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COMMENTARY

Protein Phosphatase 2A: Who Shall Regulate the Regulator?

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ABSTRACT. Protein phosphatases are responsible for keeping the signaling output of stimulus-activated protein kinases in check; but protein phosphatases are also themselves targets and conveyors of biological signals. Among the major serine/threonine phosphatases, protein phosphatase 2A (PP2A) appears to play a privileged role in the regulation of cell growth and division. How PP2A is regulated is an intriguing question. This review will focus on the role of local protein–protein interactions in PP2A control. Work from a number of laboratories has shown that the catalytic activity, substrate specificity, and subcellular targeting of PP2A are regulated by a remarkably diverse range of regulatory subunits and enzyme inhibitors. On the pathological side, DNA tumor viruses subvert PP2A function by producing proteins that compete with specific regulatory subunits. By interfering with PP2A, these viral proteins can elicit changes in the activity of specific signal transduction pathways, such as the mitogen-activated protein kinase cascade. Recent data indicate that besides classical holoenzyme forms, a fraction of PP2A molecules are associated with novel partners implicated in signal transduction. PP2A biochemically and genetically interacts with the Tap42/α4 protein, which is part of a rapamycin-sensitive pathway that connects extracellular stimuli to the initiation of mRNA translation. PP2A also binds to CK2α, the catalytic subunit of CK2 (formerly casein kinase 2), and binding is sensitive to mitogenic signaling. The potent effect of quantitatively minor PP2A partners might be explained by a general requirement for docking interactions with substrates under intracellular conditions. BIOCHEM PHARMACOL **57**;4:321–328, 1999. © 1999 Elsevier Science Inc.

KEY WORDS. phosphatase; PP2A; small t antigen; Tor; rapamycin; CK2

PP2A† AND THE REGULATION PROBLEM

The binding of extracellular ligands to membrane receptors invariably triggers changes in the phosphorylation of specific proteins. It is well established that variations (most often increases) in the activity of specific protein kinases play a fundamental role in this process. Theoretically, however, changes in the action of phosphoprotein phosphatases could also play a part; such changes have indeed been demonstrated in a number of cases, but much remains to be learned about this potentially critical aspect of intracellular signaling [1–3]. The issue of protein phosphatase regulation—does it occur? is it important? how is it accomplished?—is especially intriguing in the case of PP2A, the major soluble serine/threonine phosphatase of animal cells [1, 4, 5]. PP2A is conserved from yeast to humans. It is a primary target for a variety of natural toxins and tumor promoters [6, 7] and also viral proteins [8] (see below). *In vitro* dephosphorylation assays with growth-regulatory phosphoproteins, experiments with cell-permeant phosphatase inhibitors such as okadaic acid, and studies of mutants in genetically tractable organisms, all indicate that PP2A activity regulates signaling pathways that are critical to cell growth [3, 8, 9]. Often, PP2A is required for down-regulating activated pathways [3, 5]. For example, in at least some cell types PP2A is very likely responsible for reversing the growth factor- or hormone-induced stimulation of MAP kinase, thereby keeping the extent and duration of MAP kinase activation under control [10, 11]. Similarly, PP2A removes an activating phosphate from the mitosis-triggering protein kinase $p34^{cdc2}$ [12], thus preventing premature entry into mitosis. The very abundance of PP2A in cells (up to 0.25% of total cell protein) has raised the idea that transient inhibition of this phosphatase might be needed for MAP kinase or $p34^{cdc2}$ signaling to proceed [5]. Yet, unlike other related protein phosphatases such as PP1 or the calcium-responsive calcineurin [2, 13], total cellular PP2A activity does not display significant responses to physiological inputs such as hormonal stimulation or cell-cycle progression [14]. Moreover, it is puzzling to confront the broad range of PP2A substrates *in vitro* [1] with the exquisite time- and location-

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† *Abbreviations:* PP2A, protein phosphatase 2A; MAP kinase, mitogen-activated protein kinase; MAPKK, MAP kinase kinase; *aar*, abnormal anaphase resolution; PKC, protein kinase C; PI 3-kinase, phosphatidylinositol 3-kinase; PP1, protein phosphatase 1; and FKBP, FK506-binding protein.

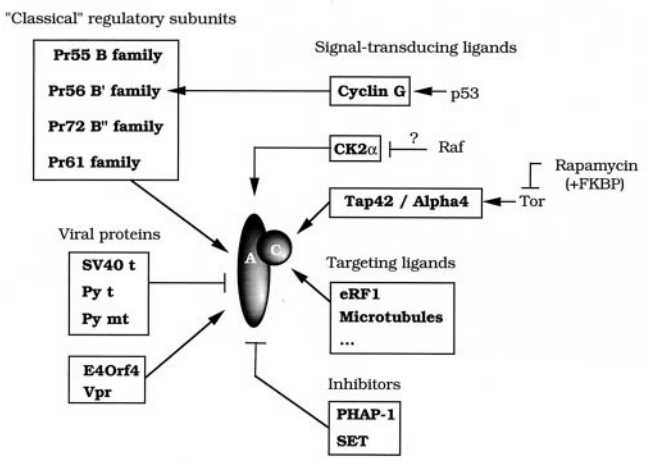

FIG. 1. Some of the proteins that bind to PP2A (shown as core A-C dimer) and regulate its activity. See text for explanation.

specificity of many PP2A-dependent dephosphorylation events *in vivo*. Possible solutions to these apparent paradoxes are now emerging out of a novel, complex scheme, in which control of PP2A by protein–protein interactions plays a major role (Fig. 1).

DIVERSITY OF PP2A SUBUNITS

The molecular structure of PP2A has been summarized in a number of reviews (see, for example, Refs. 9 and 15). Phosphatase activity is carried by a 36-kDa catalytic subunit (C subunit or PP2Ac). In cells, C is never free, but usually bound to a 65-kDa "scaffolding" subunit (A subunit or PR65), a rod-shaped molecule made of 15 imperfect sequence repeats [16]. *In vitro*, the resulting "core" (A-C) dimer is less active toward phosphoprotein substrates than is the isolated C subunit [17]. A fraction (up to 30–50%) of the PP2A isolated from cells or tissues exists as the core dimer, which according to recent experiments [18] reflects the *in vivo* situation rather than a purification artifact. However, a majority of PP2A molecules are trimers that contain a variable regulatory "B" subunit bound to the A-C dimer [1, 18]. Two striking features of the B-type subunits are their diversity, stemming from the existence of entire subunit families, and the total lack of sequence similarity between distinct families, even though they recognize similar repeated segments of the A subunit. Basic types of regulatory subunits include: B or PR55; B′ or PR56; B″ or PR72/130; and PR61 [19, 20]. Each type defines a family (e.g. B_α and B_β) encoded by several homologous genes, some of which may generate splice variants [21]. This diversity is thought to have important functional consequences: experiments with recombinant trimers demonstrate that distinct regulatory species have quantitatively different effects on enzymological properties, such as substrate preference or sensitivity to *in vitro* stimulatory agents [22]. *In vivo*, the A-C dimer is found in both the cytoplasm and the nucleus [23], but immunofluorescence studies show that certain regulatory subunit types are preferentially localized to either of these compartments [24]. Therefore, at least some regulatory subunits appear to have a targeting function, akin to that of PP1 regulatory subunits [2, 25]. Furthermore, the abundance of certain regulatory subunits varies in a tissue-specific fashion [22, 26, 27] and during *in vitro* differentiation processes [28]. Thus, the set of regulatory subunits in a cell is determined by the differentiation state, and, in turn, effectively controls the substrate- and location-specific rate of PP2A-mediated dephosphorylation. One prediction of this model is that the lack of a specific regulatory subunit *in vivo* will result in a defect in a specific range of dephosphorylation reactions, while retaining the ability to perform another set of reactions. This conclusion is supported by both genetic and biochemical data. In *Saccharomyces cerevisiae*, deficiencies in B and B′ generate qualitatively different (nonlethal) phenotypes; whereas expression of rabbit B′ isoforms can complement the yeast B′ deficiency, yeast B cannot, demonstrating that the specific functions of B and B′ are evolutionarily conserved [29]. In *Drosophila*, the *aar*^{1} mutants are deficient in B, and their early development is compromised due to deficient anaphase in the dividing embryo [30]. Compared with wild-type, the *in vitro* activity of PP2A from *aar*^{1} flies is strongly reduced when p34^{cdc2}-phosphorylated caldesmon or histone H1 is used as a substrate, but it stays normal when assayed with phosphorylase *a* (a canonical model substrate for PP2A) [31]. Therefore, it is believed that the 55-kDa B is necessary *in vivo* for allowing PP2A to dephosphorylate substrates (probably phosphorylated by p34^{cdc2} [32]) that control progression through mitosis.

PP2A AS A TARGET FOR VIRUS ACTION

The idea that subunit composition dictates the *in vivo* substrate specificity of PP2A is strongly supported by the subversive effect of certain viral proteins on the enzyme. PP2A is a privileged target for viral small t antigens, proteins that are required by DNA tumor viruses of the SV40 and polyoma types for fully expressing their transforming potential [8, 9, 33]. The best-studied case is that of SV40 small t, a protein that competes with the 55-kDa B subunit for binding to the AC complex. Compared to the normal ABC trimer, small t changes the substrate specificity of the phosphatase in such a way that it inhibits the *in vitro* dephosphorylation of many (but not all) substrates [34], including growth-controlling proteins such as MAP kinase and its upstream activator MAPKK [10]. Expression of small t in cultured cells results in mitogen-independent activation of MAPKK and MAP kinase, and stimulation of cell proliferation; studies with various small t mutants indicate that these effects require the binding of small t to PP2A [10]. Thus, *in vivo* small t appears to redirect PP2A activity away from phosphoproteins that are critical to the activation of the MAPK pathway. The identity of these putative PP2A substrates is not clear, however. Based on the *in vitro* data, small t likely blocks the dephosphorylation

of MAPKK and MAP kinase, but as these kinases are not spontaneously active, it would seem that, in addition, small t still needs to stimulate some upstream kinase to initiate the process. Of note, the classical MAPKK activator Raf is not affected by small t expression [10]. Recent experiments identified PKCζ, a nonclassical PKC, as the small t-stimulated activator of MAPKK; stimulation of PKCζ, in turn, depends on PI 3-kinase function, and again requires small t–PP2A interaction [35]. It is not known how small t causes PI 3-kinase to activate PKCζ.

Like SV40 and polyoma, adenoviruses transform cells by expressing polypeptides that bind to cellular growth-regulatory proteins, perturbing their function. Interestingly, an adenoviral protein known as E4Orf4 binds to trimeric PP2A and targets the phosphatase to specific transcription factor complexes. The outcome is inhibition of the JunB and viral E4 promoters, which apparently plays a role in preventing apoptosis of adenovirus-infected cells [36].

Another example of PP2A perturbation by viral proteins may come from the human immunodeficiency virus (HIV-1). In HIV-infected T cells, the viral protein Vpr has been shown to inhibit the G_2-M transition of the cell cycle, thereby favoring expression of the retroviral long terminal repeat (LTR) [37]. When associated with another viral protein (NCp7), Vpr has been found to bind to one of the PP2A trimer species *in vitro*, and to cause enhanced phosphatase activity toward a model substrate [38]. In view of the role of PP2A in preventing premature entry into mitosis, it is tempting to draw a connection between these two observations. It is not yet known, however, if the PP2A–Vpr interaction also occurs *in vivo*, and to what extent the interaction correlates with the cell-cycle blocking activity of Vpr.

LOCAL REGULATION OF PP2A

The effects of viral proteins provide a demonstration that changes in PP2A subunits can affect cellular signaling pathways in a highly specific fashion, without necessarily affecting the entire PP2A complement. *In vivo*, viral ligands of PP2A seem to affect very specific dephosphorylation reactions. This makes sense, as massive, nonspecific increases or decreases in PP2A activity would likely be lethal to the cell. Could physiological stimuli modulate PP2A in just as specific a fashion? Binding of PP2A to B-type regulatory subunits has not been reported to undergo modifications similar to the hormone- or cell-cycle-induced changes seen, for example, in the PP1 oligomeric state [2, 39]. However, signals may modulate specific subpopulations of PP2A molecules. While most of PP2A is soluble, distinct pools of enzyme associate with specific subcellular structures, e.g. microtubules [40], neurofilaments [41], or membranes [42]. Remarkably, the microtubule-associated pool undergoes cell-cycle dependent activity changes, with a 20-fold drop in specific activity in G_2/M [40]. Also, the nuclear and cytoplasmic fractions of soluble PP2A undergo differential changes in carboxymethylation during the cell cycle [23]. PP2A can also be targeted to particular molecules or molecular complexes, as exemplified above in the case of E4Orf4. These molecular targets may influence local PP2A activity. For example, a leukemogenic mutant of the HRX homeobox transcription factor has been shown to recruit a complex made of PP2A and the nuclear protein SET [43]. SET is a potent and specific PP2A inhibitor and can, itself, give rise to a fusion protein implicated in acute myeloid leukemia [44]. One may speculate that the leukemogenic fusion proteins deregulate the phosphorylation level of transcription factors critical to hematopoietic cell growth.

All of these data support the idea that PP2A could be regulated locally, but they do not provide the enzyme with an explicit molecular connection to stimulus-dependent signaling pathways. Such a connection clearly emerges from the work described below.

A ROLE FOR PP2A IN RAPAMYCIN-SENSITIVE PATHWAYS

New insights into the question of PP2A regulation have come recently from yeast genetics. Budding yeast has two homologues of the PP2A C subunit, known as Pph21 and Pph22 [45], whose function is absolutely required, mostly during G_2, for reorganization of the actin cytoskeleton, bud morphogenesis, and G_2-M transition [46]; in addition, *S. cerevisiae* contains a PP2A-related serine/threonine phosphatase called Sit4, required during G_1 for S phase entry and spindle pole body duplication [47]. Just like mammalian C, both Pph21/2 and Sit4 are bound to regulatory subunits, and, in particular, Pph21/2 exists in a complex with yeast homologues of A and B. The *TAP42* (for two A phosphatase associated protein) gene of budding yeast was isolated as a multicopy suppressor of the *SIT4* deficiency, and encodes a novel dimerization partner of both Sit4 and Pph21/2 [48]. Interestingly, Pph21 can bind Tap42 directly, i.e. independently of A and B subunits; this is unusual in view of the previously universal requirement for A as an anchor for PP2A partners. Tap42-associated Pph21 accounts for only about 2% of the total cellular PP2A. The Tap42–Sit4 complex is a similarly minor form of the Sit4 phosphatase. As is often the case, however, quantitatively minor does not mean unimportant, since contrary to B [29] and even A [49] subunits, Tap42 is indispensable for viability [48]. In genetic experiments, Tap42 overexpression activates a latent growth-suppressive function of both Sit4 and Pph21 [48]. It is thus possible that Tap42 stimulates the *in vivo* activities of the phosphatases against specific phosphoprotein substrates, in a fashion similar to B-type regulatory subunits.

A revealing finding is that the Tap42–PP2A and Tap42–Sit4 complexes are disrupted upon nutrient deprivation or treatment of the cells with the immunosuppressive macrolide rapamycin [48]. Both of these conditions lead to inhibition of the Tor pathway, a newly described signaling pathway that appears to play a major role in the control of

cell growth [50]. In *S. cerevisiae*, Tor (target of rapamycin), a protein kinase related to PI 3-kinase and DNA-dependent protein kinase, functions in a pathway that connects nutrient stimulation to the initiation of protein synthesis. In the presence of nutrients, Tor function is required for initiating translation of a group of mRNAs, including G_1 cyclin mRNAs [51]. Rapamycin binds to the cyclophilin FKBP, and the resulting complex specifically inhibits Tor function; the outcome is growth arrest in a G_0-like state, probably due to a lack of G_1 cyclins. The identity of the effectors that connect Tor to initiation of translation is unclear. The effect of rapamycin and starvation on the Tap42–phosphatase complexes strongly suggests that formation of these complexes necessitates Tor activity. That the complexes are disrupted in Tor-defective mutants demonstrates this point. Gain of *TAP42* function in a temperature-sensitive mutant renders cells resistant to rapamycin, confirming that Tap42 and, hence, presumably the Tap42–Sit4 and Tap42–Pph21/2 complexes are downstream effectors of Tor [48]. Thus, when associated with Tap42, PP2A effectively conveys messages issued by a stimulus-sensitive signaling pathway. Identification of targets for the Tap42–Pph21/2 and Tap42–Sit4 complexes is eagerly awaited.

The discovery of Tap42 has shed light on PP2A function in lymphocyte signaling. Tap42 has a homologue in the mouse, called the α4 protein, which is known to associate with the B cell IgM receptor [48, 52]. Upon cross-linking of the receptor with anti-IgM, α4 becomes phosphorylated on both serine and tyrosine, suggesting that α4 may be implicated in receptor-initiated signaling [52]. The discovery of the Tap42–Sit4 and Tap42–Pph21 complexes prompted a search for an α4-associated type 2A phosphatase in B cells. This was fruitful, since α4 was found to bind to the C subunit, independently of the usual A- and B-type partners [53]. *In vitro*, addition of purified α4 markedly stimulated the activity of C against MAP kinase-phosphorylated myelin basic protein, relative to activity against phosphorylase *a*. The A subunit did not confer similar substrate preference. Thus, α4 potently favors PP2A activity against specific substrates, consistent with what was predicted in the case of Tap42 [53]. Remarkably, treating B cells with rapamycin led to disruption of the α4–PP2A complex [53], suggesting that formation of the α4–PP2A complex may depend upon the activity of mTor, the mouse homologue of *S. cerevisiae* Tor [54]. mTor is thought to function in a mitogen-inducible pathway that connects PI 3-kinase to the translation inhibitor 4E-BP1 and the serine/threonine kinase $p70^{S6K}$ [50]. By phosphorylating 4E-BP1 and $p70^{S6K}$, mTor may contribute to the stimulation of protein synthesis brought about by mitogens [55–58]. In the case of B cells, antigen signaling may stimulate α4-associated PP2A and enhance the dephosphorylation of specific targets implicated in the Tor pathway. It is still unknown whether the α4–PP2A complex plays a part in the control of 4E-BP1 or $p70^{S6K}$.

Intriguingly, a possible direct relationship between PP2A and translation control has emerged from another side, with the observation that the C subunit binds to the eRF1 translation termination factor [59]. Just as with the α4 protein, the eRF1-C association can occur independently of A and B subunits. The role of PP2A in translation initiation and translation termination clearly warrants further investigation.

INTERACTION OF PP2A WITH PROTEIN KINASES

It has long been proposed that PP2A activity could be regulated by phosphorylation; this topic has been reviewed recently [4, 5]. Tyrosine kinases such as v-Src or the epidermal growth factor (EGF) receptor can phosphorylate the carboxy-terminal region of the C subunit *in vitro*; this results in inhibition of the enzyme [60]. Extensive phosphorylation of the relevant tyrosine residue occurs in mitogen-stimulated or v-Src-transformed cells [61]. Inhibitory phosphorylation of C by a novel serine/threonine kinase ("autophosphorylation-activated protein kinase") has also been reported [62]. The down-regulatory effect of mitogen-induced phosphorylation on PP2A activity may, in fact, be highly underestimated, due to the propensity of the enzyme to dephosphorylate itself during *in vitro* assays [5, 60]. *In vivo*, this self-dephosphorylation reaction is likely responsible for making down-regulation transient. The ability of mitogenic tyrosine kinases to inhibit PP2A activity is reminiscent of small t antigen-mediated inhibition, and makes sense in the light of a need to help activation of MAP kinase (and perhaps other pathways) against the powerful "dragging force" due to PP2A activity.

More recently, binding of PP2A to protein kinase subunits has been described, which opens up novel possibilities for signal-dependent regulation of PP2A. The core dimer binds to CK2α, the catalytic subunit of the ubiquitous serine/threonine kinase CK2 (formerly casein kinase 2) [63]; and one type of B subunit binds to cyclin G, the regulatory subunit of the cyclin-dependent kinase cdk5 [64]. Both of these associations seem to be modulated by signaling events, i.e. mitogenic stimulation and DNA damage, respectively.

The CK2α–PP2A complex was observed in *in vitro* assays with purified proteins, and in immunoprecipitates from serum-starved cells. The C subunit was the major coprecipitating substrate phosphorylated by CK2α in immune kinase assays performed with epitope-tagged CK2α, but whether it is a substrate for endogenous CK2α *in vivo* still needs to be established. Mitogenic stimulation or expression of v-Raf resulted in significant dissociation of the CK2α–PP2A complex. Mitogen treatment and v-Raf expression both induce phosphorylation of CK2α (unpublished data); possibly these modifications are related to the disruption of the complex. CK2α belongs to the so-called CMCG group of protein kinases, which also includes GSK-3, MAP kinases, and cyclin-dependent kinases, in

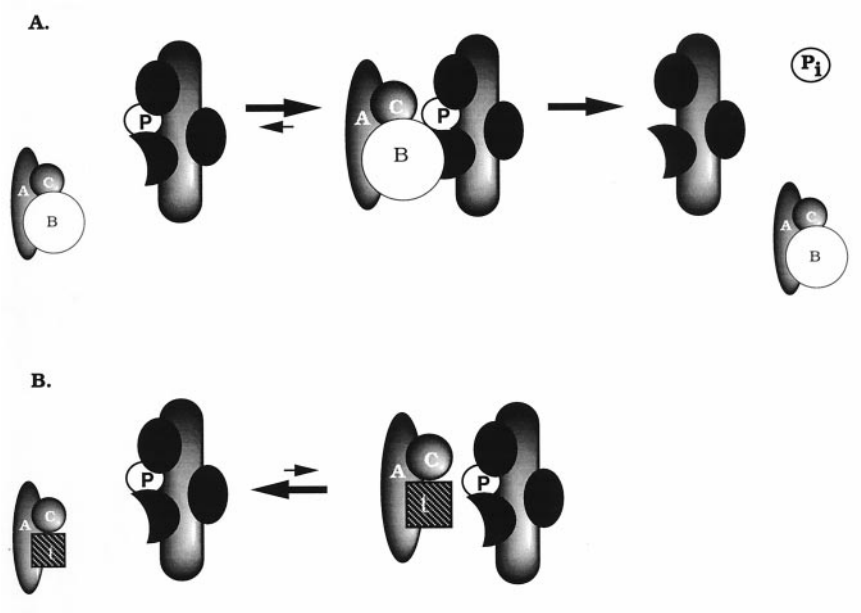

FIG. 2. Role of protein–protein interactions in intracellular substrate specificity of PP2A. (A) A regulatory subunit (B) allows transient docking and/or correct presentation of PP2A to a protein complex that includes a phosphatase substrate. Efficient dephosphorylation ensues. (B) A nonadequate subunit (t) does not allow a high frequency of productive interaction.

decreasing order of relatedness to CK2α [65]. However, unlike the former kinases, CK2α has not been known to be responsive to identified physiological inputs, and its signaling function is quite mysterious [66]. The sequence of CK2α contains a unique, phylogenetically conserved 6-residue motif (HEN/HRKL) [65] that is also found in the PP2A-binding region of the SV40 small t antigen, and a CK2α mutant carrying alanine substitutions in this motif failed to bind PP2A *in vitro*. Thus, some of the contacts between CK2α and PP2A may be homologous to those established by small t, though to our knowledge the motif has not been mutated in small t itself. CK2α might act as a signal-sensitive targeting subunit for PP2A, perhaps favoring PP2A interaction with some of the very substrates whose dephosphorylation is prevented by small t [63]. Genetic studies in both *S. cerevisiae* and *Schizosaccharomyces pombe* indicate that CK2α functions in a cell polarizing pathway [67, 68]. As PP2A is also required for yeast cell polarization [69], it may be worthwhile to look for genetic interactions between PP2A and CK2α in this system.

Two-hybrid assays have indicated that the B′ type of PP2A regulatory subunit could associate with cyclin G, a novel member of the cyclin family [64, 70]. Cyclin G is a p53-responsive gene product, induced upon activation of p53 by DNA-damaging agents [71]. Cyclin G has been shown to complex with GAK, a novel serine/threonine kinase, and cdk5, a neuron-specific member of the CDK family [72]. The cyclin G–B′ interaction suggests a number of possibilities, prominent among which is the idea that p53 may modulate PP2A composition and hence its function, perhaps by adding a targeting subunit to the PP2A oligomer. However, it is not known whether cyclin G and B′ still associate when either or both of these proteins are bound to their normal catalytic partners.

CONCLUDING REMARKS: STICKING WITH THE PURPOSE OF DEPHOSPHORYLATION

The currently accepted model of PP2A regulation calls for a diversity of regulatory subunits in order to target the enzyme to various substrates in the cell. Lending added credence to this idea is the finding that certain PP2A-dependent reactions are potently modulated by quantitatively minor oligomerization partners, such as Tap42/α4. One assumption behind the model, however, is that despite its soluble character and striking abundance, the major PP2A holoenzyme (A-B-C) is unable to dephosphorylate *in vivo* a number of substrates that it sometimes readily handles in the test tube. How is this specificity increase achieved? Several factors may be invoked. First, PP2A activity in the cell is, of course, being opposed by protein kinases [3] and also by PP2A-inhibitory molecules [44, 73, 74]; this may conceivably expand relatively modest kinetic differences between PP2A isoforms into large gaps in the stoichiometry of phosphorylation of the final substrates, especially when substrates are abundant [75]. A second, perhaps more fundamental explanation, may lie with the recent observation that many (all?) signal-transducing phosphorylation events take place in anchored supramolecular complexes [76]. Examples include the recruitment of transducing proteins to activated receptor tyrosine kinases [76], the requirement of a "catch-all" scaffolding protein for activation of the MAP kinase pathway in yeast [77], or the role of various anchoring molecules ("AKAPs," A-kinase anchoring proteins) in PKA function [78]. One may speculate that, perhaps due to steric hindrance, cytosolic PP2A does not easily reach its substrates within such complexes. Only by acquiring specific, high-affinity binding subunits could PP2A remain adsorbed long enough to increase its probability of productive interaction with its

targets (Fig. 2). The availability of some critical subunits may then, in turn, depend upon signaling events, as is seen in the case of Tap42/α4. Hence, the specificity of dephosphorylation reactions may rely on the same "proof-reading by supramolecular assembly" principle as that proposed for phosphorylation events.

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Note added in proof: complex formation between PP2A and protein kinases has just been demonstrated in novel cases, and may in fact be widespread (Westphal RS, Anderson KA, Means AR and Wadzinski BE, A signaling complex of Ca^{2+}-calmodulin-dependent protein kinase IV and protein phosphatase 2A. *Science* **280**: 1258–1261, 1998).

References

1. Cohen P, The structure and regulation of protein phosphatases. *Annu Rev Biochem* **58:** 453–508, 1989.
2. Cohen P, Signal integration at the level of protein kinases, protein phosphatases and their substrates. *Trends Biochem Sci* **17:** 408–413, 1992.
3. Hunter T, Protein kinases and phosphatases: The yin and yang of protein phosphorylation and signaling. *Cell* **80:** 225–236, 1995.
4. Brautigan DL, Flicking the switches: Phosphorylation of serine/threonine protein phosphatases. *Semin Cancer Biol* **6:** 211–217, 1995.
5. Brautigan DL, Phosphatases as partners in signaling networks. *Adv Second Messenger Phosphoprotein Res* **31:** 113–124, 1997.
6. Ishihara H, Martin BL, Brautigan DL, Karaki H, Ozaki H, Kato Y, Fusetani N, Watabe S, Hashimoto K, Uemura D and Hartshorne DJ, Calyculin A and okadaic acid: Inhibitors of protein phosphatase activity. *Biochem Biophys Res Commun* **159:** 871–877, 1989.
7. Toivola DM, Eriksson JE and Brautigan DL, Identification of protein phosphatase 2A as the primary target for microcystin-LR in rat liver homogenates. *FEBS Lett* **344:** 175–180, 1994.
8. Mumby M, Regulation by tumour antigens defines a role for PP2A in signal transduction. *Semin Cancer Biol* **6:** 229–237, 1995.
9. Walter G and Mumby M, Protein serine/threonine phosphatases and cell transformation. *Biochim Biophys Acta* **1155:** 207–226, 1993.
10. Sontag E, Fedorov S, Kamibayashi C, Robbins D, Cobb M and Mumby M, The interaction of SV40 small tumor antigen with protein phosphatase 2A stimulates the MAP kinase pathway and induces cell proliferation. *Cell* **75:** 887–897, 1993.
11. Alessi DR, Gomez N, Moorhead G, Lewis T, Keyse SM and Cohen P, Inactivation of p42 MAP kinase by protein phosphatase 2A and a protein tyrosine phosphatase, but not CL100, in various cell lines. *Curr Biol* **5:** 283–295, 1995.
12. Lee TH, Solomon MJ, Mumby MC and Kirschner MW, INH, a negative regulator of MPF, is a form of protein phosphatase 2A. *Cell* **64:** 415–423, 1991.
13. Mulkey RM, Endo S, Shenolikar S and Malenka RC, Involvement of a calcineurin/inhibitor-1 phosphatase cascade in hippocampal long-term depression. *Nature* **369:** 486–488, 1994.
14. Ruediger R, Van Wart Hood JE, Mumby M and Walter G, Constant expression and activity of protein phosphatase 2A in synchronized cells. *Mol Cell Biol* **11:** 4282–4285, 1991.
15. Shenolikar S, Protein serine/threonine phosphatases—New avenues for cell regulation. *Annu Rev Cell Biol* **10:** 55–86, 1994.
16. Ruediger R, Hentz M, Fait J, Mumby M and Walter G, Molecular model of the A subunit of protein phosphatase 2A: Interaction with other subunits and tumor antigens. *J Virol* **68:** 123–129, 1994.
17. Turowski P, Favre B, Campbell KS, Lamb NJC and Hemmings BA, Modulation of the enzymatic properties of protein phosphatase 2A catalytic subunit by the recombinant 65-kDa regulatory subunit PR65α. *Eur J Biochem* **248:** 200–208, 1997.
18. Kremmer E, Ohst K, Kiefer J, Brewis N and Walter G, Separation of PP2A core enzyme and holoenzyme with monoclonal antibodies against the regulatory A subunit: Abundant expression of both forms in cells. *Mol Cell Biol* **17:** 1692–1701, 1997.
19. McCright B and Virshup DM, Identification of a new family of protein phosphatase 2A regulatory subunits. *J Biol Chem* **270:** 26123–26128, 1995.
20. Zolnierowicz S, Van Hoof C, Andjelkovic N, Cron P, Stevens I, Merlevede W, Goris J and Hemmings BA, The variable subunit associated with protein phosphatase 2A0 defines a novel multimember family of regulatory subunits. *Biochem J* **317**(Pt 1): 187–194, 1996.
21. Zolnierowicz S, Csortos C, Bondor J, Verin A, Mumby MC and DePaoli-Roach AA, Diversity in the regulatory B-subunits of protein phosphatase 2A: Identification of a novel isoform highly expressed in brain. *Biochemistry* **33:** 11858–11867, 1994.
22. Kamibayashi C, Estes R, Lickteig RL, Yang SI, Craft C and Mumby MC, Comparison of heterotrimeric protein phosphatase 2A containing different B subunits. *J Biol Chem* **269:** 20139–20148, 1994.
23. Turowski P, Fernandez A, Favre B, Lamb NJ and Hemmings BA, Differential methylation and altered conformation of cytoplasmic and nuclear forms of protein phosphatase 2A during cell cycle progression. *J Cell Biol* **129:** 397–410, 1995.
24. McCright B, Rivers AM, Audlin S and Virshup DM, The B56 family of protein phosphatase 2A (PP2A) regulatory subunits encodes differentiation-induced phosphoproteins that target PP2A to both nucleus and cytoplasm. *J Biol Chem* **271:** 22081–22089, 1996.
25. Hubbard MJ and Cohen P, On target with a new mechanism for the regulation of protein phosphorylation. *Trends Biochem Sci* **18:** 172–177, 1993.
26. Tehrani MA, Mumby MC and Kamibayashi C, Identification of a novel protein phosphatase 2A regulatory subunit highly expressed in muscle. *J Biol Chem* **271:** 5164–5170, 1996.
27. Strack S, Zaucha JA, Ebner FF, Colbran RJ and Wadzinski BE, Brain protein phosphatase 2A: Developmental regulation and distinct cellular and subcellular localization by B subunits. *J Comp Neurol* **392:** 515–527, 1998.
28. Zhu T, Matsuzawa S, Mizuno Y, Kamibayashi C, Mumby MC, Andjelkovic N, Hemmings BA, Onoé K and Kikuchi K, The interconversion of protein phosphatase 2A between $PP2A_1$ and $PP2A_0$ during retinoic acid-induced granulocytic differentiation and a modification on the catalytic subunit in S phase of HL-60 cells. *Arch Biochem Biophys* **339:** 210–217, 1997.
29. Zhao Y, Boguslawski G, Zitomer RS and DePaoli-Roach AA, *Saccharomyces cerevisiae* homologs of mammalian B and B′ subunits of protein phosphatase 2A direct the enzyme to distinct cellular functions. *J Biol Chem* **272:** 8256–8262, 1997.

30. Mayer-Jaekel RE, Ohkura H, Gomes R, Sunkel CE, Baumgartner S, Hemmings BA and Glover DM, The 55 kd regulatory subunit of Drosophila protein phosphatase 2A is required for anaphase. *Cell* **72:** 621–633, 1993.
31. Mayer-Jaekel RE, Ohkura H, Ferrigno P, Andjelkovic N, Shiomi K, Uemura T, Glover DM and Hemmings BA, *Drosophila* mutants in the 55 kDa regulatory subunit of protein phosphatase 2A show strongly reduced ability to dephosphorylate substrates of $p34^{cdc2}$. *J Cell Sci* **107:** 2609–2616, 1994.
32. Ferrigno P, Langan TA and Cohen P, Protein phosphatase 2A1 is the major enzyme in vertebrate cell extracts that dephosphorylates several physiological substrates for cyclin-dependent protein kinases. *Mol Biol Cell* **4:** 669–677, 1993.
33. Pallas DC, Shahrik LK, Martin BL, Jaspers S, Miller TB, Brautigan DL and Roberts TM, Polyoma small and middle T antigens and SV40 small t antigen form stable complexes with protein phosphatase 2A. *Cell* **60:** 167–176, 1990.
34. Yang S-I, Lickteig RL, Estes R, Rundell K, Walter G and Mumby MC, Control of protein phosphatase 2A by simian virus 40 small-t antigen. *Mol Cell Biol* **11:** 1988–1995, 1991.
35. Sontag E, Sontag J-M and Garcia A, Protein phosphatase 2A is a critical regulator of protein kinase C ζ signaling targeted by SV40 small t to promote cell growth and NF-κB activation. *EMBO J* **16:** 5662–5671, 1997.
36. Kleinberger T and Shenk T, Adenovirus E4orf4 protein binds to protein phosphatase 2A, and the complex down regulates E1A-enhanced *junB* transcription. *J Virol* **67:** 7556–7560, 1993.
37. Goh WC, Rogel ME, Kinsey CM, Michael SF, Fultz PN, Nowak MA, Hahn BH and Emerman M, HIV-1 Vpr increases viral expression by manipulation of the cell cycle: A mechanism for selection of Vpr *in vivo*. *Nat Med* **4:** 65–71, 1998.
38. Tung HYL, De Rocquigny H, Zhao L-J, Cayla X, Roques BP and Ozon R, Direct activation of protein phosphatase-$2A_0$ by HIV-1 encoded protein complex NCp7:vpr. *FEBS Lett* **401:** 197–201, 1997.
39. Brautigan DL, Sunwoo J, Labbe JC, Fernandez A and Lamb NJ, Cell cycle oscillation of phosphatase inhibitor-2 in rat fibroblasts coincident with p34cdc2 restriction. *Nature* **344:** 74–78, 1990.
40. Sontag E, Nunbhakdi-Craig V, Bloom GS and Mumby MC, A novel pool of protein phosphatase 2A is associated with microtubules and is regulated during the cell cycle. *J Cell Biol* **128:** 1131–1144, 1995.
41. Saito T, Shima H, Osawa Y, Nagao M, Hemmings BA, Kishimoto T and Hisanaga S, Neurofilament-associated protein phosphatase 2A: Its possible role in preserving neurofilaments in filamentous states. *Biochemistry* **34:** 7376–7384, 1995.
42. Hansra G, Bornancin F, Whelan R, Hemmings BA and Parker PJ, 12-*O*-Tetradecanoylphorbol-13-acetate-induced dephosphorylation of protein kinase Cα correlates with the presence of a membrane-associated protein phosphatase 2A heterotrimer. *J Biol Chem* **271:** 32785–32788, 1996.
43. Adler HT, Nallaseth FS, Walter G and Tkachuk DC, HRX leukemic fusion proteins form a heterocomplex with the leukemia-associated protein SET and protein phosphatase 2A. *J Biol Chem* **272:** 28407–28414, 1997.
44. Li M, Makkinje A and Damuni Z, The myeloid leukemia-associated protein SET is a potent inhibitor of protein phosphatase 2A. *J Biol Chem* **271:** 11059–11062, 1996.
45. Sneddon AA, Cohen PT and Stark MJ, *Saccharomyces cerevisiae* protein phosphatase 2A performs an essential cellular function and is encoded by two genes. *EMBO J* **9:** 4339–4346, 1990.
46. Lin FC and Arndt KT, The role of *Saccharomyces cerevisiae* type 2A phosphatase in the actin cytoskeleton and in entry into mitosis. *EMBO J* **14:** 2745–2759, 1995.
47. Luke MM, Della Seta F, Di Como CJ, Sugimoto H, Kobayashi R and Arndt KT, The SAPs, a new family of proteins, associate and function positively with the SIT4 phosphatase. *Mol Cell Biol* **16:** 2744–2755, 1996.
48. Di Como CJ and Arndt KT, Nutrients, via the Tor proteins, stimulate the association of Tap42 with type 2A phosphatases. *Genes Dev* **10:** 1904–1916, 1996.
49. van Zyl W, Huang W, Sneddon AA, Stark M, Camier S, Werner M, Marck C, Sentenac A and Broach JR, Inactivation of the protein phosphatase 2A regulatory subunit A results in morphological and transcriptional defects in *Saccharomyces cerevisiae*. *Mol Cell Biol* **12:** 4946–4959, 1992.
50. Thomas G and Hall MN, TOR signalling and control of cell growth. *Curr Opin Cell Biol* **9:** 782–787, 1997.
51. Barbet NC, Schneider U, Helliwell SB, Stansfield I, Tuite MF and Hall MN, TOR controls translation initiation and early G1 progression in yeast. *Mol Biol Cell* **7:** 25–42, 1996.
52. Inui S, Kuwahara K, Mizutani J, Maeda K, Kawai T, Nakayasu H and Sakaguchi N, Molecular cloning of a cDNA clone encoding a phosphoprotein component related to the Ig receptor-mediated signal transduction. *J Immunol* **154:** 2714–2723, 1995.
53. Murata K, Wu J and Brautigan DL, B cell receptor-associated protein α4 displays rapamycin-sensitive binding directly to the catalytic subunit of protein phosphatase 2A. *Proc Natl Acad Sci USA* **94:** 10624–10629, 1997.
54. Sabers CJ, Martin MM, Brunn GJ, Williams JM, Dumont FJ, Wiederrecht G and Abraham RT, Isolation of a protein target of the FKBP12–rapamycin complex in mammalian cells. *J Biol Chem* **270:** 815–822, 1995.
55. Brunn GJ, Hudson CC, Sekulic A, Williams JM, Hosoi H, Houghton PJ, Lawrence JC Jr and Abraham RT, Phosphorylation of the translational repressor PHAS-I by the mammalian target of rapamycin. *Science* **277:** 99–101, 1997.
56. Burnett PE, Barrow RK, Cohen NA, Snyder SH and Sabatini DM, RAFT1 phosphorylation of the translational regulators p70 S6 kinase and 4E-BP1. *Proc Natl Acad Sci USA* **95:** 1432–1437, 1998.
57. Hara K, Yonezawa K, Kozlowski MT, Sugimoto T, Andrabi K, Weng QP, Kasuga M, Nishimoto I and Avruch J, Regulation of eIF-4E BP1 phosphorylation by mTOR. *J Biol Chem* **272:** 26457–26463, 1997.
58. Lawrence JC Jr, Fadden P, Haystead TA and Lin TA, PHAS proteins as mediators of the actions of insulin, growth factors and cAMP on protein synthesis and cell proliferation. *Adv Enzyme Regul* **37:** 239–267, 1997.
59. Andjelkovic N, Zolnierowicz S, Van Hoof C, Goris J and Hemmings BA, The catalytic subunit of protein phosphatase 2A associates with the translation termination factor eRF1. *EMBO J* **15:** 7156–7167, 1996.
60. Chen J, Martin BL and Brautigan DL, Regulation of protein serine-threonine phosphatase type-2A by tyrosine phosphorylation. *Science* **257:** 1261–1264, 1992.
61. Chen J, Parsons S and Brautigan DL, Tyrosine phosphorylation of protein phosphatase 2A in response to growth stimulation and v-*src* transformation of fibroblasts. *J Biol Chem* **269:** 7957–7962, 1994.
62. Guo H and Damuni Z, Autophosphorylation-activated protein kinase phosphorylates and inactivates protein phosphatase 2A. *Proc Natl Acad Sci USA* **90:** 2500–2504, 1993.
63. Hériché J-K, Lebrin F, Rabilloud T, Leroy D, Chambaz EM and Goldberg Y, Regulation of protein phosphatase 2A by direct interaction with casein kinase 2α. *Science* **276:** 952–955, 1997.
64. Okamoto K, Kamibayashi C, Serrano M, Prives C, Mumby MC and Beach D, p53-dependent association between cyclin G and the B′ subunit of protein phosphatase 2A. *Mol Cell Biol* **16:** 6593–6602, 1996.

65. Hanks SK and Hunter T, Protein kinases 6. The eukaryotic protein kinase superfamily: Kinase (catalytic) domain structure and classification. *FASEB J* **9:** 576–596, 1995.
66. Allende JE and Allende CC, Protein kinases. 4. Protein kinase CK2: An enzyme with multiple substrates and a puzzling regulation. *FASEB J* **9:** 313–323, 1995.
67. Snell V and Nurse P, Genetic analysis of cell morphogenesis in fission yeast—A role for casein kinase II in the establishment of polarized growth. *EMBO J* **13:** 2066–2074, 1994.
68. Rethinaswamy A, Birnbaum MJ and Glover CV, Temperature-sensitive mutations of the *CKA1* gene reveal a role for casein kinase II in maintenance of cell polarity in *Saccharomyces cerevisiae*. *J Biol Chem* **273:** 5869–5877, 1998.
69. Kinoshita K, Nemoto T, Nabeshima K, Kondoh H, Niwa H and Yanagida M, The regulatory subunits of fission yeast protein phosphatase 2A (PP2A) affect cell morphogenesis, cell wall synthesis and cytokinesis. *Genes Cells* **1:** 29–45, 1996.
70. Tamura K, Kanaoka Y, Jinno S, Nagata A, Ogiso Y, Shimizu K, Hayakawa T, Nojima H and Okayama H, Cyclin G: A new mammalian cyclin with homology to fission yeast Cig1. *Oncogene* **8:** 2113–2118, 1993.
71. Okamoto K and Beach D, Cyclin G is a transcriptional target of the p53 tumor suppressor protein. *EMBO J* **13:** 4816–4822, 1994.
72. Kanaoka Y, Kimura SH, Okazaki I, Ikeda M and Nojima H, GAK: A cyclin G associated kinase contains a tensin/auxilin-like domain. *FEBS Lett* **402:** 73–80, 1997.
73. Li M, Makkinje A and Damuni Z, Molecular identification of I^1_{PP2A}, a novel potent heat-stable inhibitor protein of protein phosphatase 2A. *Biochemistry* **35:** 6998–7002, 1996.
74. Shenolikar S, Protein phosphatase regulation by endogenous inhibitors. *Semin Cancer Biol* **6:** 219–227, 1995.
75. Ferrell JE Jr, Tripping the switch fantastic: How a protein kinase cascade can convert graded inputs into switch-like outputs. *Trends Biochem Sci* **21:** 460–466, 1996.
76. Pawson T and Scott JD, Signaling through scaffold, anchoring, and adaptor proteins. *Science* **278:** 2075–2080, 1997.
77. Brunet A and Pouyssegur J, Mammalian MAP kinase modules: How to transduce specific signals. *Essays Biochem* **32:** 1–16, 1997.
78. Lester LB and Scott JD, Anchoring and scaffold proteins for kinases and phosphatases. *Recent Prog Horm Res* **52:** 409–429; discussion 429–430, 1997.