Abstract

The death of different types of cells occurs in regressing or remodeling organs to transform from a tadpole to a frog in both temporally and spatially regulated manners during amphibian metamorphosis. This morphological change is drastic and visible with the naked eye. This review summarizes our current understanding of the basic mechanism of the cell death during the metamorphosis. It focuses in particular on the tail resorption and the remodeling of intestine and skin where programmed cell death is executed by thyroid hormone-signaling through the cell-autonomous response (suicide) and the degradation of the extracellular matrix (murder).

Keywords: Amphibian; Metamorphosis; Programmed cell death; Apoptosis; Thyroid hormone

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1. Introduction

Amphibian metamorphosis represents dynamic and systematic changes from a larva to an adult, involving absorption of larva-specific organs and appearance of adult type organs, and accompanying the life style change from being aquatic to being terrestrial. This reorganization is triggered simply by a surge of thyroid hormone. Thyroid hormone-induced metamorphosis is observed also in the development of sea urchin [1] and fish [2,3]. Furthermore, amphibian metamorphosis might correspond to the human perinatal period, which is marked by an abrupt increase of thyroid hormone after birth [4–6]. It is because amphibians and mammals switch hemoglobin from fetal to adult, and a major serum protein to albumin, and change the resource of oxygen for respiration to air from water through gills and from maternal blood through a placenta during post-embryonic development, respectively. Higher vertebrates retain the similar sensitivity to thyroid hormone in their developmental history. It is reflected as a key
role of thyroid hormone in brain, nerve connective tissue, and endocrine maturation [7,8]. In all processes of vertebral post-embryonic development, amphibian metamorphosis displays the most prominent transformation, which includes the typical programmed cell death in regressing or remodeling organs.

2. Induction of metamorphosis by thyroid hormone

Amphibian metamorphosis is composed of three developmental periods, premetamorphosis, prometamorphosis and climax [9]. In Xenopus laevis tadpole, premetamorphosis extends from hatching up to Nieuwkoop and Faber (NF) stage 54 [10] when the developing thyroid gland begins to secrete thyroid hormone [11]. During prometamorphosis (NF stage 55–57), endogenous thyroid hormone levels rise in the plasma, and an orderly succession of morphological changes occurs, the most obvious of which is the development of hindlimbs. At the climax (NF stage 58–66), when the concentration of thyroid hormone is at peak levels, tadpoles undergo a rapid metamorphic transition, including the eruption of frontlimbs from the opercular fold, the shrinkage of head, the resorption of gills, the remodeling of intestine and skin, and the absorption of tail in the temporally predetermined order. Almost all organs of tadpoles change during anuran metamorphosis. Three types of changes take place, which are the complete degeneration of larval-specific organs such as the tail and gills, the development of frog-specific organs-like limbs, and the remodeling of the existing larval organs into their adult forms such as intestine, skin, dorsal muscle [12] and pancreas [9]. Programmed cell death is observed in regressing or remodeling organs. Only the middle 75% of the tadpole body apparently remains without altering the size of the cranium and transforms to a frog after metamorphosis (Fig. 1A and B), suggesting that a tail, which is about two times longer than a body, and the rest of the body are absorbed by programmed cell death. Moreover, programmed cell death occurs even within the middle 75% of the body, for example, in dorsal muscle, gills, intestine, skin, pancreas and so on.

The developmental cell death during metamorphosis is caused by thyroid hormone [9,13]. Because, firstly, the morphological metamorphic changes correlate with the developmentally increasing levels of the endogenous thyroid hormone in the plasma [11] as described above. Secondly, precocious metamorphosis is observed by thyroid hormone-treatment of the premetamorphic or prometamorphic tadpoles [14], their organs such as tail [15], gills [16] and intestine [17,18], and even a myoblastic cell line derived from tail [19]. Thirdly, metamorphic changes are suppressed by the congenital defect of thyroid gland [20], inhibitors of thyroid hormone synthesis [21], the surgical removal of a pituitary gland which secretes thyroid-stimulating hormone [9], the degradation of endogenous thyroid hormone in the type III deiodinase-overexpressing transgenic tadpoles [22] and the blocking of thyroid hormone-signaling in transgenic tadpoles which overexpress a dominant negative form of thyroid hormone receptor (DNTR) [23]. Even the direct developing anuran, which hatches as a tiny frog without a tadpole stage, still undergoes a thyroid hormone-dependent metamorphosis before hatching [24].

Thyroid hormone regulates transcription by binding to heterodimers formed with thyroid hormone receptors [25,26] and 9-cis retinoic acid receptors (RXR) [27–29]. There are two TRαs [30] and two TRβ genes in Xenopus laevis [31] due to its tetraploidy [32]. The TRαs mRNA level in a whole tadpole increases throughout premetamorphosis, is maximal by prometamorphosis, and falls after the climax of metamorphosis. On the other hand, TRβ mRNA is barely detectable.
during premetamorphosis, rises in parallel with endogenous thyroid hormone, reaches a peak at the climax, and drops after it. Thyroid hormone treatment up-regulates TRβ mRNA by about 20-fold during premetamorphosis [33]. In general, organisms have higher TR mRNA at times when they are most actively transforming during metamorphosis, that is, the limb bud at NF stages 52–56 and the tail at NF stage 60 and later [33–36]. As to TR proteins [37], TRα increases to NF stage 52 in the tail and head region. Even though TRα mRNA gradually increases during metamorphosis, TRα protein remains constant, suggesting strongly that post-transcriptional events control the ultimate levels of TRα protein. In contrast, there is no detectable TRβ protein until NF stage 52, and both TRβ mRNA and protein rise along with the increase in endogenous thyroid hormone, reaching a maximum at the climax of metamorphosis.

3. Programmed cell death in regressing tails

A tadpole tail, which is about two times longer than a body, disappears completely in several days. This dramatic resorption of the tadpole tail has attracted a good deal of attention as an experimental system for a century.

3.1. The morphology of dying cells in regressing tails

The physiologically dying cells adopt one of at least three different morphological types: “apoptotic”, “autophagic” and “non-lysosomal vesiculate”. The first type of dying cell degenerates without any detectable role being played by its own lysosomes, but its fragments are destroyed in the secondary lysosomese of other cells. The second type is destroyed to a greater extent within its own lysosomes. And, the third type is destroyed without lysosomes playing any detectable role [38].

The electron microscopic study of epidermis in regressing tail revealed the aggregation of chromatin near the nuclear membrane and cytoplasmic condensation of epithelial cells, the fragmentation of the nuclei and the formation of compact apoptotic bodies, which are characteristic of apoptosis. The histological changes of dissolution of muscle cells are the condensed cytoplasm and the peripheral aggregation of condensed chromatin, which are typical of apoptosis, too. However, deletion of striated muscle fibers in tadpole tail appears to be a modification of classical apoptosis, in which the dilatation and confluence of sarcoplasmic reticulum lead to the longitudinal clefts between myofibrils, followed by fragmentation of the cytoplasm into a number of apoptotic bodies with well preserved cross-striations of myofilaments [39]. Several reports also support the observation that the space between the myofibrils within the muscle cells is occupied by tightly packed swollen and proliferated vesicles of sarcoplasmic reticulum, and the myofibrils become separated from one another by enlargement of vesicles [40–42]. These resulting muscle apoptotic bodies are engulfed by the macrophages, which are probably derived from mesenchymal cells between muscle fibers of myomeres [40]. Degradation of apoptotic bodies is presumably brought about by the action of lysosomal enzymes in macrophages.

Autophagy also occurs in the regressing tadpole tail, although autophagic vacuoles are difficult to distinguish from ingested apoptotic bodies that do not contain nuclear remnants, and no evidence shows that it plays a major part in the regressive process [39]. Autophagic vacuoles are occasionally observed between the myofibrils, and degenerating muscle cells include degraded myofilamentous tissue, suggesting some roles of autophagy in regressing tail [42].

3.2. The up-regulated genes in thyroid hormone-induced tail regression

Thyroid hormone induces involution of isolated tail tips of *Xenopus laevis* tadpole in an organ culture [15], which requires RNA and protein synthesis, suggesting that the thyroid hormone-induced proteins play a pivotal role in tissue resorption [43]. This idea is consistent with a report that thyroid hormone receptors are ligand-dependent transcription factors to regulate target gene expression through thyroid hormone–response elements [44].

Thyroid hormone-induced resorption needs about a 2-day lag before visible resgression in tail tip culture of NF stage 51/52 tadpoles in the presence of 50–500 nM 3,3′,5′-tri-iodothyronine, active form of thyroid hormone (T3) [45]. Little or no change is observed in organ culture of tails which are amputated from NF stage 54 tadpole pretreated with 100 nM T3 for 4, 8, or 24 h, while those from 48-h treated tadpoles undergo extensive resorption even in the absence of thyroid hormone. These results suggest that the complete program of gene regulation for tail absorption is conferred to tail tip between 24 and 48 h after the addition of thyroid hormone.

PCR-based subtractive hybridization has been carried out to isolate up- and down-regulated genes in regressing tails treated with thyroid hormone by the laboratory of Dr. D.D. Brown [34]. A probability analysis by Poisson distribution predicts 25 and 35 response genes up-regulated in 24 and 48 h in the presence of 100 nM T3, respectively. The up-regulated genes in the tail 48 h after thyroid hormone treatment include all genes induced in the first 24 h. During spontaneous metamorphosis, mRNA from nineteen of twenty genes up-regulated at 48 h reaches a high level at the climax of metamorphosis (NF stage 60), and remains high as a tail is undergoing resorption (NF stage 63). These genes are not regulated dramatically during normal limb development, and their mRNAs are much less abundant in limb compared to tail. The up-regulated genes encode transcription factors, proteinases, the extracellular matrix and receptors, type III iodothyronine 5-deiodinase and so on [46].

Expression of the up-regulated genes falls into two kinetic profiles. The direct response genes have a lag of from 2 to 4 h before their mRNA levels begin to increase, reach a peak
Necturus detected in thyroid hormone-resistant neotenic salamander, levels in gills, intestine and muscles, but TR/H9252 as hindlimbs\([49]\), and that TR and tail, and has less effect on the growth of adult tissues such is suggested that they commit suicide (a suicide model\([47]\). On the other hand, since in situ hybridization analysis shows very muscle cells from the extracellular matrix and induces their in degeneration of myotendinous junctions, which detaches fibers are attached. These observations lead to an idea that the up-regulated in myotendinous junctions to which the muscle enzymes are also induced in a layer of subepidermal fibroblasts, adjacent to muscle, but not in the tail muscle. These enzymes are also up-regulated in mystendinous junctions to which the muscle fibers are attached. These observations lead to an idea that the thyroid hormone-induced secretion of extracellular matrix-degrading proteinases from subepidermal fibroblasts results in degeneration of myotendinous junctions, which detaches muscle cells from the extracellular matrix and induces their programmed cell death. This is called a murder model. On the other hand, since in situ hybridization analysis shows very low expression of proteolytic enzymes in epidermal cells, it is suggested that they commit suicide (a suicide model\([47]\).

3.3. Expression patterns of thyroid hormone-induced genes in regressing tails

The regression of amputated tails requires at least 48-h pretreatment of tadpole with thyroid hormone, which shows that delayed response gene expression is necessary for tail regression\([34]\). Expression of thyroid hormone-induced genes during tail resorption has been characterized by in situ hybridization\([47]\). The observation that the highest expression of TRβ localizes to fibroblasts that strongly induce the delayed response gene expression (collagenase-3 and serine dipeptidyl peptidases), implies that TRβ which is induced by thyroid hormone\([33,48]\) may activate the delayed response genes which causes tail regression. This hypothesis is supported by two reports that a TRβ-selective ligand, GC-1, efficiently induces resorption of tadpole tissues such as the gills and tail, and has less effect on the growth of adult tissues such as hindlimbs\([49]\), and that TRβ mRNA is expressed at high levels in gills, intestine and muscles, but TRβ mRNA is not detected in thyroid hormone-resistant neotenic salamander, Necturus, which cannot be induced to metamorphose even with large doses of exogenous thyroid hormone\([50]\).

Stromelysin-3 and the delayed response proteinases including collagenase-3 and serine dipeptidyl peptidases are induced in a layer of subepidermal fibroblasts, adjacent to muscle, but not in the tail muscle. These enzymes are also up-regulated in mystendinous junctions to which the muscle fibers are attached. These observations lead to an idea that the thyroid hormone-induced secretion of extracellular matrix-degrading proteinases from subepidermal fibroblasts results in degeneration of myotendinous junctions, which detaches muscle cells from the extracellular matrix and induces their programmed cell death. This is called a murder model. On the other hand, since in situ hybridization analysis shows very low expression of proteolytic enzymes in epidermal cells, it is suggested that they commit suicide (a suicide model\([47]\).

3.4. The suicide model and the murder model

Based on the observation that thyroid hormone induces the increase of the collagenase activity\([31]\) in explants of tadpole tail fin\([52]\) and gills\([16]\) and the concomitant decrease of their sizes, it has been proposed that thyroid hormone-induced collagenase production is involved in the remodeling of collagen in these organs. The expression patterns of the thyroid hormone-induced genes in regressing tails suggest that the delayed response proteinases induce muscle cell death by proteolysis of the extracellular matrix, that is, the murder model\([47]\). This process is supported by the phenomenon "anoikis" which is programmed cell death caused by disruption of the interactions between normal epithelial cells and the extracellular matrix\([53–55]\).

To characterize the mechanism of tail muscle cell death and to facilitate in vitro analysis, we have established a myoblastic cell line derived from NF stage 57 tadpole tail of Xenopus laevis\([19]\). This cultured cell line dies in response to 10 nM of T3 and exhibits positive TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling) and internucleosomal DNA cleavage, indicating apoptotic cell death. A half-day incubation with T3 is not sufficient to provoke cell death, but a day of T3 treatment reduces cellular survival, which means that a lag time is between half a day and a day. The condition needed to induce the death of the cultured myoblastic cells is in contrast with the requirement (more than 2 days in the presence of 50–500 nM T3) for the resorption of the tail tip of NF stage 51–54 tadpoles\([45]\). Firstly, these differences may be due to the tadpole stage. The myoblastic cell line is derived from NF stage 57, just before the climax, whereas tail tips in the organ culture are prepared from NF stage 51–54. The sensitivity of isolated tail tips to thyroid hormone increases with development, as evidenced by a shortened lag period before the onset of regression\([56]\). The myoblastic cell line seems to keep the characteristics of NF stage 57. Secondary, it reflects the difference of the myoblastic cell death and tail resorption. The process of thyroid hormone-induced tail regression is composed of multiple steps including degradation of extracellular matrix, spinal cord, vessels and notochord, death of muscle cells and many types of cells, the migration of macrophages, and absorption of cell debris.

The suicide model of muscle cell death is strongly supported by the cell-autonomous death of the myoblastic cell line induced by thyroid hormone in the absence of subepidermal fibroblasts, which release extracellular matrix-degrading proteinases. Since the myoblastic cell death cannot be promoted by addition of a condition medium incubated with the cultured cells in the presence of T3 for 2 days, thyroid hormone appears not to work through a paracrine mechanism by which cells secrete death-inducing proteinases, for example, extracellular matrix-degrading proteinases to kill each other\([19]\). It is possible that the cell-autonomous death of the myoblastic cells might be an in vitro artifact due to the absence of the extracellular matrix in the cell culture condition. This possibility is excluded by the elegant experiment using a transgenic tadpole, which carries a gene of a dominant negative form of the thyroid hormone receptor under the cardiac actin promoter, and overexpresses DNTR only in muscle cells\([57]\). DNTR binds to the thyroid hormone response elements but not to thyroid hormone, and prevents transcription of thyroid hormone-induced genes by the wild-type TR in mammalian cells\([26,58]\) and Xenopus tadpoles in vivo\([59]\). At 1 week after fertilization, control and transgenic tadpoles are treated for an additional week with thyroid hormone to induce precocious metamorphosis. DNTR-overexpressing tail muscle cells of 2-week old transgenic tadpoles are protected from thyroid hormone-induced death, although tails shorten. The tail muscle cells avoid the suicide, too, in NF stage 62 transgenic tadpoles, which always die just before drastic tail
regression. Even this transgenic experiment cannot deny a possibility that tail muscle cells murder each other by thyroid hormone-dependent releasing of soluble factor. Indeed, the tail-derived myoblastic cell line secretes a gelatinase in response to thyroid hormone with the induction of cell death, raising a possibility that thyroid hormone induces muscle cells to produce and release a gelatinase for the degradation of the basement membrane between muscle cells [60].

To clarify the underlying mechanism of the death in tail muscle cells, we have performed both the transfection of the myoblastic cell line and the DNA injection into tail muscle with DNTR expression construct and a reporter gene, followed by the examination of the viability of reporter gene-expressing cells in a condition in which programmed cell death is induced [60]. The principle of our experiment is schematically drawn in Fig. 2. In the presence of thyroid hormone, the thyroid hormone-signaling pathway should be repressed only in DNTR-overexpressing cells, but not in the non-transfected surrounding cells, which are the majority. According to the murder model, all cells should be killed by soluble factors such as extracellular matrix-degrading proteinases that are synthesized by thyroid hormone-responsive non-transfected cells including subepidermal fibroblasts. In the suicide model, the minority of cells in which the thyroid hormone signaling is interrupted by the overexpression of DNTR should survive, whereas the non-transfected cells die.

A result that the transfected myoblastic cells which overexpress DNTR survive in the presence of thyroid hormone, demonstrates that the cell line commits suicide, and excludes a possibility of cell death by murder. Fig. 3A shows the process of muscle cell death during the metamorphosis in a tail injected with a GFP expression construct. Muscle cell death starts at NF stage 58 when the endogenous T3 increases abruptly and the climax of metamorphic changes begins. GFP-expressing muscle cells are almost extinguished at NF stage 64 when a tail resorbs to less than one-third of body in length. The overexpression of DNTR protects muscle cells till NF stage 61, but only delays and cannot inhibit cell death perfectly after NF stage 62 when tail regression begins (Fig. 3B). Since the similar death of DNTR-overexpressing cells is observed even in a tail injected with six times more DNA of DNTR expression construct, the death is not due to an insufficient amount of DNTR protein to compete endogenous TR. Taken together, tail muscle cells undergo DNTR-sensitive cell death (suicide) which can be inhibited completely by blocking thyroid hormone signaling till NF stage 61 before tail shortening, and then execute DNTR-resistant cell death (suicide and murder) which is only delayed even by the overexpression of DNTR and finally leads to the death of 80–90%
In this organ culture using the small intestine of NF stage culture system in the presence of thyroid hormone [17,18]. The remodeling after NF stage 62, when large macrophage-like cells with the neovascular metamorphic climax (NF stages 59–61) and decrease suddenly increase in number around the beginning of spontaneous apoptosis in the lumen [64]. The remodeling of the small intestine can be reproduced in an in vitro organ culture system in the presence of thyroid hormone [17,18]. In this organ culture using the small intestine of NF stage 62–64 when a tail regresses rapidly. It is tempting to speculate that thyroid hormone induces proteolytic enzymes to degrade the extracellular matrix and notochord, which causes losing of the cell–cell and cell–matrix interactions and relaxing the cell tension. This loss of the cell interaction and cell tension may be causative of cell death by murder.

4. Programmed cell death in remodeling intestine

As anurans transform from a omnivorous tadpole to a carnivorous frog by metamorphosing, the long small intestine, which consists of predominantly a single tubular layer of larval epithelium with very little connective tissue or muscle, reduces in its length by about 90% [61] and is replaced with the adult type of complex organ comprised of a multiply folded epithelium with elaborate connective tissue and muscle, accompanied by the programmed cell death of larval epithelium [62,63]. Both apoptotic bodies derived from larval epithelial cells and intraepithelial macrophage-like cells suddenly increase in number around the beginning of spontaneous metamorphosis (NF stages 59–61) and decrease after NF stage 62, when large macrophage-like cells with the apoptotic bodies appear in the lumen [64]. The remodeling of the small intestine can be reproduced in an in vitro organ culture system in the presence of thyroid hormone [17,18]. In this organ culture using the small intestine of NF stage 57, apoptotic cells or bodies are first detected on the second day of cultivation, while the number of macrophage-like cells begins to increase on the same day and reaches its maximum around the third day when the larval epithelial cells rapidly reduce in number. These observations imply that the degeneration of larval epithelial cells occurs by apoptosis and involves heterolysis by macrophages [64].

Thyroid hormone induces the intestinal epithelial cells from NF stage 57/58 tadpoles to undergo apoptosis in a primary culture system as in an organ culture, suggesting that the apoptotic event is cell autonomous [65]. This degeneration of larval epithelium can be inhibited by using plastic culture dishes which are coated with the extracellular matrix such as laminin, collagen type IV, and fibronectin, components of the basement membrane. However, the adult epithelial cells from NF stage 64 tadpole intestine also undergo cell death just like larval cells from NF stage 57/58 in the presence of thyroid hormone, indicating that the removal of the extracellular matrix and the underlying mesenchyme in a primary culture system alters their cell fate to the same one as larval cells.

PCR-based subtractive differential screening has isolated 22 up-regulated genes and a single down-regulated gene using intestines of NF stage 52–54 tadpoles treated with 5 nM T3 for 18 h [66]. When intestines of NF stage 52–54 tadpoles are treated with thyroid hormone for a week to induce intestinal remodeling in an organ culture, the same expression patterns of these up-regulated genes are observed as those found during spontaneous metamorphosis. Both TRβ1 and stromelysin-3 are up-regulated by thyroid hormone as direct-response genes. Their mRNAs peak in expression at the climax (NF stage 60), decrease by NF stage 66 and remain very low in frog gastrointestinal tract, while, in an organ culture, they increase to a peak level after 2 or 3 days of thyroid hormone induction, and fall after 5–6 days.

In situ hybridization analysis reveals that TRβ genes are expressed both in the larval intestinal epithelial cells prior to their apoptotic degeneration and in the proliferating cells of adult epithelium, connective tissue and muscles, and down-regulated upon the differentiation of these adult cells. The observation that the expression of TRβ genes increases before cellular death or proliferation implies that thyroid hormone signaling promotes it, depending on the cell types [67]. Stromelysin-3 mRNA is first detectable in larval fibroblasts near the muscular layer around the beginning of the climax (NF stage 58), then increasing in amount throughout connective tissue, and localized in fibroblasts just beneath the epithelium by NF stage 61, when massive cell death takes place in the larval epithelium, and adult epithelial cells proliferate rapidly. Thereafter, stromelysin-3 mRNA gradually decreases and is no longer detected after NF stage 63. The immunohistochemical analysis using anti stromelysin-3 antibody also confirms the same temporal and spatial expression pattern of stromelysin-3 protein as that of stromelysin-3 mRNA [68]. The transient expression of stromelysin-3 mRNA and protein in fibroblasts under the epithelium and
basement membrane is in good temporal accordance with the thickening and folding of the basement membrane, the apoptosis of the larval epithelial cells, and the proliferation of adult epithelial cells [69, 70].

Furthermore, a function-blocking antibody against stromelysin-3 inhibits the thyroid hormone-induced apoptosis of the larval epithelium, thickening of the basement membrane, and the invasion of the adult epithelial primordia into the connective tissue in an organ culture, although prolonged culturing eventually leads to the degeneration of larval epithelium and remodeling of extracellular matrix. This suggests that the role of extracellular matrix remodeling by stromelysin-3 lies mainly at tissue transformations but is not the determining effector for cell fate [68].

5. Programmed cell death in remodeling skin

When an anuran tadpole metamorphoses from an aquatic larval to a terrestrial frog, the conversion of the larval thin skin into the adult stratified skin is one of the important changes to adapt to the dry environment. The larval skin consists of one or two layers of cuboidal cells (basal skein cell layers) which are overlain by an outer squamous epithelium (apical cells) and lying on a thin basement membrane, a thicker collagenous lamella, and an adjacent single layer of subepithelial fibroblasts. At a middle larval stage, NF stage 55, small larval basal cells emerge in the basal skein layer. The skin drastically transforms into adult one during the climax of metamorphosis, NF stage 60-64, larval basal cells differentiating into adult basal cells, and the outer two cell layers, apical and skein cells, dying and sloughing [71-73].

The morphological study of regressing tail skin suggests that tail epidermal cells undergo apoptosis as mentioned above [39]. Moreover, the immunohistochemical analysis with active caspase-3 antibody reveals that the dying cells are observed also in the tadpole body skin during the climax of metamorphosis, restricted to the first two layers of the epidermal cells and often highly vacuolated, a characteristic of autophagy [74]. The purified tail epidermal cells do not proliferate in the presence of thyroid hormone and detached themselves from the dish within 5 days, implying the tail epidermal cells degenerate by the direct action of thyroid hormone [75].

A larval keratin is expressed from late embryogenesis throughout tadpole life in the apical and skein cells of the epidermis. Since this larval keratin promoter drives the same expression as the endogenous larval keratin, a transgene containing DNTR gene downstream of this promoter blocks the thyroid hormone-signaling specifically in those two outer layers of prepared transgenic tadpole skin [74]. They develop normally except for retaining a larval epidermis over the developing adult epithelium. This observation that DNTR expression protects larval epidermal cells from thyroid hormone-induced cell death indicates that the larval apical and skein cells are direct targets of thyroid hormone, suggesting the suicide model.

6. Apoptosis-related genes in regressing or remodeling organs

Many morphological and biochemical studies have demonstrated that apoptosis mainly mediates programmed cell death in the resorption of the larval-specific organs and the remodeling of the larval organs into their adult forms during the amphibian metamorphosis regardless of whether cell death takes place according to the suicide model or the murder model, although autophagic cell death is also observed in tail [39, 42] and skin [74]. Caspase and Bcl-2 families play an important role in apoptosis [76, 77].

Xenopus laevis tadpole expresses mRNAs encoding caspase-1, -2, -3, -6, -7, -8, -9, and -10 during the metamorphosis. These caspase mRNAs increase in regressing tail and remodeling intestine [19, 78, 79], but not in a tadpole tail-derived cultured cell line, which is induced to die by thyroid hormone, showing that the induction of caspase expression is dispensable to cell death [78]. However, the activation of caspase-3, -6 and -7 is observed in apoptotic tail-derived cells [78], regressing tail [57, 60, 79] and remodeling dorsal skin [74].

The expression of Xenopus Bcl-XL is modulated insignificantly in regressing tail during natural or thyroid hormone-induced metamorphosis [80], although it can inhibit cell death of neurons [81] and tail muscle cells [60, 79] during the spontaneous metamorphosis. A study on the expression profile of Bax mRNA [82] suggests that Bax is induced in regressing tail and thyroid hormone-treated tail, leading to muscle cell death [93].

7. Conclusions and perspectives

Programmed cell death reaches a peak level in dorsal skin, intestine and tail muscle around NF stage 60 [74], 61 [68] and 62 [60], respectively, when the endogenous T3 concentration in plasma is at the highest level [11]. The larval epithelium in skin degenerates by suicide, while the larval epithelium in intestine is suggested to die by the combination of suicide and murder. Muscle cells in tail die by suicide before tail regression (NF stage 61), and then are executed completely by both the suicide and murder mechanisms as a tail is shortening. In all cases thyroid hormone induces apoptotic cell death. In the suicide model, each cell directly responds to thyroid hormone, and expresses some proteins, which induce to kill itself. On the other hand, in the murder model, the surrounding cells are suggested to be induced by thyroid hormone to secrete extracellular matrix-degrading proteinases which disrupt the cell-cell and cell-matrix interactions, leading to the loss of cell's anchorage and apoptosis regardless of whether a dying cell responds to thyroid hormone or not. Apoptosis by murder corresponds to anokis [53, 54], the process of which is analyzed intensively in mammals. The mammalian mechanism of anokis may apply to the amphibian cell death by murder.
The molecular mechanism of apoptosis by suicide during metamorphosis seems to remain poorly elucidated in spite of the extensive PCR-based subtractive hybridization screening [34]. There are three typical examples of apoptotic pathways. Firstly, in mammals, the binding of the death receptor such as Fas to its death domain elicits the expression of the membrane-bound death factor (Fas-ligand) and death receptor (Fas), the interaction of which on the surface cell causes apoptosis in a single T h 2 myocardial cell [84,85]. It is possible that tail muscle cells die through the expression and binding of the death ligand and receptor. The cDNAs encoding caspase-8 [78], death receptors [86,87], and FADD [94] have been cloned in X e n o p u s l a e v i s . Secondly, from the studies using nematode and mammals, the induction or the processing of a proapoptotic BH3-only member of Bcl-2 family is shown to result in the activation of proapoptotic multidomain members of the same family, Bax and Bak, which are located on the mitochondria and endoplasmic reticulum, leading to the release of cytochrome c and other death-promoting proteins from the mitochondria, and Ca2+ ion from the endoplasmic reticulum in order to activate caspses [76,77]. Thyroid hormone might induce the expression of a BH3-only protein during the metamorphosis, but there is no report on Xeno p o s BH3-only protein so far. Thirdly, during the D r o s o p h i l a metamorphosis, ecdysone induces the expression of reaper and hid, and these protein products physically interact D r o s o p h i l a inhibitor of apoptosis 1 to block its ability to inhibit the effector caspses, which culminates in cell death [88–90]. The ablation of diablo/sm ac, a functional homolog of reaper and hid in mammals, in mice indicates that apoptosis can proceed in its absence [91], and its overexpression does not appear to induce apoptosis in healthy cells [92]. Although diablo/sm ac does not seem to have a potent proapoptotic activity, these three possibilities must be considered to investigate the molecular mechanism of the thyroid hormone-induced cell death.

The molecular mechanism of programmed cell death during amphibian metamorphosis should be not only shared in the resorption of the larval-specific organs and the remodeling of the tadpole organs into their adult forms, but also evolutionarily conserved in the other thyroid hormone-induced metamorphic systems of sea urchin and fish and the post-embryonic transition of higher vertebrates.

References


