Type I protein arginine methyltransferases catalyze the formation of asymmetric ω-N\textsuperscript{G},N\textsuperscript{G'}-dimethylarginine residues by transferring methyl groups from S-adenosyl-l-methionine to guanidino groups of arginine residues in a variety of eucaryotic proteins. The predominant type I enzyme activity is found in mammalian cells as a high molecular weight complex (300–400 kDa). In a previous study, this protein arginine methyltransferase activity was identified as an additional activity of 10-formyltetrahydrofolate dehydrogenase (FDH) protein. In this study, immunodepletion of FDH activity in RAT1 cells and in murine tissue extracts with antibody to FDH does not diminish type I methyltransferase activity toward the methyl-accepting substrates glutathione S-transferase fibrillarin glycine arginine domain fusion protein or heterogeneous nuclear ribonucleoprotein A1. Similarly, immunodepletion with anti-FDH antibody does not remove the endogenous methylating activity for hypomethylated proteins present in extracts from adenosine dialdehyde-treated RAT1 cells. In contrast, anti-PRMT1 antibody can remove PRMT1 activity from RAT1 extracts, murine tissue extracts, and purified rat liver FDH preparations. Tissue extracts from FDH(+/+), FDH(+/-), and FDH(-/-) mice have similar protein arginine methyltransferase activities but high, intermediate, and undetectable FDH activities, respectively. Recombinant glutathione S-transferase-PRMT1, but not purified FDH, can be cross-linked to the methyl-donor substrate S-adenosyl-l-methionine. We conclude that PRMT1 contributes the major type I protein arginine methyltransferase enzyme activity present in mammalian cells and tissues.

Arginine methylation in proteins was discovered over 30 years ago (1, 2). At least two types of protein arginine N-methyltransferase (PRMT) activities that transfer methyl groups from S-adenosyl-l-methionine (AdoMet) to the guanidino group of arginine residues exist in mammalian cells (3). Type I PRMT enzymes catalyze the formation of ω-monomethylarginine and asymmetric ω-N\textsuperscript{G},N\textsuperscript{G'}-dimethylarginine. Type I substrates include many RNA binding and transporting proteins, transcription factors, nuclear matrix proteins, and cytokines (4). Functions of type I arginine methylation in proteins may include regulation of transcription, modulation of the affinity of nucleic acid-binding proteins, regulation of interferon signaling pathways, and targeting of nuclear proteins (4–8). Type II enzymes catalyze the formation of ω-monomethylarginine and symmetric ω-N\textsuperscript{G},N\textsuperscript{G}dimethylarginine (9, 10). Myelin basic protein is the only known substrate for type II arginine methyltransferase activity (4). The type III enzyme, discovered in yeast, catalyzes the monomethylation of the internal δ-guanidino nitrogen atom of arginine residues (11).

Four enzymatically active type I protein arginine N-methyltransferases have been reported: PRMT1 (12), PRMT3 (13), and coactivator-associated arginine methyltransferase 1 (CARM1) (14) from mammalian cells and arginine methyltransferase I (RMT1) from yeast (15). Knockout of RMT1, the only type I PRMT gene in yeast, has no obvious phenotype. However, a mutant allele of RMT1 is synthetically lethal to yeast in combination with a temperature-sensitive mutant allele of NPL3 (8). NPL3 is an RMT1 substrate involved in nuclear protein import, pre-RNA processing, and export of mRNA from the nucleus (8). PRMT1, the first protein arginine N-methyltransferase in mammalian cells to be cloned, was discovered as a protein interacting with the immediate-early gene products BTG1 and TIS21 (12). BTG1 and TIS21 are negative regulators of cell growth whose overexpression in cells can lead to cell growth arrest (16, 17). BTG1 and TIS21 interact with PRMT1 and regulate its enzymatic activity (12). PRMT1 also associates with the interferon α/β receptor (5, 6). PRMT1, a predominantly nuclear protein, exists in a large complex of 300–400 kDa (12, 13) and methylates arginine residues in RGG and RXX motifs of many RNA-binding proteins and other proteins (4, 18). PRMT2 was identified because of its sequence similarity to PRMT1.
Predominant Protein Arginine N-Methyltransferase

similarity to PRMT1 (19). To date no methyltransferase activity has been demonstrated for PRMT2. PRMT3 is a monomeric cytoplasmic protein whose activity overlaps with that of PRMT1 (13). Carm1, the third active mammalian arginine methyltransferase to be discovered, was cloned as a protein interacting with the carboxyl-terminal region of p160 coactivator (14). PRMT1, PRMT2, PRMT3, Carm1, and yeast Rmt1 all contain signature regions (I, post-I, -II, and -III) that constitute the core of the AdoMet-binding site (20).

In a recent study, the predominant protein arginine N-methyltransferase was purified from rat liver (21). Sequence analysis identified the major polypeptide in this preparation as 10-formyltetrahydrofolate dehydrogenase (FDH, EC 1.5.1.6), suggesting that the major protein arginine methyltransferase may be encoded by a gene encoding an enzyme involved in folate metabolism. FDH catalyzes i) NADP⁺-dependent oxidation of 10-formyltetrahydrofolate (10-PTFH) to tetrahydrofolate, NADPH, and CO₂; ii) NADP⁺-independent hydrolysis of 10-PTFH to formate and tetrahydrofolate; and iii) NADP⁺-dependent oxidation of 2-propanal (22, 23). FDH purified from rat liver exists as a tetramer of identical 99-kDa subunits (22, 24, 25). The cDNA-deduced FDH amino acid sequence contains several domains (22, 26), including the amino-terminal phosphoribosyl-glycinamide formyltransferase homologous domain (amino acids 1–203) (27) and the carboxyl-terminal aldehyde dehydrogenase homologous domain (amino acids 417–902) (28). However, FDH does not contain any methyltransferase signature sequences (20).

To understand further the relationship between the PRMT and FDH gene products, we sought to identify the major type I protein arginine N-methyltransferase in cells and tissues. We used cultured rat cells and tissues from FDH(−/−), FDH(+/−), and FDH(−/−) mice to determine (i) which methyltransferase is the predominant type I protein arginine methyltransferase and (ii) whether the protein arginine methyltransferase activity in FDH enzyme preparations is catalyzed by the FDH enzyme or by a copurified, but distinct, methyltransferase.

**Experimental Procedures**

**Materials and Antibodies—**S-Adenosyl-l-methyl-[3H]methylthione (specific activity about 75 Ci/mmol) was obtained from NEN Life Science Products or Amersham Pharmacia Biotech. 10-Formyl-5,8-dideazafolate was obtained from Dr. John B. Hynes, Department of Pharmaceutical Chemistry, Medical University of South Carolina. 5-Formyltetrahydrofolate (5-PTFH) affinity gel was prepared by covalently linking 5-PTFH to AH-Sepharose 4B (29). Recombinant GST-PRMT1, GST-GAR, and hnRNP A1 were purified as described previously (12, 13). PRMT1 and PRMT3 antibodies were described previously (13). Anti-FDH antibody was generated in rabbits using purified rat liver FDH as the antigen (27).

**In Vitro Protein Arginine Methyltransferase Assay—**Methyltransferase activity was assayed at 37 °C for times and in final volumes as specified in figure legends. The methyl donor substrate was [3H]AdoMet. The methyl-accepting substrates were GST-GAR, hnRNP A1, or hypomethylated proteins present in lysates from adenosine diphosphate-treated RAT1 cells (13). Methylation reactions were stopped by adding SDS-PAGE sample buffer and resolved on SDS-PAGE. The methylated proteins were visualized by fluorography, as described previously (13). A separate set of assays on murine liver extracts were performed as described by Kim et al. (21, 29).

**10-Formyltetrahydrofolate Dehydrogenase Assay—**FDH activity was determined as described previously by Cook (30). The 1.0 ml reaction mixture contained 100 mM HEPES pH 7.8 buffer, 100 μM 2-mercaptoethanol, 61.6 μM 10-formyl-5,8-dideazafolate, 100 μM NADP⁺, and cell extract. The production of 5,8-dideazafolate was monitored in absorbance at 295 nm for 15 min at 23 °C.

**Purification of FDH from Rat Liver—**Rat liver FDH was purified, as described previously (29), through the 5-PTFH-Sepharose substrate affinity column step. The final purified FDH preparation contains only a major band on SDS-PAGE gel at 110 kDa, when proteins on the gel are visualized by Coomassie Blue staining.

**SDS-PAGE and Western Blot Analysis—**Protein samples were subjected to SDS-PAGE and immunoblotting analysis or silver staining as described previously (13).

**Immunoprecipitation—**Specific conditions for immunoprecipitation with anti-PRMT1, anti-PRMT3, anti-FDH, and control antibodies are described in the relevant figure legends. In general, cell lysates were incubated with antibodies and protein A-Sepharose 4B at 4 °C for 90–120 min. Supernatant and pellet fractions were recovered. The pellet fractions were further washed for 10 min with PBS containing 0.5% Triton X-100 and 0.5% Tween 20 and then incubated with 6 μg of purified FDH for 90 min at 4 °C. Then the supernatant (S) and pellet (P) fractions were recovered. The antigen-IgG-protein A-Sepharose complex (pellet fraction) was washed 3 times with PBS containing Triton X-100 and Tween 20 and then with PBS to remove residual detergents. Methyltransferase activity is assayed at 37 °C for 90 min in 90 μl of reaction mixture, which contains 2 μg of GST-GAR and 6.6 μCi of [3H]AdoMet. The methylated GST-GAR is visualized by exposing the gel to film at ~80 °C for 14 days. Panel B, FDH activity in purified FDH preparations is immunodepleted by anti-FDH antibody but not by anti-PRMT1 antibody. 6 μg of FDH is immunodepleted with 6 μl of anti-PRMT1 or anti-FDH as described in panel A. The supernatant fractions are recovered and assayed for FDH activity. The FDH activities of the supernatant fraction recovered from anti-PRMT1 (α-PRMT1) and anti-FDH (α-FDH) immunodepletions are compared with the activity of affinity purified FDH enzyme (FDH). The error bars represent the standard deviations in three independent FDH activity measurements.

**Preparation of Hypomethylated RAT1 Cell Lysates—**RAT1 cells are cultured in Dulbecco’s modified Eagle’s medium, 10% fetal bovine serum in the presence of 20 μM Adox for 48 h. Cells are washed twice with PBS and harvested in PBS with protease inhibitors (Roche Molecular Biochemicals). Cells are lysed by brief low power sonication, and cell lysate was subjected to 5 min of centrifugation. The supernatant fraction was collected as the Adox-treated RAT1 cell lysate (12).

**Preparation of Mouse Tissue Lysates—**Mouse tissues were homogenized in phosphate-buffered saline (PBS) containing protease inhibitor...
Fig. 2. PRMT1 is the predominant protein arginine methyltransferase in cultured RAT1 fibroblast cells. Panel A, RAT1 cells contain both FDH and PRMT1. Hypomethylated RAT1 cell lysates were generated as described under “Experimental Procedures” and analyzed by Western blotting. 50 μg of Adox-treated RAT1 cell lysate was subjected to SDS-PAGE and immunoblotting with antibodies against FDH and PRMT1. Panel B, anti-PRMT1, but not anti-FDH or anti-PRMT3, can immunodeplete protein arginine N-methyltransferase from lysates of Adox-treated RAT1 cells. Methyltransferase assays are performed for 60 min at 37 °C in a final volume of 90 μl of PBS in the presence of 6.6 μCi of [3H]AdoMet. The reaction is stopped by adding 30 μl of 6× SDS-PAGE loading buffer and 5 min heating at 100 °C. Methylated proteins are visualized by fluorography for 24 h. In lane 1, 40 μg of Adox-treated RAT1 cell lysate is methylated by endogenous arginine methyltransferase activity. Lanes 2–5 demonstrate methyltransferase activity in the hypomethylated cell lysate after immunodepletion by various antibodies (labeled on the top of the lanes). Immunodepletion is performed by adding IgG-protein A-Sepharose complex (the pellet fraction recovered from 8 × 100,000 g of Adox-treated RAT1 cell lysate) to 40 μg of Adox-treated RAT1 cell lysate. The reaction mixture is incubated at 4 °C for 90 min. Then the supernatant fraction is recovered and assayed for methyltransferase activity. In lanes 6–9, proteins immunoprecipitated by various antibodies from 40 μg of Adox-treated RAT1 cell lysate are assayed for methyltransferase activity, using 2 μg of GST-GAR as the methyl-accepting substrate. In lanes 10–13, methyltransferase activity was immunoprecipitated (IP) by various antibodies from 120 μg of Adox-treated RAT1 cell lysate. The precipitated proteins were then used to methylate Adox-treated hypomethylated RAT1 cell lysate that had also been immunodepleted by anti-PRMT1 antibody (the supernatant fraction of 40 μg of Adox-treated RAT1 cell lysate recovered from anti-PRMT1 immunodepletion; lane 2). Control refers to a preimmune antiserum.

RESULTS

The PRMT Activity and the FDH Activity in Substrate Affinity-purified FDH Preparations Can Be Separated by Immunoprecipitation with Antibodies to PRMT1 and FDH—FDH was previously purified as the predominant arginine methyltransferase from rat liver (21). To test whether FDH activity and protein arginine methyltransferase activity in this preparation can be further fractionated, we prepared FDH protein through the substrate affinity chromatography step. The FDH protein preparation contains a predominant polypeptide of 110 kDa. With extensive overloading of FDH proteins, some minor contaminating proteins are visible on SDS-PAGE when the gel is stained with Coomassie Blue dye (data not shown). Among these minor contaminating proteins, a polypeptide band of 45 kDa was detected. This purified FDH preparation is active in vitro methyltransferase assays, using GST-GAR as the methyl-accepting substrate (Fig. 1, panel A, lane 7). However, the specific activity of the purified FDH preparation is less than 1% of the specific activity of purified GST-PRMT1.

To determine whether the methyltransferase activity in the purified FDH protein preparation is intrinsically associated with FDH protein, we used antiserum against PRMT1 and FDH, as well as control antiserum, to immunoprecipitate the PRMT activity present in the FDH protein preparation. Neither anti-FDH antibody (panel A, lanes 5 and 6) nor the control antibody (panel A, lanes 3 and 4) can precipitate significant methyltransferase activity in the purified FDH protein preparation. In contrast, anti-PRMT1 antibody precipitates most of the GST-GAR-methylating activity in the FDH enzyme preparation (panel A, lanes 1 and 2). To confirm the ability of the anti-FDH antibody to bind and immunoprecipitate FDH, we used anti-FDH antibody to immunodeplete FDH activity from the FDH preparation (Fig. 1, panel B). Anti-FDH antibody immunodepletes 90% of the FDH activity. In contrast, anti-PRMT1 antibody does not immunoprecipitate FDH activity. We conclude that anti-PRMT1 and anti-FDH specifically immunoprecipitate PRMT1 and FDH activities; the PRMT activity present in the purified FDH protein preparation can be separated from the FDH activity.

PRMT1 Is the Predominant Protein Arginine Methyltransferase in RAT1 Cells—In our previous studies we identified two PRMTs (PRMT1 and PRMT3) in cultured RAT1 fibroblast cells (12, 13). PRMT1 is by far the most active protein arginine methyltransferase we have tested, using GST-GAR as the methyl-accepting substrate (13, 18). Because FDH was reported as the predominant PRMT in rat liver (21), we were interested in determining which protein arginine methyltrans-
FIG. 3. PRMT1 is the major protein arginine N-methyltransferase against GST-GAR in FDH +/+ and −/− mouse liver extracts. Panel A, protein arginine N-methyltransferase activity is present in tissues of both FDH(+/−) and FDH(−/−) mice and can be immunodepleted by the anti-PRMT1 antiserum but not by anti-FDH or anti-PRMT3 serum. Proteins were immunoprecipitated from 30 μl of liver extracts from FDH(+/−) mouse (protein concentration 33 μg/ml) and from FDH(−/−) mouse (protein concentration 16 μg/ml) with 8 μl of anti-PRMT1.
ferase is the predominant PRMT in RAT1 cells and contributes most to the total arginine methyltransferase activity.

We first generated a hypermethylated RAT1 cell lysate by treating cells with AdoMet, which inhibits the enzyme that metabolizes S-adenosylhomocysteine, a potent endogenous methyltransferase inhibitor (32). Western blotting with anti-PRMT1 and anti-FDH antibodies indicates that both FDH and PRMT1 are present in this hypermethylated RAT1 cell lysate (Fig. 2, panel A). The endogenous methyltransferases present in the hypermethylated RAT1 cell lysate methylate many substrates after [3H]AdoMet is added (Fig. 2, panel B, lane 1).

The control antibody-protein A Sepharose complex (lane 5) as well as the control-protein A-Sepharose complex with anti-PRMT3 (lane 3) and anti-FDH (lane 4) all deplete some PRMT activity from the cell lysate. This is presumably due to nonspecific binding of protein A-Sepharose with arginine methyltransferases and/or the hypermethylated substrates. However, of the three antisera (anti-PRMT1, anti-PRMT3, and anti-FDH), only anti-PRMT1 (lane 2) can deplete a greater degree of PRMT activity than the control serum.

Only the anti-PRMT1 immunoprecipitate (lane 6), but not the anti-PRMT3 (lane 7), anti-FDH (lane 8), or control (lane 9) immunoprecipitates, can methylate the arginine methyltransferase substrate GST-GAR. When the anti-PRMT1 immunoprecipitate is used to reconstitute the methyltransferase activity in the hypermethylated cell lysate, the protein arginine methylation pattern is restored (lane 10). In contrast, PRMT3 (lane 11), FDH (lane 12), and control (lane 13) immunoprecipitates cannot restore methylation of the PRMT substrates in hypomethylated, PRMT1-immunodepleted cell lysates. These results suggest that PRMT1 is the predominant endogenous protein arginine methyltransferase for substrates present in RAT1 cells.

**PRMT1 Contributes Most of the Protein Arginine Methyltransferase Activity in Mouse Tissues**—If FDH is the predominant protein arginine methyltransferase in liver, then livers from FDH(−/−) mice should contain much less protein arginine methyltransferase activity than livers from FDH(+/−) mice. The FDH(−/−) mouse was characterized previously (33). To test whether FDH(−/−) mice have any significant deficiency in PRMT activity, we generated mouse liver lysates from FDH(+/−) and FDH(−/−) mice and compared the protein arginine methyltransferase activities in these mouse liver lysates (Fig. 3, panel A, lanes 1 and 10). When quantitated, the methyltransferase activities against GST-GAR in the liver lysates from FDH(+/−) or FDH(−/−) mice are not significantly different (panel B). The GST-GAR-methylating activity in the liver lysates can be immunoprecipitated by anti-PRMT1 antibody (lanes 7 and 16). After immunoprecipitation with anti-PRMT1 antibody, little PRMT activity is left in the supernatant fraction (lanes 3 and 12). In contrast, anti-PRMT3 (lanes 4, 8, 13, and 17), anti-FDH (lanes 5, 9, 14, and 18), or control antibodies (lanes 2, 6, 11, and 15) do not precipitate GST-GAR-methylating activities from the FDH(+/−) or FDH(−/−) mouse liver lysates.

To confirm the genotype of the mouse livers and the FDH antibody specificity, the FDH activities from mouse liver lysates and from the supernatant fraction of the immunoprecipitation reaction were assayed (panel C). Liver extracts from FDH(−/−) mice contain no detectable FDH activity (data not shown). The FDH activity analysis confirmed that only anti-FDH, but not the anti-PRMT1, anti-PRMT3, or control antibodies, immunoprecipitates FDH activity. The genotype of the mouse livers is further confirmed by Western blot using the anti-FDH antibody (panel D). The results presented in Fig. 3 suggest that, although the FDH(−/−) mouse does not contain FDH enzyme in its liver, it has protein arginine methyltransferase activity levels similar to those found in the liver of the heterozygote FDH(+/−) mouse. The GST-GAR-methylating activity in mouse liver can only be immunoprecipitated by anti-PRMT1 antibodies. FDH activity can be immunodepleted by the anti-FDH antibody, but the immunoprecipitated FDH has no detectable GST-GAR-methylating activity.

We also analyzed whether PRMT1 contributes the bulk of the hnRNP A1 and GST-GAR-methylating activity in other tissues from FDH(+/−), FDH(+/−), and FDH(−/−) mice (Fig. 4). In this experiment, cell lysates were prepared from brains, livers, and testes of FDH(+/−), FDH(+/−), and FDH(−/−) mice. Protein arginine-N-methyltransferase activities were analyzed, using both GST-GAR and hnRNP A1 as methyl-accepting substrates. In contrast, FDH enzyme activity (Fig. 4, panel B) and antigen (Fig. 4, panel C) are detectable only in FDH(+/−) and FDH(+/−) mice. The results from these experiments indicate that it is PRMT1, not FDH, that correlates with GST-GAR- and hnRNP A1-methylating activities in tissues.

We also compared the hepatic protein arginine methyltrans-
FIG. 4. FDH activity does not correlate with GST-GAR and hnRNP A1 methylation activity in FDH(+/+), FDH(+/-), and FDH(-/-) mouse tissues. Panel A, protein arginine methyltransferase activity is present in tissues of FDH(+/+), FDH(+/-), and FDH(-/-) mice and can...
Fig. 5. UV cross-linking of [3H]AdoMet to GST-PRMT1 and FDH. Panel A illustrates the 4-week fluorograph of 5 µg of purified GST-PRMT1 (lane 1) and 5 µg of purified FDH (lane 5) enzymes UV cross-linked with 3 µM [3H]AdoMet in 250 mM Tris-HCl, pH 7.5, 5 mM dithiothreitol, for 20 min at 4 °C with a 254 nm UV light source. In lanes 2–4, UV cross-linking reactions of [3H]AdoMet to PRMT1 were performed in the presence of 10 µM unlabeled AdoMet (lane 2), 10 µM AdoHcy (lane 3), or 10 µM NADP + (lane 4). In lanes 6–8, UV cross-linking reactions of [3H]AdoMet to FDH were performed in the presence of 10 µM unlabeled AdoMet (lane 6), or 10 µM AdoHcy (lane 7), or 10 µM NADP + (lane 8). Panel B shows the Coomassie Blue-stained gel from panel A.

DISCUSSION

PRMT1 Is a Catalytic Subunit of the Predominant Protein Arginine Methyltransferase in Cells—Since type I protein arginine methyltransferase activity in cells and tissues has not been well characterized. In one study, the purest fraction demonstrated at least 8 protein bands on SDS-PAGE analysis. Two of the most prominent bands were at 100 and 45 kDa (36). The 45-kDa protein matches the molecular weight of PRMT1. In a more recent study, the predominant protein arginine methyltransferase from rat liver was purified, and the major polypeptide in the preparation, with a molecular weight of 110 kDa, was identified as FDH, suggesting that this protein may be bifunctional (21).

In this current study, however, we demonstrate that PRMT1 contributes most of the type I protein arginine methyltransferase activity, both in RAT1 cells and in murine tissues, for GST-GAR, hnRNP A1, or the hypomethylated RAT1 cell lysate (Figs. 2–4). Anti-FDH antibody does not immunoprecipitate detectable protein arginine N-methyltransferase activity from cells and tissues, using as substrates GST-GAR, hnRNP A1, or the endogenous methyl-accepting substrates in hypomethylated cell lysates. It is, therefore, unlikely that FDH contributes a significant portion of the total protein arginine N-methyltransferase activity in cells or tissues.

The native molecular mass of the cellular complex that contains PRMT1 is 300–400 kDa (12, 13). Because (i) immunodepletion experiments demonstrate that PRMT1 contributes most of the type I protein arginine N-methyltransferase activity in cells and tissues and (ii) the native molecular weight of the PRMT1 complex (12, 13) matches the molecular weight of the predominant type I protein arginine N-methyltransferase activity (9, 21, 35, 36), we conclude that PRMT1 is a catalytic subunit of the predominant protein arginine N-methyltransferase in tissues. This predominant protein arginine methyltransferase activity, which contains PRMT1 as its major catalytic subunit, has also been termed protein methylase I (9, 21, 35).

Is FDH a Protein Arginine N-Methyltransferase?—Several lines of evidence indicate that FDH is unlikely to be a protein arginine N-methyltransferase, even though FDH was identified as the predominant protein in a protein arginine N-methyltransferase purification procedure (21). The evidence includes the following observations: (i) PRMT activity can be further fractionated from FDH activity (Fig. 1), (ii) tissue ex-
tracts from FDH(−/−) mice contain normal levels of type I protein arginine N-methyltransferase activity against both GST-GAR and hnRNP A1 (Figs. 3 and 4) and normal levels of protein methylase I activity, and (iii) purified FDH does not cross-link to AdoMet, the methyl-donor substrate of protein arginine N-methyltransferase, in contrast to recombinant GST-PRMT1 protein (Fig. 5).

If FDH does not contribute significantly to protein arginine methyltransferase activity in cell lysates, why was it purified as the predominant protein arginine N-methyltransferase from rat liver? One possible explanation is that PRMT1 and FDH physically interact to form a large multisubunit protein complex. However, our results indicate that if this is the case, such a complex is difficult to demonstrate. The GST-GAR- or hnRNP A1-methylating activities in cells can be completely separated away from FDH proteins by immunodepletion (Fig. 3). No physical interactions between FDH and any GST-GAR- or hnRNP A1-methylating protein arginine N-methyltransferase have been detected in coimmunoprecipitation experiments (Figs. 1–4). Some common features shared between the predominant protein arginine N-methyltransferase and FDH proteins exist, such as their large native molecular mass of 300–400 kDa (12, 22, 24, 25) and their tight association to 5-formyltetrahydrofolate-Sepharose gels (21). These common characteristics probably contributed to the misidentification of FDH as the predominant protein arginine N-methyltransferase in rat liver. However, until recombinant FDH can be purified from a source that does not have endogenous PRMT activity (such as Escherichia coli), we cannot rule out the remote possibility that FDH has some intrinsic PRMT activity.

The Physiological Significance of Multiple Types I Protein Arginine N-Methyltransferases in Mammalian Cells—Multiple arginine methyltransferases exist in mammalian cells (4). In vitro methylation assays indicate that three known type I protein arginine N-methyltransferases (PRMT1, PRMT3, and CARM1) have overlapping, but distinct, substrate specificities (12, 13, 14). PRMT1 and PRMT3 both methylate GST-GAR, hnRNP A1, hnRNP A2, and poly(A)-binding protein II. However, the specific activity of PRMT1 for these substrates is much greater than that of PRMT3 (13, 18). The activities of PRMT1 and CARM1 overlap as well. When histone proteins were used as methyl-accepting substrates, PRMT1 prefers H4 and H2A, whereas CARM1 prefers H3, H2A, and H2B (14). Yeasts contain only one type I protein arginine N-methyltransferase, whereas mammalian cells contain multiple type I PRMT enzymes with overlapping activities but distinct regulation mechanisms and subcellular localization. The physiological significance, if any, of the difference between these methyltransferases is not known. It is possible that mammalian cells require additional regulation beyond that required in yeast. This hypothesis is supported by the observation that PRMT1 and PRMT3 have different subcellular localizations and regulatory mechanisms, despite their overlapping substrate specificities. Identification of the substrates of each protein arginine N-methyltransferase will contribute to our understanding of the functions of asymmetric arginine methylation and the role of each type I protein arginine N-methyltransferase.

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