General and Comparative Endocrinology 176 (2012) 354–360

Contents lists available at SciVerse ScienceDirect

General and Comparative Endocrinology

journal homepage: www.elsevier.com/locate/ygcen

Osmoreception: Perspectives on signal transduction and environmental modulation

A.P. Seale [a,*], S. Watanabe [b], E.G. Grau [a]

[a] Hawai'i Institute of Marine Biology, University of Hawaii, Kaneohe, HI 96744, USA
[b] Department of Aquatic Bioscience, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Bunkyo, Tokyo 113-8657, Japan

ARTICLE INFO

Article history:
Available online 20 October 2011

Keywords:
Osmoreception
Osmoregulation
Prolactin
Signal transduction
Tilapia
Oreochromis mossambicus

ABSTRACT

Osmoregulation is essential to life in vertebrates and osmoreception is a fundamental element in osmoregulation. Progress in characterizing the mechanisms that mediate osmoreception has been made possible by using a uniquely accessible cell model, the prolactin (PRL) cell of the euryhaline tilapia, *Oreochromis mossambicus*. In addition to a brief historical overview, we offer a summary of our recent progress on signal transduction and osmosensitivity in the tilapia PRL cell model. Prolactin is a central regulator of hydromineral balance in teleosts in freshwater (FW). Consistent with its essential role in FW osmoregulation, PRL release in tilapia is inversely related to extracellular osmolality, both *in vivo* and *in vitro*. Osmotically-driven changes in PRL cell volume control PRL release. A decrease in extracellular osmolality increases cell volume, leading to a rapid influx of Ca^{2+} through stretch-activated channels followed by a sharp rise in PRL release. Our recent studies also suggest that cAMP is involved in the osmotic signal transduction, and that acclimation salinity can modulate PRL cell osmosensitivity. Prolactin cells from FW tilapia show a larger rise in PRL release after a reduction in medium osmolality than those from SW fish. Paradoxically, hyposmotically-induced increase in PRL mRNA was observed only in cells from SW fish. Our studies have revealed differences in the abundance of the water channel, aquaporin 3 (AQP3), and the stretch activated Ca^{2+} channel, transient receptor potential vanilloid 4 (TRPV4) in PRL cells of FW and SW fish that may explain their differing osmosensitivity and osmoreceptive output in differing acclimation salinities.

1. Introduction

Osmoregulation is a fundamental prerequisite to physiological homeostasis in complex organisms. The function of macromolecules, such as proteins, is dependent on shape and charge, and therefore is extremely sensitive to small changes in the intracellular *milieu*. To maintain salt and water balance in the face of changing environmental salinity, euryhaline fish invest considerable energy and employ a considerable portion of the neuroendocrine system to control osmoregulatory mechanisms that respond to changes in the osmolality of body fluids. Changes in salt and water balance are detected by a network of osmoreceptors and baroreceptors found in the brain, vasculature, kidney, and likely other tissues as well [1,9,26,29,30,41,56–58,66]. These cells control a wide range of endocrine and neuroendocrine responses that maintain or restore hydromineral balance. Osmoreceptors have been defined not only by their intrinsic osmosensitivity, but by their ability to regulate systemic osmotic balance [7,9,23,45,58]. Cellular osmoreceptors can therefore be characterized by their ability to detect physiologically relevant deviations in extracellular osmolality, and to transduce these osmotic stimuli into the synthesis and release of an effector, typically a hormone, that restores osmotic equilibrium at the organismal level. During this process, the cell membrane plays a critical role in two major ways: (1) it provides an osmosensitive trigger by stretching or shrinking and (2) it selectively controls the entry of molecules, including calcium (Ca^{2+}) and phosphate (PO_4^{3-}) ions, which have filled a ubiquitous role in cell signaling. While imparting opposite charges, the binding of both Ca^{2+} and PO_4^{3-} to proteins bring about a modification in their conformation and interactions, leading to changes in cellular function that can ultimately affect transcription, translation and secretion [13,67]. To keep osmotic stability, organisms invest considerable energy in establishing the necessary gradients for intracellular signaling to take place, while maintaining precise control of intracellular and extracellular fluids.

2. A cellular model for osmoreception

For vertebrates in general, endocrine reflexes are often employed in responses that are critical for survival. For example, insulin secretion responds directly to the plasma glucose concentration it regulates. Likewise, prolactin (PRL) secretion from the tilapia pituitary responds directly to the extracellular osmolality which it regulates [45]. That is because PRL is essential for hyperosmoregulation in FW for tilapia and other euryhaline teleosts [32–34]. Without a pituitary, tilapia will not survive in FW, unless it has received PRL replacement therapy [12,39]. We have found that the direct

* Corresponding author. Fax: +1 808 236 7443.
E-mail address: seale@hawaii.edu (A.P. Seale).

0016-6480/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.ygcen.2011.10.005

regulation of PRL secretion by extracellular osmolality can be modulated by a variety of endocrine factors including urotensin II [22], somatostatin [18,22], thyrotropin-releasing hormone [2], 17β-estradiol [2], cortisol [6], chicken type II gonadotropin-releasing hormone [64], PRL-releasing peptides [46], angiotensin II [14], and natriuretic peptides [14,17]. The presence of multiple regulators of PRL release underlines its importance and suggests a diversity of signal transduction pathways available in this cell type.

Despite the critical importance of osmotic equilibrium, the transduction mechanisms that operate in osmoreceptors are not thoroughly understood. This is because the complex structure and arrangement of vertebrate osmoreceptive cells and tissues make them difficult to study. The vasopressin-secreting cells of the mammalian hypothalamus illustrate this well. Vasopressin plays a key role in osmoregulation through its actions on natriuresis and diuresis. In mice, osmosensitive neurons are located mainly in the supraoptic nucleus with axonal projections to the posterior pituitary where vasopressin is released [8,59].

Extracellular fluids of mammals are bound within a tight osmotic range, and human plasma osmolality is kept between 282 and 298 mOsmolal [35]. A deviation in plasma osmolality of 15% leads to convulsions and coma in humans [3]. In this context, it is uncertain whether a majority of studies on the responses of mammalian cells to osmotic challenges may yield insight into how osmoreception is mediated, as they employ changes in osmolality that are well beyond the physiological range [28]. By contrast, the remarkably osmosensitive PRL cell of the euryhaline tilapia, *Oreochromis mossambicus*, responds to physiological changes in extracellular osmolality of 280 to over 400 mOsmolal, well within the range measured in blood and tissue fluid *in vivo* [44]. The tilapia PRL cell has other special attributes that make it useful as a general model for studying osmoreception. Prolactin cells can be easily isolated and studied *in vitro* [20,45], as they are located in a homogeneous tissue comprising >99% of the rostral pars distalis (RPD) of the pituitary [38]. Furthermore, the tilapia PRL cell model allows the estimation of gene expression and hormone secretion simultaneously with other key parameters involved in osmoreception, such as intracellular [Ca^{2+}], cAMP and cell volume. Finally, the euryhaline nature of this species allows one to study osmoreception within the context of two interchangeable and opposing osmotic paradigms: a diluting environment in freshwater (FW) and a dehydrating environment in seawater (SW).

3. Stimulus-secretion coupling in the osmoreceptive tilapia PRL cell

Plasma PRL is elevated in FW tilapia, and this is a direct response to blood and tissue fluid osmolality [50,70]. Specifically, the transfer of fish from dilute SW to FW, whose RPD (PRL tissue) has been autotransplanted to the optic nerve, brings about an elevation in circulating PRL and RPD PRL mRNA even though hypothalamic control is eliminated [53]. *In vitro*, small decreases in extracellular osmolality elicit significant changes in PRL release from intact pituitaries or dispersed PRL cells [44,50]. Thus, the tilapia PRL cell is an osmoreceptor by virtue of its intrinsic osmosensitivity and its ability to transduce a change in extracellular osmolality into a change in the release of a hormone that is critical to FW osmoregulation.

Further studies employing the dispersed PRL cell model for osmoreception elucidated some of the mechanisms underlying stimulus-secretion coupling [42,45]. Briefly, in a hyposmotic environment, the osmotic gradient across the cell membrane, rather than a change in extracellular osmolality *per se*, leads to a rise in cell volume [65]. This rise initiates an influx of extracellular Ca^{2+} through stretch-activated channels (SACC), producing an increase in intracellular [Ca^{2+}] which stimulates PRL release [43,48,49]. This sequence of events has been supported by evidence that: (1) lowering medium osmolality from 355 mOsmolal at a rate of 2 mOsmolal/min gradually increases PRL cell volume and PRL release in a linear fashion [44,45]; (2) in the absence of extracellular Ca^{2+}, the PRL cell response to hyposmotic medium is inhibited [21,48]; (3) blocking the entry of Ca^{2+} through voltage-gated channel blockers, such as nifedipine, does not prevent the increase in PRL elicited by hyposmotic stimulation [49]; (4) gadolinium (Gd^{3+}), which is known to block stretch-activated cation channels, inhibits hyposmotically-induced PRL release without preventing cell swelling; and (5) the rapid rise in intracellular [Ca^{2+}] that evokes acute hyposmotically-induced PRL release can occur independently of the release of Ca^{2+} from intracellular stores [43]. Together, these studies suggest that medium osmolality acts in a graded fashion on a single signal transduction component across the range of extracellular osmolality that tilapia are likely to experience *in vivo*.

4. Interaction between distinct second messengers during an osmotic response

As much as Ca^{2+} has been shown to be an essential and potent second messenger in tilapia osmoreception, cAMP, another universal regulator of cell function, has also been found to increase in PRL cells after hyposmotic stimulation [19,22,24]. Cross-talk between Ca^{2+} and cAMP signaling systems has been recently reported in several cell models [4,27,69] and may play an active role in the control of PRL release. Intracellular cAMP is mainly generated via adenylyl cyclases (AC) following hormonal activation of G-protein-coupled receptors (GPCRs) linked to the $G_{\alpha s}$ subunit of the heterotrimeric G-protein. The rise in cAMP typically activates the cAMP-dependent protein kinase (PKA) which, in turn, exerts downstream effects, including changes in gene expression and protein synthesis [15]. Recently, we examined the cAMP messenger system of tilapia PRL cells, employing dispersed cell incubations to measure both PRL release and cAMP accumulation in the same preparation and time frame, and established a method to estimate AC activity in membrane and cytosolic fractions [47]. Exposure to the inhibitor of phosphodiesterase activity 3-isobutyl-1-methylxanthine (IBMX) produced a significant increase in PRL release and cAMP accumulation, which was further augmented in the presence of hyposmotic medium. In the absence of extracellular Ca^{2+}, however, these phenomena were abolished. Conversely, exposure of PRL cells to the cAMP agonist Cholera toxin (CTX), stimulated PRL release and cAMP accumulation even in the absence of extracellular Ca^{2+}. Using a PRL cell membrane preparation, we found that AC activity was reduced as extracellular Ca^{2+} was increased from 0.1 to 1.0 μM. Lastly, exposure to the Ca^{2+} ionophore A23187 stimulated PRL release up to 10-fold above the level of cells exposed to hyposmotic medium containing IBMX, but suppressed the rise in cAMP levels produced by IBMX in either hypo- or hyperosmotic medium. This suppression is likely to be a consequence of the direct inhibition of AC and stimulation of phosphodiesterase (PDE) activity by high intracellular [Ca^{2+}] [47].

Together, these experiments indicate that (1) hyposmotically-induced PRL release and cAMP formation are Ca^{2+} dependent, (2) direct agonists of the cAMP messenger system such as CTX and forskolin can stimulate PRL release and cAMP accumulation independently of extracellular Ca^{2+}, and (3) large increases in intracellular Ca^{2+} attenuate cAMP formation. These findings provide evidence that PRL cells are equipped with the means to rapidly augment PRL via pathways that are Ca^{2+}-dependent and/or cAMP dependent and Ca^{2+}-independent. Given the range of factors that regulate PRL cell function in addition to medium osmolality alone, it is not surprising that both Ca^{2+} and cAMP messenger systems interact with

each other and evoke rapid responses to control PRL release. The extent to which the signal transduction pathways triggered by rises in Ca^{2+} and cAMP are involved in differentially controlling the expression and synthesis of PRL in FW and SW-adapted fish remain to be determined.

5. Molecular triggers for cell volume changes and extracellular Ca^{2+} entry

In search for the mechanisms that mediate the inward movement of H_2O which leads to the increases in cell volume and intracellular, free [Ca^{2+}] and PRL release during hyposmotic stimulation, a closer examination of the possible membrane channels involved was carried out. Water channels, or aquaporins (AQPs), are integral membrane pores selectively permeable to H_2O. The 13 AQP isoforms found in vertebrates can be divided in two main sub-families, AQPs and aquaglyceroprins, based on their primary sequence and function [25]. While AQPs are strictly permeable to H_2O, aquaglyceroporins are also permeable to small uncharged solutes such as glycerol, CO_2, ammonia and urea. Transient receptor potential (TRP) channels, on the other hand, comprise a group of ion channels that are permeable to cations, including Na^+, Ca^{2+} and Mg^{2+}, and many are involved in mechanotransduction [72]. Three of the six channels of the TRP-vanilloid (TRPV) subfamily have been found to function in the transduction of osmotic stimuli [31]. Despite the anatomical complexity of the mammalian hypothalamus, electrophysiological and knockdown approaches targeting osmosensitive channels of the TRPV family have proven useful in clarifying the mechanisms involved in neuronal osmosensing [36,51,52]. Recently, we investigated whether AQPs and TRPVs may be functioning in PRL cell osmoreception. Expression of aquaporin 3 (AQP3), has been described in the tilapia pituitary [63] and further studies have shown that this aquaglyceroporin plays an enhancing role in PRL cell osmosensitivity [62]. The rate of hyposmotically-induced water influx and PRL cell volume increase is greatly decreased by the AQP inhibitor, mercury ($HgCl_2$), in a dose-related manner. Likewise, pre-exposure to mercury inhibited hyposmotically-induced PRL release [62].

By employing a gene specific primer set for tilapia epithelial Ca^{2+} channels (TRPV5 and/or 6), and degenerate primers for TRPV1–4, we have recently found that TRPV4 is the only gene of the TRPV family expressed in the RPD of tilapia pituitaries (Watanabe et al., unpublished data). Knowledge on the pharmacology of TRPV channels has been expanding, and Gd^{3+}, which we have previously employed as a SACC inhibitor to block hyposmotically-induced PRL release [49], has recently been described as a classical TRP inhibitor [60]. Furthermore, incubation of PRL cells with 4α-Phorbol 12,13-Didecanoate (4α-PDD), a TRPV4 agonist [61], stimulates PRL release within 20 min whereas incubation with ruthenium red (RuR), a TRPV4 antagonist [61], blocks hyposmotically-induced PRL release within the same time frame (Watanabe et al., unpublished data). The evidence supporting the involvement of AQP3 and TRPV4 in PRL cell osmoreception is therefore strengthened not only by their presence in the RPD but by blocking PRL release with inhibitors of these channels. According to the urea transport assay system employing Xenopus oocytes, tilapia AQP3 is functionally classified as an aquaglyceroporin, as it allows not only water but small non-ionic molecules, such as urea and glycerol, to permeate across the cell membrane (Fig. 1). The role of AQP3 as a membrane trigger for cell volume changes is corroborated by our previous study demonstrating that the osmoreceptive PRL cell response is tied to both water and urea movement across the cell membrane [65], a property that is exclusive to aquaglyceroporins. These recent findings also indicate that TRPV4 might function as a stretch-activated Ca^{2+} channel mediating osmoreception in the tilapia PRL cell

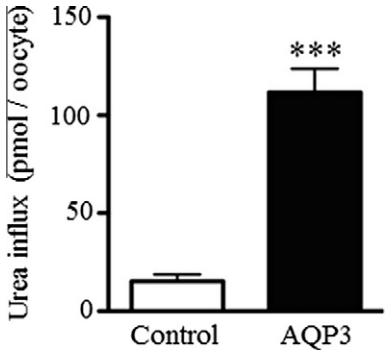

Fig. 1. Effect of AQP3 cRNA injections on the transport of urea across the cell membrane of Xenopus oocytes. AQP3-expressing and control (water-injected) oocytes were prepared as described previously Watanabe et al. [63]. Oocytes were preincubated in 200 mM mannitol, 2 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 10 mM Hepes, 5 mM Tris, pH 7.4, for 20 min at 18 °C. Then, oocytes were immersed in experimental medium (pre-incubation medium containing 6.25 mCi/mL [^{14}C] urea and 1 mM urea) for 5 min at 18 °C. The incubation was terminated by washing oocytes in ice-cold pre-incubation medium containing 1 mM unlabelled urea. Individual oocytes were dissolved in 10% SDS, and the radioactivity was measured using a liquid scintillation counter. Bars represent mean urea influx ± SEM ($n = 6$). $^{***}P < 0.001$ by Welch t-test.

as predicted by our model of stimulus-secretion coupling [45]. Taking into account our recent discoveries on the nature of PRL cell osmosensitive channels, and the interaction between Ca^{2+} and cAMP messenger systems, we offer an updated model for rapid stimulus-secretion coupling in PRL cells (Fig. 2).

6. Acclimation salinity modulates osmosensitivity

The tilapia pituitary produces two PRL molecules, designated PRL_{177} and PRL_{188}, which are encoded by separate genes, share only 130 amino acid residues (69% similarity) throughout the sequence [40,54,71]. Mentions of PRL throughout the text so far have referred to measurements of PRL_{188}. While the existence of two distinct PRL molecules in tilapia suggests the evolution of distinct actions, no clear differences in osmoregulatory action have been demonstrated [54]. Differences between both PRLs have been observed, however, in regard to osmosensitivity. Although the two PRLs have been shown to be produced and secreted from the same cells [55], studies have indicated that the processing of PRL_{177} and PRL_{188} may be differentially sensitive to environmental salinity [5]. By measuring release and content of both PRLs from isolated RPDs of FW and SW acclimated fish, Borski and co-workers have shown a greater shift in the quantity of PRL_{188} than PRL_{177} in RPDs of tilapia exposed to salinity transfers [5]. Our recent studies employing dispersed PRL cells corroborate these findings and indicate that expression of the PRL_{188} gene is more sensitive to gradual changes in medium osmolality than PRL_{177} in SW acclimated fish (Seale et al., unpublished data). These differences suggest that PRL_{188} is more osmosensitive, and therefore, may play a greater role in osmoregulation than PRL_{177}. It is also possible that the expression and release of PRL_{188} may be regulated by a separate transduction pathway from that controlling PRL_{177}.

Continued *in vitro* exposure to a hyposmotic stimulus for hours and days evokes changes in the tilapia PRL cell that extend beyond the rapid rise in PRL release and second messenger metabolism [21,37,50,68]. Since cellular stores of PRL are limited, a sustained elevation in PRL release requires the activation of PRL gene expression and synthesis. When tilapia are transferred from SW to FW, PRL mRNA content in whole pituitaries [11] and RPDs (Seale et al., unpublished data) is significantly increased as early as 6 h after transfer. Transfers from FW to SW, on the other hand, significantly decreases PRL mRNA content in tilapia RPDs when

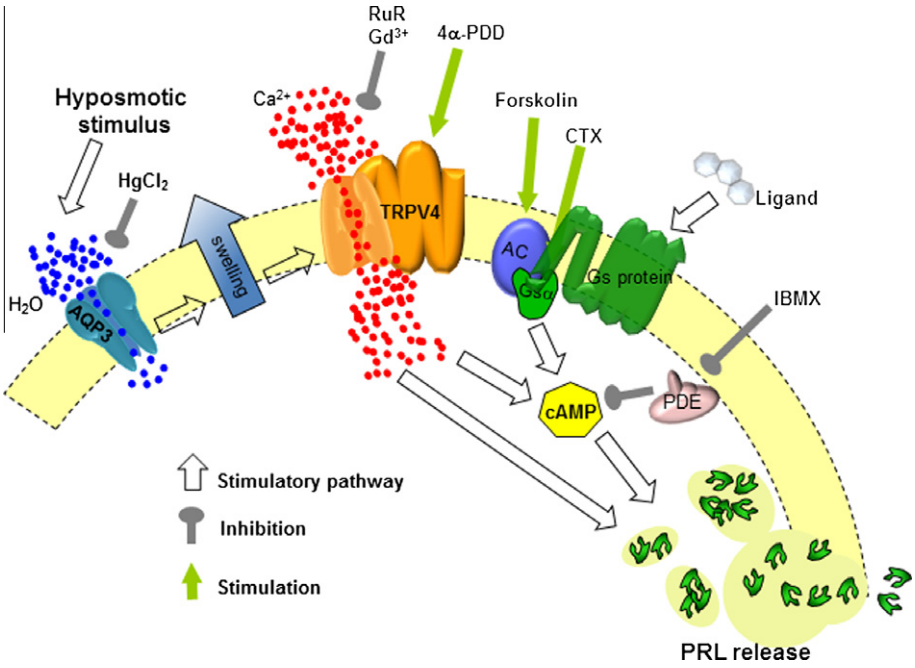

Fig. 2. Current model for rapid stimulus-secretion coupling in the tilapia osmoreceptive PRL cell. A physiologically relevant reduction in extracellular osmolality triggers water movement through AQP3 in the cell membrane. Water influx leads to an increase in cell volume which in turn triggers the opening of the mechanosensitive Ca^{2+} channel TRPV4. The stimulation of TRPV4 allows Ca^{2+} to enter the cell down its concentration gradient. The rise in intracellular [Ca^{2+}] is rapidly followed by a large spike in PRL release. In the presence of the PDE inhibitor, IBMX, a hyposmotic stimulus also induces a Ca^{2+}-dependent increase in intracellular cAMP levels. An excess of intracellular Ca^{2+} however, abolishes hyposmoticall-induced PRL release in the presence of IBMX, at least partially through direct inhibition of AC. Ligand-induced stimulation of G-protein stimulates cAMP formation. Inducing cAMP formation by stimulating AC or Gsα pharmacologically, increases PRL release independently of extracellular Ca^{2+}.

compared with SW controls (Seale et al., unpublished data). Recently, we found no significant effect of hyposmotic medium on PRL gene expression using RPDs or dispersed PRL cells from FW fish incubated for 6 h. By contrast, when cells from SW fish were employed, a reduction in medium osmolality evoked a significant increase in PRL gene expression (Fig. 3). The fact that PRL mRNA levels are 30-fold higher in FW PRL cells than in SW PRL cells may explain the differential response in gene expression with salinity, where PRL expression in FW fish could be maximal and unresponsive to further *in vitro* osmotic stimuli. Hyposmotically-induced PRL synthesis [73] and release [44], on the other hand, is more robust in fish acclimated to FW than those acclimated to SW. The lower sensitivity of short-term hyposmotically-induced PRL release from SW-acclimated tilapia may derive from the lower

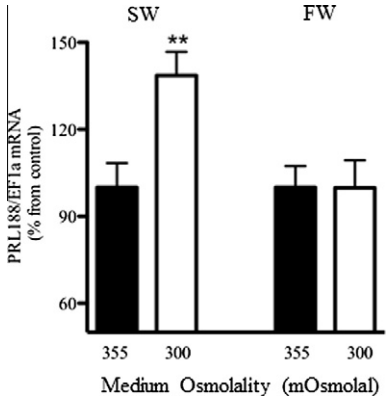

Fig. 3. Effects of hyposmotic medium (300 mOsmolal), and acclimation salinity on PRL188 expression in dispersed PRL cells and intact RPD's from two combined experiments after 6 h. Bars represent mean ± SEM (n = 13–14). While PRL cells from FW fish did not respond to medium osmolality, those from SW fish significantly increased PRL expression in hyposmotic medium. $^{**}P < 0.01$ by Students t-test.

quantity of PRL stored in the pituitaries of these fish [37]. In addition, recent evidence suggests that this discrepancy may result from the diminished ability of PRL cells from SW-acclimated tilapia to initiate osmotic signal transduction. The relative cell volume change and water influx into PRL cells following an osmotic challenge was found to be significantly lower in cells from SW tilapia compared with those in FW [62]. This discrepancy was further associated with lower AQP3 gene expression, protein content and immunofluoresence in SW PRL cells compared with those of FW fish [62]. These findings indicate that besides playing a key role in mediating PRL cell osmoreception, AQP3 may account for the acclimation salinity-dependent differences in osmosensitivity. Whether AQP3 expression is directly osmosensitive, however, remains to be determined.

We have recently found that, contrary with the pattern observed in PRL expression, TRPV4 expression *in vivo* is increased in RPDs of tilapia transferred from FW to SW and decreased in tilapia transferred from SW to FW (Seale et al., unpublished data). In dispersed PRL cells, TRPV4 expression is directly proportional to extracellular osmolality which in turn is inversely proportional to PRL release (Fig. 4). Further evidence indicates that in addition to hyposmotic medium, treatment with the Ca^{2+} ionophore A23187 and IBMX also lowers TRPV4 mRNA levels, suggesting that both Ca^{2+} and cAMP second messengers are involved in the regulation of this channel (Seale et al., unpublished data). It is possible that once extracellular Ca^{2+} enters the cell, it limits TRPV4 production to reduce further Ca^{2+} entry. Adaptively, higher levels of TRPV4 expression in PRL cells of SW fish might confer greater sensitivity by allowing increased extracellular Ca^{2+} entry when a fish moves from SW to FW.

The putative transcriptional regulator, osmotic stress transcription factor (OSTF1) has been found to be induced in response to hyperosmotic stress in tilapia branchial cells *in vitro* [16], and in response to transfer from FW to SW in tilapia gills *in vivo* [10]. Recently we have found that OSTF1 is also induced by an increase

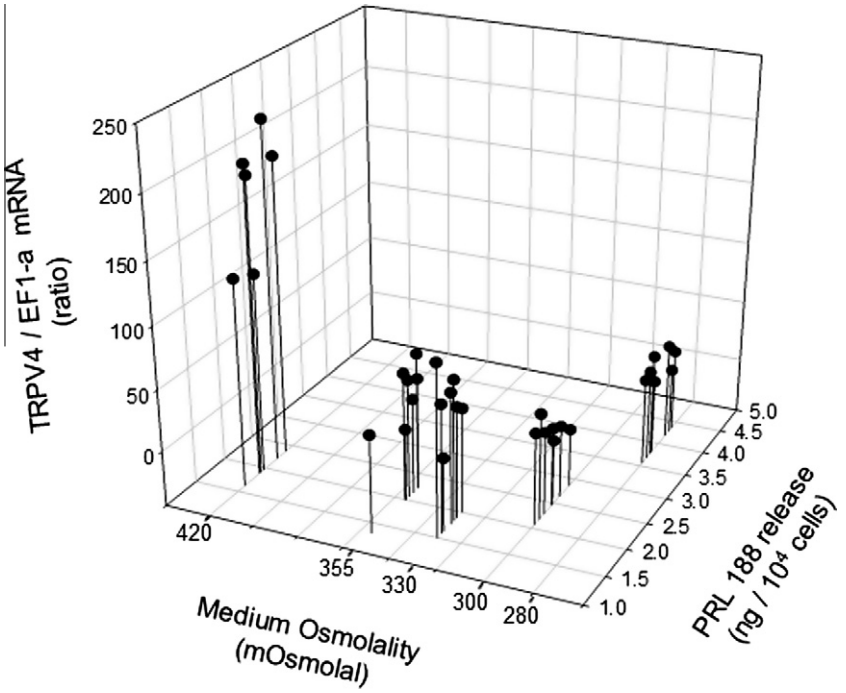

Fig. 4. Three-dimensional plot of extracellular osmolality, PRL release and TRPV4 mRNA levels from dispersed FW tilapia PRL cells incubated for 6 h in 280, 300, 330, 355 or 420 mOsmolal media. Filled circles represent individual data points. Cells incubated with medium osmolalities of 300 mOsmolal or less had the lowest TRPV4 mRNA levels and released the most PRL, while those incubated at 420 mOsmolal expressed the most TRPV4 and released the least PRL.

in extracellular osmolality in dispersed tilapia PRL cells *in vitro*, and by transfer from FW to SW in tilapia RPDs *in vivo* (Seale et al., unpublished data). Further studies are required to determine whether hyperosmotically-induced expression of OSTF1 is linked to TRPV4 and PRL in tilapia PRL cells.

Together these studies demonstrate that besides PRL, other key players involved in osmoreception, such as TRPV4 and potentially AQP3, can be directly controlled by extracellular osmolality itself. Importantly, it is possible to distinguish the short and long term osmoreceptive properties of PRL cells adapted to different ambient salinities such as FW and SW (Fig. 5). However, it remains to be determined whether the mechanisms governing an increase in AQP3 expression and a decrease in TRPV4 expression during FW acclimation are the same as those involved in the hyposmotically-induced increase in PRL release. Information is also unavailable on the downstream signaling pathways linking a rise in intracellular second messengers and the regulation of AQP3, TRPV4 and PRL gene expression as well as the possible involvement of other ion transporters and pumps, such as Na^+/K^+ ATPase, and transcription factors, such as OSTF1, during osmoreception. Further investigations on the mechanism(s) underlying the expression and production of these factors in direct response to changes in osmolality will provide a

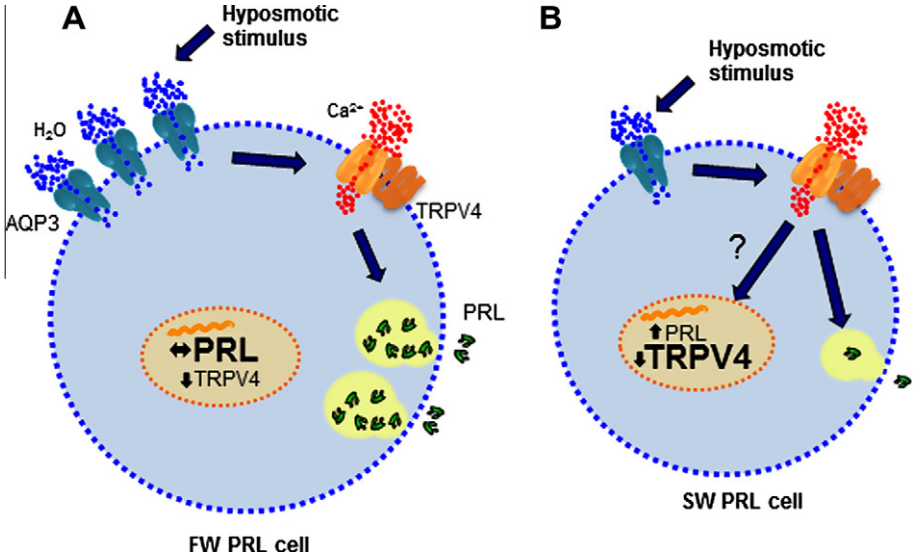

Fig. 5. PRL cells from tilapia acclimated to FW (A) and SW (B) exhibit different patterns of osmosensitivity and PRL output. The stimulus-secretion coupling in the PRL cell of FW-acclimated fish, involves AQP3 and TRPV4, where a rise in extracellular Ca^{2+} entry is directly associated to increased PRL release and takes place within minutes. This response is more robust than that of PRL cells of SW fish, which have less AQP3 and less PRL stored. PRL cells of SW fish have higher TRPV4 mRNA levels than those of FW fish, and upregulate PRL mRNA upon hyposmotic stimulation. In both acclimation salinities, hyposmotic stimulation *in vitro* decreases expression of TRPV4.

better understanding of how FW and SW acclimation affects osmosensitivity of the PRL cell.

Acknowledgments

We are grateful to Prof. Tetsuya Hirano for his assistance and encouragement during the preparation of this manuscript. This work was funded in part by grants from National Science Foundation (IOS-0517769, OISE-0852518 and IOS-1119693) and Japan Society for the Promotion of Science (21780174 and 22248021).

References

[1] L.J. Andersen, T.U. Jensen, M.H. Bestle, P. Bie, Gastrointestinal osmoreceptors and renal sodium excretion in humans, Am. J. Physiol. 278 (2000) R287–294.
[2] T.B. Barry, E.G. Grau, Estradiol-17β and thyrotropin-releasing hormone stimulate prolactin release from the pituitary gland of a teleost fish, *in vitro*, Gen. Comp. Endocrinol. 62 (1986) 306–314.
[3] R.M. Berne, M.N. Levy, B.M. Koeppen, B.A. Stanton, Physiology, Mosby, St. Louis, 1998.
[4] L.N. Borodinsky, N.C. Spitzer, Second messenger pas de deux: the coordinated dance between calcium and cAMP, Sci. STKE 2006 (2006) p. 22.
[5] R.J. Borski, M.U. Hansen, R.S. Nishioka, E.G. Grau, Differential processing of the two prolactins of the tilapia (*Oreochromis mossambicus*), in relation to environmental salinity, J. Exp. Zool. 264 (1992) 46–54.
[6] R.J. Borski, L.M.H. Helms, N.H. Richman, E.G. Grau, Cortisol rapidly reduces prolactin release, and cyclic AMP and $^{45}Ca^{++}$ accumulation in the cichlid fish pituitary, *Oreochromis mossambicus*, *in vitro*, Proc. Natl. Acad. Sci. USA 82 (1991) 2758–2762.
[7] C.W. Bourque, Central mechanisms of osmosensation and systemic osmoregulation, Nat. Rev. Neurosci. 9 (2008) 519–531.
[8] C.W. Bourque, Osmoregulation of vasopressin neurons: a synergy of intrinsic and synaptic processes, Prog. Brain Res. 119 (1998) 59–76.
[9] C.W. Bourque, S.H. Oliet, Osmoreceptors in the central nervous system, Annu. Rev. Physiol. 59 (1997) 601–619.
[10] J.P. Breves, S. Hasegawa, M. Yoshioka, B.K. Fox, L.K. Davis, D.T. Lerner, Y. Takei, T. Hirano, E.G. Grau, Acute salinity challenges in Mozambique and Nile tilapia: differential responses of plasma prolactin, growth hormone and branchial expression of ion transporters, Gen. Comp. Endocrinol. 167 (2010) 135–142.
[11] J.P. Breves, A.P. Seale, R.E. Helms, C.K. Tipsmark, T. Hirano, E.G. Grau, Dynamic gene expression of GH/PRL-family hormone receptors in gill and kidney during freshwater-acclimation of Mozambique tilapia, Comp. Biochem. Physiol. 158 (2011) 194–200.
[12] J.P. Breves, S. Watanabe, T. Kaneko, T. Hirano, E.G. Grau, Prolactin restores branchial mitochondrion-rich cells expressing Na+/Cl− cotransporter in hypophysectomized Mozambique tilapia, Am. J. Physiol. 299 (2010) R702–710.
[13] D.E. Clapham, Calcium signaling, Cell 131 (2007) 1047–1058.
[14] S.M. Eckert, T. Hirano, T.A. Leedom, Y. Takei, E.G. Grau, Effects of angiotensin II and natriuretic peptides on prolactin and growth hormone release in the tilapia, *Oreochromis mossambicus*, Gen. Comp. Endocrinol. 130 (2003) 333–339.
[15] K.M. Eyster, Introduction to signal transduction: a primer for untangling the web of intracellular messengers, Biochem. Pharmacol. 55 (1998) 1927–1938.
[16] D.F. Fiol, S.Y. Chan, D. Kultz, Regulation of osmotic stress transcription factor 1 (Ostf1) in tilapia (*Oreochromis mossambicus*) gill epithelium during salinity stress, J. Exp. Biol. 209 (2006) 3257–3265.
[17] B.K. Fox, T. Naka, K. Inoue, Y. Takei, T. Hirano, E.G. Grau, *In vitro* effects of homologous natriuretic peptides on growth hormone and prolactin release in the tilapia, *Oreochromis mossambicus*, Gen. Comp. Endocrinol. 150 (2007) 270–277.
[18] E.G. Grau, C.A. Ford, L.M.H. Helms, S.K. Shimoda, I.M. Cooke, Somatostatin and altered medium osmotic pressure elicit rapid changes in prolactin release from the rostral pars distalis of the tilapia, *Oreochromis mossambicus*, *in vitro*, Gen. Comp. Endocrinol. 65 (1987) 12–18.
[19] E.G. Grau, L.M. Helms, The tilapia prolactin cell: twenty-five years of investigation, Prog. Clin. Biol. Res. 342 (1990) 534–540.
[20] E.G. Grau, L.M.H. Helms, The tilapia prolactin cell: a model for stimulus-secretion coupling, Fish Physiol. Biochem. 7 (1989) 11–19.
[21] E.G. Grau, R.S. Nishioka, H.A. Bern, Effects of osmotic pressure and calcium ion on prolactin release *in vitro* from the rostral pars distalis of the tilapia, *Sarotherodon mossambicus*, Gen. Comp. Endocrinol. 45 (1981) 406–408.
[22] E.G. Grau, R.S. Nishioka, H.A. Bern, Effects of somatostatin and urotensin II on tilapia pituitary prolactin release and interactions between somatostatin, osmotic pressure Ca++, and adenosine 3′,5′-monophosphate in prolactin release in vitro, Endocrinology 110 (1982) 910–915.
[23] E.G. Grau, N.H. Richman, R.J. Borski, Osmoreception and a simple endocrine reflex of the prolactin cell of the tilapia, *Oreochromis mossambicus*, Persp, Comp. Endocrinol. (1994) 251–256.
[24] L.M.H. Helms, E.G. Grau, R.J. Borski, Effects of osmotic pressure and somatostatin on the cAMP messenger system of the osmosensitive prolactin cell of a teleost fish, the tilapia (*Oreochromis mossambicus*), Gen. Comp. Endocrinol. 83 (1991) 111–117.
[25] K. Ishibashi, S. Kondo, S. Hara, Y. Morishita, The evolutionary aspects of aquaporin family, Am. J. Physiol. 300 (2011) R566–576.
[26] G.C. Kember, J.A. Armour, M. Zamir, Mechanism of smart baroreception in the aortic arch, Phys. Rev. 74 (2006) 031914.
[27] L.R. Landa Jr., M. Harbeck, K. Kaihara, O. Chepurny, K. Kitiphongspattana, O. Graf, V.O. Nikolaev, M.J. Lohse, G.G. Holz, M.W. Roe, Interplay of Ca^{2+} and cAMP signaling in the insulin-secreting MIN6 beta-cell line, J. Biol. Chem. 280 (2005) 31294–31302.
[28] F. Lang, G.L. Busch, M. Ritter, H. Volkl, S. Waldegger, E. Gulbins, D. Haussinger, Functional significance of cell volume regulatory mechanisms, Physiol. Rev. 78 (1998) 247–306.
[29] S.G. Lechner, S. Markworth, K. Poole, E.S. Smith, L. Lapatsina, S. Frahm, M. May, S. Pischke, M. Suzuki, I. Ibanez-Tallon, F.C. Luft, J. Jordan, G.R. Lewin, The molecular and cellular identity of peripheral osmoreceptors, Neuron 69 (2011) 332–344.
[30] G. Leng, W.T. Mason, R.G. Dyer, The supraoptic nucleus as an osmoreceptor, Neuroendocrinology 34 (1982) 75–82.
[31] W. Liedtke, Role of TRPV ion channels in sensory transduction of osmotic stimuli in mammals, Exp. Physiol. 92 (2007) 507–512.
[32] L.A. Manzon, The role of prolactin in fish osmoregulation: a review, Gen. Comp. Endocrinol. 125 (2002) 291–310.
[33] S.D. McCormick, Endocrine control of osmoregulation in teleost fish, Am. Zool. 41 (2001) 781–794.
[34] S.D. McCormick, D. Bradshaw, Hormonal control of salt and water balance in vertebrates, Gen. Comp. Endocrinol. 147 (2006) 3–8.
[35] K. McKenna, C. Thompson, Osmoregulation in clinical disorders of thirst appreciation, Clin. Endocrinol. (Oxf) 49 (1998) 139–152.
[36] A. Mizuno, N. Matsumoto, M. Imai, M. Suzuki, Impaired osmotic sensation in mice lacking TRPV4, Am. J. Physiol. 285 (2003) C96–C101.
[37] Y. Nagahama, R.S. Nishioka, H.A. Bern, R.L. Gunther, Control of prolactin secretion in teleosts, with special reference to *Gillichthys mirabilis* and *Tilapia mossambica*, Gen. Comp. Endocrinol. 25 (1975) 166–188.
[38] R.S. Nishioka, K.M. Kelley, H.A. Bern, Control of prolactin and growth hormone secretion in teleost fishes, Zool. Sci. 5 (1988) 267–280.
[39] G.E. Pickford, J.G. Phillips, Prolactin, a factor in promoting survival of hypophysectomized killifish in fresh water, Science 130 (1959) 454–455.
[40] F. Rentier-Delrue, D. Swennen, P. Prunet, M. Lion, J.A. Martial, Tilapia prolactin: molecular cloning of two cDNAs and expression in *Escherichia coli*, DNA 3 (1989) 261–270.
[41] B.D. Rose, T.W. Post (Eds.), Clinical Physiology of Acid–base and Electrolyte Disorders, McGraw-Hill, Medical Pub. Division, New York, 2001.
[42] A.P. Seale, T. Hirano, E.G. Grau, Stimulus-secretion coupling in the osmoreceptive prolactin cell of the tilapia, in: A. Kamkin, I. Kiseleva (Eds.), Mechanosensitivity of the Cells from Various Tissues, Academia, Moscow, 2005, pp. 371–389.
[43] A.P. Seale, I.M. Cooke, T. Hirano, G.E. Grau, Evidence that IP3 and ryanodine-sensitive intra-cellular Ca^{2+} stores are not involved in acute hyposmotically-induced prolactin release in Tilapia, Cell. Physiol. Biochem. 14 (2004) 155–166.
[44] A.P. Seale, J.C. Fiess, T. Hirano, I.M. Cooke, E.G. Grau, Disparate release of prolactin and growth hormone from the tilapia pituitary in response to osmotic stimulation, Gen. Comp. Endocrinol. 145 (2006) 222–231.
[45] A.P. Seale, T. Hirano, E.G. Grau, Osmoreception: a fish model for a fundamental sensory modality, in: G. Zaccone, M. Reinecke, B.K. Kapoor (Eds.), Fish Endocrinology, vol. 1, Oxford & IBH Publishing Company, 2006, pp. 419–440.
[46] A.P. Seale, T. Itoh, S. Moriyama, A. Takahashi, H. Kawauchi, T. Sakamoto, M. Fujimoto, L.G. Riley, T. Hirano, E.G. Grau, Isolation and characterization of a homologue of mammalian prolactin-releasing peptide from the tilapia brain and its effect on prolactin release from the tilapia pituitary, Gen. Comp. Endocrinol. 125 (2002) 328–339.
[47] A.P. Seale, M. Mita, T. Hirano, E. Gordon Grau, Involvement of the cAMP messenger system and extracellular Ca^{2+} during hyposmotically-induced prolactin release in the Mozambique tilapia, Gen. Comp. Endocrinol. 170 (2011) 401–407.
[48] A.P. Seale, N.H. Richman, T. Hirano, I. Cooke, E.G. Grau, Cell volume increase and extracellular Ca^{2+} are needed for hyposmotically induced prolactin release in tilapia, Endocrinol. Metab. 23 284 (2003) C1280–C1289.
[49] A.P. Seale, N.H. Richman, T. Hirano, I. Cooke, E.G. Grau, Evidence that signal transduction for osmoreception is mediated by stretch-activated ion channels in tilapia, Endocrinol. Metab. 23 284 (2003) C1290–C1296.
[50] A.P. Seale, L.G. Riley, T.A. Leedom, S. Kajimura, R.M. Dores, T. Hirano, E.G. Grau, Effects of environmental osmolality on release of prolactin, growth hormone and ACTH from the tilapia pituitary, Gen. Comp. Endocrinol. 128 (2002) 91–101.
[51] R. Sharif-Naeini, S. Ciura, Z. Zhang, C.W. Bourque, Contribution of TRPV channels to osmosensory transduction, thirst, and vasopressin release, Kidney Int. 73 (2008) 811–815.
[52] R. Sharif-Naeini, M.F. Witty, P. Seguela, C.W. Bourque, An N-terminal variant of Trpv1 channel is required for osmosensory transduction, Nat. Neurosci. 9 (2006) 93–98.
[53] B.S. Shepherd, T. Sakamoto, S. Hyodo, R.S. Nishioka, C. Ball, H.A. Bern, E.G. Grau, Is the primitive regulation of pituitary prolactin ($tPRL_{177}$ and $tPRL_{188}$) secretion and gene expression in the euryhaline tilapia (*Oreochromis mossambicus*) hypothalamic or environmental?, J Endocrinol. 161 (1999) 121–129.
[54] J.L. Specker, D.S. King, R.S. Nishioka, K. Shirahata, K. Yamaguchi, H.A. Bern, Isolation and partial characterization of a pair of prolactins released *in vitro* by the pituitary of cichlid fish, *Oreochromis mossambicus*, Proc. Nat. Acad. Sci. USA 82 (1985) 7490–7494.

[55] J.L. Specker, M. Kishida, L. Huang, D.S. King, Y. Nagahama, H. Ueda, T.R. Anderson, Immunocytochemical and immunogold localization of two prolactin isoforms in the same pituitary cells and in the same granules in the tilapia (*Oreochromis mossambicus*), Gen. Comp. Endocrinol. 89 (1993) 28–38.
[56] E.M. Stricker, M.L. Hoffmann, Presystemic signals in the control of thirst, salt appetite, and vasopressin secretion, Physiol. Behav. 91 (2007) 404–412.
[57] S.N. Thornton, Thirst and hydration: physiology and consequences of dysfunction, Physiol. Behav. 100 (2010) 15–21.
[58] J.G. Verbalis, How does the brain sense osmolality?, J Am. Soc. Nephrol. 18 (2007) 3056–3059.
[59] D.L. Voisin, C.W. Bourque, Integration of sodium and osmosensory signals in vasopressin neurons, Trends Neurosci. 25 (2002) 199–205.
[60] J. Vriens, G. Appendino, B. Nilius, Pharmacology of vanilloid transient receptor potential cation channels, Mol. Pharmacol. 75 (2009) 1262–1279.
[61] H. Watanabe, J.B. Davis, D. Smart, J.C. Jerman, G.D. Smith, P. Hayes, J. Vriens, W. Cairns, U. Wissenbach, J. Prenen, V. Flockerzi, G. Droogmans, C.D. Benham, B. Nilius, Activation of TRPV4 channels (hVRL-2/mTRP12) by phorbol derivatives, J. Biol. Chem. 277 (2002) 13569–13577.
[62] S. Watanabe, T. Hirano, E.G. Grau, T. Kaneko, Osmosensitivity of prolactin cells is enhanced by the water channel aquaporin-3 in a euryhaline Mozambique tilapia (*Oreochromis mossambicus*), Am. J. Physiol. 296 (2009) R446–453.
[63] S. Watanabe, T. Kaneko, K. Aida, Aquaporin-3 expressed in the basolateral membrane of gill chloride cells in Mozambique tilapia *Oreochromis mossambicus* adapted to freshwater and seawater, J. Exp. Biol. 208 (2005) 2673–2682.
[64] G.M. Weber, J.F. Powell, M. Park, W.H. Fischer, A.G. Craig, J.E. Rivier, U. Nanakorn, I.S. Parhar, S. Ngamvongchon, E.G. Grau, N.M. Sherwood, Evidence that gonadotropin-releasing hormone (GnRH) functions as a prolactin-releasing factor in a teleost fish (*Oreochromis mossambicus*) and primary structures for three native GnRH molecules, J. Endocrinol. 155 (1997) 121–132.
[65] G.M. Weber, A.P. Seale, I.N. Richman, M.H. Stetson, T. Hirano, E.G. Grau, Hormone release is tied to changes in cell size in the osmoreceptive prolactin cell of a euryhaline teleost fish, the tilapia, *Oreochromis mossambicus*, Gen. Comp. Endocrinol. 138 (2004) 8–13.
[66] T. Wells, Vesicular osmometers, vasopressin secretion and aquaporin-4: a new mechanism for osmoreception?, Mol Cell. Endocrinol. 136 (1998) 103–107.
[67] F.H. Westheimer, Why nature chose phosphates, Science 235 (1987) 1173–1178.
[68] T. Wigham, R.S. Nishioka, H.A. Bern, Factors affecting *in vitro* activity of prolactin cells in euryhaline teleost *Sarotherodon mossambicus* (*Tilapia mossambica*), Gen. Comp. Endocrinol. 32 (1977) 120–131.
[69] D. Willoughby, D.M. Cooper, Ca^{2+} stimulation of adenylyl cyclase generates dynamic oscillations in cyclic AMP, J. Cell. Sci. 119 (2006) 828–836.
[70] T. Yada, T. Hirano, E.G. Grau, Changes in plasma levels of the two prolactins and growth hormone during adaptation to different salinities in the euryhaline tilapia (*Oreochromis mossambicus*), Gen. Comp. Endocrinol. 93 (1994) 214–223.
[71] K. Yamaguchi, J.L. Specker, D.S. King, Y. Yokoo, R.S. Nishioka, T. Hirano, H.A. Bern, Complete amino acid sequences of a pair of fish (tilapia) prolactins, $tPRL_{177}$ and $tPRL_{188}$, J. Biol. Chem. 263 (1988) 9113–9121.
[72] J. Yin, W.M. Kuebler, Mechanotransduction by TRP channels: general concepts and specific role in the vasculature, Cell Biochem. Biophys. 56 (2010) 1–18.
[73] J.S.M. Yoshikawa-Ebesu, R.J. Borski, N.H. Richman, E.G. Grau, Effects of acclimation salinity and *in vitro* medium osmotic pressure on the incorporatin of ^{3}H-Leucine into the two prolactins of the tilapia, *Oreochromis mossambicus*, J. Exp. Zool. 271 (1995) 331–339.