Structure of Protein Phosphatase Methyltransferase 1 (PPM1), a Leucine Carboxyl Methyltransferase Involved in the Regulation of Protein Phosphatase 2A Activity*

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The important role of the serine/threonine protein phosphatase 2A (PP2A) in various cellular processes requires a precise and dynamic regulation of PP2A activity, localization, and substrate specificity. The regulation of the function of PP2A involves the reversible methylation of the COOH group of the C-terminal leucine of the catalytic subunit, which, in turn, controls the enzyme’s heteromultimeric composition and confers different protein recognition and substrate specificity. We have determined the structure of PPM1, the yeast methyltransferase responsible for methylation of PP2A. The structure of PPM1 reveals a common S-adenosyl-L-methionine-dependent methyltransferase fold, with several insertions conferring the specific function and substrate recognition. The complexes with the S-adenosyl-L-methionine methyl donor and the S-adenosyl-L-homocysteine product and inhibitor unambiguously revealed the co-substrate binding site and provided a convincing hypothesis for the PP2A C-terminal peptide binding site. The structure of PPM1 in a second crystal form provides clues to the dynamic nature of the PPM1/PP2A interaction.

The regulation of the serine/threonine protein phosphatase 2A (PP2A), one of the most abundant protein phosphatases in eukaryotic cells, is intimately linked to the ability to modulate the composition of this multimeric enzyme (1). Although several factors such as natural small molecule substrates, other interacting proteins, and reversible phosphorylation have been implicated in the regulation of PP2A function, reversible methylation appears to be central to the regulation of PP2A assembly. Reversible methylation, like phosphorylation, is now appearing to be a fundamental process for the regulation of many cellular processes (2).

Methylation of the mammalian PP2A has been shown to be carried out by a specific methyltransferase (leucine carboxyl methyltransferase 1, also known as LCMT1) (3). Two homologues have been found in Saccharomyces cerevisiae, PPM1 and PPM2, which share, respectively, 30 and 26% sequence identity to the mammalian PP2A methyltransferase. It was subsequently shown that only PPM1 was responsible for the methylation of PP2A (4, 5). PPM1 codes for a 37-kDa protein that bears an AdoMet signature sequence motif but, overall, has weak sequence similarities to other methyltransferases. PP2A exists as a heterodimeric or heterotrimeric assembly containing A, B, or C subunits, and the methylation of PP2A occurs on the carboxyl moiety of the C-terminal leucine of the C subunit. The C subunit is the catalytically active component of the enzyme, whereas the A subunit purely acts as a scaffold for the C and B subunits (6). The A subunit first recruits the C catalytic subunit to form the core dimer. The B regulatory subunit comprises (at least) four families, each family containing several isoforms that can bind the AC dimer in a mutually exclusive manner and modulate the PP2A holoenzyme’s substrate specificity, enzymatic activity, and/or cellular localization. PP2A is therefore present under various enzymatic species (1). C subunits are well conserved, with ~70% sequence identity across different species. The six C-terminal residues (TPDYFL) are absolutely conserved in all known PP2A C subunits, and the three C-terminal residues (YFL) are also conserved in protein serine/threonine phosphatase PP4 and PP6, suggesting a role for these residues in protein phosphatase regulation.

Methylation of PP2A has been shown to influence the affinity of the AC core dimer for the different B subunits (7, 8). The regulation mechanism seems quite subtle, as some B regulatory subunits appear to bind more efficiently to an AC dimer when the catalytic C subunit has been methylated, whereas other protein partners are not influenced by the C subunit methylation state. The association of the AC core dimer with the regulatory B subunit is governed by equilibrium thermodynamics, and the methylated state of the C subunit is itself in equilibrium between the opposing actions of PPM1 and PPE1, the corresponding methyltransferase responsible for removal of the methyl group of PP2A. Variation of the ratios of methyltransferase and methyltransferase proteins or modulation of the activity of these two enzymes is therefore a dynamic method...
The Structure of PPM1

TABLE I

| Crystallographic data | Crystal form I (P6\textsubscript{3}) | Crystal form II (P2\textsubscript{1}2\textsubscript{1}2\textsubscript{1}) |
|-----------------------|-------------------------------|-------------------------------|
| Wavelength (Å)        | 0.97888 | 0.97966 | 0.97243 |
| Unit-cell parameters a, b, c (Å) | 111.3, 111.3, 162.7 | 110.7, 110.7, 165.9 | 110.7, 110.7, 165.6 |
| Resolution (Å)        | 32.8–1.97 | 52–1.80 | 24–2.58 |
| Total number of reflections | 558,140 | 261,127 | 294,159 |
| Total of unique reflections | 77,990 | 106,254 | 76,511 |
| Multiplicity           | 7.2 | 5.9 | 3.8 |
| R<sub>merge</sub> (%)  | 0.07 | 0.13 | 0.12 |
| N(hkl)                 | 16.8 | 4.2 | 5.7 |
| Overall completeness (%) | 97.2 | 100 | 98.8 |
| FoM<sup>a</sup>         | 0.5 (0.66) | 0.5 (0.66) | 0.5 (0.66) |

*<sup>a</sup> FoM, figure of merit.

MATERIALS AND METHODS

Cloning, Expression, and Purification—The YDR405w gene was amplified by PCR (from a sequenced S288C genomic template DNA) and cloned in a modified pET9 vector with an addition of six histidine codons at the 3'-extremity. The transformed XL-10 Gold expression strain (Stratagene) was grown in 2\textsuperscript{nd} YT medium (BIO101 Inc.) at 37°C up to an A\textsubscript{600} of 1. Expression was induced with 0.3 mM isopropyl-1-thio-β-D-galactopyranoside, and the cells were grown for a further 4 h at the same temperature. Cells were collected by centrifugation, resuspended in 20 mM Tris-HCl, pH 8, 200 mM NaCl, and 5 mM β-mercaptoethanol, and stored at −20°C. Cells were lysed by two cycles of freeze/thawing and sonication and were then centrifuged at 13,000 × g. The His-tagged protein was purified using a Ni<sup>2+</sup>-affinity column (Qiagen Inc.) and standard protocols. Eluted protein was further purified by gel filtration using a Superdex<sup>TM</sup> 75 (Amersham Biosciences) equilibrated against 20 mM Tris-HCl, pH 8, 200 mM NaCl, and 10 mM β-mercaptoethanol. The purity and integrity of the protein was checked by SDS-PAGE and mass spectrometry. SeMet-labeled protein was prepared as described (15, 16) and purified as the native protein.

Crystalization and Data Collection—The protein (3 mg/ml) was crystallized at 293 K by the hanging drop vapor diffusion method from 1:1 microtiter drops of protein and precipitant. Two different crystal forms were obtained. Crystal form I of the native protein grew from a mother liquor containing 15% PEG 8000 and 0.1 M KPO<sub>4</sub> or NaPO<sub>4</sub>, pH 4.6. Crystal form I of the ligand-bound PPM1 was obtained with either 5 mM AdoMet or 5 mM AdoHCys added to the protein solution and mother liquor containing 15% PEG 8000, 0.2 M ammonium sulfate, and 0.1 M MES, pH 5.6. A SeMet-labeled protein in complex with AdoMet was crystallized in 20% PEG 8000, 0.1 M sodium acetate, and 0.1 M MES, pH 5.6. All these conditions yielded rod-like crystals. Crystal form II of the PPM1/AdoHCys complex was obtained in 24% PEG 4000, 0.2 M magnesium chloride, and 0.1 M Tris-HCl, pH 8.5. All crystals were transferred to a cryoprotecting solution composed of the mother liquor and 30% glycerol prior to flash freezing in liquid nitrogen.

X-ray diffraction data on the native PPM1, the native PPM1-AdoMet complex, and the SeMet-substituted protein in complex with AdoMet were collected on the BM30-FIP beamline at the European Synchrotron Radiation Facility. The native PPM1/AdoHCys complex was recorded on the ID14-4 beamline, and the crystal form II of the PPM1/AdoHCys complex was recorded on a Rigaku rotating anode. Data were processed using MOSFLM and SCALA (17). The form I crystals belong to the P6<sub>3</sub> space group with three molecules per asymmetric unit, and crystal form II belongs to the P2<sub>1</sub>2<sub>1</sub>2<sub>1</sub> space group with one molecule per asymmetric unit. The cell parameters and data collection statistics are reported in Table I.

Structure Solution and Refinement—The structure was solved by multiwavelength anomalous diffraction (MAD) using data collected at three wavelengths on the PPM1-AdoMet complex in crystal form I at a resolution of 1.97 Å. The SOLVE (18) program retrieved 24 of 36 possible SeMet sites using the entire resolution range, yielding an interpretable electron density map. Solvent flattening was performed by RESOLVE (18), and the quality of the map allowed for 90% of the residues to be built automatically. After preliminary rebuilding cycles, the model was fully refined and completed from the high resolution...
native data recorded on the PPM1-AdoMet complex using REFMAC and O (17, 19). Structures of the apoprotein and AdoHCys complex in crystal form I were solved directly by transferring the model using one step of rigid body refinement followed by refinement and rebuilding. The structure of PPM1 in the crystal form II was solved by molecular replacement using the AMORE program (20) with a single molecule from the crystal form I as a search model. Refinement statistics are shown in Table I.

The final models in the crystal form I contain residues 2–330, and four residues belonging to the C-terminal His-tag could also be modeled in one monomer. In crystal form II, the α helix of region III could not be seen in the electron density map and is missing from the model. Structure views are generated using PyMol (pymol.sourceforge.net). The view in panel C is 90° rotated compared with that in panel B. Nter, N terminus; Cter, C terminus.

RESULTS AND DISCUSSION

Overall Structure of PPM1—The topology of PPM1 is shown in Fig. 1 along with a stereo ribbon representation from two perpendicular views. The overall structure of PPM1 shows a central β-sheet with several α-helices packing on both sides. A search for structural homologues with the Dali server (www.ebi.ac.uk/dali) revealed structural similarity with various methyltransferases that act on a wide range of substrates as diverse as small molecules, nucleic acids, and proteins. However, the structural similarity of PPM1 with the other methyltransferases is limited to a central protein core domain common to the class I AdoMet-dependent methyltransferases (blue domain in Fig. 1) (21, 22). Fig. 2 shows the superposition of the core AdoMet-dependent methyltransferase domain of PPM1 with the backbone trace of the core MTs of five structurally related enzymes, all acting on either proteins or small molecules. It can be seen that the core methyltransferase fold is well conserved, with the α-helices packing in a very similar orientation on the β-sheet. This core MT domain is characterized by alternate α/β secondary structure elements forming a central β sheet containing seven β-strands (β1 to β7) in the order β3, β2, β1, β4, β5, β7, and β6; six are parallel (β3–β5 and β6) and one is anti-parallel (β7). The six α-helices (αZ and αA–αE) are oriented roughly parallel to the β-strands, and the number of α-helices are distributed equally on each side of the β-sheet (23).

In addition to the conserved core MT fold, PPM1 contains...
several insertions and variations, a commonly used strategy by the members of this enzyme superfamily to confer diverse substrate specificity (23). However, none of these additions and insertions have a similar structural counterpart in any methyltransferase reported to date. These additions to the core domain can be divided in four regions (Fig. 1). Region I consists of three α-helices (α1, α2, and α3) at the N-terminal. The three helices pack on one side of the β-sheet, firmly fixing this subdomain to the core domain. Helix α1 interacts with helix αZ and the loop between β1 and αA. Helix α2 packs in a roughly parallel orientation on helix α1 and on helix αB of the core domain and α4 from region II. Helix α3 packs perpendicularly on helix α1, thereby clamping helix α1 between α2 and αZ. Region II is an insertion between βB and β3. Region II contains an α-helix (α4), followed by a long loop and a strand (β4') anti-parallel to β3, thereby extending the core β-sheet. Region III is a 33-residue insertion between β5 and αE containing a 12-residue α-helix (α5) that sits on top of the β-sheet. Region IV is a 34-residue insertion between β6 and β7, a common insertion site in MTs. This region contains three α-helices (α6, α7, and α8) that pack against helix αZ through hydrophobic interactions, although α7 makes additional contacts with the loop between α1 and α2, thereby completely burying the C-terminal of helix αZ inside the protein.

Comparison with PP2A MT from Other Organisms—Fig. 3 shows the alignment of the S. cerevisiae PPM1 sequence with those of PP2A methyltransferases that have been identified in other organisms, including Homo sapiens, Drosophila melanogaster, Caenorhabditis elegans, Arabidopsis thaliana, and Schizosaccharomyces pombe, as well as the related S. cerevisiae PPM2 and its homologs from Mus musculus and H. sapiens. The secondary structure of S. cerevisiae PPM1 is reported on top of the alignment. As can be expected, most of the core MT domain is conserved, with the best conserved residues located in loops or in the additional regions. One notable exception is helix α1 at the N terminus of region I, whose length and composition vary greatly in each organism and is missing in S. pombe and M. musculus. The sequence of region I corresponding to helix α2 and α3 in S. cerevisiae is also poorly conserved. The position of helix α1, which is almost totally buried inside the protein (Fig. 1C), makes it an indispensable element for the structural integrity of region I of PPM1. Removal of this helix would remove hydrophobic contacts with helices α2 and α3, thereby destabilizing the two other helices of this domain. It is therefore likely that the region I in protein phosphatase methyltransferases from different species is structurally different. In addition, region II (helix α4 and β-strand β4) and, in particular, the large loop between α4 and β4' seem to be poorly conserved. In crystal form I this loop is involved in intermolecular contacts but, nevertheless, is poorly defined. This is not thought to reflect possible intermolecular interactions occurring in vivo, notably with PP2A subunits, which are highly conserved. In contrast to the regions I and II, the sequences of regions III and IV are well conserved. The strictly conserved residues in these regions will be discussed in the context of AdoMet and PP2A binding.

AdoMet Binding Site—The crystal form I has been obtained both in the free form and in the presence of AdoMet and AdoHCys. Examination of the electron density maps during refinement (2Fo – Fc and Fc – Fo, with Fc and Fo and calculated structure factors, respectively) were obtained from data collected on the AdoMet and AdoHCys co-crystals, allowed these ligands to be modeled unambiguously in the density. For crystals of the apo form, some residual density in the binding pocket, arising from partial occupancy of some sites by a molecule co-purified from the Escherichia coli broth, was detected. The residual density could not be assigned with certainty, probably due to partial occupancy by ligands. Nevertheless, this structure will be called the apo form.

No significant differences are observed between the apo form and the AdoMet or AdoHCys bound complexes, or between the three monomers in the asymmetric unit (root mean square deviation is ~0.2 Å for all Cα positions). As illustrated in Figs. 1, 4, and 5, both ligands are bound within a deep pocket at the center of the protein, with contributions from the core MT and from region I. The loop between β1 and αA contains the conserved GXG AdoMet binding motif characteristic of class I methyltransferases (23). In PPM1, AdoMet binding follows the same mode as other methyltransferases containing this motif. The adenine base of AdoMet is stabilized by stacking interactions with Tyr-129 and hydrogen bonds between the N6 and N1 of AdoMet and, therefore, acts as a fixed by the following: 1) two hydrogen bonds between its O2' and O3' hydroxyls and the Asp-128 carboxylate oxygens; and 2) additional hydrophobic contacts with the side chains from Leu-203 and Tyr-206. Electrostatic interactions with Glu-201 and Arg-81 are preponderant with the charged tail of the AdoMet residue (Fig. 4).

Superposition of the AdoMet and AdoHCys PPM1 complexes shows that removal of the methyl group from AdoMet does not influence the conformation of the ligand in the binding pocket (not illustrated). AdoHCys occupies the same site and adopts the same conformation as AdoMet and, therefore, acts as a competitive inhibitor of PPM1. In the AdoHCys bound structure, the electron density clearly shows that the methyl group is replaced by a bound water molecule.

Although the location and general architecture of the AdoMet binding site is well preserved within the MT superfamily, considerable differences exist regarding the exact chemistry of interaction and accessibility of the bound ligand. In PPM1, the binding pocket is lined by residues from the core MT and from helix α1, which forms a cover above the bound ligand. The solvent-exposed surface of AdoMet is 8 Å² (as calculated from the program AREAIMOL, Ref. 17), which rep-
FIG. 3. Sequence alignment of leucine carboxyl methyltransferases. Aligned sequences correspond to *S. cerevisiae* PPM1, *H. sapiens* LCM1 (Q61UC8), *D. melanogaster* (Q9V3K7), *C. elegans* (P46554), *A. thaliana* (Q9VY08), *S. pombe* (O94257), *S. cerevisiae* PPM2 (Q08282), *H. sapiens* (O60294), and *M. musculus* (QSBYR1) using the program ESPript (32). The PPM1 and PPM2 subfamilies are separated by a dashed line. Members of the PPM2 family contain a C-terminal extension of ~300 residues, which has been omitted from the figure. The superposed secondary structure is extracted from the present PPM1 structure.
resents 1.3% of the total surface of AdoMet, values that range
between 2 and 100 Å² in other MTs. The core MT provides all
the specific interactions, whereas helix/H125 makes mostly non-
specific contacts with AdoMet through Ile-5, Thr-8, and Asp-9.
The helix forms a lid above the bound ligand but probably does
not contribute to binding specificity (Fig. 4). This could explain
why helix α1 is not conserved within the PPM family (see
alignment, Fig. 3). Considerable structural variability is ob-
served for the lid regions of MTs in general, which are com-
posed of α-helices, β-strands, or loops.

AdoMet is accessible in the PPM1 complex from two different
directions. First, the partially exposed N7 position of the ade-
nine ring of AdoMet defines clearly the entrance of the AdoMet
binding cavity. This entrance is occluded by the N terminus of
helix α1 and by three loops (β2/αB, β3/αC, and αD/β5). In order
for AdoMet to enter or for AdoHCys to exit the binding cavity,
a relatively large conformational change in the protein would
be necessary. In some MTs, the different conformation of bound
AdoMet and AdoHCys is thought to reflect the conformational
change that occurs after the catalytic transfer of the methyl
group from AdoMet to the substrate (24). This conformational
change would help to expel AdoHCys out of the binding site. We
have found no crystallographic evidence for conformational
flexibility in PPM1; structures obtained in various space
groups and enzyme-ligand complexes show identical and well
defined binding site conformations. This indicates a rather
rigid and stable binding site that requires large conformational
changes for substrate binding or for product release, although
it does not preclude the existence of an open apoprotein struc-
ture in a solution containing a more accessible binding site. In
crystal form II, the seven N-terminal residues of helix α1 are
disordered and might reflect a flexibility in this region that
enables the AdoMet to enter the cavity. It thus appears that
binding of the correct cofactor to PPM1 is necessary before
binding to the PP2A AC dimer, a conformational rearrange-
ment of PPM1 in the trimeric PPM1 core dimer complex being
unlikely.

Protein Phosphatase 2A Binding Site of PPM1—The pre-

Fig. 4. AdoMet and PP2A binding sites. A, detailed view of the AdoMet binding site. The 2Fo − Fc, electronic density (contoured at 1σ) is
represented around the AdoMet cofactor. Residues involved in binding are represented in sticks. Helix α1 lies on top of AdoMet and makes mostly	nonspecific hydrophobic interactions with AdoMet. No significant changes are observed between the AdoMet and AdoHCys complexes (not shown).
B, same as panel A, rotated >90°. Helix α1 has been omitted to show the more specific interactions of the other residues with AdoMet.
The flexibility of helix C-terminal TPDYFL residues of the PP2A catalytic subunit is shown. The hinge residues of helix binding site. The hinge residues of helix left PPM1 surface (Fig. 4). These conserved residues line the interior of the cavity and are therefore probably important for PP2A binding.

The structure of PPM1 in the presence of AdoMet enables us to propose a catalytic mechanism. The AdoMet carboxylate group is surrounded by a number of deeply buried charged residues, namely Arg-81, Asp-109, Arg-111, and Glu-201 (Fig. 4B). A similar surrounding for AdoMet was observed in the protein L-isoaspartyl-methyltransferase (PIMT) that also catalyzes methyl group transfer to a charged carboxylate group (26). Because AdoMet contains both a charged sulfonium atom and peptidic group, charged side chains will contribute favorably to binding. For catechol O-methyltransferase, quantum mechanical simulations have been performed on model systems mimicking the methyl transfer from the sulfur atom of an AdoMet cofactor to the hydroxyl oxygen of the catecholate substrate to assess the role of charged residues in the active site (27). These results suggest that the enzymatic acceleration of the reaction comes from the positioning of the reactants in a conformation that differs from that in solution due to the extra charges present in the active site. The positioning of the substrate in the catechol O-methyltransferase catalytic site is mediated by a decrease of electrostatic interactions using a Mg$^{2+}$ ion and the bonding of other non-polar residues (28). Arg-81, conserved in all leucine carboxyl MTs (Fig. 3), forms a salt bridge with the carboxylate group of AdoMet but is also in front of the sulfonium group of the methyl donor at the bottom of the putative peptide binding site. It could therefore play a double role: 1) stabilizing a catalytically competent conformation of the AdoMet substrate; and 2) helping to orient the incoming carboxylate of the peptide substrate for a nucleophilic attack on the sulfur atom. A number of well conserved residues are located near the methionyl group and are likely candidates for further coordination of the substrate. These include a stretch of residues between β4 and αD that are conserved in all leucine carboxyl-methyltransferases and, notably, Tyr-206 and Cys-202, which are at a distance of only 3.8 Å from the methyl-group.

Electrostatic Potential of PPM1—Assembly of the PP2A complex seems to start with the formation of AC heterodimers, which are the substrates for methylation by PPM1. Methylation of the C-terminal residue of the C subunit increases the affinity of the core dimer for some B subunits, leading to the formation of the ABC holoenzyme (8, 9). In their study on PP2A holoenzyme assembly, Strack et al. have highlighted the importance of charged residues in the binding of the By subunit to
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the A and C subunits (29). These charged residues are clustered on the surface of the Bv subunit and interact with charged residues on the A subunit surface. Whether charged residues are also involved in the binding of the PP2A core to PPM1 remains to be experimentally tested. PPM1 region IV contains a well conserved negative surface patch with a cluster of residues Asp-309, Glu-310 and Glu-312 (conserved patch on region IV in Fig. 5B). The conservation of charged surface residues indicates that these residues may be involved in intermolecular interactions. In crystal form I, these residues are involved in intermolecular interactions with the other monomers. Region IV, which is close to the proposed binding cavity of the C-terminal peptide, may therefore contribute to the interaction with the PP2A core dimer (Fig. 5).

Inactivity of PPM2—Yeast contains a second related gene, PPM2, that has 25% sequence identity with PPM1 but has no detectable methyltransferase activity on PP2A (4). PPM2 contains a C-terminal extension of ~300 residues, and homologous genes can be found in H. sapiens and M. musculus, making a second leucine carboxyl methyltransferase (LCMT) family. This C-terminal extension could interfere with binding of the PP2A core dimer. From the sequence alignment of PPM2 and PPM1 (Fig. 3), it can be concluded that both families have the same overall fold. PPM2 contains most of the conserved residues involved in AdoMet binding and also presents a putative PP2A binding site in region IV. In the peptide binding cavity most of the residues are also conserved, with the notable exception of Asn-250, Leu-251, and Arg-255 located on helix α5 in region III, which are replaced by His, Phe, and Leu/Asn respectively. Cys-202, a residue close to the catalytic site (Fig. 4B), is also mutated. The mutation of these residues in the PPM2 family could lead either to a loss of binding specificity for PP2A, or to a decreased efficiency of the methyl transfer catalysis, or to direct PPM2 activity to an unidentified target. These residues could be suggested for mutagenesis.

It has been shown that phosphorylation of the tyrosine located on the conserved TPDYFL C-terminal tail of the PP2A C subunit inhibits the enzyme (30). The peptide binding cavity of PPM1 might not be able to harbor a Tyr(P) group. Phosphorylation of this tyrosine may therefore be a mechanism for modulating the methylation of the C-terminal peptide as well as for directly modulating the binding affinity of the PP2A core dimer to the regulatory B subunits.

Conclusion—The structure of PPM1 is a convincing illustration of how the important methyltransferase superfamily uses structural variations on a common core to introduce different substrate specificities. PPM1, a representative of the methyltransferases acting on proteins, uses unique insertions and additions to the methyltransferase core domain to create a specific PP2A binding site. The biological importance of understanding PP2A methylation is warranted by the many essential functions regulated by PP2A in the cell. The structure of PPM1 also provides the molecular basis to study the involvement of PP2A methylation in tau hyperphosphorylation, neurodegeneration, dementia, and progression into Alzheimer’s disease and might help the development of anti-Alzheimer’s disease drugs.

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REFERENCES

1. Virshup, D. M. (2000) Curr. Opin. Cell Biol. 12, 180–185
2. Mumby, M. (2001) Science’s STKE http://stke.sciencemag.org/cgi/content/full/sigtrans;2001/79/pe1
3. De Baere, I., Derua, R., Janssens, V., Van Hoof, C., Waelkens, E., Merlevede, W., and Goris, J. (1999) Biochemistry 38, 16539–16547
4. Kalhor, H. R., Luk, K., Ramos, A., Zobel-Thropp, P., and Clarke, S. (2001) Arch. Biochem. Biophys. 395, 239–245
5. Wu, J., Tolstykh, T., Lee, J., Boyd, K., Stock, J. B., and Broach, J. R. (2000) EMBO J. 19, 5672–5681
6. Greves, M. R., Hanlon, N., Turovski, P., Hemmings, B. A., and Barford, D. (1999) Cell 96, 99–110
7. Bryant, J. C., Westphal, R. S., and Wadzinski, B. E. (1999) Biochem. J. 339 (Pt 2A), 241–246
8. Tolstykh, T., Lee, J., Vafai, S., and Stock, J. B. (2000) EMBO J. 19, 5682–5691
9. Wei, H., Ashby, D. G., Moreno, C. S., Ogris, E., Yeong, F. M., Corbett, A. H., and Pallas, D. C. (2001) J. Biol. Chem. 276, 1579–1577
10. Zalmorowicz, S. (2000) Biochem. Pharmacol. 60, 1225–1235
11. Janssens, V., and Goris, J. (2001) Biochem. J. 353, 417–439
12. Van Hoof, C., and Goris, J. (2003) Biochim. Biophys. Acta 1640, 97–104
13. Schonthal, A. H. (2001) Cancer Lett. 170, 1–13
14. Vafai, S. B., and Stock, J. B. (2002) FEBS Lett. 518, 1–4
15. Hendrickson, W. A., Horton, R. J., and Lemaster, D. M. (1996) EMBO J. 9, 1665–1672
16. Van Dyne, P., Ray, M. V., Bertelsen, A. H., Jackson-Mathews, D. E., Sturmer, A. M., Merkler, D. J., Consalvo, A. P., Young, S. D., Gilligan, J. P., and Shields, P. P. (1993) Biotechnology 11, 64–70
17. Collaborative Computational Project 4 (1994) Acta Crystallogr. Sect. D. Biol. Crystallogr. 50, 769–783
18. Terwilliger, T. C., and Berendzen, J. (1999) Acta Crystallogr. Sect. D. Biol. Crystallogr. 55, 849–861
19. Jones, T. A., Zou, J. H., Cowan, S. W., and Kjeldgaard, M. (1991) Acta Crystallogr. Sect. A 47, 110–119
20. Navaza, J. (1994) Acta Crystallogr. Sect. D. Biol. Crystallogr. 50, 157–163
21. Djordjevic, S., and Stock, A. M. (1997) Structure 5, 545–558
22. Schubert, H. L., Blumenthal, R. M., and Cheng, X. (2003) Trends Biochem. Sci. 28, 329–335
23. Martin, J. L., and McMillan, F. M. (2002) Curr. Opin. Struct. Biol. 12, 783–793
24. Schluckebier, G., Kozak, M., Blemming, N., Weinhold, E., and Saenger, W. (1997) J. Mol. Biol. 265, 56–67
25. Eglolf, M. P., Benaroch, D., Selbko, B., Romette, J. L., and Canard, B. (2002) EMBO J. 21, 2757–2768
26. Ryttersgaard, C., Griffith, S. C., Sawaya, M. R., MacLaren, D. C., Clarke, S., and Yeates, T. O. (2002) J. Biol. Chem. 277, 10642–10646
27. Kahn, K., and Bruce, T. C. (2000) J. Am. Chem. Soc. 122, 46–51
28. Kahn, B., and Kollman, P. A. (2000) J. Am. Chem. Soc. 122, 2586–2596
29. Strack, S., Ruediger, R., Walter, G., Dagda, R. K., Barwacz, C. A., and Cribs, J. T. (2002) J. Biol. Chem. 277, 20750–20755
30. Chen, J., Martin, B. L., and Brautigan, D. L. (1992) Science 257, 1261–1264
31. Westhead, D. R., Slidell, T. W., Flores, T. P., and Thornton, J. M. (1999) Protein Sci. 8, 897–904
32. Gouet, P., Courcelle, E., Stuatt, D. I., and Metoz, F. (1999) Bioinformatics 15, 305–308