
Preface

The purpose of *Calpain Methods and Protocols* is quite straightforward: it is to present the actual experimental methods used in many different laboratories for the study of calpain. It will provide the vital experimental detail, and the discussion of possible pitfalls, for which the standard journals no longer provide space. This will make it as easy as possible for investigators interested in calpain to adopt established methods without repeating old mistakes, and to adapt and apply these methods in novel approaches to the many outstanding calpain questions.

These questions range from purely biochemical problems of protein structure and enzyme regulation at the molecular level, through large areas of cell biology, to applied and clinical aspects of calpain function in human disease. Within this panoply of topics, a wide range of investigators will find many fascinating and as yet unanswered questions about calpain. *Calpain Methods and Protocols* will provide instant access to many essential techniques, while saving them the time and effort involved in developing a new method.

In addition to questions relating to the normal physiological roles of the calpains, there is considerable evidence that inappropriate calpain activity may have pathological effects in many tissues, for example, following ischemia. This provides a major stimulus for the development of specific calpain inhibitors for therapeutic purposes, and for the development of methods to evaluate such inhibitors.

The two most studied calpains, μ - and m-calpain, and the inhibitor calpastatin, are present in essentially every cell of a mammalian organism, although in varying relative amounts. This variation is itself a very interesting problem. Related enzymes have been described in birds, crustaceans, and insects, but not in plants or bacteria. In addition to the well characterized calpains, there is a steadily increasing family of calpain-related genes, many of them apparently tissue-specific, that have been characterized as cDNA sequences, but not yet in most cases as proteins.

Perhaps the most challenging aspect of the calpains, is that we still do not know what they do. There are fundamental difficulties in principle in proving that cleavage of a given target protein inside a cell arises entirely and

exclusively from the action of calpain; and it has proved equally difficult to find out how a particular calpain molecule is activated and directed to choose a given substrate at a given time, and how it escapes inhibition by calpastatin. The existence of many other intracellular proteases, such as caspases and the proteasome, as well as the absence of absolutely specific calpain inhibitors, add to the difficulty of designing unambiguous experiments. These obstacles have been widely recognized, and methods still need to be found to surmount them. We feel that this collection of established calpain methods will provide a solid foundation for future work.

Calpain Methods and Protocols is organized into four sections, though not every chapter can be so rigorously classified. In Section I are gathered methods of purification of the calpains, calpastatin, and the calpain activator protein, from sources ranging from beef heart and lobsters to fruit flies and *E. coli*. Section II includes analytical techniques, such as casein zymography, immunofluorescence, and calpain activity assays, for both in vivo and in vitro use, although further calpain assays are found in several other chapters. Section III presents methods of calpain research applied to specific systems, often in connection with hypoxia or other injury. These systems include neural tissue, kidney, liver, the eye, and membrane fusion in muscle and erythrocytes. Finally, in Section IV are considered some specific substrates that have been proposed for the calpains.

Several recent reviews and monographs provide excellent summaries of the theoretical and historical calpain background, and of the major outstanding problems in the calpain field. The authors in this book were therefore asked to omit all but the briefest rationale of their work, and to confine themselves simply to: "this is how we do it." Given the difficulties of the topic, controversies are bound to arise, and no attempt has been made to resolve them here. At least two examples might be mentioned: there is an extensive and contradictory literature on the physiological relevance of the interplay of calpain and protein kinase C, and another contradictory set relating to the role of calpain in apoptosis. The chapter by Dr. Shea provides methods of looking at PKC, and the chapter by Drs. Squier and Cohen provides methods of assessing apoptosis, while remaining carefully neutral on the issue of 'relevance.' We hope that chapters such as these will help future investigators to resolve these important questions.

For the purposes of *Calpain Methods and Protocols*, some methods are either too specialized or too vast for inclusion. Cloning is barely mentioned, except for the chapter by Dr. Sorimachi on calpain 3, or p94, the best known of the newer calpains, even though these methods have made major contributions to the study of calpain. X-ray crystallography and methods

relating to transgenic mice are not described, even though these areas are also likely to make major contributions to the understanding of calpain in the near future. In contrast, some methods are so widespread, for example gel electrophoresis and Western blotting, that they have been taken as known, and the details omitted to save space.

A simple search for the word 'calpain' in the Medline data base in October 1999 yielded over 2200 references, all of which I cannot claim to have read, and the list of substrates proposed for the calpains is almost as long. I have attempted to gather a wide and representative set of methods, while avoiding direct duplication of a given topic, and apologize for any real or perceived omissions. It is important to acknowledge the prompt and willing response of all those who were approached. If their wide experience distilled here is able to help other investigators to solve the various calpain problems, *Calpain Methods and Protocols* will have proved its worth.

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