

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,500

Open access books available

118,000

International authors and editors

130M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



Deciphering Protein Arginine Methylation in Mammals

Ruben Esse, Paula Leandro, Isabel Rivera, Isabel Tavares de Almeida, Henk J Blom and Rita Castro

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/51984>

1. Introduction

The myriad of different post-translation modifications (PTMs) of proteins augments the information encoded by the human genome, from about 25,000 genes to over 1 million proteins that compose the human proteome [1, 2]. PTMs involve the chemical modification of target amino acid residues and, among others, include the addition of a methyl group to arginine residues, or arginine methylation, which is one of the most extensive protein modifications occurring in mammalian cells. During the last years, arginine methylation has attracted growing attention due to its impact on cellular function. Attesting to its biological importance, protein arginine methylation appears to be an evolutionarily ancient modification. Arginine methylation is now recognized as a widespread PTM that occurs on multiple classes of proteins with distinct cellular localizations. The arginine residue is unique among amino acids, since its guanidine group contains five potential hydrogen bond donors (Figure 1) positioned for interactions with hydrogen bond acceptors as DNA, RNA and proteins. Each addition of a methyl group removes a hydrogen donor, so the methylation of arginine residues in proteins will readily modulate their binding interactions and thus their physiological functions. Here, we provide an introduction to protein arginine methylation and discuss the current state of knowledge regarding this modification in mammals. In addition, we provide insight into how protein arginine methylation relates with the homocysteine metabolism. Lastly, we briefly discuss how protein arginine methylation may be disturbed in the context of hyperhomocysteinemia, sharing some of our recent results.

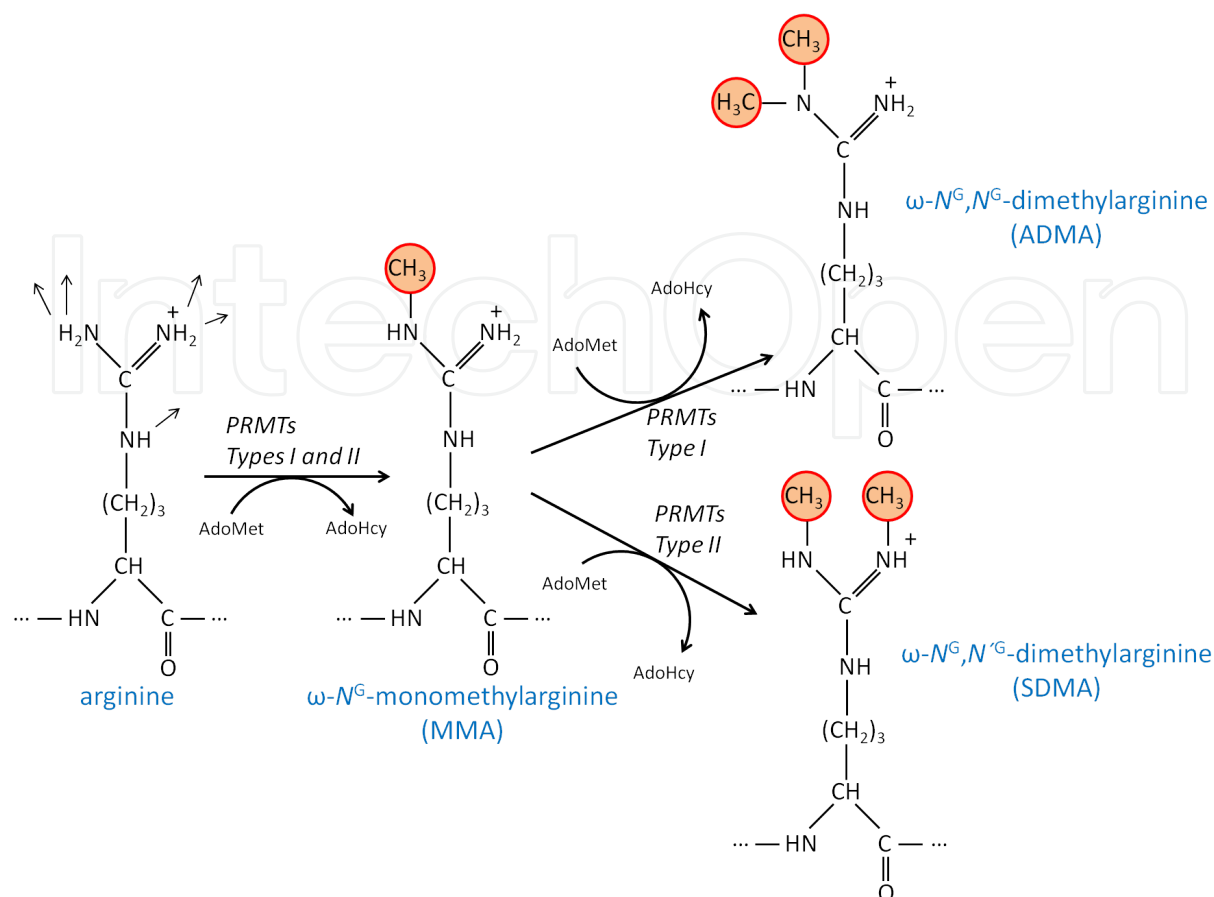


Figure 1. Methylation of the arginine side chain in proteins by protein arginine methyltransferases (PRMTs). The arginine residue holds 5 potential hydrogen bond donors. Mammalian PRMTs use the methyl group from a molecule of S-adenosyl-L-methionine (AdoMet) to form ω - N^G -monomethylarginine (MMA). Subsequently, type I PRMTs add a methyl group to the same nitrogen atom forming ω - N^G, N^G -dimethylarginine (ADMA), whereas type II PRMTs generate ω - $N^G, N^{G'}$ -dimethylarginine (SDMA).

2. The mammalian PRMTs: A brief overview

Methylation of arginine is catalyzed by protein arginine methyltransferases (PRMTs). PRMTs catalyze the transfer of methyl groups from the universal methyl donor, S-adenosyl-L-methionine (AdoMet), to the guanidinium nitrogen atom of the target arginine residues within proteins [3].

The first documented protein with arginine methylation activity, then termed protein methylase I, was purified from calf thymus [4, 5]. Additional family members were subsequently identified and purified from different tissues and cell lines [6-16]. In mammals, PRMTs are grouped in two major classes: type I and type II. Both PRMT types catalyze the transfer of a single methyl group from AdoMet to an arginine residue in the target protein, producing ω - N^G -monomethylarginine (MMA) (Figure 1). However, differences exist when adding the second methyl group: type I members add it on the same previously methylated nitrogen,

forming ω - N^G,N^G -dimethylarginine (ADMA), whereas type II members add it to the other N-terminal nitrogen of the arginine residue, yielding ω - N^G,N'^G -dimethylarginine (SDMA). Therefore, three distinct types of methylated arginine residues (MMA, ADMA and SDMA) are present on a horde of different proteins in the cytosol, nucleus and organelles of mammalian cells, ADMA being the most prevalent [3].

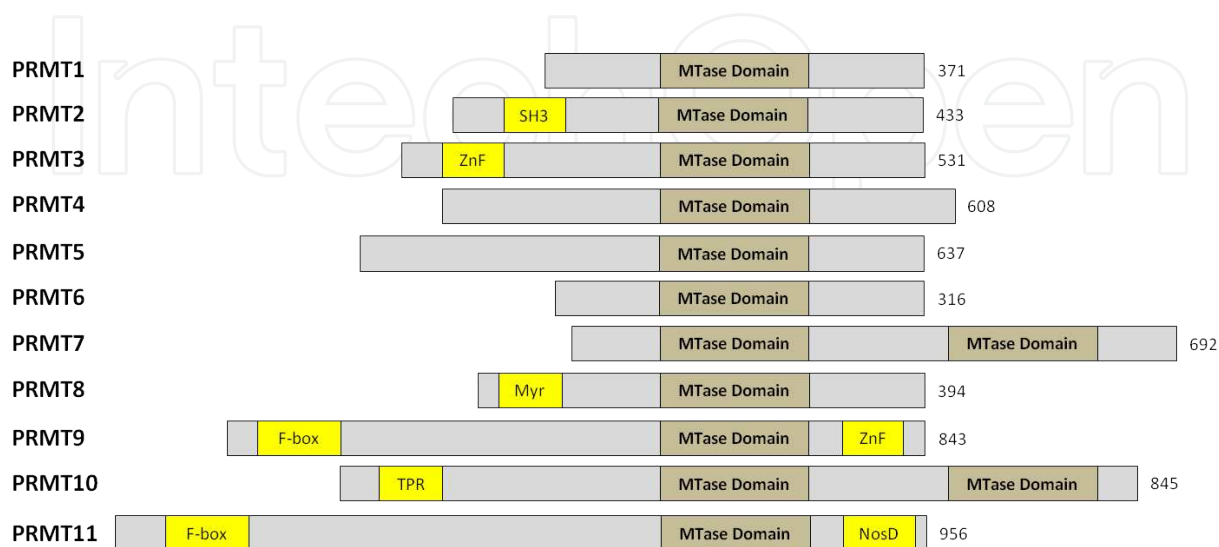


Figure 2. Schematic representation of the human protein arginine methyltransferase (PRMTs) family. For each member, the length of the longest isoform is indicated on the right. All members of the family possess at least one conserved MTase domain with signature motifs I, post-I, II, and III and a THW loop. Additional domains are marked in yellow boxes: SH3, ZnF (zinc finger), Myr (myristoylation), F-box, TPR (tetratricopeptide) and NosD (nitrous oxidase accessory protein). Adapted from [17].

To date, the human PRMT family includes eleven enzymes, termed from PRMT1 to PRMT11. Six are classified as type I (PRMT1, PRMT2, PRMT3, PRMT4, PRMT6 and PRMT8), and two as type II (PRMT5 and PRMT9). The classification of PRMT7 is controversial, as described below. The remaining two proteins, PRMT10 and PRMT11, were identified by their homology with PRMT7 and PRMT9, respectively, but their PRMT activity has not been sustained by experimental evidence [18, 19]. The genes encoding the different PRMTs are all located on different chromosomes, except the ones coding for PRMTs 1 and 4, which are both located in chromosome 19. Different splice variants coding for the corresponding isoforms are recognized for all PRMTs. Moreover, different cellular localizations are observed among them.

Although the human PRMT members vary in length from 316 to 956 amino acid residues, they all contain a highly conserved catalytic core region of around 300 residues (Figure 2). However, each PRMT presents a unique N-terminal region of variable length and distinct domain motifs, as for example PRMT3 and PRMT8 that bear a zinc finger (ZnF) and a myristoylation domain, respectively. Interestingly PRMT7 and PRMT10 exhibit a second catalytic domain. Until present, only the 3D structures of PRMT1, PRMT3 and PRMT5 were solved by X-ray crystallography [20-22]. These structures revealed that, also at the structural level, the catalytic core is highly conserved. Three structural regions

were identified, namely, a methyltransferase (MTase) domain, a β -barrel domain unique to the PRMT family and a dimerization arm. The MTase domain is located in the N-terminal region of the catalytic core. It consists of a α/β Rossmann fold, typical of AdoMet-dependent methyltransferases, containing the conserved motifs I, post-I, II and III [23]. Motif I includes in its C-terminal part the signature sequences for most classes of MTases (GxGxG) involved in AdoMet binding. A relevant structural element localized in the MTase domain is the double-E loop (containing two invariant glutamate (E) residues). The C-terminal part of the catalytic core is a β -barrel domain containing 10 β -strands and a THW (threonine-histidine-tryptophan) loop (the most highly conserved sequence of this domain). Within the overall structure, the THW loop is located next to the double-E loop in the AdoMet binding domain, forming the active site. The dimerization arm is formed by a three-helix segment, which is inserted between strands 6 and 7 of the β -barrel domain and is responsible for dimerization, essential for enzyme activity [21].

Most PRMTs methylate arginine residues localized within glycine- and arginine-rich (GAR) sequences, but there are exceptions to this rule [24]. For instance, PRMT4 cannot methylate GAR motifs, and PRMT5 methylates both GAR and non-GAR motifs. Other factors, such as the accessibility of the target arginine and the conformation of the involved sequence, also govern substrate methylation.

PRMT1 was the first mammalian PRMT to be cloned [25] and is the predominant type I enzyme [26] found in all embryonic and adult tissues examined so far [27]. Due to alternative splicing, there are seven isoforms of the protein, all varying in their N-terminal domain, which are expressed in a tissue-specific manner and have distinct subcellular localization patterns [28]. PRMT1 has broad substrate specificity with over 40 targets, most being RNA processing proteins, and multiple interacting partners [29]. Targeted PRMT1 knockout in mice results in embryonic lethality, thus showing that loss of PRMT1 activity is incompatible with life [30].

PRMT2 transcripts have been found in most human tissues, with highest levels in heart, prostate, ovary and neuronal system [10, 31]. PRMT2 was found predominantly in the nucleus and to a lower degree in the cytoplasm of mammalian cells [17]. Only recently, based on the observation that it catalyzes the formation of MMA and ADMA residues on histone H4, PRMT2 was recognized as a type I enzyme [32]. PRMT2 may act in cooperation with PRMT8, since its SH3 domain binds the N-terminal domain of the latter enzyme [33]. Some of the known targets of PRMT2, besides the aforementioned histone H4 [32], are the STAT3 (signal transducer and activator of transcription 3) protein [34], the estrogen receptor alpha [35], the androgen receptor [31], the retinoblastoma gene product [36], and the heterogeneous nuclear ribonucleoprotein (hnRNP) E1B-AP5 [37]. In contrast with PRMT1 knockouts, *PRMT2 null* mice are viable and grow normally [34].

PRMT3 was first identified as a PRMT1-binding partner. It is widely expressed in human tissues and has a predominantly cytosolic subcellular localization [9]. PRMT3 possesses a zinc finger domain that assists in its binding to ribosomal proteins, including the S2 protein of the small ribosomal subunit. Mouse embryos with a targeted disruption of PRMT3 are small in size but survive after birth and attain a normal size in adulthood [38].

PRMT4, or CARM1, was first identified as a steroid receptor coactivator [11], providing the first evidence that protein arginine methylation participates in the regulation of gene expression (please, see sections 3.2.1 and 3.2.2.1.). PRMT4 is ubiquitously expressed, exhibits a nuclear localization, and presents restricted substrate specificity. Specifically, in addition to nuclear hormone receptors and histones, it targets splicing factors [39, 40] and ATP-remodeling factors [41]. As opposed to most PRMTs, and as already referred, PRMT4 does not methylate GAR sequences. PRMT4 participates in many biological processes, including early T cell development [42], adipocyte differentiation [43], endochondral ossification [44], and proliferation and differentiation of pulmonary epithelial cells [45]. Embryos of *PRMT4 null* mice are small in size and die perinatally, thus showing the importance of PRMT4 activity to life [46].

PRMT5 was the first PRMT type II to be identified. PRMT5 is widely expressed in mammals, and more extensively in heart, muscle and testis [12]. PRMT5 is located both in the nucleus and in the cytoplasm [47]. Interestingly, PRMT5 is localized in the Golgi apparatus (GA) and its loss disrupts the GA structure, thereby suggesting that this enzyme plays a role in the maintenance of GA architecture [47]. In addition, PRMT5 is involved in the maintenance of the spliceosome integrity [48, 49]. PRMT5 targets histones, transcriptional elongation factors, chromatin remodelers and co-repressors [50]. In mice, loss of PRMT5 results in early embryonic lethality [51].

PRMT6 is the smallest family member (316 amino acids) and is localized predominantly in the nucleus [13]. Little is known about its properties, besides the fact that it produces asymmetrically dimethylated arginine residues. Similarly, PRMT6 functions are not completely understood. However, a role for PRMT6 in the regulation of cell proliferation and senescence, through transcriptional repression of tumor suppressor genes, has just been reported [52].

PRMT7 is present mainly in thymus, dendritic cells and testis [15, 53]. It has both nuclear and cytosolic localizations [15]. PRMT7 is involved in the modulation of sensitivity to DNA damaging agents [54, 55], methylation of male germline imprinted genes [56], and embryonic stem cell pluripotency [57]. Although Lee and colleagues characterized PRMT7 as a type II enzyme capable of forming SDMA [15], this finding was recently contested. In fact, Zurita-Lopez and coworkers have just reported that PRMT7 produces exclusively MMA residues, constituting the sole mammalian PRMT member with this type of methyltransferase activity [58].

PRMT8 is the type I PRMT with the highest degree of homology with PRMT1, but, unlike the latter, is mostly found in brain [14, 33]. PRMT8 possesses a myristoylation motif at its N-terminal end, which facilitates electrostatic interactions with membrane lipids and renders its distinct plasma membrane localization [14, 33]. Interestingly, its activity is regulated by the conformation of its N-terminal end, and removal of this domain results in enhancement of its enzymatic activity [33]. PRMT8 targets the pro-oncoprotein encoded by the Ewing sarcoma (EWS) gene, which contains a GAR motif in its C-terminal terminus [59].

PRMT9 was identified as a putative arginine methyltransferase and was classified as a type II PRMT due to its ability to catalyze the formation of MMA and ADMA [16]. However, there is limited sequence homology between PRMT9 and other human PRMTs [17]. Like most of the PRMTs, PRMT9 is widely expressed in mammalian tissues. PRMT9 has both nuclear and cytoplasmic localization [17]. Several PRMT9 isoforms are recognized and alternate splicing may be an important mechanism to regulate methylation by PRMT9 [16]. So far, the only known substrates for PRMT9 are histones, the maltose binding protein and several peptides [16]. Disruption of the PRMT9 activity has been associated with inflammation of the middle ear, indicating the importance of methylation in disease [60, 61].

PRMT10 and PRMT11 were identified by their homology with PRMT7 and PRMT9, respectively, but, as referred to above, their PRMT activity has not been sustained by experimental evidence.

3. Physiological roles of protein arginine methylation

Following the advent of improved sensitivity conquered with mass spectrometry, current proteomic technologies have been uncovering an increasingly large fraction of the human proteome as substrates for PRMTs. Additionally, advances in molecular biology techniques and the generation of mice with targeted deletion of the various members of the PRMTs family have been disclosing the functional relevance of this PTM. As new substrates are being characterized, a broadening spectrum of PRMT-regulated cellular processes is being realized. So far, targeted cellular processes can be loosely categorized in RNA processing, transcriptional regulation, DNA repair and signal transduction.

3.1. RNA processing

Most proteins targeted by PRMTs are RNA-binding proteins (RBPs) harboring GAR motifs, which are involved in all aspects of RNA metabolism [29]. During eukaryotic transcription, RBPs associate with the nascent pre-mRNA allowing a series of RNA processing events to take place, including 5'-end capping, splicing, 3'-end cleavage and polyadenylation [62].

The importance of protein arginine methylation for RNA processing was first revealed by the observation that in HeLa cells splicing reactions were completely inhibited by a pan SDMA-specific antibody [63]. Subsequent studies have shown that a number of spliceosomal proteins are subject to both asymmetrical and symmetrical dimethylation, uncovering the crucial role of protein arginine methylation in the assembly of the spliceosome [64-66]. Other RBPs (hnRNP1, fibrillarin, nucleolin, HuD and HuR) have also been shown to contain methylated arginine residues [67-70]. Importantly, several of these RBPs lack proper subcellular localization if hypomethylated [71, 72]. For instance, asymmetrical dimethylation of specific arginine residues comprising the nuclear poly(A) binding protein prevents its transport to the cytoplasm [73].

3.2. Transcriptional regulation

Protein arginine methylation was initially detected in histones and studied mainly in relation to transcriptional regulation. More recently, the presence of methylated arginines in a large number of non-histonic proteins that also impact on transcriptional regulation was recognized.

3.2.1. Histone arginine methylation

Methylation of histones at arginine residues has been very well documented and subject to periodic review [74-76]. Therefore, a comprehensive review of histone arginine methylation would go beyond the scope of this paper and will be depicted in general terms only.

A few arginine residues within the N-terminal tail of histones H3, H4 and H2A are subject to methylation. The N-terminal tail of histone H3 contains several arginine residues that are targeted by different PRMTs,¹ while only one residue in histone H4 (H4R3) is subject to arginine methylation.² Since the first residues of the N-terminal tails of histones H4 and H2A are identical, residue 3 of histone H2A may be methylated in the same manner as H4R3 [76]. Interestingly, histone asymmetrical dimethylation generally activates gene transcription, while generation of SDMA is normally a repressive mark, although exceptions to this rule exist [75]. Arginine methylation occurring in histone tails may either affect other PTMs or influence the docking of key transcriptional effector molecules [76].

3.2.2. Arginine methylation of non-histonic proteins

In addition to histones, PRMTs also target other proteins that are involved in the transcriptional control of gene expression in mammals. These proteins include several types of transcription factors, transcription elongation factors, methyl DNA-binding proteins, and RNA polymerase II, as presented in the following sub-sections. In each, we choose to use some illustrative examples, rather than present an exhaustive list of all non-histonic targets for PRMTs that impact transcriptional regulation.

3.2.2.1. Transcription factors

Transcriptional coregulators are well recognized targets of PRMTs. Methylation of nuclear hormone receptors (NRs) illustrate well how arginine methylation of transcriptional coregulators may affect gene transcription. NRs are a large group of structurally related transcription factors that are responsible for mediating the biological effects of hormones. Transcription activation by NRs is a multistep process involving transcriptional coactivator proteins, as CREB-binding protein (CBP) and p300. These two co-activators have similar structures, sharing one N-terminal KIX domain that displays several arginine residues that are subject of methylation by PRMT4 [83]. CBP/p300 exhibits HAT (histone acetyl transfer-

1 In histone H3, residues 17 (H3R17) and 26 (H3R26) are methylated by PRMT4 [77], H3R2 is methylated by PRMT6 [78], and H3R8 is methylated by PRMT5 [79].

2 In histone H4, one arginine residue (H4R3) is subject to methylation. Asymmetric dimethylation of this residue is catalyzed by PRMT1, PRMT6 and PRMT8 [80-82], while symmetric dimethylation is catalyzed by PRMT5 and PRMT7 [56, 79].

ase) and HMT (histone methyl transferase) activities. Both CBP/p300 and PRMT4 are recruited to DNA by the NR bound to its ligand, where they stimulate transcription by remodeling chromatin through subsequent histone acetylation and methylation in the vicinity of hormone response element (HRE). Notably, it was shown that CBP/p300 activates NR-dependent gene transcription only if methylated in their KIX domain [83].

Peroxisome proliferator-activated receptor-gamma coactivator (PGC) 1 alpha (PGC-1 α) is other example of a coactivator of NRs whose activity was clearly shown to be dependent on PRMT activity. PGC-1 α signaling is extremely important in the heart, where PGC-1 α is highly expressed and controls cardiac energy pathways during development and in response to stressors. Interestingly, it was shown that PRMT1 activates PGC-1 α through its methylation at several arginine residues [84]. Remarkably, experimentally based evidence has recently linked decreased levels of methyl-PGC-1 α due to decreased PRMT1 activity to cardiomyopathy in mice [85].

Arginine methylation may also impact transcriptional activity by targeting transcription factors that bind directly to DNA, like the STAT1 protein. STAT1 (signal transducer and activator of transcription) is a member of the STAT family and mediates the cellular response to cytokines and growth factors thus regulating many aspects of growth, survival and differentiation of cells. After activation by cytoplasmic phosphorylation, STAT1 migrates to the nucleus, where it binds to the enhancer elements of response genes. The N-terminal region of STAT1 comprises a conserved arginine residue that was shown to be a PRMT1 target [86]. Inhibition of PRMT1 activity hinders the DNA binding activity of STAT1 and STAT1-mediated transcription.

3.2.2.2. *Transcription elongation factors*

Activity of PRMTs may also affect transcription by marking transcription elongation factors, like Spt5. The Spt5 protein displays several KOW domains, which contain consensus sites for arginine methylation and overlap the RNA polymerase II binding domain. Notably, Spt5 can be methylated in its RNA polymerase II binding domain by PRMT1 or PRMT5 and this methylation may cause transcriptional pausing [87].

3.2.2.3. *Methyl DNA-binding proteins*

PRMTs may also participate in the DNA methylation system of chromatin control. DNA methylation, an important regulator of gene transcription, occurs within a CpG context in differentiated mammalian cells. MBD2 is a methyl DNA-associated protein that docks methylated CpGs in DNA and negatively affects gene transcription by recruiting proteins that repress chromatin. Interestingly, both PRMT1 and PRMT5 methylate MBD2, thereby impairing the transcription repression function of MBD2 [88].

3.2.2.4. *RNA polymerase II*

In eukaryotic cells, the C-terminal domain (CTD) of RNA polymerase II provides a platform to recruit different regulators of the transcription apparatus. Recently, arginine methylation

of a single residue in the CTD (R1810) was documented and implied in the regulation of transcription driven by RNA polymerase II [89].

3.3. DNA repair

Genome integrity is constantly subject to monitoring by a complicated and entangled network of mechanisms collectively termed as DNA damage response (DDR). Some proteins integrating this system are known targets of PRMTs. Mre11 is part of a complex that is critically implicated in homologous recombination repair of DNA double strand breaks. This protein harbors a GAR motif that is methylated by PRMT1, allowing its association with nuclear structures and recruitment to sites of DNA damage [90], as well as its exonuclease activity [91]. PRMT5-induced methylation of Rad9, a cell cycle checkpoint protein, enables activation of Chk1, an important downstream checkpoint effector, and loss of Rad9 methylation leads to S/M and G2/M checkpoint defects [92]. DNA polymerase beta, which participates in DNA base excision repair, is methylated by PRMT6, which enhances its binding to DNA and its processivity [93].

3.4. Signal transduction

In addition to regulating numerous nuclear processes, arginine methylation is involved in conveying information from the cell surface, through the cytoplasm, to the nucleus. For instance, the implication of protein arginine methylation in interferon (IFN) signaling has been substantiated by the fact that PRMT1 interacts with the cytoplasmic domain of the type I IFN receptor [94], and that its depletion impairs the biological activity of type I IFN [95]. The involvement of arginine methylation within T cell receptor signaling cascades is also gaining prominence. In fact, stimulation of T helper cells induces arginine methylation of several cytoplasmic proteins, which when inhibited causes signaling defects in CD4 T cells and severe immunosuppression [96]. PRMT1-mediated methylation also impacts insulin signaling and glucose uptake in skeletal muscle cells [97]. Estrogen rapid signaling is similarly mediated by PRMT1, which targets estrogen receptor alpha (ER- α) [98]. PRMT1 also methylates FoxO1, a member of the mammalian forkhead transcription factors of class O (FoxO) subfamily [99]. These transcription factors are involved in many vital processes (e.g. apoptosis, cell-cycle control, glucose metabolism, oxidative stress resistance and longevity) and, in response to insulin or several growth factors, are negatively regulated through phosphorylation by serine-threonine kinase Akt/protein kinase B (Akt/PKB) (that results in their export from the nucleus to the cytoplasm) [100]. PRMT1 methylates FoxO1 at arginine residues within the Akt consensus motif, blocking the Akt-mediated phosphorylation and augmenting FoxO1 target gene expression [99]. Another Akt-target protein is BCL-2, antagonist of cell death, which is also methylated by PRMT1 on its consensus phosphorylation motif [101]. Methylation-phosphorylation switches are presently regarded as paradigms operating in signal transduction pathways and other physiological processes, since numerous other examples of this crosstalk have been described. The implication of PRMTs other than PRMT1 in signaling routes has not been well studied. The association of PRMT8 with the plasma membrane provides a clue that this enzyme may facilitate the triggering of a signal-

ing pathway [14]. A recent study has uncovered that PRMT5 regulates the amplitude of the RAS to extracellular signal-regulated kinase (ERK) signal transduction cascade, impacting on cell fate decision [102].

4. Modulation of PRMTs activity

PRMTs associate with proteins that may change their activity or substrate specificity. Examples of proteins that enhance the activity of a specific PRMT are: BTG1 (B-cell translocation gene 1 protein), BTG2 (B-cell translocation gene 2 protein) and hCAF1 (chemokine receptor 4-associated factor 1), which associate with PRMT1 [25, 103]; the chromatin remodelers BRG and BRM, which enhance PRMT5 activity [79]; and BORIS (brother of the regulator of imprinted sites), which has been reported to elevate PRMT7 activity [56]. In contrast, tumor suppressor DAL-1 inhibits the activity of PRMT3 *in vitro* and *in vivo* [104]. PRMT4 association with other proteins within the nucleosomal methylation activator complex (NUMAC) enhances its ability to methylate nucleosomal histone H3, while the free enzyme preferentially methylates free core histone H3 [41]. Similarly, PRMT5 association with COPR5 shifts its preferential target from H3R8 to H4R3 [105].

PRMT activity may also be modulated by PTMs. For instance, several PRMTs are automethylated, but the relevancy of this phenomenon is not acquainted [33]. PRMT4 phosphorylation during mitosis prevents its homodimerization, thereby decreasing its activity [106]. Additionally, PRMT4 phosphorylation abolishes the binding of AdoMet and thus its activity, and promotes its cytoplasm localization [107].

5. The reversibility of protein arginine methylation: A fast-growing field of knowledge

An important question under recent investigation was whether demethylation reactions occurred to reverse the effects of protein arginine methylation. Initial studies indicated that this PTM was a permanent mark on proteins. As such, the only way to reverse the effects of the presence of this methyl mark would be to degrade the protein to its amino acid components and then make a new unmethylated version by protein synthesis [108]. However, the long-standing notion that arginine methylation is a stable covalent mark has been challenged in recent years due to the discovery of two types of enzymes that can remove methyl groups from arginine residues in histones by demethylation and citrullination (Figure 3).

The first evidence of demethylation of arginine residues came from a study by Chang and coworkers [110] that reported that JMJD6, a Jumonji domain-containing protein, demethylates H3R2 and H4R3 residues that are either asymmetrically or symmetrically dimethylated (Figure 3A). Mass spectrometry of a peptide containing dimethylated arginine residues, which was incubated with JMJD6 and then immunoprecipitated with a monomethylarginine specific antibody, revealed that the enzyme catalyzed the loss of one methyl group

from the methylated substrate. Current view holds that JMJD6 may be a bifunctional enzyme, catalyzing either demethylation or lysyl-hydroxylation reactions [111].

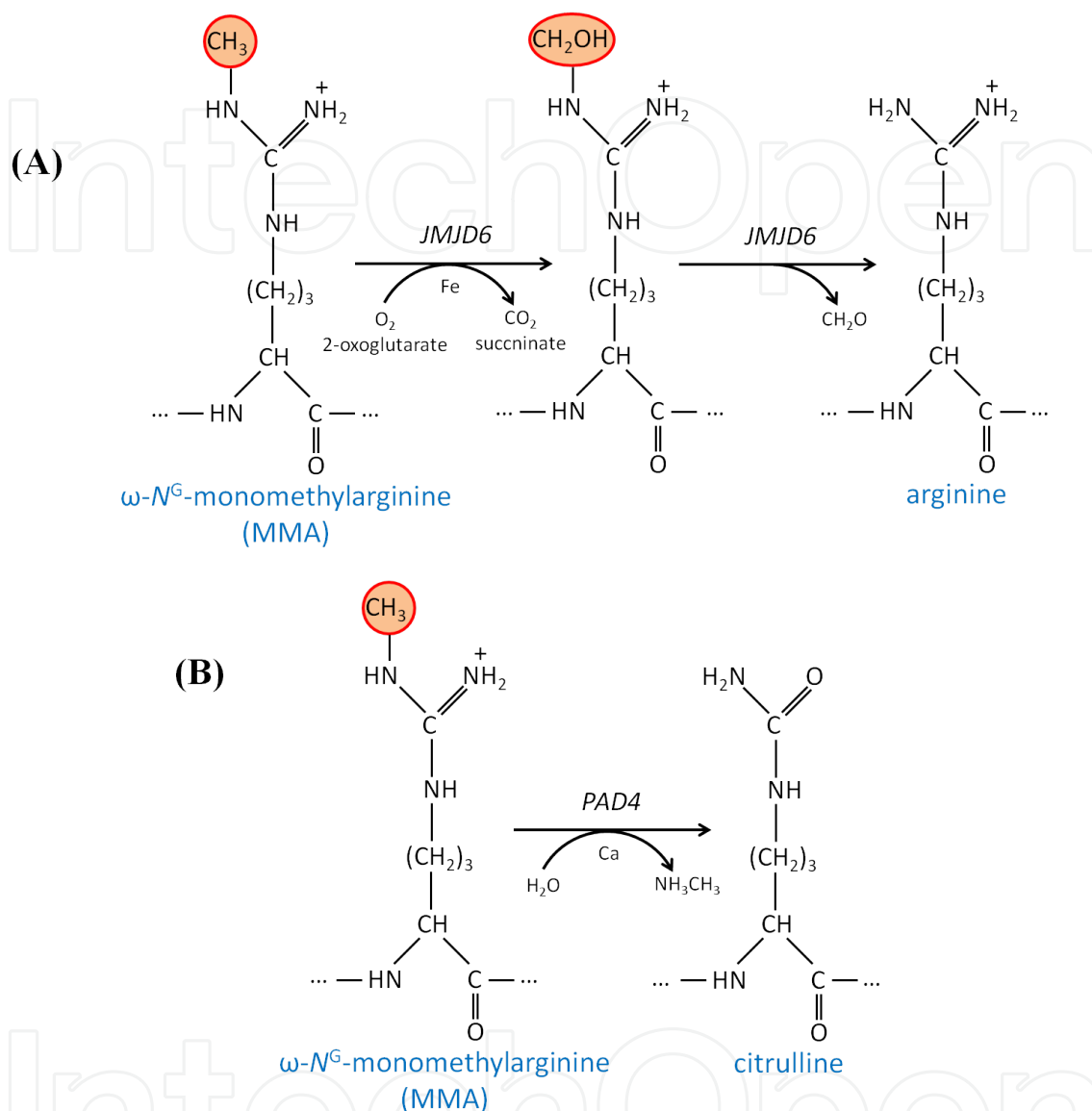


Figure 3. Reversing or annulling protein arginine methylation: demethylation and citrullination reactions. (A) Demethylation of a monomethylarginine (MMA) residue by JMJD6. (B) Citrullination of a MMA residue by PAD4. This reaction antagonizes arginine methylation, since it is not reversible. Adapted from [109].

Monomethylated arginine residues may be converted to citrulline by deimination, a modification catalyzed by peptidylarginine deiminases (PADs). In this process, the guanidinium side chain of an arginine residue is hydrolyzed and methylamine released [112] (Figure 3B). To date, five human PAD homologs have been identified and designated as PAD1-4 and PAD6. Most mechanistic studies on PADs have been performed with PAD4. PADs may be activated by calcium, which seems to be essential for the catalysis to occur [113, 114]. Citrulline residues are found with great extent in

histones H2A, H3, and H4 [115]. Importantly, the sites of deimination by PAD4 overlap those of arginine methylation on these histones [112], and an increase in histone citrulline levels correlates with a decrease in methylated arginine levels [116, 117]. However, PADs only catalyze the deimination of MMA residues, and no enzyme has been identified that can convert citrulline back to arginine. Thus, citrullination blocks remethylation, adding a new layer to the regulation of protein arginine methylation [118, 119].

6. The disposal of methylated arginine residues / ADMA and vascular disease

Proteolysis of arginine-methylated proteins results in free MMA, ADMA and SDMA, which are secreted to the extracellular space [120]. These metabolites can then be taken up by other cells via carriers of the cationic amino acid family [121] or eliminated by renal excretion [122]. In addition, ADMA can be hydrolyzed to citrulline and dimethylamine, by dimethylarginine dimethylaminohydrolase (DDAH), which is present in kidney, liver and vasculature [123]. Interestingly, MMA and ADMA are endogenous inhibitors of nitric oxide (NO) production in endothelial cells. Due to the paramount role of NO on the homeostasis of the vascular endothelium, ADMA, which is present in plasma in much larger quantities than MMA, has emerged as a novel risk factor in the setting of endothelial dysfunction and vascular disease [124]. As stated before, PRMTs are ubiquitously expressed and participate in crucial cellular processes. Thus, deregulation of these enzymes is likely implicated in the pathogenesis of a number of different diseases, including cardiovascular disease. In addition to the intricate set of potential interactions between protein arginine methylation and cardiovascular disease, we must add ADMA, an additional player that may impact the cardiovascular system.

7. Protein arginine hypomethylation in the context of hyperhomocysteinemia: “Another brick in the wall”?

As illustrated in Figure 1, the methyl group responsible for the establishment of protein arginine methylation patterns by PRMTs originates from AdoMet, an intermediate in the homocysteine metabolism. Therefore, protein arginine methylation and homocysteine metabolism are biochemically linked (Figure 4). In addition to protein arginine methylation, AdoMet serves as the methyl donor for more than one hundred cellular methyl transfer reactions, including the methylation of other residues in proteins (namely, histidine and lysine), DNA and RNA. Following the transfer of the methyl group, AdoMet is converted into S-adenosyl homocysteine (AdoHcy). AdoHcy is further converted into homocysteine and adenosine by AdoHcy hydrolase, which is widely distributed in mammalian tissues [125]. This reaction is reversible and strongly favors AdoHcy synthesis rather than its hydrolysis; however, both homocysteine and adenosine are rapidly removed under physiological condi-

tions, favoring the hydrolysis reaction [126]. Nevertheless, if homocysteine accumulates, AdoHcy will accumulate as well [125].

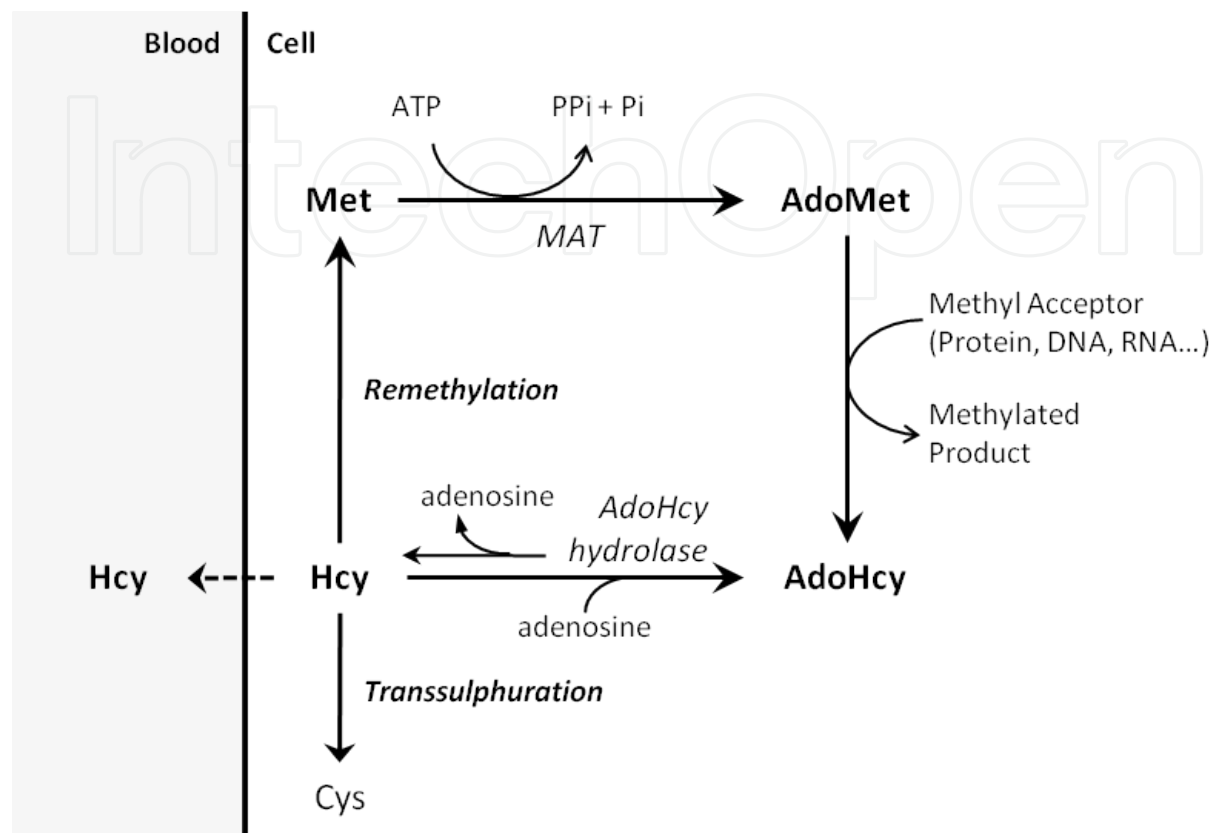


Figure 4. Simplified homocysteine metabolism. Homocysteine (Hcy) results from methionine metabolism as a by-product of cellular transmethylation reactions. Methionine (Met) is the “entry” point of this pathway and is provided by diet and protein breakdown. S-Adenosylmethionine (AdoMet) is first synthesized from Met by methionine adenosyltransferase (MAT). AdoMet is the methyl group donor in a wide variety of transmethylation reactions, being converted to S-Adenosylhomocysteine (AdoHcy). AdoHcy is subsequently hydrolyzed to Hcy and adenosine by AdoHcy hydrolase. Hcy can then be converted to cysteine (Cys) through the transsulphuration pathway, remethylated back to methionine or exported.

Despite recent controversy, elevated levels of homocysteine in plasma (or hyperhomocysteinemia) are accepted as a risk factor for cardiovascular disease [127, 128]. Notably, despite several potential mechanisms underlying this association having been the focus of intense research, the subject remains to be fully understood [125]. Our group has been actively studying whether a hypomethylating environment associated with hyperhomocysteinemia contributes to the vascular toxicity of homocysteine [129-133]. AdoHcy is a competitive inhibitor of most AdoMet-dependent methyltransferases, since it binds to their active sites with higher affinity than AdoMet [134]. As such, the ratio AdoMet/AdoHcy is taken as an index of cellular methylation capacity. As mentioned above, if homocysteine accumulates, AdoHcy will accumulate as well. Thus, increased homocysteine may be regarded as a cellular hypomethylation effector, via AdoHcy accumulation.

We have demonstrated that patients with vascular disease present increased levels of both plasma homocysteine and intracellular AdoHcy, together with decreased DNA methylation [129]. Other studies also supported a role for hyperhomocysteinemia in modulating epigenetic mechanisms [135-138]. Additionally, we confirmed, *in vitro*, the AdoHcy ability to decrease global DNA methylation status [131]. Currently, we are studying whether, in addition to DNA, protein arginine methylation extend is are also decreased by AdoHcy accumulation. Supporting this possibility, results from kinetic studies regarding competitive inhibition of several methyltransferases by AdoHcy sustain that this metabolite is a stronger inhibitor of PRMT1 than of DNA methyltransferase 1, the enzyme responsible for the maintenance of DNA methylation patterns [6, 139]. Interestingly, we observed that intracellular AdoHcy accumulation lowered ADMA production by human vascular endothelial cells accumulation [133]. This observation led us to postulate that this effect was due to AdoHcy-induced protein arginine hypomethylation. Supporting this possibility, we subsequently found decreased levels of protein-incorporated ADMA in human vascular endothelial cells under AdoHcy intracellular accumulation ([140] and Esse R *et al.*, manuscript 1 under submission). Moreover, we observed that this effect was more pronounced than the parallel reduction in global DNA methylation patterns. Therefore, we have concluded that AdoHcy build-up affects protein arginine methylation to a higher extent than DNA methylation. Importantly, this observation suggests that protein arginine methylation is more easily affected than DNA methylation in the context of hyperhomocysteinemia. Very recently, we have also assessed protein arginine methylation status in an animal model of diet-induced hyperhomocysteinemia and found that increased homocysteine is associated with protein arginine hypomethylation, although in a tissue-dependent manner ([141] and Esse R *et al.*, manuscript 2 under submission). Specifically, we found global protein arginine hypomethylation in heart and in brain from hyperhomocysteinemic rats, while methylation of hepatic proteins was not affected. Currently, we are undertaking both *in vitro* and *in vivo* studies to identify proteins sensitive to AdoHcy-induced hypomethylation, and to assess the impact of its different methylation states on vascular homeostasis. Actually, differential methylation of proteins may well play a role in pathologies related to elevated homocysteine, including vascular disease.

8. Conclusions

The field of protein arginine methylation has been enjoying widespread interest in the scientific community, owing to the recent development of molecular biology techniques and mass spectrometry instrumentation. It is now clear that this common PTM impacts crucial biological functions, including RNA processing, transcriptional regulation, DNA repair and signal transduction. Yet, information is still scant regarding some important issues of protein arginine methylation, namely its regulation by other PTMs, how it affects interaction with the protein ligands, and what are the factors governing the specificity of the different PRMTs towards the target protein. Importantly, protein arginine methylation, which was considered a static modification during a long time, is

now recognized as a reversible and dynamic modification, increasing the complexity of the interplay between DNA, proteins and vital cellular process. Nevertheless, the characterization of enzymes that reverse and block protein arginine methylation has just commenced. Moreover, the pathological implications of protein arginine methylation disturbance have not been exhaustively explored. The methyl group marking of arginine residues originates from AdoMet, an intermediate in homocysteine metabolism. Thus, the metabolism of this amino acid and protein arginine methylation are biochemically linked. Homocysteine elevation relates with an increased risk of vascular disease and others pathologies. Notably, the potential detrimental effect of increased circulating homocysteine on protein arginine methylation status may constitute an additional molecular mechanism contributing to disease, and, as such, warrants further investigation.

Acknowledgments

This work was supported by Fundação para a Ciência e a Tecnologia (FCT, Portugal) grants (SFRH/BD/48585/2008 to RE, and PTDC/SAU-ORG/112683/2009 to RC).

Author details

Ruben Esse¹, Paula Leandro^{1,2}, Isabel Rivera^{1,2}, Isabel Tavares de Almeida^{1,2},
Henk J Blom^{3,4} and Rita Castro^{1,2*}

*Address all correspondence to: rcaastro@ff.ul.pt

1 Institute for Medicines and Pharmaceutical Sciences (iMed.UL), Faculty of Pharmacy, University of Lisbon, Lisbon, Portugal

2 Department of Biochemistry and Human Biology, Faculty of Pharmacy, University of Lisbon, Lisbon, Portugal

3 Metabolic Unit, Department of Clinical Chemistry, VU University Medical Center, Amsterdam,, The Netherlands

4 Institute for Cardiovascular Research ICaR-VU, VU University Medical Center, Amsterdam, The Netherlands

References

- [1] Human Genome Sequencing Consortium. Finishing the euchromatic sequence of the human genome. *Nature*. 2004;431:931-945.

- [2] Jensen ON. Modification-specific proteomics: characterization of post-translational modifications by mass spectrometry. *Curr Opin Chem Biol.* 2004;8:33-41.
- [3] Bedford MT, Richard S. Arginine methylation an emerging regulator of protein function. *Mol Cell.* 2005;18:263-272.
- [4] Paik WK, Kim S. Enzymatic methylation of protein fractions from calf thymus nuclei. *Biochem Biophys Res Commun.* 1967;29:14-20.
- [5] Paik WK, Kim S. Protein methylase I. Purification and properties of the enzyme. *J Biol Chem.* 1968;243:2108-2114.
- [6] Ghosh SK, Paik WK, Kim S. Purification and molecular identification of two protein methylases I from calf brain. Myelin basic protein- and histone-specific enzyme. *J Biol Chem.* 1988;263:19024-19033.
- [7] Rawal N, Rajpurohit R, Paik WK, Kim S. Purification and characterization of S-adenosylmethionine-protein-arginine N-methyltransferase from rat liver. *Biochem J.* 1994;300:483-489.
- [8] Liu Q, Dreyfuss G. In vivo and in vitro arginine methylation of RNA-binding proteins. *Mol Cell Biol.* 1995;15:2800-2808.
- [9] Tang J, Gary JD, Clarke S, Herschman HR. PRMT 3, a type I protein arginine N-methyltransferase that differs from PRMT1 in its oligomerization, subcellular localization, substrate specificity, and regulation. *J Biol Chem.* 1998;273:16935-16945.
- [10] Katsanis N, Yaspo ML, Fisher EM. Identification and mapping of a novel human gene, HRMT1L1, homologous to the rat protein arginine N-methyltransferase 1 (PRMT1) gene. *Mamm Genome.* 1997;8:526-529.
- [11] Chen D, Ma H, Hong H, Koh SS, Huang SM, Schurter BT, Aswad DW, Stallcup MR. Regulation of transcription by a protein methyltransferase. *Science.* 1999;284:2174-2177.
- [12] Pollack BP, Kotenko SV, He W, Izotova LS, Barnoski BL, Pestka S. The human homologue of the yeast proteins Skb1 and Hsl7p interacts with Jak kinases and contains protein methyltransferase activity. *J Biol Chem.* 1999;274:31531-31542.
- [13] Frankel A, Yadav N, Lee J, Branscombe TL, Clarke S, Bedford MT. The novel human protein arginine N-methyltransferase PRMT6 is a nuclear enzyme displaying unique substrate specificity. *J Biol Chem.* 2002;277:3537-3543.
- [14] Lee J, Sayegh J, Daniel J, Clarke S, Bedford MT. PRMT8, a new membrane-bound tissue-specific member of the protein arginine methyltransferase family. *J Biol Chem.* 2005;280:32890-32896.
- [15] Lee JH, Cook JR, Yang ZH, Mirochnitchenko O, Gunderson SI, Felix AM, Herth N, Hoffmann R, Pestka S. PRMT7, a new protein arginine methyltransferase that synthesizes symmetric dimethylarginine. *J Biol Chem.* 2005;280:3656-3664.

- [16] Cook JR, Lee JH, Yang ZH, Krause CD, Herth N, Hoffmann R, Pestka S. FBXO11/PRMT9, a new protein arginine methyltransferase, symmetrically dimethylates arginine residues. *Biochem Biophys Res Commun*. 2006;342:472-481.
- [17] Wolf SS. The protein arginine methyltransferase family: an update about function, new perspectives and the physiological role in humans. *Cell Mol Life Sci*. 2009;66:2109-2121.
- [18] Krause CD, Yang ZH, Kim YS, Lee JH, Cook JR, Pestka S. Protein arginine methyltransferases: evolution and assessment of their pharmacological and therapeutic potential. *Pharmacol Ther*. 2007;113:50-87.
- [19] Pal S, Sif S. Interplay between chromatin remodelers and protein arginine methyltransferases. *J Cell Physiol*. 2007;213:306-315.
- [20] Zhang X, Zhou L, Cheng X. Crystal structure of the conserved core of protein arginine methyltransferase PRMT3. *EMBO J*. 2000;19:3509-3519.
- [21] Zhang X, Cheng X. Structure of the predominant protein arginine methyltransferase PRMT1 and analysis of its binding to substrate peptides. *Structure*. 2003;11:509-520.
- [22] Sun L, Wang M, Lv Z, Yang N, Liu Y, Bao S, Gong W, Xu R. M. Structural insights into protein arginine symmetric dimethylation by PRMT5. *Proc Natl Acad Sci U S A*. 2011;108:20538-20543.
- [23] Kozbial PZ, Mushegian AR. Natural history of S-adenosylmethionine-binding proteins. *BMC Struct Biol*. 2005;5:19.
- [24] Najbauer J, Johnson BA, Young AL, Aswad DW. Peptides with sequences similar to glycine, arginine-rich motifs in proteins interacting with RNA are efficiently recognized by methyltransferase(s) modifying arginine in numerous proteins. *J Biol Chem*. 1993;268:10501-10509.
- [25] Lin WJ, Gary JD, Yang MC, Clarke S, Herschman HR. The mammalian immediate-early TIS21 protein and the leukemia-associated BTG1 protein interact with a protein-arginine N-methyltransferase. *J Biol Chem*. 1996;271:15034-15044.
- [26] Tang J, Frankel A, Cook RJ, Kim S, Paik WK, Williams KR, Clarke S, Herschman HR. PRMT1 is the predominant type I protein arginine methyltransferase in mammalian cells. *J Biol Chem*. 2000;275:7723-7730.
- [27] Pawlak MR, Scherer CA, Chen J, Roshon MJ, Ruley HE. Arginine N-methyltransferase 1 is required for early post implantation mouse development, but cells deficient in the enzyme are viable. *Mol Cell Biol*. 2000;20:4859-4869.
- [28] Goulet I, Gauvin G, Boisvenue S, Cote J. Alternative splicing yields protein arginine methyltransferase 1 isoforms with distinct activity, substrate specificity, and subcellular localization. *J Biol Chem*. 2007;282:33009-33021.
- [29] Pahlich S, Zakaryan RP, Gehring H. Protein arginine methylation: Cellular functions and methods of analysis. *Biochim Biophys Acta*. 2006;1764:1890-1903.

- [30] Yu Z, Chen T, Hebert J, Li E, Richard S. A mouse PRMT1 null allele defines an essential role for arginine methylation in genome maintenance and cell proliferation. *Mol Cell Biol.* 2009;29:2982-2996.
- [31] Meyer R, Wolf SS, Obendorf M. PRMT2, a member of the protein arginine methyltransferase family, is a coactivator of the androgen receptor. *J. Steroid Biochem. Mol Biol.* 2007;107:1-14.
- [32] Lakowski TM, Frankel A. Kinetic analysis of human protein arginine N-methyltransferase 2: formation of monomethyl- and asymmetric dimethyl-arginine residues on histone H4. *Biochem J.* 2009;421:253-261.
- [33] Sayegh J, Webb K, Cheng D, Bedford MT, Clarke SG. Regulation of protein arginine methyltransferase 8 (PRMT8) activity by its N-terminal domain. *J Biol Chem.* 2007;282:36444-36453.
- [34] Iwasaki H, Kovacic JC, Olive M, Beers JK, Yoshimoto T, Crook MF, Tonelli LH, Nabel EG. Disruption of protein arginine N-methyltransferase 2 regulates leptin signaling and produces leanness in vivo through loss of STAT3 methylation. *Circ Res.* 2010;107:992-1001.
- [35] Qi C, Chang J, Zhu Y, Yeldandi AV, Rao SM, Zhu YJ. Identification of protein arginine methyltransferase 2 as a coactivator for estrogen receptor alpha. *J Biol Chem.* 2002;277:28624-28630.
- [36] Yoshimoto T, Boehm M, Olive M, Crook MF, San H, Langenickel T, Nabel EG. The arginine methyltransferase PRMT2 binds RB and regulates E2F function. *Exp Cell Res.* 2006;312:2040-2053.
- [37] Kzhyshkowska J, Schutt H, Liss M, Kremmer E, Stauber R, Wolf H, Dobner T. Heterogeneous nuclear ribonucleoprotein E1B-AP5 is methylated in its Arg-Gly-Gly (RGG) box and interacts with human arginine methyltransferase HRMT1L1. *Biochem J.* 2001;358:305-314.
- [38] Swiercz R, Cheng D, Kim D, Bedford MT. Ribosomal protein rpS2 is hypomethylated in PRMT3-deficient mice. *J Biol Chem.* 2007;282:16917-16923.
- [39] Cheng D, Cote J, Shaaban S, Bedford MT. The arginine methyltransferase CARM1 regulates the coupling of transcription and mRNA processing. *Mol Cell* 2007;25:71-83.
- [40] Feng Q, Yi P, Wong J, O'Malley BW. Signaling within a coactivator complex: methylation of SRC-3/AIB1 is a molecular switch for complex disassembly. *Mol Cell Biol.* 2006;26:7846-7857.
- [41] Xu W, Cho H, Kadam S, Banayo EM, Anderson S, Yates JR 3rd, Emerson BM, Evans RM. A methylation-mediator complex in hormone signaling. *Genes Dev.* 2004;18:144-156.

- [42] Kim J, Lee J, Yadav N, Wu Q, Carter C, Richard S, Richie E, Bedford MT. Loss of CARM1 results in hypomethylation of thymocyte cyclic AMP-regulated phosphoprotein and deregulated early T cell development. *J Biol Chem*. 2004;279:25339-25344.
- [43] Yadav N, Cheng D, Richard S, Morel M, Iyer VR, Aldaz CM, Bedford MT. CARM1 promotes adipocyte differentiation by coactivating PPAR gamma. *EMBO Rep*. 2008;9:193-198.
- [44] Ito T, Yadav N, Lee J, Furumatsu T, Yamashita S, Yoshida K, Taniguchi N, Hashimoto M, Tsuchiya M, Ozaki T, Lotz M, Bedford MT, Asahara H. Arginine methyltransferase CARM1/PRMT4 regulates endochondral ossification. *BMC Dev Biol*. 2009;9:47. (doi:10.1186/1471-213X-9-47)
- [45] O'Brien KB, Berich-Jorda M, Yadav N, Kocher O, Diruscio A, Ebralidze A, Levantini E, Sng NJ, Bhasin M, Caron T, Kim D, Steidl U, Huang G, Halmos B, Rodig SJ, Bedford MT, Tenen DG, Kobayashi S. CARM1 is required for proper control of proliferation and differentiation of pulmonary epithelial cells. *Development*. 2010;137:2147-2156.
- [46] Yadav N, Lee J, Kim J, Shen J, Hu MC, Aldaz CM, Bedford MT. Specific protein methylation defects and gene expression perturbations in coactivator-associated arginine methyltransferase 1-deficient mice. *Proc Natl Acad Sci U S A*. 2003;100:6464-6468.
- [47] Zhou Z, Sun X, Zou Z, Sun L, Zhang T, Guo S, Wen Y, Liu L, Wang Y, Qin J, Li L, Gong W, Bao S. PRMT5 regulates Golgi apparatus structure through methylation of the golg in GM130. *Cell Res*. 2010;20:1023-1033.
- [48] Friesen WJ, Paushkin S, Wyce A, Massenet S, Pesiridis GS, Van DG, Rappsilber J, Mann M, Dreyfuss G. The methylosome, a 20S complex containing JBP1 and pICln, produces dimethylarginine-modified Sm proteins. *Mol Cell Biol*. 2001;21:8289-8300.
- [49] Meister G, Fischer U. Assisted RNP assembly: SMN and PRMT5 complexes cooperate in the formation of spliceosomal UsnRNPs. *EMBO J*. 2002;21:5853-5863.
- [50] Karkhanis V, Hu YJ, Baiocchi RA, Imbalzano AN, Sif S. Versatility of PRMT5-induced methylation in growth control and development. *Trends Biochem Sci*. 2011;36:633-641.
- [51] Tee WW, Pardo M, Theunissen TW, Yu L, Choudhary JS, Hajkova P, Surani MA. Prmt5 is essential for early mouse development and acts in the cytoplasm to maintain ES cell pluripotency. *Genes Dev*. 2010;24:2772-2777.
- [52] Stein C, Riedl S, R uthnick D, N told RR, Bauer UM. The arginine methyltransferase PRMT6 regulates cell proliferation and senescence through transcriptional repression of tumor suppressor genes. *Nucleic Acids Res*. 2012; Aug 16. [Epub ahead of print]
- [53] Miranda TB, Miranda M, Frankel A, Clarke S. PRMT7 is a member of the protein arginine methyltransferase family with a distinct substrate specificity. *J Biol Chem*. 2004;279:22902-22907.

- [54] Bleibel WK, Duan S, Huang RS, Kistner EO, Shukla SJ, Wu X, Badner JA, Dolan ME. Identification of genomic regions contributing to etoposide-induced cytotoxicity. *Hum Genet.* 2009;125:173-180.
- [55] Verbiest V, Montaudon D, Tautu MT, Moukarzel J, Portail JP, Markovits J, Robert J, Ichas F, Pourquier P. Protein arginine (N)-methyl transferase 7 (PRMT7) as a potential target for the sensitization of tumor cells to camptothecins. *FEBS Lett.* 2008;582:1483-1489.
- [56] Jelinic P, Stehle JC, Shaw P. The testis-specific factor CTCFL cooperates with the protein methyltransferase PRMT7 in H19 imprinting control region methylation. *PLoS Biol.* 2006;4:e355.
- [57] Buhr N, Carapito C, Schaeffer C, Kieffer E, Van DA, Viville S. Nuclear proteome analysis of undifferentiated mouse embryonic stem and germ cells. *Electrophoresis.* 2008;29:2381-2390.
- [58] Zurita-Lopez CI, Sandberg T, Kelly R, Clarke SG. Human protein arginine methyltransferase 7 (PRMT7) is a type III enzyme forming omega-NG-monomethylated arginine residues. *J Biol Chem.* 2012;287:7859-7870.
- [59] Kim JD, Kako K, Kakiuchi M, Park GG, Fukamizu A. EWS is a substrate of type I protein arginine methyltransferase, PRMT8. *Int J Mol Med.* 2008;22:309-315.
- [60] Hardisty-Hughes RE, Tateossian H, Morse SA, Romero MR, Middleton A, Tymowska-Lalanne Z, Hunter AJ, Cheeseman M, Brown SD. A mutation in the F-box gene, *Fbxo11*, causes otitis media in the Jeff mouse. *Hum Mol Genet.* 2006;15:3273-3279.
- [61] Segade F, Daly KA, Allred D, Hicks PJ, Cox M, Brown M, Hardisty-Hughes RE, Brown SD, Rich SS, Bowden DW. Association of the *FBXO11* gene with chronic otitis media with effusion and recurrent otitis media: the Minnesota COME/ROM Family Study. *Arch Otolaryngol Head Neck Surg.* 2006;132:729-733.
- [62] Licatalosi DD, Darnell RB. RNA processing and its regulation: global insights into biological networks. *Nat Rev Genet.* 2010;11:75-87.
- [63] Boisvert FM, Cote J, Boulanger MC, Cleroux P, Bachand F, Autexier C, Richard S. Symmetrical dimethylarginine methylation is required for the localization of SMN in Cajal bodies and pre-mRNA splicing. *J Cell Biol.* 2002;159:957-969.
- [64] Brahms H, Raymackers J, Union A, de Keyser F, Meheus L, Lührmann R. The C-terminal RG dipeptide repeats of the spliceosomal Sm proteins D1 and D3 contain symmetrical dimethylarginines, which form a major B-cell epitope for anti-Sm autoantibodies. *J Biol Chem.* 2000;275:17122-17129.
- [65] Brahms H, Meheus L, de Brabandere V, Fischer U, Lührmann R. Symmetrical dimethylation of arginine residues in spliceosomal Sm protein B/B' and the Sm-like protein LSm4, and their interaction with the SMN protein. *RNA.* 2001;7:1531-1542.

- [66] Miranda TB, Khusial P, Cook JR, Lee JH, Gunderson SI, Pestka S, Zieve GW, Clarke S. Spliceosome Sm proteins D1, D3, and B/B' are asymmetrically dimethylated at arginine residues in the nucleus. *Biochem Biophys Res Commun*. 2004;323:382-387.
- [67] McBride AE, Weiss VH, Kim HK, Hogle JM, Silver PA. Analysis of the yeast arginine methyltransferase Hmt1p/Rmt1p and its in vivo function. Cofactor binding and substrate interactions. *J Biol Chem*. 2000;275:3128-3136.
- [68] Côté J, Boisvert FM, Boulanger MC, Bedford MT, Richard S. Sam68 RNA binding protein is an in vivo substrate for protein arginine N-methyltransferase 1. *Mol Biol Cell*. 2003;14:274-287.
- [69] Yu MC, Bachand F, McBride AE, Komili S, Casolari JM, Silver PA. Arginine methyltransferase affects interactions and recruitment of mRNA processing and export factors. *Genes Dev*. 2004;18:2024-2035.
- [70] Fujiwara T, Mori Y, Chu DL, Koyama Y, Miyata S, Tanaka H, Yachi K, Kubo T, Yoshikawa H, Tohyama M. CARM1 regulates proliferation of PC12 cells by methylating HuD. *Mol Cell Biol*. 2006;26:2273-2285.
- [71] Lukong KE, Richard S. Arginine methylation signals mRNA export. *Nat Struct Mol Biol*. 2004;11:914-915.
- [72] Smith WA, Schurter BT, Wong-Staal F, David M. Arginine methylation of RNA helicase a determines its subcellular localization. *J Biol Chem*. 2004;279:22795-22798.
- [73] Fronz K, Güttinger S, Burkert K, Kühn U, Stöhr N, Schierhorn A, Wahle E. Arginine methylation of the nuclear poly(a) binding protein weakens the interaction with its nuclear import receptor, transportin. *J Biol Chem*. 2011;286:32986-32994.
- [74] Banniste AJ, Schneider R, Kouzarides T. Histone methylation: dynamic or static? *Cell*. 2002;109:801-806.
- [75] Wysocka J, Allis CD, Coonrod S. Histone arginine methylation and its dynamic regulation. *Front Biosci*. 2006;11:344-355.
- [76] Di Lorenzo A, Bedford MT. Histone arginine methylation. *FEBS Lett*. 2011;585:2024-2031.
- [77] Schurter BT, Koh SS, Chen D, Bunick GJ, Harp JM, Hanson BL, Henschen-Edman A, Mackay DR, Stallcup MR, Aswad DW. Methylation of histone H3 by coactivator-associated arginine methyltransferase 1. *Biochemistry*. 2001;40:5747-5756.
- [78] Guccione E, Bassi C, Casadio F, Martinato F, Cesaroni M, Schuchlantz H, Lüscher B, Amati B. Methylation of histone H3R2 by PRMT6 and H3K4 by an MLL complex are mutually exclusive. *Nature*. 2007;449:933-937.
- [79] Pal S, Vishwanath SN, Erdjument-Bromage H, Tempst P, Sif S. Human SWI/SNF-associated PRMT5 methylates histone H3 arginine 8 and negatively regulates expression of ST7 and NM23 tumor suppressor genes. *Mol Cell Biol*. 2004;24:9630-9645.

- [80] Wang H, Huang ZQ, Xia L, Feng Q, Erdjument-Bromage H, Strahl BD, Briggs SD, Allis CD, Wong J, Tempst P, Zhang Y. Methylation of histone H4 at arginine 3 facilitating transcriptional activation by nuclear hormone receptor. *Science*. 2001;293:853-857.
- [81] Hyllus D, Stein C, Schnabel K, Schiltz E, Imhof A, Dou Y, Hsieh J, Bauer UM. PRMT6-mediated methylation of R2 in histone H3 antagonizes H3 K4 trimethylation. *Genes Dev*. 2007;21:3369-3380.
- [82] Iberg AN, Espejo A, Cheng D, Kim D, Michaud-Levesque J, Richard S, Bedford MT. Arginine methylation of the histone H3 tail impedes effector binding. *J Biol Chem*. 2008;283:3006-3010.
- [83] Xu W, Chen H, Du K, Asahara H, Tini M, Emerson BM, Montminy M, Evans RM. A transcriptional switch mediated by cofactor methylation. *Science*. 2001;294:2507-2511.
- [84] Teyssier C, Ma H, Emter R, Kralli A, Stallcup MR. Activation of nuclear receptor coactivator PGC-1 alpha by arginine methylation. *Genes Dev*. 2005;19:1466-73.
- [85] Garcia MM, Guéant-Rodriguez RM, Pooya S, Brachet P, Alberto JM, Jeannesson E, Maskali F, Gueguen N, Marie PY, Lacolley P, Herrmann M, Juillièrè Y, Malthiery Y, Guéant JL. Methyl donor deficiency induces cardiomyopathy through altered methylation/acetylation of PGC-1alpha by PRMT1 and SIRT1. *J Pathol*. 2011;225:324-335.
- [86] Mowen KA, Tang J, Zhu W, Schurter BT, Shuai K, Herschman HR, David M. Arginine methylation of STAT1 modulates IFNalpha/beta-induced transcription. *Cell*. 2001;104:731-741.
- [87] Kwak YT, Guo J, Prajapati S, Park KJ, Surabhi RM, Miller B, Gehrig P, Gaynor RB. Methylation of SPT5 regulates its interaction with RNA polymerase II and transcriptional elongation properties. *Mol Cell*. 2003;11:1055-1066.
- [88] Tan CP, Nakielny S. Control of the DNA methylation system component MBD2 by protein arginine methylation. *Mol Cell Biol*. 2006;26:7224-7235.
- [89] Sims RJ 3rd, Rojas LA, Beck D, Bonasio R, Schüller R, Drury WJ 3rd, Eick D, Reinberg D. The C-terminal domain of RNA polymerase II is modified by site-specific methylation. *Science*. 2011;332:99-103.
- [90] Boisvert FM, Hendzel MJ, Masson JY, Richard S. Methylation of MRE11 regulates its nuclear compartmentalization. *Cell Cycle*. 2005;4:981-989.
- [91] Boisvert FM, Dery U, Masson JY, Richard S. Arginine methylation of MRE11 by PRMT1 is required for DNA damage checkpoint control. *Genes Dev*. 2005;19:671-676.
- [92] He W, Ma X, Yang X, Zhao Y, Qiu J, Hang H. A role for the arginine methylation of Rad9 in checkpoint control and cellular sensitivity to DNA damage. *Nucleic Acids Res*. 2011;39:4719-4727.
- [93] El-Andaloussi N, Valovka T, Toueille M, Steinacher R, Focke F, Gehrig P, Covic M, Hassa PO, Schär P, Hübscher U, Hottiger MO. Arginine methylation regulates DNA polymerase beta. *Mol Cell*. 2006;22:51-62.

- [94] Abramovich C, Yakobson B, Chebath J, Revel M. A protein-arginine methyltransferase binds to the intracytoplasmic domain of the IFNAR1 chain in the type I interferon receptor. *EMBO J.* 1997;16:260-266.
- [95] Altschuler L, Wook JO, Gurari D, Chebath J, Revel M. Involvement of receptor-bound protein methyltransferase PRMT1 in antiviral and antiproliferative effects of type I interferons. *J Interferon Cytokine Res.* 1999;19:189-195.
- [96] Parry RV, Ward SG. Protein arginine methylation: a new handle on T lymphocytes? *Trends Immunol.* 2010;31:164-169.
- [97] Iwasaki H, Yada T. Protein arginine methylation regulates insulin signaling in L6 skeletal muscle cells. *Biochem Biophys Res Commun.* 2007;364:1015-1021.
- [98] Le Romancer M, Treilleux I, Leconte N, Robin-Lespinasse Y, Sentis S, Bouchekioua-Bouzaghrou K, Goddard S, Gobert-Gosse S, Corbo L. Regulation of estrogen rapid signaling through arginine methylation by PRMT1. *Mol Cell.* 2008;31:212-221.
- [99] Yamagata K, Daitoku H, Takahashi Y, Namiki K, Hisatake K, Kako K, Mukai H, Kasuya Y, Fukamizu A. Arginine methylation of FOXO transcription factors inhibits their phosphorylation by Akt. *Mol Cell.* 2008;32:221-231.
- [100] Kops GJ, de Rooter ND, De Vries-Smits AM, Powell DR, Bos JL, Burgering BM. Direct control of the Forkhead transcription factor AFX by protein kinase B. *Nature.* 1999;398:630-634.
- [101] Sakamaki J, Daitoku H, Ueno K, Hagiwara A, Yamagata K, Fukamizu A. Arginine methylation of BCL-2 antagonist of cell death (BAD) counteracts its phosphorylation and inactivation by Akt. *Proc Natl Acad Sci U S A.* 2011;108:6085-6090.
- [102] Andreu-Pérez P, Esteve-Puig R, de Torre-Minguela C, López-Fauqued M, Bech-Serra JJ, Tenbaum S, García-Trevijano ER, Canals F, Merlino G, Avila MA, Recio JA. Protein arginine methyltransferase 5 regulates ERK1/2 signal transduction amplitude and cell fate through CRAF. *Sci Signal.* 2011;4:ra58.
- [103] Robin-Lespinasse Y, Sentis S, Kolytcheff C, Rostan MC, Corbo L, Le Romancer M. hCAF1, a new regulator of PRMT1-dependent arginine methylation. *J Cell Sci.* 2007;120:638-647.
- [104] Singh V, Miranda TB, Jiang W, Frankel A, Roemer ME, Robb VA, Gutmann DH, Herschman HR, Clarke S, Newsham IF. DAL-1/4.1B tumor suppressor interacts with protein arginine N-methyltransferase 3 (PRMT3) and inhibits its ability to methylate substrates in vitro and in vivo. *Oncogene.* 2004;23:7761-7771.
- [105] Lacroix M, El Messaoudi S, Rodier G, Le Cam A, Sardet C, Fabbrizio E. The histone-binding protein COPR5 is required for nuclear functions of the protein arginine methyltransferase PRMT5. *EMBO Rep.* 2008;9:452-458.

- [106] Higashimoto K, Kuhn P, Desai D, Cheng X, Xu W. Phosphorylation-mediated inactivation of coactivator-associated arginine methyltransferase 1. *Proc Natl Acad Sci U S A*. 2007;104:12318-12323.
- [107] Feng Q, He B, Jung SY, Song Y, Qin J, Tsai SY, Tsai MJ, O'Malley BW. Biochemical control of CARM1 enzymatic activity by phosphorylation. *J Biol Chem*. 2009;284:36167-36174.
- [108] Bedford MT, Clarke SG. Protein arginine methylation in mammals: who, what, and why. *Mol Cell*. 2009;33:1-13.
- [109] Ng SS, Yue WW, Oppermann U, Klose RJ. Dynamic protein methylation in chromatin biology. *Cell Mol Life Sci*. 2009;66:407-22.
- [110] Chang B, Chen Y, Zhao Y, Bruick RK. JMJD6 is a histone arginine demethylase. *Science*. 2007;318:444-7.
- [111] Webby CJ, Wolf A, Gromak N, Dreger M, Kramer H, Kessler B, Nielsen ML, Schmitz C, Butler DS, Yates JR 3rd, Delahunty CM, Hahn P, Lengeling A, Mann M, Proudfoot NJ, Schofield CJ, Böttger A. Jmjd6 catalyses lysyl-hydroxylation of U2AF65, a protein associated with RNA splicing. *Science*. 2009;325:90-93.
- [112] Thompson PR, Fast W. Histone citrullination by protein arginine deiminase: is arginine methylation a green light or a roadblock? *ACS Chem Biol*. 2006;1:433-441.
- [113] Arita K, Hashimoto H, Shimizu T, Nakashima K, Yamada M, Sato M. Structural basis for Ca(2+)-induced activation of human PAD4. *Nat Struct Mol Biol*. 2004;11:777-783.
- [114] Kearney PL, Bhatia M, Jones NG, Yuan L, Glascock MC, Catchings KL, Yamada M, Thompson PR. Kinetic characterization of protein arginine deiminase 4: a transcriptional corepressor implicated in the onset and progression of rheumatoid arthritis. *Biochemistry*. 2005;44:10570-10582.
- [115] Nakashima K, Hagiwara T, Yamada M. Nuclear localization of peptidylarginine deiminase V and histone deimination in granulocytes. *J Biol Chem*. 2002;277:49562-49568.
- [116] Cuthbert GL, Daujat S, Snowden AW, Erdjument-Bromage H, Hagiwara T, Yamada M, Schneider R, Gregory PD, Tempst P, Bannister AJ, Kouzarides T. Histone deimination antagonizes arginine methylation. *Cell*. 2004;118:545-553.
- [117] Wang Y, Wysocka J, Sayegh J, Lee YH, Perlin JR, Leonelli L, Sonbuchner LS, McDonald CH, Cook RG, Dou Y, Roeder RG, Clarke S, Stallcup MR, Allis CD, Coonrod SA. Human PAD4 regulates histone arginine methylation levels via demethylimination. *Science*. 2004;306:279-283.
- [118] Hidaka Y, Hagiwara T, Yamada M. Methylation of the guanidino group of arginine residues prevents citrullination by peptidylarginine deiminase IV. *FEBS Lett*. 2005;579:4088-4092.

- [119] Raijmakers R, Zendman AJ, Egberts WV, Vossenaar ER, Raats J, Soede-Huijbregts C, Rutjes FP, van Veelen PA, Drijfhout JW, Pruijn GJ. Methylation of arginine residues interferes with citrullination by peptidylarginine deiminases in vitro. *J Mol Biol.* 2007;367:1118-1129.
- [120] Boger RH. The emerging role of asymmetric dimethylarginine as a novel cardiovascular risk factor. *Cardiovasc Res.* 2003;59:824-833.
- [121] Closs EI, Basha FZ, Habermeier A, Forstermann U. Interference of L-arginine analogues with L-arginine transport mediated by the y⁺ carrier hCAT-2B. *Nitric Oxide.* 1997;1:65-73.
- [122] Carello KA, Whitesall SE, Lloyd MC, Billecke SS, D'Alecy LG. Asymmetrical dimethylarginine plasma clearance persists after acute total nephrectomy in rats. *Am J Physiol Heart Circ Physiol.* 2006;290:H209-216.
- [123] Palm F, Onozato ML, Luo Z, Wilcox CS. Dimethylarginine dimethylaminohydrolase (DDAH): expression, regulation, and function in the cardiovascular and renal systems. *Am J Physiol Heart Circ Physiol.* 2007;293:H3227-H3245.
- [124] Antoniades C, Shirodaria C, Leeson P, Antonopoulos A, Warrick N, Van-Assche T, Cunnington C, Tousoulis D, Pillai R, Ratnatunga C, Stefanadis C, Channon KM. Association of plasma asymmetrical dimethylarginine (ADMA) with elevated vascular superoxide production and endothelial nitric oxide synthase uncoupling: implications for endothelial function in human atherosclerosis. *Eur Heart J.* 2009;30:1142-1150.
- [125] Handy DE, Castro R, Loscalzo J. Epigenetic modifications: basic mechanisms and role in cardiovascular disease. *Circulation.* 2011;123:2145-2156.
- [126] Finkelstein JD. Methionine metabolism in mammals. *J Nutr Biochem.* 1990;1:228-237.
- [127] Joseph J, Handy DE, Loscalzo J. Quo vadis: whither homocysteine research? *Cardiovasc Toxicol.* 2009;9:53-63.
- [128] Blom HJ, Smulders Y. Overview of homocysteine and folate metabolism. With special references to cardiovascular disease and neural tube defects. *J Inherit Metab Dis.* 2011;34:75-81.
- [129] Castro R, Rivera I, Struys EA, Jansen EE, Ravasco P, Camilo ME, Blom HJ, Jakobs C, Tavares de Almeida I. Increased homocysteine and S-adenosylhomocysteine concentrations and DNA hypomethylation in vascular disease. *Clin Chem.* 2003;49:1292-1296.
- [130] Castro R, Rivera I, Ravasco P, Camilo ME, Jakobs C, Blom HJ, de Almeida IT. 5,10-methylenetetrahydrofolate reductase (MTHFR) 677C-->T and 1298A-->C mutations are associated with DNA hypomethylation. *J Med Genet.* 2004; 41:454-458.

- [131] Castro R, Rivera I, Martins C, Struys EA, Jansen EE, Clode N, Graça LM, Blom HJ, Jakobs C, de Almeida IT. Intracellular S-adenosylhomocysteine increased levels are associated with DNA hypomethylation in HUVEC. *J Mol Med* 2005;83:831-836.
- [132] Castro R, Rivera I, Blom HJ, Jakobs C, Tavares de Almeida I. Homocysteine metabolism, hyperhomocysteinaemia and vascular disease: an overview. *J Inherit Metab Dis*. 2006;29:3-20.
- [133] Barroso M, Rocha MS, Esse R, Gonçalves I Jr, Gomes AQ, Teerlink T, Jakobs C, Blom HJ, Loscalzo J, Rivera I, de Almeida IT, Castro R. Cellular hypomethylation is associated with impaired nitric oxide production by cultured human endothelial cells. *Amino Acids*. 2012;42:1903-1911.
- [134] Ueland PM. Pharmacological and biochemical aspects of S-adenosylhomocysteine and S-adenosylhomocysteine hydrolase. *Pharmacol Rev*. 1982;34:223-253.
- [135] Yi P, Melnyk S, Pogribna M, Pogribny IP, Hine RJ, James SJ. Increase in plasma homocysteine associated with parallel increases in plasma S-adenosylhomocysteine and lymphocyte DNA hypomethylation. *J Biol Chem*. 2000;275:29318-29323.
- [136] Friso S, Choi SW, Girelli D, Mason JB, Dolnikowski GG, Bagley PJ, Olivieri O, Jacques PF, Rosenberg IH, Corrocher R, Selhub J. A common mutation in the 5,10-methylenetetrahydrofolate reductase gene affects genomic DNA methylation through an interaction with folate status. *Proc Natl Acad Sci U S A*. 2002;99:5606-5611.
- [137] James SJ, Melnyk S, Pogribna M, Pogribny IP, Caudill MA. Elevation in S-adenosylhomocysteine and DNA hypomethylation: potential epigenetic mechanism for homocysteine-related pathology. *J Nutr*. 2002;132:2361S-2366S.
- [138] Ingrosso D, Cimmino A, Perna AF, Masella L, De Santo NG, De Bonis ML, Vacca M, D'Esposito M, D'Urso M, Galletti P, Zappia V. Folate treatment and unbalanced methylation and changes of allelic expression induced by hyperhomocysteinaemia in patients with uraemia. *Lancet*. 2003;361:1693-1699.
- [139] Flynn J, Reich N. Murine DNA (cytosine-5-)-methyltransferase: steady-state and substrate trapping analyses of the kinetic mechanism. *Biochemistry*. 1998;37:15162-15169.
- [140] Esse R, Rocha MS, Barroso M, Goncalves Jr I, Leandro P, Teerlink T, Jakobs C, Blom HJ, Castro R, Tavares de Almeida I. S-Adenosyl homocysteine accumulation decreases global protein arginine methylation status in cultured human endothelial cells. *J Inherit Metab Dis*. 2010;33:S35.
- [141] Esse R, Imbard A, Rocha MS, Teerlink T, Castro R, Tavares de Almeida I, Blom HJ. Diet-induced hyperhomocysteinemia in rats affects protein arginine methylation in a tissue-specific manner. *J Inherit Metab Dis*. 2012;35:S51.