

Book Review

Methods in apoptosis: a four-way battle to the death

C Maisse¹, F Bernassola¹, P Salomoni² and M MacFarlane^{*2}¹ IDI-IRCCS Biochemistry lab, c/o Department Experimental Medicine, University of Rome 'Tor Vergata', Rome, Italy² MRC, Toxicology Unit, University of Leicester, Leicester, UK LE1 9HN

* Cell Death and Differentiation Editorial Office: Correspondence to: mm21@leicester.ac.uk

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Apoptosis, Methods in Enzymology, Vol. 322. Edited by JC Reed. Academic Press, San Diego, CA, USA: 2000. pp 569. ISBN: 0-12-182223-0. \$30.00**Apoptosis Methods in Pharmacology and Toxicology**. Edited by MA Davis. Humana Press, Totowa, NJ, USA: 2002. pp 176. ISBN: 0-89603-890-4. \$ 79.50**Apoptosis Techniques and Protocols**. Edited by AC LeBlanc. Humana Press, Totowa, NJ, USA: 2002. pp 263. ISBN: 1-58829-012-3**Cell Proliferation and Apoptosis**. Edited by D Hughes and H Mehmet, BIOS Scientific Publishers Ltd, UK: 2003. pp 364. ISBN: 1-85996-193-2

The last decade has seen the field of apoptosis grow from a minor footnote of cell biology to one of the most popular and prolific subjects of biomedical research. The huge progress made recently in this field is matched by an enormous, and ever-growing, array of innovative apoptosis-specific protocols. The four books covered by this review comprise a collection of protocols and methods that have the potential to be a valuable resource for any research laboratory with a focus on cell death, but are specifically targeted to those scientists with an interest in apoptosis.

The oldest of these books, *Apoptosis, Methods in Enzymology*, covers most of the standard methods that are routinely utilized in a laboratory involved in the study of apoptosis. By contrast, *Apoptosis Methods in Pharmacology and Toxicology* and *Apoptosis Techniques and Protocols* are specifically geared to the study of apoptosis in the more specialist fields of Toxicology and Neurobiology. The focus of the most recent book, *Cell Proliferation and Apoptosis*, centres around methods and protocols that can be applied to study the balance between cell growth and death, and is possibly more applicable to the field of cancer research. The strengths and weaknesses of each of these publications are discussed in detail below.

Apoptosis, Methods in Enzymology was edited by J.C. Reed, one of the most cited scientists in the field of apoptosis, who prefaced this edition with the proviso that it 'is a work in progress, which will evolve along with the field in later volumes'. Despite this caveat, the current volume provides the many scientists presently involved in apoptosis research, as well as those new to the field, with a comprehensive guide to the principle methods currently available to detect and quantify apoptosis in a number of cellular systems. The value of this book is increased by the number of quality contributors, many of whom are well-known authorities in the field of apoptosis.

The first impressions of this book are positive as it is easy to navigate and contains high-quality illustrations. The book is divided into eight sections, which are in turn subdivided into individual chapters, each beginning with a thorough but concise introduction. Each chapter explores the different aspects of apoptosis encompassing detection and quantification of cell death in lower organisms, involvement of endonucleases, proteases, mitochondria and Bcl-2 family members, as well as the role of death receptors and their downstream signal transduction events.

For the readers' convenience, references are cited at the bottom of each page. Protocols are well written and extremely detailed, and we found it particularly useful that reagent sources were included and that the advantages and disadvantages of each approach were listed at the end of each section.

One of the major challenges in studying cell death is the ability to distinguish between apoptosis and necrosis. This book directly addresses this issue in several sections. For example, in Chapter 3, Section I, Z. Darzynkiewicz and E. Bedner describe the application of flow and laser scanning cytometry to analyze apoptosis and to discriminate between apoptosis and necrosis. This technique is particularly versatile, and can be applied to both adherent and suspension cell populations. A key event in the initiation of apoptosis is the release of small proapoptotic molecules from the mitochondria. Consequently, a number of methods have been established to assess the involvement of mitochondria and their release of these molecules. In Section V, chapters contributed by a number of laboratories, including those of G. Kroemer and D.R. Green, provide a comprehensive methodological approach to the purification of mitochondria, the quantification of mitochondrial transmembrane potential and details of several assays for cytochrome *c* release.

For completeness, this book could have included a chapter on the morphological assessment of apoptosis *in situ*. However, the editor chose to focus mainly on the biochemical measurement of apoptosis, as one would expect from the series 'Methods in Enzymology'.

Apoptosis Methods in Pharmacology and Toxicology is a short book, which provides a general introduction containing a standard table describing the morphological changes characteristic of apoptosis or necrosis. Importantly, the introduction also contains a table, which lists some of the morphological, biochemical and molecular markers that can be employed as indicators of apoptosis, together with the potential drawbacks of each approach. Despite this promising beginning, the remainder of the book comprises a small number of chapters, which do not directly relate back to the issues highlighted in the introductory table.

The first three chapters focus on useful techniques, including applications of flow and laser scanning cytometry and specific methods for detecting apoptosis in tissue sections. A general overview of the most routinely used flow and laser scanning cytometry techniques is followed by a detailed and well-illustrated description of protocols for detection of phosphatidylserine exposure using Annexin V, mitochondrial transmembrane potential, caspase activation and DNA fragmentation. However, the chapter dedicated to the quantification of apoptosis in tissue sections, by analyzing classical apoptotic morphological and ultrastructural features, does not provide any experimental information, and is poorly illustrated. In Chapter 7, the authors provide a detailed and well-illustrated procedure to quantify Bcl-2 activity and caspase activation by ELISA. This method, when standardized, is likely to be the most suitable one to test the effects of a number of pharmacological agents. Despite moments of lucidity, this book lacks focus and would have done better to highlight the many protocols that are aimed at discriminating between necrosis and apoptosis, which remains a fundamental question in the field of toxicology.

Apoptosis Techniques and Protocols, edited by A.C. LeBlanc focuses on techniques and methods to analyze neuronal cell death. Neuronal loss is a major event in neurodegenerative disorders and in aging, because of the lack of replacement of lost neurons. However, the mechanisms underlying neuronal cell death are still poorly understood and the boundaries between apoptosis, necrosis and other types of cell death in neurons are still unclear. As neurons are both compartmentalized and polarized cell types, apoptotic insults can be restricted to specialized compartments of the cell, thus rendering the detection of cell death more difficult.

Most of the contributors weave together protocols with background information, allowing the reader to appreciate the methods. The protocols are well written and referenced, easy to follow and the reagent sources are often included.

This book could prove valuable in designing experiments to assess apoptosis in cultured neurons and detecting neuronal apoptosis *in situ*. Some chapters deserve particular mention: for example, two chapters are dedicated to the value of using viral vectors or microinjection techniques to assess the role of specific molecules in neuronal apoptosis. In addition, the chapter by Cole and Gylys educates the reader on synaptosis and gives important technical hints for analyzing this process

in vivo and *in vitro*. Interestingly, evidence that focal synaptic damage can occur in Alzheimer's disease and amyloid-precursor transgenic mice is discussed in relationship to synapse loss.

The chapter by Roth describes several techniques to histochemically identify apoptotic neurons in brain sections from human and experimental animals. Neuronal cell death in chronic injury and neurodegenerative diseases is troublesome to detect, since neurons undergo apoptosis in a sporadic or asynchronous fashion. Hence, the description of different methods is worthwhile since no single protocol for *in situ* detection of neuronal apoptosis can be applicable to all physiopathological conditions.

A major shortcoming of these last two books is that a number of chapters are dedicated to the description of DNA microarray technology, which is a technique that can be easily applied to any biological process and goes beyond the scope of the specialist areas covered by these publications. In *Apoptosis Techniques and Protocols*, Eastman and Loring emphasize the importance of well-designed microarray experiments. By contrast, the contributors to this topic in *Apoptosis Methods in Pharmacology and Toxicology* provide little guidance on the design of microarray experiments.

The editors introduce *Cell Proliferation and Apoptosis* as being designed 'to bring under one cover a comprehensive range of complementary cell proliferation and cell death procedures'. While five chapters are dedicated to protocols applicable to the study of cell proliferation, six focus on methods to detect apoptosis. However, the reader would benefit enormously if the protocols were more clearly grouped under specific cell proliferation and apoptosis headings. Moreover, the editors suggest that many of the procedures can be used in combination; yet no specific chapter is dedicated to highlight the circumstances when this may be possible.

Although there is some redundancy between each chapter, several methods to study apoptosis are well described and provide sufficient experimental detail. In particular, Chapter 10 contains all the necessary protocols to assess mitochondrial outer membrane permeabilization and the release of apoptogenic molecules, which are critical regulatory events in the apoptotic process. Chapter 12 highlights the power of flow cytometry in the quantitative assessment of a broad range of apoptotic features, ranging from DNA fragmentation to membrane lipid redistribution.

Like its predecessor *Apoptosis, Methods in Enzymology*, this new addition to the literature available on methods in apoptosis contains well-indexed and very detailed protocols for the guidance of even a newcomer to the field of apoptosis.

Although *Apoptosis, Methods in Enzymology* is the oldest of the four books, it still represents the most valuable resource for anyone entering the field of apoptosis, or for those aiming to establish more specialist techniques in apoptosis. The authors have successfully produced a very informative book that should certainly be on the bookshelves of those intending to get involved in this exciting field. *Apoptosis Methods in Pharmacology and Toxicology*, which is directed to the more specific fields of toxicology and pharmacology, does not provide sufficient background information and lacks several standard methods, while those that are included omit

important methodological details. Therefore, we would not recommend this book as an essential purchase for either a newcomer attempting a protocol for the first time or even an expert in the fields of toxicology and pharmacology. In contrast, the other specialist volume, *Apoptosis Techniques and Protocols*, could prove valuable for those within the field of neuronal apoptosis. Although there are some minor overlaps between certain chapters, overall, the contributors have provided detailed descriptions of relevant protocols that should contribute to increasing our understanding of apoptosis.

The most recent publication, *Cell Proliferation and Apoptosis*, is comprehensive and contains several outstanding

chapters. We are, however, unsure whether it represents a real advance beyond the information already provided within *Apoptosis, Methods in Enzymology*. Despite rapid progress in the field of apoptosis, few new technologies have been introduced since the publication of *Apoptosis, Methods in Enzymology*, in 2000. Regardless, the field would benefit from a book highlighting novel high-throughput technologies (e.g. siRNA-based screens) that have recently been established to dissect novel apoptotic pathways or to identify novel therapeutic targets. We would therefore welcome the inclusion of these new technologies in the much-anticipated second edition of *Apoptosis, Methods in Enzymology*.