PROTEIN PHOSPHORYLATION

Protein Phosphorylation

EDITED BY

Bartholomew M. Sefton

Tony Hunter

MOLECULAR BIOLOGY AND VIROLOGY LABORATORY
THE SALK INSTITUTE
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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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List of Contributors

Article numbers are in parenthesis following the names of contributors.
Affiliations listed are current.

RUEDI H. AEBERSOLD (18), *Department of Molecular Biotechnology, University of Washington, Seattle, Washington 98195*

TETSU AKIYAMA (40), *Department of Oncogene Research, Research Institute of Microbial Diseases, Osaka University, Osaka 565, Japan*

MICHAEL ANOSTARIO, JR. (10), *Department of Inflammation and Autoimmune Diseases, Hoffman-La Roche Inc., Nutley, New Jersey 07110*

ROBERT M. BELL (35), *Department of Biochemistry, Duke University Medical Center, Durham, North Carolina 27710*

ROBERT B. BOURRET (8), *Department of Microbiology and Immunology, University of North Carolina, Chapel Hill, North Carolina 27599-7290*

WILLIAM J. BOYLE (14), *Department of Molecular and Cellular Biology, AMGEN, Thousand Oaks, California 91320*

JOHN E. CASNELLIE (6), *Department of Pharmacology, University of Rochester, Rochester, New York 14642*

JONATHAN CHEN (23), *Abbott Laboratories, Pharmaceutical Products Division, Abbott Park, Illinois 60064*

PHILIP COHEN (4, 16), *Department of Biochemistry, MRC Protein Phosphorylation Unit, University of Dundee, Dundee, DD1 4HN, Scotland*

JONATHAN A. COOPER (22), *Fred Hutchinson Cancer Research Center, Seattle, Washington 98104*

JACKIE D. CORBIN (27), *Department of Molecular Physiology and Biophysics, Vanderbilt University School of Medicine, Nashville, Tennessee 37232*

ANDREW J. CZERNIK (23), *Laboratory of Molecular and Cellular Neuroscience, The Rockefeller University, New York, New York 10021*

CURTIS D. DILTZ (33), *Department of Biochemistry, University of Washington, Seattle, Washington 98195*

ARIANNA DONELLA-DEANA (20), *Dipartimento di Chimica Biologica, Universita di Padova, I-35121 Padova, Italy*

STEFANO FERRARI (25), *Friedrich Miescher-Institute, CH-4002 Basel, Switzerland*

JAMES E. FERRELL, JR. (11), *Department of Molecular Pharmacology, Stanford University School of Medicine, Stanford, California 94305*

EDMOND H. FISCHER (33), *Department of Biochemistry, University of Washington, Seattle, Washington 98195*

A. RAYMOND FRACKELTON, JR. (32), *Department of Medicine, Roger Williams Medical Center and Brown University, Providence, Rhode Island 02908*

SHARRON H. FRANCIS (27), *Department of Molecular Physiology and Biophysics, Vanderbilt University School of Medicine, Nashville, Tennessee 37232*

HIDESUKE FUKAZAWA (41), *Department of Antibiotics, National Institute of Health, Tokyo 141, Japan*

AVIV GAZIT (39), *Department of Biological Chemistry and Organic Chemistry, The Hebrew University of Jerusalem, Jerusalem 91904, Israel*

ROBERT L. GEAHLEN (10), *Department of Medicinal Chemistry and Pharmacognosy, Purdue University, West Lafayette, Indiana 47907*

BRADFORD W. GIBSON (16), *Department of Pharmaceutical Chemistry, School of Pharmacology, University of California, San Francisco, San Francisco, California 94143*

CHAIM GILON (39), *Department of Organic Chemistry, The Hebrew University of Jerusalem, Jerusalem 91904, Israel*

JEAN-ANTOINE GIRAULT (23), *INSERM U114, College de France, 75235 Paris, France*

JULIUS A. GORDON (43), *Pathology and Laboratory Medicine, University of Texas A&M School of Medicine, College Station, Texas 77843*

PAUL GREENGARD *(23), Laboratory of Molecular and Cellular Neuroscience, The Rockefeller University, New York, New York 10021*

BOYD E. HALEY (24), *Department of Biochemistry, University of Kentucky Medical Center, Lexington, Kentucky 40536*

STEVEN K. HANKS (2), *Department of Cell Biology, Vanderbilt University School of Medicine, Nashville, Tennessee 37232*

YUSUF A. HANNUN (35), *Departments of Medicine and Cell Biology, Duke University Medical Center, Durham, North Carolina 27710*

D. GRAHAME HARDIE (38), *Protein Phosphorylation Group, Department of Biochemistry, Medical Sciences Institute, University of Dundee, Dundee DD1 4H Scotland*

MARIETTA L. HARRISON (10), *Department of Medicinal Chemistry and Pharmacognosy, Purdue University, West Lafayette, Indiana 47907*

TIMOTHY A. J. HAYSTEAD (38), *Department of Pharmacology, University of Virginia, Charlottesville, Virginia 22908*

LUDWIG M. G. HEILMEYER, JR. (17), *Abteilung Biochemie Supramolekularer Systeme, Ruhr-Universität Bochum, D-4630 Bochum 1, Germany*

J. FRED HESS (8), *Department of Biochemistry, Merck Sharp & Dohme Research Laboratories, Rahway, New Jersey 07065*

HIROYOSHI HIDAKA (36), *Department of Pharmacology, Nagoya University School of Medicine, Showa-ku, Nagoya 466, Japan*

EDELTRAUT HOFFMANN-POSORSKE (17, 20), *Abteilung Biochemie Supramolekularer Systeme, Ruhr-Universität Bochum, D-4630 Bochum 1, Germany*

CHARLES F. B. HOLMES (16), *Department of Biochemistry, University of Alberta, Edmonton, Alberta T6G 2H7, Canada*

LEROY E. HOOD (18), *Department of Molecular Biotechnology, University of Washington, Seattle, Washington 98195-7730*

COLIN M. HOUSE (34), *St. Vincent's Institute of Medical Research, Fitzroy, Victoria 3065, Australia*

FREESIA L. HUANG (28), *Section on Metabolic Regulation, Endocrinology and Reproduction Research Branch, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland 20892*

KUO-PING HUANG (28), *Section on Metabolic Regulation, Endocrinology and Reproduction Research Branch, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland 20892*

TONY HUNTER (1, 14), *Molecular Biology and Virology Laboratory, The Salk Institute, San Diego, California 92186*

TAMARA R. HURLEY (15), *Molecular Biology and Virology Laboratory, The Salk Institute, San Diego, California 92138*

JILL E. HUTCHCROFT (10), *Department of Biological Sciences, Purdue University, West Lafayette, Indiana 47907*

MASAYA IMOTO (42), *Department of Applied Chemistry, Faculty of Science and Technology, Keio University, Yokohama 223, Japan*

PAUL JENÖ (26), *Department of Biochemistry, Biocenter of the University of Basel, CH-4056 Basel, Switzerland*

MARK P. KAMPS (12, 13), *Department of Pathology, School of Medicine, University of California, San Diego, La Jolla, California 92093*

B. KANNAN (32), *Georgetown University Hospital, Lombardi Cancer Research Center, Washington, D.C. 20007*

JOHN KEBABIAN (23), *Abbott Laboratories, Pharmaceutical Products Division, Abbott Park, Illinois 60064*

BRUCE E. KEMP (3, 5, 34), *St. Vincent's Institute of Medical Research, Fitzroy, Victoria 3065, Australia*

RYOJI KOBAYASHI (36), *Department of Pharmacology, Nagoya University School of Medicine, Showa-ku, Nagoya 466, Japan*

HORST KORTE (20), *Abteilung Biochemie Supramolekularer Systeme, Ruhr-Universität Bochum, D-4630 Bochum 1, Germany*

ALEXANDER LEVITZKI (39), *Department of Biological Chemistry, The Hebrew University of Jerusalem, Jerusalem 91904 Israel*

KUNXIN LUO (15), *Lawrence Berkeley Laboratory, University of California, Berkeley, California 94720*

G. STEVEN MARTIN (11), *Division of Biochemistry and Molecular Biology, Department of Molecular and Cell Biology, University of California, Berkeley, California 94720*

HARRY R. MATTHEWS (9), *Department of Biological Chemistry, University of California, Davis, California 95616*

MARIA L. MCGLONE (30), *Department of Chemistry, University of California, San Diego, La Jolla, California 92093*

F. MERMELSTEIN (32), *Department of Biochemistry, University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School, Piscataway, New Jersey 08854*

ALFRED H. MERRILL, JR. (35), *Department of Biochemistry, Emory University, Atlanta, Georgia 30322*

HELMUT E. MEYER (17, 20), *Abteilung Biochemie Supramolekularer Systeme, Ruhr-Universität Bochum, D-4630 Bochum 1, Germany*

ANGUS C. NAIRN (23), *Laboratory of Molecular and Cellular Neuroscience, The Rockefeller University, New York, New York 10021*

HIROSHI OGAWARA (40), *Department of Biochemistry, Meiji College of Pharmacy, Tokyo 154, Japan*

NIR OSHEROV (39), *Department of Biological Chemistry, The Hebrew University of Jerusalem, Jerusalem 91904, Israel*

P. J. PARKER (29), *Protein Phosphorylation Laboratory, Imperial Cancer Research Fund, London WC2A 3PX, England*

RICHARD B. PEARSON (5, 34), *St. Vincent's Institute of Medical Research, Fitzroy, Victoria 3065, Australia*

ISRAEL POSNER (39), *Department of Biological Chemistry, The Hebrew University of Jerusalem, Jerusalem 91904, Israel*

M. POSNER (32), *Department of Medicine, New England Deaconess Hospital, Boston, Massachusetts 02215*

ANNE MARIE QUINN (2), *Information Technology Services–Medicine, Yale University School of Medicine, New Haven, Connecticut 06520*

EFRAIM RACKER (7),* *Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York 14853*

PETER J. ROACH (19), *Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis, Indiana 46223*

BARTHOLOMEW M. SEFTON (15, 21), *Molecular Biology and Virology Laboratory, The Salk Institute, San Diego, California 92138*

D. SCHAAP (29), *Dutch Cancer Institute, 1066 CX Amsterdam, The Netherlands*

LEE W. SLICE (30), *Virology Department, Hoffmann-La Roche Inc., Nutley, New Jersey 07110*

ALISTAIR T. R. SIM (38), *Neurosciences Group, Faculty of Medicine, University of Newcastle, Newcastle, NSW 2308, Australia*

MELVIN I. SIMON (8), *Division of Biology, California Institute of Technology, Pasadena, California 91125*

GRETCHEN SNYDER (23), *Laboratory of Molecular and Cellular Neuroscience, The Rockefeller University, New York, New York 10021*

* Deceased.

S. STABEL (29), *Max-Delbruck-Laboratorium in der Max-Planck-Gesellschaft, Carl-von-Linne-Weg 10, D-50829*

TATSUYA TAMAOKI (37), *Kyowa Hakko Kogyo Co., Ltd., Tokyo Research Laboratories, Tokyo 194, Japan*

SUSAN S. TAYLOR (30), *Department of Chemistry, University of California, San Diego, La Jolla, California 92093*

GEORGE THOMAS (25, 26), *Friedrich Miescher-Institute, CH-4002 Basel, Switzerland*

NICHOLAS K. TONKS (33), *Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724*

YOSHIMASA UEHARA (41), *Department of Antibiotics, National Institute of Health, Tokyo 141, Japan*

KAZUO UMEZAWA (42), *Department of Applied Chemistry, Faculty of Science and Technology, Keio University, Yokohama 223, Japan*

PETER VAN DER GEER (14), *Department of Chemistry and Biochemistry, University of California, San Diego, La Jolla, California 92093*

YUHUAN WANG (19), *Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis, Indiana 46223*

MASATO WATANABE (36), *Department of Pharmacology, Nagoya University School of Medicine, Showa-ku, Nagoya 466, Japan*

YING-FEI WEI (9), *Laboratory of Toxicology, Harvard School of Public Health, Boston, Massachusetts 02115*

RICHARD E. H. WETTENHALL (18), *Department of Biochemistry, University of Melbourne, Parkville, Victoria 3052, Australia*

ANDREW F. WILKS (31), *Ludwig Institute for Cancer Research, PO Royal Hospital, Victoria 3050, Australia*

LYNN WOLFE (27), *Department of Biochemistry, University of Massachusetts Medical Center, Worcester, Massachusetts 01605*

WES M. YONEMOTO (30), *Ariad Pharmaceuticals, Cambridge, Massachusetts 02139*

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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