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Focus on:

Recent insights into Protein Phosphatase 2A structure and regulation: the reasons why PP2A is no longer considered as a lazy passive housekeeping enzyme

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Although intracellular signal transduction is often portrayed as a protein kinase "domino effect", the counterbalancing function of phosphatases, and thus the control of phosphatase activity, is equally relevant to proper regulation of cellular function. Protein Phosphatase 2A (PP2A) is a widely expressed family of protein phosphatases made of a core dimer, composed of a catalytic (C) subunit and a structural (A) subunit, in association with a third variable regulatory (B) subunit. Although viewed as a constitutive housekeeping enzyme in the past, PP2A is a highly regulated phosphatase and is emerging as an important regulator of multiple cellular processes involving protein phosphorylation. The regulation of PP2A is mainly accomplished by the identity of the regulatory B-type subunit, which determines substrate specificity, subcellular localization and catalytic activity of the PP2A holoenzyme. In agreement with this, recent findings on the structure and post-translational modifications of PP2A emphasize the importance of PP2A holoenzyme composition in its regulation and pleiotropic activities.
Keywords. Phosphatase, PP2A, structure, holoenzyme composition, regulatory subunits, regulation.

Nouvelles avancées dans la structure et la régulation de la Protéine Phosphatase 2A : les raisons pour lesquelles PP2A ne doit plus être considérée comme une enzyme passive et non spécifique. La phosphorylation réversible de protéines régulatrices intervient dans virtuellement tous les processus biologiques chez les organismes supérieurs. La Protéine Phosphatase 2A (PP2A) est une phosphatase très abondante composée d'un noyau dimérique contenant une sous-unité catalytique (C), une sous-unité structurale (A) et auquel est associé une sous-unité régulatrice (B) variable. Bien que considérée dans le passé comme une enzyme constitutive non spécifique, PP2A est une phosphatase soumise à une régulation précise et qui est importante dans le contrôle des fonctions cellulaires impliquant la phosphorylation. Cette régulation est principalement accomplie par l'identité de la sous-unité régulatrice qui détermine la spécificité de substrat, la localisation cellulaire et l'activité catalytique de l'holoenzyme PP2A. Les nouvelles avancées sur le sujet, particulièrement sur la structure et la régulation basée sur des modifications post-traductionnelles de PP2A, soulignent bien l'importance de la composition de l'holoenzyme PP2A dans les multiples rôles de cette enzyme majeure.
Mots-clés. Phosphatase, PP2A, structure, composition de l'holoenzyme, sous-unités régulatrices, régulation.

1. INTRODUCTION

Reversible protein phosphorylation is an important regulatory mechanism that controls the activities of a myriad of proteins and is thus involved in virtually every major physiological process. In the past, most of the attention was focused primarily on protein kinases and on their regulation, mainly because phosphatases were then viewed as simple housekeeping enzymes. But advances in the understanding of protein phosphatases make now clear that these enzymes are precisely regulated and are as important as kinases in the regulation of cellular processes involving protein phosphorylation.

Protein phosphatase 2A (PP2A) is a very abundant – it accounts for as much as 1% of total cellular proteins – ubiquitous and remarkably conserved enzyme. A large and still-growing number of PP2A substrates have been identified, which makes PP2A an important player in the regulation of a plethora of cellular processes.

This article will review the recent advances in the structure and regulation of this fascinating enzyme.

2. CLASSIFICATION

While proteins can be phosphorylated on nine amino acids, serine, threonine and tyrosine phosphorylation

are by far the most predominant in eukaryotic cells. The enzymes that dephosphorylate these three amino acids are classified into four groups on the basis of specific catalytic signatures/domain sequences and substrate preference. Among the 150 individual members of the protein phosphatases superfamily, more than two thirds belong to the protein tyrosine phosphatase family (PTP), which dephosphorylates phosphotyrosine and, in some cases also phosphoserine and phosphothreonine. The majority of the remaining enzymes are specific for phosphorylated serine and threonine residues and are originally divided into two families (Cohen, 2002; Moorhead et al., 2009): the phosphoprotein phosphatases (PPP) and the Mg^{2+} or Mn^{2+}-dependent protein phosphatases (PPM). Recently, the family of aspartate-based phosphatases was added to this classification. This group consists of serine and tyrosine-phosphatases with an aspartic acid signature (DXDXT/V) driving catalysis and includes the FCP/SCP [TFIIF (transcription initiation factor II)-associating C-terminal domain Phosphatase/small CTD Phosphatase] and HAD (haloacid dehalogenase) family of enzymes (Moorhead et al., 2009).

The serine-threonine phosphatases share the common property of relying on the nucleophilic attack of the phosphorus atom by a metal-activated water molecule for their catalytic mechanism (Barford, 1996). The PPM family of phosphatases is mainly represented by the protein phosphatase type 2C (PP2C) whereas the PPP family is most diverse and contains 5 subfamilies. The PPP1 subfamily includes PP1 and the PPP2/4/6 subfamily comprises PP2A, PP4 and PP6. The PPP3 subfamily contains the Ca^{2+}-activated PP2B. Two other minor families exist termed PPP5 and PPP7 which respectively comprise PP5 and PP7.

3. STRUCTURE OF PP2A

The native forms of PP2A holoenzymes are predominantly heterotrimers in which a core dimer, $PP2A_D$, made of a structural A subunit (also known as PR65) and a catalytic C subunit, $PP2A_C$, is associated with a third variable regulatory B-type subunit. In addition to the classical PP2A heterotrimer, studies demonstrated that independent $PP2A_D$ core dimers are found within cells (Kremmer et al., 1997; Janssens et al., 2001). In addition, some specific PP2A dimers, in which the PR65/A subunit is replaced by the α4 protein have been recently identified (Yang et al., 2007).

The mammalian catalytic C subunit has two isoforms (α and β) which are 97% identical, ubiquitously expressed, highly conserved. While $PP2A_{C\alpha}$ and $PP2A_{C\beta}$ seem to be interchangeable *in vitro* (Zhou et al., 2003), studies in mice suggested that both isoforms are not functionally redundant *in vivo* (Gotz et al., 1998; 2003). Within the PP2A holoenzyme, the PR65/A subunit functions as a scaffold for the recruitment of the C and B-type subunits as well as additional proteins. The structural PR65/A subunit also exists in two isoforms, α and β, which are widely expressed and 86% identical in primary sequence (Hemmings et al., 1990). Interestingly, each PR65/A isoform shows differential ability to interact with B-type and C subunits (Zhou et al., 2003).

By far, the most variable subunit of the PP2A holoenzyme is the B-type subunit. To date, about 20 different isoforms have been described that are encoded by distinct genes or result from alternative splicing of a single gene. The mammalian B-type subunits are classified into three subfamilies, called PR55/B, PR61/B′ and PR72/B″ (**Table 1**). While the PR55/B and PR61/B' families are quite evolutionary conserved, the PR72/B'' family consists of a less evolutionary conserved group of proteins, with some human gene products having no murine orthologue and vice versa (Zwaenepoel et al., 2008). **Table 1** shows the nomenclature for human PR72/B'' genes. Each B-type subunit can potentially combine with any of the two isoforms of both the A and C subunits, generating over 75 potential trimeric PP2A holoenzymes (Janssens et al., 2001; 2008). This multiple combinatorial association is central to the mechanisms that regulate PP2A activity and ensure the pleiotropic roles of this important enzyme (Ruediger et al., 1992; Li et al., 2002).

The structure of the PP2A holoenzyme has long remained elusive. The first structural information came from the isolated PR65/A scaffolding subunit, which consists entirely of 15 tandemly repeated motifs known as HEAT (huntingtin-elongation-A subunit of PP2A-TOR). Canonical HEAT motifs consist of two helixes which form a helical hairpin. In PR65/A, 15 HEAT motifs stack together to form an elongated, horseshoe-shaped molecule with a continuous hydrophobic core (Walter et al., 1989; Hemmings et al., 1990; Groves et al., 1999). However, it was more than 15 years later that the crystal structures of a $PP2A_D$ and a $PP2A_{T61\gamma1}$ holoenzymes were solved (Xing et al., 2006; Xu et al., 2006; Cho et al., 2007b). The structural analysis of the PP2A core dimer showed that the catalytic subunit contains two catalytic metal ions at the active site and adopts a globular structure with an α/β fold, typical of the serine/threonine phosphoprotein phosphatase (PPP) family of phosphatases (Barford, 1996; Xing et al., 2006). Consistent with previous mutagenesis studies (Ruediger et al., 1992), structural data also revealed that the scaffolding subunit binds to the catalytic subunit via the intra-repeat loops of one end of its HEAT-repeats. Interestingly, these studies also pointed to a remarkable conformational flexibility of the PR65/A subunit, which undergoes pronounced

Table 1. Nomenclature and corresponding gene names of PP2A various subunits — *Nomenclature et appellation génétique correspondant aux différentes sous-unités de PP2A*.

Name	Gene #	Synonyms
C subunit		
PP2A catalytic subunit α	PPP2CA	PP2A-α, PP2A-Cα
PP2A catalytic subunit β	PPP2CB	
A subunit		
PP2A structural subunit α	PPP2R1A	65kD regulatory subunit, PP2A-Aα, PR65α, R1-α
PP2A structural subunit β	PPP2R1B	
B subunit		
PP2A regulatory B subunit α	PPP2R2A	B55α, PR55α, R2α, 55kD regulatory subunit
PP2A regulatory B subunit β	PPP2R2B	
PP2A regulatory B subunit γ	PPP2R2C	
PP2A regulatory B subunit δ	PPP2R2D	
PP2A regulatory B’ subunit α	PPP2R5A	B56α, PR61α, R5α, 56kD regulatory subunit
PP2A regulatory B’ subunit β	PPP2R5B	
PP2A regulatory B’ subunit γ	PPP2R5C	
PP2A regulatory B’ subunit δ	PPP2R5D	
PP2A regulatory B’ subunit ε	PPP2R5E	
PP2A regulatory B’’ α	PPP2R3A	B72/130, PR72/130, R3α, 72/130kD regulatory subunit
PP2A regulatory B’’ β	PPP2R3B	B70/48, PR70/48, R3β, 70/48kD regulatory subunit
PP2A regulatory B’’ γ	PPP2R3C	G5PR

conformational changes when incorporated into the PP2A core enzyme.

The crystal structure of a trimeric PP2A holoenzyme containing a regulatory PR61/B'_{γ} subunit was reported independently by two laboratories (Xu et al., 2006; Cho et al., 2007b). These studies revealed that, despite lacking canonical HEAT motifs, the PR61/B'_{γ} subunit harbors a superhelical structure similar to that of PR65/A, with an apparent curvature that forms HEAT-like repeat motifs. In the $PP2A_{T61\gamma}$ trimer, the horseshoe-shaped PR65/A subunit undergoes additional conformational rearrangements, which brings the amino and carboxyl termini in close proximity. The C subunit and the convex side of the PR61/B’ pseudo HEAT bind to the intra-repeat loops of HEAT repeats 2-7 and 11-15 respectively of the scaffold PR65/A subunit. PR61/B'_{γ} also makes extensive discrete contacts with the C subunit by itself. In particular, the C-terminal tail of the C subunit docks on the interface of the PR65/A and B-type subunits, where it could regulate the recruitment of the B-type subunit.

Crystal structure analysis gave valuable insights on how B-type subunits could regulate PP2A substrate specificities. Indeed, while the active site pocket of the PP2A catalytic subunit appears accessible to substrate, the binding of the PR61/B’ subunit in the holoenzyme markedly changes the physicochemical environment near the active site and limits the accessible surface to the active site and provides novel potential substrate binding surfaces.

Crystallisation data also provided structural basis for PP2A regulation by post-translational modifications of the catalytic subunit. Methylation of the C-terminus of $PP2A_C$ selectively affects the assembly of PP2A trimers *in vivo* (see below). Nevertheless, *in vitro* holoenzyme formation is independent of $PP2A_C$ methylation since a C-terminal truncated mutant or an unmethylated catalytic subunit can still stably form a $PP2A_{T55}$ or $PP2A_{T61}$ trimeric complex (Xu et al., 2006; Ikehara et al., 2007). In addition to methylation, tyrosine phosphorylation is another modification of $PP2A_C$ C-terminal tail that regulates PP2A activity. Structural data indicate that a hydrogen bond forms between the side chain of the targeted Tyr307 residue and a carbonyl group in the peptide backbone of PR61/B′. Tyrosine phosphorylation would therefore be detrimental to the assembly of PP2A holoenzyme containing a PR61/B’ subunit. Direct interaction between phosphorylated tyrosine and the active site within the catalytic subunit could also explain why tyrosine phosphorylation of $PP2A_C$ inhibits PP2A activity (Cho et al., 2007b).

More recently, a study reported the crystal structure of a PP2A holoenzyme containing another family of regulatory subunit: the PR55/B_{α} family member (Xu et al., 2008). The sequence similarity between the various subfamilies of the regulatory subunits is very low, and in agreement with this, the structure of PR55/B_{α} subunit differs from the PR61/B’ helical structure. Instead, the PR55/B_{α} subunit forms a seven-bladed β propeller, with each blade comprising four antiparallel β strands. In addition to the propeller core, PR55/B_{α} also contains additional secondary structure elements located above the top face which contribute to the formation of a putative substrate-binding groove in

close proximity to the active site of the C subunit of PP2A. As observed for PR61/B', the regulatory PR55/B_{α} subunit recognizes the amino-terminal HEAT repeats of the PR65/A subunit. In contrast, PR55/B_{α} makes few interactions with the catalytic subunit, compared to the PR61/B' subunit, which leads to a relatively loose holoenzyme. The structural observations further suggest that the PR55/B_{α} subunit may form a relatively stable complex with the isolated A subunit, but do not seem to support the notion that the C subunit is required for interaction between the PR65/A and B-type subunits. Due to the distinct structure of the PR55/B and PR61/B' structural subunits, the conformation of the scaffold PR65/A subunit is different in the $PP2A_{T55}$ holoenzyme compared to the $PP2A_{T61}$. The intrinsic conformational plasticity of the PR65/A subunit might therefore be important in order to interact with structurally different regulatory subunits (Xu et al., 2008). Indeed, the third regulatory subunit family PR72/B″ is predicted to adopt yet a different structure and contains two calcium binding EF hands (Janssens et al., 2003).

The recent characterizations of the structures of the PP2A holoenzyme are of prime interest because they constitute a new basis to improve the understanding of some aspects of PP2A assembly, function and regulation.

4. REGULATION

PP2A has been historically regarded as a relatively non-specific and unregulated enzyme. This allegation is in direct contradiction with the discrepancy that exists between the relatively small number of Ser/Thr phosphatases and the plethora of proteins that are reversibly phosphorylated on serine or threonine residues. It is now clear that PP2A, and the other protein phosphatases, are subjected to finely tuned control mechanisms that allow cells to adequately orchestrate changes in protein phosphorylation during virtually every cellular process (**Figure 1**).

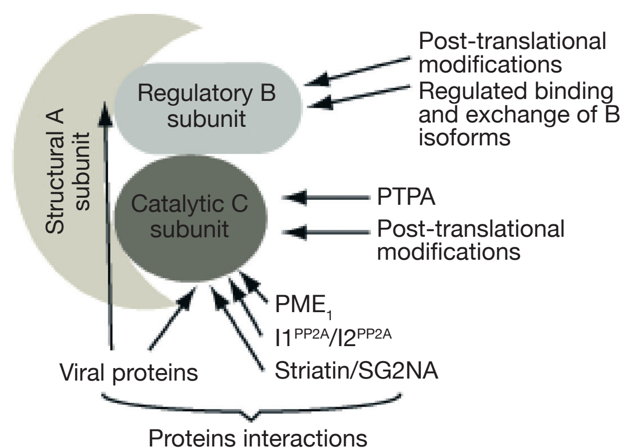

Figure 1. Summary of PP2A principal mechanisms of regulation — *Résumé des principaux mécanismes de régulation de PP2A.*

4.1. Holoenzyme composition

The composition of the holoenzyme is the most impacting determinant in the regulation of PP2A pleiotropic functions. It is now well recognized that the identity of the variable B-type subunit incorporated in the holoenzyme has specific consequences on PP2A activity. In accordance with structural data, binding of specific B-type subunit modulates the catalytic activity of PP2A *in vitro* (Sontag, 2001) and probably *in vivo*. In addition, the nature of the B-type subunit also influences substrate selectivity (Imaoka et al., 1983; Agostinis et al., 1987; 1990; 1992; Mumby et al., 1987; Cegielska et al., 1994; Mayer-Jaekel et al., 1994a; Sontag et al., 1996). Lastly, subunit composition impacts PP2A localization within the cell by targeting the phosphatase to specific subcellular compartments (Sontag, 2001).

Despite the lack of definite experimental evidence, recent data have lead to the model that the composition of the PP2A holoenzyme is not static *in vivo* and interconversions by dynamic exchange of regulatory subunits may represent a mechanism by which cells can quickly adapt to cellular demand at a given time. The observation that various viral proteins can replace specific regulatory subunits within PP2A holoenzyme *in vivo* provides a proof-of-principle that exchange between PP2A subunits is possible. In addition, B-type subunits can compete for binding to the $PP2A_{D}$ core complex *in vitro* (Kamibayashi et al., 1994) and suggest that the same phenomenon occurs within the cell. As detailed below, PP2A is subjected to diverse post-translational modifications which can have various impacts on B-type subunit binding. In this context, regulated specific post-translational modifications represent an attractive mechanism for controlling PP2A B-type subunit exchange. Alone or in combination, these post-translational modifications may constitute a "PP2A code" that dictates the formation of a specific holoenzyme or promotes the exchange between two subunits (Janssens et al., 2008).

4.2. Binding partners

The number of proteins interacting with PP2A is large and still-growing. These proteins can interact with one or more subunits and sometimes associate with a specific PP2A holoenzyme. PP2A partners play critical roles in its function and regulation. For instance, some interactors have been shown to target PP2A to specific cellular domains and regulatory functions (Sontag

et al., 1995; 1999; Kawabe et al., 1997; Takahashi et al., 1999; Turowski et al., 1999; Voorhoeve et al., 1999; Ito et al., 2000; Yan et al., 2000). Moreover, PP2A is part of multi-molecular signalling complexes through its binding to specific kinases (Westphal et al., 1998; 1999; Lebrin et al., 1999) or scaffolding proteins (Kikuchi, 1999).

Among the vast array of PP2A partners are multiple viral proteins, such as the polyoma small t and middle T, as well as with the small DNA tumour viruses simian virus 40 small t (SV40 ST) (Janssens et al., 2001; Arroyo et al., 2005; Janssens et al., 2005). By directly binding to PP2A these viral antigens inhibit its phosphatase activity (Scheidtmann et al., 1991; Yang et al., 1991; Cayla et al., 1993; Kamibayashi et al., 1994) and/or displace the B-type subunit from the holoenzyme (Pallas et al., 1990; Mumby et al., 1991; Chen et al., 2004). This impairs the prevailing cellular functions of PP2A and might explain the transforming activities of these viral proteins.

Structure of the SV40 ST/PP2A complex was recently solved and provides the basis to explain PP2A inhibition by viral proteins. One domain of SV40 ST is in a position to directly interact with the PP2A catalytic C subunit, near its active site. Therefore, it is likely that binding of SV40 ST alters PP2A phosphatase activity through direct competition with substrate for access to the catalytic site. In addition, two distinct SV40 ST domains interact with a specific region of the structural PR65/A subunit that is also recognized by PR55/B and PR61/B'. This observation could explain the competition that exists between SV40 ST and regulatory subunits for binding to the core enzyme (Chen et al., 2007; Cho et al., 2007a). However, SV40 ST has surprising little affinity for PP2A and does not efficiently displace PR55/B, PR61/B' or PR72/B'' from their respective holoenzymes *in vitro* (Chen et al., 2007; Cho et al., 2007a). It is thus likely that modulation of PP2A holoenzyme assembly through displacement of structural subunits is only a minor contributor in the inhibition of PP2A activity by SV40 ST.

The $PP2A_D$ core dimer also forms stable complexes with two calmodulin-binding scaffolding proteins, Striatin and the S/G2 nuclear autoantigen (SG2NA), which suggests that species of PP2A could be recruited to Ca^{2+}-dependent signal transduction cascades (Moreno et al., 2000). Striatin and SG2NA share some homology with PR61/B' isoforms and have sometimes been considered as a fourth regulatory subunit family. These PP2A interactors illustrate the fact that the distinction between a bona fide regulatory subunit and a binding partner is sometimes difficult. A proposition would be to consider a protein as a regulatory subunit only if it contains the canonincal PR65/A subunit-binding domain conserved in the existing regulatory subunits (Janssens et al., 2008).

Two intracellular heat stable inhibitors of PP2A, named I1PP2A/Phap and I2PP2A/SET have been identified. Both proteins inhibit specifically all holoenzyme forms of PP2A, probably by binding to the catalytic subunit (Li et al., 1996a; 1996b).

4.3. Post-translational modifications

The catalytic subunit of the phosphoprotein phosphatase (PPP) family members is very conserved both in sequence and structure. The most distinctive feature of this subunit consists in a unique C-terminal tail which extends away from the globular structure and is crucially located at the interface between the two other subunits (Xing et al., 2006; Xu et al., 2006; Cho et al., 2007b). Consistent with an important functional role for this domain, a highly conserved Thr304-Pro-Asp-Tyr-Phe-Leu309 motif is heavily post-translationally modified by methylation, tyrosine and threonine phosphorylation. These modifications are crucial for PP2A regulation and holoenzyme formation.

Leu309 residue is subjected to carboxymethylation by the S-adenosylmethionine-dependent LCMT1 (leucine carboxyl methyltransferase 1) (Lee et al., 1993; De Baere et al., 1999). The reverse demethylation is achieved through the action of a specific phosphatase methylesterase, PME-1 (Lee et al., 1996). Carboxymethylation of $PP2A_C$ has been directly implicated in the regulation of PP2A holoenzyme assembly. Indeed, several studies have shown that methylation enhances the affinity of the PP2A core enzyme for some but not all regulatory subunits. More specifically, C-terminal $PP2A_C$ methylation seems to selectively affect the assembly of PP2A trimers containing a PR55/B subunit (Ogris et al., 1997; Bryant et al., 1999; Tolstykh et al., 2000; Wu et al., 2000; Wei et al., 2001; Yu et al., 2001; Gentry et al., 2005; Longin et al., 2007; Nunbhakdi-Craig et al., 2007). In contrast, methylation of the C subunit seems to have little impact on the recruitment of other regulatory subunits (Wei et al., 2001; Gentry et al., 2005; Longin et al., 2007; Nunbhakdi-Craig et al., 2007). One indication of this selectivity relies on the observation that $PP2A_{T61}$ and $PP2A_{T72}$ can recruit a mixture of methylated and demethylated $PP2A_C$, whereas $PP2A_{T55}$ exclusively associates with methylated $PP2A_C$. Recent insights on PP2A structure suggest a plausible mechanism for how methylation could affect PP2A holoenzyme assembly. Indeed, crystal structure of a $PP2A_{T61}$ heterotrimeric PP2A holoenzyme has shown that the C-terminal $PP2A_C$ residue Leu309 does not mediate direct contact with the Aα or PR61/$B'_{\gamma 1}$ subunits but is located in a highly negatively charged environment formed by the side chains of Glu62, Asp63, Glu64 and Glu101 of the PR65/A subunit (Cho et al., 2007b). Although methylation is not strictly required for $PP2A_{T61\gamma 1}$

assembly, neutralization of the $PP2A_C$ C-terminal negative charge by carboxymethylation would promote docking of the tail in this area and, therefore, binding of $PR61/B'_{\gamma1}$ to $PP2A_D$. Methylation of the catalytic subunit is thus a crucial determinant in PP2A holoenzyme composition and could participate in the regulation of its diverse functions *in vivo*. Surprisingly, several studies argue that methylation of the C subunit is not required for the *in vitro* assembly of PP2A holoenzymes involving the PR55/B and PR61/B′ regulatory subunits (Xu et al., 2006; Ikehara et al., 2007). It is important to note that most of *in vitro* studies use an inactive form of $PP2A_C$ harboring a mutation which is known to alter the affinity of $PP2A_C$ for interacting partners (Janssens et al., 2008). Nonetheless, methylation may facilitate the assembly of the holoenzyme through enhanced binding affinity between the PP2A core enzyme and the regulatory subunit, this slight advantage being sufficient to tip the balance for holoenzyme assembly in cells but not *in vitro*. $PP2A_C$ carboxy-methylation in cells could also promote assembly of PP2A holoenzymes by recruiting assembly factors or by targeting the catalytic subunit to a specific cellular compartment where the assembly takes place.

Due to its importance in PP2A selective composition, regulation of $PP2A_C$ carboxy-methylation attracted a lot of attention these past few years. Methylation of $PP2A_C$ changes during cell cycle, suggesting a critical role in cell-cycle regulation (Janssens et al., 2001; Lee et al., 2007). In addition, differences in subcellular localizations of LCMT1 and PME-1 suggest that methylation and demethylation might be spatially controlled (Longin et al., 2008). Interestingly, structure of PME-1 in complex with PP2A reveals that PME-1 directly binds to the active site of $PP2A_C$, what is supposed to lead to the eviction of the metal ions required for the catalytic activity of PP2A (Longin et al., 2004; Xing et al., 2008). These findings indicate that, in addition to removing the methyl group from Leu309, PME-1 could directly control the phosphatase activity of PP2A. The interaction also results in the activation of PME-1 by structural rearrangement, which ensures the specificity of the methylesterase activity towards PP2A (Xing et al., 2008).

In addition to methylation at Leu309, the $PP2A_C$ tail is also subjected to phosphorylation on Tyr307 and possibly on Thr304. Tyr307 phosphorylation seems to have two striking consequences. First, it could inhibit the interaction of $PP2A_C$ with PR61/B' (Longin et al., 2007; Nunbhakdi-Craig et al., 2007) by annihilating an hydrogen bound between Tyr307 of the catalytic subunit and the carbonyl group of Val257 in the peptide backbone of the $PR61/B'_{\gamma1}$ subunit (Cho et al., 2007b). On the other hand, Tyr307 could indirectly affect the assembly of the PP2A holoenzyme containing PR55/B by preventing methylation of Leu309. Indeed, it has been suggested that Tyr307 phosphorylation might impair access to the LCMT1 cavity (Ogris et al., 1997; Yu et al., 2001; Longin et al., 2007; Nunbhakdi-Craig et al., 2007).

It should be emphasized that the above observations result from mutagenesis analysis and need to be physiologically confirmed. Mutagenesis studies have also pointed out a role for threonine phosphorylation in B-type subunit selection. Phosphorylation of Thr304 induces the selective inhibition of PR55/B subunit recruitment (Ogris et al., 1997; Wei et al., 2001; Gentry et al., 2005; Longin et al., 2007; Nunbhakdi-Craig et al., 2007) without affecting Leu309 methylation (Yu et al., 2001; Longin et al., 2007).

Evidence suggests that regulatory subunits, and in particular PR61/B' could also be subjected to phosphorylation. Phosphorylation of PR61/B′ could have opposing effects depending on the physiological context. While phosphorylation of a conserved Ser/Pro motif by extracellular signal-regulated kinase ERK would promote dissociation of PR61/B′ from the catalytic subunit (Letourneux et al., 2006; Cho et al., 2007b), phosphorylation of Ser37 by Chk1 enhances holoenzyme formation (Margolis et al., 2006).

It is now well-admitted that post-translational modifications of PP2A subunits have important roles in various aspects of holoenzyme regulation. Particularly, each B-type subunit is associated with a combination of specific post-translational modifications on $PP2A_C$. Leu309 methylation specifically favors formation of PR55/B subunit-containing PP2A holoenzyme. In contrast, Tyr307 phosphorylation is defavorable to association with PR55/B and PR61/B' and Thr304 selectively inhibits incorporation of PR55/B. This has lead to the notion of a "PP2A code" on the C-terminal tail that dictates the formation of specific PP2A holoenzymes (Janssens et al., 2008).

4.4. Substrate specificity

The reversible protein phosphorylation on proline-directed Ser/Thr motifs (Ser/Thr-Pro) is a key regulatory mechanism for the control of various cellular processes. Pro can exist in two conformations, cis and trans, in this motif. PP2A is considered as a major Pro-directed phosphatase which dephosphorylates phospho-Ser/Thr-Pro substrates. Studies of several PP2A substrates including Tau, Cdc25C, Myc and Raf1 substrates have lead to the hypothesis that a trans configuration of the proline residue adjacent to the phosphorylated residue is more favorable to dephosphorylation by PP2A. Pin1 is a peptidyl-prolyl isomerase (PPIase) which catalyses cis-to-trans isomerisation of specific pSer/Thr-Pro motifs. Studies have suggested that isomerisation of the Ser-Pro bound by Pin1 would be

required to promote dephosphorylation of substrates by PP2A (Zhou et al., 2000; Stukenberg et al., 2001; Yeh et al., 2004; Dougherty et al., 2005). Nevertheless, some observation that specific PP2A holoenzyme (especially $PP2A_{T55}$) could dephosphorylate these motifs without Pin1 requirement are not compatible with this model (Agostinis et al., 1992; Mayer-Jaekel et al., 1994b).

On the other hand, PP2A itself seems to subjected to proline isomerization. Indeed, PTPA (phosphotyrosyl phosphatase activator, newly renamed phosphatase two A phosphatase activator) can activate the classical Ser-Thr phosphatase activity of a native inactive PP2A form (Longin et al., 2004) through an isomerase activity. Isomerization induces a conformational change in PP2A which correlates with its activation (Jordens et al., 2006; Leulliot et al., 2006).

5. CONCLUSION

Genetic deletion of PP2A catalytic subunit is lethal in yeast (Sneddon et al., 1990), demonstrating the prevailing place of PP2A in homeostasis. In accordance with this and early observations (Bialojan et al., 1988), dysregulation of PP2A-regulated signalling pathways can contribute to cancer (Arroyo et al., 2005; Janssens et al., 2005; Eichhorn et al., 2009). Initial understanding of PP2A as a tumor suppressor was mainly based on the tumor-promoting activities of okadaic acid, the most famous naturally occurring PP2A inhibitor. But this loss of function approach does not discriminate between specific holoenzyme contribution and it now appears that the description of PP2A as a tumor suppressor is oversymplistic and needs more investigation (Eichhorn et al., 2009). Moreover, studies employing general inhibitory strategy, like okadaic acid, have pointed to a role for PP2A in multiple pathologies besides cancer. In this context, in order to improve our knowledge of this clinically relevant target protein, it seems important to dissect PP2A-controlled signalling pathways and, to achieve this, to precisely delineate specific cellular functions and context of each holoenzyme. Further studies on the precise role of individual PP2A B-type regulatory subunits within these signalling cascades is thus a challenging question for the future.

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