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
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
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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### References


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

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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 (T₃) regulates adult metabolism and postembryonic development in vertebrates [1]. T₃ functions mainly by binding to its receptors (TRs) to regulate gene expression. There are two TR genes, TRα and TRβ, with TRα expressed more ubiquitously and earlier during development than TRβ. TRs regulate target gene transcription in a T₃-dependent manner. For T₃-inducible genes that are positively regulated, TRs recruit coactivator and corepressor complexes in the presence and absence of T₃, 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 T₃.

During mammalian development, TRα mRNA is detected earlier than endogenous T₃, 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 T₃ 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 TRα in regulating embryonic animal development by taking advantage of the fact that annurans undergo biphasic development before and after metamorphosis. Accordingly, annurans first undergo embryogenesis to produce free-living tadpoles. After a period of growth, the tadpoles metamorphose into frogs via processes that are regulated by T₃ [4]. Similar to mammals, TRα 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, T₃-inducible genes are expressed at basal levels during early embryogenesis when there is little T₃ and TR expression. At the end of embryogenesis, these genes are no longer needed for
development; thus, a correspondingly high level of TR expression occurs during this stage in the relative absence of T3 that, in turn, leads to transcriptional repression of target genes. Later, as T3 becomes available, liganded TRs then activate the expression of T3-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 TRs during the development of the diploid anuran Xenopus tropicalis. Both groups independently designed TALENs to specifically to knock down the TRα gene. Using complementary approaches, both teams demonstrated that removing TRα 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, T3-target genes were de-repressed during the pre-metamorphic stages in TRα knockdown embryos since gene expression was higher than those in wild type embryos that expressed TRα during these same stages. Additionally, the knockdown embryos were resistant to exogenous T3 treatment and underwent delayed metamorphoses. Surprisingly, Shi’s group made the unexpected discovery that knockdown of TRα 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 TRα during anuran development as they not only showed the critical role of endogenous TRα in mediating the metamorphic effect of T3 but also revealed novel functions of unliganded TRα 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 T3 [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 T3. It is likely that unliganded TRα 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 T3. 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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