Thyroid hormone activates *Xenopus* MBD3 gene via an intronic TRE *in vivo*

Liezhen Fu¹, Christin Li¹, Wonho Na¹, Yun-Bo Shi¹

¹Section on Molecular Morphogenesis, Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD), National Institutes of Health, Bethesda, Maryland, 20892, USA

TABLE OF CONTENTS

1. Abstract
2. Introduction
3. Materials and methods
   3.1. Experimental animals
   3.2. Quantitative RT-PCR (qRT-PCR)
   3.3. Bioinformatics identification of putative TREs
   3.4. Gel mobility shift assay
   3.5. Generation of promoter reporter constructs
   3.6. Transcription assay in *Xenopus laevis* oocytes
   3.7. Chromatin immunoprecipitation (ChIP) assay
4. Results
   4.1. *Xenopus tropicalis* MBD3 is upregulated in the intestine and tail during natural and T3-induced metamorphosis
   4.2. TR/RXR heterodimer binds to a putative TRE in the first intron of MBD3 gene *in vitro*
   4.3. The intronic TRE2 mediates TR/RXR regulation of MBD3 promoter
   4.4. TR binds to the intronic TRE and mediates T3-induced recruitment of RNA polymerase and histone methylation
5. Discussion
6. Acknowledgment
7. References

1. ABSTRACT

Thyroid hormone (T3) is important for adult organ function and vertebrate development. Amphibian metamorphosis is totally dependent on T3 and can be easily manipulated, thus offering a unique opportunity for studying how T3 controls vertebrate development. T3 controls frog metamorphosis through T3 receptor (TR)-mediated regulation of T3 response genes. To identify direct T3 response genes, we previously carried out a ChIP (chromatin immunoprecipitation)-on-chip analysis with a polyclonal anti-TR antibody on the tadpole intestine and identified many putative TR target genes. Among them is the methyl-CpG binding domain protein 3 (MBD3) gene, which has been implicated to play a role in epigenetic regulation of cellular processes as a subunit of the Mi-2/NuRD (Nucleosome Remodeling Deacetylase) complex. We show here that MBD3 is upregulated in the intestine and tail by T3 and its expression peaks at stage 62, the climax of metamorphosis. We further show that a putative TRE within the first intron of the MBD3 gene binds to TR/RXR *in vitro* and *in vivo*, and mediates T3 regulation of the MBD3 promoter *in vivo*.

2. INTRODUCTION

Thyroid hormone (T3) is important for proper development and normal physiology of many adult organs/tissues in vertebrates (1; 2; 3; 4; 5). T3 deficiency during human development results in
significant pathological consequences such as the formation of human cretins, who are short in stature and severely mentally retarded (6). The most critical period of T3 action in mammals is the postembryonic period, which covers several months around birth when T3 levels are high in the plasma (1). Owing to difficulty to access and/or manipulate the uterus-enclosed late stage of mammalian embryos and neonates, it has been difficult to study how T3 affect mammalian postembryonic development.

Frog metamorphosis mimics mammalian postembryonic development (1; 2; 7). Similar to mammalian postembryonic development, frog metamorphosis involves distinct changes in different organs and tissues. During frog metamorphosis, larval specific organs, such as the tails and gills, are totally resorbed while adult specific tissues such as the limbs develop de novo. The majority of organs/tissues are present in both larval and adult stages but undergo extensive remodeling during metamorphosis. A well-studied such tissue is the intestine, which undergoes drastic changes involving apoptotic degeneration of vast majority of the larval epithelial cells and concurrent de novo formation of adult stem cells that rapidly proliferate and eventually give rise to a multiply folded adult epithelium resembling mature mammalian intestine (8; 9; 10).

Strikingly, T3 plays a causative role for anuran metamorphosis (1; 2; 7). T3 exerts its effects by regulating target gene expression through T3 receptors (TRs). For genes induced by T3, TRs can form heterodimer with 9-cis retinoic acid receptors (RXRs) and bind to T3-response promoters to repress or activate T3-inducible genes depending on the availability of T3 (5; 11; 12; 13; 14; 15; 16; 17). In premetamorphic tadpoles, T3 concentrations are low and the unliganded TRs repress T3-inducible genes by recruiting histone deacetylase-containing corepressor complexes (17; 18; 19; 20; 21; 22; 23; 24; 25; 26; 27). This helps to ensure proper premetamorphic growth and prevent premature metamorphosis. When T3 becomes available during metamorphosis, the liganded TRs recruit histone modifying coactivator complexes to the T3 response genes, leading to histone modifications, chromatin remodeling and gene activation, to effect the drastic tissue-specific metamorphic changes. Recent studies have shown that TR appears to be necessary and sufficient to mediate the metamorphic effects of T3 (17-27).

While much has been learnt about the molecular mechanism of TR action in vitro and in vivo, it is important to determine the downstream events that are responsible for the cellular and morphological changes. Arguably, the most important issue is the identification and functional characterization of direct T3 target genes during metamorphosis. Toward this end, we have been focusing on intestinal metamorphosis, a process that involves both apoptotic degeneration of the larval epithelium and concurrent de novo formation of adult stem cells (8; 9; 10). We have previously carried out a ChIP (chromatin immunoprecipitation)-on-Chip analysis of TR binding in the intestine from premetamorphic tadpoles treated with or without T3 (28). Among many thus identified putative TR target genes is the methyl-CpG binding domain protein 3 (MBD3) gene. MBD3 was originally identified as a protein bearing a highly similar domain to the methyl-CpG binding protein 2 (MeCP2) and a subunit of the Mi-2/NuRD (Nucleosome Remodeling Deacetylase) complex that has both chromatin remodeling and histone deacetylase activities (29; 30; 31). Unlike most Methyl-CpG-binding domain proteins such as MeCP2, MBD1, MBD2 and MBD4 that can bind to methylated DNA, mammalian MBD3 does not bind methylated DNA but binds to hydroxymethylated DNA instead (29; 32; 33), though a Xenopus MBD3-like protein has been shown to bind methylated DNA in vitro (34). Thus, T3 likely induces the expression of MBD3, which in turn affects downstream events via an epigenetic pathway involving the Mi-2/NuRD complex.

To determine whether and how MBD3 is regulated by TR directly at the transcriptional level, we have carried out a bioinformatics analysis of the region bound by TR from the ChIP-on-chip assay and identified two putative TREs. We show that MBD3 is indeed upregulated by T3 in the intestine during natural and T3-induced metamorphosis. In addition, T3 also regulates its expression in the tail. More importantly, we provide in vivo and in vitro evidence to show that a TRE in the first intron of the MBD3
gene mediates the T3 induction of the gene during metamorphosis.

3. MATERIALS AND METHODS

3.1. Experimental animals

*Xenopus tropicalis* and *Xenopus laevis* were purchased from Nasco (Fort Atkinson, MI). Tadpoles were staged according to Nieuwkoop and Faber (35). When indicated, premetamorphic tadpoles at stage 54 were treated with 10 nM T3 at 25 °C for 2 days. All animal procedures were done as approved by NICHD Animal Use and Care Committee.

3.2. Quantitative RT-PCR (qRT-PCR)

Total RNA was isolated from *Xenopus tropicalis* tadpole intestine and tail at indicated stages during natural metamorphosis or premetamorphic tadpoles at stage 54 treated with or without T3 for 2 days. cDNA was synthesized from 2.0 μg total RNA by using the High Capacity cDNA Archive kit (Applied Biosystems, Foster City, CA) in 40 μl reactions according to the manufacturer’s instructions. qRT-PCR was carried out by using SYBR Green PCR Master Mix on a StepOne Plus Real-Time PCR System (Applied Biosystems, Foster City, CA). The primers used were 5’-TCAAGCAACCAGTGACCAAG-3’ (forward) and 5’-TTTCCCAGAAGAGCTGCCT-3’ (reverse) for MBD3. EF1α (elongation factor 1α) was used as the normalization control as described previously (36). Each RNA preparation was from tissues pooled from at least 3 tadpoles and the qRT-PCR was done on duplicated sets of samples.

3.3. Bioinformatics identification of putative TREs

The sequence of *Xenopus tropicalis* MBD3 gene was downloaded from ENSEMBL website (https://useast.ensembl.org/index.html) and the computational analysis tool NHR-Scan (http://www.cisreg.ca/cgi-bin/NHR-scan/nhr_scan.cgi) (37) was used to identify the putative TREs.

3.4. Gel mobility shift assay

Gel mobility shift assay was performed essentially as described previously (36; 38). In brief, *Xenopus tropicalis* TRα and RXRβ proteins were made by using TnT T7 Quick Coupled Transcription/Translation System (Promega, Madison, WI). They were mixed with double-stranded, infrared dye IR700 (LI-COR, Lincoln, NE)-labelled TRE oligonucleotide of *Xenopus laevis* TRβA gene in the in vitro binding reaction in the presence or absence of unlabeled competitors containing the wild type or mutant putative TREs of *Xenopus tropicalis* MBD3 gene. The unlabeled competitors were made by annealing synthetic, complementary oligonucleotides. The oligonucleotide sequences of the upper strands were 5’-ATTTGTGGTCAGACCAATTCCATCCAT-3’ (MBD3 TRE1), 5’-ATTTGCTGAGTGGGACATCCAT-3’ (MBD3 TRE2), and 5’-ATTTGAGTCAGATGGAACATCCATCC-3’ (MBD3 mTRE2) (bold letters indicate the TRE half sites with the mutated nucleotides underlined in the mutant TRE). Each 20 μl binding reaction included 1 μl of 100 fmol of IR-700 labelled probe, 1 μl each of TR and RXR in vitro translated protein mixture, and 1 μl of the wild type or mutant TRE oligonucleotides at 400 fmol/μl, 2 pmol/μl, or 10 pmol/μl, respectively, to obtain 4x, 20x, or 100x unlabeled competitor oligonucleotides, respectively. The mixtures were incubated at room temperature for 20 min and electrophoresed on a 6% DNA retardation gel (Invitrogen, Carlesad, CA). The resulting gel was then scanned by using an Odyssey Infrared Scanner (LI-COR, Lincoln, NE). The assay was done three times with similar results.

3.5. Generation of promoter reporter constructs

The fragment encompassing 1 kb upstream of the 5’-end of the reported *Xenopus tropicalis* MBD3 cDNA, the exon 1, and part of intron 1 that contained predicted TRE2, was PCR-amplified from genomic DNA with the primer pair of 5’-cctgagctcGCTAGCGGTGATATCACTCC-3’ (Forward, bearing a NheI site at the 5’-end) and 5’-cgattgccAAGCTTgCTCTTTATTCCTC-
AGCTGCACC-3’ (Reverse, bearing a HindIII site at the 5’-end) by using high fidelity PrimeStar DNA Polymerase (Takara, Mountain View, CA). The PCR fragment was double-digested with NheI and HindIII, gel-purified, and cloned into pre-digested pGL4.10 firefly luciferase vector (Promega, Madison, WI) bearing the same restriction ends. The mutant promoter harboring a mutated TRE was PCR-amplified from the wild type promoter construct DNA by using the same forward primer for the wild type promoter fragment and 5’-tgccaagcttctctttatTCCTCCAGCTGCACCCAG CTCTGTATGTTTCCATCTGACTCAC-3’ (bearing a HindIII site at the 5’-end with the mutated nucleotides underlined). The PCR fragment was double-digested with NheI and HindIII, gel-purified and cloned into pre-digested pGL4.10 vector bearing the same restriction ends. The mutant construct was confirmed by DNA sequencing.

3.6. Transcription assay in *Xenopus laevis* oocytes

Oocyte transcription assay was performed as described (38; 39). Briefly, the plasmid constructs containing the coding region of GFP, *Xenopus tropicalis* TRα and RXRβ were linearized with EcoRI digestion and transcribed by using a mMESSAGE mMACHINE T7 Transcription Kit (Ambion, Grand Island, NY), respectively. The cytoplasm of stage VI *Xenopus laevis* oocytes was injected with 46 pg/oocyte of GFP mRNA or TR/RXR mRNA mixture. Two hour later, the reporter construct in which firefly luciferase was under the control of MBD3 promoter harboring the wild type or mutated TRE was injected into the nuclei of these oocytes (33 pg/μl) along with the internal control Renilla luciferase reporter phRG-TK (3.3 pg/μl). After incubation at 18 °C overnight in the presence or absence of 100 nM T3, groups of oocytes were collected for dual luciferase assay following the manufacturer’s instructions for the Dual-Luciferase-Reporter Assay kit (Promega, Madison, WI). The relative expression of the firefly luciferase to Renilla luciferase was determined and presented as the average of at least three groups of oocytes. The data shown were representative of a few independent experiments with similar results.

3.7. Chromatin immunoprecipitation (ChIP) assay

ChIP assay on *Xenopus tropicalis* tadpole intestines was done as described previously (40) with an antibody against TR (anti-TR) (38), RNA Polymerase II (abcam, Cambridge, MA), or methylated histone H3K79 (anti-H3K79m2, abcam, Cambridge, MA), and with IgG as a negative control, by using Chromatin Immunoprecipitation (ChIP) Assay kit (Millipore, Burlington, MA). Each sample had three replicas and each replica included at least 5 tadpoles. The immunoprecipitated DNA was analyzed by qPCR with SYBR Green PCR Master Mix on a StepOne Plus Real-Time PCR System (Applied Biosystems, Foster City, CA). For the analysis of MBD3 TRE, primers 5’-ATGCCCGCTACTCTTTATTCCTCCAGCTGCAC-3’ (forward) and 5’-GAGAGAGAGTCAGTGTGGTGGGTCAGA-3’ (reverse) were used. All ChIP experiments were done twice with similar results.

4. RESULTS

4.1. *Xenopus tropicalis* MBD3 is upregulated in the intestine and tail during natural and T3-induced metamorphosis

Our ChIP-on-Chip assay identified TR association with MBD3 gene in the tadpole intestine (28). To investigate whether MBD3 is regulated by T3 in tadpoles, we treated premetamorphic *Xenopus tropicalis* tadpoles at stage 54 with or without 10 nM T3 for 2 days and analyzed MBD3 gene expression by qRT-PCR on total RNA isolated from the intestine and tail, which undergoes resorption instead of remodeling during metamorphosis. The data showed upregulation of MBD3 expression by T3 in both organs, although less dramatically in the tail (Figure 1), suggesting that MBD3 is indeed a T3 target gene and that its expression should also increase during natural metamorphosis when T3 level is high. Indeed, when we analyzed its expression by qRT-PCR in the intestine and tail at different stages from premetamorphic (stage 56), metamorphic climax (stage 58-64), to the end of metamorphosis (stage 66), we observed that MBD3 expression was significantly upregulated in the intestine during
natural metamorphosis with a peak expression at stage 62 (Figure 2A) when T3 level in the plasma is high and the intestine undergoes drastic remodeling, including rapid larval intestinal epithelial degeneration through apoptosis and robust adult intestinal stem cell proliferation and differentiation (8; 9; 10). In addition, MBD3 expression in the tail also increased dramatically when the tail underwent rapid resorption around stage 62 to 64 (Figure 2B). Thus, MBD3 is upregulated by T3 and likely participates in the cell fate determination during intestinal remodeling and tail resorption during frog metamorphosis.

4.2. TR/RXR heterodimer binds to a putative TRE in the first intron of MBD3 gene \textit{in vitro}

As our ChIP-on-Chip analysis showed that TR was associated with MBD3 gene in the tadpole intestine, we carried out bioinformatics analysis on the sequences around the transcriptional start site, which include the region associated with TR found from the ChIP-on-Chip analysis, by using NHR-Scan and identified two putative TRES. One TRE is upstream of the predicted transcription start site (TRE1) and the other one in the first intron (TRE2, Figure 3A), with the TRE2 having a sequence more conserved with the consensus TRE made of two direct repeats of AGGTCA half site separated by 4 bp (Figure 3B). To investigate if TR/RXR heterodimer binds to these two putative TRES, we carried out \textit{in vitro} gel mobility shift assay by using as the probe of IR700-labelled TRE of \textit{Xenopus laevis} TRβA gene, a well characterized TRE consisting of two near perfect direct repeats of AGGTCA half site separated by 4 bp (Figure 3B) (39; 41), TR and RXR proteins made through \textit{in vitro} translation, and unlabeled competitor TRES made from sequences of the putative \textit{Xenopus tropicalis} MBD3 TRES (Figure 3B). The results indicated that the intronic TRE (TRE2) of the MBD3, but not the TRE1 in the upstream region, competed for binding to TR/RXR heterodimer strongly (Figure 3C). To determine if the strong binding of TRE2 to the TR/RXR heterodimer was sequence-specific, we mutated the putative TRE2 at the positions known to be important for binding to TR/RXR heterodimer (Figure 3B) and determined the effect in the mobility shift assay. The results shown in Figure 3C indicated that the mutations in the TRE2 abolished its ability to
Activation of MBD3 gene during adult stem cell development

To determine if the intronic TRE2 can mediate the transcriptional regulation of *Xenopus tropicalis* MBD3 expression by T3 *in vivo*, we cloned a genomic MBD3 fragment encompassing 1 kb upstream of the predicted transcription start site, exon 1 (276bp) and a part of intron 1 that included the putative TRE2 (265bp), upstream of the coding region of firefly luciferase gene in a reporter construct (Figure 4A). The transcription from the MBD3 promoter construct would produce an mRNA consisting of MBD3 exon 1 (152 bp for 5'-untranslated region, 108 bp for encoding a N-terminal fragment of the MBD3 protein), 265 bp of intron 1, and the coding sequence for the firefly luciferase, which encodes a fusion firefly luciferase protein containing 137 additional amino acids at its N-terminus. As a control, we also generated a mutant construct where TRE2 was mutated as in Figure 3B.

4.4. TR binds to the intronic TRE and mediates T3-induced recruitment of RNA polymerase and histone methylation

Our ChIP-on-Chip assay suggested that TR was bound to the proximity of MBD3 promoter region...
Activation of MBD3 gene during adult stem cell development

in the intestine of premetamorphic *Xenopus tropicalis*. To determine if TR is bound to the intronic TRE2 in vivo, we carried out ChIP assay on intestinal chromatin extracted from premetamorphic tadpoles at stage 54 treated with or without T3 for 2 days by using a polyclonal anti-TR antibody (anti-TR). Analysis of the ChIP DNA by using a primer pair flanking TRE2 showed that TR was bound to TRE2.
Activation of MBD3 gene during adult stem cell development

in premetamorphic tadpole intestine and T3 treatment enhanced TR binding to TRE2 (Figure 5).

To investigate if this TR binding was important for the activation of the MBD3 gene, we carried out ChIP assay with an antibody against RNA Polymerase II to measure transcription of the gene, and an antibody against methylated histone H3K79 (H3K79m2), a well-known activation histone mark associated with gene activation by TR during metamorphosis (40; 42). The results showed that T3 treatment led to strong increase in both RNA polymerase II recruitment and H3K79 methylation around TRE2 (Figure 5). In contrast, when ChIP assay was done with the negative control IgG, no signal was detected around the TRE2 region (Fig.5). Thus, the intronic TRE2 in the MBD3 gene is bound by TR in premetamorphic tadpole intestine and likely mediate the activation of MBD3 expression by T3 via histone modification during metamorphosis.

5. DISCUSSION

Amphibian metamorphosis offers an excellent opportunity to study the molecular pathways underlying postembryonic vertebrate
Activation of MBD3 gene during adult stem cell development

Development largely because of the easiness with which to access and manipulate metamorphosing tadpoles. It has been shown that TR plays an essential and sufficient role in mediating the causative effects of T3 during amphibian metamorphosis (18; 27). TR functions by recruiting cofactor complexes to activate and repress T3-inducible genes in the presence and absence of T3, respectively. Thus, it becomes critical to identify and functionally characterize T3 response genes during metamorphosis. Our earlier ChIP-on-Chip analysis has led to the discovery of MBD3 as a putative direct target gene of TR during intestinal metamorphosis. Here we have shown that MBD3 is indeed regulated by T3 during metamorphosis with its expression upregulated not only in the intestine but also in the tail during T3-induced or natural metamorphosis. More importantly, we have provided evidence to support a TRE located in the first intron of the MBD3 gene that binds to TR to facilitate the local histone modification and RNA polymerase II recruitment to activate MBD3 transcription in the presence of T3.

Earlier ChIP-on-Chip analysis showed that TR was associated with MBD3 gene in the intestine of premetamorphic *Xenopus tropicalis* tadpoles. Our bioinformatics analysis discovered two putative TRES flanking the predicted transcription start site. The putative TRE1 found in the upstream of MBD3 transcriptional start site had no detectable binding to TR/RXR heterodimer in vitro, suggesting it unlikely a functional TRE in vivo. In contrast, the TRE located in the first intron, TRE2, can bind to TR/RXR heterodimers in vitro and is required for the activation of the MBD3 promoter in the reconstituted *Xenopus* oocyte transcription system in vivo. More importantly, TRE2 is bound by TR in the tadpole intestine and T3 treatment of premetamorphic tadpoles leads to increased level of the activation histone mark H3K79 methylation and recruitment of RNA polymerase II to the TRE2 in the tadpole intestine. Thus, TRE2 is likely responsible for mediating direct transcriptional activation of the MBD3 gene by liganded TR/RXR.

Consistent with being a direct target gene
of TR, MBD3 mRNA level is upregulated in the intestine during natural metamorphosis, with peak levels occurring at the climax of metamorphosis when T3 levels are high. In addition, its mRNA level also increases dramatically during tail resorption. Furthermore, T3 treatment of premetamorphic tadpoles leads to the upregulation of MBD3 gene in both the tail and intestine. Thus, it is very likely that MBD3 is important for diverse transformations in different tissues/organs during metamorphosis, including larval cell death and adult cell development and proliferation.

MBD3 contains a domain highly similar to the methyl-CpG binding protein 2 (MeCP2) and is an essential scaffold subunit of the Mi-2/NuRD complex that can deacetylate histones and remodel chromatin upon ATP hydrolysis (29; 30; 31). The Mi-2/NuRD complex is a unique player among chromatin remodeling complexes because it couples ATP-dependent nucleosome remodeling activity with histone deacetylase activity (30; 32; 43; 44; 45). Mammalian MBD3 does not bind methylated DNA but binds to hydroxymethylated DNA instead (29; 32; 33), although a Xenopus laevis MBD3-like protein can bind methylated DNA in vitro (34). Thus, it is likely that MBD3 may affect amphibian metamorphosis through Mi-2/NuRD complex via binding to methylated or hydroxymethylated DNA.

MBD3 has been shown to affect development and stem cells in mammals. Depleting MBD3 coupled with OSKM (Oct4, Sox2, Klf4 and Myc) transduction and reprogramming result in deterministic and synchronized iPS cell programming from mouse and human cells (46), though other data have also shown that a profound reduction in reprogramming efficiency was observed from cells where MBD3 had been ablated (47). Nevertheless, MBD3 likely can affect induction of cell pluripotency and stem cell lineage commitment. In addition, regulating MBD3’s stability and MBD3/NuRD complex recruitment has been implicated to affect neural progenitor cells’ self-renewal and neuronal differentiation during mammalian corticogenesis (48). MBD3/NuRD has also been reported to be required to repress inappropriate transcription in both progenitor cells and neurons to facilitate appropriate cell lineage choice and differentiation programs during mouse neurogenesis (49). MBD3 is critical for mouse development with a distinct role from even its closely related protein MBD2. MBD3 knockout (MBD3−/−) mice die during early embryogenesis whereas MBD2 knockout (MBD2−/−) mice are viable and fertile (50). MBD3−/− embryonic stem cells misregulate a subset of pluripotency-associated genes and subsequently fail to engage in cell differentiation into embryonic lineage when self-renewal requisites are withdrawn from the culture media (43; 51; 52). The upregulation of MBD3 during both intestinal remodeling, where larval cell death and adult stem cell development take place, and tail resorption, where all tissues degenerate, would argue for a critical role of MBD3 in larval cell death as well as adult organogenesis during metamorphosis, consistent with the essential role of MBD3 during mouse development. Clearly, it would be interesting to determine if MBD3 is expressed in a cell type/tissue-specific manner during metamorphosis and what roles the endogenous MBD3 plays by taking advantage of the ability to carry out gene knockout in Xenopus tropicalis and laevis (53; 54- 56).

6. ACKNOWLEDGMENT

This work was supported by the intramural Research Program of NICHD, NIH. LF and CL conceived and designed the experiment; WN provided materials; LF and YS analyzed the data and prepared the manuscript; all authors approve the final version of the manuscript. The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

7. REFERENCES


5. P. M. Yen: Physiological and molecular basis of thyroid hormone action. Physiol Rev, 81(3), 1097-142 (2001) DOI: 10.1152/physrev.2001.81.3.1097


12. J. Wong, Y.-B. Shi and A. P. Wolfe: 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 (1997) DOI: 10.1093/emboj/16.11.3158


Activation of MBD3 gene during adult stem cell development

10.1146/annurev.bi.63.070194.002315

17. Y.-B. Shi: Dual functions of thyroid hormone receptors in vertebrate development: the roles of histone-modifying cofactor complexes. Thyroid, 19, 987-999. (2009)
DOI: 10.1089/thy.2009.0041

18. D. R. Buchholz and Y. B. Shi: Dual function model revised by thyroid hormone receptor alpha knockout frogs. Gen Comp Endocrinol (2018)
DOI: 10.1016/j.ygcen.2018.04.020

DOI: 10.1210/en.2014-2016

DOI: 10.1186/2045-3701-5-8

DOI: 10.1210/en.2016-1955

DOI: 10.1210/en.2014-1554

DOI: 10.1242/bio.030338

DOI: 10.1210/en.2017-00601

DOI: 10.1210/en.2014-1439

DOI: 10.1210/en.2016-1953

27. L. Wen and Y. B. Shi: Regulation of growth rate and developmental timing by Xenopus thyroid hormone receptor alpha. Dev Growth Differ, 58(1), 106-15 (2016)
DOI: 10.1111/dgd.12231

DOI: 10.1038/s41598-017-06679-x


36. M. Ranjan, J. Wong and Y. B. Shi: Transcriptional repression of Xenopus TR beta gene is mediated by a thyroid
hormone response element located near the start site. J Biol Chem, 269(40), 24699-705 (1994)

42. K. Matsuura, K. Fujimoto, L. Fu and Y.-B. Shi: Liganded thyroid hormone receptor induces nucleosome removal and histone modifications to activate transcription during larval intestinal cell death and adult stem cell development. Endocrinology, 153, 961-972 (2012) DOI: 10.1210/en.2011-1736


Key Words: Thyroid Hormone Receptor, Adult Organ Specific Stem Cell, Cell Proliferation And Differentiation, Small Intestine, methyl-CpG binding

Send correspondence to: Yun-Bo Shi, Section on Molecular Morphogenesis, Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD), National Institutes of Health, Bethesda, Maryland, 20892, USA, Tel:3014021004, Fax: 301-402-1323, E-mail: Shi@helix.nih.gov