nature REVIEWS MOLECULAR CELL BIOLOGY

SUPPLEMENTARY INFORMATION

In format as provided by the authors

The regulation, functions and clinical relevance of arginine methylation

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https://doi.org/10.1038/s41580-019-0155-x
Corrected: Author Correction

Supplementary Box 1: Mammalian arginine methyltransferases (writers), demethylases (erasers) and methyl-binding proteins (readers).

Writers:

There are nine S-adenosylmethionine (SAM)-dependent protein arginine methyltransferases (PRMTs), which are classified according to the products they generate [1,2]. These enzymes are known as the 'writers' of arginine methylation (**see the figure**). PRMT1, 2, 3, 4, 6 and 8 are type I PRMTs, of which PRMT1 generates most of the monomethylarginine (MMA) and asymmetrical dimethylarginine (ADMA) in cells. PRMT5 and PRMT9 are members of type II PRMTs, which generate MMA and symmetrical dimethylarginine (SDMA). PRMT5 is the major contributor of SDMA in mammalian cells. PRMT7 is a type III PRMT, known to catalyze only the formation of MMA; however, only few PRMT7 substrates have been identified and its role in generating overall cellular MMA remains unknown [3]. In addition to the PRMTs, NDUFAF7 [4] and METTL23 [5] are potential arginine methyltransferases, but further evidence of direct catalytic activity and robust biochemical validation are still required.

Type I PRMTs fold into a seven-β-stranded dimeric structure, with each monomer forming a SAM-binding and a substrate-binding pocket [6]. Of the type II PRMTs, PRMT5 dimerizes with methylosome protein 50 (MEP50) and together they form a hetero-octamer [7]; the structure of PRMT9 remains undetermined. The type III PRMT7 contains two methyltransferase domains, one truncated and one functional, which fold into a ring structure and forms a pseudo-dimer [8].

PRMT1 and PRMT5 prefer to methylate substrates with RGG or RG (RGG/RG) motifs. Thus, it is not surprising that certain residues are modified by either ADMA or SDMA. This has been observed for histone H3 Arg 2 (H3R2), H4R3 and Sm proteins. Inhibition of PRMT1 can increase the levels of MMA and SDMA, whereas inhibition of PRMT5 increases the levels of MMA [9]. PRMT7-mediated monomethylation of H4R17 and H4R19 allosterically activates PRMT5 to produce symmetrically dimethylated H4R3 (H4R3me2s) [10]. This explains why PRMT7 deficient cells have lower levels of H4R3me2s [11].

Erasers:

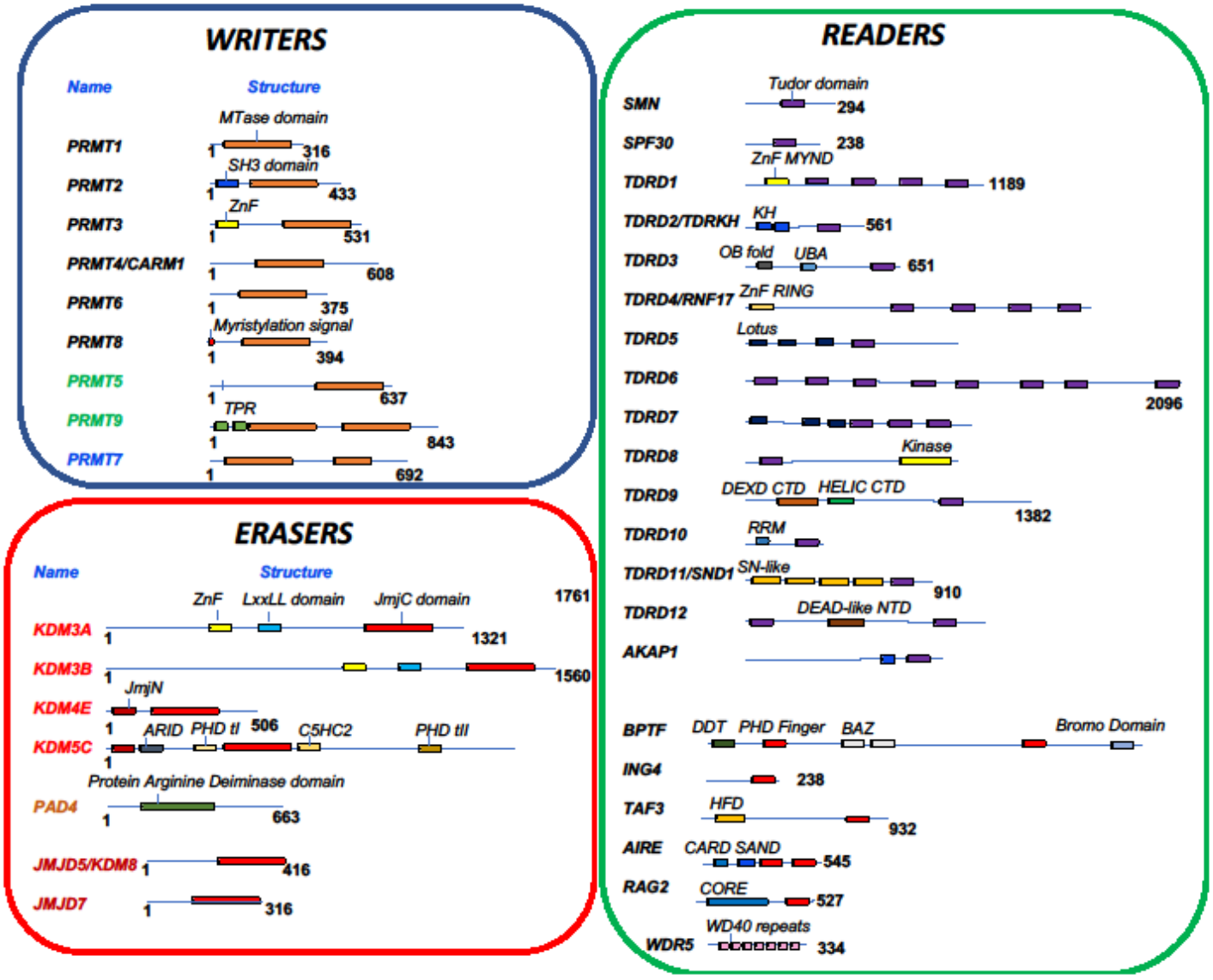
Arginine methylation is not as dynamic as protein phosphorylation, acetylation, ubiquitylation or indeed as lysine methylation and the existence of active *bona fide* arginine demethylases is still controversial. Of the Jumonji family of N^{ε}-methyllysine demethylases (KDMs), JMJD6 is a putative arginine demethylase (RDM) that was later reported to be a lysine hydroxylase [12]. KDM3A, KDM4E and KDM5C possess weak RDM activity *in vitro* [13], but the function of these dual lysine and arginine demethylases (KRDMs) *in vivo* remains to be shown. In assays *in vitro* using a histone H3 peptide where lysine 9 is substituted with arginine, KDM3A preferentially targets H3K9me2 over the methylated H3R9. KDM4E removes methyl groups from H3K9me>>H3R2me2a or H4R3me2a (*in vitro*). KDM5C demethylates H3K4me3>> H3R2me2a (*in vitro*). Another KRDM, JMJD1B (also known as KDM3B), can target both H4R3me2s and H3K9me2 at promoter regions, leading to gene activation [14]. Despite these reports, a dedicated and robust RDM remains to be identified.

Other enzymes can interfere with arginine methylation. Peptidylarginine deiminases (PADs) convert arginine into citrulline, thereby preventing methylation. MMA was thought to also be a PAD substrate, but structural analysis showed that arginine, but not MMA, fits in the PAD4 active site [15]. Finally, modified histone tails (~20 amino acids) can be proteolytically cleaved or 'clipped' by the aminopeptidase activity of JMJD5 (KDM8) and JMJD7. Trimming histone tails was postulated to weaken the nucleosome–DNA association, leading to nucleosome release and gene activation [16].

Readers:

Several protein domains preferentially bind methylated arginines: Tudor domains have hydrophobic pockets, which are able to preferentially recognize both symmetric [17,18] and asymmetric [19] methylarginine residues. Of the >36 proteins that contain Tudor domains, 15 are predicted to bind methylarginine [20] and only eight have been directly demonstrated to do so [21], whereas the majority have preference for methyllysine [22]. PHD fingers selectively recognize lysine residues in all their methylated states. Most PHD finger domains that bind the

amino-terminal tail of histone H3 make additional contacts with the neighbouring arginine residue (R2), and their binding can be either negatively affected when R2 is methylated (for example, in the case of BPTF-PHD) or positively affected when R2 is symmetrically dimethylated (for example, RAG2-PHD) [23,24]. The WD40 domain of WDR5 also possess a hydrophobic pocket, which binds well to unmethylated arginine (for example, MLL1R3765 or H3R2) [25,26], is displaced by arginine asymmetric dimethylation (for example, H3R2me2a) [27,28] and has a higher affinity for the symmetric dimethylation (for example, H3R2me2s [29]).



AIRE, (autoimmune regulator; RAG2, V(D)J recombination activating protein 2;
ARID, AT-rich interaction domain;
BPTF, bromodomain PHD finger transcription factor;
C5HC2, zinc finger C5HC2-type;
CARD, caspase activation and recruitment domain;
CORE, minimal region required for catalysis;
DDT, DNA binding homeobox and different transcription factors;
DEAD-like NTD, aspartic acid(D), glutamic acid(E), alanine(A), aspartic acid(D) N-terminal domain;
DEAF1, deformed epidermal autoregulatory factor 1 domain;

DExD CTD, aspartic acid(D), glutamic acid(E), X, aspartic acid(D), carboxy terminal domain;
HELIC CTD (helicase C_terminal domain);
ING4, inhibitor of growth family member 4;
Jmj, jumonji protein;
KH, heterogeneous nuclear ribonucleoprotein K (hnRNPK) homology;
LxxLL, L is leucine and X is any amino acid;
MTase, methyltransferase;
MYND, myeloid, nervy, and Deaf-1;
OB fold, oligonucleotide/oligosaccharide-binding fold;
PHD finger, Cys4-His-Cys3 motif;
PHD, plant homology domain finger; BAZ, bromodomain adjacent to Zinc finger domain; HFD, histone fold domain;
RING, ubiquitin binding domain;
RRM, RNA recognition motif;
SAND, Sp100, AIRE-1, NucP41/75, DEAF-1;
SH3, Src homology 3;
SMN, spinal muscular atrophy gene product;
SN-like, staphylococcal nuclease-like;
SPF30, splicing factor of 30kDa;
TAF3, TATA-box binding protein associate factor 3;
TDRD, Tudor domain containing protein;
TPR, tetratricopeptide;
UBA, ubiquitin-associated;
WD40, tryptophan(W)-aspartic acid (D) repeats;
ZnF, zinc finger

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