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.

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 TRα, they show directly that TRα is critical not only during metamorphosis but also prior to metamorphosis when most TRs are unliganded. TRα 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 TRβ (associated or not with that of TRα) 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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