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Historical perspectives

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Abstract

Apoptosis is one of the most widely studied fields in biology and accounts for over 2% of all life science publications annually. It plays a fundamental role in development, and defects in the regulation of apoptosis are directly implicated in numerous well-known diseases including cancer, neurodegenerative disorders, tissue atrophy and auto-immune diseases. However, the field of apoptosis has humble beginnings and was neglected by biologists for much of its history. This chapter reviews the history of research in apoptosis and highlights key experiments that have contributed significantly to our current understanding of apoptosis. In addition, the topics covered in later chapters are briefly introduced.

Introduction

One of the most widely researched fields in biology since the early 1990s has been the study of apoptosis, a form of programmed cell death. Apoptosis was first defined as a collection of morphological events distinct from necrosis that were observed in a diverse range of cells and tissues undergoing cell death. These included cellular events such as cell shrinkage, membrane ruffling (known as blebbing) and packaging of the dying cell into small membrane-bound vesicles called apoptotic bodies. A variety of nuclear events were also observed that include chromatin condensation and nuclear fragmentation (Figure 1) [1]. Biochemical events specific to apoptosis were discovered later, including

degradation of DNA into regular-sized fragments [2] and exposure of a lipid called phosphatidylserine on the surface of apoptotic cells [3].

These changes in morphology and biochemistry result in cell death, and in healthy adult humans around 10 million cells undergo apoptosis daily. Normal tissue homoeostasis and function depends on correct levels of apoptosis to counteract the increase in cell numbers due to cell proliferation and also to eliminate damaged or defective cells. It is a critical event during virtually every stage of development from embryogenesis right through to adulthood. Most cells retain the ability to undergo apoptosis in response to a wide range of death-inducing stimuli. Consequently, the regulation of apoptosis is tightly controlled in cells and defects in apoptosis can give rise to a number of diseases, including cancer, neurodegenerative disorders and auto-immune disease [4].

1842 to 1972: defining 'apoptosis'

Biology is littered with examples of major discoveries that drift back into obscurity because the importance of these discoveries was not fully appreciated at the time. The work done by Gregor Mendel on the inheritance of individual characteristics in the pea plant is perhaps the most widely known example. His work went unnoticed by the general scientific community for nearly 50 years even though he had independently worked out the laws of inheritance over 30 years before gene theory was developed [5]. However, no major field in biology has been rediscovered as often as the field of apoptosis, which has been independently described at least five separate times over a period of 100 years (Figure 2).

The term apoptosis was first used in 1972 to describe a form of cell death with markedly different morphology to necrosis [1]. However, the idea that cells
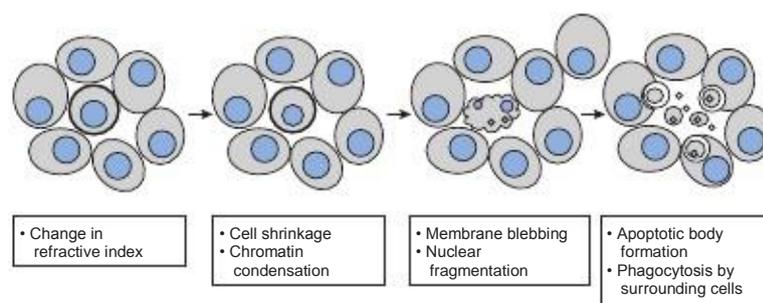

Figure 1. Apoptosis is a form of cell death with morphology distinct from necrosis
Cells undergo a number of sequential changes in morphology when dying by apoptosis and these are outlined in this figure. The earliest identifiable change in morphology is when the refractive index of the cell changes under visible light. Cell shrinkage and condensation of the chromatin on the nuclear membrane occur next and are rapidly followed by nuclear fragmentation and plasma membrane blebbing. Apoptotic body formation is the final stage in apoptosis and the entire process is believed to take about 1 h in vivo.

2002 Brenner, S., Sulston, J. and Horvitz, R.; Nobel Prize in Physiology and Medicine

2000

1990–2000 Mechanisms and biochemistry of apoptosis elucidated

1990

1988 Vaux, D.L.; BCL-2 is first protein discovered involved in apoptosis

1982 Horvitz, R.; Genetic control of PCD in C. elegans

1980   1980 Wyllie, A.H.; DNA fragmentation in apoptosis

1976 Sulston, J.; PCD in C. elegans

1972 Kerr, J.; Shrinkage necrosis is renamed as apoptisis and defined

1970   1969 Whitton, J.M.; Comparison of vertebrate and invertebrate developmental cell death

1965 Kerr, J.; First paper on cell death called shrinkage necrosis

1961 Bellairs, R.; Electron microscopy of cell death (apoptosis)

1960

1951 Glücksmann, A.; Cell death in vertebrate development

1950

1940

1930

1920

1914 Grüper, L.; Chromatolysis in tissues to counteract mitosis

1910   1908 Mechnikov, I.; Nobel Prize in Physiology and Medicine – Phagocytosis

1906 Collins, R; Developmental neuronal cell death

1900

1889 Felix, W.; Cell death in healthy mammalian tissue

1890   1885 Flemming, W.; Defines chromatolysis, equivalent to apoptosis

1880

1872 Stieda, L.; Chondrocyte death

1870

1864 Weismann, A.; Cell death in pupating Diptera

1860   1858 Virchow, R.; Defines necrobiosis as natural cell death

1850

1842 Vogt, C.; Developmental cell death in toads

1840   1839 Schleiden, M. and Schwann, T.; Cell theory developed
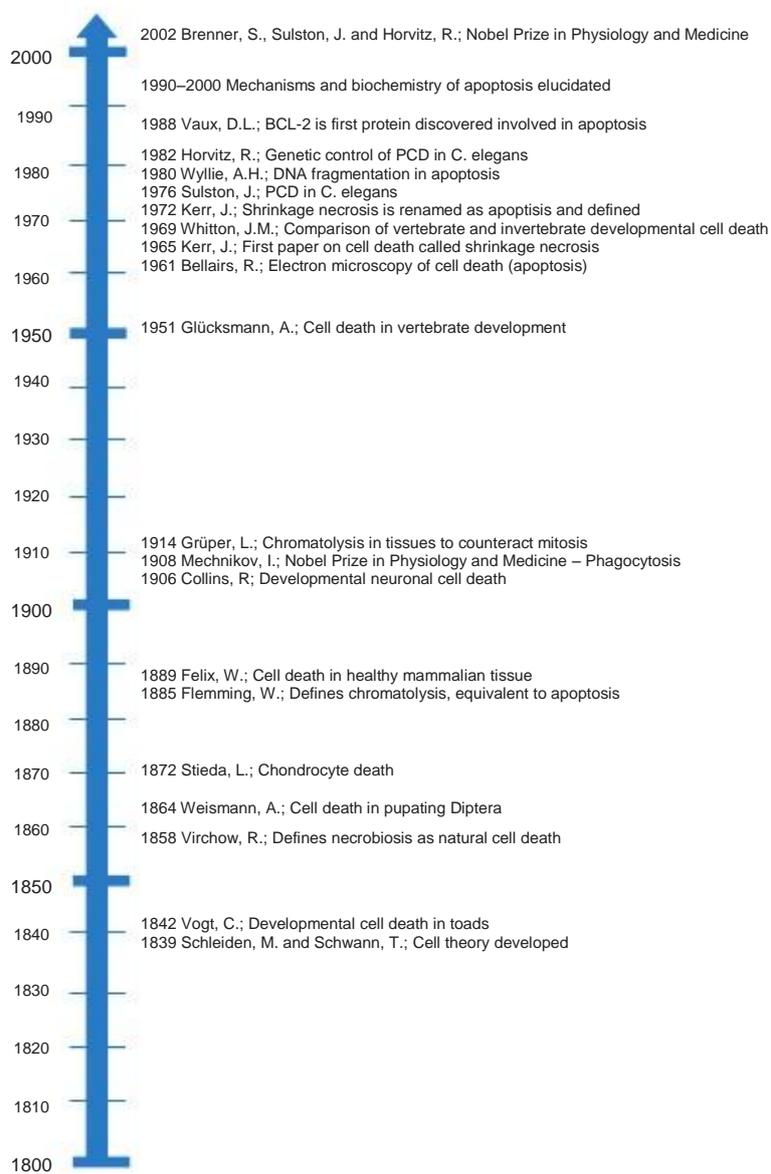
1830

1820

1810

1800

Figure 2. Timeline of apoptosis illustrating some of the major discoveries in the field
of cell death

Although the concept of natural cell death was first hypothesized in 1842, the first description of
apoptosis was in 1885 when Walther Flemming described a form of cell death that he called
chromatolysis. This was regularly described in the literature for the next 30 years, but the field
of cell death shifted in focus to phagocytosis for much of the first half of the 20th century. In the
1960s, scientists regained an interest in cell death and in 1972, apoptosis was defined for the first
time by John Kerr. Interest in the field remained low until the late 1980s when it became clear
that apoptosis played a key role in development and disease, and identification of Bcl-2 as an anti-
apoptotic molecule marked the beginning of intensive research into the mechanisms of apoptosis
throughout the 1990s. The impact that research into apoptosis had on other disciplines in bio-
logy was formally recognized when three pioneers of apoptosis research in invertebrates were
awarded the Nobel Prize in Medicine and Physiology in 2002. PCD, programmed cell death.

can die naturally is a relatively old one and was first postulated 130 years earlier in 1842 by Carl Vogt after he studied development in toads. This observation was even more remarkable when one considers that Schleiden and Schwann established cell theory, which postulates that all living matter is composed of individual units or cells, only 3 years earlier in 1839. This makes the concept of natural cell death one of the oldest theories in cell biology [6]. In 1858 Rudolph Virchow described a natural form of cell death that was distinct from necrosis and called it necrobiosis [7]. The first clear morphological description of apoptosis appeared in 1885 by Walther Flemming. Drawings of cells in regressing ovarian follicles portrayed shrinkage of cells, chromatin condensation, nuclear fragmentation and formation of apoptotic bodies, and Flemming named this natural cell death chromatolysis. These are all morphological hallmarks of apoptosis and chromatolysis was regularly reviewed in the literature for over 30 years [8]. Apoptosis continued to drift into and out of fashion throughout the first half of the 20th century until an influential review on cell death was published in 1951. In this, Glücksmann described karyorrhexis and karyopyknosis as distinct forms of cell death that we now know to be events associated with apoptosis. He also suggested that cell death plays an important role in embryogenesis and development of vertebrates [9]. Consequently, some pathologists regained an interest in the mechanisms of cell death, and in 1965, the Australian pathologist John F. Kerr observed an unusual form of cell death following ischaemic injury of liver tissue, where the constituents of cells appeared to remain intact within small vesicles. He used the term shrinkage necrosis to define this form of cell death and subsequent studies using electron microscopy identified the earliest morphological events as condensation of the cytoplasm and the nuclear chromatin [10]. Kerr went on sabbatical to Aberdeen where he collaborated with Andrew Wyllie and Professor Alister Currie. During this time, they coined the term apoptosis to replace the term shrinkage necrosis and defined this particular form of cell death in a paper published in 1972 that is widely acknowledged as being a major landmark in the field of biology [1].

1972 to 1982: the first decade in apoptosis research

Researchers were initially unaware that apoptosis had been identified and described it on numerous occasions throughout the second half of the 19th century and also during the first half of the 20th century. Consequently, much of the early research simply repeated observations already made during the 19th and 20th centuries. These papers demonstrated the important role of apoptosis in development and disease, but the results were often limited to descriptions of morphology. Studies based solely on morphology have severe limitations and the interest from the general biological community during this period remained quite low. There was a significant risk that the concept of apoptosis would fade into obscurity just as chromatolysis had done 70 years previously, but for a number of important developments in the field.

Two experiments in particular were vital in maintaining an interest during this crucial first decade of research. First was the observation that DNA was degraded into nucleosomal-sized fragments during radiation-induced cell death [2]. This is due to an endogenous nuclease that is only activated in response to apoptotic stimuli and was identified 17 years later in 1997 [11]. The presence of a DNA ladder suggested that specific biochemical alterations occur in cells during apoptosis. Throughout the 1970s and 1980s work on the nematode worm Caenorhabditis elegans yielded the second key insight into apoptosis. C. elegans proved invaluable in the study of developmental cell death and painstaking analysis of the lineage of every cell in the adult worm demonstrated that exactly 131 of the 1090 somatic cells generated during development underwent programmed cell death. Both the lineage of each dying cell and the time that it underwent programmed cell death were highly reproducible, and these observations highlighted the tightly regulated nature of developmental cell death in C. elegans [12]. Mutants of C. elegans were characterized and genetic screening quickly led to the discovery that more than 12 separate genes were involved in programmed cell death [13–15].

The identification of these specific biochemical and genetic events during apoptosis sowed the seeds for the subsequent explosion in our understanding of apoptosis. The field quickly moved away from stagnant studies of cell morphology into mainstream experimental science. Eventually, apoptosis would become a centrepiece in two of the most important fields in modern biochemistry, namely cell signalling and molecular biology. The identification of an oncogene called bcl-2 that appeared to function solely as an anti-apoptotic protein in cells was a fundamental discovery that heralded the beginning of a period of intensive research into the biochemical processes underlying apoptosis.

bcl-2: the opening act

bcl-2 was initially characterized as a proto-oncogene that enhanced transformation of human lymphoma cells [16] but further work demonstrated that over-expression of bcl-2 was found to increase the survival of haematopoietic cells by reducing the sensitivity to apoptosis. This made Bcl-2 arguably the first protein identified to be involved in apoptosis [17]. Bcl-2 was also involved in a second landmark experiment in 1992 when it was shown that over-expression could also protect against developmental cell death in C. elegans [18]. The ramifications of this experiment were enormous. For decades, biologists had compared the similar morphology of programmed cell death in invertebrates with apoptosis in vertebrates and speculated that the two processes were similar [19]. This simple experiment was the first conclusive proof that apoptosis in vertebrates and programmed cell death in invertebrate were conserved through evolution, and finally allowed genetic discoveries made in C. elegans to be applied in the study of apoptosis in mammalian cells. The unification of invertebrate and vertebrate cell death models accelerated the

identification and characterization of many biochemical components of apoptosis in mammalian cells.

Unravelling the apoptotic machinery

The morphology of vertebrate and invertebrate cells undergoing apoptosis was similar regardless of the initial apoptotic signal. This suggested that a common apoptotic pathway that is activated in response to many different initial stimuli exists in cells. Previous studies with C. elegans in the 1980s had identified about 12 different genes that were involved in apoptosis. Some mutations completely inhibited apoptosis, while others only inhibited clearance of apoptotic bodies by surrounding cells. One gene called Ced-3 was absolutely required for apoptosis and was eventually cloned in 1993. This gene was found to share sequence homology with the mammalian protease ICE (interleukin-1 -converting enzyme; caspase 1) [20]. Since then, 12 other human caspases have been described and many have been shown to play a pivotal role in apoptosis. Unlike most other proteases, caspases have very high substrate specificity. They usually only cleave target proteins at one or two conserved sites and are absolutely required for apoptosis in response to most stimuli. Initiator caspases detect apoptosis-inducing stimuli and activate effector caspases that proceed to cleave essential enzymes and structural proteins in cells in a hierarchal protease cascade (Figure 3).

The discovery that caspases are central components in apoptosis was due primarily to genetic studies in C. elegans. However, research with viruses identified an entirely new class of apoptosis regulators in mammalian cells that directly inhibit caspase activity and apoptosis by steric hindrance of caspase active sites. Some baculoviruses were shown to express a protein called IAP (inhibitor of apoptosis protein) that binds with caspases and inhibit caspase activity. Herpes virus was found to express another protein called v-FLIP (viral Flice-like inhibitory protein) that shares some sequence homology with caspase-8 and was found to inhibit only death-receptor-mediated apoptosis by binding with the FADD (Fas-associated death domain) in place of caspase-8. Cellular homologues of IAP and FLIP were quickly identified and these molecules are believed to play a crucial role in regulating the sensitivity of cells to apoptotic stimuli [21,22].

The mitochondrion is particularly sensitive to many different apoptosis-inducing stimuli and dysfunction of mitochondria is associated with pore formation and mitochondrial membrane depolarization in cells. This is a reversible step, and mitochondria can repolarize and continue to generate ATP during the early stages of apoptosis. A number of proteins were identified in mitochondria that were released into the cytosol during pore formation. Some of these molecules function as molecular guillotines in the cytosol and are sequestered in mitochondria away from their cytoplasmic targets in healthy cells. One such molecule called cytochrome c is usually involved in oxidative phosphorylation in mitochondria. However, release of cytochrome c into the cytosol stimulates the formation of a multimeric complex called the apopto-
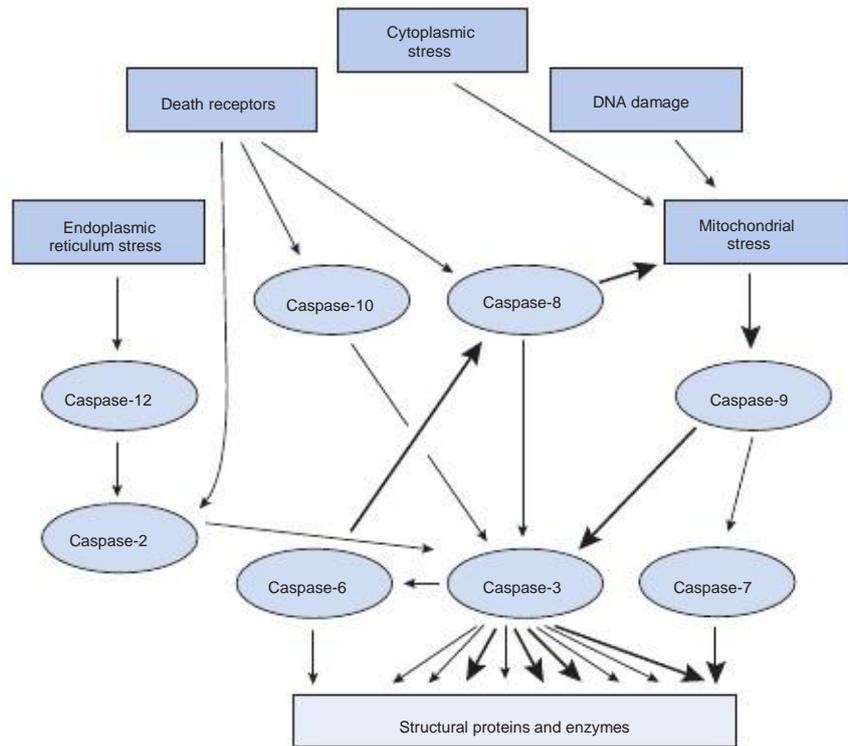
Figure 3. Caspases are central components in apoptosis
Caspases are a family of 12 aspartate proteases that have limited substrate specificity. Initiator caspases detect cellular stress signals from different sources and include caspase-2, caspase-8, caspase-9, caspase-10 and caspase-12. These initiator caspases cleave and activate effector caspases either directly or indirectly. Effector caspases, including caspase-3, caspase-6 and caspase-7, cleave a variety of downstream target proteins. This illustration highlights the central role of caspases in response to different cellular stresses.

some, where caspase-9 is recruited and can activate caspase-3 [23]. Cytochrome c release from mitochondria is regulated by Bcl-2 and this is believed to be the principal mode of action of the anti-apoptotic Bcl-2 family members [24]. Caspase-3, caspase-9 and Bcl-2 are homologous with Ced-3, Ced-4 and Ced-9 respectively in C. elegans and the conservation of this biochemical pathway helped illustrate the central role played by the mitochondrion in apoptosis.

Implicating apoptosis with disease

As the components of apoptosis were identified, it soon became clear that defects in these proteins were often associated with a number of diseases. One year after the bcl-2 gene was cloned and characterized, a cell-surface receptor called Fas was discovered and was found to induce apoptosis and cause tumour regression when bound with anti-Fas monoclonal antibodies [25]. The gene that codes for Fas

receptor was subsequently cloned and was found to share sequence homology with tumour necrosis factor receptors 1 and 2 [26]. Mutations in Fas receptor were identified in a particular strain of mice with an autosomal recessive lymphoproliferative (lpr) disorder [27]. The authors concluded correctly that these mutations were the underlying cause of auto-immune disorders in lpr mice and this was the first clear indication that apoptosis may be a key regulator of the immune system function. As a result the concept of apoptosis was introduced for the first time to many researchers studying auto-immune disease, immunosuppression, immunotolerance and AIDS.

The tumour-suppressor protein p53 had first been observed in 1979, over a decade before Fas receptor had been identified. Subsequently, p53 was found to be mutated or absent in over half of all human carcinomas [28]. While it was known that p53 could regulate transcription, the main function of p53 was not identified until researchers discovered that it induces apoptosis in response to DNA damage and defects in the cell cycle [29,30]. The prevalence of p53 mutations clearly demonstrated the importance of apoptosis during tumorigenesis. Subsequent research identified defects in apoptosis in virtually every human cancer.

Perspective: the rise and rise of apoptosis

Research in apoptosis now encompasses many diverse biological disciplines from plant science to clinical science and accounts for more than 13 000 publications annually [31]. It is remarkable that a field overlooked by the general scientific community for so long has become so important in our understanding of such a diverse range of biological processes. Early experiments identified key biochemical and genetic events and these were crucial for the subsequent explosion of research in apoptosis. Genetic studies in C. elegans contributed significantly to delineating the biochemical pathways in apoptosis during the 1990s. The scientific community formally acknowledged this when Sydney Brenner, John Sulston and Robert Horvitz shared the Nobel Prize in Physiology and Medicine in 2002. The Nobel Prize has arrived at an opportune time and heralds the beginning of a new era in apoptosis research. Defects in apoptosis have been attributed to many well-known incurable diseases. The most important question during the next decade of research will be how can we convert this new wealth of genetic and biochemical information into therapies that can prevent and cure these illnesses.

Summary

- Apoptosis was first defined in 1972, but the field of cell death is much older and was originally hypothesized in 1842. Early genetic and biochemical studies of apoptosis were essential in maintaining an interest in the nascent field.

- The first protein implicated in apoptosis was Bcl-2, when it was found that over-expression of Bcl-2 decreased apoptosis in haematopoietic cells. Bcl-2 was also shown to inhibit apoptosis in C. elegans demonstrating that evolution had conserved the mechanisms of apoptosis between invertebrates and vertebrates.

- Cloning of the C. elegans gene Ced-3 allowed researchers to identify human counterparts by homology called caspases. Caspases were subsequently shown to be central components of apoptosis in mammalian cells.

- Two key regulators of caspases, called IAP and FLIP, were originally identified in viral DNA. Cellular homologues were quickly identified and were shown to regulate the sensitivity of cells to a number of cellular insults.

- Mitochondria play a pivotal role in detecting and amplifying apoptotic stimuli from nucleus, cytoplasm, endoplasmic reticulum and death receptors. Bcl-2 family members are believed to regulate apoptosis by increasing or decreasing the sensitivity of mitochondria to these apoptotic signals.

- The importance of apoptosis in disease was highlighted when defects in Fas receptor and p53 were shown to give rise to lymphoproliferative disorders and tumorigenesis respectively.

- Robert Horvitz, who made significant contributions to the field of developmental apoptosis, was awarded the Nobel Prize in Physiology and Medicine in 2002. This represents the culmination of two decades of research into the genetic and biochemical processes involved in apoptosis.

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