

News and Commentary

Apoptosis Timeline

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This timeline of cell death (Figure 1), illustrates how independent strands of research coalesced in the field known as apoptosis – currently the hottest field of biological research. Although the fact that cells die during normal development was recognized over 150 years ago,¹ this was forgotten, only to be re-discovered several times until the influential review by Glucksmann in 1951.² Even after this time, up until the late 1980's, study of physiological cell death processes, in which an organism's cells activate intrinsic mechanisms for the purpose of killing themselves, remained relatively obscure, usually with less than 10 papers published each year.

Initially, analysis of cell death was mainly morphological, and between the late 1800's and 1960's elegant figures were published illustrating the light (see reviews by Clarke and Clarke and Lockshin)^{82,83} and electron microscopic³ features of cell death, such as cell shrinkage, chromatin condensation, break-up of the cell and its engulfment.

Even well after the proposal of the term 'apoptosis' for cell death in 1972,⁸ interest remained low. The 'modern' era of cell death research, and the explosion of interest in the field, came with the identification of the biochemical and genetic processes that implement it, beginning with recognition of the first component of the cell death system, Bcl-2, in 1988.²⁰ Since then, growth of the field has been exponential, and currently over 200 publications appear every week that refer to 'apoptosis'. A genetic understanding of cell death has primarily come from study of *C. elegans*, in which 131 of the 1090 somatic cells formed in the hermaphrodite are fated to die during development.¹⁶ This started with the recognition of cell death in the worm in 1976,¹¹ and generation of the first *ced* (cell death abnormal) mutants in 1983.¹⁴ In 1982, in a journal that unfortunately folded soon after, a paper appeared providing evidence that cell deaths in the worm were caused by a process that was specific for cell death, and had no other role, indicating that cell death in the worm is an active process whose only purpose is to remove unwanted cells.¹³ Similar conclusions were reached earlier in vertebrate systems, such as when Tata showed that cell deaths during tadpole metamorphosis could be inhibited by cycloheximide, and therefore required the cell's own proteins.⁶

At this time, the term most commonly used for the study of these cell death was 'programmed cell death', first used in 1965 to describe developmental cell deaths in insect systems by Lockshin and Williams.⁵ The term 'apoptosis' was proposed in 1972 by Kerr and colleagues,⁸ who realized that the morphology of cells dying due to toxins or hormones resembled that described for developmental cell death by Glucksmann.² For Kerr, this did not mark the beginning of apoptosis research, because he had been studying it continuously since his first publication on cell death in 1965;⁴ rather, it marked the adoption of a new terminology, because until then he had used the terms such as 'shrinkage necrosis'.

The first marker of physiological cell death that did not rely on morphology came with the recognition that cell death is usually accompanied by rapid activation of endonucleases.¹⁰ Subsequently, 'ladders' seen after electrophoresis of cleaved DNA⁹ were specifically associated with apoptosis.¹² It took a further 17 years to identify the major endonuclease responsible (DFF/CAD).^{63,64} The observation that phosphatidyl serine is exposed on dying cells³² provided another convenient marker of apoptosis, and also gave an early lead into how dead cells are recognized prior to their engulfment. Although genetic analysis of cell death progressed most rapidly in the worm, with identification of more and more *ced* mutant lines,^{16,29} biochemical analysis of cell death was faster in mammals. While Bcl-2 was cloned in 1986,^{17,18} and its role in cell death was established in 1988,²⁰ the first *ced* gene to be cloned and sequences was *ced-4* in 1992.³¹

Comparisons of the morphological and anatomical features of developmental cell deaths in invertebrates and vertebrates have been made since 1969,⁷ but unification of the molecular processes of cell death did not occur until 1992, when it was shown that the human *bcl-2* gene could inhibit developmental cell death in the worm.³⁰ This united 'apoptosis' in vertebrates with 'programmed cell death' in invertebrates, showing them to be the same, evolutionarily conserved process, and it meant that discoveries based on genetics in *C. elegans* could be applied to analysis of apoptosis in mammalian cells.

While Bcl-2 was the first component of the apoptosis mechanism to be recognized, it had been cloned not because it was a cell death gene, but because it is translocated in follicular lymphoma, one of the most common cancers of blood cells in humans. Initially, it was assumed that *bcl-2* may be like other oncogenes involved in translocations, such as *abl* and *c-myc*, and be a promoter of cell proliferation, but it turned out that when *bcl-2* was over-expressed, it did not stimulate cell division, but prevented cells from dying when growth factor was removed.²⁰ These experiments therefore not only identified Bcl-2 as a component of the apoptosis mechanism, but showed that inhibition of cell death could ultimately lead to

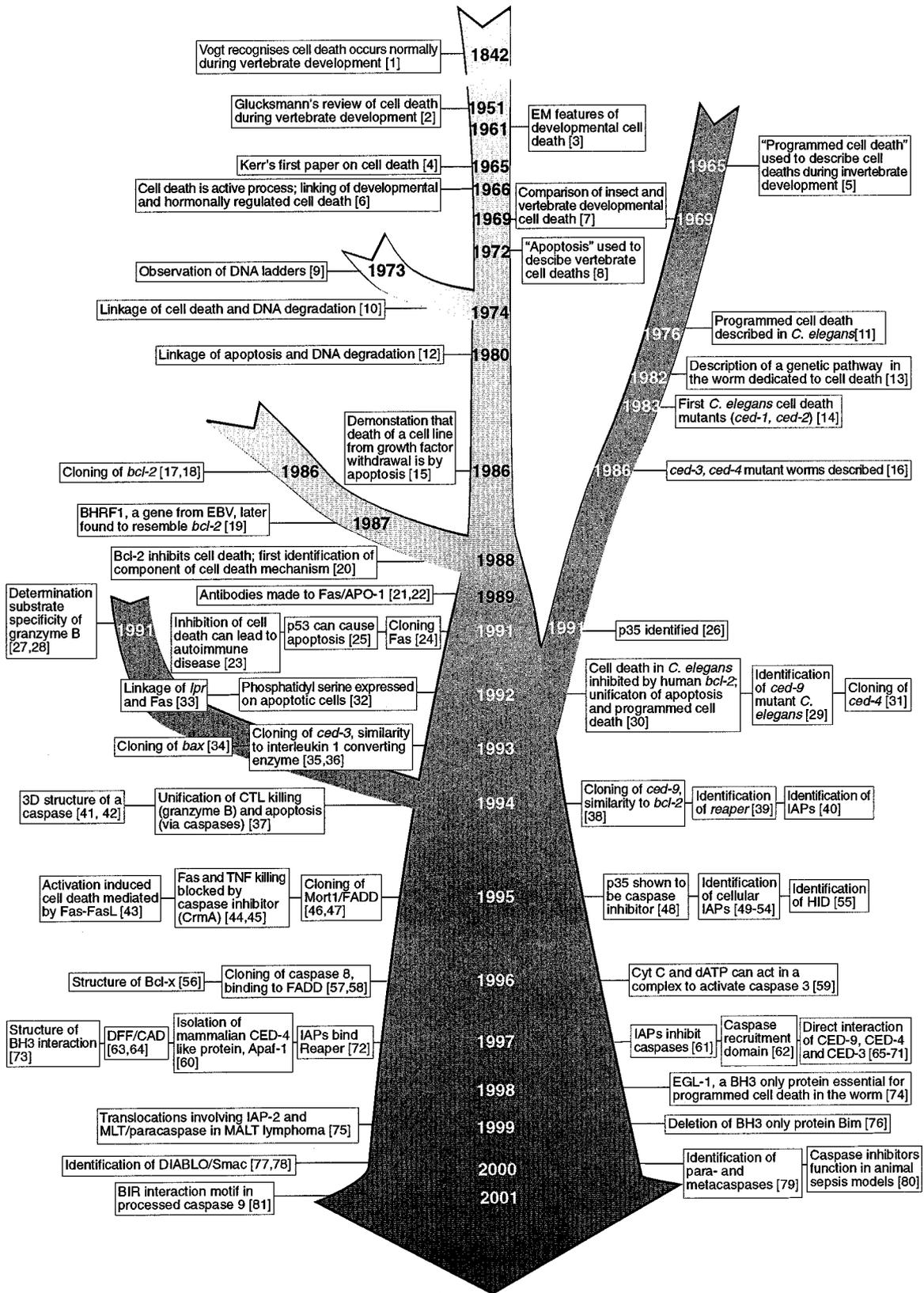


Figure 1 Over 50 000 papers have been published on apoptosis, and it is only possible to give an indication of some of them in this figure. Many, many, important papers have therefore been omitted. In most cases, only the first member of a protein family is mentioned (that is why the cloning of all the Bcl-2 family members, or all the caspases, are not listed). This figure only depicts findings that are widely accepted. There are many important molecules, or findings, whose roles or interpretations are recent or remain controversial, and have therefore not been shown (e.g. ceramide; the channel forming role of Bcl-2 family members; mitochondrial permeability transition; DAP kinase; DAP3, Survivin; DAXX; FAP1; reactive oxygen intermediaries; BAG-1; AIF; AVEN, etc., etc.). Other (non-caspase mediated) mechanisms of cell death, and cell death research on non-metazoans, has not been included

cancer in humans. The realization that one of the roles of p53, the most commonly mutated gene in human cancers, is to cause apoptosis,²⁵ further emphasized this link, as did the demonstration that p53 causes apoptosis via the mechanism that can be blocked by Bcl-2.⁸⁴ Bcl-2 also provided the first experimental evidence linking inhibition of cell death with autoimmune disease, when it turned out that on certain genetic backgrounds transgenic mice expressing *bcl-2* in their lymphocytes developed a disease resembling systemic lupus erythematosus.²³ This link was further strengthened when the gene altered in *lpr* mice, which also develop a lupus-like autoimmune syndrome, turned out to be CD95 (Fas/APO-1),³³ a TNF receptor family member²⁴ that was known to signal apoptosis when crosslinked by antibodies.^{21,22} Furthermore, mice lacking *bim*, which encodes a so-called 'BH3 only' pro-apoptotic Bcl-2 homologue, also develop autoimmune disease.⁷⁶

The effector proteases of apoptosis, now known as caspases, were first recognized when the *ced-3* gene, which is essential for programmed cell death in the worm,¹⁶ was cloned and sequenced,^{35,36} and found to resemble the mammalian gene for the cysteine protease interleukin 1 β converting enzyme, which had been cloned in 1992.^{85,86} Crystallography revealed that active caspases are heterotetramers formed from inactive zymogens.^{41,42} This focussed interest on what activates caspases, and what inhibits them.

Key findings have included the elucidation of a caspase activation pathway that originates in the plasma membrane, and proceeds from CD95, via the adaptor FADD, to activate caspase 8,^{44,45,57,58} and the findings that in *C. elegans* the adaptor CED-4 directly binds to and activates the caspase CED-3.^{67,68,70,71} Identification of mammalian homologues of these proteins (Apaf-1 and caspase 9)^{60,87} showed that a similar pathway operates in mammals, and revealed cytochrome *c* to be a molecule capable of activating Apaf-1.⁵⁹ Many of the interactions between these cell death molecules involve related protein-protein interaction motifs termed death domains, death effector domains and caspase recruitment domains.⁶²

While it is clear that anti-apoptotic Bcl-2 like proteins act upstream of caspases to prevent their activation, and pro-apoptotic Bcl-2 family members such as Bax³⁴ promote caspase activity, debate remains about exactly how they work. Biochemical experiments using *C. elegans* proteins have suggested that CED-9 (the worm homologue of Bcl-2) inhibits cell death by directly binding to CED-4,^{65–69} but it is unclear whether similar direct interactions occur between their mammalian counterparts.

Solving the structure of Bcl-x,⁵⁶ a Bcl-2 family member, raised the alternative possibility that these proteins act as membrane pores or ion channels, to somehow influence release of pro-apoptotic molecules such as cytochrome *c* from the mitochondria. From both structural studies, and genetics in *C. elegans*, it is, however, clear that anti-apoptotic Bcl-2 family members can be bound, and antagonized by, 'BH3 only' proteins such as Bim and Noxa in mammals,^{76,88} and EGL-1 in the worm,⁷⁴ thus increasing the likelihood that a cell will undergo apoptosis. BH3 only proteins are key determinants of cell death in worms and

vertebrates. All somatic developmental cell death in *C. elegans* require EGL-1,⁷⁴ and in mammals p53-dependent apoptosis seems to be signalled in large part via Noxa.⁸⁸ The discovery that the helical BH3 domain of one Bcl-2 family member can bind to a hydrophobic pocket on the surface of another⁷³ has helped explain how pro-death Bcl-2 family proteins antagonize their anti-apoptotic cousins.

Not all physiological cell deaths in animals are cell autonomous (i.e. cell 'suicide'), sometimes one cell kills another cell (i.e. cell 'murder'). In *C. elegans*, death of the male linker cell is non-cell autonomous,¹⁶ and in mammals, cytotoxic T cells (CTL) kill other host cells, especially those infected by viruses. Targets of CTL killing display the characteristic features of apoptosis,⁸⁹ and it became clear why when the mechanisms involved in CTL killing were elucidated. CTL can kill by perforin-dependent, granule exocytosis, which involves granzyme B, a serine protease with a similar substrate specificity to the caspases,^{27,28} or via CD95L-CD95 interactions, which activate caspase 8.^{57,58} Knowledge of the enzymes involved in CTL killing therefore allowed unification of cell autonomous and non-cell autonomous cell deaths, and explained the shared apoptotic appearances.³⁷

CTL killing illustrates the role of apoptosis in defense against viruses. But viruses have been selected that carry inhibitors of apoptosis. Several direct inhibitors of caspase activity were first found in viruses, and for some, cellular homologues were later identified. The first caspase inhibitor found was CrmA, a product of cowpox virus that was known to inhibit interleukin 1 β converting enzyme (caspase 1),⁹⁰ but is now known to also inhibit caspase 8, and thereby can block CD95 and TNFR signalled apoptosis.⁹¹ The gene for p35 was first found in baculoviruses,²⁶ as were the first inhibitor of apoptosis (IAP) genes.⁴⁰ Both p35 and IAPs act by binding directly to, and thereby inhibiting, active caspases.^{48,61} Several mammalian IAP homologues have been discovered,^{49–54} and one, c-IAP2, is commonly translocated in MALT lymphomas, where it is expressed as a fusion with the MLT/paracaspase gene product.^{75,79}

In insects three different proteins, Reaper, HID and Grim,^{39,55,92} promote apoptosis by antagonizing the IAPs,⁷² and a mammalian protein, Smac/Diablo, has been found that inhibits mammalian IAPs in a similar way.^{77,78} The identification of a similar BIR-interacting N-terminal motif in processed caspase 9 revealed how Smac/Diablo can displace caspase 9 from IAPs.⁸¹ A tremendous effort is now being expended to discover even more about how apoptosis works, and to resolve some of the controversies that remain. It is still not clear how Bcl-2 family members work, or how cytokines prevent default activation of the cell death mechanisms, or even whether in mammalian cells prevention of caspase activity will allow long-term survival. The answers to such questions are not trivial, but will determine to what extent these wonderful, yet curiously delayed, discoveries in basic science will be easily applied to the development of novel therapeutic agents for the treatment of diseases in which cell death fails to occur or occurs inappropriately. The first non-peptide caspase inhibitory drugs are proving useful in animal models of sepsis,⁸⁰ suggesting apoptosis-based therapies are not far away.

1. Vogt C (1842) *Untersuchungen über die Entwicklungsgeschichte der Geburtshelkroete (Alytes obstetricians)*. Solothurn: Jent und Gassman, pp 130
2. Glucksmann A (1951) Cell deaths in normal vertebrate ontogeny. *Biol. Rev.* 26: 59–86
3. Bellairs R (1961) Cell death in chick embryos as studied by electron microscopy. *J. Anat.* 95: 54–60
4. Kerr J (1965) A histochemical study of hypertrophy and ischaemic injury of rat liver with special reference to changes in lysosomes. *J. Pathol. Bacteriol.* 90: 419–435
5. Lockshin R and Williams C (1965) Programmed cell death. II. Endocrine presentation of the breakdown of the intersegmental muscles of silkworms. *J. Insect Physiol.* 11: 803–809
6. Tata JR (1966) Requirement for RNA and protein synthesis for induced regression of the tadpole tail in organ culture. *Dev. Biol.* 13: 77–94
7. Whitten JM (1969) Cell death during early morphogenesis: parallels between insect limb and vertebrate limb development. *Science* 163: 1456–1457
8. Kerr JF, Wyllie AH and Currie AR (1972) Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br. J. Cancer* 26: 239–257
9. Hewish DR and Burgoyne LA (1973) Chromatin sub-structure. The digestion of chromatin DNA at regularly spaced sites by a nuclear deoxyribonuclease. *Biochem. Biophys. Res. Comm.* 52: 504–510
10. Williams JR, Little JB and Shipley WU (1974) Association of mammalian cell death with a specific endonucleolytic degradation of DNA. *Nature* 252: 754–755
11. Sulston JE (1976) Post-embryonic development in the ventral cord of *Caenorhabditis elegans*. *Philosoph. Trans. Roy. Soc. Lond. Series B: Biological Sci.* 275: 287–297
12. Wyllie AH (1980) Glucocorticoid-induced thymocyte apoptosis is associated with endogenous endonuclease activation. *Nature* 284: 555–556
13. Horvitz HR, Ellis HM and Sternberg PW (1982) Programmed cell death in nematode development. *Neurosci. Comment.* 1: 56–65
14. Hedgecock EM, Sulston JE and Thomson JN (1983) Mutations affecting programmed cell deaths in the nematode *Caenorhabditis elegans*. *Science* 220: 1277–1279
15. Duke RC and Cohen JJ (1986) IL-2 addiction: withdrawal of growth factor activates a suicide program in dependent T cells. *Lymphokine Res.* 5: 289–299
16. Ellis HM and Horvitz HR (1986) Genetic control of programmed cell death in the nematode *C. elegans*. *Cell* 44: 817–829
17. Tsujimoto Y and Croce CM (1986) Analysis of the structure, transcripts, and protein products of bcl-2, the gene involved in human follicular lymphoma. *Proc. Natl. Acad. Sci. USA* 83: 5214–5218
18. Cleary ML, Smith SD and Sklar J (1986) Cloning and structural analysis of cDNAs for bcl-2 and a hybrid bcl-2/immunoglobulin transcript resulting from the t(14;18) translocation. *Cell* 47: 19–28
19. Pearson GR, Luka J, Petti L, Birkenbach M, Braun D and Kieff E (1987) Identification of an Epstein-Barr virus early gene encoding a second component of the restricted early antigen complex. *Virology* 160: 151–161
20. Vaux DL, Cory S and Adams JM (1988) Bcl-2 gene promotes haemopoietic cell survival and cooperates with c-myc to immortalize pre-B cells. *Nature* 335: 440–442
21. Yonehara S, Ishii A and Yonehara M (1989) A cell-killing monoclonal antibody (anti-Fas) to a cell surface antigen co-downregulated with the receptor of tumor necrosis factor. *J. Exp. Med.* 169: 1747–1756
22. Trauth BC, Klas C, Peters AM, Matzku S, Moller P, Falk W, Debatin KM and Krammer PH (1989) Monoclonal antibody-mediated tumor regression by induction of apoptosis. *Science* 245: 301–305
23. Strasser A, Whittingham S, Vaux DL, Bath ML, Adams JM, Cory S and Harris AW (1991) Enforced BCL2 expression in B-lymphoid cells prolongs antibody responses and elicits autoimmune disease. *Proc. Natl. Acad. Sci. USA* 88: 8661–8665
24. Itoh N, Yonehara S, Ishii A, Yonehara M, Mizushima S, Sameshima M, Hase A, Seto Y and Nagata S (1991) The polypeptide encoded by the cDNA for human cell surface antigen Fas can mediate apoptosis. *Cell* 66: 233–243
25. Yonish-Rouss E, Resnitzky D, Lotem J, Sachs L, Kimchi A and Oren M (1991) Wild-type p53 induces apoptosis of myeloid leukaemic cells that is inhibited by interleukin-6. *Nature* 353: 345–347
26. Clem RJ, Fechheimer M and Miller LK (1991) Prevention of apoptosis by a baculovirus gene during infection of insect cells. *Science* 254: 1388–1390
27. Poe M, Blake JT, Boulton DA, Gammon M, Sigal NH, Wu JK and Zweerink HJ (1991) Human cytotoxic lymphocyte granzyme B. Its purification from granules and the characterization of substrate and inhibitor specificity. *J. Biol. Chem.* 266: 98–103
28. Otake S, Kam CM, Narasimhan L, Poe M, Blake JT, Krahenbuhl O, Tschopp J and Powers JC (1991) Human and murine cytotoxic T lymphocyte serine proteases: subsite mapping with peptide thioester substrates and inhibition of enzyme activity and cytotoxicity by isocoumarins. *Biochemistry* 30: 2217–2227
29. Hengartner MO, Ellis RE and Horvitz HR (1992) *Caenorhabditis elegans* gene *ced-9* protects cells from programmed cell death. *Nature* 356: 494–499
30. Vaux DL, Weissman IL and Kim SK (1992) Prevention of programmed cell death in *Caenorhabditis elegans* by human *bcl-2*. *Science* 258: 1955–1957
31. Yuan J and Horvitz HR (1992) The *Caenorhabditis elegans* cell death gene *ced-4* encodes a novel protein and is expressed during the period of extensive programmed cell death. *Development* 116: 309–320
32. Fadok VA, Voelker DR, Campbell PA, Cohen JJ, Bratton DL and Henson PM (1992) Exposure of phosphatidylserine on the surface of apoptotic lymphocytes triggers specific recognition and removal by macrophages. *J. Immunol.* 148: 2207–2216
33. Watanabe FR, Brannan CI, Copeland NG, Jenkins NA and Nagata S (1992) Lymphoproliferation disorder in mice explained by defects in Fas antigen that mediates apoptosis. *Nature* 356: 314–317
34. Oltvai ZN, Millman CL and Korsmeyer SJ (1993) Bcl-2 heterodimerizes in vivo with a conserved homolog, Bax, that accelerates programmed cell death. *Cell* 74: 609–619
35. Miura M, Zhu H, Rotello R, Hartweg EA and Yuan J (1993) Induction of apoptosis in fibroblasts by IL-1 α -converting enzyme, a mammalian homolog of the *C. elegans* cell death gene *ced-3*. *Cell* 75: 653–660
36. Yuan JY, Shaham S, Ledoux S, Ellis HM and Horvitz HR (1993) The *C. elegans* cell death gene *ced-3* encodes a protein similar to mammalian interleukin 1 β converting enzyme. *Cell* 75: 641–652
37. Vaux DL, Haecker G and Strasser A (1994) An evolutionary perspective on apoptosis. *Cell* 76: 777–779
38. Hengartner MO and Horvitz HR (1994) *C. elegans* cell survival gene *ced-9* encodes a functional homolog of the mammalian proto-oncogene *bcl-2*. *Cell* 76: 665–676
39. White K, Grether ME, Abrams JM, Young L, Farrell K and Steller H (1994) Genetic control of programmed cell death in *Drosophila*. *Science* 264: 677–683
40. Birnbaum MJ, Clem RJ and Miller LK (1994) An apoptosis inhibiting gene from a nuclear polyhedrosis virus encoding a polypeptide with Cys/His sequence motif. *J. Virol.* 68: 2521–2528
41. Walker NPC, Talanian RV, Brady KD, Dang LC, NJ B, Ferenz CR, Franklin S, Ghayur T, Hackett MC, Hamill LD, Herzog L, Hugunin M, Houy W, Mankovich JA, McGuinness L, Orlewicz E, Paskind M, Pratt CA, Reis P, Summani A, Terranova M, Welch JP, Xiong L, Möller A, Tracey DE, Kamen R and Wong WW (1994) Crystal structure of the cysteine protease interleukin-1 β -converting enzyme: A (p20/p10) $_2$ homodimer. *Cell* 78: 343–352
42. Wilson KP, Black J, Thomson JA, Kim EE, Griffith JP, Navia MA, Murcko MA, Chambers SP, Aldape RA, Raybuck SA and Livingston DJ (1994) Structure and mechanism of interleukin-1 β converting enzyme. *Nature* 370: 270–275
43. Alderson MR, Tough TW, Davis ST, Braddy S, Falk B, Schooley KA, Goodwin RG, Smith CA, Ramsdell F and Lynch DH (1995) Fas ligand mediates activation-induced cell death in human T lymphocytes. *J. Exp. Med.* 181: 71–77
44. Tewari M and Dixit VM (1995) Fas- and tumor necrosis factor-induced apoptosis is inhibited by the poxvirus crmA gene product. *J. Biol. Chem.* 270: 3255–3260
45. Enari M, Hug H and Nagata S (1995) Involvement of an ICE-like protease in Fas-mediated apoptosis. *Nature* 375: 78–81
46. Boldin MP, Varfolomeev EE, Pancer Z, Mett IL, Camonis JH and Wallach D (1995) A novel protein that interacts with the death domain of Fas/APO1 contains a sequence motif related to the death domain. *J. Biol. Chem.* 270: 7795–7798
47. Chinnaiyan AM, O'Rourke K, Tewari M and Dixit VM (1995) FADD, a novel death domain-containing protein, interacts with the death domain of Fas and initiates apoptosis. *Cell* 81: 505–512

48. Xue D and Horvitz HR (1995) Inhibition of the *Caenorhabditis elegans* cell-death protease CED-3 by a CED-3 cleavage site in baculovirus p35 protein. *Nature* 377: 248–251
49. Roy N, Mahadevan MS, Mclean M, Shutler G, Yaraghi Z, Farahani R, Baird S, Besnerjohnston A, Lefebvre C, Kang XL, Salih M, Aubry H, Tamai K, Guan XP, Ioannou P, Crawford TO, Dejong PJ, Surh L, Ikeda JE, Korneluk RG and Mackenzie A (1995) The gene for neuronal apoptosis inhibitory protein is partially deleted in individuals with spinal muscular atrophy. *Cell* 80: 167–178
50. Hay BA, Wassarman DA and Rubin GM (1995) *Drosophila* homologs of baculovirus inhibitor of apoptosis proteins function to block cell death. *Cell* 83: 1253–1262
51. Rothe M, Pan MG, Henzel WJ, Ayres TM and Goeddel DV (1995) The TNFR2-TRAF signaling complex contains two novel proteins related to baculoviral-inhibitor of apoptosis proteins. *Cell* 83: 1243–1252
52. Uren AG, Pakusch M, Hawkins CJ, Puls KL and Vaux DL (1996) Cloning and expression of apoptosis inhibitory protein homologs that function to inhibit apoptosis and/or bind tumor necrosis factor receptor-associated factors. *Proc. Natl. Acad. Sci. USA* 93: 4974–4978
53. Liston P, Roy N, Tamai K, Lefebvre C, Baird S, Chertonhorvat G, Farahani R, Mclean M, Ikeda JE, Mackenzie A and Korneluk RG (1996) Suppression of apoptosis in mammalian cells by NAIP and a related family of IAP genes. *Nature* 379: 349–353
54. Duckett CS, Nava VE, Gedrich RW, Clem RJ, Vandongen JL, Gilfillan MC, Shiels H, Hardwick JM and Thompson CB (1996) A conserved family of cellular genes related to the baculovirus IAP gene and encoding apoptosis inhibitors. *EMBO J.* 15: 2685–2694
55. Grether ME, Abrams JM, Agapite J, White K and Steller H (1995) The *head involution defective* gene of *Drosophila melanogaster* functions in programmed cell death. *Genes Dev.* 9: 1694–1708
56. Muchmore SW, Sattler M, Liang H, Meadows RP, Harlan JE, Yoon HS, Nettlesheim D, Chang BS, Thompson CB, Wong SL, Ng SC and Fesik SW (1996) X-ray and NMR structure of human Bcl-x(1), an inhibitor of programmed cell death. *Nature* 381: 335–341
57. Boldin MP, Goncharov TM, Goltsev YV and Wallach D (1996) Involvement of MACH, a novel MORT1/FADD-interacting protease, in Fas/APO-1 and TNF receptor-induced cell death. *Cell* 85: 803–815
58. Muzio M, Chinnaiyan AM, Kischkel FC, O'Rourke K, Shevchenko A, Ni J, Scaffidi C, Bretz JD, Zhang M, Gentz R, Mann M, Kramer PH, Peter ME and Dixit VM (1996) FLICE, a novel FADD-homologous ICE/CED-3-like protease, is recruited to the CD95 (Fas/APO-1) death-inducing signaling complex. *Cell* 85: 817–827
59. Liu XS, Kim CN, Yang J, Jemmerson R and Wang XD (1996) Induction of apoptotic program in cell-free extracts – requirement for dATP and cytochrome c. *Cell* 86: 147–157
60. Zou H, Henzel WJ, Liu XS, Lutschg A and Wang XD (1997) Apaf-1, a human protein homologous to *C. elegans* CED-4, participates in cytochrome c-dependent activation of caspase-3. *Cell* 90: 405–413
61. Deveraux QL, Takahashi R, Salvesen GS and Reed JC (1997) X-linked IAP is a direct inhibitor of cell-death proteases. *Nature* 388: 300–304
62. Hofmann K, Bucher P and Tschopp J (1997) The CARD domain – a new apoptotic signalling motif. *Trends Biochem. Sci.* 22: 155–156
63. Liu XS, Zou H, Slaughter C and Wang XD (1997) DFF, a heterodimeric protein that functions downstream of caspase-3 to trigger DNA fragmentation during apoptosis. *Cell* 89: 175–184
64. Enari M, Sakahira H, Yokoyama H, Okawa K, Iwamatsu A and Nagata S (1998) A caspase-activated DNase that degrades DNA during apoptosis, and its inhibitor ICAD. *Nature* 391: 43–50
65. Spector MS, Desnoyers S, Hoepfner DJ and Hengartner MO (1997) Interaction between the *C. elegans* cell-death regulators CED-9 and CED-4. *Nature* 385: 6553–656
66. James C, Gschmeissner S, Fraser A and Evan GI (1997) CED-4 induces chromatin condensation in *Schizosaccharomyces pombe* and is inhibited by direct physical association with CED-9. *Curr. Biol.* 7: 246–252
67. Wu DY, Wallen HD and Nunez G (1997) Interaction and regulation of subcellular localization of CED-4 by CED-9. *Science* 275: 1126–1129
68. Chinnaiyan AM, O'Rourke K, Lane BR and Dixit VM (1997) Interaction of CED-4 with CED-3 and CED-9 – a molecular framework for cell death. *Science* 275: 1122–1126
69. Otilie S, Wang Y, Banks S, Chang J, Vigna NJ, Weeks S, Armstrong RC, Fritz LC and Oltersdorf T (1997) Mutational analysis of the interacting cell death regulators CED-9 and CED-4. *Cell Death Differ.* 4: 526–533
70. Seshagiri S and Miller LK (1997) *Caenorhabditis elegans* CED-4 stimulates CED-3 processing and CED-3-induced apoptosis. *Curr. Biol.* 7: 455–460
71. Irmier M, Hofmann K, Vaux DL and Tschopp J (1997) Direct physical interaction between the *Caenorhabditis elegans* death proteins CED-3 and CED-4. *FEBS Lett.* 406: 189–190
72. Vucic D, Kaiser WJ, Harvey AJ and Miller LK (1997) Inhibition of reaper-induced apoptosis by interaction with inhibitor of apoptosis proteins (IAPs). *Proc. Natl. Acad. Sci. USA* 94: 10183–10188
73. Sattler M, Liang H, Nettlesheim D, Meadows RP, Harlan JE, Eberstadt M, Yoon HS, Shuker SB, Chang BS, Minn AJ, Thompson CB and Fesik SW (1997) Structure of Bcl-x(l)-Bak peptide complex – recognition between regulators of apoptosis. *Science* 275: 983–986
74. Conradt B and Horvitz HR (1998) The *C. elegans* protein EGL-1 is required for programmed cell death and interacts with the Bcl-2-like protein CED-9. *Cell* 93: 519–529
75. Dierlamm J, Baens M, Woldarska I, Stefanova-Ouzounova M, Hernandez JM, Hossfeld DK, De Wolf-Peeters C, Hagemeijer A, Van den Berghe H and Marynen P (1999) The apoptosis inhibitor gene API2 and a novel 18q gene, MLT, are recurrently rearranged in the t(11;18)(q21;q21) associated with mucosa-associated lymphoid tissue lymphomas. *Blood* 93: 3601–3609
76. Bouillet P, Metcalf D, Huang DCS, Tarlinton DM, Kay TWH, Kontgen F, Adams JM and Strasser A (1999) Proapoptotic Bcl-2 relative BIM required for certain apoptotic responses, leukocyte homeostasis, and to preclude autoimmunity. *Science* 286: 1735–1738
77. Du CY, Fang M, Li YC, Li L and Wang XD (2000) Smac, a mitochondrial protein that promotes cytochrome c-dependent caspase activation by eliminating IAP inhibition. *Cell* 102: 33–42
78. Verhagen AM, Ekert PG, Pakusch M, Silke J, Connolly LM, Reid GE, Moritz RL, Simpson RJ and Vaux DL (2000) Identification of DIABLO, a mammalian protein that promotes apoptosis by binding to and antagonizing IAP proteins. *Cell* 102: 43–53
79. Uren AG, O'Rourke K, Aravind L, Pisabarro MT, Seshagiri S, Koonin EV and Dixit VM (2000) Identification of paracaspases and metacaspases: Two ancient families of caspase-like proteins, one of which plays a key role in MALT lymphoma. *Molec. Cell* 6: 961–967
80. Hotchkiss RS, Chang KC, Swanson PE, Tinsley KW, Hui JJ, Klender P, Xanthoudakis S, Roy S, Black C, Grimm E, Aspiotis R, Han Y, Nicholson DW and Karl IE (2000) Caspase inhibitors improve survival in sepsis: a critical role of the lymphocyte. *Nat. Immunol.* 1: 496–501
81. Srinivasula SM, Hegde R, Saleh A, Datta P, Shiozaki E, Chai JJ, Lee RA, Robbins PD, Fernandes-Alnemri T, Shi YG and Alnemri ES (2001) A conserved XIAP-interaction motif in caspase-9 and Smac/DIABLO regulates caspase activity and apoptosis. *Nature* 410: 112–116
82. Clarke PG and Clarke S (1996) Nineteenth century research on naturally occurring cell death and related phenomena. *Anat. Embryol.* 193: 81–99
83. Lockshin RA (1997) The early modern period in cell death. *Cell Death Differ.* 4: 347–351
84. Chiou SK, Rao L and White E (1994) Bcl2 blocks p53 dependent apoptosis. *Mol. Cell. Biol.* 14: 2556–2563
85. Thornberry NA, Bull HG, Calaycay JR, Chapman KT, Howard AD, Kostura MJ, Miller DK, Molineaux SM, Weidner JR, Aunins J, Elliston KO, Ayala JM, Casano FJ, Chin J, Ding GJ, Egger LA, Gaffney EP, Limjuco G, Paylha OC, Raju SM, Rolando AM, Salley JP, Yanin TT, Lee TD, Shively JE, MacCross JE, Mumford RA, Schmidt JA and Tocci MJ (1992) A novel heterodimeric cysteine protease is required for interleukin-1 beta processing in monocytes. *Nature* 356: 768–774
86. Cerretti DP, Kozlosky CJ, Mosley B, Nelson N, Van NK, Greenstreet TA, March CJ, Kronheim SR, Druck T, Cannizzaro LA, Huebner K and Black RA (1992) Molecular cloning of the interleukin-1 beta converting enzyme. *Science* 256: 97–100
87. Li P, Nijhawan D, Budihardjo I, Srinivasula SM, Ahmad M, Alnemri ES and Wang XD (1997) Cytochrome c and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade. *Cell* 91: 479–489

88. Oda E, Ohki R, Murasawa H, Nemoto J, Shibue T, Yamashita T, Tokino T, Taniguchi T and Tanaka N (2000) Noxa, a BH3-only member of the Bcl-2 family and candidate mediator of p53-induced apoptosis. *Science* 288: 1053–1058
89. Clouston WM and Kerr JF (1985) Apoptosis, lymphocytotoxicity and the containment of viral infections. *Med. Hypoth.* 18: 399–404
90. Ray CA, Black RA, Kronheim SR, Greenstreet TA, Sleath PR, Salvesen GS and Pickup DJ (1992) Viral inhibition of inflammation: Cowpox virus encodes an inhibitor of the interleukin-1 beta converting enzyme. *Cell* 69: 597–604
91. Muzio M, Salvesen GS and Dixit VM (1997) FLICE induced apoptosis in a cell-free system – cleavage of caspase zymogens. *J. Biol. Chem.* 272: 2952–2956
92. Chen P, Nordstrom W, Gish B and Abrams JM (1996) Grim, a novel cell death gene in *Drosophila*. *Genes Deve.* 10: 1773–1782