A Highly Specific Mechanism of Histone H3-K4 Recognition by Histone Demethylase LSD1*

Federico Forneris1‡1, Claudia Binda1‡1,2, Annachiara Dall’Aglio3, Marco W. Fraaije4, Elena Battaglioli5, and Andrea Mattevi13

From the 1Dipartimento di Genetica e Microbiologia, Università di Pavia, via Ferrata 1, 27100 Pavia, Italy, 2Dipartimento di Biologia e Genetica per le Scienze Mediche, Università di Milano, via Viotti 3/5, 20133 Milano, Italy, and 3Laboratory of Biochemistry, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Nijenborgh 4, 9747 AG Groningen, The Netherlands

Human lysine-specific demethylase (LSD1) is a chromatin-modifying enzyme that specifically removes methyl groups from mono- and dimethylated Lys4 of histone H3 (H3-K4). We used a combination of in vivo and in vitro experiments to characterize the substrate specificity and recognition by LSD1. Biochemical assays on histone peptides show that essentially all epigenetic modifications on the 21 N-terminal amino acids of histone H3 cause a significant reduction in enzymatic activity. Replacement of Lys4 with Arg greatly enhances binding affinity, and a histone peptide incorporating this mutation has a strong inhibitory power. Conversely, a peptide bearing a trimethylated Lys4 is only a weak inhibitor of the enzyme. Rapid kinetics measurements evidence that the enzyme is efficiently reoxidized by molecular oxygen with a second-order rate constant of 9.6 × 103 M⁻¹ s⁻¹, and that the presence of the reaction product does not greatly influence the rate of flavin reoxidation. In vivo experiments provide a correlation between the in vitro inhibitory properties of the tested peptides and their ability of affecting endogenous LSD1 activity. Our results show that epigenetic modifications on histone H3 need to be removed before Lys4 demethylation can efficiently occur. The complex formed by LSD1 with histone deacetylases 1/2 may function as a “double-blade razor” that first eliminates the acetyl groups from acetylated Lys residues and then removes the methyl group from Lys4. We suggest that after H3-K4 demethylation, LSD1 recruits the forthcoming chromatin remodelers leading to the introduction of gene repression marks.

Post-translational modifications on histone proteins are fundamental epigenetic marks that control chromatin state and gene expression (1, 2), and unravelling epigenetic mechanisms is a new area of biomedical research (3). Histone lysine demethylases participate in the regulation of chromatin functional states by removing methyl groups from lysine residues on histone N-terminal tails. At present two different subclasses of histone lysine demethylases have been identified: the FAD-dependent histone demethylases (4) (Fig. 1) and the JmjC domain containing histone demethylases, representing iron-dependent dioxygenases that use 2-oxoglutarate for histone lysine demethylation via hydroxylation (5). LSD1 was the first discovered histone demethylase and so far, the only one known to require a flavin cofactor (4, 6). This enzyme specifically acts on mono- and dimethylated Lys4 of histone H3 (H3-K4), and its activity induces gene repression because H3-K4 methylation is generally associated with activation (7). The enzyme is part of several multiprotein corepressors including CoREST, CtBP, and a subset of histone deacetylases 1/2 complexes (8–12). The LSD1 catalyzed reaction starts with the oxidation of the H3-K4 N-methyl group carried out by the FAD cofactor (Fig. 1) . The resulting imine intermediate is then hydrolyzed generating the demethylated histone and formaldehyde.

We used a combination of in vivo and in vitro experiments in an effort to characterize LSD1 substrate specificity and mechanism of H3-K4 recognition. We provide evidence that oxygen can be the physiological electron acceptor, and that the enzyme is likely to function through a ternary complex mechanism. In addition, our data show that LSD1 can efficiently act only after removal of the other epigenetic modifications present on the same histone H3 N-terminal tail.

EXPERIMENTAL PROCEDURES

Protein Preparation and Steady-state Kinetics Measurements—All chemicals were purchased from Sigma unless specified. Human LSD1 lacking the N-terminal 157 amino acids was expressed and purified as described (6, 13). Enzymatic activities were measured under aerobic conditions by using a peroxidase-coupled assay (13) on a Cary 100 UV/visible spectrophotometer. Peptides were purchased from Thermo Electron Corporation. Their purity was greater than 90% as checked by analytical high pressure liquid chromatography and mass spectrometry. LSD1 inhibitors were tested by using the peroxidase-coupled assay in the presence of varied concentrations (2–100 μM) of monomethylated H3-K4 peptide and of the inhibitor under analysis (global range 1–300 μM, depending on the inhibitor

states by removing methyl groups from lysine residues on histone N-terminal tails. At present two different subclasses of histone lysine demethylases have been identified: the FAD-dependent histone demethylases (4) (Fig. 1) and the JmjC domain containing histone demethylases, representing iron-dependent dioxygenases that use 2-oxoglutarate for histone lysine demethylation via hydroxylation (5). LSD1 was the first discovered histone demethylase and so far, the only one known to require a flavin cofactor (4, 6). This enzyme specifically acts on mono- and dimethylated Lys4 of histone H3 (H3-K4), and its activity induces gene repression because H3-K4 methylation is generally associated with activation (7). The enzyme is part of several multiprotein corepressors including CoREST, CtBP, and a subset of histone deacetylases 1/2 complexes (8–12). The LSD1 catalyzed reaction starts with the oxidation of the H3-K4 N-methyl group carried out by the FAD cofactor (Fig. 1). The resulting imine intermediate is then hydrolyzed generating the demethylated histone and formaldehyde.

We used a combination of in vivo and in vitro experiments in an effort to characterize LSD1 substrate specificity and mechanism of H3-K4 recognition. We provide evidence that oxygen can be the physiological electron acceptor, and that the enzyme is likely to function through a ternary complex mechanism. In addition, our data show that LSD1 can efficiently act only after removal of the other epigenetic modifications present on the same histone H3 N-terminal tail.

EXPERIMENTAL PROCEDURES

Protein Preparation and Steady-state Kinetics Measurements—All chemicals were purchased from Sigma unless specified. Human LSD1 lacking the N-terminal 157 amino acids was expressed and purified as described (6, 13). Enzymatic activities were measured under aerobic conditions by using a peroxidase-coupled assay (13) on a Cary 100 UV/visible spectrophotometer. Peptides were purchased from Thermo Electron Corporation. Their purity was greater than 90% as checked by analytical high pressure liquid chromatography and mass spectrometry. LSD1 inhibitors were tested by using the peroxidase-coupled assay in the presence of varied concentrations (2–100 μM) of monomethylated H3-K4 peptide and of the inhibitor under analysis (global range 1–300 μM, depending on the inhibitor

states by removing methyl groups from lysine residues on histone N-terminal tails. At present two different subclasses of histone lysine demethylases have been identified: the FAD-dependent histone demethylases (4) (Fig. 1) and the JmjC domain containing histone demethylases, representing iron-dependent dioxygenases that use 2-oxoglutarate for histone lysine demethylation via hydroxylation (5). LSD1 was the first discovered histone demethylase and so far, the only one known to require a flavin cofactor (4, 6). This enzyme specifically acts on mono- and dimethylated Lys4 of histone H3 (H3-K4), and its activity induces gene repression because H3-K4 methylation is generally associated with activation (7). The enzyme is part of several multiprotein corepressors including CoREST, CtBP, and a subset of histone deacetylases 1/2 complexes (8–12). The LSD1 catalyzed reaction starts with the oxidation of the H3-K4 N-methyl group carried out by the FAD cofactor (Fig. 1). The resulting imine intermediate is then hydrolyzed generating the demethylated histone and formaldehyde.

We used a combination of in vivo and in vitro experiments in an effort to characterize LSD1 substrate specificity and mechanism of H3-K4 recognition. We provide evidence that oxygen can be the physiological electron acceptor, and that the enzyme is likely to function through a ternary complex mechanism. In addition, our data show that LSD1 can efficiently act only after removal of the other epigenetic modifications present on the same histone H3 N-terminal tail.

EXPERIMENTAL PROCEDURES

Protein Preparation and Steady-state Kinetics Measurements—All chemicals were purchased from Sigma unless specified. Human LSD1 lacking the N-terminal 157 amino acids was expressed and purified as described (6, 13). Enzymatic activities were measured under aerobic conditions by using a peroxidase-coupled assay (13) on a Cary 100 UV/visible spectrophotometer. Peptides were purchased from Thermo Electron Corporation. Their purity was greater than 90% as checked by analytical high pressure liquid chromatography and mass spectrometry. LSD1 inhibitors were tested by using the peroxidase-coupled assay in the presence of varied concentrations (2–100 μM) of monomethylated H3-K4 peptide and of the inhibitor under analysis (global range 1–300 μM, depending on the inhibitor
Substrate Recognition by LSD1

![Diagram of Substrate Recognition by LSD1](image)

**FIGURE 1.** Histone H3 Lys4 demethylation reaction catalyzed by LSD1. Mono- or dimethylated H3-K4 is oxidized by the FAD cofactor (reductive half-reaction) to an imine intermediate that is hydrolyzed to yield the demethylated histone tail and formaldehyde. In the oxidative half-reaction, the FAD is reoxidized by an electron acceptor such as molecular oxygen.

**RESULTS**

Influence of Other Epigenetic Marks on Substrate Recognition and Enzymatic Activity—Substrate recognition by LSD1 is not confined to residues neighboring Lys4 but it is achieved through a network of specific interactions with the 21 N-terminal residues of histone H3 (4, 13). The histone H3 N-terminal tail has many potential sites of epigenetic post-translational modifications, and it was shown that the locus Lys9–Ser10 is particularly important in affecting LSD1 activity (13). To investigate how different epigenetic marks affect the LSD1 demethylation process, we tested several 21-amino acid peptides that in addition to monomethylated on Lys4 contain other epigenetic covalent modifications (Fig. 2, a and b). For each peptide, we measured the enzymatic activity with a peroxidase-coupled assay (Ref. 13 and Table 1), and the resulting steady-state kinetic
parameters were evaluated relatively to those obtained with the 21-amino acid monomethylated substrate (peptide 1).

We first tested the effect of arginine methylation, an epigenetic mark that has been recently shown to be dynamically modulated (15, 16). Monomethylation of Arg2 (peptide 2) decreased LSD1 activity by more than 80%, whereas a methyl group on Arg8 (peptide 3) made the peptide totally unable to function as a substrate. Arg17 monomethylation (peptide 4) had a lower effect mostly due to a decrease in binding affinity. We also found that peptide 3 hardly inhibits LSD1 ($K_i > 100 \mu M$) implying that methylation of Arg8 completely prevents binding to the enzyme.

Next, we investigated the effect of lysine hyperacetylation, a post-translational modification of histone H3 that is associated with gene activation (3). For this purpose, a monomethylated H3-K4 peptide acetylated at Lys9, Lys14, and Lys18 (peptide 5) was tested. This peptide did not exhibit any inhibitory or catalytic activity. This observation is in agreement with data reported by Shi et al. (11) showing that hyperacetylated nucleosomes are less susceptible to CoREST/LSD1-mediated demethylation. Lysine acetylation seems to have an additive effect because acetylation at Lys9 causes a 6-fold reduction in activity (13), whereas acetylation of all three Lys residues present in the 21-amino acid peptide leads to complete inactivation. Taken
Substrate Recognition by LSD1

### TABLE 1

| Peptide | $k_{cat}$ $^{\dagger}$ | $K_m$ $^\ddagger$ | $k_{cat}/K_m$ $^\ddagger$ | $K_i$ $^c$ |
|---------|------------------------|-------------------|--------------------------|-----------|
| 1. Monomethyl Lys$^4$ | 3.4 ± 0.1 | 3.4 ± 0.2 | 1.0 ± 0.1 | - |
| 2. Monomethyl Arg$^2$, monomethyl Lys$^4$ | 0.63 ± 0.03 | 5.5 ± 1.4 | 0.11 ± 0.02 | - |
| 3. Monomethyl Lys$^4$, monomethyl Arg$^4$ | 0.12 ± 0.12 | 1.2 ± 0.12 | 0.11 ± 0.02 | - |
| 4. Monomethyl Lys$^4$, monomethyl Arg$^7$ | 2.5 ± 0.2 | 5.7 ± 1.7 | 0.45 ± 0.13 | - |
| 5. Monomethyl Lys$^4$, acetyl Lys$^4$, Lys$^8$, Lys$^{18}$ | 0.12 ± 0.12 | 1.2 ± 0.12 | 0.11 ± 0.02 | - |
| 6. Monomethyl Lys$^4$, mutated G12A, G13A | No activity | No activity | No activity | - |
| 7. Monomethyl Lys$^4$, mutated P16A | 3.0 ± 0.2 | 4.9 ± 1.2 | 0.61 ± 0.15 | - |
| 8. Trimethyl Lys$^4$ | No activity | No activity | No activity | - |
| 9. Mutated K4R | No activity | No activity | No activity | 0.41 ± 0.05 |
| 10. Unmodified peptide (residues 1–21)$^f$ | No activity | No activity | No activity | 1.8 ± 0.6 |
| 11. Shorter unmodified peptide (residues 5–21)$^f$ | No activity | No activity | No activity | 87 ± 29 |

$^{\dagger}$ Apparent steady-state kinetic parameters determined as described under “Experimental Procedures.” No activity, the activity is absent or barely detectable.

$^{\ddagger}$ Inhibition assays were performed for all the peptides that showed no activity in the peroxidase-coupled assay. The inhibition constant was determined in an assay with peptide 1 as substrate. Values were determined by using competitive inhibition algorithm from the Grafit software package (Erithacus Software). Weak indicates that only a weak inhibitory effect was detectable ($K_i > 100 \mu M$). Propagation of statistical error value was carried out as described (14).

$^c$ Data taken from Forneris et al. (13).

---

**FIGURE 3.** Spectral changes observed upon mixing 10 μM fully reduced LSD1 with buffer containing 1.14 mM O$_2$. Spectra were collected every 4 ms; only spectra obtained with 20-ms intervals are shown. After deconvolution, the spectral data could be fitted with a single exponential decay function yielding a rate of 5.5 s$^{-1}$. The inset shows the absorbency at 458 nm as a function of time (black line). The gray line in the inset refers to an analogous experiment performed in the presence of 100 μM inhibitor (peptide 10).

---

Together, these experiments demonstrate that Lys$^4$ demethylation by LSD1 can be drastically reduced by other epigenetic marks present on the same histone H3 tail.

The Essential Role of Gly$^{12}$-Gly$^{13}$ of Histone H3—To investigate whether LSD1 activity is influenced by specific stereochemical and conformational properties of the histone tail, we inserted point mutations in the H3-K4 monomethylated peptide sequence (Fig. 2, a and b). We designed two peptides, one with both Gly$^{12}$ and Gly$^{13}$ mutated to alanine (peptide 6) and another with Pro$^{16}$ replaced by alanine (peptide 7). We chose glycine and proline as sites for mutagenesis because these amino acids can either restrain the peptide conformation (Pro$^{16}$) or provide the peptide with the conformational flexibility and/or adaptability required for binding (Gly$^{12}$-Gly$^{13}$). Peptide 7 exhibited normal catalytic properties suggesting that the conformational constraint imposed by Pro$^{16}$ ring was not critical for productive binding to LSD1. Conversely, peptide 6 did not function either as a substrate or as an effective inhibitor indicating that the double mutation at Gly$^{12}$-Gly$^{13}$ made the peptide unable to bind to the protein.

Specificity at the H3-K4 Site—The three-dimensional structure determination of LSD1 in the substrate-free state reveals a deep negatively charged pocket in proximity of the FAD cofactor and a shallow groove that might form the substrate-binding site (17, 18). We explored the specific properties of the Lys$^4$ binding site by using a trimethylated H3-K4 peptide and a peptide bearing an arginine at position 4. In the former, the charge on Lys$^4$ was embedded by the three methyl groups, whereas in the latter the positive charge was delocalized on the Arg guanidium group. As expected, we found that the trimethylated H3-K4 peptide (peptide 8) is not a substrate for LSD1, in agreement with the chemical nature of the flavin-dependent amine oxidation reaction that requires a free lone pair of electrons on the substrate nitrogen atom (19). Furthermore, peptide 8 was found to competitively inhibit LSD1 ($K_i$, 20 μM, Fig. 2b) but to a lower extent compared with the unmodified product ($K_i$, 2 μM; Table 1) (13). These data show that the enzyme senses the presence of the third methyl group so that LSD1 is both catalytically inactive against trimethylated Lys$^4$ and unable to bind it with high affinity. In this respect, it is worthwhile to note that LSD1 is much more efficient in discriminating among H3-K4 methylation states compared with other chromatin remodeling enzymes (20, 21).
the many peptides evaluated in this and previous studies (13, 22), peptide 9 exhibits the highest binding affinity and inhibitory power. To exclude that this tight binding simply reflects a particularly favorable interaction between the delocalized charge on the guanidinium group and LSD1 active site, we also probed guanidinium, phenyl guanidine, and arginine for potential LSD1 inhibition. None of these compounds exerted any inhibitory effect on LSD1 activity indicating that, although an Arg side chain at position 4 significantly improved peptide binding, guanidinium or the isolated arginine amino acid was unable to bind to LSD1. In a perspective of exploiting chromatin as a therapeutic target, knowledge on mechanisms of LSD1 inhibition may help future drug design studies.

**Oxygen Reactivity of LSD1**—The LSD1-catalyzed demethylation reaction is an oxidative process that requires an electron acceptor to reoxidize FAD (Fig. 1). Although it has been shown that molecular oxygen can function as the electron acceptor substrate, other molecules were also shown to efficiently reoxidize the LSD1-bound flavin (6). This raises several questions. Can dioxygen function as physiological electron acceptor? Does the reduced enzyme react with oxygen when it is still bound to the demethylated histone? What is the order of the events during the catalytic cycle? To clarify these issues, we investigated the kinetics of reduced LSD1 with oxygen at pH 7.5 and 25 °C by mixing a solution of artificially reduced enzyme with buffer solutions containing various concentrations of oxygen in the stopped-flow instrument (Fig. 3). A plot of the pseudo first-order rate constant versus oxygen concentration was linear (data not shown) giving a bimolecular rate constant of 9.6 × 10^3 M⁻¹ s⁻¹. This value is comparable with the rate constants typically found in other flavin-dependent oxidases (23) and indicates that LSD1 can be efficiently reoxidized by molecular oxygen. These observations suggest that in vivo, the enzyme likely acts as an oxidase using oxygen as the electron acceptor required for completion of the catalytic cycle. In addition, the measured rate constant value indicates that reoxidation by molecular oxygen is a relatively fast process compared with the turnover number measured in the steady-state kinetics experiments (13), implying that FAD reoxidation is not rate-limiting in the demethylation reaction. To dissect the order of events during the catalytic cycle, we analyzed the kinetics of the oxygen reaction in the presence of an unmodified histone H3 peptide known to be a LSD1 inhibitor (13) (peptide 10, Table 1). Addition of such peptide in stopped-flow measurements did not markedly influence the reoxidation rate (Fig. 3), suggesting that, after Lys⁴ demethylation, FAD can be reoxidized by oxygen before release of the demethylated product, i.e. LSD1 is likely to function through a ternary complex mechanism (23). This observation has two important implications. First, in the case of a dimethylated H3-K4 substrate, the two methyl groups can be sequentially removed, whereas the protein stays bound to the histone substrate. Second, after completion of the oxidative demethylation reaction, LSD1 can remain firmly attached to the histone, functioning as a docking element for the LSD1-containing corepressor complexes.

**In Vivo Assays of LSD1 Inhibition**—To further investigate the ability of LSD1 to bind to a demethylated histone peptide, we performed an in vivo transduction experiment by delivering peptides into cells (Fig. 4, a and b). Two peptides were selected for this experiment: the demethylated peptide consisting of residues 1–21 (peptide 10), which competitively inhibited recombinant LSD1 (Table 1); and a shorter unmodified peptide corresponding to residues 5–21 (peptide 11), which only weakly inhibited LSD1 (Ki, 90 μM; Table 1) (13). We reasoned that the presence of an excess of each peptide in HEK293 cells should compete with the cellular histone H3 tails for the binding to LSD1. To test the effect of the selected peptides on endogenous LSD1, we monitored the expression of the SCN1A and SCG10 genes that are targeted by LSD1-containing complexes (4, 24), compared with the expression levels of three different control genes, β₂-microglobulin (shown in Fig. 4b), β-actin, and β-tu-
bulin. We measured an increase in the expression of both SCN1A and SCG10 in the presence of peptide 10 (Fig. 4, a and b), whereas cells treated with peptide 11 (a very weak inhibitor) displayed no variations in the expression of target genes. These data are consistent with the notion that H3-K4 methylation is an activation mark and provides a correlation between the in vitro inhibitory properties of the peptides and their ability of affecting the LSD1 activity in vivo.

DISCUSSION

Our results highlight the sophistication and specificity of histone H3 N-terminal tail recognition by LSD1. Within the recognized segment of 21 amino acids, essential for productive binding, we found that both post-translational modifications and conformational properties of the histone H3 tail are important factors in substrate recognition and Lys4 demethylation. By using 21-amino acid peptides in biochemical assays, we previously demonstrated that phosphorylation on Ser10 drastically reduces LSD1 enzymatic activity (13). Here we show that hyperacetylation of Lys residues totally abolishes LSD1 function, whereas the strength of the effect of Arg methylation depends on the Arg residue that is modified, varying from a slight reduction in activity associated to Arg17 methylation to a complete loss of activity caused by methylation of Arg8. Methylation of Lys9 appears to be the only epigenetic modification that does not impair enzyme function (13). Taken together, these data indicate that epigenetic modifications on the H3 tail are first removed by other chromatin remodeling enzymes including histone deacetylases, arginine demethylases, and serine phosphatases that thereby “prepare” the histone tail for efficient LSD1-catalyzed Lys4 demethylation (Fig. 5). The fact that LSD1 is typically found in association with histone deacetylases 1/2 is of particular interest in that it suggests that these two enzymes might function as a sort of “double-blade razor” that first eliminates the acetyl groups from acetylated Lys residues and then removes the methyl group from Lys4, effectively pruning the histone H3 N-terminal tail.

The time-resolved stopped-flow experiments demonstrate that completion of LSD1 catalytic cycle through reoxidation of the FAD cofactor does not require release of the demethylated product. In addition, in vivo experiments showed that an unmodified 21-amino acid peptide (corresponding to the demethylated product) was able to reactivate target genes by inhibiting endogenous LSD1. On these bases we propose that after demethylating Lys4, LSD1 can complete its catalytic cycle remaining bound to the demethylated histone H3 tail. This view, LSD1 may trigger a process that leads to gene repression acting as a switch between chromatin states. We suggest that in specific contexts of genes targeted by LSD1-containing corepressor complexes, H3-K4 demethylation represents the removal of the last gene activation mark and that LSD1 recruits the forthcoming chromatin remodelers leading to the introduction of gene repression marks.

REFERENCES

1. Jenuwein, T., and Allis, C. D. (2001) Science 293, 1074–1080
2. Holbert, M. A., and Marmorstein, R. (2005) Curr. Opin. Struct. Biol. 15, 673–680
3. Biel, M., Wascholowski, V., and Giannis A. (2005) Angew. Chem. Int. Ed. Engl. 44, 3186–3216
4. Shi, Y., Lan, F., Matson, C., Mulligan, P., Whetstine, J. R., Cole, P. A., Casero, R. A., and Shi, Y. (2004) Cell 119, 941–953
5. Heinrichs, A. (2006) Nat. Rev. Mol. Cell Biol. 7, 466–467
6. Forneris, F., Binda, C., Vanoni, M. A., Mattevi, A., and Battaglioli, E. (2005) FEBS Lett. 579, 2203–2207
7. Sims, R. J., III, Nishioka, K., and Reinberg, D. (2003) *Trends Genet.* 19, 629–639
8. Humphrey, G. W., Wang, Y., Russanova, V. R., Hirai, T., Qin, J., Nakatani, Y., and Howard, B. H. (2001) *J. Biol. Chem.* 276, 6817–6824
9. Ballas, N., Battaglioli, E., Atouf, F., Andres, M. E., Chenoweth, J., Anderson, M. E., Burger, C., Moniwa, M., Davie, J. R., Bowers, W. J., Fedoroff, H. J., Rose, D. W., Rosenfeld, M. G., Brehm, P., and Mandel, G. (2001) *Neuron* 31, 353–365
10. Shi, Y., Sawada, J., Sui, G., Affar, B., Whetstine, J. R., Lan, F., Ogawa, H., Luke, M. P., Nakatani, Y., and Shi, Y. (2003) *Nature* 422, 735–738
11. Shi, Y., Matson, C., Lan, F., Iwase, S., Baba, T., and Shi, Y. (2005) *Mol. Cell* 19, 1–8
12. Metzger, E., Wissmann, M., Yin, N., Muller, J. M., Schneider, R., Peters, A. H., Gunther, T., Buettner, R., and Schule, R. (2005) *Nature* 437, 436–439
13. Forneris, F., Binda, C., Vanoni, M. A., Battaglioli, E., and Mattevi, A. (2005) *J. Biol. Chem.* 280, 41360–41365
14. Bevington, P. H. (1969) *Data Reduction and Error Analysis for the Physical Sciences*, pp. 56–62, McGraw-Hill, New York
15. Wysocka, J., Allis, C. C., and Coonrod, S. (2006) *Front. Biosci.* 11, 344–355
16. Wang, Y., Wysocka, J., Sayegh, J., Lee, Y. H., Perlin, J. R., Leonelli, L., Sonbuchner, L. S., McDonald, C. H., Cook, R. G., Dou, Y., Roeder, R. G., Clarke, S., Stallcup, M. R., Allis, C. D., and Coonrod, S. A. (2004) *Science* 306, 279–283
17. Stavropoulos, P., Blobel, G., and Hoelz, A. (2006) *Nat. Struct. Mol. Biol.* 7, 626–632
18. Yang, M., Gocke, C. B., Luo, X., Borek, D., Tomchick, D. R., Machius, M., Otwinowski, Z., and Yu, H. (2006) *Mol. Cell* 23, 377–387
19. Forneris, F., Binda, C., Vanoni, M. A., Mattevi, A., and Battaglioli, E. (2006) *The Enzymes-Protein Methyltransferases*, pp. 229–240, Academic Press, San Francisco
20. Li, H., Ilin, S., Wang, W., Duncan, E. M., Wysocka, J., Allis, C. D., and Patel, D. J. (2006) *Nature* 442, 91–95
21. Peña, P. V., Davrazou, F., Shi, X., Walter, K. L., Verkusha, V. V., Gozani, O., Zhao, R., and Kutateladze, T. (2006) *Nature* 442, 100–103
22. Cullhane, J. C., Szewczuck, L. M., Liu, X., Da, G., Marmorstein, R., and Cole, P. A. (2006) *J. Am. Chem. Soc.* 128, 4536–4537
23. Mattevi, A. (2006) *Trends Biochem. Sci.* 31, 276–283
24. Lunyak, V. V., Burgess, R., Prefontaine, G. G., Nelson, C., Sze, S. H., Chenoweth, J., Schwartz, P., Pevzner, P. A., Glass, C., Mandel, G., and Rosenfeld, M. G. (2002) *Science* 298, 1747–1752
25. Chin, H. G., Pradhan, M., Estève, P. O., Patnaik, D., Evans, T. C., Jr., and Pradhan, S. (2005) *Biochemistry* 44, 12998–13006