral de Chile. He is a Marine biologist from Universidad Austral de Chile and he did his PhD under supervision of Dr. J.M. Mancera in fish osmoregulation at Universidad de Cadiz, Spain. Currently, he is working with Dr. Steve D. McCormick in seawater adaptation in Atlantic salmon.

Dr. **David Baez-Nieto** is a biochemist from PUCV, holds a PhD in Neurosciences from Universidad de Valparaíso, and counts with a postdoctoral training in voltage activation of voltage-gated ion channel at CINV. His expertise area is electrophysiology of ion channels and ion channel biophysics.

MSc. **Amaury Pupo** is a biochemist graduated from Universidad de la Habana, he got his Master in Science from Universidad de la Habana, and at present he is a PhD student at CINV Universidad de Valparaiso. His research area includes biochemistry, protein structure and bioinformatics.

MSc. Yenisleidy Lorenzo is a biochemist graduated from Universidad de la Habana, she got his Master in Science from Universidad de la Habana, and at present she is a PhD student at CINV Universidad de Valparaiso. Her expertise is in the field of neuropsychology and neurodiagnostics, and recently in ion channel biophysics.

Dr. Karen Castillo is Molecular Biotechnology Engineer and PhD in Neuroscience and Cell Biology from University of Chile. Currently she is in a postdoctoral training at Molecular Sensors Laboratory at the CINV in the University of Valparaiso, under the supervision of Dr. Carlos Gonzalez. Research skills include fluorescence microscopy, animal experimentation, physiology, neuroscience, neurodegeneration, and ion channels biophysics.