Methyl-Specific Antibodies

At least two methylarginine-specific monoclonal antibodies were generated "accidentally" or unknowingly, when 1) the Npl3 protein was produced as an arginine methylated antigen in yeast, and used to immunize mice it generated the methyl-specific monoclonal antibody 1E4 (Siebel and Guthrie, 1996), and 2) methyl-specific autoantibodies produced in a lupus mouse model (MRL/l mice) were obtained as monoclonal antibodies when the spleens of these mice were used to generate hybridomas (Lerner et al., 1981). The Y12 monoclonal antibody was one of the antibodies generated from these MRL/l mice, and it recognizes the splicing factors Sm D1, D2 and B/B'. Almost 20 years after the Y12 hybridoma was generated, it was realized that this antibody only recognizes the symmetrically methylated form of these splicing factors, as it is not immunoreactive with these Sm proteins when they are expressed in bacteria (Brahms et al., 2000). These early studies suggest that methylated arginine peptides and proteins are good immunogens and that methyl-specific antibodies can successfully be produced to track these posttranslational modifications in the cell. Indeed, methyl-specific antibodies have been successfully raised against a number of different MMA, ADMA and SDMA motifs. A major interest currently is developing methyl-specific antibodies against motifs that are found on histone tails, and that form part of the histone code (Di Lorenzo and Bedford, 2011). There are high-quality methyl-specific antibodies, that can be used for ChIP and ChIP-seq experiments, now available for many of the methylarginine motifs that are found on histone tails (Migliori et al., 2012; Waldmann et al., 2011; Wilczek et al., 2011).

In an effort to identify and characterize arginine methylated proteins on a large scale, pan ADMA and SDMA antibodies were raised against GAR-motifs (glycine/arginine-rich motifs) by the Richard laboratory (Boisvert et al., 2003). These antibodies are now commercially available from Millipore as ASYM24 and SYM11. The characterization of these types of antibodies has recently been facilitated by the development of genetically controlled mouse cell lines. Using the Cre/lox-conditional system, we and other groups have generated 4-hydroxytamoxifen-inducible PRMT1 and CARM1 knockout MEFs (**Figure 1A**) (Yu et al., 2009). Abcam has developed a mouse monoclonal antibody, 7E6, which recognizes ADMA marks deposited by PRMT1 (**Figure 1B**). Indeed, the 7E6 antibody has been used in at least two independent mass spectrometry studies to identify arginine methylated proteins (Hung et al., 2009; Ong et al., 2004). Cell Signaling Technology (CST) has developed two rabbit monoclonal antibodies, D5A12 and Me-R^4-100, which very nicely recognize MMA motifs. In addition, there is an αH3R17me2a antibody available from Millipore that not only recognizes the methylated form of histone H3, but also cross-reacts with a host of CARM1 substrates (**Figure 1C**). The transcriptional coactivator AIB1 (Naeem et al., 2007) and the elongation/splicing factor CA150 have been identified as substrates for CARM1 using this supposedly histone-specific antibody (Cheng et al., 2007) (**Figure 1D**). The fact that methyl-arginine specific antibodies that are raised against histone marks also recognize other methylation sites is of great concern. These types of antibodies should not only be quality controlled on other histone tail methyl-marks, but also on total cell lysates.

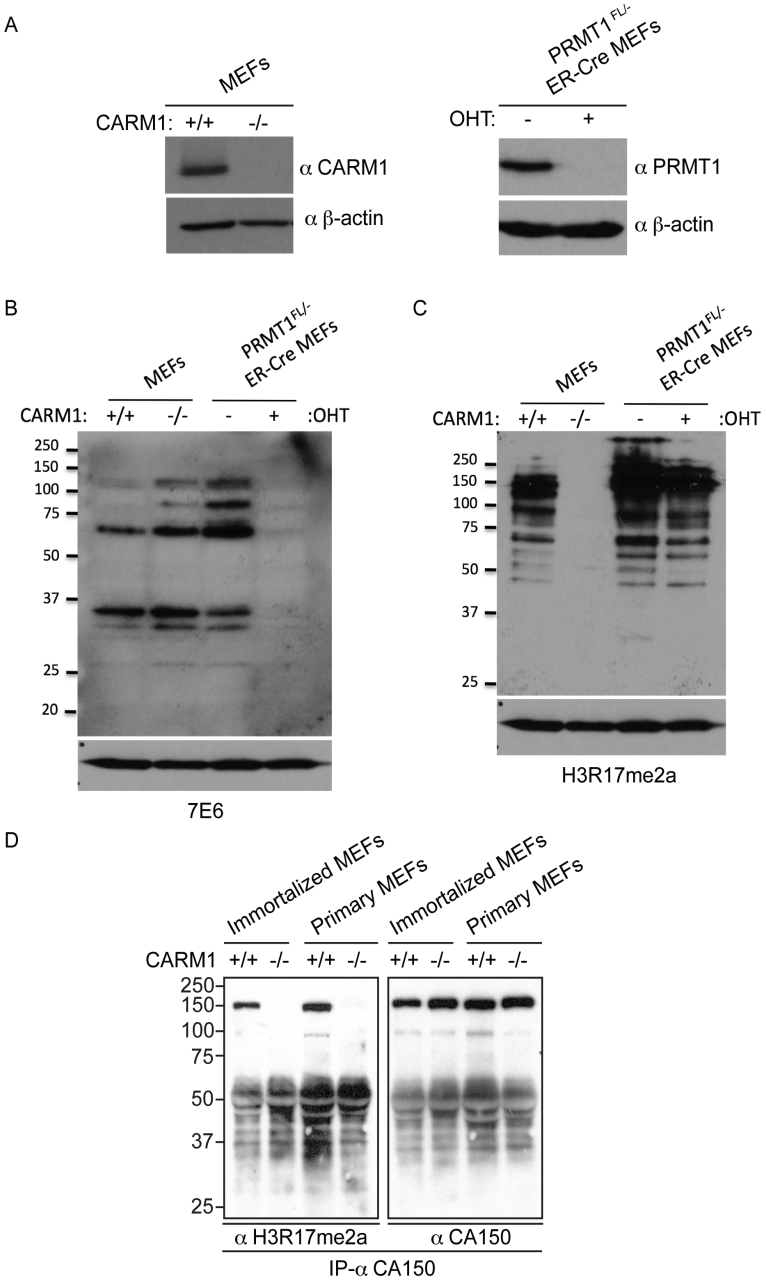

Figure 1. Characterization of the methyl-arginine motif antibodies, 7E6 and H3R17me2a. (**A**) In this experiment, CARM1 wild type (+/+) and knockout (-/-) MEFs were used. Also, PRMT1 $^{FL/-}$ ER-Cre MEFs were treated using 2 μM 4-hydroxytamoxifen (OHT) for 5 days before harvesting for cell lysate. Western analysis with αCARM1 and αPRMT1 antibodies shows the loss of these PRMT in these respective cell lines. (**B**) Cell lysates from these genetically controlled cell lines were used to perform Western analysis. The 7E6 antibody recognizes PRMT1 methylated substrates, but not CARM1 substrates. The H3R17me2a antibody recognizes CARM1 methylated substrates, but not PRMT1 substrates. (C) One of the CARM1 substrates that the H3R17me2 antibody recognizes is CA150. CA150 was IPed from both immortalized and primary CARM1 wild type and knockout MEFs and then subjected to Western blot using H3R17me2 (left panel). The same membrane was stripped and immunoblotted with αCA150 (right panel).

References

Brahms, H., Raymackers, J., Union, A., de Keyser, F., Meheus, L., Luhrmann, R., 2000. The C-terminal RG dipeptide repeats of the spliceosomal Sm proteins D1 and D3 contain symmetrical dimethylarginines, which form a major B-cell epitope for anti-Sm autoantibodies. J Biol Chem. 275, 17122-9.

Boisvert, F. M., Cote, J., Boulanger, M. C., Richard, S., 2003. A Proteomic Analysis of Arginine-methylated Protein Complexes. Mol Cell Proteomics. 2, 1319-30.

Cheng, D., Cote, J., Shaaban, S., Bedford, M. T., 2007. The arginine methyltransferase CARM1 regulates the coupling of transcription and mRNA processing. Mol Cell. 25, 71-83.

Di Lorenzo, A., Bedford, M. T., 2011. Histone arginine methylation. FEBS Lett. 585, 2024-31.

Hung, C. J., Lee, Y. J., Chen, D. H., Li, C., 2009. Proteomic analysis of methylarginine-containing proteins in HeLa cells by two-dimensional gel electrophoresis and immunoblotting with a methylarginine-specific antibody. Protein J. 28, 139-47.

Lerner, E. A., Lerner, M. R., Janeway, C. A., Jr., Steitz, J. A., 1981. Monoclonal antibodies to nucleic acid-containing cellular constituents: probes for molecular biology and autoimmune disease. Proc Natl Acad Sci U S A. 78, 2737-41.

Migliori, V., Muller, J., Phalke, S., Low, D., Bezzi, M., Mok, W. C., Sahu, S. K., Gunaratne, J., Capasso, P., Bassi, C., Cecatiello, V., De Marco, A., Blackstock, W., Kuznetsov, V., Amati, B., Mapelli, M., Guccione, E., 2012. Symmetric dimethylation of H3R2 is a newly identified histone mark that supports euchromatin maintenance. Nat Struct Mol Biol. 19, 136-44.

Naeem, H., Cheng, D., Zhao, Q., Underhill, C., Tini, M., Bedford, M. T., Torchia, J., 2007. The activity and stability of the transcriptional coactivator p/CIP/SRC-3 are regulated by CARM1-dependent methylation. Mol Cell Biol. 27, 120-34.

Ong, S.-E., Mittler, G., Mann, M., 2004. Identifying and quantifying in vivo methylation sites by heavy methyl SILAC. Narure Methods. 1, 119-26.

Siebel, C. W., Guthrie, C., 1996. The essential yeast RNA binding protein Np13p is methylated. Proc Natl Acad Sci U S A. 93, 13641-6.

Waldmann, T., Izzo, A., Kamieniarz, K., Richter, F., Vogler, C., Sarg, B., Lindner, H., Young, N. L., Mittler, G., Garcia, B. A., Schneider, R., 2011. Methylation of H2AR29 is a novel repressive PRMT6 target. Epigenetics Chromatin. 4, 11.

Wilczek, C., Chitta, R., Woo, E., Shabanowitz, J., Chait, B. T., Hunt, D. F., Shechter, D., 2011. Protein arginine methyltransferase Prmt5-Mep50 methylates histones H2A and H4 and the histone chaperone nucleoplasmin in Xenopus laevis eggs. J Biol Chem. 286, 42221-31.

Yu, Z., Chen, T., Hebert, J., Li, E., Richard, S., 2009. A mouse PRMT1 null allele defines an essential role for arginine methylation in genome maintenance and cell proliferation. Mol Cell Biol. 29, 2982-96.