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Functions and therapeutic potential of protein phosphatase 1: Insights from mouse genetics

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ABSTRACT

Protein phosphatase 1 (PP1) catalyzes more than half of all phosphoserine/threonine dephosphorylation reactions in mammalian cells. In vivo PP1 does not exist as a free catalytic subunit but is always associated with at least one regulatory PP1-interacting protein (PIP) to generate a large set of distinct holoenzymes. Each PP1 complex controls the dephosphorylation of only a small subset of PP1 substrates. We screened the literature for genetically engineered mouse models and identified models for all PP1 isoforms and 104 PIPs. PP1 itself and at least 49 PIPs were connected to human disease-associated phenotypes. Additionally, phenotypes related to 17 PIPs were clearly linked to altered PP1 function, while such information was lacking for 32 other PIPs. We propose structural reverse genetics, which combines structural characterization of proteins with mouse genetics, to identify new PP1-related therapeutic targets. The available mouse models confirm the pleiotropic action of PP1 in health and diseases.

1. Introduction

In a typical mammalian cell, more than three-quarters of all proteins are phosphorylated at serine, threonine and/or tyrosine residues during their lifetime [1]. While phosphorylation of these residues (i.e. transfer of the γ-phosphate of ATP to the hydroxyl group side chain) is catalyzed by protein kinases, protein phosphatases catalyze the hydrolytic removal of phosphate groups [2, 3]. Thus, the spatiotemporal phosphorylation state of a protein is the result of a balance between counteraction of protein kinases and protein phosphatases. Disturbance of this balance, which causes aberrant phosphorylation, is implicated in various human diseases, including cancer, cardiac hypertrophy, diabetes, and neurodegeneration [4]. Therapeutic strategies for protein phosphorylation diseases have mainly focused on drugs that target protein kinases, although protein phosphatases are equally attractive targets [4, 5]. To date, more than two dozen small-molecule protein kinase inhibitors and six therapeutic antibodies against protein tyrosine kinases have been approved by the US Food and Drug Administration (FDA) for clinical use, mainly for targeted cancer therapies [6, 7]. Along with these FDA-approved drugs, hundreds of other protein kinase inhibitors are being tested in clinical trials [6, 7].

In contrast, protein phosphatases have been neglected as potential drug targets because they were classified as ‘undruggable’, evoking the misconception that it is impossible to target a particular protein phosphatase [4, 5, 8]. In fact, multiple studies by various research groups revealed that protein phosphatases are unequivocally ‘druggable’ targets [4, 5, 8]. Various small-molecule effectors that modulate (activate or inhibit) protein phosphatase activity and exhibit therapeutic potential for various human diseases have been generated [5, 9]. For example, the immunosuppressants cyclosporine A and FK506, which are successfully used to treat acute organ transplant rejection, rheumatoid arthritis, psoriasis and Crohn’s disease, target and inhibit protein serine/threonine phosphatase PP2B, also known as PP3 or calcineurin [4, 10, 11]. Their mode of action, however, was only elucidated after their approval by the US FDA. These PP2B inhibitors do not block the active site but bind and block one of the substrate binding sites of PP2B [12, 13]. Various protein tyrosine phosphatase (PTP) inhibitors, including distinct small molecule inhibitors such as orthosteric (bonds at the enzyme active site), allosteric (bonds outside the enzyme active site) and competitive (bonds to an enzyme at the site of substrate binding)
inhibitors and biologics [8, 9, 14] have also been developed for therapeutic purposes. A few are currently undergoing clinical trials. For example, small-molecule inhibitors of vascular endothelial PTP and PTP1B are being tested for treatment of diabetic macular edema and metastatic breast cancer, respectively [8, 9, 14].

Protein phosphatase 1 (PP1) is a widely expressed and highly conserved phosphatase of ~38 kDa that is active only towards phosphoserine/threonine residues and belongs to the phosphoprotein phosphatase (PPP) family of the eukaryotic protein phosphatome [15]. Based on biochemical data, it is estimated that PP1 is responsible for more than half of all phosphoseryl/threonyl dephosphorylation reactions in eukaryotic cells [2]. PP1 itself shows a rather broad substrate specificity in vitro, but exhibits in a cellular context a much more narrow and tightly regulated substrate selectivity. This is mainly achieved through association with a wide array of PP1-interacting proteins (PIPs), thereby forming > 200 distinct multisubunit PP1:PIP(s) complexes, each of which dephosphorylates only a small subset of PP1 substrates [2, 16]. Consistent with its numerous substrates, PP1 is a key regulator of several cellular processes, including cell cycle progression, protein synthesis, pre-mRNA splicing and transcription. It is therefore not surprising that PP1 plays a crucial role in human pathologies such as cancer, heart disease, memory loss, type 2 diabetes and viral infections, suggesting a great therapeutic potential for PP1-directed drugs [21, 22]. The first breakthrough was the discovery of the phenotype of mice with a global deletion of protein tyrosine phosphatase PTP1B. Because these mice were hypersensitive to insulin and resistant to obesity, academics and pharmaceutical companies began to search for PTP1B inhibitors to treat type 2 diabetes and obesity [21, 22].

In this review, we first summarize the current knowledge about the structure and diversity of PP1 holoenzymes, and the way in which their specificity is achieved. Next, we address genetically modified mouse models of PP1 isoforms and their PIPs. Since most PIPs are multifunctional and/or interact with multiple proteins, we focus on mouse models of PIPs with a phenotype that is connected to the PP1 holoenzyme function. We review the discoveries made with the mouse models regarding the in vivo function of PP1 holoenzymes and their therapeutic potential. Finally, we examine mouse models for phenotypes that are associated with human diseases. Abbreviations of protein names and the mouse genetics nomenclature [23] are defined in Box 1 and 2, respectively.

### 2. Structure of PP1 holoenzymes

Each PP1 holoenzyme consists of PP1, the catalytic subunit, and one or two regulatory PIPs (Fig. 1). While the mammalian genome contains only three PP1 genes, > 200 PIP-encoding genes have been identified, and it is estimated that hundreds have yet to be discovered [2]. The mammalian PP1 isoforms are about 90% identical at the amino acid level, whereas PIPs are mainly structurally unrelated proteins that define where and when a PP1 holoenzyme is active, and towards which set of substrates. The binding of PIPs to PP1 is facilitated by short linear motifs (SLIMs) of 4–8 residues long that bind to specific surface grooves.

### Box 1

**Abbreviations of protein names.**

| Abbreviation | Full protein name |
|--------------|-------------------|
| CPI-17       | protein kinase C-potential protein phosphatase-1 inhibitor Mr. 17 kDa |
| CREB1        | cAMP responsive element binding protein 1 |
| CreP         | constitutive repressor of eIF2α phosphorylation |
| DARPP32      | dopamine and cyclic AMP-regulated phosphoprotein of Mr. 32,000 |
| DRD1         | dopamine receptor D1 |
| DRD2         | dopamine receptor D2 |
| E2F1         | E2F transcription factor 1 |
| eIF2α        | eukaryotic initiation factor 2α |
| GADD34       | growth arrest and DNA damage-inducible protein |
| GBP-1        | gut and brain phosphatase inhibitor 1 |
| GL           | hepatic glycogen-targeting protein phosphatase 1 regulatory subunit |
| GM           | skeletal muscle glycogen-targeting protein phosphatase 1 regulatory subunit, alias RGL |
| HSP20        | heat shock protein 20 |
| I1           | inhibitor 1 |
| I2           | inhibitor 2 |
| KEPI         | kinase C-enhanced PP1 inhibitor |
| MYPT1        | myosin phosphatase targeting subunit 1 |
| MYPT2        | myosin phosphatase targeting subunit 2 |
| MYL2         | myosin light chain 2 V |
| MYBPNC3      | cardiac myosin binding protein C |
| NIPP1        | nuclear inhibitor of protein phosphatase 1 |
| PHACTR4      | phosphatase and actin regulator 4 |
| PNL          | phospholamban |
| PP1          | protein phosphatase 1 |
| Rb           | retinoblastoma |
| SDS22        | suppressor 2 of dis2–11 mutation |
| SPZ1         | spermatogenic leucine zipper protein 1 |
**Conditional genotype:** A genotype that is dependent on the presence of a certain factor. The term often refers to Cre-mediated excision of genomic sequences flanked by loxP sites.

**Cre recombinase:** A site-specific recombination enzyme that recognizes the 34-base pair long DNA sequence, known as a loxP site.

**Cre/loxP system:** Transgenic technology based on the ability of Cre recombinase to excise the DNA sequence between two loxP sites. The promoter of the Cre-expressing transgene determines the spatiotemporal expression of Cre recombinase. Cre recombinase can be expressed wide-spread, tissue-specific and/or cell-type specific.

**Genotype:** A description of the genetic information carried by an organism. It can also refer to specific alleles and their variants.

**Genetically engineered mice:** All mice with an altered genome compared to wild-type mice, including knockouts, knockins and transgenic mice.

**Knockout (KO):** A casual term for a targeted disruption of a gene of interest in which a loss-of-function allele is produced. KOs can be created by deleting portions of the gene to make it nonfunctional or by replacing the gene with an altered sequence. Because the creation of KOs involves directed homologous recombination, KOs are here not considered to be transgenic.

**Knockin (KI):** A casual term for targeted mutation of a gene of interest in which an alteration in gene function other than a loss-of-function allele is produced. Because the creation of KIs involves directed homologous recombination, KIs are here not considered to be transgenic.

**Phenotype:** Any measurable and observable characteristic of mice that is the result of the interaction between the genetic background and the environment.

**Transgenic mice:** Mice that carry a transgene in its genome.

**Transgene (Tg):** Any DNA sequence that has been introduced into the germ line of mice by random integration. Example, Tg (Myh6-PP1α): PP1α-expressing transgene, which expression is driven by the Myh6 promoter.

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**Fig. 1.** Structure of PP1 holoenzymes. Surface representation of the PP1 catalytic domain (center). Indicated are the two metals in the active site of PP1 (red spheres) that lies at the Y-shaped intersection of three substrate-binding grooves; the acidic groove (A), the C-terminal groove (C) and the hydrophobic groove (H). Inhibitory PIP (iPIP) is depicted in yellow, a guiding PIP (gPIP) in blue and substrates in purple. Various interaction sites that can be considered for therapeutic targeting are represented by colored circles as indicated in the inset box. Bended black arrows indicate dephosphorylation of the substrate. PP1 surface structure was made using PyMOL (www.pymol.com). PIPs and substrates are schematically placed on PP1. PP1, Protein Phosphatase 1; PIP, PP1-Interacting Protein. See text for references.
of PP1 [2, 10, 16, 24]. Multiple SLiMs, also known as PP1 docking motifs, are often clustered to form an intrinsically disordered PP1-anchoring domain [13, 16, 25]. The combined binding of SLiMs establishes a high-affinity binding between PP1 and its PIP, which is often associated with (partial) structural folding of the PP1-binding domain [26]. The best-studied motif for PP1:PIP interactions is the so-called RVxF-motif, which docks into a hydrophobic groove that is remote from the catalytic pocket of PP1 (Fig. 1) [27]. This motif is present in about 70% of all known PIPs, and mutation of the valine and/or phenylala-nine into an alanine is often sufficient to at least partially disrupt the interaction with PP1. However, some SLiMs, such as the SILK-motif, are present in far fewer PIPs [13]. In addition, some binding sites are highly structured, such as leucine-rich repeats of SDS22 or ankyrin repeats of MYPT1 [28, 29]. To date, about a dozen distinct PP1-binding sites have been characterized, enabling a huge combinatorial potential for generating unique PP1 holoenzymes [2]. The number and combination of PP1-binding sites differ between PP1 holoenzymes, as confirmed by several crystal structures, explaining how PP1 can interact with numerous structurally unrelated PIPs [16].

From a therapeutic viewpoint, the most promising approach for developing PP1-directed drugs does not involve interference with the catalytic site but with specific interaction sites within the PP1 holoenzymes [13]. Drugs that bind to the catalytic site will non-selectively target all PP1 holoenzymes, possibly even other structurally related phosphatases such as PP2A and PP2B holoenzymes. In contrast, drugs that disturb specific PP1 holoenzymes will selectivity modulate PP1 activity towards a limited number of substrates [13, 16].

3. Diversity and specificity of PP1:PIP complexes

PP1 shows extreme phylogenetic and functional conservation, even though the number of PP1 genes differ between species (e.g. 7–11 genes in plants, 1 gene in S. cerevisiae, 4 genes in D. melanogaster, and 3 genes in vertebrates) [30]. The functional conservation of PP1 is illustrated by sequence identity (> 80%) between PP1 from yeast and humans, and by the observation that the lethality of PP1 loss in S. cerevisiae can be rescued by any human PP1 isoform [31]. Together, the three mammalian PP1 genes Ppp1ca, Ppp1cb and Ppp1cc encode four nearly identical PP1 isoforms (PP1α, β, γ1 and γ2) that differ primarily at their extremities [32]. PP1γ1 and γ2 isoforms arise from alternatively spliced transcripts that only differ in terms of retention or deletion of the last intron of the Ppp1cc gene. This results in PP1γ1 and γ2 isoforms with a unique C-terminal tail of 9 or 23 amino acids in mice, respectively [33]. At the biochemical level, there are no substantial differences between the PP1 isoforms [34], but genetic ablation of individual PP1 genes in mice suggests distinct and overlapping functions, as discussed in Section 4 (Table 1).

Generally, PIPs are structurally unrelated, but they share and combine various functional domains involved in PP1-anchoring, PP1-inhibition, substrate-recruitment and/or subcellular-targeting [16]. First, all PIPs contain a PP1-anchoring domain, as discussed in Section 2 (Figs. 1 and 2). Second, some PIPs have an additional PP1-inhibitory domain that covers the active site of PP1, thereby preventing substrates from binding at the catalytic site. A PP1-inhibitory domain can bind to the active site of PP1 constitutively or only after phosphorylation [35, 36]. Third, many PIPs have a specific targeting domain that mediates binding to a particular subcellular compartment or structure (e.g. sar-coplasmic reticulum or glycogen particles) [37]. This enhances the local concentration of PP1 and promotes dephosphorylation of phospho-substrates that are specifically associated with that subcellular structure [16, 37]. Fourth, some PIPs contain a binding domain for a small subset of PP1 substrates [38]. The combination of PP1 and PIP substrate-binding sites dramatically increases the affinity for these substrates, allowing their dephosphorylation at physiological levels [16, 38]. Fifth, some PIPs prevent dephosphorylation of certain substrates by occluding one of the substrate-binding grooves of PP1 [25]. Finally, it should be noted that PIPs often combine these different strategies and that multiple PIPs serve themselves as substrates for associated PP1 [2, 16].

From the list of 189 biochemically validated PIPs [37], we found genetically modified mouse models for 104 distinct PIPs (~55%), and often multiple models for one PIP, bringing the total number of PP1:PIP mouse models to > 170. Since most PIPs are multifunctional and/or do interact with multiple proteins, it is critical for the development of potential PP1-directed drugs to examine whether the observed phenotype depends on associated PP1. Therefore, we screened these mouse models to identify phenotypes connected to altered PP1 function as determined by changed PP1 activity, altered phosphorylation levels of substrates, and/or a phenotype associated with the expression of a PP1 dysfunctional mutant of a PIP. Based on these criteria, we selected 39 mouse models linked to 17 distinct PIPs (Tables 2 and 3). We functionally classify PIPs as inhibitory PIPs (iPIPs) or guiding PIPs (gPIPs) (Figs. 1 and 2). iPIPs block dephosphorylation of substrates by occupying the active site of PP1. Thus, ablation of an iPIP in mice will lead to increased dephosphorylation of physiological substrates of the PP1:iPIP holoenzyme. In contrast, gPIPs are defined as PIPs that guide PP1 towards a specific subset of substrates within a cell. Ablation of a gPIP in mice will lead to increased phosphorylation of the in vivo substrates of PP1:γgPIP complex. We analyzed genetically altered mouse models of 7 iPIPs and 10 gPIPs, which are described in Table 2 and 3, respectively.

4. Mouse models of PP1 isoforms

The global ablation of PP1α or PP1γ isoforms in mice, did not cause an overt phenotype, except that male PP1γ knockout (KO) mice were infertile due to impaired spermiogenesis (Table 1) [Agnus Nairn, personal communication, 39–41]. This indicates that other PP1 isoforms compensate for the loss of PP1α or PP1γ in all tissues except the testis, which could be explained by the distinct expression patterns of PP1 isoforms in the testis [41, 42]. More specifically, PP1α, β and γ, but not PP1γ2, are expressed in somatic testicular cells and spermatagonia. Conversely, in (post-)meiotic germ cells only PP1γ2 is detected [41, 43, 44]. Thus, the germ-cell specific deletion of the Ppp1cc gene results in male infertility and sperm with abnormal morphology or teratospermia due to the loss of entire cellular pool of PP1 in the (post-)meiotic germ cells (Table 1) [41]. Accordingly, the testis-specific transgenic expression of PP1γ2 restored the fertility of Ppp1cc null mice in two independent mouse models (Table 1) [43]. Together, the various Ppp1cc mouse models demonstrate that PP1γ2 has a unique spermiogenic function. Along with the testis-enriched PP1γ2 isoform, there are testis/sperm-enriched PIPs isoforms such as NIPPI-T (also known as NIPPIα) [45] and SPZ1 [46], accounting for the formation of unique PP1γ2:PIP complexes in testis or sperm [47]. Such complexes are attractive candidate-targets for pharmacological intervention to combat male infertility since these drugs will likely not affect cellular processes in other tissues [47].

In contrast to total PP1α and PP1γ KO mice, the global deletion of PP1β led to pre-weaning mortality (i.e. homozygotes died before an age of 3–4 weeks) as described by the International Mouse Phenotyping Consortium1 (Table 1) [48]. This lethal phenotype suggests essential PP1β-specific functions that cannot be compensated for by the remaining PP1 isoforms, likely because of the existence of PP1β selective PIPs. For example, MYPT1 has an ankyrin domain that specifically interacts with the C-terminal domain of PP1β [49]. However, biochemical data with PP1 chimera revealed that the central and N-terminal parts of PP1β also contribute to its isoform-specific functions [31, 49, 50].

1 International Mouse Phenotyping Consortium produces KO mice and phenotypes them according to fully validated and standardized protocols [48].
confirmed the existence of PP1β-specific functions. More specifically, the cardiac-specific deletion of PP1α and PP1γ by either embryonic or adult-heart specific Cre-mediated gene deletion revealed no major heart phenotype, while ablation of the PP1β isoform using the same conditional Cre/lox targeting approaches induced heart failure (Table 1) [42]. The heart-specific deletion of PP1β was associated with increased myofilament phosphorylation (myosin light chain 2V (MYL2) and cardiac myosin binding protein C (MYBPC3)). However, it did not cause changes in the phosphorylation of phospholamban (PLN), a key regulator of cardiac contractility, hinting at redundancy between PP1 isoforms in the dephosphorylation of PLN, but not of MYL2 and MYBPC3 [42]. Accordingly, the heart-specific overexpression of PP1α resulted in diminished phosphorylation of PLN at serine 16 [51]. Phenotypically, transgenic mice with overexpressed PP1α also showed reduced heart function, indicating that PP1α is a negative regulator of cardiac function (Table 1) [51]. This aligns with various studies indicating that increased PP1 activity is associated with human and experimental heart failure (reviewed in [52]). Increased PP1 activity due to increased PP1 levels and/or a decreased expression of PP1 inhibitory proteins is associated with less PLN phosphorylation at serine 16 and threonine 17 [52-55]. Collectively, the mouse models of PP1 isoforms revealed functional overlap but unique functions of PP1β in testis and of PP1β in multiple tissues, including the heart.

5. Mouse models of PP1 inhibitory PIPs

5.1. Diversity of iPIPs

Various iPIPs, including inhibitor 1 (I1) and its homolog dopamine and cyclic AMP-regulated phosphoprotein of Mr. 32,000 (DARPP32), Inhibitor 2 (I2), C-kinase potentiated protein phosphatase-1 inhibitor Mr. 17 kDa (CPI-17) and its homologs phosphoprotein holoenzyme inhibitor-1 (PHI-1), kinase C-enhanced PP1 inhibitor (KEPI) and gut and brain phosphatase inhibitor 1 (GBP1), contain a PP1-interacting domain.

Table 1

| Protein gene | Description of mouse model | Genotype of mouse model | Alterations in PP1/substrates | Phenotype [reference] |
|--------------|----------------------------|-------------------------|-------------------------------|----------------------|
| PP1α Ppp1ca  | KO                         | Ppp1ca<sup>−/−</sup>    | nd                            | No overt phenotype [A. Nairn, personal communication] |
| Heart-specific | Tg (Myh6-PPIα)            | PPI↑                   | Decreased heart function [51] |
| Tg PP1α     | Embryonic                 | Ppp1ca<sup>−/−</sup>/α | nd                            | Viable, normal lifespan [42] |
| heart-specific KO | Tg (Nkx2.5-Cre)       | PPI↑                   | Viable, normal lifespan [42] |
| heart-specific KO | Tg (Myb6-MerCreMer)     | PPI↑                   | Viable, normal lifespan [42] |
| PPIβ Ppp1cb | heart-specific KO         | Ppp1cb<sup>−/−</sup>/β | nd                            | Viable, no alteration detected in the analysed substrates [29, 40, 44] |
| Inducible    | Tg (Nkx2.5-Cre)           | PPI↑                   | Viable, no alteration detected in the analysed substrates [29, 40, 44] |
| heart-specific KO | Tg (Myb6-MerCreMer)    | PPI↑                   | Viable, no alteration detected in the analysed substrates [29, 40, 44] |
| PP1γ/γ 2 Ppp1cc | heart-specific KO       | Ppp1cc<sup>−/−</sup>  | nd                            | Viable, no alteration detected in the analysed substrates [29, 40, 44] |
| Inducible    | Tg (Nkx2.5-Cre)           | PPI↑                   | Viable, no alteration detected in the analysed substrates [29, 40, 44] |
| heart-specific KO | Tg (Myb6-MerCreMer)    | PPI↑                   | Viable, no alteration detected in the analysed substrates [29, 40, 44] |

See text for references.
Inhibitor M inhibitory without prior phosphorylation [36]. The iPIPs form stable I1, CPI-17 and their homologs, are converted into a potent PP1 inhibitor Ac, acetyl; CAMK2A (Table 2). Various trimeric PP1 complexes with PP1 alone but can also form stable trimeric complexes in II KO and I2:PP1 in II KO. Heart-specific Tg II [51, 63]. The genetically engineered mouse models of the selected I2:PP1/GADD34 [59], I2:PP1:AKAP18 [60], I1:PP1α:Spinophilin [61] and I2:PP1α/γ:Neurabin [62]. In contrast, CPI-17 and its homologs specifically inhibit MYPT1-2 containing PP1 complexes [58]. In addition to these well-known inhibitors, we also classified heat shock protein (HSP20) as an IPi based on its reported in vivo inhibitory function for PP1 [63]. The genetically engineered mouse models of the selected iPIPs disclosed their importance for heart and brain function and the response to viral infections (Table 2). This suggests that PP1 homoenzymes with an associated iPIP may serve as a therapeutic target for neurologic and psychiatric disorders, heart diseases and viral infections, as described below.

Table 2

| Inhibitor 1 | Ppp1r1a | Description of mouse model | Genotype of mouse models | Alterations in PP1/substrate | Phenotype [reference] |
|-------------|----------|-----------------------------|--------------------------|-----------------------------|----------------------|
| KO | Ppp1r1a KO | PPLN-pS16 ↓ | RVR2-pS2813 ↓ | PLN-pS16/717 ↑ | No obvious phenotype, some neurological and heart alterations [51, 64, 65]. Cardiac hypertrophy and mild cardiac dysfunction [64]. Enhanced cardiac function in long term and protection against pressure-overload-induced hypertrophy [66]. Improved cardiac contractility in young mice, but lethal after catecholaminergic stress and with aging [76]. Impaired heart function and increased arrhythmias [67]. Improved learning and enhanced memory, facilitated potentiation, impaired recovery from ischemia [77-79]. |
| Heart-specific Tg II | Tg (Myh6-II) | PP1 activity ↓ | PLN-pS16/717 ↑ | RVR2-pS2813 ↑ | |
| Heart-specific Tg II1-45, T35D (constitutive active PP1 inhibitor) | Tg (Myh6-II6, T35D) | PP1 activity ↓ | PLN-pS16/717 ↑ | RVR2-pS2813 ↑ | |
| Inducible heart-specific Tg II1-45, T35D (constitutive active PP1 inhibitor) | Tg (TetO-II1-45, T35D) | PP1 activity ↓ | PLN-pS16/717 ↑ | RVR2-pS2813 ↑ | |
| Heart-specific Tg II1-45, T35D (constitutive active PP1 inhibitor) in II KO | Tg (Myh6-tTA) | PP1 activity ↓ | PLN-pS16/717 ↑ | RVR2-pS2813 ↑ | |
| Heart-specific Tg II1-45, T35D (PP1 binding mutant) | Tg (Myh6-I1G109E) | PP1 activity ↓ | PLN-pS16/717 ↑ | RVR2-pS2813 ↑ | |
| Inducible brain-specific Tg II1-45, T35D (constitutive active PP1 inhibitor) | Tg (Myh6-tTA) | PP1 activity ↓ | PLN-pS16/717 ↑ | RVR2-pS2813 ↑ | |

Inhibitor 2 | Ppp1r2 | Description of mouse model | Genotype of mouse models | Alterations in PP1/substrate | Phenotype [reference] |
|-------------|----------|-----------------------------|--------------------------|-----------------------------|----------------------|
| KO | Ppp1r2 KO | Hsc: PP1 activity ↓ | He: CREB1-pS133 ↓ | GSK3β-pS9 ↓ | Decreased motor behavior, abolished dyskinetic behavior in response to Parkinson’s disease drug L-DOPA [85, 84]. |
| Heart-specific Tg I2-140 (constitutive active PP1 inhibitor) | Tg (Myh6-I2-140) | PP1 activity ↓ | PLN-pS16 ↓ | |
| Double heart-specific Tg I2-140 and Tg PP1α | Tg (Myh6-I2-140) | Normalized PP1 activity | Normalized heart morphology and heart function [69]. |
| CPI-17 | Ppp1r14a | KO | Ppp1r14a KO | “MYPT1-pT852 ↓” | Decreased main blood pressure [70]. |
| | Ppp1r14a KO | Normalized PP1 activity | | |
| CPI-17 | Ppp1r14a KO | Ppp1r14a KO | “MYPT1-pT852 ↓” | Decreased main blood pressure [70]. |
| | Ppp1r14a KO | Normalized PP1 activity | | |
| KEPI | Ppp1r14c | KO | Ppp1r14c KO | “MYPT1-pT852 ↓” | Decreased main blood pressure [70]. |
| | Ppp1r14c KO | “MYPT1-pT852 ↓” | Decreased response to morphine after chronic morphine injections, increased SARS coronavirus pathogenesis [88, 89]. |

Ac, acetyl; CAMK2A (Camk2a), Calcium/calmodulin-dependent protein kinase type II subunit alpha; CPI-17, protein kinase C-potentiated protein phosphatase-1 inhibitor M, 17 kDa; CREB1, cAMP responsive element-binding protein 1; DARPP32, dopamine and cyclic AMP-Regulated PhosphoProtein of Mr. 32,000; Ddr1, dopamine receptor D2; Drd2, dopamine receptor D2; ERK1/2 (Mapk3/1), extracellular signal-regulated kinase 1/2; GSK3β, gut and brain phosphatase inhibitor 1; H3, histone 3; He, heart; myocardial; Ho, salivary myosin heavy chain; nd, not determined; p, phospho; PLN, cardiac phospholamban; RP56, ribosomal protein S6; RVR2, ryanodine receptor 2; rtTA, reverse tetracycline-controlled transactivator; SARS, severe acute respiratory syndrome, TetO, tetracycline operator; Tg, transgene; TtA, tetracycline-controlled transactivator; L-DOPA, L,3,4-Dihydroxyphenylalanine. **Psychotomimetic drug-induced, ** phosphol ester-induced; #, L-DOPA-induced; ##, morphin-induced §, numbers of phospho-amino acids correspond to mouse sequence according to [https://www.phosphosite.org/].
5.2. Heart-specific functions of iPIPs

Various mouse models of iPIPs support the notion that inhibition of PP1 in the heart is an attractive therapeutic approach to treat heart failure and cardiac arrhythmia (Table 2). First, the global KO of I1 resulted in impaired heart function [51, 64, 65]. Second, cardiac-specific expression of a phosphomimetic I1 mutant (I11–65, T35D) that acts as a constitutively active PP1 inhibitor, was associated with decreased PP1 activity, increased phosphorylation of PLN at serine 16 and threonine 17 and enhanced heart function [66]. Additionally, I11–65, T35D expressing mice prevented cardiac dysfunction or hypertrophy under transverse aortic constriction conditions, suggesting that I11–65, T35D may protect against heart failure progression [66]. A heart-specific transgenic overexpression of wild-type I1 in mice resulted in a compensatory increased level of PP1 and, hence, was associated with mild heart dysfunction [64]. Third, the heart-specific expression of a transgene that encodes a human PP1 binding mutant of I1 (I1G109E) in mice also resulted in higher PP1 activity, impaired heart function and increased arrhythmia [67]. Fourth, heart-specific transgenic expression of a constitutively inhibitory fragment of I2 (I21–140, a C-terminal truncated form of I2) in mice showed enhanced cardiac contractility. This was associated with severely decreased PP1 activity and increased phosphorylation of PLN at serine 16, despite an increased level of PP1 [68]. Importantly, the heart-specific transgenic expression of PP1 in I11–140 expressing mice, rescued its phenotype and showed a normal PP1 activity, heart morphology and heart function [69]. Although transgenic expression of I11–65, T35D and I21–140 acted similarly in the heart based on reduced PLN phosphorylation, the global KO of I2, but not of I1, was embryonically lethal. This suggests that I1 and I2 have distinct functions in other tissues. Fifth, global KOs of CPI-17 or its homolog GBP1, were associated with decreased blood pressure and abnormal heart morphology, respectively (Table 2) [46, 70]. Decreased blood pressure was also observed in mice expressing only a non-phosphorylatable CPI-17 mutant (CPI-17T35A) that acts as a constitutively inactive PP1 inhibitor, confirming the importance of PP1:CPI-17 interaction for this phenotype. Sixth, heart-specific transgenic expression of HSP20 was cardioprotective and associated with increased phosphorylation of PLN at serine 16 and threonine 17 and enhanced sarcoplasmic reticulum calcium cycling [63, 71]. Elevated levels of HSP20 also improved cardiac function and angiogenesis in diabetic mice [72].

Together, these different mouse models suggest that PP1 inhibition holds great potential for the treatment of heart failure. However, this approach should be considered cautiously. First, a long-term inhibition of PP1 with I11–140 worsened heart failure under conditions of pressure overload (i.e. an experimental model for cardiac hypertrophy) [73, 74]. Second, ablation of I1 was cardioprotective under conditions of increased adrenergic drive [64, 75]. Third, the inducible heart-specific expression of I11–65, T35D in I1-deficient mice improved heart function in young mice but was deleterious after catecholaminergic stress and with increased age [76]. Thus, it is still uncertain whether I1 is beneficial or detrimental to the development of heart disease [74].

5.3. Brain-specific functions of iPIPs

Four iPIPs (I1, DARPP32, I2 and KEPI) are associated with neurological phenotypes (Table 2). Inducible expression of the constitutively active PP1 inhibitor I11–9, 54, T35D in the brain improved learning and memory but impaired recovery from an ischemic stroke [77–79]. Thus, PP1 constrains learning and memory formation but positively regulates recovery of brain damage [77, 78]. DARPP32, which is a homolog of I1 that is highly enriched in the dopaminergic systems in the striatum, is turned into a potent PP1 inhibitor upon phosphorylation at T34 e.g. by protein kinase A. DARPP32 plays a pivotal role in various neurological processes, including dopamine neurotransmission. Medium spiny neurons are classified as striatonigral and striatopallidal neurons, which are morphologically similar but differ in their expression of different subtypes of dopamine receptors. Striatonigral neurons primarily express dopamine receptors of type 1 (DRD1), while striatopallidal neurons primarily express dopamine receptors of type 2 (DRD2) [80]. Various neurological and psychiatric disorders such as drug addiction, schizophrenia and Parkinson’s disease are characterized by a disturbed balance between DRD1 and DRD2 signalling, which is often associated with altered DARPP32 function [80]. Global deletion of DARPP32 in mice led to alterations in dopaminergic neurotransmission and an impaired behavioral response to psychostimulants (e.g. cocaine), and antipsychotic drugs (e.g. haloperidol) [81]. Cell type-specific deletion of DARPP32 or expression of differentially-tagged DARPP32 in DRD1- or DRD2-expressing neurons of mice revealed neuron-specific pathways in the brain coupled to opposite functional outputs [80, 82–85]. Psychostimulants and antipsychotic drugs exert differential effects on DARPP32 phosphorylation in striatongiral and striatopallidal neurons, explaining their opposing behavioural and clinical effects [80, 82–85]. Mice that carried the T34A mutation in the endogenous DARPP32 gene, and thus express a constitutively inactive inhibitor of PP1, largely phenocopied DARPP32 KO both functionally and biochemically. For example, DARPP32T35A or mice with DARPP32 specifically ablated in striatongiral neurons featured phosphosubstrates with the same alterations (Table 2). This underscores the importance of the PP1:DARPP32 holoenzyme for the observed phenotypes [86].

While genetic deletion of both I1 alleies was embryonic lethal, I2 heterozygous mice exhibited no overt phenotype but showed increased memory formation, decreased PP1 activity and increased phospho-CREB1 [87]. Importantly, this model showed that, despite its presumed function as a PP1 inhibitor, I2 positively regulates PP1 in memory function in vivo [87]. KEPI is yet another iPIP linked to a neurological phenotype. KEPI inhibits PP1 after phosphorylation by protein kinase C. Global deletion of KEPI was associated with increased PP1 activity and decreased responsiveness to morphine after chronic morphine injections [88], suggesting that the selective inhibition of PP1 enhances morphine analgesia. Collectively, the neurological phenotypes associated with the different iPIPs revealed that PP1 constrains learning and memory formation and plays a key role in dopamine neurotransmission and morphine signaling. Therefore, targeting a particular PP1:iPIP holoenzyme can be a valuable therapeutic approach for the treatment of various neurological disorders. However, a cell type-selective method may be required to target, for example PP1:DARPP32 [80].

5.4. The role of iPIPs in viral infections

A global KO of KEPI disclosed the importance of iPIPs for the treatment of viral infections (Table 2) [89]. KEPI-null mice were more susceptible to the pathogenesis of severe acute respiratory syndrome coronavirus (SARS-CoV), suggesting that KEPI may protect against this virus [89]. Independently, some studies identified the importance of PP1 in other types of viral infections, including Human Immunodeficiency Virus (HIV) 1, hepatitis B and adenoassociated virus [90–92]. However, these findings have not yet been validated by mouse models and have been coupled to other PIPs such as trans-activator of transcription (TAT), hepatitis B regulatory protein x (HBx) and nuclear inhibitor of PP1 (NIPP1), respectively [90–92].

6. Mouse models of guiding PIPs

6.1. Diversity of gPIPs

gPIPs employ various strategies to guide PP1 to a subset of substrates (Fig. 1 and 2). Many gPIPs contain subcellular-targeting domains that bind to specific organelles or macromolecular complexes, such as glycogen particles, plasma membranes or the endoplasmic reticulum [16, 37]. For example, various glycogen-targeting G subunits (e.g. hepatic glycogen-targeting protein phosphatase 1 regulatory subunit (GL),
sphorylation by associated PP1 [94]. One PIP alone can combine phosphorylated at threonine-proline dipeptide motifs, for regulated dephosphorylation [93]. Other gPIPs have substrate-recruitment domains that bind to associated substrates such as glycogen synthase and glycogen phosphatase subunit (GM), protein targeting to glycogen (PTG) target PP1 toward skeletal muscle glycogen-targeting protein phosphatase 1 regulatory subunit. The Ppp1r3a, Ppp1r3b and Ppp1r3c genes encode GM, GL and PTG isoforms, respectively. GM is primarily expressed in muscle and GL in the liver, while PTG is ubiquitously expressed.

### Table 3

| Protein/gene | Description of mouse model | Genotype of mouse model | Alters in PP1/substrate | Phenotype [reference] |
|--------------|----------------------------|-------------------------|-------------------------|-----------------------|
| GM (Rca) Ppp1r3a | KO | Ppp1r3a<sup>−/−</sup> | PP1 level/activity; GS-pS641; GP-pS15↑ | Decreased glycogen content; prediabetic phenotype in 129/Ola derived background but no obvious phenotype in C57Bl/6, 129s2/sv and 129SvJ background [97-100] |
| GL Ppp1r3b | (SR binding mutant) | Tg (Alb-Cre); Tg<sup>−−</sup> | GP activity↑ | Decreased muscle glycogen content [102] |
| NIPP1 Ppp1r8 | Inducible brain-specific Tg | Tg<sup>−/−</sup> | Nuclear | Early embryonic lethality [107, 113] |
| Neurabin I Ppp1r9a | KO | Ppp1r9a<sup>−/−</sup> | PP1 levels↑ | Abnormal psychostimulant response and dopamine signaling transduction, reduced anxiety- and depression-related behaviors in middle-aged mice and associative learning homeostasis [145-147] |
| Spinophilin Ppp1r9b | KO | Ppp1r9b<sup>−/−</sup> | PP1 activity↑ | Embryonic lethality [150] |
| MYP1 Ppp1r12a | Smooth muscle-specific KO | Tg<sup>−/−</sup> | PP1β level↑ | Moderate alteration in bladder contractile responses [153] |
| MYPT2 Ppp1r12b | Cardiac-specific | Tg<sup>−/−</sup> | PP1β level↑ | Left ventricular heart enlargements with heart dysfunctions [154] |
| GADD34 Ppp1r15a | Early-embryonic specific KO | Ppp1r15a<sup>−/−</sup> | elF2α-pS52↑ | No overt phenotype, reduced hepatocarcinogenesis in chemical-induced tumorigenesis [122, 126] |
| CREP Ppp1r15b | KO | Ppp1r15b<sup>−/−</sup> | elF2α-pS52↑ | Hypersplenism, erythrocyte abnormalities, resembling mild thalassemia syndromes [125] |
| PHACTR4 Phactr4 | (PP1 binding mutant) | Tg<sup>−/−</sup> | elF2α-pS52↑ | Perinatal lethality, impaired erythropoiesis [123] |

For example, GM has separate glycogen and membrane-targeting domains (Fig. 2 [95]), while the stress-induced protein GADD34 binds to the endoplasmic reticulum and elF2α via its subcellular-targeting and substrate-recruitment domains, respectively [96]. Various gPIPs have a number of isoforms with often a different tissue expression pattern. For example, the mammalian genome harbors seven Ppp1r3a-g genes (Ppp1r3a-g), which each encode an isoform of the glycogen-targeting GM subunit. The Ppp1r3a, Ppp1r3b and Ppp1r3c genes encode GM, GL and PTG isoforms, respectively. GM is primarily expressed in muscle and GL in the liver, while PTG is ubiquitously expressed.

skeletal muscle glycogen-targeting protein phosphatase 1 regulatory subunit (GM), protein targeting to glycogen (PTG) target PP1 toward glycogen, thereby enhancing the dephosphorylation of glycogen-associated substrates such as glycogen synthase and glycogen phosphorylase [93]. Other gPIPs have substrate-recruitment domains that bind to specific subsets of substrates. For example, the forkhead-associated domain of NIPP1 binds a specific subset of proteins that are phosphorylated at threonine-proline dipeptide motifs, for regulated dephosphorylation by associated PP1 [94]. One PIP alone can combine multiple subcellular-targeting and/or substrate-recruitment domains.

{sphorylation by associated PP1 [94]. One PIP alone can combine phosphorylated at threonine-proline dipeptide motifs, for regulated dephosphorylation [93]. Other gPIPs have substrate-recruitment domains that bind to associated substrates such as glycogen synthase and glycogen phosphatase subunit (GM), protein targeting to glycogen (PTG) target PP1 toward skeletal muscle glycogen-targeting protein phosphatase 1 regulatory subunit. The Ppp1r3a, Ppp1r3b and Ppp1r3c genes encode GM, GL and PTG isoforms, respectively. GM is primarily expressed in muscle and GL in the liver, while PTG is ubiquitously expressed.
We identified 10 gPIP-encoding genes (Ppp1r3a/b, Ppp1r8, Ppp1r9a/b, Ppp1r12a/b, Ppp15a/b, Phactr4) that have been functionally studied using genetically modified mouse models and showed PP1-dependent phenotypes (Table 3). While all mouse models showed alteration in PP1 activity and/or altered phosphorylation levels of substrates, six gPPIs (GM, GL, GADD34, CReP, PHACTR4 and NIPP1) had at least one mouse model with the expression of a PP1 mutant that alters associated PP1 function. Phenotypical analyses of expression of these mutants in mice enable the identification of interaction sites with huge therapeutic potential. We selected these six gPPIs for further review.

6.2. PP1-dependent functions of glycogen-targeting subunits GM and GL

GM- and GL-deficient mice showed increased phosphorylation of their substrates glycogen synthase and glycogen phosphorylase, resulting in the inactivation of glycogen synthase but activation of glycogen phosphorylase and thus decreased glycogen content (Table 3) [97–100]. Furthermore, the striated-muscle specific transgenic expression of GM was associated with increased muscle glycogen content and the abolition of glycogen synthase activation in response to exercise (Table 3) [101]. A third GM mouse model expressed a C-terminally truncated form of GM (GM224) with intact glycogen-targeting and PP1-anchoring domains, but lacking its sarcoplasmic-targeting domain (Fig. 2). These mice demonstrated impaired glycogen synthesis associated with the inactivation of glycogen synthase (i.e. hyperphosphorylation), thus phenocopying the GM-null mice (Table 3) [102]. This result suggests that a disturbance of the binding of a gPIP to its subcellular structure can lead to a nonfunctional PP1 holoenzyme. Therefore, interference with the subcellular targeting of a gPIP is a potential therapeutical target for altering PP1 function (Fig. 1, Table 3). Interestingly, phenylalanine mutation of GL at tyrosine 284 prevented the allosteric inhibitory binding of phospho-glycogen-phosphorylase to GL, resulting in the dephosphorylation and activation of glycogen synthase [103, 104]. Thus, knockin mice that express mutant GL284F displayed an enhanced hepatic glycogen synthase activity and improved glucose tolerance, supporting the notion that pharmacological inhibition of this particular GL:phospho-glycogen-phosphorylase interaction can be used to combat diabetic hyperglycaemia [93, 103, 105, 106].

6.3. Cellular-context dependent functions of NIPP1

NIPP1, encoded by the Ppp1r18 gene, is a major nuclear PIP that is ubiquitously expressed, but its expression level is cell-type dependent [107, 108]. The N-terminal forkhead-associated domain of NIPP1 recruits PP1 substrates that are phosphorylated at threonine-proline dipeptide motifs, including the pre-mRNA splicing factor SAP155 and the methyltransferase EZH2 [38, 94, 109, 110]. NIPP1 binds PP1 via a central PP1-anchoring domain but lacking its sarcoplasmic-targeting domain (Fig. 2). These mice demonstrated impaired glycogen synthase associated with the inactivation of glycogen synthase (i.e. hyperphosphorylation), thus phenocopying the GM-null mice (Table 3) [102]. This result suggests that a disturbance of the binding of a gPIP to its subcellular structure can lead to a nonfunctional PP1 holoenzyme. Therefore, interference with the subcellular targeting of a gPIP is a potential therapeutical target for altering PP1 function (Fig. 1, Table 3). Interestingly, phenylalanine mutation of GL at tyrosine 284 prevented the allosteric inhibitory binding of phospho-glycogen-phosphorylase to GL, resulting in the dephosphorylation and activation of glycogen synthase [103, 104]. Thus, knockin mice that express mutant GL284F displayed an enhanced hepatic glycogen synthase activity and improved glucose tolerance, supporting the notion that pharmacological inhibition of this particular GL:phospho-glycogen-phosphorylase interaction can be used to combat diabetic hyperglycaemia [93, 103, 105, 106].

6.4. PP1-dependent functions of GADD34 and CReP

Ppp1r15a and Ppp1r15b genes encode for growth-arrest and DNA-damage-inducible transcript 34 (GADD34) and constitutive repressor of eIF2α phosphorylation (CReP), respectively. They both target the eukaryotic initiation factor (eIF) 2α for dephosphorylation of phospho-serine 52 by associated PP1. The phospho-serine 52 in mouse corresponds to the well-known phospho-serine 51 site of eIF2α. GADD34 and CReP share structural homology in their C-termini which harbors their PP1-binding domain [118]. The expression of GADD34 is mainly induced in stress conditions, while CReP is constitutively expressed and its levels are unchanged by stress [119–121]. Stress signaling in eukaryotic cells depends critically on the phosphorylation of eIF2α at serine 52, which occurs for example in response to the accumulation of misfolded proteins in the endoplasmic reticulum (also known as the unfolded protein response UPR), amino acid deprivation and viral infections [120, 121]. Upon stress, eIF2α gets hyperphosphorylated at serine 52, attenuates global protein synthesis and induces the expression of GADD34 (but not CReP) at the transcript and protein levels. GADD34 expression generates a feedback loop that reverses the stress-induced phosphorylation of eIF2α, thereby restoring normal protein synthesis. Thus, under basal conditions, the dephosphorylation of eIF2α-pS52 is tightly regulated by PP1-CReP, while PP1-GADD34 is responsible for the dephosphorylation of eIF2α-pS52 in stress condition.

Mice lacking either GADD34 or CReP exhibited enhanced basal and/or stress-induced eIF2α-pS52 signals, indicating that both isoforms are crucial for eIF2α dephosphorylation at serine 52 (Table 3) [122, 123]. Two other genetically engineered GADD34 mice models confirmed the increased stress-induced hyperphosphorylation of eIF2α at serine 52 (Table 3). First, a conditional KO model in which GADD34 was ablated at the early embryonic stages using the Cre/loxP system confirmed the altered phosphorylation of eIF2α. Second, mice that expressed a truncated form of GADD34 (GADD34–224) that was no longer able to form a functional phosphatase complex but still interacted with eIF2α, displayed the similar effect on eIF2α-pS52 [123–125]. The latter mouse model established the importance of the PP1:GADD34 interaction for eIF2α dephosphorylation [96, 123]. Phenotypically, mice lacking functional GADD34 exhibited mild phenotypes with no visible abnormalities but demonstrated enhanced obesity when fed a high-fat diet, impaired hepatocarcinogenesis in chemically-induced tumorigenesis models and erythrocyte defects that resembled mild thalassemia syndromes [122, 125, 126]. In contrast, the global deletion of CReP causes perinatal lethality associated with severe anemia [123]. Furthermore, mice expressing GADD34–143 and lacking CReP displayed early embryonic lethality, demonstrating that GADD34 and CReP have overlapping as well as distinct functions [123]. Together, this suggests functional redundancy between the two isoforms during embryonic development, but not throughout the neonatal stage. Importantly, early embryonic lethality could be rescued by alanine mutation of eIF2α at serine 52 (eIF2α224S), indicating that eIF2α is the...
only important substrate for PP1:CReP and PP1:GADD34 [123].

Some viruses encode proteins (e.g. ICP34.5) that are structurally related to the C-terminal domain of GADD34/CReP and also promote PP1-mediated dephosphorylation of eIF2α-pS52 [96, 118, 127]. This enables these viruses to reverse the translational block that is instigated when they enter the host cell [96, 127]. Finally, there is evidence at the biochemical level for the existence of trimeric complexes such as PP1:GADD34:II and PP1:GADD34:ACTIN, hinting at additional levels of regulation of eIF2α phosphatases [59, 128, 129]. To gain further insight into the physiological importance of II in eIF2α-pS52 dephosphorylation, it would be worthwhile to analyze the stress-induced eIF2α phosphorylation in II-null mice. From a therapeutic perspective, drugs that maintain eIF2α phosphorylation by inhibiting eIF2α phosphatases hold great promise for the treatment of protein misfolding disorders (e.g. neurodegenerative diseases) and viral infections [5].

6.5. PP1-dependent functions of PHACTR4

The family of phosphatase and actin regulators (PHACTR) comprises four structurally related members (PHACTR1-4) [130]. These isoforms exhibit different spatiotemporal expression patterns in the brain of mice, but contain each a highly conserved C-terminal PP1-anchoring domain that is preceded by an actin-targeting domain. Mice with a missense mutation in their Phactr4 gene, known as Phactr4humdy, express the PHACTR4R650P mutant, which binds actin, but not PP1 (Table 3) [131]. This indicates that the phenotype of Phactr4humdy is caused by deficient targeting of PP1. The Phactr4humdy mutant mouse embryos had defective neural tubes and optic fissure closures, resulting in exencephaly (fetal malformation with protruding brain tissues) and retinal coloboma (eye abnormalities). This fetal neural phenotype accords with the specific expression of PHACTR4 in the neural stem cells of developing and adult mouse brains. This phenotype was rescued by a loss of the transcription factor E2F1, indicating that PHACTR4 regulates PP1 during E2F1-regulated cell cycle progression in neurulation and eye development [131]. At the molecular level, disruption of the PP1:PHACTR4 interaction led to increased inhibitory phosphorylation of PP1 at threonine 320, resulting in retinoblastoma (Rb) hyperphosphorylation and the derepression of E2F targets [131]. The Phactr4humdy phenotype also seems to mimic Hirschsprung disease in humans, which is a congenital disorder of the distal colon characterized by the absence of the enteric nervous system [132, 133]. Indeed, the Phactr4humdy mutants showed intestinal hypoganglionosis (a reduced number of nerves in the intestinal wall) as well as defects in cell migration and lamellipodia of the enteric neural crest cells. This suggests that the PP1:PHACTR4 holoenzyme plays a role in the directional migration of enteric neural crest cells.

7. Mouse models with human disease-associated phenotypes

Lastly, we screened the > 170 mouse models that were connected to 3 PP1- and 104 PIP-encoding genes for their association with human diseases. First, we examined the PP1 and PIP-related mouse models that

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**Fig. 3.** Classification of PP1 and PIPs according their biological functions and their connection to at least one human disease-associated phenotypes. The figure shows PP1 isoforms and PIPs that are linked to specific pathologies reminiscent of several human diseases: cardiovascular systems to heart failure, heart arrhythmia and/or hypertension; behavioural/neurological to drug addiction, schizophrenia, and/or Parkinson’s disease, homeostatic/metabolic to type 2 diabetes and/or bile duct hyperplasia; reproductive to male infertility; haematological to thalassemia and respiratory system to SARS-CoV pathogenesis. See text for references. PP1, protein phosphatase 1; PIPs, PP1 interacting proteins; SARS-CoV, severe acute respiratory syndrome coronavirus.
are functionally linked to PP1 (3 PP1s, 7 iPIPs and 10 gPIPs; Tables 1–3) for their connection to human diseases using only the Mouse Genome Informatics database. We found 32 PIPs that were affiliated to human disease-associated phenotypes and connected to eight different disease areas: 2 in cardiovascular, 15 in behavioural/neurological, 3 in homeostasis/metabolism, 3 in reproductive, 1 in haematological, 2 in renal, 1 in the immune system, 5 in muscle and 5 in cancer predisposition disorders, as documented in Table S1. A few PIPs were associated with two human disease areas. Although these PIPs are biochemically proven PP1 interactors, their phenotypes are not yet connected to PP1 functions, indicating that we cannot exclude that the molecular basis of human disease phenotypes is PP1-independent.

None of the mouse models that are functionally linked to PP1 (Tables 1-3) displayed a spontaneous cancer phenotype, although various PP1:PIP complexes have been linked to cell cycle progression and cancer [17]. However, mouse models from five non-functionally validated PIPs (tumor suppressors APC, BRCA1 and Rb; protein kinase Aurora A and chromatin remodeling factor SNF5) are connected to cancer predisposition (Table S1). Various genetically engineered mouse models of these PIPs, showed increased susceptibility to develop tumors, hinting at an opportunity to target PP1:PIP complexes also for cancer therapy [134–140].

Since most of the PP1 binding sites of these 32 PIPs have been mapped [37, 141], their connection to PP1 can be explored by structural reverse genetics, i.e. a genetically engineered mouse model that expresses a PIP mutant that alters associated PP1 function but does not affect the interaction with other ligands [142]. A PP1-binding mutant can be generated through the deletion of the PP1-binding domain (e.g. GADD34/549), or the introduction of a point mutation in its PP1-binding domain (e.g. PHACTR4/550), while non-inhibitory (e.g. DARPP32/548, CPI17/588) or constitutively-inhibitory PP1 mutants (e.g. N1–65, T35D) can be generated by mutation of a critical residue in the PP1-inhibitory domain. Generation of these mutants in mice endorses the functional involvement of PP1 in that particular mouse’s phenotype (Tables 2 and 3). Importantly, other interactions within functional PP1 holoenzyme complexes such as the interaction of PIPs with subcellular structures can also be exploited as targets for therapeutically intervention as example by GM and GL, for instance, and have overlapping and distinct functions (e.g. GADD34 and CReP).

The examination of > 170 mice connected with 3 PP1- and 104 PIP-encoding genes revealed that the disease-associated phenotypes of 20 proteins were dependent on PP1 function (Tables 1-3 and Fig. 3). While the 32 others have not yet been functionally linked to PP1 (Table S1). We propose the use of structural reverse genetics, which combines the structural characterization of proteins with mouse genetics, as a powerful and attractive tool to establish whether human-disease-associated phenotypes are physiologically dependent on PP1 functions [142]. Various interaction sites, including PP1:PIP, PP1:substrate and PP1:subcellular interaction site, can be considered for therapeutic targeting (see colored circles in Fig. 1). We believe that structural reverse genetics provides exciting opportunities for new discoveries as an initial step to identify novel PP1-related therapeutic targets.

In conclusion, mice models have contributed enormously to understanding the in vivo functions of PP1 holoenzymes and have confirmed the pleiotropic role of PP1s in health and diseases, especially in the heart and brain. The discovery that PP1 itself and at least 49 PIPs proteins are associated with human disease-associated phenotypes suggests a huge potential for targeting PP1 holoenzymes and indicates that PP1:PIP-directed drugs hold great potential for treatment of human diseases.

Conflict of interest

The authors declare no conflict of interests.

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Transparency document

The Transparency document associated with this article can be found, in online version.

Appendix A. Supplementary data

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