General and Comparative Endocrinology 271 (2019) 91–96

Contents lists available at ScienceDirect

General and Comparative Endocrinology

journal homepage: www.elsevier.com/locate/ygcen

Involvement of epigenetic modifications in thyroid hormone-dependent formation of adult intestinal stem cells during amphibian metamorphosis

Liezhen Fu, Jessica Yin, Yun-Bo Shi*

Section on Molecular Morphogenesis, Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD), National Institutes of Health (NIH), 49 Convent Dr., Bethesda, MD 20892, United States

ARTICLE INFO

Keywords:
Thyroid hormone receptor
Adult stem cell
Amphibian metamorphosis
Xenopus laevis
Xenopus tropicalis
Histone methylation
Histone acetylation
Intestinal remodeling

ABSTRACT

Amphibian metamorphosis has long been used as model to study postembryonic development in vertebrates, a period around birth in mammals when many organs/tissues mature into their adult forms and is characterized by peak levels of plasma thyroid hormone (T3). Of particular interest is the remodeling of the intestine during metamorphosis. In the highly-related anurans *Xenopus laevis* and *Xenopus tropicalis*, this remodeling process involves larval epithelial cell death and *de novo* formation of adult stem cells via dedifferentiation of some larval cells under the induction of T3, making it a valuable system to investigate how adult organ-specific stem cells are formed during vertebrate development. Here, we will review some studies by us and others on how T3 regulates the formation of the intestinal stem cells during metamorphosis. We will highlight the involvement of nucleosome removal and a positive feedback mechanism involving the histone methyltransferases in gene regulation by T3 receptor (TR) during this process.

1. Introduction

Adult organ-specific stem cells are critical for tissue homeostasis, repair, and regeneration in vertebrates. These stem cells are often developed as organs mature into their adult forms. For many organs, this takes place during postembryonic development in mammals, a period about 4 months before to several months after birth in human when plasma thyroid hormone (T3) concentration is high. Among such organs is the intestine. The adult mammalian intestine has a self-renewing epithelial system, where the stem cells reside in the crypts while most of the differentiated cells are located in the villi. The offspring of the stem cells migrate along the crypt-villus axis as they gradually differentiate into different types of epithelial cells and undergoes apoptosis, mostly at the tip of the villus, to complete the self-renewal cycle (MacDonald et al., 1964; Toner et al., 1971; van der Flier and Clevers, 2009; Shi et al., 2011). Early studies have shown that the mouse intestine matures into the adult form during the first three weeks or so after birth as the plasma thyroid hormone (T3) level rises to a peak level (Matsuda and Shi, 2010; Harper et al., 2011; Muncan et al., 2011; Sun and Shi, 2012). In particular, the neonatal mouse intestine after birth lacks any crypts. The crypts are formed during the first few weeks after birth to establish the self-renewing adult epithelium. Increasing evidence suggest that T3 plays a critical role in the formation and/or function of adult intestinal stem cells in mammals (Plateroti et al., 1999; Plateroti et al., 2001; Flamant et al., 2002; Plateroti et al., 2006; Kress et al., 2009; Yakut et al., 2011; Bochukova et al., 2012; van Mullem et al., 2012; Moran and Chatterjee, 2015; Sun et al., 2016). On the other hand, it is difficult to alter T3 levels in the uterus-enclosed mammalian embryos and reduce T3 levels in the neonates since T3 synthesis begins during embryogenesis and neonates are dependent on maternal supply of nutrients for survival and development. Thus, how T3 affects adult intestinal stem cell development in mammals remains to be determined.

Intestinal remodeling during amphibian metamorphosis offers an opportunity to study how T3 regulates adult intestinal stem cell development. The adult *Xenopus* intestine resembles adult mammalian intestine with a self-renewing system in the form of epithelial folds, with the stem cells localized in the trough of the fold, similar to the crypt-villus structure in mammals (Fig. 1) (Shi and Ishizuya-Oka, 1996; Sterling et al., 2012). The tadpole intestine, however, has only a single epithelial fold, the typhlosole, and the epithelium is surrounded by thin layers of connective tissue and muscles (Fig. 1). During metamorphosis, the larval epithelial cells undergo programmed cell death while adult progenitor/stem cells are developed *de novo* and exist as clusters of proliferating cells (also referred to as cell nests or islet cells) (Fig. 1) (Shi and Ishizuya-Oka, 1996). These cells subsequently give rise to the adult epithelium, with concurrent development of the connective tissue and muscles. Like any other processes during amphibian metamorphosis, intestinal remodeling is totally dependent on T3 and can even

* Corresponding author.
E-mail address: Shi@helix.nih.gov (Y.-B. Shi).

https://doi.org/10.1016/j.ygcen.2018.11.012
Received 24 September 2018; Received in revised form 19 November 2018; Accepted 21 November 2018
Available online 22 November 2018
0016-6480/ Published by Elsevier Inc.

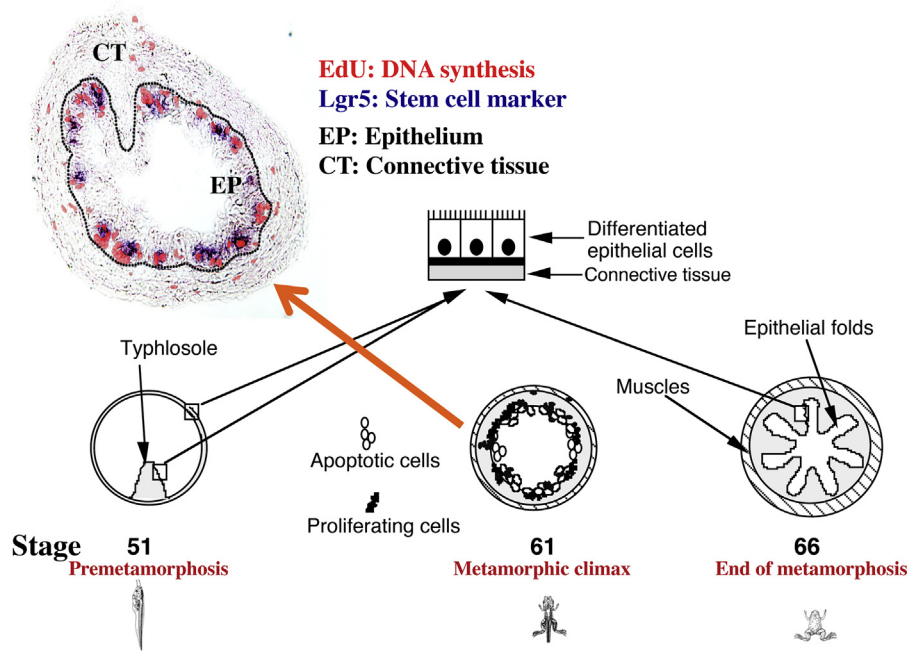

Fig. 1. *Xenopus* intestinal metamorphosis serves as a model for studying adult organ-specific stem cell development in vertebrates. In premetamorphic tadpoles (e.g., at stage 51), the intestine has only a single fold, the typhlosole, where connective tissue is abundant, and is structurally similar to the mammalian embryonic intestine. At the metamorphic climax around stage 61, the vast majority of the larval epithelial cells undergo apoptosis (the open circles). A small fraction of the larval epithelial cells undergo dedifferentiation into cells that rapidly proliferate (EdU positive) and express the adult stem cell marker Lgr5 (black dots in the stage 61 diagram). By stage 66 (the end of metamorphosis), these cells differentiate to form a multiply folded epithelium surrounded by elaborate connective tissue and thick muscle layers. See (Okada et al., 2015) for EdU labeling and Lgr5 in situ hybridization.

be reproduced in tadpole intestinal organ cultures with T3 treatment (Ishizuya-Oka and Shimozawa, 1991; Shi and Ishizuya-Oka, 1996), making it a unique system to study the development of adult organ-specific stem cells.

2. T3-induced formation of adult intestinal stem cells during metamorphosis

Earlier studies have failed to identify any progenitor or adult stem cells in the premetamorphic *Xenopus* intestinal epithelium (McAvoy and Dixon, 1977; Shi and Ishizuya-Oka, 1996). The tadpole epithelium consists of a monolayer of differentiated cells that are yet mitotically active. During metamorphosis, the larval epithelial cells undergo apoptosis. Organ culture and primary cell culture studies have shown that during metamorphosis, T3 induces the larval epithelial cell death via two distinct mechanisms, directly inducing apoptosis within the epithelial cells or indirectly through T3 action in the underlying non-epithelial tissues, in part by activating the expression of matrix metalloproteinases to degrade/modify the extracellular matrix (Ishizuya-Oka and Shimozawa, 1992b; Su et al., 1997a; Su et al., 1997b; Fu et al., 2007; Ishizuya-Oka et al., 2009; Mathew et al., 2009; Ishizuya-Oka et al., 2010; Mathew et al., 2010; Hasebe et al., 2011). The adult epithelial progenitor/stem cells are formed *de novo*, organ-autonomously in response to T3 (Ishizuya-Oka and Shimozawa, 1992b). Recombinant organ culture studies with wild type and transgenic-GFP expressing tadpoles have revealed that the adult stem cells originate from the larval epithelium (Ishizuya-Oka et al., 2009), suggesting that some larval epithelial cells are induced by T3 to undergo dedifferentiation to become the adult epithelial stem cells.

T3 can act via both genomic and non-genomic pathways, with the latter through the binding of T3 to cell surface or cytoplasmic proteins. Molecular and transgenic studies in *Xenopus laevis* have shown that the metamorphic effects of T3 are due to transcriptional regulation of gene expression through nuclear T3 receptors (TRs) (Shi, 1994; Sachs et al., 2000; Schreiber et al., 2001; Buchholz et al., 2003; Nakajima and Yaoita, 2003; Buchholz et al., 2004; Buchholz et al., 2006; Brown and Cai, 2007; Bagamasbad et al., 2008; Denver et al., 2009; Schreiber et al., 2009; Shi, 2009; Shi et al., 2012). More recently, gene knockout studies have provided direct evidence for the important role of TRs during *Xenopus tropicalis* development (Choi et al., 2015; Sachs, 2015; Wen and Shi, 2015; Yen, 2015; Wen and Shi, 2016; Choi et al., 2017; Wen et al., 2017b; Buchholz and Shi, 2018; Nakajima et al., 2018; Sakane et al., 2018). To determine if T3 induces the formation of the adult stem cells tissue-autonomously, we have used recombinant organ-cultures made of tissues from wild type and transgenic animals expressing a dominant positive TR (dpTR) (Buchholz et al., 2004; Hasebe et al., 2011). The dpTR functioned as a T3-bound TR but without a need to actually bind to T3 and was placed under the control of a heat shock-inducible promoter. Heat shock-treatment of the organ cultures showed that the expression of the dpTR in all tissues of the intestine in the absence of T3 was sufficient to induce intestinal metamorphosis, showing that T3 action through TR is sufficient for intestinal metamorphosis, including stem cell formation (Hasebe et al., 2011). More importantly, such experiments also revealed that the formation of adult stem cells requires T3 action not only in the epithelium but also in the underlying non-epithelial tissues. In particular, the expression of dpTR in the larval epithelium alone was able to induce the dedifferentiation of larval epithelial cells and upregulate sonic hedgehog gene, which is highly expressed in the proliferating adult epithelial progenitor/stem cells, in these cells. However, such cells failed to express markers of adult intestinal stem cells. In addition, the expression of dpTR in only the non-epithelial tissues lead to only epithelial cell death without the formation of any adult stem cells. Thus, the formation of the adult stem cells requires both T3 action in the tadpole epithelial cells and T3-induced cell-cell interactions between the epithelium and non-epithelial tissues (Hasebe et al., 2011), in agreement with previous findings for a role of cell-cell interaction during intestinal metamorphosis (Ishizuya-Oka and Shimozawa, 1992a; Schreiber et al., 2005; Schreiber et al., 2009).

2.1. Chromatin remodeling and histone modification by TR during intestinal metamorphosis

TR is a T3-dependent, DNA binding transcription factor. TR can form a heterodimer with 9-cis retinoic acid receptor (RXR), which is also a member of the nuclear hormone receptor superfamily (Lazar, 1993; Tsai and O'Malley, 1994; Mangelsdorf et al., 1995; Yen, 2001; Laudet and Gronemeyer, 2002). TR/RXR heterodimers bind to T3-response elements (TREs) in T3-inducible genes both in the presence and absence of T3 in chromatin (Lazar, 1993; Tsai and O'Malley, 1994;

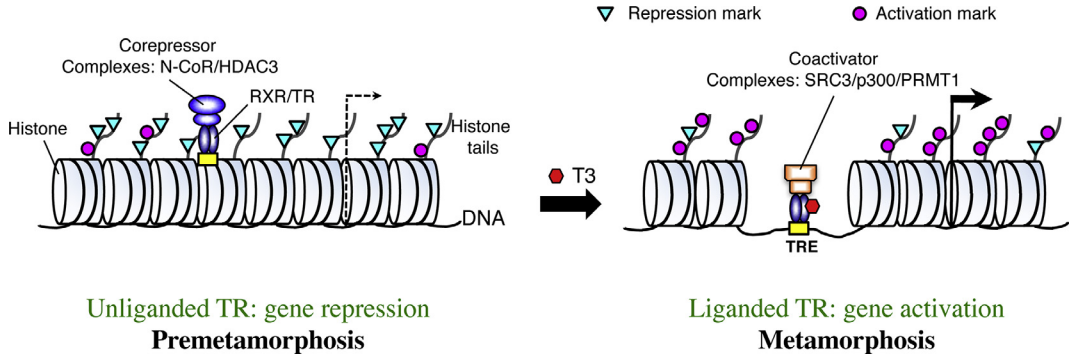

Fig. 2. Regulation of T3-inducible genes by TR during *Xenopus* development. In premetamorphic tadpoles, there is little T3 and TR is unliganded. The unliganded TR/RXR heterodimer binds to TREs in the target genes and recruits corepressor complexes such as the N-CoR-HDAC3 complex, resulting in the reduction in the levels of activation histone marks and increase of repression marks, and consequently gene repression. During metamorphosis, high levels of T3 leads to T3-binding to TR. Liganded TR/RXR recruits coactivator complexes such as SRC complexes as shown to disrupt chromatin and modify histones, leading to increased levels of activation histone marks and gene activation. N-CoR: nuclear corepressor, HDAC: histone deacetylase, SRC3: steroid receptor coactivator 3 (a histone acetyltransferase), p300: a histone acetyltransferase, PRMT1: protein arginine methyltransferase 1.

Mangelsdorf et al., 1995; Wong et al., 1995; Wong et al., 1997; Wong et al., 1998; Yen, 2001; Shi, 2009). This binding leads to repression or activation of the T3-inducible genes in the absence or presence of T3 via the recruitment of corepressor or coactivator complexes, respectively. Interestingly, the corepressor complexes contain histone deacetylases (HDACs) while coactivator complexes contain histone acetyltransferases, histone methyltransferases, and/or chromatin remodeling enzymes, implicating a role of chromatin remodeling and histone modification in gene regulation by TR.

Indeed, chromatin immunoprecipitation (ChIP) studies on the intestine and tail during metamorphosis have shown that T3 activation of gene expression involves increased levels of so-called activation histone marks, i.e., those histone modifications associated with high levels of mRNA expression, and reduction in repression histone marks, i.e., those histone modifications associated with repressed genes (Fig. 2) (Sachs and Shi, 2000; Sachs et al., 2002; Havis et al., 2003; Tomita et al., 2004; Paul et al., 2005a; Paul et al., 2005b; Paul et al., 2007; Matsuda et al., 2009; Bilesimo et al., 2011; Matsuura et al., 2012b; Shi et al., 2012; Grimaldi et al., 2013). In addition, ChIP analyses of total histones at the TR binding sites in target genes have revealed that liganded TR causes the removal of approximately 2 nucleosomes in each TR binding region during metamorphosis (Matsuura et al., 2012b), in agreement with earlier studies using T3-responsive reporter plasmid minichromosomes in the reconstituted frog oocyte transcription system (Wong and Shi, 1995; Wong et al., 1995; Wong et al., 1997; Hsia and Shi, 2002). These findings suggest that in the premetamorphic tadpole intestine, TR/RXR heterodimers bind to TREs in chromatin and recruit HDAC-containing corepressors to remove activation histone marks and add repression histone marks, leading to gene repression (Fig. 2). During metamorphosis, T3 binds to TR and leads to the removal of the corepressor complexes and recruitment of the coactivator complexes. Such complexes cause the removal of up to two nucleosomes near the TR binding region, increasing activation histone marks, and reducing in repression histone marks, and eventually lead to gene activation and induce the intestinal remodeling process.

2.2. A positive feedback loop involving histone methyltransferases in gene regulation by TR during intestinal stem cell development

The studies as reviewed above have provided a detailed understanding on how TR regulates gene expression in the context of chromatin *in vivo* and revealed a requirement of T3-induced gene expression changes in both the epithelium and non-epithelial tissues for the formation of adult stem cells during intestinal metamorphosis. The next key issue toward understanding the development of the stem cells is thus to identify and functionally characterize the genes that are regulated by T3 in different intestinal tissues during metamorphosis. A lot of efforts have been made in this regard and many genes have been identified and found to be involved in adult stem cell formation/proliferation (Shi and Brown, 1993; Amano and Yoshizato, 1998; Ishizuya-Oka et al., 2001; Buchholz et al., 2007; Das et al., 2009; Heimeier et al., 2010; Luu et al., 2013; Miller et al., 2013; Sun et al., 2013; Fu et al., 2017; Okada et al., 2017; Okada and Shi, 2018).

Of particular interest among the genes are two encoding histone methyltransferases, protein arginine methyltransferase (PRMT) 1 and Dot1L (Dot1-like) (Matsuda et al., 2009; Matsuura et al., 2012b). PRMT1 is a histone H4R3 methyltransferase and is a well-known TR coactivator (Chen et al., 1999; Matsuda et al., 2009). PRMT1 is induced by T3 during intestinal metamorphosis in *Xenopus laevis*. Mechanistically, T3 appears to activate the transcription factor cMyc in the developing stem cells and cMyc in turn activates the PRMT1 promoter (Fujimoto et al., 2012; Okada et al., 2017). These findings suggest that T3 induces the expression of PRMT1, although indirectly, and PRMT1 in turn feeds back positively on T3 action by functioning as a TR coactivator to further enhance T3 signaling to promote intestinal stem cell development.

Consistently, PRMT1 has little expression in premetamorphic intestine and is upregulated in the developing stem cells but not the dying larval epithelial cells during metamorphosis, just like cMyc, which activates PRMT1 promoter (Fujimoto et al., 2012; Okada et al., 2017). More importantly, transgenic overexpression of PRMT1 enhances the activation of T3 response genes by T3 and increases the number of stem cells during metamorphosis (Matsuda and Shi, 2010). Conversely, knockdown the expression of PRMT1 in the tadpole intestine results in reduced number of stem cells during metamorphosis (Matsuda and Shi, 2010). These findings support a role of PRMT1 in adult stem cell formation and/or proliferation during intestinal remodeling.

The other histone methyltransferase, Dot1L, is the only enzyme that can methylate histone H3K79 *in vitro* (Nguyen and Zhang, 2011) and is directly regulated by T3 at the transcription level through the binding of TR to a TRE in its promoter (Matsuura et al., 2012a). Importantly, H3K79 methylation at TREs of T3 response genes is increased during metamorphosis in the intestine, raising the possibility that Dot1L is a TR coactivator that functions through H3K79 methylation, a known activation histone mark (Matsuura et al., 2012b). Indeed, transcription studies in the reconstituted frog oocyte system showed that Dot1L could enhance transcriptional activation by TR (Wen et al., 2017a). This might be in part due to increased TR binding to the TRE when Dot1L was overexpressed in the oocyte (Wen et al., 2017a). No recruitment of Dot1L to the TRE of the reporter plasmid minichromosome was detected in the frog oocyte, although it remains possible that Dot1L was weakly/transiently recruited by liganded TR to the TRE (Wen et al.,

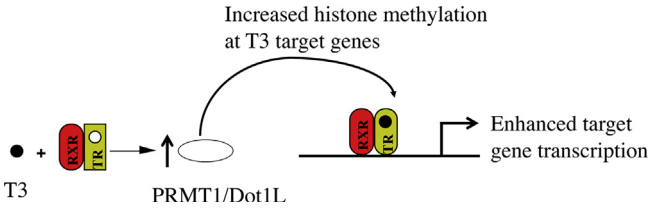

Fig. 3. A positive feedback mechanism to enhance T3 activation of gene transcription through histone methylation. T3 induces the expression of Dot1L directly at the transcription level (Matsuura et al., 2012a) and PRMT1 indirectly via transcriptional activation of cMyc by TR in the developing stem cells (Fujimoto et al., 2012; Okada et al., 2017). Dot1L and PRMT1 in turn function as TR coactivators to increase local histone methylations to enhance transcription (Fujimoto et al., 2012; Wen et al., 2017a). It is worth pointing out that there has been no direct evidence for the recruitment of Dot1L to TREs, although PRMT1 has been shown to be recruited by TR to TREs in the presence of T3 during metamorphosis (Matsuda et al., 2009).

2017a).

In addition, Dot1L overexpression also enhances T3-induction of gene expression in frog cell lines as well as in transgenic tadpoles (Wen et al., 2017a). Finally, a TALEN-nuclease was found to be able to cause up to 90% mutation in feeding stage tadpoles upon injecting its mRNAs into fertilized *Xenopus tropicalis* eggs and caused a correspondingly 80–90% reduction in H3K79 methylation of total H3 in the tadpoles, suggesting that in the developing tadpoles, Dot1L is the only methyltransferase for H3K79 (Wen et al., 2015a). Importantly, upon T3 treatment of such knockdown tadpoles, the induction of endogenous T3 target genes were found to be reduced, indicating that endogenous Dot1L contributes to gene activation by TR. Collectively, these findings support a role of Dot1L as a TR coactivator during intestinal remodeling and adult stem cell development via a positive feedback mechanism (Fig. 3).

3. Conclusion

Intestinal remodeling during amphibian metamorphosis offers a unique opportunity to study the development of adult organ-specific stem cells and the mechanism of gene regulation by TR *in vivo*. Studies on this model system have revealed that during intestinal stem cell development and subsequent formation of the adult epithelium, T3 activates the expression of genes in a number of pathways that are known to be important for stem cells and cell proliferation, including the hedgehog, Notch, BMP, and Wnt signal pathways, in the development and/or proliferation of adult intestinal stem cells during this T3-dependent process (Stolow and Shi, 1995; Ishizuya-Oka et al., 2001; Ishizuya-Oka et al., 2006; Hasebe et al., 2008; Hasebe et al., 2012; Ishizuya-Oka and Hasebe, 2013; Ishizuya-Oka et al., 2014; Wen et al., 2015b; Hasebe et al., 2016; Hasebe et al., 2017). Thus, these pathways are involved both in the formation of adult stem cells and their subsequent function and/or maintenance in the adult. While some of the T3-regulated genes in these pathways, such as sonic hedgehog (Stolow and Shi, 1995), appear to be directly regulated by TR at the transcription level, others are likely regulated by T3 indirectly through the regulation of other genes by TR.

Like most DNA-binding transcription factors, TR functions by recruiting cofactor complexes upon binding to specific DNA elements in the target genes. *In vitro* and cell culture studies have identified many TR-cofactor complexes containing histone modifying enzymes and/or chromatin remodelers. Studies on *Xenopus* intestinal remodeling have provided one of the few pieces of *in vivo* evidence supporting a role of epigenetic modifications in gene regulation by TR during vertebrate development. First, most, although not all, histone marks analyzed so far correlate with gene regulation by TR during *Xenopus* intestinal remodeling as well as tail resorption (Bilesimo et al., 2011; Matsuura et al., 2012b; Grimaldi et al., 2013). That is, the levels of activation histone marks are increased at target genes during metamorphosis when T3 is present, supporting that TR utilizes such epigenetic modifications to control gene expression during adult stem cell development in the intestine. Second, liganded TR causes drastic chromatin remodeling, leading to the loss of two nucleosomes around the TR/RXR binding region. Finally and perhaps most interestingly, T3 activates the expression of at least two histone modifying enzymes, PRMT1 and Dot1L, that can also function as TR co-activators, demonstrating the existence of positive feedback mechanisms involving histone modifications to further enhance T3 action during metamorphosis (Fig. 3). Such mechanisms are likely important to ensure spatiotemporal coordination of the transformations of different tissues during metamorphosis. While a lot have been learnt, most of the studies so far have been correlative. Functional analysis, especially by using gene editing technologies (Young et al., 2011; Lei et al., 2012; Lei et al., 2013), are required to determine roles of the endogenous histone modifying enzymes and chromatin remodelers in chromatin remodeling and histone modification in regulating gene expression and adult stem cell development.

Competing interests

None.

Authors' contributions

All authors participated in the writing of the review.

Acknowledgement

This work was supported by the intramural Research Program of NICHD, NIH.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygcen.2018.11.012.

References

Amano, T., Yoshizato, K., 1998. Isolation of genes involved in intestinal remodeling during anuran metamorphosis. Wound Repair Regen. 6, 302–313.
Bagamasbad, P., Howdeshell, K.L., Sachs, L.M., Demeneix, B.A., Denver, R.J., 2008. A role for basic transcription element-binding protein 1 (BTEB1) in the autoinduction of thyroid hormone receptor beta. J. Biol. Chem. 283, 2275–2285.
Bilesimo, P., Jolivet, P., Alfama, G., Buisine, N., Le Mevel, S., Havis, E., Demeneix, B.A., Sachs, L.M., 2011. Specific histone lysine 4 methylation patterns define TR-binding capacity and differentiate direct T3 responses. Mol. Endocrinol. 25, 225–237.
Bochukova, E., Schoenmakers, N., Agostini, M., Schoenmakers, E., Rajanayagam, O., Keogh, J.M., Henning, E., Reinemund, J., Gevers, E., Sarri, M., et al., 2012. A mutation in the thyroid hormone receptor alpha gene. N. Engl. J. Med. 366, 243–249.
Brown, D.D., Cai, L., 2007. Amphibian metamorphosis. Dev. Biol. 306, 20–33.
Buchholz, D.R., Heimeier, R.A., Das, B., Washington, T., Shi, Y.-B., 2007. Pairing morphology with gene expression in thyroid hormone-induced intestinal remodeling and identification of a core set of TH-induced genes across tadpole tissues. Dev. Biol. 303, 576–590.
Buchholz, D.R., 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.
Buchholz, D.R., Paul, B.D., Fu, L., Shi, Y.B., 2006. Molecular and developmental analyses of thyroid hormone receptor function in Xenopus laevis, the African clawed frog. Gen. Comp. Endocrinol. 145, 1–19.
Buchholz, D.R., Shi, Y.B., 2018. Dual function model revised by thyroid hormone receptor alpha knockout frogs. Gen. Comp. Endocrinol.
Buchholz, D.R., Tomita, A., Fu, L., Paul, B.D., 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.
Chen, D., Ma, H., Hong, H., Koh, S.S., Huang, S.M., Schurter, B.T., Aswad, D.W., Stallcup, M.R., 1999. Regulation of transcription by a protein methyltransferase. Science 284, 2174–2177.
Choi, J., Ishizuya-Oka, A., Buchholz, D.R., 2017. Growth, development, and intestinal remodeling occurs in the absence of thyroid hormone receptor alpha in tadpoles of

Xenopus tropicalis. Endocrinology 158, 1623–1633.
Choi, J., Suzuki, K.I., Sakuma, T., Shewade, L., Yamamoto, T., Buchholz, D.R., 2015. Unliganded thyroid hormone receptor alpha regulates developmental timing via gene repression as revealed by gene disruption in Xenopus tropicalis. Endocrinology 156, 735–744.
Das, B., Heimeier, R.A., Buchholz, D.R., Shi, Y.B., 2009. Identification of direct thyroid hormone response genes reveals the earliest gene regulation programs during frog metamorphosis. J. Biol. Chem. 284, 34167–34178.
Denver, R.J., Hu, F., Scanlan, T.S., Furlow, J.D., 2009. Thyroid hormone receptor subtype specificity for hormone-dependent neurogenesis in Xenopus laevis. Dev. Biol. 326, 155–168.
Flamant, F., Poguet, A.L., Plateroti, M., Chassande, O., Gauthier, K., Streichenberger, N., Mansouri, A., Samarut, J., 2002. Congenital hypothyroid Pax8(-/-) mutant mice can be rescued by inactivating the TRalpha gene. Mol. Endocrinol. 16, 24–32.
Fu, L., Das, B., Matsuura, K., Fujimoto, K., Heimeier, R.A., Shi, Y.B., 2017. Genome-wide identification of thyroid hormone receptor targets in the remodeling intestine during Xenopus tropicalis metamorphosis. Sci. Rep. 7, 6414.
Fu, L., Hasebe, T., Ishizuya-Oka, A., Shi, Y.-B., 2007. Roles of matrix metalloproteinases and ECM remodeling during thyroid hormone-dependent intestinal metamorphosis in Xenopus laevis. Organogenesis 3, 14–19.
Fujimoto, K., Matsuura, K., Hu-Wang, E., Lu, R., Shi, Y.B., 2012. thyroid hormone activates protein arginine methyltransferase 1 expression by directly inducing c-Myc transcription during xenopus intestinal stem cell development. J. Biol. Chem. 287, 10039–10050.
Grimaldi, A., Buisine, N., Miller, T., Shi, Y.B., Sachs, L.M., 2013. Mechanisms of thyroid hormone receptor action during development: lessons from amphibian studies. BBA 1830, 3882–3892.
Harper, J., Mould, A., Andrews, R.M., Bikoff, E.K., Robertson, E.J., 2011. The transcriptional repressor Blimp1/Prdm1 regulates postnatal reprogramming of intestinal enterocytes. Proc. Natl. Acad .Sci. U.S.A. 108, 10585–10590.
Hasebe, T., Buchholz, D.R., Shi, Y.B., Ishizuya-Oka, A., 2011. Epithelial-connective tissue interactions induced by thyroid hormone receptor are essential for adult stem cell development in the Xenopus laevis intestine. Stem Cells 29, 154–161.
Hasebe, T., Fujimoto, K., Kajita, M., Fu, L., Shi, Y.B., Ishizuya-Oka, A., 2017. Thyroid hormone-induced activation of notch signaling is required for adult intestinal stem cell development during xenopus laevis metamorphosis. Stem Cells 35, 1028–1039.
Hasebe, T., Fujimoto, K., Kajita, M., Ishizuya-Oka, A., 2016. Thyroid hormone activates Wnt/beta-catenin signaling involved in adult epithelial development during intestinal remodeling in Xenopus laevis. Cell Tissue Res. 365, 309–318.
Hasebe, T., Kajita, M., Fu, L., Shi, Y.B., Ishizuya-Oka, A., 2012. Thyroid hormone-induced sonic hedgehog signal up-regulates its own pathway in a paracrine manner in the Xenopus laevis intestine during metamorphosis. Dev. Dyn. 241, 403–414.
Hasebe, T., Kajita, M., Shi, Y.B., Ishizuya-Oka, A., 2008. Thyroid hormone-up-regulated hedgehog interacting protein is involved in larval-to-adult intestinal remodeling by regulating sonic hedgehog signaling pathway in Xenopus laevis. Dev. Dyn. 237, 3006–3015.
Havis, E., Sachs, L.M., Demeneix, B.A., 2003. Metamorphic T3-response genes have specific co-regulator requirements. EMBO Rep. 4, 883–888.
Heimeier, R.A., Das, B., Buchholz, D.R., Fiorentino, M., Shi, Y.B., 2010. Studies on Xenopus laevis intestine reveal biological pathways underlying vertebrate gut adaptation from embryo to adult. Genome Biol. 11, R55.
Hsia, V.S.-C., Shi, Y.-B., 2002. Chromatin Disruption and Histone Acetylation in the Regulation of HIV-LTR by Thyroid Hormone Receptor. Mol. Cell. Biol. 22, 4043–4052.
Ishizuya-Oka, A., Hasebe, T., 2013. Establishment of intestinal stem cell niche during amphibian metamorphosis. Curr. Top. Dev. Biol. 103, 305–327.
Ishizuya-Oka, A., Hasebe, T., Buchholz, D.R., Kajita, M., Fu, L., Shi, Y.B., 2009. Origin of the adult intestinal stem cells induced by thyroid hormone in Xenopus laevis. FASEB J. 23, 2568–2575.
Ishizuya-Oka, A., Hasebe, T., Shi, Y.B., 2010. Apoptosis in amphibian organs during metamorphosis. Apoptosis 15, 350–364.
Ishizuya-Oka, A., Hasebe, T., Shimizu, K., Suzuki, K., Ueda, S., 2006. Shh/BMP-4 signaling pathway is essential for intestinal epithelial development during Xenopus larval-to-adult remodeling. Dev. Dyn. 235, 3240–3249.
Ishizuya-Oka, A., Kajita, M., Hasebe, T., 2014. Thyroid hormone-regulated Wnt5a/Ror2 signaling is essential for dedifferentiation of larval epithelial cells into adult stem cells in the Xenopus laevis intestine. PLoS One 9, e107611.
Ishizuya-Oka, A., Shimozawa, A., 1991. Induction of metamorphosis by thyroid hormone in anuran small intestine cultured organotypically in vitro. Vitro Cell Dev. Biol. 27A, 853–857.
Ishizuya-Oka, A., Shimozawa, A., 1992a. Connective tissue is involved in adult epithelial development of the small intestine during anuran metamorphosis in vitro. Roux's Arch. Dev. Biol. 201, 322–329.
Ishizuya-Oka, A., Shimozawa, A., 1992b. Programmed cell death and heterolysis of larval epithelial cells by macrophage-like cells in the anuran small intestine in vivo and in vitro. J. Morphol. 213, 185–195.
Ishizuya-Oka, A., Ueda, S., Inokuchi, T., Amano, T., Damjanovski, S., Stolow, M., Shi, Y.-B., 2001. Thyroid hormone-induced expression of Sonic hedgehog correlates with adult epithelial development during remodeling of the Xenopus stomach and intestine. Differentiation 69, 27–37.
Kress, E., Rezza, A., Nadjar, J., Samarut, J., Plateroti, M., 2009. The frizzled-related sFRP2 gene is a target of thyroid hormone receptor alpha1 and activates beta-catenin signaling in mouse intestine. J. Biol. Chem. 284, 1234–1241.
Laudet, V., Gronemeyer, H., 2002. The nuclear receptor FactsBook. Academic Press, San Diego.
Lazar, M.A., 1993. Thyroid hormone receptors: multiple forms, multiple possibilities. Endocr. Rev. 14, 184–193.
Lei, Y., Guo, X., Deng, Y., Chen, Y., Zhao, H., 2013. Generation of gene disruptions by transcription activator-like effector nucleases (TALENs) in Xenopus tropicalis embryos. Cell Biosci. 3, 21.
Lei, Y., Guo, X., Liu, Y., Cao, Y., Deng, Y., Chen, X., Cheng, C.H., Dawid, I.B., Chen, Y., Zhao, H., 2012. Efficient targeted gene disruption in Xenopus embryos using engineered transcription activator-like effector nucleases (TALENs). Proc. Natl. Acad. Sci. U.S.A. 109, 17484–17489.
Luu, N., Wen, L., Fu, L., Fujimoto, K., Shi, Y.B., Sun, G., 2013. Differential regulation of two histidine ammonia-lyase genes during Xenopus development implicates distinct functions during thyroid hormone-induced formation of adult stem cells. Cell Biosci. 3, 43.
MacDonald, W.C., Trier, J.S., Everett, N.B., 1964. Cell proliferation and migration in the stomach, duodenum, and rectum of man: Radioautographic studies. Gastroenterology 46, 405–417.
Mangelsdorf, D.J., Thummel, C., Beato, M., Herrlich, P., Schutz, G., Umesono, K., Blumberg, B., Kastner, P., Mark, M., Chambon, P., 1995. The nuclear receptor superfamily: the second decade. Cell 83, 835–839.
Mathew, S., Fu, L., Fiorentino, M., Matsuda, H., Das, B., Shi, Y.-B., 2009. Differential regulation of cell type specific apoptosis by stromelysin-3: a potential mechanism via the cleavage of the laminin receptor during tail resorption in Xenopus laevis. J. Biol. Chem. 284, 18545–18556.
Mathew, S., Fu, L., Hasebe, T., Ishizuya-Oka, A., Shi, Y.B., 2010. Tissue-dependent induction of apoptosis by matrix metalloproteinase stromelysin-3 during amphibian metamorphosis. Birth Defects Res. C Embryo Today 90, 55–66.
Matsuda, H., Paul, B.D., Choi, C.Y., 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.
Matsuda, H., Shi, Y.B., 2010. An essential and evolutionarily conserved role of protein arginine methyltransferase 1 for adult intestinal stem cells during postembryonic development. Stem Cells 28, 2073–2083.
Matsuura, K., Fujimoto, K., Das, B., Fu, L., Lu, C.D., Shi, Y.B., 2012a. Histone H3K79 methyltransferase Dot1L is directly activated by thyroid hormone receptor during Xenopus metamorphosis. Cell Biosci. 2, 25.
Matsuura, K., Fujimoto, K., Fu, L., Shi, Y.-B., 2012b. 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.
McAvoy, J.W., Dixon, K.E., 1977. Cell proliferation and renewal in the small intestinal epithelium of metamorphosing and adult Xenopus laevis. J. Exp. Zool. 202, 129–138.
Miller, T.C., Sun, G., Hasebe, T., Fu, L., Heimeier, R.A., Das, B., Ishizuya-Oka, A., Shi, Y.B., 2013. Tissue-specific upregulation of MDS/EVI gene transcripts in the intestine by thyroid hormone during Xenopus metamorphosis. PLoS One 8, e55585.
Moran, C., Chatterjee, K., 2015. Resistance to thyroid hormone due to defective thyroid receptor alpha. Best Pract. Res. Clin. Endocrinol. Metab. 29, 647–657.
Muncan, V., Heijmans, J., Krasinski, S.D., Buller, N.V., Wildenberg, M.E., Meisner, S., Radonjic, M., Stapleton, K.A., Lamers, W.H., Biemond, I., et al., 2011. Blimp1 regulates the transition of neonatal to adult intestinal epithelium. Nat. Commun. 2, 452.
Nakajima, K., Tazawa, I., Yaoita, Y., 2018. Thyroid hormone receptor alpha- and beta-knockout xenopus tropicalis tadpoles reveal subtype-specific roles during development. Endocrinology 159, 733–743.
Nakajima, K., Yaoita, Y., 2003. Dual mechanisms governing muscle cell death in tadpole tail during amphibian metamorphosis. Dev. Dyn. 227, 246–255.
Nguyen, A.T., Zhang, Y., 2011. The diverse functions of Dot1 and H3K79 methylation. Genes Dev. 25, 1345–1358.
Okada, M., Miller, T.C., Wen, L., Shi, Y.B., 2017. A balance of Mad and Myc expression dictates larval cell apoptosis and adult stem cell development during Xenopus intestinal metamorphosis. Cell Death Dis. 8, e2787.
Okada, M., Shi, Y.B., 2018. EVI and MDS/EVI are required for adult intestinal stem cell formation during postembryonic vertebrate development. FASEB J. 32, 431–439.
Okada, M., Wen, L., Miller, T.C., Su, D., Shi, Y.B., 2015. Molecular and cytological analyses reveal distinct transformations of intestinal epithelial cells during Xenopus metamorphosis. Cell Biosci. 5, 74.
Paul, B.D., Buchholz, D.R., Fu, L., Shi, Y.-B., 2005a. Tissue- and gene-specific recruitment of steroid receptor coactivator-3 by thyroid hormone receptor during development. J. Biol. Chem. 280, 27165–27172.
Paul, B.D., Buchholz, D.R., Fu, L., Shi, Y.-B., 2007. SRC-p300 coactivator complex is required for thyroid hormone induced amphibian metamorphosis. J. Biol. Chem. 282, 7472–7481.
Paul, B.D., Fu, L., Buchholz, D.R., Shi, Y.-B., 2005b. Coactivator recruitment is essential for liganded thyroid hormone receptor to initiate amphibian metamorphosis. Mol. Cell. Biol. 25, 5712–5724.
Plateroti, M., Chassande, O., Fraichard, A., Gauthier, K., Freund, J.N., Samarut, J., Kedinger, M., 1999. Involvement of T3Ralpha- and beta-receptor subtypes in mediation of T3 functions during postnatal murine intestinal development. Gastroenterology 116, 1367–1378.
Plateroti, M., Gauthier, K., Domon-Dell, C., Freund, J.N., Samarut, J., Chassande, O., 2001. Functional interference between thyroid hormone receptor alpha (TRalpha) and natural truncated TRDeltaalpha isoforms in the control of intestine development. Mol. Cell. Biol. 21, 4761–4772.
Plateroti, M., Kress, E., Mori, J.I., Samarut, J., 2006. Thyroid hormone receptor alpha1 directly controls transcription of the beta-catenin gene in intestinal epithelial cells. Mol. Cell. Biol. 26, 3204–3214.
Sachs, L.M., 2015. Unliganded thyroid hormone receptor function: amphibian metamorphosis got TALENs. Endocrinology 156, 409–410.
Sachs, L.M., Damjanovski, S., Jones, P.L., Li, Q., Amano, T., Ueda, S., Shi, Y.B., Ishizuya-

Oka, A., 2000. Dual functions of thyroid hormone receptors during Xenopus development. Comp. Biochem. Physiol. B: Biochem. Mol. Biol. 126, 199–211.
Sachs, L.M., Jones, P.L., Havis, E., Rouse, N., Demeneix, B.A., 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.
Sachs, L.M., Shi, Y.-B., 2000. Targeted chromatin binding and histone acetylation in vivo by thyroid hormone receptor during amphibian development. PNAS 97, 13138–13143.
Sakane, Y., Iida, M., Hasebe, T., Fujii, S., Buchholz, D.R., Ishizuya-Oka, A., Yamamoto, T., Suzuki, K.T., 2018. Functional analysis of thyroid hormone receptor beta in Xenopus tropicalis founders using CRISPR-Cas. Biol. Open 7.
Schreiber, A.M., Cai, L., Brown, D.D., 2005. Remodeling of the intestine during metamorphosis of Xenopus laevis. Proc. Natl. Acad. Sci. U.S.A. 102, 3720–3725.
Schreiber, A.M., Das, B., Huang, H., Marsh-Armstrong, N., Brown, D.D., 2001. Diverse developmental programs of Xenopus laevis metamorphosis are inhibited by a dominant negative thyroid hormone receptor. PNAS 98, 10739–10744.
Schreiber, A.M., Mukhi, S., Brown, D.D., 2009. Cell-cell interactions during remodeling of the intestine at metamorphosis in Xenopus laevis. Dev. Biol. 331, 89–98.
Shi, Y.-B., 1994. Molecular biology of amphibian metamorphosis: a new approach to an old problem. Trends Endocrinol. Metab. 5, 14–20.
Shi, Y.-B., 2009. Dual functions of thyroid hormone receptors in vertebrate development: the roles of histone-modifying cofactor complexes. Thyroid 19, 987–999.
Shi, Y.-B., Brown, D.D., 1993. The earliest changes in gene expression in tadpole intestine induced by thyroid hormone. J. Biol. Chem. 268, 20312–20317.
Shi, Y.-B., Ishizuya-Oka, A., 1996. Biphasic intestinal development in amphibians: embryogensis and remodeling during metamorphosis. Curr. Top. Develop. Biol. 32, 205–235.
Shi, Y.B., Hasebe, T., Fu, L., Fujimoto, K., Ishizuya-Oka, A., 2011. The development of the adult intestinal stem cells: insights from studies on thyroid hormone-dependent amphibian metamorphosis. Cell Biosci. 1, 30.
Shi, Y.B., Matsuura, K., Fujimoto, K., Wen, L., Fu, L., 2012. Thyroid hormone receptor actions on transcription in amphibia: the roles of histone modification and chromatin disruption. Cell Biosci. 2, 42.
Sterling, J., Fu, L., Matsuura, K., Shi, Y.-B., 2012. Cytological and morphological analyses reveal distinct features of intestinal development during Xenopus tropicalis metamorphosis. PLoS One 7, e47407.
Stolow, M.A., Shi, Y.B., 1995. Xenopus sonic hedgehog as a potential morphogen during embryogenesis and thyroid hormone-dependent metamorphosis. Nucl. Acids Res. 23, 2555–2562.
Su, Y., Shi, Y., Shi, Y.-B., 1997a. Cyclosporin A but not FK506 inhibits thyroid hormone-induced apoptosis in xenopus tadpole intestinal epithelium. FASEB J. 11, 559–565.
Su, Y., Shi, Y., Stolow, M., Shi, Y.-B., 1997b. Thyroid hormone induces apoptosis in primary cell cultures of tadpole intestine: cell type specificity and effects of extracellular matrix. J. Cell Biol. 139, 1533–1543.
Sun, G., Heimeier, R.A., Fu, L., Hasebe, T., Das, B., Ishizuya-Oka, A., Shi, Y.-B., 2013. Expression profiling of intestinal tissues implicates tissue-specific genes and pathways essential for thyroid hormone-induced adult stem cell development. Endocrinology 154, 4396–4407.
Sun, G., Roediger, J., Shi, Y.B., 2016. Thyroid hormone regulation of adult intestinal stem cells: implications on intestinal development and homeostasis. Rev. Endocr. Metab. Disord. 17, 559–569.
Sun, G., Shi, Y.-B., 2012. Thyroid hormone regulation of adult intestinal stem cell development: mechanisms and evolutionary conservations. Int. J. Biol. Sci. 8, 1217–1224.
Tomita, A., Buchholz, D.R., 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.
Toner, P.G., Carr, K.E., Wyburn, G.M., 1971. The Digestive System: An Ultrastructural Atlas and Review. Butterworth, London.
Tsai, M.J., O'Malley, B.W., 1994. Molecular mechanisms of action of steroid/thyroid receptor superfamily members. Ann. Rev. Biochem. 63, 451–486.
van der Flier, L.G., Clevers, H., 2009. Stem cells, self-renewal, and differentiation in the intestinal epithelium. Annu. Rev. Physiol. 71, 241–260.
van Mullem, A., van Heerebeek, R., Chrysis, D., Visser, E., Medici, M., Andrikoula, M., Tsatsoulis, A., Peeters, R., Visser, T.J., 2012. Clinical phenotype and mutant TRalpha1. N. Engl. J. Med. 366, 1451–1453.
Wen, L., Fu, L., Guo, X., Chen, Y., Shi, Y.B., 2015a. Histone methyltransferase Dot1L plays a role in postembryonic development in Xenopus tropicalis. FASEB J. 29, 385–393.
Wen, L., Fu, L., Shi, Y.B., 2017a. Histone methyltransferase Dot1L is a coactivator for thyroid hormone receptor during Xenopus development. FASEB J. 31, 4821–4831.
Wen, L., Hasebe, T., Miller, T.C., Ishizuya-Oka, A., Shi, Y.B., 2015b. A requirement for hedgehog signaling in thyroid hormone-induced postembryonic intestinal remodeling. Cell Biosci. 5, 13.
Wen, L., Shi, Y.B., 2015. Unliganded thyroid hormone receptor alpha controls developmental timing in Xenopus tropicalis. Endocrinology 156, 721–734.
Wen, L., Shi, Y.B., 2016. Regulation of growth rate and developmental timing by Xenopus thyroid hormone receptor alpha. Dev. Growth Differ. 58, 106–115.
Wen, L., Shibata, Y., Su, D., Fu, L., Luu, N., Shi, Y.-B., 2017b. Thyroid hormone receptor α controls developmental timing and regulates the rate and coordination of tissue specific metamorphosis in Xenopus tropicalis. Endocrinology 158, 1985–1998.
Wong, J., Patterton, D., Imhof, D., Guschin, D., Shi, Y.-B., Wolffe, A.P., 1998. Distinct requirements for chromatin assembly in transcriptional repression by thyroid hormone receptor and histone deacetylase. EMBO J. 17, 520–534.
Wong, J., Shi, Y.-B., 1995. Coordinated regulation of and transcriptional activation by Xenopus thyroid hormone and retinoid X receptors. J. Biol. Chem. 270, 18479–18483.
Wong, J., Shi, Y.-B., Wolffe, A.P., 1997. Determinants of chromatin disruption and transcriptional regulation instigated by the thyroid hormone receptor: hormone-regulated chromatin disruption is not sufficient for transcriptinal activation. EMBO J. 16, 3158–3171.
Wong, J., Shi, Y.B., Wolffe, A.P., 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.
Yakut, M., Üstün, Y., Kabacan, G., Soykan, I., 2011. Thyroid disorders in patients with inflammatory bowel diseases. Int. J. Clin. Med. 2.
Yen, P.M., 2001. Physiological and molecular basis of thyroid hormone action. Physiol. Rev. 81, 1097–1142.
Yen, P.M., 2015. Unliganded TRs regulate growth and developmental timing during early embryogenesis: evidence for a dual function mechanism of TR action. Cell Biosci. 5, 8.
Young, J.J., Cherone, J.M., Doyon, Y., Ankoudinova, I., Faraji, F.M., Lee, A.H., Ngo, C., Guschin, D.Y., Paschon, D.E., Miller, J.C., et al., 2011. Efficient targeted gene disruption in the soma and germ line of the frog Xenopus tropicalis using engineered zinc-finger nucleases. Proc. Natl. Acad. Sci. U.S.A. 108, 7052–7057.