The mixed lineage leukemia protein-1 (MLL1) catalyzes histone H3 lysine 4 methylation and is regulated by interaction with WDR5 (WD-repeat protein-5), RbBP5 (retinoblastoma-binding protein-5), and the Ash2L (absent, small, homeotic discs-2-like) oncoprotein. In the accompanying investigation, we describe the identification of a conserved arginine containing motif, called the “Win” or WDR5 interaction motif, that is essential for the assembly and H3K4 dimethylation activity of the MLL1 core complex. Here we present a 1.7-A crystal structure of WDR5 bound to a peptide derived from the MLL1 Win motif. Our results show that Arg-3765 of MLL1 is bound in the same arginine binding pocket on WDR5 that was previously suggested to bind histone H3. Thermodynamic binding experiments show that the MLL1 Win peptide is preferentially recognized by WDR5. These results are consistent with a model in which WDR5 recognizes Arg-3765 of MLL1, which is essential for the assembly and enzymatic activity of the MLL1 core complex.

Histone H3 lysine 4 methylation catalyzed by the mixed lineage leukemia protein-1 (MLL1) is important for the regulation of *hox* genes in hematopoiesis and development (1–3). MLL1 belongs to a family of SET domain histone methyltransferases that are regulated by a core complex of proteins that are conserved from yeast to humans (4–13). Previous studies have shown that WDR5 interacts directly with the catalytic subunits of MLL1, MLL3, and MLL4 and mediates interactions with the other components of SET1 complexes (6, 12, 14). In addition, previous crystal structures have shown that WDR5 recognizes arginine in a histone H3R2 context (15–18) (PDB codes 2h13, 2g99, 2oco, 2h9n, 2cnx, 2o9k, 2g9a, 2h6k, 2h6n, 2h9p, 2h9n, and 2h6q), which has been suggested to mediate histone methylation by SET1 family complexes (12, 17). In the accompanying investigation (22), we describe the identification of a WDR5 interaction motif, or “Win” motif, which is located in the N-SET region of MLL1. We demonstrate that the MLL1 Win motif is highly conserved among metazoan SET1 family members and is recognized by WDR5 in a mechanism that is crucial for the assembly and enzymatic activity of the MLL1 core complex in vitro. Intriguingly, our functional results suggest that WDR5 preferentially recognizes the conserved Arg-3765 within the MLL1 Win motif using the same arginine binding site as suggested previously for histone H3. To test this hypothesis, we determined the crystal structure of WDR5 bound to a peptide derived from the MLL1 Win motif. Our results are consistent with a model in which WDR5 preferentially recognizes Arg-3765 of MLL1 using the same binding site previously suggested for histone H3. These results suggest that the previously observed histone H3-WDR5 interaction may not be relevant for the enzymatic activity of the MLL1 core complex.

**EXPERIMENTAL PROCEDURES**

**Crystallization and Structure Determination**—An N-terminally truncated form of WDR5 (residues 23–335, AN-WDR5) was expressed and purified as described previously (14). As a final step of purification and for buffer exchange, all proteins were passed through a gel filtration column pre-equilibrated with 20 mM Tris(7.5), 300 mM NaCl, 1 mM (tris(2-carboxyethyl)phosphine, and 1 mM ZnCl$_2$. The protein was concentrated to 9 mg/ml and mixed with a stock solution of the MLL1 Win peptide (acetylGSARAEVHLRKSNH$_2$) dissolved in the same buffer. The final concentration of AN-WDR5 and peptide was 8.2 mg/ml and 0.9 mM, respectively. The hanging drop vapor diffusion method was used for crystallization, using as mother liquor 30 mM ammonium sulfate, 30% (w/v) polyethylene glycol-col-3350, and 100 mM HEPES (pH 7.5). Crystals were flash-frozen in mother liquor containing 40% polyethylene glycol-col-3350. Diffraction data were collected at the National Synchrotron Light Source (NSLS) on beamline X6A. Data were collected with a Quantum 210 CCD detector and reduced with HKL-2000 and CCP4. Data collection statistics are given in Table 1. The MLL1 Win peptide-WDR5 complex structure was determined by molecular replacement with MOLREP (19) using the coordinates of the previously determined structure of WDR5 (PDB code: 2H68) as a search model (17). After an initial rigid body refinement, the structure was further refined with rounds of simulated annealing, energy minimization, and individual B-factor refinement with a maximum likelihood target using CNS (20). Difference Fourier maps were calculated with CNS and used to locate electron density corresponding to
bound peptide, and the structure was built using O (21). The peptide position was verified with simulated annealing omit maps. Final refinement statistics are given in Table 1. All structural figures were generated with PyMOL (23).

Isothermal Titration Calorimetry—Isothermal titration calorimetry (ITC) experiments were performed using VP-ITC calorimeter (MicroCal). All experiments were performed at 20 °C in a sample buffer containing 20 mM Tris (pH 7.5), 150 mM sodium chloride, and 1 mM (tris(2-carboxyethyl)phosphine. A 0.05 mM solution of full-length WDR5 diluted in sample buffer was made up in sample buffer was loaded into the injection syringe. For each experiment, a 180-s delay at the start of the experiment was placed in the sample cell, and a 0.45 mM solution of peptide made up in sample buffer was loaded into the injection syringe. The sample was stirred at 300 rpm throughout. Blank injections of the peptide into buffer were subtracted from the experimental titrations, and binding isothersms were fit to a theoretical titration curve describing one binding site per titrant. A nonlinear best-fit binding isotherm for the data was used to calculate the protein–titrant stoichiometry, dissociation constant, and standard change in enthalpy using the supplied manufacturer’s software Origin 7.0 (Origin-Lab Corp.).

RESULTS AND DISCUSSION

We co-crystallized WDR5 with the MLL1 Win peptide consisting of amino acid residues 3762–3773 of MLL1 and determined the x-ray structure of the complex at 1.72-Å resolution (Fig. 1, Table 1). The overall structure of WDR5 is highly similar to that of previously reported structures of WDR5 bound to histone H3 peptides (15–18), which superimpose with average root mean square differences in Ca positions of 0.1- and 0.3-Å for structures previously determined in space groups C2221, (15) and P212121 (17), respectively. Like previously reported structures of WDR5, the present structure is formed by seven WD40 repeats or β-propeller blades that are organized around a central cavity. A water-filled tunnel connects the two openings of the cavity at the top and bottom of the structure (Fig. 1b), with the top opening having a smaller diameter. Previous structures of WDR5 bound to H3 peptides show that the N terminus of histone H3 forms a partial 310-helix when bound to the smaller opening at the top of WDR5, which is anchored by the insertion of H3R2 into the central tunnel (15–18). In the present structure, the initial difference electron density map clearly identifies the location of the MLL1 Win peptide, which, consistent with our prediction, is located in the same position as suggested previously for histone H3 peptide binding (15–18) (Fig. 1a).

Nine residues from the N terminus could be modeled into the density, with the last three residues of the C terminus being disordered. Consistent with the tight binding observed in ITC experiments (see below), the electron density from simulated annealing Fo – Fe omit maps covers most of the modeled peptide, even when contoured at 3σ (Fig. 2).

Like histone H3 bound to WDR5, the N terminus of the MLL1 Win peptide forms a 310-helix that fits snugly into the outer opening of the central cavity, like a cork in a bottle (Fig. 1). Conserved MLL1 residues GSARAE all participate in the formation of the 310-helix, which is stabilized by intramolecular i → i + 3 main-chain hydrogen bonds between Ser-3763 and Ala-3766 and between Ala-3764 and Glu-3767. This configuration is further stabilized by an intramolecular hydrogen bond between the carboxylate side chain of Glu-3767 and the amide nitrogen of Gly-3762 (Fig. 2). This intramolecular hydrogen bond gives the sequence a cyclical conformation that allows the 310-helix to fit precisely into the outer opening of the central cavity (Fig. 1a).

An extensive network of direct and water-mediated intermolecular

![FIGURE 1. Crystal structure of WDR5 in complex with the MLL1 Win peptide.](image)
hydrogen bonds and van der Waals interactions stabilizes the binding of the $3_{10}$-helix to the opening of the central cavity. In previous structures of WDR5 bound to histone H3 peptides, Asp-107 of WDR5 forms a salt bridge with the N terminus of the H3 peptide and coordinates a network of water molecules that fill part of the central cavity (15–18). In the present structure, these water molecules are displaced by Gly-3762 and Ser-3763 of the MLL1 peptide, which participate in multiple hydrogen bond and van der Waals interactions with Ala-3764 and Arg-3765 of the MLL1 Win peptide (Fig. 2). It is likely that these newly observed interactions account for the high affinity of WDR5 for the MLL1 Win peptide (Fig. 3). Indeed, the high quality of density at the N-terminal end of the peptide suggests that additional residues at the N terminus could show additional interactions. However, ITC binding experiments with a peptide containing six additional residues on the N terminus shows identical affinity with that of the shorter peptide used for crystallization (Fig. 3), suggesting that the peptide used in this investigation captures most if not all of the important interactions.

Ala-3764 and Arg-3765 of the MLL1 Win peptide occupy similar positions as Ala-1 and Arg-2 of the H3 peptide in the previously reported H3-WDR5 complexes (Fig. 4). As in previous structures, the side chain of Arg-3765 of MLL1 inserts into the central tunnel of WDR5 and is stabilized by an extensive network of hydrogen bond, $\pi$-$\pi$ and cation-$\pi$, and hydrophobic interactions with WDR5 residues: Ser-91, Phe-133, Ser-175, Ser-218, Cys-261, Phe-263, and Ile-305 (Fig. 2). As previously noted for Arg-2 of histone H3 (15), it is likely that the sum of these interactions accounts for the majority of the binding energy between MLL1 and WDR5, which explains why mutation of Arg-3765 in MLL1 is sufficient to abolish the assembly and dimethylation activity of the MLL1 core complex in vitro (22). This suggests a reason for the conservation of Arg-3765 in MLL1 orthologs and other SET1 family members. In addition, the guanidinium moiety of Arg-3765 is sandwiched between the conserved aromatic side chains of Phe-133 and Phe-263 of WDR5, which are important for the interaction. This probably explains why the replacement of Phe-133 with alanine significantly diminishes the interaction between MLL1 and the WDR5-RbBP5-Ash2L subcomplex in vitro (22) and in vivo (12).

Amino acids on the C-terminal side of Arg-3765 in the MLL1 Win peptide also participate in a number of intermolecular interactions with WDR5. Ala-3766 and Cys-261 of MLL1 interacts with Ala-47 and Tyr-260 of WDR5. The aromatic ring of Tyr-260 lines the outer edge of the central cavity and makes van der Waals contacts with Ala-3766, Cys-261, and Val-3768 of the MLL1 Win peptide (Fig. 2). Glu-3767 and of the MLL1 Win peptide occupies a similar position as Lys-4 of the histone H3 peptide in previous structures with WDR5 (Fig. 4). However, in contrast to previous structures in which the side chain of H3K4 was poorly ordered (15), the side chain of Glu-3767 is well ordered in the present structure (Fig. 2). This is due to direct and water-mediated intramolecular hydrogen bonds involving the side chain carboxylate of Glu-3767 and the main chain amides of Gly-3762 and Val-3768. This may explain the high sequence
conservation of Glu-3767 in SET1 family members (22). In addition, the carbonyl oxygen of Glu-3767 forms a water-mediated hydrogen bond with the side-chain of Tyr-191.

The last two residues of the MLL1 Win peptide that could be modeled into the density include His-3769 and Leu-3770, the latter of which was less ordered. The main chain of His-3769 participates in water-mediated hydrogen bonds with Lys-259, whereas the side chain of His-3769 forms hydrogen bond, van der Waals, and T-stacking interactions with WDR5 residues: Phe-149, Asp-172, Pro-173, and Tyr-191. This results in theimidazole of His-3769 being well ordered, even when consoured at 3-σ. Leu-3770 is less ordered than previous residues and does not appear to make any interactions with WDR5.

It is likely that these newly observed interactions account in part for the greater affinity of the MLL1 Win peptide when compared with previously published values for histone H3 peptide binding to free WDR5. ITC measurements show that the MLL1 Win peptide binds to WDR5 with an equilibrium dissociation constant $K_d$ of 1.7 ± 0.1 μM (Fig. 3), which is between 3- and 45-fold lower than that previously reported for the binding of dimethylated histone H3K4 peptides to WDR5 using a similar technique (15, 18). However, the affinity observed in ITC experiments does not appear to completely recapitulate the full interaction surface between MLL1 and WDR5, which interact with a $K_d$ of ~0.12 μM in solution when measured by sedimentation velocity analytical ultracentrifugation (22). Nevertheless, as shown in the accompanying investigation, the same MLL1 Win peptide specifically inhibits the dimethylation activity of the MLL1 core complex by dissociating the catalytic SET domain of MLL1 from the regulatory WDR5-RbBP5-Ash2L subcomplex (22).

These results are consistent with our hypothesis that the recognition of Arg-3765 of MLL1 by WDR5 is crucial for the interaction between WDR5 and MLL1. As demonstrated in the accompanying investigation (22), this interaction is essential for the assembly and activity of the MLL1 core complex. Because the MLL1 Win motif is highly conserved among metazoan SET1 family members (22), these results can probably be generalized for the interaction of WDR5 with other SET1 family histone methyltransferases.

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REFERENCES

1. Milne, T. A., Briggs, S. D., Brock, H. W., Martin, M. E., Gibbs, D., Allis, C. D., and Hess, J. L. (2002) Mol. Cell 10, 1107–1117
2. Terranova, R., Agerbi, H., Boned, A., Meresse, S., and Djabali, M. (2006) Proc. Natl. Acad. Sci. U. S. A. 103, 6629–6634
3. Yu, B. D., Hess, J. L., Hornung, S. E., Brown, G. A., and Korsmeyer, S. J. (1995) Nature 378, 505–508
4. Yokoyama, A., Wang, Z., Wysocka, J., Sanyal, M., Auferio, D. J., Kitabayashi, I., Herr, W., and Cleary, M. L. (2004) Mol. Cell Biol. 24, 5639–5649
5. Steward, M. M., Lee, J. S., O’Donovan, A., Wyatt, M., Bernstein, B. E., and Shilatifard, A. (2006) Nat. Struct. Mol. Biol. 13, 852–854
6. Dou, Y., Milne, T. A., Tackett, A. J., Smith, E. R., Fukuda, A., Wysocka, J., Allis, C. D., Chait, B. T., Hess, J. L., and Roeder, R. G. (2005) Cell 121, 873–885
7. Wysocka, J., Myers, M. P., Laherty, C. D., Eisenman, R. N., and Herr, W. (2003) Genes Dev. 17, 896–911
8. Nakamura, T., Morit, T., Tada, S., Krajecki, W., Rozovskaia, T., Wassell, R., Dubois, G., Mazo, A., Croce, C. M., and Canaan, E. (2002) Mol Cell 10, 1119–1128
9. Nagy, P. L., Griesenbeck, J., Kornberg, R. D., and Cleary, M. L. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 90–94
10. Roguev, A., Schaft, D., Shevchenko, A., Pijnappel, W. W., Wilm, M., Aasland, R., and Stewart, A. F. (2001) EMBO J. 20, 7137–7148
11. Miller, T., Krogan, N. J., Dover, J., Erdjument-Bromage, H., Tempst, P., Johnston, M., Greenblatt, J. F., and Shilatifard, A. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 12902–12907
12. Dou, Y., Milne, T. A., Ruthenburg, A. J., Lee, S., Lee, J. W., Verdine, G. L., Allis, C. D., and Roeder, R. G. (2006) Nat. Struct. Mol. Biol. 13, 713–719
13. Lee, J. H., Tate, C. M., You, J. S., and Skalnik, D. G. (2007) J. Biol. Chem. 282, 13419–13428
14. Cho, Y. W., Hong, T., Hong, S., Guo, H., Yu, H., Kim, D., Gusczynski, T., Dressler, G. R., Copeland, T. D., Kalkum, M., and Ge, K. (2007) J. Biol. Chem. 282, 20395–20406
15. Couture, J. F., Collazo, E., and Trivel, R. C. (2006) Nat. Struct. Mol. Biol. 13, 698–703
16. Han, Z., Guo, L., Wang, H., Shen, Y., Deng, X. W., and Chai, J. (2006) Mol Cell 22, 137–144
17. Ruthenburg, A. J., Wang, W., Graybosch, D. M., Li, H., Allis, C. D., Patel, D. J., and Verdine, G. L. (2006) Nat. Struct. Mol. Biol. 13, 704–712
18. Schuetz, A., Allali-Hassani, A., Martin, F., Loppnau, P., Vedadi, M., Buchkarev, A., Plotnikov, A. N., Arrowsmith, C. H., and Min, J. (2006) EMBO J. 25, 4245–4252
19. Vagin, A. T., and Teplyakov, A. (1997) J. Appl. Crystallogr. 30, 1022–1025
20. Brunger, A. T., Adams, P. D., Clore, G. M., DeLano, W. L., Gros, P., Grosse-Kunstleve, R. W., Jiang, S., Kuszewski, J., Nilges, M., Pannu, N. S., Read, R. J., Rice, L. M., Simonson, T., and Warren, G. L. (1998) Acta Crystallogr. Sect. D Biol. Crystallogr. 54, 905–921
21. Jones, T. A., Zou, J. Y., Cowan, S. W., and Kjeldgaard, M. (1991) Acta Crystallogr. Sect. A 47, 110–119
22. Patel, A., Vought, V. E., Dharmarajan, V., and Cosgrove, M. S. (2008) J. Biol. Chem. 283, 32162–32175
23. DeLano, W. L. (2002) The PyMOL Molecular Graphics System, DeLano Scientific, San Carlos, CA.