Characteristic analysis of prolactin and its receptor genes from *Rana chensinensis* and expression pattern during metamorphosis

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Abstract: In order to explore the role of prolactin (PRL) and the prolactin receptor (PRLR) during metamorphosis of amphibians, both cDNAs from the Chinese brown frog *Rana chensinensis* were identified. A PRL cDNA consisting of a 645-bp open reading frame and a PRLR cDNA consisting of a 1854-bp open reading frame were obtained. The amino acid sequence analysis indicated that there were 2 pairs of conserved Cys residues in both mature rcPRL and rcPRLR, and that their positions were conserved as well. Comparison of our results with those for other vertebrates implies that receptor recognition in anurans may not be pH-dependent. The relations of identity between rcPRL and other vertebrate PRLs resembled those of the identity between rcPRLR and other vertebrate PRLRs, suggesting that PRL may have coevolved with PRLR in *R. chensinensis*. In addition, rcPRL and rcPRLR mRNA was detected via RT-PCR in various tissues of *R. chensinensis* tadpoles. By using qRT-PCR, rcPRLR mRNA in the liver, skin, tail, and kidney tissue of different stages (stages 33–43) during metamorphosis was analyzed. The results showed that the expressional tendencies of rcPRLR increased throughout stages 33–43 in the four tissues, which was probably related to tissue remodeling.

Key words: Prolactin, prolactin receptor, expression pattern, Amphibia, metamorphosis

1. Introduction

Prolactin (PRL), a protein hormone synthesized and secreted by the anterior pituitary, widely acts on various tissues and organs of vertebrates. PRL belongs to the same superfamily as growth hormone (GH) and shares similar structures and functional features (Niall et al., 1971; Kitamura et al., 1994). PRL, like GH, is associated with almost all aspects of functional regulation, including reproduction, osmotic pressure, growth and development, cell proliferation and differentiation, and immune, metabolic, and behavioral traits (Bole-Feysot et al., 1998; Matsukawa et al., 2004). Recent studies show that some extrapituitary tissues can also secrete PRL. For example, breast, prostate, brain, skin, and immune cells in mammals have been shown to secrete PRL, but the mechanism remains to be fully characterized (Ormandy et al., 1997; Kulkarni et al., 2010; Bouilly et al., 2012). PRL signaling is modulated by binding to a specific membrane receptor, the prolactin receptor (PRLR), a protein with a single transmembrane domain. Therefore, PRLR is composed of 3 parts, an intracellular domain (ICD), a transmembrane domain (TMD), and an extracellular domain (ECD). In mammals, it is well known that there are 3 PRLR isoforms, namely long, intermediate, and short forms. Although the 3 isoforms are identical in ECD, they differ in the length of the ICD, expression patterns, and mechanisms of signal transduction (Bouilly et al., 2012). In vertebrates, PRLR gene expression has been identified in almost all organs (Bole-Feysot et al., 1998). However, most studies have focused on mammals; limited information is available for amphibians.

The life cycle of amphibians, especially anurans, proceeds through a process of metamorphosis, the change of the body from a larva into a juvenile. There are still some controversies about the role PRL plays in amphibian metamorphosis. Some researchers think that PRL plays a similar role in amphibians as the juvenile hormone (JH) plays in insects, counteracting the effects of thyroid hormone (TH) and suppressing the metamorphic process (Etkin and Gona, 1967). Others think that PRL differs from JH (Yamamoto and Kikuyama, 1982; Niinuma et al., 1991). Recent research has shown that overexpression of PRL stimulates growth of fibroblasts in the tail but does not affect the time it takes *Xenopus laevis* tadpoles to reach metamorphic climax (Huang and Brown, 2000). However, the latter work did not preclude PRL from having an effect on the development of other organs during metamorphosis.
Larval amphibians undergo extensive remodeling during metamorphosis, including changes to the skin, liver, brain, respiratory surfaces, and much of the skeleton, thereby adapting the organism to changes in its living environment.

In metamorphosis, the osmoregulatory role of the skin and kidney shifts, corresponding to changes in the living environment. Thus, PRL stimulates epithelial sodium channel proliferation in adult frog skin (Greenlee et al., 2014); many factors in the liver are activated during the turnover of hepatocytes, including PKC and MAP kinase. These processes are all affected by PRL (Takada, 1989; Hasunuma et al., 2004).

There have been few attempts to research the roles of PRL and PRLR in various organs during the metamorphosis of amphibians. Studies have shown that PRL plays roles in the regulation of hydromineral balance and maintenance of lung function (Takada, 1989; Oguchi et al., 1994). Epithelial sodium channel activity is regulated by PRL in A6 cells, and PRL has the ability to both stimulate and inhibit vascular function in vertebrates, including amphibians (Greenlee et al., 2014). In addition, PRL has a synergistic effect with growth hormone in amphibian metamorphosis, regulating the morphological and functional integrity of skin and influencing osmoregulation processes from the gills to the kidneys in amphibians; it plays an important role in the adaptation of amphibians during winter by thereby adapting the organism to changes in its living environment. Thus, PRL stimulates epithelial sodium channel proliferation in adult frog skin (Greenlee et al., 2014); many factors in the liver are activated during the turnover of hepatocytes, including PKC and MAP kinase. These processes are all affected by PRL (Takada, 1989; Hasunuma et al., 2004).

2.2. RNA extraction and cDNA synthesis
Total RNA was extracted from the brain and liver using RNAiso Plus according to the manufacturer's protocol (TaKaRa, Japan). Concentration of total RNA from the tissue was quantified by a Nucleic Acid Analyzer (NANO-200). cDNA was then synthesized with PrimeScript RT Master Mix (TaKaRa) using 1 µg of RNA in a 20-µL mixture. In order to clone the PRL in brain tissue and PRLR in liver tissue from *R. chensinensis*, specific primers (Table 1) were designed referring to the relevant cDNA sequences of *Rana catesbeiana* (CAA34199.1, BAD14941.1) and *Xenopus laevis* (AAH75216.1, AA170439.1). PCR was carried out in 25-µL mixtures containing 3 µL of cDNA, 0.5 µL each of F and R primer (10 µM), 2.5 µL of 10X PCR buffer, 3 µL of dNTP mix, 15 µL of ddH₂O, and 0.5 µL of rTap (TaKaRa). For all PCR reactions above, amplifications were performed in a thermocycler (Thermo, USA) with an initial denaturation at 94 °C for 4 min, followed by 40 cycles of 94 °C for 30 s, 48–59 °C for 45 s, and 72 °C for 1 min; the reaction was completed by a further 10 min at 72 °C. The amplified products were analyzed on a 1.0% agarose gel and purified using the TaKaRa MiniBEST Agarose Gel DNA Extraction Kit Ver. 4.0, followed by cloning into the pGEM-T Easy Vector (Promega, USA). At least 3 different individual positive clones containing expected inserts were sequenced to ensure the accuracy of the sequence information. SeqMan software was used for sequence assembling.

2.3. Sequence and phylogenetic analysis
Full-length amino acid (aa) sequences of rcPRL and rcPRLR were deduced from the obtained nucleotide sequences. N-linked glycosylation and tyrosine phosphorylation sites were predicted using the NetNGlyc 1.0 (http://www.cbs.dtu.dk/services/NetNGlyc/) and NetPhos 2.0 servers (Blom et al., 1999) in the CBS prediction servers, respectively. Signal peptiwas predicted using the SignalP 4.1 server (Petersen et al., 2011). Transmembrane domain was predicted using the TMpred server (Hofmann, 1993). Secondary structure was predicted using PSIPRED v3.3 (http://bioinf.cs.ucl.ac.uk/psipred/). Molecular weight and pI were predicted by Compute pI/Mw (http://web.expasy.org/compute_pi/). Sequence alignments were carried out using Clustal X (Thompson et al., 1997) and DNAman with default settings. Phylogenetic trees were constructed by the neighbor-joining method using MEGA v5.1. Reliability of the phylogenetic trees was assessed by using a bootstrap with 1000 replicates.

2.4. RT-PCR
cDNA was synthesized in a 20-µL reaction mixture using 1 µg of total RNA of the brain, liver, skin, tail, and kidneys. Gene-specific primers (Table 1), PRL5F and PRL5R (PRL) or PRLR4F and PRLR4R (PRLR), were used for PCR with the synthesized cDNA. rpl8 (GenBank accession no.
KP274078) was used as a reference gene to standardize the PRL and PRLR mRNA level, with primers rpl8F and rpl8R (Table 1) (Santillo et al., 2012; Lou et al., 2014). PCR reactions were performed and the amplified products were analyzed as described in Section 2.2.

2.5. Quantitative real-time PCR
For rcPRL mRNA expression analysis in various tissues at different stages, quantitative real-time PCR (qRT-PCR) was conducted using SYBR Premix Ex Taq II (TaKaRa) with the CFX96 Reaction System (Bio-Rad, USA). PRLR5F and PRLR5R were designed as specific primers for rcPRLR. rpl8 was used as a reference gene to normalize mRNA expression of rcPRLR (Table 1). PCR conditions were as follows: 95 °C for 30 s and then 40 cycles of 95 °C for 5 s and 59 °C for 30 s, followed by a melting curve analysis. rcPRLR expression in a specific stage or tissue was determined by 2−ΔΔCt method and normalized with respect to rpl8. The values obtained were compared using one-way analysis of variance (ANOVA) with SPSS 20.0. All data are shown as mean ± SD. P < 0.05 was regarded as statistically significant.

3. Results
3.1. Identification of rcPRL and rcPRLR
Using specifically designed primers, we obtained a cDNA of 645 bp from R. chensinensis, named rcPRL (GenBank accession no. KP260562). The rcPRL sequence was composed of a 645-bp open reading frame (ORF) encoding 214 aa residues with a 27-aa signal peptide. Thus, the mature rcPRL is a polypeptide of 187 aa (Figure 1). The molecular weight of rcPRL was around 24313.6 Da with a pI of 6.07.

In addition, a cDNA of 2120 bp from R. chensinensis named rcPRLR (GenBank accession no. KJ490947) was obtained. The rcPRLR sequence was composed of a 29-bp 5'-UTR, 237-bp 3'-UTR, and 1854-bp ORF encoding 617 aa residues. The deduced protein contained a signal peptide of 26 aa, followed by a 212-aa ECD, a 22-aa TMD, and a 357-aa ICD. Thus, the mature rcPRLR is a polypeptide of 595 aa (Figure 2). The molecular weight of rcPRLR was about 69298.6 Da with a pI of 5.59.

3.2. Characteristic analysis of rcPRL and rcPRLR
Three pairs of conserved cysteine residues were found in rcPRL, including 2 pairs of cysteine residues at positions 38, 48, 77, and 88; a WS motif; 3 potential N-linked glycosylation sites at positions 61, 114, and 134 in the ECD; and boxes 1

Table 1. Oligonucleotide primers used for PCR.

<table>
<thead>
<tr>
<th>Target genes</th>
<th>Sequence of forward primer (5’ to 3’)</th>
<th>Sequence of reverse primer (5’ to 3’)</th>
<th>Product size (bp)</th>
</tr>
</thead>
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<td>GCACGGTCAAAGAGAGCAG</td>
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<tr>
<td>PRL2</td>
<td>AAATGCTCAATATCCCCAG</td>
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</tr>
<tr>
<td>PRL3</td>
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<td>CGAAGGAGTGGAGTAGA</td>
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</tr>
<tr>
<td>PRL4</td>
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</tr>
<tr>
<td>PRL5</td>
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<td>GAGGGACTGGAGTAGATT</td>
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<tr>
<td>PRL6</td>
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<td>PRL7</td>
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<tr>
<td>PRLR1</td>
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<td>115</td>
</tr>
</tbody>
</table>
Figure 1. Nucleotide and deduced aa sequences of rcPRL. The predicted site of signal peptide cleavage is marked by a triangle. The conserved cysteine residues are circled and the potential phosphorylation site is boxed, while the four α-helical domains are boxed in shaded rectangles. The stop codon is marked by an asterisk.

Figure 2. Nucleotide and deduced aa sequences of rcPRLR. The predicted site of signal peptide cleavage is marked by a triangle. The conserved cysteine residues are circled. Three potential N-glycosylation sites are underlined by dotted lines. The WS motif is boxed and TMD is underlined with a solid line. Within the ICD, boxes 1 and 2 are underlined with a double line and a wavy line, respectively. The stop codon is marked by an asterisk.
and 2 in the ICD (Figure 2). All of these features correspond to the long-form PRLR of mammals. Moreover, alignment of various PRLRs showed that a conserved Tyr609 and a DSGRGS motif were also found in rcPRLR. However, there was a His found for Urodela, fishes, mammals, birds, and reptiles, but not for rcPRLR and other anurans (Figure 4).

3.3. Phylogenetic analysis of rcPRL and rcPRLR
The multialignments of PRL and PRLR aa sequences from *R. chensinensis* and other species were performed by DNAman software. The results showed that the overall identity of rcPRL with other amphibians was 80%–97%; birds and reptiles, 73%–75%; mammals, 48%–67%; and fishes, 38%–39%. The overall identity of rcPRLR with Amphibia was 53%–94%; birds and reptiles, 45%–47%; mammals, 43%–44%; and fishes, 35%–39%. Th ECD was more conservative than the ICD: the values ranged from 46% to 92% in the ECD and 30% to 95% in the ICD (Table 2).

Phylogenetic analysis by neighbor-joining tree indicated that rcPRL and rcPRLR were respectively grouped in the clades of amphibious PRLs and PRLRs. *R. chensinensis* bore the closest homology to *R. catesbeiana*, being close also to other species of amphibians such as *X. laevis* and *B. japonicus*, but relatively distant from reptiles, birds, and mammals, such as *C. mydas*, *M. gallopavo*, and *M. musculus* (Figure 5). In both trees, rcPRL and rcPRLR were largely consistent in the evolutionary position.

3.4. Detection of rcPRL and rcPRLR in tadpoles
The rcPRL and rcPRLR mRNA signals were detected in tadpoles by RT-PCR. The rcPRL mRNA level in brain tissue was low at stage 40, but it subsequently increased to peak at stage 46 (Figure 6A). Weak but significant signals were seen in the kidneys, while strong signals of rcPRLR were detected in the liver, skin, and tail at stage 43 (Figure 6B).

3.5. Expression analysis of PRLR mRNA in different stages by qRT-PCR
Hepatic rcPRLR mRNA levels were low at stage 33 and slightly decreased at stage 37, but rose again to peak at stage 43 (Figure 7A). In the skin, rcPRLR expression was weak at stage 33, thereafter gradually strengthening (Figure 7B). In the tail, rcPRLR expression was hardly detectable at stage 33, but it increased dramatically at stage 42 to peak at stage 43 (Figure 7C). In the kidneys, rcPRLR mRNA level changed modestly throughout stages 33–43 (Figure 7D).

4. Discussion
4.1. Characteristic analysis of rcPRL and rcPRLR
In this study, we obtained PRL cDNA from *R. chensinensis*. Three pairs of conserved cysteine residues were found, and their relative positions were the same in PRL throughout the species investigated. Mature PRLs of nonteleost fishes and tetrapods contain 3 disulfide bonds (Rand-Weaver et al., 1993). In contrast, mature rcPRL has only 2 disulfide bonds, due to the removal of the first pair of cysteines within the signal sequence. This condition is seen in several teleosts, including *Sparus aurata* and *Epinephelus coioides* (Chang et al., 1992; Doliana et al., 1994; Zhang et al., 2004).

There is an N-linked glycosylation site reported in human and many nonrodent mammal PRLs (Lewis et al., 1985; Kato et al., 2005). The glycosylation of hPRL leads to reduced hPRLR binding capacity (Pellegrini et al., 1988). In contrast, no putative N-linked glycosylation site was discerned for rcPRL, indicating that rcPRL is likely to have a higher binding capacity without hindering the binding of rcPRL and its receptor. This is similar to some fishes such as *Epinephelus coioides* (Zhang et al., 2004).

Consistent with human and other vertebrate PRLs (Bole-Feyset et al., 1998; Freeman et al., 2000), prediction of the secondary structure demonstrates that rcPRL
also contains 4 α-helices. In addition, rcPRL possesses 2 conserved motifs, motif 1 and motif 2, which exist in most PRL/GH family members (Yao and Ding-Zong, 2007). The 2 motifs are composed of 34 aa and 18 aa, respectively, and they are more conserved compared to the others.

We also obtained an rcPRLR sequence corresponding to the long-form PRLR of mammals. Two pairs of conserved Cys residues are found in the ECD of rcPRLR. Similarly, the GH receptor, a member of the class I cytokine receptor superfamily, also has 2 pairs of highly conserved Cys residues (Bazan, 1989; Rozakis-Adcock and Kelly, 1991). Cys38–Cys48 and Cys77–Cys88 form 2 disulfides, improving the structural stability of PRLR and playing a role in the interaction between PRLR and PRL. Replacement of any one of the 4 Cys residues causes failure of ligand-binding. In addition, residue mutation leads to misfolding of the receptor (Rozakis-Adcock and Kelly, 1991). The relative positions of the 2 pairs of Cys residues are also conserved in all species. Interestingly, and similar to the receptor, there are also 2 pairs of conserved

Figure 4. Alignment of aa sequences of various PRLRs. Conserved cysteines are circled; 3 potential N-glycosylation sites (His 212; WS motif, Box 1; and DSGRGS motif) are marked by boxes; Trp165 and Tyr609 are marked by triangles. Dots (·) represent spaces inserted to maximize similarity, and small letters represent the conserved amino acids in that position.
Cys residues existing in mature rcPRL, and their relative positions are conserved as well, implying that they play a role in the structural stability and activity of both rcPRL and rcPRLR.

The WS motif exists in almost all known class I cytokine receptors with the exception of the GH receptor (Dagil et al., 2012). It is required for the correct folding and transportation of PRLR protein in vivo (Rozakis-Adcock and Kelly, 1992; Goffin et al., 2002). Compared with other residues, the third of the WS motifs has the lowest conservation. In R. chensinensis, the third residue is E, which is mimicked by R. catesbeiana and X. laevis; the last residue has the lowest conservation, as seen for T in sea bream and goldfish (Santos et al., 2001), G in the mouse (Tse, et al., 2000), and S in R. chensinensis and most other species.

Table 2. Comparison of identities among PRLs and PRLRs from various species.

<table>
<thead>
<tr>
<th>Species</th>
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<th>PRLR</th>
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<td></td>
<td>ECD</td>
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<tr>
<td>Amphibians</td>
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<tr>
<td>Rana chensinensis</td>
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<td>Rana catesbeiana</td>
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<td>Bufo japonicus</td>
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<td>Xenopus laevis</td>
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</tr>
<tr>
<td>Cynops pyrrhogaster</td>
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<td>54</td>
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<tr>
<td>Reptiles</td>
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<td></td>
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<td>Chelonia mydas</td>
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<td>Birds</td>
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<td>53</td>
</tr>
<tr>
<td>Homo sapiens</td>
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<td>50</td>
</tr>
<tr>
<td>Danio rerio</td>
<td>39</td>
<td>46</td>
</tr>
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</table>

The values are % aa identities of rcPRL and rcPRLR, respectively.

Figure 5. Construction of neighbor-joining tree based on aa sequences of PRL (A) and PRLR (B). The sequences used above are: Rana catesbeiana (CA34199.1, BAD14941.1), Xenopus laevis (AAH75216.1, AAI70439.1), Chelonia mydas (XP_007059983.1, EMP31801.1), Anser anser (ADG03649.1, ABW74516.1), Sus scrofa (NP_999091.1, ABA41035.1), Mus musculus (P06879.1, CAA51789.1), Homo sapiens (NP_001157030.1, NP_000940.1), Oncorhynchus mykiss (NP_001118205.1, NP_001118071.1), Danio rerio (AAH92358.1, AA163012.1), Bufo japonicus (BAF75354.1), and Cynops pyrrhogaster (BAB61107.1). Bootstrap percentage values are indicated for each node.
Figure 6. Detection of rcPRL and rcPRLR in tadpoles by RT-PCR. (A) and (B): Upper panel, RT-PCR using rcPRL and rcPRLR gene-specific primers, respectively; lower panel, control RT-PCR using rpl8 gene-specific primers.

Figure 7. Expression analysis of rcPRLR by qRT-PCR in liver (A), skin (B), tail (C), and kidneys (D) of different stages. The data for rcPRLR mRNA levels were normalized by those for rpl8 mRNA levels and expressed relative to the value for stage 43 tissue, respectively. The experiments were repeated 3 times using 3 independent biological samples. Each bar represents mean ± SD. Those with different letters were regarded as statistically significant at the 5% level (Duncan test).
Box 1 in the ICD of rcPRLR has a high identity with other PRLRs. Box 1 is the most conserved motif for PRLRs (Bouilly et al., 2012), and it plays an essential role in signal transduction (Lebrun et al., 1995a; Bole-Feyos et al., 1998; Demmer, 1999; Santos et al., 2001). On the other hand, Tyr609 at the C-terminal was highly conserved among all the species. Tyr609 promotes the activation of the β-casein promoter in response to PRL, indicating that Tyr609 is directly engaged in signal transduction (Lebrun et al., 1995a; Bole-Feysot et al., 1998; Demmer, 1999; Santos et al., 2001). The facts above suggest that the signal transduction mechanism of PRLR is highly conserved in evolution, although the overall identity of aa sequences among R. chensinensis and mammals is low. This agrees with previous research (Yamamoto et al., 1998; Tse et al., 2000; Santos et al., 2001).

Studies show that a conserved DSGRGS motif is closely linked to the ubiquitin-dependent degradation of long-form PRLR (Li et al., 2004). It is thought that the degradation of PRLR is prevented by deleting the ICD, resulting in the increase of PRLR expression (Rozakis-Adcock and Kelly, 1991). We infer that it results from the absence of the DSGRGS motif, which is found in most species. Thus, the degradation of rcPRLR is probably also ubiquitin-dependent.

In mature hPRLR, His-188 is important for pH-dependent receptor binding and activation (Kulkarni et al., 2010). However, no His is found in anurans at the same position, e.g., R. chensinensis, Rana catesbeiana, Bufo japonicas, or Xenopus laevis. It is noteworthy that a His is found in Cynops pyrrhogaster, a Urodela, which is common with other species such as Homo, but not with the closer 4 species above. This may imply that the receptor recognition in R. chensinensis and other anurans is not pH-dependent.

4.2. Expressional analysis of rcPRL and rcPRLR in tadpoles during metamorphosis

The metamorphosis of amphibians is a complex process in which most organs are remodeled to adapt to the transition from aquatic to terrestrial life. Our results show that rcPRL mRNA in the brain is weak at prometamorphosis (stage 40); thereafter, it increases until late metamorphosis, suggesting that it plays a role during metamorphosis that is closely related to the distribution of PRLR.

In mice, the turnover of hepatocytes and the activation of many factors, including PKC and MAP kinase, are affected by PRL (Bole-Feyos et al., 1998). In this study, rcPRLR expression in the liver was low and expressed a fluctuation before metamorphosis (stages 33–37), but it kept increasing thereafter until climax (stage 43), which is probably related to the reconstruction of hepatocytes and adaptation to a carnivorous diet. Considering that rcPRLR is highly expressed at the climax, the main effect of PRL on hepatocytes may occur at metamorphosis or later.

Researchers have confirmed that PRL is involved in osmoregulation in the skin and kidneys (Takada, 1989; Hasunuma et al., 2004). Clearly, effective osmoregulation is essential for aquatic tadpoles. During our study, rcPRLR expression changed from light to strong expression during metamorphosis, indicating that the skin is a principal target tissue for PRL in tadpoles. Before metamorphosis, the regulation of salt and water balance is mainly dependent on the gills and skin. However, the gills disappear during metamorphosis and the organizational structure of the skin needs to adapt to terrestrial living. Therefore, the high expression of rcPRLR in the skin during metamorphosis may be closely related to the skin's reconstruction. On the other hand, in kidney tissue, rcPRLR is expressed at a low level at premetamorphosis (stages 33–37), but it increases obviously as metamorphosis proceeds, indicating that rcPRLR is also connected to morphologic changes of the kidney.

The tail is an important target organ for PRL (Hasunuma et al., 2004); based on Gosner stages, it disappears during stages 43–46 (Gosner, 1960). rcPRLR expression remains low at premetamorphosis but increases sharply from the onset of metamorphosis to climax, at which time considerable tail degradation is seen. At this time, TH levels are low but continue to rise to a peak at climax, with the TH receptor being highly expressed at the same time (Denver, 1997; Lou et al., 2014). Since PRL has an anti-TH effect (Tata et al., 1991), it is possible that PRL regulates the rate of tail resorption. Tadpoles do not eat during later-stage metamorphosis since the mouth undergoes reconstruction. Thus, the regressing tail becomes the primary energy source (Hasunuma et al., 2004). Without inhibition of TH by PRL, the tail might absorb too fast, resulting in malnutrition or deformed juveniles.

Since PRL resembles JH, a hormone that counteracts insect metamorphosis, some researchers hold the viewpoint that PRL is a JH equivalent in anurans (Etkin and Gona, 1967). However, our results contradict this supposition, since rcPRL is continuously expressed in brain tissue from prometamorphosis to late metamorphosis; additionally, the mRNA level of rcPRL is low at premetamorphosis, and it increases to a peak around the climax in all tissues examined. This expression pattern corresponds to findings in the pituitary of X. laevis: the mRNA level of PRL is very low at pre- and prometamorphosis, increases sharply in late prometamorphosis, and continues to rise thereafter (Buckbinder and Brown, 1993). In addition, overexpression of PRL in transgenic X. laevis did not prolong tadpole life (Huang and Brown, 2000).

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