nature structural & molecular biology

Swc2 is a widely conserved H2AZ-binding module essential for ATP-dependent histone exchange

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The histone variant H2AZ is incorporated preferentially at specific locations in chromatin to modulate chromosome functions. In *Saccharomyces cerevisiae*, deposition of histone H2AZ is mediated by the multiprotein SWR1 complex, which catalyzes ATP-dependent exchange of nucleosomal histone H2A for H2AZ. Here, we define interactions between SWR1 components and H2AZ, revealing a link between the ATPase domain of Swr1 and three subunits required for the binding of H2AZ. We discovered that Swc2 binds directly to and is essential for transfer of H2AZ. Swc6 and Arp6 are necessary for the association of Swc2 and for nucleosome binding, whereas other subunits, Swc5 and Yaf9, are required for H2AZ transfer but neither H2AZ nor nucleosome binding. Finally, the C-terminal α-helix of H2AZ is crucial for its recognition by SWR1. These findings provide insight on the initial events of histone exchange.

The condensation of eukaryotic DNA in arrays of nucleosomes and the higher-order folding of nucleosome arrays in chromatin fibers has an important influence on gene function and metabolism[1,2]. In the nucleosome core particle, the fundamental unit of chromatin compaction, the wrapping of ~146 base pairs (bp) of DNA over the histone octamer occludes about half of the DNA surface[3], rendering it poorly accessible to the transcriptional machinery. To counteract constraints imposed by nucleosome architecture, cells deploy two major classes of multiprotein enzymes. The first class covalently modifies the nucleosome core histones, whereas the second class catalyzes nucleosome mobility or reorganization in an ATP-dependent fashion. These enzyme complexes, when targeted to or associated with chromatin, have major effects on DNA-dependent processes including transcription, DNA repair and recombination[4,5].

Although conventional histones constitute the bulk of nucleosomal proteins, variants of histone H2A and histone H3 that are incorporated into chromatin also have important roles in gene expression and chromosome metabolism[6,7]. The histone H2A.F/Z (H2AZ) variant is a functionally distinct, highly conserved histone subgroup that seems to represent a separate evolutionary lineage of histone H2A proteins[6,8]. Histone H2AZ replaces the major histone H2A in a fraction of the nucleosomes isolated from chromatin[9] and reconstitutes a structure similar to the canonical nucleosome[10], but with distinctive surface and biophysical properties[11–13]. In budding yeast, the incorporation of H2AZ has positive and negative effects on gene-specific transcription[14,15]. In transcriptionally active domains near yeast telomeres and flanking the HMR mating-type locus, H2AZ is able to counteract gene silencing caused by the spread of heterochromatin proteins. By contrast, vertebrate H2AZ is enriched in pericentric heterochromatin and has a role in chromosome segregation[16,17], and the *Drosophila melanogaster* H2AZ counterpart, H2AvD, is required for the formation of heterochromatin[18].

Genetic and biochemical studies of the means by which H2AZ in budding yeast (called Htz1) is incorporated into chromatin have revealed a requirement for the Swi2/Snf2-related (Swr1) ATPase, which is contained in a complex of 12–14 subunits[19–21]. The purified SWR1 complex catalyzes displacement of histone H2A from conventional nucleosome arrays and its replacement with histone Htz1 (ref. 19). Similarly, the *Drosophila* Tip60 complex catalyzes exchange of phospho-H2AvD with unmodified H2AvD *in vitro*[22]. Histone exchange entails major alterations of nucleosome architecture, involving the eviction and reassembly of core histones, and it contrasts with the activities of other ATP-driven chromatin-remodeling enzymes (such as ISWI complexes) that catalyze nucleosome mobility without irretrievable histone displacement[23–26].

Unlike the relatively simple two- to four-subunit composition of many ISWI complexes, the *S. cerevisiae* SWR1 complex contains up to fourteen distinct components, eight of which—Swr1, Swc2 (also called Vps72), Swc3, Swc5 (also called Aor1), Swc6 (also called Vps71), Swc7, Arp6, Yaf9 and Bdf1—are encoded by genes that are nonessential for cell viability[19–21,27]. Other subunits of the complex—Act1 (actin), Arp4, Swc4 (also called God1), Rvb1 and Rvb2—are essential. Some of these have functions apart from the SWR1 complex or are shared components of the INO80 chromatin-remodeling complex and the NuA4 histone acetyltransferase complex[28–31], providing a basis for the mutant phenotype. The way in which essential and nonessential

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Received 17 August; accepted 19 October; published online 20 November 2005; doi:10.1038/nsmb1023

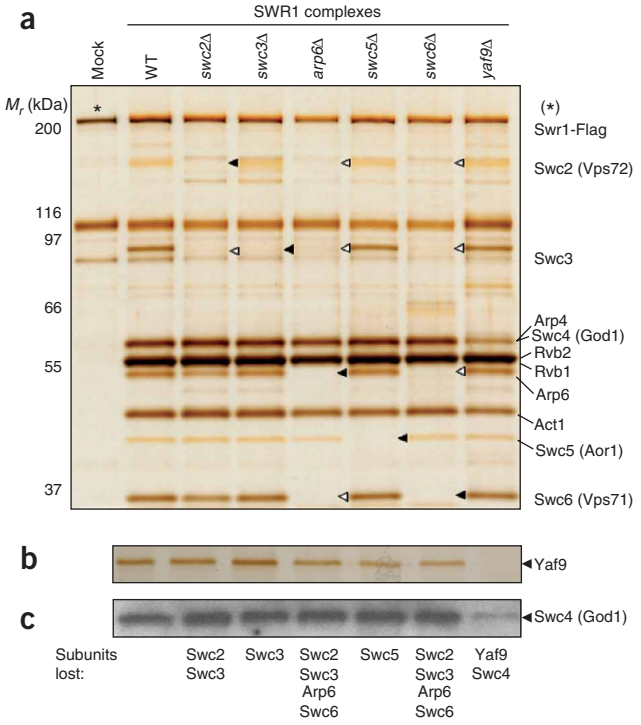


Figure 1 Subunits required for integrity of the SWR1 complex. (**a**) SDS-PAGE (8% gel) and silver staining analysis of partial SWR1 complexes purified from wild-type and mutant strains as indicated. Filled arrows, proteins eliminated by gene deletion; open arrows, additional missing subunits. The mock purification shows three major contaminants, one of which (asterisk) migrates with Swr1-Flag. (**b**) SDS-PAGE (14% gel) and silver staining analysis of Yaf9 in partial SWR1 complexes. A horizontal gel strip encompassing the molecular size of Yaf9 is shown. (**c**) SDS-PAGE (10% gel) and western blot analysis of Swc4 in partial SWR1 complexes. The PVDF membrane was probed with antibody to Swc4.

components of SWR1 collectively operate to carry out histone exchange is poorly understood. Genetic analysis shows that strains deleted for individual nonessential subunits display phenotypes similar to a *swr1* deletion and do not undergo site-specific deposition of histone Htz1 *in vivo*[20,21]. Hence, these nonessential components should be involved in the process of histone replacement, but the way in which they are involved has been obscure.

Because viable mutants are amenable to biochemical analysis, we have sought to understand the functions of nonessential SWR1 components in the histone H2AZ exchange pathway. In principle, these subunits could be used at any step of the exchange process, including complex assembly and regulation, substrate binding and histone displacement and replacement. In view of such complexity, we first explored the initial and end stages of the reaction, that is, the requirements for the association of SWR1 subunits in a stable complex, for nucleosome binding and for binding and transfer of histone Htz1. These studies have led to an understanding of roles for six SWR1 components and the discovery of Swc2 as a binding module for Htz1.

RESULTS

Subunits required for integrity of the SWR1 complex

To define associations between components of the SWR1 complex that are required for its overall integrity, we examined proteins associated with Flag-tagged Swr1 (Swr1-Flag) in cells deficient for a nonessential subunit. Complexes were purified by anti-Flag agarose chromatography and analyzed by SDS-PAGE and silver staining. We found that elimination of either the Swc3 or Swc5 subunit had no effect on association of the remaining SWR1 components, indicating that the integrity of the complex is independent of Swc3 and Swc5 (**Fig. 1**, compare WT, *swc3*Δ and *swc5*Δ). However, removal of Swc2 resulted in the additional loss of Swc3 from complex (**Fig. 1**, *swc2*Δ). Hence, the association of Swc3 is dependent on Swc2. Moreover, removal of Yaf9 resulted in reduced levels of Swc4 from complex, as revealed by western blotting (to circumvent masking by the comigrating Arp4; **Fig. 1**, *yaf9*Δ). Hence, the association of Swc4 in the SWR1 complex is dependent on Yaf9, consistent with a recent finding that Yaf9 binds directly to Swc4[32].

Notably, removal of either Arp6 or Swc6 in single-mutant strains resulted in the reciprocal loss of the other subunit and of Swc2 and Swc3 as well—a total loss of four subunits from complex (**Fig. 1**, *arp6*Δ and *swc6*Δ). Given that gene expression for the affected components is not appreciably altered in *arp6*Δ and *swc6*Δ mutant strains (**Supplementary Fig. 1** online), we conclude that Arp6 and Swc6 are mutually dependent for association with Swr1 and that they are also required for the association of Swc2 and Swc3. These results suggest that Swc2, Swc3, Arp6, Swc6 and Swr1 could be organized as a subcomplex, with Swc6 and Arp6 possibly acting as a bridge between Swc2 and Swr1.

Multiple SWR1 components are required for histone exchange

We next examined whether the partial SWR1 complexes derived from mutant cells were able to catalyze the incorporation of Htz1 into chromatin. In a standard *in vitro* histone-exchange reaction containing SWR1 enzyme at a ~1:50 ratio to nucleosomes, catalytic activity is measured by the transfer of Htz1-Flag (from an affinity-purified, native Htz1-Flag–H2B preparation) to immobilized, conventional nucleosome arrays (preassembled from bacterially expressed histone

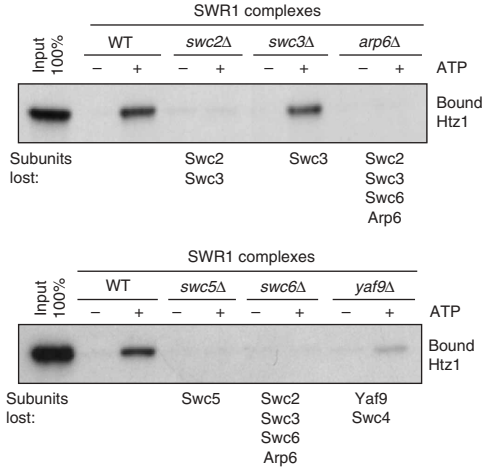


Figure 2 Most nonessential subunits are required for functional replacement of Htz1. SDS-PAGE and western blotting analysis, showing Htz1-Flag transfer from solution to immobilized, conventional nucleosome arrays. Histone-exchange reactions (100 μl) contained 20 ng of wild-type or partial SWR1 complexes derived from wild-type or mutant strains (equivalent levels to wild-type Swr1-Flag), 150 ng DNA and 200 ng of purified native Htz1-H2B, in the presence or absence of 1 mM ATP. The level of Htz1-H2B used here was two-fold in excess of standard conditions, leading to a somewhat lower ratio of histone transfer than previously reported[19].

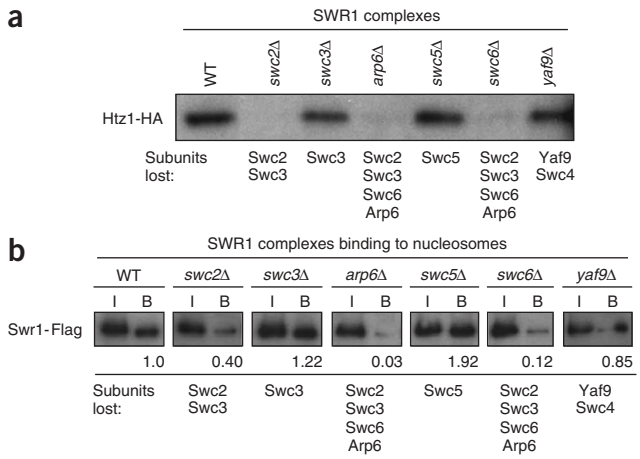


Figure 3 SWR1 components required for Htz1 or nucleosome binding. (**a**) Htz1-HA binding to partial SWR1 complexes. SDS-PAGE (14% gel) and western blotting analysis of wild-type and partial SWR1 complexes purified from mutant strains (samples normalized to Swr1-Flag). The PVDF membrane was probed with antibody to HA. (**b**) Nucleosome binding is dependent on several SWR1 components. SDS-PAGE and western blotting analysis showing wild-type or partial SWR1 complexes binding immobilized, conventional nucleosome arrays. Binding reactions (100 μl) contained wild-type or partial SWR1 complexes derived from wild-type or mutant strains (20 ng, equivalent to wild-type Swr1-Flag) and nucleosome arrays (equivalent to 150 ng DNA). I, input Swr1-Flag; B, bound Swr1-Flag. Numerical values indicate normalized binding relative to wild-type (WT; set to 1.0).

octamers)[19]. We found that the partial complex purified from *swc3*Δ cells showed active transfer of Htz1-Flag to immobilized nucleosomes, indicating that histone exchange *in vitro* is not highly dependent on Swc3 (**Fig. 2**). By contrast, partial SWR1 complexes purified from *swc2*Δ, *swc5*Δ, *swc6*Δ, *arp6*Δ or *yaf9*Δ strains had strikingly reduced histone-exchange activity, as indicated by little or no transfer of Htz1-Flag to immobilized nucleosomes (**Fig. 2**).

Because removal of one SWR1 component can lead to loss of one or several subunits from complex, we have interpreted our observations of histone exchange accordingly. As only Swc5 is absent from the SWR1(*swc5*Δ) complex, the lack of histone exchange with this complex indicates simply that Htz1 transfer is dependent on Swc5. Furthermore, although Swc2 and Swc3 subunits are both absent from the histone exchange–deficient SWR1(*swc2*Δ) complex, Htz1 transfer should be dependent on Swc2, because it is independent of Swc3 in the *swc3*Δ complex. The inclusion of Swc2 among four missing subunits when either Arp6 or Swc6 is eliminated might be a sufficient explanation for the inactivity of SWR1(*arp6*Δ) and SWR1(*swc6*Δ) complexes. However, additional dependencies on Swc6, Arp6 or both for Htz1 transfer should not be excluded. Finally, because Yaf9 and Swc4 are both absent from the SWR1(*yaf9*Δ) complex, inactivity of this complex indicates that Htz1 transfer is also dependent on Yaf9, Swc4 or both. Hence, these findings establish functional requirements for Swc2, Swc5, Yaf9 and/or Swc4, and possibly Swc6 and/or Arp6 in the SWR1 complex.

Components involved in H2AZ-binding and postbinding steps

The observation that partial SWR1 complexes do not catalyze histone transfer could be caused by defective binding to the Htz1-H2B dimer at an initial step of the exchange process or by deficiencies at steps after Htz1 binding. The association of Htz1 with wild-type SWR1 enzyme has previously been shown by immunopurification of Swr1-Flag from yeast extracts[19]. For the partial SWR1 complexes derived from *swc3*Δ, *swc5*Δ and *yaf9*Δ strains, we observed essentially wild-type levels of Htz1 associating with Swr1-Flag (**Fig. 3a**; Htz1 is detectable as Htz1-hemagglutin (HA) owing to the introduction of an HA-epitope tag). Hence, binding to Htz1 is not dependent on Swc3, Swc5, Yaf9 or Swc4. Given that Swc5 and Yaf9/Swc4 are still necessary for the Htz1 transfer, we conclude that these components are involved in separate step(s) of the histone-exchange reaction after Htz1 binding.

In contrast, we did not observe association of Htz1-HA when Swr1-Flag was purified from extracts derived from *swc2*Δ, *swc6*Δ and *arp6*Δ cells (**Fig. 3a**). This was not a consequence of indirect effects on transcription, as *HTZ1* gene expression was not appreciably affected in the mutant strains (**Supplementary Fig. 1**). Inspection of partial SWR1 complexes purified from *swc2*Δ, *swc6*Δ and *arp6*Δ strains indicated that Swc2 and Swc3 were commonly absent. As Htz1 binding is independent of Swc3, it follows that Swc2 is minimally necessary for the association of Htz1 with the SWR1 complex.

Nucleosome binding is dependent on Arp6 and Swc6

To address the role of individual components in binding of the SWR1 complex to the canonical nucleosome substrate, we examined interactions between partial SWR1 complexes and immobilized nucleosome arrays. As revealed by western blot analysis of Swr1-Flag, partial SWR1 complexes purified from *swc3*Δ and *yaf9*Δ cells showed binding to nucleosomes at nearly wild-type levels (**Fig. 3b**). By contrast, partial complexes from *swc6*Δ and *arp6*Δ cells showed a one-order-of-magnitude decrease in nucleosome binding. In addition, the partial SWR1 complex purified from *swc2*Δ cells showed an approximately two-fold decrease in nucleosome binding, whereas the complex purified from *swc5*Δ cells had an approximately two-fold increase in binding (**Fig. 3b**). These results indicate that nucleosome binding is substantially dependent on Swc6 and Arp6, and to a lesser extent on Swc2. Thus, the loss of histone-exchange activity for SWR1 complexes derived from *swc6*Δ and *arp6*Δ cells can be attributed to the impairment of both H2AZ and nucleosome binding. Notably, Swc5 seems to have a suppressive effect on nucleosome binding.

The Swc2 subunit binds to H2AZ

To further examine the role of Swc2 in H2AZ binding, we used a pull-down assay to analyze the binding of Htz1 in yeast extracts without

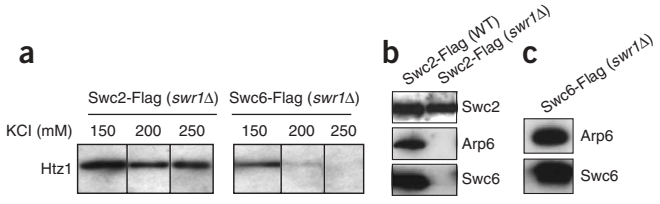


Figure 4 Swc2 binds to Htz1. (**a**) Htz1 binding to Swc2 or Swc6 in a *swr1*Δ mutant background. SDS-PAGE (14% gel) and western blotting analysis of purified Swc2-Flag or Swc6-Flag complexes (equimolar Swc2-Flag and Swc6-Flag). The PVDF membrane was probed with antibody to Htz1. (**b**) Swc2 is dissociated from Arp6 and Swc6 in a *swr1*Δ mutant background. Western blot shows protein pull-down by Swc2-Flag using the mutant yeast extract. The membrane was probed with antibodies to Flag, Arp6 and Swc6. (**c**) Swc6 is complexed with Arp6 in a *swr1*Δ mutant background. Western blot shows protein pull-down by Swc6-Flag using the mutant yeast extract. The membrane was probed with antibodies to Arp6 and Swc6.

protein overexpression. To avoid complications arising from the presence of Swr1, which provides a scaffold for assembly of the entire SWR1 complex (see later), we performed the assay on cell extracts derived from an *swr1*Δ mutant strain. Flag-tagged Swc2 was expressed in this strain, and the endogenous Htz1 was detected by western blotting with a specific antibody. The pull-down assay revealed a robust interaction between Swc2-Flag and Htz1 that was maintained at 0.15, 0.2 and 0.25 M KCl (**Fig. 4a**). In the absence of Swr1, we found that Swc2 was associated with neither Swc6 nor Arp6 (**Fig. 4b**). Hence, the interaction between Swc2 and Htz1 is, at a minimum, independent of those two SWR1 components. Moreover, in the absence of Swr1, Swc6 and Arp6 were found to be tightly associated (**Fig. 4c**), and an equivalent pull-down of Swc6-Flag showed substantially weaker interactions with Htz1 at 0.15 M KCl and none at 0.2 and 0.25 M KCl (**Fig. 4a**). Together, the results indicate that Swc2 is the major component of the SWR1 complex that is capable of associating with Htz1.

To examine the properties of Swc2 in the complete absence of any other SWR1 component, we analyzed the binding of bacterially expressed, immobilized Swc2 to purified, native preparations of Flag-tagged Htz1-H2B or H2A-H2B. Portions of His_6-tagged Swc2 containing residues 1–281, 1–345 and 345–795 were purified for analysis (as no viable clones expressing full-length protein could be obtained). We found that Htz1-Flag was appreciably associated with bacterially expressed Swc2(1–281) under conditions of 0.15, 0.3 and even 0.5 M KCl (**Fig. 5a**). Notably, Swc2(1–281) also showed binding to H2A-Flag, although this binding was abolished in 0.3 and 0.5 M KCl (**Fig. 5a**). Like Swc2(1–281), Swc2(1–345) also showed binding to Htz1-Flag over H2A-Flag under 0.3 M KCl conditions, whereas Swc2(345–795) showed poor binding to both Htz1-Flag and H2A-Flag (**Fig. 5b**). Finally, bacterially expressed Swc6 or Yaf9 proteins did not show binding to Htz1-Flag, even in low-salt conditions (0.15 M KCl) (**Fig. 5c**). These results indicate that the crucial domain for selective binding to Htz1 is contained within Swc2 (residues 1–281).

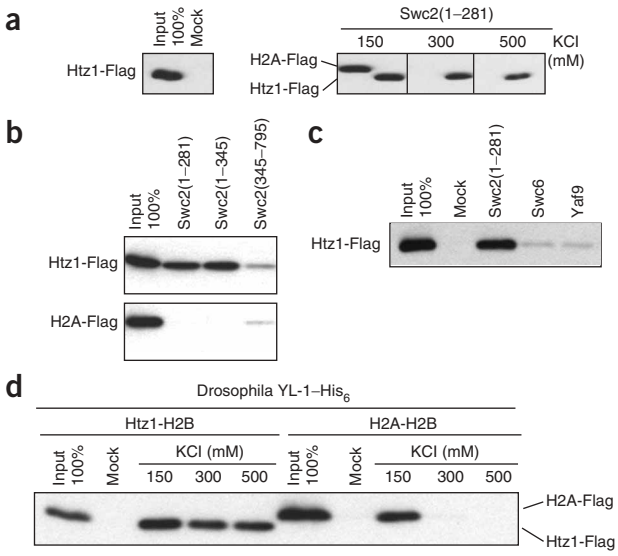


Figure 5 Bacterially expressed Swc2 binds Htz1. (**a**) SDS-PAGE results (14% gel) and western blot showing Htz1-Flag–H2B or H2A-Flag–H2B binding to immobilized, His_6-tagged Swc2, as indicated. The membrane was probed with antibody to Flag. (**b**) Immobilized, bacterially expressed Swc2(1–281), Swc2(1–345) and Swc2(345–795) binding to Htz1, as in **a**, in 0.3 M KCl. (**c**) Immobilized, bacterially expressed Swc6 and Yaf9 binding to Htz1, as in **a**, in 0.15 M KCl. Equivalent levels of Htz1-Flag–H2B and H2A-Flag–H2B were used in the binding reactions. (**d**) Bacterially expressed *Drosophila* YL-1 binds to yeast Htz1. SDS-PAGE results (14% gel) and western blot showing Htz1-Flag–H2B or H2A-Flag–H2B binding to immobilized, His_6-tagged *Drosophila* YL-1, as indicated. The membrane was probed with antibody to Flag. Equivalent levels of Htz1-Flag–H2B and H2A-Flag–H2B were used in the binding reactions.

Swc2 is conserved in metazoans as YL-1

The amino acid sequence of yeast Swc2 (particularly residues 1–281, the Htz1-binding domain) is conserved in *Drosophila* and mammals (**Supplementary Fig. 2** online). YL-1, the metazoan counterpart of Swc2, was first identified as an anchorage-independent growth suppressor of Kirsten sarcoma virus–transformed NIH3T3 cells[33]. YL-1 is a shared component of two mammalian multiprotein assemblies, the TRAPP (or Tip60) histone acetyltransferase complex and the SRCAP complex[34,35], and it is a subunit of the *Drosophila* Tip60 complex[22]. Notably, mammalian SRCAP and *Drosophila* Tip60 complexes are associated with histone H2AZ or its fly counterpart H2AvD. These similarities suggest that YL-1 may serve as a binding module for histone H2AZ in metazoans, as does Swc2 in yeast. Consistent with this hypothesis, we found that *Drosophila* YL-1 is capable of binding to Htz1 selectively over H2A (**Fig. 5d**).

Moreover, the amino acid composition of yeast Swc2 (pI 4.93) is unusually enriched for charged residues, with a prevalence of acidic (29%) over basic residues (15%), and includes clusters of acidic residues. This composition is reminiscent of a number of unrelated histone chaperones such as nucleoplasmin, N1, Nap1, Asf1, CAF-1 and FACT, which mediate ordered nucleosome assembly or disassembly under physiological conditions[36–38]. Although the means by which nuclear chaperones facilitate histone transactions and the role of acidic tracts in particular remains unclear, the similarity with Swc2 is notable and may suggest a common mechanism of action.

Swr1 maintains the integrity of the enzyme complex

The activity of the Swr1 ATPase is known to be essential for the functional exchange of Htz1. However, a role for Swr1 in maintaining structural integrity of the multiprotein complex has not been explored. To address this issue, we analyzed N- and C-terminal truncations of Swr1 that leave intact the ATPase domain. Affinity-purification of Flag-tagged Swr1 truncations showed that mutants lacking the extreme N- and C-terminal regions of Swr1 (**Fig. 6**, ΔN1 and ΔC) retained the subunit composition of the wild-type protein complex. Furthermore, these Swr1ΔN1-Flag and Swr1ΔC-Flag complexes carried out functional histone exchange, like wild-type enzyme (**Supplementary Fig. 3** online).

In contrast, an N-terminal truncation ending just before the conserved ATPase domain resulted in loss from the complex of Arp4, Act1, Swc4, Swc5 and Yaf9 (**Fig. 6a–c**, ΔN2). Hence, the association of these five subunits is dependent on the N2 region of Swr1. Moreover, the results indicate that association of the other SWR1 components is dependent on the ATPase domain including the unique 'Insert or Spacer' that distinguishes Swr1 (and its close relative Ino80) from other branches of the Swi2/Snf2 family. Consistent with these findings, deletion of the Insert region within the ATPase domain resulted in loss of another six subunits—Swc2, Swc3, Rvb1, Rvb2, Arp6 and Swc6—indicating that the association of these subunits is dependent on either the Insert region or the overall integrity of the ATPase domain (**Fig. 6a–c**, ΔIns). As might be anticipated from the foregoing observations, the loss of multiple crucial subunits from truncated Swr1 complexes compromised functional exchange of Htz1 (**Supplementary Fig. 3**).

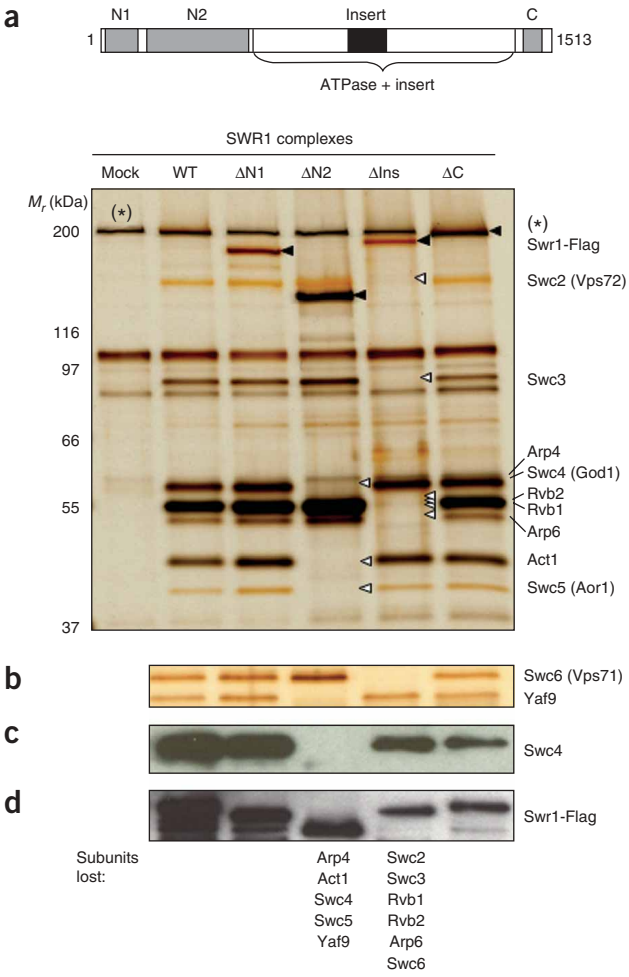
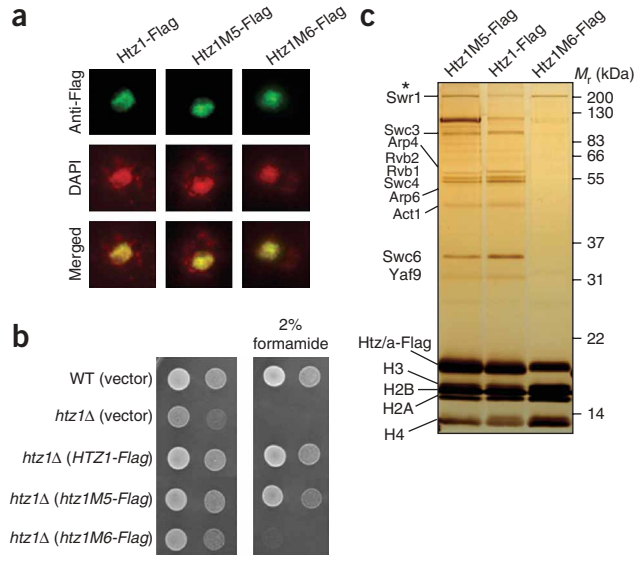


Figure 6 Swr1 is a scaffold for the complex. (**a**) Top, schematic diagram of Swr1 domains. Bottom, SDS-PAGE (8% gel) and silver staining results, showing subunit composition of purified complexes assembled from Swr1-Flag truncations. Filled arrows, truncated Swr1-Flag proteins; open arrows, missing subunits; asterisk, contaminating protein that migrates with wild-type Swr1-Flag. (**b**) SDS-PAGE (14% gel) and silver staining for presence of Swc6 and Yaf9 in mutant complexes. A gel strip encompassing the two proteins is shown. (**c**) SDS-PAGE (10% gel) and western blot analysis for Swc4 in mutant complexes. The membrane was probed with antibody to Swc4.

The C-terminal α-helix of Htz1 is essential for recognition

We compared the protein sequences of Htz1 and H2A to address how the SWR1 complex selectively recognizes the variant histone. Previously, a systematic replacement of seven regions containing the entire *Drosophila* H2AZ (H2AvD) polypeptide with corresponding segments of histone H2A showed that a crucial element (M6), which encompasses the extra C-terminal α-helix of H2AZ, is required for viability[39] (**Supplementary Fig. 4** online). We constructed a similar replacement of yeast Htz1 with the corresponding region of H2A and expressed the Flag-tagged *htz1M6* mutant under native promoter regulation. Despite robust expression and correct targeting to the cell nucleus, the yeast *htz1M6* mutant also did not complement *htz1*Δ when assayed for growth under restrictive conditions (**Fig. 7a**,**b**). By contrast, expression of wild-type *HTZ1* or of a *htz1M5* mutant in which the adjacent M5 segment was replaced resulted in functional complementation of the null mutant (**Fig. 7b**). Hence, as in *Drosophila*, the M6 region of yeast H2AZ is functionally essential.

To further elucidate the role of the C-terminal α-helix of Htz1, we analyzed binding of the chimeric Htz1M6 to the SWR1 complex. Affinity purification of the Flag-tagged chimera (Htz1M6-Flag) showed a striking loss of copurifying SWR1 components (**Fig. 7c**). By contrast, the Htz1M5-Flag chimera showed association with SWR1 components, similarly to wild-type Htz1 (**Fig. 7c**; an additional, unknown ~100-kDa protein interacts with Htz1M5-Flag). These results indicate that the M6 region of Htz1 is necessary for selective association of the histone variant. Notably, in addition to pull-down of the SWR1 complex, affinity purification of Htz1-Flag from whole-yeast extracts enriched a population of variant nucleosomes, as observed by the association of histones H2B, H3 and H4.



DISCUSSION

In this report, we have distinguished roles for six of fourteen components of the SWR1 complex (Swc2, Swc6, Arp6, Swc5, Yaf9 and Swr1) in the process of Htz1 replacement. The histone-exchange reaction can be viewed as a multistep process that initiates with the assembly of the SWR1 complex and its binding to Htz1-H2B and the canonical nucleosome, followed by ATP-driven disruption of DNA-histone interactions, displacement of an H2A histone, release of Htz1 from the SWR1 complex into the vacant site and restoration of DNA contacts. The sequential or simultaneous processing of a second H2A on the histone octamer would reconstitute a fully variant nucleosome containing two Htz1 histones. Given that individual histones lack defined structure, it is likely that the units of histone exchange are

Figure 7 The M6 domain in Htz1 is crucial for binding to the SWR1 complex. (**a**) The indicated transformants of BY4741 were analyzed by immunofluorescence microscopy. 4′,6-diamidino-2-phenylindole (DAPI) staining (red) identifies the nucleus. Flag epitope–tagged Htz1 is colored green. (**b**) *htz1*Δ mutants transformed with plasmids carrying the indicated Htz1 constructs were spotted onto SD-URA plates supplemented with or without 2% formamide. Ten-fold greater cell numbers were spotted in the left column for each panel. (**c**) SDS-PAGE and silver staining of proteins associated with the indicated Htz1-Flag upon anti-Flag purification. Asterisk indicates a contaminant comigrating with Swr1. Htz/a-Flag indicates the position of wild-type or mutant Htz1.

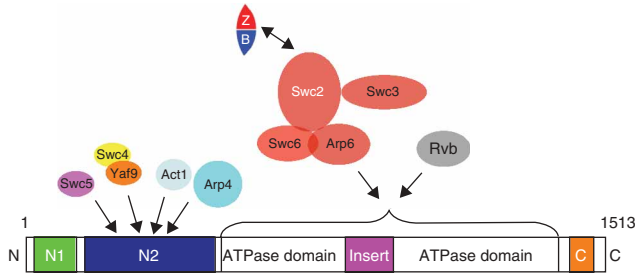


Figure 8 Summary of subunit interactions in the SWR1 complex. SWR1 subunits associate with N-terminal segment (N2) or the ATPase domain of Swr1 as shown. Swc2 interacts with Htz1 directly.

H2A-H2B and Htz1-H2B dimers rather than Htz1 and H2A monomers. Of the seven components we have investigated by biochemical analysis of mutants—Swc2, Swc3, Arp6, Swc5, Swc6, Yaf9 and Swr1—all except Swc3 evidently participate in at least one step of the histone-exchange pathway. Among these, only Swc5 is not involved in complex assembly: Swc2, Arp6, Swc6, Yaf9 and Swr1 are each required for the association of other subunits. We also note that Htz1 itself is not required for assembly of the SWR1 complex (W.-H.W., S.A., E.L. and C.-H.W., unpublished data).

Besides providing the ATPase that drives histone exchange, Swr1 also presents the main scaffold for the assembly of the multisubunit complex (**Fig. 8**). As shown by analysis of Swr1 truncations, the scaffold can be divided into two main regions: an N-terminal segment (N2, Swr1 residues 278–681) and the ATPase domain itself (Swr1 residues 699–1359), including the distinctive Insert (Swr1 residues 1002–1221), which bisects the ATPase domain. At least five components—Swc5, Yaf9, Swc4, Arp4 and actin—associate with the N-terminal scaffold. Adjoining the N-terminal scaffold of Swr1, the ATPase domain plus Insert directs the association of Rvb1, Rvb2 and the four-subunit module Swc2, Swc3, Arp6 and Swc6. Within this group of four, Swc6 and Arp6 are mutually responsible for the association of Swc2. In addition, Swc6 and Arp6 are also required for binding to the canonical nucleosome substrate.

Swc2 is notable in its ability to bind to histones Htz1-H2B with greater affinity than H2A-H2B. Demonstration of selective Htz1 binding by bacterially expressed, purified Swc2 (residues 1–281) indicates that Swc2 interacts directly with Htz1, providing the first insight into how this histone variant is recognized by the SWR1 enzyme. (It should be noted that the bacterially expressed polypeptide seems to bind less efficiently to Htz1-H2B than does the native SWR1 complex; other regions of Swc2 or components of SWR1 such as Swc6 and Arp6 may contribute to or modulate Htz1 binding.) The tethering of Swc2-Swc6-Arp6 to the ATPase domain of Swr1 may reflect topography constraints for the delivery of Htz1 in the vicinity of ATP-driven DNA alterations.

Swc2 residues 1–281 demarcate the conserved region (26% identity, 40% similarity) between yeast Swc2 and YL-1, a subunit of the mammalian SRCAP and *Drosophila* TIP60 complexes, which are also found in association with H2AZ. Our finding that *Drosophila* YL-1 binds yeast Htz1 suggests that YL-1 may be responsible for H2AZ binding in metazoans. In addition, the acidic nature of Swc2 residues 1–281 is reminiscent of a diverse group of histone chaperones that have generally acidic character and commonly mediate transactions of histones H2A-H2B or H3-H4 during chromatin assembly and disassembly[36–38]. Thus, the coupling of a core DNA-translocating Swi2/Snf2-like ATPase[40,41] with a histone chaperone–like subunit may be a defining feature of ATP-driven histone-exchange enzymes. Given previous demonstrations of histone binding by Arp4 and Arp8 (refs. 42,43), two Arp proteins in the SWR1 complex, Arp4 and Arp6, may also mediate histone transactions.

Although we have begun to define roles for many components of the SWR1 complex, the role of Swc3 has been elusive. Given that *in vivo* deposition of Htz1 is somewhat affected in a *swc3*Δ strain, our inability to detect appreciable effects on the histone-exchange reaction was probably due to assay conditions. However, it should be noted that the Swc3 requirement for *in vivo* deposition is quite subtle when compared to those observed for all other components of the SWR1 complex[20]. In summary, the binding of the SWR1 complex to Htz1 involves at least four components: the SWR1 ATPase domain, the Htz1 receptor Swc2 and the Swc6-Arp6 bridge.

We have further defined functional roles for Yaf9/Swc4, which are required for Htz1 transfer, but not for Htz1 and nucleosome binding. Yaf9 and/or Swc4 could be important for activation of the Swr1 ATPase or for histone transactions. Likewise, Swc5 has no effect on complex integrity, but is required for histone exchange. The increased nucleosome binding of the SWR1(*swc5*Δ) complex may offer a clue to its normal role in the exchange reaction. It should be noted that the remaining subunits of the SWR1 complex—the enigmatic subunit Act1 (actin) and the nonessential components Swc7 and Bdf1—have not been investigated in this report. How they affect the association of other SWR1 components and the activity of the complex are important questions for future studies. In addition, potential roles of SWR1 components in modulating the ATPase activity of Swr1 remain to be determined. Given the low ATPase activity of SWR1 relative to other SWI/SNF-type enzymes[19], definitive characterization will require the development of a more robust ATPase assay.

A crucial part of the histone signal by which the SWR1 complex recognizes histone Htz1 over H2A can be attributed to the extra C-terminal α-helix of Htz1. Its replacement by the corresponding region of histone H2A (a 7-residue change) results in a *htz1* phenotype, loss of binding to the SWR1 complex and presumably loss of site-specific incorporation in chromatin. This region is included within the 'docking domain'[10] that mediates interactions between H2A-H2B and the $(H3\text{-}H4)_2$ tetramer within the histone octamer, and it has been shown previously to be essential for viability in *Drosophila*[39] and for interactions with heterochromatin protein-1 in mammals[44]. In preliminary work, we have observed that a reciprocal swap of the corresponding region of histone H2A for Htz1 sequences restores only partial binding to the SWR1 complex and does not complement an *htz1*Δ mutation. As sequences differ over other regions of H2A and Htz1, the definition of H2AZ signals that confer full recognition by SWR1 awaits systematic swapping of yeast H2A for Htz1 sequences. In addition, as yeast and mammalian H2AZ are only 70% identical, extrapolation of results obtained from yeast to metazoans should be cautiously exercised. Finally, although mutant Htz1M6 did not associate with the SWR1 complex, it nonetheless did copurify with other core histones, particularly H3 and H4 (despite extensive MNase digestion to remove adjacent, contaminating nucleosomes). Given evidence from multiple sources that the SWR1 complex is the major catalyst for the site-specific incorporation of Htz1, this finding raises the intriguing possibility of an alternative pathway for the nonspecific deposition of Htz1 in chromatin.

METHODS

Yeast strain construction. Strains used in this study are listed in **Supplementary Table 1** online. The *swc2*Δ, *swc3*Δ, *arp6*Δ, *swc5*Δ, *swc6*Δ and *yaf9*Δ strains were generated using the HphMX4 dominant drug-resistance cassette[45]. For

details, see **Supplementary Methods** online. All deletion or tagging strains were confirmed by colony PCR amplification of the targeting cassette that was used for gene disruption or gene tagging.

Media and phenotypes. YPD, synthetic complete and synthetic dropout (SD-URA) media were prepared according to standard recipes[19]. Formamide medium was prepared by adding 1% or 2% (v/v) formamide after sterilization[46]. For the spotting assay, 10 µl of cells diluted to an A_{600} of 1.0 or 0.1 were spotted onto SD-URA plates in the presence or absence of formamide. Cells were grown at 30 °C for 2 d.

Protein purification. Protein purifications were performed essentially as described in ref. 19, using one-step anti-Flag immunoaffinity purification of yeast whole-cell extracts. Mock purifications were done using two strains: an isogenic wild-type strain without a Flag tag or an isogenic wild-type strain transformed with a Flag vector. In either case, we observed similar contaminating protein bands. The mock purifications used in **Figures 1** and **7** were purified from an untagged strain. Wild-type and mutant HTZ1 protein complexes were similarly purified. In the case of Swc2-Flag and Swc6-Flag immunopurification in *swr1*Δ cells, 5 ml of 0.15-M-KCl whole-cell extracts were used for each purification, and four washes were then performed with buffer B (see **Supplementary Methods**) containing 0.15, 0.2 or 0.25 M KCl, followed by 3× Flag–peptide (0.5 mg ml^{-1}) elution. In general, protein complexes purified and washed in buffer B containing 0.5 M KCl were used for analysis of protein composition by 8% and 14% SDS-PAGE, and for most western blotting analyses. By contrast, protein complexes purified and washed in buffer B containing 0.2 M KCl were used for Htz1 binding. Wild-type Htz1 complexes were further fractionated by 15–40% glycerol gradient sedimentation as described in ref. 19. Fractions 5 and 6 were combined and used in subsequent histone-transfer assays (see below).

Histone-exchange assay. The amounts of wild-type and mutant SWR1 complexes used were normalized for the Flag-tagged Swr1 subunit by western blotting using horseradish peroxidase (HRP)-conjugated antibody to Flag (Sigma). Procedures for nucleosome assembly and Htz1 transfer were as described in refs. 19,47.

Antibody preparation and western blotting. Recombinant Htz1 protein was expressed and purified by conventional histone purification[48] and used as immunogen for rabbit polyclonal antibodies. For analysis of Htz1 by western blotting, the amounts of wild-type and mutant SWR1 protein eluates were normalized by western blotting of the Flag-tagged Swr1 subunit using HRP-conjugated antibody to Flag. Antibody to Htz1 was used at 1:4,000 dilution. Antibody to HA (Covance) was used at 1:1,000 dilution. Recombinant His_6-Swc4 was expressed from pET28c (Novagen) and purified by HisBind Resin and the HisBind buffer kit under denaturing conditions (Novagen). After dialysis to remove imidazole, the purified protein in solution was used as immunogen for preparation of chicken polyclonal antibody. For western blotting, antibody to Swc4 was used at 1:10,000 dilution. Antibody to histone H3 (Abcam) was used at 1:500 dilution and HRP-conjugated antibody to Flag M2 (Sigma) was used at 1:2,000 dilution.

Protein interaction assays. Swc2 (residues 1–281) and full-length *Drosophila* YL-1 were cloned into pET28c, whereas Swc2 (residues 1–345) and Swc2 (residues 345–795) were cloned into pET100 (Invitrogen). All three plasmids were transformed, expressed and purified from the BL-21 DE3 pLys strain (Stratagene). The His_6-tagged proteins were purified using HisBind Resin and the HisBind buffer kit (Novagen). Proteins were retained on the beads and stored in buffer B containing 0.1 M KCl with protease inhibitors (Roche Applied Science) at 4 °C.

Each binding reaction contained 3 µg of bead-bound His_6-tagged recombinant protein (**Supplementary Fig. 5** online) and 60 ng of Htz1-H2B or Hta1-H2B glycerol-gradient fractions or unfractionated, immunopurified eluates of Htz1-Flag or Hta1-Flag. The reaction mix (50 µl) was gently rotated for 1 h at 4 °C in buffer B containing 0.1 M KCl, then washed four times with 1 ml buffer B containing 0.15, 0.3 or 0.5 M KCl. Sample beads were resuspended with 2× SDS sample-loading buffer and incubated at 65 °C for 5 min. The supernatants were analyzed with SDS-PAGE (14% gel) and then western blotted.

Nucleosome array–binding assay. The amounts of wild-type and mutant SWR1 complexes used were normalized for the Flag-tagged Swr1 subunit by western blotting. Procedures for nucleosome assembly were as described in refs. 19,47. The binding assay was essentially the same as that for Htz1 transfer, except that neither Htz1-H2B nor ATP was included.

Immunostaining. BY4741 cells transformed with the plasmid expressing Htz1-Flag, Htz1M6-Flag or Htz1M6-Flag were grown in SD-URA medium to an A_{600} of 0.9. Immunofluorescence microscopy was conducted as described previously[49] except that fixed cells were digested with 1.5 µg $µl^{-1}$ Zymolyase 20T (ICN) for 10 min at 30 °C. Wild-type and mutant Htz1-Flag were probed with the mouse primary antibody to Flag M2 (Sigma) at a dilution of 1:125, then with a 1:1,000 dilution of anti-mouse conjugated to Alexa488 (Molecular Probes). Fluorescence microscopy was carried out on a Zeiss Axiophot microscope at ×1,000 magnification.

Note: Supplementary information is available on the Nature Structural & Molecular Biology website.

ACKNOWLEDGMENTS

We thank X. Shen (University of Texas, M.D. Anderson Cancer Center) for the pHtz1-2Flag plasmid, T. Tsukiyama (Fred Hutchinson Cancer Research Center) for the yeast strain W1544-4C and the 3× Flag–tagging plasmid and J. Landry and H. Xiao for helpful discussions. This work was supported by the Intramural Research Program of the US National Cancer Institute.

COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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