HORMONE NUCLEAR RECEPTORS AND THEIR LIGANDS: ROLE IN PROGRAMMED CELL DEATH

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Programmed cell death (PCD) represents a highly efficient and very sophisticated system for removing cells from the surrounding environment. As deadly as it may be, PCD is essential for elimination of aberrant cells and the survival of the living organism as a whole. Therefore, PCD is meticulously controlled, and among major regulatory actors belong small lipophilic hormones acting as ligands of the members of a nuclear receptor superfamily. In general, these hormones which include steroids, thyroids, retinoids, vitamin D_3 derivatives, serve a critical role in the maintenance of homeostasis. For example, steroids regulate metabolism, reproduction, and development in animals that are as different as insects and humans. During animal development, steroids trigger distinct responses including cell differentiation and programmed cell death. Thus, hormones have been linked to numerous human health problems, and defects in hormone triggered programmed cell death may result *e.g.* in the survival of tumor cells or degenerative disorders. In vertebrate and invertebrate organisms where steroids including androgens, estrogens, progesterone, glucocorticoids and ecdysteroids regulate cell death, intensive study of this processes has resulted in a wealth of new information regarding how small lipophilic hormones contribute to cell demise within an organism. There is a great knowledge on the execution phase of apoptosis, the most frequent form of programmed cell death, and on the variety of its inducers. Even though we will review also recent advances on the topics various small ligands in the role of inducers, nevertheless we want to highlight the mechanisms that links action of hormones to the activation of apoptotic execution, the complex of processes which are poorly understood so far.

1. Introduction

By repeated cell divisions and differentiation, a fertilized egg produces millions or even billions of cells to create body of multicellular eukaryotic organisms. During this process and even in adults, many superfluous, harmful or unwanted cells are generated, and they must be eliminated [115]. The senescent cells are removed and replaced by newly generated cells to maintain homeostasis by the process of programmed cell death (PCD), the most frequent form of which is termed apoptosis. Deviations from the physiological levels of cell death during adult life may result in either proliferative or degenerative disorders [235, 141]. The picture emerging from studies on PCD suggests a complex interplay between factors that promote cell death and those that prevent cell death, the end result being life or death of the cell (for reviews, see [107, 206]). The remarkable conservation of physiological cell death mechanisms from nematodes to humans has allowed the genetic pathways of programmed cell death determined in *Caenorhabditis elegans* to act as a framework for understanding the biology of apoptosis in other invertebrates as well as vertebrates including mammals. The most downstream compo-

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nents of the cell-death machinery identified so far are proteases known as caspases, a class of cysteine proteases that cleave substrates following aspartate residues. However, prior to activation of caspases, there is a set of upstream regulatory networks that require to be turned on or off to achieve final cell death execution, and these pathways can be controlled at different levels. This multilevel control system that has evolved during animal evolution opens numerous possibilities for involvement of divergent signaling pathways which can contribute to regulation of PCD. It is becoming apparent that tight control of death/survival signals plays a fundamental role in various developmental processes including embryogenesis, pattern formation or metamorphosis of animals. The process of programmed cell death is often triggered by natural inducers, such as steroids, thyroids or retinoids, a group of small lipophilic ligands that mediate their own action through the family of highly conserved nuclear receptors.

Genetic studies of developmentally-linked PCD on mammals including mouse, even using knock-out techniques, have not been very informative thus far. However, with the power of fruit fly genetics, *Drosophila* started to make significant contributions to the understanding of pathways that mediate developmental programmed cell death. Steroid hormones appear to regulate programmed cell death by a variety of mechanisms. Most studies have reported that steroids serve as survival factors, and that hormone withdrawal results in the activation of programmed cell death. Examples of this mechanism include mammalian androgens in the prostate [109], and ecdysteroids in the insect nervous system [197, 237, 6]. Alternatively, increases in steroids also activate programmed cell death. In *Drosophila*, increases in ecdysteroids trigger cell death in larval midguts and salivary glands [116, 70]. Besides genetic and molecular studies that have identified homologues of most of the genes involved in mammalian and *C. elegans* programmed cell death playing crucial role during embryogenesis and patterning, recent studies of *Drosophila* are providing insights into the genetic mechanisms underlying steroid-triggered and developmentally-associated cell death. Since steroids, thyroids, retinoids and other small lipophilic ligands have been linked to numerous human health disorders whereas some defects in hormone signaling may results in the survival of tumour cells, in the present review several times we will focus on how recent *Drosophila* developmental genetics research contributes to our knowledge of hormone-triggered PCD, and how these studies can provide a novel entry to understanding human disorders and disease processes.

2. Why PCD and why not just apoptosis ?

Waste majority of papers on programmed cell death (PCD) and its induction with various factors including hormones, use term apoptosis although many of these reports clearly deal with atypical variations of apoptosis or even with non-apoptotic cell death. Original and so far undiscredited definition of apoptosis is based on mostly morphological criteria that are characterized by initial condensation of the nuclear chromatin followed or accompanied by nucleosomal fragmentation of DNA, cascade activation of caspases, then formation of apoptotic bodies which eventually are eliminated by phagocytic macrophages [125, 126, 127, 128, 255]. Nucleosomal fragmentation nowadays is easily detected by widespread technique called TUNEL assay [83, 12], nevertheless its positive signal is often misused as unambiguous hallmark of apoptosis [166]. However, several workers noticed that there are morphologically distinct types of cell death [210, 170, 79] with no or significantly delayed nucleosomal fragmentation [261, 153, 23], and that TUNEL positivity not always corresponds to active nucleosomal fragmentation which itself should be assayed and proven by agarose gel electrophoresis of extracted DNA [201, 243, 123]. On the other hand, most of the known cases of regulated programmed cell death are characterized by activation of caspases [137, 132, 134, 178, 236, 48, 175, 136] what however should not serve as a rule for naming various types of cell death with the same name. This situation just shows that regardless signalling pathways involved in preparative or regulatory phase of cell death, all organisms and cell types during evolution has converged probably on one highly efficient mechanism of proteolysis for the execution phase of death program. Formighli *et al*. [76] described aponecrosis as special combination of two antagonistic types of cell death when molecular and morphological signs of apoptosis coexists with degenerative necrotic features, in-

cluding cytoplasmic swelling and plasma membrane disruption. In addition, there are cases of necrosis with positive TUNEL signal [32], and thus most of the authors came to the conclusion that identification of cell death type requires multiple technical approaches providing complex appraisal of morphological, biochemical, molecular and where possible also genetic criteria [47, 163, 176].

As will be shown below, cell death induced by steroids, retinoids and other small lipophilic hormones is not an exception and displays variety of morphological, biochemical and molecular features that do not allow oversimplified classification. Therefore, we will use term apoptosis only in the cases where there was enough evidence accumulated by researchers on the particular type of cell death. In all other cases we will prefer generally accepted term programmed cell death or PCD. This circumstance evokes several questions that need to be asked regarding hormonally controlled PCD: Can we relate steroid-induced apoptosis *e.g*. in glucocorticoid-thymocyte system and steroid-triggered non-apoptotic cell death *e.g*. in larval salivary glands of *Drosophila* ? Do share hormonally controlled cases of cell death in various systems something in common except activation of caspases ? Is hormonally-induced cell death just a consequence of the imbalance between death and survival factors or it is final unavoidable and committed program of the particular cell or tissue ? And finally, are caspases really indispensable for PCD execution or can cell death be issued in the absence of their activation ? In the following text we will try to answer or at least discuss each of these points and present clues on the role of hormones in cell death.

3. Variety of inducers

3a. Steroids

Steroid hormones are potent regulators of programmed cell death in many mammalian steroid-dependent cell types and tissues such as the mammary gland, prostate, ovary and testis where they can affect or facilitate apoptotic process either by their presence or absence [234, 129]. In spite of general believe about direct steroid hormone action, steroid-facilitated apoptotic events can be initiated not only by direct action of a steroid hormone on target cells but also indirectly by altering expression of paracrine effectors in the affected or in supporting stromal cells [234]. For many years, glucocorticoid regulation of programmed lymphocyte death has served as a paradigm for steroid activation of apoptosis, and this response is dependent on glucocorticoid receptor function [254, 45, 45, 57, 98]. Steroid hormones appear to regulate programmed cell death by a variety of mechanisms. Most studies have reported that steroids serve as survival factors, and that hormone withdrawal results in the activation of programmed cell death. Examples of this mechanism include androgens in the prostate [109], and estrogens in endometrium or ovarian granulosa cells [106, 18, 87]. Glucocorticoid regulation of thymocyte cell death is more complex and has been reported to be under both positive and negative control by this hormone, but recent *in vivo* studies indicate that a decrease in steroid titer regulates thymocyte apoptosis [239]. Nevertheless, in cases like this we have to take in account the fact that decrease in a steroid hormone follows after its previous increased levels.

Although generally the effect of glucocorticoid hormones is mediated by a member of the nuclear receptor superfamily, the role of multiple forms of the glucocorticoid receptor (GR), generated as multiple splice variants, in cell death process are not clearly understood [103, 223, 84, 80]. The unstimulated glucocorticoid receptor is maintained in inactive form by its integration within a multiple protein complex consisting of heat shock proteins/chaperons/imunophilins. When the hormone binds to its receptor, the complex dissociates and the receptor migrates to the nucleus. Hence, the activated receptor provokes an upregulation or downregulation of target gene expression. DNA recognition elements for steroid hormone receptors or hormone response elements (HRE) are often located among binding sites for other trans acting factors, and thus gene expression can be regulated by direct receptor-DNA interaction or by DNA-binding independent mechanisms which may involve protein-protein interaction of a receptor with other transcription factors [49, 10, 9, 102, 5]. Interestingly, one splice variant of the GR has been associated with the expression and presentation of a membrane form of this receptor [37, 38, 81, 247] that is thought to be responsible for the

switching between proliferative and death pathways, thus resulting in eventual cell cycle activation or cell elimination. In addition, there is direct evidence that the membrane glucocorticoid receptor is involved in apoptosis of CCRF-CEM human leukemic cell line [204].

Studies in a number of experimental systems including glucocorticoid negative mice indicate that action of glucocorticoid hormones on T lymphocyte apoptosis requires dimerization of the GR and direct binding to glucocorticoid hormone response element (GRE) on DNA [10, 9, 31, 89]. This way of glucocorticoid signal transduction also contributes to some other actions as antileukemic effect, thymus atrophy and, at least in part, to the declining of T cell number in the peripheral blood. However, only until recently we missed a link between described glucocorticoid effects and specific targets of their action. For example, glucocorticoid-induced TNFR family-related gene (GITR) [177] and glucocorticoid-induced leucine zipper gene (*GILZ*) [50] can inhibit apoptosis through T cell survival regulation that involves the NF-κB transcription activity and the expression of the Fas/Fas ligand (FasL) system [195]. Importantly, Robertson *et al*. [196] have found that activation of the apoptotic mediator CPP32 is a critical event in glucocorticoid-induced apoptosis of thymocytes and that this pathway is inhibited at or upstream of CPP32 by baculovirus anti-apoptotic protein P35. We can consider this finding either as one which is in strike contrast to P35 function in *Drosophila* where it prevents cell death by inhibiting caspase-3 and caspase-9 [95, 96, 61, 238] or as a new function of P35 coopted during evolution. The caspase-inhibiting function of P35 remains conserved also during thymocyte apoptosis which requires, among others, also proteolytic processing of human poly (ADP-ribose) polymerase (PARP), a 116 kDa protein, that is catalyzed by members of the ICE/Ced-3 family and this process is efficiently blocked by the expression of baculovirus P35 [196]. Additionally, Cifone *et al*. [43] demonstrated that caspase activity in dexamethasone (Dex)-induced apoptosis of normal mouse thymocyte is downstream of acidic sphingomyelinase (aSMase) activation required for early ceramide generation, the central molecule of the sphingomyelin cycle acting as endogenous regulator of apoptosis [191, 29, 216]. But prior to these events, early Dex action rapidly induces diacylglycerol (DAG) generation through a protein kinase C (PKC) and G-protein dependent phosphatidylinositol-specific phospholipase C (PI-PLC), two steps which are required for aSMase activation.

While little is known about the glucocorticoid-regulated genes that control thymocyte and steroid-regulated genes in other vertebrate cell deaths, recent studies in *Drosophila* are providing insights into the genetic mechanisms underlying hormone-triggered programmed cell death. Also in *Drosophila* there are studies reporting that moulting hormones, ecdysteroids, serve as survival factors, and their withdrawal or natural decline results in the activation of apoptosis in a specific subsets of neurons within central nervous system [197, 198, 237, 6]. From these studies it not only become clear that ecdysone or ecdysteroids are factors required for apoptosis of specific neurons, but also that ecdysone receptor (EcR) encoded by *EcR*, and its heterodimerization partner Ultraspiracle (Usp) encoded by the homologue of human retinoid X receptor, *usp*, are essential for transducing hormonal signal evoking apoptotic process. However, it was not until the advent of using *Drosophila* larval salivary glands [116, 117, 70, 146] that the longer and systemic line of genes involved in steroid-triggered PCD has started to be revealed. Metamorphosis in *Drosophila* and many other insects is a complex ecdysone-controlled process during which future imaginal tissues such as imaginal disks or abdominal histoblasts proliferate and differentiate into adult structures forming at eclosion a fly-shaped adult whereas obsolete larval tissues like gut and salivary glands undergo programmed histolysis. Cell death process of larval salivary glands is thought to be inducible by elevation of ecdysone titer, rather than by its decline, although it seems to require two hormonal pulses. An important characteristic of *Drosophila* salivary gland cell death is that even originally termed as apoptosis [116, 70] it has very little apoptotic features. Although there was an attempt to name it autophagic cell death [146], and salivary glands in fact display some features of autophagy shortly after pupariation, evidence shows that one can observe strong signs of necrosis and so far unspecified histolysis process in preparative and executive phase [70]. Regardless of this unclarity on cell death type, research on salivary glands was fruitful in determining genes involved in cell death program.

Originally work of RESTIFO and WHITE [194] has shown that *rbp* mutations, alleles of the *Broad-Complex* (*BR-C*) locus, fail to undergo programmed cell death of salivary glands, dorso-ventral class of indirect flight muscles, and gut. The *BR-C* locus is ecdysone-inducible complex locus encoding family of related BTB and Zn-finger containing transcription factors [56] which are necessary for transducing ecdysone signal at initiation of metamorphosis about 16 to 10 hr prior to pupariation. Their results not only identified first particular gene downstream of ecdysone receptor involved in control of hormonally-triggered PCD but also show that factor(s) required for development of histolytic characters and execution phase of cell death programme which take place 12-13 hr after pupariation (AP) are present or expressed much earlier than anticipated from phenotypic appearance. Study of JIANG *et al*. [116] has indicated that second and small pulse of ecdysone about 10 hr AP triggers in salivary glands an induction of *reaper* (*rpr*) and *head involution defective* (*hid*) expression, activation of caspases, and downregulation of *diap1* and *diap2*, two *Drosophila inhibitors of apoptosis* described by HAY *et al*. [95]. Genes *rpr*, *hid* and also *grim* belongs to the single genetic interval named *Df(3L)H99* that has been recovered in the genetic screen for defects in programmed cell death during embryogenesis [1], and encode distantly related caspase inducers [35, 90, 250]. Two other steroid-regulated *E74A* and *E93* early-inducible genes also function in the salivary gland cell death, although their mutations result in different cell death defects, and function in processes other than cell death including pupal and adult differentiation [72, 11, 25]. In contrast to *BR-C* and *E74*, *E93* appears to function more specifically in destruction of larval tissues [145]. However, all early genes like *EcR*, *usp*, *BR-C*, *E74* and *E93* impact on the transcription of programmed cell death genes including *rpr*, *hid*, *croquemort* (*crq*; CD36 homologue), *Ark* (*Ced4/Apaf-1* homologue) and *dronc* (*Nc; Nedd2 like caspase*) during larval tissue destruction [77, 78, 120, 199, 263, 60, 117, 145], suggesting a potential mechanism for steroid-triggered cell death. Direct induction of Dronc caspase by ecdysone in contrast to other caspases which are activated via Rpr and Hid remains physiologically unclear, although Dronc with its unusually long prodomain [36] may work as initiator of caspase cascade. It was shown that competence of these late or „executive" genes to respond to the second pulse of ecdysone is mediated by mid ecdysone responsive gene β*FTZ-F1* encoding a member of the nuclear hormone receptor superfamily and that act almost at the top of this signaling hierarchy [253, 25, 117].

As recently shown by LEE and BAEHRECKE [146], *E93* might have specific role in steroid-triggered cell death machinery in *Drosophila* salivary glands and also in other tissues. The E93 protein, which shares homology with putative transcription factors, if expressed ectopically, is sufficient to cause rapid and widespread cell death in embryos of *Df(3L)H99* genetic background, thereby eliminating any potential contribution from caspase inducers *rpr*, *hid*, and *grim*. However, these abilities of E93 are probably not exclusively used by doomed larval tissues like salivary glands or gut, because it works in a complex with many other factors within ecdysone hierarchy.

Unexpected link between steroid-regulated transcriptional hierarchy and the role of cytoskeletal proteins in the implementation of programmed cell death comes from the finding that tumour suppressor gene *lethal (2) giant larvae* (*l(2)gl*) coding for cytoskeletal protein p127 has dosage-dependent effect on the speed of ecdysone-triggered salivary gland histolysis in *Drosophila* [70]. Salivary glands of null mutation of *l(2)gl* locus fails to undergo PCD in response to ecdysone in similar way as *rbp* alleles of *BR-C*. Using transgenic animals it was revealed that reduced levels of p127 expression delays disintegration whereas overexpression accelerates process of PCD without affecting the duration of third larval instar and pupal development, or affecting speed or intensity of cell death in imaginal discs. Striking effect of $l(2)gl^{-}$ null mutation is in prevention of nuclear uptake of BR-C transcription factor, which is caused to accumulate in the cytoplasm. Overexpression of nonmuscle myosin II heavy chain (nmMHC), the contractility of which is negatively regulated by p127 protein, results in a retention of BR-C Z1 in the cytoplasm of salivary gland cells and considerably delayed their histolysis. Use of the $zip^{E(br)}$ allele, a neomorphic mutation of the *zipper* gene encoding nmMHC, revealed that a large proportion of the *l(2)gl* +/+ $zip^{E(br)}$ transheterozygotes were developmentally arrested at the larval-pupal transition phase. These larvae displayed a phenotype similar to that observed in *l(2)gl* larvae with tumours in the imaginal discs

and the brain hemispheres. In the salivary glands of these animals, BR-C Z1 was predominantly detected in the cytoplasm, although both protein components of functional ecdysone receptor, EcR and Usp, were present in the nuclei.

Another but not least feature of accelerated cell death in transgenic animals overexpressing *l(2)gl* is significant change in the pattern of puffs on polytene chromosomes [70]. Shortly after pupariation of transgenic animals normal puffing pattern disappears, and surprisingly it is not replaced by any kind of „abnormal" puffs, but one particular puff at locus 52A is induced prematurely. In wild type siblings this puff is active during puff stages 18 to 20 about 10-11 hr AP. Molecular cloning and sequence analysis of DNA from this locus revealed presence of subunit D of the vacuolar ATPase (*vATPase-D*), which was found to be inducible by ecdysone and normally expressed in the time of puff appearance. This coincides with massive extrusion of protein components from cells into lumen of salivary glands, the process strongly resembling necrosis. vATPase, a membrane-bound proton pump, is known to be responsible for acidification of the cytoplasm, and might be necessary for regulated extrusion of proteins as a prerequisite of forthcoming execution phase of cell death programme. In transgenic larvae or prepupae with ectopically increased amounts of p127 protein also process of protein extrusion into lumen is greatly accelerated, and salivary gland histolysis is completed 2 to 3 hr AP, *i.e.* 8 to 9 hr prior second and small pulse of ecdysone which is thought to be required as trigger for cell death program. Thus, these data not only show that the cytoskeletal components can play a direct and critical role in the implementation of programmed cell death triggered by steroids via interaction with transcription factors, but also may provide sufficient milieu to execute whole cell death programme without second exposure to the hormone.

Studies on *Drosophila* larval salivary glands have been emphasized because utility of this tissue for studies of steroid signaling in combination with genetic tools has provided the most comprehensive information on transduction pathways involved in developmental cell death, and also can be even more fruitful in near future with using techniques like DNA-microarrays [251].

3b. Thyroids

It was shown as early as in 1960s that thyroid hormone-induced RNA and protein synthesis are required for the destruction of the tadpole tail during *Xenopus* metamorphosis [229]. Destruction of tadpole tail and well-known precocious induction of amphibian metamorphosis by exogenous thyroid hormone are associated with the process of cell death [232]. Recent studies on amphibian metamorphosis have revealed that processes of thyroid hormone receptor (TR) gene regulation along with cell death play an important role in the maturation of the central nervous system and organolysis [14]. Tadpole larval intestinal epithelial cells are prone to apoptosis at the time of metamorphosis, and prior to their degeneration they express high levels of a novel transcription factor bZip which is directly induced by triiodothyronine (T3) via TRβ receptor isoform [111]. However, how pro-apoptotic signal of bZip is further transduced in these cells remains unclear. Shi and Ishizuya-Oka [111] and Shi *et al*. [214] have shown that effects of thyroid hormone on *Xenopus* embryonic development requires functional heterodimer between TRβ and the retinoid X receptor (RXR), the natural ligand of which is 9-*cis* retinoic acid and can bind this heterodimer complex along with thyroid hormone. Interesting feature of this tadpole metamorphosis model is that T3 hormone not only induce to 30-fold levels the expression of TRβ but also downregulates at least by 50% expression of TRγ isoform which is not involved neither in metamorphosis-associated cell differentiation nor in apoptosis [114]. As it was shown by Berry *et al*. [15] process of tadpole tail resorption, which involves also apoptosis, uses multiple programs and TR and RXR isoforms. Besides above mentioned TRβ, the expression of TRα isoform is also required, and TR heterodimers with either RXRα or RXRβ receptor isoforms act functionally during tail resorption while upregulating expression of *stromelysin-3* gene more-less equivalently [110]. Vertebrate brain differentiation requires precisely regulated death of glial cells which is triggered by T3 and mediated by TRα receptor isoform heterodimerizing with RXR. Unusual step in this process is specific suppression of protein kinase C (PKC) activity and *pkC* plus *bcl-2* gene expression by T3/TRα pathway using distinct but

not well characterized mechanisms [152]. In this context, downregulation of PKC activity and suppression of *pkC* gene are logical events since growth factors including TGFβ, FGF and platelet-derived growth factor (PDGF) are all strong inducers of PKC and thus act as potent survival factors which are almost permanently available. On the other hand, T3-triggered apoptosis of neurons in the developing cerebellum is mediated mostly by TRα isoform of the receptor without participation of TRβ [42], suggesting that cell-specific machinery of signaling receptors is used to implement death program in the developing organism. This notion is further supported by similar situation found for EcR isoforms in various tissues of metamorphosing *Drosophila*. Tissues which will proliferate and/or differentiate into pupal and adult structures predominantly express EcR A isoform of ecdysone receptor, while obsolete larval tissues doomed to die predominantly express EcR B1 isoform [197, 228, 237]. In the light of these data, it is reasonable to conclude that speciation of nuclear hormone receptors to function-linked isoforms or isoform dimers had to happen quite early in the evolution of eukaryotic metazoans.

Another level of regulation was observed in the control of TR isoform expression during *Xenopus* larval epithelial apoptosis. As in tadpole tail, also in larval epithelium T3 is an universal trigger for both the cell death and the subsequent proliferation. These two events appears to represent two independent phases of T3 action during which hormone induces increased expression of TRβ receptor isoform, but not TRα [212, 213]. Expression of TRβ occurs in two peaks corresponding to two separate developmental phases, apoptosis of larval epithelium and proliferation of adult epithelial cells, thus concluding that the same receptor isoform may be implicated within the same tissue in two different processes. Besides this regulation, both TRα and TRβ isoforms play dual function in the control of tail and larval epithelium apoptosis. First, unliganded TRs function initially as repressors of T3-inducible genes in premetamorphic tadpoles to prevent precocious metamorphosis ensuring a proper period of tadpole growth, and later in liganded status as activators of these genes to initiate metamorphic process [203]. Repressing function of TR-RXR heterodimers is due to the ability of unliganded TR-RXR heterodimers to bind to TR response elements (TRE) present in the target genes, while repressing their basal transcription [73, 74, 192].

Metamorphosis promoting and cell death-triggering effects of thyroid hormone in amphibians are prevented by action of the peptide hormone prolactin [233]. Prolactin prevents the rapid T3-induced upregulation of TRα and TRβ mRNAs in *Xenopus* tadpole tails, followed by the inhibition of the *de novo* activation of 63-kDa *keratin* gene [8] as well as collagen and collagenase genes expression [182]. This was suggesting that prolactin exerts its juvenilizing action by preventing the upregulation of TR by its autoinduction by T3 [230, 231, 232]. Nevertheless, this conclusion seems to be oversimplification or it explains just one aspect of prolactin action, since question remains what mechanisms are involved in these preventive effects of prolactin ? The first step in prolactin action involves its interaction with a specific membrane receptor that belongs to the rapidly growing family of cytokine receptors that currently includes also receptors for growth hormone, erythropoietin, granulocyte colony-stimulating factor, interleukins 2-7, 9-13 and 15, interferons, thrombopoietin, leptin, oncostatin M and some other factors [21, 28]. Upon receptor dimerization, an early and most likely initiating event for prolactin action, and other members of this family, is the activation of one or more members of the Janus (or JAK/Src/Fyn/Tec) family of tyrosine kinases, which are not a part of the receptor but associated proteins that subsequently phosphorylate prolactin receptor and other cellular proteins [108, 211, 241]. Other molecules recruited to the activated prolactin receptors are phosphatase SHP-2, guanine nucleotide exchange factor Vav, and signaling suppressor SOCS [44]. Among direct JAK substrates is also the transcription factor Stat5 whose phosphorylation mediates the transcriptional activation of several target genes like those coding for β-casein, sodium-potassium cotransporter NKCC1, X-linked inhibitor of apoptosis protein (XIAP), connexin 32, and genes involved in cell cycle control [135, 167]. Thus, induction of mitogenic factors and XIAP during G1 and S phases of cell cycle can explain, at least in part, antiapoptotic effects of prolactin that is contradictory to action of T3 and TR receptors. Involvement of G-protein coupled receptors as targets of prolactin [143] broadens potential signalling ramification of this hormone even

more, leading to activation of p53 tumor suppressor that upregulates expression of proapoptotic Bax protein which is not known to be suppressible by T3 [2, 130].

In addition, thyroid hormones have been implicated also in facilitation of human lymphocyte apoptosis *in vivo* as well as *in vitro*. Treatment of T lymphocytes with thyroid hormones is accompanied by reduction in Bcl-2 protein expression, production of reactive oxygen species, and reduction of mitochondrial delta/psi transmembrane potential, finally resulting in apoptotic death [165]. One of the several mechanisms which has already been confirmed to work also for cell death, but being used widely by nuclear receptors, is regulation of histone deacetylase, an enzyme implicated in transcriptional repression, and shown to be recruited by unliganded TR [202]. In addition to its own effects, T3 hormone can act synergistically on apoptosis by potentiating effects of *all-trans*-retinoic acid (see below) in promyeloleukemic HL-60 cells [92]. As it was shown, this synergism is not a simple mechanistic results of TRα and TRβ isoforms action in heterodimer complex with the RXR partner. T3 potentiates arrest in G1 phase of cell cycle only in the presence of RAR-specific agonists but fails to do so in the presence of RXR-specific agonists suggesting that arrest in G1 which eventually results in apoptosis is mediated by TR/RXR heterodimer exclusively binding only T3 within TR moiety; RXR partner remains unliganded. Besides G1 arrest, the TR/RXR-bound T3 selectively induces expression of *bfl-1* and represses expression of *bcl-2* genes, respectively, thus enhancing apoptotic effects of *all-trans*-retinoic acid. This mechanism explains how T3 in combination with RAR-specific agonists may be used as a chemotherapeutic agent in acute leukemias.

Quite specific case of PCD is represented by autoimmune and degenerative diseases which are caused by excessive apoptosis of a specific group or type of cells. Graves' disease and Hashimoto's thyroiditis, two chronic inflammatory organ-specific disorders of the endocrine system, are resulting from Fas-mediated thyrocyte destruction by infiltrating T lymphocytes due to altered susceptibility of the thyroid [22, 169, 63]. Fas is an apoptotic receptor found on the surface of a number of cell types. Malfunction of the Fas system accelerates autoimmune diseases, whereas its exacerbation may cause tissues destruction. Soluble Fas (sFas) molecule is a receptor that lacks the transmembrane domain due to alternative splicing, and therefore blocks Fas-mediated apoptosis. The concentration of serum sFas in Graves' disease and Hashimoto's thyroiditis patients correlated with free thyroxine. By other words, thyroid hormones via their TR receptors can modulate apoptosis of target tissues by switching between Fas and sFas expression [215, 222]. In this case, TRs influence splicing of *fas* primary transcript by so far unknown mechanism, and it remains to be investigated whether there are differences between T3, T4 and synthetic agonists or antagonists of TRs on this process.

3c. Retinoids

Retinoids, vitamin A-related compounds that are able to prevent cancer, have been shown to cause cell death and affect multiple signaling like IGF-, TGFβ, and AP1-pathways which all have been found to modulate cell death under specific circumstances [258]. It is believed that retinoid's ability to suppress proliferative growth and prevent development of breast cancer or myeloma in animal models is due to their effects on cell death [180, 219]. However, combination of retinoids with steroids like dexamethasone is not only more efficient in inhibitory action on myeloma cells growth but also cause myeloma and other cancer cells to become less able to overcome action of interleukin 6 (IL-6). Because under specific experimental conditions antiproliferative and proapoptotic activities of retinoids are separable, their therapeutic effect is considered to be caused by dual mechanism involving both decrease in a proliferative fraction and increase in apoptotic fraction of tumor cells [226, 40, 39]. Since IL-6 is the most important growth factor of cytokine family for multiple myeloma cells, along with retinoids its signalling pathway is another potential target for therapy, increasing thus chances for successful treatment [149, 104].

In spite of the fact that retinoids are ligands for retinoid receptors, the retinoid X receptor (RXR) class of which specifically plays a role of universal promiscuous dimerization partners for a number of nuclear receptors [30, 121, 161], *9-cis*-RA, *all-trans*-RA, and their synthetic agonists or antagonists have profound effects of their own on programmed cell

death. Independent molecular and *in vitro* studies have established that RXR homodimers bind preferentially *9-cis*-RA, while retinoic acid receptor (RAR) homodimers bind *all-trans*-RA, and for a long time it was appreciated that RXR and RAR homodimer receptors transduce *9-cis*-RA and *all-trans*-RA signaling, respectively [101, 190, 158, 159, 160, 174, 30]. However, present work established that *9-cis*-RA is a pan-agonist binding with high affinity to both type of receptors, RARs and RXRs [88, 161, 240, 85, 91]. Moreover, genetic studies using knockout mice indicate that functional *in vivo* receptors for both *9-cis*-RA and *all-trans*-RA are heterodimers between RAR and promiscuous RXR receptors rather than homodimers [121, 161, 91]. All these properties of retinoid receptors are crucial for transducing their cell-specific apoptotic signals. Various carcinoma cell lines including osteosarcoma cells OOS and HOS, acute promyelocytic leukemia cells NB4, non-small cell lung carcinoma, and squamous carcinoma are prone to undergo apoptosis after treatment with retinoids, however the response appear to be cell line and receptor specific [4]. For example, human ovarian cancer cell lines Ovcar-3 and Ovcar-8 respond by induction of apoptosis not only to *9-cis*-RA and *all-trans*-RA but also to *13-cis*-RA while all 3 ligands require presence of RARβ receptor; RARα and RARγ are negligible [221]. Different situation was described for osteosarcoma cells in which *9-cis*-RA required both RARs and RXRs receptors whereas apoptotic action of *all-trans*-RA required only presence of RARs for binding [105]. Gastric cancer cells undergo apoptosis in response only to *9-cis*-RA and presence of RXRα receptor [207] whereas oral squamous carcinoma cell lines HSC-4 and Ho-1-N-1 for the same ligand required sufficient expression of RARβ receptor [97]. On the other hand, human breast cancer cells responding by cell death to *9-cis*-RA require sufficient level of RARα, RARγ as well as RXRα, but two RARα-selective synthetic ligands, AM80 and AM580, either strongly potentiate effects of *9-cis*-RA or are sufficient to induce apoptosis in its absence [209]. In the NB4 acute promyelocytic leukemia line *9-cis*-RA, *all-trans*-RA, TTNPB, pan-RAR agonist, and AM580, RARα-selective agonist, all induced growth arrest and apoptosis, but RXR-selective and RARβ-selective agonists have poor if any apoptotic-inducing activity [85]. In several carcinomas, RARγ-selective agonist, CD437, shows very powerful effect on facilitation of cell death while RARα-selective agonists fail to induce any apoptotic activity [118, 224].

In contrast to wealth of information on steroid hormone triggered cell death pathways and target genes, targets of retinoid action leading to activation of cell death machinery are just being revealed in very recent years. Retinoids were shown to cause inactivation of NF-κB, activation of Retinoblastoma (Rb) protein, upregulation of *c-myc* and ornithine decarboxylase expression, increased activity of transglutaminase II followed by G1 arrest [88, 82, 225, 59]. Differential display study of *all-trans*-RA-induced genes during apoptosis of T-cell lymphoma line has identified following genes: prothymosin, p78 serine/threonine kinase, interleukin-1β-stimulating protein, glucocorticoid receptor, gastrin-binding protein, heat shock protein 90, chloride ion channel protein-3, ezrin, and vimentin [244]. In leukemic HL-60 cells RARγ-selective agonist induce lysosomal leakage as revealed by using lyososomotropic probes [262]. Quite unexpected was finding or Pendino *et al*. [187] that retinoids potentiate apoptosis of acute promyelocytic leukemic cells by downregulation of telomerase activity which results in shortening telomere length.

Receiving or perception of apoptosis-triggering signal does not account for automatic execution of the process. There are multilevel control mechanisms that can either revert, enhance or attenuate apoptotic process. This has been well documented for action of RA on apoptosis via RAR proteins. Liu *et al*. [151] demonstrated that antiapoptotic BAG-1 protein (also known as RAP46) can regulate *all-trans*-RA activities through the interaction with retinoic acid receptor (RAR), so explaining how elevated levels of BAG-1 are able contribute to resistance of cancer cells to retinoids. BAG-1 is capable bind directly RARs, but not RXRs, and effectively inhibit binding of RAR/RAR homodimers or RAR/RXR heterodimers to RAR-response elements (RAREs) in the *bcl-2* promoter, thus preventing downregulation of its expression. On the contrary, insulin-like growth factor binding protein 3 (IGFBP-3) binds only RXR receptors, with highest affinity for RXRα, and does not interfere with RARs. IGFBP-3 mediates apoptotic signals on its own, however, RXR-selective

ligands potentiate its effects, and interaction between IGFBP-3 and RXRα enhances RXRα binding to RXREs, although molecular mechanism of this activity needs to be elucidated [150].

Most if not all above described cases of retinoid-triggered PCD miss integrity of the organism where also a therapeutic treatment would take place and might have very different outcomes. Therefore, study of developmental cell death within an integral organism should provide clues as to how retinoid signalling is transduced to result in cell-specific apoptosis. It should be stressed that PCD is one of the major driving forces that shape and pattern the organs and tissues of a developing organism or during postembryonic development like in metamorphosis. Rodriguez-Leon *et al*. [200] have analyzed effects of *all-trans*-RA on the interdigital necrotic zones (INZ), apoptotic form of cell death, that occurs during the outgrowth of the vertebrate limb. They have shown that *all-trans*-RA can control chick INZ by promoting bone morphogenetic proteins (BMPs) gene expression and simultaneously repressing the chondrogenic potential of BMPs. The appearance of apoptotic features was preceded by upregulation of *bmp-4* and *bmp-7* genes, and was effectively blocked by specific *all-trans*-RA antagonists; these antagonists had no effect on cell death induced by BMP treatment, indicating that *all-trans*-RA is a physiological regulator of INZ, acting upstream of BMP signalling. The BMPs belong to the family of transforming growth factors-β (TGFβ) the signalling pathway of which has been elucidated in detail in *Drosophila* where it is encoded by the single *decapentaplegic* (*dpp*) gene [183, 252]. Dpp acts as secretory morphogen at short distance in the role of a ligand for membrane serine/threonine protein kinase receptors which mediate its signalling to two nuclear proteins, Schnurri and Brinker, required for dorso-ventral axis determination, alimentary track, eye, leg and wing morphogenesis, and definition of the compartment boundaries [51, 168, 3].

Identical process, interdigital webbing, is regulated by retinoids also in mice where in the knockout mutants it was shown that RARγ and RARβ or RARγ/RARβ genotypes fail to transduce *all-trans*-RA signal resulting to persistence of the fetal interdigital mesenchyme due to absence of apoptosis [62]. In wild type mouse several genes are targeted by *all-trans*-RA via RARs: *bmp-4* and *bmp-7*, *bcl-2*, *bax*, *p53* and members of *Hox* gene family. The *bmp-4*, *bmp-7*, *bax* and *p53* are not induced whereas *bcl-2* is not downregulated in RARγ, RARβ or RARγ/RARβ knockouts, thus linking INZ cell death to their preceded regulation of expression. The *Hox* genes, vertebrate homologues of *Drosophila* homeotic genes, are well known targets of retinoid signalling and play a key role in hindbrain and neural crest patterning, lung and limb morphogenesis [179, 131, 205, 246, 155] but their function in cell death need yet to be firmly established.

Therapeutic application of retinoids has brought potentially new ideas into basic research of their signalling pathways. For example, Piacentini *et al*. [189] have investigated the effect of cisplatin and retinoic acid (RA) on apoptosis of human neuroblastoma cells in relation to the cell cycle. Their results suggest that RA and cisplatin have two separate cell cycle sensitive periods for issuing programmed cell death: G1 phase for action of RA, and G2/M phase for cisplatin.

3d. Vitamins D

Vitamin D is a cholesterol derivative generated by photolysis, which is essential for normal bone structure and the maintenance of serum calcium homeostasis [64, 24, 122, 257]. Its active metabolite is 1,25-dihydroxyvitamin D_3 (VD_3), also known as calcitriol, the genomic actions of which are mediated through the nuclear receptor, VDR, sharing strong homology to steroid/thyroid/retinoid superfamily of receptors [69, 112]. The VDR functions as homodimer or as heterodimer with the RXR to bind vitamin D responsive elements (VDREs) within target gene sequences [19, 148, 113]. In addition to its role in calcium homeostasis and bone metabolism VD_3 exhibits anti-inflammatory and immunomodulatory properties, therefore VD_3 and its analogues are potential therapeutics in psoriasis, multiple sclerosis, rheumatoid arthritis, diabetes and transplantation [16, 94, 112]. The discovery of VDR expression in peripheral blood monocytes and activated T-lymphocytes, and the observation that T-cell mediated delayed hypersensitivity response is impaired in vitamin D deficiency but suppressed by VD_3 suggests a role in modulating cellular immune response

[147, 17, 259]. Proapoptotic properties of VD_3 were disclosed in mid 1990s as result of its potent inhibitory action against growth of MCF-7 breast cancer cells [248]. Growth inhibition, at least in part, was due to induction of MCF-7 cell death, as revealed from occurrence of pyknotic nuclei, chromatin and cytoplasmic condensation. The apoptotic action of VD_3 was strongly potentiated by addition of tamoxifen, an antiestrogenic compound. Some newly synthesized epi-analogues of VD_3 were shown to have even stronger pro-apoptotic effects than natural VD_3 [65], and apoptosis-triggering action of VD_3 has been extended also to other tumour-derived cell lines including promyeloleukemic HL-60 [26], monoblastoid U937 cells [100], human prostate cancer LNCaP line [264], skin basal cell carcinomas [193], and bladder carcinoma [133]. Upon treatment with VD_3, MCF-7 cells respond by upregulation of clusterin and cathepsin B and downregulation of *bcl-2* expression [171]. The *bcl-2* gene seems to be a direct and more general target of VD_3/VDR action because its ectopic expression is sufficient to prevent VD_3-inducible apoptosis of LNCaP prostate carcinoma cells [20]. Using various VD_3 analogues, Danielsson *et al*. [52, 53] have found that VDR can show promoter selectivity based on type of ligand; even though two new analogues, CB1093 and EB1089, are both very potent inhibitors of proliferation of breast MCF-7 and melanoma WM1341 lines, the CB1093 is more effective inducer of apoptosis than EB1089 due to stronger downregulation of *bcl-2* expression at 10-fold lower concentration in comparison to EB1089. As shown by Narvaez and Welsh [172], induction of apoptosis by VD_3 is not associated with changes in p53, Rb and p21 regulation. Transfection of VDR into rat C6 glioma VD_3-resistant cells is sufficient to restore their susceptibility to VD_3 and to induce apoptosis accompanied by increased expression of *p53*, *c-myc* and *gadd45* genes [55]. Human glioblastoma cell line HU197 responds to VD_3 treatment by activation of sphingomyelin pathway leading to production of ceramide and execution of apoptosis [157], resembling dexamethasone action in thymocytes [43]. Apoptosis inducing action of VD_3 in WM1341 cells requires not only VDR but also RXRα indicating that not VDR homodimer but VDR/RXR heterodimer is employed. Additionally, VD_3 apoptotic effects are significantly boosted by cotreatment with CD437, RARγ selective agonist [54], although type of interaction between retinoid and VD_3 signaling pathways was not outlined. Cross-talk between noradrenergic and vitamin D pathways was observed in glioma cells were noradrenaline has inhibited programmed cell death induced by VD_3, but its molecular mechanism remains elusive [27].

Above depicted VD_3/VDR-cell death system deals exclusively with transformed carcinoma cells and there is very limited information on the role of VD_3/VDR in apoptosis of normal tissue. Work of Narvaez *et al*. [173] indicated that lowered expression of VDR in mammary gland causing an increase in VD_3 resistance can be a prerequisite for development of breast cancer. Impairment of human osteoblast function may be reduced by VD_3 treatment leading to increased apoptosis of osteoblastic cells which otherwise show significantly reduced levels of alkaline phosphatase, osteocalcin and collagen type I mRNAs [140]. Expression of these genes, however, may serve as a marker of VD_3 insufficiency rather than any causal relationship to VD_3-induced osteoblast apoptosis. Potentially more reasonable mediator of VD_3/VDR apoptotic action appears to be Wilms' tumor gene product, WT1, required for normal kidney development [242]. Exceptional effect of VD_3 was observed in normal human thyrocytes where inhibits their apoptosis by up-regulating *bcl-2* expression [245].

3e. Peroxisome proliferators, prostanoids and fatty acids

Peroxisome proliferators (PPs) are a diverse group of nongenotoxic chemicals that in rodents cause hepatic peroxisome proliferation, liver enlargement, increased replicative DNA synthesis, suppression of cell death and development of cancer [34]. PPs are ligands for peroxisome proliferator-activated receptors (PPARs) that have been implicated in metabolic diseases, such as obesity, diabetes, and atherosclerosis, due to their activity in liver and adipose tissue on genes involved in lipid and glucose homeostasis [41]. PPARs are members of steroid/thyroid/retinoid receptor superfamily of nuclear receptors and for their action require dimerization with RXRs [75]. PPARα and PPARγ represent related but distinct members of this family; PPARα signaling is modu-

lated by long-chain fatty acids, whereas PPARγ ligands are potent antidiabetic agents including natural 15-deoxy-Δ-12,14-prostaglandin J_2 (15Δ-PGJ_2). Human and mice breast cancer cell lines are sensitive to 15Δ-PGJ_2 and synthetic PPARγ ligand troglitazone (TGZ) treatment, and respond by inhibition of proliferation and lipid accumulation. Concurrent treatment with 15d-PGJ_2 or TGZ and *all-trans*-RA, an RAR ligand, induces apoptosis associated with dramatic decrease in Bcl-2 [66]. Similar effect of 15d-PGJ_2 and other PPARγ agonist was observed in normal and malignant B-lineage cells [184], astrocytes [33], cerebellar granule cells [99], vascular smooth muscle cells [181], human trophoblasts [208], synoviocytes under arthritic conditions [124], human colon cancer cell line HT-29 [260], and mouse T-lymphocytes [93], thus documenting that PPARγ and its agonist can induce cell death in various organs outside liver and adipose tissue. There was also a report on induction of vascular smooth muscle cells apoptosis by PPARα and its ligand docosahexanoic acid via stimulation of p38 mitogen-activated protein kinase [58]. With regard to the recent research interest in PPARs, there is significantly less knowledge on the mechanisms how these ligand binding receptors transduce or amplify apoptotic signals. PPs and PPARγ can enhance tumor necrosis factor (TNF)-related apoptosis [86], cause arrest cell cycle in G1 phase and downregulate ornithine decarboxylase [222] or cyclooxygenase-2 [260]. Very recently Park *et al*. [185] suggested that specific subset of PPAR receptors, PPARΔ, are downstream target of the adenomatous polyposis coli (APC)/β-catenin oncogenic pathway in colorectal carcinogenesis which itself if mutated has strong antiapoptotic consequences. Intracellular signaling messengers mediating cross-talk between APC/β-catenin and PPARΔ pathways remain to be identified.

3f. No ligands, just receptor(s)

Human genome contains about 50 nuclear receptors, about half of which are orphan receptors; *Drosophila* genome encodes 21 nuclear receptors and only one, EcR, has known ligand; *Caenorhabditis* genome is supposed to code for over 270 nuclear receptors, and all of them are considered to be orphans *i.e*. without known ligand. If there are so many orphan receptors, what is their part in controlling PCD? Until now we have been discussing ligand-mediated triggering of PCD via nuclear receptors, and question arises whether nuclear receptors can contribute to regulation of PCD also without ligands? An example is orphan steroid receptor Nur77, also called NGFI-B, that plays an important role in steroidogenesis and testicular cell death [220]. Increased expression of Nur77 is implicated also in glucocorticoid independent T-cell apoptosis [256, 162]. Activity of Nur77 receptor is regulated by protein kinase B (PkB) or Akt that phosphorylates Nur77 at Ser350 to decrease its transcriptional transactivation potential [186]. The Nur77-related PCD of T cells can be inhibited by retinoids via facilitation of increased heterodimerization of Nur77 with RARs or RXRs receptors [119].

Retinoids negatively affect also apoptotic properties of TR2-11, another nuclear orphan receptor implicated in apoptosis of P19 cells [144]. Promoter of *TR2-11* gene contains several RAR/RXR response elements which attenuate its expression. Retinoid-related orphan receptor γ (RORγ) has opposite function on lymphoid apoptosis which is strongly increased in RORγ null mice along with upregulated expression of anti-apoptotic Bcl-X protein [139]. Information on orphan nuclear receptor function in PCD is very sporadic to date, and thus we can only speculate if they promote their effects solely or in coordination with larger hierarchic cascade as in the case of ecdysone-triggered cell death in *Drosophila*. In this model, expression of two orphan receptors, βFTZ-F1 and DHR3, swithin ecdysone cascade ensures acquisition of the competence to respond to the ecdysone and also ensures that responses to the second pulse of ecdysone will be distinct from first pulse [142].

4. Conclusions and future directions

Picture emerging from above described data raises the simple question whether there exist any general or integral mechanisms underlying action of lipophilic hormones and their receptors on programmed cell death ? Existence of cell death machinery triggered by steroids, thyroids, retinoids and other lipophilic ligands indicates that it is involvement or participation of nuclear receptors as transcription

factors that mediate death/survival signals rather than the property of these ligands. However, it remains to be answered whether participation of nuclear receptors in implementation of cell death was ancient receptor's property before acquiring ability to bind ligands. Based on comparison of nuclear receptors from all variety of organisms from nematodes to mammals, the recent studies conclude that binding of ligands to nuclear receptors was acquired later during their evolution [67, 68, 164], holding the same to be true for their role in programmed cell death when considering evolutionary aspect. This viewpoint is strengthened through the lack of any evidence for involvement of *C. elegans* orphan receptors in PCD, the genome of which encodes over 270 nuclear receptors, and none of them is known to take part in the nematode's apoptosis and to bind any ligand [218, 156, 217]. Nevertheless, few mammalian orphan receptors, Nur77, TR2-11 and RORγ, were found to trigger cell death but their function in apoptotic signaling need to be established. Still, this situation strongly supports notion that participation of nuclear receptors in PCD is their innate property independent of ligand.

Such a conclusion is not surprising given the following facts. Regardless of the type of the ligand, in all cases, homo- or heterodimers of nuclear receptor proteins were found to be required to mediate survival or death signaling. It appears that after binding a ligand, functional receptor complexes become trapped within mechanisms common to most if not all members of this superfamily *e.g.* interaction with BAG-1/RAP46 anti-apoptotic proteins or repression of transcription through histone deacetylase *etc* [202]. The integration of nuclear receptors into specific functional protein complexes are their innate property and are not principally affected by ligands the role of which becomes limited to switching between given possibilities like modification or facilitation of any of these particular mechanisms. Given these data together, they suggest that nuclear receptors and their ligands seem to act in two major and closely related functions: (1) stimulate or facilitate terminal differentiation of tissues during development or as a part of adult homeostasis, and (2) then participate in the elimination of these terminally differentiated tissues via tightly regulated process of PCD.

Clearly, a vast amount of data has been accumulated on induction of cell death in various systems by different ligands, but much of the work offers only a small glimpse of deeper understanding how many intracellular cascades are involved in implementation of the signal. Numerous cases described above rely upon the simple model in which nuclear receptor(s) after binding a ligand transactivate/transrepress expression of target genes the products of which are then shifting a balance between death and survival factors to one direction. However, it is very difficult to comprehend how general or specific these findings and conclusions may be. A good examples are interaction of BAG-1/RAP46 anti-apoptotic protein with a number of nuclear hormone receptors, including receptors for glucocorticoids, estrogen, thyroid hormone and retinoids (Liu *et al.* [151], or timing of cell death program execution in dependance on the dosage of *Drosophila* tumour suppressor gene *l(2)gl* coding for a cytoskeletal protein (Farkas and Mechler [70]). To gain further insight into the mechanism of hormone-triggered cell death it will be required to consider participation of several regulatory pathways that could modulate cellular responses, and thus increase the complexity of steroid signaling. For example, there is limited if any knowledge on the role of mitochondrial systems in steroid, thyroid or retinoid-triggered apoptosis. On the other hand, Bergmann *et al.* [13] and Kurada and White [138] in *Drosophila* have shown that even though generally known death inducers *rpr*, *hid* and *grim* are all transcriptionally regulated by a variety of inducing stimuli, Hid protein function can be repressed by active Ras signalling, independently of transcriptional regulation. The role Ras or other signaling pathways on hormone triggered cell death was not seriously addressed in any study until now.

Research on *Drosophila* provided clues about direct transcriptional regulation of at least one of the caspases, but in vertebrate models we lack information on the role of granzyme and similar death effectors in cell death implementation. On the contrary to the situation in mammals, especially humans, where multiple drugs are being systematically tested for their efficiency against various diseases including cancer, in *Drosophila* we miss systemic study on the various ecdysone ago-

nists and antagonists in their ability to display different efficiency on cell death induction. Recent study of Farkas and Sláma [71] on induction of puffing, salivary gland secretion and imaginal disc evagination by nonsteroidal ecdysone agonists just indicated that potentially also subsequent processes including PCD can be triggered by these agents, but cell death response to acylhydrazine compounds may bear different sensitivity or affinity to these ligands and shed more light on the role of ligands and their metabolites in preparative and execution phases of this process.

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