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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

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