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Review

The CASBAH: a searchable database of caspase substrates

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Apoptosis is coordinated by members of the caspase family of aspartic acid-specific proteases. Other members of this protease family also play essential roles in inflammation where they participate in the maturation of pro-inflammatory cytokines. To date, almost 400 substrates for the apoptosis-associated caspases have been reported and there are likely to be hundreds more yet to be discovered. Thus, the fraction of the proteome that is degraded (the degradome) by caspases during the demolition phase of apoptosis appears to be quite substantial. Despite this, we still know surprisingly little concerning how caspases provoke some of the signature events in apoptosis, such as membrane phosphatidylserine externalization, cellular retraction, chromatin condensation and apoptotic body production. The inflammatory caspases appear to be much more specific proteases than those involved in apoptosis and only two confirmed substrates for these proteases have been described to date. Here, we have compiled a comprehensive list of caspase substrates and describe a searchable web resource (The Casbah; www.casbah.ie) which contains information pertaining to all currently known caspase substrates. We also discuss some of the unresolved issues relating to caspase-dependent events in apoptosis and inflammation.
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Since the discovery that the major effectors of programmed cell death are members of the caspase family of proteases, significant efforts have been directed towards understanding the multifarious roles that members of this evolutionary-conserved family of proteases play in apoptosis. Although the mechanisms of activation of the worm, fly and mammalian caspases are now relatively well understood,[1,2] much less is known about how these proteases contrive to kill cells. Although hundreds of mammalian caspase substrates have now been identified – and therein lies the root of the problem – we still know surprisingly little about how caspases coordinate the terminal events in apoptosis that result in death of the cell.

Discriminating Innocent Bystanders from Legitimate Targets

For this overview, we have searched the literature for caspase substrates and have compiled a somewhat daunting list of 390 proteins that have been reported to be cleaved by caspases, either *in vitro*, during apoptosis or inflammation (see Supplementary Table 1). Owing to this profusion of caspase substrates, the major challenge now is to identify the subset of these proteins that have functional significance for the process of apoptosis and/or inflammation. Although it is within the bounds of possibility that all proteins that are cleaved by caspases during apoptosis contribute to the demise of the cell on some level, it seems highly unlikely that all caspase substrates play equally significant roles in this process. On the contrary, it seems rather more likely that many proteins are simply caught up in the proteolytic maelstrom that envelops a cell during apoptosis and become cleaved by caspases– by accident rather than design – during the terminal phase of cell death. Thus, the nub of the problem is to discriminate what we have previously termed the 'innocent bystanders' from the 'legitimate targets' of caspase-mediated attack.[3]

Unfortunately, there is no easy way to identify functionally important caspase substrates from the proteolytic noise that almost inevitably accompanies a process where several proteases become activated within a short time frame.[4–6] Painstaking analysis of individual caspase substrates – involving the mapping of precise caspase cleavage sites, the creation and expression of non-cleavable mutants and/or cleaved forms of the substrate, the generation of gene knockouts or non-cleavable knock-ins – is required to generate a definitive link between proteolysis of an individual substrate and a specific 'apoptotic parameter'. For the vast majority of substrates that have been described to date (see Supplementary Table 1), such analyses have not been carried out. Rather, the consequences of proteolysis of individual substrates has either been inferred or in some cases, the cleaved form of the substrate has been overexpressed and the functional effects of this have been evaluated in otherwise healthy cells. In many cases, we also await confirmation that putative caspase substrates are actually cleaved by caspases

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at physiological concentrations of enzyme. For example, the recently identified cytokine, IL-33, has been reported to be a substrate for caspase-1.[7] However, the latter proposal is based solely upon the observation that this cytokine is cleaved *in vitro* by recombinant caspase-1 at arbitrary concentrations of this enzyme.[7]

One way of approaching the question of whether a caspase substrate is potentially more relevant than others is to ask whether this protein is also cleaved by caspases in other species. However, this approach has not been adopted in many cases and has led to generalizations concerning the significance of some substrates for apoptosis that are unlikely to be borne out upon more detailed analysis. Although it is certainly possible that some caspase substrates may be cleaved in a species-specific manner, it seems implausible that the same functional endpoints are achieved through proteolysis of a completely different array of proteins in different species. This view is buttressed by observations that many of the same morphological endpoints are seen in apoptotic cells from organisms as divergent as flies and man.[8] Thus, it is probable that a conserved cohort of caspase substrates are cleaved by worm, fly and mammalian caspases, and that these represent the legitimate targets that ensure controlled cell destruction, as well as the morphological and functional hallmarks of apoptosis.

Exceptions to the above are likely to be caspase substrates that play roles in signal transduction cascades that operate only in certain organisms. For example, nematodes do not appear to possess death receptors; therefore, signalling intermediates in death receptor pathways, such as BID and RiP-1, which are important caspase substrates in mammals are not present in this organism. Similarly, it is also possible that tissue-specific proteins may be targeted by caspases to regulate some unique aspect of apoptosis in such tissues.

Caspases are Required for the Apoptotic Phenotype

There has been an ongoing debate as to whether caspases are essential for apoptosis of mammalian cells or whether other proteases can step into the breach to achieve the same end points when caspase activity is stymied.[9–12] Some investigators hold the view that essentially similar cell death endpoints can be achieved regardless of whether caspase activity is muzzled. Under conditions where caspase activity is experimentally disabled – either through genetic inactivation of specific caspases or caspase-activating proteins such as Apaf-1 or through exposure of cells to poly-caspase inhibitors, such as Z-VAD-fmk – cell death still occurs in response to many pro-apoptotic triggers and this is taken as evidence that caspases are not required for apoptosis. However, in the vast majority (if not all) of these cases, the morphological end points seen when caspases are prevented from participating are not what are commonly regarded as hallmarks of apoptotic cell death.[11,12] Rather, caspase inhibition typically converts the phenotype of the dying cell from apoptosis into necrosis.[11,12]

So why does caspase inhibition/inactivation fail to protect cells from stimuli that provoke apoptosis? In mammals, this is owing to events upstream of caspase activation that provoke the release of mitochondrial intermembrane space proteins such as cytochrome *c*. Although this does not apply to all pro-apoptotic stimuli (engagement of death receptors for example), it applys to stimuli where BH3-only proteins play instrumental roles in promoting mitochondrial permeabilisation and assembly of the Apaf-1/caspase-9 apoptosome downstream.[13] In such cases, owing to the rapid decline in mitochondrial function, most cells are irreversibly committed to die irrespective of whether caspases are activated downstream. However, in the absence of caspase activation, the phenotype of the resulting death is necrotic rather than apoptotic. The exception seems to be certain post-mitotic cells such as neurons and cardiomyocytes, as these cells can recover from mitochondrial permeabilisation as long as caspase activation is prevented downstream.[14,15]

In the case of the nematode worm, *Caenorhabditis elegans*, it is clear that the CED-3 caspase is essential for all developmental-related programmed cell deaths in this organism.[16,17] Strong loss-of-function mutations in CED-3 block all programmed cell deaths in the worm, thereby illustrating that a default cell death programme is not present in this organism. Similar observations have also been made with respect to mutations in the CED-3 – activating adaptor molecule, CED-4.[16]

In the fruitfly, it is also the case that loss-of-function mutations associated with the CED-4 homologue, ARK (dApaf-1/HAC-1)[18] or its caspase-binding partner, DRONC, lead to extensive disruption of the normal patterns of cell death that are seen during development.[19–21] Furthermore, despite intensive genetic screening using both worm and fly, no other proteases have emerged as playing influential roles in programmed cell death in these organisms.

In mammals, there is also good genetic evidence to argue that caspases and their activators play central roles in apoptosis. To date, knockouts have been generated for the apoptosis-associated caspases, caspase-2, caspase-3, caspase-6, caspase-7, caspase-8 and caspase-9, as well as the caspase-9 adaptor protein Apaf-1.[22–24] In several of these cases, programmed cell death is severely disrupted and embryonic lethality is observed. However, as discussed above, mutation or inactivation of caspases rarely confers clonogenic rescue upon a cell owing to the fact that one of the most commonly utilised routes to apoptosis in mammals, the mitochondrial pathway, involves permeabilization of mitochondria before caspase activation.[13] Because of this, blocking caspase activation downstream of mitochondrial outer membrane permeabilization is typically futile as cells die as a result of mitochondrial dysfunction. However, death without caspase participation rarely, if ever, manifests the normal spectrum of changes that define apoptosis.[25] Nonetheless, some investigators have labeled this type of cell death 'atypical apoptosis' or 'caspase-independent apoptosis' or 'aponecrosis' which can give rise to misunderstandings concerning whether caspases are really required for apoptosis.

Global Perspectives on Caspase-Mediated Substrate Proteolysis

Given the embarassment of riches presented by approximately 400 mammalian caspase substrates that have now

been described (see Supplemental Table 1), it is unfortunately not yet possible to paint a clear picture of how caspases achieve the sterotypical changes that are seen during apoptosis. Indeed because most of the known caspase substrates have been identified serendipituously, it is very likely that hundreds of additional caspase substrates are likely to emerge upon systematic proteomics-based analyses of the proteomes of apoptotic cells.

Although we have tabulated essentially all of the caspase substrates that have been reported in the literature to date, it is important to point out that many of these substrates are simply reported to be cleaved during apoptosis and data pertaining to their functional significance have yet to emerge. Moreover, in most cases the caspase cleavage sites within these proteins have not been mapped, nor has it been tested whether these proteins are also targeted for proteolysis by caspases in other mammalian species, such as the mouse. Therefore, the precise role of the majority of caspase substrates in apoptosis remains speculative, at best, and many are almost certainly insignificant for the completion of the process. However, some caspase substrates have been definitively linked to specific cell death end points as we will discuss below.

The CASBAH: The CAspase Substrate DataBAse Homepage

Because of the expanding list of caspase substrates, we have created an online database containing all of the reported mammalian caspase substrates (http://www. casbah.ie). This database is fully searchable and contains linkouts to the UniProt (the Universal Protein Resource) database, which contains extensive additional information on each protein. In addition, where the caspase cleavage site(s) with a substrate have been mapped, this information is included within the CASBAH entry, along with relevant references to the primary papers that first reported a particular caspase substrate. We hope that this will be a useful resource for investigators working in this area.

Relative contributions of Caspases to Cell Demolition

The majority of studies indicate that caspases are certainly not equal in terms of their ability to cleave cellular substrates. The apoptosis-associated initiator caspases (caspase -8, -9 and -10 and possibly also caspase-2) appear to be highly specific proteases that cleave few substrates apart from their own precursors and other caspases downstream. Apart from other caspases, the only well-established substrates for the initiator caspases, which have been identified to date, is the BH3-only protein BID and RIP kinase. BID is cleaved by caspases -8 and -10 upon stimulation of death receptors and plays an important role in the propagation of pro-apoptotic signals through promoting mitochondrial outer membrane permeabilization and cytochrome *c* release.[26,27] RIP is recruited to the tumour necrosis factor and Fas receptor complexes and controls nuclear factor-kappa B (NFκB) activation via recruitment of the regulatory subunit of the IκB kinases complex.[28,29] However, RIP can be inactivated through proteolysis by caspase-8 and this facilitates propagation of the death signal as opposed to NFκB-mediated survival signals.[28,29]

There is little evidence that the initiator caspases can contribute to the generalised proteolysis seen during apoptosis. However, it is useful to point out that high (i.e. nonphysiological) concentrations of recombinant caspases will cleave practically any caspase substrate *in vitro*. Clearly, this does not offer proof that this protease is responsible for this event *in vivo*, although it is often implied that this is the case. So, which proteases are responsible for most of the substrate proteolysis seen during the demolition phase of apoptosis? Evidence from caspase-deficient cells and cell lines, as well as immunodepleted cell-free extracts, point towards caspase-3 as the major effector caspase.[25,30,31] Loss of caspase-3 abolishes or dramatically delays the kinetics of the majority of substrate proteolysis seen during apoptosis. Thus, the vast majority of substrates listed in Supplementary Table 1 are most likely preferentially cleaved by caspase-3 during apoptosis. Caspase-7 possesses similar specificity to caspase-3 towards synthetic tetrapeptide substrates and these enzymes are sometimes regarded as functionally redundant.[32,33] However, activity towards synthetic tetrapeptide-based substrates does not give information concerning interactions with full-length substrate proteins, particularly at surfaces outside of the catalytic pocket of the enzyme. Therefore, it is certainly plausible that caspases -3 and -7 exhibit distinct activities towards full length substrate proteins. Indeed, gene knockout and immunodepletion experiments argue that caspase-7 cannot readily substitute for the absence of caspase-3, although this may also relate to the relative expression levels of either caspase within cells.[4,31,34–37]

Few cellular substrates for caspase-6 have been identified thus far. Although several substrates for caspase-6 have been reported, the majority of these claims rest upon data generated *in vitro* using high concentrations of recombinant caspases. The most well-established substrate for caspase-6 appears to be the nuclear lamins A and C,[31,38,39] whereas lamin B is cleaved by caspase-3.[31] Caspase-6 is also responsible for the proteolytic maturation of caspases -2 and -8 within the caspase cascade that is initiated downstream of assembly of the Apaf-1 apoptosome.[4,40,41] However, loss of caspase-6 appears to have no overt effect on developmental apoptosis.[22,36] Thus, the contribution of caspase-6 to the demolition phase of apoptosis remains enigmatic.

How do Caspases Coordinate Apoptosis?

Of the hundreds of caspase substrates that have been reported to date, a small subset of these have been convincingly linked with specific alterations to the cellular architecture that are generally accepted to be hallmarks of apoptosis. The list of these ‘intended victims’ is surprisingly short at present.

It is worth reminding ourselves that apoptosis is a morphologically defined mode of cell death that is associated with several distinct features.[42,43] Of course, there are likely to be other modes of programmed cell death that display different characteristics, but these modes of cell death are, almost by definition, not apoptotic in nature. On the basis of the original studies of Kerr *et al.*,[42] apoptotic cells typically

display pronounced compaction of chromatin and, in the majority of cases, the nucleus undergoes progressive fragmentation and dispersal throughout the cell body. The latter events are highly distinctive and can be completely blocked by inhibition of caspase activity.[44,45] Studies on the serine/threonine kinase rho-associated kinase I (ROCK I) have strongly implicated this kinase in apoptosis-associated nuclear fragmentation (Figure 1).[46,47] Caspase-mediated proteolysis of ROCK I leads to loss of the C-terminal autoinhibitory region of this molecule and produces a constitutively active kinase which is capable of contributing to myosin light chain phosphorylation and other events leading to increased actin–myosin contractility (Figure 1). This increased contractility of the actin cytoskeleton, in tandem with caspase-mediated proteolysis of the nuclear lamins that contribute to weakening of the nuclear envelope,[48] has been linked with the dismembering of the nucleus into several fragments as typically occurs during apoptosis.[46,47]

Cells undergoing apoptosis also retract from neighbouring cells, losing contact with other cells and the substratum and usually undergo extensive plasma membrane blebbing which can also lead to collapse of the cell into numerous small fragments, termed apoptotic bodies.[42] However, the production of apoptotic bodies seems to be very dependent on cell type, as some cell types fragment to a greater or lesser degree than others. Once again, neutralisation of caspase activity is a very efficient means of blocking apoptosis-associated cellular retraction, plasma membrane blebbing and apoptotic body production.[30,49] Although it remains unclear precisely how caspases coordinate cellular retraction, this very likely relates to the ability of caspases to cleave several cytoskeletal proteins such as actin, tubulin, fodrin and vimentin. Many other proteins with structural roles within the cell are also cleaved during apoptosis (see Table 1) and it is likely that proteolysis of such proteins contributes to the dramatic morphological rearrangements that take place during this mode of cell death.

ROCK I has also been heavily implicated in the production of plasma membrane blebs during apoptosis, again through reorganisation of the actin–myosin cytoskeleton owing to the caspase-dependent loss of ROCK I-auto-inhibition (Figure 1).[46,47,49,50] In agreement with this, depolymerisation of the actin cytoskeleton using fungal toxins efficiently abolishes the production of apoptotic bodies.[51]

Although not a morphological feature of apoptosis, one of the earliest biochemical signatures of this process to be

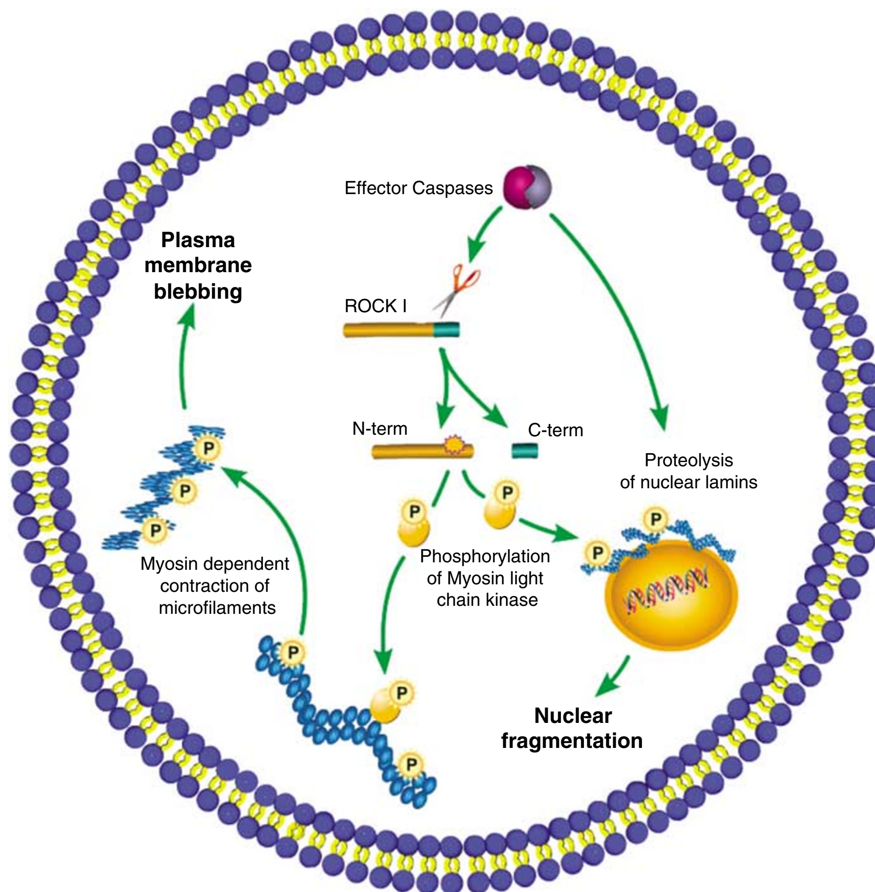

Figure 1 Caspase-dependent proteolysis of ROCK I contributes to plasma membrane blebbing and nuclear fragmentation. Caspase-dependent cleavage of ROCK I removes the regulatory C terminus of the molecule, resulting in a constitutively active kinase. Active ROCK I promotes phosphorylation of myosin light chain kinase which, in turn, regulates myosin-dependent actin rearrangements. Rearrangement of actin microfilaments can contribute to plasma membrane blebbing and, in tandem with caspase-dependent cleavage of nuclear lamins, nuclear fragmentation

Table 1 Caspase substrates: structural proteins

Name	UniProt	Consequences of caspase-dependent proteolysis (proposed)	Site(s)
α-Actin	ACTC_HUMAN	Putative: myofibrillar damage	Unknown
β-Actin	ACTB_HUMAN	Unknown	ELPD[244]
α-Actinin	ACTN1_HUMAN	Putative: myofibrillar damage	Unknown
α-Adducin	ADDA_HUMAN	Putative: reduction of adducin from adherens junctions and possibly cell detachment	DDSD[633]
AIIx	APC_HUMAN	Separates β-catenin binding region and armadillo repeat	DVID[777]
BAP31	BAP31_HUMAN	Downregulation of surface CD9/CD80 and reduction of cell attachment to fibronectin.	AAVD[163]
BAT3	BAT3_HUMAN	Overexpressed C-terminal fragment leads to morphological changes similar to apoptosis	DEQD[1001]
β-Catenin	CTNB1_HUMAN	Reduced α-catenin binding. Putative: loss of cell adhesion	YQDD[145], NDED[164], SYLD[32], ADID[83], TQFD[115], YPVD[751]
Cadherin 2	CADH2_HUMAN	Putative: loss of cell adhesion	Unknown
Cas	BCAR1_HUMAN/ MOUSE	Contributes to disassembly of focal adhesion complexes	DVPD[416], DSPD[748]
CALM	Q6GMQ6_HUMAN	Unknown	Unknown
CD-IC	DC1I1_HUMAN	Destroys the cytoplasmatic dynein complex and stops dynein dependent membrane motility	DSGD[116]
Cortactin	Q96H99_HUMAN	Putative: disruption of cytoskeletal reorganisation	Unknown
Desmocollin-3	DSC3_HUMAN	Putative: loss of cell–cell contact	Unknown
Desmoglein-1	DSG1_HUMAN	Decreased cell surface expression of desmoglein 1	DLRD[888]
Desmoglein-3	DSG3_HUMAN	Putative: loss of cytoskeleton structure and cell–cell contact	DYAD[781]
Desmoplakin	DESP_HUMAN	Putative: loss of cytoskeleton structure and cell–cell contact	Unknown
DLG1	DLG1_HUMAN	Putative: disruption of cell–cell contact	QSVD[427]
Dynactin-1	DYNA_HUMAN	Destroys the cytoplasmatic dynein complex and stops dynein dependent membrane motility	Unknown
E-cadherin	CADH1_HUMAN/ MOUSE	Release of intracellular fragment	DTRD[750]
N-cadherin	CADH2_HUMAN	Putative: loss of cell adhesion	Unknown
P-cadherin	CADH3_HUMAN	Possibly disruption of cell to cell contact	Unknown
Emerin	EMD_HUMAN	Putative: involved in nuclear envelope breakdown	Unknown
Filamin-A	FLNA_HUMAN	Putative: disruption of cytoskeletal reorganisation	Unknown
FAK	FAK1_HUMAN	Contributes to disassembly of focal adhesion complexes	DQTD[772]
Gas2	GAS2_HUMAN	Regulation of microfilament and cell shape changes	SRVD[37]
γ-Catenin	Q86W21_HUMAN	Inactivated, involved in cell dismantling. Putative: loss of cell adhesion	Unknown
Gelsolin	GELS_HUMAN	Loss of monomeric actin binding and triggering of F-actin depolymerisation, membrane blebbing	DQTD[403]
Helicard	IFIH1_MOUSE	C-terminal fragment accelerated DNA degradation. Putative: involved in chromatin remodelling	DNTD[208], SCTD[251], SHRD[216]
HIP-55	DBNL_HUMAN	Dissociates the actin binding from the SH3 domain, Putative: cytoskeletal reorganisation	EHID[361]
HS1	HCLS1_HUMAN	Putative: cytoskeletal reorganisation	Unknown
Lamin A /C	LMNA_HUMAN	Breakdown of nuclear envelope	VEID[230]
Lamin B1	LMNB1_HUMAN	Nuclear lamina disassembly	VEID[231]
Lamin B2	LMNB2_HUMAN	Putative: nuclear lamina disassembly	Unknown
LAP2-α	LAP2A_HUMAN	Loss of chromatin association. Putative: detachment of chromatin from the nuclear lamina	Unknown
LBR	LBR_HUMAN	N-terminal fragment relocalises to cytosol. Putative: nuclear lamina disassembly	Unknown
Myosin heavy chain	MYH2_HUMAN	Unknown	Unknown
Myosin-14	MYH14_HUMAN	Unknown	Unknown
Myosin-9	MYH9_HUMAN	Unknown	DTLD[1152]
P-cadherin	CADH3_HUMAN	Possibly disruption of cell to cell contact	Unknown
Plakophilin-1	PKP1_HUMAN	Putative: loss of cytoskeleton structure and cell–cell contact	Unknown
Plectin	PLEC1_HUMAN	Putative: reorganisation of microfilament system	ILRD[2395]
ROCK-1	ROCK1_HUMAN	Constitutively activates kinase activity and drives cell contraction and blebbing.	DETD[1113]
α-Spectrin	SPTA2_HUMAN	Disrupts cytoskeleton, possibly involved in membrane blebbing	DETD[1185]
β-Spectrin erythroid	SPTB1_HUMAN	Disruption of cortical cytoskeleton, possibly involved in membrane blebbing	DSLD[1478], DEVD[1457], ETVD[2146]
β-Spectrin nonerythroid	SPTB2_HUMAN	Disrupts cytoskeleton, possibly involved in membrane blebbing	DEVD[1457]
SLK	SFRS9_HUMAN	N-terminal fragment: kinase promotes cytoskeletal rearrangement. C-terminal: disassembles actin fibres	Unknown
Troponin T	TNNT2_HUMAN	Involvement in myofibrillar damage and contractile dysfunction	VDFD[96]
α-Tubulin 1	TBA1_HUMAN	Unknown	Unknown
Vimentin	VIME_HUMAN	Disrupts intermediate filaments, proapoptotic	DSVD[85], IDVD[259], TNLD[429]
Vinculin	VINC_HUMAN	Putative: disruption of cytoskeletal assembly	Unknown
vMLC1	MYL3_HUMAN	Reduced myocyte contractile performance	DFVE[135]

Table 2 Caspase substrates: regulators of transcription/translation

Name	UniProt	Consequences of caspase-dependant proteolysis (proposed)	Site(s)
AP2 α	AP2A_HUMAN	Putative: loss of DNA binding capability, reduction in endocytosis	DRHD[19]
ATM	ATM_HUMAN	Loss of kinase activity. Fragment still binds DNA and acts as a dominant-negative inhibitor	DYPD[863]
BCAR1	BCAR1_HUMAN/ MOUSE	Overexpressed C-terminal fragment binds E2A and inhibits p21$^{WAF1/Cip}$ transcription	DSPD[650]
BLM	BLM_HUMAN	Interaction with topoisomerase 3α is impaired but the C-terminal fragment retains helicase activity	TEVD[415]
BTF3	BTF3_HUMAN	Unknown	Unknown
c-Rel	REL_HUMAN	Loss of transcriptional activity	Unknown
CREB	CREB1_HUMAN	Putative: abolishes antiapoptotic effect	Unknown
Nuclear DNA helicase II	DHX9_HUMAN	Putative reduction of transcription	EEVD[167]
eIF2a	IF2A_HUMAN	Putative: N-terminal fragment might not be phosphorylated by PKR	AEVD[301], DGDD[304]
eIF3	IF3A_HUMAN	Unknown	DLAD[242], DYED[256]
eIF3S1	IF31_HUMAN	Unknown	DSWD[15]
eIF4B	IF4B_HUMAN	Loss of poly(A)-binding and translation	DETD[45]
eIF4E type 3	IF4E3_HUMAN	Unknown	TQKD[27]
eIF4E-BP1	4EBP1_HUMAN	Dominant-negative inhibitor of CAP-dependent translation	VLGD[25]
eIF4G 1	IF4G1_HUMAN	Inhibition of translation	DLLD[492], DRLD[1136]
eIF4G 2	IF4G2_HUMAN	DAP5 still binds to eIF4A and eIF3 and has an increased translation from IRES sites	DETD[790]
eIF4G 3	IF4G3_HUMAN	Dominant-negative inhibitor of CAP-dependant translation	Unknown
eIF4H	IF4H_HUMAN	Unknown	DEVD[92]
FHOD1	FHOD1_HUMAN	Overexpressed C-terminal fragment translocates to nucleus and inhibits transcription	SVPD[615]
FLI-1	FLI1_MOUSE	Putative: loss of transcription factor function to ensure apoptosis	SLFD[20], MEID[155], SHTD[209]
GATA-1	GATA1_HUMAN	Loss of transcriptional activity which leads to impaired erythropoiesis	EDLD[125]
Helicard	IFIH1_MOUSE	Overexpressed C-terminal fragment translocates to the nucleus and accelerated DNA degradation	DNTD[208], SCTD[251], SHRD[216]
hnRNP A0	ROA0_HUMAN	Reduced RNA processing	HAVD[73]
hnRNP A1	ROA1_HUMAN	Unknown	Unknown
hnRNP A2/B1	ROA2_HUMAN	Reduced RNA processing	Unknown
hnRNP A3	ROA3_HUMAN	Reduced RNA processing	Unknown
hnRNP C1/C2	HNRPC_HUMAN	Unknown	Unknown
hnRNP F	HNRPF_HUMAN	Unknown	Unknown
hnRNP G	HNRPG_HUMAN	Unknown	Unknown
hnRNP I	PTBP1_HUMAN	Reduced RNA processing	IVPD[7], AAVD[172]
hnRNP K	HNRPK_HUMAN	Reduced RNA processing	Unknown
hnRNP R	HNRPR_HUMAN	Reduced RNA processing	Unknown
hnRNP U	HNRPU_HUMAN	Loss of DNA binding and translocation to cytoplasm	Unknown
HSF	HSF1_HUMAN	Putative: loss of protective heat shock response	Unknown
ICAD/DFF45	DFFA_MOUSE	Release of active CAD endonuclease	DETD[117], DAVD[224]
La	LA_HUMAN	Putative: accumulation in nucleus and reduction of Pol III transcription	DEHD[374]
LEDGF	PSIP1_HUMAN	Loss of antiapoptotic function under conditions of serum starvation	EVPD[30], WEID[85], DAQD[486]
Max	MAX_HUMAN	Higher DNA-binding affinity	IEVE[10], SAFD[135]
MEF2A	MEF2A_HUMAN	Putative: reduced transcriptional activity	SSYD[466], STTD[215], TLTD[176]
MEF2C	MEF2C_HUMAN	Putative: reduced transcriptional activity	SSYD[442]
MEF2D	MEF2D_HUMAN	Loss of the transactivation domain. Putative: reduced transcriptional activity	LTED[288], DHLD[291]
MCM 2	MCM2_HUMAN	Putative: inhibition of replication	Unknown
MCM 3	MCM3_HUMAN	Putative: inhibition of replication	Unknown
NAC α	NACA_HUMAN	Unknown	Unknown
NF-κB p50	RELB_HUMAN	Loss of DNA binding	Unknown
NF-κB p65	TF65_HUMAN	Dominant-negative fragment, proapoptotic	Unknown
NONO	NONO_HUMAN	Unknown	Unknown
Nucleolin	NUCL_HUMAN	Unknown	Unknown
NRF2	NF2L2_HUMAN	Overexpressed C-terminal fragment is proapoptotic	TEVD[208], EELD[366]
PABP4	PABP2_HUMAN	Unknown	VEGD[107]
Pol ε	DPOE1_HUMAN	Loss of catalytic subunit binding capability	DQLD[216], DEMD[1214]
RAR α	RARA_HUMAN	Loss of transcriptional activity	Unknown
Relish	NFKB1_DROME	Putative: loss of transcriptional activity	Unknown
RHA	DHX9_HUMAN	Putative reduction of transcription	EEVD[167]
RIP-1	RIPK1_HUMAN	Inhibits NF-κB activation	LQLD[324]
SATB1	SATB1_HUMAN	Putative: loss of transcriptional activity	Unknown
Serum response factor	SRF_HUMAN	Putative: loss of DNA binding and loss of survival signalling	Unknown
SnRNP70	RU17_HUMAN	Reduced RNA processing	DGPD[341]
SP1	SP1_HUMAN	Loss of DNA binding	NSPD[590]
Splicing factor 45	SPF45_HUMAN	Unknown	TEKD[286]
SREBP-1	SRBP1_HUMAN	Unknown	SEPD[460]
SREBP-2	SRBP2_HUMAN	Unknown	DEPD[486]

Table 2 *(Continued)*

Name	UniProt	Consequences of caspase-dependant proteolysis (proposed)	Site(s)
SRP72	SRP72_HUMAN/ MOUSE	Unknown	SELD[614]
SSRP1	SSRP1_HUMAN	C-terminal fragment: loss of chromatin binding. Putative: impairment of transcription/replication	DQHD[450]
STAT1	STAT1_HUMAN	Blocks interferon and cytokine signalling	MELD[694]
STAT3	STAT3_HUMAN	Reduced DNA binding and hence reduction in transcription factor activity	Unknown
TAF6	TAF6_HUMAN	Alteration of transcription of gadd45 and p21	Unknown
TRAF1	TRAF1_HUMAN	Inhibits NF-κB activation, proapoptotic	LEVD[163]
Vav-1	VAV_HUMAN	Reduced activation of AP-1, NF-κB, NF-AT, p38 but not JNK	DQID[150], DLYP[161]

Table 3 Caspase substrates: kinases and signalling intermediaries

Name	UniProt	Consequences of caspase-dependant proteolysis (proposed)	Site(s)
AKT	AKT1_HUMAN	Loss of kinase activity, Putative: loss of survival signalling	ECVD[462], TVAD[108], EEMD[119]
Bid	BID_HUMAN	Translocates to mitochondria and induces apoptosis through MOMP	LQTD[59], IEAD[74]
BMX	BMX_HUMAN	Increased kinase activity and proapoptotic upon overexpression of cleaved fragment	DFPD[242]
CaMKII-α	KCC2A_RAT	Unknown	Unknown
CaMKIV	KCC4_MOUSE	Loss of kinase activity	PAPD[176]
CaMKLK	MP2K1_HUMAN / MOUSE	N-terminal fragment is proapoptotic, C-terminal fragment loses kinase activity	DEND[62]
PPP3CA	PP2BA_HUMAN	Constitutively active phosphatase which triggers NF-AT activation and IL-2 release	DFGD[386]
Cdc42	CDC42_HUMAN	Overexpression of non-cleavable mutant is antiapoptotic	DLRD[121]
Claspin	CLSPN_HUMAN	C-terminal fragment inhibits Chk1 phosphorylation and checkpoint signalling	DEYD[1072]
CDK inhibitor 1	CDN1A_HUMAN	Reduced association with cyclin-cdk2 complexes and increased cdk2 activity	DHVD[112]
ρ-GDI 2	GDIS_HUMAN	Translocation to nucleus. Loss of ρ- GTPase signalling	DELD[19]
DNA-PKCs	PRKDC_HUMAN	Loss of catalytic protein kinase activity	DEVD[2713]
EGF-R	EGFR_HUMAN	Impaired tyrosine phosphorylaion of PLC-γ 1 and hence impaired survival signalling	Unknown
ErbB-2	ERBB2_HUMAN	Putative: loss of kinase activity	SETD[1125], DVFD[1087]
Fyn	FYN_HUMAN	Relocalisation and increased kinase activity	EERD[19]
GrpL/Gads	GRAP2_HUMAN	Desensitisation of antigen receptor signalling	DIND[235]
HEF1	CASL_HUMAN	Putative: disrupts antiapoptotic integrin signalling	DLVD[363], DDYD[630]
HPK-1	M4K1_MOUSE	Increased kinase activity	DDVD[385]
IPLA2	PA2G6_HUMAN	Increased phospholipid turnover, release of LPC which attracts monocytic cells	DVTD[183]
IRS-1	IRS1_HUMAN	Putative: suppresses IGF-mediated survival signalling	Unknown
IRS-2	IRS2_HUMAN	Putative: suppresses IGF-mediated survival signalling	Unknown
Kinectin	KTN1_HUMAN	Putative: disruption of the membrane trafficking pathway	Unknown
Lyn	LYN_HUMAN	Relocalisation and increased kinase activity	DGVD[18]
MEK	MP2K1_MOUSE	Reduced Erk1/2 phosphorylation	Unknown
MEKK1	M3K1_MOUSE	Increased kinase activity	DTVD[874] (not conserved)
Mst1	STK4_HUMAN	Kinase constitutive active.	DEMD[326]
Mst2	STK3_HUMAN	Kinase constitutive active	DELD[322]
Mst3	STK24_HUMAN	Kinase constitutive active.	AETD[313]
$p27^{Kip1}$	CDN1B_HUMAN	Reduced binding to cyclin cdk2 and increased cdk2 activity	DPSD[139], ESQD[108]
PAK2	PAK2_HUMAN	Kinase constitutive active and activates c-Jun N-terminal kinase pathway.	SHVD[212]
PDE6	PDE6A_HUMAN	Reduced cGMP-hydrolyzing activity	Unknown
Phosphorylase b kinase	KPB1_HUMAN	Putative: slight increased phoshphorylase activity	DWMD[646]
PKC δ	KPCD_HUMAN	Kinase constitutive active. Proapoptotic upon overexpression of fragment	DMQD[329]
PKC ε	KPCE_HUMAN/ MOUSE	Kinase constitutive active	SSPD[383]/SATD[383]
PKC η	KPCL_HUMAN	Kinase constitutive active. Proapoptotic upon overexpression of fragment	Unknown
PKC μ	KPCD1_HUMAN	Increased sensitivity to genotoxic stress	CQND[378]
PKC θ	KPCT_HUMAN/ MOUSE	Kinase constitutive active.	DEVD[354]/DDND[355], DQED[397]
PKC ζ	KPCZ_HUMAN	Kinase constitutive active	EETD[210], DGVD[239]
PKR	E2AK2_HUMAN	Kinase constitutive active, phosphorylates eIF2a which leads to translational inhibition	DLPD[251]
PLC γ1	PLCG1_HUMAN	Facilitates apoptosis	AEPD[770]
PMCA4	AT2B4_HUMAN	Conflicting reports. Enzyme activity is lost or kept	Unknown

Table 3 *(Continued)*

Name	UniProt	Consequences of caspase-dependant proteolysis (proposed)	Site(s)
PP2A	2AAA_HUMAN	Increased phosphatase activity	DEQD[218]
PPP3CA	PP2BA_HUMAN	Constitutively active phosphatase which triggers NF-AT activation and IL-2 release	DFGD[386]
PRK1	PKN1_HUMAN	Kinase constitutive active	Unknown
pro-IL-16	IL16_HUMAN	Induces T-cell chemotaxis	SSTD[510]
pro-IL-18	IL18_HUMAN	Activates IL-18 and induces IFN-γ production	LESD[36]
pro-IL-1β	IL1B_HUMAN	Activated IL-1β and Induces inflammatory response	YVHD[116]
pro-IL-33	IL33_HUMAN	Putative: Activated IL-33 which sensitises towards a TH2 response	Unknown
Rabaptin-5	RABE1_HUMAN	Block of endosome fusion	DESD[438]
Raf-1	RAF1_HUMAN	Overexpressed C terminus translocates to mitochondrial fraction and has a higher enzymatic activity	Unknown
Ran-GAP1	RGP1_HUMAN	Putative: altered nuclear pore transport	DEGD[157], DTVD[459]
Ras-GAP	RASA1_HUMAN	Low-caspase activity generates antiapoptotic N terminus. High-caspase activity generates proapoptic fragments	DTVD[455], DEGD[157]
RET	RET_HUMAN	Proapoptotic	VSVD[707], DYLD[1017]
ρ-GDI 2	GDIS_HUMAN	Translocation to nucleus. Loss of ρ- GTPase signalling	DELD[19]
ROCK 1	ROCK1_HUMAN	Constitutively activates kinase activity and drives cell contraction and blebbing.	DETD[1113]
SLK	SLK_HUMAN	Unknown	Unknown
SPAK	STK39_HUMAN/ MOUSE	Removal of substrate binding domain. Downregulation sensitises to apoptosis	Murine: ANED[455], DTAD[491]
Src	SRC_HUMAN	Putative: loss of antiapoptotic signalling downstream of EGFR	Unknown
SRPK1	SRPK1_HUMAN	Decreased kinase activity	Unknown
SRPK2	SRPK2_HUMAN	Decreased kinase activity	Unknown
STAT1	STAT1_HUMAN	Blocks interferon and cytokine signalling	MELD[694]
SLK	SFRS9_HUMAN	N terminus: kinase promotes apoptosis and cytoskeletal rearrangement. C terminus: disassembles actin fibres	Unknown
TCR ζ chain precursor	CD3Z_HUMAN	Putative: degradation of protein	GLLD[28] or YLLD[36],DTYD[153]
TNF-R1	TNR1A_HUMAN	Unknown	Unknown
TRAF1	TRAF1_HUMAN	Inhibits NF-κB activation, proapoptotic	LEVD[163]
TRAF3	TRAF3_HUMAN	Altered cellular distribution	EEAD[348], ESVD[368]
Tax1-binding protein 1	TAXB1_HUMAN	Loss of antiapoptotic effect	Unknown
Wee1	WEE1_HUMAN	$>$20-fold reduction of kinase activity	Unknown

identified was the internucleosomal scission of DNA into multiples of approximately 200 bp,[43] an event that is also under the control of caspases. Caspase inhibitors very efficiently block this extensive DNA hydrolysis, apparently through delaying the caspase-3 -mediated inactivation of inhibitor caspase-activated DNase (ICAD)/DNA fragmentation factor (DFF)45, the inhibitory subunit of the caspase-activated DNase (CAD)/DFF40 nuclease.[52,53] Multiple transcription factors are also cleaved by caspases and a range of translation initiation factors and ribosomal proteins are also affected (see Table 2). Predictably, this results in transcriptional and translation shut down relatively early in the process. Similar to DNA hydrolysis during apoptosis, it is not clear why the transcriptional and translational machineries are targeted by caspases. A plausible explanation is that this may guard against the possibility that these machineries could be used to replicate viruses that might have provoked apoptosis in the first place. Similarly, apoptosis-associated DNA hydrolysis may also safeguard against the persistence of viral DNA. Alternatively, the latter event may simply render chromatin more manageable for subsequent removal by phagocytes.

Numerous kinases and other signaling intermediaries have also been reported to be cleaved by caspases during apoptosis (see Table 3). In many cases, this results in the production of constitutively active forms of such kinases but, with the exception of ROCK I as discussed above, it is likely that many of these events are not especially relevant for apoptosis.

A major goal of programmed cell death is to facilitate the swift clearance of dying cells while avoiding release of cellular contents. A number of alterations to the composition of the membranes of apoptotic cells have been documented,[54] perhaps the most well known of which is the externalisation of phosphatidylserine on the outer leaflet of the plasma membrane.[55] Although this event is also blocked by inhibition of caspase activity,[56] it remains unclear how phosphatidylserine externalisation or other apoptosis-associated membrane events, is regulated by caspases. In addition to becoming licensed for removal by phagocytes, apoptotic cells may also actively attract the attentions of such cells by secreting molecules with chemotactic properties. This may occur through the caspase-dependent release of a chemoattractant lipid, lysophosphatidylcholine (LPC), possibly mediated by cleavage and activation of calcium independent phospholipase A (iPLA) by caspase-3 (Figure 2).[57]

Concluding Remarks

The knowledge that there are in excess of 400 proteins that become targeted for proteolysis by caspases during the terminal phase of apoptosis suggests that we are still some way from fully understanding how these proteases coordinate this cell death paradigm. It seems rather unlikely that failure to cleave individual substrates is likely to have any impact on the kinetics of cell death in the great majority of cases. Rather, failure to cleave particular substrates may well alter certain

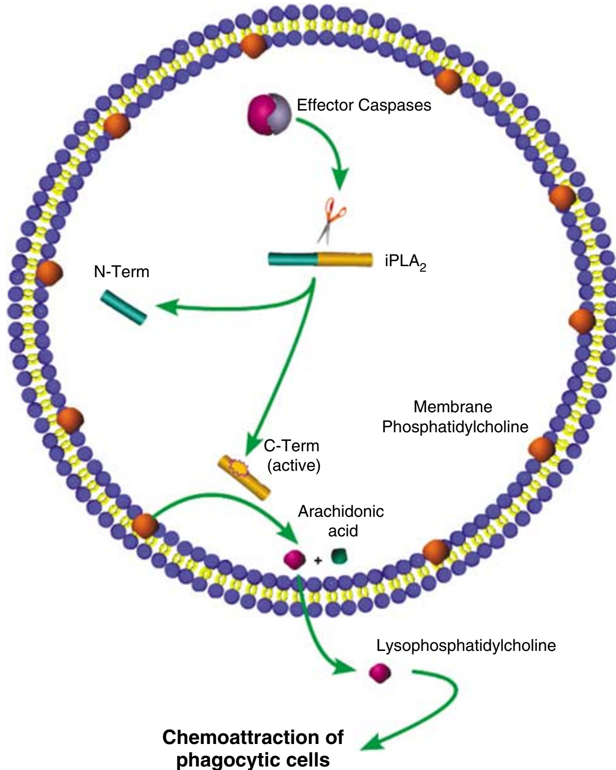

Figure 2 Caspases promote the release of chemotactic factors via iPLA2 Activated effector caspases cleave the calcium independent phospholipase A_2 resulting in a constitutively active C-terminal fragment. Active $iPLA_2$ hydrolyses membrane bound phosphatidylcholine to bioactive LPC and arachidonic acid. LPC is released from the cell to the extracellular space and acts as a chemoattractant for phagocytic cells, which engulf and remove the apoptotic cell

aspects of the apoptotic phenotype; a good example of which is the pronounced delay in the kinetics of DNA fragmentation upon disablement of the ICAD/CAD system. However, discovery of especially influential caspase substrates appears to be the exception rather than the rule, with the majority of substrates that have been reported to date playing relatively minor roles in the process. Owing to the sheer number of proteins that are cleaved by caspases during the throes of death, it has thusfar proved difficult to identify a subset of proteins that have particular relevance for the signature events that define apoptosis. Nonetheless, a blueprint for how caspases kill is slowly emerging from the dense thicket of caspase substrates that have been identified to date.

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