REVIEW ARTICLE
Serine/threonine protein phosphatases

Stefaan WERA* and Brian A. HEMMINGS†
Friedrich Miescher-Institut, Postfach 2543, CH-4002 Basel, Switzerland

INTRODUCTION

Phosphorylation of structural and regulatory proteins is a major intracellular control mechanism in eukaryotes. The phosphorylation state of a protein is a dynamic process controlled by both protein kinases and protein phosphatases [1]. This review deals with those enzymes that dephosphorylate serine (Ser) and threonine (Thr) residues in proteins, whereas the tyrosine (Tyr) phosphatases and dual-specificity protein phosphatases will be discussed in a future issue of this journal. The structure of each Ser/Thr protein phosphatase will be discussed, followed by its regulation and probable physiological roles.

Based on biochemical parameters, Ser/Thr protein phosphatases were initially divided into two classes: type-1 phosphatases (PP1) are inhibited by two heat-stable proteins, termed inhibitor-1 (I-1) and inhibitor-2 (I-2), and preferentially dephosphorylate the β-subunit of phosphorylase kinase, whereas type-2 phosphatases are insensitive to the heat-stable inhibitors and preferentially dephosphorylate the α-subunit of phosphorylase kinase [1,2]. Type-2 phosphatases can be further subdivided into spontaneously active (PP2A), Ca^{2+}-dependent (PP2B) and Mg^{2+}-dependent (PP2C) classes. The use of okadaic acid, a specific phosphatase inhibitor, further facilitated the discrimination between different classes [3].

Although still widely in use, this classification does not reflect the actual phylogenetic relationship between the different Ser/Thr protein phosphatases. Molecular cloning revealed that PP2A was in fact much more closely related to PP1 than to PP2C. Moreover, in the past few years many novel protein phosphatases have been identified that do not fit into the above classification. Many of these protein phosphatases are closely related to the existing classes or are intermediates between classes. From a phylogenetic point of view, it would be more reasonable to group PP1, PP2A and PP2B in a family I and PP2C in a family II. Recently a detailed comparison of the primary structures of 44 different protein Ser/Thr phosphatases, excluding PP2C, was carried out [4]. This revealed a common core structure that comprises two domains. The first domain is predicted to fold as a single β sheet flanked by α helices, whereas the second is predominantly helical [4]. This multiple alignment also shows that family I can be subdivided into PP1-like, PP2A-like and PP2B-like enzymes.

TOXIN INHIBITORS OF PROTEIN PHOSPHATASES

The discovery of many low-molecular-mass protein phosphatase inhibitors, which are able to penetrate living cells, has revolutionized the study of the function of protein phosphatases [5]. The best studied, and most widely used, is okadaic acid. This polyether fatty acid is produced by marine dinoflagellates and is the causative agent of diarrhoetic shellfish poisoning. Due to its polyether structure it is cell-permeable. It is a potent inhibitor of PP2A (K_i = 0.2 nM) and PP1 (K_i = 20 nM), but is a much less efficient inhibitor of PP2B (K_i = 10 μM). In spite of these different sensitivities, okadaic acid cannot be easily used to discriminate between PP1 and PP2A *in vivo* [3,6]. Furthermore, okadaic acid does not rapidly penetrate cell membranes, but accumulates slowly, making it difficult to control the actual concentration of the compound *in vivo*. With cell-free extracts, however, the above-mentioned limitations do not exist, and okadaic acid is a valuable tool used to distinguish between different phosphatases acting upon a given substrate [3].

Since the discovery of okadaic acid as a specific phosphatase inhibitor, a number of other phosphatase inhibitors (tautomycin, dinophysistoxin, calyculin, microcystin and nodularin) have also been described [5]. We wish to draw attention to yet another phosphatase inhibitor, cantharidin [7,8]. This toxin is derived from blister beetles (a traditional Chinese medicine and aphrodisiac) and inhibits PP2A (IC_{50} = 0.16 μM) more potently than PP1 (IC_{50} = 1.7 μM). Although less potent than okadaic acid, it has been successfully used recently to study the dephosphorylation of hsp26 *in vivo* [9].

PROTEIN PHOSPHATASE 1

Structure of PP1

Several recent reviews deal with the structure and function of PP1 [1,10–12].The catalytic subunit of PP1, termed PP1c, was originally isolated as a 33 kDa protein, but this turned out to be a proteolytic fragment of a 37 kDa protein. PP1c has been crystallized in the presence of the phosphatase inhibitor microcystin [13], but to date no details of the atomic structure have been published.

Initially two isoforms of PP1c were cloned, termed α and β [14]. PP1cβ was later shown to be a cloning artefact [15]. In rat, cDNA cloning revealed the existence of at least four isoforms, termed α, γ1, γ2 and δ [16]. Interestingly, the γ1 and γ2 isoforms are produced by alternative splicing of the same primary transcript. The mouse homologues, derived from a brain library [17], were termed dis2m1 (for the γ1 isoform) and dis2m2 (for the δ isoform). Later a homologue of the δ isoform was found in a rabbit cDNA library and termed β [18].

PP1cα expressed in insect cells, using a baculovirus vector, is mainly found as an insoluble protein. However, denaturation followed by rapid renaturation in the presence of Mn^{2+} results in reactivation of the enzyme [15]. Bacterially expressed PP1c (α, β and γ isoforms) differs in some aspects (sensitivity to phosphatase

Abbreviations used: PP1, type-1 protein phosphatase; PP2A, PP2B and PP2C, type-2 protein phosphatase class A, B and C respectively; I-1 and I-2, inhibitor-1 and -2 respectively; GSK3, glycogen synthase kinase 3; NIPP-1, nuclear inhibitor of PP1; cAMP, cyclic AMP; cAMP-PK, cAMP-dependent protein kinase; SV40, simian virus 40; MAP kinase, mitogen-activated protein kinase; PTPA, protein tyrosine phosphatase activator; CAK, cdk1-activating kinase; CREB, cAMP response element binding protein; NF, nuclear factor; IκB, inhibitor of NF-κB; NMDA, *N*-methyl-D-aspartate.

Present address: Afdeling Biochemie, Fakulteit Geneeskunde, Herestraat 49, B-3000 Leuven, Belgium.
† To whom correspondence should be addressed.

inhibitors, binding to regulatory subunits, requirement for Mn^{2+} and specific activity) from native PP1c. The recombinant PP1c isoforms regain their native characteristics following incubation with I-2 and subsequent phosphorylation of the latter by glycogen synthase kinase 3 (GSK3) [19]. A recent study of deletion mutants of PP1c [20] indicated that removal of the C-terminus of the protein (up to 33 residues) has no effect on the activity of the enzyme, nor on the binding of either okadaic acid or I-2. A PP1c mutant was constructed in which residues 274–277 (-Gly-Glu-Phe-Asp-) of PP1c were replaced with residues 267–270 (-Tyr-Arg-Cys-Gly-) of PP2A. Interestingly, this mutant displays a 10-fold increased sensitivity towards okadaic acid. One possibility to explain these results is that binding of okadaic acid and other toxins occurs at or near this site [21]. Alternatively, the effect of mutating residues 274–277 might cause a modest conformational change in the enzyme. This could also explain the 7-fold increased K_m and 2-fold decreased $V_{max.}$ of the mutant enzyme for its substrate phosphorylase.

The native structure of PP1 is a 1:1 complex between the catalytic and a number of different regulatory subunits [22]. The role of these regulatory subunits is to target the catalytic subunit towards specific subcellular locations (hence the name targeting subunits), and to increase activity towards particular substrates. Furthermore PP1c can associate with proteins that inhibit its activity. These inhibitors can also be considered as regulatory subunits. The holoenzymes are named according to their apparent subcellular location [10]. For example, the glycogen-bound PP1 holoenzyme (PP1G) is a heterodimer of PP1c and a glycogen-binding subunit (G-subunit) [23]. The G-subunit anchors the phosphatase to glycogen and increases its activity towards the glycogen-bound substrates: glycogen synthase and glycogen phosphorylase [24]. These substrates are activated and inactivated respectively by dephosphorylation. The G-subunit was purified from skeletal muscle [23]. Molecular cloning revealed a molecular mass of 124 kDa [25]. The *Saccharomyces cerevisiae* homologue of the G-subunit (encoded by the *GAC1* gene) has also been identified [26].

The PP1 holoenzyme isolated from muscle sarcoplasmic reticulum has the same subunit composition as PP1G. It therefore appears that the G-subunit can target PP1c to either glycogen or the sarcoplasmic reticulum [27]. The G-subunit has a region of 32 hydrophobic residues at its C-terminus, which is probably responsible for the interaction of the G-subunit with membranes [25]. Sarcoplasmic reticulum-located PP1G is the major phosphatase that acts upon phospholamban [28], a protein involved in Ca^{2+} uptake into the cardiac SR and in the regulation of the rate of cardiac muscle relaxation.

Highly purified preparations of the glycogen-bound protein phosphatase from rat liver ($PP1G_l$) were shown to contain PP1c and polypeptides of 54 kDa and 161 kDa [29]. Based on immunological data, activity measurements and partial peptide sequences, the 54 kDa polypeptide is identical or highly related to liver amylase, and most likely is not essential for the regulatory properties of the enzyme [10,29]. The structure of the 161 kDa component has not yet been described, but could be functionally related to the skeletal muscle G-subunit (see below).

The PP1 bound to the myosin fraction in smooth muscle was purified as a trimer consisting of PP1c and polypeptides of 130 kDa and 20 kDa. The latter subunits are responsible for enhanced activity towards heavy meromyosin or isolated myosin light chains [30]. The 58 kDa component found in some preparations of myosin-bound PP1 from smooth muscle seems to be a proteolytic derivative of the 130 kDa component [31]. The myosin-bound form of PP1 in skeletal muscle (PP1M) was purified as a dimeric enzyme consisting of PP1c and a regulatory subunit that increased dephosphorylation of the myosin light chain, present in heavy meromyosin [32].

High levels of PP1 activity are also present in the nuclei of eukaryotic cells [33]. Strikingly, upon subnuclear fractionation more than 90% of this phosphatase activity is found in the chromatin fraction [34], and immunofluorescence reveals that PP1 co-localizes with individual chromosomes during mitosis [35]. Part of the nuclear PP1 is present in a latent form (termed $PP1N_x$), and is composed of PP1c complexed to an inhibitory (NIPP-1) polypeptide [36]. Two highly related forms of NIPP-1 (termed a and b) have been purified from bovine thymus [34] and migrate on SDS/PAGE as 18 kDa and 16 kDa proteins respectively.

A cytosolic form of PP1, termed PP1S, is composed of PP1c complexed to I-2 (also termed the modulator subunit). Upon purification of PP1S, the enzyme gradually converts to an inactive conformation (reviewed in [10,11]). After proteolysis of I-2 in this complex, PP1c remains in the inactive conformation. *In vitro*, phosphorylation of I-2 on Thr-72 (by GSK3) initiates a reactivation process (most likely a conformational change of the catalytic subunit). The phosphorylation stoichiometry never exceeds 0.2 mol/mol, indicating that rapid autodephosphorylation occurs. After dephosphorylation of I-2, PP1S becomes active against exogenous substrates but slowly reverts to its inactive conformation. Thr-72 is the only phosphorylation site for GSK3, but prior phosphorylation of I-2 at Ser-86 by casein kinase II enhances phosphorylation at Thr-72 and thereby promotes the reactivation of PP1S.

It was suggested that PP1S serves as a pool of inactive phosphatase, from which the catalytic subunit could be recruited if needed in other subcellular compartments. I-2 would then function as a chaperone that folds the nascent PP1c in the correct way [19]. It is possible, however, to prevent the inactivation of PP1S by the inclusion of low concentrations of fluoride in the isolation buffers during the purification [37]. Under these conditions PP1S can be isolated as an active phosphatase, with no evidence found for a pool of inactive enzyme. These recent observations argue strongly against a role for I-2 as a chaperone *in vivo*.

PP1cγ2, an isoform of PP1c that is particularly enriched in testis, was purified as a complex with proteins of 78 kDa and 55 kDa. Partial amino acid sequence data suggest that the 78 kDa subunit is identical to the 78 kDa glucose-regulated protein, a member of the 70 kDa heat-shock protein family [38]. The physiological significance of the association of PP1cγ2 with these proteins is unclear at the moment.

Using the yeast two-hybrid system, it was shown that the retinoblastoma gene product binds to PP1cα2, a variant of PP1c with an N-terminal 11-amino-acid insert. PP1cα2 binds preferentially to the hypophosphorylated forms of retinoblastoma protein. The association seems to occur during early G1. The authors suggest that binding of PP1 to retinoblastoma keeps the latter in the hypophosphorylated state [39].

Heat-stable inhibitors of PP1

I-1 is a 18.7 kDa protein which becomes inhibitory to PP1 after phosphorylation on Thr-35 by cyclic AMP (cAMP)-dependent protein kinase (cAMP-PK). Phosphorylation of I-1 occurs *in vivo* in skeletal muscle after adrenaline injection and also in rabbit liver after glucagon administration (reviewed in [1,10]).

DARPP-32 is an isoform of I-1 that inhibits PP1 with the same potency. Although the expression pattern of DARPP-32 differs between species, in most species it is typically expressed in brain regions with dopaminergic innervation and striatonigral neurons,

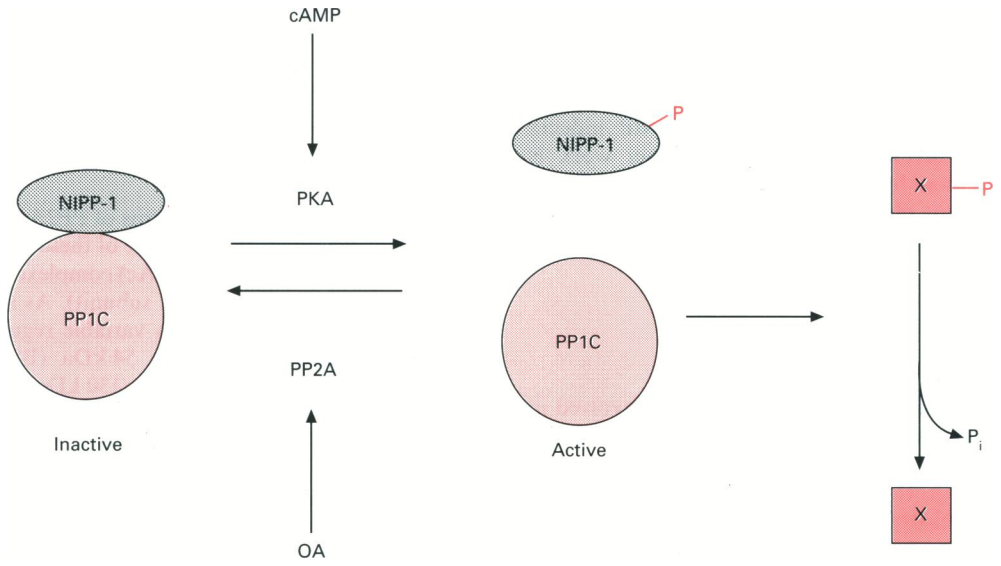

Figure 1 Possible mechanism for the control of nuclear PP1

Part of the nuclear PP1 activity is present as a latent enzyme consisting of PP1c complexed with the inhibitor NIPP-1. Phosphorylation of NIPP-1 by cAMP-PK (PKA) relieves the inhibition of PP1c [34,36] and leads to dephosphorylation of nuclear proteins (X). PP2A reverses the effect. Inhibition of nuclear PP2A with okadaic acid (OA) is expected to activate PP1.

but it is also found in renal tubule cells, adrenal medulla, pineal gland, choroid plexus and brown adipose tissue.

I-2 (or modulator subunit) is a 22.8 kDa protein without sequence identity to I-1. The inhibition of PP1c by I-2 is clearly different from the above described inactivation, since it requires much higher I-2 concentrations, and is reversible upon I-2 destruction [10]. I-2 is encoded by two transcripts that differ in the 3′ untranslated region, probably due to the use of alternative polyadenylation signals [40].

Regulation of PP1

The activity of PP1c in the different holoenzyme complexes is primarily mediated through the regulatory subunits [22].

Regulation of the glycogen-bound PP1 from skeletal muscle

Phosphorylation of the G-subunit by cAMP-PK at site-2 results in the release of PP1c [41]. At physiological salt concentrations, free catalytic subunit is about 5-fold less active in dephosphorylating glycogen phosphorylase and glycogen synthase [24] and will presumably be further inhibited by I-1. Phosphorylation of site-2 occurs *in vivo* in response to adrenaline (acting via cAMP) and the resulting dissociation of PP1G contributes significantly to the inactivation of glycogen synthase [42,43]. Conversely, dephosphorylation of site-2 *in vitro* is catalysed by PP2A, PP2B and PP2C [41]. Dephosphorylation of site-2 by PP2B, after an intracellular Ca^{2+} rise *in vivo*, would also lead to reactivation of PP1G in response to Ca^{2+}-mobilizing stimuli. Whether such a mechanism operates *in vivo* remains to be established.

Insulin, acting through the mitogen-activated protein (MAP) kinase pathway, stimulates the activity of an insulin-stimulated protein kinase-1 or MAPKAP kinase-1 [44], which is identical to $rsk^{mo\text{-}2}$ and S6 kinase II [45]. This kinase phosphorylates site-1 on the G-subunit, which does not lead to dissociation of PP1G, but to a 3-fold increase in its glycogen synthase phosphatase activity [44]. This mechanism explains the stimulatory effect of insulin on glycogen synthesis in skeletal muscle. G-subunit phosphorylated at site-1 is an excellent substrate for GSK3, but the physiological consequences of this phosphorylation are unclear at present [46,47]. GSK3 also phosphorylates and inactivates glycogen synthase directly. Furthermore, GSK3 is inactivated through MAPKAP-kinase-1-mediated phosphorylation [48,49]. Thus insulin administration not only activates the synthase phosphatase (PP1G), but also inactivates an important synthase kinase (GSK3).

Regulation of the glycogen-bound protein phosphatase from liver

The glycogen-bound PP1 from liver ($PP1G_l$) is regulated differently from its muscle counterpart. Liver glycogen synthase phosphatase activity is strongly inhibited by phosphorylase *a* through an allosteric mechanism [50,51]. This mechanism most likely replaces the direct regulation through G-subunit phosphorylation which is observed in skeletal muscle. Agents that promote phosphorylation (activation) of liver phosphorylase *a* lead to inhibition of the synthase phosphatase activity of $PP1G_l$, and thus stimulate glycogenolysis while at the same time inhibiting glycogen synthesis.

Regulation of nuclear PP1

Latent $PP1N_x$ can be activated *in vitro* through phosphorylation of NIPP-1 by cAMP-PK or casein kinase II [36,52]. PP2A can reverse this activation. It remains to be determined whether PP2A exerts the same effect *in vivo* but, if so, PP2A could control PP1 activity in the nucleus, as shown in Figure 1. This might

explain why inhibition of PP2A (through okadaic acid treatment) leads to dephosphorylation of certain nuclear phosphoproteins [53], although an indirect effect of okadaic acid on protein kinases cannot be excluded. In much the same way, the activation of $PP1N_{\alpha}$ by cAMP-PK might explain why cAMP induces dephosphorylation of certain proteins [54]. Further work is necessary to validate these hypothetical regulatory mechanisms. The regulation of nuclear phosphatases is poorly understood at the moment and could be a fruitful avenue of research in the future.

Physiological role of PP1

From the previous discussion, it is clear that PP1 is involved in many different cellular processes, as diverse as glycogen metabolism, calcium transport, muscle contraction, protein synthesis and intracellular transport (reviewed in [1,10,12]). In some cellular processes the substrates for PP1 (e.g. glycogen synthase, glycogen phosphorylase, myosin light chain, phospholamban, ribosomal protein S6) have been identified (reviewed in [1,10–12]), whereas for other phosphoproteins (such as transcription factors) the contribution of PP1 to their dephosphorylation remains to be further clarified.

Recent evidence from the study of cell cycle mutants has revealed that PP1 also plays an important role in the regulation of mitosis. The temperature-sensitive *Bim G11* mutant of *Aspergillus nidulans* carries a mutation in the PP1c gene. At the restrictive temperature, mutants are unable to complete anaphase successfully [55]. The fission yeast (*Schizosaccharomyces pombe*) gene *dis2*$^+$ also encodes a PP1c homologue, and mutations in this gene lead to a block in mitotic chromosome disjoining [17]. The *dis2*$^+$ gene is identical to the *bws*+ gene, which is involved in the initiation of mitosis in fission yeast [56]. Further, in budding yeast (*Saccharomyces cerevisiae*) the *DIS2S1/GLC7* gene, which encodes a PP1 catalytic subunit, is an essential gene. Recent experiments indicate that PP1 plays a role in chromosome segregation [57]. This conclusion was reached through the study of the *IPL1* gene, which apparently encodes a protein kinase that is required for high-fidelity chromosome segregation. PP1 acts in opposition to the Ipl1 kinase, since mis-segregation of chromosomes caused by a mutation in the kinase is partially suppressed by expression of a truncated *DIS2S1/GLC7* gene. The data suggest that the truncated catalytic subunit is expressed and, although catalytically inactive, it is able to bind to regulatory subunits. As a consequence, overexpression of the truncated catalytic subunit competitively inhibits binding of the native catalytic subunit to its regulatory subunits. Interestingly, overexpression of the full-length gene in wild-type cells also results in chromosome mis-segregation.

Further evidence for a role of PP1 in mitosis has been obtained from the study of *Drosophila* mutants [58,59]. A mutation in the PP1 *87B* gene (one of four genes that encode PP1 homologues) leads to flies that die at the larval–pupal boundary. Analysis of neuroblasts from such flies showed defective spindle organization, abnormal sister chromatid segregation, hyperploidy and excessive chromosome condensation, as well as a delay in progression through mitosis. That PP1 is required for mitotic progression was confirmed by reintroduction of the PP1 *87B* gene, which resulted in normal mitosis.

Further evidence for a role for PP1 during mitosis was demonstrated by microinjection experiments using rat fibroblasts [60]. Recently it was found that PP1 is phosphorylated and inhibited by cdk1 complexed with either cyclin A or B [61]. Whether cdk1 phosphorylates PP1 *in vivo* remains to be established. Further work is necessary to define the role of PP1 during cell cycle progression.

PROTEIN PHOSPHATASE 2A

Structure of PP2A

Several trimeric holoenzyme forms of PP2A exist and have been extensively characterized. The core of these structures consists of a 36 kDa catalytic subunit (PP2Ac) complexed with a regulatory subunit of 65 kDa (PR65 or A subunit). As shown in Figure 2, this core dimer associates with variable regulatory subunits of 55 kDa (PR55 or B subunit), 54 kDa (B′ subunit), 72 kDa (PR72), 74 kDa (B″ subunit) or 130 kDa (PR130) [62,63]. *In vivo*, PP2A is probably only present as a trimer, but this remains a controversial issue because the core dimer has been purified from many different tissues. This suggests the possibility of subunit rearrangements where the variable subunits are able to associate and dissociate from the core dimer.

The exact function of the regulatory subunits is not yet completely resolved, but they probably influence substrate specificity and/or subcellular localization. It is noteworthy in this respect that PR72 contains a potential nuclear localization signal in its primary sequence [64], possibly explaining the presence of PP2A in the nucleus [65].

Molecular cloning revealed the existence of several isoforms of each subunit (reviewed in [58]). Two isoforms of the catalytic subunit (α, β), two isoforms of PR65 (α, β), three isoforms of

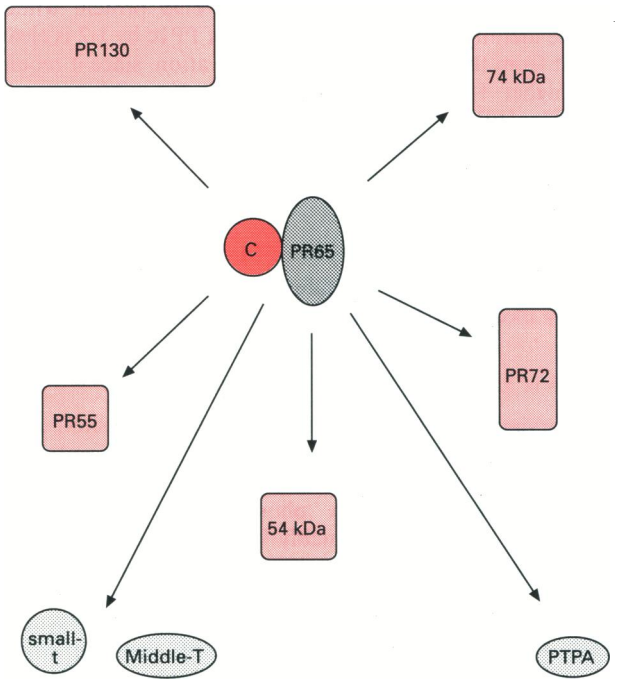

Figure 2 Different PP2A holoenzymes

The core dimer of PP2Ac (C) and the PR65 regulatory subunit associate with variable or B subunits of 55 kDa (PR55), 54 kDa, 72 kDa (PR72), 130 kDa (PR130) or 74 kDa [62,63]. PR55, PR72 and PR130 refer to cloned gene sequences; the 54 and 74 kDa subunits have not been cloned. In addition, the core dimer can interact with PTPA [88,89] and associates with SV40 and polyoma small-t and polyoma middle-T antigens [97].

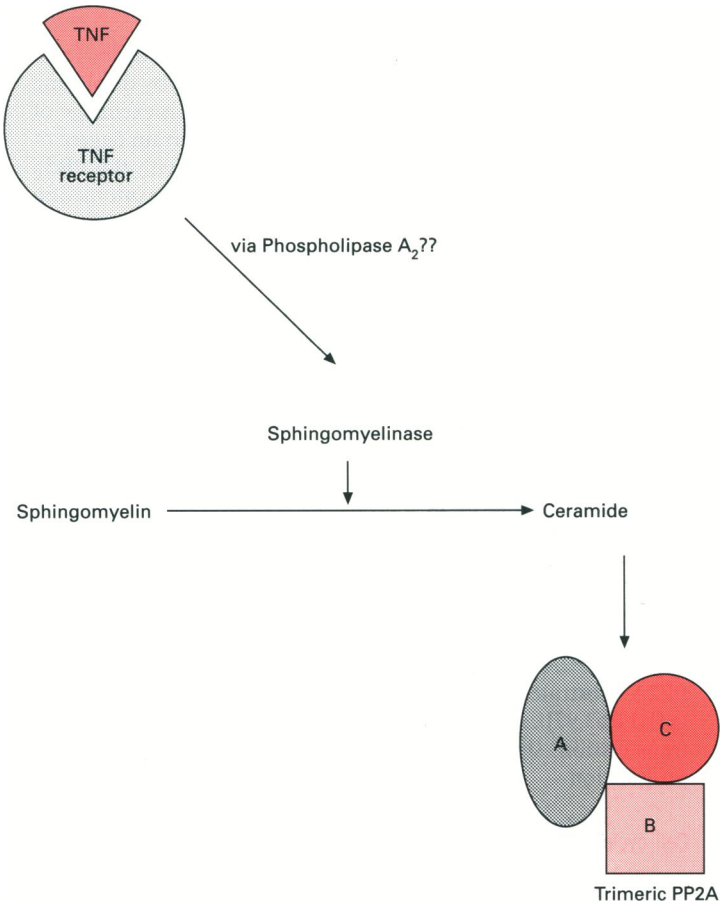

Figure 3 Ceramide activates PP2A

Binding of tumour necrosis factor (TNF) to its receptor triggers a series of events (possibly involving phospholipase A_2 activation) that leads to activation of sphingomyelinase and the formation of ceramide [72,73] that in turn activates the trimeric PP2A.

PR55 (α, β, γ) and two isoforms of PR72 exist [60]. Most PP2A subunits appear to be ubiquitously expressed. Interestingly, expression of PR55β and γ is mainly limited to neuronal tissues [62,66], whereas PR72 transcripts are only found in muscle tissue [64].

In *Drosophila* the situation is less complex, since every subunit cloned so far appears to be encoded by a single gene (see [62]). In budding yeast (*Saccharomyces cerevisiae*) PP2A catalytic subunit is encoded by two genes (*PPH21* and *PPH22*) [67,68]. A related gene (*PPH3*) can provide some PP2A activity in the absence of *PPH21* and *PPH22*. PP2A-deficient yeast is not viable, but can be rescued by *PAM1* overexpression [69]. PAM1 has no homology to known protein phosphatases, but might activate a PP2A-like phosphatase. *TPD3* and *CDC55* genes (see below) encode the yeast homologues of PR65 and PR55 respectively [70,71].

Regulation of PP2A

Activation by ceramide

The binding of tumour necrosis factor-α or γ-interferon to their receptors leads to the activation of a sphingomyelinase, resulting in the formation of ceramide (see Figure 3). The mechanisms that connect receptor activation to activation of sphingomyelinase are poorly understood (reviewed in [72]). *In vitro* ceramide activates those trimeric forms of PP2A that contain either the PR55α or the 54 kDa (B′) variable regulatory subunit. In contrast, the PP2A dimer and the catalytic subunit are insensitive to ceramide [73]. The observations that dihydroceramide (which is physiologically inactive) is unable to activate the PP2A trimer and that okadaic acid can overcome the effects of ceramide on apoptosis and c-*myc* down-regulation suggest that PP2A is the most important mediator of ceramide action [72,74]. The effect of ceramide on protein kinases might then be indirect [72]. For a recent review on this topic, see Kolesnick and Golde [75].

Inhibition through phosphorylation of the catalytic subunit

The catalytic subunit of PP2A can be phosphorylated *in vitro* by the tyrosine kinases $p60^{v\text{-}src}$, $p56^{lck}$, epidermal growth factor receptor and insulin receptor [76]. Phosphorylation occurs on Tyr-307 at the C-terminus of the protein and leads to a 90% loss in activity; dephosphorylation reactivates the phosphatase. This reactivation is prevented by okadaic acid, suggesting an autodephosphorylation reaction.

Furthermore, serum stimulation or transformation of cells by $p60^{v\text{-}src}$ apparently promotes the transient phosphorylation of PP2A on Tyr, whereas serum starvation has the opposite effect [77]. The *in vivo* phosphorylation of the catalytic subunit has not been correlated with activity changes. Nevertheless, it has been suggested that activation of growth factor receptors leads to transient inactivation (Tyr phosphorylation) of PP2A, and thereby amplifies and prolongs activation of protein kinases [76]. This is an attractive model that places PP2A in a pivotal position to modulate signal transduction cascades. In support of this idea, Srinivasan and Begum [78] have recently shown that addition of insulin to rat skeletal muscle cells leads to a decrease in PP2A activity by 40–80%. Furthermore, the fact that okadaic acid treatment of cells can lead to activation of some protein kinases points in the same direction [79]. Taken together the data suggest that the activity of PP2A can be modulated by extracellular signals and thereby can modulate signal transduction pathways.

In vitro phosphorylation of the catalytic subunit on threonine leading to a loss of activity has also been reported [80]. This reaction is catalysed by an autophosphorylation-activated protein kinase, and leads to about 80% inactivation of PP2A [80]. This has the elegance of amplifying the signal propagated by the kinase. Strikingly, the kinase itself is dephosphorylated and inactivated by PP2A. Mechanistically these data are difficult to rationalize at the moment.

Carboxymethylation of the catalytic subunit

Recently a carboxymethyltransferase, different from the known type II and type III methyltransferases, has been purified [81,82]. The major substrate of this enzyme is a 36 kDa protein, subsequently identified as the catalytic subunit of PP2A. The purified methyltransferase catalyses methylation of the α-carboxyl group of the C-terminal Leu-309 residue of PP2Ac *in vitro* [82]. Methylation has moderate stimulatory effects on phosphatase activity and impairs binding of peptide-specific antibodies to the C-terminus of PP2Ac [83]. Strikingly, the C-terminal sequence of PP2Ac (Thr-Pro-Asp-Tyr-Phe-Leu) is conserved from yeast to man, suggesting that modifications of the C-terminus of PP2Ac may be a conserved regulatory mechanism [84].

In *Xenopus* egg extracts, addition of cAMP increases methylation of PP2A, whereas okadaic acid inhibits methylation [85]. In view of the moderate effects of methylation on PP2A activity, it was suggested that this modification might influence the interaction of PP2Ac with its regulatory subunits [83]. C-

terminal methylation of PP2Ac was also inhibited *in vitro* by okadaic acid or microcystin [86], suggesting that the inhibitory effect is mediated by binding to PP2Ac.

Tyrosine phosphatase activity of PP2A

Although PP2A was originally purified as a Ser/Thr protein phosphatase it can, under certain conditions, dephosphorylate Tyr residues. The tyrosine phosphatase activity of PP2A towards exogenous substrates is increased in the presence of ATP or PP_i, tubulin and MAP-2 [87]. Furthermore, a 37 kDa protein (termed PTPA) was isolated and characterized which, in the presence of ATP and Mg^{2+}, activates the Tyr phosphatase activity of PP2A. This effect is limited to the dimeric form of PP2A, and to a lesser extent to the isolated catalytic subunit [88,89]. The tyrosine phosphatase activity of PP2A after PTPA stimulation is of the same order of magnitude as its Ser/Thr (phosphorylase) phosphatase activity. The PTPA-induced tyrosine phosphatase activity of PP2A is stabilized by small-t or middle-T antigen [90]. Intriguingly, the mechanism whereby PTPA stimulates the tyrosine phosphatase activity of PP2A remains to be established.

Interaction with regulatory subunits

The interaction of PP2Ac with the regulatory subunits PR65, PR55, PR72 and others influences its substrate specificity [91–93]. This is clear from studies with cdk1-phosphorylated substrates [91]. Dephosphorylation of a cdk1-phosphorylated histone H1 peptide occurs efficiently with PP2A trimers that contain either the PR55 or PR72 subunit, but not with the dimer or with PP2Ac. Furthermore, the presence of PR55, but not PR72, in the PP2A holoenzyme allows efficient dephosphorylation of a cdk1-phosphorylated retinoblastoma peptide. The observation is extended by the finding that 2 nM okadaic acid inhibits the dephosphorylation of cdk1-phosphorylated histone H1 in diluted extracts from *Xenopus* eggs, human fibroblasts and rat liver [92].

Further evidence that PP2A holoenzymes display different substrate specificities comes from the observation that the PP2A trimer containing PR72 preferentially dephosphorylates Ser-120 and Ser-123 in simian virus 40 (SV40) large-T antigen, whereas dephosphorylation of Thr-124 (the cdk1 target site) is only efficiently catalysed by the PP2A trimer containing PR55 [93].

Analysis of yeast and *Drosophila* mutants lacking one particular PP2A subunit confirmed the importance of the regulatory subunits. Yeast carrying a mutation in the *TPD3* gene (encoding a PR65 homologue) shows defects in tRNA gene transcription and the cells become multinucleated [70]. Mutations in the *CDC55* gene, encoding the homologue of PR55, lead to defects in cell septation and separation in yeast [71] and to abnormal anaphase resolution in the *Drosophila* aar^1 mutant [94]. The PP2A holoenzyme in brain extracts from aar^1 homozygous flies displays decreased activity towards the cdk1-phosphorylated substrates histone H1 and caldesmon, whereas activity towards other substrates is not affected [95].

The *Drosophila* $twins^P$ mutant carries a P-element insertion in the same gene as the aar^1 mutant but is a stronger allele [95,96]. The mechanoreceptor of wild-type flies is composed of a single neuron and three accessory cells, which are derived from a single precursor cell. In the $twins^P$ mutants only accessory cells are present in the mechanoreceptor. Stepwise reduction of PR55 (*twins* protein) enhances this transformation [96]. From this study it can be concluded that the PP2A containing the PR55 subunit is crucial for neural cell identity specification.

Interaction with tumour antigens

SV40 and polyoma small-t and polyoma middle-T antigens form complexes with the PR65/PP2Ac dimer, probably by displacing the variable third subunit (reviewed in [67,97]). This exchange of a regulatory subunit for a tumour antigen has a certain degree of specificity, as small-t antigen is able to exchange with the PR55 subunit but not with the 54 kDa (B′) subunit [98]. The interaction of PP2A with tumour antigens has an inhibitory effect on the dephosphorylation of *in vitro* substrates such as myosin light chain, myelin basic protein, large-T antigen and p53 [97].

When analysed in cells the interaction of small-t with PP2A results in inhibition of PP2A-mediated dephosphorylation of MEK (ERK kinase) and ERK (extracellular signal regulated kinase; also termed MAP kinase) and thus in receptor-independent activation of these two kinases. This leads to cell proliferation without affecting Raf kinase activity [98].

Physiological role of PP2A

Through the use of okadaic acid and related inhibitors it is possible to specifically inhibit PP2A activity in cell-free extracts. Although one cannot exclude the possibility that PP2A-like enzymes are affected, this approach, often in combination with purified PP2A preparations, has been used to identify potential PP2A substrates in a wide variety of cellular processes (reviewed in [1,11,63,97]), such as metabolism, muscle contraction, synaptic transmission, signal transduction, RNA splicing and cell cycle progression.

Cell cycle regulation

Progression of eukaryotic cells through the cell division cycle is primarily controlled by the activity of the cyclin-dependent protein kinases. The best studied event is the transition from G2 to M phase, which is controlled by cdk1 (also referred to as cdc2 or p34) complexed with cyclin B (reviewed in [99]). In frog oocytes this complex is termed maturation promoting factor.

In mammalian cells, the activity of cdk1 is controlled by the phosphorylation state of three sites (Thr-161, Thr-14 and Tyr-15; see Figure 4). Binding of cyclin B to cdk1 allows this triple phosphorylation. Phosphorylation on Thr-161, which is absolutely required for kinase activity, is catalysed by cdk1-activating kinase (CAK). cdk1 remains inactive, however, because Thr-14 and Tyr-15 are also phosphorylated at this time. These latter phosphorylations are catalysed by the wee1 kinase and possibly the mik1 kinase. At a certain threshold level of cyclin, cdc25, a dual-specificity protein phosphatase, is activated. This phosphatase then dephosphorylates Thr-14 and Tyr-15, but not Thr-161, and thus activates cdk1. Interestingly, cdc25 itself is activated through cdk1-mediated phosphorylation. (More recently it has been reported that raf can activate cdc25 and thus links a mitogenic signalling pathway to cell cycle control.) This positive feedback loop leads to rapid cdk1 activation and entry into mitosis. cdk1 is subsequently deactivated through the proteolytic destruction of cyclin B.

PP2A has a negative effect on the progression from G2 to M phase. This was concluded from the observation that inhibition of PP2A with okadaic acid leads to premature entry into M phase [100,101]. Furthermore INH, an inhibitor of activation of maturation promoting factor from frog oocytes, was shown to be identical to a PP2A holoenzyme consisting of PP2Ac/PR65/PR55 [102]. This trimeric PP2A acts by inhibiting the pathway leading to Thr-161 phosphorylation [102]. PP2A is unable to dephosphorylate Thr-161 directly, suggesting that the effect of PP2A is mediated indirectly via the CAK kinase. The activity of

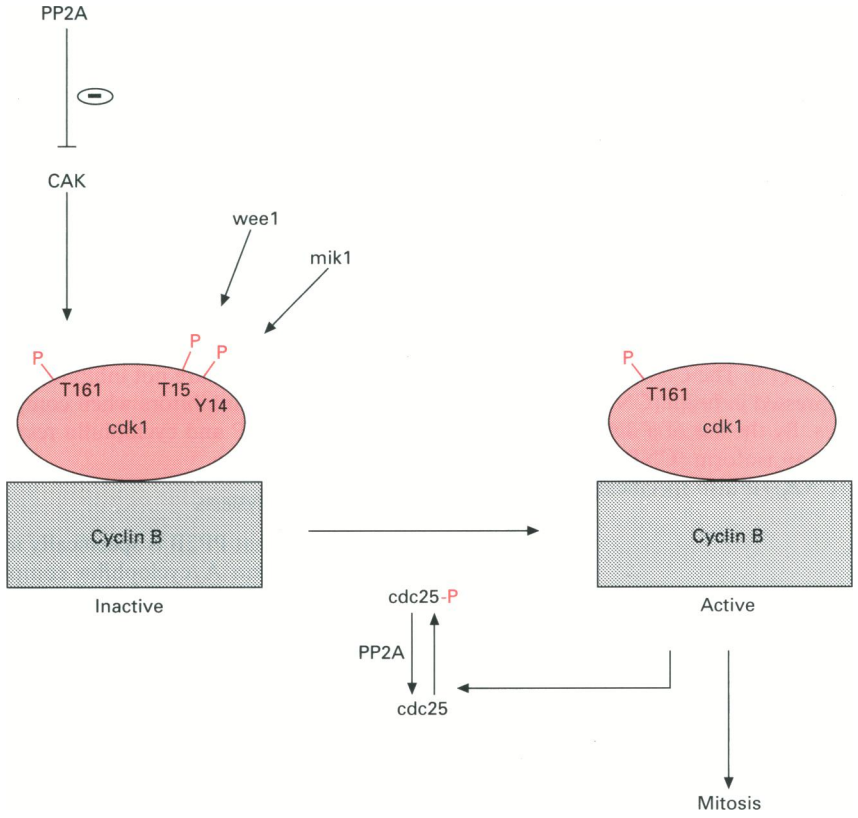

Figure 4 Role of PP2A in cell cycle regulation

Phosphorylation of cdk1 on Thr-161 is absolutely required for activity, whereas phosphorylation of Thr-15 and Tyr-14 is inhibitory. Phosphorylation of Thr-161 is catalysed by CAK, and the pathway leading to Thr-161 phosphorylation is inhibited by PP2A. Thr-161 is not a direct PP2A substrate [99,102]. cdk1 activation requires dephosphorylation of Thr-15 and Tyr-14 by cdc25. cdc25 is only active in the phosphorylated form and is kept inactive by PP2A [104].

CAK, however, does not vary between interphase and mitosis [103]; thus the significance of the hypothetical PP2A-mediated dephosphorylation of CAK is not immediately obvious. Moreover, PP2A dephosphorylates cdc25, keeping it in a low-activity state [104]. This also contributes to the negative effect of PP2A on the G2 to M transition.

PP2A and transcription factors

Phosphorylation of transcription factors is a major mechanism utilized to control their intracellular location, trans-activating potential or DNA-binding properties (reviewed in [105]). Although progress has been made in identifying the kinases responsible for these phosphorylations, much less is known about the phosphatases that act upon transcription factors.

The best studied case is that of the transcription factor CREB, which binds to the cAMP-responsive element. CREB is phosphorylated on Ser-133, and this phosphorylation increases its trans-activation activity without affecting its DNA-binding properties. An initial study using isolated phosphatase catalytic subunits suggested that PP1 was the major CREB phosphatase [106], whereas a later study showed that CREB dephosphorylation in nuclear extracts is catalysed by a specific holoenzyme of PP2A [107]. Furthermore, the presence of small-t antigen inhibits CREB dephosphorylation both *in vitro* and in intact cells [108]. Microinjection of an expression vector that encodes a constitutively active form of I-1, however, leads to an increase in the phosphorylation state of CREB, again indicating a role for PP1 in CREB dephosphorylation [109].

One explanation for the discrepancy between these studies might be the use of different cell types (PC12 cells and NIH 3T3 fibroblasts versus HepG2 hepatoma cells). Alternatively, PP2A might regulate PP1 activity in the nucleus and thus some of the observed effects might be indirect.

PROTEIN PHOSPHATASE 2B

Structure of PP2B

PP2B or calcineurin was first identified as a major calmodulin-binding protein from brain, where it accounts for up to 1% of the total protein (reviewed in [110]). Later it was shown to display Ser/Thr protein phosphatase activity. PP2B is a heterodimer of calcineurin A (molecular mass 60 kDa) and calcineurin B (19 kDa). Calcineurin A is the catalytic subunit and also binds to calmodulin, whereas calcineurin B is the regulatory, Ca^{2+}-binding subunit. Calcineurin B is a member of the 'EF hand' family of Ca^{2+}-binding proteins. It contains four Ca^{2+}-binding loops and shows 35% sequence identity to calmodulin.

The use of monoclonal antibodies revealed the presence of different isoforms of PP2B catalytic subunit in brain compared with other tissues [111]. These results were confirmed by molecular cloning and Northern blot analysis. The catalytic subunit

is encoded by three separate genes (*CNAα*, *CNAβ* and *CNAγ*). The *CNAα* gene gives rise to two transcripts (α1 and α2). The corresponding isoforms differ by a 10-amino-acid insertion in the C-terminal domain of α1. The *CNAβ* gene gives rise to three transcripts (β1, β2 and β3). Compared with CNAβ2, the CNAβ1 isoform lacks a 10-amino-acid insertion at the C-terminus, but carries an 18-amino-acid insertion at residue 137. The CNAβ3 isoform lacks the 10-amino-acid insertion, but its sequence is otherwise identical to that of CNAβ2. CNAα1 and CNAβ2 are the predominant forms in brain, whereas CNAγ is a testis-specific form.

The situation for the B-subunit of calcineurin was long thought to be less complex. It has now, however, become clear that two genes encode this subunit [112,113]. The *CNBα* gene gives rise to an isoform that is highly expressed in brain (CNBα1), but is also present in most other tissues. By the use of a different promoter the *CNBα* gene gives rise to an isoform (CNBα2) that is testis-specific. The second gene (*CNBβ*) is also specifically expressed in testis.

Regulation of PP2B

PP2B is dependent upon Ca^{2+} for activity and is stimulated by calmodulin, but the purified enzyme also requires bivalent metal ions such as Mn^{2+}, Mg^{2+} or Ni^{2+} (reviewed in [110]). Interestingly, calcineurin becomes activated at much lower Ca^{2+} concentrations (K_d = 0.1 nM) than the Ca^{2+}/calmodulin-dependent protein kinase II (K_d = 45 nM).

Partial proteolysis of calcineurin converts it to a form which no longer requires Ca^{2+} for activity. This is due to destruction of the C-terminus of the catalytic subunit, which contains an auto-inhibitory domain [114]. Binding of calmodulin to a nearby site and binding of Ca^{2+} to the B-subunit allows the enzyme to overcome this auto-inhibition.

Inhibition of calcineurin activity towards model substrate peptides by the immunosuppressant/immunophilin complex (FK506/FKBP or cyclosporin A/cyclophilin) is mediated by both calcineurin subunits [115]. Association of calcineurin with FK506/FKBP is dependent on Ca^{2+}, unless the auto-inhibitory domain on the catalytic subunit is removed [115]. More recent studies [116,117] indicate that both the FK506/FKBP and cyclosporin A/cyclophilin interact with the latch region of the CNB subunit but that the association of CNB with CNA is required for the former to adopt the correct conformation to permit interaction with drug/immunophilin complexes. Interestingly, the so-called latch region allosterically activates the CNA catalytic subunit.

PP2B has a rather restricted substrate specificity. The α subunit of phosphorylase kinase, I-1, DARPP-32 and the type II regulatory subunit of cAMP-PK (RII) are excellent *in vitro* substrates.

Physiological role of PP2B

PP2B and T-cell activation

T-cell activation, in response to the interaction of the T-cell receptor with antigen, is mediated by induction of interleukin-2 gene expression. Activation of at least two signalling pathways is needed for this induction (reviewed in [118]). One pathway can be activated with Ca^{2+} ionophores, and the other with phorbol esters. At the level of the promoter, induction of interleukin-2 gene expression requires co-operative interactions of several transcription factors.

The Ca^{2+}-dependent pathway is mediated via activation of calcineurin. This leads to dephosphorylation of the cytosolic subunit of the transcription factor NF-AT, as is shown in Figure 5. The dephosphorylated subunit then migrates to the nucleus where it combines with nuclear factors (probably jun and fos) to form the transcription factor NF-AT [119]. NF-AT binds to, and activates, the interleukin-2 promoter.

Calcineurin also appears to mediate effects on other Ca^{2+}-sensitive elements within the interleukin-2 promoter (see Figure 5), such as the binding sites for Oct-1 and NF-κB. Surprisingly, the effect of calcineurin on NF-κB is mediated by phosphorylation (inactivation) of IκB, the cytosolic inhibitor of NF-κB. As depicted in Figure 5, this might be due to calcineurin-mediated activation of an IκB kinase [120].

The immunosuppressants FK506 and cyclosporin A block the activation of T-cells by inhibiting calcineurin. Although FK506 and cyclosporin A are not inhibitory to calcineurin as such, they become potent inhibitors when complexed to their intracellular receptors FKBP and cyclophilin respectively [121].

PP2B in other systems

The finding that PP2B is specifically inhibited by FK506/FKBP and cyclosporin A/cyclophilin complexes made it possible to investigate the function of PP2B in a variety of cellular systems. In these experiments a control is provided by the overexpression of PP2B (which overcomes the effects of immunosuppressants), or by the use of antagonists such as rapamycin, which binds to FKBP without affecting PP2B. Using this approach, Schwaninger et al. [122] showed that, in a pancreatic acinar cell line, calcineurin was responsible for mediating Ca^{2+}-induced gene expression, driven by the cAMP-responsive element. In isolated pancreatic acini, a role for PP2B in the release of amylase in response to cholecystokinin and Ca^{2+}-mobilizing agents was suggested [123,124].

Inhibition of PP2B results in stimulation of corticotropin secretion in a rat pituitary tumour cell line [125], and protects thymocytes and immature B-cells against Ca^{2+}-induced apoptosis [126,127]. Furthermore, PP2B mediates the α-adrenergic stimulation of Na^+/K^+-ATPase in renal tubule cells [128] and regulates K^+ channels in guard cells from the plant *Vicia faba* [129].

FK506 protects primary cortical cultures against *N*-methyl-D-aspartate (NMDA) neurotoxicity [130], and PP2B functions in a negative feedback mechanism regulating the channel opening of the NMDA subtype of the glutamate receptor [131]. Calcineurin was shown to mediate the effect of activation of the NMDA receptor in the hippocampus [132] and the striatum [133].

In most of these studies, however, the direct substrates of calcineurin were not determined. It appears that activation of calcineurin in response to Ca^{2+} is an early event. This is reflected by the model substrates of PP2B (I-1, RII, DARPP-32), which themselves are mostly inhibitors of protein phosphatases and protein kinases. It appears likely that calcineurin functions in a cascade system [1] and is the key enzyme controlling the activity state of other enzymes with a much broader substrate specificity. This view is confirmed by the study of calcineurin function in NMDA receptor signalling [123,124], where calcineurin activation leads to dephosphorylation (inactivation) of I-1/DARPP-32 and thus indirectly to PP1 activation.

The budding yeast *Saccharomyces cerevisiae* turns out to be a good model system for the study of PP2B function. In this organism, PP2B is encoded by two genes (*CMP1* and *CMP2*). A double mutant lacking both genes was isolated that grows normally under standard conditions [134]. High NaCl concentrations, however, inhibit growth of this double mutant, indicating that PP2B is responsible for the stimulation of Na^+ export [135]. The same phenotype was observed in a mutant lacking a functional B subunit of calcineurin [136]. The authors suggested

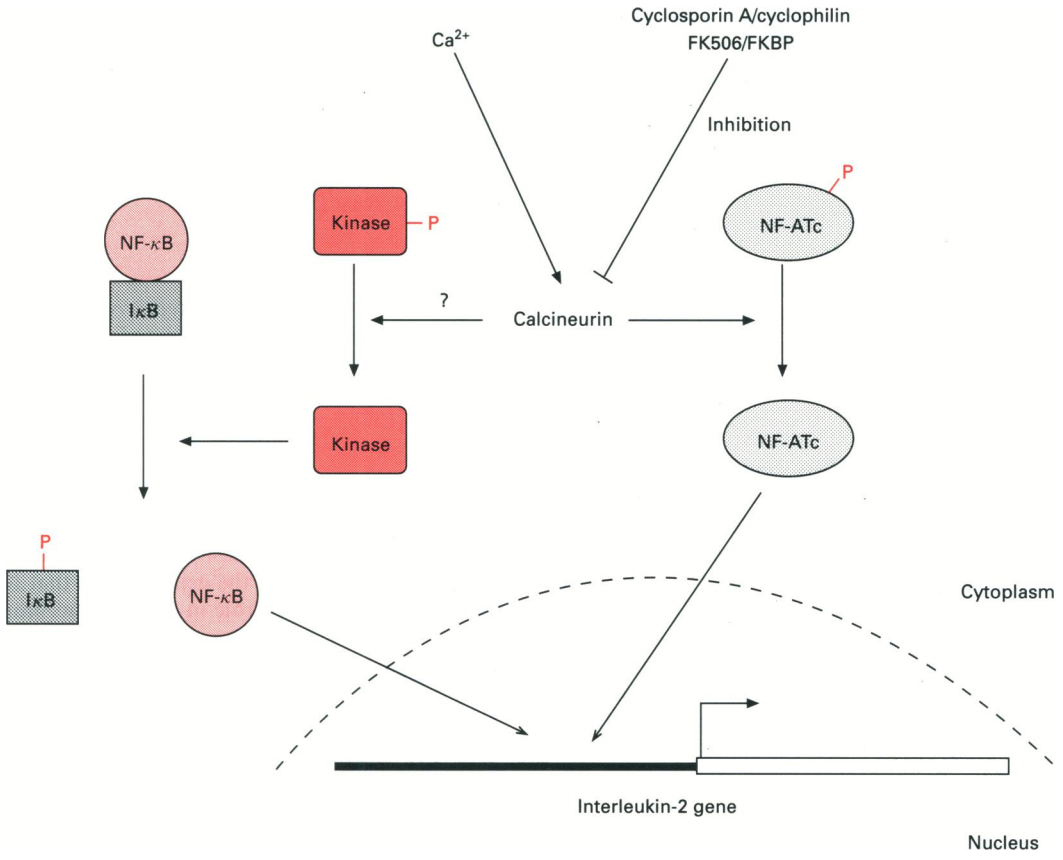

Figure 5 Role of calcineurin in T-cell activation

T-cell activation is triggered in part by activation of calcineurin, which directly or indirectly leads to dephosphorylation of NF-ATc. Dephosphorylated NF-ATc migrates to the nucleus where it assembles with other subunits into the transcription factor NF-AT, leading to interleukin-2 gene expression [118–121]. Activated calcineurin inactivates IκB, possibly by activating an IκB kinase [120]. Phosphorylated IκB dissociates from NF-κB, which then also migrates to the nucleus and binds to the interleukin-2 promoter. The FK506/FKBP complex blocks these events by inhibiting calcineurin [121].

that this mutated B subunit could act as a dominant negative. They further showed that the phenotype was due to reduced expression of *ENA1*, a gene encoding a P-type ATPase involved in Li^+ and Na^+ efflux. Again PP2B seems to act indirectly, in this case by controlling gene expression.

In *Schizosaccharomyces pombe*, PP2B is encoded by a single gene, termed *ppb1*$^+$ [137]. Disruption of this gene leads to defects in cytokinesis and to virtually sterile cells. In agreement with this, defects in cytokinesis were also observed following treatment of fission yeast with cyclosporin A.

PROTEIN PHOSPHATASE 2C

PP2C was originally identified as a Mg^{2+}-dependent protein phosphatase (half-maximal activity at 1 mM Mg^{2+}). The enzyme is monomeric with a molecular mass of 43–48 kDa (reviewed in [1]). Two major isoforms (α, β) of PP2C have been cloned. Further analysis revealed the existence of subtypes for both isoforms. Three α subtypes have been identified, with $\alpha 1$ being the most dominant species. Two subtypes of the β isoform appear to be the result of alternative splicing, since they differ only at their C-terminus. Whereas $\beta 1$ is ubiquitously expressed, $\beta 2$ transcripts are only found in brain and heart [138,139]. The latter observation might be important, since PP2C was suggested to play a role in Ca^{2+}-dependent signal transduction in brain. More specifically, PP2C might catalyse the dephosphorylation of autophosphorylated Ca^{2+}/calmodulin-dependent protein kinase II in granule cells of the cerebellum [140].

Interestingly, Peruski et al. [139] identified a region of sequence identity between PP2C and PP1/PP2A/PP2B that is located in the putative catalytic domain and spans 80 amino acid residues. This suggests, as pointed out by these authors, that all of these enzymes are derived from a common ancestor.

In both *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* three genes encoding PP2C homologues have been identified [141–143]. The analysis of yeast strains carrying deletions of these three genes suggests the existence of a fourth isoform [143]. Mutations in the *TPD1/PTC1* gene of *Saccharomyces cerevisiae* [142,144] cause a temperature-sensitive growth defect (failure of cell separation during mitosis) and are accompanied by the accumulation of unspliced tRNA species. In *Schizosaccharomyces pombe*, the PP2C isoform, encoded by the *ptc1*$^+$ gene, is important in the heat shock response. mRNA levels of *ptc1*$^+$ increase 5–10-fold upon heat shock, and deletion mutants have greatly reduced ability to survive heat shock [145].

Recent analysis of both yeast strains has provided clues on the *in vivo* targets of PP2C [142,143]. The results from both of these studies implicate PP2C in signal transduction processes involved in osmoregulation. It appears that PP2C negatively regulates an osmosensing MAP kinase cascade comprising the PBS2 and

HOG1 kinases. Whether PP2C directly regulates the kinases or their downstream targets still remains to be established.

In *Arabidopsis thaliana* the *abi1* gene, essential for the response to the plant hormone abscisic acid, was shown to encode a protein homologous to PP2C. Interestingly, this PP2C homologue has a putative Ca^{2+}-binding site [146,147].

OTHER PROTEIN PHOSPHATASES

Many novel protein phosphatases have been recently identified by molecular cloning. These are summarized in Table 1 and discussed below.

Protein phosphatase λ

The N-terminal halves of the proteins encoded by *orf221* of bacteriophage and the homologous gene of $\pi 80$ show 35% identity to PP1 and PP2A repectively [148]. *Escherichia coli* infected with λgt10 contains a casein phosphatase activity which is absent from uninfected cells or from cells infected with λgt11, lacking part of *orf221* [149]. More recently, the *orf221* gene was cloned and expressed in bacteria, and the recombinant protein was purified and shown to posses Ser/Thr phosphatase activity. Characterization of the enzyme revealed that it was resistant to okadaic acid, I-1 and I-2, but absolutely required Mn^{2+} for activity [150].

Mitochondrial protein phosphatases

Labelling of mitochondria with radioactive phosphate results in the detection of two major phosphoproteins: pyruvate dehydrogenase and branched-chain 2-oxo-acid dehydrogenase (reviewed in [151]). Dephosphorylation of these proteins appears to be catalysed by highly specific phosphatases. The phosphatase acting upon pyruvate dehydrogenase consists of a 97 kDa flavoprotein and a catalytic subunit of about 50 kDa [151]. The latter has been cloned and expressed in bacteria. The activity of the recombinant protein towards pyruvate dehydrogenase was Mg^{2+}-dependent and Ca^{2+}-stimulated [152]. Some identity with the primary sequence of PP2C is observed, suggesting a common origin of the two phosphatases.

Protein phosphatase Z

The *Saccharomyces cerevisiae* enzyme PPZ exists in two isoforms (PPZ1 and PPZ2) that show about 60% identity to yeast PP1 and 40% identity to PP2A [153,154]. Characteristic of these phosphatases is a large Ser-rich N-terminal extension. Both isoforms of PPZ are involved in the maintenance of osmotic stability in yeast [155,156]

Protein phosphatase Q1

PPQ1 is a 61 kDa *Saccharomyces cerevisiae* Ser/Thr protein phosphatase that contains a C-terminal domain which is 60% similar to PP1, and a Ser/Asp-rich N-terminal domain. Deletion of the gene encoding PPQ1 results in a reduced rate of protein synthesis [157].

Protein phosphatase G

PPG from *Saccharomyces cerevisiae* is related to PP2A and is characterized by an extension of 50 amino acids at the C-terminus. Disruption of the gene showed that it is not essential for growth, but plays a role in glycogen accumulation [158].

Protein phosphatase X

A cDNA encoding PPX was isolated from a rabbit liver library [159]; it encodes a 35 kDa protein whose amino acid sequence shows 65% identity to PP2A and 45% identity to PP1. The sensitivity of PPX to okadaic acid is similar to that of PP2A. Purified PPX does not, however, form a complex with the PR65 subunit of PP2A. Immunofluorescence revealed that PPX is predominantly associated with centrosomes [160], but its function at this location is at present unclear.

Protein phosphatase SIT-4

SIT-4 was identified as a suppressor of a mutation that leads to defects in the transcription of the *HIS4* gene in *Saccharomyces cerevisiae* [161]. Later SIT-4, a PP2A-like protein phosphatase, was shown to play a role in glycogen metabolism. SIT-4 mutants show much higher levels of glycogen phosphorylase *a* and have a much lower glycogen synthase activity ratio compared with wild-type cells [162]. Furthermore SIT-4 is important for the G1

Table 1 Novel protein phosphatases

See the text for references.

Name	Source	Most closely related 'classical' phosphatase	Function
PPλ	Phage λ	PP1/PP2A	Lysis/lysogeny decision
Pyruvate dehydrogenase PP	Mitochondria	PP2C	Pyruvate dehydrogenase dephosphorylation
PPZ	Budding yeast	PP1	Osmotic stability
PPQ	Budding yeast	PP1	Protein synthesis
PPG	Budding yeast	PP2A	Glycogen metabolism
PPX	Rabbit liver	PP2A	Localized to centrosomes
SIT-4	Budding yeast	PP2A	Glycogen metabolism; G1 to S transition
PPV	*Drosophila*	PP2A	*Drosophila* SIT-4 homologue?
PPY	*Drosophila*	PP1	
rdgC	*Drosophila*	PP2B	Prevents light-induced retinal degradation
PPT	Budding yeast	PP1/PP2A/PP2B	Nuclear phosphatase
PP5	Human	PP1/PP2A/PP2B	Human PPT homologue

to S transition during cell cycle in yeast [163]. Part of this function of SIT-4 is explained by its effect on the accumulation of G1 cyclin mRNAs [164].

Protein phosphatases V and Y

PPV and PPY, both isolated from *Drosophila*, are related to PP1 and PP2A respectively [165]. Overexpression of *Drosophila* PPV can complement SIT-4 mutants of *Saccharomyces cerevisiae* [166]. A chimaeric protein phosphatase composed of the catalytic region of PP1 fused to the N-terminus of PPV has the same effect, suggesting that this region is involved in substrate specificity or in binding to regulatory subunits.

Protein phosphatase rdgC

The product of the *Drosophila* retinal degradation C (*rdgC*) gene is a protein phosphatase [167] that, within its predicted catalytic domain, displays 30% identity to the PP2B family. It also contains a regulatory domain with multiple (potential) Ca^{2+}-binding sites. The *rdgC* gene is necessary to prevent light-induced retinal degradation.

Protein phosphatase T (PP5)

A PPT (or PP5) cDNA was isolated from a rat fat-cell library [168]. The gene *PPT1* encodes the *Saccharomyces cerevisiae* homologue [169]. The enzyme is predominantly nuclear, but is apparently excluded from the nucleolus. PPT is related to PP1, PP2A and PP2B, but has an N-terminal extension of 200 amino acids. This extension contains tetratricopeptide repeat (TPR) motifs, also found in several other nuclear proteins.

PERSPECTIVES

Protein phosphatases specific for phosphoserine and phosphothreonine residues are involved in virtually all aspects of cellular regulation. Based on biochemical characteristics they have been subdivided into four main classes. PP1 and PP2A catalytic subunits interact with a number of different regulatory subunits, resulting in a wide variety of holoenzymes, a feature that could explain the pleiotropic actions of these enzymes. PP2B (calcineurin), a Ca^{2+}-dependent phosphatase, is crucial as a mediator of the Ca^{2+}-signalling pathway in a variety of cell types. At present, only limited information is available on the function of the Mg^{2+}-dependent protein phosphatase (PP2C). Further insights into the role of Ser/Thr protein phosphatases will undoubtedly come through the study of the 'non-classical' enzymes, recently revealed by molecular cloning techniques. In this respect, the *Caenorhabditis elegans* genome sequencing project has provided valuable information on the magnitude of this problem, as recently pointed out by Hunter [170]. It is predicted that the mammalian genome encodes 1000 protein phosphatases (including both Ser/Thr and Tyr phosphatases). If many of these new species are as complex as PP1/PP2A in terms of regulatory subunits then the total number of phosphatase holoenzymes is likely to be higher than this figure. We are therefore approaching a situation where there is one phosphatase for every kinase. This may not be the beginning of the end, but certainly it is the end of the beginning (with apologies to W. Churchill!).

Until recently, protein phosphatases were perceived as rather unsophisticated components in the cellular tapestry of phosphorylation/dephosphorylation reactions. It was also thought that there are only a few relatively unspecific phosphatases capable of resetting the work of numerous protein kinases. It is now apparent that both of these ideas were oversimplistic. What should also be appreciated is that the mechanisms of regulation of the Ser/Thr protein phosphatases will be as complex and varied as the enzymes themselves.

The picture that appears to be emerging is either that the regulation of phosphatases is mediated by the assembly of complex holoenzymes consisting of catalytic and regulatory subunits, or that the catalytic subunits are fused regulatory domains. Both of these types of regulation are very reminiscent of those adopted by the protein kinase superfamily.

Apart from identifying other members of the protein phosphatase family, the challenge in the future will be to understand how the different regulatory subunits function in terms of responding to the appropriate signals to bring about the orderly dephosphorylation of target proteins and enzymes. The example of the PR55 mutant from *Drosophila* is particularly illustrating in this respect. In this case the loss of a single regulatory subunit leads to a dramatic phenotype, i.e. neuroblasts fail to complete the metaphase/anaphase transition. This result suggests that a subset of substrates is not regulated (dephosphorylated?) correctly, and that the PR55 subunit is crucial in identifying these proteins.

Identification of substrates is likely to be an important aspect of future studies. This could also involve finding the appropriate kinase to phosphorylate a specific substrate. The use of the two-hybrid system will undoubtedly help in this identification process.

Another exciting problem with respect to the oligomeric phosphatases is how the cell regulates the assembly of the different subunits into the correct holoenzyme of a particular cell type or tissue. Part of this control may be at the transcriptional level, e.g. binding sites for brain-specific transcription factors in the promoter of the *PR55β* and *PR55γ* genes. It seems likely, however, that translational and post-translational control mechanisms also contribute to this regulation.

Overall it can be stated that our vision of the protein phosphatase field has been greatly enhanced through the use of molecular genetic techniques, but there is still some way to go. We are indeed in a new era for this group of enzymes.

We thank Natasa Andjelkovic, Aleyde Van Eynde, Tom Millward, Dr. Stanislaw Zolnierowicz, Dr. Thomas Boller, Dr. Mathieu Bollen and Dr. Jozef Goris for comments on this review. S.W. is the recipient of a long-term EMBO fellowship.

REFERENCES

1 Cohen, P. (1989) Annu. Rev. Biochem. **58**, 453–508
2 Ingebritsen, T. S. and Cohen P. (1983) Eur. J. Biochem. **132**, 255–261
3 Cohen, P., Klumpp, S. and Schelling, D. L. (1989) FEBS Lett. **250**, 596–600
4 Barton, G. J., Cohen, P. T. W. and Barford, D. (1994) Eur. J. Biochem. **220**, 225–237
5 Holmes, C. F. B. and Boland, M. P. (1993) Curr. Opin. Struct. Biol. **3**, 934–943
6 Goris, J., Hermann, J., Hendrix, P., Ozon, R. and Merlevede, W. (1989) Adv. Protein Phosphatases **5**, 579–592
7 Li, Y. M. and Casida, J. E. (1992) Proc. Natl. Acad. Sci. U.S.A. **89**, 11867–11870
8 Honkanen, R. E. (1993) FEBS Lett. **330**, 283–286
9 Cairns, J., Qin, S., Philp, Y. H. T. and Guy, G. R. (1994) J. Biol. Chem. **269**, 9176–9183
10 Bollen, M. and Stalmans, W. (1992) Crit. Rev. Biochem. Mol. Biol. **27**, 227–281
11 DePaoli-Roach, A. A., Park, I. K., Cerovsky, V. et al. (1994) Adv. Enzyme Regul. **34**, 199–224
12 Shenolikar, S. and Nairn, A. C. (1990) Adv. Second Messenger Phosphoprotein Res. **23**, 1–121
13 Barford, D. and Keller, J. C. (1994) J. Mol. Biol. **235**, 763–766
14 Cohen, P. T. W. (1988) FEBS Lett. **232**, 17–23
15 Berndt, N. and Cohen, P. T. W. (1990) Eur. J. Biochem.**190**, 291–297
16 Sasaki, K., Shima, H., Kitagawa, Y., Irino, S., Sugimara, T. and Nagao, M. (1990) Jpn. J. Cancer Res. **81**, 1272–1280
17 Okhura, H., Kinoshita, N., Miyatani, S., Toda, T. and Yanagida, M. (1989) Cell **57**, 997–1007

18 Barker, H. M., Brewis, N. D., Street, A. J., Spurr, N. K. and Cohen, P. T. W. (1994) Biochim. Biophys. Acta **1220**, 212–218
19 Alessi, D. R., Street, A. J., Cohen, P. and Cohen, P. T. W. (1993) Eur. J. Biochem. **213**, 1055–1066
20 Zhang, Z., Zhao, S., Bai, G. and Lee, E. Y. C. (1994) J. Biol. Chem. **269**, 13766–13770
21 Zhang, Z., Zhao, S., Long, F., Zhang, L., Bai, G., Shima, H., Nagao, M. and Lee, E. Y. C. (1994) J. Biol. Chem. **269**, 16997–17000
22 Hubbard, M. J. and Cohen, P. (1993) Trends Biochem. Sci. **18**, 172–177
23 Stralfors, P. Hiraga, A. and Cohen, P. (1985) Eur. J. Biochem. **149**, 295–303
24 Hubbard, M. J. and Cohen, P. (1989) Eur. J. Biochem. **186**, 711–716
25 Tang, P. M., Bondor, J. A., Swiderek, K. M. and DePaoli-Roach, A. A. (1991) J. Biol. Chem. **266**, 15782–15789
26 François, J. M., Thompson-Jaeger, S., Skroch, J., Zellenka, U., Spevak, W. and Tatchell, K. (1992) EMBO J. **11**, 87–96
27 Hubbard, M. J., Dent, P., Smythe, C. and Cohen, P. (1990) Eur. J. Biochem. **189**, 243–249
28 MacDougall, L. K., Jones, L. R. and Cohen, P. (1991) Eur. J. Biochem **196**, 725–734
29 Wera, S., Bollen, M. and Stalmans, W. (1991) J. Biol. Chem. **266**, 339–345
30 Alessi, D., MacDougall, L. K., Sola, M. M., Ikebe, M. and Cohen, P. (1992) Eur. J. Biochem. **210**, 1023–1035
31 Okubo, S., Ito, M., Takashiba, Y. et al. (1994) Biochem. Biophys. Res. Commun. **200**, 429–434
32 Dent, P., MacDougall, L. K., MacKintosh, C., Campbell, D. G. and Cohen, P. (1992) Eur. J. Biochem. **210**, 1037–1044
33 Kuret, J., Bell, H. and Cohen, P. (1986) FEBS Lett. **203**, 197–202
34 Beullens, M., Van Eynde, A., Bollen, M. and Stalmans, W. (1992) J. Biol. Chem. **267**, 16538–16544
35 Fernandez, A., Brautigan, D. L. and Lamb, N. J. C. (1992) J. Cell Biol. **116**, 1421–1430
36 Beullens. M., Van Eynde. A., Bollen. M. and Stalmans. W. (1993) J. Biol. Chem. **268**, 13172–13177
37 Bollen, M., DePaoli-Roach, A. A. and Stalmans, W. (1994) FEBS Lett. **344**, 196–200
38 Chun, Y. S., Shima, H., Nagasaki, K., Sugimura, T. and Nagao, M. (1994) Proc. Natl. Acad. Sci. U.S.A. **91**, 3319–3323
39 Durfee T., Becherer K., Chen P. L. et al. (1993) Genes Dev. **7**, 555–569
40 Park, I. K., Roach, P., Bondor, J., Fox, S. P. and DePaoli-Roach, A. A. (1994) J. Biol. Chem. **269**, 944–954
41 Hubbard, M. J. and Cohen, P. (1989) Eur. J. Biochem. **186**, 701–709
42 Dent, P., Campbell, D. G., Caudwell, F. B. and Cohen, P. (1990) FEBS Lett. **259**, 281–285
43 Nakielny, S., Campbell, D. G. and Cohen, P. (1991) Eur. J. Biochem. **199**, 713–722
44 Dent, P., Lavoinne, A., Nakielny, S., Caudwell, F. B., Watt, P. and Cohen, P. (1990) Nature (London) **348**, 302–308
45 Sutherland, C., Campbell, D. G. and Cohen, P. (1993) Eur. J. Biochem. **212**, 581–588
46 Fiol C. F., Haseman J. H., Wang Y. et al. (1988) Arch. Biochem. Biophys. **267**, 797–802
47 Dent, P., Campbell, D. G., Hubbard, M. J. and Cohen, P. (1989) FEBS Lett. **248**, 67–72
48 Cross, D. A. E., Alessi, D. R., Vandenheede, J. R., McDowell, H. E., Hundal, H. S. and Cohen, P. (1994) Biochem. J. **303**, 21–26
49 Eldar-Finkelman, H., Seger, R., Vandenheede, J. R. and Krebs, E. G. (1995) J. Biol. Chem. **270**, 987–990
50 Stalmans, W., De Wulf, H. and Hers, H. G. (1971) Eur. J. Biochem. **18**, 582–587
51 Alemany, S. and Cohen, P. (1986) FEBS Lett. **198**, 194–202
52 Van Eynde, A., Beullens, M., Stalmans, W. and Bollen, M. (1994) Biochem. J. **297**, 447–449
53 Paulson, J. R., Ciesielski, W. A., Schram, B. R. and Mesner, P. W. (1994) J. Cell Sci. **107**, 267–273
54 Kiss, Z. and Steinberg, R. A. (1985) J. Cell. Physiol. **125**, 200–206
55 Doonan, J. H. and Morris, N. R. (1989) Cell **57**, 987–996
56 Booher, R. and Beach, D. (1989) Cell **57**, 1009–1016
57 Francisco, L., Wang, W. and Chan, C. S. M. (1994) Mol. Cell. Biol. **14**, 4731–4740
58 Axton, J. M., Dombradi, V., Cohen, P. T. W. and Glover, D. M. (1990) Cell **63**, 33–46
59 Dombradi, V., Axton, J. M., Barker, H. M. and Cohen, P. T. W. (1990) FEBS Lett. **275**, 39–43
60 Fernandez, A., Brautian, D. L. and Lamb, N. J. C. (1992) J. Cell Biol. **116**, 1421–1430
61 Dohadwala M., Da Cruz e Silva E. F., Hall F. L. et al. (1994) Proc. Natl. Acad. Sci. U.S.A. **91**, 6408–6412
62 Mayer-Jaekel, R. E. and Hemmings, B. A. (1994) Trends Cell Biol. **4**, 287–291
63 Mumby, M. C. and Walter, G. (1993) Physiol. Rev. **73**, 673–699
64 Hendrix, P., Mayer-Jaekel, R. E., Cron, P., Goris, J., Hofsteenge, J., Merlevede, W. and Hemmings, B. A. (1993) J. Biol. Chem. **268**, 15267–15276
65 Turowski, P., Fernandez, A., Favre, B., Lamb, N. J. C. and Hemmings, B. A. (1995) J. Cell. Biol. **129**, 397–410
66 Zolnierowicz, S., Csortos, C., Bondor, J., Verin, A., Mumby, M. C. and DePaoli-Roach, A. A. (1994) Biochemistry **33**, 11858–11867
67 Sneddon, A. A., Cohen, P. T. W. and Stark, M. J. R. (1990) EMBO J. **9**, 4339–4346
68 Ronne, H., Carlberg, M., Hu, G. Z. and Nehlin, J. O. (1991) Mol. Cell. Biol. **11**, 4876–4884
69 Hu, G. Z. and Ronne, H. (1994) J. Biol. Chem. **269**, 3429–3435
70 van Zyl, W., Huang, W., Sneddon A. A. et al. (1992) Mol. Cell. Biol. **12**, 4946–4959
71 Healy, A. M., Zolnierowicz, S., Stapleton, A. E., Goebl, M., DePaoli-Roach, A. A. and Pringle, J. R. (1991) Mol. Cell. Biol. **11**, 5767–5780
72 Hannun, Y. A. (1994) J. Biol. Chem. **269**, 3125–3128
73 Dobrowski, R. T., Kamibayashi, C., Mumby, M. C. and Hannun, Y. A. (1993) J. Biol. Chem. **268**, 15523–15530
74 Wolff, R. A., Dobrowsky, R. T., Bielawska, A., Obeid, L. M. and Hannun, Y. A. (1994) J. Biol. Chem. **269**, 19605–19609
75 Kolesnick, R. and Golde, D. W. (1994) Cell **77**, 325–328
76 Chen, J., Martin, B. L. and Brautigan, D. (1992) Science **257**, 1261–1264
77 Chen, J., Parsons, S. and Brautigan, D. L. (1994) J. Biol. Chem. **269**, 7957–7962
78 Srinivasan, M. and Begum, N. (1994) J. Biol. Chem. **269**, 12514–12520
79 Haystead, T. A. J. and Krebs, E. G. (1991) Adv. Protein Phosphatases **6**, 331–346
80 Guo, H. and Damuni, Z. (1993) Proc. Natl. Acad. Sci. U.S.A. **90**, 2500–2504
81 Lee, J. and Stock, J. (1993) J. Biol. Chem. **268**, 19192–19195
82 Xie, H. and Clarke, S. (1994) J. Biol. Chem. **269**, 1981–1984
83 Favre, B., Zolnierowicz, S., Turowski, P. and Hemmings, B. A. (1994) J. Biol. Chem. **269**, 16311–16317
84 Zolnierowicz, S., Favre, B. and Hemmings, B. A. (1994) Adv. Protein Phosphatases **8**, 355–369
85 Floer, M. and Stock, J. (1994) Biochem. Biophys. Res Commun. **198**, 372–379
86 Li, M. and Damuni, Z. (1994) Biochem. Biophys. Res. Comm. **202**, 1023–1030
87 Jessus, C., Goris, J., Staquet, S., Cayla, X., Ozon, R. and Merlevede, W. (1989) Biochem. J. **260**, 45–51
88 Cayla, X., Goris, J., Hermann, J., Hendrix, P., Ozon, R. and Merlevede, W. (1990) Biochemistry **29**, 658–667
89 Cayla, X., Van Hoof, C., Bosch, M. et al (1994) J. Biol. Chem. **269**, 15668–15675
90 Cayla, X, Ballmer-Hofer, K., Merlevede, W. and Goris, J. (1993) Eur. J. Biochem. **214**, 281–286
91 Agostinis, P., Derua, R., Sarno, S., Goris, J. and Merlevede, W. (1992) Eur. J. Biochem. **205**, 241–248
92 Ferrigno, P., Langan, T. A. and Cohen, P. (1993) Mol. Biol. Cell **4**, 669–677
93 Cegielska, A., Shaffer, S., Derua, R., Goris, J. and Virshup, D. M. (1994) Mol. Cell. Biol. **14**, 4616–4623
94 Mayer-Jaekel, R. E., Okhura, H., Gomes, R., Sunkel, C. E., Baumgartner, S., Hemmings, B. A. and Glover, D. M. (1993) Cell **72**, 621–633
95 Mayer-Jaeke, R. E., Okhura, H., Ferrigno, P. et al. (1994) J. Cell Sci. **107**, 2609–2616
96 Shiomi, K., Takeichi, M., Nishida, Y., Nishi, Y. and Uemura, T. (1994) Development **120**, 1591–1599
97 Walter, G. and Mumby, M. (1993) Biochim. Biophys. Acta **1155**, 207–226
98 Sontag, E., Fedorov, S., Kamibayashi, C., Robbins, D., Cobb, M. and Mumby, M. (1993) Cell **75**, 887–897
99 Dunphy, W. G. (1994) Trends Cell Biol. **4**, 202–207
100 Goris, J., Hermann, J., Hendrix, P., Ozon, R. and Merlevede, W. (1989) FEBS Lett. **245**, 91–94
101 Felix, M. A., Cohen, P. and Karsenti, E. (1990) EMBO J. **9**, 675–683
102 Lee, T. H., Turck, C. and Kirschner, M. W. (1994) Mol. Biol. Cell **5**, 323–338
103 Solomon, M. J., Lee, T. and Kirschner, M. W. (1992) Mol. Biol. Cell **3**, 13–27
104 Clarke, P. R., Hoffmann, I., Draetta, G. and Karsenti, E. (1993) Mol. Biol. Cell **4**, 397–411
105 Hunter, T. and Karin, M. (1992) Cell **70**, 375–387
106 Hagiwara, M., Alberts, A., Brindle, P. et al. (1992) Cell **70**, 105–113
107 Wadzinsky, B. E., Wheat, W. H., Jaspers, S., Peruski, L. F., Lickteig, R. L., Johnson, G. L. and Klemm, D. J. (1993) Mol. Cell. Biol. **13**, 2822–2834
108 Wheat, W. H., Roesler, W. J. and Klemm, D. J. (1994) Mol. Cell. Biol. **14**, 5881–5890
109 Alberts, A. S., Montminy, M., Shenolikar, S. and Feramisco, J. R. (1994) Mol. Cell. Biol. **14**, 4398–4407
110 Stemmer, P. and Klee, C. B. (1991) Curr. Opin. Neurobiol. **1**, 53–64
111 Yokoyama, N., Furuyama, S. and Wang, J. H. (1990) J. Biol. Chem. **265**, 8170–8175
112 Ueki, K., Muramatsu, T. and Kincaid, R. L. (1992) Biochem. Biophys. Res. Commun. **187**, 537–543
113 Chang, C. D., Mukai, H., Kuno, T. and Tanaka, C. (1994) Biochim. Biophys. Acta **1217**, 174–180

114 Hashimoto, Y., Perrino, B. A. and Soderling, T. R. (1990) J. Biol. Chem. **265**, 1924–1990
115 Parsons, J. N., Wiederrecht, G. J., Salowe, S., Burbaum, J. J., Rokosz, L. L., Kincaid, R. L. and O'Keefe, S. J. (1994) J. Biol. Chem. **269**, 19610–19616
116 Clipstone, N. A., Fiorentino, D. F. and Crabtree, G. R. (1994) J. Biol. Chem. **269**, 26431–26437
117 Milan, D., Griffith, J., Su, M., Price, E. R. and McKeon, F. (1994) Cell **79**, 437–447
118 Nolan, G. P. (1994) Cell **77**, 795–798
119 Jain, J., McCaffrey, P. G., Miner, Z. et al. (1993) Nature (London) **365**, 352–355
120 Frantz, B., Nordby, E. C., Bren, G. et al. (1994) EMBO J. **13**, 861–870
121 Liu, J., Farmer, J. D., Lane, W. S., Friedman, J., Weissman, I. and Schreiber, S. L. (1991) Cell **66**, 807–815
122 Schwaninger, M., Blume, R., Oetjen, E., Lux, G. and Knepel W. (1993) J. Biol. Chem. **268**, 23111–23115
123 Doi, R., Inoue, K., Chowdury, P. and Rayford, P. L. (1993) J. Pharmacol. Exp. Ther. **264**, 873–878
124 Groblewski, G. E., Wagner, A. C. C. and Williams, J. A. (1994) J. Biol. Chem. **269**, 15111–15117
125 Antoni, F. A., Shipstone, M. J. and Smith, S. M. (1993) Biochem. Biophys. Res. Commun. **194**, 226–233
126 Bonnefoy-Berard, N., Genestier, L., Flacher, M. and Revillard, J. P. (1994) Eur. J. Immunol. **24**, 325–329
127 Shi, Y., Sahai, B. M. and Green, D. R. (1989) Nature (London) **339**, 625–626
128 Aperia, A., Ibarra, F., Svensson, L. B., Klee, C. and Greengard, P. (1992) Proc. Natl. Acad. Sci. U.S.A. **89**, 7394–7397
129 Luan, S., Li, W., Rusnak, F., Assmann, S. M. and Schreiber, S. L. (1993) Proc. Natl. Acad. Sci. U.S.A. **90**, 2202–2206
130 Dawson, T. M., Steiner, J. P., Dawson, V. L., Dinerman, J. L., Uhl, G. R. and Snyder, S. H. (1993) Proc. Natl. Acad. Sci. U.S.A **90**, 9808–9812
131 Lieberman, D. N. and Mody, I. (1994) Nature (London) **369**, 235–239
132 Mulkey, R. M., Endo, S., Shenolikar, S. and Malenka, R. C. (1994) Nature (London) **369**, 486–488
133 Halpain, S., Girault, J. A. and Greengard, G. (1990) Nature (London) **343**, 369–372
134 Nakamura, T., Tsutsumi, H., Mukai, H., Kuno, T. and Miyakawa, T. (1992) FEBS Lett. **309**, 103–106
135 Nakamura, T., Liu, Y., Hirata, D., Namba, H., Harada, S., Hirokawa, T. and Miyakawa, T. (1993) EMBO J. **12**, 4063–4071
136 Mendoza, I., Rubio, F., Rodriguez-Navarro, A. and Pardo, J. M. (1994) J. Biol. Chem. **269**, 8792–8796
137 Yoshida, T., Toda, T. and Yanagida, M. (1994) J. Cell Sci. **107**, 1725–1735
138 Terasawa, T., Kobayashi, T., Murakami, T., et al. (1993) Arch. Biochem. Biophys. **307**, 342–349
139 Peruski, L. F., Wadzinsky, B. E. and Johnson, G. L. (1993) Adv. Protein Phosphatases **7**, 9–30
140 Fukunaga, K., Kobayashi, T., Tamura, S. and Miyamoto, E. (1993) J. Biol. Chem. **268**, 133–137
141 Murakami, T., Kobayashi, T., Terasawa, T. et al. (1994) J. Biochem. (Tokyo) **115**, 762–766
142 Maeda, T., Susannah, M., Wurgler-Murphy and Saito, H. (1994) Nature (London) **369**, 242–245
143 Shiozaki, K. and Russel, P. (1995) EMBO J. **14**, 492–502
144 Robinson, M. K., van Zyl, W. H., Phizicky, E. M. and Broach, J. R. (1994) Mol. Cell. Biol. **14**, 3634–3645
145 Shiozaki, K., Akhavan-Niaki, H., McGowan, C. H. and Russel, P. (1994) Mol. Cell. Biol. **14**, 3742–3751
146 Meyer, K., Leube, M. P. and Grill, E. (1994) Science **264**, 1452–1455
147 Leung, J., Bouvier-Bertrand, M., Morris, P. C., Guerrier, D., Chefdor, F. and Giraudat, J. (1994) Science **264**, 1448–1452
148 Cohen, P. T. W., Collins, J. F., Coulson, A. F. W., Berndt, N. and da Cruz e Silva, O. B. (1988) Gene **69**, 131–134
149 Cohen, P. T. W. and Cohen, P. (1989) Biochem. J. **260**, 931–934
150 Barik, S. (1993) Proc. Natl. Acad. Sci. U.S.A. **90**, 10633–10637
151 Bradford, A. P. and Yeaman, S. J. (1986) Adv. Protein Phosphatases **3**, 73–106
152 Lawson, J. E., Niu, X. D., Browning, K. S., Trong, H. L., Yan, J. and Reed, L. J. (1993) Biochemistry. **32**, 8987–8993
153 Da Cruz e Silva, E. F., Hughes, V., McDonald, P., Stark, M. J. R. and Cohen, P. T. W. (1991) Biochim. Biophys. Acta **1089**, 269–272
154 Posas, F., Casamayor, A., Morral, N. and Ariño, J. (1992) J. Biol. Chem. **267**, 11734–11740
155 Posas, F., Casamayor, A. and Ariño, J. (1993) FEBS Lett. **318**, 282–286
156 Hughes, V., Müller, A., Stark, M. J. R. and Cohen, P. T. W. (1993) Eur. J. Biochem. **216**, 269–279
157 Chen, M. X., Chen, Y. H. and Cohen, P. T. W. (1993) Eur. J. Biochem. **218**, 689–699
158 Posas. F., Clotet. J., Muns. M. T., Corominas. J., Casamayor. A. and Ariño. J. (1993) J. Biol. Chem. **268**, 1349–1354
159 Da Cruz e Silva, O. B., Da Cruz e Silva, E. F. and Cohen, P. T. W. (1988) FEBS Lett. **242**, 106–110
160 Brewis, N. D., Street, A. J., Prescott, A. R. and Cohen, P. T. W. (1993) EMBO J. **12**, 987–996
161 Arndt, K. T. Styles, C. A. and Fink, G. R. (1989) Cell **56**, 527–537
162 Posas, F., Clotet, J. and Ariño, J. (1991) FEBS Lett. **279**, 341–345
163 Sutton, A., Immanuel, D. and Arndt, K. T. (1991) Mol. Cell. Biol. **11**, 2133–2148
164 Fernandez-Sarabia, M. J., Sutton, A., Zhong, T. and Arndt, K. T. (1992) Genes Dev. **6**, 2417–2428
165 Cohen, P. T. W., Brewis, N. D., Hughes, V. and Mann, D. J. (1990) FEBS Lett. **268**, 355–359
166 Mann, D. J., Dombradi, V. and Cohen, P. T. W. (1993) EMBO J. **12**, 4833–4842
167 Steele, F. R., Washburn, T., Rieger, R. and O'Tousa, J. E. (1992) Cell **69**, 669–676
168 Becker, W., Kentrup, H., Klumpp, S., Schultz, J. E. and Joost, H. G. (1994) J. Biol. Chem. **269**, 22586–22592
169 Chen, M. X., McPartlin, A. E., Brown, L., Chen, Y. H., Barker, H. M. and Cohen, P. T. W. (1994) EMBO J. **13**, 4278–4290
170 Hunter, T. (1995) Cell **80**, 225–236