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Molecular and genetic studies suggest that thyroid hormone receptor is both necessary and sufficient to mediate the developmental effects of thyroid hormone

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ABSTRACT

Thyroid hormone (TH) affects diverse biological processes and can exert its effects through both gene regulation via binding the nuclear TH receptors (TRs) and non-genomic actions via binding to cell surface and cytoplasmic proteins. The critical importance of TH in vertebrate development has long been established, ranging from the formation of human cretins to the blockage of frog metamorphosis due the TH deficiency. How TH affects vertebrate development has been difficult to study in mammals due to the complications associated with the uterus-enclosed mammalian embryos. Anuran metamorphosis offers a unique opportunity to address such an issue. Using *Xenopus* as a model, we and others have shown that the expression of TRs and their heterodimerization partners RXRs (9-cis retinoic acid receptors) correlates temporally with metamorphosis in different organs in two highly related species, *Xenopus laevis* and *Xenopus tropicalis*. *In vivo* molecular studies have shown that TR and RXR are bound to the TH response elements (TREs) located in TH-inducible genes in developing tadpoles of both species. More importantly, transgenic studies in *X. laevis* have demonstrated that TR function is both necessary and sufficient for mediating the metamorphic effects of TH. Thus, the non-genomic effects of TH have little or no roles during metamorphosis and likely during vertebrate development in general.

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1. Introduction

Thyroid hormone (TH)[1] plays an important role in vertebrate development and human pathology (Oppenheimer, 1979; Yen, 2001). The critical effects of TH on human development have been well documented. The most obvious and earliest known human abnormalities due to TH deficiency are the goiter (a lump in the neck due to thyroid gland enlargement) and cretinism (a form of severe mental deficiency together with retarded skeletal growth) (Hetzel, 1989). In humans, many of the developmental defects caused by TH deficiency prior to birth can be reversed if TH replacement is initiated shortly after birth (Larsen, 1989), indicating that TH influences neonatal development mainly by acting directly on the foetus, not through the mother. The most important period of TH action is the so-called postembryonic development, a few months before and several months after birth when TH levels are high (Howdeshell, 2002; Tata, 1993). This period bears many similarities to anuran metamorphosis (Shi, 1999; Tata, 1993), including the presence of high levels of TH. Such similarities coupled with the difficulties to manipulate the uterus-enclosed mammalian embryos have made anuran metamorphosis a highly valuable model to study TH action during vertebrate development. Here, we will review some of the studies on the role of TH receptor (TR) during amphibian metamorphosis, with an emphasis on our own work in *Xenopus laevis* as presented at the meeting.

2. Mechanisms of TH action

To affect target cells, circulating TH in the plasma needs to be taken up by cells through active transport (Hennemann et al., 2001; Shi et al., 2002). Upon entering the cells, TH can bind to a number of cytosolic proteins and enter the nucleus where it binds to TRs (Shi et al., 1996). In addition, TH can also bind to cell surface proteins, such as integrins (Bassett et al., 2003; Davis et al., 2005). Thus, it is not surprising that TH can affect cells through both the so-called non-genomic action via the binding to cell surface and cytoplasmic proteins and transcriptional regulation via TRs.

2.1. Non-genomic action of TH

TH affects diverse biological processes. Some of the effects of TH are too fast to be mediated through transcriptional regulation via TR in the nucleus (Bassett et al., 2003; Davis and Davis, 1996).

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[1] *Abbreviations used:* TH, thyroid hormone; TR, thyroid hormone receptor; HDAC, histone deacetylase; HAT, histone acetyl transferase; RXR, 9-cis retinoic acid receptor; TRE, TH response element; ChIP, chromatin immunoprecipitation.

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For example, TH administration leads to acute improvement in cardiac output in human patients, TH can alter myocardial contractility and reduce systemic vascular resistance within minutes. At the cellular level, TH can affect cell morphology, respiration (mitochondrial function), and ion homeostasis, etc. TH appears to exert diverse non-genomic effects through multiple pathways (Bassett et al., 2003; Davis and Davis, 1996, 2002; Davis et al., 2005; Shi et al., 1996). First, it has long been known that TH can bind to cell surface proteins (Davis et al., 2005). The identities of these proteins are largely unknown except the integrin αVβ3, which binds strongly T4 (3,3′,5,5′-tetraiodothyronine) and to a lesser extent T3 (3,3′,5-triiodothyronine) (Davis et al., 2005). This binding of TH to the integrin is expected to affect cell-extracellular matrix interactions and trigger intracellular signaling processes rapidly. Second, within the cell, a number of cytosolic proteins are known to bind to TH (Davis and Davis, 1996, 2002; Parkison et al., 1991; Shi et al., 1994, 1996). Most of these cytosolic proteins have additional functions, often as enzymes. TH binding may thus affect the enzymatic functions of these proteins and conversely, regulating their enzymatic activity may influence their binding to TH. For example, a cytosolic thyroid hormone binding protein is the monomer form of M2 pyruvate kinase (Parkison et al., 1991). TH binding prevents the formation of the enzymatically active tetramer and conversely, the formation of the tetramer inhibits its binding to TH (Ashizawa and Chen, 1992; Ashizawa et al., 1991). Finally, while TR is predominantly nuclearly localized even in the absence of TH, a small fraction is present in the cytoplasm. It has been shown that one of the two TR isoforms, TRβ, can form a complex with the signaling kinase MAPK in TH treated cells, which is likely responsible for the rapid activation of MAPK by TH (Davis et al., 2005). In addition, unliganded TRβ can interact with phosphatidylinositol 3 kinase (PI3K) to activate the signaling pathway (Guigon and Cheng, 2009; Storey et al., 2006). Thus, TRβ can also function as a mediator of the non-genomic effects of TH by interacting with these and other cytosolic proteins (Guigon and Cheng, 2009).

2.2. Nuclear action of TH

There are two types TRs in all vertebrates, TRα and TRβ, both of which bind TH with high affinities (Davey et al., 1994; Puzianowsak-Kuznicka et al., 1996; Sap et al., 1986; Weinberger et al., 1986). TRs belong to the superfamily of nuclear hormone receptors (Evans, 1988; Laudet and Gronemeyer, 2002; Mangelsdorf et al., 1995; Tsai and O'Malley, 1994; Yen and Chin, 1994). TH can both activate and repress transcription through TRs. The mechanism for gene repression by TH is not well understood and thus will not be discussed here. Transcriptional activation by TH requires the binding of TRs, most likely as heterodimers with RXRs (9-cis-retinoic acid receptors), to the TH response elements (TREs) in TH-inducible genes. TR/RXR heterodimers bind to TREs constitutively, even in the context chromatin (Perlman et al., 1982; Tsai and O'Malley, 1994; Wong et al., 1995). They repress or activate transcription in the absence or presence of TH, respectively.

In vitro and cell culture studies involving different animal species by many laboratories have led to a fairly detailed understanding of the mechanisms of the gene regulation by TR. TR functions by recruiting cofactors. Many such cofactors have been isolated and characterized (Burke and Baniahmad, 2000; Glass and Rosenfeld, 2000; Huang et al., 2003; Ito and Roeder, 2001; Jones and Shi, 2003; McKenna et al., 1999; McKenna and O'Malley, 2001; Meng et al., 2003; Rachez and Freedman, 2000, 2001; Sato et al., 2009; Wahlstrom et al., 1999; Xu et al., 1999; Zhang and Lazar, 2000). In the absence of TH, TR recruits corepressors, such as the highly related proteins SMRT and N-CoR, which form multimeric complexes containing histone deacetylases (HDACs) (Burke and Baniahmad, 2000; Glass and Rosenfeld, 2000; Jones and Shi, 2003; Zhang and Lazar, 2000) (Fig. 1). This leads to the deacetylation of the promoter regions of the target genes to facilitate gene repression. When TH is present, the corepressor complexes are released and replaced by coactivator complexes. Many diverse groups of coactivators have been identified. Among them include ATP-dependent chromatin remodeling proteins, histone acetylases (HATs) such as p300 and SRCs, protein arginine methyltransferases, and TRAP/DRIP/mediator complex that associates with the recruitment and activation of RNA polymerase II (Chen et al., 1999; Demarest et al., 2002; Heimeier et al., 2008; Huang et al., 2003; Ito and Roeder, 2001; Li et al., 2000; Matsuda et al., 2009, 2007; McKenna and O'Malley, 2001; Rachez and Freedman, 2001; Sheppard et al., 2001; Yen, 2001; Zhang and Lazar, 2000) (Fig. 1). The recruitment of such cofactors to the target genes leads to histone acetylation, methylation, and chromatin remodeling, resulting in transcriptional activation.

3. Roles of TR in *Xenopus* metamorphosis

3.1. A model of TR in frog development

Early expression studies showed that the mRNA levels of TR, especially TRα, are upregulated shortly after hatching at stage 35 in *X. laevis*, reaching peak levels by tadpole feeding stage (stage 45), when a free living tadpole is developed, although TRβ expression parallels with plasma TH concentrations (Fig. 2) (Shi et al., 1994; Yaoita and Brown, 1990). In addition, RXR genes, in particular, RXRα, are also expressed in premetamorphic *X. laevis* tadpoles (Fig. 2) (Wong and Shi, 1995). Similar expression patterns for TR and RXR genes have also been observed in *X. tropicalis* (Wang et al., 2008). Based on these and the transcriptional properties of TR/RXR heterodimers, we have previously proposed a dual function model for TR during *X. laevis* development (Fig. 2) (Sachs et al., 2000; Shi et al., 1996). According to the model, the unliganded TR expressed in premetamorphic tadpoles between stage 45 when a free feeding tadpole is formed (Nieuwkoop and Faber, 1956) and stage 55, just when endogenous TH becomes detectable (Fig. 2) (Leloup and Buscaglia, 1977), forms a heterodimer with RXR and the TR-RXR heterodimer binds to the TREs of TH-inducible genes, leading to the repression of their expression. This then ensures proper tadpole growth before metamorphic organ transformations. After stage 55, availability of TH allows the binding of TH to chromatin-bound TR and the TH-bound TR-RXR then activates these target genes to initiate metamorphosis in different organs and tissues (Fig. 2).

3.2. TR binds to the TREs of endogenous target genes during frog development

We used the chromatin immunoprecipitation assays (ChIP) to analyze the binding of TR to target genes during *X. laevis* development (Sachs and Shi, 2000). As the model predicted, there is little or no TR present at the TREs of two known direct TH-inducible genes, TRβ and TH/bZIP genes, in embryos but TR is present on the TREs in premetamorphic tadpoles when analyzed either in whole animals or in individual organs like the intestine and tail (Buchholz et al., 2005; Havis et al., 2003; Matsuda et al., 2009; Paul et al., 2005a,b; Sachs et al., 2002; Sachs and Shi, 2000; Tomita et al., 2004). Furthermore, quantitative ChIP assay showed that during metamorphosis or after TH-treatment of premetamorphic tadpoles, the binding of TR to the TREs increases, especially on the TH/bZIP TRE, which has a weaker affinity to TRs compared to the TRE in the TRβ in direct DNA binding assays *in vitro* (Buchholz et al., 2005; Matsuda et al., 2009). Similar results were also observed in *X. tropicalis* (Wang et al., 2008), a species highly related