Substrate Sources for *In vitro* Methylation Assays

PRMTs can methylate histones, RNA binding proteins, transcription factors, transcription coactivators, and growth factor receptors (Bedford and Clarke, 2009; Lee and Stallcup, 2009). As new screening methods are developed, an increasing number of specific substrates of PRMTs have been identified. A more thorough understanding of the physiological substrates of PRMTs would aid in elucidating the physiological and pathological roles of PRMTs. An *in vitro* methylation reaction can be used to determine if a newly discovered methyltransferase is active as fusion protein, and to determine its substrate specificity. The following substrate sources can be used for *in vitro* methylation assays:

(1) Recombinant proteins (purified as in **Purification of Recombinant Methyltransferase Enzymes**). (2) Histones purified by acid extraction (Yadav et al., 2003), or obtained from commercial sources. (3) Myelin basic protein (MBP) from commercial sources is good for evaluating the activity of type II enzymes. (4) Total cell lysates can also be used as substrates. Usually, hypomethylated substrates are the most efficient methyl-acceptors. Hypomethylated substrates of interest can be specifically IPed, either from cell lines that are null for a specific PRMT, or from cells treated with global-methylation inhibitors to generate, as described in subheading **7**. (5) Synthetic peptides are also often used as substrates to query the specificity of a specific PRMT. Because peptides can be synthesized to carry specific modifications, they are particularly suitable for investigating the crosstalk between arginine methylation and other types of modifications (**Figure 1**).

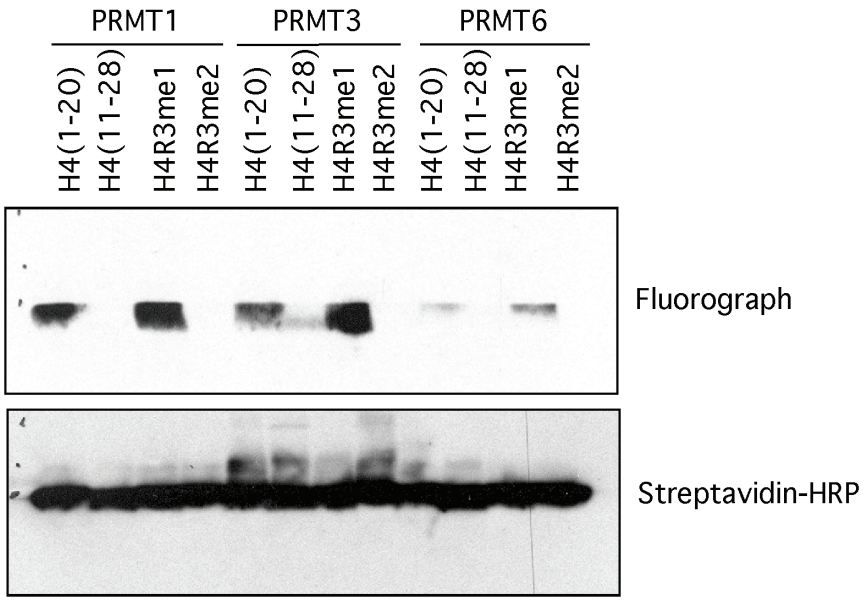

Figure 1. Using peptides to identify specific methylation site on histones by PRMTs. Recombinant PRMT1, 3, and 6 (0.2-0.5 µg) were incubated with 1 µg of various peptides *in vitro* in the presence of 1 µL of S-adenosyl-L-[methyl-3H]methionine (85 Ci/mmol from a 0.5 mCi/ml stock solution) for 90 min at 30 ºC in a final volume of 30 µL of PBS. The methylated peptides were separated by SDS-PAGE, transferred to a PVDF membrane, sprayed with EN^{3}HANCE, and exposed to X-ray film overnight. PRMT1, 3, and 6 all methylate the H4R3 site, to varying degrees.

References

Bedford, M. T., Clarke, S. G., 2009. Protein arginine methylation in mammals: who, what, and why. Mol Cell. 33**,** 1-13.

Lee, Y. H., Stallcup, M. R., 2009. Minireview: protein arginine methylation of nonhistone proteins in transcriptional regulation. Mol Endocrinol. 23**,** 425-33.

Yadav, N., Lee, J., Kim, J., Shen, J., Hu, M. C., Aldaz, C. M., Bedford, M. T., 2003. Specific protein methylation defects and gene expression perturbations in coactivator-associated arginine methyltransferase 1-deficient mice. Proc Natl Acad Sci U S A. 100**,** 6464-8.