Greengard and Robison

Series Editors

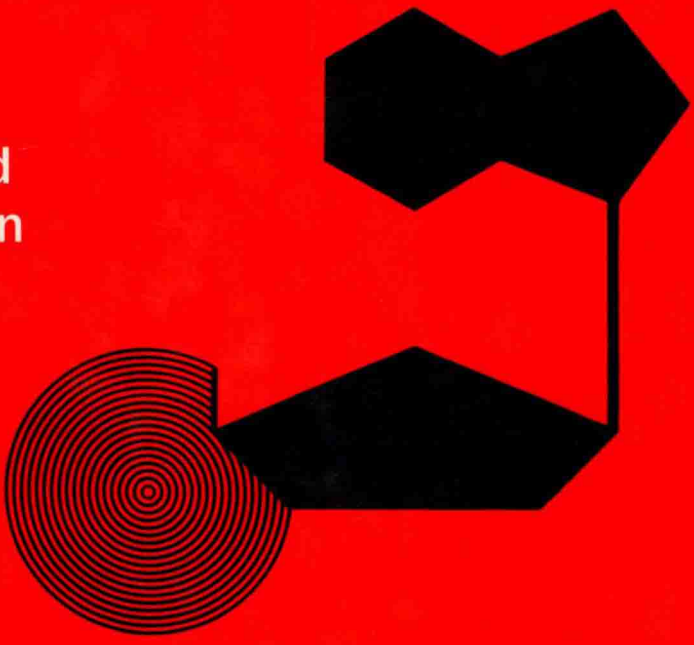
ADVANCES IN SECOND MESSENGER AND PHOSPHOPROTEIN RESEARCH

Formerly
ADVANCES IN
CYCLIC NUCLEOTIDE
AND PROTEIN
PHOSPHORYLATION
RESEARCH

VOLUME 23

Volume Editors

Paul Greengard
G. Alan Robison



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Advances in Second Messenger and Phosphoprotein Research

Volume 23

formerly *Advances in Cyclic Nucleotide and Protein Phosphorylation Research*

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Preface

With this volume of *Advances in Second Messenger and Phosphoprotein Research* we would like to rededicate ourselves to the proposition with which this series began some 18 years ago. The purpose was to provide a forum for thoughtful review articles that would not only help to make the current scientific literature more meaningful but that might also stimulate further productive research. To that end we have always encouraged our authors to engage in as much informed speculation as they felt would contribute to those goals. We have not always agreed with all of the speculations they came up with over the years, but we have almost always agreed that those speculations were interesting and worth considering.

Every third year the proceedings of the International Conferences on Second Messengers and Phosphoproteins are published as part of this series. Volume 24, covering the 7th Conference held this past fall in Kobe, has already appeared. The 8th Conference will be held in 1992 in Glasgow. The volumes in intervening years feature longer and more comprehensive critical reviews of the literature. Some of these volumes will cover a variety of topics, as in the case of this one, but it seems likely that our occasional practice of inviting others to serve as editors of more highly focused volumes will occur more frequently in the future.

Earl Sutherland predicted, at the time of the first conference in Milan, that cyclic nucleotides would continue to provide a helpful unifying theme for the biomedical sciences for another 25 years. We feel that our decision to expand the subject area to other second messengers and to phosphoproteins, whether related to cyclic nucleotides or not, has expanded the useful life of our theme considerably beyond the quarter of a century originally envisioned by Earl.

We are pleased to announce that beginning with Volume 26 we will be joined by two younger colleagues as Associate Editors, Angus Nairn in New York and Shirish Shenolikar in Houston. These two friends and colleagues have collaborated to produce the opening review in this volume, the first comprehensive review we have ever featured on the subject of protein phosphatases.

Almost all of the previous volumes in this series have included a mixture of old and new topics, and this one is no exception. The serine protein kinases, reviewed here by Tuazon and Traugh, and the regulation of ciliary motility, reviewed by Bonini, Nelson, and colleagues, are new to the series this year. Our knowledge of signal transduction in *Dictyostelium* was first reviewed in this series by Theo Konijn in Volume 1 and has been reviewed on several subsequent occasions by others. The subject is updated in this volume by one of

Professor Konijn's most distinguished students, Peter Van Haastert. Finally, Randall Kincaid's review on signaling mechanisms in microorganisms reflects our increasingly sophisticated understanding of how cell regulatory mechanisms have evolved in lower organisms. We hope to feature an entire volume on this subject in the near future.

PAUL GREENGARD
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Contents

Advances in Second Messenger and Phosphoprotein Research, Vol. 23,
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Protein Phosphatases: Recent Progress

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I. INTRODUCTION

Reversible protein phosphorylation is widely recognized as an important mechanism for the control of a wide variety of cellular processes (81,82). Phosphorylation of key proteins with associated changes in their biological activity accounts for the physiological response. The phosphate content of these proteins reflects a net balance of the protein kinases and protein phosphatases acting on them. Biochemical as well as recombinant DNA studies have identified a large number of protein kinases (184). In comparison it has been widely accepted that fewer enzymes are involved in the dephosphorylation of cellular proteins. This viewpoint was based on the biochemical properties of mammalian serine/threonine protein phosphatases. Early studies focused on determining the number of these protein phosphatases, their subunit composition, substrate specificity, and potential regulation by endogenous and exogenous compounds. The role of subcellular distribution of these enzymes in dictating their physiological actions was also examined. The initial perception of protein phosphatases has now been modified for several reasons. It is clear from molecular cloning studies that the number of protein phosphatases has been greatly underestimated. The functional roles of serine/threonine protein phosphatases has also been expanded with the discovery that they are essential components regulating gene transcription and cell division in eukaryotic cells. In addition, the identification of membrane-associated receptor-linked tyrosine protein phosphatases points to a new intracellular signaling pathway mediated by protein dephosphorylation, controlling cell proliferation and cell-cell communication. A number of cytoplasmic tyrosine protein phosphatases have also been identified. These enzymes most likely play a role in the control of signal transduction by the tyrosine kinase activities of receptors for peptide hormones and growth factors. The recent progress in the serine/threonine protein phosphatases and the tyrosine protein phosphatases will be the topic of this review. Over the past decade there have been several excellent reviews on serine/threonine protein phosphatases (34,82,302,512), which document progress during this period. We have chosen not to reiterate the content of these reviews. Thus, the current discussion will be focused primarily on data obtained over the past 4 years.

II. STRUCTURAL COMPARISON OF SERINE/THREONINE PROTEIN PHOSPHATASES

As we review the structural and functional information on serine/threonine protein phosphatases, for convenience, we will adhere to the nomenclature proposed by Ingebritsen and Cohen (237), which has greatly facilitated the characterization of these enzymes. The methods used by Ingebritsen and Cohen identified two major classes of protein phosphatases (Table 1). These were called type-1 (PrP-1) and type-2 (PrP-2) protein phosphatases. The type-2 enzymes were further subdivided into three groups, termed PrP-2A, PrP-2B, and PrP-2C. The application of this system has highlighted the considerable functional conservation in protein phosphatases in all types of organisms, including animals (83), plants (314), yeast (86), and bacteria (88,89). However, there are examples of protein phosphatases that are not adequately accommodated by these guidelines. Their discussion should serve to emphasize the considerable progress that has been made in our understanding of the structure and regulation of protein phosphatases.

TABLE 1. *Serine/threonine protein phosphatases*

	PrP-1	PrP-2A	PrP-2B	PrP-2C
Substrates				
Phosphorylase kinase *a*	β	α	α	α
Phosphorylase *a*	Yes	Yes	No	No
Histone H1 (cA-PK)[a]	Yes	Yes	No	No
Histone H1 (PK-C)	No	Yes	No	No
Histone H2B	Yes	Yes	No	Yes
Myosin light chain	Yes	Yes	Yes	Yes
Activators				
Polyamines/polycations	No	Yes	No	No
Mg^{2+}	No	No	No	Yes
Ca^{2+}/calmodulin	No	No	Yes	No
F_a/GSK-3	Yes	No	No	No
Inhibitors				
I_1	Yes	No	No	No
I_2	Yes	No	No	No
Heparin	Yes	No	No	No
Okadaic acid	Yes	Yes[b]	No	No
Calyculin A	Yes[c]	Yes	No	No
NaF	Yes	Yes	Yes	No
Orthovanadate	Yes	Yes	No	Yes
Phenothiazines	No	No	Yes	No

[a]Histone H1 phosphorylated by cAMP-dependent protein kinase (cA-PK) and by protein kinase C (PK-C) demonstrates a different capacity to be dephosphorylated by these protein phosphatases (246).

[b]PrP-$2A_c$ is inhibited with 10-fold lower K_i than PrP-1_c (82).

[c]Calyculin A shows similar apparent inhibition of both PrP-1_c and PrP-$2A_c$ (241).

Recent progress concerning serine/threonine protein phosphatases has come largely from molecular cloning of the cDNAs for the catalytic subunits of these enzymes. The various PrP-1_c sequences provide a striking example of the conservation of this class of protein phosphatases through evolution (Fig. 1). Indeed, more than 80% structural identity has been retained between the enzymes from fungi (*Schizosaccharomyces pombe, Saccharomyces cerevisiae,* and *Aspergillus nidulans*) and mammalian tissues. There is also a very high degree of conservation among the various forms of PrP-$2A_c$ (Fig. 2). Greater than 96% identity in the amino acid sequences of either the α or β isoforms of PrP-$2A_c$ from rat, rabbit, porcine, bovine, and human tissues has been noted. The complete cDNA for the catalytic subunit of PrP-2B (the A subunit) has so far been cloned only from rat, mouse, bovine, and human brain. Initial analysis suggests that the primary structure of this enzyme will also be highly conserved. Only one complete primary sequence for PrP-2C (deduced from the cDNA) is available at this time.

Analysis of the primary structures of these catalytic subunits indicates substantial structural homology between PrP-1, PrP-2A, and PrP-2B (Fig. 3). In comparison, PrP-2C, a broad substrate specificity enzyme, which dephosphorylates some protein substrates in common with PrP-1, PrP-2A, and PrP-2B, shows relatively little homology with these enzymes. There are six regions of the catalytic subunits of PrP-1, PrP-2A, and PrP-2B, which are very highly conserved (Fig. 3, boxed regions) and could form structural elements of the catalytic domain. Visual comparison of these domains with the sequence of PrP-2C shows that several amino acids are conserved in domains II, III, IV, and V. It is not known at this time if these amino acids are essential for catalysis. Interestingly, conserved cysteine residues are found in domains IV and V in all classes of enzyme (24). It has been suggested that cysteine residues are essential for the catalytic activity of several protein phosphatases (60,274). In this regard, we would predict that site-directed mutagenesis of the cysteine residues in domains IV and V should prove particularly valuable in determining the role of these regions of homology in protein phosphatase activity. So far analysis of the amino acid sequences of the various protein phosphatases, with the exception of the calmodulin-binding domain of PrP-2B (see below), has failed to indicate the structural basis for their functional differences, such as their substrate specificities or their unique interactions with endogenous inhibitors, subunits, and other targeting proteins. The predicted primary structures of tyrosine protein phosphatases show no discernible similarity with the serine/threonine protein phosphatases (473). There is also no apparent identity between the serine/threonine protein phosphatases and acid or alkaline phosphatases.[1]

[1]Acid (504,538) and alkaline phosphatases (145,342) as well as mitochondrial protein phosphatases (399) constitute different groups of enzymes and will not be discussed further in this review.

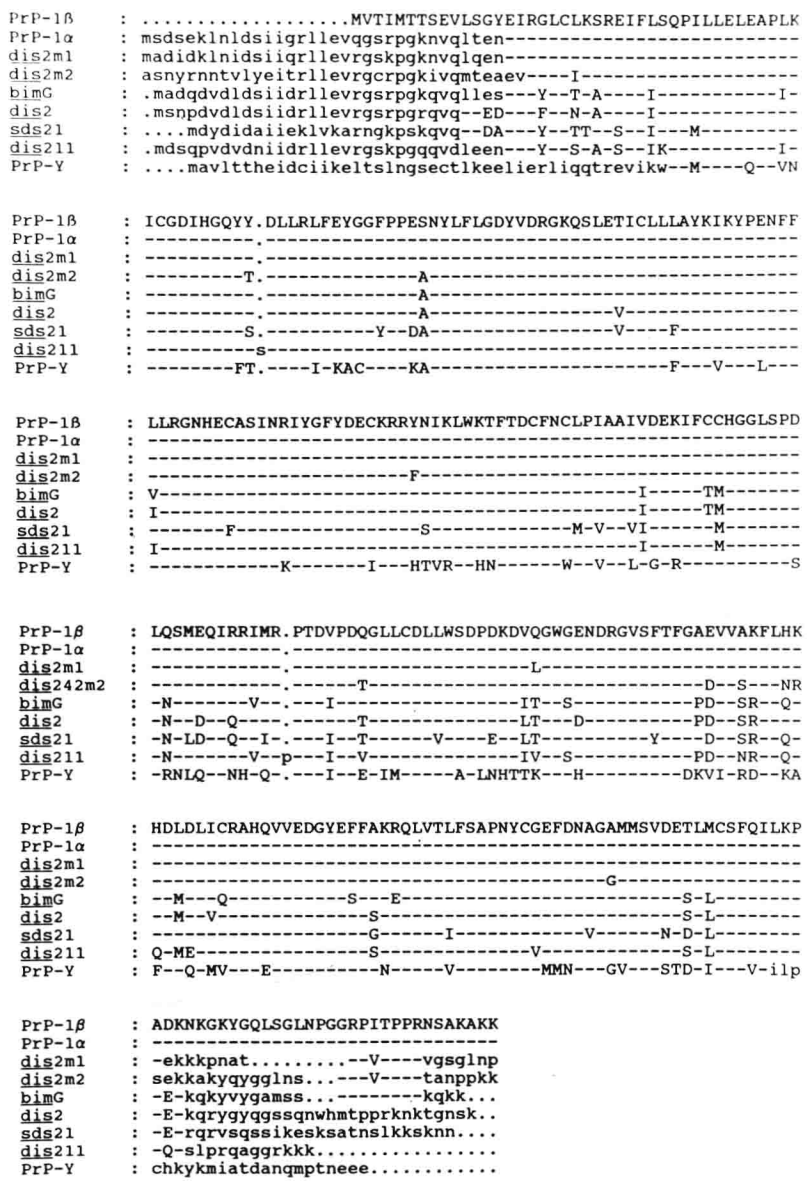

FIG. 1. Type-1 protein phosphatases. The deduced protein sequence of protein phosphatases, PrP-$1_c\beta$ (87) and PrP-$1_c\alpha$ (38) from rabbit skeletal muscle were compared with sequences deduced by molecular cloning of the cDNAs: *dis*2m1 and *dis*2m2 from mouse brain (367), *bim*G from *Aspergillus nidulans* (115), *dis*2 and *sds*21 from *Schizosaccharomyces pombe* (367), *dis*211 from *Saccharomyces cerevisiae* (367), and PrP-Y from *Drosophila melanogaster* (114) using a modification of the SS2 algorithm of Altschul and Erickson (19). The opening cost penalty was set to minimize gaps. *Upper case* indicates aligned nonidentical amino acids (or conservative substitutions). *Lower case* represents unaligned residues. Identical residues are shown as dashes (–) and gaps are represented by dots (·).