In Vitro and in Vivo Analyses of a Phe/Tyr Switch Controlling Product Specificity of Histone Lysine Methyltransferases

Robert E. Collins, Emory University
Makoto Tachibana, Kyoto University
Hisashi Tamaru, University of Oregon
Kristina M. Smith, University of Oregon
Da Jia, Emory University
Xing Zhang, Emory University
Eric U. Selker, University of Oregon
Yoichi Shinkai, Kyoto University
Xiaodong Cheng, Emory University

Journal Title: Journal of Biological Chemistry
Volume: Volume 280, Number 7
Publisher: American Society for Biochemistry and Molecular Biology | 2005-02-18, Pages 5563-5570
Type of Work: Article | Final Publisher PDF
Publisher DOI: 10.1074/jbc.M410483200
Permanent URL: http://pid.emory.edu/ark:/25593/fjmx4

Final published version: http://www.jbc.org/content/280/7/5563.long

Copyright information:
© 2005, by the American Society for Biochemistry and Molecular Biology

Accessed October 30, 2019 1:56 PM EDT
In Vitro and in Vivo Analyses of a Phe/Tyr Switch Controlling Product Specificity of Histone Lysine Methyltransferases*

The functional significance of mono-, di-, and tri-methylation of lysine residues within histone proteins is under investigation. Evidence from several model organisms suggests that different methylated states of H3 Lys9 (H3K9) are generated by specific histone methyl transferases (MTases) to mark distinct types of silent chromatin. Sequence alignment of all histone lysine MTases with known product specificity suggested that a key residue in the active site determines how many methyl groups they add. We examined this possibility both in vitro and in vivo and found that a Phe at the position equivalent to Phe281 of Neurospora crassa DIM-5 or Phe1205 of human G9a allows the enzyme to perform di and tri-methylation, whereas a Tyr at this position is restrictive, inhibiting tri-methylation and thus yielding a mono- or di-MTase. Phe to Tyr mutants of both DIM-5 and G9a restrict product specificity in vitro and in vivo without compromising overall catalysis. These mutants were employed to probe the biological significance of mono-, di-, and tri-methylation of H3K9 in both mouse embryonic stem cells and N. crassa. G9a F1205Y, when expressed in G9a (+/−) embryonic stem cells, rescued only H3K9 mono-methylation, but not di-methylation, to wild-type levels yet silenced Mage-a gene expression. When expressed in dim-5 strains, DIM-5 F281Y generated significant levels of mono- and di-H3K9 methylation (which are not observed in wild type Neurospora) as well as tri-methyl H3K9. The altered DIM-5 rescued the growth defect characteristic of dim-5 N. crassa but did not fully rescue the gross DNA hypomethylation of dim-5 strains.

Histones are subject to diverse post-translational modifications including acetylation, phosphorylation, ubiquitination, methylation, and sumoylation. Evidence accumulated over the past few years suggests that such modifications constitute a "histone code" that directs a variety of processes involving chromatin (1, 2). Considering just methylation of lysines, there are at least six modification sites (Lys4, Lys9, Lys27, Lys36, and Lys79 of histone H3 and Lys20 of histone H4), and in principle each site can have zero, one, two, or three methyl groups. It has been suggested that methylation at these sites, in combination with other nearby modifications, generates "modification cassettes" (3), yielding distinct patterns on chromatin for signaling downstream events (reviewed in Refs. 3 and 4).

With only one known exception, histone lysine methyltransferases (HKMTs) contain a SET domain of ~130 amino acids. SET proteins can be grouped into families according to the sequence around this distinctive domain (5, 6) (see Table I). In this study we focus on two members of the SUV family that methylate Lys9 of histone H3, G9a characterized in mammals and DIM-5 characterized in the filamentous fungus Neurospora crassa. DIM-5 provided the first evidence that histone methylation can direct DNA methylation (7). DIM-5 normally generates primarily tri-methyl-Lys9 on histone H3 (8, 9). G9a is essential for early embryogenesis and is the predominant H3 Lys9 HKMT that directs euchromatic mono- and di-methylation (10–12). G9a apparently plays key roles in later development, because its activity is required for PRDI-BF1 (BLIMP-1) silencing in terminal B-cell differentiation (13), for neuron-restrictive silencing factor/REST-mediated silencing of neuronal genes in non-neuronal lineages (14), and for CCAAT displacement protein/cut-mediated silencing of many diverse genes involved in cell proliferation and differentiation (15). G9a and its close relative GLP-1 (G9a-like protein-1, also called EuHMT1) are also known to reside in CtBP co-repressor (16) and E2F-6.com-1 complexes (17). Like other members of the SUV family, DIM-5 of N. crassa (7), KYP of Arabidopsis (18), and Suv39h of mouse (19), G9a has been implicated in DNA methylation, because G9a (−/−) cells lack DNA methylation of the Prader-Willi syndrome imprinted center (20).

We wished to investigate the mechanism and consequences of different product specificities (mono-, di-, or tri-methylation) of HKMTs. With the advent of antibodies specific for mono-, di-, or tri-methylation of various lysines, it became increasingly evident that product specificity can be important for generating distinct regulatory signals (11, 12). For example, in Saccharomyces cerevisiae, SET-1 can di- or tri-methylate H3 Lys4, but only tri-methylation is associated with the early stage of active transcription; di-methylation is globally distributed (21, 22). Human SET7/9 protein, on the other hand, generates mono-methylation, because SET7/9 is specific for both mono- and dimethylation of Lys6 and 9.
methyl Lys$^3$ of H3 (9, 23). Mouse Suv39h specifically tri-methylates H3K9 at pericentric heterochromatin, whereas centromeric regions display Suv39h-independent di-methylation (4, 19). Tri-methylated H3 Lys$^7$ but not di-methylated Lys$^7$ is associated with X inactivation (24). In contrast to DIM-5, which tri-methylates Lys$^9$ of histone H3 to mark chromatin regions for DNA methylation (8), in Arabidopsis thaliana, the KRYPTONITE HKMT dimethylates Lys$^9$ (25).

By comparing the active sites of SETT9 (a mono-MTase) and DIM-5 (a tri-MTase), we designed point mutations in both enzymes that profoundly altered their product specificities without affecting their catalytic activities (9). This led us to propose that a few key residues in the active site of HKMTs determine how many methyl groups they add, and the product specificity may be predictable from their primary sequences. We show that G9a forms mono- and di-methyl Lys$^9$ as initial products and then slowly adds the third methyl group, consistent with reports that G9a is a global mono- and di-MTase (11, 12) but can generate trimethyl-H3K9 in some situations (26). We also demonstrate that a single point mutation, F1205Y, converts G9a to a mono-MTase. When expressed in G9a (−/−) ES cells, the F1205Y mutant rescues mono-, but not di-methyl H3K9 levels, yet represses Mage-a gene expression to wild-type levels. Similarly, the F281Y mutant was fully sequenced to confirm the desired mutation and the corresponding gene was amplified from a N. cassa wild-type strain (1) by PCR with Herculase polymerase (Stratagene) and a pair of primers, oligonucleotide 1282 (5’-CGAAGTCCTACACGACACTGCTGCATC-3’) and oligonucleotide 1285 (5’-CCGATTGATGATCCTCTTCCATCTTTCA-3’). The resulting PCR product was digested with EcoRI and BamHI, gel-purified, and cloned into a his-3 targeting vector, pBM61 (28), yielding pH15. A BglIII fragment carrying the wild-type dim-5 sequence in pH15 was replaced with the corresponding dim-5 fragment from pXC379 (29) or from pXC379 (19), yielding pH16 and pH17, respectively. The dim-5 null strain was derived by allelic exchange (28) with Ndel-linearized pH15, pH16, or pH17, and his-3 transformants were isolated. Correctly targeted constructs at his-3 in the resulting transformant strains were verified by Southern hybridizations probed with a his-3 fragment.

**PCR Primers**—Pairs of PCR primers used to amplify fragments of pcr (470 bp), His4 (425 bp), A221 (355 bp), A486 (302 bp), and S810 (316 bp) were: 560 pcr-FWD (ATGCTTGAAGACGTGGTGA), 561 pcr-REV (ACAAGTTATATGTTCTCAGC), and 562 H4-REV (ATCGCCGACCGTGTTGTAAC); Id121-FWD (5’-GATCCGGTCTCTGCTCTGCTTT-3’) and Id121-REV-2 5’-CCGCG CGA ACA TAT TAG TAT CAC TG-3’, 8A6-FWD 5’-GGA TGG CCG ATC CTA AAA AAT A-3’, and 8A6-REV 5’-TAA CCG CCG TTT AAA ATT AGG A-3’. The final spectra are compatible with the function of DIM-5 in maintaining normal DNA cytosine methylation. In contrast, the remaining tri-methylation and/or the increased levels of mono- and di-methylation by the F281Y mutant is sufficient to rescue the characteristic growth defect of the dim-5 strain.

**MATERIALS AND METHODS**

**Protein Expression and Purification**—A fragment encoding the C-terminal 280 residues of human G9a was amplified from an expressed sequence tag clone BC002686 (Resgen) and subcloned between the BamHI and EcoRI sites of pGEX2T (Amersham Biosciences), yielding pXC428. BL21(DE3) Codon-Plus REL (Stratagene) cells harboring pXC428 were grown in LB supplemented with 100 mg/liter ampicillin, 50 mg/liter chloramphenicol, and 25 μg/ml ZnSO$_4$ at 37 °C until A$_{600}$ = 0.5. The culture was shifted to 16 °C and induced by the addition of 0.4 mM isopropyl β-D-thiogalactopyranoside for 16 h. The cells were collected by centrifugation, lysed with a French press, and applied to glutathione-Sepharose 4B (Amersham Biosciences) in a batch slurry. Thrombin cleavage of the glutathione S-transferase tag was performed on the beads, yielding the G9a catalytic domain following four residues: GSH$q$ from the vector. Eluted protein was concentrated to 1 mg/ml, flash frozen, and stored at −80 °C in 25 ml Tris, pH 8.5, 50 ml NaCl, 2 ml dithiothreitol, and 5% glycerol. DIM-5 was expressed and purified as described (27).

**Mutagenesis**—Mutagenesis was performed with the QuikChange site-directed mutagenesis kit (Stratagene). The human G9a amino acid, corresponding to 1205 of the G9a mouse long isoform (10), in pXC428 was converted from Phe (TTT) to Tyr (TAT), yielding pXC444. The construct was fully sequenced to confirm the desired mutation and the lack of unintended mutations. Generation of DIM-5 mutants Y178V and F281Y has been described (9).

**Mass Spectrometry Analysis**—Methylation reactions for G9a were performed in 25 ml Tris, pH 8.5, 5 ml dithiothreitol, 250 μg S-adenosyl-l-methionine, 10 μM N-acetyllysine (H3 amino acids 1–15, 1–24, or 21–34), and 0.1 mg/ml enzyme at room temperature. For DIM-5, 20 μM of peptide and 0.02 mg/ml of the enzyme were used. The reactions were stopped at various times by the addition of triffuoroacetic acid to 0.5%. MALDI-TOF mass spectrometry was performed on an Applied Biosystems Voyager System 4258 (Empory University Chemistry Department) with a-cyano-4-hydroxycinnamic acid as matrix. The final spectra are the averages of 200 shots/position at 10 different positions.

**Construction of Neurospora Strains That Carry a dim-5 Null Mutation**—DNA methylation and/or the increased levels of mono- and di-methyl H3K9 that are not observed in the wild-type Neurospora. The mono- and di-methyl marks is incompatible with the function of DIM-5 in maintaining normal DNA methylation. In contrast, the remaining tri-methylation and/or the increased levels of mono- and di-methylation by the F281Y mutant is sufficient to rescue the characteristic growth defect of the dim-5 strain.
G9a ES Cells—Undifferentiated ES cells were maintained in 10% fetal calf serum and leukemia-inducing factor (500 units/ml)-containing medium. G9a long isoform (G9a-L) cDNA carrying the F1205Y mutation was generated by conventional double PCR mutagenesis and subcloned into the expression vector, pCAGGS. The G9a-L F1205Y expression vector was introduced into ES cells using Lipofectamine 2000 reagent (Invitrogen) according to the manual.

**RESULTS**

**G9a Is a Fast Monodi-MTase and a Slow Tri-MTase**—We have previously used MALDI-TOF mass spectrometry to monitor the kinetic progression of methylation reactions and determined that DIM-5 is a tri-MTase and SET7/9 is a mono-MTase (8, 9) (Fig. 1A). Here we report a similar investigation of the product specificity of the G9a catalytic domain by monitoring the methylation of a synthetic histone H3 peptide, residues H3 1–24 (Fig. 1B) or H3 1–15 (Fig. 2, left panels), by mass spectrometry. G9a produced both mono- and di-methylated peptides early in the reaction. Di-methylated H3K9 was the dominant form after about 1 min on both the 1–24 and 1–15 peptides, shortly after a burst of mono-methyl product coincident with the rapid disappearance of unmethylated peptides. Notably, tri-methylated H3K9 was first detected only after the di-methyl product was maximal and both un-modified and mono-methylated peptides had disappeared. The conversion of di- to tri-methyl lysine was much slower, not becoming complete until after 30 min in both cases. These observations suggest that mono and di-methyl H3K9 are the initial products of G9a. This is in sharp contrast to DIM-5, which shows a direct progression to tri-methyl lysine without significant accumulation of di-methyl lysine (8, 9) (Fig. 1A) and SET7/9, which produces only mono-methyl lysine plus a trace of di-methyl lysine after overnight incubation (9). These biochemical findings are consistent with *in vivo* observations that G9a is a global mono- and di-HKMT; specifically, G9a (−/−) ES cells lose global euchromatic mono and di-methyl H3K9 immunostaining (11, 12). Interestingly, our results suggest G9a could generate tri-methyl H3K9 at regions where it has high local concentrations. In fact, G9a tethered to a synthetic mini-locus as a Gal4DBD fusion generates tri-methyl lysine *in vivo* (26). Whether G9a makes trimethyl-H3K9 under normal circumstances *in vivo* remains to be determined.

Considering that G9a has also been reported to have activity on H3K27 (10), we also tested G9a product specificity on a H3 peptide containing Lys27 (Fig. 1C). We found that G9a has very poor activity on this lysine, some 100-fold worse than on peptides of similar length that include Lys9. Nevertheless, G9a did produce mono- and di-methyl H3K27 simultaneously in the early stages of the reaction and very slowly generated trimethyl Lys27 (−/−) (9). This low activity is consistent with the finding that methyl-H3K27 levels are unaffected in G9a (−/−) ES cells (11) and that G9a is a strong H3K9 MTase but has low or nondetectable H3K27 MTase activity, even when assayed as a member of a complex isolated by co-immunoprecipitation (13, 15). We conclude that G9a has poor activity on H3K27 *in vitro*, and it is unclear whether it has any significant effect on H3K27 methylation *in vivo*.

**Replacement of Phe1205 with Tyr Converts G9a to a Mono-MTase**—Comparison of the crystal structures for DIM-5 and SET7/9 led us to generate mutants (F281Y in DIM-5 and Y305F in SET7/9) with altered product specificity (9). To test the hypothesis that the corresponding residue Phe1205 in G9a (Table I) is the major determinant of product specificity, we replaced this residue with Tyr (F1205Y). The mutation did not significantly affect the catalytic activity of G9a, because the rate of loss of unmethylated peptide was roughly equal to that of wild-type (WT) enzyme (Fig. 2D, right panel). However, the reaction by F1205Y stalled at the mono-methyl stage (compare 1.5 and 10 min; Fig. 2, A and B), supporting our hypothesis. A trace amount of di-methylated product was observed only after
FIG. 2. Generation of mono-methyl H3K9 (H3 amino acids 1–15) by G9a F1205Y variant without impaired catalytic ability. A–C, representative mass spectra (from a single reaction) at various time points for WT G9a (left panels) and F1205Y (right panels). Unmodified, mono-, di-, and tri-methyl peptides are labeled. D, the relative amount of each peptide species over the full time courses of the reactions, expressed as a percentage of the sum of intensity of all related peaks. a.i., absolute intensity.
prolonged incubation (45 min; Fig. 2C), which indicated that di-methylation by the altered enzyme was actually slower than the tri-methylation by the WT G9a. We conclude that the F1205Y mutation changed the product specificity of G9a from a fast mono/di-MTase with a slow tri-MTase activity to a predominantly mono-MTase without affecting overall catalytic activity, analogous to the F281Y mutation for DIM-5 (9).

Monomethyl G9a Silences Mage-a Gene Expression—G9a (-/-) ES cells lack global mono- and di-methyl H3K9 and show aberrant expression of Mage-a genes (11, 12, 31). A G9a transgene with the F1205Y mutation was expressed as a full-length G9a (long isoform) transgene in G9a (-/-) mouse ES cells. When expressed at WT levels (Fig. 3A), the F1205Y mutant rescued H3K9 mono- but not di-methylation to WT levels (Fig. 3B), consistent with our biochemical data. Interestingly, Northern hybridization results showed that this mono-methylation of Lys9 was sufficient to repress Mage-a (Fig. 3C). This result may reflect an unknown repressive lysine-binding module that recognizes mono- and/or di-H3K9, or it may be that mono- and di-H3K9 can both block H3K9 acetylation and neighboring marks required for transcription of the Mage genes.

F281Y DIM-5 Partially Rescues Phenotypes of dim-5 Mutant—A lack of DIM-5 HKMT leads to gross hypomethylation of H3K9, which is a required mark for DNA methylation in *Neurospora* (7, 8). In addition, dim-5 strains are characterized by growth defects and poor fertility. *Neurospora* strains that lack all DNA methylation because of a null mutation in the DNA MTase gene *dim-2* do not show these defects, suggesting that DIM-5, or its product trimethyl-H3K9, has functions beyond DNA methylation. To determine whether DNA methylation and other functions of DIM-5 activity rely on tri-methylation of H3K9, we introduced the F281Y substitution, which, in *vitro*, dramatically changed the product specificity of the enzyme from tri- to mono-H3K9 (with slight residual di-methyl activity) into a *Neurospora* strain bearing a nonsense mutation in *dim-5*. We then tested the transformed strains for mono-, di-, and tri-methylation and scored for physiological effects. As controls, we also transformed the *dim-5* host strain with the WT *dim-5* gene as well as with a second mutated gene (Y178V), resulting in a catalytically inactive variant (~0.5% WT activity) (9). All of the constructs were targeted to the *his-3* locus. The ectopic WT and F281Y mutant expressed at similar levels as wild-type DIM-5, as determined by Western blot (Fig. 4A), but the Y178V mutant was not detected, presumably because of instability of the mutant protein.
FIG. 4. DIM-5 F281Y variant partially rescues defects of dim-5 mutant. A, DIM-5 expression is similar from both the ectopic dim-5WT allele and the dim-5HT1 transformants carrying dim-5WT or dim-5F281Y alleles at his-3. The dim-5Y178V allele, however, is either not expressed or the resulting mutant protein is unstable. 

B, expression of DIM-5F281Y variant in N. crassa produces mono-, di-, and tri-methylated H3K9, whereas the WT DIM-5 produces exclusively trimethylated H3K9. Western blot analysis of N. crassa histone H3 using antibodies against mono-, di-, or tri-methylated H3K9. Coomassie Blue staining of a replica gel confirmed that the amount of histone H3 was approximately even between the WT, dim-5Y178V, and dim-5F281Y protein samples. 

C, chromatin immunoprecipitation experiments with chromatin from dim-5HT1 transformants carrying dim-5Y178V (inactive), dim-5WT, or dim-5F281Y alleles at his-3 were carried out using the indicated antibodies. Total DNA and immunoprecipitated DNA were subject to duplex PCR to amplify pairs of unmethylated (hH4) and methylated (1d21, 8A6, and 8F10; underlined) DNA regions of N. crassa. Note that the F281Y mutant, but not the inactive and wild-type alleles, show significant mono- and di-methyl-H3K9 signals in the regions that are subject to DNA methylation. The specificity mutant (F281Y) also shows a tri-methyl H3K9 signal, although it is somewhat weaker than in the ChIP with the wild-type allele (most obvious in the 1d21 region). In one experiment (right panel), regions were probed for dimethyl-H3K4, which is underrepresented at heterochromatic regions. 

D, DIM-5F281Y variant poorly complements DNA methylation defect in dim-5HT1 strain. Genomic DNA samples from two independently isolated dim-5HT1 transformants (1 and 2) carrying dim-5WT, dim-5F281Y, or dim-5Y178V alleles at his-3 were digested with DpnII (D) or Sau3AI (S) and analyzed by gel electrophoresis and Southern hybridization using probes for the indicated regions. A photograph of the ethidium bromide-stained gel (total) shows partial complementation on a global level. Note that Sau3AI and DpnII are isoschizomers, but only Sau3AI is sensitive to DNA cytosine methylation.
Western blots showed that the dim-5 strain transformed with the WT gene showed strong tri-methylation of H3K9, but no noticeable mono- or di-methylation of H3K9, as expected (Fig. 4B). The catalytically impaired enzyme also lacked mono- or di-methylated forms of H3K9 but showed a background of tri-methyl H3K9 (as does the dim-5 null strain (8)), possibly because of an unknown HKMT that is not preferentially targeted to regions that are subject to DNA methylation.

Interestingly, results of Western blot and ChIP experiments showed that the F281Y mutant produced mono-, di-, and tri-methyl H3K9 (Fig. 4, B and C). Although the anti-trimethyl antibody has been well characterized (8), we carried out an additional peptide competition experiment that demonstrated that the signal detected in the F281Y transformant is truly tri-methyl H3K9 (data not shown). Although the overall levels of trimethyl-H3K9 seem similar in the F281Y mutant and WT based on the semi-quantitative Western analysis, repeated duplex ChIP experiments, in which a region of the genome that is normally subject to DNA methylation (1d21, SA6, or 8F10) was compared with a region not subject to DNA methylation (pen or h4), revealed modest reductions of tri-methyl Lys9, especially in the 1d21 and 8F10 regions (Fig. 4C). That the F281Y mutant produced substantial levels of tri-methyl H3K9, in addition to mono- and di-methylated H3K9, may be due to strong localization at heterochromatic loci, allowing its very slow residual di- and mono-methyl H3K9, in addition to in the 1d21 and 8F10 regions (Fig. 4C).

That the F281Y mutant produced mono- and di-methylated forms of H3K9 but showed a background of tri-methyl H3K9 (as does the dim-5 null strain (8)), possibly because of an unknown HKMT that is not preferentially targeted to regions that are subject to DNA methylation.

The Y178V mutant completely failed to complement the DNA methylation defect of dim-5 strains, as expected. More interestingly, F281Y poorly restored DNA methylation compared with ectopically expressed WT DIM-5 (Fig. 4D). We conclude that either the presence of mono- and di-methyl K9 or the slight reduction of tri-methyl H3K9 significantly compromised signals for DNA methylation. Curiously, although the F281Y mutant poorly restored DNA methylation, it rescued the dim-5 growth defect to the same degree as WT DIM-5 (Fig. 5). The catalytically impaired mutant (Y178V) did not complement the growth defect but because this mutant failed to produce stable protein (Fig. 4A), we could not determine whether the full rescue of the growth defect is due to some function of DIM-5 not dependent on its methyltransferase activity.

**DISCUSSION**

The multiplicity of methylation levels at a given residue is a newly recognized elaboration of the histone code. Each methylation mark (mono-, di-, or tri-) could serve to establish or mask unique binding sites of chromodomain proteins or associated proteins, thereby greatly expanding the complexity of the histone code. Variable degrees of methylation could also serve to tune the output of the “code” by modulating the affinity of lysine binding by chromodomains. The extent to which mono-, di-, and tri-methylation of a single lysine generates a unique mark or act to govern the signal strength of a single mark remains to be seen. The ability to predict the product of each HKMT will be useful in creating engineered mutants to test the biological impact of the variable methylation states of histone lysines. In this study, we found evidence that distinct processes may differ in their reading of a methyl mark. Specifically, in Neurospora, introduction of lower methylation states (mono- and di-methyl) at H3K9 appeared to preclude full complementation of the DNA methylation defect of dim-5 but did not hamper full complementation of the growth and fertility defects. The effect of mono- and tri-methyl H3K9 on DNA methylation may reflect a requirement for a high local concentration of tri-methyl H3K9 and perhaps both H3 molecules in the nucleosome must be tri-methylated. This is consistent with the finding that the ectopic expression of histone H3 with K9R or K9L mutations in a wild-type background has the dominant effect of bringing about gross DNA hypomethylation (7). Additional work will be required to determine whether the striking difference in complementation reflects differences in the molecules that read the marks. It is conceivable, for example, that HP1, which is known to bind both di- and tri-methylated forms of H3K9 in other organisms (32, 33) and has been demonstrated to be essential for DNA methylation in Neurospora (34), forms distinct complexes that are differentially sensitive to the methylation state.

As previously reported, Phe^281 of DIM-5 is critical in determining its product specificity (9). A Tyr at this position generates a mono/di-MTase. Sequence alignment including all HK-
MTs with known product specificity suggests that this rule may be generalized (Table I). Arabidopsis KYP and SUVH6 both have a tyrosine and they are both mono/di-MTases (25). From a structural perspective, it appears the tyrosine hydroxyl can block substrate lysines with methyl group(s) attached from rotating into a position where they can be further methylated. In this work, we demonstrated that G9a, which has a Phe at this position, is a fast mono/di-MTase and slow tri-MTase. Interestingly, substituting the Phe of G9a with a Tyr also resulted in a mono-MTase, analogous to DIM-5. This mutant with “switched” product specificity rescued only mono-methyl H3K9 yet represses Mage-a gene expression. It is unknown whether di-methyl H3K9 is critical for other G9a-dependent processes (e.g., maintenance of DNA methylation at imprinting centers, PRDI-BF1 silencing in terminal B-cell differentiation, and neuron-restrictive silencing factor/REST-mediated silencing of neuronal genes).

At face value, S. cerevisiae SET1 protein appears to conflict with our Phe/Tyr switch hypothesis. It has a tyrosine at the position comparable with Phe281 of DIM-5, and yet results of ChIP experiments suggest that it can both di- and tri-methylate H3K4 (21, 22). It should be noted, however, that the published studies did not reveal whether SET1 also produces di-methylated peptide, consistent with it being a mono/di-MTase (38) have robust HKMT activity, but no activity on a ALL1 fragments of C-terminal 224 residues (39) or 377 residues (38) have robust HKMT activity, but no activity on a SET domain HKMTs suggests that a Phe/Tyr switch controls their product specificity and that alteration of this product specificity can have important biological consequences. It appears the histone code may show rigidity in some cases and surprising plasticity in others.

Acknowledgments—We are indebted to T. Jenuwein and P. B. Singh for the use of the H3K9 methyl-specific antibodies described in this manuscript.

REFERENCES
In Vitro and in Vivo Analyses of a Phe/Tyr Switch Controlling Product Specificity of Histone Lysine Methyltransferases
Robert E. Collins, Makoto Tachibana, Hisashi Tamaru, Kristina M. Smith, Da Jia, Xing Zhang, Eric U. Selker, Yoichi Shinkai and Xiaodong Cheng

doi: 10.1074/jbc.M410483200 originally published online December 6, 2004

Access the most updated version of this article at doi: 10.1074/jbc.M410483200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 45 references, 16 of which can be accessed free at http://www.jbc.org/content/280/7/5563.full.html#ref-list-1