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REVIEW

Protein arginine methylation: a prominent modification and its demethylation

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Abstract Arginine methylation of histones is one mechanism of epigenetic regulation in eukaryotic cells. Methylarginines can also be found in non-histone proteins involved in various different processes in a cell. An enzyme family of nine protein arginine methyltransferases catalyses the addition of methyl groups on arginines of histone and non-histone proteins, resulting in either mono- or dimethylated-arginine residues. The reversibility of histone modifications is an essential feature of epigenetic regulation to respond to changes in environmental factors, signalling events, or metabolic alterations. Prominent histone modifications like lysine acetylation and lysine methylation are reversible. Enzyme family pairs have been identified, with each pair of lysine acetyltransferases/deacetylases and lysine methyltransferases/demethylases operating complementarily to generate or erase lysine modifications. Several analyses also indicate a reversible nature of arginine methylation, but the enzymes facilitating direct removal of methyl moieties from arginine residues in proteins have been discussed controversially. Differing reports have been seen for initially characterized putative candidates, like peptidyl arginine deiminase 4 or Jumonji-domain containing protein 6. Here, we review the most recent cellular, biochemical, and mass spectrometry work on arginine methylation and its reversible nature with a special focus on putative arginine demethylases, including the enzyme superfamily of Fe(II) and 2-oxoglutarate-dependent oxygenases.

Keywords Histone modifications · Post-translational modifications · KDM · KMT · KDM2A · KDM3A · KDM4E · KDM5C · KDM6B · PHF8 · KDM7B · Liquid chromatography–tandem mass spectrometry

Introduction

Protein methylation has mainly been detected on lysine and arginine residues [1]. *N*-ε-lysine methylation emerged as one of the most abundant histone marks in eukaryotic chromatin [2]. In addition, hundreds of non-histone proteins, including for instance p53 and NFκB, are methylated at lysine residues [3, 4]. Here, lysine methylation can regulate protein–protein interaction or protein stability [4]. Lysine can be mono- (Kme1), di- (Kme2) or tri-methylated (Kme3) on its ε-amino group (Fig. 1a). Lysine methylation has been revealed to be a reversible modification, participating in a dynamic network of regulation of protein function and gene expression [5]. Similar to kinases and phosphatases for phosphorylations, an enzyme family pair with contrary activities also for lysine methylation has been identified (Fig. 1a). *N*-ε-lysine methylation is catalyzed by an enzyme family of lysine methyltransferases (KMTs), that up to date harbors more than 25 members in humans [6, 7]. The removal of methyl moieties from lysine is achieved by lysine demethylases (KDMs), including the Fe(II) and 2-oxoglutarate (2OG) dependent JmjC-subfamily (2OG

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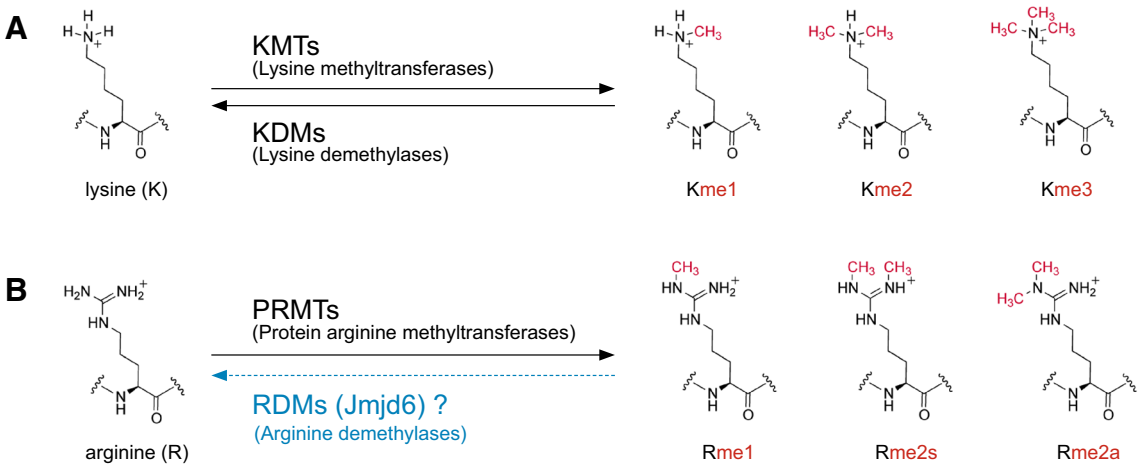


Fig. 1 Counteracting pair of enzyme families regulates methylation of lysine residues in proteins. Mono-, di-, or tri-methylation (Kme1, Kme2, and Kme3) of lysine at the ε-amino group is catalyzed by lysine methyltransferases (KMTs) (**a**). This modification is reversible and can be erased by lysine demethylases (KDMs) (**a**). The protein arginine methyltransferases (PRMTs) can modify the guanidinium group of arginine residues and result in either mono-methylated (Rme1) or symmetric (Rme2s) or asymmetric (Rme2a) di-methylated arginine (**b**). Presently, an arginine demethylation activity in vivo has been described for Jumonji domain containing protein 6 (Jmjd6) (**b**)

oxygenases) and the flavin-dependent LSD1-subfamily [6, 8]. A recent mass-spectrometry analysis identified several thousand methylated-arginine residues in cellular proteomes, which indicates a ubiquitous role also for arginine methylation in histone and non-histone proteins [9]. However, arginine methylation is different. Although nine mammalian protein arginine methyltransferases (PRMTs) have been identified [10], in contrast so far just one enzyme, the Jumonji domain containing protein 6 (Jmjd6), has been reported to have a potential arginine demethylation activity in vivo [11]. However, this activity has been challenged by several other studies [12–16]. Indeed, arginine methylation seems to be reversible. Several analyses including a large-scale mass spectrometry approach support the idea of direct arginine demethylation. Very recently, several lysine demethylases of the 2OG oxygenase family have also shown arginine demethylation activity in vitro [17]. Here, we review recent analyses about the distribution of methylarginine in proteomes and emerging data on reversibility and demethylation of methylarginine residues.

Arginine methylation

Arginines in peptides can be either mono-methylated on one of the ω-amino groups to produce mono-methylarginine (MMA, Rme1) or they can be di-methylated. Two methyl groups on one ω-amino group result in an asymmetric di-methylated arginine (ADMA, Rme2a), whereas a symmetric di-methylarginine (SDMA, Rme2s) has one methyl group on each ω-amino group (Fig. 1b). Arginine exhibits a side chain with a planar guanidinium group with five potential hydrogen bond donor sites and an overall positive charge at physiological conditions. This makes it ideally suitable for binding of negatively charged molecules, like for instance nucleic acids. Addition of methyl moieties does not alter the overall positive charge of arginine but occupies hydrogen bond donor sites.

Arginine methylation has been identified down to the earliest branches of eukaryotes [18, 19]. In *Saccharomyces cerevisiae*, an additional mono-methylation on the δ-amino group of arginine (δ-MMA) has been described [20]. Initially thought to be a yeast-specific modification, a recent analysis of plasma samples with triple stage mass spectrometry reported δ-MMA in human as well [21]. Methylation of arginine is catalyzed by a group of nine protein arginine methyltransferases (PRMT1-9), which were thoroughly reviewed in [10]. The PRMT family of enzymes is well conserved within multicellular organisms ranging from cnidarians to humans [22]. Even in yeast (*S. cerevisiae*), four protein arginine methyltransferases have been identified [23].

In eukaryotes, methylated arginines have been found in histone and non-histone proteins, involved in functions such as RNA processing, DNA repair, and transcription [24]. In histones, the best-characterized methylated-arginine residues include R2, R8, R17, and R26 of histone H3 and R3 in histone H4 and H2A [25]. Due to the methylation state of individual arginine residues, the adjacent chromatin region is either transcriptional active or repressed. Asymmetric di-methylation of H3R2, for instance, has been linked to inactive chromatin regions. In contrast, mono-methylation or symmetric di-methylation of H3R2 is associated with an active chromatin state [26, 27].

Proteomic analyses of methylarginine residues

In recent years, advances in liquid chromatography–tandem mass spectrometry (LC-MS/MS) enabled comprehensive large-scale proteomic analyses, including detection of post-translational modifications (PTMs) on a proteome-wide scale. This gave rise to several analyses of global PTM patterns and may help to characterize respective enzymes catalyzing these modifications. However, several challenges are associated with the LC-MS/MS detection of PTMs in general and arginine methylation in particular. The peptides containing regulatory PTMs are often low abundant peptides [28]. To characterize those PTMs in whole proteomes, a peptide immunoaffinity purification step has been established prior to LC-MS/MS analysis [29, 30] (Fig. 2). The use of the PTM-specific antibody enrichment technology has led to several large-scale LC-MS/MS analyses of arginine methylation in different organisms [31–41]. The diversity and specificity of those antibodies must be taken into account when analyzing methylarginine (meR) data from global mass spectrometry approaches. In each individual experiment, a different anti-meR antibody might have been used for enrichment of meR-peptides (Fig. 2). Another issue of specific identification of methylated-arginine peptides in LC-MS/MS might be the fact that the 14 Da mass shift accompanied with the addition of a methyl group corresponds also to differences between amino-acid side chains such as G to A, D to E, and E to Q or to some other amino-acid substitutions [39]. Therefore, several groups developed applications of the stable-isotope labeling with amino acids in cell culture (SILAC) technology to improve confidence in identified methylated peptides [34, 38]. In the 'heavy methyl' SILAC approach (hmSILAC), methyl groups in PTMs are directly labeled in vivo. This has been achieved by growing cells in medium, which contains heavy methionine, carrying one ^{13}C and three deuterium atoms ([$^{13}CD_3$]methionine). The [$^{13}CD_3$]methionine is metabolically converted into labeled 'heavy' SAM, which is the methyl group donor for PRMTs [38].

About a decade ago, an initial LC-MS/MS approach (without SILAC) identified 200 proteins with putatively methylated arginines in the proteome of Hela cells. Immunoaffinitiy enrichment with antibodies detecting symmetric and asymmetric di-methylated arginines (Rme2s and Rme2a) revealed mainly proteins related to transcription and RNA processing, signal transduction, and DNA repair [31]. Application of the first hmSILAC experiment coupled to LC-MS/MS pinpointed 59 specific methylation sites to 33 proteins in Hela cells in 2004 [38]. Separate analyses of cytosolic and nucleosolic fractions of Hela cells further expanded the discovery to 323 methylated arginines [32]. They detected a higher occurrence of methylated arginines in nuclear proteins as compared to cytosolic fractions [32]. Over time, proteomic analyses of several different mammalian cell lines, including Jurkat cells [34, 42], HCT116 colon cancer cells [35], and human embryonic kidney 293 cells [9, 40] raised the amount of detected arginine methylation sites up to 8030 in 3300 proteins of 293 cells [9]. Arginine methylation now appears as a widespread and abundant post-translational modification. Studies in mouse [35], yeast [37, 39, 41, 43–45], and trypanosomas [33, 36] confirmed arginine methylation as an evolutionary conserved PTM mechanism (summary of MS analyses in Table 1).

Reversibility of arginine methylation?

Arginine methylation was initially thought to be a rather permanent modification [46], but there are several analyses supporting a reversible nature of methylarginine. The estrogen receptor α (ERα) is methylated in an RGG motif

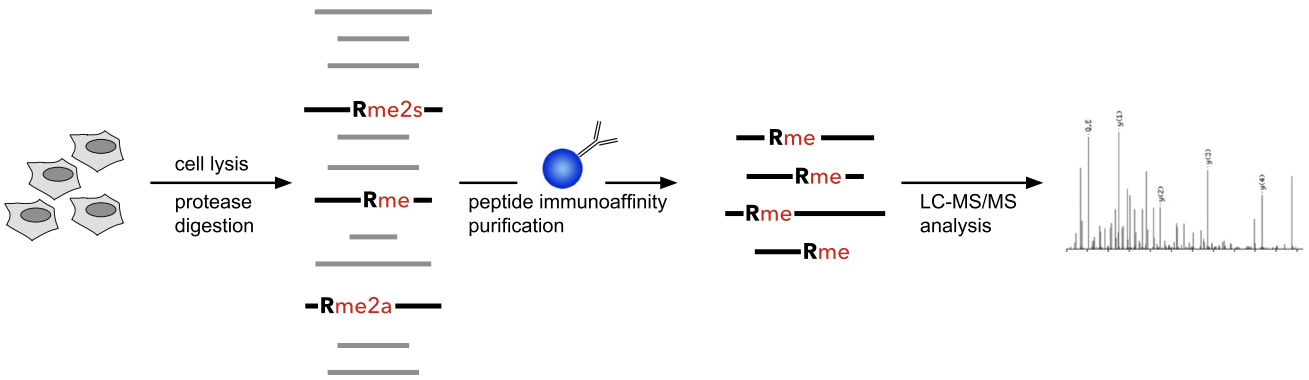


Fig. 2 General procedure of large-scale mass spectrometry analysis of cellular arginine methylomes. The first step of the proteomic workflow is protein extraction and digestion with a sequence-specific protease. Cells might be pretreated with stable-isotope labeling methods (SILAC). To enrich the usually low abundant peptides with post-translational modifications, an immunoaffinity purification step has been established. Prior to LC-MS analysis, the peptide mixture is incubated with antibodies detecting specific PTMs of interest

Table 1 Comprehensive summary of whole-proteome analyses on methylated arginines from different cells, tissues, and organisms, including human cells, mouse, yeast (*Saccharomyces cerevisiae*), and *Trypanosoma brucei*

	Cells/tissue/organism	SILAC	Protease	Antibodies	Methylarginines	References
Human	HeLa S3	–	Trypsin	Asym24 (Rme2a) Asym25 (Rme2a) Sym10 (Rme2s) Sym11 (Rme2s)	200 proteins	Boisvert et al. [31]
	HeLa S3	SILAC	Trypsin	ab412 [7E6] (Rme, RRme2s, Rme2a)	59 arginine methylation sites in 33 proteins	Ong et al. [38]
	Jurkat, human primary CD4+ lymphocytes	hmSILAC	Trypsin, Chymotrypsin	ab412, [7E6] (Rme, RRme2s, Rme2a)	249 arginine methylation sites in 131 proteins	Uhlmann et al. [42]
	HeLa S3	hmSILAC	Trypsin	CS Rme-I (Rme) CS Rme-ll (Rme) EG-Rsym (Rme2s) MP-Rsym (Rme2s) EG-Rasym (Rme2a) MP-Rasym (Rme2a)	323 arginine methylation sites	Bremang et al. [32]
	HCT116 cells	–	Trypsin	D5A12 (Rme) Me-R4-100 (Rme) D6A8 (Rme2) D4H5 (Rme2)	1106 MMA sites in 570 proteins	Guo et al. [35]
	293T	SILAC	LysC, Trypsin	Me-R4-100 (Rme) D5A12 (Rme)	1027 MMA sites in 494 proteins	Sylvestersen et al. [40]
	Jurkat, primary T-cells	iMethyl-SILAC	Trypsin, Chymotrypsin, GluC	Me-R4-100 (Rme) D5A12 (Rme)	2505 MMA sites in 1257 proteins	Geoghegan et al. [34]
	293	SILAC	LysC, Trypsin	PTMScan® Mono-Methyl Arginine Motif [mme-RG] Kit	8030 MMA sites in 3300 proteins	Larsen et al [9]
Mouse	Mouse brain (3-month-old mice)	–	Trypsin	D5A12 (Rme) Me-R4-100 (Rme) D6A8 (Rme2) D4H5 (Rme2)	807 MMA sites from 453 proteins	Guo et al. [35]
	Mouse embryo (E16-17)	–	Trypsin	D5A12 (Rme) Me-R4-100 (Rme) D6A8 (Rme2) D4H5 (Rme2)	598 MMA sites from 331 proteins	Guo et al. [35]
Yeast	*S. cerevisiae*	–	Trypsin	ICP0801 (anti-MMA)	90 proteins	Low et al. [23]
	S. cerevisiae	hmSILAC	Trypsin	–	8 arginine methylation sites	Wang et al. [41]
	S. cerevisiae	hmSILAC	Trypsin	Me-R4-100 (Rme) D5A12 (Rme)	41 arginine methylation sites in 13 proteins	Plank et al. [39]

Table 1 (continued)

	Cells/tissue/organism	SILAC	Protease	Antibodies	Methylarginines	References
Trypanosoma	*T. brucei*	–	Trypsin GluC	Asym24 Sym11 mRG	1332 methylarginine sites in 676 proteins	Lott et al. [36]
	T. brucei (mitochondria)	–	Trypsin GluC	Asym24 Sym10 Sym11 mRG	167 mitochondrial proteins with methylarginine	Fisk et al. [33]

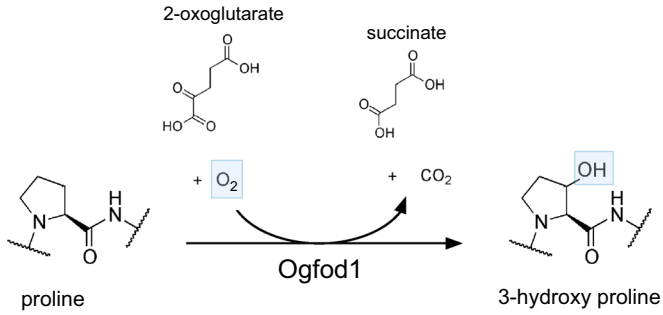
at R260 by PRMT1 [47]. Treatment of MCF-7 cells with estrogens resulted in an increase in ERα R260 methylation within 5 min, followed by a rapid decrease of methylation level at R260 within another 10 min [47]. These results point towards an active demethylation of ERα. Another example is kinetic ChIP experiments on the promoter of the estrogen-inducible pS2 gene. In this case, dimethylation of R17 in histone H3 (H3R17me2) increases and declines again within 20 min cycles periodically [48]. H3R17me2 has also been shown to change during cell cycle progression. Extracted histones from synchronized HeLa cells exhibited an increased H3R17me2 level in M-phase, whereas in G1 phase, the anti-H3R17me2 signal in Western blots fell back to basal levels [49].

In 2014, a large-scale mass spectrometry SILAC analysis revealed dynamic MMA alterations in U2OS cells after blocking of transcription with Actinomycin D (ActD) [40]. A time course of one to 16 h of ActD treatment revealed MMA sites with a continuous downregulation in this period, whereas others only decreased after a prolonged ActD treatment. An early decrease of MMAs was identified within 3 h and overall protein levels of those MMA sites did not change within this time frame. Sylvestersen et al. suggested an active demethylation could account for MMA alterations instead of protein degradation and re-synthesis [40].

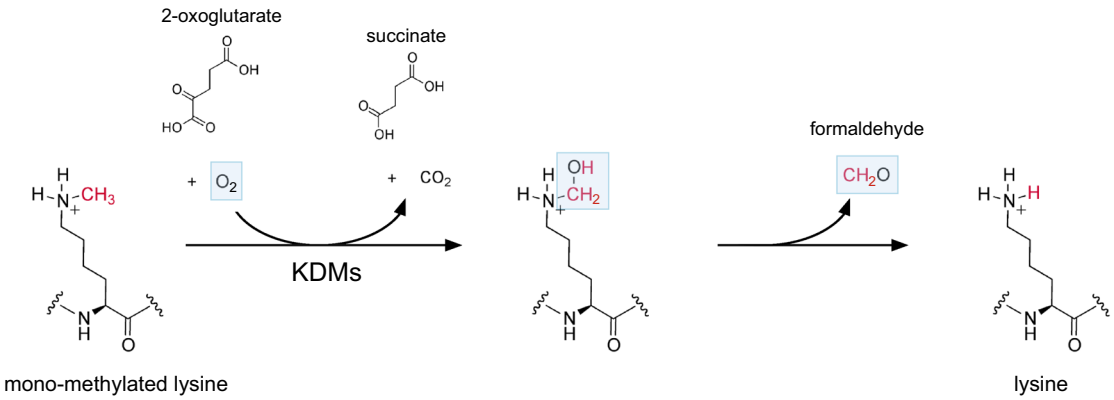
More recently, stress granule (SG) assembly has been linked to arginine demethylation in the Ras-GAP SH3 Binding Protein 1 (G3BP1) [50]. Arginines in the RGG domain of the SG-nucleating protein G3BP1 were demonstrated to be methylated by PRMT1 and 5. Monitoring methylated arginines (ADMA) in G3BP1 upon arsenite stress revealed a decrease in anti-ADMA antibody signal in western blots of 20% after 30 min and 50% after 1 h [50]. Liquid chromatography–mass spectrometry (LC-MS) analysis identified demethylated residues R435, R447, and R460 in G3BP1 in stress conditions [50].

In summary, whole proteome analyses revealed prominent arginine methylation levels, and indicated reversibility of this modification. However, the 'arginine demethylases' (RDMs), proteins, which can directly remove methyl groups from arginine, are not very well characterized. There are only two candidate proteins, including the Peptidylarginine deiminase 4 (PADI4) and Jumonji domain containing protein 6 (Jmjd6). Results describing their RDM-activities have been challenged in the meantime. In addition, several KDMs of the Fe(II) and 2OG-dependent oxygenase family have been shown to also demethylate arginines [17]. These results are summarized below.

A **Fe(II) & 2-oxoglutarate dependent oxygenases ► protein hydroxylation**



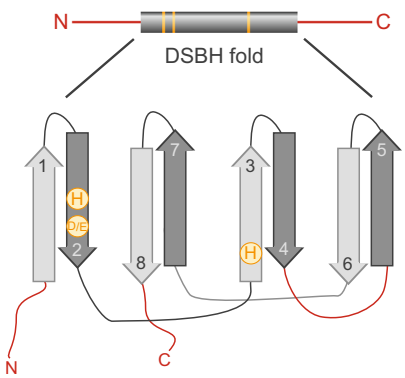
B **Fe(II) & 2-oxoglutarate dependent oxygenases ► protein lysine (oxidative) demethylation**



C

human protein hydroxylases	human KDMs
LEPRE1	KDM2A
LEPREL1	KDM2B
LEPREL2	KDM3A
P4HA1	KDM3B
P4HA2	JMJD1C
P4HA3	KDM4A
ASPH	KDM4B
P4HTM	KDM4C
PHD1	KDM4D
PHD2	KDM4E
PHD3	KDM5A
PLOD1	KDM5B
PLOD2	KDM5C
PLOD3	KDM5D
OGFOD1	KDM6A
FIH1	KDM6B
MINA53	KDM6C
NO66	KDM7A
JMJD4	KDM7B
JMJD6	KDM7C

D



E Putative arginine demethylases:

JMJD6	Arginine demethylase activity Lysine hydroxylation activity
KDM2A KDM3A KDM4E KDM5C KDM6B KDM7B	*in vitro* arginine demethylation activity on peptides

◂**Fig. 3** Fe(II) and 2-oxoglutarate-dependent oxygenases. The protein superfamily of Fe(II) and 2-oxoglutrate-dependent oxygenases (2OG oxygenase) transfers oxygen (O_2) onto a substrate. This can either result in a stable protein hydroxylation (**a**) or in a lysine demethylation (**b**). Substrate oxidation is always coupled to decarboxylation of the co-substrate 2-oxoglutarate, leading to succinate and CO_2. 2OG oxygenase-catalyzed protein hydroxylation is exemplified by Ogfod1, which hydroxylates proline 62 in the human ribosomal protein RPS23 [58, 59] (**a**), but has also been described for Arg, Asp, Asn, His, and Lys in different proteins [57]. Alternatively, hydroxylation can occur on a methyl group of methylated lysine, which then results in release of formaldehyde and demethylation of the lysine residue (**b**). Protein hydroxylases and lysine demethylases (KDMs) comprise two distinct subgroups of 2OG oxygenases (**c**). They all share one common structural motif, the double stranded β-helix (DSBH) fold (or jumonji fold) consisting of eight β-sheets. A conserved HxD/E…H motif coordinates the binding of co-factor Fe(II) (**d**). Some members of the 2OG oxygenase family have been assigned a putative arginine demethylation activity (**e**)

Peptidyl arginine deiminase 4 (PAD4)

Peptidyl arginine deiminases (PADs or PADIs) are Ca^{2+}-dependent enzymes, which catalyse the hydrolytic deimination of arginine to citrulline in proteins [51]. Initially, PAD4, a nuclear protein, has been proposed to also convert mono-methylarginine (MMA) to citrulline, including modifications in histones H3 and H4 [52]. However, later studies revealed that methylation of arginine, in fact, interferes with PAD-catalyzed conversion to citrulline [53, 54]. This was corroborated by kinetic analysis of PAD4 activity identifying methylarginine as a very poor PAD4 substrate compared to the unmodified arginine residue [55]. Recently, a large-scale mass spectrometry detection of MMA peptides by the Nielsen lab showed that increased levels of PAD4 in 293T cells had no effect on regulation of the MMA sites [40].

Fe(II) and 2-oxoglutarate-dependent oxygenases

All enzymes, including Jmjd6 and several KDMs, with a proposed arginine demethylation activity described to date are part of the enzyme superfamily of Fe(II) and 2-oxoglutarate-dependent oxygenases (2OG oxygenases). Members of this enzyme family catalyse the transfer of molecular oxygen (O_2) onto a substrate [56]. With respect to protein substrates, 2OG oxygenases can either hydroxylate amino acids for post-translational modification (Fig. 3a) or they can demethylate lysine residues [57] (Fig. 3b). In the latter case, the initial hydroxylation targets the methyl group and this results in a subsequent removal of the methyl-moiety via release of formaldehyde. More than 20 2OG oxygenases have been assigned as histone lysine demethylases (KDMs) [8], whereas the other 2OG oxygenases, the protein hydroxylases, are known to generate a stable hydroxylated product [57] (Fig. 3c).

The 2OG oxygenases might also be promising candidates for the identification of arginine demethylases. Conflicting results on Jmjd6 and emerging candidates within the KDMs are described in the following sections.

Jumonji domain containing protein 6 (Jmjd6)

The first description of an enzyme directly demethylating arginine residues occurred in 2007. Chang et al identified the 2OG oxygenase Jumonji-domain containing protein 6 (Jmjd6) as a histone arginine demethylase, specifically demethylating histone H3 at arginine 2 (H3R2) and histone H4 at arginine 3 (H4R3) [11]. The authors showed demethylation of di-methylated H3R2 (H3R2me2) and di-methylated H4R3 (H4R3me2) after incubating bulk histones with purified recombinant Jmjd6 and subsequent staining of western blots with several histone modification-specific antibodies. H3R2me2 and H4R3me2 signals also decreased in immunofluorescence upon transient overexpression of Jmjd6 in HeLa cells, whereas an iron-binding Jmjd6 mutant did not show demethylation. Finally, a H4 peptide including the di-methylated R3 was incubated with recombinant wild-type Jmjd6 or iron-binding mutant. Following immunoprecipitation of the potential product with an α-H4R3me1 antibody, mass spectrometry analysis was performed. Besides from mono-methylated product, Chang et al also detected hydroxylated lysine residues (K5 and K8) in the peptides incubated with Jmjd6, but not in the control experiments [11]. Similar results have been demonstrated for a H3 peptide harboring a di-methylated R2 residue [11]. This initial discovery of a putative arginine demethylase sparked a controversial discussion in literature about the enzymatic activity of Jmjd6. On one hand, several results support an arginine demethylation activity, but, on the other hand, a lysine hydroxylation activity has been identified.

Jmjd6-catalyzed arginine demethylation of H4R3me2 peptides has been confirmed by Liu and colleagues, although they were not able to detect demethylation of H3R2me2 in this case [60]. In 2014, the estrogen receptor α (ERα) and RNA helicase A (RHA) were shown to be demethylated by Jmjd6 in several antibody-based analyses [61, 62]. Recently, Jmjd6 has been suggested to demethylate tumor necrosis factor receptor-associated factor 6 (TRAF6) and Hsp70 [63, 64]. Immunoprecipitation with an anti-methylarginine antibody was performed on cells overexpressing Jmjd6. Subsequent immunoblotting for TRAF6 displayed a weaker signal upon overexpression of Jmjd6 [63]. Demethylation of mono-methylated R469 (R469me1) in Hsp70 was detected on peptides upon incubation with recombinant Jmjd6 in immunoblots and in MALDI-TOF analysis [64]. A mild increase of anti-R469me1 signal in immunoblots of lysates from cells

with siRNA-mediated knockdown of Jmjd6 also suggested a Jmjd6 arginine demethylation activity [64]. An arginine demethylation activity has also been detected in *Arabidopsis thaliana* [65]. In western blot analyses with anti-H3R2me2, anti-H4R3me1, and H4R3me2s antibodies, the levels of corresponding modifications decreased upon incubation of bulk histones with recombinant His-tagged *A. thaliana* protein JMJ20. No alterations in signal were detected for methylated lysines [65]. Sequence comparison has revealed human jumonji domain containing protein 4 (Jmjd4), a lysyl-hydroxylase of eukaryotic release factor 1 (eRF1) and Jmjd6 to be most similar to JMJ20 [65, 66].

Despite the described range of potential substrates for Jmjd6-catalyzed arginine demethylation, the enzymatic activity of Jmjd6 has been discussed controversially. The ability of Jmjd6 to demethylate arginine has been challenged by several groups, reporting instead a lysine hydroxylation activity for Jmjd6 [12, 14–16]. Webby et al demonstrated hydroxylation of lysines in peptides of the splicing factors U2 small nuclear ribonucleoprotein auxiliary factor 65-kilodalton subunit (U2AF65) and Luc7-like2 (Luc7l2) with recombinant Jmjd6 in vitro using MALDI mass spectrometry [16]. In addition, endogenous hydroxylation of lysine residues K15 and K276 in U2AF65 has been shown in HeLa cells [16]. In this case, arginine demethylation of histone H3 and H4 peptides could not be demonstrated, although the presence of hydroxylated lysines in the histone peptides upon Jmjd6 incubation, as described by Chang et al [11], was detectable [16].

Further NMR and amino-acid analyses showed that Jmjd6-catalyzed lysine hydroxylation occurs at the C-5 position and gives a 5 S stereochemistry [13, 67]. In 2013, Unoki and colleagues confirmed Jmjd6-catalyzed lysine hydroxylation of histone peptides and identified 5-hydroxylysine in histones H2A/H2B, H3, and H4 in vivo [14]. Analysis of hydroxylated lysine levels in histones of wild-type and Jmjd6 knockout embryos by amino-acid composition analysis revealed a lower abundance of 5-hydroxylysine in knockout embryos [14]. The p53 protein has also been described as a substrate of Jmjd6 [15]. Lysine 382 (K382) in recombinant p53 was hydroxylated in vitro upon incubation with recombinant Jmjd6. Analysis with liquid chromatography–tandem mass spectrometry (LC-MS/MS) revealed an absence of hydroxylation in p53 samples without Jmjd6 incubation [15]. MALDI-TOF analysis of p53 peptides including the K382 incubated with recombinant Jmjd6 corroborated the K-hydroxylation activity of Jmjd6. A catalytically inactive Jmjd6 mutant resulted in no K382 modification. Hydroxylated K382 was also detectable in endogenous p53 in HCT116 cells and transient overexpression of Jmjd6 in these cells resulted in a sixfold increase of K382-hydroxylation level [15].

The role of Jmjd6 as an arginine demethylase is still a subject of debate. However, methylated lysines in histones appear to not be a substrate of Jmjd6 [68]. A western blot analysis for mono-, di-, and tri-methylated H3K4, H3K9, H3K27, H3K36, and H4K20 signals did not show any changes upon either Jmjd6 overexpression in 293T cells or in Jmjd6 homozygote knockout mouse embryonic fibroblasts (MEFs) [68].

KDMs with arginine demethylation activity

Very recently, a first evidence for arginine demethylation activity of some KDMs has been provided by in vitro analyses of the Schofield lab. Here, they tested several KDMs, including KDM2A, KDM3A, KDM4E, KDM5C, KDM6B, and PHF8/KDM7B, for an arginine demethylation (RDM) activity in vitro [17]. In this approach, recombinant KDM proteins were incubated with histone H3 peptides. Methylated lysine residues known to be demethylated by the corresponding KDMs had been replaced by methylated-arginine residues (MMA, ADMA, and SDMA) in these H3 peptides. RDM activity was analyzed by MALDI-TOF mass spectrometry [17]. KDM3A, KDM4E, KDM5C, and KDM6B displayed RDM activity in this setting, whereas KDM2A and PHF8/KDM7B did not [17]. RDM activity for KDM4E and KDM5C could be confirmed in a similar approach while using histone peptides with methylated arginines at positions naturally known to occur in cells. In contrast, KDM3A and KDM6B displayed no activity on the peptides representing cellular methylarginine patterns. An RDM activity on histone peptides was also detected with immunoprecipitated Flag-tagged KDMs from cells, whereas enzymatically inactive KDM mutants displayed no RDM activity [17]. The results suggest an RDM activity for some of the known KDMs, in a sequence-specific and modification-specific manner. Whether the described in vitro activity of these KDMs accounts for arginine demethylation events in cells too has to be investigated in future experiments.

Future perspectives

Arginine methylation in proteins has emerged as an abundant PTM in mammalian cells with roles in several biological functions, including epigenetic regulation. An intriguing research subject is the analysis of protein arginine methylation patterns and their regulation in physiological and pathophysiological settings [69, 70]. Abnormal regulation and altered PRMT and Jmjd6 expression levels are associated with several disease states, including cancers [15, 71–77]. Thus, those investigations might offer the possibility for novel therapeutic interventions. However,

further research is needed to unravel the functional roles of individual methylarginine events. The writers of arginine methylation, the PRMT protein family, have been identified. The existence of erasers is likely. Due to the dynamic appearance of arginine methylation marks in some contexts, a direct arginine demethylation is very probable. Candidates for this are found in the enzyme family of 2OG oxygenases, most notably KDMs. Current advances in SILAC-based mass spectrometry detection of arginine methylation might aid identification and cellular validation of further RDM candidates.

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