Methods in ENZYMOLOGY

Volume 200

Protein Phosphorylation

Part A

Protein Kinases: Assays, Purification, Antibodies, Functional Analysis, Cloning, and Expression

Edited by

Tony Hunter

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Preface

The field of protein phosphorylation has grown and changed considerably since it was covered in 1983 in Volume 99 on Protein Kinases in the *Methods in Enzymology* series. At that time fewer than five protein kinase amino acid sequences were known. The number of identified protein kinases and the number of processes known to be regulated by protein phosphorylation have both increased enormously since then, and the end is not yet in sight. Fundamental to this proliferation has been the ability to isolate novel genes encoding protein kinases using the techniques of molecular biology. Equally important is the fact that the similarity in amino acid sequence of the catalytic domains of the protein kinases allows the instantaneous realization that a molecular clone isolated on the basis of biological function or partial amino acid sequence encodes a protein kinase. It is now clear that many, and perhaps most, aspects of growth regulation are controlled by a complex network of protein kinases and phosphatases.

The techniques that have already defined the unexpectedly large size and degree of complexity of the protein kinase gene family, and will continue to do so, are described in Volumes 200 and 201. These two volumes were consciously entitled Protein Phosphorylation, rather than Protein Kinases. This decision had two origins. One was the emerging realization that the protein phosphatases may prove to be of as much regulatory significance as the protein kinases. The other was that the study of protein kinases is sterile in the absence of the identification and characterization of both upstream regulators and downstream polypeptide substrates, many of which will not be protein kinases.

Of necessity, the first protein kinases identified and studied were those whose activity was prominent in tissues that could be obtained in large quantities. Most of the protein kinases that are important in growth control, however, are present at extremely low levels in cells. The development of sensitive techniques to study nonabundant proteins was, therefore, imperative. Considerable attention is given in these volumes to the use of recombinant DNA techniques for the preparation of large quantities of protein kinases, to means by which to detect trace quantities of specific polypeptides in complex mixtures of proteins, and to techniques with which to perform protein chemistry on vanishingly small quantities of phosphoproteins.

What does the future of this field hold? A major "watershed" will be the determination of the three-dimensional structure of a protein kinase.

Techniques useful for the crystallization of cyclic AMP-dependent protein kinase are presented in Volume 200, but solution of the structure of the enzyme at atomic resolution has not yet been achieved. Knowledge of the structure of one or more protein kinases will almost certainly alter the study of these enzymes very significantly.

To date, with few exceptions, the study of protein phosphorylation has involved the study of the phosphorylation of proteins on serine, threonine, or tyrosine. The lack of attention paid to protein kinases generating acid-labile phosphoamino acids reflects not a lack of biological importance of these enzymes, since they clearly play a central role in bacterial chemotaxis, but rather the fact that methods for their study are few and poorly developed. Unanticipated and important roles for protein kinases may well become apparent if simple and reliable means with which to detect and study proteins containing labile phosphorylated amino acids are devised. No doubt the future will also hold other surprises, but we can only hope that four volumes are not needed the next time protein phosphorylation is covered in this series!

These volumes would never have seen "the light of day" without the diligence of Karen Lane. We thank her for her cheerful and tireless help.

BARTHOLOMEW M. SEFTON
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