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Effects of carboxyl-terminal methylation on holoenzyme function

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Abstract

Phosphoprotein Phosphatases (PPPs) are enzymes highly conserved from yeast and human and catalyze the majority of serine and threonine dephosphorylation in cells. To achieve substrate specificity and selectivity, PPPs form multimeric holoenzymes consisting of catalytic, structural/ scaffolding, and regulatory subunits. For the Protein Phosphatase 2A (PP2A)-subfamily of PPPs, the holoenzyme assembly is at least in part regulated by an unusual carboxyl-terminal methyl-esterification, commonly referred to as “methylation”. Carboxyl-terminal methylation is catalyzed by leucine carboxyl methyltransferase-1 (LCMT1) that utilizes S-adenosyl-methionine (SAM) as the methyl donor and removed by protein phosphatase methylesterase 1 (PME1). For PP2A, methylation dictates regulatory subunit selection and thereby downstream phosphorylation signaling. Intriguingly, there are four families of PP2A regulatory subunits, each exhibiting different levels of methylation sensitivity. Thus, changes in PP2A methylation stoichiometry alters the complement of PP2A holoenzymes in cells and creates distinct modes of kinase opposition. Importantly, selective inactivation of PP2A signaling through the deregulation of methylation is observed in several diseases, most prominently Alzheimer’s disease (AD). In this review, we focus on how methylation of the carboxyl-terminus of the PP2A subfamily (PP2A, PP4, and PP6) regulates holoenzyme function and thereby phosphorylation signaling, with an emphasis on AD.

Introduction

Reversible protein phosphorylation regulates most cellular processes. In mammalian cells, ~98% of phosphorylation occurs on serine and threonine residues ^[1,2]. There are more than 400 protein kinases encoded in the human genome that catalyze the phosphorylation of serine and threonine residues ^[3]. Conversely, the family of Phosphoprotein Phosphatases (PPPs) carry out the majority of the opposing dephosphorylation reactions ^[4,5]. The PPP family consists of PP1, PP2A, PP2B, PP4, PP5, PP6, and PP7, with 90% of all serine and threonine dephosphorylation being attributed to PP1 and PP2A ^[4,5]. Most PPPs achieve

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Declaration of Interests

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substrate specificity and selectivity through the formation of holoenzymes of catalytic and non-catalytic subunits ^[4,5] (Figure 1A).

PP2A, PP4, and PP6 holoenzymes

The PP2A subfamily of serine/threonine phosphatases includes PP2A, PP4, and PP6, whose catalytic subunits contain a phosphatase domain that is highly conserved with other members of the PPP family from yeast to human ^[6,7]. The PPP catalytic subunits form heterodimeric or heterotrimeric holoenzyme complexes with scaffolding and regulatory subunits ^[4,8]. Despite the high sequence similarities between the catalytic subunits, PP2A, PP4, and PP6 have their own unique sets of structural and regulatory subunits. The PP2A holoenzyme is formed by a structural scaffolding ‘A’ subunit (PPP2R1A), a catalytic ‘C’ subunit (PPP2CA), and a regulatory B subunit. The A and the C subunits both have two isoforms, α and β , and form a high affinity dimer (AC) that binds to a regulatory B subunit or other interacting proteins. There are 15 human genes encoding regulatory B subunits, which belong to four distinct families: B55 (B/PPP2R2/PR55), B56 (B'/PPP2R5/PR61), B72 (B''/PPP2R3/PR72), and the striatin family (B'''/STRN/PR93/PR110) ^[9,10] (Figure 1B). Alternative splicing of several regulatory subunit genes generates additional isoforms resulting in the formation of up to 100 different PP2A holoenzymes with defined substrate specificity, subcellular localization, and phosphatase activity. The PP4 catalytic subunit (PPP4C) forms both heterodimers and heterotrimers with five different regulatory subunits: PPP4R1, PPP4R2, PPP4R3A, PPP4R3B, and PPP4R4 ^[11] (Figure 1C), while the PP6 catalytic subunit (PPP6C) forms heterotrimeric complexes with one of three Sit4-associated protein subunits (SAPS1-3 or PPP6R1-3) and one of three Ankyrin repeat (ANKRD28/44/52) subunits ^[12,13] (Figure 1D).

Carboxyl-terminal methylation of the catalytic subunits of the PP2A subfamily

The carboxyl-terminal regions of PPP2CA, PPP4C, and PPP6C play an important role in facilitating the interactions with regulatory subunits. The last six amino acids of PPP2CA (TPDYFL-309) and the last three amino acids of PPP2CA, PPP4C, and PPP6C are identical from yeast to human and are post-translationally modified to regulate holoenzyme function (Figure 1E). Unusually, the α -carboxyl group of the carboxyl-terminal leucine (Leu 309 in human PPP2CA, Leu 307 in human PPP4C, and Leu 305 in human PPP6C) of the catalytic subunits undergoes methyl-esterification ^[14-17], hereafter referred to as “methylation” as is done by convention in the field. In mammalian cells, the majority of PPP2CA is methylated. For example, in NIH3T3 cells more than 90% of PPP2CA is methylated ^[18] while in transformed human embryonic kidney (HEK) cells ^[19] about 74% of PPP2CA is methylated ^[20].

For all members of the PP2A subfamily, reversible methylation is catalyzed by the enzyme Leucine carboxyl methyltransferase-1 (LCMT1), which utilizes S-adenosyl-methionine (SAM) as the methyl donor ^[14,15,17,21,22]. The modification is removed by protein phosphatase methylesterase 1 (PME1), at least in the case of PPP2CA and PPP4C ^[23-25] (Figure 2A). For PPP6C, the role of PME1 in removing the methyl group has not yet

been established. The mechanisms of methylation and hydrolysis are best understood for PP2A. The substrate for LCMT1 and PME1 is the PP2A AC heterodimer, not the B subunit-containing heterotrimer [23,26]. Substrate recognition by both enzymes requires structural features beyond the PPP2CA carboxyl-terminus as peptides based on the sequence of the PPP2CA carboxyl-terminus are not modified by either enzyme [15,23]. Additionally, a peptide corresponding to the last eight amino acids of PPP2CA failed to co-crystallize with LCMT1 or bind to the active site when LCMT1 crystals were soaked with it [27]. Indeed, further investigations revealed that interactions of LCMT1 and PME1 with the active site of the PPP2CA catalytic subunit are required to facilitate the reaction. Consequently, mutations that reduce PPP2CA activity (H59Q, H118Q, D85N, and R89A) also reduce carboxyl-terminal methylation of PPP2CA to levels ranging from 3% to 13% depending on the mutations [18]. Consistently, inhibition of phosphatase activity using PPP inhibitors such as okadaic acid inhibits the methylation and demethylation reactions [23,28,29]. Structural analysis of LCMT1 bound to S-adenosyl-homocysteine (SAH), a co-product of the methylation reaction, and PPP2CA revealed that LCMT1 makes contacts with the PPP2CA active site, and the PPP2CA carboxyl-terminus binds to the LCMT1 active site (Figure 2B) [27]. The interaction of LCMT1 with the PPP2CA active site suppresses PP2A activity and promotes a conformation of LCMT1 essential for the methylation reaction [27]. Thus, these observations explain why a peptide composed of the last eight amino acids of PPP2CA is not a substrate of LCMT1. Additional contacts between LCMT1 and PPP2CA are needed to activate LCMT1 and facilitate the methylation reaction. Although the name suggests otherwise, the carboxyl-terminal leucine is not the only amino acid that can be methylated [30]. While deletion of Leu309 abolishes methylation, substitutions of the leucine with alanine or valine are still methylated *in vitro* [30].

PME1 also binds to the PPP2CA active site, which results in a structural rearrangement of PME1 active site residues and its activation [31]. This rearrangement places the PME1 active site close to the PPP2CA carboxyl-terminus, allowing the latter to reach into the PME1 active site (Figure 2C). Interestingly, structural analysis of the PME1 and PPP2CA interaction revealed that *in vitro*, PME1 displaces the two catalytic bivalent cations from the PPP2CA active site, which are required for the catalysis of the dephosphorylation reaction [31]. Thus, over time the phosphatase activity of PPP2CA declines. This is not an immediate process upon binding of PPP2CA to PME1. In cells, the interaction of PME1 and PP2A did not affect PPP2CA activity [32], suggesting that the duration of the PPP2CA-PME1 interaction determines if only demethylation or also inactivation occurs. These observations that LCMT1 and PME1 both require the interaction with the phosphatase active site explain why LCMT1 and PME1 can modify all members of the PP2A subfamily. While PP2A, PP4, and PP6 have unique non-catalytic subunits and hence distinct quaternary structures, their active sites are highly conserved, providing the structural basis for their interactions with LCMT1 and PME1.

The regulatory function of carboxyl-terminal methylation

The role of the carboxyl-terminus of PPP2CA in PP2A holoenzyme formation and activity was first identified by a mutational analysis deleting the last eight amino acids [33], in which the Thr301stop mutant does not bind to B55 subunits. However, it binds to the polyomavirus

middle tumor antigen (MT), a viral protein that can interact with the AC dimer in place of B-type subunits. Single point mutations of L309Q^[34] or L309A^[35] result in the recovery of mostly AC dimers, indicating disruption of the ABC trimer by lack of methylation. Deletion of Leu 309 (L309⁻) abolished the interaction of PPP2CA with B55, but not B56, B72, and the binding of striatin and S/G2 nuclear antigen are increased^[18,30]. Furthermore, MT and other viral tumor antigens such as polyomavirus small (PyST) tumor antigen and the SV40 small tumor antigen (SVST) bound to PPP2CA L309⁻ as well as the catalytically inactive, unmethylated PPP2CA mutant D85N^[18,20]. Consistently, endogenous B55 isoforms have been shown to exclusively bind to methylated PPP2CA (Figure 2D)^[26,30]. However, B56 containing trimers have been reported to contain methylated as well as unmethylated catalytic subunit (Figure 2D)^[26,30]. For both B55 and B56 subunits, methylation increases the affinity of the AC dimer. While methylation per se does not reduce PP2A activity, the methylation-dependent recruitment of B subunits does^[26]. B72 subunits also bind both methylated and unmethylated PPP2CA (Figure 2D)^[30]. The depletion of LCMT1 reduces PPP2CA methylation over time and results in preferential incorporation of the remaining methylated PPP2CA into B55-containing trimers, as compared to B56- or B72-containing holoenzymes^[30]. Once methylation decreases below a threshold needed to incorporate available B55 subunits into trimeric holoenzymes, non-complexed B55 is degraded^[30]. The role of methylation in holoenzyme assembly is conserved to yeast, which express B55 and B56 regulatory subunits^[36,37]. This methylation-dependent selection of regulatory subunits, specifically of B55 and B56, is of major consequence to cellular signaling. PP2A-B55 and PP2A-B56 have unique and non-overlapping substrate preferences^[38]. While PP2A-B55 nearly exclusively dephosphorylates proline-directed serine and threonine phosphorylation sites, PP2A-B56 mostly dephosphorylates basophilic motifs, thereby generating unique modes of kinase opposition. In this way, changes in the relative composition of the repertoire of PP2A-B55 and -B56 holoenzymes differentially impacts cellular signaling pathways. For instance, a decrease in PP2A methylation will first affect PP2A-B55 holoenzymes, resulting in a stabilization and a prolonged half-life of cyclin-dependent kinase- and mitogen-activated protein kinase-dependent phosphorylation sites.

PPP4C and PPP6C are also highly methylated by LCMT1 in mouse embryonic fibroblasts^[17]. For PPP4C, loss of methylation reduces PPP4R1 binding, while PP6 holoenzyme components bind PPP6C methylation-independently^[17]. Taken together, these observations suggest that methylation is an essential regulatory mechanism in the assembly of PP2A holoenzymes, and to a certain degree of PP4 holoenzymes, that determines the cellular repertoire of PP2A and PP4 trimeric complexes.

Challenges in the detection of carboxyl-terminal methylation

PPP2CA methylation is most commonly detected using methyl-specific antibodies, and methylation stoichiometry is determined by hydrolysis of the carboxyl-terminal ester using base followed by detection using demethyl-specific antibodies. However, recently the specificity of several PP2A antibodies has come under scrutiny. Antibodies employed to detect and immunoprecipitate PPP2CA for activity assays have been shown to exhibit differential preferences for methylated and demethylated PPP2CA^[39]. Many antibodies against PPP2CA were raised against demethylated peptides from the carboxyl-terminus

of PPP2CA and preferentially recognize and precipitate demethylated PPP2CA. Thus, methylation-based biases in the detection of PPP2CA can significantly impact the interpretation of existing data. Because of the high occupancy of PPP2CA methylation and the methylation-dependent regulation of regulatory subunit binding, analyses using these antibodies provides an incomplete presentation of the PP2A holoenzyme. Furthermore, because of the high conservation of the carboxyl-terminus between PPP2CA, PPP4C, and PPP6C, some PP2A antibodies cross-react with PP4 and PP6 [39]. In addition, the carboxyl-terminus of PPP2CA (TPDYFL-309) can be modified by phosphorylation. For PPP2CA, phosphorylation has been reported to occur on threonine (Thr 304 in human PPP2CA α) and tyrosine (Tyr 307 in human PPP2CA α) and was found to impact PPP2CA recognition by demethyl-specific antibodies [30,39-41]. Interestingly, some monoclonal antibodies used to detect Tyr 307 phosphorylation do not discriminate between phosphorylated and unphosphorylated Tyr 307, but are sensitive to phosphorylation of Thr 304 or the methylation state of the carboxyl-terminus [42,43]. Thus, the selection of antibodies for the characterization of PPP2CA expression, its modification states, and immunoprecipitation of PP2A holoenzyme complexes must be carefully considered. Correspondingly, the existing literature on PP2A might require a reinterpretation of results, depending on the antibodies used in the respective study.

Regulation of PP2A carboxyl-terminal methylation

The regulation of LCMT1 and PME1 and thereby the methylation status of the PP2A subfamily is less well understood. Some reports suggest that the stoichiometry of PPP2CA methylation changes during the cell cycle and in a subcellular location-specific manner [44,45]. LCMT1 is mainly localized in the cytoplasm, while PME1 is enriched in the nucleus [46]. This distribution correlates with an enrichment of demethylated PPP2CA in the nucleus [46]. In mouse neuroblastoma (N2A) cells, LCMT1, methylated PPP2CA and PP2A-B55 are enriched in membrane rafts and cholesterol- and sphingolipid-enriched microdomains (CEMs), while demethylated PPP2CA and PME1 are present in non-raft membrane microdomains [47]. LCMT1 and PME1 themselves are further regulated by post-translational modifications. PME1 is phosphorylated by Ca²⁺/calmodulin-dependent kinase 1 (CaMK1) [48] and salt-inducible kinase 1 (SIK1) [49], both of which regulate the binding of PME1 to PP2A.

Intriguingly, it was recently shown that the availability of the LCMT1 co-substrate SAM is a major regulatory factor of the methylation reaction. SAM is the universal methyl-donor in cells for proteins, nucleic acids, lipids, and secondary metabolites. Upon methyl-transfer, SAM is converted to S-adenosylhomocysteine (SAH), which is recycled into SAM through the methionine cycle (Figure 3). SAH is cleaved in a reversible reaction by the enzyme SAH hydrolase into adenosine and homocysteine (Hcy). Hcy is remethylated to methionine by the betaine-homocysteine S-methyltransferase (BHMT) using betaine as a co-substrate (Figure 3) [50]. Alternatively, 5,10-methylene-tetrahydrofolate (5,10-methylene-THF) is converted to 5-methyl-THF by the methylene-tetrahydrofolate reductase (MTHFR), which functions as a co-substrate in the remethylation of Hcy by methionine synthase (MS) in a vitamin B12-dependent reaction (Figure 3) [50]. Finally, methionine is converted to SAM by the S-adenosylmethionine synthase (Figure 3) [50]. The availability of methionine

and thereby SAM dictates cellular methylation levels, including that of PPP2CA [51,52]. Indeed, the treatment of mammalian cells with SAH reduced PPP2CA methylation by inhibiting LCMT1 [53]. In yeast, transfer from nutrient-rich to minimal growth media can induce non-nitrogen starvation (NNS)-induced autophagy, which depends on methionine and SAM availability [51]. Methionine starvation activates NNS autophagy and results in the demethylation of PPP2CA, which increases the phosphorylation of proteins involved in NNS autophagy and other growth-related processes [51]. Furthermore, methionine starvation-induced PPP2CA demethylation and inactivation leads to increased phosphorylation and activation of histone H3K36 demethylases and histone demethylation [54]. Thus, PPP2CA acts as an amino acid sensor as its methylation status can be linked to amino acid availability.

The role of PP2A carboxyl-terminal methylation in Alzheimer's disease

The regulation of the methylation cycle and thereby PPP2CA methylation and PP2A holoenzyme formation have been linked to the neuropathological symptoms of Alzheimer's disease (AD). AD is pathologically characterized by amyloid-beta (A β) containing senile plaque deposition, accumulation of neurofibrillary tangles (NFTs), neuronal loss, and severe cognitive impairment [55]. Misfolded protein aggregates are a common feature in AD, including hyperphosphorylated Tau protein deposits in the intraneuronal NFTs [56-58]. Tau is a microtubule-associated protein (MAP) that is highly enriched in neurons and plays a crucial role in maintaining microtubule stability. It has been previously shown that Tau protein can be reversibly phosphorylated on over 40 serine/threonine residues by multiple protein kinases, including glycogen synthase kinase 3 beta (GSK3 β) and cyclin-dependent kinase 5 (CDK5), among others [59-61].

PP2A and PP1 phosphatases have been associated with Tau dephosphorylation, with PP2A-B55 α containing holoenzymes acting as the major Tau phosphatases [62]. Low levels of Tau phosphorylation contribute to microtubule assembly and stability. However, hyperphosphorylation of Tau leads to its dissociation from microtubules, aggregation, and formation of NFTs. Tau hyperphosphorylation in AD is often due to decreased PP2A-B55 α activity [55,63]. Specific inhibition of PP2A alone is sufficient to form hyperphosphorylated Tau aggregates *in vitro* and *in vivo* [64]. Inhibition of serine/threonine phosphatases with a selective PPP inhibitor okadaic acid induces Tau hyperphosphorylation and A β deposition, leading to neurodegeneration and cognitive impairment. Moreover, fibroblasts and affected brain regions from AD patients have been shown to have decreased PP2A activity with reduced protein expression of PPP2CA as well as B55 regulatory protein [63,65,66]. Deregulation of PP2A-B55 holoenzymes has been associated with enhanced Tau phosphorylation, microtubule stability and neurite outgrowth in neuroblastoma cells [57].

Reduced PPP2CA methylation has been associated with Tau hyperphosphorylation and correlates with the severity of phospho-Tau pathology in AD brains [67,68]. LCMT1 has been shown to be downregulated in AD neurons with neurofibrillary tangles [68]. Changes in the methionine cycle have been linked to AD through the regulation of PPP2CA methylation. Elevated Hcy plasma levels are a risk factor for AD and patients with high Hcy plasma levels show more rapid neural atrophy than those with lower levels of plasma Hcy [69].

Higher Hcy levels have also been shown to be a global inhibitor of cellular methylation through increased SAH production, affecting PP2A methylation and holoenzyme formation and in turn affecting Tau phosphorylation and microtubule binding ability [70]. As noted earlier, Hcy is converted to methionine by MTHFR using 5-methyl-THF as a co-substrate in a vitamin B12-dependent reaction [50]. Increased levels of folate and vitamin B12 can decrease Hcy plasma levels by increasing methionine and SAM synthesis. This increased PPP2CA methylation can lead to activation of PP2A-B55 preventing hyperphosphorylation of Tau [71]. Conversely, vitamin B12 and folate deficiency decrease methionine and SAM production and reduce cellular methylation through product inhibition by increased SAH levels. Thus, dietary intervention by supplementation of folic acid and vitamin B12 can decrease plasma Hcy levels, which may help reduce AD occurrence or slow its progression via modulating PP2A methylation. Furthermore, a functional polymorphism in MTHFR (*Mthfr* 677C→T) causes mild hyperhomocysteinemia due to reduced enzymatic activity and is associated with an increased risk of AD in Asian populations [72,73]. *Mthfr* deficient mice show reduced LCMT1 expression and PPP2CA methylation, and hyperphosphorylation of Tau in the hippocampus and cerebellum, and to a lesser degree in the cortex [58]. Thus, reactivation of PP2A-B55 through increased methylation of PPP2CA represents a therapeutic option in the treatment of AD. Besides dietary interventions using folic acid and vitamin B12, specific inhibitors of PME1 have been developed to inhibit methyl-hydrolysis. ABL127 [74] and AMZ30 [75] inhibit PME1 at nanomolar concentrations by covalently binding to an active site serine. However, only limited cellular effects have been observed upon PME1 inhibition [76], potentially due to the high stoichiometry of carboxyl-terminal methylation of PPP2CA or the lack of inhibition of the second mechanism of PPP2CA inactivation by displacement of the two catalytic bivalent cations from the active site.

Conclusion

The PP2A-subfamily of PPPs, specifically PP2A, are major cellular serine/threonine phosphatases whose substrate selection and specificity are determined by the regulatory subunits to which they bind. Thus, determining the mechanisms controlling holoenzyme assembly is essential for understanding phosphorylation signaling by PPPs. While phosphorylation of the carboxyl-terminus of the catalytic subunit can regulate B subunit binding, the modulation of the affinity of the PP2A AC dimer for different B-type regulatory subunits through methylation adds an orthogonal, phosphorylation-independent mode of regulation. Utilizing SAM as the methyl-donor, the methylation reaction connects phosphorylation signaling with the metabolic state of the cell, making PP2A a sensor of amino acid and energy availability. This is specifically important in the context of AD, where metabolic changes lead to hyperphosphorylated states due to changes in PP2A holoenzyme composition.

In most mammalian cells and tissues, the majority of PP2A is methylated. The high methylation stoichiometry suggests that rather than dynamically changing PPP holoenzyme composition, methylation establishes a steady-state repertoire of PPP holoenzymes that disconnects B subunit abundance from holoenzyme incorporation. While B55 subunits are degraded when not part of PP2A holoenzymes, other B subunits are stable, and only a portion of them is incorporated into PP2A holoenzymes. Thus, PP2A methylation might

serve as a rheostat, fine-tuning the affinity of AC dimers to ensure a sufficient supply of each B regulatory subunit-containing holoenzyme to balance kinase activities. Further investigations into how B regulatory subunits are incorporated into holoenzyme complexes are needed to elucidate this mechanism. PP2A-B55 and -B56 holoenzymes exhibit distinct substrate recruitment mechanisms and phosphorylation site preferences. Thus, understanding the mechanisms governing the cellular abundances of specific B-subunit containing holoenzymes is essential for elucidating kinase opposition in the regulation of phosphorylation signaling.

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Perspectives

- Importance of the field: The complex regulation of protein dephosphorylation and the specificity of the dephosphorylation reaction has only recently been appreciated. Carboxy-terminal methyl-esterification is a unique modification that connects the control of PP2A-dependent dephosphorylation with the metabolic state of cells.
- Summary of the current thinking: Carboxyl-terminal methylation modulates the affinity of the PP2A AC dimer for different B regulatory subunits and their incorporation into holoenzymes. The deregulation of methylation contributes to the pathogenesis of several diseases, most prominently Alzheimer's disease.
- Future directions: Outstanding questions concern the identification of the molecular mechanisms governing LCMT1 and PME1 regulation. Furthermore, extending our current knowledge on PP2A methylation to PP4 and PP6 is critical to understand its regulatory role fully.

Figure 1: Regulation of holoenzyme formation of PPP family of phosphatases.

A. Phospho-protein phosphatases (PPPs) can work as heterodimers with a catalytic and a regulatory subunit as in case of PP1 and PP2B, or as heterotrimers with a catalytic, a regulatory and a scaffolding subunit as in case of PP2A, PP4 and PP6. Some catalytic members of the PPP family can also work alone to dephosphorylate substrates as in case of PP5 and PP7. **B.** The heterotrimeric PP2A holoenzyme consists of a catalytic subunit (PPP2CA), a scaffolding subunit (PPP2R1A) and one member of the four regulatory protein families: B (B55/PPP2R2/PR55), B' (B56/PPP2R5/PR61), B'' (B72/PPP2R3/PR72), B''' (STRN/PR93/PR110). **C.** PP4 can form both heterotrimers and heterodimers. Trimeric PP4 holoenzyme consists of the catalytic subunit (PPPP4C) with a scaffolding subunit (PPP4R2) and one of the regulatory subunits PPP4R3A or PPP4R3B. PP4 dimeric holoenzyme contains PPP4C catalytic subunit with PPP4R4 regulatory subunit. **D.** PP6 phosphatase also forms heterotrimers with a catalytic subunit (PPPP6C), a scaffolding subunit (ANKRD28 or ANKRD44 or ANKRD52) and a regulatory subunit (SAPS1/PPP6R1

or SAPS2/PPP6R2 or SAPS3/PPP6R3). **E.** Multiple sequence alignment of C-terminal amino acids of PP2A subfamily catalytic subunits including yeast and human sequences with highly conserved residues highlighted. The alignment was generated using T-Coffee^[77] and formatted using Boxshade.

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Figure 2: C-terminal methylation of Leucine 309 in PP2A regulates holoenzyme composition.

A. Reversible methyl esterification of Leucine 309 in PP2A is catalyzed by the enzyme leucine carboxyl methyltransferase 1 (LCMT1) and removed by the enzyme protein phosphatase methylesterase 1 (PME1). **B.** Crystal structure of LCMT1 (purple) in complex with PPP2CA (yellow) (PDB ID: 3P71)^[27]. The C-terminal tail of PP2A (302RRTPDYFL₃₀₉), highlighted in red with side chains in gray, occupies the deep active site pocket of LCMT1 as shown in the inset. **C.** Crystal structure of catalytic PPP2CA (yellow)- scaffold PPP2R1A (gray) dimer with PME1 (blue) (PDB ID: 3C5W)^[31]. The C-terminal tail of PP2A (304TPDYFL₃₀₉), highlighted in red with side chains in gray, is placed in the active site pocket of PME1 as shown in the inset. The sequence from

P293-T304 did not show any electron density in these crystals and are not shown in the structure. **D.** Methylation of Leucine 309 regulates the formation of PP2A holoenzymes. The binding of all members of B55 family of regulatory proteins to PP2A core enzyme is dependent on the methylation of PP2A. B56, B72 and Striatin families of regulatory proteins can form holoenzyme complexes both with methylated and unmethylated forms of the catalytic subunit.

Figure 3: Metabolic control of PP2A C-terminal methylation.

LCMT1 utilizes SAM (S-adenosyl-methionine) as a methyl donor to methylate the Leucine 309 in PP2A A-C dimer which allows for the regulatory B55 subunit family members to bind PP2A forming the active holoenzyme complex. This PPP2CA-PPP2R1A-B55 trimer can dephosphorylate Tau, a microtubule associate protein, hyperphosphorylation of which can lead to microtubule instability and formation of neurofibrillary tangles in AD. After the methyl transfer, SAM is converted to SAH (S-adenosylhomocysteine), which is hydrolyzed by the enzyme SAHH (SAH hydrolase) to form Homocysteine (Hcy). Hcy is converted into methionine by the enzyme BHMT (betaine-homocysteine S-methyltransferase) using betaine as a co-substrate. Alternatively, Hcy can also be remethylated to methionine by MS (methionine synthase) using vitamin B12 as a cofactor. This reaction also uses 5-methyl-THF as a co-substrate, which is produced from 5,10-methylene-THF by the enzyme MTHFR (methylene-tetrahydrofolate reductase). Methionine is then converted into SAM through adenylation by MAT (Methionine adenosyl transferase).