

## Widespread Collaboration of Isw2 and Sin3-Rpd3 Chromatin Remodeling Complexes in Transcriptional Repression

THOMAS G. FAZZIO,<sup>1,2</sup> CHARLES KOOPERBERG,<sup>3</sup> JESSE P. GOLDMARK,<sup>1</sup> CASSANDRA NEAL,<sup>4</sup>  
RYAN BASOM,<sup>4</sup> JEFFREY DELROW,<sup>4</sup> AND TOSHIO TSUKIYAMA<sup>1\*</sup>

*Division of Basic Sciences,<sup>1</sup> Division of Public Health Sciences,<sup>3</sup> and DNA Array Facility,<sup>4</sup> Fred Hutchinson Cancer Research Center, Seattle, Washington 98109-1024, and Molecular and Cellular Biology Program, Fred Hutchinson Cancer Research Center and University of Washington, Seattle, Washington 98195<sup>2</sup>*

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**The yeast Isw2 chromatin remodeling complex functions in parallel with the Sin3-Rpd3 histone deacetylase complex to repress early meiotic genes upon recruitment by Ume6p. For many of these genes, the effect of an *isw2* mutation is partially masked by a functional Sin3-Rpd3 complex. To identify the full range of genes repressed or activated by these factors and uncover hidden targets of Isw2-dependent regulation, we performed full genome expression analyses using cDNA microarrays. We find that the Isw2 complex functions mainly in repression of transcription in a parallel pathway with the Sin3-Rpd3 complex. In addition to Ume6 target genes, we find that many Ume6-independent genes are derepressed in mutants lacking functional Isw2 and Sin3-Rpd3 complexes. Conversely, we find that *ume6* mutants, but not *isw2 sin3* or *isw2 rpd3* double mutants, have reduced fidelity of mitotic chromosome segregation, suggesting that one or more functions of Ume6p are independent of Sin3-Rpd3 and Isw2 complexes. Chromatin structure analyses of two nonmeiotic genes reveals increased DNase I sensitivity within their regulatory regions in an *isw2* mutant, as seen previously for one meiotic locus. These data suggest that the Isw2 complex functions at Ume6-dependent and -independent loci to create DNase I-inaccessible chromatin structure by regulating the positioning or placement of nucleosomes.**

The regulation of RNA synthesis is a complex process affected by many factors that influence the initiation, elongation, and termination of transcription. These factors include transcriptional activators and repressors, as well as general transcription factors. In eukaryotes, the compaction of DNA into chromatin provides an additional level of complexity, due to the fact that chromatin structure is inhibitory to many protein-DNA interactions required for transcription (43). Consequently, chromatin structure must be dynamically regulated for many genes whose transcription is repressed or induced at different times of the cell cycle, at different phases of development, or by changes in growth conditions.

A large number of proteins from several organisms have been found to modify chromatin structure. These factors can be grouped into two classes based on their biochemical activities: histone-modifying enzymes and ATP-dependent chromatin remodeling complexes (for reviews, see references 29, 30, 36, 42, 45, 54, and 55). Histone-modifying enzymes use several different enzymatic mechanisms to covalently modify histone proteins. These modifications include acetylation, phosphorylation, ubiquitination, and methylation. The second class of factors uses the energy of ATP hydrolysis to alter histone-DNA contacts, often leading to repositioning of histone octamers on DNA (56).

The role of histone acetylation in the regulation of transcription has been studied extensively (11, 42, 57). With some exceptions, high levels of histone acetylation correlate with transcriptionally active regions of chromatin, while lower levels of

acetylation are found in transcriptionally inactive regions. Histone acetylation is regulated by the activities of histone acetyltransferase and histone deacetylase (HDAC) complexes, many of which function as transcriptional activators or repressors, respectively (42, 44, 45).

Three major families of HDACs have been conserved in eukaryotes, represented by the Rpd3, Hda1, and Sir2 proteins (12, 32). The *RPD3* gene was first identified genetically in yeast as a transcriptional repressor of several genes (52, 53). It was later found that Rpd3 proteins in several species associate with Sin3p and other Sin3-associated proteins to form a multiprotein complex (1, 24, 59). Recruitment of this complex by transcriptional repressors leads to local deacetylation and repression of transcription (14, 15, 24, 26, 33, 37, 59).

A variety of ATP-dependent chromatin remodeling factors have also been identified from several organisms and can be grouped into three classes based on the ATPase subunit present in each complex: SWI/SNF, ISWI, and CHD1 (6, 22, 29, 38). Members of the ISWI class of chromatin remodeling factors were first identified biochemically in *Drosophila*, following their ATP-dependent activity in disrupting nucleosomes or imparting regular spacing on nucleosome arrays in vitro. These studies led to the identification of three ISWI-containing complexes, NURF (48, 50), CHRAC (51), and ACF (20). Subsequently, several other ISWI-containing complexes were identified in humans (3, 34, 35, 39), yeasts (49), and *Xenopus* (13, 28), based on sequence homology to *Drosophila* ISWI.

Although the biochemical activities of several ISWI complexes have been studied in detail, the in vivo functions of this class of factors are only beginning to be identified. The *Drosophila* *ISWI* gene is essential for development and cell viability (9). *Drosophila* *ISWI* mutants also have reduced expression of

\* Corresponding author. Mailing address: Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Mail Stop A1-162, 1100 Fairview Ave. North, Seattle, WA 98109-1024. Phone: (206) 667-4996. Fax: (206) 667-6497. E-mail: ttsukiya@fhcrc.org.

the *Ubx* and *engrailed* genes, suggesting the requirement of one or more ISWI-containing complexes for the expression of these genes. In addition, *ISWI* mutants are compromised for male X chromosome integrity (9).

It was recently found that one of the two yeast ISWI complexes, the Isw2 complex, functions during vegetative growth to repress genes induced early in meiosis (10). Many early meiotic genes are repressed under these conditions by the Sin3-Rpd3 HDAC complex upon recruitment by the sequence-specific DNA-binding protein, Ume6p (24, 25, 40). Like the Sin3-Rpd3 complex, the Isw2 complex appears to be recruited to the promoters of early meiotic genes by Ume6p; however, repression by the Isw2 complex occurs independently of the Sin3-Rpd3 complex. Analysis of chromatin structure of one Ume6 target gene in wild-type and various mutant cells reveals that the Isw2 complex functions to create DNase I-inaccessible chromatin structure by altering the positions of nucleosomes upstream of the Ume6p-binding site. These data reveal that the Isw2 complex functions in parallel with the Sin3-Rpd3 complex to repress the transcription of common target genes.

While it is clear that Isw2 and Sin3-Rpd3 complexes collaborate to repress Ume6 target genes, it is not clear whether this collaboration extends to any Ume6-independent genes. To investigate this possibility, we compared the full genome transcriptional profiles of mutants defective in one or more of these factors. We also competed single mutants of *sin3* and *rpd3* with *isw2 sin3* and *isw2 rpd3* double mutants directly on microarray slides to identify genetic interactions between Isw2 and Sin3-Rpd3 complexes that are not always detectable in traditional mutant versus wild-type competitions. We find that while Ume6 target genes represent a major group of genes repressed by these complexes, a large number of Ume6-independent genes are derepressed in mutants defective in Sin3-Rpd3 and Isw2 complex functions. A comparison of *isw2* and *rpd3* deletion and catalytically inactive mutants reveals differences in both phenotype and transcriptional profiles which may result from functions of these proteins independent of their known catalytic activities, inhibition of related factors by catalytically inactive proteins, or a combination of the two. We also find that *ume6* mutants have a reduced fidelity of chromosome segregation which results in higher rates of chromosome gains and losses relative to those in wild-type cells, suggesting a role for Ume6p in chromosome segregation that is independent of Isw2 and Sin3-Rpd3 complexes. Chromatin structure analyses of two Ume6-independent genes that require Sin3-Rpd3 and Isw2 complexes for proper regulation reveal that *ISW2* function is required in both instances for the formation of DNase I-inaccessible chromatin structure, as previously observed for the Ume6 target gene *REC104* (10). These data suggest that Sin3-Rpd3 and Isw2 complexes collaborate to repress the transcription of Ume6-dependent and some Ume6-independent genes and that the Isw2 complex functions to create DNase I-inaccessible chromatin structure at the promoters of many of these genes.

#### MATERIALS AND METHODS

**Strains.** Unless indicated, all yeast strains were derived from W1588-4C. This strain is congenic to W303-1A, except that a weak *rad5* mutation in the original strain W303 is repaired (60). Deletion and catalytically inactive mutations of the *ISW2*, *SIN3*, *RPD3*, and *UME6* genes were described previously (10). The CFIII

minichromosome (41) was introduced into isogenic wild-type and mutant strains by crossing an *isw2 ume6* double mutant (YTT1065) with SBY475 (courtesy of Sue Biggins, Fred Hutchinson Cancer Research Center). The colony sectoring assay for measuring rates of minichromosome loss was done as previously described (16, 58).

**RNA isolation.** RNA samples were prepared from mutant and wild-type cells grown at 30°C in YEPD medium (2% Bacto Peptone, 1% yeast extract, 2% glucose) to early log phase (optical density at 660 nm, 0.7) using acid phenol extraction. To ensure identical growth conditions, strains were grown in aliquots of media from a common preparation. mRNA was prepared using Oligotex beads (Qiagen) as described by the manufacturer.

**Production of spotted microarrays.** Microarray construction and hybridization protocols were modified from those described elsewhere (8). Yeast microarrays were constructed using a set of ~6,200 open reading frame (ORF)-specific PCR primer pairs (Research Genetics, Huntsville, Ala.), which were used to amplify each ORF of the yeast genome. Individual PCR products were verified as unique via gel electrophoresis and purified using ArrayIt 96-well PCR purification kits (TeleChem International, Sunnyvale, Calif.). Purified PCR products in 3× SSC (1× SSC is 0.15 M sodium chloride plus 0.015 M sodium citrate [pH 7.0]) were mechanically spotted onto polylysine-coated microscope slides using an Omni-Grid high-precision robotic gridded (GeneMachines, San Carlo, Calif.).

**Microarray hybridizations and data analysis.** The protocol used for cDNA labeling was a modification of a protocol described elsewhere (<http://cmgm.stanford.edu/pbrown/protocols/aadUTPCouplingProcedure.htm>). Briefly, labeled cDNA targets were prepared by reverse transcription of 2 µg of mRNA using oligo(dT)<sub>18</sub> primer in the presence of 0.2 mM 5-(3-aminoallyl)-2'-deoxyuridine 5'-triphosphate (Sigma-Aldrich Company, St. Louis, Mo.), 0.3 mM dTTP, and 0.5 mM each dATP, dCTP, and dGTP. Following cDNA synthesis, either Cy3 or Cy5 monoreactive fluor (Amersham Life Sciences, Arlington Heights, Ill.) was covalently coupled to the cDNA-incorporated aminoallyl linker in the presence of 50 mM sodium bicarbonate (pH 9.0). Two color expression profiles were generated using microarrays in which reference and experimental cDNA targets were labeled with different fluors. Following cohybridization to the chip, a fluorescence image of a microarray was collected at both emission wavelengths using a GenePix 4000 fluorescence scanner (Axon Instruments, Inc., Foster City, Calif.), and image analysis was performed using GenePix Pro microarray acquisition and analysis software.

Four microarray hybridizations were carried out for each comparison of mutant versus wild type or mutant versus mutant (two sets of two reverse fluor combinations). We carried out a Bayesian background correction, described in detail by Kooperberg et al. (31). Briefly, we assume that both the foreground and the background intensities of both channels for each spot come from a normal distribution with an unknown mean and variance. After estimating the variance parameters from the data, we can estimate for each channel the posterior distribution of the difference between the foreground and the background means as well as their ratio, which is the quantity of interest. To do this, we assume an uninformative uniform prior on the intensities. The effect of this procedure is that for genes where the intensities are high, the estimated ratio is very similar to the traditional estimate, but for spots where the foreground intensity is close to the background intensity, the estimate for the ratio is shrunk slightly toward one, yielding a substantial reduction in variance for these spots.

All calculations involving expression ratios were carried out on the (natural) log scale by computing averages and standard errors (SEs). Averages and SEs were then converted to ratios by assuming that the average of the log ratios follows the normal distribution (which seems reasonable given both the error distribution of log ratios and the central-limit theorem). The average ratio then has a log-normal distribution. This average and its SE are related, as described previously (21), to the average and the SE of the log ratios by the following equations:

$$\text{average}_{\text{ratio}} = \exp[\text{average}_{\log \text{ratio}} + (\text{SE}_{\log \text{ratio}}^2/2)]$$

$$\text{SE}_{\text{ratio}} = (\text{average}_{\text{ratio}})[\exp(\text{SE}_{\log \text{ratio}}^2) - 1]^{1/2}$$

For analysis of the genomic locations of misregulated genes and *cis* element searches of promoter sequences, we used the GENESPRING software package (Silicon Genetics).

**Chromatin structure analysis.** Digestion of chromatin was performed with crude preparations of nuclei (17) as described previously (10). After digestion with micrococcal nuclease or DNase I, DNA was purified, digested with *EcoRI* and *PstI* (for *POT1*) or *EcoRI* and *XbaI* (for *SUC2*), and subjected to indirect end labeling. The probe used for indirect end labeling of the *POT1* locus was a PCR product extending from +406 to +623 with respect to the initiation codon.

TABLE 1. Genes with increased expression in various deletion mutants<sup>a</sup>

Deletion mutant	No. of genes with the following fold increases in expression <sup>b</sup>		
	>3	>5	>10
<i>isw2</i>	3 (0)	0	0
<i>rpm3</i>	35 (8)	9 (3)	1 (1)
<i>isw2 rpm3</i>	114 (21)	24 (13)	6 (2)
<i>sin3</i>	42 (10)	8 (3)	2 (1)
<i>isw2 sin3</i>	93 (22)	31 (12)	9 (5)
<i>ume6</i>	113 (40)	45 (20)	17 (10)
<i>isw2 ume6</i>	320 (52)	80 (24)	22 (13)

<sup>a</sup> A total of 720 genes were more than 3-fold derepressed in one or more of the seven mutants listed above; of these, the expression ratios for 630 were statistically significantly higher than 2 at the 0.05 level. The ratios of 178 of the 197 genes that were more than 5-fold derepressed were statistically significantly higher than 3 at the 0.05 level; and the expression ratios of 53 of the 57 genes that were more than 10-fold derepressed were statistically significantly higher than 5 at the 0.05 level. <sup>b</sup> Values in parentheses are the numbers of genes that contain the URS1 core sequence (GGCGGC) within 500 bp upstream of their initiation codons.

The probe used for indirect end labeling of the *SUC2* locus was a PCR product extending from -885 to -661 with respect to the initiation codon.

## RESULTS

### Overlapping functions of Isw2 and Sin3-Rpd3 complexes.

Recently, it was found that the Isw2 complex functions during mitotic growth in Ume6p-dependent repression of early meiotic genes in a pathway parallel to that of the Sin3-Rpd3 HDAC complex (10). While it is clear that Sin3-Rpd3 and Isw2 complexes function in the repression of Ume6 target genes, it is not known whether this collaboration extends to genes not regulated by Ume6p. To determine the full extent to which Sin3-Rpd3 and Isw2 complexes function in parallel pathways of transcriptional repression, we used cDNA spotted microarrays representing >96% of all yeast genes to analyze the genome-wide expression profiles for mutants defective in one or both of these complexes. For each mutant, four independent mutant versus wild-type microarray hybridizations were carried out, and the average expression ratio and standard error for each spot were determined (see Materials and Methods for details; the full data set can be obtained at <ftp://milano.fhrc.org/ArrayLab/Fazzio/>). Initially, we focused on *isw2*, *rpm3*, and *sin3* single null mutants, as well as *isw2 rpm3* and *isw2 sin3* double null mutants. In addition, we analyzed expression profiles for *ume6* and *isw2 ume6* null mutants; these data are discussed below. We observed relatively few changes in transcript levels for the *isw2* single mutant, as previously described for an *isw2/isw2* homozygous mutant diploid strain (18). Because it was previously found that Isw2 and Sin3-Rpd3 complexes can each partially compensate for the lack of the other in repression of common target genes, we focused on genes that require defects in both complexes for moderate levels of derepression. As shown in Table 1, larger numbers of genes are moderately derepressed (>3-fold) in *isw2 rpm3* and *isw2 sin3* double mutants than in single mutants. Similarly, more genes are derepressed to higher levels (>5-fold, >10-fold) in double mutants than in single mutants, revealing that Sin3-Rpd3 and Isw2 complexes function in parallel pathways to repress the transcription of a considerable group of genes.

As expected, many genes found to be repressed by Sin3-Rpd3 and Isw2 complexes include known targets of Ume6p and genes predicted to be Ume6 targets by virtue of an upstream Ume6p-binding site (URS1) (Table 1). We also searched for a second Ume6-binding sequence previously identified in the promoter of the *PHR1* gene (46); however, this sequence was not overrepresented in the promoters of genes derepressed in any of our mutants. In addition to genes repressed by Ume6p, we observed many genes that require Sin3-Rpd3 and Isw2 complexes for repression that lack URS1 sequences. The majority (82%) of these genes are not substantially derepressed in the *ume6* mutant, suggesting that derepression in *isw2 sin3* and *isw2 rpm3* mutants is not a secondary consequence of the upregulation of early meiotic genes (see the full data set for details). Together, these data reveal that a substantial group of genes requires Sin3-Rpd3 and Isw2 complexes for repression, independent of Ume6p.

In addition to the role of the Isw2 complex in repression, we also found that the transcription of a small number of genes is reduced in the *isw2* mutant (see the full data set for details). For these genes, the relationship between the Isw2 complex and Sin3-Rpd3 complex in the activation or maintenance of basal transcription is less clear. Few genes have significantly reduced expression in the *isw2* deletion mutant (zero genes reduced >3-fold and one gene reduced >2-fold). In addition, many genes with reduced expression in the *sin3* or *rpm3* single mutant are unaffected or oppositely affected by the addition of an *isw2* mutation in these backgrounds. These data suggest a relatively minor role of the Isw2 complex in the transcriptional activation or maintenance of basal transcription under the conditions used (logarithmic growth in rich glucose medium).

**Hidden functions of the Isw2 complex revealed in *sin3* and *rpm3* mutant backgrounds.** The data in Table 1 suggest that many targets of Isw2-mediated repression may be masked in *isw2* mutant cells by the presence of a functional Sin3-Rpd3 complex. To identify *ISW2* target genes affected in this way, we performed two experiments. First, we divided *isw2 rpm3*/wild-type expression ratios by *rpm3*/wild-type ratios for all genes (Table 2); similarly, *isw2 sin3*/wild-type expression ratios were divided by *sin3*/wild-type ratios (Table 2). In theory, the ratios obtained through these calculations should reveal defects in transcriptional regulation resulting from the loss of Isw2 function in an *rpm3* or *sin3* mutant background. However, when we compared these data to those obtained by Northern blotting (Table 2), we noticed several inconsistencies. For several meiotic genes previously found to be targets of Isw2-dependent repression, the ratios derived from one or both calculations described above do not reflect known derepression due to the *isw2* mutation (cf. *HOP1*, *SPO11*, *REC104*, and *SPO1*). Under the growth conditions used for this experiment (logarithmic growth of haploid cells in rich glucose medium), many genes are tightly repressed in wild-type cells. In microarray experiments, expression ratios for genes with a low signal in the wild-type channel are often underestimated relative to ratios measured by Northern blotting (31). As a result, *isw2 rpm3*/wild-type expression ratios for these genes measured using microarrays can be similar to *rpm3*/wild-type ratios; likewise, *isw2 sin3*/wild-type expression ratios can be similar to *sin3*/wild-type ratios. Consequently, when double mutants are divided by

TABLE 2. Hidden functions of the Isw2 complex revealed by direct competition of double mutants with single mutants

Gene	Ratio <sup>a</sup> for:					
	<i>isw2 rpd3/rpd3</i> <sup>b</sup>	<i>isw2 rpd3</i> vs wt/ <i>rpd3</i> vs wt <sup>c</sup>	<i>isw2 rpd3/rpd3</i> <sup>d</sup>	<i>isw2 sin3/sin3</i> <sup>b</sup>	<i>isw2 sin3</i> vs wt/ <i>sin3</i> vs wt <sup>c</sup>	<i>isw2 sin3/sin3</i> <sup>d</sup>
<i>SPO11</i>	<b>2.0</b>	<b>1.1 (0.3)</b>	<b>1.6 (0.2)</b>	1.6	1.4 (0.3)	1.6 (0.3)
<i>SPO1</i>	<b>3.3</b>	<b>1.3 (0.2)</b>	<b>1.7 (0.2)</b>	2.5	1.6 (0.1)	1.8 (0.2)
<i>HOP1</i>	<b>4.1</b>	<b>1.5 (0.1)</b>	<b>1.9 (0.1)</b>	2.4	1.8 (0.1)	2.2 (0.3)
<i>REC104</i>	5.5	2.5 (0.2)	2.9 (0.4)	<b>4.0</b>	<b>1.4 (0.4)</b>	<b>3.3 (0.3)</b>
<i>IME2</i>	2.8	2.2 (0.5)	1.8 (0.1)	1.6	1.8 (0.2)	1.9 (0.2)
<i>SGA1</i>	1.7	1.5 (0.2)	1.8 (0.1)	1.4	1.9 (0.2)	1.8 (0.2)
<i>SIP4</i>	2.7	1.9 (0.3)	2.9 (0.4)	2.0	1.6 (0.3)	1.9 (0.4)
<i>POT1</i>	3.7	3.1 (0.5)	6.1 (3.2) <sup>e</sup>	3.2	3.3 (0.7)	4.7 (0.8)
<i>SPO13</i>	4.7	4.2 (1.1)	3.4 (0.3)	6.8	2.9 (0.4)	3.0 (0.4)
<i>INO1</i>	7.3	6.4 (0.8)	8.3 (0.4)	2.9	3.6 (0.7)	5.0 (0.6)

<sup>a</sup> Means (SEs) of ratios were computed as means (SEs) of log ratios and backtransformed to the ratio scale assuming that the log ratios have a normal distribution, so that the ratios have a log-normal distribution (21). Bold type indicates genes for which derepression due to the *isw2* mutation is more evident in direct hybridization experiments than in array-array comparisons. wt, wild type.

<sup>b</sup> Ratios of Northern quantitations of genes. mRNA levels measured in *isw2 rpd3* and *isw2 sin3* double mutants were divided by mRNA levels measured in *sin3* and *rpd3* single mutants, respectively.

<sup>c</sup> Ratios were obtained by dividing the results from one mutant-wild type microarray experiment by those from another (array-array comparisons). *isw2 sin3*/wild-type ratios were divided by *sin3*/wild-type ratios for each gene. Similarly, *isw2 rpd3*/wild-type ratios were divided by *rpd3*/wild-type ratios for each gene.

<sup>d</sup> *isw2 sin3/sin3* expression ratios were measured directly on microarray slides (direct hybridization). Similarly, *isw2 rpd3/rpd3* expression ratios were measured directly on microarray slides.

<sup>e</sup> For one of the replicates for POT1, the background level was very high, and as a result the posterior variance of the estimate of the expression log ratio was very high. If this one realization is excluded, the estimate of the expression ratio changes to 3.4, with an SE of 0.8.

single mutants to compare their expression profiles, some differences observed by Northern blotting are not revealed.

To circumvent this problem, we measured *isw2 rpd3/rpd3* ratios and *isw2 sin3/sin3* ratios directly by labeling single- and double-mutant RNA samples with opposing dyes and hybridizing them to the same microarray slide. In contrast to the typical method of measuring mutant/wild-type ratios, this approach uses *rpd3* or *sin3* single mutants as "references" against which double-mutant expression levels are compared. As a result, the problem of underestimated expression ratios resulting from minimal wild-type expression is significantly reduced for genes derepressed in *rpd3* or *sin3* mutants. For both the *isw2 rpd3-rpd3* and the *isw2 sin3-sin3* comparisons, the expression ratio obtained from the direct (mutant-versus-mutant) hybridization is higher for most genes examined than that obtained by dividing two different mutant/wild-type expression ratios (Table 2). In addition, when expression ratios obtained by direct hybridization are compared to those calculated from mutant-versus-wild-type experiments, we find that direct hybridization more accurately detects derepression observed by Northern blotting. While the values of expression ratios measured for mutant-versus-mutant experiments were not always identical to those calculated from Northern data, this method qualitatively identified derepression due to the *isw2* mutation for all 10 genes analyzed in both *rpd3* and *sin3* mutant backgrounds.

In an attempt to categorize the targets of Isw2-dependent repression, we analyzed more closely the set of genes identified above that are derepressed in *isw2 rpd3* or *isw2 sin3* double mutants relative to *sin3* or *rpd3* single mutants. This class of genes requires the Isw2 complex for repression in the absence of a functional Sin3-Rpd3 complex. We focused on the set of genes derepressed at least 1.7-fold in one or both of the direct hybridization experiments, as this cutoff level allowed for inclusion of ~90% of genes known to be repressed by the Isw2 complex (as measured by Northern blotting) (Table 2) but did not include any genes found by Northern blotting to be unaf-

fected in *isw2* mutant cells (data not shown). By these criteria, 315 genes (~5% of all yeast genes) belonging to many different functional categories were found to be derepressed when an *isw2* mutation was present in *rpd3* and/or *sin3* mutant backgrounds; representatives of this group of genes are listed in Table 3. For comparison, only 112 genes met this 1.7-fold threshold in the *isw2* single mutant. As expected, a significant portion (~20%) of the 315 genes in this group contained the core Ume6p-binding site, 5'-GGCGGC-3', in the 500 bp upstream of their initiation codons; the majority (~72%) of these genes were also found to be derepressed in *ume6* and *isw2 ume6* mutant backgrounds (see the full data set). No other sequences were significantly overrepresented in the promoters of these genes. We also observed 80 genes whose expression is decreased more than 1.7-fold in one or both double mutants relative to *rpd3* or *sin3* single mutants, consistent with a lesser role for the Isw2 complex in the activation of transcription. The majority of these genes are uncharacterized ORFs, and no functional category of genes is highly represented in this group.

**Catalytically inactive *isw2* and *rpd3* mutants differ from deletion mutants in phenotype and transcriptional profiles.** Isw2p has nucleosome-stimulated ATPase activity that is required for both chromatin remodeling in vitro (49) and repression of early meiotic genes in vivo (10). Similarly, HDAC activity of Rpd3p is required for normal levels of repression of target genes (7, 10, 23). However, several deacetylase-defective mutants of *rpd3* were previously found to be only partially defective in the repression of a LexA reporter construct (23), suggesting one or more functions of Rpd3p that are independent of its deacetylase activity. In addition, overexpression of catalytically inactive Rpd3 protein in wild-type cells results in a partial dominant-negative phenotype (23). It is therefore possible that catalytically inactive mutants of *isw2* and *rpd3* exhibit defects in transcriptional regulation not seen in deletion mutants. In deletion mutants, related chromatin remodeling factors may partially compensate for the deleted proteins, whereas catalytically inactive proteins may prevent these fac-

TABLE 3. Subset of genes that require *ISW2* function for repression in *sin3* or *rpd3* mutant background<sup>a</sup>

Category	Gene	Category	Gene
ATP synthesis .....	<i>STF1</i> <i>ATP10</i> <i>ATP20</i>	Meiosis or sporulation .....	<i>SPO13</i> <i>MEK1</i> <i>SPO1</i> <i>HOP2</i> <i>NDJ1</i> <i>MSH4</i> <i>REC104</i> <i>HOP1</i> <i>MEI5</i> <i>SHC1</i> <i>SPS100</i> <i>SGA1</i> <i>SPS19</i> <i>NOS1</i>
Secretion .....	<i>BFR2</i> <i>SED5</i> <i>NCE103</i>	Tricarboxylic acid cycle .....	<i>IDP1</i> <i>IDP2</i> <i>CIT3</i>
Transcriptional regulation .....	<i>SMP1</i> <i>CAT8</i> <i>SIP4</i> <i>MTH1</i> <i>KAR4</i> <i>SPO1</i> <i>RTG1</i> <i>PHD1</i> <i>YAP6</i> <i>CIN5</i> <i>CYC8</i> <i>SUB1</i> <i>NRD1</i> <i>FUR4</i>	Transport .....	<i>FUR4</i> <i>AGP2</i> <i>BPH1</i> <i>SIT1</i> <i>FTR1</i> <i>JEN1</i> <i>TPO1</i> <i>SUL2</i> <i>THI7</i> <i>FET3</i> <i>ALP1</i> <i>YOR071C</i> <i>CTP1</i> <i>AQY2</i> <i>AQY1</i>
Cu <sup>2+</sup> ion homeostasis .....	<i>SLF1</i> <i>CUP1-1</i> <i>CUP1-2</i>	Unknown .....	<i>BOP2</i> <i>BTN2</i> <i>COS1</i> <i>COS4</i> <i>COS7</i> <i>COS8</i> <i>CTL1</i> <i>EBP2</i> <i>FKS3</i> <i>FRE7</i> <i>GIT1</i> <i>KIN2</i> <i>PGU1</i> <i>PIR3</i> <i>SIP18</i> <i>SMT4</i> <i>SOL4</i> <i>SPI1</i> <i>YCL035C</i> <i>YDR374C</i> <i>YER037W</i> <i>YET1</i> <i>YFR026C</i> <i>YGL117W</i> <i>YGR079W</i> <i>YIL037C</i> <i>YIR043C</i> <i>YJL045W</i> <i>YKL071W</i> <i>YLR179C</i> <i>YMR269W</i>
Cell cycle .....	<i>PCL5</i> <i>PCL1</i> <i>SYF2</i> <i>CDC26</i>		
DNA repair .....	<i>MSH5</i> <i>MAG1</i> <i>RNR3</i> <i>DIN7</i> <i>ALK1</i> <i>MGT1</i> <i>RAD51</i>		
Ethanol utilization .....	<i>ALD3</i> <i>ALD2</i> <i>ALD4</i>		
Glucose metabolism .....	<i>HSP12</i> <i>GIP2</i> <i>PIG2</i> <i>GPH1</i> <i>GPM2</i>		
Amino acid synthesis .....	<i>GCV1</i> <i>LYS1</i> <i>MET3</i>		
Protein degradation or modification .....	<i>SMT3</i> <i>RPN4</i> <i>SRT1</i>		
Cytoskeleton .....	<i>SRO9</i> <i>TPM2</i> <i>BNR1</i> <i>HSP42</i>		
Phosphate metabolism .....	<i>PHO11</i> <i>PHO89</i> <i>PHO12</i>		

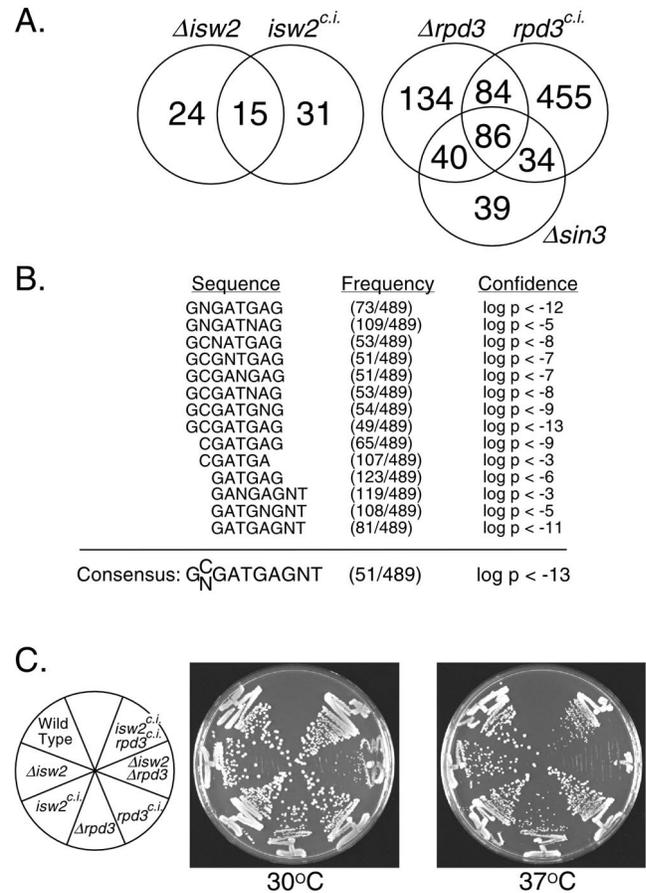
<sup>a</sup> For a full list of genes that require *ISW2* function for repression in *sin3* and *rpd3* mutant backgrounds, see [ftp://milano.fhcr.org/ArrayLab/Fazzio/](http://milano.fhcr.org/ArrayLab/Fazzio/).

tors from accessing chromatin. In addition, accessory proteins shared by multiple chromatin remodeling factors may be titrated by catalytically inactive proteins. Alternatively, deletion mutants may have defects in transcriptional regulation not observed in catalytically inactive mutants due to functions of these proteins that are independent of their known catalytic activities.

To determine the differences between catalytically inactive and deletion mutants of *isw2* and *rpd3*, we analyzed the transcriptional profiles of *isw2* and *rpd3* single catalytically inactive mutants as well as the *isw2 rpd3* double catalytically inactive mutant. For this purpose, we analyzed an *rpd3* mutation by which a conserved histidine residue at position 151 is changed to alanine; this mutation was previously found to eliminate the deacetylase activity of Rpd3p (23). Similarly, we analyzed a previously characterized *isw2* substitution mutation (which changes lysine 214 to arginine), known to eliminate the ATPase activity of Isw2p.

While many of the same genes were misregulated in deletion and catalytically inactive mutants of *isw2* and *rpd3*, in each instance there were considerable differences in transcriptional profiles between the two types of mutants (Fig. 1A). While relatively few genes are derepressed at least twofold in either *isw2* mutant, a substantial amount of nonoverlap is apparent for the two transcriptional profiles. A moderate number of genes are derepressed at least twofold in the *rpd3* deletion mutant but not in the catalytically inactive mutant. More strikingly, a large number of genes that are derepressed at least twofold in the *rpd3* catalytically inactive mutant do not meet this threshold in the deletion mutant (Fig. 1A, right panel). Most of these genes are not significantly derepressed in *sin3* mutant cells, suggesting that Rpd3p is required for the repression of this group of genes independent of Sin3p. In contrast, most genes derepressed in the *sin3* mutant are also derepressed in one or both *rpd3* mutants, with the largest single group of *sin3*-responsive genes being those derepressed in all three mutants. These data suggest that Sin3p acts primarily in conjunction with Rpd3p to repress the transcription of common target genes, whereas Rpd3p appears to be required for the repression of some genes independent of Sin3p.

To further investigate the differences in transcriptional profiles between deletion and catalytically inactive mutants of *rpd3*, we searched the 489 genes derepressed in the catalytically inactive mutant but not in the deletion mutant for common sequences within their presumed regulatory regions (within 500 bp upstream of their initiation codons). While we did not find any known regulatory elements, several elements with overlapping sequences were overrepresented in this group to a high level of significance (Fig. 1B). The consensus for these sequences, 5'-GNGATGAGNT-3', is present in the upstream 500 bp of 174 yeast genes. Of these, 56 (32%) are derepressed at least 2-fold and 131 (75%) are derepressed at least 1.5-fold in the *rpd3* catalytically inactive mutant. This sequence was previously identified by two independent computational approaches and found to be overrepresented in the regulatory regions of a group of genes with a common expression pattern during the cell cycle (5, 47). These data cannot distinguish whether this sequence functions directly in Rpd3-mediated repression or whether the *rpd3* catalytically inactive mutation indirectly leads to derepression of these genes. However, the



**FIG. 1.** Catalytically inactive and deletion mutations of *isw2* and *rpd3* have different effects on transcription and growth. (A) The numbers of genes derepressed at least twofold in catalytically inactive (c.i.) and deletion ( $\Delta$ ) mutants of *isw2* and *rpd3* are compared in Venn diagrams. The number of genes derepressed at least twofold in the  $\Delta$ *sin3* mutant is included for comparison. It should be noted that the Venn diagrams tended to overestimate the differences between two mutants, since genes derepressed in both mutants can be derepressed slightly more than twofold in one mutant and slightly less than twofold in the other mutant. Nevertheless, real differences in transcriptional profiles between deletion and catalytically inactive mutants are evident for both *isw2* and *rpd3*. (B) Summary of a regulatory element search of the 489 genes derepressed at least twofold in the *rpd3* catalytically inactive mutant but not in the *rpd3* deletion mutant. The search parameters allowed for oligonucleotides of eight bases or fewer, permitting degeneracies. Highly significant elements belonging to the same consensus were aligned, and their frequencies and confidence estimates (calculated with the GENESPRING software package) are indicated. (C) Wild-type, deletion, and catalytically inactive mutant yeast cells were streaked on rich (YEPD) plates and incubated at 30 or 37°C. The genotypes of the yeast cells are shown to the left.

fact that this sequence is overrepresented in the regulatory regions of genes derepressed in the same mutant as well as coexpressed during the cell cycle suggests that it may function in transcriptional regulation. Among the genes that contain this sequence in their upstream regulatory regions are those encoding components of the RNA processing and degradation machinery, as well as subunits of RNA polymerases I and III (47) (data not shown). This element may therefore coordinate the regulation of genes involved in several different aspects of RNA metabolism.

Consistent with the differences in transcriptional profiles, we also observed differences in synthetic growth phenotypes between deletion and catalytically inactive mutants of *isw2* and *RPD3*. It was previously found that the *isw2 rpd3* double deletion mutant exhibits a slow-growth phenotype at 30°C and fails to form colonies at 37°C (10). In contrast, the double catalytically inactive mutant grows at a rate similar to that of the wild type at 30°C and grows slightly more slowly than the wild type at 37°C (Fig. 1C). We also find that the *RPD3* single deletion mutant grows slightly more slowly than the wild type at 37°C, while growth of the single catalytically inactive mutant is unaffected. These results suggest that the *isw2 rpd3* double deletion mutant is impaired in one or more functions required for cell growth or division and that this defect is enhanced at higher temperatures. The phenotype of the double catalytically inactive mutant suggests that it is less severely impaired in these functions. The partial growth defect of the double catalytically inactive mutant at 37°C is very similar to that observed previously for an *isw2 sin3* double mutant (10). These data, combined with the transcriptional data discussed above, suggest one or more functions of Rpd3p that require neither deacetylase activity nor Sin3p. However, the fact that many more genes are derepressed in the *RPD3* catalytically inactive mutant than in the deletion mutant suggests that the catalytically inactive protein may inhibit other transcription or chromatin remodeling factors. The differences in growth phenotypes and transcriptional profiles between deletion and catalytically inactive mutants may therefore result from a combination of these two effects.

**Genomic instability of *ume6* mutants.** The Isw2 complex is recruited by Ume6p and functions in repression of genes that contain the Ume6p-binding site, URS1 (10). Since repression of early meiotic genes by Isw2 and Sin3-Rpd3 complexes is fully dependent on Ume6p, we wished to determine whether *UME6* function is required for repression of nonmeiotic genes by these complexes. To determine the extent to which Isw2 and Sin3-Rpd3 complexes depend on Ume6p, as well as the extent to which Ume6p functions through these complexes, we analyzed the expression profiles for *ume6* and *isw2 ume6* mutants.

Surprisingly, more genes were derepressed to high levels in *ume6* and *isw2 ume6* mutants than in *isw2 rpd3* or *isw2 sin3* mutants (Table 1), suggesting the presence of a number of Ume6 targets that are not regulated by Isw2 and Sin3-Rpd3 complexes. In addition, we found that many genes with no nearby URS1 sequence were derepressed in *ume6* and *isw2 ume6* mutants (Table 1). Upon closer inspection, a much higher-than-average density of derepressed genes was located on chromosome 16 (for the *ume6* mutant) or chromosomes 9 and 16 (for the *isw2 ume6* double mutant) (data not shown). Recently, Hughes et al. observed similar chromosomal expression biases in ~8% of a large number of expression studies (19). This group examined the nature of the expression biases for each of these mutants and found, for nearly every one examined, that the biases resulted from duplications or deletions of chromosomal segments or whole chromosomes.

To determine whether *ume6* and *isw2 ume6* mutants contained chromosomal duplications, we isolated DNA from mutant and wild-type cells, labeled each, and performed competitive hybridizations on microarray slides as described previously (19). We analyzed the DNA contents of two inde-

TABLE 4. Chromosomal duplications in *ume6* mutants

Strain <sup>a</sup>	Background	Genotype	Chromosomal duplication(s)	
			Partial <sup>b</sup>	Full <sup>c</sup>
YTT570	W303	<i>ume6</i>	1, 6 1, 3, 6 1, 3, 6, 9 1, 3, 6 1, 3, 5, 6, 9	9, 16 9, 16 16 16 16
RSY431	W303 <sup>d</sup>	<i>ume6</i>	1, 3, 6	2, 9
YTT622	S288C	<i>ume6</i>	1, 6	9
YTT572	W303	<i>isw2 ume6</i>	3, 6 1, 3, 6 1, 3, 6 1, 3, 6 1, 3, 6	1 9, 16 9, 16 9, 16 9, 16
YTT624	S288C	<i>isw2 ume6</i>	1, 6	9
YTT625	S288C	<i>isw2 ume6</i>	1, 6	3, 8, 9

<sup>a</sup> Five independent DNA isolates were analyzed for YTT570 (*ume6*) and YTT572 (*isw2 ume6*).

<sup>b</sup> Chromosomes with DNA contents that were moderately higher than normal were considered partial duplications. In every instance, the DNA contents of genes along the entire length of the partially duplicated chromosome were moderately increased, suggesting that the entire chromosome was duplicated in a portion of cells from which DNA was prepared, rather than that a portion of the chromosome was duplicated in all cells.

<sup>c</sup> Chromosomes with DNA contents that were substantially higher than normal were considered full duplications (duplicated in all or nearly all cells).

<sup>d</sup> RSY431 is a *ume6* mutant in the W303 yeast background (obtained from Randy Strich).

pendent *ume6* mutants and three independent *isw2 ume6* mutants constructed in two different strain backgrounds in our laboratory, as well as one *ume6* mutant obtained from another laboratory (Table 4). For all of the mutants, we found evidence that at least one and sometimes two or more chromosomes were duplicated. During the course of this work, independent expression data for a *ume6* mutant were published (2); analysis of these data reveals chromosomal expression biases that strongly suggest the presence of chromosomal duplications (data not shown), supporting our findings. Independent DNA isolates from the same *ume6* mutant revealed variability in which chromosomes were duplicated, suggesting that duplicated chromosomes were not always maintained during growth (Table 4). While the most common duplications were of chromosomes 9 and 16, we also observed, less frequently, duplications of chromosomes 1, 2, 3, and 8. Hughes et al. (19) found that for several mutants, the duplicated chromosomes or chromosomal segments contained genes homologous to the mutated gene, suggesting that selection for extra copies of homologous genes might partially compensate for the lack of the mutated gene. However, for *UME6*, we have found no close homolog within the yeast genome. In addition, the high degree of variability in chromosomes duplicated in *ume6* mutants argues against the possibility that these mutants are selected for extra copies of particular genes.

Alternatively, chromosomal duplications in *ume6* mutants may result from a general defect in chromosome segregation. In this scenario, chromosome loss would be predicted to occur

TABLE 5. Minichromosome loss in *ume6* mutants

Genotype	No. of colonies:			Rate of loss/generation <sup>a</sup>
	Red	Half-sector	Total	
Wild type	19	4	6,944	$5.8 \times 10^{-4}$
<i>isw2</i>	47	5	5,248	$9.6 \times 10^{-4}$
<i>ume6</i>	183	36	1,225	$3.6 \times 10^{-2}$
<i>isw2 ume6</i>	154	26	1,924	$1.5 \times 10^{-2}$

<sup>a</sup> Half-sector colonies/(total colonies – red colonies) (58).

in addition to chromosomal duplications. However, in our expression studies, we would only have observed chromosomal duplications, since chromosome loss is lethal to haploid cells. To test this possibility, we constructed strains containing a minichromosome harboring the *SUP11* gene, which suppresses the *ade2-101* mutation in our yeast strains, rendering colonies white. Because this chromosome is dispensable for cell viability, chromosome loss rates can easily be measured by monitoring differences in colony color after nonselective growth (16, 41). With this assay, we found that *ume6* and *isw2 ume6* mutant cells lose the minichromosome roughly 26- to 62-fold more often than wild-type cells (Table 5). These results suggest that chromosomal duplications observed in *ume6* mutants result from chromosome missegregation events.

**The Isw2 complex is required for the formation of DNase I-inaccessible chromatin structure at two Ume6-independent loci.** Chromatin structure analysis of one gene (*REC104*) targeted for repression by Isw2 and Sin3-Rpd3 complexes via Ume6p revealed that the Isw2 complex forms DNase I-inaccessible chromatin structure upstream of the Ume6p-binding site (URS1) (10). In *isw2* mutants, increased accessibility of chromatin near the URS1 sequence appeared to be due to a shift in the positions of two or three nucleosomes directly upstream of this site. The formation of an inaccessible chromatin structure at this site by the Isw2 complex requires a functional *UME6* gene but is unaffected by mutation of the *RPD3* gene.

Because the formation of DNase I-inaccessible chromatin structure by the Isw2 complex occurs directly adjacent to the Ume6p binding site, it is possible that Ume6p modifies or regulates the activity of the Isw2 complex upon recruitment, in a manner unique to Ume6 target genes. If this theory is correct, the Isw2 complex may modify chromatin structure differently at loci where it functions independently of Ume6p. Alternatively, the Isw2 complex may function similarly to create inaccessible chromatin structure at all genes for which it regulates chromatin structure, regardless of the mechanism of recruitment. To distinguish between these possibilities, we analyzed the chromatin structure of the *POT1* gene using micrococcal nuclease and DNase I digestions of chromatin, followed by indirect end labeling (Fig. 2A). The *POT1* gene was selected because it is repressed in parallel pathways by Isw2 and Sin3-Rpd3 complexes, yet data from both microarray and Northern blotting experiments confirmed little or no change in *POT1* expression in a *ume6* mutant (Fig. 2A) (see the full microarray data set). In addition, no Ume6p-binding site (URS1) is present in the *POT1* upstream regulatory region. As previously observed for the *REC104* gene, the positions of three nucleosomes at the *POT1* locus appear to change in the *isw2* mutant,

while no change is observed in the *rpm3* mutant. As with the *REC104* gene, these changes are accompanied by an increase in DNase I hypersensitivity near the promoter. In contrast to the situation for the *REC104* locus, chromatin structure changes at the *POT1* locus extend well into the coding region in *isw2* mutant cells. Nevertheless, DNase I-inaccessible chromatin structure established at the *POT1* locus in the presence of the Isw2 complex is very similar to chromatin structure at the *REC104* locus. These data suggest that the Isw2 complex may function similarly to remodel chromatin at these two loci, despite the fact that repression of *REC104* by the Isw2 complex requires *UME6* function, while repression of *POT1* transcription is *UME6* independent.

Although the Isw2 complex appears to have only a small role in transcriptional activation or maintenance of basal transcription, we found that transcription of one gene (*SUC2*) was significantly reduced in *isw2*, *sin3*, *rpm3*, *isw2 sin3*, and *isw2 rpm3* mutants (see the full data set). The level of expression of *SUC2* was lower in *isw2 sin3* and *isw2 rpm3* double mutants than in any single mutant, indicating that Isw2 and Sin3-Rpd3 complexes affect *SUC2* transcription independently. These data were confirmed by Northern blotting (Fig. 2B, Relative expression). Under the conditions of RNA isolation used for these studies (2% glucose media), the *SUC2* gene is actively repressed. Therefore, our results suggest that Isw2 and Sin3-Rpd3 complexes are required for low levels of basal *SUC2* transcription. The chromatin structure of the *SUC2* promoter has been studied extensively, revealing a role for the Swi-Snf complex in the creation of nuclease-accessible chromatin during transcriptional activation (17). Since increased transcription is often associated with a more accessible chromatin structure, it is possible that the Isw2 complex also functions to make chromatin more accessible at the *SUC2* locus, in contrast to its functions to create DNase I-inaccessible chromatin structure at the *REC104* and *POT1* loci.

To test this possibility, we probed the chromatin structure of the *SUC2* locus using DNase I digestions of chromatin, followed by indirect end labeling (Fig. 2B). The DNase I digestion patterns of wild-type chromatin and *isw2* mutant chromatin are very similar for the *SUC2* ORF and much of the upstream regulatory region. However, a notable increase in DNase I cleavage is observed approximately 500 bp upstream of the initiation codon in the *isw2* mutant. As previously found for the *REC104* and *POT1* loci, the DNase I digestion pattern of *rpm3* mutant chromatin was very similar to that of wild-type chromatin. Thus, at the *SUC2* locus, as at the *REC104* and *POT1* loci, the Isw2 complex, but not the Sin3-Rpd3 complex, is required for the creation of DNase I-inaccessible chromatin structure. However, unlike the situation for the *REC104* and *POT1* genes, the increase in DNase I accessibility is associated with a decrease in *SUC2* transcription in the *isw2* mutant. These data suggest that the Isw2 complex functions by a common mechanism at Ume6-dependent and -independent loci to create DNase I-inaccessible chromatin structure and that *ISW2*-dependent inaccessible chromatin structure can affect transcription both positively and negatively.

## DISCUSSION

Because it was previously found that the functional Sin3-Rpd3 complex could often compensate for the loss of Isw2

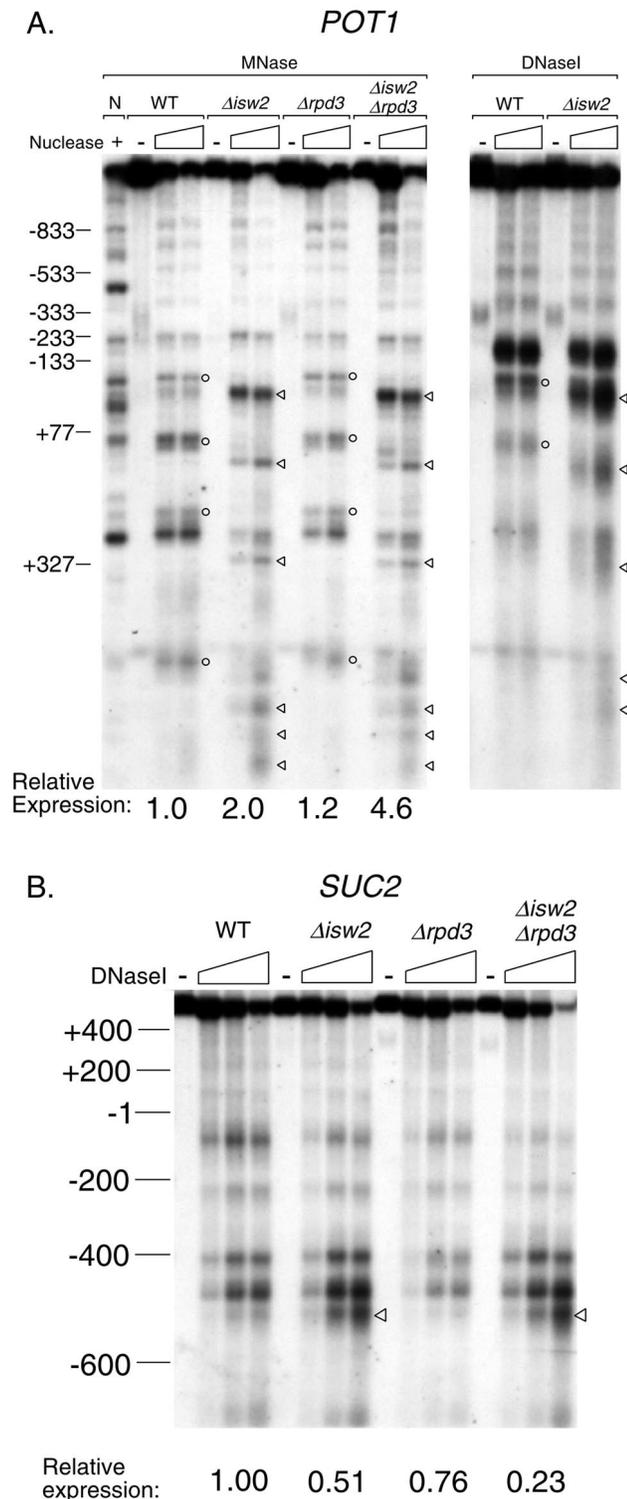


FIG. 2. The Isw2 complex is required for the formation of DNase I-inaccessible chromatin structure at two Ume6-independent loci. (A) Increased DNase I accessibility and improperly positioned nucleosomes at the *POT1* locus in *isw2* mutant cells. Chromatin structure analysis was performed using micrococcal nuclease (MNase) or DNase I digestion followed by indirect end labeling. MNase and DNase I cleavage sites enhanced in wild-type (WT) or *rpd3* mutant cells are marked with circles; those enhanced in *isw2* mutant cells are marked with triangles. N, naked DNA control. Relative expression levels indi-

function in repression of common target genes, we compared the transcriptional profiles of mutants defective in one or both complexes to determine the full extent of their overlap. In addition, we wished to determine the extent to which the repressive functions of Isw2 and Sin3-Rpd3 complexes depend on Ume6p. Our data indicate four main conclusions. First, the Isw2 complex functions in a pathway parallel to that of the Sin3-Rpd3 complex to repress transcription of a substantial number of genes. We find that synthetic phenotypes (those observed only when an *isw2* mutation is combined with a *sin3* or *rpd3* mutation) are more consistently revealed using double mutant versus single mutant hybridizations, especially for genes that are tightly repressed in wild-type cells. While the largest single group of genes repressed by these complexes consists of Ume6 target genes, the majority of genes that require Isw2 and Sin3-Rpd3 complexes for repression are Ume6 independent.

Second, chromatin structure analyses suggest that the Isw2 complex is required to create DNase I-inaccessible chromatin structure in the upstream regulatory regions of two Ume6-independent genes. At this time, we cannot exclude the possibility that the observed changes in chromatin structure and transcription are due to indirect effects of *isw2* mutation, since we have been unable to localize the Isw2 complex to specific chromosomal loci. The *rpd3* mutant is defective in regulation of the *POT1* and *SUC2* genes to a similar extent as the *isw2* mutant. However, only cells with the *isw2* mutation show the mutant nuclease digestion patterns at these loci, arguing against the possibility that chromatin structure defects in *isw2* mutant cells result from misregulation of transcription. Because *SUC2* transcription is decreased in *isw2* mutant cells, it appears that Isw2-dependent DNase I-inaccessible chromatin structure can have different effects on transcription at different loci. While it is not clear why inaccessible chromatin structure results in increased transcription of the *SUC2* gene, one possibility is that it partially inhibits the binding of a transcriptional repressor of *SUC2*. Consistent with this possibility, a binding site for the Mig1 repressor is located very close (at -499 with respect to the initiation codon) (4) to the DNase I-hypersensitive site present only in *isw2* mutant cells.

Recently, Kent et al. showed that the Isw2 complex is required for the formation of wild-type chromatin structure upstream of three randomly selected Ume6-independent genes, *FIG1*, *MET17*, and *PHO3*, as revealed by differences in their MNase cleavage patterns in *isw2* mutant cells relative to wild-type cells (27). However, transcription of the *FIG1* and *PHO3* genes is unaffected by the *isw2* mutation, and *MET17* transcription is only slightly increased in *isw2* mutant cells (see the full data set). It is therefore possible that regulation of chromatin structure at these loci by the Isw2 complex serves a function

cated below each mutant were measured by Northern blotting. For reference, *POT1* expression measured by Northern blotting in a *ume6* mutant is 1.2-fold that in the wild type. (B) Decreased expression of the *SUC2* gene in *isw2* mutants despite increased DNase I accessibility of the upstream regulatory region. Chromatin structure analysis was performed using DNase I digestion followed by indirect end labeling. The DNase I cleavage site enhanced in *isw2* mutant cells is marked with triangles. Relative expression levels indicated below each mutant were measured by Northern blotting.

other than transcriptional regulation. Alternatively, additional chromatin remodeling factors may function in parallel to regulate the transcription of these genes. In this case, loss of Isw2 function may be masked by these parallel factors, analogous to the relationship between Isw2 and Sin3-Rpd3 complexes in the repression of some early meiotic genes. Taken together, the data suggest that the Isw2 complex functions by a common mechanism at Ume6-dependent and -independent loci to create nuclease-inaccessible chromatin structure and that the ISW2-dependent inaccessible chromatin structure can affect transcription positively, negatively, or not at all, depending on the specific context.

Third, there are considerable differences in transcriptional profiles between deletion and catalytically inactive mutants of *isw2* and *RPD3*. These differences are most evident for *RPD3* mutants, suggesting the presence of deacetylase-independent and Sin3-independent functions of Rpd3p, as well as the possibility that catalytically inactive Rpd3p may inhibit other repressors of transcription. Consistent with these differences, *RPD3* deletion mutations show a synthetic growth defect in an *isw2* mutant background, whereas catalytically inactive mutations of *RPD3* and deletion mutations of *SIN3* show less severe synthetic growth defects in this background. In contrast, a recently published comparison of the transcriptional profiles of *RPD3* and *SIN3* deletion mutants suggests that virtually all Rpd3p functions require Sin3p (2). The major difference between this report and our data is our finding that a large number of genes require *RPD3* but not *SIN3* for repression. These conflicting conclusions may be explained by differences in strain background or conditions used for the growth of yeast cells. However, we previously observed phenotypic differences between *RPD3* and *SIN3* deletion mutants in two different yeast strain backgrounds with regard to their synthetic growth defect in an *isw2* mutant background (10). These data support the conclusion that Rpd3 has some Sin3-independent functions in vivo.

Fourth, Ume6p has one or more roles in chromosome segregation during haploid mitotic growth that are independent of Isw2 and Sin3-Rpd3 complexes. As a result, haploid *ume6* mutants tend to accumulate chromosomal duplications. For this reason, expression data for the *ume6* and *isw2 ume6* mutants should be interpreted with caution, since chromosomal duplications result in artificial inflation of genes on the duplicated chromosomes and may indirectly affect the expression of additional genes. Despite this fact, we find that a large group of genes appears to be regulated by Isw2 and Sin3-Rpd3 complexes independently of Ume6p. It is noteworthy that the expression profiles for *SIN3*, *RPD3*, *isw2 SIN3*, and *isw2 RPD3* mutants show no signs of chromosomal duplications in these mutants. In contrast, Hughes et al. previously found chromosomal duplications associated with *RPD3/RPD3* and *SIN3/SIN3* homozygous mutant diploids (19). These differences may result from differences in strain background or growth media or possibly from different effects of *SIN3* and *RPD3* mutations in haploid and diploid cells. Nevertheless, our data suggest that Ume6p has one or more functions in chromosome segregation during haploid mitotic growth that are independent of Sin3-Rpd3 and Isw2 complexes under the conditions tested. It is possible that Ume6p regulates the transcription of specific genes independently of Sin3-Rpd3 and Isw2 complexes. Alternatively, Ume6p may function more directly in some aspect of chromo-

some segregation. The bias observed for duplications of chromosomes 9 and 16 may reflect a growth advantage associated with extra copies of these chromosomes in *ume6* mutant cells. However, it is also possible that these chromosomes are more susceptible than others to missegregation in *ume6* mutants.

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