

METHODS IN MOLECULAR BIOLOGY

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Caspases, Paracaspases, and Metacaspases

Methods and Protocols

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Preface

Among a plethora of known proteases, caspases are perhaps the ones that have attracted and continue to attract much more research than any other group of proteolytic enzymes. The reason for such an extraordinarily high interest to caspases is their pivotal regulatory role in cell death, cell differentiation, and inflammatory responses, with broad implications for human health and disease. However, caspases are just a tip of the iceberg, representing an apical and relatively small group of animal-specific enzymes within a huge superfamily of structurally related proteases found in all living organisms.

The discovery of caspase-related and apparently ancestral proteins called metacaspases and paracaspases in bacteria, protists, slime molds, fungi, and plants has initiated a “post-caspase” wave of research in studying the biochemistry and function of these proteins in the contexts of development, aging, stress response, pathogenicity, and disease resistance. This field of research moves very rapidly and has a motley pattern due to a wide evolutionary conservation and multifunctionality of para- and metacaspases, reflecting their diversity in molecular structure and enzymatic properties.

When planning this book, we pursued two opportunities. Firstly, as strange as it may seem, this is in fact the first collection of laboratory protocols to study caspases published in single cover. Secondly, we intended to break inter-kingdom barriers by including protocols for para- and metacaspases and in this way to support the rapid progress in these areas by providing common protocols that can be useful for distinct members of the caspase fold. Accordingly, the book consists of two parts. The first part presents methods to measure, detect, and inhibit activation and activity of a subset of or specific caspases *in vitro* and in several model systems and organisms, primarily in the context of programmed cell death. In addition, two chapters describe recently established protocols for high-throughput analysis of caspase substrate specificity and caspase substrates by employing chemistry and proteomics. The second part of the book provides experimental protocols for purification and *in vitro* and *in vivo* analysis of yeast, protozoan, and plant metacaspases, as well as of a human paracaspase MALT1.

Each technique in *Caspases, Paracaspases, Metacaspases Methods and Protocols* is described in an easy-to-follow manner with details so that the beginner can succeed with challenging techniques. The Notes section provides the researcher with valuable hints and troubleshooting advice. We wish to thank the authors for their valuable time in preparing these diligently written chapters.

Uppsala, Sweden
La Jolla, CA

Peter V. Bozhkov
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