Point of VIEW

ACS chemical biology

The 3D Structure of Protein Phosphatase 2A: New Insights into a Ubiquitous Regulator of Cell Signaling

Marc Mumby*

Department of Pharmacology, University of Texas Southwestern Medical Center, Dallas, Texas 75390

ABSTRACT Protein phosphatase 2A (PP2A) is a serine/threonine phosphatase implicated in cancer. Three new crystal structures of PP2A show how it interacts with inhibitory toxins and with one of its regulatory subunits. The structures also explain how specific site mutations may lead to cancer and suggest a novel role for PP2A methylation in the formation of PP2A holoenzymes.

Protein phosphatase 2A (PP2A) is a protein serine/threonine phosphatase that is an important component of a wide variety of signal transduction pathways. The term PP2A refers to a group of phosphatases containing a common catalytic subunit that functions within oligomeric complexes formed by interaction with a large and diverse group of regulatory proteins (*1*–*3*). The most common forms of PP2A contain a highly active core dimer composed of the catalytic subunit and a scaffold protein termed the A subunit (the AC core dimer). The scaffold subunit mediates the formation of heterotrimeric holoenzymes by binding additional regulatory subunits that dictate the functions of individual forms of the enzyme (Figure 1). The lack of structural information on this group of phosphatases has been a major impediment to defining the molecular basis of PP2A action. Consequently, a series of recent reports on the 3D structures of multimeric forms of PP2A have had a major impact on our understanding of this important signaling molecule. A report by Shi and colleagues solved the structure of the AC core dimer bound to inhibitory toxins (*4*). Two additional papers, one by Cho and Xu (*5*) and a second from Shi and colleagues (*6*), coincidently report the structure of the same PP2A holoenzyme composed of the AC core dimer associated with the B56γ regulatory subunit.

The structure of the catalytic subunit of PP2A presented in these reports is one of the last of the serine/threonine phosphatases to be solved, even though the protein was purified and cloned 20 years ago. The results described in these reports are major technical achievements because PP2A has been notoriously difficult to express and purify, let alone crystallize, despite the efforts of many groups. The primary sequence of the PP2A catalytic subunit has 50% identity with the catalytic domains of other members of the serine/threonine phosphatase family. Consistent with this homology, the structure of the PP2A catalytic subunit is very similar to the catalytic subunits of PP1, PP2B/calcineurin, and PP5. These enzymes have a characteristic α/β fold and contain two catalytic metal ions in the active site. Xing and colleagues showed that the two metal ions in PP2A are manganese as suggested, but never proven, by previous studies (*4*). The most distinctive feature of the catalytic subunits of the serine/threonine phosphatases is their unique carboxy-terminal (C-terminal) tail. Consistent with unique functions for these domains, the C-terminal tail of catalytic subunit (residues 296–309) extends away from the main globular protein phosphatase domain and nestles into a surface groove generated by the interface of the scaffold and regulatory subunits (Figure 2). This arrangement of the C-terminal tail, along with interacting residues within the helical domain and a loop between β-strands 12 and 13, accounts for the speci-

*Corresponding author, marc.mumby@utsouthwestern.edu.

Published online February 16, 2007
10.1021/cb700021z CCC: $37.00

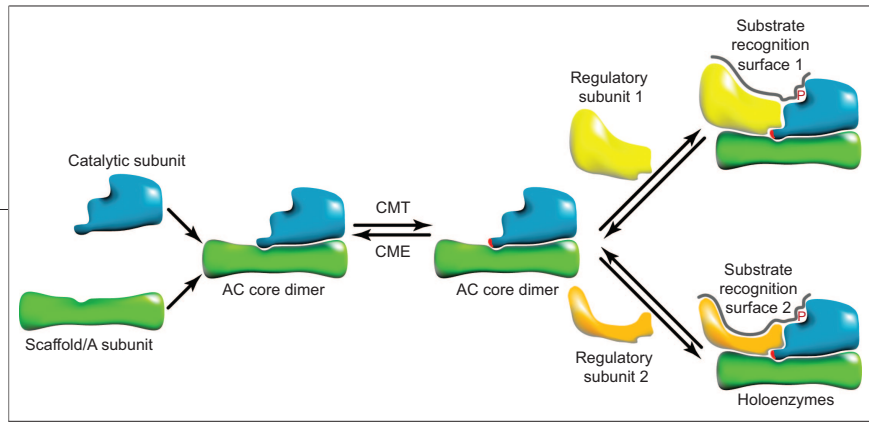

Figure 1. Assembly of PP2A holoenzymes. This diagram shows the current view of the steps involved in assembly of PP2A holoenzymes. The scaffold/A (green) and the catalytic (blue) subunits initially form an active AC core dimer. The AC core dimer can be modified by reversible carboxymethylation on the C-terminal leucine residue of the catalytic subunit (shown as a red cap). Methylation is catalyzed by a C-terminal leucine methyltransferase (CMT), and demethylation is catalyzed by a PP2A-specific carboxymethyl esterase (CME). Methylation of Leu309 facilitates the binding of the regulatory subunits and the formation of holoenzymes. Association of different regulatory subunits (shown in yellow and orange) generates distinct substrate recognition surfaces. These composite recognition surfaces, containing elements from both the catalytic and regulatory subunits, dictate which substrates are recognized by individual PP2A holoenzymes. These surfaces are also likely to play an important role in orienting the phosphorylated amino acids (red P) to the vicinity of the active site of the catalytic subunit.

ficity of the PP2A catalytic subunit for its scaffold protein.

The catalytic subunits of PP2A and PP1 are the only known targets of a set of toxins produced by a variety of organisms (*7*). The cell-permeable toxins, particularly okadaic acid, have been used extensively to study the biology of PP1 and PP2A. Microcystin is an equipotent inhibitor of PP1 and PP2A (IC_{50} of 0.2 nM), whereas okadaic acid is a more potent inhibitor of PP2A than PP1 (IC_{50}'s of 0.2 and 20 nM, respectively). The structures in these reports all contain toxins at the active site. Xing *et al.* (*4*) determined the structure of the AC core dimer bound with either okadaic acid or microcystin. Although the chemical structures of the two toxins are very different, they bind to the same surface pocket on PP2A and interact with an identical set of amino acids. The greater affinity of PP2A for okadaic acid is due to a "hydrophobic cage" within the binding pocket of PP2A that is not conserved in PP1. This cage surrounds the hydrophobic portion of okadaic acid and is the structural feature responsible for the 100-fold difference in affinity of okadaic acid for the two enzymes. The identification of compounds that exploit the hydrophobic cage in PP2A could provide more effective inhibitors for differentiating PP2A and PP1 action *in vivo*.

The PP2A holoenzyme structures show that the A subunit of PP2A is a remarkably adaptable scaffold that alters its conformation to accommodate multiple proteins. The previous structure of the free A subunit described by Barford and colleagues showed that the entire protein was composed of 15 tandem HEAT repeats (*8*). HEAT repeats are a loosely conserved sequence motif originally identified in the Huntingtin protein, elongation factor 3, the A subunit of PP2A, and the target of rapamycin (TOR) protein kinase. Each HEAT repeat is composed of ~40 amino acids that fold into two antiparallel α-helices connected by an intrarepeat loop. Adjacent HEAT repeats are connected by short inter-repeat turns and stack together to form an elongated molecule. Rotations at several inter-repeat interfaces generate an L-shaped superhelical conformation. In contrast to the free protein, the A subunit adopts a horseshoe or C-shaped conformation in the PP2A holoenzyme. Part of the dramatic structural rearrangement is due to the binding of the C subunit with an additional contribution caused by binding of the B56γ subunit. The rearrangements are due to conformational changes within HEAT repeats 11 and 12 that occur in the AC core dimer and the holoenzyme. An important contribution to the rearrangement is made by the breakage of a hydrogen bond in HEAT repeat 11 that is present in the AC core dimer but not the holoenzyme (*6*). The structural flexibility of the PP2A scaffold subunit is probably an important feature of the enzyme. The AC core dimer must be able to accommodate multiple families of regulatory subunits. The structure of the A subunit in the holoenzyme helps form a relatively compact structure, where each subunit makes multiple contacts with both of the others (Figure 2). The conformational flexibility of the A subunit is consistent with a general role for HEAT repeats in the formation of flexible scaffolds capable of interacting with multiple proteins. Other good examples of this are the nuclear transport factors of the importin-β superfamily of HEAT-repeat proteins, where structural flexibility is essential for their ability to transport multiple cargo proteins (*9*).

As predicted by modeling and mutagenesis experiments by Walter and colleagues (*10, 11*), amino acid residues in the A subunit that interact with the catalytic and B56γ subunits lie within the intrarepeat loops that form a ridge running along one side of the A subunit (Figure 2). Intrarepeat loops within HEAT repeats 11–15 interact with the catalytic subunit, whereas the intrarepeat loops in repeats 2–8 interact with the B56γ regulatory subunit. The interaction between the A and C subunits is dominated by an extensive hydrogen-bonding network that probably accounts for the high affinity of this interaction. The association of B56γ with the A subunit involves contacts between the intrarepeat loops of HEAT repeats 2–5 of the A subunit and α-helices in B56γ and intrarepeat loops 7 and 8 of A with a long intrahelical loop in B56γ. Although the association of B56γ with the A subunit buries a fairly large surface area, the interaction is still relatively weak, and this accounts for the much lower stability of A–B56γ complexes compared with A–C complexes.

Point mutations of several amino acids in the A subunit of PP2A have been associated with multiple types of cancer. Although a causal role of these alterations in transfor-

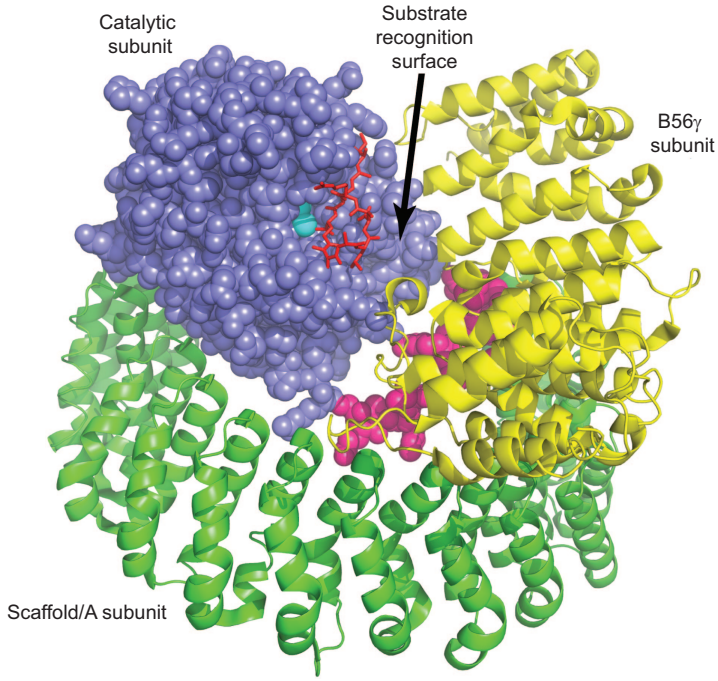

Figure 2. The 3D structure of the PP2A holoenzyme containing the B56γ regulatory subunit. In this view of the PP2A holoenzyme, the scaffold/A subunit is shown as a green ribbon diagram. The C-shaped A subunit is lying on its side with the open mouth of the C facing toward the rear. The paired α-helices of the HEAT repeats, along with the intrarepeat loops that contact the B56γ and catalytic subunits, can be seen running along the top of the protein. The HEAT-like α-helical repeats in the B56γ subunit are shown as a yellow ribbon diagram. B56γ rests on top of the A subunit with one of its edges against the catalytic subunit (in blue). The B56γ subunit has a curved surface that tilts toward the catalytic subunit. The catalytic subunit is shown in blue in space-filling representation. The catalytic manganese atoms (cyan) can be seen sitting at the bottom of the active site. The inhibitory toxin microcystin-LR (in red) is shown bound in a pocket at the active site. The unique C-terminal tail of the catalytic subunit (magenta) is bound in a groove formed at the interface of the A and B56γ subunits, which is behind the B56γ subunit in this view. The interface between the catalytic and B56γ subunit generates a broad groove-like surface that is likely to be involved in substrate recognition. This figure was generated with PyMOL (http://pymol.sourceforge.net) with coordinates from PDB entry 2NPP.

mation has not been established, a number of them disrupt interaction between the A subunit and the catalytic or regulatory subunits *in vitro* (*12, 13*). The crystal structures show that most of the cancer-associated mutations involve amino acids that directly participate in subunit interactions. The new structural data provide important support for the conclusion that the cancer-associated mutations can disrupt PP2A holoenzymes. Because PP2A has an established role in cellular growth control, loss of normal regulation of PP2A activity by disrupting formation of holoenzymes could contribute to cancer phenotypes.

There are four families of PP2A regulatory subunits that are each composed of multiple isoforms and splice variants. Both of the reported PP2A holoenzyme structures contain the B56γ1 subunit, which is a member of the PPP2R5 gene family. Previous biochemical and genetic analyses have indicated that the regulatory subunits control PP2A specificity by bridging the AC core dimer to phosphorylated substrate proteins. The holoenzyme structures reported in these papers support this model and provide the first molecular insights into how regulatory subunits control PP2A function. A totally unexpected finding was that B56γ is a completely α-helical protein composed of paired α-helices that closely resemble HEAT repeats. Although the HEAT-like repeats of B56γ do not have a common consensus sequence, they form sets of two antiparallel α-helices that assemble into a curved superhelical structure remarkably similar to the A subunit. B56γ binds to the same side of the A subunit as the catalytic subunit and interacts with both the A and C subunits. Interactions with the A subunit are mediated by intrahelical residues in HEAT-like repeats 4 and 6 on the convex surface of B56γ (the lower surface in Figure 2), which interact with residues in intrarepeat loops in HEAT repeats 2–7 of the A subunit. Contacts with the C subunit involve three sets of interactions. One set involves intrarepeat loops in HEAT-like repeats 6, 7, and 8 and the helical domain of the catalytic subunit. The second involves the long intrarepeat loop in repeat 2 of B56γ and the β12–β13 loop of the C subunit. The third involves interactions between residues in HEAT-like repeats 4–6 at the B56γ–A subunit interface with the C-terminal tail of the catalytic subunit. B56γ forms an exposed concave surface that sits adjacent to the catalytic site of the C subunit (Figure 2). The active site lies partway up the side of a broad groove formed by the B56γ–C subunit interface. This structure suggests that association of substrates with the B56γ-containing holoenzyme will likely require interactions with both the B56γ and catalytic subunits. Thus, the exposed concave surface of the B56γ subunit is likely to be a major determinant in substrate recognition. The B56γ subunit probably also plays an important role in orienting substrates so that the phosphorylated amino acid is near the catalytic site.

The effects of the B56γ regulatory subunit on the activity of the AC core dimer are likely to differ from those caused by binding

of the PP1 catalytic subunit to its regulatory subunits. The myosin phosphatase targeting (MYPT1) regulatory subunit that targets PP1 to myosin is a member of a large group of regulatory proteins that bind to the PP1 catalytic subunit. The contact between MYPT1 and PP1 is more intimate than that between B56γ and the PP2A catalytic subunit. MYPT1 wraps around much of the surface of PP1 and reshapes the catalytic cleft by extending an acidic groove important for binding of the light chain of myosin (*14*). The contacts between B56γ and the PP2A catalytic subunit are less extensive, presumably because the interaction is buttressed by the scaffold subunit. There are no contacts between B56γ and residues near the active site of the catalytic subunit. The structures suggest that the major role of the PP2A regulatory subunits is to tether the holoenzyme complex to its substrates rather than modify the catalytic site to accommodate different substrates. At the same time, the regulatory subunits should also reduce promiscuous activity of the AC core dimer by burying a considerable amount of exposed surface on both the A and C subunits (*5*).

The striking similarity between the 3D structure of B56γ and the A subunit suggests that B56γ may also have structural flexibility in order to accommodate binding to a variety of substrate proteins. This possibility is supported by data showing that B56γ interacts with multiple substrates and is involved in multiple regulatory processes (*15*). A very high degree of sequence identity exists within the conserved B56 domain in all the members of the family. Nearly all of the residues involved in interaction with the A and C subunits are conserved. It seems highly likely that all of the B56 subunits interact with the AC core dimer in a manner similar to B56γ. The exposed concave surface of the B56γ subunit is negatively charged and is proposed to recruit substrates containing a positively charged patch (*6*). Therefore, another possibility is that multiple members of the B56 family interact with some of the same substrates. Targeting of multiple isoforms of the B56 family to the same substrate or signaling complex is supported by data showing the interaction of both B56β and B56γ with the immediate early response factor X1 (IEX-1) early gene product (*16*), the presence of each member of the B56 family in complexes with the shugoshin protein (*17*), and data showing that any member of the B56 family is capable of regulating the Wnt pathway (*18*). Members of the B56 family also appear to have unique functions. For example, B56δ but not other B56 subunits associates with the cell division cycle 25 (Cdc25) cell cycle protein (*19*) and the HAND basic helix-loop-helix transcription factors (*20*). Recognition of some substrates may require interaction with isoform-unique sequences outside the common B56 domain.

The catalytic subunit of PP2A undergoes an important post-translational modification involving methylation of the C-terminal leucine residue (Leu309). This appears to be a reversible process that occurs in the AC core dimer. Methylation of Leu309 is catalyzed by a specific leucine carboxyl methyltransferase and removal of the methyl group catalyzed by a PP2A-specific CME (Figure 1). Methylation of Leu309 facilitates formation of holoenzymes containing multiple classes of PP2A regulatory subunits, including the B56 family (reviewed in ref 4). Although it is not clear whether reversible methylation plays a role in regulating the distribution of AC core dimers or PP2A holoenzymes *in vivo*, the loss of carboxyl methyltransferase activity reduces but does not abolish formation of PP2A holoenzymes in yeast (*21*). Until now, the molecular basis for the effects of Leu309 methylation on holoenzyme assembly was unknown.

The structures presented in the PP2A holoenzyme papers suggest a plausible mechanism for how Leu309 carboxymethylation promotes formation of PP2A holoenzymes. As mentioned earlier, the C-terminal tail of the PP2A catalytic subunit nestles in a groove generated by the interface of the A and B56γ subunits. This binding groove is very acidic, and both reports suggest that methylation of the carboxyl group of Leu309 neutralizes its negative charge and promotes docking of the C-terminal tail into the acidic groove (*5, 6*). Although this mechanism helps explain how methylation could enhance formation of holoenzymes, Xu *et al.* (*6*) provide convincing evidence that carboxymethylation is not required, at least for B56γ-containing holoenzymes. A preparation of the AC core enzyme containing nonmethylated catalytic subunits formed holoenzyme complexes *in vitro* just as readily as those with methylated catalytic subunits (*6*). In fact, AC core dimers containing a truncated form of the catalytic subunit that is missing the entire C-terminal tail (residues 295–309) also readily formed holoenzymes *in vitro*. Thus, while the PP2A structures provide a good model for how methylation could affect holoenzyme assembly, the role of methylation remains enigmatic. It will be important to test the charge neutralization model, especially in intact cells, where there may be additional methylation-sensitive factors that regulate holoenzyme assembly.

Acknowledgment: The author wishes to thank Hongtao Yu for helpful discussions and critical reading of this manuscript.

REFERENCES

1. Virshup, D. M. (2000) Protein phosphatase 2A: a panoply of enzymes, *Curr. Opin. Cell Biol. 12*, 180–185.
2. Janssens, V., and Goris, J. (2001) Protein phosphatase 2A: a highly regulated family of serine/threonine phosphatases implicated in cell growth and signalling, *Biochem. J. 353*, 417–439.
3. Mumby, M.C. (2003) Protein phosphatase 2A: a multifunctional regulator of cell signaling, in *Topics in Current Genetics* (Arino, J., and Alexander, D. R., Eds.) 5th ed., pp 45–72, Springer, Berlin/Heidelberg.
4. Xing, Y., Xu, Y., Chen, Y., Jeffrey, P. D., Chao, Y., Lin, Z., Li, Z., Strack, S., Stock, J. B., and Shi, Y. (2006) Structure of protein phosphatase 2A core enzyme bound to tumor-inducing toxins, *Cell 127*, 341–353.
5. Cho, U. S., and Xu, W. (2007) Crystal structure of a protein phosphatase 2A heterotrimeric holoenzyme, *Nature 445*, 53–57.

6. Xu, Y., Xing, Y., Chen, Y., Chao, Y., Lin, Z., Fan, E., Yu, J. W., Strack, S., Jeffrey, P. D., and Shi, Y. (2006) Structure of the protein phosphatase 2A holoenzyme, *Cell 127*, 1239–1251.
7. Dawson, J. F., and Holmes, C. F. (1999) Molecular mechanisms underlying inhibition of protein phosphatases by marine toxins, *Front. Biosci. 4*, D646–D658.
8. Groves, M. R., Hanlon, N., Turowski, P., Hemmings, B. A., and Barford, D. (1999) The structure of the protein phosphatase 2A PR65/A subunit reveals the conformation of its 15 tandemly repeated HEAT motifs, *Cell 96*, 99–110.
9. Conti, E., Muller, C. W., and Stewart, M. (2006) Karyopherin flexibility in nucleocytoplasmic transport, *Curr. Opin. Struct. Biol. 16*, 237–244.
10. Ruediger, R., Hentz, M., Fait, J., Mumby, M., and Walter, G. (1994) Molecular model of the A subunit of protein phosphatase 2A: interaction with other subunits and tumor antigens, *J. Virol. 68*, 123–129.
11. Ruediger, R., Fields, K., and Walter, G. (1999) Binding specificity of protein phosphatase 2A core enzyme for regulatory B subunits and T antigens, *J. Virol. 73*, 839–842.
12. Ruediger, R., Pham, H. T., and Walter, G. (2001) Alterations in protein phosphatase 2A subunit interaction in human carcinomas of the lung and colon with mutations in the A beta subunit gene, *Oncogene 20*, 1892–1899.
13. Ruediger, R., Pham, H. T., and Walter, G. (2001) Disruption of protein phosphatase 2A subunit interaction in human cancers with mutations in the Aα subunit gene, *Oncogene 12*, 10–15.
14. Terrak, M., Kerff, F., Langsetmo, K., Tao, T., and Dominguez, R. (2004) Structural basis of protein phosphatase 1 regulation, *Nature 429*, 780–784.
15. Janssens, V., Goris, J., and Van, H. C. (2005) PP2A: the expected tumor suppressor, *Curr. Opin. Genet. Dev. 15*, 34–41.
16. Letourneux, C., Rocher, G., and Porteu, F. (2006) B56-containing PP2A dephosphorylate ERK and their activity is controlled by the early gene IEX-1 and ERK, *EMBO J. 25*, 727–738.
17. Kitajima, T. S., Sakuno, T., Ishiguro, K., Iemura, S., Natsume, T., Kawashima, S. A., and Watanabe, Y. (2006) Shugoshin collaborates with protein phosphatase 2A to protect cohesin, *Nature 441*, 46–52.
18. Seeling, J. M., Miller, J. R., Gil, R., Moon, R. T., White, R., and Virshup, D. M. (1999) Regulation of beta-catenin signaling by the B56 subunit of protein phosphatase 2A, *Science 283*, 2089–2091.
19. Margolis, S. S., Perry, J. A., Forester, C. M., Nutt, L. K., Guo, Y., Jardim, M. J., Thomenius, M. J., Freel, C. D., Darbandi, R., Ahn, J. H., Arroyo, J. D., Wang, X. F., Shenolikar, S., Naim, A. C., Dunphy, W. G., Hahn, W. C., Virshup, D. M., and Kornbluth, S. (2006) Role for the PP2A/B56delta phosphatase in regulating 14-3-3 release from Cdc25 to control mitosis, *Cell 127*, 759–773.
20. Firulli, B. A., Howard, M. J., McDaid, J. R., McIlreavey, L., Dionne, K. M., Centonze, V. E., Cserjesi, P., Virshup, D. M., and Firulli, A. B. (2003) PKA, PKC, and the protein phosphatase 2A influence HAND factor function: a mechanism for tissue-specific transcriptional regulation, *Mol. Cell 12*, 1225–1237.
21. Wu, J., Tolstykh, T., Lee, J., Boyd, K., Stock, J. B., and Broach, J. R. (2000) Carboxyl methylation of the phosphoprotein phosphatase 2A catalytic subunit promotes its functional association with regulatory subunits *in vivo*, *EMBO J. 19*, 5672–5681.