Death from within: apoptosis and the secretory pathway

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Recent studies have highlighted the importance of the secretory pathway in stress-induced apoptotic signaling. Sensing stress at the endoplasmic reticulum and Golgi might first trigger recovery mechanisms, followed by apoptosis if repair is unsuccessful. Cleavage of endoplasmic-reticulum- or Golgi-resident proteins can signal repair or apoptosis and promote organelle disassembly during apoptosis. Initiation of apoptosis from the secretory pathway requires components of the death machinery localized to these membranes. Extensive trafficking between compartments of the secretory pathway might allow the cell to integrate signals and to determine the proper response to a particular stress.

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Abbreviations

DR	death receptor
GRASP	Golgi reassembly and stacking protein
IAP	inhibitor of apoptosis
TNF	tumor necrosis factor
TNFR	TNF receptor
UPR	unfolded protein response

Introduction

The secretory pathway is poised to sense a variety of stresses because it communicates with the entire cell and the extracellular environment. Secretory stresses include an excess of misfolded proteins, virus infection, and changes in endomembrane dynamics caused by changes in lipid composition or transmembrane protein interactions. By initiating signaling events at different locations in the secretory pathway, cells might be able to sense specific kinds of stress and either adapt or eventually undergo apoptosis if the stress is too severe for recovery (reviewed in [1]). Apoptosis is a conserved process designed to remove damaged or extraneous cells from an organism without inducing inflammation. During apoptosis, cells are disassembled and cellular contents are packaged into membrane-bound blebs bearing signals that stimulate surrounding cells to phagocytose and degrade the apoptotic blebs.

In this review, we examine the role of the secretory pathway in sensing stress and transducing apoptotic signals, along with the fate of secretory pathway components during the execution phase of apoptosis.

General aspects of apoptosis

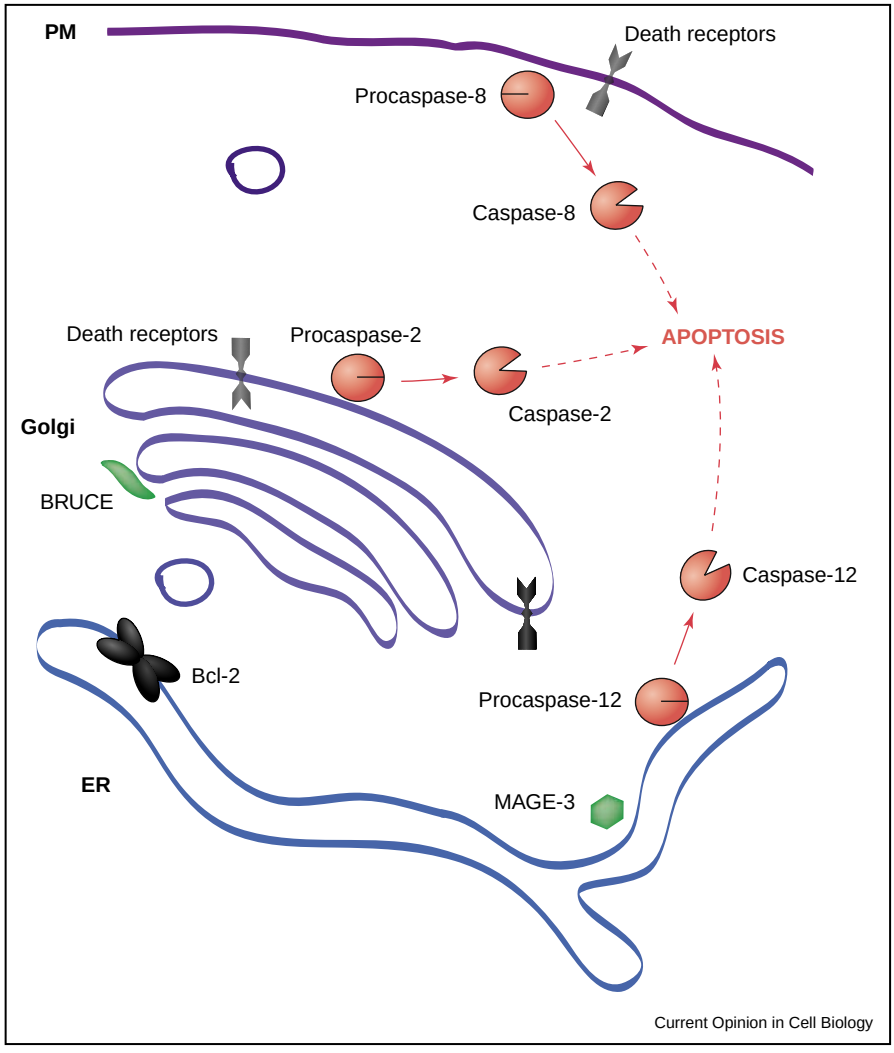
Death machinery

Caspases are a family of proteases that acts as central mediators of apoptosis (reviewed in [2]). They cleave selected cellular proteins at key sites, thereby modifying the function of each target protein. Cleavage can cause protein complexes to disassemble, eliminate target protein function, alter target activity level, or uncover an alternative function for the target protein. Caspases are synthesized as zymogens with low intrinsic activity, and are themselves activated by cleavage at specific sites to release the inhibitory prodomain and separate the protease subunits. There are two classes of caspases, initiators and effectors; initiator caspases have a long prodomain with protein interaction motifs, which mediate initiator caspase oligomerization, allowing autoactivation. Once activated, initiator caspases can proteolytically activate effector caspases, which mediate downstream cleavage events.

Triggering the apoptotic pathway involves tipping the balance between pro- and anti-apoptotic regulators. Regulators of apoptosis include the Bcl-2 family of proteins, which can be either pro- or anti- apoptotic. Pro-apoptotic Bcl-2 family members are thought to contribute to formation of a pore or channel in cell membranes. If this occurs in the mitochondria, other pro-apoptotic factors that are normally sequestered in the mitochondria can be released [3]. Anti-apoptotic Bcl-2 family members antagonize the activity of pro-apoptotic Bcl-2 family members. Bcl-2 family members have also recently been implicated in apoptotic response to ER stress [4].

Death receptors (DRs), including the tumor necrosis factor (TNF) receptors (TNFRs) and Fas, are also important mediators of apoptotic signaling. DRs are activated by ligand binding, which induces receptor oligomerization to signal a variety of cellular responses, including apoptosis [5,6]. Other regulators of apoptosis include the inhibitor of apoptosis (IAP) family of proteins. IAPs are typically identified by the presence of a baculovirus IAP repeat domain, and can inhibit caspase activity by directly binding to activated caspases or by blocking caspase

Figure 1



Components of the death machinery that localize to the secretory pathway. Bcl-2 family members, MAGE-3 and caspase-12 all associate with the ER, caspase-2 and BRUCE localize to the Golgi complex, and caspase-8 is recruited to the plasma membrane (PM). Death receptors, including TNFR-1 and Fas, populate both the Golgi complex and plasma membrane. Machinery that promotes apoptosis is shown in red; negative regulators of apoptosis are green. Death receptors and Bcl-2-family members can be pro- or anti-apoptotic depending on the identity of the family member and the context in which it is activated.

activation [7]. Several components of the death machinery, including caspase-2, caspase-12, DRs and Bcl-2 family members, are localized to membranes of the secretory pathway (Figure 1).

Disassembly and packaging of secretory pathway organelles

During apoptosis, secretion ceases (M Lowe M, VJ Allan *et al.*, personal communication) and the cell is ultimately packaged into membrane-bound blebs. Striking morphological changes take place in the secretory pathway as the cell is dismantled. The ER swells and is vesiculated [8]; however, specific events mediating ER disassembly are unknown.

During apoptotic disassembly of the Golgi complex, individual stacks of cisternal membranes lose their juxtanuclear position and are disassembled into vesicles and tubular clusters, perhaps similarly to mitotic disassembly [8]. Several proteins with proposed roles in Golgi structure and membrane trafficking have recently been reported to be caspase substrates, including golgin-160 [9], GRASP65 (Golgi reassembly and stacking protein 65) [10•], giantin ([11] M Lowe M, VJ Allan *et al.*, personal communication), p115 [12•], and GM-130 ([11]; M Lowe M, VJ Allan *et al.*, personal communication). The t-SNARE (target membrane soluble *N*-ethylmaleimide sensitive factor attachment protein receptor) syntaxin-5 (M Lowe M, VJ Allan *et al.*, personal communication) and

two components of the dynein–dynactin motor complex [13•], specifically the intermediate chain of dynein and $p150^{Glued}$ subunit of dynactin, are also caspase substrates during apoptosis. Cleavage of these proteins might contribute to membrane traffic arrest and promote exposure of lipids and carbohydrates that serve as phagocytosis signals on the surface of the cell (M Lowe M, VJ Allan *et al.*, personal communication).

Cleavage of Golgi structural proteins is required for efficient Golgi disassembly during apoptosis: expression of non-cleavable mutants of GRASP65, p115 or golgin-160 delays apoptotic Golgi disassembly [9,12•,13•]. Interestingly, cells expressing non-cleavable golgin-160 demonstrate a delay in apoptosis induced by secretory pathway stress (CE Machamer *et al.*, unpublished data), suggesting that golgin-160 cleavage might be involved in signaling apoptotic progression as well as being required for efficient disassembly of the Golgi (see below).

The plasma membrane is also disassembled during apoptosis as membrane-bound blebs containing cellular contents pinch off of the cell. This process is largely regulated by phosphorylation of the myosin light chain [14] Caspase cleavage of the Rho-dependent kinase ROCK I increases its kinase activity, elevating myosin light chain phosphorylation and inducing membrane blebbing at the cell surface [15•,16•]. Inhibition of ROCK I activity blocks plasma membrane blebbing during apoptosis; however, other apoptotic events are unaffected, including phagocytosis, phosphatidylserine exposure and the removal of sialic acid from the cell surface [17], suggesting that plasma membrane blebbing is independent of other apoptotic events.

Stress responses in the secretory pathway

What induces secretory pathway stress?

There are several physiologically relevant inducers of ER stress, including accumulation of unfolded or misfolded proteins, depletion of lumenal calcium stores, and β-amyloid production [18]. Although there are no well-characterized Golgi-specific stresses, the structure of Golgi membranes might be particularly sensitive to changes in lipid composition. For example, increased ceramide at the Golgi complex perturbs membrane trafficking [19].

Other potential inducers of Golgi stress are likely to include virus assembly and budding at the Golgi, or disruptions in pH or cation content. Extensive trafficking between the ER and the Golgi might aid in the integration of stress signals sensed in these compartments.

Endoplasmic reticulum stress can lead to repair

The unfolded protein response (UPR) in the ER is well understood. In mammalian cells, the major players are two transmembrane sensor proteins, ATF6 and the protein kinase R-like ER kinase PERK (reviewed in [20]). When unfolded proteins accumulate in the ER, they displace the chaperone protein BiP/GRP78 from ATF6, which is then transported to the Golgi, where it is cleaved by the same proteases that release sterol-regulated transcription factors from the membrane [21,22]. The cytoplasmic portion of ATF6 then translocates to the nucleus and induces expression of chaperone proteins, including BiP/GRP78 and GRP94.

Activation of PERK also induces phosphorylation of the translation initiation factor eIF2α and global downregulation of protein synthesis. This reduces the load of proteins in the ER, giving the accumulated proteins a chance to fold, potentially rescuing the cell from stress. Mutant proteins that cannot fold are usually translocated to the cytoplasm for proteasome-mediated degradation [23]; a prolonged UPR that cannot be repaired leads to apoptosis.

The ER also plays a central role in calcium homeostasis, serving as the major reservoir for free Ca^{2+} ions, which bind to calcium-buffering proteins in the ER lumen. Free Ca^{2+} is pumped across the ER membrane by Ca^{2+}-dependent ATPases, including the SERCA pump. Various stresses, including oxidative stress [24], trigger Ca^{2+} release from the ER. Calnexin and calreticulin are calcium-binding chaperones in the ER that monitor protein folding, and decreases in ER calcium levels disrupt the function of calnexin and calreticulin, contributing to the UPR [25].

Apoptotic signaling at the endoplasmic reticulum leads to cell death

Caspase-12, found on the cytoplasmic face of the ER [26], is important for inducing apoptosis in response to prolonged ER stress in mice. The mechanism of caspase-12 activation is currently unknown, but elucidation might provide clues to why cells eventually abandon repair and initiate apoptosis. For example, there could be procaspase-12 binding proteins in the ER that mediate procaspase-12 oligomerization and autoactivation in response to ER stress. One such candidate is the TNFR-associated protein TRAF2, which binds to and promotes clustering of procaspase-12 in cells overexpressing both proteins [27]. Procaspase-12 can be cleaved by itself or by calpain [26] and caspase-7 [28]. It is also possible that ER stress disrupts an interaction between procaspase-12 and an inhibitor, such as MAGE-3 (melanoma-associated antigen-3), which blocks proteolytic activation of procaspase-12 *in vitro* [29]. Stable expression of MAGE-3 makes mouse cells more resistant to ER-stress-induced apoptosis [29].

Although caspase-12 appears to be crucial for activation of apoptosis in response to ER stress in mice, no functional human orthologue of caspase-12 has been identified. Human cDNAs putatively encoding caspse-12 have both a premature stop codon in all splice variants, and an

additional loss-of-function mutation in a conserved region essential for caspase activity [30]. In the absence of caspase-12, therefore, humans must have an alternative mechanism to activate apoptosis in response to ER stress. For example, a caspase with overlapping function might compensate for the lack of caspase-12. One attractive candidate is caspase-2 (discussed below).

In addition to activation of caspase-12, stress can cause calcium release, depleting ER calcium stores. Thapsigargin irreversibly inhibits the SERCA pump and causes depletion of ER calcium stores. Cells treated with thapsigargin upregulate expression of DR5, which can activate apoptotic signaling in response to TNF-related apoptosis-inducing ligand [31], suggesting a connection between depletion of ER calcium stores and the apoptotic machinery.

Depletion of ER calcium stores can induce apoptosis, possibly by increasing mitochondrial calcium levels. The anti-apoptotic protein Bcl-2 is partially localized to ER membranes and can regulate ER calcium homeostasis. Different effects of Bcl-2 have been reported, however; and it is likely that cell type and context can influence the outcome of Bcl-2 activity (reviewed in [4]). Interestingly, treatment of cells with TNF-α, which activates the DR TNFR-1, also activates ER calcium release; this effect is blocked by overexpression of Bcl-2 [32]. The mechanism by which an extracellular ligand causes calcium release from the ER is unclear, but might involve DR trafficking and/or acid sphingomyelinase-induced changes cellular lipid metabolism (see also Update).

Golgi stress and apoptotic signaling

The Golgi complex appears to have an important role in apoptosis. Caspase-2 is localized to the Golgi complex and the nucleus [9,33]. While the caspase-2-null mouse is viable and shows only minor developmental defects [34], recent experiments have provided evidence that caspase-2 plays a pivotal role in apoptotic signaling ([35]; CE Machamer *et al.*, unpublished data). Caspase-2-deficient mouse fibroblasts are resistant to apoptosis induced by pro-apoptotic drugs that induce either the UPR or other secretory stress, and expression of non-cleavable golgin-160 (a Golgi-localized caspase-2 substrate) impairs apoptosis in human cells induced by the same stimuli (CE Machamer *et al.*, unpublished data). The requirement for Golgi-localized caspase-2 might indicate that the UPR-inducing stimuli affect the Golgi in addition to the ER, or that continuous trafficking between the ER and Golgi allows integrated apoptotic signaling from these compartments.

As for capsase-12, the mechanism of activation of caspase-2 at the Golgi complex is unknown. One potential negative regulator of caspase-2 activation is the baculoviral IAP-repeat-containing ubiquitin-conjugating enzyme (BRUCE), which is localized to the Golgi complex [36]. The *Drosophila* homologue of BRUCE can suppress cell death induced by the pro-apoptotic proteins Reaper and Grim [37], suggesting that it is an IAP.

Caspase cleavage products of Golgi-resident proteins have potentially intriguing roles in induction of apoptosis. Constructs that mimic caspase cleavage fragments of golgin-160 and p115 localize to the nucleus [12•,38], where they have the potential to signal transcriptional activation of repair or apoptotic machinery. Apoptotic cleavage and relocalization of Golgi proteins might provide the missing link between stress at the Golgi and the cellular response, analogous to UPR activation in response to ATF6 cleavage.

DRs demonstrate another potential link between the Golgi and induction of apoptosis. Most research has focused on DRs present at the plasma membrane. Interestingly, the bulk populations of these same DRs localize to the Golgi complex [39,40]. Trafficking of the DR Fas to the plasma membrane is stimulated by GD3 [41], a ganglioside produced at the Golgi that has been implicated as an important apoptotic signaling molecule (reviewed in [42]). Furthermore, Golgi-localized caspase-2 appears to be required for apoptosis following the ligation of the DRs TNFR-1 and Fas in mouse fibroblasts (CE Machamer *et al.*, unpublished data). These findings suggest that, at least in some cell types, the apoptotic response to death ligands might depend on Golgi-mediated regulation of membrane trafficking within the cell.

Conclusions

Signaling from membranes in the secretory pathway allows cells to sense specific types of stress. Communication between secretory compartments might integrate stress signals, allowing activation of specific repair pathways, and eventually initiating apoptosis in response to irreparable damage. Understanding the activation mechanisms for caspases-2 and -12 will be critical for deciphering apoptotic signaling in the secretory pathway. Determining how stresses are sensed at secretory pathway membranes and how these signals reach the rest of the cell are the next challenges for understanding cell death pathways involving the secretory pathway.

Update

A potential link between ligation of DRs and ER calcium release might be provided by the transmembrane ER-resident protein BAP31. BAP31 is cleaved by caspase-8 during apoptosis to produce an integral membrane p20 fragment and a soluble carboxy-terminal fragment [43]. Cells expressing a caspase-resistant BAP31 are resistant to Fas ligation-induced apoptosis [44]. A recent paper shows that cells expressing the p20 caspase-cleavage fragment of BAP31 undergo apoptosis and are sensitized to caspase-8-induced cytochrome *c* release from mitochondria [45•].

This process is blocked by treatment with calcium chelators or depletion of ER calcium stores before addition of an apoptotic stimulus, indicating that calcium-mediated signaling between the ER and mitochondria is required for this communication [45•].

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