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Metamorphosis in Amphibians and the Role of Thyroid Hormone

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INTRODUCTION: THE FOUNDATIONS OF AMPHIBIAN METAMORPHOSIS ANALYSIS

The remarkable natural transition of a pupal or juvenile life form to a highly complex adult form is a phenomenon that has inspired hundreds of years of academic thought; this dramatic physical transformation has encouraged researchers to investigate the biological and chemical origins and mechanisms behind the magnificence of metamorphosis. These spectacular shifts in morphology and physiology have been extensively studied in a variety of vertebrates, ranging from the shell-enveloped embryonic development of the domesticated chicken or *Gallus gallus domesticus*, to smoltification in young salmon, to metamorphosis in amphibians (Sachs and Buchholz, 2019). The latter serves as one of the most accessible instances of life history transitions, specifically when studying anurans like the African clawed frog, *Xenopus laevis*, or even the odd case of the common coquí, *Eleutherodactylus coqui*, which lacks a tadpole stage (Callery and Elinson, 2000). Through these centuries of academic investigation, an impressive understanding of the process of metamorphosis has been established. However, there are many facets of metamorphosis that have yet to be thoroughly investigated including the precise chronology and operation of certain signaling pathways or the synchronized interaction of several endocrine organs, including but not limited to the thyroid gland.

The amphibian metamorphosis process involves many dramatic anatomical and physiological changes typically arranged into three periods including premetamorphosis, prometamorphosis, and the climax with the usage of standardized numerical stages created by Faber and Nieuwkoop (Dodd and Dodd, 1976; Faber and Nieuwkoop, 2020). In premetamorphosis, normal differentiation and growth take place prior to the presence of a fully formed thyroid gland, ending at about stage 54 (Faber and Nieuwkoop, 2020; Yaoita and Brown, 1990). At stage 54, the growing thyroid gland commences thyroid hormone secretion, acknowledged as the main proponent of metamorphosis which will be discussed at length (Leloup and Buscaglia, 1977). From stages 55-57 (Faber and Nieuwkoop, 2020), prometamorphosis takes place in an organized series of morphological alterations, most notably with limb development as the front legs form out of the opercular fold (Yaoita and Brown, 1990). By the climax, the concentration of thyroid hormone is at its maximum as the former tadpole begins to take shape as a minute frog; at stage 62 (Faber and Nieuwkoop, 2020), the tail is rapidly resorbed (Yaoita and Brown, 1990). Each transition of tissue structure and function is incredibly influenced by the concentration of thyroid hormone at that particular location (Kollros, 1961), and this is further supported by both early observational experiments (Allen, 1917, 1918, 1938; Gudernatsch, 1912) and later in-depth studies (Becker et al., 1997; Brown and Cai, 2007; Buchholz et al., 2006; Leloup and Buscaglia, 1977; Sachs et al., 2000; Yaoita and Brown, 1990).

One of the earliest published studies performed by J. F. Gudernatsch was quick to recognize the pivotal role played by thyroid hormone, TH, in the post-embryonic development of Amphibia when conducting food experiments (Gudernatsch, 1912). Gudernatsch found that out of several organs, the thyroid organ triggered a noticeable, premature differentiation of the tadpole into more advanced stages of development but inhibited additional growth. The converse of this interaction was observed by later studies by removing both the pituitary gland (Allen, 1917) and the rudimentary thyroid gland (Allen, 1918) from the tadpole prior to metamorphosis. The former study focused on the pituitary gland, which produces thyrotrophic hormone (TSH), a hormone that plays a direct role in regulating the production and release of TH (Manzon and Denver, 2004); meanwhile, the latter study focused on the thyroid gland itself, the direct source of TH, which is studied in its active form as T_3 or tri-iodothyronine (Wen et al., 2017). As a result of these studies and related investigations, it could be reasonably concluded that the removal of TH or TSH, a related hormone centered around enabling TH, prevented metamorphosis but did not inhibit further growth of the juvenile tadpole (Allen, 1938).

After these initial discoveries laid the foundations of research surrounding TH and amphibian metamorphosis,

dozens of subsequent studies have been conducted investigating the key proponents of the amphibian metamorphic process involving TH (Atkinson et al., 1998; Bonett et al., 2010; Buchholz, 2018; Buchholz et al., 2003; Buchholz et al., 2006; Buchholz et al., 2004; Cai and Brown, 2004), the molecular compounds which interact with TH (Becker et al., 1997; Cai and Brown, 2004; Ishizuya-Oka et al., 2006; Marsh-Armstrong et al., 1999; St Germain et al., 1994), the paradoxical response (Yaoita and Brown, 1990) to TH across the amphibian body plan, and several other enlightening topics of study. With this chronological context for discoveries in mind, it is evident that developing technologies ushered in a new wave of research surrounding the role of TH in amphibian metamorphosis, which will be dissected in this paper.

Through this analysis of the role of thyroid hormone in amphibian metamorphosis, the complexity and breadth of metamorphosis and related biological factors are succinctly explained to offer a greater sense of understanding regarding the intricacy and specificity required for successful transformational development, as well as the consistent involvement of the thyroid hormone in growth and development across many species. The primary focuses of this investigation will be the cellular and molecular mechanisms taking place between TH, respective thyroid hormone receptors (TRs), and other interactive molecules (Becker et al., 1997; Buchholz, 2018; Buchholz et al., 2003; Buchholz et al., 2004; Cai and Brown, 2004; Kawahara et al., 1991; Manzon and Denver, 2004; Marsh-Armstrong et al., 1999; Rastinejad et al., 1995; Sachs et al., 2000; Sachs and Shi, 2000; Schreiber et al., 2001; Shi et al., 2012; St Germain et al., 1994; Wen et al., 2019; Yaoita and Brown, 1990) and the specialized role of TH within several organs and organ systems of studied amphibians (Cai and Brown, 2004; Ishizuya-Oka et al., 2006; Kawahara et al., 1991; Wen et al., 2019). Considering that all vertebrates require functional TH for successful growth and differentiation (Kollros, 1961; Schreiber et al., 2001), continuous detailed investigation in the role of TH in amphibian metamorphosis allows researchers to study the hormonal parallels between both post-embryonic amphibian development and the general biological development of mammals.

THE CELLULAR AND MOLECULAR MECHANISMS OF THE THYROID HORMONE

After completion of the foundational studies of Gudernatsch, Allen, and other biologists of the time, it was clear that TH played a critical role in amphibian metamorphosis (Allen, 1917, 1918, 1938; Dodd and Dodd, 1976; Gudernatsch, 1912; Kollros, 1961; Leloup and Buscaglia, 1977; Tata, 1966). Upon this discovery, the molecular forms and structure of TH were investigated, resulting in the isolation of two forms of TH: T_3 (3,5,3'-triiodothyronine) and T_4 (3,5,3',5'-tetraiodothyronine or thyroxine (Becker et al., 1997; Bonett et al., 2010). Through TH plasma analysis in *Xenopus laevis*, it was discovered that in the premetamorphosis-to-prometamorphosis stage of amphibian metamorphosis, the presence of T_4 in the blood plasma of larvae following the generation of the thyroid fell slightly behind the levels of T_3 (Leloup and Buscaglia, 1977). As the plasma concentrations of both T_3 and T_4 increase (Leloup and Buscaglia, 1977), the organism further develops and differentiates from the aquatic tadpole to the terrestrial amphibian (Gudernatsch, 1912), noted by the formation of the hindlimb, an early sign of morphogenesis and metamorphic change (Becker et al., 1997). At the climax, both T_3 and T_4 are at their maximal concentrations, specifically around stage 60 (Leloup and Buscaglia, 1977). Despite the continuous presence of T_4 during amphibian metamorphosis, it is actually its conversion to T_3 that gives it the power to stimulate the process; thus, T_3 is the more potent form of TH and is depended upon for the induction of metamorphic development (Becker et al., 1997; Bonett et al., 2010; Galton, 1990; Wen et al., 2019).

Since early investigation into the thyroid gland's role in amphibian metamorphosis, it has been universally recognized that the hypothalamus-pituitary-thyroid axis plays an important role in the dramatic developmental and physiological process (Allen, 1938; Gudernatsch, 1912; Manzon and Denver, 2004). One prominent enzyme involved in the transition between T_3 and T_4 and the general activation and deactivation of TH is the deiodinase, appearing in three relevant forms during metamorphosis: deiodinase type I (DI), deiodinase type II (DII), and deiodinase type III (DIII) (Manzon and Denver, 2004; St Germain et al., 1994). DI and DII primarily convert T_4 to active T_3 by removing an iodine atom (Becker et al., 1997; Ishizuya-Oka, 2011). DIII transforms T_3 into an inactive compound, T_2, by removing an iodine atom in a different location on the active TH (Ishizuya-Oka, 2011). The expression of these deiodinases, particularly DII and DIII, have been experimentally proven to increase concurrently with the increase in TH levels during metamorphosis, supporting the idea that deiodinases play a critical role in the tactical usage and regulation of TH concentrations and distributions in the developing organism (Becker et al., 1997; Cai and Brown, 2004; Manzon and Denver, 2004; Marsh-Armstrong et al., 1999; St Germain et al., 1994).

When TH is secreted from the thyroid gland, it travels through the blood via carrier proteins or various transportation routes and acts on target cells in highly specialized local mechanisms (St. Germain et al., 2009). After secretion and dispersal throughout the organism,

TH interacts with thyroid hormone receptors (TRs), transcriptional factors that are part of the nuclear receptor family, thus creating a biological change in target cells (Buchholz et al., 2004; Sachs and Shi, 2000; Wen et al., 2019). Both in vitro and tissue culture investigations provide evidence that when active TH interacts with TRs, the receptors heterodimerize with retinoid X receptors, RXRs (Rastinejad et al., 1995; Wong and Shi, 1995), and then bind to thyroid hormone response elements, TREs, located in T_3 response genes (Buchholz et al., 2003). This binding of the heterodimers to TREs is not dependent on T_3 concentration, and this is adequately expressed using the infamous dual function model (Buchholz et al., 2003). Throughout the past few decades of academic study, TRs have been commonly referred to as dual function regulators of transcription; when unliganded by T_3, they act as repressors of genes that induce metamorphosis with the assistance of corepressors, and when liganded by T_3, enhance transcription of genes that induce metamorphosis with the assistance of coactivators (Buchholz, 2018; Buchholz et al., 2003; Buchholz et al., 2004; Sachs et al., 2000; Schreiber et al., 2001). Examples of these findings linking the role of TH and TRs involve dominant-negative receptors that block amphibian metamorphosis through the inhibition of TRE activation (Buchholz et al., 2003; Schreiber et al., 2001), as well as dominant-positive thyroid receptors that allow for TRE activation via mediation by TR (Buchholz et al., 2004).

Analogous to TH, TRs appear in two separate, highly conserved isoforms (Schreiber et al., 2001), TRα and TRβ, both of which begin to be expressed following hatching of the *Xenopus laevis* embryo (Yaoita and Brown, 1990). Even prior to premetamorphosis and the formation of the thyroid gland, TRα is widespread throughout the tissues of amphibians, increasing in concentration as metamorphosis progresses (Schreiber et al., 2001; Yaoita and Brown, 1990). As for TRβ, mRNA assays revealed that it is scarcely detected during premetamorphosis (Yaoita and Brown, 1990). Instead, TRβ presence rises in concentration in conjunction with an increase in the secretion of endogenous TH, eventually reaching a maximum concentration at the final climax stage of the process; this climax is promptly followed by a dramatic decrease in TRβ concentration throughout the organism at the conclusion of metamorphosis (Schreiber et al., 2001; Yaoita and Brown, 1990). It has been demonstrated that TRα is compulsory for the premature development of tadpoles into adult amphibians when providing exogenous TH in vitro (Schreiber et al., 2001; Wen et al., 2019). However, recent in vivo studies experimenting with TRα knockout genes show that TRα is not required for the general mechanism of amphibian metamorphosis (Fu et al., 2018; Wen et al., 2019; Wen et al., 2017); instead, TRα plays a more prominent role in the regulation of the rate and timing of metamorphosis, as seen with the ubiquitous sequential formation or resorption of certain organs and tissues (Wen et al., 2017). Without TRα, amphibians were shown to enter and complete the metamorphic process at younger stages in life, ultimately resulting in smaller, reduced froglets; this success despite the absence of TRα could be explained by TRβ partially compensating for TRα, but this requires further study with TRβ knockout gene analysis (Wen et al., 2017). In general, both TRs and their interactions with TH are vital in carrying out three general actions that take place on tissues during metamorphosis: de novo development, degeneration, and remodeling—each of which will be explored further when discussing TH-dependent tissue-specificity during metamorphosis in future sections of this analysis (Cai and Brown, 2004; Das et al., 2002; Dodd and Dodd, 1976; Kawahara et al., 1991; Wen et al., 2017).

One prominent general molecular mechanism involved in the suppression and activation of metamorphic changes is the recruitment of a histone deacetylase complex in the absence of a ligand and the recruitment of a histone acetylase complex in the presence of a ligand, respectively (Sachs and Shi, 2000); the dual function model proposed by previous studies supports these findings (Buchholz, 2018; Buchholz et al., 2003; Sachs and Shi, 2000). In terms of amphibian metamorphosis, histone deacetylase complex recruitment is more common during premetamorphosis when there is less TH in the plasma, and therefore more unliganded TRs repressing transcription (Sachs and Shi, 2000). Meanwhile, histone acetylase complex recruitment is more common during prometamorphosis and the climax when there is much more circulating TH, and therefore more liganded TRs activating gene expression and allowing for metamorphic processes to take place in the developing organism (Sachs and Shi, 2000). These modifications in chromatin availability also involve a number of cofactor complexes that enable these histone acetylation and deacetylation complexes in a variety of specialized ways depending on organs, tissues, and particular genes (Buchholz et al., 2003; Fu et al., 2018; Sachs and Shi, 2000). Small-scale conformational changes and molecular modifications allow for the dramatic, diverse physiological transformations across the body axes of the organism including organ reformation, extensive cell proliferation and cell death (Buchholz et al., 2003; Buchholz et al., 2004; Sachs and Shi, 2000; Schreiber et al., 2001; Tata, 1966; Wong and Shi, 1995).

MEDIATION OF TISSUE-SPECIFICITY BY THE THYROID HORMONE

With the help of deiodinases, TRs, coactivators, corepressors, and other interactive molecules and

complexes, TH is able to facilitate the highly specialized and complex tissue-specific morphogenesis of amphibians (Becker et al., 1997; Buchholz et al., 2003; Cai and Brown, 2004; Kollros, 1961; Sachs and Shi, 2000; Schreiber et al., 2001). This is seen in de novo tissue development including the lungs and limb buds (Becker et al., 1997; Das et al., 2002; Veldhoen et al., 2015), restructured tissues such as the liver, eye, skin, intestines, and central nervous system (Atkinson et al., 1998; David Furlow et al., 1997; Ishizuya-Oka et al., 2006; Kawahara et al., 1991; Schreiber and Brown, 2003; Schreiber et al., 2001; Wen et al., 2019), and eliminated or regressed structures such as the tail, gills, and select muscles (Cai and Brown, 2004; Schreiber et al., 2001; Wen et al., 2017; Yaoita and Brown, 1990; Yaoita and Nakajima, 1997); through these studies, it has been demonstrated that several tissues undergo a combination or sequential usage of these strategies of metamorphosis and functional differentiation, and the role played by TH varies distinctly from tissue to tissue despite a ubiquitous presence throughout the organism overtime. For example, extensive remodeling is experienced in the tadpole retina as it shifts from an aquatic to terrestrial lifestyle (Kawahara et al., 1991; Marsh-Armstrong et al., 1999). In this metamorphic transition, DIII acts as an important enzyme locally expressed in the dorsal marginal region of the retina (Marsh-Armstrong et al., 1999). As a result, DIII inhibits TH-driven gene expression by converting it to its inactive form, thus only allowing for ventral marginal cells to express TH-activated genes and promoting asymmetrical cell proliferation and differentiation (Marsh-Armstrong et al., 1999); this eventually leads to the characteristic ipsilateral eye position of adult amphibians post-metamorphosis (Kawahara et al., 1991; Marsh-Armstrong et al., 1999).

Similar to the development of the retina, the intestines experience extensive remodeling at the climax stage, but undergo a different mechanism of change (Schreiber et al., 2005; Shi and Brown, 1993). This involves a dramatic transition from a long mesenchymal tube lined by a single endothelial cell layer to a shortened multicellular-layered intestinal tract; in this instance, the tadpole's epithelial cells act as progenitors for the adult epithelial tissue (Brown and Cai, 2007; Faber and Nieuwkoop, 2020; Ishizuya-Oka et al., 2006; Schreiber et al., 2005; Shi and Brown, 1993). In this intestinal transition at the climax of metamorphosis, TH down-regulates specialized genes in the larval mesenchyme by engaging with TRs, inducing alterations in the digestive tract and contributing to the formation of the intestines more characteristic of adult amphibians (Ishizuya-Oka et al., 2006; Kawahara et al., 1991; Shi and Brown, 1993). Through RT-PCR and in situ hybridization analyses, it has been demonstrated that TH directly induces sonic hedgehog (Shh), which up-regulates the connective tissue-specific expression of bone morphogenic protein-4 (BMP-4), which represses cell proliferation of the larval mesenchymal connective tissue and promotes cell differentiation of the more developed intestinal lining (Ishizuya-Oka et al., 2006). In addition to the extensive restructuring of the digestive tract, the intestines undergo apoptotic pathways to eliminate the inner layer of larval epithelial cells, serving as an example of a combination or specialized sequence of metamorphic strategies in order to achieve a revised adult tissue (Buchholz et al., 2003; Buchholz et al., 2004; Ishizuya-Oka et al., 2006; Sachs and Shi, 2000; Yaoita and Brown, 1990).

Many similarities have been drawn between the tail and intestines of the larval stage amphibian. Both of these structures transform during the climax of metamorphosis and involve the presence of apoptosis to induce dramatic physiological changes; it has been suggested that tail resorption occurs via the same TH-dependent Shh/BMP-4 signaling pathway in order to encourage extensive cell death (Brown and Cai, 2007; Buchholz et al., 2006; Ishizuya-Oka et al., 2006; Sachs and Shi, 2000; Yaoita and Brown, 1990; Yaoita and Nakajima, 1997). Based on experimental findings regarding an increase in TR mRNA concentration in tail cells during the climax of metamorphosis, it has been established that high levels of TH are required to stimulate tail resorption; this maximal concentration of TH at the climax leads to the expression of DII in the organs and tissues that develop later in the metamorphic timeline (tail, intestines, anterior pituitary, etc.) (Brown and Cai, 2007; Cai and Brown, 2004; Kawahara et al., 1991; Yaoita and Brown, 1990). These studies and other extensive in vitro and in vivo research regarding the role of TH in tail resorption show that TH plays a direct role in inducing tail resorption within a matter of days, leading to apoptosis of a variety of tail cells (Wen et al., 2017; Yaoita and Nakajima, 1997). Though the exact tissue-specific mechanisms that link TH and particular apoptotic pathways like those involving caspase-9 or mitochondrial factors is not known, it is largely agreed upon that these mechanisms are intrinsically linked to increasing TH levels during metamorphosis (Ishizuya-Oka, 2011; Ishizuya-Oka et al., 2010; Rowe et al., 2005). Both the intestines and the tail experience some impact from apoptotic pathways, and this lays the groundwork for a new developed anatomy and physiology to take shape in the form of an adult amphibian (Cai and Brown, 2004; Das et al., 2002; Kawahara et al., 1991; Sachs and Buchholz, 2019; Wen et al., 2017; Yaoita and Brown, 1990; Yaoita and Nakajima, 1997).

The metamorphosis of the central nervous system from the larval stage to adult stage exhibits all three metamorphic strategies of de novo development,

remodeling, and apoptosis and regression, providing an elucidative research focus (Cai and Brown, 2004; Das et al., 2006; Schreiber et al., 2001; Wen et al., 2019). In terms of de novo development, one of the earliest TH-induced changes in amphibian metamorphosis involves DNA replication at low levels of TH, which allows for the formation of cells lining brain ventricles to proliferate and differentiate to form the spinal cord and motor neurons; this same DNA replication is involved in limb bud formation (Cai and Brown, 2004; Das et al., 2006; Marsh-Armstrong et al., 2004; Schreiber et al., 2001; Wen et al., 2019). Microarrays have provided evidence that in both the nervous tissue and limb tissues, increasing levels of TH up-regulate proteins involved in all stages of the cell cycle using several means of signaling (Cai and Brown, 2004; Das et al., 2006). However, only in the brain has there been evidence of Notch and OTX2 homeobox protein expression, both being utilized in a variety of cellular processes like cell proliferation and cell differentiation (Das et al., 2006). In addition to slowly increasing levels of TH, there are also increasing levels of TRα, RXRs, and DII, demonstrating their fundamental activity with TH (Becker et al., 1997; Cai and Brown, 2004). In terms of tissue remodeling, TH presence at the climax stage of metamorphosis stops DNA replication and initiates the resorption of gill arches, the creation of a prominent lower jaw, the development of a widened brain, and the relocation of the nose towards the olfactory bulb (Schreiber et al., 2001). In addition to nervous tissue remodeling at the climax, the brain also undergoes tissue regression due to the presence of TH; this has been observed in the extensive regression of the Rohon-Beard and Mauthner neurons via an apoptotic pathway, but the mechanistic details remain to be investigated (Coen et al., 2001; Das et al., 2006; Schreiber et al., 2001). Throughout the formation of the neuroectoderm, the interaction between TH and TRs, specifically TRα, is vital for maximal gene regulation responses to TH in the brain (Kawahara et al., 1991; Wen et al., 2019). While proliferating cells in the subventricular regions of the larval brain show high levels of TRα mRNA instead of TRβ mRNA, cells distal from the brain ventricles that will eventually remodel or experience apoptosis show high levels of TRβ mRNA, thus demonstrating a localized specialization of the amphibian brain (Buchholz et al., 2006; Schreiber et al., 2001; Wen et al., 2019).

In summary, each tissue and organ system undergoes their own specialized metamorphosis process due to differential tissue sensitivity to TH, and this is demonstrated by dozens of means and mechanisms ranging from the dual function model of TRs to the action of DII and DIII (Becker et al., 1997; Buchholz, 2018; Cai and Brown, 2004; Kawahara et al., 1991; Marsh-Armstrong et al., 1999; Schreiber et al., 2001; St Germain et al., 1994; St. Germain et al., 2009; Yaoita and Brown, 1990). One specific example of the complexity of the role of TH in tissue-specific morphogenesis is the opposite mechanism involved in tail regression and limb cell proliferation; in this example, genes that express mitochondrial electron transport chain proteins are down-regulated in the tail but up-regulated in the limbs due to the action of TREs or the interaction of TRs with coactivators and corepressors (Das et al., 2006). In general, early developments such as limb growth and neural DNA replication occur during premetamorphosis while TH and TRβ levels are low, being primarily mediated by TRα (Cai and Brown, 2004; Schreiber et al., 2001). Eventually, the TH concentration increases to a maximum at the climax of metamorphosis, triggering the final developments of metamorphosis such as intestinal remodeling and gill and tail resorption with more influence from TRβ (Schreiber et al., 2001). It is evident that each of these tissue transitions are TH-dependent, relying on the feedback loop connecting the pituitary and thyroid gland to supply TH throughout the process of amphibian metamorphosis (Kawahara et al., 1991; Kollros, 1961; Yaoita and Brown, 1990).

CONCLUSION: THE DUALITY OF AMPHIBIAN METAMORPHOSIS & APPLICATION TO MAMMALIAN SYSTEMS

On the surface, the process of amphibian metamorphosis is characterized by a consistent theme of duality as seen in the infamous dual function model, the utilization of two TRs, the two prominent forms of TH—even the term “amphibian” translates to “double life” as it transitions from aquatic to terrestrial lifestyles— but this process is characterized by much more molecular complexity than initially proposed by J.F. Gudernatsch in 1912 (Buchholz et al., 2003; Leloup and Buscaglia, 1977; Sachs et al., 2000; Schreiber et al., 2001; St. Germain et al., 2009; Yaoita and Brown, 1990). The activation of TH through deiodinases and its interaction with TRs creates histone modifications that either suppress or activate gene expression that characterizes metamorphic changes in amphibians, and these developments are highly localized and specialized within each tissue of the organism (Becker et al., 1997; Cai and Brown, 2004; Marsh-Armstrong et al., 1999; Sachs and Shi, 2000; St Germain et al., 1994; Wen et al., 2019). The strong reliance of amphibian metamorphosis on the fluctuating levels of TH in the organism makes it an ideal model of study when examining metamorphosis or mechanisms of TH in general (Buchholz et al., 2003; Kollros, 1961; Schreiber et al., 2001; Wen et al., 2019). It has been emphasized by many researchers that the dual function of TRs and the role of TH in tissue specificity can be found in both amphibians and mammals during postembryonic

development and can help explain extensive evolutionary diversity across species (Buchholz et al., 2003; Buchholz et al., 2004; Wen et al., 2019). Future directions of research involving the mechanisms of TH in postembryonic developments could investigate more tissue-specific molecular mechanisms of TH or work toward preventing thyroid deficiency during human gestation or in the development of cretinism (Buchholz et al., 2004; Wen et al., 2017).

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