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Tissue-dependent developmental expression of a cytosolic thyroid hormone protein gene in *Xenopus*: its role in the regulation of amphibian metamorphosis

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Abstract We have cloned the cDNA encoding the *Xenopus laevis* homolog of mammalian cytosolic thyroid hormone binding protein (CTHBP). We found that while its mRNA level varies little in whole animals during development, the expression of CTHBP is inversely correlated with tissue-specific transformations during metamorphosis. A high level of its mRNA was observed in the tail of premetamorphic tadpoles. However, the expression is dramatically repressed with the onset of rapid tail resorption. In the hindlimb, the expression of CTHBP is very low during morphogenesis. Subsequently, its expression continuously increases during the period of limb growth. In contrast, a low level of CTHBP expression was detected in the intestine throughout metamorphosis. These results suggest that CTHBP could function to modulate the metamorphic process by regulating the level of intracellular thyroid hormones.

Key words: Thyroid hormone; Amphibian metamorphosis; Binding of thyroid hormone; Gene regulation; Expression of a cytosolic protein

1. Introduction

Amphibian metamorphosis is a developmental process that is regulated by thyroid hormone (TH) [1]. The transition from a tadpole to a frog brings about systematic transformations in tissues. These changes are apparently autonomous as an individual tissue/organ can undergo metamorphosis even when cultured in vitro. During development, while the increase of plasma thyroid hormone correlates with metamorphosis in general, different tissues/organs metamorphose systematically but at different developmental stages. Thus, limb development is one of the earliest observable morphological changes and tail resorption is one of the last [1,2]. Recently, four thyroid hormone receptor genes, two TRβs and two TRαs, have been cloned from *Xenopus*. TRs are TH-dependent transcription factors. Evidence has been presented to indicate that the TH-induced morphological changes are mediated by TRs through regulation of gene expression [3]. However, it is still unknown how the thyroid hormone-induced temporal development of various tissues is regulated.

Cytosolic thyroid hormone binding proteins (CTHBPs) are present in many tissues [4]. We have previously cloned a human cDNA encoding CTHBP. Analysis of its cDNA sequence indicates that it is a subunit of pyruvate kinase, subtype M_2 (PKM_2). The monomeric CTHBP binds T_3 with high affinity and specificity, whereas the tetrameric PKM_2 does not bind T_3. The monomer-tetramer interconversion is modulated in vivo by fructose 1, 6-bisphosphate, a metabolite of the glycolytic pathway. Using fructose 1,6-bisphosphate to modulate the monomer–tetramer concentration in cultured cells, we have recently found that CTHBP plays a critical role in the regulation of the transcriptional activity of the TRs by modulating the cytoplasmic level of the thyroid hormone, 3,3′,5-triiodo-L-thyronine (T_3) [5].

To investigate whether CTHBP plays a similar regulatory role in tadpoles, thereby affecting metamorphosis, we have cloned the CTHBP gene from *Xenopus laevis*. We show that its expression is regulated in a tissue-dependent manner during metamorphosis. High levels of its mRNA are present in tails of the premetamorphic tadpoles and post-morphogenic hindlimbs, suggesting a potential buffering effect of CTHBP for TH in these tissues.

2. Materials and methods

2.1. Treatment of tadpoles and RNA analysis

Tadpoles staged according to Nieuwkoop and Faber [2] were treated with 5 nM of T_3 as described previously [6]. RNA from total tadpoles at different stages or the individually dissected organs/tissues was analysed by Northern blot hybridization as described [6]. To show that equivalent amounts of total RNA were present, the same filters were hybridized with a cDNA probe for ribosomal protein L8 (rpL8), a gene whose mRNA level does not change in developing frog tissues [7].

2.2. Cloning of the cDNA for a Xenopus laevis CTHBP

A 1.8 kb fragment of the human CTHBP (also known as PKM2) cDNA [8] was used to screen 10^6 clones of an amplified cDNA library made from mRNA isolated from stage 52–54 tadpole intestine. The filters were hybridized as above but washed 3 times at room temperature in 2× SSC and 0.2% SDS for 10 min each and then twice at 50°C in 0.5× SSC and 0.5% SDS for 25 min each. Of the three positive clones isolated, one contained a 2 kb insert, close to the size of the mRNA and was sequenced. The sequence has been deposited to GenBank with Accession Number U03878.

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Abbreviations: CTHBP, cytosolic thyroid hormone binding protein; TH, thyroid hormone; T_3, 3,5,3′-triiodothyronine; T_4, L-thyroxine; PK, pyruvate kinase.

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3. Results

3.1. Cloning and sequence of a Xenopus laevis CTHBP gene

A cDNA fragment of the human CTHBP gene [8] was initially used as a probe for Northern blot analysis. A single RNA of about 2 kb in size was detected in RNAs from whole tadpoles at stage 52/54 or from the intestine alone under reduced stringency conditions (not shown), indicating the presence of a homologous frog gene. This probe was then used to screen an intestinal cDNA library. A full-length cDNA clone was isolated and sequenced (GenBank U03878). It contains a 5′-untranslated region of 28 bases, an open reading frame encoding 527 amino acids, and a 3′-unstranslated region of 431 bases followed by a poly(A) tail.

Fig. 1 compares the deduced protein sequences of CTHBP from human and *Xenopus*. An 89% identity was found between these two proteins. The X-ray crystallographic structure of the cat PKM_2 has been determined [9]. The active site of the enzyme consists of an eight-stranded α/β-barrel structure. The sequences of each of the eight β-strands that are close to the center of the active site are conserved. Recently the hormone binding domain of human TR subtype $\beta 1$ has been modeled as an eight-stranded α/β barrel and the T_3 binding site was proposed to be in the barrel [10], suggesting that the α/β barrel of CTHBP is involved in T_3 binding. Furthermore, key residues deduced from the crystal structure in the binding of metal ions, substrate, and catalysis are also present in the frog CTHBP (Fig. 1). This high degree of conservation suggests that the frog protein is not only a T_3 binding protein but also a pyruvate kinase.

3.2. Tissue-dependent developmental regulation of Xenopus CTHBP gene

The expression of *Xenopus* CTHBP gene was first analyzed using RNA from whole tadpoles at different stages. The CTHBP mRNA was detected in all tadpoles from pre- (stages 50–54) to post-metamorphic (stage 66) stages and its level in whole tadpoes changed very little during development (data not shown). However, dramatically different developmental expression patterns were found in different tissues. Fig. 2 shows the expression of CTHBP mRNA from tail, limb and intestine. In the tail, a high level of CTHBP was expressed in the premetamorphic stage (stages 54–60). Its expression was dramatically repressed during tail resorption (stages 62–64). In the hindlimb, a low expression was found in stages 54–56 when morphogenesis occurs. However, increasing expression was observed begin-

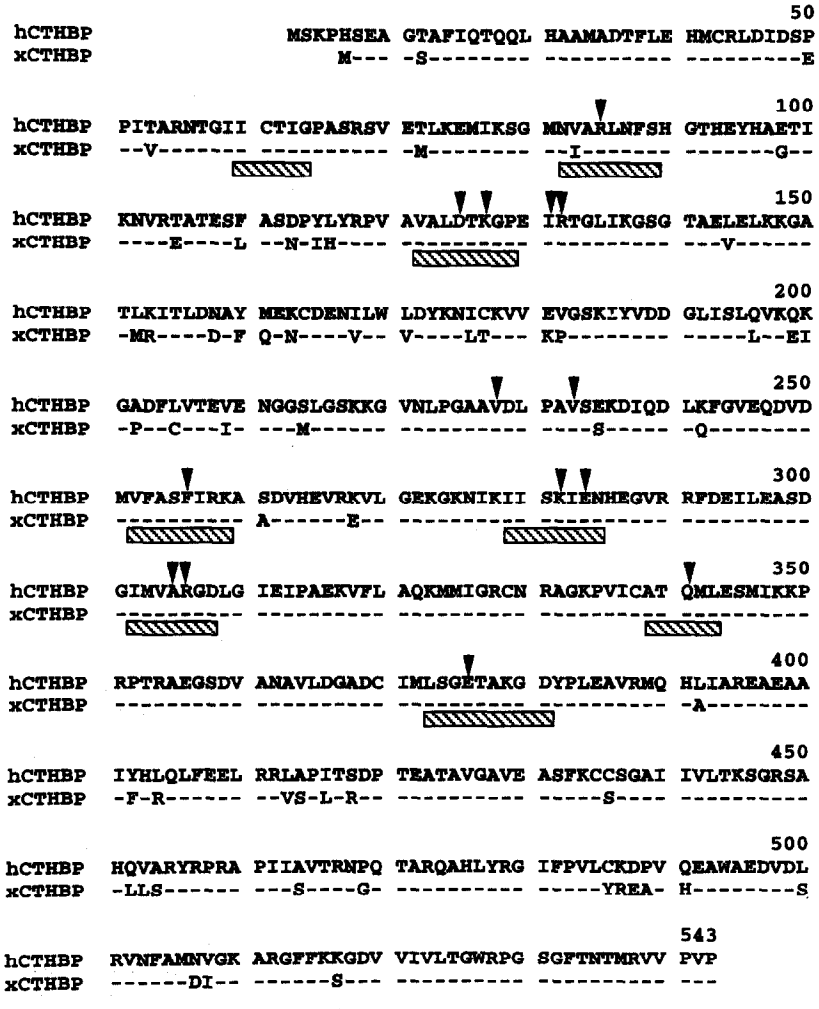

Fig. 1. Sequence comparison of CTHBP from *Xenopus* and human. Sequences in the eight β-strands that are close to the center of the active site are conserved (underlined with hatched bars), so are the key residues involved in the binding of metal ions, substrate, and catalysis (pointed by arrow heads, [9]).

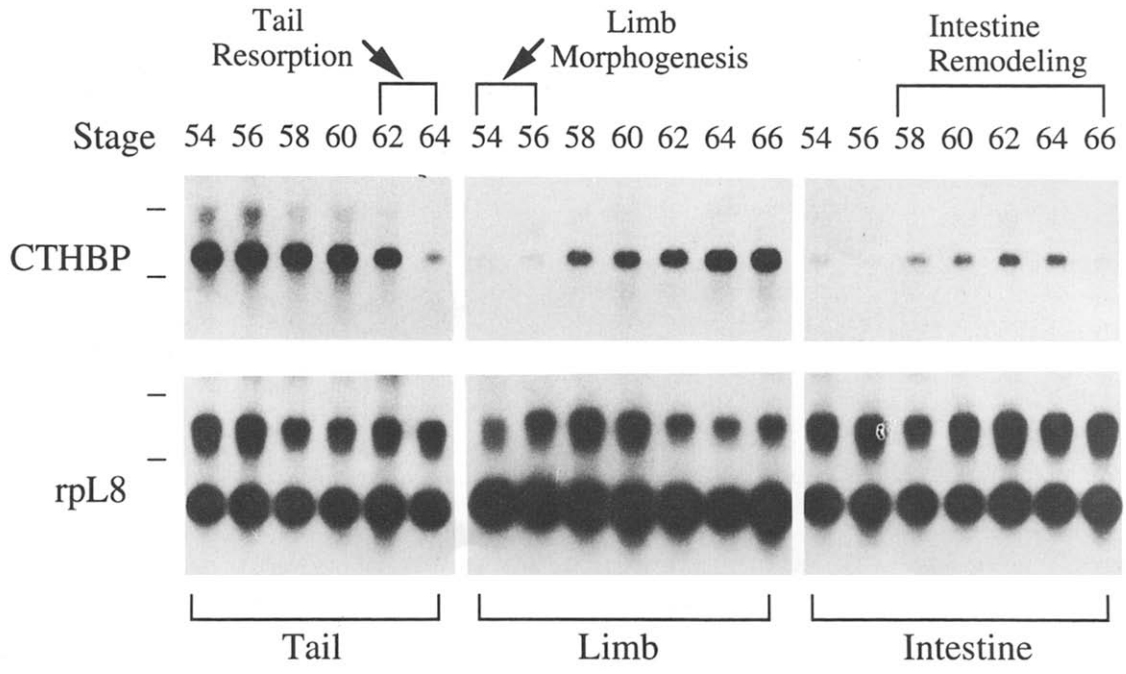

Fig. 2. Differential expression of CTHBP in intestine, tail, and hindlimb during metamorphosis. Two μg total RNA from different organs at the indicated stages per lane were analyzed by Northern blot hybridization. The probe rpL8 served as a loading control. The positions of 28S and 18S rRNAs are indicated by the upper and lower bars, respectively.

ning at stage 58 and continued to stage 66 during limb growth. In contrast, the expression of CTHBP was generally low in the intestine.

3.3. *CTHBP mRNA level is inversely correlated with the expression of TRα genes and with the morphological changes in the tadpole tail and hindlimb*

Recently, many lines of evidence have been presented to suggest that the T_3-induced metamorphosis is mediated by TR-dependent gene regulation [3]. The TRα genes are expressed at a high level during metamorphosis. The expression of TRα genes was also found to be regulated in a tissue-dependent manner during metamorphosis. However, the expression profile of TRα genes contrasts sharply with that of CTHBP in the tail and hindlimb (Fig. 3A,B). In contrast, the expression patterns of TRα and CTHBP genes are similar in the intestine (Fig. 3C). In the tail, the TRα mRNA level continues to increase between stages 56 and 64. This is in contrast to that shown for the expression of CTHBP. The latter is down-regulated, which is in concurrence with the tail resorption ([11]; Fig. 3A). Similarly, while the expression of CTHBP gradually increases after stage 56 in the hindlimb, TRα mRNA is dramatically down-regulated. The high level of TRα expression was observed only during the early period of limb development when CTHBP mRNA is low (Fig. 3B). In the intestine, the mRNA levels of both TRα and CTHBP increase only slightly during metamorphosing (stages 60–64, Fig. 3C).

4. Discussion

The present studies have shown that CTHBP is expressed in a tissue-specific and developmentally regulated manner. Limb development is one of the earliest changes in a tadpole. While the hindlimb buds are formed around stage 48, the most dramatic morphological differentiation occurs around stages 53–56 when relatively low levels of plasma T_3 and T_4 are present (Fig. 3C; [12]), which is followed by limb growth [2]. The CTHBP gene is minimally expressed during the period of morphogenesis in the hindlimb. Tail resorption, by contrast, is one of the last events to occur. The reduction in tail length starts around stage 62 and its completion marks the end of metamorphosis [2]. The CTHBP expression is down-regulated during this period in tail. Thus, a high level of CTHBP mRNA is present in tissues undergoing few morphological changes, while reduced expression is found in the tissues undergoing morphogenesis.

Amphibian metamorphosis involves coordinated transformations of different organs. Even though all the changes are controlled by TH, different tissues undergo the transition at different developmental stages. While the molecular mechanism for this temporal regulation is unknown at present, the tissue-specific differential expression of TRα and CTHPB mRNAs observed in the present studies suggest two possibilities. The first possibility is that the temporal regulation is mediated by the expression of TRα. A high level of TRα expression detected in the hindlimbs of stage 54–56 enables the limb morphogenesis to occur. Similarly, the high level of TRα could also mediate the tail resorption by affecting the genes necessary for such morphological changes. Such a correlation between the TRα expression and metamorphosis has also been reported recently by Wang and Brown [13].

The second possibility is that the temporal regulation is indirectly mediated by CTHBP as suggested by the excellent inverse correlation between the expression level of CTHBP and the morphogenesis in the tail and hindlimb. Previously, we have demonstrated that in cultured cells, CTHBP regulates the

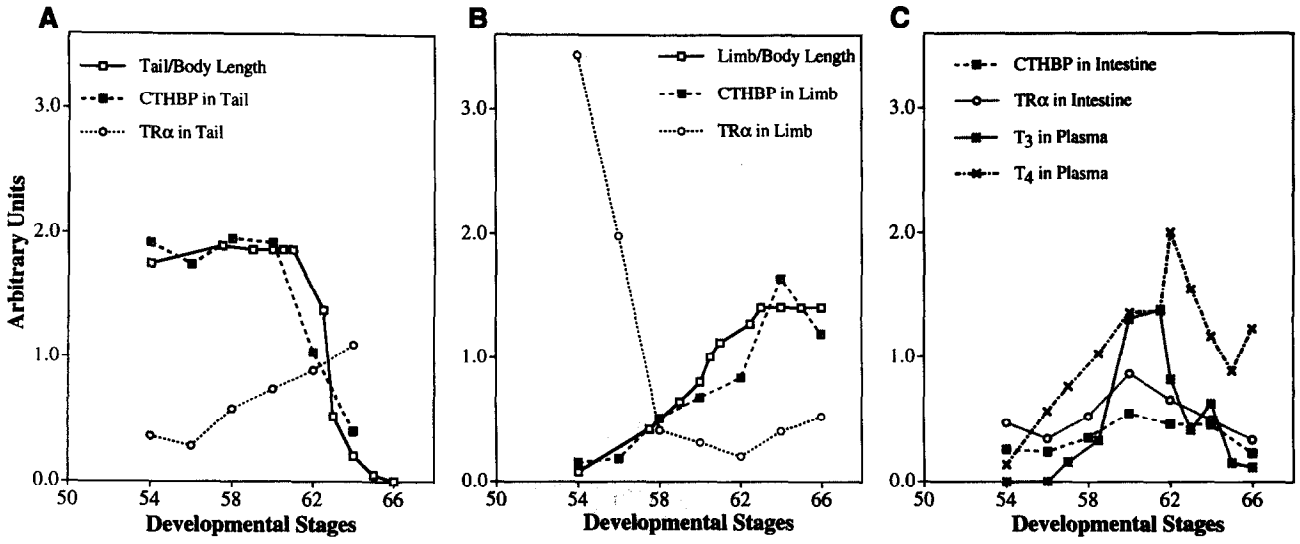

Fig. 3. Comparison of the expression of TRα and CTHBP mRNAs during development of tails (A), hindlimb (B), and intestine (C). The plasma concentrations of thyroid hormones T_4 and T_3 were from Leloup and Buscaglia [12]. The CTHBP mRNA levels were determined from the hybridized filters shown in Fig. 2 using a Phosphoimager and TRα mRNA levels were determined using a Phosphoimager after hybridization of the same filters in Fig. 2 with a TRα cDNA probe. Both were normalized using rpL8 as a loading control. The tail/body or hindlimb/body length ratio was from Atkinson [11], normalized to the staging of Nieuwkoop and Faber [2].

transcriptional activity of TR by affecting the intracellular concentration of TH [5]. The present studies show that *Xenopus* CTHBP is a homolog of human CTHBP. Taken together, it is reasonable to assume that the *Xenopus* CTHBP could function as a regulator for the intracellular level of TH.

A potentially cooperative effect between the repression of CTHBP and up-regulation of TRα genes could result in the observed temporal development of various tissues. Thus, in the hindlimb at stages 54–56, even though the plasma TH levels are low, the reduced expression of CTHBP could increase free intracellular hormone concentration. This elevated level of intracellular TH, together with the very high level of TRα could in turn lead to the induction of limb morphogenesis. Similarly, in the tails of tadpoles up to stage 60, the high level of CTHBP expression reduces the available intracellular TH. This leads to the delay in tail resorption, even though high levels of plasma TH and TRα are present. The repression of CTHBP gene at and after stage 62 results in a higher level of intracellular free TH, thereby activating the resorption process. A similar cooperative effect is also found in the intestine. No dramatic changes for the expression of CTHBP and TRα genes occur during remodeling of the intestine. The low level of CTHBP and the high level of TRα in intestine are comparable to those in the tail during resorption and in the limb during morphogenesis. These, together with the high level of TH enable the remodeling of intestine to occur. Thus, CTHBP appears to play an important role in the temporal regulation of metamorphosis.

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