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Rafael Pulido *Editor*

Protein Tyrosine Phosphatases

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Preface

Protein tyrosine phosphatases (PTPs) are major direct regulators of the phosphotyrosine cellular content and essential drivers of the tyrosine-phosphorylation status of key cell signaling proteins. Tyrosine phosphatases include proteins from the Cys-based PTP superfamily (containing a PTP catalytic domain and a CxxxxxR signature catalytic motif) as well as enzymes from other gene families (Asp- and His-based phosphatases) that have converged to perform dephosphorylation of biological moieties by a two-step, nucleophile-based catalytic mechanism. Such convergence illustrates the adaptive relevance and the wide variety of the dephosphorylation functions mediated by these enzymes, whose manipulation could be important for specific therapeutic targeting in human disease, including cancer, neurodevelopmental, and metabolic diseases. Moreover, since mutations in many PTP genes are associated with hereditary diseases, several PTP family members are currently relevant in disease prevention and early molecular diagnosis. Tyrosine phosphatases are versatile enzymes in terms of substrate specificity and regulatory properties. Classical PTPs dephosphorylate specific phosphotyrosine residues from protein substrates, whereas dual-specificity PTPs dephosphorylate phosphotyrosine, phosphoserine, and phosphothreonine residues, as well as non-proteinaceous substrates, including phosphoinositides (the tumor suppressor PTEN being a hallmark) and carbohydrates, among others. In addition, several PTPs have impaired catalytic activity as a result of amino acid substitutions at their active sites but retain regulatory functions related to phosphotyrosine or phosphoinositide signaling. The substrate specificity and biological function of PTPs, as well as their regulation during cell homeostasis, is facilitated by a diverse array of protein-interaction and protein-targeting domains, and reversible oxidation of their active sites is a major physiological regulatory mechanism of the catalysis of many Tyr phosphatases.

This book is aimed to provide coverage, methodology, and laboratory protocols on the more essential aspects of PTP function and regulation, including the use of standardized in vitro functional assays, suitable cell systems, and animal and microorganism models. Chapters covering state-of-the-art technical approaches suitable to decipher the physiologic roles of PTPs, and their involvement in tissue-specific functions, are also included, which will be of utility for both newcomers and experienced researchers in the field of tyrosine- and phosphoinositide-phosphorylation/dephosphorylation. I wish to thank all authors for their valuable input and contribution to this issue of *Methods in Molecular Biology*. We think the book will be of interest to chemists, biochemists, molecular biologists, and cell biologists, as well as to clinicians focusing their attention on the role of protein kinases and phosphatases in human disease. It is our hope that the methods and protocols from the chapters of this book will help researchers to better define the common and individual features of the PTP family members, and how this knowledge can translate into PTP-based therapy for human disease.

Barakaldo, Bizkaia, Spain ***Rafael Pulido***

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Chapter 1

The Extended Family of Protein Tyrosine Phosphatases

Andrés Alonso, Caroline E. Nunes-Xavier, Yolanda Bayón, and Rafael Pulido

Abstract

In higher eukaryotes, the Tyr phosphorylation status of cellular proteins results from the coordinated action of Protein Tyrosine Kinases (PTKs) and Protein Tyrosine Phosphatases (PTPs). PTPs have emerged as highly regulated enzymes with diverse substrate specificity, and proteins with Tyr-dephosphorylation or Tyr-dephosphorylation-like properties can be clustered as the PTPome. This includes proteins from the PTP superfamily, which display a Cys-based catalytic mechanism, as well as enzymes from other gene families (Asp-based phosphatases, His-based phosphatases) that have converged in protein Tyr-dephosphorylation-related functions by using non-Cys-based catalytic mechanisms. Within the Cys-based members of the PTPome, classical PTPs dephosphorylate specific phosphoTyr (pTyr) residues from protein substrates, whereas VH1-like dual-specificity PTPs dephosphorylate pTyr, pSer, and pThr residues, as well as nonproteinaceous substrates, including phosphoinositides and phosphorylated carbohydrates. In addition, several PTPs have impaired catalytic activity as a result of amino acid substitutions at their active sites, but retain regulatory functions related with pTyr signaling. As a result of their relevant biological activity, many PTPs are linked to human disease, including cancer, neurodevelopmental, and metabolic diseases, making these proteins important drug targets and molecular markers in the clinic. Here, a brief overview on the biochemistry and physiology of the different groups of proteins that belong to the mammalian PTPome is presented.

Key words Tyrosine phosphatase, Lipid phosphatase, Asp-phosphatase, His-based phosphatase, Phosphorylation, Dephosphorylation

1 Tyr Phosphatases: Positive and Negative Protein Regulators of Cell Signaling

Tyr phosphorylation/dephosphorylation is a profuse regulatory mechanism of the responses of the cells to physiologic and pathologic changes in their environment, and it is exerted in holozoan organisms by the coordinated action of Protein Tyrosine Kinases (PTKs) and Protein Tyrosine Phosphatases (PTPs) [1, 2]. Unlike protein kinases, PTPs have evolved independently of the Ser/Thr Phosphatases, displaying a characteristic PTP domain, a CxxxxxR conserved catalytic loop (where C is the catalytic Cys, x is any amino acid, and R is an Arg), and a Cys-based catalysis [1, 3–7].

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Beyond that, the mammalian PTPome, considered as the cluster of proteins with Tyr-dephosphorylation or Tyr-dephosphorylation-like activity, includes proteins distributed in several families (Cys-based, His-based, Asp-based), among which the PTP family itself contributes with most of the members. In line with this, we have defined the concept of an open and extended PTPome whose members fulfill the following criteria: (a) harboring of a structurally defined PTP domain; *or* (b) presence of a CxxxxxR signature catalytic motif within a non-PTP phosphatase domain; *or* (c) displaying experimentally validated Tyr phosphatase activity; *or* (d) displaying high sequence similarity to members with demonstrated Tyr phosphatase activity. This updated human PTPome contains 125 genes, which encode both catalytically active and inactive (pseudophosphatases) proteins [8] (Fig. 1 and Table 1).

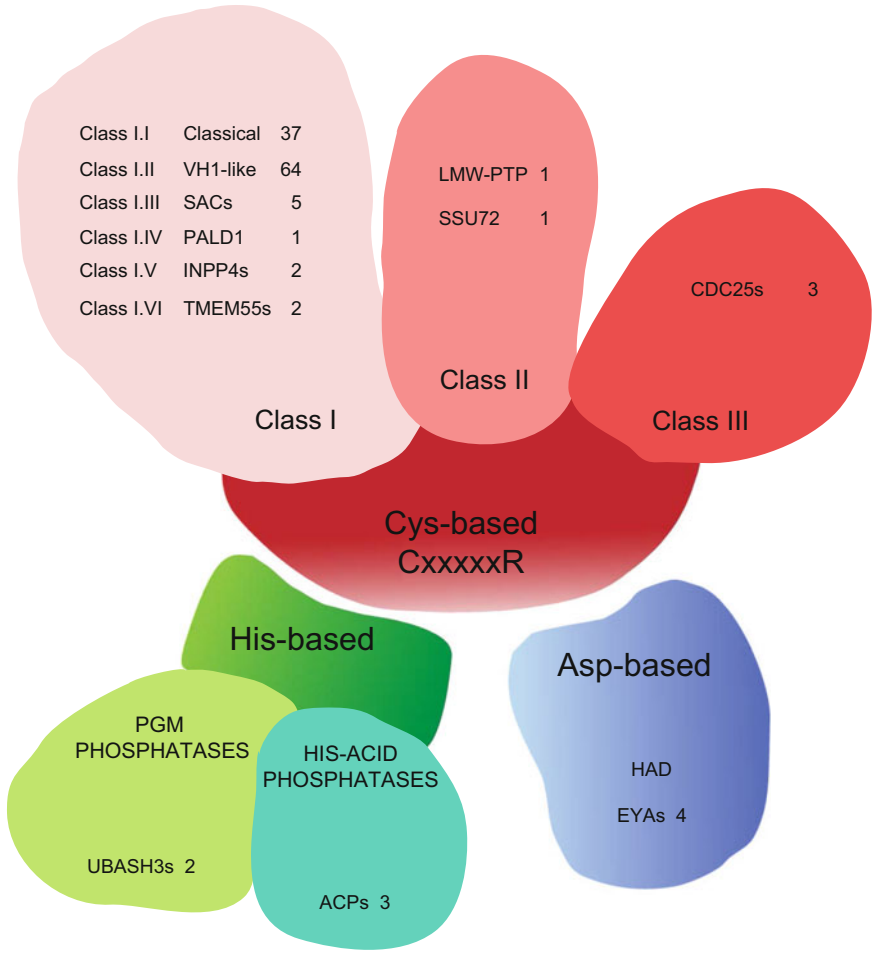

Fig. 1 Scheme of the extended family of Tyr phosphatases (extended PTPome). The classification is based on the nucleophilic catalytic residue (Cys, Asp, or His) and on protein topology. *Numbers* indicate the members included in each group. *See* Table 1 for a complete list of the members of the extended PTPome. *HAD* haloacid dehalogenase, *PGM* phosphoglyceromutase