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Review

Mechanisms of thyroid hormone receptor action during development: Lessons from amphibian studies☆

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ABSTRACT

Background: Thyroid hormone (TH) receptor (TR) plays critical roles in vertebrate development. However, the in vivo mechanism of TR action remains poorly explored.
Scope of review: Frog metamorphosis is controlled by TH and mimics the postembryonic period in mammals when high levels of TH are also required. We review here some of the findings on the developmental functions of TH and TR and the associated mechanisms obtained from this model system.
Major conclusion: A dual function model for TR in Anuran development was proposed over a decade ago. That is, unliganded TR recruits corepressors to TH response genes in premetamorphic tadpoles to repress these genes and prevent premature metamorphic changes. Subsequently, when TH becomes available, liganded TR recruits coactivators to activate these same genes, leading to metamorphic changes. Over the years, molecular and genetic approaches have provided strong support for this model. Specifically, it has been shown that unliganded TR recruits histone deacetylase containing corepressor complexes during larval stages to control metamorphic timing, while liganded TR recruits multiple histone modifying and chromatin remodeling coactivator complexes during metamorphosis. These complexes can alter chromatin structure via nucleosome position alterations or eviction and histone modifications to contribute to the recruitment of transcriptional machinery and gene activation.
General significance: The molecular mechanisms of TR action in vivo as revealed from studies on amphibian metamorphosis are very likely applicable to mammalian development as well. These findings provide a new perspective for understanding the diverse effects of TH in normal physiology and diseases caused by TH dysfunction. This article is part of a Special Issue entitled Thyroid hormone signalling.

1. Introduction

Thyroid hormones (TH) regulate multiple developmental and physiological functions in vertebrates. At the cellular level, 3,5,3′ triiodothyronine (T_3), the active form of TH, controls cell metabolism, proliferation, and commitment to differentiation or apoptosis. TH is synthesized in the thyroid gland. This organ has attracted the attention of physicians since antiquity. It was only 100 years ago that TH had begun to be characterized. In 1912, Friedrich Gudernatsch fed premetamorphic tadpoles with several horse organs and found that thyroid gland but none of the others could accelerate amphibian metamorphosis [1].

Amphibian development is a biphasic process. Embryonic stages and juvenile life are separated by a larval (tadpole) period that ends with metamorphosis. Metamorphosis is a switch that results in the reprogramming of the morphological and biochemical characteristics of nearly all tadpole organs, including de novo development (limbs), tissue remodeling (nervous system) and organ resorption (tail) [2]. These transformations involve apoptosis of larval cells and concurrent proliferation and differentiation of adult cell types.

Amphibian metamorphosis bears strong similarities with the perinatal (postembryonic) development in mammals at molecular and morphological levels [3]. First, both take place in a period when plasma TH levels peak during development. Second, both mammals and

Abbreviations: TH, Thyroid hormone; TR, Thyroid hormone receptor; T_3, 3,5,3′ triiodothyronine; TRE, TH response element; DR, direct repeat; IR, inverted repeat or palindrome; ER, everted repeat; RXR, retinoic acid X receptor; dp, dominant positive; dn, dominant negative; NCoR, Nuclear receptor CoRepressor; SMRT, Silencing Mediator for RAR and TR; ChIP, chromatin immunoprecipitation; HDAC, histone deacetylase; TBL1, transducin beta like protein 1; SRC, steroid receptor coactivator; CBP, p300/CREB binding protein; CARM1, coactivator associated arginine methyltransferase 1; PRMT1, Protein arginine methyltransferase 1; DRIP, vitamin D receptor interacting protein complex; TRAP, TR associated protein complex; ARC, activator recruited cofactor complex; BRG1, Brahma related gene 1; BAF57, BRG1 associated factor 57; HDM, histone demethylase; HAT, histone acetyltransferase; HMT, histone methyltransferase; ezh2, enhancer of zeste 2; H3, histone H3; H4, histone H4; Me2, Dimethyl; Me3, Trimethyl; K, Lysine; R, Arginine; X. laevis, Xenopus leavis; X. tropicalis, Xenopus tropicalis

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(most) anurans change their living habitat during this period, from an aquatic (amniotic) to a terrestrial environment. Third, many processes that occur during anuran metamorphosis resemble those occurring during postembryonic development in mammals [4,2]. These include skin keratinization, the induction of urea cycle enzymes, the switching of the hemoglobin genes from larval (tadpole) or fetal types to frog or adult types, respectively, and the developmental progression and restructuring of the central and peripheral nervous systems, etc. One of the best studied among them is the development of the adult intestine. Recent studies have shown that both the metamorphic intestinal remodeling in *Xenopus laevis* (X. laevis) and postembryonic intestinal maturation in mouse are dependent on TH and involve the formation of adult stem cells from pre-existing tadpole/neonatal intestinal epithelial cells [5,6]. Such findings suggest conserved molecular mechanisms governing a TH-dependent postembryonic development in vertebrates. Thus, amphibian metamorphosis can serve as a model to investigate the effects of TH on vertebrate development. More importantly, although TH is clearly important for embryogenesis in humans and other mammals, it has been difficult to investigate the mechanisms in mammals. This is in part due to the difficulty to manipulate the uterus-enclosed mammalian embryos and to separate the direct effects of TH on embryos from the indirect maternal effects caused by TH. The metamorphic changes in tadpoles occur in a free-living organism and can be easily controlled by adding TH or its inhibitors to the rearing water.

The diverse effects of TH suggest the existence of tissue specific and developmental stage specific control of gene expression by TH to coordinate different transformations in various organs. TH can affect gene expression by binding to TH receptors (TRs). TRs are transcription factors that belong to the superfamily of nuclear receptors [7,8]. TH is a versatile player, not only upregulating the expression of some genes but also downregulating expression of other genes. It is generally believed that both effects are mediated by TR. Studies on gene repression by TR in the presence of TH are very limited [9]. To date, most of the studies on the mechanisms of TR action have been carried out on positively regulated TH response genes. Extensive in vitro and cell culture analyses have shown that TRs bind to specific sequences present in the promoter regions of their target genes to regulate their expression. Molecular and genetic studies on postembryonic development in frogs coupled with in vivo analysis in the reconstituted frog oocyte transcription system have provided important insights on transcriptional activation and repression by TR during vertebrate development.

2. Gene regulation by thyroid hormone receptor

During the sixties, J.R. Tata showed that TH induced mRNA and protein synthesis in mammals [10] as well as in amphibians [11]. Several studies in mammals subsequently showed the existence of nuclear binding sites for TH [12] that led to the concept of TR. Later, the isolation of complementary DNA (cDNA) coding for *verbA* oncogene from avian erythroblastosis virus led to the cloning of the proto-oncogene *cerbA* that was identified as a TR [13,14]. A few years later the *X. laevis* TR, first from an amphibian, was cloned [15,16].

2.1. Amphibian thyroid hormone receptors

As in mammals there are two types of TR, α and β [16–20]. The amino acid sequences of the amphibian TRs are well conserved in evolution compared to their homologs described in mammals, fishes and chickens. Additionally, alternative splicing yields multiple mRNA isoforms similar to those found in mammals [16].

Xenopus TR, as most other nuclear receptors, can be organized from the amino to the carboxyl terminus into four domains that indicate differing functions, A/B, C, D and E; however, there can be overlap between the functions of each domain. The A/B domain is generally involved in the transcriptional activation and is highly variable in sequence and length, the shortest being in *X. laevis* TRβ1 where this domain is absent [16]. The recent isolation of TRβ2 from *Xenopus tropicalis* (X. tropicalis) led to the identification of an A/B domain with high sequence identity to the mammalian domain [LM Sachs unpublished data], further confirming the conservation of this domain's function in some amphibian isoforms.

The C domain is one of the signatures of all nuclear receptors. This highly conserved region is involved in DNA binding and thus in the specific recognition of TH response elements (TRE) present in TH response genes. The consensus TRE consists of two repeats of the hexameric AGGTCA sequence. In mammals, this half site can be configured in direct repeat (DR), inverted repeat or palindrome (IR) and everted repeat (ER). The hormonal specificity is dictated by the number of nucleotide spacing the two half sites as well as its direction. A DR with 4 nucleotide spacing most strongly binds to TR and is thus considered the highest affinity TRE. However, an IR with no nucleotide between the two half sites or an ER with 6 or 8 nucleotide spacing can also function as a TRE. In Xenopus, all the identified TREs are DR4 [21]. TRs bind to DNA as monomer, homodimer or heterodimer. The most frequent partner is the retinoic acid X receptor (RXR), a nuclear receptor that binds 9-cis retinoic acid. As in mammals, three heterodimeric partners (RXRα, RXRβ and RXRγ) have been cloned in Xenopus [22,23]. Binding of RXR ligand is not necessary for heterodimerization with TR or response to TH. However, RXR is a critical requirement for the developmental function of TR in amphibian. Without RXR, TR alone has little effect on TH response gene transcription in vivo [24].

The D domain is a hinge region that influences surrounding domains C and E and is involved in nuclear localization. The E domain is a complex region with multiple functions. Aside from its important role in ligand binding, it is also involved in the receptor dimerization (interaction with RXR), transcriptional activation in the presence of ligand and transcriptional repression in the absence of ligand. The E domain also interfaces with transcriptional machinery.

Thus, the amphibian TR behaves similarly to the mammalian and avian TR in terms of their secondary structure organization, ligand and DNA binding properties, and their requirement to heterodimerize with RXR for high affinity DNA binding [25]. Likewise, TH action appears conserved in amphibians. TH is versatile and while most direct target genes are upregulated, TH can also suppress gene expression. However, TR can bind to DNA in the presence and the absence of ligand. In the context of a positive TRE, transcription is activated by TH and repressed by TR binding in the absence of TH [26].

2.2. The dual function model

By activating or repressing transcription in a TH dependent manner, TR has dual functions. Considering the importance of TR function in *X. laevis* development, this model has provided a good system to examine the dual function of TR in vivo. Moreover, the relatively rapid and developmentally critical transition from low to high TH levels at the onset of metamorphosis makes Xenopus a unique and important model to study the dual functions of the receptor in vivo. In Xenopus, the duplicated TRα and TRβ genes are differentially regulated during development (Fig. 1; green and dark blue lines, respectively) [29,30]. TRβ genes were found to be direct response genes (Fig. 1) [31,32,20]. Furthermore, TR and RXR genes are coordinately regulated in different tissues during amphibian development [25,20]. The tissue distribution has shown a correlation between TR/RXR expression and organ transformation during metamorphosis [33,30,34]. While TR (such as TRα) is present during both larval and metamorphic periods, T_3 is present only during metamorphosis. Thus, according to the dual function model, TR functions in these two phases are distinct. First, during the larval period, unliganded TR should repress TH response genes (Fig. 1). Second, during the metamorphic period, liganded TR should activate TH target genes