

Protein Phosphorylation

EDITED BY

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Front cover photograph: Structure of a large fragment of Hck, one member of the Src family of cytoplasmic, nonreceptor tyrosine protein kinases, as determined by Sicheri, F., Moarefi, I., and Kuriyan, J. (1997) *Nature* **385**:602–609. Hck depicted here lacks its amino-terminal 81 residues. The SH3 domain (residues 82 to 150) is shown in yellow. The SH2 domain (residues 155 to 245) is shown in blue. The catalytic domain (residues 259 to 517) is shown in red. Linker regions and the carboxy-terminal tail are shown in white. In this form, Tyr-527 near the carboxy terminus (shown as a ball and stick model) is phosphorylated and bound to the SH2 domain. The SH3 domain is complexed with a polyproline type-II helix present in the linker between the SH2 domain and the catalytic domain. These two intramolecular interactions cause the kinase to exhibit relatively low catalytic activity. The second major site of tyrosine phosphorylation, Tyr-416, is not phosphorylated in this form and the activation loop in the catalytic domain containing it is disordered.

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Preface

Protein phosphorylation has been found to play a more central and essential role in the regulation of cellular growth and function than almost anyone could have imagined. The study of protein phosphorylation has simultaneously expanded into new areas of biology and begun to address much more sophisticated questions as a result of a number of recent advances. First, mass spectroscopy is being used more and more frequently to identify sites of phosphorylation. Where this technique can be employed, it simplifies site identification enormously. Second, a suite of antisera specific for the phosphorylated form of proteins involved in signal transduction has become widely available. The phosphorylation of specific proteins at specific sites *in vivo* can thereby be detected fairly directly and unambiguously. Third, three-dimensional structures of a number of protein kinases and phosphatases are now known. It is, therefore, now possible to understand both catalysis and enzyme regulation in atomic detail. Fourth, gene knockout techniques are now revealing biological roles for specific protein kinases and protein phosphatases in larger eukaryotes. This builds on the substantial base of genetic knowledge already obtained with yeast, *Drosophila*, and *C. elegans*. Finally, because the sequencing of the genomes of increasingly larger eukaryotes continues apace, the number, size, and diversity of the protein kinase and protein phosphatase gene families are becoming much clearer.

Indeed, with the completion of the sequencing of mammalian genomes just over the horizon, it should soon be possible to identify and study any and every protein kinase and protein phosphatase in a cell. The field will then reach a remarkable maturity. The identification of substrates of the protein kinases and phosphatases is unfortunately less straightforward and will probably advance more slowly. The use of antibodies to phosphotyrosine, on one hand, and SH2 and PTB domains, on the other, to fish out tyrosine phosphorylated proteins should continue to facilitate the study of the substrates of the tyrosine protein kinases and phosphatases. There is clearly a need, however, for the development of comparable approaches for the isolation of proteins that are phosphorylated on serine and threonine.

Although the study of the role of protein phosphorylation in cell function often addresses more sophisticated questions now than it did five years ago, many of the techniques that are brought to bear on the subject today were well established some time ago. Assembled in this book are articles from *Methods in Enzymology* Volumes 200 and 201 which have been chosen

for their contemporary relevance and their usefulness to scientists new to the study of protein phosphorylation.

Note that any cross-reference in this collection refers to a paper or volume actually in the *Methods in Enzymology* series. Where only volume and paper numbers are mentioned, these too refer to the *Methods in Enzymology* series.

BARTHOLOMEW M. SEFTON
TONY HUNTER

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