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

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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.

Keywords

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

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(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 play 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 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.

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).

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; 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.

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 cMyc7 in the developing stem cells and cMyc in turns activate 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. 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).

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.

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Highlights:

Thyroid hormone induces adult intestinal stem cell formation via dedifferentiation of larval epithelial cells.

Unliganded thyroid hormone receptor recruits histone deacetylase-containing complexes to target genes to establish a repressive state.

Liganded thyroid hormone receptor causes the loss of nucleosomes at target genes in tadpole.

Liganded thyroid hormone receptor recruits histone acetyltransferase and methyltransferase-containing complexes to target genes to add activation histone marks.

Thyroid hormone upregulates the expression of two histone methyltransferases, which in turn feedback positively to enhance thyroid hormone receptor function via histone methylation.
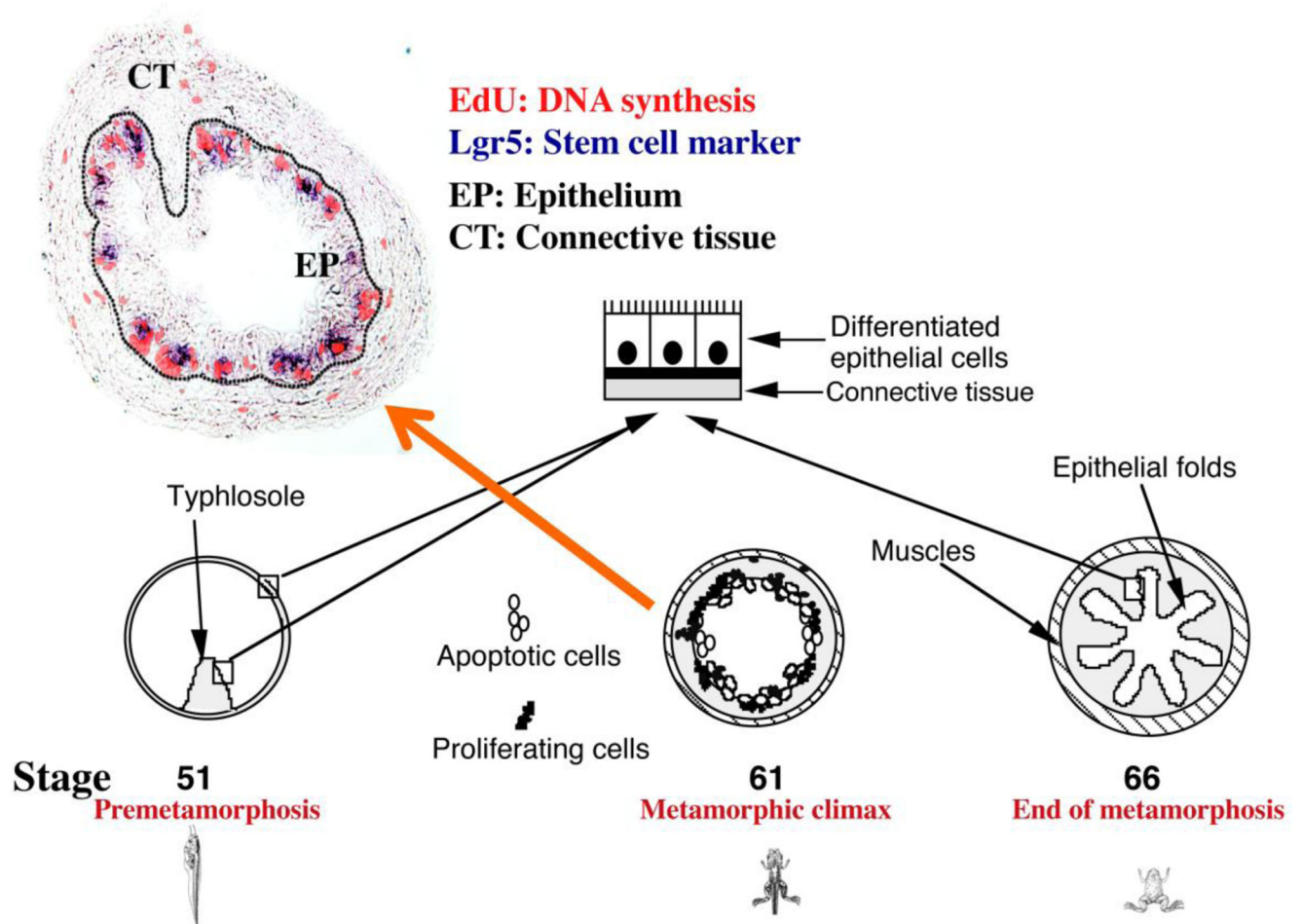
Fig. 1.

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.
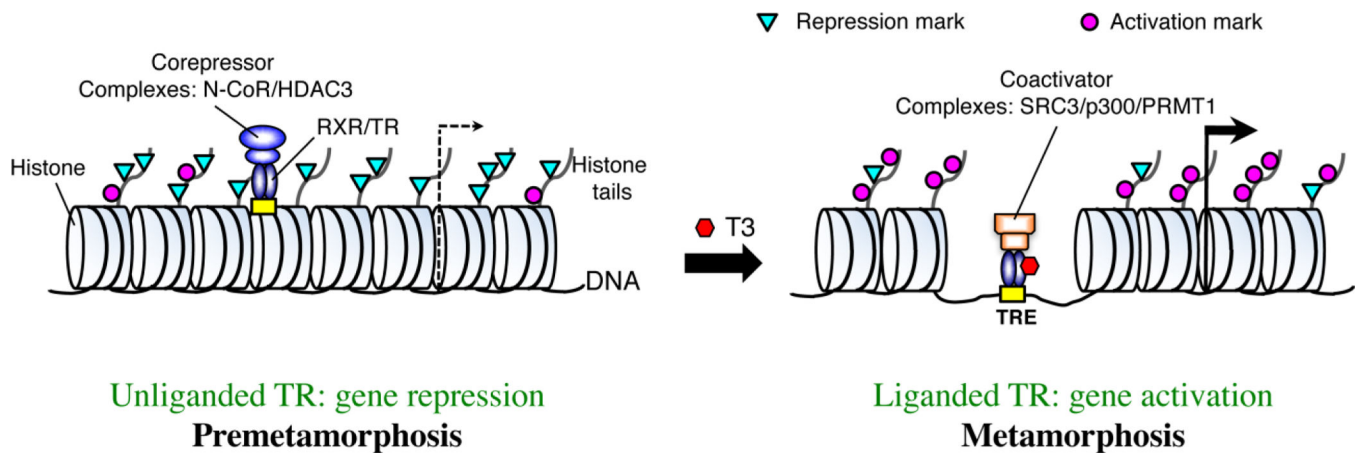
Fig. 2. Regulation of T3-inducible genes by TR during *Xenopus* development.
In premetamorphic tadpoles, there is little T3 and TR is unliganded. The unliganded TR/RXR heterodimer binds to TREs in the target genes and recruits corepressor complexes such as the N-CoR-HDAC3 complex, resulting in the reduction 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.
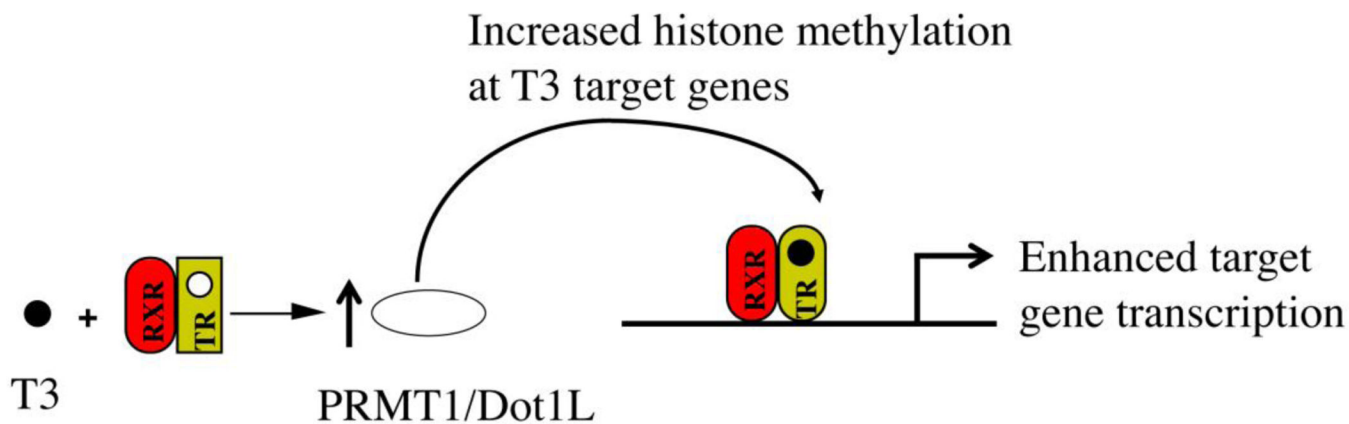
Fig. 3. A positive feedback mechanism to enhance T3 activation of gene transcription through histone methylation.

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).