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Xenopus metamorphosis as a model to study thyroid hormone receptor function during vertebrate developmental transitions

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ABSTRACT

A hormone-dependent developmental transition from aquatic to terrestrial existence occurs in all tetrapod vertebrates, such as birth, hatching, and metamorphosis. Thyroid hormones (TH) and their receptors (TRs) are key players in the tissue transformations comprising vertebrate developmental transitions. The African clawed frog, *Xenopus*, is a premier model for the role of TRs in developmental transitions because of the numerous and dramatic TH-dependent tissue transformations during metamorphosis and because of the endocrine, molecular, and genomic resources available. TRs are nuclear receptors that repress TH-response genes when plasma TH is minimal and that activate those same genes to induce tissue-specific gene regulation cascades when TH plasma levels increase. Tissue-specific TR expression levels help determine tissue sensitivity and responsivity to TH thereby regulating the initiation and rate of developmental change in TH-sensitive tissues which govern the tissue developmental asynchrony observed during metamorphosis. This review highlighting *Xenopus* presents the key experimental findings underpinning the roles TRs play in control of vertebrate developmental transitions.

1. Developmental transitions

A developmental transition from aquatic to terrestrial existence is a physical requirement for all terrestrial vertebrates. Vertebrate eggs and embryos all begin in an aqueous environment, either inside the mother, in a shelled egg, or free-living in water or moist areas. The transition to terrestrial living is often but not always coincident with lung maturational events and air breathing (mammals breath first at birth, but functional lung development in tadpoles occurs well before metamorphosis (Pronych and Wassersug, 1994; Rose and James, 2013)). In mammals in addition to breathing, physiological preparations for the abrupt loss of placental functions at birth include increased liver gluconeogenesis and glycogen storage, kidney glomerular filtration and sodium reabsorption, acid and digestive enzyme secretion in the gut, and switching from fetal to adult hemoglobin and liver to bone marrow hematopoiesis (Fowden et al., 1998; Liggins, 1994). Frog metamorphosis represents an extreme, where nearly all aspects of the tadpole change to accommodate the terrestrial existence, including gain of limbs, loss of tail and gills, switching of liver metabolism, and remodeling of the cranial cartilages, the intestine, and the skin (Dodd and Dodd, 1976). Aquatic tetrapods, e.g., marine mammals and reptiles and many salamanders (e.g., *Crypotbranchus*, *Dicamptodon, Rhyacotriton*, *Amphiuma*) and some frogs (e.g., *Telmatobius*, pipids including *Xenopus*), still have a developmental transition, where they retain numerous ancestral features of the aquatic to terrestrial transition. No tetrapod lacks such a developmental transition, except perhaps neotenic salamanders though they too have cryptic/subtle hormone-dependent post-embryonic physiological changes not related to sexual maturity (Laudet, 2011; Vlaeminck-Guillem et al., 2006).

2. Hormonal control of developmental transitions

An important aspect of all vertebrate aquatic to terrestrial developmental transitions is coordination in the timing of the changes among tissues. Whereas many features of morphology and physiology are compatible with both aquatic and terrestrial lifestyles, some changes must occur in preparation for or immediately at the aquatic to terrestrial transition, such as air breathing, skin cornification, and switching of hemoglobin and liver metabolism (Fowden et al., 1998; Liggins, 1994). Vertebrate species vary in the set of tissues/organs that need strict coordination at the

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developmental transition depending on their life history strategies, such that some terrestrial features may be present in the aquatic phase well before the transition without negative consequences. For example, human intestine histogenesis is complete at seven months and skin cornifies six months prior to birth (Carlson, 2014), whereas frog intestine and skin remodeling events are tied to the metamorphic transition (Dodd and Dodd, 1976). In addition, some tissue transformations within the developmental transition do not occur exactly at the same time, a phenomenon called tissue developmental asynchrony (Furlow and Neff, 2006; Shi et al., 1996). For example, tadpole tail resorption occurs after hind limb development and outgrowth to maintain locomotor ability.

The main mechanism to coordinate developmental events among tissues during the aquatic to terrestrial transition is afforded by hormones traveling in the blood providing a signal to all organs simultaneously. The main hormones inducing developmental changes are thyroid hormone (TH) and a glucocorticoid hormone (GC, either cortisol or corticosterone) (Dodd and Dodd, 1976; Fowden and Forhead, 2013; Hillman et al., 2012). In all vertebrates, a peak in TH and GC occurs at the developmental transition and is determined by the neuroendocrine system based on internal and external inputs (Buchholz, 2015; Denver, 2013; Laudet, 2011). When the developmental timing of terrestrial features is not critical, development might not be under hormonal control, e.g., skin cornification and intestine histogenesis still occurs in humans with cretinism or severely reduced TH production (Delange, 2005).

Even though both TH and GC seem to be involved in all aquatic to terrestrial transitions, this review focuses on the role of TH and their receptors (TRs). TRs are nuclear receptors that constitutively bind TH response elements located in the genome in and around TH-regulated genes to control their transcription, where TH-response genes may be induced or repressed in response the TH (Cheng et al., 2010; Yen, 2001). The molecular mechanisms of gene repression by TH is not well understood, but in the case of TH-induced genes, absence of TH in the cell nucleus allows TRs to recruit co-repressors to reduce transcription of TH-response genes, and the presence of TH causes a conformational change in TR to allow co-activators to replace co-repressors and induce transcription of TH-response genes (Das et al., 2010; Wen and Shi, 2016). All tetrapods have two isoforms of TR, namely TRα and TRβ, which have different tissue distributions and developmental expression profiles (Laudet and Gronemeyer, 2002; Ng and Forrest, 2006; Shi et al., 1996). Distinct developmental roles attributable to the two TR isoforms are due mostly to their differential expression and perhaps to possible isoform-specific gene regulation (Cheng et al., 2010; Denver et al., 2009; Nunez et al., 2008).

3. Utility of *Xenopus* as a model for TR function in development

Model organisms provide exaggerated and/or simplified systems that are accessible and easily manipulated and can reveal the basic operating principles that are nearly the same across vertebrates. For elucidating the roles of TH signaling during development, the African clawed frogs, *Xenopus laevis* and *X. tropicalis*, have several intrinsic experimental advantages that make them an excellent model (Buchholz, 2015; Harland and Grainger, 2011; Sachs and Buchholz, 2017; Shi, 1999, 2009). First, the dramatic TH-dependent molecular and morphological changes that occur during metamorphosis are unrivaled in scope among terrestrial vertebrates (Dent, 1968), and signaling via TH and their receptors is necessary and sufficient to initiate virtually all metamorphic events (Das et al., 2010). Second, mechanisms of TH signaling in gene regulation and development are highly conserved between frogs and other vertebrates (Furlow and Neff, 2006; Sachs and Buchholz, 2017). Indeed, TH-dependent metamorphosis is comparable to perinatal stages in mammals and hatching in birds (Buchholz, 2015; McNabb, 2007). Third, tadpoles are large and accessible throughout their development, including TH-dependent stages, and *Xenopus* produces large numbers of free-living eggs and embryos that are easy to culture without specialized media or temperature requirements making tadpole studies fast, easy, and cheap with respect to comparable, perinatal stages in mammals. Fourth, plasma levels of TH undetectable by radioimmunoassay occur naturally during the frog larval period prior to metamorphosis (Leloup and Buscaglia, 1977), indicating that virtually all TH receptors *in vivo* are in the unliganded condition, such that simple exogenous addition of TH to the rearing water during pre-metamorphosis enables precise temporal control of TH receptors to the liganded state that can mimic natural metamorphosis (Shi, 1999). Fifth, *Xenopus* has all the modern tools of a genetic model system, such as a sequenced genome (Hellsten et al., 2010; Session et al., 2016), an ORFeome (Grant et al., 2015), a model organism database (James-Zorn et al., 2015), and established methods for gene knockout (Tandon et al., 2016) and transgenesis (Buchholz, 2012; Das and Brown, 2004; Ishibashi et al., 2012). Studies to elucidate developmental mechanisms of TH signaling are constrained in mammalian systems by the difficulty of observing relatively subtle or cryptic TH-dependent changes and of obtaining samples from fetuses *in utero*. An additional difficulty is that fetal tissues are constantly exposed to maternal hormones through the placenta (Forhead and Fowden, 2014), such that manipulation of fetal endocrine signaling to examine receptor function in plus or minus hormonal states is difficult to achieve without potentially introducing artefacts from altered maternal endocrine physiology. Thus, *Xenopus* development is a particularly compelling model system for use in elucidating TH signaling applicable to developmental transitions in other vertebrates.

4. Early breakthroughs in frog metamorphosis

Early work in ranid frogs, beginning over 100 years ago, identified the first developmental action of TH in vertebrates (i.e., induction of morphological changes of frog metamorphosis) and the role of the hypothalamus and pituitary in control of TH production (Allen, 1938; Etkin, 1964; Gudernatsch, 1912; Lynn and Wachowski, 1951). Later, biochemical changes induced by TH in terms of overall DNA, RNA, and protein synthesis and enzyme activity were examined in ranid frogs and in *Xenopus* (Frieden and Just, 1970; Galton, 1983; Tata, 1965). Cloning of the TRs in *Xenopus* and bullfrogs launched the molecular phase of analysis of TH and development by revealing that TH receptors (TRs) are ligand-activated nuclear receptors and that TH-induced genes constitute a gene regulation cascade (Brown et al., 1995; Davey et al., 1994; Helbing et al., 1992; Shi, 1999; Yaoita et al., 1990). We are currently in the era of continued molecular examination uncovering additional molecular mechanisms of gene regulation *in vivo* by using transgenic overexpression, ChIP assay, and bioinformatics (Buchholz et al., 2003, 2004; Das et al., 2006; Sachs and Shi, 2000). The remaining sections address the current state of molecular analysis performed predominantly in *Xenopus* that support a dual function model for the role of TRs in development and the contributions of TRs to developmental transitions.

5. Dual function model

The dual function model describes the role of TRs in control of gene regulation and developmental timing during ontogeny (Buchholz et al., 2006; Sachs et al., 2000; Shi, 2009; Wen and Shi, 2016). The dual function model was developed based on the

knowledge of the role of ligand in control of TR-mediated transcription and the developmental expression profiles of TRα and TRβ with respect to the peak of plasma TH at the developmental transition. TRα is expressed in most tissues when plasma TH is minimal, prompting the question of its function before TH signaling is known to occur. The dual function model states that TRs act as transcriptional repressors in the absence of plasma TH to keep genes important for metamorphosis turned off and allow tadpole growth until TH becomes available, at which point TRs become transcriptional activators to induce TH-response genes and consequent metamorphic changes. Extensive evidence from ChIP assays and transgenic overexpression studies support the gene induction role of TRs during development (Buchholz et al., 2006; Shi, 2009). However, support for a role of TRs actively repressing genes having a developmental role has been equivocal.

Recently, two studies using TRα knockout *Xenopus* provided strong evidence for gene repression by TR prior to circulating TH (Choi et al., 2015b; Wen and Shi, 2015). In frogs, hind limb development and outgrowth is nearly completely dependent on signaling via TRα (Cai and Brown, 2004; Furlow et al., 2004). Knockout of TRα led to precocious hind limb development with early increased expression of TH-response genes important for metamorphosis due to lack of TRα-mediated repression, in complete agreement with the dual function model (Choi et al., 2015b; Wen and Shi, 2015). Further studies in intestine showed similar results of de-repressed levels of TH response genes when plasma TH is undetectable (Choi et al., 2017). Results from knockout studies in mice are consistent with the dual function model (Bernal and Morte, 2013; Morte et al., 2002), but few mouse studies have examined such roles for TR, likely due to the inherent difficulties for these experiments in mammals.

6. Application of the dual function model to developmental transitions

The aquatic to terrestrial developmental transition in vertebrates comprises the coordination of hormone-dependent tissue transformations within a relatively short post-embryonic period. The dual function model explains the function of TRs on gene regulation in any one tissue at any one time, but the relative timing of the switch from repression to activation among tissues is controlled by mechanisms other than those addressed by the dual function model. Also, coordination of tissue transformations does not come from communication among transforming organs, as no such communication is known to exist, especially considering that tadpole organs from *Xenopus* and other species can be induced to transform independently in organ culture *in vitro*, including tail, hind limbs, skin, intestine, liver, and lung (Buchholz and Hayes, 2005; Derby, 1968; Helbing et al., 1992; Ishizuya-Oka and Shimozawa, 1991; Mathisen and Miller, 1989; Tata et al., 1991; Veldhoen et al., 2015). Similarly, transgenic overexpression of a dominant negative TR, which constitutively represses TH-response genes and fails to induce them even in the presence of TH, reveals striking tissue autonomy when expressed in individual tissues, where nerves, muscle, cartilage, and skin of the hind limb transform independently of one another (Brown et al., 2005; Das et al., 2002; Marsh-Armstrong et al., 2004).

Coordination of tissue transformations during the developmental transition comes from the tissue autonomous amount of TH signaling as determined by a combination of 1) plasma TH levels and 2) tissue-specific sensitivity and responsivity to plasma TH levels. Tissue sensitivity and responsivity ties the tissue-specific timing of the transformation of each tissue to the developmental profile of plasma TH levels. The dual function model explains what TRs do, not when they do it. Thus, to understand the role of TRs in developmental transitions, we need to understand how TRs contribute to control of the hormone profile and tissue sensitivity and responsivity (Fig. 1).

7. Global timing of the developmental transition and role of TR

Aquatic to terrestrial developmental transitions are associated with elevated plasma TH levels around birth, hatching, and metamorphosis. The developmental TH profile is controlled centrally by the hypothalamus - pituitary - thyroid gland axis. In tadpoles, brain processing of internal signals and external cues cause release of corticotropin releasing hormone from the hypothalamus that stimulates release of thyroid stimulating hormone from the pituitary that signals the thyroid to secrete TH into the blood (Denver, 2013). The duration of elevated THs is highly variable among vertebrates but tends to be at least 1–2 weeks or longer (Forhead and Fowden, 2014; Leloup and Buscaglia, 1977) The mechanisms controlling the shape, duration, and peak height of the plasma TH profile are largely unknown. Early studies in ranid frogs suggested that TRs contribute to the plasma TH profile by their action on vascularization and maturation of the median eminence to increase the ability of the hypothalamus to signal to the pituitary (Etkin, 1965, 1968).

Recently, it was found using TRα knock out *Xenopus* that overall duration of the metamorphic transition was shortened, via unknown mechanisms (Choi et al., 2017). During the developmental stages that are dependent on TH (i.e., from limb outgrowth to tail resorption in wild-type animals), TRα knock out animals achieved those stages and completed metamorphosis earlier in time compared to wild-type siblings. Untested possibilities include 1) the median eminence was able to develop precociously from lack of TRα-mediated repression allowing precocious rise in plasma TH, 2) unknown other precocious development of the hypothalamus - pituitary - thyroid axis development, 3) accelerated transformation among virtually all tadpole organs (which express TRα and TRβ) due to de-repression of metamorphic genes including TRβ, in effect "priming" them for increased responsivity once TH is present in circulation.

8. Role of TR in tissue sensitivity regulating timing of tissue transformations

Tissue sensitivity refers to the threshold level of plasma TH at which a tissue is able to respond to the TH signal. The ability of different tissues to initiate development in thyroidectomized ranid tadpoles was dependent on the concentration of exogenously administered TH, showing differential tissue sensitivity (Kollros, 1961). As examined in *Xenopus*, tissue sensitivity is determined by expression levels and activities of TH signaling genes, which include TH cytoplasmic transporters, deiodinases, cytoplasmic TH binding proteins, TRs, and TR transcriptional co-factors (Brown, 2005; Choi et al., 2015a; Matsuda et al., 2009; Nakajima et al., 2012; Paul et al., 2007; Shi et al., 1996). TH transporters, deiodinases, and cytoplasmic proteins regulate the amount of active TH that gets to the nucleus from outside the cell (Bianco and Kim, 2006; Brown, 2005; van Mullem et al., 2016; Visser et al., 2010; Yamauchi and Tata, 1997), and TR and TR co-factor expression levels determine degree of altered TH-response gene regulation based on the intracellular TH level (Matsuda et al., 2009; Nakajima et al., 2012; Paul et al., 2007). Collectively, the actions of these TH signaling genes within any one cell or tissue control whether or not TH signaling can occur. Coordination of timing of transformation among tissue then comes from each tissue having tissue-specific expression levels of TH signaling genes that trigger tissue

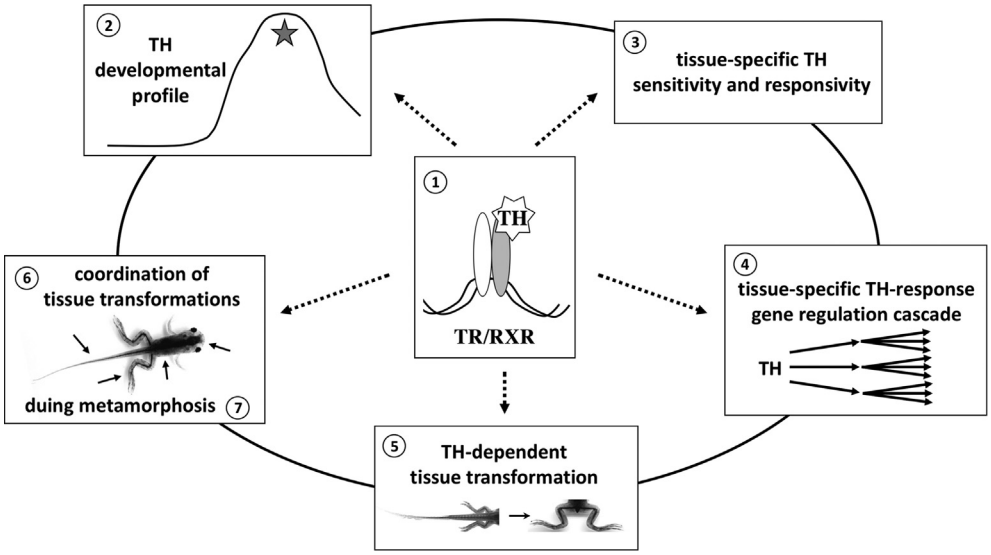

Fig. 1. Levels of TH involvement in developmental transitions. (1) Thyroid hormone (TH) receptors (TRs) regulate metamorphic progression at endocrine, molecular, developmental, and organismal levels (dashed arrows). TRs bind TH and heterodimerize with RXR (retinoid-X receptor) and constitutively bind DNA to regulate the expression of TH response genes. (2) Rise in plasma TH under neuroendocrine control initiates metamorphosis. The star indicates the peak of plasma TH levels achieved during metamorphosis. TRs may regulate several aspects of neuroendocrine control of plasma TH levels. (3) Tissue sensitivity and responsivity enable tissues to receive and react to the TH signal from the blood. TR expression levels directly contribute to a tissue's ability to respond to TH. (4) Tissue-specific gene regulation cascades are the first response of tissues to TH signaling. Interactions of TR with tissue-specific transcription factors and chromatin modifications determine the specific gene regulation cascades. (5) Tissue transformations result as a consequence of altered TH-response gene regulation. Signaling through TR is required for tissue transformations to occur in wild-type animals. (6) Coordination of tissue transformations occur within a relatively short period of time, including leg outgrowth, tail and gill resorption, and brain and intestine remodeling (little arrows). Coordination is affected by TRs through their contribution to tissue sensitivity and responsivity. (7) Metamorphosis is the sum total of the coordinated tissue transformations.

transformation corresponding to each tissue's threshold plasma TH level.

Direct experimental evidence for the role of TRs in tissue sensitivity during development stems from experiments in tadpoles of *Xenopus*. *In-vivo* gene transfection to overexpress TR in tadpole tail muscle cells decreased the TH concentration required (and thus increased the sensitivity) for the transfected cells to die by apoptosis (Nakajima et al., 2012). Also, *Xenopus* transgenesis methods lead to variable expression levels of transgenes due to position site variegation, such that transgenesis to overexpress dominant negative TRs leads to variable amounts of resistance to TH-induce transformation (Schreiber et al., 2001), showing that TR signaling capacity can control the TH level required to initiate tissue transformation.

9. Role of TR in tissue responsivity regulating timing of tissue transformations

Tissue responsivity refers to the rate of TH-induced gene regulation and phenotypic change. For example, tadpole tail tips in culture shrink faster when treated with higher doses of TH in a dose-dependent manner, as shown in *Xenopus* and desert spadefoot toads (Buchholz and Hayes, 2005; Derby, 1968). Tissue responsivity and sensitivity are related in that more capacity for TH signaling (i.e., higher expression of TH signaling genes) explains higher tissue sensitivity as well as higher tissue responsivity. It is not clear if higher tissue responsivity is reflected in a higher maximum expression level of TH response genes or only achieves the maximum earlier. Correlational and experimental studies have shown that TR expression level can affect tissue responsivity directly. In *Xenopus*, hind limbs and brain express higher levels of TRα than other tissues before metamorphosis starts (Wang et al., 2008), and hind limb outgrowth and brain neuron proliferation are among the earliest morphological changes evident in tadpoles in natural and TH-induced metamorphosis, shown first in ranid frogs (Dodd and Dodd, 1976; Kollros, 1981). Experimental overexpression of TR by *in-vivo* gene transfer in *Xenopus* tail muscle cells increased the rate of muscle cell apoptosis in response to exogenous TH treatment (Hollar et al., 2011; Nakajima et al., 2012). In TRα knock out *Xenopus* tadpoles, their tissues showed extreme reduction in ability to respond to TH, again showing the TR expression levels affects responsivity to TH (Choi et al., 2015b). In addition, TRs regulate tissue responsivity by upregulating TH signaling genes. For instance, many tissues have autoinduction of TRβ and induction of the TH transporter, LAT1 (Baker and Tata, 1990; Ritchie et al., 2003; Shi et al., 2002; Tata et al., 1993). Increased expression of these TH signaling genes increases the TH signaling capacity and thus the responsivity of the tissue.

10. TR actions in developmental transitions beyond regulating sensitivity and responsivity

10.1. Sharpness of developmental transitions

Regulation of tissue transformation by TR is expected to cause their transformation to be of shorter duration, thereby producing a sharp transition from aquatic to terrestrial state. Control of development by TRs may have this effect because of the molecular mechanism of gene regulation by TR. Prior to the developmental transition with low or no plasma TH, TR-mediated repression keeps metamorphic genes turned off thus blocking any initiation of transformation (Choi et al., 2015b; Shi, 2009; Wen and Shi, 2015). As soon as a tissue-specific threshold level of plasma TH occurs, TRs then induce metamorphic genes previously repressed to immediately initiate developmental events. The sharpness in timing of transformation is predicted to be increased by autoregulation of TRβ and LAT1 (a TH transporter), which increases tissue responsivity and thus rate of response to TH (Choi et al., 2015a; Nakajima et al., 2012; Ritchie et al., 2003). Indeed, increasing signaling by treating tadpoles with exogenous TH reduces the total time

required for a tissue to transform for all known frog species (Dodd and Dodd, 1976; Shi, 1999). Sharp transitions can be shown experimentally by blockade of TH signaling, via throidectomy in ranids and transgenic overexpression in *Xenopus* of the TH-degrading enzyme deiodinase type 3, which each can inhibit progress of transformation, such that not even slow rates of transformation occur (Dodd and Dodd, 1976; Huang et al., 1999). Addition of TH to thyrodectomized tadpoles immediately initiates tissue transformation, creating a sharp temporal boundary for timing of TH-dependent transformations (Dodd and Dodd, 1976). In tissues not under TH control, developmental timing is not affected by lack of TH, e.g., the gonads in ranids (Hoskins and Hoskins, 1919; Swingle, 1918). However, studies using TRα knockout *Xenopus* fail to support a role for TR in controlling sharpness of developmental timing (Choi et al., 2017). Lack of TRα allowed expression of low levels of metamorphic genes with rate of progress through tissue transformation in TRα-dependent tissues, e.g., hind limb comparable to wild-type tadpole, where the same number of days were required to pass through TH-dependent hind limb stages (~15 days from NF54-57). Additional studies on other TH-dependent tadpole tissues in TRα and TRβ double knockout tadpoles are needed to identify a potential role of TR in control of sharpness of the transition. Such mechanisms may be informative in evolutionary studies, where evolutionary alteration of the degree or dependence of TH signaling may underlie phenotypic differences in TH-dependent traits among species (Buchholz and Hayes, 2005; Hollar et al., 2011).

10.2. TH-response gene induction and cell fate determination

The dual function model indicates that gene induction by TH signaling is required for developmental progression of TH-dependent events. However, hind limbs, which are believed to be dependent on TRα only, developed to their full extent in TRα knock out animals (Choi et al., 2017). This result implies that levels of gene induction afforded by TH/TR during normal wild-type development is not necessarily required to achieve tissue transformation. Rather, de-repressed levels of TH-response genes appear to be sufficient to allow transformation to proceed to completion, at least in hind limb. Whether or not these effects of TRα in hindlimb generalize to other TH-dependent tissues that express significant levels of TRβ in addition to TRα will await examination using TRα/β double knockouts.

Another implication from the complete hind limb development in TRα knock out animals is that TH signaling apparently allows a predetermined fate to occur rather than determining the nature of the transformation. In wild-type animals, lack of TH signaling (due to thyroidectomy in ranids or methimazole treatment in *Xenopus*) puts developmental fate virtually on hold rather than allowing the tissue/organ to develop along a different developmental path (Brown et al., 2005; Brown and Cai, 2007; Hoskins and Hoskins, 1919). Also, inducible overexpression of a dominant positive TR in transgenic *Xenopus* induced normal transformation in the many tissues examined, suggesting that TR signaling is sufficient to allow development to proceed (Buchholz et al., 2004). Thus, TH signaling in a tissue seems to act like opening a flood gate rather than swiveling a railroad switch telling tissues not what to do, but when to do it.

On the other hand, in some cases, treatment with exogenous TH does not recapitulate natural metamorphosis. A clear example is lung development in bullfrogs, where TH treatment causes thickened connective tissue rather than a thin tissue for gas exchange observed in natural metamorphosis (Atkinson and Just, 1975). However, the reason for this lack of induction of normal development by TH signaling alone is not known. Possible explanations are that other hormones are required to interact with TH in some tissues, the tissues may not be fully competent to respond to TH appropriately, or the doses of TH may be pharmacological resulting in unnatural phenotypes. Speaking to the possibility of other hormone involvement, a microarray analysis of gene expression after TH and/or corticosterone treatment showed that regulation of nearly 20% of the genes regulated by TH was antagonized by corticosterone, indicating that the nature of tissue transformation may not simply be characterized as a switch to allow development to occur (Kulkarni and Buchholz, 2012). Also, hypophysectomized tadpoles of *Alytes* treated only with TH failed to metamorphose unless stress hormone was also added (Remy and Bounhiol, 1971).

11. Conclusions and future directions

Numerous insights into the roles of TRs in developmental transitions have come from studies on metamorphosis in *Xenopus* and other frog species. TRs contribute to aquatic to terrestrial developmental transitions at several levels by regulating TH-response genes underlying tissue transformations and by controlling the timing of these transformations throughout the organism via their roles in tissue sensitivity and responsivity to TH (Fig. 1). TH signaling seems to affect only timing rather than fate of tissue transformations, though there is evidence that TH alone is not sufficient to complete metamorphosis (Atkinson and Just, 1975; Remy and Bounhiol, 1971). Additional fundamental aspects of TR in development will come through continued molecular, endocrine, and developmental analyses using transgenic and knockout frog models. Promising directions particularly suitable for *Xenopus* studies include 1) using global methods of genome and network analysis to better understand mechanisms of gene regulation particularly as it relates to the epigenetic mechanisms involved (Sachs and Buchholz, 2017), 2) examining new indications that thyroxine (T4), triiodothyronine (T3), and additional iodothyronines may have distinct and relevant biological roles (Helbing et al., 2007; Maher et al., 2016; Mendoza et al., 2013), 3) determining how multiple hormones interact, such as TH and stress hormones, to accomplish the developmental transition, especially how the environment may modulate the timing of developmental transitions (Denver, 2009; Kaltenbach, 1996; Kulkarni and Buchholz, 2014), 4) elucidating mechanisms and consequences of endocrine disrupting chemicals on development (Hayes et al., 2006; Helbing, 2012; Opitz et al., 2005), and 5) providing insights into how evolutionary changes in control of developmental transitions by TH signaling may explain divergent phenotypes among species (Buchholz et al., 2011).

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References

Allen, B.M., 1938. The endocrine control of Amphibian metamorphosis. Biol. Rev. 13, 1–19.
Atkinson, B.G., Just, J.J., 1975. Biochemical and histological changes in the respiratory system of *Rana catesbiana* larvae during normal and induced metamorphosis. Dev. Biol. 45, 151–165.
Baker, B.S., Tata, J.R., 1990. Accumulation of proto-oncogene c-erb-A related transcripts during Xenopus development: association with early acquisition of response to thyroid hormone and estrogen. Embo J. 9, 879–885.
Bernal, J., Morte, B., 2013. Thyroid hormone receptor activity in the absence of ligand: physiological and developmental implications. Biochimica Biophysica Acta 1830, 3893–3899.

Bianco, A.C., Kim, B.W., 2006. Deiodinases: implications of the local control of thyroid hormone action. J. Clin. Invest 116, 2571–2579.
Brown, D.D., 2005. The role of deiodinases in amphibian metamorphosis. Thyroid 15, 815–821.
Brown, D.D., Cai, L., 2007. Amphibian metamorphosis. Dev. Biol. 306, 20–33.
Brown, D.D., Cai, L., Das, B., Marsh-Armstrong, N., Schreiber, A.M., Juste, R., 2005. Thyroid hormone controls multiple independent programs required for limb development in Xenopus laevis metamorphosis. Proc. Natl. Acad. Sci. U. S. A. 102, 12455–12458.
Brown, D.D., Wang, Z., Kanamori, A., Eliceiri, B., Furlow, J.D., Schwartzman, R., 1995. Amphibian metamorphosis: a complex program of gene expression changes controlled by the thyroid hormone. Recent Prog. Horm. Res. 50, 309–315.
Buchholz, D.R., 2012. Tet-On binary systems for tissue-specific and inducible transgene expression. Methods Mol. Biol. 917, 265–275.
Buchholz, D.R., Moskalik, C., Kulkarni, S., Hollar, A., Ng, A., 2011. Chapter 7: hormone regulation and the evolution of frog metamorphic diversity. In: Flatt, T., Heyland, A. (Eds.), Mechanisms of Life History Evolution. Oxford University Press, Oxford, 87–87-97.
Buchholz, D.R., 2015. More similar than you think: frog metamorphosis as a model of human perinatal endocrinology. Dev. Biol. 408, 188–195.
Buchholz, D.R., Hayes, T.B., 2005. Variation in thyroid hormone action and tissue content underlies species differences in the timing of metamorphosis in desert frogs. Evol. Dev. 7, 458–467.
Buchholz, D.R., Hsia, S.C., Fu, L., Shi, Y.B., 2003. A dominant-negative thyroid hormone receptor blocks amphibian metamorphosis by retaining corepressors at target genes. Mol. Cell. Biol. 23, 6750–6758.
Buchholz, D.R., Paul, B.D., Fu, L., Shi, Y.B., 2006. Molecular and developmental analyses of thyroid hormone receptor function in Xenopus laevis, the African clawed frog. Gen. Comp. Endocrinol. 145, 1–19.
Buchholz, D.R., Tomita, A., Fu, L., Paul, B.D., Shi, Y.B., 2004. Transgenic analysis reveals that thyroid hormone receptor is sufficient to mediate the thyroid hormone signal in frog metamorphosis. Mol. Cell. Biol. 24, 9026–9037.
Cai, L., Brown, D.D., 2004. Expression of type II iodothyronine deiodinase marks the time that a tissue responds to thyroid hormone-induced metamorphosis in Xenopus laevis. Dev. Biol. 266, 87–95.
Carlson, B.M., 2014. Human Embryology and Developmental Biology, fifth ed. Elsevier/Saunders, Phladelphia, PA.
Cheng, S.-., Leonard, J.L., Davis, P.J., 2010. Molecular aspects of thyroid hormone actions. Endocr. Rev. 31, 139–170.
Choi, J., Ishizuya-Oka, A., Buchholz, D.R., 2017. Growth, development, and intestinal remodeling occurs in the absence of thyroid hormone receptor alpha in tadpoles of *Xenopus tropicalis*. Endocrinology. http://dx.doi.org/10.1210/en.2016-1955.
Choi, J., Moskalik, C.L., Ng, A., Matter, S.F., Buchholz, D.R., 2015a. Regulation of thyroid hormone-induced development in vivo by thyroid hormone transporters and cytosolic binding proteins. Gen. Comp. Endocrinol. 222, 69–80.
Choi, J., Suzuki, K.T., Sakuma, T., Shewade, L., Yamamoto, T., Buchholz, D.R., 2015b. Unliganded thyroid hormone receptor alpha regulates developmental timing via gene repression as revealed by gene disruption in Xenopus tropicalis. Endocrinology 156, 735–744.
Das, B., Brown, D.D., 2004. Controlling transgene expression to study Xenopus laevis metamorphosis. Proc. Natl. Acad. Sci. U. S. A. 101, 4839–4842.
Das, B., Cai, L., Carter, M.G., Piao, Y.L., Sharov, A.A., Ko, M.S., Brown, D.D., 2006. Gene expression changes at metamorphosis induced by thyroid hormone in Xenopus laevis tadpoles. Dev. Biol. 291, 342–355.
Das, B., Matsuda, H., Fujimoto, K., Sun, G., Matsuura, K., Shi, Y.B., 2010. Molecular and genetic studies suggest that thyroid hormone receptor is both necessary and sufficient to mediate the developmental effects of thyroid hormone. Gen. Comp. Endocrinol. 168, 174–180.
Das, B., Schreiber, A.M., Huang, H., Brown, D.D., 2002. Multiple thyroid hormone-induced muscle growth and death programs during metamorphosis in Xenopus laevis. Proc. Natl. Acad. Sci. U. S. A. 99, 12230–12235.
Davey, J.C., Schneider, M.J., Galton, V.A., 1994. Cloning of a thyroid hormone-responsive Rana catesbeiana c-erbA-β gene. Dev. Genet. 15, 339–346.
Delange, F.M., 2005. Endemic cretinism. In: Braverman, L.E., Utiger, R.D. (Eds.), Werner & Ingbar's the Thyroid: a Fundamental and Clinical Text, ninth ed. Lippincott Williams and Wilkins, Philadelphia, pp. 731–744.
Dent, J.N., 1968. A survey of amphibian metamorphosis. In: Etkin, W., L.I.G (Eds.), Metamorphosis. A Problem in Developmental Biology. Appleton-Crofts, New York, pp. 271–311.
Denver, R.J., 2013. Neuroendocrinology of Amphibian metamorphosis. Curr. Top. Dev. Biol. 103, 195–227.
Denver, R.J., 2009. Stress hormones mediate environment-genotype interactions during amphibian development. Gen. Comp. Endocrinol. 164 (1), 20–31.
Denver, R.J., Hu, F., Scanlan, T.S., Furlow, J.D., 2009. Thyroid hormone receptor subtype specificity for hormone-dependent neurogenesis in Xenopus laevis. Dev. Biol. 326, 155–168.
Derby, A., 1968. An in vitro quantitative analysis of the response of tadpole tissue to thyroxine. J. Exp. Zool. 168, 147–156.
Dodd, M.H.I., Dodd, J.M., 1976. The biology of metamorphosis. In: Lofts, B. (Ed.), Physiology of the Amphibia. Academic Press, New York, pp. 467–599.
Etkin, W., 1964. Metamorphosis. In: Moore, J.A. (Ed.), Physiology of the Amphibia. Academic Press, New York, pp. 427–468.
Etkin, W., 1965. The Phenomena of Amphibian Metamorphosis. IV. The development of the median eminence. J. Morph 116, 371–378.
Etkin, W., 1968. Hormonal control of amphibian metamorphosis. In: Etkin, W., Gilbert, L.I. (Eds.), Metamorphosis: a Problem in Developmental Biology, pp. 313–348.
Forhead, A.J., Fowden, A.L., 2014. Thyroid hormones in fetal growth and prepartum maturation. J. Endocrinol. 221, R87–R103.
Fowden, A.L., Forhead, A.J., 2013. Endocrine interactions in the control of fetal growth. In: Bhatia, J., Bhutta, Z.A., Kalhan, S.C. (Eds.), Maternal and Child Nutrition: the First 1,000 Days. Karger, Basel, pp. 91–102.
Fowden, A.L., Juan Li, J., Forhead, A.J., 1998. Glucocorticoids and the preparation for life after birth: are there long-term consequences of the life insurance? Proc. Nutr. Soc. 57, 113–122.
Frieden, E., Just, J.J., 1970. Hormonal Responses in Amphibian metamorphosis. In: Litwack, G. (Ed.), Biochemical Actions of Hormones. Academic Press, New York, pp. 1–52.
Furlow, J.D., Neff, E.S., 2006. A developmental switch induced by thyroid hormone: Xenopus laevis metamorphosis. Trends Endocrinol. Metab. 17, 40–47.
Furlow, J.D., Yang, H.Y., Hsu, M., Lim, W., Ermio, D.J., Chiellini, G., Scanlan, T.S., 2004. Induction of larval tissue resorption in Xenopus laevis tadpoles by the thyroid hormone receptor agonist GC-1. J. Biol. Chem. 279, 26555–26562.
Galton, V.A., 1983. Thyroid hormone action in amphibian metamorphosis. In: Oppenheimer, J.H., Samuels, H.H. (Eds.), Molecular Basis of Thyroid Hormone Action. Academic Press, New York, pp. 445–483.
Grant, I.M., Balcha, D., Hao, T., Shen, Y., Trivedi, P., Patrushev, I., Fortriede, J.D., Karpinka, J.B., Liu, L., Zorn, A.M., Stukenberg, P.T., Hill, D.E., Gilchrist, M.J., 2015. The Xenopus ORFeome: a resource that enables functional genomics. Dev. Biol. 408, 345–357.
Gudernatsch, J.F., 1912. Feeding experiments on tadpoles. I. Tile influence of specific organs given as food on growth and differentiation. Arch. F. Entwcklngsmechn, D. Organ 35, 457–457-484.
Harland, R.M., Grainger, R.M., 2011. Xenopus research: metamorphosed by genetics and genomics. Trends Genet. 27, 507–515.
Hayes, T.B., Case, P., Chui, S., Chung, D., Haeffele, C., Haston, K., Lee, M., Mai, V.P., Marjuoa, Y., Parker, J., Tsui, M., 2006. Pesticide mixtures, endocrine disruption, and amphibian declines: are we understanding the impact? Environ. Health Perspect. 114, 40–50.
Helbing, C.C., 2012. The Metamorphosis of Amphibian Toxicogenomics. Frontiers in Genetics. Article 37, vol. 3.
Helbing, C.C., Bailey, C.M., Ji, L., Gunderson, M.P., Zhang, F., Veldhoen, N., Skirrow, R.C., Mu, R., Lesperance, M., Holcombe, G.W., Kosian, P.A., Tietge, J., Korte, J.J., Degitz, S.J., 2007. Identification of gene expression indicators for thyroid axis disruption in a *Xenopus laevis* metamorphosis screening assay Part 1. Eff. brain. Aquatic Toxicol 82, 227–241.
Helbing, C.C., Gergely, G., Atkinson, B.G., 1992. Sequential up-regulation of thyroid hormone β receptor, ornithine transcarbamylase, and carbamyl phosphate synthetase mRNAs in the liver of Rana catesbeiana tadpoles during spontaneous and thyroid hormone-induced metamorphosis. Dev. Genet. 13, 289–289-301.
Hellsten, U., Harland, R.M., Gilchrist, M.J., Hendrix, D., Jurka, J., Kapitonov, V., Ovcharenko, I., Putnam, N.H., Shu, S., Taher, L., Blitz, I.L., Blumberg, B., Dichmann, D.S., Dubchak, I., Amaya, E., Detter, J.C., Fletcher, R., Gerhard, D.S., Goodstein, D., Graves, T., Grigoriev, I.V., Grimwood, J., Kawashima, T., Lindquist, E., Lucas, S.M., Mead, P.E., Mitros, T., Ogino, H., Ohta, Y., Poliakov, A.V., Pollet, N., Robert, J., Salamov, A., Sater, A.K., Schmutz, J., Terry, A., Vize, P.D., Warren, W.C., Wells, D., Wills, A., Wilson, R.K., Zimmerman, L.B., Zorn, A.M., Grainger, R., Grammer, T., Khokha, M.K., Richardson, P.M., Rokhsar, D.S., 2010. The genome of the Western clawed frog Xenopus tropicalis. Science 328, 633–636.
Hillman, N.H., Kallaour, S.G., Jobe, A., 2012. Physiology of transition from intrauterine to extrauterine life. Clin. Perinatalogy 39, 769–783.
Hollar, A.R., Choi, J., Grimm, A.T., Buchholz, D.R., 2011. Higher thyroid hormone receptor expression correlates with short larval periods in spadefoot toads and increases metamorphic rate. Gen. Comp. Endocrinol. 173, 190–198.
Hoskins, E.R., Hoskins, M.M., 1919. Growth and development of amphibia as affected by thyroidectomy. J. Exp. Zool. 29, 1–69.
Huang, H., Marsh-Armstrong, N., Brown, D.D., 1999. Metamorphosis is inhibited in transgenic Xenopus laevis tadpoles that overexpress type III deiodinase. Proc. Natl. Acad. Sci. U. S. A. 96, 962–967.
Ishibashi, S., Love, N.R., Amaya, E., 2012. A simple method of transgenesis using I-SecI meganuclease in Xenopus. Methods Mol. Biol. 917, 205–218.
Ishizuya-Oka, A., Shimozawa, A., 1991. Induction of metamorphosis by thyroid hormone in anuran small intestine cultured organotypically in vitro. In Vitro Cell. Dev. Biol. 27A, 853–857.
James-Zorn, C., Ponferrada, V.G., Burns, K.A., Fortriede, J.D., Lotay, V.S., Liu, Y., Karpinka, B.J., Karimi, K., Zorn, A.M., Vize, P.D., 2015. Xenbase: core features, data acquisition, and data processing. Genesis 53, 486–497.
Kaltenbach, J., 1996. Endocrinology of Amphibian metamorphosis. In: Gilbert, L.I., Tata, J.R., Atkinson, B.G. (Eds.), Metamorphosis: Postembryonic Reprogramming of Gene Expression in Amphibian and Insect Cells. Academic Press, San Diego, CA, pp. 403–431.
Kollros, J.J., 1961. Mechanisms of amphibian metamorphosis: Hormones. Amer. Zool. 1, 107–114.
Kollros, J.J., 1981. Chapter 13: transitions in the nervous system during amphibian metamorphosis. In: Gilbert, L.I., Frieden, E. (Eds.), Metamorphosis: a Problem in Developmental Biology, second ed. Plenum Press, New York, pp. 445–460.
Kulkarni, S.S., Buchholz, D.R., 2012. Beyond Synergy: corticosterone and thyroid

hormone have numerous interaction effects on gene regulation in *Xenopus tropicalis* tadpoles. Endocrinology 153, 5309–5324.
Kulkarni, S.S., Buchholz, D.R., 2014. Corticosteroid signaling in frog metamorphosis. Gen. Comp. Endocrinol. 203, 225–231.
Laudet, V., 2011. The origins and evolution of vertebrate Review metamorphosis. Curr. Biol. 21, R726–R737.
Laudet, V., Gronemeyer, H., 2002. The Nuclear Receptor Facts Book. Academic Press, San Diego.
Leloup, J., Buscaglia, M., 1977. La triiodothyronine, hormone de la metamorphose des Amphibies. C. R. Acad. Sci. Paris, Ser. D. 284, 2261–2263.
Liggins, G.C., 1994. The role of cortisol in preparing the fetus for birth. Reprod. Fertil. Dev. 6, 141–150.
Lynn, W.G., Wachowski, H.E., 1951. The thyroid gland and its function in cold-blooded vertebrates. Quart. Rev. Biol. 26, 123–168.
Maher, S.K., Wojnarowicz, P., Ichu, T.A., Veldhoen, N., Lu, L., Lesperance, M., Propper, C.R., Helbing, C.C., 2016. Rethinking the biological relationships of the thyroid hormones, l-thyroxine and 3,5,3'-triiodothyronine. Comp. Biochem. Physiol. Part D. 18, 44–53.
Marsh-Armstrong, N., Cai, L., Brown, D.D., 2004. Thyroid hormone controls the development of connections between the spinal cord and limbs during Xenopus laevis metamorphosis. Proc. Natl. Acad. Sci. U. S. A. 101, 165–170.
Mathisen, P.M., Miller, L., 1989. Thyroid hormone induces constitutive keratin gene expression during Xenopus laevis development. Mol. Cell Biol. 9, 1823–1831.
Matsuda, H., Paul, B.D., Choi, C.Y., Hasebe, T., Shi, Y.B., 2009. Novel functions of protein arginine methyltransferase 1 in thyroid hormone receptor-mediated transcription and in the regulation of metamorphic rate in Xenopus laevis. Mol. Cell. Biol. 29, 745–757.
McNabb, F.M.A., 2007. The hypothalamic-pituitary-thyroid (HPT) Axis in birds and its Role in bird development and reproduction. Crit. Rev. Toxicol. 37, 163–193.
Mendoza, A., Navarrete-Ramírez, P., Hernández-Puga, G., Villalobos, P., Holzer, G., Renaud, J.P., Laudet, V., Orozco, A., 2013. 3,5-T2 is an alternative ligand for the thyroid hormone receptor beta 1. Endocrinology 154, 2948–2958.
Morte, B., Manzano, J., Scanlan, T., Vennström, B., Bernal, J., 2002. Deletion of the thyroid hormone receptor alpha 1 prevents the structural alterations of the cerebellum induced by hypothyroidism. Pnas 99, 3985–3989.
Nakajima, K., Fujimoto, K., Yaoita, Y., 2012. Regulation of thyroid hormone sensitivity by differential expression of the thyroid hormone receptor during Xenopus metamorphosis. Genes Cells 17, 645–659.
Ng, L., Forrest, D., 2006. Developmental roles of thyroid hormone receptor a and b genes. Adv. Dev. Biol. 16, 1–31.
Nunez, J., Celi, F.S., Ng, L., Forrest, D., 2008. Multigenic control of thyroid hormone functions in the nervous system. Mol. Cell. Endocrinol. 287, 1–12.
Opitz, R., Braunbeck, T., Bögi, C., Pickford, D.B., Nentwig, G., Oehlmann, J., Tooi, O., Lutz, I., Kloas, W., 2005. Description and initial evaluation of a Xenopus metamorphosis assay for detection of thyroid system-disrupting activities of environmental compounds. Environ. Toxicol. Chem. 24, 653–664.
Paul, B.D., Buchholz, D.R., Fu, L., Shi, Y.B., 2007. SRC-p300 coactivator complex is required for thyroid hormone-induced amphibian metamorphosis. J. Biol. Chem. 282, 7472–7481.
Pronych, S., Wassersug, R., 1994. Lung use and development in Xenopus laevis tadpoles. Can. J. Zool. 72, 738–743.
Remy, C., Bounhiol, J.J., 1971. Normalized metamorphosis achieved by adrenocorticotropic hormone in hypophysectomized and thyroxined Alytes tadpoles. C. R. Acad. Sci. Hebd. Seances. Acad. Sci. D. 272, 455–458.
Ritchie, J.W., Shi, Y.B., Hayashi, Y., Baird, F.E., Muchekehu, R.W., Christie, G.R., Taylor, P.M., 2003. A role for thyroid hormone transporters in transcriptional regulation by thyroid hormone receptors. Mol. Endocrinol. 17, 653–661.
Rose, C.S., James, B., 2013. Plasticy of lung development in the amphibian, *Xenopus laevis*. Biol. Open 2, 1324–1335.
Sachs, L.M., Buchholz, D.R., 2017. Frogs Model Man: in-vivo thyroid hormone signaling during development. Genesis 55, e23000.
Sachs, L.M., Shi, Y.B., 2000. Targeted chromatin binding and histone acetylation in vivo by thyroid hormone receptor during amphibian develoment. Pnas 97, 13138–13143.
Sachs, L.M., Damjanovski, S., Jones, P.L., Li, Q., Amano, T., Ueda, S., Shi, Y.B., Ishizuya-Oka, A., 2000. Dual functions of thyroid hormone receptors during Xenopus development. Comp. Biochem. Physiol. B. Biochem. Mol. Biol. 126, 199–211.
Schreiber, A.M., Das, B., Huang, H., Marsh-Armstrong, N., Brown, D.D., 2001. Diverse developmental programs of Xenopus laevis metamorphosis are inhibited by a dominant negative thyroid hormone receptor. Proc. Natl. Acad. Sci. U. S. A. 98, 10739–10744.
Session, A.M., Uno, Y., Kwon, T., Chapman, J.A., Toyoda, A., Takahashi, S., Fukui, A., Hikosaka, A., Suzuki, A., Kondo, M., van Heeringen, S.J., Quigley, I., Heinz, S., Ogino, H., Ochi, H., Hellsten, U., Lyons, J.B., Simakov, O., Putnam, N., Stites, J., Kuroki, Y., Tanaka, T., Michiue, T., Watanabe, M., Bogdanovic, O., Lister, R., Georgiou, G., Paranjpe, S.S., van Kruijsbergen, I., Shu, S., Carlson, J., Kinoshita, T., Ohta, Y., Mawaribuchi, S., Jenkins, J., Grimwood, J., Schmutz, J., Mitros, T., Mozaffari, S.V., Suzuki, Y., Haramoto, Y., Yamamoto, T.S., Takagi, C., Heald, R., Miller, K., Haudenschild, C., Kitzman, J., Nakayama, T., Izutsu, Y., Robert, J., Fortriede, J., Burns, K., Lotay, V., Karimi, K., Yasuoka, Y., Dichmann, D.S., Flajnik, M.F., Houston, D.W., Shendure, J., DuPasquier, L., Vize, P.D., Zorn, A.M., Ito, M., Marcotte, E.M., Wallingford, J.B., Ito, Y., Asashima, M., Ueno, N., Matsuda, Y., Veenstra, G.J., Fujiyama, A., Harland, R.M., Taira, M., Rokhsar, D.S., 2016. Genome evolution in the allotetraploid frog *Xenopus laevis*. Nature 538, 336–343.
Shi, Y., 1999. Amphibian Metamorphosis: from Morphology to Molecular Biology. Wiley-Liss, Inc, New York.
Shi, Y.B., 2009. Dual functions of thyroid hormone receptors in vertebrate development: the roles of histone-modifying cofactor complexes. Thyroid 19, 987–999.
Shi, Y.B., Ritchie, J.W., Taylor, P.M., 2002. Complex regulation of thyroid hormone action: multiple opportunities for pharmacological intervention. Pharmacol. Ther. 94, 235–251.
Shi, Y.B., Wong, J., Puzianowska-Kuznicka, M., Stolow, M.A., 1996. Tadpole competence and tissue-specific temporal regulation of amphibian metamorphosis: roles of thyroid hormone and its receptors. Bioessays 18, 391–399.
Swingle, W.W., 1918. The acceleration of metamorphosis in frog larvae by thyroid feeding, and the effects upon the alimentary tract and sex glands. J. Exp. Zool. 24, 521–525.
Tandon, P., Conlon, F., Furlow, J.D., Horb, M.E., 2016. Expanding the genetic toolkit in Xenopus: approaches and opportunities for human disease modeling. Dev. Biol. http://dx.doi.org/10.1016/j.ydbio.2016.04.009.
Tata, J.R., 1965. Turnover of nuclear and cytoplasmic ribonucleic acid at the onset of induced amphbian metamorphosis. Nature 204, 378–381.
Tata, J.R., Baker, B.S., Machuca, I., Rabelo, E.M., Yamauchi, K., 1993. Autoinduction of nuclear receptor genes and its significance. J. Steroid Biochem. Mol. Biol. 46, 105–119.
Tata, J.R., Kawahara, A., Baker, B.S., 1991. Prolactin inhibits both thyroid hormone-induced morphogenesis and cell death in cultured amphibian larval tissues. Dev. Biol. 146, 72–80.
van Mullem, A.A., van Gucht, A.L.M., Visser, W.E., Meima, M.E., Peeters, R.P., Visser, T.J., 2016. Effects of thyroid hormone transporters MCT8 and MCT10 on nuclear activity of T3. Mol. Cell. Endocrinol. 437, 252–260.
Veldhoen, N., Stevenson, M.R., Helbing, C.C., 2015. Comparison of thyroid hormone-dependent gene responses in vivo and in organ culture of the American bullfrog (Rana (Lithobates) catesbeiana) lung. Comp. Biochem. Phys. - Part D Genomics Proteomics 16, 99–105.
Visser, W.E., Friesema, E.C., Visser, T.J., 2010. Minireview: thyroid hormone transporters: the knowns and the unknowns. Mol. Endocrinol. 25, 1–14.
Vlaeminck-Guillem, V., Safi, R., Guillem, P., Leteurtre, E., Duterque-Coquillaud, M., Laudet, V., 2006. Thyroid hormone receptor expression in the obligatory paedomorphic salamander Necturus maculosus. Int. J. Dev. Biol. 50, 553–560.
Wang, X., Matsuda, H., Shi, Y.B., 2008. Developmental regulation and function of thyroid hormone receptors and 9-cis retinoic acid receptors during Xenopus tropicalis metamorphosis. Endocrinology 149, 5610–5618.
Wen, L., Shi, Y., 2015. Unliganded thyroid hormone receptor α controls developmental timing in *Xenopus tropicalis*. Endocrinology 156, 721–734.
Wen, L., Shi, Y., 2016. Regulation of growth rate and developmental timing by Xenopus thyroid hormone receptor a. Dev. Growth Differ. 58, 106–115.
Yamauchi, K., Tata, J.R., 1997. Tissue-dependent and developmentally regulated cytosolic thyroid-hormone-binding proteins (CTBPs) in Xenopus. Comp. Biochem. Physiol. C. Pharmacol. Toxicol. Endocrinol. 118, 27–32.
Yaoita, Y., Shi, Y., Brown, D., 1990. Xenopus laevis alpha and beta thyroid hormone receptors. Proc. Natl. Acad. Sci. U. S. A. 87, 8684.
Yen, P.M., 2001. Physiological and molecular basis of thyroid hormone action. Physiol. Rev. 81, 1097–1142.