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Review

Molecular and developmental analyses of thyroid hormone receptor function in *Xenopus laevis*, the African clawed frog

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Abstract

The current review focuses on the molecular mechanisms and developmental roles of thyroid hormone receptors (TRs) in gene regulation and metamorphosis in *Xenopus laevis* and discusses implications for TR function in vertebrate development and diversity. Questions addressed are: (1) what are the molecular mechanisms of gene regulation by TR, (2) what are the developmental roles of TR in mediating the thyroid hormone (TH) signal, (3) what are the roles of the different TR isoforms, and (4) how do changes in these molecular and developmental mechanisms affect evolution? Even though detailed knowledge of molecular mechanisms of TR-mediated gene regulation is available from in vitro studies, relatively little is known about how TR functions in development in vivo. Studies on TR function during frog metamorphosis are leading the way toward bridging the gap between in vitro and in vivo studies. In particular, a dual function model for the role of TR in metamorphosis has been proposed and investigated. In this model, TRs repress genes allowing tadpole growth in the absence of TH during premetamorphosis and activate genes important for metamorphosis when TH is present. Despite the lack of metamorphosis in most other vertebrates, TR has important functions in development across vertebrates. The underlying molecular mechanisms of TR in gene regulation are conserved through evolution, so other mechanisms involving TH-target genes and TH tissue-sensitivity and dependence underlie differences in role of TR across vertebrates. Continued analysis of molecular and developmental roles of TR in *X. laevis* will provide the basis for understanding how TR functions in gene regulation in vivo across vertebrates and how TR is involved in the generation of evolutionary diversity.
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1. Hormonal context of TR function in frog development

Metamorphosis in frogs is a post-embryonic developmental process (Tata, 1999) that transforms aquatic, herbivorous tadpoles into terrestrial (usually), carnivorous juveniles (Dodd and Dodd, 1976). Many review articles and books have been written on various aspects of this subject, including hormonal control, molecular and developmental mechanisms, and biochemical and histological metamorphosis of skin, brain, intestine, blood, immune system, liver, and other organs (Allen, 1938; Atkinson, 1994; Balcells, 1955; Brown et al., 1996; Dent, 1988; Denver et al., 2002; Dodd and Dodd, 1976; Etkin, 1964; Galton, 1983; Gilbert and Frieden, 1981; Gilbert et al., 1996; Hourdry, 1993; Kikuyama et al., 1993; Kollros, 1961; Rose, 2005; Sachs et al., 2000; Shi, 1999; Tata, 1996; Wakahara and Yamaguchi, 2001). The intent of this review is to highlight recent molecular studies on the developmental roles of thyroid hormone receptor (TR) in the frog *Xenopus laevis* and how this research informs comparative studies in vertebrate diversity.

Metamorphosis is completely dependent on thyroid hormone (TH) (Gudernatsch, 1912; Allen, 1929). The activities of TH and TR occur in a hormonal context that can be divided into "central" and "peripheral" control of metamorphosis (Fig. 1) (Denver et al., 2002). A key component

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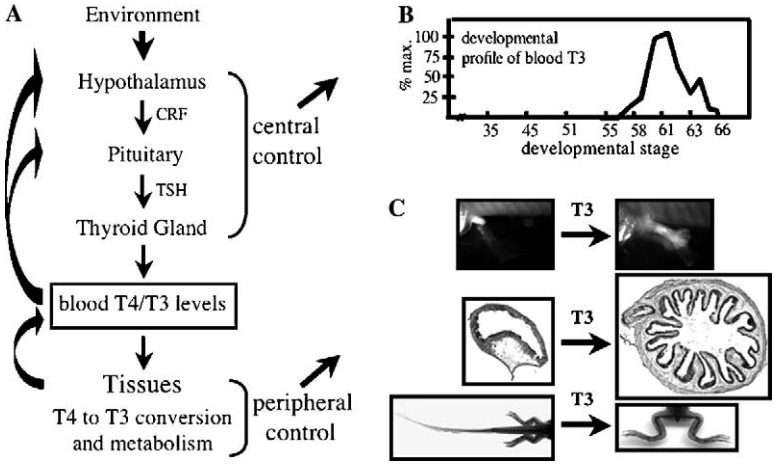

Fig. 1. Endocrine context of TR action and levels of control of metamorphosis. (A) Production of TH by the thyroid gland and tissues responses to TH during metamorphosis constitute the endocrine context of TR. TH is produced by the thyroid gland and secreted into the blood in response to thyroid stimulating hormone (TSH) from the pituitary, which is stimulated by corticotropin releasing factor (CRF) from the hypothalamus. Central control of metamorphosis is the regulation of the levels of circulating TH across development by the hypothalamus, pituitary, and thyroid gland in response to a combination of environmental and nutritional signals and hormonal feedback. Peripheral control of metamorphosis is the tissue-specific responses to TH of tissues outside the hypothalamic–pituitary–thyroid gland axis. (B) The blood levels of TH across development, derived from the thyroid gland and tissue metabolism of T4 to T3, are first detectable in the blood at the point when tadpoles begin to metamorphose at NF stage 55, i.e., when the limbs begin to grow out, and then rise to a peak in the middle of metamorphosis when the morphological transition is most dramatic (Leloup and Buscaglia, 1977). (C) Tissue-specific responses to TH include predominance of cell proliferation in developing limbs (NF stages 51 and 56 are shown). Intestinal remodeling involves both cell proliferation and death in the same tissue (NF stages 54 and 66 are shown in cross-section just posterior to bile duct entry, stained with methyl green pyronine Y). Cell death is predominant during tail resorption (NF stages 57 and 66 are shown).

of metamorphosis, the timing of the peak in TH plasma levels during development, is under central control by the hypothalamus–pituitary–thyroid gland axis (Figs. 1A and B). The neurosecretory hypothalamus, which coordinates environmental and nutritional signals, secretes corticotropic releasing factor into the median eminence, a vascular tissue that supplies the pituitary (Denver, 1999). TH is thought to control the functional development of the median eminence, whose integrity is important for metamorphosis (Etkin, 1965). Corticotropic releasing factor stimulates pituitary thyrotrope secretion of thyroid stimulating hormone (TSH) (Okada et al., 2004), which, in turn, stimulates thyroid follicle cell proliferation and production of TH by the thyroid gland (Kaye, 1961; Sakai et al., 1991). The thyroid gland secretes into the blood mostly thyroxine (T4) and very little of the more active form triiodothyronine (T3) (White and Nicoll, 1981), both forms collectively called TH. TH negatively feeds back on pituitary secretion of TSH (Kaye, 1961; Denver, 1996; Manzon and Denver, 2004). Changes in gene expression of TRs, deiodinases, and receptors for hypothalamic and pituitary hormones likely influence the effectiveness of this feedback (Huang et al., 2001; Manzon and Denver, 2004).

TH target organs outside the central axis, including limbs, intestine, and tail, are collectively known as the "periphery" and respond to T4 and T3 circulating in the blood in a tissue-specific manner (Fig. 1C) (Dodd and Dodd, 1976; Shi, 1999). Some organs develop de novo, such as the limbs, whereas others are completely resorbed, such as the tail and gills (Nakajima et al., 2005). However, most organs, such as intestine and skin, are remodeled from the larval form to the adult version (Fox, 1981; McAvoy and Dixon, 1977; Shi and Ishizuya-Oka, 2001; Suzuki et al., 2002). The only developmental event not known to be affected by TH physiology is primary gonad differentiation (Hoskins and Hoskins, 1919; Gruca and Michalowski, 1961; Ogielska and Kotusz, 2004; Rot-Nikcevic and Wassersug, 2004). Physiological changes accompany the morphological changes, e.g., the transition from ammonotelism to ureotelism, from larval to adult hemoglobins, and from larval to adult immune systems (Gilbert et al., 1996). These tissue-specific developmental events occur asynchronously, e.g., intestine transforms after the limbs and before the tail, and this asynchrony is thought to be due to tissue-specific control of effective intracellular T3 levels (Shi et al., 1996). T4 to T3 conversion by the deiodinase DII in target tissues is one such peripheral mechanism that increases tissue and organ sensitivity to TH (Becker et al., 1997; Cai and Brown, 2004). In addition, T4 and T3 degradation by the deiodinase DIII present in target tissues reduces cellular levels of T3 (Becker et al., 1997). Indeed, transgenic overexpression of DIII blocks TH-induced metamorphic events (Huang et al., 1999; Marsh-Armstrong et al., 1999). Additional cellular proteins controlling intracellular T3 levels and thereby tissue sensitivity to circulating TH, include cytosolic TH binding protein (Shi et al., 1994; Yamauchi and Tata, 1994), TRα (Shi et al., 1996), and TH transporters (Ritchie et al., 2003). TRα expression levels may affect how sensitive cells are to circulating TH. The levels of cytosolic TH binding proteins likely affect free TH within the cells available to bind TR. Furthermore, higher TH transporter expression levels