

Guest Editor's Introduction

## Emerging new techniques for studying protein phosphatases

Protein phosphorylation reactions are reversible and dynamic and are governed by the opposing activities of protein kinases, which catalyze the transfer of the  $\gamma$ -phosphoryl moiety from ATP to the side chains of Ser/Thr and Tyr residues in proteins, and protein phosphatases, which are responsible for the removal of the phosphate group from the protein molecules. Protein kinases and phosphatases and their corresponding substrates are integrated within elaborate signal transducing networks that are essential for cell growth, differentiation, metabolism, progression through the cell cycle, cell–cell communication, cell adhesion and migration, gene transcription, ion channel activity, the immune response, and apoptosis/survival decisions. Defective or inappropriate operation of these networks often leads to aberrant protein phosphorylation, which contributes to the development of many human diseases including cancers, diabetes, and inflammatory disorders. Major insights into the protein phosphorylation-mediated cellular processes have been derived from studies of protein kinases and it is common to view signaling pathways as cascades of kinase reactions. However, because the levels of protein phosphorylation are controlled by the activities of both kinases and phosphatases, comprehension of the physiological roles of protein phosphorylation, and its potential as a mechanism for reversible modulation of protein function and cell physiology, must necessarily encompass the characterization of protein phosphatases in addition to the protein kinases.

The present issue of *Methods* highlights a number of contemporary chemical, structural, genomics, and proteomics techniques that have been developed to study protein phosphatases. These new techniques are complementary to the traditional genetic approaches, including gene knock-out and overexpression, and should greatly enhance our ability to probe the function of protein phosphatases. To provide insight into the specific roles of protein phosphatases in cellular signaling, it is important to have detailed understanding of the molecular basis for substrate recognition by these enzymes. Anne-Sophie Wavreille, Mathieu Garaud, Yanyan Zhang, and Dehua Pei describe a “one-bead–one-compound” phosphotyrosine-containing combi-

natorial peptide library method that can be used to systematically assess the sequence specificity of both SH2 domains and protein tyrosine phosphatases (PTPs). The beads that carry the tightest binding sequences against the SH2 domain or the most efficient substrates of the PTP are selected by an enzyme-linked assay and individually sequenced by partial Edman degradation/mass spectrometry. Luo Sun, Inca Ghosh, Tanya Barshevsky, Samvel Kochinyan, and Ming-Qun Xu outline an effective method for assay protein phosphatase activity using phosphoprotein substrates generated by ligating synthetic phosphopeptides to a carrier protein by the intein-mediated protein ligation technique. The facile production of a diversity of pure protein substrates containing well-defined structural motifs opens a new avenue for the investigation of protein phosphatase substrate specificity. To address the question of how protein phosphatases discriminate between multiple structurally diverse substrates that they encounter in the cell, X-ray crystallography is frequently employed to reveal the intimate structural details for molecular interaction. Unfortunately, structures of higher order PTP-substrate complexes are often difficult to obtain. Bo Zhou and Zhong-Yin Zhang illustrate the use of hydrogen/deuterium exchange mass spectrometry to define the molecular basis of substrate/ligand recognition by the PTPs. In addition to mapping protein–protein interfaces, hydrogen/deuterium exchange mass spectrometry can also be used to probe conformational changes and dynamics associated with protein–protein interactions.

Like those of many enzymes, the activities of protein phosphatases may also be influenced by phosphorylation. However, it has been difficult to evaluate the effect of phosphorylation on protein phosphatases, primarily because of their intrinsic phosphatase activities. Kui Shen summarizes the application of expressed protein ligation and semisynthetic proteins to incorporate nonhydrolyzable phosphotyrosine analogs into PTPs to delineate the effect of phosphorylation on the PTPs. In addition to phosphorylation, PTP activity can also be modulated by various highly reactive species such as nitric oxide (NO). Yi-Yun Chen, Yi-Fen Huang, Kay-Hooi Khoo, and Tzu-Ching Meng

describe the development of a novel mass spectrometry-based method that enables the identification and characterization of PTP1B *S*-nitrosylation. This study also establishes a direct link between *S*-nitrosylation of the catalytic Cys residue and reversible inactivation of the PTPs.

Chemical approaches using small molecules to interrogate components of signal transduction pathways offer an invaluable means of biological investigation complementary to many genetic techniques. Thus, small-molecule inhibitors that are specific for each protein phosphatase would be valuable tools in dissecting protein phosphatase-mediated signaling networks and for validating protein phosphatases as therapeutic targets. Lutz Tautz and Tomas Mustelin outline a number of strategies for developing PTP inhibitors, starting from high-throughput chemical library screening, to hit confirmation and prioritization, and *in silico* or virtual screening methods. Most high-throughput screening campaigns for PTP inhibitor development employ PTP activity-based assays. Unfortunately, these assays suffer from a major weakness, in that the reactivity of the active site Cys can cause serious problems as highly reactive oxidizing and alkylating agents may surface as hits. Sheng Zhang, Lan Chen, Sanjai Kumar, Li Wu, David S. Lawrence, and Zhong-Yin Zhang introduce a fluorescence polarization-based displacement assay that makes use of the active site Cys to Ser mutant PTP that retains the wild-type binding affinity. The potency of library compounds is assessed by their ability to compete with the fluorescently labeled active site ligand for binding to the Cys to Ser PTP mutant. The substitution of the active site Cys by a Ser renders the mutant PTP insensitive to oxidation and alkylation and thus will likely eliminate “false” positives due to modification of the active site Cys that destroy the phosphatase activity. Andreas Vogt and John S. Lazo provide a step-by-step protocol for the implementation of high-content assay for inhibitors of mitogen-activated protein kinase phosphatases. These cell-based high-content methods should enable investigators to probe for selective inhibitors of disease-relevant protein phosphatases. As an alternative small-molecule approach, enzyme/inhibitor interface engineering can be used to generate selective PTP inhibitors. Anthony C. Bishop, Xin-Yu Zhang, and Anna Mari Lone describe the current state of the PTP-sensitization strategy, with emphases on the

methodology of identifying PTP-sensitizing mutations and synthesizing compounds that have been found to target PTPs in an allele-specific manner.

Significant progress in our knowledge of protein phosphatase biology can also be made through application of advanced proteomic and genomic methods. The rapidly developing field of various omics-based technologies enables us to investigate biological systems on a global scale. Minjie Guo, Jacob Galan, and W. Andy Tao describe a novel soluble nanopolymer-based phosphoproteomics strategy that can be used to selectively isolate phosphopeptides for mass spectrometric analysis, allowing global analysis of protein phosphorylation and dephosphorylation. Association of protein phosphatases with regulatory proteins is a key component contributing to their specificity, and the identification of these binding partners is critical to understanding phosphatases function and regulation. Ginny I. Chen and Anne-Claude Gingras review the basic principles behind affinity purification coupled to mass spectrometry to identify interacting proteins. They then provide two basic protocols that have been successfully employed in the lab for the affinity purification of serine/threonine phosphatases. Finally, with the development of genome-wide RNAi and shRNA libraries it is now possible to carry out high-throughput functional screens for protein phosphatases in various cellular pathways. Amanda M. Opaluch, Pedro Aza-Blanc, Torkel Vang, Scott Williams, Lutz Tautz, Loribelle Milan, and Tomas Mustelin outline the basics of PTPome-wide functional RNA interference screening methods and key issues to consider in analyzing the entire PTP family in an unbiased manner.

I thank all of the contributors of this issue of *Methods*, who made it possible to bring together some of the state-of-the-art technologies currently used in the study of protein phosphatases. I hope that the readers of this special issue will enjoy these articles, and find them useful in facilitating their own examination of the function of the various members of the protein phosphatase families for years to come.

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