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Histone arginine methylations: their roles in chromatin dynamics and transcriptional regulation

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Synopsis

PRMTs (protein arginine N-methyltransferases) specifically modify the arginine residues of key cellular and nuclear proteins as well as histone substrates. Like lysine methylation, transcriptional repression or activation is dependent upon the site and type of arginine methylation on histone tails. Recent discoveries imply that histone arginine methylation is an important modulator of dynamic chromatin regulation and transcriptional controls. However, under the shadow of lysine methylation, the roles of histone arginine methylation have been under-explored. The present review focuses on the roles of histone arginine methylation in the regulation of gene expression, and the interplays between histone arginine methylation, histone acetylation, lysine methylation and chromatin remodelling factors. In addition, we discuss the dynamic regulation of arginine methylation by arginine demethylases, and how dysregulation of PRMTs and their activities are linked to human diseases such as cancer.

Keywords

arginine demethylase; cancer; chromatin insulator; histone cross-talk; protein arginine N-methyltransferase (PRMT); transcriptional regulation

INTRODUCTION

Post-transcriptional modifications, like phosphorylation, acetylation, methylation, sumoylation and ubiquitination, play important roles in the regulation of cellular and nuclear protein function. Changes in these modifications significantly alter the activity and function of targeted proteins. In eukaryotes, arginine methylation is a prevalent post-transcriptional modification that occurs in both cytoplasmic and nuclear proteins (Figure 1). Arginine

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residues can be methylated symmetrically or asymmetrically. These reactions are catalysed by a specific family of enzymes called PRMTs (protein arginine N-methyltransferases). These proteins and arginine methylation are implicated in a variety of cellular processes, including RNA processing, ribosomal biosynthesis, DNA repair, signal transduction and transcriptional regulation (Figure 1) [1].

Most PRMTs are ubiquitously expressed and have been found in fungi, plants, *Caenorhabditis elegans*, *Drosophila* and vertebrate animals [2], suggesting that arginine methylation is evolutionarily conserved. To date, eleven mammalian PRMTs have been identified. They are classified into two classes depending on the nature of the modification introduced. Although both type I and type II PRMTs catalyse the formation of the monomethylarginine intermediate, they differ in the final type of arginine modification (Figure 2). The type I enzymes (PRMT1, PRMT3, PRMT4, PRMT6 and PRMT8) further catalyse the intermediate to asymmetrical dimethylarginine, whereas the Type II enzymes (PRMT5, PRMT7 and PRMT9) transfer methyl groups to the ω -nitrogen of the arginine residue, resulting in symmetrical dimethylarginine [3–5]. PRMT7 was first identified as an enzyme that produces only monomethylarginine (Figure 2). It was shown previously to catalyse the formation of symmetrical dimethylarginine on peptide and histone H4 substrates [4,6]. PRMT2, PRMT10 and PRMT11 have not yet been shown to possess enzymatic activity [2].

Growing evidence indicates that the methylation of arginine residues at histone N-terminal tails affects high-order chromatin structures, leading to the recruitment of protein complexes that regulate transcription and chromatin insulator activity [7]. The potential functional activity of arginine methylation depends on the residue being modified and the type of methylation which is introduced. In the present review, we focus on the mechanisms by which PRMTs regulate the dynamic chromatin structure and its link to transcriptional regulation.

THE MEMBERS OF THE PRMT FAMILY

Except for PRMT3, all PRMTs that possess detectable methyltransferase activity are able to methylate a histone substrate. The present review summarizes what is known about the function of each of these PRMTs regarding histone modification as well as transcriptional regulation (Figure 3).

PRMT1

PRMT1 was initially identified as a binding partner of TIS21 (PMA-inducible sequence 21) and the related protein BTG1 (B-cell translocation gene 1). Both of these proteins belong to a family of cellular factors involved in the negative regulation of cell growth [8]. PRMT1, which is the most predominant PRMT in eukaryotic cells, exists in cells as a large protein complex with a variety of proteins [8,9]. Homozygous mutation of *prmt1* in mice resulted in early embryonic lethality at the E6.5 (embryonic day 6.5) stage [10]. Although the loss of PRMT1 did not affect the viability of mouse ES cells (embryonic stem cells), the level of arginine N-methyltransferase activity was reduced by 85% in the *prmt1* null ES cells, suggesting that PRMT1 is a major type I enzyme in cells [10]. Several different types of

regulatory proteins are modified by PRMT1; consequently, it is involved in many different types of cellular processes (Figure 1). The fact that it methylates a number of the GAR-motif-harboring hnRNPs (heterogeneous nuclear ribonucleoproteins) suggests that it plays an important role in RNA biosynthesis and processing [1]. PRMT1 methylation of NFAT (nuclear factor of activated T-cells) and STAT (signal transducer and activator of transcription) implicate it in signal transduction and methylation of MRE11 (meiotic recombination protein 11), which alters the exonuclease activity of MRE11 in the double-strand break repair protein complex, MRE11–RAD50 (homologue of scRAD50)–NBS1 (Nijmegen breakage syndrome 1) [11], implicating it in DNA repair. Another key substrate of PRMT1 methylation is H4R3 (Arg³ of histone H4) tails [12,13]. Asymmetrical dimethylation of H4R3 potentiates subsequent histone acetylation and contributes to the establishment and maintenance of an active euchromatin structure, suggesting that this histone modification can function as a transcriptional activation mark [12,14]. Indeed, as a nuclear receptor co-activator, the arginine methyltransferase activity of PRMT1 is essential for its co-activation. In addition, PRMT1 can be targeted to the promoter by several transcription factors and plays a critical role in transcriptional regulation via methylating H4R3 or transcription factors [15–19].

PRMT2

Prmt2-knockout mice are viable and grow normally [20]. Although no enzymatic activity has been detected, PRMT2 is a co-activator for the oestrogen receptor (Figure 1) [21]. It also binds to RB (retinoblastoma protein) 1 and regulates E2F activity in cellular growth and proliferation [20].

PRMT3

PRMT3, which was identified as a PRMT1-interacting partner, harbours a zinc finger domain in its N-terminus as a substrate-recognition motif. This unique structure makes it different from PRMT1 in its oligomerization, subcellular localization and substrate specificity [22]. PRMT3 is involved in ribosomal biosynthesis through methylating the 40S ribosomal protein S2 (Figure 1) [23].

PRMT4

PRMT4, which is referred to as CARM1 (co-activator-associated arginine methyltransferase 1), was identified as a binding partner for the p160 transcriptional co-activator GRIP1 (glucocorticoid receptor-interacting protein 1) [24] and is involved in the transcriptional activation of NR (nuclear hormone receptor)-mediated transcription [25]. Evidence indicates that CARM1 is involved in NR-mediated transcription by co-operating with the histone acetyltransferases, p300/CBP [CREB (cAMP-response-element-binding protein)-binding protein] and PCAF (p300/CBP-associated factor), and methylating H3R2 and H3R17 tails [26]. Interestingly, CARM1 can also target H3R26. This modification is correlated with stem cell fate and potency in the early mouse embryo [27]. Consistent with the role of CARM1 in early development, *Carm1*-knockout mice are much smaller than their wild-type littermates and die soon after birth [28].

PRMT5

Like PRMT1, PRMT5 also methylates H4R3. Unlike PRMT1, PRMT5 catalyses symmetrical methylation and is associated with transcriptional repression [1]. PRMT5 is normally present in the histone deacetylase co-repressor complexes, such as NuRD (nucleosome remodelling and deacetylase), which negatively regulate transcription [29]. In addition, PRMT5 directs the symmetrical methylation of H2AR3 and/or H4R3. These modifications are associated with the B-cell specific transcription repressor Blimp1 in PGCs (primordial germ cells) [30]. PRMT5 directly represses the RB family of tumour-suppressor genes by targeting the hypermethylation of H3R8 and H4R3 to the promoters of the RB family genes [31]. Differing from other symmetrical arginine methylation marks, the methylation of H3R8 tails by PRMT5 facilitates the ATP-dependent SWI/SNF chromatin remodelling activity and potentiates transcription associated with myogenic differentiation [32]. This suggests that symmetrical arginine methylation may have a context-dependent function and is not solely associated with transcriptional silencing.

PRMT6

PRMT6 is the only type I enzyme for which expression is restricted to the nucleus and undergoes auto-methylation [33]. The targets of PRMT6 include HMG (high-mobility group) 1a and HMG1b [34]. These factors bind to the DNA minor groove, potentially affecting chromatin architecture. Like CARM1, PRMT6 also catalyses asymmetrical dimethylation of H3R2 tails. However, a genome-wide ChIP (chromatin immunoprecipitation)-on-DNA chip study showed that this methyl mark is enriched in heterochromatic or inactive euchromatic regions and is specifically depleted from active promoters, making it a mark for silent chromatin [35,36]. PRMT6 also methylates DNA polymerase β . The methylation stimulates DNA polymerase activity and therefore has strong implications for this enzyme in regulating DNA base-excision repair (Figure 1) [37].

PRMT7

PRMT7 is a type II enzyme that targets histones H2A and H4 [6]. PRMT7 contains two SAM (*S*-adenosylmethionine)-binding domains, neither of which is dispensable for its enzymatic activity. Recently, it has been shown that, in male germ cells, PRMT7 was recruited by a testis-specific protein CTCFL (CCCTC-binding factor-like)/Boris (brother of the regulator of imprinted sites), a paralogue of the enhancer blocker CTCF (CCCTC-binding factor), which binds to the imprinting control region of the *Igf2/H19* (where *Igf2* is insulin-like growth factor 2) locus. PRMT7 then creates a symmetrical dimethylated H4R3 tail that disengages CTCFL from the complex and allows a downstream enhancer to activate the paternally expressed *Igf2* gene [4].

PRMT8, PRMT9, PRMT10 and PRMT11

PRMT 8 is exclusively expressed in the brain and is localized to the plasma membrane [38]. PRMT9, also called FBXO11 (F-box protein 11), consists of four splice variants. The smallest isoform, isoform 4, possesses type II methyltransferase activity and is able to methylate histones H2A and H4 *in vitro* [5]. PRMT10 and PRMT11 are the most recent members of the PRMT protein family to be identified, and were identified based on their

homology to PRMT7 and PRMT9 respectively [2]. The biological functions of these four PRMTs are currently unknown.

REGULATION OF HISTONE ARGININE METHYLATION

Arginine methylation of histone tails has been correlated with chromatin dynamics and gene expression. In order to maintain the steady-state balance of individual modifications, arginine methylation needs to be regulated dynamically. Consequently, as well as methylating an arginine residue, there needs to be a means of unmethylating. Although several protein methyltransferases have been identified, little is known about their demethylase. Therefore several studies have focused on the search for demethylases that remove methyl groups from arginine residues at histone tails. To date, at least two proteins are implicated in arginine demethylation pathways: PAD4 (peptidyl arginine deaminase 4) and JMJD6 (jumonji domain-containing protein 6) (Figure 4) [39,40,42].

Human PAD4 was shown to catalyse a deamination reaction that converts both arginine and monomethylarginine to citrulline [39,40], but which cannot convert symmetrical or asymmetrical dimethylarginine (Figure 4A). Although this enzyme can prevent methylation on an arginine residue, it is unable to directly convert methylarginine to arginine by removing the methyl group, so it is not a true demethylase.

Recently, a family of the Jumonji domain-containing proteins exhibited methyl type- and site-specific demethylase activities toward different lysine residues [41]. Among them, JMJD6 is a JmjC-containing iron- and 2-oxoglutarate-dependent dioxygenase that specifically removes methyl groups from the monomethylated or dimethylated H3R2 and H4R3 *in vitro* and *in vivo* (Figure 4B) [42]. JMJD6 was unable to demethylate H3R8, H3R17, H3R26 or H2A sites. *Jmjd6*^{-/-} knockout mice exhibited numerous developmental defects during embryogenesis, suggesting that arginine demethylation plays an essential role in cellular differentiation and proliferation programmes during development [43–45]. The fact that H3R8, H3R17, H3R26 and H2A are methylated by different PRMTs and linked to different cellular functions suggests that demethylation of these residues by other arginine demethylase proteins may play an important role in the dynamic regulation of arginine methylation. However, it remains to be determined whether additional arginine-specific demethylases are required for reversing other sites of arginine methylation.

In addition to PAD4 and JMJD6, which regulate arginine methylation through removal of arginine residues or methyl groups respectively, certain proteins can regulate arginine methyltransferase activity by a direct interaction with PRMTs. For example, hCAF1 [CCR4 (CC chemokine receptor 4)-associated factor-1] can selectively inhibit PRMT-1-mediated methylation of H4R3 in a substrate- and dose-dependent manner, whereas hCAF1 has no effect on the methylation of hnRNPA1, another target of PRMT1 [46]. The methyltransferase activities of PRMT3 and PRMT5 are modulated by their interaction with the tumour-suppressor protein DAL-1 (differentially expressed in adenocarcinoma of the lung)/4.1B [47,48]. Furthermore, the nuclear protein COPR5 (cooperator of PRMT5) specifically modulates the recruitment of PRMT5 to chromatin at PRMT5 target genes and restricts PRMT5 enzymatic activity by preferentially methylating histone H4R3 but not

H3R8 [49]. Therefore it is clear that the enzymatic activities of PRMTs are regulated in a substrate-specific fashion by PRMT-interacting partners, even though they often do not possess enzymatic activity.

CROSS-REGULATION OF HISTONE ARGININE METHYLATION WITH OTHER HISTONE MODIFICATIONS IN TRANSCRIPTIONAL REGULATION

Chromatin carries numerous histone modifications, the majority of which are involved in transcription. Arginine methylation has been closely linked to histone acetylation (Figure 5). Methylation of Arg³ by PRMT1 facilitates the subsequent acetylation of histone H4 tails by p300. This acetylation is essential for the NR-mediated transcriptional activation on the MMTV (mouse mammary tumour virus)-based reporter [12]. A mutation that crippled the HMT (histone methyltransferase) activity of PRMT1 attenuates reporter activity, suggesting that the HMT activity of PRMT1 is critical for its co-activator function [12]. In the chicken β -globin locus, PRMT1-mediated arginine methylation is also necessary for the establishment and maintenance of both histone H3 and H4 acetylation patterns, implying a link with active chromatin structures and gene activation [14]. The interplay of arginine methylation and histone acetylation appears to be important for the transcription of a p53-dependent reporter gene. Roeder and colleagues showed that, in a cell-free system with reconstituted chromatin templates, the order of recruitment and the subsequent histone modification by PRMT1, p300 and CARM1 are the keys to stimulate transcription [15]. These previous studies suggest that arginine methylation regulates transcription in concert with histone acetylation.

ROLE OF PRMTS IN TRANSCRIPTIONAL ACTIVATION AND REPRESSION

Activation of transcription mediated by the NR is a complex multi-step process that requires co-operative action among multiple co-activators [50,51]. Hormone-activated nuclear receptors bind to a specific HRE (hormone-responsive enhancer element) and recruit the p160 family of co-activators [SRC1 (steroid receptor co-activator 1), ACTR (activator for thyroid hormone and retinoid receptors) and GRIP1]. These co-activators then recruit the secondary co-activators, histone acetyltransferases p300/CBP and PCAF, and PRMT1 and CARM1. CARM1 has also been found to associate with the ATP-dependent chromatin remodelling complex SWI/SNF (Figure 6). Activation of hormone-responsive genes clearly requires the co-operation of these different histone-modifying and remodelling enzymes to modify and remodel local chromatin structures [52,53]. In contrast to H4R3 methylation by PRMT1, which stimulates histone acetylation, the acetylation of H3K18 (Lys¹⁸ of histone H3) and H3K23 tails by CBP potentiates the methylation of H3R17 tails by CARM1 (Figure 5) [15,26]. In reverse, CARM1 then methylates the arginine residue in the KIX domain of p300/CBP and blocks co-activator-mediated CREB activation [54].

PRMT 1 and CARM1 are often targeted to the same target genes by transcription factors [25]. A previous study which used siRNA (short interfering RNA)-mediated single or double knockdowns for PRMT1 and/or CARM1 revealed that a group of 46 target genes are co-operatively regulated by these two enzymes. Among them, STAT5-dependent transcription in response to IL4 (interleukin 4) signalling is specifically stimulated by the co-operation

between PRMT1 and CARM1 [17]. In this case, PRMTs play a role in connecting the IL4 signalling pathway with the function of STAT5 by directly interacting with STAT5 in a cytokine-dependent fashion and enhancing STAT5-mediated transcription.

Symmetrical or asymmetrical methylation can both occur at H4R3, but exhibit opposite effects on transcription regulation. For example, PRMT1-mediated asymmetrical modification correlates with transcriptional activation, whereas PRMT5-mediated symmetrical methylation is linked to transcriptional repression [1]. It was reported that PRMT5 stably associates with the MBD2 (methyl-DNA-binding protein 2)/NuRD complex, which contains histone deacetylases. In this case, PRMT5 is recruited to CpG islands in a DNA-methylation-dependent manner and symmetrically methylates H4R3 at the promoters, co-operatively repressing the target genes [29]. In addition, PRMT5 can interact with SWI/SNF chromatin remodelling complexes and negatively regulate the ST7 and NM23 tumour-suppressor genes (Figure 7A). These repressive effects are accompanied by histone H3R8 methylation and H3K9 deacetylation [55].

The most direct evidence of cross-talk between arginine methylation and other histone modifications is two recent back-to-back reports in which the authors showed that the methylation of H3R2 controls the deposition of di- or tri-methylation of histone H3K4 by the Set1 [su(var), enhancer of zeste trithorax domain 1]/MLL (mixed-lineage leukaemia) HMT [35,36]. PRMT6-mediated H3R2 methylation abrogates the trimethylation of H3K4 by Set1/MLL complexes and is selectively marked at inactive promoters and heterochromatin regions (Figure 7B). It is particularly interesting that the interplay of CARM1 and PRMT6 on H3R2 methylation might dictate cell fate by marking transcriptionally active or repressive chromatin landscapes respectively.

ARGININE METHYLATION IN CHROMATIN INSULATOR FUNCTION

The chicken β -globin insulator 5'-HS4 (5'-hypersensitive site 4) protects genes from position-effect silencing by establishing and maintaining a local active chromatin modification [56]. PRMT1 plays an important role in organizing the local array of histone modifications. The suppression of asymmetrical dimethylation of H4R3 on the insulator sites results in the inhibition of histone acetylation and the loss of chromatin barrier functions, suggesting that PRMT1-mediated arginine methylation is involved in the regulation of dynamic chromatin structures [14,57]. However, the precise mechanisms by which the insulator initiates the active chromatin domain and how different histone modifications, including H4R3 methylation, are cross-regulated to achieve barrier protection remain to be elucidated.

The differential binding of the zinc finger protein CTCF to a chromatin insulator in the ICR (imprinting control region) also regulates paternal *Igf2* and maternal *H19* expression respectively [58,59]. It was reported that in the male germline, CTCFL/Boris, a testis-specific paralogue of CTCF, recruits PRMT7 to the ICR of the imprinted locus. As a result, PRMT7 symmetrically methylates H4R3 at nearby nucleosomes, facilitating the recruitment of the *de novo* Dnmt3 (DNA methyltransferase 3). This methylates the ICR DNA region, eliminating the binding of CTCFL and allowing the activation of *Igf2* in the testis [4]. The

question remains, however, of whether or not symmetrical methylation of H4R3 is the cause of differential activation of imprinted *Igf2* in the testis, and whether CTCF is regulated in the same way in adult tissues.

ARGININE METHYLATION IN HUMAN CANCERS

Histone modifying enzymes that mediate epigenetic modifications have been recognized as key regulators in gene regulation. There is growing evidence that the aberrant expression and activity of these enzymes contributes to oncogenesis [60,61]. In a certain acute myeloid leukaemia which features the MLL–EEN translocation, PRMT1 is a component of the MLL–EEN oncoprotein complex, in which H4R3 dimethylation and histone acetylation are important for leukaemic transformation [62]. Cheung et al. [62] further showed that the knockdown of PRMT1 suppresses MLL-mediated transformation, suggesting a direct and convincing role of PRMT1 in MLL-mediated leukaemogenesis. Furthermore, PRMT1 is involved in myeloid differentiation by methylating the haematopoietic-specific regulator RUNX1 (Runt-related transcription factor 1)/AML1 (acute myeloid leukaemia 1), which is frequently involved in chromosomal translocations in human acute myeloid leukaemia. The methylation of RUNX1 at residues Arg²⁰⁶ and Arg²¹⁰ abrogates the interaction with the SIN3A (SWI-independent 3A) co-repressor and releases the repression of target genes. Interestingly, these arginine methylation sites are lost in the leukaemia-associated chromosomal translocation that generates the RUNX1–ETO (eight-twenty-one) fusion protein. Consequently, the absence of SIN3A-mediated transcriptional repression contributes to its leukaemic activity [63].

Previous evidence indicates that the incidence of prostate and breast cancers are associated with changes in hormones. Several studies have shown a correlation between oestrogen and breast cancer [64,65]. The fact that PRMT1 and CARM1 are critical co-activators for nuclear-receptor-mediated transcription makes them possible oncogenic targets for these specific types of cancers. This is supported by some previous studies. For example, CARM1 has been shown to be overexpressed in primary prostate cancer. The expression of CARM1 is essential for the prostate cancer cell line LNCaP growth and proliferation [66]. Similarly, the overexpression of PRMT5 has been correlated with the inhibition of two tumour-suppressor genes. In these cases, PRMT5 recruits the hSWI/SNF chromatin remodelling complex and BRG1 [BRM (brahma)-related gene 1]- and hBRM (human BRM)-associated PRMT5, then symmetrically methylates H3R8 or H4R3 to stimulate cancer cell proliferation by altering the expression of genes involved in tumour suppression [31,55].

CONCLUDING REMARKS

Increasing evidence supports the idea that epigenetic marks laid by histone-modifying enzymes regulate transcription through the recruitment of effector protein complexes. These effector proteins then further shape the chromatin structure or directly affect transcription. One issue in this field is the interplay of these histone modifications and how cross-regulation establishes a chromatin structure that changes transcriptional activity. Specifically, it is important to determine how histone arginine methylation regulates other histone modifications or chromatin remodelling complexes and further links to

transcriptional regulation. Another issue involves the inheritance of these epigenetic marks. Several questions remain about how these marks are maintained and established through cellular diversions. These questions should be a major subject of future studies.

Finally, there has been only one ‘true’ arginine demethylase identified so far. Given that PRMTs can methylate different arginine residues on histone H2A, H3 and H4 tails that are linked to distinct functions, it is conceivable that reversible arginine demethylation would play a critical role in the regulation of PRMT functions. However, there are many unanswered questions as to whether or not there are other arginine demethylases that are specific for individual dimethylarginine residues on histone tails or that specifically remove methyl groups from asymmetrical compared with symmetrical modifications. This is an intriguing question since each arginine methylation site probably performs a distinct biological function. Finally, it will be interesting to determine if separate demethylases convert monomethylarginine to arginine.

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Abbreviations used

Boris	brother of the regulator of imprinted sites
BRM	brahma
CARM1	co-activator-associated arginine methyltransferase 1
CREB	cAMP-response-element-binding protein
CBP	CREB-binding protein
CTCF	CCCTC-binding factor
CTCFL	CCCTC-binding factor-like
ES cell	embryonic stem cell
GRIP1	glucocorticoid receptor-interacting protein 1
HnKx etc	Lys ^x of histone Hn etc
HnRx etc	Arg ^x of histone Hn etc
hCAF1	CCR4 (CC chemokine receptor 4)-associated factor-1
HMG	high-mobility group
HMT	histone methyltransferase

hnRNP	heterogeneous nuclear ribonucleoprotein
ICR	imprinting control region
Igf2	insulin-like growth factor 2
IL4	interleukin 4
JMJD6	jumonji domain-containing protein 6
MLL	mixed-lineage leukaemia
MRE11	meiotic recombination protein 11
NR	nuclear hormone receptor
NuRD	nucleosome remodelling and deacetylase
PAD4	peptidyl arginine deaminase 4
PCAF	p300/CBP-associated factor
PRMT	protein arginine N-methyltransferase
RB	retinoblastoma protein
RUNX1	Runt-related transcription factor 1
Set	su(var), enhancer of zeste trithorax domain
SIN3A	SWI-independent 3A
STAT	signal transducer and activator of transcription

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Figure 1. Role of PRMTs in cellular processes

PRMTs are found in both the cytoplasm and the nucleus. PRMT1 is found in higher concentrations in the cytoplasm than in the nucleus. Only PRMT6 is found exclusively in the nucleus. Arginine methylation of protein modulates several cellular processes, including signal transduction, transcriptional regulation, DNA repair and RNA processing. The red lines indicate modifications that are inhibitory. The green lines indicate modifications that are stimulatory. Green circles labelled Me indicate a protein modified by a PRMT. ER, oestrogen receptor; 53BP1, p53-binding protein 1; 40S/rpS2, 40S ribosomal protein S2; SPT5, Suppressor of Ty 5; TAT, HIV transactivator; TF, transcription factor.

Figure 2. Arginine methylation by PRMTs

Both type I and type II enzymes produce monomethylarginine. Asymmetrical dimethylated arginine is generated by type I enzymes, and symmetrical dimethylarginine is generated by type II enzymes. AdoHey, *S*-adenosylhomocysteine; AdoMet, *S*-adenosylmethionine.

PRMTs:		Type	Histone substrate	Biological Function
PRMT1		I	H4R3	NR, chromatin dynamic, transcription activation
PRMT2		?		Coactivator for ER, Cellular proliferation
PRMT3		I		ribosomal biosynthesis
PRMT4		I	H3R2, 17, 26 (Rare)	NR, transcription activation, epigenetic reprogram in embryos
PRMT5		II	H4R3, H3R8	Stem cell function, transcription repression, repressive chromatin
PRMT6		I	H3R2	Repressive chromatin, supression of H3K4 methylation
PRMT7		II	H2A, H4R3	Potentiating DNMT3 binding, regulation of imprinting genes
PRMT8		I	H4?	?
PRMT9 Isoform 4		II	H4, H2A	?
PRMT10		?		?
PRMT11		?		?

Figure 3. Family of PRMT enzymes
There are currently 11 members of the PRMT family that share conserved catalytic domains (black). PRMT9 has four isoforms. PRMT7 and PRMT10 harbour two catalytic domains. The histone substrate of each enzyme, the type of modification that it catalyses, and the biological functions of modification are shown. DNMT3, DNA methyltransferase 3; ER, oestrogen receptor.

Figure 4. Histone arginine demethylation

(A) PAD4 can convert methylated or unmethylated arginine to citrulline. The reaction then blocks arginine methylation from occurring on histone tails. (B) JMJD6 is an Fe(II)- and 2-oxoglutarate (α -KG)-dependent dioxygenase which removes methyl groups from symmetrical dimethyl H4R3 (SDMA) or asymmetrical dimethyl H4R3 (ADMA) to generate monomethylarginine (H4R3me1).

Figure 5. Cross-regulation between arginine methylation and histone lysine acetylation on histone H3 and H4 tails

Asymmetrical or symmetrical dimethylation of H4R3 can either potentiate or inhibit H4 tail acetylation (Ac) respectively. Methylation of H3R2 by PRMT6 negatively controls the deposition of H3K4 trimethylation. In contrast, H3K18 and H3K23 acetylation stimulates methylation of H3R17 by CARM1. aMe, asymmetrical methylation; sME, symmetrical methylation.

Figure 6. Transcriptional activation of hormone-response genes via arginine methylation

The picture illustrates a simplified model of the transcriptional activation of a hormone-response gene. PRMT1 and CARM1 are important regulators of the hormone-response genes. One of the first steps in transcriptional activation is asymmetrical dimethylation of H4R3 by PRMT1. This is followed by acetylation of H4K8, H4K12 and H4K16 by HATs (histone acetyltransferases), as well as acetylation of H3K18 and H3K23 by CBP/p300. Acetylation of H3K18 and H3K23 is preceded by asymmetrical dimethylation of H3R17 by CARM1. This results in an increase in the number of transcripts. Subsequent recruitment of SWI/SNF complex further increases the specificity/activity of CARM1 on nucleosomal substrates and remodels chromatin to facilitate the formation of transcription complexes. CARM1 also methylates the KIX domain of CBP. This methylation acts to inhibit the activity of the HAT complex and to initiate the process of transcriptional deactivation [26]. HRE, hormone-responsive enhancer element; Me, methylation; NR, nuclear hormone receptor; POLII, RNA polymerase II; TBP, TATA-box-binding protein; TFIIB, transcription factor IIB.

Figure 7. Transcriptional repression by PRMT5 and PRMT6

(A) The picture depicts a model of the transcriptional repression of Hox genes and Myc-target genes, cad and nuc, by PRMT5 [67]. PRMT5 is associated with the hSWI/SNF complex. PRMT5 catalyses the symmetrical dimethylation of H4R3 and H3R8 (denoted by red circles labelled Me R3 and Me R8). Symmetrical dimethylation of H3R8 is associated with deacetylation of histones H3 and H4 (denoted by the grey circle labelled K9). (B) The picture depicts a model of the transcriptional repression of Hox genes and Myc-dependent genes by PRMT6 [35,68]. Asymmetrical dimethylation of H3R2 (denoted by the red circle labelled Me R2) is mutually exclusive of the di- and tri-methylation of H3K4 (denoted by the green circle labelled Me K4). The presence of asymmetrical dimethylation of H3R2 inhibited binding of the Ash2 (absent, small, or homeotic disc 2)/WDR5 (WD40 repeat-containing protein 5)/MLL-family methyltransferase complex and methylation of H3K4. Brg1, BRM-related gene 1; mSin3A; mammalian SIN3A; HDAC2, histone deacetylase 2; RpBP5, retinoblastoma binding protein 5.