Role of a DEF/Y motif in histone H2A-H2B recognition and nucleosome editing

Yan Huang[a,b], Lu Sun[c], Leonidas Pierrakeas[c], Linchang Dai[a], Lu Pan[a], Ed Luk[c,d,1], and Zheng Zhou[a,b,1]

[a]National Laboratory of Biomacromolecules, CAS Center for Excellence in Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences, 100101 Beijing, China; [b]University of Chinese Academy of Sciences, 100049 Beijing, China; [c]Department of Biochemistry and Cell Biology, Stony Brook University, NY 11794-5215; and [d]Renaissance School of Medicine, Stony Brook University, NY 11794-5215

Edited by Song Tan, The Pennsylvania State University, University Park, PA, and accepted by Editorial Board Member Michael F. Summers December 30, 2019 (received for review August 19, 2019)

The SWR complex edits the histone composition of nucleosomes at promoters to facilitate transcription by replacing the two nucleosomal H2A-H2B (A-B) dimers with H2A.Z-H2B (Z-B) dimers. Swc5, a subunit of SWR, binds to A-B dimers, but its role in the histone replacement reaction was unclear. In this study, we showed that Swc5 uses a tandem DEF/Y motif within an intrinsically disordered region to engage the A-B dimer. A 2.37-Å X-ray crystal structure of the histone binding domain of Swc5 in complex with an A-B dimer showed that consecutive acidic residues and flanking hydrophobic residues of Swc5 form a cap over the histones, excluding histone–DNA interaction. Mutations in Swc5 DEF/Y inhibited the nucleosome editing function of SWR in vitro. Swc5 DEF/Y interacts with histones in vivo, and the extent of this interaction is dependent on the remodeling ATPase of SWR, supporting a model in which Swc5 acts as a wedge to promote A-B dimer eviction. Given that DEF/Y motifs are found in other evolutionary unrelated chromatin regulators, this work provides the molecular basis for a general strategy used repeatedly during eukaryotic evolution to mobilize histones in various genomic functions.

ATP-dependent chromatin remodeler | Swc5 | nucleosome editing | DEF/Y motif | X-ray crystal structure

Eukaryotes use histone proteins to package their genomic DNA by forming repeating units of nucleosome structures (1). A typical nucleosome has a protein core made up of two copies of histones H2A, H2B, H3, and H4 (2). The core is in turn wrapped by ∼150 base pairs of DNA with linker DNA of various lengths protruding on both ends (3). But at specific genomic locations, such as promoters and enhancers, histone H2A is replaced by its variant H2A.Z (4, 5). These H2A.Z-containing nucleosomes present specific binding surfaces for transcriptional and chromatin regulators, alter biophysical properties of chromatin, and fine-tune transcriptional responses (6, 7). H2A.Z is required for life in metazoans, and for adaption and fitness in yeast (8–10).

The site-specific deposition of H2A.Z requires a 14-polypeptide complex, called SWR, which is an ATP-dependent chromatin remodeler (8, 11, 12). SWR deposits H2A.Z by replacing the two H2A-H2B (A-B) dimers within a nucleosome with two H2A.Z-H2B (Z-B) dimers in a sequential manner (11, 13). In yeast, the histone chaperone Chz1 delivers Z-B dimers to SWR, which bears chaperone-like domains in the Swr1 and Swc2 subunits to accept the histones (14–18). The ATPase motor of SWR, which is located in the C-terminal half of the Swr1 core subunit, translocates the nucleosomal DNA at a site two helical turns from the dyad called Superhelical Location 2 (SHL2) to disrupt the histone–DNA contacts on the adjacent A-B dimer (19–21). The Swc6 and Arp6 subunits of SWR partially unwrap the nucleosomal DNA near the entry site to expose the H2A L2 loop and H2B L1 loop of the outgoing A-B dimer to facilitate its eviction (20). The exit of A-B and entry of Z-B are mechanistically coupled (13, 21). Rewrapping of the DNA around Z-B restores the nucleosomal structure with no net change in translational position (19).

Swc5 is a subunit of SWR essential for its H2A.Z deposition activity in vivo and in vitro (22–25). Swc5 preferentially binds to A-B dimer over Z-B dimer in solution and is involved in eviction of the A-B dimer during SWR-mediated histone exchange (22). But how Swc5 engages the outgoing A-B dimer at the mechanistic level remains unclear. In this study, we determined a 2.37-Å structure of Swc5 N-terminal region in complex with H2A-H2B dimer, using X-ray crystallography. Structural and biochemical studies showed that Swc5 uses a tandem DEF/Y motif, which has previously been shown to bind to histones, to engage the A-B dimer in the L2-L1 region of H2A and H2B, respectively, suggesting it acts as a wedge between the unwrapped DNA and the outgoing A-B dimer to facilitate eviction. Part of the Swc5-(A-B) structure resembles the DEF/Y motif found in other histone chaperones, including Spt16, YL1, Swr1, ANP32e, and Chz1, reinforcing a paradigm that DEF/Y is a universal histone binding motif involved in a wide spectrum of histone transaction processes (14–16, 18, 26, 27).

Results

A Tandem DEF/Y Motif within the Intrinsically Disordered Region of Swc5 Is Required for A-B Dimer Binding. Swc5 consists of an intrinsically disordered region (IDR) on the N-terminal half of the 303-amino acid (aa) polypeptide and a highly conserved BCNT

Significance

Eukaryotic cells mobilize histones at specific locations along the chromosome to regulate genomic processes, such as transcription. DEF/Y motifs are short amino acid sequences that are known to bind to histones. We found that a tandem DEF/Y motif in the Swc5 subunit of the SWR complex is required for SWR's nucleosome editing function, a process that involves the replacement of H2A within nucleosomes with the variant histone H2A.Z. Swc5 DEF/Y preferentially binds to histone H2A over H2A.Z. In yeast, the DEF/Y–histone interaction is partially dependent on SWR's ATPase activity. We solved an X-ray crystal structure of Swc5 DEF/Y in complex with H2A, providing the structural basis for how Swc5 contributes to the directionality of the H2A-to-H2A.Z nucleosome editing process.

Author contributions: Y.H., L.S., E.L., and Z.Z. designed research; Y.H., L.S., L. Pierrakeas, L.D., and L. Pan performed research; Y.H., E.L., and Z.Z. analyzed data; and Y.H., E.L., and Z.Z. wrote the paper.

The authors declare no competing interest.

This article is a PNAS Direct Submission. S.T. is a guest editor invited by the Editorial Board.

Data deposition: The atomic coordinate and structure factor of the Swc5/H2A-H2B complex has been deposited in the Protein Data Bank (accession no. 6KBB).

[1]To whom correspondence may be addressed. Email: ed.luk@stonybrook.edu or zhouzh@ibp.ac.cn.

This article contains supporting information online at https://www.pnas.org/lookup/suppl/doi:10.1073/pnas.1914313117/-/DCSupplemental.

First published January 30, 2020.

domain (72 aa in length) near the C terminus (Fig. 1*A*). A 79-aa, D/E-rich region at the N terminus has previously been shown to interact with A-B dimer and participate in the eviction step of the H2A-to-H2A.Z exchange reaction (22). To identify structural elements involved in interaction between Swc5 and its histone substrate, recombinant yeast Swc5 and its mutant variants were interrogated in binding assays against A-B dimers, using isothermal titration calorimetry (ITC). The A-B dimers used in the binding studies were recombinantly produced in *Escherichia coli* as a single-chain fusion of yeast H2A (Hta1) and H2B (Htb1; termed yeast scAB hereafter), which has been shown to behave structurally and biochemically similar to the wild-type A-B dimer (14, 15, 28). Consistent with our earlier conclusion, the 147-aa IDR at the N-terminal half of Swc5 (swc5 [1-147]) binds to scAB with a robust affinity (K_d = 23.6 nM), but the C-terminal half of Swc5 (swc5[147-303]) exhibits no detectable binding under the experimental conditions. Additional truncation mutants showed that the D/E-rich region is required and sufficient for interaction with scAB, as indicated by strong binding of swc5(1-79) (K_d = 16.1 nM) and loss of binding in swc5(79-303) (Fig. 1*B*). In addition, ITC analysis showed that swc5(1-79) and swc5(1-147) bind to scAB with saturation curves centered around 1 molar relative to scAB, suggesting that Swc5 and A-B dimer bind with a 1:1 stoichiometry (Fig. 1*B* and *SI Appendix*, Table S1). Our analytical ultracentrifugation analysis also supports a 1:1 binding stoichiometry, as swc5(1-79) and scAB together sedimented at 34.7 kDa, which have theoretical molecular weights of 8.8 and 29.5 kDa, respectively (*SI Appendix*, Fig. S1). In contrast, scAB alone sedimented at 26.5 kDa (*SI Appendix*, Fig. S1).

The D/E-rich region of Swc5 contains two consecutive DEF/Y motifs (defined here as 5 to 6 D or E residues followed by an aromatic F or Y residue). Because DEF/Y motifs found in other histone chaperones are known to physically interact with free histones including A-B and Z-B dimers (14–16, 18, 26, 27), we hypothesize that the Swc5 DEF/Y motifs and the neighboring elements are involved in A-B binding. To pare down the residues critical for its histone binding function, we performed sequence alignment analysis of Swc5 homologs and found that the second DEF/Y in the tandem motif (namely, DEF/Y-2) is more conserved than the first motif (DEF/Y-1), and that a conserved proline is present two residues from the C-terminal side of DEF/Y-2 (Fig. 1*C*). The spacing between DEF/Y-1 and DEF/Y-2 is also somewhat conserved, with one or two residues separating the two motifs. To evaluate how this conserved region in the tandem DEF/Y motif is involved in A-B dimer binding, aa 21 to 31 were ablated in yeast swc5(1-79), the minimal fragment for binding A-B dimers. The resulting deletion mutant, swc5(Δ21–31), was titrated against scAB in the ITC assay, using swc5(1-79) as reference (which was replotted in Fig. 1*D* in red with the data from Fig. 1*B*). Indeed, this 11-aa region containing DEF/Y-2 is required for binding to A-B dimer, as swc5(Δ21–31) failed to bind to scAB (Fig. 1*D*). In contrast, another mutant lacking the acidic residues of DEF/Y-1, swc5(Δ15–20), only marginally decreased scAB binding as compared with swc5(1-79) (Fig. 1*D*). Since the internal truncation of swc5(Δ21–31) could cause misalignment of histone-binding elements elsewhere, leading to indirect loss of binding, the residues of 21 to 31 in swc5(1-79) were substituted with A residues (swc5[21-31→A]) and the mutant was interrogated by ITC (Fig. 1*D*). Consistent with the result observed for swc5(Δ21–31), swc5(21-31→A) also failed to interact with scAB, underscoring the role of DEF/Y-2 and its neighboring elements in binding to histone A-B dimers.

To test whether the histone binding activity of the DEF/Y motif of Swc5 is conserved, the human Swc5 homolog called Craniofacial Development Protein 1 (CFDP1) was purified from

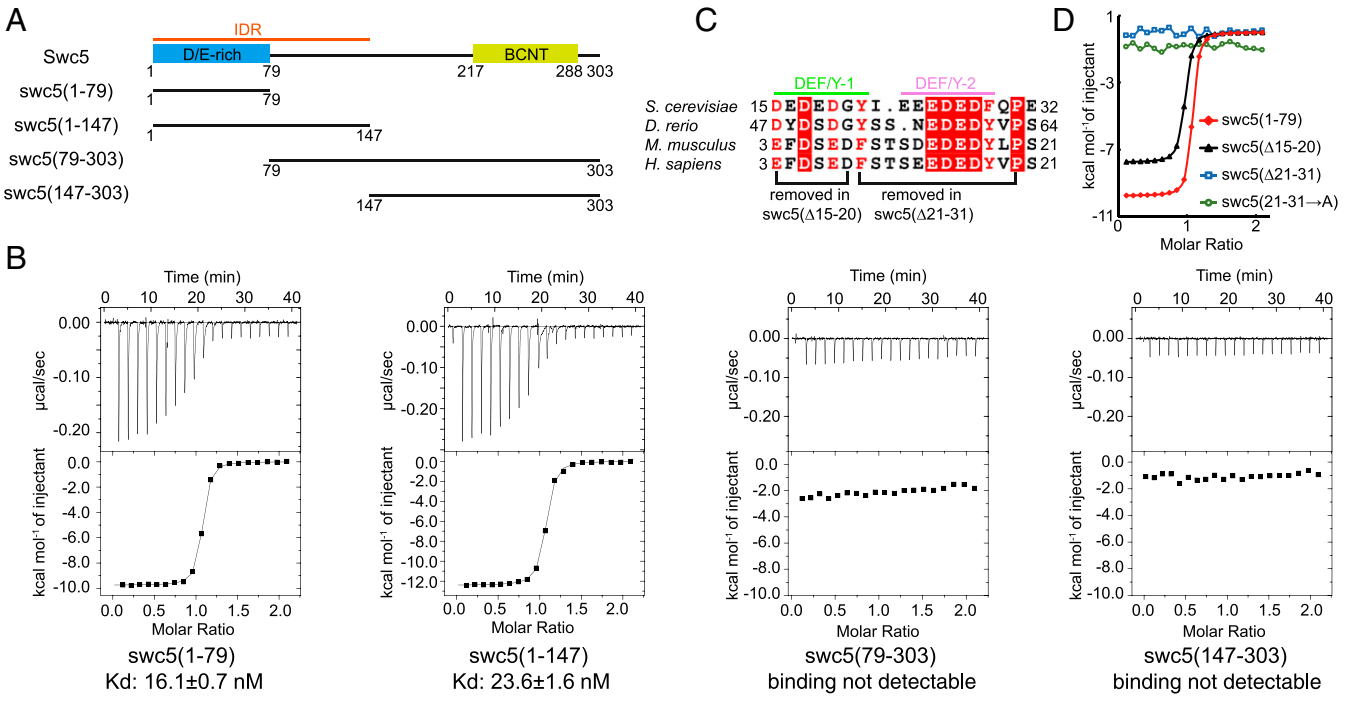

Fig. 1. Domain analysis of Swc5 in A-B binding. (*A*) A schematic representation of yeast Swc5 and the mutant variants used in domain analysis. D/E-rich: asparate/glutamate-rich region. BCNT, Bucentaur domain; IDR, intrinsically disordered region (37). (*B*) ITC analysis of the Swc5 fragments against scAB. (*C*) Multiple sequence alignment of the DEF/Y motif region of Swc5 in yeast and other species. White fonts in red background: identical in the four species analyzed. Red and black fonts: similar and unique residues in the four species analyzed. Alignment analysis was performed using Uniprot and ESPript 3.0 (38). (*D*) Integrated thermal curves of Swc5 fragments binding to scAB. ITC was performed in the presence of 0.2 M NaCl. swc5(1-79), red; swc5(Δ15–20), black; swc5(Δ21–31), blue; and swc5(21-31→A), green.

E. coli and interrogated using ITC by titrating against human A-B dimers (which were reconstituted from full-length H2A and H2B). CFDP1 binds to human A-B dimers (K_d of 95.2 nM), but not the alanine mutant variant, CFDP1(9-20→A), in which the DEF/Y-2 motif and the neighboring P and F residues were substituted with consecutive A residues (*SI Appendix*, Fig. S2 and Table S1). Therefore, the histone binding activity of Swc5/CFDP1 is conserved.

The Swc5 DEF/Y Motif Binds to Histones with Additional Contacts Not Previously Observed in Other DEF/Y-Containing Histone Chaperones. To uncover the structural basis of the interaction between Swc5 and its histone substrate, the structure of yeast swc5(1-79) in complex with human A-B was solved by X-ray crystallography and molecular replacement at 2.37 Å resolution. Note that crystallization screening was performed for both yeast and human histones, but only the human A-B dimers yielded crystals with yeast swc5(1-79). The structure showed that H2A T16-G98 and H2B R31-S123, which encompass the conserved histone-fold domains, fold into a conformation resembling the nucleosomal A-B dimer (Fig. 2*A*). Importantly, additional electron density corresponding to Swc5 D15-E32 was observed near the L2 loop of H2A and the L1 loop of H2B, indicating that DEF/Y-1, DEF/Y-2, and the conserved P within the IDR of Swc5 became structured upon binding to the A-B dimer (Fig. 2*A* and *SI Appendix*, Fig. S3). The refined model fits well with the crystallographic data exhibiting geometric statistics of 0.23/0.19 for R_{free}/R_{work} (Table 1).

Although Swc5 DEF/Y-2 engages its histone partner in a manner similar to the DEF/Y motifs of other histone chaperones, including Chz1, Spt16, ANP32e, Swr1, and YL1, additional contacts were found in the Swc5-(A-B) interaction (14–16, 18, 26, 27). A short helix formed between E23 and D26 of DEF/Y-2 $(EEEDEDF)_{23–29}$ grips the H2A L2 and the H2B L1 by engaging the E23 and D26 side chains in electrostatic interactions with H2A K75 and R77 (corresponding to K76, R78 of yeast H2A) and H2B S55, S56, K57 (corresponding to S58, Q59, K60 of yeast H2B; Fig. 2*C*). Swc5 E27 and D28 reinforce this interaction by forming additional contacts with H2B S56 and H2A R77, respectively (Fig. 2*C*). As such, the acidic residues of DEF/Y-2 form an electrostatic cap over key residues involved in histone–DNA binding within a nucleosome, implying that Swc5 binding would preclude the A-B dimer from interacting with the nucleosomal DNA (*SI Appendix*, Fig. S4*A*). Similar to the DEF/Y motifs of other histone chaperones, the F29 of DEF/Y-2 is inserted into a hydrophobic cradle of H2B. However, unlike the other DEF/Y–histone structures, Swc5 P31 also participates in hydrophobic interaction. As such, Swc5 F29 and P31 interact with S38, Y42, I54, and M59 residues in the α1-L1-α2 region of H2B (corresponding to S41, Y45, I57, and M62 of yeast H2B), resulting in a 187-$Å^2$ interface. (Fig. 2*D*). Also unique to Swc5 is that the DEF/Y-1 Y21 participates in A-B binding through a hydrophobic contact with H2A (Fig. 2*B*). Altogether, the Swc5 fragment $(YIEEEDEDFQP)_{21–31}$ interacts with the L2-L1 region of the A-B dimer, using an electrostatic cap flanked by hydrophobic anchors.

To assess the relative contribution of the interacting residues at the Swc5–(A-B) interface, alanine substitutions were introduced in and around the DEF/Y-2 motif of swc5(1-79) and the resulting mutants, along with the wild-type control (i.e., swc5[1-79]), were purified and interrogated by the ITC binding assay against yeast scAB. Consistent with the structural data, swc5(1-79) D26A and F29A exhibited strong binding defects compared with swc5(1-79) with a ~23- and 15-fold decrease of affinity for scAB (Fig. 2*E* and *SI Appendix*, Table S1). The double D26A/F29A

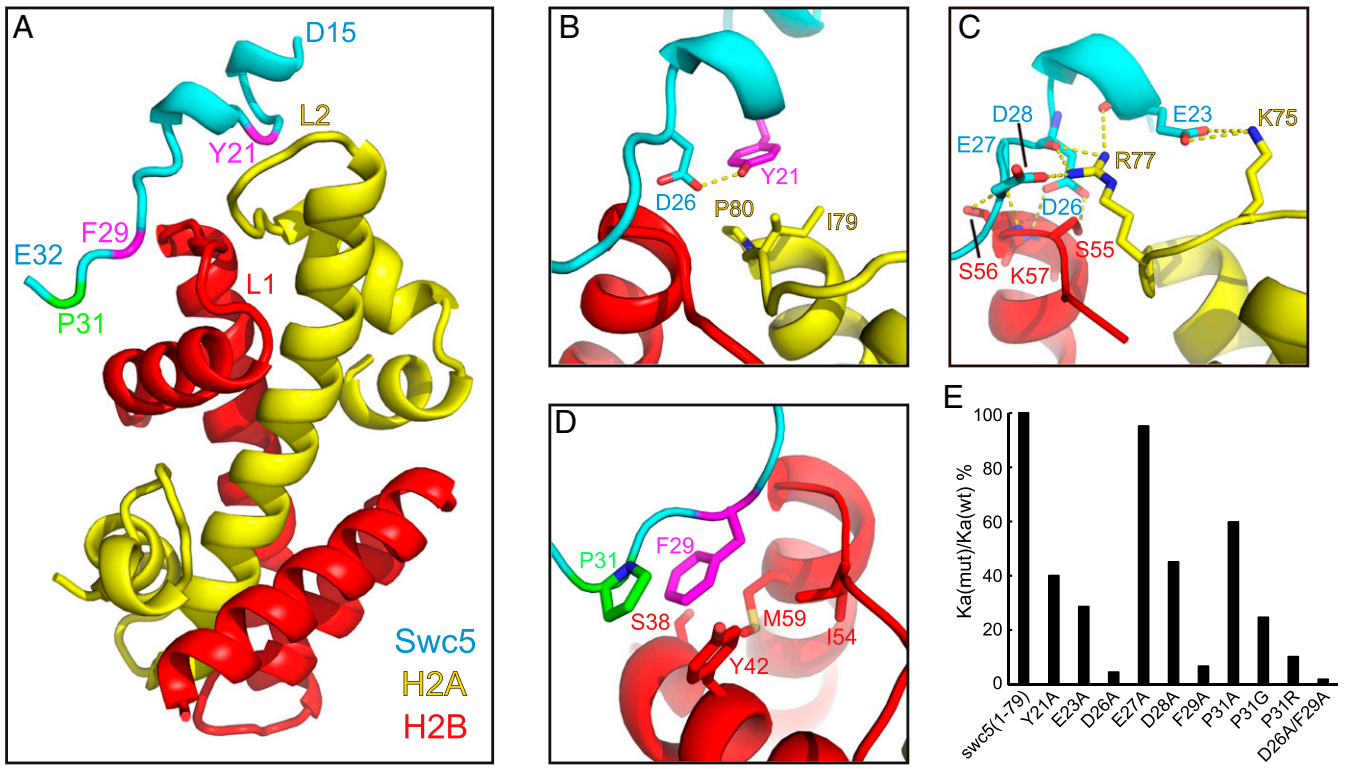

Fig. 2. Swc5 binds to the A-B dimer using a tandem DEF/Y motif. (*A*) X-ray crystal structure of yeast swc5(1-79) in complex with human A-B. Regions outside of D15-E32 of swc5(1-79) are not visible in the structure. H2A, yellow; H2B, red; Swc5, cyan. (*B–D*) Close-up views highlight the hydrophobic interaction between Swc5 Y21 and nonpolar side chains of H2A in *B*, the electrostatic interaction between the acidic residues in DEF/Y-2 and the L2-L1 region of A-B in *C*, and the interaction between Swc5 DEF/Y-2 motif and the hydrophobic pocket of H2B. (*E*) Relative binding affinity of Swc5 mutants as compared with swc5(1-79).

Table 1. Data collection and refinement statistics

Structure	$Swc5_{1–79}$-$H2A_{13–106}$-$H2B_{26–125}$
PDB ID code	6KBB
Data collection	
Space group	C 1 2 1
Cell dimensions	
a, b, c (Å)	107.07 65.52 84.82
α, β, γ (°)	90.00 122.83 90.00
Resolution (Å)	50.00–2.37(2.49–2.37)*
Rmerge	0.150 (0.953)
I/σ I	12.2 (2.0)
Completeness (%)	99.9 (99.5)
Redundancy	6.5 (5.7)
Refinement	
Resolution (Å)	44.99–2.37(2.49–2.37)
No. reflections	19,642
R_{work}/R_{free}	0.19/0.23
No. atoms	
Protein	3,008
Water	59
B factors	
Protein	50.72
Water	45.97
r.m.s. deviations	
Bond lengths (Å)	0.004
Bond angles (°)	0.793

*Statistics for the highest-resolution shell are shown in parentheses.

mutant further decreased the affinity by ∼61-fold, underscoring the significance of the acidic cap and the hydrophobic anchor in DEF/Y-2 in binding to the A-B dimer, consistent with a previous report (27). To assess the contribution of Swc5 P31 to the hydrophobic anchor, P31G, P31R, and P31A mutations were introduced into swc5(1-79). Indeed, binding to scAB was severely interfered by the charged P31R mutation, but less so by the nonpolar P31A (Fig. 2*E* and *SI Appendix*, Table S1). P31G exhibited an intermediate binding defect to scAB, suggesting that conformational rigidity provided by P31 could play a role in stabilizing the Swc5-(A-B) interaction (Fig. 2*E* and *SI Appendix*, Table S1).

Interestingly, not all D/E residues in the acidic cap of DEF/Y-2 are critical. For example, the defects of D28A and E23A are moderate (2.2- and 3.5-fold decrease, respectively; Fig. 2*E* and *SI Appendix*, Table S1), whereas E27A is virtually normal in scAB binding. The latter is supported by the structure as the main chain carbonyl oxygen of Swc5 E27 hydrogen bonds with H2B S56, whereas the side chain of E27 points away from the A-B dimer (Fig. 2*C*). Notwithstanding the varying degree of contributions of the DEF/Y motif acidic residues, the role of R78 of H2A (corresponding to R77 in human) is clear; the yeast H2A mutant R78A reduced binding by ∼15-fold, further emphasizing the electrostatic component of the Swc5-(A-B) interaction.

To evaluate the biological relevance of the structural model of Swc5-(A-B), the VivosX approach was employed to probe the interaction between Swc5 and A-B dimers inside yeast cells. VivosX uses strategically placed cysteine residues at the binding interface of two proteins and site-specific disulfide cross-linking to infer in vivo protein–protein interaction (Fig. 3 *A* and *B*) (29). Swc5 E27 was selected for cysteine substitution, as alanine substitution at this site exhibited the least binding defect with A-B dimers (Fig. 2*E*). In addition, Swc5 E27 is in proximity (5.8 Å Cα-Cα distance) to a relatively unconserved Q59 on yeast H2B (corresponding to S56 of human H2B). Yeast cells expressing *HTB1(Q59C)* as the sole source of H2B exhibited no observable growth defects, suggesting that the Htb1(Q59C) protein is functional (*SI Appendix*, Fig. S5). An oxidizing agent 4,4′-dipyridyl disulfide was added to *HTB1(Q59C) swc5(E27C)* yeast to induce in vivo cross-linking. Consistent with our structural data, Swc5(E27C) cross-linked to Htb1(Q59C) as indicated by the presence of Swc5-Htb1 adducts in anti-Swc5 and anti-H2B immunoblots resolved under nonreducing sodium dodecyl sulfate (SDS)/polyacrylamide gel electrophoresis (PAGE; Fig. 3 *C* and *D*). The Swc5-Htb1 adducts were cleaved by beta-mercaptoethanol treatment, confirming the disulfide nature of the cross-links (Fig. 3 *E* and *F*). Furthermore, when VivosX was performed in the *swr1(K727G)* genetic background, which encodes an ATPase-dead Swr1, the relative cross-linking between Swc5(E27C) and Htb1(Q59C) was diminished (Fig. 3 *C*–*G*). Therefore, we conclude that E27 of Swc5 is within disulfide cross-linking distance to Q59 of Htb1 in yeast, and that the interaction between Swc5 and Htb1 is partially dependent on the remodeling activity of SWR.

The SWR Complex Requires Swc5 DEF/Y-2 for Optimal Remodeling Function. The histone exchange activity of the SWR complex requires the Swc5 subunit (23). To investigate the functional role of the Swc5 DEF/Y motif, a biochemical complementation assay was performed to evaluate the ability of recombinant Swc5 and its mutant variants in rescuing the histone exchange activity of a Swc5-deficient SWR complex (SWR[swc5Δ]) (22). To monitor histone exchange activity, native PAGE was used to detect the mobility shift of a Cy3-labeled nucleosomal substrate caused by the stepwise replacement of the A-B dimers with epitope-tagged Z-B dimers (which are fused with a 3xFLAG tag at the C terminus of H2B). As such, the fastest migrating species represents the homotypic H2A/H2A nucleosomes, the middle band is the intermediate (i.e., heterotypic H2A/H2A.Z nucleosomes), and the top band is the homotypic H2A.Z/H2A.Z product (Fig. 4*A*) (13, 22).

Consistent with our earlier results, SWR(swc5Δ) was defective in depositing H2A.Z, but its activity was complemented by recombinant wild-type Swc5 (Fig. 4*B*, *lanes 1–3*) (22). However, a Swc5 mutant that lacks the D/E-rich N-terminal region (swc5 [Δ2–78]) was deficient in complementing SWR(swc5Δ) relative to wild-type Swc5, suggesting that the tandem DEF/Y motif in the deleted region is important for histone exchange (Fig. 4*B*, *compare lanes 3 and 4*). To directly test the contribution of DEF/Y-1 and DEF/Y-2 in the histone exchange process, critical DEF/Y motif residues identified in our binding studies were mutated to alanines; the resulting mutants were then tested for complementation of SWR(swc5Δ). Indeed, a Swc5 mutant containing alanine substitutions at D26 and F29 of DEF/Y-2 demonstrated a strong defect in H2A.Z deposition (Fig. 4*B*, *compare lanes 3 and 5*), consistent with the critical role of these residues in histone binding (Fig. 2*E*). In comparison, the H2A.Z deposition defect of the E18A, Y21A mutant in DEF/Y-1 was marginal, suggesting that these residues are largely dispensable for H2A.Z deposition (Fig. 4*B*, *compare lanes 3 and 6*). The E18A, Y21A, D26A, F29A quadruple mutant is similar to the D26A, F29A double mutant, consistent with DEF/Y-2 playing a more prominent role than DEF/Y-1 in histone exchange (Fig. 4*B*, *compare lanes 5–7*).

Structural Basis of Preferential Binding of Swc5 to H2A over H2A.Z. SWR replaces nucleosomal A-B dimers with Z-B dimers in a unidirectional manner (13). The directionality relies on the ability of SWR to recognize specific amino acids in H2A and H2A.Z during the histone transaction process. Previous studies showed the Swr1 and Swc2 subunits of SWR bind preferentially to the incoming Z-B dimers (14–16, 24, 30). How SWR recognizes A-B dimers during the eviction step is unclear.

H2A shares ∼60% sequence identity with H2A.Z. To understand how Swc5 recognizes H2A, we focused on the L2 loop of H2A where extensive contacts are made with Swc5 (Fig. 2*A*).

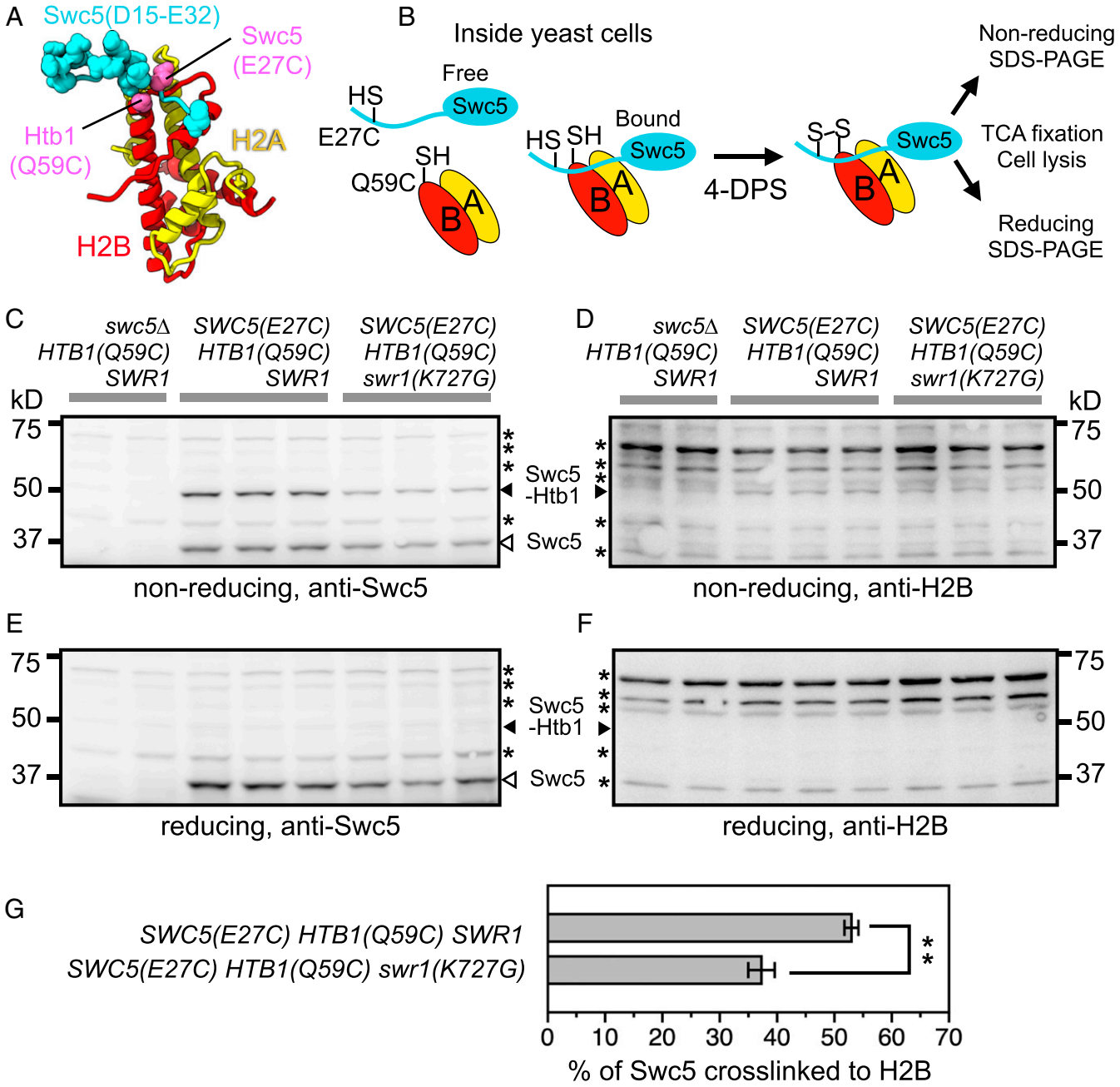

Fig. 3. Using VivosX to detect site-specific interactions between Swc5 and the A-B dimer in yeast cells. (*A*) The sites selected for cysteine substitutions are highlighted in pink. (*B*) A cartoon depicting the VivosX strategy. 4-DPS, 4,4′-dipyridyl disulfide. Red, H2B; yellow, H2A; blue, Swc5. (*C* and *D*) Nonreducing SDS/PAGE followed by anti-Swc5 western in *C* and anti-H2B western in *D*. (*E* and *F*) Same as *C* and *D*, except that the protein samples were reduced with beta-mercaptoethanol before SDS/PAGE. The different lanes of each strain represent biological replicates or triplicates. (*G*) Quantification of the amount of Swc5 that is cross-linked to H2B (at ∼50 kDa, solid triangles) over the uncross-linked species (at ∼37 kDa, open triangles). Asterisks indicate nonspecific bands. Note that there are more nonspecific bands in the nonreducing H2B western than the reducing H2B western. Some of the cross-linked adducts may represent disulfide adducts of H2B with other cysteine-containing chromatin factors.

L2 contains a disproportionately high number of H2A- and H2A.Z-specific residues that are conserved among eukaryotes (Fig. 5*A*). The side-chains of H2A K75 and R77, which are involved in DNA binding within the nucleosome, reorient to engage Swc5 through electrostatic interactions with E23 of Swc5 (Fig. 5*B*). K75 and R77 then contribute to form a shallow hydrophobic pocket together with H2A P80 and H82 and Swc5 Y21 and E23 to envelop the H2A-specific residue I79 (Fig. 5 *B* and *C*). The corresponding position of H2A I79 is T82 of H2A.Z (corresponding to T87 of yeast H2A.Z), whereas the DNA binding K75 of H2A is shifted one position downstream in H2A.Z next to the common R residue (R77 in H2A and R80 in H2A.Z). We hypothesize that this 5-aa region in H2A L2 (KTRII, aa 75–79) is the critical region distinguished by Swc5 from the corresponding region in H2A.Z L2 (VKRIT, aa 78–82) to allow preferential interaction with the outgoing A-B dimer. To test this hypothesis, we replaced the H2A KTRII motif in yeast scAB with the corresponding H2A.Z sequence VKRIT, generating the scAB^{VKRIT} mutant. In parallel, the H2A.Z VKRIT motif of a single-chain yeast H2A.Z-H2B protein (scZB) was

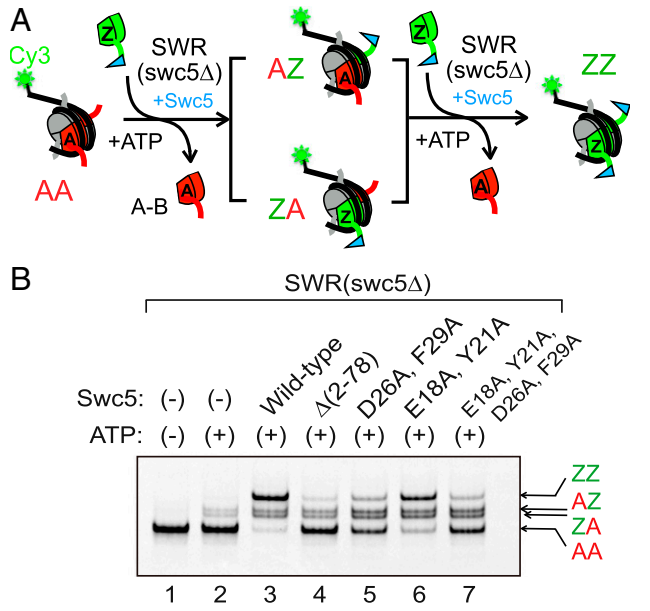

Fig. 4. The role of Swc5 DEF/Y in nucleosome editing. (*A*) A cartoon depicting the in vitro strategy used to monitor the progress of histone exchange catalyzed by the SWR complex. Green, Z-B dimers; red, A-B dimers; blue triangles, FLAG tags. AA, homotypic H2A nucleosome; AZ or ZA, heterotypic H2A/H2A.Z nucleosomes; ZZ, homotypic H2A.Z nucleosome. (*B*) Histone exchange assay. SWR purified from a yeast strain lacking *SWC5* (i.e., SWR [swc5Δ]) was added to histone exchange reactions containing recombinant wild-type Swc5 or its mutant variants. The AA, AZ/ZA, and ZZ nucleosomes were separated by native PAGE due to different copy number of FLAG-tagged Z-B dimers. The gel was scanned for Cy3 fluorescence as the longer linker of the nucleosomal substrate was conjugated to a Cy3 fluor.

replaced with the corresponding H2A sequence KTRII, generating scZB^{KTRII}. The binding affinities of these L2 swap mutants and the wild-type controls were analyzed by ITC. Consistent with our earlier results, swc5(1-79) preferentially binds to scAB (K_d of 117.2 nM) over scZB (K_d of 237.0 nM; Fig. 5 *D* and *E* and *SI Appendix*, Table S2) (22). Importantly, the binding preference for Swc5 was reversed for scAB^{VKRIT} and scZB^{KTRII} (Fig. 5*E*). The affinity of scAB^{VKRIT} mutant to Swc5(1-79) decreased by ∼4-fold, whereas the scZB^{KTRII} mutant increased by ∼3-fold (Fig. 5 *D* and *E* and *SI Appendix*, Table S2). Together, these data indicate that the conserved, unique residues in H2A L2 are critical for directing the binding preference of Swc5 to the outgoing A-B dimer during SWR-mediated histone exchange.

The 2-fold binding preference of swc5(1-79) for A-B dimers over Z-B dimers is modest, contrasting the strong preference previously observed in a chromatographic binding assay when full-length Swc5 was used (22). The discrepancy could be a result of contribution of H2A recognition by structural elements outside of swc5(1-79). To test this, the full-length Swc5 was mixed with scAB or scZB in the ITC assay. Indeed, full-length Swc5 binds more tightly to scAB (K_d of 37.9 nM) when compared with swc5(1-79) (K_d of 117.2 nM), whereas the affinities of full-length Swc5 and swc5(1-79) for scZB are virtually the same (K_d of ∼237 nM), resulting in a >6-fold H2A preference when full-length Swc5 was used (*SI Appendix*, Fig. S6 and Table S2).

While the DEF/Y motif of the human homolog CFDP1 is critical for A-B binding, it is not sufficient to distinguish A-B from Z-B dimers, according to our ITC analysis (*SI Appendix*, Table S1). On the basis of the structural model of Swc5, the lack of binding preference could be due to the absence of E23 (S12 in CFDP1) in the acidic cap and/or the misalignment of the aromatic anchor Y21 (F9 in CFDP1) as a result of a single aa insertion (*SI Appendix*, Fig. S7*A*). To test this, the structural determinants for H2A recognition were introduced into CFDP1, thereby substituting T11 and S12 of CFDP1 with a glutamate. The resulting CFDP1(TS→E) mutant indeed gained binding preference for A-B (K_d of 26.5 nM) over Z-B dimers (K_d of 74.1 nM; Fig. 5*F* and *SI Appendix*, Table S1). Conversely, when the corresponding characteristics of CFDP1 were introduced into Swc5, the resulting E23S and E-insertion (Eins) mutants lost binding preference for A-B dimers (*SI Appendix*, Fig. S7*B* and Table S2). Altogether, we conclude that the Swc5-specific aromatic anchor on the N-terminal side of DEF/Y-2 and the acidic cap are critical for H2A recognition.

Discussion

The Molecular Role of Swc5 DEF/Y Motif in SWR-Mediated Histone Exchange. SWR catalyzes a unique nucleosome remodeling reaction that involves coordinated transaction of histones in the expense of ATP hydrolysis, generating H2A.Z-containing nucleosomes that are critical for accurate transcriptional response (6, 31). The directional H2A-to-H2A.Z exchange is characteristic of the remodeling mechanism of SWR. The directionality is ensured by both the ability of SWR to preferentially bind to the correct histone substrates (i.e., H2A-containing nucleosomes and Z-B dimers) and the subsequent activation of the remodeling ATPase upon substrate binding (13, 19, 24, 30). This study suggests the Swc5 component of the SWR complex further contributes to a third component for histone exchange directionality through binding to the outgoing A-B dimer in the DNA-unwrapped state.

At this time, it is unclear whether Swc5 actively assists the eviction of A-B by binding to the outgoing dimer while it is still associated with a partially unfolded nucleosome, or whether Swc5 binds to the ejected A-B to prevent passive nucleosome reassembly. It is tempting to speculate that Swc5 actively assists A-B eviction in light of the recent cryo-EM structure of the SWR-nucleosome complex (20). Upon binding to the nucleosome in the presence of a nonhydrolysable ATP analog, the Arp6-Swc6 subunits of SWR unwraps ∼2.5 turns of DNA from the entry site of the nucleosome, exposing the L2-L1 site of the outgoing A-B dimer. Coincidently, the exposed site is where Swc5 DEF/Y motif makes contacts, suggesting that Swc5 acts as a wedge between nucleosomal DNA and core histones to facilitate A-B dimer removal during SWR-mediate Z-B insertion (*SI Appendix*, Fig. S4*B*). Our in vivo cross-linking data support this model, as Swc5 interacts less with its histone substrate in yeast where SWR remodeling activity was impaired. However, despite Swc5's binding preference for H2A over H2A.Z, the affinity of Swc5 for Z-B dimers is nontrivial. Therefore, we cannot eliminate the possibility that Swc5 could play a dual role in facilitating A-B eviction and Z-B insertion.

Deciphering the Interaction between the DEF/Y Motif and Histones. The seemingly simple DEF/Y motif has been shown to bind to the chromatin-free, dimeric form of the lysine-rich histones H2A, H2A.Z, and H2B in a growing list of evolutionary unrelated histone chaperones and chromatin remodeling factors, including Swc5, Chz1, Spt16, YL1, ANP32e, and Swr1. In fact, the consensus DEF/Y motif can be found in >50 yeast nuclear proteins. For example, the ATP-dependent chromatin remodelers Chd1 and Fun30, as well as components of the histone-modifying complexes, Sas3, Jhd2, and Hos3, all possess at least two consensus DEF/Y motifs. The structural similarity of the DEF/Y-histone interactions of the seven available structures (PDB: 6KBB [Swc5], 6AE8 [Chz1], 4WNN [Spt16], 5CHL [dYL1], 5FUG [hYL1], 4CAY [ANP32e], and 4M6B [Swr1]) and the presence of DEF/Y in other chromatin regulators underscore the notion that the DEF/Y motif is a general ligand for chromatin-free histone A-B or Z-B dimers.

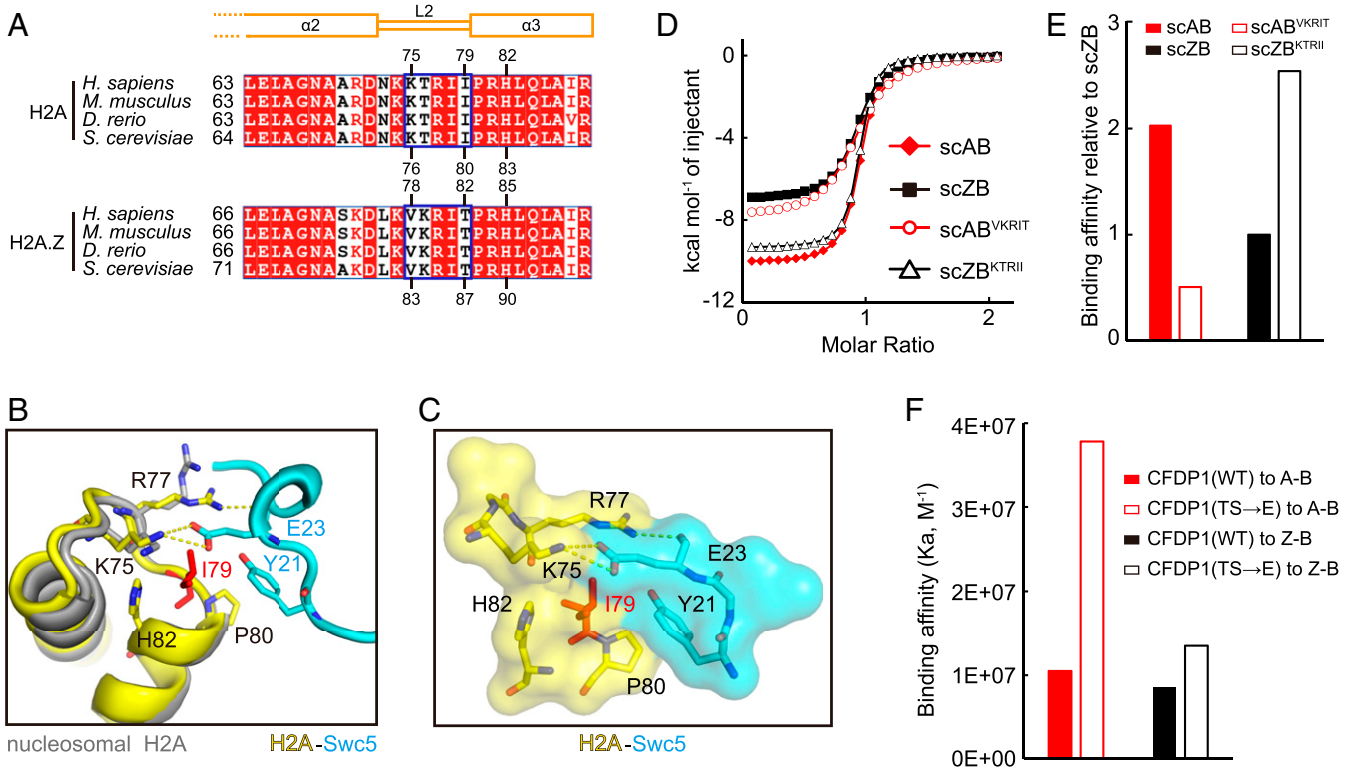

Fig. 5. H2A-specific residues in L2 confer binding preference of Swc5 to A-B over Z-B dimers. (*A*) Sequence alignment analysis of H2A and H2A.Z. White fonts in red background: residues identical between H2A and H2A.Z. Red and black fonts: similar and unique residues between H2A and H2A.Z. Blue boxes: region critical for recognition by Swc5. α2 and α3, alpha 2 and alpha 3 helices of the histone fold domain of H2A/H2A.Z. L2, Loop 2 region. (*B*) Comparison of H2A in complex with Swc5 (yellow) or in the context of a nucleosome (gray, PDB: 2CV5). Cyan, Swc5. The side chain of H2A I79 is highlighted in red. (*C*) Stick and surface models highlighting sidechain interaction of Swc5 DEF/Y with H2A L2. (*D* and *E*) Binding assay of swc5(1-79) against scAB, scZB, and mutant variants containing a swapped L2 region. Integrated ITC curves were shown in *D*. Relative binding affinities of the histone proteins to swc5(1-79) were normalized to scZB. (*F*) Binding affinities of the CFDP1 (wild-type [WT] or mutant) against histones (A-B or Z-B) show that CFDP1(TS→E) gains H2A preference.

Previous studies showed that the DEF/Y motif of Spt16, Swr1, YL1, ANP32e, and Chz1 engages histone dimers in a bidentate mode, such that the acidic residues form a cap over the DNA-binding residues on H2A/H2A.Z and the aromatic residue anchor in the hydrophobic pocket of H2B (27). This interaction, however, is not sufficient to provide specificity to distinguish H2A from H2A.Z. In the case of Swr1, YL1, Chz1, and ANP32e, regions outside of DEF/Y are required to differentiate H2A.Z from H2A (15, 16, 18, 26, 32). The tandem DEF/Y motif of Swc5 is therefore unique in that the motif itself contributes to the preferential binding of H2A over H2A.Z. The specificity is provided in part by Y21 in DEF/Y-1, as this aromatic residue participates in the formation of a second hydrophobic pocket to envelop the H2A-specific I80 (I79 in human). Therefore, the tandem DEF/Y motif of Swc5 binds to A-B dimer in a tridentate mode, contrasting the DEF/Y motif found in other histone chaperones. Note that the full-length Swc5 has a stronger H2A-to-H2A.Z bias than the DEF/Y-containing swc5(1-79) fragment, suggesting that a structural element in the C-terminal half of Swc5 is contributing directly or indirectly to H2A recognition.

It is noteworthy that the DEF/Y motifs with histone binding activity demonstrated thus far are all located within regions that are predicted to be intrinsically disordered (28, 33). The correlation raises the possibility that the DEF/Y motif requires structural flexibility to engage its histone partner. In addition, recent studies have shown that IDRs are involved in the recruitment of transcription factors and RNA polymerase II into phase-separated condensates (34, 35). Whether the IDRs in histone chaperones and chromatin remodeling factors play similar roles is unclear. However, if true, the histone-binding DEF/Y motifs may recruit or retain histones within phase-separated liquid droplets where transcription factors and chromatin remodeling factors coalesce to regulate transcription. Future work will be geared toward testing these possibilities.

Materials and Methods

Protein Expression and Purification. Swc5 and histones were expressed in *E. coli* cells and further purified by affinity chromatography and ion exchange chromatography.

Crystallization and Structure Determination. Crystals of yeast swc5(1-79)-human H2A-H2B ($H2A_{13–106}$-$H2B_{26–125}$) complex were harvested using the hanging-drop vapor-diffusion method at 16 °C. The atomic structure of Swc5-H2A-H2B complex was determined by molecular replacement and refined to 2.37 Å.

ITC. To quantify the binding affinity between Swc5 (or CFDP1) and histones, ITC assays were conducted on MicroCal ITC 200 or VP-ITC microcalorimeter, by which Swc5 or CFDP1 was titrated into histones at 25 °C.

VivosX Assay. The VivosX protocol was performed according to a previous protocol (29). Briefly, logarithmically growing yeast cells were treated with 4,4′-dipyridyl disulfide (Sigma catalog no. 143057) for 20 min before they were fixed with 20% trichloroacetic acid. The fixed cells were lysed by bead beating and the proteins were extracted at 30 °C for 60 min with the TUNES buffer, which contains 100 mM Tris at pH 7.2, 6 M urea, 10 mM EDTA, 1% SDS, 0.4 M NaCl, 10% glycerol and 50 mM *N*-ethylmaleimide. The extracts were cleared by centrifugation before being analyzed by SDS/PAGE. Swc5 and H2B western analyses were conducted using custom anti-Swc5 (Covance) and anti-H2B (gift from Carl Wu) antibodies.

Histone Exchange Assay. The SWR(swc5Δ) complex and the histone substrates for the histone exchange reaction were, respectively, purified from yeast and reconstituted with recombinant components according to previous studies (22, 36). Histone exchange reactions were conducted at room temperature with 16 nM Cy3-labeled canonical nucleosomes, 115 nM FLAG-tagged Z-B dimers, 1.5 nM SWR(swc5Δ) complex, and 15 nM of Swc5 or mutant variants, where indicated. The reactions were initiated with 1 mM ATP and were allowed to proceed for 1 h at room temperature before quenching with 25 ng lambda phage DNA (NEB) and 35 pmol Nap1. The reactions were resolved on a 6% polyacrylamide/0.5×TBE gel and imaged with Cy3 fluorescence using a Typhoon FLA9500 imager (GE Life Sciences).

Data Availability. The atomic coordinates and structure factors have been deposited in the Protein Data bank (http://www.rcsb.org/) with PDB ID 6KBB.

ACKNOWLEDGMENTS. We thank our colleagues in the Shanghai Synchrotron Radiation Facility for technical support on crystallographic data collection; Yuanyuan Chen, Zhenwei Yang, and Bingxue Zhou for advice on ITC data analysis; Xiaoxia Yu for assistance in analytical ultracentrifugation (AUC) analysis; Shan Shan for advice on data processing; Zimeng Zhou for sample preparation; Carl Wu for anti-H2B antibody; Fred Winston for the yeast strain FY406; and members of the Z.Z. and E.L. laboratories for helpful discussion and critical comments of the manuscript. This work was supported by research grants from the United States National Institutes of Health (GM104111 to E.L.), grants from the National Key Research and Development Program of China (2019YFA0508902 to Z.Z.), grants from the Natural Science Foundation of China (31521002, 31671344, 31871318 to Z.Z.; 31801070 to L. Pan), and grants from the Strategic Priority Research Program (XDB08010104 to Z.Z.).

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