Human histone demethylase LSD1 is a flavin-dependent amine oxidase that catalyzes the specific removal of methyl groups from mono- and dimethylated Lys4 of histone H3. The N-terminal tail of H3 is subject to various covalent modifications, and a fundamental question in LSD1 biology is how these epigenetic marks affect the demethylase activity. We show that LSD1 does not have a strong preference for mono- or dimethylated Lys4 of H3. Substrate recognition is not confined to the residues neighboring Lys4, but it requires a sufficiently long peptide segment consisting of the N-terminal 20 amino acids of H3. Electrostatic interactions are an important factor in protein-substrate recognition, as indicated by the high sensitivity of $K_m$ to ionic strength. We have probed LSD1 for its ability to demethylate Lys4 in presence of a second modification on the same peptide substrate. Methylation of Lys9 does not affect enzyme catalysis. Conversely, Lys9 acetylation causes an almost 6-fold increase in the $K_m$ value, whereas phosphorylation of Ser10 totally abolishes activity. LSD1 is inhibited by a methylated peptide with an inhibition constant of 1.8 $\mu$M, suggesting that LSD1 can bind to H3 independently of Lys4 methylation. LSD1 is a chromatin-modifying enzyme, which is able to read different epigenetic marks on the histone N-terminal tail and can serve as a docking module for the stabilization of the associated corepressor complex(es) on chromatin.

Histones play a fundamental role in the control of a variety of cellular processes, including gene expression, DNA replication, and repair. Histone function is modulated through covalent modifications by acetylation, methylation, ubiquitination, and sumoylation (1, 2). These modifications have specific effects and act in a combinatorial manner defining the so-called histone code (1, 3). Very recently, Shi et al. (4) and our group (5) have reported on the discovery of the first enzyme able to specifically demethylate Lys4 of histone H3. The protein was therefore named LSD1 (for lysine-specific demethylase; it is also known as KIAA0601 and BHC110). The existence of histone demethylating activity was predicted that several histone demethylases are likely to exist in mammalian and other eukaryotic organisms (4). From a biochemical and structural standpoint, LSD1 belongs to the class of flavin-dependent amine oxidases, which typically catalyze the oxidation of an amine-containing substrate using molecular oxygen as the electron acceptor (5, 12). Indeed, LSD1 catalyzes the demethylase reaction through an oxidative process (5) (Fig. 1a). The amino group of the methylated Lys is oxidized presumably to generate the corresponding imine compound, which is subsequently hydrolyzed to produce formaldehyde. Substrate oxidation leads to the two-electron reduction of the protein-bound FAD cofactor, which is regenerated to its oxidized form by molecular oxygen to produce hydrogen peroxide.

LSD1 catalyzes the specific removal of methyl groups from mono- and dimethylated Lys4 of histone H3 (H3-K4) (4, 5), although an androgen receptor-controlled activity on H3-K9 has also been reported (13). The histone H3 N-terminal tail is a region characterized by extreme density of covalent modifications with diverse biological meanings (reviewed in Refs. 1, 2, and 6). Among them, of special significance are positions Lys4, Lys9, and Ser10, which are the most extensively and widely studied epigenetic marks of H3. Methylation of Lys4 is generally known to activate transcription, and, therefore, the demethylase activity of LSD1 removes an activation mark. Lys9 can be either acetylated or methylated, resulting in opposite effects; acetylation promotes formation of euchromatin, whereas methylation leads to repression of transcription. Phosphorylation of Ser10 is a pivotal activating signal and prevents the recruitment of the transcriptional repressors by methylated Lys9. A key problem is now to define the biological properties of LSD1, especially in relation to its ability to “read and interpret” these epigenetic marks on H3. We addressed this problem through an in vitro study that probed human LSD1 (Fig. 2a) for its ability to act on histone peptides bearing different covalent modifications.

EXPERIMENTAL PROCEDURES

Protein Purification—Escherichia coli cells overproducing a truncated form of LSD1 lacking the N-terminal 184 amino acids (Δ184) were grown as described (5). The recombinant protein was purified following the protocol of Forneris et al. (5), modified by omitting the cation exchange chromatography step. The purity of the protein was monitored by SDS-PAGE (Fig. 2a) and UV-visible absorption spectroscopy.

5 The abbreviations used are: H3-K4, Lys4 of histone 3; H3, histone 3; Δ184, human LSD1 mutant harboring a deletion of the N-terminal 184 amino acids; Mes, 2-N-morpholinoethanesulfonic acid; Taps, 3-(2-hydroxy-1,1-bis(hydroxymethyl)methyl)amino)-1-propanesulfonic acid.
The recombinant protein carries an N-terminal His<sub>6</sub> tag to facilitate purification. Control experiments have shown that removal of the His<sub>6</sub> tag by proteolysis (using TEV protease) did not alter the enzyme activity by more than 5%. Protein aliquots were stored in 50 mM sodium phosphate buffer, pH 7.5, and 50% (w/v) glycerol at −80 °C. Protein concentrations were routinely measured by absorption spectroscopy using an extinction coefficient of 10,790 M<sup>−1</sup> cm<sup>−1</sup> at 458 nm, which was determined based on absorbance changes observed after protein denaturation in 50 mM sodium phosphate buffer, pH 7.5, plus 0.3% (w/v) SDS (14).

Activity Assays—Peptides were purchased from Thermo Electron Corp. Their purity was greater than 90% as checked by analytical high pressure liquid chromatography and mass spectrometry. All other chemicals were from Sigma. Initial velocity measurements were performed using a peroxidase-coupled assay, which monitors hydrogen peroxide production (5). The time courses of the reaction were measured under aerobic conditions by using a Cary 100 UV-visible spectrophotometer equipped with thermostated cell holder (T = 25 °C). Reactions were started by adding 2 μl of protein solution (40 μM protein in 50 mM sodium phosphate buffer, pH 7.5, and 5% (w/v) glycerol) to reaction mixtures (150 μl) consisting of 50 mM Hepes/NaOH buffer, pH 7.5, 0.1 mM 4-aminophenylpyridine, 1 mM 3,5-dichloro-2-hydroxybenzenesulfonic acid, 0.35 μM horseradish peroxidase, and variable concentrations (2–100 μM) of methylated H3-K4 peptide. Absorbance changes were monitored at 515 nm, and an extinction coefficient of 26,000 M<sup>−1</sup> cm<sup>−1</sup> was used to calculate the initial velocity values. After visual inspection of doubled reciprocal plots, which were linear, the initial velocity values, expressed as apparent turnover values, were directly fitted to the Michaelis-Menten equation (Fig. 2b) using Grafit (Erithacus Software), which provides the values of apparent K<sub>cat</sub> and K<sub>m</sub> along with their associated errors.

Effect of Ionic Strength and pH on LSD1 Activity—To evaluate the effects of ionic strength on LSD1 activity, assays were performed by using increasing amounts of NaCl and KCl in the reaction mixture, starting from 1 mM up to 500 mM. In the determination of the pH dependence of steady-state parameters, the pH range was covered using Mes/NaOH (pH 6.5–7.0), Hepes/NaOH (7.0–8.0), Taps/NaOH (8.0–9.0) buffers at a final concentration of 50 mM. The ionic strength was kept constant at 48 mM by the addition of an appropriate amount of NaCl (up to 32 mM). We checked that the extinction coefficient of the chromophore generated by the reaction did not change as a function of pH. The K<sub>cat</sub> and K<sub>cat</sub>/K<sub>m</sub> data were best fit to the following equation, which assumes that a given parameter (P) decreases at low and high pH to a value as groups with pK<sub>cat</sub> and pK<sub>m</sub> dissociate (15): log P = log P<sub>max</sub> − log(1 + 10<sup>(pK<sub>cat</sub>−pH)/2</sup>) + 10<sup>(pK<sub>m</sub>−pH)/2</sup>.

Inhibition Studies—LSD1 inhibitors were tested by using the peroxidase-coupled assay in the presence of varied concentrations (2–100 μM) of methylated H3-K4 peptides and of the inhibitor under analysis (global range 1–300 μM, depending on the inhibitor strength). Initial velocity values were fit to equations describing competitive, uncompetitive, and noncompetitive inhibition patterns using Grafit. In all cases, the best fit was obtained with the equation describing a competitive inhibition, which allowed us to apply in some instances the Dixon plot for a graphical estimate of the K<sub>i</sub> values. We have attempted to measure peptide binding affinities also by isothermal titration microcalorimetry; however, we found that upon the addition of the peptides, the protein solution became transiently turbid, and this feature made the usage of microcalorimetry unfeasible.

RESULTS

Activities with Monomethylated and Dimethylated 21-Amino Acid Peptides—Biochemical analyses were performed using a recombinant N-terminally truncated form of human LSD1 (5) lacking the first 184 amino acids (we refer to the sequence deposited in the NCBI data base with accession code BAA25527; 886 amino acids). This protein form comprises the SWIRM and polyamine oxidase domains of LSD1 (9); analysis of the amino acid sequence by bioinformatic tools predicts that the 184 truncated amino acids do not fold into an ordered well defined conformation (5). Furthermore, control experiments (not shown) demonstrated that the catalytic properties of Δ184 are indistinguishable from those of longer variants lacking the first 157 and 34 residues, respectively, the latter being the protein variant used by Shi et al. (4). The Δ184 mutant was chosen, because it can be overproduced in E. coli cells and readily purified to a stable and homogeneous form in the amounts required to perform the described experiments (Fig. 2a). LSD1 activity was monitored using histone peptides, which allowed us to quantitatively evaluate the binding and catalytic parameters and the differential effects of various covalent modifications.

The first question we addressed in our study was the difference (if any) in the catalytic efficiency toward mono- and dimethylated H3-K4 substrates. We performed steady-state kinetic analysis using peptides consisting of the H3 N-terminal 21 amino acids modified by mono- or di-methylation of Lys<sup>4</sup>. These were the peptides employed in the early studies that led to the discovery of LSD1 function (4, 5). The catalytic efficiency using these two substrates turned out to be very similar (TABLE ONE and Fig. 2b), demonstrating that LSD1 does not exhibit a strong preference for mono- versus dimethylated H3-K4 substrate. An additional value of the steady-state enzymatic parameters measured with the 21-amino acid peptides was that they provided a benchmark to...
evaluate the activity of LSD1 toward peptides of different lengths or bearing additional covalent modifications (TABLE ONE).

The peptide substrate is rich in charged and titratable groups (the N-terminal amino group; three Arg and four Lys side chains) that in many cases are sites of epigenetic marks (Fig. 1b). We have therefore evaluated the effect of pH and ionic strength on the enzymatic activity. Both apparent \( k_{\text{cat}} \) and \( k_{\text{cat}}/K_m \) exhibit a bell-shaped pH dependence approaching 0 at low and high pH values (Fig. 3). The \( k_{\text{cat}}/K_m \) value increases as a group with \( pK_a \) of about 7.2 dissociates and decreases as a group with \( pK_a \) of about 8.9 becomes deprotonated. \( k_{\text{cat}}/K_m \) shows a similar profile, with \( pK_a \) values of 7.4 and 9.2, respectively. The similar \( pK_a \) values observed in the \( k_{\text{cat}}/K_m \) and \( k_{\text{cat}}/K_m \) profiles suggest that they reflect the dissociation state of the same groups and that such groups belong to the enzyme rather than to the peptide substrate (15). However, the possibility cannot be ruled out that the \( pK_a \) value of 8.9 observed in the \( k_{\text{cat}}/K_m \) profile actually reflects the dissociation of one of the Arg or Lys residues of the substrate. Despite the fact that the \( k_{\text{cat}}/K_m \) and \( k_{\text{cat}}/K_m \) profiles exhibit maximal values at pH 8.0 and 8.4, respectively, the data reported in this paper refer to assays performed at pH 7.5, which is of physiological significance and close to the optimal pH for activity.

![FIGURE 3. pH dependence of the steady-state kinetic parameters of the 21aa-[dimethyl]K4 demethylation reaction catalyzed by LSD1. Filled circles, \( k_{\text{cat}}/K_m \) values; open circles, \( k_{\text{cat}}/K_m \). The curves show the best fit of the data to a double pK model (for \( k_{\text{cat}}/K_m \) pK1 = 7.2 ± 0.1, and pK2 = 8.9 ± 0.1; for \( k_{\text{cat}}/K_m \) pK1 = 7.4 ± 0.2, and pK2 = 9.2 ± 0.3). Propagation of statistical error value was carried out as described (22).]

![TABLE ONE Substrate specificity of human LSD1.

| Peptides of 21 amino acids\(^{a}\) | \( k_{\text{cat}} \) \( \mu \text{M}^{-1} \text{min}^{-1} \) | \( K_m \) \( \mu \text{M} \) | \( k_{\text{cat}}/K_m \) \( \mu \text{M}^{-1} \text{min}^{-1} \) | Percentage \(^{c}\) |
|---------------------------------|-----------------|----------------|----------------------------|-------------|
| 21aa-[monomethyl]K4             | 3.2 ± 0.1       | 3.0 ± 0.3      | 1.1 ± 0.12                 | 100         |
| 21aa-[dimethyl]K4               | 8.1 ± 0.2       | 4.2 ± 0.5      | 1.9 ± 0.23                 | 173         |
| 21aa-[dimethyl]K4 in 80 mM NaCl | 7.1 ± 0.6       | 12.8 ± 2.8     | 0.55 ± 0.13                | 50          |
| Peptides of different lengths   |                 |                |                           |             |
| 30aa-[monomethyl]K4             | 2.9 ± 0.1       | 3.4 ± 0.5      | 0.8 ± 0.12                 | 73          |
| 16aa-[monomethyl]K4             | ND              | ND             | ND                        | ND          |
| 12aa-[monomethyl]K4             | ND              | ND             | ND                        | ND          |
| 8aa-[monomethyl]K4              | ND              | ND             | ND                        | ND          |
| Epigenetic marks                |                 |                |                           |             |
| 21aa-[monomethyl]K4-[monomethyl]K9 | 5.6 ± 0.2       | 3.9 ± 0.5      | 1.4 ± 0.19                 | 130         |
| 21aa-[monomethyl]K4-[phospho]S10 | ND              | ND             | ND                        | ND          |
| 21aa-[monomethyl]K4-[acetyl]K9  | 4.1 ± 0.3       | 17.5 ± 4.0     | 0.23 ± 0.06                | 21          |

\(^{a}\) Apparent steady-state kinetic parameters were determined in air-saturated buffer (50 mM Hepes/NaOH, pH 7.5, at 25 °C) by a horseradish peroxidase-coupled assay (5).

\(^{b}\) Propagation of statistical error was carried out as described (22).

\(^{c}\) The percentage of activity with reference to that measured with 21aa-[monomethyl]K4.

\(^{d}\) As a control, the \( K_m \) values for 21aa-[dimethyl]K4 and 21aa-[monomethyl]K4 peptides were measured also by means of a ferricenium assay (5), with ferricenium being a nonphysiological monoelectronic acceptor that reacts directly with the reduced LSD1-bound FAD cofactor. The resulting values were essentially identical to those measured with the peroxidase-coupled assay (not shown).

\(^{e}\) The assay was performed in 80 mM NaCl, 50 mM Hepes/NaOH, pH 7.5, at 25 °C.

\(^{f}\) ND, not determined. The activity is absent or barely detectable.
A crucial role of ionizable groups is suggested also by the analysis of the effect of the ionic strength on LSD1 catalytic function. The enzyme activity remains essentially constant at NaCl or KCl concentrations up to 50 mM, but it decreases sharply at higher concentrations so that only 10% of residual activity is measured at concentrations greater than 150 mM. The observed decrease of activity is due to a $K_m$ increase, as shown by the fact that at 80 mM NaCl, the $K_m$ value is 3-fold higher than that found in the absence of added salt, whereas the measured $k_{cat}$ values at 0 and 80 mM NaCl are essentially identical (see TABLE ONE). In this respect, the 4-fold lower specific activity previously reported for LSD1 (5) can be ascribed to the ionic strengths of the initial assays, which were carried out in 50 mM sodium phosphate buffer, pH 7.5 (ionic strength = 132 mM) as opposed to 50 mM Hepes/NaOH, pH 7.5 (ionic strength = 22 mM).

### TABLE ONE

| Peptide                        | $K_m$  |
|-------------------------------|--------|
| 21aa (product)                | 1.8 ± 0.6 |
| 12aa                         | 199 ± 22 |
| Nter-Δ5–21aa                  | 87 ± 29  |
| 21aa-[monomethyl[K4]-phospho]S10 | 31 ± 5   |

* Enzymatic activities were evaluated in air-saturated buffer (50 mM Hepes, pH 7.5, at 25 °C) by a horseradish peroxidase-coupled assay using a 21-amino acid peptide monomethylated on Lys4 (5). In all cases, the inhibition was of the competitive type.

**Enzyme Inhibition**—The finding that LSD1 recognizes a long H3 peptide segment suggested the possibility that the reaction product (i.e., the demethylated peptide) could bind to the protein. Experimental verification of this hypothesis revealed that the unmodified 21-amino acid peptide is indeed a competitive inhibitor with a $K_i$ of 1.8 μM (TABLE TWO). Consistent with the substrate specificity studies, a shorter 12-amino acid peptide exhibited a >100-fold reduction in affinity (TABLE TWO). Likewise, deletion of the four N-terminal residues from the 21-amino acid peptide resulted in a 40-fold reduction of the inhibitory power (TABLE TWO). These data imply that methylation of Lys9 is not essential for binding, whereas the affinity is critically dependent on the presence of a sufficiently long segment of the H3 N-terminal tail.

Recently, Metzger et al. (13) have reported on an androgen receptor-dependent activity of LSD1. The interaction with the receptor appears to switch the substrate specificity, making LSD1 able to demethylate Lys9 of H3 (isolated LSD1 does not act on Lys9) (4, 5). This activity was shown to be inhibited by pargyline, deprenyl, and clorgyline, which are specific inhibitors of human monoamine oxidase A and/or B (17). We have tested whether these inhibitors act on LSD1, interfering with its H3-K4 demethylase function. None of these compounds induced the characteristic changes in the absorption spectrum of the protein-bound flavin that are found in inhibited monoamine oxidases (data not shown).

**Readout of Multiple Epigenetic Marks**—The N-terminal tail of H3 is subject to various covalent modifications, and a crucial question in LSD1 biology is how these epigenetic marks affect the demethylase activity. We have probed LSD1 for its ability to demethylate Lys9 in the presence of a second modification on the same 21-amino acid peptide substrate. As shown in TABLE ONE, we tested the effects of methylation and acetylation of Lys9 and phosphorylation of Ser10. Methylation of Lys9 did not affect activity, whereas Lys9 acetylation caused an almost 6-fold increase in the $K_m$ value, indicating that this covalent modification significantly affects the substrate binding affinity. Even more dramatic is the effect of Ser10 phosphorylation, which totally suppressed the ability of the peptide to function as substrate. Taken together, these data are consistent with the notion that electrostatic interactions are especially relevant in the enzyme-substrate recognition. Methylation of Lys9, which does not neutralize the Lys positive charge, has no effect on enzymatic reaction. Conversely, neutralization of Lys9 positive charge by acetylation reduces affinity, whereas introduction of the negatively charged phosphate group on Ser10 completely abolishes activity. These findings predict that the Lys9-Ser10 pair binds to a cluster of hydrogen bond acceptors and/or negatively charged groups on LSD1.
phate group on Ser\textsuperscript{10} appears to prevent the catalytically competent positioning of the methylated Lys\textsuperscript{4} inside the active site (Fig. 4).

**DISCUSSION**

The main theme emerging from these data is that LSD1 is finely tuned and highly specific. This finding has profound implications for the control of histone H3 demethylation and its role in chromatin biology. A first important observation is that LSD1 does not have a strong preference for mono- or dimethylated H3-K4. This feature contrasts with the properties of many lysine methyltransferases that specifically perform the addition only of the first, second, or third methyl group on a Lys residue (18). The fact that LSD1 acts with similar efficiency (TABLE ONE) on mono- and dimethylated substrates indicates that the \textit{in vivo} function of LSD1 is to reset H3-K4 to its demethylated “ground” state.

We have shown that an H3 peptide must contain the first \textemdash 20 N-terminal amino acids of H3 in order to be a substrate. The sequence of this polypeptide stretch has many charged residues, and electrostatic interactions are likely to dominate the protein-substrate association, providing a rationale for the high sensitivity of \(K_m\) to ionic strength. This ability of recognizing a “long” and “heavily charged” segment of the histone N-terminal tail enables LSD1 to detect the presence of multiple post-translational modifications written in the H3 tail (Fig. 5). In this regard, several important functional properties have been unraveled. (i) LSD1 has an exquisite specificity. Although the site of oxidative attack is Lys\textsuperscript{4}, the enzyme senses covalent modifications on neighboring residues. (ii) The charge distribution on the histone N-terminal tail appears to be a key determinant in the readout of the histone code by LSD1, as exemplified by the differential effects of methylation and acetylation of Lys\textsuperscript{9} on enzyme activity. (iii) The Lys\textsuperscript{4} demethylase activity of LSD1 is not affected by methylation of Lys\textsuperscript{9}, which is generally known to cause gene repression. (iv) Acetylated Lys\textsuperscript{9} and phosphorylated Ser\textsuperscript{10} cross-talk and act synergistically in the Lys\textsuperscript{4} demethylation process. Ser\textsuperscript{10} dephosphorylation is a prerequisite for LSD1 function, whereas Lys\textsuperscript{9} deacetylation significantly improves activity mainly through an increase in substrate affinity. (v) These features provide insight into the order and interplay of the events that lead to transcriptional repression. Dephosphorylation of Ser\textsuperscript{10} by a phosphatase must precede H3 demethylation catalyzed by LSD1, emphasizing the role of Ser\textsuperscript{10} phosphorylation/dephosphorylation in triggering the cascades of events that lead to transcriptional activation/repression. Lys\textsuperscript{9} deacetylation by HDAC1/2 (which are typically associated with LSD1) makes H3 more susceptible to LSD1-catalyzed demethylation.

The activity of LSD1 can be modulated by its associated protein factors (11, 13, 16), and the enzyme specificity observed on free peptides might be different when chromatin and nucleosomes are used as substrates. However, it is of notice that the lower efficiency observed with a peptide acetylated on Lys\textsuperscript{9} is consistent with the finding by Shi \textit{et al.} (11) that LSD1 is more active toward hypoacetylated nucleosomes.

LSD1 binds with significant affinity (TABLE TWO) to a demethylated H3 peptide, as indicated by an inhibition constant that compares with the \(K_m\) for the peptide substrates. The measured affinity is similar to the affinity measured for other H3-binding proteins such as the chromodomain-containing proteins HP1 (19) and Polycomb (20). This finding raises the intriguing hypothesis that LSD1 plays a double role, namely that of Lys\textsuperscript{4}-specific demethylase and of H3 N-terminal tail binder. LSD1 is member of the co-repressor complex CoREST-HDAC1/2, a module that has been found in association with several large multiprotein complexes. The finding that LSD1 has significant affinity for the H3 peptide indicates that it could serve as a docking module for the stabilization of the associated corepressor complex(es) on chromatin. At least in one case, it has been demonstrated that the CoREST corepressor complex remains bound to chromatin after the release of the DNA-binding protein responsible for its initial recruitment (21).

The main conclusion from these studies is that LSD1 is not only a chromatin-modifying enzyme, but it is able to interpret histone marks on the Lys\textsuperscript{2}-Ser\textsuperscript{10} locus and to stabilize the co-repressor complex by binding to the demethylated H3. Knowledge of LSD1 substrate specific-
ity properties will provide a framework for future inhibitor design studies.

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REFERENCES

1. Biel, M., Wascholowski, V., and Giannis, A. (2005) Angew. Chem. Int. Ed. Engl. 44, 3186–3216
2. Fischle, W., Wang, Y., and Allis, C. D. (2003) Nature 425, 475–479
3. Jenuwein, T., and Allis, C. D. (2001) Science 293, 1074–1080
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. Forneris, F., Binda, C., Vanoni, M. A., Mattevi, A., and Battaglioli, E. (2005) FERS Lett. 579, 2203–2207
6. Sims, R. J. 3rd, Nishioka, K., and Reinberg, D. (2003) Trends Genet. 19, 629–639
7. Bannister, A. J., and Kouzarides, T. (2005) Nature 436, 1103–1106
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., Atoud, F., Andres, M. E., Chenoweth, J., Anderson, M. E., Burger, C., Moniwa, M., Davie, J. R., Bowers, W. J., Federoff, 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., Shi, Y. (2005) Mol. Cell. 19, 857–864
12. Binda, C., Mattevi, A., and Edmondson, D. E. (2002) J. Biol. Chem. 277, 23973–23976
13. 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
14. Aliverti, A., Curti, B., and Vanoni, M. A. (1999) Methods Mol. Biol. 131, 9–23
15. Segel, I. H. (1975) in Enzyme Kinetics, John Wiley & Sons, Inc., New York
16. Lee, M. G., Wynder, C., Cooch, N., and Shiekhattar, R. (2005) Nature 437, 432–435
17. Edmondson, D. E., Mattevi, A., Binda, C., Li, M., and Hubalek, F. (2004) Curr. Med. Chem. 11, 1983–1993
18. Cheng, X., Collins, R. E., and Zhang, X. (2005) Annu. Rev. Biophys. Biomol. Struct. 34, 267–294
19. Jacobs, S. A., Taverna, S. D., Zhang, Y., Briggs, S. D., Li, J., Eissenberg, J. C., Allis, C. D., and Khorasanizadeh, S. (2001) EMBO J. 20, 5232–5241
20. Fischle, W., Wang, Y., Jacobs, S. A., Kim, Y., Allis, C. D., and Khorasanizadeh, S. (2003) Genes Dev. 17, 1870–1881
21. Ballas, N., Grunseich, C., Lu, D. D., Speh, J. C., and Mandel, G. (2005) Cell 121, 645–657
22. Bevington, P. H. (1969) Data Reduction and Error Analysis for the Physical Sciences, pp. 56–62, McGraw-Hill, New York