Biochimica et Biophysica Acta xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbagrm

Review

Readers of histone methylarginine marks ☆

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ARTICLE INFO

Article history:
Received 17 November 2013
Received in revised form 31 January 2014
Accepted 14 February 2014
Available online xxxx

Keywords:
Arginine methylation
Tudor domain
CARM1
PRMT1
Histone code

ABSTRACT

Arginine methylation is a common posttranslational modification (PTM) that alters roughly 0.5% of all arginine residues in the cells. There are three types of arginine methylation: monomethylarginine (MMA), asymmetric dimethylarginine (ADMA), and symmetric dimethylarginine (SDMA). These three PTMs are enriched on RNA-binding proteins and on histones, and also impact signal transduction cascades. To date, over thirty arginine methylation sites have been cataloged on the different core histones. These modifications alter protein structure, impact interactions with DNA, and also generate docking sites for effector molecules. The primary "readers" of methylarginine marks are Tudor domain-containing proteins. The complete family of thirty-six Tudor domain-containing proteins has yet to be fully characterized, but at least ten bind methyllysine motifs and eight bind methylarginine motifs. In this review, we will highlight the biological roles of the Tudor domains that interact with arginine methylated motifs, and also address other types of interactions that are regulated by these particular PTMs. This article is part of a Special Issue entitled: Molecular mechanisms of histone modification function.

1. Introduction

Arginine has the longest side chain of the 20 naturally occurring amino acids, and the end of the side chain bears a positive charge — properties that make it a good anchor for potential protein–protein interactions. Its guanidine group contains five potential hydrogen bond donors that can be used to stabilize interactions with DNA, RNA and proteins [1]. The methylation of arginine changes its shape, does not alter the charge, but removes potential hydrogen bond donors, which would potentially inhibit certain interactions [2]. Importantly, the methylation of arginine residues can also increase their affinity to aromatic rings in cation–pi interactions, thus promoting other interactions [3]. So, protein arginine methylation can both positively and negatively regulate protein–protein interactions, examples of which will be highlighted here.

Three distinct types of methylated arginine residues occur in mammalian cells (Fig. 1A). The most abundant type is omega-N^G,N^G-dimethylarginine [4]. In this case, two methyl groups are placed on one of the terminal nitrogen atoms of the guanidino group, and this derivative is commonly referred to as asymmetric dimethylarginine (ADMA). Two other derivatives occur at levels less than 50% that of ADMA. These include the symmetric dimethylated derivative, where one methyl group is placed on each of the terminal guanidino nitrogens (omega-N^G,N'^G-dimethylarginine; commonly referred to as SDMA) and the monomethylated derivative with a single methyl group on the terminal nitrogen atom (omega-N^G-monomethylarginine; commonly referred to as MMA). The three types of arginine methylation are catalyzed by a family of nine AdoMet-dependent enzymes called the protein arginine methyltransferases (PRMTs). Arginine demethylation activity has been reported for the JmjC-domain-containing protein JMJD6 [5,6].

The PRMTs are classified according to the type of methylation they are able to catalyze. Types I, II and III are able to generate a MMA. Type I enzymes (PRMT1, PRMT2, PRMT3, PRMT4/CARM1, PRMT6, and PRMT8) perform a second methylation step to generate the ADMA mark, and the Type II enzyme (PRMT5) generates the SDMA mark. The Type III enzyme (PRMT7) only generates a MMA mark. Most MMA marks are presumed to serve as precursors for the subsequent methylation by Type I and II PRMTs, but certain proteins exist in a heavily monomethylated state [7]. Sequence analysis of all PRMTs shows a highly conserved catalytic core region, containing the signature methyltransferase motifs I, post-I, II and III, which are characteristic of the super-family of seven-beta strand methyltransferases. They also harbor additional "double E" (two glutamate residues) and "THW" (threonine–histidine–tryptophan) sequence motifs, which are particular to the PRMT subfamily of methyltransferases [1]. The catalytic core is highly conserved at the structural level, as revealed by the crystal structure of PRMT1, PRMT3, PRMT4 and PRMT5 [8–14].

It should be noted that the metabolic cost of arginine methylation is high, requiring the use of 12 ATP molecules per methylation event [15]. The fact that such an "expensive" PTM is abundant and has not been lost to evolutionary pressure underscores the biological importance of the methylated motifs that have survived.

☆ This article is part of a Special Issue entitled: Molecular mechanisms of histone modification function.

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http://dx.doi.org/10.1016/j.bbagrm.2014.02.015
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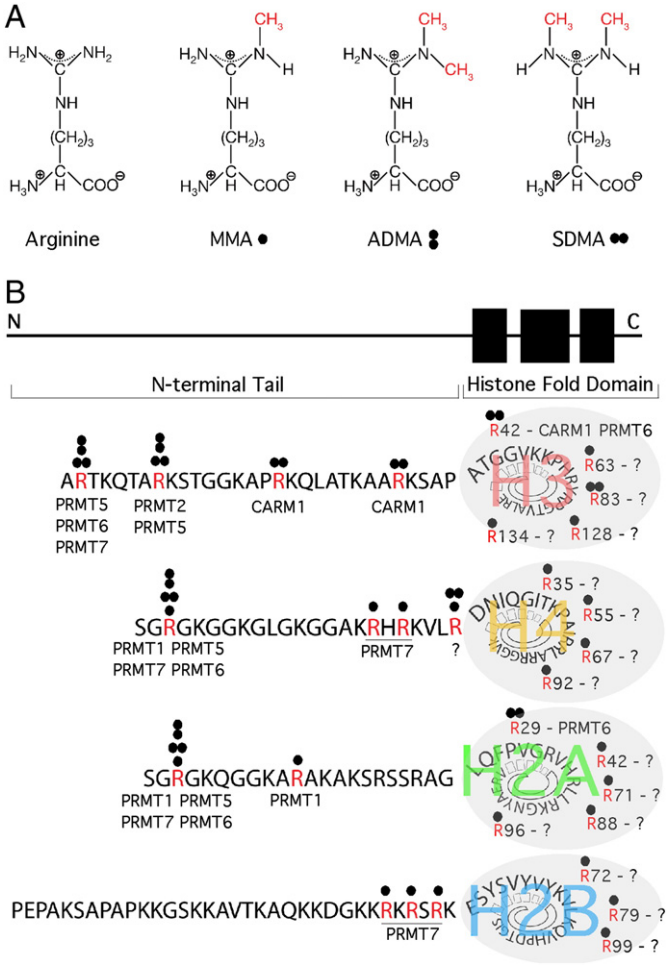

Fig. 1. Types and sites of arginine methylation on histones. (A) Arginine residues in the tails of histones can be monomethylarginines (MMA — ●), asymmetric dimethylarginines (ADMA — ●●), or symmetric dimethylarginines (SDMA — ●●). Methyl groups are marked in red. (B) Positioning of the unstructured histone tail relative to the structure C-terminal core region. The reported sites of histone H3, H4, H2A, and H2B arginine methylation are shown. The references that first reported these methylated sites are listed in Table 1. A number of arginine methylated sites have been identified by mass spectrometric methods, but it has yet to be established which PRMTs modify them, and these sites are thus assigned question marks.

2. Sites of arginine methylation on histones

Arginine methylation is an abundant posttranslational modification (PTM), with about 0.5% of arginine residues methylated in mammalian tissues [4,16], and roughly 2% of arginine residues methylated in rat liver nuclei [17]. The large majority of this type of protein methylation occurs on non-histone proteins and most of these substrates are methylated on Glycine/Arginine-Rich (GAR) motifs. Many of these substrates have recently been cataloged by mass spectrometric analysis [18–20]. Importantly, a number of sites on histone tails are methylated [21], and there is emerging evidence for the existence of arginine-methylated sites within the histone core region [22] (Fig. 1B). We have compiled a list of the arginine methylation sites that are found on histone, along with the reference that reported each particular PTM (Table 1). We must emphasize that many of these sites are not very well characterized, and often their existence has not been confirmed by alternative approaches, like methyl-specific antibodies or in vitro methylation assays. Furthermore, the enzymes that methylate many of these sites have yet to be elucidated. Finally, modulation of the levels of one type of arginine modification can alter the levels of the other two methylarginine types [7]. This is important, because manipulation of one type of arginine methylation reaction by PRMT knockout, small molecule inhibition or overexpression, may impact the occurrence of other types of arginine methylation.

Table 1
Arginine methylation sites on histone tails and cores.

Site & type	Enzyme	Ref	Site & type	Enzyme	Ref
Histone H3			*Histone H4*		
R2me2a	PRMT6	[86–88]	R3me2a	PRMT1	[120]
R2me2s	PRMT5 PRMT7	[94]	R3me2a	PRMT6	[87]
R8me2a	PRMT2	[121]	R3me2s	PRMT5	[97]
R8me2s	PRMT5	[97]	R3me2s	PRMT7	[122]
R17me2a	CARM1	[123]	R17me1/me2	PRMT7	[124,125]
R26me2a	CARM1	[123]	R19me1/me2	PRMT7	[124,125]
R42me2a	CARM1 PRMT6	[22]	R23me1/me2	Unknown	[124]
R63me1	Unknown	[126]	R35me1	Unknown	[127,126]
R83me1/me2	Unknown	[124]	R55me1	Unknown	[127,126]
R128me1	Unknown	[126]	R67me1	Unknown	[127,126]
R134me1	Unknown	[124]	R92me1	Unknown	[128]
H2A			*H2B*		
R3me2a	PRMT6	[87]	R29me1	PRMT7	[125]
R3me2s	PRMT5	[129]	R31me1	PRMT7	[125]
R3me2s	PRMT7	[122]	R33me1	PRMT7	[125]
R11me1	PRMT1	[130]	R72me1	Unknown	[127]
R29me2a	PRMT6	[130]	R79me1	Unknown	[126]
R42me1	Unknown	[126]	R99me1	Unknown	[128]
R71me1	Unknown	[124]			
R88me1	Unknown	[126]			
R96me1	Unknown	[127]			

3. Tudor domains

The seminal discovery, made by Tony Pawson over twenty years ago, that SRC homology 2 (SH2) domains bind to short protein motifs that are tyrosine phosphorylated [23], led to the realization that different modular domains bind distinct types of PTMs [24]. For example, lysine methylated motifs are bound by at least eight different domain types — Chromo, PHD, MBT, Tudor, PWWP, Ank, BAH and WD40 domains. Currently, the only protein domain family known to bind methylated arginine motifs is the Tudor family (although individual PHD and WD40 domains also harbor this ability). Tudor domains were identified simultaneously by two research groups, which both realized that the *Drosophila melanogaster* Tudor protein contains previously unrecognized repeating domains, which were found in a number of other proteins in many different species [25,26]. Interestingly, the fly Tudor gene was discovered in a genetic screen for maternally expressed genes that result in lethality or sterility of the progeny [27]. The Tudor gene was named after the English Tudor dynasty because of the fertility issues that plagued Henry VIII, who was desperate for a male heir to continue the Tudor line, and whose many wives had repeated stillbirths and miscarriages. Tudor domains are roughly 60 amino acids in size and fold into four antiparallel β-strands. The Tudor domain is the founding member of the 'Royal Family' of domains, which also includes Chromo, MBT and PWWP domains [28]. All 'Royal Family' domains with methyl-binding properties have an aromatic cage to facilitate the methyl-dependent protein–protein interaction.

4. Tudor domains bind methyllysine and methylarginine motifs

The pioneering work on Tudor domain biochemistry and structure involved studies using the human survival motor neuron (SMN) protein [29,30], which is mutated in spinal muscular atrophy syndrome [31]. SMN harbors a single Tudor domain and was one of the first proteins identified to interact with a methylated motif [29,32], along with the chromo domain-containing protein, HP1 [33,34]. It soon became clear that Tudor domains bind not only methylarginine motifs, but also methyl-lysine marks [35–37]. In humans, there are at least thirty-six proteins that harbor Tudor domains, but there are over 60 Tudor