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State of the Arg: Protein Methylation at Arginine Comes of Age

Minireview

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Posttranslational modification of proteins allows the cell to expand its repertoire beyond the constraints imposed by the twenty encoded amino acids. Methylation at arginines, although discovered over 30 years ago, has only recently come to the attention of cell biologists through a combination of genetic and molecular biology experiments that have implicated arginine methylation in processes from signaling and transcription activation to protein sorting. The panoply of arginine methylated substrates suggests that this specifically eukaryotic modification may parallel phosphorylation in its level of complexity. We will summarize much of the recent information about the function of methylation and the methyltransferase enzymes that modify arginines.

Many Proteins Are Targets of Arginine Methylation

Three main forms of methylarginine have been identified in eukaryotes: N^G-monomethylarginine (MMA), N^GN^G (asymmetric) dimethylarginine (aDMA), and $N^GN'^G$ (symmetric) dimethylarginine (sDMA), all of which involve modification of guanidino nitrogen atoms (Figure 1). Although early purification of mammalian protein arginine methyltransferases used methylation of histones to track activity, the majority of nuclear asymmetric dimethylarginine residues are found in heterogeneous nuclear ribonucleoproteins (hnRNPs), which play roles in pre-mRNA processing and nucleocytoplasmic RNA transport. Subsequent work on numerous hnRNPs and other RNA binding proteins has revealed that they are methylated on arginine residues, frequently in the context of RGG tripeptides. Notably, all methylarginine residues within RGG motifs have been shown to be MMA or aDMA rather than sDMA. Proteins have also been identified that are asymmetrically dimethylated at RXR and RG motifs.

Myelin basic protein, which was one of the first arginine-methylated proteins identified, stands in contrast to most methylated RNA binding proteins in that it contains symmetrically dimethylated arginine residues in addition to monomethylarginine. Recently, however, two RNA binding proteins, spliceosomal snRNP proteins SmD1 and SmD3, have been shown to be symmetrically dimethylated (Brahms et al., 2000). Intriguingly, the context of methylated residues in these proteins (GRG) differs from the original RGG consensus for asymmetrically dimethylated proteins, suggesting the importance of residues in the −1 to +2 positions for recognition of the substrate arginine (for a detailed compilation of methylarginine contexts, see Gary and Clarke, 1998).

An Emerging Family of Arginine Methyltransferases

Recent interest in arginine methylation has been sparked by the identification of multiple arginine methyltransferase encoding genes. Although these genes have been uncovered in a variety of screens designed to identify proteins involved in different cellular processes (see below), the most striking characteristic of this emerging family of enzymes is the presence of an S-adenosyl methionine (AdoMet) binding motif (Figure 2A), which is closely related to the motif found in nucleic acid and small molecule methyltransferases that use AdoMet as a methyl donor. In addition to the AdoMet binding motif, four of five putative mammalian arginine methyltransferases studied to date also share a less conserved C-terminal domain, which is presumably involved in arginine substrate interactions.

The majority of arginine methylation in eukaryotic cells appears to be performed by a specific methyltransferase subfamily, which includes mammalian PRMT1 and its functional homolog, yeast Hmt1/Rmt1. The enzymes in this subfamily contain few residues outside the core region. In contrast, Carm1/PRMT4 contains both N- and C-terminal extensions to the methyltransferase core region. Other family members have N-terminal extensions, several of which contain additional motifs such as an SH3 domain (PRMT2) and a zinc finger motif (PRMT3).

Whereas all arginine methyltransferase activities identified to date can monomethylate arginine in the context of a protein substrate, methyltransferases have been classified as type I or type II enzymes according to whether further dimethylation is asymmetric (type I) or symmetric (type II). Most PRMT genes discovered to date encode type I enzymes, but recent data have revealed that PRMT5/JBP1 (Janus kinase binding protein 1) is a type II methyltransferase (Branscombe et al., 2001). Although in vivo substrates for the type I PRMT1/Hmt1 enzymes have been defined, the substrate specificity of the majority of arginine methyltransferases remains mysterious. In the case of PRMT3, however, its N-terminal zinc finger domain has been shown to influence its substrate specificity (Frankel and Clarke, 2000).

The three-dimensional structures of the core regions of yeast Hmt1 and human PRMT3 have been determined by X-ray crystallography and a comparison of these structures underscores the structural similarity between these enzymes (Figure 2B) (Weiss et al., 2000; Zhang et al., 2000). Whereas the AdoMet binding domains are virtually superimposable, the C termini contain divergent loops, suggesting that this region is involved in interactions with different methyltransferase substrates and regulators. The N termini are also variable and not completely resolved in the structures. Indeed, genetic and biochemical experiments in yeast, combined with the Hmt1 crystal structure, have suggested a specific binding pocket for its hnRNP substrate Npl3 that includes the N and C termini (Weiss et al., 2000). In addition, PRMT3 was cocrystallized with the inhibitor S-adenosyl

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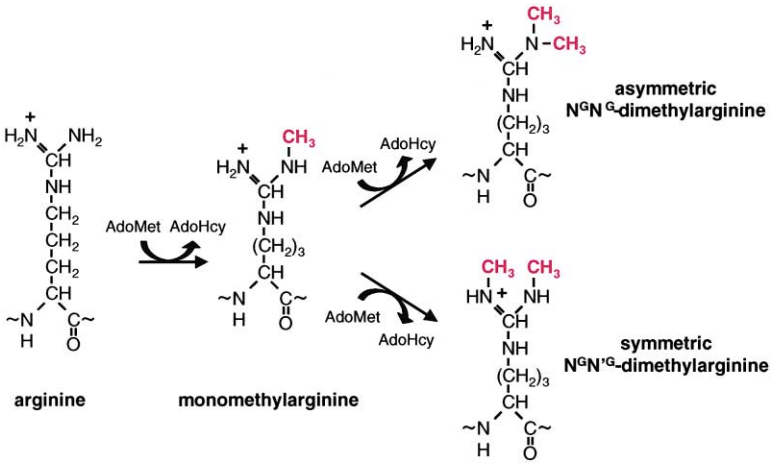

Figure 1. Arginine Methylation

Methylation of arginine residues within the context of a protein requires the methyl donor S-adenosyl methionine (AdoMet), which is converted into S-adenosyl homocysteine (AdoHcy).

homocysteine (AdoHcy), indicating the importance of an N-terminal X helix in stabilizing cofactor binding (Zhang et al., 2000).

One remarkable feature of both crystal structures is the presence of methyltransferase dimers and, in the case of Hmt1, dimerization correlates with enzymatic function (Weiss et al., 2000). The ability of these enzymes to multimerize is tantalizing as it may reflect a mechanism for regulation of methyltransferase activity (see below). Future studies of cocrystallized methyltransferase-substrate pairs may also unveil specific interactions that could clarify both the mechanism of methylation and how it might be regulated.

Methylation Affects Protein-Protein Interactions

Although methylation does not change the overall charge on an arginine residue, addition of methyl groups increases steric hindrance and removes amino hydrogens that might be involved in hydrogen bonds. Therefore, methylation could serve to modulate intra- or intermolecular interactions of target proteins. In spite of the prevalence of RNA binding proteins with RGG motifs, few studies have directly addressed the effect of arginine methylation on binding of target proteins to RNA ligands. Although methylation of hnRNPA1 had a slight effect on nonspecific binding to homopolymeric RNA (Rajpurohit et al., 1994), methylation of yeast hnRNP Hrp1 did not affect its affinity for a specific RNA ligand (Valentini et al., 1999). RGG motifs may play an auxiliary role in RNA binding, since RGG proteins frequently contain other RNA binding domains such as RNA-recognition motifs (RRMs) or hnRNP K homology (KH) domains.

Instead, recent studies have implicated arginine methylation in modulating protein-protein interactions. Sam68, a mitotic substrate for the Src kinase, is thought to act as an adaptor protein in signaling pathways and binds to both WW domain and SH3 domain-containing proteins through proline-rich regions. Interestingly, methylation of Sam68 at arginine residues within these regions decreases Sam68 binding to SH3 proteins but not to WW domain proteins (Bedford et al., 2000). These results suggest that methylation may be involved in switching the function of Sam68 by altering specific protein-protein contacts. Similarly, arginine methylation of the Stat1 (signal transducer and activator of transcription) transcription factor decreases its affinity for its inhibitor PIAS1 (Mowen et al., 2001). In contrast to these asymmetrically dimethylated proteins, SmD1 and SmD3 have been shown to require symmetric dimethylation for effi-

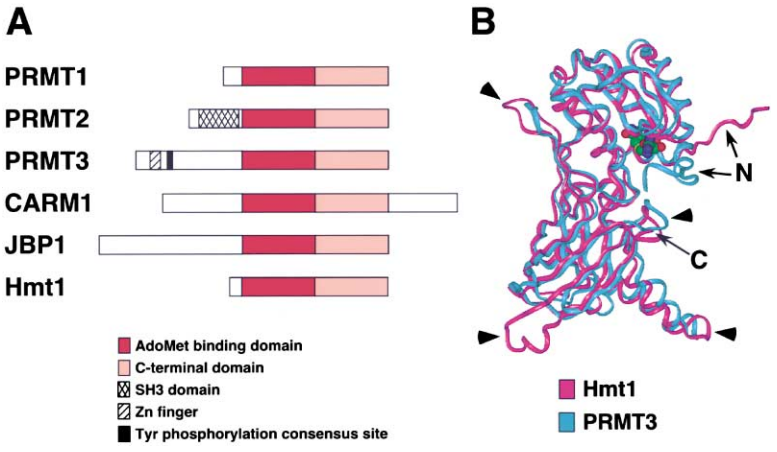

Figure 2. The Structure of Protein Arginine Methyltransferases

(A) Primary structure comparison. All family members share a core arginine methyltransferase region, composed of a conserved AdoMet binding domain (red) and a more divergent C-terminal domain (pink). Mammalian methyltransferases (PRMT1-3, CARM1, and JBP1) and *S. cerevisiae* Hmt1, the budding yeast PRMT1 homolog, are shown.

(B) Comparison of the yeast Hmt1 and human PRMT3 crystal structures. Arrowheads denote divergent loops in the C-terminal domains of yeast Hmt1 and human PRMT3 methyltransferases. The carboxyl termini of the two proteins are superimposed (C), whereas the amino terminus of Hmt1 is unstructured and that of PRMT3 contains an additional X helix (N), which allows binding of AdoHcy (shown in space-filling model). Figure by V. Weiss from PDB files 1G6Q and 1F3L (Weiss et al., 2000; Zhang et al., 2000).

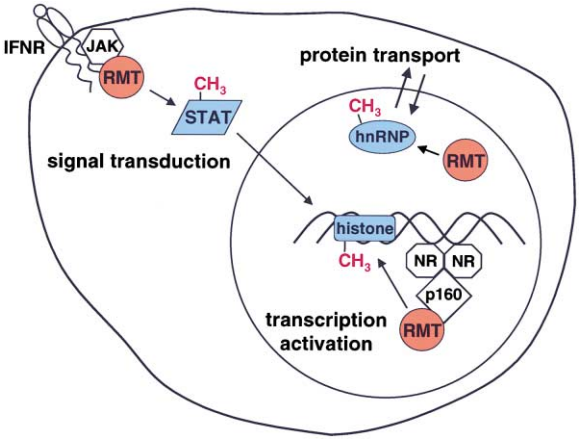

Figure 3. Possible Cellular Roles of Arginine Methylation

Arginine methyltransferases (RMT) have been implicated in signaling by interactions with the interferon receptor (IFNR; Abramovich et al., 1997), Janus kinase (JAK; Pollack et al., 1999), and Stat transcription factor (Mowen et al., 2001). Methyltransferase interactions with the p160 family of transcriptional coactivators of nuclear hormone receptors (NR) have also suggested a role in gene expression (Chen et al., 1999). Methylation of certain heterogeneous ribonucleoproteins (hnRNPs) in yeast affects their nucleocytoplasmic transport (Shen et al., 1998; Yun and Fu, 2000). Methylation substrates are shown in blue.

cient binding to SMN, the product of the spinal muscular atrophy gene (Friesen et al., 2001). Therefore, although Sam68, Stat1, SmD1, and SmD3 are all nucleic-acid binding proteins, methylation appears to affect their binding to protein partners in a nucleic-acid-independent manner.

Roles of Methylation: Protein Sorting, Transcription, and Signaling

Whereas hnRNP proteins, myelin basic protein, and histones have long been known to be substrates for arginine methylation, the cellular processes affected by methylation have only recently begun to be elucidated (Figure 3). Studies in *Saccharyomyces cerevisiae* revealed that the yeast arginine methyltransferase Hmt1/Rmt1 is critical for efficient nuclear export of its substrates, Npl3 and Hrp1 (Shen et al., 1998). Methylation of Npl3 may also regulate its nuclear import (Yun and Fu, 2000). A null mutation in mouse PRMT1, the mammalian homolog of Hmt1, resulted in embryonic lethality, although embryonic stem cells bearing this mutation were viable (Pawlak et al., 2000), suggesting the importance of PRMT1 in basic cellular processes.

Arginine methyltransferases have also been implicated in signal transduction. PRMT1 binds to the cytoplasmic domain of the type I interferon receptor (Abramovich et al., 1997), and methylation of a conserved arginine in the Stat1 transcription factor was shown to be essential for interferon α/β-induced signaling (Mowen et al., 2001). Interestingly, the most divergent putative arginine methyltransferase family member, PRMT5/JBP1, was identified by its interaction with the Janus kinase, which is required for signaling between the interferon receptor and Stats (Pollack et al., 1999). JBP1 binds AdoMet and, when isolated from cell lysates, can methylate histones H2A and H4 as well as myelin basic protein (Pollack et al., 1999). However, it is not yet clear whether these proteins or the symmetrically dimethylated Sm proteins are in vivo substrates of JBP1 or its yeast homolog Hsl7 (histone synthetic lethal 7).

The identification of a *c*oactivator-*a*ssociated *a*rginine *m*ethyltransferase (Carm1) led to studies that showed that this methyltransferase could stimulate transcriptional activation by nuclear receptors in combination with p160 family coactivators (Chen et al., 1999). PRMT1 has also been shown to have coactivator activity (Wang et al., 2001). The abilities of Carm1 to methylate histone H3 and of PRMT1 to methylate histone H4 suggest that this posttranslational modification of histones may be involved in transcriptional coactivation (Chen et al., 1999; Wang et al., 2001). Indeed, mutations within the AdoMet binding domain of both Carm1 and PRMT1 reduce both histone methylation and coactivator activities (Chen et al., 1999; Wang et al., 2001).

Can Methylation Be Regulatory?

Although it is tempting to compare protein methylation to phosphorylation, arginine methylation is generally believed to be irreversible. In the absence of an identified arginine demethylase protein or enzymatic activity, the increasing stability of the amine bond as arginine is mono- and then dimethylated suggests that this modification is likely to persist over the lifetime of a protein. Therefore, regulation of arginine methylation would presumably occur at the level of modulating methyltransferase activity toward unmethylated substrates, by sequentially increasing the amount of methylation over the lifetime of a protein, or by selective turnover of methylated proteins. Indeed, the first mammalian enzyme identified, PRMT1, was discovered by virtue of its physical interaction with immediate-early protein TIS21 and the related BTG1 protein and these proteins were shown to modulate the activity and substrate specificity of PRMT1 (Lin et al., 1996). Many arginine methyltransferase genes have been cloned similarly in yeast two-hybrid screens with a variety of proteins of interest, many of which are involved in cell signaling (e.g., PRMT1 [Abramovich et al., 1997], Carm1 [Chen et al., 1999], JBP1 [Pollack et al., 1999]). The plethora of methyltransferase-interacting proteins likely includes both upstream regulators and downstream targets or effectors of arginine methyltransferases.

Methyltransferase activity may also be regulated by self-association or interaction with other methyltransferase family members. Although PRMT3 was initially identified as a PRMT1-interacting protein and the PRMT3 core crystal structure revealed a dimer interface, PRMT3 itself does not appear to dimerize in mammalian cell lysates (Tang et al., 1998). In contrast, yeast Hmt1 and its mammalian homolog PRMT1 both oligomerize in cell lysates, and mutations that disrupt dimerization of Hmt1 eliminate its activity in vivo (Tang et al., 1998; Weiss et al., 2000). The reduced activity of PRMT3 compared to PRMT1 in vitro may reflect a role for multimerization in regulation of PRMT3 enzymatic function, perhaps dependent on specific cellular environments.

Protein Methylation and Disease

Methylarginine and methylated proteins have been connected to a number of diseases. Monomethylarginine and asymmetric dimethylarginine are potent inhibitors of nitric oxide synthase (NOS), and increased levels of

these species have been found in patients with various cardiovascular and noncardiovascular disorders. The likely irreversible nature of arginine methylation raises the possibility that protein arginine methylation and subsequent metabolism of target proteins may influence NOS activity.

Several targets for arginine methylation have been implicated in autoimmune diseases. Anti-myelin basic protein antibodies have been found in multiple sclerosis (MS) patients and, in a mouse model for MS, myelin basic protein provokes an autoimmune response. In addition, multiple ribonucleoproteins, many of which are methylated, have been found as antigens in autoimmune diseases. In particular, epitopes recognized by anti-Sm autoantibodies in lupus erythematosis patients include symmetrical dimethylarginines in SmD1 and SmD3 (Brahms et al., 2000).

Conclusions

The recent identification of multiple arginine methyltransferase family members and targets for arginine methylation has implicated protein methylation in a variety of cellular processes from signaling to protein transport. The full characterization of each methyltransferase and its in vivo substrates will serve as a next step in understanding the role of arginine methylation in eukaryotes. In addition, specific inactivation of particular enzymes may facilitate experiments to define how methylation affects target proteins, including, for example, their protein-protein interactions. Such studies will address the importance of methylation for protein function and may therefore eventually lead to new insights into disease processes.

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