ISSN 0006-2979, Biochemistry (Moscow), 2010, Vol. 75, No. 5, pp. 535-548. © Pleiades Publishing, Ltd., 2010.
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REVIEW

Lysine Methylation of Nonhistone Proteins Is a Way to Regulate Their Stability and Function

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Received July 15, 2009

Abstract—This review is devoted to the dramatically expanding investigations of lysine methylation on nonhistone proteins and its functional importance. Posttranslational covalent modifications of proteins provide living organisms with ability to rapidly change protein activity and function in response to various stimuli. Enzymatic protein methylation at different lysine residues was evaluated in histones as a part of the "histone code". Histone methyltransferases methylate not only histones, but also many nuclear and cytoplasmic proteins. Recent data show that the regulatory role of lysine methylation on proteins is not restricted to the "histone code". This modification modulates activation, stabilization, and degradation of nonhistone proteins, thus influencing numerous cell processes. In this review we particularly focused on methylation of transcription factors and other nuclear nonhistone proteins. The methylated lysine residues serve as markers attracting nuclear "reader" proteins that possess different chromatin-modifying activities.

DOI: 10.1134/S0006297910050019

Key words: lysine, methylation of nonhistone proteins, p53, posttranslational modification, Stellate

Fifty years ago, Ambler and Rees discovered an ε-lysine methylation on *Salmonella typhimurium* flagellar protein [1]. Several years later methylated lysine residues were revealed in histones [2, 3]. These early findings predetermined success in understanding epigenetic mechanisms of histone methylation achieved much later, to the beginning of XXI century, when Strahl, Allis, and Jenuwein proposed a "histone code" concept that assumed that various modifications of histone "tails" protuberating from nucleosomes influenced each other enhancing or inhibiting interactions, as well as recruiting various protein complexes altering the properties of chromatin sites [4, 5]. Histones can be methylated at lysine (K) and arginine (R) residues. Eight main lysine methylation sites are known in histones (H3K4, H3K9, H3K14, H3K27, H3K36, H3K79, H4K20, and H4K59) [6], in which the same residue can be mono-, di-, or trimethylated. Di- and trimethylation of histone H3K4 (H3K4me2 and H3K4me3) is revealed in genomic transcriptionally active euchromatin sites, whereas di- and trimethylation of K9 (H3K9me2 and H3K9me3) is associated with dense heterochromatin packing [7, 8]. Covalent posttranslational protein modifications, such as lysine methylation, provide living things with the ability to rapidly change activity and function of proteins in response to various stimuli. Histone methylation and its consequences are regulated by a complex protein network including specific histone methyltransferases (HMT), demethylases, and "reader" proteins interacting with methyl markers. SUV39H1 was the first discovered HMT [9] containing the earlier described SET-domain (suppressor of variegation, enhancer-of-zeste and trithorax) responsible for methylation [10]. "Histone code" is realized in the control of expression of genetic information via remodeling chromatin structure and altering transcription level [6, 11]. Unlike other modifications, such as phosphorylation, lysine methylation was previously considered as a very stable marker until the discovery of histone demethylases [12, 13].

However, methylation of lysines in proteins occurs not only in histones. The modification is revealed in nonhistone nuclear and cytoplasmic proteins, and the number of such proteins keeps growing. Some HMTs methylate not only histones but also nonhistone proteins [14, 15]. Regulatory functions of protein methylation are not

Abbreviations: AML1, acute myeloid leukemia 1 protein; CaM, calmodulin; ERα, estrogen receptor α; ESC, embryonic stem cell; HMT, histone methyltransferase; PCAF, p300/CBP-associated factor; PTM, posttranslational modification; RARα, retinoic acid receptor α; TAF, TBP-associated factor.

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Table 1. Lysine methylation on transcription factors and other nuclear proteins

Substrate	Methylation site	Methyl-transferase	Function	Species	Reference
1	2	3	4	5	6
p53	K370me1	Smyd2	suppression of target gene transcription	*Homo sapiens*	[23]
	K370me2	unknown	transcription activation, interaction with 53BP1; regulated by demethylation executed by LSD1	–"–	[20, 29]
	K372me1	SET7/9	transcription activation, interaction with acetyltransferase Tip60, enhancement of p53 acetylation, stabilization	–"–	[18, 22, 24]
	K382me1	SET8/ PR-SET7	suppression of target gene transcription	–"–	[19]
	K382me2	unknown	stabilization, interaction with 53BP1	–"–	[20]
	K386me1, K386me2	–"–	unknown	–"–	[20]
TAF10	K189me1	SET7/9	increase in affinity to RNA-polymerase II, transcription activation	–"–	[32]
TAF7	K5me1	SET7/9	unknown	–"–	[33]
ERα	K302me1	SET7/9	accumulation in the nucleus, stabilization, transcription activation of ER-dependent genes	–"–	[34]
RARα	K109me1	G9a	facilitates RARα activation by retinoic acid, improves heterodimerization of RXR-RAR receptors	*Mus musculus*	[35, 36]
	K171me1	unknown	unknown	–"–	[35]
	K347me3	–"–	facilitates RARα activation by retinoic acid, improves heterodimerization of RXR-RAR receptors, facilitates binding to PCAF	–"–	[35]
NF-κB (RelA)	K314me1, K315me1	SET7/9	negative control of target gene transcription, acceleration of proteolytic degradation	*H. sapiens*	[39]
RIP140	K591me1, K653me1, K757me1	unknown	activation, improvement of interaction with HDAC3	mammalian cell cultures	[42]
G9a	K114me2 (K167me2)	G9a	unknown	*H. sapiens* (*M. musculus*)	[48, 50]
	K185me3 (K239me3)	G9a	interactions with HP1 and CDYL1	–"–	[48-50]
Dnmt1	K70me2	G9a	unknown	*H. sapiens*	[49]
	K142me1	SET7/9	destabilization, acceleration of proteolytic degradation	–"–	[53]

Table 1. (Contd.)

1	2	3	4	5	6
	K1096me1	SET7/9	destabilization; regulated by demethylation executed by LSD1	*M. musculus*	[54]
PCAF	K89me1	SET7/9	unknown	*H. sapiens*	[56]
Dam1	K233me2	SET1	inhibition of phosphorylation of neighboring serines, regulation of kinetochore assembly	*Saccharomyces cerevisiae*	[57]
AML1	not determined	unknown	unknown	*H. sapiens*	[61]
Stellate	K92me3	–"–	unknown; promotion of crystal assembly?	*Drosophila melanogaster*	[62]
Tat	K50, K51	SETDB1	Tat inactivation, suppression of transcription from viral promotor	HIV-1	[71]

restricted to the "histone code", but are implicated in a number of other cellular processes.

This review aims to describe the rapidly growing field of studies on nonhistone protein methylation on lysine residues and analyze its functional importance. Attention is focused on methylation of nuclear nonhistone proteins (Table 1). Their methylation is realized within the regulatory system encompassing both histone and nonhistone protein modifications and serving for directed regulation of chromatin structure and target gene transcription.

METHYLATION OF TRANSCRIPTION FACTORS AND OTHER NUCLEAR PROTEINS

p53. The most studied nonhistone protein being methylated at lysine residues is the tumor suppressor p53. It is a transcription activator playing a crucial role in control of cell cycle, apoptosis, and DNA repair in response to various genotoxic stresses [16, 17]. Activity of p53 is principally regulated at the posttranslational level, particularly by methylation of the C-terminal regulatory domain of the protein at K370, K372, K382, and K386 residues, by multiple phosphorylations at serine residues, and by acetylation at lysine residues [18-20]. Overall, more than 36 amino acid residues of p53 are modified in some way. The control of p53 intracellular level normally occurs via its ubiquitinylation by Mdm2 ubiquitin ligase followed by proteasomal degradation, whereas multiple p53 phosphorylations and acetylations are induced in response to stress signals leading to stabilization of the protein and enhancement of its DNA-binding ability [16]. The activity of p53 can also be enhanced or suppressed depending on methylation sites and degree of methylation (Fig. 1).

HMT SET7/9, that methylates H3K4 [21], monomethylates p53K372 in response to DNA lesions [18, 22]. SET7/9 can also dimethylate K372 *in vitro* [23]. The monomethylation of K372 leads to considerable transcription enhancement of known p53 target genes, particularly *p21* and *PUMA*, whose products are crucial for cell cycle arrest at G1 phase and induction of apoptosis [16]. Moreover, monomethylation of K372 stabilizes p53 due to modulation of other C-terminal modifications; in particular it promotes p53 acetylation *in vivo* [22, 24]. Acetylation of C-terminal lysines increases both transcriptional activity and stability of p53 due to counteracting its Mdm2-dependent ubiquitinylation and degradation [16, 25]. Recently, it was reported that p53K372me1 is directly recognized by the chromodomain of acetyltransferase Tip60 responsible for acetylation of p53 and implicated in control of its apoptotic function [24]. Hence, the methylated K372 is a marker attracting acetylase by interaction with its chromodomain (Fig. 1a, below), which resembles processes involving the "histone code". For instance, HMT Su(var)3-9 trimethylating H3K9 possesses a chromodomain [10] by which it is targeted to nucleosomes methylated at H3K9 to form heterochromatin domains [26, 27].

HMT Smyd2, a member of the Smyd (SET and MYND domain) family, dimethylates H3K36 [28] and monomethylates p53K370 [23]. The K370 methylation suppresses the binding of p53 with promoters of the *p21* and *mdm2* genes controlled by p53 (Fig. 1d), whereas a decrease in Smyd2 level in cells enhances p53-mediated apoptosis. Smyd2-dependent K370 methylation is inhibited by SET7/9-dependent K372 methylation [23], thus suggesting an intracellular balance: K370me1 provides dissociation of p53–DNA complex, whereas K372me1 contrariwise elevates the affinity of p53 to a promoter by

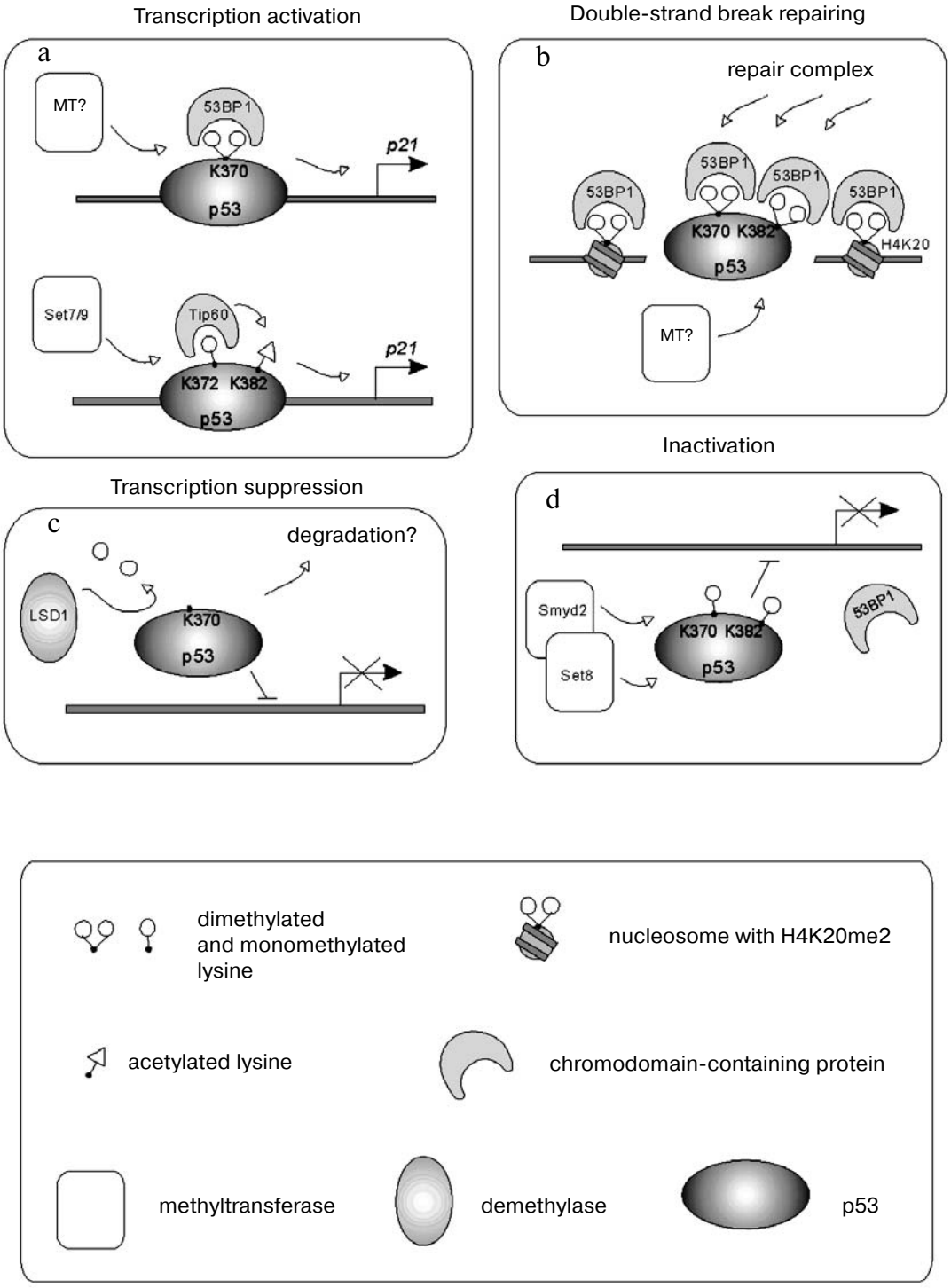

Fig. 1. Regulation of p53 activity by methylation at lysine residues. a) p53K370me2 activates p53 target gene transcription by implicating 53BP1 (above). p53K372me1 involves acetyl transferase Tip60 responsible for p53 acetylation and regulation of its apoptotic function (below). b) p53K370me2 and p53K382me2 involve 53BP1 facilitating its homooligomerization, as well as stabilization of repair complex proteins. 53BP1 simultaneously interacts with H4K20me2 and dimethylated p53 sites, which allows its implementation of adaptor function in damaged DNA sites. c) Lysine demethylase LSD1 specifically demethylates p53K370me2, thus exercising negative control over the interaction between p53 and 53BP1 and suppressing p53 transcriptional activity. d) p53K370me1 and p53K382me1 suppress the ability of p53 to bind with promoters of the genes under its control, which results in repression of p53-mediated transcription. MT, methyltransferase.

blocking the Smyd2-mediated transcription factor methylation and enhancing transcription of target genes. Dimethylation of K370 *in vivo* by an unidentified methylase was also discovered [29], but, unlike K370me1, K370me2 is a p53-activating modification providing both the interaction with coactivator 53BP1 (p53 binding protein 1) containing a tandem Tudor domain and enrichment of the *p21* gene promotor with p53 (Fig. 1a, above) [20, 29]. Generally, non-proteolytic negative control of p53 by K370 monomethylation creates a pool of inactive p53, but does not directly lead to its degradation, thus providing an opportunity for immediate response to DNA damage.

The first discovered histone lysine-specific demethylase 1 LSD1/KDM1 (demethylating both H3K4me1 and H3K4me2 [12], as well as H3K9me1 and H3K9me2 [13]) specifically demethylates p53K370me2, thus negatively controlling the interaction between p53 and 53BP1 and suppressing the p53 transcriptional activity (Fig. 1c) [27].

HMT SET8 (also called SET8/PR-Set7), that monomethylates H4K20 [30], also monomethylates p53 at K382, which leads to its inhibition and repression of p53-dependent transcription (Fig. 1d) [19]. The increase in both total p53 and acetylated p53K382 levels and decrease in the level of p53K382me1 are normally observed in response to induced DNA lesions. So, the monomethylation of K382 seems to compete with its acetylation. Later, the same research group discovered p53 dimethylation at K382 by an unidentified methylase. It was shown that, like p53K370me2, p53K382me2 is a high-affinity ligand for the tandem Tudor domain of the 53BP1 [20]. Both p53 and 53BP1 are key mediators of cell response to DNA lesions. When double-strand breaks appear, 53BP1 is rapidly relocated to damage sites and seems to attract and stabilize the repair complex proteins. Homooligomerization of 53BP1 allows it to fulfill adaptor functions in the damage sites due to simultaneous interaction with histone H4 dimethylated site, H4K20me2 [31], and p53 dimethylated sites (Fig. 1b). DNA lesions lead to increase in the level of endogenous p53K382me2 resulting in strengthening of the interaction between p53 and 53BP1 that provides stabilization and accumulation of the intracellular p53 pool in the sites of damage, though it does not influence p53-mediated *trans*-activation [20].

Lan and Shi [15] suggested the certain order of events occurring in the course of p53 methylation. First, p53K370me2 attracts 53BP1 and activates transcription of p53 target genes; dimethylation of K382 creates an additional binding site for 53BP1, which can result in p53–53BP1 complex formation and consequent stabilization of p53. Note that the amino acid sequence surrounding K382 (RHK**K**L) is highly homologous to that surrounding histone H4K20 (RHR**K**V), and 53BP1 also interacts with H4K20me2 [31]. However, methyltransferases dimethylating p53 at K370 and K382 have not yet been found, so a distinct mechanism of p53 regulation by methylation remains unknown. Nonetheless, it is obvious that methylases, demethylases, and "reader" proteins recognizing specific methylated sites are implicated in regulation of the C-terminal methylation of p53. The same enzymatic apparatus is implicated in both observed processes and regulation of histone "tail" methylation. This allows drawing a direct analogy between functions of p53 and realization of the "histone code".

TAF10. Beside histones and p53, HMT SET7/9 also methylates TAF10 ($TAF_{II}30$), a subunit of basal eukaryotic transcription factor TFIID [32]. TFIID is composed of TATA box-binding protein TBP and more than ten TBP-associated factors (TAFs). TFIID binds to TATA box on a promoter, thus determining the transcription initiation site for TATA-containing genes and promoting assembly of preinitiation complex. SET7/9 monomethylates the K189 lysine residue localized in a domain with implied histone-like fold, which is necessary for proper architecture and stability of the TFIID complex. Methylation of TAF10 at K189 enhances its affinity to RNA polymerase II. Moreover, the loss of SET7/9 methyltransferase activity or mutation in the TAF10 methylation site leads to reduction of transcription activity of reporter genes and specific endogenous genes. However, experiments with a TAF10 mutant (K189Q) on cell cultures revealed no effect of TAF10 methylation on cell viability. One can hypothesize that K189me1 is necessary for "fine tuning" of transcription complexes under certain physiological conditions.

TAF7. SET7/9 also methylates TAF7 ($TAF_{II}55$), another subunit of the TFIID complex, at the K5 lysine residue *in vitro* [33]. However, this fact has not yet been experimentally confirmed *in vivo*.

ERα. Another target for SET7/9 is estrogen receptor α (ERα) [34]. The nuclear receptor ERα is a ligand-dependent transcription factor that, following its binding with the steroid hormone estrogen, is attracted to target genes and recruits coactivator complexes possessing either acetyl- or methyltransferase activity. SET7/9 monomethylates the ERαK302 lysine residue resulting in receptor accumulation and stabilization in the nucleus, which is necessary for effective attraction of ERα to target genes. ERαK302 is localized in a loop between the DNA-binding and ligand-binding domains that can undergo multiple posttranslational modifications. The close surroundings of K302 correspond to the consensus sequence recognized by SET7/9, (R/K)(S/T)**K** [33]. Some amino acid residues disposed between K299 and S305 undergo acetylation (K299, K302, and K303), ubiquitinylation (K302), and phosphorylation (S305). Hence, a direct competition must exist between ERα methylation, acetylation, and ubiquitinylation, along with a cross-regulation of neighboring amino acid modifications. Also, one cannot exclude that K302me1 might serve as a marker for some proteins implicated in metab-

olism of ERα. The methylation of K302 can counteract ubiquitinylation with following degradation of ERα – by formation of its complexes with calmodulin or proteins recognizing the methylated lysine residue. It was shown that mutations at position K303, such as K303R, associated with the development of breast cancer, deteriorate methylation at K302 both *in vitro* and *in vivo* [34]. Further studies are necessary for elucidating the role of ERα methylation in oncogenic transformation of cells.

RARα. Another nuclear receptor, the retinoic acid receptor α (RARα), possesses three sites of lysine methylation: K109me1, K171me1, and K347me3 [35, 36]. In the absence of hormonal ligand, the heterodimeric nuclear receptors RXR-RAR are in association with their target gene promoters, thereby inhibiting transcription. The RAR receptors are activated by retinoic acid and regulate genes implicated in growth, development, differentiation, and apoptosis [37]. K347 is localized in the RARα ligand-binding domain, and its trimethylation positively regulates RARα activation by retinoic acid [35]. It was shown that K347me3 provides the receptor with local hydrophobicity required for effective binding to the coactivator PCAF, as well as for heterodimerization of RXR-RAR receptors. Besides, RARα undergoes monomethylation at lysines K109 and K171 that are localized in the DNA-binding domain and in the receptor loop area, respectively [36]. The loss of K109 methylation leads to significant impairment of the *trans*-activation of the target gene. Mutations at position 109 make RARα insensitive to activation by retinoic acid and hinder RXR-RAR heterodimerization that depends on this activation. Since the substitution of K109me1 with hydrophobic phenylalanine does not lead to restoration of RARα activity, one can suppose that the methyl marker is to be recognized by a specific "reader" protein, as it was shown for p53 [20]. According to preliminary data, the K109 methylation is catalyzed by HMT G9a [36].

NF-κB. Another transcription factor regulated by lysine methylation, NF-κB (RelA subunit), fulfils important functions in regulation of immune and inflammatory responses, apoptosis, cell proliferation, differentiation, and oncogenic transformation [38]. SET7/9 monomethylates RelA at K314 and K315 in response to treatment of human osteosarcoma U2OS cells with tumor necrosis factor TNF-α. The methylation launches a proteasomal degradation of the promoter-bound NF-κB and negatively regulates transcription of its target genes, *IL-8* and *IL-6* [39]. Besides methylation at lysine residues, RelA undergoes other posttranslational modifications at amino acid residues localized in the same small region: K310 acetylation [40] and S311 phosphorylation [41]. RelA acetylated at K310 is a poor substrate for methylation by SET7/9 (Yang and Chen, unpublished data). It is supposed that acetylation and methylation can be competitive modifications occurring at different stages of NF-κB functioning.

RIP140. RIP140, a corepressor of many nuclear receptors, is monomethylated at K591, K653, and K757 residues by an unidentified methyltransferase [42]. Methylation at lysine residues enhances the repressor activity of RIP140 by facilitating its association with histone deacetylase HDAC3. HDAC3 suppresses transcription by histone deacetylation in nucleosomes resulting in decrease in promoter accessibility for transcription factors. Besides methylation at K591, K653, and K757, RIP140 is subjected to multiple phosphorylation and acetylation, as well as methylation at arginine residues. In particular, the phosphorylation of RIP140 facilitates its acetylation at lysine residues [43]; however, both acetylation and methylation enhance the repressor function of RIP140. Mutations at lysine methylation sites lead to somewhat enhanced methylation at arginine residues, but not vice versa [42]. Moreover, methylation at arginine residues stimulates the export of RIP140 into the cytoplasm at late stages of cell differentiation [44]. It is possible that RIP140 is constitutively methylated at lysine residues, whereas methylation at arginines increases at late stages of differentiation, thus regulating the corepressor behaviour [42].

G9a. G9a, a member of the SET domain-containing HMT family, can trimethylate histone H3K9 lysine *in vitro* [45], mono- and dimethylate it *in vivo* in genome euchromatin regions [46], and methylate H3K27 [45, 47]. Recently it was shown that HMT G9a could also methylate nonhistone proteins [48, 49]. Moreover, G9a is capable of automethylation at K114 (dimethylation) and K185 (trimethylation) in human and *M. musculus* (corresponding lysines are K167 and K239) [48-50]. Methylation does not influence the catalytic activity of the enzyme. The automethylation site G9aK185 is homologous to the methylation site H3K9 by surrounding amino acid residues (AR**K**T and AR**K**S, respectively) and, like H3K9me3, interacts with the chromodomain of heterochromatin protein HP1 [48, 50]. Phosphorylation of the neighboring threonine T186 impairs the interaction between G9a and HP1 [50]. Similarly, phosphorylation of H3S10 by Aurora B protein kinase leads to release of HP1 from its complex with chromatin formed due to the interaction with H3K9me3 (methyl–phos switch) [51]. It is an impressive example of histone mimicry demonstrated by the nonhistone protein. Nonetheless, impairment of the G9aK185 methylation leads to insignificant increase in the amount of HP1 associated with pericentromeric heterochromatin and does not influence expression of multiple examined genes. It was shown that the G9aK185 methylation is necessary for interaction with another chromodomain-containing protein, CDYL1, *in vivo* [50].

Using methylation of peptide libraries, new putative targets for G9a were identified including nuclear proteins CDYL1 (K135), WIZ (K305), ACINUS (K654), and Dnmt1 (K70). Coexpression of WIZ and CDYL1 with

G9a in human and *Escherichia coli* cells led to methylation of these proteins [49], but the biological consequence of these modifications is still unclear, and no definite confirmation of methylation of the endogenous proteins in cells has been obtained.

Dnmt1. DNA methyltransferase 1 (Dnmt1), which is responsible for maintenance of methylation of DNA CpG-sites [52], is monomethylated at K142 and K1096 in humans [53] and mice [54], respectively. In both cases the methylation is catalyzed by HMT SET7/9. Dnmt1 methylation does not influence DNA methylase activity and nuclear localization, but it decreases the enzyme stability, thereby facilitating its proteasomal degradation in murine embryo stem cells (ESC) [54], as well as in various human cells, in which Dnmt1K142 methylation was shown to be a signal for Dnmt1 polyubiquitinylation [53]. Besides, Dnmt1K1096me1 undergoes demethylation by LSD1 *in vitro* [54]. A substantial decrease in Dnmt1 level together with the decrease in total level of DNA methylation is observed in murine LSD1 knockout ESC. It is likely that demethylation by LSD1 stabilizes Dnmt1. This study was the first to reveal a direct interrelationship between histone methylation and DNA methylation. Both LSD1 and Dnmt1 are known to participate in transcription suppression. It seems that LSD1, demethylating both histones and Dnmt1, coordinates the processes of DNA and histone methylation.

PCAF. Quite recently, a new nonhistone substrate for SET7/9 was found, namely PCAF (p300/CBP-associated factor). PCAF is a histone acetyltransferase implicated in many cellular processes [55]. SET7/9 monomethylates PCAF *in vitro* at lysines K78, K89, K638, K671, K672, and K692. In cell culture, SET7/9 monomethylates PCAF at K89 and K638, and the methylated protein is translocated into the nucleus [56]; however, the functional role of this modification remains unclear.

Dam1. Dam1 kinetochore protein of *S. cerevisiae* is dimethylated at K233 by HMT SET1 [57] that also methylates H3K4 [58]. The Dam1K233 lysine residue is surrounded by conserved S232, S234, and S235 serines that are phosphorylated *in vivo*, which is essential for kinetochore formation and subsequent chromosomal segregation [59]. However, excessive phosphorylation can affect Dam1 functions and kinetochore integrity. Dam1 dimethylation suppresses phosphorylation at one or more adjacent serine residues. The K233A mutation at the methylation site is lethal, but it can be suppressed by the S235A mutation at the phosphorylation site; that is, the region of 194-235 amino acid residues of the Dam1 molecule is apparently an important regulator managed via phosphorylation/methylation events [57]. The authors of the study also suppose that Dam1 methylation likely provides the cell with a "memory" mechanism allowing discrimination between the kinetochores already implicated in mitosis and the newly synthesized ones. This resembles the situation with epigenetic "memory" of methylated histones, enabling maintenance of a distinct transcriptional level through generations of cell divisions [11].

AML1. AML1 (acute myeloid leukemia 1) transcription factor belongs to the RUNX family of highly homologous DNA-binding proteins and regulates expression of multiple genes important for hematopoiesis. In human lymphoid and myeloid leukemia, the *AML1* gene becomes a target for chromosomal translocations and point mutations leading to the loss of its *trans*-activation potential and imbalance between differentiation, proliferation, and apoptosis in hematopoietic cell lines. Chimeric oncoproteins, the products of these translocations, are responsible for aberrant expression of genes crucial for development and differentiation of stem cells. AML1 activity can be modulated by multiple posttranslational modifications, such as phosphorylation and acetylation [60]. AML1 is methylated *in vivo* in human cell culture [61]. The methyltransferase executing this modification, as well as functional importance of AML1 methylation, remain unknown, but the interaction between HMT SUV39H1 and AML1 leading to suppression of both *trans*-activation activity and DNA-binding capability of AML1 was demonstrated.

Stellate. Recently, we demonstrated that Stellate protein expressed in *Drosophila melanogaster* testes is trimethylated *in vivo* [62]. In the absence of Y-chromosome or Y-linked *Suppressor of Stellate* (*Su*(*Ste*)) locus, derepression of *Stellate* genes occurs leading to accumulation of needle- or star-like Stellate crystals in testes, as well as to chromosomal malsegregation in meiosis and either partial or total sterility [63, 64]. Normally, expression of tandem clusters of testis-specific *Stellate* genes localized in X-chromosome is suppressed by short RNAs produced by the antisense transcription of highly homologous *Su*(*Ste*) genes according to the mechanism of RNA-silencing specific for eukaryotic germinal tissues (piRNA pathway) [65, 66]. The pathogenetic mechanism of Stellate overexpression remains unknown. Stellate is homologous to the regulatory β-subunit of protein kinase CK2 (CK2β) and interacts with the catalytic α-subunit of CK2 *in vivo* [62]. Two forms of Stellate are present in *D. melanogaster* spermatocytes: the soluble one (in nucleoplasm) and insoluble crystalline one (preferentially revealed in cytoplasm). The data obtained in our study suggest that both soluble and crystalline Stellate forms are trimethylated at lysine residue K92. Interestingly, Stellate K92me3 is specifically recognized by antibodies against H3K9me3 (Fig. 2) suggesting structural mimicry of H3K9me3 epigenetic modification, which is "read" by HP1 protein possessing a chromodomain and responsible for the formation of transcriptionally silent heterochromatin sites [26, 27]. We suppose that Stellate can recruit HP1 and other chromodomain-containing proteins, thus disturbing regulatory processes at the heterochromatin organization level. However, a direct interaction of trimethylated Stellate with HP1 has still not been demon-

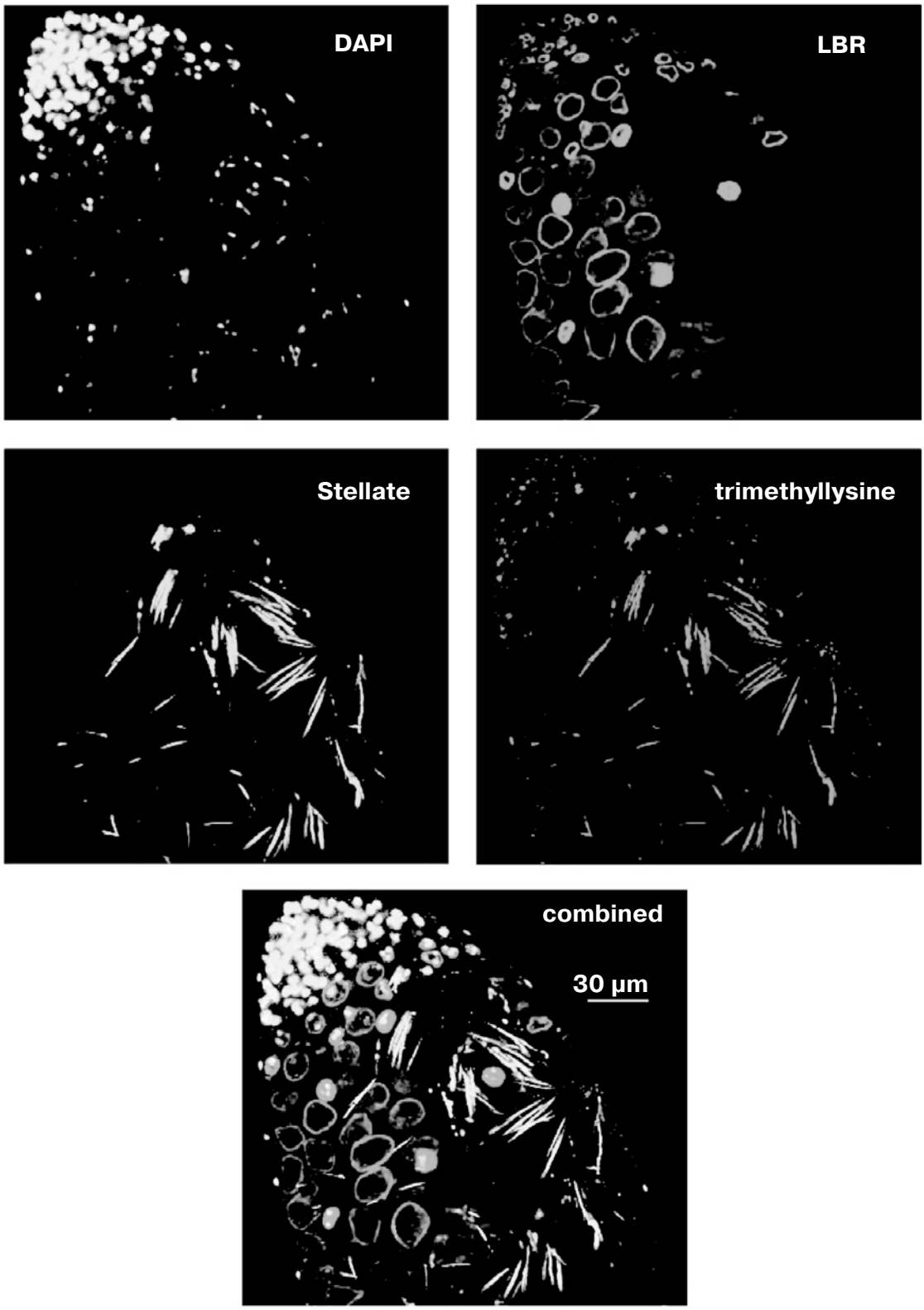

Fig. 2. Trimethylation of the Stellate protein in *Drosophila melanogaster* testes. A complete colocalization of signals from Stellate and trimethylated lysines is observed in fluorescently labeled testes of the fruit flies carrying a deletion of the *Su*(*Ste*) locus (*cry*^{1} line). A confocal section of the apical tip of the testis is shown. Chromatin was stained with DAPI (4′,6-diamidino-2-phenylindole), nuclear envelope with antibodies against LBR (lamin B receptor), Stellate with antibodies against the Stellate protein, and trimethyllysine with antibodies against the trimethylated lysine residue.

strated experimentally. The methyltransferase realizing the Stellate modification is also unknown, but one can hypothesize that it is one of the HMTs trimethylating H3K9, for instance, Su(var)3-9 or DSETDB1 [67, 68].

We also suppose that trimethylation can be a factor facilitating effective assembly of Stellate crystals *in vivo*. Methylation of lysine ε-amino groups favors the crystallization of the protein *in vitro* [69]. This modification increases hydrophobicity of the methylated sites, decreases their flexibility, decreases entropy, and leads to ordering of water molecules around methyls exposed at the surface of the protein molecule [69, 70]. It is likely that trimethylation favors rapid transition of Stellate into the crystalline form resulting in formation of biologically inert crystals and removal of the active soluble Stellate from regulatory nuclear processes (Fig. 2). If confirmed, this will be the first example of posttranslational regulation of protein activity via its transition into an inert crystalline form *in vivo*.

Tat. The first case of viral protein methylation at lysine residues was demonstrated for Tat protein of human immunodeficiency virus HIV-1 [71]. Tat acts as an activator of transcription initiation and elongation from the exclusive HIV-1 promoter incorporated into the genome in the area of the long terminal repeat. Tat interacts with specific RNA-enhancer element TAR in the 5′-region of viral transcript and recruits host transcription factors to the promoter [72]. Tat undergoes multiple posttranslational modifications catalyzed by host proteins: phosphorylation, acetylation, ubiquitinylation, and methylation at lysine and arginine residues. A positively charged Tat domain (49-57 amino acid residues) responsible for the binding to TAR is subjected to acetylation, which is necessary for Tat-mediated *trans*-activation. Methylation of K50 and K51 by HMT SETDB1 competes with their acetylation and represses transcription of viral mRNA [71]. It was shown earlier that methylation of adjacent R52 and R53 arginine residues by PRMT 6 also weakens the interaction between Tat and TAR and suppresses viral transcription [73]. Further studies will help to elucidate whether the Tat methylation results from intracellular immune defense aimed to restriction of viral replication.

METHYLATION OF TRANSLATIONAL APPARATUS PROTEINS

Ribosomal proteins and elongation factors. Posttranslational modifications, including methylation, occur in various components of the translational apparatus. Not only rRNAs and tRNAs, but also some ribosomal proteins and translation factors undergo methylation. Methylation of ribosomal proteins, mainly at arginine and lysine residues, has been found in prokaryotes, archaea, and eukaryotes. Moreover, methylation patterns are similar in different eubacteria and partially overlap those of archaea and eukaryotes, although some substantial differences are also present, for instance, more frequent arginine methylation in eukaryotes [74]. We summarize the main data on methylation of ribosomal proteins and translational factors in Table 2. Generally, mutations at methylation sites do not cause significant defects in ribosomal assembly and do not influence viability [75]; however, one cannot exclude that synergism of several posttranslational modifications can exist, and the loss of one of them does not induce any substantial alteration in ribosomal architecture and function. Is has also been demonstrated that methylated lysine residues in Rpl23a interact with RNA rather than with other proteins [76]. Nevertheless, the function of translational apparatus protein methylation is not yet understood.

Methylation of lysine residues is found in bacterial elongation factor Tu (EF-Tu) [85] and its eukaryotic homolog EF-1α [74, 86-88]. EF-Tu is highly conserved among all taxa from bacteria to humans. However, EF-Tu posttranslational modifications are less conserved than its amino acid sequence (Table 2).

METHYLATION OF OTHER NONHISTONE PROTEINS

Calmodulin. Calmodulin (CaM) is a highly conserved calcium sensor modulating activity of many enzymes. Trimethylation of CaM at K115 is found in various species including humans [89, 90]. Methyltransferase CLNMT trimethylates K115 [91, 92]. This posttranslational modification decreases NAD kinase-activating ability of CaM [93] and, possibly, also protects CaM from proteasomal degradation [94]. *Drosophila melanogaster* CaM is not methylated at K115, but a CaM isoform subjected to mono- and dimethylation at K94 was found in the eye tissue, although this modification was not found in other tissues. In CaM crystals, K94 is localized in the vicinity of the Ca^{2+}-binding site; the methylation of K94 putatively enhances interaction of CaM with photoreceptor proteins [95].

Rubisco. The SET domain-containing methyltransferase LSMT found in some plants trimethylates ribuloso-1,5-bisphosphate carboxylase/oxygenase (Rubisco) large subunit at lysine K14 residue [96, 97]. The role of this modification is still unclear.

Cytochrome *c*. Trimethylation of *S. cerevisiae* cytochrome *c* at K72 [98, 99] is catalyzed by Ctm1p methyltransferase that does not contain SET-domain and is localized in the cytoplasm [100]. Cytochrome *c* methylation strengthens its attachment to the mitochondrial membrane [101] and elevates import into the mitochondria [102]. K72me3 is necessary for defense of cytochrome *c* against proteolytic degradation *in vivo* [103], like in the case of p53K372me1 [22]. However, no

Table 2. Methylation of translation apparatus proteins

Substrate	Methylation site	Methyltransferase	Function	Species	Reference
Rpl1ab	unknown	unknown	unknown	*S. cerevisiae*	[78]
L10aA, L10aB	K90me3	–"–	–"–	*Arabidopsis thaliana*	[79]
Rpl3	unknown	–"–	–"–	*S. cerevisiae*	[78]
L11	K3me3, K39me3	PrmA	–"–	*E. coli*	[80]
L11	K3me3, K16me3, K39me3	PrmA	–"–	*Thermus thermophilus*	[81]
Rpl12ab	K10me3 K3me2	Rkm2 unknown	–"– –"–	*S. cerevisiae*	[75]
Rpl12	K3me3	SET11	–"–	*Schizosaccharomyces pombe*	[77]
L12	K3me3	unknown	–"–	*A. thaliana*	[79]
Rpl23ab	K105me2, K109me2	Rkm1	–"–	*S. cerevisiae*	[76]
L29	K4me1	unknown	–"–	*H. sapiens*	[82]
L29	K4me1	–"–	–"–	*Rattus norvegicus*	[83]
L40	K22me3	–"–	–"–	–"–	[83]
L36a	K55me1	–"–	regulation of ribosomal structure	*A. thaliana*	[79]
Rpl42ab	K40me1, K55me1	Rkm3 Rkm4	–"–	*S. cerevisiae*	[78, 84]
EF-Tu	K56me1, K56me2	unknown	deceleration of translation by inhibition of tRNA-dependent GTPase activity	*E. coli*	[85]
EF1A	K79me3, K316me2, K390me	–"–	stimulation of protein synthesis, regulation of GTPase activity	*S. cerevisiae*	[86]
EF1α	K36me3, K55me2, K79me3, K165me2, K318me3	–"–	unknown	*Oryctolagus cuniculus*	[87]
EF1α	K35me3, K54me1, K78me3, K218me3, K317me3	–"–	–"–	*Artemia salina*	[88]

dysfunction was detected in mutant Δ*ctm1* yeast line [100]. Methylation of cytochrome *c* at lysine residues was found in some plants and fungi, but not in higher animals. For instance, K72 and K82 are trimethylated in wheat germ, and K72 in *Neurospora* [104]. Similar cases of trimethylation are also found in other plants [105, 106].

ATP-synthase. Batten disease, a juvenile neurodegenerative disorder, is characterized by the buildup of lipofuscins in all tissues. The major protein component of these accumulating bodies is the mitochondrial ATP-synthase *c* subunit that is completely trimethylated at K43 in humans, as well as in other mammals [107-109]. However, the role of this modification in neurodegeneration is not understood.

VEGFR1. Vascular endothelial growth factor receptor 1 (VEGFR1) is an endoplasmic receptor tyrosine kinase implicated both in physiological and pathological angiogeneses. Smyd3, the SET domain-containing HMT that di- and trimethylates H3K4 [110], also dimethylates K831 localized in the cytoplasmic VEGFR1 kinase domain [111]. Dimethylation of VEGFR1 elevates its kinase activity *in vitro* as well as stimulates its ability for ligand-dependent autophosphorylation *in vivo* [111]. Smyd3 overexpression detected in hepatocellular and colorectal carcinomas and breast cancer might be responsible for pathological enhancement of VEGFR1 kinase activity resulting in tumor progression and metastasis. Hence, suppression of Smyd3 methyltransferase activity is regarded as a potential target for anticancer therapy.

PROTEINS RECOGNIZING METHYLATED LYSINE RESIDUES (METHYL-LYSINE "READERS")

Exact mechanisms of deciphering "posttranslational modification code" ("PTM code") including "histone code" are not yet completely defined. However, two main models, the "direct model" and the "effector-mediated model", have been introduced [5]. According to the first model, PTMs directly alter local properties of a protein molecule, thus influencing its spatial structure; the second model assumes existence of effector proteins, or "readers", specifically recognizing PTM and then recruiting some particular activities to the site of interaction. The discovery of these "reader" proteins is an evidence of the second model. For instance, bromodomains recognize acetylated lysine residues in histones, whereas chromodomains recognize H3K9me2/3 and H3K27me2/3 [11, 70]. Histone methylation at lysine residues is one of the most studied modifications of the "histone code". Methylation at distinct lysine residues represents a specific marker of distinct chromatin sites and functionally correlates with gene activation or silencing. Methylation does not alter the charge of a lysine residue in the protein molecule, but introduction of each new methyl group elevates hydrophobicity of the methylammonium group of lysine, simultaneously decreasing its ability for hydrogen bond formation. Trimethyllysine is a cation surrounded by hydrophobic methyl groups. Recognition of methylated lysine residues is realized via the contacts between the methylammonium group and aromatic residues of a "reader" protein, which form surrounding structure resembling a "cage" [70]. Various cases of lysine residue methylation are recognized by different effector domains, thus providing site-specific PTM "reading".

Methylated lysine residues are preferentially recognized by protein domains of two classes – Royal and PHD (plant homeodomain) fingers. Chromodomains (chromatin modifier, chromo), double chromodomain, Tudor, double Tudor, and MBT (malignant brain tumor) comprise the Royal superfamily. For instance, heterochromatin protein HP1 and HMT Su(var)3-9 carry chromodomains recognizing H3K9me2/3 [10, 112], whereas the chromodomain of Polycomb protein specifically interacts with H3K27me3 [113, 114]. A chromodomain consists of an incomplete "β-barrel" of four β-strands, at one terminus of which is a site for methylated lysine residue binding [70].

Chromodomains were shown to recognize methylation not only in histones, but also in some nonhistone proteins. In particular, Tip60 acetyltransferase recognizes p53K372me1, thus promoting acetylation of p53 [24], and HMT G9a automethylated at K185 interacts with the HP1 chromodomain [48, 50].

The 53BP1 protein, containing tandem Tudor domains, recognizes H4K20me1/2 [31] and interacts with two methylated sites in p53 – p53K370me2 and p53K382me2 [20]. The interaction with H4K20me2 recruits 53BP1 to double-strand breaks in DNA [31], and homooligomerization of 53BP1 in these sites enables its simultaneous interaction with H4K20me2 and p53 dimethylated sites (Fig. 1b). Its interaction with p53K370me2 is known to promote activation of p53 target genes (Fig. 1a) [20, 29].

Although interactions with other "reader" domains have not yet been shown for nonhistone proteins methylated at lysine residues, one can anticipate these interactions to be discovered soon, which in turn would shed light on the "PTM code" principles.

The interest in methylation of nonhistone proteins gained new impetus at the beginning of XXI century due to two circumstances: first, due to success in understanding the epigenetic information management principles realized through posttranslational modifications of histones and known as "histone code"; and second, due to the power of modern technologies, such as introduction of mass-spectrometry into routine laboratory practice and development of commercially available antibodies

recognizing lysine residues in various contexts. The last decade is characterized by doubling of the number of published experimental studies on this subject in comparison with the previous period.

The bulk of proteins carrying methylated lysine residues has been identified in various species from viruses to humans. A significant group is nuclear proteins associated with transcription and regulation of chromatin structure. All these proteins are methylated by enzymes identified earlier as SET-domain containing histone methyltransferases, such as SET7/9, Smyd2, SET8, and G9a. Homology has been shown between many methylation sites in nonhistone proteins and those in histones [18, 34, 49]. Keeping the distinct substrate specificity, these methyltransferases apparently possess much broader spectrum of targets than has been supposed earlier.

Methylation at lysine residues facilitates modulation of transcriptional activity and apoptotic functions of the p53 tumor suppressor, regulation of its stability, as well as interaction with the 53BP1 partner protein and acetyltransferase Tip60 [19, 20, 28, 29, 34]. Multiple modifications of p53 in the cell – phosphorylation, acetylation, methylation, and ubiquitinylation – reveal a complex character of interactions and, as in the case of modification of histones comprising nucleosomes, require "reading" by proteins capable of integration of a complex informational code. Like a number of other nuclear nonhistone proteins regarded here, p53 uses the cellular epigenetic apparatus for its regulation: methyltransferases, demethylases, and "reader" proteins recruited to the methylated sites. Methylation of DNA-methylase Dnmt1 at a lysine residue promotes its degradation, whereas demethylation, on the contrary, stabilizes the enzyme and influences the total level of methylation at CpG-sites. Thus, necessity of the common enzymatic apparatus for regulation of both histone and DNA methylation was first demonstrated [54]. Methylation of ribosomal proteins apparently facilitates regulation of ribosomal structure. Methylation of VEGFR1 tyrosine kinase domain regulates the pathway of signal transduction, when endothelial growth factor interacts with the cellular receptor [111]. Yet another potential function of protein methylation at lysine residues was found for *D. melanogaster* Stellate [62]. Trimethylation at lysine residue putatively facilitates effective formation of insoluble Stellate crystals in spermatocytes due to the increase in hydrophobicity of sites exposed at the surface and decrease in entropy factor.

Thus, methylation of nonhistone proteins at lysine residues influences their stability and functionality, regulates their interaction with DNA and partner proteins, and prevents other modifications at the same lysine residues. The "histone code" previously considered as a unique system for regulation of gene expression by histone posttranslational modifications seems to represent only a particular case of such regulation.

This study was supported by the Russian Academy of Sciences Presidium Program for Fundamental Research "Molecular and Cellular Biology", a grant for "Leading Scientific Schools" (NSh-3464.2008.4), the Russian Foundation for Basic Research (grant No. 08-04-00087a), and State Contract (02.740.11.0288).

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