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[REVIEW]

Regulation of the Ion-Transporting Mitochondrion-Rich Cell during Adaptation of Teleost Fishes to Different Salinities

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ABSTRACT—The mitochondrion-rich cells (MRCs) in teleost gill and equivalent tissues are important osmoregulatory sites in maintaining ionic balance. These cells express a variety of ion pumps, transporters, and channels, which play central roles in ionic regulation. Recently, two types of MRCs have been identified in euryhaline fishes: seawater (SW)-type MRCs extrude Na and Cl ions in SW conditions; freshwater (FW)-type MRCs take up at least Cl⁻. Long-term development/differentiation of the two types of MRCs during adaptation to different salinities appears to be regulated mainly by endocrine factors. Osmolality, Ca²⁺, neurotransmitters, and fast-acting hormones rapidly regulate the SW MRCs. Recent information is assembled in this review and suggests the functional plasticity of highly specialized MRCs.

Key words: adaptation, chloride cell, fish, mitochondrion-rich cell, osmoregulation

INTRODUCTION

Mechanisms of teleost osmoregulation have been described in several reviews (e.g., Silva *et al.*, 1977; Evans, 1979, 1993). Briefly, SW fishes lose water and gain ions through the body surface, mainly through the gills. In order to compensate for the osmotic loss of water, they drink the surrounding SW and absorb both ions and water from the intestine. Excess Na⁺ and Cl⁻ ions, which enter the body through the surface as well as via the intestine, are excreted by the gills. In contrast, FW fishes continuously need to dispose of water that enters through the body surface. The latter type of fish produces a large amount of hypotonic urine and they drink very little water. The passive loss of ions in the urine and across the body surface is compensated for by active ion uptake through the gills. Thus, the ionic exchange required for teleost osmoregulation is mainly located in the gill epithelium.

About 50 years ago, Keys and Willmer (1932) suggested that a certain type of gill epithelial cell might be responsible for Cl⁻ excretion in the SW-adapted eel. Later, Copeland (1948)

described such cells, presumably for the first time, and referred to them as “chloride cells” in the killifish, *Fundulus heteroclitus*. These cells contain elaborate basolateral infoldings that produce an extensive intracellular tubular system associated with the ion-transporting enzyme Na⁺,K⁺-ATPase (Karnaky *et al.*, 1976) and numerous prominent mitochondria (e.g., Laurent, 1984). Therefore, these cells are also often referred to as “mitochondrion-rich cells (MRCs)”. Mature MRCs that come into contact with their external water via the apical membrane are involved in ion transport, though the tubular system is already developed in immature MRCs (Wendelaar Bonga and van der Meij, 1989; Goss *et al.*, 1998). MRCs are interspersed among pavement cells which occupy more than 90% of the gill surface (Perry and Walsh, 1989). Tight junctions between MRCs and adjacent pavement cells are also considered to be “deep junctions” because of their multi-strand connections (Sardet *et al.*, 1979; Sardet, 1980; Karnaky, 1992).

MRCs are found especially in the interlamellar epithelium and in the trailing edge of the filament epithelia of the gills. In some species, a considerable number of MRCs are observed in the gill lamellar epithelia (e.g., Laurent, 1984). MRCs are not necessarily confined to gill epithelia; they are also found in the inner surface of the operculum of the killifish

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Fundulus heteroclitus (e.g., Degnan *et al.*, 1977), tilapia *Oreochromis mossambicus* (Foskett *et al.*, 1981), and goldfish (Fujimoto, personal communication), and in the skin of gobies (Marshall and Nishioka, 1980; Yokota *et al.*, 1997). In the embryos and larvae of several teleost species, MRCs have been detected in the epithelia covering the yolk and body surface (see Kaneko *et al.*, 2002 for review). Most of these extrabranchial MRCs are found in the vascularized epithelia of the body surface; they may compensate for insufficient ion-transport in undeveloped or vestigial gills.

Regulation of MRCs is critical for euryhaline fish during movement between FW and SW. Reviews on this general topic have appeared previously (Foskett *et al.*, 1983; McCormick, 1995; Marshall, 1995; Perry, 1997; Marshall and Bryson 1998; Evans *et al.*, 1999). However, recent advances in molecular biology methods have allowed the determination of the function and regulation of MRCs. Such techniques involve the use of antibodies and molecular probes for ion-transporting proteins and hormonal factors. This review first considers current models of NaCl transport systems in MRCs in teleosts, especially those at the molecular and cellular levels, and then focuses primarily on recently obtained important evidence regarding the regulation of MRCs when teleost fish are exposed to different osmotic environments. Possible involvement of the gill MRCs in acid-base regulation, nitrogen excretion, and Ca²⁺ regulation will not be addressed here; these topics have been reviewed in detail elsewhere (Flik *et al.*, 1995, 1996; Claiborne, 1998; Walsh, 1998; Evans *et al.*, 1999).

MITOCHONDRION-RICH CELLS EXTRUDE NaCl IN SW

In marine teleosts, and euryhaline species acclimated to SW, the mucosal surface of MRCs is usually invaginated below the pavement cells; this forms "apical crypts" between pavement cells. The MRCs usually display multicellular complexes and a well-developed intracellular tubular network (Hossler *et al.* 1979; Laurent 1984). Adjacent MRCs share an apical crypt and a single-stranded shallow junction. The same paracellular pathways are also observed between MRCs and accessory cells which is considered to be partially differentiated MRCs (e.g., Laurent, 1984). These "leaky" paracellular pathways are thought to be the morphological basis for the relatively high ionic permeability of gills in SW teleosts (e.g., Karnaky, 1992).

Inhibition of the efflux of Na⁺ and Cl⁻ by basolateral application of ouabain (an inhibitor of Na⁺,K⁺-ATPase) suggests that Na⁺,K⁺-ATPase generates an electrochemical gradient for Na⁺ from the plasma to the cytoplasm of the MRC to drive Na⁺ inward across the basolateral membrane (Silva *et al.*; 1977; Degnan *et al.*; 1977). Studies with the opercular membrane demonstrated that, under short-circuited conditions, the net Cl⁻ extrusion rate (serosa to mucosa) was equal to the short-circuit current, but there was no net extrusion of Na⁺. Basolateral application of furosemide (an inhibitor of the Na⁺-K⁺-2Cl⁻ cotransporter family) inhibited the net extrusion of Cl⁻ (e.g., Degnan *et al.*, 1977; Eriksson and Wistrand, 1986; Marshall,

1995; Payne and Forbush, 1995; Kaplan *et al.*, 1996). Using the vibrating probe technique, Foskett and Scheffey (1982) demonstrated that the MRCs are the definite site of active Cl⁻ extrusion. An apical Cl⁻ channel seems to be a member of the cystic fibrosis transmembrane conductance regulator (CFTR) family because of its electrical characteristics and stimulation by cyclic AMP (Marshall *et al.*, 1995). Ba²⁺ sensitivity of the serosal surface suggests the presence of a basolateral K⁺ channel (Degnan, 1985). Apical K⁺ secretion was also observed in short-circuited skin (Marshall and Bryson, 1998). The fine-tuned current model for NaCl extrusion by the teleost gill epithelium resulting from these studies is best described in detail in a review by Marshall (1995): The Na⁺ gradient, which is produced across the basolateral membrane by Na⁺,K⁺-ATPase, drives the Na⁺-K⁺-2Cl⁻ cotransporter; K⁺ enters via the basolateral Na⁺,K⁺-ATPase and Na⁺-K⁺-2Cl⁻ cotransport, and the K⁺ is thought to be recycled from the cell via K⁺ channels; Cl⁻ exits the cell via an apical Cl⁻ channel and K⁺ via a basolateral K⁺ channel, resulting in a serosa-positive trans-epithelial potential that moves Na⁺ through the leaky paracellular pathway between adjacent cells (see also Fig. 2, SW-type).

Recent reports have shown that Na⁺-K⁺-2Cl⁻ cotransporter immunoreactivity is localized on the basolateral membrane of the MRCs; such studies have also demonstrated the presence of a CFTR-like anion channel in the apical crypt (Singer *et al.*, 1998; Wilson *et al.*, 2000b). A cDNA for an inward rectifier K⁺ channel in the basolateral membrane has been identified in SW-adapted eels as an inducible mRNA (Suzuki *et al.*, 1999). Miyazaki *et al.* (1999) have cloned two Cl⁻ channels (CLC-3 and 5) as intracellular Cl⁻ channels from the tilapia gill.

INVOLVEMENT OF MITOCHONDRION-RICH CELLS IN NaCl UPTAKE IN FW CONDITIONS

In FW teleosts, MRCs in the gill filament epithelium are as abundant as in SW fish, but they may also appear on the lamellar epithelium in several species (e.g., Uchida *et al.*, 1996; Perry, 1997; Hirai *et al.*, 1999; Sasai *et al.*, 1999). The MRCs observed in FW fish generally have apical microvilli, which presumably increase the mucosal surface area and extensive tight junctions between adjacent cells (Hwang, 1988; Perry *et al.*, 1992; Marshall *et al.*, 1997). In addition, MRCs in FW fish contain a moderately developed tubular system in the cytoplasm. Despite several exceptions, FW MRCs are often reported to be singular with their mucosal surface above the adjacent pavement cells (Hwang, 1988; Van Der Heijden *et al.*, 1997; Marshall *et al.*, 1997). Accessory cells are also found in several species in FW, although they are more typically found in SW fishes (Pisam *et al.*, 1989; Cioni *et al.*, 1991).

It is generally accepted that Cl⁻ uptake occurs via the MRCs because the morphological characteristics of the MRCs correlate well with Cl⁻ uptake rates (e.g., Perry and Laurent, 1989; Goss *et al.*, 1994; Wood and Marshall, 1994; Marshall *et al.*, 1997). Apical Cl⁻/HCO₃⁻ exchange is assumed to medi-

ate Cl^- uptake across the gill in FW conditions; inhibitors of the $\text{Cl}^-/\text{HCO}_3^-$ exchanger reduce Cl^- uptake and produce a metabolic alkalosis in fish, as does the removal of external Cl^- (reviewed in Perry, 1997; Goss *et al.*, 1998). Furthermore, the $\text{Cl}^-/\text{HCO}_3^-$ exchanger was shown to be localized in MRCs using *in situ* hybridization and immunocytochemical staining (Sullivan *et al.*, 1996; Wilson *et al.*, 2000a). It remains unclear what drives this exchanger, since the Cl^- gradient between the cytoplasm and FW does not favor uptake of Cl^- ions from FW, and the true apical HCO_3^- gradient is unknown. Presumably, net Cl^- movement across the gill may be mediated via a basolateral Cl^- channel, driven by the inside-negative membrane potential for regular cells. The intracellular generation of H^+ and HCO_3^- necessary for these apical extrusion mechanisms is probably derived from the hydration of CO_2 , since carbonic anhydrase has been localized in the opercular epithelium MRCs in the killifish (Lacy, 1983), and inhibition of carbonic anhydrase by acetazolamide reduced proton excretion (Lin and Randall, 1991).

Branchial uptake of Na^+ is most probably via the apical

Na^+ channel, and down an electrochemical gradient generated by an apical vacuolar H^+ -ATPase, although the Na^+/H^+ exchange mechanism cannot be ignored (see Evans *et al.*, 1999; Fenwick *et al.* 1999). There is some debate about the cellular localization of H^+ -ATPase and Na^+ channels. With *in situ* hybridization and/or immunocytochemistry, the H^+ -ATPase has been reported to be localized in the pavement cells of the gill epithelium of rainbow trout (Sullivan *et al.*, 1995, 1996) and of the yolk-sac membrane of the tilapia (Hiroi *et al.*, 1998). Recently, immunoreaction for H^+ -ATPase and Na^+ channel in both tilapia and rainbow trout were co-localized in the pavement cells (Wilson *et al.*, 2000a), although apical labeling was also found in the MRCs of FW trout whose environmental pH and ionic strength are lower than those reported by Sullivan *et al.* (1995, 1996). The amiloride-sensitive Na^+/H^+ exchanger immunoreactivity is associated with the accessory cells and with a small population of pavement cells in tilapia (Wilson *et al.*, 2000a) and with MRCs in Japanese dace (Kaneko, personal communication).

In order to determine the cellular site of Na^+ uptake, and

Table 1. Time course of adaptations to different salinities and regulations of mitochondrion-rich cells (MRCs)

Adaptation to SW		
Time course	Related events	Mitochondrion-rich cells
Minutes ~ hours	Environmental osmolality and Ca^{2+} ↑	Apical pit open
	Plasma osmolality ↑ Plasma cortisol, angiotensin II and ANP ↑	Na^+, K^+ -ATPase ↑ Cl^- secretion ↑ c-Jun modification Hsps ↑
		mRNA of CFTR and K^+ channel ↑
Days ~ weeks	Plasma GH/IGF-I ↑	IGF-I mRNA ↑
	Plasma PRL ↓ Plasma osmolality ↓	$\text{Na}^+/\text{K}^+/\text{2Cl}^-$ cotransporter and cytoskeletons ↑ SW MRC ↑ FW MRC ↓
Adaptation to FW		
Time course	Related events	Mitochondrion-rich cells
Minutes ~ hours	Environmental osmolality and Ca^{2+} ↓	Apical pit close
	Sympathetic nerve stimulation Plasma osmolality ↓	Cl^- secretion ↓ c-Jun modification
Days ~ weeks	Plasma PRL ↑	Cl^- uptake and FW MRC ↑
	Plasma osmolality ↓	SW MRC ↓

Note: Reports using several euryhaline species (see text) are assembled, and the universality is uncertain.

also to advance our understanding of MRC ion-transport, more species should be examined under a variety of physiological conditions using the antibodies and the molecular probes for ion-transporting proteins described above. Another powerful tool for the measurement of ion movement includes the use of ion-sensitive fluorescent dyes in combination with confocal laser scan microscopy (see Li *et al.*, 1997).

CONTROL OF MITOCHONDRION-RICH CELLS BY DIFFERENT SALINITIES

When euryhaline teleosts adaptable to both FW and SW are transferred to different salinities, they show a sharp change in the rate of NaCl flux during the first hour of transition. The initial rapid change is followed by a more protracted change (hours - days) in the rate and direction of ion movement (e.g., Motais *et al.*, 1966; Wood and Marshall, 1994). Therefore, the above-mentioned two functions of MRCs are likely to be skillfully regulated during adaptation to different salinities (see Table 1).

Rapid Regulation (minutes to hours)

Euryhaline teleosts, especially intertidal species, need to regulate the rate of NaCl transport in the MRCs within several hours. River mouth intertidal habitats are subject to extreme tidal changes that result in rapid and frequent alternations in environmental salinity.

Marshall (1995) has reviewed the role of neurotransmitters and classical rapid-acting hormones on MRC function. Urotensins, eicosanoids, glucagon, and vasoactive intestinal polypeptides influence Cl⁻ secretion by MRCs, although it is not clear whether or not the MRCs are exposed to these hormones during adaptation to different salinities. Marshall *et al.* (1993, 1998) have shown that a portion of the stress-induced rapid reduction in Cl⁻ secretion may be mediated by the α_2 -adrenergic receptor activated by the sympathetic nervous system in killifish. This adrenergic receptor acts *via* phospholipase C, inositol triphosphate and intracellular Ca²⁺. Scheide and Zadunaisky (1988) showed that atrial natriuretic peptide (ANP), recognized as a SW-adapting hormone (Takei, 2000), directly increases Cl⁻ secretion. The role of other natriuretic peptides should be examined, since three types of natriuretic peptide receptors have been identified in the gills of eels (see Takei, 2000). Angiotensin II is also a SW-adapting hormone; it increases gill MRC Na⁺,K⁺-ATPase in the eel within 30 min. and receptors for angiotensin II are present in the MRCs (Marsigliante *et al.*, 1997; Russel *et al.*, 2001). There are also several instances where rapid activation of gill Na⁺,K⁺-ATPase has been reported after transfer of the killifish, mullet, or tilapia to conditions of higher salinity (Towle *et al.*, 1977; Hossler, 1980; Hwang *et al.*, 1989; Mancera and McCormick, 2000). The activation of the Na⁺,K⁺-ATPase in killifish is induced 3 hr. after SW transfer by hyperosmolality *in vitro*, and is dependent on transcriptional and translational processes (Mancera and McCormick, 2000). Cortisol, which increases rapidly following exposure to SW (see Shreck, 1981; Wendelaar Bonga,

1997), seems to directly activate gill MRC Na⁺,K⁺-ATPase in the eel within 2–6 hr. (Marsigliante *et al.*, 2000). Borski *et al.* (2000) have suggested that cortisol may act on teleost target cells through membrane-associated effector systems, as well as more slowly via changes in gene expression. Cyclic AMP-mediated phosphorylation by the activity of protein kinases seems to play a role in the rapid modulation of Na⁺,K⁺-ATPase (Tipsmark and Madsen, 2001)

Furthermore, both increases and decreases in the osmolality of the basolateral side of the opercular epithelia *in vitro* (simulating early events during adaptation) evoke immediate increases and decreases, respectively, in the rate of Cl⁻ secretion in killifish from SW (Zadunaisky *et al.* 1995; Marshall *et al.*, 2000). This regulation seems to be mediated by tyrosine phosphorylation of the CFTR upon MRC shrinkage and swelling, accompanied by epithelial conductance changes (see also Daborn *et al.*, 2001).

In this regard, we have shown that the MRC apical crypts of the estuarine mudskipper close 30 min. after transfer from SW to FW in order to shut down salt secretion and passive ion loss. Such responses are reversible when fish are returned to SW (Sakamoto *et al.*, 2000c). This morphological oscillation seems to be triggered by differences in osmolality and Ca²⁺ concentration between FW and SW. Increases and decreases in osmolality of the basolateral side of killifish opercular epithelia *in vitro* also evoke similar morphological changes, and the actin cytoskeleton is required to maintain crypt opening (Daborn *et al.*, 2001; Yasunaga *et al.*, 2001). Via these morphological alterations, generally, MRCs seem to control the availability of ion channel/transporters at the apical membrane to the external water; hence, MRCs appear to affect the rate of ion transport (see Goss *et al.*, 1998; Pisam *et al.*, 1990).

The combination of these events, both the regulation of active ion transport and the modification of ion diffusion, could account for the full regulation of NaCl flux during rapid adaptation. It is of note that cross-talk between intracellular mechanisms of these regulations occurs. In addition to physiological approaches involving inhibitors of the signal transduction and the measurement of the secondary messenger levels, new approaches of molecular and cellular biological should be used to elucidate the candidate protein kinases and other related enzymes (e.g., Sakamoto *et al.*, 2000b; Hashimoto *et al.*, 1997, 1998, 2000). Surprisingly, phosphorylation of these proteins has not been widely analyzed in MRCs. However, antibodies against phosphorylated amino acids and the mammalian enzymes are currently available. Breakthroughs may proceed from studies involving the rapid, simultaneous measurement of ion transport and morphological or biochemical changes in MRCs. Caged second messengers may also be useful in this regards.

Importantly, such rapid regulation suggests the functional plasticity of highly differentiated MRCs, not only at the molecular level but also at the morphological level. It should be noted that most of these rapid regulatory processes have been observed in intertidal species.

Long-term Regulation (days to weeks)

For most teleost species examined to date, Cl⁻-secretory MRCs in hyperosmotic environments increase in number (e.g., Shirai and Utida, 1970; Foskett *et al.*, 1983) and size (e.g., Shirai and Utida, 1970; Pisam, 1981; Pisam *et al.*, 1988). The apical area of the MRC is enlarged, and accessory cells gradually intrude into the MRCs and form a multicellular complex (e.g., Shiraishi *et al.*, 1997; Hiroi *et al.*, 1999). These morphological changes are accompanied by increased expression and activity of Na⁺,K⁺-ATPase (Kirschner, 1980; McCormick, 1995; Seidelin *et al.*, 2000; Cutler *et al.*, 2000; Sakamoto *et al.*, 2001), several days after transfer of the fish from FW to SW. The Na⁺,K⁺-ATPase α -subunit gene is considered to be AP-1 responsive (Shull *et al.*, 1990). Moreover, Kültz (1996) has reported the modification of the AP-1 transcriptional factor c-Jun in the gills after transfer of a goby *Gillichthys mirabilis* to different salinities. Expression of the CFTR, Na⁺-K⁺-2Cl⁻ cotransporter and cytoskeletal elements (e.g., actin-binding protein and a member of the Rho family known to control actin) was also shown to be elevated and seems to be involved in MRC function in SW conditions (Singer *et al.*, 1998; Suzuki *et al.*, 1999; Pelis *et al.*, 2001; Yasunaga *et al.*, 2001). It has recently become clear that actin directly regulates Na⁺,K⁺-ATPase, the Cl⁻ channel, and the Na⁺-K⁺-2Cl⁻ cotransporter in various cells (Nelson and Hammerton, 1989; Suzuki *et al.*, 1993; Mills *et al.*, 1994; Shapiro *et al.* 1991; Matthews *et al.* 1992).

Pisam and coworkers (1987) have described two types of MRCs, α - and β , present in the gill filament of FW species (loach and gudgeon) and euryhaline species (salmonids, guppy, and tilapia) in FW. The α -type MRCs are activated in the filamental epithelium of euryhaline fishes acclimated to SW and are thought to be the homologue of the Cl⁻-secretory SW MRCs (Pisam *et al.*, 1987, 1995). On the other hand, the β -type MRCs are observed only in FW-adapted fish, and these cells disappeared during SW adaptation. Two different types of MRCs were also identified in the gill filament and lamellar epithelia of salmonids (Uchida *et al.*, 1996, 1997; Seidelin *et al.*, 2000), guppy (Shikano and Fujino, 1998), seabass (Hirai *et al.*, 1999), and eel (Sasai *et al.*, 1999), on the basis of their location and response to SW/FW transfer. Filament MRCs were activated after exposure to SW, and inactivated in FW conditions. The incorporation of 5-bromo-2'-deoxyuridine into filament MRCs increased after SW transfer, suggesting that filament MRCs play important roles in SW conditions (Uchida and Kaneko, 1996). In contrast, lamellar MRCs were mainly observed in FW conditions and practically disappeared by apoptosis during SW adaptation. Fish exposed to low ion concentrations in FW displayed extensive proliferation of the MRCs on the lamellar epithelium (e.g., Perry and Laurent, 1993; Perry, 1997). These results suggest that lamellar MRCs are the possible site of ion uptake in FW conditions. Although the relationship between the β -type MRCs in the filament and lamellar MRCs is unclear, Hirai *et al.* (1999) suggest that the latter originates from the filament and migrates to the lamellae during FW adaptation. Recently, Wong and Chan (1999)

confirmed by flow cytometry the heterogeneity of MRCs and they hypothesized that stem cells, but not FW MRCs, differentiate into SW-type MRCs in the adult eel gill. On the other hand, Hiroi *et al.* (1999) observed *in vivo* sequential changes in the MRCs of the tilapia yolk-sac membrane, and indicated that FW-type MRCs are transformed into SW-type MRCs during SW adaptation, thus suggesting the plasticity of MRCs. Further research using these sequential observations may show the inverse transformation of SW-type cells into FW-type cells and should also address the functional plasticity of the MRCs using ion-sensitive dyes. However, the plasticity of MRCs may be a characteristic of those cells in the transient yolk sac during early development.

Although Shiraishi *et al.* (2001) have recently showed that the MRCs of this yolk-sac membrane can differentiate independently of endocrine factors, they have been believed to mediate most of the above-mentioned slow responses of MRCs to different salinities. Since McCormick (1995) provides an excellent review of the hormonal regulation of MRCs, only the more recent research will be considered here.

Prolactin (PRL)

Prolactin, a FW-adapting hormone in teleosts (see Hirano *et al.*, 1986), inhibits the development Cl⁻-secretory SW-type MRCs and promotes the development of FW-type MRCs. Foskett *et al.* (1982) have postulated that PRL reduced MRC numbers and active transport of ions in SW-adapted fish. PRL treatment of SW-adapted tilapia resulted in a reduction of MRC size (Herndon *et al.*, 1991). Pisam *et al.* (1993) reported that PRL injection into SW-adapted tilapia resulted in the appearance of the putative FW-type β MRCs, whereas the SW-form α MRCs were reduced in size. Although mammalian PRL sometimes increased gill Na⁺,K⁺-ATPase activity possibly through growth hormone (GH) receptors, homologous PRLs decrease the activity of Na⁺,K⁺-ATPase in tilapia (Flik *et al.*, 1994; Sakamoto *et al.*, 1997). Prolactin receptors have been found in gill MRCs (Auperin *et al.*, 1994; Weng *et al.*, 1997; Sandra *et al.*, 2000; Prunet *et al.*, 2000; Santos *et al.*, 2001), suggesting the direct action of PRL on MRCs.

Growth hormone/insulin-like growth factor (IGF) axis

Despite being structurally related to PRL, GH, one of the essential SW-adapting hormones in salmonids, activates gill Na⁺,K⁺-ATPase activity and SW MRCs (see Sakamoto *et al.*, 1993; Prunet *et al.*, 1994; Seidelin and Madsen, 1999). This GH role may be a common feature of euryhaline teleosts such as killifish, tilapia, striped bass, silver seabream and mudskipper (see Sakamoto *et al.*, 1997, 2000a, 2002; Mancera and McCormick, 1998; Kelly *et al.*, 1999).

One important pathway for the GH action is through its major influence on IGF-I secretion. IGF-I, especially plasma IGF-I from the liver, seems to be primarily induced by GH (see Moriyama *et al.*, 2000). In the gill epithelium, IGF-I seems to be localized in the interlamellar epithelium (Fig. 1; Richardson *et al.*, 1995), and it is also induced by GH after transfer of trout and tilapia to SW (Sakamoto and Hirano, 1993; Sakamoto

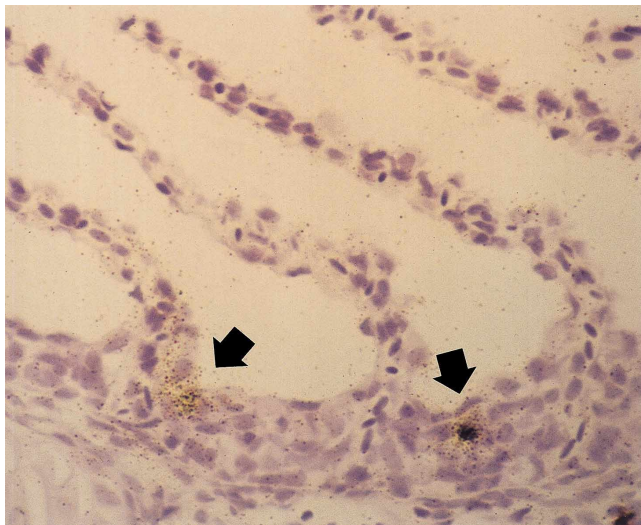


Fig. 1. Localization of IGF-I mRNA at interlamellar space of the gill filament of rainbow trout, presumably in some of the MRCs (arrows).

et al., 1995). The GH receptor has been characterized in rainbow trout gills, although there is no evidence for a direct action of GH on gill MRCs (Sakamoto and Hirano, 1991). IGF-I has been shown to increase Na^+, K^+ -ATPase activity, SW MRCs, and/or salinity tolerance in salmonids and killifish (see Mancera and McCormick, 1998; Seidelin *et al.*, 1999; Seidelin and Madsen, 1999). When coho salmon were pretreated with GH, IGF-I directly stimulated gill Na^+, K^+ -ATPase activity (Madsen and Bern, 1993). Thus, at least among salmonids, GH may stimulate differentiation of MRCs via the local production of IGF-I, whereas systemic IGF-I may act on the differentiated cells. This hypothesis is similar to the dual effector model for the promotion of growth (Green *et al.*, 1985; Gray and Kelley, 1991).

Although IGF-II is another member of the IGF family expressed in gills (Chan *et al.*, 1994; Chen *et al.*, 1994; Duguay *et al.*, 1996), human IGF-II had no effect on killifish osmoregulation (Mancera and McCormick, 1998). Additional experiments using homologous peptides may be necessary to demonstrate a possible action of IGF-II on gill MRC function. IGF-binding proteins play several biological roles along the GH/IGF axis; IGF-binding proteins have also been identified in teleosts (see Siharath and Bern, 1993). Although the growth-inhibiting role of IGF-binding protein 2 in zebrafish has been reported recently (Duan *et al.*, 1999), there is no report about the possible functions of IGF-binding proteins in MRCs. The role of IGF-binding proteins during the adaptation of teleosts to different salinities should be examined using cDNA probes and proteins.

Cortisol

In teleosts, cortisol, the major corticosteroid secreted by interrenal glands, used to be understood as the central hormone for SW adaptation. Cortisol directly stimulates gill Na^+, K^+ -ATPase activity and differentiation of MRCs (McCormick and Bern, 1989; McCormick, 1990; Ayson *et al.*, 1995). A min-

eralocorticoid/glucocorticoid response element has been identified in the human Na^+, K^+ -ATPase α gene (Kolla *et al.*, 1999). Presence of the cortisol receptor has been demonstrated by steroid-binding assay in the gill cytosol and nucleus of several euryhaline species (e.g., Sandor *et al.*, 1984; Chakraborti *et al.*, 1987). Translocation of the cortisol receptor to the nucleus seemed to be rapidly stimulated by the plasma cortisol increase (Weisbart *et al.*, 1987) and regulated by a heat shock protein (Hsp90) (Pan *et al.*, 2000; Yasunaga *et al.*, 2001). By means of *in situ* hybridization and immunocytochemical staining of chum salmon gills, Uchida *et al.* (1998) found that the cortisol receptor was expressed in the filament MRCs of the SW fish more than in the FW fish, suggesting the involvement of cortisol in the maintenance of their function in SW conditions.

There is a strong interaction between GH and cortisol in the regulation of SW MRCs. GH/IGF-I and cortisol act in synergy to increase Na^+, K^+ -ATPase activity, MRC number, and/or salinity tolerance (see Mancera and McCormick, 1998). GH stimulated gill cortisol receptor, and directly increased the sensitivity of the interrenal tissue to adrenocorticotropin (ACTH) in coho salmon (Young, 1988; Shrimpton *et al.*, 1995; Shrimpton and McCormick, 1998). On the other hand, cortisol stimulates GH release in tilapia (Nishioka *et al.*, 1985).

Cortisol seems to be involved in ion uptake in FW fish as well. Cortisol treatment of FW fish stimulated the whole-body uptake of Na^+ and Cl^- ions, possibly by increasing gill H^+ -ATPase activity as well as cell number, apical surface areas, and/or Na^+, K^+ -ATPase density in MRCs (Perry *et al.*, 1992; Dang *et al.*, 2000). Cortisol receptors were also localized in the MRCs in the gill lamellar epithelium of FW chum salmon, as well as in undifferentiated cells at the interlamellar regions near the central venous sinus (Uchida *et al.*, 1998). Thus, cortisol seems to have a dual and fundamental role, acting not only on SW-type MRC but also on FW-type MRCs.

Other slow-acting hormones

Thyroid hormones have been hypothesized to play a role in many developmental processes including that of MRCs (see Hoar, 1988). However, the reported roles of these hormones on MRCs are equivocal. In salmonids, though still contradictory, thyroid hormones seem to stimulate the activity of gill Na^+, K^+ -ATPase and MRCs, possibly through their interaction with the GH/IGF-I axis and cortisol (Miwa and Inui, 1985; Young and Lin, 1988; Moav and McKeown, 1992; Leloup and Lebel, 1993; Shrimpton and McCormick, 1998). These processes may be a part of the smoltification process, which may be essentially regulated by thyroid hormones. However, in tilapia and summer flounder, thyroid hormones enhance the Na^+, K^+ -ATPase and MRCs in FW conditions, favoring hyperosmoregulatory capacity (Dange, 1986; Schreiber and Specker 2000; Subash Peter *et al.*, 2000).

Sex steroids have been shown to have a negative effect on the activity of SW MRCs, Na^+, K^+ -ATPase, and salinity tolerance of salmonids (see Madsen and Korsgaard, 1991). This response may be related to the FW migration of sexually

mature salmonids. Although receptors for thyroid hormones and sex steroids have been found in the gills (Bres and Eales, 1988; Lebel and Leloup 1989; MacLatchy and Eales, 1992; Pinter and Thomas, 1995), the cellular localization and direct action of these hormones are currently unknown; further research similar to the case of cortisol is clearly warranted.

CONCLUSIONS: AN INTEGRATED MODEL FOR REGULATION OF MITOCHONDRION-RICH CELLS DURING ADAPTATION TO DIFFERENT SALINITIES

Any summary of MRC regulation in teleost fishes must confront the diverse habitat (FW, SW, estuarine) and life history (sedentary, anadromous, catadromous, diadromous) of this large group. Our literature search may have revealed contradictory results among the findings. Nevertheless, we present an integrated model of salinity regulation of MRCs, although the universality of the model remains uncertain (Fig. 2). MRCs possess a suite of transport proteins for salt excretion in SW conditions and Cl^- uptake in FW conditions. The cellular junctions and/or cytoskeletal components such as tight junctions and actin have been suggested to play a role in the ion trans-

port. However, as there is currently little information on this topic, future investigations will hope fully shed more light on their involvement.

Evidence to date indicates that rapid regulations occur at least in Cl^- -secretory SW MRCs. Hyperosmolality, Ca^{2+} , angiotensin II, ANP, and cortisol rapidly activate the SW MRCs, whereas sympathetic nerve and hypoosmolality inactivate the cells at rest. Important advances in this area may come from the rapid, simultaneous measurement of ion transport as well as of morphological and biochemical changes in MRCs. Ion-sensitive dyes and fluorescent probes may be particularly valuable in this regard.

Long-term development/differentiation of MRCs seems to be regulated mainly by endocrine factors. Cortisol seems to play a fundamental role in promoting the development of both FW and SW-type MRCs. PRL inhibits SW MRCs and activates FW-type MRCs, whereas GH/IGF-I stimulates SW MRCs. Receptors for cortisol, angiotensin II, and PRL are localized in MRCs. FW-type MRCs can be transformed into SW-type MRCs, suggesting the plasticity of MRCs. One question of particular interest for further study would be to determine the intracellular cues for the *de novo* development of

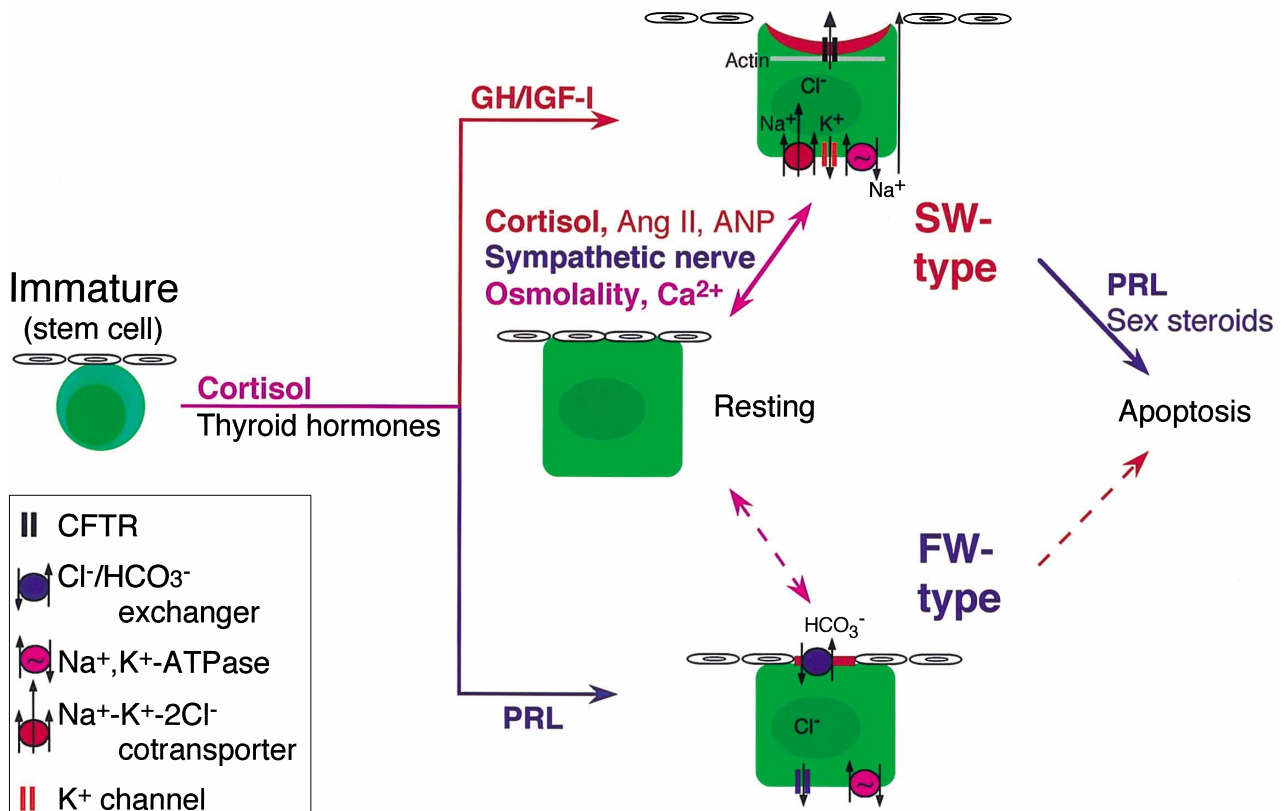


Fig. 2. Integrated model of regulation of MRCs during adaptation to different salinities. Arrows and letters in red denote the regulations during SW adaptation, and those in blue during FW adaptation; broken arrows denote possible pathways. There are reports on FW MRCs containing apical H^+ -ATPase and Na^+ channels. Evidence to date indicates that rapid regulation by osmolality, neurotransmitters, fast-acting hormones, and Ca^{2+} occurs in SW MRCs. Long-term development/differentiation of MRCs from immature cells (resting or stem cells) is regulated by endocrine factors. Cortisol seems to play a basic role in activating both FW and SW-type MRCs. PRL inhibits SW MRCs and promotes FW-type MRCs, whereas GH/IGF-I stimulates SW MRCs. Receptors for cortisol, PRL, and angiotensin II (Ang II) are localized in MRCs, suggesting direct action. See text for details.

FW and SW MRCs, or for the changeover from one cell type to another. Transcriptional regulations of ion-transport proteins should be examined in order to answer these questions. Recently-developed DNA arrays containing cDNAs of various transcriptional factors may prove useful for such studies. Translocation of transport proteins may also be possible (see Nielsen *et al.*, 1993).

Continued development of preparations with MRCs (e.g., Fletcher *et al.*, 2000; Shiraishi *et al.*, 2001), as well as combinations of the various ideas and methods from molecular biology, histology, and physiology will be especially powerful approaches to advancing our understanding of MRC regulation.

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REFERENCES

- Auperin B, Rentier-Delrue F, Martial JA, Prunet P (1994) Characterization of a single prolactin (PRL) receptor in tilapia (*Oreochromis niloticus*) which binds both PRLI and PRLII. *J Mol Endocrinol* 13: 241–251
- Ayson FG, Kaneko T, Hasegawa S, Hirano T (1995) Cortisol stimulates the size and number of mitochondrion-rich cells in the yolk-sac membrane of embryos and larvae of tilapia (*Oreochromis mossambicus*) *in vitro* and *in vivo*. *J Exp Zool* 272: 419–425
- Borski RJ (2000) Nongenomic membrane actions of glucocorticoids in vertebrates. *Trends Endocrinol Metab* 11: 427–436
- Bres O, Eales JG (1988) High-affinity, limited-capacity triiodothyronine-binding sites in nuclei from various tissues of the rainbow trout (*Salmo gairdneri*). *Gen Comp Endocrinol* 69: 71–79
- Chakraborti PK, Weisbart M, Chakraborti A (1987) The presence of corticosteroid receptor activity in the gills of the brook trout, *Salvelinus fontinalis*. *Gen Comp Endocrinol* 66: 323–332
- Chan SJ, Steiner DF (1994) Structure and expression of insulin-like growth factor genes in fish. In "Fish Physiology, vol XIII" Ed by WS Hoar, D Randall, Academic Press, New York, pp 213–224
- Chen TT, Marsh A, Shambloot M, Chan K-M, Tang Y-L, Cheng CM, Yang B-Y (1994) Structure and evolution of fish growth hormone and insulin-like growth factor genes. In "Fish Physiology, vol XIII" Ed by WS Hoar, D Randall, Academic Press, New York, pp 179–209
- Cioni C, De Merich D, Cataldi E, Sataudella S (1991) Fine structure of chloride cells in freshwater-and seawater-adapted *Oreochromis niloticus* (Linnaeus) and *Oreochromis mossambicus* (Peters) *J Fish Biol* 39: 197–209
- Claiborne JB (1998) Acid-base regulation. In "The Physiology of Fishes" Ed by DH Evans, CRC Press, Boca Raton, pp 177–198
- Copeland DE (1948) The cytological basis of chloride transfer in the gills of *Fundulus heteroclitus*. *J Morphol* 82: 201–227
- Cutler CP, Brezillon S, Bekir S, Sanders IL, Hazon N, Cramb G (2000) Expression of a duplicate Na,K-ATPase beta(1)-isoform in the European eel (*Anguilla anguilla*). *Am J Physiol Regul Integr Comp Physiol* 279: R222–R229
- Daborn K, Cozzi RRF, Marshall WS (2001) Dynamics of pavement cell? Chloride cell interactions during abrupt salinity change in *Fundulus heteroclitus*. *J Exp Biol* 204: 1889–1899
- Dang Z, Balm PH, Flik G, Wendelaar Bonga SE, Lock RA (2000) Cortisol increases Na(+)/K(+)-ATPase density in plasma membranes of gill chloride cells in the freshwater tilapia *Oreochromis mossambicus*. *J Exp Biol* 203: 2349–2355
- Dange AD (1986) Branchial Na⁺-K⁺-ATPase activity in freshwater or saltwater acclimated tilapia, *Oreochromis (Sarotherodon) mossambicus*: effects of cortisol and thyroxin. *Gen Comp Endocrinol* 62: 341–343
- Degnan KJ (1985) The role of K⁺ and Cl⁻ conductances in chloride secretion by the opercular membrane. *J Exp Zool* 231: 11–17
- Degnan KJ, Karnaky KJ Jr, Zadunaisky J (1977) Active chloride transport in the *in vitro* opercular skin of a teleost (*Fundulus heteroclitus*), a gill-like epithelium rich in chloride cells. *J Physiol* 271: 155–191
- Duan C, Ding J, Li Q, Tsai W, Pozios K (1999) Insulin-like growth factor binding protein 2 is a growth inhibitory protein conserved in zebrafish. *Proc Natl Acad Sci U S A* 96: 15274–15279
- Duguay SJ, Lai-Zhang J, Steiner DF, Funkenstein B, Chan SJ (1996) Developmental and tissue-regulated expression of IGF-I and IGF-II mRNAs in *Sparus aurata*. *J Mol Endocrinol* 16: 123–132
- Eriksson O, Wistrand PJ (1986) Chloride transport inhibition by various types of 'loop' diuretics in fish opercular epithelium. *Acta Physiol Scand* 126: 93–101
- Evans DH (1979) Fish. In "Comparative Physiology of Osmoregulation in Animals Vol 1" Ed by GMO Malooy, Academic Press, Orlando, pp 305–390
- Evans DH (1993) Osmotic and ionic regulation. In "The Physiology of Fishes" Ed by DH Evans, CRC Press, Boca Raton, pp 315–341
- Evans DH, Piermarini PM, Potts WTW (1999) Ionic transport in fish gill epithelium. *J Exp Zool* 283: 641–652
- Fenwick JC, Wendelaar Bonga SE, Flik G (1999) *In vivo* bafilomycin-sensitive Na(+) uptake in young freshwater fish. *J Exp Biol* 202: 3659–3666
- Fletcher M, Kelly SP, Part P, O'Donnell MJ, Wood CM (2000) Transport properties of cultured branchial epithelia from freshwater rainbow trout: a novel preparation with mitochondria-rich cells. *J Exp Biol* 203: 1523–1537
- Flik G, Klaren PHM, Schoenmakers TJM, Bijvelds MJC, Verbost PM, Wendelaar Bonga SE (1996) Cellular calcium transport in fish: unique and universal mechanisms. *Physiol Zool* 69: 403–417
- Flik G, Rentier-Delrue F, Wendelaar Bonga SE (1994) Calcitropic effects of recombinant prolactins in *Oreochromis mossambicus*. *Am J Physiol* 266: R1302–1308
- Flik G, Verbost PM, Wendelaar Bonga SE (1995) Calcium transport processes in fishes. In "Cellular and Molecular Approaches to Fish Ionic Regulation" Ed by CM Wood, TJ Shuttleworth, Academic Press, San Diego, pp 317–342
- Foskett JK, Bern HA, Machen TE, Conner M (1983) Chloride cells and hormonal control of teleost fish osmoregulation. *J Exp Biol* 100: 255–281
- Foskett JK, Logsdon CD, Turner T, Machen TE, Bern HA (1981) Differentiation of the chloride extrusion mechanisms during seawater adaptation of a teleost fish, the cichlid *Sarotherodon mossambicus*. *J Exp Biol* 93: 209–224
- Foskett JK, Machen TE, Bern HA (1982) Chloride secretion and conductance of teleost opercular membrane: effects of prolactin. *Am J Physiol* 242: R380–389
- Foskett JK, Scheffey C (1982) The chloride cell: definitive identification as the salt-secretory cell in teleosts. *Science* 215: 164–166
- Goss G, Perry S, Fryer J, Laurent P (1998) Gill morphology and acid-base regulation in freshwater fishes. *Comp Biochem Physiol* 119A: 107–115
- Goss GG, Laurent P, Perry SF (1994) Gill morphology during hypercapnia in brown bullhead (*I. nebulosus*): role of chloride cells and pavement cells in acid-base regulation. *J Fish Biol* 45: 705–

718

- Gray ES, Kelley KM (1991) Growth regulation in the gobiid teleost, *Gillichthys mirabilis*: roles of growth hormone, hepatic growth hormone receptors and insulin-like growth factor-I. *J Endocrinol* 131: 57–66
- Green H, Morikawa M, Nixon T (1985) A dual effector theory of growth-hormone action. *Differentiation* 29: 195–198
- Hashimoto H, Fukuda M, Matsuo Y, Yokoyama Y, Nishida E, Toyohara H, Sakaguchi M (2000) Identification of a nuclear export signal in MKK6, an activator of the carp p38 mitogen-activated protein kinases. *Eur J Biochem* 267: 4362–4371
- Hashimoto H, Matsuo Y, Yokoyama Y, Toyohara H, Sakaguchi M (1997) Structure and expression of carp mitogen-activated protein kinases homologous to mammalian JNK/SAPK. *J Biochem (Tokyo)* 122: 381–386
- Hashimoto H, Yokoyama Y, Matsuo Y, Toyohara H, Kohno M, Sakaguchi M (1998) Existence of two isoforms of extracellular signal-regulated kinase in fish. *J Biochem (Tokyo)* 123: 1031–1035
- Herndon TM, McCormick SD, Bern HA (1991) Effects of prolactin on chloride cells in opercular membrane of seawater-adapted tilapia. *Gen Comp Endocrinol* 83: 283–289
- Hirai N, Tagawa M, Kaneko T, Seikai T, Tanaka M (1999) Distributional changes in branchial chloride cells during freshwater adaptation in Japanese sea bass *Lateolabrax japonicus*. *Zool Sci* 16: 43–49
- Hirano T (1986) The spectrum of prolactin action in teleosts. *Prog Clin Biol Res* 205: 53–74
- Hiroi J, Kaneko T, Uchida K, Hasegawa S, Tanaka M (1998) Immunolocalization of vacuolar-type H⁺-ATPase in the yolk-sac membrane of tilapia (*Oreochromis mossambicus*) larvae. *Zool Sci* 15: 447–453
- Hiroi J, Kaneko T, Tanaka M (1999) *In vivo* sequential changes in chloride cell morphology in the yolk-sac membrane of Mozambique tilapia (*Oreochromis mossambicus*) embryos and larvae during seawater adaptation. *J Exp Biol* 24: 3485–3495
- Hoar WS (1988) The physiology of smolting salmonids. In "Fish Physiology Vol XIB" Ed by WS Hoar, DJ Randall, Academic Press, Orlando, pp 275–343
- Hossler FE (1980). Gill arch of the mullet, *Mugil cephalus*, III: rate of response to salinity change. *Am J Physiol* 238: R160–R164
- Hossler FE, Ruby JR, McIlwain TD (1979) The gills arch of the mullet, *Mugil cephalus*: II. Modification in surface ultrastructure and Na⁺/K⁺-ATPase content during adaptation to various salinities. *J Exp Zool* 208: 399–405
- Hwang PP (1988) Multicellular complex of chloride cells in the gills of freshwater teleosts. *J Morphol* 196: 15–22
- Hwang PP, Sun CM, Wu SM (1989) Changes of plasma osmolality, chloride concentration and gill Na⁺-K⁺-ATPases activity in tilapia *Oreochromis mossambicus* during seawater acclimation. *Mar Biol* 100: 295–299
- Kaneko T, Shiraishi K, Katoh F, Hasegawa S, Hiroi J (2002) Chloride cells in early life stages of fish and their functional differentiation. *Fish Sci* 68 (in press)
- Kaplan MR, Mount DB, Delpire E, Gamba G, Hebert SC (1996) Molecular mechanisms of NaCl cotransport. *Ann Rev Physiol* 58: 649–668
- Karnaky KJ Jr (1992) Teleost osmoregulation: changes in the tight junction in response to the salinity of the environment. In "Tight junctions" Ed by M Cereijido, CRC Press, Boca Raton, pp 175–185
- Karnaky KJ Jr, Kinter LB, Kinter WB, Stirling CE (1976) Teleost chloride cell. II. Autoradiographic localization of gill Na⁺-K⁺-ATPase in killifish *Fundulus heteroclitus* adapted to low and high salinity environments. *J Cell Biol* 70: 157–177
- Keys AB, Willmer EN (1932) "Chloride-secreting cells" in the gill of fishes with special reference to the common eel. *J Physiol* 76: 368–378
- Kelly SP, Chow IN, Woo NY (1999) Effects of prolactin and growth hormone on strategies of hypoosmotic adaptation in a marine teleost, *Sparus sarba*. *Gen Comp Endocrinol* 113: 9–22
- Kirschner LB (1980) Comparison of vertebrate salt-excreting organs. *Am J Physiol* 238: R219–R223
- Kolla V, Robertson NM, Litwack G (1999) Identification of a mineralocorticoid/glucocorticoid response element in the human Na⁺/K⁺-ATPase alpha1 gene promoter. *Biochem Biophys Res Commun* 266: 5–14
- Kültz D (1996) Plasticity and stressor specificity of osmotic and heat shock responses of *Gillichthys mirabilis* gill cells. *Am J Physiol* 271: C1181–C1193
- Lacy ER (1983) Histochemical and biochemical studies of carbonic anhydrase activity in the opercular epithelium of the euryhaline teleost, *Fundulus heteroclitus*. *Am J Anat* 166: 19–39
- Laurent P (1984) Gill internal morphology. In "Fish physiology vol XA" Ed by WS Hoar, DJ Randall, Academic Press, Orlando, pp 73–183
- Lebel JM, Leloup J (1989) Triiodothyronine binding to putative solubilized nuclear thyroid hormone receptor in liver and gill of the brown trout (*Salmo trutta*) and the European eel (*Anguilla anguilla*). *Gen Comp Endocrinol* 75: 301–309
- Leloup J, Lebel JM (1993) Triiodothyronine is necessary for the action of growth hormone in acclimation to seawater of brown trout (*Salmo trutta*) and rainbow trout (*Onchoryhnchus mykiss*). *Fish Physiol Biochem* 11: 165–173
- Li J, Eygensteyn J, Lock R, Bonga S, Flik G (1997) Na⁺ and Ca²⁺ homeostatic mechanisms in isolated chloride cells of the teleost *Oreochromis mossambicus* analyzed by confocal laser scanning microscopy. *J Exp Biol* 200: 1499–1508
- Lin H, Randall D (1991) Evidence for the presence of an electrogenic proton pump on the trout gill epithelium. *J Exp Biol* 161: 119–134
- MacLachy DL, Eales JG (1992) Intra- and extra-cellular sources of T3 binding to putative thyroid hormone receptors in liver, kidney, and gill nuclei of immature rainbow trout, *Oncorhynchus mykiss*. *J Exp Zool* 262: 22–29
- Madsen SS, Bern HA (1993) *In-vitro* effects of insulin-like growth factor-I on gill Na⁺,K⁺-ATPase in coho salmon, *Oncorhynchus kisutch*. *J Endocrinol* 138: 23–30
- Madsen SS, Korsgaard B (1991) Opposite effects of 17 beta-estradiol and combined growth hormone-cortisol treatment on hypo-osmoregulatory performance in sea trout presmolts, *Salmo trutta*. *Gen Comp Endocrinol* 83: 276–282
- Mancera JM, McCormick SD (1998) Osmoregulatory actions of the GH/IGF axis in non-salmonid teleosts. *Comp Biochem Physiol B* 121: 43–48
- Mancera JM, McCormick SD (2000) Rapid activation of gill Na(+),K(+)-ATPase in the euryhaline teleost *Fundulus heteroclitus*. *J Exp Zool* 287: 263–274
- Marshall WS (1995) Transport processes in isolated teleost epithelia: opercular epithelium and urinary bladder. In "Cellular and Molecular Approaches to Fish Ionic Regulation" Ed by CM Wood, TJ Shuttleworth, Academic Press, San Diego, pp 1–23
- Marshall WS, Nishioka RS (1980) Relation of mitochondria-rich chloride cells to active chloride transport in the skin of a marine teleost. *J Exp Zool* 214: 147–156
- Marshall WS, Bryson SE (1998) Transport mechanisms of seawater teleost chloride cells: an inclusive model of a multi-functional cell. *Comp Biochem Physiol* 119A: 97–106
- Marshall WS, Bryson SE, Darling P, Whitten C, Patrick M, Wilkie M, Wood CM, Buckland-Nicks J (1997) NaCl transport and ultrastructure of opercular epithelium from a freshwater-adapted euryhaline teleost, *Fundulus heteroclitus*. *J Exp Zool* 277: 23–37
- Marshall WS, Bryson SE, Garg D (1993) Alpha 2-adrenergic inhibition of Cl⁻ transport by opercular epithelium is mediated by intra-

- cellular Ca²⁺. *Proc Natl Acad Sci U S A* 90: 5504–5508
- Marshall WS, Bryson SE, Midelfart A, Hamilton WF (1995) Low-conductance anion channel activated by cAMP in teleost Cl⁻-secreting cells. *Am J Physiol* 268: R963–969
- Marshall WS, Bryson SE, Luby T (2000) Control of epithelial Cl⁻ secretion by basolateral osmolality in the euryhaline teleost *Fundulus heteroclitus*. *J Exp Biol* 203: 1897–1905
- Marshall WS, Duquesnay RM, Gillis JM, Bryson SE, Liedtke CM (1998) Neural modulation of salt secretion in teleost opercular epithelium by 2-adrenergic receptors and inositol 1,4,5-trisphosphate. *J Exp Biol* 201: 1959–1965
- Marsigliante S, Muscella A, Vilella S, Storelli C (2000) Dexamethasone modulates the activity of the eel branchial Na⁺/K⁺ATPase in both chloride and pavement cells. *Life Sci* 66: 1663–1673
- Marsigliante S, Muscella A, Vinson GP, Storelli C (1997) Angiotensin II receptors in the gill of sea water- and freshwater-adapted eel. *J Mol Endocrinol* 18: 67–76
- Matthews JB, Awtrey CS, Madara JL (1992) Microfilament-dependent activation of Na⁺/K⁺/2Cl⁻ cotransport by cAMP in intestinal epithelial monolayers. *J Clin Invest* 90: 1608–1613
- McCormick SD (1990) Cortisol directly stimulates differentiation of chloride cells in tilapia opercular membrane. *Am J Physiol* 259: R857–R863
- McCormick SD (1995) Hormonal control of gill Na⁺,K⁺-ATPase and chloride cell function. In "Cellular and Molecular Approaches to Fish Ionic Regulation" Ed by CM Wood, TJ Shuttleworth, Academic Press, San Diego, pp 285–315
- McCormick SD, Bern HA (1989) *In vitro* stimulation of Na⁺-K⁺-ATPase activity and ouabain binding by cortisol in coho salmon gill. *Am J Physiol* 256: R707–R715
- Mills JW, Scwiebert EM, Stanton BA (1994) Evidence for the role of actin filaments in regulating cell swelling. *J Exp Zool* 268: 111–120
- Miyazaki H, Uchida S, Takei Y, Hirano T, Marumo F, Sasaki S (1999) Molecular cloning of CLC chloride channels in *Oreochromis mossambicus* and their functional complementation of yeast CLC gene mutant. *Biochem Biophys Res Commun*. 255: 175–181
- Miwa S, Inui Y (1985) Effects of L-thyroxin and ovine growth hormone on smoltification of amago salmon (*Oncorhynchus rhodurus*). *Gen Comp Endocrinol* 58: 436–42
- Moav B, McKeown BA (1992) Thyroid hormone increases transcription of growth hormone mRNA in rainbow trout pituitary. *Horm Metab Res* 24: 10–14
- Moriyama S, Ayson FG, Kawauchi H (2000) Growth regulation by insulin-like growth factor-I in fish. *Biosci Biotechnol Biochem* 64: 1553–1562
- Motais R, Garcia-Romeu F, Maetz J (1966) Exchange diffusion effect and euryhalinity in teleosts. *J Gen Physiol* 50: 391–422
- Nelson WJ, Hammerton RW (1989) A membrane-cytoskeleton complex containing Na⁺,K⁺-ATPase, ankryrin, and fodrin in Madin-Darby canine kidney (MDCK) cells: Implications for the biogenesis of epithelial cell polarity. *J Cell Biol* 108: 893–902
- Nielsen S, DiGiovanni SR, Christensen EI, Knepper MA, Harris HW (1993) Cellular and subcellular immunolocalization of vasopressin-regulated water channel in rat kidney. *Proc Natl Acad Sci U S A* 90: 11663–11667
- Nishioka RS, Grau EG, Bern HA (1985) *In vitro* release of growth hormone from the pituitary gland of tilapia, *Oreochromis mossambicus*. *Gen Comp Endocrinol* 60: 90–94
- Pan F, Zarate JM, Tremblay GC, Bradley TM (2000) Cloning and characterization of salmon hsp90 cDNA: upregulation by thermal and hyperosmotic stress. *J Exp Zool* 287: 199–212
- Payne JA, Forbush B III (1995) Molecular characterization of the epithelial Na-K-Cl cotransporter isoforms. *Curr Opin Cell Biol* 7: 493–503
- Pelis RM, Zydlewski J, McCormick SD (2001) Gill Na(+)-K(+)-2Cl(-) cotransporter abundance and location in Atlantic salmon: effects of seawater and smolting. *Am J Physiol* 280: R1844–1852
- Perry SF (1997) The chloride cell: structure and function in the gills of freshwater fishes. *Annu Rev Physiol* 59: 325–347
- Perry SF, Goss GG, Laurent P (1992) The interrelationships between gill chloride cell morphology and ionic uptake in four freshwater teleosts. *Can J Zool* 70: 1775–1786
- Perry SF, Laurent P (1989) Adaptational responses of rainbow trout to lowered external NaCl concentration: Contribution of the branchial chloride cell. *J Exp Biol* 147: 147–168
- Perry SF, Laurent P (1993) Environmental effects on fish gill structure and function. In "Fish ecophysiology" Ed by JC Rankin, JB Jensen, Chapman & Hall, London, pp 231–264
- Perry SF, Walsh PJ (1989) Metabolism of isolated fish gill cells: contribution of epithelial chloride cells. *J Exp Biol* 144: 507–520
- Pinter J, Thomas P (1995) Characterization of a progesterone receptor in the ovary of the spotted seatrout, *Cynoscion nebulosus*. *Biol Reprod* 52: 667–675
- Pisam M (1981) Membranous systems in the "chloride cell" of teleostean fish gill; their modifications in response to the salinity of the environment. *Anat Rec* 200: 401–414
- 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
- Pisam M, Boeuf G, Prunet P, Rambourg A (1990) Ultrastructural features of mitochondria-rich cells in stenohaline freshwater and seawater fishes. *Am J Anat* 187: 21–31
- Pisam M, Caroff A, Rambourg A (1987) Two types of chloride cells in the gill epithelium of a freshwater-adapted euryhaline fish: *Lebistes reticulatus*; their modifications during adaptation to saltwater. *Am J Anat* 179: 40–50
- Pisam M, Le Moal C, Auperin B, Prunet P, Rambourg A (1995) Apical structures of "mitochondria-rich" alpha and beta cells in euryhaline fish gill: their behaviour in various living conditions. *Anat Rec* 241: 13–24
- Pisam M, Prunet P, Boeuf G, Rambourg A (1988) Ultrastructural features of chloride cells in the gill epithelium of the Atlantic salmon, *Salmo salar*, and their modifications during smoltification. *Am J Anat* 183: 235–44
- Pisam M, Prunet P, Rambourg A (1989) Accessory cells in the gill epithelium of the freshwater rainbow trout *Salmo gairdneri*. *Am J Anat* 184: 311–320
- Prunet P, Pisam M, Claireaux JP, Boeuf G, Rambourg A (1994) Effects of growth hormone on gill chloride cells in juvenile Atlantic salmon (*Salmo salar*). *Am J Physiol* 266: R850–R857
- 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
- Richardson NA, Anderson AJ, Rimmer MA, Sara VR (1995) Localization of insulin-like growth factor-I immunoreactivity in larval and juvenile barramundi (*Lates calcarifer*). *Gen Comp Endocrinol* 100: 282–292
- Russell MJ, Klemmer AM, Olson KR (2001) Angiotensin signaling and receptor types in teleost fish. *Comp Biochem Physiol A Mol Integr Physiol* 128: 41–51
- Sakamoto T, Agustsson T, Björnsson BTh, Ando M (2000a) Roles of growth hormone and prolactin during adaptation of the gobies to various environments. In "Growth and Growth Regulation in Fish" Ed by Th Björnsson, D MacKinlay, American Fisheries Society, Vancouver, pp 29–32
- Sakamoto T, Hirano T (1991) Growth hormone receptors in the liver and osmoregulatory organs of rainbow trout: characterization and dynamics during seawater adaptation. *J Endocrinol* 130: 425–433
- Sakamoto T, Hirano T (1993) Expression of insulin-like growth factor

- I gene in osmoregulatory organs during seawater adaptation of the salmonid fish: Possible mode of osmoregulatory action of growth hormone. *Proc Natl Acad Sci USA* 90: 1912–1916
- Sakamoto T, Iwata K, Ando M (2002) Growth hormone and prolactin expression during environmental adaptation of gobies. *Fish Sci* (in press)
- Sakamoto T, Kozaka T, Takahashi A, Kawauchi H, Ando M (2001) Medaka, *Oryzias latipes*, as a model for hypoosmoregulation of euryhaline fishes. *Aquaculture* 193: 347–654
- Sakamoto T, McCormick SD, Hirano T (1993) Osmoregulatory actions of growth hormone and its mode of action in salmonids: A review. *Fish Physiol Biochem* 11: 155–164
- Sakamoto T, Ojima N, Yamashita M (2000b) Induction of mRNAs in response to acclimation of trout cells to different osmolalities. *Fish Physiol Biochem* 22: 255–262
- Sakamoto T, Shepherd BS, Nishioka RS, Madsen SS, Siharath K, Bern HA, Grau EG (1995) Osmoregulatory actions of growth hormone in an advanced teleost. *Am. Zool* 34: 43A
- Sakamoto T, Shepherd BS, Nishioka RS, Madsen SS, Siharath K, Richman NH III, Bern HA, Grau EG (1997) Osmoregulatory actions of growth hormone and prolactin in an advanced teleost. *Gen Comp Endocrinol* 106: 95–101
- Sakamoto T, Yokota S, Ando M (2000c) Rapid morphological oscillation of mitochondrion-rich cell in estuarine mudskipper following salinity changes. *J Exp Zool* 286: 666–669
- Sandor T, DiBattista JA, Mehdi AZ (1984) Glucocorticoid receptors in the gill tissue of fish. *Gen Comp Endocrinol* 53: 353–64
- Sandra O, Le Rouzic P, Cauty C, Ederly M, Prunet P (2000) Expression of the prolactin receptor (tiPRL-R) gene in tilapia *Oreochromis niloticus*: tissue distribution and cellular localization in osmoregulatory organs. *J Mol Endocrinol* 24: 215–224
- Santos CR, Ingleton PM, Cavaco JE, Kelly PA, Ederly M, Power DM (2001) Cloning, Characterization, and Tissue Distribution of Prolactin Receptor in the Sea Bream (*Sparus aurata*). *Gen Comp Endocrinol* 121: 32–47
- Sardet C (1980) Freeze fracture of the gill epithelium of euryhaline teleost fish. *Am J Physiol* 238: R207–212
- Sardet C, Pisam M, Maetz J (1979) The surface epithelium of teleostean fish gills: cellular and junctional adaptations of the chloride cell in relation to salt adaptation. *J Cell Biol* 80: 96–117
- Sasai S, Kaneko T, Hasegawa S, Tsukamoto K (1999) Morphological alteration in two types of gill chloride cells in Japanese eel (*Anguilla japonica*) during catadromous migration. *Can J Zool* 76: 1480–1487
- Scheide JI, Zadunaisky JA (1988) Effect of atriopeptin II on isolated opercular epithelium of *Fundulus heteroclitus*. *Am J Physiol* 254: R27–R32
- Schreck CB (1981) Stress and compensation in teleostean fishes: Response to social physical factors. In "Stress and Fish" Ed by AD Pickering, Academic Press, New York, pp 295–321
- Schreiber AM, Specker JL (2000) Metamorphosis in the summer flounder, *Paralichthys dentatus*: thyroidal status influences gill mitochondria-rich cells. *Gen Comp Endocrinol* 117: 238–250
- Seidelin M, Madsen SS (1999) Endocrine control of Na⁺,K⁺-ATPase and chloride cell development in brown trout (*Salmo trutta*): interaction of insulin-like growth factor-I with prolactin and growth hormone. *J Endocrinol* 162: 127–135
- Seidelin M, Madsen SS, Byrialsen A, Kristiansen K (1999) Effects of insulin-like growth factor-I and cortisol on Na⁺,K⁺-ATPase expression in osmoregulatory tissues of brown trout (*Salmo trutta*). *Gen Comp Endocrinol* 113: 331–342
- Seidelin M, Madsen SS, Blenstrup H, Tipsmark CK (2000) Time-course changes in the expression of Na⁺,K⁺-ATPase in gills and pyloric caeca of brown trout (*Salmo trutta*) during acclimation to seawater. *Physiol Biochem Zool* 73: 446–453
- Shapiro M, Matthews J, Hecht G, Delp C, Madara JL (1991) Stabilization of F-actin prevents cAMP-elicited Cl⁻ secretion in T84 cells. *J Clin Invest* 87: 1903–1909
- Shikano T, Fujio Y (1998) Relationships of salinity tolerance to immunolocalization of Na⁺,K⁺-ATPase in the gill epithelium during seawater and freshwater adaptation of the guppy, *Poecilia reticulata*. *Zool Sci* 15: 35–41
- Shirai N, Utida S (1970) Development and degeneration of the chloride cell during seawater and freshwater adaptation of the Japanese eel, *Anguilla japonica*. *Z Zellforsch Mikrosk Anat* 103: 247–64
- Shiraishi K, Hiroi J, Kaneko T, Matsuda M, Hirano T, Mori T (2001) *In vitro* effects of environmental salinity and cortisol on chloride cell differentiation in embryos of Mozambique tilapia, *Oreochromis mossambicus*, measured using a newly developed 'yolk-ball' incubation system. *J Exp Biol* 204: 1883–1888
- Shiraishi K, Kaneko T, Hasegawa S, Hirano T (1997) Development of multicellular complexes of chloride cells in the yolk-sac membrane of tilapia (*Oreochromis mossambicus*) embryos and larvae in seawater. *Cell Tissue Res* 288: 583–590
- Shrimpton JM, Devlin RH, McLean E, Byatt JC, Donaldson EM, Randall DJ (1995) Increases in gill cytosolic corticosteroid receptor abundance and saltwater tolerance in juvenile coho salmon (*Oncorhynchus kisutch*) treated with growth hormone and placental lactogen. *Gen Comp Endocrinol* 98: 1–15
- Shrimpton JM, McCormick SD (1998) Regulation of gill cytosolic corticosteroid receptors in juvenile Atlantic salmon: interaction effects of growth hormone with prolactin and triiodothyronine. *Gen Comp Endocrinol* 112: 262–274
- Shull MM, Pugh DG, Lingrel JB (1990) The human Na, K-ATPase alpha 1 gene: characterization of the 5'-flanking region and identification of a restriction fragment length polymorphism. *Genomics* 6: 451–460
- Siharath K, Bern HA (1993) The physiology of insulin-like growth factor (IGF) and its binding proteins in teleost fishes. *Proc Zool Soc Haldane Commun Vol* 1993 113–124
- Silva P, Solomon R, Spokes K, Epstein F (1977) Ouabain inhibition of gill Na-K-ATPase: relationship to active chloride transport. *J Exp Zool* 199: 419–426
- Singer TD, Tucker SJ, Marshall WS, Higgins CF (1998) A divergent CFTR homologue: highly regulated salt transport in the euryhaline teleost *F. heteroclitus*. *Am J Physiol* 274: C715–C723
- Subash Peter MC, Lock RA, Wendelaar Bonga SE (2000) Evidence for an osmoregulatory role of thyroid hormones in the freshwater mozambique tilapia *Oreochromis mossambicus*. *Gen Comp Endocrinol* 120: 157–167
- Sullivan G, Fryer J, Perry S (1995) Immunolocalization of proton pumps (H⁺-ATPase) in pavement cells of rainbow trout gill. *J Exp Biol* 198: 2619–2629
- Sullivan GV, Fryer JN, Perry SF (1996) Localization of mRNA for proton pump (H⁺-ATPase) and Cl⁻/HCO₃⁻ exchanger in rainbow trout gill. *Can J Zool* 74: 2095–2103
- Suzuki M, Miyazaki K, Ikeda M, Kamaguchi Y, Sakai O (1993) F-actin network may regulate a Cl⁻ channel in renal proximal tubule cells. *J Memb Biol* 134: 31–39
- Suzuki Y, Itakura M, Kashiwagi M, Nakamura N, Matsuki T, Sakuta H, Naito N, Takano K, Fujita T, Hirose S (1999) Identification by differential display of a hypertonicity-inducible inward rectifier potassium channel highly expressed in chloride cells. *J Biol Chem* 274: 11376–11382
- Takei Y (2000) Structural and functional evolution of the natriuretic peptide system in vertebrates. *Int Rev Cytol* 194: 1–66
- Tipsmark CK, Madsen SS (2001) Rapid modulation of Na⁺/K⁺-ATPase activity in osmoregulatory tissues of a salmonid fish. *J Exp Biol* 204: 701–709
- Towle DW, Gilman ME, Hempel JD (1977) Rapid modulation of gill Na⁺+K⁺-dependent ATPase activity during rapid acclimation of the killifish *Fundulus heteroclitus* to salinity change. *J Exp Zool* 202: 179–186

- Uchida K, Kaneko T (1996) Enhanced chloride cell turnover in the gills of in chum salmon fry in seawater. *Zool Sci* 13: 655–660
- Uchida K, Kaneko T, Miyazaki H, Hasegawa S, Hirano T (2000) Excellent salinity tolerance of Mozambique tilapia (*Oreochromis mossambicus*): elevated chloride cell activity in the branchial and opercular epithelia of the fish adapted to concentrated seawater. *Zool Sci* 17: 149–160
- Uchida K, Kaneko T, Tagawa M, Hirano T (1998) Localization of cortisol receptor in branchial chloride cells in chum salmon fry. *Gen Comp Endocrinol* 109: 175–185
- Uchida K, Kaneko T, Yamauchi K, Hirano T (1996) Morphometrical analysis of chloride cell activity in the gill filaments and lamellae and changes in Na⁺,K⁺-ATPase activity during seawater adaptation in chum salmon fry. *J Exp Zool* 276: 193–200
- Uchida K, Kaneko T, Yamauchi K, Ogasawara T, Hirano T (1997) Reduced hypoosmoregulatory ability and alteration in gill chloride cell distribution in mature chum salmon (*Onchorhynchus keta*) migrating upstream for spawning. *Marine Biol* 129: 247–253
- Van Der Heijden AJH, Verbost PM, Eygensteyn J, Li J, Wendelaar Bonga SE, Flik G (1997) Mitochondria-rich cells in gills of tilapia (*Oreochromis mossambicus*) adapted to fresh water or seawater: quantification by confocal laser scanning microscopy. *J Exp Biol* 200: 55–64
- Walsh PJ (1998) Nitrogen excretion and metabolism. In "The physiology of fishes" Ed by DH Evans, CRC Press, Boca Raton, pp 199–214
- Weisbart M, Chakraborti PK, Gallivan G, Eales JG (1987) Dynamics of cortisol receptor activity in the gills of the brook trout, *Salvelinus fontinalis*, during seawater adaptation. *Gen Comp Endocrinol* 68: 440–448
- Wendelaar Bonga SE (1997) The stress response in fish. *Physiol Rev* 77: 591–625
- Wendelaar Bonga S, van der Meij CJM (1989) Degeneration and death, by apoptosis and necrosis, of the pavement and chloride cells in the gills of the teleost *Oreochromis mossambicus*. *Cell Tiss Res* 255: 235–243
- Weng CF, Lee TH, Hwang PP (1997) Immune localization of prolactin receptor in the mitochondria-rich cells of the euryhaline teleost (*Oreochromis mossambicus*) gill. *FEBS Lett* 405: 91–94
- Wilson JM, Laurent P, Tufts BL, Benos DJ, Donowitz M, Vogl AW, Randall DJ (2000a) NaCl uptake by the branchial epithelium in freshwater teleost fish: an immunological approach to ion-transport protein localization. *J Exp Biol* 203: 2279–2296
- Wilson JM, Randall DJ, Donowitz M, Vogl AW, Ip AK (2000b) Immunolocalization of ion-transport proteins to branchial epithelium mitochondria-rich cells in the mudskipper (*Periophthalmodon schlosseri*). *J Exp Biol* 203: 2297–2310
- Wong CKC, Chan DKO (1999) Chloride cell subtypes in the gill epithelium of Japanese eel *Anguilla japonica*. *Am J Physiol* 46: R517–R522
- Wood CM, Marshall WS (1994) Ion balance, acid-base regulation and chloride cell function in the common killifish, *Fundulus heteroclitus*—a freely euryhaline estuarine teleost. *Estuaries* 17: 34–52
- Yasunaga H, Sakamoto T, Yokota S, Ando M (2001) Differential display of skin mRNAs regulated during adaptation of mudskipper to different environments. *J Comp Physiol B* (in press)
- Yokota S, Iwata K, Fujii Y, Ando M (1997) Ion transport across the skin of the mudskipper *Periophthalmus modestus*. *Comp Biochem Physiol* 118A: 903–910
- Young G (1988) Enhanced response of the interrenal of coho salmon (*Oncorhynchus kisutch*) to ACTH after growth hormone treatment *in vivo* and *in vitro*. *Gen Comp Endocrinol* 71: 85–92
- Young G, Lin RJ (1988) Response of the interrenal to adrenocorticotrophic hormone after short-term thyroxin treatment of coho salmon (*Oncorhynchus kisutch*). *J Exp Zool*. 245: 53–58
- Zadunaisky JA, Cardona S, Au L, Roberts DM, Fisher E, Lowenstein B, Cragoe EJ Jr, Spring KR (1995) Chloride transport activation by plasma osmolarity during rapid adaptation to high salinity of *Fundulus heteroclitus*. *J Membr Biol* 143: 207–217

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