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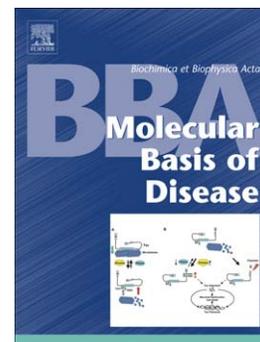
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Multifaceted deaths orchestrated by mitochondria in neurones

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Abstract

Neurones undergo diverse forms of cell death depending on the nature and severity of the stress. These death outcomes are now classified into various types of programmed cell death, including apoptosis, autophagy and necrosis. Each of these pathways can run in parallel and all have mitochondria as a central feature. Recruitment of mitochondria into cell death signalling involves either (or both) induction of specific death responses through release of apoptogenic proteins into the cytosol, or perturbation in function leading to loss of mitochondrial energisation and ATP synthesis. Cross-talk between these signalling pathways, particularly downstream of mitochondria, determines the resultant pattern of cell death. The differential recruitment of specific death pathways depends on the timing of engagement of mitochondrial signalling. Other influences on programmed cell death pathways occur through stress of the endoplasmic reticulum and the associated ubiquitin-proteasome system normally handling potentially neurotoxic protein aggregates. Based upon contemporary evidence apoptosis is a relatively rare in the mature brain whereas the contribution of programmed necrosis to various neuropathologies has been underestimated. The death outcomes that neurones exhibit during acute or chronic injury or pathological conditions considered here (oxidative stress, hypoxic-ischaemic injury, amyotrophic lateral sclerosis, Parkinson's and Huntington's diseases) fall within a spectrum of the diverse death types across the apoptosis-necrosis continuum. Indeed, dying or dead neurones cells may simultaneously manifest characteristics of more than one of death pathway. Understanding neuronal death pathways and their cross-talk not only informs the detailed pathobiology but also suggests novel therapeutic strategies.

Keywords

Neurones, Mitochondria, Programmed Cell Death, Apoptosis, Necrosis, Autophagy, ER stress

Overview and general principles of diverse neuronal cell death modalities

Neurons die in a variety of ways and mitochondria play a key role in such outcomes. This topical review explores the recent literature underlying the nature of neuronal death and its contextualisation in injury and disease. Particular emphasis is placed on mitochondria as organelles that, on the one hand, provide bioenergetic sustenance to the cell and, on the other, act as the focal point of cellular signalling leading to various types of death outcome.

Various markers of cell death have been used to identify the outcome of cellular injury or neurodegeneration in the nervous system. Thus, in stroke-affected brain (similar to hypoxic-ischaemic (HI) injury) necrosis clearly occurs at the core of the injury site. There is a penumbra surrounding this core, which is characterised by cells with apoptotic markers such as caspase activation and nuclear DNA fragmentation [1]. In neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS), markers of apoptosis are observed, together with evidence of cellular stress responses including endoplasmic reticulum (ER) stress and markers of autophagy [2].

In these and other pathological settings, mitochondria in the affected cells, being central to cellular homeostatic and energy-yielding activities, play key regulatory roles in death processes. Thus, these organelles receive signals from pro-apoptotic members of the Bcl-2 family, which are antagonised by other pro-survival (anti-apoptotic) members of the same protein family. In situations where the pro-apoptotic Bcl-2 family proteins predominate, outer mitochondrial membrane permeabilisation (OMMP) results, thereby allowing redistribution to the cytosol of a number of apoptogenic proteins [3-5]. These proteins enable progression of programmed cell death (PCD), initially defined as apoptosis. However, as will be discussed later in this review, mitochondria influence not only apoptosis (PCD-Type I) but also can engender a more recently identified death outcome called programmed necrosis (PCD-Type III), typified by lack of caspase

involvement [6]. Autophagy operates for the most part as a routine homeostatic mechanism for turnover of various cellular constituents, but under some stress conditions may become part of the death program leading to autophagic cell death (PCD-type II) [6-8].

Neurons are complex cells relative to many other cell types. They have the characteristic morphology of a cell body, with greatly elongated processes (axons and dendrites), with consequential transport issues for intracellular trafficking. Moreover they have a plethora of surface ion channels, and are very susceptible to injury (and death) through deprivation of energy or molecular ATP, or via severe excitation by neurotransmitters producing excitotoxicity. Finally, neurons function and are sustained in an adaptive cellular milieu comprised of a majority of glial cells such as astrocytes [12, 13]. Neurons may also be exposed to cytokines produced by microglia and astrocytes in the inflammatory phase of injury in the central nervous system [12, 14], which can activate extrinsic pathways to cell death. These aspects of the cellular milieu of neurons are not dealt with in detail in this review.

Whilst post mortem human brain tissue or those from animal models of injury or disease provide clues as to the diversity of neuronal death, the clearest information about molecular mechanisms leading to death comes from laboratory studies at the cellular level using cell models of injury or disease. Thus, detailed studies of the molecular cell biology of appropriate cultured primary neurons, or immortalized cells with neuronal properties, have enabled the dissection of cellular responses and death outcomes schematically represented in Fig.1. There is an initial primary response to the trigger that may be external (e.g. toxic molecules impinging on the cell) or internal (e.g. inappropriate intracellular protein aggregation). The primary distortion of cellular biochemistry and physiology, if not rectified by the cell promptly and effectively, activates signalling pathways (secondary response) that converge on mitochondria. Such signalling upstream of mitochondria includes cooperative and antagonistic interactions between Bcl-2

family members. These mechanisms, together with any perturbation in function that mitochondria may have directly sustained, culminate in an overall processed signal (or the decision) that activates the mitochondria to signal further downstream initiating one or more of the death pathways [3, 5, 15]. In neurodegenerative disease involving protein aggregation, ER stress may play an important role in pathways to death. In this setting, damaged proteins also need to be eliminated through the ubiquitin-proteasome system (UPS).

All these diverse signalling pathways, both upstream and downstream of mitochondria, seem to exhibit cross-talk. In other words, the progression in one pathway may influence (positively or negatively) progression of other pathways simultaneously. The outcome of these complex interactions maybe such that in one initially homogeneous population of cultured neurons exposed to a given treatment, there is likely to be a heterogeneous set of responses between different cells of that population. The recognition that individual cells may display a subset of markers characteristic of explicitly defined cell death paradigms (e.g. PCD Types I, II or III) (Fig. 2) adds a further level of complexity. This concept needs to be considered in concert with the general notion of the potential of stressors to induce injury across an insult-dependent apoptosis-necrosis continuum [16, 17] and as a consequence individual cells in the same treated population can lie at a different end point within the continuum.

Severe injury or cellular damage may lead to unregulated necrosis, called “accidental” [15, 18] because cells subjected to extensive ionic and water imbalance undergo rapid swelling leading to gross cellular debilitation [17, 19, 20]. This severe cytotoxic outcome is distinguished from programmed necrosis where there are distinct regulated pathways (separate from both apoptosis and autophagy). Those cells undergoing PCD-Type III bear some morphological resemblance to truly necrotic cells but undergo a discrete loss of permeability of the plasma membrane [18]. Cells undergoing either programmed or unregulated necrosis seem both to lose mitochondrial

energisation and ATP production. Two proposed subsets of PCD-Type III, designated “necroptosis” [21] and “parthanatos” [22] are briefly considered herein below.

In the sections that follow, molecular and cellular details of the pathways relevant to mitochondria in relation to neuronal cell death are discussed. This mechanistic coverage is followed by consideration of several scenarios of neurodegeneration and neuronal injury, in which both the general principles above and the molecular pathways discussed below are considered in the context of clinical conditions that present discrete neuropathologies.

2. Molecular aspects of pathways involving mitochondria that can lead to neuronal cell death

2.1 Apoptosis

Most studies on cell death have focused on apoptosis (PCD-Type I) which is dependent on caspase activation. Apoptosis is composed of two main pathways, the extrinsic (death receptor pathway) and intrinsic pathways. Whilst the extrinsic pathway can bypass mitochondria, the intrinsic pathway is defined by the involvement of mitochondria. In the latter pathway, apoptogenic proteins are redistributed to the cytosol from the mitochondrial intermembrane space (IMS), leading to activation of caspases that directly activate cell death. The initiation of OMMP is regulated by the Bcl-2 family of proteins.

This family consists of three subfamilies, which are categorised according to function and the presence of up to four Bcl-2 homology domains (BH1-4) (reviewed in [23]). A hierarchy exists within the Bcl-2 family ensuring that initiation of OMMP is a tightly controlled process. At the top of the hierarchy is the pro-apoptotic BH3-only subfamily, whose members are responsible for initiating the activation of other Bcl-2 proteins. These are divided into pro-apoptotic and anti-apoptotic (pro-survival) subfamilies. The anti-apoptotic Bcl-2 proteins are the intermediaries

between the BH3-only proteins and the pro-apoptotic Bcl-2 family members, which include Bax and Bak. One view is that the anti-apoptotic Bcl-2 proteins antagonise Bax and Bak until the pro-survival proteins are deactivated by the BH3-only proteins. Others argue that some BH3-only proteins also act as facilitators, directly interacting with Bax and Bak to initiate their activation (reviewed by [23]). Once activated, Bax and Bak change conformation, oligomerise and form pores in the outer mitochondrial membrane (OMM). The “pore” is likely to be lipidic in nature, thus allowing transit of proteins from the IMS to the cytosol and vice versa [5].

The key executioners that relocate to the cytosol from the IMS include cytochrome c (cyt c), the IAP inhibitors “second mitochondria-derived activator of caspases/direct inhibitor of apoptosis-binding protein with low pI” (Smac/DIABLO) and HtrA2/Omi that permit general caspase activation, and the caspase-independent proteins, apoptosis inducing factor (AIF) and Endonuclease G (Endo G) [4, 25-27]. Once redistributed to the cytosol, cyt c forms a complex with Apaf-1 and ATP, named the apoptosome which is responsible for activating caspase-9. Caspase-9 in turn activates downstream effector caspase-3, caspase-6 and caspase-7, ultimately causing chromatin condensation and DNA fragmentation (reviewed in [24]). The redistribution of AIF and Endo G during apoptosis is often observed as a late event, relative to cyt c and Smac/DIABLO redistribution [25, 26]. The activation of both AIF and Endo G results in nuclear breakdown and DNA cleavage apparently without the requirement of caspases [28]. Moreover, in an apoptotic setting, AIF and Endo G are thought to act as part of a feed-forward mechanism whereby their redistribution from mitochondria is triggered by downstream caspase activation (reviewed in [29]).

In some settings (discussed below for programmed necrosis), the release of AIF tethered onto the inner mitochondrial membrane (IMM) requires calpains rather than caspases [30]. Furthermore other IMS proteins are also tethered, such that cyt c needs to be released from

cardiolipin embedded in the IMM and Smac/DIABLO may be bound to survivin in the IMS itself [5]. Therefore OMMP does not allow unbridled redistribution of IMS proteins to cytosol, but other factors may also be needed to disrupt tethering mechanisms.

A further feature of the systematic mitochondrial changes leading to OMMP is the topological reorganisation of the IMM in some situations. The IMM is normally impermeable to ions (including protons) and when energized sustains an electrical potential ($\Delta\Psi_m$). There is a complex apparatus in the IMM, the MPT pore, which is able to undergo wide amplitude opening to totally dissipate $\Delta\Psi_m$. This results in loss of energisation of the IMM, leading to an inability of the mitochondria to sustain ion gradients and ATP production. This complete MPT event arises from various stressors acting on mitochondria and often characterises necrotic types of death (see below), but is not a defining feature of apoptosis. However, small amplitude swelling (or MPT pore “flickering”) can lead to transient depolarization. This event is accompanied by gentle swelling of the IMM allowing IMS proteins, such as cyt c (trapped deep inside cristal invaginations of the IMM), to now become physically closer to the OMM as the invaginations disappear with IMM expansion (reviewed by [5]).

The features outlined above are derived from several mammalian systems and apply generally to the intrinsic pathway observed in neurones. Particular members of the Bcl-2 family are recognised as prominent in neurones, such that Bax rather than Bak often induces apoptosis [31]. Further, in neurones, p53 has been implicated in up-regulating the BH3-only proteins PUMA, Noxa and Bid as well as Bax (reviewed in [32]). While Noxa does not appear critical for neuronal apoptosis [33-35], PUMA and Bid evidently often initiate OMMP.

In neurones of various types, including cortical, hippocampal and sympathetic neurones, PUMA has been shown to localise to mitochondria during apoptosis and interacts specifically with Bcl-X_L and Bax [33, 35, 36]. Under oxidative stress conditions PUMA is upregulated and

controls the activation of Bax [33, 36]. Likewise p53 activation resulting from DNA damage also triggers activation of PUMA and apoptosis in superior cervical ganglion neurones [35]. PUMA and p53 are also engaged during ER-stress induced neuronal apoptosis [37, 38]. However PUMA is not involved in apoptosis induced by excitotoxicity, highlighting the specificity of PUMA recruitment to the type of insult involved [37, 38].

Bid is another BH3-only protein regulated by p53 and is also responsible for the activation of Bax at the mitochondrial level during OMMIP in neurones [39]. Such Bid activation requires it to be truncated (generating tBid), carried out by active caspase-8 or caspase-2 [40, 41], as with other mammalian cell types. In neurones tBid is critical for apoptosis to occur under conditions of focal cerebral ischaemia and oxygen/glucose deprivation [42, 43]. Once translocated to the mitochondria, tBid is directly involved in the redistribution of AIF to the nucleus in cultured neurons [44, 45]. However, recently it was demonstrated that full length Bid is also capable of inducing caspase-independent cell death in neurones [40, 46]. These findings imply that Bid does not require truncation to be activated in neurones, and that it may have an important role in the redistribution of AIF during cell death in the absence of caspase activity.

Amongst the various apoptogenic IMS proteins that are redistributed during neuronal apoptosis, cytochrome c and AIF appear to play deterministic roles [25, 26]. The role of cytochrome c in activating caspase-9 (and subsequently caspase-3) has been well documented in neurones [25, 26, 47]. Likewise, particular attention has been paid to the role of AIF in inducing caspase-independent apoptosis [48]. Indeed, AIF is a critical component of neuronal apoptosis, as shown by studies utilising AIF-deficient harlequin mice and cyclophilin A $-/-$ mice (the latter being required for AIF translocation) [49, 50]. Recent studies have suggested that AIF may also be a major player in programmed necrosis (see below). Under conditions that induce caspase-dependent death (PCD-Type I), AIF involvement has been noted as a late event, following the initial activation of

caspase-3 in cortical neurones and cerebellar granule cells [25, 26]. This is presumably due to the need for caspases to untether AIF from the IMM. The redistribution of both Smac/DIABLO and HtrA2/Omi has been documented in neurones, during apoptosis [25]. However, neither is critical for neuronal apoptosis, indicating that these apoptogenic proteins are merely facilitators of neuronal cell death [51-54]. Similarly, Endo G has been shown to relocate from the IMS during neuronal apoptosis, yet does not appear to be a critical factor in apoptotic induction [27]. Overall, recruitment of the intrinsic mitochondrial pathway to effect pure apoptosis appears a less common event than initially believed in neuronal injury (see below), being more likely to occur at low stress intensity in the insult-dependent apoptosis-necrosis continuum [16, 17]. However, apoptosis is induced by discrete forms of stress, particularly in the immature brain and during trophic factor-dependent developmental PCD [6, 19, 26, 28].

2.2 Autophagy

Autophagy is best known as the process by which molecules and organelles undergo lysosomal clearance/degradation in order to help maintain cellular homeostasis. While autophagy can manifest itself via several different pathways (reviewed in [56]), macroautophagy (MA) is the type most commonly associated with mitochondria and cell death and will be considered in detail here. Other autophagic pathways include microautophagy (autophagy without vesicles) and chaperone-mediated autophagy (CMA), and consideration of these processes is left till relevant sections below. “Autophagy” will be used here in reference to MA except where specifically identified otherwise. Autophagy involves the formation of a double membrane vesicle known as an autophagosome, which sequesters and engulfs organelles or molecules and transports them to lysosomes for degradation. This process has been widely studied by monitoring the recruitment to these vesicles of microtubule-associated protein 1-light

chain 3 (LC3), an essential protein component of the autophagosome membrane [57]. Due to the post-mitotic nature of neurones, there is thought to be heightened emphasis for autophagy to maintain homeostasis of functional organelles, such as mitochondria [58]. The prominence of autophagy in neurones can be attributed to the lack of cellular division, which contributes to the build-up of dysfunctional mitochondria and protein aggregates (i.e. β -amyloid or α -synuclein (α SN) in Alzheimer's disease (AD) and Parkinson's disease (PD), respectively), thought to lead to neuronal cell death (reviewed in [58, 59]). There are at least two routes involving autophagy to cell death in neurones. Firstly, when the autophagic process is insufficient to deal with toxic protein aggregates, as possibly occurs in AD and PD (reviewed in [60]). Secondly, the up-regulation of autophagy that is a direct contributor to cell death as in PCD-Type II. Adding further complexity to this paradigm has been the recent discovery of cross-talk between autophagy and the apoptotic pathways (Fig.1).

The recent recognition of autophagy as a cell death mechanism has seen a huge expansion of effort in this area. However, it has been noted that an enhancement of autophagic activity alone during cell death is insufficient to constitute PCD-Type II [60]. To meet the test of authentic autophagic cell death [15], it is necessary to demonstrate that down-regulation of key proteins essential for autophagy arrest cell death, as has been shown in various mammalian cell systems with Beclin-1, Atg5 or Atg7 [61, 62].

The importance of PCD-Type II in neurones has only recently been appreciated. Autophagic neuronal cell death was first suspected a decade ago with the discovery that an alternative death pathway was activated in the absence of apoptosis in sympathetic neurones [63]. However, while there have been several neuronal studies implicating PCD-Type II since then, most are not definitive in the above-mentioned terms in defining death as autophagic. Such studies have reported formation of multiple vesicles [64], the up-regulation of autophagic

proteins [65] or inhibition of death with autophagic inhibitors [63, 64]. Use of these inhibitors, such as 3-methyladenine or wortmannin, may also block apoptotic pathways [66]. Thus, many neuronal studies to date have lacked the sophistication required to confirm PCD-Type II as an independent cell death mechanism (see section 4.1).

The concept that mitochondria are central to the cell death decision process (Fig.1) also has relevance to autophagic cell death, specifically in relation to the process of mitochondrial autophagy or mitophagy (reviewed in [67]). Mitophagy is defined as the selective degradation of mitochondria by autophagy, but the mechanism of this process remains unclear. For example, it is not well understood how mitophagy is able to selectively target mitochondria, and the cellular role needs clarification, i.e. whether mitophagy is beneficial or detrimental. Nevertheless, some insight into the role of mitophagy in neurones and other mammalian cell types has been obtained. Mitochondria which are depolarised, or those associated with Bax, have been suggested to possess a propensity to be cleared via mitophagy in the absence of caspase activation during cell death (reviewed in [67]).

The process of mitophagy has been suggested in rat cortical neurones, with increased observations of autophagy associated with oxidative damage to mitochondria [68]. Moreover, the clearance of mitochondria that contain the ubiquitin ligase Parkin, a protein implicated in familial PD, provides evidence for mitophagy in a pathological neuronal setting [69]. The relocalisation of Parkin from the cytoplasm to the mitochondria was followed by the disappearance of mitochondrial markers concomitantly with the appearance of numerous lysosomes within cells. A further link between PD and mitophagy has been shown in neuroblastoma cells treated with 1-methyl-4-phenylpyridinium (MPP^+) that is the active species of the PD toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). This treatment resulted in an increase in autophagy and a loss

of the mitochondrial proteins, pyruvate dehydrogenase [70]. Here, mitophagy appeared to be regulated by MEK/ERK signalling pathway downstream from mitochondria.

Another example of cross-talk involving mitochondrial regulation, autophagic players and cell death involves Beclin-1. This protein is a central component of the autophagic pathway and itself contains a BH3-only domain [71]. A role for Beclin-1 in PCD-Type II is directly supported by evidence that its over-expression induces massive autophagy followed by cell death [72]. Beclin-1 is negatively regulated by interacting with the anti-apoptotic Bcl-2 or Bcl-X_L proteins; autophagy in parallel is induced by the competitive disruption of this interaction by BH3-only proteins such as Bad [73]. Moreover, disruption of these Bcl-2 family/Beclin-1 interactions by mutation of the BH3-only domain within Beclin-1 led to active autophagic death [72]. The identification of such interactions modulating autophagy has rendered earlier notions that Beclin-1 directly regulates apoptosis as being less likely. The current view is that cross-talk is unidirectional [73, 74], such that Bcl-2 family influences autophagy but Beclin-1 does not influence apoptosis. An interesting side-play to these interactions can be noted in relation to autophagy under starvation conditions in which autophagy can be beneficial to cellular homeostasis, rather than leading to cell death. Thus, in starved HeLa cells, ABT737 (a BH3-only peptide mimetic) induced mitophagy but not reticulophagy (also known as ERphagy), yet only ER-targeted Bcl-2/Bcl-X_L (not mitochondrially targeted Bcl-2/Bcl-X_L) was capable of inhibiting starvation-induced autophagy [75]. These interactions may be relevant to ER-stress/autophagic cross-talk discussed below.

Further interplay between the apoptotic and autophagic pathways exists via death signalling molecules such as the autophagy protein Atg5, as well as the BH3-only proteins BNIP3 and BIK. In its capacity as an autophagy protein, Atg5 is essential for autophagosome formation [76]. However, Atg5 may undergo truncation by calpains, converting it to an effective

apoptotic inducer [77]. Once truncated, Atg5 has been shown to translocate from the cytosol to mitochondria, where it associates with Bcl- X_L , triggering cyt c redistribution and downstream caspase activation [77]. Hypoxia in HEK293 cells up-regulates BNIP3 as well as effecting cell death [78]. Overexpression of BNIP3 induced autophagy, while silencing its expression resulted in a reduced level of hypoxia-induced autophagy and cell death. Interestingly, knockdown of Beclin-1 and Atg5 in this same system also reduced hypoxia-induced cell death, suggesting that all three proteins may be involved in the same cell death mechanism. BIK, another BH3-only protein, has also been linked to PCD-Type II in Bcl-2 $-/-$ murine embryonic fibroblasts. In this model, cell death triggered by ectopic expression of BIK was attenuated by autophagic inhibitors and silencing of Beclin-1 and Atg5 [79]. Autophagy may be activated by relevant Bcl-2 family members during cell death as a means for triggering additional supply of energy required to drive apoptosis to completion.

2.3 Programmed necrosis

The concept of programmed necrosis (or PCD-Type III) is less well characterised than the other two types of PCD. Programmed necrosis, as mentioned in the introductory overview (section 1), has similar hallmarks to “accidental” necrosis with the major distinction being that the former is a regulated process which can be arrested. As with PCD-Type I and PCD-Type II, mitochondria are also the hub of cell death regulation in programmed necrosis. PCD-Type III is not dependent on caspase activation [15], unlike PCD-Type I (Fig. 1). Programmed necrosis implicates the MPT and is reliant on AIF and Endo G redistribution [18, 27]. A family of cysteine proteases, called calpains, which are dependent on Ca^{2+} for activation are often involved in PCD-Type III [15, 80]. Finally, a subclass of programmed necrosis, coined “necroptosis” [21]

has been identified which requires Receptor interacting protein-1 (RIP-1) [81], which has also been implicated in MPT activation.

Through studies focusing on the role of cyclophilin D (CypD) as a component of the MPT pore, this protein has been shown to have a specific role in regulating necrotic death, but not apoptosis. In non-neuronal cells, MPT (and consequently the loss of $\Delta\Psi_m$) is blocked in cells from mice deficient in CypD; under these conditions some types of necrotic cell death, but not apoptosis were prevented [82, 83]. Moreover, over-expression of CypD in B50 neuronal cells promoted MPT and necrosis after nitric oxide (NO) treatment, but inhibited staurosporine-induced apoptosis [84]. In non-neuronal studies RIP-1 has been demonstrated to translocate to the mitochondria where it can initiate necrotic cell death, regulating the interaction between CypD and adenine nucleotide translocase [85].

As mentioned necroptosis, a subtype of PCD-Type III, involves the serine/threonine kinase RIP-1 [86]. Necroptosis has been demonstrated to be involved in delayed cell death after ischaemic brain injury in mice, which can be blocked by the necroptosis inhibitor, Necrostatin-1 (Nec-1) [21]. Nec-1 has been shown to inhibit RIP-1 [81], as well as reducing ROS levels in neuronal cells, acting upstream of the ROS production, rather than scavenging ROS directly [21, 87]. Necroptosis has also been shown to be induced by NMDA-excitotoxicity in rat cortical neurones [88]. In this study a subpopulation of neurones were thought to undergo necroptosis in response to NMDA-excitotoxicity which was inhibited by Nec-1. As with PCD-Type I and PCD-Type II, Bcl-2 family members can modulate PCD-Type III. Thus, it has recently been shown that necroptosis may also be regulated by the BH3-only protein Bmf [89]. Knockdown of Bmf in L929 cells directly inhibited necroptosis but not apoptosis, although Bmf has also been reported as a pro-apoptotic protein in other studies [90, 91].

Calpains are Ca^{2+} -dependent proteases, which have been implicated in all the PCD pathways that exist across the apoptosis-necrosis continuum (Fig. 1). While calpains have been reported to be involved in apoptosis, for example in studies on motor neurones (MNs) [92-94], these proteases are more generally prominent in programmed necrosis, where intracellular Ca^{2+} levels are elevated [95-97]. Calpains include several tissue-specific isoforms (n-calpains) and two ubiquitous isozymes, calpain I (μ -calpain) and calpain II (m-calpain) that are activated in vitro by micromolar and millimolar concentrations of Ca^{2+} , respectively [30]. Compared with caspases, calpains do not have strict cleavage specificity, and their activity is tightly regulated by cofactors, such as the endogenous inhibitor, calpastatin, and phospholipids [98]. Some cross-talk between caspase-dependent and caspase-independent PCD pathways is evident from observations that calpastatin is cleaved by caspase-3 and that this cleavage is essential for regulation of calpain activity during PCD-Type I [99, 100]. In a model of experimental hypoglycaemia, neuronal death is dependent on both MPT and redistribution of cyt c and AIF, and apparently involves both caspase-3 and calpain activation [101]. Furthermore, calpain-I is directly involved in the redistribution of AIF from isolated mitochondria [30].

Much attention has been paid to the role of AIF during neuronal cell death. Many studies reported involvement of AIF in the completion of caspase-independent cell death in neurones [25, 26, 48-50, 102-104]. Only recently has AIF-dependent death (in the absence of caspase activation) been recognised as programmed necrosis [18]. Other studies of neuronal cell death in which AIF redistribution occurred early, associated with calpain activation, and relatively weak caspase involvement, may now be redefined as PCD-Type III [95]. Whether Bid is involved in the redistribution of AIF under PCD-Type III conditions, as has been reported in apoptosis or whether calpain-I acts alone in its relocation is currently not clear [30]. Interestingly, herein calpain inhibition prevented AIF release from isolated mitochondria, underscoring the

significance of calpains in caspase-independent cell death. In another study, over-expression of calpastatin, an inhibitor of calpains, was observed to reduce kainate-mediated excitotoxicity in vivo, which was directly associated with a reduction in cleavage of Bid to tBid and the redistribution of AIF and Endo G from mitochondria [28]. Poly(ADP-ribose) polymerase 1 (PARP-1)-mediated neuronal injury was recently defined as a caspase-independent, AIF-dependent type of PCD termed “parthanatos” [22] and its characteristics are suggestive of programmed necrosis [18]. Ca^{2+} dysregulation may be a key event here, but it remains unresolved whether calpain triggers AIF redistribution in intact neurones, although potentially calpain-dependent or calpain-independent pathways could operate in parallel [105, 106].

Until recently, the role of Endo G in PCD has been unclear, due to conflicting reports on its significance in apoptosis [107-109]. In neurones Endo G translocates to the nucleus during cell death, although it is uncertain whether it is active in these systems [28, 110, 111]. Recently, Endo G was shown to be required for PCD-Type III in cortical neurons under oxidative stress on the basis of siRNA-mediated down regulation of Endo G [27]. This finding has brought new focus to the role of Endo G in PCD, because suppression of Endo G did not affect cell progression to PCD-Type I induced by staurosporine [27]. The co-existence of the two pro-death proteins AIF and Endo G offers multiple routes to demise and thus greater certainty of elimination of “at risk” neurones, raising the possibility that each might be responsible for eliciting cell death under varying conditions. Indeed, one possibility is that Endo G redistribution has a specific role in completing PCD-Type III, but is a passive event during PCD-Type I.

3. ER stress

In neurones, as in all eukaryotic cells, the ER is important for the synthesis, folding and post-translational modification of transmembrane and secreted proteins [112]. The ER also serves as

the major cellular Ca^{2+} store and the site of biosynthesis of steroids, cholesterol and other lipids, thus playing a crucial role in organelle biogenesis and signalling [113]. ER stress is caused by a perturbation of any of these major functions of the ER, and accumulation of unfolded proteins in the ER lumen is a shared hallmark of disturbance of these functions. Normally, the protein folding capacity of the ER is dynamic and alters according to the flux of unfolded polypeptide chains entering the ER. Such homeostatic control is achieved through the combined actions of signal transduction pathways named the unfolded protein response (UPR) [114]. Cellular insults that can lead to ER stress include nutrient deprivation or alterations in the redox state of the ER, secretory protein synthesis or intracellular Ca^{2+} concentrations [115].

The principles of the UPR are now relatively well defined and have been detailed in recent reviews [112, 114]. At least three distinct signalling pathways are involved, and each one is mediated by ER integral membrane proteins that sense the protein folding status in the lumen and transmit this information to the cytosol. These UPR sensor proteins are activating transcription factor 6 (ATF6), PRK-like ER kinase (PERK), and inositol-requiring kinase 1 (IRE1) [116-118]. The ER chaperone, immunoglobulin-binding protein (BiP), plays a central role in activation of each UPR sensor by detecting misfolded proteins in the ER. Under normal conditions, BiP binds to each UPR sensor. However, when misfolded proteins are present in the lumen [112], BiP binds preferentially to these proteins, and is released from each UPR sensor, resulting in their activation.

The first two responses of the UPR act to relieve ER stress and to thus promote survival of the neurone. Firstly, activation of PERK results in a reduction in the load of protein entering the ER by inhibition of cellular protein translation [119]. This step prevents further protein synthesis when the ER lumen is already overloaded and compromised in its folding capacity. The second pro-survival response, mediated by both IRE1 and ATF6 pathways, results in an increase in the capacity of the ER to handle unfolded proteins. UPR chaperones, such as BiP, calreticulin,

calnexin and the protein disulphide isomerase (PDI) family members, are upregulated to enhance the protein folding capacity of the ER [112, 114]. The UPR also removes misfolded proteins from the ER by enhancing proteolytic degradation. There are two possible routes; (i) ER-associated protein degradation (ERAD), whereby proteins are retro-translocated from the ER lumen into the cytosol for degradation by the UPS [112, 114], and (ii) autophagy, whereby regions of the ER are targeted to lysosomes or vacuoles, as discussed in more detail (section 2.2) There is a functional link between ER stress and the UPS; induction of the UPR leads to an accumulation of ERAD substrates and compromised UPS [120]. Autophagy may serve as a mechanism to eliminate portions of damaged ER under stress conditions or to control the rate of ER expansion [33] and thus promote survival upon ER stress in mammalian cells [121, 122]. However, it is not yet clear how much autophagy contributes to the clearance of misfolded proteins from the ER during UPR induction [123]. Also this process appears to be a novel, ER-specific type of autophagy that has been termed ERphagy [121], in which the autophagosomes containing sequestered ER are not degraded by vacuolar proteases until the stress is abolished. Beclin-1 is essential for the initiation of autophagy and is regulated at the ER membrane by Bcl-2 proteins, suggesting that signalling events originating from the ER are essential for autophagy.

The third response of the UPR occurs when the ER stress is prolonged or severe and homeostasis cannot be re-established. PCD is triggered, presumably to protect the organism from cells containing a potentially toxic load of misfolded proteins. The molecular mechanisms that link protein misfolding at the ER to PCD have not been fully defined. However, it is clear that PCD pathways that are either dependent on, or independent of, mitochondria are triggered by ER stress, originating from all three branches of the UPR [127].

Accumulating evidence indicates that members of the Bcl-2 family are located at the ER membrane and mediate PCD induced by ER stress [128]. The BH3-only proteins PUMA and NOXA are transcriptionally induced during ER stress, through the tumour suppressor p53 [127, 129-131]. Further, Bim is upregulated in response to ER stress both transcriptionally and at the protein level, by inhibition of proteasomal degradation [131]. The C/EBP homologous protein (CHOP) is a pro-apoptotic transcription factor strongly induced by ER stress (reviewed in [132]). Expression of CHOP leads to translocation of Bax from the cytosol to mitochondria [133], down-regulation of Bcl-2 [133] and up-regulation of BIM mRNA [131]. Moreover, in vivo studies have cells deficient in any of Bim, CHOP or Bax/Bak are all resistant to ER stress-induced apoptosis [128]. Other BH3-only proteins, Bik and Spike, also localise to the ER [134]. Together these data demonstrate a direct connection between activation of the UPR and the core mitochondrial PCD program.

All three UPR pathways activate both protective and apoptosis-like responses and, as a consequence, the initial triggers that activate PCD in ER stressed cells are not fully understood. Recently, it has been proposed that the balance between cell survival and cell death is facilitated by the relative levels of pro-apoptotic and pro-survival Bcl-2 proteins at the ER membrane [114, 135]. Tilting the balance towards PCD may take time, perhaps explaining why prolonged UPR is necessary for cell death. An alternative explanation is that the levels of CHOP mediate the transition from pro-survival to pro-apoptosis. CHOP mRNA and protein are both relatively unstable compared to other UPR proteins; hence, prolonged ER stress may be required before a critical threshold for cell death is reached [136]. Another possible mechanism for the control of ER stress-induced PCD is direct protein-protein interactions involving IRE1. Bax and Bak bind directly to IRE1 at the ER, promoting its activation, and this interaction modulates cell survival [137]. This action is in contrast to the pro-apoptotic effect of Bax and Bak at the mitochondria,

suggesting a compartmentalisation of function. Recently it was demonstrated that Bax inhibitor-1 (BI-1) also physically interacts and inhibits IRE1 [135], thus facilitating further control and cross talk between the UPR and PCD.

PCD may also result from proteolytic activation of specific caspases located in the ER. Caspase-12 in rodents is bound to the ER membrane and may be activated by a similar process to the death receptor pathway. Activated IRE1 recruits TNF receptor-associated factor 2 (TRAF2) from pro-caspase-12, resulting in its activation, leading to subsequent cleavage of caspase-9 and caspase-3 independent of classical activation of the intrinsic mitochondrial pathway. In humans, caspase-4 may substitute for caspase-12 because deletions are present in the human caspase-12 gene homologue [138, 139]. However, although caspase-12 and caspase -4 are widely used as ER stress markers, their role as initiators of ER stress-induced cell death has been refuted [140, 141]. Although poorly understood, activated IRE1 has also been implicated in ER stress-induced activation of the c-Jun N-terminal kinase (JNK), ERK and NF- κ B pathways, leading to PCD [142-144].

The ER represents the primary store for Ca^{2+} in the cell, and within the ER Ca^{2+} functions in protein folding by binding and modulating the activity of ER chaperones such as calnexin and calreticulin. Mitochondria act as an important intracellular buffer for Ca^{2+} signals emanating from the ER and recycle Ca^{2+} back to the ER [134]. Reciprocally, adjustments in Ca^{2+} levels within the mitochondria can regulate respiratory activity. Hence, the ER and mitochondria are closely coupled at several contact points.

ER stress induces the release of Ca^{2+} from the ER lumen, leading to PCD via Ca^{2+} -dependent mechanisms involving ER channels such as IP_3R [147, 148], or the ryanodine receptor [149, 150]. ER stress can also trigger prolonged Ca^{2+} accumulation in the mitochondrial matrix, leading to PCD via a voltage-dependent anion channel and IP_3R -dependent process [151].

UPR mechanisms have mainly been elucidated in cultured non-neuronal cells expressing high levels of protein, although a handful of studies have been specific to neurones [152, 153] and reviewed in [154]. The exact role of the UPR in the central nervous system in normal physiology is unknown but probably involves the early, protective components of the UPR. Due to their unique properties, neurones may be more susceptible to ER stress triggered by specific events, such as dysfunction of intracellular trafficking or Ca^{2+} buffering, compared to other cell types. Neurones also contain ER not only in the soma, but also in the dendrites [153]. The role of the ER stress in influencing cellular life/death decisions has led generally to much interest in the link between UPR signalling and human diseases that arise from pathological conditions eliciting ER stress. Despite their differing phenotypes, neurodegenerative diseases such as ALS, AD and PD share common neuropathological features, including the presence of insoluble aggregates of abnormally misfolded proteins. While the misfolded protein differs in each disorder, the common feature is protein misfolding involving neurones. [155, 156]. There is now substantial evidence that UPR is involved in the death of neurones in many neurological conditions (reviewed in [157] and discussed in more detail below), and also in acute brain disorders such as ischaemia [154].

Proteins are also synthesized and folded in the mitochondria as well as in the ER and cytosol and a similar pathway responds to mitochondrial stress, the mitochondrial unfolded-protein response (UPR_{mt}) [158]. The molecular mechanisms underlying this process are unclear, although recent studies suggested that the pathway starts with stress detection in the mitochondrial matrix followed by activation of UPR_{mt} targets in the nucleus [159].

4. Multifaceted PCD in acute and chronic neurodegeneration

4.1 Oxidative injury

Oxidative stress has been reported to occur in many different pathological conditions within the brain. Such circumstances include chronic neurodegenerative disorders such as PD (reviewed in [160]) and more acute settings such as the period directly following ischaemia-reperfusion injury (reviewed in [161]). The contribution that oxidative stress makes in triggering cell death in the brain remains unclear and it is uncertain if it alone is a sufficient trigger in pathological conditions. A major internal source of ROS in neurones is thought to result from escaping electrons from the respiratory chain within mitochondria reacting with oxygen (reviewed in [162]). These ROS in turn cause damage to DNA, proteins and lipids. Other contributions to the oxidative load may be through metal ion-associated Fenton reactions, lipid peroxidation, and nitric oxide-induced protein nitrosylation [10, 162, 163]. The type of ROS and the load of individual reactive molecules are generally accepted to play a determinant role in the death outcome. Indeed, it is now apparent that ROS have the capacities to induce diverse patterns of PCD.

In response to small or relatively low doses of oxidative stress, neurones are capable of activating the intrinsic apoptotic pathway [25]. In neurones, ROS can elicit p53 activation which in turn can upregulate the BH3-only protein PUMA [36], which has been demonstrated to translocate to the mitochondria and activate Bax under these conditions [33, 36]. The initiation of OMMP results in the redistribution of pro-apoptotic factors such as cyt c, Smac/DIABLO and HtrA2/Omi which are essential for the activation of downstream effector caspase-3 and caspase-7, which ultimately lead to cell death [25, 164]. However, under higher levels of oxidative stress, which may in itself inactivate caspases [165-167] or lead to a decrease in ATP production due to mitochondrial damage (reviewed in [9]), activation of alternate cell death pathways to apoptosis, including PCD-Type II or PCD-Type III, is a more likely outcome [19, 20].

The induction of autophagy during oxidative insult has been well documented in other cell types (reviewed in [168]). NGF-deprived sympathetic neurones can accumulate mitochondrial ROS, which results in lipid peroxidation and the loss of IMM lipid cardiolipin and ultimately autophagic cell death [169]. Oxidative stress has also been reported to induce lysosomal dysfunction resulting in neuronal death (reviewed in [170]). However these limited neuronal studies involving oxidative stress have not explored autophagic cell death as an alternative death mechanism to apoptosis. We have shown that in response to oxidative insults such as hydrogen peroxide and superoxide, there is minimal caspase activity, while autophagy activity is elevated as shown by autophagosome formation and conversion of LC3-I to LC3-II (Higgins, G.C., Devenish, R.J., Beart, P.M. and Nagley, P., unpublished data). Furthermore this increase in autophagic activity correlated with the increase in loss of membrane integrity associated with cell death. Here, autophagic cell death was confirmed by the inhibition of cell death with the autophagic inhibitor 3-methyladenine and by silencing the autophagic proteins Beclin-1 and Atg7 with siRNA.

The most pertinent of the three PCD pathways to oxidative stress is likely to be PCD-Type III [171], particularly since necrosis is the most commonly reported morphological outcome to oxidative insult. While it is possible that unregulated necrosis has occurred in many previous neuronal studies involving oxidative stress, the recognition of programmed necrosis (PCD-Type III) has driven our recent work demonstrating that under hydrogen peroxide insult the death of mouse cortical neurones is dependent on Endo G in the absence of downstream caspase activity [27]. While AIF has been widely implicated as a caspase-independent cell death signal in neurones [48, 102, 172], it has not been reported to act as a death executioner under oxidative stress conditions. This may be the result of AIF acting primarily as an antioxidant under these

conditions (due to its oxidoreductase function which allows it to clear H₂O₂ [172]) which thereby obstructs it from performing its cell death function.

4.2 Hypoxic-ischaemic injury

Stroke is one of the most common forms of acute neurological insult in adults and, while different from HI injury in infants and children, they appear to share common but differentially recruited patterns of PCD. Ischaemia involves complete arrest of circulation, but in middle cerebral artery occlusion blood flow is differently reduced in various brain areas [173], so the pathobiochemical cascade and cellular response would be expected to differ markedly across brain regions. Injury in human brain and in animal models of HI has been considered for some 10 years to involve a mix of types across the apoptosis-necrosis continuum, with apoptosis in the penumbra and necrosis in the core of the injury [174]. Only recently, with our improved understanding of the complexities of PCD, have the complexities of recruitment of diverse injury cascades, often in parallel or overlapping, been appreciated in the evaluation of HI injury [19, 20].

In the few studies of injury profiles in human stroke, apoptotic neuronal changes are considered to predominate in the infarct periphery [175, 176]. However, apoptotic cell damage (DNA fragmentation and apoptotic morphology), concomitant with necrotic cell loss, was found in autopsied brain tissue from a cohort of human ischaemic stroke patients [177]. Recent evidence in autopsied brain tissue from stroke patients suggests involvement of mitochondrial signalling in the pathobiology [178]. The first large-scale microarray report showed alterations of many genes following human stroke, including genes involved in apoptosis and cytoprotection, and although differences were found compared with data from HI rat brain many overlapping genes were identified [179]. In hippocampus and cortex of neonatal rat, HI-induced injury

involved neurones predominantly exhibiting mixed features of apoptosis-necrosis despite the presence of caspase-3. Early mitochondrial swelling with Ca^{2+} deposits could, by analogy to adult brain, be associated with cyclosporine-sensitive MPT [180]. However, classical apoptotic morphologies were rare observations in neonatal HI models, whereas older animals all exhibited predominantly necrosis [181]. However, others believe the pattern of death to be neither apoptotic nor necrotic, but to be novel and the morphology was not that of rupture, but rather a shrinkage-type of necrosis [182].

Interruption of cerebral blood by HI insults produces reduced glucose supply to the brain, marked reduction of mitochondrial respiration and ATP deficiency leading to energy crisis. Subsequent disruption of membrane ion pumps, plus increased cellular Ca^{2+} mobilization after its entry through over-activation of L-glutamate receptors, can potentially elevate mitochondrial Ca^{2+} resulting in MPT [183], generation of ROS, NAD^+ depletion and PARP-1 activation [145, 184]. Although the involvement of $\Delta\psi_m$ and subsequent rupture of the OMM remains controversial in PCD, elevated mitochondrial Ca^{2+} can induce MPT and some forms of apoptosis can be inhibited by cyclosporine, which binds to CypD in the MPT pore. Moreover, cyclosporine inhibits MPT and is neuroprotective against ischaemic brain injury [185, 186]. CypD-knockout mice display a dramatic reduction in the size of brain infarcts [187]. Collectively, events consequent to elevated cellular and mitochondrial Ca^{2+} will activate the intrinsic mitochondrial pathway and the redistribution of Ca^{2+} to cytosol activating various processes, including ER-based mechanisms and calpain-mediated redistribution of AIF [30]. While $\Delta\psi_m$ may contribute here, Bax and Bid are known to redistribute and be recruited to mitochondria during HI [44, 188] and can be sufficient signals to initiate PCD. Emerging evidence indicates that both caspase-dependent and caspase-independent signalling contributes to HI-induced cell death, which occurs across the apoptosis-necrosis continuum dependent on cell type and energy availability [49, 103, 189-193].

Many components of UPR (CHOP, , PDI, caspase-12) are activated in ischaemic injury [194]. ER-mediated injury with up-regulation of UPR markers has been studied during ischaemic injury in cultured neurones [20, 194]. In models of either focal or global ischaemia, up-regulation of UPR markers is dependent upon the model, severity of insult and the neuronal population under study [195-199]. Indeed, it has been suggested that the level of UPR response after brain ischaemia and reperfusion may be important in the extent of reperfusion neuronal death [200].

Autophagy is an adaptive strategy by which cells survive bioenergetic crisis and thus should be especially relevant to HI injury where ATP depletion occurs. Therefore, as a mechanism autophagy could be theoretically cytoprotective on the one hand, or destructive on the other, if stress becomes so advanced that cellular elimination is required. After the initial report of autophagy during HI injury, when autophagic expression of LC-3-II was found to be more prominent in older animals than in young rat brain where cell death (both caspase-dependent and caspase independent) was more pronounced [193], there has been recent increased interest in the role of autophagic mechanisms in HI [201]. In focal ischaemia there is increased formation of autophagosomes and autolysosomes, and elevated expression of LC-3, cathepsin B, and Beclin-1 [202, 203]. In the ischaemic penumbra approximately 30% of cells show caspase-3 activation and up-regulation of Beclin-1 suggesting that the two pathways may be occurring in parallel and independently in different neuronal populations, and that autophagy could have both protective and destructive outcomes [204]. In neonatal HI, Beclin-1-positive cells were also TUNEL-positive and were increased in both cortex and hippocampus. Further, rapamycin in vivo increased expression of Beclin-1, decreasing both necrosis and injury [205]. Autophagy may also occur concurrently with necroptosis during transient focal cerebral ischaemia [21].

4.3 Amyotrophic lateral sclerosis

ALS is characterized by the death of upper MNs in the motor cortex and of lower MNs in the brainstem and spinal cord [206]. The aetiology of most cases of ALS is unknown but proposed mechanisms of neurotoxicity include misfolded protein aggregation, ER stress, mitochondrial dysfunction, excitotoxicity, oxidative damage from ROS or reactive nitrogen species (collectively RONS), UPS dysfunction, and axonal transport defects [207]. Most cases arise sporadically (sporadic ALS (SALS)) but 2% of all ALS cases are familial (FALS) linked to mutations in Cu/Zn superoxide dismutase 1 (SOD1). Transgenic rodents over-expressing mutant human SOD1 develop symptoms and pathological features that recapitulate human disease and are the most widely accepted animal model [208, 209].

There is substantial evidence that molecular components of mitochondrial pathways of PCD play a role in neurodegeneration in both SOD1 rodents and in mutant SOD1 over-expressed in cell culture (reviewed in [206, 210]). The death receptor pathway has also been implicated in experimental models of ALS via Fas/CD95 receptor stimulated caspase-8 activation [211] or TNFR1 and p75 neurotrophin receptor [212, 213]. However, in humans the evidence is more conflicting, perhaps reflecting the extensive degeneration, scarcity of MNs and notorious difficulty in detecting PCD in post-mortem tissues [214]. In some studies, morphological features of PCD have been described in human ALS tissues, including condensed nuclei, DNA fragmentation and fusiform cells devoid of processes [215, 216]. However contradictory findings have been obtained in other studies, in which degenerating human MNs did not demonstrate apoptotic morphology [217-219].

Accumulating evidence suggests that mitochondrial dysfunction is involved in the pathogenesis of ALS and possible mechanisms include Ca^{2+} -mediated excitotoxicity, increasing generation of ROS, and initiation of PCD by various pathways. Morphological and ultrastructural abnormalities of mitochondria are well documented in human ALS post-mortem

anterior horns and proximal axons [220-222], and in muscle biopsy tissue [223]. Concomitantly, the activities of mitochondrial respiratory chain in human SALS tissues are reduced in complex I [224] and complex IV [225] in the skeletal muscle and in the spinal cord of SALS patients [226-228], correlating with disease progression [227]. Oxidative damage [229-231], excessive mitochondrial Ca^{2+} accumulation, and calpain activation (reviewed in [232]) are also features of human ALS MNs. More recently our laboratory implicated ER stress as an important pathway in neuronal cell death in human ALS. The full spectrum of ER stress mediators, including UPR sensors, ER chaperones and apoptotic effectors (CHOP, caspase-3, JNK kinase, p38 and caspase-4) were upregulated in ALS post-mortem lumbar spinal cords in comparison to controls [233]. PDI was also significantly upregulated in cerebrospinal fluid from SALS patients and in the surviving MNs of SALS patient tissues. In these tissues PDI was widely distributed and associated with intracellular inclusions, thus linking the UPR and misfolded protein aggregation with neurotoxicity in ALS. Consistent with our observations, another study reported the up-regulation of PDI and phosphorylation of eIF2 α in human SALS patient cords. Furthermore, an interplay between mitochondrial function, proteasome activity, and ER stress was also implicated in sporadic disease [229].

Whilst studies using human tissues are informative, detailed investigations in experimental models allow pathogenic mechanisms to be dissected. Morphological abnormalities and dysfunction of mitochondria are well documented in cellular and animal models of ALS, and in mice over-expressing mutant, these irregularities are identified prior to the onset of symptoms, thus implying an active role for mitochondria in pathogenesis. A portion of mutant SOD1 is mislocalised to mitochondria, where it forms aberrant aggregates and protein interactions [234-236] that are spinal cord-selective and age-dependent [237]. Vacuolation derived from degenerating mitochondria is a striking pathological characteristic in transgenic SOD1^{G93A} or

SOD1^{G37R} mice, which increases dramatically at the onset of disease [238]. A higher level of RONS [239] and impairment of mitochondrial energetics [235] are also observed in SOD1^{G93A} mice, and in cells expressing SOD1 mutants in vitro [240]. More recently, the MPT pore was causally implicated in MN degeneration in SOD1 mice [241]. Improper transport of mitochondria through long MN axons (up to 1 m in length) could also contribute to neurodegeneration, and disruption of both fast and slow axonal transport is well documented in ALS (reviewed in [242] and [243]). Inhibition of anterograde fast axonal transport of mitochondria was recently detected in MNs of mutant SOD1 mice, leading to their asymmetric redistribution [244]. Furthermore, mitochondrial dysfunction correlated with impaired mitochondrial transport in ALS (reviewed in [243]). This unequal distribution of mitochondria may limit their local availability in some cellular regions, leading to a deficit in energy utilization or Ca²⁺ buffering capacity, which may have important consequences due to the particularly low Ca²⁺ buffering capacity of MNs compared to other cell types [245].

Recent studies have documented convincingly a role for ER stress and UPR-induced PCD of MNs in ALS. ER stress also has close connections with other pathways implicated in ALS, involving triggers such as excitotoxicity and oxidative stress, and responses including autophagy and the UPS [121, 126, 229, 246]. Our laboratory demonstrated that up-regulation of the whole spectrum of UPR mediators occurs in lumbar spinal cords of SOD^{G93A} mice [247], including caspase-12, CHOP and downstream effectors caspase-9 and caspase-3. Other reports also describe the up-regulation of UPR proteins [229, 248-250] or dysfunction of ERAD as a trigger for ER stress [251] in animal and cellular experimental models of ALS. We and other workers also detected ER stress in pre-symptomatic animals [152, 229, 233] and induction of the UPR correlated with the fast fatiguable MNs that are targeted first in disease [152]. Together these studies imply that ER stress is an active participant in pathogenesis in ALS. This hypothesis is

borne out by studies demonstrating that modulation of the UPR in SOD1 mice by either administration of ER stress inhibitor salubrinal [152] or deletion of ER stress PCD effectors, BIM [252], ASK1 [251] or PUMA [37] has a beneficial effect on either motor performance or disease onset or both.

The presence of inclusion bodies containing aggregated and potentially harmful ubiquitinated proteins is a typical feature in MNs of both ALS patients and animal models. The UPR chaperone PDI was found to be physically associated with mutant SOD1 inclusions in SOD1^{G93A} mice and in cell culture, implying a relationship between the UPR and intracellular inclusion formation [247]. This link is borne out by the finding that pharmacological agents that modulate ER stress also alter the formation of inclusions [253]. A recent study demonstrated that mutant SOD1 inclusions are closely associated with neurotoxicity in ALS. Only cells bearing inclusions underwent PCD-Type I via the mitochondrial pathway when mutant SOD1 was expressed [254]. We also demonstrated that ER stress occurs upstream of the formation of inclusions of mutant SOD1, suggesting that oligomeric forms of SOD1 trigger the UPR (Atkin, J.D., unpublished data). We also identified an important role for Bim in linking ER stress to mitochondrial apoptotic signalling (Soo, K.Y. and Nagley, P., unpublished data). There is also evidence that dysfunction of the UPS [255] or autophagy contributes to inclusion formation and MN death in ALS models [256, 257]. These studies include those based on TDP-43 [126], which has recently received much of attention due to its potential significance in sporadic disease (reviewed in [258]).

4.4 Parkinson's disease

Long before genes implicated in familial PD had been identified, the vulnerability of dopaminergic (DA) neurones of the substantia nigra pars compacta (SNc) had been recognised,

oxidative stress documented and the existence noted of proteinaceous inclusions termed Lewy bodies [259]. Together this evidence was suggestive of injury mechanisms involving mitochondrial dysfunction, in association with inappropriate handling of abnormal proteins [260, 261], and considered to contribute to the predominant sporadic forms of the disease. Early analyses in post-mortem PD tissue revealed reduced glutathione and oxidative damage to proteins, lipids and DNA [262] in association with decreases in mitochondrial complex I activity by 30-40% [263]. DNA fragmentation reported in autopsied brain tissue may be downstream of apoptosis-like injury mediated by Bax and caspases [264]. Autophagosomes have been identified also in SNc in post-mortem PD brain [265, 266].

The case for mitochondrial dysfunction in PD was greatly strengthened by epic work that found MPTP produced PD-like symptoms in humans [267]. Further a metabolite of MPTP, MPP^+ , is transported into DA neurones where it selectively inhibited mitochondrial respiration at complex I of the electron transport chain. Subsequent investigations, using both cellular and animal models confirmed the pathological mechanism of MPP^+ . Moreover, rotenone and paraquat also produced PD-like actions via mitochondrial complex I [268-270]. The function of complex I is critical to the maintenance of $\Delta\psi_m$ and, consequently, to ATP synthesis. Since MPP^+ blocks complex I, its action dissipates $\Delta\psi_m$, predisposing mitochondria to MPT [260]. Whether MPP^+ and rotenone induce DA neuronal injury dependent or independent of caspases [271] may depend on the cellular milieu (especially redox and energetic status), but recent evidence indicates mitochondria-mediated injury favouring caspase-independent apoptotic mechanisms involving preferential redistribution of AIF [272, 273]. MPP^+ also possesses the capacity to activate autophagy in DA cell lines and neurones [70]. Paraquat can produce caspase-dependent and proteasome-dependent PCD via ER stress [274]. Finally, we should note that genes causing

familial forms of PD encode mitochondrial proteins such as PTEN-induced kinase 1 (PINK1) and DJ-1, or are linked to mitochondrial dysfunction (α SN and Parkin) [261, 275-277].

α SN is a constituent of Lewy bodies a hallmark of PD, which is just one of a wide group of synucleinopathies [278]. The neurobiology of α SN remains poorly understood - it is localized to various intracellular components, including synaptic vesicles where its association is activity-dependent [279]. Only with the detection of a mutant species of α SN in a familial form of PD [280] was its pathological role suspected, providing the first evidence for a gene causing the disease. Other forms of PD are associated with the gene encoding α SN and with variants around the gene. Expression of α SN in cellular systems has detrimental effects, but its over-expression or gene delivery produces animal models where loss of SNc DA cells is generally more modest than in the human disease [276, 279]. The mitochondrial import of mutant α SN depends on ATP and $\Delta\psi_m$, and its association with complex I was recently reported in post-mortem PD tissue [281, 282]. Recent advances in our understanding of differential involvement of PCD in neuronal injury have led to renewed interest in whether accumulation of α SN preferentially exposes neurones to elimination by ER stress and/or autophagy [261]. Mild accumulation of α SN induces the protective phases of UPR and autophagy (Tinsley, R.B. and Horne, M.K., personal communication). Oligomeric α SN triggers Ca^{2+} entry and toxicity in cell lines, but controversy remains as to the toxic contributions of fibrillary, oligomeric and aggregated α SN. Effects of α SN on ER-Golgi trafficking may underlie ER stress and Golgi fragmentation [276, 279]. In cell lines, mutant α SN induces ER stress with increased expression of caspase-12 and , which are preceded first by reduced proteasomal activity and ROS generation, and second by activation of the intrinsic mitochondrial pathway [283]. Soluble α SN is degraded by CMA whereas its aggregates induce turnover by autophagy [261].

There is far less evidence linking other more recently discovered genes to mitochondrial dysfunction and PCD. Although sporadic forms of PD are much more common than genetic PD, the identification of autosomal dominant and negative genes has allowed unprecedented insights into pathogenic mechanisms that might contribute the panoply of clinical symptoms grouped under PD. An extensive range of studies on various genes implicated in familial forms of PD has established the importance of the UPS and inappropriate protein handling as key pathological features of the disease [261, 276, 279]. Three proteins encoded by such genes are considered here, namely, Parkin, PINK1 and DJ-1.

Parkin exhibits E3 ubiquitin ligase activity *in vitro* and catalyses various types of ubiquitylation [284] but whether its ligase activity is essential for a protective role remains unknown [275, 276]. Parkin appears to have an evolutionary conserved function and the knockout mouse exhibits reduced mitochondrial respiratory activity promoting oxidative stress [285]. Recent evidence in cell lines indicates that Parkin is recruited to dysfunctional mitochondria in an ATP-independent, voltage-dependent manner promoting their mitophagy [69].

PINK1 is imported and activated in mitochondria where it is localized to the IMM, and its deficiency results in morphologically abnormal mitochondria that produce less ATP. There is mitochondrial fragmentation and reduced OMMP in PINK1-deficient human cell lines and the phenotype is reduced by expression of Parkin [286], which might activate autophagy of dysfunctional mitochondria resulting from loss of PINK1 function. Knockdown of PINK1 produced mitochondrial fragmentation, oxidative stress, mitophagy and more general autophagy in cultured cells, whilst its over-expression was cytoprotective [287]. The interaction of PINK1 and the IMS protein HtrA2/Omi via the same stress pathway is currently controversial [288, 289].

Nevertheless HtrA2/Omi, a mediator of caspase-dependent PCD, has been implicated in PD on molecular genetic grounds [290].

DJ-1 is partially localized to mitochondria and appears to possess activity as an anti-oxidant protein or redox sensor preventing cell death [291]. DJ-1-null mice possess altered striatal DA function [292] and are hypersensitive to MPTP [293]. Paraquat produces autophagy in cell lines with silenced DJ-1 [294].

These three genes affecting mitochondrial function appear to be part of an ubiquitin E3 ligase complex promoting unfolded protein degradation. Decreased expression of PINK1 or DJ-1 reduced ubiquitinylation of Parkin suggesting their cooperativity in an E3 ligase complex is vital to UPS activity and dysfunction pathogenic in PD [295]. A further link to the UPS may be via mutation of ubiquitin-C-terminal hydrolase-L1 (UCH-L1), which is associated with familial PD [276]. Its expression increases α SN apparently via CMA [296]. Aberrant activities of several kinases may contribute to the pathogenesis of PD, notably through mutations in LRRK2 (leucine-rich repeat kinase 2, PARK8), which are associated with a monogenetic form of PD, and cause tau-positive, α SN-negative inclusions of affected neurones. Mutant LRRK2 affects neuritic length and branching causing apoptosis [297], and its knockdown has a gain-of-function effect [261]. LRRK2 may be linked to the Akt/mTOR pathway, a key regulatory step in autophagy [298]. Akt itself has various influences, especially through the Bcl-2 family of proteins, and inhibition of Akt-mediated signalling by PD toxins could underlie effects on SNc neurones in post-mortem PD tissue [299].

4.5 Huntington's disease

Unlike ALS and PD, Huntington's disease (HD) is caused by mutation of a single gene, Huntingtin (Htt) [300], where expansion of the polyglutamine CAG repeats beyond 40

determines whether the disease is of juvenile or adult-onset [301]. This dominant gain of function results in pathological changes, which were initially considered to involve predominantly the caudate-putamen (corpus striatum), but have now been identified to involve degenerative changes in other brain regions, especially the cortex. Although, the disease-susceptibility of striatal and cortical neurones remains unexplained [302, 303], the restriction of Htt expression to glutamatergic pyramidal neurones in mouse models of HD fails to produce the full pathology and motor deficits, implying that multiple processes involving both cell and non-cell autonomous mechanisms work in concert [304]. Nevertheless, the striatum undergoes appreciable shrinkage where its medium-sized spiny GABAergic neurones are a principal target of neurodegeneration, although whether the direct or indirect output pathway from the striatum is preferentially affected remains unresolved [302, 303, 305].

There is clear evidence from diverse studies employing post-mortem brain tissue that PCD contributes to the neuropathology of HD, although its temporal patterns of recruitment and how the Htt aggregate load influences the cell death mechanisms need to be fully delineated. These findings include striatal neurones displaying shrinkage of their neuritic tree, alterations of various intracellular organelles including the ER, increased caspase and calpain activities, the presence of potentially toxic aggregates and inclusions, an activated UPS and evidence for autophagosomes [303, 305, 306].

Striatal cells expressing mutant Htt display increased Ca^{2+} sensitivity of $\Delta\Psi_m$ and are more vulnerable to the complex II inhibitor, 3-nitropropionic acid [307, 308]. Reported deficits in mitochondrial complex II in the striatum of symptomatic HD patients would in theory generate destructive ROS, which have detrimental effects on $\Delta\Psi_m$ impairing ATP production and ROS-scavenging mechanisms [302]. Although the relevance of this facilitation of Ca^{2+} -mediated $\Delta\Psi_m$ in immortalized striatal cells has remained unclear, recent findings of a large change in $\Delta\Psi_m$

downstream of NMDA receptor activation in mutant Htt versus wild-type striatal neurones [309] are consistent with older evidence for altered NMDA receptors and NMDA receptor-mediated excitotoxicity emergent from studies in autopsy brain tissue from HD patients [310]. Moreover, since the commonly accepted view is that impairments of mitochondrial respiratory enzyme activities are likely to be secondary rather than primary events in disease aetiology [302], these data offer a potential link in early disease between excitotoxicity and mutant Htt-induced mitochondrial and energetic defects. Mutant Htt shifts the mitochondria-mediated injury pattern induced by 3-nitropropionic acid away from PCD-type I to a caspase-independent form of cell death apparently via altered Ca^{2+} -mediated $\Delta\Psi_m$ [311]. Other evidence supports such involvement of alternative, caspase-independent, modes of PCD. In HD caudate nucleus, despite evidence for DNA fragmentation, minor caspase-3 labelling but enhanced expression of PARP have been described [312]. Our own group undertook the first detailed study in striatal neurones of mitochondria-mediated redistribution of IMS proteins by pathological stressors and these GABAergic neurones display a remarkable propensity for caspase-independent injury on the apoptosis-necrosis continuum with prominent calpain, but variable insult-dependent caspase-3 activation [95].

Mobilisation of intracellular Ca^{2+} is important for the activation of death signalling and the ER has primary roles in intracellular Ca^{2+} storage, and in protein folding and post-translational modification. Thus evidence of Htt targeting the ER and translocation to the nucleus upon ER stress is suggestive of its involvement in cellular injury in HD [306]. Additionally, findings in various cellular models suggest triggering of the ER stress response by mutant Htt [313-315]. Recently ER stress has been reported in the striatum of the Htt knock-in mouse where it precedes and persists during the formation of N-terminal Htt inclusions. Moreover, BiP and CHOP mRNAs were found to be up-regulated two-fold in the parietal cortex of HD post-mortem tissue

providing the first evidence for ER stress associated with the impact of mutant Htt [316]. Such evidence of early activation of ER stress cascades, with consequent effects on the UPR, suggests these events could initiate PCD with recruitment of mitochondria-mediated redistribution of IMS proteins and downstream activation of destructive proteases. As a consequence of ER stress and UPR, there appears to be a further interface of PCD mechanisms in that Htt redistributes from ER and can shuttle between nucleus and cytoplasm to play a role in autophagy. Indeed the co-existence of Htt in endosomal-lysosomal compartments is consistent with older evidence for the presence of autophagosomes and their role in the handling of protein aggregates [317] and autophagic cell death [306].

5. Conclusions and perspectives

Extensive evidence from research on both animal and cellular systems indicates that neurones experience a variety of stresses relevant to acute or chronic neurodegeneration. The stresses recruit death pathways that can no longer be simply classified as either apoptosis or necrosis. Not only are there various types of PCD but also cells may show features of different types of PCD due to the multiple death pathways that can operate in parallel and show considerable extents of cross-talk, including sharing components of the intrinsic mitochondrial pathway and recruitment of various proteases. Mitochondria are involved in most, if not all, of such multifaceted death pathways. Moreover, mitochondria may be recruited early such as in cellular responses leading to apoptotic outcomes or they may have later engagement such as when ER stress occurs. This review has emphasised the range of mitochondrial responses, including what is now recognised as classical apoptotic signalling (PCD-Type I) through redistribution of cyt c and apoptosome formation, or the signalling that leads to early AIF and Endo G redistribution as primary players

in programmed necrosis (PCD-Type III). Mitochondria may also show deficits in energisation and consequent loss of ATP synthetic capacity, and thus be subject to mitophagy in parallel with other autophagic events leading to cell death (PCD-Type II).

Overall, these considerations have led us to a general schema (Fig. 3) to represent the balance (or imbalance) between the forces that cause stress on the one hand (left side of fulcrum) and ameliorative or restorative factors on the other (right side of fulcrum). In neuronal homeostasis these ameliorative forces are in balance with stress, but as the stresses become more pronounced and the restorative factors less able to cope, the cells undergo greater degrees of debilitation until death ensues. The factors detailed Fig. 3 are those appraised in this review and which are especially involved in neuronal stress and death. We have emphasised here that the death outcomes are indeed multifaceted and we have thus represented this new understanding as a Venn diagram (Fig. 4) in which the main classifications of PCD are displayed. The death outcome(s) for any given cell under a particular stress in an acute injury or chronic disease may fall within the zones indicated in Fig. 4. Central to this diversity of death patterns is the primary player, the mitochondrion, plus the two parallel processes that are known to have important roles as discussed above, ER stress and the UPS (see also Fig. 1).

Whilst cellular models enable an incisive dissection of the specific molecular details of cell death pathways and their cross-talk, animal models can provide a closer link to the “authentic” pathobiological scenario as occurs in clinically presented acute or chronic neurodegenerative disease. This review has integrated such investigative approaches to encompass acute situations such as oxidative stress or HI injury, and the chronic neurodegenerative conditions ALS, PD and HD. The general principles of multifaceted neuronal death and the role of mitochondria have been emphasised in each case. Indeed, it is likely that many of these general principles may extend, at least in part, to other pathologies, including AD [318-320].

Animal models directed towards neurodegenerative diseases include pharmacological models, genetic knockouts affecting particular genes, and also transgenic animals that over-express specific proteins. Each model has its own advantages and disadvantages. Extrapolation of findings obtained from studies using animals to the situation in humans must be approached with caution. Therefore it is necessary to check all symptomatic features and disease progression in such animal models. The degree of correlation may thus lead to tempering of conclusions drawn from animals in their application to the clinical situations.

Notwithstanding the detailed information obtained to date about the cell death pathways in individual neuronal model systems, a further context that requires much deeper analysis is the relationship between neurones and juxtaposed glial cells in the physiological milieu. A specific issue is whether a particular neurodegenerative disease is cell-autonomous, where neurones form the primary defective entities in isolation from surrounding cells. Additionally, there are also functional changes in glial cells, such as astrocytes, which normally sustain neurones. Such glial perturbations (non-cell-autonomous) are thought to apply in ALS and several other neurodegenerative disorders [13, 206, 321]. Thus, whilst we can analyse multifaceted death in neurones under various stresses that replicate a relevant aspect of a particular disease, the full understanding of the pathobiological processes requires us to learn much more about all the cells that such neurones may interact with. Astrocytes, for example, display very different biological profiles when they become “reactive” and an emergent literature indicates the normal homeostatic or sustenance relationships, are disrupted turning them instead into “collaborators in neurotoxicity” [13].

Finally, brief mention can be made of the potential for development of new therapeutic agents based on understanding of the processes leading to the multifaceted deaths that neurones may undergo. Appreciation of the complexity of interactions affecting mitochondria and the range of

downstream outcomes and parallel factors, as represented in Fig. 1 and Fig. 3, may lead one to conclude that approaches based on suppression of just one death outcome (e.g. PCD-Type I, using small molecule caspase inhibitors) may not work effectively. The death pathways may be shifted under such a regime of treatment [171]. In order to achieve effective results in neuroprotection it might be better to address the upstream signalling processes and the mitochondria themselves. The issues here are perhaps opposite to those in much of cancer research where activating death of “rogue” cells is paramount. Neuroprotective strategies should be addressed to preventing cell death while preserving optimal neuronal function. Indeed, one can present a strong case for “polypharmacy” strategies targeting multiple upstream mechanisms and cellular compartments such as the ER and proteasome. Identification of the relevant death pathways and in particular diseases and learning how to block their initiation (rather than their execution) will be key to future therapeutic strategies.

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Figure legends

Fig. 1. Diverse pathways leading to cell death. The schematic indicates key stages that occur after a cell experiences a particular stressor from either outside or within the cell (the death trigger). The primary response is elicited by the immediate perturbation of cellular structures and functions. Subsequent intracellular signalling pathways are activated (secondary response), which, after processing (e.g. through interaction of competing members of the Bcl-2 family of proteins), lead to a “decision”. This determines the adoption of one or more death pathways that result in the possible outcomes listed at the foot of the schematic. Mitochondria play a key role in many of these death pathways, which may operate through redistribution to the cytosol of apoptogenic proteins. Other aspects of mitochondrial involvement (not shown here) include disrupted energisation of these organelles (and consequent imbalances in ion fluxes and loss of ATP production) and generation of secondary fluxes of ROS. Endoplasmic reticulum (ER) stress is sometimes invoked, for example in protein aggregation which becomes determinant when putative toxic protein intermediates are not destroyed by the UPS. Cross-talk between pathways (double-headed horizontal arrows) signifies the upstream and downstream interactions between pathways. Upstream cross-talk may influence the signal processing phase, ; downstream cross-talk affects the final death mechanism and morphological indications that typify the various death modes. Caspase involvement is obligatory for “pure” apoptosis (PCD Type I), but is less evident in other apoptosis-like outcomes. The currently applied various death classification systems are summarised below the schematic, embracing the PCD Type nomenclature and the more recently defined mechanistic terms for cell death modes [12]. The concept of the apoptosis-necrosis continuum integrates the differential involvement of these various death pathways and outcomes, to help explain the often complex patterns of neuronal death (mix of PCD-Types I, II and/or III) as described in more detail in the text. In unregulated (accidental) necrosis, there is a rapid

general breakdown of many aspects of cellular integrity. This characteristically includes dissipated mitochondrial membrane potential ($\Delta\Psi_m$) with consequential losses of both ion gradients across the IMM and ATP production.

Fig. 2. Typical markers of cell death in neurones undergoing various types of PCD. Cells illustrated here are primary cortical neurones from embryonic mice. For markers of PCD-Type I cells were either untreated (controls) or treated with staurosporine (STS). Cyt c localisation (green) is observed in controls (A) as mitochondrial but in treated cells (B) as redistributed throughout the cell; nuclei are stained with DAPI (blue). Further, caspase-3 activation (red) is not seen in controls (C) but is observed prominently in treated cells (D). DAPI staining shows normal nuclei in controls (C) but condensed and fragmented nuclei in treated cells (D). For markers of PCD-Type II, cells were either untreated (controls) or treated with hydrogen peroxide. GFP-LC3 (green) is seen as diffusely distributed throughout the cell in controls (E) but as numerous puncta in treated cells (F). In cells showing GFP-LC3 puncta, analysis of nuclear morphology after staining with propidium iodide (red), which diffuses into cells once the plasma membrane becomes permeabilised (G and H), shows that cells may either contain condensed fragmented nuclei (G) or condensed but not fragmented nuclei (H). For markers of PCD-Type III, cells were either untreated (controls) or treated with hydrogen peroxide. Endo G localisation (red) is observed in controls (I) as mitochondrial but in treated cells (J) as redistributed diffusely throughout the cells, which have become rounded. Plasma membrane permeabilisation does not occur in controls (K) as evidenced by exclusion of propidium iodide (red); the differential interference contrast image is shown to reveal the cell population in this field. However, at relatively early times (4 h) of treatment (L), uptake of propidium iodide occurs in the majority of cells. Bars in all micrographs indicate 10 μm . Arrows indicate index cells that demonstrate the

described features. Detailed methods for cell treatment, analysis, microscopy and imaging are as published [27, 322]. GFP-LC3 images are from Higgins, G.C. and Nagley, P., unpublished data.

Fig. 3. Neuronal cellular welfare as a function of the balance between homeostatic and deleterious factors. In healthy functional neurones (A) homeostasis is maintained by cellular feedback mechanisms. Under conditions of minor stress (B) homeostasis is disturbed by deleterious factors (represented by spheres at left) such as RONS and misfolded proteins (MP). These factors have deleterious influences on energy production (ATP) and intracellular Ca^{2+} , the latter being elevated in both cytoplasm and mitochondria by entry via various channels and transporters. Neurones possess inbuilt capacities to respond to minor stress (spheres at right, upward arrows) by activation of the UPR (in its initial, pro-survival phase) and CMA. With greater imbalances (indicated by an increased number of vertical arrows; RONS, ATP, and Ca^{2+}), deleterious factors overwhelm cellular homeostatic processes (C). The UPR switches to its destructive mode via induction of apoptotic effector proteins such as CHOP, caspase-12 and JNK. The UPS becomes increasingly dysfunctional as neither it nor CMA can adequately handle the increased load of MP, leading to formation of intracellular aggregates of MP. At this stage there is a strong commitment to death; at which point mitochondrial membrane potential ($\Delta\Psi_m$) usually declines. Finally, the neurone is overwhelmed by the total level of stress (D), and the cell advances to PCD (type-I, type-II or type-III, or combinations thereof, as represented in Fig. 4).

Fig. 4. Composite death outcomes in neurones. The death outcomes may show a range of properties in terms of both biochemical mechanisms and cellular morphologies (see text and Figs 1 and 2, herein). The relative emphasis of each type of death process is dependent upon the neuronal cell type and the stresses undergone. The icons in the central common zone indicate key

players considered in this review, namely, mitochondria, ER stress and the UPS (icons as in Fig. 1).

Table 1

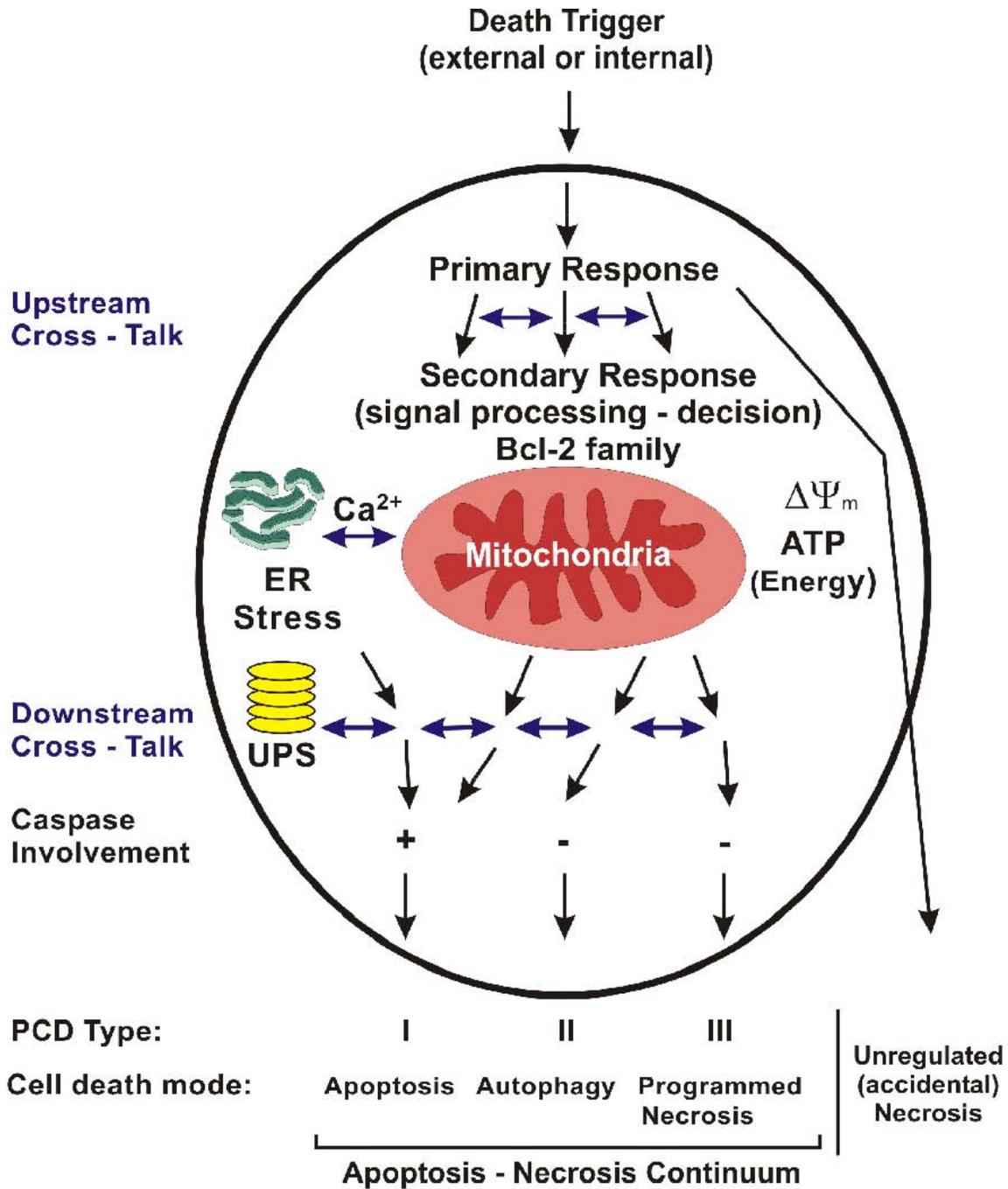
List of abbreviations

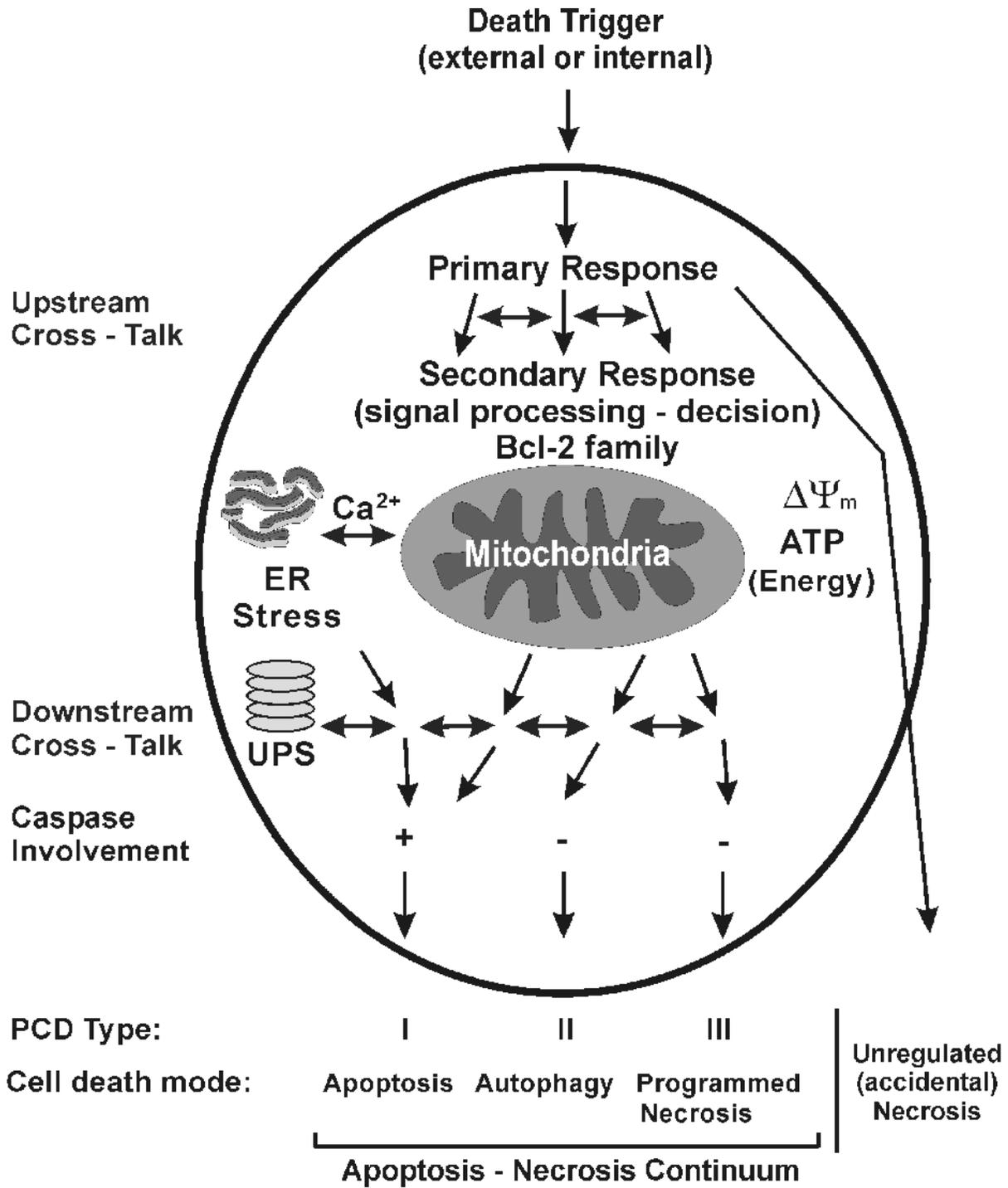
Abbreviations	Definitions
AD	Alzheimer's disease
AIF	Apoptosis inducing factor
ALS	Amyotrophic lateral sclerosis
ATF6	Activating transcription factor 6
BH-3	Bcl-2 Homology domain 3
BiP	Immunoglobulin-binding protein
CHOP	C/EBP homologous protein
CMA	Chaperone-mediated autophagy
CypD	Cyclophilin D
Cyt c	Cytochrome <i>c</i>
DA	Dopaminergic
Endo G	Endonuclease G
ER	Endoplasmic reticulum
ERAD	ER-associated protein degradation
FALS	Familial ALS
HD	Huntington's disease

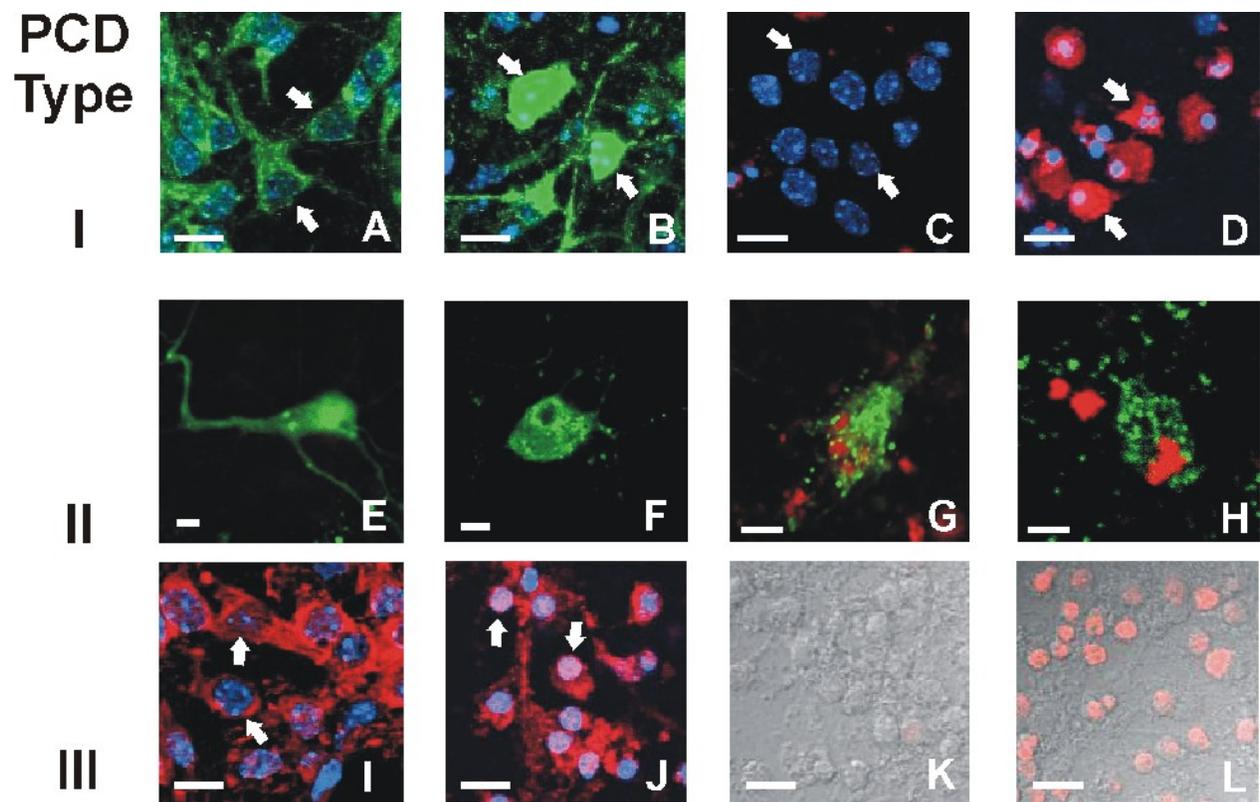
HI	Hypoxia-ischemia
Htt	Huntingtin
IMM	Inner mitochondrial membrane
IMS	Intermembrane space
IP ₃	Inositol-1,4,5-triphosphate
IP ₃ R	Inositol-1,4,5-triphosphate receptor
IRE1	Inositol-requiring kinase 1
JNK	c-Jun N-terminal kinase
LC3	Microtubule-associated protein 1-light chain 3
MA	Macroautophagy
MN	Motor neurones
MP	Misfolded proteins
MPP ⁺	1-methyl-4-phenylpyridinium
MPT	Membrane permeability transition
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
Nec-1	Necrostatin-1
OMM	Outer mitochondrial membrane
OMMP	Outer mitochondrial membrane permeabilization
PARP-1	Poly(ADP-ribose) polymerase 1
PCD	Programmed cell death
PD	Parkinson's disease
PDI	Protein disulphide isomerase
PERK	PRK-like endoplasmic reticulum kinase PTEN-induced kinase 1

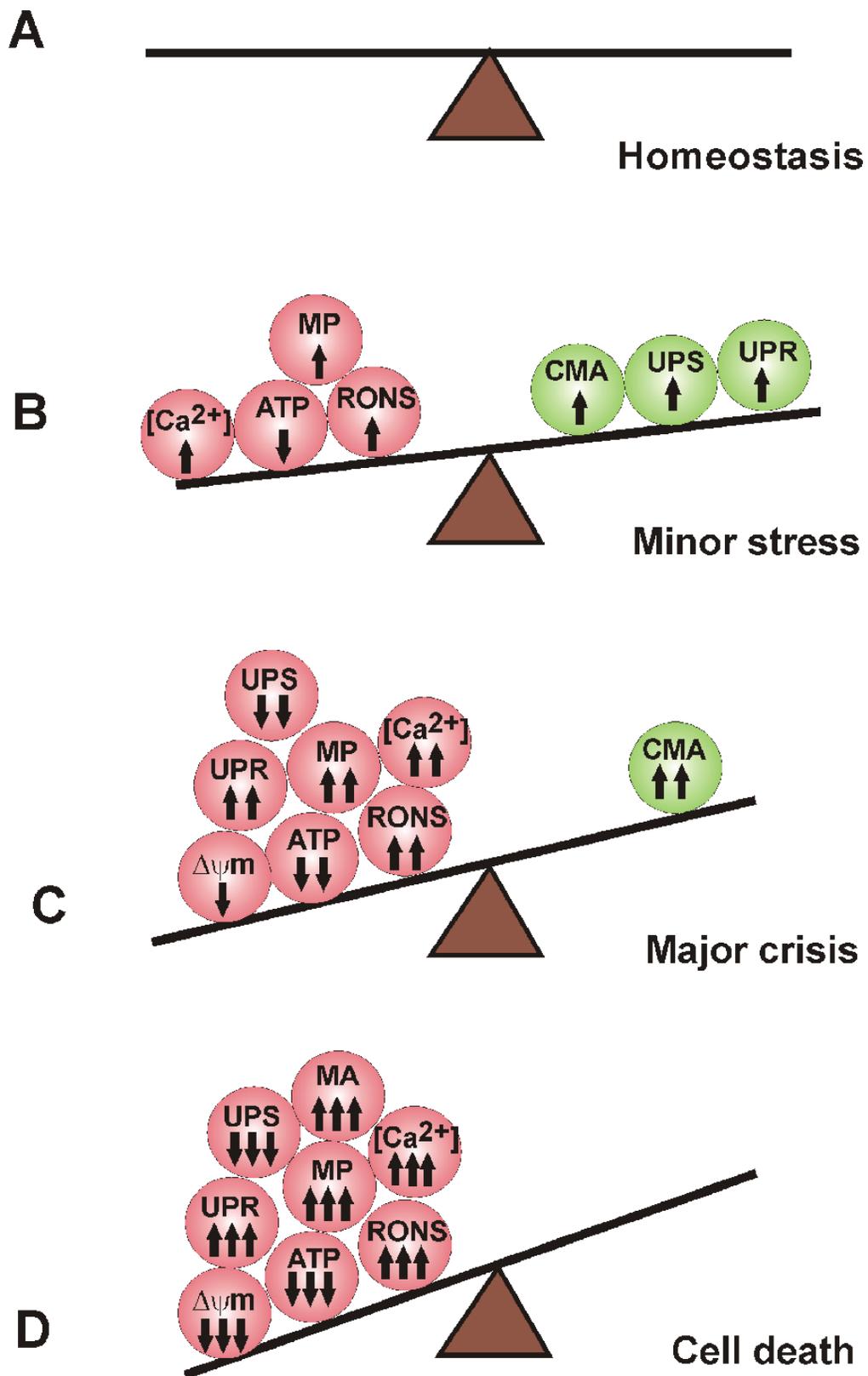
PINK1	
RIP-1	Receptor interacting protein-1
RONS	Reactive oxygen and nitrogen species
ROS	Reactive oxygen species
SALS	Sporadic ALS
Smac/DIABLO	Second mitochondria-derived activator of caspases/direct inhibitor of apoptosis-binding protein with low PI
SNC	Substantia nigra pars compacta
SOD1	Superoxide dismutase 1
TRAF2	TNF receptor-associated factor 2
UPR	Unfolded protein response
UPRmt	Mitochondrial unfolded protein response
UPS	Ubiquitin-proteasome system
α SN	α -Synuclein
$\Delta\Psi_m$	Mitochondrial membrane potential

List shows abbreviations used more than once after the initial definition in the text.







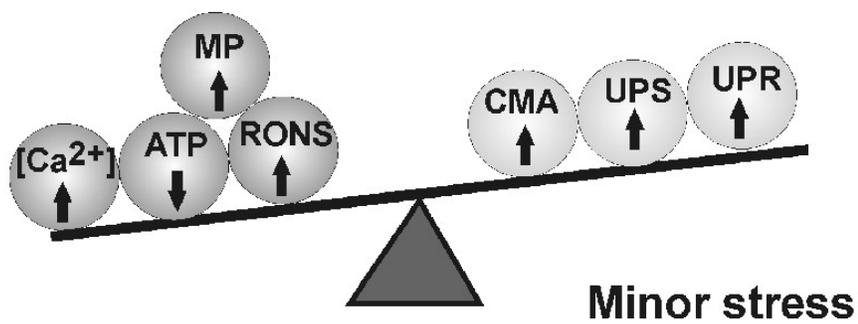
Balance of life
and death

Balance of life
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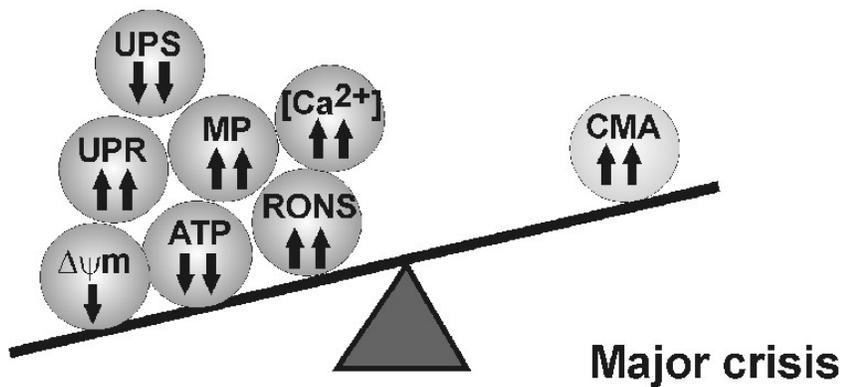
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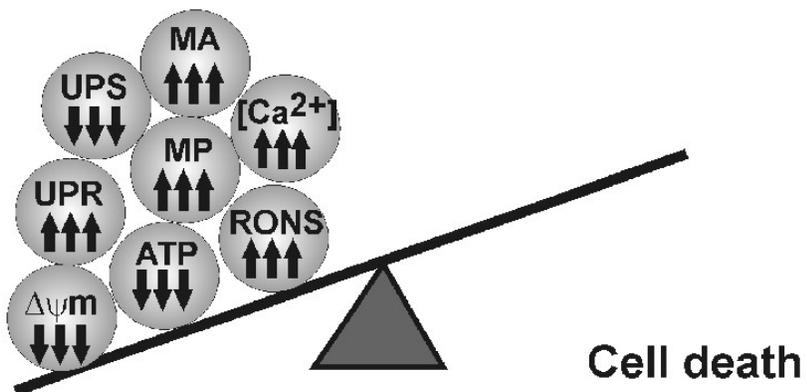
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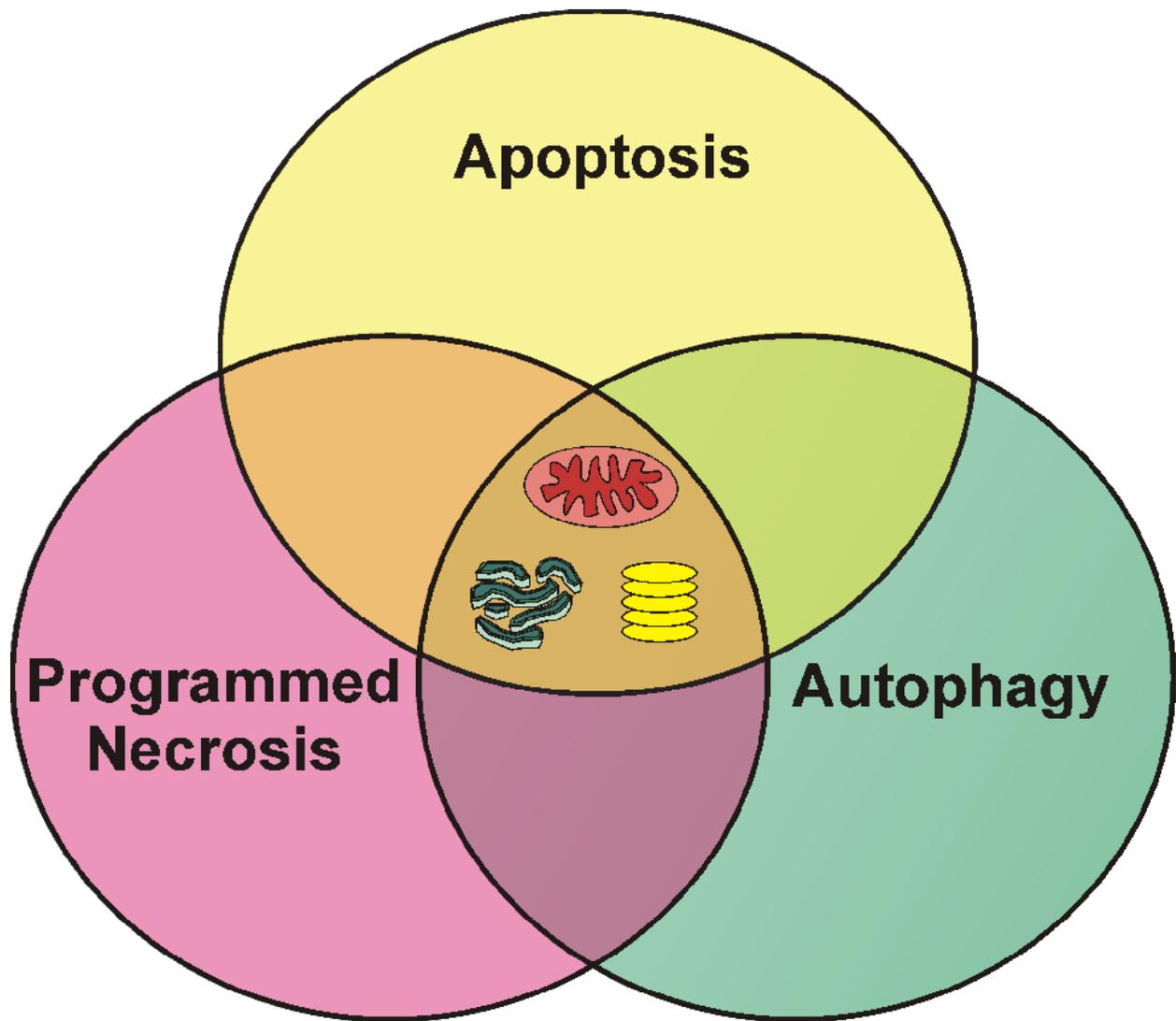


C



D





AC

