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Type 2A Protein Phosphatase, the Complex Regulator of Numerous Signaling Pathways

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ABSTRACT. Type 2A protein phosphatase (PP2A) comprises a diverse family of phosphoserine- and phosphothreonine-specific enzymes ubiquitously expressed in eukaryotic cells. Common to all forms of PP2A is a catalytic subunit (PP2Ac) which can form two distinct complexes, one with a structural subunit termed PR65/A and another with an alpha4 protein. The PR65/A–PP2Ac dimer may further associate with a regulatory subunit and form a trimeric holoenzyme. To date, three distinct families of regulatory subunits, which control substrate selectivity and phosphatase activity and target PP2A holoenzymes to their substrates, have been identified. Other molecular mechanisms that regulate PP2Ac function include phosphorylation, carboxyl methylation, inhibition by intracellular protein inhibitors (I_1^{PP2A} and I_2^{PP2A}), and stimulation by ceramide. PP2A dephosphorylates many proteins *in vitro*, but *in vivo* protein kinases and transcription factors appear to represent two major sets of substrates. Several natural compounds can inhibit PP2A activity and are used to study its function. Mutations in genes encoding PR65/A subunits have been identified in several different human cancers and the PP2A inhibitor, termed fostriecin, is being tested as an anticancer drug. Thus, a more thorough understanding of PP2A structure and function may lead to the development of novel strategies against human diseases. BIOCHEM PHARMACOL **60**;8:1225–1235, 2000. © 2000 Elsevier Science Inc.

KEY WORDS. protein phosphatase 2A; protein phosphorylation; protein phosphatases; protein kinases; signal transduction; phosphatase inhibitors; cell cycle; transcription factors

Reversible protein phosphorylation catalysed by protein kinases and phosphoprotein phosphatases represents the most common regulatory mechanism in eukaryotic cells. In an average eukaryotic cell, every third protein undergoes reversible phosphorylation. The high frequency of reversible phosphorylation is in agreement with the high abundance of genes encoding protein kinases, as revealed by the sequenced genomes of *Saccharomyces cerevisiae*, *Caenorhabditis elegans*, and *Drosophila melanogaster*. As listed in Table 1, between 1.7% and 2.2% of genes present in each of these three sequenced genomes [1–3] encode protein kinases. In contrast, the number of genes encoding protein phosphatases is 2.4- to 3.4-fold lower than those for protein kinases (Table 1). However, since catalytic subunits of protein phosphatases often associate with different regulatory subunits to form distinct holoenzymes, the number of functional phosphatases should at least be equal to the number of kinases. All protein kinases appear to descend from one primordial gene, whereas protein phosphatases have been classified into 3 families based on amino acid sequence identity and similarity of their three-dimensional structures. These include one family specific against phosphotyrosine residues (PTP) and two families specific against phosphoserine and phosphothreonine residues, termed PPP and PPM (Mg^{2+}-dependent) [4]. In the PTP family, there also exist dual specificity protein phosphatases, which are able to dephosphorylate all three phosphoresidues. Finally, a novel family of mammalian protein phosphatases, specific against phosphohistidine residues, has recently been identified [5].

STRUCTURE OF TYPE 2A PROTEIN PHOSPHATASE

As depicted in Fig. 1, PP2A† in mammalian cells forms either dimers or trimers [reviewed in 4, 6–12]. Phosphatase activity resides in PP2Ac (a member of the PPP family) of 36–38 kDa. The closest relatives of PP2Ac include PP4c and PP6c [4], which are 66% and 59%, respectively, identical to PP2Ac. PP2Ac is a metalloenzyme containing

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† *Abbreviations:* PP2A, type 2A protein phosphatase; PP2Ac, the catalytic subunit of PP2A; PR65/A, the structural subunit of PP2A; PR55/B, PR61/B′, and PR72/B″, three families of regulatory subunits of PP2A; PR59 and PR48, regulatory subunits of PP2A belonging to the PR72/B″ family; PP1/PP4/PP6/PP2B, type 1/4/6/2B protein phosphatase, respectively; PP4c and PP6c, catalytic subunits of PP4 and PP6; HEAT motif, repeating protein structure present in PR65/A structural subunit; TF-23A, thyrsiferyl 23-acetate; CDK, cyclin-dependent protein kinase; CDC2, cyclin-dependent protein kinase, also termed both $p34^{cdc2}$ and CDK1; GSK3β, glycogen synthase kinase 3β; CDC6, eukaryotic regulator of DNA replication initiation; CREB, cAMP response element binding protein; SV40, simian virus 40; and STAT, signal transducer and activator of transcription.

TABLE 1. Number of protein kinases and phosphoprotein phosphatases identified in sequenced eukaryotic genomes

	S. cerevisiae	*C. elegans*	*D. melanogaster*
Total number of proteins/genes	~6100*	~18400*	~13600†
Protein kinases	124	411	236
Protein phosphatases	37	174	93

The numbers presented for protein kinases and protein phosphatases are from [1] and [2]. Phosphoinositol phosphatases are not counted among the protein phosphatases in *S. cerevisiae* and *C. elegans* [1].

*Number of proteins for yeast and *C. elegans* are from [3].

†For *D. melanogaster*, the total number of genes are from [2].

Zn^{2+} and Fe^{2+} [13]. Two types of PP2A dimers have been described: a PR65/A–PP2Ac 'core dimer' [reviewed in 4, 6–12] and a more recently identified alpha4–PP2Ac dimer [14, 15] (Fig. 1). The level of the PR65/A–PP2Ac dimer has been estimated to be at least 30% of all cellular PP2A in murine 10T1/2 fibroblasts [16]. The 'core dimer' contains a protein of 61–65 kDa termed the constant regulatory subunit of PP2A and abbreviated PR65 or A, hence PR65/A. The PR65/A contains 15 leucine-rich, non-identical repeats of 39 amino acids [17]. An axial ratio of 11:1 has been determined for PR65/A, corresponding to its very elongated shape [18]. This has recently been confirmed by the three-dimensional structure solved from recombinant PR65/A expressed in *Escherichia coli* [19]. The structure, which has been determined at 2.3 Å resolution, revealed an elongated molecule built of 15 pairs of antiparallel α-helices, termed HEAT motifs. Analysis of the PR65/A three-dimensional structure has also revealed that exposed hydrophobic surfaces are localised at the intrarepeat turns connecting two helices present within a single HEAT motif [19]. The intrarepeat turns of the PR65/A most probably represent the sites of interaction with the PP2Ac and regulatory subunits.

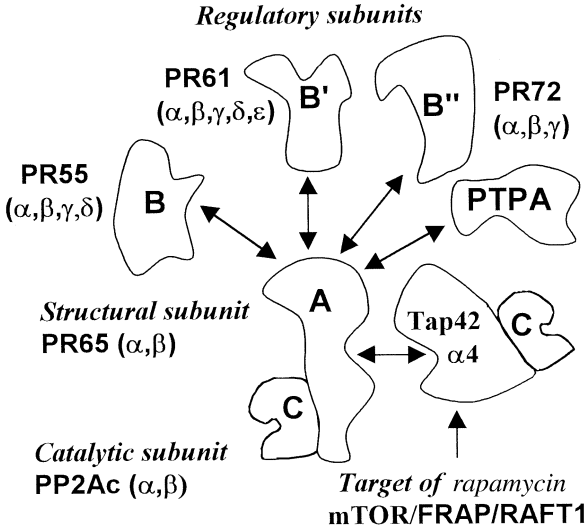

FIG. 1. Mammalian protein phosphatase 2A (PP2A) holoenzymes. Isoforms encoded by different genes are indicated by Greek letters. The PR72/B″ family comprises a longer variant encoded by the PR72/B″ gene and termed PR130 as well as products of two other genes: PR59 (β isoform) and PR48 (γ isoform). Names assigned according to human genome nomenclature are the following: PPP2C for PP2Ac/C, PPP2R1 for PR65/A, PPP2R2 for PR55/B, PPP2R3 for PR72/B″, PPP2R4 for PTPA, and PPP2R5 for PR61/B′. The PR61/B′ is also termed B56. The interconversion between PR65/A–PP2Ac/C and alpha4–PP2Ac/C dimers is purely hypothetical. PTPA, phosphotyrosine phosphatase activator; mTOR, mammalian target of rapamycin; FRAP, FKBP12-rapamycin-associated protein; RAFT1, rapamycin and FKBP12 target 1.

More recently identified is an alpha4–PP2Ac dimer containing an alpha4 protein previously known as a component of the B lymphocyte receptor [14]. Initially, the alpha4–PP2Ac complex was identified in *S. cerevisiae*, where homologues of PP2A, namely Sit4, Pph21, and Pph22, form complexes with yeast alpha4 termed Tap42 protein [20]. Interestingly, phosphorylation of Tap42, triggered by TOR (target of rapamycin) kinases, results in the binding of this molecule to PP2A [21]. The presence of a similar signalling pathway has been suggested to exist in mammalian cells [14], but is still a controversial issue [22]. The alpha4/Tap42–PP2Ac complex has also been identified in plants [23]. In contrast to the PR65/A subunit, which associates exclusively with PP2Ac, alpha4 also associates with the PP2A-related phosphatases PP4c and PP6c [24]. It still remains to be established whether there is an exchange of regulatory subunits between PR65/A- and alpha4-containing dimers (Fig. 1).

The PR65/A–PP2Ac core dimer associates further with a third protein, a regulatory subunit forming a trimeric holoenzyme. In these trimeric complexes, the PR65/A serves as a scaffold protein: HEAT motifs from 1 to 10 are engaged in binding regulatory subunits, whereas PP2Ac occupies HEAT motifs from 11 to 15 [25, 26]. To date, three unrelated protein families of PP2A regulatory subunits, termed B/PR55, B′/PR61, and B″/PR72 and encoded by 12 distinct genes, have been described in mammalian cells [10, 11, 27, 28] (Fig. 1). Regulatory subunits display tissue-specific distribution. For instance, PR55/Bα and PR55/Bδ isoforms are distributed evenly in different mice tissues, whereas PR55/Bβ and PR55/Bγ are highly brain-enriched [27]. Taking into account that PP2Ac and PR65/A are also encoded by two genes termed α and β in mammals, one can calculate that 50 distinct PP2A holoenzymes may exist in mammalian cells (This number would be doubled if one takes into account regulatory subunits encoded by all possible mRNA splicing variants.). Interestingly, PP2Ac also displays phosphotyrosine phosphatase activity that can be up-regulated by the association of the PR65/A–PP2Ac core dimer with a protein termed phosphotyrosine phosphatase activator (PTPA) [29] (Fig. 1). Studies on the yeast homologue of PTPA have demonstrated its role in protecting against oxidative DNA damage [30]. Another group of proteins known to associate with the PR65/A–PP2Ac dimer are those encoded by small DNA viruses, namely the small t antigen of SV40 as well as small t and middle T antigens encoded by the polyomavirus [4, 8–12]. Association of polyomavirus small and middle T

antigens encoded with the PR65/A–PP2Ac dimer increases the phophotyrosine phosphatase activity of PP2A and decreases its activity against retinoblastoma (Rb) peptide phosphorylated by CDC2–cyclin B kinase [31]. Regulatory subunits control substrate selectivity and phosphatase activity and target PP2A holoenzymes to their substrates [10, 11, 32]. It is possible that other unidentified families of PP2A regulatory subunits may exist. Two candidates termed striatin and SG2NA, which are highly homologous Ca^{2+}/calmodulin (CaM)-binding proteins, form stable complexes with PR65/A–PP2Ac dimers [33]. Alternatively, striatin and SG2NA, which form complexes with other so-far-unidentified polypeptides, may represent scaffold proteins responsible for targeting a set of proteins to the same location. It remains to be established how Ca^{2+}-dependent signalling pathways regulate the assembly and activity of striatin-SG2NA–PP2A complexes.

REGULATION OF PP2A ACTIVITY AND CELLULAR LOCALISATION

The major regulators of PP2A are regulatory subunits that control PP2A activity and substrate specificity and target holoenzymes to specific intracellular compartments. Many examples of different substrate specificity, displayed by distinct PP2A holoenzymes, are described in the literature. For instance, *in vitro* reassociation of the PR65/A subunit with PP2Ac results in a decrease of its activity against phosphorylated 40S ribosomal subunits, while at the same time causing an increase in the dephosphorylation of a eukaryotic translation initiation factor phosphorylated in its α subunit [18]. The PR65–PP2Ac dimer displays very low activity against CDC2–cyclin B kinase-phosphorylated histone H1, whereas trimers containing PR55/B subunits utilise this substrate very efficiently [34]. Trimers containing PR72/B″ preferentially dephosphorylate Ser-120 and Ser-123 in the SV40 large T antigen, whereas PR55/B-containing trimers can only efficiently dephosphorylate the CDC2–cyclin B kinase-phosphorylated site corresponding to Thr-124 [35]. Furthermore, PP2A trimers associated with the cytoskeleton contain the PR55/B subunit, whereas nuclear PP2A contain regulatory subunits from the PR61/B′ family [36, 37]. In agreement with this, putative nuclear localisation signals (NLS) have been identified in several members of the PR61/B′ family of regulatory subunits [37, 38]. Molecular mechanisms of PP2A nuclear import and export remain to be determined.

PP2A has been demonstrated to undergo reversible carboxyl methylation on the last C-terminal leucine residue [39–41]. This methylation reaction is catalysed by a purified and unidentified carboxyl methyltransferase and reversed by phosphatase methylesterase [42, 43]. This methylesterase has recently been identified by its ability to form stable complexes with H59Q and H118Q mutants of PP2Ac [42]. Contrary to initial data [41], methylation does not affect PP2A activity [44]. Instead, it seems to play a role in the conversion of dimeric into trimeric forms of phosphatase. In agreement with this, PP2Ac associated with trimeric PP2A has been found to display a greater degree of methylation than PP2A associated with dimeric phosphatase [44].

PP2Ac also undergoes reversible phosphorylation. Tyrosine phosphorylation of PP2A occurs *in vivo* in response to growth stimulation and *v-src* transformation of fibroblasts [45]. Another laboratory has also demonstrated that PP2Ac undergoes phosphorylation on an unidentified threonine residue in response to autophosphorylation-activated protein kinase [46]. In both cases, phosphorylation inhibited PP2A activity. More research will be required in order to elucidate both the identity of phosphorylated residues as well as the physiological conditions leading to PP2Ac phosphorylation.

PP2A activity is also regulated by cellular inhibitory proteins. Two protein inhibitors of PP2Ac, termed I_1^{PP2A} and I_2^{PP2A}, have been isolated and characterised [47, 48]. Transfection of cells with constructs encoding these inhibitors can be applied to regulate PP2A *in vivo*. Interestingly, in the presence of Mn^{2+}, these inhibitors are able to regulate the activity of another type 1 protein phosphatase (PP1) [49]. Thus, PP1 activity against myelin basic protein and histone H1, but not against phosphorylase *a*, is stimulated by I_1^{PP2A} and I_2^{PP2A} [49]. This phenomenon suggests that certain signalling pathways may utilise I_1^{PP2A} and I_2^{PP2A} for the concurrent regulation of different phosphatases.

PP2A has also been reported to undergo stimulation in response to ceramide [50, 51]. This lipid second messenger is produced by sphingomyelinase activated in response to binding tumour necrosis factor-α, interferon-γ, and interleukin-1 to corresponding membrane receptors. Ceramide inhibits cell growth and induces apoptosis. Recently, a molecular mechanism of ceramide-induced apoptosis involving Bcl2 has been presented [52]. It was established that phosphorylation of Bcl2 at Ser-70 is required for its antiapoptotic action. Stimulation of mitochondria-bound PP2A by ceramide results in Bcl2 dephosphorylation, followed by its inactivation and initiation of apoptosis.

PP2A is mainly a soluble enzyme present in the cytosol of eukaryotic cells. Another pool of soluble PP2A is present inside the nucleus. In addition, PP2A has been found in mitochondrial, cytoskeletal, and membrane compartments [36, 53, 54]. For example, the intermediate filament protein vimentin was demonstrated to associate with PR55/B-containing trimeric PP2A [55] in mammalian fibroblasts. In addition, PR55/B trimers associate with microtubules via a targeting protein which is thought to be tubulin itself [56] or to an elusive heat-labile protein that is not related to classical microtubule-associated proteins [57]. Another form of PP2A is bound to membranes. In mast cells stimulated by antigens, the activity and membrane association of PP2A increases 2-fold [58]. Therefore, in addition to nuclear translocation and cytoskeletal association, PP2A can also associate with membranes upon reception of the appropriate activating signal.

TABLE 2. Inhibitors of protein phosphatase 2A (PP2A)

Inhibitor	Source	Inhibitory potency
Okadaic acid	*Dinoflagellates*	PP2A ~ PP4 > PP1 ~ PP5 >>> PP2B*
Dinophysistoxin-1	*Dinoflagellates*	PP2A > PP1 >>> PP2B
Microcystins	cyanobacteria	PP2A ~ PP1 >>> PP2B
Nodularins/Motuporin	cyanobacteria	PP2A ~ PP1 >>> PP2B
Calyculin A	isolated from marine sponges	PP2A > PP1 >>> PP2B
Tautomycin	*Streptomyces spiroventricillatus*	PP1 > PP2A >>> PP2B
Cantharidin	blister beetles	PP2A > PP1 >>> PP2B
Endothall	synthetic compound	PP2A > PP1 >>> PP2B
Fostriecin	*Streptomyces pulveraceus* subsp. *fostreus*	PP2A ~ PP4*
TF-23A	isolated from marine red alga	PP2A
Cytostatin	*Streptomyces sp.* MJ654-NF4	PP2A
I_1^{PP2A}	cellular inhibitor	PP2A
I_2^{PP2A} (SET, PHAP-II, TAF-1β)	cellular inhibitor	PP2A

*According to available data, the L7 loop is a target for the low molecular non-protein inhibitors listed in this table. PP2Ac and PP4c, which have the same L7 loop sequence, CVYRCG, are most probably affected in a similar manner, not only by okadaic acid and fostriecin but also by other inhibitors. Furthermore, PP6c containing the same L7 loop sequence may be affected similarly to PP2Ac and PP4c by the inhibitors listed in this table.

PP2A AS A MOLECULAR TARGET OF TOXINS

A plethora of low molecular mass inhibitors of PP2A and other protein phosphatases has been identified in nature (Table 2). Okadaic acid, 38-carbon polyether fatty acid, was isolated from marine sponges of the *Halichondria* species including *Halichondria okadaii* [59]. Okadaic acid is produced by several species of marine dinoflagellates and accumulates in filter feeding organisms. In laboratory practice, this compound, which can penetrate cell membranes and potently inhibit PP2Ac, became a revolutionary tool for the study of protein phosphatases [reviewed in 59 and 60]. Okadaic acid also inhibits PP1, but with a 100-fold lower potency. However, when applied to living cells at 1 μM concentration, it does not inhibit PP1 [61]. After the identification of other protein phosphatases, it became clear that the inhibitory effects assigned to PP2A might have actually resulted from okadaic acid inhibition of PP4c [IC_{50}: 0.1 nM, 62] or PP5c [IC_{50}: 1–10 nM, 63].

Both the mutagenesis of PP2A/PP1 [64–66] and the three-dimensional structure of PP1 [67] indicate that the L7 loop in this region, which lies between β-strands 12 and 13 of PP1, is responsible for okadaic acid binding. Based on the identity of the L7 loop sequences (CYRCG) between PP2Ac, PP4c, and PP6c, one can predict that PP6c should also be very sensitive to okadaic acid. Many other PP2A-inhibiting toxins are listed in Table 2, several of which, e.g., microcystins, calyculin A, tautomycin, cantharidin, and endothal are commercially available. Since many inhibitors can affect the activity of several different protein phosphatases, the application of several different inhibitors in parallel experiments may help to distinguish which phosphatases are involved in cellular processes. However, one must realise that from the many protein phosphatases predicted to exist in mammalian cells, just a handful have been identified and characterised to date.

In addition to serving as a laboratory tool, okadaic acid is a causative agent of a form of gastroenteritis, termed diarrhetic shellfish poisoning, which results from eating contaminated mussels. Even more serious health problems can result from the ingestion of microcystins. Based on epidemiological studies, these compounds have been shown to induce liver cancer in humans [59]. Higher doses of these toxins lead to rapid liver damage and eventually death. For example, microcystin-contaminated water used for dialysis resulted in the death of 50 patients in 1996 [68].

The addition of PP2A inhibitors to cells grown in culture often results in apoptosis. Studies on TF-23A, which inhibits PP2A but not PP1, PP2C, and PP2B, brought forth some interesting data. TF-23A induces apoptosis in various leukaemic T- and B-cell lines. However, TF-23A analogues that do not inhibit PP2A still induce apoptosis, indicating that these two properties of TF-23A are not connected [69]. Similar studies done with tautomycin demonstrated that its phosphatase-inhibiting activity is located within the C22–C26 region of the molecule, whereas the C1–C18 region was essential for induction of apoptosis [69]. This could also be the case for other inhibitors used in laboratory practice. Another novel inhibitor, cytostatin, also specifically inhibits PP2A [70]. Interestingly, cytostatin acts as an inhibitor of cell adhesion to the extracellular matrix.

In addition to being used for the inhibition of protein phosphatases, the inhibitors listed in Table 2 can also be applied as affinity reagents for the purification of phosphatases, their regulatory subunits, and phosphatase-interacting proteins. This in most cases involves the immobilisation of microcystin to Sepharose [71]. Microcystin–Sepharose is now commercially available.

CELL CYCLE

Cell cycle progression is regulated by the activity of CDKs that are controlled by reversible phosphorylation. PP2A has been implicated in the regulation of different cell cycle events, due to its multiplicity and different substrate specificity of trimeric holoenzymes (Fig. 2). A significant amount of genetic evidence points towards PP2A as a

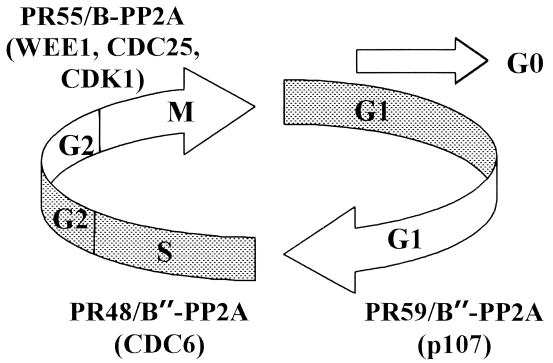

FIG. 2. Holoenzymes of type 2A protein phosphatase (PP2A) involved in the regulation of cell cycle. For simplicity, a PR65/A–PP2Ac dimer was abbreviated to PP2A. Therefore, PR55/B–, PR48/B″–, and PR59/B″–PP2A should be read as trimers composed of PR55/B, PR48/B″, and PR59/B″ in complex with the PR65/A–PP2Ac dimer. Substrates of PP2A holoenzymes are indicated in brackets.

regulator of cell cycle progression. For example, strains of *S. cerevisiae*, which carry a mutation in yeast PR55/B, display a cold-sensitive phenotype with a defect of cell septation and separation [72]. Further analysis of this mutant revealed that the PR55/B subunit is required for a kinetochore/spindle checkpoint [73]. In *Drosophila*, the absence of the PR55/B protein results in an abnormal sister chromatid separation called abnormal anaphase resolution (*aar*) [74]. Other studies also point to a negative role for PP2A in the transition from G2 to M phase, which is controlled by the CDC2–cyclin B complex [reviewed in 11]. The kinase activity of CDC2 kinase is absolutely dependent on the phosphorylation of Thr-161, whereas phosphorylation of two additional sites, Thr-14 and Tyr-15, inhibits the activity of this enzyme. PP2A has been suggested to be an active phosphatase at Thr-161 of CDC2 (Fig. 2). However, other data suggest that CDK-associated protein phosphatase, termed KAP, is phosphatase-active at this site [reviewed in 11]. In contrast, PP2A can inactivate CDC2 indirectly by both the activation of WEE1 kinase, which phosphorylates CDC2 at Tyr-15, and by the inactivation of dual-specificity phosphatase CDC25, which removes phosphate from both Thr-14 and Tyr-15 of CDC2 [reviewed in 11] (Fig. 2).

As discussed above, different regulatory subunits impose substrate specificity on PP2A. In agreement with this, two members of the PR72/B″ family of regulatory subunits, namely PR59 and PR48, have recently been implicated in the dephosphorylation of cell cycle regulators. PR59 specifically targets retinoblastoma protein (pRb)-related p107 for dephosphorylation by PP2A [75]. Similarly as for pRb, phosphorylation of p107 in late G1 phase results in the release and activation of E2F transcription factors. Another member of the PR72/B″ family, PR48, targets PP2A to dephosphorylate CDC6 [28]. CDC6 binds to the origin of replication via interactions with the origin recognition complex. Phosphorylation of CDC6 by CDK2 is required for DNA replication and the export of CDC6 from the nucleus. Since PR48 resides in the cell's nucleus, the role of PR48–PP2A could be to maintain levels of dephosphorylated CDC6 until replication is triggered by CDK2. In agreement with this, experiments performed with a cell-free replication system derived from *Xenopus laevis* eggs demonstrated that PP2A is required for the initiation of chromosomal DNA replication [76].

PP2A-INTERACTING PROTEINS IDENTIFIED BY CO-PURIFICATION

Purification of PP2A from rabbit skeletal muscle resulted in the isolation of a trimeric holoenzyme containing a PR55/Bα regulatory subunit together with the translation termination factor 1 (eRF1) and another protein of 55 kDa [77]. Yeast two-hybrid system analysis demonstrated interactions between PP2Ac and eRF1. Co-immunoprecipitation and isolation of polysomes also revealed interactions between eRF1 and a PR65/A–PP2Ac dimer. At present, the role of PP2A in the termination of protein translation has not been demonstrated. It is therefore possible that eRF1 is a substrate of PP2A or that it takes part in the disassembly of ribosomes, a process which occurs after termination of translation [78]. It remains unknown whether the identified protein of 55 kDa plays any role in PP2A regulation or translational termination.

PROTEIN KINASES AS SUBSTRATES FOR PP2A

PP2A regulates a diverse set of cellular processes, such as metabolism, transcription, RNA splicing, translation, DNA replication, cell cycle progression, signal transduction, differentiation, and oncogenic transformation by reversing the actions of protein kinases [6–12]. However, many protein kinases themselves are regulated by reversible phosphorylation. In recent years, protein kinases have emerged as an important group of substrates for PP2A. This issue has recently been the subject of a thorough review [11] and will as such be discussed only briefly here. *In vitro*, more than 30 protein kinase activities are regulated by PP2A-catalysed dephosphorylation [11]. Most of these are inactivated by dephosphorylation, with the exception of casein kinase 1, GSK-3, MST1, and WEE1 kinases, which are all activated upon dephosphorylation [11]. It remains to be established how many of these protein kinases are *in vivo* substrates of PP2A. Several lines of evidence, including the application of different phosphatase inhibitors, indicate that CDKs specific for G1 phase are substrates of PP2A [79]. In addition, as discussed above, WEE1 is most probably regulated by PP2A. Fewer protein kinases have been demonstrated to interact with PP2A *in vivo*. These include Ca^{2+}/calmodulin-dependent (CaM) kinase IV (CaMKIV), casein kinase 2α (CK2α), Janus kinase 2 (JAK2), p70S6 kinase, and p21-activated kinases (PAK1 and PAK3) [reviewed in 11]. Apparently, complexes containing both protein kinase and phosphoprotein phosphatase are important in maintaining the phosphorylation state of intracel-

lular substrates. It was shown that in a CaM kinase IV–PP2A complex, phosphatase, both dephosphorylates and inactivates kinase, acting as a negative regulator of CREB-mediated transcription in Jurkat T cells. CK2α, the catalytic subunit of CK2, forms a complex with PR65/A–PP2Ac dimers and activates phosphatase activity. As a result, activity of the mitogen-activated protein kinase pathway is deactivated, which leads to suppression of cell growth. JAK2 has been shown to transiently associate with PP2A, phosphatidylinositol 3-kinase (PI-3K), and Yes (a member of the Src tyrosine kinase family) upon interleukin-11 stimulation of adipocytes. It remains to be established whether JAK2 is a substrate for PP2A. PAK1, PAK3, as well as p70S6 kinase, which controls translation, are associated with PP2A *in vivo*. Although the physiological significance of these complexes is currently unknown, they must be important regulators of respective signalling pathways. Several lines of evidence point towards PP2A as a regulator of MAP kinase cascades. Transient expression of SV40 small t induces activation (and transformation) of mitogen-activated protein kinase/ERK (MEK) and extracellular signal-regulated (ERK) kinases, implicating PP2A in their regulation. However, other data suggest that the dual-specificity phosphatase mitogen-activated protein kinase phosphatase-1 (MKP-1) can act as ERK phophatase [11]. Different cells are possibly equipped with different sets of protein phosphatases that regulate MAP kinase pathways.

AKAPs (A kinase-anchoring proteins) have been shown to target both protein kinases and protein phosphatases to the same location [80]. Recently, two such complexes containing PP2A together with different protein kinases have been identified in mammalian cells. A giant scaffolding protein of 450 kDa termed CG-NAP (centrosome- and Golgi-localised PKN-associated protein) anchors four enzymes: PKN (a serine/threonine protein kinase), cAMP-dependent protein kinase (PKA), PP1, and PP2A [81]. Interestingly, PR130, a regulatory subunit belonging to the PR72/B″ family, seems to be responsible for the association of PP2A with CG-NAP. The function of these anchored enzymes located at the centrosome and the Golgi apparatus is not yet known. Another type of multiprotein complex containing PP2A is associated with the Wnt/β-catenin signaling pathway and involved in the regulation of growth and development in vertebrates [82–84]. As a result of this signaling pathway, β-catenin is released from the adenomatous polyposis coli (APC)–axin–GSK3β complex, binds to a member of the LEF/TCF family of transcription factors, and activates the transcription of target genes. On the contrary, β-catenin, which remains anchored to APC/axin, is phosphorylated by GSK3β and undergoes ubiquitin-mediated proteasomal degradation. Inhibition of Wnt/β-catenin signaling is associated with multiple human malignancies, including colon cancer, melanoma, hepatocellular carcinoma, ovarian cancer, endometrial cancer, medulloblastoma pilomatricomas, and prostate cancer. Members of PR61/B′ regulatory subunits of PP2A have been shown to interact with APC protein by analysis with the yeast two-hybrid system [82]. Other studies suggest that axin is a molecular partner of PP2Ac [83, 84]. Obviously, physiological signaling requires an appropriate balance between GSK3β-catalysed phosphorylation of APC complex components including β-catenin, axin, and APC, and PP2A-catalysed dephosphorylation. PP2A may therefore regulate β-catenin stability in response to Wnt signaling.

TRANSCRIPTION FACTORS AS SUBSTRATES OF PP2A

Phosphorylation of transcription factors regulates their trans-activating potential, DNA-binding properties, and intracellular location. As for other cellular processes, much more is known about protein kinases than about phosphoprotein phosphatases regulating transcription. Furthermore, in many studies, the main criterion used to link PP2A involvement to the regulation of transcriptional activity was the application of okadaic acid [85]. Only those studies that utilised multiple experimental approaches for the analysis of PP2A involvement in the regulation of transcriptional factor activity will be discussed in this review. CREB, one of the best-studied transcription factors, is activated by cAMP-dependent kinase phosphorylation at the Ser-133 residue. Based on co-purification and transfection of cells with SV40 small t antigen, PP2A has been implicated in the inactivation of CREB [86, 87]. However, other studies indicate that PP1 functions as a phospho-CREB phosphatase [88]. These observed differences may be due to the different cell lines used to study CREB deactivation. In addition, both phosphatases may also be involved in CREB dephosphorylation, acting either within the same or different signaling pathways.

Microinjection studies with purified PP2A [89] and I_2^{PP2A} expression in HEK 293 cells [90] both demonstrate that this phosphatase regulates c-Jun activity. Contrary to CREB, which is inactivated by PP2A, dephosphorylation of c-Jun induces c-Jun and activator protein-1 (a complex of c-Jun and c-Fos) transcriptional activity. The site of PP2A-catalysed dephosphorylation was preliminarily mapped to c-Jun Ser-63 [90].

E2F transcription factors, which are important for the activation of a variety of genes essential for DNA synthesis, are maintained in an inactive state by members of the retinoblastoma protein family (pRb, p107, and p130) [75]. As already pointed out in the cell cycle section above, PR59-containing trimeric PP2A dephosphorylates p107 and indirectly inactivates E2F by reversing the action of CDK5–cyclin D1 kinase, which phosphorylates p107 [75].

Expression of the SV40 small t antigen is currently being used as a tool in the search for PP2A-dependent processes in mammalian cells. Using this approach, PP2A has recently been implicated in the regulation of transcription factor Sp1 activity. Both PR65/A–PP2Ac dimers as well as trimers containing PR55/B (without PR61/B′ subunits) dephosphorylate Sp1 [91]. PP2A has also been implicated

in the regulation of STAT3. This transcription factor is activated by tyrosine phosphorylation by JAK kinases. In addition, STAT3 serine phosphorylation by mitogen-activated protein (MAP) kinases is induced upon angiotensis II stimulation of vascular smooth muscle cells [92]. Complete transcriptional activity of STAT3 requires both tyrosine and serine phosphorylation. Apparently, PP2A is involved in dephosphorylation of STAT3 at a serine residue [93]. Thus, PP2A can down-regulate STAT3 trans-activating potential.

Both PP1c and PP2Ac are involved in the regulation of HOX11, a transcription factor from a homeobox family that controls spleen formation [94]. Both yeast two-hybrid system analysis and immunoprecipitation were applied in an attempt to identify the N-terminal region of HOX11 as a possible interaction site. Microinjection of HOX11 into *Xenopus* oocytes induced cell cycle arrest at the G2 phase of the cell cycle and promoted progression to the M phase, implying that HOX11 acts as a cellular oncogene [94]. However, it remains to be established whether PP2Ac and/or PP1c controls transcriptional activity or HOX11 DNA binding.

ROLE OF PP2A IN HUMAN DISEASES

PP2A regulates a diverse set of cellular processes such as metabolism, transcription, translation, cell cycle, signal transduction, differentiation, and oncogenic transformation [reviewed in 6–12]. Thus, one may predict that any dysfunction of PP2A would have severe consequences on cell physiology. In agreement with this, it has been demonstrated by gene knockout technology that mice with a disrupted gene, encoding an α isoform of PP2Ac, die *in utero* at embryonic days 5–6.5. This indicates that PP2Acα is indispensable for embryogenesis and cannot be replaced by PP2Acβ [95]. Recent data suggest that the embryonic lethality results from defects in cell adhesion caused by insufficient levels of membrane-associated E-cadherin and β-catenin [5], which is in agreement with a proposed role of PP2A in Wnt/β-catenin signaling [82–84]. Several examples point out that PP2A also plays a very important role in human cells: (i) Naturally occurring inhibitors of PP2A, which are produced by microorganisms, are causative agents of human diseases, such as diarrhetic shellfish poisoning (okadaic acid) and hepatocarcinoma (microcystins and nodularins) [59]; (ii) Fostriecin, which is an inhibitor of PP2A and topoisomerase II, is currently being tested clinically as a possible anticancer drug [96]; (iii) Pathogenic viruses, such as human adenovirus type 5 and HIV-1, encode PP2A-interacting proteins [97, 98]; (iv) Human cytomegalovirus, which is one of the principal causes of congenital malformation, carries host-cell-derived PP2A, associated with the nuleocapsid fraction [99]; (v) Mutations identified in the human *PP2R1B* gene, which encodes the β isoform of PR65/A, were found in samples of primary colon and lung tumours, indicating that PR65/Aβ may act as a tumour suppressor [100]. More recently, mutations in the *PPP2R1B* have also been identified in breast cancer [101]. In addition, mutations in the gene termed *PPP2R1A*, encoding an α isoform of the PR65/A, have been identified in breast and lung carcinoma and melanoma [101]. Although the frequency of these mutations is low, they clearly implicate PP2A as a participant in tumorogenesis; and (vi) The myeloid leukaemia-associated protein SET is a potent inhibitor of PP2A [47, 48]. Obviously, the examination of phenotypes of mice lacking functional genes encoding various regulatory subunits of PP2A will shed more light on the physiological functions of this enzyme.

CONCLUDING REMARKS

Currently, 119 genes encoding protein phosphatases have been identified in the human genome, including 21 belonging to phosphoserine-phosphothreonine-specific (PPP/PPM) families and 98 members of the phosphotyrosine-specific (PTP) family, and obviously this number is going to increase [1]. Why is PP2A such a biologically important and frequently studied phosphoprotein phosphatase? The answer may be trivial—it is very abundant (0.05–0.1% of cellular proteins) and its activity is easy to measure with artificial substrates such as ^{32}P-labelled phosphorylase *a*. In addition, there are several easily available inhibitors to study its function. However, examples presented in this review point out that PP2A is also a very unique phosphatase that regulates basic cellular functions and is indispensable for the survival of eukaryotic organisms. In recent years, several protein kinases have been isolated in complexes with PP2A, and protein kinases appear to be an important group of PP2A substrates. In addition, PP2A regulates the activity of several transcription factors. In order to understand PP2A function in complexes with protein kinases and transcription factors as well as other PP2A-interacting proteins, a three-dimensional structure of PP2Ac is awaited. Apparently, PP2Ac overexpressed in prokaryotic host does not display phosphatase activity. This may be due to the absence of appropriate posttranslational modification/s of recombinant PP2Ac and/or lack of chaperones necessary to fold this phosphatase into an active conformation. Obviously, the chase is on to produce amounts of PP2Ac sufficient for crystallisation using eukaryotic hosts such as insect cells or yeast *Pichia pastoris*. One may predict that after solving the three-dimensional structure of the catalytic subunit, structures of two- and three-subunit complexes of PP2A will be elucidated. In addition, complexes of PP2A with different low molecular weight inhibitors will be studied in an attempt to answer a long-standing puzzle concerning the ability of structurally unrelated chemical compounds to inhibit this phosphatase. Several data accumulated in recent years point out that PP2A associated with subcellular structures plays as important role as a soluble form of this enzyme and that different intracellular pools of PP2A are in strictly controlled equilibrium. As described in this review, PP2A forms complexes with a plethora of regulatory subunits, but it remains to be

demonstrated whether any of them is responsible for targeting this phosphatase to intracellular membranes and/or mitochondria. To the contrary, posttranslational modification/s of already identified regulatory subunits may be responsible for intracellular trafficking of PP2A. In order to gain insights into functions of PP2A regulatory subunits, several laboratories are currently in the process of making and characterising mice knockouts. Finally, one of the most difficult questions which remains is to identify *in vivo* substrates of PP2A. This challenge may be solved with the help of dominant negative mutants of PP2Ac applied as molecular traps. But even if it is known that a particular phosphoprotein is a substrate of PP2A, it will remain to be demonstrated whether other protein phosphatases may act on the same protein removing, for instance, different phosphate groups. This hypothetical sequential dephosphorylation would resemble a process of sequential phosphorylation described for protein kinases. Furthermore, if a particular protein is a substrate of PP2A, one may ask whether in a different cellular context other phosphatases may remove this particular phospho-residue instead of PP2A. Taking into account the above conclusions and questions, one may argue that even though numerous newly discovered phosphatases have emerged in this new era of human proteomics, PP2A remains one of the most important phosphoprotein phosphatases studied today.

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