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REVIEW

The Role of Prolactin in Fish Osmoregulation: A Review

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The protein hormone prolactin (PRL) was first discovered as an anterior pituitary factor capable of stimulating milk production in mammals. We now know that PRL has over 300 different functions in vertebrates. In fish, PRL plays an important role in freshwater osmoregulation by preventing both the loss of ions and the uptake of water. This paper will review what is currently known about the structure and evolution of fish PRL and its mechanisms of action in relation to the maintenance of hydromineral balance. Historically, functional studies of fish PRL were carried out using heterologous PRLs and the results varied greatly between experiments and species. In some cases this variability was due to the ability of these PRLs to bind to both growth hormone and PRL receptors. In fact, a recurring theme in the literature is that the actions of PRL cannot be generalized to all fish due to marked differences between species. Many of the effects of PRL on hydromineral balance are specific to euryhaline fish, which is appropriate given that they frequently experience sudden changes in environmental salinity. Much of the recent work has focused on the isolation and characterization of fish PRLs and their receptors. These studies have provided the necessary tools to obtain a better understanding of the evolution of PRL and its role in osmoregulation.

Key Words: prolactin; prolactin receptor; osmoregulation; fish; tilapia; evolution.

INTRODUCTION

A pituitary factor capable of stimulating milk secretion in rabbits was discovered in 1928 by Stricker and Grueter. Shortly thereafter, another group identified this factor as a hormone that could also stimulate growth of the pigeon crop sac and called this hormone "prolactin" (Riddle *et al.*, 1933). Since these discoveries, a wealth of knowledge has accumulated regarding prolactin (PRL), earning it the distinction of being the most versatile hormone with respect to its array of actions in vertebrates. The numerous functions of PRL were first summarized by Nicoll and Bern (1972) and Nicoll (1974). They classified the 85 different actions of PRL into five categories: (1) reproduction, (2) osmoregulation, (3) growth, (4) integument, and (5) synergism with steroids. However, over the next two decades, several hundred more actions were described. In fact, over 300 separate biological activities have been attributed to PRL; this number exceeds the total for all other pituitary hormones combined (see Bole-Feysot *et al.*, 1998 for review). The discovery of these new PRL actions prompted a reclassification into the following areas: (1) water and electrolyte balance, (2) growth and development, (3) endocrinology and metabolism, (4) brain and behaviour, (5) reproduction, and (6) immunoregulation and protection (Bole-Feysot *et al.*, 1998).

The importance of PRL in fish osmoregulation was first demonstrated in the 1950s by studies on the killifish (*Fundulus heteroclitus*), a euryhaline teleost. Bur-

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den (1956) discovered that *F. heteroclitus* were unable to survive in fresh water (FW) following hypophysectomy, indicating that a pituitary factor was involved in FW adaptation in this species. Subsequently, Pickford and Phillips (1959) found that treatment with PRL allowed hypophysectomized killifish to survive FW transfer. This latter finding spawned an intensive investigation into the osmoregulatory role of PRL in FW and euryhaline species such as the killifish (*Fundulus* sp.), stickleback (*Gillichthyes mirabilis*), molly (*Poecilia latipinna*), and tilapia (*Oreochromis mossambicus* and *Oreochromis niloticus*) (see Horseman, 1987).

It is generally accepted that PRL is the FW-adapting hormone in most euryhaline teleosts, but this is not the case for teleosts in general (reviewed in Clarke and Bern, 1980; Bern, 1983). The importance of PRL in FW adaptation varies both between and within species: FW species can survive in FW following hypophysectomy, anadromous teleosts such as salmon are less dependent on PRL for FW osmoregulation, and although hypophysectomized *F. heteroclitus* perishes shortly after FW transfer, its congener *Fundulus kansas* survives indefinitely in FW following pituitary removal (see Bern, 1983; Horseman, 1987). Pituitary and plasma PRL levels increase during adaptation to FW and PRL regulates hydromineral balance by decreasing water uptake and increasing ion retention (especially Na^+ and Cl^-). These effects are a result of alterations in both the permeability of osmoregulatory surfaces (gill, skin, kidney, intestine, and urinary bladder) and ion transport mechanisms (Hirano *et al.*, 1986). This paper will review what is currently known about fish PRL and its role in osmoregulation. The following areas will be discussed: (1) PRL structure, distribution, and evolution, (2) PRL receptors (PRLRs) and signaling pathways, and (3) osmoregulatory targets of PRL action.

1. STRUCTURE, DISTRIBUTION, AND EVOLUTION OF PRL IN FISH

A. Structure

The tropic hormones of the adenohypophysis were first grouped into families based on their staining properties (i.e., acidophilic, basophilic, neutral) (Norris, 1997) and it was later found that members of each family also shared structural similarities (Wallis, 1984). PRL is a member of the protein hormone family that includes growth hormone (GH), mammalian placental lactogen (PL), and teleostean somatolactin (SL) (Wallis, 1984; Nicoll *et al.*, 1986; Wallis, 1992; Rand-Weaver *et al.*, 1993). Mammalian PRL (mPRL) and GH (mGH) are single polypeptide chains of approximately 190–200 amino acids (aa), but they differ with respect to their tertiary structures (Norris, 1997). There are three disulfide bridges in mPRL, one at the N-terminal, one in the middle, and one at the C-terminal of the protein, whereas mGH lacks the N-terminal disulfide bridge (Norris, 1997) (Fig. 1). Mammalian PRL is synthesized as a prohormone containing a signal peptide of approximately 28 aa (Bole-Feysot *et al.*, 1998).

Piscine PRLs are also synthesized as prohormones with signal peptides of 23–24 aa. The full-length aa sequence of PRL has been determined for a variety of teleost and nonteleost fish (Table 1). Both of the nonteleost representatives, the sturgeon and lungfish, have PRLs with three disulfide bridges (Noso *et al.*, 1993a,b) (Fig. 1) and are longer than those of teleosts, but similar in length to mPRLs (Table 1). In contrast, all teleost PRLs lack the N-terminal disulfide bond due to the absence of 12–14 aa at the N-terminus (Rand-Weaver *et al.*, 1993) (Fig. 1). It is possible that the loss of the N-terminal disulfide bridge in teleosts may be related to the central role of PRL as an osmoregulatory hormone in these organisms. This idea is supported by the fact that disruption of the N-terminal disulfide bond of ovine PRL greatly enhanced its potency in a teleost osmoregulation bioassay (Doneen *et al.*, 1979).

Despite the structural differences between teleost and nonteleost PRLs, four conserved domains have been identified based on aa sequence alignments with other vertebrate PRLs (see Chen *et al.*, 1994). Two of these four domains exhibit an especially high degree of conservation, leading to the suggestion that these regions may be responsible for the functions of PRL that are common to all vertebrates (Rand-Weaver *et al.*, 1993), however, the identification of these shared biological activities of PRL is hampered by both the diversity of PRL's effects and the unique roles of PRL in some vertebrate species. Perhaps these conserved domains contain binding determinants for either

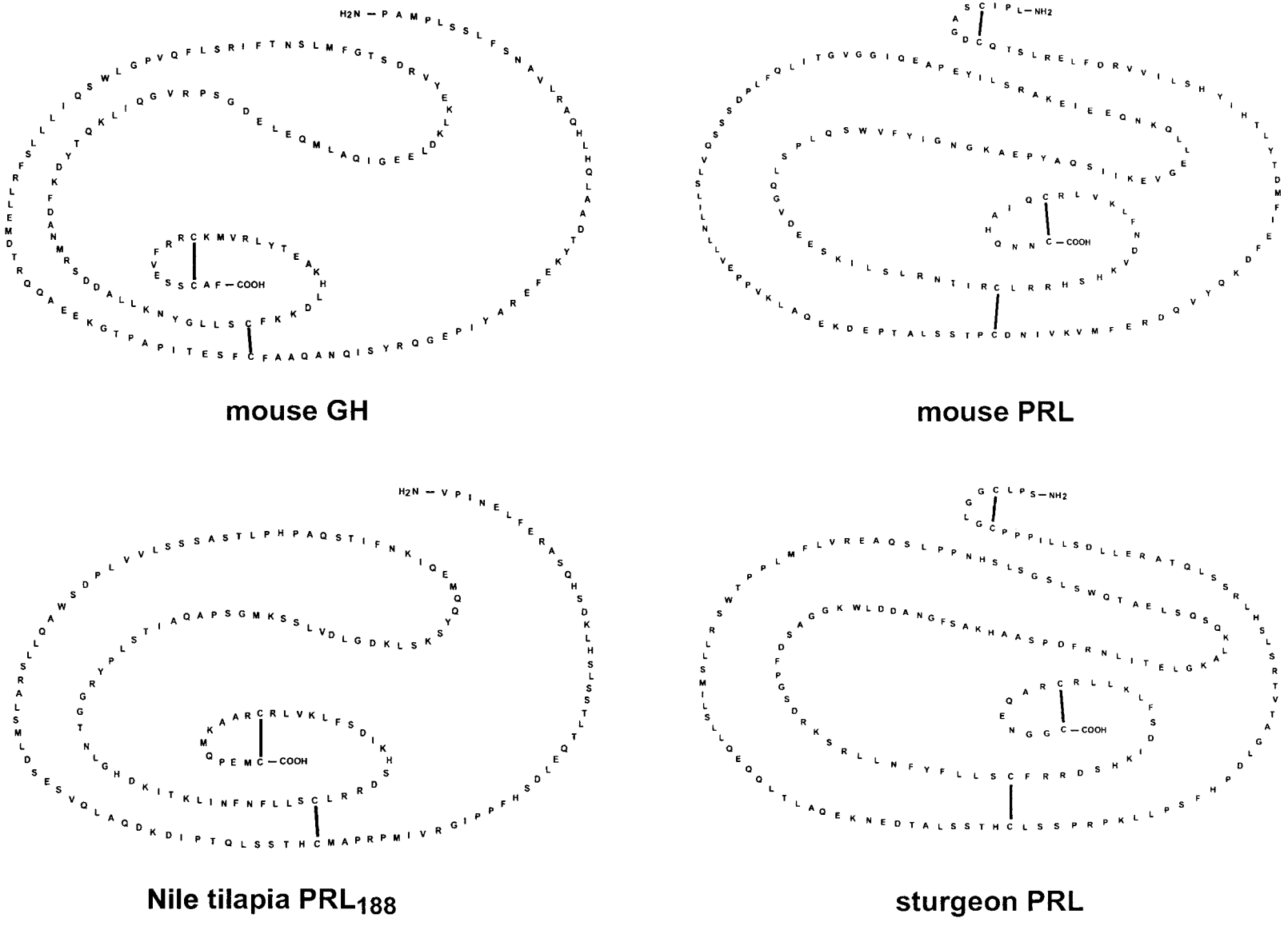

FIG. 1. Structural comparison of fish PRLs with mammalian (mouse) PRL and GH. Teleost PRLs (i.e., Nile tilapia PRL_{188}) lack the N-terminal disulfide bridge that is present in the mouse PRL and appear to be more similar in structure to mouse GH. Conversely, nonteleost fish like the sturgeon have retained the N-terminal bridge and more closely resemble mouse PRL. The loss of the N-terminal disulfide bond by teleosts may be related to the central role of PRL in teleost osmoregulation. References for amino acid sequences presented in this figure: mouse GH, Linzer and Talamantes, 1985; mouse PRL, Harigaya *et al.*, 1986; Nile tilapia PRL_{188}, Rentier-Delrue *et al.*, 1989; sturgeon PRL, Noso *et al.*, 1993.

TABLE 1
Species of Fish for Which the Full-Length PRL Amino Acid Sequence Has Been Determined

Classification	Species name	Common name	Size (aa)	Reference
Nonteleosts				
Chondrostei	*Acipenser gueldenstaedtii*	Russian sturgeon	204	Noso *et al.*, 1993a
Dipnoi	*Protopterus aethiopicus*	Marbled lungfish	200	Noso *et al.*, 1993b
Teleosts				
Elapomorpha	*Anguilla anguilla*	European eel	185	Querat *et al.*, 1994
Ostiophysi	*Cyprinus carpio*	Common carp	186	Yasuda *et al.*, 1987; Chao *et al.*, 1988
	Hypophthalmichthys molitrix	Silver carp	187	Chang *et al.*, 1992
	Hypophthalmichthys nobilis	Noble carp	187	Chang *et al.*, 1992
	Ictalurus punctatus	Channel catfish	186	Watanabe *et al.*, 1992
	Carassius auratus	Goldfish	187	Chan *et al.*, 1996
	Heteropneustes fossilis	Indian catfish	186	Anathy *et al.*, 2001
Salmoniformes	*Oncorhynchus keta I*	Chum salmon	188	Yasuda *et al.*, 1986; Song *et al.*, 1988
	Oncorhynchus keta II	Chum salmon	187	Yasuda *et al.*, 1986; Kuwana *et al.*, 1988
	Oncorhynchus mykiss	Rainbow trout	187	Mercier *et al.*, 1989
	Oncorhynchus tschawytscha II	Chinook salmon	187	Xiong *et al.*, 1992
	Coregonus autumnalis	Baikal omul	187	Trofimova *et al.*, 1993
	Salmo salar	Atlantic salmon	187	Martin *et al.*, 1995
Neoteleostei	*Oreochromis mossambicus I* ($tPRL_{188}$)	Mozambique tilapia	188	Specker *et al.*, 1985a; Yamaguchi *et al.*, 1988
	Oreochromis mossambicus II ($tPRL_{177}$)	Mozambique tilapia	177	Specker *et al.*, 1985a; Yamaguchi *et al.*, 1988
	Oreochromis niloticus I	Nile tilapia	188	Rentier-Delrue *et al.*, 1989
	Oreochromis niloticus II	Nile tilapia	177	Rentier-Delrue *et al.*, 1989
	Dicentrarchus labrax	European sea bass	188	Doliana *et al.*, 1994
	Paralichthys olivaceus	Japanese flounder	187	Kim and Lee, 1998
	Sparus aurata	Gilthead sea bream	188	Santos *et al.*, 1999

transmembrane receptors (PRLRs; discussed in Section 2 below), or soluble forms of these membrane receptors that are found in the plasma (i.e., PRL-binding proteins). Although PRL-binding proteins have not yet been isolated in fish, they do exist in humans (Kline and Clevenger, 2001) and GH-binding proteins have been isolated from rainbow trout (*Oncorhynchus mykiss*) (Sohm *et al.*, 1998a). PRL-binding proteins may serve to protect circulating PRL from degradation, thus providing a "ready-reserve" of hormone to allow for rapid responses to salinity changes.

The existence of two PRL forms has been shown in some teleosts, namely chum salmon (*Oncorhynchus keta*), common carp (*Cyprinus carpio*), Japanese eel (*Anguilla japonica*) (Suzuki *et al.*, 1991), Mozambique tilapia (*O. mossambicus*), and Nile tilapia (*O. niloticus*) (see Table 1 for references). The two salmon, carp, and eel PRLs are highly homologous, whereas the two tilapia PRLs share only 69% aa identity (Yamaguchi *et al.*, 1988). These two tilapia PRLs (tPRLs) are classified as long (188 aa; PRL I or $tPRL_{188}$) and short (177 aa; PRL II or $tPRL_{177}$) forms and $tPRL_{188}$ is more similar than $tPRL_{177}$ to the PRLs of other fish (Yamaguchi *et al.*, 1988). Two small stretches of aa that are present in $tPRL_{188}$ are absent from $tPRL_{177}$ and these deleted regions (the five aa N-terminal to C46 and the six aa N-terminal to C161) are immediately adjacent to the two cysteines (C46 and C161) that are thought to form the first disulfide bridge of teleost PRLs (Yamaguchi *et al.*, 1988).

These deletions are not without consequence, as the biological activities of the two tPRLs are different. Both tPRLs appear to be equally efficient at preventing the loss of plasma Na^+ and Cl^- and at maintaining the plasma osmolality and whole-animal transepithelial potential of hypophysectomized *O. mossambicus* in FW (Specker *et al.*, 1985a; Young *et al.*, 1988). However, in *O. niloticus* adapted to brackish water (BW), injection of $tPRL_{188}$ resulted in a clear, dose-dependent effect on ion retention as evidenced by an increase in plasma Na^+ and Cl^-, but injection of $tPRL_{177}$ caused only slight increases in plasma Na^+ and Cl^- that were not dose dependent (Auperin *et al.*, 1994a). The different results obtained for tilapia adapted to FW vs those

adapted to BW indicate that the effects of $tPRL_{188}$ and $tPRL_{177}$ may differ depending on the environmental salinity and the species being investigated. Auperin *et al.* (1994a) suggest that $tPRL_{188}$ is primarily involved in the regulation of plasma Na^+ and Cl^-, while $tPRL_{177}$ acts to decrease water permeability in Nile tilapia adapted to hyperosmotic environments.

The somatotropic actions of tPRLs have also been examined in *O. mossambicus*. The first study to demonstrate that the two tPRLs have different effects on growth was conducted by Specker *et al.* (1985b). They reported that injections of $tPRL_{188}$, but not $tPRL_{177}$, were able to promote growth as measured by an increase in length and weight of the fish. However, the $tPRL_{188}$ preparation used in this study did have some GH contamination (<20%) (Specker *et al.*, 1985b). Conversely, Shepherd *et al.* (1997) found that $tPRL_{177}$, but not $tPRL_{188}$, had somatotropic activity as evidenced by an increase in [^{3}H]thymidine and [^{35}S]sulfate incorporation into ceratobranchial cartilage and a stimulation of hepatic IGF-I mRNA synthesis. In addition, radioreceptor assays demonstrated that $tPRL_{177}$ has the ability to displace tilapia GH from its receptor. The differences observed between $tPRL_{177}$ and $tPRL_{188}$ may be due to the presence of a unique cartilage or liver receptor that only binds $tPRL_{177}$. Alternatively, under conditions where $tPRL_{177}$ levels are high (i.e., in FW), $tPRL_{177}$ may influence growth by competing with GH for binding sites (Shepherd *et al.*, 1997). One possible explanation for the differing results obtained in these two studies may lie in the indices used to measure growth. Perhaps the 21-day $tPRL_{177}$ treatment regime used by Specker *et al.* (1985b) was not sufficient to detect changes in morphometric parameters.

Not only do $tPRL_{188}$ and $tPRL_{177}$ differ with respect to their biological activities, their levels are also differentially regulated during development (*O. mossambicus:* Ayson *et al.*, 1994) and in response to alterations in environmental salinity (*O. mossambicus:* Borski *et al.*, 1992; Yada *et al.*, 1994; Yoshikawa-Ebesu *et al.*, 1995; *O. niloticus:* Auperin *et al.*, 1994a). *In vitro* studies carried out using the isolated rostral pars distalis of *O. mossambicus* showed that the content, release, and synthesis of both tPRLs was greater, but the ratio of $tPRL_{177}$:$tPRL_{188}$ was lower, in FW vs seawater (SW)-adapted fish (Borski *et al.*, 1992; Yoshikawa-Ebesu *et al.*, 1995). Similarly, the transfer of *O. niloticus* from FW to either BW or SW resulted in a decrease in plasma and pituitary levels of the two tPRLs and an increase in the ratio of $tPRL_{177}$:$tPRL_{188}$ (Auperin *et al.*, 1994a). The results of these studies indicate that the disparate levels of the two tPRLs are a result of differences in production, rather than secretion, in response to salinity changes. Also, regulation appears to be posttranscriptional, as changes in the ratio of $tPRL_{177}$ mRNA:$tPRL_{188}$ mRNA were not observed following the adaptation of *O. niloticus* to BW (Auperin *et al.*, 1994a). Secretagogues of PRL do not seem to differentially regulate the release of these two hormones (see Nishioka *et al.*, 1988; Borski *et al.*, 1992), although most studies did not examine the response of both FW and SW-adapted animals. Future studies should examine the possible mechanisms that mediate the differential production of pituitary tPRLs.

B. Distribution

The primary cell type involved in the synthesis and secretion of PRL in mammals is the lactotroph or mammotroph of the anterior pituitary gland, however, there is also an intermediate cell population known as mammosomatotrophs (MS) which have the ability to secrete both PRL and GH and can differentiate into lactotrophs in the presence of estrogen (reviewed by Freeman *et al.*, 2000). In addition to these pituitary sites, PRL has also been detected in the brain, placenta, uterus, mammary gland, and lymphocytes of mammals (Freeman *et al.*, 2000).

PRL-secreting cells form a definitive mass within the rostral pars distalis of most teleost pituitaries, a feature that greatly facilitates the study of PRL (Clarke and Bern, 1980). For example, 95% of the cells in the rostral pars distalis of *O. mossambicus* are PRL-secreting cells and this region can be isolated to yield a nearly homogenous population of PRL-secreting cells devoid of GH-secreting cells (Nishioka *et al.*, 1993). However, some PRL-immunoreactive cells have also been found in the proximal pars distalis and the pars intermedia of teleosts (see Huang and Specker, 1994 for references). Immunocytochemical examination of the distribution of $tPRL_{177}$ and $tPRL_{188}$ revealed that they were colocalized in the same rostral pars distalis cells of *O. niloticus* (Ayson *et al.*, 1993) and *O. mossambicus* (Specker *et al.*, 1993). Cellular colocalization of the two tilapia PRL mRNAs was also demonstrated in *O. mossambicus* using *in situ* hybridization (Nishioka *et*

al., 1993). Furthermore, immunogold staining indicated that both tPRLs were found in the same secretory granules in *O. mossambicus* (Specker *et al.,* 1993). The results of these studies show that differences in the biological activities of $tPRL_{188}$ and $tPRL_{177}$ are not due to the independent regulation of different cell types or secretion granules.

Recently, MS cells have been identified in the pituitary of the gilthead sea bream (*Sparus aurata*) during its development (Villaplana *et al.,* 2000). This study found that the MS cells were first observed in newly hatched *S. aurata* and cells containing only GH or PRL were not present. Conversely, GH cells and PRL cells, but not MS cells, were observed by 2 days posthatch. Curiously, the MS cells reappeared in older larvae and juveniles, but were absent in adults (Villaplana *et al.,* 2000). When the MS cells reappeared in older larvae and juveniles, they were located in the region of the rostral pars distalis where the PRL cells were first detected. Based on these results, Villaplana *et al.* (2000) suggest that the MS cells seen in newly hatched larvae may differentiate into GH and PRL cells and the location of the MS cells within the pituitary dictates which cell type (GH or PRL) will be formed. The reappearance of the MS cells in the older larvae and juveniles may be to provide an additional source of GH during these periods of development when vigorous growth is occurring (Villaplana *et al.,* 2000). Thus, it appears that MS cells may be pluripotent, having the ability to differentiate into either GH or PRL cells, depending on their location and the physiological needs of the organism. It would be of interest to determine whether these MS cells are present in euryhaline fish species and if they are involved in adaptation to hypoosmotic environments, perhaps by providing an additional source of PRL.

In contrast to the teleosts, the lungfish pituitary is not clearly delineated into zones and the different adenohypophyseal cells are more intermingled as in tetrapods (Bentley, 1998). The PRL-secreting cells tend to be distributed throughout the pars distalis with no apparent regionalization (Clarke and Bern, 1980). The existence of PRL-secreting cells in Agnatha (Myxinoidea, the hagfishes; Petromyzontidae, the lampreys) has also been examined. Researchers have failed to detect PRL in the hagfish pituitary by both bioassay and immunocytochemistry (see Specker *et al.,* 1984), but immunoreactive cells were detected in the proximal pars distalis of the adult sea lamprey (*Petromyzon marinus*) with rat PRL antibodies (Wright, 1984). In addition, human PRL-immunoreactive cells were detected in the neural gland of an ascidian (*Styela plicata*) (Pestrarino, 1984).

The existence of PRL at extrapituitary sites has also been demonstrated in some fish. Immunocytochemical studies have detected PRL-like molecules in the preoptic nuclei of a polypteran, *Calamoichthys calabaricus* (Hansen and Hansen, 1982) and an agnathan, *P. marinus* (Wright, 1986). Recently, expression of the PRL gene has been demonstrated in the pituitary, liver, intestine, and gonads, but not in the brain, of the sea bream (*S. aurata*) (Santos *et al.,* 1999) and in the pituitary, liver, kidney, spleen, gill, muscle, gonads, and brain, but not in the intestine of goldfish (*Carassius auratus*) (Imaoka *et al.,* 2000). PRL may act in an autocrine or paracrine manner in these extrapituitary tissues and poses an interesting area for future research.

C. Evolution

It is thought that the GH / PRL / PL / SL gene family evolved from a common ancestral gene approximately 850 million years ago (see Rand-Weaver *et al.,* 1993 for discussion). This estimate suggests that the genes for PRL and GH diverged prior to the emergence of metazoans, and gains support from the fact that PRL and GH-like proteins have been found in invertebrates (Pestrarino, 1984; Rand-Weaver *et al.,* 1993). When SL is added to the equation, the interpretation becomes more complex. Because SL is equally similar (23–28%) to both PRL and GH, it is likely that it diverged independently from the ancestral gene, either coincident with, or perhaps prior to the divergence of PRL and GH (Rand-Weaver *et al.,* 1993). In order to establish these three branches, at least two gene duplications must have occurred early in the evolution of these protein hormones (Wallis, 2000).

The origin of the ancestral gene that gave rise to the GH / PRL family has also been examined (see Rand-Weaver *et al.,* 1993 for discussion) and two contrasting models have been proposed. The first model was developed based on the examination of vertebrate history and the study of ancient chordates and suggests that the primary function of the ancestral gene was to regulate somatic growth (i.e., was GH-like) (Specker *et al.,* 1984). The cells that expressed this ancestral gene

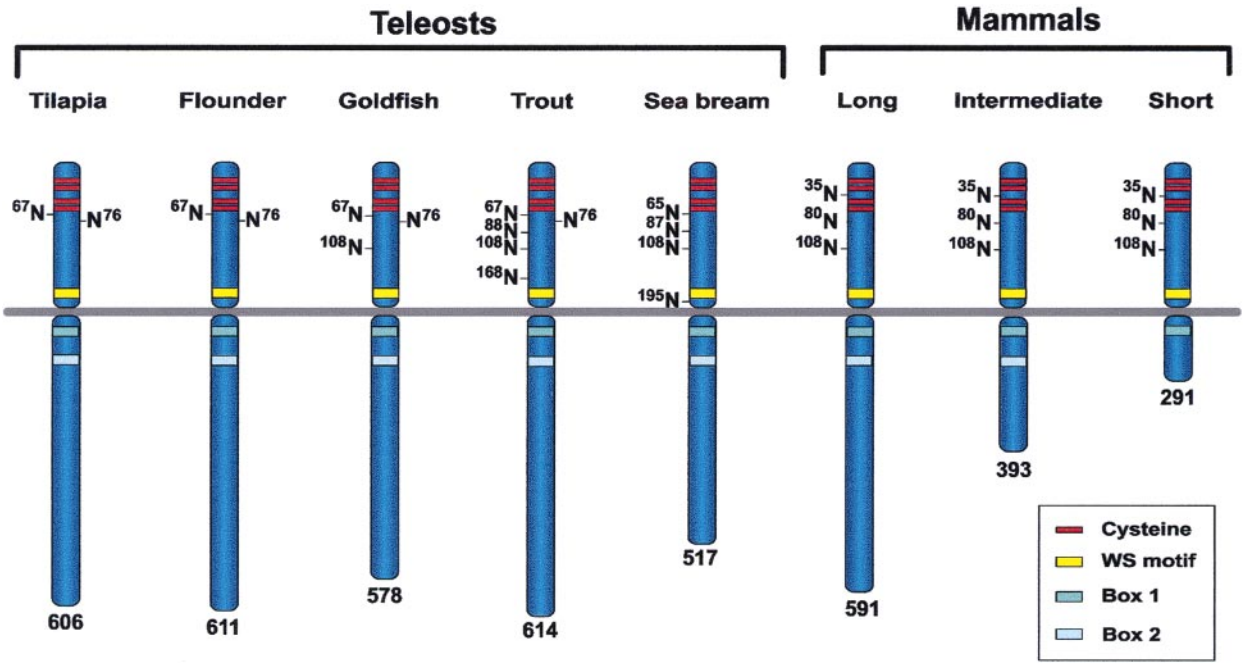

FIG. 2. Comparison of fish and mammalian (rat) PRLRs. Receptor lengths are indicated below the individual receptors. The three mammalian isoforms have identical extracellular domains and differ in the length of their intracellular domains and signal transduction abilities. Teleost PRLRs are most similar in appearance to the mammalian long form and several functional domains have been conserved between mammalian and teleost PRLRs: the two pairs of cysteines and the WS motif of the extracellular domain and the intracellular boxes 1 and 2. Note that the teleost PRLRs lack the N-glycosylation site (^{35}N) that is present in all mammalian PRLRs between the second and third cysteines. Sequence references: Nile tilapia, Sandra *et al.*, 1995; goldfish, Tse *et al.*, 2000; trout, Le Rouzic *et al.*, 2001; sea bream, Santos *et al.*, 2001; rat long, Shirota *et al.*, 1990; rat intermediate, Ali *et al.*, 1991; rat short, Boutin *et al.*, 1988.

may have been "paraneuronal" in nature, regulated directly by changes in the external environment (Specker *et al.*, 1984). PRL activity evolved later, perhaps to allow for the colonization of FW habitats. The opposing view states that the ancestral gene was involved in osmoregulation, because this one function is common to fish PRL, GH, and SL (Chen *et al.*, 1994). Further comparative studies of PRL, GH, and SL will shed light on the origins of these structurally and functionally related hormones.

With respect to the evolution of PRL in fishes, two lineages have been proposed based on the structural characteristics of PRL: a tetrapod lineage and a teleost lineage (Kawauchi and Yasuda, 1988, 1989). In this model, the nonteleost fish occupy strategic points: the lobe-finned fish (Crossopterygii and Dipnoi) are part of the lineage that gave rise to the tetrapods and the ray-finned fish (Polypteri, Chondrostei, and Holostei) are part of the lineage that gave rise to the teleosts. Subsequent studies on the PRLs of lobe-finned (lungfish, a Dipnoan) and ray-finned (bowfin, an Holostean) fish provided support for this dual-lineage model of PRL evolution.

Lungfish (*Protopterus aethiopicus*) PRL has three disulfide bonds, and is more similar to tetrapod PRLs (66% aa identity) than to teleost PRLs (38% aa identity) (Noso *et al.*, 1993a). In contrast, the bowfin (*Amia calva*) PRL lacks the N-terminal disulfide bond characteristic of tetrapod PRLs and thus appears to be more similar to the PRLs of teleosts (Dores *et al.*, 1993). However, the PRL of a chondrostean (the sturgeon, *Acipenser gueldenstaedti*) appeared to be more closely related to the tetrapod lineage than to the teleost lineage (Noso *et al.*, 1993b). This sturgeon PRL had three disulfide bridges, but its aa sequence was slightly more homologous to the PRLs of teleosts (35–46%) than to those of tetrapods (30–40%) (Noso *et al.*, 1993b). Because Chondrostei are considered to be the most ancient ray-finned fishes, it is likely that the common ancestor of the lobe- and ray-fins had three disulfide bonds, a

characteristic that was retained by the tetrapods. At some point after the divergence of chondrosteans and holosteans, one disulfide bond was lost and this feature was retained in the teleost lineage (Noso *et al.*, 1993b). The isolation of agnathan and chondrichthyian PRLs would further our understanding of PRL evolution in fish.

2. FISH PROLACTIN RECEPTORS (PRLRs) AND SIGNALING PATHWAYS

The effects of PRL on target tissues are mediated by a receptor. The PRLR is a member of the class 1 cytokine receptor superfamily that includes receptors for GH, erythropoetin, leptin, and the interleukins (Bole-Feysot *et al.*, 1998). All of these members are single-pass, transmembrane chains and are classified as class 1 cytokine receptors based on the presence of several highly conserved, functionally important aa sequences in their extracellular and intracellular domains. In mammals, there are long, intermediate, and short PRLR isoforms (Fig. 2) that are generated by alternative splicing and promoter usage (Freeman *et al.*, 2000). These isoforms have identical extracellular domains, but differ with respect to the length and composition of their intracellular domains, their expression patterns, and their mechanisms of signal transduction (see Bole-Feysot *et al.*, 1998) (Fig. 2).

A. Fish PRLR Binding Studies

Initial studies of fish PRLRs were hampered by the lack of homologous PRLs to use in binding assays and tissue localization studies (reviewed by Prunet and Auperin, 1994). Ovine PRL was extensively used to characterize PRLRs in lower vertebrates based on its ability to bind to bullfrog kidney membranes and its performance in inducing PRL-specific effects in biological assays (Clarke and Bern, 1980; Prunet and Auperin, 1994). However, it was later determined that ovine PRL probably could not distinguish between fish GH and PRL receptors and therefore was not suitable for the molecular and physiological characterization of fish PRLRs (see Prunet and Auperin, 1994 for discussion).

The isolation of PRLs from a variety of fish (Table 1) has provided the homologous ligands needed to facilitate the study of PRLRs. Most of the research on the molecular and physiological characterization of fish PRLRs has been carried out in Nile tilapia using homologous PRLs ($tPRL_{188}$ and $tPRL_{177}$). The specific binding of radiolabelled, recombinant $tPRL_{188}$ and $tPRL_{177}$ was examined in the gill, kidney, gut, skin, and liver of *O. niloticus* (Auperin *et al.*, 1994b). $tPRL_{188}$ binding was greater than $tPRL_{177}$ in all tissues examined and the highest levels of binding were observed in the kidney followed by the gill, with low levels observed in the other three tissues (Auperin *et al.*, 1994b). Scatchard analysis and competitive binding studies were utilized to analyze the tPRL binding sites in kidney and gill in more detail and the results indicated that there was one PRLR that had a higher affinity for $tPRL_{188}$ than $tPRL_{177}$ (Auperin *et al.*, 1994b). Interestingly, the K_a values calculated from the Scatchard plots of $tPRL_{177}$ binding were significantly different in gill and kidney, indicating that the PRLRs in these two tissues may not be identical (Auperin *et al.*, 1994b).

The effects of transfer to a hyperosmotic environment on the regulation of gill PRLRs in *O. niloticus* were examined to determine whether salinity had an effect on the types of PRLRs present in this tissue (Auperin *et al.*, 1995). The results of this study indicated that one PRLR was present regardless of the environmental salinity, but transfer from FW to BW increased the binding of $tPRL_{188}$ and $tPRL_{177}$ to receptors, the receptor affinity for $tPRL_{188}$, and the number of PRLRs (Auperin *et al.*, 1995). The observed correlation between increased PRLR affinity and number and decreased plasma PRL levels suggests that *O. niloticus* PRLs may exert inhibitory control over their own receptor. This will be discussed in further detail in the following section.

B. Isolation and Characterization of PRLR cDNAs from Fish

It became apparent that molecular biological approaches would be needed in order to better understand the structure and function of fish PRLRs. In 1995, the first full-length fish PRLR cDNA was isolated from the tilapia (*O. niloticus*) (Sandra *et al.*, 1995). Since this initial discovery, complete PRLR cDNAs have been isolated from goldfish (*C. auratus*) (Tse *et al.*, 2000), rainbow trout (*O. mykiss*) (Le Rouzic *et al.*, 2001),

TABLE 2
Comparison of Amino Acid Sequence Identities of Various Vertebrate PRLRs[a]

	Human	Rat	Ovine	Chicken	*Xenopus*	Tilapia	Goldfish	Trout	Sea bream	Flounder
A. Full-length PRLR										
Human	—	67	69	48	44	32	34	33	30	32
Rat	—	—	65	50	42	31	32	33	31	31
Ovine	—	—	—	49	44	35	33	35	27	36
Chicken	—	—	—	—	44	34	33	34	29	31
Xenopus	—	—	—	—	—	33	33	32	29	32
Tilapia	—	—	—	—	—	—	44	50	34	64
Goldfish	—	—	—	—	—	—	—	54	34	46
Trout	—	—	—	—	—	—	—	—	38	52
Sea bream	—	—	—	—	—	—	—	—	—	34
B. Extracellular domain										
Human	—	69	70	58	50	45	44	48	43	47
Rat	—	—	71	61	52	47	47	51	44	47
Ovine	—	—	—	62	50	47	45	47	45	48
Chicken	—	—	—	—	56	52	49	52	42	48
Xenopus	—	—	—	—	—	47	44	48	43	46
Tilapia	—	—	—	—	—	—	60	70	50	75
Goldfish	—	—	—	—	—	—	—	70	52	60
Trout	—	—	—	—	—	—	—	—	56	71
Sea bream	—	—	—	—	—	—	—	—	—	52
C. Intracellular domain										
Human	—	65	66	44	39	23	28	23	20	23
Rat	—	—	60	42	35	21	22	22	20	21
Ovine	—	—	—	39	38	27	25	26	14	28
Chicken	—	—	—	—	36	23	22	24	19	22
Xenopus	—	—	—	—	—	24	27	22	19	24
Tilapia	—	—	—	—	—	—	35	41	21	56
Goldfish	—	—	—	—	—	—	—	43	20	37
Trout	—	—	—	—	—	—	—	—	23	40
Sea bream	—	—	—	—	—	—	—	—	—	20

[a] Original references: human, Boutin *et al.*, 1989; rat, Shirota *et al.*, 1990; ovine, Bignon *et al.*, 1997; chicken, Tanaka *et al.*, 1992; *Xenopus*, Yamamoto *et al.*, 2000; Nile tilapia, Sandra *et al.*, 1995; goldfish, Tse *et al.*, 2000; trout, Le Rouzic *et al.*, 2001; sea bream, Santos *et al.*, 2001; flounder, Higashimoto *et al.*, 2001.

sea bream (*S. aurata*) (Santos *et al.*, 2001), and Japanese flounder (*Paralichthys olivaceus*) (Higashimoto *et al.*, 2001). All fish PRLR cDNAs encode for a mature protein of approximately 600 aa in length and are most similar in appearance to the long form of mammalian PRLRs (Fig. 2). Overall, the aa identity between the fish PRLRs is 34–64%, as compared to only 27–36% identity with the PRLRs of other vertebrates (Table 2A). The fact that the homology between fish PRLRs and those of higher vertebrates (mammals and birds) is similar to the homology (~37%) between fish and mammalian PRLs, suggests that there may have been a coevolution of PRL and its receptor (Sandra *et al.*, 1995).

The greatest aa identities are found in the extracellular (ligand-binding) domain, which is approximately 52–75% identical between fish species and 42–52% identical between fish and mammals (Table 2B). The extracellular domain of the mammalian PRLR (mPRLR) has two pairs of disulfide-linked cysteine residues and a C-terminal, membrane-proximal pentapeptide sequence (W-S-X-W-S) termed the "WS motif"; both of these features are required for the proper folding and trafficking of the receptor, and may participate in ligand binding (Bole-Feysot *et al.*, 1998; Freeman *et al.*, 2000) (Fig. 2). The two pairs of cysteine residues are conserved in fish PRLRs, but there are subtle differences in the sequences of the WS motif.

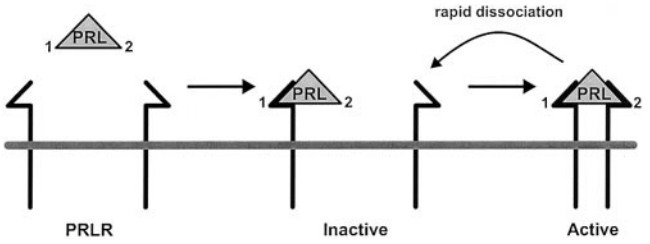

FIG. 3. Mechanism of activation of the PRLR. Binding site 1 on the PRL molecule interacts with one PRLR to form an inactive complex. A second receptor is recruited and binds to the PRL binding site 2 to form an unstable, active trimeric unit of 2 PRLRs:1 PRL. A rapid dissociation of this 2:1 complex to the inactive form has been observed in all organisms studied with the exception of *O. niloticus* (modified from Bole-Feysot *et al.*, 1998).

The WS motif of fish PRLRs is highly homologous to the mammalian sequence, but there is a conservative substitution (S to T) in the fifth position of the goldfish (Tse *et al.*, 2000) and sea bream (Santos *et al.*, 2001) PRLRs. The most variable component of the extracellular domain is the number and position of N-glycosylation sites (Fig. 2). Notably, all fish PRLRs studied to date lack an N-glycosylation site that is present in mPRLRs between the second and third cysteine residues (Fig. 2).

The mechanism of ligand binding and receptor activation is also conserved between fish and mammals. Activation of the mPRLR (and the mGH receptor) involves a ligand-induced, sequential, receptor dimerization (Fig. 3). One binding site on the PRL molecule binds with one PRLR to form an inactive complex necessary for the interaction of a second PRL binding site with another PRLR to form the active, trimeric complex of 2 PRLRs:1 PRL (Bole-Feysot *et al.*, 1998) (Fig. 3). Bell-shaped response curves were obtained for *O. niloticus* (Sohm *et al.*, 1998b) and *C. auratus* (Tse *et al.*, 2000) PRLRs in gene transactivation studies, indicating a ligand-induced PRLR homodimerization as seen in mammals, but the dynamics of this homodimerization event appear to differ between *O. niloticus* and other vertebrates. Studies on mammalian (Gertler *et al.*, 1996) and trout (*O. mykiss*; Sandowski *et al.*, 2000; Le Rouzic *et al.*, 2001) PRLRs have shown that the 2:1 complex is transient and rapidly dissociates to an unstable 1:1 complex (Fig. 3). Furthermore, the use of heterologous ligands (ovine PL, ovine PRL, and human GH) resulted in hormone–PRLR interactions that were more stable than those obtained using homologous PRLs (Gertler *et al.*, 1996; Sandowski *et al.*, 2000; Le Rouzic *et al.*, 2001). Although the *O. niloticus* PRLR (tPRLR) also acts via a homodimerization mechanism (Sohm *et al.*, 1998b), the fact that tPRL exhibits a strong, specific binding to its receptor in standard radioreceptor assays suggests that the 2:1 tPRLR:tPRL complex may be stable (Prunet *et al.*, 2000).

However, it is unlikely that an increase in the stability of the 2:1 complex results in an increased biological response. In fact, the formation of a stable trimeric complex may be detrimental, as rapid dissociation to the individual components would liberate PRL and the PRLR for further interactions (Gertler *et al.*, 1996). Furthermore, in a mammalian study examining the effects of GH analogues with an increased affinity for the second GH receptor of the dimeric complex on biological activity, there was no increase in either cell proliferation or the activation of signal transduction (Pearce *et al.*, 1999). Helman *et al.* (2001) propose that there is a "minimal time of homodimer existence" required for the activation of signal transduction pathways. Perhaps this "minimal time" is greater for tilapia than for other vertebrates, thus the 2:1 complex must persist longer in order to elicit a biological response. Future research should be directed toward gaining a better understanding of what effects the formation of a stable 2:1 complex may have on signal transduction and osmoregulation in tilapia.

The intracellular domain of mPRLRs has two conserved regions: an eight aa, membrane-proximal region rich in prolines and hydrophobic aa called box 1 that contains recognition sites for transducing molecules and is present in all mPRLRs, and a less conserved region, box 2, that is not found in the short PRLR isoforms and is comprised of hydrophobic, negatively charged, then positively charged residues (Bole-Feysot *et al.*, 1998) (Fig. 2). The intracellular domain does not possess intrinsic enzyme activity, but the membrane-proximal region, including box 1, is constituitively associated with a tyrosine kinase called Janus kinase 2 (JAK2) and ligand-induced activation results in the phosphorylation of the long and intermediate forms of mPRLR and several other cellular proteins (Bole-Feysot *et al.*, 1998; Freeman *et al.*, 2000). The primary pathway activated by mPRLRs is the JAK / Stat (signal transducer and activator of transcription) pathway, but other downstream cascades include the MAPK (mitogen-activated protein kinase)

pathway, the activation of ion channels, and Src kinases (see Freeman *et al.*, 2000 for details).

Although the intracellular domain of the PRLR is the region of lowest aa identity between vertebrates (Table 2C), the sequence of box 1 is completely conserved between mammalian and fish PRLRs and box 2 is more variable in sequence, but is still well conserved. The conservation of these intracellular domains suggests that the signal transduction mechanisms used by fish and mammals may have been conserved during evolution. Indeed, the results of a study on the tPRLR found that both forms of tPRL were able to activate the transcription of a Stat5-responsive reporter gene (Sohm *et al.*, 1998b). However, the concentration of $tPRL_{177}$ required to induce activation was 33 times greater than that needed by $tPRL_{188}$, a result that is consistent with the results of tPRL-binding studies in *O. niloticus* (Sohm *et al.*, 1998b). Using a mutant tPRLR that lacked the majority of the intracellular domain, this study also showed that box 1 is not sufficient for activation of the Stat5 pathway (Sohm *et al.*, 1998b). Thus, it appears that the C-terminal region of the tPRLR intracellular domain is required for Stat5 activation, as is the case for mammals (see Bole-Feysot *et al.*, 1998; Freeman *et al.*, 2000). Whether the PRLRs of other fish activate the same signal transduction pathways as *O. niloticus* and the ability of fish PRLRs to activate other signal pathways such as Stat1 or MAPK should be investigated.

The tissue distribution of fish PRLRs has also been examined. Northern blotting has revealed differences between fish with respect to the number and size of PRLR transcripts: *O. niloticus*, 3.2 kb (Sandra *et al.*, 1995), *O. mykiss*, 3.4 kb (Le Rouzic *et al.*, 2000), *P. olivaceus*, 3.5 kb (Higashimoto *et al.*, 2001), *C. auratus*, 4.6 kb (major transcript) and 3.5 kb (Tse *et al.*, 2000), and *S. aurata*, 3.2, 2.8, 1.9, 1.3, and 1.1 kb (Santos *et al.*, 2001). Generally, the highest PRLR expression levels were observed in the primary osmoregulatory organs (kidney, gill, and intestine) (Sandra *et al.*, 1995, 2000; Le Rouzic *et al.*, 2000; Prunet *et al.*, 2000; Tse *et al.*, 2000; Higashimoto *et al.*, 2001; Santos *et al.*, 2001). However, PRLR transcripts were also detected in other tissues such as the brain, gonad, liver, muscle, skin, spleen, head kidney, lymphocytes, and bone of some fish (Sandra *et al.*, 1995, 2000; Tse *et al.*, 2000; Higashimoto *et al.*, 2001; Santos *et al.*, 2001). The wide distribution of fish PRLRs in tissues other than those involved in osmoregulation argues for a phylogenetic conservation of the physiological effects of PRL throughout the vertebrates (Sandra *et al.*, 2000). Further investigations of FW-reared *O. niloticus* by *in situ* hybridization (Sandra *et al.*, 2000) and *S. aurata* by immunohistochemistry (Santos *et al.*, 2001), localized the PRLR to the chloride cells of the gill and the mucosal layer of the intestine, consistent with the roles of these cells in osmoregulation (discussed below in Section 3).

Changes in PRLR expression have been detected during *O. mossambicus* development (Shiraishi *et al.*, 1999) and in response to changes in environmental salinity (*O. niloticus:* Sandra *et al.*, 1995, 2000; *O. mossambicus*: Shiraishi *et al.*, 1999). PRLR mRNA was present in fertilized *O. mossambicus* eggs and levels increased steadily as the embryo grew regardless of the environmental salinity, however, receptor levels were higher in those larvae maintained in FW than in those transferred to SW 3 days before hatching (Shiraishi *et al.*, 1999). An increase in environmental salinity also resulted in a decrease in the expression of the PRLR in the gills of adult *O. niloticus* (Sandra *et al.*, 1995, 2000) and *O. mossambicus* (Shiraishi *et al.*, 1999). Thus, it appears that PRLR mRNA expression is up-regulated in both species of tilapia by the higher circulating PRL levels present in FW (Ayson *et al.*, 1993; Auperin *et al.*, 1994a).

However, the number of PRLRs and specific binding of tPRL increased following transfer of *O. niloticus* to a hyperosmotic environment (Section 2B and Auperin *et al.*, 1995), suggesting a down-regulation of the PRLR protein in the presence of elevated PRL levels in FW, perhaps due to an increase in internalization and degradation of the PRLR under these conditions, as has been reported for mammals (Genty *et al.*, 1994). In agreement with the results of Auperin *et al.* (1995), an immunofluorescence study failed to detect PRLRs in the mitochondria-rich cells of the gills of FW-adapted *O. mossambicus*, but PRLRs were abundant in this cell type in SW-adapted fish (Weng *et al.*, 1997). The increase in the number of PRLRs in *O. niloticus* transferred to BW may provide a "ready-reserve" of receptors to allow for rapid responses to decreases in environmental salinity that these fish cope with on a daily basis (Auperin *et al.*, 1995). Conversely, fish that are not euryhaline do not need to respond rapidly to changes in salinity, and are thought to have low concentrations of high-affinity PRLRs (Prunet and Au-

perin, 1994). The regulation of PRLR by PRL should be examined in other species of fish to determine whether this feature is specific to euryhaline species.

3. OSMOREGULATORY TARGETS OF PRL ACTION

Fish that reside in a FW environment face two primary challenges: preventing the loss of ions to the external hypoosmotic environment and preventing the influx of water. PRL plays a central role in the control of these activities during the adaptation of fish to FW, as evidenced by its ability to increase plasma ion concentrations (primarily Na^+ and Cl^-) and decrease the permeability of osmoregulatory organs (gill, kidney, intestine, urinary bladder, skin) to water (see Section 1A). Furthermore, it has been demonstrated that PRL binds to PRLRs that are present in these osmoregulatory organs, indicating that these are the sites of PRL action (see Sections 2B and C). As is the case for other aspects of fish PRL physiology, there are marked species differences in the actions of PRL on target organs. Although this variability makes generalizations difficult, an overview of the osmoregulatory effects of PRL on its target organs is presented in Table 3. Many of these effects apply mainly to euryhaline species.

TABLE 3
Overview of the Effects of PRL on Osmoregulatory Organs

Target	Effect of PRL
Gill	↓ Na^+ and Cl^- efflux
	↓ Na^+, K^+-ATPase activity
	↓ α chloride cell size and number
	↑ β chloride cell number
	↓ H_2O influx/permeability
	↑ Mucus secretion
Kidney	↑ Glomerular size and filtration rate
	↑ Na^+ reabsorption
	↑ Na^+, K^+-ATPase activity
	↑ Urine volume
Intestine	↓ H_2O absorption
	↓ Na^+ and Cl^- absorption
	↓ Na^+, K^+-ATPase activity
	↑ Mucus secretion
Urinary bladder	↓ H_2O absorption
	↑ Na^+, K^+-ATPase activity
	↑ Na^+ absorption
Skin	↓ H_2O permeability
	↓ Ion transport
	↑ Mucus secretion

Note. Some of these actions are not observed in all fish and many apply primarily to euryhaline species.

A. Gill

The gill is one of the major sites for control of ion and water exchange owing to its large surface area and direct contact with the external environment. Although the gill plays an important role in Ca^{2+} transport (reviewed by Flik *et al.*, 1995), this discussion will be limited to the effects of PRL on the transport of Na^+, Cl^-, and water across the gill. A recent model (Fig. 4) for the maintenance of ion balance in FW fish has been proposed by Lin and Randall (1995): (1) Na^+ uptake from the water occurs in response to the activities of a H^+-ATPase located in the apical membranes of gill epithelia. The extrusion of H^+ from the cells is ATP dependent and results in the establishment of a negative potential inside the cell that drives the passive transport of Na^+ into the cell via Na^+ channels. (2) the active transport of Na^+ out of cells and into the blood occurs via Na^+, $K^+(NH_4^+)$-ATPases located on the basolateral membranes. The transport of NH_4^+ into the cells provides an additional source of H^+ and fuels the aforementioned active transport of Na^+ into the cell. (3) CO_2 that enters the cell is hydrated to HCO_3^- which provides another source of H^+ and can also be extruded into the external environment in exchange for Cl by an apical Cl^--HCO_3^- exchanger.

(i) Effects of PRL on ion balance. There is no clear consensus with respect to the effects of PRL on gill Na^+, K^+-ATPase activity. Studies have shown that PRL either decreases (Pickford *et al.*, 1970; Gallis *et al.*, 1979; Madsen and Bern, 1992; Madsen *et al.*, 1997; Sakamoto *et al.*, 1997; Shepherd *et al.*, 1997; Kelly *et al.*, 1999), increases (Boeuf *et al.*, 1994; Leena and Oommen, 2000), or has no effect (Young *et al.*, 1988; Herndon *et al.*, 1991; Madsen *et al.*, 1995; Seidelin and Madsen, 1997, 1999; Eckert *et al.*, 2001) on Na^+, K^+-ATPase activity in the gill. These discrepancies may be

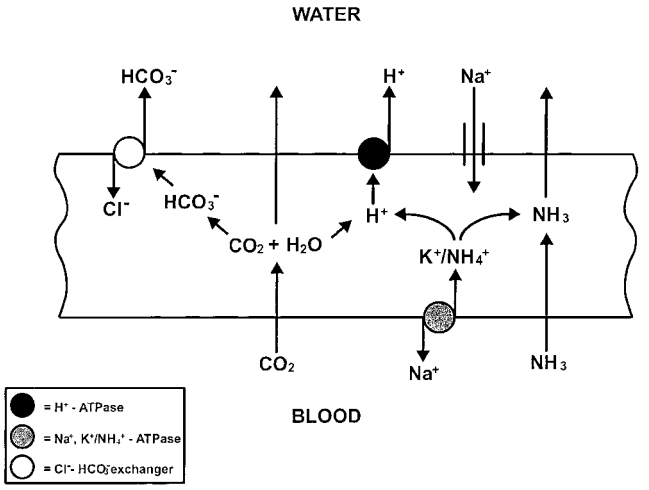

FIG. 4. Model of ion transfer across the gill epithelium of freshwater fish. The primary driving force for this model is the active extrusion of H^+ by a H^+-ATPase located on the apical side of the cell. This activity creates a negative potential inside the cell resulting in an influx of Na^+ via apical membrane channels. The hydration of CO_2 and deprotonation of NH_4^+ (that entered the cell via the Na^+, K^+/NH_4^+-ATPase) are the source of protons to fuel the apical proton pump. The hydration of CO_2 also produces HCO_3^- that is exchanged for Cl^- at the apical surface (adapted from Lin and Randall, 1995).

related to the use of heterologous hormones, species variability, or developmental differences (McCormick, 1995). Recent studies have examined the effects of ovine PRL on the expression of the catalytic α subunit and the β subunit of Na^+, K^+-ATPase in two different species of fish (Seidelin and Madsen, 1999; Deane *et al.*, 1999). PRL caused a significant reduction in gill α subunit, but not in β subunit mRNA levels in silver sea bream (*Sparus sarba*) (Deane *et al.*, 1999); conversely, α subunit mRNA levels were not affected by PRL in the brown trout (*Salmo trutta*) (Seidelin and Madsen, 1999). To fully elucidate the effects of PRL on gill Na^+, K^+-ATPase, studies should be carried out on different species of fish using homologous hormones.

The gill epithelium contains four distinct cell types: chloride cells (CC), pavement (respiratory) cells, mucous cells, and neuroepithelial cells (Perry, 1997). The CC of marine fish are essential for eliminating excess Na^+ and Cl^- due to the fact that their basolateral membranes contain the enzyme Na^+, K^+-ATPase (reviewed by McCormick, 1995). Given that FW fish do not have the need to eliminate excess Na^+ and Cl^-, the role of CC (also called mitochondria-rich cells) in these fish is not well established (see Perry, 1997 for review). There are two subpopulations of CC (α and β cells) in FW fish that differ with respect to their locations and morphologies (reviewed by Perry, 1997). The α cells are located at the base of gill lamellae, are strongly Na^+, K^+-ATPase immunoreactive (Shikano and Fujio, 1998), and are thought to be the precursors of the characteristic CC seen in SW-adapted fish (Perry, 1997). In contrast, the β cells are generally found in the interlamellar regions, are weakly immunoreactive with Na^+, K^+-ATPase antibodies (Shikano and Fujio, 1998), and are only found in FW teleosts (Perry, 1997).

PRL appears to have a marked effect on the morphology, distribution, and number of CC (see McCormick, 1995) and PRLRs have been localized to the CC in the gills of *O. mossambicus* (Weng *et al.*, 1997) and *S. aurata* (Santos *et al.*, 2001). PRL has been reported to either reduce (Foskett *et al.*, 1982; Madsen *et al.*, 1997) or have no effect (Herndon *et al.*, 1991; Seidelin and Madsen, 1999) on CC numbers and to decrease CC size (Herndon *et al.*, 1991; Madsen *et al.*, 1997). However, the effects of PRL on CC size and number may vary with environmental salinity, as Kelly *et al.* (1999) showed that the number and size of CC in *S. sarba* were reduced by PRL in a hypoosmotic environment, but not in SW.

Pisam *et al.* (1993) examined the effects of PRL on the α and β subpopulations of CC in SW-adapted tilapia (*O. niloticus*). This study found that PRL injections caused a decrease in α cell size and a dedifferentiation to the FW α cell morphology. These changes are thought to contribute to the Na^+-retaining effects of PRL by obstructing the paracellular pathway thought to be involved in the extrusion of Na^+ in SW fish and causing a degeneration of the basolateral tubular system where Na^+, K^+-ATPase is located (Pisam *et al.*, 1993). There was also a concomitant appearance of FW β cells following PRL administration to SW-adapted *O. niloticus*. The biological function of FW β cells has not been determined, but it is thought that they are involved in either Na^+ and Cl^- uptake (Seidelin and Madsen, 1999) or Ca^{2+} regulation during FW-adaptation (Pisam *et al.*, 1993). A recent study has determined the cellular locations of active transport enzymes and ion exchangers in FW tilapia (*O. mossambicus*) and rainbow trout (*O. mykiss*) gills (Wilson *et al.*, 2000). It would be useful to examine the effects of PRL on the activity of these ion transporters

to obtain a clearer picture of how PRL regulates ion exchange in the gill. The actions of PRL on active ion uptake systems would be of particular significance.

(ii) Effects of PRL on water permeability. The main threat to fish that occupy a FW environment is the loss of ions by diffusion into the external hypoosmotic medium, rather than the elimination of excess water (Clarke and Bern, 1980). Even though the regulation of water balance may be secondary in importance with regards to FW survival, the effects of PRL on water permeability should not be dismissed as inconsequential. Studies have demonstrated that PRL decreases the osmotic permeability of the gills (see Clarke and Bern, 1980; Brown and Brown, 1987 for reviews). In addition, PRL acts on the mucous cells of the gill to increase mucus secretion (see Horseman, 1987) that may contribute to the regulation of ion and water balance in FW by impeding the passage of molecules in and out of gill cells (Bentley, 1998).

B. Kidney

Earlier studies suggested that PRL acts on the kidney to cause increases in Na^+ reabsorption and water excretion, however, the mechanisms by which PRL exerts these effects are poorly understood (see Clarke and Bern, 1980). A specific action of PRL on the glomerulus was evidenced by an increase in glomerular size following PRL treatment and an increase in urine output was also observed (reviewed by Braun and Dantzler, 1987). The demonstration of specific PRL binding and the presence of PRLRs in the kidney clearly indicate that PRL is acting directly on this tissue (see Sections 2B and C). Pickford *et al.* (1970) found that PRL injections increased renal Na^+, K^+-ATPase activity in hypophysectomized *F. heteroclitus*, and decreases in activity have been observed during SW-acclimation in *F. heteroclitus* (Epstein *et al.*, 1969) and *Chelon labrosus* (Gallis *et al.*, 1979). These results are consistent with the Na^+-retaining functions of PRL.

However, recent studies have shown that PRL activation of renal Na^+, K^+-ATPase may not be a universal response among fish. Na^+, K^+-ATPase activity was unaltered in the kidney in response to either PRL treatment or SW acclimation in several salmonids (*O. mykiss*: Jürss *et al.*, 1985; *Salmo salar*: McCormick *et al.*, 1989; *S. trutta*: Seidelin and Madsen, 1997; Madsen *et al.*, 1995) and in the silver sea bream (*S. sarba*: Kelly *et al.*, 1999). In the saffron cod (*Eleginus gracilis*), PRL decreased plasma osmolality, but Na^+ and Cl^- levels were not altered (Ogawa *et al.*, 1997). This decrease in plasma osmolality is attributed to the ability of PRL to increase the glomerular filtration rate, resulting in an increased clearance of an antifreeze glycoprotein from the blood (Ogawa *et al.*, 1997).

In addition to increasing urine output by increasing the glomerular filtration rate, PRL also decreases urine osmolality, presumably by decreasing the permeability of kidney tubules to water (see Clarke and Bern, 1980). These effects of PRL on urine output and concentration are consistent with the observation that FW fish excrete copious amounts of dilute urine to counteract the influx of water through the gills. Perhaps the actions of PRL on the kidney are primarily related to water balance and not ion balance in some fish, such as salmonids. This would explain the inability of PRL to influence renal Na^+, K^+-ATPase activity in these fish. Insights into the effects of PRL on the kidney could be provided by immunohistochemical or *in situ* hybridization studies aimed at determining the specific renal cell types that interact with PRL. Further examination of the renal effects of PRL in other fish species using homologous PRLs is needed.

C. Intestine

Previous studies have shown that PRL decreases the intestinal absorption of Na^+, Cl^-, and water and that these effects may be due to decreases in both the permeability of the intestine to water and salts and intestinal Na^+, K^+-ATPase activity (reviewed by Collie and Hirano, 1987). However, as is the case for other target tissues, there is species variability with respect to the actions of PRL on the intestine, making generalizations difficult. Injections of ovine PRL decreased fluid and salt absorption in SW-adapted Japanese eel (*A. japonica*: Utida *et al.*, 1972) and trout (*O. mykiss*: Morley *et al.*, 1981), but increased these parameters in the anterior intestine of SW-adapted *O. mossambicus* (Mainoya, 1982). In agreement with the tilapia results, Madsen *et al.* (1997) demonstrated that injections of either ovine PRL or $tPRL_{188}$ increased the water transport capacity of the intestine of SW-acclimated striped bass (*Morone saxatilis*). It has been proposed for *M. saxatilis* that PRL acts to decrease salt excretion from

the gills and increase water and salt uptake from the intestine and that this increased intestinal water absorption may be a compensatory response to dehydration caused by decreased drinking in FW (Madsen *et al.*, 1997).

Recent studies have shown that ovine PRL increases intestinal Na^+, K^+-ATPase activity in *S. sarba* (Kelly *et al.*, 1999), but not in *S. trutta* (Seidelin and Madsen, 1999). One of the reasons for these conflicting results may be due to the amounts of ovine PRL used in each study. *Sparus sarba* were injected with 6 μg PRL/g body weight for seven consecutive days, while *S. trutta* were injected with 2 μg PRL/g body weight on alternate days for 4 days. Moreover, although treatment of *S. trutta* presmolts with ovine PRL resulted in the appropriate increases in Na^+ and Cl^- concentrations and plasma osmolality following SW transfer, perhaps the intestine is not competent to respond to PRL by altering Na^+, K^+-ATPase activity at this life history stage. In fact, Seidelin and Madsen (1999) state that the developmental stage of *S. trutta* used in their study may have been a factor in the failure to observe a change in gill Na^+, K^+-ATPase activity after GH treatment.

D. Urinary Bladder and Skin

The effects of PRL on the urinary bladder and skin have not received much attention in recent literature, perhaps due to the fact that the contributions of these organs to fish osmoregulation are minor compared to those of the gill, kidney, and intestine. Not all fish have a permeable urinary bladder and the surface area of the skin is relatively small when compared to that of the gill (Clarke and Bern, 1980). Nonetheless, the contributions of these tissues are significant and previous studies on the urinary bladders of fish found that PRL decreased the osmotic permeability, either increased or had no effect on Na^+ transport, increased Na^+, K^+-ATPase levels, and elicited an expansion of the epithelial intercellular spaces (reviewed in Clarke and Bern, 1980; Bentley, 1987). The species specificity of the effects of PRL on the urinary bladder was clearly demonstrated by Hirano *et al.* (1973), who found that the ability of PRL to alter urinary bladder permeability was limited to euryhaline fishes of marine origin and that the urinary bladders of stenohaline or FW euryhaline species were impermeable. The elaboration of mucus from mucous cells in the skin is thought be involved in osmoregulation by reducing the permeability of the skin to ions and water. Injections of PRL increased the number of mucous cells and mucus secretion in the skin of some fish, but had no effect in others (see Clarke and Bern, 1980 for review). Other epithelia, such as those of the gills and intestine, also possess mucous cells whose number and secretory activity are modified by PRL in some species (Clarke and Bern, 1980).

SUMMARY

PRL is a protein hormone secreted by the pituitary gland that belongs to the GH/SL gene family. In fish, PRL plays an important role in FW osmoregulation by promoting the conservation of ions (primarily Na^+ and Cl^-) and decreasing water uptake. Fish PRLs are structurally similar to those of higher vertebrates, but differences exist between teleosts and nonteleosts with respect to their tertiary structures. Given these structural differences, the importance of using homologous vs heterologous PRLs to conduct functional studies has been established. Homologous PRLs have been isolated from several species of fish and there are two distinct PRLs in tilapia that differ significantly from one another with respect to their lengths, aa compositions, biological activities, receptor affinities, and regulation. MS cells have been identified in the sea bream pituitary and appear to have the ability to differentiate into either PRL-secreting or GH-secreting cells depending on their location and the physiological state of the organism. PRL has also been detected at extrapituitary sites in some fish and may be acting in an autocrine or paracrine manner in these tissues. The isolation and characterization of fish PRLRs has revealed that several important functional domains, receptor activation mechanisms, and signal transduction pathways have been conserved between fish and mammals. Generally, the major osmoregulatory organs such as the gill, kidney, and intestine have the highest PRLR levels, although there are significant differences between species with respect to the actions of PRL on ion and water transport machinery.

The importance of PRL is evident from the wide spectrum of functions it performs in vertebrates and

its degree of conservation throughout evolution. The isolation of homologous PRLs, PRLRs, and ion transport proteins from a variety of fish species will allow us to achieve a better understanding of the evolution of the PRL/GH/SL gene family, the role of PRL in osmoregulation, and its mechanisms of action on target organs. Although not discussed in this review, the complexity of PRL's actions is mirrored by the complexity of factors involved in regulating PRL synthesis and secretion and the interactions of PRL with other endocrine systems. The next decade should prove to be an exciting and fruitful period for PRL research.

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