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Sci. STKE **2005** (271), re2. [DOI: 10.1126/stke.2712005re2]

Protein Interfaces in Signaling Regulated by Arginine Methylation

François-Michel Boisvert, Carol Anne Chénard, and Stéphane Richard*

(Published 15 February 2005)

Posttranslational modifications are well-known effectors of signal transduction. Arginine methylation is a covalent modification that results in the addition of methyl groups to the nitrogen atoms of the arginine side chains. A probable role of arginine methylation in signal transduction is emerging with the identification of new arginine-methylated proteins. However, the functional consequences of arginine methylation and its mode of regulation remain unknown. The identification of the protein arginine methyltransferase family and the development of methylarginine-specific antibodies have raised renewed interest in this modification during the last decade. Arginine methylation was mainly observed on abundant proteins such as RNA binding proteins and histones, but recent advances have revealed a plethora of arginine-methylated proteins implicated in a variety of cellular processes, including signaling by interferon and cytokines, and in T cell signaling. We discuss these recent advances and the role of arginine methylation in signal transduction.

Methylated Arginines

Arginine is a positively charged amino acid that often mediates hydrogen bonding and amino aromatic interactions with proteins and nucleic acids. Arginines may be posttranslationally modified to contain methyl groups in a process termed arginine methylation (*1*). Arginines can also be cleaved by enzymes such as peptidylarginine deiminases (PADs) and dimethylarginine dimethylaminohydrolase (DDAH) to generate citrulline (*2*) and ornithine (*3*). Protein arginine methylation is a posttranslational modification that adds monomethyl or dimethyl groups to the guanidino nitrogen atoms of arginine (*1*). Three main forms of arginine methylation have been identified in eukaryotes: ω-N^G, monomethylarginines (MMA), ω-N^G,N^G-asymmetric dimethylarginines (aDMA), and ω-N^G,N'^G-symmetric dimethylarginines (sDMA) (Fig. 1). The modification does not alter the positive charge of the arginine, rather, it increases its bulkiness, blocks hydrogen bonding, and increases its hydrophobicity.

Protein Arginine Methyltransferases

In humans, protein arginine methyltransferases (PRMTs) represent a family of eight enzymes that use *S*-adenosylmethionine (SAM) as a methyl donor [(*4*), Table 1]. The characteristics of PRMTs include the presence of a highly conserved methyltransferase domain that oligomerizes into a ring-like structure (*5*–*7*). Little structure-function analysis has been performed on the PRMTs to identify their regulatory domains.

The PRMTs are classified into two groups: type I and type II enzymes. There are five known type I PRMTs that catalyze the formation of MMA and aDMA: PRMT1 (*8*), PRMT2 (*9*), PRMT3 (*10*), PRMT4 or CARM1 for coactivator methyltransferase (*11*), and PRMT6 (*12*). There are two type II enzymes that catalyze the formation of MMA and sDMA: PRMT5 (*13*) and PRMT7 (*14*). PRMT7 was originally identified in a genetic screen for targets conferring resistance to a topoisomerase II inhibitor (*15*). PRMT7 catalyzes the formation of MMA (*16*) and can also catalyze the formation of sDMA (*14*). HRMT1L3 (GenBank accession number AAF91390), herein called PRMT8, has ~80% sequence identity with PRMT1 and is most likely a type I enzyme because of its similarity with PRMT1. Arginine methyltransferase activity was detected in the Golgi. Q9DD20 is the enzyme proposed to mediate this activity (*17*). However, its amino acid sequence diverges from that of the known arginine methyltransferases, and it remains to be demonstrated whether or not it is a bona fide PRMT.

Saccharomyces cerevisiae has one major type I enzyme, hnRNP (heterogeneous nuclear ribonucleoprotein) methyltransferase 1 (HMT1), that accounts for 85% of all arginine methylation (*18*, *19*). HMT1 is not essential for cell growth, as *hmt1*Δ yeast are viable, but such cells fail to export RNA binding proteins (*20*–*22*). *S. cerevisiae* also has an enzyme with type II activity called histone synthetic lethal-7 (HSL7) (*23*). Type I and II activities were also identified in *Schizosaccharomyces pombe*. Type I enzymes include the homologs of PRMT1 and PRMT3, herein called spPRMT1 and spPRMT3. *S. pombe* lacking spPRMT3 are viable and display an accumulation of free 60*S* ribosomal subunits, which implies a role for spPRMT3 in ribosome biosynthesis (*24*). The role of PRMT3 in ribosomal function has been confirmed in higher eukaryotes, where PRMT3 interacts with and methylates the ribosomal protein rpS2 (*25*). *S. pombe* also contains type II activity, and the methyltransferase is called SKB1 (Shk1 kinase–binding protein), the homolog of PRMT5 (*26*). Other eukaryotes exhibit type I and type II arginine methyltransferase activity, including plants (*27*), the nematode *Caenorhabditis elegans*, the puffer fish *Fugu rubripes,* the zebrafish *Danio rerio* (*28*), *Trypanosome brucei* (*29*), and the fruit fly *Drosophila melanogaster* (*30*). The fruit fly PRMTs are named DART1 to DART9 for *Drosophila* arginine methyltransferases 1 to 9 (*30*).

To compare the evolution of type I and type II PRMTs, we constructed a phylogenetic tree with mammalian, yeast, and *Drosophila* enzymes (Fig. 2). The PRMTs are distributed in three major branches. The first branch (top, Fig. 2) represents the type I enzymes, including hPRMT1, hPRMT8, DART1, HMT1, spPRMT1, hPRMT3, DART3, spPRMT3, DART9, DART2, and DART6. The second branch represents the type II enzymes including hPRMT5, DART5, SKB1, HSL7, hPRMT7,

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and DART7. The last branch represents enzymes that have evolved separately from the other PRMTs and include the type I enzymes CARM1, DART4, hPRMT2, hPRMT6, and DART8. On the basis of the phylogenic tree, it appears that DART8 is the homolog of PRMT6. The phylogenic analysis demonstrates that the type I and II enzymes, represented by PRMT1 and PRMT5, are conserved in all eukaryotes. The members of the lower branch, including CARM1 and PRMT6, are only found in multicellular eukaryotes.

PRMT Consensus Sites. The preferred methylation consensus sequence of PRMT1 was determined to be a glycine- and arginine-rich (GAR) motif (*31*). PRMT3 methylates substrates within GAR motifs (*10*) and also methylates arginines next to small hydrophobic residues in the sequence Arg-Ala-Arg (*32*). CARM1 is not a GAR motif–specific enzyme, and the alignment of peptides methylated by CARM1 has defined a loose consensus of Arg-Pro-Ala-Ala-Pro-Arg (*33*, *34*). More substrates need to be identified to define an accurate methylation consensus sequence. PRMT5 methylates arginines within GAR motifs and arginines outside the GAR motif (*13*, *35*, *36*). The specificity of PRMT2 and of PRMT6 through PRMT8 remains to be defined, as no physiological substrates have been identified for these enzymes.

PRMT Substrates. Techniques to detect methylarginines include mass spectrometry, the incorporation of labeled methyl groups in vivo and in vitro with purified PRMTs, amino acid substitution analysis, and, more recently, assays with general and site-specific antibodies to methylarginine (*37*, *38*). Over the years, many proteins have been shown to contain methylarginines (Table 2). In 1967, histones were identified as methylarginine-containing proteins (*39*, *40*), and a few other proteins were identified before 1990, including the myelin basic proteins (MBPs) (*41*). The number of known methylated proteins increased after 1996, owing to the identification and cloning of the PRMTs. Moreover, proteomic studies using antibodies specific to methylated GAR identified more than 200 putative methylated proteins, which showed that arginine methylation may be a broad post-translational modification implicated in many cellular processes including signal transduction, the cytoskeleton, DNA repair, transcription, translation, and apoptosis (*37*). It is now known that arginine methylation is an abundant modification, as some of the methylarginine containing proteins identified to date are abundant proteins such as histones, MBP, and RNA binding proteins (*1*). Most of the known methylated proteins from Table 2 are substrates of PRMT1, which is consistent with this finding that this enzyme is the major PRMT in the cell (*42*) and with the fact that the other methyltransferases are less well characterized.

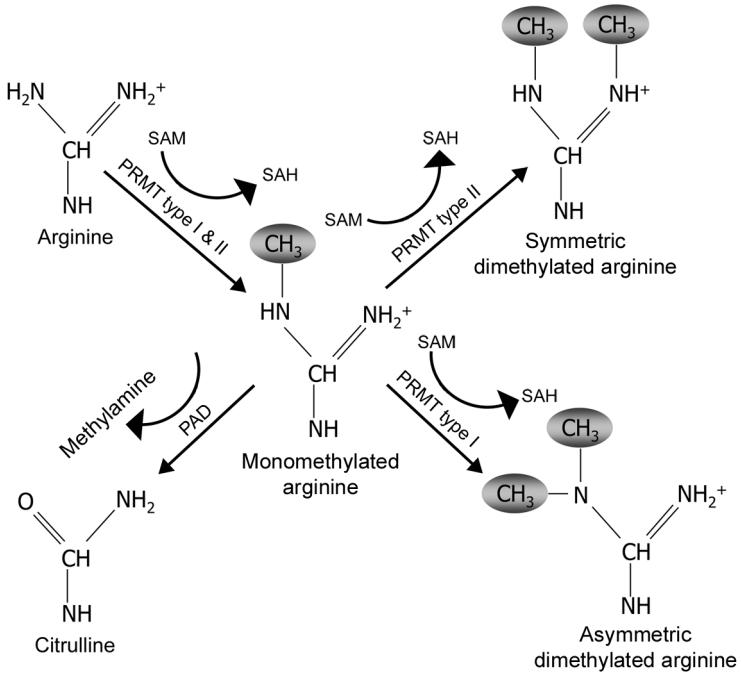

Fig. 1. Methylation of arginines. Arginine can be methylated on a guandino nitrogen atom by type I and II protein arginine methyltransferases (PRMT) to become monomethylated. Symmetrically dimethylated arginines result from the addition of a second methyl group to the opposite nitrogen atom by type II enzymes, whereas asymmetrically dimethylated arginines result from the addition of a second methyl group to the same nitrogen with type I enzymes using SAM as a methyl donor releasing *S*-adenosylhomocysteine (SAH). Monomethylarginines can also be deimidated by peptidylarginine deiminases (PADs) resulting in the formation of a citrulline.

Homo sapiens		*S. cerevisiae*		*S. pombe*		*D. melanogaster*	
PRMT1	Q99873	HMT1	P38074	PRMT1	NP_594825	DART1	NP_650017
PRMT2	P55345	–	–	–	–	–	–
PRMT3	O60678	–	–	PRMT3	NP_595572	DART3	NP_650434
PRMT4/CARM1	NP_954592	–	–	–	–	DART4	NP_649963
PRMT5/JBP1	O14744	HSL7	P38274	SKB1	P78963	DART5	NP_477184
PRMT6	Q96LA8	–	–	–	–	DART8	NP_609478
PRMT7	NP_061896	–	–	–	–	DART7	NP_611753
PRMT8/HRMT1L3	AAF91390	–	–	–	–	–	–
PRMT9	Q9DD20	–	–	–	–	–	–
–	–	–	–	–	–	DART2	NP_608821
–	–	–	–	–	–	DART6	NP_650322
–	–	–	–	–	–	DART9	NP_650321

Table 1. Arginine methyltransferases.

Arginine Methylation Modulates Protein-Protein Interactions

Posttranslational modifications can alter protein-protein interactions. The classical example is binding of the Src-homology 2 domain (SH2) to tyrosine residues modified by phosphorylation (*43*). Arginine methylation can both negatively and positively regulate protein-protein interactions. Arginines are especially important in hydrogen bonding. For example, a critical aspartic acid within the Src-homology 3 domain (SH3) of the tyrosine kinase Fyn mediates an Asp-Arg salt bridge between Fyn and the HIV-1 (human immunodeficiency virus-1) Nef protein (*44*). The methylation of this arginine results in collision with the SH3-interacting surface and blocks the interaction (*45*). The substrate of Src in mitosis, having a mass of 68 kD, Sam68, is an adaptor protein that binds to several proteins containing SH3- and the WW domain (two-tryptophan domain) (Fig. 3H) (*46*). The methylation of arginines neighboring proline motifs in Sam68 actually prevents association with SH3 domains, but not with WW domains, as demonstrated using blot overlays and BIAcore experiments (*45*). These data were further corroborated by using a protein microarray spotted with SH3 and WW domains and probed with methylated and nonmethylated peptides (*47*). Arginine methylation prevents the interaction of the transcription factor STAT1 (signal transducer and activator of transcription 1) with its inhibitor PIAS1 (protein inhibitor of activated STAT1) (*48*). Similarly, the interaction of the transcription elongation factor SPT5 (Suppressor of Ty–5) with RNA polymerase II is stimulated under conditions where SPT5 is hypomethylated (*49*). The interaction of Npl3 (nuclear protein localization 3) with the TREX (transcription/export) mRNA export machinery is also inhibited by arginine methylation (*21*). In constrast to the methylation of Sm proteins, the arginine methylation of Ewing sarcoma (EWS) protein decreases its interaction with SMN, the spinal muscular atrophy gene product (*50*). However, the interface and the molecular details of this interaction have not been examined in any detail.

Contrary to the interaction of EWS with SMN, arginine methylation positively regulates the interaction between SMN and the small nuclear ribonucleoproteins SmB/B′, SmD1, and SmD3 (*51*). The Tudor domain of SMN is likely to mediate this direct interaction (*51*, *52*). The proposed function of SMN is to participate in ribonucleoprotein complex assembly (*53*). It is noteworthy that arginine methylation of the C terminus of the SmB and SmD proteins (*54*) enhances their association with SMN (*55*). Thus, a model was proposed where arginine methylation signals the assembly of ribonucleoproteins. Consistent with this model, pre-mRNA splicing reactions were inhibited when nuclear extracts from HeLa cells were treated with methyltransferases inhibitors (*56*). Moreover, nuclear extracts incubated with methylarginine-specific antibodies were inefficient in pre-mRNA splicing in vitro (*56*). Another interaction may be positively regulated by arginine methylation, because the interaction between the nuclear factor of activated T cells (NF-AT) and the NF-AT–interacting protein of 45 kD, NIP45, decreased in the presence of methylase inhibitors (*57*).

Methylation can also selectively impair certain signaling pathways. The cAMP response element–binding protein (CREB)-binding protein (CBP) is arginine methylated in its CREB kinase–inducible interacting KIX domain (*58*), as well as in its N terminus. CBP and p300 function as important transcriptional coactivators in multiple pathways (Fig. 3E). Arginine methylation of CBP within the KIX domain by CARM1 reduces association of CBP with CREB through its kinase-inducible domain (KID domain), which results in a decrease in cAMP-dependent gene expression (*58*). CBP methylation outside of the KIX domain can also affect the glucocorticoid receptor interacting protein–1 (GRIP1)–dependent and hormone-induced transcriptional activation (*59*, *60*).

The Role of Arginine Methylation in Signaling Through the JAK-STAT Pathway

Cytokine and interferon (IFN) signaling are known to transduce intracellular signals through the JAK-STAT (Janus kinase–STAT) pathway (*61*). The concept that arginine methylation could regulate cytokine signaling was suggested from the observation that a mutation resulting in an inositol-requiring

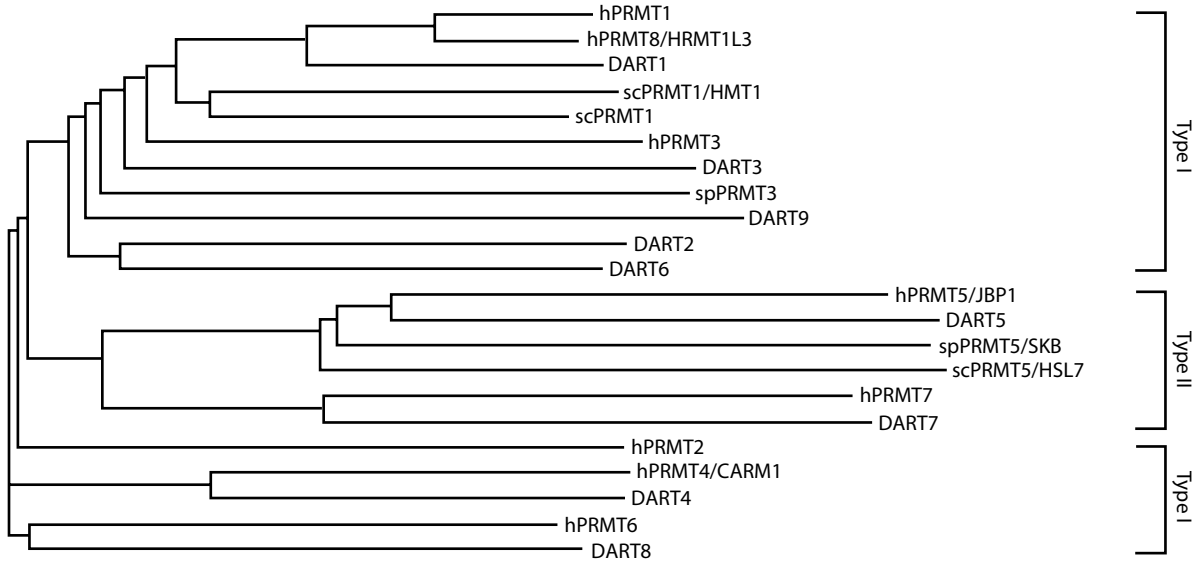

Fig. 2. Phylogenetic analysis of yeast, *Drosophila* and human PRMTs. Multiple sequence alignments were performed using clustalW (http://www.ebi.ac.uk/clustalw/) and a phylogenetic tree analysis of the PRMT amino acid sequences from human (hPRMT1 to hPRMT8), *Drosophila* (DART1 to DART9), and the yeasts *S. cerevisiae* [scPRMT1(HMT) and scPRMT5 (HSL7)] and *S. pombe* (spPRMT1, spPRMT13, and spPRMT5 (SKB)] was plotted.

	Substrate	Enzyme	Reference
1	Histone H4	PRMT1	(*40*)
2	Fibrillarin	PRMT1	(*101*)
3	Nucleolin	PRMT1	(*101*)
4	hnRNP A1	PRMT1	(*105*)
5	hnRNP B	PRMT1	(*105*)
6	hnRNP D	PRMT1	(*105*)
7	hnRNP E	PRMT1	(*105*)
8	hnRNP G	PRMT1	(*105*)
9	hnRNP H	PRMT1	(*105*)
10	hnRNP J	PRMT1	(*105*)
11	hnRNP K	PRMT1	(*105*)
12	hnRNP P	PRMT1	(*105*)
13	hnRNP Q	PRMT1	(*105*)
14	hnRNP R	PRMT1	(*105*)
15	hnRNP U	PRMT1	(*105*)
16	TIS21	PRMT1	(*103*)
17	FGF-2	PRMT1	(*104*)
18	hnRNP A2	PRMT1	(*105*)
19	STAT1	PRMT1	(*48*)
20	Adenovirus E1B-AP5	PRMT1	(*106*)
21	Ewing's sarcoma (EWS)	PRMT1	(*107*)
22	Hepatitis C virus NS3 Helicase	PRMT1	(*108*)
23	CIRP2	PRMT1	(*109*)
24	ZF5	PRMT1	(*110*)
25	GRY-RBP	PRMT1	(*110*)
26	TLS-FUS	PRMT1	(*110*)
27	TAFII-68	PRMT1	(*110*)
28	SAMT1	PRMT1	(*110*)
29	p137GPI	PRMT1	(*110*)
30	RBP58	PRMT1	(*110*)
31	hnRNP R	PRMT1	(*110*)
32	hnRNP K	PRMT1	(*110*)
33	Sam68	PRMT1	(*111*)
34	QKI-5	PRMT1	(*111*)
35	SLM-1	PRMT1	(*111*)
36	SLM-2	PRMT1	(*111*)
37	GRP33	PRMT1	(*111*)
38	SPT5	PRMT1	(*49*)
39	NIP45	PRMT1	(*57*)
40	Adenovirus L4-100 kD	PRMT1	(*112*)
41	FKBP 12	PRMT1	(*113*)
42	RNA helicase A	PRMT1	(*114*)
43	Npl3p	HMT1	(*115*)
44	Hrp1p	HMT1	(*116*)
45	Nab2p	HMT1	(*22*)
46	Gar1p	HMT1	(*117*)
47	Nor1p	HMT1	(*117*)
48	Nsr1p	HMT1	(*117*)
49	Yra1	HMT1	(*21*)
50	PABP II	PRMT3	(*32*)
51	Ribosomal S2 protein	PRMT3	(*24*)
52	Histone H3	CARM1	(*40*)
53	p300	CARM1	(*58*)
54	CBP	CARM1	(*58*)
55	ILF3	CARM1	(*34*)
56	PABP1	CARM1	(*34*)
57	HuR	CARM1	(*90*)
58	Squid	DART4	(*30*)
59	Vasa	DART4	(*30*)
60	TARPP	CARM1	(*74*)

	Substrate	Enzyme	Reference
61	Histone H2A	PRMT5	(*65*)
62	Histone H3	PRMT5	(*118*)
63	Histone H4	PRMT5	(*118*)
64	Sm B	PRMT5	(*55*)
65	Sm B'	PRMT5	(*55*)
66	Sm D1	PRMT5	(*55*)
67	Sm D3	PRMT5	(*55*)
68	LSm4	PRMT5	(*54*)
69	p80 Coilin	PRMT5	(*56*)
70	Epstein-Barr virus EBNA-2	PRMT5	(*120*)
71	SPT5	PRMT5	(*49*)
72	PRMT6	PRMT6	(*12*)
73	HIV-1 TAT	PRMT6	(*121*)
74	MBP	N/D	(*41*)
75	Myosin	N/D	(*122*)
76	Herpes simplex virus ICP27	N/D	(*123*)
77	RBP16	N/D	(*29*)
78	HMGA1a	N/D	(*124*)
79	STAT6	N/D	(*67*)
80	Integral membrane protein Tmp21-I (p23)	N/D	(*17*)
81	p24B, *cis*-Golgi protein	N/D	(*17*)
82	Putative transmembrane methyltransferase	N/D	(*17*)
83	TGN38, *trans*-Golgi network protein 1	N/D	(*17*)
84	Golgin-84	N/D	(*17*)
85	*cis*-Golgi SNARE (p28)	N/D	(*17*)
86	Mannoside acetylglucosaminyltransferase 1	N/D	(*17*)
87	Mannoayl (-1,3-)-glycoprotein-1,4-*N*-acetylglucosaminyltransferase 2	N/D	(*17*)
88	Mannosidase 1, alpha	N/D	(*17*)
89	GRASP55	N/D	(*17*)
90	Cytochrome P450 2d2	N/D	(*17*)
91	Cytochrome P450 2c29	N/D	(*17*)
92	Epoxide hydrolase 1	N/D	(*17*)
93	Protein disulfide isomerase A3	N/D	(*17*)
94	Flavin containing monooxygenase 5	N/D	(*17*)
95	Transferrin	N/D	(*17*)
96	EMP70, member 2	N/D	(*17*)
97	EMP70 member 3	N/D	(*17*)

Table 2. Known arginine-methylated proteins.

phenotype in yeast (*ire15*) could be rescued by genetic complementation with either the tumor growth factor–β receptor or the HMT1 methyltransferase (*62*). A direct link arose from the identification in a yeast two-hybrid screen of an interaction between PRMT1 and the cytoplasmic domain of the IFN-α receptor (*63*). PRMT1 is constitutively associated with the IFN-α receptor, and its expression correlates with IFN-α action (*63*). HeLa cells stably transfected with an expression plasmid encoding PRMT1 antisense were resistant to growth arrest induced by type 1 interferons. These cells no longer mounted an efficient antiviral response when challenged with vesicular stomatitis virus (VSV) (*64*).

Evidence for the involvement of arginine methylation in the JAK-STAT signaling pathway also comes from the identification of JBP1 (JAK2-binding protein 1) as a JAK2-interacting protein in a yeast two-hybrid assay (*13*). JBP1 is the mammalian homolog of SKB1; it is a type II enzyme and was renamed PRMT5 (*65*). PRMT5 binds to a protein known as pICln (a chlorine channel regulator) (*66*) and is part of a large complex dubbed the methylosome (*35*, *36*). Although PRMT5 was identified as a JAK2-interacting protein, there are no functional data linking PRMT5 with the JAK protein kinases or other tyrosine kinases. Moreover, a link between arginine methylation and tyrosine phosphorylation has yet to be established.

Another link between arginine methylation and the JAK-STAT pathway was revealed in studies showing that STAT1 is asymmetrically dimethylated by PRMT1 on Arg^{31} (*48*). Hypomethylation of this arginine results in a decrease in transcriptional activation by STAT1 of an interferon-responsive gene in cells stimulated with IFN-α (*48*). Arginine methylation blocks the association of STAT1 with PIAS1 (protein inhibitor of activated STAT1), but not other properties of STAT1 such as DNA binding activity, tyrosine phosphorylation, or nuclear translocation (*48*). Arginine methylation is also implicated in the regulation of STAT6 function. Inhibition of methylation of Arg^{27} of STAT6 results in reduction of STAT6 tyrosine phosphorylation and failure of STAT6 to translocate to the nucleus and to bind DNA (*67*). There is no known PIAS1 equivalent for STAT6. However, a mutant form of STAT6 containing an amino acid substitution of Arg^{27} to alanine was less stable than its methylated wild-type counterpart, which suggests that methyla-

tion may enhance stability of this protein (*67*). Because all STAT family members contain a conserved Arg at the N terminus (Arg^{31} for STAT1 and Arg^{27} for STAT6), it is possible that all STATs can be methylated. Nevertheless, the finding that arginine methylation of STAT1 and STAT6 has distinct consequences indicates that arginine methylation may have multiple roles in the JAK-STAT signaling cascades (Fig. 3D). The methylation of STAT1 on Arg^{31} has been called into question (*68*) by studies in which no peaks corresponding to methylated STAT1 were detected by mass spectrometry, and no incorporation of radioactive methyl groups could be detected in STAT1 immunoprecipitated after methylation in vivo (*68*). In these experiments, treatment of cells with the methyltransferase inhibitor MTA (5′-deoxy-5′(methylthio)adenosine diminished IFN-induced phosphorylation of STAT1, which is contradictory to results mentioned above (48) and shows that the methylation status of STAT1 does not affect the levels of its phosphorylation (*48*). Further studies are required to clarify these discrepancies.

Arginine methylation of STAT1 and signaling through the JAK-STAT pathway are exploited in chronic viral infection. The hepatocytes of transgenic mice expressing the entire hepatitis C virus (HCV) open reading frame and of patients infected with

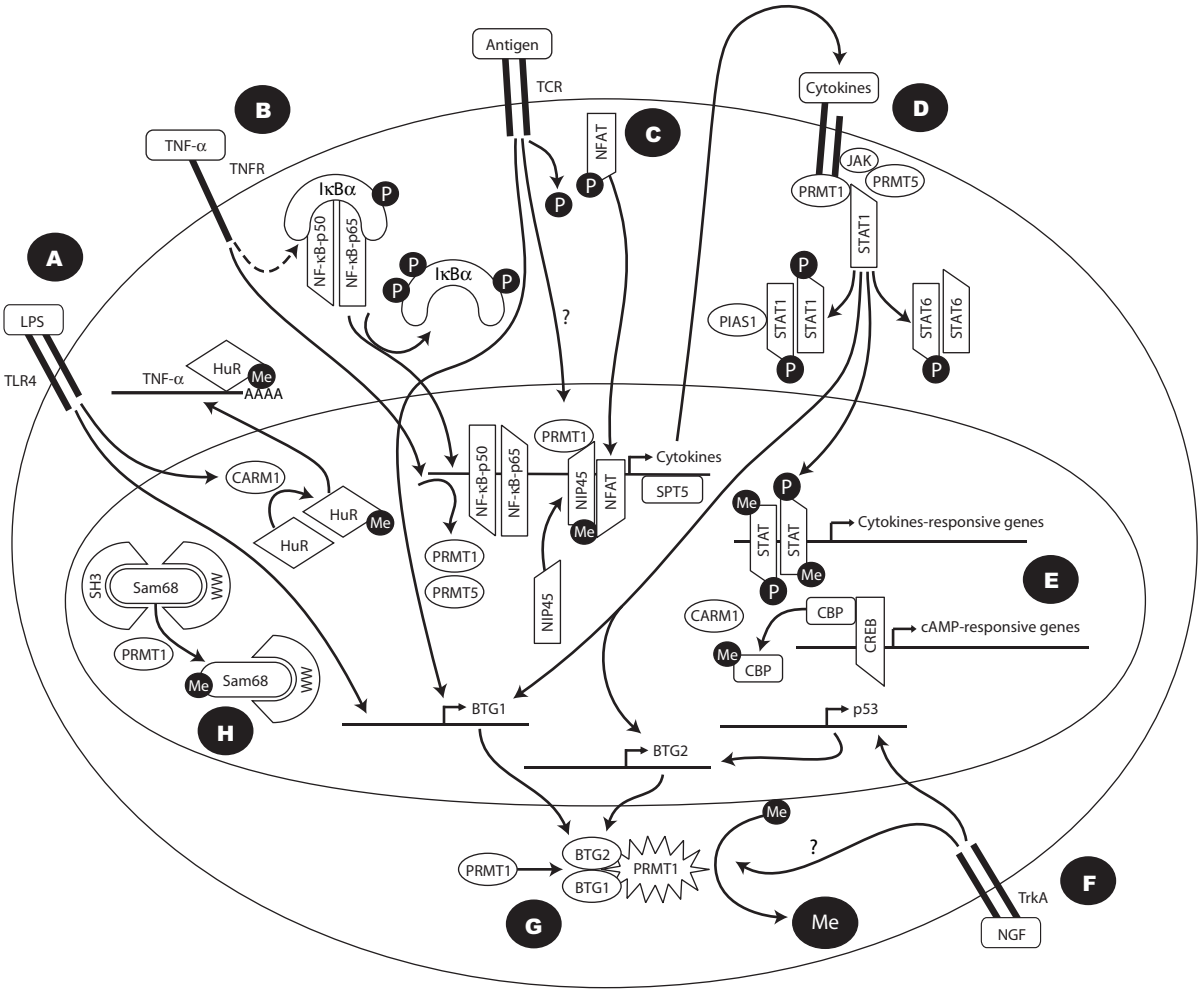

Fig. 3. Arginine methylation in signaling cascades. (**A**) Binding of lipopolysaccharide (LPS) to the toll-like receptor 4 (TLR4) receptor results in increased methylation of HuR by CARM1, which in turn stabilize the mRNA of TNF-α (*90*). (**B**) TNF-α then activates the NF-κB pathway, resulting in transcriptional activation of several genes (including genes encoding cytokines) through transcriptional activation by NF-κB (*92*) and through the elongation factor for polymerase II–mediated transcription SPT5 (*49*). (**C**) Binding of antigen to the TCR, also results in transcriptional activation of cytokine genes through an increase in the amount of PRMT1 and CARM1 coactivators. Methylated NIP45 stimulates cytokine gene expression by associating with NF-AT (*57*). (**D**) Activation of the JAK-STAT pathway by cytokines depends on arginine methylation of STAT1 and STAT6 by PRMT1 in order to achieve proper gene expression responses (*48*, *67*). (**E**) Arginine methylation of the transcriptional coactivator CBP by CARM1 reduces its association with CREB, resulting in a decrease in cAMP-dependent gene expression. (**F**) Amounts of cellular arginine methylation are increased in response to NGF (*89*). (**G**) LPS, TCR, and cytokines increase expression of BTG1 (*83*–*85*), and cytokines increase expression of BTG2 (*86*, *87*). BTGs increased PRMT1 activity (*8*). (**H**) The RNA binding protein and Src adaptor protein, Sam68, binds to SH3 and WW domains (*46*). Arginine methylation of Sam68 by PRMT1 inhibits its binding to SH3, but not to WW, domains (*45*).

chronic HCV show reduced IFN-α gene expression. This signaling defect results from increased expression level of the catalytic subunit of protein phosphatase 2A and the hypomethylation of STAT1 Arg*31* (*69*). These observations suggest arginine methylation may have antiviral effects and that viruses such as HCV may neutralize this line of defense to help establish a chronic infection.

On the basis of these observations, it would be predicated that PRMT1$^{-/-}$ and PRMT5$^{-/-}$ mice would be defective in interferon and cytokine signaling. PRMT1$^{-/-}$ mice have been generated, but they die during early embryogenesis (*70*). PRMT5$^{-/-}$ animals also die as embryos (*71*). Thus, the physiological role of PRMT1 and PRMT5 in the JAK-STAT pathway may be elucidated after the generation of mice with conditional alleles of PRMT1 and PRMT5.

Regulation of the NF-AT Pathway by Methylarginines

T lymphocytes incubated with methylase inhibitors have a reduced ability to stimulate cytokine release after activation (*57*). T cell receptor (TCR) stimulation increases the abundance of mRNAs for PRMT1 and CARM within 1 hour. These studies suggest that increased arginine methylation may be associated with TCR activation; however, this remains to be determined. The NF-AT pathway (which results in activation of the transcription factor NF-AT) is one regulated cascade that appears to be modulated by arginine methylation [reviewed in (*72*)]. TCR signaling triggers a cascade of events that leads to increased cytokine gene expression. The secreted cytokines then bind their receptors through a paracrine or autocrine loop and induce STAT-dependent gene activation (Fig. 3C). NF-AT transcription factors likely determine the spectrum of cytokines that are produced by stimulated T helper cells (*73*). The NF-AT coactivator NIP45 is arginine-methylated by PRMT1, and the NF-AT–NIP45 interaction is enhanced with arginine methylation by an unknown mechanism (*57*). Differentiated T helper cells (T_H1 and T_H2) had increased levels of PRMT1 and CARM1 compared with T helper progenitors, and when stimulated with antibodies against CD3 and CD28, PRMT1 transcripts and the amount of protein increased. Overexpression of PRMT1 resulted in an increase in transcription of the IFN-γ gene and of NF-AT–mediated transcription of the interleukin 4 gene (*57*). The importance of CARM1 in T cell development was observed in CARM1$^{-/-}$ mice, which die shortly after birth because of breathing problems. These animals have T lymphocyte defects because thymocytes remain at an early progenitor stage (*74*). Collectively, these studies represent early work in the area of T cell signaling, and the future will likely show that arginine methylation participates in other key regulatory steps in lymphocytes.

Regulation of Other Signaling Cascades

PRMT1 was identified in a yeast two-hybrid screen for proteins that interact with the immediate early B cell translocation gene (BTG) products BTG1 and BTG2 [BTG2 is also called PC3 (NGF-induced gene 3 in PC12 cells) or TIS21 (tetradecanoyl phorbol acetate–inducible sequences)] (*8*). The human antiproliferative BTG1 gene was cloned from a chromosomal translocation of a B cell chronic lymphocytic leukemia (*75*), and BTG2 was identified as an immediate early gene expressed in response to nerve growth factor (NGF) in PC12 cells (*76*). BTG2 expression is increased by p53 in cells exposed to agents that cause DNA damage. Such cells, but not cells lacking functional BTG2, undergo cell cycle arrest, which suggests that BTG2 might function in control of the cell cycle (*77*). BTG1 and BTG2 activate PRMT1 activity in vitro (Fig. 3G) (*8*), but the PRMT1-interacting domain alone of BTG1 or BTG2 (the BoxC domain) has an inhibitory effect (*78*). BTG1 is a target gene for the forkhead box transcription factor Foxo3a during erythroid differentiation. The increased expression of BTG1 during erythroid differentiation coincided with increased arginine methylation, whereas inhibition of methylation blocked erythroid differentiation (*79*). These results demonstrate that PRMT1 activity may be modulated by changes in expression of BTG1 and BTG2 and that regulation through these proteins may influence cell differentiation. Considering that NGF causes a change in subcellular localization of the p53 tumor suppressor protein and an increase in the overall amount of the protein (*80*, *81*), as well as that BTG2 is a p53-transcriptional target (*82*), it seems possible that the increased expression of p53 in response to NGF and its subsequent stimulation of BTG2 synthesis, which can cause an increase in methylation, could explain the increase in methylation seen upon neuronal differentiation (Fig. 3F). However, it is still unclear what mechanism or which specific target proteins account for the observed effects. Lipopolysaccharide (LPS), cytokines, and TCR activation can all increase the expression of BTG1 (*83*–*85*), and the fact that cytokines increase the expression of BTG2 (*86*, *87*) suggests an additional mechanism that explains the increase in methyltransferase activity observed after activation of cytokine signaling pathways.

Amounts of cellular arginine methylation increase after stimulation of PC12 cells with NGF (Fig. 3F) (*88*, *89*). Treatment of cells with methyltransferase inhibitors or in vivo delivery of a domain having an inhibitory effect on arginine methyltransferases, the BoxC domain, of BTG1 or BTG2 inhibited differentiation of PC12 cells induced by NGF (*78*), but not epidermal growth factor (EGF) (*88*). Therefore, arginine methylation appears to be required during cellular differentiation of PC12 cells.

A role for arginine methylation was observed in cells exposed to LPS. The RNA binding protein HuR (Hu antigen protein R) was observed to be methylated on Arg^{217} by CARM1 in response to LPS treatment (*90*). HuR binds AU-rich elements and stabilizes mRNAs in response to cellular signals including cytokines and LPS (*91*), and how methylation regulates these functions is unknown. Liver macrophages pretreated with methylase inhibitors had a decrease in TNF-α and nuclear factor κB (NF-κB) promoter activity in response to LPS (Fig. 3, A and B) (*92*, *93*). Taken together these findings suggest that methylation on arginines may influence transcriptional and posttranscriptional events that modulate TNF-α and NF-κB function.

In several cases, phosphorylation and methylation events appear to be coregulated (*94*). For example, arginine methylation of the Npl3p RNA binding protein prevents its phosphorylation and nuclear import (*95*). Methylation of STAT6 also influences its phosphorylation and activation (*67*). In contrast, STAT1 phosphorylation was not altered by methylation, but rather its activity is regulated by PIAS1 (*48*). Arginine methylation of STAT1 controls the rate of STAT1 dephosphorylation by the nuclear TcPTP (T cell protein tyrosine phosphatase) protein tyrosine phosphatase (*96*).

Demethylases: Reversing Arginine Methylation

Arginine methylation was recently demonstrated to be reversible (*97*). Peptidylarginine deiminase 4 (PAD4) converts MMA into citrulline and releases methylamine (*98*, *99*). Thus, a

balance may exist between methylation and demethylation, much like the balance that occurs with kinases and phosphatases. PADs were identified decades ago (*100*). However, their demethylation activity may have eluded discovery because of their inability to reverse a dimethylarginine residue, the major type of arginine methylation that was studied over the past decades. It is interesting that PADs only convert MMA and not DMA to citrulline (*98*, *99*). Therefore, the conversion of MMA to citrulline by PADs would prevent its methylation into aDMA and sDMA by PRMTs. Also the methylation of MMA by PRMTs into aDMA would protect these residues against PAD activity. Therefore, PADs and PRMTs may have antagonistic activities. Further work will surely be aimed at understanding this balance in signaling pathways.

Conclusion

The studies documented herein demonstrate that methylated residues are critical for regulating protein function such as protein-protein interactions. The fact that methylarginines are present in key signaling proteins suggests that arginine methylation represents an additional level of complexity in signal transduction. The growing evidence describing roles for arginine methylation in different T cell signaling and cytokine responses and the identification of methylated arginines in proteins involved in other signaling cascades suggest that arginine methylation may play additional roles in many cellular signaling processes.

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Citation: F.-M. Boisvert, C. A. Chénard, S. Richard, Protein interfaces in signaling regulated by arginine methylation. *Sci. STKE* **2005**, re2 (2005).