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Histone Fold Protein Dls1p Is Required for Isw2-Dependent Chromatin Remodeling In Vivo

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We report the identification of two new subunits of the Isw2 chromatin-remodeling complex in *Saccharomyces cerevisiae*. Both proteins, Dpb4p and Yjl065cp (named Dls1p), contain histone fold motifs and are homologous to the two smallest subunits of ISWI-containing CHRAC complexes in higher eukaryotes. Dpb4p is also a subunit of the DNA polymerase epsilon (polε) complex, and Dls1p is homologous to another polε subunit, Dpb3p. Therefore, these small histone fold proteins may fulfill functions that are required for both polε and Isw2 complexes. We characterized the role of Dls1p in known roles of the Isw2 complex in vivo. Transcriptional analyses reveal that the Isw2 complex requires Dls1p to various degrees at a wide variety of loci in vivo. Consistent with this, Dls1p is required for Isw2-dependent chromatin remodeling in vivo, although the requirement for this protein varies among Isw2 targets. Dls1p is likely required for functions of the Isw2 complex at steps subsequent to its interaction with chromatin, since a *dls1* mutation does not affect cross-linking of Isw2 with chromatin.

Chromatin structure in eukaryotic nuclei in vivo imposes restrictions on a wide variety of protein-DNA interactions, causing inhibition of cellular processes that depend upon these interactions. As a consequence, the regulation of chromatin structure can modulate these processes both positively and negatively. Consistent with this idea, it has become clear that chromatin-remodeling factors play critical roles in the regulation of a wide variety of nuclear processes. There are two major classes of chromatin-remodeling factors, histone-modifying enzymes and ATP-dependent chromatin-remodeling complexes. Among the histone modifications described so far, histone acetylation, methylation, and phosphorylation have been the most extensively studied because of their close correlation with transcription, DNA repair, and chromatin dynamics (23, 24, 33, 37).

Biochemical and genetic studies have identified several ATP-dependent chromatin-remodeling complexes from a wide variety of eukaryotic organisms (4, 31, 45). These complexes exhibit numerous biochemical activities, including ATPase activity, nucleosome sliding activity, and nucleosome assembly activity, and the ability to modify histone-DNA contacts. They are grouped into different classes according to the ATPase subunit found in each complex, including SWI/SNF, ISWI, CHD1, and INO80. The ISWI class of ATPases has been evolutionarily well conserved. It has been identified in mammals (5, 6, 34, 39), *Xenopus laevis* (10, 18, 26, 30), *Drosophila melanogaster* (21, 41, 43), and the budding yeast *Saccharomyces cerevisiae* (42). This high degree of evolutionary conservation strongly argues for critical functions of ISWI chromatin-remodeling factors in vivo.

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The biological functions of some ISWI chromatin-remodeling factors have just begun to be uncovered. In *Drosophila*, the *ISWI* gene is essential for cell viability, and *ISWI* mutant embryos die at late larval or early pupal stages (11). One of the most striking phenotypes of *ISWI* mutants is a massive deformation of the X chromosome in male flies (11), which requires the presence of an active dosage compensation complex (8). NURF, one of three ISWI-containing complexes in *Drosophila*, is responsible for both cell viability and maintenance of chromosome architecture in males (3). The connection between ISWI remodeling factors and higher-order chromatin structure appears to be evolutionarily conserved. In mammalian cells, ISWI-containing complexes are targeted to heterochromatin (6) and are required for its replication (7) and silencing (38). In addition, ISWI complexes are implicated in loading of cohesin complex (19) and functions of matrix association regions (46).

ISWI-containing CHRAC complexes in higher eukaryotes contain two small histone fold proteins that are evolutionarily conserved (9, 34). Recent studies revealed that the human CHRAC and DNA polymerase epsilon (polε) complexes share a small histone fold subunit (28, 34). This finding led to the proposal that functions of ISWI complexes in heterochromatin may be mediated by the subunits containing histone fold motifs (7). However, the roles of these histone fold proteins in vivo have not been identified.

Our laboratory has shown that one of the two ISWI proteins in yeast, Isw2p, forms a complex that is required for repression of early meiotic genes during mitotic cell growth (17). Our group further demonstrated that Isw2 and Rpd3-Sin3 histone deacetylase complexes collaborate to repress a wide variety of genes (13, 17). In addition, the Isw2 complex is required for repression of Mat**a**-specific genes in Matα cells (40; M. E. Gelbart and T. Tsukiyama, unpublished data). Consistent with these results in yeast, *Drosophila* ISWI protein and RNA polymerase II localize in a mutually exclusive manner on polytene

TABLE 1. Strains used in this study

Strain	Description
W1588-4C	*MAT***a** *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1*
W1588-4A	*MATα ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1*
YTT219	*MAT***a** *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 isw2::HisG*
YTT550	*MAT***a** *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 rpd3::KanMX*
YTT552	*MAT***a** *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 isw2::HisG rpd3::KanMX*
YTT717	*MAT***a** *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 isw2::HisG rpd3::KanMX*
YTT966	*MAT***a** *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 ISW2-3FLAG-KanMX*
YTT970	*MAT***a** *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 ISW2-TAP-klTRP1*
YTT1080	*MAT***a** *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 isw2::NAT*
YTT1083	*MATα ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 isw2::NAT*
YTT1409	*MATα ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 ISW2-3FLAG-KanMX*
YTT1423	*MATα ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 isw2::HisG rpd3::KanMX*
YTT2133	*MAT***a** *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 ISW2-13MYC-KanMX POL2-3FLAG-Hyg*
YTT2172	*MAT***a** *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 ds1l::KanMX*
YTT2173	*MAT***a** *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 ds1l::KanMX*
YTT2174	*MATα ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 dls1::KanMX*
YTT2186	*MAT***a** *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 DPB4-6HA-klTRP ISW2-13MYC-KanMX POL2-3FLAG-Hyg*
YTT2200	*MAT***a** *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 DSL1-3FLAG-Hyg*
YTT2346	*MAT***a** *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 dls1::Hyg rpd3::KanMX*
YTT2347	*MAT***a** *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 dls1::Hyg rpd3::KanMX*
YTT2348	*MAT***a** *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 dls1::Hyg rpd3::KanMX*
YTT2351	*MATα ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 dls1::Hyg rpd3::KanMX*
YTT2492	*MATα ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 dls1::Nat Isw2-3FLAG-KanMX*
YTT2527	*MAT***a** *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 DLS1-3FLAG-Hyg ISW2-6HA-klTRP1 ITC1-13MYC-KanMX*
YTT2529	*MAT***a** *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 ISW2-6HA-klTRP1 ITC1-13MYC-KanMX*
YTT2532	*MATα ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 DLS1-3FLAG-Hyg*
YTT2722	*MATα ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 dls1::Nat isw2::HisG rpd3::KanMX*
YTT2756	*MAT***a** *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 DPB2-3FLAG-HYG*
YTT2757	*MAT***a** *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 DPB2-3FLAG-HYG ISW2-13MYC-KanMX*

chromosomes (11), and a mammalian ISWI complex, No-RC, represses ribosomal DNA transcription (48). Isw1p, another ISWI protein in yeast, forms two complexes (44) that affect nucleosome position at a number of loci in vivo (25) and are involved in transcriptional termination together with the Isw2 complex and another chromatin-remodeling factor, Chd1p (1).

In this work, we describe the identification of two new subunits of the Isw2 complex, Dpb4p and Yjl065cp (named Dls1p). Dpb4p has previously been identified as a nonessential subunit of polε, whereas Dls1p has not been previously characterized. However, Dls1p is homologous to another nonessential subunit of polε, Dpb3p. Dpb4p and Dls1p have histone fold motifs and are homologous to the smallest subunits of CHRAC complexes in higher eukaryotes. Our studies reveal that Dls1p plays critical roles in Isw2-dependent repression of a wide variety of genes in vivo, including early meiotic genes. However, Dls1p is only partially required for repression of some genes, including *POT1*, and is dispensable for repression of Mat**a**-specific genes in Matα cells. Chromatin analyses reveal that Dls1p is required for formation of normal repressive chromatin structure at each of these loci, although the degree to which the Isw2 complex depends on Dls1p varies among Isw2 targets. Chromatin immunoprecipitation analyses suggest that Dls1p is likely required for functions of Isw2 at steps subsequent to its interaction with chromatin.

MATERIALS AND METHODS

Yeast strains. All strains are congenic to W303 and were derived from W1588-4A and W1588-4C (47). The strains used in this study are listed in Table 1. The following epitope tags were used to mark Isw2 and polε subunits as previously described: three-FLAG (16), TAP (36), six-hemagglutinin (HA) (27), and 13-Myc (29) tags.

Purification of Isw2 complex. Isw2p was purified via the TAP tag (36) or three-FLAG tag (16) followed by a Source Q column (42) or a glycerol gradient (15 to 35% in buffer H [42], 20 h at 49,000 rpm in an SW55 rotor). Dls1p was tagged by a three-FLAG epitope and purified by immunoaffinity chromatography, followed by a Source Q column (0.2 ml) as described previously (42).

Coimmunoprecipitation assay. Yeast cells grown to mid-log phase (2 liters) were harvested, and extracts were prepared by breaking frozen cell pellets in coffee grinders, followed by thawing frozen cell powder in buffer H-0.3. Extracts were then clarified by ultracentrifugation, yielding approximately 1.5 ml in volume. For immunoprecipitation, 12 (FLAG) or 20 (HA) μg of antibodies was conjugated with 20 or 80 μl, respectively, of Protein G Dynabeads (Dynal) and mixed with approximately 400 μg (100 to 150 μl) of extract for 3 h at 4°C. After beads were washed in buffer H-0.3 five times for 5 min each, bound proteins were eluted by boiling in 20 μl of sodium dodecyl sulfate-polyacrylamide gel sample buffer. Five microliters of samples was loaded per lane.

RNA analysis. Northern blotting was done as described previously (17) using 30 μg of total RNA per lane. For DNA microarray analysis, RNA was prepared in triplicate (YTT2346, YTT2347, and YTT2348 for the *dls1 rpd3* mutant and YTT552 and YTT717 [duplicated] for the *isw2 rpd3* mutant). To make data comparable to published results (13), Mat**a** cells were used. Direct comparison of transcriptional profiles of mutants was done as follows. Each RNA sample was labeled separately with Cy3 and Cy5 dyes, and samples from the two mutants were directly competed on PCR-based spotted arrays as described previously (13). For each pair of mutants, analysis was done using a dye swap pair. This was done for three independent RNA samples from each genotype (total of six hybridizations), and the results were normalized by GeneSpring 5.03 software (Silicon Genetics). The full data set for DNA microarray analysis is available at http://www.fhcrc.org/labs/tsukiyama/mcb-supplementary-data/. All DNA microarray analyses were performed at the DNA Array Facility at the Fred Hutchinson Cancer Research Center.

Nucleosome mapping. Nucleosome mapping was done by digestion of chromatin with micrococcal nuclease (MNase) followed by indirect end labeling as described elsewhere (14).

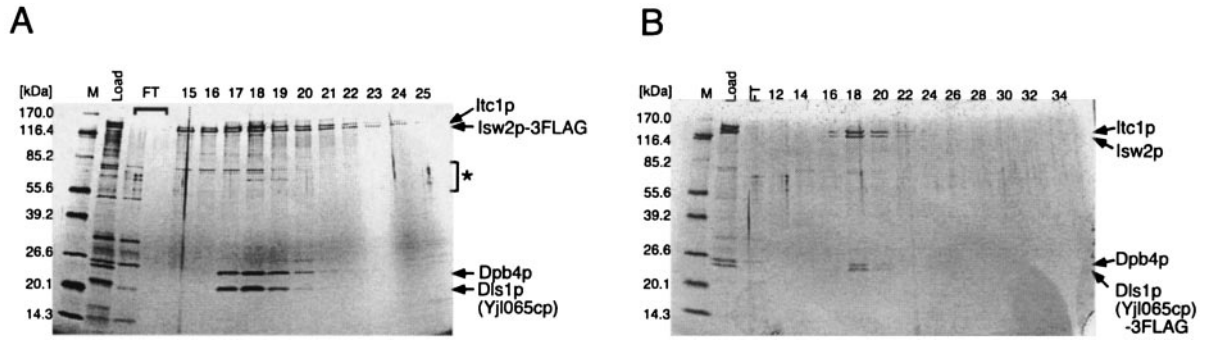

FIG. 1. Identification of two previously unidentified subunits of the Isw2 complex. (A) Silver-stained gel of Isw2p purified by FLAG immunoaffinity chromatography followed by Source Q anionic-exchange chromatography. The identities of Isw2 complex subunits are shown on the right. The asterisk shows contaminants in the preparation. The numbers on the top indicate the fractions from the Source Q column. "Load" corresponds to the fraction from the FLAG immunoaffinity purification step, and "FT" represents the flowthrough fractions. (B) Silver-stained gel of Dls1p (Yjl065cp) purified by FLAG immunoaffinity chromatography followed by the Source Q column. The identities of Isw2 complex subunits are shown on the right. The numbers indicate the fractions from the Source Q column.

Chromatin immunoprecipitation (CHIP) assay. The CHIP assay was done as previously described (17) with minor modifications. Briefly, cells (300 ml of culture in yeast extract-peptone-dextrose) were fixed at an optical density at 600 nm of 0.5 by addition of 30 ml of 11% formaldehyde–0.1 M NaCl–1 mM EDTA–50 mM HEPES-KOH, pH 7.6, for 20 min. After quenching for 5 min by addition of 54 ml of 2.5 M glycine, cells were washed and broken by glass beads. Chromatin was precipitated by centrifugation (1 min at 20,800 × *g* in a microcentrifuge) and washed twice with 1 ml of FA lysis buffer (50 mM HEPES-KOH [pH 7.6], 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% sodium deoxycholate). Chromatin fragments (average, ~500 bp) were solubilized by sonication, and insoluble materials were removed by centrifugation. Chromatin corresponding to approximately 40 ml of culture was used per immunoprecipitation with 4 μg of FLAG M2 antibody (Sigma) conjugated with 20 μl of Dynabeads Protein G (Dynal) as described previously (35). After beads were washed three times with FA lysis buffer, twice with FA lysis buffer with 500 mM NaCl, and once with radioimmunoprecipitation assay buffer (10 mM Tris [pH 8.0], 0.25 M LiCl, 0.5% NP-40, 0.5% sodium deoxycholate, 1 mM EDTA), bound proteins were eluted twice with 50 μl of 1-mg/ml peptide (Met-Asp-Tyr-Lys-Asp-His-Asp-Gly-Asp-Tyr-Lys-Asp-His-Asp-Ile-Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys) in radioimmunoprecipitation assay buffer. Cross-linking was reversed by incubation of samples at 75°C for 6 h, and DNA was purified by proteinase K digestion followed by organic extractions. A detailed protocol for the CHIP assay is available upon request.

For PCR, the following primers were used: REC104 (+), GTCCTTTAGCT AATAGAGTAAGCC; REC104 (−), ATGGACATGTTGTCCAAGTTGCTG; POT1 (+), TGCTAGTTTTGAACCTATGCCAC; POT1 (−), TATTCACTCT GTACTCAGAGCCAC; STE6 (+), GCGACATAGCTGTTATTACCTAC TAG; STE6 (−), GATGAACGGCAATAATGCAACAGT; YPL025C (+), GA CAGATACTTGAGCAGATTTTGTGG; and YPL025C (−), CACAGTTTTA GTAGGGTCACCGATA.

YPL025C was identified in a preliminary CHIP analysis on DNA microarray as a locus where Isw2p cross-links significantly less efficiently than at most other chromosomal loci (T. Tsukiyama, unpublished results). PCR was done in the presence of a radioactive nucleotide, and signals were quantified by a phosphorimager (Molecular Dynamics). The signal at each Isw2 target locus was measured relative to that at the *YPL025C* locus. Three independent immunoprecipitations were performed using two independent chromatin preparations, and the average and standard deviation of the signals were calculated at each locus.

RESULTS

Identification of two previously unidentified Isw2 subunits. A large-scale purification of the Isw2 complex by FLAG immunoaffinity chromatography followed by a Source Q anionic-exchange column showed Isw2p to copurify with two previously unidentified proteins (Fig. 1A). A FLAG tag on Isw2p does not disturb the functions of the Isw2 complex, as Isw2 target genes remain repressed in the tagged strain (data not shown). These proteins were identified as Dpb4p and Yjl065cp by mass spectrometry. Association of these two proteins with Isw2p was confirmed by an independent purification of Isw2p via TAP tag followed by mass spectrometry analysis, as well as by an independent purification of the Isw2 complex by FLAG immunoaffinity chromatography and a glycerol gradient (data not shown). To further confirm the association of Dpb4p and Yjl065cp with Isw2, we directly purified Yjl065cp via FLAG immunoaffinity chromatography followed by a Source Q anionic-exchange column (Fig. 1B), which yielded a four-subunit Isw2 complex (Itc1p, Isw2p, Dpb4p, and Yjl065cp) and no other proteins at a detectable level. This result confirms the presence of Yjl065cp and Dpb4p in the Isw2 complex. In addition, it suggests that Yjl065cp is predominantly present in the Isw2 complex in the conditions under which cells were grown. It is likely that our group (42) and others (15) previously failed to identify these small subunits due to their low molecular weights and because they do not stain well by silver staining. Dpb4p and Yjl065cp were independently identified as Isw2 subunits by Iida and Araki (20), and Yjl065cp was named Dls1p since it is homologous to a DNA polymerase epsilon (polε) subunit, Dpb3p (Dpb3-like subunit of Isw2 complex 1). This name will be used in the rest of this paper.

While Dls1p has been previously uncharacterized, Dpb4p was identified as a nonessential subunit of polε (32), suggesting that this subunit is shared between polε and Isw2 complexes. We tested this possibility by a coimmunoprecipitation experiment using a strain in which Dpb4p, Isw2p, and Pol2p were epitope tagged. Pol2p is the catalytic subunit of polε. As shown in Fig. 2A, immunodepletion of Dpb4p resulted in quantitative precipitation of both Isw2p and Pol2p, showing that Dpb4p is indeed shared by Isw2 and polε complexes. Since no polε subunits other than Dpb4p were detected in any large-scale purification of Isw2 described above, we concluded that polε and Isw2 complexes are not stably associated with each other in vivo. We further confirmed this conclusion by an immunoprecipitation assay. As shown in Fig. 2B, no detectable amount of Isw2p coprecipitated when the polε subunit Dpb2p was immunoprecipitated from whole-cell extract. These results also suggest that, despite the homology, Dpb3p does not substitute

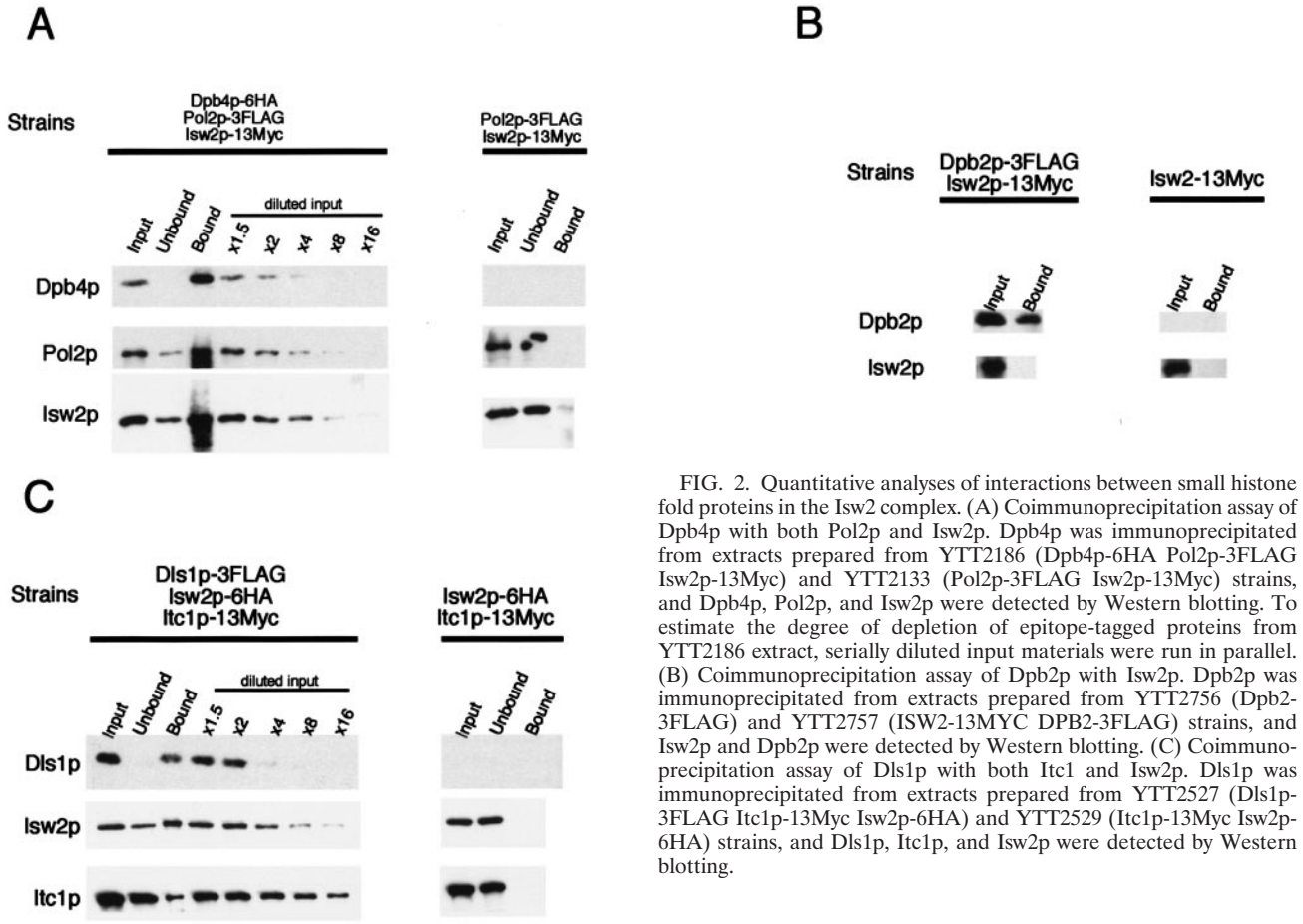

FIG. 2. Quantitative analyses of interactions between small histone fold proteins in the Isw2 complex. (A) Coimmunoprecipitation assay of Dpb4p with both Pol2p and Isw2p. Dpb4p was immunoprecipitated from extracts prepared from YTT2186 (Dpb4p-6HA Pol2p-3FLAG Isw2p-13Myc) and YTT2133 (Pol2p-3FLAG Isw2p-13Myc) strains, and Dpb4p, Pol2p, and Isw2p were detected by Western blotting. To estimate the degree of depletion of epitope-tagged proteins from YTT2186 extract, serially diluted input materials were run in parallel. (B) Coimmunoprecipitation assay of Dpb2p with Isw2p. Dpb2p was immunoprecipitated from extracts prepared from YTT2756 (Dpb2-3FLAG) and YTT2757 (ISW2-13MYC DPB2-3FLAG) strains, and Isw2p and Dpb2p were detected by Western blotting. (C) Coimmunoprecipitation assay of Dls1p with both Itc1 and Isw2p. Dls1p was immunoprecipitated from extracts prepared from YTT2527 (Dls1p-3FLAG Itc1p-13Myc Isw2p-6HA) and YTT2529 (Itc1p-13Myc Isw2p-6HA) strains, and Dls1p, Itc1p, and Isw2p were detected by Western blotting.

for Dls1p in the Isw2 complex. Our results are consistent with those recently reported by Iida and Araki (20).

Figure 2A also suggests the presence of Isw2p and Pol2p populations that are not associated with Dpb4p, as a significant portion of Pol2p and Isw2p remained soluble after Dpb4p was immunodepleted. To address if this is the case for Isw2p, we next tested the extent to which Isw2p and Itc1p stably associate with Dls1p by a coimmunoprecipitation experiment using a strain in which Itc1p, Isw2p, and Dls1p were epitope tagged. As shown in Fig. 2C, immunodepletion of Dls1p resulted in a partial depletion of both Isw2p and Itc1p, suggesting the presence of Isw2p and Itc1p populations that do not stably interact with Dls1p. This is consistent with the data in Fig. 1A, in which the two-subunit (fractions 15 and 16) and four-subunit (fractions 17 to 20) Isw2 complexes were separated by anion-exchange chromatography. These results collectively suggest that Isw2p is likely present in two forms in vivo, the four-subunit (Itc1p-Isw2p-Dpb4p-Dls1p) and two-subunit (Itc1p-Isw2p) complexes. The four- and two-subunit Isw2 complexes resemble CHRAC (9, 43) and ACF (21, 22) complexes, respectively, in higher eukaryotes that share ISWI and ACF1 subunits. We, however, cannot rule out the possibility that the four-subunit complex was destabilized by epitope tagging, immunoprecipitation, or chromatographic procedures to form the two-subunit complex.

Dls1p is required for Isw2-dependent transcriptional repression of some target genes. To gain some insight into the in vivo function of Dls1p, we sought to test its role in Isw2-dependent transcriptional repression. A *dls1* null mutant can be used to specifically test the role of this small subunit in the Isw2 complex in vivo, since no other complex containing Dls1p was detected. We first compared transcriptional profiles in *isw2* and *dls1* mutants by DNA microarray analysis in order to obtain a comprehensive view of the requirement for Dls1p in Isw2 functions in vivo. We have previously reported that the Isw2 complex represses transcription of a wide variety of genes in parallel with the Rpd3-Sin3 complex (13, 17). As a result, the effects of an *isw2 rpd3* mutation on transcription are more prominent than those of an *isw2* single mutant (13, 17). We therefore used an *rpd3* mutant background to sensitize the system. RNA was prepared from independent *isw2 rpd3* and *dls1 rpd3* strains, and labeled cDNA was directly competed on PCR-based spotted DNA microarrays (see Materials and Methods for details). Data from three independent labeling and hybridization assays were normalized by the GeneSpring software and graphed on a scatter plot to directly compare the profiles of the two mutant strains. As shown in Fig. 3A, signals were generally distributed along a diagonal line, and the vast majority of spots stayed within a twofold difference between the mutants. This result demonstrates that *isw2* and *dls1* mu-

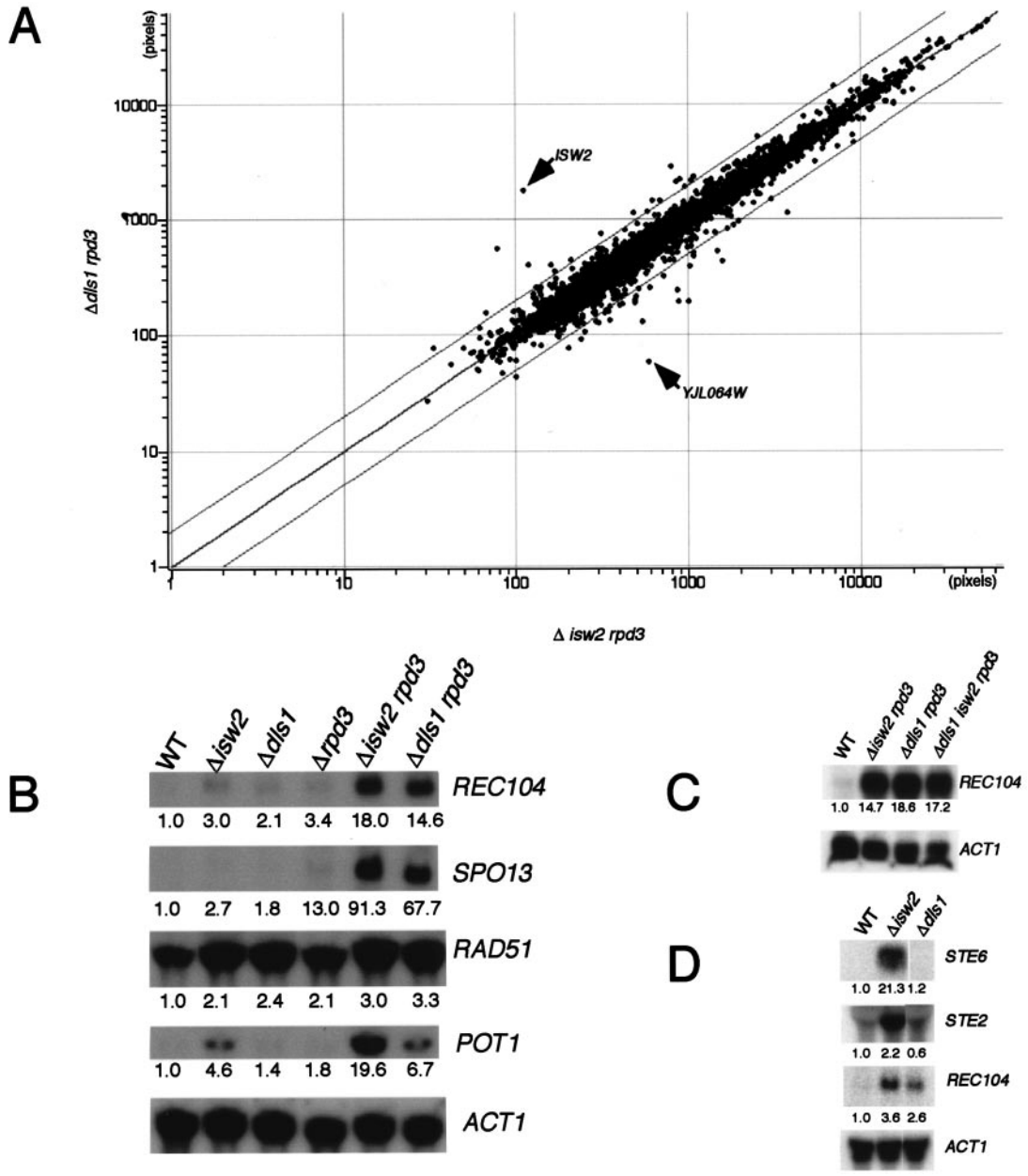

FIG. 3. Requirement for Dls1p in Isw2-dependent effects on transcription. (A) Direct comparison of transcriptional profiles in *isw2 rpd3* and *dls1 rpd3* cells by DNA microarray analysis. Signals (pixels) normalized from six independent hybridizations were plotted. *x* axis, *isw2 rpd3* mutant; *y* axis, *dls1 rpd3* mutant. Note that the signals are represented by the number of pixels detected, rather than the ratio of expression to that of wild-type cells. The numbers of pixels detected are proportional to the strength of signals, hence the abundance of RNA molecules. The line in the center represents identical signals from each mutant. The two outside lines represent a twofold difference between the mutants. The two dots that are most distant from the diagonal line represent *ISW2* and *YJL064W* genes. *YJL064W* is a dubious open reading frame that overlaps with the *DLS1* gene (*YJL065C*). The *DLS1* gene itself was eliminated from this analysis because of high local backgrounds in the batch of DNA microarrays used. (B) Northern blot analysis of Isw2 target genes. The genotypes of the cells used are indicated on the top. Listed vertically on the right of each blot is the gene being probed. The number under each band indicates the fold change in expression level relative to wild-type cells as determined with a phosphorimager. Signals were normalized for a loading control, *ACT1*. (C) Epistasis analysis of *ISW2* and *DLS1* genes by Northern blotting. (D) Northern blot analysis of Mat**a**-specific genes *STE2* and *STE6* in Matα *isw2* and *dls1* mutants. As an internal positive control, the *REC104* gene was also probed. WT, wild type.

tations affect transcription in a similar manner, indicating that Dls1p is required for the Isw2 complex to be fully functional at a wide variety of target genes in vivo. Signals for many known Isw2 targets, including early meiotic genes *REC104* and *SPO13*, for example, landed on the diagonal line. The signals for many non-Isw2 targets, such as *ACT1*, also stayed on the diagonal line as expected. However, Dls1p is not absolutely required for Isw2 to function at all loci, and the degree to which the Isw2 complex depends on Dls1p varies among target genes. For example, we observed that Isw2 target *POT1* is

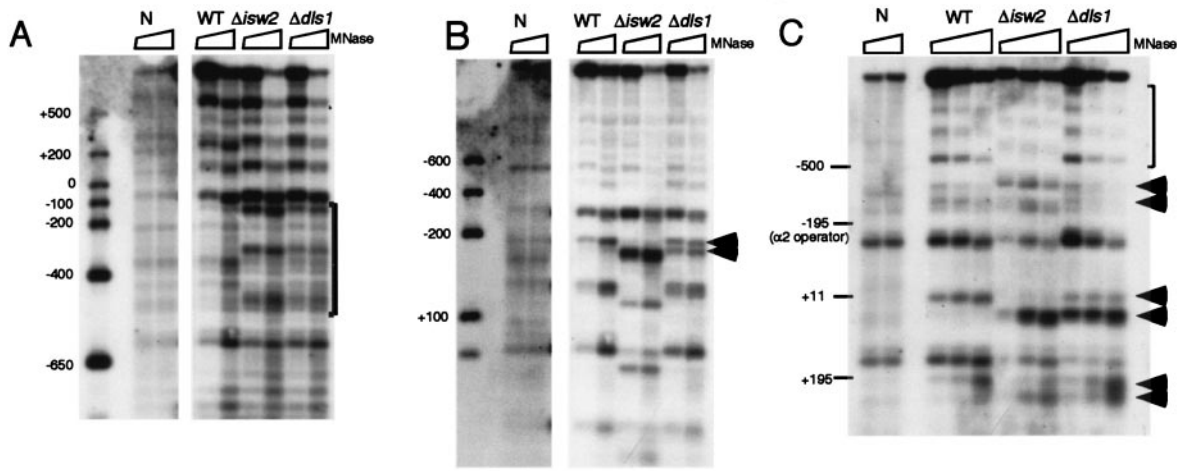

FIG. 4. Requirement for Dls1p in Isw2-dependent chromatin regulation. (A) Nucleosome mapping at the *REC104* locus. The genotypes of cells used are indicated on the top. "N" stands for naked DNA control. The bracket on the right shows the region where differences in MNase sensitivities were observed between *isw2* and *dls1* mutants. (B) Nucleosome mapping at the *POT1* locus. The arrowheads indicate the sites where a *dls1* mutant exhibits a mixture of mutant and wild-type patterns. (C) Nucleosome mapping at the *STE6* locus. The arrowheads indicate the sites where a *dls1* mutant exhibits a mixture of mutant and wild-type patterns. The bracket shows the region where wild-type and *dls1* cells have the same MNase digestion pattern, which is distinct from that of an *isw2* mutant. WT, wild type.

expressed at a lower level in a *dls1 rpd3* mutant than in an *isw2 rpd3* mutant. No particular common functions or regulatory pathways were found among genes whose transcription levels showed more than a twofold difference between the two mutants.

Transcription of some Isw2 targets was confirmed by Northern blot analysis using *isw2*, *dls1*, *isw2 rpd3*, and *dls1 rpd3* mutants. Figure 3B shows that *dls1* and *isw2* mutations caused comparable derepression of *REC104* and *SPO13* in both single and double mutants. A nonmeiotic *ISW2* target, *RAD51* (13), was also similarly affected by *dls1* and *isw2* mutations. Consistent with DNA microarray results, *POT1* was significantly less affected by a *dls1* mutation than by an *isw2* mutation, showing a partial requirement for Dls1p in Isw2 function at this locus. Our epistasis analysis shown in Fig. 3C revealed that the Isw2 target gene *REC104* was similarly derepressed in an *isw2 dls1 rpd3* triple mutant and in both *isw2 rpd3* and *dls1 rpd3* double mutants. This result is consistent with our conclusion from biochemical analyses that Dls1p exists exclusively in the Isw2 complex.

It was recently found that the Isw2 complex is also required for repression of Mat**a**-specific genes in Matα cells (40; Gelbart and Tsukiyama, unpublished) (Fig. 3D). However, a *dls1* mutation did not derepress the Mat**a**-specific genes *STE2* and *STE6* to detectable levels (Fig. 3D), thus showing that Mat**a**-specific genes are exceptions to the general requirement of Dls1p for Isw2 function in vivo.

Dls1p is partially required for Isw2-dependent chromatin remodeling in vivo. The differential effects of a *dls1* mutation on the expression of Isw2 target genes prompted us to test the effects of this mutation on the chromatin structure of these genes. The positions of nucleosomes were determined at these loci by MNase digestion of chromatin followed by indirect end labeling. At the *REC104* locus, a few nucleosomes were shifted in position in an *isw2* mutant compared to wild-type cells as previously described (17). A *dls1* mutant showed nucleosome positions similar to those of an *isw2* mutant (Fig. 4A). The most prominent difference between the two mutants was that MNase digestion was less intense in the promoter region of the *dls1* mutant (note the bracketed region in Fig. 4A). This result demonstrates that the Isw2 complex requires Dls1p for much of its function at this locus.

In contrast to the *REC104* locus, two major differences in chromatin structure were observed in comparing *dls1* and *isw2* mutants at the *POT1* locus (Fig. 4B). As reported previously (13), the positions of three nucleosomes are changed in an *isw2* mutant in comparison to wild-type cells. The position of only one MNase cleavage site was affected in a *dls1* mutant, however. In addition, MNase cleavage at the edge of the affected nucleosome showed a mixture of the wild-type and mutant patterns as marked by arrows. This suggests that mutation of *DLS1* results in a mixed population of cells, approximately half of which harbor the wild-type chromatin structure while the other half have one nucleosome shifted in position.

At the *STE6* locus, we have found that chromatin structure around the promoter region is different in wild-type and *isw2* cells (Gelbart and Tsukiyama, unpublished) (Fig. 4C). While *STE6* transcription was not affected in a *dls1* mutant (Fig. 3D), MNase digestion at the promoter region exhibited a mixture of the wild-type and *isw2* mutant patterns, as indicated by arrows. This result demonstrates a partial requirement for Dls1p in Isw2-dependent chromatin remodeling around the promoter region of this gene. The *isw2* mutant showed an altered chromatin structure in an upstream region of *STE6*, as indicated by a bracket. However, the *dls1* mutant exhibited wild-type chromatin structure in this region, showing that the requirements for Dls1p in Isw2 complex function can differ at two loci separated by a short distance. These results collectively show differing requirements for Dls1p in Isw2-dependent chromatin remodeling at various loci in vivo.

Dls1p is not required for the Isw2 complex to interact with chromatin. We next employed CHIP assays to test whether the

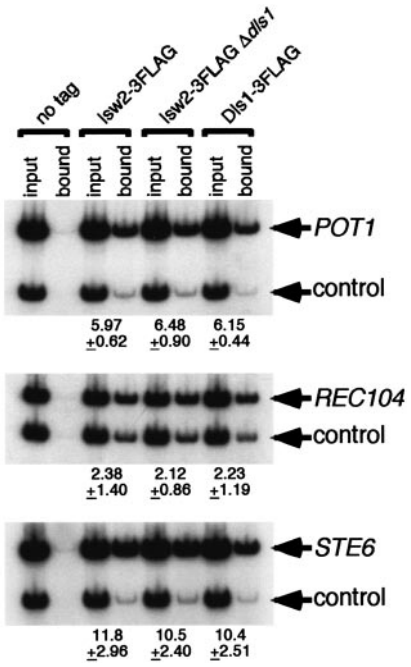

FIG. 5. CHIP analysis of Isw2p and Dls1p. The relevant genotypes of the strains are shown on the top. The numbers indicate the averages and standard deviations of the CHIP signals at each locus relative to those at a control (*YPL025C*) locus. See Materials and Methods for the selection of the *YPL025C* locus as a control locus. As a control 100-fold (*POT1* and *STE6*)- or 400-fold (*REC104*)-diluted input DNA from each strain was used.

Isw2 complex requires Dls1p for interaction with chromatin or, rather, at steps following chromatin interaction in vivo. To this end, we used strains carrying Isw2p with three copies of the FLAG epitope tag (Isw2-3FLAG) in wild-type and *dls1* mutant backgrounds, as well as a strain with a FLAG-tagged Dls1p (Dls1-3FLAG). As shown in Fig. 5, CHIP signals above background were detected at the *REC104*, *POT1*, and *STE6* genes with both Isw2-3FLAG and Dls1p-3FLAG strains, and a *dls1* mutation did not significantly affect the CHIP signals of Isw2-3FLAG at these loci. This result shows that Dls1p does not affect the interaction of the Isw2 complex with chromatin as measured by the CHIP assay. In addition, the strength of the CHIP signals of Isw2p and Dls1p did not correlate with the strength of the effects of a *dls1* mutation on chromatin structure or on transcriptional repression at Isw2 targets. Taken together, the results of the CHIP analyses suggest that Dls1p does not affect interaction of the Isw2 complex with chromatin and is likely required for functions of the Isw2 complex subsequent to chromatin binding. We, however, cannot rule out the possibility that Dls1p affects association of Isw2 with chromatin at loci that are yet to be tested.

DISCUSSION

In this paper, we described the identification of new subunits of the *S. cerevisiae* Isw2 complex that contain histone fold motifs. The two proteins, Dpb4p and Dls1p, are homologous to small subunits of *Drosophila* and mammalian CHRAC complexes. Therefore, Dpb4p and Dls1p are the only ISWI subunits, with the exception of the ISWI ATPases, that are highly conserved from yeast to mammalian cells. Another Isw2 subunit, Itc1p (16), shares the WAC motif with *Drosophila* and mammalian ACF1, a subunit of CHRAC and ACF complexes (22). However, no significant homology was detected outside of the WAC motif between Itc1p and ACF1. None of the three Isw1 subunits, Ioc2p, Ioc3p, and Ioc4p, share detectable homology with ISWI subunits in higher eukaryotes, with the exception of a PHD finger-like structure in Ioc2p (44). Conservation of small histone fold proteins in ISWI complexes during evolution is consistent with the possibility that these proteins play important roles in vivo. However, the functions of these proteins have not been reported either in vivo or in vitro.

As Dpb4p is shared by Isw2 and polε complexes, and Dls1p and Dpb3p are homologous, it is possible that these small histone fold proteins play similar roles in the two complexes. Dpb3p and Dpb4p form a heterodimer in the polε complex that interacts with a heterodimer of Dpb2p and Pol2p, the subunits essential for cell viability (12). The precise functions of Dpb3p and Dpb4p in polε in vivo have not been identified, but recent reports suggest their roles in DNA replication and repair: transcription of the *DPB3* gene is cell cycle regulated, peaking during S phase (2), and a *dpb4* mutation shows strong synthetic growth defects when combined with mutations in DNA replication and repair enzymes (32). Taken together with the fact that the *DPB3* and *DPB4* genes are not essential for cell viability, these results suggest regulatory roles for Dpb3p and Dpb4p in polε functions. Given the high degree of homology between the small histone fold proteins in ISWI complexes and polε, one possibility is that these proteins fulfill similar functions required for the two complexes, such as interactions with DNA or with specific sets of chromatin proteins.

Our transcriptional profiling and Northern blot analyses revealed that Dls1p is required for the Isw2-dependent transcriptional effect on most genes. However, the extent of this requirement varies among different Isw2 target genes. For example, repression of early meiotic genes by Isw2 is largely dependent on Dls1p, but the *POT1* gene only partially requires Dls1p for repression. A *dls1* mutation did not cause any detectable derepression of *STE2* and *STE6* in Matα cells. On the other hand, chromatin structure near the promoter of the *STE6* gene was affected in a *dls1* mutant, albeit to a lesser degree than in an *isw2* mutant. This implies that the reason that *STE6* transcription is not affected in a *dls1* mutant is not because Dls1p is dispensable for chromatin remodeling at this gene but rather because chromatin changes at *STE6* in a *dls1* mutant are not sufficient to cause detectable derepression. In contrast to the *STE6* locus, changes in chromatin structure at the *REC104* and *POT1* genes were consistent with changes in transcriptional levels of these genes. At *REC104*, chromatin structure was similar in *isw2* and *dls1* mutants, whereas the *POT1* gene was less affected in a *dls1* mutant than in an *isw2* mutant. Chromatin was more locally affected at the *POT1* gene in a *dls1* mutant than in an *isw2* mutant (one nucleosome versus three). The basis for the differential requirements for Dls1p in Isw2 function at various loci is currently unknown. Since only a part of Isw2p forms a complex with Dpb4p and Dls1p (Fig. 2), one possibility is that the four-subunit (Itc1p-Isw2p-Dpb4p-Dls1p) and two-subunit (Itc1p-Isw2p) Isw2 complexes are differentially targeted to these loci. For example, if

the two-subunit complex is preferentially recruited to function at *POT1* and *STE6* loci, the effects of a *dls1* mutation would be more subtle than those of an *isw2* mutation. However, the results of the CHIP assay in Fig. 5 are not consistent with this possibility.

CHIP analysis showed that a *dls1* mutation does not affect the CHIP signals of Isw2p at any of the three loci tested. CHIP signals of Dls1p did not correlate with the requirement of Dls1p in chromatin regulation at these loci either. These results suggest that Dls1p is not required for association of the Isw2 complex with its target genes as measured by the CHIP assay. Rather, it is likely that Dls1p affects the activity of the Isw2 complex at steps following its association with chromatin at these loci. Therefore, identification of the steps at which Dls1p is required for Isw2 function will help us to further dissect the process of Isw2-dependent chromatin remodeling.

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