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Extended Abstract

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Structure-based screening for protein phosphatase-1 interactome mapping

Julia de Vasconcellos Castro

Columbia University Medical Center, USA

E-mail: jd3393@cumc.columbia.edu

Protein phosphatase-1 (PP1) is a prominent member of the Phosphoprotein Phosphatases family, and it catalyzes the majority of Ser/Thr dephosphorylation reactions. This broad range of functions is tightly regulated by its ability to form hundreds of holoenzymes by swapping a variety of regulatory subunits known as PP1-interacting proteins (PIPs). PIPs are seemingly unrelated in sequence and structure, but share a number of PP1-binding motifs (PP1-BMs). This common trait allows PIPs to combine multiple motifs and bind distinctive sites on PP1 surface to assemble unique holoenzymes. Although the majority of known PP1-BMs are unstructured short linear motifs (SLIMs), some are highly structured. Previous PP1 interactome mapping derives from high-throughput techniques combined with bioinformatics approaches that exploit SLIM PP1-BMs in proteome-wide screens based on sequence homology. Even though the number of known structured PP1-BMs is scarce when compared with the number of established SLIMs, it seems reasonable to expect that structural homology of proteins subunits (domains) could also be applied to expand the PP1 interactome. The present study explores structure-based PrePPI predictions to identify new PP1 interactors. PrePPI combines structural information with different sources of non-structural evidence to predict high-confidence interaction models. PrePPI predicts 17 models that recapitulate known holoenzymes and 127 novel interactions between PP1 and 70 putative PIPs. The analysis suggests that several proteins interact with PP1 via their ankyrin repeat domains, a known structured PP1-BM. Similarly, various proteins were proposed to interact with PP1through their PDZ domains in a manner previously unexplored. Most of the predicted PIPs contain established SLIM PP1-BMs, providing support for their physiological relevance, and additional novel PP1-BMs are proposed based on the analysis of the interaction models. This structural approach facilitates the mapping of a more complete PP1 interactome and provides the basis for novel therapeutic approaches to selectively modulate particular signaling cascades. Among posttranslational modifications, reversible protein phosphorylation mediates most of signal transduction pathways in living cells, through the action of protein kinases and phosphatases. These events occur under tight and transient regulation and abnormal phosphorylation mechanisms lead to disorders such as cancer, diabetes, heart failure,and neurological degeneration. Of all Ser/Thr protein phosphatases, Protein Phosphatase 1 (PP1) forms a major class and is highly conserved among all eukaryotes. Three genes are known to encode PP1 catalytic subunits, termed PP1α, PP1β, and PP1γ, with diversity increased by alternative splicing. PP1 regulates a variety of cellular events through the dephosphorylation of multiple substrates and its multifunctionality is due to its association with different regulators and/or targeting subunits known as PP1 Interacting Proteins (PIPs). The PP1 isoforms are highly conserved across their large catalytic domain, but are divergent at the N and C termini. Thus, PIPs bind to the unique C terminus to direct their isoform specific activities. To exert their dephosphorylation reactions that are important in time and space, the diverse functions of PP1 must be independently regulated. For this reason, PIPs are believed to be much more specific for individual functions and are therefore better targets for specific pathways. The large majority of PIPs contain a degenerate, so-called RVxF-motif that conforms to the consensus sequence [R/K]-X0-1-[V/I]-[F/W], where X denotes any residue except proline. This motif binds with high affinity to a hydrophobic channel that is remote from the catalytic site of PP1 . The binding of the RVxF-motif by itself has no major effects on the conformation or activity of PP1. However, RVxF-mediated anchoring of PP1 promotes the occupation of secondary, lower affinity binding sites, and this often does affect the activity and/or substrate specificity of PP1. The RVxF-motif is present in about one-third of all eukaryotic proteins but only a small fraction are PIPs. It seems that RVxF-consensus sequences function as PP1 interaction sites only when they are present in a flexible and exposed loop that can be modeled into a β-strand. Other PP1-binding motifs (PP1-BMs) have been described, F-X-X-R-X-R, present in several PP1 interactors, and the MyPhoNE motif, RXXQ[VIL][KR]X[YW], present in MYPT-1. An additional generic PP1-binding motif was identified, the SILK-motif: [GS]-IL-[KR]. It was first described for I2, a specific PP1 inhibitor. This motif is present in nearly 10% of proteins containing the RVxF-motif and is normally N-terminal to it. The SILK and RVxF-motifs are functionally interchangeable and can both be essential for PP1 anchoring. More recently, work from Bollen and coworkers allowed the redefinition of the RVxF motif and its flanking residues based on the sequences of 143 PIPs: [KRL]-[KRSTAMVHNQ]-[VI]-{FIMYDP}-[FW]. The existence of common binding sites for PIPs explains why a relatively small protein such as PP1 can interact with numerous different regulatory proteins and why the binding of most regulatory subunits is mutually exclusive. The relative abundance of each PP1 isoform may be an important factor in determining the composition of numerous PP1 holoenzymes and the relative contribution of each PP1 isoform to different biological functions. The broad *in vitro* substrate specificity of PP1 leads to the idea that the enzymatic specificity is mainly dictated by the PIPs. Thus, a complete understanding of PP1 function requires the identification of the associated subunits that direct PP1 specific functions, as well as functional analysis of PP1 holoenzymes. A variety of approaches has identified more than 100 mammalian proteins known to interact with PP1.

These PIPs function as inhibitors, substrate specifiers, and substrate targeting proteins, or a combination thereof. Sometimes PP1 interactors are themselves substrates for associated. Given the number of protein phosphatases and phosphoprotein substrates encoded in the human genome, a large number of PIPs surely remain to be discovered. Moreover, relatively little is known about isoform specific PP1 regulators. Recently, we have characterized the human testis PP1γ interactome and have shown that there are isoform tissue-specific PIPs. Some PIPs were identified when PP1γ1 was used as bait while others were only obtained when the bait was PP1γ2. Even more interesting was the fact that the majority of PIPs obtained with a single bait were with the unique C-terminal of PP1γ2. Thus, clearly, there exists a PP1 isoform specificity in what concerns PIPs binding that is highly relevant for PP1 isoform particular function. The majority of the putative PP1 interactions proposed derived primarily from biochemical approaches, high-throughput Yeast Two-Hybrid (YTH) screens, mass spectrometry and *in silico* screenings. The YTH system provides a sensitive method for detecting relatively weak and transient protein interactions. High-throughput YTH screens, which generated most of the binary protein interaction data currently available, are providing samples of complete interactomes. Even though the resulting interaction mapping lacks sufficient coverage and dynamic information for a complete interactome, they greatly increased our knowledge, although understanding the global organization of proteomes is still far from complete.

Bottom Note: *This work is partly presented at* 6th World Congress on NATURAL PRODUCT & SYNTHETIC CHEMISTRY June 24-25, 2019 | New York, USA.