A developmental switch induced by thyroid hormone: *Xenopus laevis* metamorphosis

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Thyroid hormone induces the complete metamorphosis of anuran tadpoles into juvenile frogs. Arguably, anuran metamorphosis is the most dramatic effect of a hormone in any vertebrate. Recent advances in pharmacology and molecular biology have made the study of this remarkable process in the frog *Xenopus laevis* attractive to developmental biologists and endocrinologists alike. In particular, the availability of a straightforward transgenesis assay and the near completion of the *Xenopus tropicalis* genome are enabling significant advances to be made in our understanding of the major remaining problems of metamorphosis: the extraordinary tissue specificity of responses, the precise timing of morphological changes, the degree of cell autonomy of hormone responses and developmental competence. We argue that *X. laevis* metamorphosis presents an exciting opportunity for understanding the role of thyroid hormone in vertebrate development.

**Introduction**

Almost 100 years ago, J.F. Gudernatsch made the remarkable discovery that equine thyroid extracts could accelerate the metamorphosis of tadpoles into juvenile frogs [1]. Subsequent work demonstrated that removal of the tadpole thyroid gland or treatment with inhibitors of thyroid hormone (TH) synthesis prevents metamorphosis. Indeed, THs that are identical to those produced by the human thyroid gland, thyroxine (T4) and the more potent 3,5, 3′-triiodothyronine (T3), are detected in increasing amounts as tadpole metamorphosis proceeds [2].

A hypothalamic–pituitary–thyroid axis controls the production of TH, in the same way as in mammals, although amphibians appear to use corticotropin-releasing hormone instead of thyrotropin-releasing hormone to induce pituitary thyroid-stimulating hormone (TSH) secretion at the onset of metamorphosis [3]. Since the findings of Gudernatsch and others established TH as the developmental signal that triggers the onset of metamorphosis, the system has attracted investigators from various disciplines, including those studying life cycle evolution, environmental toxicology and basic mechanisms of TH action. The central question of how such a simple molecule, two coupled tyrosines decorated by iodines, could coordinate such a wide range of functional and morphological changes remains a fascinating issue.

Most anurans (frogs and toads) undergo indirect development, hatching as free-living larvae capable of substantial growth but incapable of reproduction or survival in a nonaquatic environment. Upon metamorphosis into juvenile frogs, virtually every organ system undergoes extensive morphological and/or functional changes as circulating TH levels rise (Box 1). These changes can be grouped into three categories: (i) death and resorption of larval tissues used only by the tadpole; (ii) *de novo* growth and differentiation of new adult tissues; and (iii) remodeling of larval tissues to create a new adult function [4]. The two most visually striking changes are complete resorption of the tail and the formation of the hind- and fore-limbs from nests of undifferentiated mesenchyme. Most tissues and organs, such as the skin, skeletal muscle, digestive tract and the central nervous system, undergo an extensive remodeling process involving spatially and temporally coordinated apoptosis of the larval cell component combined with proliferation and differentiation of adult precursor cells. The degree of ‘transdifferentiation’ of larval cells into new adult cells has not been unequivocally documented in amphibians to date, although this clearly occurs in specific tissues during insect metamorphosis [5].

In this review, we discuss the transcriptional control of metamorphosis by TH via its well-conserved TH receptors (TRs), with a focus on a comparison between the tail resorption versus limb growth programs. Although digestive tract remodeling has been the subject of extensive investigation, this process has been reviewed extensively elsewhere, so will not be discussed in detail here [6,7]. We also revisit some major long-standing questions that are now approachable using recently developed molecular and pharmacological tools. Most of this work has been performed using the South African clawed frog *Xenopus laevis*. Owing to its complete external development, conserved mechanism of TH action and large collection of TH-response genes, *X. laevis* represents an ideal system for ascertaining the developmental roles of TH and its receptors. Importantly, a transgenesis method in this organism has been developed that promotes integration of DNA into sperm chromatin before the first cleavage of the fertilized egg, so that all of the
Transcriptional control of metamorphosis by thyroid hormone

Several lines of evidence demonstrate that most, if not all, of the morphological and functional changes of metamorphosis are the result of changes in the transcription of specific sets of genes induced by TH. In this section, we summarize our current understanding of TH control of transcription during metamorphosis via the thyroid hormone receptors and associated coactivators.

TRs are structurally and functionally conserved

Early studies by Tata [13] showed that inhibitors of RNA or protein synthesis could block TH-induced morphological changes in organ culture. Further evidence for the role of transcriptional control of metamorphosis comes from the fact that TRs belong to the nuclear receptor superfamily of transcription factor genes; lipophilic ligand binding regulates the activity of many members of the family [14]. X. laevis, in common with all vertebrates, has two TR gene loci, designated TR-α and TR-β. Both X. laevis TRs (xTRs) show over 85% amino acid identity with their human homologs, with highest conservation in their ligand-binding and DNA-binding domains [15] (Figure 1a). The two xTRs are also highly similar (85% identity), with the most significant difference being in the amino-terminal activation function (AF) 1 domains. The

![Figure 1](https://example.com/figure1.png)

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<th>TR isoform</th>
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Cells of the resulting tadpole carry the transgene in a nonmosaic fashion [8]. Integrated reporter constructs faithfully reproduce the pattern of expression of endogenous genes [9]; a variety of mammalian promoters are active in transgenic X. laevis [10], and transgenes are faithfully transmitted through the germ line [11]. In addition, genetic resources for the Xenopus community continue to develop at a rapid pace, including complementary DNA (cDNA) sequencing and microarray production. A particularly exciting development is the potential of a close relative of X. laevis, Xenopus tropicalis, to be used in traditional genetic experiments to identify key regulatory genes during embryogenesis and metamorphosis [12]. X. tropicalis is a diploid organism with a genome half the size of that of the tetraploid X. laevis and achieves sexual maturity in about four months. A first draft of the entire X. tropicalis genome will have been completed before the end of 2005. Thus, the Xenopus metamorphosis model combines well-understood endocrine physiology, ease of hormonal manipulation of the externally developing tadpole and potent molecular tools for the discovery of important information about tissue- and developmental stage-specific responses to TH.
mammalian TR-β gene has two major splicing isoforms, which create new amino-terminal transactivation domains, designated TR-β1 and TR-β2. In X. laevis, the major expressed TR-β isoform contains a very short amino-terminal sequence and thus is more similar to the avian TR-β0 isoform than to mammalian TR-β1 [15]. The mammalian TR-α2 isoform does not bind ligand, and has been described only in mammals. In X. laevis, xTR-α is expressed after the embryo hatches, and mRNA levels in whole tadpoles peak several days before the rise in TH at metamorphosis [16] (Figure 2). xTR-α is detectable in all cells examined so far, although the highest levels are found in those cells that proliferate upon TH treatment, such as the limb buds, basal layers of the skin, specific head cartilages and subventricular zones of the brain [17]. xTR-β is expressed at low levels during embryogenesis and premetamorphosis but then is strongly induced during metamorphosis [16]. xTR-β is highly induced in resorbing tissues such as the tail but at relatively low levels in most proliferating tissues [18].

TRs regulate specific target genes by binding to cis-acting DNA sites known as TH response elements (TREs). TRs can bind to TREs as monomers or homodimers but bind most efficiently as heterodimers with another family of nuclear receptors, the retinoid-X receptors (RXRs) [14]. X. laevis has three RXR genes, α, β and γ [19,20], which are also highly conserved with their mammalian counterparts (Figure 1b). Each RXR gene shows distinct expression patterns [12,20] (Figure 2). RXR-α expression essentially mirrors that of TR-α expression—that is, rising after hatching through metamorphosis—and is particularly highly expressed in the brain and limb buds; RXR-γ is only significantly expressed in the fertilized egg and early embryos and RXR-β expression peaks during neurulation and tail bud stages, when retinoids are important for nervous system patterning. The prototypical TRE, a synthetic element selected for maximal strength in transfection assays and TR-specific regulation, consists of two AGGTCA half-sites in the same orientation, separated by four nucleotides. Many endogenous TH-responsive genes contain consensus TREs, including the X. laevis TR-β gene [21,22] and two other transcription factor genes, encoding the X. laevis basic transcription element binding protein BTEB [23] and the Xenopus laevis homolog of the basic region leucine zipper protein E4BP4 termed TH/bZIP [24]. Unlike the promoter-proximal TR-β and TH/bZIP TREs, the BTEB gene TRE was found to be located ~6.5 kilobases from the transcription start site in a highly conserved 200 base pair region in both duplicated BTEB gene sequences [23]. Because genome duplication in the Xenopus genus occurred 30 million years ago, this remote island of homology implies the existence of an enhancer region that is important for TH control of this gene. Distal and evolutionarily conserved enhancer regions have been discovered in mammalian estrogen-regulated genes, and have led to the discovery of FoxA1 as an important auxiliary factor for transcripational control by the estrogen receptor [25]. Similar analysis for a larger group of TH-regulated genes might reveal a combinatorial enhancer code important for stage- and tissue-specific TH-mediated transcriptional control during development.

**TR corepressors and coactivators are structurally and functionally conserved**

Similarly to their mammalian counterparts, the xTRs interact with coregulatory proteins that mediate their potent transcriptional activation or repression functions [14]. In vivo chromatin immunoprecipitation (ChIP) assays using isolated tail or intestine nuclei demonstrated that the unliganded xTR-α interacts with the TR-β and TH/bZIP TREs in tadpole tissues before the onset of metamorphosis [26] and is found in a complex with the X. laevis homolog of the nuclear receptor corepressor NCoR [27]. The TR complex also contains chromatin-silencing machinery such as histone deacetylases (e.g., Rpd3 or histone deacetylase 1) and accessory factors Sin3 and transducin-β-like protein 1 [28,29]. Addition of TH disrupts recruitment of corepressors to target gene promoters, and concomitant increases in acetylation of histones H3 and H4 are observed. X. laevis NCoR shows 68% identity and 80% similarity overall compared with human NCoR, including 17 of 18 amino acids forming each TR-interacting corepressor nuclear receptor (CoRNR) box (Figure 1c). The related silencing mediator of retinoid and thyroid hormone receptors (SMRT) corepressor is also expressed in X. laevis tissues before and during metamorphosis but only a partial complementary DNA sequence has been reported.

Multiple proteins interact with the TRs upon ligand binding and contribute to transcriptional activation by a variety of mechanisms. Members of the p160/steroid receptor coactivator (SRC) family of coactivators contain intrinsic histone acetyltransferase activity and recruit
additional histone acetyltransferases such as cAMP response element-binding protein-binding protein (CBF/p300 and p300/CBP-interacting protein (p/CIP).

SRC-3 and transcription intermediary factor 2 (also known as SRC-2) have been identified in X. laevis [30,31]; however, only SRC-3 has been analyzed in detail at metamorphosis. X. laevis SRC-3 shows 77% overall identity compared with its human counterpart, including three conserved TR-interacting LXXLL motifs (Figure 1d). The SRC-3 gene is induced by TH in the tail and intestine, and this coactivator is recruited to the TH/bZIP promoter TRES in a TH-dependent manner [32]. Recruitment of SRC-3 to the TR-β TRE is TH dependent in the intestine but not in the tail, despite significant increases in histone acetylation on both promoters in both tissues [28,32]. The significance of this promoter- and tissue-selective constitutive SRC-3 binding is unclear. For functional analysis in vivo, a dominant negative form of SRC-3 (dnSRC-3) containing only the central receptor-interacting LXXLL motifs was expressed in transgenic tadpoles and competitively displaced endogenous SRC-3 from target genes, inhibiting their induction [33]. The transgene strongly inhibited TH-induced gill and tail resorption, intestinal remodeling and Meckel’s cartilage (lower jaw) outgrowth, and partially inhibited hindlimb development. Although most animals died during spontaneous metamorphosis, some survivors retained their tails for several months after metamorphosis was complete [33]. Thus, coactivator recruitment to the ligand-bound TR appears to be crucial for larval tissue resorption programs and might also contribute to the growth and differentiation of adult tissues.

Additional coactivators and associated complexes could have important roles in metamorphosis. Indeed, multiple coactivators use the same interaction surface that would be competitively occupied by the dnSRC-3 protein. For example, the X. laevis homolog of the nucleosome-binding Trip7 coactivator was identified as a TH-inducible gene in X. laevis [33]. However, only SRC-3 has been analyzed in detail at metamorphosis. X. laevis TR-α shows 77% overall identity compared with its human counterpart, including three conserved TR-interacting LXXLL motifs (Figure 1d). The SRC-3 gene is induced by TH in the tail and intestine, and this coactivator is recruited to the TH/bZIP promoter TRES in a TH-dependent manner [32]. Recruitment of SRC-3 to the TR-β TRE is TH dependent in the intestine but not in the tail, despite significant increases in histone acetylation on both promoters in both tissues [28,32]. The significance of this promoter- and tissue-selective constitutive SRC-3 binding is unclear. For functional analysis in vivo, a dominant negative form of SRC-3 (dnSRC-3) containing only the central receptor-interacting LXXLL motifs was expressed in transgenic tadpoles and competitively displaced endogenous SRC-3 from target genes, inhibiting their induction [33]. The transgene strongly inhibited TH-induced gill and tail resorption, intestinal remodeling and Meckel’s cartilage (lower jaw) outgrowth, and partially inhibited hindlimb development. Although most animals died during spontaneous metamorphosis, some survivors retained their tails for several months after metamorphosis was complete [33]. Thus, coactivator recruitment to the ligand-bound TR appears to be crucial for larval tissue resorption programs and might also contribute to the growth and differentiation of adult tissues.

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TR activation is essential for metamorphosis

A role for TRs in mediating TH-induced metamorphosis has been demonstrated by two different approaches. The first approach used transgenic overexpression of a potent dominant negative form of X. laevis TR-α (DNTR) that lacks the last 17 amino acids of the ligand-binding domain [36]. This truncation strongly inhibits ligand-binding activity and prevents recruitment of coactivators that rely on an intact AF-2 domain (i.e. the p160/SRC class). As expected, the corepressor NCoR is retained on promoters occupied by the DNTR even in the presence of TH, thus maintaining target genes in a repressed state [37]. Ubiquitous expression of the DNTR prevents a broad spectrum of TH-induced morphological changes, ranging from proliferation of the jaw and brain, resorption of the gills and tail, and remodeling of the intestinal tract [36] (Figure 3). A twist on the dominant negative approach was to attach both a strong viral activation domain and the carboxy-terminus of NCoR containing only the receptor-
interacting domains to the AF-2 truncated receptor, with the dual goal of interfering with NCoR recruitment and promoting hormone-independent activation of target genes [38]. Heat shock-inducible, ubiquitous expression of this unique ‘dominant positive’ receptor resulted in TH-independent cell death, hindlimb outgrowth, intestinal remodeling and upregulation of several TH-response genes.

A second approach to probe the importance of the liganded TR in metamorphosis was to use a synthetic TR antagonist, NH-3, which competitively inhibits TH-induced transcription in transient transfection assays [39]. NH-3 was designed rationally with a nitrophenyl group extension to prevent proper helix-12 folding and coactivator recruitment; indeed, the compound induces a distinct conformation of the receptor, as judged by protease protection assays [40]. Importantly, the compound strongly inhibits TH-induced morphological changes in treated tadpoles as effectively as does DNTR expression in transgenic tadpoles (Figure 3); it also inhibits TH-induced transcription of endogenous genes and completely and reversibly inhibits spontaneous metamorphosis [40]. Therefore, NH-3 is the first TR antagonist to show potent in vivo inhibition of TH action and further demonstrates the clear dependence on TR transactivation properties for metamorphosis to proceed.

Major questions of metamorphosis
Although significant progress has been made on the role of TRs and associated coregulators in metamorphosis, several key unanswered questions remain. Addressing each of these questions has important ramifications for hormone action in all animals.

Tissue specificity
Perhaps the most remarkable aspect of metamorphosis is that the same simple molecule (i.e. TH) can induce completely opposite morphological responses in distinct tissues, ranging from growth and differentiation of limbs to the complete resorption of the tail, even though both organs contain similar tissue types (e.g. skin, nerves, blood vessels and muscle). Differences in TR isoform expression suggest specific roles in metamorphosis, and correlate with distinct gene expression programs (Box 2). Supporting this observation, the synthetic TR agonist GC-1 activates TR-β with tenfold greater potency compared with TR-α, and induces gill and tail resorption with little effect on limb growth [41]. In Drosophila metamorphosis, distinct ecdysone receptor isoforms that differ only in their aminoterminal AF-1 domains control specific tissue responses, as demonstrated by isofrom-specific mutations [42,43]. Such unequivocal genetic evidence for distinct TR function in amphibians is not currently possible; even so, the exact means of receptor isofrom-specific control would still be unclear, as it is in Drosophila. Indeed, tissue specificity might be programmed during embryogenesis, long before TR isoforms and TH first appear. Further dissection of highly specific TR response genes using transgenic reporter gene assays should reveal important enhancer and silencer elements and lead to the identification of key transcription factors involved in the exquisite degree of tissue-specific responses induced by TH.

Timing
Timing is a crucial issue in metamorphosis. For example, the animal must complete the differentiation of the hind- and fore-limbs before the tail is lost, and the lungs must become fully functional before the gills are resorbed. A simple explanation for timing is based on the differential sensitivity of tissues to TH [44]. Low doses of TH will induce limb outgrowth, whereas several-fold higher concentrations are required for tail resorption. Accordingly, during spontaneous metamorphosis, limbs grow when there are low levels of circulating TH and the tail resorbs only when TH levels are highest [14]. How is differential tissue sensitivity achieved? One important mechanism is the differential expression of type III deiodinase (D3), which removes an inner-ring iodine from both T4 and T3, creating inactive metabolites [44] (Box 2). D3 is expressed in the tail before metamorphosis and is induced by TH, followed by a decline at the onset of resorption [45]. D3 expression is initially undetectable in the limb buds and rises modestly as the limbs complete morphogenesis. Dorsal retina-specific D3 expression enables asymmetric proliferation of the ventral ciliary marginal zone and ipsilateral projection of retinal ganglion cells from that region to the optic tectum, thus establishing binocular vision in the predatory frog [46]. Overexpression of D3 in transgenic X. laevis delays metamorphosis, including ventral retinal proliferation, and it is particularly effective at blocking tail resorption, resulting in ‘tailed’ froglets [47]. By contrast, the type II deiodinase (D2), which converts T4 to T3, is expressed at high levels before metamorphosis in the subventricular zones of the brain and the hindlimb buds. Thus, these tissues are poised to respond to very low circulating T4 levels [48]. At metamorphic climax, when the final changes of metamorphosis, such as tail resorption, commence, D2 levels rise abruptly in the tail and in the TSH-secreting cells of the pituitary [49]. Although the capacity for negative feedback exists before climax [50], the onset of D2 expression leads to a dramatic increase in the ability of T4 to suppress pituitary TSH expression and thus sets up a classical negative feedback loop in the adult. D2 overexpression in transgenic tadpoles, either
ubiquitously or under specific promoters, has not yet been reported.

Differences in TR and coregulator expression levels in specific tissues might also contribute to precise timing (Box 2). Limb buds express high levels of TR-α and SRC-3 before metamorphosis, whereas tails express much lower levels of each [18,32]. Coincident induction of TR-β and a coactivator in the tail would substantially increase target gene responsiveness, leading to its timely demise. Perhaps counterintuitively, NCoR and SMRT levels are also high in the limb buds and low in the tails before metamorphosis [27]. Unoccupied TRs bound to highly expressed NCoR and SMRT corepressors might compete with low levels of TH-occupied and coactivator-bound TRs at the beginning stages of metamorphosis. Elevated corepressor levels might be important to prevent precocious proliferation without adequate amounts of TH, or might be used by other nuclear receptors or transcription factors to maintain adult precursor cells in an undifferentiated state. In addition, corepressor localization and receptor interaction might be altered by growth factor activity [51,52]. The loss of TR interaction with NCoR or SMRT in rapidly growing cells would shift the TH dose–response curve leftward and thereby amplify the sensitivity of the tissue.

Cell autonomy
A useful feature of amphibian biology is the ability to culture various organs and tissues in vitro, or transplant tissues onto various locations of the developing embryo and tadpole. Such experiments first demonstrated the tissue autonomy of TH responses; isolated tails in culture will resorb, and isolated limb buds will grow and differentiate, in a TH-dependent manner [14]. To determine the degree of cell autonomy, or whether the fate of one cell is dependent on TH influence on adjacent cells, DNTR was expressed in specific cell types using transgenic assays or by direct injection of expression vectors. Epidermal larval cell-specific DNTR expression prevents TH-induced cell death via apoptosis and repression of larval skin-specific genes but enables adult skin to develop normally underneath [53]. Using multiple tissue specific-promoters driving DNTR, several cell types in the developing limbs were shown to develop autonomously. Neural-specific DNTR expression resulted in the absence of motor neurons and paralysis, with apparently normal muscle, cartilage and bone formation; skeletal muscle-specific DNTR expression also resulted in paralysis owing to the complete lack of muscle despite normal limb size, cartilage and bone [54]. The fate of motor neurons in the absence of muscle fiber development was not reported. However, most cell autonomy experiments have investigated whether skeletal muscle in the resorbing tail dies via an intrinsic TH-induced program (‘suicide’) or as the result of degradation of the surrounding extracellular matrix by TH-responsive fibroblasts (‘murder’). The first evidence that larval muscle dies autonomously came from isolation of a myoblast cell line from tadpole tails [55]. These cells differentiated into myotubes that would undergo features of apoptosis and induce caspase-3 expression upon TH treatment. Consistent with these findings, skeletal muscle-specific DNTR expression by transgenesis or direct plasmid injection strongly inhibits muscle loss during metamorphosis [56,57]. Therefore, larval muscle responds directly to TH to activate its own death-inducing cascade, although complete extracellular matrix breakdown and loss of the vasculature might accelerate the process during the latter stages of tail resorption [57]. The intrinsic larval muscle death program involves activation of the apoptotic machinery including multiple caspases and the proapoptotic gene bax [58,59]. Although the limb, skin and larval muscle results using DNTR expression are fairly clear, other tissues and organs might not show the same degree of cell autonomy.

Competence
A crucial concept in developmental biology is competence, or the development of the ability of a cell to respond maximally to a given inducer. Adequate receptor levels are taken for granted as a primary mechanism of hormonal competence. In X. laevis, many tadpole tissues, such as the gills and the lower jaw, can respond to TH one week after fertilization [60]. In general, the ability of tadpoles to respond at least partially to exogenous TH correlates with rising expression of xTR-α and xRXR-α [16]. Injection of synthetic mRNAs encoding xTR-α and xRXR-α into fertilized eggs enabled the precocious upregulation of two early TH-response genes along with some associated morphological changes; however, these changes were not identical to those observed during natural metamorphosis [61]. Tissue competence does not develop uniformly — tail resorption competence develops later than that of most other tissues. In addition, different gene classes acquire transcriptional competence at distinct developmental stages [18]. For example, when hindlimbs are still small buds, the tail is poorly responsive to exogenous T3, and only early genes such as TR-β can be significantly induced. Several days later, when the hindlimbs are beginning to grow and differentiate, TH addition causes a dramatic increase in the tail resorption rate and the ability to upregulate late-response genes such as that encoding collagenase-3. Early genes are induced to the same degree at both stages of development [18]. Therefore, adequate receptor expression is necessary but not sufficient to obtain maximal tissue sensitivity to TH, and late genes might require the expression of an additional competence factor for full induction. Stage-specific competence in Drosophila metamorphosis requires a network of orphan nuclear receptor activity [62]. Although the Xenopus genome encodes most, if not all, of the vertebrate nuclear receptor family, few have been studied at metamorphosis. Certainly, other mechanisms for TR competence can be envisioned, such as quantitative or qualitative differences in coactivator expression and activity and/or rearrangement of target gene chromatin.

Conclusion
X. laevis has been a classical model system for embryogenesis for decades. With the application of advanced molecular tools, detailed molecular dissection of vertebrate postembryonic development, such as metamorphosis, is now possible. The essential role of ligand-occupied TRs in the induction of metamorphosis has been clearly
established by both ubiquitous and cell type-specific expression of a dominant negative TR, and the ability of a rationally designed TH antagonist, NH-3, to block induced and spontaneous metamorphosis. Many cell types appear to have autonomous, independent gene expression pathways leading to death or growth and differentiation in response to TH. A large number of TH-responsive genes have been identified in both death and growth pathways; however, significant work to determine their functional roles in metamorphosis remains to be carried out. One TH-response gene that has been analyzed in detail – that encoding the D3 enzyme – is a major contributor to the timing of tissue responses to TH [47]. D3 function was established through over- and mis-expression studies via transgenesis and the use of pharmacological inhibitors. Ultimately, full understanding of metamorphosis will be enhanced by the development of efficient gene interference assays suitable for use in tadpoles. Morpholino, or traditional antisense oligonucleotides and RNAs, dilute out rapidly or are degraded after injection into fertilized eggs; however, successful direct injection of certain tadpole tissues with these reagents has recently been demonstrated [58]. Tissue-specific and inducible expression of RNA interference or ribozyme constructs would be useful but such experiments are not yet routine in any transgenic vertebrate system. The continued development of the X. tropicalis system is especially important because it could provide a powerful genetic handle on gene function at metamorphosis [63,64]. Continued efforts using a variety of approaches to investigate the major questions of anuran metamorphosis outlined in this review (i.e. tissue specificity, timing, cell autonomy and competence) will have wide-ranging implications for important biological problems as diverse as worldwide declines in amphibian populations to the role of TH in human development.

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