

Invited Review

Molecular and cellular basis of tissue remodeling during amphibian metamorphosis

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Summary. Amphibian metamorphosis involves systematic transformations of various tadpole organs/tissues. Three major types of changes take place during this process. These are remodeling, resorption, and de novo development, all of which appear to involve both cell proliferation and apoptosis (programmed cell death). All metamorphic changes are controlled by thyroid hormone (T_3) and are organ-autonomous. Recent studies using primary cell cultures and a stably transformed cell line from tadpole tissues have implicated that T_3 induces apoptosis cell-autonomously. This T_3 -induced, metamorphosis-associated apoptosis is similar to cell death in other animal species and involves similar cell death executioners. Both the activation of these executioners and the pathways leading to cell proliferation and differentiation are believed to be through transcriptional regulation by T_3 receptors (TRs). TRs can activate or repress target gene transcription depending upon the presence or absence of T_3 , respectively. Many direct T_3 -response genes have been isolated and found to encode a variety of proteins that can affect both intra- and extra-cellular events. The determinations of the identities of these response genes through sequence analyses and studies on their expression profiles during development have provided strong clues toward their roles in metamorphosis. However, future studies using organ and cell culture systems and/or transient or stable transgenic technologies are required to understand how these genes transduce the T_3 signal to activate the downstream cell death and proliferation/differentiation pathways.

Key words: Apoptosis, Thyroid hormone, Amphibian metamorphosis, Transcriptional regulation, Development

Introduction

Amphibian metamorphosis offers an excellent opportunity to study the molecular basis of organogenesis and tissue remodeling during postembryonic vertebrate development (Gilbert and Frieden, 1981; Tata, 1993; Gilbert et al., 1996). This process involves systematic transformations of different organs/tissues of a tadpole to prepare the animal for adult living as a frog (Dodd and Dodd, 1976). Often, the animal changes from being herbivorous and aquatic to being carnivorous and terrestrial. Despite the extensive and dramatic changes involved, amphibian metamorphosis is largely under the control of a single hormone, the thyroid hormone (Dodd and Dodd, 1976; Galton, 1983; Kikuyama et al., 1993).

As early in 1912 (Gudernatsch, 1912), it was discovered that a substance(s) in the thyroid gland could induce precocious metamorphosis when added to rearing water of premetamorphic tadpoles. Conversely, removing the thyroid of the tadpole blocked the transformation (Dodd and Dodd, 1976). Later, the active substance was found to be thyroid hormone (Dodd and Dodd, 1976; Gilbert and Frieden, 1981). Either one of the two natural occurring thyroid hormones, T_4 (thyroxine or 3,3',5,5'-tetraiodothyronine) and T_3 (3,3',5-triiodothyronine) are effective in inducing precocious metamorphosis, although it is very likely that T_4 needs to be converted to T_3 by 5'-deiodinases present in most, if not all tissues (St Germain, 1994; St Germain and Galton, 1997). The discovery of the controlling agent of this developmental process has triggered extensive morphological, biochemical, and molecular studies in the past several decades. This makes it difficult to provide a comprehensive review on the subject. In this article, we will focus mostly on the more recent molecular investigations on *Xenopus laevis*.

Morphological changes

There are three major types of changes taking place during metamorphosis (Dodd and Dodd, 1976). The first

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one is the complete resorption of tadpole specific organs such as the tail (Fig. 1) and gill. On the other extreme end, frog specific organs such as the limb develop de novo from undifferentiated blastema cells (Fig. 1). Limb development involves first the proliferation of undifferentiated blastema cells. Subsequently, morphogenesis takes place to form the digits when these cells undergo differentiation. Finally the limb increases in size without major morphological changes as the tadpole gradually complete metamorphosis.

Most of the organs undergo the third type of change, partial but drastic remodeling of the existing organs into their adult forms. Perhaps the two best studied organs in this class are the liver and intestine. Although no major morphological changes take place in the liver, the organ alters dramatically its gene expression program (Dodd and Dodd, 1976; Atkinson et al., 1994). Most noticeably is the activation of the genes encoding urea cycle enzymes to prepare to animal from being ammonium-excreting to urea-excreting.

The change in liver gene expression appears to be through reprogramming of the existing hepatocytes in the liver (Atkinson et al., 1994). On the other hand, intestinal remodeling involves the complete degeneration of larval epithelial cells (Fig. 1; Yoshizato, 1989; Shi and Ishizuya-Oka, 1996). During metamorphosis, the larval epithelium undergoes complete degeneration. Concurrently, adult epithelial cells, whose origin is yet unknown, rapidly proliferate. Toward the end of metamorphosis, the adult epithelial cells differentiate to form a multiply folded epithelium, which

is underlined by a well-developed connective tissue. Such an organization resembles that in adult mammalian and avian intestine (Glass, 1968; Segal and Petras, 1992; Shi and Ishizuya-Oka, 1996). The fully differentiated adult epithelial cells are localized at the crests of the epithelial folds. In contrasts, the less differentiated or stem cells of the epithelium lie at the troughs of the folds. As the daughter cells of these stem cells differentiate, they gradually migrate toward the crest. After a finite period of time, the fully differentiated epithelial cells at the crest are sloughed off into the lumen and are replaced by the newly arrived differentiated epithelial cells. Such a self renewal process is similar to that in higher vertebrates (Glass, 1968; Segal and Petras, 1992).

T₃-induced apoptosis during tissue remodeling

Selective removal of specific cells plays important roles in many developmental and pathological process. However, due to the short-lived nature of such cells, it had been difficult to study them. On the other hand, the resorption of the tadpole tail involves complete removal of all cell types within a short developmental period. This has served as an excellent model system to study cell death. In fact, Kerr et al. (1974) reported more than 25 years ago that cell death occurs with distinct morphological changes, including membrane blebbing, chromatin condensation, cytoplasmic and nuclear fragmentation to form membrane-enclosed vesicles containing chromatin fragments. Such cellular

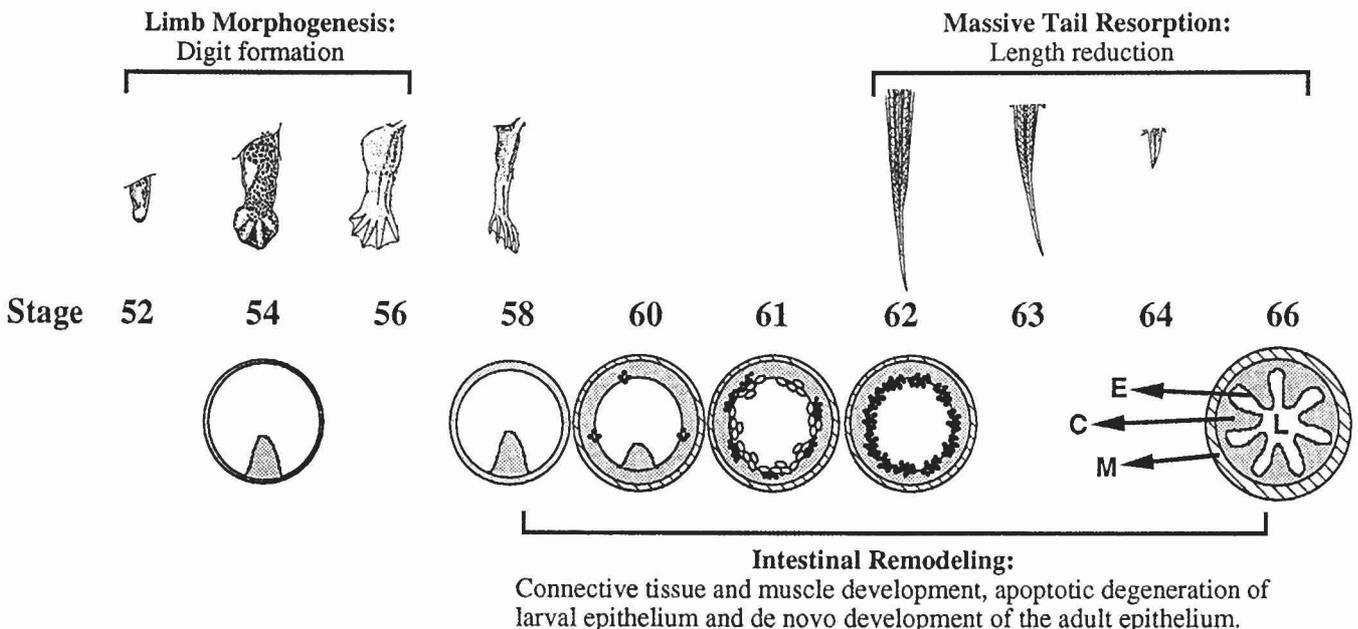


Fig. 1. Three major types of organ transformations during amphibian metamorphosis: de novo development (e.g., hindlimb), total resorption (e.g., tail), and remodeling (e.g., intestine). The staging refers to *Xenopus laevis* (Nieuwkoop and Faber, 1956). The tails are drawn to the same scale to show the resorption while the hindlimbs and intestinal cross-sections are not. Tadpole intestine has a single fold, where connective tissue (C) is abundant. The frog has a multiply folded epithelium (E). M: muscles; L: Lumen; Open circles: apoptotic epithelial cells; filled circles: proliferating adult epithelial cells.

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morphological changes led to the name “apoptosis” to describe programmed cell death; and the vesicles containing chromatin fragments are referred as “apoptotic bodies” (Kerr et al., 1972).

Apoptosis is not unique to the resorbing organs like the tail. Similar microscopic examination of the remodeling intestine during *Xenopus laevis* metamorphosis have revealed that the degeneration of larval

epithelial cells also occur through apoptosis (Fig. 2) and the resulting apoptotic bodies are removed at least in part through phagocytosis by the macrophages from the underlying connective tissue (Ishizuya-Oka and Shimozaawa, 1992a). While the mechanism which triggers these macrophages to migrate across the basal lamina into the degenerating larval epithelium is unclear, these macrophages engulfing the apoptotic bodies appear to be excreted into the intestinal lumen.

Various biochemical and cytological methods have been developed to detect apoptosis. One of them, the TUNEL assay, labels in situ the DNA ends generated during chromatin fragmentation. Using this method, Ishizuya-Oka and Ueda (1996) reported that DNA fragmentation can be detected even before cellular fragmentation and that apoptosis occurs throughout the larval intestinal epithelium during *Xenopus laevis* metamorphosis. However, little apoptotic cells are present in the other intestinal tissues of tadpoles or frogs. In frogs, cell death is limited to the fully differentiated epithelial cells located at the crest of an intestinal fold. These observations are in full agreement with early microscopic studies and analyses of DNA synthesis in intestinal cells of tadpoles and frogs (McAvoy and Dixon, 1977; Ishizuya-Oka and Shimozaawa, 1992a).

The apoptosis observed during metamorphosis is thyroid hormone-dependent. In fact, it can be induced even when individual organs such as the tail and intestine from premetamorphic tadpoles are cultured in vitro in the presence of physiological levels of T_3 (Tata et al., 1991; Ishizuya-Oka and Shimozaawa, 1992a). This in vitro T_3 -induced apoptosis accompanies specific tissue transformations, i.e., the resorption of the tail and remodeling of the intestine, that closely reflect the in vivo events.

By culturing primary cells from the tadpole tail, Yoshizato and colleagues showed that cells of the tadpole tail could be induced to die when treated with T_3 (Izutsu and Yoshizato, 1993). Interestingly, by co-culturing different cell types, they obtained evidence to suggest the involvement of non-T lymphocytes in triggering skin cell death in vitro (Izutsu et al., 1996). On the other hand, by establishing a stable cell line from tadpole tail muscles, Yaoita and Nakajima (1997) showed that the tadpole tail muscle cells could respond to T_3 directly to undergo apoptosis. Thus, at least in some circumstances, metamorphic apoptosis is cell autonomous.

Our own studies on intestinal cells also support a cell autonomous response to T_3 . We have isolated partially purified intestinal epithelial cells and fibroblasts from tadpoles of *Xenopus laevis* and cultured them in vitro (Fig. 3, Su et al., 1997a,b). Both cell types can proliferate in vitro but with limited life spans, especially for the epithelial cells. When the cells are treated with T_3 , both the epithelial and fibroblastic cells increase their DNA synthesis, suggesting that T_3 can stimulate the proliferation of both cell types. However, the epithelial cells are induced to undergo rapid apoptosis by

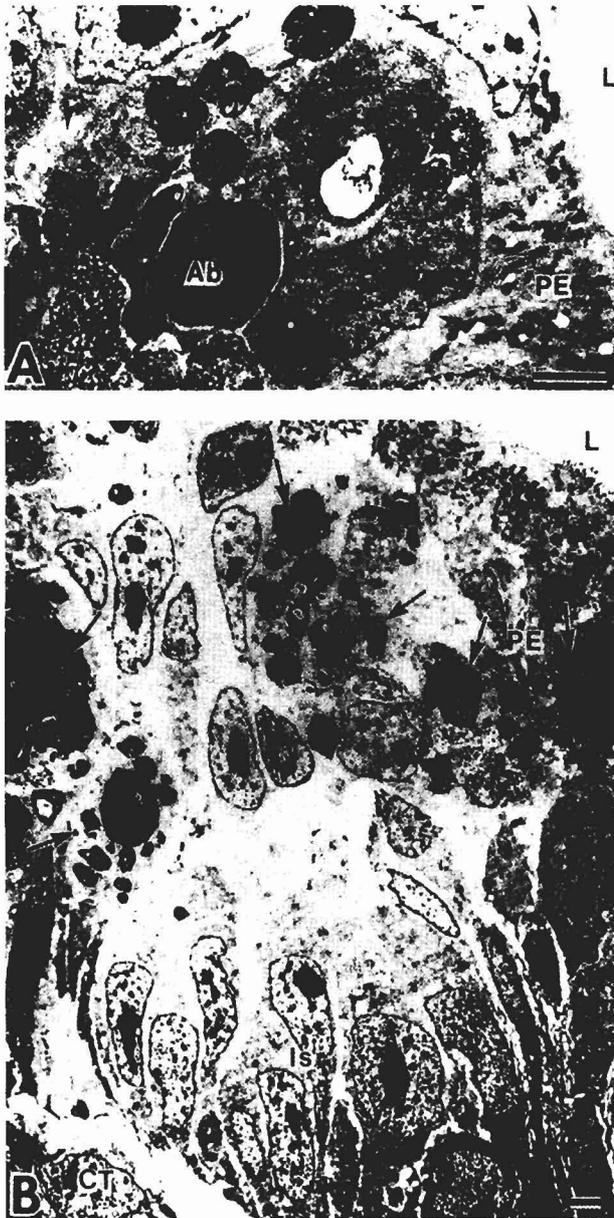


Fig. 2. Degeneration of the primary epithelium (PE) of the *Xenopus* intestine occurs through apoptosis. **A.** An intra epithelial macrophage (arrowheads) including an apoptotic body. **B.** Many macrophages (arrow) are localized in the primary epithelium, but not in the proliferating islet (IS) of adult epithelial cells. CT: connective tissue; L: Lumen. Scale bar: 2 μ m. Adapted from Shi and Ishizuya-Oka (1996).

T_3 while the fibroblasts are refractory to this T_3 -induced cell death. Thus, T_3 -treatment of primary cells in culture appears to accurately reproduce the in vivo behaviors of these cells during metamorphosis.

The signal transduction mechanisms leading to apoptosis are likely distinct for different cell types. Few details are known about such mechanisms for the vast majority of the apoptotic systems. On the other hand, extensive studies with various model systems have firmly established the involvement of ICE-like enzymes or caspases as one of the late executioner of apoptosis and members of the bcl-2 superfamily as regulators of apoptosis (White, 1996; Wang and Reed, 1998). Some of the bcl-2 family members like bax are cell death promoters while others like bcl-2 itself have anti-apoptotic activities. A critical balance of the two types of bcl-2 members appears to be important for cell fate determination. Although their exact mechanisms of action are still unknown, they may function as ion channels or adaptors (or docking proteins) to regulate cell death (Vaux, 1997; Wang and Reed, 1998). Two bcl-2 family members have been cloned in *Xenopus laevis*, and are expressed in tadpole tissues (Cruz-Reyes and Tata, 1995). However, their expression levels do not change during metamorphosis. On the other hand, studies on *Xenopus* tadpole tail muscle degeneration have implicated the involvement of bax in muscle cell death (Sachs et al., 1997). Clearly, further studies on these and other possible bcl-2 members are required to define the role of bcl-2 family members during metamorphosis.

Strong evidence, however, exists for the involvement of caspases in T_3 -induced apoptosis during amphibian metamorphosis. First, the *Xenopus laevis* homolog of caspase-3 or CPP32 has been cloned and found to be up-regulated during both natural and T_3 -induced tail resorption (Yaoita and Nakajima, 1997).

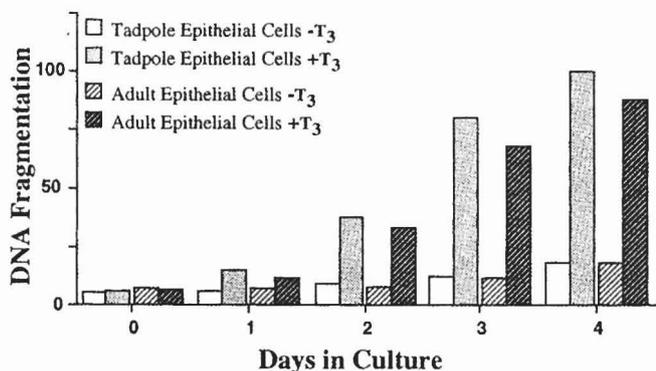


Fig. 3. Both primary and adult type epithelial cells undergo apoptosis when treated with T_3 in vitro. Epithelial cells were isolated from stage 57 tadpoles (tadpole epithelial cells) or stage 64 tadpoles (adult type epithelial cells) and cultured in vitro in the presence or absence of 100 nM T_3 . Apoptosis was measured by using a DNA fragmentation ELISA assay (higher levels of DNA fragmentation indicate higher levels of cell death). Based on the data of Su et al. (1997b).

Second, inhibitors of caspases can block the T_3 -induced apoptosis of in vitro cultured larval intestinal epithelial cells or the cell line derived from the tadpole tail muscles (Yaoita and Nakajima, 1997; Su et al., 1997b). Thus, the T_3 -induced cell death employs similar apoptotic execution mechanisms as cell death in other animal species, a conclusion also supported by the ability of a known apoptosis-inhibiting nuclease inhibitor to block T_3 -induced intestinal epithelial cell DNA fragmentation in vitro (Su et al., 1997b).

While the intestinal epithelial cells can respond to T_3 cell-autonomously in vitro, the T_3 -induced apoptosis can be influenced by other factors. In particular, cell-cell and cell-ECM interactions have been implicated by earlier studies to be important for at least adult epithelial development (Ishizuya-Oka and Shimozaawa, 1992b). Interestingly, when intestinal epithelial cells from *Xenopus laevis* tadpoles at stage 64 are cultured in the presence of T_3 in vitro, they too undergo T_3 -dependent apoptosis (Fig. 3. Su et al., 1997b). The intestinal epithelial cells at stage 64 are of adult type and are differentiated or differentiating. In vivo, they do not undergo apoptosis in spite of the presence of T_3 in the metamorphosing tadpole. The contrasting response of these cells to T_3 in vitro suggests that dissociating the cells from the underlying basal lamina might have altered their properties. Consistently, when tadpole epithelial cells are cultured on plastic dishes coated with various ECM components, they are more resistant to T_3 -induced apoptosis. Although no differences are observed among different ECM components, the results support a role of the ECM in intestinal epithelial transformation (Su et al., 1997b).

In addition to ECM, other factors also appear to influence epithelial cell behavior. Organ culture studies on intestinal remodeling have shown that the development of adult tissues can be induced by treating intestinal fragments but not the epithelium alone with T_3 (Ishizuya-Oka and Shimozaawa, 1992b). T_3 -treatment of the dissociated epithelium alone leads to only apoptosis. Interestingly, when epithelium is cocultured with the connective tissue, adult epithelium is produced upon prolonged T_3 -treatment. Furthermore, this effect of the connective tissue is preserved even if the connective tissue is separated from the epithelium by a permeable membrane. Thus, a diffusible factor(s) produced by the connective tissue is required for adult epithelial development while larval epithelial cell death is a cell-autonomous event, even though extracellular factors such as the ECM can regulate this apoptotic process.

Molecular mechanism of T_3 action during metamorphosis

Thyroid hormone regulates gene expression through its nuclear receptors or thyroid hormone receptors (TRs). TRs belong to the growing family of nuclear hormone receptors, which contains at least over 150 members, and

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includes the receptors for glucocorticoid, estrogen, retinoic acid, and vitamin D, etc. (Mangelsdorf et al., 1995). In general, each receptor can be divided into 5 domains, A/B, C, D, E, and F, respectively, from the amino- to carboxyl-end. The DNA binding domain (C) is located in the amino terminal half and the large ligand binding domain (E) is located in the carboxyl terminal half of the protein. The rest of the domains are involved in various functions such as DNA and hormone binding, dimerization, and transcriptional activation.

Extensive evidence have strongly implicated that TRs functions as heterodimers formed with RXRs (9-cis retinoic acid receptors) (Tsai and O'Malley, 1994; Yen and Chin, 1994; Shi et al., 1996a). In the presence of T_3 , TR/RXR heterodimers can activate the transcription of their target genes. However, they function as transcriptional repressors when the ligand is absent. Many TR-interacting cofactors have been isolated and implicated in the transcriptional activation or repression (Halachmi et al., 1994; Baniahmand et al., 1995; Burris et al., 1995; Chen and Evans, 1995; Horlein et al., 1995; Lee et al., 1995a,b; Onate et al., 1995; Chakravarti et al., 1996; Kamei et al., 1996; Zamir et al., 1996). How these cofactors participate in T_3 -dependent transcriptional regulation remains unknown. However, transcriptional activation by T_3 -bound TR/RXR is accompanied by extensive chromatin remodeling (Wong et al., 1995, 1997) and some of the coactivators have histone acetylase activity (e.g. CBP/p300 and SRC-1, Ogryzko et al., 1996; Spencer et al., 1997) while the corepressors (e.g. SMRT, Nagy et al., 1997) have the ability to recruit histone deacetylases. Thus, histone modification and other chromatin remodeling may be among the mechanisms by which these cofactors influence T_3 -dependent transcriptional regulation.

Both TR and RXR genes are expressed during amphibian metamorphosis (Yaoita and Brown, 1990; Kawahara et al., 1991; Schneider and Galton, 1991;

Helbing et al., 1992; Wang and Brown, 1993; Davey et al., 1994; Eliceiri and Brown, 1994; Shi et al., 1994; Wong and Shi, 1995; Fairclough and Tata, 1997; Shi and Ishizuya-Oka, 1997). In fact their expression levels in different organs correlate with organ specific transformations during metamorphosis and at least in *Xenopus laevis*, the TR genes are up-regulated by T_3 treatment of premetamorphic tadpoles (Yaoita and Brown, 1990; Kawahara et al., 1991). In particular, the *Xenopus* TR β genes have been shown to be directly regulated at the transcriptional level by T_3 through at least one thyroid hormone response element (TRE) in their promoters (Ranjan et al., 1994; Machuca et al., 1995; Wong et al., 1995). Thus, TRs auto-regulate their own expression to facilitate the drastic metamorphic changes needed within a short developmental period.

The presence of TRs and RXRs in metamorphosing tissues suggest that they mediate the regulatory effects of T_3 by regulating the transcription of various target genes, i.e., the so-called direct T_3 -response genes. The products of these genes in turn affect either directly, i.e., as transcription factors, or indirectly, the downstream T_3 -response genes, which then influence the expression of genes further downstream. Such a gene regulation cascade eventually lead to tissue specific metamorphosis (Fig. 4).

The key to understand the T_3 -dependent metamorphosis is, therefore, to identify and functionally characterize the T_3 -response genes in the cascade. Of particular interests are the direct T_3 -response genes as they are likely the ones that initiate, together with other existing factors, the tissue or cell specific responses to T_3 . To systematically isolate these genes, Wang and Brown (1991) developed a PCR-based subtractive differential screening method. This allowed them to isolate many genes which are either up or down-regulated in the tail within 24 hr of the T_3 -treatment of premetamorphic *Xenopus* tadpoles. Most of the genes

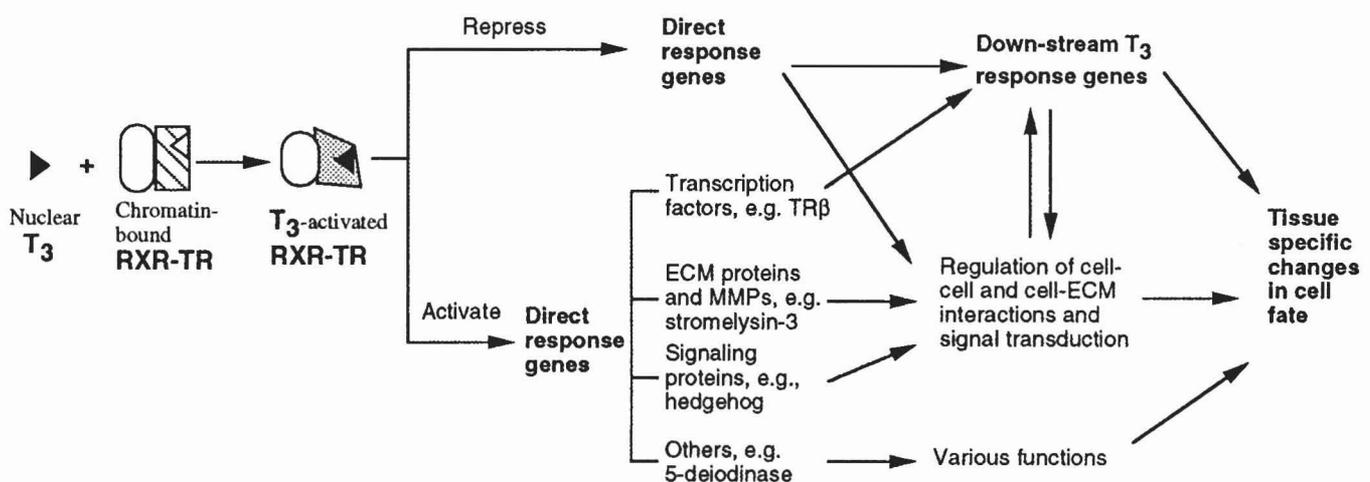


Fig. 4. A gene regulation cascade model for T_3 -regulation of tissue remodeling. The binding of T_3 to TR/RXR heterodimer is presumed to induce a conformational change(s) (Tsai and O'Malley, 1994; Shi et al., 1996a) that leads to the activation of the receptor complex.

were found to be regulated by T_3 directly as their regulation is independent of new protein synthesis (Wang and Brown, 1991, 1993).

Such a subtractive screen has since been applied to several different organs/tissues of *Xenopus laevis* tadpoles, including the limb (Buckbinder and Brown, 1992), the intestine (Shi and Brown, 1993); and the brain (Denver et al., 1997). In general, the expression profiles of the isolate genes during both natural and T_3 -induced metamorphosis are consistent with their potential roles during organ transformation. Cloning and sequence analysis of the full length cDNAs have revealed that the early T_3 -response genes (regulated by T_3 within 24 hr belong to several different groups (Fig. 4.; Brown et al., 1996; Shi, 1996; Denver et al., 1997). The first group includes genes encoding transcription factors, such as TR β , NF- κ B, and a leucine-zipper containing factor (TH-bZip), etc. These genes are expected to directly regulate the expression of downstream genes, thus influencing cell behavior during metamorphosis. For example, detailed in situ analyses of the *Xenopus* TR β (Shi and Ishizuya-Oka, 1997) and TH-bZip (Ishizuya-Oka et al., 1997) gene expression during intestinal remodeling suggest that high levels of these proteins lead to apoptosis of the larval epithelium but proliferation of the stem cells of the adult epithelium, connective tissue, and muscles. However, upon the differentiation of these adult type cells, the expression of both TR β and TH-bZip is downregulated in these cells, implicating that high levels of these proteins are incompatible with differentiated phenotypes. The understanding of exactly how the TR β and TH-bZip or other TH-regulated transcription factors influence cell transformations during metamorphosis will require the identification of their own target genes in different metamorphosing tissues.

The second group contains genes encoding ECM components and matrix metalloproteinases (MMPs), which degrade various components of the ECM. ECM remodeling and degradation have long been observed during metamorphosis (Dodd and Dodd, 1976; Shi and Ishizuya-Oka, 1996). For example, the basal lamina separating the intestinal epithelium and the underlying connective tissue is known to be thicker but more permeable during metamorphosis (Shi and Ishizuya-Oka, 1996); and collagenases, which degrade collagen in the ECM, were first discovered in the resorbing tadpole tail (Gross, 1966). It is very likely that the TH-regulated MMP genes and ECM component-encoding genes are involved in the ECM-remodeling and degradation which in turn influence cell behavior (Fig. 4). In fact, expression analyses have implicated the possible involvement of the MMP stromelysin-3 in apoptosis during metamorphosis while some other MMPs in post-apoptosis ECM degradation (Patterton et al., 1995; Ishizuya-Oka et al., 1996; Stolow et al., 1996).

The third class of genes encode protein which participate in signal transduction processes involved in cell-cell and cell-ECM interactions (Fig. 4). These proteins include the putative morphogen hedgehog, an

ECM receptor integrin, and a transmembrane protein of yet unknown function (Stolow and Shi, 1995; Brown et al., 1996; Shi, 1996; Liang et al., 1997). Relatively little is known about the spatial and temporal expression of these genes and their roles in metamorphosis remain to be defined.

The rest of the genes regulated by T_3 within the first 24 hr of treatment of premetamorphic tadpoles include genes encoding various other enzymes and cell specific proteins. Their functions during metamorphosis are likely to vary depending on the particular genes and/or the cell types in which they are expressed. Of particular interest is the gene encoding 5-deiodinase (St. Germain et al., 1994), which inactivate thyroid hormone. Its expression profiles in various organs suggests that the 5-deiodinase participates in regulating the levels of thyroid hormone in metamorphosing organs (Wang and Brown, 1993; St. Germain et al., 1994). Such a role has been suggested to be a critical factor in determining the temporal regulation of tissue-specific metamorphosis (Shi et al., 1996b).

The existence of multiple classes of T_3 -response genes suggest that T_3 simultaneously induces many intra- and extra-cellular events during metamorphosis (Fig. 4). It is unclear how these events cooperate to affect downstream gene expression. Currently, no systematic search has been carried out to isolate the downstream genes. Various late T_3 -response genes, i.e., genes requiring more than 24 hr of treating premetamorphic tadpoles with T_3 to alter their expression, have been identified over the years (Gilbert et al., 1996; Shi, 1996). The up- or down-regulation of these genes represents one of the late events during T_3 -induced tissue transformation and likely requires one or more of the direct T_3 -response genes.

Conclusions

Amphibian metamorphosis represents a unique system to study postembryonic vertebrate development (Tata, 1993; Atkinson, 1994). First, it involves systematic, drastic transformations of different organs but is largely under the simple control of one hormone. Second, T_3 regulates gene transcription through TRs and many of the direct T_3 -response gene have been isolated. Third, extensive morphological, cellular, and biochemical studies have been carried out on the transformations of different organs/tissues. Finally, in vivo and in vitro systems are available for studying the T_3 -induced cell death and proliferation/differentiation involved in tissue remodeling.

The transformations of various organs involves not only the proliferation and differentiation of adult cell types, but also selective elimination of certain larval cells through programmed cell death or apoptosis. Cell death is important for both resorbing tissues like the tail and remodeling organs like the intestine. It is even an important aspect for the de novo development of certain adult organs like the limb as the interdigital cells

undergo apoptosis during limb morphogenesis. At least some of the T₃-induced apoptosis is cell-autonomous and involves similar executioners such as caspases and nucleases as those employed in the apoptosis of other animal species. What is unclear is how these death executioners are activated in a temporal and cell-type specific manner during metamorphosis.

Both cell proliferation/differentiation and apoptosis are initiated by the transcriptional regulation induced by T₃ through its nuclear receptors. Currently, many of the direct T₃-response genes have been cloned and found to encode a wide variety of proteins predicted to affect both intra- and extra-cellular processes. In addition, some late T₃-response genes that are associated with late or terminal transformations during metamorphosis have also been isolated and characterized. The future challenge lies at understanding how the T₃-induced early genes (events) transduce the T₃ signal to the downstream events like apoptosis and cell proliferation or the regulation of late T₃-response genes. This will require the isolation of the target genes affected by the early or direct T₃-response genes and/or the genes which are immediately upstream of the late T₃-response genes and the cell death executioners.

Perhaps the most important study is to investigate directly the roles of various T₃ response genes during metamorphosis. The existing organ and cell culture systems will be very useful for this purpose, especially for genes which encode extracellular proteins as over-expressed proteins or function-blocking antibodies and inhibitors can be added to the culture systems to modulate the levels and function of the encoded proteins.

The ability to manipulate *Xenopus* embryos through microinjection of DNA or RNA encoding proteins of interests to fertilized eggs serves as another means to study gene function. In fact, by over-expressing wild type and mutant TRs and RXRs in developing embryos, we have shown that both TRs and RXRs are required for efficiently mediating the developmental effects of T₃ in embryos (Puzianowska-Kuznicka et al., 1997). Further-more, the over-expressed TRs and RXRs regulate specifically the expression of several genes which are known to be regulated by T₃ during metamorphosis. Thus, the embryo-injection method and the more recently developed transgenic *Xenopus* technology (Kroll and Amaya, 1996) hold great promises for future functional dissections of the molecular mechanisms underlying the actions of T₃-response genes during metamorphosis.

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