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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<sup>-</sup>. Long-term development/differentiation of the two types of MRCs during adaptation to different salinities appears to be regulated mainly by endocrine factors. Osmolality, Ca<sup>2+</sup>, 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<sup>+</sup> and Cl<sup>-</sup> 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<sup>-</sup> 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<sup>+</sup>,K<sup>+</sup>-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 Ca<sup>2+</sup> 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<sup>+</sup> and Cl<sup>-</sup> by basolateral application of ouabain (an inhibitor of Na<sup>+</sup>,K<sup>+</sup>-ATPase) suggests that Na<sup>+</sup>,K<sup>+</sup>-ATPase generates an electrochemical gradient for Na<sup>+</sup> from the plasma to the cytoplasm of the MRC to drive Na<sup>+</sup> 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<sup>-</sup> extrusion rate (serosa to mucosa) was equal to the short-circuit current, but there was no net extrusion of Na<sup>+</sup>. Basolateral application of furosemide (an inhibitor of the Na<sup>+</sup>-K<sup>+</sup>-2Cl<sup>-</sup> cotransporter family) inhibited the net extrusion of Cl<sup>-</sup> (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<sup>-</sup> extrusion. An apical Cl<sup>-</sup> 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). Ba<sup>2+</sup> sensitivity of the serosal surface suggests the presence of a basolateral K<sup>+</sup> channel (Degnan, 1985). Apical K<sup>+</sup> 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<sup>+</sup> gradient, which is produced across the basolateral membrane by Na<sup>+</sup>,K<sup>+</sup>-ATPase, drives the Na<sup>+</sup>-K<sup>+</sup>-2Cl<sup>-</sup> cotransporter; K<sup>+</sup> enters via the basolateral Na<sup>+</sup>,K<sup>+</sup>-ATPase and Na<sup>+</sup>-K<sup>+</sup>-2Cl<sup>-</sup> cotransport, and the K<sup>+</sup> is thought to be recycled from the cell via K<sup>+</sup> channels; Cl<sup>-</sup> exits the cell via an apical Cl<sup>-</sup> channel and K<sup>+</sup> via a basolateral K<sup>+</sup> channel, resulting in a serosa-positive trans-epithelial potential that moves Na<sup>+</sup> through the leaky paracellular pathway between adjacent cells (see also Fig. 2, SW-type).

Recent reports have shown that Na<sup>+</sup>-K<sup>+</sup>-2Cl<sup>-</sup> 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<sup>+</sup> 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<sup>-</sup> channels (CLC-3 and 5) as intracellular Cl<sup>-</sup> 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<sup>-</sup> uptake occurs via the MRCs because the morphological characteristics of the MRCs correlate well with Cl<sup>-</sup> uptake rates (e.g., Perry and Laurent, 1989; Goss *et al.*, 1994; Wood and Marshall, 1994; Marshall *et al.*, 1997). Apical Cl<sup>-</sup>/HCO<sub>3</sub><sup>-</sup> exchange is assumed to medi-

ate  $\text{Cl}^-$  uptake across the gill in FW conditions; inhibitors of the  $\text{Cl}^-/\text{HCO}_3^-$  exchanger reduce  $\text{Cl}^-$  uptake and produce a metabolic alkalosis in fish, as does the removal of external  $\text{Cl}^-$  (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  $\text{Cl}^-$  gradient between the cytoplasm and FW does not favor uptake of  $\text{Cl}^-$  ions from FW, and the true apical  $\text{HCO}_3^-$  gradient is unknown. Presumably, net  $\text{Cl}^-$  movement across the gill may be mediated via a basolateral  $\text{Cl}^-$  channel, driven by the inside-negative membrane potential for regular cells. The intracellular generation of  $\text{H}^+$  and  $\text{HCO}_3^-$  necessary for these apical extrusion mechanisms is probably derived from the hydration of  $\text{CO}_2$ , 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  $\text{Na}^+$  is most probably via the apical

$\text{Na}^+$  channel, and down an electrochemical gradient generated by an apical vacuolar  $\text{H}^+$ -ATPase, although the  $\text{Na}^+/\text{H}^+$  exchange mechanism cannot be ignored (see Evans *et al.*, 1999; Fenwick *et al.* 1999). There is some debate about the cellular localization of  $\text{H}^+$ -ATPase and  $\text{Na}^+$  channels. With *in situ* hybridization and/or immunocytochemistry, the  $\text{H}^+$ -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  $\text{H}^+$ -ATPase and  $\text{Na}^+$  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  $\text{Na}^+/\text{H}^+$  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  $\text{Na}^+$  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 $\text{Ca}^{2+}$ ↑	Apical pit open
	Plasma osmolality ↑ Plasma cortisol, angiotensin II and ANP ↑	$\text{Na}^+, \text{K}^+$ -ATPase ↑ $\text{Cl}^-$ secretion ↑ c-Jun modification Hsps ↑
		mRNA of CFTR and $\text{K}^+$ 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 $\text{Ca}^{2+}$ ↓	Apical pit close
	Sympathetic nerve stimulation Plasma osmolality ↓	$\text{Cl}^-$ secretion ↓ c-Jun modification
Days ~ weeks	Plasma PRL ↑	$\text{Cl}^-$ 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<sup>-</sup> 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<sup>-</sup> secretion may be mediated by the  $\alpha_2$ -adrenergic receptor activated by the sympathetic nervous system in killifish. This adrenergic receptor acts *via* phospholipase C, inositol triphosphate and intracellular Ca<sup>2+</sup>. Scheide and Zadunaisky (1988) showed that atrial natriuretic peptide (ANP), recognized as a SW-adapting hormone (Takei, 2000), directly increases Cl<sup>-</sup> 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<sup>+</sup>,K<sup>+</sup>-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<sup>+</sup>,K<sup>+</sup>-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<sup>+</sup>,K<sup>+</sup>-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<sup>+</sup>,K<sup>+</sup>-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<sup>+</sup>,K<sup>+</sup>-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<sup>-</sup> 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 Ca<sup>2+</sup> 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<sup>-</sup>-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<sup>+</sup>,K<sup>+</sup>-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<sup>+</sup>,K<sup>+</sup>-ATPase  $\alpha$ -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<sup>+</sup>-K<sup>+</sup>-2Cl<sup>-</sup> 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<sup>+</sup>,K<sup>+</sup>-ATPase, the Cl<sup>-</sup> channel, and the Na<sup>+</sup>-K<sup>+</sup>-2Cl<sup>-</sup> 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,  $\alpha$ - and  $\beta$ , present in the gill filament of FW species (loach and gudgeon) and euryhaline species (salmonids, guppy, and tilapia) in FW. The  $\alpha$ -type MRCs are activated in the filamental epithelium of euryhaline fishes acclimated to SW and are thought to be the homologue of the Cl<sup>-</sup>-secretory SW MRCs (Pisam *et al.*, 1987, 1995). On the other hand, the  $\beta$ -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  $\beta$ -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.

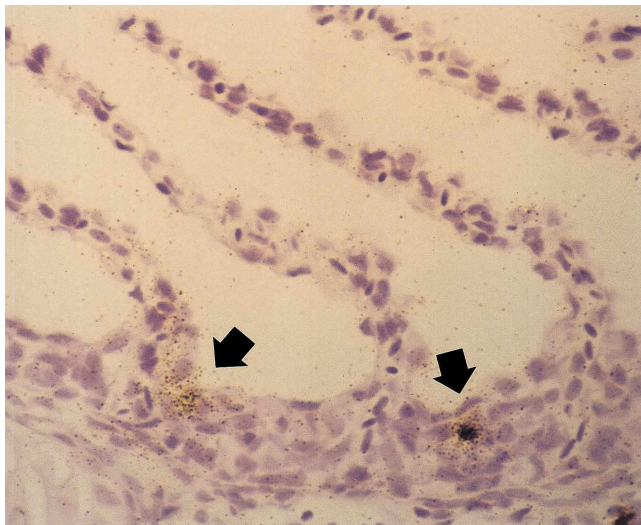
### Prolactin (PRL)

Prolactin, a FW-adapting hormone in teleosts (see Hirano *et al.*, 1986), inhibits the development Cl<sup>-</sup>-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  $\beta$  MRCs, whereas the SW-form  $\alpha$  MRCs were reduced in size. Although mammalian PRL sometimes increased gill Na<sup>+</sup>,K<sup>+</sup>-ATPase activity possibly through growth hormone (GH) receptors, homologous PRLs decrease the activity of Na<sup>+</sup>,K<sup>+</sup>-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<sup>+</sup>,K<sup>+</sup>-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  $\text{Na}^+, \text{K}^+$ -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  $\text{Na}^+, \text{K}^+$ -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  $\text{Na}^+, \text{K}^+$ -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  $\text{Na}^+, \text{K}^+$ -ATPase  $\alpha$  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  $\text{Na}^+, \text{K}^+$ -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  $\text{Na}^+$  and  $\text{Cl}^-$  ions, possibly by increasing gill  $\text{H}^+$ -ATPase activity as well as cell number, apical surface areas, and/or  $\text{Na}^+, \text{K}^+$ -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  $\text{Na}^+, \text{K}^+$ -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  $\text{Na}^+, \text{K}^+$ -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,  $\text{Na}^+, \text{K}^+$ -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.

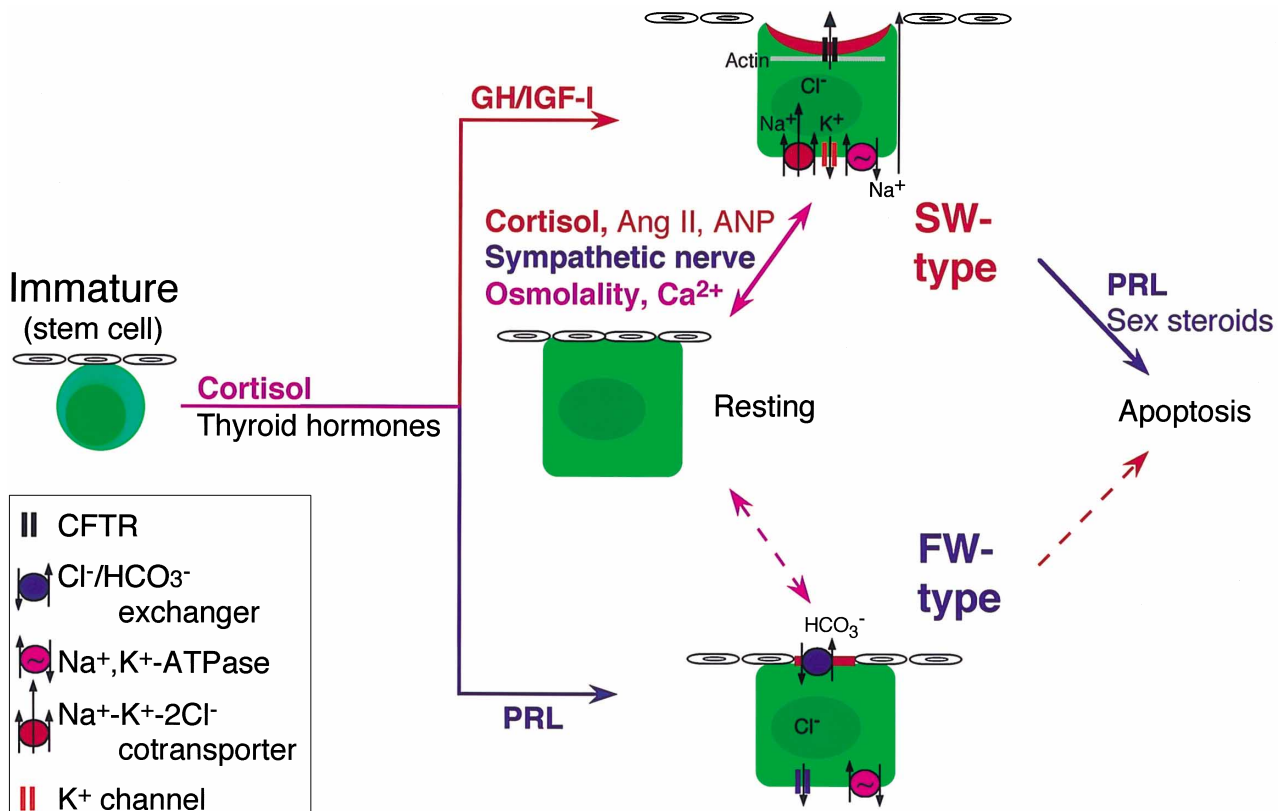
### CONCLUSIONS: AN INTEGRATED MODEL FOR REGULATION OF MITOCHONDRION-RICH CELLS DURING ADAPTATION TO DIFFERENT SALINITIES

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  $\text{Cl}^-$  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  $\text{Cl}^-$ -secretory SW MRCs. Hyperosmolality,  $\text{Ca}^{2+}$ , 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  $\text{H}^+$ -ATPase and  $\text{Na}^+$  channels. Evidence to date indicates that rapid regulation by osmolality, neurotransmitters, fast-acting hormones, and  $\text{Ca}^{2+}$  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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