

Review

The molecular basis of thyroid hormone-dependent central nervous system remodeling during amphibian metamorphosis¹

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Abstract

Tadpole metamorphosis involves a coordinated series of changes in virtually every tissue of the body. This developmental process is induced by the single morphogen, thyroid hormone (TH). The amphibian central nervous system (CNS) is a primary target for TH, and it undergoes dramatic morphological and cytoarchitectural changes in response to the hormone. TH acts by regulating gene expression and its actions in metamorphosis are thought to result from its ability to induce tissue-specific genetic programs. Receptors for TH are ligand-dependent transcription factors whose mRNA expression is upregulated by TH during metamorphosis (receptor autoinduction). Studies on the tadpole CNS have identified four general classes of early TH response genes. These genes code for: (1) transcription factors, that are likely to be required for the expression of downstream genes (i.e. secondary response genes), (2) cellular enzymes, which carry out hormone conversions, energy transformations and may possibly mediate extranuclear effects of TH on neural cells, (3) cytoskeletal elements required for axonal development, and (4) secreted signaling molecules that control the production of TH. Recent studies suggest a critical, evolutionarily conserved role for the TH-induced transcription factor genes in controlling neural cell proliferation and differentiation. © 1998 Elsevier Science Inc. All rights reserved.

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1. Introduction

The life histories of amphibians that have a larval stage are characterized by a discrete phase of morphogenesis referred to as metamorphosis. In anurans, this metamorphosis is characterized by the transition from an aquatic, fish-like, herbivorous larvae (in many species) to a tetrapodal, exclusively carnivorous, and often

terrestrial adult. Because of the dramatic changes that occur during metamorphosis (which are readily observed due to the accessibility of the organism compared with amniotes) the process is often referred to as a transformation. However, this usage often obscures the commonalities that the developmental processes that occur during metamorphosis have to postembryonic development in vertebrates generally. During metamorphosis there is organogenesis and tissue remodeling, each involving a coordinated process of cell proliferation, differentiation and death. Recent findings show that the cellular and molecular bases of these postembryonic morphogenetic processes are highly conserved among diverse animal species. In this paper recent molecular studies of hormone action in amphibian neural development will be examined.

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2. Hormonal control of amphibian metamorphosis

Metamorphosis of the amphibian tadpole involves a coordinate series of changes in virtually every tissue. The entire suit of changes that occur during metamorphosis is induced by the hormone thyroxine (T_4) [72,75,86]. Enzymatic conversion of T_4 (monodeiodination) in target tissues results in the production of 3,5,3'-triiodothyronine (T_3), which has ten times or greater the biological potency of T_4 [31]. Throughout the remainder of this paper T_3 will be used to refer to thyroid hormone (TH). Plasma T_3 levels rise during metamorphosis and reach a peak at climax [54,96]. In addition to T_3 , plasma levels of corticosteroids (produced by the interrenal glands) rise during metamorphosis and have been shown to synergize with T_3 to promote metamorphosis [20,54,96].

While T_3 is the major stimulus for metamorphosis, pituitary prolactin (PRL) is thought to exert antimetamorphic actions and perhaps promote larval growth [20,54,96]. Etkin [24] predicted that PRL production would be high during premetamorphosis (before limb morphogenesis), when larval growth is maximal. Levels of PRL would then decline during prometamorphosis (when morphogenesis is accelerating in response to rising titers of plasma T_3) and be lowest at metamorphic climax (the most rapid phase of morphogenesis). However, recent findings have shown that plasma PRL and pituitary PRL mRNA levels show the exact opposite relationship; that is, levels are very low during premetamorphosis but rise during late prometamorphosis and peak at metamorphic climax [14,54]. Nevertheless, it has been proposed that even low levels of PRL during premetamorphosis could antagonize the metamorphic effects of T_3 [14,86]. A potential mechanism for PRL antagonism of metamorphosis is the blockade of thyroid hormone receptor (TR) autoinduction [86]. The surge in PRL at metamorphic climax may serve to balance the morphogenic actions of TH [14,64].

Secretory activity of the thyroid and interrenal glands is controlled by pituitary hormones (Fig. 1). Pituitary thyrotropin (TSH; a glycoprotein hormone composed of two subunits) controls T_3 production and adrenocorticotropic (ACTH; a 39-amino acid polypeptide derived from processing of proopiomelanocortin) controls corticosteroid production [14,54]. The biosynthesis and secretion of TSH and ACTH (and also PRL) are controlled by neurohormones produced in the hypothalamus. The neuroendocrine system is central to the control of the endocrine changes that drive metamorphosis. An intact pituitary gland and hypothalamus are both required for metamorphosis [20,37,54,10,64].

The secretion of hormones by the adenohypophysis during metamorphosis is controlled by specific release and release-inhibiting factors produced by modified neurons in the hypothalamus and released into the

pituitary portal circulation (Fig. 1). The primary neurohormonal regulator of TSH secretion in mammals is thyrotropin-releasing hormone (TRH; a tripeptide) [62]. However, despite increased TRH immunoreactivity in tadpole brain during metamorphosis [61], TRH does not appear to control thyroid secretion during metamorphosis [14,64]. Instead, corticotropin-releasing hormone (CRH; a 41-amino acid peptide) may be the larval amphibian TSH-releasing factor, and CRH may control both TSH and ACTH secretion during metamorphosis [13–17,32,49,54,89].

3. Metamorphosis of the amphibian central nervous system

TH controls metamorphosis and thus plays an important role in the developmental changes in the nervous system that occur during metamorphosis. Primary among these changes is the extensive remodeling of regions of the CNS that are necessary for the shift from larval to adult life. Metamorphosis of the amphibian tadpole is a unique system for examining the effects of T_3 on cell growth, differentiation and death in the CNS, and for correlating these actions with functional changes that lead to the development of adult behaviors [50].

During CNS remodeling, certain larval structures are eliminated; for example, the Mauthner neurons found on either side of the medulla, and the sensory and motoneurons supplying the tail [20,29,55]. While larval structures are disappearing, adult-specific neural struc-

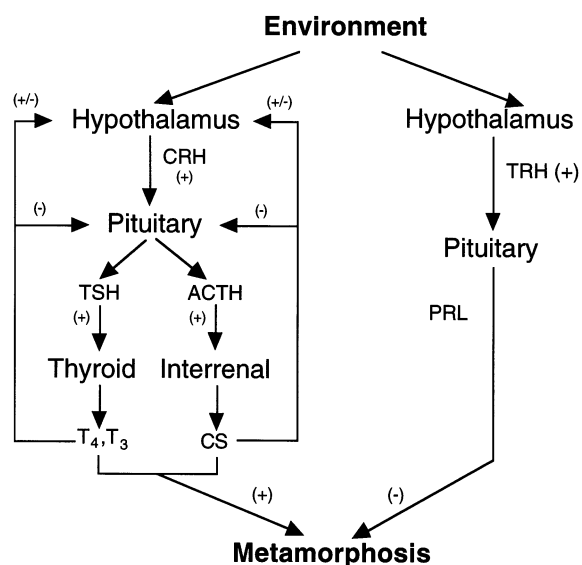


Fig. 1. Schematic representation of the endocrine systems controlling amphibian metamorphosis. Pluses designate stimulatory, and minuses inhibitory control pathways. Pluses and minuses together indicate that the effect can be either stimulatory or inhibitory depending on the developmental stage. CS, corticosteroids.

tures develop. For instance, the major portion of the retina arises along with the associated visual projections in the di- and mesencephalon. This involves major changes in retinal circuitry and projections that will subservise binocular vision (compared with the panoramic vision of the tadpole) [41]. Other changes include development of the mesencephalic nucleus of the trigeminal nerve (mesencephalic V nucleus) [56], development of the cerebellum [35], and development of spinal cord segments connected to the limbs [3,42].

As described above, an intact, functional neuroendocrine system is required for metamorphosis. TH is known to influence the development of neurosecretory structures in the diencephalon, and thus can exert a positive influence on further production of the hormone. The median eminence is the structure containing neurosecretory nerve terminals that contact capillaries that drain into the pituitary portal vessels. This structure is critical for the delivery of neurohormones to the pituitary gland, and its development during prometamorphosis is dependent upon T_3 [23,24,37,79,93].

In addition to the modified nerve terminals in the median eminence, the development of hypothalamic neurosecretory perikarya is dependent upon T_3 [36–39]. In *Xenopus* tadpoles the neurosecretory neurons of the preoptic nucleus (the major neurosecretory center in the tadpole diencephalon) develop in parallel with the development of the thyroid follicles [37]. The development of hypothalamic monoaminergic neurons are also dependent on T_3 [53]. Support for the hypothesis that TH is required for the maturation of neurosecretory centers was provided by Norris and Gern [65] who showed that neotenic tiger salamanders could be induced to metamorphose by intrahypothalamic T_4 injections; whereas, injection of the same dose intraperitoneally did not influence metamorphosis.

This is only a partial description of the many changes induced by T_3 during metamorphosis of the amphibian brain. Complete coverage of these changes is beyond the scope of this paper, and the reader is referred to several excellent review papers for further information [20,24,29,35,37,41,46].

4. Molecular basis of thyroid hormone action on CNS development

In addition to inducing metamorphic changes in amphibia, TH is required for normal development of the brain of all vertebrates. TH deficiency during fetal life in mammals results in severe mental retardation (i.e. cretinism) and growth defects postnatally [21]. On a global scale, TH deficiency is one of the most pervasive and detrimental human health problems, resulting in entire populations of mentally impaired and physically stunted individuals in certain areas of the world.

TH exerts pleiotropic actions on the developing mammalian brain, influencing diverse processes such as neuronal maturation, neurite outgrowth, synapse formation, timing of cell differentiation, and myelination [66]. Cytoarchitectural abnormalities in the hypothyroid brain have been studied most in the rat, and these analyses have revealed multiple morphological and biochemical defects. The neurodevelopmental abnormalities caused by insufficient TH during fetal and neonatal development result in behavioral abnormalities postnatally. Studies with fetal brain cell cultures clearly demonstrate that the action of T_3 on the morphological and functional development of neural cells is direct [2,34,67,68]. Despite the profound changes induced by T_3 in the developing brain, relatively little is known about the molecular basis of T_3 action in neural development.

4.1. Thyroid hormone receptors

TRs are ligand-dependent transcription factors that belong to the steroid receptor superfamily [60,90]. Thus, T_3 is thought to control metamorphosis primarily by regulating gene expression [75]. The T_3 -TR complex interacts with specific TH response elements (TRE) present in the target gene and can either enhance or repress gene transcription. The TRs bind TREs as either homo- or heterodimers, and while TRs can heterodimerize with receptors for retinoic acid, vitamin D and retinoid X, the most effective dimerization partner appears to be retinoid X receptor (RXR) which binds 9-*cis* retinoic acid [40,97].

TRs are encoded by at least two genes (α and β) in all vertebrates that have been examined [60,90]. *Xenopus* possesses two $TR\alpha$ and two $TR\beta$ genes (owing to its pseudotetraploidy), each of which are expressed during metamorphosis [100,101]. In mammals, alternative splicing of the primary $TR\alpha$ mRNA transcript produces a functional TR ($\alpha 1$), and a variant that does not bind T_3 ($TR\alpha 2$; this protein may have dominant negative activity on certain TREs) [67,90]. Alternative splicing of the $TR\beta$ mRNA produces two functional TRs ($TR\beta 1$ and $TR\beta 2$). The $TR\alpha 1$ is the first TR isoform to be expressed in the brain of rat embryos; whereas, the $TR\beta 1$ becomes the predominant TR isoform after the second week of life [28,67]. While splicing variants of TRs exist in *Xenopus* [76,101], there is currently no evidence for their differential expression or for dominant negative activity of their protein products.

In *Xenopus* tadpoles the $TR\alpha$ gene is constitutively expressed from early in development, while the $TR\beta$ gene is induced in response to rising titers of T_3 (autoinduction) [86]. The highest concentration of both $TR\alpha$ and $TR\beta$ mRNA transcripts is found in the tadpole central nervous system (CNS) [51]. The tadpole CNS

becomes 'competent' to respond to T_3 earlier and exhibits a greater response to exogenous T_3 than any other tadpole tissue [14,29,55,83]. Despite considerable information on TR expression in other tadpole tissues [22,51,97,100], little is currently known about the regional distribution or the regulation of TRs in tadpole brain.

At metamorphosis, it is predicted that the induction of gene expression by TH is dependent on the upregulation (autoinduction) of TR β [86]. As change is initiated in target tissues, the synthesis of TR β probably accounts for the increased receptor content of the responding tissue [22]. This receptor autoinduction is thought to be responsible for the activation of downstream genes [86], although this has not been tested directly. To examine the role of receptor autoregulation in metamorphosis, Ulisse et al. [91] introduced dominant negative TR β mutants into tadpole tail muscle and assessed their effects on the expression of coinjected reporter genes. The TR β mutants blocked the activation by T_3 of a reporter gene (chloramphenicol acetyl transferase; CAT) driven by the TR β promoter. This study represents a first step towards understanding the role of TR β autoinduction in gene regulation. However, this type of analysis has not yet been extended to the effects of mutant TRs on endogenous gene expression, which will be necessary to fully test the hypothesis that TR β autoinduction is required to activate downstream genes.

4.2. Thyroid hormone target genes in the developing CNS

Because TH exerts its effects through changes in gene expression, the pleiotropy of the effects of the hormone suggests an underlying molecular developmental process of great complexity. However, only a relatively small number of genes have been shown to be regulated by T_3 in the developing mammalian nervous system. These include the transcription factor NGFI-A [63], RC3, a brain-specific gene encoding a protein kinase C substrate [47], prostaglandin D2 synthetase [33], neurotrophin-3 [59], Purkinje cell protein-2 [81], myelin basic protein (MBP) [25,26], and myelin-associated glycoprotein (MAG) [69]. Recent attempts to isolate T_3 -regulated genes in neonatal rodent brain by differential screening techniques have met with limited success. Genes identified by these methods include several mitochondrial genes (12S and 16S rRNAs, cytochrome c oxidase subunit III and NADH dehydrogenase subunit 3) [43,92], α -tubulin and NCAM [44], a novel synaptotagmin-related protein (involved with regulating neurotransmitter release) and a putative zinc finger protein related to the product of a recently identified mouse gene, hairless [88].

4.3. Gene regulation programs induced by thyroid hormone during amphibian development

Metamorphosis involves a coordinated process of cell replacement, cell death and functional reorganization, all of which is controlled by TH [84]. Because TH acts through ligand-dependent transcription factors, it is predicted that the hormone induces a gene regulation cascade. Following the autoinduction of TR β by T_3 , a series of gene expression changes is induced which drive the transformation of larval into adult tissues. The hypothesis that this results in the induction of tissue-specific genetic programs is supported by studies of gene expression in tadpole tail, hind limb, intestine and brain [4,5,18,73,95]. The induction of such gene regulation programs is a universal phenomenon in the development of eukaryotes; similar gene regulation cascades are induced by steroid (and thyroid) hormones during mammalian development [12].

Using a PCR-based gene expression screen (subtractive hybridization) [94], Denver et al. [18] isolated 34 cDNAs that correspond to mRNAs that are regulated by T_3 in the brain of *Xenopus* tadpoles within the first 20 h after exposure to T_3 (most of the genes are upregulated, although 14 show a complex, biphasic pattern of expression in response to T_3). Seven of the fragments share significant sequence similarity to known genes; five other T_3 -response genes were identified by hybridization. Kinetic analyses showed that most of these genes are regulated by TH within 4–8 h and their regulation is independent of protein synthesis, suggesting that most of them are regulated at the transcriptional level. Furthermore, 13 are regulated by TH only in the brain.

5. The early gene regulation program induced by T_3 in the developing CNS

How can a single morphogen (T_3) induce asynchronous, organ-specific transformation (i.e. involving cell growth, differentiation and death in different tissues)? Recent studies that identified T_3 target genes in different tadpole tissues suggest that the hormone induces tissue-specific genetic programs that underlie organ-specific transformation [4,5,18,73,95]. While some of the early T_3 -response genes are common to all tadpole tissues, others are tissue-specific in their expression patterns. Considering our studies on *Xenopus* tadpole brain, the early gene regulation program induced by T_3 can be divided into four classes of genes (Table 1): (1) transcription factors, (2) cellular enzymes, (3) structural proteins, and (4) secreted signaling molecules.

Table 1
Early T₃ response genes identified in premetamorphic tadpole brain [18]

Class	Gene	T ₃ regulation ^a
Transcription factors	TR β	U
	Glucocorticoid receptor	B
	BTEB	U
	bZIP	U
	HMG-box protein (homolog of rat Hbp1)	B
Cellular enzymes	Type III monodeiodinase	U
	Creatine kinase	B
	Cytochrome c oxidase (subunit I)	U
	PDI	B
Cytoskeletal proteins	Neural-specific β -tubulin	B
Secreted signaling molecules	TRH	B
	CRH	D

^a U, upregulated; B, biphasic response pattern (up–down–up regulation); D, downregulated.

5.1. Transcriptional regulatory networks induced by T₃ in tadpole brain

Several of the early T₃-regulated genes isolated from tadpole brain code for transcription factors [18]. The protein products of these genes are likely to be critical to the induction of a secondary wave of gene expression. These secondary response genes are predicted to code for proteins that define the adult neural cell phenotype (for example, extracellular matrix proteins, neurotransmitter metabolizing and synthesizing enzymes, receptor molecules, etc.). Two of the transcription factors (TR β and GR) belong to the steroid receptor gene superfamily. The upregulation of TR β expression by T₃ in the brain is consistent with results from studies of other tissues [74,85,86]. As described earlier, TR β autoregulation is likely to be critical to the induction of downstream genes required for tissue-specific transformation. The GR exhibits complex biphasic regulation, but the sustained effect of T₃ is upregulation. Crossregulation of nuclear hormone receptors may be one mechanism to explain the observed interactions between thyroid and corticosteroid hormones during metamorphosis (see above) [54,85].

The basic transcription element-binding protein (BTEB) gene was first cloned from rat [45] and was recently identified as a TH-regulated gene in tadpole tail [4]. We isolated BTEB from tadpole brain as a T₃-regulated gene [18]. BTEB belongs to the Sp1 family of proteins that binds to GC box motifs to activate transcription [45]. The rat proteins differ significantly in size (244 amino acids for BTEB versus 788 amino acids for Sp1) [45], and it is only in the zinc finger region

(DNA-binding domain) where significant sequence similarity (72%) with Sp1 is observed. The zinc finger region is identical between rat and *Xenopus* BTEB [4]. Interestingly, while BTEB mRNA is ubiquitously expressed in the rat, the mRNA is translated only in the brain [46]. The gene is strongly upregulated in tadpole brain by T₃; thus, it is likely that BTEB plays a central role in the transduction of the T₃ signal in neural development.

Other T₃-regulated transcription factors in tadpole brain include the basic leucine zipper protein (bZIP), first isolated from tail [4,95], and the *Xenopus* homolog of the rat HMG-box transcription factor Hbp1 [58]. Both of these transcription factors may function as transcriptional repressors. For instance, the *Xenopus* bZIP exhibits the greatest sequence similarity to human E4BP4 [11], a protein with demonstrated transcriptional repressor activity [10]. The rat Hbp1 was recently shown to interact (by protein–protein interaction) with the retinoblastoma family of growth suppressors and is upregulated in cells undergoing differentiation [87]. Transcriptional repression plays a key role in cell differentiation pathways, and these two transcription factors may be central to the growth arrest and differentiation of neural cells.

5.2. T₃-dependent energy conversions during neurogenesis

Several of the T₃-regulated genes isolated from tadpole brain code for cellular enzymes involved in energy metabolism (cytochrome c oxidase subunit I and brain-type creatine kinase). Similarly, in mammals, several genes which code for energy-converting enzymes were isolated as T₃-regulated in neonatal rodent brain [43,92]. Cytochrome c oxidase is an important mitochondrial proton-pumping respiratory protein that catalyzes the transfer of electrons from cytochrome c to O₂ [82]. Brain-type creatine kinase catalyzes the transfer of phosphoryl groups from phosphocreatine to ATP [82]. While T₃ does not affect brain metabolism in adult mammals, it does increase oxygen consumption of neonatal neural cells [48]. The regulation by T₃ of these energy-converting enzymes could subserve an adaptive function by providing sufficient energy for developmental processes.

5.3. Hormone conversion

The monodeiodinase cloned from tadpole brain by subtractive hybridization [18] was previously isolated from tail [95] and later shown to be a type III deiodinase [80]. This enzyme catalyzes the conversion of T₄ to reverse T₃ and T₃ to T₂ (i.e. it degrades TH). A potential role for this enzyme is to maintain levels of active TH within a range appropriate for the coordinate actions of the hormone during development.

5.4. Extranuclear actions of thyroid hormone

Several investigators have demonstrated the existence of plasma membrane and cytosolic binding sites for T_3 in mammalian cells [7–9,71,99], and two such cases in *Xenopus* [77,98]. At least two roles for these proteins have been suggested: (i) to regulate the bioavailability of T_3 for binding to TRs [1], or (ii) to modulate the rapid, extranuclear effects of T_3 on neural cells. For instance, T_4 promotes actin polymerization in astrocytes [78] and regulates laminin–integrin interactions [27] by a mechanism that is independent of nuclear TRs.

Early studies on extranuclear T_3 -binding identified a 55-kDa protein in various tissues that was plasma-membrane associated and was responsible for 85% of the binding of *N*-bromoacetyl-3- $[^{125}I]T_3$ [8,9]. This protein has subsequently been identified as a monomer of protein disulfide isomerase (PDI) [7,70,99]. PDI is a multifunctional protein; its primary functions are to assist the folding of proteins containing disulfide bonds and to serve as a subunit for more complex enzyme systems [30]. However, the mechanisms by which PDI functions are still not clear. Human PDI has two active sites that share a high degree of similarity to the redox protein thioredoxin [30]. PDI exhibits a T_3 -dependent association to the F-actin microfilaments in cultured astrocytes [71] and could thus be involved in the effects of T_4 on actin polymerization.

We isolated a PDI-like protein (PDI-LP) from *Xenopus* tadpole brain as a T_3 -regulated gene [18]. The *Xenopus* PDI-LP may be critical for the successful expression of the secondary gene regulation cascade induced by T_3 in neuroendocrine cells. For instance, while the transcription factors that are rapidly induced in response to T_3 are expected to regulate the transcription of the secondary response genes (see above), the xPDI-LP could be essential for expression of the functional proteins (i.e. correct folding and disulfide bond formation). Given the multifunctional nature of PDIs, it is also possible that xPDI-LP could modulate the development of the neural cell cytoskeleton. The protein could control polymerization of actin and tubulin, the monomeric subunits of the microfilaments and microtubules, respectively (for example, axon development and process formation in neuroglial cells) [70,71,78]. As we have shown, T_3 regulates the expression of the neural-specific β -tubulin in tadpole diencephalon [18]; polymerization of tubulin could be catalyzed by the xPDI-LP.

5.5. Secreted signaling molecules

Neuropeptides are transported to the anterior pituitary gland via the pituitary portal vessels where they influence the secretion of hormones that control the

thyroid and the interrenal glands [14] (see above). Current data support the view that CRH is the primary regulator of the thyroid and interrenal axes (regulating pituitary TSH and ACTH), and that TRH has no effect on tadpole thyroid function, but may play a role in controlling the secretion of PRL [13–17,29]. Earlier studies showed that T_3 is required for the differentiation of the hypothalamus and median eminence [14,23,36–39]. T_3 induces the accumulation of neurosecretory material in the preoptic nucleus and a molecular basis for this effect may be through upregulation of neuropeptide gene expression. For instance, we have shown that the TRH gene is upregulated by T_3 in the tadpole diencephalon [18].

In addition to exerting a positive, differentiative effect on the neuroendocrine system, T_3 also exerts a well-known negative feedback action on hypothalamic and pituitary hormone production in vertebrates [14]. Indirect measures of TSH using histological analyses of thyroid and pituitary glands and thyroidal radioactive iodine uptake analyses suggest that T_3 can exert a negative feedback action on pituitary TSH production in premetamorphic and early prometamorphic tadpoles [14,19,36–39,99]. There have been no direct measures of TSH gene expression or secretion in tadpoles except for the study of a single hypothyroid tadpole by Buckbinder and Brown [6], in which $TSH\beta$ mRNA was greatly elevated, thus supporting the existence of negative feedback on TSH. Whether the feedback is at the level of the pituitary gland or the hypothalamus has not been established, but our results suggest that both mechanisms may be operative. For instance, $TSH\beta$ mRNA levels were reduced by T_3 in a dose-dependent manner in pituitaries of prometamorphic *X. laevis* tadpoles cultured in vitro (Fig. 2). A negative feedback action of T_3 at the hypothalamic level is suggested by the downregulation of hypothalamic CRH mRNA in stage 52 *X. laevis* tadpoles treated with 5 nM T_3 in the aquarium water for 24 h [14,18]. However, still unexplained is how the dramatic rise in plasma TH concentration is achieved during late prometamorphosis and metamorphic climax given that functional negative feedback appears to be operative. In adult vertebrates, negative feedback would prohibit such a continuous, sustained rise in TH production. It is possible that the feedback sensitivity changes during metamorphosis, with feedback being suspended during metamorphic climax when thyroid activity is maximal as suggested by Kaye [52]. This hypothesis awaits a rigorous test.

6. Evolutionary conservation of T_3 -regulated neural genes

TH regulation of neural genes is evolutionarily conserved. For example, TR β , COX I and neural-specific

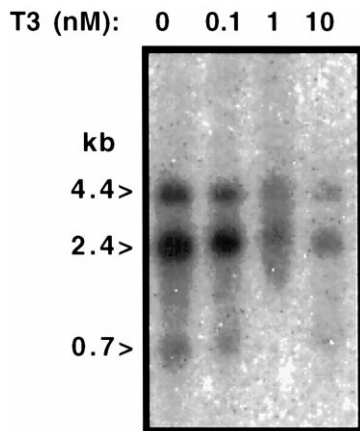


Fig. 2. Downregulation of TSH β mRNA expression by T₃ in pituitaries of prometamorphic (stage 58) *X. laevis* tadpoles. Pituitary glands were removed and cultured in vitro (10 glands/well) in Dulbecco's modified Eagle's medium (DMEM; diluted 1:2 for amphibian tissues) with or without different doses of T₃ for 24 h. A Northern blot was prepared from total pituitary RNA as described [18] and the blot was hybridized with a ³²P-labeled xTSH β cDNA probe [6]. The blot was stripped and reprobed with a ³²P-labeled cDNA for the *X. laevis* ribosomal L8 gene [18]. This analysis, and the analysis of methylene blue-stained ribosomal RNA bands confirmed equal RNA loading in each lane.

β -tubulin have been shown to be T₃-regulated in developing frog and rat brain [18]. Furthermore, our preliminary studies have demonstrated that T₃ regulates BTEB gene expression in fetal rat brain [19]. In addition, our results show that T₃ regulates rat BTEB mRNA levels in primary neurons and astrocytes and a neuroblastoma cell line (Neuro2a cells) that has been engineered to overexpress TR β_1 [19,57]. Thus, T₃-regulated transcriptional pathways are evolutionarily conserved from frogs to mammals. Future studies employing cross-species hybridizations should identify novel T₃-regulated genes in mammal brain. Furthermore, the exploitation of these two powerful model systems should allow the elucidation of the molecular basis of T₃ action in vertebrate neural development.

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