

# Arginine Methylation: An Emerging Regulator of Protein Function

## Review

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Arginine methylation is now coming out of the shadows of protein phosphorylation and entering the mainstream, largely due to the identification of the family of enzymes that lay down this modification. In addition, modification-specific antibodies and proteomic approaches have facilitated the identification of an array of substrates for the protein arginine methyltransferases. This review describes recent insights into the molecular processes regulated by arginine methylation in normal and diseased cells.

### Introduction

The primary sequence of a protein is dictated by the genetic code, and functional diversity can then be achieved by the lamination of different posttranslational modifications. Phosphorylation remains by far the most studied and understood posttranslational modification due to the identification of large families of kinases, phosphatases, and proteins containing phosphoprotein-interacting modules (Pawson, 2004). As early as 1967, arginine was shown to contain methyl groups (Paik and Kim, 1967), but the distinguished status of arginine methylation is only now ascending into prominence. Arginine is a positively charged amino acid known to mediate hydrogen bonding and amino-aromatic interactions. The nitrogens of arginine within polypeptides can be posttranslationally modified to contain methyl groups, a process termed arginine methylation. Protein arginine methylation results in the addition of one or two methyl groups to the guanidino nitrogen atoms of arginine (Gary and Clarke, 1998). There are three main forms of methylated arginine identified in eukaryotes:  $\omega$ -N<sup>G</sup>, monomethylarginines (MMA);  $\omega$ -N<sup>G</sup>,N<sup>G</sup>-asymmetric dimethylarginines (aDMA); and  $\omega$ -N<sup>G</sup>,N<sup>G</sup>-symmetric dimethylarginines (sDMA) (Figure 1).

### The Protein Arginine Methyltransferase Family

It has been predicted that over 1% of genes in the mammalian genome encode methyltransferases (Katz

et al., 2003). Methyltransferases catalyze the addition of methyl groups to nitrogen, carbon, sulfur, and oxygen atoms of small molecules, lipids, protein, and nucleic acids. The methyltransferase family encompasses the enzymes that catalyze the protein arginine N-methylation reactions (Gary and Clarke, 1998). Currently, eight mammalian protein arginine methyltransferases (PRMT) have been identified (Figure 1A). Six have been shown to catalyze the transfer of a methyl group from S-adenosylmethionine (AdoMet) to a guanidino nitrogen of arginine, resulting in S-adenosylhomocysteine (AdoHcy) and methylarginine (Figure 1B). No activity has been demonstrated for PRMT2 and PRMT8. Arginine methyltransferases have been identified in yeast (Gary and Clarke, 1998; McBride and Silver, 2001), *Drosophila melanogaster* (Boulanger et al., 2004), plants, *Caenorhabditis elegans*, and fish (Hung and Li, 2004).

PRMTs are classified as either type I or type II enzymes (Figure 1B). Both types catalyze the formation of MMA as an intermediate, and type I PRMTs (PRMT1, PRMT3, PRMT4, and PRMT6) lead to the production of aDMA, whereas type II PRMTs (PRMT5 and PRMT7) catalyze the formation of sDMA. PRMT1, a predominant type I enzyme, was identified by homology to the yeast arginine methyltransferase Hmt1/Rmt1 and as an interacting protein of the immediate early genes *TIS1/BTG1* (Lin et al., 1996). PRMT2 was identified by homology, and PRMT3 was identified by its association with PRMT1 (Scott et al., 1998; Tang et al., 1998). CARM1 (also referred to as PRMT4) was identified in the yeast two-hybrid to associate with GRIP1, the p160 steroid receptor coactivator (Chen et al., 1999). PRMT5 was cloned as Jak2 binding protein and was shown to have type II activity (Branscombe et al., 2001; Pollack et al., 1999). Recently PRMT6 and PRMT8 were identified by homology (Frankel et al., 2002; Zhang and Cheng, 2003). PRMT7 was identified in a genetic screen looking for suppressor elements conferring resistance to a topoisomerase II inhibitor (Gros et al., 2003) and was shown to catalyze the formation of MMA (Miranda et al., 2004b) and sDMA (Lee et al., 2004b).

The PRMTs are ubiquitously expressed enzymes, and they may achieve a degree of tissue specificity by alternative splicing (Scorilas et al., 2000). Little data exist about the regulation of the expression or the stability of the PRMTs. Gene ablation experiments of PRMT1 and CARM1 have revealed that these enzymes are vital for existence, as PRMT1 null embryos die at approximately embryonic day 6.5, and CARM1 null mice die at birth (Pawlak et al., 2000; Yadav et al., 2003). These observations indicate that CARM1 and PRMT1 serve unique, nonoverlapping functions, which is further attested by their distinct substrate preference. Yeast lacking Hmt1/Rmt1 and mammalian cells without PRMT1 and CARM1 are viable but contain numerous hypomethylated substrates. In addition, *CARM1*<sup>-/-</sup> animals have a defect in thymocyte maturation at an early progenitor stage (Kim et al., 2004).

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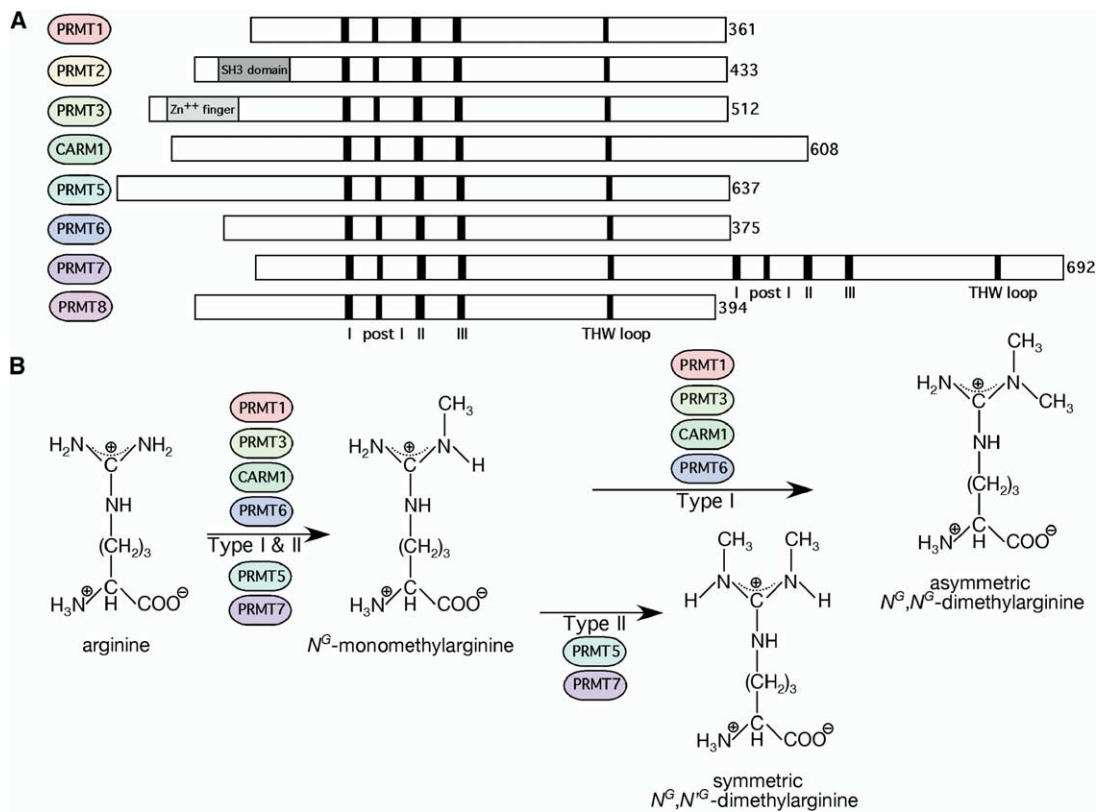


Figure 1. The Protein Arginine Methyltransferase Family

(A) There are currently eight mammalian members of the PRMT family, which harbor signature motifs I; post I, post II, and post III; and the conserved THW loop (in black). PRMT7 has a duplication of these motifs. PRMT2 and PRMT3 have an SH3 domain and a Zn<sup>2+</sup> finger, respectively, which likely facilitate substrate recognition. The accession numbers for the PRMTs are as follows: AAF62893 for hPRMT1, AAH00727 for hPRMT2, AAC39837 for hPRMT3, NP\_954592 for CARM1, AAF04502 for hPRMT5, Q96LA8 for hPRMT6, NP\_061896 for hPRMT7, and DAA01382 for mPRMT8. The number of residues is indicated at the C terminus of the PRMTs.

(B) Type I and type II PRMTs generate monomethylarginine. The generation of asymmetric dimethylarginine is catalyzed by type I, and the production of symmetric dimethylarginine is catalyzed by the type II enzymes.

### PRMT Substrates

It has long been known that proteins that harbor glycine and arginine-rich (GAR) motifs are often targets for PRMTs (Najbauer et al., 1993). Specific PRMT substrates have been identified through candidate approaches (Chen et al., 1999; Li et al., 2002), through serendipitous discovery (Wong et al., 1992; Xu et al., 2001), and more recently, through focused in vitro substrate screens (Kim et al., 2004; Lee and Bedford, 2002; Wada et al., 2002) and by using proteomic-based mass spectrometric approaches (Boisvert et al., 2003; Ong et al., 2004). The type I enzymes, PRMT1, PRMT3, and PRMT6, generally recognize GAR motif-containing substrates, whereas CARM1 displays a higher degree of specificity and does not methylate GAR motifs. There is no obvious motif that is recognized by CARM1, making it difficult to predict potential substrates with in silico searches of primary protein sequences. The type II enzymes, PRMT5 and PRMT7, methylate isolated arginine residues as well as arginines within GAR motifs. A selection of characterized methylated proteins is listed in Figure 2, and more substrates are likely to be identified that deviate from the GAR motif. Three enzymes

that methylate GAR motifs have been crystallized, and their core structures have proven very similar (Weiss et al., 2000; Zhang and Cheng, 2003; Zhang et al., 2000). The crystal structure of PRMT1 in complex with the reaction product AdoHcy and a GAR motif has been described (Zhang and Cheng, 2003), and it reveals three different peptide binding channels, possibly reflecting alternate docking orientations for different GAR motif-containing substrates.

### Cellular Processes Regulated by Arginine Methylation

#### RNA Processing

RNA binding proteins (RBPs) fulfill numerous tasks to ensure the proper processing and folding as well as the stabilization and localization of RNAs and mRNA translation (Figure 3). RBPs represent major targets for PRMTs because most hnRNPs (A1, A2, K, R, and U) harbor GAR motifs. Indeed many RBPs have been identified to be arginine methylated (Herrmann et al., 2004; Liu and Dreyfuss, 1995). It was proposed that arginine methylation might serve as a maturation signal (Cote et al., 2003), as several RBPs including Sam68 are mislo-

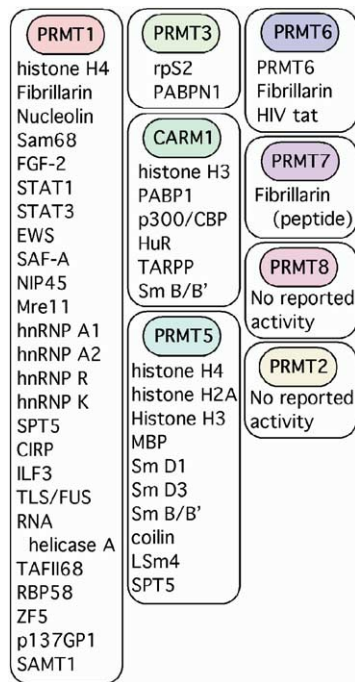


Figure 2. A List of Known PRMT Substrates

A large number of additional substrates has been identified using mass spectrometry approaches (Boisvert et al., 2003; Ong et al., 2004; Wu et al., 2004), but in many of these cases the enzyme responsible for the arginine methylation remains unknown. Abbreviations: EWS, Ewing Sarcoma; SAF-A, hnRNP; CIRP, cold-inducible RNA binding protein; ILF-3, interleukin enhancer binding factor 3; TLS/FUS, Translocated in liposarcoma; ZF5, Zn<sup>2+</sup> finger 5; p137GP1, GPI-anchor protein p137; SAMT1, substrate of arginine methyl transferase 1; TARPP, thymocyte cyclic AMP-regulated phosphoprotein.

calized in their hypomethylated state (Lukong and Richard, 2004; Smith et al., 2004). It is also considered that the methylation of RBPs, Sm B, B', D1, and D3, mediates their assembly into mature small nuclear ribonucleoprotein particles (snRNPs) (Brahms et al., 2001; Friesen et al., 2001a). The methylation of ribosomal protein S2 is conserved from yeast to man and has been shown to influence ribosomal biosynthesis (Bachand and Silver, 2004; Swiercz et al., 2005). The functional consequences of poly(A) binding protein (PABP) methylation has yet to be determined (Lee and Bedford, 2002; Smith et al., 1999).

The extensive secondary and tertiary structures of RNA, with its numerous non-Watson-Crick base pairing, represents unique interfaces for recognition by RBPs (Jones et al., 2001). Arginines within the active sites of RBPs have been recognized as key amino acids in RNA-protein interactions (Calnan et al., 1991). The guanidino nitrogens of arginine favor hydrogen bonding and van der Waal contacts (Jones et al., 2001). The affinity of a particular RBP for its RNA targets may be altered negatively, as the methyl group would prevent hydrogen bonding by sterically hindering interactions. Alternatively, arginine methylation may positively regulate RNA-protein interactions, as the arginine becomes

more “hydrophobic” in nature with methyl groups, and this may facilitate stacking with the bases of the RNA. However, it remains to be determined whether arginine methylation will dramatically influence the specific RNA binding activity of RBPs.

#### Transcriptional Regulation

It was recognized early on that histones were substrates of methyltransferases (Paik and Kim, 1967), and it is now known that histones are substrates of PRMT1, CARM1, and PRMT5 (reviewed in McBride and Silver, 2001; Pal et al., 2004). The posttranslational modification of histones is known to regulate gene expression and contribute to the histone code (Jenuwein and Allis, 2001). There are numerous transcription factors, including p53 (An et al., 2004), YY1 (Rezai-Zadeh et al., 2003), and NF-κB (Covic et al., 2004), that contribute to the recruitment of the PRMTs to promoters, as illustrated in Figure 3 (reviewed by Lee et al., 2004a). In addition to the histones, PRMTs have been shown to methylate coactivators including CBP/p300 (Chevallard-Briet et al., 2002; Xu et al., 2001) as well as transcriptional elongation factors SPT5 (Kwak et al., 2003), HIV Tat (Boulangier et al., 2005), and hnRNPs to promote the packaging of mRNPs (Xu and Henry, 2004; Yu et al., 2004). Thus, arginine methylation regulates the initiation and elongation steps of transcription and may synchronize with mRNP packaging and export.

The high-mobility group proteins (HMGs) are modulators of chromatin structure and transcription. A subfamily of HMGs, the HMGA proteins, contains three positively charged motifs called AT hooks, which bind DNA and other transcription factors. The AT hooks of HMGA1a harbor RG sequences that are arginine methylated (Edberg et al., 2004; Sgarra et al., 2003). The sites of methylation and the degree of methylation within the AT hooks vary from cell line to cell line. It has been proposed, but not yet demonstrated, that the methylation of AT hooks may regulate protein-DNA or protein-protein interactions.

#### Signal Transduction

Signaling is governed by posttranslational modifications that alter protein function in part by altering protein-protein interactions. Methylated arginines have been shown to block some interactions and to promote others (McBride and Silver, 2001). Interactions mediated by SH3 domains are observed to be sensitive to arginine methylation, whereas WW and Tudor domain interactions are unaffected or are enhanced by arginine methylation (Bedford et al., 2000a; Friesen et al., 2001a; Selenko et al., 2001). There are several pathways that have been shown to utilize arginine methylation as a tag for signal transduction downstream of the interferon receptor, the T cell receptor, cytokine receptors, and nerve growth factor (NGF) receptor. In addition, many cell surface receptors and signaling proteins were identified by proteomic analysis (Boisvert et al., 2003). PRMT1 was the first enzyme in this family to be linked to signal transduction, with the finding that it binds the cytoplasmic region of the type I interferon receptor (Abramovich et al., 1997). In this case, arginine methyltransferase activity is a positive signal as anti-sense-mediated reduction of PRMT1 levels bestows resistance to cell growth inhibition by IFN. NGF treatment of PC12 cells activates PRMT1-like activity, but the

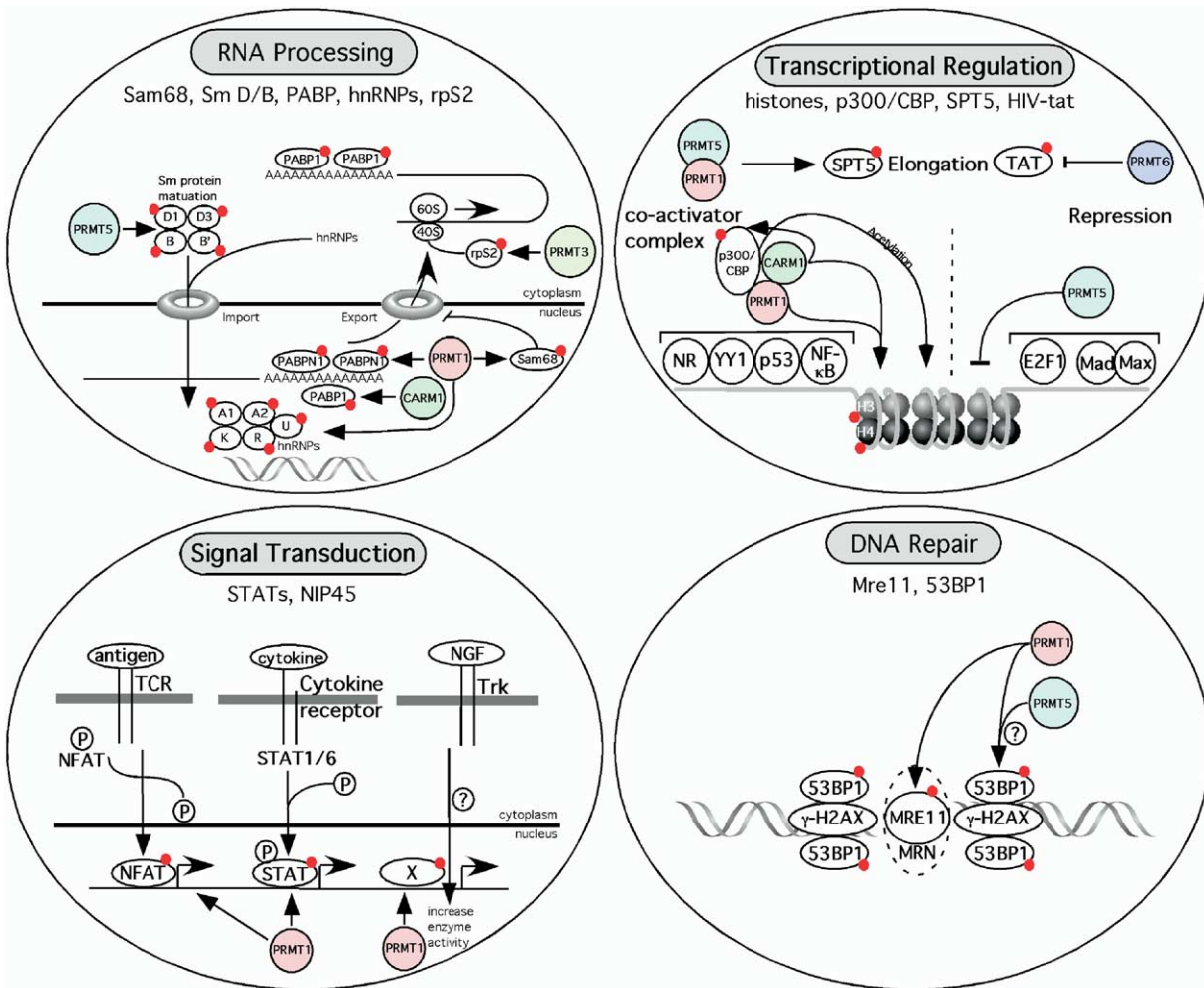


Figure 3. Cellular Processes Regulated by Arginine Methylation

PRMTs have been implicated in a number of basic cellular actions, including RNA processing, transcriptional regulation, signal transduction, and DNA repair. Methylarginines are denoted by a red dot.

molecular mechanism remains unknown (Cimato et al., 1997). The NF-AT pathway has also been identified recently as a target of PRMT1. Arginine methylation facilitates the interaction of NIP45 with NF-AT and stimulates cytokine gene expression (Mowen et al., 2004). The JAK-STAT pathway downstream of cytokine receptors has been proposed to be regulated through direct arginine methylation of STAT1 and STAT6 transcription factors (Chen et al., 2004; Mowen et al., 2001). However, this has recently been a subject of controversy (Meissner et al., 2004).

#### DNA Repair

The role of protein methylation in the DNA response pathway is largely unexplored. The identification of the MRE11-RAD50-NBS1 double-strand break-repair protein complex with ASYM25, an ADMA-specific antibody, suggested that at least one of its components is arginine methylated (Boisvert et al., 2003). It was recently demonstrated that the GAR motif of MRE11 is arginine methylated by PRMT1 (Boisvert et al., 2005). The methylation of MRE11 did not affect the MRE11-RAD50-

NBS1 complex formation. However, mutation of the arginines within the GAR domain abrogated the exonuclease activity of MRE11, suggesting that arginine methylation of these arginines regulates the enzymatic activity of MRE11. Because the exonuclease domain resides at the N terminus of the protein, these data suggest that the GAR motif is a regulatory region that may adjust the activity of MRE11 in response to signals that modulate arginine methylation.

Cell cycle arrest is a common feature of normal cells that undergo DNA damage. This permits the cell to monitor and repair the damaged DNA or to abort and undergo apoptosis. Cells with hypomorphic alleles of MRE11 are known to display cell cycle checkpoint defects similar to cells derived from patients with ataxia-telangiectasia and continue to cycle in response to DNA damage (Stewart et al., 1999). Cells treated with methylase inhibitors or PRMT1 siRNA also contained cell cycle-checkpoint defects in response to DNA damage (Boisvert et al., 2005). This effect was partially rescued with purified methylated baculovirus-produced

MRE11-RAD50-NBS1. These findings suggest that PRMTs play a significant role in signaling DNA damage and identifies one of its substrates as the MRE11-RAD50-NBS1 complex.

53BP1 is another central mediator of the DNA damage checkpoint, and it also harbors a GAR motif, which has been shown to interact with its Tudor domains (Charier et al., 2004). It is likely that 53BP1 will be a substrate of either PRMT1 or PRMT5. Recently it was shown that the 53BP1 Tudor domain targets 53BP1 to sites of DNA damage by association with methylated lysines within histones (Huyen et al., 2004). These findings suggest that DNA damage induces conformational changes in DNA that render methylated lysine 79 of histone H3 accessible to 53BP-1. Further studies are required to determine whether faulty methylation leads to genomic instability.

### Regulation of Arginine Methylation

#### PRMT Binding Proteins Regulate Their Activity

PRMTs have been found to interact with proteins and to associate with protein complexes. These binding proteins are often not substrates, and in some circumstances they alter the methyltransferase activity of the PRMTs (Figure 4A). The related proteins BTG1 and TIS21/BTG2 bind PRMT1 and stimulate its activity toward selected substrates (Lin et al., 1996). Binding of the tumor suppressor DAL-1 to PRMT3 acts as an inhibitor of enzyme activity, both in *in vitro* reactions and in cell lines (Singh et al., 2004). CARM1 is found in a complex of at least ten proteins called the nucleosomal methylation activator complex (NUMAC) (Xu et al., 2004). CARM1 within NUMAC acquires the ability to methylate nucleosomal histone H3, whereas recombinant CARM1 preferentially methylates free histone H3. PRMT5 is found in at least three different protein complexes—two nuclear and the other cytoplasmic. In the cytoplasm, PRMT5 is found in the “methylosome,” where it is involved in the methylation of Sm proteins, thus implicating PRMT5 in snRNP biogenesis (Friesen et al., 2001b). Nuclear PRMT5 associates with the regulator of transcriptional elongation, SPT4 and SPT5, and pICln, which is also a component of the methylosome (Kwak et al., 2003). Nuclear PRMT5 also complexes with the hSWI/SNF chromatin remodelers BRG and BRM, and this association enhances PRMT5 methyltransferase activity (Pal et al., 2004). Furthermore, PRMT1, Hmt1/Rmt1, and CARM1 can homodimerize (Weiss et al., 2000; Xu et al., 2004; Zhang and Cheng, 2003) through a hydrophobic face called the antenna region. It is possible that the regulation of this dimerization could affect the activity of these enzymes. Indeed, if dimerization of PRMT1 or Hmt1/Rmt1 is experimentally inhibited, no enzymatic activity is seen (Weiss et al., 2000; Zhang and Cheng, 2003). As yet, there is no evidence that PRMTs themselves are regulated by post-translational events, although PRMT6 is automethylated (Frankel et al., 2002).

#### Conversion of Arginine and Monomethylarginine to Citrulline by Deimination

A major group of deiminated proteins are the core histones H2A, H3, and H4 (Nakashima et al., 2002). The peptidyl arginine deiminase PAD4 can block methyla-

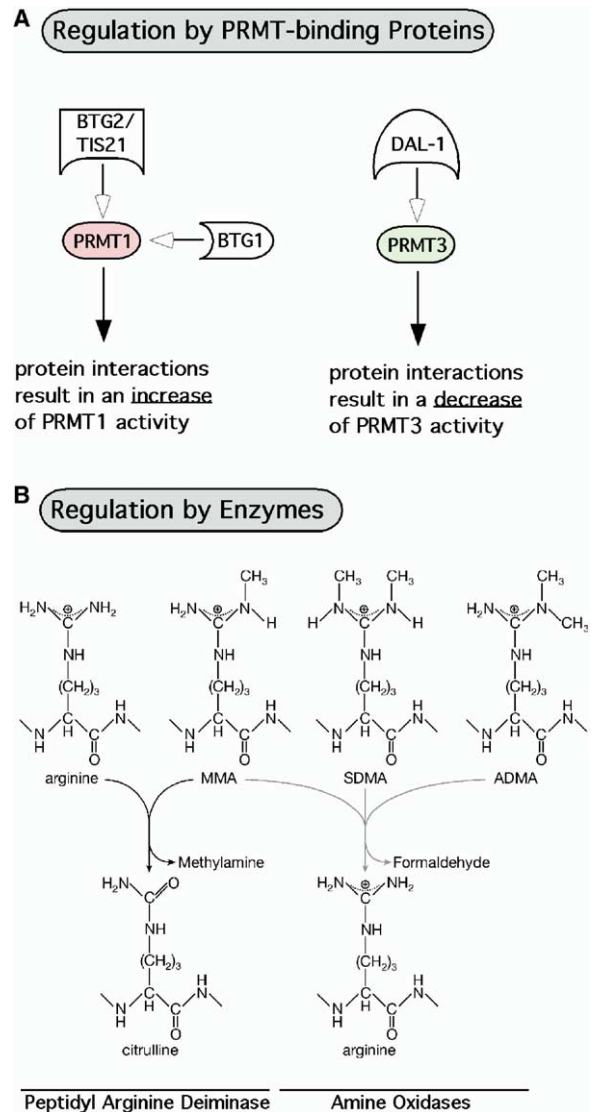


Figure 4. Molecular Mechanisms Regulating Arginine Methylation (A) PRMT specificity and/or activity may be influenced by binding proteins. (B) The peptidyl arginine deiminases can block methylation by converting arginine (and MMA) to citrulline. In addition, a family of amine oxidases may be able to demethylate arginine residues in the same fashion as they demethylate lysine residues. Abbreviations: BTG/TIS21, B cell translocation gene/tetradecanoyl phorbol acetate-inducible sequences; DAL-1, differentially expressed in adenocarcinoma of the lung.

tion on an arginine residue by converting it to citrulline (Cuthbert et al., 2004; Wang et al., 2004). PAD4 catalyzes the deimination of both arginine and monomethylarginine, but not dimethylarginine, to citrulline (Figure 4B). Peptidyl arginine deiminases are not true “demethylases,” as they do not convert monomethylarginine back to arginine. However, these enzymes may carry out a preemptive strike on key sites of arginine methylation, thereby preventing the proposed recruitment of binding proteins to H3-R17 or H4-R3 upon dimethylation by CARM1 and PRMT1, respectively.

### **Possible Arginine Demethylation by Amine Oxidases**

Recently, it was demonstrated that a common component of a number of corepressor complexes possesses lysine demethylase activity (Shi et al., 2004). The enzyme responsible for this activity, LSD1, is an amine oxidase that can specifically remove the methyl groups from dimethylated H3-K4. Although not yet confirmed, members within this family of enzymes may also be able to demethylate arginine residues (Figure 4B). These enzymes are very likely the long sought after, bona fide lysine and arginine demethylases.

### **Adjacent Posttranslational Modifications**

#### **Can Mask Arginine Methylation Motifs**

The interplay between lysine methylation at lysine 9 (H3-K9) and phosphorylation at serine 10 (H3-S10) on the N-terminal tail of histone H3 has been observed (Rea et al., 2000). In this scenario, phosphorylation of H3-S10 prevents the methylation of H3-K9 by the methyltransferase SUV39H1. In the same region of histone H3, acetylation of K9 prevents the methylation of H3-R8 by PRMT5 (Pal et al., 2004). Thus, preexisting modifications close to a site of methylation can alter the recognition motif of protein methyltransferases. A similar prediction can be made with HIV Tat, where the known lysine acetylation sites about the PRMT6 methylation motif (Boulanger et al., 2005; Kiernan et al., 1999).

#### **Type I and Type II Enzymes Share Methylation Sites**

There is mounting evidence that the same arginines within substrates are methylated by both type I and type II PRMTs. The ability for the same site to carry an aDMA or sDMA modification may have opposing biological consequences. The snRNP core proteins, SmB/B', are methylated by PRMT5 in an sDMA fashion (Friesen et al., 2001b). In addition, these splicing factors can be asymmetrically methylated in vitro by CARM1 (Boulanger et al., 2004; Cheng et al., 2004). Recently, it was shown that SmB, known to shuttle between the cytosol and nucleus, harbors sDMA in the cytosol and both sDMA and aDMA in the nucleus (Miranda et al., 2004a). It has yet to be determined if common arginine residues in the SmB C-terminal region are targeted by PRMT5 and CARM1. The methylation motif in SmB also has the capacity to bind both WW and SH3 domains (Bedford et al., 1998, 2000b; Espejo et al., 2002), which raises the possibility that the different types of arginine methylation (aDMA or sDMA) may compete for similar substrates with GAR motifs and regulate distinct protein-protein interactions.

There is accumulating data that PRMT1 and PRMT5 can vie for the same arginine residues. Histone H4 is asymmetrically methylated at arginine 3 by PRMT1 (Wang et al., 2001) and is symmetrically methylated at the same residue by PRMT5 (Pal et al., 2004). PRMT1 is a transcriptional coactivator and PRMT5 is generally regarded as a corepressor. Thus, the type of methylation at arginine 3 of histone H4 may dictate the accessibility of a locus to transcription factors. Furthermore, a single arginine residue (R698) in the RNA polymerase II binding domain of the transcriptional elongation factor SPT5 can also be methylated by both PRMT1 and PRMT5 (Kwak et al., 2003). This again raises the possi-

bility that aDMA and sDMA may antagonize each other and differentially regulate protein function.

### **Arginine Methyltransferases and Disease Cancer**

Prostate and breast cancers are common tumors that are often hormone dependent. The fact that PRMTs are known coactivators for nuclear receptors makes them likely candidates to be overexpressed in these cancer types. Indeed, it has been found that increased expression of CARM1 correlates with androgen independence in human prostate carcinoma (Hong et al., 2004). Importantly, small molecules that inhibit both PRMT1 and CARM1 can suppress estrogen and androgen receptor-mediated transcriptional activation (Cheng et al., 2004), and, in mouse embryonic fibroblast cell lines derived from *Carm1* null embryos, estrogen receptor-mediated transactivation is dramatically attenuated (Yadav et al., 2003). In addition, the ability of PRMT5, when overexpressed, to promote anchorage-independent cell growth also points to this enzyme as a candidate for deregulation in transformed cellular states (Pal et al., 2004). PRMT5 may achieve this task by inhibiting the expression of tumor suppressors. Although the PRMTs have not been convincingly identified as oncogenes or tumor suppressors, a precedent has been set by the lysine methyltransferases for the involvement of protein methylation in transformation (Hamamoto et al., 2004; Marmorstein, 2003). Moreover, the involvement of arginine methylation in the DNA damage response may identify examples of cancer in which there is genomic instability caused by the deregulation of arginine methylation.

#### **Cardiovascular Diseases**

Nitric oxide (NO), which is synthesized from L-arginine by nitric oxide synthase (NOS), plays multiple roles in the cardiovascular system. The arginine analogs MMA and aDMA, but not sDMA (Figure 1), inhibit the activity of NOS, leading to atherosclerosis (Stuhlinger et al., 2001). Proteolysis of methylated proteins provides the cellular source of free methylarginine residues. This methylarginine pool size is controlled by dimethylarginine dimethylaminohydrolase (DDAH) enzymes, which specifically hydrolyze MMA and aDMA, but not sDMA. The NOS inhibitory properties of free methylarginine are thus under tight enzymatic control, and deregulated levels may result in cardiovascular disease (Vallance and Leiper, 2002).

#### **Viral Pathogenesis**

The human immunodeficiency virus (HIV) transactivator protein (Tat) was identified to be the first HIV protein to contain methylated arginines (Boulanger et al., 2005). More importantly, inhibition of protein arginine methylation leads to increased HIV gene expression (Boulanger et al., 2005; Kwak et al., 2003). These findings suggest that increasing arginine methylation may offer some protection against HIV infection. Furthermore, inhibition of arginine methylation prevents hepatitis delta replication, suggesting that blocking methylation may offer protection against certain viruses (Li et al., 2004).

#### **Multiple Sclerosis**

The nervous system has long been recognized to be sensitive to levels of methylation, as diets poor in folate

and vitamin B12 required for AdoMet production cause demyelination (Kim et al., 2003). The myelin basic proteins (MBP) are known to contain a single sDMA (Brostoff and Eylar, 1971), but the physiological relevance of this modification and the PRMT(s) that catalyze it in vivo remain unknown. The deimination and the arginine methylation of MBP are increased in multiple sclerosis, suggesting the importance of posttranslational modification of arginines in this disease (Kim et al., 2003). It is possible that the methylated MBP may serve as an autoantigen, as is the case with methylated Sm and coilin in lupus erythematosus (Carroll, 2004).

#### **Spinal Muscular Atrophy**

Spinal muscular atrophy (SMA) is an autosomal recessive disease resulting from deletions or loss-of-function mutations within the *SMN1* gene (Lefebvre et al., 1995). SMN is required for RNP assembly, and this function requires arginine methylation (Friesen et al., 2001b). The fact that the lack of SMN leads to failure in motor neuron axonal guidance suggests that SMN may be required for the assembly, transport, and translation of mRNPs during motor neuron development (Bassell and Kelic, 2004). Recently, point mutations within the SMN Tudor domain have been identified in SMA patients, confirming the need for a functional Tudor domain (Cusco et al., 2004). Because the SMN Tudor domain associates with methylarginines (Selenko et al., 2001) and sDMA-containing proteins are mislocalized in cells from SMA patients (Boisvert et al., 2002), these findings demonstrate a role for arginine methylation in this disease.

#### **PRMTs as Drug Targets**

Two types of compounds are used to hamper methyltransferase activity. First, small molecules that inhibit AdoHcy-hydrolase, like AdOx, result in a marked intracellular accumulation of AdoHcy. Most methylation reactions are affected through feedback inhibition by elevated levels of AdoHcy. Second, analogs of AdoMet, like sinefungin and MTA (methylthioadenosine), also function as inhibitors of methylation. The inhibition of AdoHcy hydrolase by small molecules and the use of AdoMet analogs can affect cellular methylation of phospholipids, proteins, DNA, and RNA. Thus, current methyltransferase inhibitors display limited specificity, indiscriminately targeting all enzymes that use AdoMet.

A “bump-and-hole” approach has been used to generate analogs of AdoMet that selectively inhibit a mutant form of the yeast protein methyltransferase Hmt1p (Lin et al., 2001). This approach demonstrates the feasibility of generating specific methyltransferase inhibitors.

Small molecule inhibitors of PRMT were recently identified (Cheng et al., 2004). AMIs (arginine methyltransferase inhibitors) selectively inhibit PRMTs, not lysine methyltransferases. The AMIs display no specificity for individual PRMTs, demonstrating that further primary or analog screens are required to identify PRMT-specific inhibitors. Importantly, one of these compounds, AMI1, is able to inhibit the coactivator function of PRMTs, as nuclear receptor regulated transcription from estrogen- and androgen-response elements was inhibited in the presence of this compound.

These studies show that PRMT inhibitors can operate as a brake on certain hormone actions and provide encouraging evidence that specific PRMT inhibitors may be useful to treat hormone-responsive tumors and to interfere with hormone-regulated processes in general.

#### **Future Challenges**

The study of protein modification is experiencing a renaissance thanks to recent discoveries in the disciplines of arginine and lysine methylation. Much progress has been achieved in the field due to the identification of the family of PRMTs. Proteomic and methylarginine antibodies have helped expand the repertoire of methylated proteins. However, there are likely many other substrates to be identified that defy the GAR consensus. The specificity of each PRMT needs to be characterized, and both gain- and loss-of-function mouse models remain to be generated for many of the PRMTs. These studies will define the physiological roles of the PRMTs in vivo. The regulation of arginine methylation remains the biggest challenge. The identification and characterization of the enzymes that reverse and block arginine methylation will have far reaching ramifications in the methylation field in general. Finally, methylation binding domains, possibly tudor domains, or new domain types will likely be identified and characterized in the near future.

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