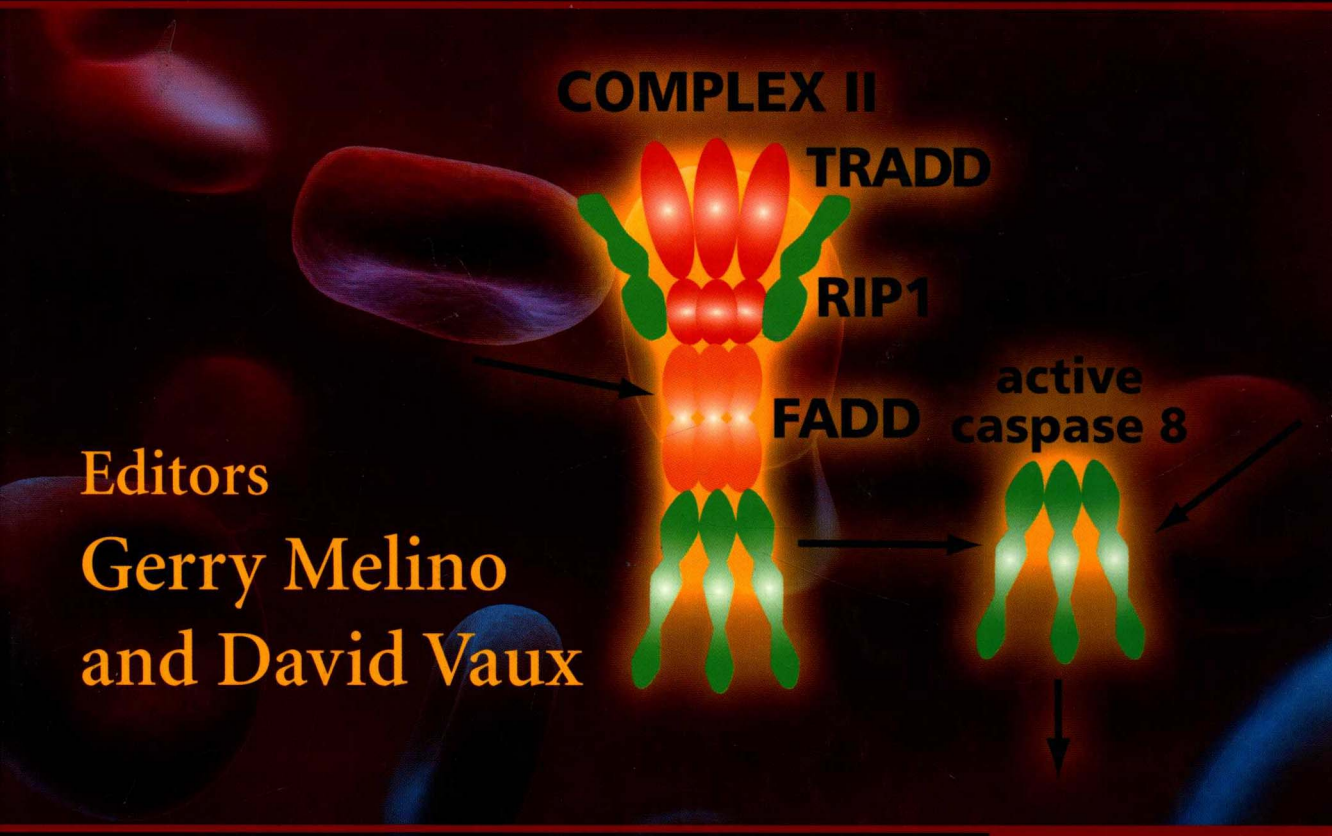
Cell Death



Editors
Gerry Melino
and David Vaux

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Editors

Gerry Melino
MRC Toxicology Unit, University of Leicester, Leicester, UK

David Vaux
La Trobe University, Victoria, Australia

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Contributors

Jean Claude Ameisen Université Paris-Diderot, Faculté de Médecine Xavier Bichat, Paris, France
The Origin and Evolution of Programmed Cell Death; The Siren's Song: This Death That Makes Life Live

Eric H Baehrecke University of Massachusetts Medical School, Worcester, Massachusetts, USA
Autophagy in Nonmammalian Systems

Giovanni Benard University of Maryland Biotechnology Institute, Baltimore, Maryland, USA
Mitochondria Fusion and Fission

Brent Butts Merck Research Laboratories, Rahway, New Jersey, USA
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Eleonora Candi University of Tor Vergata, Department of Experimental Medicine, Rome, Italy
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Francesco Cecconi Department of Biology, University of Rome Tor Vergata, and IRCCS Fondazione Santa Lucia, Rome, Italy
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María Isabel Colombo IHEM-CONICET- Facultad de Ciencias Médicas, Universidad Nacional de Cuyo, Mendoza, Argentina
Autophagy

Catherine L Day University of Otago, Dunedin, New Zealand
Structure and Function of IAP and Bcl-2 Proteins

Wim Declercq Molecular Signaling and Cell Death Unit, Department for Molecular Biomedical Research, VIB-Ghent University, Ghent, Belgium
Death Receptor-induced Necroptosis

Paula Deming University of Vermont, Burlington, Vermont, USA
Dismantling the Apoptotic Cell

Vishva M Dixit Genentech Inc., South San Francisco, California, USA
Drug Discovery in Apoptosis

Elisabetta Ferraro Department of Biology, University of Rome Tor Vergata, and IRCCS Fondazione Santa Lucia, Rome, Italy
The Apoptosome: The Executioner of Mitochondria-mediated Apoptosis

Douglas R Green St. Jude Children's Research Hospital, Memphis, Tennessee, USA
Mitochondrial Outer Membrane Permeabilization

Georg Häcker Institute for Medical Microbiology, Technische Universität München, Munich, Germany
Microbial Inhibitors of Apoptosis

Rikinari Hanayama Department of Medical Chemistry, Kyoto University Graduate School of Medicine, Kyoto, Japan
Engulfment of Apoptotic Cells and its Physiological Roles

Lina Happo The Walter and Eliza Hall Institute of Medical Research, Melbourne, Victoria, Australia
BH3-only Proteins

Sue Haupt The Peter MacCallum Cancer Centre, Melbourne, Victoria, Australia
P53 and Cell Death

Ygal Haupt Lautenberg Center for General and Tumor Immunology, The Hebrew University Hadassah Medical School, Jerusalem, Israel
P53 and Cell Death

Michael O Hengartner Institute of Molecular Biology, University of Zurich, Zurich, Switzerland
Cell Death in C. elegans

Lorraine D Hernandez Merck Research Laboratories, Rahway, New Jersey, USA
Caspases and Cell Death

Franky Van Herreweghe Molecular Signaling and Cell Death Unit, Department for Molecular Biomedical Research, VIB-Ghent University, Ghent, Belgium
Death Receptor-induced Necroptosis

Jahda H Hill University of Maryland Biotechnology Institute, College Park, Maryland, USA
Autophagy in Nonmammalian Systems

Mark G Hinds Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, Australia
Structure and Function of IAP and Bcl-2 Proteins

Maarten Hoek Merck Research Laboratories, Rahway, New Jersey, USA
Caspases and Cell Death

Caroline Houde Merck Research Laboratories, Rahway, New Jersey, USA
Caspases and Cell Death

Mariusz Karbowski University of Maryland Biotechnology Institute, Baltimore, Maryland, USA
Mitochondria Fusion and Fission

Contributors

Richard A Knight MRC Toxicology Unit, University of Leicester, Leicester, UK *and* University College London, London, UK
Cornification of the Skin: A Non-apoptotic Cell Death Mechanism; The Siren's Song: This Death That Makes Life Live

Sally Kornbluth Duke University, Durham, North Carolina, USA
Dismantling the Apoptotic Cell

Peter H Krammer German Cancer Research Center, Heidelberg, Germany
Death Receptors

Inna N Lavrik German Cancer Research Center, Heidelberg, Germany
Death Receptors

Philippe M LeBlanc McGill University, Montreal, Canada
Caspases in Inflammation and Immunity

Michael J Lenardo National Institutes of Health, Bethesda, Maryland, USA
Apoptosis: Inherited Disorders

Stuart A Lipton Burnham Institute for Medical Research, La Jolla, California, USA
From Reactive Oxygen and Nitrogen Species to Therapy

Yu-Chih Lo Weill Cornell Medical College, New York, USA
Structures, Domains and Functions in Cell Death (DD, DED, CARD, PYD)

Peter D Mace University of Otago, Dunedin, New Zealand
Structure and Function of IAP and Bcl-2 Proteins

Marion MacFarlane MRC Toxicology Unit, University of Leicester, Leicester, UK
Death Receptors at the Molecular Level: Therapeutic Implications

Seamus J Martin Department of Genetics, The Smurfit Institute, Trinity College, Dublin, Ireland
Caspases, Substrates and Sequential Activation

Scott R McKercher Burnham Institute for Medical Research, La Jolla, California, USA
From Reactive Oxygen and Nitrogen Species to Therapy

Huseyin Mehmet Merck Research Laboratories, Rahway, New Jersey, USA
Caspases and Cell Death

Gerry Melino MRC Toxicology Unit, University of Leicester, Leicester, UK *and* University of Tor Vergata, Department of Experimental Medicine, Rome, Italy
Cornification of the Skin: A Non-apoptotic Cell Death Mechanism; The Siren's Song: This Death That Makes Life Live

Masanori Miyanishi Department of Medical Chemistry, Kyoto University Graduate School of Medicine, Kyoto, Japan
Engulfment of Apoptotic Cells and its Physiological Roles

Shigekazu Nagata Department of Medical Chemistry, Kyoto University Graduate School of Medicine, Kyoto, Japan
Engulfment of Apoptotic Cells and its Physiological Roles

Tomohiro Nakamura Burnham Institute for Medical Research, La Jolla, California, USA
From Reactive Oxygen and Nitrogen Species to Therapy

Donald W Nicholson Merck Research Laboratories, Rahway, New Jersey, USA
Caspases and Cell Death

Tom O'Brien Genentech Inc., South San Francisco, California, USA
Drug Discovery in Apoptosis

Melissa J Parsons St. Jude Children's Research Hospital, Memphis, Tennessee, USA
Mitochondrial Outer Membrane Permeabilization

Guihong Peng University of Maryland Biotechnology Institute, Baltimore, Maryland, USA
Mitochondria Fusion and Fission

Maya Saleh McGill University, Montreal, Canada
Caspases in Inflammation and Immunity

Clare L Scott The Walter and Eliza Hall Institute of Medical Research, Melbourne, Victoria, Australia
BH3-only Proteins

Karin A Sedelies Apoptosis and Natural Toxicity Laboratory, Peter MacCallum Cancer Centre, Melbourne, Victoria, Australia
Immunity, Granzymes and Cell Killing

Ataman Sendoel Institute of Molecular Biology, University of Zurich, Zurich, Switzerland
Cell Death in C. elegans

John Silke La Trobe University, Melbourne, Victoria, Australia
Apoptosis: Regulatory Genes and Disease

Hans-Uwe Simon Institute of Pharmacology, University of Bern, Bern, Switzerland
Autophagy

Andreas Strasser The Walter and Eliza Hall Institute of Medical Research, Melbourne, Victoria, Australia
BH3-only Proteins

Helen C Su National Institutes of Health, Bethesda, Maryland, USA
Apoptosis: Inherited Disorders

Olivia Susanto Apoptosis and Natural Toxicity Laboratory, Peter MacCallum Cancer Centre, Melbourne, Victoria, Australia
Immunity, Granzymes and Cell Killing

Jun Suzuki Department of Medical Chemistry, Kyoto University Graduate School of Medicine, Kyoto, Japan
Engulfment of Apoptotic Cells and its Physiological Roles

Joseph A Trapani Cancer Cell Death Laboratory, Peter MacCallum Cancer Centre, Melbourne, Victoria, Australia
Immunity, Granzymes and Cell Killing

Tom Vanden Berghe Molecular Signaling and Cell Death Unit, Department for Molecular Biomedical Research, VIB-Ghent University, Ghent, Belgium
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Peter Vandenabeele Molecular Signaling and Cell Death Unit, Department for Molecular Biomedical Research, VIB-Ghent University, Ghent, Belgium
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David L Vaux La Trobe University, Victoria, Australia
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James E Vince The University of Lausanne, Epalinges, Switzerland
Apoptosis: Regulatory Genes and Disease

John G Walsh Department of Genetics, The Smurfit Institute, Trinity College, Dublin, Ireland
Caspases, Substrates and Sequential Activation

Nigel J Waterhouse Apoptosis and Natural Toxicity Laboratory, Peter MacCallum Cancer Centre, Melbourne, Victoria, Australia
Immunity, Granzymes and Cell Killing

Kamil Wolyniec The Peter MacCallum Cancer Centre, Melbourne, Victoria, Australia
P53 and Cell Death

Hao Wu Weill Cornell Medical College, New York, USA
Structures, Domains and Functions in Cell Death (DD, DED, CARD, PYD)

Hiroshi Yamaguchi Department of Medical Chemistry, Kyoto University Graduate School of Medicine, Kyoto, Japan
Engulfment of Apoptotic Cells and its Physiological Roles

Preface

Individual cells face three choices: to divide (mitosis), to specialize (differentiate) or to commit suicide (cell death). The balance between these processes ensures that the number of cells in an organism remains essentially in functional equilibrium. While mitosis and differentiation have received detailed attention from cell and molecular biologists for well over a century, physiological cell death has become a major interest only in the last twenty years. Until recent times, most reports of cell death focussed on "accidental" cell death, or "cell killing", where a cell dies because a vital metabolic process necessary for its continued survival is blocked. We now know that in multicellular organisms cell death by suicide is far, far, more common than death of cells because they have been killed.

In retrospect, it is surprising that for a long time biologists never questioned the fate of so many duplicating cells in our body. If we imagine an 80-year-old person in which mitosis proceeded unopposed by any balancing homeostatic death process, he would have around two square km of skin, two tons of bone marrow and lymph nodes, and a gut 16 km long. Indeed, mitosis unchecked by cell death results in neoplastic pathology. Ironically, it was study of just such a neoplasia - follicular lymphoma - that led to identification of the first component of the mechanism for cell suicide. While determining more about the mechanisms for cell death have continued to reveal much about the origins of malignant disease, they have also provided new insights into diseases caused by too much or unregulated cell suicide, such as neurodegenerative diseases.

Searching journal articles in the last twelve months using the terms "cell death" or "apoptosis" yields about 20,000 publications, yet the same search in the year 1987 identifies only 439 publications. The reason for this tremendous growth in interest in cell death research is that many of the molecular mechanisms by which cells kill themselves have been discovered, and abnormalities in the regulation of cell death have been linked to human disease.

Unfortunately, because of the rapid growth of the field, many of the publications on cell death are not totally reliable, have been contradicted, or remain controversial, which can be misleading for students or clinicians new to the area. Nonetheless, several new drugs have been developed based on our current understanding of the molecular mechanisms of death, and many advanced clinical trials look highly promising.

Since cell death has now become translational, and therefore of interest to clinicians, pharmacologists and medical chemists, as well as to basic biologists, it seems an appropriate time to produce this book. In it, we have attempted to clarify the inconsistencies in the literature, in particular by referring to more definitive studies now available using transgenic or gene deleted mice. We have also tried to highlight those as yet unexploited molecular pathways susceptible to therapeutic intervention.

Our thanks go first to our publisher, who stimulated us in this endeavour, and to the scientists who dedicated some of their precious time writing the individual chapters. Our apologies go to our many colleagues who could not be mentioned and properly credited because of time and space limitations. We hope our readers find our efforts insightful and rewarding.

Gerry Melino and David Vaux
Leicester, UK and
Victoria, Australia
February 2010

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The Siren's Song: This Death That Makes Life Live

Keynote article

Gerry Melino, *MRC Toxicology Unit, University of Leicester, Leicester, UK*

Richard A Knight, *MRC Toxicology Unit, University of Leicester, Leicester, UK*

Jean Claude Ameisen, *Université Paris-Diderot, Faculté de Médecine, Xavier Bichat, Paris, France*

Individual cells can divide (mitosis), specialize (differentiate) or undergo programmed cell death (apoptosis). The balance between these processes ensures that the number of cells in an organism remains essentially constant. In the past 30 years, the molecular mechanisms of cell death have been identified (caspases, Bcl-2 family, death receptors and apoptosome), with their clinical implications and therapeutic exploitation. Here, we review the entire process from a philosophical and historical viewpoint.

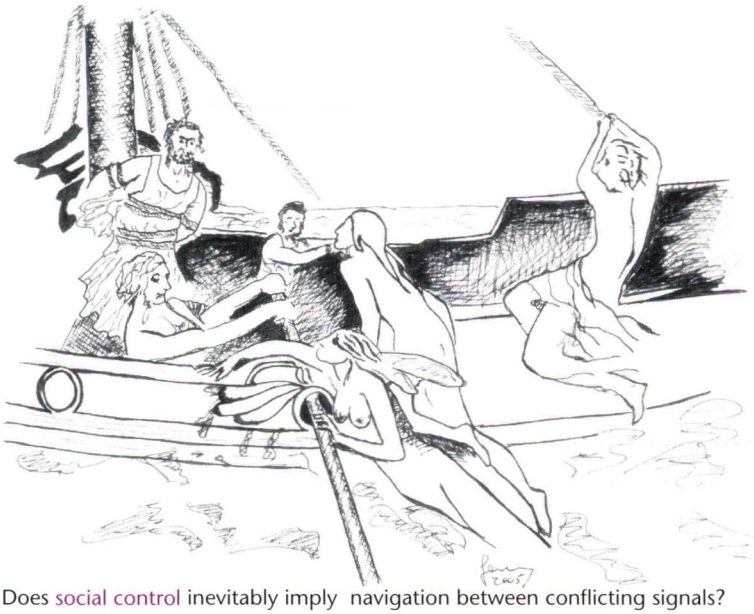
Thereby no ship of men ever escapes that comes thither, but the planks of ships and the bodies of men confusedly are tossed by the waves of the sea and the storms of ruinous fire.

'There is only one serious philosophical problem. It is suicide. It is to judge whether life is or is not worth living'. Thus Albert Camus, following Homer (**Figure 1**), Novalis and Kierkegard puts suicide at the centre of thinking. Since almost 3% of all 650 000 papers published annually in the life sciences are related to apoptosis (cell suicide), it would seem that biology has also entered an existentialist phase.

Individual cells face three choices; to divide (mitosis), to specialize (differentiate) or to commit suicide (apoptosis). The balance between these processes ensures that the number of cells in an organism remains essentially constant (**Figure 2**). However, while mitosis and differentiation have received detailed attention from cell and molecular biologists for well over a century, a morbid fascination with cell death has only recently become a major interest. Indeed, it is surprising that for a long time biologists never questioned the fate of so many duplicating cells in our body. This is particularly true, since if mitosis proceeded unopposed by any balancing homeostatic death process, an 80-year-old person would have *c*.2 Km^2 of skin, 2 tons of bone marrow and lymph nodes, and a gut 16 Km long – the radius of a major world city, including the unpleasant last 500 m (Melino, 2001).

Nevertheless, it took a long time for apoptosis to enter its current position in the limelight. Part of the explanation for this historical imbalance of interest lies in the apoptotic process itself. It is usually 20 times faster than mitosis and sightings of dying cells are rare as they are rapidly absorbed and degraded (phagocytosed) by neighbouring cells. This very speed makes scientific observation and description more difficult. Indeed, *in vivo*, in a given time frame, 20% of mitotic cells are equilibrated by 1% of apoptotic cells, the limit of detection. Hence, in contrast to passive cell death (necrosis) – where leakage and inflammation are distinctive features – apoptotic cells are engulfed and degraded by neighbouring cells without a trace (**Figure 3**). The apoptotic cell and its fragments effectively become surrounded by a dynamically remodelled but impermeable cocoon which prevents extracellular leakage of any potentially harmful intracellular contents, and which would otherwise cause surrounding inflammation and scarring (as observed in toxic death and necrosis). The introduction of the concept of apoptosis/programmed cell death and its molecular interpretation, which is still only partially understood, now allows us to understand how these cellular choices are made and how the entire process evolved (Ameisen, 2002; Koonin and Aravind, 2002). **See also**: Apoptosis: Regulatory Genes and Disease; Engulfment of Apoptotic Cells and its Physiological Roles; The Origin and Evolution of Programmed Cell Death

However, there may be deeper reasons for the late development of interest in cell death. To paraphrase



The Siren's song

1. Sirens evoke a death desire [death receptors]
2. To survive, Odyssesus uses wax in his sailor's ears [block of death receptors]
3. ...and ties himself to the mast [block of signalling, DISC]
4. Orpheus plays his lyre [survival factors, NGF]

Does social control inevitably imply navigation between conflicting signals?

Figure 1 Odysseus is tempted by the Sirens. Homer first describes the death wish of the Siren's song, and the way Odysseus resists to survive. Indeed, Homer describes death (point 1 on the right) and two survival mechanisms (points 2 and 3). Similarly Orpheus (point 4) counteracts death signals by playing survival signals with his song.

Hermann Hesse's views: 'The Orient is pervaded with a totality or religiosity encompassing death, whereas the West is focused on logic and technology which breed divisiveness and which to a degree negate or even ignore death'. Death is therefore marginalized in the Occident, which may therefore account for its late incorporation into scientific consciousness. Although it may have been expected that the study of death would therefore emerge earlier in the East, the lack of development of necessary technology must also be borne in mind. Death (not Being) raises the question of what is, in fact, Being (see **Box 1**). Another German philosopher, Martin Heidegger, asked a related question; what does the verb 'to be' mean? We know that a table and my colleague 'are', but what constitutes their 'areness' as opposed to their absence? What is the Being, and its related modification that we call 'persistence during time'? One possible answer, more applicable to the animate situation, is to regard 'being' as a process, by which we imply at the level of the cell, the individual and even the species, both an identity and a sequence of finite adaptive change over time, and which becomes 'non-being' when the process is ended by fragmentation or death or extinction. In fact, beside technical difficulties, we were not philosophically ready to study 'Death', and in particular 'death from within'.

Thomas Kuhn argued that a scientific revolution begins with the perception of an anomaly. Accordingly, Gunther Stent believed that a scientific discovery starts when a series of implications could not be linked in a logical structure based on current knowledge – a process which can take a long time. Cell death clearly shows such a long gestation (Ameisen, 1999; **Figure 4** and **Figure 5**). Some morphological observations which we would now regard as apoptotic have been made since the middle of the nineteenth century without their biological significance being appreciated until recently. In 1842, Vogt recognized a form of physiological cell death, whereas Flemming, in 1855, used the term chromatolysis to describe the nuclear fragmentation seen during cell death – a characteristic still used, among others, as a hallmark of apoptosis. Other similar descriptions occurred occasionally in the nineteenth and early twentieth centuries. Recently the embryologist Glucksman (1951), the haematologist Bessis (1955) and the biologist Tata (1960) clearly described the morphological phases of apoptosis. In the 1960s, working on insect development, Richard Lockshin recognized the coordinated death of sheets of cells – a process he termed programmed cell death – and which he showed to be energy dependent and to require gene transcription (Lockshin and Williams, 1965). In 1966,

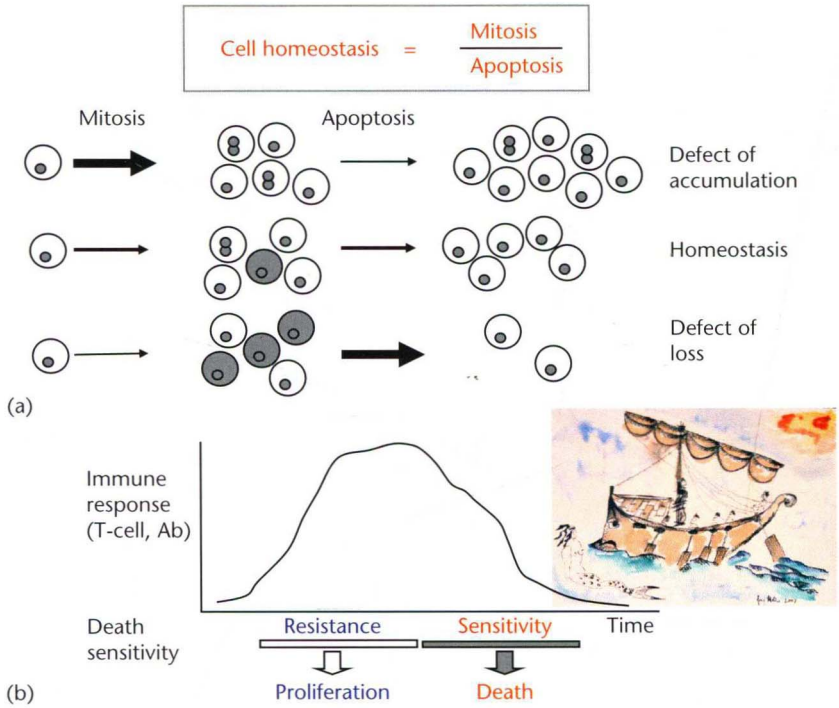


Figure 2 Death and homeostasis. (a) The basic importance of cell death is in counteracting mitosis to regulate homeostasis of cell number in tissues as well as in the entire organism. Consequently, unbalance of mitosis versus apoptosis results in pathologies with accumulation (e.g. cancer) or loss (e.g. neurodegeneration and AIDS) of cell numbers. (b) Physiological events such as immune responses require a tight regulation between death sensitivity and resistance.

John Saunders is able to review 'Death in embryonary systems', showing the role of cell death in moulding the body during development. It was not until the 1970s, when the young Australian pathologist John F Kerr start analysing the morphology of dying cells in histopathology, first alone in Brisbane (1964–1968), then in Edinburgh (1972–1980) where, in collaboration with Andrew H Wyllie and Alistair Currie, the term 'apoptosis' was first used (Kerr *et al.*, 1972). Lockshin and Kerr deserve credit for creating an intellectual framework for all previous observations, moving from scattered observations to interpretation, thus playing a pivotal role in the creation and diffusion of the concept, which has been highly conducive to its development.

It was not until apoptosis moved from the morphological to the mechanistic that it fully acquired scientific credibility and began to provide an intellectual framework for the previous scattered observations (Hengartner, 2000; Krammer, 2000; Meier *et al.*, 2000; Nicholson, 2000; Rich *et al.*, 2000; Savill and Fadok, 2000; Yuan and Yankner, 2000). For this, the credit is largely due to Sydney Brenner, John Sulston and mainly H Robert Horvitz and his collaborators (Michael Hengartner and Junying Yuan) (Metzstein *et al.*, 1998; **Figure 3**). Working with the nematode, *Caenorhabditis elegans*, Sulston first mapped the fate of every cell in the organism, including those that were to commit apoptosis. At first sight, it may seem bioenergetically pointless to generate cells which are then programmed to die. However, the vast majority of cell division is asymmetrical, according, for example, to an anterior–posterior axis of division, generating from a cell A two daughters A and B. If, for example we need two B (e.g. 'neuronal'), a first division generates A–B, and a further division of A, generates A–B, by killing A, we then obtain two B instead of one A and two B (Horvitz and Herskowitz, 1992). This illustrates one of the roles of cell death in the generation of differentiation during development. **See also**: Cell Death in *C. Elegans*

By 1990, Horvitz had shown that apoptosis was determined by several genes, including ced-9 (*The Good*, which blocks apoptosis), ced-3 (*The Bad*, which executes apoptosis) and ced-4 (*The Ugly*, which is an activator of apoptosis) (Avery and Horvitz, 1987;

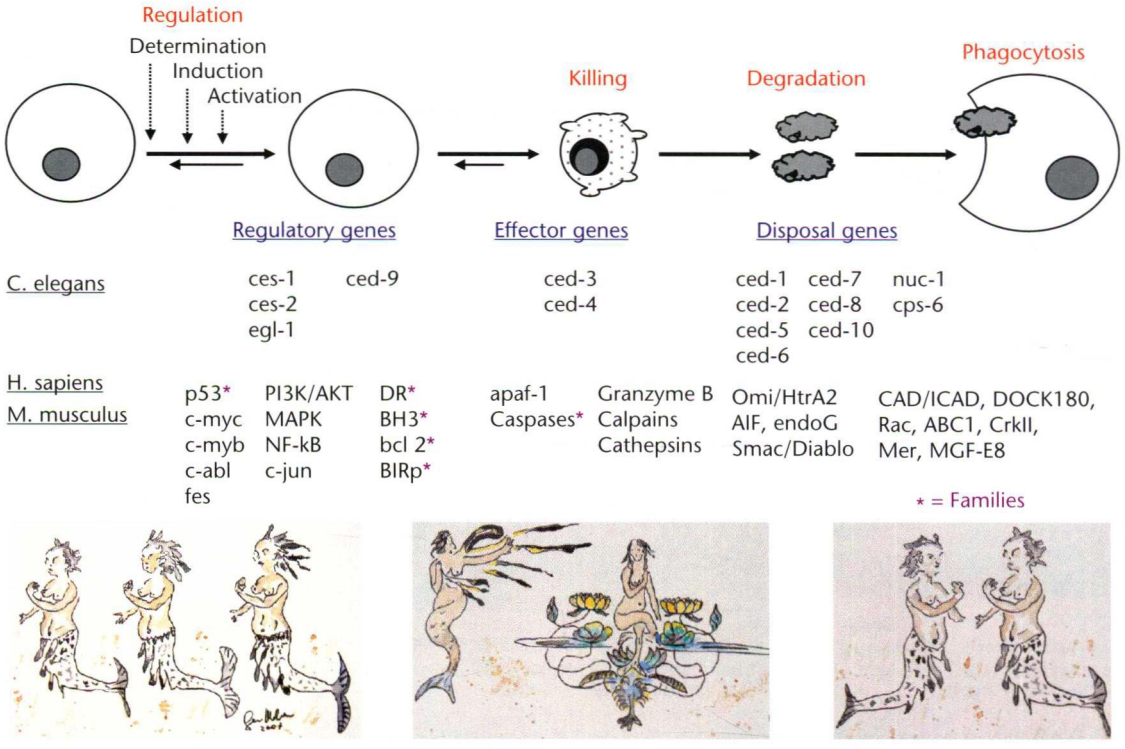


Figure 3 Mechanisms of cell death. Compared to living cells, apoptotic cells show cell shrinkage, smoothness of the cell membrane which remains intact, detachment of the nuclear membrane and condensation of chromatin (with fragmentation of DNA). The dead cell is recognized and phagocytosed by neighbouring cells, thus disappearing from the tissue. The entire process occurs within minutes. The genes involved can be distinguished into regulatory, effector (killing and degradation) and disposal genes, as indicated for the nematode and mammals. (* indicates families of proteins). The basic core mechanism of cell death requires a killer protease (ced-3/caspases) always ready to act, which requires an activator (ced-4/apaf-1) which in turn is repressed by a regulator (ced-9/Bcl-2, related to mitochondria): *ced-9* —| *ced-4* → *ced-3* → death. This core mechanism is activated by an activator (Egl-1/BH-3), and followed by the rapid disposal of the dead corps: Elg-1 —| *ced-9* —| *ced-4* → *ced-3* → death → phagocytosis.

Yuan and Horvitz, 1990; Hengartner *et al.*, 1992). In describing a molecular mechanism, this work provided a new intellectual stimulus. That these molecular developments were crucial is evident from the number of genes and pathways now identified in insects, mammals, as well as other species and their emerging physiological and pathological roles. Crucial, in this process, was the identification of the function of the ced-9 equivalent, Bcl-2 before the sequencing of ced-9 (Vaux *et al.*, 1988). These three main *C. elegans* genes have been highly conserved throughout evolution (Koonin and Aravind, 2002; Ameisen, 2002), such that they, or rather their corresponding gene families, still determine the apoptotic process in mammals. Thus in man, there are 21 *Goods* (the Bcl-2 family), 14 *Bads* (the caspase family), but still (so far) only one *Ugly* (Apaf-1 homologues) (**Figure 3**). **See also**: Caspases and Cell Death; The Apoptosome: The Executioner of Mitochondria-mediated Apoptosis; The Bcl-2 Family Proteins - Key Regulators and Effectors of Apoptosis

Caspases can be the target of viral (as first shown by Lois Miller) or cellular (BIR) inhibitory proteins modulating cell death, and are now being actively exploited for pharmaceutical purposes, for example by Don Nicholson's work (Nicholson *et al.*, 1995). Their molecular effector mechanisms were clarified by the brilliant work of Xiaodong Wang (Liu *et al.*, 1996) and the definition of a novel dedicated cellular organelle, the apoptosome, which activates the *Bads*. These 'master switches' have been highly conserved in evolution so that they, or, again, their equivalent families, still orchestrate apoptosis in mammals (**Figure 3** and **Figure 6**). Like all biological systems, however, death (like life) is not this simple, and apoptosis following mitochondrial damage or death receptor binding may not be inevitable. A number of regulatory sites have

Box 1 Being, Not-being and Death

Being is a fundamental theme in philosophy: the Soul is, in a certain way, the Being [*ens*] (Aristotle): *ens, quod natum est convenire cum omni ente* (soul) (Thomas Aquinas); the *res cogitans* from the *Coito ergo sum* (Descartes). A theme, perhaps, not fully elucidated as yet. Returning to our field, science could be defined as the possibility of interconnections founded on true propositions (Heidegger); and as behaviour/acts of man. Science has the way of being of this Being, defined as Being-in [*Insein*]. Hence, the comprehension of the Being is also the determination of the being of the Being-in. Consequently, science is a way of being of the Being-in, on which the Being relates (being, of the Being-in-the-World in Heidegger In-der-Welt-Sein). Therefore, the fundamental ontology must be found in the existential analysis of the Being-in-the-World, both in an ontic (determined in his being by existence) and ontologic (for its being-determined by existence) sense. Thus, biology, as the science of life, is found on the ontology of the Being-in-the-World. In a modern sense, science results in a phenomenology, from *Φεινομενον* (*id est*, manifest, bring to light, apparent) and *Λογοσ* (*id est*, discuss, be true, be false – thus including the possibility that science, as a phenomenology, might result in false assertions). Following Heidegger, the fundamental structural character or mode of the Being-there/here [*Dasein*] is not that of a subject or that of the object, but that of the coherence of the Being-in-the-world [*Insein*], with its related modifications, a concept that we define as time [*weltanshaung, zeitlichkeit, temporalitaet*]; its modalities are the emotional situation (fear, anguish) and the understanding (interpretation, assertion, discussion, curiosity, chat). For Heidegger, the anguish call of the ethical conscience is silence (see also Wittgenstein) and its sense is 'Death' (Being-toward-detah, in Heidegger Sein-zum-Tode).

been described which can interfere with pro-apoptotic signalling, and even some caspase-like molecules have been shown to have antiapoptotic effects. The *Bads* are not always so? This multiplicity of interacting pro- and antiapoptotic factors implies that, as in mitosis, a number of checks and balances are present in the apoptotic programme, and that suicide is carefully considered by the cell as it was by Camus, although a suicidal cell can scarcely be accorded the thoughtful dimension that Camus applied to human suicide. **See also**: Inhibitor of Apoptosis (IAP) and BIR-containing Proteins

There are further complications. How did these molecules and pathways evolve to their current genomic status without having been counter-selected (Ameisen, 1999)? Most likely, the genetic/biochemical subroutines have evolved from other pathways, for example related to mitosis or deoxyribonucleic acid (DNA) damage. It is necessary, moreover, to consider the danger of transferring definitions from one discipline to another, without also bringing confusing implications. Despite the fact that it is suggestive and attractive, the use of the words 'death' or 'suicide' carries implications that are different from those if we would have instead used 'dismantling' or 'disaggregating'. 'Death', for example, implies that there is only one death, that there is nothing after death, and that it is the final event. However, dead cells might 'die' more than once (erythroblasts 'die' when they lose their nuclei and mitochondria to become erythrocytes, and then 'die' again when they are eliminated from circulation; keratinocytes 'die' when differentiated and lose their nuclei and mitochondria, and then 'die' again during desquamation; the same applies to megakaryocytes and platelets). These cells remain active and functional after 'partial death' (e.g. erythrocytes transport oxygen, keratinocytes guarantee the barrier function of the epidermis, platelets provide aggregation and clotting), and death is not the final event (but it precedes differentiation in all earlier examples). Accordingly, if we use the term 'suicide', we bring in, subliminally, anthropological implications derived from the social and philosophical field. We could say that cells commit suicide for the benefit of the organism (altruistic death with social implications). We could also say that the organism kills innocent cells for its own selfish interest (egotistic death). Here, we should consider the definition of 'self' of the cell (I, cell, kill myself for the benefit of the organism), or 'self' of the gene/organism (I, gene/organism, kill the innocent cell for the survival of the genome/organism). But do genes, cells and organisms have a 'self'? **See also**: Cornification of the Skin: a Non-apoptotic Cell Death Mechanism

It is therefore only within the closing years of the last millennium, *The Golden Age*, that apoptosis has been given the scientific interest that its biological significance deserves, providing important research guidelines for the next decade. Recently, differences in the execution of apoptosis are emerging. Possibly, these new emerging facets of the original concept are related to cell-to-cell variation due to the fact that several molecular constituents of the process exists as large

redundant molecular families which are differentially expressed in different tissues.

The concept of apoptosis has now reached the level of a fashion, **Figure 4**. With over 19 000 papers annually and three dedicated journals, it may be that overemphasis is leading to distortion. The concentration on apoptotic death diminishes the physiological and pathological role of necrosis, and fields such as toxicology are desperately searching for a new identity. At the same time, the definition is changing, and acquiring subtle differences. Originally, the term 'programmed cell death' was a definition of a process (genetically and developmentally programmed) whereas 'apoptosis' implied a biochemical character (apoptosis = caspases). Now, the two terms are generally used as synonymous, as opposites to necrosis (passive, nongenetically programmed death). Recently, the term 'cell death' has become more general, including not just apoptosis and necrosis but also other types of death such as: autophagy (Kourtis and Tavernarakis, 2009), caspase-independent cell death, keratinization, Wallerian degeneration and erythrocyte karyorexis. Until definitions are linked to biochemical pathways, in other words, to process, however, these subdivisions remain semantic.

In stating that it is not possible to enter the same river twice, Heraclitus expressed the irreversibility of time. We too, like a flowing river, undergo continuous changes. If the molecules forming our body are continuously changing, what is the meaning of permanence? What controls the changes in the molecules that form our bodies? How do our cells socially interact between themselves to constitute a unified whole? Gradually, the idea emerged that equilibrium and stability of the body is maintained in a dynamic way by signals controlling life and death of single cell. In 1992, Martin Raff developed the concept of 'social control of life and death' (**Figure 7**). This is a concept of enormous power, implying that there are specific survival and death signals, and corresponding receptors on cells. In

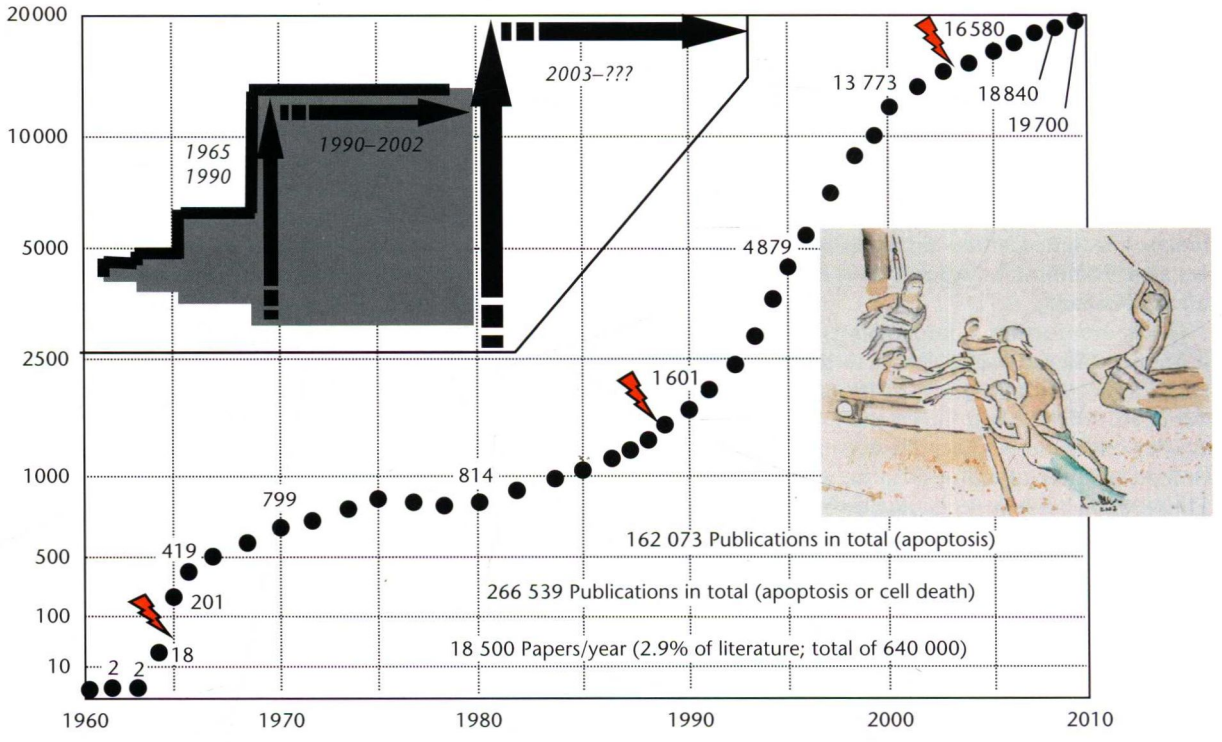


Figure 4 Scientific papers on cell death. A large number of scientific publications have focused on cell death. We might distinguish three phases, from scattered observations before 1965, when the original work in invertebrates and embryology described the phenomenon. From 1990, culminating with the 2002 Nobel Prize, the molecular events were identified. Recently, the detailed mechanisms have been investigated, whereas the clinical relevance, with its potential therapeutic exploitation is being explored. *Inset*, advancement occurs in steps, with pioneering explorative and controversial work, followed by consolidation and refining research.