

By Derek Bagley

UNLIGANDED THYROID HORMONE RECEPTOR May Play Role in Development

A novel animal study has uncovered clues to embryo developmental timing and growth rates. The study, recently published in *Endocrinology*, should prompt further research and improve the understanding on the role of the thyroid hormone (T3) signaling pathway during early development, according to lead author Yun-Bo Shi, PhD, of the Eunice Kennedy Shriver National Institute Child Health and Human Development.

T3 affects adult metabolism and postembryonic development in vertebrates by binding to thyroid hormone receptors (TRs) to regulate gene expression. Of the two TR genes, TR α and TR β , TR α is more ubiquitously expressed. The authors wrote, “During development, TR α expression appears earlier than T3 synthesis and secretion into the plasma. This and the ability of TRs to regulate gene expression both in the presence and absence of T3 have implicated a role of unliganded TR during vertebrate development.” The researchers also noted that it is difficult to study the role of unliganded TR during development in mammals due to the difficulty to manipulate the uterus-enclosed, late-stage embryos.

Since it was recently shown that transcriptional activator like effector nucleases (TALENs) can be used to knockout/knockdown genes in amphibians and zebrafish, the investigators used the amphibian *Xenopus tropicalis* (tadpole) to study how unliganded TR affects its development. That animal’s embryogenesis produces a free feeding tadpole in the absence of T3. “Subsequently,” the authors wrote, “as endogenous T3 becomes available, the tadpole is transformed into a frog in a metamorphic process that changes essential every organ/tissue of the animal.”

The scientists designed TALENS to mutate the TR α gene in the tadpoles and found that knockdown TR α enhances tadpole growth in premetamorphic tadpoles, likely through increased growth hormone gene expression. “More importantly,” the authors wrote, “the knockdown also accelerates animal development, with the knockdown animals initiating metamorphosis at a younger age and with a smaller body size. On the other hand, such tadpoles are resistant to exogenous T3 treatment and have delayed natural metamorphosis.”

Shi and his team concluded that their studies have not only directly demonstrated a critical role of endogenous TR α in mediating the metamorphic effect of T3 but also revealed novel functions of unliganded TR α during postembryonic development — regulating both tadpole growth rate and the timing of metamorphosis. The authors wrote that “mammalian development likely also utilizes unliganded TR, especially during early embryogenesis when T3 levels are low, to coordinate organ development and maturation, similar to premetamorphic tadpole development.”

“Given the conservation in vertebrate development,” Shi says, “our findings suggest that it is very likely that unliganded TR will affect mammalian embryo growth and development during the early stages when there is little or no circulating T3.”



Time-Restricted Feeding May Prevent Metabolic Diseases

Confining access to food to a set time may prevent metabolic diseases, a recent animal study published in the journal *Cell* has shown.

Researchers led by Satchidananda Panda, PhD, of the Salk Institute for Biological Studies, say that novel interventions are needed to treat

obesity, since current therapeutics are limited and offer only modest improvements. “Lifestyle interventions,” they wrote, “including changes in diet, reduced caloric intake, and increased exercise, have been the first-line therapy in efforts to combat obesity and metabolic diseases. However, these lifestyle changes require constant attention to nutrient quality and quantity and physical activity.” Panda and his team note that success with lifestyle

Unliganded Thyroid Hormone Receptor Function: Amphibian Metamorphosis Got TALENs

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Thyroid hormones (THs) are capital for development and cell homeostasis. THs act by regulating gene expression through TH receptors (TRs), which are transcription factors that belong to the nuclear receptors superfamily (1). THs and TRs are versatile players because they can not only up-regulate, but also down-regulate, gene expression in target cells. It is generally believed that TRs mediate both effects with their ability to bind DNA, regardless of the presence of its ligand. Unfortunately, the details of the mechanisms involved in transcriptional repression by THs remain enigmatic. However, most studies have focused on the mechanism of TR action on up-regulated TH-response genes. In brief, TRs repress the transcription in the absence of THs, whereas TRs lead to gene activation in the presence of THs. The fact that unliganded TR seems to work as an active repressor raises one main question. For instance, what is the physiological role of this active repression through unliganded TR?

Only few developmental roles of unliganded TRs have been described in mammals despite TRs expression when TH levels are absent or minimal (2). This physiological status is encountered during pregnancy when the thyroid gland is not yet functional. In this case, unliganded TRs may serve as a competence factors that enable tissues to respond upon TH release later. However, this is difficult to observe in mammals because THs may pass through the placenta, and thus, low levels of THs are present in the fetus. Low/no TH availability also occurs through the modulation of deiodinases activity in target cells (3). Deiodinases are enzymes involved in the activation of TH precursor but also in TH degradation that force the accumulation of unliganded TRs. The roles of unliganded TRs are not fully defined yet. Alternative models can then be

attractive. The Anura amphibians are a pertinent choice. In this issue of *Endocrinology*, two manuscripts from Wen and Shi (4) and Choi et al (5) present elegant works that manages to address the aforementioned question in amphibians.

Anura development is indirect with embryogenesis and adult stages separated by a larval period (corresponding to tadpole growth) that end with metamorphosis. Metamorphosis is a spectacular postembryonic transition. This developmental phase, which is strictly triggered by THs, corresponds to the perinatal period in mammals or to hatching in sauropsids, periods also marked by high levels and strong action of THs (6). In amphibians, a dual-function model for TR during development was proposed (7). In premetamorphic tadpoles in which TRs are expressed and TH levels are barely detectable, unliganded TRs represses transcription of target genes. During metamorphosis, endogenous THs allow TRs to activate gene expression, which leads to tadpole transformation. Although this model is strongly supported by mechanistic evidences (7), the *in vivo* function of unliganded TRs in the premetamorphic tadpoles is unknown. The work of Wen and Shi (4) and Choi et al (5) using a powerful promising technology clearly reach a breakthrough.

New genome editing technologies are popular tools to dissect developmental mechanism in a wide variety of organisms. *Xenopus* is a classical model widely used in the study of development, but its usefulness has been strongly limited by the lack of genetic tools (homologous recombination and embryonic stem cell derivation) that prevent gene targeting. Currently transcription activator-like effector nucleases (TALENs) have become effective tools for target gene editing/knockout in *Xenopus* (8). TALENs are

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Abbreviations: TALEN, transcription activator-like effector nuclease; TH, thyroid hormone; TR, TH receptor.

For articles see pages 721 and 735

artificial restriction enzymes generated by fusing a DNA binding domain engineered to bind practically any desired DNA sequence to a DNA cleavage domain, which then cut specifically DNA strands of any desired DNA sequence. Cells respond to double-strand breaks by DNA repair mechanisms, which introduce errors/indel at the repaired breakpoint, often resulting in a nonfunctional gene product. Because this method may introduce unwanted cleavage, genome integrity has to be controlled. This is especially important in *Xenopus tropicalis*, for which the genome sequence did not receive as much effort as human or mouse genome, although it is improving quickly.

The use of TALENs is clearly a key and powerful element of the work of Wen and Shi (4) and Choi et al (5). Their clever works illustrate the importance of nonconventional animal models in showing how they can reveal important biological processes before they can be verified and extended to mammals. Overall, using TALENs to disrupt TR α , they show directly that TR α is critical not only during metamorphosis but also prior to metamorphosis when most TRs are unliganded. TR α knockout leads to the increase of tadpole growth associated with an acceleration of development. Accordingly, metamorphosis initiates earlier but with a smaller body size, and metamorphosis occurs at a slower pace. At the molecular level, the expression of TH-regulated genes mirrors the phenotypes. The repression by unliganded receptors and the activation by liganded receptors were lost in the mutants compared with control animals. Thus, TH-response genes are expressed at a basal level in mutants. This explains nicely the earlier initiation of metamorphosis and its concomitant slow-down. Consequently, the novel functions of unliganded TR are linked to tadpole growth rate and the timing of metamorphosis. This being said, these works come with a lot of new challenges and questions.

A first point that now needs to be addressed is to determine the relative contribution of each TR isoform and their tissues specific effects. This is important because each isoform can have specific and/or redundant functions. Analysis of TALENs-mediated knockout of TR β (associated or not with that of TR α) is highly expected. A second question is related to the role of TRs during embryogenesis and organogenesis. This was suggested previously (9), but it was not observed in the present studies. The sensitivity of phenotype detection can be a concern because TRs could have many discrete and tissue-specific effects. The community working on amphibians must develop new

tools to detect not only anatomical phenotypes but also the effects on behavior, memory, or metabolism, to name a few. Lastly, it is generally considered that unliganded TR binds DNA but in a tissue- or gene-specific manner. What are the molecular mechanisms underlying this diversity? This is clearly a future challenge, and it is most likely that at least some of these answers may come from works with amphibian models using in part TALEN technology.

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COMMENTARY

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Unliganded TRs regulate growth and developmental timing during early embryogenesis: evidence for a dual function mechanism of TR action

Paul M Yen

Abstract

Recent genetic studies in the anuran *Xenopus tropicalis* reveal surprising new roles of thyroid hormone receptor in regulating growth and developmental timing in the absence of thyroid hormone.

Keywords: Thyroid hormone receptor, Vertebrate postembryonic development, Developmental timing, Organogenesis

Text

Thyroid hormone (T_3) regulates adult metabolism and postembryonic development in vertebrates [1]. T_3 functions mainly by binding to its receptors (TRs) to regulate gene expression. There are two TR genes, $TR\alpha$ and $TR\beta$, with $TR\alpha$ expressed more ubiquitously and earlier during development than $TR\beta$. TRs regulate target gene transcription in a T_3 -dependent manner. For T_3 -inducible genes that are positively regulated, TRs recruit coactivator and corepressor complexes in the presence and absence of T_3 , respectively. This leads to chromatin remodeling and changes in histone modifications to activate and repress target gene transcription [1-3]. This dual function mechanism suggests that both liganded and unliganded TRs have distinct physiological roles, particularly during embryonic stages when the fetus is exposed to little or no T_3 .

During mammalian development, $TR\alpha$ mRNA is detected earlier than endogenous T_3 , suggesting that TRs are mainly unliganded during these early stages. However, it has been challenging to ascertain whether unliganded TRs have transcriptional or physiological functions in a convincing manner since the transplacental supply of maternal T_3 to the fetus complicates any analysis in mammalian systems. In addition, it is difficult

to determine the functional role of unliganded TRs since it is not easy to manipulate their actions pharmacologically or genetically due to the uterine enclosure of mammalian embryos.

In two recent articles published in *Endocrinology*, the laboratories of Yun-Bo Shi of Eunice Kennedy Shriver National Institute Child Health and Human Development, and Daniel R. Buchholz of University of Cincinnati used *Xenopus tropicalis* as a model system to overcome some of these problems in studying the action of unliganded TRs during embryogenesis. Their findings have revealed novel functions for unliganded $TR\alpha$ in regulating embryonic animal development by taking advantage of the fact that anurans undergo biphasic development before and after metamorphosis. Accordingly, anurans first undergo embryogenesis to produce free-living tadpoles. After a period of growth, the tadpoles metamorphose into frogs via processes that are regulated by T_3 [4]. Similar to mammals, $TR\alpha$ mRNA is expressed very early and reaches high levels of expression by the end of embryogenesis when the feeding tadpole is formed and before synthesis of TH by the mature tadpole. Based on these and other observations, Shi's group proposed a dual function model for TR during frog development over a decade ago [5]. Initially, T_3 -inducible genes are expressed at basal levels during early embryogenesis when there is little T_3 and TR expression. At the end of embryogenesis, these genes are no longer needed for

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development; thus, a correspondingly high level of TR expression occurs during this stage in the relative absence of T_3 that, in turn, leads to transcriptional repression of target genes. Later, as T_3 becomes available, liganded TRs then activate the expression of T_3 -inducible genes that will play key roles in metamorphosis. Although extensive studies have demonstrated a critical role for liganded TRs in anuran development [6], there has been little direct evidence supporting the involvement of unliganded TRs in the transcriptional regulation of target genes *in vivo*.

To address this issue, the Shi and Buchholz groups ingeniously made use of the recently developed TALEN (transcriptional activator-like effector nuclease) method to investigate the role of TR α during the development of the diploid anuran *Xenopus tropicalis*. Both groups independently designed TALENs to specifically to knock down the TR α gene. Using complementary approaches, both teams demonstrated that removing TR α affected developmental timing in premetamorphic tadpoles, causing the animals to initiate metamorphosis earlier than their wild type siblings [7,8]. Consistent with the dual function model for TR, T_3 -target genes were de-repressed during the pre-metamorphic stages in TR α knockdown embryos since gene expression was higher than those in wild type embryos that expressed TR α during these same stages. Additionally, the knockdown embryos were resistant to exogenous T_3 treatment and underwent delayed metamorphoses. Surprisingly, Shi's group made the unexpected discovery that knockdown of TR α enhanced tadpole growth in premetamorphic tadpoles. This growth most likely occurred due to de-repression (*i.e.*, increase) of growth hormone gene expression. These two studies independently demonstrated the validity of the dual function mechanism of TR α during anuran development as they not only showed the critical role of endogenous TR α in mediating the metamorphic effect of T_3 but also revealed novel functions of unliganded TR α , that control both tadpole growth rate and timing of metamorphosis during post-embryonic development.

Vertebrate development is evolutionally conserved with frog metamorphosis resembling the postembryonic development period in mammals as the latter occurs shortly after birth and is accompanied by a burst of high production and circulating levels of T_3 [4]. Likewise, during early embryogenesis the fetus is in a relatively "hypothyroid" state as the fetal thyroid gland has not been formed yet and placental deiodinase III protects the fetus from exposure to maternal T_3 . It is likely that unliganded TR α plays major roles in repressing the transcription of important target genes during this period before they are activated later during pregnancy and the neonatal period. Therefore, the novel findings from these two studies strongly suggest that unliganded

TRs may play similar roles in the regulation of embryo growth and the timing of organ development and maturation in mammals during the stages when TR is expressed but there is little or no fetal exposure to T_3 . They also provide new insight into some of the developmental consequences within the fetus during iodine deficiency during pregnancy, congenital hypothyroidism, and maternal thyroid dysfunction.

Competing interests

The author declares that he has no competing interests.

Authors' contributions

Wrote commentary.

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