**Cellular and Molecular Life Sciences**

REVIEW

# The protein arginine methyltransferase family: an update about function, new perspectives and the physiological role in humans

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**Abstract** Information about the family of protein arginine methyltransferases (PRMTs) has been growing rapidly over the last few years and the emerging role of arginine methylation involved in cellular processes like signaling, RNA processing, gene transcription, and cellular transport function has been investigated. To date, 11 PRMTs gene transcripts have been identified in humans. Almost all PRMTs have been shown to have enzymatic activity and to catalyze arginine methylation. This review will summarize the overall function of human PRMTs and include novel highlights on each family member.

## Introduction

Posttranslational modification of proteins is a hallmark of signal transduction where cells are able to react quickly to changes or events of the surrounding cells thereby expanding the structural and functional diversity of the proteome. The role of acetylation and phosphorylation of proteins have been extensively studied as highly reversible reactions for fine-tuning gene expression in response to external stimuli or changes in the environmental conditions. Recently, the importance of other types of protein modifications, including ubiquination and methylation,

have begun to be recognized [1]. The methylation of proteins and the enzymes that carry out these reactions increased the dimensions of the regulation of gene transcription by marking genes to be or not to be transcribed [2]. Protein methylation can occur on amino acids such as lysine, arginine, histidine, or proline, and on carboxy groups [3]. Arginine methylation of mainly nuclear proteins is an important posttranslational modification process involved in structural remodeling of chromatin, signal transduction, cellular proliferation, nucleocytoplasmatic shuttling, translation, gene transcription, DNA repair, RNA processing, or mRNA splicing [4–7]. Here, I will focus on the family members of the human protein arginine methyltransferases (PRMTs) which modify the characteristics of cellular proteins by transferring methyl residues to the basic amino acid arginine in target proteins and which are important for many benign or malign cellular processes.

## The discovery of protein arginine methyltransferases

The proteins included in the PRMT family are evolutionarily conserved between organisms but differ in the number of its members. Arginine methylation activity was discovered in nuclear thymus extracts over 40 years ago and, later, different family members were identified and purified from calf brain, rat liver, and different cell lines [8–12]. Shortly after the discovery of the proteins, the corresponding cDNAs were isolated from mammalian tissues [13–15]. It was only recently that novel proteins of this enzyme family were found using sequence comparison, database searches, or homology studies [16, 17]. From the group around W. K. Paik [18], the discover of the first methylase enzyme, an excellent historical review describing the multifaceted role of protein methylation was

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published. Currently, the following enzymes have been found: in yeast (*Saccharomyces cerevisiae*), there are four enzymes (*Rmt*1/*Hmt*1, *Rmt*2, *Rmt*3, *Hsl*7/*Skb*1); in the mold (*Aspergillus nidulans*), three enzymes (RMTA–C); in the fruit fly (*Drosophila melanogster*), nine enzymes (DART1–9); in the sea squirt (*Ciona intestinalis*), six enzymes (*Ci*1, *Ci*3–7); and in the zebrafish (*Danio rerio*), seven PRMT enzymes (*Zf*1–7). They are described in detail elsewhere [16, 19–22]. As new sequencing projects using many different species have been performed, the combination of relevant database mining with homology searches may reveal novel proteins and extend the PRMT family even further. So far in humans, 11 PRMT proteins have been identified based on differences of the primary protein sequence and of the specificity for distinct substrates. With the exception of PRMT2 and two recently identified putative PRMT genes (PRMT10 and PRMT11), all remaining proteins of the family possess enzymatic and catalytic arginine methylation activity.

## Biochemical reaction of PRMTs

The posttranslational modification of protein arginine methylation is widely appreciated, playing a vital role in cellular function. The PRMT enzymes remove one residue, the methyl group, from the donor molecule *S*-adenosyl-L-methionine (AdoMet) to generate the product *S*-adenosyl-L-homocystein (AdoHcy), and hereby transferring the residue to an acceptor molecule which is the terminal nitrogen atom of the guanidinium side chain of an individual arginine residue in the target protein [23, 24]. As there are three nitrogens in the guanidine group, putatively all of them could be methylated; the two $\omega$-guanidino nitrogen atoms and the internal $\delta$-guanidino nitrogen atom [25, 26]. Indeed, mono- and dimethylation reactions of arginine are found to occur in mammalians: $\omega$-$N^G$-monomethylarginine (MMA), symmetric $\omega$-$N^G,N'^G$-dimethylarginine (sDMA), or asymmetric $\omega$-$N^G,N^G$-dimethylarginine (aDMA) (Fig. 1). The third methylated arginine is generated by monomethylation of the internal $\delta$-guanidino nitrogen atom of arginine ($\delta$-$N$-methyl-L-arginine) and has so far been documented only for yeast proteins [27]. According to their methylation status, the PRMT enzymes were classified into different group types. While the type-I PRMT enzymes catalyze the formation of MMA and aDMA, the type-II PRMT enzymes form MMA and sDMA (Fig. 1). The enzymes PRMT1, PRMT3, PRMT4, PRMT6, or PRMT8 belonging to the type-I and PRMT5, PRMT7, or PRMT9 to the type-II enzymes [17, 22, 26]. The type-III PRMT enzymes which catalyzes methylation at the $\delta$-guanidino group in yeast are discussed elsewhere [27, 28]. Type-I and type-II PRMT enzymes were responsible for the arginine methylation of human proteins, and the list of proteins known to be methylated has been growing rapidly over the past decade detecting hundreds of proteins which include methylarginine residues. Interestingly, some of them have implications for disease processes and are involved in certain cancer types, cardiovascular disease, multiple sclerosis, and spinal muscular atrophy [24, 29, 30]. The first arginine methylated proteins identified in humans were histones and heterogenous nuclear ribonucleoproteins (hnRNP) [9, 10]. Recently, many additional proteins such as nucleolin, fibrillarin, and helicases have been found to be methylated by PRMT enzymes [18, 26]. There is also a growing list of biochemical and biological processes, such as signal transduction, proliferation, transcriptional regulation, and RNA splicing, where arginine methylation is involved. Interestingly, for many years it was unknown if the methylation process would be reversible by removing the methyl group from the corresponding amino acid in the target proteins. Only very recently have different enzymes been identified which counteract the methylation process by catalyzing a demethylation step and so remove methyl residues from the target proteins (e.g., LSD1, JMJD6) [31–33]. It should be pointed out that JMJD6, a Jumonji-domain-containing protein, is the only arginine specific demethylase so far identified [32]. These findings clearly indicate that the process of protein methylation is also, like acetylation or phosphorylation, a reversible step in the modification of active and repressed states of chromatin function [34].

## Structure and functional analysis of the PRMTs

The length of the PRMT proteins vary between 316 and 956 amino acids (Fig. 2, Table 1). All PRMTs have a common catalytic methyltransferase domain which consists of a highly conserved core region of around 310 amino acids and subdomains important for binding to the methyl donor and to the substrate [25]. The individual PRMT family members differ in unique N-terminal regions of variable length and distinct domain motifs (Fig. 2). Some of the enzymes, like PRMT2, PRMT3, or PRMT8, include SH3, zinc finger (ZnF), or myristoylation (Myr) motifs, respectively. In addition, PRMT4 has an unique C-terminal region, PRMT7 or PRMT10, a second catalytic domain, and PRMT9 or PRMT11, an additional module important for protein interaction (F-box). As all the functions of the N- or C-terminal regions of PRMTs are not fully understood, deletion analysis elucidated an impairment in stability as well as a decline in PRMT enzymatic activity [35–37]. Different regions of PRMT5 were able to interact with each other indicating the importance in formation of homomeric protein complexes [38]. Furthermore, an

**Fig. 1** Methylation of the arginine side chain by PRMTs. Depicted is the amino acid arginine in target proteins. Type-I (*TypeI*) and type-II (*TypeII*) enzymes catalyze the formation of MMA by transfer of a methyl group to the $\omega$-guanidino group. In addition, transfer of an additional methyl group results in aDMA (type-I enzymes), or sDMA (type-II enzymes). Type-III (*TypeIII*) enzymes transfer the methyl group to the internal $\delta$-nitrogen
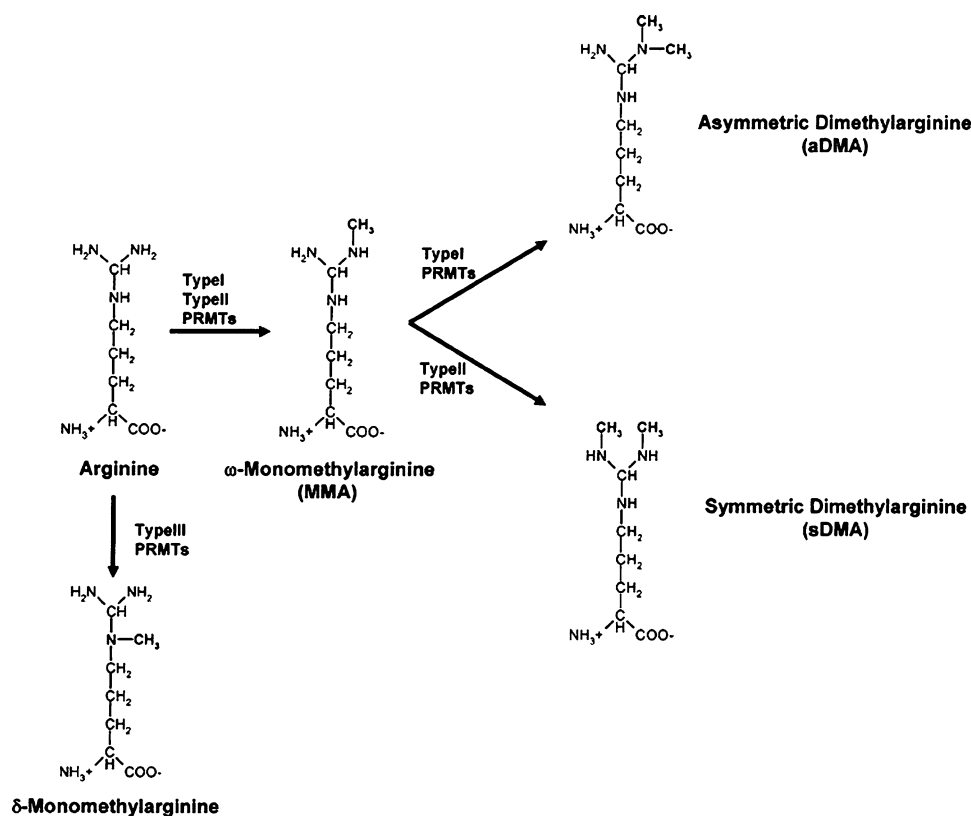
**Fig. 2** The human PRMT family. The different members are indicated as *boxes* and the protein length by the numbers of amino acids. Only the longest isoforms are shown. All PRMTs have at least one conserved Catalytic Domain (*shaded gray*). Different additional domains are highlighted (*green*): *SH3, ZnF* zinc finger, *Myr* myristoylation, *F-box, TPR* tetratricopeptide, *NosD* nitrous oxidase accessory protein
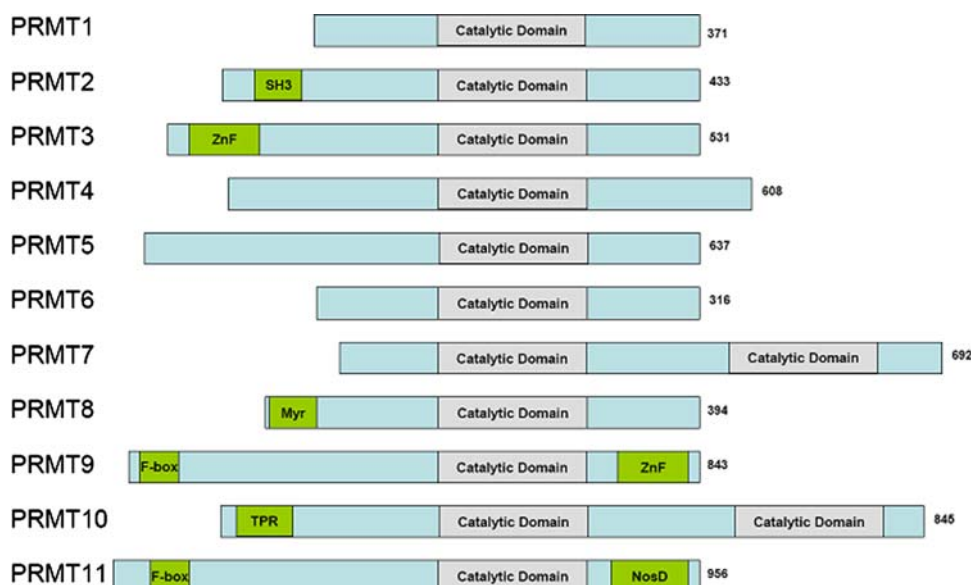
autonomous activation domain in the C-terminus of PRMT4 was important for the coactivator function of the protein [39]. At the time, only the structures of type-I PRMTs were solved using X-ray crystallography studies such as PRMT1, PRMT3, or *HMT*1p [36, 37, 40].

## The PRMT family members

Until now, 11 PRMTs have been identified in humans which differ in sequence and length (Fig. 2). In addition, different splice variants coding for the corresponding

**Table 1** The human PRMT family members

| Name | PRMT1 | PRMT2 | PRMT3 | PRMT4 | PRMT5 | PRMT6 | PRMT7 | PRMT8 | PRMT9 | PRMT10 | PRMT11 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Other aliases/synonyms | ANM1<br>HCP1<br>IR1B4<br>HRMT1L2 | MGC11137<br>HRMT1L1 | HRMT1L3 | CARM1 | JBP1<br>SKB1<br>IBP72<br>SKB1hs<br>HRMT1L5 | FLJ10559<br>HRMT1L6 | FLJ10640<br>KIAA1933 | HRMT1L4 | FBXO11<br>VIT1<br>UBR6<br>FLJ12673<br>MGC44383 | LOC90826<br>FLJ46629 | FBX10<br>FBXO10<br>FLJ41992<br>MGC149840 |
| Enzyme type | I | Unknown | I | I | II | I | II | I | II | Unknown | Unknown |
| Chromosomal localization | 19q13.3 | 21q22.3 | 11p15.1 | 19p13.2 | 14q11.2 | 1p13.3 | 16q22.1 | 12p13.3 | 2p16.3 | 4q31.23 | 9p13.2 |
| RefSeq transcript(s) | | | | | | | | | | | |
| Variant 1 | NM_001536 | NM_206962 | NM_005788 | NM_199141 | NM_006109 | NM_018137 | NM_019023 | NM_019854 | NM_025133 | NM_138364 | NM_012166 |
| Variant 2 | NM_198319 | NM_001535 | | | NM_001039619 | | | | NM_018693 | | |
| Variant 3 | NM_198318 | | | | | | | | NM_012167 | | |
| Variant 4 | | | | | | | | | AK022735 | | |
| RefSeq protein(s) | | | | | | | | | | | |
| Isoform 1 | NP_001527 | NP_996845 | NP_005779 | NP_954592 | NP_006100 | NP_060607 | NP_061896 | NP_062828 | NP_079409 | NP_612373 | NP_036298 |
| Isoform 2 | NP_938075 | NP_001526 | | | NP_001034708 | | | | NP_061163 | | |
| Isoform 3 | NP_938074 | | | | | | | | NP_036299 | | |
| Isoform 4 | | | | | | | | | BAB14214 | | |
| Length Isoform 1 (aa) | 371 | 433 | 531 | 608 | 637 | 316 | 692 | 394 | 843 | 845 | 956 |
| Length Isoform 2 (aa) | 347 | 433 | | | 620 | | | | 686 | | |
| Length Isoform 3 (aa) | 353 | | | | | | | | 585 | | |
| Length Isoform 4 (aa) | | | | | | | | | 561 | | |
| Cellular localization | | | | | | | | | | | |
| Nucleus | + | + | − | + | − | + | + | − | + | Unknown | Unknown |
| Free cytoplasm | + | + | + | − | + | − | + | − | + | Unknown | Unknown |
| Plasma membrane | − | − | − | − | − | − | − | + | − | Unknown | Unknown |

Listed are the PRMT family members, their aliases and synonyms, the enzyme types, and the chromosomal localization in the human genome. The RefSeq transcripts of the variants and the RefSeq protein isoforms are indicated as described in GenBank. The length of the major isoforms are included with the corresponding amino acids (aa). The cellular localization of the PRMTs are indicated as positive (+) or negative (−) in the nucleus, free cytoplasm, or the plasma membrane

PRMT isoforms are recognized (Table 1). With the exception of the localization of PRMT1 and PRMT4 on human chromosome 19, all other PRMTs are located on different chromosomes (Table 1). In humans, five PRMTs belong to the type-I and three to the type-II enzymes. For the remaining three proteins (PRMT2, PRMT10, PRMT11) the enzymatic activity as well as the methylation function have not so far been determined (Table 1). PRMT proteins were found in the nucleus and/or the cytoplasm, or even attached to the plasma membrane (Table 1). Phylogenetic studies of the PRMT protein sequences using guide-tree calculation based on the sequence distant method and applying the neighbor-joining algorithm indicated a close relationship between the PRMT isoforms and the different PRMTs (Fig. 3) [41]. So the proteins PRMT1 and PRMT8 were more closely related to PRMT3, whereas PRMT11 and PRMT9 were closer to PRMT5 (Fig. 3). PRMT2, PRMT4 and PRMT6 were more distant to the other PRMTs (Fig. 3). Subsequently, we describe a detailed description of all the individual family members which are known up to now.

## PRMT1

PRMT1 as a type-I enzyme is involved in a variety of processes including gene transcription, DNA repair, and signal transduction [42–44]. PRMT1 was the first protein arginine N-methyltransferase in mammalian cells to be cloned and discovered independently from different groups as a protein interacting with the mammalian intermediate-early TIS21 protein and the leukemia-associated BTG1 protein, or with the intracellular domain of the IFNα receptor [13, 15]. In addition, PRMT1 was found by direct sequence comparison of human expressed sequence tags (ESTs) with the previously cloned homolog protein from yeast (HMT1p) [45]. PRMT1 as the predominant type-I PRMT present in mammalian cells and tissues is expressed in every cell type investigated [46–48]. There are at least three different transcript variants described coding for

isoform between 371 and 353 amino acids: isoform 1 (371aa), isoform 2 (347aa), and isoform 3 (353aa) (Table 1). Interestingly, the relative prevalence of alternatively spliced variants were different between normal and cancerous breast tissues making this a valuable tool as a prognostic marker in breast cancer [42]. Close inspection of the genomic organization of the PRMT1 gene revealed up to seven protein isoforms that are expressed in a tissue-specific manner [49]. The variants 4–7 are expressed in distinct tissues such as heart, pancreas, or muscle only, whereas variants 1–3 are expressed ubiquitously. In addition, the different variants are also localized predominantly in the nucleus (variants 1 and 7), the cytosol (variant 2), or both compartments (variants 3–6). Because it is the predominant enzyme in mammalian cells and accounting for most of the cellular activity, PRMT1 was the first arginine methylase discovered. Therefore, it is not surprising that particularly for this enzyme over 40 substrates are already described and discussed elsewhere [26, 48]. The methylation of histone H4 to regulate gene transcription and of the elongation factor SPT5 which regulates its interaction with RNA polymeraseII were two important processes mediated by PRMT1 [11, 50]. Furthermore, PRMT1 methylates proteins involved in RNA processing such as poly(A)-binding proteins and proteins in DNA repair and checkpoint control [51–53]. Besides ribosomal and RNA-binding proteins, a kinase adaptor protein (SAM68) is methylated by PRMT1 indicating a role in cell cycle regulation [54]. Recently, it was shown that PRMT1 played a role in rapid estrogen signaling by methylating arginine residues within the estrogen receptor α (ERα) in breast cells [55]. PRMT1 is also involved in interferon signaling by transferring methyl residues to the protein inhibitor of activated STAT1 (PIAS1) [56]. In addition, PRMT1 was able to cooperate with other PRMTs to regulate gene transcription [57]. The PRMT1 binding proteins such as the antiproliferative protein BTG1 or the deadenylase hCAF1 are able to interact with PRMT1 and thereby regulate the activity of the methylase in a substrate-dependent manner [58, 59]. Interestingly, as most PRMT1 variants are predominantly



Fig. 3 Phylogenetic analysis of the protein sequences of all PRMTs using guide-tree calculations. The length of the *lines* indicate the relationship and distance between the PRMT proteins. The sequences for the different PRMTs and the isoforms (indicated by the additional *number*) are from Table 1
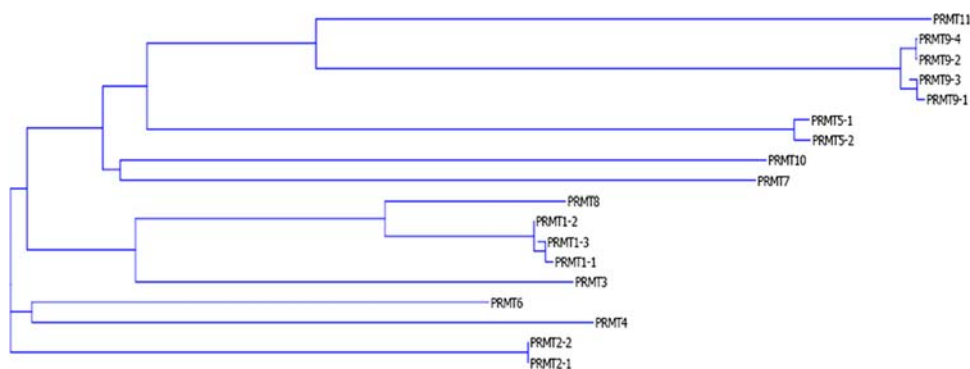
localized in the nucleus and some in the cytoplasm as well, an independent role of the protein in both compartments is likely [35, 49, 60]. The structure of the mammalian PRMT1 revealed a two-domain structure composed of the AdoMet binding domain and a barrel-like domain letting the active site be situated inbetween [40].

## PRMT2

PRMT2 was identified by sequence homology with the human PRMT1 and the yeast methyltransferase [45, 47]. PRMT2 transcripts were detected in most human tissues with an increased expression in heart, prostate, ovary, and the neuronal system [47, 61]. There are two splice variants described in the human genome which differ only in the second exon (Table 1). As the translation start site of PRMT2 is localized in the following exon (exon 3), both variants encoded the identical protein of 433 amino acids. Interestingly, the C-terminus of PRMT2 possessed homology to the SH3 domain of the human SRC oncogene which was essential for the interaction with the heterogeneous nuclear ribonucleoprotein E1B-AP5 (Fig. 2) [62]. Analysis using two-hybrid screening approaches identified PRMT2 as interacting with different nuclear hormone receptors such as the ERα [63] and the androgen receptor (AR) [61]. It is of importance to note that, in contrast to the publication of Qi et al. [63], the binding of PRMT2 to the C-terminal AF2 domain of ERα was dependent on the presence of estrogen [61]. The amplification of ER signaling by PRMT2 strongly depends on the cellular background and differs between neuroblastoma and prostate cells [61]. Furthermore, even if a nuclear localization signal in the PRMT2 protein sequence could not be detected, PRMT2 was found predominantly in the nucleus and to a lower degree also in the cytoplasm of mammalian cells [62]. Furthermore, trafficking of PRMT2 between different cellular compartments was ligand-dependent because an AR agonist was able to translocate PRMT2 together with the nuclear receptor from the cytosol to the nucleus, whereas an AR antagonist could not induce this effect [61]. At the time, the enzymatic activity of PRMT2 could not be demonstrated and therefore no specific substrates were revealed. The high homology between the sequences of PRMT1 and PRMT2 especially in the catalytic core and the substrate binding domain indicated that similar enzymatic activity between both proteins may exist [45]. Also, the interaction and the colocalization of both PRMT2 and the E1B-AP5 protein in the nucleus implied that the protein may be methylated by PRMT2 [62]. In addition, PRMT2 was able to bind its methyl donor indicating that a methylation step would be conceivable [61, 63]. Future studies will have to elucidate this aspect further.

## PRMT3

It was suggested that PRMT1 was predominantly present as a component of a polypeptide complex and therefore a two-hybrid screening approach identified PRMT3 as a novel protein binding to PRMT1 [35]. One variant was isolated encoding a protein with 531 amino acids (Table 1). PRMT3 belongs to the type-I enzyme and is expressed widely in human tissues with subcellular localization in the cytoplasm [25, 35]. An important feature of PRMT3 is the ZnF domain in the amino-terminal part of the protein (Fig. 2). Using deletion studies of this motif, it was concluded that this domain confers substrate specificity and appears to be required for the enzyme to bind and methylate target proteins associated with RNA [35]. PRMT3 was able to transfer methyl groups to ribosomal and RNA-binding proteins [65]. Interestingly, the interaction of a tumor suppressor gene important in lung carcinomas (DAL-1/4.1B) with PRMT3 inhibited its ability to methylate cellular substrates and modulated its enzymatic activity negatively. This suggested an important mechanism through which the suppressor gene was able to affect tumor cell growth [66]. The crystal structure of the conserved core revealed a two-domain structure with the substrate binding, and the barrel-like domain provides a mechanism for the methylation reaction and proposing dimer formation of PRMT3 [37].

## PRMT4

PRMT4 first described from the laboratory of M. Stallcup as CARM1 (coactivator associated arginine methyltransferase1) was identified using a yeast two-hybrid approach as a novel protein interacting with GRIP1, a member of the p160 family of proteins which enhance transcriptional activation by nuclear receptors [67]. A single variant was isolated encoding a protein with 608 amino acids (Table 1). PRMT4 belongs to the type-I class of PRMT enzymes and its gene is expressed in all tissues investigated with an increased expression in heart, kidney, and testis [67]. The p160 coactivators, a family of three related p160 kDa proteins SRC1, SRC2 (GRIP1, TIF1), and SRC3 (p/CIP, RAC3, ACTR, ABI, TRAM1), serve primarily as coactivators by binding directly to the relevant nuclear receptors, and are able to recruit additional secondary proteins to mediate gene transcription. The PRMT4 protein is able to bind directly to the p160 family of coactivators and in doing so amplifying the nuclear receptor mediated transactivation of target genes [68]. Furthermore, PRMT4 can synergistically enhance the nuclear receptor function and influence gene activation of ER or AR regulated genes [61, 68–70]. In addition, the coactivator function of PRMT4

relies on its ability to transfer the methyl group to the amino-terminal tail of histone H3 after recruitment to the promoter by nuclear receptors and p160 coactivators. This is believed to link the process of methylation directly with transcriptional function [67, 68, 71]. Besides histones, mRNA stabilizing proteins are also methylated by PRMT4 indicating a possible role in post-transcriptional gene regulation [72, 73]. The identification of the methylation of a variety of splicing factors by PRMT4 couples transcription with mRNA processing [74]. A positive regulation of cell cycle gene like cyclin E and an involvement in estrogen-stimulated breast tumors was described for PRMT4 [75, 76]. The methylation of SRC3 by PRMT4 decreased the ER-mediated transactivation suggesting that PRMT4 not only activates transcription but also terminates hormone signaling by disassembly of the coactivator complex [77]. Interestingly, the methyltransferase activity of PRMT4 itself is negatively regulated through phosphorylation at a conserved serine residue in the substrate binding domain of the protein [78]. The nuclear localization of PRMT4 propose an involvement in muscle differentiation where PRMT4 plays a fundamental role during skeletal myogenesis by activating specifically myogenic genes [79]. Embryos with a targeted disruption of PRMT4 were small in size, died perinatally and had a defect during T-cell development [80, 81]. In these animals, estrogen-responsive gene expression was aberrant indicating genetic evidence for an important role of PRMT4 in hormone-mediated transcriptional regulation [80]. Recently, it was shown that PRMT4 is involved in lipid metabolism by promoting adipocyte differentiation, suggesting an important role in adipose tissue biology [82]. PRMT4 has both chromatin and nonchromatin substrates, and methylation can function as a unique transcriptional switch by selectively impairing cAMP-induced transcription while stimulating nuclear receptor target genes [83]. Because these signaling pathways are developmentally and physiologically important, PRMT4 methylation might have unexpectedly broad biological significance [83]. Finally, it was described that PRMT4 was able to cooperate with *PRMT*1 and be involved in STAT5- and NF-$\kappa$B-dependent gene expression or in transcriptional activation by the tumor suppressor p53 [57, 84, 85].

## PRMT5

PRMT5 was isolated in a two-hybrid search for proteins interacting with the Janus tyrosine kinase (Jak2), implying a role in cytokine-activated transduction pathways [86]. In human tissues, PRMT5 is widely expressed with some higher level in heart, muscle, and testis [86]. Two variants which differ in the first exon are described and code two

isoforms with 637 and 620 amino acids in length, respectively (Table 1). PRMT5 was the first type-II enzyme identified that can result in the formation of sDMA residues [87]. PRMT5 plays a significant role in control and modulation of gene transcription, as the proteins methylated by PRMT5 are important in the regulation of genes such as IL-2 and cyclin E1 [88, 89]. PRMT5 was able to methylate histones and transcriptional elongation factors thereby affecting gene transcription [50, 86, 90]. In addition, PRMT5 is involved in the shaping of spliceosomes by forming methylosomes, complexes involved in the assembly of snRNA core particles where proteins were methylated symmetrically to be able to interact properly [91, 92]. In contrast to PRMT1, which is known to be mainly found in the nucleus, human PRMT5 protein is localized in the cytoplasm and able to form homo-oligomers [38]. Recently, it was shown that the histone-binding and selective adaptor protein COPR5 (cooperator of PRMT5), was able to bind PRMT5 thereby modulating the enzyme substrate specificity and influencing the regulation of genes [93]. In addition, PRMT5 was able to transfer methyl residues to the tumor suppressor p53 and help to discriminate between the cell cycle response and the apoptotic response [94]. Furthermore, as arginine methylation has the potential to alter the effects of p53 activation, it may therefore provide a suitable drug target for the manipulation of the p53 pathway [94]. Finally, knock-out studies in animals show that animals heterozygous for a PRMT5 deletion were viable without obvious pathologies whereas homozygous animals lacking PRMT5 led to immediate death of the zygotes [17].

## PRMT6

PRMT6 was identified by searching the human genome for PRMT family members using the characteristic structure of the methyltransferase motif [95]. One splice variant expressed predominantly in kidney, brain, and testis was isolated and encodes for a protein with 316 amino acids making PRMT6 the smallest protein in the PRMT family (Table 1). It was shown that PRMT6 displays a strong nuclear localization, as does PRMT4 [95]. PRMT6 belongs to the type-I enzyme of PRMTs catalyzing the formation of aDMA (Table 1). Interestingly, the methylation of selected proteins of the human immunodeficiency virus type-1 (HIV-1) by PRMT6 down-regulated gene expression by acting as a restriction factor for viral replication representing a form of innate cellular immunity [96–98]. In addition, PRMT6 methylated proteins from the high mobility group A (HMGA1a) family of architectural nuclear factors which were important in chromatin dynamics, placing PRMT6 in the context of chromatin

structure organization [99, 100]. Recently, PRMT6 methylation of arginine in histone H3 has also been shown to play an important role in post-translational modification [101].

## PRMT7

PRMT7 was cloned independently from two groups by sequences homology search that match known PRMT enzymes and by comparison of motifs containing the consensus substrate binding site of PRMT enzymes [102, 103]. One splice variant mainly expressed in dentritic cells, in the thymus and the reproductive system, codes for a protein of 692 amino acids (Table 1) [104]. It was shown that the PRMT7 protein is localized in both cellular compartments: the nucleus and the cytosol of mammalian cells [102]. PRMT7 was initially characterized in hamster cells as a protein that modulates drug sensitivity to DNA-damaging agents [104]. PRMT7 is unusual among the other family members in that two PRMT core domains are present [103]. The gene seems to be derived from a gene duplication event resulting in two putative substrate binding motifs (Fig. 2). For the functionality of the enzyme, both domains are required as each separate domain was unable to function alone [102]. Beside PRMT5, PRMT7 was characterized as a type-II methyltransferase that was able to synthesize sDMA residues in proteins and was able to methylate proteins such as histones, myelin basic protein, fibrillarin, and spliceosomal proteins [17, 102, 103]. PRMT7 is involved in cancer treatment as inhibitor of the enzyme activity sensitizing cancer cells to chemotherapeutics [105].

## PRMT8

PRMT8 was revealed by searching of the public human genomic, cDNA, and EST databases. This enzyme shares over 80% sequence identity with the predominant PRMT1 protein making this the highest degree of homology within the enzyme family of PRMTs (Fig. 3) [106, 107]. Expression analysis using Northern analysis revealed a unique tissue-specific expression as PRMT8 transcripts were largely found in human brain [106]. Only a few PRMT8 ESTs were identified in cDNA libraries from lung, testes, pharynx, and kidney origin making it possible that minor expression occurs in these tissues [106]. That feature lacks all the other enzymes of the methyltransferase family as they are more widely expressed in human tissues [106]. The single variant of PRMT8 codes for a protein of 394 amino acids (Table 1). The PRMT8 protein sequence revealed a Myr motif at the N-terminal end of the protein in

proximity with some basic amino acids that facilitate electrostatic interactions with membrane lipids (Fig. 2). Indeed, by using PRMT8 fusion or mutation constructs, it could be shown that the cellular localization of PRMT8 is not the nucleus or the cytoplasm but its association with the plasma membrane using the unique Myr motif in the protein [106, 107]. PRMT8 belongs to the type-I enzymes of methytransferases such as PRMT1, PRMT3, PRMT4, and PRMT6. Because of the close sequence homology of PRMT8 with PRMT1, both proteins have some overlapping substrates such as histones, RNA-binding proteins (e.g., hnRNPs) or the EWS protein responsible for causing Ewing sarcoma family tumor [108, 109]. Over 20 proteins able to bind to PRMT8 were identified which contain putative methylation motifs and could be potential substrates for the enzyme, such as the cytoplasmic phosphoprotein caprin which plays a significant role in cell proliferation [109]. In addition, it was shown that the interaction between the methyltransferase PRMT8 and its potential substrate protein is maintained, although the substrate is completely methylated [109]. This suggests that PRMT8 has, besides the methyltransferase activity, another additional functional activity on the plasma membrane [109]. Interestingly, PRMT8 can interact with PRMT1 to form dimers, and its enzymatic activity is regulated by altering the conformation of the protein at the N-terminal end [106, 107].

## PRMT9

PRMT9, also known as F-box only protein 11 (FBXO11), was identified by motif search using part of the sequence of the conserved methyl donor binding domain of PRMTs [110]. PRMT9 has little sequence similarities with other PRMTs but the core domains of PRMT9 are more similar to PRMT5 and PRMT7 suggesting that PRMT9 may also belong to the type-II class of the enzyme (Fig. 3). Indeed, biochemical analysis determined that PRMT9 was able to transfer methyl residues to arginine in target proteins [110]. PRMT9 is, like most of the PRMTs, widely expressed and found in a variety of human tissues and associated with pigment loss in a chronic skin disease [111]. There are four major transcripts in the PRMT9 locus in humans encoding four isoforms between 843 and 561 amino acids: isoform 1 (843aa), isoform 2 (686aa), isoform 3 (585aa), and isoform 4 (561aa). All isoforms of PRMT9 contain the putative donor binding domain and, except for the shortest isoform, a F-box in the N-terminal region (Fig. 2) [110]. The F-box is a protein motif of around 50 amino acids that functions as an important side for protein–protein interaction [112, 113]. The longest isoform of PRMT9 includes a ZnF motif at the C-terminus indicating possible interaction with

nucleic acids, but no functional information is presently known (Fig. 2) [110]. Only for the shortest PRMT9 isoform 4 is a direct enzymatic activity described, and methylation of histones, the maltose binding protein (MBP) and several peptides, could be demonstrated [110]. Furthermore, PRMT9 has been identified as a type-II methyltransferase as it synthesizes MMA and sDMA [110]. By using transfection experiments to localize PRMT9 in mammalian cells, it was determined that PRMT9 is found throughout the cell including the nucleus and the cytoplasm [110]. Recently, an association between polymorphism in the PRMT9 gene and inflammation of the middle ear was shown, indicating the importance of methylation in disease [114, 115]. Interestingly, besides regulating development, PRMT9 was identified as an adaptor protein to be responsible for posttranslational modification of the tumor suppressor gene p53 by inhibiting the transcriptional activity without affecting its stability [116, 117]. As PRMT9 is a Nedd8 ligase for p53, a direct relationship between neddylation of the suppressor protein and the methyltransferase activity is unknown [116].

## PRMT10

PRMT10 was predicted by its homology to PRMT7 [16]. One human transcript encoding a protein with 845 amino acids is described (Table 1). No biochemical activity or substrates have been determined but the resemblance to PRMT7 suggested that PRMT10 may also belong to the type-II arginine methyltransferases [17]. A second catalytic domain at the C-terminus propose similar activity with PRMT7 but no experimental data about enzyme activity, substrate specificity, or function is presently known [16, 17]. In PRMT10, the N-terminal tetratricopeptide repeat (TRP) motif consists of 34 amino acids and, as a protein–protein interaction module, it is involved in different cellular functions such as cell cycle control, transcription, and splicing [118]. The TRP motif is conserved in evolution indicating functional and fundamental importance, but the link with methylation is presently unknown [118].

## PRMT11

PRMT11 was found by homology search using the sequence of PRMT9. One variant, encoding for 956 amino acids, is described and makes PRMT11 the longest member of the enzyme family [16, 17]. At the N-terminus of the PRMT11 protein, a F-box motif was identified which enables other proteins to bind, similar as described for PRMT9 (Fig. 2) [114]. GenBank analysis shows a nitrous

oxidase accessory protein (NosD) conserved C-terminal motif that may be important for inorganic ion transport and metabolism (Fig. 2). Because of the similarity with PRMT9, it is predicted that PRMT11 also has a methyltransferase activity and may belong to the type-II of PRMT enzymes, but no biochemical data and functional information are known at this time [17].

## Concluding remarks

The transfer of methyl groups to the arginine residues in target proteins is a process involved in posttranslational modification and is being recognized as a very important step for the modification of proteins and their subsequent function. At this time, there are 11 family members of the human protein arginine methyltransferase (PRMTs) described which are expressed in many tissues. For most of the proteins, the transfer of methyl residues to the basic amino acid arginine in target proteins can be directly shown and different enzyme types have been classified. The importance of the PRMT protein family is underlined by the presence of additional isoforms in humans and the evolutionary conservation in a wide variety of eukaryotic genomes. Besides the methylation activity, many PRMTs are involved in transcriptional regulation, can interact with other proteins, or can replace each other's function. Further work will be necessary to elucidate additional novel functions of the enzyme family. Finally, as some new enzymes are already being described, more proteins may be found which reverse the methylation and demethylate arginine residues.

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