Neuron
Review

New Views on the Misconstrued: Executioner Caspases and Their Diverse Non-apoptotic Roles

Nicolas Unsain[1] and Philip A. Barker[2,*]
[1]Laboratorio de Neurobiología, Instituto de Investigación Médica Mercedes y Martín Ferreyra, Instituto Nacional de Investigación Médica Córdoba-Consejo Nacional de Investigaciones Científicas y Técnicas, Universidad Nacional de Córdoba, Friuli 2434, Córdoba (5016), Argentina
[2]Irving K. Barber School of Arts and Sciences, University of British Columbia, Kelowna, BC V1V 1V7, Canada
*Correspondence: phil.barker@ubc.ca
http://dx.doi.org/10.1016/j.neuron.2015.08.029

Initially characterized for their roles in apoptosis, executioner caspases have emerged as important regulators of an array of cellular activities. This is especially true in the nervous system, where sublethal caspase activity has been implicated in axonal pathfinding and branching, axonal degeneration, dendrite pruning, regeneration, long-term depression, and metaplasticity. Here we examine the roles of sublethal executioner caspase activity in nervous system development and maintenance, consider the mechanisms that locally activate and restrain these potential killers, and discuss how their activity be subverted in neurodegenerative disease.

The pioneering studies that led to the identification of CED3 as an executioner caspase in *Caenorhabditis elegans* heralded an explosion of activity that established caspases and apoptosis as crucial developmental effectors in essentially all metazoan species (Putcha and Johnson, 2004; Yuan et al., 1993). In the last quarter century, the biochemistry and biology of the caspase family have been under intense study, and the roles of caspases in developmental, injury-induced, and disease-associated cell death have been well characterized (McIlwain et al., 2013). There are 18 caspases present in mammals, but only a subset of these function in the core pathways that mediate apoptosis. Termed the apoptotic caspases, these are further categorized into initiator caspases and executioner caspases. Initiator caspases act as signal integrators that translate receptor- or sensor-based cues into activation signals for the executioner caspases that mediate most of the proteolytic cleavages required to kill the cell.

The executioner caspases are very good at what they do. In almost every cell, from worms to man, their destructive power can be unleashed to kill the cell. However, over the last several years, it has become clear that executioner caspases also carry out a wide array of sublethal functions. Using such potent killers for non-lethal tasks is a risky proposition, and mechanisms that locally activate, target, and limit executioner caspase activity play a key role, ensuring that caspase activity is kept in check. Indeed, a primary function of caspase regulatory mechanisms is to ensure that executioner caspases are employed in sublethal settings.

Because executioner caspases are under tight temporal and spatial control, their actions are particularly well suited for neurons that must integrate local signals and then drive rapid structural and morphological change in regions far from the cell body and nucleus. In this review, we first examine cellular settings in which roles for sublethal caspase roles have been established, taking a phylogenetic view when possible, and then focus on the roles of sublethal executioner caspase activity in nervous system development, maintenance, and disease. We then discuss the mechanisms that regulate sublethal caspase activity and close with future perspectives.

Executioner Caspase Activation Pathways

In mammals, the ultimate effectors of the cascade that drives the apoptotic program are the executioner caspases 3, 6, and 7 (Fuentes-Prior and Salvesen, 2004). The executioner caspases are zymogens that are activated by upstream initiator caspases such as caspase-8 and caspase-9. After proteolytic cleavage, the large (α) and small (β) subunits assemble into catalytically active oligomers, with $\alpha\beta\beta'\alpha'$ symmetry, that have two active sites per complex. The accumulation of cleaved forms of an executioner caspase is often considered a hallmark of an irreversible commitment to apoptosis, and cleavage-specific antibodies that detect the active forms of caspase-3, -6, or -7 are often used to identify apoptotic cells.

Two main pathways of executioner caspase activation have been identified (Chowdhury et al., 2008; Li and Yuan, 2008). The extrinsic pathway is initiated by activation of a group of cell surface receptors collectively referred to as death receptors (DDs). In the presence of ligand, trimeric receptor complexes bind TRADD and/or FADD and, thereby, engage pro-caspase-8 to form the death-inducing signaling complex (DISC). Caspase-8 undergoes an autocatalytic activating cleavage event in the DISC and, thereby, initiates the caspase cascade, either indirectly through cleavage of BID and activation of the intrinsic pathway or directly via direct cleavage and activation of caspases-3,-6, and -7. The intrinsic pathway of apoptosis converges on mitochondria to induce formation of the mitochondrial permeability transition pore (MPTP) (reviewed in Gillies and Kuwana, 2014; Westphal et al., 2014). Bax and Bak play key roles in the formation of the MPTP, and their activities are normally suppressed through the action of anti-apoptotic Bcl2 family members. The task of relieving the Bcl2-dependent suppression of Bax and Bak is mediated by activation of BH3 domain-only proteins such as Bid, Bad, and Noxa (reviewed in Happo et al.,

2012). Generation of the MPTP allows cytochrome *c* to leave the mitochondrion and bind Apaf1, thereby creating the apoptosome, in which autocatalytic cleavage of caspase-9 allows executioner caspase activation to proceed.

In *Drosophila*, the main initiator caspase is the caspase-9 ortholog Dronc. Activation of Dronc requires association with Dark, the *Drosophila* homolog of Apaf-1, and, in some but not all tissues, a specific cytochrome *c* isoform is also required for Dronc activation (Arama et al., 2006; Dorstyn et al., 2002; Mendes et al., 2006). Dronc and Apaf1 are mutually suppressive. When Dronc levels are high, Apaf1 levels are reduced, and when Apaf1 level are high, Dronc levels are reduced. It has been proposed that this mutually suppressive regulation allows a low level of apoptosome activity to be generated for use in sublethal contexts, after which it is rapidly suppressed (Shapiro et al., 2008). The fly executioner caspases are reasonably well conserved with their mammalian counterparts, with Dcp1 and Drice resembling caspase-3, Damm appearing most orthologous to caspase-7, and Decay aligning best with caspase-6 (Steller, 2008).

Sublethal Executioner Caspase Activity in Cellular Remodeling, Differentiation, and Survival

Initial evidence that executioner caspases play sublethal roles emerged from an examination of the remodeling events that drive the loss of cellular material during spermatid individualization in *Drosophila*. During fly spermatogenesis, syncytial spermatids are resolved into sperm through the action of the individualization complex (IC), a specialized cytoskeletal structure that depletes spermatid cytoplasm and then encapsulates sperm in the plasma membrane (Zheng et al., 1999). Individualization complex function relies on the action of the Apaf-1 homolog *Dark* and the fly caspase *Dronc*, and flies containing mutations in either of these genes have spermatogenesis defects and male sterility (Arama et al., 2006; Huh et al., 2004; Rodriguez et al., 1999). Subsequent studies have demonstrated that apoptotic caspases also play key roles in several mammalian cell remodeling events, including the enucleation of erythroblasts (Carlile et al., 2004), the terminal differentiation of keratinocytes and lens cells (Ishizaki et al., 1998; Lippens et al., 2000; Yamamoto-Tanaka et al., 2014), and the production of platelet cells from megakaryocytes (De Botton et al., 2002).

Induction of myogenesis correlates with large increase in caspase-3 and -9 activity (Murray et al., 2008), and myotube formation is reduced by caspase-3 knockdown in primary myoblasts derived from caspase-3-null animals (Fernando et al., 2002; Hunter et al., 2007; Murray et al., 2008). Interestingly, mitochondria do not lose membrane potential or release cytochrome *c* during myogenesis, suggesting that caspase-9 and -3 activation may not require assembly of the apoptosome in this context. An intriguing feature of myocyte differentiation is the importance of DNA double-strand breaks (DSBs), which promote the expression of regulatory genes that drive the differentiation program (Larsen and Megeney, 2010; Sjakste and Sjakste, 2007). These DSBs are produced through caspase-dependent cleavage of iCAD, the inhibitory protein that normally restrains caspase-activated DNase (CAD) activity. Myocytes depleted of CAD fail to produce DSBs, and, as a result, myogenic differentiation is blocked (Larsen et al., 2010). It is intriguing to note that transient DSBs also accumulate in neurons within multiple brain regions of rodents exploring novel environments (Suberbielle et al., 2013), raising the possibility that caspase-dependent iCAD cleavage may drive gene expression changes in other cellular contexts.

Caspase-3 activity is also required for stem cell differentiation (Fujita et al., 2008; Janzen et al., 2008), for the production of pluripotent stem cells from human fibroblasts (Li et al., 2010a), and for neural stem cell differentiation (Fernando et al., 2005). In addition to driving DNA double-strand breaks, caspase-3 can cleave and constitutively activate kinases (Fernando et al., 2002, 2005; Kanuka et al., 2005) or destroy proteins that are required for the maintenance of self-renewal and pluripotency (Fujita et al., 2008; Janzen et al., 2008).

Surprisingly, caspase-3 activity can also contribute to cell survival. Initial results suggesting this showed that neuroprotection conferred by a preconditioning ischemic event was lost in the presence of caspase inhibitors (McLaughlin et al., 2003). More recently, Khalil et al. (2012) have generated compelling genetic evidence showing that moderate caspase-3 activation results in cleavage of RasGAP, which, in turn, increases Akt activation and promotes cell survival. Therefore, mice lacking caspase-3 are defective in Akt activation and display increased cell death and tissue damage in response to modest levels of cell stress (Khalil et al., 2012). These counter-intuitive results demonstrate the diverse activity of the executioner caspases and emphasize that they cannot simply be considered apoptotic proteases.

Executioner caspases can also affect cellular behavior via non-cell-autonomous effects (King and Newmark, 2012; Ryoo and Bergmann, 2012). For example, apoptotic cells can generate mitogens that induce cell division in surrounding cells (Bergmann and Steller, 2010; Mollereau et al., 2013). This phenomenon has been established in decapitated *Hydra*, where caspase activity in cells at the injury site generates Wnt3, which then induces proliferation of neighboring cells and drives head regeneration (Chera et al., 2009). Similarly, in *Planaria*, caspase activity in dying cells drives production of Wnt family members, which induce stem cell division and drive regenerative responses in surrounding cells (Gurley et al., 2010; Pellettieri et al., 2010).

Apoptosis-induced proliferation has also been observed in mammalian cells where caspase-3 and -7-dependent activation of phospholipase A_2 and the subsequent production of arachidonic acid and prostaglandins mediate the proliferation of neighboring progenitor cells (Li et al., 2010b). Apoptosis can also exert non-cell-autonomous survival effects. For example, when *Drosophila* wing imaginal discs undergo apoptosis, they release a factor that induces activation of the receptor tyrosine kinase Tie on neighboring cells. This, in turn, induces an anti-apoptotic microRNA termed Bantam, making surviving cells harder to kill (Bilak et al., 2014). Therefore, in an act of cellular altruism, executioner caspases generate factors that act non-cell-autonomously to promote proliferation and survival in neighbors while ensuring host cell death.

Turning to the Nervous System: Caspases in Neuronal Growth and Regeneration

Because the activation, localization, and control of caspase activity are regulated tightly, these enzymes are well suited to mediate local proteolytic events in response to extracellular

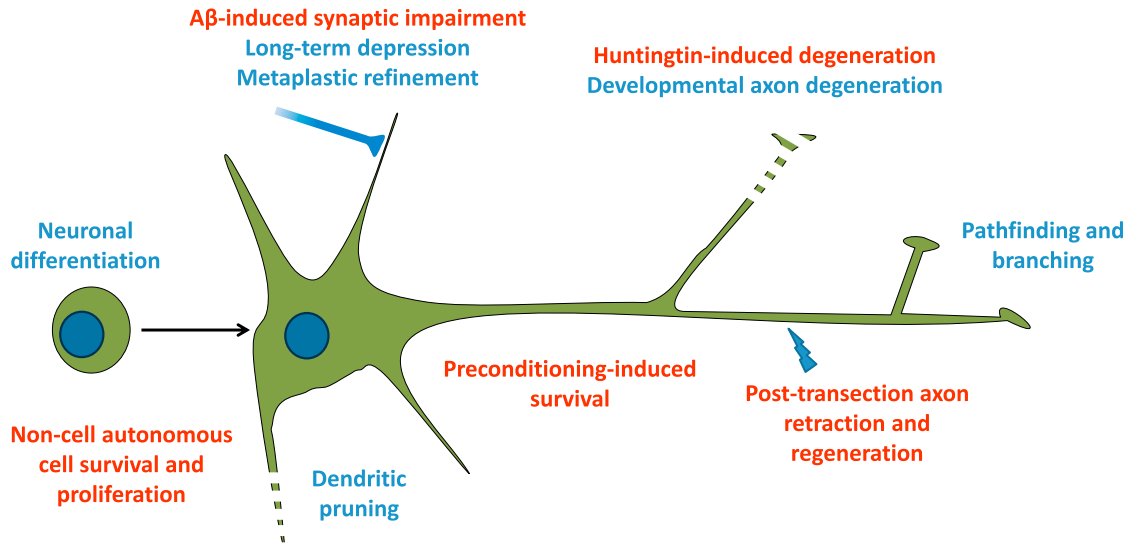

Figure 1. Sublethal Caspase Activation Regulates Several Aspects of Neuron Biology
Neuronal activities in which sublethal caspase activity plays a significant role are indicated. Activities shown in blue text indicate caspase functions that represent normal physiological roles, and those in red indicate roles for sublethal caspase activity after injury or in disease. See text for details.

cues. However, in the context of a developing neuron, the cellular consequences of caspase activity are diverse (Figure 1). In some circumstances, caspases facilitate growth cone formation and promote pathfinding, whereas, in other situations, caspase activity reduces or even blocks growth.

The first indication that caspase activity plays a significant role in neuronal growth and pathfinding came from Campbell and Holt (2003), who found that retinal neurons exposed to netrin-1 or lysophosphatidic acid (LPA) displayed a rapid rise in caspase-3 activity and showed that caspase inhibitors prevented chemotropic responses to these factors. A physiological role of sublethal caspase activity in directing retinotectal projections has been confirmed recently by Campbell and Okamoto (2013). By imaging caspase activity in vivo, they showed that the Slit-Robo cascade activates caspase-3 and -9 specifically at retinal ganglion cell branch points.

The patterning of olfactory sensory neurons also relies on sublethal caspase activity, with caspase-9- or Apaf1-null animals displaying defects in axonal projection and synapse formation (Ohsawa et al., 2010). Ohsawa et al. (2010) have also shown that at least part of this defect reflects reduced cleavage and release of Semaphorin 7A, a membrane-anchored semaphorin required for the proper targeting of these projections. Taken together with recent work showing that caspase-3 activity is required for NCAM-dependent neurite outgrowth in hippocampal neurons (Westphal et al., 2010), these data suggest that ligand-receptor systems traditionally associated with pathfinding (Netrin, Slit, Semaphorins) or adhesion (NCAM) mediate at least part of their effects through sublethal caspase activation, which, in this context, likely targets cytoskeletal elements with the growth cone or growing axon.

The early events that occur after axon transection include axon sealing, retraction, formation of the growth cone, and then axon extension. Interestingly, in dorsal root sensory neurons transected in vivo, caspase-3 inhibition drastically reduces axon retraction, prevents growth cone formation, and blocks regeneration (Öztürk et al., 2013; Verma et al., 2005). Similarly, after laser transection of sensory or motor axons in *Caenorhabditis elegans* lacking CED3 (the worm caspase 3 homolog), growth cone initiation was reduced, and, of the few sprouts produced, most were defective. This resulted in a dramatic defect in regenerative outgrowth in CED3-null axons. The authors also found that calcium transients, calreticulin, and Ced-4 (the worm form of Apaf1) played key roles in regeneration. Intriguingly, they noted that Ced-4 has two EF-hand like domains and proposed that Ca^{2+} binding to these may induce Ced-4 activation in this setting (Pinan-Lucarre et al., 2012).

Taken together, these studies show that orthologs of caspase-3 and -9 play a key role in developmental pathfinding decisions and regeneration, most likely by acting locally at or near the distal tip of the neurite. In most cases, caspases seem to have a positive impact, facilitating turning, growth cone formation, and neuronal extension. It seems likely that they achieve this by cleaving regulatory elements that control growth cone and/or branch point formation and outgrowth dynamics, but this remains to be demonstrated experimentally.

Caspases in Developmental Axon and Dendrite Degeneration

The facilitation of growth by caspases is contrasted dramatically by their role in the destruction of axons and dendrites. Unequivocal evidence for a role of caspases in neurite remodeling emerged from analyses of the remodeling of *Drosophila* class IV dendritic arborization sensory neurons, which produce an elaborate dendritic arbor in the larval stage that is destroyed during morphogenesis. The cell body remains intact and gives rise to new dendrites that are maintained into maturity (Truman, 1990). The dendritic arbor destruction relies on the activity of DRONC, which is present in an active form in the dendrite but

suppressed initially by an inhibitor of apoptosis protein termed DIAP1. In this setting, DRONC becomes active only after DIAP1 is phosphorylated, ubiquitinated, and proteolyzed (Kuo et al., 2006; Kuranaga et al., 2006; Williams et al., 2006).

The role of caspases in developmental pruning in mammals has been best studied using NGF-dependent dorsal root sensory neurons (DRGs). In vivo, these neurons extend axons to the periphery, and those that arrive late or are mistargeted do not receive adequate NGF and undergo rapid degeneration (Ernsberger, 2009). This degeneration can be replicated in vitro simply by plating these neurons in NGF, allowing them to establish exuberant axons, and then subjecting them to NGF withdrawal (Unsain et al., 2014). Although early studies using chemical caspase inhibitors ruled out a role for caspases in NGF deprivation-induced axonal degeneration (Finn et al., 2000; Raff et al., 2002), more recent analyses of DRG neurons derived from mice null for BAX, caspase-9, or caspase-3 have demonstrated that the intrinsic pathway plays a key role (Nikolaev et al., 2009; Simon et al., 2012; Unsain et al., 2013). Importantly, these studies confirmed that chemical caspase inhibitors had a minor effect on NGF deprivation-induced axonal degeneration in *CASP3*$^{+/+}$ axons but strongly suppressed generation in *CASP3*$^{+/-}$ axons. This suggest that, under normal circumstances, the amount of inhibitor that accumulates in axons is simply too low to block caspase activated upon NGF withdrawal (Nikolaev et al., 2009; Unsain et al., 2013).

Caspases were reconsidered as effectors of mammalian axonal degeneration in large part because of a 2009 paper that indicated that a signaling pathway activated by death receptor 6 (DR6), a TNF receptor superfamily member, induced local caspase activation in DRG axons deprived of NGF (Nikolaev et al., 2009). The scheme presented indicated that NGF deprivation activated BACE-dependent cleavage of the amyloid precursor protein (APP) and that an extracellular fragment of APP generated by this cleavage functioned as the activating ligand for DR6 (Nikolaev et al., 2009). As noted above, the notion that caspases play a crucial role in DRG axon degeneration induced by NGF deprivation has now been repeated by many labs, but other elements of this proposed pathway have not stood up to genetic scrutiny performed by the authors of the original study. Their subsequent work has shown that animals rendered null for DR6, APP, or BACE show little or no protection from NGF withdrawal-induced axon degeneration and that many of the pharmacological inhibitors and function-blocking antibodies used in the study by Nikolaev et al. (2009) have protective effects even on mice rendered null for their molecular targets (Olsen et al., 2014). Therefore, the activities ascribed to the BACE inhibitors and function-blocking antibodies against APP or DR6 in Nikolaev et al. (2009) likely reflect their interaction with alternative targets.

Compartmentalized cultures (Campenot chambers or microfluidic devices) can be used to provide cell bodies with NGF while examining the local events that occur in distal axons upon NGF withdrawal. Using this approach with sympathetic neurons, Cusack et al. (2013) showed that caspase-3 and -9 play essential roles in local axon degeneration induced by NGF withdrawal, whereas Apaf1 was not required, suggesting that uncharacterized post-mitochondrial caspase activation pathways must be activated in these axons.

The precise role of caspase-6 in axonal degeneration has been controversial. The 2009 paper by Nikolaev et al. (2009) indicated that caspase-6 was selectively activated within axons of NGF-deprived DRG neurons and that caspase-3 was activated only in cell bodies. However, subsequent genetic and biochemical analyses have established that caspase-9 and -3 are highly active in axons of NGF-deprived neurons and that they play essential and dominant roles in NGF withdrawal-induced degeneration (Cusack et al., 2013; Simon et al., 2012; Unsain et al., 2013). Interestingly, axons of *CASP6*$^{-/-}$-sympathetic neurons subjected to "whole-cell" NGF withdrawal undergo rapid degeneration, but, when maintained in compartmented cultures and subjected to local NGF deprivation, *CASP6*$^{-/-}$ axons are protected from degeneration (Cusack et al., 2013). Therefore, caspase cascades that are activated by local deprivation can differ from those activated when the entire cell is subjected to trophic factor withdrawal. Discerning the specific events that occur in these settings is certain to provide insights into novel mechanisms that locally activate executioner caspases.

To date, detailed biochemical analyses have been performed only on NGF-dependent peripheral neurons, but genetic analyses have suggested that caspases also play an important role in developmental pruning of central populations. For example, pruning is defective in projections of retinocollicular axons to the superior colliculus in both caspase-3 and caspase-6 knockout mice (Simon et al., 2012), and both caspase-3 and -9 have been implicated in developmental pruning of olfactory neurons (Cowan and Roskams, 2004; Cowan et al., 2001). Furthermore, although mechanistic details are lacking, genetic data indicate that APP and DR6 (but not BACE) play significant roles in the regulation of retinocollicular axon pruning during development and in experience-dependent cortical plasticity (Kallop et al., 2014; Marik et al., 2013; Olsen et al., 2014). Taken together, these data indicate that executioner caspases have a fundamental phylogenetically conserved role in axon and dendrite pruning and raise the possibility that specific, largely uncharacterized local signaling cascades drive selective caspase activation events in these settings.

Caspases in Neuronal Plasticity

The hypothesis that caspases may function as modifiers of synaptic plasticity was first advanced after α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic (AMPA) receptor (AMPAR) subunits were identified as substrates for caspase cleavage (Chan et al., 1999; Lu et al., 2002). Subsequent studies using pharmacological inhibitors raised the possibility that caspase activity plays a role in hippocampal long-term potentiation (LTP) (Gulyaeva et al., 2003; Lu et al., 2006), in long-term sensitization in snails (Bravarenko et al., 2006; Kozyrev et al., 2007), and in the maintenance of long-term spatial memory in rats (Dash et al., 2000). In a particularly elegant and thorough study, Huesmann and Clayton (2006) showed that levels of active caspase-3 were increased post-synaptically in the bird auditory cortex during song habituation and that caspase blockade prevented acquisition of song-specific memory in the adult zebra finch.

More recently, caspase-3 activation has been shown to play an essential role in long-term depression in the hippocampus,

acting to accelerate AMPA receptor internalization (Jiao and Li, 2011; Li et al., 2010c). The activation of caspase-3 that occurred in this setting appeared to rely on the mitochondrial activation pathways because mice lacking *BAD* or *BAX* were similarly defective in NMDA receptor-dependent long-term depression (LTD) (Jiao and Li, 2011). This was a selective effect. Despite a profound defect in NMDA receptor-dependent LTD, both LTP and mGluR-LTD were normal in the *BAD, BAX*, and *CASP3* mutants.

There are at least two ways in which caspase-3 activation can lead to LTD, the first involving a caspase-3, Akt, and GSK3 cascade that contributes to AMPAR internalization. GSK3β activity is normally suppressed by Akt, but caspase-3 dependent cleavage of Akt ablates its kinase activity, freeing GSK3β from inhibition. PSD95 can then be phosphorylated directly by GSK3β, which leads to dispersal of PSD95 clusters and destabilization of PSD95 within post-synaptic densities (Asselin et al., 2001; Li et al., 2010c; Nelson et al., 2013; Peineau et al., 2008).

The second involves calcineurin, the calcium-regulated protein phosphatase that is activated by NMDA receptor (NMDAR)-dependent calcium influx. Calcineurin mediates AMPR dephosphorylation and, thereby, facilitates AMPA receptor internalization (Beattie et al., 2000; Esteban et al., 2003). Interestingly, calcineurin can also dephosphorylate BAD to promote caspase activation (Wang et al., 1999), and calcineurin itself can be activated by caspase-3 cleavage (Mukerjee et al., 2000). Therefore, it is possible that caspase- and calcineurin-dependent feedforward mechanisms facilitate the generation of LTD.

Using a proteomics approach, Han et al. (2013) identified several caspase-3 cleavage substrates in neurons undergoing apoptotic cell death. One of these was GAP43, a protein well known for its roles in axonal biology. Surprisingly, GAP43 was found to be enriched in the post-synapse, and overexpression of GAP43 mutants resistant to caspase-3 cleavage reduced NMDA-induced AMPAR endocytosis and blocked LTD (Han et al., 2013). Several other neuronal caspase-3 substrates identified in this study have been directly or indirectly linked to synaptic biology, raising the possibility that other caspase-dependent AMPAR internalization routes may exist.

Numerous studies have shown that LTD is associated with spine shrinkage and elimination (Nägerl et al., 2004; Oh et al., 2013; Zhou et al., 2004), and it is reasonable to predict that these spine-specific morphological changes are produced by sub-lethal caspase activity. This has not yet been demonstrated under physiological circumstances, but, by inducing local caspase activity in defined dendritic regions of cultured hippocampal neurons, Ertürk et al. (2014) induced local spine elimination and dendritic pruning, consistent with this notion. Interestingly, this local activation of caspases went on to kill the cell when endogenous caspase inhibitors were blocked, suggesting that, under normal circumstances, dendritic caspases must be tightly controlled to enable local morphological change while preventing widespread cellular damage.

Stimuli that induce long-term potentiation of synaptic strength within one population of synapses can induce heterosynaptic LTD at less active synapses on the same cell (Lo and Poo, 1991; Scanziani et al., 1996). Recent studies have provided compelling evidence that heterosynaptic plasticity produces changes in synaptic structure that reflect a competitive balance between active and inactive synapses (Bourne and Harris, 2011; Lee et al., 2013). By monitoring the dendritic morphology of individual spines at or close to a site of glutamate uncaging, Oh et al. (2015) established that input-specific synaptic potentiation induces shrinkage and weakening of nearby unstimulated synapses. They rule out the possibility that this local synaptic involution reflects a competition for limited resources within the dendrite but provide evidence that the synaptic weakening reflects an activity-dependent shrinkage signal produced from spines undergoing potentiation. The nature of the signal(s) produced and the signaling pathways evoked remain unknown, but it seems likely that cell surface signaling cascades that induce local caspase activation will emerge as an important mediator of spine shrinkage.

Initial neuronal activity can alter subsequent synaptic plasticity events through metaplastic changes (Huang et al., 1992). Recently, Chen et al. (2012) established a paradigm for examining metaplasticity in vivo, using natural stimuli (patterned or unpatterned light) to stimulate tadpole retinal neurons and then examining the morphology and synaptic activity of tectal projections. After establishing that patterned stimuli cause LTP, whereas non-variant ambient light results in LTD, the authors examined the impact of a metaplastic stimulation (i.e., unpatterned visual stimuli) on the activity and dendritic structures evoked by subsequent patterned or non-variant ambient light. Interestingly, after metaplastic stimulation, patterned stimuli evoked LTD instead of LTP, and this switch relied on an NMDA-dependent activation of caspase-9 and caspase-3/7. MEF2, a transcription factor that functions as a key regulator of activity-dependent synapse development, was identified as a caspase target required for the metaplastic effect (Chen et al., 2012; Dunfield and Haas, 2009). Therefore, caspases can alter synaptic physiology locally by altering AMPA receptor internalization and spine morphology and through long-range effects by cleaving transcription factors that are transported retrogradely to alter gene expression.

Caspases have also been implicated in other forms of synaptic remodeling, most notably for the removal or dispersal of inactive synapses at neuromuscular junctions during development. In developing muscle, acetylcholine receptors (AChRs) form presumptive clusters before innervation, and those that fail to associate with neuronal inputs disperse and then disappear (Moody-Corbett, 1986). Intriguingly, dispersion of the non-innervated AChR clusters is regulated by locally activated caspase-3 (Wang et al., 2014), which acts to cleave disheveled 1 (Dsv1), a protein that normally associates with MuSK, which acts to promote AChR clustering. Cleavage of Dsv1 by caspase-3 ablates its pro-clustering activity, allowing AChR cluster dispersion to proceed.

Taken together, these data indicate that caspase-9 and -3 play a key role in synapse modifications and that they use a variety of context-specific mechanisms to accomplish this. Their targets include cytoskeletal structural elements, phosphatases, kinases, and transcription factors in which cleavage can cause gain or loss of target function. Novel caspase targets involved in synaptic changes are emerging, and sorting out precisely who does what in specific synaptic contexts will be a fascinating challenge in the coming years.

Synaptic Caspases in Neurological Disease

Caspase activity is well suited to drive the major structural modifications required to produce changes in plasticity. However, caspases are powerful proteases, and it can be expected that small changes in their activity can result in pathophysiological consequences. Consistent with this, several recent studies have demonstrated that caspase activity can drive synaptic loss in models of neurodegenerative disease.

More than a century ago, Cajal (1911) proposed that dementia reflects the weakening of synapses. This concept has been confirmed in Alzheimer's disease (AD), where post-mortem analyses of hippocampi from AD patients have revealed a substantial decrease in dendritic spine density compared with age-matched controls (Ferrer et al., 1990), along with early changes in synaptic function (Ingelsson et al., 2004; Masliah et al., 1994).

Emerging evidence from animal models examining the roles of APP in AD pathogenesis strongly suggest that caspase activity plays a key role driving synaptic defects induced by this protein. It is clear that the Aβ fragment of APP can inhibit hippocampal LTP and facilitate LTD (Cheng et al., 2009; Kim et al., 2001; Rowan et al., 2005; Shankar et al., 2008) through activation of a BAX- and caspase-3-dependent pathway (Chen et al., 2013; Olsen and Sheng, 2012) and that mice expressing mutant forms of APP associated with familial AD display increased cleaved caspase-3 in the post-synaptic compartment, along with reduced spine density (D'Amelio et al., 2011; Lanz et al., 2003). Importantly, when these same mice were established in an Apaf1-null background, they did not show elevated cleaved caspase-3, and pharmacological inhibition of caspase-3 activity rescued the reduction in spine density seen in these animals (D'Amelio et al., 2011).

Several studies have shown that Aβ42-induced synaptic removal of AMPAR relies on the activation of calcineurin (Hsieh et al., 2006; Wu et al., 2010, 2012; Zhao et al., 2010), and, as noted above, calcineurin can be activated by caspase cleavage (Mukerjee et al., 2000). Therefore, it is possible that APP products produced in the Tg2576 strain activate caspase-3-dependent calcineurin cleavage, and, consistent with this, abnormally high levels of calcineurin proteolytic fragments are observed in mice expressing mutant forms of APP, and these return to baseline levels after administration of a pharmacological caspase inhibitor (D'Amelio et al., 2011).

Although the generation of LTP is caspase-independent, the suppression of LTP induced by Aβ42 does not occur in caspase-3-null mice (Jo et al., 2011). Furthermore, mice with the Danish mutation of BRI2/ITM2b, which have increased APP processing, display a reduction in LTP that is accompanied by enhanced caspase-9 activity in synaptic fractions derived from the hippocampus (Tamayev and D'Adamio, 2012; Tamayev et al., 2012b). Interestingly, inhibition of caspase-9 activity rescues the LTP defect and the progressive memory loss that occurs in these animals (Tamayev et al., 2012a).

Executioner caspase activity has also been implicated in the neurodegeneration that occurs in Huntington disease (HD). HD is caused by an expansion of the CAG repeat in the *huntingtin* gene (Albin et al., 1990; Vonsattel et al., 1985), and the resulting mutant protein (mHTT) is a substrate for proteolysis. The generation of toxic N-terminal fragments from mHTT are thought to play a crucial role in the pathogenesis of HD (Gafni et al., 2004; Graham et al., 2006), and caspase-6 has emerged as an important mHTT protease. Graham et al. (2006) found that mutation of the caspase-6 cleavage site within mHTT blocked generation of the toxic N-terminal fragments, and they and others have found that YAC128 mice bearing a mutation in the caspase-6 cleavage site have a delay in disease onset (Graham et al., 2006, 2010, 2012; Metzler et al., 2010; Milnerwood et al., 2010; Pouladi et al., 2009). Therefore, cleavage of mHTT at this site (amino acid 586) is sufficient and required for pathogenesis in mouse models.

Interestingly, cleavage of mHTT at amino acid 586 still occurs in caspase-6-null animals, albeit at a reduced level (Gafni et al., 2012; Landles et al., 2012; Wong et al., 2015) and Wong et al. (2015) have shown that caspase-8 mediates mHTT cleavage under these circumstances. Detailed phenotypic analyses indicate that some, but not all, HD-like phenotypes are ameliorated in YAC128 mice lacking caspase-6 (Wong et al., 2015), suggesting that that toxic mHTT fragments have a dose-dependent effect. Given this, therapies that specifically target caspase-6 may have the potential to elicit a partial therapeutic benefit.

Keeping Caspases Sublethal

Executioner caspases can be readily and permanently switched from an inactive to an active state, are highly processive, and have a broad target range. Over the last two decades, a wide array of potential caspase regulators have emerged, but these regulatory mechanisms do not seem to play a significant role in regulating apoptosis under physiological circumstances (Galbán and Duckett, 2010; Martins et al., 2004; Okada et al., 2002). This is not surprising because the one circumstance under which the cell requires unrestrained caspase activity is when it is trying to rapidly destroy itself. Rather, it seems likely that the major physiological role of the regulatory mechanisms that spatially or temporally limit caspase activity is to regulate the sublethal caspase activity (Figure 2).

One way to ameliorate the dangerous effects of caspases is to ensure that activation occurs only when and where it is required. Mechanisms that allow caspase activation in specific subcellular compartments are beginning to emerge. One example is Tango7, a phylogenetically conserved apoptosome component that binds DRONC and DARK and directs the *Drosophila* apoptosome to the individualization complex, where sublethal caspase activity is required for sperm individualization (D'Brot et al., 2013). Another involves the local activation of Apaf1 and sublethal caspase activity induced by local calcium fluxes that occur after transection of sensory and motor axons in *Caenorhabditis elegans* (Pinan-Lucarre et al., 2012). In other cases, such as the sublethal activation of caspase-3 that occurs during LTD, caspase-9 and caspase-3 activation occur only within specific dendritic spines (Li et al., 2010c). The precise mechanisms that mediate this local activation remain uncertain, but Bax-dependent engagement of mitochondria plays a crucial role in this process (reviewed in Brusco and Haas, 2015).

When an executioner caspase is activated, the cell can draw upon an array of overlapping mechanisms to reduce its activity or destroy it. The best characterized cellular caspase inhibitors are members of the inhibitor of apoptosis (IAP) family. Of the eight mammalian IAP proteins, XIAP, cIAP1, and cIAP2 have

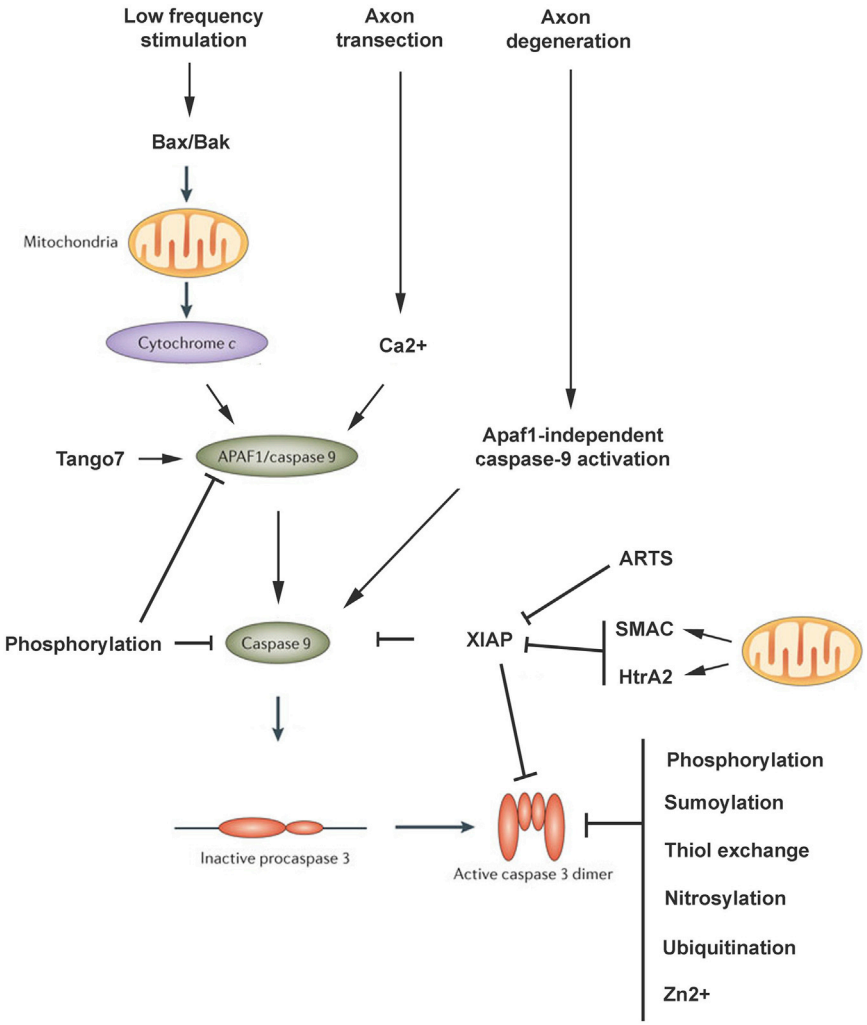

Figure 2. Regulation of Sublethal Caspase Activity
Schematic showing distinct pathways that have been implicated in caspase-9 activation in sublethal settings as well as negative regulatory elements that may facilitate or suppress sublethal caspase activation. See text for details.

been best characterized for their roles in caspase regulation (Galbán and Duckett, 2010; Lopez and Meier, 2010; O'Riordan et al., 2008). These can each bind caspases via their baculovirus inhibitor of apoptosis protein repeat (BIR) domains, with their C-terminal RING domain conferring E3 ligase activity (Yang et al., 2000). All three proteins contain a central ubiquitin-associated (UBA) domain (Gyrd-Hansen et al., 2008), and cIAP1 and cIAP2 also contain a caspase activation and recruitment domain (CARD) that regulates auto-ubiquitination (Lopez et al., 2011). *Drosophila melanogaster* has four IAPs, and one of these, DIAP1, structurally resembles XIAP and potently suppresses several caspases.

IAPs can directly bind and inhibit caspases, or they can target caspases for ubiquitination and proteolytic removal. In flies, DIAP1 suppresses caspase activity and developmental apoptosis through the latter mechanism (Goyal et al., 2000; Hawkins et al., 1999; Lisi et al., 2000; Wilson et al., 2002; Yan et al., 2004). cIAP1, cIAP2, and XIAP can also target mammalian caspases for ubiquitination (Choi et al., 2009; Huang et al., 2000; Suzuki et al., 2001b), but it is not certain whether this mechanism is employed to remove caspases under physiological circumstances. Of the mammalian IAPs, only XIAP can directly block caspase-3, -7, and -9 enzymatic activity (Chai et al., 2001; Riedl et al., 2001; Salvesen and Duckett, 2002; Scott et al., 2005; Shiozaki et al., 2003; Sun et al., 1999, 2000; Vaux and Silke, 2005). It is important to note that IAPs do not block caspase activation. Rather, they inhibit activated caspases. This is because IAPs interact with caspases via a tetrapeptide motif that is revealed at the amino terminus of the small caspase subunit of caspase-3, -7, or -9 after proteolytic processing (Salvesen and Duckett, 2002; Scott et al., 2005). The amino terminus of the small subunit of caspase-6 does not contain this IAP-binding motif and, therefore, cannot bind or be inhibited by the IAPs (Riedl et al., 2001).

IAPs can regulate sublethal caspase activity in vivo. As noted above, *Drosophila* class IV dendritic arborization sensory neurons initially produce complex dendritic arbors during pupation that are subsequently destroyed by the sublethal activation of DRONC (Kuo et al., 2006; Williams et al., 2006). Before morphogenesis, DRONC activity is constrained through the action of DIAP1. This inhibition is relieved when IAP is phosphorylated by the kinase IK2, which triggers its ubiquitination and subsequent proteolysis in the proteasome (Kuo et al., 2006; Kuranaga et al., 2006; Oshima et al., 2006). Similarly, XIAP must be removed by proteosomal degradation for axonal degeneration to proceed in DRG sensory axons that have been withdrawn from NGF (Unsain et al., 2013). The pathway driving this event in axons has not been established, but it is notable that IKKε, the mammalian homolog of IK2, phosphorylates XIAP, leading to its ubiquitination and proteasomal destruction in non-neuronal cells (Kuranaga et al., 2006; Nakhaei et al., 2012).

A role for IAPs in regulating sublethal caspase activity in synapses has not been established directly, but they are likely to play a substantive role. Huesmann and Clayton (2006) showed that IAP overexpression blocked song acquisition in finches and argued that active caspase-3 is always present in synapses but is normally sequestered by inhibitors that release a limited amount of active caspase-3 only for non-sublethal functions. This implies that synaptic caspase activity is regulated mainly by tuning caspase inhibition. IAPs seem to play an important

role in this context because IAP antagonists allow caspase activity that is normally limited to the dendrite to spread throughout the neuron and ultimately kill the cell (Ertürk et al., 2014).

IAP transcripts are strongly regulated at transcriptional and translational levels. cIAP2 mRNA expression is strongly induced by the canonical and non-canonical nuclear factor κB (NF-κB) pathways, whereas XIAP mRNA harbors an internal ribosome entry site (IRES) in its 5′ UTR that is utilized during periods of cellular stress. The Holcik groups (Durie et al., 2011; Thakor and Holcik, 2012) have studied the regulation of the XIAP IRES in detail and have shown that, when eIF2α is phosphorylated and bulk translation is blocked, XIAP is recruited into polysomes and translated in a cap-independent manner. Because eIF2α phosphorylation is required for some forms of LTD (Costa-Mattioli et al., 2007), it will be interesting to test whether XIAP produced via cap-independent IRES translation regulates caspase activity in dendrites.

Several IAP antagonists have been characterized in mammals and in *Drosophila*. SMAC (second mitochondria-derived activator of caspase; Fulda and Vucic, 2012) and HTRA2 (second homolog of high temperature requirement-like; Gray et al., 2000) are produced in the cytosol and then transported into mitochondria, where they are cleaved to produce N-terminal neo-epitopes that resemble the IAP binding motifs present in caspase-3, -7, and -9. Upon MPTP, these are released from mitochondria and can function as competitive antagonists that release caspases from their IAP blockade. They can also destroy their IAP clients, with SMAC inducing IAP auto-ubiquitination and proteasomal destruction (Galbán and Duckett, 2010; Yang and Du, 2004) and HTRA2 utilizing its intrinsic serine protease activity to cleave the IAPs (Srinivasula et al., 2003; Suzuki et al., 2001a; Yang et al., 2003).

A similar mechanism of IAP antagonism is employed in *Drosophila*. The *reaper*, *hid*, and *grim* (RHG) genes are transcriptionally activated in response to many different proapoptotic signals, and, when translated, they bind to DIAP1 to induce its autoubiquitination and proteosomal destruction (Steller, 2008). Interestingly, although the RHG-DIAP1 pathway plays a crucial role in regulating developmental and injury-induced apoptosis in flies, mice rendered null for SMAC, HtrA2, or both do not display defects in apoptosis during development or in adulthood (Jones et al., 2003; Martins et al., 2004; Okada et al., 2002). Although it seems likely that SMAC or HTRA2 will have relevance for the regulation of caspases in sublethal settings, their involvement remains to be demonstrated experimentally.

ARTS is not structurally or genetically related to the IAPs, but, nonetheless, it directly binds XIAP and inhibits its anti-apoptotic activity, apparently by stimulating XIAP autoubiquitination (Gottfried et al., 2004; Larisch et al., 2000). Importantly, ARTS-null mice have elevated XIAP protein levels in some tissues and display defects in spermatocyte cytoplasm clearing and hair follicle stem cell apoptosis (Fuchs et al., 2013; Kissel et al., 2005). Importantly, the defect in hair follicle stem cell apoptosis in *ARTS*$^{-/-}$ mice can be rescued in *XIAP*$^{-/-}$ mice, demonstrating that the ARTS-XIAP regulatory loop plays a critical role in regulating caspase-3 in vivo (Fuchs et al., 2013). Whether ARTS regulates caspases in the nervous system remains unknown, and this is clearly an interesting topic for future studies.

IAP-independent Caspase Regulatory Mechanisms

Caspases can also be regulated by a range of post-translational modifications (PTMs). These range from phosphorylation, ubiquitination, and sumoylation to thiol exchange reactions and nitrosylation. In several cases, these PTMs have been shown to have a significant impact on executioner caspase activation and enzymatic activity. Caspases can also bind divalent cations or associate with non-IAP protein partners, and these may also contribute to sublethal caspase regulation.

Direct phosphorylation of caspases can have a major impact on caspase activity. For example, phosphorylation of caspase-9 on Thr125 or Ser144 blocks its activation in the apoptosome (Brady et al., 2005; Seifert et al., 2008), whereas phosphorylation of caspase-6 on Ser257 prevents auto-activation and blocks caspase-3-mediated cleavage (Cao et al., 2012; Suzuki et al., 2004; Velázquez-Delgado and Hardy, 2012a). Recent studies have examined the consequences of Ser257 phosphorylation in caspase-6 from a structural perspective and determined that phosphorylation at this site flips an active site loop with a consequent 1,000-fold reduction in activity (Cao et al., 2012; Velázquez-Delgado and Hardy, 2012a). The notion that caspase phosphorylation may act as a switch that regulates caspase activity is appealing, in part because several kinases converge to target active loop sites (e.g., CDK1, Erk1, Erk1, PKA, DYRK1A, and PKCζ can each target caspase-9) and because dephosphorylation at these sites can recover caspase-9 activity (Allan et al., 2003; Brady et al., 2005; Martin et al., 2005, 2008; Seifert et al., 2008).

Several caspases bind, and are inhibited by, zinc but not other divalent cations. Of the executioner caspases, zinc inhibition is strongest for caspase-6 (inhibitory constant [K_i] = 0.3 μM) and weakest for caspase-3 (K_i = 8.8 μM). The site responsible for zinc-mediated inhibition has recently been identified in caspase-9 and shown to be comprised of His237, Cys239, and Cys287, a spaced amino acid triad that spans the caspase active site and is conserved throughout the caspase family (Huber and Hardy, 2012; Velázquez-Delgado and Hardy, 2012b). In most tissues, zinc ions are trapped within proteins as structural or catalytic cofactors, but in the hippocampus, cortex, striatum, and amygdala, free zinc is highly enriched in a subset of glutamatergic neurons through the action of the zinc transporter ZnT3 (Sensi et al., 2009). Interestingly, zinc has been implicated in caspase-dependent apoptotic cell death in the developing brain (Cho et al., 2010), and ZnT3-null mice show a decrease in neuronal spine density, have defects in synaptic protein levels, and display deficits in learning and memory (Adlard et al., 2010).

Caspases are cysteine proteases and, as such, are sensitive to various forms of redox reactions. For example, when the active cysteine in caspase-3 is S-nitrosylated by nitric oxide, enzymatic activity is reduced sharply (Liu and Stamler, 1999; Tenneti et al., 1997). Interestingly, apoptotic stimuli that activate caspase-3 also lead to its denitrosylation (Mannick et al., 1999), and recent work has shown that neuronal XIAP is also S-nitrosylated (Tsang et al., 2009). When this occurs, the ability of XIAP to inhibit caspases is attenuated. This may have physiological relevance because S-nitrosylated XIAP accumulates in neurons subjected to NMDA excitotoxicity and is prevalent in brains of patients with AD, Parkinson's disease and HD (Nakamura

et al., 2010; Tsang et al., 2009). Assessing the role of NO-mediated PTMs in the regulation of sublethal caspase control under physiological and pathophysiological settings will clearly be an interesting topic for future studies.

Conclusions and Future Directions

Over the last 20 years, the mechanisms of apoptosis and the role of caspases in this process have been studied intensively, and we now have a detailed picture of the signaling cascades that converge to activate these proteases and induce apoptosis. In addition, numerous studies have demonstrated that executioner caspases participate in a wide variety of sublethal roles, and many of these are critical for normal nervous system function during development and in adulthood. We are now beginning to understand the regulatory constraints that control sublethal caspase activity in normal settings and identify pathophysiological settings in which these constraints may be reduced or lost.

It is now certain that sublethal caspase activity plays a critical role in regulating neuronal pathfinding, pruning, and synaptic plasticity. Emerging data suggest that these crucial pathways may be subverted in a wide range of nervous system disorders to promote pathological changes, but our knowledge of the mechanisms that activate, localize, and suppress this activity is rudimentary. Learning when, where, and how sublethal executioner caspase activity controls nervous system function is an important priority and a major challenge for future studies. Fortunately, most of the pathways characterized to date are well conserved across the phylogeny. This suggests that generating and controlling sublethal caspase activity may be a prerequisite for the development of a functional nervous system and provides the means for rapidly generating new insights into the mechanisms that regulate sublethal caspase activity.

Although significant insights have been gleamed from "grind and find" biochemistry, new findings will be facilitated by the development of new model systems and tools. Here the future looks bright because several groups have produced Förster resonance energy transfer (FRET)-based biosensors to detect caspase activity in vitro and in vivo (Ai et al., 2008; Nguyen and Daugherty, 2005; Takemoto et al., 2003; Xu et al., 1998; Yamaguchi et al., 2011; Zhou et al., 2010) or developed GFP isoforms that undergo a dark-to-bright transition in response to caspase-dependent cleavage (Nicholls et al., 2011). These approaches have been used mainly to capture caspase activation in the context of apoptosis but are likely to provide important spatial and temporal information on sublethal caspase activation, particularly when they are produced as fusion proteins that allow them to be localized to specific subcellular compartments (Figueroa et al., 2011) or to specific caspase substrates. Super-resolution microscopy, proximity ligation assays, and other emerging techniques will prove to be useful for identifying the partners that associate with executioner caspases in specific subcellular locales, and high-resolution proteomics will define post-translational modifications in caspases that regulate activity in sublethal settings and will clarify the signaling pathways that converge to regulate them.

Previously misconstrued as dedicated assassins, it is now certain that the executioner caspases play important roles in a wide array of sublethal cellular activities. Our knowledge of when, where, and how their sublethal activities are controlled within the cell remains rudimentary, but the stage is set for rapidly expanding our knowledge of this intriguing and therapeutically important area.

ACKNOWLEDGMENTS

N.U. is supported by a grant from the International Society from Neurochemistry (ISN-CAEN Return Home Award), and P.A.B. is a Tier 1 Canadian Research Chair in Molecular Neuroscience. This work was supported by grants to P.A.B. from the Canadian Foundation for Health Research (funding references 38942 and 325420). We thank Dr. Wayne Sossin for critically reading the manuscript.

REFERENCES

Adlard, P.A., Parncutt, J.M., Finkelstein, D.I., and Bush, A.I. (2010). Cognitive loss in zinc transporter-3 knock-out mice: a phenocopy for the synaptic and memory deficits of Alzheimer's disease? J. Neurosci. *30*, 1631–1636.

Ai, H.W., Hazelwood, K.L., Davidson, M.W., and Campbell, R.E. (2008). Fluorescent protein FRET pairs for ratiometric imaging of dual biosensors. Nat. Methods *5*, 401–403.

Albin, R.L., Young, A.B., Penney, J.B., Handelin, B., Balfour, R., Anderson, K.D., Markel, D.S., Tourtellotte, W.W., and Reiner, A. (1990). Abnormalities of striatal projection neurons and N-methyl-D-aspartate receptors in presymptomatic Huntington's disease. N. Engl. J. Med. *322*, 1293–1298.

Allan, L.A., Morrice, N., Brady, S., Magee, G., Pathak, S., and Clarke, P.R. (2003). Inhibition of caspase-9 through phosphorylation at Thr 125 by ERK MAPK. Nat. Cell Biol. *5*, 647–654.

Arama, E., Bader, M., Srivastava, M., Bergmann, A., and Steller, H. (2006). The two Drosophila cytochrome C proteins can function in both respiration and caspase activation. EMBO J. *25*, 232–243.

Asselin, E., Mills, G.B., and Tsang, B.K. (2001). XIAP regulates Akt activity and caspase-3-dependent cleavage during cisplatin-induced apoptosis in human ovarian epithelial cancer cells. Cancer Res. *61*, 1862–1868.

Beattie, E.C., Carroll, R.C., Yu, X., Morishita, W., Yasuda, H., von Zastrow, M., and Malenka, R.C. (2000). Regulation of AMPA receptor endocytosis by a signaling mechanism shared with LTD. Nat. Neurosci. *3*, 1291–1300.

Bergmann, A., and Steller, H. (2010). Apoptosis, stem cells, and tissue regeneration. Sci. Signal. *3*, re8.

Bilak, A., Uyetake, L., and Su, T.T. (2014). Dying cells protect survivors from radiation-induced cell death in Drosophila. PLoS Genet. *10*, e1004220.

Bourne, J.N., and Harris, K.M. (2011). Coordination of size and number of excitatory and inhibitory synapses results in a balanced structural plasticity along mature hippocampal CA1 dendrites during LTP. Hippocampus *21*, 354–373.

Brady, S.C., Allan, L.A., and Clarke, P.R. (2005). Regulation of caspase 9 through phosphorylation by protein kinase C zeta in response to hyperosmotic stress. Mol. Cell. Biol. *25*, 10543–10555.

Bravarenko, N.I., Onufriev, M.V., Stepanichev, M.Y., Ierusalimsky, V.N., Balaban, P.M., and Gulyaeva, N.V. (2006). Caspase-like activity is essential for long-term synaptic plasticity in the terrestrial snail Helix. Eur. J. Neurosci. *23*, 129–140.

Brusco, J., and Haas, K. (2015). Interactions between mitochondria and the transcription factor myocyte enhancer factor 2 (MEF2) regulate neuronal structural and functional plasticity and metaplasticity. J. Physiol. *593*, 3471–3481.

Cajal, R. (1911). Histologie du systeme nerveux (New York, NY: Oxford University Press).

Campbell, D.S., and Holt, C.E. (2003). Apoptotic pathway and MAPKs differentially regulate chemotropic responses of retinal growth cones. Neuron *37*, 939–952.

Campbell, D.S., and Okamoto, H. (2013). Local caspase activation interacts with Slit-Robo signaling to restrict axonal arborization. J. Cell Biol. *203*, 657–672.

Cao, Q., Wang, X.J., Liu, C.W., Liu, D.F., Li, L.F., Gao, Y.Q., and Su, X.D. (2012). Inhibitory mechanism of caspase-6 phosphorylation revealed by crystal structures, molecular dynamics simulations, and biochemical assays. J. Biol. Chem. *287*, 15371–15379.

Carlile, G.W., Smith, D.H., and Wiedmann, M. (2004). Caspase-3 has a nonapoptotic function in erythroid maturation. Blood *103*, 4310–4316.

Chai, J., Shiozaki, E., Srinivasula, S.M., Wu, Q., Datta, P., Alnemri, E.S., and Shi, Y. (2001). Structural basis of caspase-7 inhibition by XIAP. Cell *104*, 769–780.

Chan, S.L., Griffin, W.S., and Mattson, M.P. (1999). Evidence for caspase-mediated cleavage of AMPA receptor subunits in neuronal apoptosis and Alzheimer's disease. J. Neurosci. Res. *57*, 315–323.

Chen, S.X., Cherry, A., Tari, P.K., Podgorski, K., Kwong, Y.K., and Haas, K. (2012). The transcription factor MEF2 directs developmental visually driven functional and structural metaplasticity. Cell *151*, 41–55.

Chen, X., Lin, R., Chang, L., Xu, S., Wei, X., Zhang, J., Wang, C., Anwyl, R., and Wang, Q. (2013). Enhancement of long-term depression by soluble amyloid β protein in rat hippocampus is mediated by metabotropic glutamate receptor and involves activation of p38MAPK, STEP and caspase-3. Neuroscience *253*, 435–443.

Cheng, L., Yin, W.J., Zhang, J.F., and Qi, J.S. (2009). Amyloid beta-protein fragments 25-35 and 31-35 potentiate long-term depression in hippocampal CA1 region of rats in vivo. Synapse *63*, 206–214.

Chera, S., Ghila, L., Dobretz, K., Wenger, Y., Bauer, C., Buzgariu, W., Martinou, J.C., and Galliot, B. (2009). Apoptotic cells provide an unexpected source of Wnt3 signaling to drive hydra head regeneration. Dev. Cell *17*, 279–289.

Cho, E., Hwang, J.J., Han, S.H., Chung, S.J., Koh, J.Y., and Lee, J.Y. (2010). Endogenous zinc mediates apoptotic programmed cell death in the developing brain. Neurotox. Res. *17*, 156–166.

Choi, Y.E., Butterworth, M., Malladi, S., Duckett, C.S., Cohen, G.M., and Bratton, S.B. (2009). The E3 ubiquitin ligase cIAP1 binds and ubiquitinates caspase-3 and -7 via unique mechanisms at distinct steps in their processing. J. Biol. Chem. *284*, 12772–12782.

Chowdhury, I., Tharakan, B., and Bhat, G.K. (2008). Caspases - an update. Comp. Biochem. Physiol. B Biochem. Mol. Biol. *151*, 10–27.

Costa-Mattioli, M., Gobert, D., Stern, E., Gamache, K., Colina, R., Cuello, C., Sossin, W., Kaufman, R., Pelletier, J., Rosenblum, K., et al. (2007). eIF2alpha phosphorylation bidirectionally regulates the switch from short- to long-term synaptic plasticity and memory. Cell *129*, 195–206.

Cowan, C.M., and Roskams, A.J. (2004). Caspase-3 and caspase-9 mediate developmental apoptosis in the mouse olfactory system. J. Comp. Neurol. *474*, 136–148.

Cowan, C.M., Thai, J., Krajewski, S., Reed, J.C., Nicholson, D.W., Kaufmann, S.H., and Roskams, A.J. (2001). Caspases 3 and 9 send a pro-apoptotic signal from synapse to cell body in olfactory receptor neurons. J. Neurosci. *21*, 7099–7109.

Cusack, C.L., Swahari, V., Hampton Henley, W., Michael Ramsey, J., and Deshmukh, M. (2013). Distinct pathways mediate axon degeneration during apoptosis and axon-specific pruning. Nat. Commun. *4*, 1876.

D'Amelio, M., Cavallucci, V., Middei, S., Marchetti, C., Pacioni, S., Ferri, A., Diamantini, A., De Zio, D., Carrara, P., Battistini, L., et al. (2011). Caspase-3 triggers early synaptic dysfunction in a mouse model of Alzheimer's disease. Nat. Neurosci. *14*, 69–76.

D'Brot, A., Chen, P., Vaishnav, M., Yuan, S., Akey, C.W., and Abrams, J.M. (2013). Tango7 directs cellular remodeling by the Drosophila apoptosome. Genes Dev. *27*, 1650–1655.

Dash, P.K., Blum, S., and Moore, A.N. (2000). Caspase activity plays an essential role in long-term memory. Neuroreport *11*, 2811–2816.

De Botton, S., Sabri, S., Daugas, E., Zermati, Y., Guidotti, J.E., Hermine, O., Kroemer, G., Vainchenker, W., and Debili, N. (2002). Platelet formation is the consequence of caspase activation within megakaryocytes. Blood *100*, 1310–1317.

Dorstyn, L., Read, S., Cakouros, D., Huh, J.R., Hay, B.A., and Kumar, S. (2002). The role of cytochrome c in caspase activation in Drosophila melanogaster cells. J. Cell Biol. *156*, 1089–1098.

Dunfield, D., and Haas, K. (2009). Metaplasticity governs natural experience-driven plasticity of nascent embryonic brain circuits. Neuron *64*, 240–250.

Durie, D., Lewis, S.M., Liwak, U., Kisilewicz, M., Gorospe, M., and Holcik, M. (2011). RNA-binding protein HuR mediates cytoprotection through stimulation of XIAP translation. Oncogene *30*, 1460–1469.

Ernsberger, U. (2009). Role of neurotrophin signalling in the differentiation of neurons from dorsal root ganglia and sympathetic ganglia. Cell Tissue Res. *336*, 349–384.

Ertürk, A., Wang, Y., and Sheng, M. (2014). Local pruning of dendrites and spines by caspase-3-dependent and proteasome-limited mechanisms. J. Neurosci. *34*, 1672–1688.

Esteban, J.A., Shi, S.H., Wilson, C., Nuriya, M., Huganir, R.L., and Malinow, R. (2003). PKA phosphorylation of AMPA receptor subunits controls synaptic trafficking underlying plasticity. Nat. Neurosci. *6*, 136–143.

Fernando, P., Kelly, J.F., Balazsi, K., Slack, R.S., and Megeney, L.A. (2002). Caspase 3 activity is required for skeletal muscle differentiation. Proc. Natl. Acad. Sci. USA *99*, 11025–11030.

Fernando, P., Brunette, S., and Megeney, L.A. (2005). Neural stem cell differentiation is dependent upon endogenous caspase 3 activity. FASEB J. *19*, 1671–1673.

Ferrer, I., Guionnet, N., Cruz-Sánchez, F., and Tuñón, T. (1990). Neuronal alterations in patients with dementia: a Golgi study on biopsy samples. Neurosci. Lett. *114*, 11–16.

Figueroa, R.A., Ramberg, V., Gatsinzi, T., Samuelsson, M., Zhang, M., Iverfeldt, K., and Hallberg, E. (2011). Anchored FRET sensors detect local caspase activation prior to neuronal degeneration. Mol. Neurodegener. *6*, 35.

Finn, J.T., Weil, M., Archer, F., Siman, R., Srinivasan, A., and Raff, M.C. (2000). Evidence that Wallerian degeneration and localized axon degeneration induced by local neurotrophin deprivation do not involve caspases. J. Neurosci. *20*, 1333–1341.

Fuchs, Y., Brown, S., Gorenc, T., Rodriguez, J., Fuchs, E., and Steller, H. (2013). Sept4/ARTS regulates stem cell apoptosis and skin regeneration. Science *341*, 286–289.

Fuentes-Prior, P., and Salvesen, G.S. (2004). The protein structures that shape caspase activity, specificity, activation and inhibition. Biochem. J. *384*, 201–232.

Fujita, J., Crane, A.M., Souza, M.K., Dejosez, M., Kyba, M., Flavell, R.A., Thomson, J.A., and Zwaka, T.P. (2008). Caspase activity mediates the differentiation of embryonic stem cells. Cell Stem Cell *2*, 595–601.

Fulda, S., and Vucic, D. (2012). Targeting IAP proteins for therapeutic intervention in cancer. Nat. Rev. Drug Discov. *11*, 109–124.

Gafni, J., Hermel, E., Young, J.E., Wellington, C.L., Hayden, M.R., and Ellerby, L.M. (2004). Inhibition of calpain cleavage of huntingtin reduces toxicity: accumulation of calpain/caspase fragments in the nucleus. J. Biol. Chem. *279*, 20211–20220.

Gafni, J., Papanikolaou, T., Degiacomo, F., Holcomb, J., Chen, S., Menalled, L., Kudwa, A., Fitzpatrick, J., Miller, S., Ramboz, S., et al. (2012). Caspase-6 activity in a BACHD mouse modulates steady-state levels of mutant huntingtin protein but is not necessary for production of a 586 amino acid proteolytic fragment. J. Neurosci. *32*, 7454–7465.

Galbán, S., and Duckett, C.S. (2010). XIAP as a ubiquitin ligase in cellular signaling. Cell Death Differ. *17*, 54–60.

Gillies, L.A., and Kuwana, T. (2014). Apoptosis regulation at the mitochondrial outer membrane. J. Cell. Biochem. *115*, 632–640.

Gottfried, Y., Rotem, A., Lotan, R., Steller, H., and Larisch, S. (2004). The mitochondrial ARTS protein promotes apoptosis through targeting XIAP. EMBO J. *23*, 1627–1635.

Goyal, L., McCall, K., Agapite, J., Hartwieg, E., and Steller, H. (2000). Induction of apoptosis by Drosophila reaper, hid and grim through inhibition of IAP function. EMBO J. *19*, 589–597.

Graham, R.K., Deng, Y., Slow, E.J., Haigh, B., Bissada, N., Lu, G., Pearson, J., Shehadeh, J., Bertram, L., Murphy, Z., et al. (2006). Cleavage at the caspase-6 site is required for neuronal dysfunction and degeneration due to mutant huntingtin. Cell *125*, 1179–1191.

Graham, R.K., Deng, Y., Carroll, J., Vaid, K., Cowan, C., Pouladi, M.A., Metzler, M., Bissada, N., Wang, L., Faull, R.L., et al. (2010). Cleavage at the 586 amino acid caspase-6 site in mutant huntingtin influences caspase-6 activation in vivo. J. Neurosci. *30*, 15019–15029.

Graham, R.K., Deng, Y., Pouladi, M.A., Vaid, K., Ehrnhoefer, D., Southwell, A.L., Bissada, N., Franciosi, S., and Hayden, M.R. (2012). Caspase-6-Resistant Mutant Huntingtin Does not Rescue the Toxic Effects of Caspase-Cleavable Mutant Huntingtin in vivo. J. Huntingtons Dis. *1*, 243–260.

Gray, C.W., Ward, R.V., Karran, E., Turconi, S., Rowles, A., Viglienghi, D., Southan, C., Barton, A., Fantom, K.G., West, A., et al. (2000). Characterization of human HtrA2, a novel serine protease involved in the mammalian cellular stress response. Eur. J. Biochem. *267*, 5699–5710.

Gulyaeva, N.V., Kudryashov, I.E., and Kudryashova, I.V. (2003). Caspase activity is essential for long-term potentiation. J. Neurosci. Res. *73*, 853–864.

Gurley, K.A., Elliott, S.A., Simakov, O., Schmidt, H.A., Holstein, T.W., and Sánchez Alvarado, A. (2010). Expression of secreted Wnt pathway components reveals unexpected complexity of the planarian amputation response. Dev. Biol. *347*, 24–39.

Gyrd-Hansen, M., Darding, M., Miasari, M., Santoro, M.M., Zender, L., Xue, W., Tenev, T., da Fonseca, P.C., Zvelebil, M., Bujnicki, J.M., et al. (2008). IAPs contain an evolutionarily conserved ubiquitin-binding domain that regulates NF-kappaB as well as cell survival and oncogenesis. Nat. Cell Biol. *10*, 1309–1317.

Han, M.H., Jiao, S., Jia, J.M., Chen, Y., Chen, C.Y., Gucek, M., Markey, S.P., and Li, Z. (2013). The novel caspase-3 substrate Gap43 is involved in AMPA receptor endocytosis and long-term depression. Mol. Cell. Proteomics *12*, 3719–3731.

Happo, L., Strasser, A., and Cory, S. (2012). BH3-only proteins in apoptosis at a glance. J. Cell Sci. *125*, 1081–1087.

Hawkins, C.J., Wang, S.L., and Hay, B.A. (1999). A cloning method to identify caspases and their regulators in yeast: identification of Drosophila IAP1 as an inhibitor of the Drosophila caspase DCP-1. Proc. Natl. Acad. Sci. USA *96*, 2885–2890.

Hsieh, H., Boehm, J., Sato, C., Iwatsubo, T., Tomita, T., Sisodia, S., and Malinow, R. (2006). AMPAR removal underlies Abeta-induced synaptic depression and dendritic spine loss. Neuron *52*, 831–843.

Huang, Y.Y., Colino, A., Selig, D.K., and Malenka, R.C. (1992). The influence of prior synaptic activity on the induction of long-term potentiation. Science *255*, 730–733.

Huang, Hk., Joazeiro, C.A., Bonfoco, E., Kamada, S., Leverson, J.D., and Hunter, T. (2000). The inhibitor of apoptosis, cIAP2, functions as a ubiquitin-protein ligase and promotes in vitro monoubiquitination of caspases 3 and 7. J. Biol. Chem. *275*, 26661–26664.

Huber, K.L., and Hardy, J.A. (2012). Mechanism of zinc-mediated inhibition of caspase-9. Protein Sci. *21*, 1056–1065.

Huesmann, G.R., and Clayton, D.F. (2006). Dynamic role of postsynaptic caspase-3 and BIRC4 in zebra finch song-response habituation. Neuron *52*, 1061–1072.

Huh, J.R., Vernooy, S.Y., Yu, H., Yan, N., Shi, Y., Guo, M., and Hay, B.A. (2004). Multiple apoptotic caspase cascades are required in nonapoptotic roles for Drosophila spermatid individualization. PLoS Biol. *2*, E15.

Hunter, A.L., Zhang, J., Chen, S.C., Si, X., Wong, B., Ekhterae, D., Luo, H., and Granville, D.J. (2007). Apoptosis repressor with caspase recruitment domain (ARC) inhibits myogenic differentiation. FEBS Lett. *581*, 879–884.

Ingelsson, M., Fukumoto, H., Newell, K.L., Growdon, J.H., Hedley-Whyte, E.T., Frosch, M.P., Albert, M.S., Hyman, B.T., and Irizarry, M.C. (2004). Early Abeta accumulation and progressive synaptic loss, gliosis, and tangle formation in AD brain. Neurology *62*, 925–931.

Ishizaki, Y., Jacobson, M.D., and Raff, M.C. (1998). A role for caspases in lens fiber differentiation. J. Cell Biol. *140*, 153–158.

Janzen, V., Fleming, H.E., Riedt, T., Karlsson, G., Riese, M.J., Lo Celso, C., Reynolds, G., Milne, C.D., Paige, C.J., Karlsson, S., et al. (2008). Hematopoietic stem cell responsiveness to exogenous signals is limited by caspase-3. Cell Stem Cell *2*, 584–594.

Jiao, S., and Li, Z. (2011). Nonapoptotic function of BAD and BAX in long-term depression of synaptic transmission. Neuron *70*, 758–772.

Jo, J., Whitcomb, D.J., Olsen, K.M., Kerrigan, T.L., Lo, S.C., Bru-Mercier, G., Dickinson, B., Scullion, S., Sheng, M., Collingridge, G., and Cho, K. (2011). Aβ(1-42) inhibition of LTP is mediated by a signaling pathway involving caspase-3, Akt1 and GSK-3β. Nat. Neurosci. *14*, 545–547.

Jones, J.M., Datta, P., Srinivasula, S.M., Ji, W., Gupta, S., Zhang, Z., Davies, E., Hajnóczky, G., Saunders, T.L., Van Keuren, M.L., et al. (2003). Loss of Omi mitochondrial protease activity causes the neuromuscular disorder of mnd2 mutant mice. Nature *425*, 721–727.

Kallop, D.Y., Meilandt, W.J., Gogineni, A., Easley-Neal, C., Wu, T., Jubb, A.M., Yaylaoglu, M., Shamloo, M., Tessier-Lavigne, M., Scearce-Levie, K., and Weimer, R.M. (2014). A death receptor 6-amyloid precursor protein pathway regulates synapse density in the mature CNS but does not contribute to Alzheimer's disease-related pathophysiology in murine models. J. Neurosci. *34*, 6425–6437.

Kanuka, H., Kuranaga, E., Takemoto, K., Hiratou, T., Okano, H., and Miura, M. (2005). Drosophila caspase transduces Shaggy/GSK-3beta kinase activity in neural precursor development. EMBO J. *24*, 3793–3806.

Khalil, H., Peltzer, N., Walicki, J., Yang, J.Y., Dubuis, G., Gardiol, N., Held, W., Bigliardi, P., Marsland, B., Liaudet, L., and Widmann, C. (2012). Caspase-3 protects stressed organs against cell death. Mol. Cell. Biol. *32*, 4523–4533.

Kim, J.H., Anwyl, R., Suh, Y.H., Djamgoz, M.B., and Rowan, M.J. (2001). Use-dependent effects of amyloidogenic fragments of (beta)-amyloid precursor protein on synaptic plasticity in rat hippocampus in vivo. J. Neurosci. *21*, 1327–1333.

King, R.S., and Newmark, P.A. (2012). The cell biology of regeneration. J. Cell Biol. *196*, 553–562.

Kissel, H., Georgescu, M.M., Larisch, S., Manova, K., Hunnicutt, G.R., and Steller, H. (2005). The Sept4 septin locus is required for sperm terminal differentiation in mice. Dev. Cell *8*, 353–364.

Kozyrev, S.A., Nikitin, V.P., and Sherstnev, V.V. (2007). Synapse-specific plasticity in command neurons during learning of edible snails under the action of caspase inhibitors. Bull. Exp. Biol. Med. *144*, 755–759.

Kuo, C.T., Zhu, S., Younger, S., Jan, L.Y., and Jan, Y.N. (2006). Identification of E2/E3 ubiquitinating enzymes and caspase activity regulating Drosophila sensory neuron dendrite pruning. Neuron *51*, 283–290.

Kuranaga, E., Kanuka, H., Tonoki, A., Takemoto, K., Tomioka, T., Kobayashi, M., Hayashi, S., and Miura, M. (2006). Drosophila IKK-related kinase regulates nonapoptotic function of caspases via degradation of IAPs. Cell *126*, 583–596.

Landles, C., Weiss, A., Franklin, S., Howland, D., and Bates, G. (2012). Caspase-6 does not contribute to the proteolysis of mutant huntingtin in the HdhQ150 knock-in mouse model of Huntington's disease. PLoS Curr. *4*, e4fd085bfc9973.

Lanz, T.A., Carter, D.B., and Merchant, K.M. (2003). Dendritic spine loss in the hippocampus of young PDAPP and Tg2576 mice and its prevention by the ApoE2 genotype. Neurobiol. Dis. *13*, 246–253.

Larisch, S., Yi, Y., Lotan, R., Kerner, H., Eimerl, S., Tony Parks, W., Gottfried, Y., Birkey Reffey, S., de Caestecker, M.P., Danielpour, D., et al. (2000). A novel

mitochondrial septin-like protein, ARTS, mediates apoptosis dependent on its P-loop motif. Nat. Cell Biol. *2*, 915–921.

Larsen, B.D., and Megeney, L.A. (2010). Parole terms for a killer: directing caspase3/CAD induced DNA strand breaks to coordinate changes in gene expression. Cell Cycle *9*, 2940–2945.

Larsen, B.D., Rampalli, S., Burns, L.E., Brunette, S., Dilworth, F.J., and Megeney, L.A. (2010). Caspase 3/caspase-activated DNase promote cell differentiation by inducing DNA strand breaks. Proc. Natl. Acad. Sci. USA *107*, 4230–4235.

Lee, K.J., Park, I.S., Kim, H., Greenough, W.T., Pak, D.T., and Rhyu, I.J. (2013). Motor skill training induces coordinated strengthening and weakening between neighboring synapses. J. Neurosci. *33*, 9794–9799.

Li, J., and Yuan, J. (2008). Caspases in apoptosis and beyond. Oncogene *27*, 6194–6206.

Li, F., He, Z., Shen, J., Huang, Q., Li, W., Liu, X., He, Y., Wolf, F., and Li, C.Y. (2010a). Apoptotic caspases regulate induction of iPSCs from human fibroblasts. Cell Stem Cell *7*, 508–520.

Li, F., Huang, Q., Chen, J., Peng, Y., Roop, D.R., Bedford, J.S., and Li, C.Y. (2010b). Apoptotic cells activate the "phoenix rising" pathway to promote wound healing and tissue regeneration. Sci. Signal. *3*, ra13.

Li, Z., Jo, J., Jia, J.M., Lo, S.C., Whitcomb, D.J., Jiao, S., Cho, K., and Sheng, M. (2010c). Caspase-3 activation via mitochondria is required for long-term depression and AMPA receptor internalization. Cell *141*, 859–871.

Lippens, S., Kockx, M., Knaapen, M., Mortier, L., Polakowska, R., Verheyen, A., Garmyn, M., Zwijsen, A., Formstecher, P., Huylebroeck, D., et al. (2000). Epidermal differentiation does not involve the pro-apoptotic executioner caspases, but is associated with caspase-14 induction and processing. Cell Death Differ. *7*, 1218–1224.

Lisi, S., Mazzon, I., and White, K. (2000). Diverse domains of THREAD/DIAP1 are required to inhibit apoptosis induced by REAPER and HID in Drosophila. Genetics *154*, 669–678.

Liu, L., and Stamler, J.S. (1999). NO: an inhibitor of cell death. Cell Death Differ. *6*, 937–942.

Lo, Y.J., and Poo, M.M. (1991). Activity-dependent synaptic competition in vitro: heterosynaptic suppression of developing synapses. Science *254*, 1019–1022.

Lopez, J., and Meier, P. (2010). To fight or die - inhibitor of apoptosis proteins at the crossroad of innate immunity and death. Curr. Opin. Cell Biol. *22*, 872–881.

Lopez, J., John, S.W., Tenev, T., Rautureau, G.J., Hinds, M.G., Francalanci, F., Wilson, R., Broemer, M., Santoro, M.M., Day, C.L., and Meier, P. (2011). CARD-mediated autoinhibition of cIAP1's E3 ligase activity suppresses cell proliferation and migration. Mol. Cell *42*, 569–583.

Lu, C., Fu, W., Salvesen, G.S., and Mattson, M.P. (2002). Direct cleavage of AMPA receptor subunit GluR1 and suppression of AMPA currents by caspase-3: implications for synaptic plasticity and excitotoxic neuronal death. Neuromolecular Med. *1*, 69–79.

Lu, C., Wang, Y., Furukawa, K., Fu, W., Ouyang, X., and Mattson, M.P. (2006). Evidence that caspase-1 is a negative regulator of AMPA receptor-mediated long-term potentiation at hippocampal synapses. J. Neurochem. *97*, 1104–1110.

Mannick, J.B., Hausladen, A., Liu, L., Hess, D.T., Zeng, M., Miao, Q.X., Kane, L.S., Gow, A.J., and Stamler, J.S. (1999). Fas-induced caspase denitrosylation. Science *284*, 651–654.

Marik, S.A., Olsen, O., Tessier-Lavigne, M., and Gilbert, C.D. (2013). Death receptor 6 regulates adult experience-dependent cortical plasticity. J. Neurosci. *33*, 14998–15003.

Martin, M.C., Allan, L.A., Lickrish, M., Sampson, C., Morrice, N., and Clarke, P.R. (2005). Protein kinase A regulates caspase-9 activation by Apaf-1 downstream of cytochrome c. J. Biol. Chem. *280*, 15449–15455.

Martin, M.C., Allan, L.A., Mancini, E.J., and Clarke, P.R. (2008). The docking interaction of caspase-9 with ERK2 provides a mechanism for the selective inhibitory phosphorylation of caspase-9 at threonine 125. J. Biol. Chem. *283*, 3854–3865.

Martins, L.M., Morrison, A., Klupsch, K., Fedele, V., Moisoi, N., Teismann, P., Abuin, A., Grau, E., Geppert, M., Livi, G.P., et al. (2004). Neuroprotective role of the Reaper-related serine protease HtrA2/Omi revealed by targeted deletion in mice. Mol. Cell. Biol. *24*, 9848–9862.

Masliah, E., Mallory, M., Hansen, L., DeTeresa, R., Alford, M., and Terry, R. (1994). Synaptic and neuritic alterations during the progression of Alzheimer's disease. Neurosci. Lett. *174*, 67–72.

McIlwain, D.R., Berger, T., and Mak, T.W. (2013). Caspase functions in cell death and disease. Cold Spring Harb. Perspect. Biol. *5*, a008656.

McLaughlin, B., Hartnett, K.A., Erhardt, J.A., Legos, J.J., White, R.F., Barone, F.C., and Aizenman, E. (2003). Caspase 3 activation is essential for neuroprotection in preconditioning. Proc. Natl. Acad. Sci. USA *100*, 715–720.

Mendes, C.S., Arama, E., Brown, S., Scherr, H., Srivastava, M., Bergmann, A., Steller, H., and Mollereau, B. (2006). Cytochrome c-d regulates developmental apoptosis in the Drosophila retina. EMBO Rep. *7*, 933–939.

Metzler, M., Gan, L., Mazarei, G., Graham, R.K., Liu, L., Bissada, N., Lu, G., Leavitt, B.R., and Hayden, M.R. (2010). Phosphorylation of huntingtin at Ser421 in YAC128 neurons is associated with protection of YAC128 neurons from NMDA-mediated excitotoxicity and is modulated by PP1 and PP2A. J. Neurosci. *30*, 14318–14329.

Milnerwood, A.J., Gladding, C.M., Pouladi, M.A., Kaufman, A.M., Hines, R.M., Boyd, J.D., Ko, R.W., Vasuta, O.C., Graham, R.K., Hayden, M.R., et al. (2010). Early increase in extrasynaptic NMDA receptor signaling and expression contributes to phenotype onset in Huntington's disease mice. Neuron *65*, 178–190.

Mollereau, B., Perez-Garijo, A., Bergmann, A., Miura, M., Gerlitz, O., Ryoo, H.D., Steller, H., and Morata, G. (2013). Compensatory proliferation and apoptosis-induced proliferation: a need for clarification. Cell Death Differ. *20*, 181.

Moody-Corbett, F. (1986). Formation of the vertebrate neuromuscular junction. Dev. Biol. (N. Y.) *2*, 605–635.

Mukerjee, N., McGinnis, K.M., Park, Y.H., Gnegy, M.E., and Wang, K.K. (2000). Caspase-mediated proteolytic activation of calcineurin in thapsigargin-mediated apoptosis in SH-SY5Y neuroblastoma cells. Arch. Biochem. Biophys. *379*, 337–343.

Murray, T.V., McMahon, J.M., Howley, B.A., Stanley, A., Ritter, T., Mohr, A., Zwacka, R., and Fearnhead, H.O. (2008). A non-apoptotic role for caspase-9 in muscle differentiation. J. Cell Sci. *121*, 3786–3793.

Nägerl, U.V., Eberhorn, N., Cambridge, S.B., and Bonhoeffer, T. (2004). Bidirectional activity-dependent morphological plasticity in hippocampal neurons. Neuron *44*, 759–767.

Nakamura, T., Wang, L., Wong, C.C., Scott, F.L., Eckelman, B.P., Han, X., Tzitzilonis, C., Meng, F., Gu, Z., Holland, E.A., et al. (2010). Transnitrosylation of XIAP regulates caspase-dependent neuronal cell death. Mol. Cell *39*, 184–195.

Nakhaei, P., Sun, Q., Solis, M., Mesplede, T., Bonneil, E., Paz, S., Lin, R., and Hiscott, J. (2012). IκB kinase ε-dependent phosphorylation and degradation of X-linked inhibitor of apoptosis sensitizes cells to virus-induced apoptosis. J. Virol. *86*, 726–737.

Nelson, C.D., Kim, M.J., Hsin, H., Chen, Y., and Sheng, M. (2013). Phosphorylation of threonine-19 of PSD-95 by GSK-3β is required for PSD-95 mobilization and long-term depression. J. Neurosci. *33*, 12122–12135.

Nguyen, A.W., and Daugherty, P.S. (2005). Evolutionary optimization of fluorescent proteins for intracellular FRET. Nat. Biotechnol. *23*, 355–360.

Nicholls, S.B., Chu, J., Abbruzzese, G., Tremblay, K.D., and Hardy, J.A. (2011). Mechanism of a genetically encoded dark-to-bright reporter for caspase activity. J. Biol. Chem. *286*, 24977–24986.

Nikolaev, A., McLaughlin, T., O'Leary, D.D., and Tessier-Lavigne, M. (2009). APP binds DR6 to trigger axon pruning and neuron death via distinct caspases. Nature *457*, 981–989.

O'Riordan, M.X., Bauler, L.D., Scott, F.L., and Duckett, C.S. (2008). Inhibitor of apoptosis proteins in eukaryotic evolution and development: a model of thematic conservation. Dev. Cell *15*, 497–508.

Oh, W.C., Hill, T.C., and Zito, K. (2013). Synapse-specific and size-dependent mechanisms of spine structural plasticity accompanying synaptic weakening. Proc. Natl. Acad. Sci. USA *110*, E305–E312.

Oh, W.C., Parajuli, L.K., and Zito, K. (2015). Heterosynaptic structural plasticity on local dendritic segments of hippocampal CA1 neurons. Cell Rep. *10*, 162–169.

Ohsawa, S., Hamada, S., Kuida, K., Yoshida, H., Igaki, T., and Miura, M. (2010). Maturation of the olfactory sensory neurons by Apaf-1/caspase-9-mediated caspase activity. Proc. Natl. Acad. Sci. USA *107*, 13366–13371.

Okada, H., Suh, W.K., Jin, J., Woo, M., Du, C., Elia, A., Duncan, G.S., Wakeham, A., Itie, A., Lowe, S.W., et al. (2002). Generation and characterization of Smac/DIABLO-deficient mice. Mol. Cell. Biol. *22*, 3509–3517.

Olsen, K.M., and Sheng, M. (2012). NMDA receptors and BAX are essential for Aβ impairment of LTP. Sci. Rep. *2*, 225.

Olsen, O., Kallop, D.Y., McLaughlin, T., Huntwork-Rodriguez, S., Wu, Z., Duggan, C.D., Simon, D.J., Lu, Y., Easley-Neal, C., Takeda, K., et al. (2014). Genetic analysis reveals that amyloid precursor protein and death receptor 6 function in the same pathway to control axonal pruning independent of β-secretase. J. Neurosci. *34*, 6438–6447.

Oshima, K., Takeda, M., Kuranaga, E., Ueda, R., Aigaki, T., Miura, M., and Hayashi, S. (2006). IKK epsilon regulates F actin assembly and interacts with Drosophila IAP1 in cellular morphogenesis. Curr. Biol. *16*, 1531–1537.

Öztürk, G., Cengiz, N., Erdoğan, E., Him, A., Oğuz, E.K., Yenidünya, E., and Ayşit, N. (2013). Two distinct types of dying back axonal degeneration in vitro. Neuropathol. Appl. Neurobiol. *39*, 362–376.

Peineau, S., Bradley, C., Taghibiglou, C., Doherty, A., Bortolotto, Z.A., Wang, Y.T., and Collingridge, G.L. (2008). The role of GSK-3 in synaptic plasticity. Br. J. Pharmacol. *153* (*Suppl 1*), S428–S437.

Pellettieri, J., Fitzgerald, P., Watanabe, S., Mancuso, J., Green, D.R., and Sánchez Alvarado, A. (2010). Cell death and tissue remodeling in planarian regeneration. Dev. Biol. *338*, 76–85.

Pinan-Lucarre, B., Gabel, C.V., Reina, C.P., Hulme, S.E., Shevkoplyas, S.S., Slone, R.D., Xue, J., Qiao, Y., Weisberg, S., Roodhouse, K., et al. (2012). The core apoptotic executioner proteins CED-3 and CED-4 promote initiation of neuronal regeneration in Caenorhabditis elegans. PLoS Biol. *10*, e1001331.

Pouladi, M.A., Graham, R.K., Karasinska, J.M., Xie, Y., Santos, R.D., Petersen, A., and Hayden, M.R. (2009). Prevention of depressive behaviour in the YAC128 mouse model of Huntington disease by mutation at residue 586 of huntingtin. Brain *132*, 919–932.

Putcha, G.V., and Johnson, E.M., Jr. (2004). Men are but worms: neuronal cell death in C elegans and vertebrates. Cell Death Differ. *11*, 38–48.

Raff, M.C., Whitmore, A.V., and Finn, J.T. (2002). Axonal self-destruction and neurodegeneration. Science *296*, 868–871.

Riedl, S.J., Renatus, M., Schwarzenbacher, R., Zhou, Q., Sun, C., Fesik, S.W., Liddington, R.C., and Salvesen, G.S. (2001). Structural basis for the inhibition of caspase-3 by XIAP. Cell *104*, 791–800.

Rodriguez, A., Oliver, H., Zou, H., Chen, P., Wang, X., and Abrams, J.M. (1999). Dark is a Drosophila homologue of Apaf-1/CED-4 and functions in an evolutionarily conserved death pathway. Nat. Cell Biol. *1*, 272–279.

Rowan, M.J., Klyubin, I., Wang, Q., and Anwyl, R. (2005). Synaptic plasticity disruption by amyloid beta protein: modulation by potential Alzheimer's disease modifying therapies. Biochem. Soc. Trans. *33*, 563–567.

Ryoo, H.D., and Bergmann, A. (2012). The role of apoptosis-induced proliferation for regeneration and cancer. Cold Spring Harb. Perspect. Biol. *4*, a008797.

Salvesen, G.S., and Duckett, C.S. (2002). IAP proteins: blocking the road to death's door. Nat. Rev. Mol. Cell Biol. *3*, 401–410.

Scanziani, M., Malenka, R.C., and Nicoll, R.A. (1996). Role of intercellular interactions in heterosynaptic long-term depression. Nature *380*, 446–450.

Scott, F.L., Denault, J.B., Riedl, S.J., Shin, H., Renatus, M., and Salvesen, G.S. (2005). XIAP inhibits caspase-3 and -7 using two binding sites: evolutionarily conserved mechanism of IAPs. EMBO J. *24*, 645–655.

Seifert, A., Allan, L.A., and Clarke, P.R. (2008). DYRK1A phosphorylates caspase 9 at an inhibitory site and is potently inhibited in human cells by harmine. FEBS J. *275*, 6268–6280.

Sensi, S.L., Paoletti, P., Bush, A.I., and Sekler, I. (2009). Zinc in the physiology and pathology of the CNS. Nat. Rev. Neurosci. *10*, 780–791.

Shankar, G.M., Li, S., Mehta, T.H., Garcia-Munoz, A., Shepardson, N.E., Smith, I., Brett, F.M., Farrell, M.A., Rowan, M.J., Lemere, C.A., et al. (2008). Amyloid-beta protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. Nat. Med. *14*, 837–842.

Shapiro, P.J., Hsu, H.H., Jung, H., Robbins, E.S., and Ryoo, H.D. (2008). Regulation of the Drosophila apoptosome through feedback inhibition. Nat. Cell Biol. *10*, 1440–1446.

Shiozaki, E.N., Chai, J., Rigotti, D.J., Riedl, S.J., Li, P., Srinivasula, S.M., Alnemri, E.S., Fairman, R., and Shi, Y. (2003). Mechanism of XIAP-mediated inhibition of caspase-9. Mol. Cell *11*, 519–527.

Simon, D.J., Weimer, R.M., McLaughlin, T., Kallop, D., Stanger, K., Yang, J., O'Leary, D.D., Hannoush, R.N., and Tessier-Lavigne, M. (2012). A caspase cascade regulating developmental axon degeneration. J. Neurosci. *32*, 17540–17553.

Sjakste, N., and Sjakste, T. (2007). Possible involvement of DNA strand breaks in regulation of cell differentiation. Eur. J. Histochem. *51*, 81–94.

Srinivasula, S.M., Gupta, S., Datta, P., Zhang, Z., Hegde, R., Cheong, N., Fernandes-Alnemri, T., and Alnemri, E.S. (2003). Inhibitor of apoptosis proteins are substrates for the mitochondrial serine protease Omi/HtrA2. J. Biol. Chem. *278*, 31469–31472.

Steller, H. (2008). Regulation of apoptosis in Drosophila. Cell Death Differ. *15*, 1132–1138.

Suberbielle, E., Sanchez, P.E., Kravitz, A.V., Wang, X., Ho, K., Eilertson, K., Devidze, N., Kreitzer, A.C., and Mucke, L. (2013). Physiologic brain activity causes DNA double-strand breaks in neurons, with exacerbation by amyloid-β. Nat. Neurosci. *16*, 613–621.

Sun, C., Cai, M., Gunasekera, A.H., Meadows, R.P., Wang, H., Chen, J., Zhang, H., Wu, W., Xu, N., Ng, S.C., and Fesik, S.W. (1999). NMR structure and mutagenesis of the inhibitor-of-apoptosis protein XIAP. Nature *401*, 818–822.

Sun, C., Cai, M., Meadows, R.P., Xu, N., Gunasekera, A.H., Herrmann, J., Wu, J.C., and Fesik, S.W. (2000). NMR structure and mutagenesis of the third Bir domain of the inhibitor of apoptosis protein XIAP. J. Biol. Chem. *275*, 33777–33781.

Suzuki, Y., Imai, Y., Nakayama, H., Takahashi, K., Takio, K., and Takahashi, R. (2001a). A serine protease, HtrA2, is released from the mitochondria and interacts with XIAP, inducing cell death. Mol. Cell *8*, 613–621.

Suzuki, Y., Nakabayashi, Y., and Takahashi, R. (2001b). Ubiquitin-protein ligase activity of X-linked inhibitor of apoptosis protein promotes proteasomal degradation of caspase-3 and enhances its anti-apoptotic effect in Fas-induced cell death. Proc. Natl. Acad. Sci. USA *98*, 8662–8667.

Suzuki, A., Kusakai, G., Kishimoto, A., Shimojo, Y., Miyamoto, S., Ogura, T., Ochiai, A., and Esumi, H. (2004). Regulation of caspase-6 and FLIP by the AMPK family member ARK5. Oncogene *23*, 7067–7075.

Takemoto, K., Nagai, T., Miyawaki, A., and Miura, M. (2003). Spatio-temporal activation of caspase revealed by indicator that is insensitive to environmental effects. J. Cell Biol. *160*, 235–243.

Tamayev, R., and D'Adamio, L. (2012). Inhibition of γ-secretase worsens memory deficits in a genetically congruous mouse model of Danish dementia. Mol. Neurodegener. *7*, 19.

Tamayev, R., Akpan, N., Arancio, O., Troy, C.M., and D'Adamio, L. (2012a). Caspase-9 mediates synaptic plasticity and memory deficits of Danish dementia knock-in mice: caspase-9 inhibition provides therapeutic protection. Mol. Neurodegener. *7*, 60.

Tamayev, R., Matsuda, S., Arancio, O., and D'Adamio, L. (2012b). β- but not γ-secretase proteolysis of APP causes synaptic and memory deficits in a mouse model of dementia. EMBO Mol. Med. *4*, 171–179.

Tenneti, L., D'Emilia, D.M., and Lipton, S.A. (1997). Suppression of neuronal apoptosis by S-nitrosylation of caspases. Neurosci. Lett. *236*, 139–142.

Thakor, N., and Holcik, M. (2012). IRES-mediated translation of cellular messenger RNA operates in eIF2α- independent manner during stress. Nucleic Acids Res. *40*, 541–552.

Truman, J.W. (1990). Metamorphosis of the central nervous system of Drosophila. J. Neurobiol. *21*, 1072–1084.

Tsang, A.H., Lee, Y.I., Ko, H.S., Savitt, J.M., Pletnikova, O., Troncoso, J.C., Dawson, V.L., Dawson, T.M., and Chung, K.K. (2009). S-nitrosylation of XIAP compromises neuronal survival in Parkinson's disease. Proc. Natl. Acad. Sci. USA *106*, 4900–4905.

Unsain, N., Higgins, J.M., Parker, K.N., Johnstone, A.D., and Barker, P.A. (2013). XIAP regulates caspase activity in degenerating axons. Cell Rep. *4*, 751–763.

Unsain, N., Heard, K.N., Higgins, J.M., and Barker, P.A. (2014). Production and isolation of axons from sensory neurons for biochemical analysis using porous filters. J. Vis. Exp. Published online July 8, 2014. http://dx.doi.org/10.3791/51795.

Vaux, D.L., and Silke, J. (2005). IAPs, RINGs and ubiquitylation. Nat. Rev. Mol. Cell Biol. *6*, 287–297.

Velázquez-Delgado, E.M., and Hardy, J.A. (2012a). Phosphorylation regulates assembly of the caspase-6 substrate-binding groove. Structure *20*, 742–751.

Velázquez-Delgado, E.M., and Hardy, J.A. (2012b). Zinc-mediated allosteric inhibition of caspase-6. J. Biol. Chem. *287*, 36000–36011.

Verma, P., Chierzi, S., Codd, A.M., Campbell, D.S., Meyer, R.L., Holt, C.E., and Fawcett, J.W. (2005). Axonal protein synthesis and degradation are necessary for efficient growth cone regeneration. J. Neurosci. *25*, 331–342.

Vonsattel, J.P., Myers, R.H., Stevens, T.J., Ferrante, R.J., Bird, E.D., and Richardson, E.P., Jr. (1985). Neuropathological classification of Huntington's disease. J. Neuropathol. Exp. Neurol. *44*, 559–577.

Wang, H.G., Pathan, N., Ethell, I.M., Krajewski, S., Yamaguchi, Y., Shibasaki, F., McKeon, F., Bobo, T., Franke, T.F., and Reed, J.C. (1999). Ca2+-induced apoptosis through calcineurin dephosphorylation of BAD. Science *284*, 339–343.

Wang, J.Y., Chen, F., Fu, X.Q., Ding, C.S., Zhou, L., Zhang, X.H., and Luo, Z.G. (2014). Caspase-3 cleavage of dishevelled induces elimination of postsynaptic structures. Dev. Cell *28*, 670–684.

Westphal, D., Sytnyk, V., Schachner, M., and Leshchyns'ka, I. (2010). Clustering of the neural cell adhesion molecule (NCAM) at the neuronal cell surface induces caspase-8- and -3-dependent changes of the spectrin meshwork required for NCAM-mediated neurite outgrowth. J. Biol. Chem. *285*, 42046–42057.

Westphal, D., Kluck, R.M., and Dewson, G. (2014). Building blocks of the apoptotic pore: how Bax and Bak are activated and oligomerize during apoptosis. Cell Death Differ. *21*, 196–205.

Williams, D.W., Kondo, S., Krzyzanowska, A., Hiromi, Y., and Truman, J.W. (2006). Local caspase activity directs engulfment of dendrites during pruning. Nat. Neurosci. *9*, 1234–1236.

Wilson, R., Goyal, L., Ditzel, M., Zachariou, A., Baker, D.A., Agapite, J., Steller, H., and Meier, P. (2002). The DIAP1 RING finger mediates ubiquitination of Dronc and is indispensable for regulating apoptosis. Nat. Cell Biol. *4*, 445–450.

Wong, B.K., Ehrnhoefer, D.E., Graham, R.K., Martin, D.D., Ladha, S., Uribe, V., Stanek, L.M., Franciosi, S., Qiu, X., Deng, Y., et al. (2015). Partial rescue of some features of Huntington Disease in the genetic absence of caspase-6 in YAC128 mice. Neurobiol. Dis. *76*, 24–36.

Wu, H.Y., Hudry, E., Hashimoto, T., Kuchibhotla, K., Rozkalne, A., Fan, Z., Spires-Jones, T., Xie, H., Arbel-Ornath, M., Grosskreutz, C.L., et al. (2010). Amyloid beta induces the morphological neurodegenerative triad of spine loss, dendritic simplification, and neuritic dystrophies through calcineurin activation. J. Neurosci. *30*, 2636–2649.

Wu, H.Y., Hudry, E., Hashimoto, T., Uemura, K., Fan, Z.Y., Berezovska, O., Grosskreutz, C.L., Bacskai, B.J., and Hyman, B.T. (2012). Distinct dendritic spine and nuclear phases of calcineurin activation after exposure to amyloid-β revealed by a novel fluorescence resonance energy transfer assay. J. Neurosci. *32*, 5298–5309.

Xu, X., Gerard, A.L., Huang, B.C., Anderson, D.C., Payan, D.G., and Luo, Y. (1998). Detection of programmed cell death using fluorescence energy transfer. Nucleic Acids Res. *26*, 2034–2035.

Yamaguchi, Y., Shinotsuka, N., Nonomura, K., Takemoto, K., Kuida, K., Yosida, H., and Miura, M. (2011). Live imaging of apoptosis in a novel transgenic mouse highlights its role in neural tube closure. J. Cell Biol. *195*, 1047–1060.

Yamamoto-Tanaka, M., Makino, T., Motoyama, A., Miyai, M., Tsuboi, R., and Hibino, T. (2014). Multiple pathways are involved in DNA degradation during keratinocyte terminal differentiation. Cell Death Dis. *5*, e1181.

Yan, N., Wu, J.W., Chai, J., Li, W., and Shi, Y. (2004). Molecular mechanisms of DrICE inhibition by DIAP1 and removal of inhibition by Reaper, Hid and Grim. Nat. Struct. Mol. Biol. *11*, 420–428.

Yang, Q.H., and Du, C. (2004). Smac/DIABLO selectively reduces the levels of c-IAP1 and c-IAP2 but not that of XIAP and livin in HeLa cells. J. Biol. Chem. *279*, 16963–16970.

Yang, Y., Fang, S., Jensen, J.P., Weissman, A.M., and Ashwell, J.D. (2000). Ubiquitin protein ligase activity of IAPs and their degradation in proteasomes in response to apoptotic stimuli. Science *288*, 874–877.

Yang, Q.H., Church-Hajduk, R., Ren, J., Newton, M.L., and Du, C. (2003). Omi/HtrA2 catalytic cleavage of inhibitor of apoptosis (IAP) irreversibly inactivates IAPs and facilitates caspase activity in apoptosis. Genes Dev. *17*, 1487–1496.

Yuan, J., Shaham, S., Ledoux, S., Ellis, H.M., and Horvitz, H.R. (1993). The C. elegans cell death gene ced-3 encodes a protein similar to mammalian interleukin-1 beta-converting enzyme. Cell *75*, 641–652.

Zhao, W.Q., Santini, F., Breese, R., Ross, D., Zhang, X.D., Stone, D.J., Ferrer, M., Townsend, M., Wolfe, A.L., Seager, M.A., et al. (2010). Inhibition of calcineurin-mediated endocytosis and alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors prevents amyloid beta oligomer-induced synaptic disruption. J. Biol. Chem. *285*, 7619–7632.

Zheng, T.S., Hunot, S., Kuida, K., and Flavell, R.A. (1999). Caspase knockouts: matters of life and death. Cell Death Differ. *6*, 1043–1053.

Zhou, Q., Homma, K.J., and Poo, M.M. (2004). Shrinkage of dendritic spines associated with long-term depression of hippocampal synapses. Neuron *44*, 749–757.

Zhou, F., Xing, D., Wu, S., and Chen, W.R. (2010). Intravital imaging of tumor apoptosis with FRET probes during tumor therapy. Mol. Imaging Biol. *12*, 63–70.