PRMT7, a New Protein Arginine Methyltransferase That Synthesizes Symmetric Dimethylarginine*

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The cDNA for PRMT7, a recently discovered human protein-arginine methyltransferase (PRMT), was cloned and expressed in Escherichia coli and mammalian cells. Immunopurified PRMT7 actively methylated histones, myelin basic protein, a fragment of human fibrillarin (GAR) and spliceosomal protein SmB. Amino acid analysis showed that the modifications produced were predominantly monomethylarginine and symmetric dimethylarginine (SDMA). Examination of PRMT7 expressed in E. coli demonstrated that peptides corresponding to sequences contained in histone H4, myelin basic protein, and SmD3 were methylated. Furthermore, analysis of the methylated proteins showed that symmetric dimethylarginine and relatively small amounts of monomethylarginine and asymmetric dimethylarginine were produced. SDMA was also produced when a GRG tripeptide was methylated by PRMT7, indicating that a GRG motif is by itself sufficient for symmetric dimethylation to occur. Symmetric dimethylation is reduced dramatically compared with monomethylation as the concentration of the substrate is increased. The data demonstrate that PRMT7 (like PRMT5) is a Type II methyltransferase capable of producing SDMA modifications in proteins.

Protein methylation has been shown to occur in a diverse number of biological processes such as signal transduction (1, 2), chromatin remodeling (3), RNA splicing (4, 5), RNA decay (6, 7), gene regulation (8, 9), nuclear export of proteins (10), inhibition of oncogenic ras signal transduction (11), and bacterial chemotaxis (12, 13). The methylation of protein substrates is catalyzed by enzymes that transfer a methyl group from S-adenosylmethionine to a protein acceptor, a process that occurs in many organisms. The residues that are modified by methylation include arginine, histidine, lysine, and aspartic acid.

Protein-arginine methyltransferases (PRMTs)1 catalyze the formation of methylarginine residues. Four types of protein arginine methyltransferases have been described (14). Type I PRMTs form ω-N2-,ω-N3-monomethylarginine and asymmetric ω-N2,G,N3-dimethylarginine (ADMA) residues; type II PRMTs form ω-N2-monomethylarginine and symmetric ω-N2,G,N3-dimethylarginine (SDMA) residues; type III and type IV PRMTs synthesize only ω-N2-monomethylarginine (MMA) and δ-N2,G-dimethylarginine, respectively. Although the presence of SDMA in eukaryotic cells has been known for a number of years (4, 15), an enzyme that could synthetize SDMA was first identified by Pollack et al. (16) and subsequently characterized (17, 18) and designated PRMT5. PRMT5 was found to methylate histones H2A and H4, myelin basic protein (MBP), and several spliceosomal Sm proteins (SmD1, SmD3 and SmB) in vitro (4, 15, 16–21). Methylation of spliceosomal components by PRMT5 is a prerequisite for their assembly into the spliceosome (20–22). PRMT5 also has been shown to be associated with the cyclin E gene and to be involved with other transcriptional events as well (8, 23).

Although a large number of proteins have been found to contain SDMA residues (24), for several years PRMT5 was the only known Type II PRMT. Here we report the discovery of another Type II protein arginine methyltransferase, PRMT7, that can synthesize SDMA. This protein was initially characterized in hamster cells as a protein that modulates drug sensitivity to DNA-damaging agents (25).

**EXPERIMENTAL PROCEDURES

Plasmid Constructs—The cDNA of PRMT7 was cloned with primers specific to the open reading frame of FLJ10640, a cDNA we identified with homology to PRMT5 and other PRMTs (Fig. 1 and Table I). Of the three splice variants of this cDNA, the longest variant was chosen. The cDNA was amplified by PCR at 95 °C for 2 min followed by 30 cycles of denaturing at 95 °C for 45 s and then annealing at 55 °C for 45 s and elongation at 72 °C for 2 min. After 30 cycles, the reactions were then incubated at 72 °C for 10 min. The PCR product was digested with EcoRI and XbaI restriction endonucleases, gel-purified, and ligated at 16 °C for 21 h into vector pEF2 (16), which had been digested with EcoRI and XbaI restriction endonucleases. The primers used were 5'-GGAATTCCATGGACTACAAGGACGACGATGACAAGAAGAAGATC-3' and 3'-GCTCTAGAGCTCAGTCTGGGGTAT/H11032. The resulting constructs were ligated into pet-GRG-Isocodon in the vector pT7-7 (Stratagene) at the EcoRI and XbaI sites. The pet-GRG-Isocodon plasmid was kindly provided by Dr. Carol Kehoe. Plasmids were isolated from E. coli (Stratagene). The DNA sequence was determined by a genetic analysis services at the University of Medicine and Dentistry of New Jersey-Robert Wood Johnson Medical School.

The abbreviations used are: PRMT, protein-arginine methyltransferase; ADMA, asymmetric dimethylarginine; SDMA, symmetric dimethylarginine; MMA, monomethylarginine; MBP, myelin basic protein; GST, glutathione S-transferase; PBS, phosphate-buffered saline; Fmoc, N-(9-fluorenyl)methoxycarbonyl.

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1 The abbreviations used are: PRMT, protein-arginine methyltransferase; ADMA, asymmetric dimethylarginine; SDMA, symmetric dimethylarginine; MMA, monomethylarginine; MBP, myelin basic protein; GST, glutathione S-transferase; PBS, phosphate-buffered saline; Fmoc, N-(9-fluorenyl)methoxycarbonyl.
FIG. 1. Pileup of the known human PRMTs. Protein sequences of PRMTs 1–7 were aligned by the GCG (Exceleris) pileup program. The Boxshade Web site was used to format the results. Comparisons were made with full-length proteins. The asterisk over position 163 of PRMT1 indicates the methionine residue in Type I PRMTs, which is replaced by cysteine and alanine in PRMT5 and PRMT7, respectively. White letters in black boxes represent identity; white letters in gray boxes represent similarity; dots represent gaps in the sequence. GenBankTM accession numbers for the PRMT proteins are as follows: PRMT1, Q99873; PRMT2, P55345; PRMT3, O60678; PRMT4, NP_954592; PRMT5, AF167572, O14744; PRMT6, Q96LA8; PRMT7, AAH00146.
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for the PCR reaction was a plasmid obtained from the American Type Culture Collection (ATCC, Manassas, VA; catalog number MGC-5331) that contains the FLJ10640 cDNA. The FLJ10640 open reading frame was also amplified by PCR and digested with EcoRI restriction endonucleases and cloned into pGEX-3X at the EcoRI site to form pGEX-PRMT7. The primers used were 5'-GGAAATTCGATGAAAGATCTTTGCAGAC and 5'-GGAAATTCTCAAGTGCCGGGTGGTT. The reactions were then incubated at 72 °C for 10 min. To create the plasmid pEF2-Myc-PRMT7, the pEF2-FLAG-PRMT7 plasmid was amplified by PCR at 95 °C for 2 min followed by 20 cycles of denaturing at 95 °C for 45 s, annealing at 55 °C for 45 s, and elongation at 72 °C for 10 min with primers that encode the Myc epitope: 5'-GTAACCCGCGCAATGGCTGGGAGATGAAGATCTTCTGTCGGGC and 5'-GGCCCGACTGCAGAAGATCTTCATGGTCCTCCTC-3'. The product had an m.p. 143–144 °C (uncorrected) (theory: m.p. 150–153 °C) and was used directly in the next step.

Preparation of Monomethylarginine (ω-N²-Monomethylarginine)—Conversion of Fmoc-OcocI to Fmoc-Osou was performed as described (28, 29). HOSu (0.633 g, 5.5 mmol) was added to a solution of Fmoc-OcocI (1.29 g, 5.0 mmol) in dioxane (12.5 ml) in a 50-ml round-bottomed flask with a magnetic stirrer. It was cooled in an ice bath, and triethylamine (0.70 ml, 5.0 mmol) was added over a 5-min period with stirring. Stirring then continued at room temperature for 2 h. The precipitated triethylamine hydrochloride was filtered off (weight = 0.63 g), and the filtrate was evaporated to ~5 ml and added portion-wise to an Erlenmeyer flask containing 25 ml of diethyl ether. An oil separated out and was placed in the refrigerator overnight. The next day the supernatant solution was decanted and separated from the oil. This filtrate immediately began to crystallize and afforded 430 mg of white crystals (28% yield). Additional product left in the oil was not isolated.

The product was insoluble in water and ethyl acetate; soluble in acetonitrile and N,N-dimethylformamide. Deprotection of an aliquot with 15% piperidine in N,N-dimethylformamide followed by a ninhydrin test was positive and in agreement with the expected structure. The peptide, SGRGKGGKGLGKKGARRHKH, was prepared by solid phase synthesis as described below for the other peptides (R²⁶-representing ω-N²-monomethylarginine).

Peptides—The following peptides were synthesized chemically as substrates for PRMT7: P-SmD3, AGGRGKKA1LQAVARGGRGGRGSRGHGRRHRRH-GGKGGH; P-MBP, SQGKGRLSLSFSWGEAE-NH₂; M1, SGGRGGKGGGRHKHH-NH₂; M8, SGAGGKKGLGKKGGAK-HARH-NH₂ with all arginines substituted with alanine; MM, SGRGKGGKGLGKKGARRHKH; SGRGKGGKGLGKKGARRHKH; ω-N²-Monomethylarginine. The peptides were prepared on solid phase by the Fmoc/Bt-butyryl-strategy and O-benzotriazole-N,N,N',N'-tetramethyluronium hexafluorophosphate-activation on a SYRO multiple peptide synthesizer (MultiSynTech GmbH, Witten, Germany) as reported (30). The methylated peptide MM was synthesized until the previous glycine (position 4 of peptide MM) on a 433A batch synthesizer (Applied Biosystems, Weiterstadt, Germany), and the remaining three residues were coupled manually with 20 mg of peptide resin after cleavage with trifluoroacetic acid the crude peptides were purified by reversed-phase high pressure liquid chromatography with an aqueous acetonitrile gradient with 0.1% trifluoroacetic acid. The purified peptides were characterized by matrix-assisted laser desorption/ionization mass spectrometry and stored dry at ~20 °C until use.

In Vitro Methylation Reactions—The 30-µl reaction mixtures included 1–10 µg of substrate protein or peptide as noted in the figure legends, 5 µl of [H³]adenosine methionine (81 Ci/mmol, PerkinElmer Life Sciences), 0.1–1 µg of PRMT7, or 15 µl of protein A/G PLUS beads (containing the immunopurified FLAG-PRMT7 isolated from ~1 x 10⁷ HeLa or COS cells), plus buffer (50 mM sodium phosphate, pH 7.6). The 30-µl reaction mixtures were incubated at 37 °C for 5–21 h as described in the figure legends. To determine the incorporation, aliquots of the reaction mixtures were precipitated with cold 10% trichloroacetic acid onto 0.45 µm nitrocellulose filters (HA, Millipore) as described (31).

Gel Electrophoresis of Methylated Proteins—Aliquots of the in vitro methylation reactions were electrophoresed on precast 15% polyacryl-
PRMTs were compared two at a time with the Bestfit program (GCG). The percentages of identity and similarity (in parentheses) are shown in the table. Comparisons were made with full-length sequences for each PRMT. Accession numbers of PRMTs are noted in Fig. 1.

| Identity (similarity) | PRMT1 | PRMT2 | PRMT3 | PRMT4 | PRMT5 | PRMT6 | PRMT7 |
|----------------------|-------|-------|-------|-------|-------|-------|-------|
| PRMT1                | 100   | 38.1  | 52.0  | 37.4  | 33.9  | 36.0  | 28.3  |
| PRMT2                | 38.1  | 100   | 38.8  | 40.1  | 30.5  | 39.7  | 27.0  |
| PRMT3                | 52.0  | 38.8  | 100   | 38.7  | 26.6  | 37.0  | 25.6  |
| PRMT4                | 37.4  | 40.1  | 38.7  | 100   | 32.4  | 30.8  | 35.7  |
| PRMT5                | 33.9  | 30.5  | 26.6  | 32.4  | 100   | 30.8  | 26.3  |
| PRMT6                | 36.0  | 39.7  | 37.0  | 40.8  | 30.8  | 100   | 30.5  |
| PRMT7                | 28.3  | 27.0  | 25.6  | 35.7  | 30.5  | 100   | 30.5  |

**FIG. 3.** *In vitro* methylation of substrates by FLAG-PRMT7 immunopurified from HeLa cells. A, *in vitro* methylation reactions were performed as described under "Experimental Procedures" with histones (10 μg), MBP (10 μg), GAR (2 μg), and SmB (1 μg) proteins for 5 h. Trichloroacetic acid-precipitable counts and picomoles incorporated are the means of duplicate determinations. Background without substrate was 30,769 cpm. B, top, Coomassie Blue-stained gel. B, bottom, autoradiograph of ³H-labeled proteins produced by *in vitro* methylation. Exposure time was 14 days.

**FIG. 4.** PRMT7 is not associated with PRMT5. COS cells were transfected with the indicated plasmids. After 48 h, the cells were lysed and either lysate (from 1 × 10⁵ cells) or immunoprecipitate (from 1 × 10⁶ cells) was separated by PAGE (12.5%) and blotted. The blots were probed with anti-hemagglutinin antibody.

| Sample | Lysate | Lysate | FLAG IP | HA IP |
|--------|--------|--------|---------|-------|
| Transfection | Flag-PRMT7 HA-PRMT5 | HA-PRMT5 | Flag-PRMT7 HA-PRMT5 | HA-PRMT5 |
| BLOT: HA | 1 | 2 | 3 | 4 |
| HA-PRMT5 | → |

amide gels (Novex, Invitrogen). Gels were stained with 50% methanol, 10% acetic acid, and 0.25% Coomassie Brilliant Blue in water and destained with 30% methanol and 10% acetic acid in water and then dried and exposed to Biomax film at −80 °C for 14 days.

**TLC—*In vitro* methylation reactions (30 μl) were hydrolyzed with 250 μl of 6 × HCl at 110 °C for 21 h in a sealed glass ampule. The hydrolyzed amino acids were dried in an oven after opening the ampule. Thirty μl of water was added to the dried residue, and then 10 μl of the solution was applied to each lane of a Silica 60 TLC plate (Whatman). The solvent of 30% ammonium hydroxide, chloroform, methanol, and water (2:0.5:4.5:1) was used for the chromatographic separations of the amino acids (5). Color was developed with a ninhydrin spray (Sigma). Standards (MMA, ADMA, and SDMA) were purchased from Calbiochem. Chromatographs were coated with three applications of EN3HANCE Spray (PerkinElmer Life Sciences) and then exposed to film at −80 °C for 7–21 days.

**Analysis of the Distribution of PRMT7 in Cells—** COS-1 cells were plated at 3 × 10⁵ cells per chamber in LabTek II 4-well chamber slides (VWR) on the day before transfection. Plasmid pEF2-Myc-PRMT7 was transfected into COS-1 cells with Superfectamine transfection reagent (Qiagen) as described above under "Immunopurification of FLAG-PRMT7." Plasmid pEF2-Myc-PRMT5 or pEF2-Myc vector was transfected at the same time as positive and negative controls, respectively. 24–36 h after transfection, the cells were washed three times with PBS, pH 7.4 and fixed in 3.7% formaldehyde in PBS for 15 min at room temperature (32). The cells were then washed three times in PBS and permeabilized in 0.2% Triton X-100 plus 1% normal goat serum in PBS.
for 5 min on ice. The cells were washed three times in PBS plus 1% normal goat serum and incubated in 1:200 diluted rabbit anti-Myc antibody (Santa Cruz Biotechnology, Inc.) for 1 h at room temperature. The cells were then washed three times in PBS plus 1% normal goat serum followed by incubation in rhodamine-conjugated goat anti-rabbit antibody (Santa Cruz Biotechnology, Inc.) at a dilution of 1:150 for 1 h at room temperature. The cells were subsequently washed three times in PBS. The chamber was removed from the slide and then mounting medium (Vector Laboratories, Inc., Burlingame, CA) containing 4',6-diamidino-2-phenylindole was added to cells. A coverslip was placed over the cells on the slide and sealed with clear nail polish. The stained cells were visualized under a Nikon eclipse TE2000 microscope with ×200 magnification, and photographs were taken by a CoolSNAP Pro digital camera (Media Cybernetics, Houston, TX) with Image Pro Plus software.

RESULTS
The Sequence Motif Search Program (Kyoto University) was used to search for proteins containing consensus S-adenosylmethionine binding sites. One protein identified was encoded by the cDNA designated FLJ10640, which contained a motif for an S-adenosylmethionine binding site and other methyltransferase motifs suggesting that it might be a PRMT. We designated the protein encoded by the FLJ10640 cDNA PRMT7. When the protein sequence of PRMT7 was compared with the other known human PRMTs, PRMT7 seemed to be a member of the human PRMT family (Fig. 1). Table I shows a summary of the homology of all the known protein arginine methyltransferases (PRMT1 through PRMT7) with respect to each other. PRMT7 is most similar to PRMT4.

To determine whether PRMT7 contains methyltransferase activity, FLAG-PRMT7 was expressed in HeLa cells and immunopurified with anti-FLAG antibody. The immunopurified protein was assayed in vitro for methylation activity with four different substrate proteins. Fig. 3A shows that the FLAG-PRMT7 preparation has significant methyltransferase activity with histones, MBP, GAR (a fragment of human fibrillarin), and SmB (a spliceosomal Sm protein) as substrates. Fig. 3B shows both a Coomassie Blue-stained gel and the autoradiograph of the proteins methylated in these reactions. Of the five histones (H1, H2A, H2B, H3, and H4) in the preparation that was assayed only two were methylated. Based on their size and the results of a control experiment in which the five histones were methylated individually (data not shown), we concluded that the methylated (labeled) histones are H2A and H4. MBP was also methylated. Although low quantities of GAR and SmB were present in the methylation reactions, their methylation was nevertheless appreciable judging from the intensity of the autoradiographic bands. Because PRMT5 methylates these same proteins in vitro, these data suggested that there might be some functional similarity between PRMT7 and PRMT5 (16, 18). Alternatively, the similarity of substrates for PRMT5 and PRMT7 could be caused by association of PRMT5 with PRMT7.

To rule out this possibility, hemagglutinin-PRMT5 and FLAG-PRMT7 were expressed in COS cells. After the cells were lysed, FLAG-PRMT7 was immunoprecipitated as described under “Experimental Procedures.” The GST-PRMT7 fusion protein was used to methylate various peptides in vitro. The GST-PRMT7 fusion protein was used to methylate various peptides in vitro. Fig. 6A shows that methylation occurred with peptides M1, P-SmD3, and P-MBP as substrates. Because the GRG tripeptide was not trichloroacetic acid-precipitated because of its small size, it was analyzed with the other peptides after hydrolysis and TLC. As is shown in Fig. 6B (lane 1), GST-PRMT7 alone did not produce SDMA, ADMA, or MMA in detectable quantities. With peptides M1 (lane 2), P-SmD3 (lane 3), P-MBP (lane 4), and GRG (lane 5), however, a large amount of SDMA was generated with relatively much

![Fig. 5. TLC analysis of methylation of peptide substrates by immunopurified FLAG-PRMT7 isolated from COS cells. Sequences of peptides are shown under “Experimental Procedures.” In vitro methylation reactions were performed for 18 h as described under “Experimental Procedures.” The reaction products were hydrolyzed and run on TLC plates with MMA, ADMA, and SDMA standards as described under “Experimental Procedures.” Lane 1, FLAG-PRMT7 immunoprecipitate; lane 2, FLAG-PRMT7 immunoprecipitate plus 10 μg histones; lane 3, FLAG-PRMT7 immunoprecipitate plus 5 μg P-MBP peptide. Exposure time was 10 days.](http://www.jbc.org/content/163/5/3660/F5)
less ADMA and MMA, indicating that PRMT7 is in fact a Type II PRMT. The fact that the GRG tripeptide was methylated by PRMT7 was surprising. Nevertheless, this result demonstrates that the GRG motif is sufficient for methylation by PRMT7.

To confirm that SDMA is synthesized by PRMT7, we used GST-PRMT7 produced in E. coli with protein (Fig. 7A) and peptide (Fig. 7B) substrates. GST-PRMT7 methylated the proteins H2A, MBP, and SmD1, although SmD1 was methylated at a low level. GST-PRMT7 also methylated the peptides M1 and MM. Peptide MM contained a G-RMe-G (where RMe is methylarginine) instead of the usual GRG motif present in peptide M1. PRMT7 methylated MM significantly greater than it labeled M1 (Fig. 7B). When TLC was performed to determine the products of the methylation, it was evident that GST-PRMT7 alone produced no detectable SDMA, ADMA, or MMA (Fig. 7C, lane 1). However, PRMT7 yielded mostly SDMA with the M1 peptide (lane 2). When the monomethylated peptide (MM) was tested, once again SDMA was synthesized (lane 3), indicating that PRMT7 is a Type II PRMT. When all three of the arginines in peptide M1 were substituted with alanines, this peptide designated M8 was not methylated (lane 4) showing that PRMT7 methylates only arginine residues to synthesize SDMA predominantly.

Because Miranda et al. (33) found that PRMT7 synthesizes MMA but no dimethylarginines with a peptide substrate, we tested the effects of peptide concentration on the relative levels of MMA and SDMA produced by PRMT7. Whereas Miranda et al. (33) used 50 μg of peptide we used 3 μg or less of several peptides. Therefore we hypothesized that higher concentrations of peptides would reduce the quantity of SDMA, whereas the level of MMA would increase. As shown in Fig. 8, the ratio of SDMA to MMA was progressively reduced as the quantity of P-SmD3 substrate was increased from 1 to 50 μg in the methylation reaction. As the increased concentration of substrate increased the amount of MMA and decreased the level of SDMA, we propose a model of peptide substrate methylation by PRMT7 in which the monomethyl peptide is released from PRMT7 after methylation (Fig. 9). Increasing the substrate concentration would increase monomethylation if the substrate concentration was not saturating. However, because the ratio of monomethylated to unmethylated peptides would be smaller at higher substrate concentrations, the methylation of monomethylated peptides is reduced because the unmethylated peptides compete with the released monomethyl peptides. Thus, less dimethylation occurs at increasing concentrations of peptide substrate. In the case of PRMT7, 10 μg or more of P-SmD3 reduced the formation of SDMA to undetectable levels (Fig. 8) consistent with our model (Fig. 9).

To determine the distribution of PRMT7 in cells, Myc-PRMT7 was expressed in COS cells (Fig. 10). The data show
that PRMT7 is present in the nucleus and the cytosol in comparison to PRMT5 that is present primarily in the cytosol (19, 34).

DISCUSSION

Of the six known human PRMTs, only PRMT5 has thus far been identified as a Type II methyltransferase capable of synthesizing SDMA in proteins. In this report, we describe a new protein arginine methyltransferase, PRMT7. Our data demonstrate that PRMT7 is also capable of forming SDMA so that it is the second PRMT able to synthesize SDMA. While this paper was in preparation, we became aware of a publication in press that also described PRMT7 (33). However, the authors reported that PRMT7 synthesized only ω-N⁵-monomethylarginine but did not observe any ADMA or SDMA. In addition, the report noted that a peptide was methylated but that no protein substrate tested was methylated. In contrast, our results with PRMT7 demonstrated robust methylation of proteins and significant methylation of peptides, even the tripeptide GRG. Histone H2A, MBP, and GST-GAR were methylated by PRMT7 that we prepared (Figs. 3, 5, and 7) but were not observed to be methylated by Miranda et al. (33). Furthermore, we observed that the methylation yielded SDMA predominantly with peptides and proteins consistent with the designation of PRMT7 as a Type II PRMT. The fact that the tripeptide GRG alone can be

![Figure 7](image-url)

**FIG. 7.** *In vitro* methylation of protein substrates and peptides M1, MM, and M8 by GST-PRMT7 isolated from *E. coli*. A, *in vitro* methylation reactions with substrates histone H2A (5 μg), MBP (5 μg), and SmD1 (0.5 μg) were performed as described under “Experimental Procedures” for 18 h. Trichloroacetic acid-precipitable counts and picomoles incorporated are means of duplicate determinations. B, trichloroacetic acid-precipitated counts and picomoles incorporated from *in vitro* methylation reactions with peptide substrates M1, MM, and M8 (3 μg each) are shown. The sequences of these peptides are given under “Experimental Procedures.” Reaction time was 18 h. C, TLC analysis of hydrolyzed [³H]methyl peptides produced by GST-PRMT7 *in vitro*. Lane 1, GST-PRMT7; lane 2, GST-PRMT7 plus M1 peptide; lane 3, GST-PRMT7 plus MM peptide; lane 4, GST-PRMT7 plus M8 peptide. B and C were samples from the same reaction mixtures.
and then released.

Once the monomethylated peptide binds to PRMT7, it is dimethylated peptide competes with the unmethylated peptide for binding to PRMT7. The released peptide binds to PRMT7, is monomethylated, and then is released. The released methylated indicates that at least one motif for methylation by PRMT7 is the GRG sequence. The discrepancies between our observations and the report of Miranda et al. (33) are likely because of the concentrations of the peptides used. We demonstrated that at high concentrations of substrate there was no formation of SDMA but only MMA (Fig. 8), whereas at low peptide concentrations SDMA formation was significant. This result is consistent with our model (Fig. 9). It is thus likely that after formation of MMA, the peptide is released from PRMT7. This may not apply to protein substrates that have binding sites that keep them attached specifically to PRMT7 during methylation. Furthermore, assessing proteins that complex with PRMT7 may assist in the recognition of protein substrates. It should be noted that previously no purified PRMT5 was shown to synthesize SDMA or ADMA with small peptide substrates as we have shown here, although Brahms et al. (20) observed SDMA synthesis with a BSA-peptide conjugate by a cytosolic lysate of HeLa cells. The ability to use peptide substrates readily enables the definition of the motifs and the residues that are methylated as we showed above.

SDMA has been found in Sm proteins (4, 20) and coilin (35). Because PRMT5 was found in these complexes and was the only known Type II PRMT, it was concluded that PRMT5 was the enzyme that carried out synthesis of SDMA residues in the relevant proteins. However, the discovery that PRMT7 is a Type II enzyme suggests that it is likely responsible for synthesis of SDMA residues in some proteins. In addition, PRMT5 and PRMT7 could assist each other, substitute for each other, or possibly function in different pathways by methylating different sets of proteins in vivo. Possibly PRMT5 and PRMT7 may methylate the same proteins under different physiological circumstances or in different cellular compartments. Isolation of proteins with antibodies to SDMA has identified over 100 circumstances or in different cellular compartments. Isolation of proteins with antibodies to SDMA has identified over 100 proteins with SDMA residues (24). It was assumed that PRMT5 was responsible for SDMA residues that are methylated as we showed above.

However, it is likely that PRMT7 is responsible for methylation of some of these proteins.

The activity of GST-PRMT7 is lower than that of immunopurified PRMT7. A similar phenomenon was noted for PRMT5 when we found that a great deal of methyltransferase activity was associated with the immunoprecipitated protein but very little or no methylation could be obtained with GST-PRMT5 isolated from E. coli.2 This difference is likely because of the requirement of accessory proteins for full activity of PRMT5 prepared from mammalian cells, and the lack of these proteins in preparations from bacteria could account for the lower activity of PRMT5 expressed in E. coli. It is possible that PRMT7 also requires one or more associated proteins for activity. The

2 Y. Kim, L. Izotova, Z. Yang, and S. Pestka, unpublished data.
methylolation of the GRG tripeptide by GST-PRMT7 (Fig. 6b) is strong evidence that PRMT7 methylates substrates at arginine residues that are flanked by two glycine residues. Methylation of GRG-containing proteins (MBP, GAR, SmB, H2A, and H4, Fig. 3, A and B) and peptides (P-SmD3, P-MBP, Fig. 6A; and M1, MM, Fig. 7, B and C) but not a peptide without an intact GRG (M8, Fig. 7, B and C) is further evidence in support of this conclusion. Additional work will be required to determine whether the residues flanking the arginine residues contribute to the extent of methylation or to the type of modification produced.

As PRMT5 and PRMT7 are the only known Type II PRMTs, it is informative to compare their sequences to begin to determine which residues in the protein are required to form SDMA. In a previous study, we and colleagues hypothesized that differences in the residues residing in the catalytic pocket of Type I and Type II PRMTs may account for the different modifications produced (18). It was predicted that substitution of a serine for methionine in the active site would allow SDMA to be synthesized rather than ADMA (18). In our sequence alignments of the PRMTs, including PRMT7 (Fig. 1), however, a cysteine is substituted for the methionine in PRMT7 and an alanine in PRMT5. Both of these substitutions could allow for SDMA synthesis because of the shorter side chains of cysteine and alanine compared with the methionine that is present in all Type I PRMTs. Other sequence differences between the Type I and Type II PRMTs may contribute to the formation of SDMA (Fig. 1). These structure/function relationships could be defined further by site-specific mutagenesis.

PRMT7 predominantly produces SDMA and small amounts of MMA and ADMA. The same phenomenon has been observed with PRMT5 (36). This indicates that the production of ADMA, albeit in small quantities, might be a consistent property of Type II PRMTs. Because the GRG tripeptide becomes methylated with ADMA slightly and because only one arginine is available to methylate, PRMT7 synthesizes both SDMA and ADMA on the same arginine residue. Thus, it appears that synthesis of SDMA and ADMA is a function of the enzyme rather than the substrate. One consequence of the production of both ADMA and SDMA by Type II PRMTs is that the presence of ADMA in proteins is not a guarantee that the methylation was produced by a Type I methyltransferase. Our experiments with PRMT1 have not detected any SDMA in proteins methylated by this Type I protein arginine methyltransferase in vitro.3

Elevated plasma SDMA has been implicated in kidney disease (37). In addition, plasma ADMA levels have been shown to be elevated in various cardiovascular diseases (37–41). These observations suggest that the relative amounts of SDMA and ADMA may have significant ramifications in health and disease and that both PRMT5 and PRMT7 may have specific roles in controlling these levels.

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3 J.-H. Lee, J. R. Cook, and S. Pestka, unpublished data.
PRMT7, a New Protein Arginine Methyltransferase That Synthesizes Symmetric Dimethylarginine

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