Binding of the Concave Surface of the Sds22 Superhelix to the α4/α5/α6-Triangle of Protein Phosphatase-1*

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† The abbreviations used are: PP1, protein phosphatase-1; BLAST, basic local alignment search tool; CC, cysteine containing; EGFP, enhanced green fluorescent protein; LRR, leucine-rich repeat; RI, ribonuclease inhibitor; X-gal, 5-bromo-4-chloro-3-indolyl-β-D-galactoside.

Functional studies of the protein phosphatase-1 (PP1) regulator Sds22 suggest that it is indirectly and/or directly involved in one of the most ancient functions of PP1, i.e. reversing phosphorylation by the Aurora-related protein kinases. We predict that the conserved portion of Sds22 folds into a curved superhelix and demonstrate that mutation to alanine of any of eight residues (Asp168, Phe170, Glu182, Phe214, Asp280, Glu289, Trp302, or Tyr327) at the concave surface of this superhelix thwarts the interaction with PP1. Furthermore, we show that all mammalian isoforms of PP1 have the potential to bind Sds22. Interaction studies with truncated versions of PP1 and the yeast PP1-like protein phosphatase Ppz1 suggest that the site(s) required for the binding of Sds22 reside between residues 43 and 173 of PP1γ. Within this region, a major interaction site was mapped to a triangular region delineated by the α4-, α5-, and α6-helices. Our data also show that well known regulatory binding sites of PP1, such as the RVXF-binding channel, the β12/β13-loop, and the acidic groove, are not essential for the interaction with Sds22.

Among the protein phosphatases that occur in all studied eukaryotic lineages, the Ser/Thr-specific protein phosphatases of type-1 are the best conserved, with more than 70% of their residues nearly invariant (1). This conservation extends well beyond structurally and catalytically important residues to include exposed residues involved in the binding of regulatory proteins. As a catalytic subunit, PP11 depends on the interaction with one or two regulatory subunits for subcellular localization, substrate specificity, and activity regulation (1, 2). Eukaryotic cells contain a large variety of regulatory subunits of PP1, which account for the diversified action of this phosphatase. We have recently proposed that PP1 acquired an essential function during early eukaryotic evolution by the development of sites for interaction with a primate regulatory subunit(s) (1). This essential primordial function and the sequential acquirement of additional interaction sites and functions would then have impeded further mutation of the corresponding portion(s) of the surface. The phylogenetic distribution of PP1 indicates that this primordial function must have been acquired before the divergence of the extant eukaryotic lineages. One of the most ancient functions of PP1 is to dephosphorylate substrates of the Aurora-related protein kinases, and this is essential for the completion of mitosis (3). The regulatory subunit(s) associated with this function of PP1 remain unknown, but the protein Sds22 (38 kDa) has emerged as a prime candidate. First, both yeast and mammalian Sds22 have been shown to interact with PP1 and to be part of a complex with PP1 that is enriched in the nucleus (4–7). Second, the Sds22 encoding gene was identified independently in fission and in budding yeast as an extra-copy suppressor of the temperature-sensitive mitotic arrest phenotypes that are associated with certain mutations of PP1 (4, 5, 8). Deletion of the Sds22-encoding gene caused a similar mitotic arrest, and this phenotype could be complemented by the overexpression of PP1 (4, 5, 8). Third, the conditionally lethal phenotype in budding yeast that was conferred by a loss-of-function mutation of the Aurora-related kinase Ipl1 (Ipl1–2) was largely relieved by the expression of certain temperature-sensitive mutant versions of Sds22 or PP1 (9, 10). The mutant Sds22 version that rescued the Ipl1–2 phenotype showed a decreased ability to interact with PP1. The expression of this mutant Sds22 did not affect the cellular levels of PP1 or Sds22, but drastically reduced the nuclear level of PP1 and caused a redistribution of the nuclear pool of PP1 (9).

Hitherto, little is known about the mechanism of interaction between Sds22 and PP1. Most known PP1 regulators contain a so-called “RVXF” motif with the consensus sequence [RK][X]0−1,[V/I][X][FW], which binds to a hydrophobic channel of the catalytic subunit (1, 11). Sds22 lacks an RVXF motif but consists of a tandem array of leucine-rich repeats (LRRs), which are established protein interaction modules (12). In this paper, we demonstrate that the LRR-repeats of Sds22 are indeed essential for binding to PP1 and we propose that the LRRs assume the conformation of a curved superhelix ending in a C-terminal so-called LRR cap (13). Guided by this three-dimensional model and by the crystal structure of PP1, we have been able to map determinants of the Sds22-PP1 interaction at the concave surface of the Sds22 superhelix and in a triangle composed of α-helices 4, 5, and 6 of PP1.
Binding of Sds22 to the a41/a5/a6-Triangle of Protein Phosphatase-1

EXPERIMENTAL PROCEDURES

Data Base Searches and Structural Modeling—BLAST searches (14) were launched via the web-interface of the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov/BLAST). Protein-structure files were obtained from the European mirror site of the Protein Data Bank (pdb.ccdc.cam.ac.uk/pdb/). For structural analysis and the modeling of the backbone of Sds22, Windows versions 3.53 and 3.7 of the DeepView/Swiss-PdbViewer program (15) were used. The side-chains were added and energy-minimized using the dead-end elimination method (16) run on a dual processor Octane 2 work station (Silicon Graphics). The scene represented in Fig. 5A was constructed in DeepView and rendered with version 3.1 of the program POV-Ray. Fig. 2 was produced using version 1.0 of the ICM lite program (MolSoft).

Plasmid Construction—The full-length and truncated versions of the coding sequence of human Sds22 were PCR-amplified using Pwo polymerase (Roche Molecular Biochemicals) and introduced between the NcoI and BamHI sites of the pACT-II vector (17) at the 3' end of a cassette encoding the transcription-activation domain of Gal4. Similarly, the full-length and truncated coding sequences of rabbit PP1 and rat PP1γ, and budding yeast Ppz1 were subcloned in the NdeI and BamHI sites of the pAS-2 vector (18), downstream of a cassette encoding the DNA-binding domain of Gal4. The PP1/Ppz1 chimeras were constructed by consecutive introduction of a fragment from one parent molecule between the Smal and BamHI sites of pAS-2 and of the complementary fragment from the other parent molecule between the NdeI and Smal sites.

The QuikChange protocol (Stratagene) was applied for site-directed mutagenesis. Human Sds22-T356A was used as the parent plasmid for the introduction of other point mutations in Sds22 because substitution of alanine for Thr356 in full-length Sds22 occasionally yielded a moderately enhanced interaction with PP1. PP1γ-Δ286–323 was chosen as the parent plasmid for site-directed mutagenesis of PP1 since disruption of the RVXF-binding channel dramatically improved the interaction with Sds22. The construction of pAS-2-based plasmids carrying wild-type and mutant alleles of the budding yeast PP1 gene (GLC7) has been described elsewhere (19). All mutations were verified by DNA sequencing, and the expression of the mutant versions of Sds22 and PP1 was confirmed by Western analysis in crude yeast lysates.

For expression in mammalian cells, wild-type, and mutated coding sequences of Sds22 were cloned in the BamHI site of a pSG5 vector (Stratagene) with a triple FLAG tag cassette inserted in its EcoRI site. The full-length or truncated coding sequences of rabbit PP1 and PP1β/δ and of rat PP1γ were introduced between the XhoI and BamHI sites of pEGFP-C1 (Clontech), downstream of the enhanced green fluorescent protein (EGFP) cassette.

Yeast Two-hybrid Assays—The Y190 reporter strain was transformed with the bait and prey vectors using a lithium acetate transformation protocol adapted as described in Ref. 20. Briefly, a 350-ml culture of the reporter strain was harvested from agar plates with the appropriate synthetic dropout medium in 1 ml of 10 mM Tris-HCl and 1 mM EDTA at pH 7.5. After sedimentation of the cells by centrifugation (5 min at 16,000 × g), the cells were lysed by a 10-min incubation on ice with an equal volume of 0.1× KOH solution.

Cos-1 cells were grown in Dulbecco’s modified Eagle’s medium with 10% fetal calf serum. FuGENE 6 (Roche Molecular Biochemicals) was used for transfection with mammalian expression vectors encoding FLAG-labeled Sds22 and an EGFP-PP1 fusion protein. 48 h after transfection, the cells were washed twice with ice-cold phosphate buffered saline and lysed in 50 ml Tris/HCl at pH 7.5, 0.5 mM dithiothreitol, 0.5% Triton X-100, 0.3 M NaCl, 0.5 mM phenylmethylsulfonyl fluoride, 0.5 mM benzamidine, and 5 μM leupeptin. After centrifugation (5 min at 5000 × g), the FLAG-tagged (mutated versions of) Sds22 in the supernatant were immunoprecipitated with the monoclonal anti-FLAG antibodies and protein-G-Sepharose (Amersham Biosciences). The immunoprecipitates were washed twice in Tris-buffered saline containing 0.5% Igepal CA-630 (Sigma) and twice in pure Tris-buffered saline.

After addition of Laemmli loading buffer to the lysates or immunoprecipitates and boiling, the samples were separated by SDS-PAGE and electroblotted onto polyvinylidene difluoride membranes. Enhanced chemiluminescence (PerkinElmer Life Sciences) was used for the visualization of epitopes after incubation with the appropriate primary and peroxidase-labeled secondary antibodies.

RESULTS

Optimization of the Detection of Interaction between Sds22 and PP1—Because the yeast two-hybrid system has been successful in demonstrating the interaction between Sds22 and PP1 in yeast (5), we decided to use this technique for the mapping of the interaction sites involved. Preliminary experiments showed that human Sds22 (Sds22-WT) interacted rather weakly with full-length yeast or mammalian PP1 isoforms (Fig. 1). We wondered whether this weak interaction could be accounted for by the sequestration of the PP1 hybrid by endogenous yeast PP1 regulators. Because Sds22 lacks a canonical PP1-binding RVXF sequence, we reasoned that disruption of the RVXF-binding channel of PP1 might improve the interaction with Sds22 by eliminating competition with endogenous RVXF-containing regulators. To test this hypothesis, PP1γ1 was truncated just before (PP1γ1-Δ286–323) and just after (PP1γ1-Δ297–323) the last β-strand, β14, which contributes half of the residues that line the RVXF-binding channel (11).
As expected, the interaction signal of PP1γ₁-Δ297–323 was roughly equal to that of wild-type PP1γ₁, whereas PP1γ₁-Δ286–323 interacted much more strongly with Sds22 (Fig. 1). PP1γ₁-Δ286–323 also failed to interact with the yeast protein Gac1, an RVXF-containing glyogen targeting subunit of PP1 (not shown). Collectively, these observations indicate that it is not the deletion of the flexible C terminus of PP1 but the disruption of the RVXF-binding channel that improves the detection of interaction between Sds22 and PP1. Therefore, PP1γ₁-Δ286–323 was used for the further mapping of PP1-binding residues of Sds22 and served as the starting point for further mutational studies of PP1.

**Mapping of Sds22-binding Sites of PP1**—A first issue to address was whether or not the interaction with Sds22 is isofrom- or subtype-specific. The PP1α-subtype encompasses three of the four mammalian PP1 isoforms, i.e. PP1α, PP1γ₁, and PP1γ₂ (21). The latter two are products from a single gene and differ only in their C terminus. The fourth isoform, PP1β/δ, makes up the β-subtype. Interestingly, some regulatory subunits are selectively associated with a particular PP1 isoform or subtype, such as the PP1β/δ-specific myosin-targeting subunits (22) or the α-subtype-specific neurabin (23). Thus far, the only mammalian PP1 isoform that has been shown to interact with Sds22 is PP1γ₁ (24). We found that human Sds22 interacted with PP1α and PP1γ₁ in a two-hybrid assay (Fig. 1). Unexpectedly, after double transformation of the yeast with pAS-2-PP1β/δ and either pACT-II-Sds22-WT or empty pACT-II no colonies were obtained. This observation suggests that the constitutive expression of PP1β/δ in yeast is lethal, possibly due to the uncontrolled dephosphorylation of critical substrates and/or the sequestration of essential endogenous PP1 regulators. Intriguingly, transformants expressing the same PP1β/δ hybrid in conjunction with a Gac1 hybrid were viable, indicating that the interaction with Gac1 neutralized the toxicity of PP1β/δ. C-terminally truncated versions of all mammalian PP1 isoforms (PP1α-Δ286–330, PP1β/δ-Δ285–327, and PP1γ₁-Δ286–323) gave a similarly strong interaction signal, which was enhanced in comparison with that of the wild type (Fig. 1). Moreover, fusions of full-length PP1α, PP1β/δ, and PP1γ₁ to EGFP all co-sedimented with FLAG-tagged Sds22 (see below), which leads to the conclusion that Sds22 can bind to all mammalian PP1 isoforms. In accordance with previous reports on the activity of the mammalian and yeast PP1 holoenzymes that contain Sds22 (7, 25), Sds22-associated PP1 showed little or no spontaneous phosphatase activity with glycogen phosphorylase as a substrate (not shown).

Using PP1γ₁-Δ286–323 as a starting point, we then set out to evaluate the effect of additional deletions on the ability to bind Sds22. N-terminal deletions proved to be ill tolerated; loss of the first nine amino acids (PP1γ₁-Δ1–9/Δ286–323) already resulted in a drastic reduction of the interaction signal, and amputation of the N-terminal 12 or 40 residues (PP1γ₁-Δ12/Δ286–323 and PP1γ₁-Δ1–40/Δ286–323) abolished interaction altogether (Fig. 1). This suggests that the N terminus of PP1 is required for the binding of Sds22, either as a site for interaction or for consolidation of the required structural conformation. By contrast, the C-terminal removal of an additional 16 or 37 residues, yielding PP1γ₁-Δ270–323 and PP1γ₁-Δ249–323, respectively, had no appreciable effect (Fig. 1), which rules out the β12/β13-loop (Fig. 2) as an essential Sds22-binding site. The latter loop has been reported to intervene in the binding of other PP1 regulators, such as Inhibitor 2 and NIPP1 (26). PP1γ₁-Δ249–323 moreover lacks an entire flank of the acidic groove (Fig. 2), another interaction site of PP1 that has been proposed to accommodate a basic stretch of the PP1 regulators Inhibitor 1 and DARPP-32 (27). Considering that residues 41–269 of PP1α have been shown to constitute the minimal fragment that retains phosphatase activity (28), these results also indicate that catalytic activity is no requirement for the binding of Sds22.

Further C-terminal deletions of PP1, such as in PP1γ₁-Δ208–323 and PP1γ₁-Δ174–323, were inconclusive as fusions of the Gal4 DNA-binding domain, and these PP1-fragments already induced reporter gene activity in the absence of the Sds22 hybrid. However, following their expression as tagged proteins in COS-1 cells, EGF/T-fused PP1γ₁-Δ208–323 and PP1γ₁-Δ174–323, like wild-type PP1γ₁ and PP1γ₁-Δ286–323, co-precipitated from the cell lysates with FLAG-tagged Sds22 (data not shown). To corroborate these data, two chimeric proteins were constructed that combine one-half of PP1γ₁ with its complementary half of the catalytic domain of Ppz1. The latter is a type-1-like protein phosphatase from budding yeast that comprises an N-terminal regulatory domain and a catalytic domain. The catalytic domain of Ppz1 is 67% identical to the conserved portion of PP1 (Fig. 3). Several regulators of PP1, such as Inhibitor 2 and Inhibitor 3, also interact with Ppz1 (29). However, we could not detect any interaction between the catalytic domain of Ppz1 (Ppz1-Δ1–360) and Sds22 (Fig. 1). The chimeric protein Ppz1-361-528/Ppz1γ₁-174–323 also failed to bind Sds22, but in agreement with the co-precipitation results its counterpart Ppz1γ₁-1–173/Ppz1-529–692 yielded a strong interaction signal (Fig. 1). A third chimeric protein, consisting of the 39 N-terminal residues of the catalytic domain of Ppz1 fused to residues 43–323 of PP1γ₁ (Ppz1-361-399/PP1γ₁-42–323), interacted equally well as did wild-type PP1γ₁ (Fig. 1). Collectively, these results suggest that the essential Sds22-binding site(s) reside between residues 43 and 173 and, consequently, that the presence of the N terminus of PP1 may be required mainly for conformational reasons. Surprisingly, 79% (104/131) of the residues in the essential fragment of PP1γ₁ are identical in Ppz1, which is substantially more than the 67% identity overall (Fig. 3). Nevertheless, the 27 differing residues apparently prohibit binding of Sds22 to the catalytic domain of Ppz1.
phenotype (19), no viable transformants were recovered that interact with Sds22 in the two-hybrid system. The corresponding residues lie between the yeast counterparts of Lys111 and Lys131, which are typically 22 amino acids, as already reported for yeast and mammalian Sds22 (4, 32). However, the previous delineation of the LRRs in Sds22 was based on the limited amount of insight into the LRR-architecture that were obtained from crystal and NMR structures of LRR-containing proteins (33, 34). Fitting of the redefined LRR-profile onto the Sds22 sequence shifts the earlier described 11 LRRs to the C terminus by 8 residues and reveals a twelfth, incomplete C-terminal repeat that ends in a largely conserved C terminus (Fig. 4). The latter represents a so-called LRR-cap motif of 16–17 amino acids, which also terminates the array of LRRs in many other LRR-containing proteins (13).

Crystal structures of LRR proteins show that each LRR adopts a hairpin-like conformation. The first or consensus fragment of the repeat corresponds to a β-strand of the form LXXLXL, followed by a sharp turn of the form XX[N/C]XXL, which is stabilized by the hydrogen-bonded Asn or Cys side-chain. The second fragment of the LRRs is much more variable. Generally consists of 9 to 18 residues that form a helicaloid fragment and two loops that connect the helicaloid fragment with the flanking consensus fragments. Convenienently, the sequence and length of the variable C-terminal part of the repeats has been used to discern at least six distinct classes of LRRs (33, 34). All the repeats in an array stack as turns of a curved superhelix with a parallel β-sheet at the concave side and the helicoidal fragments at the convex side. The side-chains of the hallmark Leu (or Ile) residues make up the hydrophobic core of the protein. In several LRR-proteins, the C-terminal end of the hydrophobic core is shielded from the solvent by an LRR-cap (13). The Asn and Cys side-chains at the [NC] position form a hydrogen-bonded ladder that prevents contact of the buried hydrophobic side-chains with polar main chain moieties.

Two classes of LRRs, the ribonuclease inhibitor-like (RI-like) and the cysteine-containing (CC) repeats, are clearly distinct from the combined remaining classes. RI-like and CC repeats tend to be longer than repeats from other classes and often have a Cys residue at the [NC] position, whereas this position is invariably occupied by an Asn residue in the other classes. Interestingly, these observations correlate with a more pro...
The arrangement of the 12 redefined LRRs of Sds22. The LRR-cap sequence is underlined and the boxes in thick and thin lines encompass the residues conserved in LRRs in general and in Sds22 repeats, respectively.

The conservation of the consensus residues among Sds22 repeats in general points at a structural role for these residues, which was considered in the modeling of Sds22. On the other hand, residues that are conserved in a particular Sds22 repeat most likely serve a functional purpose. Strikingly, these residues are almost exclusively exposed at the concave surface of the LRR superhelix (Fig. 5B). As described below, we could indeed experimentally confirm that the prime PP1 interaction site of Sds22 corresponds to the region that could be modeled confidently in terms of general architecture and curvature.

Mapping of PP1-binding Residues of Sds22—Using the C-terminally truncated PP1(1-4286-323) in yeast two-hybrid assays, we first explored the importance of the ill-conserved N terminus of Sds22 in the interaction with PP1. A truncated version of fission yeast Sds22 that lacked the ill-conserved N terminus and the first half of the first LRR, has been reported to co-precipitate with PP1 (6). Using a similarly truncated version of human Sds22 (Sds22Δ1-88), we confirmed that the conserved C-terminal three-fourths of Sds22, i.e. the LRR-array and the LRR-cap, suffice for the interaction with PP1 (not illustrated). However, the N-terminally truncated Sds22 interacted considerably weaker with PP1 than did wild-type Sds22.

To study the participation of the concave surface of the Sds22 superhelix in the binding to PP1, 36 exposed residues of repeats 2–12 were separately targeted for mutation to alanine, or in the case of Asp/Asp to valine, and the effect of these mutations on the interaction with PP1 was evaluated by two-hybrid assays (Fig. 5B). The mutation of Asp148, Glu192, Phe214, Glu230, Trp232, or Tyr237 completely abolished the interaction with PP1 while mutation of Phe217 or Asp260 severely impaired the binding of PP1 (Fig. 5B). These eight residues are conserved and cluster in two patches. The same eight mutations also impeded

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A. Anti-FLAG

![Image](http://www.jbc.org/)

**Fig. 6. Co-precipitation of the EGFP-PP1β/δ fusion protein with FLAG-tagged wild-type and mutated versions of Sds22.** Western analysis with anti-FLAG (A) or anti-PP1β/δ (B) antibodies of anti-FLAG precipitates from lysates of COS-1 cells expressing an EGFP-PP1β/δ fusion protein and the indicated FLAG-tagged human Sds22 mutants.

The interaction of Sds22 with full-length PP1 in the yeast-two hybrid system (not shown).

To verify some key findings of the two-hybrid assays in a mammalian expression system, four mutant versions of Sds22 with a triple N-terminal FLAG tag were co-expressed with an EGFP-PP1β/δ fusion protein in COS-1 cells. In complete agreement with the results from the yeast two-hybrid assays, Western analysis revealed co-sedimentation of EGFP-PP1β/δ with immunoprecipitated FLAG-tagged wild-type Sds22, Sds22-V172A, and Sds22-S236A, but not with Sds22-E192A or Sds22-W302A (Fig. 6).

**DISCUSSION**

The present study established that the conserved C-terminal three-fourths of Sds22, which are proposed to form a curved LRR superhelix fused to a C-terminal LRR-cap, suffice for binding to PP1. Nevertheless, the affinity of Sds22 for PP1 was significantly reduced by removal of the N terminus of Sds22. Strikingly, a similar N-terminal amputation of fission yeast Sds22 confers a temperature-sensitive mitotic defect (6), which may also correlate with compromised binding to PP1. Possibly, the N terminus of Sds22 folds into an N-terminal LRR-cap, like the one observed in the structure of Internalin B, a prokaryotic LRR protein of the Sds22-like family (38). Deletion of such a shielding cap may induce a distortion of some of the LRRs to avoid exposure of the hydrophobic core of the LRR-superhelix. Furthermore, a major bipartite interaction site for PP1, comprising residues Asp148, Phe170, Glu192, Phe214, Asp280, Glu300, Trp302, and Tyr327 of Sds22, was mapped to the concave surface of the superhelix. Indeed, mutation of any of these residues severely or completely compromised the interaction with PP1. The reproducibility of a selection of these yeast two-hybrid results was confirmed in co-immunoprecipitation experiments from mammalian cell lysates. Interestingly, in the four available crystal structures of LRR proteins in complex with macromolecular ligands, the concave side of the LRR-superhelix also functions as a binding site (35, 37, 40, 41).

Conveniently, the concave surface can be quite confidently modeled in terms of general architecture and curvature because the proposed rigid ladder of hydrogen-bonded asparagine residues also restricts the curvature of the attached parallel β-sheet. It must, however, be noted that some uncertainty remains in terms of the twist of the sheet. While we have opted for a virtually untwisted sheet as observed in other eukaryotic proteins with Sds22-like LRRs and an LRR-cap, a modest right-handed twist of the sheet cannot be excluded. Such a twist occurs in the proteins Internalin B, which consists largely of Sds22-like LRRs (38), and YopM, which has shorter LRRs of the so-called bacterial class (42). The right-handed twist of these proteins has been tentatively explained by the repulsion of exposed negatively charged side-chains that occupy a fixed position in the second half of neighboring repeats (34). The Glu position in the consensus sequence of the Sds22 repeats may therefore induce a similar twist.

We have also demonstrated that Sds22 can bind all mammalian isoforms of PP1. Given that most of the variance between these isoforms is concentrated in the N-terminal 40 and C-terminal 30 residues, this observation is in line with our conclusion that the N terminus of PP1 does not function as a binding site for Sds22. Nevertheless, removal of the N terminus proved detrimental to the interaction, probably because of a structural distortion of the truncated protein. Further deletion and alanine-scanning studies and experiments with chimeric PP1/PP1 proteins suggest that the essential Sds22-binding sites are located between residues 43–173 of PP1γ1, which rules out several classical interaction sites such as the RVXF-binding channel, the β12/β13-loop, and the acidic groove as essential determinants of the interaction. In accordance with our results, it has recently been shown that point mutations in the RVXF-binding channel that impeded binding of RVXF-containing PP1 regulators did not compromise the interaction with Sds22 (43). In fact, for our mapping we exploited the observation that PP1γ1–Δ286–323 yielded a stronger interaction signal in yeast two-hybrid assays than did the wild-type. Because no stronger signal was obtained with PP1γ1–Δ297–323, this effect cannot be ascribed to the deletion of the flexible C terminus of PP1 but is indeed caused by the deletion of residues 286–296 that include the β14-strand. Given that RVXF-binding is disrupted by this deletion, it is likely that the binding of Sds22 to this truncated version of PP1 is facilitated by the elimination of interactions with RVXF-containing sub-units that are incompatible with Sds22 binding. However, it cannot be ruled out that deletion of residues 286–296 relieves some sterical hindrance of the Sds22-PP1 interaction. A third explanation, namely that this deletion introduces an artificial binding site for Sds22 is highly unlikely. Indeed, such a site would comprise previously unexposed elements, and the crystal structure of PP1 (11) shows that these elements must have been eliminated in PP1γ1–Δ270–323 and PP1γ1–Δ249–323, which yield a similarly enhanced interaction signal. Moreover, the point mutations in Sds22 that compromise the binding to PP1γ1–Δ286–323 also impede binding to wild-type PP1γ1. The observation that the C-terminal half of PP1, including all residues that contribute to the RVXF-binding channel, is not required for the interaction with Sds22 raises the interesting possibility that in Sds22-associated PP1 this channel is free for interaction with a specific additional RVXF-containing subunit. For instance, the unidentified phosphoprotein of 25 kDa that copurifies with Sds22 and PP1 from fission yeast (6), may constitute a third component of the Sds22 complex. Other trimeric PP1 holoenzymes are known that contain an RVXF-containing and an RVXF-less regulator, such as the CPI-17/Mypt1-PP1 complex (30).
We also confirmed earlier observations that Sds22-associated PP1 largely lacks phosphorylase phosphatase activity (7, 25). This, however, does not necessarily imply that Sds22 is a negative regulator of PP1 since the effects of various regulators is well known to be substrate-dependent (2). It can indeed be envisaged that the binding of Sds22 to the α4/α5/α6-triangle of PP1 restricts the accessibility of the catalytic site to its physiological substrate(s) and thus blocks out other PP1 substrates, such as phosphorylase α.

In summary, we have gained insight into the complex interaction mechanism that governs the binding of Sds22 to PP1 and have charted major binding sites on both interaction partners.

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Binding of the Concave Surface of the Sds22 Superhelix to the α4/α5/α6-Triangle of Protein Phosphatase-1
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