

TIMELINE

Programmed cell death and apoptosis: origins of the theory

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Interest in the study of apoptosis grew with the recognition that it is a highly regulated process. Such a change in attitude allowed the intellectual and technical breakthroughs that led to the explosive development of this subject.

The subject of programmed cell death — or apoptosis — now boasts almost 80,000 publications, and most scientists presume that the subject was born in the 1970s. As in most fields with assumptions of this sort, we have spent a substantial portion of our careers reinventing wheels: by the mid-nineteenth century, histologists and particularly developmental biologists were well aware of physiological cell deaths (BOX 1).

Interest in the subject was modest, but began to grow rapidly in about 1990, when a changed attitude — that cell death is not an incidental part of life, but that it is a highly controlled and medically important element of existence — caught hold. In other words, we began to invert a standard description of cell turnover — ‘cells die and are replaced’ — by changing the emphasis to the first phrase. So, by saying that ‘cells die, and they are then replaced’ the death was recognized as an interesting and biological event.

Much of the history surrounding cell death at various times has been reviewed elsewhere^{1–2}, and here we summarize the main milestones (TIMELINE). This article addresses only the earlier parts of the history of cell death, and the number of references is restricted. So, many important findings of recent years — including the expansion of

research into caspases and the role of mitochondria in apoptosis — are mentioned only briefly.

Cell death in the nineteenth century
In 1996, Clarke and Clarke¹ undertook the Herculean task of searching the nineteenth century literature, and found many instances in which anatomists commented on cell death. Usually this related to the metamorphosis of tadpoles and insects, but transient embryonic structures such as the notochord were also mentioned. Many of these studies were done in the context of evolving and improving histological techniques, and many involved bulky tissues with large cells such as muscles or cartilage transforming into bone. Many of these tissues undergo a form of cell death that differs from paradigmatic apoptosis (BOX 1),

and, for the most part, the illustrations of cell death were used to categorize, but not emphasize, certain features. These included the margination and coalescence of the chromatin, or the shrinkage of cells — both defined then as chromatolytic cell death and now considered hallmarks of apoptosis.

What we would today call truly apoptotic cells were seen by Walther Flemming^{3,4} in 1885 and by others^{1,5}. Other types of cell death were also seen⁶, such as those that were more vacuolar (probably lysosomal^{7–10}), but at the end of the nineteenth century many scientists were more interested in phagocytosis. The variants of cell death are pictured in FIGS 1 and 2. Furthermore, other than an interest in the possibility that phagocytes directly attacked healthy cells, and attempts to categorize the types seen, there was little effort (or capability) to study causes or controls.

The first half of the twentieth century
During the early twentieth century, cell death remained a subject of some interest in insect physiology and perhaps elsewhere. For instance, Ilya Mechnikov, resident in France, won a Nobel prize in 1908 for his discovery of phagocytosis, and Charles Pérez¹⁰ wrote a long treatise on the metamorphosis of the blowfly *Calliphora erythrocephala*, emphasizing

Box 1 | Types of cell death

‘Programmed cell death’ originally referred specifically to instances in which a sequence of events could be established that lead to death of the cell. Later, a requirement for protein synthesis to initiate cell death was identified in most developmental situations. ‘Apoptosis’ refers to a particular morphology in which the chromatin condenses or coalesces to heterochromatin in one or more masses in the nucleus. It usually settles along the still-intact nuclear membrane (referred to as margination of the chromatin, and illustrated in FIG. 1). The cells also shrink and become denser as determined by staining or flow cytometry, and often fragment into several pieces. This morphology is now considered to derive from the activation of caspases, and is common to many but not all cell deaths. The terms ‘physiological’ or ‘regulated’ cell death are more general and cover the different morphologies and sequences. These terms emphasize the idea that such deaths are part of the normal function of the organism, while calling attention to the fact that there are different signals and different evoked pathways. All of these are directly or indirectly genetically regulated, as opposed to necrosis or oncosis, which is accidental and in which the cell has no active role. Necrotic cells most typically lyse, provoking a substantial inflammatory response.

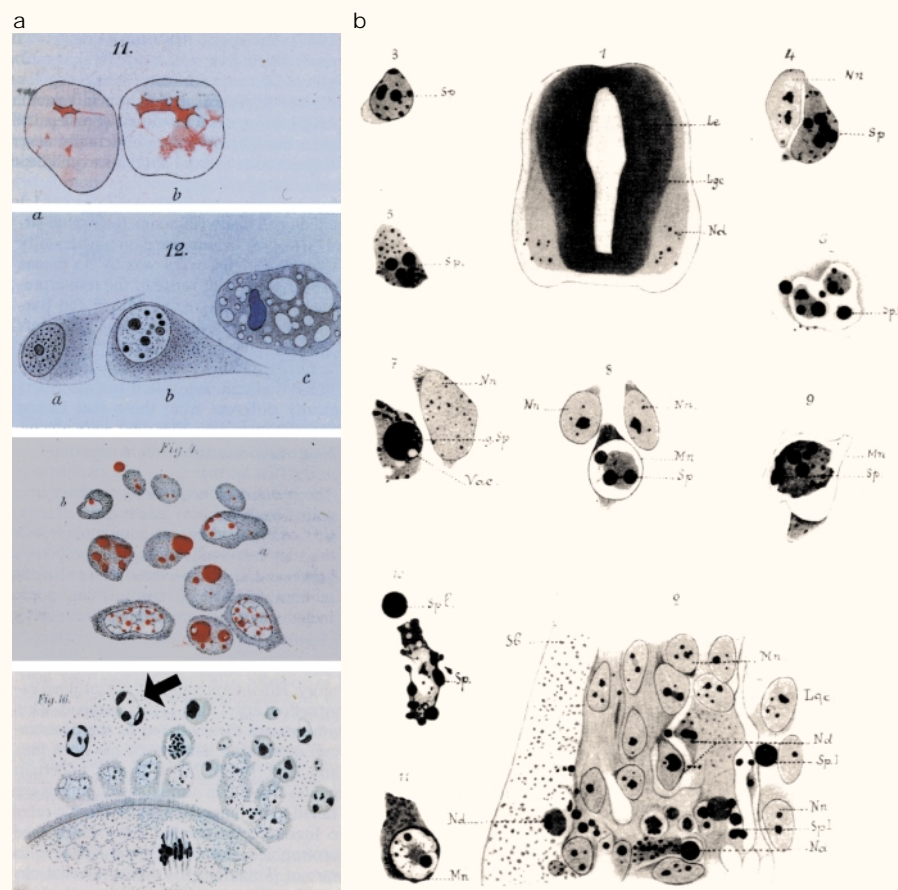


Figure 1 | Older images of cell death. **a** | The top image is axons from an involuting tadpole tail. The second image is from the spinal ganglion. The centre cell might be undergoing apoptosis and the rightmost cell is vacuolating. The lower two frames are from rabbit ovarian follicles from REF. 3, also illustrated in REF. 1. The cell indicated by an arrow shows margination of the chromatin. **b** | Dying chick spinal motor neurons, taken from REF. 52, also illustrated in REF. 1. The neurons marked 'Nd' are degenerating and images of the nuclei are enlarged. Adapted with permission from REF. 1. © (1996) Springer-Verlag.

ing his belief that the larva was destroyed by phagocytes. (There is some validity to this argument: in the 1970s, A. C. Crossley in Australia noted that blowfly muscles are attacked by phagocytes when they seem to be in good condition, and, in amphibia, phagocytes contain pieces of muscle a few sarcomeres in size.)

Many others, relying on inadequate histological techniques, noted the 'liquefaction' of larval tissues or the disappearance of muscle fibres⁷⁻¹⁰. Otherwise, there was little attempt to define the mechanism of cell death. In general, the cells reported in these treatises did not look apoptotic in the sense of today's definition. Up to this time, then, efforts in this field tended to be those of systematists, who were interested in all their new observations, cataloguing deaths in an effort to extract meaning from the weight of the data. This effort culminated in the masterful compilation of A. Glücksmann^{11,12}, who recognized that cell death serves morphogenetic (embry-

onic), histogenetic (as in metamorphosis) and phylogenetic (vestigial or larval organs) purposes. In fact, in the earlier article¹¹, he clearly described several modes of cell death, including an apoptotic appearance of nuclei (karyopyknosis) and nuclear fragmentation (karyorrhexis). He intemperately went on to note, "these three stages have been described in detail" and cited two of his previous publications, suggesting that his earlier work had not been taken into account.

By the mid-1950s, the soluble enzymes of glycolysis were understood and differential centrifugation had been developed to analyse the enzymes of the particulate fractions. By this time, Christian De Duve and his collaborators had recognized the existence of lysosomes¹³. Lysosomes were named because of artefacts associated with their discovery. Carbon tetrachloride poisoning of the liver liberated acid hydrolases from particulate form and thereby increased their activity, leading to the hypothesis that cell death was a

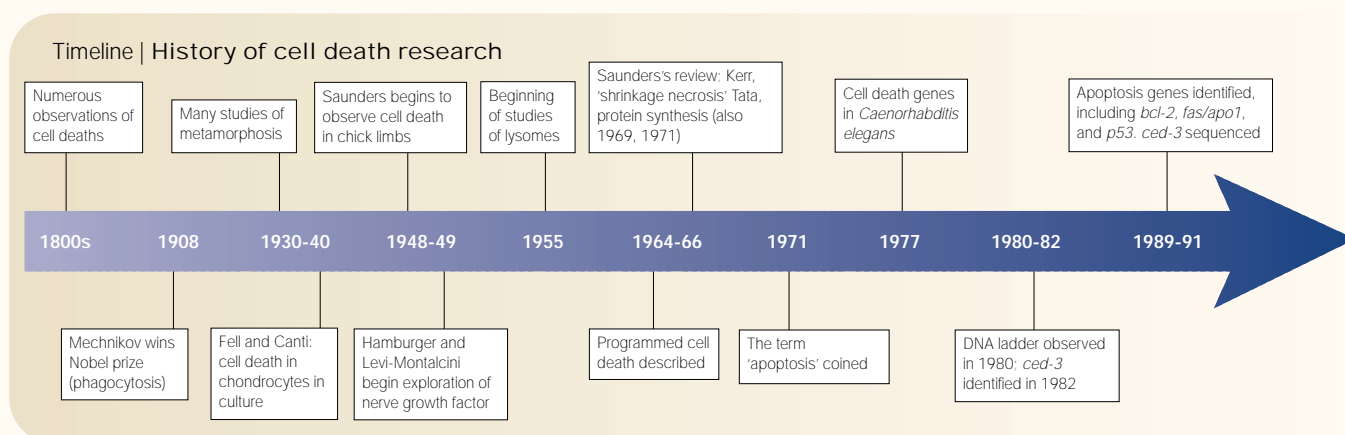
matter of lysosomal rupture. Today we know that lysosomal rupture occurs only in pathological conditions (and, perhaps, other rare circumstances), but the rise of cell biology directed attention to the role of lysosomes in cell death.

Cell death in the 1960s

The 1960s were an exciting time to be in biology. Developmental biologists marvelled at the new levels of sensitivity that were possible using electron microscopy, recently improved histological and histochemical techniques, and miniaturized biochemical techniques including differential centrifugation. These were, in fact, several orders of magnitude less sensitive than techniques today, but nevertheless held the promise of exciting discoveries. The embryologist John W. Saunders Jr, at Albany in the United States, had recognized that there were reproducible patterns of cell death in chick embryos¹⁴ and had been thinking about it since 1948. And insect physiologists, including Zyuiti Kuwana in Japan and L. H. Finlayson in Carroll M. Williams's laboratory at Harvard, had begun to examine metamorphic cell deaths that occurred well after pupation, so could be examined without the messiness and low survival that accompanies the handling of moulting insects. Saunders and other scientists active in the field at this time (and since) are illustrated in FIG. 3.

Williams's group was modestly large at the time, and he enjoyed a habit he had picked up during a stay in England — a late afternoon tea at which everyone gathered and discussed the ideas of the day. He also had a marvellous sense of language, creating juxtapositions of vocabulary and image that were always startling, frequently hilarious, and often provocative and imaginative. It was in this context that we began to refer, in the developing doctoral thesis of one of us (R.A.L.), to 'programmed cell death', meaning that cells followed a sequence of controlled (and thereby implicitly genetic) steps towards their own destruction. The thesis was defended in 1963 and one of several papers entitled "Programmed Cell Death" was published¹⁵ in 1964.

Lockshin and Williams found their argument to be consistent with that of Saunders, who had described the posterior necrotic zone in the axilla of chick limbs and the interdigital death zones in the hand and foot palettes of mice. Saunders was demonstrating that explanted posterior necrotic zone cells of chicks would die on schedule in culture but could be rescued by transplanting them to



the back of other chick embryos. He therefore suggested the existence of a controlled regulation of cell death. As he described in his observations in 1966, "the death clock is ticking"¹⁴. (Fell and Canti¹⁶ had observed much earlier, in 1934, that cells can progress to death *in vitro*, in this instance during differentiation of cartilage, and the fascinating story of nerve growth factor and growth factors in general had started as a cell death story¹⁷; BOX 2.) Saunders's and Lockshin's findings enunciated the possibility that cell death might be regulated, and focused on the genetic pathways and the biochemical mechanisms by which this might occur. Later, they established that the cells remained functional during the early part of their involution¹⁸.

The 1970s

The bulk of publications in the 1970s related cell death to lysosomal activity. For instance, Rudolf Weber and others measured cathepsin levels in metamorphosing tadpole tails¹⁹, and Jan Ericsson and colleagues showed the role of lysosomes in post-lactational involution of the mammary gland²⁰. The increasing ascendancy of thymocytes and lymphocytes as models for the study of cell death gradually wiped out this line of research, as these cells typically undergo a more classically defined apoptosis (BOX 1). Nevertheless, the lysosomal (autophagic) forms of cell death are returning to vogue today — to some extent as a rediscovery of the work done in the 1970s. In essence, several groups are rediscovering the

fact that programmed or genetically controlled cell death takes many forms, and hence may follow different pathways; that is, autophagic or apoptotic pathways.

Apoptosis

John Kerr is an Australian pathologist, now retired, who, during the late 1960s, observed a consistent and not particularly explicable pattern of cell death, which he termed 'shrinkage necrosis'. It was relatively easy to see how necrotic cells could rupture — if their ionic pumps failed they would accumulate lactate, leading to osmotic entry of water and acidification, and swelling and lysis of the cell. It was harder to see how they would shrink. Shrinkage could come about only by loss of solute²¹, a difficulty finally interpreted by Benjamin Trump, who recognized the importance of ATP or energy resources as the cell began to fail²². When Kerr joined Andrew Wyllie and A. R. Currie in Edinburgh, the three generalized the idea that cell deaths followed a specific pattern that included shrinkage of the cell, apparently little damage to organelles, coalescence and margination of chromatin, and fragmentation of the cell and the nucleus. They coined the term 'apoptosis' to focus attention on the yin-yang relationship of death to birth (that is, homeostasis is not maintained unless the loss of cells equals the birth of cells). The three argued that the ritualistic nature of cell death implied an organized and conserved mechanism: cell death or apoptosis was an aspect of life like any other. In other words, cell death was as much a part of cell biology as mitosis, extension of an axon, the enzymatic sequence of glycolysis, or secretion²³.

Coining the term was insightful, but there was a risk that it might fall into the limbo of neologisms — noted but ignored. However, further developments came along. First, Arends and colleagues²⁴, expanding on Wyllie's earlier observation and picking up on

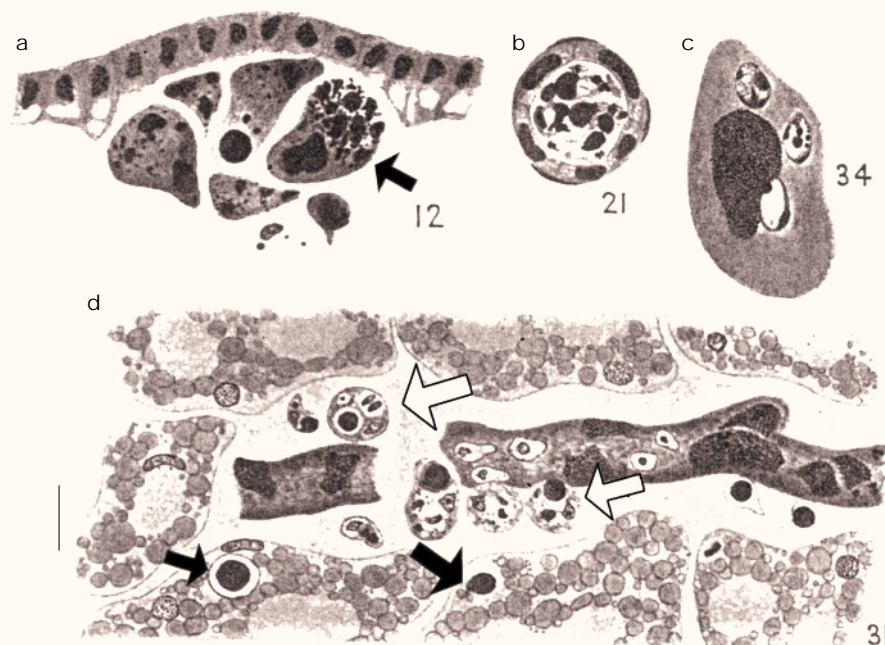


Figure 2 | **Apoptotic and other types of cell death in a metamorphosing weevil.** **a** | Gland cell disrupting, with a leukocyte (arrowhead) present. **b** | Regenerating Malpighian tubule with degenerate cells in lumen. **c** | Degenerating oenocyte. **d** | Degenerating salivary gland, with both leukocytes and surrounding fat participating in the phagocytosis of the gland. The white arrows show leukocytes, and the black arrows show phagocytic vacuoles. Adapted from REF. 5.



Figure 3 | **Scientists active since 1945 in the field of cell death.** Top panel: Viktor Hamburger (photo services, Washington University); John W. Saunders Jr; J.R. Tata; R.A.L. at the 1995 Gordon conference. Bottom panel: H. Robert Horvitz at the 1995 Gordon conference; John Kerr; A. R. Currie (reproduced with permission from Lothian Health Services Archive); Andrew Wyllie, at the 1995 Gordon conference. Photos from the 1995 Gordon conference are courtesy of the Gordon Research Conferences Archives.

the observations of others, realized that apoptosis is characterized by a specific pattern of internucleosomal DNA degradation. This created an interesting problem, but also provided a simple means of identifying and measuring the amount of cell death. This was an important issue because, whereas an instance of mitosis can be identified months later by labelling, a cell that has disappeared (in the space of an hour or so) is gone. In this case, the identification of fragmented DNA was readily applicable to the study of thymocytes and lymphocytes undergoing cell death in culture. This was important as the roles of cytokines and helper cells, and the peregrinations, expansions and contractions of molecules in the immune system, were now being recognized. In fact, this model so overshadowed others that degradation of DNA was considered to be either diagnostic or even causal of apoptosis. It was recognized only later that in many cells the internucleosomal degradation of DNA is delayed or absent²⁵. In fact, a more comprehensive terminology would be to consider apoptosis as one of several types of regulated cell death.

The genetics of cell death
A heritable pattern must be at least indirectly genetic in origin, but, by the late 1960s, a more direct link was detected for cell death. Three laboratories demonstrated that inhibi-

tion of protein synthesis could prevent cell death in, respectively, metamorphosing tadpole tail²⁶, metamorphosing insect muscle²⁷ or glucocorticoid-treated thymocytes²⁸. Later, this blockage would be confirmed for developing neurons, and the synthesis of specific proteins would be detected in dying insect muscle and prostate epithelium that degenerates after castration.

These stories attracted modest attention — the time was not yet ripe, and the models provided little potential for true genetic analysis. The embryologists and developmental biologists were not always geneticists, and the available biochemical and surgical techniques were not readily adaptable to animals as small as *Drosophila melanogaster*. At

the time, studies on mutant mice and chicks indicated that cell death could be regulated but gave no insight into the mechanisms of this process²⁹.

A breakthrough came with the application of modern molecular genetic techniques to the question of how cell death is controlled. In 1976, *Caenorhabditis elegans* was developed as an organism useful for genetics, and John Sulston and H. Robert Horvitz demonstrated that ~13% of somatic cells in the embryo die predictably, shortly after appearing³⁰. This provided a simple model to study the genetic basis of cell death.

Thus, deaths could be considered to be genetically based, and the *C. elegans* model was suitable for the analysis of cell death. By

Box 2 | Nerve growth factor and cell death

Viktor Hamburger, from 1934 onwards, published articles on the removal of limbs from developing chicks. These experiments fascinated Rita Levi-Montalcini who, at the end of the Second World War, came to Hamburger's laboratory in St Louis, Missouri, to continue those experiments. They demonstrated that sympathetic and sensory ganglia are larger in the regions of the shoulder and hip girdles, and that this difference in size was eliminated if the limb was removed. They then showed that there was far less cell death in the limb girdles, leading them ultimately to their recognition of a factor from peripheral tissue that supported survival of the ganglion cells. Later, a fortunate turn of events led them to a rich source of the factor — the salivary glands from males of some strains of mice. Levi-Montalcini and Stanley Cohen isolated this first growth factor, now known as nerve growth factor, and won the Nobel prize in 1986. This is well described in Levi-Montalcini's Nobel speech⁴⁶.

Box 3 | Conservation of cell death sequence

The sequence of CED-3 indicated that it is related to a mammalian protease known at that time as interleukin-converting enzyme. Subsequent investigation revealed the existence of a family of these proteases, now known as caspases (for cysteinyl-aspartate-cleaving proteases) because they are cysteinyl endoproteases that cleave a limited number of amino-acid sequences terminating in an aspartic acid. More than a dozen caspases are now known, and most are associated with one or more steps of apoptosis. In mammalian systems, **caspase 8** or **caspase 9** usually initiates a sequence of steps leading to the activation of the effector caspases, which digest many crucial cytoplasmic and nuclear proteins. The sequence and function of these enzymes is conserved from nematodes to humans. Furthermore, the general structure of this death sequence, including an adaptor protein involved in caspase activation that must be dislodged to permit the activation of caspase 9 (**CED-4** in nematodes, which is related to apoptotic protease-activating factor 1 (**APAF-1**) in mammals), and an inhibitory protein (**CED-9** in nematodes, related to **Bcl-2** in mammals) that prevents premature or inappropriate activation of the caspase cascade. (See, for instance, the review by Cryns and Yuan⁴⁷, an earlier review by Hengartner and Horvitz⁴⁸, and other articles^{49,50}.)

1982, the existence of genes that controlled essentially all the somatic cell deaths, such as **ced-3**, was established³¹.

Despite such advances, however, the field remained as it had been — modestly prominent but not spectacular, averaging 800–1,000 papers per year, as it had at least since 1969. Indeed, the field did not really start to grow until about 1990, when the number of publications began to rise at slightly over 20% per year, a pace that has been maintained for the past decade. Major reviews began to appear in the *Annual Reviews* and elsewhere at about this time, and, although the number of articles published is an imperfect measure of

“...today we are witnessing an explosive development of the field; numerous biotechnology companies have been founded ... and the first true clinical uses of our knowledge are within sight.”

progress, it is a reflection of growing interest.

Eugene Garfield and Gerry Melino have undertaken an interesting analysis based on the citation index, and have traced several influential papers and their descendants. It seems most likely, however, that three or four discoveries nearly simultaneously brought the subject above the pre-clinical horizon in the fields of immunology, oncology and neurology, leading to the birth of societies, journals, reviews and meetings on the subject. In one discovery, David Vaux and colleagues identified the B-cell lymphoma gene **bcl-2** as

an anti-apoptosis gene³², thereby relating apoptosis to the differentiation and maintenance of the immune system. The biology of the **bcl-2** gene was aggressively pursued by Stanley Korsmeyer and others. In another discovery, Elisheva Yonish-Rouach and others^{33,34} identified one function of **p53** as a pro-apoptosis regulator, and others emphasized the same for **c-Myc**^{35,36}, further establishing apoptosis as a field important in cancer research — just as Samuil Umansky³⁷ and Andrew Wyllie³⁸ had predicted. A third discovery was the identification of **Fas/Apo-1** as a death-transducing cell-surface receptor^{39,40}. Jean-Claude Ameisen presented the argument that AIDS might be a disease of cell death⁴¹, John Reed produced many antibodies that proved invaluable in tracing the mechanisms of cell death, and Arnold Greenberg first reported mechanisms of killing by cytotoxic T cells. Finally, at about this time, Martin Raff succinctly summarized an idea that was not rare among embryologists in an aphorism, “the social control of apoptosis”⁴². The prominence and aptness of the expression led to a new awareness of the subject, and substantial influence of the article.

Fas/Apo-1 at first seemed to be a spectacular tumour suppressor. Later, the tumour suppression was connected with the immune system in particular, and it was recognized that uncontrolled interactions between the Fas receptor and its ligand could lead to the destruction of healthy cells. The realization that both **Bcl-2** and **Fas** have powerful effects on the regulation of the immune system tied the concept of apoptosis to the dynamic fields of immunology, including autoimmune disease, immunosuppression, immunotolerance and AIDS. Neurologists quickly followed with the recognition that programmed cell death is

important in developmental neurology, and, in 1993, the sequencing⁴³ of **ced-3** led to the discovery of the apoptosis-related proteases or caspases (BOX 3). Furthermore, the conservation of this cell death gene sequence, as well as the discovery that human **Bcl-2** could substitute for **CED-9** in *C. elegans*⁴⁴ (BOX 3), forced us to consider its importance as a cell function, and this led to greater interest in what the invertebrates can tell us. These discoveries led quickly to the explosion in investigations and knowledge of caspases, a topic that is beyond the scope of this article but was reviewed recently⁴⁵. Symposia and meetings began to appear although, interestingly, those who had the foresight to organize those meetings were not, for the most part, the scientists already mentioned.

Although some feel that the study of regulated cell death, including apoptosis, has focused only on post-mortem changes, the result is that today we are witnessing an explosive development of the field; numerous biotechnology companies have been founded, the philosophical implications of the concept have reached both working scientists and the lay public, and the first true clinical uses of our knowledge are within sight.

Update — added in proof
Viktor Hamburger passed away in June 2001, leaving behind a list of publications that spanned 76 years and defined many aspects of modern developmental biology and neurology.

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 Links

DATABASE LINKS **ced-3** | **bcl-2** | **p53** | **c-Myc** | **Fas/Apo-1** | **CED-9** | **caspase 8** | **caspase 9** | **CED-4** | **APAF-1**

FURTHER INFORMATION **Lockshin lab** | **Zakeri lab** | **Mechnikov Nobel prize** | **Levi-Montalcini Nobel prize**

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OPINION

XIAP, the guardian angel

Martin Holcik and Robert G. Korneluk

Controlling the activity of caspases is essential for the appropriate execution of cell death and the regulation of cell survival. One cellular inhibitor of apoptosis, XIAP, has emerged as a crucial regulator of caspases, and is itself subject to complex negative regulation.

It is now well established that cellular suicide (apoptosis or programmed cell death) is an essential part of the maintenance of homeostasis and the survival of multicellular organisms. Apoptosis is central to several physiological cellular processes, from host defence against viral infections to the sculpting of organs and tissues during embryonic development.

Equally, or perhaps even more, important is the role of apoptosis in the pathogenesis of human disease. An uncontrolled rate of cellular death can have dire consequences — too much cell death is often associated with the destruction of healthy cells and tissues, as exemplified in neurodegenerative disorders and autoimmune disease. Conversely, too little cell death is suspected to be partly responsible for the uncontrolled proliferation of cancer cells. It therefore follows that

tight control of the apoptotic machinery is critical for cellular survival. Indeed, several decades of research into programmed cell death have identified numerous genes and pathways that control and influence, either positively or negatively, the progression of apoptosis from the initial death trigger to the final demise of the cell (see the Timeline article by Lockshin and Zakeri on page 545 for more details).

One group of apoptotic regulators, the inhibitor of apoptosis (IAP) genes (BOX 1), the products of which block apoptosis, are particularly interesting as they are the most powerful intrinsic inhibitors of cell death and could potentially be used therapeutically. Among the IAPs, the X-chromosome-linked inhibitor of apoptosis (*XIAP*) is the most potent and versatile regulator of cell death. Furthermore, the mechanisms by which XIAP interferes with apoptotic pathways have now been worked out at the genetic, biochemical and structural levels, allowing a model to be proposed that accounts for the centrality of XIAP in the regulation of cellular suicide.

Inhibition by XIAP

Apoptosis is facilitated through a family of