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## Cellular Calcium Transport in Fish: Unique and Universal Mechanisms

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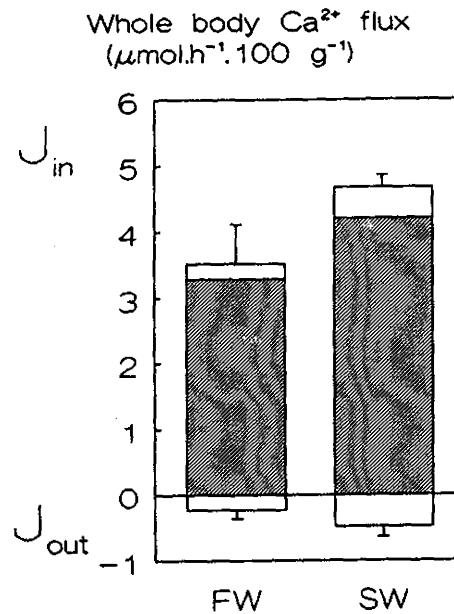
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### Abstract

*The tilapia, Oreochromis mossambicus, is a truly euryhaline species in that it lives, grows, and reproduces in freshwater as well as in full-strength seawater. The gills, intestine, and kidneys show ionoregulatory adaptations fundamental for the calcium balance of this fish in these vastly different ionic media. This review focuses on calcium flows in these ionoregulatory organs and the changes that occur in the  $Ca^{2+}$ -transporting mechanisms in the basolateral plasma membrane compartment of the cells that make up their ion-transporting epithelia. Influx of  $Ca^{2+}$  via the gills is comparable in freshwater and seawater; also, the  $Ca^{2+}$ -ATPase and  $Na^+/Ca^{2+}$  exchanger in branchial epithelial plasma membranes have comparable activities in fish adapted to freshwater and in those adapted to seawater. Ussing chamber experiments with isolated opercular membranes (a flat epithelium with chloride cells) suggest that chloride-cell-mediated, inward  $Ca^{2+}$  transport is largely dependent on  $Na^+$ -dependent mechanisms. The  $Ca^{2+}$ -ATPase is thought to play a "housekeeping" role in cellular  $Ca^{2+}$  regulation. Intestinal epithelial  $Ca^{2+}$  flux is lower in seawater fish than in freshwater fish, and this may reflect adaptation to the imminent overload of calcium in seawater, where for the uptake of water the fish drinks a 10-millimolar Ca solution. Intestinal  $Ca^{2+}$  transport is fully dependent on serosal  $Na^+$ . Accordingly, a powerful  $Na^+/Ca^{2+}$  exchanger operates in the basolateral plasma membrane of the enterocyte, and in particular the capacity of this transporter decreases in seawater fish. The kidney of freshwater fishes produces a typical dilute and hypocalcic urine; in seawater, urine production decreases and the urine calcium concentration is always higher than that of the plasma. Exchange activity of  $Na^+$  and  $Ca^{2+}$  is undetectably low or absent in renal tissue plasma membranes. However, a high-affinity, high-capacity  $Ca^{2+}$ -ATPase activity appears to correlate with  $Ca^{2+}$*

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*Fig. 2. Whole-body  $\text{Ca}^{2+}$  fluxes in tilapia. Tilapia were adapted to freshwater or seawater for at least 4 wk. Whole-body, unidirectional  $\text{Ca}^{2+}$  influx ( $J_{in}$ ) and  $\text{Ca}^{2+}$  efflux ( $J_{out}$ ) (in  $\mu\text{mol}/(\text{h} \cdot 100\text{ g})$ ) were determined as described in detail elsewhere (Flik et al. 1985a, 1994). The hatched area reflects the net influx of  $\text{Ca}^{2+}$  calculated as the difference between  $J_{in}$  and  $J_{out}$ . Mean values for six fish are given; bars indicate standard errors of the mean. No statistically significant differences were observed. The flux ratio in both cases far exceeds 1 and indicates that active  $\text{Ca}^{2+}$  transport occurs in the branchial epithelium (Perry and Flik 1988).*

is exposed to solutions containing ultrafiltered, millimolar concentrations of calcium. The great variety in nephric structures among fishes and the limited number of studies on calcium handling by fish nephrons (or nephric segments) make it difficult to appreciate the role of the kidney in the calcium metabolism of fish in general. Fish, again, and in particular their kidneys, should be appreciated as versatile models for (comparative) renal physiology.

Active (i.e., cell-mediated)  $\text{Ca}^{2+}$  transport implies that calcium ions have to cross two plasma membrane barriers, namely, the apical and the basolateral plasma membranes. This leaves two plasma membrane sites requiring different forms of regulation (Murer and Gmaj 1986; van Os et al. 1988; Flik and Verbost 1993; Friedman and Gesek 1993). Proceeding from the reasonable assumption that the movement of calcium over biomembranes

is not diffusive but mediated through carrier proteins, we may advance distinct and carrier-specific kinetic criteria for  $\text{Ca}^{2+}$  transporters in the different plasma membrane domains. For all three epithelia mentioned, mucosal concentrations of calcium are mostly in the millimolar range. Intracellular free  $\text{Ca}^{2+}$  concentrations (resting levels) are in the submicromolar range (typical value 100 nmol/L; Carafoli 1987; Schoenmakers et al. 1993). We predict, therefore, that carriers in the apical membrane of a calcium transporting epithelium will have a half-maximum activation concentration of  $\text{Ca}^{2+}$  ( $K_m$ ) in the millimolar range, those in the basolateral plasma membrane one in the nanomolar range. As the interior of the cell is electrically negative relative to the exterior, it follows that cellular  $\text{Ca}^{2+}$  influx is passive, down an electrochemical gradient, and that  $\text{Ca}^{2+}$  extrusion to the serosal compartment requires energy to overcome the steep electrochemical gradient.

In recent years a steady flow of papers has advanced evidence that  $\text{Ca}^{2+}$  influx, at least in branchial and intestinal (and likely in renal)  $\text{Ca}^{2+}$  transporting cells, is controlled by stanniocalcin (STC; Butler 1993; Flik and Verbost 1993; Verbost et al. 1993). Stanniocalcin is a hormone restricted to holostean and teleostean fish species. In gills and intestine, STC inhibits  $\text{Ca}^{2+}$  entry (Flik and Verbost 1993; Verbost et al. 1993), and it thus controls the permeability to  $\text{Ca}^{2+}$  of the apical membrane through a pathway dependent on a second messenger (cyclic AMP), most likely a calcium channel operated by a second messenger (Verbost et al. 1993). Stanniocalcin is a fast-acting hormone providing for the fish a tool for the rapid control of  $\text{Ca}^{2+}$  flow. A higher STC metabolic clearance rate and secretion rate in seawater eels compared to that in freshwater eels (Hanssen et al. 1993) indicates that seawater may impose a greater calcium challenge on the animal than freshwater.

Studies on  $\text{Ca}^{2+}$  transport in isolated apical membranes of fish tissues are restricted to intestinal brush border membranes. Apical membranes of branchial or renal epithelium have not been isolated for this purpose. We have obtained good evidence for a carrier-mediated transport of  $\text{Ca}^{2+}$  in tilapia intestinal brush border vesicles (Klaren et al. 1993). Although there are indications that intestinal  $\text{Ca}^{2+}$  transport is controlled by STC (Flik and Verbost 1993), we have failed so far to show an influence of STC (or a second messenger) on this particular transport. A consistent finding is that calcium transport in brush border vesicles, also in tilapia (P. H. M. Klaren, personal observation), is linked to a purinergic, P2-type (suramin-sensitive) receptor, assumed to be a calcium channel (Wiley, Chen, and Jamieson 1993). The possible link between STC and suramin-sensitive  $\text{Ca}^{2+}$  transport is presently under investigation in our lab.

The main goal of this review is to focus on some long-term (on the order of days) adaptational responses of tilapia exposed to freshwater or seawater and treated with prolactin and to show that such responses include specific changes in extrusion mechanisms of the basolateral plasma membrane compartment opposite the apical membrane. These studies show that  $\text{Ca}^{2+}$  transporter densities reflect the  $\text{Ca}^{2+}$  flows in the epithelium.

### Gills

Analysis of the  $\text{Ca}^{2+}$  flux ratio in intact trout and trout isolated head preparations has unequivocally demonstrated that active  $\text{Ca}^{2+}$  transport mechanisms must underlie branchial uptake of  $\text{Ca}^{2+}$  from the water (Perry and Flik 1988). In the complex epithelium of the gills, chloride cells are found—cells specialized for ion transport and the likely site of  $\text{Ca}^{2+}$  uptake (McCormick, Hasegawa, and Hirano 1992; Perry, Goss, and Fenwick 1992).

Studies on active  $\text{Ca}^{2+}$  uptake mechanisms in fish gills started in the seventies with the search for an energized  $\text{Ca}^{2+}$  pump. In early studies a  $\text{Ca}^{2+}$ -ATPase with a low (millimolar) affinity for  $\text{Ca}^{2+}$  was postulated to provide the driving force. This "ATPase" was unmasked as a nonspecific phosphatase (Flik, Wendelaar Bonga, and Fenwick 1983). Subsequently, a high-affinity  $\text{Ca}^{2+}$ -ATPase with characteristics of P-type ATPases (Strehler 1991) was demonstrated in the basolateral plasma membrane of these cells (Flik, van Rijs, and Wendelaar Bonga 1985*b*). Such a calcium pump has been demonstrated now in trout species, tilapia species, eel species, carp, and killifish and seems, therefore, of general occurrence in fish.

A series of specific criteria were advanced to positively identify the calcium pump in the basolateral plasma membrane fraction of gills of fishes. First, the location of the calcium pump in the basolateral plasma membrane of an ion-transporting cell implies that the pump is co-located with markers for this membrane compartment. The  $\text{Na}^+/\text{K}^+$ -ATPase activity is such a marker (Mircheff and Wright 1976), and this enzyme is more abundant in the ionocytes with their elaborate invaginations of the basolateral plasma membrane (Flik and Verbost 1994). Accordingly, a membrane isolation procedure based on a specific purification of  $\text{Na}^+/\text{K}^+$ -ATPase is likely to yield a membrane preparation enriched in basolateral plasma membranes and, in particular, plasma membranes of the ionocytes of the branchial epithelium. Procedures have been described that allow the isolation of membrane fractions that are essentially devoid of membranes from blood cells (saline perfusion of the gills strongly decreases their number in scrapings of branchial epithelium collected at the start of an isolation procedure) and

subcellular membranes from endoplasmic reticulum, Golgi apparatus, or mitochondria. The development of such procedures appears to be a prerequisite for the demonstration of a plasma membrane  $\text{Ca}^{2+}$  pump, as  $\text{Ca}^{2+}$  pumps are present in all membranes that face the cytosol, and only the plasma membrane calcium pumps mediate extrusion for epithelial calcium uptake.

Kinetic criteria may further contribute to the identification of a plasma membrane calcium pump. Analysis of enzymes with submicromolar affinity for  $\text{Ca}^{2+}$  require proper  $\text{Ca}^{2+}$  buffering of the assay media (when constant  $\text{Ca}^{2+}$  concentrations below  $50 \mu\text{mol/L}$  need to be realized). The principle behind the use of calcium buffers is not different from that of pH buffers. However, the chemistry relating to  $\text{Ca}^{2+}$  buffering in physiological media is complex. When ionic strength, pH, and temperature of the medium are taken into account, free  $\text{Ca}^{2+}$  levels for complex combinations of ligands (e.g., ATP, ethylene glycol-bis-( $\beta$ -aminoethyl ether) [EGTA], N-(2-hydroxyethyl)-ethylenediamine- $N',N',N'$ -triacetic acid [HEDTA], and nitrilotriacetic acid [NTA]) and metals (Ca and Mg) can be calculated. The computer program Chelator (Schoenmakers et al. 1992) provides the researcher with good estimates in excellent agreement with actual (measured) concentrations.

Another problem encountered in the study of fish branchial plasma membranes is an often exuberant nonspecific phosphatase activity: in a large pool of phosphatases one has to identify the  $\text{Ca}^{2+}$ -transporting enzyme, of which ATP-hydrolytic activity contributes only a small percentage (typically less than 5% in our preparations) to the total hydrolysis of ATP. Specific ATP preference, submicromolar affinity for  $\text{Ca}^{2+}$ , and Michaelis-Menten kinetics of phosphatase activity are tedious to assess in such assays, but these criteria were successfully advanced to discriminate a high-affinity  $\text{Ca}^{2+}$ -ATPase in gill plasma membranes from nonspecific phosphatases (Flik et al. 1983, 1985b). Yet homogeneous kinetics, high affinity for  $\text{Ca}^{2+}$ , and ATP dependence still do not guarantee the discrimination of an actual transporting ATPase. Lin and Russell (1988) elegantly showed that the rat liver plasma membrane contains two  $\text{Ca}^{2+}$ -ATPase activities with comparable kinetics, one of which is the molecular correlate of a calcium pump, the other an ecto-ATPase involved in regulating extracellular levels of adenosine nucleotides. It follows that in addition to copurification with  $\text{Na}^+/\text{K}^+$ -ATPase and proper  $\text{Ca}^{2+}$  kinetics, more criteria should be advanced to identify a calcium pump involved in epithelial transport than ATP-dependent and  $\text{Ca}^{2+}$ -stimulated phosphate release, and this is realized in transport assays with membrane vesicles.

With preparations of resealed plasma membrane vesicles (typical configuration: 30% inside-out-oriented vesicles, 30% right-side-out-oriented vesicles, and 40% leaky membrane fragments), in accordance with the same criteria as mentioned above for the ATPase assays, the activity of the calcium pump can be assayed as ATP-driven  $^{45}\text{Ca}^{2+}$  accumulation into the vesicular space. This "vectorial" assay has unambiguously revealed the activity of a calcium pump. The plasma-membrane-associated calcium pump of gills proved to be calmodulin dependent (Perry and Flik 1988)—a characteristic of plasma membrane calcium pumps (P-type  $\text{Ca}^{2+}$ -ATPases) in general (Strehler 1991). In the plasma membrane preparations used for these studies, thapsigargin (Thastrup et al. 1990; Favero and Abramson 1994) did not inhibit  $\text{Ca}^{2+}$  pump activity in gill plasma membrane preparations or affect its kinetics, and this observation confirmed the purity of this preparation. It seems justified then to state that fish gill plasma membranes contain a P-type  $\text{Ca}^{2+}$ -ATPase, which could be the driving force for  $\text{Ca}^{2+}$  transport in the tissue.

The  $\text{Na}^+/\text{Ca}^{2+}$  exchanger is a second mechanism for energized transport of  $\text{Ca}^{2+}$  across plasma membranes. This carrier was first assumed to be of particular importance in excitable tissues, where  $\text{Na}^+$  and  $\text{Ca}^{2+}$  countercurrents underlie the events related to the generation of action potentials. More recently this carrier was demonstrated in nonexcitable cells, and thus it may function in  $\text{Ca}^{2+}$  transport in these cells. Understanding  $\text{Ca}^{2+}$  extrusion in cells now requires the simultaneous analysis of ATP- and  $\text{Na}^+$ -dependent  $\text{Ca}^{2+}$  pumps. The carrier exchanges three  $\text{Na}^+$  for one  $\text{Ca}^{2+}$  and participates in  $\text{Ca}^{2+}$  extrusion in the tilapia enterocyte (Flik et al. 1990; Schoenmakers et al. 1993), where  $\text{Na}^+/\text{K}^+$ -ATPase activity maintains an inward sodium gradient as the driving force. However, in rat renal and intestinal tissue no clear function in  $\text{Ca}^{2+}$  extrusion could be attributed to the exchanger (Ghijsen, de Jong, and van Os 1982; Heeswijk, Geertsen, and van Os 1984; van Os et al. 1988).

The kinetic parameters of the exchanger were compared to those of the ATP-driven calcium pump (table 1) and this biochemical comparison led us to conclude that in the gills the ATP-driven  $\text{Ca}^{2+}$  pump may play an equal or even more pronounced role in active  $\text{Ca}^{2+}$  transport in branchial epithelium. We calculated an almost twofold higher activity of the ATPase than of the exchanger at prevailing cytosolic  $\text{Ca}^{2+}$  levels (Verbost et al. 1994). Anticipating a higher turnover for  $\text{Na}^+$  (Maetz and Bornancin 1975) in the gills of seawater fish than of freshwater fish, we predicted a more pronounced role for the exchanger in calcium handling by seawater fish gills. However, kinetic analyses revealed that the relative densities of the two calcium transporters do not differ in tilapia well-acclimated to either freshwater or sea-

TABLE 1  
*Ca<sup>2+</sup> pumps and their kinetic parameters in tilapia gill, intestine, and kidney plasma membranes*

Pump	Freshwater $V_{\max}$ ( $K_m$ )	Seawater $V_{\max}$ ( $K_m$ )
Gill:		
Ca <sup>2+</sup> -ATPase .....	4.7 (0.36)	6.8 (.49)
Na <sup>+</sup> /Ca <sup>2+</sup> exchange .....	12.3 (2.0)	15.6 (1.9)
Intestine:		
Ca <sup>2+</sup> -ATPase .....	.8 (.19)	.6 (.14)
Na <sup>+</sup> /Ca <sup>2+</sup> exchange .....	18.2 (2.3)	7.9 <sup>a</sup> (1.8)
Kidney:		
Ca <sup>2+</sup> -ATPase .....	4.5 (.06)	2.9 <sup>a</sup> (.06)
Na <sup>+</sup> /Ca <sup>2+</sup> exchange .....	n.d.	n.d.

Note. Maximum velocities ( $V_{\max}$ ) of Ca<sup>2+</sup> pump activities are expressed in nmol/(min · mg protein), and the half-maximal activation concentrations ( $K_m$  in  $\mu$ M) for Ca<sup>2+</sup> are given in parentheses. n.d., Nondetectable. Data are taken from Bijvelds et al. (1995), Schoenmakers et al. (1993), and Verbost et al. (1994).

<sup>a</sup> Significantly different from freshwater data.

water. The involvement and role of the exchanger in calcium transport in branchial epithelium awaited physiological studies. Such studies have recently been realized with opercular membranes used as a model for fish gills. Transport of Ca<sup>2+</sup> in opercular epithelium of freshwater- as well as seawater-adapted *Fundulus* is for the most part Na<sup>+</sup>-dependent (P. M. Verbost, S. E. Bryson, S. E. Wendelaar Bonga, and W. S. Marshall, personal observations). This would imply that the Ca<sup>2+</sup>-ATPase in branchial and opercular tissues is more a "housekeeping" enzyme to guarantee cellular Ca<sup>2+</sup> homeostasis than a transepithelial transport (vectorial) extrusion mechanism. We realize, however, that extrapolation of data on *Fundulus* to the tilapia and vice versa is speculation. Recently, however, Ca<sup>2+</sup>-ATPase and Na<sup>+</sup>/Ca<sup>2+</sup> exchange activity were also demonstrated in *Fundulus* gills (P. M. Verbost, personal observation), which means that the mechanisms present in tilapia and killifish are comparable.

Prolactin produces hypercalcemic actions in fishes (Hirano et al. 1986): it stimulates Ca<sup>2+</sup> influx from the water and specifically enhances ATP-driven Ca<sup>2+</sup> pump activity in the plasma membranes of branchial epithelium (Flik et al. 1986, 1994; Flik, Fenwick, and Wendelaar Bonga 1989; Ayson et al. 1993). It thus appears that the calcium pump capacity of the branchial ep-



ithelium is adjusted to the calcium uptake from the water under the control of this hormone. At this moment we cannot exclude that this increased  $\text{Ca}^{2+}$ -ATPase activity relates to an increased  $\text{Ca}^{2+}$  turnover in the epithelium and thus reflects a housekeeping task of the enzyme rather than a transepithelial transport function. In tilapia, homologous prolactin (Swennen et al. 1991) increases the branchial ionocyte density in a dose-dependent manner (Flik et al. 1994), and the branchial  $\text{Ca}^{2+}$  influx in parallel. Therefore, we assume that in prolactin-treated fishes new populations of chloride cells become active. Moreover, the density of the  $\text{Ca}^{2+}$ -ATPase relative to the  $\text{Na}^+/\text{K}^+$ -ATPase increases upon prolactin treatment, and this suggests that the expression of this  $\text{Ca}^{2+}$  pump in fish gills is specifically enhanced by this pituitary hormone.

### Intestine

The tilapia, *Oreochromis mossambicus*, is an omnivorous fish with a long (up to 10 body lengths) intestinal tract. The intestinal epithelium shows no macroscopically distinct sections, but gets thinner along an aboral gradient. The intestinal epithelium is homogeneous and does not contain crypts such as are observed in higher vertebrates. The majority of the cells are enterocytes; mucocytes are not particularly abundant (1%–2% of the cells), and endocrine cells are scarce. The enterocytes are elongated cells with well-developed brush border membranes and an extensive basolateral labyrinth, composed of basolateral plasma membrane invaginations and mitochondria. A particular advantage of the tilapia intestine is that the intestinal epithelium can be easily stripped off its submucosa (Flik et al. 1990; Schoenmakers et al. 1993) for Ussing chamber studies as well as biochemical processing. Considering the leaky character of intestinal epithelia and the significant resistance imposed on the tissue by the submucosa and muscle layers, stripping of the epithelium for electrophysiological studies allows a proper evaluation of the active transport component in the calcium movements over this epithelium. For practical reasons (amount of epithelium, manageability) we routinely use the proximal one-third of the intestine for our biochemical transport studies. Therefore, we cannot evaluate the contributions of more distal parts of the intestine of this fish to  $\text{Ca}^{2+}$  transport or assess whether, overall, the intestine is a calcium secretory organ. We have been successful so far in measuring calcium transport and magnesium transport (Flik et al. 1993) in proximal, stripped epithelium, and we have analyzed calcium transport mechanisms in vesicles of basolateral and apical membrane compartments of the enterocyte.

Influx of  $\text{Ca}^{2+}$  over this epithelium is  $\text{Na}^+$ -dependent, as measured in an Ussing chamber setup. The rate of ATP-driven  $\text{Ca}^{2+}$  transport in the intestinal basolateral plasma membrane vesicles is very low, and too low to explain the  $\text{Ca}^{2+}$  fluxes that occur in this epithelium. However, in the tilapia enterocyte basolateral plasma membrane a powerful  $\text{Na}^+/\text{Ca}^{2+}$  exchange activity may fulfill this function, an explanation that is supported by the  $\text{Na}^+$  dependence in the Ussing chamber experiments (Flik et al. 1990).

Once adapted to seawater, it appears that the intestine has changed remarkably. The seawater fish drinks water containing around 10 mmol/L of calcium. In vitro, a net  $\text{Ca}^{2+}$  influx could no longer be measured in the intestine of seawater fish. The  $\text{Na}^+/\text{K}^+$ -ATPase activity in the basolateral plasma membrane had increased in line with a water transport function (solute-linked water transport) for the intestine in seawater. Coincidentally, the activities of the  $\text{Ca}^{2+}$ -ATPase and, more pronounced, of the  $\text{Na}^+/\text{Ca}^{2+}$  exchanger showed very significant decreases in seawater fish compared to freshwater fish (table 1). It thus appears that a physiological adaptation evokes an adjustment of  $\text{Ca}^{2+}$  carrier densities in the basolateral plasma membrane compartment, where the extrusion step of transepithelial  $\text{Ca}^{2+}$  transport is realized (Schoenmakers et al. 1993).

## Kidney

As reviewed in the introduction, a hallmark in the adaptation of fish to seawater is a decreased renal  $\text{Ca}^{2+}$  reabsorption (Schmidt-Nielsen and Renfro 1975; Björnsson and Nilsson 1985; Elger et al. 1987). Also, renal membrane preparations of several euryhaline species exhibited lower  $\text{Na}^+$  pump activity when the fish was exposed to seawater (Trombetti et al. 1990; Doneen 1993). The hypothesis subsequently tested was that in seawater, less transcellular  $\text{Ca}^{2+}$  transport would be required and that this should be reflected by a decrease in  $\text{Ca}^{2+}$  extrusion activity. Biochemical analyses on fish renal tissue are compromised by the mixed character of the tissue. Hematopoietic and renal tissues are intermingled, and the nephron is morphologically and thus in all likelihood functionally segmented (Elger et al. 1987). One can, therefore, even if the blood cells are removed from the renal tubules before membrane isolation, never be sure about the role a mechanism plays in the physiology of the tissue (e.g., is the mechanism involved in vectorial transport, in cellular homeostasis, or both?). Yet two remarkable observations lead us to conclude that a  $\text{Ca}^{2+}$ -ATPase in the basolateral plasma membrane of renal cells must be involved in  $\text{Ca}^{2+}$  reabsorption. First, we cannot demonstrate  $\text{Na}^+/\text{Ca}^{2+}$  exchange activity in tilapia renal membrane preparations

(Bijvelds et al. 1995). This observation is in line with studies suggesting that in flounder, renal  $\text{Ca}^{2+}$  reabsorption depends on intracellular ATP but is largely  $\text{Na}^+$ -independent (Renfro et al. 1982). Second, a high-affinity, high-capacity  $\text{Ca}^{2+}$ -ATPase is easily demonstrated in renal plasma membranes, and the activity of this enzyme decreases significantly on seawater adaptation (table 1; Doneen 1993; Bijvelds et al. 1995). Proceeding from a homogeneous  $\text{Ca}^{2+}$ -ATPase activity (indicated by kinetic analyses), similar purification numbers, and specific activities for  $\text{Na}^+/\text{K}^+$ -ATPase in freshwater and seawater renal membrane preparations, we conclude that the relative density of a single class of ATP-driven  $\text{Ca}^{2+}$  pumps decreased on seawater adaptation. Assuming that requirements for cellular  $\text{Ca}^{2+}$  homeostasis do not differ drastically in kidney cells of freshwater or seawater tilapia, it must be the decreased need for  $\text{Ca}^{2+}$  reabsorption (i.e., transcellular  $\text{Ca}^{2+}$  transport) that is apparently reflected by this decreased  $\text{Ca}^{2+}$ -ATPase activity.

### Conclusions

Four examples of adaptations in tilapia concerning the  $\text{Ca}^{2+}$  extrusion mechanisms in the basolateral plasma membrane compartment of branchial, intestinal, and renal epithelium have been given. In all cases sustained changes in  $\text{Ca}^{2+}$  flow correlated with similar changes in  $\text{Ca}^{2+}$  pump activity. The  $\text{Na}^+$  dependence of  $\text{Ca}^{2+}$  transport in chloride-cell-rich epithelium favors the thesis that in gills a  $\text{Na}^+$ -gradient-driven  $\text{Ca}^{2+}$  pump is the primary mechanism securing vectorial, transcellular  $\text{Ca}^{2+}$  transport. Prolactin specifically controls the density of the ATP-driven  $\text{Ca}^{2+}$  pump activity in branchial epithelial plasma membranes. Considering that prolactin is a hormone vital for survival of fish in freshwater environments and that prolactin cell activity in tilapia is inversely related to ambient calcium levels (Wendelaar Bonga, Löwik, and van der Meij 1983), we speculate that ATP-driven  $\text{Ca}^{2+}$  pumps may be specifically recruited under prolactin control in (low-calcium) freshwater. Seawater adaptation resulted in decreased  $\text{Ca}^{2+}$  pump activity in proximal intestinal and renal epithelium. In the renal tissue  $\text{Ca}^{2+}$ -ATPase activity decreases with the need for  $\text{Ca}^{2+}$  reabsorption. In the enterocyte, the  $\text{Na}^+/\text{Ca}^{2+}$  exchanger activity decreases in parallel with  $\text{Ca}^{2+}$  flux.

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