

PRMT8, a New Membrane-bound Tissue-specific Member of the Protein Arginine Methyltransferase Family*

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Protein arginine methylation is a common post-translational modification that has been implicated in signal transduction, RNA processing, transcriptional regulation, and DNA repair. A search of the human genome for additional members of the protein arginine *N*-methyltransferase (PRMT) family of enzymes has identified a gene on chromosome 12 that we have termed PRMT8. This novel enzyme is most closely related to PRMT1, although it has a distinctive N-terminal region. The unique N-terminal end harbors a myristoylation motif, and we have shown here that PRMT8 is indeed modified by the attachment of a myristate to the glycine residue after the initiator methionine. The myristoylation of PRMT8 results in its association with the plasma membrane. The second singular property of PRMT8 is its tissue-specific expression pattern; it is largely expressed in the brain. A glutathione *S*-transferase fusion protein of PRMT8 has type I PRMT activity, catalyzing the formation of ω -*N*^G-monomethylated and asymmetrically ω -*N*^G,*N*^G-dimethylated arginine residues on a recombinant glycine- and arginine-rich substrate. PRMT8 is thus an active arginine methyltransferase that is membrane-associated and tissue-specific, two firsts for this family of enzymes.

Arginine methylation is a widespread post-translational modification that is mediated by the protein arginine methyltransferase (PRMT)⁴ family of enzymes (1). In mammals, the family of protein arginine *N*-methyltransferases falls into two groups, type I enzymes that ω -*N*^G-monomethylate and asymmetrically ω -*N*^G,*N*^G-dimethylate arginine residues (ADMA) and type II enzymes that ω -*N*^G-monomethylate and symmetrically ω -*N*^G,*N*^G-dimethylate arginine residues. PRMT1, -3, -4, and -6 are type I enzymes, and PRMT5 is a type II enzyme. The post-translational methylation of arginine residues has been shown to modify protein function by regulating protein-protein interactions, both negatively and positively (2–4). Arginine methylation is involved in a number of cellular systems, including DNA repair, RNA transcription, signal transduction, protein compartmentalization, and possibly also protein translation (5).

The first PRMT to be identified and characterized was the yeast enzyme hnRNP methyltransferase/arginine methyltransferase 1 (6, 7). Shortly thereafter, PRMT1 was identified as the predominant mammalian type I enzyme (8). PRMT2 was recognized because of its homology to known arginine methyltransferases (9); however, activity for this enzyme has yet to be demonstrated. PRMT3 was isolated as a PRMT1-binding partner in a yeast two-hybrid screen (10). CARM1 (PRMT4) was also identified in a yeast two-hybrid screen as an enzyme that binds the steroid receptor coactivator GRIP1 (11). PRMT5 was cloned as a Jak2-binding protein (12). PRMT6 was identified by homology to other members of this enzyme family (13). PRMT7 was isolated in a genetic screen for suppressor elements that confirm resistance to a topoisomerase II inhibitor (14).

The different PRMTs display varying degrees of substrate specificity. PRMT1, -3, -5, and -6 all methylate a glycine/arginine-rich (GAR) motif (10, 13, 15, 16). CARM1 methylates relatively few substrates, none of which harbor a GAR motif, and currently, the CARM1 methylation motif remains ill-defined (11, 17). There is emerging evidence that the type I and type II enzymes can methylate some of the same substrates. This occurrence has been shown for the splicing factor SmB (18), the transcription elongation factor SPT5 (19), and for histone H4 (20). Thus, there are PRMT substrates that carry both symmetric ω -*N*^G,*N*^G-dimethylarginine and ADMA residues at the same time, as well as substrates on which the same arginine residue can be either symmetrically or asymmetrically methylated.

Here we characterized the eighth member of the PRMT family, PRMT8. This enzyme displays type I activity and is able to methylate a GAR motif from fibrillarin. The primary amino acid sequence of PRMT8 is very similar to ubiquitously expressed PRMT1, but PRMT8 is specifically expressed in the brain. Also unique to PRMT8 is an N-terminal extension that harbors a myristoylation motif and a patch of basic residues, which facilitate the membrane targeting of this enzyme.

EXPERIMENTAL PROCEDURES

Full-length PRMT8 Plasmid Construction—To generate a full-length construct of PRMT8, we used the reported Hrmt113 (GenBankTM accession number BK001349) sequence to design primers for PCR amplification. Human “full-length” cDNA (Panomics) was used to amplify PRMT8 by PCR using the primers 5'-CTT GAA TTC ACC ATG GGC ATG AAA CAC TCC-3' and 5'-TCA GTC GACT GA CGC ATT TTG TAG TCA TTA-3'. The PCR product was then digested with EcoRI and SalI and used to construct both GST-PRMT8 and GFP-PRMT8 by subcloning in-frame into pGEX-6P-1 (Amersham Biosciences) and pEGFP-N1 (Clontech, Inc.). FLAG-PRMT8 was generated by PCR with the primers 5'-CAT GAA TTC ATG GGC ATG AAA CAC TCC-3' and 5'-TAA GTT TAA ACT TAA CGC ATT TTG TAG TCA TT-3'. The product was digested with EcoRI and Pml and subcloned into a modified pTracer-EF vector (Invitrogen). The modified pTracer-EF vector contains a Kozak sequence, and ATG start codon,

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⁴ The abbreviations used are: PRMT, protein arginine *N*-methyltransferase; ADMA, asymmetric ω -*N*^G,*N*^G-dimethylarginine; CARM1, coactivator-associated arginine methyltransferase 1; GST, glutathione *S*-transferase; GAR, glycine/arginine-rich; GST-GAR, GST fusion protein containing the GAR N-terminal region of fibrillarin; GFP, green fluorescent protein; FLAG, peptide epitope DYKDDDDK; PVDF, polyvinylidene fluoride; EST, expressed sequence tag.

and a FLAG-tagged sequence. The site-directed mutant GFP-PRMT8(G→A) was generated using *Pfu* Advantage polymerase (Clontech, Inc.) and the primers 5'-CTT GAA TTC AAG ATG GCC ATG AAA CAC TCC-3' and 5'-TCA GTC GAC TGA CGC ATT TTG TAG TCA TTA-3'. The resulting product was digested with EcoRI and SalI and subcloned into pEGFP-N1 (Clontech, Inc.) and confirmed by sequencing.

Northern Blot Analysis—A human poly(A)⁺ RNA blot (Origene Technologies, Inc.) was probed with DNA corresponding to the PRMT8 open reading frame, which was liberated from the appropriate GFP fusion vector by restriction enzyme digestion. As the loading control, human β -actin DNA was used as a probe. DNA probes were labeled with [³²P]dCTP using a Prime-it II® kit (Stratagene). Northern blot analysis was performed following standard procedures as previously described (13). Probed blots were exposed either 7 days (PRMT8) or 4 h (β -actin) at -80 °C.

In Vitro Methylation—GST-GAR, GST-Npl3, and GST-PABPf have been described previously (13). Histones H3 and H4 were purchased from Roche Applied Science. Recombinant PRMT1 (1 μ g), CARM1 (1 μ g), and PRMT8 (1 μ g) were incubated with GST (1 μ g), GST-GAR (1 μ g), GST-Npl3 (1 μ g), histone H4 (1 μ g), and histone H3 (1 μ g) in the presence of 0.5 μ Ci *S*-adenosyl-L-[methyl-³H]methionine (79 Ci/mmol from a 0.5 mCi/ml stock solution) (Amersham Biosciences) for 1 h at 30 °C in a final volume of 30 μ l of phosphate-buffered saline. Reaction samples were boiled and separated on 15% SDS-PAGE and transferred onto PVDF membrane. Methylated proteins were visualized by fluorography after the membrane was sprayed with Enhance (PerkinElmer Life Sciences).

Amino Acid Analysis of Methylated GST-GAR—Methylation reactions were quenched by the addition of 60 μ l of 25% (w/v) trichloroacetic acid to precipitate proteins in 6 × 50-mm glass vials. Precipitated proteins were centrifuged at 4000 × *g* for 40 min at 25 °C. The supernatant was discarded, and the pellets were washed once with an equal volume of cold acetone (-20 °C). After an additional centrifugation for 20 min, the acetone was discarded and the pellets were allowed to dry. Acid hydrolysis was carried out on the dried pellet in a Waters Pico-Tag vapor-phase apparatus containing 200 μ l of 6 N HCl for 20 h *in vacuo* at 110 °C. The hydrolyzed samples were resuspended in 50 μ l of water mixed with 1 μ mol each of ω -N^G-monomethylarginine (Sigma product M7033; acetate salt) and asymmetric ω -N^G,N^G-dimethylarginine (Sigma product D4268; hydrochloride) as standards. Hydrolyzed amino acids and standards were loaded onto a Beckman AA-15 sulfonated polystyrene cation exchange column (0.9 diameter × 11 cm height) that was pre-equilibrated with Na⁺ citrate buffer (0.35 M in Na⁺, pH 5.27) at 55 °C and regenerated with 0.2 N NaOH. Approximately 1 ml/min column fractions were collected for analysis. ³H radioactivity was detected by adding 200 μ l from each fraction to 400 μ l of water, mixing with 5 ml of fluor, and counting on a scintillation counter. Unlabeled methylarginine standards were detected by analyzing 100 μ l of every other fraction by a ninhydrin method previously described (21).

Subcellular Localization—GFP-PRMT1- (13) and GFP-PRMT8-expressing plasmids were transiently transfected into HeLa cells. After 24 h, the cells were fixed with 2% formaldehyde solution in phosphate-buffered saline. The cells then were washed and stained with Texas Red[®]-X-phalloidin solution (Molecular Probes). Nuclei were stained with 4',6-diamidino-2-phenylindole solution. Images were captured using a confocal microscope (Nikon), and three-dimensional reconstruction was performed with MetaMorph imaging software (Universal Imaging Corporation).

In Vitro Myristoylation—HeLa cells were transiently transfected with plasmids expressing GFP, GFP-PRMT1, GFP-PRMT8WT, and GFP-

PRMT8(G→A). After 24 h, each plate was labeled with 50 μ Ci of [9,10 (*n*)-³H]myristic acid [51 Ci/mmol from a 1 mCi/ml stock solution] (Amersham Biosciences) for 5 h. This labeling is modified from a previously described protocol (22). After labeling, the cells were lysed and immunoprecipitated with an anti-GFP antibody (Molecular Probes). The samples were then separated on 12% SDS-PAGE and transferred onto PVDF membrane. Myristoylated proteins were visualized by fluorography. Using the same membrane, a Western blot was performed with an anti-GFP antibody (Covance Research Products, Inc.) to make sure each sample had roughly the same amount of immunoprecipitation.

Heterodimerization Experiment—The cloning of GFP-PRMT1, GFP-PRMT3, GFP-PRMT4, and GFP-PRMT6 expression plasmids has been described previously (13). These plasmids were individually transiently cotransfected with FLAG-PRMT8-expressing plasmids into HeLa cells. After 24 h, the cells were lysed and immunoprecipitated by anti-FLAG M2-agarose beads (Sigma). The beads were washed, boiled, and separated in duplicate on 12% SDS-PAGE and transferred onto PVDF membranes. Western blotting was performed with either an anti-GFP antibody (Covance Research Products, Inc.) or an anti-FLAG antibody (Sigma).

RESULTS

Characterization of a Novel PRMT—The members of the PRMT family all harbor the set of four conserved amino acid sequence motifs (I, post-I, II, and III) common to the seven β -strand methyltransferases (21, 23) as well as motifs specific for this class of enzymes (13, 24). A search of the publicly available human genomic, cDNA, and expressed sequence tag (EST) databases revealed a novel PRMT that has not yet been characterized. This PRMT has been annotated in the National Center for Biotechnology Information (NCBI) data base as hnRNP methyltransferase-like 4 (Hrmt114) (NM_019854) and Hrmt113 (BK001349). The later sequence has a slightly larger open reading frame. This enzyme shares a high degree of homology (over 80% identity) with the predominant arginine methyltransferase PRMT1 (Fig. 1A). The existence of this PRMT1-like molecule has been noted previously (24, 25), although the unique N-terminal region of 76 amino acids was not recognized. This N-terminal region is conserved between mouse and man (Fig. 1B), and an orthologue also exists in fish (*fugu*L3) (25). This PRMT1-like enzyme has recently been termed PRMT8 (5, 26), and we will use this nomenclature. Analysis of DNA databases generated from the human and mouse genome projects reveals that PRMT1 and PRMT8 are localized on different chromosomes. In mouse, mPRMT8 is on chromosome 6 and mPRMT1 is on chromosome 7, and in man, hPRMT8 maps to the subtelomeric region of chromosome 12 and hPRMT1 is on chromosome 19. The exon/intron boundaries of PRMT8 and PRMT1 are identical in both man and mouse, suggesting a recent duplication of an ancestral locus.

PRMT8 Is Expressed in Brain Tissue—To determine the expression pattern of PRMT8, Northern analysis was performed using RNA from a number of different human tissues. PRMT8 expression was only detected in the brain as a major transcript of 2.6 kb and two minor transcripts of 2.2 and 2.9 kb (Fig. 2). This same Northern blot has been previously probed with PRMT1 (13). Although this stripped Northern blot harbored no residual PRMT1 signal, we do see a faint band at 1.4 kb that corresponds to the size of PRMT1 after probing with PRMT8 cDNA. The cross reactivity of the PRMT8 probe with PRMT1 is not surprising, as they share 81% identity over 420 bp at the RNA level. In addition, a number of ESTs were identified for mouse and human PRMT8, and almost all of these sequences were generated from brain cDNA libraries or pooled libraries that contain brain cDNA. In the case of the mouse, a few ESTs were also seen in cDNA libraries that used eye

PRMT8 Is a Type I Arginine Methyltransferase

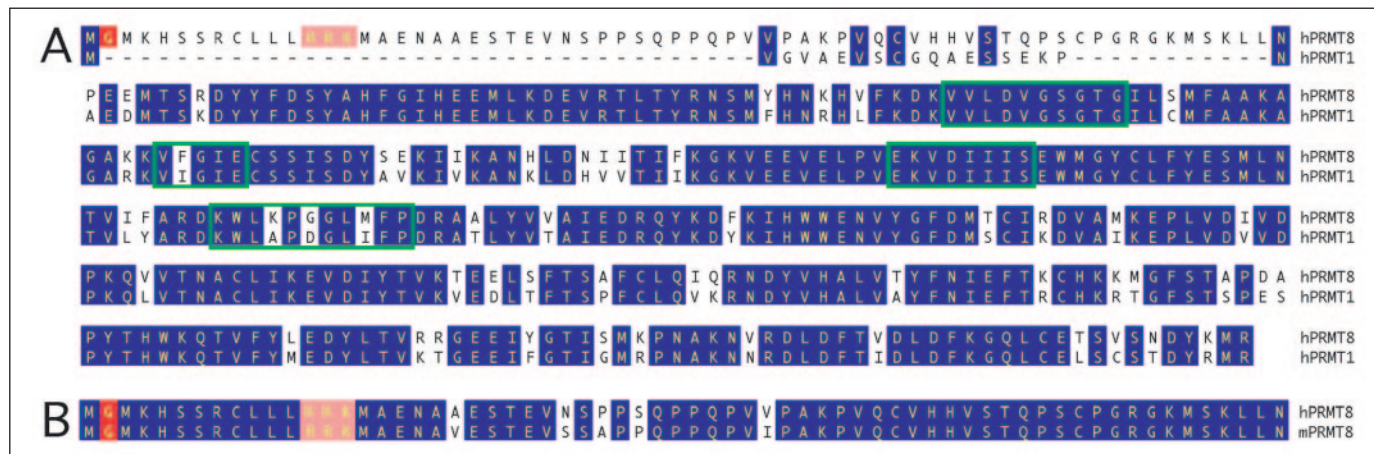


FIGURE 1. Amino acid sequence alignment of human PRMT8 and PRMT1. A, the full-length amino acid sequences of PRMT8 and PRMT1 are compared. Matching amino acids are boxed in solid blue. The signature methyltransferase motifs are boxed with green lines, which appear in the following order: I, post-I, II, III. B, the unique N-terminal ends (first 76 amino acids) of mouse and human PRMT8 are compared. The N-terminal glycine residue that functions as the site of myristoylation is highlighted in solid red. The cluster of basic amino acids close to the myristoylation motif is boxed in solid pink.

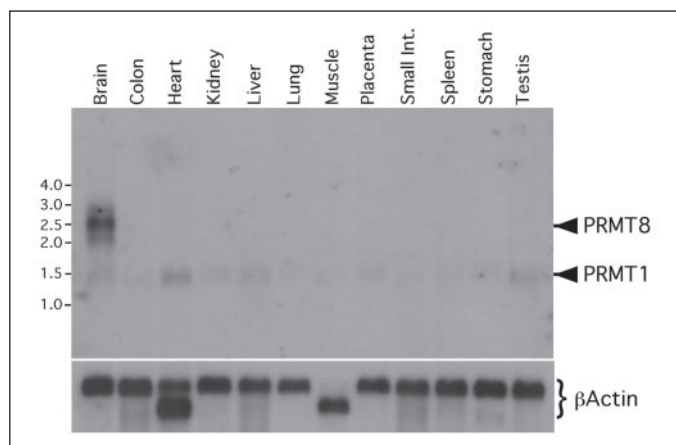


FIGURE 2. Northern analysis identifies PRMT8 as a brain-specific transcript. The expression level of PRMT8 in various human tissues was examined by hybridization to a multiple tissue Northern blot. The same membrane was probed with β -actin to confirm equal mRNA loading of the Northern blot.

and retina as a tissue source but in no other tissue. In humans, a few PRMT8 ESTs were also identified in cDNA libraries from lung, testes, nasopharynx, and kidney origin. Furthermore, the laboratory of Austin Smith recently identified PRMT8 (Hrmt113) as a transcript that is selectively expressed in neuroepithelial cells (27). This screen made use of Sox1-GFP knock-in mice to isolate neural precursor cells from embryos by flow cytometry. These cells were then used as a source of RNA for microarray analysis, and PRMT8 was identified as one of fifteen transcripts that were selectively expressed in neural progenitor cells from mid-gestation mouse embryos. Thus three independent lines of evidence suggest that PRMT8 is largely an enzyme of the brain: 1) Northern analysis reveals PRMT8 expression in brain and not in other tissues, 2) the majority of PRMT8 ESTs are from brain libraries, and 3) fluorescence-activated cell sorter-purified neural precursor cells initiate PRMT8 expression and identified it as an early marker.

PRMT8 Has Type I Protein Arginine N-Methyltransferase Activity—To date, four arginine methyltransferases have been shown to possess type I activity, which results in the generation of ω - N^G -monomethylarginine and ADMA residues. These enzymes include PRMT1 (8), PRMT3 (10), PRMT4 (28), and PRMT6 (13). Type II activity has been clearly shown for PRMT5 (16), and this activity may also be present for PRMT7 (29); PRMT7 has also been shown to display a strong propensity to monomethylate arginine residues (30). To establish whether PRMT8

is active and, if so, what type of activity it possesses, we generated a GST-PRMT8 fusion and assayed its methyltransferase activity using a panel of known arginine-methylated substrates. The substrates tested included GST-GAR, GST-Npl3, and histone H4, which are methylated by PRMT1, PRMT3, and PRMT6 (8, 13, 31). The CARM1-specific substrates GST-PABP and histone H3 (11, 17) were also included in the panel. PRMT8 preferentially methylated histone H4 and the recombinant forms of GAR and Npl3, thus displaying PRMT1-like substrate specificity (Fig. 3A).

To determine the type of activity of PRMT8, GST-PRMT8 was assayed for methyltransferase activity using GST-GAR as a substrate. GST-PRMT8 catalyzed the formation of both ω - N^G -monomethylarginine and ADMA (Fig. 3B), thus establishing that PRMT8 is a type I enzyme. No obvious automethylation activity is seen for PRMT8 (Fig. 3C). The fact that the catalytic regions of PRMT1 and PRMT8 are extremely similar (Fig. 1A) supports the findings that PRMT8 can methylate PRMT1 substrates *in vitro* and, similar to PRMT1, it possess Type I activity.

PRMT8 Is a Plasma Membrane-associated Enzyme—Arginine methyltransferases have been localized to both the cytoplasm and the nucleus of the cell. PRMT6 and CARM1 are primarily found in the nucleus, whereas PRMT3 and PRMT5 are mostly excluded from the nucleus (13, 32). PRMT1 and PRMT7 are found in both of these cellular compartments (13, 29). To determine the subcellular localization of PRMT8, we fused it to the N-terminal end of GFP. Due to its high degree of homology to PRMT1, we expected to observe a similar pattern of fluorescence signal. However, to our surprise, GFP-PRMT8 colocalized with phalloidin (Fig. 4A), which binds F-actin, and not with GFP-PRMT1 (Fig. 4B). A different field shows the same plasma membrane-associated pattern of GFP-PRMT8 localization (Fig. 4, C–F), which can be modeled using a three-dimensional rendering program to clearly demonstrate the exclusion of GFP-PRMT8 from both the nuclear and the free cytoplasmic compartments (Fig. 4G).

N-terminal Myristoylation Targets PRMT8 to the Membrane—Proteins can be concentrated on the plasma membrane by a number of possible mechanisms including: 1) a transmembrane region that has an α -helical fold covering 20–25 amino acids and is enriched in hydrophobic residues (33); 2) the covalent linkage of the protein to a fatty acid (prenylation, palmitoylation, myristoylation) that is incorporated into the lipid bilayer (34); or 3) the presence of a modular protein domain (PH, PX, FYVE, ENTH) that can bind the phospholipid membrane (35). Close scrutiny of the PRMT8 sequence revealed a myristoylation motif

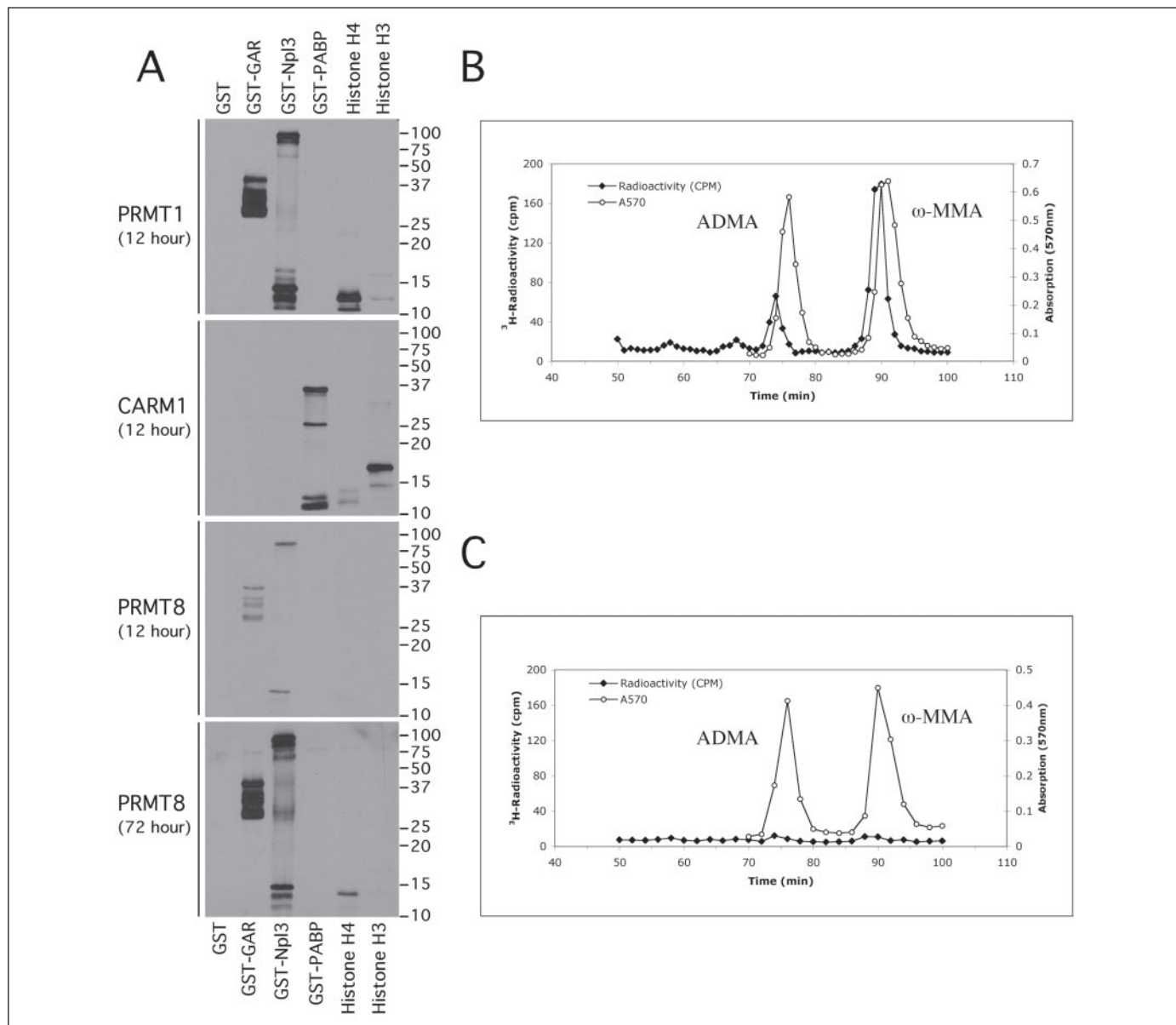


FIGURE 3. GST-PRMT8 is a type I protein arginine N-methyltransferase. *A*, recombinant PRMT8 (1 μg), PRMT1 (1 μg), and CARM1 (1 μg) arginine methyltransferases were incubated with a set of potential substrates (1 μg) in the presence of 0.5 μM 5-adenosyl-L-[methyl- ^3H]methionine for 30 min at 37 $^{\circ}\text{C}$ in a final volume of 30 μl of phosphate-buffered saline. The selected substrates included GST, GST-GAR, GST-Npl3, GST-PABP, and histones H3 and H4. *B*, purified GST-PRMT8 (2 μg) was incubated with GST-GAR (10 μg) and 5-adenosyl-L-[methyl- ^3H]methionine (1 μM) for 1 h at 37 $^{\circ}\text{C}$, trichloroacetic acid-precipitated, acid-hydrolyzed with 6 M HCl at 110 $^{\circ}\text{C}$ for 20 h, and loaded on a cation exchange column with unlabeled methyl-arginine standards ADMA and ω - N^{G} -monomethylarginine. *C*, methylation reaction in *B* was performed on GST-PRMT8 in the absence of the GST-GAR methyl-accepting substrate. Unlabeled asymmetric ω - N^{G} , N^{G} -dimethylarginine and ω - N^{G} -monomethylarginine standards reacted with ninhydrin to produce Ruheman's Purple (open circles) and radioactivity (diamonds) detected using 200 μl of fractions added to 400 μl of water and 5 ml of fluor (Safety Solve, Research Products International) three times for 3 min each.

at the unique N-terminal end of the protein. This consensus motif (MGXXX(S/T)) (36) is conserved between mouse and man (Fig. 1*B*) and the puffer fish (25). In addition, there is a patch of basic amino acids close to this motif that likely facilitates electrostatic interactions with the membrane lipids and contributes to the membrane association of myristoylated PRMT8. *N*-myristoylation occurs cotranslationally with the addition of a myristate to the N-terminal glycine (Gly-2) residue after the removal of the initiating methionine. For all myristoylated proteins studied, a glycine to alanine (G \rightarrow A) mutation at the Gly-2 position abolishes myristoylation (22, 36). To test whether PRMT8 harbors a functional myristoylation motif that is required for its membrane localization, we generated a G \rightarrow A mutant in GFP-PRMT8. The GFP moiety is fused to the C-terminal end of PRMT8, thus leaving the N terminus free for cotranslational modification. The GFP constructs

were transfected into HeLa cells and viewed under a confocal microscope. We clearly observed that, by altering a single amino acid (G \rightarrow A) at the N-terminal end of PRMT8, the fusion protein loses its ability to associate with the plasma membrane (Fig. 5, *A–D*) and acquires a localization similar to GFP-PRMT1.

To confirm that PRMT8 is indeed myristoylated at the Gly-2 position, HeLa cells were transfected with GFP, GFP-PRMT1, GFP-PRMT8, and GFP-PRMT8(G \rightarrow A) and metabolically labeled with [^3H]myristic acid for 5 h. The immunoprecipitated fusion proteins were separated by SDS-PAGE, transferred to a PVDF membrane, and subjected to fluorography (Fig. 5*E*). GFP-PRMT8 is labeled under these conditions, and the conversion of the acceptor glycine residue (Gly-2) to an alanine residue results in a loss of label. Thus PRMT8 is a myristoylated protein.

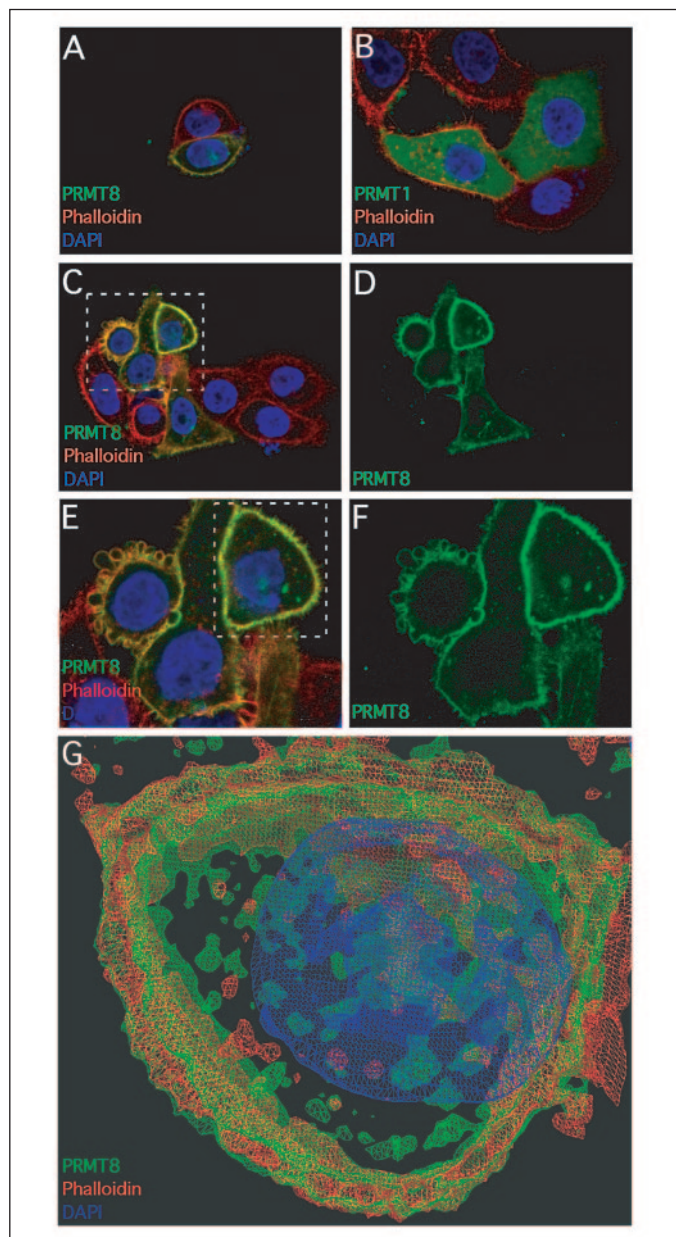


FIGURE 4. GFP-PRMT8 localizes to the plasma membrane of the cell. The GFP fusion constructs were transfected into HeLa cells, and protein localization was observed by confocal fluorescence microscopy. The subcellular localization of GFP-PRMT8 and GFP-PRMT1 are compared (A and B). A different view of GFP-PRMT8 localization, with (C and E) and without co-stain (D and F). G, the boxed region in E was serially scanned, and the sandwiched images were modeled using three-dimensional reconstruction to show the GFP-PRMT8 localization. Cells were also stained with 4',6-diamidino-2-phenylindole (blue) to mark the nuclei and with fluorescence-labeled phalloidin (red), which binds F-actin and decorates the plasma membrane and cytosol.

PRMT8 Homodimerizes and Heterodimerizes with PRMT1—The arginine methyltransferases PRMT1, hnRNP methyltransferase/arginine methyltransferase 1, and CARM1 can homodimerize (24, 37, 38) through a hydrophobic face called the antenna region. When dimerization of PRMT1 or hnRNP methyltransferase/arginine methyltransferase 1 is experimentally inhibited, no enzymatic activity is seen (24, 37). To investigate whether PRMT8 also exists as a homodimer in the cell, we cotransfected HeLa cells with FLAG-tagged PRMT8 with either GFP-PRMT8 or GFP. FLAG-PRMT8 was then immunoprecipitated, separated by SDS-PAGE, transferred to a PVDF membrane, and subjected to Western analysis with anti-GFP antibodies (Fig. 6A), demonstrating that PRMT8 can homodimerize. Next, using the same

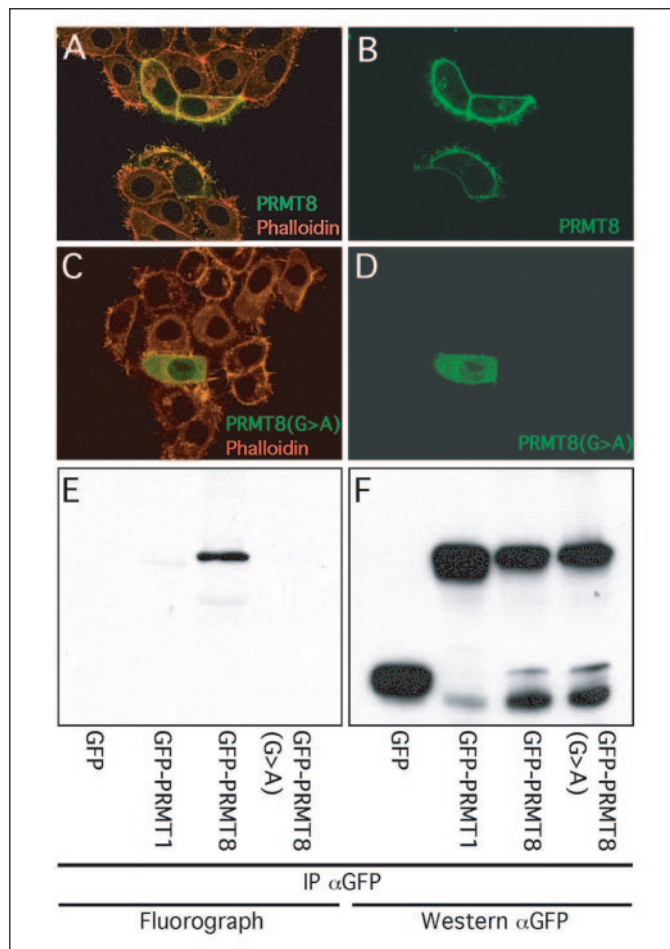


FIGURE 5. Myristoylation of PRMT8 facilitates its plasma membrane localization. The GFP-PRMT8 (A and B) and GFP-PRMT8(G→A) (C and D) constructs were transfected into HeLa cells and visualized by confocal fluorescence microscopy. E, GFP-PRMT8 was labeled with [³H]myristic acid. HeLa cells were transfected with GFP, GFP-PRMT1, GFP-PRMT8, and GFP-PRMT8(G→A) and metabolically labeled with [³H]myristic acid for 5 h. The GFP fusion proteins were immunoprecipitated with αGFP antibodies, separated by SDS-PAGE, transferred to a PVDF membrane, sprayed with Enhance (PerkinElmer Life Sciences), and subjected to fluorography for 3 days. F, after fluorography, the same membrane was subjected to Western analysis with αGFP antibodies to demonstrate the presence of similar amounts of GFP fusion protein in each lane.

approach, we tested the ability of PRMT8 to heterodimerize with other PRMTs. FLAG-PRMT8 could not only coimmunoprecipitate itself, but also GFP-PRMT1 (Fig. 6B), which is likely because of the high degree of sequence similarity between PRMT8 and PRMT1 (Fig. 1A). Thus, PRMT8 can both homodimerize and recruit PRMT1 activity (but not other PRMT activities) to the plasma membrane.

DISCUSSION

A comparison of mammalian PRMTs reveals that PRMT1 and PRMT8 share the highest degree of identity within this enzyme family. PRMT8 has orthologues in both puffer fish and zebra fish (25). However, a PRMT8 orthologue is not present in the sea squirt, which is an invertebrate chordate. This suggests that PRMT8 arose through a gene duplication of an ancestral PRMT1-like gene after the divergence of the vertebrates from other chordates. PRMT8 has a unique N-terminal region that bestows on it the ability to associate with the plasma membrane (Fig. 1B). The use of a non-conserved 5' exon has ensured that PRMT8 has evolved an exclusive property (myristoylation) that PRMT1 does not possess.

Apart from being the only PRMT to be localized to the plasma membrane, PRMT8 is also the only member of this family of enzymes that

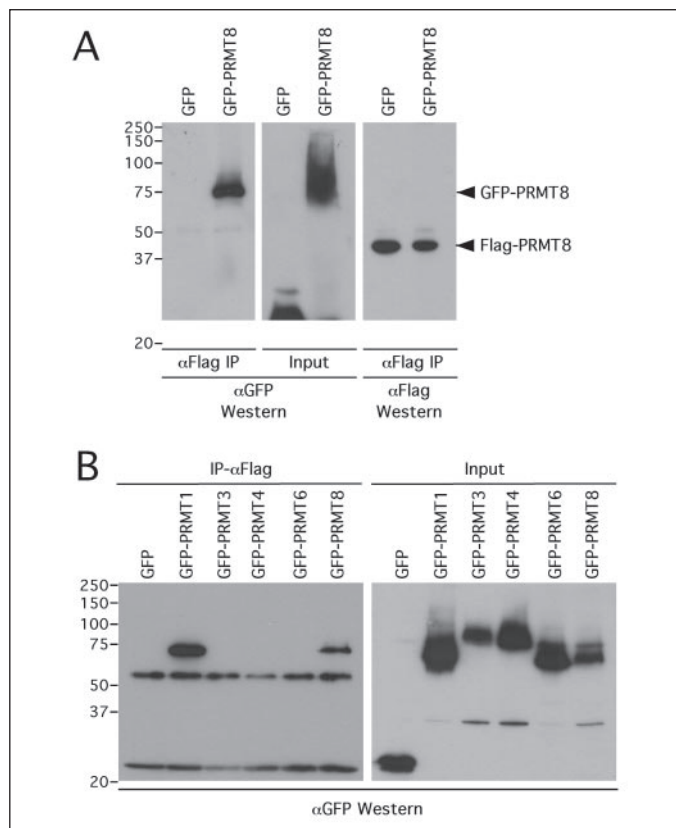


FIGURE 6. PRMT8 coimmunoprecipitates itself and PRMT1. A, FLAG-PRMT8 was cotransfected into HeLa cells with either GFP or GFP-PRMT8. An aliquot of the total cell lysate was subjected to Western analysis with α GFP antibodies to demonstrate the efficiency of transfection (*middle panel*). We immunoprecipitated the remainder of the total cell lysate with α FLAG antibodies and then performed Western analysis with α GFP (*left panel*) and α FLAG (*right panel*). B, a set of GFP-PRMT fusion proteins was cotransfected into HeLa cells with FLAG-PRMT8. The efficiency of transfection was assessed by α GFP Western analysis prior to immunoprecipitation (*right panel*). The ability of PRMT8 to coimmunoprecipitate other PRMTs was assessed by α GFP Western analysis after immunoprecipitation with α FLAG antibodies (*left panel*).

displays a tissue-specific expression pattern (Fig. 2). The largely brain-specific expression of PRMT8 suggests that it may play a role in neuronal differentiation. Indeed, protein methylation was first shown to play a role in nerve growth factor-mediated PC12 cell differentiation over 20 years ago (39, 40), when it was demonstrated that methyltransferase inhibitors interfere with nerve growth factor signal transduction. This observation was further expanded upon with the findings that methyltransferase inhibitors decrease neurite outgrowth (41) and that PRMT1-like activity is increased after the treatment of PC12 cells with nerve growth factor (42). PRMT8 may play an important role in the mediation of a nerve growth factor signal, and gene targeting studies will be required to dissociate the roles of PRMT8 from PRMT1 in this process.

The catalytic activity of PRMT8 is very similar to PRMT1, with both enzymes methylating the same panel of substrates (GST-GAR, GST-Np13, and histone H4) (Fig. 3A), and similar to PRMT1, PRMT8 possesses type I activity (Fig. 3B). In addition, PRMT8 can heterodimerize with PRMT1 and thereby tag PRMT1 activity to the plasma membrane. Thus, if there are subtle differences between the substrate specificities of these two enzymes, then a broader set of proteins will be methylated by the heterodimer. It is possible to speculate what these targets may be. PRMT8 will likely methylate GAR-motif-containing proteins that are both brain-specific and membrane-associated. A genetically controlled knock-out system will be required to validate putative substrates as PRMT8-dependent targets.

Arginine methylation is emerging as a major regulator of protein function (5). Inspection of the mouse and human genomes reveals that most mammalian PRMTs have now been characterized. There remains an uncharacterized gene on human 4q31 (AAH64403) that is most closely related to PRMT7. This gene is a candidate for PRMT9, and judging from its EST expression pattern, it is relatively well expressed. Now that we have the players in this field, we can begin to look at issues such as redundancy, substrate specificity, and regulation of arginine methylation.

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