Cell. Mol. Life Sci. (2015) 72:2041–2059
DOI 10.1007/s00018-015-1847-9

Cellular and Molecular Life Sciences

REVIEW

The PRMT5 arginine methyltransferase: many roles in development, cancer and beyond

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Received: 15 November 2014 / Revised: 10 January 2015 / Accepted: 29 January 2015 / Published online: 7 February 2015

Abstract Post-translational arginine methylation is responsible for regulation of many biological processes. The protein arginine methyltransferase 5 (PRMT5, also known as Hsl7, Jbp1, Skb1, Capsuleen, or Dart5) is the major enzyme responsible for mono- and symmetric dimethylation of arginine. An expanding literature demonstrates its critical biological function in a wide range of cellular processes. Histone and other protein methylation by PRMT5 regulate genome organization, transcription, stem cells, primordial germ cells, differentiation, the cell cycle, and spliceosome assembly. Metazoan PRMT5 is found in complex with the WD-repeat protein MEP50 (also known as Wdr77, androgen receptor coactivator p44, or Valois). PRMT5 also directly associates with a range of other protein factors, including pICln, Menin, CoPR5 and RioK1 that may alter its subcellular localization and protein substrate selection. Protein substrate and PRMT5–MEP50 post-translation modifications induce crosstalk to regulate PRMT5 activity. Crystal structures of *C. elegans* PRMT5 and human and frog PRMT5–MEP50 complexes provide substantial insight into the mechanisms of substrate recognition and procession to dimethylation. Enzymological studies of PRMT5 have uncovered compelling insights essential for future development of specific PRMT5 inhibitors. In addition, newly accumulating evidence implicates PRMT5 and MEP50 expression levels and their methyltransferase activity in cancer tumorigenesis, and, significantly, as markers of poor clinical outcome, marking them as potential oncogenes. Here, we review the substantial new literature on PRMT5 and its partners to highlight the significance of understanding this essential enzyme in health and disease.

Keywords Protein arginine methyltransferase · Histones · Spliceosome · Development · Cancer

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Introduction

Protein arginine methyltransferases (PRMTs) transfer methyl groups from *S*-adenosylmethionine (AdoMet or SAM) to a guanidine nitrogen of protein arginine resulting in the reaction products methylarginine and *S*-adenosylhomocysteine (SAH) (reviewed in [1]). There are four types of PRMTs: type I PRMTs catalyze ω-N^G-monomethylarginine (MMA) and asymmetric ω-N^G, N^G-dimethylarginine (aDMA); type II PRMTs catalyze MMA and symmetric ω-N^G, N'^G-dimethylarginine (sDMA); type III PRMTs are capable of only monomethylation; and Type IV generates δ-N^G-monomethylarginine (Fig. 1; type IV activity, limited to yeast Rmt2 [2], is not shown). PRMT1, 2, 3, 4, 6, and 8 are Type I, while PRMT5 and possibly PRMT7 are Type II PRMTs [3–6]. Recent proteomic analysis of human tissues reveals differences in PRMT family protein expression (Fig. 2) [7], with higher expression in fetal tissues for all PRMTs. PRMT2, 3, 6, 7, and 8 exhibit tissue-specific expression patterns, while PRMT1, 4, and 5 exhibit more universal expression. PRMT5's partner methylosome protein 50 (MEP50) has similar

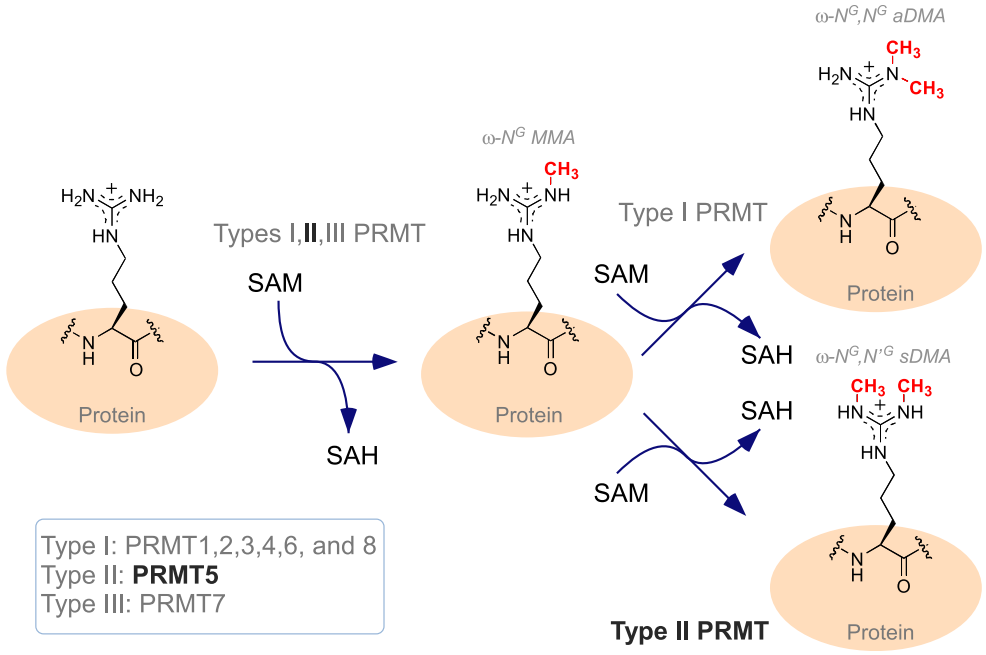


Fig. 1 Arginine methylation states catalyzed by the family of protein arginine methyltransferases (PRMTs). The guanidinium side chain of arginine residues in proteins is positively charged. It can accept a monomethyl addition, catalyzed by the family of Type I, II, and III PRMTs through transfer from the *S*-adenosylmethionine (SAM or AdoMet) cosubstrate, resulting in a ω-N^G monomethylated arginine (MMA) and *S*-adenosylhomocysteine (SAH). Type I PRMTs, comprising the majority of PRMT enzymes, can further catalyze the ω-N^G monomethylation to ω-N^G, N^G asymmetric dimethylarginine (aDMA), consuming SAM and producing SAH. PRMT5, a Type II enzyme, catalyzes the ω-N^G monomethylation to ω-N^G, N'^G asymmetric dimethylarginine (sDMA), also consuming SAM and producing SAH. Type III enzymes are incapable of processing to dimethylation. Methylation does not alter the positive charge on the arginine guanidinium side chain

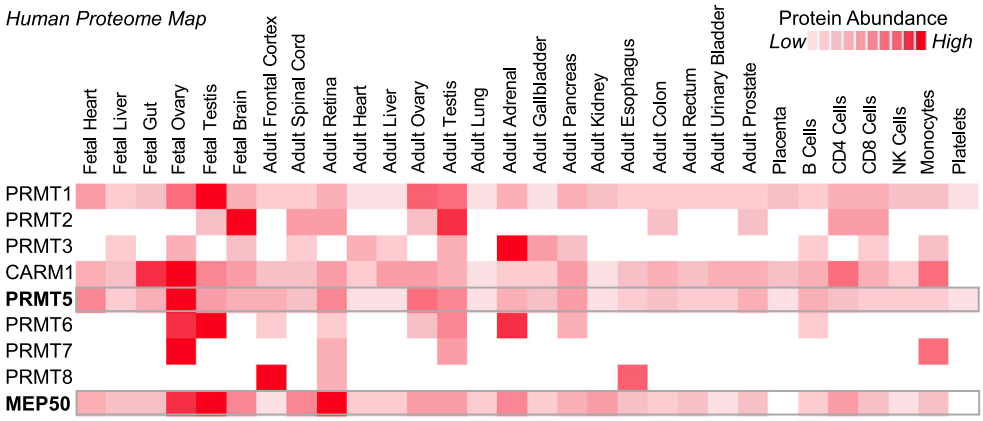


Fig. 2 PRMT5 and MEP50 are broadly expressed in somatic and embryonic tissues. The human proteome map, analyzed by total proteome mass spectrometry (http://www.humanproteomemap.org [7]), was queried for the PRMT family of proteins which showed that they are distinctly expressed in a range of human tissues and cells. The relative protein abundances for the PRMT1-8 (CARM1 is the name for PRMT4) are shown in a heatmap, with white representing low protein abundance and dark red representing higher abundance, with a ten-step range indicated in the legend. PRMT5 is *bolded* and *boxed*, as is its MEP50 cofactor. Note that PRMT5 and MEP50 are most highly expressed in fetal tissue and that their expression patterns are quite similar

expression to PRMT5. PRMT9, newly annotated in NCBI, is still undescribed. The initially annotated PRMT9 is now correctly identified as an F-box protein, FBXO11 [8].

PRMT5 is the primary Type II arginine methyltransferase and found in all eukaryotic species investigated (Fig. 3a). The *S. cerevisiae* homolog of PRMT5 is histone synthetic lethal 7 (Hsl7); the *S. pombe* homolog is Shk1 kinase-binding protein 1 (Skb1) [9, 10]. *Hsl7*'s synthetic lethality with histones that led to its name likely had no connection with histone methylation, as no evidence of

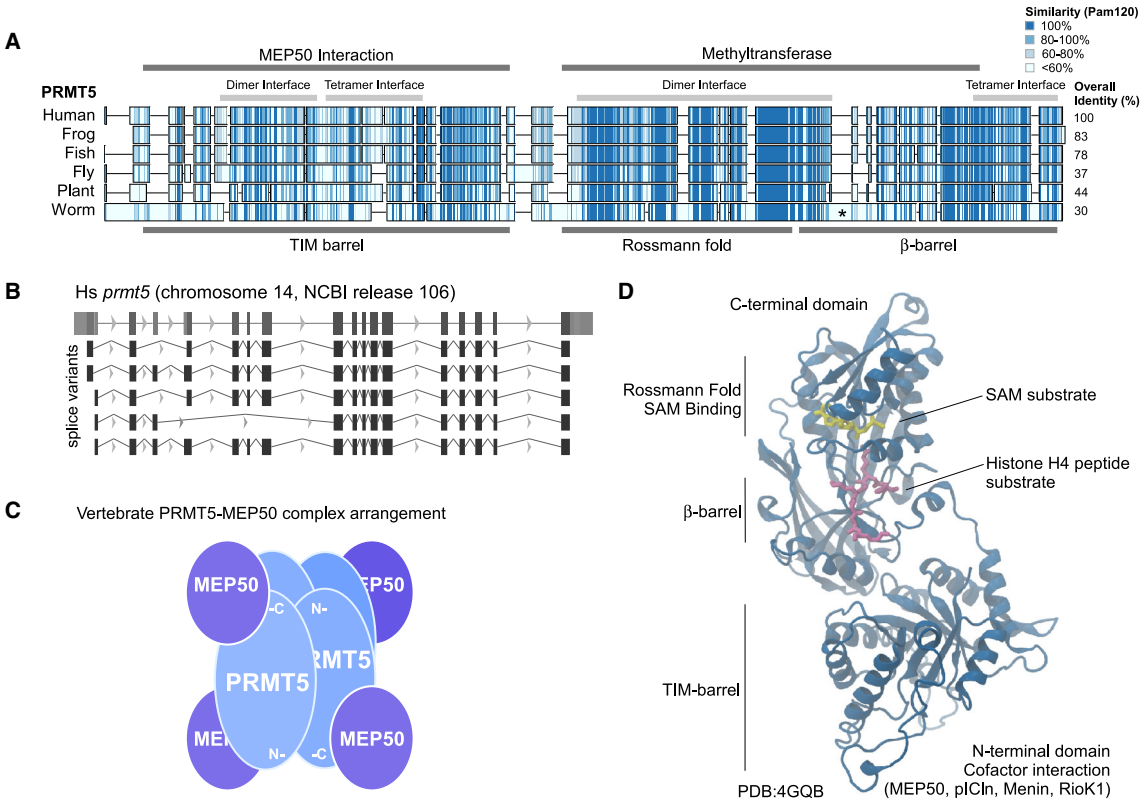


Fig. 3 PRMT5 domain organization and structure are evolutionarily conserved. **a** A range of PRMT5 protein sequences across eukaryotic species [*Homo sapiens* (human), *Xenopus laevis* (frog), *Danio rerio* (fish), *Drosophila melanogaster* (fly), *Arabidopsis thaliana* (plant), *Caenorhabditis elegans* (worm)] was aligned using the MAFFT algorithm and the Pam120 similarity index and represented in a heatmap from *white* (<60 % similarity) to *dark blue* (100 % similarity). Alignment gaps are indicated by a *line*, and overall identity is shown on the *right*. The major domains and interfaces are indicated *above* and *below* the sequences. *Asterisk* indicates sequence insertion in *C. elegans* PRMT5 that prohibits tetramerization. **b** The human *prmt5* gene has multiple splice variants, as shown from the NCBI human genome sequence. All the variants are in the N-terminal domain of the encoded protein. **c** Subunit arrangement of the hetero-octameric PRMT5–MEP50 structure shown in *cartoon* form, with the head-to-tail N-terminal and C-terminal PRMT5 arrangement shown by "N-" and "-C". **d** *Ribbon* diagram of a monomer of human PRMT5 (PDB:4GQB) with the domains and substrate-binding sites as indicated

histone arginine methylation exists in *S. cerevisiae*. Human PRMT5 was first identified as Jak-binding protein 1 (JBP1), and shown to methylate, among many cellular proteins, histones H2A and H4 on Arg3 and histone H3 on Arg8 [11–13] (Table 1). Histones H2A and H4 share a conserved targeted N-terminal sequence: SGRGK…. Multiple PRMT5 splice variants are found in human cells, although evidence for translated proteins from these shorter mRNAs is lacking (Fig. 3b).

In this review, we highlight and interpret the literature on PRMT5, its partners, targets, structure, and enzymology. We address PRMT5's role in stem cells and primordial germ cells, differentiation, and animal development. In the context of PRMT5's wide-ranging biological roles, we explore the extensive literature implicating PRMT5 in a large number of cancers. While hints of PRMT5's significance for tumorigenesis have been apparent for some time, we argue here that the sheer abundance of evidence shows that PRMT5 is now a compelling target for clinical screening and, hopefully, for future chemotherapeutic approaches. A recent review of the function of all PRMTs in chromatin organization provides a complementary view of the specific function of arginine methylation in nuclear function [14].

MEP50: a critical PRMT5 cofactor

The majority of vertebrate PRMT5 complexes contain MEP50, a 7-bladed WD40 repeat (tryptophan, aspartic acid) β-propeller protein. MEP50 is also known as Wdr77 or androgen receptor coactivator p44, by which it is referred to in the cancer literature [15–24]. MEP50 directly binds PRMT5 and greatly enhances PRMT5's histone methyltransferase ability, primarily through increased

Table 1 Major PRMT5 protein substrates and their function

PRMT5 substrate	Biological function of arginine methylation by PRMT5	References
Histone H2A and H4 R3	Transcriptional repression	[54, 56, 61, 62, 64, 65, 67, 68, 76, 77, 103, 162]
Histone H3 R2	Transcriptional repression	[4, 80, 163]
Histone H3 R8	Transcriptional repression	[13, 57, 60, 82, 122, 164]
Spliceosome Sm proteins	Facilitates spliceosomal assembly	[41, 44, 49, 108, 165–169]
Ribosomal protein RPS10	Facilitates ribosomal assembly	[170]
p53	Facilitates survival and cell cycle arrest over apoptosis	[71, 143]
FEN1	Facilitates PCNA binding and DNA replication and repair	[171]
Nucleoplasmin	Enriched in early development; unknown function	[37]
Nucleolin	RNA binding; unknown function	[108, 109]
EGFR	Reduces autophosphorylation and EGFR activation	[145]
EBNA	Methylation stimulates Epstein–Barr nuclear antigen promoted transcription	[153, 154]

affinity for protein substrate (D.S., manuscript under review). The arrangement of MEP50 in complex with PRMT5 is illustrated in Fig. 3c.

Structure and enzymology of PRMT5 and MEP50

Structural insight into general PRMT mechanisms was recently reviewed [25]. The *C. elegans*, *Xenopus*, and human PRMT5 all contain a triosephosphate isomerase (TIM) barrel on the N-terminus, a middle Rossmann-fold, and a C-terminal β-barrel containing a dimerization domain (Fig. 3d). *Ce*PRMT5 forms a homodimer in which the dimerization arm of one monomer interacts with residues contained in the TIM barrel of the other monomer, forming a ring [26]. This head-to-tail ring-shaped homodimer is conserved in all of the solved Type I PRMT structures [27–33]. In contrast, the human and *Xenopus* PRMT5s form a heterooctomeric complex composed of four PRMT5 proteins and four MEP50 proteins (Fig. 3c) [34, 35]. The PRMT5 molecules form two dimers in the head-to-tail arrangement typical of PRMTs. One of the two dimers in the human and *Xenopus* PRMT5 tetramer is similar to the *C. elegans* dimer and contains a number of conserved hydrogen bonds. The second dimer interface, unique to the human and *Xenopus* PRMT5 tetramer, contains hydrogen bonds not seen in the *C. elegans* dimer. Furthermore, a sequence insertion found in *C. elegans* would prevent this dimerization of PRMT5 to a tetramer (noted by asterisk in Fig. 3a). The PRMT5 tetramer forms the core of the complex and MEP50 interacts with PRMT5 through the N-terminal TIM barrel domains. A monomer of human PRMT5 is illustrated in Fig. 3d, showing the domain structures as well as the locations of the SAM and histone peptide substrates within the crystal.

The PRMT5–MEP50 complex has a higher level of methyltransferase activity compared to PRMT5 alone [35]. This could be due to MEP50 having a positive allosteric effect on the binding of cofactor and protein or SAM substrates by PRMT5 and/or MEP50 being necessary to present protein substrate to PRMT5. The latter is supported by experiments demonstrating MEP50 interaction with H2A and H4 [34, 36], and that excess MEP50 inhibits methyltransferase activity, consistent with MEP50 sequestering substrate from the enzyme [34]. The PRMT5 catalytic site is also oriented toward the cross-dimer paired MEP50 and electron microscopy-localized substrate density on MEP50 [34].

PRMT5–MEP50 is nonprocessive, as production of the dimethylated H4 peptide product is dependent on the concentration of the monomethylated peptide exceeding that of the unmethylated substrate [35, 37]. Thompson and colleagues [38] demonstrated that CePRMT5 is truly distributive. This is in contrast to PRMT1, for which monomethylated and dimethylated products are observed despite the presence of excess unmodified substrate, indicating PRMT1 uses a partially processive mechanism [39].

A conserved phenylalanine in the *C. elegans* PRMT5 catalytic site is essential for specifically catalyzing symmetric dimethylation by structural orientation of the monomethylated arginine substrate [26]. Mutation of a catalytic site Met to Phe remodels PRMT1 to produce symmetric dimethylation, although production of the symmetric dimethylarginine has a higher energy barrier [40]. This reveals that the catalytic mechanisms for production of the various methylarginine products are similar and are regulated through structural and energetic means.

PRMT5 and the major spliceosome

PRMT5–MEP50, along with PRMT7, play important roles in the splicing of mRNA through methylating spliceosomal proteins [41]. Sm proteins D1, D3, and B/B′ are symmetrically dimethylated on their C-terminus by the methylosome, PRMT5–MEP50 in complex with pICln (chloride channel nucleotide sensitive 1A, Fig. 4) [42, 43]. pICln binds the Sm domain and acts as an assembly chaperone [44–47]. PRMT5-catalyzed sDMA of Sm D1, D3, and B/B′ dramatically increases binding of these three proteins to the Tudor domain-containing protein SMN (-survival of motor neuron), the product of the spinal muscular atrophy gene [42, 43]. SMN is part of a complex consisting of at least six other subunits, and is responsible for loading the seven Sm proteins onto the snRNA [48–51]. There is some evidence the snRNPs can assemble without the SMN complex in vitro [52], leading to some debate as to whether the symmetric dimethylation of Sm proteins is necessary. However, in vivo the SMN–PRMT5 relationship most likely acts as a chaperone that prevents the misassembly of Sm proteins to non-target RNAs and blocks the aggregation of Sm proteins [51]. A conditional PRMT5 knockout in mouse neural stem/progenitor cells (NPCs) shows PRMT5 is necessary for correct splicing: absence of PRMT5 leads to selective retention of introns and skipping of exons with weak 5′ donor sites [53].

Histone methylation by PRMT5 and its function in transcriptional regulation

Histone tail modifications are major components of the epigenetic regulation of gene transcription. PRMT5 symmetrically dimethylates H2AR3, H4R3, H3R2, and H3R8 in vivo, all of which are linked to a range of transcriptional regulatory events (Fig. 5) [11, 13, 54–60]. Specific gene targets include cyclin E1 [59], Rb [57], and ribosomal genes [61]. In *Arabidopsis,* PRMT5 is recruited to the CORYNE locus to down-regulate its expression and regulate shoot apical meristem phenotypes [62] and the FLOWERING LOCUS C to control flowering time [63]. PRMT5 coordinates with a range of Mediator complex subunits to dimethylate H4R3 at promoter regions of immune response genes and C/EBPβ target genes [64]. Conversely, PRMT5 methylation of histone H3R2 recruits Wdr5 and the MLL complex, stimulating H3K4 methylation and euchromatin maintenance [4].

PRMT5 selectively methylates cytosolic H2AR3 in ES cells, but not H4R3 [65]. The distinction between roles for

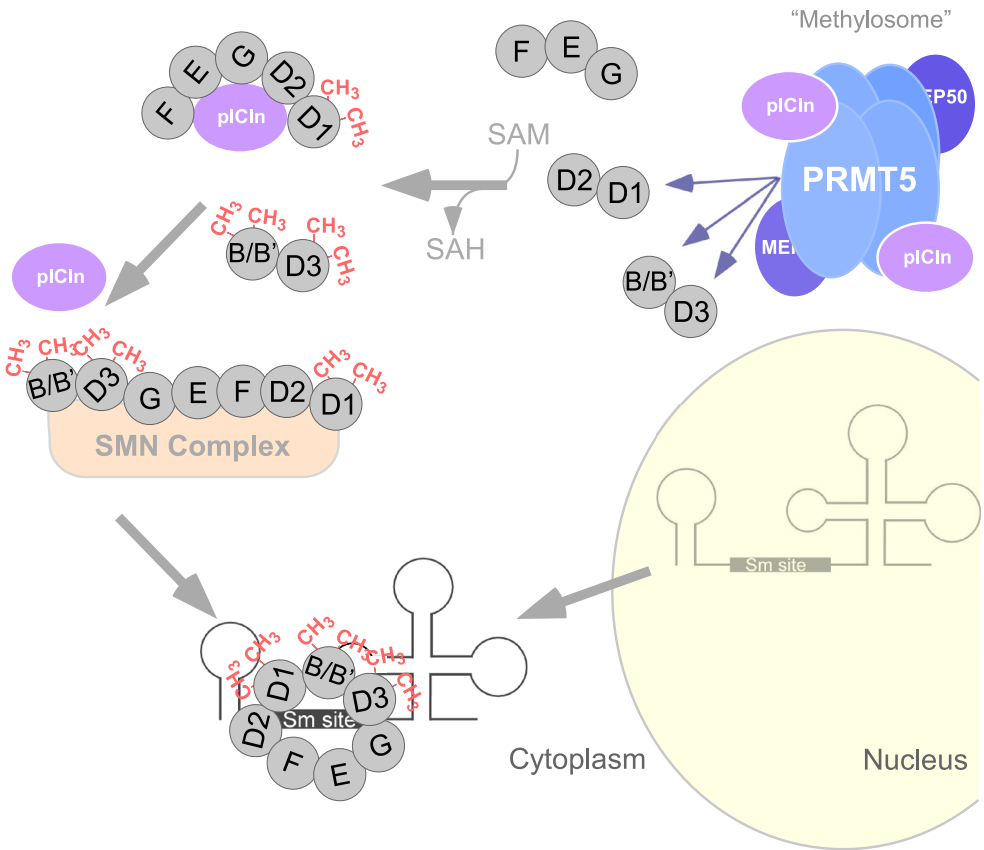


Fig. 4 PRMT5 methylation and regulation of the spliceosome. A *cartoon* representation of the function of PRMT5 methylation of splicing proteins in the cytoplasm. Methylated substrates are represented with a *red* "–CH_3". PRMT5, in complex with MEP50 and pICln, form the methylosome that targets spliceosomal subunits for methylation. pICln then chaperones the subunits to the SMN complex, resulting in proper targeting of RNAs to be spliced

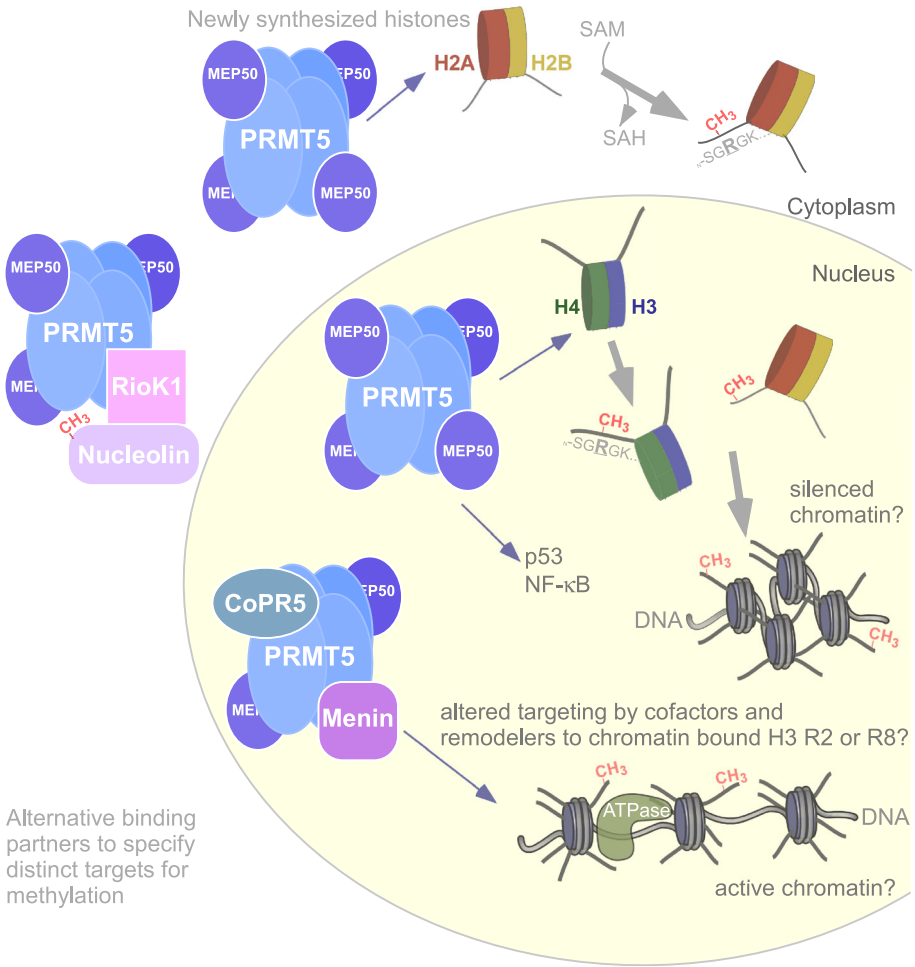


Fig. 5 PRMT5 is targeted to multiple histone and nuclear targets by cofactors. A *cartoon* representation of the function of PRMT5 methylation of nuclear proteins (nucleus represented by *pale yellow*). Methylated substrates are represented with a *red* "–CH_3". Histones, the protein component of chromatin, are synthesized and then transported to the nucleus. PRMT5–MEP50 targets newly synthesized histone H2A in the cytoplasm and may target soluble H4 in the nucleus (both H2A and H4 are methylated on R3 in the sequence N-SGRGK… as shown in the *cartoon*), as well as transcription factors such as p53 and NF-κB. PRMT5-methylated H2A and H4 are then deposited into chromatin (DNA wrapped around histone proteins, with histone N-terminal tails indicated in the *cartoon*). Alternative binding partners for PRMT5 (RioK1 in the cytoplasm, CoPR5 and Menin in the nucleus) may displace one or more MEP50 molecules and alter the targeting of PRMT5 toward substrates as shown, including histone H3 on R2 or R8 in the sequence N-ARTKQTARKST…

H2A and H4 R3 methylation by PRMT5 suggests that each histone tail and targeted arginine has a unique function and will require future work to disentangle. However, since H2A and H4 have the same "NH_2-SGRGK…" site of methylation, most available antibodies recognize both methylated histones making discrimination difficult. The few genome-wide studies of PRMT5-catalyzed histone methylation on H2A/H4 R3me2s demonstrate global enrichment [66], with specific enrichment at GC-rich promoter regions in mouse embryonic fibroblasts [67]. In contrast, enrichment on non-GC satellite DNA [68] as well as a modest anti-correlation with H3K36me3 [56] is observed in other studies. Girardot et al. [67] used an antibody lot that specifically recognizes H4R3me2s but not H2AR3me2s, possibly explaining these distinct observations. Future experimentation with a range of highly specific histone methylarginine antibodies, including monomethylarginine, and performed in a range of cell types and organisms, will help clarify the function of histone arginine methylation in gene regulation.

PRMT5 also regulates transcription and many downstream events through methylation of transcription factors, such as NF-κB [69, 70], p53 [71], and E2F-1 [72]. PRMT1- and PRMT5-catalyzed asymmetric and symmetric dimethylarginine have distinct roles in activating or suppressing apoptotic activity, respectively, of E2F-1 through recruitment of the p100-TSN Tudor domain to symmetric dimethylarginine [72].

Readers of symmetric dimethylation

Methylated arginine is translated into a meaningful cellular signal through recognition ("reading") by effector proteins or by inhibiting binding of effector proteins (recently reviewed in [73]). Tudor domain-containing proteins are the primary direct readers of methylarginine. The splicing factors methylated by PRMT5 are recognized by SMN proteins containing Tudor domains [74] while PRMT5-methylated PIWI proteins are recognized by the SND1 Tudor-containing protein [75]. Histone H4R3me2s specifically recruits the DNA methyltransferase DNMT3A to chromatin domains via its ADD (ATRX-DNMT3-DNMT3L) domain to suppress gene expression [76, 77]. However, another report was unable to reproduce this interaction [78], so further study is necessary. In contrast, H4R3me2s or H4R3me2a can interfere with the ability of the Signal Recognition Particle (SRP) proteins SRP68 and SRP72 to bind the H4 tail [79].

PRMT5 also methylates histone H3R2 and recruits Wdr5, a WD40-repeat protein and essential component of MLL (mixed lineage leukemia lysine methyltransferase) complexes, to promote H3K4 methylation and downstream gene activation [4, 80]. Wdr5 quantitatively binds H3R2me2s, but does not bind H3R2me2a, providing a unique switch between recruitment states based on the change in methylarginine. The crystal structure of Wdr5 bound to H3R2me2s demonstrates that the symmetric dimethylarginine displaces water within the binding cavity, substantially enhancing the interaction and suggesting that WD-repeat proteins may function to distinguish between different post-translation modification (PTM) states [4].

Interaction of PRMT5 with ATP-dependent chromatin remodelers: function in transcriptional regulation

PRMT5 methylates histones and interacts with ATP-dependent chromatin remodelers to either enable or repress gene expression, depending on the cellular context (Fig. 5) (reviewed in [81]). PRMT5 localizes to the promoter of the early MyoD-induced gene *myogenin*, and also coimmunoprecipitates with MyoD and the chromatin remodeler ATPase Brg1 [82]. Furthermore, H3R8 dimethylation catalyzed by PRMT5 at the *myogenin* promoter is a necessary prerequisite for the binding and chromatin remodeling activity of Brg1, which in turn is necessary for the binding of MyoD. Antisense-mediated knockdown of PRMT5 positively and negatively regulated many genes, including several with antiproliferative and tumor suppressor activity [13]; in this study, PRMT5 was shown to associate with the BRG1 and BRM chromatin remodelers and methylate promoter H3R8 to inhibit tumor suppressors. PRMT5 also associates with the NuRD remodeling complexes that contain the methyl-CpG-binding domain protein 2 (MBD2) [83]. Together these studies suggest that gene repression or activation by PRMT5 is context dependent.

Other PRMTs associate with chromatin remodeling complexes as well. PRMT4 is required to facilitate SWI/SNF chromatin remodeling activity for late but not early gene expression in skeletal muscle differentiation, in contrast to PRMT5 promotion of early gene expression [84, 85]. These data demonstrate that arginine methyltransferases sequentially cooperate with chromatin remodeling complexes.

Role of PRMT5 in development

PRMT5 participates in both early and late developmental pathways. In murine early development, PRMT5 is maternally inherited in the oocyte cytoplasm until the first cellular differentiation event when it translocates to the nucleus [65]. *Prmt5*$^{-/-}$ murine embryos suffer early embryonic lethality and are incapable of producing embryonic stem ES cells. RNAi knockdown of PRMT5 in ES cells results in down-regulation of pluripotency-associated genes and up-regulation of differentiation-associated genes [65]. In human stem cells, PRMT5 is only required for proliferation, and not pluripotency, through methylation of the cell cycle-regulated p57 [86]. *Mep50* null mice are similarly embryonic lethal [21, 24], further supporting the essential function of the intact PRMT5–MEP50 complex.

In *Xenopus laevis* embryos, *prmt5* is abundant until zygotic stage 8, when transcript levels drop precipitously coincident with the onset of zygotic transcription [37]. PRMT5-methylated histones and histone chaperones are heavily enriched in early frog embryos [87–89]. PRMT5–MEP50 methylates pre-deposition histones H2A/H2A.X-F and H4 and the maternal histone chaperone nucleoplasmin on a conserved motif ("GRGxK") [37]. These observations are consistent with a maternal and early zygotic role for PRMT5–MEP50 in regulating embryonic chromatin assembly and globally repressing zygotic transcription.

PRMT5 function in primordial germ cell and keratinocyte differentiation

PRMT5 also plays a role in a number of tissue-specific differentiation pathways, including primordial germ cells, keratinocyte, muscle, and nerve cell differentiation [81, 82, 84, 90–94].

In germ cell development, PRMT5 methylates Piwi proteins and regulates their subsequent binding to Tudor domain-containing proteins in an sDMA-dependent fashion

[95–99]. Piwi proteins are primarily expressed in the germline lineage and interact with small non-coding RNAs, piRNAs [100]. piRNAs complement transposable DNA elements and other genes, leading to their silencing, which is essential for normal gametogenesis [101]. For example, in *Drosophila,* either a *prmt5* homozygous null mutant or a loss of function *Tudor* mutation causes transposon up-regulation [102]. PRMT5 histone methylation is also required for suppressing transposable elements during murine PGC demethylation [103]. PRMT5 interacts with the transcriptional repressor Blimp1, an essential component of primordial germ cell (PGC) induction [54, 104]. Association of PRMT5 and Blimp1 in the nucleus of PGCs results in increasing levels of H2A/H4 R3me2s and upon the subsequent translocation of PRMT5 and Blimp1 to the cytoplasm H2A/H4 R3me2s is almost completely lost [54]. This coincides with the down-regulation of pluripotency genes and the expression of Dhx38, an RNA helicase, which may recruit PRMT5 and Blimp1 to specific DNA sequences [54, 105]. These results suggest that the Blimp1/PRMT5 complex has an essential role in maintaining the PGC lineage during the migration of the cells into the gonads [106]. Alternatively, PRMT5's function may be at the end of PGC programming to regulate RNA splicing [107].

In human keratinocyte differentiation involucrin gene expression is partially controlled by PKC-δ suppression of PRMT5 [92]. PRMT5 is part of the p38-δ complex and functions through suppression of p38-δ phosphorylation and sDMA modification of an as yet unidentified protein [92].

Modulation of PRMT5 activity through binding partners, post-translational modification crosstalk, and subcellular localization

PRMT5 activity and localization are regulated in multiple ways, including binding partners (Table 2), PTMs, subcellular localization, and microRNAs (miRNA).

Binding partner regulation of PRMT5

PRMT5 binds to pICln or the Rio domain-containing protein RioK1 in a mutually exclusive manner on PRMT5's N-terminal domain, and likely serves to specify substrate choice [108] (Fig. 5). The RNA-binding protein nucleolin interacts only with the C-terminus of RioK1, and not with PRMT5 or MEP50. RioK1 functions similarly to pICln and MEP50 by acting as an adaptor protein [108]. In further support of the biological connection between PRMT5 and nucleolin, the AS1411 aptamer that targets nucleolin alters the subcellular localization of the PRMT5–nucleolin complex within prostate cancer cells, potentially providing a molecular basis for some AS1411 effect on cancer cell proliferation [109]. RioK1 is exclusively located in the cytoplasm, which may further control the temporal and spatial activity of PRMT5. Therefore, coupled subcellular localization of adaptor proteins could be an important mechanism to regulate PRMT5 activity.

Other vertebrate-specific binding partners also regulate or target PRMT5 activity to specific substrates, including Menin/Men1, pICln, RioK1, and CoPR5 [44, 45, 93, 108, 110–114]. CoPR5 (cooperator of PRMT5), to date only found in mammals, binds histones in the nucleus and recruits PRMT5 to nucleosomes [114]. CoPR5 binding to PRMT5 is necessary for myogenic differentiation, possibly through altered targeting of PRMT5 [93]. Menin, a unique adapter protein found in MLL complexes to target histone K4 trimethylation and frequently mutated in endocrine tumors, was shown to directly bind to the N-terminus of PRMT5 and target H4R3me2s at a specific promoter [110].

One compelling hypothesis supported by published interaction data and our structural modeling is that RioK1 and Menin may displace one or more MEP50 molecules from the PRMT5 complex, altering PRMT5 targeting while maintaining MEP50 in part of the heterocomplex to promote histone or other methylation (Fig. 5). This hypothesis could explain why PRMT5 forms a tetramer in vertebrates: to maintain MEP50 interaction and allow simultaneous binding of additional cofactors. Another mechanism for regulation of PRMT5 binding is via splicing. Alternative transcripts of PRMT5 missing exons in the N-terminus of PRMT5, which binds MEP50, Menin, Riok1 and plCln, are known (Fig. 3b) [115]. Future studies may reveal altered PRMT5 protein production from these transcripts that alter partner binding.

PTM crosstalk modulation of PRMT5

PRMT5–MEP50 substrate PTMs can affect methyltransferase activity. SWI/SNF-associated PRMT5 methylates hypoacetylated H3 and H4 more efficiently than hyperacetylated H3 and H4 [58]. Neighboring H4 lysine acetylation marks stimulate PRMT5 activity in contrast to their inhibition of PRMT1 activity [116], while high-density histone peptide arrays document an elaborate crosstalk of activity regulation [34]. We modeled acetylation on H4K5 in the crystal structure of human PRMT5 and demonstrate that it would likely be stabilized in position compared to the hydrogen bonding with the structural water molecule in the unacetylated H4K5 in the structure (Fig. 6a, b). H2AS1 and H4S1 phosphorylation also inhibit PRMT5 activity [34]; as shown in Fig. 6c, the bulkier S1ph may be hindered from binding and/or may be electrostatically repulsed from the neighboring PRMT5 Y304.

Table 2 Major PRMT5 interacting proteins and their function

PRMT5 binding partner[a]	Biological function	References
MEP50 (also known as Wdr77/Androgen Coactivator p44)	Essential for PRMT5 histone methylation; always found bound to PRMT5 in metazoans	[16, 22, 23, 34–37, 65, 83, 118, 125, 129, 167, 172, 173]
pICln	Contributes to spliceosome assembly and directs PRMT5 methylation to Sm proteins	[44, 45, 47, 113, 167]
RioK1	Competes with pICln for PRMT5 binding and recruits nucleolin for methylation	[108]
Menin/MEN1	Adapter protein for MLL methyltransferase that targets PRMT5 to chromatin	[110–112]
CoPR5	Mammalian nuclear protein that targets PRMT5 to chromatin	[93, 174]
hSWI/SNF Chromatin remodeling complexes	Targets PRMT5 to chromatin and methylation of Histone H3	[13, 57, 58, 60, 82, 84, 122, 164]
JAK kinases	Mutant Jak2 found in leukemia phosphorylates PRMT5 and reduces its activity	[11, 117]
Blimp1	Localization of PRMT5 in primordial germ cells	[54]
AJUBA	Coordinates PRMT5 interaction with SNAIL	[120]
Piwi	Recruitment via Tudor domain proteins to piRNA pathways	[95–97, 101]

[a] Caution is warranted when considering PRMT5 interacting proteins identified in the literature by anti-FLAG precipitation (not shown here) as PRMT5 was shown to directly interact with FLAG antibodies [175]

PTMs on PRMT5 or MEP50 also modulate methyltransferase activity. Although PRMT5 was first identified through its interaction with Jak2 protein in humans [11], the functional significance of this finding was not fully realized until recently. Mutant Jak2, common in certain types of leukemia, phosphorylates PRMT5 on its N-terminus in a region that is highly conserved from human to *Xenopus* (Y304 shown in Fig. 6c) [117]. This may abolish the interaction of PRMT5 with the histone substrate by clashing with its N-terminal Ser1 and thus significantly impairs the ability of PRMT5 to methylate histones H2A or H4 on R3 (similarly to H2A/H4 S1ph, Fig. 6c) [35, 117]. Conversely, phosphorylation of MEP50 on T5 increases the methyltransferase activity of PRMT5–MEP50 toward H4 [118], potentially by increased affinity for histone substrates. Finally, PRMT5 can influence the activity of other enzymes, as PRMT5 methylation of the transcription factor GATA4 inhibits p300-mediated GATA4 acetylation [119].

Subcellular localization and other regulation of PRMT5

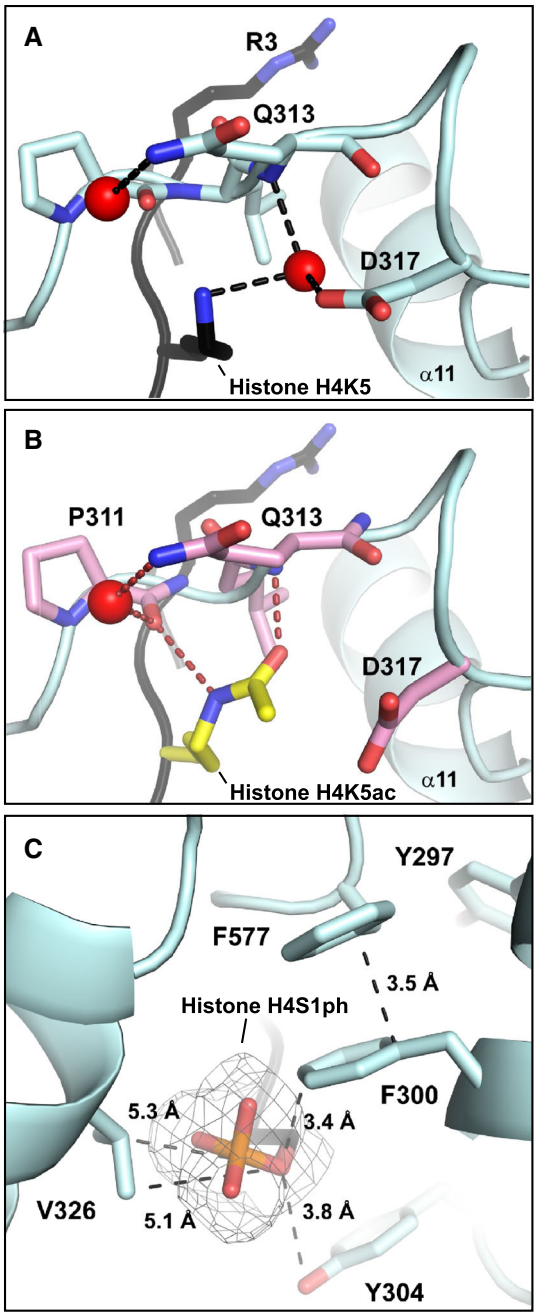
In a variety of somatic cells, PRMT5 predominantly localizes to the cytoplasm [120–122] and as noted above the translocation of PRMT5 appears to play a role in controlling pluripotency in early development of mouse embryos [65]. PRMT5 has three novel nuclear exclusion signals (NES) that are unlike the conventional leucine-rich NES [123].

PRMT5 localization is also regulated by binding partners. The transcription factor SNAIL forms a complex with PRMT5–MEP50 mediated by the LIM protein AJUBA [120] and promotes translocation of the primarily cytoplasmic AJUBA and PRMT5 to the nucleus. SNAIL recruits the complex to the *E-cadherin* proximal promoter, resulting in increased methylation of H4R3. PRMT5 knockdown or inhibition results in expression of *E-cadherin*, suggesting transcriptional repression of *E-cadherin* by the SNAIL complex is dependent on PRMT5 methyltransferase activity. The SNAIL-induced epithelial-to-mesenchymal transition is essential during development and a major contributor to metastasis and tumor progression [124].

PRMT5 translation is regulated by miRNAs in mantle cell lymphoma (MCL) cells, in which a global increase in PRMT5 protein and H3R8 and H4R3 methylation appears despite less mRNA and slower transcription compared to normal B lymphocytes [122]. Re-expression of miRNAs that normally bind the 3′UTR of PRMT5 results in a strong decrease in PRMT5 protein levels. Similar results were obtained in transformed B cell chronic lymphocytic leukemia (B-CLL) cell lines [57]. Intriguingly, a *prmt5* antisense RNA is found embedded within the *prmt5* gene in the human genome possibly causing a similar effect on translation (NCBI Entrez Gene ID 100505758).

PRMT5–MEP50 in cancer

PRMT5's regulation of proliferation and its direct interaction with proteins commonly misregulated or mutated in



◀**Fig. 6** Structural basis for modification crosstalk regulation of PRMT5 activity. The crystal structure of PRMT5–MEP50 complexed with H4 (1–8) tail peptide (PDB:4GQB) provided insight into activity crosstalk by other histone PTMs. **a** The histone H4 Lys 5 (H4K5, *black stick*) interacts with PRMT5 through a hydrogen bond between a structural water molecule (*red ball*) and its ε-NH_2. **b** Modeled interactions between an acetylated histone H4 Lys 5 (H4K5ac, *yellow stick*) within the HsPRMT5 active site. The oxygen-carbonyl occupies the position of the structural water molecule shown in **a**. Acetylation of the peptide at the K5 position increases the enzyme/substrate affinity through enhanced hydrogen bonding. **c** Modeled potential interactions between a phosphorylated histone H4 Ser 1 (H4S1ph) and the enzyme. The potential occupied space of the phosphorylated residue is shown in mesh, and may either sterically block histone peptide interaction, electrostatically repel PRMT5 Y304 in an active site pH-dependent fashion, or alternatively enhance interaction with enzyme and reduce turnover

cancer indicate that PRMT5 may play a role in cancer as an oncogene [21–24, 57, 123, 125–129]. Cancer etiology is now highly correlated with alterations in the histone code signaling of epigenetic information [130, 131]. Yang and Bedford [132] provide an overall literature review of the role of the family of PRMTs in cancer.

Increased expression and mutation of PRMT5 and MEP50 are found in a wide range of cancers, as we extracted from The Cancer Genome Atlas project database (Fig. 7a) [133]. PRMT5 overexpression appears to be an important factor in its tumorigenicity and occurs in a large number of cancers, including ovarian, lung, lymphoid, lymphoma, glioblastoma multiforme, melanoma, colon, gastric, bladder cancer and germ cell tumors [57, 122, 123, 127–129, 134–138]. In epithelial ovarian cancer, elevated PRMT5 correlates with decreased patient survival [128]. Elevated PRMT5 and MEP50 expression in non-small cell lung cancer (NSCLC) is highly correlated (logrank $P \sim 2 \times 10^{-6}$) with poorer survival in a large sample of patients, as we extracted from a clinical database of published data (http://www.kmplot.com, Fig. 7b, c) [139].

Mechanistic insight into this elevated expression in lung adenocarcinoma was shown by studies in which high cytoplasmic expression of PRMT5 was directly correlated with poor prognosis, possibly mediated through the epithelial-to mesenchymal transition [140] and histone methylation [141]. PRMT5 overexpression causes the formation of tumors in nude mice [135]. MEP50 had significant parallel roles in enhancing PRMT5 methylation of PI3-kinase to promote lung cancer tumorigenesis [142]. PRMT5 overexpression also results in increased proliferation and induced anchorage-independent colony growth [13, 135]. Conversely, PRMT5 knockdown significantly reduces cellular proliferation and colony formation in breast and lung cancer cells [13, 135, 143]. PRMT5 depletion inhibits proliferation in a majority of metastatic melanoma cell lines but accelerates growth in others [129]. These results suggest cell type might be an important factor in determining if overexpression leads to increased growth. However, no effect on cellular proliferation is observed when PRMT5 is overexpressed in MCF-7 breast cancer cells [143]. PRMT5 overexpression in cancer may in part be mediated by the NF-Y transcription factor, known to directly control cell cycle genes and other proliferative and cell survival factors [144]. PRMT5-catalyzed methylation of the growth factor receptor EGFR reduces its autophosphorylation, attenuating its activation and potentially playing a role in tumorigenesis [145].

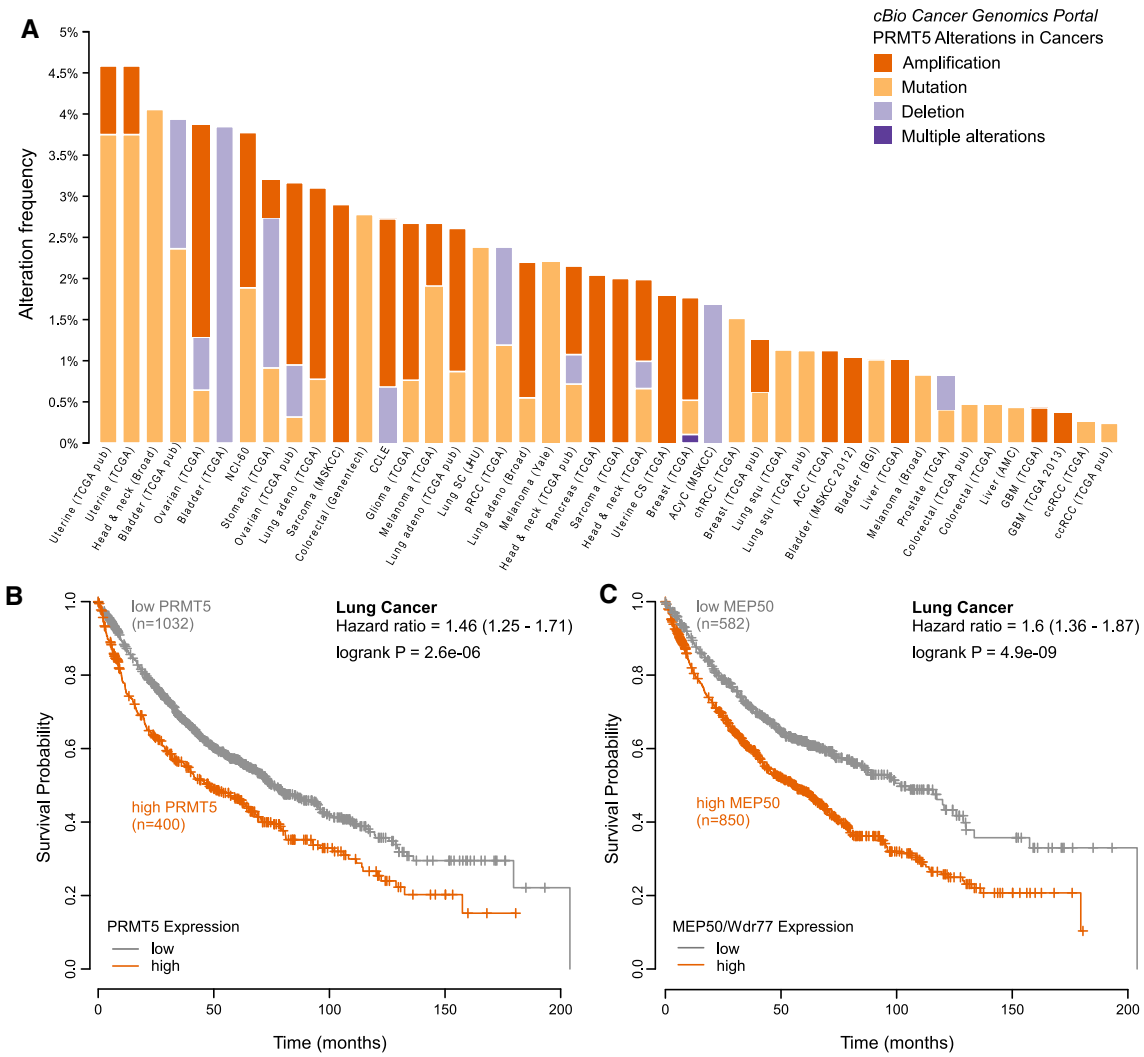


Fig. 7 PRMT5 is altered in a range of cancers and its expression is correlated with poor prognosis. **a** The alteration frequency of *prmt5* gene amplification, mutation, and deletions in a wide range of human cancers cataloged in The Cancer Genome Atlas (TCGA, accessed through the cBio Cancer Genomics Portal; http://www.cbioportal.org) was plotted in a histogram, ranging up to 4.5 % alteration in uterine cancer. This analysis did not include increased gene expression or protein abundance. **b** A Kaplan–Meier survival probability plot for high (*orange*) versus low (*gray*) *prmt5* gene expression/mRNA level for lung cancer is shown, with high *prmt5* expression resulting in a ~1.5-fold worse survival (hazard ratio) at very high significance. **c** A Kaplan–Meier survival probability plot for high (*orange*) versus low (*gray*) *mep50* gene expression/mRNA level for lung cancer is shown, with high *prmt5* expression resulting in a ~1.6-fold worse survival (hazard ratio) at very high significance. Survival data obtained from http://www.kmplot.com

The effect of PRMT5 overexpression on cellular proliferation suggests a role for PRMT5 in regulating cell cycle progression. PRMT5 knockdown slows the cell cycle in NIH3T3 cells and induces G1 arrest in 293T and MCF7 cells [135, 143]. PRMT5 overexpression increases the protein levels of the positive regulators of G1 phase cyclin D1, cyclin D2, cyclin E1, CDK4, and CDK6, and decreases the protein level of the negative regulator of G1 phase Rb protein [135]. Loss of PRMT5 leads to the increased expression of the cell cycle regulator $p27^{Kip1}$ [129].

PRMT5 is also linked to the expression of the oncogenes p53, eukaryotic translation initiation factor (eIF4E), and microphthalmia-associated transcription factor (MITF) [129, 143, 146]. Knockdown of PRMT5 causes a significant decrease in both p53 and eIF4E [143]. Overexpression of eIF4E, a translational regulator, results in rapid proliferation, suppression of apoptosis, and malignant transformation [147, 148]. Expression of eIF4E rescues short-term loss of cellular proliferation caused by PRMT5 knockdown, consistent with eIF4E functioning as a critical downstream effector of PRMT5 activity [140].

In the human osteosarcoma cell line U2OS, PRMT5, Strap and p53 form a complex in response to DNA damage [71]. DNA damage-induced apoptosis is greater

concomitant with PRMT5 knockdown, indicating that arginine methylation is a part of the p53 response. This apoptotic response could possibly be linked to PRMT5s role in splicing, such as in cell cycle genes with weak 5′ donor sites. One of these mRNAs is *Mdm4*, which senses defects in the spliceosomal machinery and transfers the signal to activate the p53 response [53]. Furthermore, PRMT5 monomethylates p53 within its oligomerization domain on a similar "GRG$^{R}/_{K}$" sequence to that found in histones, modestly influencing p53 tetramer formation and its target selection [71].

PRMT5 activity is modulated by the DAL-1/4.1B tumor suppressor which is known to function in pro-apoptotic pathways in breast cancer cells [149, 150] and is essential for the growth of lung cancer cells [123, 135]. The programmed cell death 4 (PDCD4) tumor suppressor protein conversely functions to promote cell growth and tumor formation when overexpressed with PRMT5 [126, 151]. Menin/MEN1 interacts with PRMT5 to alter its activity, and cancer-associated Menin mutations appear to block this interaction, possibly altering the targeting of PRMT5 and promoting tumorigenesis [110, 111].

In developing fetal testes, both PRMT5 and MEP50 were nuclear in Leydig cells and in adult nonneoplastic testes; in contrast, testicular cancers exhibited reduced nuclear PRMT5 and MEP50 with enhanced cytoplasmic localization [125]. Similarly, cytoplasmic expression of MEP50 in prostate cancer cells promotes both androgen- and estrogen-mediated transcriptional activity and tumorigenesis [17, 23], while forced nuclear localization of MEP50 inhibited prostate cancer cell proliferation [24]. Consistently, targeting PRMT5 to the nucleus by fusing a nuclear localization signal (NLS) to the N-terminus of PRMT5 also results in inhibition of growth of LNCaP cells.

In contrast, MEP50 was nuclear in invasive ovarian and breast cancer cells while mainly cytoplasmic in normal cells [22]. Consistent with this observation, overexpression of MEP50 in the nucleus stimulated proliferation and invasion only in the presence of estrogen or androgen [19]. Part of the role of MEP50 in hormone-responsive tumors may be independent of PRMT5, mediated through interaction and recruitment of the Smad1 transcription factor [16].

PRMT5 in additional diseases and future drug design outlook

Host and microbe PRMTs are involved in infectious disease pathways. Parasitic protozoa with PRMTs have a conserved Type I PRMT with homology to PRMT1 and a conserved Type II PRMT with homology to PRMT5 [152]. PRMT5 also binds and methylates the Epstein–Barr Nuclear Antigen protein and stimulates EBNA-dependent transcription, possibly indicating that host PRMT5 plays a role in latent EB infection [153, 154]. Retroviral infections may also be regulated by PRMT5. Human T lymphotropic viruses encode accessory proteins p30 and p28, which were shown to interact specifically with PRMT5, while reduction of host cell PRMT5 levels decreased HTLV-2, but not HTLV-1, viral gene expression [155]. The HIV Tat protein is known to be methylated and regulated by PRMT6, and contains a long stretch of "GR" residues, suggesting that it may also be a target of PRMT5 [156].

PRMT5 may also have significance for heart disease. PRMT5, along with PRMT3, was shown to bind to and methylate the voltage-gated sodium channel NaV1.5. Strikingly, this arginine methylation enhanced NaV1.5 cell surface localization and current density, showing that this regulation may be a previously unknown component of heart health and disease [157]. PRMT5 also was shown to interact with GATA4 in cardiomyocytes and methylated it on three Arg residues, inhibiting the ability of GATA4 to promote transcriptional activation [119].

A number of other arginine and lysine methyltransferases have also been implicated in cancer and other diseases [132, 158, 159]. This makes PRMT5, and protein methyltransferases in general, a prime target for drug development and diagnostics [159]. Though no pharmacological treatments directly targeting PRMT5 are available yet, research into PRMT5 inhibitors has greatly increased within the last several years, with a number of inhibitors currently being developed specifically for application to cancer, β-thalassemia, or sickle cell disease. Interestingly, the epizyme inhibitor EPZ004777 directed against the Dot1L lysine methyltransferase also inhibits PRMT5, but not the PRMT5–MEP50 complex, suggesting that some of its activity may be due to PRMT5 inhibition [160, 161].

Concluding remarks

Mono- and symmetric dimethylation of arginine is versatile and commonly utilized PTMs that until recently were under-recognized. An ever-greater number of proteins and cellular pathways are now known to be regulated by these modification states, including the splicing machinery and histones that are the foundation of many essential biological functions. Here, we focused on PRMT5 and highlighted its mechanisms of catalysis and substrate recognition, the somatic and cancerous biological processes that PRMT5 and its partner MEP50 participate in or are essential for, and showed the role PRMT5 and MEP50 play in early development. Current and forthcoming insights into PRMT5's molecular mechanisms of targeting specific proteins and catalyzing mono- and dimethylation

will provide crucial information for the development of specific small molecule inhibitors. Future research will clarify the role of PRMT5 in development and disease, while the development of specific small molecule inhibitors of PRMT5 may lead to novel chemotherapeutic approaches for cancer. However, caution is necessary in the potential use of specific PRMT5 inhibitors due to their multiple biological roles, suggesting possible toxicity from its inhibition. New studies targeting PRMT5, and redundancy with other methyltransferases such as PRMT7, and their multiple biological roles are necessary to fully understand how PRMT5 functions in health and disease. New tools, such as better methylarginine antibodies that can distinguish histone substrates and mono- and dimethylation states, as well as conditional knockouts in cell culture and animals will be essential for future elucidation of the important biological roles of PRMT5.

Acknowledgments N.S. and J.K. were supported by NIH/NIGMS [P20GM103395]. D.S. is funded by an NIH/NIGMS grant [R01GM108646] and by The American Cancer Society—Robbie Sue Mudd Kidney Cancer Research Scholar Grant [124891-RSG-13-396-01-DMC]. We are grateful to Emmanuel Burgos for structural and enzymatic insight and for rendering Fig. 6. The analysis shown in Fig. 7 is based upon data generated by the TCGA Research Network: http://cancergenome.nih.gov and from KM-plotter: http://kmplot.com. We thank the specimen donors to these projects for their essential contributions. We thank the many investigators studying PRMT5 and we apologize to the authors whose work on PRMT5 was not included due to space limitations.

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