Amino Acids (2005) 28: 357–362
DOI 10.1007/s00726-005-0197-6

Amino Acids
Printed in Austria

S-Adenosylmethionine and protein methylation

Review Article

M. A. Grillo and **S. Colombatto**

Dipartimento di Medicina e Oncologia Sperimentale, Sezione di Biochimica, Università di Torino, Torino, Italy

Received December 12, 2004
Accepted February 2, 2005
Published online April 21, 2005;

Summary. The enzymes responsible for protein methylation by S-adenosylmethionine, both at the carboxyl groups and at the nitrogen groups, are reviewed. The possibility that the reactions involved may be reversible is also considered.

Keywords: Protein methyltransferases – Isoaspartyl residues – Protein phosphatase 2A – Histones – Isoprenylcysteine

Introduction

S-adenosylmethionine (SAM) is one of the most commonly used and very versatile enzyme substrates. Its sulfonium group enables it to be employed as both a methyl donor (and it is recognized as the major methyl donor in all living organisms) and a monoalkyl donor in the synthesis of polyamines, and in the synthesis of ethylene in plants. It is also involved in the formation of 5′-deoxyadenosyl radicals, these being part of a family of iron-sulfur enzymes that utilize SAM to initiate the radical catalysis involved, for example, in the synthesis of lipoate and biotin.

As a methyl donor, SAM is used in many reactions to transfer the methyl group to the nitrogen or the oxygen of many different compounds. When proteins are methylated, too, methyltransferases transfer the methyl group to basic or acid amino acids, in this case forming an ester group. In either case, the function of the protein may be changed and the process is implicated, for instance, in signal transduction (Stock et al., 1992), regulation of transcription (Chen et al., 1999), heterogeneous nuclear ribonucleoprotein export (Shen et al., 1998) and possibly splicing (Friesen et al., 2001).

The protein methyltransferases currently known will now be described. Consideration will be mainly to those of animal origin.

Protein nitrogen groups methylation

Protein arginine methylation

Methylation of protein arginine has attracted particular attention because methylarginines are inhibitors of nitric oxide synthase (NOS), and therefore thought to participate in the regulation of the NO's many effects. Moreover, arginine methylation promotes specific protein–protein interactions (Friesen et al., 2001). In some cases, involvement in such interactions is due to inhibition of the binding of proline-rich ligands (Bedford et al., 2000). It has also been shown that DAL-1/4.1B, a tumor suppressor differentially expressed in adenocarcinoma of the lung, interacts with one of the arginine methyltransferases and inhibits its activity both *in vitro* and *in vivo* (Singh et al., 2004).

Distinct Protein Arginine (R) Methyl Transferases (PRMT) activities have been found in mammalian cells and divided into four types: type I activity gives rise to monomethylarginine (NMA) and asymmetric dimethylarginine (ADMA); type II to NMA and symmetric dimethylarginine; type III enzymes catalyze only the formation of NMA; type IV enzymes form δ-N^8-monomethylarginine. PRMT 1, 3 and 4 are responsible for type I methylation, while PRMT5 catalyzes type II methylation.

A novel gene coding for a sixth human PRMT enzyme has been identified and called PRMT 6. It resides in the nucleus, whereas PRMT3 and 5 are located in the cytoplasm. It has a type I activity, can methylate itself and has different substrate specificity (Frankel et al., 2002). A PRMT 7 has also been described very recently. This, according to Miranda et al. (2004), is a type III enzyme, whereas according to Lee et al. (2005b) it synthesizes symmetric dimethylarginine and is thus of type II. These workers suggest that differences in the conditions of the assay are responsible for these results.

PRMT 5 seems to have a role in cell cycle regulation.

Arginines which can be methylated have been shown in histones. Histone H3, for example, is methylated *in vitro* at Arg 2, 17 and 26 (Schurter et al., 2001) by PRMT4, now also called coactivator-associated arginine methyltransferase (CARM1), though it is not known which residues are methylated *in vivo*. PRMT4 is widely expressed in adult mouse tissues, whereas PRMT1 preferentially methylates histone H4 and thus facilitates its subsequent acetylation (Wang et al., 2001).

Methylarginines cannot be retransformed to arginine. However, protein-arginine-iminohydrolase (PAD), a Ca^{++} dependent enzyme that catalyzes the conversion of protein arginine residues to citrulline residues in a post-translational modification (called citrullination), has been detected in several tissues. One form of this enzyme (PAD2) is widely distributed and particularly abundant in the muscles and brain (Moscarello et al., 2002). PAD4 is abundant in granulocytes (Arita et al., 2004; Hagiwara et al., 2002). It also converts histone methylarginine to histone citrulline and releases methylamine (Wang et al., 2004). It is thought that this reaction participates in gene expression.

When methylated proteins are degraded by proteases, methylarginines are formed. ADMA and NMA are potent endogenous inhibitors of NOS. Free methyl arginines are found in differing concentrations in the cell cytosol, plasma and tissues. Both ADMA and the symmetric isomer are eliminated by renal excretion, whereas ADMA alone is degraded to citrulline and methylamines by dimethylarginine dimethylaminohydrolase (DDAH), a zinc containing protein (Bogumil et al., 1998). Two DDAH isoforms with distinct tissue distribution and some relationship to NOS isoforms have been identified: DDAH I is found in tissues that express nNOS, whereas high levels of DDAH II are found in tissues with eNOS (Leiper et al., 1999). These results support the view that methyl arginine concentration is actively regulated. According to Tsikas et al. (2000) NOS inhibition by endogenous methylarginines, with ADMA as the most potent, is responsible for the so-called arginine paradox, i.e. the observation that excess arginine has no effect on NOS *in vitro*, but enhances its activity *in vivo* by competition with the inhibitor. Plasma ADMA levels are high in many disorders characterized by endothelial dysfunction (McCarty, 2004). According to Böger (2004), ADMA is a cardiovascular risk factor.

Protein lysine methylation

Lysine methylation mainly occurs on histone H3 and H4 and to a lesser extent on histone H1. Six lysines within histone H3 (4, 9, 14, 27, 36, 79) and two within histone H4 (20 and 59) are methylated by a specific histone methyltransferase (Lee et al., 2005a). Each residue can accept up to three methyl groups, resulting in mono-, di-, and trimethylated Lys. Moreover, Lys 9 in H3 can be both acetylated or methylated.

Initially only CARM1 appeared to display a stringent specificity for histone (Chen et al., 1999). Another enzyme has since been identified (Rea et al., 2000) as the mammalian homolog of a gene product encoded by the suppressor gene *Su(var)3-9* of Drosophila and hence called SUV39H1. The SUV39HMTases are characterized by the presence of one invariant protein motif, namely the SET domain. The approximately 130-residue SET module is generally flanked by distinct pre-SET and post-SET domains at its N and C termini. The C-flanking domain is needed to generate the access channel that connects the cofactor-binding site on one surface of the domain with the substrate-binding site on the opposite surface. This access channel regulates whether the enzyme produces mono-, di- or tri-methylated lysine (Xiao et al., 2003). Dimethylated and trimethylated lysines on Lys9 or Lys4 of histone H3 have distinct roles (Tamaru et al., 2003; Santos-Rosa et al., 2002; Lee et al., 2005a).

Other enzymes, including G9a (Tachibana et al., 2002) and Set9 (Nishioka et al., 2002) have been shown later to methylate lysine in histones. Set9 contains a SET domain, but lacks the pre- and post-SET domains. Chuikov et al. (2004) have demonstrated that Set9 can methylate the tumor suppressor p53 *in vivo*, a process that seems necessary for its stabilization. The authors suggest that Set9 may also regulate the function of other factors. Another enzyme, SMYD3, has been postulated as a key factor in human carcinogenesis (Hamamoto et al., 2004).

We know now that lysine methylation regulates specific gene expression and organization of the chromosomal regions. However, the position of the methylated lysine within H3 and H4 marks a gene to be activated or repressed.

Methylation of histone, once regarded as irreversible, is now seen as less permanent, though proteins responsible for its demethylation have not been identified.

The function of lysine methylation in histones has been examined in recent reviews (Sims et al., 2003; Lachner et al., 2002; Lee et al., 2004).

Protein carboxyl methylation

In bacteria, methylation and demethylation of protein glutamate regulate the output of certain chemoreceptors (Stock et al., 1992). Methylation of a C-terminal leucine, isoprenylated C-terminal cysteine residues and abnormal aspartyl residues on age-damaged proteins have been demonstrated in eukaryotic cells.

Leucine carboxyl methylation

A specific methyltransferase has been shown to methylate the mammalian protein phosphatase 2A (PP2A) at the C-terminal carboxyl group, i.e. at leucine, this being the C-terminus of the 36 kD cytosolic polypeptide Thr^{304}-Pro-Asp-Tyr-Phe-Leu^{309} (Lee et al., 1993). Since PP2A is involved in both carbohydrate, amino acid and lipid metabolism and cell cycle control, its methylation has attracted attention as a possible regulator of metabolism (Xie et al., 1994). PP2A is a heterodimeric or heterotrimeric assembly containing A, B, or C subunits. Methylation occurs on the catalytic C subunit. The A subunit binds the C subunit to form the dimer AC; one isoform of the regulatory B subunit binds the AC dimer and modulates substrate specificity, enzyme activity and/or cellular localization. Methylation of PP2A, influences the affinity of the AC dimer for the different B subunits (Tolstykh et al., 2000), some of which appear to bind more efficiently to an AC dimer when the catalytic C subunit has been methylated, whereas others are not influenced by methylation (Yu et al., 2001).

However, while it is known that phosphorylation of $Tyrosine^{204}$ promotes inactivation of the enzyme, conflicting data exist on the effect of methylation of $Leucine^{309}$ on the catalytic activity. It has thus been suggested that methylation of PP2A mainly affects other characteristics of the enzyme.

Studies on the structure of the corresponding yeast enzyme enabled Leulliot et al. (2004) to propose a catalytic mechanism for this methylation, in which an arginine stabilizes the conformation of SAM and helps to orient the incoming carboxylate of the peptide substrate for nucleophilic attack on the sulphur atom.

The methyl group is removed by a specific PP2A-methylesterase. The enzyme purified from bovine brain is a 46 kD monomer (Lee et al., 1996).

PP2A expression decreases with age in the mouse brain (Jiang et al., 2001) and significantly in hippocampus of Alzheimer patients (Vogelsberg-Ragaglia et al., 2001). This is thought to lead to hyperphosphorylation of tau protein. As PP2A heterotrimer formation is necessary for efficient dephosphorylation of this protein, Vafai et al. (2002) suggested that inhibition of methylation by homocysteine participates in the decrease in PP2A activity, since homocysteine is increased in the plasma of Alzheimer patients.

Isoprenylated C-terminal carboxyl methylation

Another carboxyl methyltransferase acts on proteins formed by their precursors synthesized with a C-terminal CAAX tetrapeptide motif (C, cysteine, A generally an aliphatic residue, X a variable residue) after receiving a 15-carbon farnesyl or a 20-carbon geranylgeranyl group on the sulfur atom of cysteine and removing the three AAX amino acids. When the X is Ser, Met, Cys, Ala, or Gln, the CAAX box is modified by a farnesyl isoprenoid, or by a geranylgeranyl group when X is leucine. The C-terminal isoprenylcysteine residue is esterified with SAM by a membrane-bound enzyme of the endoplasmic reticulum (Dai et al., 1998). It appears that prenylation is important for attachment of proteins to membranes, while methylation of the carboxyl terminal of the prenylated cysteine enhances lipid bilayer association of farnesylated peptides.

Several proteins are modified in this way, such as cGMP phosphodiesterase and several small G-proteins involved in cell signaling, such as the Ras and the Rho proteins (Bergo et al., 2004). Processing of these CAAX proteins has attracted interest due to the role of activated Ras proteins in the development of cancer. The enzymes responsible for the posttranslational modifications of CAAX proteins have therefore been considered as potential targets in blocking the growth of Ras-induced malignancies. Ras methylation is decreased by antifolate methotrexate and its signaling functions are impaired (Winter-Vann et al., 2003). Methotrexate may thus additionally decrease methyltransferase activity. Other recent results by Bergo et al. (2004) also support the suggestion that this methyltransferase could be a therapeutic target for treating cancer.

Other proteins involved are some nuclear lamins. In the case of prelamin A, a farnesylated nuclear lamin A precursor, there is an additional upstream proteolysis after

the CAAX box modification (Beck et al., 1990). This modification removes 18 amino acid residues at C terminal and is required for lamin A assembly in the nuclear lamina (Lutz et al., 1992). A genetic disease is known with deletion of this protease target, resulting in an aberrant lamin A molecule (Eriksson et al., 2003). According to Corrigan et al. (2004), the two proteolytic cleavages are effected by the same enzyme, namely Zmpste24.

Isoaspartylmethylation

Another carboxymethyltransferase is involved in the repair of age-damaged proteins. Spontaneous alterations of proteins occur mainly at their asparagine and aspartyl residues. Nucleophilic attack of the peptide-bond nitrogen atom of the following residue results in the formation of a succinimide ring, an unstable five-member ring. This reaction is the rate-limiting step in the transformation of these residues. Aspartyl or isoaspartyl residues are formed by spontaneous hydrolysis (Fig. 1) and D-succinimidyl, D-aspartyl- and D-isoaspartyl-forms by racemization of succinimide. The L-isoaspartyl-form is generally predominant (Clarke, 2003).

These new amino acids are recognized by a specific enzyme, L-isoaspartyl methyltransferase, which initiates their conversion to the normal aspartyl form by methylation with SAM, followed by spontaneous demethylation. Succinimide is re-formed and this is followed, at least in part, by hydrolysis to form L-aspartate. Moreover, methylation sometimes results in partial recovery of protein function, though the efficacy of its repairs differs from one protein to another and depends on its structure (Athmer et al., 2002) or other factors: for instance, the enzyme is particularly sensitive to inhibition by S-adenosylhomocysteine. Therefore all the conditions which give rise to an increase of this compound, such as homocystinuria, may be accompanied by dysfunction of the repair system.

Other carboxylmethyltransferases

Carboxyl methylation of the β-subunit of the epithelial Na^+ channels has also been claimed to regulate Na^+

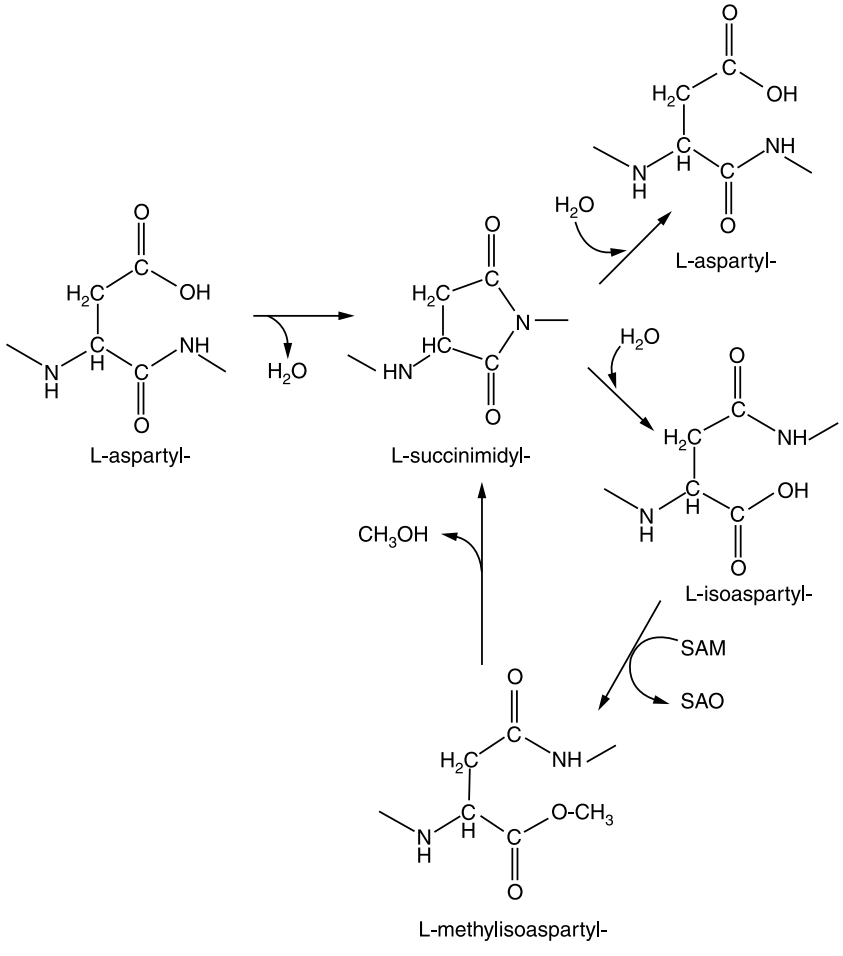

Fig. 1. Spontaneous degradation of aspartyl residues in proteins and methyltransferase mediated repair

channel activity in A6 cells, though the carboxyl involved is not known (Rokaw et al., 1998).

Structure of the methyltransferases

Enzymes which use SAM as methyl donor are one of the large structural families. During evolution, they have been shaped into distinct structures with a common catalytic role. The study of such structures has led to the demonstration of the existence of five classes: in Class I there are many enzymes with similar structure, comprising a seven-stranded beta sheet, flanked by helices. This Class comprises protein arginine methyltransferase, though here strands 6 and 7 are absent, and isoaspartylmethyltransferase, where these two strands are reversed (Schubert et al., 2003). Other methyltransferases have different structures (Type II, III, IV). Now another type has been shown, Class V. This is observed in the histone-lysine methyltransferase containing the SET domain. Here there are three small β-sheets.

Conclusions

Methylation would seem to have a role in protein function regulation along with phosphorylation, acetylation and ubiquitination. This is demonstrated for methylation of the carboxyl groups, as this reaction can be easily reversed by a methylesterase. For the amino group, it is less so, as the reaction is not easily reversible. However, in some cases the amino acid methylated is degraded and in other cases it has been suggested that the protein may be somehow recovered. More studies are needed to see whether this occurs, and how.

References

Arita K, Hashimoto H, Shimizu T, Nakashima K, Yamada M, Sato M (2004) Structural basis for Ca^{2+}-induced activation of human PAD4. Nat Struct Mol Biol 11: 777–783

Athmer L, Kindrachuk J, Georges F, Napper S (2002) The influence of protein structure on the products emerging from succinimide hydrolysis. J Biol Chem 277: 30502–30507

Beck LA, Hosick TJ, Sinensky M (1990) Isoprenylation is required for the processing of the lamin A precursor. J Cell Biol 110: 1489–1499

Bedford MT, Frankel A, Yaffe MB, Clarke S, Leder P, Richard S (2000) Arginine methylation inhibits the binding of proline-rich ligands to Src homology 3, but not WW, domains. J Biol Chem 275: 16030–16036

Bergo MO, Gavino BJ, Hong C, Beibneux AP, McMahon M, Casey PJ, Young SG (2004) Inactivation of *Icmt* inhibits transformation by oncogenic K-Ras and R-Raf. J Clin Invest 113: 539–550

Böger RH (2004) Asymmetric dimethylarginine, an endogenous inhibitor of nitric oxide synthase, explains the ''L-arginine paradox'' and acts as a novel cardiovascular risk factor. J Nutr 134: 2842S–2847S

Bogumil R, Knipp M, Fundel SM, Vasak M (1998) Characterization of dimethylargininase from bovine brain: evidence for a zinc binding site. Biochemistry 37: 4791–4798

Chen D, Ma H, Hong H, Koh SS, Huang S-M, Schurter BT, Aswad DW, Stallcup MR (1999) Regulation of transcription by a protein methyltransferase. Science 284: 2174–2177

Chuikov S, Kurash JK, Wilson JR, Xiao B, Justin N, Ivanov GS, McKinney K, Tempst P, Prives C, Gamblin SJ, Barlev NA, Reinberg D (2004) Regulation of p53 activity through lysine methylation. Nature 432: 353–360

Clarke S (2003) Aging as war between chemical and biochemical processes: protein methylation and the recognition of age-damaged proteins for repair. Ageing Res Rev 2: 263–285

Corrigan DP, Kuszczak D, Rusinol AE, Thewke DP, Hrycyna CA, Michaelis S, Sinensky MS (2004) Prelamin A endoproteolytic processing *in vitro* by recombinant Zmpste24. Biochem J (in press)

Dai Q, Choy E, Chiu V, Romano J, Slivka SR, Steitz SA, Michaelis S, Philips MR (1998) Mammalian prenylcysteine carboxyl methyltransferase is in the endoplasmic reticulum. J Biol Chem 273: 15030–15034

Eriksson M, Brown WT, Gordon LB, Glynn MW, Singer J, Scott L, Erdos MR, Robbins CM, Moses TY, Berglund P, Dutra A, Pak E, Durkin S, Csoka AB, Boehnke M, Glower TW, Collins FS (2003) Recurrent de novo point mutations in lamin A cause Hutchinson-Gilford progeri syndrome. Nature 423: 293–298

Frankel A, Yadav N, Lee J, Branscombe TL, Clarke S, Bedford MT (2002) The novel human protein arginine N-methyltransferase PRMT6 is a nuclear enzyme displaying unique substrate specificity. J Biol Chem 277: 3537–3543

Friesen WJ, Massenet S, Paushkin S, Wyce A, Dreyfuss G (2001) SMN, the product of the spinal muscular atrophy gene, binds preferentially to dimethylarginine-containing protein targets. Mol Cell 7: 1111–1117

Hagiwara T, Nakashima K, Hirano H, Senshu T, Yamada M (2002) Deimination of arginine residues in nucleophosmin/B23 and histone in HL-60 granulocytes. Biochem Biophys Res Commun 290: 979–983

Hamamoto R, Furukawa Y, Morita M, Iimura Y, Pittella Silva F, Li M, Yagyu R, Nakamura Y (2004) *SMYD3* encodes a histone methyltransferase involved in the proliferation of cancer cells. Nat Cell Biol 6: 731–740

Jiang CH, Tsien JZ, Schultz PG, Hu Y (2001) The effect of aging on gene expression in hypothalamus and cortex of mice. Proc Natl Acad Sci 98: 1930–1934

Lachner M, Jenuwein T (2002) The many faces of histone lysine methylation. Curr Opin Cell Biol 14: 286–298

Lee J, Stock J (1993) Protein phosphatase 2A catalytic subunit is methylesterified at its carboxyl terminus by a novel methyltransferase. J Biol Chem 268: 19192–19195

Lee J, Chen Y, Tolstykh T, Stock J (1996) A specific carboxyl methylesterase that demethylatesphosphoprotein phosphatase 2A in bovine brain. Proc Natl Acad Sci 93: 6043–6047

Lee DY, Teyssier C, Strahl BD, Stallcup MR (2005a) Role of protein methylation in regulation of transcription. Endocr Rev 26: 147–170

Lee JH, Cook JR, Yang ZH, Mirochnitchenko O, Gunderson S, Felix AM, Herth N, Hoffmann R, Pestka S (2005b) PRMT7: a new protein arginine methyltransferase that synthesizes symmetric dimethylarginine. J Biol Chem 280: 3656–3664

Leuillot N, Quevillon-Chereul S, Sorel I, de La Sierra-Gallay, Collinet B, Graille M, Blondeau K, Bettache N, Poupon A, Janin J, van Tilbeurgh H (2004) Structure of protein phosphatase methyltransferase 1 (PPM1), a leucine carboxyl methyltransferase involved in the regulation of protein phosphatase 2A activity. J Biol Chem 279: 8351–8358

Leiper JM, Santa Maria J, Chubb A, MacAllister RJ, Charles IG, Whitley GS, Vallance P (1999) Identification of two human dimethylarginine dimethylaminohydrolases with distinct tissue distributions

and homology with microbial arginine deiminase. Biochem J 343: 209–214

Lutz RJ, Trujillo MA, Denham KS, Wenger L, Sinensky M (1992) Nucleoplasmic localization of prelamin A: implications for prenylation-dependent lamin A assembly into the nuclear lamina. Proc Natl Acad Sci 89: 3000–3004

McCarty MF (2004) Vascular endothelium is the organ chiefly responsible for the catabolism of plasma asymmetric dimethylarginine – an explanation for the elevation of plasma ADMA in disorders characterized by endothelial dysfunction. Med Hypotheses 63: 699–708

Miranda TB, Miranda M, Frankel A, Clarke S (2004) PRMT7 is a member of the protein arginine methyltransferase family with a distinct substrate specificity. J Biol Chem 279: 22902–22907

Moscarello MA, Pritker L, Mastronardi FG, Wood DD (2002) Peptidylarginine deiminase: a candidate factor in demyelinating disease. J Neurochem 81: 335–343

Nishioka K, Chuikov S, Sarma K, Erdiument-Bromage H, Allis CD, Temest P, Reinberg D (2002) Ser9, a novel histone H3 methyltransferase that facilitates transcription by precluding histone tail modifications required for heterochromatin formation. Genes Dev 16: 479–489

Rea S, Eisenhaber F, O'Carroll D, Strahl BD, Sun Z-W, Schmid M, Opravil S, Mechtler K, Ponting CP, Allis CD, Jenuwein T (2000) Regulation of chromatin structure by site-specific histone H3 methyltransferases. Nature 406: 593–599

Rokaw MD, Wang J-M, Edinger RS, Weisz OA, Hui D, Middleton P, Shlyonsky V, Berdiev BK, Ismailov I, Eaton DC, Benos DJ, Johnson JP (1998) Carboxylmethylation of the ß subunit of xENaC regulates channel activity. J Biol Chem 273: 28746–28751

Santos-Rosa H, Schneider R, Bannister AJ, Sherriff J, Bernstein BE, Emre NC,Schreiber SL, Mellor J, Kouzarides T (2002) Active genes are trimethylated at K4 of histone H3. Nature 419: 407–411

Schubert H, Blumenthal RM, Cheng X (2003) Many paths to methyltransfer: a chronicle of convergence. Trends Biochem Sci 28: 329–335

Schurter BT, Koh SS, Chen D, Bunick GJ, Harp JM, Hanson BL, Henschen-Edman A, Mackai DR, Stallcup MR, Aswad DW (2001) Methylation of histone H3 by coactivator-associated arginine methyltransferase 1. Biochemistry 40: 5747–5756

Shen EC, Henry MF, Weiss VH, Valentini SR, Silver PA, Lee MS (1998) Arginine methylation facilitates the nuclear export of hnRNP proteins. Genes Dev 12: 679–691

Sims RJ 3rd, Nishioka K, Reinberg D (2003) Histone lysine methylation: a signature for chromatin function. Trend Gen 19: 629–639

Singh V, Branscombe Miranda T, Jiang W, Frankel A, Roemer ME, Robb VA, Gutmann DH, Herschman HR, Clarke S, Newsham IF (2004) 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 23: 7761–7771

Stock JB, Surette MG, McCleary WR, Stock AM (1992) Signal transduction in bacterial chemotaxis. J Biol Chem 267: 19753–19756

Tachibana M, Sugimoto K, Nozaki M, Ueda J, Ohta T, Ohki M, Fukuda M, Takeda N, Niida H, Kato H, Shinkai Y (2002) G9a histone methyltransferase plays a dominant role in euchromatic histone H3 lysine 9 methylation and is essential for early embryogenesis. Genes Dev 16: 1779–1791

Tamaru H, Zhang X, McMillen D, Singh PB, Nakayama J, Grewal SI, Allis CD, Cheng X, Selker EU (2003) Trimethylated lysine 9 of histone H3 is a mark for DNA methylation in Neurospora crassa. Nat Genet 34: 75–79

Tolstykh T, Lee J, Vafai S, Stock JB (2000) Carboxyl methylation regulates phosphoprotein phosphatase 2A by controlling the association of regulatory B subunit. EMBO J 19: 5682–5691

Tsikas D, Böger RH, Sandmann J, Bode-Böger SM, Frölich JC (2000) Endogenous nitric oxide synthase inhibitors are responsible for the Larginine paradox. FEBS Lett 478: 1–3

Vafai SB, Stock JB (2002) Protein phosphatase 2A methylation: a link between elevated plasma homocysteine and Alzheimer's disease. FEBS Lett 518: 1–4

Vogelsberg-Ragaglia V, Schuck T, Trojanowski JQ, Lee VM (2001) PP2A mRNA expression is quantitatively decreased in Alzheimer's disease hippocampus. Exp Neurol 168: 402–412

Wang H, Huang Z-Q, Xia L, Feng Q, Erdjument-Bromage H, Strahl BD, Briggs SD, Allis CD, Wong J, Tempst P, Zhang Y (2001) Methylation of histone H4 at arginine 3 facilitating transcriptional activation by nuclear hormone receptor. Science 293: 853–857

Wang Y, Wysocka J, Sayegh J, Lee Y-H, Perlin JR, Leonelli L, Sonbuchner LS, McDonald CH, Cook RG, Dou Y, Roeder RG, Clarke S, Stallcup MR, Allis CD, Coonrod SA (2004) Human PAD4 regulates histone arginine methylation levels via demethylimination. Science 306: 279–283

Winter-Vann AM, Kamen BA, Bergo MO, Young SG, Melnyk S, James SJ, Casey PJ (2003) Targeting Ras signalling through inhibition of carboxyl methylation: an unexpected property of methotrexate. Proc Natl Acad Sci 100: 6529–6534

Xiao B, Wilson JR, Gamblin SJ (2003) SET domains and histone methylation. Curr Opin Struct Biol 13: 699–705

Xie H, Clarke S (1994) Protein phosphatase 2A is reversibly modified by methyl esterification at its C-terminal leucine residue in bovine brain. J Biol Chem 269: 1981–1984

Yu XX, Du X, Moreno CS, Green RE, Ogris E, Feng Q, Chou L, McQuoid MJ, Pallas DC (2001) Methylation of the protein phosphatase 2A catalytic subunit is essential for association of Ba regulatory subunit but not SG2NA, striatin, or polyomavirus middle tumor antigen. Mol Biol Cell 12: 185–199

Authors' address: Maria A. Grillo, Dipartimento di Medicina e Oncologia Sperimentale, Sezione di Biochimica, Università di Torino, Via Michelangelo, 27, 10126 Torino, Italy,
E-mail: mariaangelica.grillo@unito.it