Mol Neurobiol (2008) 37:1–6
DOI 10.1007/s12035-008-8021-4

# Cell Death in Brain Development and Degeneration: Control of Caspase Expression May Be Key!

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Received: 26 February 2008 / Accepted: 9 April 2008 / Published online: 1 May 2008


**Keywords** Apoptosis · Caspases · Brain · Development · Neurodegeneration

The study of apoptosis is classically linked to that of development, where developmental biologists noted that cell death occurred during the development of insects, amphibians, birds, and mammals [1, 2, 63] before the term apoptosis was even coined [3]. In this review, we specifically examine the involvement of apoptosis in the development of the brain as well as its involvement in neurodegeneration.

The initial discovery of the genes that control apoptosis occurred during a study of metazoan development. A genetic study of the development of the nematode worm, *Caenorhabditis elegans*, revealed that *cell-death abnormality-3* (*ced-3*) was responsible for the programmed death of certain cells during the development of the worm [4]. This study was furthered by the demonstration that *ced-3* was homologous to the mammalian cysteine protease interleukin-1β-converting enzyme (ICE) [5]. As increasing numbers of homologous mammalian cysteine proteases were identified, it was decided to name them caspases, in reference to their ability to cleave substrates after aspartate residues [6]. Since then, at least 14 mammalian caspases have been identified [7] and a number of them and their regulators have been shown to be vital for mammalian development [8–14]. Caspases are expressed as inactive zymogens that become cleaved during apoptosis. Initiator caspases autoactivate and self-process upon recruitment to adaptor proteins. They then proceed to cleave and thereby activate the executioner caspases. Activated executioner caspases proceed to process key structural and nuclear proteins and thereby cause the disassembly and death of the cell [7, 15]. Two major caspase pathways have been described: the intrinsic pathway, which is initiated by cytochrome *c* release from the mitochondrion [7, 15], and the extrinsic pathway, which is initiated by the binding of ligands to plasma-membrane death receptors [16]. Caspase knockout studies have demonstrated that the intrinsic pathway is vitally important for fetal murine brain development.

The intrinsic caspase pathway is initiated by the release of cytochrome *c* from the mitochondrion into the cytosol (Fig. 1). Mitochondrial integrity is regulated by members of the Bcl-2 protein family, and this family is therefore of central importance to the health of the cell and its regulation is the subject of numerous studies as a result [17–20]. Once released into the cytosol, cytochrome *c* binds Apaf-1 along with a molecule of 2′-deoxyadenosine 5′-triphosphate (dATP) and induces Apaf-1 to oligomerize into a heptamer. This large protein complex is called the apoptosome. The apoptosome proceeds to recruit caspase-9, which subsequently self-activates and self-processes. Active caspase-9 triggers a cascade of caspase activation, which leads to the death of the cell [7, 15]. The precise order of this pathway was determined using cytosolic extracts derived from human cell-lines, which were treated with cytochrome *c*, following the immunodepletion of specific caspases. It was determined that caspase-9 was essential for mediating the

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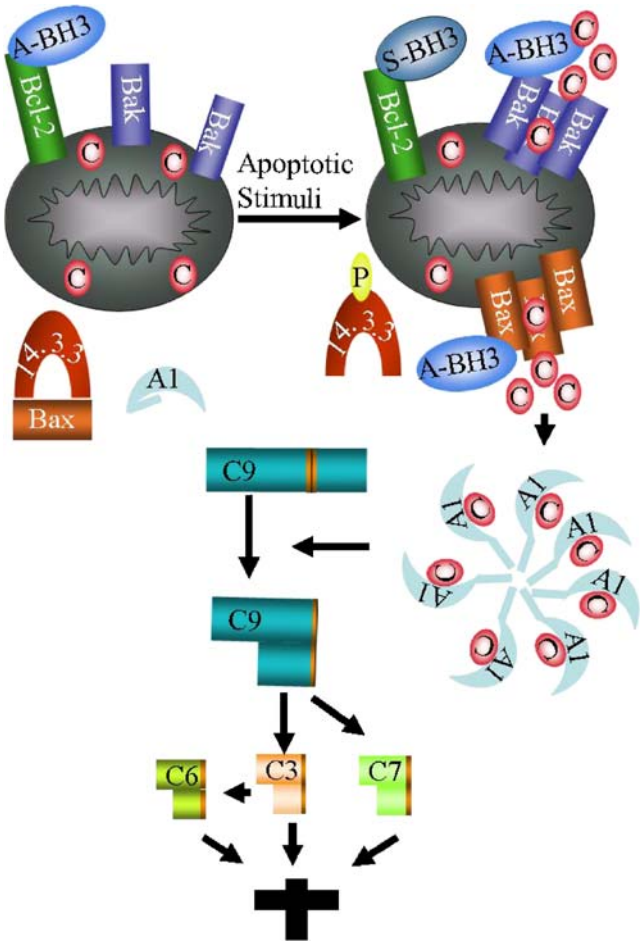


**Fig. 1** The intrinsic apoptosis pathway: cytochrome c release is regulated by the Bcl-2 family of proteins. Apoptotic stimuli induce their modification and induce cytochrome *c* release from channels formed by Bax/Bak. Cytochrome *c* induces Apaf-1 to form the apoptosome, which recruits and activates caspase-9. Caspase-9 cleaves and activates the executioner caspases. A1, Apaf-1, C9, caspase-9, C6, caspase-6, C3, caspase-3, C7, caspase-7

cytochrome *c*-induced signaling cascade, and that it was the only caspase recruited by the apoptosome [21]. Slee and colleagues also determined that caspase-3 was the main executioner of the pathway [22].

## Postnatal Brain Development

The brain develops from the ectoderm in the embryo and continues to develop postnatally. After gastrulation, the notochord forms from the mesoderm in an anteroposterior axis. It then signals the overlying ectoderm to form the neural plate, which folds and forms the neural tube, from which the structures of the brain develop. As well as being vital for the development of the embryo, apoptosis is also central to the postnatal development of the brain.

During postnatal brain development neurons migrate into the cortical regions and form synaptic connections with each other and neuronal processes from other areas of the brain. This period of plasticity is unique and allows children to recover from brain surgery and brain trauma quicker than adults and also makes learning musical instruments and languages easier for them than for adults [64]. Incorrect synaptic connection at this period can have life-long effects, the best characterized condition being amblyopia, a condition that arises from the failure of an individual eye to make strong connections in the visual cortex during postnatal development, which results in poor vision through that eye throughout the life span, even if the lenses and retina are functioning adequately [30]. It is thought that the developing brain overproduces neurons to ensure that a sufficient amount of synaptic connections are formed and that the surplus neurons that fail to make synaptic connections are removed by apoptosis through the deprivation of neurotrophic factors. In a similar way, glial cells are also thought to be overproduced during development and the excess cells removed by apoptosis [23–25].

The occurrence of apoptosis has been demonstrated in the postnatal rat cerebrum where a wave of cell death peaking between postnatal day (P)7 and P9 has been demonstrated in the somatosensory cortex and the medial cortical regions [31] and in the subicular complex and the hippocampus [32]. The authors of these two studies suggested that, as cell death occurred in the subcortical plate in correlation with the penetration of the cortex by thalamocortical afferents, then the cortical cell death may have been caused by the removal of target neurons that had failed to connect to the penetrating afferents during this period. Cell death in the hippocampus may also be attributed to the failure of neurons to connect to afferents, as the cell death period also coincided with the arrival of afferents to the hippocampus. Apoptosis also occurs in the developing cerebellum of the postnatal rat, during which the anatomy of the cerebellum changes markedly (Fig. 2). Neuronal granule cells proliferate in the external granular layer (EGL) and proceed to migrate through the molecular layer, past the Purkinje cell layer and into the internal granular layer (IGL). The EGL eventually shrinks completely as the IGL thickens. Pyknotic cells have been observed during this remodeling in the rat, both in the EGL and the IGL, where the apoptotic cells of the EGL were identified as proliferating granule neurons [33], and the apoptotic cells of the IGL and the molecular layer were mainly astrocytes [34].

Oligodendrocyte apoptosis was also observed in the postnatally developing optic nerve [35]. We have recently shown that Apaf-1, caspase-3 and the proapoptosis Bcl-2 family member, Bim, all become dramatically downregulated after P10 in the mouse brain [36]. We have also previously shown that these proteins are also downregulated in the same time frame in the postnatal retina [37, 38],

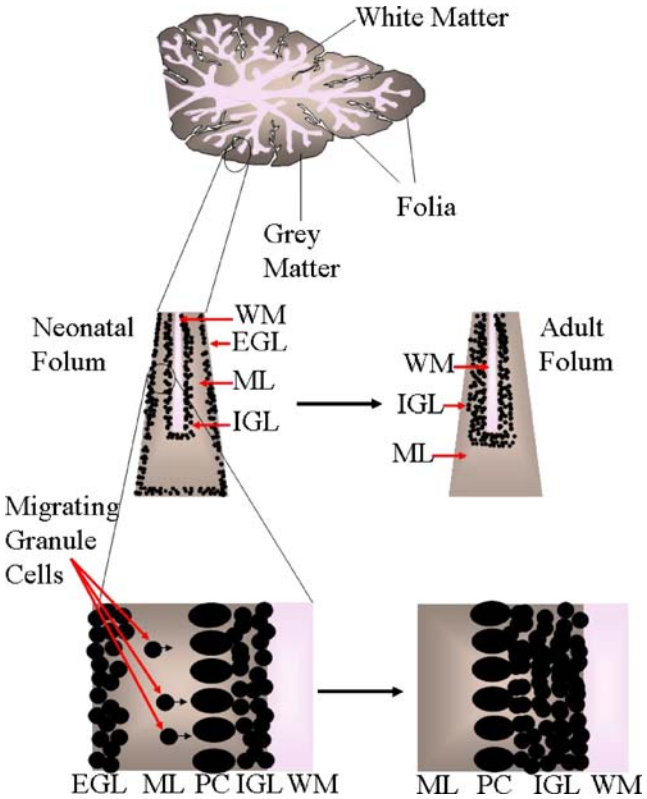


**Fig. 2** Postnatal cerebellar development. Cerebellum is divided into a series of lobes, which are divided into a series of folia. In the neonate, each folium consists of two granule cell layers; the EGL and the IGL. The intervening layer is termed the molecular layer. In addition, the outer margin of the IGL is bordered by a single layer of Purkinje cells. During postnatal development, granule cells migrate from the EGL across the molecular layer and past the Purkinje cell layer into the IGL. Granule cells that remain in the EGL die by apoptosis. In the adult, the EGL is completely absent and the IGL is thicker. *WM* white matter, *EGL* external granular layer, *ML* molecular layer, *IGL* internal granular layer, *PC* Purkinje cells

a neural tissue that also undergoes waves of apoptosis during postnatal development [39]. Furthermore, we have hypothesized that the downregulation of proapoptosis proteins may serve to protect the adult tissue from the loss of irreplaceable cells. However, as mediators of the intrinsic apoptosis pathway have been implicated in a range of neurodegenerative disorders, it therefore appears that the downregulation proapoptosis mediators during postnatal development is not enough to protect the brain during disease and trauma.

## Caspase Knockout Mice and Brain Development

While a number of proteases seem to be involved in pathological cell death, the necessity of caspases and adaptor proteins for apoptosis pathways and for murine development has been validated by the use of targeted gene deletion. The importance of the intrinsic apoptosis pathway for fetal brain development was demonstrated through the deletion of the genes that encode the proteins that regulate the pathway. Deletion of Apaf-1, caspase-9, and caspase-3 genes result in malformed mice with strikingly similar phenotypes: perinatal lethality, craniofacial abnormalities, and enlarged brains [8–12]. In some cases, the brain was enlarged so severely that it actually protruded through the skull! While playing a role throughout brain development, apoptosis is responsible for matching the numbers of neurons and oligodendrocytes to the number of target cells then innervate and myelinate, respectively [23–25]. As there is a marked reduction in apoptotic cells in the developing brain in the knock-out models of Apaf-1, caspase-9, and caspase-3, the phenotypically enlarged brains of these models were attributed to a lack of normal developmental apoptosis, rather than neuronal hyperplasia and tumor formation.

The caspase knockout models have also presented some surprising results. In particular, the caspase-$3^{-/-}$ mice generated from certain genetic backgrounds developed normally into adulthood, were fertile and presented minimal brain pathology [26]. This unexpected result indicated that the requirement of caspase-3 for murine development was strain-dependant. It was later shown that the caspase-$3^{-/-}$ strain, which developed normally, expressed higher levels of caspase-7, in comparison to the developmentally sensitive caspase-$3^{-/-}$ strain. It was further demonstrated that in contrast to human enzymes, mouse caspase-7 could induce DNA fragmentation as efficiently as caspase-3 and that levels of caspase-7 expression and activity correlated with the presence of fragmented DNA and apoptotic cells in caspase-$3^{-/-}$ strains [27]. This study indicated that caspase-3 is redundant to caspase-7, especially where caspase-7 is expressed at relatively high levels. It has been reported that the targeted deletion of caspase-7 results in embryonic lethality [28], whereas a later study demonstrated that caspase-$7^{-/-}$ mice exhibited normal appearance and organ morphology [29]. The contrast between the results of both studies may again be caused by strain-dependant differences, however, it is difficult to determine as the initial study was not published, but merely reported in a review article. The later study investigated the effect of both caspase-3 and -7 by using the caspase-$7^{-/-}$ mice to generate double knockout caspase-$7^{-/-}$/caspase-$3^{-/-}$ mice. Interestingly, the resulting phenotype was more similar to the caspase-$8^{-/-}$ [13] and Fadd$^{-/-}$ [14] phenotypes than to the Apaf-$1^{-/-}$, caspase-$9^{-/-}$ and caspase-$3^{-/-}$ phenotypes, as caspase-$7^{-/-}$/caspase-$3^{-/-}$ mice died shortly after birth and exhibited defects in cardiac development. In addition, only 10% of the caspase-$7^{-/-}$/caspase-$3^{-/-}$ mice displayed exencephally, in contrast to the severe brain malformation of the original caspase-3 knockout mice. The authors attributed this divergence to strain-dependent differences. In contrast

to the conclusions of Houde and colleagues, the differing phenotypes could certainly not be attributed to the redundant activity of caspase-7 in this study [27]. A complete characterization of the differences in gene expression between the various caspase knockout strains would help to elucidate the factors behind the divergent phenotypes. The differences between various caspase knock-out models may also be explained by the existence of cell death pathways independent of caspases. For example, there is evidence that programmed cell death can occur through the activation of other families of proteases, such as calpains [60] and cathepsins [61]. Furthermore, triggering the endoplasmic reticulum stress pathway induces cell death. We have previously observed programmed cell death in the absence of caspase expression in murine retinas [37]. It is therefore possible that cell death may occur through alternative pathways in the caspase-3 knockout strains that exhibit minimal neuronal phenotypes.

## Intrinsic Apoptosis Mediators in Neurodegeneration

Caspase-9 activity has been specifically implicated in the pathology of neurodegenerative diseases such as Alzheimer's disease (AD) and amyotrophic lateral sclerosis (ALS), where active caspase-9 was detected in the brains of AD patients [40, 41] and in the motor neurons of ALS patients and in an ALS mouse model [42]. This is interesting in the context of our research, as we have shown that both the activator of caspase-9, Apaf-1, and its executioner, caspase-3, are downregulated in the adult brain. This suggests that caspase-9 may be activated by an alternative pathway and that it can induce cell death in the absence of caspase-3. In fact, caspase-9 has been shown to be directly activated by endoplasmic reticulum (ER) stress in the absence of cytochrome *c* [43] and of Apaf-1 [44]. ER stress is caused by the aggregation of misfolded proteins, which is a central feature of AD and ALS and a range of other neurodegenerative diseases [45–47]. In addition, caspase-9 is also activated independently of Apaf-1 in lysosome-mediated cell death [48], where lysosomal dysfunction is also thought to occur in neurodegenerative disorders [49]. It therefore seems possible that caspase-9 can be activated in the absence of Apaf-1 during the progression of AD and ALS. Caspase-9 was also shown to directly cleave amyloid precursor protein, a protein that is central to the pathology of AD, into a proapoptotic peptide [40]. It appears that caspase-9 may be capable of inducing cell death in AD brains through the direct processing of APP, independently of caspase-3. Further study of the pathways that activate caspase-9 in the absence of Apaf-1 and an examination of alternative caspase-9 substrates in the adult brain may present a caspase-9 pathway that is alternative to the classical intrinsic apoptosis pathway, but is relevant in AD and ALS.

Despite the downregulation of Apaf-1 and caspase-3 in the adult brain, both apoptosis-inducing proteins have been implicated in neurodegenerative diseases. A dominant negative inhibitor of Apaf-1 was shown to protect neuron loss in the substantia nigra in a model of Parkinson's disease [50]. Active caspase-3 has been detected in the apoptotic neurons [51] and in the neurofibrillary tangles [52] of AD brain. This implies that both proteins can become upregulated during adult brain disorders. In fact, this has already been demonstrated, as Apaf-1 and caspase-3 were both upregulated after brain trauma in the rat [53]. The regulation of Apaf-1 and caspase-3 expression may therefore be central to the mechanism of neurodegeneration in many cases. Apaf-1 gene expression is regulated independently by the transcription factors E2F-1 and p53 [54–56]. E2F-1 also positively regulates caspase protein expression, where the overexpression of E2F-1 induced the upregulation of caspase-3, -7, -9, and -8 [57]. E2F-1 is negatively regulated by retinoblastoma (Rb) [55] and by histone deacetylase (HDAC) [58]. We have previously demonstrated that the inhibition of HDAC induces Apaf-1 and caspase-3 upregulation in retina and induces widespread apoptosis [59]. It therefore appears that the E2F-1/Rb/HDAC is a good candidate system for the regulation of Apaf-1 and caspase-3 expression during neural tissue development. We are currently investigating the role of these transcription regulators in disease models of the adult retina.

In conclusion, the intrinsic apoptosis pathway is required for fetal and postnatal brain development, but is downregulated through the suppression of the expression of its key mediators, Apaf-1 and caspase-3, in the adult. However, after trauma and during disease, these two pro-death proteins are re-expressed and cell death ensues. Study of the mechanisms that control the expression of these two proteins may yield therapeutic targets. In addition, caspase-9 expression is sustained in the adult brain. This pro-death mediator may facilitate cell death during the process of neurodegenerative disease either through uncharacterized cell death pathways or through the classical intrinsic apoptosis pathway upon the reexpression of Apaf-1 and caspase-3. A study of the function of this enzyme in the adult brain may further our understanding of its role in neurodegeneration.

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