![R] Cell biology in neuroscience

Death of developing neurons: New insights and implications for connectivity

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The concept that target tissues determine the survival of neurons has inspired much of the thinking on neuronal development in vertebrates, not least because it is supported by decades of research on nerve growth factor (NGF) in the peripheral nervous system (PNS). Recent discoveries now help to understand why only some developing neurons selectively depend on NGF. They also indicate that the survival of most neurons in the central nervous system (CNS) is not simply regulated by single growth factors like in the PNS. Additionally, components of the cell death machinery have begun to be recognized as regulators of selective axonal degeneration and synaptic function, thus playing a critical role in wiring up the nervous system.

Why do so many neurons die during development?

Programmed cell death occurs throughout life, as cell turnover is part of homeostasis and maintenance in most organs and tissues. The situation in the nervous system is principally different, as the vast majority of neurons undergo their last round of cell division early in development. Soon after exiting the cell cycle, neurons start elongating axons to innervate their targets. It is during this period that they are highly susceptible to undergo programmed cell death: a large percentage, as much as 50% in several ganglia in the peripheral nervous system (PNS) as well as in various central nervous system (CNS) areas, is eliminated around the time that connections are being made with other cells. Later in development, the propensity of neurons to

initiate apoptosis progressively decreases. The likelihood for a neuron to undergo apoptosis seems to be determined by a tightly regulated apoptotic machinery (summarized in Fig. 1). Therefore, modulation of the expression levels or the activity of components of this apoptotic balance changes the sensitivity to death-promoting cues, allowing temporal restriction of cell death.

Programmed cell death eliminates many neurons during development, even in organisms comprised of only few cells, such as *Caenorhabditis elegans*. As neurons and their targets are initially separated, it is possible that the initial generation of an overabundance of neurons is simply part of a mechanism to ensure that distal targets are adequately innervated (Oppenheim, 1991; Conradt, 2009; Chen et al., 2013). In various tissues other than the nervous system, programmed cell death is used to eliminate cells that are no longer needed, defective, or harmful to the function of the organism. However, there is strong evidence that the elimination of superfluous neurons in the developing nervous system is not essential. For example, early work in *C. elegans* revealed that preventing programmed cell death does not result in significant behavioral alterations (Ellis and Horvitz, 1986). In the C57BL/6 mouse strain, deletion of the executor caspases 3 and 7 (Fig. 1) has a remarkably limited neuropathological and morphological impact in the CNS (Leonard et al., 2002; Lakhani et al., 2006) compared with the 129X1/SvJ strain, in which deletion of these caspases causes neurodevelopmental defects (Leonard et al., 2002). Similar conclusions were reached by blocking the Bcl-2–associated X protein (BAX)–dependent pathway in many neuronal populations, including motoneurons (Buss et al., 2006a). A recent study in the developing retina showed that in mice lacking the central apoptotic regulator BAX, the normal mosaic distribution of intrinsically photosensitive retinal ganglion cells (ipRGCs) was perturbed (Chen et al., 2013). Although this abnormal distribution is dispensable for the intrinsic photosensitivity of the ipRGCs, it is required for establishing proper connections to other neurons in the retina, which is necessary for rod/cone photo-entrainment (Chen et al., 2013). Even though this finding highlights a physiological role for
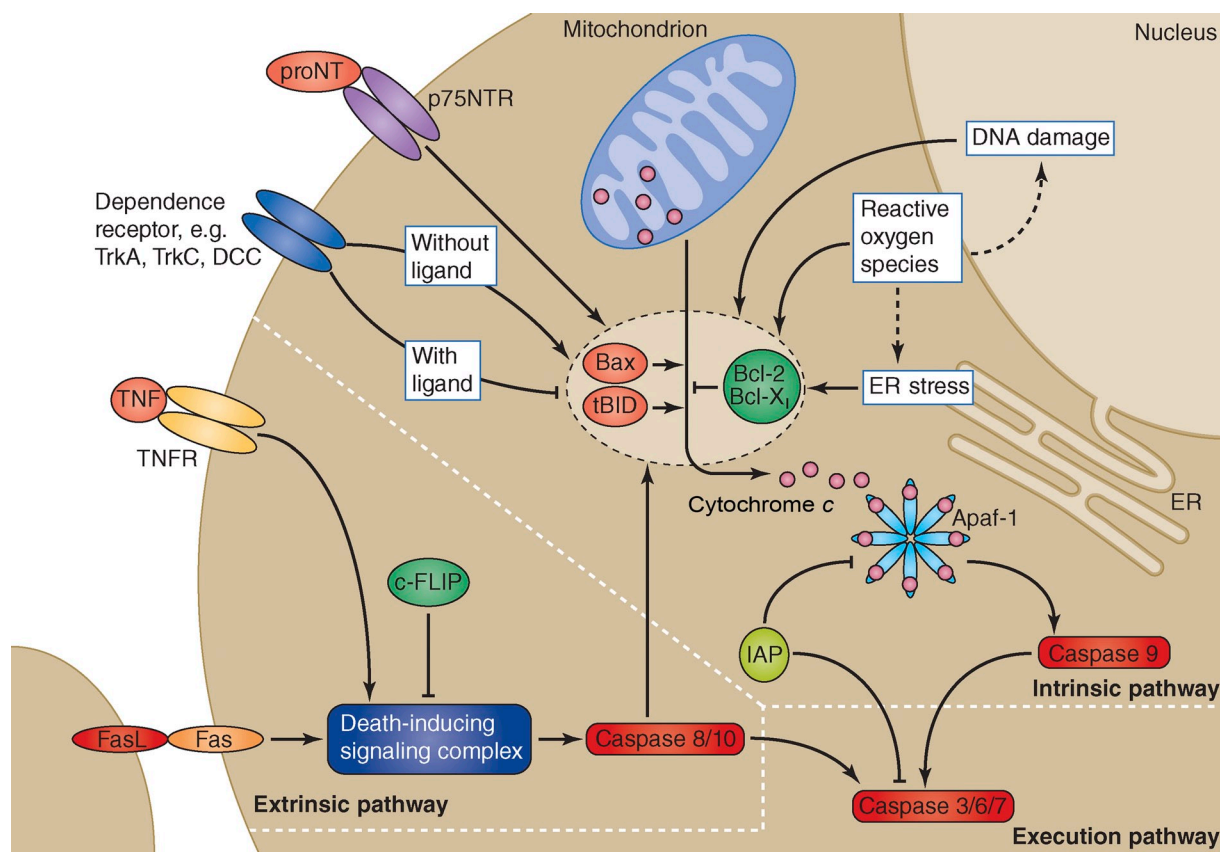
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Figure 1. **Core components of the apoptotic machinery.** The likelihood that a neuron undergoes apoptosis is determined by the interplay of the tightly interlinked apoptotic machinery, many components of which are highly conserved between species. The critical, and often terminal, step in programmed cell death is the proteolytic activation of the executor caspases (such as caspase 3, 6, 7) by the initiator caspases (i.e., caspase 8, 9, and 10; Riedl and Salvesen, 2007). In mammalian cells, initiation of the executor caspases is regulated by two distinct protein cascades: the intrinsic pathway, also known as the mitochondrial pathway, and the extrinsic pathway. The intrinsic pathway integrates a number of intra- and extracellular signal modalities, such as redox state (for example, the reactive oxygen species; Franklin, 2011), DNA damage (Sperka et al., 2012), ER stress (Puthalakath et al., 2007) and growth factor deprivation (Deckwerth et al., 1998; Putcha et al., 2003; Bredesen et al., 2005), or activation of the p75NTR neurotrophin receptor by pro-neurotrophins (Nykjaer et al., 2005). The stressors converge onto pro- and anti-apoptotic members of the Bcl-2 protein family (for example: BCL-2, BCL-X$_I$, BAX, and tBID; Youle and Strasser, 2008). These proteins regulate the release of cytochrome c from mitochondria, which activates the initiator caspase 9 through Apaf1 (Riedl and Salvesen, 2007). The extrinsic pathway links activation of ligand-bound death receptors (such as Fas/CD95 and TNFR) to the initiator caspase 8 and 10, through formation of the death-inducing signaling complex (DISC; LeBlanc and Ashkenazi, 2003; Peter and Krammer, 2003). Together with additional regulatory elements (including the Inhibitors of apoptosis proteins [IAP]; Vaux and Silke, 2005) and cFLIP (Scaffidi et al., 1999; Wang et al., 2005), the apoptotic machinery forms a balance that determines the propensity of the neuron to undergo apoptosis.

programmed cell death in the CNS, the functional consequences remain rather underwhelming in the face of a process that eliminates such large numbers of neurons (Purves and Lichtman, 1984; Oppenheim, 1991; Miller, 1995; Gohlke et al., 2004). It thus appears that apoptotic removal of the surplus neurons generated during development mainly serves the purpose to optimize the size of the nervous system to be minimal, but sufficient.

A molecular substrate for the neurotrophic theory

Quantitatively, programmed cell death of neurons in the PNS and CNS is most dramatic when neurons start contacting the cells they innervate. Because experimental manipulations such as target excision typically lead to the death of essentially all innervating neurons (Oppenheim, 1991), the concept emerged that the fate of developing neurons is regulated by their targets. This notion is often referred to as the "neurotrophic theory" (Hamburger et al., 1981; Purves and Lichtman, 1984; Oppenheim, 1991), but it is important to realize that it evolved in the absence of direct

mechanistic or molecular support (Purves, 1988). Originally described as a diffusible agent promoting nerve growth, the eponymous NGF later provided a strong and very appealing molecular foundation for this theory (Korsching and Thoenen, 1983; Edwards et al., 1989; Hamburger, 1992). The tyrosine kinase receptor tropomyosin receptor kinase A (TrkA), which was initially identified as an oncogene (Martin-Zanca et al., 1986), was fortuitously discovered to be the critical receptor necessary for the prevention of neuronal cell death by NGF (Klein et al., 1991). Both the remarkable expression pattern of TrkA in NGF-dependent neurons and the onset of its expression during development (Martin-Zanca et al., 1990) provided further additional support for the neurotrophic theory. However, for a surprisingly long time, the question was not asked as to why only specific populations of neurons strictly depend on NGF for survival, while others do not. Indeed, it was only recently shown that TrkA causes cell death of neurons by virtue of its mere expression, and that this death-inducing activity is prevented by addition of NGF (Nikoletopoulou et al., 2010). These findings thus

indicate that TrkA acts as a "dependence receptor," a concept introduced after observations that various cell types die when receptors are expressed in the absence of their cognate ligands (Bredesen et al., 2005; Tauszig-Delamasure et al., 2007). Accordingly, embryonic mouse sympathetic or sensory neurons survive in the absence of NGF when TrkA is deleted (Nikoletopoulou et al., 2010). The closely related neurotrophin receptor TrkC also acts as a dependence receptor (Tauszig-Delamasure et al., 2007; Nikoletopoulou et al., 2010). Here, it is interesting to note a series of older, convergent results indicating that deletion of neurotrophin-3 (NT3), the TrkC ligand, leads to a significantly larger loss of sensory and sympathetic neurons in the PNS than the deletion of TrkC (Tessarollo et al., 1997). This phenotypic discrepancy fits well with the idea that inactivation of the ligand of a dependence receptor is expected to yield a more profound phenotype than inactivation of the receptor itself (Tauszig-Delamasure et al., 2007). How TrkA and TrkC induce apoptosis remains to be fully elucidated. It seems that proteolysis is involved, either of TrkC itself (Tauszig-Delamasure et al., 2007), as was suggested for other dependence receptors (Bredesen et al., 2005), or by the proteolysis of the neurotrophin receptor p75NTR, which associates with both TrkA and TrkC (Fig. 2; Nikoletopoulou et al., 2010). Surprisingly, although TrkA and TrkC cause cell death, the structurally related TrkB receptor does not (Nikoletopoulou et al., 2010), a difference that appears to be accounted for by their differential localization in the cell membrane. TrkA and TrkC colocalize with p75NTR in lipid rafts, whereas TrkB, which also associates with p75NTR (Bibel et al., 1999), is excluded from lipid rafts (Fig. 2; unpublished data). Interestingly, the transmembrane domains of TrkA and TrkC are closely related, and differ clearly from that of TrkB. It turns out that a chimeric protein of TrkB with the transmembrane domain of TrkA causes cell death, which can be prevented by the addition of the TrkB ligand brain-derived neurotrophic factor (BDNF; unpublished data). The suggestion that the lipid raft localization of TrkA and TrkC is important for their death-inducing function is in line with a number of reports indicating that certain apoptotic proteins preferentially localize in lipid rafts in the plasma membrane. After activation of the extrinsic apoptosis pathway, translocation of the activated receptors to lipid rafts in the membrane is required for assembling the death-inducing signaling complex (DISC; Davis et al., 2007; Song et al., 2007). Indeed, regulators of the extrinsic pathway (e.g., cFLIP; Fig. 1) prevent this translocation, explaining how they attenuate cell death induction (Song et al., 2007). Similarly, the localization of the dependence receptor DCC (deleted in colorectal cancer) in lipid rafts is a prerequisite for its pro-apoptotic activity in absence of its ligand, Netrin-1 (Furne et al., 2006).

Despite the fact that TrkB does not act as a dependence receptor, its activation by BDNF is required for the survival of several populations of cranial sensory neurons (Ernfors et al., 1995; Liu et al., 1995). It appears that other death-inducing receptors predispose these neurons to be eliminated, such as p75NTR, which is expressed at high levels in some of these ganglia, or TrkC in vestibular neurons (Stenqvist et al., 2005). This latter case is of special interest, as NT3 is known not to be required for the survival of these neurons (Stenqvist et al., 2005).
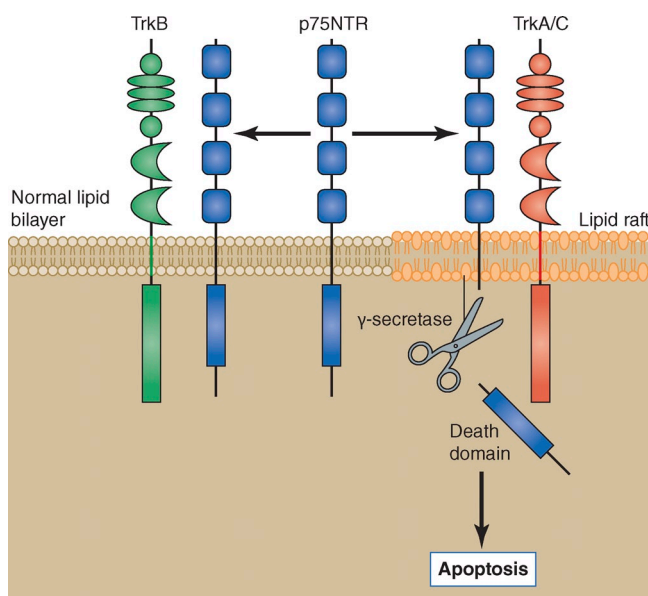

Figure 2. **TrkA and TrkC as dependence receptors: mode of action and contrast with TrkB.** All Trk receptors associate with the pan-neurotrophin receptor p75NTR (Bibel et al., 1999). A critical step in the induction of apoptosis by TrkA is the release of the intracellular death domain of p75NTR by the protease γ-secretase (Nikoletopoulou et al., 2010), which is localized in lipid rafts (Urano et al., 2005). Our membrane fractionation studies indicate that while TrkA and TrkC associate with p75NTR in lipid rafts, TrkB associated with p75NTR is excluded from this membrane domain (unpublished data). The 24–amino acid transmembrane domain of the Trk receptors may be responsible for this differential localization (see text).

In addition to inducing apoptosis in the absence of their ligand, TrkA and TrkC have long been recognized to have a pro-survival function similar to TrkB, as can be inferred from the loss of specific populations of peripheral sensory neurons in mutants lacking these receptors (Klein et al., 1994; Smeyne et al., 1994).

Cell death in the CNS

Although TrkA is primarily expressed in peripheral sympathetic and sensory neurons, it is also found in a small population of cholinergic neurons in the basal forebrain (Sobreviela et al., 1994), a proportion of which requires NGF for survival (Hartikka and Hefti, 1988; Crowley et al., 1994; Müller et al., 2012). Selective deletion of TrkA was recently shown not to cause the death of these neurons (Sanchez-Ortiz et al., 2012). This supports the notion that TrkA acts as a dependence receptor for this small population of CNS neurons, like for peripheral sensory and sympathetic neurons. TrkA activation by NGF is essential for the maturation, projections, and function of these neurons (Sanchez-Ortiz et al., 2012), as was previously described for sensory neurons in the PNS as well (Patel et al., 2000).

Whether or not receptors other than TrkA act as dependence receptors in the CNS is an important open question, particularly because TrkB, which is expressed highly by most CNS neurons, does not act as a dependence receptor (Nikoletopoulou et al., 2010). In retrospect, the structural similarities between TrkA and TrkB, just like those between NGF and BDNF (Barde, 1989), have substantially misled the field by suggesting that BDNF would act in the CNS like NGF in the PNS. Adding to the confusion were early findings showing that BDNF supports

the growth of spinal cord motoneurons in vitro or in vivo after axotomy (Oppenheim et al., 1992; Sendtner et al., 1992; Yan et al., 1992). However, in the absence of lesion, deletion of BDNF does not lead to significant losses of neurons in the developing or adult CNS (Ernfors et al., 1994a; Jones et al., 1994; Rauskolb et al., 2010), unlike the case in some populations of PNS neurons. The poor correlation of the role of BDNF in CNS development and in axotomy and in vitro experiments is surprising, especially because the role of NGF in vivo could in essence be recapitulated by in vitro experiments. Although the reasons for this discrepancy are not fully understood, the strong up-regulation of death-inducing molecules such as p75NTR after axotomy (Ernfors et al., 1989) may be a part of the explanation. At present, most of the growth factors promoting the survival of PNS neurons fail to show significant survival properties for developing neurons in the CNS, as for example was shown for NT3 (Ernfors et al., 1994b; Fariñas et al., 1994), glial cell line–derived neurotrophic factor (GDNF; Henderson et al., 1994), ciliary neurotrophic factor (CNTF; DeChiara et al., 1995), and several others.

In the developing CNS, neuronal activity and neurotransmitter input seem to play a more significant role than single growth factors in regulating neuronal survival. In particular, it has been known for a long time that blocking synaptic transmission at the neuromuscular junction has a pro-survival effect on spinal cord motoneurons (Pittman and Oppenheim, 1978; Oppenheim et al., 2008). By contrast, surgical denervation of afferent connections leads to increased apoptosis of postsynaptic neurons (Okado and Oppenheim, 1984), whereas inhibiting glycinergic and GABAergic synaptic transmission has both pro- and anti-apoptotic effects on motoneurons (Banks et al., 2005). Throughout the developing brain, blocking glutamate-mediated synaptic transmission involving NMDA receptors markedly increases normally occurring neuronal death (Ikonomidou et al., 1999; Heck et al., 2008). The mechanism involves a reduction of neuronal expression of anti-apoptotic proteins, such as B-cell lymphoma 2 (BCL-2; Hansen et al., 2004). Conversely, a limited increase in neuronal activity leads to down-regulation of the pro-apoptotic genes BAX and caspase 9 (Léveillé et al., 2010), thereby reducing the propensity of these cells to initiate programmed cell death (Hardingham et al., 2002). In addition to directly modulating the expression of apoptotic proteins, neuronal activity affects the expression of several secreted growth factors, such as BDNF (Hardingham et al., 2002; Hansen et al., 2004) and GDNF (Léveillé et al., 2010). So, even though BDNF is not a major survival factor in the developing CNS, it appears to be critical for activity-dependent neuroprotection (Tremblay et al., 1999). A recent publication revealed that certain populations of neurons in the CNS do not follow the predictions of the neurotrophic theory and showed that apoptosis of cortical inhibitory neurons is independent of cues present in the developing cerebral cortex (Southwell et al., 2012). This study indicates that programmed cell death of a large proportion of interneurons in the CNS is regulated by intrinsic mechanisms that are largely resistant to the presence or absence of extrinsic cues (Dekkers and Barde, 2013).

Taken together, even though the extent of naturally occurring cell death in the different regions of the CNS is not nearly as well characterized as in the PNS, let alone quantified, it appears that its regulation may significantly differ. Although single secreted neurotrophic factors seem to be largely dispensable for survival, neuronal activity and other intrinsic mechanisms drive the propensity of the neurons in the CNS to undergo apoptosis. An important open question in this context is a possible involvement of non-neuronal cells, such as glial cells (see Corty and Freeman, in this issue).

The apoptotic machinery as a regulator of connectivity

Activation of the executor caspases has been most studied in cell bodies and typically results in the demise of the entire cell (Williams et al., 2006). However, recent evidence shows that caspases are also activated locally in neuronal processes and branches destined to be eliminated, for example in axons overshooting their targets that are subsequently pruned back to establish the precise adult connectivity (Finn et al., 2000; Raff et al., 2002; Luo and O'Leary, 2005; Buss et al., 2006b). Initially, axonal degeneration and axon pruning were thought to be independent of caspases (Finn et al., 2000; Raff et al., 2002). Later work in *Drosophila melanogaster* (Kuo et al., 2006; Williams et al., 2006) and in mammalian neurons (Plachta et al., 2007; Nikolaev et al., 2009; Vohra et al., 2010) demonstrated that interfering with the apoptotic balance or the executor caspases can prevent or at least delay axonal degeneration. Simon et al. (2012) have found that a caspase 9 to caspase 3 cascade is crucial for axonal degeneration induced by NGF withdrawal, with caspase 6 activation playing a significant but subsidiary role. Upstream of the caspases, BCL-2 family members such as BAX and BCL-X$_l$ are required (Nikolaev et al., 2009; Vohra et al., 2010). It is conceivable that the failure of ipRGCs in BAX-deficient mice to form appropriate connections to other cells in the retina (Chen et al., 2013) may be in part attributable to defective axonal degeneration. Surprisingly, Apaf1 appears not to be involved in this process (Cusack et al., 2013), suggesting that axon degeneration depends on the concerted activation of the intrinsic initiator complex in a different way from apoptosis.

Strikingly, a series of recent studies showed that several caspases and components of the intrinsic pathway also affect normal synaptic physiology in adulthood (Fig. 3, A–D). Here, pro-apoptotic proteins are predominantly involved in weakening the synapses, whereas the anti-apoptotic proteins have been mainly associated with synaptic strengthening (Fig. 3 B). In particular, caspase 3 promotes long-term depression (LTD), a stimulation paradigm that results in a period of decreased synaptic transmission (Li et al., 2010), and also prevents long-term potentiation (LTP), the converse situation leading to strengthened synaptic transmission (Jo et al., 2011). Likewise, the proapoptotic BCL-2 family members BAX and BAD stimulate LTD (Jiao and Li, 2011). By contrast, the anti-apoptotic protein BCL-X$_l$ increases synapse numbers and strength (H. Li et al., 2008), and the inhibitor of apoptosis protein (IAP) family member survivin was reported to be involved in LTP in the hippocampus (Iscru et al., 2013) and in activity-dependent gene regulation (O'Riordan et al., 2008).
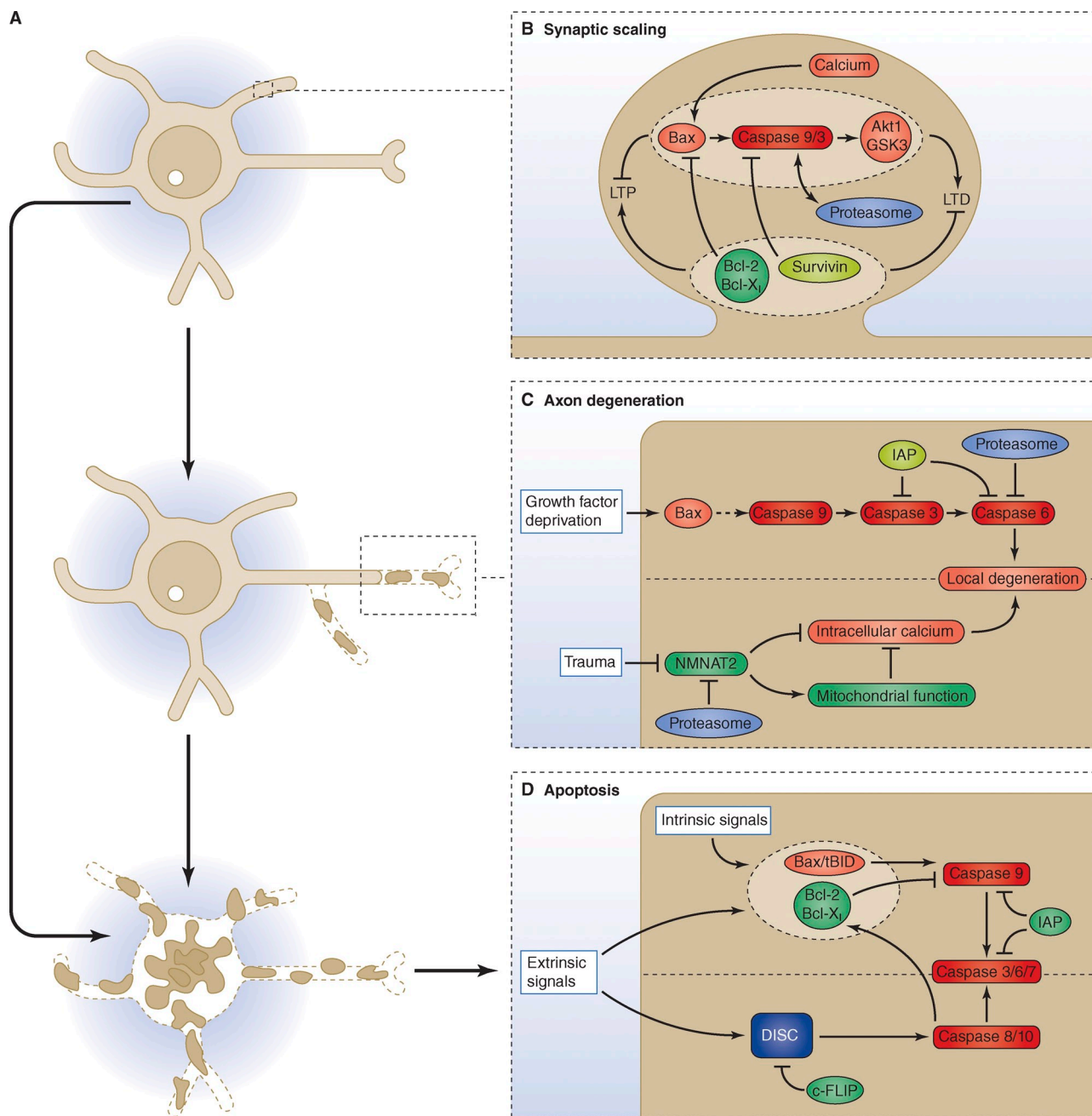
Figure 3. Canonical and noncanonical functions of the apoptotic machinery. (A) The apoptotic machinery is not only involved in eliminating cells destined to die, but is also a central player in refining neuronal connectivity, by regulating synaptic transmission and by generating the adult connectivity through axon pruning (Luo and O'Leary, 2005; Hyman and Yuan, 2012). But how the canonical and noncanonical roles of the apoptotic machinery are interlinked and spatially restricted is not well understood. (B) In the adult nervous system, the pro-apoptotic proteins BAX, caspase 9, and caspase 3 promote weakening of synapses (long-term depression [LTD]; Li et al., 2010; Jiao and Li, 2011; Jo et al., 2011), while the anti-apoptotic proteins Bcl-X_l and the IAP survivin promote synaptic strengthening (long-term potentiation [LTP]; Li et al., 2008a; Iscru et al., 2013). It is unclear how the activation of these pathways is restricted to a single synapse, but a recent review suggested that the proteasomal degradation of activated caspases may prevent their diffusion (Hyman and Yuan, 2012). (C) Caspase activation is now known to be required for axon pruning during development to generate the adult refined connectivity (Luo and O'Leary, 2005; Simon et al., 2012). Different pathways are activated depending on the stimulus leading to degeneration. Growth factor deprivation during development leads to activation the executor caspases 3 and 6 (Simon et al., 2012) through the intrinsic apoptotic pathway, although its core protein Apaf1 does not seem to be required for this process (Cusack et al., 2013). On the other hand, a traumatic injury leads to reduced influx of NMNAT2 into the axon, which negatively affects the stability and function of mitochondria and leads to an increased calcium concentration (Wang et al., 2012). The effector caspase, caspase 6, is dispensable for this form of axonal degeneration (Vohra et al., 2010; Simon et al., 2012). Regulatory proteins such as the IAPs and also the proteasome seem to play a role in limiting the extent of activation to the degenerating part of the axon (Wang et al., 2012; Cusack et al., 2013; Unsain et al., 2013). (D) Simplified schematic of the main pro- and anti-apoptotic components. DISC, death-induced signaling complex. IAP, inhibitor of apoptosis protein. See Fig. 1 for details.

These findings indicate that the apoptotic machinery acts at different levels in the cell, ranging from driving sub-lethal degradation of a compartment (Fig. 3 C) and attenuating synaptic transmission at the neuronal network level (Fig. 3 B) to destroying the entire cell during development or in disease (Fig. 3 D). How the cell spatially restricts the extent of activation of the apoptotic machinery is yet unclear. For example, elimination of the somata of developing neurons after neurotrophin deprivation is preceded by axonal degeneration, but not all instances of axonal degeneration lead to the death of the neuron (Campenot, 1977; Raff et al., 2002). Local regulation of caspase activation by IAPs is well established as a means for ensuring the elimination of neuronal processes in *D. melanogaster* (Kuo et al., 2006; Williams et al., 2006). Recent findings suggest a similar role for IAP in mammalian neurons, where it limits caspase activation to the degenerating axon (Fig. 3 C; Cusack et al., 2013; Unsain et al., 2013). The spontaneous mutation Wallerian degeneration slow (WldS; Lunn et al., 1989) has been instrumental to understand that trauma-induced axon degeneration is a regulated process different from, and independent of, cell body degeneration (Wang et al., 2012), but also distinct from axon pruning (Hoopfer et al., 2006). Work on the chimeric protein encoded by the WldS mutation also led to the identification of the protein NMNAT2 (nicotinamide mononucleotide adenylyltransferase 2) as a labile axon survival factor (Gilley and Coleman, 2010). How the WldS chimeric protein and NMNAT2 result in axon protection is unclear, but several lines of evidence seem to converge on local regulation of mitochondrial function and motility (Avery et al., 2012; Fang et al., 2012).

Related to the spatial limiting of apoptotic activity is the question of how a local source of neurotrophins leads to the rescue of a developing peripheral neuron. When neurons encounter a source of neurotrophins, only the receptors close to the target will be activated, whereas the others, located further away, are not. The cell, therefore, needs to integrate a pro-survival signal from the activated receptors, and death-inducing signals from the nonactivated dependence receptors. The continued signaling of activated neurotrophin receptors that are retrogradely transported to the soma (Grimes et al., 1996; Howe et al., 2001; Wu et al., 2001; Harrington et al., 2011) likely play a role in counteracting the pro-apoptotic signaling proximal to the source of neurotrophins. It will be interesting to investigate whether similar mechanisms play a role in axon pruning and traumatic axon degeneration as well.

Programmed cell death in the adult brain

Most of the nervous system becomes post-mitotic early in development. In rodents, two brain areas retain the capacity to generate new neurons in the adult: the sub-ventricular zone, which generates neurons that migrate toward the olfactory bulb, and the sub-granular zone of the dentate gyrus of the hippocampus, where neurons are generated that integrate locally. Similar to what is observed during embryonic development, these adult-generated neurons are produced in excess, and a large fraction undergoes apoptosis when contacting its designated targets (Petreanu and Alvarez-Buylla, 2002; Kempermann et al., 2003; Ninkovic et al., 2007). Preventing apoptosis of adult-generated neurons in the

olfactory bulb only has limited functional consequences (Kim et al., 2007), whereas a similar maneuver in the dentate gyrus does lead to impaired performance in memory tasks (Kim et al., 2009). Why superfluous hippocampal neurons would need to be eliminated for proper function is a matter of speculation, but may be linked with the fact that these are excitatory projection neurons, whereas in the olfactory bulb only axon-less inhibitory granule cells are integrated. The extent of survival in both these areas critically depends on the activity of the neuronal network in which these newly born neurons have to integrate (Petreanu and Alvarez-Buylla, 2002; Kempermann et al., 2006; Ninkovic et al., 2007). In this context, BDNF, the expression level of which is well known to be regulated by network activity, supports the survival of young adult–generated neurons and possibly even stimulates the proliferation of neural progenitors (Y. Li et al., 2008; Waterhouse et al., 2012). Interestingly, in young adult mouse mutants that exhibit spontaneous epileptic seizures, significantly higher levels of BDNF have been measured (Lavebratt et al., 2006; Heyden et al., 2011). Concomitantly, the entire hippocampal formation is considerably enlarged by as much as 40% (Lavebratt et al., 2006; Angenstein et al., 2007), which in turn is dependent on the epileptic seizures (Lavebratt et al., 2006). Whether or not there is a causal relationship between increased BDNF levels and hippocampal volume remains to be established.

Conclusion

Now that is has become clear that action of the apoptotic machinery can be limited spatially and temporally, several questions need to be addressed: how do neurons integrate intrinsic and extrinsic pro- and anti-apoptotic signals; and how they are spatially restricted to allow degradation of a dendrite or axon, or modulation of synaptic transmission? Another important issue is the regulation of cell death by intrinsic mechanisms in the central nervous system of vertebrates, not least because programmed cell death is observed in the CNS in a number of neurodegenerative diseases (Vila and Przedborski, 2003). Indeed, several of the central apoptotic components discussed here are also involved in these disorders (Hyman and Yuan, 2012). New insights in the regulation of programmed cell death in the developing nervous system may therefore continue to help to better understand the pathophysiological mechanisms of neurodegenerative disorders.

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