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Review

Organelle dynamics and membrane trafficking in apoptosis and autophagy

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Summary. The accurate control of cell death is a vital aspect of development in metazoans and plays crucial roles in the prevention of disease. Apoptosis is the main form of regulated cell death in multicellular organisms, although there are other contributory pathways. During apoptosis, mammalian cells undergo dramatic changes in organelle structure ad organisation that define the apoptotic execution phase. Although the roles of apoptotic protease machinery (the caspases) in these rearrangements are quite well understood, the purpose of organelle disruption during cell death is not yet entirely appreciated. Indeed, recent evidence implicates caspase targeting of organellar proteins and subsequent organelle disruption upstream of apoptotic execution proper, suggesting the existence of pathways linking organelle damage to cell death. In this review, we describe the changes to the endomembrane system that are inherent during the apoptotic execution phase, and examine the evidence for endomembrane-mediated pathways towards apoptotic execution. We also discuss aspects of the molecular control of autophagy - an important contributor to a cell's response to stress, and a membrane trafficking process whose regulation is linked to the apoptotic machinery at multiple levels.

Key wods: Apoptosis, Autophagy, Autophagosome, Atg proteins, Golgi apparatus, Endoplasmic reticulum, Mitochondria, Mitophagy

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Introduction

Regulated cell death is an essential process during development and for tissue homeostasis (Penaloza et al., 2006). Over recent years several distinct cell death pathways have been described in studies of cellular and organelle morphology in isolated cells, some of which have also been observed in intact tissues; however, their relative contributions to development, homeostasis and tissue remodelling remain unclear. Apoptosis is the primary, regulated cell death mechanism in mammals, although necrosis - previously considered unregulated and non-physiological - has recently emerged as a coordinated process with a molecular basis (Galluzzi and Kroemer, 2008). In healthy tissues and during pathology the types of cell death pathways initiated will depend upon the cell-type, its tissue context and the nature of the death stimulus - an important consideration when studying cell death mechanisms. Importantly, distinct cell death processes can be triggered in parallel (Lockshin and Zakeri, 2002; Gonzalez-Polo et al., 2005), so the exact mechanism of cell death is not always easily defined.

A good example of how classifying cell death purely on morphological grounds can lead to confusion is cell death by overstimulation of autophagy - a process that has commonly become known as autophagic or type II programmed cell death (Lockshin and Zakeri, 2002). Autophagy is a catabolic membrane trafficking process that requires novel membrane biogenesis, membrane remodelling and vesicular trafficking, and is important in a number of diseases (Levine and Kroemer, 2008). Its primary role is to promote cell survival during episodes of starvation or hypoxia, although in mammalian cells, there is strong evidence for dramatic upregulation of autophagy in dying cells; however, it is considered more

likely that this reflects an attempt to survive, meaning that the phrase "cell death with autophagy" is a more accurate description (Kroemer and Levine, 2008). Importantly, evidence from invertebrates suggests that autophagy does indeed contribute to cell death in certain tissues during development (Baehrecke, 2005; Bergmann, 2007; Hou et al., 2008). Notwithstanding this confusion, evidence is accumulating to suggest that the molecular regulatory components of the autophagy pathway can indeed influence cell death through direct regulation of apoptosis signalling pathways (Martin and Baehrecke, 2004; Yousefi et al., 2006; Maiuri et al., 2007a,b; Hou et al., 2008; Betin and Lane, 2009a,b; Cho et al., 2009), meaning that it is becoming increasingly clear that these distinct regulatory pathways cannot be considered in isolation.

In this review we discuss the roles of organelles and membrane trafficking pathways during apoptotic signalling and execution, and also explore how organelles of the secretory and endocytic systems regulate apoptotic commitment through the control of death receptor trafficking and signalling. Finally we describe the molecular relationships between the autophagy and apoptosis pathways, and discuss how these complex cellular responses to cell stress converge to dictate the fate of a given cell.

Apoptosis: signalling and execution

Apoptosis is essential during the development of multicellular organisms (Kuida et al., 1998). It also eradicates damaged or virally infected cells throughout the life of an organism in the absence of an inflammatory response (Savill et al., 2002). It does this by engaging a series of coordinated changes in cell structure/behaviour that culminate in the presentation of surface markers that flag the dying cell for engulfment by professional or non-professional phagocytes (Savill et al., 2002). For this to occur, dying cells must retain the capacity to control the organisation/positioning of membranes and proteins long enough to bring about their recognition by phagocytes. Most observers consider the execution phase of apoptosis - the time during which gross apoptotic morphological/behaviour changes are observed and before the cell begins the process of secondary necrosis - to last for around 1-2 hours (e.g. (Mills et al., 1999; Moss and Lane, 2006)). Hence, a dying cell has a limited time window during which it must coordinate both the changes in morphology that are required for its isolation and the exposure of novel and/or altered surface moieties that are needed for efficient recognition and engulfment.

Apoptotic signalling: basic principles

Apoptosis can be induced by an array of toxic insults, physiological and non-physiological, which all converge on the activation of a family of proteases called caspases (Earnshaw et al., 1999). These enzymes are present in the cytosol of all viable cells as zymogens with low intrinsic activity, providing cells with the capability to rapidly trigger apopotic protease action without the need for new protein synthesis. Apoptotic caspases fall into two general classes: the initiator caspases that are required for initial apoptotic signalling and the executioner caspases whose roles are to cleave a variety of important structural and regulatory proteins at conserved aspartic acid residues to alter their functions irreversibly (Earnshaw et al., 1999; Taylor et al., 2008). Members of the Bcl-2 family of pro- and anti-apoptotic signalling molecules integrate these signals, and their interactions dictate whether or not a cell enters the apoptotic execution phase. Importantly, many of these are known to associate with organelles of the endomembrane system or with cytoskeletal components (Betin and Lane, 2007). Of particular importance are the interactions between Bcl-2 family members and the mitochondria which harbour an array of apoptotic signalling molecules whose release via Bcl-2 family engagement triggers a robust apoptotic response; although relationships between Bcl-2 family members and the endoplasmic reticulum (ER) are also significant particularly with respect to calcium signalling or in cells subject to oxidative stress (Ferri and Kroemer, 2001).

Induction of mitochondrial outer membrane permeabilisation (MOMP) via Bcl-2 family members causes release of cytochrome c, which, in turn, activates the initiator caspase-9 by inducing assembly of the "apoptosome" - a complex consisting of multiple copies of caspase-9, cytochrome c, dATP and the adaptor APAF-1. Caspase-9, in turn, activates effector caspases that cleave target proteins to initiate the apoptotic execution phase proper. Mitochondria are therefore central to a cell's apoptotic response. The other major mechanism for activating caspases in response to death stimuli is via cell surface engagement of death receptors such as those of the TNF family (e.g. Fas receptor/CD95). Death receptors can activate caspases directly, or via mitochondrial amplification. As transmembrane proteins that exert their influence at the plasma membrane, their trafficking through the secretory and endocytic systems is a key feature of death receptor signalling. It has become apparent that both the secretory and endocytic systems are disrupted as an early consequence of caspase activation, but it is unclear how this impacts upon death receptor distribution, trafficking and signalling.

Apoptotic cell remodelling

The dramatic changes in cell behaviour observed during the apoptotic execution phase (Fig. 1) are orchestrated by caspases. In response to targeted protein cleavage, apoptotic cells begin a process of remodelling which includes cell retraction, plasma membrane blebbing and often fragmentation of the cell into membrane-bound apoptotic bodies (Fig.1). In epithelia the execution phase also invokes the actions of neighbouring cells that respond to early changes in the dying cell (possibly allied to cytoskeletal deregulation

and changes in lateral tensile resistance) to initiate its extrusion (Rosenblatt et al., 2001). Extrusion then follows a regulated sequence of events involving the redistribution of actin and microtubule cytoskeletons within neighbouring cells to squeeze the dying cell (usually apically) via p115 RhoGEF-mediated actin contractility (Slattum et al., 2009). Fragmentation is also largely actin/myosin II driven (Mills et al., 1999; Moss and Lane, 2006), and probably aids cell engulfment (Moss and Lane, 2006) while also providing carriers for the dispersal of potent autoimmune factors (Savill et al., 2002).

Live-cell imaging of cells treated with a variety of apoptosis inducing factors suggests that surface blebbing is one of the earliest morphological features of the execution phase (Lane et al., 2005). Surface blebbing is a regulated process requiring caspase cleavage and activation of the Rho effector, ROCK1 and remodelling of the actin cytoskeleton (Coleman et al., 2001; Sebbagh et al., 2001). Its exact roles are not understood, but these may include apoptotic cell recognition, cytoplasmic mixing and energy depletion. In adherent cells, blebbing and retraction begin simultaneously, although addition of myosin II inhibitors (such as blebbistatin) prevents blebbing but does not block cell retraction, suggesting that these processes are not mutually dependent (Lane et al., 2005). Close inspection of adherent cells in isolation suggests that blebbing is biphasic (Fig. 1): the early blebbing phase begins concomitant with cell retraction, is characterised by numerous, small, dynamic blebs and lasts for around 40 minutes; the second phase is initiated after a brief pause, and is associated with fewer, large blebs that decorate the cell surface asymmetrically (Lane et al., 2005). Importantly, non-adherent cell-lines demonstrate only a single phase of blebbing, and these blebs are equivalent to the late blebs in adherent cells on account of their morphology and timing in relation to exposure of the plasma membrane inner leaflet lipid, phosphatidyl serine (PS) (Lane et al., 2005). These observations suggest that early blebbing is initiated during cell retraction and is restricted to adherent cell-types - an important consideration when comparing the apoptotic phenotype across different cell lineages. Equally, our observations that the active redistribution of membranes and organelle fragments into surface blebs correlates with late blebbing (Lane et al., 2005), suggest that it is this stage of remodelling that is important for the final stages of apoptotic cell partitioning and eventual disposal.

Organelle remodelling during the apoptotic execution phase

Several early morphological studies and a number of recent focussed investigations have provided strong evidence for a global restructuring of cellular organelles during the apoptotic execution phase (Taylor et al., 2008). For some organelles, direct links between caspase cleavage of key structural residents and membrane remodelling have been proposed (see below), although in most cases it is unclear why this occurs. One possibility is that organelle breakdown facilitates the active enrichment of organelle remnants into apoptotic surface blebs destined to bud off as apoptotic bodies (Lane et al., 2005), to aid the process of non-phlogistic corpse engulfment by accelerating phagocytosis of potential autoimmune protein moieties (e.g. (White and Rosen, 2003)). Otherwise, organelle remodelling might play a more active role in coordinating the exposure of altered surface moieties at the plasma membrane to flag the dying cell for phagocytosis. Early electron micrographs of apoptotic immune cells reveal surface

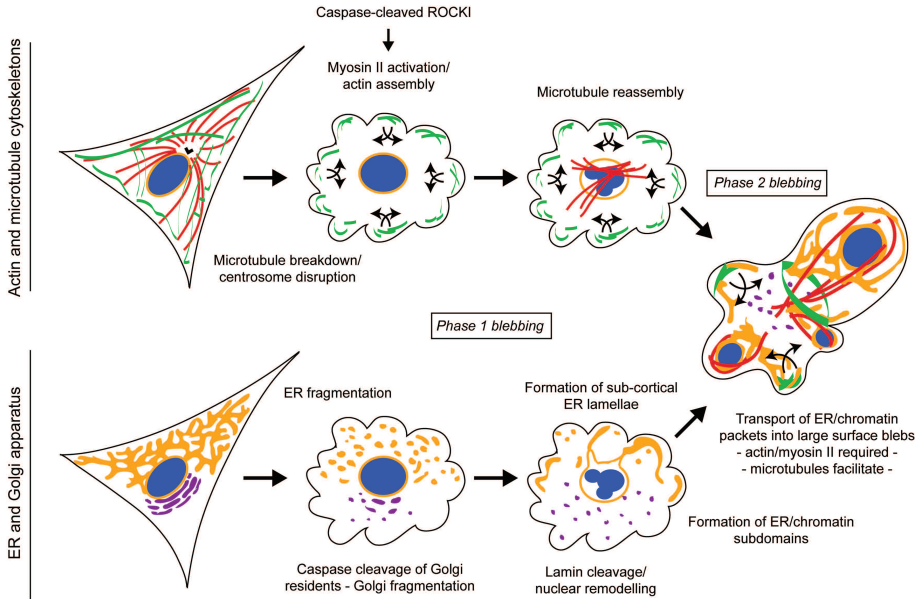

Fig. 1. Organelle and cytoskeletal rearrangements during the apoptotic execution phase. Cartoon of an epithelial cell undergoing apoptosis. Cytoskeletal components (Actin: green; microtubules: red) are depicted to the top; organelles (ER: orange; Golgi: magenta) are shown at the bottom, with a combined image of actin, microtubules and organelles in a late apoptotic cell shown to the right. Chromatin is shown in blue.

pits proposed to represent sites of localised fusion of ER membranes with the plasma membrane (Wyllie et al., 1980), while much more recent data suggest that deposition of the ER chaperone calnexin on the plasma membrane constitutes an important phagocytosis flag (Ogden et al., 2001; deCathelineau and Henson, 2003). There are several plausible mechanisms to explain the deposition of calnexin on the surface of the dying cell (Fig. 2). ER membranes might fuse with the plasma membrane, allowing direct transfer of ER-resident membrane proteins to the plasma membrane. This would likely require specialised membrane tethering and fusion factors or the deregulation of factors involved at other membrane trafficking interfaces, and would also result in the release of luminal ER proteins into the extracellular space. Direct fusion of the ER with the plasma membrane has been observed during phagocytosis, where the ER has been proposed to provide additional membrane for the expanding phagophore (Desjardins, 2003). Indeed, the involvement of the luminal ER chaperone, calreticulin, in the recognition of apoptotic cells by phagocytes (Ogden et al., 2001), is indicative of a general pathway for direct delivery of ER proteins to the plasma membrane - a process that might be enhanced during apoptosis. There is increasing evidence for unconventional secretion pathways that bypass the Golgi and in some cases involve autophagosomes and endocytic compartments (Nickel and Rabouille, 2009). Whether these pathways are upregulated or usurped during apoptosis has not been investigated.

Calreticulin is deposited at the surface of apoptotic tumour cells, and this dictates the immunogenicity of the dying cell (Panaretakis et al., 2008). Mechanistically, this requires PERK-mediated phosphorylation of the eukaryotic initiation factor 2α(eIF2α), and involves partial cleavage of the ER protein BAP31 leading to the release of calreticulin via the Golgi apparatus (Kepp et al., 2009; Panaretakis et al., 2009). This is suggestive of a regulated mechanism of protein redistribution in apoptotic cells, and is further evidence of how the execution phase constitutes a coordinated process of cellular reorganisation, although how this fits with other data showing a profound block in secretory trafficking in apoptosis is at present unclear (Lowe et al., 2004). A possible alternative process leading to the accumulation of ER proteins at the plasma membrane would be a failure to remove errant ER proteins that leak to the plasma membrane as a normal consequence of anterograde traffic (Fig. 1). There is evidence for the disruption of endocytosis early during apoptosis (Cosulich et al., 1997), although whether this would be sufficient to bring about the accumulation of mislocalised proteins at the cell surface within the allotted time is uncertain.

In viable cells, organelle positioning and morphology depend upon the underlying cytoskeleton and the actions of molecular motor proteins (e.g. (Lane and Allan, 1998)). During apoptosis, gross changes in cellular structure (blebbing, fragmentation) are driven by the cytoskeleton (Mills et al., 1999; Moss and Lane, 2006), and it is highly likely that the cytoskeleton also contributes to changes in organelle structure during the execution phase. The fates of the different cytoskeletal components differ during apoptosis, though, and their roles are altered accordingly. Intermediate filaments (cytokeratins, vimentin, nuclear lamins) are all early targets for irreversible, caspase-mediated apoptotic disassembly (Caulin et al., 1997; Byun et al., 2001), while the actin cytoskeleton is remodelled to control aspects of cellular retraction, blebbing and fragmentation (Mills et al., 1999). Microtubules undergo complex changes in stability/organisation, beginning with their disassembly early in apoptosis (Bonfoco et al., 1996; Mills et al., 1998a, 1999; Moss et al., 2006), by a process that that requires caspases (Gerner et al., 2000; Adrain et al., 2006), followed by their reformation into non-centrosomal bundles (Moss et al., 2006; Moss and Lane, 2006). Filamentous actin is remodelled during the execution phase from its typical collection of stress fibres and cross-linked cortical networks to form a

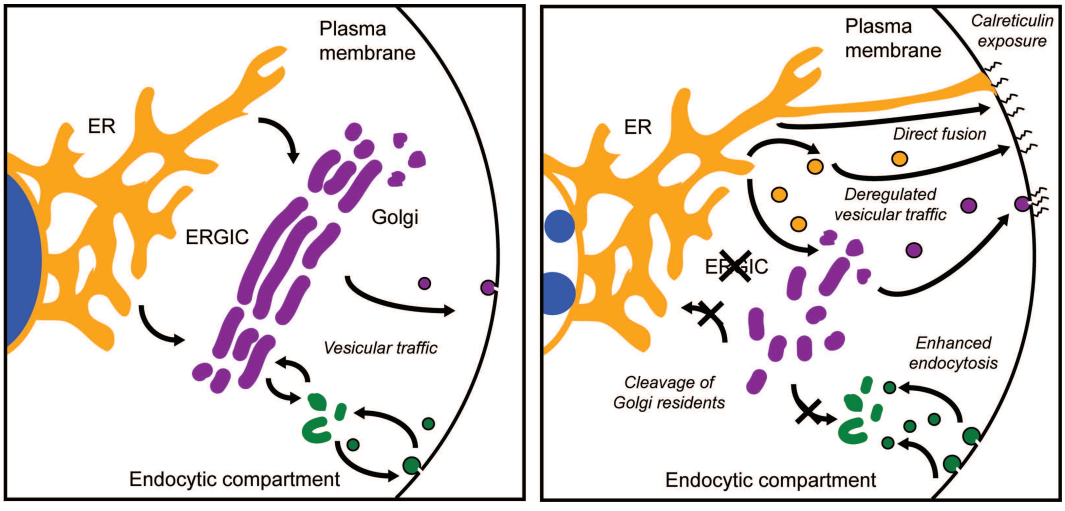

Fig. 2: Possible mechanisms for the delivery of calreticulin and other atypical proteins to the plasma membrane of an apoptotic cell. To the left, generalised membrane trafficking pathways in a healthy cell. To the right, possible outcomes of caspase-mediated organelle disruption and deregulated membrane fusion leading to calreticulin exposure.

cortical basket whose contraction is required for surface blebbing and cellular fragmentation (see below; (Mills et al., 1998b; Moss and Lane, 2006)).

Several membrane proteins that control the structure, localisation and function of organelles are targets for cleavage by caspases. In some cases, the reasons for cleavage of any given membrane protein are uncertain, whereas for others there is a direct link between protein cleavage and changes in organelle structure, distribution or function (e.g. (Ferri and Kroemer, 2001; Maag et al., 2003; Hicks and Machamer, 2005)). There is also evidence for redundancy, with caspase cleavage of several proteins in the same pathway sometimes evident (e.g. cleavage of the Golgi membrane tethers giantin (Lowe et al., 2004), p115 (Chiu et al., 2002) and GM130 (Walker et al., 2004), and of the GM130 Golgi receptor, GRASP65 (Lane et al., 2002)). Aside from assisting in wholesale cellular degradation, there is accumulating evidence for an active role for organelle disruption in apoptotic signalling (Maag et al., 2003; Hicks and Machamer, 2005). Organelles, or more accurately the cytoplasmic faces of organelle membranes can be considered to act as apoptotic signalling centres (Ferri and Kroemer, 2001; Maag et al., 2003; Hicks and Machamer, 2005), so understanding the contributions of different organelles during apoptotic signal transduction and their fates during the execution phase remains an important objective. In this section, we explore the fates of various organelles during the apoptotic execution phase, and how caspase action influences organelle structure and function.

Mitochondrial positioning and dynamics during apoptosis

Most of the known apoptotic signalling pathways within mammalian cells converge upon mitochondria. These propagate the apoptotic response through the release of downstream apoptotic signalling factors. Mitochondria have also emerged as key players in other non-apoptotic cell death processes, most notably necrosis. Meanwhile, their tendency to generate reactive oxygen species (ROS) as a normal consequence of respiration means that mitochondria impact upon cellular homeostasis and can contribute to many different diseases and accelerate the ageing process (Cuervo, 2008; Tolkovsky, 2009). Fittingly, cells have evolved mechanisms to monitor, repair and eradicate damaged or redundant mitochondria to lessen the cumulative effects of oxidative damage (Tolkovsky, 2009). Mitophagy - the process of targeted mitochondrial autophagy - selectively removes damaged mitochondria, and by reducing the effective mitochondrial mass, impacts upon the capability of cells to mount a robust apoptotic response. In healthy cells, continual mitochondrial remodelling by fusion, fission, mitophagy and new biogenesis impacts upon cellular homeostasis. Hence, evidence suggests that the status of the mitochondrial network has important consequences for the eventual fate of a cell in response to stresses. Mitochondria lose their tubulo-reticular distribution during apoptosis, becoming more fragmented (see (Perfettini et al., 2005)). Under certain pro-apoptotic stimuli, increased mitochondrial fission is observed before MOMP, and this is required for caspase activation and apoptotic execution (e.g. (Frank et al., 2001)). Correspondingly, overexpression of mitofusins increases mitochondrial fusibility and inhibits MOMP (Sugioka et al., 2004). Hence, the presence of extensive networks of interconnected mitochondria raises the apoptotic threshold, while the reverse is true in cells with fragmented mitochondria. Interestingly, recent data suggest that mitochondria can exchange content through transient "kiss-and-run" fusion events - a process that is important for overall mitochondrial homeostasis (Liu et al., 2009). Mitochondria use microtubules and their associated motor proteins for long range movements (Lane and Allan, 1998) and for productive encounters (Liu et al., 2009), meaning that disruption of the mitochondrial network as an early consequence of caspase action (Moss et al., 2006) might lower the apoptotic threshold by priming mitochondria for cytochrome c release.

In response to caspase action during the execution phase, mitochondria cluster at the perinuclear region. Why this takes place is uncertain, although it has been proposed that this process concentrates ATP generation in the cell centre or facilitates transfer of pro-apoptotic factors to the nucleus (Desagher and Martinou, 2000). In epithelial cells, mitochondria - but not the ER - are excluded from surface blebs (Lane et al., 2005), so the observed clustering may be to contain mitochondria within the body of the apoptotic cell, perhaps to limit the potential for their release from a ruptured cell corpse. Inhibition of the plus end-directed microtubule motor, kinesin via phosphorlyation of its light chains, has been reported following treatment of cells with tumour necrosis factor, and this leads to coalescence of mitochondria at the cell centre (De Vos et al., 1998, 2000). Apoptotic mitochondrial clustering downstream of caspase activation might be facilitated by the reorientation of the microtubule network during apoptosis (Bonfoco et al., 1996; Mills et al., 1998a; Moss et al., 2006), although this has not been formally demonstrated.

Remodelling of the ER during apoptosis

The ER is the largest organelle within the cell. It extends tubules and lamellae throughout the peripheral cytoplasm, and is the site for synthesis and translocation of membrane-bound and secreted proteins. It also has roles in calcium homeostasis and is the principle site for lipid synthesis. Its dynamic structure is governed by a variety of proteins including microtubule motors (Lane and Allan, 1998, 1999; Waterman-Storer and Salmon, 1998; Wozniak et al., 2009), resident microtubule binding proteins (e.g. CLIMP-63 (Klopfenstein et al., 2001; Vedrenne et al., 2005)), microtubule tip attachment complexes (TACs (Waterman-Storer et al., 1995); now known to comprise STIM1 and EB1

(Grigoriev et al., 2008)), reticulons (e.g. (Yang and Strittmatter, 2007)), and fusion factors (Vedrenne and Hauri, 2006). During the apoptotic execution phase the ER is dramatically reorganised (Sesso et al., 1999; Lane et al., 2005). In UV-induced apoptotic keratinocytes the ER is not well preserved and small ER vesicles are observed within large apoptotic surface blebs (Casciola-Rosen and Rosen, 1997), whereas in late anisomycin-treated apoptotic HeLa cells the tubulo-reticular arrangement of the ER is reasonably well maintained (Lane et al., 2005; Betin and Lane, 2007). In these cells, the ER first breaks up into large vesicles that later reform into tubules and lamella structures that abut against the underside of the plasma membrane (Lane et al., 2005). The molecular pathways underpinning this extensive reorganisation have not been described, although we do know that cytoplasmic dynein - the minus-end directed microtubule motor that contributes to microtubule-based ER motility (Allan, 1995; Lane and Allan, 1999; Wozniak et al., 2009) - and its regulator, dynactin (Allan, 2000), are both inhibited by caspase cleavage of key subunits (Lane et al., 2001).

Fragmentation of the Golgi apparatus during apoptosis

The Golgi apparatus receives material from the ER, which it modifies before dispatching to its final destination within the cell or as a secreted product. It comprises a series of stacked membrane cisternae that in mammalian cells are linked laterally to form a contiguous juxtanuclear ribbon. During mitosis the Golgi apparatus is disassembled to facilitate stoichiometric inheritance by daughter cells (Sesso et al., 1999; Lowe and Barr, 2007). This is coordinated by reversible phosphorylation of many important Golgi proteins (e.g. GM130 (Lowe et al., 2000), GRASP65 (Barr et al., 1997), GRASP55 (Xiang and Wang, 2010)), and the actions of membrane fission factors (Colanzi et al., 2007; Lowe and Barr, 2007). In mammalian cells, accurate Golgi fragmentation/partitioning is required for mitotic progression (Sutterlin et al., 2002), suggesting pathways for cells to monitor Golgi integrity during the cell cycle (Lowe and Barr, 2007). During apoptosis the Golgi is also disassembled (Sesso et al., 1999); however, unlike mitosis, this is an irreversible process that is driven by caspase cleavage of structural Golgi residents (e.g. GRASP65 (Lane et al., 2002), GM130 (Walker et al., 2004), giantin (Lowe et al., 2004), syntaxin-5 (Lowe et al., 2004), p115 (Chiu et al., 2002) and Golgin-160 (Mancini et al., 2000). Apoptotic Golgi fragments do not retain the characteristic cis-, medial-, trans-membrane asymmetry that is observed in fragmented mitotic Golgi clusters (Lane et al., 2002), suggesting a more profound process of Golgi disassembly, and these fragments are excluded from surface blebs (Lane et al., 2005). Exactly why the Golgi is dismantled to such an extent is uncertain, but one obvious consequence will be a block in productive membrane trafficking (see next Section). Whether this event contributes to the exposure of novel apoptotic surface phagocytosis flags remains untested.

Disruption of membrane trafficking pathways during apoptosis

The secretory pathway comprises sequential membrane compartments that regulate the synthesis, post-translational processing and delivery of soluble and membrane proteins to their final destinations. Endocytosis describes the uptake of soluble and plasma membrane proteins into membrane-bound compartments within the cell. These may be recycled to the plasma membrane, returned to the trans-Golgi network (TGN) or degraded within the lysosome. Maintaining the correct distribution of lipids and proteins between the organelles of the secretory and endocytic pathways is essential for cellular function and this is largely achieved through the actions of vesicular-tubular transport intermediates that traffic between compartments, and factors that control the targeting and fusion of these with acceptor membranes (e.g. Rabs, tethers and SNAREs).

During apoptosis, both the secretory and endocytic pathways are profoundly disrupted, both structurally and functionally (Lowe et al., 2004) (Figs. 1, 2). For example, the Golgi apparatus is fragmented (Lane et al., 2002), and this accompanies the loss of the ER-to-Golgi intermediate compartment determined by solubilisation of COPI membrane coats (unpublished observations). Mechanistically, secretory cargo cannot exit the ER (Lowe et al., 2004), due to caspase cleavage of many important downstream trafficking factors (including giantin (Lowe et al., 2004), GRASP65 (Lane et al., 2002), GM130 (Walker et al., 2004), p115 (Chiu et al., 2002), syntaxin 5 (Lowe et al., 2004), the intermediate chain of cytoplasmic dynein (CDIC; (Lane et al., 2001)), and the $p150^{Glued}$ subunit of the dynein regulatory complex, dynactin (Lane et al., 2001)). In addition, endosomal fusion is reduced during apoptosis due to caspase cleavage of the fusion factor rabaptin-5, leading to decreased transferrin internalisation in apoptotic HL60 cells (Cosulich et al., 1997). Hence there is widespread inhibition of regulated transport between membrane compartments, although it has been proposed that cleavage of the Golgi SNARE syntaxin-5 and removal of its auto-inhibitory domain may increase membrane fusion during apoptosis (Lowe et al., 2004). Whether this event leads to non-canonical membrane fusion (Fig. 2) has not yet been tested, although a generalised process of membrane intermixing has been proposed (Ouasti et al., 2007).

One obvious question that arises from these observations is whether an arrest in membrane traffic during cell death has any role to play beyond simple shutdown of productive cellular events. Evidence for a role for PERK and caspases in the regulated deposition of calnexin at the cell surface (Kepp et al., 2009; Panaretakis et al., 2009) strongly suggests that membrane traffic disruption has mechanistic consequences, meanwhile studies of upregulated clathrin-independent endocytosis in response to exposure to Fas ligand (FasL) (Degli Esposti et al., 2009) suggests a general shift towards membrane

internalisation as a prelude to apoptotic induction in this context (Fig. 2). Further studies are needed to dissect the roles of membrane trafficking in cell death signalling.

Roles of organelles of the secretory and endocytic pathways in apoptotic signalling

Whilst it is clear that many organelles of the secretory and endocytic endomembrane systems are disrupted during the apoptotic execution phase, there is accumulating evidence to suggest that many of these organelles play active roles in engaging apoptotic pathways upstream of their restructuring. The principal organelles involved in apoptotic signal transduction are the mitochondria which harbour many pro- and anti-apoptotic signalling factors and are triggered to release these by Bcl-2 family members to potentiate a strong apoptotic response. The links between mitochondrial function and cell death are described in detail elsewhere (Ferri and Kroemer, 2001), and will not be covered here. The ER monitors and mediates oxidative stress signalling pathways, and also interacts with some Bcl-2 proteins to determine cellular fate. Less well understood are the roles of the Golgi apparatus in apoptotic signalling and the relationships between autophagosomal and lysosomal compartments and cellular viability.

The ER and apoptosis

In addition to its known associations with mitochondria, the anti-apoptotic protein Bcl-2 has been shown to localise to the cytoplasmic face of the ER. Importantly, evidence suggests that Bcl-2 family members are important regulators of ER Ca^{2+} homeostasis. Maintaining the appropriate balance of ER Ca^{2+} ($[Ca^{2+}]_{ER}$) is essential for cellular function, and the ER can act as a Ca^{2+} buffer in cells to modulate downstream cellular responses to Ca^{2+}. Importantly, Ca^{2+} efflux from the ER lumen can trigger MOMP, meaning that the amount or duration of Ca^{2+} released during an episode of ER stress can determine the fate of a stressed cell. Significantly, Bax/Bak double knock-out mice display reduced resting $[Ca^{2+}]_{ER}$ and can thus tolerate levels of oxidative stress that would normally lead to Ca^{2+}-mediated MOMP (Scorrano et al., 2003). Evidence suggests that Bax/Bak can be antagonised by Bcl-2 at the ER, such that cells overexpressing Bcl-2 phenocopy the Bax/Bak null lines (Pinton et al., 2000). This suggests $[Ca^{2+}]_{ER}$, and therefore the strength of any cellular response to ER stress is directly controlled by the relative balance of Bcl-2 and Bax/Bak at the level of the ER.

Apoptosis can also be triggered by signalling pathways at the ER in response to a build up of misfolded proteins; a process known as the unfolded protein response (UPR). Inhibitors of glycosylation (such as tunicamycin) or drugs that block membrane traffic (such as brefeldin A) induce the UPR (Ferri and Kroemer, 2001), which either removes the unfolded proteins via retrograde translocation and proteasome action, or triggers translocation to the nucleus of ER mediators of protein translation (e.g. Ire1-b (Iwawaki et al., 2001)). These increase the transcription of stress genes such as the transcription factor CHOP/GADD153 which suppresses Bcl-2 and sensitises cells to apoptosis (McCullough et al., 2001). In addition, it has been proposed that the UPR can trigger the direct activation of caspase-12 at the ER to initiate a robust caspase cascade (Nakagawa et al., 2000). More recently, a role for the Bax inhibitor, BI-1 in UPR-mediated apoptosis has been proposed (Lisbona et al., 2009; Madeo and Kroemer, 2009). It does this by inhibiting IRE1α to influence cellular stress responses and chaperone expression (Madeo and Kroemer, 2009), further evidence for direct interplay between the ER and apoptosis signalling pathways.

The Golgi apparatus and apoptosis

The Golgi apparatus has been proposed to play a role in apoptotic signalling in response to abnormal pH variations, or to disruptions in glycosylation or lipid metabolism (see (Maag et al., 2003; Hicks and Machamer, 2005)). One pathway involves the translocation of the ceremide-derived ganglioside GD3 from the Golgi to mitochondria via vesicular intermediates and the actin and microtubules networks (Garcia-Ruiz et al., 2002). At the mitochondrial level, GD3 triggers MOMP, subsequently inducing an apoptotic response that can be inhibited by Bcl-2 overexpression (Rippo et al., 2000).

As discussed in section: Fragmentation of the Golgi apparatus during apoptosis, the Golgi apparatus is profoundly disrupted during apoptosis. This is a caspase-dependent process and fittingly expression of caspase-resistant mutants of the Golgi caspase targets GRASP65 (Lane et al., 2002), p115 (Chiu et al., 2002) and Golgin-160 (Mancini et al., 2000) delays Golgi fragmentation. At first glance, caspase cleavage of these Golgi residents might be expected to simply advance apoptotic Golgi fragmentation and cripple membrane traffic; however, there is evidence for an active role for caspase action at the Golgi in downstream apoptotic signalling. Overexpression of caspase-resistant Golgin-160 delays apoptosis in HeLa cells but only in response to cell surface death ligands or reagents that increase secretory pathway stress (Maag et al., 2005). Caspase-2 is localised to the Golgi and cleaves Golgin-160 (Mancini et al., 2000), but the mechanisms that couple caspase cleavage of Golgin-160 and resultant Golgi fragmentation to the apoptotic signalling pathways remain obscure. One plausible pathway involves the by-products of the cleavage of Golgi caspase targets acting as signalling factors. For example, caspase cleavage products of p115 (Chiu et al., 2002) and Golgin-160 (Sbodio et al., 2006) accumulate in the nucleus to propagate apoptotic signalling, but their modes of action have yet to be resolved.

Cell surface death receptor trafficking and apoptotic signalling

The coordinated transport of proteins and lipids between organelles of the secretory and endocytic systems is crucial for maintaining organelle identity and for regulating the location and signalling of many classes of receptor molecules (Miaczynska et al., 2004; van Meer and Sprong, 2004; Behnia and Munro, 2005). Of particular importance are the cell surface receptors of the tumour necrosis factor family (TNF-R). These can stimulate cell proliferation or apoptosis upon ligand binding, via proliferative MAPK and IKB signalling pathways, or via assembly of the death inducing signalling complex (DISC: containing activated receptor; adaptor, e.g. FADD; caspase-8/-10). The events determining which of these opposing signalling pathways is triggered are beginning to be understood, and a central involvement of membrane trafficking has emerged. In healthy cells, significant populations of TNF-R molecules are retained in the Golgi region at steady state (TNF-R1, for example, possesses a C-terminal TGN localisation signal (Storey et al., 2002)), and in the case of Fas/CD95, the Golgi/TGN pool can be rapidly mobilised for delivery to the cell surface to enhance the cell's sensitivity to the Fas ligand (CD178/FasL) (Bennett et al., 1998) (although the physiological significance of these data has been challenged (O'Connor and Strasser, 1999)). Recent data strongly suggest that the internalisation of receptor/ligand complexes into endocytic compartments is a key step in effective apoptotic TNF-R signalling (Schutze et al., 1999; Algeciras-Schimnich et al., 2002; Algeciras-Schimnich and Peter, 2003; Lee et al., 2006; Feig et al., 2007): ligand binding to TNF-R members at the cell surface initially favours proliferation, but subsequent receptor clustering/capping triggers internalisation of receptor/ligand complexes into endocytic compartments where DISC assembly can occur. Hence, our understanding of TNF-R function will require a complete appreciation of the transfer of receptors and receptor/ligand complexes between membrane compartments of the biosynthetic and endocytic systems. Importantly, the inherent ability to control the synthesis and trafficking of TNF-R molecules can be exploited by cells during tumourigenesis: cancer cells can down-regulate expression and/or surface presentation of their own TNF-R to evade immune surveillance (Debatin and Krammer, 2004), and can also up-regulate expression of FasL to become toxic to infiltrating immune (the "Fas counterattack" (O'Connell et al., 1999; Ryan et al., 2005)); however, the underlying mechanisms remain obscure.

Mathematical modelling suports the notion that Fas operates by a thereshod mechanism in which there is a critical ratio of FasL:Fas receptor that determines whether a cell undergoes or escapes/avoids apoptosis (Bentele et al., 2004). The principle behind this is that sufficient numbers of activated receptor are required to overcome inhibition of DISC function the anti-apoptotic protein C-FLIP, allowing for downstream autocatalytic processing of pro-caspase8. Thus one possible method of controlling apoptotic signalling is through regulating death receptor trafficking from an internal store to its site of action, or vice versa. This is exemplified by treatment of rat hepatoma cells with bile salts which promote translocation of Golgi-associated Fas receptor to the plasma membrane, thus elevating sensitivity to FasL-mediated apoptosis. In accordance to this, anti-Fas antibody tratment (which causes Fas receptor clustering and subsequent internalization) has also been shown as sufficient to drive translocation of Fas receptor from intracellular stores to the cell surface (Ungefroren et al.., 2001). In fact, Fas associated phosphatase (Fap-1) has been implicated by numerous studies t be involved in the retention of Fas at the Golgi (e.g. (Ungefroren et al., 2001; Ivanov et al., 2003)). Over-expression of Fap-1 lead to the reduction of surface expressed Fas recpetor accompanied by an increase in intracellular stores while depletion of Fap-1 resulted in the opposite (Ivanov et al., 2003). It is unclear how Fap-1 regulates Fas-mediated signal transduction, but is thought that via interacting with the cytoplasmic C-terminus of Fas receptor, it prevents its trafficing from intracellular stores to the plasma membrane (Meinhold-Heerlein et al., 2001; Ungefroren et al., 2001; Ivanov et al., 2003).

The importance of coordinated regulation of Fas receptor trafficing is highlighed by the fact that impaired Fap-1 function and/or its overexpression has been documented in many cancers that are resistance to Fas-mediated cell death (Lee et al., 1999; Elnemr et al., 2001; Meinhold-Heerlein et al., 2001; Ungefroren et al., 2001). Furthermore, mobilization of intracellular stores to potentiate the death response is not only restricted to death receptors but is also applicable to death receptor ligands. For example, in neutrophils TRAIL (TNF-related apoptosis-inducing ligand) is sequestered in intracellular secretory vesicles which cna be mobilized following exposure to pro-inflammatory factors (Cassatella et al., 2006). Furthermore, TRAIL-induced apoptosis is regulated by the expression/presence of "decoy" receptors DcR1 and DcR2 at the cell surface. These can bind death ligands, but are not capable of downstream apoptotic signalling due to the absence of an intact cytoplasmic death domain. Competitition for ligand binding between "funcional" and "decoy" receptors at the cell surface modulate TRAIL-mediated apoptosis (Ashkenazi and Dixit, 1998). Notably, immunoprecipitation studies reveal that receptor clusters can contain mixed populations of death receptors and decoys within the same complex, thereby preventing the ativation of initiator caspases independent of ligand binding (Merino et al., 2006). Interestingly, in melanoma cells (which are largely resistant to TRAIL-induced apoptosis). DR4 and DR5 are present in the TGN whereas decoy receptors DcR1 and DcR2 are nuclear localized (Cassatella et al., 2006). Internalization of DR4 and DR5 within endosomes following TRAIL apoptotic induction triggers translocation of decoy reeptors from the nucleus to the cytoplasm and cell surface conferring

resistance to TRAIL (Cassatella et al., 2006). Conversely, cells can be sensitized to TRAIL-induced apoptosis by downregulation of dcR1 and an increase in TRAIL (Screaton et al., 1997) as seen in CD8+T lymphocytes following stimulation (Mongkolsapaya et al., 1988). Together, evidence suggests it is possible that differential localization of decoy and functional receptors could allow for co-ordinated trafficking from distinct intracellular stores in response to survival or apoptotic signals though direct evidence is lacking.

Autophagy: regulation, function and crosstalk with apoptosis

Autophagy is an essential catabolic process with roles in development, homeostasis and disease. To date, four forms of autophagy have been described: macroautophagy, whereby novel double membrane-bound organelles assemble in the cytoplasm; microautophagy, which describes delivery of cytoplasm into the lysosome by membrane invagination; chaperone-mediated autophagy, in which substrates with specific targeting motifs are imported directly into the lysosome; and non-canonical/alternative (Atg5/7-independent) macroautophagy (Klionsky, 2007; Nishida et al., 2009; Klionsky and Lane, 2010). For the sake of brevity, and because it has the greatest relevance to the current topic, this section will deal solely with macroautophagy which from herein will be described simply as "autophagy".

Molecular regulation of autophagosome biogenesis

Autophagy is coordinated by a family of autophagy related proteins (Atg proteins) that control all aspects of autophagosomes biogenesis. ATG genes have been initially identified and characterised in yeast, while their mammalian homologues are being actively studied (Yang and Klionsky, 2010). In yeast, autophagosomes are assembled at a unique site known as the pre-autophagosomal structure (PAS), whilst mammalian cells instead assemble autophagosomes at multiple peripheral sites where autophagosomal isolation membranes are established. There are four subsets of core autophagy proteins that are responsible for autophagosome biogenesis: the Atg1/ULK complex; the Vps34 class III phosphatidylinositol 3-kinase complex (PI3K); parallel ubiquitin-like (Atg12 and Atg8) conjugation systems; and two trans-membrane proteins, Atg9 and VMP1 (Yang and Klionsky, 2010). The main signalling pathway that stimulates autophagy in mammalian cells is the mTOR (mammalian target of rapamycin; TORC1) pathway. This regulates the activity of Atg1, a protein kinase that in yeast exists in complex with Atg13 and Atg17. Binding of Atg13 to Atg1 and Atg17 requires Atg13 to be in its dephosphorylated form, a property that depends indirectly upon TORC1 activity (Yang and Klionsky, 2010). In mammalian cells, ULK1 and ULK2 are the suspected Atg1 orthologues, and these form a complex with Atg13 and the mammalian Atg17 orthologue, FIP200, which recruits TORC1 depending upon nutrient status (Jung et al., 2009). This system provides a mechanism to couple nutrient sensing to the autophagosome biogenesis system; however, the molecular pathways linking these processes remain unclear (Orsi et al., 2010; Yang and Klionsky, 2010).

Membrane trafficking in autophagy

The Vps34 class III PI3K operates downstream of the Atg1/ULK1 signalling complex to define the site of autophagosome biogenesis. Its role is to phosphorylate the 3' position of the inositol ring of phosphatidyl inositol (PI) to generate PI(3)P, and this triggers recruitment of effectors to initiate autophagosome assembly (Fig. 3). In mammalian cells, it is comprised of Vps34, Beclin 1 (the mammalian Atg6 homologue), and Barkor (a mammalian Atg14-like protein) (see (Orsi et al., 2010) for details). Other potential regulators of the mammalian Vps34 class III PI3K complex include UVRAG, Ambra1, Rubicon and Bif-1 (Endophilin B1). Of these, Bif-1 is an interesting player since it contains a BAR (Bin/Amphiphysin/Rvs) domain; a feature that has been implicated in sensing and conferring membrane curvature (Peter et al., 2004), and one that is needed for its autophagy roles (Takahashi et al., 2009). This perhaps provides clues as to how the remodelling of existing membrane structures might contribute to the generation of a concave autophagosomal isolation membrane (Fig. 3). Our understanding of the initiation of autophagosome assembly in mammals has benefitted greatly from studies of DFCP-1 (double FYVE domain containing protein-1); a protein that binds to PI(3)P enriched membranes thereby providing insight into the appearance and localisation of the proposed mammalian isolation membrane (Axe et al., 2008). In starved cells, DFCP-1 forms cup shaped structures called omegasomes on or adjacent to ER membranes, and these represent the sites for recruitment of PI(3)P effectors and downstream autophagy molecules such as Atg5 and LC3 (one mammalian Atg8 paralogue) (Axe et al., 2008). These observations strongly implicate the ER as a source of at least some of the membrane contributing to the nascent autophagosome. Interestingly, recent tomographic electron microscopy data support the notion that the isolation membrane is indeed established in continuity with the ER (Hayashi-Nishino et al., 2009; Yla-Anttila et al., 2009), although whether this is true for all forms of autophagy (e.g. starvation induces vs. mitophagy) remains to be clarified.

Two complementary pathways involving Atg5-12-16L and Atg8 are required for expansion and completion of the isolation membrane to form the characteristic double-membrane autophagosomal structure (Fig. 3). For each, a ubiquitin-like modification process is involved: for the first, Atg12 is covalently linked to Atg5 via the sequential actions of Atg7 (E1-like) and Atg10 (E2-like); for the second, Atg8 is a modifier of the lipid, phosphatidyl ethanolamine (PE) (here Atg7 is the E1-

like enzyme while At3 id the E2-like enzyme). To enable lipidation (PE linkage), Atg8 is first cleaved at its C-terminus by the C54 endopeptidase Atg4 to reveal a Glycine residue that is the site for attachment to PE. Interestingly, Atg4 also acts as the Atg8 delipidation enzyme (Fig. 3). Regulated Atg8 delipidation is thought to be particularly important for autophagosome formation since over expression of wild-type (Tanida et al., 2004; Betin and Lane, 2009b) or active site mutant Atg4 (Fujita et al., 2008; Betin and Lane, 2009b; Hayashi-Nishino et al., 2009) inhibits autophagosome formation or causes formation of stalled autophagosomal structures, respectively. The Atg5-Atg12 complex interacts with Atg16L to form a large multimeric complex which is somehow required for Atg8 recruitment to the nascent autophagosome. Importantly, Atg8 proteins are adapters for ubiquitin binding/sequestering proteins such as p62 and NBR1 (via conserved LIR domains), allowing misfolded protein aggregates and other ubiquitylated structures to be recruited to and sequestered within the expanding autophagosome membrane (Fig. 3) (Kirkin et al., 2009; Lamark et al., 2009).

If it is indeed true that the ER acts as the site for the initiation of autophagosome assembly in mammalian cells, it might be expected that the ER provides all of the membrane required to form an autophagosome. Interestingly, though, studies of the multi-spanning transmembrane protein Atg9 suggest that the trans-Golgi network (TGN)/Rab7/9-positive late endosomal compartment plays an active role in autophagosome expansion (see (Orsi et al., 2010)). Mammalian Atg9 cycles between juxtanuclear TGN/endosomal membranes and the sites of autophagosome assembly, becoming more dispersed upon starvation in an Ulk1-dependent manner (Young et al., 2006). One possibility is that in mammalian cells Atg9 cycles between these membrane compartments to deliver and/or retrieve factors (possibly including membrane) to/from the site of autophagosome assembly (Orsi et al., 2010; Yang and Klionsky, 2010). Also implicated are the mammalian orthologues of yeast Atg18 and Atg2, the former most

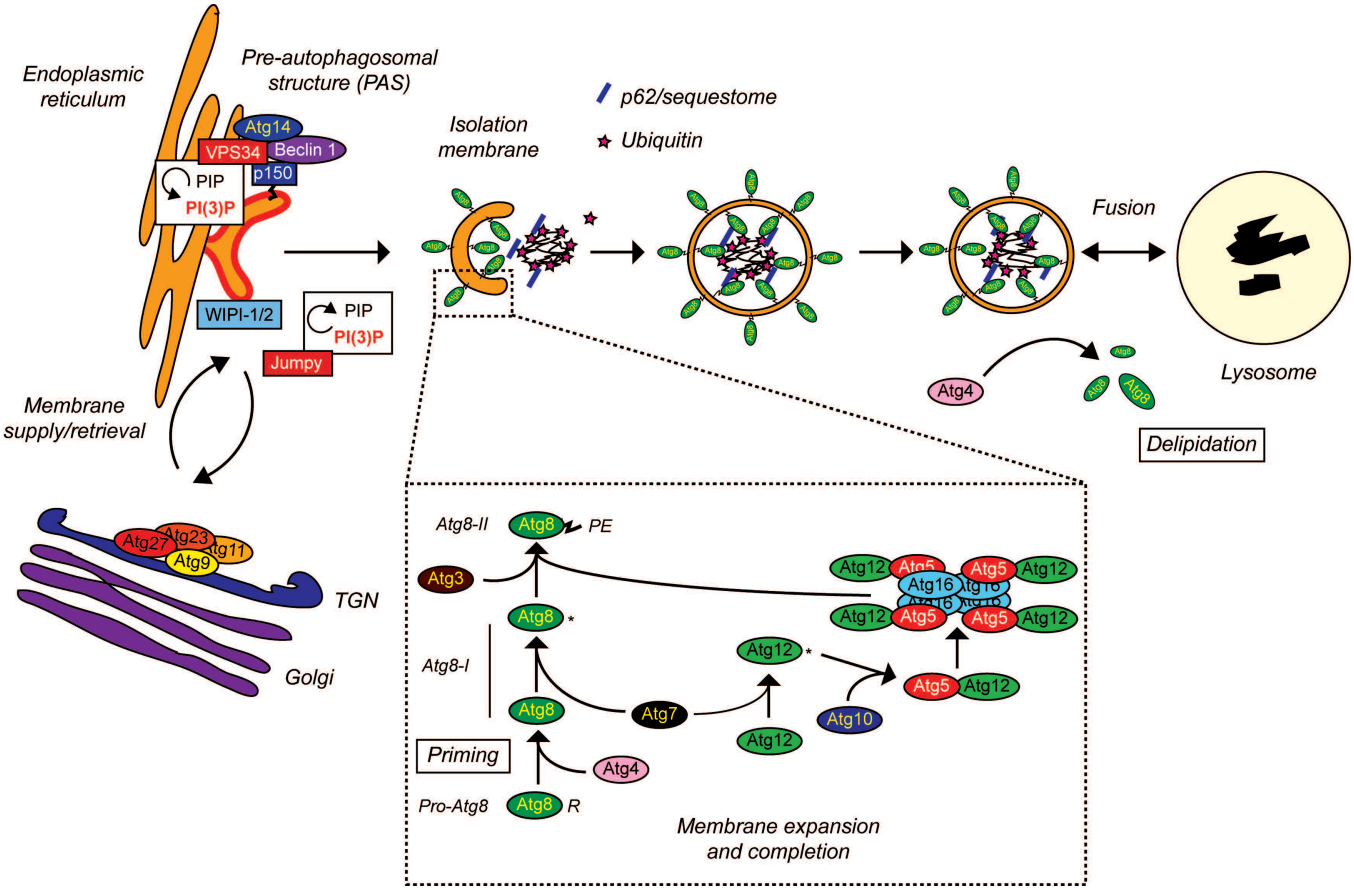

Fig. 3. Autophagosome biogenesis. Cartoon of the regulatory factors controlling autophagosome assembly. The formation of a sub-domain of the ER that is enriched in PI(3)P (the omegosome) via the actions of the Beclin 1 PI3K complex sets in motion a series of molecular interactions leading to the recruitment of the Atg5-12-16 complex and lipidated Atg8 to the nascent autophagosome (hatched box). In this example, ubiquitinylated misfolded protein aggregates are sequestered by the autophagosome via interactions between Atg8 and p62. The C54 endopeptidase Atg4 controls the lipidation status of Atg8 via its priming and delipidation activities.

likely represented by the PI(3)P effector, WIPI-1 (WD-repeat protein interacting with phosphoinositides), and the latter represented by two presumed orthologues that await characterisation (see (Longatti and Tooze, 2009)). Four models for autophagosome formation have been proposed (Longatti and Tooze, 2009), all of which require expansion and closure (fusion/hemifusion) of the autophagosomal membrane. Interestingly, Atg8 has been implicated in the process of autophagosome closure (Nakatogawa et al., 2007), suggesting that these proteins act in the expansion and completion of the nascent autophagosome (Longatti and Tooze, 2009). Once formation of the autophagosome is initiated, membrane trafficking factors allied to the endosomal system contribute to the maturation, trafficking and eventual fusion of the autophagosome with the late endosome/lysosome compartment (e.g. (Razi et al., 2009)). Rabs - Ras superfamily GTPases involved in many aspects of membrane trafficking - are implicated at several stages of autophagosome formation and maturation. Early endosomal Rab5 is required during autophagosome biogenesis (Ravikumar et al., 2008), while Rab7 is required for autophagosomal fusion with the late endosomal/lysosomal compartments (Gutierrez et al., 2004; Jager et al., 2004). Interestingly, Rab33 - a Golgi resident Rab implicated in Golgi-to-ER transport - has been shown to interact with Atg16L and to facilitate early stages of autophagosome biogenesis (Itoh et al., 2008). Clearly, much remains to be resolved about the processes of autophagosomal biogenesis, maturation and trafficking, including the fusion factors (SNARE proteins) required for mammalian autophagy.

Selective mitochondrial autophagy (mitophagy)

Autophagy can be a non-selective process for the random delivery of cytoplasm into the lysosomal system; however, work on the p62/NBR1-dependent clearance of ubiquitinylated protein aggregates (e.g. (Lamark et al., 2009)) and the removal of damaged mitochondria (Kim et al., 2007; Tolkovsky, 2009) demonstrates that autophagy can be selective. Our understanding of the pathways that control the selective removal of damaged mitochondria by autophagy (mitophagy) has greatly improved in recent years. Mitophagy has emerged as an extremely important process for the prevention of diseases that result from the cumulative effects of reactive oxygen species (ROS) generated by dysfunctional mitochondria (Kim et al., 2007; Tolkovsky, 2009). In theory, continual removal/replenishment of aged, damaged or redundant mitochondria can protect organisms from the effects of ageing and from diseases such as neurodegeneration and cancer (Kim et al., 2007; Cuervo, 2008; Tolkovsky, 2009). Yeast genetics was recently used to identify candidate genes involved in mitophagy. Two groups identified Atg32 as a gene required for autophagy in yeast (Kanki et al., 2009; Okamoto et al., 2009); however, there is no direct homologue in mammalian cells. Instead, two independent pathways are thought to be involved in mitophagy in different mammalian cell-types. Parkin, an ubiquitin E3 ligase linked to Parkinson's disease, is a cytosolic factor that is recruited to uncoupled mitochondria, triggering their selective elimination (Narendra et al., 2008). Parkin recruitment is dependent upon the protein kinase PINK1 (Geisler et al., 2010; Vives-Bauza et al., 2010), and evidence suggests that one key substrate for its E3 ligase activity is the voltage-dependent anion channel (VDAC1) (Geisler et al., 2010). During erythropoiesis, removal of mitochondria from the nascent reticulocyte is an essential step for the generation of viable cells (Mortensen et al., 2010), and a pathway involving the mitochondrial protein BNIP3/Nix is required (Novak et al. 2010; Schweers et al., 2007; Sandoval et al., 2008; Zhang and Ney, 2008, 2009; Schwarten et al., 2009). Nix binds to the Atg8 orthologue, GABARAP-L1 with particularly high affinity via its LIR domain, and in doing so triggers recruitment of the autophagosome biogenesis machinery to damaged mitochondria (Novak et al., 2010). How the structure/properties of Nix are altered to engage this process remains undetermined.

Molecular interplay between autophagy and apoptosis

The first evidence for molecular crosstalk between autophagy and apoptosis came from studies of the haploinsufficient tumour suppressor Beclin 1. It has emerged that Beclin 1 binds to anti-apoptotic Bcl-2 and Bcl-X_L by virtue of its Bcl-2 homology domain 3 (BH3 domain); an interaction that can be disrupted by BH3-only Bcl-2 family members and BH3 mimetic drugs (Pattingre et al., 2005; Maiuri et al., 2007a,b). These observations are highly significant because they suggest that the Beclin 1/Bcl-2(Bcl-X_L) interaction constitutes an apoptosis/autophagy rheostat: simply put, Bcl-2(Bcl-X_L) inhibits autophagy by sequestering Beclin 1; meanwhile pro-apoptotic BH3-only proteins have the capacity to trigger autophagy by competing for Bcl-2(Bcl-X_L) (Maiuri et al., 2007a). This is perhaps one explanation as to why many pro-apoptotic stimuli concomitantly trigger autophagy in cultured cell-lines, and is a plausible reason for the prevalence of autophagic structures in dying cells. To add to the complexity, it has recently been demonstrated that Beclin 1 is itself a caspase target whose cleavage renders it both incapable of regulating autophagy and pro-apoptotic (Wirawan, 2010) (Luo and Rubinsztein, 2009; Djavaheri-Mergny et al. 2010). Intriguingly, proteolysis of several autophagy proteins has now been reported, leading to different autophagy and cell death responses. In flies, several apoptosis genes - including caspase homologues - are required for developmentally regulated autophagy (Martin and Baehrecke, 2004; Hou et al., 2008), suggesting that in flies apoptosis and autophagy are strongly connected at the molecular level. Atg5 undergoes calpain-mediated cleavage in mammalian cells treated with staurosporine, a process that inhibits autophagy while concomitantly releasing a pro-apoptotic

Atg5 fragment that is reported to interact with Bcl-X_L at the mitochondrial surface to trigger apoptosis (Yousefi et al., 2006). Cell death mediated by Atg5 fragments may also involve an interaction with Fas-associated protein with a death domain (FADD), although in this case the mode of cell death appears to be upregulation of autophagy rather than apoptosis (Pyo et al., 2005). Caspase-3 mediated cleavage of human Atg4D has also been demonstrated (Betin and Lane, 2009b). Here, cleavage stimulates Atg4D endopeptidase action by removal of an autoinhibitory N-terminal domain, and is proposed to stimulate autophagy (Betin and Lane, 2009a, b). Interestingly, Atg4D is itself cytotoxic, and its cell death-inducing capabilities are linked to its recruitment to mitochondria and the presence of a C-terminal BH3-like domain (Betin and Lane, 2009a,b), further demonstrating how apoptosis and autophagy pathways converge.

Concluding remarks

Regulated cell death mechanisms are essential facets of developing organisms and are vital for tissue homeostasis. Indeed, many diseases are known to result from inadequate or inappropriate stimulation of cell death in tissues. Evidence is emerging that the involvement of cellular organelles and membrane trafficking pathways during the major physiological cell death mechanism, apoptosis, extend beyond simple bystander roles. Mitochondria are well-established mediators of apoptosis (and indeed other cell death mechanisms), whilst the roles of the ER and the Golgi apparatus in apoptotic signalling are being unravelled. One membrane trafficking process that impacts directly upon cell death/survival is autophagy. Once considered a death mechanism in its own right, it is now becoming apparent that autophagy contributes to apoptosis signalling at multiple levels, further demonstrating how the molecules involved in organelle remodelling and organelles themselves can dictate the fate of a given cell. As our appreciation of the roles and fates of cellular organelles during cell death signalling advances, our understanding of the control of cell death in health and disease will improve. With this will emerge novel strategies to control cell survival/death in the context of disease.

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