Histone fold proteins Dpb4 and Dls1 are components of the yeast ISW2 chromatin remodeling complex that resemble the smaller subunits of the CHRAC (Chromatin Accessibility Complex) complex found in Drosophila and humans. DNA photoaffinity labeling found that the Dpb4 subunit contacts extranucleosomal DNA 37–53 bp away from the entry/exit site of the nucleosome. Binding of Dpb4 to Isw2 and Itc2, the two largest subunits of ISW2, was found to require Dls1. Even after remodeling and nucleosome movement, Dpb4 tends to remain bound to its original binding site and likely serves as an anchor point for ISW2 on DNA. In vitro, only minor differences can be detected in the nucleosome binding and mobilization properties of ISW2 with or without Dpb4 and Dls1. Changes in the contacts of the largest subunit Itc1 with extranucleosomal DNA have, however, been found upon deletion of the Dpb4 and Dls1 dimer that may affect the nucleosome spacing properties of ISW2.

Chromatin is a repressive structure obstructing various cellular processes, including transcription, replication, recombination, and DNA repair. Chromatin is a dynamic structure regulated by many enzymes including ATP-dependent remodeling complexes that mobilize nucleosomes to expose or occlude various promoter regions for transcription (1). ATP-dependent chromatin remodeling complexes are classified into major subfamilies based on their catalytic ATPase subunits, such as SNF2, ISWI (Imitation Switch),4 CHD (Chromodomain helicase DNA binding), and INO80 (1, 2). Different subfamily members show distinct substrate specificities and nucleosome remodeling activities. For instance, the ATPase activity of SWI/SNF is stimulated by either free or nucleosomal DNA, whereas the ISWI and CHD proteins are stimulated primarily by nucleosomes (3–7). ISWI proteins require the flexible N-terminal tail of histone H4 to efficiently dock its translocase on the nucleosome (25) and for stimulating the ATPase activity and inducing nucleosome mobility (9). Histone tails have no apparent role in activating the CHD proteins (6). The SWI/SNF complexes can mobilize nucleosomes (10, 11), create a stably remodeled nucleosome intermediate (12), and transfer a histone octamer from one DNA fragment to another (13). In contrast, the ISWI complexes mediate movement of nucleosomes in cis without permanent displacement from DNA or disrupting the final nucleosome structure (14, 15).

In Saccharomyces cerevisiae, two ISWI ATPases have been identified and characterized, Isw1 and Isw2 (16). Initially, Isw2 (130 kDa) was found to bind to Itc1 (145 kDa), and later two histone fold proteins Dpb4 and Dls1 were also identified to be components of the ISW2 complex (17, 18). Dpb4 (22.0 kDa) is a homolog of human HuCHRAC-17 and Drosophila CHRAC-14, and Dls1 (18.8 kDa) is a homolog of human HuCHRAC-15 and Drosophila CHRAC-16. ISW2 appears to be an ortholog of human and Drosophila CHRAC complexes, which is further supported by the limited homology of Itc1 with human and Drosophila ACF1. Dpb4 is also part of DNA polymerase ε and is involved in the processive synthesis of both the leading and lagging strands of DNA (19). Dls1 (Dpb3-like subunit 1) is homologous to Dpb3, another subunit of DNA polymerase ε, that interacts with Dpb4. These proteins show a striking similarity to the histone fold domains of the mammalian transcription factors NF-YC (CBF-C) and NF-YB (CBF-A), respectively, and supposedly interact with each other via the “handshake” motif similar to the H2A/H2B histone dimer (19).

Similar to the CHRAC complex, yeast ISW2 slides mononucleosomes from the end to the center of DNA (15, 20, 21) and requires the acidic patch 17RHR19 of the histone H4 tail (9, 22–24). ISW2 has been shown to preferentially bind nucleosome substrates containing ~70 bp of extranucleosomal DNA at one entry/exit site of the nucleosome (25). ISW2 interacts with three nucleosomal and extranucleosomal regions. Itc1 and Isw2 bind to the region 2 helical turns (SHL2) from the dyad axis and a region closest to the entry/exit site. The Itc1 subunit makes extensive contacts with extranucleosomal DNA. The interactions of Itc1 with extranucleosomal DNA are likely to be involved in the directional binding and sliding of nucleosomes as well as the spacing of nucleosome arrays. An optimal length of extranucleosomal DNA has been shown to act in concert with the histone H4 tail to direct ISW2 to the critical internal contact site on the nucleosome for efficient nucleosome mobilization (8).

In vivo, ISW2 functions in parallel with the Sin3-Rpd3 histone deacetylase complex to repress early meiotic genes upon recruitment by Ume6 and creates a DNase I-inaccessible chromatin structure through the regulation of nucleosome positioning (26). The histone fold dimer Dpb4/Dls1 has been shown
to be required for generation of some ISW2-specific chromatin structures, repression of many ISW2-regulated genes, and the function of ISW2 at telomeric regions (17). Tsukiyama and colleagues (18) have shown that Dis1p is not required in vivo for binding of ISW2 to chromatin but is required for ISW2 remodeling and for repression of ISW2 target genes. The extent to which these histone fold subunits are required for transcription and nucleosome mobilization seems to vary among different targets.

In vitro, the *Drosophila* and human CHRAC histone fold dimer was found to interact with the ACF1 subunit (21). More importantly, excess histone dimer has been shown to enhance nucleosome binding and movement by hACF1-SNF2h (27). Recombinant human CHRAC histone fold dimer binds to DNA but not nucleosomes (28). The roles of the histone fold dimer of yeast ISW2 in nucleosome binding and sliding remain unexplored.

We have shown that Dpb4 specifically interacts with the extranucleosomal DNA at least 37 bp away from the edge of the nucleosome by site-specific DNA photoaffinity labeling and has no detectable contact with nucleosomal DNA. During remodeling by ISW2, Dpb4 did not readily track along DNA as nucleosomes are moved on to extranucleosomal DNA. These data suggest that Dpb4, likely in concert with Dls1, act as an anchor point on DNA for ISW2. We have compared ISW2 binding and remodeling with ISW2, lacking the two histone-fold subunits to test whether the histone dimer plays a role in nucleosome binding and mobilization by ISW2. Although some minor effects can be detected for nucleosome binding affinity and sliding efficiency, changes in Ltc1 contacts with extranucleosomal DNA have been observed. These data suggest the histone fold dimer may help to stabilize the interaction of Ltc1 with extranucleosomal DNA.

**MATERIALS AND METHODS**

**DNA Probes**—The nucleosome positioning sequence “601” was obtained from the pGEM-3Z/601 plasmid (from Jon Widom) (29). DNA probes with 601 and various extents of flanking extranucleosomal DNA were synthesized by PCR with HotMaster Mix from Eppendorf AG. One of the PCR primers (20 pmol) was radiolabeled using 40 units of OptiKinase (US Biochemicals) and 5 pmol of [γ-32P]ATP (6000 Ci/mmol, 10 mCi/ml, PerkinElmer Life Sciences) in a 40-μl reaction. The PCR products were purified with the QIAquick PCR purification kit (Qiagen) and analyzed by native PAGE and denaturing PAGE containing 8 M urea.

**DNA Photoaffinity Cross-linking and Immunoprecipitation**—Site-specific photoreactive and radiolabeled DNA probes were synthesized using a template containing a 601-nucleosome positioning sequence as described previously (20, 25, 29). Mononucleosomes were reconstituted using purified photoreactive probes by the salt dilution method (20). Mononucleosomes (2.8 pmol) were incubated with or without FLAG-purified ISW2 at 30 °C for 30 min in a 25-μl reaction containing 30 mM Na-HEPES, pH 7.8, 65 mM NaCl, 5 mM MgCl2, 5% glycerol, and 0.1 mg/ml bovine serum albumin. Four microliters were analyzed on a 4% native polyacrylamide gel (acrylamide:bisacrylamide, 38.9:1.1, in 0.5× TBE) to assess ISW2 binding. In all experiments, >90% of nucleosomes were bound to ISW2. Reactions were irradiated for 2 min (310 nm, 2.65 milliwatt/cm2) and then digested sequentially with 4.6 units of DNase I (Ambion) and 20 units of S1 nuclease (US Biochemicals) (30). A final concentration of 0.4% SDS was added to the samples after DNase I digestion to disrupt protein-DNA complexes. Samples were analyzed by 14 or 4–20% SDS-PAGE and cross-linked ISW2 subunits visualized by phosphorimaging. Colloidal blue staining (Owl Pro-Blue) was performed for the 14% SDS-PAGE prior to phosphorimaging.

For immunoprecipitation of Dpb4, binding reactions were scaled up to 100 μl. After DNase I and S1 nuclease digestion, the pH of the reaction was adjusted to 7.0 with 7 μl of 0.5 M Tris base and reactions diluted 4-fold with precooled immunoprecipitation buffer containing 25 mM Na-HEPES, pH 7.8, 300 mM NaCl, 10% glycerol, 0.1% Nonidet P-40, 10 mM 2-mercaptoethanol, 1 μg/ml leupeptin, 1 μg/ml pepstatin A, and 1 mM phenylmethylsulfonyl fluoride. One microliter of anti-Dpb4 antibody (from Akio Sugino) was added and incubated at 4 °C overnight. Samples were incubated for another hour at 4 °C with 10 μl of equilibrated EZview protein A-agarose (Sigma) and gentle agitation. Agarose beads were washed three times with immunoprecipitation buffer and resuspended in 35 μl of SDS gel loading buffer. Samples were denatured and analyzed on a 4–20% SDS-polyacrylamide gel.

Remodeling reactions that were cross-linked were cooled to 20 °C for 5 min before the addition of ATP. ATP was added to a final concentration of 90 μM and incubated for an additional 5 min. Samples with or without ATP were irradiated for 5 s and digested as described above and analyzed by 4–20% SDS-PAGE and phosphorimaging.

**Generation of DLS1 Deletion Strain**—The open reading frame YIL065C (DLS1) was deleted in the strain YTT480 (MATa ade2-1 can1–100 his3–3,15 leu2–3,112 trp1–1 ura3–1 RAD5+ pep4::HIS3 isw2-2FLAG) containing FLAG-tagged Isw2 by PCR-based gene deletion using URA3 as the selection marker and is referred to as YMK1010 (31). Transformation was performed using the yeast-TRAFO procedure as described previously (32) and appropriate colonies selected on uracil minus plates. Knock-out of the DLS1 gene was confirmed by PCR for deletion at both junctions.

**Affinity Purification of ISW2 and ISW2ΔDls1 Complexes**—The ISW2 and ISW2ΔDls1 complexes were purified from *Saccharomyces cerevisiae* strain YTT480 and YMK1010, respectively, both bearing a FLAG epitope-tagged version of the ISW2 gene. The ISW2 complex was affinity-purified from S100 extract by batch binding to anti-FLAG M2-agarose beads (Sigma) (10 μl of anti-FLAG M2 beads/ml of whole cell extract) and eluted as described previously (16, 25).

**Hydroxyl Radical Footprinting**—ISW2 binding reactions were carried out in a 25-μl reaction as described previously (25) without the addition of ATP. The cleavage reaction was performed as described previously (33), except that the final concentrations of Fe(II), H2O2, ascorbate, and EDTA were 280 μM, 0.17%, 5.7 mM, and 220 μM, respectively, and terminated by the addition of 100 μl of 5 M ammonium acetate, 5 mM thiourea, and 10 mM EDTA.
**The Histone Fold Subunits of ISW2**

**A.** DNA (1 mg/ml). Binding and remodeling reactions were analyzed by % native PAGE (acylamide:bisacrylamide ratio of 36:1, 0.5× TBE, 4 °C) and phosphorimaging. All lanes were normalized based on total signal and the fraction bound or mobilized calculated by comparing the amount of unbound or immobilized nucleosomes in each lane to nucleosomes in the control lane with no ISW2 added.

**Exonuclease III Footprinting—**

Saturating amounts of ISW2 or ISW2ΔDls1 were incubated with nucleosomes containing 70 bp of extranucleosomal DNA at one entry/exit site in a typical ISW2 reaction at 30 °C for 30 min. A titration of exonuclease III (0.5–4 units, New England Biolabs) was added to 12.5-μl of the ISW2 binding reaction. Digestion was performed at 30 °C for 30 s and stopped by the addition of 95% formamide containing 0.0625% bromphenol blue and 0.0625% xylene cyanol FF. Samples were denatured by heating at 90 °C for 3 min and analyzed by denaturing PAGE containing 8 M urea.

**RESULTS**

**Dpb4 Interacts with Extranucleosomal DNA in the Nucleosome—**

DNA photoaffinity labeling was used to detect contacts of the two histone fold subunits of ISW2 with DNA due to binding of the ISW2 complex to nucleosomes. Twenty-three different DNA photoaffinity probes were used to topologically map the location of the histone fold subunits of ISW2 with nucleosomal and extranucleosomal DNA (25). Cross-linking of one of the histone fold proteins was detected within extranucleosomal DNA from 126 to −110 bp from the dyad axis or 37–53 bp from the entry/exit site of the nucleosome (Fig. 1, A and C). The identity of the cross-linked histone fold protein was verified by comparison of the electrophoretic mobilities of the cross-linked protein with that of the stained smaller subunits (data not shown). Cross-linked Dpb4 was further verified by immunoprecipitation (as shown in Fig. 1B, comparing lanes 4 and 5 with lanes 1–3 and 6). No cross-linked Dpb4 was detected in regions other than extranucleosomal DNA, indicating that it likely does not interact with nucleosomal DNA.
The Histone Fold Subunits of ISW2

A.

Before Remodeling

After Remodeling

310 bp

-169
-138
-111/110

251 bp

-169
-138
-111/110

222 bp

-169
-138
-111/110

B. Probe Length (bp)

Probe Position

ATP

Dpb4

Histones

C. Relative Intensity of Dpb4

Probe Position

ATP

Dpb4

Histones

FIGURE 2. Histone fold dimer acts as an anchor point during ISW2 remodeling. A, a diagram is shown of the sites probed by DNA cross-linking in nucleosomes assembled with 222, 251, and 310 bp of DNA before and after remodeling with ISW2. The distance of the sites from the dyad axis is also shown underneath. B, photoaffinity labeling was performed the same as in Fig. 1 with or without ATP (90 μM) and nucleosomes containing different lengths of DNA. The probe position number shown above the lanes are the same as in Fig. 1. C, the cross-linking intensities of Dpb4 were quantified and plotted from three independent experiments. The corresponding probe positions, probe DNA length, and extranucleosomal DNA (E. DNA) length are shown. Also indicated are the distances the nucleosomes are moved with particular lengths of DNA probe and extranucleosomal DNA and the corresponding new positions (with reference to the new dyad position) of the cross-linker after nucleosome mobilization.

110/111 bp from the dyad axis was moved 50–60 or 20–30 bp from the dyad axis, respectively (Fig. 2A). Under these conditions, the efficiency of Dpb4 cross-linking was reduced 3.5-fold (50–60 bp from the dyad axis) or 6-fold (20–30 bp from the dyad axis) after remodeling (Fig. 2B, lanes 7–10, and Fig. 2C). These data suggest that Dpb4 is partially displaced when the DNA to which it is bound is pushed inside the nucleosome, but remarkably even then some Dpb4 is still retained at the original position on DNA.

DNA cross-linking of nucleosomes with 104 bp of extranucleosomal DNA showed no change in the efficiency of Dpb4 cross-linking at a position that starts out 138 bp from the dyad axis and was moved to 78–88 bp from the dyad axis (Fig. 2B, lanes 5 and 6). This result is again consistent with Dpb4 remaining close to its original bound position rather than moving along with the nucleosome to keep a distance of 37 bp from the edge of the nucleosome. Cross-linking of Dpb4 increased in these nucleosomes when initial cross-linking 169 bp from the dyad is relocated 109–119 bp from the dyad axis due to remodeling (Fig. 2B, lanes 3 and 4). Changes in the translational position of nucleosomes are noticeable at these positions as evident by changes in histone cross-linking (Fig. 2B). The Dpb4 subunit appears to act as an anchor point for the ISW2 complex on extranucleosomal DNA that retains its position on DNA during nucleosome mobilization.

The Dpb4 and Dls1 Histone Fold Dimer Makes Minor Contributions to Nucleosome Binding and Mobilization by ISW2—A complex of Isw2 and Itc1 without the two histone fold proteins was made by deleting the DLS1 gene and FLAG tagging ISW2 and is referred to as ISW2ΔDls1. The absence of the histone fold subunits was confirmed by SDS-PAGE and Western analysis (Fig. 3A). The nucleosome binding activity of ISW2 and ISW2ΔDls1 were compared by gel shift assays with nucleosomes that were assembled with the 601-nucleosome positioning sequence and 70 bp of extranucleosomal DNA. ISW2ΔDls1 missing the histone fold subunits had only slightly reduced binding affinity for nucleosomes compared with wild-type ISW2 (Fig. 3, B and C). Subtle changes of <2-fold were observed with nucleosomes containing different lengths of extranucleosomal DNA (data not shown). Efficiency of nucleosome mobilization with ISW2ΔDls1 was similar to that observed for wild-type ISW2 with 70 bp of extranucleosomal DNA (Fig. 3, D and E) or

The association of Dpb4 with extranucleosomal DNA corresponded to the same region that of strong Itc1 cross-linking (25).

Dpb4 Does Not Retain Its Position Relative to the Edge of the Nucleosome after Remodeling—Photocross-linking of ISW2 subunits was used to monitor the location of Dpb4 after ISW2 remodeling of nucleosomes containing 75, 104, and 163 bp of extranucleosomal DNA at one entry/exit site. These nucleosomes are moved 30–90 bp depending on the length of extranucleosomal DNA, and nucleosome movement was earlier determined by site-directed mapping (Fig. 2A and results not shown) (34). If Dpb4 maintains the same distance from the edge of the nucleosome after remodeling, then cross-linking of Dpb4 should be eliminated at the DNA position originally 110 and 111 bp from the dyad axis. After ISW2 remodeling of nucleosomes with 75 bp of extranucleosomal DNA, this DNA position is moved 30–40 bp, such that it is 70–80 bp from the dyad axis (Fig. 2, A and C). Contrary to expectations, cross-linking of Dpb4 at the original position 110 and 111 bp from the dyad axis was not reduced when ISW22 remediated nucleosomes with 75 bp of extranucleosomal DNA (Fig. 2B, lanes 1 and 2, and Fig. 2C). This position was moved even closer until it was inside the nucleosome by increasing extranucleosomal DNA to 104 and 163 bp, such that after remodeling, the original position...
other lengths of extranucleosomal DNA (data not shown). More detailed analysis of the interactions of ISW2ΔDls1 with nucleosome was done by hydroxyl radical footprinting (25). ISW2ΔDls1 had the same tripartite interaction with nucleosomes as wild-type ISW2, with extensive protection of extranucleosomal DNA, a 10–20 bp region at the entry site of nucleosomes, and a 10-bp region at SHL2 (Fig. 4). The histone fold subunits did not appear to be critical for efficient nucleosome binding, mobilization, or for the proper interaction of ISW2 with the three different extranucleosomal and nucleosomal regions.

The Absence of the Histone Fold Subunits Compromises the Interaction of ISW2 at the Far Edge of Extranucleosomal DNA—The absence of the histone fold dimer from the ISW2 complex could cause a change in binding to extranucleosomal DNA that might not be as readily detected by hydroxyl radical footprinting. Exonuclease III mapping was used to determine the extent of ISW2 interactions with extranucleosomal DNA. Wild-type ISW2 protected a region up to 52 bp from the edge of the nucleosome, whereas ISW2ΔDls1 protected less well with its strongest site of protection only 39 bp from the edge of the nucleosome (Fig. 5, compare lanes 12 and 16, asterisks). Exonuclease III may more readily displace ISW2 lacking the histone fold subunits from DNA at the edge of ISW2 bound to extranucleosomal DNA than wild-type ISW2, thus accounting for differences in pausing from 39 to 52 bp from the edge of the nucleosome (Fig. 5, compare lanes 12 and 16). The 13-bp region in which this difference occurs remarkably coincides with the region bound to Dpb4, 37–53 bp from the edge of the nucleosome, as earlier shown by DNA photoaffinity labeling.

Loss of the Histone Fold Dimer Changes the Interactions of Itc1 with Extranucleosomal DNA—The effect of the Dpb4-Dls1 histone fold
dimer on the interactions of Itc1 and Isw2 with extranucleosomal DNA was examined by comparing the photoaffinity labeling patterns of ISW2 and ISW2/Dls1. The most efficient cross-linking of Itc1 to DNA corresponded to the same region of Dls1-Dpb4 cross-linking (Fig. 1A, lanes 4, 6, and 8). These data and the fact that Itc1 is required for binding of Dls1-Dpb4 to the ISW2 complex5 indicate that Itc1 interacts with the Dls1/Dpb4 complex. Loss of the histone fold dimer caused a reduction of Itc1 cross-linking at the more distal extranucleosomal DNA region 169 bp from the dyad axis (Fig. 6A, lanes 1 and 2, and Fig. 6B). At regions more proximal to the nucleosome, 122 and 111/110 bp from the dyad axis, loss of Dls1/Dpb4 caused an increase in cross-linking of Itc1 (Fig. 6A, lanes 7–8), with no significant changes evident at 138 and 149 bp from the dyad axis (lanes 3–6). One of the two possible explanations is that Itc1 adopted a more extended conformation in the presence of the histone fold subunits, such that Itc1 can be cross-linked to more distal extranucleosomal DNA. The other explanation may be that the histone fold subunits, similar to the histone H2A/H2B dimer, bends the extranucleosomal DNA and brings the distal extranucleosomal DNA closer to the nucleosome and to other parts of the ISW2 complex.

**DISCUSSION**

We have shown that Dpb4 interacts with extranucleosomal DNA starting ~37 bp from the entry/exit site of the nucleosome and proceeding farther out on extranucleosomal DNA as shown by cross-linking. The DNA sites of strongest cross-linking of Dpb4 correlate with the region of strongest cross-linking of Itc1 with extranucleosomal DNA and implicate that the Dpb4/Dls1 dimer interacts with Itc1. Dpb4 likely forms a tight heterodimer with Dls1 based on their homology with the heterodimers from CHRAC and similarity to other histone fold proteins. The interaction of Dpb4 with Dls1 was also shown by the deletion of Dls1, causing the loss of both Dls1 and Dpb4 from ISW2 when purified using FLAG-tagged Isw2. It is not clear why Dls1 was not also cross-linked to DNA in the same way that Dpb4 was to extranucleosomal DNA; but instead, Dpb4 anchors itself to a region of extranucleosomal DNA.

We have demonstrated that removing the histone fold dimer reduced the extent of interaction between Itc1 and extranu-
cleosomal DNA as well as destabilizing the interaction of ISW2 with extranucleosomal DNA. Reduced cross-linking of Itc1 at the distal sites in the extranucleosomal DNA was accompanied by increased Itc1 cross-linking to regions closer to the edge of the nucleosome. The overall affinity and remodeling activity of ISW2 was, however, not dependent on Dpb4 or Dls1 as shown.

The binding affinity of ISW2 for nucleosomes is unchanged in the absence of Dpb4/Dls1, because most of the interactions of ISW2 were intact in the absence of Dpb4/Dls1 as shown by hydroxyl radical footprinting of the ISW2-nucleosome complex. These results are different from those found previously for the human and Drosophila CHRAC complexes. Nucleosome binding and remodeling of recombinant human ACF1-SNF2h was significantly enhanced by the addition of an excess of recombinant CHRAC-15/CHRAC-17 dimer (21). Nucleosome mobilization was also shown to be enhanced by the addition of excess heterodimer of Drosophila CHRAC complex as well in an ACF1-mediated fashion. It is not clear as to the need for an excess of the heterodimer, because in the intact complex, there is only stoichiometric amounts of the heterodimer. In these studies, a much larger amount of the recombinant human ACF1-SNF2h was required for binding or remodeling than that observed in our studies for the Itc1-Isw2 complex (ISW2ΔDls1).

During chromatin remodeling by ISW2, we have found that the Dls1/Dpb4 dimer did not continuously track along the DNA as the nucleosome is mobilized. Instead, Dpb4 remained bound to its original binding site until partly displaced by the edge of the moving nucleosome. These results suggest that the histone fold dimer Dpb4-Dls1 acts as an anchor point on the extranucleosomal DNA for the ISW2 complex. Further experiments will be required to determine whether the Dls1/Dpb4 dimer facilitates in the processivity of ISW2.

Tsukiyama and colleagues (18) show that the histone fold subunits are required for chromatin remodeling from various loci and that their effect can be quite varied. They suggest that some genes may require ISW2 containing Dpb4/Dls1, whereas the alternative form of ISW2 missing the two subunits is sufficient for other genes. The in vivo binding of ISW2 was shown not to be affected by the absence of Dpb4/Dls1 at any of these target genes, consistent with our data that Dpb4/Dls1 is not required for efficient ISW2 binding. However, the interaction of Dpb4 with extranucleosomal DNA and its modulation of Itc1 interactions with extranucleosomal DNA suggest that it might affect its nucleosome spacing activity, the ability to sense an adjacent nucleosome and stop remodeling. These kinds of effects could explain those observed in vivo with the loss of Dpb4 and Dls1. Changes in chromatin structure, as detected by indirect labeling, were in several instances as severe as isw2, whereas others were either intermediate between that of isw2 and wild type or were completely unaltered (18). Our results suggest these differences could be due to variable requirements for spacing nucleosomes at these different promoter regions. Our data points to the heterodimer of Dpb4 and Dls1 contacting extranucleosomal DNA and influencing the interaction of Itc1, the largest subunit of ISW2 that makes extensive contact with extranucleosomal DNA. These changes would likely modulate the spacing activity of ISW2 and could account for the observed changes in chromatin structure upon their loss in vivo.

Recently Johansson and colleagues (35) have solved the cryo-electron microscopy structure of yeast DNA polymerase ε, and they suggest that the Dpb4 portion of DNA polymerase ε forms an extended tail of the complex and faces the double-stranded DNA that feeds into the Pol2 subunit of the polymerase. The Dpb-Pol2 connection is flexible enough to allow the polymerase to track along the DNA. We envision that the Dpb4-Dls1 subunits of yeast ISW2 may serve such a role in securing the DNA that is being translocated by ISW2.

Acknowledgments—We thank Toshi Tsukiyama for providing us with the yeast strain YTT7480 and Aki Sugino for anti-Dpb4 antiserum. We also thank Swetansu Hota for technical assistance in the construction of the strain YMK1010 and the purification of ISW2ΔDls1 and other members of the Bartholomew laboratory for critical review and comments on the manuscript.

REFERENCES
The Dpb4 Subunit of ISW2 Is Anchored to Extranucleosomal DNA
Weiwei Dang, Mohamedi N. Kagalwala and Blaine Bartholomew

doi: 10.1074/jbc.M700640200 originally published online May 9, 2007

Access the most updated version of this article at doi: 10.1074/jbc.M700640200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 35 references, 15 of which can be accessed free at
http://www.jbc.org/content/282/27/19418.full.html#ref-list-1