Apoptosis: controlled demolition at the cellular level

Rebecca C. Taylor, Sean P. Cullen and Seamus J. Martin

Abstract | Apoptosis is characterized by a series of dramatic perturbations to the cellular architecture that contribute not only to cell death, but also prepare cells for removal by phagocytes and prevent unwanted immune responses. Much of what happens during the demolition phase of apoptosis is orchestrated by members of the caspase family of cysteine proteases. These proteases target several hundred proteins for restricted proteolysis in a controlled manner that minimizes damage and disruption to neighbouring cells and avoids the release of immunostimulatory molecules.

Phagocyte
A cell that can engulf and ingest foreign material, such as an apoptotic cell corpse, which then undergoes digestion within lysosomes.

Caspase
One of a family of proteases that have an essential Cys residue in their active site and a requirement for an Asp residue in the substrate cleavage site. Initiator caspases are typically activated in response to particular stimuli, whereas effector caspases are particularly important for the ordered dismantling of vital cellular structures.

Apoptotic body
During apoptosis, cells collapse into small intact fragments that exclude vital dyes. Such fragments are termed apoptotic bodies.

Molecular Cell Biology Laboratory, Department of Genetics, The Smurfit Institute, Trinity College, Dublin 2, Ireland.
Correspondence to S.J.M.
e-mail: martinsj@tcd.ie
doi:10.1038/nrm2312
Published online
12 December 2007

Apoptosis is a mode of cell death that is used by multicellular organisms to dispose of unwanted cells in a diversity of settings[1,2]. In many ways, what happens during apoptosis is akin to how large buildings are demolished to make way for new developments. During demolition, it is important that the process is carried out in a safe and controlled manner to ensure that neighbouring structures remain unaffected. To achieve this, a specialized demolition squad is called in and, all being well, these experts carry out the task in a precise and highly efficient manner. After demolition has been completed, the debris is removed and a new structure takes the place of the old one within a short time.

Similar to the scenario outlined above, cells that undergo apoptosis are also dismantled from within, in a controlled manner that minimizes damage and disruption to neighbouring cells[1]. The resulting cellular debris is then removed, typically by professional phagocytes[1], and a new cell typically takes the place of the old one in a matter of hours. Here, we focus on the events that take place during the demolition phase of apoptosis that result in the controlled dismantling of a range of key structures within the cell and its subsequent disposal. These events are orchestrated primarily, but not exclusively, by members of a family of cysteine proteases known as caspases[3,4].

Cellular demolition: a broad perspective

So what actually happens during apoptosis? From the outside, it appears that cells that undergo this form of cell death initially become rounded and retract from neighbouring cells, which is reminiscent of what also happens when cells undergo mitosis (FIG. 1a). This is accompanied, or followed closely, by a prolonged period of dynamic plasma membrane blebbing, which frequently culminates in the 'pinching off' of many of these blebs as small vesicles that have been named apoptotic bodies (FIG. 1a; Supplementary information S1 (movie)).

For reasons that will be discussed below, cells that undergo apoptosis are readily recognized as being different from their viable counterparts and are rapidly engulfed by phagocytes for recycling of their contents[1,2,5]. This event is particularly remarkable when it is considered that phagocytes are normally engaged in the business of recognizing and removing foreign, or 'non-self', entities. However, in this instance, the dying cell becomes licensed for removal, despite having been a part of 'self' only minutes earlier. Indeed, for controlled cell elimination to operate, the minimum requirement seems to be for the chosen cell to undergo changes that attract the attentions of phagocytes. However, apoptotic cells also exhibit many other alterations to their internal architecture, which probably increase the efficiency of the disposal process and minimize activation of the immune system.

Looking inside the cell, one of the most noticeable features of apoptosis is condensation of the nucleus and its fragmentation into smaller pieces[1,2,6] (FIG. 1b), a highly distinctive event that is not seen under any other circumstances. Indeed, early studies on apoptosis relied heavily on this characteristic of apoptotic cells for their identification in the absence of specific molecular markers[1,2,6,7]. Another defining characteristic of this mode of cell death is the extensive hydrolysis of nuclear DNA into numerous fragments, often down to multiples of 200 bp[8]. Although more subtle, the Golgi, endoplasmic reticulum (ER) and mitochondrial networks also undergo pronounced fragmentation during apoptosis, and numerous proteins are released from the mitochondrial intermembrane

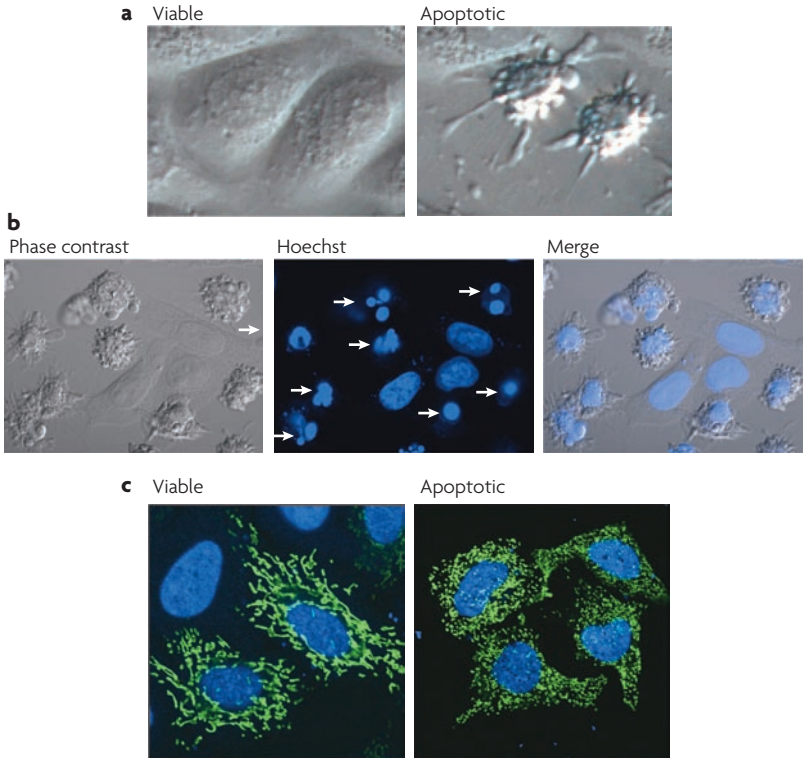


Figure 1 | **Morphology of apoptosis. a** | The morphology of apoptotic cells. HeLa cells were induced to die by exposure to daunorubicin (10 μM) for ~12 hours. The left panel shows cells after 2 h of exposure, when both cells in the field of view still appear healthy. By ~4.5 h (right panel), both cells display typical apoptotic morphology, with cell retraction and dynamic plasma membrane blebbing being evident. The original movie that these frames were taken from can be viewed in Supplementary information S1 (movie). **b** | Features of apoptosis-associated nuclear condensation and fragmentation. HeLa cells were induced to undergo apoptosis by treatment with actinomycin D (5 μM) followed by staining of nuclei (blue) with Hoechst dye. For comparison, a mixture of viable and apoptotic cells is shown. Apoptotic cells (arrows) exhibit extensive plasma membrane blebbing and contain nuclei that are condensed and/or fragmented into several pieces. **c** | Fragmentation of mitochondrial networks. To visualize mitochondria, cells were transfected with a mitochondrially targeted green fluorescent protein construct, and then either left untreated (left panel) or treated with 5 μM actinomycin D for 12 h to induce apoptosis (right panel). Note that the mitochondrial network is extensively interconnected and appears filamentous in viable cells. By contrast, mitochondrial networks become highly fragmented in the early stages of apoptosis. Images in parts **b** and **c** courtesy of P. Delivani, Trinity College, Dublin, Ireland.

space[9,10]. This feature has special relevance for how cellular demolition is initiated because one of these intermembrane space proteins, cytochrome *c*, can trigger the assembly of a caspase-activating complex (called the apoptosome) on release into the cytosol (FIG. 2).

At the level of the proteome, literally hundreds of proteins undergo restricted proteolysis during apoptosis (see the caspase substrate database for a comprehensive list of caspase substrates)[11]. Members of the caspase family of proteases carry out much of this proteolysis and indeed, most of the events described above are initiated as a result of caspase-mediated cleavage of particular substrate proteins. However, it is curious that so many proteins are targeted for proteolysis by caspases during apoptosis because the relevance of most of these proteolytic events remains unclear.

Apoptosome
A large protein complex that comprises cytochrome *c* and apoptotic protease-activating factor-1 (APAF1), and forms in the presence of ATP or dATP. The apoptosome recruits pro-caspase-9 and results in the allosteric activation of caspase-9.

Death, danger and the hidden self

Before we discuss the details of apoptosis-associated cell demolition, it is worth considering the consequences of failing to control the process of cell death and disposal. It has long been recognized that necrosis (that is, uncontrolled cell death) is accompanied by a rapid loss of membrane integrity and the release of cellular contents into the extracellular space[1,2]. Apart from the damage to neighbouring cells that such release may provoke, there is also ample evidence to suggest that the immune system responds to apoptotic and necrotic cells in fundamentally different ways[12–14].

Necrotic cells almost invariably trigger inflammation by neutrophils, macrophages and other cells of the innate immune system, and recent evidence suggests that this is caused by the release of molecules (collectively called danger-associated molecular patterns (DAMPs) or alarmins) that stimulate one or more pattern-recognition receptors on macrophages, dendritic cells and natural killer cells[15,16]. Stimulation of pattern-recognition receptors on cells of the innate immune system, particularly dendritic cells, equips these cells with the ability to activate T cells productively and to initiate immune responses[17]. Thus, the presence of necrotic cells in a tissue is frequently interpreted by the immune system as dangerous and therefore acts as a signal to initiate an immune response[18].

In biological terms, immune responses are costly and can lead to further cell death and persistence or even escalation of the initial damage. The idea that necrotic cell death alerts the immune system to danger is an appealing one, not only because many pathogens provoke necrotic cell death, but also because many types of trauma that provoke necrosis (for example, burns, cuts and compression injuries) also facilitate infection.

The full spectrum of molecules that are released by necrotic cells and can engage pattern-recognition receptors on dendritic cells still await definition, but early indications suggest that these include the high mobility group protein B1 (HMGB1), uric acid, certain heat shock proteins, single-stranded RNA and genomic DNA[14,15,19]. Many more endogenous alarmins probably await discovery, and this is an active area of investigation at present. What these alarmins have in common is that they represent 'hidden self' because these molecules are not normally found in the extracellular space. Thus, their presence in this compartment is indicative of a departure from normality. Because apoptotic cells are equipped with plasma membrane alterations (see below) that facilitate their rapid removal from tissues before rupture and release of their cytoplasmic contents[5], such cell deaths typically do not attract the attention of innate immune cells.

Therefore, apart from limiting direct cell damage due to release of cytoplasmic contents, one of the major benefits of controlled cell death by apoptosis may be to prevent the unmasking of hidden self, thereby preventing unwanted immune responses. Where this has been investigated, it has been found that alarmins such as HMGB1, genomic DNA and heat shock proteins are typically not released from apoptotic cells unless they

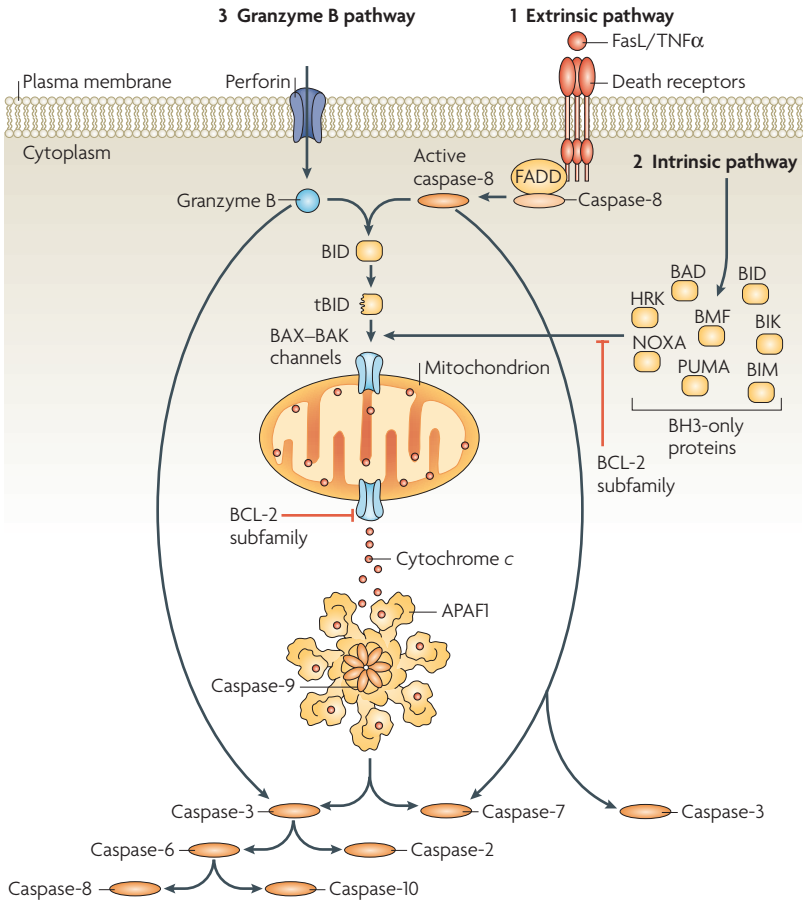


Figure 2 | **Caspase activation pathways.** Caspase activation by the extrinsic pathway (route 1) involves the binding of extracellular death ligands (such as FasL or tumour necrosis factor-α (TNFα)) to transmembrane death receptors. Engagement of death receptors with their cognate ligands provokes the recruitment of adaptor proteins, such as the Fas-associated death domain protein (FADD), which in turn recruit and aggregate several molecules of caspase-8, thereby promoting its autoprocessing and activation. Active caspase-8 then proteolytically processes and activates caspase-3 and -7, provoking further caspase activation events that culminate in substrate proteolysis and cell death. In some situations, extrinsic death signals can crosstalk with the intrinsic pathway through caspase-8-mediated proteolysis of the BH3-only protein BID (BH3-interacting domain death agonist). Truncated BID (tBID) can promote mitochondrial cytochrome *c* release and assembly of the apoptosome (comprising ~7 molecules of apoptotic protease-activating factor-1 (APAF1) and the same number of caspase-9 homodimers). In the intrinsic pathway (route 2), diverse stimuli that provoke cell stress or damage typically activate one or more members of the BH3-only protein family. BH3-only proteins act as pathway-specific sensors for various stimuli and are regulated in distinct ways (BOX 1). BH3-only protein activation above a crucial threshold overcomes the inhibitory effect of the anti-apoptotic B-cell lymphoma-2 (BCL-2) family members and promotes the assembly of BAK–BAX oligomers within mitochondrial outer membranes. These oligomers permit the efflux of intermembrane space proteins, such as cytochrome *c*, into the cytosol. On release from mitochondria, cytochrome *c* can seed apoptosome assembly. Active caspase-9 then propagates a proteolytic cascade of further caspase activation events. The granzyme B-dependent route to caspase activation (route 3) involves the delivery of this protease into the target cell through specialized granules that are released from cytotoxic T lymphocytes (CTL) or natural killer (NK) cells. CTL and NK granules contain numerous granzymes as well as a pore-forming protein, perforin, which oligomerizes in the membranes of target cells to permit entry of the granzymes. Granzyme B, similar to the caspases, also cleaves its substrates after Asp residues and can process BID as well as caspase-3 and -7 to initiate apoptosis. BAD, BCL-2 antagonist of cell death; BAK, BCL-2-antagonist/killer-1; BAX, BCL-2-associated X protein; BID, BH3-interacting domain death agonist; BIK, BCL-2-interacting killer; BIM, BCL-2-like-11; BMF, BCL-2 modifying factor; HRK, harakiri (also known as death protein-5); PUMA, BCL-2 binding component-3.

appear in numbers that overwhelm the ability of phagocytes to dispose of them quickly[14,19]. Thus, what happens to a cell as it undergoes apoptosis may be as much geared towards preventing inappropriate immune activation as it is towards terminating the life of the cell.

Caspases: agents of demolition

Caspases are normally present in healthy cells as inactive precursor enzymes (zymogens) with little or no protease activity[3,4,20,21] (BOX 2). However, all stimuli that trigger apoptosis seem to do so by initiating events that culminate in caspase activation, albeit in somewhat different ways. To date, three main routes to apoptosis-associated caspase activation have been firmly established in mammals[4,22] (FIG. 2), and one or two other possible routes have been proposed but remain controversial. Irrespective of the actual route to caspase activation, all pathways lead to the activation of the major effector caspases, caspase-3, caspase-6 and caspase-7, and these enzymes carry out much of the proteolysis that is seen during the demolition phase of apoptosis.

Weakening key structures

Just as it is important to weaken the major structural components of a building, such as load-bearing walls, when carrying out its controlled demolition — a task often achieved through the strategic positioning of small explosive charges at key points along these structures — a similar strategy seems to be in operation during apoptosis. Here, the caspases take the place of explosives and their actions undermine the fabric of several key cellular structures (FIG. 3).

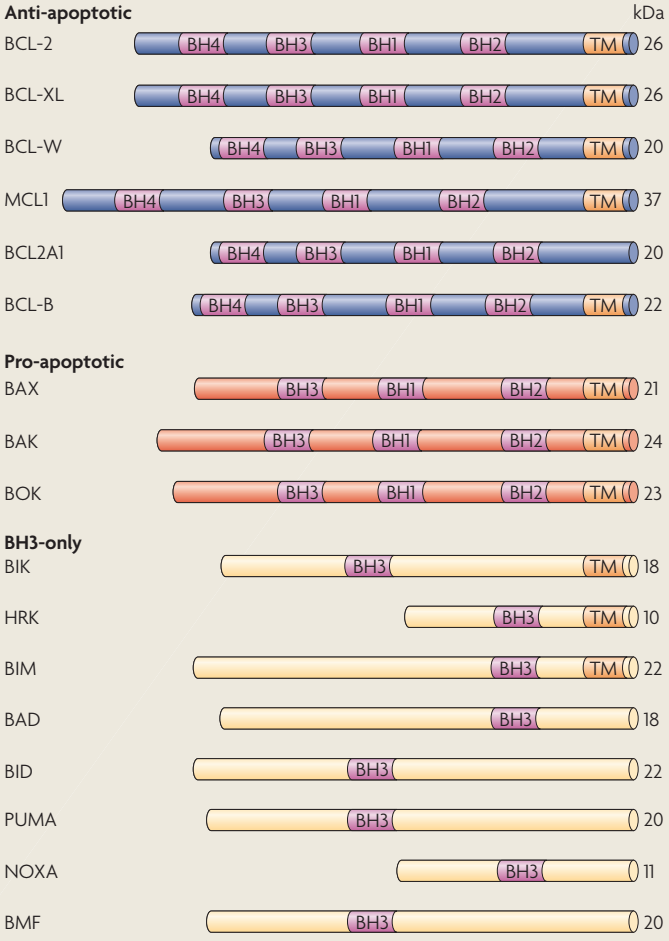
Components of the cytoskeleton. On activation, caspases cleave many of the major constituents of the cell cytoskeleton. These substrates include components of actin microfilaments, such as actin itself, and actin-associated proteins, including myosin, spectrins, α-actinin, gelsolin and filamin[23–28]. Several microtubular proteins are also substrates for caspases, including tubulins and microtubule-associated proteins such as tau, cytoplasmic dynein intermediate chain and p150^{Glued} (REFS 29–31). In addition, intermediate filament proteins such as vimentin, keratins and nuclear lamins are also targeted[32–34]. Proteolysis of these cytoskeleton constituents probably contributes to the rounding and retraction of the cell that is seen in the early stages of apoptosis (FIG. 1a).

Another consequence of weakening of the cytoskeleton is likely to be dynamic membrane blebbing (a distinctive feature of apoptosis) as the cell cytoplasm flows against unsupported areas of the plasma membrane. Indeed, dissolution of the actin network typically abrogates this feature of apoptosis, so it seems that membrane blebbing requires at least some intact actin filaments[35]. Pharmacological inhibitors of caspases efficiently block apoptosis-associated plasma-membrane blebbing.

A caspase substrate that is strongly implicated in this phase of apoptosis is the Rho effector ROCK1, a regulator of actin cytoskeleton dynamics. Removal of the C-terminus of ROCK1 through caspase-mediated proteolysis results in constitutive activation of this kinase,

Box 1 | The BCL-2 family

B-cell lymphoma-2 (BCL-2)-family proteins have a crucial role in the regulation of apoptosis through their ability to regulate mitochondrial cytochrome *c* release. The BCL-2 family comprises three subfamilies that contain between one and four BCL-2 homology (BH) domains (see figure). The anti-apoptotic subfamily comprises proteins that contain four BH domains. Most members of this subfamily also contain transmembrane domains (TM) and are therefore typically associated with membranes. The pro-apoptotic BAX-like subfamily lacks BH4 domains and promotes apoptosis by forming pores in mitochondrial outer membranes. The BH3-only subfamily is a structurally diverse group of proteins that only display homology within the small BH3 motif.



BH3-only proteins

The mammalian BH3-only protein family currently comprises eight members (BID, BAD, BIM, BIK, BMF, NOXA, PUMA and HRK), all of which promote apoptosis when overexpressed. These proteins share little sequence homology apart from the BH3 motif, and are regulated in distinct ways.

NOXA, PUMA and, to a lesser extent, BID are transcriptionally upregulated by p53. DNA damage therefore results in the increased synthesis of these BH3-only proteins in a p53-dependent manner. BID is activated through proteolysis by caspase-8. BAD is inactivated through growth-factor-receptor signals that result in phosphorylation of this BH3-only protein at several sites, leading to sequestration by 14-3-3 proteins. BIM is tethered to microtubules and BMF is tethered to actin microfilaments, possibly through interactions with dynein light chain-1 and dynein light chain-2, respectively, although this remains to be conclusively demonstrated. Disruption of the cytoskeleton can liberate these proteins to promote apoptosis. Growth factors stimulate the ERK-dependent phosphorylation of BIM, and this results in proteasome-mediated degradation of this BH3-only protein. Conversely, growth factor withdrawal results in a decline in ERK signalling and elevated levels of BIM.

Anti-apoptotic BCL-2-family members

BCL-2 and its close relatives (BCL-XL, MCL1, BCL2A1 , BCL-W and BCL-B) have four BCL-2 homology (BH) domains and all block apoptosis. The anti-apoptotic BCL-2 proteins block apoptosis by preventing BH3-only protein-induced oligomerization of the pro-apoptotic BCL-2-family members BAX and/or BAK in mitochondrial outer membranes, which would otherwise lead to the efflux of cytochrome *c* and other mitochondrial intermembrane space proteins. The anti-apoptotic BCL-2 proteins differentially bind to the BH3-only proteins. Some BH3-only proteins (for example, BID and BIM) interact with essentially all anti-apoptotic BCL-2 proteins, whereas others (for example, NOXA) interact only with certain BCL-2-family members.

BAD, BCL-2 antagonist of cell death; BAK, BCL-2-antagonist/killer-1; BAX, BCL-2-associated X protein; BID, BH3-interacting domain death agonist; BIK, BCL-2-interacting killer; BIM, BCL-2-like-11; BMF, BCL-2 modifying factor; BOK, BCL-2-related ovarian killer; ERK, extracellular signal-regulated kinase; HRK, harakiri (also known as death protein-5); PUMA, BCL-2 binding component-3.

Granzyme B
A Ser protease contained within the secretory granules of cytotoxic lymphocytes and natural killer cells. Granzyme B cleaves its protein substrates after Asp residues, and can promote caspase activation and apoptosis.

Myosin
One of a family of actin-associated motor proteins that bind to actin fibres by their head domain and use ATP hydrolysis to move along fibres. The tail domain of myosin associates with cargo or other myosin molecules.

Nuclear lamin
One of a family of intermediate filament proteins that form the proteinaceous nuclear lamina structure, which surrounds the nucleus.

ROCK1
An effector of the small GTPase Rho, ROCK1 is a kinase that can phosphorylate myosin light chain, which alters the dynamics of the actin cytoskeleton.

14-3-3 protein
One of a family of small phosphopeptide-binding dimeric proteins that typically act as adaptors or localize components of signalling pathways.

leading to myosin light chain phosphorylation and contraction of actin bundles[36,37] (FIG. 3). Therefore, the extensive membrane blebbing that is seen during apoptosis might result from myosin-dependent contraction of cortical bundles of actin, pushing the cytosol against other areas of the cell cortex and causing blebs in areas where the cytoskeleton has been weakened.

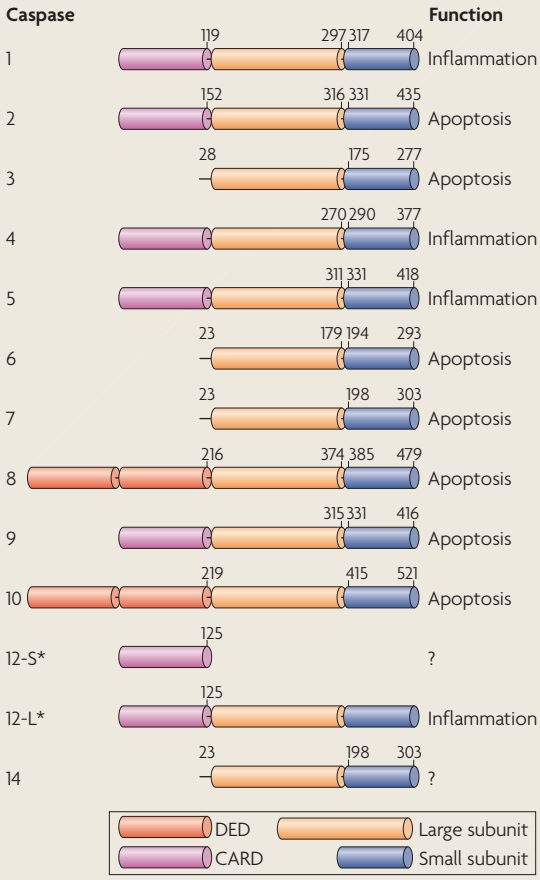
Interestingly, the actin–myosin network has also been suggested to have an important role in the removal of apoptotic cells from epithelia[38]. In the early stages of apoptosis, dying epithelial cells stimulate their neighbours to form a network of actin–myosin cables and these effectively extrude the apoptotic cell from the epithelium as the cables contract. This process permits

Box 2 | **The caspases**

Caspases (cysteine aspartic acid-specific proteases) are highly specific proteases that cleave their substrates after specific tetrapeptide motifs (P4-P3-P2-P1) where P1 is an Asp residue. The caspase family can be subdivided into initiators, which are able to auto-activate and initiate the proteolytic processing of other caspases, and effectors, which are activated by other caspase molecules. The effector caspases cleave the vast majority of substrates during apoptosis.

All caspases have a similar domain structure comprising a pro-peptide followed by a large and a small subunit (see figure). The pro-peptide can be of variable length and, in the case of initiator caspases, can be used to recruit the enzyme to activation scaffolds such as the APAF1 apoptosome. Two distinct, but structurally related, pro-peptides have been identified; the caspase recruitment domain (CARD) and the death effector domain (DED), and these domains typically facilitate interaction with proteins that contain the same motifs. Caspase activation is usually initiated through proteolytic processing of the caspase between the large and small subunits to form a heterodimer. This processing event rearranges the caspase active site into the active conformation. Caspases typically function as heterotetramers, which are formed through dimerization of two caspase heterodimers. Initiator caspases exist as monomers in healthy cells, whereas effector caspases are present as pre-formed dimers.

Not all mammalian caspases participate in apoptosis. For example, caspase-1, caspase-4, caspase-5 and caspase-12 are activated during innate immune responses and are involved in the regulation of inflammatory cytokine processing (for example, IL1β and IL18). Interestingly, caspase-12 is expressed as a truncated, catalytically inactive protein in most humans (caspase-12S*). However, a subset of individuals of African descent express full-length caspase-12 (caspase-12L*) and these individuals appear to be more susceptible to inflammatory diseases. To date, ~400 substrates for the mammalian caspases have been identified, but the significance of many of these cleavage events remains obscure.



surrounding neighbouring cells to fill the gap left by the dead cell and to maintain epithelial integrity[38]. The nature of the signal(s) emanating from the dying cell that trigger the formation of actin cables within its neighbours remain unclear, although this process appears to be caspase independent[38].

The nuclear envelope and nuclear fragmentation. Although nuclear fragmentation is a major hallmark of apoptosis[1,2], it remains unclear why the nucleus fragments and disperses throughout the cell body during this mode of cell death. This may contribute to the efficient removal of potentially immunogenic chromatin, as mentioned above, and to the irreversibility of the death process.

Nuclear fragmentation relies on the disintegration of the nuclear lamina and the collapse of the nuclear envelope. The first of these events involves the proteolysis of lamins A, B and C by caspases[39]. The expression of mutant forms of lamins, which are resistant to caspase-mediated proteolysis, can prevent the loss of laminar integrity and delay the onset of DNA fragmentation[39]. Although several other nuclear membrane and laminar proteins have been identified as caspase substrates, none of these proteolytic events has been convincingly linked to nuclear fragmentation[11].

The actin cytoskeleton also has a role in nuclear fragmentation. The nuclear lamina is surrounded by a mesh of actin, which is associated with the nuclear envelope. Inhibition of either ROCK1, myosin light chain kinase or disruption of actin filaments prevents apoptosis-associated nuclear fragmentation[40]. The loss of the C terminus of ROCK1 through caspase-mediated proteolysis (as described above) constitutively activates this kinase and provokes reorganization of the actin–myosin system[36,37] (FIG. 3). Because of the attachments between the actin cytoskeleton and the nuclear envelope, this literally tears the nucleus apart during apoptosis[40]. Lamin cleavage alone is not sufficient to cause nuclear fragmentation in the absence of the contractile force of the actin cytoskeleton but might weaken the nuclear lamina, allowing the nuclear envelope to tear[40]. On fragmentation of the nucleus, the microtubule-based cytoskeleton has been implicated in the dispersal of nuclear fragments into plasma membrane blebs[41].

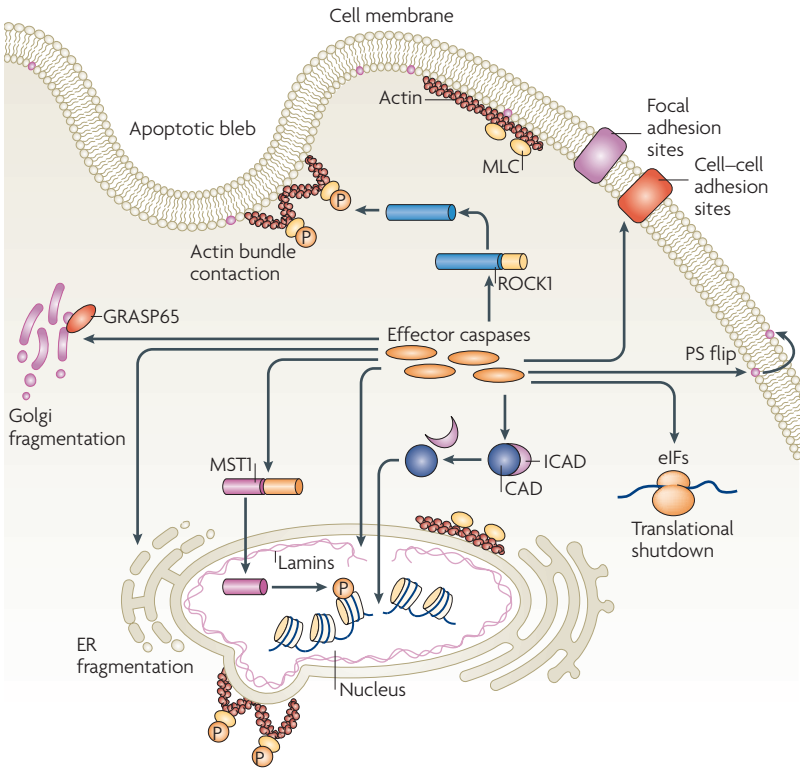


Figure 3 | **Caspases coordinate demolition of key cellular structures and organelles.** Effector caspases (such as caspase-3, -6 and -7 in mammals) orchestrate the dismantling of diverse cell structures through cleavage of specific substrates. Collectively, these proteolytic events produce the phenotypic changes to the cell that are characteristic of apoptosis, and some examples are shown here. Cleavage of ICAD (inhibitor of caspase-activated DNase) releases CAD (caspase-activated DNase), which can then catalyse inter-nucleosomal DNA cleavage. Caspase-mediated cleavage of nuclear lamins weakens the nuclear lamina, allowing nuclear fragmentation, and nuclear envelope proteins are also proteolysed. Proteolysis of proteins at focal adhesion sites and cell–cell adhesion sites allows cell detachment and retraction. Caspase activity is required for the exposure of phosphatidylserine (PS) and other phagocytic signals on the cell surface. Proteolysis of the Rho effector ROCK1 leads to contraction of the actin cytoskeleton and plasma membrane blebbing as well as nuclear fragmentation, whereas cleavage of tubulins and microtubule-associated and motor proteins leads to changes in the microtubule cytoskeleton that may contribute to apoptotic body formation (not shown). Caspases also cleave the Golgi-stacking protein GRASP65 and other Golgi proteins, causing fragmentation of the Golgi apparatus. Proteolysis of the mammalian sterile-20 kinase MST1 results in translocation of a catalytically active fragment of this kinase to the nucleus where it phosphorylates histone H2B to provoke chromatin condensation. Finally, important cellular functions such as translation are disrupted through caspase-mediated proteolysis of multiple translation initiation factors (eIFs). ER, endoplasmic reticulum; MLC, myosin light chain.

Detachment from the extracellular matrix. Cells in the early stages of apoptosis typically retract from neighbouring cells and lose contact with the extracellular matrix (FIG. 1a; Supplementary information S1 (movie)). This detachment process involves the caspase-dependent dismantling of cell–matrix focal adhesion sites, as well as cell–cell adhesion complexes, and probably facilitates the subsequent removal of apoptotic cells by phagocytes. Furthermore, apoptotic cells may be actively extruded by their neighbours due to the formation of contractile actin filaments within neighbouring cells, as discussed above[38].

Several components of focal adhesion sites have been reported to be substrates for caspases, including focal adhesion kinase, p130^{cas} and tensin[42–44]. However, although proteolysis of all of these substrates correlates with a loss of protein localization to focal adhesions, the key proteolytic events that precipitate the breakdown of cell–matrix contacts remain unclear. Similarly, several components of cell–cell adherens junctions are cleaved by caspases, including β- and γ-catenins[45,46]. Cleavage of β-catenin by caspase-3 *in vitro* abrogates its ability to bind to α-catenin, an association that is necessary for the linkage of cadherins at the adhesion site to the actin cytoskeleton. This suggests that this event might be instrumental in disrupting these junctions[45,46]. The fact that γ-catenin has a similar function and is similarly cleaved perhaps lends weight to the potential importance of this mechanism. Cadherins also undergo proteolysis by caspases[46,47]. Desmosomes, a second type of cell–cell adhesion site, are also disassembled during apoptosis through caspase-dependent proteolysis of several desmosome-associated proteins[48,49].

Shutting off the life-support systems

Caspases also target many proteins that are involved in essential housekeeping functions within the cell[11]. Proteins that function in transcription (for example, nuclear factor of activated T cells (NFAT), nuclear factor-κB (NFκB) p50 and p65, and La ribonucleoprotein) and translation (for example, the eukaryotic translation initiation factors eIF2a, eIF3, eIF4 and the β-subunit of the nascent polypeptide-associated complex (βNAC)) come under caspase-mediated attack during apoptosis[11] and ribosomal RNA is also degraded[50]. Genomic DNA becomes extensively hydrolysed[8] and the Golgi, ER and mitochondrial networks undergo fragmentation[9,10]. Indeed, all of the major cell organelles become extensively remodelled during apoptosis and, once again, caspases orchestrate much of this (FIG. 3).

DNA condensation and degradation. One of the first biochemical hallmarks of apoptosis to be identified was the degradation of genomic DNA into a ladder of fragments[8,51]. This pattern of fragmentation is the result of endonuclease-mediated chromatin cleavage at internucleosomal sites, and is typically accompanied by condensation of the chromatin. The purpose of DNA fragmentation during apoptosis is, like many events of the demolition phase, open to speculation because it does not seem to be essential for cell death[52]. This act certainly precludes any further possibility of cell division, but its real purpose might be to render the chromatin more manageable for subsequent disposal by phagocytic cells. Indeed, in *Caenorhabditis elegans*, DNA degradation does not occur within the dying cell itself, but instead occurs in the macrophage that engulfs it[53]. Additionally, DNA degradation might also represent an antiviral strategy that is designed to destroy the genomes of viruses that provoke apoptosis on entry into cells.

Evidence that DNA degradation is important for avoiding unwanted immune activation has come from studies of mice that are deficient in nucleases involved

in DNA fragmentation within the dying cell (caspase-activated DNase (CAD)), as well as within the phagocyte (DNase II). In these animals, failure to degrade DNA from apoptotic cells results in activation of the innate immune system, leading to defective thymic development[54]. This suggests that apoptosis-associated DNA degradation helps to prevent the accumulation of DNA that, if released into the extracellular space, could provoke autoimmune responses[55]. Indeed, anti-DNA antibodies are frequently observed in several autoimmune conditions, such as systemic lupus erythematosus, and can contribute to disease pathology by forming immune complex depositions within the kidneys that can lead to renal failure[55].

Cells that are deficient in CAD exhibit little cell-autonomous DNA degradation and chromatin condensation during apoptosis, which implies an important role for this enzyme[56,57]. In healthy cells, CAD is found in complex with its inhibitor, ICAD (inhibitor of CAD), which represses the activity of this endonuclease[57]. However, during apoptosis, ICAD is cleaved by caspases, which results in the liberation of CAD and fragmentation of chromatin[58,59] (FIG. 3). Some residual endonuclease activity remains in the absence of CAD, however, and this promotes the hydrolysis of DNA into high-molecular-weight fragments, an event that precedes low-molecular-weight DNA hydrolysis and indicates the existence of other apoptotic nucleases that are active earlier in the process[55]. The identity of these enzymes has been the subject of some debate and remains unresolved at present[60].

Early-stage chromatin condensation proceeds normally in CAD-deficient cells[56], suggesting that CAD activation is unrelated to the initial chromatin condensation that is also characteristic of apoptosis. Eukaryotic chromatin is composed of histone proteins wrapped around DNA to form nucleosomes, and recent work has suggested that histone 2B (H2B) phosphorylation is closely correlated with apoptotic chromatin condensation[61]. The kinase responsible for this modification, mammalian sterile-20 (MST1), is cleaved and activated by caspase-3 during apoptosis and this also permits shuttling of MST1 to the nuclear compartment[62]. Mutation of the caspase cleavage site within MST1 reduced apoptosis-associated chromatin condensation, as did expression of a kinase-dead form of this enzyme[62]. Interestingly, sterile-20 kinase, a yeast homologue of MST1, has also been strongly implicated in death-associated chromatin condensation in yeast, suggesting that H2B phosphorylation may represent a conserved mechanism of chromatin compaction during cell death, from yeast to mammals[63].

Transcription and translation. Multiple transcription factors (such as AP-2α, BTF3, NFATc1 and NFATc2, NFκBp65 and SP1) are cleaved by caspases, and a range of translation initiation factors (including eIF2a, eIF3, eIF4B, eIF4E, eIF4G and eIF4H) and ribosomal proteins (for example, RPP0 and p70S6K) are also affected[11]. Predictably, this results in the shutdown of transcription and translation relatively early in the process, although this is probably not what delivers the fatal blow. It is not clear why the transcriptional and translational machineries are targeted by caspases, but a plausible explanation is that it guards against these machineries being used to replicate viruses that might have provoked apoptosis in the first place. In such circumstances, it would seem appropriate that the means to produce additional viral particles would be incapacitated at the earliest opportunity.

Golgi and ER fragmentation. Other events that are characteristic of apoptosis are the fragmentation of cellular organelles, such as the Golgi apparatus and the ER, and the subsequent packaging of these organelles into apoptotic bodies. The mechanism by which the Golgi apparatus is fragmented appears to involve caspase-mediated cleavage of the Golgi-stacking protein GRASP65, because introduction of a proteolysis-resistant form of this protein delays Golgi breakdown[18]. Cleavage of other substrates, such as golgin-160, p115, cytoplasmic dynein intermediate chains (CD-IC), p150, syntaxin-5 and giantin, might also have roles in the breakdown of the Golgi and the inhibition of secretory traffic[31,64–66]. At a late stage of the apoptotic programme, the ER is also extensively remodelled and redistributed to apoptotic blebs to form a membrane around enclosed chromatin, a process that seems to require actin and the microtubule cytoskeleton[67]. Although firm evidence for this is lacking, it has been speculated that blocking ER–Golgi membrane trafficking might contribute to one or more of the membrane alterations that occur on apoptotic cells due to alteration of the carbohydrate composition of the plasma membrane[66].

Mitochondrial fragmentation. The mitochondrial network also becomes extensively fragmented during apoptosis (FIG. 1c), although in this instance caspases are not the main effectors. BAX (BCL-2-associated X protein) and/or BAK (BCL-2-antagonist/killer-1) become activated early in apoptosis through the actions of one or more BH3-only proteins, and this results in permeabilization of mitochondrial outer membranes and release of mitochondrial intermembrane space proteins[68,69] (FIG. 2). Coincident with this, mitochondria — which are frequently organized into interconnected networks within many cell types — fragment into much smaller organelles that are dispersed throughout the cell (FIG. 1c). Although it has been proposed that mitochondrial fragmentation is linked with the release of intermembrane space proteins from these organelles[9,70], other evidence suggests that these events are merely coincident and this issue has yet to be resolved[71,72]. Apoptosis-associated mitochondrial fragmentation seems to be caused primarily by the conformational changes that occur in BAX and BAK during their assembly into a mitochondrial pore or channel, leading some to speculate that these proteins also have a role in the normal ongoing process of mitochondrial fission and fusion in healthy cells, which is antagonized during apoptosis[71,73].

Although caspases do not contribute to apoptosis-associated mitochondrial fragmentation, they do contribute to the subsequent shutting down of mitochondrial function during this process. Caspase-mediated

BH3-only protein
A member of a class of pro-apoptotic proteins in the larger B-cell lymphoma-2 (BCL-2) family of proteins. BH3-only proteins share a short (~12 amino acid) motif known as the BH3 domain that exhibits homology with a region within BCL-2.

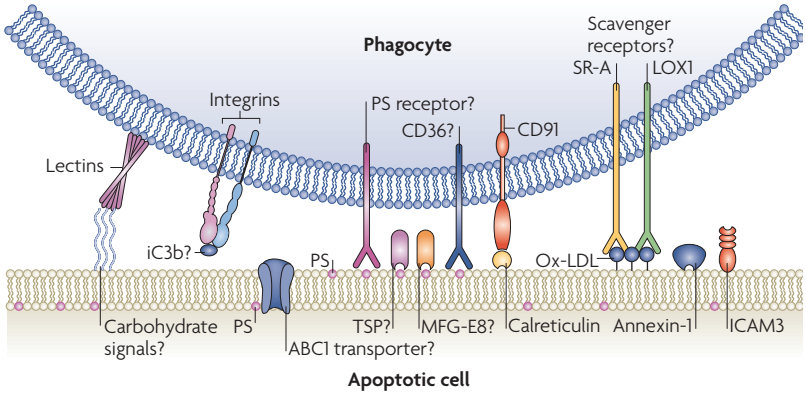


Figure 4 | **Apoptotic cells display ligands that promote their engulfment by phagocytes.** Several molecules have been proposed to act as signals for the engulfment of apoptotic cells by macrophages, dendritic cells and other cells with phagocytic capacity. On the apoptotic cell, phosphatidylserine (PS) is exposed on the outer plasma membrane leaflet and can be recognized by a specific receptor, possibly involving thrombospondin (TSP), milk fat globule–EGF factor-8 protein (MFG-E8) or CD36. PS exposure can also promote opsonization of apoptotic cells by the complement factor iC3b and this may promote the uptake of apoptotic cells by integrins (complement receptors CR3 and CR4). Calreticulin that is exposed on the dying cell can be bound by CD91, whereas oxidized low-density lipoprotein (ox-LDL)-like sites can be recognized by other scavenger receptors, including scavenger receptor A (SR-A) and oxidized low-density lipoprotein-1 (LOX1). Further apoptotic cell ligands may include annexin-1 and intercellular adhesion molecule-3 (ICAM3), and receptors on the phagocyte that do not have known ligands include lectins and members of the integrin family. ABC1, ATP-binding cassette transporter.

proteolysis of the p75 subunit of complex I of the electron transport chain was found to be partially required for the swelling and destructive morphological changes that are suffered by mitochondria during apoptosis[74]. In addition, apoptosis-associated loss of mitochondrial transmembrane potential, decline in cellular ATP levels and the production of reactive oxygen species have also been linked to p75 proteolysis[74]. The delayed cell death that is observed on introduction of a cleavage-resistant p75 mutant suggests that caspase-mediated disruption of mitochondrial function contributes to timely cell death and apoptotic corpse clearance[74].

Other proteolytic events. Caspases also cleave numerous other proteins during apoptosis, and almost 400 mammalian caspase substrates have been identified to date (see the caspase substrate database)[11]. Although many of the caspase cleavage events discussed thus far are linked to a specific feature of apoptosis, it is likely that many proteins targeted by caspases contribute to death of the cell but have no significant impact on the phenotypic changes seen during this process. For example, the multi-subunit proteasome complex is essential for normal cell function, and several subunits of this complex are cleaved by caspases[75,76]. Similarly, several metabolic enzymes are also cleaved by caspases and it is likely that these events contribute to the demise of the cell on some level[11]. Conversely, it is also probable that many caspase substrates are simply proteins that have become caught up in the proteolytic mayhem that breaks out on caspase activation. Conceptually, these caspase substrates can be likened to innocent bystanders that simply have the appropriate caspase cleavage motifs and are, therefore, of little significance to the completion of the process.

Because proteins involved in diverse cell functions are cleaved by caspases, it seems reasonable to speculate that these enzymes use a 'death by a thousand cuts' strategy to ensure that a cell dies. In other words, death by caspase activation probably results from many potentially lethal injuries rather than a single fatal blow. In addition to terminating cell viability, the cleavage of certain caspase substrates might plausibly modify the antigenicity of such proteins. As discussed earlier, certain intracellular constituents can act as danger signals when released from their normal location inside the cell. Proteolysis of such proteins might render these immunologically silent to preclude activation of the innate immune system.

Clearing the debris: apoptotic cell disposal

The terminal event of the demolition phase — consumption of the dead cell by phagocytes — is perhaps the most important aspect of the entire process[5]. This allows cells to be eliminated with their plasma membranes intact and precludes the potentially damaging release of cellular constituents into the surrounding milieu. The collapse of apoptotic cells into numerous apoptotic bodies is likely to make this task easier for the phagocytes. Importantly, phagocytes behave differently towards apoptotic cells than they do towards bacteria, for example, by minimizing the production of pro-inflammatory cytokines such as tumour necrosis factor-α (TNF-α)[77,78]. However, antigens from ingested apoptotic cells can be presented on the surface of major histocompatibility complex (MHC) molecules of dendritic cells, particularly when combined with a signal that both indicates danger (such as the presence of microorganism-derived peptides) and elicits T-cell responses[79–81]. This might allow the immune system to respond to pathological infections that trigger apoptosis.

The generation of binding sites for phagocytes and the release of chemoattractant molecules represent the last acts of the dying cell (FIG. 4). The focus then shifts to cells of the immune system, which coordinate the final removal of the corpse. To achieve this, phagocytes are equipped with a panoply of receptors that specifically detect engulfment signals on the apoptotic cell.

Apoptotic body production. The formation of apoptotic bodies is thought to be a means of safely breaking the cell apart into more manageable pieces for engulfment. This is likely to minimize the potential for failing to phagocytose the dead cell and thus releasing danger signals that may provoke activation of the innate immune system. However, it is important to note that many cell types, such as thymocytes and neutrophils, do not undergo apoptotic body formation. The process might be necessary only for larger cells that are difficult to engulf when intact.

The formation of apoptotic bodies probably follows on naturally from the actin–myosin-driven process of membrane blebbing, in which membrane-bound extrusions of cytosol become pinched off into independent vesicles[35,82]. However, a role has also been suggested for the microtubule cytoskeleton. Contracted microtubules have been observed during the late stages of the demolition phase extending into stable late blebs[41]. Disruption of the microtubule cytoskeleton prevents the formation of these blebs and also of apoptotic bodies, implicating microtubules in driving apoptotic body formation[41].

Attractant release for phagocytes by apoptotic cells. As well as becoming licensed for removal by phagocytes, apoptotic cells appear to be capable of attracting the attention of such cells by secreting molecules with chemotactic properties. An intriguing report has suggested that this occurs through the release of a chemoattractant lipid, lysophosphatidylcholine, in a caspase-3-dependent manner. In this context, lysophosphatidylcholine appears to be generated through the hydrolysis of membrane phosphatidylcholine by an active form of iPLA2 (Ca^{2+}-independent phospholipase-A2) that is generated through caspase-3-mediated cleavage[83]. Other suggested chemoattractant molecules include the S19 ribosomal protein and aminoacyl-tRNA synthetases, although these reports have yet to be substantiated[84,85].

Membrane changes. Despite the importance of phagocytosis, how apoptotic cells coordinate their recognition by phagocytes is far from clear. The best-characterized example of a phagocytic ligand is the membrane phospholipid phosphatidylserine (PS). PS is confined to the inner plasma membrane leaflet in healthy cells, but is translocated to the outer membrane leaflet in response to pro-apoptotic stimuli, where it induces phagocytosis[86,87] (FIG. 4). This translocation is caspase dependent, but how caspases promote PS externalization is unclear[88]. Likewise, the transporter that is involved is also uncertain, although the ATP-binding cassette transporter ABC1 has been implicated in this process[89].

A specific PS receptor is thought to exist, but the protein that was originally implicated now seems to have other roles; thus, the identity of the real receptor remains a mystery[90,91]. One candidate is milk fat globule–EGF factor-8 protein, a secreted glycoprotein that is produced by activated macrophages, which can bind to aminophospholipids (including PS) and induce cell uptake[92]. Extracellular thrombospondin is another potential receptor, as is the scavenger receptor CD36, which can recognize oxidized PS on apoptotic cells[93,94].

Oxidized low-density lipoprotein is another potential ligand on the surface of apoptotic cells, and antibodies against it attenuate phagocytosis by macrophages[95]. These molecules might also be recognized by scavenger receptors including SR-A and LOX1 (oxidized low-density lipoprotein-1)[96,97] (FIG. 4). A further scavenger receptor, CD91 (also known as LRP), can bind to the ER chaperone protein calreticulin. Calreticulin becomes localized to the surface of apoptotic cells, and its ligation by CD91 triggers cell engulfment[98]. The adhesion molecule ICAM3, a glycosylated member of the immunoglobulin superfamily, is also a possible ligand, as is annexin-1, which is exposed on the surface of dying cells in a caspase-dependent manner[99,100]. Furthermore, the ability of macrophage lectins, such as mannose-binding lectin and lung surfactant proteins A and D, to act as receptors for apoptotic cells indicates the existence of carbohydrate signals[101,102]. Several integrins (such as complement receptors CR3 and CR4) have also been implicated in the recognition of dying cells through the bridging receptors thrombospondin and the complement component iC3b[103,104].

Cross-presentation of peptides from apoptotic cells. Although the recognition and engulfment of apoptotic cells by phagocytes was previously thought to represent the final event in apoptosis, recent studies suggest that peptides derived from engulfed apoptotic cells can enter the MHC class I antigen presentation pathway[79–81]. This pathway is normally reserved for peptides that are synthesized within the antigen-presenting cell itself. However, a growing body of evidence now suggests that peptides derived from engulfed apoptotic cells can be presented on MHC class I molecules and prime for cytotoxic T-cell responses[79–81]. Because caspases cleave hundreds of proteins within dying cells, it seems plausible that these proteolytic events might influence, either positively or negatively, the repertoire of peptides that subsequently become presented on MHC class I molecules. Therefore, even after burial within the phagocyte, apoptotic cells may be capable of reaching out beyond the grave to influence the likelihood that their constituent proteins may be presented to cells of the adaptive immune system.

Conclusions

It is more than 30 years since Kerr, Wyllie and Currie proposed that many programmed cell deaths in higher organisms are characterized by a common set of morphological features, coining the term 'apoptosis' for cells that display these features. Much progress has been made towards unravelling the molecular basis of this death ritual in the intervening years. However, as we have pointed out above, significant gaps remain in our knowledge of this process. In particular, we have little information concerning how caspases initiate the membrane changes that are required for phagocytosis of apoptotic cells, even in the case of relatively well-characterized ligands such as PS. Furthermore, we are only now beginning to understand the mechanisms by which dying cells attract and influence the behaviour of phagocytes. Further study of these events is likely to prove fruitful for understanding how cells coordinate and shape the response to their own disposal. In addition, accumulating evidence also suggests that caspases participate in other cellular events, such as differentiation, although the details remain scanty at present. As Isaac Asimov once noted: "Life is pleasant. Death is peaceful. It's the transition that's troublesome".

Phosphatidylserine
A membrane phospholipid that is usually localized to the inner leaflet of the plasma membrane, but which is translocated to the outer leaflet during apoptosis.

Scavenger receptor
A type of cell-surface receptor that recognizes modified low-density lipoprotein or other negatively charged macromolecules, and often functions to remove foreign substances or waste materials from the body.

1. Kerr, J. F. R., Wyllie, A. H. & Currie, A. R. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br. J. Cancer* **24**, 239–275 (1972).
This seminal study introduced the term apoptosis, providing a detailed description of the features of apoptotic cells and some of the biological contexts in which this mode of cell death is observed.
2. Wyllie, A. H., Kerr, J. F. R. & Currie, A. R. Cell death: the significance of apoptosis. *Int. Rev. Cytol.* **68**, 251–305 (1980).
3. Nicholson, D. W. Caspase structure, proteolytic substrates and function during apoptotic death. *Cell Death Differ.* **6**, 1028–1042 (1999).
4. Creagh, E. M., Conroy, H. & Martin, S. J. Caspase-activation pathways in apoptosis and immunity. *Immunol. Rev.* **193**, 10–21 (2003).
5. Savill, J. & Fadok, V. Corpse clearance defines the meaning of cell death. *Nature* **407**, 784–788 (2000).
6. Robertson, A. M., Bird, C. C., Waddell, A. W. & Currie, A. R. Morphological aspects of glucocorticoid-induced cell death in human lymphoblastoid cells. *J. Pathol.* **126**, 181–187 (1978).
7. Martin, S. J., Bradley, J. G. & Cotter, T. G. HL-60 cells induced to differentiate towards neutrophils subsequently die via apoptosis. *Clin. Exp. Immunol.* **79**, 448–453 (1990).
8. Williams, J. R., Little, J. B. & Shipley, W. U. Association of mammalian cell death with a specific endonucleolytic degradation of DNA. *Nature* **252**, 754–755 (1974).
9. Frank, S. *et al.* The role of dynamin-related protein 1, a mediator of mitochondrial fission, in apoptosis. *Dev. Cell* **1**, 515–525 (2001).
10. Lane, J. D. *et al.* Caspase-mediated cleavage of the stacking protein GRASP65 is required for Golgi fragmentation during apoptosis. *J. Cell Biol.* **156**, 495–509 (2002).
11. Lüthi, A. U. & Martin, S. J. The CASBAH: a searchable database of caspase substrates. *Cell Death Differ.* **14**, 641–650 (2007).
A comprehensive survey of the ~400 caspase substrates identified to date (which can be found in the caspase substrate database.
12. Gallucci, S., Lolkema, M. & Matzinger, P. Natural adjuvants: endogenous activators of dendritic cells. *Nature Med.* **5**, 1249–1255 (1999).
A provocative study that provides evidence that necrotic, but not apoptotic, cells activate dendritic cells.
13. Shi, Y., Zheng, W. & Rock, K. L. Cell injury releases endogenous adjuvants that stimulate cytotoxic T cell responses. *Proc. Natl Acad. Sci. USA* **97**, 14590–14595 (2000).
14. Shi, Y., Evans, J. E. & Rock, K. L. Molecular identification of a danger signal that alerts the immune system to dying cells. *Nature* **425**, 516–521 (2003).
15. Oppenheim, J. J. & Yang, D. Alarmins: chemotactic activators of immune responses. *Curr. Opin. Immunol.* **17**, 359–365 (2005).
16. Chen, C. J. *et al.* Identification of a key pathway required for the sterile inflammatory response triggered by dying cells. *Nature Med.* **13**, 851–856 (2007).
17. Trinchieri, G. & Sher, A. Cooperation of Toll-like receptor signals in innate immune defence. *Nature Rev. Immunol.* **7**, 179–190 (2007).
18. Matzinger P. The danger model: a renewed sense of self. *Science* **296**, 301–305 (2002).
An excellent update of the 'danger model' by its originator.
19. Scaffidi, P., Misteli, T. & Bianchi, M. E. Release of chromatin protein HMGB1 by necrotic cells triggers inflammation. *Nature* **418**, 191–195 (2002).
20. Stennicke, H. R. & Salvesen, G. S. Properties of the caspases. *Biochim. Biophys Acta.* **1387**, 17–31 (1998).
An excellent overview on the biochemical characteristics of caspases.
21. Stennicke, H. R. *et al.* Pro-caspase-3 is a major physiologic target of caspase-8. *J. Biol. Chem.* **273**, 27084–27090 (1998).
22. Adrain, C. & Martin, S. J. The mitochondrial apoptosome: a killer unleashed by the cytochrome seas. *Trends Biochem. Sci.* **26**, 390–397 (2001).
23. Communal, C. *et al.* Functional consequences of caspase activation in cardiac myocytes. *Proc. Natl Acad. Sci. USA* **99**, 6252–6256 (2002).
24. Gerner, C. *et al.* The Fas-induced apoptosis analysed by high throughput proteome analysis. *J. Biol. Chem.* **275**, 39018–39026 (2000).
25. Martin, S. J. *et al.* Proteolysis of fodrin (non-erythroid spectrin) during apoptosis. *J. Biol. Chem.* **270**, 6425–6428 (1995).
26. Thiede, B., Treumann, A., Kretschmer, A., Sohike, J. & Rudel, T. Shotgun proteome analysis of protein changes in apoptotic cells. *Proteomics* **5**, 2123–2130 (2005).
27. Kothakota, S. *et al.* Caspase-3-generated fragment of gelsolin: effector of morphological change in apoptosis. *Science* **278**, 294–298 (1997).
28. Browne, K. A., Johnstone, R. W., Jans, D. A. & Trapani, J. A. Filamin (280 kDa actin-binding protein) is a caspase substrate and is also cleaved directly by the cytotoxic T lymphocyte protease granzyme B during apoptosis. *J. Biol. Chem.* **275**, 39262–39266 (2000).
29. Adrain, C., Brumatti, G., Duriez, P., Delivani, P. & Martin, S. J. The cytotoxic lymphocyte protease, granzyme B, targets the cytoskeleton and perturbs microtubule polymerization dynamics. *J. Biol. Chem.* **281**, 8118–8125 (2006).
30. Canu, N. *et al.* Tau cleavage and dephosphorylation in cerebellar granule neurons undergoing apoptosis. *J. Neurosci.* **18**, 7061–7064 (1998).
31. Lane, J. D., Vergnolle, M. A. S., Woodman, P. G. & Allan, V. J. Apoptotic cleavage of cytoplasmic dynein intermediate chain and $p150^{Glued}$ stops dynein-dependent membrane motility. *J. Cell Biol.* **153**, 1415–1426 (2001).
32. Morishima, N. Changes in vimentin morphology during apoptosis correlate with vimentin cleavage by different caspases located either upstream or downstream of Bcl-2 action. *Genes Cells* **4**, 401–414 (1999).
33. Ku, N.-O., Liao, J. & Omary, M. B. Apoptosis generates stable fragments of human type I keratins. *J. Biol. Chem.* **272**, 33197–33203 (1997).
34. Orth, K., Chinnaiyan, A. M., Garg, M., Froelich, C. J. & Dixit, V. M. The CED-3/ICE-like protease Mch2 is activated during apoptosis and cleaves the death substrate lamin A. *J. Biol. Chem.* **271**, 16443–16446 (1996).
35. Cotter, T. G., Lennon, S. V., Glynn, J. M. & Green, D. R. Microfilament-disrupting agents prevent the formation of apoptotic bodies in tumour cells undergoing apoptosis. *Cancer Res.* **52**, 997–1005 (1992).
The first study to show that actin microfilaments are required for the formation of apoptotic bodies.
36. Coleman, M. L. *et al.* **Membrane blebbing during** apoptosis results from caspase-mediated activation of ROCK I. *Nature Cell Biol.* **3**, 339–345 (2001).
37. Sebbagh, M. *et al.* Caspase-3-mediated cleavage of ROCK I induces MLC phosphorylation and apoptotic membrane blebbing. *Nature Cell Biol.* **3**, 346–352 (2001).
This study, along with reference 36, strongly implicates ROCK1 in apoptosis-associated membrane blebbing.
38. Rosenblatt, J., Raff, M.C. & Cramer, L.P. An epithelial cell destined for apoptosis signals its neighbors to extrude it by an actin- and myosin-dependent mechanism. *Curr. Biol.* **11**, 1847–1857 (2001).
39. Rao, L., Perez, D. & White, E. Lamin proteolysis facilitates nuclear events during apoptosis. *J. Cell Biol.* **135**, 1441–1455 (1996).
40. Croft, D. R. *et al.* Actin-myosin-based contraction is responsible for apoptotic nuclear disintegration. *J. Cell Biol.* **168**, 245–255 (2005).
41. Moss, D. K., Betin, V. M., Malesinski, S. D. & Lane, J. D. A novel role for microtubules in apoptotic chromatin dynamics and cellular fragmentation. *J. Cell Sci.* **119**, 2362–2374 (2006).
42. Levkau, B., Hrren, B., Koyama, H., Ross, R. & Raines, E. W. Caspase-mediated cleavage of focal adhesion kinase $pp125^{FAK}$ and disassembly of focal adhesions in human endothelial cell apoptosis. *J. Exp. Med.* **187**, 579–586 (1998).
43. Kim, W., Kook, S., Kim, D. J., Teodorof, C. & Song, W. K. The 31 kDa caspase-generate cleavage product of $p130^{cas}$ functions as a transcriptional repressor of E2A in apoptotic cells. *J. Biol. Chem.* **279**, 8333–8342 (2004).
44. Kook, S. *et al.* Caspase-dependent cleavage of tensin induces disruption of actin cytoskeleton during apoptosis. *Biochem. Biophys. Res. Comm.* **303**, 37–45 (2003).
45. Brancolini, C., Lazarevic, D., Rodriguez, J. & Schneider, C. Dismantling cell–cell contacts during apoptosis is coupled to a caspase-dependent proteolytic cleavage of β-catenin. *J. Cell Biol.* **139**, 759–771 (1997).
46. Bannerman, D. D., Sathyamoorthy, M. & Goldblum, S. E. Bacterial lipopolysaccharide disrupts endothelial monolayer integrity and survival signalling events through caspase cleavage of adherens junction proteins. *J. Biol. Chem.* **273**, 35371–35380 (1998).
47. Steinhusen, U. *et al.* Cleavage and shedding of E-cadherin after induction of apoptosis. *J. Biol. Chem.* **276**, 4872–4980 (2001).
48. Dusek, R. L. *et al.* The differentiation-dependent desmosomal cadherin desmoglein 1 is a novel caspase-3 target that regulates apoptosis in keratinocytes. *J. Biol. Chem.* **281**, 3614–3624 (2006).
49. Weiske, J. *et al.* The fate of desmosomal proteins in apoptotic cells. *J. Biol. Chem.* **276**, 41175–41181 (2001).
50. Houge, G., Doskeland, S. O., Boe, R. & Lanotte, M. Selective cleavage of 28S rRNA variable regions V3 and V13 in myeloid leukaemia cell apoptosis. *FEBS Lett.* **315**, 16–20 (1993).
51. Wyllie, A. H. Glucocorticoid-induced thymocyte apoptosis is associated with endogenous endonuclease activation. *Nature* **284**, 555–556 (1980).
The first study to explicitly link internucleosomal DNA fragmentation to apoptosis.
52. Schulze-Osthoff, K., Walczak, H., Droge, W. & Krammer, P. H. Cell nucleus and DNA fragmentation are not required for apoptosis. *J. Cell Biol.* **127**, 15–20 (1994).
53. Wu, Y. C., Stanfield, G. M. & Horvitz, H. R. NUC-1, a *Caenorhabditis elegans* DNase II homolog, functions in an intermediate step of DNA degradation during apoptosis. *Genes Dev.* **14**, 536–548 (2000).
54. Kawane, K. *et al.* Impaired thymic development in mouse embryos deficient in apoptotic DNA degradation. *Nature Immunol.* **4**, 138–144 (2003).
55. Napirei, M. *et al.* T. Features of systemic lupus erythematosus in DNase1-deficient mice. *Nature Genet.* **25**, 177–181 (2000).
References 54 and 55 suggest that DNase-mediated degradation of genomic DNA from apoptotic cells is essential to ward off inappropriate activation of the immune system, which can lead to autoimmunity.
56. Samejima, K., Tone, S. & Earnshaw, W. C. CAD/DFF40 nuclease is dispensable for high molecular weight DNA cleavage and stage I chromatin condensation in apoptosis. *J. Biol. Chem.* **276**, 45427–45432 (2001).
57. Liu, X., Zou, H., Slaughter, C. & Wang, X. DFF, a heterodimeric protein that functions downstream of caspase-3 to trigger DNA fragmentation during apoptosis. *Cell* **89**, 175–184 (1997).
58. Enari, M. *et al.* A caspase-activated DNase that degrades DNA during apoptosis, and its inhibitor ICAD. *Nature* **391**, 43–50 (1998).
This study, along with reference 57, reported the identification of the nuclease (called CAD or DFF40) that is responsible for internucleosomal DNA fragmentation during apoptosis.
59. Sakahira, H., Enari, M. & Nagata, S. Cleavage of CAD inhibitor in CAD activation and DNA degradation during apoptosis. *Nature* **391**, 96–99 (1998).
60. Samejima, K. & Earnshaw, W. C. Trashing the genome: the role of nucleases during apoptosis. *Nature Rev. Mol. Cell Biol.* **6**, 677–688 (2005).
61. Cheung, W. L. *et al*. Apoptotic phosphorylation of histone H2B is mediated by mammalian sterile twenty kinase. *Cell* **113**, 507–517 (2003).
62. Ura, S., Masuyama, N., Graves, J. D. & Gotoh, Y. Caspase cleavage of MST1 promotes nuclear translocation and chromatin condensation. *Proc. Natl Acad. Sci. USA* **98**, 10148–10153 (2001).
This study, together with reference 61, links proteolysis of MST1 with apoptotic chromatin condensation.
63. Ahn, S. H. *et al*.Sterile 20 kinase phosphorylates histone H2B at serine 10 during hydrogen peroxide-induced apoptosis in *S. cerevisiae*. *Cell* **120**, 25–36 (2005).
64. Mancini, M. *et al.* Caspase-2 is localized at the Golgi complex and cleaves golgin-160 during apoptosis. *J. Cell Biol.* **149**, 603–612 (2000).
65. Chiu, R., Novikov, L., Mukherjee, S. & Shields, D. A caspase cleavage fragment of p115 induces fragmentation of the Golgi apparatus and apoptosis. *J. Cell Biol.* **159**, 637–648 (2002).
66. Lowe, M., Lane, J. D., Woodman, P. G. & Allan, V. J. Caspase-mediated cleavage of syntaxin 5 and giantin accompanies inhibition of secretory traffic during apoptosis. *J. Cell Sci.* **117**, 1139–1150 (2003).

67. Lane, J. D., Allan, V. J. & Woodman, P. G. Active relocation of chromatin and endoplasmic reticulum into blebs in late apoptotic cells. *J. Cell Sci.* **118**, 4059–4071 (2005).
68. Letai, A. *et al.* Distinct BH3 domains either sensitize or activate mitochondrial apoptosis, serving as prototype cancer therapeutics. *Cancer Cell* **2**, 183–192 (2002).
Provided the first evidence that BH3-only proteins can act either as sensitizers or as direct activators of the BAX–BAK channel.
69. Kuwana, T. *et al.* BH3 domains of BH3-only proteins differentially regulate Bax-mediated mitochondrial membrane permeabilization both directly and indirectly. *Mol. Cell* **17**, 525–535 (2005).
70. Karbowski, M. *et al.* Spatial and temporal association of Bax with mitochondrial fission sites, Drp1, and Mfn2 during apoptosis. *J. Cell Biol.* **159**, 931–938 (2002).
71. Delivani, P., Taylor, R. C., Adrain, C., Duriez, P. & Martin, S. J. Role for CED-9 and Egl-1 as regulators of mitochondrial fission and fusion dynamics. *Mol. Cell* **21**, 761–773 (2006).
72. Arnoult, D., Grodet, A., Lee, Y. J., Estaquier, J. & Blackstone, C. Release of OPA1 during apoptosis participates in the rapid and complete release of cytochrome *c* and subsequent mitochondrial fragmentation. *J. Biol. Chem.* **280**, 35742–35750 (2005).
73. Karbowski, M., Norris, K. L., Cleland, M. M., Jeong, S.-Y. & Youle, R. J. Role of Bax and Bak in mitochondrial morphogenesis. *Nature* **443**, 658–662 (2006).
This provocative study, along with reference 71, suggests that certain BCL-2 family proteins may have roles in mitochondrial fission and fusion dynamics that are unrelated to their roles within the cell death machinery.
74. Ricci. J. E. *et al.* Disruption of mitochondrial function during apoptosis is mediated by caspase cleavage of the p75 subunit of complex I of the electron transport chain. *Cell* **117**, 773–786 (2004).
75. Sun, X. M. *et al.* Caspase activation inhibits proteasome function during apoptosis. *Mol. Cell* **14**, 81–93 (2004).
76. Adrain, C., Creagh, E. M., Cullen, S. P. & Martin, S. J. Caspase-dependent inactivation of proteasome function during programmed cell death in *Drosophila* and man. *J. Biol. Chem.* **279**, 36923–36930 (2004).
77. Voll, R. E, Hermann, M., Roth, E. A., Stach, C. & Kalden, J. R. Immunosupressive effects of apoptotic cells. *Nature* **390**, 350–351 (1997).
78. Fadok, V. *et al.* Macrophages that have ingested apoptotic cells *in vitro* inhibit proinflammatory cytokine production through autocrine/paracrine mechanisms involving TGF-β, PGE_2, and PAF. *J. Clin. Invest.* **101**, 890–898 (1998).
79. Bellone, M. *et al.* Processing of engulfed apoptotic bodies yields T-cell epitopes. *J. Immunol.* **159**, 5391–5399 (1997).
80. Albert, M. I., Sauter, B. & Bhardwaj, N. Dendritic cells acquire antigen from apoptotic cells and induce class-restricted CTLs. *Nature* **392**, 86–89 (1998).
This study presents the first evidence that peptides from apoptotic cells may be cross-presented to class-I-restricted cytotoxic T lymphocytes.
81. Steinman, R. M., Turley, S., Mellman, I. & Inaba, K. The induction of tolerance by dendritic cells that have captured apoptotic cells. *J. Exp. Med.* **191**, 411–416 (2000).
82. Orlando, K. A., Stone, N. L. & Pittman, R. N. Rho kinase regulates fragmentation and phagocytosis of apoptotic cells. *Exp. Cell Res.* **312**, 5–15 (2006).
83. Lauber, K. *et al.* Apoptotic cells induce migration of phagocytes via caspase-3-mediated release of a lipid attraction signal. *Cell* **113**, 717–730 (2003).
This intriguing study suggested that apoptotic cells can secrete molecules with chemotactic properties.
84. Horino, K. *et al.* A monocyte chemotactic factor, S19 ribosomal protein dimer, in phagocytic clearance of apoptotic cells. *Lab. Invest.* **78**, 603–617 (1998).
85. Wakasugi, K. & Schimmel, P. Two distinct cytokines released from a human aminoacyl-tRNA synthetase. *Science* **284**, 147–151 (1999).
86. Fadok, V. A. *et al.* Exposure of phosphatidylserine on the surface of apoptotic lymphocytes triggers specific recognition and removal by macrophages. *J. Immunol.* **148**, 2207–2216 (1992).
This was the first report to suggest that phosphatidylserine might be exposed on the surface of apoptotic cells.
87. Martin, S. J. *et al.* Early redistribution of plasma membrane phosphatidylserine is a general feature of apoptosis regardless of the initiating stimulus: inhibition by overexpression of Bcl-2 and Abl. *J. Exp. Med.* **182**, 1545–1556 (1995).
This report directly demonstrates that phosphatidylserine exposure is a universal event during apoptosis of various cell types. It also introduced the annexin-5 labelling method.
88. Martin, S. J., Finucane, D. M., Amarante-Mendes, G. P., O'Brien, G. A. & Green, D. R. Phosphatidylserine externalization during CD95-induced apoptosis of cells and cytoplasts requires ICE/CED-3 protease activity. *J. Biol. Chem.* **271**, 28753–28756 (1996).
89. Hamon, Y. *et al.* ABC1 promotes engulfment of apoptotic cells and transbilayer redistribution of phosphatidlyserine. *Nature Cell Biol.* **2**, 399–406 (2000).
90. Fadok, V. A. *et al.* A receptor for phosphatidylserine-specific clearance of apoptotic cells. *Nature* **405**, 85–90 (2000).
91. Mitchell, J. E. *et al.* The presumptive phosphatidylserine receptor is dispensable for innate anti-inflammatory recognition and clearance of apoptotic cells. *J. Biol. Chem.* **281**, 5718–5725 (2006).
92. Hanayama, R. *et al.* Identification of a factor that links apoptotic cells to phagocytes. *Nature* **417**, 182–187 (2002).
93. Manodori, A. B., Barabino, G. A., Lubin, B. H. & Kuypers, F. A. Adherence of phosphatidylserine-exposing erythrocytes to endothelial matrix thrombospondin. *Blood* **95**, 1293–1300 (2000).
94. Greenberg, M. E. *et al.* Oxidized phosphatidylserine-CD36 interactions play an essential role in macrophage-dependent phagocytosis of apoptotic cells. *J. Exp. Med.* **203**, 2613–2625 (2006).
95. Chang, M.-K. *et al.* Monoclonal antibodies against oxidised low-density lipoprotein bind to apoptotic cells and inhibit their phagocytosis by elicited macrophages: evidence that oxidation-specific epitopes mediate macrophage recognition. *Proc. Natl Acad. Sci. USA* **96**, 6353–6358 (1999).
96. Oka, K. *et al.* Lectin-like oxidised low-density lipoprotein receptor 1 mediates phagocytosis of aged/apoptotic cells in endothelial cells. *Proc. Natl Acad. Sci. USA* **95**, 9535–9540 (1998).
97. Platt, N., Suzuki, H., Kurihara, Y., Kodama, T. & Gordon, S. Role for the class A macrophage scavenger receptor in the phagocytosis of apoptotic thymocytes in vitro. *Proc. Natl Acad. Sci. USA* **93**, 12456–12460 (1996).
98. Gardai, S. J. *et al.* Cell-surface calreticulin initiates clearance of viable or apoptotic cells through *trans*-activation of LRP on the phagocyte. *Cell* **123**, 321–334 (2005).
99. Moffatt, O. D., Devitt, A., Bell, E. D., Simmons, D. L. & Gregory, C. D. Macrophage recognition of ICAM-3 on apoptotic leukocytes. *J. Immunol.* **162**, 6800–6810 (1999).
100. Arur, S. *et al.* Annexin I is an endogenous ligand that mediates apoptotic cell engulfment. *Dev. Cell* **4**, 587–598 (2003).
101. Hall, S. E., Savill, J. S., Henson, P. M. & Haslett, C. Apoptotic neutrophils are phagocytosed by fibroblasts with participation of the fibroblast vitronectin receptor and involvement of a mannose/fucose-specific lectin. *J. Immunol.* **153**, 3218–3227 (1994).
102. Ogden, C. A. *et al.* C1q and mannose-binding lectin engagement of cell surface calreticulin and CD91 initiates macropinocytosis and uptake of apoptotic cells. *J. Exp. Med.* **194**, 781–795 (2001).
103. Savill, J., Hogg, N., Ren, Y. & Haslett, C. Thrombospondin cooperates with CD36 and the vitronectin receptor in macrophage recognition of neutrophils undergoing apoptosis. *J. Clin. Invest.* **90**, 1513–1522 (1992).
104. Takizawa, F., Tsuji, S. & Nagawa, S. Enhancement of macrophage phagocytosis upon iC3b deposition on apoptotic cells. *FEBS Lett.* **397**, 269–272 (1996).

Acknowledgements
Work in the Martin laboratory is supported by an award from Science Foundation Ireland. We thank P. Delivani for providing the images for Figure 1. We apologize to colleagues for citing reviews instead of primary papers in several places owing to space constraints.

DATABASES
OMIM: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM
systemic lupus erythematosus
UniProtKB: http://beta.uniprot.org/uniprot
BAK | BAX | CAD | caspase-1 | caspase-3 | caspase-4 | caspase-5 | caspase-6 | caspase-7 | caspase-8 | caspase-9 | caspase-12 | GRASP65 | HMGB1 | ICAD | MST1 | ROCK1

FURTHER INFORMATION
Seamus Martin's homepage:
http://www.tcd.ie/genetics/martin_research.php
Caspase substrate database homepage:
http://www.casbah.ie

SUPPLEMENTARY INFORMATION
See online article: S1 (movie)

ALL LINKS ARE ACTIVE IN THE ONLINE PDF