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Programmed Cell Death in the Nervous System – a Programmed Cell Fate?

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Abstract

Studies of developmental cell death in the nervous system have revealed two different modes of programmed cell death (PCD). One results from competition for target-derived trophic factors and leads to the stochastic removal of neurons and/or glia. A second, hard-wired form of PCD involves the lineage-specific, stereotypical death of identifiable neurons, glia or undifferentiated cells. Although traditionally associated with invertebrates, this “programmed PCD” can also occur in vertebrates. Recent studies have shed light on its genetic control, and have revealed that activation of the apoptotic machinery can be under the same complex, combinatorial control as the expression of terminal differentiation genes. This review will highlight these findings, and will suggest why such complex control evolved.

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Introduction

Programmed cell death (PCD) is essential for the development and homeostasis of the nervous system. As more complex nervous systems evolved, so did the need for this powerful mechanism of “polishing away” the unwanted, supernumerary or abnormal. Indeed, there appears to be a positive correlation between neural cell number and the prevalence of apoptosis in different organisms: from 10% in the nematode *Caenorhabditis elegans* (*C. elegans*) [1,2] and 25% in the ventral nerve cord of the fruit fly *Drosophila melanogaster* (*Drosophila*) [3], to about 50% in mammalian nervous systems [4,5].

There are two fundamentally different ways in which PCD occurs in developing nervous systems: stochastic PCD and stereotyped PCD, herein referred to as “programmed PCD”. In stochastic PCD, typically large numbers of cells compete for a limiting factor. This trophic factor is required for the survival of the cells and can be presented to their cell bodies, axons or dendrites. A classic example of stochastic PCD is the removal of large numbers of sensory neurons in mammalian peripheral ganglia upon competition for limiting neurotrophic factors present in their target organs ([5]; Figure 1A). Although stochastic PCD has traditionally been associated with vertebrates, recent studies indicate that this form of PCD also occurs in invertebrates such as *Drosophila* [6-8]. Given the existence of many excellent reviews concerning “stochastic PCD” (see, for example [5,9]), this review will focus on programmed PCD.

Figure 1. Stochastic vs. programmed PCD. (A) In stochastic PCD, a group of initially equivalent cells fated to die compete for a non-cell autonomous trophic factor. Only the cells that receive this trophic factor will survive, and experimentally induced increases or decreases in the amount of trophic factor leads to accompanying changes in neuronal cell numbers. (B) In programmed PCD, the cell-autonomous expression of “cell death determinants” in specific cells (E and F) leads to their activation of the core cell death pathway.

In contrast to the unpredictable survival of a subpopulation of initially equivalent cells, only cells that are predetermined to die by lineage determinants do so in programmed PCD. The classic example is the death of specific and identifiable cells within the developing *C. elegans* embryo [2] (Figure 1B; see below). Reproducible cell death has also been observed in other invertebrates such as *Drosophila* [3], and recent studies suggest that similar deaths take place in vertebrate nervous systems (reviewed below). Thus, it appears that programmed PCD is an evolutionarily conserved mechanism used to remove specific cells at very precise time points during development.

The intense study of the apoptotic core machinery during the last three decades has led to the elucidation of the genetic and molecular pathways effecting PCD, and has revealed a key role for a family of molecules known as caspases (see [10] for a recent review). Less is known, however, about the upstream regulatory events that confine activation of the core cell death pathway to subpopulations of cells. This is particularly true for programmed PCD; indeed, even in well-characterized PCD model systems such as *C. elegans*, the regulatory events that lead to caspase activation have only been investigated in 10 out of the 131 programmed deaths in the hermaphrodite [11]. In this review, we will summarize recent studies suggesting that programmed PCD is controlled in a variety of organisms by cell-specific, primarily intrinsic and combinatorial codes of transcription factors. We propose that programmed PCD is akin to any unique and programmed cell fate within the nervous system, and discuss why such complex control of specific apoptotic events may have evolved.

Programmed PCD I: death of precursors or newborn cells in defined lineages

The elucidation of the complete cell lineage of *C. elegans* almost 30 years ago revealed remarkably reproducible patterns of proliferation and apoptotic cell deaths [1,2]. About 10% of the 1090 cells that are generated to form an adult hermaphrodite undergo PCD, and most of these cells differentiate as supernumerary neurons when apoptosis is prevented [12,13]. The vast majority of cells that are fated to die appeared to do so within 30 minutes after their birth. Interestingly, a recent study has shown that in the *C. elegans* embryo, the asymmetric division of a precursor and the apoptosis of one of its two daughter cells are regulated by the same three genes, thereby providing a possible mechanism by which subsets of progeny undergo PCD soon after their generation [14]. The presence of orthologous genes in vertebrates suggests that the link between these two processes may be evolutionary conserved.

PCD was also known to occur in the developing CNS of the *Drosophila* embryo [15], but the lack of detailed lineage information in this model system had precluded its systematic investigation. Subsequent studies characterized the different precursors in the embryonic ventral nerve cord, their segmental differences as regards lineage composition, as well as the number and kind of progeny to which they give rise [16-19]. Together with the identification of pro-apoptotic genes in *Drosophila* [20,21], these studies greatly facilitated the investigation of defined apoptotic events. Reproducible patterns of PCD were observed for both embryonic and postembryonic precursors [22,23] as well as newly generated cells [24]. These studies were followed by a comprehensive study of the clonal origin of all deaths in the embryonic ventral nerve cord [3]. Together, they reveal that there are reproducible apoptotic events in most embryonic lineages, and that such deaths are often segment-specific. The dying cells may be precursors, undifferentiated cells, glia and/or mature neurons (see next section for a detailed description of the latter).

Does programmed PCD also occur in vertebrate nervous systems? It is well established that PCD occurs during earlier stages of vertebrate neural development [25,26]. Although less extensive than the later neurotrophic death, this PCD occurs within populations of proliferating neural precursors and newly postmitotic neuroblasts, and appears to be essential for the normal development of the nervous system. Indeed, precise removal of mitotically competent secondary progenitors from within a lineage may be of great importance to ensure the generation of adequate numbers of each unique cell type. Precise understanding of lineage relationships between progenitors and cell identities will be required to unequivocally establish the reproducibility of apoptotic events. This has been achieved in the developing mammalian forebrain, where time-lapse analysis of identified cortical progenitor cells has revealed the existence of distinct lineages [27]. Strikingly, this study also

finds evidence for specific patterns of PCD within each type of lineage. Given the complexity of the nervous system, the scarcity of markers for distinct cell types and the partial redundancy of PCD genes, the importance of programmed PCD in vertebrates may have been underestimated. Newly developed techniques [28,29] may facilitate clonal analysis of neuronal populations and thereby reveal the extent and reproducibility of programmed PCD in the mammalian nervous system. The zebrafish is also emerging as a powerful vertebrate system in which to study PCD. There are several advantages to this system, such as its smaller size and cell number, and the genetic amenability of its embryos, which are transparent. Together with the availability of genetic tools such as fluorescent reporter transgenes, these properties should make the zebrafish an attractive tool for future studies of programmed PCD in vertebrates [30].

Programmed PCD II: death of differentiated cells

Differentiated neurons can also undergo PCD in a stereotypical fashion. A couple of well characterized sexually dimorphic deaths in *C. elegans* occur after some hallmarks of differentiation: the hermaphrodite-specific neurons (HSNs) migrate anteriorly in males before undergoing PCD, whereas the male-specific CEM neurons die in hermaphrodites after forming desmosomes [2].

Clear examples of late neuronal death were first reported in the grasshopper [31], where FETi and DUMETi neurons extend growth cones before undergoing apoptosis in segments where their muscle targets are absent. Importantly, ablation of these muscles in segments where these neurons normally survive did not result in neuronal death, thereby arguing against a target-derived signal regulating their segment-specific survival [32]. PCD of differentiated neurons appears to occur in a segmental fashion also in other species. This includes all four identified neurons undergoing late PCD in *Drosophila*: the dMP2 and MP1 pioneer/visceral neurons and the GW and anterior NB2-4t motor neurons [33,34]. The death of the dMP2 pioneer neuron is probably the clearest case of a late neuronal death that is not under the control of target-derived survival signals: once dMP2 neurons have carried out their embryonic function in guiding follower axons in the ventral nerve cord, they undergo apoptosis in anterior segments [33,34]. The survival of posterior dMP2 neurons does not appear to depend on cell-extrinsic signals, although their later differentiation into insulinergic neurons innervating the hindgut does [35].

In mammals, it is technically challenging to identify single apoptotic events in fully differentiated neurons and/or glia. Consequently the prevalence of late programmed PCD is unclear. However, late deaths have been observed in other vertebrates such as fish and amphibians, where a simple network of early (primary) neurons and its characteristic touch-induced motor output has been extensively studied [36]. The Rohon-Beard cells, one particular sub-class of primary neurons with cell bodies in the spinal cord and dendrites in the skin, mediate primary sensory input into the circuitry. Rohon-Beard neurons later undergo apoptosis and are replaced by more mammalian-like, secondary sensory neurons in the developing dorsal root ganglia [37]. Since many other sub-classes of primary neurons survive, as yet unidentified regulatory cues are likely to act exclusively within the Rohon-Beard neurons to trigger their late death.

Combinatorial regulation of the “Cell Death Fate”

The precision and reproducibility of apoptotic events, whether they occur in early-born or differentiated cells, indicate fine genetic control. In line with this notion, studies in *C. elegans* have identified several regulators that control specific subsets of apoptotic deaths, such as

those of the NSM sister cells, P11.aap and the sexually dimorphic CEM and HSN neurons [11,38]. They have also revealed an important role for homeobox genes, which are emerging as key regulators of the PCD core machinery in both vertebrates and invertebrates. In *C.elegans*, the Hox gene *lin-39* is required for the survival of the six VC neurons [39], whereas expression of the Hox protein MAB-5 leads to the death of some cells generated in the posterior ventral nerve cord [40]. In this case, the Hox co-factor and Pbx homologue *ceh-20* provides some of the context dependency that confines the pro-apoptotic action of MAB-5 to a subset of cells [41]. In *Drosophila*, Hox genes have been shown to regulate PCD of both precursors and differentiated neurons – in both cases resulting in segmental differences in neuronal composition. A pulse of expression of the Hox protein Abdominal-A is used to schedule the end of proliferation of postembryonic precursors, thereby limiting the size of their progeny in abdominal segments [22]. By contrast, the Hox gene *Abd-B* prevents death of differentiated dMP2 and MP1 neurons in posterior segments, where it then functions to promote differentiation of dMP2 neurons into neurosecretory insulin-producing cells [33,35]. Whether Hox genes act in a pro- or anti-apoptotic fashion in *Drosophila* does not seem to depend on the specific Hox gene or the timing (progenitor vs. differentiated neuron). Indeed, *Abd-B* can cause death of other fairly differentiated neurons (M. Bate, personal communication), and the segmental death of GW motor neurons results from the antagonistic function of two different Hox genes: *Antennapedia* and *Ultrabithorax* [34]. Importantly, the action of Hox genes on apoptotic genes has been shown to be direct, at least in two cases, in both *C. elegans* (see next section) and *Drosophila* [42], and some evidence exist for the involvement of Hox genes in death of postmitotic neurons in vertebrates [43]. Thus, a picture is emerging of Hox genes as important members of the terminal codes regulating programmed PCD. However, many of the cell-specific partners that may explain the context dependency of their actions remain to be isolated.

Other homeobox genes also appear to play an evolutionarily conserved role in the regulation of the apoptotic machinery. The Bar class of homeodomain transcription factors is required for the survival of sensory neurons in both *C.elegans* and mice, and it seems to be a positive regulator of apoptosis in the neural plate of *Xenopus* [44-47]. Furthermore, the homeobox gene *hb9* acts in a pro-apoptotic fashion in *Drosophila* dMP2 neurons [35]. Although *hb9* is more broadly expressed in motor neurons that survive, a small subset of uncharacterized *hb9*-positive neurons also seems to undergo apoptosis [8,34], and *hb9* misexpression in interneurons can lead to ectopic caspase activation (I. M.-A., unpublished). It is tempting to speculate that in addition to contributing to motor neuron identity, *hb9* may sensitize the motor neuron compartment to PCD, thereby making it more dependent on a target-derived signal.

Two other gene families also seem to play conserved roles in the regulation of PCD: bHLH and Snail family zinc-finger transcription factors act antagonistically to regulate the death of NSM neurons in *C. elegans* [11]. Intriguingly, manipulating the levels of bHLH proteins involved in neural development in vertebrates affects the level of PCD occurring in progenitors [26], and *slug/SNAI2* regulates the PCD of hematopoietic cell lineages in mice [38].

Two important features have emerged from these studies. Firstly, the expression of the transcription factors found to activate death is relatively broad and not confined to dying cells. This implies context dependency and suggests combinatorial control of cell death gene(s). Secondly, many of identified transcription factors belong to families of genes with important roles in neuronal differentiation. This raises the intriguing possibility that the combinatorial codes for neuronal and PCD fates partly overlap. This has been demonstrated in the *Drosophila* dMP2 neurons, where three genes (*hb9*, the winged-helix/forkhead box gene *fork head* and *Abd-B*) co-regulate death and differentiation [35]. The ability of Hb9 and Fkh

to activate death or promote insulinergic differentiation is regulated in a segment-specific fashion by Abd-B. In the absence of Abd-B in anterior segments, expression of Hb9 and Fkh results in death of dMP2 neurons. In posterior segments, Abd-B prevents the Hb9/Fkh-dependent activation of the apoptotic machinery by repressing expression of the RHG domain gene *reaper* (*rpr*). Strikingly, all three genes then act combinatorially to promote the expression of an insulin-like peptide. Hence, two different but partly overlapping codes result from the segmental expression of one single gene, which thereby acts as a switch between death and neuronal differentiation.

From code to death

Less is known about how these “death codes” lead to activation of the core cell death pathway. Here we suggest two possible combinatorial models (Figure 2). One involves different transcription factors binding to the regulatory region of an upstream component of the core cell death pathway. Expression of this gene would be the limiting step that determines whether or not to activate the core cell death pathway (Figure 2B). Until recently, this was believed to be the main mode of death specification in *C. elegans*, where the expression of the BH3-only (Bcl-2 homology region 3) protein EGL-1 is restricted to those cells that are going to die, and its transcriptional regulation underlies a life/death decision in several lineages [11]. Transcriptional regulation of *egl-1* expression has been shown to be combinatorial, at least in the case of the posterior ventral nerve cord progeny, where a complex between the Hox protein MAB-5 and the Pbx homologue CEH-20 directly regulates *egl-1* transcription [41]. However, recent evidence suggests there are also EGL-1-independent ways to activate PCD in *C. elegans*, and these may be more relevant to the more infrequent late deaths [48].

Figure 2. Cell death as a combinatorial cell fate. (A) The classic paradigm of cell fate specification, whereby several more broadly expressed transcription factors act combinatorially on specific genes to impart a unique cell fate. (B and C) Similarly, combinatorial codes of transcription factors lead to the activation of the core cell death pathway in programmed PCD. In (B), combinatorial activation of one key PCD gene is sufficient to trigger cell death. In (C), combinatorial coding occurs at the level of PCD genes. Expression of multiple PCD genes is required to activate death, and each PCD gene is differentially regulated by one or several transcription factors. In both (B) and (C), combinatorial codes could also trigger cell death by repressing PCD inhibitory genes.

An alternative to this model would involve different transcription factors regulating expression of different cell death genes. Co-expression of multiple cell death activators would then be required to trigger death (Figure 2C). Some support for this model

comes from *Drosophila*, where several RHG domain proteins play a key role in activating the apoptotic machinery by binding to and antagonizing inhibitor of apoptosis (IAP) proteins (see [49] for a recent review). Given that not all RHG-domain genes are simultaneously expressed in every cell fated to die, their expression is likely to be differentially regulated. However, two or more RHG-domain genes are sometimes co-expressed in the same cells, and in these cases they appear to function in an additive manner. Indeed, the normal pattern of PCD in midline glia requires the three RHG-domain genes *head involution defective*, *rpr* and *grim* [50]. Furthermore, both *rpr* and *grim* cooperate to trigger the late, segment-specific death of both dMP2 and MP1 pioneer neurons [33].

Death codes: why and when?

Many reviews have discussed the role of stochastic PCD in adjusting cell numbers to their targets and optimizing connectivity in complex nervous systems (see [4] for a recent and comprehensive review). The roles of programmed PCD may initially appear less obvious, especially in simple organisms where it would seem more parsimonious not to generate the cells fated to die in the first place. One obvious and previously suggested reason for programmed PCD is to regulate the size of progenitor populations or newly born cells, so as to control the number and kind of progeny to which they give rise [4]. This can be done in a segmental fashion to generate substantial differences in neuronal composition along the anteroposterior axis, which is of obvious functional utility if the targets of neural action also differ in a segmental fashion. In this case, programmed PCD would fulfil a patterning role, for instance enabling the formation of region-specific neuromuscular circuits. In the case of early postmitotic deaths, it may also be easier to generate cells at unnecessary locations and then delete them by PCD. This would be consistent with the observed genetic link between asymmetric cell division and PCD in *C.elegans* [4,14].

The late deaths of differentiated neurons are more puzzling. It has been suggested that some differentiated neurons die because they are no longer needed, such as pioneer neurons after carrying out their function [51]. However, it does not seem particularly parsimonious to evolve considerable transcriptional complexity only to remove “harmless” cells with no specific function. Alternatively, it is conceivable that some late deaths fall into the abovementioned “patterning category”. These would include, for example, the segmental PCD of a fairly differentiated motor neuron in segments where its target muscle is absent, or the sexually dimorphic deaths of *C. elegans* neurons. The activity of these “unmatched” neurons in the wrong sex or segment might have undesirable consequences, making their removal necessary. However, there are some late deaths that cannot be easily accounted for by regional or sexual differences in their target. One such case is the segmental death of dMP2 pioneer/visceral neurons: a case of complex combinatorial control of a death fate. If apoptosis of anterior dMP2 neurons is prevented, supernumerary neurons will exit in the same nerve and innervate the same target as the ones that normally survive (I. M.-A., unpublished). An alternative and very speculative explanation is inspired by recent findings in the *Drosophila* wing and eye imaginal discs. As a result of apoptotic stimuli, dying cells in the disc will signal to neighbouring ones to trigger compensatory proliferation [52-54]. This raises the intriguing possibility that what is an induced response in the disc functions as a developmental signal in the nervous system: namely, the death of differentiated neurons may signal transiently to affect the development of other neurons/glial cells. In this case, death would have a purpose in itself.

Conclusions

Increasing amounts of evidence suggest that there are no mechanistic differences between specifying unique cell fates and programmed PCDs; evolution has used the same combinatorial strategy to ensure the most appropriate outcome (death or differentiation) for any given cell in its particular temporal and spatial contexts. The complexity of the transcriptional codes specifying death fates suggests that programmed PCD may be playing important roles, many of which remain to be established. Important questions remain to be addressed, such as how complex death codes are or what targets they regulate. Furthermore, detailed analysis of the terminal neuronal identities and behavioural repertoires of invertebrates should shed light on the functional relevance of cell death specification. In parallel, future characterization of defined lineages and their specification in vertebrates will reveal the extent and reproducibility of programmed PCD in more complex organisms.

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