Dual Functions of Thyroid Hormone Receptors in Vertebrate Development: The Roles of Histone-Modifying Cofactor Complexes

Yun-Bo Shi

Thyroid hormone (TH) receptor (TR) plays critical roles in vertebrate development. Transcription studies have shown that TR activates or represses TH-inducible genes by recruiting coactivators or corepressors in the presence or absence of TH, respectively. However, the developmental roles of these TR cofactors remain largely unexplored. Frog metamorphosis is totally dependent on TH and mimics the postembryonic period in mammalian development during which TH levels are also high. We have previously proposed a dual function model for TR in the development of the anuran *Xenopus laevis*. That is, unliganded TR recruits corepressors to TH-inducible genes in premetamorphic tadpoles to repress these genes and prevent premature metamorphic changes and subsequently, when TH becomes available, liganded TR recruits coactivators to activate these same genes, leading to metamorphosis. Over the years, we and others have used molecular and genetic approaches to demonstrate the importance of the dual functions of TR in *Xenopus laevis*. In particular, unliganded TR has been shown to recruit histone deacetylase-containing corepressor complexes in premetamorphic tadpoles to control metamorphic timing. In contrast, metamorphosis requires TH-bound TR to recruit coactivator complexes containing histone acetyltransferases and methyltransferases to activate transcription. Furthermore, the concentrations of coactivators appear to regulate the rate of metamorphic progression. Studies in mammals also suggest that the dual function model for TR is conserved across vertebrates.

Introduction

THYROID HORMONE (TH) has long been known to be important for organ function and metabolism in adult vertebrates and for embryo development (1–10). The most obvious and earliest known abnormalities of human body and behavior associated with TH deficiency are goiter and cretinism (3,6). In humans, the most important period of TH action responsible for cretinism appears to be the so-called postembryonic period, a few months before and several months after birth, which is critical for growth and maturation of many organs, including the brain (3,6).

During human development, TH begins to function very early (3,6,11). The thyroid gland is developed within the first 12 weeks of gestation. *In vitro* organ culture studies of human thyroid tissue suggest that fetal TH production begins as early as 10 weeks of gestation, although significant TH production probably does not occur until 20 weeks or later (11). In the fetal plasma, TH levels, especially 3,5,3'-triiodothyronine (T₃), remain low until about 4 months prior to birth when endogenous synthesis of TH leads to a rapid increase in plasma TH

levels (3,11). The TH levels reach a peak at birth and remain high several months after birth. Thus, it may not be surprising that TH deficiency during the neonatal period leads to irreversible, profound neurological deficit and mental retardation (6,12,13). In addition to fetal TH, maternal TH also plays an important role in early embryogenesis (14,15). On the other hand, excess TH also impairs embryo development, leading to increased miscarriages and lower birth weights (16). Although TH is clearly important for embryogenesis in humans and other mammals, it has been difficult to investigate the underlying mechanisms in mammals. This is in part due to the difficulty to manipulate the uterus-enclosed mammalian embryos and to separate the direct effects of TH on embryos from the indirect maternal effects caused by altering TH levels.

Amphibian Metamorphosis as a Model for Vertebrate Development

Amphibian metamorphosis is a process that is totally dependent on TH. The metamorphosis of anurans has been

studied as a model of TH action for about a century (17,18). This process involves extensive changes in essentially every single organ/tissue of the tadpole (4,19). Tadpole-specific organs such as the tail and gills are completely resorbed, while adult specific organs such as the limbs develop *de novo*. Most of the organs and tissues such as the intestine and brain are present in both the tadpole and frog but undergo drastic remodeling often involving degeneration of larval tissues and development of the adult ones. Despite the diverse changes, all are under the control of TH in an organ-autonomous manner for most, if not all organs (3,4,19).

Anuran metamorphosis bears strong similarities to the postembryonic development in mammals at morphological and molecular levels. For example, the adult intestines of both frogs and mammals have similar structural organizations with extensive epithelial folding to increase the luminal surface for nutrient absorption (20). Anuran and mammalian intestines also share similar developmental pathways. They both initially develop into a simple tubular structure consisting of mostly endoderm-derived epithelial cells. While this initial simple organ is more developed in anuran tadpoles for it to be functional in an independent living tadpole, both the amphibian and mammalian larval/embryonic intestines subsequently undergo similar morphogenetic transformations to form the complex adult organ (20,21).

Likewise, many other processes that occur during amphibian metamorphosis also take place during postembryonic development in mammals (3–5). These include the switching of the hemoglobin genes from larval (tadpole) or fetal types to frog or adult types, respectively, the dramatic increase in the serum albumin levels, skin keratinization, the induction of urea cycle enzymes, and the developmental progression and restructuring of the central and peripheral nervous systems, etc. Additionally and most obviously, both mammals and (most) anurans change their living habitat after this postembryonic period, from an aquatic (amniotic) to a terrestrial environment, accompanied by the maturation of the lung for air breathing. All these changes occur in the presence of high levels of plasma TH during both metamorphosis and mammalian postembryonic development. More importantly, the metamorphic changes in tadpoles occur in a free-living organism and can be induced by the addition of physiological levels of TH to the rearing water, making study of postembryonic development in frogs an ideal model.

Gene Regulation by TH Receptor

The biological effects of TH are mainly mediated through gene regulation by TH receptors (TRs), although nongenomic effects of TH have been well documented (1,2,22–25). There are two evolutionary-conserved TR genes, TR α and TR β , in all vertebrate species and both are members of the nuclear hormone receptor superfamily (1,2,4,22,24,26). TRs are capable of functioning as monomers, homodimers, and heterodimers with 9-*cis*-retinoic acid receptors (RXRs), which belong to the same superfamily. For genes induced by TH, TR-RXR heterodimers bind to TH response elements (TREs) in/around the promoters of target genes even in the context of chromatin (27). In the absence of TH, TR represses transcription from their promoters; when TH is present, TR activates their transcription, accompanied by chromatin disruption (27–29). There are also genes that are repressed by the presence of TH.

Much less is known about how TH represses these genes. Thus, the discussions here will focus only on TH-induced gene expression.

TR accomplishes gene regulation by recruiting corepressor or coactivator complexes to the target promoters, respectively (Fig. 1A). Many TR-interacting proteins have been isolated and characterized through *in vitro* and cell culture studies (2,30–45). Two highly related proteins N-CoR (nuclear corepressor) and SMRT (silencing mediator of retinoid and thyroid hormone receptors) are among the best-studied TR-binding corepressors. Both bind to unliganded TR and form large complexes containing histone deacetylase 3 (HDAC3), transducin beta-like protein 1 (TBL1)/TBL1-related protein 1 (TBLR1), and G-protein pathway suppressor 2 (GPS2) (Fig. 1A) (30–34,36,46–50), suggesting that unliganded TR represses transcription in part through deacetylation of the local histones at target promoters.

In the presence of TH, TR releases corepressors and recruits coactivators. The known TR coactivators include ATP-dependent chromatin-remodeling proteins, histone acetyltransferases such as CREB-binding protein (CBP) and p300, and TRAP/DRIP/mediator complex that associates with the recruitment and activation of RNA polymerase II (2,35–42,51,52). Among the coactivators, the role of the SRC family (SRC1, 2, 3) in gene regulation by TR has been studied extensively. SRCs bind to TR in the presence of TH and form complexes with other cofactors, including CBP/p300, protein arginine methyltransferase 1 (PRMT1), and coactivator-associated arginine methyltransferase 1 (CARM1 or PRMT4) (38,40–42,53–58), suggesting that SRCs activate transcription in part through acetylation or methylation of local histones in the promoter region (Fig. 1A).

TR Function During Frog Development

A dual function model

Based on the developmental expression of TRs and RXRs and the ability of TR to activate and repress TH-inducible genes in a TH-dependent manner, we have previously proposed a dual function model for TR during the development of the South African clawed toad *Xenopus laevis* (Fig. 1B) (59,60). According to the model, TH-inducible genes are not regulated by TH or TR when the hormone and receptor levels are low or absent. Thus, the lack of activation or repression by the thyroid system allows for normal embryonic development. By stage 45 when a free feeding tadpole is formed upon the completion of embryogenesis (61), the embryonic roles of these genes have past. The increased expression of TR and RXR genes just after embryogenesis, especially TR α and RXR α , leads to the formation of TR-RXR heterodimers that bind to the TREs of these genes, leading to the recruitment of corepressor complexes containing N-CoR or SMRT due to the lack of TH. This results in the repression of the genes via histone deacetylation. The hypothesis here is that this gene repression is important to ensure proper tadpole growth and to prevent premature metamorphic organ transformations.

After stage 55, the synthesis and secretion of endogenous TH elevate the levels of TH in the plasma and tadpole tissues. The binding of TH to chromatin-bound TR releases the corepressor complexes from the target genes with concurrent recruitment of the coactivator complexes, such as those containing SRC-p300-PRMT1 (Fig. 1B). This results in local his-

FIG. 1. (A) Mechanisms of transcriptional regulation by thyroid hormone (TH) receptor (TR). For TH-inducible genes, TR heterodimerized with 9-*cis*-retinoic acid receptors (RXR) constitutively binds the TH response elements (TREs) in their promoters or enhancers. In the absence of TH, TR binds corepressor complexes, such as those containing histone deacetylase HDAC3 and the highly related protein N-CoR (nuclear corepressor) or SMRT (silencing mediator of retinoid and thyroid hormone receptors) to inhibit transcription from the promoters through deacetylation of lysine residues of histone H3 and H4 to induce a “closed” chromatin state, as suggested by the folding of histone tails (red beaded structure) on to the DNA helix because of the charge–charge interaction between the positively charged histone tails and negatively charged DNA. The binding by TH induces a conformational change in TR, leading to the binding of coactivator complexes, such as those containing the related coactivators SRC 1, 2, or 3, which in turn binds p300 and PRMT1. SRCs and p300 have histone acetyltransferase (HAT) activity to acetylate histones H3 and H4, and PRMT1 can

methylate histone H4. Histone acetylation is believed to facilitate the formation of an “open” chromatin state, as diagrammed by the unfolding of histone tails (red beaded structure) away from the DNA helix due to the neutralization of the positive charges on the histone tails by acetylation. There are other corepressors and coactivators that can participate in this process, including those involved in chromatin disruption upon transcriptional activation by TR (27,28,37). For simplicity, these are not shown here. (B) A dual function model of TR in frog development. TH-inducible genes are expressed at the basal levels during embryogenesis when the levels of TH and TRs are low. After tadpole feeding begins at stage 45, the increased expression of TR α and RXRs, e.g., RXR α as shown in the figure, leads to the formation of TR-RXR heterodimers that bind to the TREs in the target genes and recruit corepressor complexes to repress gene expression. After stage 55, the synthesis of endogenous TH leads to the binding of TH to TR, causing the release of the corepressor complexes and recruitment of coactivator complexes to the promoters. This results in the activation of the genes and initiates metamorphosis.

tone acetylation and methylation and gene activation, leading to metamorphic transformations in the tadpoles.

In vivo evidence for the dual function model

The first *in vivo* functional study in *X. laevis* showed that in early *Xenopus* embryos lacking endogenous TR and TH, overexpression of TR upon microinjection of its mRNA into fertilized eggs was capable of repressing or activating endogenous gene expression (62). Interestingly, overexpression of TR and RXR together, but not either one alone, represses endogenous TH response genes while the addition of TH

leads to the reversal of the repression and further activation of the TH response genes. This suggests that TRs function as heterodimers with RXR *in vivo* and that endogenous TR and RXR normally expressed later at tadpole stages should repress or activate these genes in the absence or presence of TH, respectively.

To determine whether TR binds to endogenous target genes during development, we adapted the chromatin immunoprecipitation assays (ChIP) to analyze the binding of endogenous TR to endogenous target genes in whole embryos/tadpoles or tadpole tissues (63). This assay provided us with direct evidence that there is little or no binding of TR

FIG. 2. TH-dependent recruitment of coactivator SRC3 and histone acetylation at TH-responsive promoters, TR β A and TH/bZIP, in the intestine of tadpoles during natural metamorphosis. **(A)** Schematic diagrams showing the location of the transcription start site (thick arrow) for the two TH-response genes and the locations of the TREs and the primers (thin arrows) used to analyze the DNA in the ChIP assay (110–112). **(B)** ChIP assays on intestinal samples from premetamorphic (stage 54, when TH level is low) and metamorphosing (stage 62, when endogenous TH is at the peak level) tadpoles. The Input represents DNA prior to immunoprecipitation with antibodies against TR, SRC3, or acetylated histone H4 (AcH4) for the ChIP assay. Note that TR binds to the TREs at both stages while SRC3 recruitment is high when TH level is high at stage 62, accompanied by increased histone acetylation (65).

and RXR to endogenous TREs during embryogenesis while both are bound to TREs in tadpoles as predicted by the model. Subsequently, we and others have shown that TRs bind to target promoters in different organs independently of TH in both *X. laevis* and a related species *X. tropicalis* and more importantly recruit coactivators and corepressors as the model predicts (Fig. 2) (57,63–70).

Several different laboratories have investigated the requirement for TR-dependent gene activation during metamorphosis by using a dominant negative TR. This mutant TR contains a C-terminal deletion that prevents ligand binding and coactivator recruitment. Overexpression of this dominant negative TR through transgenesis or *in vivo* gene transfer inhibits TH-induced gene activation and metamorphosis in *X. laevis* (71–75). The dominant negative TR expressed in transgenic animals has been shown to compete against endogenous TR for binding to endogenous target genes, leading to the retention of corepressors at the target genes even in the presence of TH (75). These findings indicate an essential role of TH-bound TR in gene regulation and metamorphosis.

To determine whether TR is sufficient to mediate the metamorphic effects of TH, we have generated a dominant positive TR that cannot bind to TH (due to a small deletion at the C-terminus) but constitutively activate transcription (due to the N-terminal fusion of the strong viral activator VP16) (76). As early embryogenesis is adversely affected by inappropriate TR function, especially the activated (TH-bound) form of TR (62), we introduced the dominant positive TR under the control of a heat shock-inducible promoter into developing animals through transgenesis. When premetamorphic transgenic tadpoles and their wild-type siblings were subjected to heat shock treatment to induce the transgene expression, within several days the transgenic tadpoles underwent metamorphic changes typical of TH-treated premetamorphic tadpoles, a phenomenon not observed in the wild-type siblings (Fig. 3A) (76). Furthermore, ChIP assays have shown that the dominant positive TR is bound to endogenous target genes (76). This leads to the activation of the same genes that are normally induced by TH (Fig. 3B) (76). Thus, TR is not only necessary but also sufficient to mediate the effects of TH on all the parameters that were measured.

Roles of TR Cofactors During Frog Development

Coactivator recruitment is essential for metamorphosis

There are two components in the transcriptional activation by TH, the relief of the repression caused by unliganded TR and additional activation due to liganded TR, with the former representing the major fraction of the gene induction by TH in many reporter assays. The relative contributions from corepressor release and coactivator recruitment upon TH binding to TR have been difficult to determine *in vivo*. Cofactor knockout mice often have relatively mild phenotypes due to cofactor redundancy or embryonic lethal phenotypes, thus revealing little information about their roles in development. In addition, many cofactors function with multiple transcription factors and this makes it difficult to pinpoint the role of a particular cofactor to a nuclear hormone receptor pathway even when gene knockout and transgenesis result in easily identifiable phenotypes, such as mice deficient in N-CoR, p300, SRC1-3, or TRAP220 (77–82). Frog metamorphosis, however, is totally dependent on TH. There has been no evidence that any ligands for other nuclear hormone receptors can induce morphological changes in premetamorphic tadpoles in the absence of TH, although some can modulate TH-induced changes (4,83). Furthermore, TR is both necessary and sufficient for mediating the effects of TH. This makes it possible to correlate developmental phenotypes during metamorphosis with specific cofactor function in the TR signaling pathway. We have focused our effort on SRC coactivator complexes. We have previously shown that the coactivators SRC3, p300, PRMT1, and CARM1 are expressed during *X. laevis* metamorphosis with the expression of SRC3 and PRMT1 upregulated indirectly by TH (57,58,84). Furthermore, ChIP assays by us and others have shown that SRC3, p300, and PRMT1 are recruited by TR to endogenous target genes in a TH-dependent manner (Fig. 2) (57,64–66,85). Surprisingly, SRC3 recruitment to target genes is gene and organ dependent. It is recruited to the promoter regions of both of the two endogenous target genes analyzed, TH/bZIP (a transcription factor gene) and TR β , in the intestine but only to the TH/bZIP gene in the tail (65), in agreement with those reported by Havis *et al.* (66). These results suggest that tissue content and promoter context affect cofactor specificity.

FIG. 3. Expression of a TH-independent, constitutively active, dominant positive TR in transgenic tadpoles initiates metamorphosis. **(A)** Premetamorphic wild type tadpoles and sibling tadpoles transgenic for the dominant positive TR under the control of a heat shock-inducible promoter were reared together in methimazole to block endogenous TH synthesis and were heat-shocked daily for 8 days or treated with 5 nM 3,5,3'-triiodothyronine (T₃) for 3 days. Note that expression of the dominant positive TR in transgenic animals upon heat shock treatment induced the same metamorphic changes, e.g., the gills (white brackets) and the hind limbs (white arrow heads), in the absence of T₃, as those induced by T₃ in wild-type tadpoles. **(B)** The dominant positive TR regulates gene expression in the animal intestine just like TH treatment in wild type tadpoles. Premetamorphic wild-type and dominant positive TR transgenic tadpoles were heat shocked for 4 days or treated with 5 nM T₃ for 3 days. RT-PCR analysis was carried out on RNA isolated from the intestine for the expression of TH-response genes with the TH-independent gene rpl8 as an internal control. The genes analyzed were TR β and TH/bZIP, ubiquitously expressed, direct TH response genes; stromelysin-3 (ST3), a fibroblast-specific, direct TH-response gene; sonic hedgehog (xhh), an

intestinal epithelium-specific, direct TH-response gene; and bone morphogenic protein (BMP)-4, a fibroblast-specific, late upregulated gene. See Buchholz *et al.* (76) for more details.

FIG. 4. Transgenic, constitutive expression of a Flag-tagged dominant negative coactivator SRC3, containing the receptor interaction domain of *Xenopus laevis* SRC3, inhibits natural and TH-induced metamorphosis by competing for recruitment to endogenous target genes. **(A)** Transgenic dominant negative SRC3 competes with endogenous wild-type SRC3 for binding to liganded TR at TH-regulated promoters in the intestine, leading to reduced histone acetylation, but without an effect on TH-induced corepressor release. Premetamorphic wild type (WT) and transgenic (Tg) animals were treated with 10 nM T₃ for 2 days. Intestinal nuclei were isolated and ChIP assays performed using anti-SRC3 (for endogenous wild-type SRC3), anti-acetylated histone H4 (AcH4), anti-SMRT (for endogenous corepressor SMRT), and anti-Flag (for the dominant negative SRC3) antibodies. The TRE regions of TH/bZIP and TR β A promoters in the ChIP DNA were analyzed by real-time PCR. Note the reduced or absence of endogenous SRC3 recruitment and the corresponding histone acetylation in TH-treated transgenic tadpoles compared to wild type ones. On the other hand, as expected, there was no difference in the release of the corepressor SMRT upon TH treatment. **(B)** Overexpression of the dominant negative SRC3 inhibits TH-induced metamorphosis. Premetamorphic wild-type and transgenic tadpoles at stage 54 were treated with 5 nM T₃ for 3 days. The characteristic TH-induced changes such as resorption of gills (bracketed) and the morphogenesis of the hind limbs (arrows) were induced in wild-type animals treated with TH. These changes were all inhibited in transgenic animals. **(C)** Transgenic tadpoles overexpressing the dominant negative SRC3 fail to complete metamorphosis. Left: a wild type tadpole completed metamorphosis about 2 months after fertilization. Right, two transgenic siblings retained their tail and some gills (the top one) even at 4 months of age. See Paul *et al.* (64) for more details.

To investigate the role of coactivator recruitment during frog metamorphosis, we generated transgenic tadpoles constitutively overexpressing a Flag-tagged dominant negative form of SRC3, which contains only the receptor interacting domain of *Xenopus* SRC3 under the control of the ubiquitously expressing CMV promoter (64). The mutant SRC3 is recruited by liganded TR to endogenous target genes, thus competing away the endogenous wild type SRC3 and presumably other TR-binding coactivators as well (Fig. 4A) (64). As expected, it does not affect the TH-induced release of corepressors (Fig.

4A). Interestingly, despite the release of the corepressors upon TH treatment of the transgenic tadpoles, there is little change in histone acetylation at the target genes, indicating that the release of histone deacetylase-containing corepressors is not sufficient to cause significant increases in local histone acetylation in developing tadpoles (Fig. 4A). Surprisingly, but consistent with the histone acetylation result, there is little induction of TH-inducible genes upon TH treatment of the transgenic animals (64). Thus, corepressor release has relatively small contributions to the TH-induced increases in

FIG. 4. Continued

histone acetylation and transcription of endogenous target genes in developing tadpoles, unlike those observed in many studies with reporter genes in cell cultures and other model systems. In addition, morphological analyses have shown that the dominant negative SRC3 inhibits essentially all aspects of TH-induced metamorphosis (Fig. 4B) and also inhibits or delays natural metamorphosis (Fig. 4C) (64). These results indicate that coactivator recruitment is required for gene activation by TH and metamorphosis. Our findings are consistent with those from a study with a TR antagonist by Lim *et al.* (86), where the TR antagonist NH-3 was shown to prevent the binding of SRC coactivators to TR and inhibits metamorphosis as well as gene activation by TR in tadpoles. They further suggest that corepressor release is a minor component of TH signaling needed to effect postembryonic organ remodeling during metamorphosis. On the other hand, as described below, premature release of corepressors in premetamorphic tadpoles can alter development.

Since the dominant negative SRC3 blocks the binding of all coactivators to liganded TR, it remains possible that SRC family members do not play an essential role in gene regulation by TR and metamorphosis. To determine whether and how SRCs participate in metamorphosis, we have investigated the role of SRC-binding protein p300 during metamorphosis. As stated above, *Xenopus* p300 is recruited to TH-responsive promoters in a TH-dependent manner (66,85). To investigate whether complexes containing SRCs and p300 or its related protein CBP are required for metamorphosis, we have generated a Flag-tagged dominant negative form of p300 that contains only the SRC-binding domain, which is expected to bind to SRCs and thus disrupt the function of SRC-p300/CBP complexes but not other coactivator complexes such as TRAP/DRIP/mediator complex. Transgenic expression of this dominant negative form of p300 under the control of the CMV promoter has similar effects as the dominant negative form of SRC3 (85). That is, it inhibits

FIG. 5. Cofactors regulate developmental rate and timing. **(A)** Transgenic tadpoles (Tg) overexpressing PRMT1 under the control of a heat shock-inducible promoter develop to more advanced metamorphic stages than wild-type siblings (Wt) when treated with TH. Wt and Tg animals were heat-shocked for 30 minutes twice daily starting at stage 56. Three days after the first heat-shock treatment, all the animals were treated with 2 nM T₃ and continuously subjected to heat shock treatment daily. The Wt and Tg animals were kept in the same container and analyzed morphologically every 3 days throughout the 15-day experiment. The average developmental stages were plotted. See Matsuda *et al.* (57) for more details. **(B)** Tg tadpoles expressing a dominant negative N-CoR under the control of a heat shock-inducible promoter initiate metamorphosis earlier than Wt siblings. Wt and Tg sibling tadpoles were heat-shocked every day starting from stage 46, the onset of tadpole feeding. Developmental stages of the tadpoles were examined every 5 days for 30 days. The average developmental stages were plotted. Note that at the end of the 30-day experiments, Tg animals had initiated metamorphosis (passed stage 55) while Wt ones were one stage behind. It would take Wt animals about 7 more days to begin metamorphosis. See for Sato *et al.* (93) more details. * $p < 0.05$ when Tg animals were compared to the Wt ones.

TH-induced gene activation and metamorphosis as well as natural metamorphosis. Since this dominant negative form of p300 does not interfere with the direct binding of coactivators to liganded TR but specifically interferes with the function of SRC-p300/CBP complexes, these results demonstrate an essential role of such complexes in gene regulation by TR and metamorphosis.

Coactivator levels regulate rate of metamorphic progression

To further analyze the role of SRC-p300 complexes during metamorphosis, we have taken a complementary approach to the above function-inhibition experiments. We have analyzed the role of another component of the SRC-p300 complexes, namely, PRMT1, a histone methyltransferase (57). In the presence of TH, PRMT1 is highly upregulated in the tadpole intestine and is recruited to endogenous TH-target genes during metamorphosis. When a Flag-tagged wild-type *Xenopus* PRMT1 was introduced into transgenic tadpoles under the control of a heat shock-inducible promoter, heat shock treatment led to ubiquitous overexpression of the Flag-tagged transgenic PRMT1 and its recruitment to target genes in a TH-dependent manner, accompanied by increased target gene expression. More importantly, overexpression of PRMT1 also accelerated both natural and TH-induced metamorphosis (Fig. 5A) (57). Thus, coactivator levels appear to influence the rate of the progression of metamorphosis in different organs/tissues.

Corepressor binding to unliganded TR controls metamorphic timing

The dual function model for TR predicts that gene repression by unliganded TR is important for proper development and this repression utilizes corepressors. Many studies have firmly established that N-CoR/SMRT complexes participate in gene repression by unliganded nuclear receptors including TRs in cultured cells (32–34,36,46–50). Furthermore, transgenic overexpression of a dominant negative N-CoR in the liver relieves the repression by unliganded TR in mouse (87). In addition, specific gene targeting that mutated the receptor interacting domain of N-CoR, which resulted in a mutant N-CoR that cannot bind to TR, in the liver also abrogates the repression of TH-inducible genes in hypothyroid mice (88), demonstrating the importance of N-CoR in gene repression by unliganded TR in mouse liver *in vivo*. During *Xenopus* development, low levels of TRs are expressed during embryogenesis (89–91). A recent study suggests that corepressor binding by unliganded receptor expressed during this period is essential for eye development (92). As reviewed above, upon the completion of embryogenesis and the formation of a free living tadpole by stage 45, TR α is highly expressed in *X. laevis*. When we injected dominant negative N-CoR peptides into the tail of premetamorphic *X. laevis* tadpoles, the expression of a co-injected, TR-dependent reporter gene was upregulated in the absence of TH (69), suggesting that endogenous TRs and corepressors are capable of repressing gene expression in tadpoles.

To directly investigate the role of corepressors in pre-metamorphic tadpoles, we have generated transgenic animals expressing a dominant negative form of *Xenopus* N-CoR, which contains two copies of the receptor interacting domain of N-CoR (93), under the control of a heat shock-inducible

promoter. This dominant negative N-CoR competes effectively against endogenous N-CoR for binding to unliganded TR in the frog oocyte, leading to the derepression of a reporter gene injected into the oocyte nucleus (93). As this dominant N-CoR binds only to unliganded TR, it is expected to affect TR function only during the developmental stages 45 to 55 when TR expression is high while TH levels are low (Fig. 1B), a window period of about 30 days. Thus, we subjected transgenic tadpoles carrying the dominant negative N-CoR and their wild type siblings to daily heat shock treatment, starting at stage 46. Heat shock treatment led to overexpression of the dominant negative N-CoR and upregulation of most of the TH-response genes analyzed, although the extent of the increase (about twofold or less) was much less than that due to TH treatment (indicating that derepression represents only a minor fraction of the gene activation induced by TH in the developing tadpoles, consistent with the dominant negative coactivator studies above). More importantly, transgenic animals developed faster, reaching stage 55, the onset of metamorphosis, by as much as 7 days earlier than the wild-type siblings within the 30-day experiment (93) (Fig. 5B). These findings demonstrate that unliganded TR indeed recruits corepressors to repress gene expression in the premetamorphic tadpole to regulate the timing for the initiation of metamorphosis, thereby ensuring proper growth of the tadpole before its transformation into a frog, an important measure for the growth and survival of the postmetamorphic animal.

Conclusions

Anuran metamorphosis is one of the most dramatic events mediated by TR-dependent gene regulation. It serves as an excellent model to understand the function and molecular mechanism of transcriptional regulation by TR *in vivo* during postembryonic development in vertebrates. Studies by us and others have provided strong evidence to show that TR is both necessary and sufficient to mediate the metamorphic effects of TH. Furthermore, TR has dual functions during development, as an unliganded repressor to repress gene expression to ensure a proper premetamorphic tadpole growth period and later as a TH-bound activator to induce metamorphosis. These dual roles are exerted by recruiting corepressors and coactivators, respectively. While this basic dual function model is well supported, the detailed molecular pathways controlling metamorphosis in different tissues are likely to be much more complex. For example, the two TRs, TR α and TR β , are likely to have different functions, in part due to their distinct expression patterns and in part due to the differences in their molecular properties.

Indeed, studies with a TR-specific agonist have provided evidence to support different roles for the receptors during development in *X. laevis* (94,95). In addition, much remains to be learned about the roles of TH target genes during metamorphosis. Metamorphosis in insects, particularly *Drosophila*, is also controlled by a member of the nuclear receptor family, the ecdysone receptor. Extensive genetic and molecular analyses have demonstrated that a complex gene regulation cascade regulates the metamorphic transformations and the existence of feedback mechanisms involving transcription factors encoded by ecdysone target genes (96,97). Such feedbacks appear also to exist during amphibian metamorphosis as a recent study has shown that a TH-induced transcription

factor can bind to the TH-dependent promoter of the *X. laevis* TR β A genes to regulate TR β expression (98). There are a number of other TH-induced transcription factors during *Xenopus* metamorphosis. It would be interesting to study how they participate in the process of metamorphosis.

While the dual function model has been substantiated during frog development, it is very likely to exist during higher vertebrate development as well. During mammalian development, TRs are expressed prior to the synthesis of endogenous TH (3,11). While some maternal TH is likely to enter the fetus, low levels of TH during early embryogenesis will undoubtedly leave many of the TRs unliganded in many organs/tissues. However, as shown during frog development, the effects of derepression are relatively mild, leading to relatively small increases in gene expression compared to the much bigger contribution from activation through ligand-dependent coactivator recruitment. On the other hand, such mild effects can have an important influence on development, e.g., controlling the timing of metamorphosis. Thus, one would expect that disrupting the repression by unliganded TR may also have relatively mild effects on development in mammals, which is consistent with the weak phenotypes of TR knockout mice (99,100).

Most genetic and molecular studies in mice have focused on the activating effects of liganded TR and demonstrated an essential role of TH in development. The role of unliganded TR in mammalian development has been difficult to ascertain (101). On the other hand, several studies suggest a role of unliganded TR in the development of at least some mouse organs. First, in mouse embryos where TH levels are low, TR α knockout increases the expression of several TH-response genes in the heart, and this is accompanied by a significant increase in heart rate of the embryos (102). In postnatal mice when TH levels are high, the expression of these same TH-response genes are lower in the knockout mice compared to the wild-type siblings and the heart rate is also lower in the knockout animals, opposite of those observed in the embryos. Thus, TR α appears to repress the target genes and to keep the heart rate low in the unliganded state in the embryos and does the opposite when TH levels are high after birth. Similarly, it has been reported that TR β deletion leads to hearing loss in both mouse and human and causes major defects in the photoreceptor cells in mouse, while mutations in TR β that result in the formation of dominant negative forms of TR β have relatively mild effects on the auditory and visual systems (103–108). These findings suggest that unliganded TR β is important for the development of the auditory and visual systems in higher vertebrates. In support of this, deleting the gene encoding the TH-inactivating enzyme type 3 deiodinase also leads to auditory deficits (109), suggesting unliganded TRs are important for the development of the auditory system. Thus, like frog development, mammalian development also utilizes the dual functions of TR to coordinate organ development and maturation. It would be of interest in the future to determine what corepressors and coactivators are recruited by TR to endogenous target genes to regulate the developmental processes in different organs/tissues in mammals.

Acknowledgments

I would like to thank Dr. D. Buchholz and current members in my laboratory for comments on the manuscript. The work in

the author's laboratory has been supported by the Intramural Research Program of National Institute of Child Health and Human Development, National Institutes of Health.

Disclosure Statement

No competing financial interests exist.

References

- Lazar MA 1993 Thyroid hormone receptors: multiple forms, multiple possibilities. *Endocr Rev* **14**:184–93.
- Yen PM 2001 Physiological and molecular basis of thyroid hormone action. *Physiol Rev* **81**:1097–142.
- Tata JR 1993 Gene expression during metamorphosis: an ideal model for post-embryonic development. *Bioessays* **15**:239–48.
- Shi Y-B 1999 Amphibian Metamorphosis: From Morphology to Molecular Biology. John Wiley & Sons, Inc., New York.
- Atkinson BG 1994 Metamorphosis: model systems for studying gene expression in postembryonic development. *Dev Genet* **15**:313–319.
- Hetzel BS 1989 The Story of Iodine Deficiency: An International Challenge in Nutrition. Oxford University Press, Oxford.
- Freake HC, Oppenheimer JH 1995 Thermogenesis and thyroid function. *Annu Rev Nutr* **15**:263–91.
- Dillman WH 1996 Thyroid hormone and the heart: basic mechanistic and clinical issues. *Thyroid* **9**:1–11.
- Franklyn JA, Gammage MD 1996 Thyroid disease: effects on cardiovascular function. *Trends Endocrinol Metab* **7**:50–54.
- Silva JE 1995 Thyroid hormone control of thermogenesis and energy balance. *Thyroid* **5**:481–492.
- Howdeshell KL 2002 A model of the development of the brain as a construct of the thyroid system. *Environ Health Perspect* **110**:337–348.
- Porterfield SP, Hendrich CE 1993 The role of thyroid hormones in prenatal and neonatal neurological development—current perspectives. *Endocr Rev* **14**:94–106.
- Hsu JH, Brent GA 1998 Thyroid hormone receptor gene knockouts. *Trends Endocrinol Metab* **9**:103–112.
- de Escobar GM, Obregon MJ, del Rey FE 2004 Maternal thyroid hormones early in pregnancy and fetal brain development. *Best Pract Res Clin Endocrinol Metab* **18**:225–48.
- de Escobar GM, Obregon MJ, del Rey FE 2007 Iodine deficiency and brain development in the first half of pregnancy. *Public Health Nutr* **10**:1554–70.
- Anselmo J, Cao D, Karrison T, Weiss RE, Refetoff S 2004 Fetal loss associated with excess thyroid hormone exposure. *JAMA* **292**:691–695.
- Allen BM 1918 The results of thyroid removal in the larvae of *Rana pipiens*. *J Exp Zool* **24**:499–519.
- Gudernatch JF 1912 Feeding experiments on tadpoles. I. The influence of specific organs given as food on growth and differentiation: a contribution to the knowledge of organs with internal secretion. *Arch Entwicklungsmech Org* **35**:457–483.
- Gilbert LI, Tata JR, Atkinson BG 1996 Metamorphosis: Post-Embryonic Reprogramming of Gene Expression in Amphibian and Insect Cells. Academic Press, New York.
- Shi Y-B, Ishizuya-Oka A 1996 Biphasic intestinal development in amphibians: Embryogenesis and remodeling during metamorphosis. *Curr Top Dev Biol* **32**:205–235.
- Glass GB J 1968 Introduction to Gastrointestinal Physiology. Prentice-Hall, Inc., Englewood Cliffs, NJ.
- Evans RM 1988 The steroid and thyroid hormone receptor superfamily. *Science* **240**:889–895.
- Davis PJ, Davis FB 1996 Nongenomic actions of thyroid hormone. *Thyroid* **6**:497–504.
- Tsai MJ, O'Malley BW 1994 Molecular mechanisms of action of steroid/thyroid receptor superfamily members. *Ann Rev Biochem* **63**:451–486.
- Buchholz DR, Paul BD, Fu L, Shi YB 2006 Molecular and developmental analyses of thyroid hormone receptor function in *Xenopus laevis*, the African clawed frog. *Gen Comp Endocrinol* **145**:1–19.
- Laudet V, Gronemeyer H 2002 The Nuclear Receptor FactsBook. Academic Press, San Diego, CA.
- Wong J, Shi YB, Wolffe AP 1995 A role for nucleosome assembly in both silencing and activation of the *Xenopus* TR beta A gene by the thyroid hormone receptor. *Genes Dev* **9**:2696–2711.
- Wong J, Shi Y-B, Wolffe AP 1997 Determinants of chromatin disruption and transcriptional regulation instigated by the thyroid hormone receptor: hormone-regulated chromatin disruption is not sufficient for transcriptional activation. *EMBO J* **16**:3158–3171.
- Wong J, Patterson D, Imhof D, Guschin D, Shi Y-B, Wolffe AP 1998 Distinct requirements for chromatin assembly in transcriptional repression by thyroid hormone receptor and histone deacetylase. *EMBO J* **17**:520–534.
- Hörlein AJ, Näär AM, Heinzel T, Torchia J, Gloss B, Kurokawa R, Ryan A, Kamei Y, Söderström M, Glass CK, Rosenfeld MG 1995 Ligand-independent repression by the thyroid hormone receptor mediated by a nuclear receptor co-repressor. *Nature* **377**:397–404.
- Chen JD, Evans RM 1995 A transcriptional co-repressor that interacts with nuclear hormone receptors. *Nature* **377**:454–457.
- Burke LJ, Baniahmad A 2000 Co-repressors 2000. *FASEB J* **14**:1876–88.
- Jones PL, Shi Y-B 2003 N-CoR-HDAC corepressor complexes: Roles in transcriptional regulation by nuclear hormone receptors. In: Workman JL (ed) Current Topics in Microbiology and Immunology: Protein Complexes that Modify Chromatin, vol. 274. Springer-Verlag, Berlin, pp 237–268.
- Glass CK, Rosenfeld MG 2000 The coregulator exchange in transcriptional functions of nuclear receptors. *Genes Dev* **14**:121–141.
- Ito M, Roeder RG 2001 The TRAP/SMCC/Mediator complex and thyroid hormone receptor function. *Trends Endocrinol Metab* **12**:127–134.
- Zhang J, Lazar MA 2000 The mechanism of action of thyroid hormones. *Annu Rev Physiol* **62**:439–66.
- Huang Z-Q, Li J, Sachs LM, Cole PA, Wong J 2003 A role for cofactor-cofactor and cofactor-histone interactions in targeting p300, SWI/SNF and mediator for transcription. *EMBO J* **22**:2146–2155.
- McKenna NJ, O'Malley BW 2001 Nuclear receptors, coregulators, ligands, and selective receptor modulators: making sense of the patchwork quilt. *Ann NY Acad Sci* **949**:3–5.
- Rachez C, Freedman LP 2001 Mediator complexes and transcription. *Curr Opin Cell Biol* **13**:274–80.
- Chen H, Lin RJ, Schiltz RL, Chakravarti D, Nash A, Nagy L, Privalsky ML, Nakatani Y, Evans RM 1997 Nuclear receptor coactivator ACTR is a novel histone acetyltransferase and forms a multimeric activation complex with P/CAF and CBP/p300. *Cell* **90**:569–580.

41. Demarest SJ, Martinez-Yamout M, Chung J, Chen H, Xu W, Dyson HJ, Evans RM, Wright PE 2002 Mutual synergistic folding in recruitment of CBP/p300 by p160 nuclear receptor coactivators. *Nature* **415**:549–553.
42. Onate SA, Tsai SY, Tsai MJ, O'Malley BW 1995 Sequence and characterization of a coactivator for the steroid hormone receptor superfamily. *Science* **270**:1354–1357.
43. Meng X, Yang YF, Cao X, Govindan MV, Shuen M, Hollenberg AN, Mymryk JS, Walfish PG 2003 Cellular context of coregulator and adaptor proteins regulates human adenovirus 5 early region 1A-dependent gene activation by the thyroid hormone receptor. *Mol Endocrinol* **17**:1095–1105.
44. Wahlstrom GM, Vernstrom B, Bolin MB 1999 The adenovirus E1A protein is a potent coactivator for thyroid hormone receptors. *Mol Endocrinol* **13**:1119–1129.
45. Sato Y, Ding A, Heimeier RA, Yousef AF, Mymryk JS, Walfish PG, Shi Y-B 2009 The adenoviral E1A protein displaces corepressors and relieves gene repression by unliganded thyroid hormone receptors *in vivo*. *Cell Res* **19**:783–792.
46. Yoon H-G, Chan DW, Huang ZQ, Li J, Fondell JD, Qin J, Wong J 2003 Purification and functional characterization of the human N-CoR complex: the roles of HDAC3, TBL1 and TBLR1. *EMBO J* **22**:1336–1346.
47. Zhang J, Kalkum M, Chait BT, Roeder RG 2002 The N-CoR-HDAC3 nuclear receptor corepressor complex inhibits the JNK pathway through the integral subunit GPS2. *Mol Cell* **9**:611–623.
48. Ishizuka T, Lazar MA 2003 The N-CoR/histone deacetylase 3 complex is required for repression by thyroid hormone receptor. *Mol Cell Biol* **23**:5122–5131.
49. Guenther MG, Lane WS, Fischle W, Verdin E, Lazar MA, Shiekhhattar R 2000 A core SMRT corepressor complex containing HDAC3 and TBL1, a WD40-repeat protein linked to deafness. *Genes Dev* **14**:1048–1057.
50. Li J, Wang J, Wang J, Nawaz Z, Liu JM, Qin J, Wong J 2000 Both corepressor proteins SMRT and N-CoR exist in large protein complexes containing HDAC3. *EMBO J* **19**:4342–4350.
51. Heimeier RA, Hsia VS-C, Shi Y-B 2008 Participation of BAF57 and BRG1-containing chromatin remodeling complexes in thyroid hormone-dependent gene activation during vertebrate development. *Mol Endocrinol* **22**:1065–77.
52. Sheppard HM, Harries JC, Hussain S, Bevan C, Heery DM 2001 Analysis of the steroid receptor coactivator 1 (SRC1)-CREB binding protein interaction interface and its importance for the function of SRC1. *Mol Cell Biol* **21**:39–50.
53. Li J, O'Malley BW, Wong J 2000 p300 requires its histone acetyltransferase activity and SRC-1 interaction domain to facilitate thyroid hormone receptor activation in chromatin. *Mol Cell Biol* **20**:2031–2042.
54. Torchia J, Rose DW, Inostroza J, Kamei Y, Westin S, Glass CK, Rosenfeld MG 1997 The transcriptional co-activator p/CIP binds CBP and mediates nuclear-receptor function. *Nature* **387**:677–684.
55. Chen D, Ma H, Hong H, Koh SS, Huang SM, Schurter BT, Aswad DW, Stallcup MR 1999 Regulation of transcription by a protein methyltransferase. *Science* **284**:2174–2177.
56. Koh SS, Chen DG, Lee YH, Stallcup MR 2001 Synergistic enhancement of nuclear receptor function by p160 coactivators and two coactivators with protein methyltransferase activities. *J Biol Chem* **276**:1089–1098.
57. Matsuda H, Paul BD, Choi CY, Hasebe T, Shi Y-B 2009 Novel functions of protein arginine methyltransferase 1 in thyroid hormone receptor-mediated transcription and in the regulation of metamorphic rate in *Xenopus laevis*. *Mol Cell Biol* **29**:745–757.
58. Matsuda H, Paul BD, Choi CY, Shi Y-B 2007 Contrasting effects of two alternative splicing forms of coactivator-associated arginine methyltransferase 1 on thyroid hormone receptor-mediated transcription in *Xenopus laevis*. *Mol Endocrinol* **21**:1082–1094.
59. Shi Y-B, Wong J, Puzianowska-Kuznicka M, Stelow M 1996 Tadpole competence and tissue-specific temporal regulation of amphibian metamorphosis: roles of thyroid hormone and its receptors. *BioEssays* **18**:391–399.
60. Sachs LM, Damjanovski S, Jones PL, Li Q, Amano T, Ueda S, Shi YB, Ishizuya-Oka A 2000 Dual functions of thyroid hormone receptors during *Xenopus* development. *Comp Biochem Physiol B Biochem Mol Biol* **126**:199–211.
61. Nieuwkoop PD, Faber J 1956 Normal Table of *Xenopus laevis*, 1st edition. North Holland Publishing, Amsterdam, The Netherlands.
62. Puzianowska-Kuznicka M, Damjanovski S, Shi Y-B 1997 Both thyroid hormone and 9-cis retinoic acid receptors are required to efficiently mediate the effects of thyroid hormone on embryonic development and specific gene regulation in *Xenopus laevis*. *Mol Cell Biol* **17**:4738–4749.
63. Sachs LM, Shi Y-B 2000 Targeted chromatin binding and histone acetylation *in vivo* by thyroid hormone receptor during amphibian development. *Proc Natl Acad Sci U S A* **97**:13138–13143.
64. Paul BD, Fu L, Buchholz DR, Shi Y-B 2005 Coactivator recruitment is essential for liganded thyroid hormone receptor to initiate amphibian metamorphosis. *Mol Cell Biol* **25**:5712–5724.
65. Paul BD, Buchholz DR, Fu L, Shi Y-B 2005 Tissue- and gene-specific recruitment of steroid receptor coactivator-3 by thyroid hormone receptor during development. *J Biol Chem* **280**:27165–27172.
66. Havis E, Sachs LM, Demeneix BA 2003 Metamorphic T3-response genes have specific co-regulator requirements. *EMBO Rep* **4**:883–888.
67. Buchholz DR, Paul BD, Shi YB 2005 Gene-specific changes in promoter occupancy by thyroid hormone receptor during frog metamorphosis. Implications for developmental gene regulation. *J Biol Chem* **280**:41222–41228.
68. Tomita A, Buchholz DR, Shi Y-B 2004 Recruitment of N-CoR/SMRT-TBLR1 corepressor complex by unliganded thyroid hormone receptor for gene repression during frog development. *Mol Cell Biol* **24**:3337–3346.
69. Sachs LM, Jones PL, Havis E, Rouse N, Demeneix BA, Shi Y-B 2002 N-CoR recruitment by unliganded thyroid hormone receptor in gene repression during *Xenopus laevis* development. *Mol Cell Biol* **22**:8527–8538.
70. Wang X, Matsuda H, Shi Y-B 2008 Developmental regulation and function of thyroid hormone receptors and 9-cis retinoic acid receptors during *Xenopus tropicalis* metamorphosis. *Endocrinology* **149**:5610–5618.
71. Nakajima K, Yaoita Y 2003 Dual mechanisms governing muscle cell death in tadpole tail during amphibian metamorphosis. *Dev Dyn* **227**:246–255.
72. Schreiber AM, Brown DD 2003 Tadpole skin dies autonomously in response to thyroid hormone at metamorphosis. *Proc Natl Acad Sci U S A* **100**:1769–1774.
73. Das B, Schreiber AM, Huang H, Brown DD 2002 Multiple thyroid hormone-induced muscle growth and death programs during metamorphosis in *Xenopus laevis*. *Proc Natl Acad Sci U S A* **99**:12230–12235.

74. Schreiber AM, Das B, Huang H, Marsh-Armstrong N, Brown DD 2001 Diverse developmental programs of *Xenopus laevis* metamorphosis are inhibited by a dominant negative thyroid hormone receptor. *Proc Natl Acad Sci U S A* **98**:10739–10744.
75. Buchholz DR, Hsia V S-C, Fu L, Shi Y-B 2003 A dominant negative thyroid hormone receptor blocks amphibian metamorphosis by retaining corepressors at target genes. *Mol Cell Biol* **23**:6750–6758.
76. Buchholz DR, Tomita A, Fu L, Paul BD, Shi Y-B 2004 Transgenic analysis reveals that thyroid hormone receptor is sufficient to mediate the thyroid hormone signal in frog metamorphosis. *Mol Cell Biol* **24**:9026–9037.
77. Xu J, Liao L, Ning G, Yoshida-Komiya H, Deng C, O'Malley BW 2000 The steroid receptor coactivator SRC-3 (p/CIP/RAC3/AIB1/ACTR/TRAM-1) is required for normal growth, puberty, female reproductive function, and mammary gland development. *Proc Natl Acad Sci U S A* **97**:6379–6384.
78. Gehin M, Mark M, Dennefeld C, Dierich A, Gronemeyer H, Chambon P 2002 The function of TIF2/GRIP1 in mouse reproduction is distinct from those of SRC-1 and p/CIP. *Mol Cell Biol* **22**:5923–5927.
79. Yao TP, Oh SP, Fuchs M, Zhou ND, Ch'ng LE, Newsome D, Bronson RT, Li E, Livingston DM, Eckner R 1998 Gene dosage-dependent embryonic development and proliferation defects in mice lacking the transcriptional integrator p300. *Cell* **93**:361–372.
80. Wang Z, Rose DW, Hermanson O, Liu F, Herman T, Wu W, Szeto D, Gleiberman A, Krones A, Pratt K, Rosenfeld R, Glass CK 2000 Regulation of somatic growth by the p160 coactivator p/CIP. *Proc Natl Acad Sci U S A* **97**:13549–13554.
81. Jepsen K, Hermanson O, Onami TM, Gleiberman AS, Lunyak V, McEvilly RJ, Kurokawa R, Kumar V, Liu F, Seto E, Hedrick SM, Mandel G, Glass CK, Rose DW, Rosenfeld MG 2000 Combinatorial roles of the nuclear receptor co-repressor in transcription and development. *Cell* **102**:753–763.
82. Ito M, Yuan CX, Okano HJ, Darnell RB, Roeder RG 2000 Involvement of the TRAP220 component of the TRAP/SMCC coactivator complex in embryonic development and thyroid hormone action. *Mol Cell* **5**:683–693.
83. Hayes TB 1997 Steroids as potential modulators of thyroid hormone activity in anuran metamorphosis. *Am Zool* **37**:185–194.
84. Paul BD, Shi Y-B 2003 Distinct expression profiles of transcriptional coactivators for thyroid hormone receptors during *Xenopus laevis* metamorphosis. *Cell Res* **13**:459–464.
85. Paul BD, Buchholz DR, Fu L, Shi Y-B 2007 SRC-p300 coactivator complex is required for thyroid hormone induced amphibian metamorphosis. *J Biol Chem* **282**:7472–7481.
86. Lim W, Nguyen NH, Yang HY, Scanlan TS, Furlow JD 2002 A thyroid hormone antagonist that inhibits thyroid hormone action in vivo. *J Biol Chem* **277**:35664–35670.
87. Feng X, Jiang Y, Meltzer P, Yen PM 2001 Transgenic targeting of a dominant negative corepressor to liver blocks basal repression by thyroid hormone receptor and increases cell proliferation. *J Biol Chem* **276**:15066–15072.
88. Astapova I, Lee LJ, Morales C, Tauber S, Bilban M, Holtenberg AN 2008 The nuclear corepressor, NCoR, regulates thyroid hormone action *in vivo*. *Proc Natl Acad Sci U S A* **105**:19544–19549.
89. Kanamori A, Brown DD 1992 The regulation of thyroid hormone receptor beta genes by thyroid hormone in *Xenopus laevis*. *J Biol Chem* **267**:739–745.
90. Banker DE, Bigler J, Eisenman RN 1991 The thyroid hormone receptor gene (c-erbA alpha) is expressed in advance of thyroid gland maturation during the early embryonic development of *Xenopus laevis*. *Mol Cell Biol* **11**:5079–5089.
91. Yaoita Y, Brown DD 1990 A correlation of thyroid hormone receptor gene expression with amphibian metamorphosis. *Genes Dev* **4**:1917–1924.
92. Havis E, Le Mevel S, Dubois GM, Shi D-L, Scanlan TS, Demeneix BA, Sachs LM 2006 Unliganded thyroid hormone receptor is essential for *Xenopus laevis* eye development. *EMBO J* **25**:4943–4951.
93. Sato Y, Buchholz DR, Paul BD, Shi Y-B 2007 A role of unliganded thyroid hormone receptor in postembryonic development in *Xenopus laevis*. *Mech Dev* **124**:476–488.
94. Denver RJ, Hu F, Scanlan TS, Furlow JD 2009 Thyroid hormone receptor subtype specificity for hormone-dependent neurogenesis in *Xenopus laevis*. *Dev Biol* **326**:155–168.
95. Furlow JD, Yang HY, Hsu M, Lim W, Ermio DJ, Chiellini G, Scanlan TS 2004 Induction of larval tissue resorption in *Xenopus laevis* tadpoles by the thyroid hormone receptor agonist GC-1. *J Biol Chem* **279**:26555–26562.
96. Thummel CS 1996 Flies on steroids—*Drosophila* metamorphosis and the mechanisms of steroid hormone action. *Trends Genet* **12**:306–310.
97. Thummel CS 1990 Puffs and gene regulation—molecular insights into the *Drosophila* ecdysone regulatory hierarchy. *BioEssays* **12**:561–568.
98. Bagamasbad P, Howdeshell KL, Sachs LM, Demeneix BA, Denver RJ 2008 A role for basic transcription element-binding protein 1 (BTEB1) in the autoinduction of thyroid hormone receptor beta. *J Biol Chem* **283**:2275–85.
99. Gothe S, Wang Z, Ng L, Kindblom JM, Barros AC, Ohlsson C, Vennstrom B, Forrest D 1999 Mice devoid of all known thyroid hormone receptors are viable but exhibit disorders of the pituitary-thyroid axis, growth, and bone maturation. *EMBO J* **13**:1329–1341.
100. Flamant F, Samarut J 2003 Thyroid hormone receptors: lessons from knockout and knock-in mutant mice. *Trends Endocrinol Metab* **14**:85–90.
101. Chassande O 2003 Do unliganded thyroid hormone receptors have physiological functions? *J Mol Endocrinol* **31**:9–20.
102. Mai W, Janier MF, Allioli N, Quignodon L, Chuzel T, Flamant F, Samarut J 2004 Thyroid hormone receptor alpha is a molecular switch of cardiac function between fetal and postnatal life. *Proc Natl Acad Sci U S A* **101**:10332–10337.
103. Griffith AJ, Szymko YM, Kaneshige M, Quinonez RE, Kaneshige K, Heintz KA, Mastroianni MA, Kelley MW, Cheng SY 2002 Knock-in mouse model for resistance to thyroid hormone (RTH): an RTH mutation in the thyroid hormone receptor beta gene disrupts cochlear morphogenesis. *J Assoc Res Otolaryngol* **3**:279–288.
104. Brucker-Davis F, Skarulis MC, Pikus A, Ishizawa D, Mastroianni M-A, Koby M, Weintraub BD 1996 Prevalence and mechanisms of hearing loss in patients with resistance to thyroid hormone (RTH). *J Clin Endocrinol Metab* **81**:2768–2772.
105. Refetoff S, DeWind LT, DeGroot LJ 1967 Familial syndrome combining deaf-mutism, stippled epiphyses, goiter and

- abnormally high PBI: possible target organ refractoriness to thyroid hormone. *J Clin Endocrinol Metab* **27**:279–294.
106. Ng L, Hurley JB, Dierks B, Srinivas M, Saltó C, Vennström B, Reh TA, Forrest D 2001 A thyroid hormone receptor that is required for the development of green cone photoreceptors. *Nat Genet* **27**:94–98.
107. Jones I, Srinivas M, Ng L, Forrest D 2003 The thyroid hormone receptor beta gene: structure and functions in the brain and sensory systems. *Thyroid* **13**:1057–1068.
108. Forrest D, Erway LC, Ng L, Altschuler R, Curran T 1996 Thyroid hormone receptor beta is essential for development of auditory function. *Nat Genet* **13**:354–357.
109. Ng L, Hernandez A, He W, Ren T, Srinivas M, Ma M, Galton VA, St Germain DL, Forrest D 2009 A protective role for type 3 deiodinase, a thyroid hormone-inactivating enzyme, in cochlear development and auditory function. *Endocrinology* **150**:1952–1960.
110. Furlow JD, Brown DD 1999 In vitro and in vivo analysis of the regulation of a transcription factor gene by thyroid hormone during *Xenopus laevis* metamorphosis. *Mol Endocrinol* **13**:2076–2089.
111. Wong J, Liang VC, Sachs LM, Shi YB 1998 Transcription from the thyroid hormone-dependent promoter of the *Xenopus laevis* thyroid hormone receptor betaA gene requires a novel upstream element and the initiator, but not a TATA Box. *J Biol Chem* **273**:14186–14193.
112. Ranjan M, Wong J, Shi YB 1994 Transcriptional repression of *Xenopus* TR beta gene is mediated by a thyroid hormone response element located near the start site. *J Biol Chem* **269**:24699–24705.

Address correspondence to:

Yun-Bo Shi, Ph.D.

Building 18 T, Rm 106

Laboratory of Gene Regulation and Development

PCRM

National Institute of Child Health and Human Development

National Institutes of Health

Bethesda, MD 20892

E-mail: shi@helix.nih.gov