HHS Public Access

Author manuscript

Drug Discov Today. Author manuscript; available in PMC 2021 September 01.

Published in final edited form as:

Drug Discov Today. 2020 September ; 25(9): 1735–1743. doi:10.1016/j.drudis.2020.06.031.

Modulating the modulators: regulation of protein arginine methyltransferases by post-translational modifications

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Abstract

The therapeutic potential of targeting protein arginine methyltransferases (PRMTs) is inextricably linked to their key roles in various cellular functions, including splicing, proliferation, cell cycle regulation, differentiation, and DNA damage signaling. Unsurprisingly, the development of inhibitors against these enzymes has become a rapidly expanding research area. However, effective targeting of PRMTs requires a deeper understanding of the mechanistic details behind their regulation at multiple levels, involving those mechanisms that alter their activity, interactions, and localization. Recently, post-translational modifications (PTMs) of PRMTs have emerged as another crucial aspect of this regulation. Here, we review the regulatory role of PTMs in the activity and function of PRMTs, with emphasis on the contribution of these PTMs to pathological states, such as cancer.

Keywords

arginine methylation; automethylation; phosphorylation; post-translational modifications; PRMTs

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Teaser: The complex regulation of protein arginine methyltransferases (PRMTs) remains incompletely understood. A growing number of reports suggest that the activity, stability, and protein–protein interactions of PRMTs can be dynamically altered by post-translational modifications, creating new opportunities for drug discovery in cancer treatment and beyond.

Overview of protein arginine methyltransferases

PTMs have evolved as focal points of signal transduction [1]. They are crucial to initiating and augmenting signaling cascades by fine-tuning the function of the molecules they alter [2]. Moreover, these PTM events underlie, in part, the transcriptional and epigenetic-driven changes in gene expression at the heart of various normal cellular requirements [3]. Unsurprisingly, in pathological states, the enzymes that catalyze the addition and removal of PTMs are frequently dysregulated and have emerged as major drug targets for a variety of diseases, including cancer [4,5].

Arginine methylation continues to garner intense interest as a key PTM, largely because of its role as a regulator of both histone and nonhistone proteins involved in diverse cellular processes [6,7]. These range from splicing, RNA processing, transcription, development, and DNA damage repair, to proliferation and signal transduction, among others [8,9] (Table 1). The result of arginine methylation is the addition of methyl groups to the guanidino nitrogen atoms of the arginine residue of proteins, the complexity of which is further augmented by the addition of either one or two methyl groups [10]. Specifically, there are three main types of arginine methyl mark: ω-N^G, monomethylarginines (MMA); ω-N^G,N^G-asymmetric dimethylarginines (ADMA); and ω-N^G,N'^G-symmetric dimethylarginines (SDMA). These marks are deposited by the family of PRMTs that comprise nine members, classified based on which mark they deposit [8]. For instance, type I, II, and III PRMTs all catalyze the formation of MMA intermediates, whereas type I PRMTs [PRMT1, 2, 3, 4 (also known as Coactivator Associated Arginine Methyltransferase 1, CARM1), 6, and 8] further catalyze the production of ADMA. Meanwhile, type II PRMTs (PRMT5 and PRMT9) catalyze the formation of SDMA, whereas PRMT7 was described as exhibiting type III enzymatic activity because of its preferential formation of MMA on histones, its only known substrate (Figure 1) [10]. More recently, a type IV enzyme, which remains to be fully characterized, was identified in yeast as monomethylating the internal (or δ) guanidino nitrogen atom [11].

Role of arginine methylation

Although MMA, ADMA, and SDMA all potentially have different functional consequences, the overall main effect of arginine methylation is alteration of protein–protein, protein–DNA, and protein–RNA interactions of the methylated protein [12]. This is evidenced in the structural effects conferred by this PTM. For example, each arginine residue of a protein has five potential hydrogen-bond donors, which interact with the hydrogen-bond acceptors of its interacting partners [13]. Therefore, the addition of a methyl group will sterically affect these interactions by providing increased hydrophobicity without changing the overall charge of the residue [14]. Ultimately, this results in either positive or negative effects on the interaction of the methylated proteins with other molecules [14].

Most PRMTs share similar substrates, which are inclusive of both histone and nonhistone proteins. Other extensive reviews have highlighted the increasing list of PRMT substrates and, thus, we do not cover this topic at length here [6,8]. Briefly, most PRMT substrates harbor conserved glycine (G)- and R-rich (GAR) motifs. A notable exception is PRMT4 (CARM1), which methylates arginine residues within proline and glycine-rich motifs

(PGM). Others, such as PRMT5, can methylate arginine residues located within both GAR and PGM motifs [6,8].

Interestingly, it also not uncommon for type I and II PRMTs to share the same substrates with differential outcomes. For example, a recent study demonstrated that, PRMT5-mediated symmetric methylation of p65 at R30 (R30me2) positively regulated the DNA-binding ability of NF-κB to drive transcriptional activation of NF-κB target gene expression [15]. Moreover, predicted modeling revealed a stabilizing effect of R30me2 via van der Waals contacts that indirectly increased the affinity of p65 for DNA [15]. Conversely, Reintjes *et al.* reported a mechanism by which TNFα induces asymmetric dimethylation of RelA by PRMT1 (R30me2a), resulting in impaired binding of NF-κB to gene promoters [16].

In summary, this suggests a complex and possibly context-specific structural effect of ADMA versus SDMA modifications. Arginine methylation has also been shown to affect other crucial aspects of a protein, including its stability [17], subcellular localization [18], and enzymatic activity [19]. For instance, CARM1-mediated methylation of steroid receptor coactivator-3 (SRC-3) was shown to enhance its turnover, whereas methylation of RNA helicase A was required for its nuclear import [20]. Undoubtedly, these findings reveal only a fraction of the roles of arginine methylation in the regulation of protein function. It is possible that more roles will be uncovered in the near future, particularly because of the involvement of this PTM in the pathogenesis of disease, such as cancer [21].

Regulation of arginine methylation

Similar to other types of PTM, arginine methylation of proteins is under the control of several different regulatory mechanisms. Theoretically, arginine methylation is reversible and, to date, only a few enzymes have been identified as putative arginine methylation demethylases [22]. In 2007, Jumonji domain-containing 6 protein (JMJD6) was identified as the first JmjC-containing iron- and 2-oxoglutarate-dependent dioxygenase to demethylate histone H3 at arginine 2 (H3R2) and histone H4 at arginine 3 (H4R3) [22,23]. Another pertinent enzyme is protein arginine deiminase 4 (PADI4), which converts arginine in proteins to citrulline by deamination [24]. Given that deamination effectively blocks further methylation of arginine residues, it remains to be elucidated whether citrulline can be converted back to arginine by an aminotransferase, or whether citrullinated proteins are replaced by their resynthesized counterparts. Finally, regulation of arginine methylation is also frequently accomplished via regulating the PRMTs themselves. These regulatory mechanisms include fine-tuning PTMs [25], alteration of the interactions with cofactor proteins [26], modulation of subcellular compartmentalization [27], and miRNA-mediated targeted degradation [8,28]. Here, we provide the first extensive review of the role of PTMs in regulating PRMTs. Other regulatory mechanisms are covered by other reports and are not discussed in further detail here [6,8].

PTMs of PRMTs

PRMT1

PRMT1 is the main methyltransferase catalyzing the ADMA of proteins and acts as a transcriptional co-activator via methylation of H4R3 (H4R3me2a) [29,30]. Additionally, PRMT1 displays wide substrate specificity by methylating nonhistone proteins, such as RNA-binding and DNA damage repair proteins [31]. As a transcriptional co-activator, it is no surprise that deregulation of the expression levels of PRMT1 is frequently observed in diseases, such as cancer [32]. For instance, PRMT1 is highly overexpressed in cancers of the prostate and bladder, and has a requisite role in cell proliferation [33]. The regulation of PRMT1 is complex and includes mechanisms such as alternative spicing into seven isoforms, miRNAs, oxidation, and PTMs [28,34]. Here, we focus on the effects of identified PTMs on regulating PRMT1 activity, primarily phosphorylation (Table 2).

Using mass spectrometry, Rust *et al.* identified Y291 of PRMT1 as being phosphorylated. Moreover, mutagenesis studies in K562 myelogenous leukemia cells revealed that Y291 phosphorylation altered the substrate specificity of PRMT1 by inhibiting its interaction with the RNA-binding proteins, heterogeneous nuclear ribonucleoprotein A1 and 3 (hnRNPA1 and hnRNPH3) [35]. Another study demonstrated an important role of phosphorylation of PRMT1 in the maintenance of cell stemness. Not only were higher PRMT1 levels observed in epidermal progenitor cells, but, this expression was also significantly lower in differentiated cells, indicating a role for PRMT1 in suppressing differentiation. Interestingly, the authors also demonstrated a concerted role for PRMT1 and Casein kinase 1 alpha 1 (CSNK1a1) in maintaining progenitor self-renewal through CSNK1a1-mediated phosphorylation of PRMT1 [36]. Specifically, CSNK1a1 catalyzed the phosphorylation of PRMT1 on S/T residues within the amino acid regions 55–57, 102–105, and 284–289. Alanine substitution of S/T residues within these regions impaired the self-renewing function of PRMT1 and its ability to suppress premature cell differentiation [36]. Collectively, these data underscore the crucial role of phosphorylation in the regulation of PRMT1 at multiple levels, including substrate specificity in leukemia cells, as well as its epigenetic functions related to controlling genes involved in sustaining tissue self-renewal. Finally, PRMT1 was identified as a substrate of the ubiquitin E3 ligase E4B and was shown to undergo proteasomal degradation as a result of polyubiquitylation [37]. However, the physiological relevance of this finding is still unknown, highlighting the ongoing need to not only identify, but also experimentally validate PTMs apart from phosphorylation.

PRMT3

Similar to PRMT1, PRMT3 is classified as a type I PRMT ancl catalyzes the formation of mono- and asymmetric dimethylation marks on arginine residues [6]. Interestingly, PRMT3 was initially identified in 1998 as a PRMT1-interacting protein from a yeast two-hybrid screen and shares 67% sequence similarity with PRMT1 within its methyltransferase domain [38]. In contrast to other PRMTs, PRMT3 is structurally distinct in that it contains a well-defined zinc-finger domain in its N terminus, which has been implicated in directing substrate specificity [39]. To date, few substrates of PRMT3 have been identified. These range from RNA-binding proteins to sodium channels. For example, PRMT3-mediated

methylation of its earliest known substrate, ribosomal protein S2 (RPS2), has been linked to the maintenance of ribosomal biosynthesis. Cells lacking PRMT3 accumulate free 60S ribosomal subunits, resulting in an imbalance in the 40S:60S ratio [40]. Other validated substrates of PRMT3 include hnRNPA1 and NaV1.5. Hsu *et al.* showed that PRMT3-mediated methylation of hnRNPA1 promoted the interaction of hnRNPA1 with ATP binding cassette subfamily G Member 2 (ABCG2) to enhance gemcitabine drug resistance in pancreatic cancer cells [41]. In HEK293 cells, coordinated methylation of NaV1.5 by PRMT3 and 5 was shown to increase NaV1.5 current density by enhancing NaV1.5 cell surface expression [42].

Although the activity of PRMT3 has been shown to be regulated by interacting proteins, such as the tumor suppressor DAL-1/4.1B [43], evidence for the post-translational regulation of PRMT3 is still lacking. Of the many possible phosphorylation sites, most are merely predicted or only identified by mass spectrometry. Furthermore, none of these reported sites have been shown to confer functional consequences. Tyr87 was found to be a highly conserved phosphorylatable residue but concrete evidence of its phosphorylation has not yet been demonstrated (Table 2). Substitution of Tyr87 with a residue mimicking phosphorylation (Y87G) led to markedly decreased affinity of PRMT3 towards RPS2, thus impairing its enzymatic activity [44]. However, this might be attributed to the impact of glutamate on local surface properties given that the nonphosphorylated mutant (Y87F) remained unaffected compared with wild type. Interestingly, using mass spectrometry, the same study identified S25 and 27 (Table 2) as being phosphorylated, but further exploration is needed to elucidate the regulatory role of these phosphoserines [44].

CARM1/PRMT4

As the most prominent type I arginine methyltransferase, CARM1/PRMT4 catalyzes methylation of H3R17 and H3R26 [10]. Importantly, PRMT4 also acts as a transcriptional coactivator, and is well known for its role in recruiting nuclear receptors and transcription factors [45,46]. Furthermore, through its interacting partners, PRMT4 cooperates with a range of nuclear receptors, such as p160, to promote transcriptional activation via the recruitment of other epigenetic enzymes, such as p300/ CREB-binding protein (CBP) [47].

Apart from its role in transcription, PRMT4 is involved in a diverse array of cellular and biological functions, ranging from splicing, T cell development, adipocyte and muscle cell differentiation, to the maintenance of embryonic stem cell pluripotency [10]. Considering these complex roles, the activity of PRMT4 is tightly regulated at many levels. Its expression is regulated by various miRNAs, including miR181c, miR-223, and miR-15 [28]. Additionally, several types of PTM have important yet differential regulatory roles. For instance, the coactivator function of PRMT4 was shown to be regulated positively by automethylation on R551 (Table 2) [48,49]. R-to-K substitution at this site impaired PRMT4-medatied transcriptional activation, as well as pre-mRNA splicing processes, while leaving its enzymatic activity intact. Conversely, phosphorylation at S217 (Table 2) promoted CARM1 cytoplasmic localization and abolished PRMT4 S-adenosyl methionine (SAM)-binding ability, thus inhibiting its methyltransferase activity during mitosis without impairing its dimerization or coactivator function [50]. A separate study also demonstrated

an inhibitory effect of phosphorylation of residue S229 (Table 2); by blocking SAM binding, modification of this site impaired PRMT4 enzymatic activity [51]. However, unlike S217 phosphorylation, S229 significantly abrogated PRMT4 dimerization and, ultimately, transactivation of estrogen receptor-dependent transcription. Intriguingly, a different modification, glycosylation by *O*-linked-b-*N*-acetylglucosaminyltransferase (OGT) (Table 2) was shown to determine PRMT4 substrate specificity by preventing its phosphorylation and altering its subcellular localization during mitosis [52].

Although the exact biological function of these sites is still unclear, these data collectively provide strong support for the role of phosphorylation and rarer PTMs, such as glycosylation, in regulating various properties of PRMT4. Furthermore, we provide evidence for the differential outcomes of the same type of PTM on different sites and demonstrate how crosstalk between diverse PTMs can concertedly fine-tune the localization and activity of PRMT4. However, future studies are needed to identify the enzymes mediating these modifications, which could serve as promising targets for modulating PRMT4 in pathogenic states.

PRMT5

PRMT5 is the main type II methyltransferase and is responsible for 95% of the symmetric dimethylation observed in cells [26,53-57]. In addition to histones, PRMT5 methylates a variety of nonhistone substrates, such as Sm proteins, transcription factors (e.g., p65 and p53) and signaling effector proteins (e.g., RAF) [41]. Importantly, these methylation events impact several biological processes, including transcriptional control, proliferation, DNA repair, splicing, signal transduction, differentiation, and development, among others [24].

PRMT5 can be regulated at multiple levels and, as part of a multimeric complex, it associates with a range of interacting partners that regulate its activity and/or substrate specificity. For example, the most prominent cofactor, methylosome protein 50 (MEP50), forms a hetero-octameric complex with PRMT5 *in* vitro, which serves to define its substrate specificity and distributive catalytic mode of action [58]. However, whether MEP50 is obligatory for PRMT5 function *in vivo* remains uncertain. Nevertheless, the MEP50–PRMT5 interaction along with various mutually exclusive partners, including plCln and the kinase RioK1, appear to be primarily involved in contextually modulating substrate recognition [59]. A second level of regulation occurs post transcriptionally, wherein research shows that PRMT5 is subject to regulation by miRNAs [28].

Similar to other methyltransferases, the activity of PRMT5 is also modified by PTMs, such as phosphorylation, methylation, and ubiquitination (Table 2 and Figure 2). Tyrosine (Y) phosphorylation constitutes some of the earliest evidence of post-translational regulation of PRMT5. Phosphorylation of Y304 and Y307 by oncogenic JAK2V617F mutant (Table 2 andFigure 2) downregulates the methyltransferase activity of PRMT5 via disruption of the PRMT5–MEP50 interaction to promote myeloproliferation [60]. Structural predictions suggest that phosphorylation at these two tyrosine sites disrupts the substrate pocket because of their occurrence on a short linker between the TIM barrel and catalytic domain of neighboring PRMT5 monomers [61].

In addition to tyrosine phosphorylation, S/T modification of PRMT5 has also been shown to regulate other aspects of its functions. For instance, T634 phosphorylation (Table 2 and Figure 2) has been linked to a unique function of targeting PRMT5 to the plasma membrane via modulation of a PRMT5 interaction switch involving PDZ and 14-3-3 [62]. Recently, a novel regulatory role was revealed for serine phosphorylation of PRMT5 within the TIM-Barrel of PRMT5. Specifically, it was shown that IL-1β-inducible phosphorylation of S15 regulates its methyltransferase activity and interaction with the p65 subunit of NF-κB [63]. Moreover, compared with wild type, the S15A mutant form of PRMT5 downregulated a subset of NF-κB target genes and attenuated the growth, migratory, and colony-forming abilities of colorectal cancer cells. Another recent reported by Lattouf *et al.* also identified LKB1-mediated phosphorylation of T132, 139, and 144 residues (Table 2 and Figure 2) within the TIM-Barrel domain of PRMT5 in breast cancer cells [64]. Phosphorylation of these sites resulted in dissociation of the interaction with regulatory cofactors MEP50, pICln, and RiOK1, thus maintaining low PRMT5 activity [64]. Overall, these studies highlight a potentially new regulatory mechanism involving phosphorylation of residues in the unique TIM-Barrel of PRMT5, with implications for drug design that targets the N-terminal region of PRMT5.

Nie *et al.* showed another type of PTM that enhanced PRMT5 activity. CARM1 was shown to directly interact with, and asymmetrically dimethylate, PRMT5 on R505 (Table 2 and Figure 2), to positively regulate PRMT5 oligomerization and, thus, enhance its methyltransferase activity [19]. Besides phosphorylation and methylation, polyubiquitination of PRMT5 by the E3 ligase CHIP was found to be essential for the negative regulation of PRMT5 expression via K48-linked ubiquitin-dependent proteasomal degradation (Table 2 and Figure 2) [65]. Taken together, these findings support a complex model of the regulation of PRMT5 by PTMs that define methyltransferase activity, co-factor binding, and protein–protein interactions (PPIs). Indeed, further understanding of the extracellular clues that underlie these regulatory PTMs could serve as the basis for designing novel therapeutic approaches for PRMT5-driven diseases, including cancer.

PRMT6

PRMT6 catalyzes both monomethylarginine and ADMA marks and has been implicated in roles in cell fate determination during early embryonic development [6]. Similar to other PRMTs, PRMT6 methylates both histone and nonhistone proteins. Methylation of H3R2 by PRMT6 was identified as an important transcriptional repressive mark that competes with lysine trimethylation of K4 of the histone H3 209 tail [66]. Importantly, PRMT6-mediated repression of genes such as *TP53* is linked to a blockade of cellular senescence [67]. Additionally, PRMT6 methylates nonhistone proteins, such as DNA polymerase beta (POLB), CRTC2, and HIV-1 proteins Tat, Rev, and Nucleocapsid protein p7 (NC), thus linking it to DNA base excision during DNA repair, gluconeogenic programs, and innate immunity, respectively [68].

Similar to reports for PRMT4 and 8, Singhroy *et al.* showed that PRMT6 also undergoes automethylation (Table 2). Specifically, this mark, which occurs on R35, was shown to have a requisite role in maintaining its stability and anti-HIV-1 activity [69]. Interestingly, this

automethylation site did not affect the ability of PRMT6 to methylate its substrate HIV-1 Rev. Overall, knowledge regarding the regulation of PRMT6 by PTMs or other mechanisms has been limited, warranting further efforts to uncover these mechanisms under both physiological and pathological conditions.

PRMT8

PRMT8 is a cell type-restricted PRMT found mainly within spinal cord motoneurons (MNs) [70]. In contrast to the other arginine methyltransferase family members, this PRMT is dynamically regulated through its N-terminal domain [71]. Deletion studies of the first 60 residues in the N-terminal domain of PRMT8 significantly enhanced its enzymatic activity compared with the full-length protein. Furthermore, automethylation at two sites (Table 2) within the N-terminal domain negatively regulates activity by decreasing affinity for AdoMet, suggesting that methylation functions as an autoregulatory switch for controlling PRMT8 enzymatic activity [72]. Importantly, it is possible that proteolysis or interaction with one or more binding partners masks these automethylation sites to alter PRMT8 activity, but further studies are needed to shed light on this point.

Concluding remarks and perspectives

The development of PRMTs as novel drug targets is fundamentally based on a deep mechanistic understanding of their regulation in various cellular and pathological contexts [73,74]. In this review, we have summarized advances made regarding the regulatory role of PTMs on PRMT activity, function, stability, and PPIs. Although the effect of several PTMs, including phosphorylation, ubiquitination, and methylation, have been demonstrated in detail, whether other types of PTM, such as acetylation and sumoylation, also exist on any of the PRMTs is unknown and requires further investigation. Another interesting possibility is whether evolutionary conservation of PTM residues across different PRMT family members is among the PRMTs discussed in this review. Interestingly, as expected, alignment of the PRMT sequences showed most similarity in the highly conserved catalytic domain (Figure 3). However, although there are many highly conserved putative phosphorylatable residues, most have not yet been experimentally confirmed as modified sites. Further studies are needed to probe this idea, which could help to inform the design of novel inhibitors against this important domain based on the pre-existence of PTMs in this region.

Furthermore, to date, preclinical studies geared towards targeting PTMs on any of the PRMTs for therapeutics have not yet been developed. Arguably, the potential of targeting PTMs is inherently complex. Theoretically, one could simply target upstream enzymes that catalyze these modifications as one approach. However, as shown in Table 2, the identity of these upstream modifiers might not always be known. Selective drug design often relies on a better understanding of the structural changes that PTMs impart, such as those exposing or masking active sites or binding interfaces necessary for PPIs. Given the dynamic and labile nature of some PTMs, capturing the modified enzyme can also be challenging. Hence, future studies that involve synthesizing and purifying recombinant PRMTs containing the modified residue(s) combined with mass spectrometry and molecular dynamic simulation studies will be crucial to expanding our limited comprehension of the structural and molecular

consequences of these important modifications. Along these same lines, structural analyses can also be used to understand whether certain PTMs within the highly conserved catalytic domain of different PRMTs alter the binding affinity of AdoMet and, thus, serve as universal mechanisms for modulating the activity of this family of epigenetic enzymes.

Importantly, PTMs are often sequential and occur in a stepwise manner to properly create the right 'PTM code' on the protein surface. Major challenges for the future will involve improving our understanding of the positive and negative consequences of PTM crosstalk on PRMTs, including the existence of motifs, such as phosphodegrons, and identifying how certain PTM combinations trigger effectors to either initiate or inhibit specific downstream events. Ultimately, the hope is that results from these studies will create previous unexplored avenues for novel therapeutic intervention, including the development of anticancer agents for PRMT-driven tumors.

Acknowledgments

This publication was made possible, in part, with support from V Foundation Kay Yow Cancer Fund (#4486242 to T.L.), NIH-NIGMS Grant (#1R01GM120156-01A1 to T.L.), and 100 VOH Grant (#2987613 to T.L.), as well as NIH-NCI Grant (#1R03CA223906-01 to T.L.).

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Highlights

- Development of small molecule inhibitors against PRMTs is a hot research area.
- PTMs of PRMTs has emerged as a critical aspect of the regulation of PRMTs.
- Understanding the molecular consequences of PTMs is arguably important.
- PTMs studies will create novel therapeutic intervention for PRMT-driven tumors.

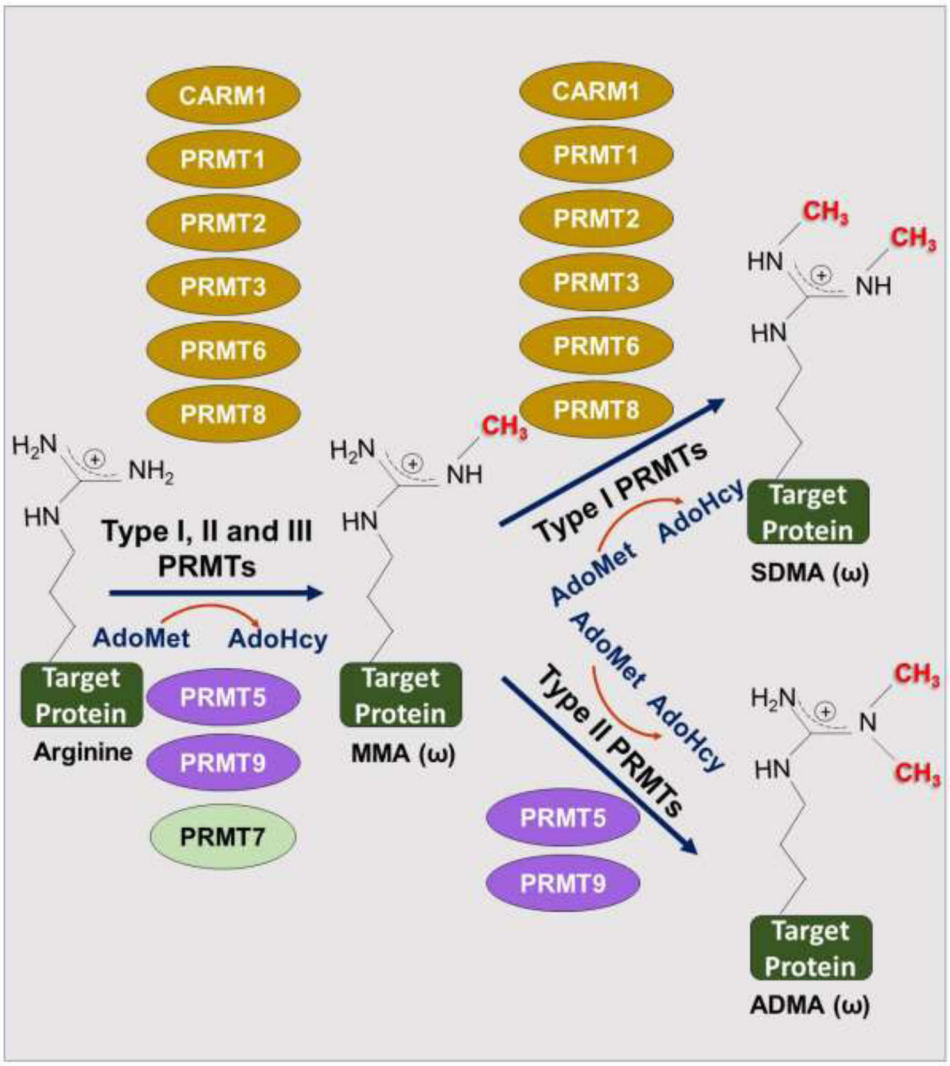

Figure 1.

Classification of protein arginine methyltransferase (PRMT) family members based on methylation pattern [8]. Three distinct types of methylated arginine residue exist in mammalian cells: monomethylarginine (MMA), asymmetric dimethylarginine (ADMA), and symmetric dimethylarginine (SDMA). All PRMT family members catalyze the formation of MMA as an intermediate by adding one methyl group to the terminal (ω) guanidine nitrogen atoms. ADMA marks are catalyzed by Type I enzymes, of which CARM1 is the most prominent. ADMA marks are generated by the addition of two methyl groups on the same terminal (ω) guanidine nitrogen atom, whereas SDMA marks are formed by the addition of a methyl group on each of the two terminal nitrogens. This is catalyzed by Type II PRMTs, of which PRMT5 is the most prominent. PRMT7 is the only type III enzyme, which mainly generates MMA. The main enzymes for each type of methylation pattern are indicated.

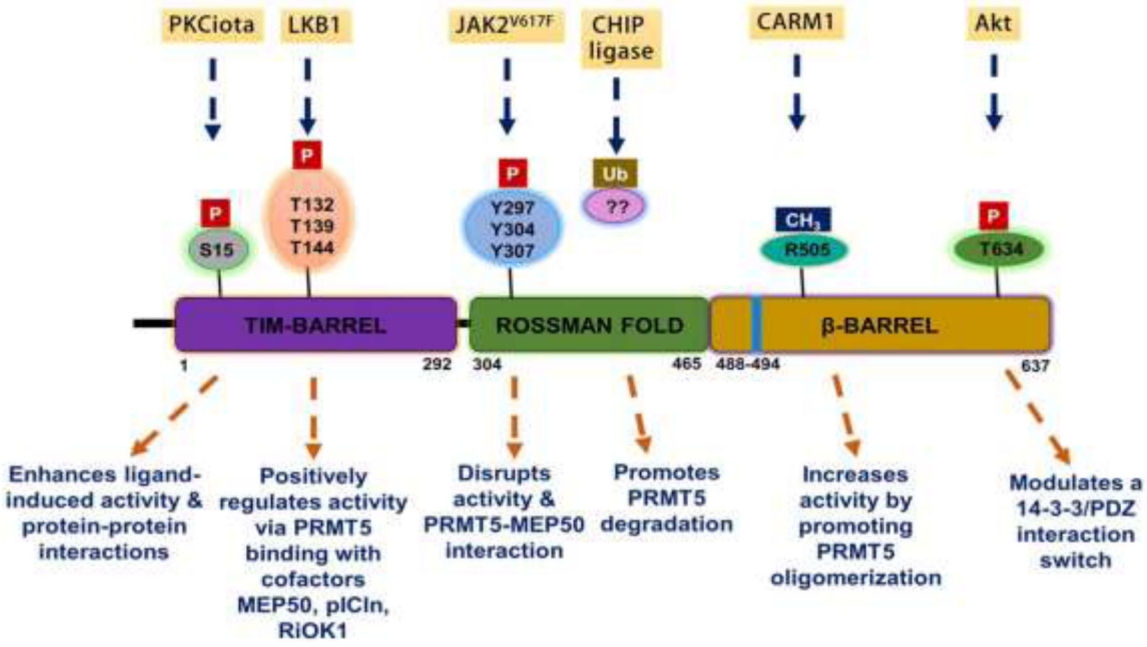

Figure 2.

Functional effects of post-translational modifications (PTMs) within different structural domains of protein arginine methyltransferase 5 (PRMT5). Architecture and PTMs of the three major domains: (i) the N-terminal TIM barrel, which is known to mediate substrate and cofactor [e.g., methylosome protein 50 (MEP50)] binding, harbors both serine and threonine phosphorylation sites. These PTMs appear to affect the known functions of this domain, suggesting a potential regulatory role for these modifications; (ii) the Rossman fold or catalytic domain harbors three tyrosine (Y) phosphorylation sites, which affect both methyltransferase activity and cofactor interactions. Although the exact sites are still known, CHIP ligase-mediated K48-linked ubiquitination of PRMT5 near the Rossman fold triggers degradation of PRMT5; (iii) the β-barrel contains a small dimerization domain (amino acids 488–494), which aids in the formation of higher-order multimeric complexes. Interestingly, a nearby methylation event mediated by CARM1 affects PRMT5 oligomerization, which in turn increases PRMT5 activity. The C-terminal β-barrel is also modified at a T634 site, which modulates a 14-3-3/PDZ interaction switch.

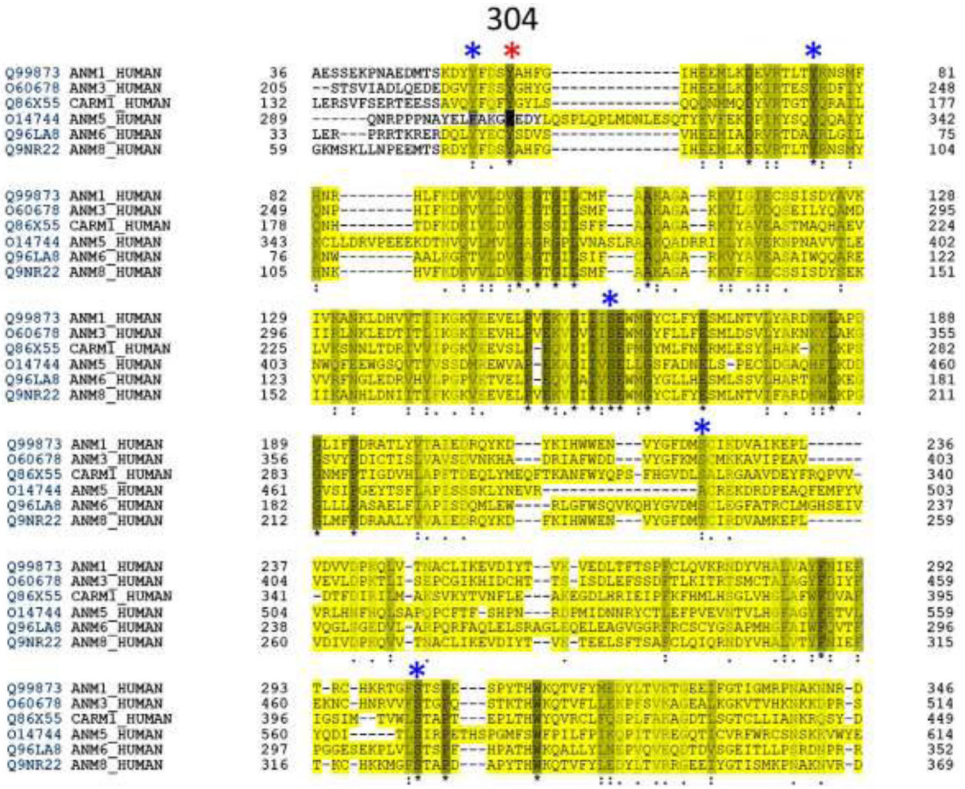

Figure 3.

Amino acid sequence alignment of the catalytic (methyltransferase) domain of six protein arginine methyltransferase (PRMT) family members with previously reported post-translational modifications (PTMs). Sequence alignment of six PRMT family members from humans is shown for the catalytic domain (yellow highlight): PRMT 1 (ANM1_HUMAN), PRMT3 (ANM3_HUMAN), PRMT4 or CARM1 (CARM1_HUMAN), PRMT5 (ANM5_HUMAN), PRMT6 (ANM6_HUMAN), and PRMT8 (ANM8_HUMAN). Similar amino acids are highlighted in dark gray with black asterisks underneath the indicated residues. Lighter gray-highlighted residues with one to two dots below are less similar but conserved. Larger black asterisks shown on top indicate highly conserved serine (S) or tyrosine (Y) residues that are phosphorylatable but have not been experimentally confirmed as such. Finally, a large blue asterisk shows a highly conserved tyrosine (Y) residue across all six PRMT family members that was experimentally identified and confirmed in PRMT5 (Y304) as being phosphorylated [60]. Alignment and shading were generated using www.uniprot.org/align.

Table 1.

PRMT classification and their broad roles

PRMT classification	Broad roles	Refs
Type I: PRMT1, 2, 3, 4 (CARM1), 6, and 8	Transcription, mRNA splicing, DNA repair, signal transduction, ribosomal assembly, cell differentiation	[6,8-10,53]
Type II: PRMT5 and PRMT9	Transcription, ribosomal assembly, cell proliferation, pluripotency, DNA repair	[6,8-10,53]
Type III: PRMT7	Transcription	[6,8-10,53]

Table 2.

Effect of various PTMs on members of the PRMT family

PRMT	Predicted modifie sites	Experimentally confirmed sites	Type of PTM	Effect of PTM	Enzymes catalyzing the modification	Refs
PRMT1	Y291	Y291	Phosphorylation	Alters substrate specificity and PPIs	Unidentified	[35]
	Likely multiple sites (S284–S289; S55–S57; S102–S105)	--	Phosphorylation	Positively regulates function of PRMT1 in suppressing premature differentiation of keratinocytes	CSNK1a1	[36]
	Unknown	--	Polyubiquitination	Promotes proteasomal degradation	E4B	[37]
PRMT3	S25, S27	--	Phosphorylation	Unclear	Unidentified	[44]
CARM1/ PRMT4	S217, S228, S229 S447	S217, S229	Phosphorylation	Inactivating	Unidentified	[50,51]
	--	--	OGT-glycosylation	Inactivating	OGT	[52]
	Unknown	--	Automethylation	Inactivating	CARM1	[47,48]
PRMT5	Y297, Y304, Y307	Y297, Y304, Y307	Phosphorylation	Decreases activity	JAK mutant $JAK2^{V617F}$	[60]
	T132, T139, T144	T132, T139, T144	Phosphorylation	Maintains methyltransferase activity	LKB1	[64]
	Unknown, likely multiple sites	--	Polyubiquitination	Negative regulation of PRMT5 expression via K48-linked ubiquitin-dependent proteasomal degradation	CHIP ligase	[65]
	T634	T634	Phosphorylation	Modulates 14-3-3/PDZ interaction switch	Akt/serum- and glucocorticoid-inducible kinases	[62]
	R505	R505	Methylation	Modulates oligomerization, increases activity	CARM1	[19]
	S15	S15	Phosphorylation	Mediates NF-κB activation, target gene expression; modulates IL-1β-inducible PRMT5 activity	PKCiota	[63]
	Cys125	--	S-nitrosylation	Promotes methyltransferase activity in *Arabidopsis thaliana*	Not specified	[74]
PRMT6	R35	--	Automethylation	Not specified	PRMT6	[69]
PRMT8	R58, R73	--	Automethylation	Not specified	PRMT8	[72]