Identification of thyroid hormone receptors α and β genes and their expression profiles during metamorphosis in *Rana chensinensis*^

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Abstract: To explore the role of thyroid hormones in organ remodeling during the metamorphosis of amphibians, the cDNAs of two thyroid hormone receptors (TRα and TRβ) in *Rana chensinensis* were identified. The 1257 bp of *rcTRα* cDNA encoding 418 amino acids and the 1122 bp of *rcTRβ* cDNA encoding 373 amino acids were cloned. Their polypeptide sequences contain two highly conserved cysteine-rich zinc fingers in the DNA-binding domain, while *rcTRβ* is 42 amino acids shorter in its A/B domain when compared with *rcTRα*. Fifty-five amino acids differed between the TRα and TRβ domains. The *rcTRα* and *rcTRβ* mRNA levels in the liver, brain, skin, and tail of tadpoles were analyzed via qRT-PCR during metamorphosis. The results showed that the changes of *rcTRA* mRNA levels were moderate while those of *rcTRβ* were dramatic in all 4 tissues during metamorphosis. In addition, the levels of *rcTRβ* mRNA were relatively low at premetamorphosis and reached a peak around the climax. Tissue-specific expression of *rcTRα* and *rcTRβ* suggests that thyroid hormones play various roles in different organs or tissues at different developmental stages of metamorphosis.

Key words: Thyroid hormone receptors, molecular characterization, gene expression, amphibian, metamorphosis

1. Introduction
The metamorphosis of amphibians is a complex process, in which various organs and tissues undergo extensive remodeling during transformation from larva to juvenile (Atkinson et al., 1998; Huang et al., 2001). It is well established that this process is closely related to thyroid hormones. Therefore, amphibians represent an ideal model to study the role of thyroid hormones and their receptors (TRs) in regulating metamorphosis (Shi, 2000; Buchholz et al., 2006; Tata 2006). In addition, understanding the roles of thyroid hormones during amphibian development may shed light on our appreciation of the mechanisms of these hormones’ actions during human perinatal development (Buchholz, 2015).

Thyroid hormones exert their functions by binding to their receptors acting as DNA-binding transcription factors. In the metamorphosis of amphibians, the levels of thyroid hormones may be sufficient, but different levels of TRs in various tissues and organs contribute to their diverse effects (Forrest et al., 1990; Ogilska and Kotasz, 2004; Wang et al., 2008; Duarte-Guterman and Trudeau, 2010; Duarte-Guterman et al., 2012a, 2012b; Navarro-Martin et al., 2012; Lou et al., 2014). Therefore, understanding the profiles of TR expression in different organs is important for studying the roles of this hormone in regulating metamorphosis. TRs are members of the nuclear receptor super family and act as hormone-regulated transcriptional factors. They are composed of similar domain structures as found in other nuclear receptors: an amino-terminal A/B domain, a central DNA-binding domain (DBD) containing two zinc fingers, a D domain (hinge region) containing the nuclear localization signal, and a carboxyl-terminal ligand-binding domain (LBD) through which thyroid hormones bind and activate their TRs (Zhang and Lazar, 2000; Yen, 2001; Oetting and Yen, 2007). TRs usually activate the target gene transcription when binding with thyroid hormones but act as repressors in the absence of a ligand. There are two major TR subtypes in vertebrates, TRα and TRβ, which are encoded by the TRα and TRβ genes, respectively. The functions of TRα and TRβ have been well investigated in transgenic mice and it is clear that both have common functions in some aspects. However, they also have isoform-specific roles. For example, TRα plays significant tasks in heart, gastrointestinal tract, and temperature regulation, whereas TRβ is of greater importance to the inner ear, liver, and homeostasis (Forrest et al., 1996; Flamant and Samarut, 2003; Mai et al., 2004).

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The slight biochemical differences between TRα and TRβ in vivo may cause significant functional differences (Guissouma et al., 2002). However, TRα and TRβ gene expressions in the livers of TR knockout mice indicate that the two subtypes regulate almost the same set of genes (Yen et al., 2003). Hence, some functional differences between TRα and TRβ may be reflected by the differences in their expression patterns. TRα and TRβ have been identified in many species of vertebrates, such as teleosts (Conger myriaster, Kawakami et al., 2003; Oncorhynchus kisutch, Harada et al., 2008), amphibians (Xenopus laevis, Yaoita et al., 1990; Xenopus tropicalis, Wang et al., 2008; Physalaemus pustulosus, Duarte-Guterman et al., 2012b; Lithobates sylvaticus, Navarro-Martin et al., 2012), reptiles (Alligator mississippiensis, Helbing, et al., 2006), as well as in chicken and mice (Sechman et al., 2009; Dettling et al., 2014).

Previous studies also showed that TRα and TRβ are differentially expressed in various tissues of different species. Some studies, especially with transgenic animals, emphasized the importance of differential expression of TRs (Buchholz et al., 2004, 2006; Hogan et al., 2007; Duarte-Guterman and Trudeau, 2010; Duarte-Guterman et al., 2012a). Recent reports show that in X. tropicalis, knockdown of TRα could enhance tadpole growth while delaying natural metamorphosis (Wen and Shi, 2015). Unliganded TRα could delay developmental progression by repressing TH-response genes (Choi et al., 2015). However, the regular pattern of TRα and TRβ gene expressions during amphibian metamorphosis still need to be supported in more amphibian species. The Chinese brown frog, Rana chensinensis, a unique amphibian species in China, is widely distributed in northern China. This species is considered to be a potential candidate indicator for its high sensitivity to chemical pollution in aquatic environments, and as a native species it can be conveniently used to research the ecotoxicology of local amphibians (Li et al., 2014, 2016).

In the current experiments, the cDNAs of TRα and TRβ from R. chensinensis were cloned and their sequences were analyzed in order to investigate the expression profiles of TR genes during metamorphosis. Furthermore, the expressions of TRα and TRβ in the liver, brain, skin, and tail of tadpoles during different stages of development were examined, and the functions of thyroid hormones in the reconstruction of organs during metamorphosis were analyzed.

2. Materials and methods
2.1. Animals
Chinese forest frog (Rana Chensinensis) tadpoles were collected from the Qinling Mountains, China, during March and April of 2014. They were raised in tanks at room temperature (20 ± 2 °C) and fed with boiled lettuce every other day (ad libitum). Tap water (pH 7.2) was used after standing for 48 h. The light/dark cycle was 14 h/10 h. At Gosner stages 33, 37, 38, and 41–46 (Gosner, 1960), 12 tadpoles for each stage in triplicate were used. Their livers, brains, skin, and tails (only stages 33–44) were removed immediately following ether anesthesia. Samples were frozen in liquid nitrogen and stored at −80 °C for RNA isolation. The gonads of tadpoles were identified by dissecting microscope at stages 41–46. All animal procedures were performed according to protocols approved by the Animal Care Advisory Committee of Shaanxi Normal University.

2.2. RNA isolation and cDNA synthesis
Total RNA was extracted using RNeasy Plus according to the manufacturer’s protocol (Takara, Japan). The concentrations of total RNA from the liver, brain, skin, and tail tissues were quantified respectively by a nucleic acid analyzer (NANO-200). cDNAs were then synthesized by PrimeScript RT Master Mix (Takara) using 1 µg of RNA in 20 µL of mixture. In order to clone the TRα and TRβ from R. chensinensis, specific primers (Table 1) were designed referring to the relevant cDNA sequences of Rana catesbeiana (L06064.1, L27344.1). PCR was carried out in mixtures of 25 µL containing 4 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 35 cycles of 94 °C for 30 s, annealing at 62 °C/56 °C for 45 s, and extension at 72 °C for 1 min; the reaction was ended by a further 10 min at 72 °C. The amplified products were analyzed on a 1.0% agarose gel, purified using the MiniBEST Aгарose Gel DNA Extraction Kit Ver. 4.0 (Takara), 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 assembly.

2.3. Sequence and phylogenetic analysis
All sequencing results were spliced with DNASTAR; then the open reading frame (ORF) was analyzed with NCBI ORF Finder and Blast and the signal peptide was analyzed with SignalP. Functional domains, molecular weight, theoretical pl, and secondary structures were predicted using SMART, ProtParam, and SWISS-MODEL. Multiple sequence alignments were made with ClustalW2. A BLAST search was used to identify sequences (Gish and States, 1993). The phylogenetic tree was constructed with MEGA 5.1 by the neighbor-joining method (Tamura, 1992). The confidence of each node was assessed by 2000 bootstrap replicates.
2.4. RT-PCR
The primers specific to TRα2 and TRβ2, respectively, were used in RT-PCR, and all the PCR products were shown as described in Section 2.3. The mRNA levels were normalized with rpl8 (Table 1) (Radek et al., 2006; Navarro-Martin et al., 2012).

2.5. Quantitative real-time PCR
The specific primers TRα5 and TRβ2 were used in qRT-PCR with rpl8 as the reference gene (Table 1). PCR was performed in a reaction volume of 20 µL using 10 µL of SYBR Premix Ex Taq II (2X), 0.4 µL of ROX Reference Dye (50X), 0.8 µL each of forward and reverse primer (10 µM), 6 µL of ddH2O, and 2 µL of cDNA. Amplification was carried out in a thermal cycler (Bio-Rad, USA), including 30 s at 95 °C and 40 cycles of 5 s at 95 °C and 30 s at 58 °C for TRα or 57 °C for TRβ, followed by a melting curve analysis. Each sample was run in triplicate.

The relative expression levels of TR mRNA at a specific stage or in tissues were analyzed by 2 –ΔΔCt method and normalized with respect to rpl8. The values were compared using one-way analysis of variance (ANOVA) with SPSS 20.0. All data were shown as mean ± SD (n = 6). P < 0.05 was regarded as statistically significant.

3. Results
3.1. Molecular characterization of rcTRs
The assembled cDNA from R. chensinensis was named as rcTRα (GenBank Accession No. KJ579109.1). The coding sequence (CDS) of rcTRα was 1257 bp, which included an ORF coding 418 amino acids. The deduced amino acid sequence does not include an N-terminal signal peptide, which showed that rcTRα is a nonsecretory protein. Its molecular weight is 47.79 kDa and its theoretical pI is 7.08. Three mainly functional domains in rcTRα were predicted, including the ZnF_C4 domain (58–131), coiled coil domain (137–165), and HOLI domain (228–386) (Figure 1). The secondary structure contains 48.09% α-helix, 4.31% extended strand, and 46.89% random coil.

Another assembled cDNA was named as rcTRβ (GenBank Accession No. KJ579110.1). The coding sequence of rcTRβ is 1122 bp, which includes an ORF coding 373 amino acids. rcTRβ does not include an N-terminal signal peptide either. Its molecular weight is 42.28 kDa and its theoretical pI is 6.76. The ZnF_C4 domain (16–89) and HOLI domain (186–344) are found in rcTRβ (Figure 1). The secondary structure contains 50.94% α-helix, 6.17% extended strand, and 40.48% random coil.

Alignment of predicted polypeptide sequences revealed the modular characteristics of rcTRα and rcTRβ, with an N-terminal A/B domain, a conserved DBD, a D domain, and a LBD. Moreover, each sequence contained two putative cysteine-rich zinc fingers in the DBD, which are characteristic of nuclear hormone receptors. However, rcTRβ had a deletion of 42 amino acids in the A/B domain when compared with rcTRα. In addition, there was a difference of 51 amino acids between rcTRα and rcTRβ in the DBD, D domain, and LBD (Figure 1).

3.2. Phylogenetic analysis of rcTRs
Analysis of nucleotide and polypeptide sequences showed that rcTRα and rcTRβ have high identity similarity with other species of anurans, while being relatively low in similarity to reptiles, birds, and mammals. Compared with TRα, TRβ showed a higher homology between R. chensinensis and other species. Moreover, the identity of nucleotide sequences between rcTRα and rcTRβ was 72%, and the identity of polypeptide sequences between them was 87%. Similar identity in nucleotide and amino acid sequences between rcTRα and rcTRβ also exists in other species of vertebrates (Table 2).

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Forward primer (5’ to 3’)</th>
<th>Reverse primer (5’ to 3’)</th>
<th>Size (bp)</th>
<th>Tm (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRα1</td>
<td>TGGAATTACGTTGAATGGACCAG</td>
<td>TCCCGATTGGTTCTCAATCAGC</td>
<td>466</td>
<td>57</td>
</tr>
<tr>
<td>TRα2</td>
<td>TGGCAGGGTACATCCCAAGCTA</td>
<td>ATACATTTCTTTAAAGGAGGAGA</td>
<td>231</td>
<td>57</td>
</tr>
<tr>
<td>TRα3</td>
<td>GTAAGGCAAGGATCAAAC</td>
<td>CAGGCTCAATCATCGGAGA</td>
<td>968</td>
<td>58</td>
</tr>
<tr>
<td>TRα4</td>
<td>CTGCTGTCGTTAGATGCAA</td>
<td>CTTCACTGCTGTTTGGTAGTC</td>
<td>458</td>
<td>56</td>
</tr>
<tr>
<td>TRα5</td>
<td>TTACTGCTCCGCTGCTGAAC</td>
<td>CTTCACTGCTGCTGTTTAGTC</td>
<td>214</td>
<td>57</td>
</tr>
<tr>
<td>TRα6</td>
<td>CTCTGTGCATGAGACT</td>
<td>TTTGAGATTGGAGAGGCTA</td>
<td>397</td>
<td>52</td>
</tr>
<tr>
<td>TRβ1</td>
<td>ATGCCTAGCAGATCGGAGG</td>
<td>AAAGCTGCTGTCGTGGCAGA</td>
<td>293</td>
<td>62</td>
</tr>
<tr>
<td>TRβ2</td>
<td>ACAGGGTAGTATGATGATG</td>
<td>GACAACGGTGATTAGCTCC</td>
<td>333</td>
<td>58.5</td>
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<tr>
<td>TRβ3</td>
<td>CATGGAACAGAGTTGCTTT</td>
<td>CACTCGACTTTCATGTGCAA</td>
<td>819</td>
<td>52</td>
</tr>
<tr>
<td>rpl8</td>
<td>GCTGCTGACTTGGAGAAGGCA</td>
<td>ACCGTAAGGGTGACGAGGAGCA</td>
<td>115</td>
<td>57/58.5</td>
</tr>
</tbody>
</table>
A phylogenetic tree was constructed from rcTRs and various species of vertebrates. The phylogenetic tree showed that the TRα and TRβ sequences are clearly separated. In both TRα and TRβ clades, *R. chensinensi* is grouped with *R. catesbeiana*, *R. rugosa*, and *P. nigromaculatus*, which is consistent with these species being members of Ranidae. In addition, rcTRα and rcTRβ have similar positions in the phylogenetic tree (Figure 2).

3.3. Expression of rcTRs in different stages of tadpole

RT-PCR showed that the products of rcTRα, rcTRβ, and *rpl8* had 231 bp, 333 bp, and 115 bp, respectively. rcTRα and rcTRβ mRNA were detected in the liver, brain, skin, and tail of *R. chensinensis* tadpoles at stages 33–46. In the four tissues, the expression of rcTRα changed moderately while rcTRβ dramatically changed during larval development. In addition, the expression of *rpl8* changed during all stages (Figure 3).

The results of qRT-PCR were similar to that of RT-PCR. In liver tissue, rcTRα mRNA levels at stage 33 was significantly higher than in other stages; thereafter, its levels decreased during development towards stage 46, but its levels were higher at stages 37 and 43, respectively. rcTRβ mRNA levels obviously increased at stage 43; thereafter, its levels decreased during development. In brain tissue, rcTRα expression retained a higher level and reached a peak at stage 43 in comparison with rcTRβ. The trend of rcTRβ expression was similar to that found in liver. In skin tissue, rcTRα and rcTRβ levels were higher at stages 42 and 43, and levels were lower at other stages. In tail tissue, the levels of rcTRα at stages 41 and 42 and the levels of rcTRβ at stage 43 increased significantly. There was no sex difference detected (Figure 4).

4. Discussion

4.1. Analysis of rcTRα and rcTRβ

In this study, we cloned the cDNA sequences of rcTRα and rcTRβ from *R. chensinensis*. In alignment analysis, the high homology of TRα and TRβ suggests that both sequences are relatively well conserved between *R. chensinensis* and other species, especially two highly conserved cysteine-rich zinc fingers in the DBD, signifying that fundamental functions of TRα and TRβ are preserved stably in phylogeny. The differences between rcTRα and rcTRβ, including the deletion of 42 amino acids in the A/B domain of rcTRβ.
Table 2. Sequence identities (%) of TRs from *R. chensinensis* and other species.

<table>
<thead>
<tr>
<th>Species</th>
<th>TRα</th>
<th>TRβ</th>
<th>TRα/TRβ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nucleotide</td>
<td>Polypeptide</td>
<td>Nucleotide</td>
</tr>
<tr>
<td><em>Rana chensinensis</em></td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td><em>Rana catesbeiana</em></td>
<td>99</td>
<td>99</td>
<td>99</td>
</tr>
<tr>
<td><em>Rana rugosa</em></td>
<td>98</td>
<td>99</td>
<td>98</td>
</tr>
<tr>
<td><em>Pelophylax nigromaculatus</em></td>
<td>98</td>
<td>99</td>
<td>98</td>
</tr>
<tr>
<td><em>Xenopus laevis</em></td>
<td>87</td>
<td>98</td>
<td>—</td>
</tr>
<tr>
<td><em>Xenopus tropicalis</em></td>
<td>—</td>
<td>—</td>
<td>90</td>
</tr>
<tr>
<td><em>Eublepharis macularius</em></td>
<td>82</td>
<td>88</td>
<td>83</td>
</tr>
<tr>
<td><em>Alligator mississippiensis</em></td>
<td>81</td>
<td>95</td>
<td>84</td>
</tr>
<tr>
<td><em>Alligator sinensis</em></td>
<td>83</td>
<td>89</td>
<td>84</td>
</tr>
<tr>
<td><em>Gallus gallus</em></td>
<td>82</td>
<td>90</td>
<td>84</td>
</tr>
<tr>
<td><em>Sarcophilus harrisii</em></td>
<td>82</td>
<td>89</td>
<td>82</td>
</tr>
</tbody>
</table>

GenBank accession numbers of sequences as follows (TRα: nucleotide, polypeptide; TRβ: nucleotide, polypeptide): *R. catesbeiana* (AAA16902.1, AAA53658.1; L06064.1, L27344.1), *P. nigromaculatus* (AGT55994.1, AGT55995.1; KC139354.1, KC139355.1), *R. rugosa* (BAM15695.1, BAM15696.1; AB683466.1, AB683467.1), *E. macularius* (BAF03080.1, BAF03081.1; AB204861.1, AB204862.1), *S. harrisii* (XP_003768307.1, XP_003772079.1; XM_003768259.1, XM_003772031.1), *G. gallus* (NP_990644.1, CAA90566.1; NM_205313.1, Z50188.1), *A. sinensis* (XP_006022601.1, XP_006021255.1; XM_006022539.1, XM_006021193.1), *A. mississippiensis* (ABD48806.1, ABD48808.1; DQ386683.1, DQ386685.1), *X. laevis* (NP_001081595.1; NM_001088126.1) and *X. tropicalis* (ACE07029.1; EU723572.1). TRα/TRβ represents the identity between TRα and TRβ.
and a mismatch of 51 amino acids in the remaining domains, indicate functional differences between the two subtypes. In the phylogenetic tree, rcTRα and rcTRβ were located in two clearly separated clades (i.e. TRα and TRβ), and in each clade, their respective positions were similar. These findings agree with the speculation that TRα and TRβ may be the products of an ancient gene duplication event during evolution (Laudet et al., 1992; Laudet, 1997).

4.2. Analysis of rcTRα and rcTRβ during metamorphosis

The primary morphogens controlling amphibian metamorphosis are thyroid hormones, which regulate gene transcription by binding to TRs. During amphibian metamorphosis, most organs and tissues have to go through remodeling to adapt to the transition in living environments from aquatic to terrestrial. In the presence of TRs, thyroid hormones play key roles in cell proliferation and differentiation (Buchholz et al., 2004; Pascual and Aranda, 2013). Therefore, to determine the expression of TRs in tadpoles at different stages is important for exploring the roles of thyroid hormones in organ remodeling (Yaoita and Brown, 1990). Previous studies show that there are no endogenous thyroid hormones or sex differentiation in tadpoles at stages 27–30. Thereafter, thyroid hormones are produced and gonads commence differentiation at stages 31–36, with sex distinction being complete at stages 37–42. Finally, the tail is resorbed and the juvenile frog forms at stages 43–46 (Gosner, 1960; Ogielska and Kotusz, 2004; Hogan et al., 2008). In this study, the expressions of two

![Figure 3. Organ-specific expression of rcTRα and rcTRβ in tadpoles during stages 33–46 by RT-PCR. M: DL2000 DNA marker.](image-url)
Figure 4. The levels of rcTRα and rcTRβ mRNA in several (n = 6) tadpoles as assessed by qRT-PCR. Different letters indicate significant differences (P < 0.05) between developmental stages.
$rcTR$s were detected in four tissues of tadpoles; $rcTRα$ was higher while $rcTRβ$ was lower during premetamorphosis (stages 33–37), and $rcTRβ$ expression reached a peak at climax (stage 43), suggesting that expression of $rcTRα$ started in premetamorphosis, whereas emergence of $rcTRβ$ occurs later than $rcTRα$. This agrees with a report that $rcTRα$ is expressed throughout the larval period, whereas the presence of $TRβ$ is correlated with thyroid hormone levels (Luria and Furlow, 2004). Our results indicate that $TRβ$ appears later than $TRα$, with the earliest time point at which $TRβ$ occurs being after stage 31. At stage 33, endogenous thyroid hormones are produced to prepare for the next metamorphosis. Nevertheless, many studies have shown fold changes in TR expression relative to particular developmental stages, but the criteria used for defining the stages are inconsistent (Ogielska and Kotusz, 2004; Wang et al., 2008; Navarro-Martin et al., 2012; Lou et al., 2014).

In the liver of tadpoles at premetamorphosis, the high expression of $rcTRα$ may be related to the proliferation and differentiation of hepatocytes (Paik and Cohen, 1960). The expression of $rcTRα$ and $rcTRβ$ at climax may be related to both the changes of feeding habits from herbivore to omnivore and the transition of the organism from an ammonotelic larva to a ureotelic juvenile (Atkinson et al., 1998; Li et al., 2014).

Thyroid hormones have major effects on brain development via regulating neural proliferation, migration and apoptosis, synapse formation, and neurotransmitter receptors (Oofusa and Yoshizato, 1991; Bernal, 1999). Additionally, there is no single critical period of thyroid hormone action in the developmental brain of the fetal rat (Dowling et al., 2000). In the tadpole of Lithobates sylvaticus, $TRβ$ expression levels in the brain at climax increased 5-fold compared with premetamorphic stages (Navarro-Martin et al., 2012). In the brain of X. laevis tadpoles, $TRβ$ mRNA was detected earliest at Nieuwkoop stage 58 and maximal expression occurred at Nieuwkoop stage 60 (which corresponds to Gosner stage 41; Gosner, 1960), and in the juvenile frog, $TRβ$ levels remained at a late metamorphic level (Krain and Denver, 2004). Recently researchers compared the developmental profiles between $TRα$ and $TRβ$ in the brain of X. tropicalis, R. pipiens, and Physalaemus pustulosus and concluded that $TRα$ does not significantly change during development but $TRβ$ dramatically increases at climax (Duarte-Guterman et al., 2012a). Our results showed that $rcTRα$ expression maintained a high level and changed only moderately in the brain throughout metamorphosis, whereas $rcTRβ$ expression changed markedly around climax (Gosner stages 42–44). These expression profiles of $TRα$ and $TRβ$ in the brain are similar to variations reported for L. sylvaticus and X. laevis, X. tropicalis, R. pipiens, and P. pustulosus (Duarte-Guterman et al., 2012a). These findings suggest that the expression profiles of $TRα$ and $TRβ$ in developing brains have common characteristics for most species of tailless amphibians (Furlow and Neff, 2006; Hogan et al., 2006; Denver et al., 2009). These results suggest that the sustained expression of $TRα$ is associated with thyroid hormone-induced cell proliferation and neuronal maturation, whereas $TRβ$ is associated with cell differentiation, migration, and tissue reconstruction in developmental brain.

It is reported that the fibroblasts begin to synthesize collagen and elastic fibers under the regulation of thyroid hormones at metamorphosis (Coen et al., 2001). In this study, $rcTRα$ and $rcTRβ$ were expressed in tadpole skin during metamorphosis, suggesting that this may be associated with the structural changes of skin to adapt to terrestrial life. In addition, the tadpole tail is an important target organ for thyroid hormones during metamorphosis (Berry et al., 1998). Our result show that $rcTRα$ and $rcTRβ$ were expressed in the tail. In comparison with $rcTRα$, $rcTRβ$ had a higher expression level at climax, suggesting that $rcTRβ$ plays a more important role than $rcTRα$ in tail regression. Moreover, this is in concordance with previous observations that $TRβ$ gene expression in X. laevis tails demonstrates more marked changes at the time of tissue remodeling (Krain and Denver, 2004; Opitz et al., 2006; Wang et al., 2008). In addition, our prior studies illustrated the high expression of the prolactin receptor gene ($PRLR$) in the skin and tail at metamorphic climax (Wang et al., 2016). In view of prolactin having an antithyroid hormone effect, the high expression of TR and PRLR simultaneously occurred during tail degradation, indicating the potential for the rate of tail resorption being coordinated by both thyroid hormones and prolactin.

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