

## Review

# Many cuts to ruin: a comprehensive update of caspase substrates

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## Abstract

Apoptotic cell death is executed by the caspase-mediated cleavage of various vital proteins. Elucidating the consequences of this endoproteolytic cleavage is crucial for our understanding of cell death and other biological processes. Many caspase substrates are just cleaved as bystanders, because they happen to contain a caspase cleavage site in their sequence. Several targets, however, have a discrete function in propagation of the cell death process. Many structural and regulatory proteins are inactivated by caspases, while other substrates can be activated. In most cases, the consequences of this gain-of-function are poorly understood. Caspase substrates can regulate the key morphological changes in apoptosis. Several caspase substrates also act as transducers and amplifiers that determine the apoptotic threshold and cell fate. This review summarizes the known caspase substrates comprising a bewildering list of more than 280 different proteins. We highlight some recent aspects inferred by the cleavage of certain proteins in apoptosis. We also discuss emerging themes of caspase cleavage in other forms of cell death and, in particular, in apparently unrelated processes, such as cell cycle regulation and cellular differentiation.

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**Abbreviations:** See Table 1

## Introduction

In 1998, we published a list of caspase substrates comprising 65 different proteins that were cleaved by proteases of the caspase family.<sup>1</sup> Most of the substrates known at that time could be categorized into a few functional groups, including proteins involved in scaffolding of the cytoplasm and nucleus,

signal transduction and transcription-regulatory proteins, cell cycle controlling components and proteins involved in DNA replication and repair. Since then, the number of caspase substrates has considerably increased, more recently in particular because of a systematic proteome analysis of apoptotic cells.<sup>2–4</sup> To date, more than 280 caspase targets are identified. Various methods have been employed to search for caspase substrates, including direct cDNA pool expression strategies or two-hybrid cloning approaches.<sup>5,6</sup> By comparative two-dimensional (2D) gel electrophoresis of healthy and apoptotic cells, often a few hundred altered protein spots can be detected. Although not all of them have been confirmed as caspase targets, such proteomic approaches will certainly lead to the identification of numerous additional substrates in the near future (Table 1).

Already now, a bewildering number of substrates are cleaved by caspases. However, it should be kept in mind that some proteins might be cleaved very late and less completely during apoptosis, or not in all cell types. For example, it has been reported that  $\beta$ -actin can be cleaved by caspases in pheochromocytoma and ovarian carcinoma cells,<sup>7,8</sup> whereas in many other cell types no cleavage was detected.<sup>9</sup> Thus, it is possible that certain protein cleavages are cell type-specific, which may be because of variations in the expression of individual caspases. Also, caspase cleavage sites are not always conserved in different species. For instance, cyclin A is cleaved during apoptosis of *Xenopus* oocytes,<sup>10</sup> but the caspase cleavage site is not present in homologues of mammalian cells. Some proteins, such as DNase-X, contain one or more classical cleavage sites in their sequence. However, the protein is virtually not cleaved inside apoptotic cells despite massive caspase activation.<sup>11</sup> Moreover, in some cases, a first cut by caspases unleashes additional cleavage sites for other types of proteases. Cleavage of acinus, for instance, by caspase-3 is necessary but not sufficient to activate its DNA-condensing activity. For full activation, an additional, still unknown serine protease has to intervene. Only the combined action of both proteases generates the mature fragment, which, when added to purified nuclei, causes chromatin condensation.<sup>12</sup>

For many of the identified substrates, the functional consequences of their cleavage are unknown and have only been inferred from their normal functions. In other cases, the role of caspase cleavage has been experimentally assessed by expressing substrate proteins that have mutant caspase cleavage sites or by expressing protein fragments of the caspase-cleaved products. Given the high conservation of the apoptotic phenotype, from worms to mammals, it is highly likely that a conserved group of crucial caspase substrates exist. Proteolysis of the latter substrates presumably leads to the stereotypical destructive alterations that we call apoptosis.

**Table 1** List of known caspase substrates

Substrate <sup>b</sup>	Physiological Function	Cleavage Effect	Consequences of Cleavage	Cleavage Sites <sup>a</sup>	References
<b>1. Apoptosis regulators</b>					
Apaf-1	Apoptosome component	Inactivated?		SVTD (271) and a second unknown site	43, 44
Bad	Proapoptotic Bcl-2 protein	Activated	Cleavage product proapoptotic, if overexpressed	Human: EQED (14) Mouse: SATD (61)	45
Bax Bcl-2	Proapoptotic Bcl-2 protein Apoptosis inhibitor	Unknown Inactivated	Generation of a proapoptotic fragment	FIQD (33) DAGD (34)	46, 47 48
Bcl-x <sub>L</sub>	Apoptosis inhibitor	Inactivated	Generation of a proapoptotic fragment	HLAD (61), SSLD (76)	49, 50
Bid	Apoptosis activator	Activated	Generation of a proapoptotic fragment that is myristoylated; phosphorylation inhibits cleavage	LQTD (59)	14, 16, 51, 52
c-FLIP c-IAP1	Caspase-8 inhibitor Caspase inhibitor	Inactivated	Generation of a proapoptotic fragment	LEVD (376) ENAD (372)	53 54
Procaspases	Procaspase-1-14	Activated	Activation by proteolytic processing	XXXD	For a review see Earnshaw <i>et al.</i> <sup>55</sup>
XIAP	Caspase inhibitor	Inactivated?	Generation of two fragments with distinct inhibitory activity for caspase-3, -7 and -9. Cleaved XIAP is less antiapoptotic and ineffective to activate NF- $\kappa$ B	SESD (242)	56, 57
<b>2. Cell adhesion</b>					
APC	Adenomatous polyposis coli protein		Cleavage separates $\beta$ -catenin binding region and N-terminal armadillo repeat	DNID (777)	58, 59
CALM	Clathrin assembly protein of myeloid leukemia (syn. AP180), promotes assembly of clathrin triskelia into clathrin cages	Inactivated		Unknown	60
Cas	Crk-associated substrate (p130cas), associates with FAK, paxillin, involved in integrin signaling	Inactivated	Contributes to disassembly of focal adhesion complexes, interrupts extracellular survival signals	DVPD (416), DSPD (748)	61, 62
$\beta$ -Catenin	Cell adhesion and WNT/wingless signaling pathway, constituent of adherens junctions	Inactivated	Reduced $\alpha$ -catenin binding and cell-cell contact, reduced transcriptional activity, relocalization to the cytoplasm	SYLD (32), ADID (83), TQFD (115), YPVD (751), DLMD (764)	61, 63–65
$\gamma$ -Catenin	Adherens junction protein (syn. plakoglobin)	Inactivated	Relocalization to the cytoplasm, involved in cell dismantling	Unknown	61, 64, 66
Desmoglein-3	Major transmembrane component of desmosomes	Inactivated	Loss of cell-cell contacts	DYAD (781) and additional unknown sites	67
Desmocollin 3 Desmoplakin	Component of desmosomes Desmoplakin-1, -2, components of desmosomes	Inactivated Inactivated	Loss of cell-cell contacts Loss of cell-cell contacts	Unknown Unknown	67 67
E-cadherin	Calcium-dependent adhesion protein in adherens junctions	Inactivated	Rather late cleavage may contribute to disruption of cell-cell contacts	DTRD (750)	68, 69
N-cadherin	Calcium-dependent cell adhesion protein	Inactivated		Unknown	70
P-cadherin	Cell adhesion protein in adherens junctions	Inactivated?	Rather late cleavage may be involved in loss of cell-cell contacts	Putative site: ETAD (695)	69
FAK	Focal adhesion kinase, tyrosine kinase involved in formation of contact sites to extracellular matrix	Inactivated	Cleavage leads to disassembly of the focal adhesion complex, cell detachment and interruption of survival signals	DQTD (772)	71–74
HEF1	Human enhancer of filamentation 1, member of the docking protein family, involved in integrin signaling	Inactivated	Disruption of antiapoptotic integrin signaling	DLVD (363), DDYD (630)	75, 76
Connexin 45.6	Lens gap junction protein	Inactivated	Cleavage at a noncanonical site; phosphorylation by casein kinase II prevents degradation	DEVE (367)	77
Paxillin	Component of the focal adhesion complex	Inactivated	Cleavage results in focal adhesion disassembly and detachment	Early: NPQD (102), SQLD (301) Late: DDL D (5), SELD (146), FPAD (165), SLLD (222)	78, 79
Plakophilin-1	Component of desmosomes	Inactivated	Loss of cell-cell contacts	Unknown	67
<b>3. Cytoskeletal and structural proteins</b>					
$\alpha$ -Actin	Cardiac actin, myofilament protein	Inactivated	Rather inefficient cleavage by caspase-3, involved in myofibrillar damage	Unknown	80

Table 1 (continued)

Substrate	Physiological Function	Cleavage Effect	Consequences of Cleavage	Cleavage Sites <sup>a</sup>	References
$\beta$ -Actin	Cytoskeleton	Inactivated	Cleavage has not been observed in all cell types	ELPD (244)	7–9, 81
$\alpha$ -Actinin	Cardiac myofilament protein, component of the Z band	Inactivated	Cleavage results in myofibrillar damage	Unknown	80
$\alpha$ -Adducin	Actin-capping protein involved in actin network organization	Inactivated	Cleavage results in loss of adducin from adherens junctions and may contribute to cell detachment	DDSD (633)	82
CD-IC	Cytoplasmic dynein intermediate chain, mediates dynein/dynactin interaction	Inactivated	Cleavage destroys the cytoplasmic dynein complex and stops dynein-dependent membrane motility	DSGD (99) and an additional unknown site	83
Cortactin	Actin-binding and SH3-containing protein	Inactivated?	Probably involved in cytoskeletal reorganization	Unknown	84
Filamin	Actin-binding protein that crosslinks actin filaments and anchors membrane proteins to the cytoskeleton	Inactivated	Probably involved in cytoskeletal reorganization	Unknown	85, 86
Fodrin	Component of the membrane cortical cytoskeleton (syn. $\alpha$ II-spectrin)	Inactivated	Cleavage results in disruption of the cortical cytoskeleton and may contribute to membrane blebbing	$\alpha$ -II-Fodrin: DETD (1185) $\beta$ -II-Fodrin: DEVD (1457)	87–90
Gas2	Growth-arrest-specific 2 gene, involved in microfilament organization	Inactivated	Cleaved form specifically regulates microfilament and cell shape changes	SRVD (37)	91
Gelsolin	Actin-severing protein	Inactivated	Cleaved fragment triggers F-actin depolymerization and membrane blebbing	DQTD (403)	92, 93
HIP-55	Actin-binding protein with SH3 domain, interacts with hematopoietic progenitor kinase-1	Inactivated?	Cleavage dissociates the actin binding from the SH3 domain and leads to cytoskeletal reorganisation	EHID (361)	84
HS1	Hematopoietic-specific protein 1 (syn. Lck-binding protein)	Inactivated?	Probably involved in cytoskeletal reorganization	Unknown	84
Keratins	Cytokeratin-14, -17, -18, -19, intermediate filament proteins	Inactivated	Cleavage may contribute to cellular collapse	Keratin 18: VEVD(238), DALD (397)	94–97
MHC	Myosin heavy chain	Inactivated?	Identified by 2D gel electrophoresis, not confirmed by <i>in vitro</i> cleavage	Unknown	3
vMLC	Ventricular essential myosin light chain, cardiac myofilament protein	Inactivated	Cleavage at a noncanonical site results in myofibrillar damage, presumably involved in contractile dysfunction	DFVE (135)	98
p150 <sup>Glued</sup>	Mediates dynein/dynactin interaction	Inactivated	Cleavage destroys the cytoplasmic dynein complex and stops dynein-dependent membrane motility	Unknown	83
Plectin	Abundant crosslinking protein of cytoplasmic filament systems	Inactivated	Reorganization of the microfilament system	ILRD (2395)	99
$\beta$ -Spectrin	Component of the membrane cortical cytoskeleton	Inactivated	Cleavage results in disruption of cortical cytoskeleton and may contribute to membrane blebbing	DSL D (1478), DEVD (1457), ETV D (2146)	100
Tau	Neuronal microtubule-associated protein	Inactivated	Cleavage generates a proapoptotic fragment, may be involved in neuronal disorders	DMVD (421)	101, 102
Troponin T	Cardiac troponin, myofilament protein	Inactivated	Cleavage contributes to myofibrillar damage and contractile dysfunction	VDFD (96)	80
$\alpha$ -Tubulin	Component of microtubuli	Inactivated?	Identified by 2D gel electrophoresis, not confirmed by <i>in vitro</i> cleavage	Unknown	3
Vimentin	Intermediate filament specific for mesenchymal cells	Inactivated	Disruption of intermediate filaments and promotion of apoptosis	DSVD (85), IDVD (259), TNLD (429)	103, 104
<b>4. Nuclear structural and abundant proteins</b>					
Emerin	Nuclear membrane-anchored protein mutated in Emery–Dreifuss muscular dystrophy, related to LAP2 $\alpha$	Inactivated	Cleavage may contribute to nuclear envelope breakdown	Unknown	105
LBR	Lamin B receptor; chromatin and lamin-binding protein in the inner nuclear membrane	Inactivated	Nuclear lamina disassembly	Unknown	106
Lamin A	Nuclear envelope protein	Inactivated	Nuclear lamina disassembly	VEID (230)	107–109
Lamin B1	Nuclear envelope protein	Inactivated	Nuclear lamina disassembly	VEVD (231)	109

**Table 1** (continued)

Substrate	Physiological Function	Cleavage Effect	Consequences of Cleavage	Cleavage Sites <sup>a</sup>	References
Lamin C LAP2 $\alpha$	Nuclear envelope protein Lamin-associated polypeptide 2 $\alpha$ , involved in nuclear structure organization	Inactivated Inactivated	Nuclear lamina disassembly Impaired chromatin-binding properties	VEID (230) Putative sites: KRID (413), EERD (441), SQHD (483)	109 110
Nup153	Nucleoporin 153, component of the nuclear pore and binding site for importin- $\beta$	Inactivated	Impaired nuclear transport, redistribution of importins	DITD (343)	111, 112
Nup214	Nucleoporin 214, binding site for exportin	Inactivated	Impaired nuclear transport	Unknown	112
RanBP2 (Nup358)	Nucleoporin 358, Ran-binding protein-2 with SUMO E3 ligase activity	Inactivated	Impaired nuclear transport	Unknown	112
SAF-A	Scaffold attachment factor-A, DNA- binding protein involved in nuclear matrix stabilization	Inactivated	Loss of DNA binding and detachment from nuclear structural sites	SALD (100)	113, 114
SATB1	Special AT-rich sequence-binding protein 1, T-cell-specific DNA- binding protein for nuclear matrix- associating DNAs, involved in gene expression	Inactivated	Cleaved protein dissociates from chromatin, may be involved in high molecular weight DNA fragmentation	VEMD (254)	115, 116
Tpr	Nuclear pore-associated filament protein; binding site for importin- $\beta$	Inactivated	Impaired nuclear transport	Putative sites: DSQD (1892), DGTD (1999), DDED (2117), DDGD (2250), DESD (2285)	112
<b>5. ER and Golgi-resident proteins</b>					
p28BAP31	Bcl-2 adaptor at the ER, originally identified as B-cell receptor- associated protein	Inactivated	BAP31 is cleaved by and recruits caspase-8 to the ER. Expression of cleaved product is proapoptotic and causes disturbed transport of proteins from ER to Golgi	AAVD (164)	117–119
Golgin-160	Golgi autoantigen, Golgin-3 (GOLGA3), located at the rims of cisternas	Inactivated	Cleavage by caspase-2 results in disintegration of the Golgi complex	ESPD (59), CSTD (139), SEVT (311)	120
GRASP65	Golgi reassembly and stacking protein of 65 kDa	Inactivated	Golgi disassembly and loss of integrity	SLLD (320), SFPD (375), TLPD (393)	121
Kinectin	ER-resident receptor for molecular motor kinesin, involved in micro- tubule-based vesicle transport	Unknown	Preferentially cleaved by caspase-7	Unknown	122
<b>6. Cell cycle</b>					
c-Abl	Tyrosine kinase involved in cell cycle arrest	Inactivated	Cleavage-mediated inactivation may suppress erythropoiesis	Putative sites: DTTD (546), DTAD (655)	123
Bcr-Abl	Constitutively active fused gene product of c-Abl and Bcr in chronic myeloid leukemia	Inactivated		See c-Abl	123
Cdc6	Required for prereplicative complex formation	Inactivated	Cleavage results in loss of chromatin binding	LVRD (99), SEVD (442)	124
CDC27	Cdc2 and Cdk-inhibitory kinase of the anaphase-promoting complex	Inactivated	Cleavage results in increased Cdk activity	Unknown	35
Cyclin A	<i>Xenopus</i> cyclin A	Inactivated	The cleavage site of <i>Xenopus</i> Cyclin A2 is not present in mammals	DEPD (90)	10
Cyclin E	Regulator of G1/S cell cycle progression	Inactivated	Elimination of Cdk2 interaction, results in inactivation of cdk kinase. Overexpression of the p18 fragment triggers apoptosis	Unknown	125
MDM2/HDM2	Mouse/human double minute chromosome oncogene 2, controls degradation of p53	Inactivated	The cleaved MDM2 loses the ability to promote p53 degradation and functions in a dominant-negative fashion to stabilize p53	DVPD (361)	126, 127
MDMX	p53-binding protein homologous to MDM2, which promotes degradation of p53.	Inactivated	In analogy to MDM2, cleaved MDMX does not degrade p53 and functions in a dominant- negative fashion to stabilize p53	DVPD (361)	128
NuMA	Nuclear mitosis apparatus protein, translocates to spindle poles at mitosis	Inactivated	Cleavage causes redistribution of NuMA and contributes to nuclear disruption	DSL D (1712)	129, 130
p21 <sup>Waf1</sup>	Cdk2 inhibitor involved in G1/S arrest	Inactivated	Loss of N-terminal cdk- inhibitory domain results in a reduced association with cyclin-cdk2 complexes and increased cdk2 activity	DHVD (112)	131, 132

Table 1 (continued)

Substrate	Physiological Function	Cleavage Effect	Consequences of Cleavage	Cleavage Sites <sup>a</sup>	References
p27 <sup>Kip1</sup>	Cdk2 inhibitor	Inactivated	Cleavage results in reduced association with cyclin-cdk2 complexes and increased cdk2 activity	DPSD (139), ESQD (108)	132, 133
PITSLRE	Cell cycle-regulatory cdc2-like kinase	Activated?	Several isoforms are cleaved and presumably activated	YVPD (391)	134, 135
Prothymosin- $\alpha$	Involved in cell proliferation	Inactivated	Expression of a fragment-like mutant induces apoptosis	Three overlapping sites at the C-terminus: DDEDVVVD(101)	136, 137
Rb	Retinoblastoma protein, phosphorylation-controlled cell cycle regulator that binds to E2F-1	Inactivated	Cleavage prevents nuclear localization, proliferation-inducing ability is abolished	DEAD (886)	138
Wee1	Inhibitory kinase of cdc2 and cdk2	Inactivated	Rb is cleaved in its hypophosphorylated form which results in unopposed E2F-1 action and reduced antiapoptotic activity	Unknown	35
<b>7. DNA synthesis, cleavage and repair</b>					
Acinus	'Apoptotic chromatin condensation inducer in the nucleus'	Activated	Cleavage-mediated inhibition results in elevated cdk activity	DEL (1093)	12
ATM	Ataxia telangiectasia mutated protein; kinase involved in the p53 DNA repair pathway	Inactivated	Essential mediator of chromatin condensation	DYPD (863)	139
BLM	RECQ-like helicase, defective in Bloom's syndrome, involved in DNA replication and repair	Inactivated	Cleavage abrogates kinase activity. Fragment is DNA binding and functions as a dominant-negative inhibitor	TEVD (415)	140, 141
BRCA-1	Breast cancer suppressor protein, mediates cell cycle arrest and DNA repair	Inactivated	Fragment retains helicase activity, albeit interaction with topoisomerase III $\alpha$ is impaired	DLLD (1154)	142
DNA-PKcs	DNA-dependent protein kinase catalytic subunit; involved in repair of DNA breaks and nucleotide excision repair	Inactivated	Expression of a noncleavable BRCA-1 attenuates apoptosis	DEVD (2713)	143, 144
ICAD	Inhibitor of caspase-activated DNase (syn. DFF45)	Inactivated	Loss of catalytic activity	DETD (117), DAVD (224)	145, 146
Helicard	CARD-containing DNA helicase	Activated	Cleavage liberates the active CAD endonuclease	DNTD (208), SCTD (251)	147
MCM3	'Minichromosome maintenance protein 3', replication factor of the MCM complex, restricts replication to one round per cell cycle	Inactivated	Involved in chromatin remodeling. Cleavage separates the CARD from the helicase domain and induces nuclear translocation	Unknown	148
PARG	Poly(ADP-ribose) glycohydrolase; removes poly(ADP-ribose) residues from proteins	Unknown	Probably destruction of the MCM complex, prevention of replication	DEID (256), MDVD (307)	149
PARP-1	Poly(ADP-ribose) polymerase-1; involved in DNA repair and gene expression	Inactivated	Cleavage does not alter enzymatic activity	DEVD (214)	24, 150–152
PARP-2	Poly(ADP-ribose) polymerase-2; involved in DNA repair	Inactivated	Cleavage results in loss of catalytic activity and may prevent depletion of ATP which is required for apoptosis.	LQMD (186)	153
Pol $\epsilon$	DNA polymerase epsilon (Pol $\epsilon$ ) catalytic subunit	Inactivated	Cleavage between DNA binding and catalytic domain	DQLD (189), DMED (1185)	154
RAD21	Component of the cohesin complex	Inactivated	Cleavage dissociates the N-terminal catalytic core from the C-terminus; can no longer bind PCNA or other Pol $\epsilon$ subunits	DSPD (279)	155
RAD51	Human recombinase HsRad51 (homologous to RecA). Involved in homologous recombination and DNA repair	Inactivated	Proapoptotic cleavage product	DVLD (187)	156
RFC140	Replication factor C (syn. DSEB), DNA-dependent ATPase of the replication factor complex, involved in DNA replication and repair	Inactivated	Cleavage products lack recombinase activity	DEVD (722)	157–159
Topo I	Topoisomerase I, breaks and rejoins DNA single strands	No effect?	Cleavage separates the DNA binding from its association domain and impairs DNA replication	PEDD (123), DDVD (146), EEED (170)	160, 161
Topo II $\alpha$	Topoisomerase II $\alpha$	Unknown	Unconventional cleavage sites. Fragment still binds and cleaves DNA	Unknown	162
XRCC4	X-ray repair, complementing defective, in Chinese hamster 4; involved in DNA double-strand break repair and V(D)J recombination	Inactivated	Inhibition of DNA repair	Unknown	163

Table 1 (continued)

Substrate	Physiological Function	Cleavage Effect	Consequences of Cleavage	Cleavage Sites <sup>a</sup>	References
<b>8. DNA-binding and transcription factors</b>					
AP-2 $\alpha$	Inducible transcription factor	Inactivated	Loss of DNA-binding activity	DRHD (19)	164
CREB	cAMP response element-binding protein	Inactivated	Antiapoptotic function is abolished	Putative site: ILND (140) or LSSD (144)	165
c-Rel	NF- $\kappa$ B subunit	Inactivated	Loss of transcriptional activity	Unknown	166
GAL4	Yeast transcription factor used in two-hybrid assays	Inactivated	Cleavage results in loss of transcription in reporter gene assays	Unknown C-terminal cleavage sites	167
GATA-1	Erythropoietic transcription factor	Inactivated	Loss of transcriptional activity results in impaired erythroblast development	EGLD (42), EDLD (125), LSPD (144)	168
HSF	Heat shock factor	Inactivated	Protective induction of heat shock response genes is abolished	Unknown	169
hTAF(II)80 $\delta$	Specialized isoform of basal transcription factor TFIID subunit hTAF80	Unknown	Elevated expression of hTAF(II)80 $\delta$ triggers apoptosis	Unknown	170
I $\kappa$ B $\alpha$	Inhibitor of NF- $\kappa$ B	Activated	Cleavage generates a constitutive inhibitor	DRHD (32)	171
LEDGF	Lens epithelium-derived growth factor, transcriptional coactivator	Inactivated	Cleavage abolishes survival function	EVPD (30), WEID (85), DAQD (486)	172
Max	Myc-associated factor	Inactivated	Cleavage by caspase-5 and -7 at an unusual glutamic acid residue	IEVE (10), SAFD (135)	19
MEF2A, C, D	Myocyte enhancer factor 2, isoforms A, C and D	Inactivated	Caspase cleavage generates a proapoptotic fragment with decreased transcriptional activity	MEF2A: SSYD (466), MEF2C: SSYD (422), MEF2D: LTED (288), DHLD (291)	173, 174
NF- $\kappa$ B p50	Subunit of NF- $\kappa$ B	Inactivated	Loss of DNA binding	Unknown	175
NF- $\kappa$ B p65	Subunit of NF- $\kappa$ B (RelA)	Inactivated	Cleavage generates a dominant-negative proapoptotic fragment	VFTD (465)	175, 176
NRF2	Basic leucine-zipper transcription factor of the NF-E2 family; binds to antioxidant response elements	Inactivated	Overexpression of C-terminal fragment induces apoptosis; gene induction of detoxifying enzymes is abolished	TEVD (208), EELD (366)	177
PML-RAR $\alpha$	Fused oncogenic transcription factor in acute promyelocytic leukemia	Inactivated	Cleavage results in retargeting of PML to nuclear bodies	PHLD (523)	178, 179
RAR $\alpha$	Retinoid acid receptor- $\alpha$	Inactivated	Loss of transcriptional activity	Unknown	179
Relish	Drosophila NF- $\kappa$ B homolog involved in innate immunity	Inactivated	Loss of transcriptional activity	Unknown	180
Sp1	Constitutive transcription factor	Inactivated	DNA-binding activity abolished	NSPD (590)	181
SREBP-1/-2	Sterol-regulatory element-binding protein-1/-2 involved in cholesterol metabolism	Activated	Nonphysiological cleavage by caspases	SREBP-2: DEPD (468)	182
SRF	Serum response factor	Inactivated	DNA binding abolished; loss of, for example, c-fos expression	Unknown	183
STAT1	Signal transducer and activator of transcription-1	Inactivated	Blockade of interferon and other cytokine signaling	MELD (694)	184
<b>9. RNA synthesis and splicing</b>					
BTF3	Transcription initiation factor of RNA polymerase II	Unknown	Identified by 2D gel electrophoresis and <i>in vitro</i> cleavage	Putative site: QSVD (175)	4
hnRNPs (A0, A2/B1, A3, C1, C2, I, K, R)	Heterogeneous nuclear ribonucleoproteins involved in pre-mRNA-splicing and transport	Inactivated	Reduced RNA processing	hnRNP A1/B2: SYND (262), putative site. hnRNP A2/B1: KLTD (49), VMRD (55), AEVD (76), putative sites. hnRNP C1, C2: NKTD (10), EGED (295), DDRD (298), GEDD (305), putative sites. hnRNP I: IVPD (7), LKTD (139), AAVD (172). hnRNP R: RAID (66) and DYYD (472) or KESD (87) and DYHD (481), putative sites	2, 4, 185, 186
KHSRP	KH-type splicing regulatory protein (syn. FUSE-binding protein 2), part of a complex that binds to an intronic splicing enhancer	Unknown	Identified by 2D gel electrophoresis and <i>in vitro</i> caspase-3 cleavage	Putative sites: IRKD (72), AFAD (76), IGGD (91), STPD (102), QLED (114), EDGD (116), SOGD (128)	4
NONO/ p54 <sup>nrb</sup>	Non-Pou domain-containing octamer-binding protein (syn. nuclear RNA-binding protein 54-kD, p54 <sup>nrb</sup> ), splicing factor	Unknown	Identified by 2D gel electrophoresis and <i>in vitro</i> caspase-3 cleavage	Putative site: MMPD (421)	4

Table 1 (continued)

Substrate	Physiological Function	Cleavage Effect	Consequences of Cleavage	Cleavage Sites <sup>a</sup>	References
NS1-associated protein1	RNA-binding protein that interacts with the nonstructural NS1 parvovirus protein	Unknown	Identified by 2D gel electrophoresis	Unknown	4
Nucleolin	Abundant protein, involved in rRNA transcription, ribosome maturation and assembly	Unknown		Putative sites: TEID (455), and AMED (629) or GEID (633)	2, 4
RHA	RNA helicase A, mediates interactions between RNA polymerase II and transcription factors	Inactivated	Cleavage results presumably in reduced transcription of particular genes	EEVD (167)	187, 188
SFRS1	Member of the SR (serine- and arginine-rich) family of non-snRNP splicing factors, (syn. alternative splicing factor-2 or SRp30a)	Unknown	Identified by 2D gel electrophoresis and <i>in vitro</i> caspase-3 cleavage	Putative sites: DLKD (139), CYAD (151), VYRD (155), RKLD (176)	4
SFRS9	Member of the SR (serine- and arginine-rich) family of non-snRNP splicing factors, involved in alternative splicing (syn. SRp30c)	Unknown	Identified by 2D gel electrophoresis	Putative site: GWAD (6)	4
SRPK1	Serine/arginine splicing factor protein kinase 1	Inactivated?		Unknown	See Utz and Anderson <sup>189</sup>
SRPK2	Serine/arginine splicing factor protein kinase 2	Inactivated?		Unknown	See Utz and Anderson <sup>189</sup>
SS-B/La-autoantigen	Involved in RNA biogenesis; Sjogren's syndrome autoantigen	Inactivated	Cleavage presumably results in disturbed Pol III transcription	DEHD (371) or DEHD (374)	190
U1-70-kDa snRNP	Component of the U1 small nuclear ribonucleoprotein complex, involved in pre-mRNA-splicing	Inactivated	Reduced RNA processing	DGPD (341)	191–193
<b>10. Protein translation</b>					
60S acidic ribosomal protein P0	Component of the ribosome	Unknown	Identified by 2D gel electrophoresis, not confirmed by <i>in vitro</i> cleavage	Putative sites: PRED (5), EESD (308), SDED (310)	2, 4
DAP5	Death-associated protein 5 (syn. p97, NAT1); member of the eIF4G-family	Activated	Cleavage product stimulates translation from the IRES sites of c-Myc, Apaf-1, DAP5 and XIAP, supporting translation of apoptosis-related proteins	DETD (792)	17, 194
eIF2 $\alpha$	Eukaryotic translation initiation factor 2 $\alpha$	Inactivated?	Generation of C-terminally truncated protein might result in protection of protein synthesis from PKR-mediated phosphorylation of eIF2 $\alpha$	AEVD (301) or DGDD (304)	195, 196
eIF3	p35 subunit of translation initiation factor eIF3	Inactivated?		DLAD (242), DYED (256)	197
eIF4B	Eukaryotic translation initiation factor 4B	Inactivated	Generation of N-terminal truncated cleavage product, loss of poly(A)-binding and translation	DETD (45)	197, 198
eIF4E-BP1	Eukaryotic translation initiation factor 4E-binding protein 1	Inactivated	Fragment functions as a dominant-negative inhibitor of CAP-dependent translation	VLGD (25)	197, 199
eIF4GI	Eukaryotic translation initiation factor 4GI, binds to the 5' cap structure of mRNAs and facilitates binding of capped mRNA to 40S ribosomal subunits	Inactivated	Inhibition of translation	DLLD (492), DRLD (1136)	200–202
eIF4GII	Eukaryotic translation initiation factor 4GII, binds to the 5' cap structure of mRNAs and facilitates their binding to 40S ribosomal subunits	Inactivated	Shut-off of cap-dependent translation	Unknown	197, 203–205
NAC $\alpha$	Nascent polypeptide-associated complex $\alpha$ ; subunit of a complex that binds newly synthesized polypeptides and prevents them from incorrect translocation to the ER	Unknown	Identified by 2D gel electrophoresis	Unknown	4
PABP4	Poly(A)-binding protein 4 required for poly(A) shortening and translation initiation	Unknown	Identified by 2D gel electrophoresis	Unknown	4
SRP72	72-kDa signal recognition particle protein	No effect?	Cleaved SRP72 still transports signal peptide-containing proteins to the ER	SELD (614)	206
<b>11. Cytokines</b>					
pro-IL-1 $\beta$	Interleukin-1 $\beta$ precursor	Activated	Essential proinflammatory mediator	YVHD (116)	207–209
pro-IL-16	Interleukin-16 precursor	Activated	Induces T-cell chemotaxis	SSTD (510)	210

Table 1 (continued)

Substrate	Physiological Function	Cleavage Effect	Consequences of Cleavage	Cleavage Sites <sup>a</sup>	References
pro-IL-18	IFN- $\gamma$ -inducing factor	Activated	Induces IFN- $\gamma$ production	LESD (36)	211–213
pro-EMAP-II	Endothelial monocyte-activating polypeptide-II	Activated	Pro-EMAP-II is identical to the p43 component of the aminoacyl-tRNA synthetase complex	Mouse: ASTD (144) Human: site not conserved	214, 215
<b>12. Membrane Receptors</b>					
DCC	Deleted in colorectal cancer, tumor suppressor gene	Inactivated	Cleavage product is proapoptotic	LSVD (794)	216
EGF-R	Epidermal growth factor receptor	Inactivated	Cleavage inactivates EGF-R and triggering of survival signals	Putative sites: DEED (1006), DMDD (1009)	217
ErbB-2	Receptor tyrosine kinase, functions as a coreceptor with ligand-occupied members of the EGF receptor, ErbB-3 or -4	Inactivated?	Cleavage of the cytoplasmic part presumably deletes signaling capacity	SETD (45)	218
Glutamate receptor	Receptor family involved in neurotransmission	Inactivated	Cleavage of the glutamate receptor subunits GluR1, 2, 3, 4, but not of NMDA receptor subunits results in modified responsiveness to glutamate	Asp 865	219, 220
RET	Tyrosine kinase receptor, proto-oncogene involved in Hirschsprung disease and multiple endocrine neoplasia type 2	Inactivated	RET induces apoptosis via its own cleavage by caspases through the liberation of a proapoptotic domain of RET	VSVD (707), DYLD (1017)	221
TCR $\zeta$	T-cell receptor zeta chain	Inactivated	Cleavage of the cytoplasmic part results in loss of $\zeta$ chain expression	GLLD (28) or YLLD (36), and DTVD (153)	222
TNF-R1	Tumor necrosis factor receptor-1 (p60)	Inactivated	Cleavage of the cytoplasmic tail at a nonconsensus motif by caspase-7	GELE (260)	223
<b>13. Adapter proteins</b>					
GrpL/Gads	Adapter of the Grb2 family in hematopoietic cells, couples to the T-cell receptor and SLP-76 to regulate transcription factors such as NF-AT	Inactivated	Deletion of the C-terminal SH3 domain prevents recruitment of SLP-76 and leads to desensitization of antigen receptor signaling	DIND (235)	224, 225
TRAF1	TNF-R-associated factor 1	Inactivated	C-terminal cleavage product blocks NF- $\kappa$ B activation and promotes apoptosis	LEVD (163)	226–228
TRAF3	TNF-R-associated factor 3	Inactivated?	Altered cellular distribution of the cleavage product	EEAD (348), ESVD (368)	229
TXBP151	HLTLV-1 Tax-binding protein, antiapoptotic A20-binding protein	Inactivated	Loss of antiapoptotic effect of TXBP15	Unknown	230
<b>14. Tyr protein kinases</b>					
ETK/BMX	Member of the Btk/Tec family of kinases	Activated	Overexpression of the fragment induces apoptosis	ETK: DFPD (242) and a second unknown site	231
Fyn	T-cell Src kinase	Activated	Removal of N-terminal myristoylation sites leads to relocalization and increased activity	EERD (19)	232, 233
Lyn	B-cell Src kinase	Activated	Removal of N-terminal myristoylation sites leads to relocalization and increased activity	DGVD (18)	233
Src	pp60(c-Src), proto-oncogene	Inactivated?	Antiapoptotic effect is abolished	Unknown	234
<b>15. Ser/Thr-Protein kinases in signal transduction</b>					
AKT	Important survival kinase (syn. PKB)	Inactivated	Loss of kinase activity and antiapoptotic function	TVAD (108), EEMD (119), ECVD (462)	235–238
CaMK II $\alpha$	Calcium/calmodulin-dependent kinase II $\alpha$	Inactivated?	Effect on kinase activity not tested	Unknown	239
CaMK IV	Calcium/calmodulin-dependent kinase IV	Inactivated	Cleavage within catalytic domain results in loss of activity	YWID (31), PAPD (176)	239
CaMKK	CaMK kinase	Inactivated?	Effect on kinase activity not tested	Unknown	239
CaMKLK	Ca <sup>2+</sup> /calmodulin-dependent protein kinase (CaMK)-like kinase	Dysregulated	C-terminal fragment retains kinase activity, while N-terminal fragment promotes apoptosis	Rat: DEND (62), (Human, mouse: putative DEND site at 369)	240
HPK-1	Hematopoietic progenitor kinase-1, Ste20-related protein kinase	Dysregulated	Proapoptotic cleavage converts an activator into an inhibitor of NF- $\kappa$ B, product fails to bind to Grb2	DDVD (385)	241, 242



Table 1 (continued)

Substrate	Physiological Function	Cleavage Effect	Consequences of Cleavage	Cleavage Sites <sup>a</sup>	References
MASK	Mst3 and SOK1-related kinase (MASK) of the germinal center kinase family	Activated	Proapoptotic, if overexpressed	DESD (305)	243
MEK	MAP kinase kinase	Inactivated	Direct cleavage by caspases uncertain. Proteolysis results in reduced Erk1/2 phosphorylation	Unknown	244
MEKK1	MEK kinase-1; involved in stress signaling	Activated	Cleavage product is constitutively active, intracellularly redistributed and proapoptotic	Mouse: DTVD (874) (Human and rat: not conserved)	245–247
Mst1	Mammalian STE20-related kinase-1 (Krs2); involved in stress signaling	Activated	Removal of C-terminal regulatory domain results in constitutive activity, relocalization and activation of stress kinases and caspases.	DEMD (326)	248–250
Mst2	Mammalian STE20-related kinase (Krs1), involved in stress signaling	Activated	Cleavage results in a constitutively active kinase	DELD (322)	250
Mst3	Mammalian STE20-related kinase, involved in stress signaling	Activated	Cleavage results in a constitutively active kinase. Overexpression of the C-terminal kinase fragment induces apoptosis.	AETD (313)	251
PAK2	P21-activated kinase 2 (syn. PAK65; PAK $\gamma$ )	Activated	Constitutive activation by separation of N-terminal regulatory and C-terminal catalytic domain, induces apoptotic morphology	SHVD (212)	252, 253
PKC $\delta$	Protein kinase C delta	Activated	Constitutively active kinase, proapoptotic	DMQD (329)	254, 255
PKC $\epsilon$	Protein kinase C epsilon	Activated	Constitutively active kinase	Human: SSPD (383), Mouse: SATD (383)	256–258
PKC $\eta$	Protein kinase C eta	Activated	Kinase-active fragment is proapoptotic	Unknown site in or upstream of the V3 region	259
PKC $\mu$	Protein kinase C mu	Activated	Increased sensitivity to genotoxic stress	CQND (378)	260, 261
PKC $\theta$	Protein kinase C theta	Activated	C-terminal fragment is constitutively active and proapoptotic	DEVD (354)	257, 262
PKC $\zeta$	Protein kinase C zeta	Activated	Constitutively active kinase	EETD (210), DGVD (239)	256, 263, 264
PKR	Double-stranded RNA-dependent protein kinase, involved in antiviral response	Activated	Caspase-dependent activation leads to eIF2- $\alpha$ phosphorylation and translation inhibition	DLPD (251)	265
PRK1	PKC-related kinase-1 (syn. PKN)	Activated	Constitutively active kinase	Unknown	266
PRK2	PKC-related kinase-2	Activated?	Proapoptotic; C-terminal fragment inhibits AKT and PDK-1	DITD (117)	5, 267
Raf-1	'Ras-associated factor 1', important kinase in mitogenic signaling	Inactivated	Cleavage results in loss of Raf-1 antiapoptotic function	Unknown	235
RIP-1	Receptor-interacting kinase-1, component of the TNF-R1 DISC	Inactivated	Proapoptotic cleavage by caspase-8 results in inhibition of NF- $\kappa$ B activation	LQLD (324)	268, 269
ROCK-1	Rho-associated kinase-1	Activated	Caspase-mediated activation results in activation of myosin light chain kinase and membrane blebbing	DETD (1113)	270, 271
SLK	STE20-related kinase, JNK-pathway	Activated	Two cleavage products with distinct activities: N-terminal kinase promotes apoptosis and cytoskeletal rearrangement, the C-terminal fragment disassembles actin fibers	Mouse: DTQD (436) (site not conserved in human)	272
SPAK	STE20/SPS1-related, proline alanine-rich kinase of STE20 kinase family	Unknown		Rat: DEMD (398) Human: DEMD (392) Mouse: DEMD (402)	273
p70 <sup>S6K</sup>	p70 form of S6 kinase	Inactivated	Direct cleavage by caspases uncertain	Unknown	274
<b>16. Protein phosphatases</b>					
Calcineurin	Calmodulin-dependent phosphatase involved in NFAT activation and cytokine synthesis	Activated	Caspase-mediated constitutive activation triggers NF-AT activation and IL-2 release	DFGD (386)	275, 276
PP2A	Protein phosphatase 2A	Activated	Caspases cleave regulatory $\alpha$ -subunit of PP2A and increase its activity	DEQD (218)	277

Table 1 (continued)

Substrate	Physiological Function	Cleavage Effect	Consequences of Cleavage	Cleavage Sites <sup>a</sup>	References
<b>17. Protein modification</b>					
FTase	Farnesyltransferase, attaches farnesyl groups to cysteine residues of proteins	Inactivated	Cleavage of $\alpha$ -subunit (common to FTase and GTTase), expression of cleavage product induces cell death	VSLD (59)	278
GGTase I	Geranylgeranyltransferase I, attaches geranylgeranyl groups to cysteine residues	Inactivated	Cleavage of $\alpha$ -subunit (common to FTase and GGTase), expression of cleavage product induces cell death	VSLD (59)	278
O-GlcNAcase	$\beta$ -O-linked-N-acetylglucosaminidase; releases O-GlcNAc residues from peptides	No effect	Cleavage has no effect on enzyme activity <i>in vitro</i>	Unknown	279
tTG	Tissue transglutaminase (TG-2) crosslinks proteins and assembles scaffolds that prevent leakage of intracellular components	Inactivated	Cleaved late in apoptosis, results in loss of crosslinking activity	Unknown	280
<b>18. Protein degradation</b>					
Calpastatin	Calpain inhibitor	Inactivated	Decreased inhibition of calpain	ALDD (137), LSSD (203), ALAD (404)	281, 282
Cbl	Adapter protein with ubiquitin ligase activity, negative regulator of T-cell activation, downregulates receptor tyrosine kinases by ubiquitinylation	Unknown		Unknown	235
Cbl-b	Cbl-related protein with ubiquitin ligase activity, downregulates receptor tyrosine kinase and PI3K signaling	Unknown		Unknown	235
Nedd4	'Neural-expressed developmentally downregulated gene4 protein', ubiquitin protein ligase	Unknown	Cleavage products do not affect apoptosis, enzyme activity of NEDD4 presumably not impaired	DQPD (206)	283
PA28 $\gamma$	Proteasome activator 28 $\gamma$ -subunit	Unknown		DGLD (80)	284
PAI-2	Plasminogen activator inhibitor type 2	Inactivated	Function as putative cytoprotective protease inhibitor may be abolished	Unknown	285
UFD2	Ubiquitin fusion degradation protein-2, with E3 ligase activity	Inactivated	E3 activity is abolished <i>in vitro</i>	MDID (109), VDVD (123)	286
<b>19. G protein signaling</b>					
Cdc42	Ras-related GTP-binding protein, provides survival signals and controls cytoskeletal architecture	Inactivated	Antiapoptotic function abolished. Mutation of the cleavage site of Cdc42 provides protection	DLRD (121)	287
D4-GDI	D4-GDP dissociation inhibitor (syn. Rho-GDI 2; Ly-GDI), inhibitor of Rho GTPases	Inactivated	Cleavage product translocates to the nucleus, defective Rho GTPase signaling	DELD (19)	288, 289
Rabaptin-5	Small GTPase, rate-limiting component in membrane fusion in the early endocytotic pathway	Inactivated	Cleavage blocks endosome fusion	DESD (438)	290
Rac	Ras-related GTP-binding protein	Inactivated?		DLRD (121)	287
Ran-GAP1	Ran GTPase activating protein 1, involved in nuclear transport	Unknown	May be involved in alterations of nuclear pore transport. Cleavage not confirmed <i>in vitro</i>	Unknown	291
Ras-GAP	Ras GTPase-activating protein	Activated, inactivated	Limited caspase cleavage: N-terminal fragment is antiapoptotic by activating the PI3K pathway. Increased caspase levels: further cleavage into two proapoptotic fragments	DEGD (157), DTVD (459)	235, 292, 293
TIAM1	Rac-specific guanine nucleotide exchange factor	Inactivated	Functional inactivation, cannot stimulate GDP loading of Rac	DETD (993)	294
Vav-1	Hematopoietic proto-oncogene, guanine nucleotide exchange factor	Inactivated	Fails to induce IL-2 transcription; diminished capacity to activate AP-1, NF- $\kappa$ B, NF-AT; can still activate JNK; but not p38	DQID (150), DLYD (161)	295
<b>20. Calcium, c-AMP, c-GMP and Lipid metabolism</b>					
CCT- $\alpha$	CTP: phosphocholine cytidyltransferase $\alpha$ , involved in phosphatidyl choline synthesis	Activated	Cleavage results in nuclear export	TEED (28)	296

Table 1 (continued)

Substrate	Physiological Function	Cleavage Effect	Consequences of Cleavage	Cleavage Sites <sup>a</sup>	References
IP(3)R-1/-2	Inositol 1,4,5-triphosphate receptor-1 and -2	Inactivated	Decrease in IP(3)-gated Ca <sup>2+</sup> channel activity	Mouse IP(3)R-1: DEVD (1892)	297–299
PIP5K-1 $\alpha$	Phosphatidylinositol phosphate 5-kinase-1 $\alpha$ , synthesizes phosphatidyl-inositol 4,5-bisphosphate which inhibits caspases	Inactivated	Inactivation contributes to progression of apoptosis	DIPD (279)	300
PDE4A5	cAMP-specific phosphodiesterase 4A5	Dysregulated	Cleavage removes SH3-binding domain and results in altered intracellular targeting and Lyn kinase interaction	Mouse: DAVD (72)	301
PDE5A1	cGMP-binding phosphodiesterase 5A1	Inactivated?		Unknown	302
PDE6	cGMP-binding phosphodiesterase 6	Inactivated?	Reduced cGMP-hydrolyzing activity	Putative site: DFVD (167)	302
PDE10A2	cGMP-binding phosphodiesterase 10A2	Inactivated?		Putative site: Rat: DLFD (315), Human: DLFD (333)	302
PMCA-2	Neuron-specific plasma membrane Ca <sup>2+</sup> ATPase isoform 2	Unknown		Putative site: EEID (1072)	28
PMCA-4	Ubiquitous plasma membrane Ca <sup>2+</sup> ATPase isoform 4	Inactivated/activated?	Cleavage-mediated inactivation may result in calcium overload and secondary necrosis.	DEID (1080)	28, 303
iPLA(2)	Calcium-independent phospholipase A <sub>2</sub>	Activated	Fragment accelerates phospholipid turnover and contributes to apoptotic membrane changes	DVTD (183)	304
cPLA(2) $\alpha$	Cytosolic phospholipase A <sub>2</sub> $\alpha$ (type IVA), involved in arachidonic acid metabolism	Inactivated	Cleavage blocks PLA2 activity and prevents production of lipid mediators; may have immunosuppressive function	DELD (336)	305, 306
PLC- $\gamma$ 1	Phospholipase C- $\gamma$ 1, involved in mitogenic signaling	Inactivated	Phosphorylated PLC is resistant against cleavage. Cleavage facilitates apoptosis	AEPD (770)	307
<b>21. Neurodegeneration</b>					
Androgen receptor	Polyglutamine tract protein, defective in spinal bulbar muscular atrophy (Kennedy's disease)	Aggregates	Aggregation of the truncated protein may result in neurodegeneration	DEDD (155)	308, 309
APLP1	Amyloid precursor protein-like protein -1, related to APP	Aggregates?	Cleavage generates a cytotoxic C-terminal fragment similar to C31 in APP	VEVD (620)	310
APP	$\beta$ -Amyloid precursor protein, involved in Alzheimer's disease		Cleavage results in generation of the proapoptotic C-terminal C31-peptide	VEVD (739)	31, 311
Ataxin-3	Polyglutamine tract protein defective in spinocerebellar ataxia type 3	Aggregates	Aggregation of the truncated protein may result in neurodegeneration	Putative sites: LISD (145), DLPD (171), LDED (225), DEED (228)	308, 312
Atrophin-1	Polyglutamine tract protein defective in Dentatorubral pallidalsian atrophy (syn. DRPLA protein)	Aggregates	Aggregation of the truncated protein may result in neurodegeneration	DSL D (109)	308, 312, 313
Calsenilin	Member of the recoverin family of calcium-binding proteins, interacts with presenilins	Inactivated	May be involved in Alzheimer's disease	DSSD (64)	314
Huntingtin	Polyglutamine tract protein defective in Huntington's disease	Aggregates	N-terminal fragment is cytotoxic and triggers caspase activation	DSVD (513), DEED (530), IVLD (586)	30, 315
Parkin	Involved in Parkinson's disease	Inactivated	Protein degradation abolishes antiapoptotic function	LHTD (126)	316
Presenilin-1	Involved in Alzheimer's disease	Inactivated	Cleavage abolishes interaction with $\beta$ -catenin and antiapoptotic function	AQRD (345)	317, 318
Presenilin-2	Involved in Alzheimer's disease	Inactivated	Cleavage abolishes interaction with $\beta$ -catenin and disables antiapoptotic function	DSYD (329)	317–319
<b>22. Viral proteins</b>					
Bcl-2 homologs	Viral Bcl-2-homolog encoded by $\gamma$ -Herpesvirus 68	No effect	Unlike mammalian Bcl-2, most viral Bcl-2 proteins are not cleaved. $\gamma$ -HSV68 Bcl-2 is cleaved, but not converted to a proapoptotic form	DCVD (31)	50
CrmA	Cytokine response modifier A, serpin-like caspase inhibitor of poxvirus	Activated	Unlike IAPs, CrmA requires peptide bond hydrolysis for caspase-inhibitory action	LVAD (303)	320

Table 1 (continued)

Substrate	Physiological Function	Cleavage Effect	Consequences of Cleavage	Cleavage Sites <sup>a</sup>	References
M2 (influenza A)	Virus-specific ion channel membrane protein of influenza A virus	Unknown	Cleavage may attenuate virus production	DVDD (88)	321
NP (influenza A and B)	nucleocapsid protein of influenza A and B viruses	Unknown	Cleavage may attenuate virus production	Influenza A: METD (16), Influenza B: MDID (7), SEAD (61)	321, 322
NS5A (HCV)	Nonstructural protein 5A of hepatitis C virus	Unknown	N-terminal deleted protein translocates to the nucleus and has transactivating function	Putative sites: TEVD (154), SGVD (396)	323, 324
Nucleoporin (TGEV)	Transmissible gastroenteritis coronavirus (TGEV) nucleocapsid protein	Unknown	Cleavage may limit virus production	VVPD (359)	325
p35	pancaspase inhibitor of baculovirus	Activated	Cleavage is required for caspase inhibition by p35	DQMD (87)	326, 327
<b>23. Other substrates</b>					
AHNAK	Autoantigen in systemic lupus Erythematosus. DNA-binding phosphoprotein	Unknown		Unknown	328
CPSII	Carbamoyl phosphate synthetase II, required for pyrimidine nucleotide synthesis	Inactivated		EAVD (1371) in catalytic B2 domain and VACD (1143) in allosteric B3 domain	329
F1A $\alpha$	Mammalian homolog of FEM-1 (syn. FEM1 $\beta$ ), ankyrin repeat-containing protein	Activated?	F1A $\alpha$ is proapoptotic and binds to death receptors in 2-hybrid assays. Loses apoptosis-inducing ability upon cleavage	DNID (342)	330
FEM-1	Involved in sex-specific cell elimination in <i>C. elegans</i> , necessary for male phenotype	Activated?	Caspase cleavage promotes apoptosis-inducing property of FEM-1, which interacts with Ced-4	ELLD (320)	331
FKBP46	FK506 binding protein 46, insect nuclear immunophilin	Unknown		Unknown	332
GCL	Glutamate-L-cysteine ligase, rate-limiting enzyme in glutathione synthesis	Inactivated	Cleavage of the catalytic subunit results in loss of the antioxidant glutathione	AVVD (499)	333
Hsp90		Unknown		DEED (259)	96
PDC-E2	Pyruvate dehydrogenase complex E2, autoantigen.	Unknown	Cleavage confirmed by <i>in vitro</i> caspase-3 cleavage	Unknown	334
SET $\beta$	Product of the putative set oncogene	Unknown		SNHD (18)	335

<sup>a</sup>If not otherwise indicated, the cleavage sites refer to the human sequence.

<sup>b</sup>During typesetting of this manuscript additional caspase substrates have been identified including the large subunit of RNA polymerase II,<sup>336</sup> the vesicle-tethering Golgi protein p. 115,<sup>337</sup> the neuronal Ras-guanine nucleotide exchange factor GRASP-1,<sup>338</sup> the hematopoietic transcription factor FL1-1,<sup>339</sup> SRPK1 and SRPK2, two kinases of the serine/arginine splicing factors,<sup>340</sup> the K10 retroviral polyprotein HERV-K10<sup>gag</sup>,<sup>341</sup> adenovirus early region 1A proteins,<sup>342</sup> and baculovirus apoptotic suppressor protein p 49.<sup>343</sup>

The search for caspase substrates has brought several major questions into focus. For instance, is there a critical death substrate or what is the minimal set of proteins that must be cleaved in order to induce the phenotypic hallmarks of apoptosis? How is caspase substrate cleavage coordinated with other cellular processes, such as removal of dead cells, or presumably unrelated events including cell proliferation and differentiation? Although the significance of cleavage is not well understood for many substrates, the intense study of caspase substrates has recently shed some light on these questions. Here, we discuss several topics that have emerged from the accumulating knowledge regarding the role of caspase substrates in different biological processes.

### Key morphological alterations are determined by caspase substrate cleavage

For most proteins, the consequences of their cleavage are poorly understood. In a few cases, however, proteolysis of

certain components can be linked to discrete morphological changes of cell death. A classical example is the DNase inhibitor ICAD. Cleavage of ICAD by caspase-3 liberates the active CAD nuclease that mediates apoptotic DNA fragmentation (for references, see Table 1). In addition, the cleavage of acinus and helicard, a DNA helicase, contributes to chromatin condensation and nuclear remodeling. The cleavage of several other substrates, including gelsolin as well as the kinases ROCK-1 and PAK2, has been implicated in membrane blebbing, a classical morphological feature. Gelsolin is cleaved by caspase-3 to generate a constitutively active fragment that can depolymerize F-actin. Gelsolin-deficient neutrophils exhibit greatly delayed membrane blebbing during apoptosis, implying that membrane blebbing requires actin reorganization mediated by caspase-activated gelsolin. Caspases also cleave and thereby activate ROCK-1 leading to the phosphorylation of myosin light chains, which finally results in membrane blebbing.

Caspases destroy several proteins involved in maintenance of the cytoskeletal architecture such as the intermediate filaments cyokeratin-18 and vimentin, or Gas2 and plectin,

two proteins involved in filament organization. These cleavages may directly contribute to apoptotic changes in cell shape. Caspases attack targets of the cortical actin network such as fodrin, and several components of the focal adhesion complex which links cortical actin filaments and membrane proteins to the extracellular matrix. Examples of this kind of substrates are focal adhesion kinase, Cas or paxillin. Cleavage of these proteins presumably contributes to cell shrinkage and cell detachment and, importantly, will interrupt antiapoptotic integrin signaling. A large percentage of caspase substrates are involved in cell adhesion or mediate cell–cell communication in adherens and gap junctions, or in desmosomes. Examples are  $\beta$ -catenin, E-cadherin, plakoglobin or desmoglein.

In the course of apoptosis, disruption of the endoplasmic reticulum and Golgi apparatus also takes place. Cleavage of golgin-160 and GRASP65 was suggested to cause disassembly of the Golgi complex, and proteolysis of Bap31 disrupts the transport between the ER and the Golgi complex. During apoptosis, vesicle transport processes are also impaired, for instance by the cleavage of rabaptin-5 or kinectin.

Caspases initiate the destruction of the nucleus where a huge variety of different proteins are cleaved. By 2D gel electrophoresis it has been recently determined that approximately 70 nuclear matrix proteins are consistently degraded or translocated during apoptosis, irrespective of the cell type or apoptotic stimulus.<sup>13</sup> Many cleavages lead to nuclear lamina disassembly, and the cleavage of several components of the nuclear pore results in impaired nuclear transport. Inhibition of DNA repair, for instance by the cleavage of PARP-1 or the kinases ATM and DNA-PK, has been long thought to promote the apoptosis process. Other targeted factors are involved in DNA synthesis and replication, such as DNA polymerase Pol  $\epsilon$ , MCM3 or replication factor RFC140. In addition, various proteins that bind to chromatin, and either fulfill a transcriptional role or have structural functions in the nuclear matrix, are destroyed. In almost all cases, these cleavages result in the generation of proteins that are no longer able to bind to DNA or to stabilize chromatin in the nuclear matrix. With a few exceptions that are discussed below, virtually all pathways of macromolecular synthesis are impaired by caspases. Cleavage of RNA helicase A and multiple splicing factors, including U1 70-kDa snRNP and at least eight different heterogeneous nuclear ribonucleoproteins (hnRNPs), leads to a general shut-off of RNA synthesis, processing and transport. Moreover, protein synthesis is blocked either by the inactivation of translation initiation factors, including eIF2 $\alpha$ , eIF3 and eIF4G proteins, or by the activation of PKR kinase that blocks protein synthesis through eIF2- $\alpha$  phosphorylation.

## Caspase substrates in signal transduction

A tremendous variety of proteins involved in signal transduction are cleaved by caspases. The proteolytic cleavage can either lead to the functional inhibition or to the activation of these mediators. In some cases, it has been established that caspase-mediated activation of these molecules is involved in

transduction and amplification of the apoptotic signal. Caspases turn off cell-protective mechanisms and activate pathways that lead to cell destruction. Classical apoptosis inhibitors that are cleaved by caspases are Bcl-2 proteins or the caspase-8 inhibitor c-FLIP. The cleavage of Bcl-2 and Bcl-x<sub>L</sub> resulting in the removal of the N-terminal BH4 domain not only leads to a loss of their antiapoptotic function, but even converts them to proapoptotic proteins. Similarly, during death receptor-mediated apoptosis caspase-8 cleaves the Bcl-2 member Bid generating an active C-terminal fragment that induces the proapoptotic release of cytochrome *c* from mitochondria. The conversion of antiapoptotic into proapoptotic regulators constitutes a positive feedback loop in the terminal phase of apoptosis, removing apoptotic inhibitors and promoting caspase activation. It is interesting to note that certain viral Bcl-2 proteins can also be cleaved by caspases, but in these cases no proapoptotic fragments are generated.

Several kinases and transcription factors with antiapoptotic activity are inactivated during apoptosis. Akt and Raf-1 provide two examples of antiapoptotic kinases that are cleaved by caspase-3. As both kinases can inactivate proapoptotic molecules such as Bad, their degradation presumably constitutes a positive feedback loop in apoptosis. Antiapoptotic transcription factors inhibited by caspases include the cAMP-responsive factor CREB, heat-shock factor HSF-1 and NF- $\kappa$ B. The NF- $\kappa$ B pathway is a paradigm of how caspase cleavage may result in a complete loss of the transcription factor's antiapoptotic function: (i) Cleavage of NF- $\kappa$ B subunit p65 (RelA) generates a dominant-negative fragment that is still able to bind to DNA but loses its transactivating activity, and therefore functions as a dominant-negative inhibitor. (ii) The NF- $\kappa$ B inhibitor I $\kappa$ B- $\alpha$  is normally inducibly degraded by the proteasome. The N-terminal cleavage of I $\kappa$ B- $\alpha$  by caspases generates a constitutive super-repressor that can no longer be removed by the proteasome. (iii) The cleavage of the adapter proteins TRAF-1 and RIP-1 that are involved in receptor-mediated pathways also contributes to impaired NF- $\kappa$ B activation and antiapoptotic capacity. Thus, cells have elaborate mechanisms in order to interrupt antiapoptotic signaling efficiently.

While some substrates are functionally inactivated upon caspase-mediated cleavage, other proteins and enzymes can be activated, mostly by removing an inhibitory or regulatory domain within the caspase target. The physiological consequence of this gain-of-function cleavage for apoptosis remains mostly unclear. Several members of the PKC family and MAP kinase pathway are constitutively activated by the separation of an N-terminal regulatory and the C-terminal catalytic domain. Examples are the p21-activated kinase PAK2 as well as ROCK-1. As described above, activation of PAK2 and ROCK-1 is important for cytoskeletal reorganization and plasma membrane blebbing. In the case of MEKK1, expression of the caspase-cleaved kinase fragment induces caspase activation, thereby providing a positive feedback loop for apoptosis. Epithelial cells undergo apoptosis if they are detached from the basement membrane, a process called anoikis. MEKK1 is activated following cell detachment, and blockade of either MEKK1 or caspase activity blocks anoikis. Cleavage of several MST kinases by caspase-3 also yields

constitutively active molecules and potent inducers of apoptosis. Apoptosis induction by all these upstream kinases in the SAPK/JNK pathway may be explained in part by their ability to activate JNK, which then phosphorylates and inactivates Bcl-2.

Most kinase pathways exert antiapoptotic functions. It is thus not unexpected that a major cellular protein phosphatase, PP2A, which counteracts the survival function of kinases, is activated by caspases. Protein phosphorylation can also protect caspase substrates from proteolysis. This has been convincingly demonstrated for Bid that is protected from caspase-8 cleavage through phosphorylation by casein kinases I and II.<sup>14</sup> Another example is Max, a transcription factor in the c-Myc network, which can be cleaved only if dephosphorylated. A very intriguing finding has been recently made for C/EBP $\beta$ . The transcription factor itself is not cleaved by caspases, but curiously acts as caspase inhibitor upon phosphorylation.<sup>15</sup> Threonine phosphorylation of C/EBP $\beta$  within a KTVD sequence creates a noncleavable mimic of an XEXD cleavage site, which binds caspases and thereby inhibits caspase action. Hence, such dummies of caspase substrates may represent a novel survival mechanism.

### Some peculiarities of substrate cleavage

Caspase cleavage can also result in the cellular redistribution and dislocation of signaling mediators. In some cases, such as the Grb2 adapter protein GrpL or the phosphodiesterase PDE4A5, an SH3-domain within the substrate is removed causing its inability to bind to physiological interaction partners. A change of subcellular localization following caspase cleavage has also been observed for the kinases Fyn and MEKK1. Another notable example is Bid. Upon cleavage by caspase-8, the proapoptotic p15 fragment of Bid undergoes post-translational rather than the classical cotranslational N-myristoylation at a glycine residue that becomes newly exposed by the cleavage.<sup>16</sup> This postproteolytic N-myristoylation then enables Bid to target mitochondria and serves as an activating switch, which strongly enhances cytochrome *c* release.

Apoptosis is generally associated with a shut-down of cap-dependent protein translation, which is mediated by caspase cleavage of several translation factors. Interestingly, it has been recently observed that during apoptosis, translation of a subset of mRNAs prevails. The reason for this is presumably a switch from cap-dependent to internal ribosome entry site (IRES)-mediated protein translation. DAP-5, a member of the eIF4G family, is activated by caspases and stimulates translation from the IRES sites of c-Myc, Apaf-1, and its own mRNA.<sup>17</sup> Thus, DAP-5 is a rather unique caspase-activated factor that supports cap-independent translation of apoptosis-related proteins and thereby may amplify the apoptosis cascade.

Most caspase substrates identified so far are cleaved by caspase-3. This has been convincingly shown in the system of MCF-7 breast carcinoma cells that lack caspase-3, and caspase-3 re-expressing derivatives.<sup>18</sup> Nevertheless, several substrates that are efficiently cleaved by caspase-3 can also be targeted by caspase-7, suggesting an at least partial

redundance of both caspases. Caspase-7 activity is upregulated in cells of caspase-3-deficient mice, where it might compensate for the loss of caspase-3. Caspase-7 and -5, but not caspase-3, cleave transcription factor Max. Interestingly, in this case Max is not cleaved at the classical aspartate residue in the P1 position, but at an unusual glutamate residue.<sup>19</sup> Cleavage of the cytoplasmic tail of TNF-R1, the cardiac myosin light chain vMLC and connexin 45.6 at a glutamate instead of an aspartate residue are further examples. Cleavage at these noncanonical sites suggests that the specificity of caspases may in fact be broader than generally thought. Also, the *Drosophila* caspase DRONC can cleave substrates following glutamate residues.<sup>20</sup> Caspase-7 not only cleaves substrates at atypical motifs, but can be activated itself by a rather unusual processing event. It has been reported that various serine proteases can trigger the proteolytic activity of the caspase-7 zymogen.<sup>21</sup> For instance, cathepsin G activates caspase-7 by cleaving at a glutamate bond, indicating that the cleavage specificity at aspartic acid is not strictly required for caspase activation.

The interaction of caspases with other classes of proteases, including calpains, cathepsins or the proteasome, is poorly understood. When searching for caspase substrates, it must be considered that high concentrations of caspase inhibitors, such as the fluoromethylketone zVAD-fmk, are less specific than often anticipated, because calpains are inhibited as well. Several substrates of caspases are also cleaved by calpains including structural proteins, such as fodrin, keratins and  $\beta$ -actin, and proteins involved in signal transduction, such as Bid, Bax, focal adhesion kinase and many others. It has been found that caspases and calpains interfere with each other, resulting in mutual protease activation. Caspases can indirectly activate calpain by cleavage and inactivation of its inhibitor calpastatin, and thereby turn on downstream events leading to cellular destruction. However, it is still controversial as to whether calpains function upstream or downstream of caspases. It has also been reported that calpains cleave procaspases to generate proteolytically inactive caspase fragments.<sup>22</sup>

Caspases are not only involved in apoptosis but also in the induction of inflammation. In fact, the former notion that apoptosis and inflammation are exclusive processes should be replaced, as both processes are linked at various levels. Caspase-1 processes and matures the cytokine precursors pro-IL-1 $\beta$  and pro-IL-18, also known as IFN- $\gamma$ -inducing factor. Although caspase-1 is required mainly for induction of inflammation, it can process the effector caspases-3, -6 and -7 and may initiate apoptosis under certain conditions. Effector caspases can also activate pro-IL-16 and pro-EMAP-II, an endothelial-monocyte-activating polypeptide. This precursor of EMAP-II is an intriguing substrate, because it exerts a dual function:<sup>23</sup> Pro-EMAP-II is identical to the p43 cofactor of the aminoacyl-tRNA synthetase complex. After cleavage, preferentially by caspase-7, its t-RNA binding capacity is lost and protein translation is blocked. The translation arrest is accompanied by the release of the EMAP-II cytokine that may play a role in the engulfment of apoptotic cells by phagocytes. Caspase-mediated substrate cleavage therefore has multiple effects summarized as (i) a halt of cell cycle progression, (ii) disabling of repair

mechanisms, (iii) disassembly of molecular structures, (iv) cell detachment, and (v) maturation of cytokine precursors.

## Substrate cleavage at the balance between necrosis and apoptosis

Although caspases are presumably not essential for necrotic cell death, recent evidence suggests that the cleavage of certain substrates may determine the form of cell death. One of the first death substrates found to be cleaved by caspases was PARP-1, which catalyzes the transfer of ADP-ribose polymers to nuclear proteins and thus presumably facilitates DNA repair.<sup>24</sup> Owing to its role in DNA repair, it was originally hypothesized that the cleavage of PARP may lead to lethal DNA damage and compromise most of its DNA repair activity, and thus may contribute to the demise of the cell. However, PARP<sup>(-/-)</sup> mice neither reveal a phenotype which would indicate a crucial role in apoptosis nor is the sensitivity towards CD95- and TNF-R1-mediated apoptosis affected.<sup>25</sup> Thus, cleavage of PARP may be a characteristic event, but is presumably dispensable for most apoptotic pathways.

New evidence, however, suggests that PARP inactivation by caspase-3 is important for turning off an energetically expensive DNA repair pathway and for maintaining ATP levels that are required for the execution of apoptosis. PARP is rapidly activated during oxidative stress and DNA damage. Activated PARP then transfers more than 100 ADP-ribose moieties to each acceptor site in target proteins, and each cycle of ADP-ribosylation is coupled with consumption of one NAD molecule, which is metabolically equivalent to four ATP molecules. Hence, it can be imagined that excessive activation of PARP will quickly deplete cellular energy stores. In the absence of an energy pool sufficient to execute apoptosis or to maintain ionic homeostasis, cells can die quickly by necrosis. Indeed, when cells engineered to express caspase-resistant PARP are treated with apoptotic stimuli, they undergo extensive necrosis instead of apoptosis.<sup>26</sup> Consistent with the requirement of maintaining cellular energy during apoptosis, cells artificially depleted of ATP undergo necrosis instead of apoptosis under conditions that would normally trigger caspase activation.<sup>27</sup> Thus, cleavage of PARP prevents depletion of the cellular energy needed for apoptosis and thus may function as a molecular switch between apoptotic and necrotic cell death. Similar to PARP, also the cleavage of other substrates may provide a link between apoptosis and necrosis. For instance, cleavage and inactivation of the plasma membrane calcium ATPase PMCA-4, which removes calcium from the cytosol, disturbs ion homeostasis.<sup>28</sup> The subsequent cellular calcium overload may be responsible for the secondary necrosis that is observed in the late stages of apoptosis.

## Role of caspase substrates in disease progression

Increased caspase activation has been recently demonstrated in various diseases. However, the cleavage of several substrates may not only contribute to increased tissue damage, but may also play an active role in disease

progression. Such a direct role of substrate cleavage has been most intensively studied in neurodegeneration and autoimmune diseases. Autoimmunity to intracellular proteins has been identified as an important factor in autoimmune diseases. Massive apoptosis or defective clearance may lead to an accumulation of apoptotic cells that concentrate caspase-cleaved proteins in their apoptotic bodies and membrane blebs. The presence of autoantibodies against caspase substrates, such as lamins, fodrin, DNA-PK, PARP or NuMA, has been demonstrated in several autoimmune diseases.<sup>29</sup> Cleavage of these autoantigens presumably enhances their immunogenicity by exposing cryptic neoepitopes. The cleaved proteins are then processed and presented by dendritic cells to circulating autoreactive T cells, triggering an autoimmune response.

The cleavage of specific substrates can be directly linked to the pathogenesis of certain neurodegenerative disorders. Huntington's disease, a genetically determined neurodegenerative disease, results from the expansion of CAG triplets at the 5'-primed end of the gene encoding huntingtin, a protein with a long polyglutamine stretch. Huntingtin is cleaved by caspase-3 and results in an N-terminal fragment, which aggregates and forms nuclear inclusions that are directly cytotoxic for neurons.<sup>30</sup> Huntington's disease manifests only if huntingtin exceeds 35 glutamine residues. Because the rate of caspase cleavage of huntingtin correlates with the length of the polyglutamine stretch, accumulation of the fragment may cause a vicious cycle. A pathogenic role of caspase cleavage has also been implicated in other neurodegenerative disorders. Similar to huntingtin, the polyglutamine tract proteins atrophin-1, androgen receptor and ataxin-3 are caspase substrates. Indeed, mutations of the caspase recognition sites in atrophin-1 and androgen receptor abrogate their cytotoxicity *in vitro*.

Alzheimer's disease is characterized by brain lesions of neurofibrillary tangles, and senile plaques built of aggregates of the  $\beta$ -amyloid peptide. Aggregates of  $\beta$ -amyloid peptide induce neuronal apoptosis, and increased production of  $\beta$ -amyloid peptide has been postulated as an important pathologic mechanism in early-onset familial Alzheimer's disease. Effector caspases presumably increase  $\beta$ -amyloid production by several mechanisms. Loss-of-function mutations in the presenilin-1 and -2 genes are responsible for the majority of familial Alzheimer's disease and are thought to increase  $\beta$ -amyloid production. Caspase-3 can cleave and inactivate presenilins, which may mimic the effect of pathologic presenilin mutations. The 40- to 42-amino-acid  $\beta$ -amyloid peptide is derived from proteolytic processing of the amyloid precursor protein (APP) at two sites by the  $\beta$ - and  $\gamma$ -secretase. Caspase-3 cleaves APP at a site different from the  $\gamma$ -secretase site.<sup>31</sup> Nevertheless, the N-terminal caspase cleavage product of APP strongly facilitates the production of  $\beta$ -amyloid peptide, and appears itself to be a component of senile plaques found in Alzheimer patients. Because caspase-3 activation and APP cleavage are also induced *in vitro* after ischemic brain injury, a risk factor for Alzheimer's disease, these results provide another example of a positive feedback loop between caspase substrate cleavage and neurodegeneration. Neuronal apoptosis from ischemia or other causes activates caspase-3 and stimulates APP

cleavage, which increases the propensity for  $\beta$ -amyloid peptide production. In turn, increased extracellular  $\beta$ -amyloid peptide production may induce neuronal apoptosis, leading to further deposition of senile plaques. The cytotoxic properties of their cleavage products illustrate that specific caspase substrates are not only involved in cell destruction, but also fulfill an active role in the exacerbation of disease processes.

## Caspases: more than just killers?

A strikingly large number of caspase targets are involved in cell cycle regulation. This has led to speculations that caspases are not only involved in cell death but also in proliferative events.<sup>32</sup> Supportive, yet indirect evidence for a role of caspases in cell growth is the observation that proliferation of primary T cells is inhibited by cell-permeable caspase inhibitors.<sup>33,34</sup> Moreover, interference with pathways leading to caspase processing, as in FADD-deficient or Bcl-2-transgenic mice, also results in impaired mature T-cell proliferation.

Several negative regulators including Wee1, an inhibitor of the cell cycle-regulatory kinases CDK2 and CDC2, as well as CDC27, a component of the anaphase-promoting complex, are cleaved by caspases. Wee1 is a critical component of the G2/M cell cycle checkpoint machinery and mediates cell cycle arrest by phosphorylation of CDC2. Therefore, cleavage of Wee1 in proliferating lymphocytes could lead to its inactivation, thus allowing cell cycle progression. Of note, Wee1 processing by caspases during apoptosis in Jurkat T cells correlated with a strong decrease in Wee1 activity and an increase in CDC2 activity.<sup>35</sup> Moreover, the cyclin inhibitors p21<sup>Waf1</sup> and p27<sup>Kip1</sup> are targeted by caspases resulting in increased CDK2 activity that could allow cell cycle progression.

If caspases are activated during mitosis, a critical question is then, how could caspase cleavage be restricted to those cell cycle regulators, while leaving other vital proteins intact? The answer could lie in a specific subcellular compartmentalization of caspases, the existence of scaffold proteins or a different accessibility of cleavable substrates. Some caspases are translocated to a certain organelle during activation, and in some cell types certain caspases have been localized in the nucleus. Interestingly, it has been found that, although caspases were activated and Wee1 was cleaved after mitogenic T-cell stimulation, neither DNA replication factor RFC140 nor ICAD were cleaved in proliferating T cells.<sup>33</sup> Cleavage of RFC140 and ICAD would lead to inhibition of DNA replication and fragmentation of genomic DNA, events that are not compatible with cell proliferation. Thus, selective substrate processing could explain why nonapoptotic cells survive and proliferate despite caspases being activated.

Certainly, there exist many links, also at the morphological level, between the processes of cell death and proliferation. However, it must be emphasized that the view of a potential involvement of caspases in proliferation is largely based on indirect evidence and therefore remains highly speculative. Because cleavage of cell cycle regulators occurs late in apoptosis by caspase-3-like activities in parallel with the dismantling of the transcription and translation machinery,

caspase activation cannot trigger the normal mitotic program. For example, mitotic spindles do not form in apoptotic cells, distinguishing apoptosis from a mitotic catastrophe.

## Limited substrate cleavage in terminal differentiation and hematopoiesis

In contrast to the rather speculative involvement of caspases in proliferation, there is an increasing body of evidence suggesting that caspases might act in cellular differentiation. A physiological role of caspases in this process has first been suggested for keratinocytes and lens fiber cells, in which the characteristic enucleation of the cells could be regarded somehow as a caspase-mediated incomplete apoptotic process.<sup>36,37</sup> Caspases have also been implicated in erythropoiesis, because caspase inhibitors suppressed the nuclear extrusion process and consequent erythrocyte formation.<sup>38</sup> Furthermore, caspase activation can be detected during thrombopoiesis and the fragmentation of proplatelets from megakaryocytes, without a concomitant induction of cell death.<sup>39</sup> Both the incubation with peptide caspase inhibitors and the overexpression of Bcl-2 blocked proplatelet formation. Interestingly, in transgenic mice overexpressing Bcl-2 under the control of a hematopoietic cell-specific promoter, also a reduction in platelet formation is found, whereas the number of megakaryocytes remains unchanged. Finally, caspases might be required for differentiation processes also of nucleated cells such as macrophages and muscle cells. Elevated caspase activation is detectable in monocytes when they undergo M-CSF-stimulated macrophage differentiation.<sup>40</sup> This is not only prevented by pharmacological caspase inhibitors, but also by the overexpression of Bcl-2 and p35. In myoblasts, homologous deletion of caspase-3 leads to a dramatic reduction in myofiber formation and decreased expression of muscle-specific proteins.<sup>41</sup> Thus, all these lines of evidence suggest that caspases are not only required for cell death processes, but might also be capable of regulating nonapoptotic functions in certain cell types.

It is obvious that differentiation-related caspase activation must be tightly regulated to prevent cells from dying by apoptosis. During cellular differentiation, caspase activation is apparently either very limited, transient or localized. For instance, during megakaryocyte differentiation, the limited caspase activation is confined to dot-like structures.<sup>39</sup> When senescent megakaryocytes die, however, caspase activation switches from a localized to a diffused and largely increased cytosolic activation. Also, little is known about the proteins cleaved by caspases during differentiation processes. Only a limited number of distinct substrates seem to be cleaved. For instance, in erythroblasts cleavage of PARP, lamin B and acinus was found, while the ICAD and GATA-1, a transcription factor essential for erythrocyte formation, remained intact. Interestingly, MST1 kinase was identified as a crucial caspase-3 effector in myoblast differentiation.<sup>41</sup> As mentioned above, MST1 is cleaved and activated by caspase-3, and serves to enhance the activity of downstream MAP kinases that promote skeletal muscle differentiation. Expression of the truncated active kinase restored the differentiation phenotype in caspase-3 deficient myoblasts.



As discussed above, it remains currently unexplained as to how caspases could selectively cleave some targets without cleaving others. The compartmentalization of caspases, the duration of the caspase signal, or the coordinated expression of antiapoptotic molecules might play a role in the selectivity of caspase cleavage. It is also conceivable that low levels of caspase activity, such as those observed in differentiating cells, are associated with protective mechanisms. For instance, it was reported that the partial cleavage of Ras-GAP, a GTPase in the Ras signaling pathway, owing to low caspase activity first generates an N-terminal fragment that is antiapoptotic by activating the PI3K pathway.<sup>42</sup> Increased caspase levels, in contrast, result in the further cleavage of Ras-GAP into two proapoptotic fragments. Thus, caspase cleavage of intracellular target proteins may strongly depend on the cellular context including the differentiation status. Clearly, much remains to be learned about a potential dual role of caspases in apoptosis and cellular differentiation. Characterization of the molecules that regulate this limited caspase activation and the relevant substrates will certainly provide exciting new insights into processes that, beyond cell death, might link caspase cleavage to important nonapoptotic biological processes.

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