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Functional Diversity of Protein Phosphatase-1, a Cellular Economizer and Reset Button

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Ceulemans, Hugo, and Mathieu Bollen. Functional Diversity of Protein Phosphatase-1, a Cellular Economizer and Reset Button. *Physiol Rev* 84: 1–39, 2004; 10.1152/physrev.00013.2003.—The protein serine/threonine phosphatase protein phosphatase-1 (PP1) is a ubiquitous eukaryotic enzyme that regulates a variety of cellular processes through the dephosphorylation of dozens of substrates. This multifunctionality of PP1 relies on its association with a host of function-specific targetting and substrate-specifying proteins. In this review we discuss how PP1 affects the biochemistry and physiology of eukaryotic cells. The picture of PP1 that emerges from this analysis is that of a "green" enzyme that promotes the rational use of energy, the recycling of protein factors, and a reversal of the cell to a basal and/or energy-conserving state. Thus PP1 promotes a shift to the more energy-efficient fuels when nutrients are abundant and stimulates the storage of energy in the form of glycogen. PP1 also enables the relaxation of actomyosin fibers, the return to basal patterns of protein synthesis, and the recycling of transcription and splicing factors. In addition, PP1 plays a key role in the recovery from stress but promotes apoptosis when cells are damaged beyond repair. Furthermore, PP1 downregulates ion pumps and transporters in various tissues and ion channels that are involved in the excitation of neurons. Finally, PP1 promotes the exit from mitosis and maintains cells in the G_1 or G_2 phases of the cell cycle.

I. INTRODUCTION

About one-third of all eukaryotic proteins are controlled by phosphorylation of specific serine, threonine, and/or tyrosine residues. Most phosphorylations are reversible, implying that the phosphorylation level of a protein reflects the balance between the activities of the involved protein kinases and phosphatases and that alterations in the phosphorylation state can result from changes in the activities of either of these enzymes. Eukaryotic cells express a large variety of protein kinases and phosphatases, each with their own substrate specificity, subcellular localization, and regulation. Mammalian genomes encode ~100 protein tyrosine kinases and protein tyrosine phosphatases. However, the numbers of protein serine/threonine kinases (~400) and protein serine/threonine phosphatases (~25) are hugely different (294), and this has been accounted for by distinct diversification strategies during evolution (74). Indeed, while the number of protein kinases has steadily increased during eukaryotic evolution, serine/threonine phosphatases have not flourished to the same extent, but the diversity of their interacting polypeptides has increased enormously. Thus the true diversity of protein serine/threonine phosphatases is only seen at the holoenzyme level and largely stems from the variety of regulators that can interact with a given catalytic subunit. When holoenzymes are considered, protein serine/threonine kinases and phosphatases show a similar diversity.

Protein serine/threonine phosphatases are currently divided into three structurally unrelated families. The PPM family comprises Mg^{2+}-dependent enzymes, including protein phosphatase (PP) 2C. The FCP family contains only one member, which is also Mg^{2+} dependent. All other protein serine/threonine phosphatases are classified in the PPP family, consisting of the subfamilies PP1, PP2A (including PP4 and PP6), PP2B, and PP5, which all have a structurally related core and a similar catalytic mechanism. This review only deals with PP1, in particular with its functions in various cellular processes. Other recent reviews on PP1 have mainly focused on the structure of the enzyme and the diversity of its regulators (3, 33, 48, 74, 86).

II. THE STRUCTURE OF PROTEIN PHOSPHATASE-1

A. The Catalytic Subunit

PP1 (35–38 kDa) is one of the most conserved eukaryotic proteins. This is nicely illustrated by the early branching eukaryote *Giardia lamblia*, which expresses an isoform of PP1 that is 72% identical to the mammalian PP1 isoforms (74). Also, the phenotypes associated with mutations of PP1 in fungi could be (partially) complemented by expression of mammalian PP1 (113, 311), indicating that PP1 is also functionally conserved. Eukaryotic genomes contain one (*Saccharomyces cerevisiae*) to eight genes (*Arabidopsis thaliana*) encoding PP1 isoforms. More than 70% of the residues in the central three-quarters of these isoforms are virtually invariant, yet the flanking NH_2- and COOH-terminal sequences show more divergence. Mammals have three PP1 genes, encoding the isoforms PP1α, PP1γ, and PP1β/δ. Two splice variants can be generated from the PP1γ gene, PP1γ_1 and PP1γ_2. With the exception of the testis-enriched PP1γ_2, the mammalian isoforms are ubiquitously expressed.

The crystal structure of PP1 shows a compact fold with a central β-sandwich that excludes only the COOH terminus and the extreme NH_2 terminus (Fig. 1). A number of invariant residues coordinate two metals, presumably Fe^{2+} and Zn^{2+}, near the front edge of the β-sandwich, and these metals are thought to contribute to catalysis by enhancing the nucleophilicity of metal-bound water and the electrophilicity of the phosphorus atom (117, 148). The active site is situated at the bifurcation point of an extended Y-shaped surface depression. The arms of this depression are denoted as the COOH-terminal groove, the acidic groove, and the hydrophobic groove (Fig. 1). Crystallographic studies also suggested the mechanism of inhibition of PP1 by some cell-permeable toxins that are widely used for functional studies. Thus the cyclic heptapeptide microcystin LR interacts with two of the metal-bound water molecules and thereby blocks the binding of substrates to the catalytic site. Furthermore, it interacts with the hydrophobic groove and binds covalently to Cys-273 in the β12-β13 loop, which overhangs the catalytic site. The polyether fatty acid okadaic acid binds to the hydrophobic groove and forms hydrogen bonds with Tyr-272 in the β12-β13 loop and with basic residues in the catalytic site (247). Another polyether fatty acid, calyculin A, contains a phosphate group that interacts with the metal binding site, but calyculin A also forms a tight network of interactions with the hydrophilic and acidic grooves (207).

The COOH-terminal fragment of PP1 (~30 residues) is excluded from the globular structure but contains threonine residues that are phosphorylated in a cell cycle-dependent manner, resulting in a reduced activity of PP1 (see sects. III*C* and IV). It has been suggested that this inhibition is caused by the binding of phosphothreonine at the catalytic site and the interaction of basic residues in the COOH terminus with acidic residues that surround the catalytic site (117).

B. Protein Interactors of PP1

The catalytic subunits of PP1 do not exist freely in the cell, but they associate with a host of different regu-

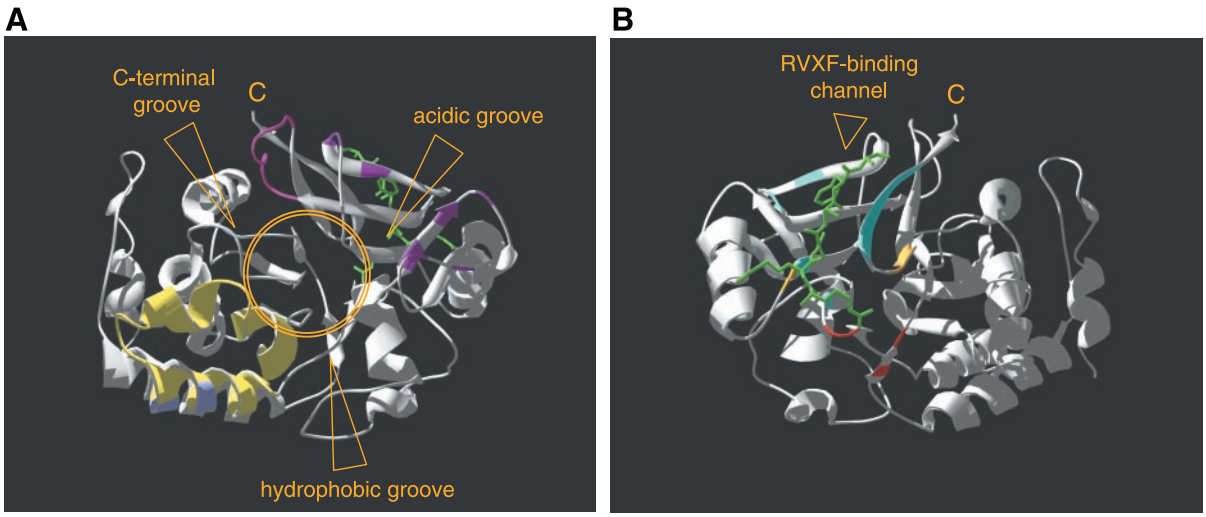

FIG. 1. The crystal structure of protein phosphatase-1γ (PP1γ) (ribbons) bound to an RVXF-containing peptide (118). *A*: frontal view of PP1 with the catalytic site (encircled) and the three grooves that emanate from the catalytic site. The β12/β13 loop is drawn in magenta and the α4/α5/α6 triangle in yellow, with the exception of the blue-colored Lys-147 and Lys-150 that are pivotal in the binding of Sds22. The conserved acidic residues that give the acidic groove its name are indicated in purple. *B*: dorsal view of the same structure. The RVXF-containing peptide is rendered as a green sticks representation. The accommodating RVXF-binding channel is lined by residues of the last β-strand, β14, and by adjacent residues (cyan). The protein surface near the entrance of the channel, which is thought to bind the basic residues preceding the V-position of the RVXF motif, is negatively charged due to the presence of conserved acidic residues (red and orange). Half of these residues (red) have also been implicated in the binding of the K[GS]ILK-motif of inhibitor 2. The depicted scenes were constructed in DeepView3.7 and rendered with POV-Ray3.1.

latory (R) polypeptides (Table 1) to form a variety of distinct multimeric holoenzymes. Thus many of the identified interactors of PP1 have been characterized as regulators. For other interactors, such as phosphofructokinase, the retinoblastoma protein, and Sla1, it is not yet clear whether they are regulators and/or substrates of PP1, or whether they bind directly to PP1 or via another interactor. Regulators of PP1 can be divided in primary and secondary regulators (74), according to whether they originated as regulators of PP1 or acquired this PP1 binding function only later in evolution. Primary regulators (e.g., inhibitor-2, NIPP1, and Sds22) typically contain (putative) PP1-binding sites in all eukaryotic lineages where they occur. Secondary regulators (e.g., AKAP149, Nek2, Bcl2), on the other hand, share functional domains with homologs that lack binding sites for PP1, which indicates that these sites were acquired later in evolution by proteins with an originally unrelated function. Some PP1 interactors appear to have evolved late in the evolution of a particular eukaryotic lineage, as no homologs can be identified in other lineages. For example, the PKA-activated inhibitors are vertebrate specific, while some *Drosophila* (Bifocal, Klp38B) or fungal (Reg1/2, Gip1) regulators have no obvious vertebrate counterparts. The protein interactors of PP1 can also be classified based on their function (48) into substrate-independent activity regulators [e.g., inhibitor-1, dopamine and cAMP-regulated phosphoprotein of 32 kDa (DARPP-32), and inhibitor-2], targetting subunits/substrate specifiers (e.g., G subunits, Mypts) or substrates (Aurora kinases, Nek2). The limitations of the latter classification are that the exact function of many protein interactors is still unknown and that some interactors, e.g., Reg1, function both as a targetting subunit and as a substrate.

An intriguing question is how a relatively small protein like PP1 can interact with a large variety of R subunits that are not structurally related and that have distinct effects on the activity and substrate specificity of the phosphatase. Work by many groups has revealed that *1*) the R subunits typically bind to PP1 via short (4–6 residues), degenerate sequence motifs; *2*) most R subunits have multiple points of interaction with PP1; and *3*) the R subunits can share PP1 interaction sites. This led us to propose that PP1 is subject to a combinatorial control that relies on the competition of its different regulators for a combination of interaction sites. Even with a limited number of interaction sites for the R subunits, the latter can thus "combine" with PP1 in many different ways and form a large variety of holoenzymes with distinct specific activities and substrate specificities. The combinatorial control model also provides a framework for an understanding of the hormonal and metabolic control of PP1, which is largely mediated by phosphorylation of the R subunits or by their interaction with allosteric effectors, and results in altered affinities of specific interaction sites for PP1 (48).

Some regulatory binding sites of PP1 have been mapped (Fig. 1). The best characterized is the so-called

TABLE 1. *Interactors of PP1*

Interactors	Aliases, Isoforms, and Homologs	Section or Reference
AKAIs	Inhibitor-1, DARPP-32, PPP1R1C product	VI*C*; IX*A1*
AKAP149		III*D*
AKAP220		316
AKAP450	AKAP450/AKAP350/CG-NAP/Yotiao	III*B*; VIII*C/D*
ASPPs	ASPP1, ASPP2/p53BP2	IV
Aurora kinases	Aurora-A, Aurora-B, Ipl1 (Y)	III*A1*; III*B*
Bcls	Bcl-2, Bcl-x_L, Bcl-w	IV
BH-protocadherin		401
Bifocal (D)		VII*C*
Focal adhesion kinase		VII*B*
GADD34 related	GADD34, ICP34.5/$\gamma_1$34.5, PPP1R16B product	VII*A/C*
Gip1 (Y)		III*D*
Grp78		83
G substrate		156
G subunits	See Table 2, Gac1 (Y), Pig1 (Y), Pig2 (Y), Gip2 (Y)	V*B*; VI*A*; VIII*A2*; VIII*C/D*
Host cell factor		4
Hox11		198
PP2A inhibitors	I_1^{PP2A}/PHAP-I; I_2^{PP2A}/SET/PHAP-II/TAF-1β	196
Inhibitor-2	Inhibitor-2, Glc8 (Y)	III*B*; IX*B*
Inhibitor-3	Inhibitor-3/HCG-V, Yfr003c (Y)	409
Klp38B (D)		III*B*
Mypts	Mypt1/M110/M130, Mypt2/PP-1bp55/M20/M21, $Mypt_{p85}$	III*A2*; VII*B*
N-CoR		168
Nek2		III*B*
Neurabins	Neurabin-I, neurabin-II/spinophilin	VII*A*; VIII*A2/3*; VIII*C*
Neurofilament L		361
NKCC1		VIII*D*
NIPP1	NIPP1/Ard1	VI*A/B*
Pan1 (Y)		VII*C*
PHIs	PHI-1/2, CPI-17, KEPI, PPP1R14D product	IX*A2*
Phosphofructokinase		412
PRIP-1	PRIP-I/p130	402
Protein kinase R		VI*C*
PNUTS	PNUTS/R111/p99	VI*B*
PP-1bp80		95
PSF		VI*B*
Regs (Y)	Reg1, Reg2	V*A*
Retinoblastoma protein		III*C*; IV
Ribosomal protein L5		VI*C*
RIPP1		VI*C*
SARAs	SARA, endofin	VIII*B*
Scd5 (Y)		VII*C*
Sds22	Sds22/Egp1	III*A4*
Sla1 (Y)		VII*C*
SNF5	SNF5/INI1	VI*A*
SNP70	SNP70/NpwBP/SIPP1	VI*B*
Staufen		VI*B*
Tau		223
Trithorax		307
TIMAPs	TIMAP, MYPT3	70
Vitamin D receptor		IV; VII*A*

The first column shows, in alphabetical order, either general names for families of protein phosphatase-1 (PP1) interactors or the name of a representative. PP1 interactors from yeast and *Drosophila* are followed by (Y) and (D), respectively. In the second column, the names of isoforms and homologs are separated by a comma, while synonyms and the names of splice variants and fragments are separated by a slash. The third column refers either to a key reference or to the section(s) where the interactors are discussed. AKAIs, A-kinase-activated inhibitors; AKAP, A-kinase-anchoring protein; Ard, activator of RNA decay; ASPP, apoptosis stimulating protein of p53; Bcl-2, B-cell lymphoma 2; CG-NAP, centrosome and Golgi-localized PKN-associated protein; CPI-17, C-kinase-dependent phosphatase inhibitor of 17 kDa; DARPP-32, dopamine and cAMP-regulated protein of 32 kDa; Egp1, extra-copy suppressor of glc7, gpp1; Gac1, glycogen accumulation 1; GADDs, growth arrest and DNA damage-inducible proteins; Gip1/2, Glc7-interacting protein 1/2; G subunits, glycogen targeting subunits; Glc8, glycogen-deficient 8; Grp78, glucose-regulated protein of 78 kDa; HCG-V, hemochromatosis candidate gene V; Hox11, homeobox 11; $I_{1/2}^{PP2A}$, inhibitor-1/2 of PP2A; INI1, integrase interactor 1; Klp38B, kinesin-like protein at 38B; Ipl1, increased ploidy; Mypts, myosin phosphatase targeting subunit; KEPI, kinase-enhanced protein phosphatase type-1 inhibitor; N-CoR, nuclear receptor corepressor; Nek2, NIMA-related protein kinase 2; NKCC1, Na-K-Cl cotransporter 1; NIPP1, nuclear inhibitor of PP1; NpwBP, Npw38 binding protein; Pan1, poly(A) ribonuclease-1; p53BP, p53 binding protein; PHAP-II, putative class II human histocompatibility leukocyte-associated protein II; PHI, phosphatase holoenzyme inhibitor; Pig1/2, protein interacting with Gsy2 1/2; PRIP-1, phospholipase C-related inactive protein 1; PNUTS, phosphatase 1 nuclear targeting subunit; PSF, polypyrimidine tract-binding protein-associated splicing factor; RIPP1, ribosomal inhibitor of PP1; SARA, Smad anchor for receptor activation; Scd5, suppressor of clathrin heavy-chain deficiency 5; Sds22, suppressor of the dis2 mutant; Sla1, synthetically lethal with ABP1; SNF5, sucrose nonfermenting 5; SNP70, SH_3 domain binding protein of 70 kDa; SIPP1, splicing factor that interacts with PQBP1 and PP1; TIMAP, TGF-β-inhibited membrane-associated protein.

"RVXF" binding channel, which is a hydrophobic groove remote from the catalytic site and is formed by the top rear edges of the two central β-sheets (118). Most regulators of PP1 contain an RVXF motif, which actually conforms to the consensus sequence [RK]x_{0-1}[VI]{P}[FW], where x can be any residue and {P} refers to any residue but proline (74, 118, 378, 411). Binding of the RVXF motif per se is not associated with major conformational changes of PP1 and does not have significant effects on the catalytic activity. The available data rather suggest that the RVXF motif serves as an anchor for the initial binding of the R subunits to PP1 and thereby promotes, sometimes cooperatively, the binding of secondary sites, which often bind with lower affinity but affect the activity and substrate specificity of PP1 (48, 378). The β12-β13 loop forms a second, flexible binding site of PP1, one that is essential for inhibition of PP1 by both toxins (see sect. II*A*) and protein inhibitors (inhibitor-1, DARPP-32, inhibitor-2, and NIPP1) (91). Still another interaction site for R subunits is the triangular region delineated by the α_4-, α_5-, and α_6-helices of PP1, which we have recently identified as a major interaction site for Sds22 (75). Finally, an interaction site for the conserved NH_2-terminal K-[GS]-I-L-K motif of inhibitor-2 has been mapped near the entrance of the RVXF-binding channel (90). Some R subunits, such as the Neurabins and the Mypts (see sect. VII, *A* and *B*), interact with PP1 in an isoform-specific manner, indicating that PP1 also contains isoform-specific regulatory binding sites.

The R subunits bring PP1 in close proximity to its substrates by anchoring the phosphatase in specific cellular compartments via targetting motifs or domains. Some R subunits block the activity of PP1 by acting as pseudosubstrates (see sects. VII*B* and IX*A*) or by inducing conformational changes (see sect. IX*B*). The substrate-specifying effect of some R subunits (G subunits, Mypts, AKAP149) implies both an increased activity toward some substrates and a decreased activity toward other substrates. The surface of PP1 is relatively open, and no peptide binding cleft is evident, in accordance with its broad substrate specificity (33). One can therefore envisage that the binding of R subunits to PP1 restricts the accessibility of the catalytic site, either by causing steric hindrance or by inducing conformational changes. At least in some instances, the substrate-specifying activity may stem from the fact that the R subunits are themselves substrates (see sects. III*B* and VI*C*) or have binding sites for specific substrates (see sect. V*B*).

III. CELL DIVISION AND MEIOSIS

Mutations of PP1 in various fungi and in the fruitfly, or microinjection of PP1-neutralizing antibodies or antisense PP1 oligonucleotides in cultured mammalian cells, all result in a mitotic arrest or a deficient cytokinesis (24, 31, 79, 114, 131, 172, 286, 329). The phenotypical heterogeneity of various M phase-arrested PP1 mutants in yeast (31, 329) suggests that PP1 has multiple substrates during the M phase. A pleiotropic action of PP1 in mitosis in mammals is also supported by the observed targetting of PP1 to multiple mitotic structures such as the chromosomes, the centrosomes, and the spindle (15, 47).

A. Reversal of Signaling by Protein Kinase Aurora(-B)

1. Mitotic substrates of aurora(-B) and PP1

Protein kinases of the Aurora family have multiple mitotic substrates (281), and increasing evidence suggests that PP1 reverses the action of these protein kinases. One of these substrates is histone H3 (Fig. 2), which is phosphorylated on Ser-10 by the unique Aurora protein kinase in yeast and the Aurora-B protein kinase in animals (1, 146), and is an established mitotic substrate of PP1 (176, 269). Various studies have reported a correlation between the phosphorylation of histone H3 along chromosomes in G_2 and chromosome condensation (146, 176, 375), and also between chromosome decondensation in telophase and PP1 activity or histone H3 dephosphorylation (24, 151, 368). These observations have led to the hypothesis that chromosome (de)condensation requires histone H3 (de)phosphorylation. Accordingly, in fission yeast and in animals, phosphorylation of histone H3 is involved in the recruitment to chromosomes of a component of the heteropentameric condensin complex (Fig. 2), which has been implicated in chromosome condensation (146, 191, 257). However, mutation of Ser-10 of histone H3 did not cause any observable growth defect in budding yeast (176), and neither Ser-10 nor the entire NH_2-terminal tail of *Xenopus* histone H3 is essential for chromosome condensation (100). An alternative hypothesis proposes that a checkpoint labels chromosomes that are ready to go through anaphase and telophase by phosphorylation of histone H3 and that this checkpoint impinges on the balanced activity of Aurora(-B) and PP1 (100). The histone H3 kinase activity of *Xenopus* Aurora-B depends on the latters' phosphorylation by an unknown kinase, which may well be Aurora-B itself, as its yeast counterpart autophosphorylates (44, 269). Interestingly, this Aurora-B activation is antagonized by PP1 (269), and PP1 interacts physically with Aurora-B (337).

Recent complementary work in yeast and in animals suggests that Aurora(-B) and PP1 may also act antagonistically in the complex control of the layered protein interface between the centromeres and the mitotic spindle that ensures biorientation of sister kinetochores, spindle integrity, and chromosome segregation (Fig. 2). First, the histone H3 homolog CENP-A, which substitutes for his-

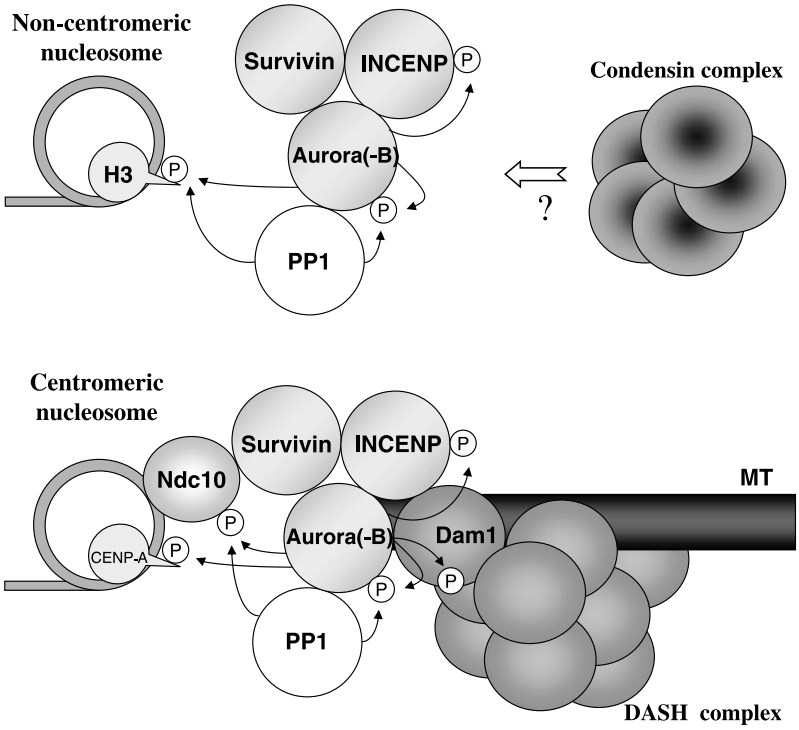

FIG. 2. Aurora(-B) and PP1 act antagonistically during mitosis. The open arrow indicates recruitment to noncentromeric chromatin. MT, microtubules.

tone H3 in centromeric nucleosomes, is phosphorylated by Aurora-B at a site similar to that of H3 (407). CENP-A phosphorylation starts in mitotic prophase and decreases in anaphase and appears to be correlated with kinetochore maturation. It remains to be explored whether CENP-A is also a substrate of PP1. Second, yeast PP1 and Aurora control the phosphorylation state of the kinetochore protein Ndc10, which binds directly to the centromere (44, 314). Hyperphosphorylation of Ndc10 impairs the attachment of microtubules to the kinetochore (314). Third, both in yeast and in animals Aurora(-B) phosphorylates the inner centromere protein INCENP (194). Strikingly, the temperature-sensitive mitotic defects of a yeast INCENP mutant are attenuated by overexpression of a dominant-negative truncated version of PP1 (204), in accordance with the proposed antagonism between Aurora(-B) and PP1. Together with Survivin, which interacts with Ndc10 (400), Aurora(-B) and INCENP form the chromosomal passenger complex. Like PP1, this complex has been implicated in chromosome segregation and cytokinesis. The passenger complex migrates from the centromeres to the spindle midzone and the cleavage furrow after the transition to anaphase (1, 47, 387, 407). Interestingly, disruption of the phosphorylation site of CENP-A disturbs the subcellular localization of Aurora(-B), INCENP, and PP1 in the latter half of mitosis (407). Given that PP1 and Aurora(-B) interact physically (337), these findings lead to the enticing hypothesis that PP1 is a component of the chromosomal passenger complex.

The Aurora substrate Dam1 is a component of the multimeric spindle-associated DASH complex that is required for biorientation of sister kinetochores and for mitotic spindle integrity (185, 222). Dam1 binds to Aurora and INCENP (Fig. 2). Interestingly, overexpression of PP1 exacerbates the temperature-sensitive growth defect of *dam1* mutant cells, indicating that Dam1 may also be a substrate of PP1 (194).

2. Aurora(-B) and PP1 in cytokinesis

Although considerable progress has been made in deciphering the role of Aurora(-B) and PP1 in spindle integrity and chromosome segregation, their function in cytokinesis remains largely elusive. It is known that both the passenger complex and PP1γ_1 are present at the cleavage furrow at the end of the M phase (47, 407). A conditional mutation of PP1 in yeast was associated with various cell cycle defects, including a perturbed cytokinesis, which correlated with the absence of an actin ring at the bud neck (16). Furthermore, functional deficiencies of the passenger complex (1, 190, 221) or microinjection of antisense PP1γ_1 oligonucleotides (79) resulted in a severe defect in cytokinesis. Only a single candidate target has thus far been identified, i.e., the regulatory light chain of myosin II, which is an in vitro Aurora-B substrate (267). The regulatory light chain is also a well-established substrate of Mypt-containing holoenzymes of PP1 (see sect. VII*B*). However, the latter holoenzymes contain the β-isoform of PP1 rather than the γ_1-isoform (256). Furthermore, a functional depletion of Mypt in *Caenorhab-*

ditis resulted in a rather mild cytokinetic phenotype with ectopic furrowing and an accelerated furrow ingression (293). Therefore, it is likely that Aurora-B and PP1 share still other substrates that play an important and conserved role in cytokinesis.

3. Meiotic substrates of Aurora(-B) and PP1

In addition to their involvement in the progression of mitosis and cytokinesis, Aurora(-B) and PP1 have also been implicated in meiosis. *Caenorhabditis* oocytes depleted of Aurora-B (or Survivin) by RNA interference fail to separate homologous chromosomes in meiosis I and sister chromatids in meiosis II (304). It has been proposed that Aurora-B promotes chromosome separation by the phosphorylation of the meiosis-specific cohesin Rec8 and that this phosphorylation results in the cleavage of Rec8 by Separase. Accordingly, Rec8 is an in vitro substrate for Aurora-B (304), and Aurora-B is targetted to the remaining points of contact between separating chromosomes in metaphase I and II (191, 304). Interestingly, the latter subchromosomal regions also exhibit a pronounced phosphorylation of histone H3 on Ser-10 (191). Aurora-B could thus be the meiotic counterpart of the Polo-like kinase, which phosphorylates the mitotic cohesin and thereby marks it for Separase-dependent cleavage (9). Like most other Aurora-B functions, this role as meiotic cohesin kinase appears to be conserved and antagonized by PP1. Thus fission yeast Rec8 is phosphorylated during meiosis I and II (290), and PP1 depletion by RNA interference causes precocious separation of sister chromatids at the onset of anaphase I (191, 304). The latter effect correlated with an increased presence of Aurora-B on meiotic chromosomes and a decrease in the level of chromosomal Rec8 (304). It remains to be studied whether PP1 directly dephosphorylates Rec8 or impinges on the targetting or activity of Aurora-B.

4. R subunits that target PP1 to Aurora(-B) substrates

The R-subunit(s) that are involved in the dephosphorylation of Aurora(-B) substrates by PP1 remain(s) unknown, but Sds22, an established interactor of PP1 in both yeast and mammals (75, 107, 171, 234, 334), seems an attractive candidate. Indeed, the Sds22 encoding gene was identified independently in fission and in budding yeast as an extra-copy suppressor of temperature-sensitive mitotic arrest phenotypes that are associated with particular mutations of PP1 (171, 234, 286). Deletion of the Sds22 encoding gene caused a similar arrest, and this phenotype could be complemented by the overexpression of PP1 (171, 234, 286). Also, the conditionally lethal phenotype in budding yeast that was conferred by a loss-of-function mutation of the yeast Aurora kinase was largely relieved by the expression of certain temperature-sensitive mutant versions of Sds22 or PP1 (136, 291). The mutant Sds22 version that rescued the conditional Aurora phenotype showed a decreased ability to interact with PP1. The expression of this mutant Sds22 did not affect the cellular levels of PP1 or Sds22, but drastically reduced the nuclear level of PP1 and caused a redistribution of the nuclear pool of PP1 (291). Whether Sds22 is also involved in meiosis is not known, but it can certainly not be ruled out as Sds22 has been identified in a ternary complex with the mammalian PP1γ_2 isoform (82, 179).

B. Delay of Centrosome Splitting Until the G_2/M Transition

Centrosomes duplicate during S phase, but they remain paired and continue to function as a single microtubule-organizing center during G_2 (281). Shortly before the onset of mitosis, the duplicated centrosomes separate and form the poles of the bipolar spindle apparatus. At least two kinases that have been implicated in the induction of this separation are inactivated by PP1, which suggests that PP1 may prevent precocious splitting of the centrosomes. One of these is Aurora-A, a homolog of Aurora-B (195). While Aurora-A is required for centrosome separation in animals (147), the unique yeast Aurora kinase does not appear to subserve this function, as a conditional loss-of-function mutation of this enzyme did not affect spindle pole body separation (356). Like Aurora-B, Aurora-A interacts with PP1, and this interaction peaks at mitosis. A mechanism of regulation of Aurora-A by PP1 that is similar to that of Aurora-B (269) is suggested by the observation that PP1 dephosphorylates and thereby inactivates Aurora-A in vitro. Interestingly, PP1 is also an in vitro substrate for Aurora-A, and this phosphorylation results in the inactivation of the phosphatase.

A second kinase involved in centrosome splitting is Nek2, a member of the NIMA family of protein kinases. Nek2 is activated by autophosphorylation and is thought to phosphorylate the centrosomal protein C-Nap1, resulting in the dissolution of the structure that keeps the centrosomes together. The counterplayer of Nek2 is PP1, which dephosphorylates both C-Nap1 and Nek2 itself (165). Furthermore, Nek2, PP1, and C-Nap1 can form a ternary complex in vitro, and Nek2 contains an RVXF motif that is essential for its interaction with PP1 (165). Recently, it was reported that inhibitor-2 also interacts with the Nek2/PP1 complex via PP1 and that the expression of inhibitor-2 increases Nek2 kinase activity and promotes centrosome splitting (124). Conversely, the overexpression of PP1 strongly suppresses Nek2-mediated centrosome splitting (250). Interestingly, a parallel can be drawn between the regulatory relationships of PP1 with Aurora-A and Nek2, as PP1 is also a substrate for the associated Nek2 and phosphorylation of COOH-terminal site(s) reduces its phosphatase activity (165). This sug-

gests that the separation of centrosomes may depend on both the activation of the inducing kinases and the inactivation of associated PP1. In this respect, it is worthy of note that PP1 is also inactivated through phosphorylation by cyclin-dependent protein kinase 1 (Cdk1) in early to mid-mitosis at a COOH-terminal site that is different from the Nek2 and Aurora-A phosphorylation site(s) (195, 213, 226, 297).

The splitting of the centrosomes is accompanied by the recruitment of γ-tubulin ring complexes, which function as nucleation sites for microtubules (281). The recruitment of these complexes is mediated by AKAP450, which also contains binding sites for a host of different protein kinases and phosphatases, including PP1 (348, 349). The functions of these AKAP450-associated signaling enzymes remain unknown.

C. PP1 at the M/G_1 Transition

PP1 contributes to the reassembly of the nuclear envelope at the end of mitosis by acting as a lamin-B phosphatase (362). Lamin-B is a component of the nuclear lamina, and its phosphorylation at the onset of mitosis leads to the disassembly of the nuclear lamina. More recently, Collas and co-workers (331) showed in an elegant series of experiments that PP1 is targetted to Lamin-B by AKAP149, an integral membrane protein of the endoplasmic reticulum and the nuclear envelope. They also found that the recruitment of PP1 by AKAP149 is a prerequisite for the reassembly of the nuclear lamina and that a failure to recruit PP1 results in apoptosis (330). Human AKAP149 binds PP1 via an RVXF motif and, importantly, also functions as a lamin-B specifying subunit (329a).

The burst of protein dephosphorylation at the M/G_1 transition not only involves proteins that function in the execution of mitosis per se, but also affects numerous proteins that play a role in such diverse processes as replication, transcription, pre-mRNA splicing, cell survival, and cell cycle progression (49). One of these is the antiapoptotic protein Bcl-2, an integral membrane protein of the mitochondria and the endoplasmic reticulum (see also sect. IV), which is targetted for proteasome-mediated degradation by dephosphorylation (60). A late-mitotic Bcl-2 phosphatase was biochemically identified as PP1 and, moreover, PP1 was found to coimmunoprecipitate with mitochondrial Bcl-2 during late mitosis. Furthermore, it has been shown that Bcl-2 contains a functional PP1-binding RVXF motif (26). Another late-mitotic substrate of PP1 is the retinoblastoma protein (Rb), which is hyperphosphorylated from the S phase until the end of mitosis. During G_1, in contrast, Rb is hypophosphorylated, and this allows sequestration of key stimulators of the G_1/S transition transition, such as the E2F transcription factors. PP1 was found to function as the Rb phosphatase in mitotic cell lysates (276), and Rb was shown to bind PP1 in two-hybrid (116) and coprecipitation assays (297, 306, 352). The sensitivity of the Rb phosphatase in intact cells to various cell-permeable cytotoxins also points to PP1 (396).

D. Exit From the Pachytene Stage in Yeast Meiosis

In yeast, a premature exit from the pachytene stage after the initiation of meiotic recombination is prevented by the so-called "pachytene checkpoint" (reviewed in Ref. 303). An active checkpoint results in the phosphorylation and activation of protein kinase Mek1, which keeps its substrate Red1 phosphorylated (29, 102). When recombination has ended in late pachytene, the checkpoint is inactivated by the dephosphorylation of Red1 by PP1 (30). Overexpression of PP1 bypasses the checkpoint precociously.

The nature of the regulatory subunit(s) associated with this meiotic function of PP1 remains unclear. A number of findings originally pointed to Gip1, a PP1-binding protein that is specifically expressed in middle meiosis and that is essential for sporulation (372). Thus it was reported that *1*) Gip1 was required for the targetting of PP1 to chromosomes in late pachytene, *2*) yeast cells lacking Gip1 displayed a pachytene arrest that was similar to that of cells with constitutively active Mek1 or with a deficient version of PP1, and *3*) this arrest was alleviated by overexpression of PP1 (30). However, in a more recent study, deletion of the Gip1-encoding gene was found not to affect meiotic progression, but instead to interfere with the normal localization of sporulation-specific septins and the deposition of spore wall material (347). Strikingly, replacement of PP1 by a mutant version that fails to interact with Gip1 yielded a similar phenotype.

IV. CELL CYCLE ARREST AND APOPTOSIS

PP1 not only activates the Rb protein at the M/G_1 transition (see sect. III*C*), but it is also implicated in the control of Rb at the G_1/S transition and in Rb-mediated cell cycle arrest. In late G_1, the Rb protein is inactivated through phosphorylation by Cdks (226). Equally important for the Rb phosphorylation is the inactivation of the Rb-associated pool of PP1α, which results from the Cdk-mediated phosphorylation on Thr-320. This is strikingly illustrated by the observation that the expression of the constitutively active T320A mutant of PP1α, but not that of the wild-type PP1α, prevented the Rb phosphorylation in late G_1 and caused cell cycle arrest (35). Moreover, expression of the PP1α mutant T320A in Rb-negative cells did not impede cell cycle progression, indicating that this effect on cell cycle progression was Rb dependent. Cell

cycle arrest and/or apoptosis induced by genotoxins is also correlated with a dephosphorylation of the Rb protein (115, 122, 211, 274, 382). Under these conditions, Rb dephosphorylation is accounted for by a decreased activity of Cdks and by an activation of PP1 via the dephosphorylation of the inhibitory COOH-terminal Cdk site (49, 150). PP1 inhibitors such as calyculin A or inhibitor-2 prevent the induction of cell cycle arrest and apoptosis, which underlines the crucial role of PP1 in this cellular response to stress (115, 382).

It has recently been demonstrated that both PP1 and the p70 S6 kinase interact with the vitamin D receptor (37). However, the p70 S6 kinase was only recruited in its phosphorylated form and in the absence of ligand. The binding of PP1 was ligand independent, but PP1 activity increased in a ligand-dependent manner. Ligand-activated PP1 was shown to dephosphorylate p70 S6 kinase, resulting in the inactivation of the kinase and its dissociation from the receptor/phosphatase complex. Because p70 S6 kinase is essential for the G_1/S transition, it was argued that its inactivation by PP1/PP2A contributes to the vitamin D-induced cell cycle arrest.

The Bcl-2, Bcl-x_L, and Bcl-w proteins have mainly been described as positive regulators of cell survival, but in conjunction with a dephosphorylated form of the proapoptotic protein Bad, they can also induce apoptosis via the activation of proteases of the caspase family. Bcl-2/x_L/w contain a PP1-binding RVXF motif, and they can occur in a ternary complex with PP1 and Bad (25–27). Furthermore, the dephosphorylation of Bad and the apoptosis induced by interleukin deprivation from hematopoietic cells were both alleviated by the inhibition of PP1. Combined with the observation that the Bad phosphatase is mainly associated with Bcl-2/x_L/w, these data suggest that the Bcl-2/x_L/w proteins target Bad for dephosphorylation by PP1.

Recently, PP1 has also been implicated in the ceramide-induced shift of the splicing pattern of the Bcl-x and caspase 9-encoding genes, causing these genes to produce the proapoptotic splice variants Bcl-x_S and caspase 9 rather than the antiapoptotic variants Bcl-x_L and caspase 9b (77). Increased ceramide levels are thought to induce the dephosphorylation by PP1 of splicing factors of the SR family, which are indeed involved in the regulation of alternative splicing (76).

The COOH-terminal half of another interactor of Bcl-2, ASPP2, contains a PP1-binding RVXF motif of its own. However, the binding of PP1 and Bcl2 to ASPP2 are mutually exclusive (163, 273). ASPP2 and its RVXF-containing homolog ASPP1 also bind to p53 and thereby specifically stimulate the transactivation of proapoptotic genes by p53 (310), but addition of PP1 dissociated p53 from the COOH-terminal half of ASPP2 (163), leaving the function of the PP1-ASSP interaction unknown.

V. METABOLISM

A. Reversal of Starvation-Induced Metabolic Shifts

An ancient eukaryotic response to nutrient starvation and hypoxia (reviewed in Refs. 72 and 202) is orchestrated by conserved trimeric protein kinases that consist of a catalytic α-subunit, a substrate-defining and targeting β-subunit (249, 317), and a regulatory γ-subunit. These protein kinases, termed Snf1 in yeast and AMP-activated kinase (AMPK) in animals, have a common mechanism of regulation by reversible phosphorylation. Glucose deprivation and other stress factors bring about phosphorylation of the α-subunit on a conserved threonine residue by an upstream kinase (72). Subsequently, the γ-subunit binds to the autoinhibitory domain of the α-subunit and thereby activates the catalytic domain. Activated Snf1/AMPK in turn promotes *1*) glucose import; *2*) gluconeogenesis; *3*) respiration; *4*) the use of alternative sugars and other carbon sources like fatty acids, ethanol, glycerol, pyruvate, and lactate; and *5*) the downregulation of anabolic pathways (72, 158). These effects are achieved via direct phosphorylation or transcriptional control of key metabolic enzymes. Two mechanisms are involved in the transcriptional control: phosphorylation-dependent nuclear exclusion of transcriptional repressors (106) and phosphorylation at specific promotors of serine-10 of histone H3, which facilitates acetylation of lysine-14 and transcription (230).

The phosphatase that reverts the α-subunit of AMPK to its inactive state in vivo remains unknown, but Snf1 is dephosphorylated by a PP1 holoenzyme. Two noncatalytic subunits have been identified in this PP1 complex, namely, Reg1 and Sip5 (371). The RVXF-containing Reg1 binds constitutively to PP1 and to Sip5 (Fig. 3), and this ternary complex is targetted to the activated Snf1 at limiting glucose concentrations (313). The Snf1 kinase then phosphorylates Reg1. The phosphorylation of Reg1 is antagonized by Reg1-associated PP1, but at low glucose concentrations, the balance is tipped in favor of a net phosphorylation of Reg1 by the hexokinase Hxk2, which is itself phosphorylated on Ser-15 in these conditons (299). Hxk2 interacts (weakly) with both Snf1 and Reg1, but it is not clear yet whether Hxk2 promotes the phosphorylation of Reg1 by the stimulation of Snf1 and/or by the inhibition of the associated PP1 (313). When the availability of glucose increases, an hitherto unidentified trigger promotes the net dephosphorylation of Snf1 by the associated PP1 complex, resulting in the release of the latter complex from Snf1 (313). Phosphorylation of Reg1 is a prerequisite for the dephosphorylation of Snf1 and for the release of the phosphatase complex from the kinase complex. Indeed, deletion of the Hxk2 encoding gene, which is associated with a hypophosphorylation of Reg1,

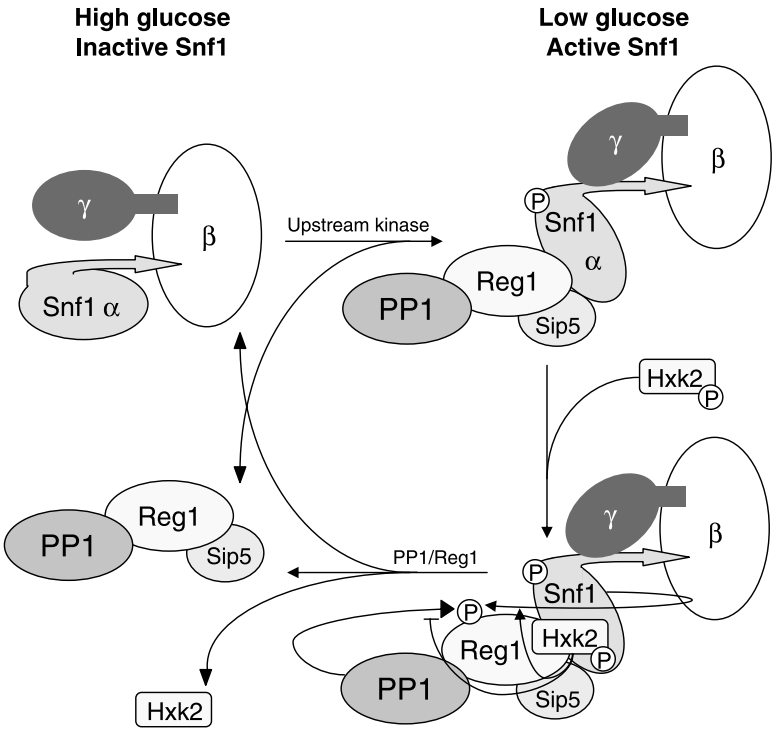

FIG. 3. The regulation of Snf1 kinase in response to the availability of nutrients. Hxk2, hexokinase 2.

renders the Snf1 complex constitutively active. Also, increased glucose levels fail to dissociate Reg1 from a genetically inactivated Snf1. Interestingly, Hxk2 is also dephosphorylated by PP1 in a Reg1- and glucose-dependent manner (13). After its release from the Snf1 complex, PP1 rapidly reverts Reg1 to its unphosphorylated state.

By the dephosphorylation of Snf1, Reg1-associated PP1 reestablishes glucose as the preferred source of energy and reinitiates anabolic pathways. Because PP1 is also a well-established histone H3 phosphatase (see sect. III*A1*), it may also downregulate Snf1 signaling by directly dephosphorylating Snf1 substrates, such as histone H3. As histone H3 phosphorylation on Ser-10 has also been proposed as a mechanism for the regulation of transcription by other histone H3 kinases, such as Msk1 (363), this would add to the importance of transcriptional repression as a function for PP1. In *Caenorhabditis* too, PP1 was found to antagonize more than one histone H3 kinase (191).

One of the effects of glucose-induced Snf1 inactivation is a loss of maltose permease activity, both by transcriptional repression and by a posttranscriptional mechanism termed glucose(-induced) inhibition (177). Ubiquitin-mediated proteolysis of maltose permease constitutes a third, Snf1-independent mechanism for the downregulation of maltose import. More recently, a novel function in the proteolysis of maltose permease has been proposed for Reg1 and for the distantly related PP1 interactor Reg2, which is not involved in Snf1-mediated signaling (186). Reg1- and Reg2-associated PP1 have been suggested to promote the proteolysis of maltose permease, possibly via the regulation of an as of yet unidentified maltose permease kinase.

B. Glycogen Metabolism

The study of glycogen metabolism has contributed enormously to our understanding of the structure and regulation of PP1 (53, 54, 85, 180). For example, these studies have led to the concepts that R subunits of PP1 function as targetting and substrate-specifying subunits and that the activity of PP1 is largely controlled by phosphorylation and allosteric regulation of its R subunits. Also, to this day, glycogen phosphorylase is by far the most widely used substrate for the assay of PP1 in vitro.

The rate-limiting enzymes of glycogen synthesis and breakdown are glycogen synthase and phosphorylase, respectively. The phosphorylation of glycogen synthase is generally associated with an inactivation of the enzyme, whereas phosphorylase is activated by phosphorylation. A host of protein kinases phosphorylate multiple residues in the extremities of glycogen synthase, but phosphorylase is only phosphorylated on one NH_2-terminal serine by a single protein kinase, namely, phosphorylase kinase. The latter is itself activated by phosphorylation of its regulatory α- and β-subunits and by the binding of Ca^{2+} to the regulatory δ-subunit, which is identical to calmodulin. Glycogen synthase, phosphorylase, and, to a lesser extent, phosphorylase kinase are bound to the glycogen particles, and their dephosphorylation is believed to be

(partially) mediated by species of PP1 that are anchored to the glycogen particles via glycogen-targetting subunits (G subunits). The dephosphorylation of the rate-limiting enzymes of glycogen metabolism by PP1 results in the storage of glycogen, in accordance with the proposed function of PP1 as an energy conserving enzyme (see sect. X).

1. Glycogen-associated substrates of PP1

Overwhelming evidence implicates PP1 in the activation of glycogen synthase in vivo. In budding yeast, mutants of PP1 with a reduced affinity for the G subunit Gac1 or loss-of-function mutants of Gac1 were glycogen deficient and had a low activity of glycogen synthase (298, 336). The additional deletion of Pig1, one of the three other yeast G subunits, exacerbated the glycogen deficiency of a Gac1 null strain (80). Conversely, a higher expression level of Gac1 was associated with an increased activity of glycogen synthase. In rat hepatocytes, a loss of glycogen-associated PP1, as seen for example in insulin-dependent diabetes, was associated with an impaired glucose-induced activation of glycogen synthase (54). On the other hand, a superactivation of glycogen synthase was noted in liver cells from rats with hyperthyroidism, which showed an increased level of glycogen-associated PP1 (217). Furthermore, the overexpression of various G subunits in cultured cells correlated with an activation of glycogen synthase (144, 219, 395). Finally, mice lacking the skeletal muscle specific G_M subunit had a low basal activity of glycogen synthase and showed a deficient exercise-induced activation of the enzyme (22).

PP1 is also likely to function as a phosphorylase phosphatase in vivo, since alterations in the expression levels of PP1 or of specific G subunits resulted in corresponding changes in the activity of phosphorylase (22, 84, 144, 395). However, activated phosphorylase (phosphorylase *a*) is also known as an excellent in vitro substrate for PP2A, and it cannot be ruled out that the latter also contributes significantly to the dephosphorylation of phosphorylase in vivo. There is no information available on the protein phosphatases that dephosphorylate phosphorylase kinase in vivo. It should be noted, however, that only the β-subunit of phosphorylase kinase is an in vitro substrate for dephosphorylation by PP1.

2. The mammalian G subunits

Mammalian genomes contain no less than seven genes that encode G subunits, but only four of these have been characterized at the protein level (74). For the purpose of conformity, we suggest that the G subunits are differentiated by a capital subscript, which refers to their gene name, except for G_M and G_L where the subscript refers to the tissues (striated muscle and liver, respectively) where they are expressed most abundantly (Table 2). With the exception of G_M and G_L, the G subunits are expressed ubiquitously, albeit at variable levels (20, 110, 215). G_L displays a remarkable species-dependent distribution in that it is absent from sketal muscle of rats while it is highly expressed in human skeletal muscle (263).

TABLE 2. *Human G subunits*

Protein Name(s)	Mass, kDa	Gene Symbol
G_M, R_{GL}	124	*PPP1R3A*
G_L, FLJ14005	33	*PPP1R3B*
G_C, PTG, R5, U5	36	*PPP1R3C*
G_D, R6	33	*PPP1R3D*
G_E, FLJ00089	31	*PPP1R3E*
G_F, H2bE	79	*PPP1R3F*
G_G	38	*PPP1R3G*

Accession numbers for the gene products can be found on www.gene.ucl.ac.uk/nomenclature/.

Two modules are conserved in all G subunits, i.e., a PP1-binding RVXF motif and a targetting module with binding sites for glycogen and PP1 substrates (21, 80, 134, 228, 392, 393). The binding of the G subunits to both PP1 and its substrates seems to be required as the disruption of either binding site abolished the activation of glycogen synthase that is associated with the expression of the G_M or G_C subunits in cultured cells (228, 393). Interestingly, the binding of glycogen synthase to G_M was reported to be modulated by phosphorylation of glycogen synthase (228). The better characterized G_M and G_L subunits have also been shown to contain a COOH-terminal domain that is involved in the binding to phospholamban in the membranes of the sarcoplasmic reticulum (see sect. VII*A2*) and to the allosteric inhibitor phosphorylase *a*, respectively. Furthermore, G_M and G_L modulate the substrate specificity of PP1 in that they inhibit the dephosphorylation of phosphorylase but increase the specific glycogen synthase phosphatase activity (6, 110, 180). Likewise, the G_C subunit decreases the phosphorylase phosphatase activity of PP1 (111).

3. Control of hepatic glycogen metabolism

The liver functions as a glucose sensor, and hepatic glycogen metabolism contributes to the control of the blood glucose homeostasis (53). A postprandial rise in blood glucose results in the inactivation of phosphorylase and activation of glycogen synthase. Conversely, when glucose levels drop below a given threshold, phosphorylase is activated and glycogen synthase is inactivated. The glucose-induced inactivation of phosphorylase is at least in part explained by the binding of glucose to phosphorylase *a*, which turns the latter into a better substrate for dephosphorylation (53). The glucose-induced activation of glycogen synthase is associated with a translocation of glycogen synthase to the cell periphery (142) and may be partially mediated by a phosphatidylinositol 3-kinase-de-

pendent signaling pathway (209, 217). However, glucose also elicits hepatic synthase phosphatase activity, both by the removal of the allosteric inhibitor phosphorylase *a* and by the generation of the stimulator glucose 6-phosphate (66, 328). Glucose 6-phosphate probably acts via an allosteric increase of the substrate quality of glycogen synthase. Phosphorylase *a* is only inhibitory to G_L-associated PP1, the major glycogen-associated synthase phosphatase (63). A glucose-induced activation of glycogen synthase by the latter phosphatase is also in accordance with reports that the loss of G_L in the liver of diabetic or adrenalectomized and starved rats (63, 109) is associated with an impaired activation of glycogen synthase by glucose (51, 52). Moreover, the restoration of the G_L level by the administration of insulin or by refeeding closely correlates with an improved activation of glycogen synthase.

It seems unlikely that G_L-associated PP1 also contributes to the glucose-induced inactivation of phosphorylase in vivo, since the loss of G_L in diabetic and in adrenalectomized starved rats did not hamper the inactivation of phosphorylase by glucose in hepatocytes (51, 52) and had but a moderate effect on the glycogen-associated phosphorylase phosphatase activity (54, 109). Moreover, G_L is inhibitory to the phosphorylase phosphatase activity of associated PP1, and the allosteric G_L inhibitor phosphorylase *a* does not affect its own dephosphorylation by hepatic protein phosphatases (21, 110). It thus appears that the control of glycogen synthase and phosphorylase by glucose is mediated by different protein phosphatases. The nature of the G subunit(s) that targets PP1 to phosphorylase in the liver remains to be explored. The loss of the G_C subunit from the diabetic liver, which retains its ability to inactivate phosphorylase in response to a glucose load, argues against an involvement of G_C in this process (63). A role for the G_D subunit is also unlikely given that this protein is only expressed at very low levels in the liver (53). Perhaps one of the poorly characterized G subunits (G_E, G_F, or G_G) is involved in the targetting of PP1 to phosphorylase. Alternatively, hepatic phosphorylase *a* may be dephosphorylated by species of PP1 (or PP2A) that are not or only transiently associated with glycogen.

In view of the contribution of the G subunit(s) to the hepatic uptake of glucose, it has been proposed that the therapeutic expression of (fragments of) these proteins may serve to lower blood glucose in diabetes (279). One benefit of (over)expressing G subunits rather than glycolytic enzymes is that they are not expected to increase the circulating level of lipids. As further support for their proposal, Newgard and co-workers (398) noted that the (over)expression of G subunits in cultured hepatocytes or in rat liver stimulated glycogen deposition, albeit with different sensitivity to glycogenolytic agents and potency. For example, the hepatic overexpression of G_C resulted in a 70% increase in the hepatic storage of glycogen and an improved whole body glucose homeostasis, but the deposited glycogen was not broken down during fasting. However, the glycogen pool that was synthesized as a result of the expression of a truncated version of G_M was responsive to glycogenolytic stimuli, and the expresssion of this G_M fragment moreover normalized glucose tolerance in rats on a high-fat diet (143). Whether the (over) expression of (fragments of) G subunits will ever be used in the treatment of diabetes will of course depend on the availability of suitable gene delivery methods. Drugs that promote the functions of endogenous G subunits would constitute an alternative strategy. For example, a drug that alleviates the allosteric inhibition of G_L by phosphorylase *a* would have great potential in this respect (21).

4. Glycogen metabolism in skeletal muscle

Glycogen in skeletal muscle serves as a source of energy to sustain contractions. In the period following exercise the glycogen stores are replenished, which correlates with an increased glucose uptake and activation of glycogen synthase. Mice lacking G_M had a very low basal activity of glycogen synthase and an increased level of phosphorylase *a* (340). Conversely, G_M-overexpressing mice showed an increased activity of muscle glycogen synthase, but their phosphorylase activity was not affected (22). Importantly, the G_M null mice failed to activate glycogen synthase following exercise or electrically induced muscle contraction. These data clearly show that G_M is essential for the regulation of glycogen synthase under basal conditions and in response to contractile activity. The mechanism by which muscle contraction affects G_M remains to be explored.

Epinephrine promotes glycogenolysis and inhibits glycogen synthesis in skeletal muscle. This hormone elevates cAMP and activates protein kinase A (PKA), which in turn promotes glycogenolysis via the phosphorylation of phosphorylase kinase. In addition, PKA functions as a glycogen synthase kinase, and it has been shown to dissociate and thereby inactivate the G_M/PP1 complex via the phosphorylation of Ser-67 of G_M (380), which occupies position X of the RVXF motif. PKA also phosphorylates Ser-48 of G_M, which increases the synthase phosphatase activity of G_M-associated PP1. However, the phosphorylation of Ser-67 has an overriding effect since it disrupts the holoenzyme structure. It has been suggested that the phosphorylation of Ser-48 serves as a mechanism for maximal glycogen synthesis in the recovery period after adrenergic stimulation, when PP1 reassociates with G_M (380).

Insulin is a major stimulator of glycogen synthesis in skeletal muscle, in particular in the postprandial phase (340). This insulin effect is partially accounted for by signaling via protein kinase B, which results in the inhibition of glycogen synthase kinase 3 (GSK-3). In addition, insulin activates a glycogen-associated species of PP1 that dephosphorylates glycogen synthase (101, 340). Anal-

ysis of two, independently generated G_M null mice led to different conclusions as to the role of G_M in the insulin-mediated control of glycogen metabolism. Suzuki et al. (340) concluded that the G_M-PP1 complex is unlikely to mediate the control of glycogen synthesis by insulin since their G_M null mice were lean, glucose tolerant, and still responded normally to insulin with an activation of glycogen synthase. In contrast, the G_M knock-out mice that were generated by Delibegovic et al. (101) were obese, glucose intolerant, and insulin resistant, suggesting a key role for the G_M protein in the metabolic control by insulin. The latter group also reported that insulin still caused a mild activation of glycogen synthase in the G_M-deficient mice and that this activation was correlated with a stimulation of the G_C-PP1 complex, a response that was not seen in the wild-type animals.

VI. PROTEIN SYNTHESIS

A. Transcription

The transcription of protein-encoding genes by RNA polymerase II relies on the reversible multisite phosphorylation of heptapeptide repeats in the COOH-terminal domain (CTD) of the largest subunit of the polymerase. Phosphorylation of the CTD domain by the cyclin-dependent protein kinases Cdk7 and Cdk9 is needed for promotor clearance, transcriptional elongation, and recruitment of mRNA processing factors, while its dephosphorylation is required for the regeneration of initiation-competent RNA polymerase II. Although originally the phosphoserine phosphatase FCP1 had been characterized as the CTD phosphatase, recent studies have suggested that PP1 may also contribute to the dephosphorylation of the CTD domain (383). Thus CTD dephosphorylation in cultured cells was inhibited by okadaic acid, which blocks PP1 but does not affect FCP1. Moreover, PP1 was shown to act as a major CTD phosphatase in nuclear extracts and to affinity-purify with RNA polymerase II. Both PP1 and the nuclear regulator NIPP1 have also been identified as components of the Tat-associated RNA polymerase II complex, which regulates transcription from the human immunodeficiency virus type 1 promoter (40, 275).

The transcription factor CREB mediates the expression of cAMP-induced genes by binding to a conserved cAMP-responsive element. Phosphorylation of CREB on Ser-133, e.g., by PKA, promotes the recruitment of the histone acetyltransferase CBP, which facilitates access of the promoter region to the transcriptional machinery. Attenuation of CREB signaling results from the dephosphorylation of CREB by PP1 (5, 45, 154), but the involved targetting subunit is unknown. Interestingly, it was recently reported that the histone deacetylase HDAC1 is part of a CREB-associated complex that also includes PP1 and that promotes the dephosphorylation of CREB (69). The importance of PP1 as a CREB phosphatase is illustrated by the finding that brain-targetted genetic inhibition of PP1 in mice correlated with an enhanced learning capability that involved the hyperphosphorylation of a number of proteins, including CREB (145) (see also sect. VIII*C*). In normal conditions, two additional serines that control the stability of CREB are kept dephosphorylated by PP1 (359). However, the decreased expression of PP1γ following hypoxia results in the hyperphosphorylation and subsequent ubiquitin-mediated degradation of CREB.

The heat shock factor (HSF) is a key transcriptional activator of stress-inducible genes and is activated by phosphorylation (174). The yeast homolog interacts with the G subunit Gac1 (224), suggesting that Gac1 may contribute to the recovery from stress by promoting the PP1-mediated dephosphorylation of HSF.

Initial evidence also implicates PP1 in the regulation of chromatin remodeling. Indeed, PP1 was identified as an antagonistic regulator of the trithorax protein in *Drosophila*, a component of a protein complex that is required for the maintenance of normal expression of homeotic genes (307). We have recently found that the nuclear PP1 interactor NIPP1 also binds to Eed (186a) which, as a member of the Polycomb group proteins, acts antagonistically to the trithorax protein and maintains transcriptional repression of homeotic genes by histone deacetylation and methylation. Moreover, like Eed, NIPP1 functioned as a transcriptional repressor of targetted genes. NIPP1, Eed, and PP1 can form a ternary complex, suggesting that NIPP1 targets Eed or an Eed-associated protein for dephosphorylation by PP1. Another trimeric complex that is presumably involved in chromatin remodeling consists of PP1, GADD34, and the SNF5 protein (391). GADD34 is a stress-induced protein that facilitates cell cycle arrest, while SNF5 is a component of a SWI/SNF chromatin remodeling complex that acts by repositioning nucleosomes. Both SNF5 and GADD34 interact directly with PP1, and SNF5 functions as a positive regulator of the GADD34/PP1 complex in vitro. GADD34 binds to PP1 via a canonical RVXF motif that is also required for binding of SNF5. Nevertheless, SNF5 does not compete with PP1 for the same binding site on GADD34. By analogy with the established targetting function of GADD34 in translation (see sect. VI*C*), these data may reflect that GADD34 promotes the dephosphorylation of a component of a SWI/SNF remodelling complex by PP1.

B. mRNA Processing

At least four PP1 interactors are established splicing factors or are known to colocalize with splicing factors. One of these is the splicing factor PSF, which is involved in the second catalytic step of splicing (169) but has

recently also been implicated in the control of transcription and gene silencing (319). The nuclear PP1 targetting subunits PNUTS (11) and SNP70 (93) show a punctate nuclear distribution typical for splicing factors and coprecipitate with spliceosomes during splicing in nuclear extracts (M. Lloriam, M. Beullens, I. Andrés, J.-M. Ortiz, and M. Bollen, unpublished observations). The PP1 interactor NIPP1 contains a forkhead-associated domain that is associated with phosphorylated forms of the splicing factors Cdc5L (57), SAP155 (58), and Melk, a novel protein kinase of the AMP-activated kinase family that blocks pre-mRNA splicing in nuclear extracts (V. Vulsteke, M. Beullens, and M. Bollen, unpublished data). We have recently identified NIPP1 as a splicing factor that is involved in a late step of spliceosome assembly but, surprisingly, this function appears to be unrelated to its ability to bind PP1 (38).

The inhibition of PP1 does not affect pre-mRNA splicing in nuclear extracts (38), indicating that PP1 is not involved in spliceosome assembly and splicing catalysis per se. Initial evidence suggests that PP1 may be involved in alternative 5′-splice site selection, possibly by dephosphorylating splicing factors of the SR family (71, 76, 77) (see also sect. IV). PP1 has also been proposed to contribute to spliceosome disassembly and/or the shuttling of splicing factors from the spliceosomes to the splicing factor compartments, which are thought to be storage or assembly sites for splicing factor complexes (252, 270). Consistent with this proposal, it was reported that the addition of either PP1 or inhibitors of PP1 to permeabilized cells interferes with the subnuclear distribution of splicing factors (253).

The RNA binding protein Staufen is a component of ribonucleoprotein complexes that move bidirectionally along dendritic microtubules and are believed to represent local storage compartments for mRNAs under translational arrest (210). Recently, Staufen has been identified as an RVXF-containing interactor of PP1, indicating that the Staufen complexes may be subject to regulation by reversible protein phosphorylation and that this regulation involves PP1 (255).

C. Translation

The phosphorylated form of the eukaryotic translation initiation factor eIF2α integrates general translational repression with the induction of stress-responsive genes in various stress conditions (284, 391). Phosphorylated eIF2α inhibits the assembly of translation initiation complexes by sequestering eIF2B, a translation factor that is needed for the regeneration of GTP-bound eIF2α. Paradoxically, phosphorylated eIF2α enhances the translation of the activating-transcription-factor-4, which is involved in the induction of stress-responsive genes. At least four structurally related protein kinases, each activated by specific stress stimuli, phosphorylate eIF2α. One of these is protein kinase R, which is activated by autophosphorylation and dimerization following the binding of double-stranded RNA. Recently, it was reported that protein kinase R binds directly to PP1 and is dephosphorylated by associated PP1 (354). In accordance with protein kinase R being a physiological substrate of PP1, these investigators found that the activation of the kinase is prevented by the coexpression of PP1.

Considerable evidence indicates that PP1 also contributes to the cellular recovery from stress by acting as an eIF2α phosphatase. PP1 was first identified as an eIF2α phosphatase in reticulocyte lysates (121). In yeast, a genetic antagonism was established between PP1 and an eIF2α kinase (385). More recently, the hyperphosphorylation of mammalian eIF2α during stress has been linked to a reduction in a PP1-derived eIF2α phosphatase activity (262). The targetting of PP1 to eIF2α appears to be mediated by GADD34 (92, 284). Indeed, the GADD34/PP1 complex is an efficient eIF2α phosphatase in vitro, and the level of phospho-eIF2α is diminished in GADD34-overexpressing cells. Remarkably, a heterotrimeric complex was identified consisting of GADD34, inhibitor-1, and PP1 (92). In this complex, GADD34 interacted directly with PP1 and with inhibitor-1 regardless of the latters' phosphorylation state, but PP1 only interacted with inhibitor-1 when the latter was phosphorylated by PKA. Hence, it was argued that the earlier described PKA-mediated inhibition of translational initiation in reticulocyte lysates could potentially be explained by the phosphorylation of inhibitor-1, resulting in the inhibition of the GADD34/PP1/inhibitor-1 complex and a hyperphosphorylation of eIF2α. Connor et al. (92) also reported that the GADD34/PP1 and GADD34/inhibitor-1 interactions in the brain of ground squirrels were lost during hibernation, and this correlated with a hyperphosphorylation of eIF2α and an inhibition of protein synthesis. These data suggest that the loss of GADD34-associated PP1 contributes to an eIF2α-mediated inhibition of translation during hibernation and that the GADD34/PP1/inhibitor complex is involved in the recovery from hibernation by dephosphorylating eIF2α.

Many viruses have evolved a mechanism for circumventing the translational inhibition in the host cell that results from the double-stranded RNA-induced activation of protein kinase R and the associated phosphorylation of eIF2α. ICP34.5, a protein encoded for by the Herpes simplex virus-1 genome, is structurally related to the PP1 binding COOH terminus of GADD34. This viral protein was shown to recruit PP1 from the host cell and to redirect the phosphatase to dephosphorylate eIF2α, enabling protein synthesis in spite of the presence of active protein kinase R (161, 162). Lysates from virus-infected cells contained a 3,000-fold higher eIF2α phosphatase activity than the lysates from noninfected cells. Interest-

ingly, some natural variants of ICP34.5 are nuclear and are also associated here with PP1, suggesting that ICP34.5 may also promote the dephosphorylation of nuclear substrates of PP1.

Phosphorylation of the ribosomal S6 protein allows the 40S ribosomal subunit to form translation initiation complexes more efficiently. The S6 protein has been identified as a likely substrate of PP1 (54). The involved targetting/regulatory subunit(s) are not known, but obvious candidates are the inhibitor RIPP1 (39) and the activator L5 (170), both of which interact with PP1 and are associated with the ribosomal fraction.

VII. ACTIN AND ACTOMYOSIN REORGANIZATION

The organization and dynamics of the actin cytoskeleton is tightly controlled by reversible protein phosphorylation, and this regulation clearly involves PP1 (132) and at least two families of R subunits, i.e., the Neurabins and Mypts. Neurabins and Mypts are most abundant and hence best studied in neural and muscle tissue, respectively, but lower concentrations of these R subunits can be detected in most other tissues as well (12, 65, 287). Judging from the phylogenetic distribution of the Neurabins and Mypts, the role of PP1 in actin reorganization gained importance during the evolution of metazoan multicellularity (74). However, as detailed below, recent work in yeast suggests that an important function for PP1 in actin reorganization had been acquired much earlier.

A. Neurabin-Associated PP1

Neurabins are fairly large and complex R subunits of PP1 (Fig. 4). They contain multiple protein binding modules and appear to act as scaffold proteins that promote the interaction between Neurabin-binding proteins by increasing their local concentration. Neurabins occur predominantly as membrane-associated proteins at cadherin-based adherens junctions, postsynaptic densities, and growth cones (288, 309), but are also detected in the cytoplasm (201, 333). Both vertebrate isoforms, Neurabin-I and Neurabin-II, contain an NH_2-terminal F-actin cross-linking domain (Fig. 4) that accounts largely, but not exclusively, for their localization near membranes (272, 288, 315). Invertebrate Neurabins, however, appear to lack this actin-binding domain. Neurabin-II, in contrast to Neurabin-I, interacts with the third intracellular loop of various G protein-coupled receptors of the biogenic amine-binding type, namely, the D2 dopamine receptor (322) and the α_{2A}-, α_{2B}-, and α_{2C}-adrenergic receptors (300). The central portion of Neurabins contains a PP1-binding module centered by an RVXF module, and a PDZ domain that can recruit the COOH terminus of the p70 S6 kinase (65, 130). This central portion is also involved in the reported Neurabin-II-mediated targetting of PP1 to ryanodine receptors (242) (see sect. VII*A1*). Notably, the PP1 binding module of Neurabins discriminates between PP1 isoforms, in that it prefers the α-subtype isoforms PP1γ_1 and PP1α over PP1β (87, 236, 360). Finally, the COOH-terminal third of Neurabins includes a putative coiled-coil module and that of vertebrate Neurabin-I and of invertebrate Neurabins also a sterile α-motif. Given that isolated Neurabin-I coiled-coil modules are capable of oligomerization (332), they presumably underlie the observed homo- and heterodimerization of Neurabin-I and -II (236, 272, 288, 315). Furthermore, dimerized Neurabin coiled-coil modules constitute a site for interaction with the *trans*-Golgi network protein TGN38 (333).

The involvement of Neurabins in the remodeling of actin and actomyosin and in the formation of cell projections is well documented. Thus treatment of hippocampal cells with antisense Neurabin-I oligonucleotides impedes neurite formation (272), and overexpression of Neurabin-I in kidney cells induces a collapse of the stress fiber network and the projection of filopodia (288). These effects are dependent on the presence of the F-actin binding domain and the PP1 binding module and are furthermore influenced by elements in the latter half of Neurabin-I (288), which underlines the functional importance of the juxtaposition of the various interaction sites in Neurabins. Intriguingly, an increase in the number of otherwise normal dendritic spines was observed in Neurabin-II knock-

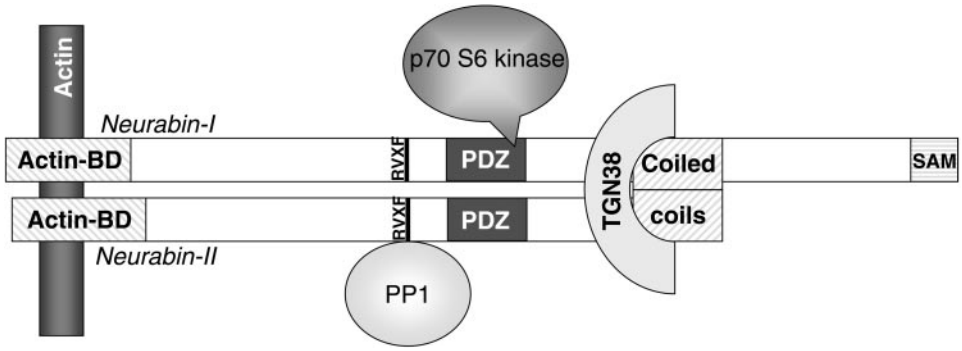

FIG. 4. Diagram of a Neurabin-I/Neurabin-II heterodimer with its ligands. The Neurabin bar diagrams were drawn to scale. Only ligands that can bind to both isoforms are included. Binding of PP1 and of p70 S6 kinase is mutually exclusive. Actin-BD, actin-binding domain; PDZ, PSD-95, disks large, Z0–1; SAM, sterile α-motif; TGN38, *trans*-Golgi network protein of 38 kDa.

out mice (130). This may hint at distinct roles in F-actin remodeling for the two vertebrate isoforms.

While Neurabin-associated PP1 induces the disassembly of stress fibers, the active, phosphorylated form of the p70 S6 kinase has been shown to promote the assembly of stress fibers (94). The p70 S6 kinase interacts with the PDZ domain of Neurabins but, strikingly, the binding of PP1 and p70 S6 kinase to Neurabin-I is mutually exclusive, possibly because these ligands compete for overlapping binding sites (288). However, if the interaction of p70 S6 kinase with Neurabins would be restricted to the phosphorylated form of the kinase, Neurabin-associated PP1 could also prevent the recruitment of the kinase by keeping it dephosphorylated. The latter mechanism is similar to the recently proposed regulation of the p70 S6 kinase by vitamin D signaling (see sect. IV). The p70 S6 kinase only binds to the vitamin D receptor in its phosphorylated form and is kept dephosphorylated by receptor-associated PP1 as long as vitamin D is bound (37). Whatever the mechanism of the mutually exclusive binding of PP1 and p70 S6 kinase to the Neurabin complex, the dissociation of PP1 from the complex may represent a key event in stress-fiber formation. It has been reported that the affinity of PP1 for Neurabin-I is drastically reduced in vitro and in vivo by the PKA-mediated phosphorylation of the RVXF-flanking Ser-461 of Neurabin-I (248, 288). However, given that PKA functions as a negative regulator of stress fiber formation (227), it is unlikely that PKA-induced dissociation of PP1 from Neurabin-I is involved in this process.

Surprisingly, although the RVXF-flanking serine of Neurabin-I is conserved in Neurabin-II, PKA does not reduce the latter's affinity for PP1, but instead phosphorylates two serine residues in and near the NH_2-terminal actin binding domain of the targetting protein. This phosphorylation promotes the dislocation of Neurabin-II from the membrane-associated fraction to the cytosol (175).

B. Mypt-Associated PP1

Type II myosin is a major generator of tension and/or contraction in eukaryotic cells. This actin-associated motor protein consists of two myosin II heavy chains that are each associated with an essential and a regulatory light chain. In animals the phosphorylation of the regulatory light chains relieves their complex inhibitory action on the contraction of actomyosin fibers (305). Conversely, dephosphorylation of the regulatory light chains by myosin phosphatases favors the relaxation of these fibers.

1. Myosin phosphatase

All known myosin phosphatases consist of PP1β (7, 256, 353) and both a large and a small myosin phosphatase targetting (Mypt) subunit (7, 19, 321). The large Mypt targets PP1 to myosin and determines the substrate specifity of the phosphatase (189). The function of the small Mypt remains unclear, but it is known to interact directly with myosin and the large Mypt (188). There is some variation in the composition of the holoenzymes in vertebrates, due to the existence of three Mypt-encoding genes and to splice variance. All three genes give rise to large Mypts, termed Mypt1 (350), Mypt2 (256), and $Mypt_{p85}$ (353), respectively, whereas all known small Mypts are derived from the Mypt2-encoding gene (19, 256). In contrast to the nearly ubiquitous Mypt1 (287), which is the prime large Mypt in smooth muscle and in nonmuscle tissues, Mypt2 has been proposed to be the major large Mypt in striated muscle (19, 138, 256). However, Mypt2 is also expressed in brain (138). $Mypt_{p85}$ has thus far only been described in brain and in testis (353).

Large Mypts contain three conserved modules (Fig. 5). The NH_2-terminal module binds PP1 and defines the substrate specificity of the phosphatase. Binding of the pivotal RVXF motif to PP1 is a prerequisite for the interaction of the phosphatase with two other elements of this conserved module (365), namely, an NH_2-terminal fragment that promotes the dephosphorylation of the myosin regulatory light chain and an array of ankyrin repeats that suppresses the dephosphorylation of other substrates (189). An ill-conserved fragment of Mypt1 COOH terminal of the ankyrin array has also been reported to interact with PP1 (365), and phosphorylation within this fragment by an unidentified early mitotic kinase stimulates the activity of the myosin phosphatase (367). The second conserved module encompasses the phosphorylation inhibitory motif (PIM). Phosphorylation of the PIM on a specific threonine by one of several Mypt kinases (see below) inhibits the myosin phosphatase (129, 231, 264, 265, 353), possibly by functioning as a pseudosubstrate. In agreement with this hypothesis, the PIM module only interacts with the catalytic subunit when phosphorylated (353). Finally, the third conserved module of large Mypts is COOH terminal and roughly corresponds to the small Mypts (Fig. 5). It comprises a myosin binding site (188) and a putative coiled coil region that has been proposed to function as the site for Mypt dimerization (214). Recently, it has been found that phosphorylation of Mypt1 in or near the myosin binding site by Rho-kinase dissociates the subunit from myosin (377). In some isoforms of large and small Mypts, the COOH-terminal module is extended by a leucine zipper that engages in a zipper interaction with cGMP-dependent protein kinase Iα (339). When activated, the latter enzyme stimulates the activity of myosin phosphatase, presumably via the phosphorylation of the large Mypt on an as yet unknown site.

2. Myosin and Mypt kinases

In contrast to the relative homogeneity of the myosin phosphatases, more than a dozen protein kinases have

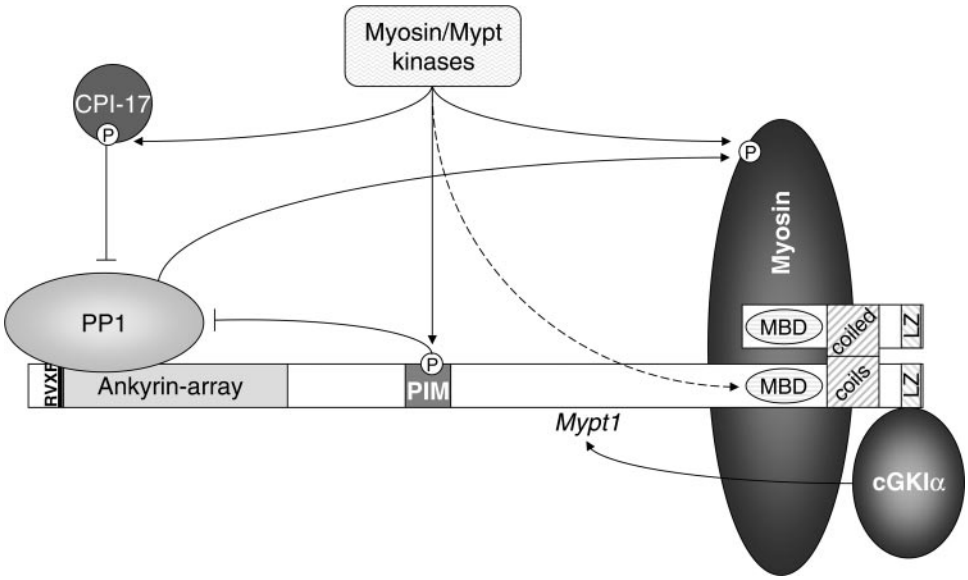

FIG. 5. Diagram of a myosin phosphatase holoenzyme with its ligands. The Mypts were drawn to scale. The arrows indicate enzymatic activity, whereas the blocked lines denote inhibition. The dashed arrow represents the phosphorylation of Mypt1 by Rho-kinase on a residue at or near the myosin-binding site. MBD, myosin-binding domain; LZ, leucine zipper; PIM, phosphorylation inhibitory motif; cGKIα, cGMP-dependent kinase Iα.

been implicated in promoting the phosphorylation of the regulatory light chain and/or inhibit the myosin phosphatases via the phosphorylation of the PIM module of the large Mypts (Fig. 5). A first group of related protein kinases encompasses death-associated protein kinase (187), zipper-interacting protein kinase (ZIPK) (266, 268), ZIPK-like protein kinase (231), Ca^{2+}/calmodulin-dependent kinase I (338), and the skeletal and smooth muscle myosin light-chain kinases (MLCKs) (192), which have all been proposed to function as genuine kinases of the regulatory light chains. Interestingly, these protein kinases belong to a subfamily with many other members that act on the myosin regulatory light chain in vitro (183, 199, 312). ZIPK (206, 264) and ZIPK-like protein kinase (55, 231) also phosphorylate large Mypts. The best studied representative of a second group of protein kinases that control actomyosin contraction is Rho kinase, which acts on both large Mypts and the regulatory light chains (14, 129, 366). The related myotonic dystrophy protein kinase (265) and myotonic dystrophy protein kinase-related Cdc42-binding kinase (MRCK) (220) both function as in vitro Mypt kinases, and MRCK also phosphorylates the myosin regulatory light chain (353). A fourth member of the Rho-kinase subfamily, citron kinase, phosphorylates the regulatory light chain, but not large Mypts (245). Other protein kinases implicated in the control of actomyosin function are the integrin-linked kinase (ILK), which acts both on the regulatory light chains and on large Mypts (206, 264); Aurora-B, which functions as a regulatory light chain kinase in vitro (267); and Raf-1, which phosphorylates large Mypts (61). Rho-kinase (208), ILK (103), and ZIPK-like kinase (232) also phosphorylate and activate the myosin phosphatase inhibitor CPI-17 (see sect. IX*A2*).

3. Actomyosin (de)phosphorylation in cytokinesis

The complex regulation and variable composition of the myosin phosphatases, and the multitude of protein kinases involved in the control of the phosphorylation state of the myosin regulatory light chains underlines the importance of type II actomyosin contractility in various cell processes. Perhaps the most ancestral function of actomyosin is its role in cytokinesis, which is conserved in all studied eukaryotes. However, regulation of the contraction of the cytokinetic actomyosin ring by phosphorylation of the regulatory light chain has only been observed in animals (reviewed in Ref. 245). Rho-kinase (293), MLCK (296), citron kinase (245), and Aurora-B (267) have been implicated as the protein kinases involved. The myosin phosphatase has been proposed to be inactivated by Rho-kinase after the metaphase/anaphase transition (200, 367), mainly via phosphorylation of the PIM module and of a residue at or near the myosin binding site. That the myosin phosphatase is also involved in the regulation of cytokinesis (see also sect. III*A2*) is indicated by the findings that a temperature-sensitive allele of the unique *Caenorhabditis* Mypt-encoding gene correlated with ectopic furrowing and accelerated furrow ingression at the restrictive temperature (293).

4. Regulation of cell shape and cell adhesion by myosin kinases/phosphatase

A second function for type II actomyosin, which was acquired during the development of metazoan multicellularity, is its contribution to the cell shape and the adhesion of cells to each other and to the extracellular matrix. Central to this function is the contractility and tension of the actomyosin stress fibers that connect focal adhesions

and of the cortical actomyosin fibers that underlie adhesion belts. Both the formation of stress fibers and the composition of focal adhesions are induced by promoting the phosphorylation of the myosin regulatory light chain via the activation of protein kinases and the inactivation of myosin phosphatases (81). Various signals can cause the contraction of distinct stress fiber populations. For instance, Rho-activated Rho-kinase phosphorylates myosin in central stress fibers, whereas Ca^{2+}/calmodulin-activated MLCK or Cdc42-activated MRCK act on peripheral stress fibers (112, 197, 366). These highly regulated pathways intervene in the physiology and pathophysiology of several tissues. Indeed, the inhibition of myosin phosphatase by the Rho-mediated phosphorylation of Mypt1 has been implicated in *1*) the change of the shape of platelets activated by thrombin or thromboxanes (341); *2*) the migration of macrophages, neutrophils, and fibroblasts during the development of lung fibrosis (320); *3*) the dissemination of prostatic cancer cells (326); and *4*) the loss of endothelial integrity during gram-negative sepsis (123). A similar regulatory mechanism involving Rho-kinase and myosin phosphatase has also been observed in the developmental elongation of cells in *Caenorhabditis* (388) and *Drosophila* (254).

Recently, evidence has emerged that the myosin regulatory light chain is not the only actin-associated target of myosin/Mypt kinases and the myosin phosphatase. Thus members of the ezrin/radixin/moesin family, which link actin filaments to integral membrane proteins and are involved in the formation of microvilli, are phosphorylated by Rho-kinase and MRCK and are dephosphorylated by myosin phosphatase (205, 244, 271). Other common substrates of Rho-kinase and myosin phosphatase are adducin (205), which promotes the formation of actin/spectrin complexes at cell-cell contact sites, and elongation factor 1α (184), which is an actin-binding protein as well as a cofactor in protein synthesis (89). Finally, a potential additional substrate of myosin phosphatase is found in focal adhesion kinase (FAK). The latter enzyme, which is indeed an in vitro substrate for PP1, is phosphorylated on a serine residue in early prophase and dephosphorylated after cytokinesis (394). PP1β but no other PP1 isoforms coprecipitated with FAK between 1 and 8 h after release from mitosis, in accordance with the proposed role of myosin phosphatase in the dephosphorylation of FAK (137). The dephosphorylation of FAK is proposed to allow reattachment of focal adhesions to the substrate.

5. *Myosin (de)phosphorylation and muscle contraction*

The contractile function of actomyosin culminates in muscle tissue. Phosphorylation of the myosin regulatory light chains is sufficient to trigger contraction in smooth muscle (192). This phosphorylation is promoted by raising the concentration of Ca^{2+}, which activates MLCK. The Ca^{2+}-independent net increase in phosphorylation of the regulatory light chains induced by Rho-kinase (139), ILK (264), ZIPK (264), and ZIPK-like kinase (55, 231) sensitizes muscles to Ca^{2+}. Muscle contractility is also influenced by variations in the composition of myosin phosphatase. Thus the sensitivity of vascular smooth muscles to regulation by nitric oxide via the activation of cGMP-dependent protein kinase 1α depends on the presence of leucine zippers in the splice variants of the large and small Mypt that are expressed in this tissue (339). In chicken gizzard, a developmental switch between leucine zipper-positive and -negative Mypts correlates with the loss of cGMP-mediated myosin relaxation at hatching (203). Other forms of splice variance of the Mypt1-encoding gene have also been implicated in the developmental regulation of muscle contractility (108). The importance of the enzymes that control the contractility of smooth muscles in arteries (193), airways (182), and corpora carvenosa (381) makes them potential targets in the treatment of cardiac and cerebral vascular spasms, asthma, and erectile dysfunction.

In striated muscle, contraction is triggered by membrane depolarization. Even though phosphorylation of the myosin regulatory light chain is not required for contraction, it does positively affect the speed and force of contraction (346). Thus a gradient of myosin regulatory light chain phosphorylation correlates with the pattern of cardiac contraction (99). The role of the Mypt2-based myosin phosphatase in the contraction of striated muscle has been poorly studied.

C. Scd5-Associated PP1

Fungi lack Mypt or Neurabin homologs, yet in budding yeast PP1 has also been implicated in the organization of cortical actin. Thus, after shifting of the temperature-sensitive PP1 mutant *glc7–10* to a restrictive temperature, the actin ring at the bud neck disappeared, actin cables became rare, and actin no longer showed its typical polarized localization (16). This mutant was also deficient in vacuolar fusion and in secretory and endocytotic vesicular transport (292). Strikingly similar phenotypes were observed after the functional disruption of Scd5, Rvs167, or Sla2 (32, 59, 167, 259, 277). Scd5 has been identified as a PP1-binding protein in various screens (173, 372, 374), and recently, it was found that disruption of the RVXF motif of Scd5 that mediates the interaction with PP1 severely disturbed endocytosis and actin organization (78). Rvs167 and Sla2 interact physically and genetically with Scd5 (32, 59, 167, 259). Also, mutation of either Rvs167 or Sla2, like that of PP1, compromised the integrity of the cell wall at high temperatures, presumably because of a disruption in the transport of vesicles with

cargoes required for the construction of the cell wall (16, 59, 259).

Collectively, the available data indicate that PP1, Scd5, Rvs167, and Sla2 function together in a signaling pathway that regulates vesicular transport and the polarized distribution of actin patches. Possibly, Scd5 targets PP1 to actin patches and vesicles. Potential substrates of Scd5-associated PP1 include the phosphoproteins Sla2, Sla1, and Pan1. The latter two proteins have both been found to interact with PP1 in yeast two-hybrid screens (372, 374) and to be components of a complex involved in actin organization, endocytosis, and cell wall morphogenesis (357). Because homologs of various proteins introduced in this section have been identified in animals, where they have also been implicated in actin organization and endocytosis (167), it is tempting to speculate that the function of PP1 in these processes may have been conserved as well.

One example of an animal PP1-interacting protein that is potentially involved in these pathways is the *Drosophila* protein Bifocal. The localization of this protein at the apical membrane of embryonic, epithelial, and neuronal cells overlaps with that of actin and of the *Drosophila* Rvs167 homolog Amphiphysin (28, 408). Moreover, the disturbed rhabdomere morphogenesis that is the main phenotypical characteristic of disruption of the Bifocal encoding gene resembles that of overexpression of Amphiphysin (408). Importantly, Bifocal interacts with PP1 via a canonical RVXF motif and, unlike expression of exogenous wild-type Bifocal, expression of a mutated version with a disrupted RVXF motif failed to compensate for the loss of endogenous Bifocal, which indicates a role of PP1 in this process (164). Scd5 and Bifocal do not display significant sequence similarity, so their modest functional similarity does not necessarily reflect an evolutionary relationship.

VIII. RECEPTORS, ION CHANNELS, AND ION PUMPS

A. Intracellular Ca^{2+} Release Channels and Ca^{2+} Pumps

1. The ryanodine and inositol trisphosphate receptors

In both excitable and nonexcitable cells, two distantly related types of tetrameric receptors, the ryanodine receptors (RyRs) and the inositol 1,4,5-trisphosphate receptors (IP_3Rs), function as Ca^{2+} release channels at the endoplasmic reticulum (327). RyR-dependent Ca^{2+} release from intracellular stores is of particular importance for the excitation-contraction coupling in muscles. The open-state probability of RyR channels is decreased by the binding of the prolyl isomerases FKBP12 or FKBP12.6 (364) (Fig. 6*A*), but this interaction is blocked by phosphorylation of the RyRs by PKA (243). Conversely, RyR-associated PP1 and/or PP2A are thought to counteract PKA and to promote thereby the recruitment of FKBP12/12.6 (Fig. 6*A*). In agreement with this view, PKA-phosphorylated RyR subunits were dephosphorylated by PP1 in vitro (155, 410), and the conductance of RyR2 channels was increased in the presence of the PP1/PP2A inhibitor okadaic acid (243). It has been proposed that the association of RyR2 channels with their regulators PP1, PP2A, and PKA is mediated by specific targetting molecules that bind via leucine zipper interactions (242). In the case of PP1, it is Neurabin-II that functions as the targetting subunit. It is puzzling, however, that the suggested leucine zipper motif in Neurabin-II partially overlaps with the well-documented PDZ domain (242). Given the substantially different three-dimensional structures adopted by these interaction modules, the binding of RyR2 to this putative leucine zipper motif of Neurabin-II would implicate a dramatic conformational change.

Recently, it has been demonstrated that PP1α also occurs in a complex with IP_3R1 (105, 358). The conductance of the IP_3R1 channel, like that of RyR channels, is increased after phosphorylation by PKA (105), but it remains controversial whether this regulation also involves FKBP12 (64, 68, 96). PP1α on the other hand dephosphorylated and thereby inhibited the IP_3R1 channel (358).

2. Sarcoplasmic reticulum Ca^{2+}-ATPase

The uptake of Ca^{2+} in intracellular stores is mediated by sarcoplasmic or endoplasmic reticulum Ca^{2+}-ATPases (SERCAs). In the heart, in smooth muscle, and in slow-twitch skeletal muscle, the activity of SERCA pumps is inhibited by the associated membrane protein phospholamban in its unphosphorylated state, but this inhibition is alleviated by the phosphorylation of phospholamban on Ser-16 and/or Thr-17 (88). Ser-16 is phosphorylated by PKA in response to stimulation of β_2-adrenergic receptors, whereas the phosphorylation of Thr-17 by Ca^{2+}/calmodulin-dependent kinase II (CaMKII) is frequency dependent (reviewed in Ref. 153). PP1 dephosphorylates phospholamban and thereby reduces the activity of the associated SERCA pump (233). Thus overexpression of PP1 led to a hypophosphorylation of Ser-16, and the increased PP1 activity in inhibitor-1 null mice correlated with a hypophosphorylation of both Ser-16 and Thr-17 (73). In vitro assays suggested that the glycogen-targetted pool of PP1 represents the major fraction of the phospholamban phosphatase activity in the heart (233). Interestingly, the muscle-type glycogen-targetting subunit G_M contains a COOH-terminal transmembrane domain that interacts directly with the transmembrane domain of phospholamban (36). This raises the intriguing possibility

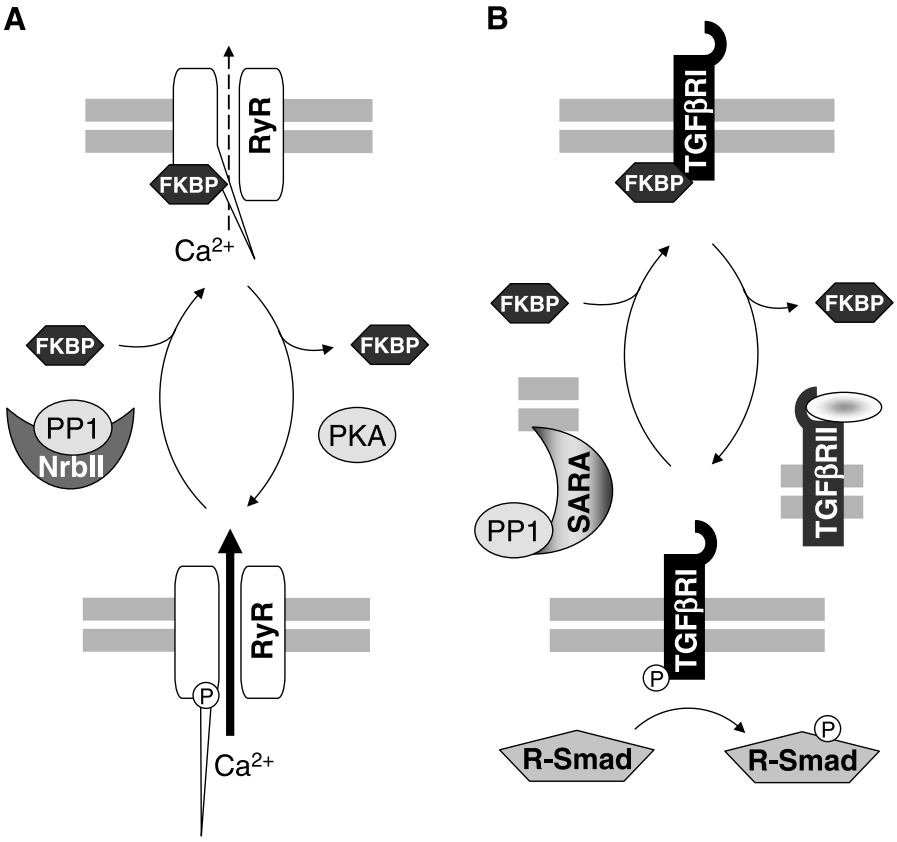

FIG. 6. Similarities in the regulation of ryanodine receptor (RyR) channels (*A*) and transforming growth factor-β receptor (TGFβR-I) (*B*) by PP1. NrbII, Neurabin-II; FKBP, FK506 binding protein.

that G_M functions as the phospholamban-targetting subunit of PP1.

3. PP1 and cardiac (dys)function

In physiological conditions, increased β-adrenergic stimulation of the heart upregulates adenylyl cyclase and hence PKA. The latter promotes contractility by a dual effect on Ca^{2+} cycling. On the one hand, PKA increases RyR2-dependent Ca^{2+} release during contraction and indirectly promotes SERCA2a-mediated Ca^{2+} uptake during relaxation via the phosphorylation of phospholamban. On the other hand, by phosphorylation of inhibitor-1, PKA also reduces the antagonistic downregulation of Ca^{2+} cycling by PP1. Accordingly, inhibitor-1 knock-out mice displayed a mildly depressed cardiac function (73). Moreover, a cardiomyocyte-restricted overexpression of PP1 was even associated with a severely impaired cardiac function, dilated cardiomyopathy, and increased mortality from heart failure (73). In both cases, hypophosphorylation of phospholamban was demonstrated. The effects on RyR2 phosphorylation were not explored.

From a medical point of view, it is interesting to note that the downregulated β-adrenergic signaling in human heart failure correlated with decreased cAMP levels and hypophosphorylation of inhibitor-1 and phospholamban (73, 251). This indicates a downregulation of SERCA-mediated Ca^{2+} reuptake and thus constitutes a maladaptive response. Unexpectedly, RyR2 channels in failing hearts were found to be hyperphosphorylated, and the levels of attached PP1 and PP2A were decreased (243). In accordance with these changes, heart failure has also been correlated with a decrease in RyR2-associated FKP12.6 (399). The hyperphosphorylated state of the RyR2 channels presumably reflects an adaptive mechanism to maximize Ca^{2+} release from the sarcoplasmic reticulum when Ca^{2+} uptake is suboptimal. The molecular mechanism underlying the decreased targetting of PP1 to RyR2 channels in failing hearts has not been explored but may involve the phosphorylation of Neurabin-II by RyR2-attached PKA. Indeed, PKA-dependent phosphorylation of the related Neurabin-I has been shown to dissociate PP1 (248, 288). Alternatively, PP1 may be displaced from the RyR2 channels by competition with phospholamban-targetting subunit(s).

B. Transforming Growth Factor-β Receptor-I

Transforming growth factor-β (TGF-β) signaling involves two types of receptors, TGFβR-I and TGFβR-II, which both contain an intracellular serine/threonine kinase domain. Upon binding of the dimeric ligand, two TGFβR-I/TGFβR-II pairs are formed, and TGFβR-II acti-

vates TGFβR-I via phosphorylation on at least three residues in the short so-called "GS" motif just NH_2 terminal to the kinase domain (181). GS phosphorylation promotes the activity of TGFβR-I in two ways (181). First, it causes the dissociation of the prolyl isomerase FKBP12, which keeps TGFβR-I in an inactive conformation (Fig. 6*B*). Second, (some of) the phosphorylated residues may function as docking sites for the Mad homology 2 (MH2) domain of the TGFβR-I substrates Smad2 and Smad3. Recruitment of Smad2/3 is also promoted by Smad anchor for receptor activation (SARA), which contains binding sites for both the TGFβR-I receptor and the MH2 domain of Smad2/3 (370). SARA is also targetted to phosphatidylinositol 3-phosphate in the membrane through a Fab1p/YotB/Vac1p/Eea1 (FYVE) domain (370). Once phosphorylated, Smad2/3 dissociates from the receptor kinase and from SARA, forms a complex with Smad4, and is targetted to the nucleus, where it acts as a transcription activator.

Recently, it was found that SARA, via an RVXF motif near the MH2-binding module, also recruits PP1 to the TGFβR-I complex (34). It was furthermore proposed that PP1 dephosphorylates TGFβR-I and thereby antagonizes TGFβR-II (Fig. 6*B*). This would imply that PP1 resets the signaling complex to a basal state, which may serve to prevent unwarranted signaling in the absence of ligand. Interestingly, TGFβR-I serves as a working paradigm for a whole family of metazoan receptor kinases that all contain a GS motif. Two branches of these receptor kinases can be discerned (23). The first one, including TGFβR-I, has TGF-β or activin-related ligands and signals via Smad2 or Smad3 or orthologous Smads, whereas the second one binds bone morphogenic proteins (BMPs) or Decapentaplegic (Dpp) and signals via Smad1, Smad5, Smad8, or their orthologs. The observation that PP1 negatively regulates Dpp signaling (34) suggests that its role extends to both branches of the receptor family. As human SARA binds to Smad2 and Smad3, but not to other Smads, another PP1 targetting subunit is likely to be involved in the downregulation of BMP/Dpp signaling. Interestingly, a recently characterized human SARA paralog called Endofin also contains the FYVE domain, the RVXF motif, and the large COOH-terminal domain that mediates receptor binding, but Endofin does not bind Smad2 (318). Possibly, Endofin is the PP1 partner in the regulation of the BMP/Dpp branch of receptor kinases. It is also intriguing to note that the overexpression of either SARA or Endofin induces endosome fusion (318). This may reflect a function of SARA and Endofin-associated PP1 in membrane fusion and vesicular transport, reminiscent of the function of PP1 in vesicular fusion that has been established in yeast (292) (see also sect. VII*C*).

A remarkable analogy can be drawn between the regulation of RyR channels and that of TGFβR-I receptor kinases (Fig. 6). Both types of receptors are activated by phosphorylation and the concomitant dissociation of FKBP12, and in both cases PP1 restores the unphosphorylated, inhibited state. The main difference is the nature of the kinase involved, i.e., PKA in the case of RyR channels and TGFβR-II in that of TGFβR-I. However, it should be pointed out that *Caenorhabditis* SARA was originally identified as the A-kinase (PKA) anchoring protein $AKAP_{Ce}$ (17), indicating that TGF-β signaling is also controlled by PKA.

C. Regulation of Ionotropic Glutamate Receptors

PP1 has been linked to the regulation of at least two out of the three types of ionotropic glutamate receptors, namely, the *N*-methyl-D-aspartate (NMDA) and the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors. Conventional NMDA receptors reside in the plasma membrane and consist of two glycine-binding NR1 subunits and two glutamate-binding NR2 subunits. When activated by their dual ligands, they function as cation channels (216). The currents through NR1A-containing receptors have been shown to be regulated by PKA and PP1, which are both recruited to NR1A by the scaffold protein Yotiao (386). In HEK293 cells expressing exogeneous NMDA receptors and Yotiao, currents through the NMDA receptor were augmented by the stimulation of PKA, by the inhibition of PP1 with okadaic acid, or by the addition of an RVXF-containing peptide that disrupts the interaction between PP1 and Yotiao (386). Similarly, NMDA receptor currents in acutely dissociated hippocampal neurons were enhanced by the inhibition of PP1 with calyculin A (130). Remarkably, the latter effect was not observed in Neurabin-II null mice (130), which implicates this PP1-targetting scaffold protein in the regulation of at least some NMDA receptor complexes. While these studies suggested that PP1 constitutively downregulates NMDA currents, it has been recently proposed that synaptic NMDA receptor currents in hippocampal CA1 pyramidal cells are only downregulated by PP1 after the receptors have been activated (258). Additional studies will be required to determine whether these apparent differences in regulation are accounted for by distinct NMDA receptor complexes, e.g., synaptic versus extrasynaptic or Yotiao-associated versus Neurabin-II-associated complexes.

NMDA receptors are pivotal in synaptic plasticity, the long-term strengthening or reduction of synaptic efficacy in response to patterns of synaptic discharge, which is thought to be intrinsic to the learning process (Fig. 7). The processes involved are most extensively studied at hippocampal Schaffer collateral-CA1 synapses. In this model, repetitive high frequency (100-Hz) pulse trains induce long-term potentiation (LTP) of synaptic strength, whereas extended low-frequency stimulation (1–5 Hz) results in long-term depression (LTD) of synaptic strength.

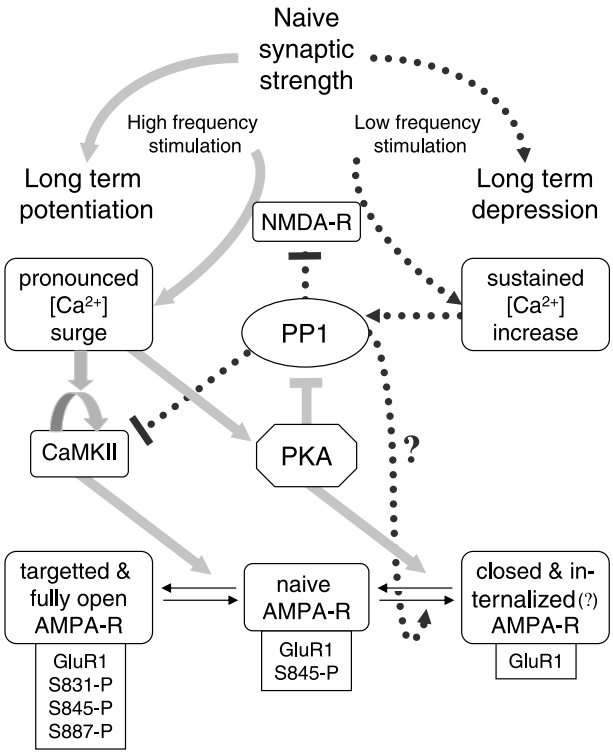

FIG. 7. Synopsis of proposed mechanisms for the induction of long-term potentiation (full light gray arrows) and long-term depression (dotted dark gray arrows) of synaptic strength. NMDA-R, NMDA receptor; AMPA-R, AMPA receptor; GluR1, subunit of AMPA receptor.

High-frequency stimulation relieves the Mg^{2+}-mediated blockade of the NMDA receptor channel at resting membrane potential and thereby induces an inward Ca^{2+} and Na^{+} flux (237). The resulting transient surge in Ca^{2+}, possibly amplified by Ca^{2+} release from intracellular stores, promotes the autophosphorylation of CaMKII on Ser-286 (237), and thereby its activation and recruitment to the postsynaptic density (405). There, the kinase phosphorylates numerous substrates, including the GluR1 subunit of the tetrameric AMPA receptors (403). The CaMKII-mediated phosphorylation of the latter protein on two sites stimulates AMPA receptor signaling in two ways: phosphorylation of GluR1 on Ser-831 increases the conductance of the AMPA receptor cation channel (295), while phosphorylation on Thr-887 is required for the observed postsynaptic targetting of AMPA receptors at the onset of LTP (104, 160). PP1 has been proposed to dephosphorylate CaMKII, resulting in its inactivation and dissociation from the postsynaptic density (335, 404).

The Ca^{2+} surge that triggers LTP also activates the postsynaptic adenylyl cyclase and hence PKA, resulting in an inhibition of PP1 (46) and an increased CaMKII activity. It has been proposed that the PKA-dependent inhibition of PP1 is mediated by inhibitor-1 (46), but this seems at variance with the report that LTP at Schaffer collateral-CA1 pyramidal cell synapses is unaffected in inhibitor-1 null mice (10). Nevertheless, the reduced PP1 activity after inducible expression of constitutively active inhibitor-1 in the hippocampus and the cerebral cortex correlated with a hyperphosphorylation of CaMKII and an augmented efficacy of learning (145). The observed downregulation of AMPA receptor currents after stimulation of 5-HT_{1A} serotonin receptors has also been proposed to be mediated by PP1-modulated CaMKII (67).

The modest Ca^{2+} increase induced by low-frequency stimulation does not reach the treshold required for the induction of LTP but, if sustained, instead leads to LTD via a poorly understood mechanism that is also dependent on the NMDA receptor and that involves NMDA receptor-mediated postsynaptic recruitment of PP1 (258). Furthermore, LTD is more pronounced after addition of exogeneous PP1 and is reduced in Neurabin-II knock-out mice and after the inhibition of PP1 by okadaic acid, calyculin A, or exogeneous inhibitor-1 (130, 260, 261). LTD is in part caused by the endocytosis of AMPA receptors, but the role of PP1 in this process remains controversial (reviewed in Ref. 238). Alternatively, PP1 may be involved in the proposed dephosphorylation of Ser-845 of the GluR1 subunit at the onset of LTD, which reduces conductance of the AMPA receptor channel (218). Under basal conditions, this residue is thought to be kept in a phosphorylated state by PKA. This hypothesis is supported by the observation that the reduction of PP1 activity caused by the induced expression of constitutively active inhibitor-1 correlated with a hyperphosphorylation of Ser-845 (145).

A direct dephosphorylation of the PKA-phosphorylated Ser-845 of GluR1 and/or an inhibition of CaMKII-mediated phosphorylation of GluR1 on Ser-831 may account for the finding that inhibition of PP1 or interference with its targetting impedes the normal gradual decline in time of the amplitude of kainate-induced AMPA receptor currents (397). Indeed, this rundown has all but disappeared after inhibition of PP1 by okadaic acid (397) or after the injection of an RVXF-containing peptide that disrupts the association with most R subunits (397). The rundown of AMPA receptor currents is also largely absent in Neurabin-II knock-out mice (130). This may reflect that Neurabin-II targets PP1 directly to AMPA receptors or that it is involved in the dephosphorylation of CaMKII.

D. Regulation of Other Channels and Transporters

Yotiao also targets PKA and PP1 to the pore-forming KCNQ1 subunits of the voltage-gated I_{Ks} K^{+} channel (241). In a regulatory mechanism reminiscent of that of the above-discussed receptors, the slow outward K^{+} currents of the I_{Ks} channel are increased through phosphorylation of a conserved serine residue of KCNQ1 (Ser-27 of mouse KCNQ1) by PKA, and this upregulation is enhanced by application of the PP1/PP2A inhibitor okadaic

acid (241). Interestingly, mutations in KCQN1 are responsible for more than half of the cases of congenital LQT syndrome, a potentially life-threatening cardiac arrythmia (302). One such mutation is located in the leucine zipper motif that constitutes the interaction site with Yotiao, and accordingly occludes binding of Yotiao (241).

The vertebrate NKCC1 Na^+-K^+-Cl^- cotransporter ensures coupled inward movement of Na^+, K^+, and Cl^- that is crucial for maintaining cell volume. Decreases in cell volume or intracellular Cl^- concentrations result in the phosphorylation of the NH_2-terminal regulatory region of NKCC1 by an unknown kinase, which in turn promotes transport (97). PP1 binds directly to the RVXF motif of NKCC1 and counteracts the kinase-induced activation (97).

PP1 has also been implicated in the upregulation of the heteropentameric $GABA_A$ receptor Cl^- channels. While PKA phosphorylates $GABA_A$ receptors containing β_1- or β_3-subunits, and thereby reduces GABA-evoked currents, the reduced inhibition of PP1 in DARPP-32 null mice (see below) largely undoes the PKA-mediated phosphorylation of these receptors (133).

Another Cl^- channel that has been proposed to be regulated by PP1 is the tetrameric ClC-2 channel. ClC-2 interacts with PP1 isoforms in the yeast two-hybrid system, and ClC-2 currents that were increased by the cyclin-dependent kinase inhibitor olomoucine were again reduced by the PP1/PP2A inhibitor calyculin A (140). Moreover, it has recently been shown that the maturation and swelling-induced activation of the *Caenorhabditis* ortholog of ClC-2 during meiosis is mediated by PP1 (308). Interestingly, the phenotype of ClC-2 knock-out mice (56) is reminiscent of that of PP1γ null mice (376); both mice combine a normal gross anatomy with exclusively male infertility, which is correlated with a reduced diameter of the seminiferous tubules and a loss of germ cells. Even though the testicular histopathology is more pronounced in the ClC-2 knock-out mice, it is tempting to conjecture that the phenotype of PP1γ null mice is caused by the loss of PP1-dependent activation of ClC-2.

IX. INHIBITION AND MATURATION OF PROTEIN PHOSPHATASE-1

From the various examples discussed above, it is evident that by far the majority of PP1 regulators act as substrate specifiers and/or targetting proteins. Indeed, their function is to increase the local concentration of PP1 and to adapt the catalytic subunit to appropriate substrates. Often such adaptation results in the exclusion of other substrates from the catalytic site, which explains why a positive regulator of PP1 can simultaneously act as an inhibitor of the phosphatase activity toward standard assay substrates. However, there are also regulators that block the access to the catalytic site for all substrates. The best-studied examples of these genuine inhibitors are the PKA-activated inhibitors (inhibitor-1, DARPP-32) and the phosphatase holoenzyme inhibitors or PHIs (CPI-17, PHI1/2, KEPI). They have all been proposed to use a pseudosubstrate mechanism and to bind in their phosphorylated state to the catalytic site as nondephosphorylatable substrates. As true inhibitors of an enzyme that in general resets pathways to basal and economical states of activity, these regulators facilitate the induction and maintenance of high activity states. For instance, DARPP-32 and CPI-17 both promote locomotor activity, the former on a neurological level and the latter at the level of the muscle tissue (see below). PP1 is regulated in a completely different manner by inhibitor-2, which induces conformational changes that are thought to play a role in the maturation of PP1.

A. Inhibition

1. The PKA-activated inhibitors

The PKA-activated inhibitors of PP1 are vertebrate specific and embody, in evolutionary terms, a recent expansion of the interplay between PKA and PP1 signaling (74). Other illustrations of this interplay are the phosphorylation and regulation by PKA of several vertebrate interactors of PP1 (G_M subunit, NIPP1, and the Neurabins) and the interaction of some AKAPs with both PKA and PP1. Two of the three mammalian PKA-activated inhibitors, DARPP-32 and inhibitor-1, are among the best-characterized regulators of PP1. The mammalian PKA-activated inhibitors share an NH_2-terminal PP1-regulating module headed by an RVXF motif. Although the latter enables binding of the inhibitors to PP1 irrespective of their phosphorylation status, the unphosphorylated proteins are poor inhibitors with an IC_{50} in the micromolar range (120, 212). A 1,000-fold more potent inhibition is unleashed by the cAMP-induced phosphorylation of a conserved threonine in the PP1-regulating module by PKA (or by PKG in response to increased cGMP levels), which is thought to turn the module into a pseudosubstrate.

A) DARPP-32. As suggested by its name, DARPP-32 is mainly expressed in dopaminoceptive brain areas and peripheral tissues (166). Within the brain, DARPP-32 as well as PP1α and PP1γ_1 are particularly enriched in the striatum (289, 379). Because of the medical importance of the striatum, both as a target for therapeutic and abusive drugs and as an afflicted area in motoric disorders such as Huntington's disease, most functional studies of DARPP-32 have focused on this region.

I) The neurophysiological context and general regulation of striatal DARPP-32. The striatum is one of the neural centers of the so-called extrapyramidal system, a network of subcortical circuits involved in the control of voluntary motor activity by the cerebral cortex. Under the

control of extrinsic and intrinsic afferents, the axons of the predominantly GABAergic and inhibitory medium-sized spiny neurons of the striatum convey signals to mainly two other extrapyramidal centers, the globus pallidus and the substantia nigra, the degeneration of which leads to Parkinson's disease. Dopaminergic fibers stemming from the substantia nigra and from other midbrain cell groups, and excitatory glutamatergic fibers originating in the cortex and the thalamus, form the main extrinsic afferents. Intrinsic control is exerted by collaterals of neighboring medium-sized spiny neurons and by various types of striatal aspiny neurons secreting GABA, acetylcholine, adenosine, serotonin, neurotensin, endorphins, somatostatin, vasoactive intestinal peptide, cholecystokinin, and neuropeptide Y (149). Upon binding to their respective receptors, this host of extrinsic and intrinsic ligands regulates the levels of the secondary messengers cAMP (or cGMP) and Ca^{2+} (Fig. 8) and thereby the activities of PKA and PP2B, respectively (149). The antagonistic activities of this kinase/phosphatase pair then determine the phosphorylation state of Thr-34 of DARPP-32, which controls the inhibitory potency of the protein towards PP1. Remarkably, phosphorylation of DARPP-32 on Thr-75 by the cyclin-dependent kinase Cdk5 turns the protein into an inhibitor of PKA and thereby impedes phosphorylation on Thr-34 (43, 282). Via an ill-understood pathway, the ligands that stimulate PKA also promote the dephosphorylation of Thr-75 by PP2A (225, 282). Thus DARPP-32 accounts for the inverse coupling of the striatal PKA and PP1 activities, which in turn govern the expression of neuropeptides as well as the ion channels and pumps that determine the excitation state of the medium-sized spiny neurons (Fig. 8).

II) The regulation of striatal DARPP-32 by dopaminergic signaling. Dopamine-bound D_1 and D_2 receptors of the medium-sized spiny neurons have opposite effects on PKA signaling (283). Dopamine has an excitatory effect on striatonigral neurons, which are rich in D_1 receptors, and causes a cAMP-mediated activation of PKA that is amplified by the PP2A-dependent dephosphorylation of Thr-75 of DARPP-32, and an inhibition of PP1 via the phosphorylation of DARPP-32 on Thr-34. In contrast, striatopallidal neurons express predominantly D_2 receptors and are inhibited by dopamine, on the one hand by the inhibition of adenylyl cyclase and hence PKA, and on the other hand by a Ca^{2+}-and PP2B-mediated release of

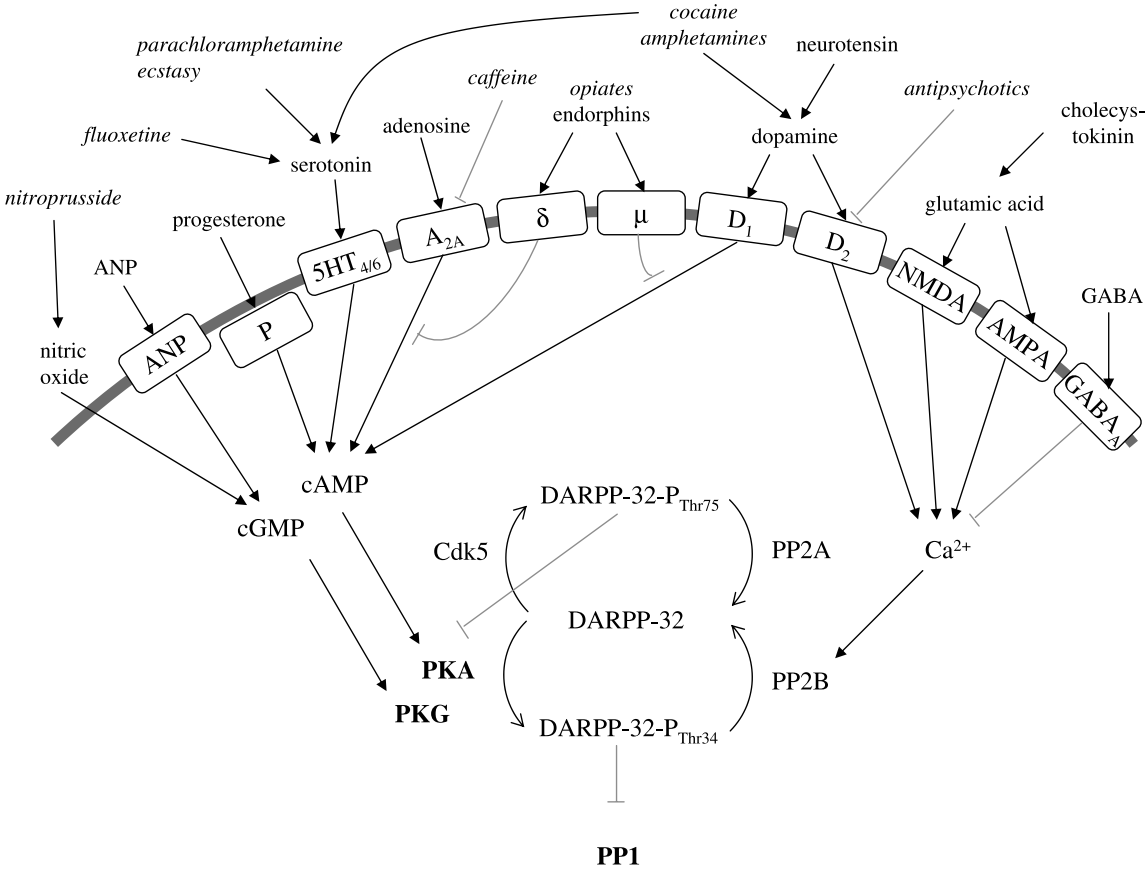

FIG. 8. Signaling pathways impinging on DARPP-32. Neurotransmitters and drugs are written upright and in italic, respectively, and their receptors are drawn as boxes. Stimulatory and inhibitory effects are represented as arrows and gray blocked lines, respectively.

the inhibition of PP1 by DARPP-32. Various neurotransmitters, therapeutic agents, and drugs of abuse impinge on dopamine signaling (reviewed in Ref. 149). Neurotensin, for instance, stimulates the presynaptic release of dopamine and thus promotes the D_1-induced phosphorylation of DARPP-32 on Thr-34 (149, 246). Conversely, the binding of endorphins or opiates to μ-opioid receptors, which are selectively expressed in striatonigral neurons, causes an inhibition of the D_1-mediated DARPP-32 phosphorylation (149). Nearly all antipsychotics function primarily as D_2-receptor blockers, and in accordance, haloperidol (Haldol), clozapine, raclopride, and eticlopride promote the phosphorylation of DARPP-32 on Thr-34 (149, 283, 342). The acute administration of amphetamines or cocaine stimulates locomotor activity via an increased release and inhibited reuptake, respectively, of biogenic amines, including dopamine and serotonin. This stimulatory effect is accompanied by an increased phosphorylation of DARPP-32 on Thr-34 and is attenuated in DARPP-32 knock-out mice (149). Chronic exposure to cocaine, however, upregulates the expression of Cdk5 and attenuates normal D_1 and PKA signaling via the phosphorylation of DARPP-32 on Thr-75 (41). Finally, in line with the established positive correlation between dopaminergic signaling and ethanol consumption, DARPP-32 knock-out mice were found to be less sensitive to ethanol reinforcement and to self-administer less ethanol than wild-type mice, even though they displayed a higher sensitivity to an ethanol-induced increase of locomotor activity (301).

III) The regulation of striatal DARPP-32 by adenosine. Upon activation, the striatal A_{2A} adenosine receptor, which is thought to be primarily expressed in striatopallidal neurons, appears to function like the D_1 dopamine receptor in striatonigral neurons in that it stimulates adenylyl cyclase, PKA, and PP2A and inhibits PP1 via the phosphorylation of DARPP-32 on Thr-34 (149, 343). Accordingly, the stimulatory effect of caffeine and other A_{2A} antagonists on locomotion is in part accounted for by the loss of stimulation of adenylyl cyclase and PKA, and by the unopposed Cdk5-dependent phosphorylation of DARPP-32 on Thr-75 (225). Moreover, it was found that this stimulatory effect was significantly reduced in DARPP-32 null mice. The double function of DARPP-32 as an inhibitor of both PP1 and PKA was nicely demonstrated by a similar attenuation in DARPP-32 knock-out mice of the reduced motor activity following selective stimulation of the A_{2A} receptor. The pathway of the A_{2A} receptor, like that of the D_1 receptor, is inhibited by endorphins and opiates, but in this case, these substances act through δ-opioid receptors, which are specific for striatopallidal neurons.

IV) The regulation of striatal DARPP-32 by serotonergic signaling. Like adenosine and dopamine, serotonin promotes the activity of adenylyl cyclase, PKA, and PP2A and hence the phosphorylation of striatal DARPP-32 on Thr-34 (344, 345). It was demonstrated that these effects result from the binding of serotonin to 5-HT_4 and 5-HT_6 receptors. Binding of the neurotransmitter to 5-HT_2 receptors adds to the phosphorylation of DARPP-32 on Thr-34 via the activation of the phospholipase C pathway and hence protein kinase CK1. The latter kinase catalyzes the phosphorylation of DARPP-32 on Ser-137, which is known to lower the rate of dephosphorylation of Thr-34 by PP2B. In line with these findings, *para*-chloroamphetamine, an amphetamine derivative that induces a more selective release of serotonin, promotes the phosphorylation of DARPP-32 on Thr-34 and Ser-137, decreases that on Thr-75, and stimulates locomotor activity (344). These pathways may also underlie the increased locomotor activity in similarly serotonin-selective drugs such as methylenedioxymethamphetamine, commonly known as ecstasy. Interestingly, serotonin reuptake inhibitors, such as the antidepressant fluoxetine (Prozac), induced similar changes in the phosphorylation of DARPP-32 in the striatum, and also in the prefrontal cortex and in the hippocampus (345). Chronic administration of fluoxetine increased the expression of DARPP-32 in the prefrontal cortex and in the hippocampus, but not in the striatum. Finally, compared with wild-type mice, DARPP-32 null mice exhibited an attenuated effect of fluoxetine in learned-helplessness tests that are used as a predictor of clinical antidepressant efficacity. Collectively, these data indicate an involvement of DARPP-32 in the therapeutic effects of the antidepressant fluoxetine.

V) Glutamatergic and GABAergic signaling and the striatal DARPP-32 pathway. Stimulation of the AMPA and NMDA ionotropic glutamate receptors oppose the PKA-mediated phosphorylation of DARPP-32 on Thr-34 and, in the case of the NMDA receptor, this effect is largely counteracted by the PP2B inhibitor cyclosporin A (reviewed in Ref. 149). An NMDA receptor antagonist also blocks the negative effect of cholecystokinin on DARPP-32 phosphorylation on Thr-34 (325). Importantly, via the inhibition of PP1 (see also sect. VII*C*), DARPP-32 is in its turn involved in the regulation of the AMPA and NMDA receptors. Accordingly, the serotonin or fluoxetine-induced phosphorylation of DARPP-32 on Thr-34 correlated with an increased phosphorylation of Ser-831 and Ser-845 of the GluR1 subunit of the AMPA receptor (345). Moreover, the hyperphosphorylation of Ser-845 was reduced in the striatum, the prefrontal cortex, and the hippocampus of DARPP-32 null mice, and that of Ser-831 was attenuated in the striatum of these mice. Similarly, acute cocaine or methamphetamine administration induced a partly DARPP-32-mediated hyperphosphorylation of Ser-845 but not of Ser-831 of GluR1 (323).

The transient GABA-induced decrease of the phosphorylation of DARPP-32 on Thr-34 is thought to be me-

diated by the activation of PP2B by the $GABA_A$ receptor (324). Conversely, activation of PKA and the concurrently increased phoshorylation of DARPP-32 on Thr-34 led to a hyperphosphorylation of the β_1-subunit of the $GABA_A$ receptor and enhanced $GABA_A$ currents (133). These effects were significantly attenuated in DARPP-32 knock-out mice, suggesting a mechanism of DARPP-32-mediated inhibition of PP1.

VI) Hypothalamic DARPP-32 and sexual receptivity. The administration of progesterone, dopamine, or serotonin enhances the sexual receptivity in rodents (240). The intracellular progestin receptor is essential for the effects of both dopamine and progesterone, indicating overlapping pathways (239). In agreement with this hypothesis, cAMP production, PKA activity, and phosphorylation of DARPP-32 on Thr-34 in the hypothalamus of ovariectomized, estradiol benzoate-primed mice were stimulated by dopamine or progesterone but not by serotonin (240). Furthermore, the effect on sexual receptivity of dopamine and progesterone, but not that of serotonin, was largely absent in DARPP-32 knock-out mice. The stimulation of the cAMP/PKA/DARPP-32 pathway by progesterone was not inhibited by a D_1 antagonist, suggesting that the effects of dopamine and progesterone on this pathway are additive. Dopamine moreover activates the progestin receptor through a direct, ligand-independent mechanism (239).

VII) DARPP-32 and blood pressure homeostasis. Two lines of evidence suggest a role for DARPP-32 in maintaining a normal blood pressure. First, nitroprusside, a nitric oxide-producing antihypertensive drug, stimulates the phosphorylation of DARPP-32 on Thr-34, via the activation of guanylyl cyclase and PKG (369). This raises the possibility that DARPP-32 is involved in the antihypertensive properties of nitric oxide. Second, in contrast to wild-type mice, DARPP-32 null mice failed to induce natriuresis in response to the atrial natriuretic peptide, a well-established regulator of ion and blood pressure homeostasis (119). Like nitric oxide, the atrial natriuretic peptide was found to stimulate the cGMP/PKG/DARPP-32 pathway. Presumably via the inhibition of PP1, Thr-34-phosphorylated DARPP-32 in turn promotes the inactive hyperphosphorylated state of the renal tubular Na^+-K^+-ATPase (18). The loss of responsiveness to the atrial natriuretic peptide is likely to account for the 10% increase in arterial blood pressure of DARPP-32 knock-out mice, compared with that of wild-type animals (119). Interestingly, the cGMP phosphodiesterase-5 inhibitor zaprinast induces a similar DARPP-32-dependent natriuresis.

B) INHIBITOR-1. As suggested by the high degree of sequence conservation, the PP1-inhibiting module of inhibitor-1 is controlled in much the same way as that of DARPP-32. Thus Thr-35 is phosphorylated by cAMP-activated PKA and dephosphorylated by PP2B when Ca^{2+} is available. However, Thr-35-phosphorylated inhibitor-1 is also a substrate for PP2A. Like DARPP-32, inhibitor-1 also harbors a phosphorylation site for Cdk5 (42, 178). It has recently been reported that, contrary to an earlier study (178), phosphorylation at this site does not convert the regulator into a potent PP1 inhibitor, but instead induces a fivefold increase in the Michaelis constant for its phosphorylation by PKA, thereby reducing the fraction of PP1-inhibiting inhibitor-1 (42). Like Thr-35, Ser-67 is dephosphorylated by PP2A and PP2B (42).

Our knowledge of the physiological functions of inhibitor-1, in contrast to that of DARPP-32, is fairly limited in spite of the frequent use of this AKAI as a specific PP1 inhibitor. Indeed, while the addition or expression of inhibitor-1 can be used to demonstrate a participation of PP1 in a specific cellular process, the observed effects do not necessarily imply that endogenous inhibitor-1 is also involved in that process. This is clearly illustrated by the observation that the expression of mammalian inhibitor-1 in yeast downregulated a number of endogenous PP1 holoenzymes, although yeast itself does not express an inhibitor-1 homolog (413). Nevertheless, in recent years progress has been made in unraveling some functions of inhibitor-1, in particular thanks to the availability of an inhibitor-1 knock-out mouse (10). Thus inhibitor-1 has been proposed to counteract the negative effect of PP1 on myocardial function (see sect. VIII*A3*). In agreement with this suggestion, the β-adrenergic agonist isoproterenol, which has a positive inotropic effect on heart contractility, promoted the cAMP-induced phosphorylation of inhibitor-1 on Thr-35 in the cardiac ventricles (152). Moreover, inhibitor-1 null mice displayed a mildly depressed cardiac function (73). Interestingly, β-adrenergic stimulation also induces the PKA-dependent phosphorylation of inhibitor-1 in many other tissues (135, 278). Thus DARPP-32 and inhibitor-1 may be the selective PKA-activated inhibitor for dopaminoceptive and adrenoceptive tissues, respectively.

Another suggested function of inhibitor-1, namely, that of a mediator of LTP of synaptic strength and memory enhancement (see sect. VIII*C*) through the inhibition of hippocampal PP1, remains more equivocal. On the one hand, conditions that precipitate LTP, such as high-frequency (30–100 Hz) stimulation or combined θ frequency (5–12 Hz) and adrenergic stimulation, also promote the cAMP- and PKA-mediated phosphorylation of inhibitor-1 on Thr-35 in hippocampal CA1 pyramidal cells (46, 62). On the other hand, LTP at Schaffer collateral-CA1 pyramidal cell synapses is unaffected in inhibitor-1 knock-out mice, although LTP at lateral perforant path-dentate gyrus granule cell synapses in the hippocampal formation is deficient in these mice (10). It also remains unclear to what extent endogenous inhibitor-1 is involved in relieving the recently demonstrated PP1-mediated constraint on learning and memory (145). Recently emerged functions of inhibitor-1 include its role as an inhibitor of GADD34-

associated PP1 (see sect. VI*A*), which is linked to the regulation of translation (92), and an involvement in the rewarding effects of drugs such as cocaine that is similar to that of DARPP-32 (406).

2. The PHIs

Only one out of four mammalian PHIs, the C-kinase-dependent phosphatase inhibitor CPI-17, has been studied in detail. The main target of this inhibitor is the myosin phosphatase in smooth muscles, where it is highly expressed (127, 390), and in some nonmuscle cells, such as blood platelets (384). That CPI-17 mainly acts on myosin phosphatase is surprising, since the Mypts themselves contain a PIM module that also functions as a pseudosubstrate when phosphorylated (see sect. VII*B*). For potent inhibition, CPI-17 must be phosphorylated on Thr-38 by one of several possible protein kinases, including protein kinase C (PKC) (127), the closely related PKN (157), and furthermore the ILK (103), ZIPK-like (232), and Rho-kinases (208), which also act on the large Mypt subunit and on the myosin regulatory light chain, the prime substrate of the myosin phosphatase (see sect. VII*B*). Agonists of smooth muscle contraction such as histamine and phenylephrine have been proposed to promote the phosphorylation of CPI-17 by PKC and Rho-kinase, respectively (126, 381). It should be noted, however, that the actions of the latter two kinases may overlap, as the alledgedly Rho-kinase-specific inhibitor Y27632, which has been used in a number of studies to identify Rho-kinase as a CPI-17 kinase, also inhibits the activity of PKC towards CPI-17 (126). Dephosphorylation of CPI-17 and the concomitantly increased myosin phosphatase activity contribute to the vasodilatation effected by nitroprusside and presumably also by other drugs that give rise to nitric oxide (128). In contrast to the dephosphorylation-resistant PIM module of the large Mypts, CPI-17 is readily dephosphorylated in vitro and in vivo and has therefore been implicated in the acute control of the myosin phosphatase (351).

While CPI-17 is an excellent substrate for PP2A, PP2B, and PP2C, it is an extremely poor substrate for PP1 (351). However, mutation of Tyr-41 of CPI-17 to alanine converts into an even better substrate for myosin phosphatase than the latters' prime substrate, the regulatory light chain (159). This lends weight to the hypothesis that phosphorylated CPI-17 binds to PP1 as a pseudosubstrate, that is shielded from dephosphorylation by the side chain of Tyr-41 and to a lesser extent by other adjacent residues. A fragment of CPI-17 encompassing residues 35–120 retains its full inhibitory potency and has been shown to fold into a bundle of four helices, the first of which is reoriented after phosphorylation of the adjacent Thr-38 (285). This fragment is conserved in all other PHIs.

PHI-1 not only inhibits myosin phosphatase but also glycogen-bound PP1 holoenzymes (125). Nevertheless, its inhibitory potency is weaker than that of CPI-17, which may indicate that the endogenous target of this inhibitor is yet another PP1 holoenzyme. The functions of the two other PHIs also remain elusive. The expression of one of these, a presumably membrane-associated protein termed KEPI, is upregulated under morphine treatment (229).

B. Maturation

The earliest in vitro studies characterized inhibitor-2 as a negative regulator of PP1 (reviewed in Ref. 54). Inhibitor-2 blocks the activity of PP1 by two different mechanisms, namely, inhibition and inactivation. Inhibition occurs instantaneously at a high inhibitor-2-to-PP1 ratio and is readily reversible by the removal of the regulator, whereas the slower process of inactivation is observed at roughly equimolar concentrations of the regulator and the phosphatase. Inactivation is not reversed by the removal of inhibitor-2, which indicates that it involves a conformational change. However, once phosphorylated on a conserved threonine, inhibitor-2 can reactivate the associated PP1. The activated PP1 then dephosphorylates inhibitor-2.

In vivo, both negative and positive effects of inhibitor-2 on PP1 activity have been observed (280, 373). Thus overexpression of inhibitor-2 correlated with a decreased PP1 activity (373), presumably due to inhibition. On the other hand, at endogenous concentrations, inhibitor-2 was found to function as an activator of PP1 (280, 373), suggesting that inactivation does not occur in vivo. This view is supported by the finding that the in vitro inactivation of PP1 by inhibitor-2 is blocked by physiological salt concentrations (50). Like the in vitro reactivation of PP1, the in vivo activation of PP1 by inhibitor-2 is phosphorylation dependent (355), indicating a single underlying mechanism. Interestingly, phosphorylated inhibitor-2 also induced a near-native behavior of bacterially expressed mammalian PP1, which normally differs from the native protein in that it has a broader substrate specificity and a reduced sensitivity to inhibitor-1 and okadaic acid (8, 235). Collectively, these observations support the proposal that the prime function of this ancient regulator may be to promote a conformational change that converts the newly formed, premature PP1 into a fully functional enzyme. Accordingly, the phosphorylation mechanism involved is conserved. In yeast, the prime inhibitor-2 kinase is the cyclin-dependent kinase Pho85 (355), which also phosphorylates glycogen synthase. In mammals, inhibitor-2 is phosphorylated by the Pho-85 ortholog Cdk5 (2) and by glycogen synthase kinase-3 (389).

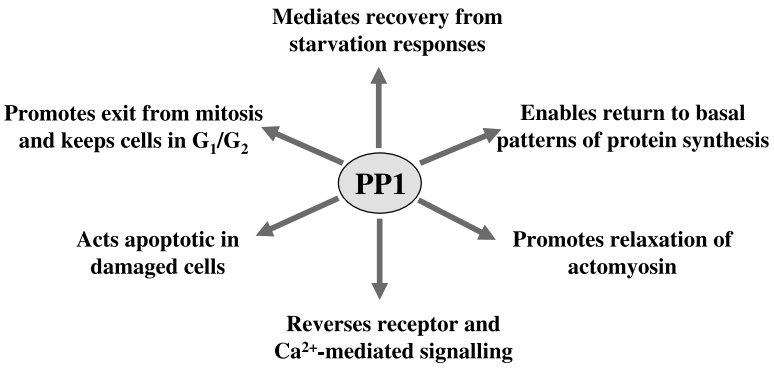

FIG. 9. Functions of PP1.

X. CONCLUSIONS

We have discussed here ~50 proteins as likely substrates of PP1. Yet, these probably represent only a minor fraction of the total pool of physiological PP1 substrates. An often cited estimate puts the number of mammalian phosphoproteins at one-third of the proteome or ~10,000 (98), an order of magnitude that is in agreement with proteome electrophoresis experiments in yeast (141). Although a considerable number of these phosphoproteins are phosphorylated exclusively on tyrosine residues, are intermediates of phosphotransfer reactions, or are permanently phosphorylated, the large majority of phosphoproteins are reversibly phosphorylated on serine/threonine, often even phosphorylated at multiple sites. This estimate implies that each of the ~25 protein serine/threonine phosphatases has on average hundreds of substrates. Moreover, it is conceivable that protein phosphatases such as PP1 or PP2A, which interact with a large variety of targetting subunits, have a relatively larger share of substrates. Currently, ~65 mammalian genes are known to encode protein interactors of PP1 (Table 1). It is difficult to estimate how many PP1 interactors remain to be discovered, but with the assumption that the number of protein serine/threonine phosphatase holoenzymes approximates that of the ~400 protein serine/threonine kinases and given that PP1 and PP2A are the only known serine/threonine phosphatases associated with a wide variety of targetting subunits, it seems fair to predict that PP1 and PP2A each occurs in 100–200 different holoenzymes. Hence, up to two-thirds of the protein interactors of PP1 may still be unknown. There are likely to be many more PP1 substrates than targetting subunits, since the latter can direct PP1 to multiple substrates that, surprisingly, can function in unrelated cellular process. For example, G subunits are implicated in the targetting of PP1 to glycogen-associated substrates (see sect. V*B*), to the membrane-associated phospholamban (see sect. VIII*A*), and to transcription factors (see sect. VI*A*). Equally, polyfunctional targetting subunits are the Neurabins, which may target PP1 to actin-associated proteins (see sect. VII*A*), to RyR (see sect. VIII*A1*), and to the NMDA/AMPA receptors (see sect. VIII*C*).

The picture that emerges from all the PP1 functions described is that of a "green" enzyme that *1*) reduces energy expenditure and even stockpiles energy in the form of glycogen, *2*) promotes the recycling of specific proteins, and *3*) returns the cell to a basal state (see Fig. 9). Starvation forces cells to use any available energy source but, once the nutrient supply rebounds, PP1 enables the cell to shift to its most energy-efficient and hence preferred fuels, and even to store energy in the form of glycogen (see sect. V). PP1 also reverses translational control mechanisms induced by other forms of stress, inactivates specific transcription factors, and promotes the recycling of transcription and splicing factors (see sect. VI). PP1 resets highly specialized muscle and neural tissues to a more relaxed and energy-conserving state. Thus muscle contraction is downregulated, both by promoting muscle relaxation and by reducing the Ca^{2+} cycles that are pivotal in excitation-contraction coupling (see sects. VII and VIII). PP1-induced actomyosin relaxation, however, also occurs in nonmuscle cells. Via its effect on ion channels, PP1 clamps down on the excitability of neural cells (see sect. VIII). In various tissues, PP1 also downregulates the activity of ATP-consuming ion pumps and transporters, such as the SERCA Ca^{2+}-ATPase, the NKCC1 Na^+-K^+-Cl^- cotransporter, and the Na^+-K^+-ATPase. PP1 furthermore reverses TGFβR-I to its inactive basal state, when the concentrations of the activating ligand drops. Following irreparable cell damage, PP1 coinduces apoptosis (see sect. IV), which is a energy-conserving measure, as it eliminates the use of nutrients by these inviable cells. Finally, PP1 promotes the exit from mitosis and contributes to the resetting of the cell cycle (see sects. III and IV). In conclusion, PP1 functions as both an economizer and as a reset button.

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