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Review Article

Biogenesis and activity regulation of protein phosphatase 1

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Protein phosphatase 1 (PP1) is expressed in all eukaryotic cells and catalyzes a substantial fraction of phosphoserine/threonine dephosphorylation reactions. It forms stable complexes with PP1-interacting proteins (PIPs) that guide the phosphatase throughout its life cycle and control its fate and function. The diversity of PIPs is huge (≈200 in vertebrates), and most of them combine short linear motifs to form large and unique interaction interfaces with PP1. Many PIPs have separate domains for PP1 anchoring, PP1 regulation, substrate recruitment and subcellular targeting, which enable them to direct associated PP1 to a specific subset of substrates and mediate acute activity control. Hence, PP1 functions as the catalytic subunit of a large number of multimeric holoenzymes, each with its own subset of substrates and mechanism(s) of regulation.

Introduction

Protein phosphatase 1 (PP1) belongs to the phosphoprotein phosphatase (PPP) superfamily of hydrolases [1–3]. It catalyzes the hydrolysis of serine/threonine-linked phosphate monoesters by a nucleophilic attack of the incoming phosphorus atom with a metal-activated water molecule. PP1 increases the reaction rate by a staggering 10^{21}-fold, making it one of the most proficient of all known enzymes [4]. It also ranks among the structurally and functionally most conserved proteins: PP1 from yeast and man shows >80% sequence identity and human PP1 can rescue the lethal phenotype associated with the deletion of PP1 in yeast [5]. PP1 is expressed in all eukaryotic cells at moderately high levels. Human U2OS and HeLa cancer cells, for example, contain ≈250 000 copies of PP1 isoforms (α, β and γ), corresponding to a calculated concentration of ≈0.2 μM [6,7]. Biochemical data indicate that PP1 catalyzes a major fraction of all protein dephosphorylation events in eukaryotic cells and regulates a wide array of processes [8]. Consistent with its pleiotropic action, PP1 displays a broad substrate specificity. However, PP1 is not completely aspecific and shows a substrate preference that is different from that of the other PPP-type phosphatases, namely PP2A, PP2B and PP4–7 [8]. The recently published structure of a PP5–substrate complex sheds some light on the molecular basis of substrate recognition by PPP phosphatases [9]. The side chains of the peptide substrate engage in water-mediated hydrogen bonds with residues in pockets that radiate from the catalytic site and are known as the hydophobic and C-terminal grooves (Figure 1). These pockets are spacious and can accommodate highly divergent sequences, accounting for the sequence plasticity of PPP substrates. Most phosphatase residues that mediate substrate binding are highly conserved among PPP phosphatases. However, a residue that interacts with the substrate −2 position differs between PPP members and therefore probably functions as a substrate-specifying element.

There is no evidence for the existence of cellular pools of unbound PP1. In fact, artificially generated free PP1 causes uncontrolled protein dephosphorylation and results in cell death [10]. Cells prevent the accumulation of unleashed PP1 by expressing PP1-interacting proteins (PIPs) in a large molar excess [3,6,7]. From a biological perspective, it is, therefore, only meaningful to discuss the properties and regulation of PP1 as the catalytic subunit of a large array of multisubunit complexes or holoenzymes. In general, PIPs guide PP1 throughout its life cycle and determine when and where the

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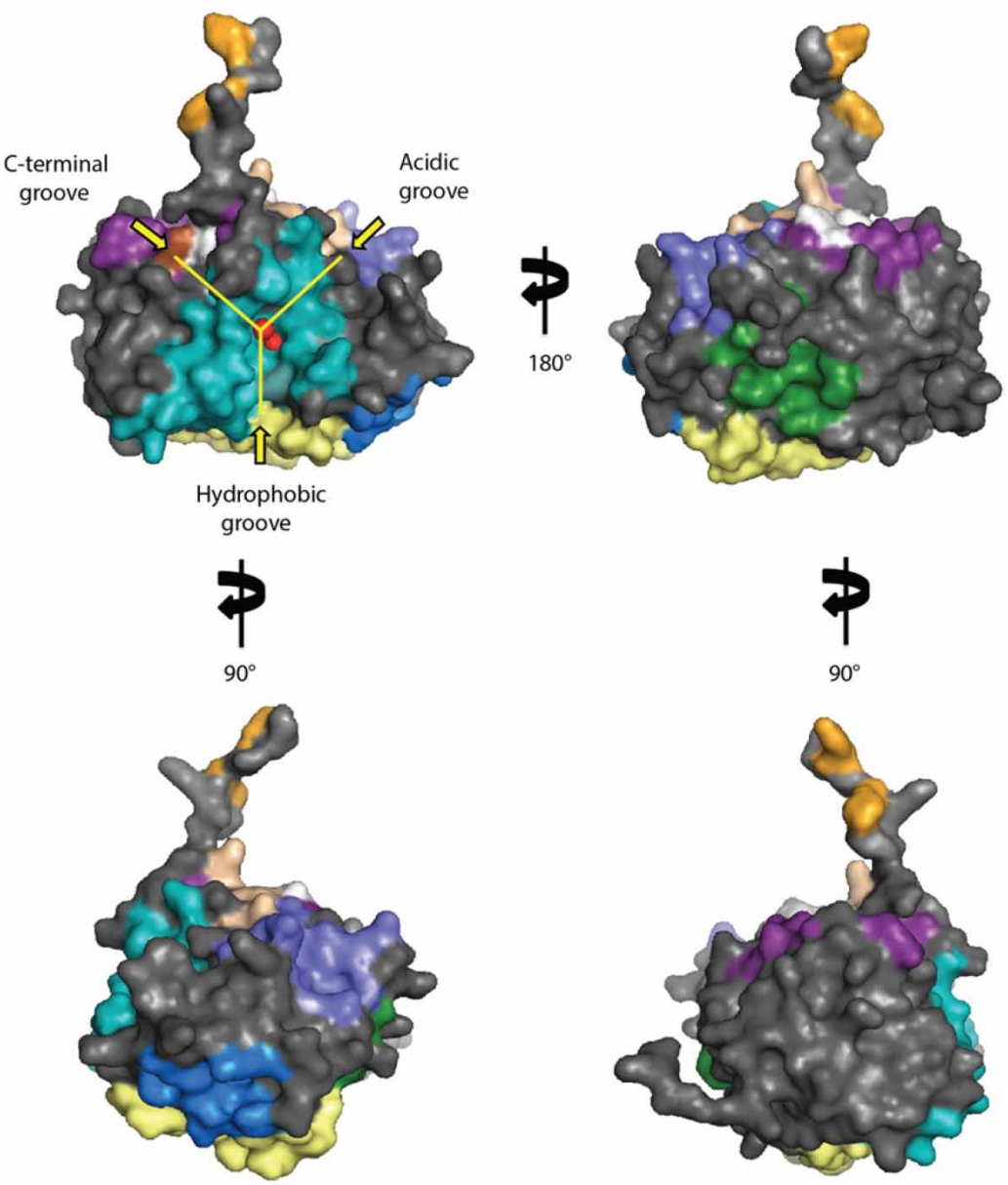

Figure 1. PIP docking sites on PP1.

The figure shows a surface model of four different orientations of mammalian PP1β (PDB 1s70). Indicated are the two metals in the active site (red circles) and the substrate-binding channels that emanate from the active site. The residues of PP1 that mediate binding to SLiMs are colored: violet, RVxF motif; magenta, Ki67–RepoMan SLIM (KiR-SLiM) motif; green, SILK motif; dark blue, myosin phosphatase N-terminal element (MyPHONE) motif; yellow, NIPP1 α-helix motif; wheat, ϕϕ motif; cyan, Inhibitor-2 SLiM for docking at the hydrophobic and acidic grooves (IDoHA); brown, Spinophilin SLiM for docking at the C-terminal groove (SpiDoC). Also shown are the residues in the C-terminus of PP1β that interact with the ankyrin-repeat domain (AnkCap) of MYPT1 (orange). Overlapping binding residues for the SpiDoC, KiR-SLiM and ϕϕ motifs are depicted in white. Figures were made using *PyMOL* (www.pymol.com).

phosphatase acts. In the following sections, we will consecutively describe PP1–PIP interaction modes, the involvement of PIPs in the biogenesis and turnover of PP1, their role in substrate selection and their contribution to holoenzyme abundance and activity regulation. The available data suggest that most PIPs serve a dual function: they restrain PP1 and enable the controlled dephosphorylation of a small subset of PP1 substrates.

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PP1–PIP interaction modes

More than 200 mammalian genes encode validated PIPs [1,3]. Some are ubiquitously expressed (e.g. Inhibitor-2); others show a more restricted expression (e.g. Spinophilin in neurons) or are expressed conditionally (e.g. Ki67 in proliferating cells). Most PIPs have an intrinsically disordered domain of 40–60 residues that mediates binding to PP1 with high affinity, as reflected by K_d values of 5–200 nM [11–16]. These PP1-anchoring domains contain short linear motifs (SLiMs) that dock to surface grooves of PP1 (Figure 1). PIPs typically combine several SLiMs to create an interaction area of 1500–5000 $Å^2$, thereby covering 5–20% of the surface of PP1. Nearly a dozen of PP1-binding SLiMs have already been identified, but it seems likely that additional SLiMs remain to be discovered that bind to surface areas of PP1 that have no known interaction partner (Figure 1). Some of the well-characterized SLiMs are present in many PIPs, but others are less widespread. For example, the RVxF-type PP1-binding motif is shared by 70% of all known PIPs, while the recently discovered KiR-SLiM motif is only found in the nuclear proteins RepoMan and Ki67 [3,14,16].

The diversity and concomitance of PP1-binding SLiMs creates a huge combinatorial potential that has been referred to as the 'PP1-binding code' [1,3,17]. This code enables PIPs to create unique interaction interfaces with PP1 and has particular properties (for references and more details, see ref. [3]). First, the code is *specific* in that PP1-binding SLiMs do not interact with other phosphatases. Second, it is *universal* and applies to all eukaryotes. Third, the code is partially *overlapping* as it excludes combinations of SLiMs that bind to the same PP1 surface residues. Fourth, it is *degenerate*, implying that SLiMs come in sequence variants that differ in their affinity for PP1. Fifth, the code is *nonexclusive*, allowing two PIPs to bind simultaneously to the same molecule of PP1 as long as they have at least one nonshared SLiM. Sixth, it is *dynamic* and tolerates competition between PIPs within and between PP1 holoenzymes for the same binding sites. The elucidation of the SLiM-based PP1-binding code is yielding structural insights that gradually make it feasible to predict the PP1-interaction mode of poorly characterized PIPs. It can also be expected that the obtained insights will inspire investigators to design artificial PIPs that can be used as tools to explore PP1 signaling and its therapeutic potential.

In addition to the SLiMs in PP1-anchoring domains, some PIPs also have PP1-binding SLiMs in regulatory domains. Biochemically well-defined examples are the phosphorylation-regulated PP1-inhibitory SLiMs of Inhibitor-1 and NIPP1 [18,19]. The prevalence and importance of PP1-binding SLiMs in regulatory PIP domains has probably been grossly underestimated, because they contribute little to the overall binding affinity for PP1 and are therefore easily overlooked using classical mapping strategies for PP1-binding domains. SLiMs in PP1-regulatory domains add to the flexibility of the PP1-binding code and are important mediators of acute activity regulation (see below). Further diversification of PP1–PIP interaction modes comes from the existence of highly structured PP1-binding domains. For example, Sds22 has a PP1-binding domain that consists of an array of well-folded leucine-rich repeats [20]. Another example is the ankyrin-repeat domain of MYPT1, which specifically binds to the intrinsically disordered C-terminus of PP1β (Figure 1), accounting for the PP1 isoform binding specificity of this myosin-targeting PIP [11]. Interestingly, recent data show that the PP1 isoform selectivity of some PIPs is not only achieved through interactions with the C-terminus but also through interactions with the structured catalytic domain [16]. Thus, the L1 loop of PP1β/γ is ordered by an arginine-mediated salt bridge (Arg19 for PP1β and Arg20 for PP1γ), making it more available for binding of Ki67 and RepoMan. The corresponding residue of PP1α (Gln20) does not order this pocket, explaining why Ki67 and RepoMan preferentially bind to the β/γ isoforms.

PIPs in the biogenesis and turnover of PP1

At an early point in the PP1 biogenesis process, during or shortly after translation, the metal ions Fe^{2+} and Zn^{2+} are incorporated into the active site to generate a catalytically competent enzyme [21]. Eukaryotes probably have dedicated chaperones for Fe^{2+} and Zn^{2+} loading of PP1 that are absent from bacteria because the latter can express eukaryotic PP1, but erroneously incorporate two Mn^{2+} ions in the active site, even when Fe^{2+} and Zn^{2+} are abundant. The nature of the incorporated metals is important because PP1 with Mn^{2+} in its active site has a manifold lower specific activity than native PP1 and is less specific, as it also dephosphorylates tyrosine residues and even nonprotein substrates [21]. The identity of the putative PP1 metal-loading chaperones is still unknown, but Inhibitor-2 is an excellent candidate (Figure 2). Actually, an *in vitro* reconstituted inactive complex of PP1 and Inhibitor-2, known as the MgATP-dependent phosphatase, can be reactivated by the transient

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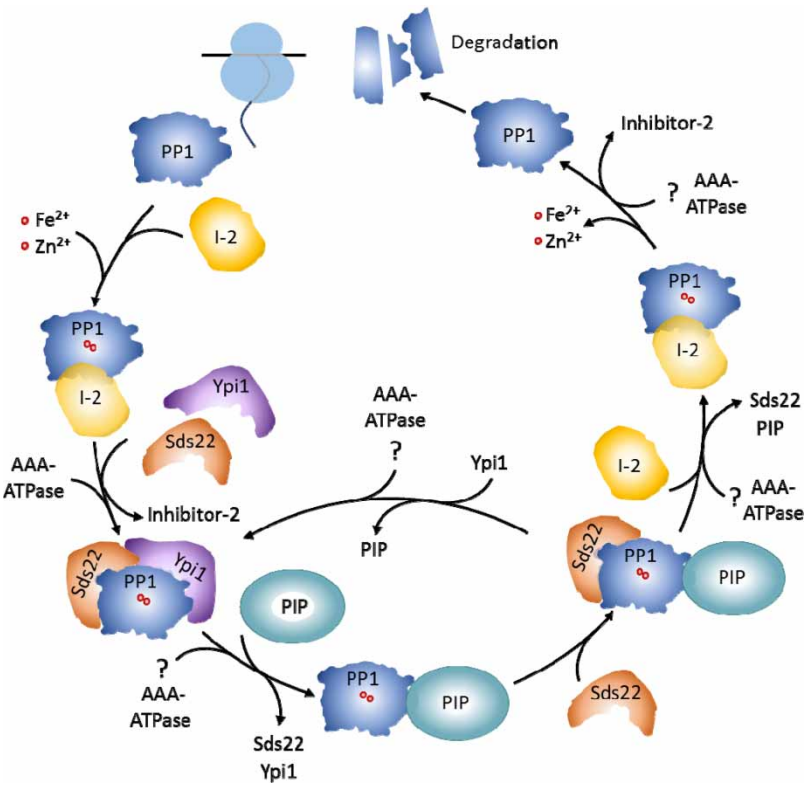

Figure 2. The hypothetical life cycle of PP1.

During or shortly after the translation of PP1, the metals Fe^{2+} and Zn^{2+} are incorporated into the active site by a mechanism that probably involves the transient phosphorylation of Inhibitor-2 by protein kinase GSK-3. Subsequently, PP1 is extracted by an AAA-type ATPase (Cdc48 and the cofactor Shp1 in yeast) to form a soluble, inhibited trimeric complex with Sds22 and Ypi1 (Inhibitor-3 in vertebrates). This complex serves as the source of PP1 for the assembly of PP1 holoenzymes. At least some PP1 holoenzymes can recruit Sds22 as a third subunit. It is suggested that Sds22 mediates the recruitment of an AAA–ATPase to extract PP1 from these holoenzymes, either for (phosphorylation-independent) metal unloading by Inhibitor-2 and its subsequent proteolytic degradation or for recycling to form a trimeric complex with Sds22 and Ypi1/Inhibitor-3.

phosphorylation of Inhibitor-2, and considerable biochemical evidence suggests that this activation process involves the incorporation of metal(s) in the active site (reviewed in ref. [22]).

In yeast, also Sds22 and Ypi1 have been identified as PIPs that are required for an early step in the biogenesis of PP1 [23]. In the absence of functional Sds22 or Ypi1, newly translated PP1 forms aggregates that require the proteasome for clearance. Sds22 and Ypi1 (or its ortholog Inhibitor-3 in vertebrates) form a heterotrimeric complex with PP1, both *in vitro* and *in vivo* (Figure 2) [24,25]. Sds22 and Ypi1 also interact with each other. Intriguingly, the assembly of the Sds22–PP1–Ypi3 complex requires a chaperone complex consisting of the AAA–ATPase Cdc48 and its adaptor Shp1, which transiently binds to Sds22 [23]. Sds22 has a structured PP1-binding domain, but the binding of Ypi1/Inhibitor-3 to PP1 is SLiM-based [3,20]. The combined binding of Sds22 and Ypi1/Inhibitor-3 is expected to cover a large part of the surface of PP1. Ypi1/Inhibitor-3 inhibits PP1 [24,25], while Sds22 stabilizes a partially unfolded form of PP1 [24], hinting at its preferential binding to newly translated, incompletely folded PP1. Hence, Sds22 and Ypi1/Inhibitor-3 probably serve to keep newly synthesized PP1 soluble and inhibited. We speculate that the resulting heterotrimeric complex is used as a source of PP1 for the assembly of functional holoenzymes.

Virtually nothing is known about the mechanisms underlying the formation and turnover of PP1 holoenzymes. Possibly, the biogenesis factors, such as Sds22, Ypi1/Inhibitor-3 and Inhibitor-2, are also implicated in these processes. It is indeed striking that Sds22 can be present as a 'third' subunit in at least some PP1 holoenzymes and that Inhibitor-3 competes with other PIPs for binding to PP1–Sds22 [26]. Does Sds22 in these

complexes serve to recruit an AAA–ATPase complex that extracts PP1 for recycling or degradation (Figure 2)? *In vitro*, Inhibitor-2 removes Fe^{2+} from the active site of PP1 [21] and the bacterially expressed PP1/Inhibitor-2 complex lacks one or both metals [27]. Does Inhibitor-2 remove metals from PP1 once the catalytic subunit is extracted from a holoenzyme and does this represent a key step in its degradation process (Figure 2)?

Sds22, Inhibitor-2 and Ypi1/Inhibitor-3 are the most ancient PIPs [28], suggesting that their functions in the biogenesis and turnover of PP1 are possibly also phylogenetically conserved. Strikingly, the much better studied biogenesis factors of the PPP-type PP2A phosphatase are structurally unrelated to those of PP1, but nevertheless appear to fulfill similar functions. During or shortly after its translation, the catalytic subunit of PP2A also forms a heterotrimeric complex with polypeptides that stabilize its inactive conformation (the α4 protein, similar to Sds22 for PP1) and inhibit its activity (the TIPRL protein, similar to Inhibitor-3 for PP1) [29–32]. Moreover, a chaperone complex (TriC/CCT, similar to AAA–ATPases for PP1) may be involved in the assembly of this complex [30]. In addition, the PP2A interactor PTPA appears to be functionally equivalent to Inhibitor-2 as it has been demonstrated to play a role in the metal loading of PP2A [33].

PIPs as substrate specifiers

Eukaryotic cells contain hundreds if not thousands of distinct PP1 substrates, in various amounts. A key function of PIPs is to limit the action of associated PP1 to a subset of substrates or (transiently) inhibit PP1 altogether. PIPs have evolved multiple strategies to restrain PP1 (Figure 3A). The SpiDoC SLiM of Spinophilin docks to the C-terminal groove of PP1 (Figures 1 and 3A) and sterically hinders the dephosphorylation of substrates that are recruited via this groove [12]. PNUTS occludes the same groove using a different SLiM that has, however, an Arg in common with the SpiDoC motif and was therefore termed the Arg motif [14]. The PP1-anchoring central domain of NIPP1 inhibits the dephosphorylation of many but not all PP1 substrates

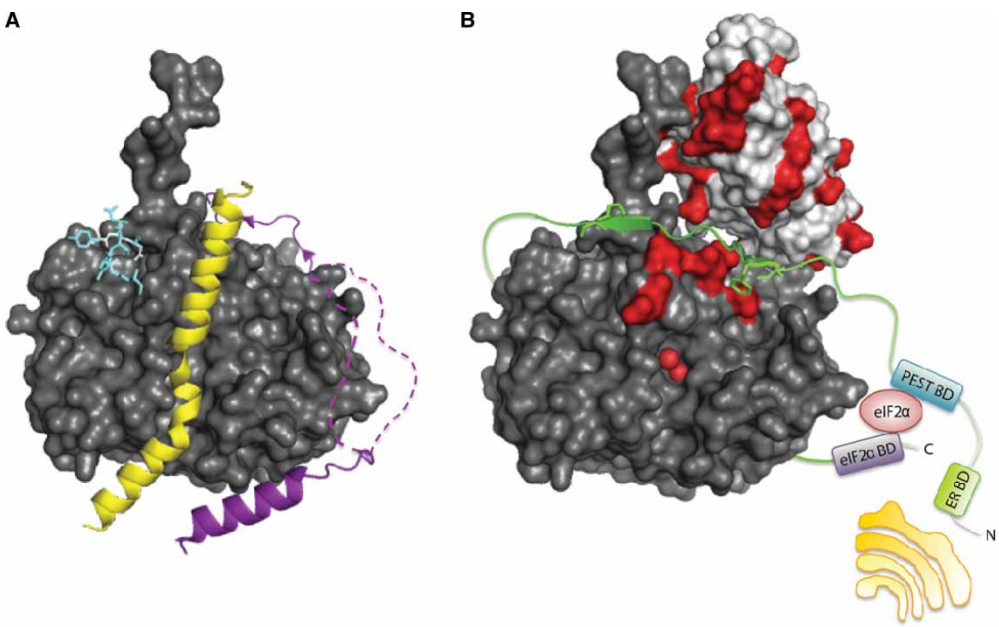

Figure 3. Mechanisms of substrate selection by PP1.

The figure shows strategies for restricted (**A**) and facilitated (**B**) substrate recruitment by PIPs. (**A**) The SpiDoC motif (cyan) sterically hinders the recruitment of PP1 substrates via the C-terminal groove. The IDoHA motif of Inhibitor-2 (yellow) prevents the dephosphorylation of all substrates by occluding the hydrophobic and acidic grooves as well as the active site. A polybasic stretch in the PP1-anchoring domain of NIPP1 (purple) hampers the dephosphorylation of a large subset of substrates through dynamic electrostatic interactions, as suggested by the dotted lines. (**B**) Some ankyrin repeats of MYPT1 (light gray) may promote the binding of a subset of PP1 substrates through extension of the acidic groove (acidic residues highlighted in red). GADD34 (green) promotes the dephosphorylation of eIF2α by providing binding domains (BDs) for the endoplasmic reticulum (ER-BD) and eIF2α itself (PEST-BD + eIF2α BD).

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[19]. A key inhibitory element in this domain was mapped to a polybasic region close to the PP1-binding RVxF-type SLiM. Interestingly, this polybasic stretch of residues was not visible in the electron density map of the PP1–NIPP1 heterodimer, suggesting that it remains flexible in the complex and prevents the dephosphorylation of a subset of substrates through dynamic electrostatic interactions with PP1 (Figure 3A). NIPP1 also has a PP1-regulatory C-terminal domain that prevents the dephosphorylation of all substrates, possibly because it binds at or near the catalytic site [19]. The IDoHA motif of Inhibitor-2 adopts a largely α-helical structure that occupies the acidic and hydrophobic grooves of PP1, but also occludes the active site (Figures 1 and 3A) [27]. In addition, local interactions at the active site cause the displacement of one or both metals. Other PIPs (e.g. Inhibitor-1, CPI-17 and MYPT1) have a PP1-regulatory domain that inhibits PP1 but only when it is phosphorylated [1,18,34,35]. Probably, these PIPs inhibit PP1 by binding as pseudosubstrates.

Many PIPs have also acquired structural features for positive substrate selection (Figure 3B). They often contain a domain that mediates binding to a specific subcellular compartment. This enhances the local concentration of PP1 and thereby promotes the dephosphorylation of resident substrates. PIPs target PP1 to a wide range of subcellular structures [3], including centrosomes (e.g. Cep192), chromosomes (e.g. RepoMan), endoplasmic reticulum (e.g. GADD34), glycogen particles (e.g. PTG), microtubules (e.g. Kif18A), actin (e.g. Spinophilin), myofibrils (e.g. MYPT1), nuclear speckles (e.g. NIPP1), nucleoli (e.g. NOM1) and the plasma membrane (e.g. TIMAP). Some PIPs have multiple subcellular-targeting domains (e.g. a few glycogen-targeting subunits also have a membrane-targeting domain), which enable them to function as signal integrators [1].

Substrate-recruitment domains of PIPs also contribute to substrate selection and dephosphorylation (Figure 3B). This is because substrates bind relatively poorly to PP1 itself (K_m in the micromolar range, often far above the cellular concentration of the substrate), but their binding affinity and dephosphorylation rate are massively increased if the associated PIP contains an additional substrate-binding site. Examples of substrate-recruitment domains are the ForkHead-associated (FHA) domain of NIPP1 and two eIF2α-binding elements of GADD34 [13,15,36]. Subcellular-targeting and substrate-recruitment domains may be different or the same. Thus, the targeting of NIPP1 to the nuclear speckles is mediated by its substrate-binding FHA domain [37], but GADD34 has distinct binding domains for the endoplasmic reticulum and eIF2α (Figure 3B) [15,36,38]. Some PIPs may simply enhance the affinity for a subset of substrates by extending a substrate-binding groove of PP1. For example, some ankyrin repeats of MYPT1 lengthen the acidic groove of PP1, which has been suggested to promote the recruitment of a subset of substrates (Figure 3B) [11].

Determinants of PP1 holoenzyme abundance

PIPs compete with each other for binding to the limited cellular pool of PP1 (Figure 4). This is nicely illustrated by repeated observations that the overexpression of a single PIP results in a reduced association of PP1 with endogenous PIPs (Figure 4A) [39–41]. The measles virus escapes sensing by the host cell using a similar competition strategy. Indeed, the viral V protein titrates PP1 away from the sensor protein MDA5, thereby preventing its PP1-mediated activation [42]. Similarly, a prolonged unfolded protein response triggers the assembly of the PP1–GADD34 complex at the endoplasmic reticulum [43]. This reduces the nuclear accumulation of PP1, resulting in the hyperphosphorylation of the Hippo signaling effector Yap and apoptosis. There are also examples of competition between PP1 and other signaling molecules for binding to overlapping PIP-binding sites (Figure 4B). Thus, PP1 and cyclin-dependent kinases compete for an overlapping binding motif on the retinoblastoma protein [44]. Likewise, PP1 and protein tyrosine phosphatase Shp1 compete for binding to Spinophilin [45].

Since the global cellular level of PP1 is kept more or less constant during the cell cycle [46,47], its distribution between PIPs is determined in the first place by the relative abundance of PIPs. Numerous data show that the concentration of PIPs is tightly regulated at multiple levels. Their expression is regulated in a cell-type (e.g. glycogen-targeting G-subunits, [48]) or cell-cycle (e.g. PNUTS, [49]) dependent manner, but can also be induced by specific stimuli (e.g. GADD34 by stress signals, [15]). In addition, the level of PIPs can be adjusted post-translationally through regulated proteolysis by caspases (e.g. Inhibitor-3, [50]), the proteasome (e.g. MYPT1, [51]) or lysosomes (e.g. glycogen-targeting R6 [52]) (Figure 4C). The abundance of specific PP1–PIP complexes is also affected by the binding affinity of the components, which is subject to regulation (Figure 4D). Many PIPs show a reduced affinity for PP1 after phosphorylation of residues in or near PP1-binding SLiMs in PP1-anchoring domains (e.g. RepoMan, CENP-E and KNL1 in the first half of mitosis [16,53–55]). Conversely, microtubule binding by the spindle- and kinetochore-associated (SKA) complex possibly serves to promote the recruitment of PP1 by SKA [56].

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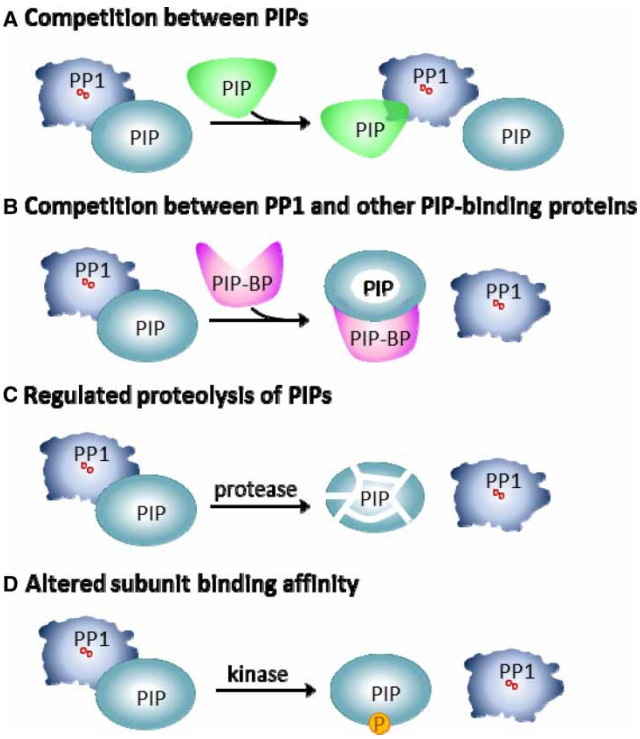

Figure 4. Regulation of PP1 holoenzyme abundance.

The concentration of PP1 holoenzymes is regulated by different mechanisms. (**A**) PIPs compete with each other for binding to PP1. (**B**) PP1 can compete with other PIP-binding proteins (PIP-BPs) for binding to PIPs. (**C**) The cellular abundance of PIPs can be modulated post-translationally by proteolysis. (**D**) The binding affinity of PIPs for PP1 is regulated by phosphorylation (P).

Acute activity regulation of PP1 holoenzymes

Most if not all PP1–PIP complexes, similar to the PPP phosphatases PP2B and PP5 [9,57], are (largely) inactive under basal circumstances. Phosphatase assays have indeed revealed that a majority of bacterially expressed PP1-anchoring domains are inhibitory [40]. In addition, many PIPs also have a PP1-regulatory domain that is inhibitory under basal conditions [18,19]. Furthermore, at least some PP1 holoenzymes can recruit inhibitory proteins (e.g. Inhibitor-1 and CPI-17) as a second regulatory subunit [58–60]. If PP1–PIP complexes are mostly kept inactive in the cell, specific signaling mechanisms must exist for their transient and controlled (in) activation. It indeed appears that multiple, holoenzyme-specific strategies have evolved for acute phosphatase activity regulation (Figure 5), although the underlying molecular mechanisms are often still poorly understood.

Some PP1 holoenzymes are activated by the (de)phosphorylation-regulated release of an inhibitory subunit (e.g. CPI-17, [35]) or dissociation of an inhibitory SLiM (e.g. NIPP1, [19]; Figure 5A,B). Other holoenzymes are activated by the transfer of PP1 from an inhibitory to an activatory PIP within the same complex. For example, the dephosphorylation of protein kinase Raf by the SHOC2–PP1–SCRIB complex is regulated by internal competition between the inhibitory SCRIB and activatory SHOC2 for binding to PP1 (Figure 5C) [61]. Activation of PP1–PNUTS requires both its recruitment to target genes via non-coding RNAs and the reversal of PP1 inhibition through binding of PNUTS to acetylated histones [62]. Other (in)activation mechanisms target the catalytic subunit itself. GADD34 can recruit the NADPH oxidase 4, which inhibits GADD34-associated PP1 via oxidation of active site metal(s) [63]. It is not clear whether such oxidation is reversible but if it is, this would be an elegant mechanism for acute activity regulation. In addition to metal oxidation, metal (un)loading by Inhibitor-2 may also represent an efficient mechanism for the transient (in)activation of PP1 holoenzymes (Figure 5D) [22]. Finally, the inhibitory phosphorylation of the C-terminus of PP1 by cyclin-dependent kinases can be reversed by autodephosphorylation, which appears to be modulated by Inhibitor-2 (Figure 5E) [64].

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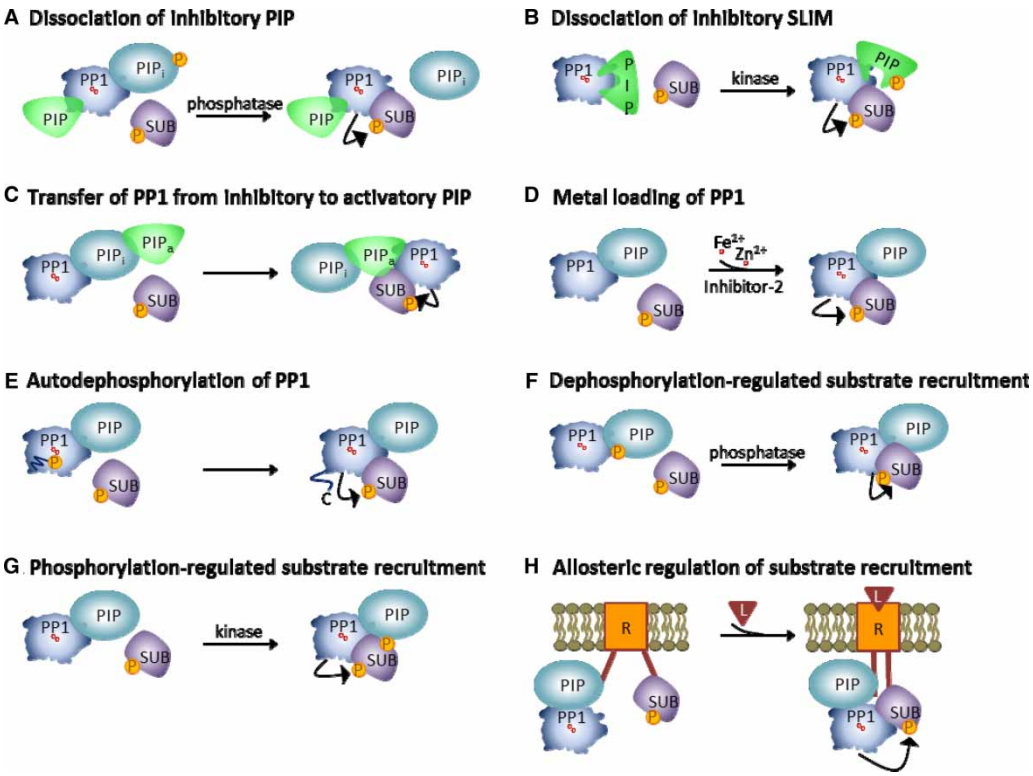

Figure 5. Activation mechanisms of PP1 holoenzymes.

Different modes of PP1 holoenzyme activation are depicted. (**A**) Dephosphorylation of an inhibitory PIP causes its dissociation and activation of a PP1 holoenzyme. (**B**) Phosphorylation-dependent dissociation of an inhibitory SLiM activates a PP1 complex. (**C**) A PP1 holoenzyme can be activated by transfer of PP1 from an inhibitory to an activatory PIP. (**D** and **E**) Targeting of the catalytic subunit of PP1 itself can modulate activation, for example, by metal loading of PP1 (**D**) or by autodephosphorylation of an inhibitory site in the C-terminus of PP1 (**E**). (**F**) The phosphorylation state of PIPs determines their binding affinity for substrates. (**G**) Substrate recruitment can depend on its prior phosphorylation state. (**H**) Ligand binding to a receptor complex can induce conformational changes that bring PP1 within reach of its substrate. P, phosphorylation; SUB, substrate; i, inhibitory; a, activatory; R, receptor; L, ligand.

Another type of PP1 holoenzyme regulation concerns substrate recruitment. Phosphorylation of PIPs has been associated with an altered substrate-binding affinity (e.g. binding of phospholamban to glycogen-targeting G_M in the heart [65]; Figure 5F). Sometimes, substrate recruitment depends on covalent modifications or allosteric regulation of the substrates themselves. For example, the FHA domain of NIPP1 only binds substrates for dephosphorylation by associated PP1 when they are phosphorylated on a threonine that is followed by a proline (Figure 5G) [66]. The recruitment of the glycogen-degrading enzyme phosphorylase *a* by the liver-specific PP1–G_L phosphatase is enhanced by the glucose-induced acetylation of phosphorylase *a*, which increases its affinity for the substrate-binding site of G_L [67]. Interestingly, the G_L subunit also has a second, higher affinity binding site for phosphorylase *a*, and the occupation of this site allosterically prevents the dephosphorylation (and activation) of glycogen synthase by PP1–G_L [68]. This substrate-level control mechanism serves to prevent glycogen synthesis as long as the glycogenolytic phosphorylase *a* is present. Yet, another mechanism of substrate-recruitment regulation relates to conformational changes within a preexisting complex. For example, ligand binding to the NMDA–receptor complex induces conformational changes that bring PP1 within reach of its substrate protein kinase CaMKII (Figure 5H) [69].

Conclusions

The ubiquitous expression and low *in vitro* substrate specificity of PP1 originally led to the widespread belief that it is a constitutively active phosphatase that only serves to end kinase signaling. This is clearly a

misconception as it is now firmly established that PP1 forms stable complexes with a large variety of PIPs that direct the phosphatase to a small subset of substrates and tightly regulate its activity. PP1 holoenzymes have turned out to be as specific and tightly regulated as any protein kinase. Recently acquired insights into PP1–PIP interaction modes and mechanisms of activity regulation and substrate recruitment offer exciting perspectives for the development of PP1 holoenzyme-specific small-molecule inhibitors or activators that can be used therapeutically. Once named an ugly duckling [70], PP1 has truly become a beautiful swan.

Abbreviations

FHA, ForkHead associated; PIPs, PP1-interacting proteins; PP1, protein phosphatase 1; PPP, phosphoprotein phosphatase; SKA, spindle- and kinetochore-associated; SLiMs, short linear motifs.

Competing Interests

The Authors declare that there are no competing interests associated with the manuscript.

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