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In vivo chromatin remodeling by yeast ISWI homologs Isw1p and Isw2p

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Isw1p and Isw2p are budding yeast homologs of the *Drosophila* ISWI chromatin-remodeling ATPase. Using indirect-end-label and chromatin immunoprecipitation analysis, we show both independent and cooperative Isw1p- and Isw2p-mediated positioning of short nucleosome arrays in gene-regulatory elements at a variety of transcription units in vivo. We present evidence that both yeast ISWI complexes regulate developmental responses to starvation and that for Isw2p, recruitment by different DNA-binding proteins controls meiosis and haploid invasive growth.

[*Key Words*: Yeast; chromatin; transcription; Isw1p; Isw2p; chromatin-remodeling ATPase]

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Eukaryotic genomes are packaged into chromatin, a nucleoprotein complex with a hierarchy of condensed structures. Transcription, replication, recombination, and repair of DNA require enzymes and *trans*-acting factors to gain access to DNA in chromatin, and the facilitation of this by remodeling chromatin structure has emerged as an important theme in the regulation of these processes. A range of recent genetic and biochemical experiments have identified mechanisms that act to remodel the primary repeating unit of chromatin, the nucleosome core particle that consists of 146 bp of DNA wrapped around two copies of each histone protein H2A, H2B, H3 and H4. These mechanisms fall into two main categories. The first class involves covalent modification of histone protein N-terminal tails, typically by acetylation, methylation, and phosphorylation. The second class appears to use ATP hydrolysis to alter the conformation or location of the nucleosome. These two mechanisms are not mutually exclusive, and both may interact in regulating chromatin fluidity (Xue et al. 1998; for review, see Cairns 1998; Kingston and Narlikar 1999).

ATP-dependent chromatin remodeling is catalyzed by a variety of multi-subunit protein complexes that all contain members of a superfamily of ATPase enzymes conserved throughout the *Eukaryota*. The various ATP-dependent chromatin-remodeling complexes can be grouped into three main families (SWI/SNF-, ISWI- and Mi-2/CHD-like) on the basis of the primary structure of their ATPase subunits and in vitro biochemical activities (for review, see Kingston and Narlikar 1999; Brehm et al. 2000). Although the ISWI-like complexes have undergone extensive biochemical analysis, relatively little is known of their physiological role. In *Drosophila*, the ISWI ATPase purifies as a component of three compositionally and functionally distinct chromatin-remodeling complexes termed NURF, ACF, and CHRAC. Immunolocalization of ISWI in *Drosophila* cells suggests that one or more of these complexes is abundantly distributed over both mitotic and polytene chromosomes. Analysis of null alleles suggests that ISWI activity is essential for development and is required for normal X chromosome structure. The ISWI protein appears to localize to nontranscribed regions of polytene chromosomes but is also required for *engrailed* and *ultrabithorax* expression, suggesting participation in both gene repression and activation processes (Deuring et al. 2000). A *Xenopus* ISWI activity has recently been implicated in the chromatin remodeling that takes place when somatic nuclei are transplanted into oocytes (Kikyo et al. 2000). Budding yeast contains two ISWI homologs: Isw1p, which purifies in a complex with three other proteins, and Isw2p, which purifies separately with one other protein. Both complexes show ATP-dependent nucleosome spacing activities in vitro, and the Isw1p complex also shows in vitro nucleosome disruption activity similar to NURF (Tsukiyama et al. 1999). Although disruption of both *ISW1* and *ISW2* genes has very little phenotypic effect in vegetatively growing yeast (Tsukiyama et al. 1999), a recent transcriptome analysis suggests that both Isw1p and Isw2p are involved in gene activation and repression (Hughes et al. 2000). Diploid *isw2* homozygotes also appear to be unable to undergo premeiotic DNA replication, suggesting that Isw2p activity might be required for activation of meiosis/sporulation-specific genes (Trachtulcova et al. 2000). Nevertheless, it remains unclear how the nucleosome remodeling activity of the ISWI proteins manifests in real chromatin.

We therefore decided to look for ISWI-dependent chromatin structures directly in vivo. Nucleosome position

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at a specific locus can be inferred by comparing micrococcal nuclease (MNase) cleavage patterns in digests of chromatin with those obtained for purified DNA (Livingstone-Zatchej and Thoma 1999). We have been using indirect-end-label analysis for rapid MNase digestion of chromatin in whole yeast cells, previously employed to study SWI/SNF nucleosome-remodeling activity in vivo (Kent and Mellor 1995; Wu and Winston 1997), to screen panels of yeast loci for changes in chromatin structure in strains deficient in various chromatin-remodeling activities. The indirect-end-label methodology allows relatively large regions of chromatin to be visualized in terms of nucleosome footprints in MNase digestion patterns, allowing identification of target regions for chromatin modulators and providing information on mechanistics, targeting, and remodeling factor abundance. Here we show that during vegetative growth in rich media, both Isw1p- and Isw2p-dependent chromatin structures are common in yeast regulatory DNA.

Results and Discussion

We took two approaches in our search for Isw1/2p-dependent chromatin structures. Initially we analyzed a panel of seven yeast loci including *DRS2* and *GDH3*, which were picked at random from Chromosome I (Avedano et al. 1997; Chen et al. 1999), and *HIS3*, *MET16*, *MET17*, *PHO5*, and *PHO3*, which were chosen because their chromatin structure has been characterized previously (Struhl 1983; Almer et al. 1986; Kent et al. 1994; O'Connell et al. 1995; Svaren and Hörz 1997). During these experiments, an *isw1/2* mutant transcriptome became available (Hughes et al. 2000). We therefore also analyzed the *FIG1* gene (Erdman et al. 1998), which is induced in *isw1* mutant yeast, and the *INO1* gene (Jackson and Lopes 1996), which is induced in *isw2* yeast, with the prediction that we might observe Isw1p- and Isw2p-dependent changes in chromatin, respectively.

Chromatin structures were examined by comparing MNase cleavage patterns in *isw1 isw2* double mutants and isogenic wild-type strains during exponential vegetative growth in rich medium. No changes in MNase cleavage pattern were detected at *PHO5* or *HIS3* (Fig. 1A; data not shown). Figure 1A shows that both repressed and activated chromatin structures at *PHO5* (Svaren and Hörz 1997) occur normally in the absence of Isw1p and Isw2p. However, at *DRS2*, *FIG1*, *GDH3*, *INO1*, *MET16*, *MET17*, and *PHO3*, we observed changes in chromatin MNase cleavage patterns in *isw1 isw2* yeast, which localized to potential regulatory DNA in both promoters and 5′ ORF regions (Fig. 1; data not shown). These results suggest that yeast ISWI factors act widely throughout the yeast genome. The loci with ISWI-dependent MNase cleavage patterns were then analyzed further in *isw1* and *isw2* single mutants to determine the contribution of each ISWI complex.

Isw1p- and Isw2p-dependent chromatin structures

At the loci we sampled, we found three types of contribution from Isw1p and Isw2p; examples of each are presented in Figure 1. The *DRS2* locus showed a chromatin structure that altered only in cells lacking Isw1p, that is, where the MNase cleavage pattern in the *isw1* single mutant is identical to the *isw1 isw2* pattern but the *isw2* pattern is identical to wild type (Fig. 1B). Chromatin structures at the *GDH3* promoter and within the 5′ ORF of *MET16* were similarly Isw1p dependent (data not shown). The *INO1* and *PHO3* promoters, however, showed chromatin structures that altered only in the absence of Isw2p, that is, where MNase cleavage patterns in the *isw2* single mutant are identical to the *isw1 isw2* pattern but *isw1* patterns are identical to wild type (Fig. 1C,D). These results therefore suggest that the Isw1p and Isw2p complexes can function independent of each other. However, analysis of MNase cleavage patterns at the *FIG1* and *MET17* loci revealed regions of chromatin that appear to require the action of both Isw1p and Isw2p together, that is, where neither single-mutant MNase cleavage pattern is identical to the *isw1 isw2* double-mutant pattern (Fig. 1E,F). Figure 1E illustrates the Isw1p-dependent MNase cleavage patterns within the 5′ ORF of *FIG1* and an Isw2p-dependent cleavage site within the promoter, close to the *Dra* I site at –279 bp. At the *MET17* locus (Fig. 1F), the ISWI-dependent region of MNase cleavage is located not within the regulatory DNA of *MET17* but over a solo Ty1 retroposon δ element (a sequence equivalent to a retroviral

Figure 1. Isw1p- and Isw2p-dependent changes in MNase accessibility in yeast chromatin mapped by indirect-end-label analysis. All chromatin digestions were performed with MNase at concentrations of 75, 150, and 300 units/mL. Naked DNA controls are marked "DNA." Marker restriction digests (MK) are shown in relation to schematic maps of each locus. Restriction enzyme positions are relative to the labeled ORF. Indirect-end-label probes are marked with a bar at the bottom right of the locus map. ISWI-dependent changes in MNase cleavage patterns, where present, are highlighted next to blots with black diamonds and grey boxes. (*A*) *PHO5* chromatin structure is independent of Isw1p and Isw2p: Chromatin was analyzed in the strains indicated (CEN.PK2 background). Yeast were grown in rich media, which has a high phosphate concentration, for the left panel. Yeast for the right panel were grown in synthetic media with 1 mM KH2PO4 (+Pi) or phosphate free (–Pi). The region showing the characteristic *PHO5* chromatin transition on phosphate starvation (Almer et al. 1986) is indicated by the dotted line. (*B*) Isw1p-dependent chromatin structure at *DRS2* analyzed in CEN.PK2 strains. (*C*) Isw2p-dependent chromatin structure at *INO1* analyzed in CEN.PK2 strains. (*D*) Isw2p-dependent chromatin structure at *PHO3* analyzed in CEN.PK2 strains. (*E*) Isw1p- and Isw2p-dependent chromatin structure at *FIG1*. The left panel shows chromatin analyzed in CEN.PK2 strains. Center and right panels show YTT strain chromatin and illustrate separate Isw1p- and Isw2-dependent MNase cleavage patterns. (*F*) Isw1p- and Isw2p-dependent chromatin structure at a solo Ty δ element 5′ to the *MET17* gene in YTT strains. (*G*) Isw1/2p-dependent chromatin structure at 5′ δ element of YARCTy1-1 on Chromosome I in YTT strains.

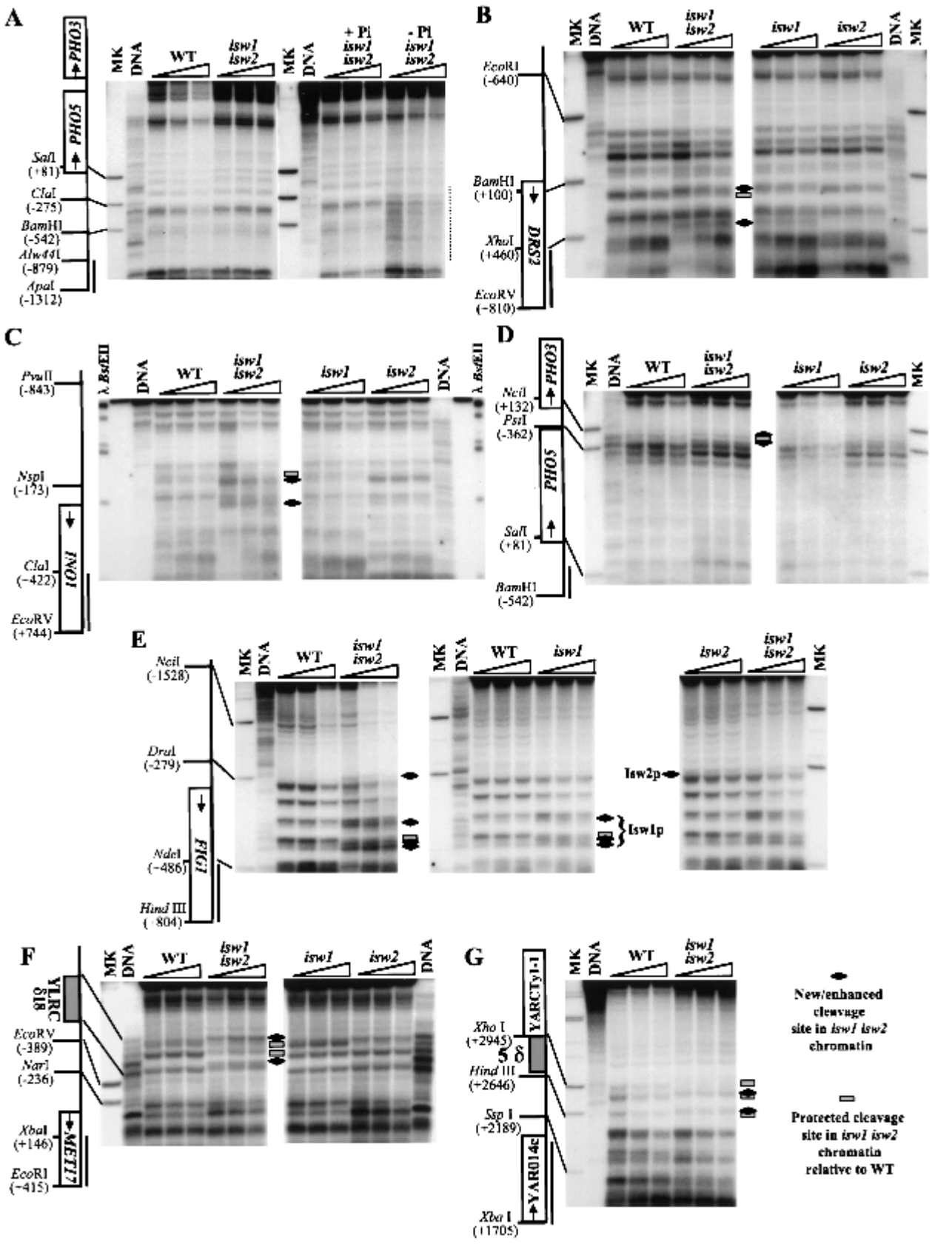

Figure 1. (*See facing page for legend.*)

long terminal repeat) termed YLRCδ18. At this locus, the *isw1* pattern is essentially the same as the wild-type structure, and the *isw2* pattern only shows one cleavage site characteristic of the *isw1 isw2* double mutant. This result suggests that both Isw1p and Isw2p act on chromatin in this region, although Isw2p alone is sufficient for the wild-type structure as far as we can detect with MNase. To test whether the ISWI-dependent chromatin structure that we observe at YLRCδ18 might be a general feature of Ty1δ elements, we picked a full-length Ty1 retroposon, YARCTy1-1, at random from the yeast genome and analyzed the MNase cleavage pattern of its 5′ δ in wild-type and *isw1 isw2* strains. Figure 1G shows that an ISWI-dependent change in MNase accessibility also occurs within this δ element. Therefore, it appears likely that Isw1p and Isw2p together affect δ element chromatin.

Drosophila ISWI- and both yeast Isw1p- and Isw2p-containing complexes show similar ATP-dependent transdisplacement activity toward in vitro assembled nucleosomes (Hamiche et al. 1999; Längst et al. 1999; Tsukiyama et al. 1999). The in vivo ISWI-dependent chromatin structures we observe are all consistent with this observed activity: In all cases, MNase cleavage sites characteristic of deproteinized DNA are protected in wild-type and *isw1/2* mutant chromatin, implying the presence of nucleosomes in both states. However, chromatin-specific cleavage sites in wild-type and *isw1/2* yeast differ in relative position (typically by 50–100 bp), suggesting that two to three nucleosomes at each of these loci adopt alternative translational positions in the absence of Isw1p and/or Isw2p (Fig. 2).

To test whether Isw1p and Isw2p are acting directly on the nucleosomal templates we have analyzed, we next performed chromatin immunoprecipitation (ChIP) experiments with strains expressing tagged Isw1p and Isw2p. Chromatin structures at *FIG1* and *INO1* are identical to wild type in the Isw1p–myc- and Isw2p-myc-tagged strains, respectively (data not shown), indicating that both tagged proteins are functional. ChIP samples were analyzed with PCR primers designed to amplify the regions associated with the ISWI-dependent chromatin structures shown in Figure 1. Figure 3A shows anti-myc–tag specific ChIP of regions of Isw1p-dependent chromatin in the Isw1p–-myc strain and similarly specific ChIP of regions of Isw2p-dependent chromatin in the Isw2p-myc strain. Loci such as *FIG1* and the Ty δ element at *MET17* that show chromatin structures that are dependent on both Isw1p and Isw2p show ChIP of DNA in both marked strains. We note here that ChIP with Isw1p–-myc recovers about fourfold less DNA than an equal input of Isw2p-myc chromatin. Although this could be a consequence of differential myc tag accessibility, it might also suggest that Isw1p and Isw2p work in different ways, with Isw1p being associated with DNA more transiently.

The results presented above suggest that the chromatin structures we observe are a direct consequence of the action of Isw1p and/or Isw2p and that in vivo Isw1p and Isw2p both use their transdisplacement activity to move

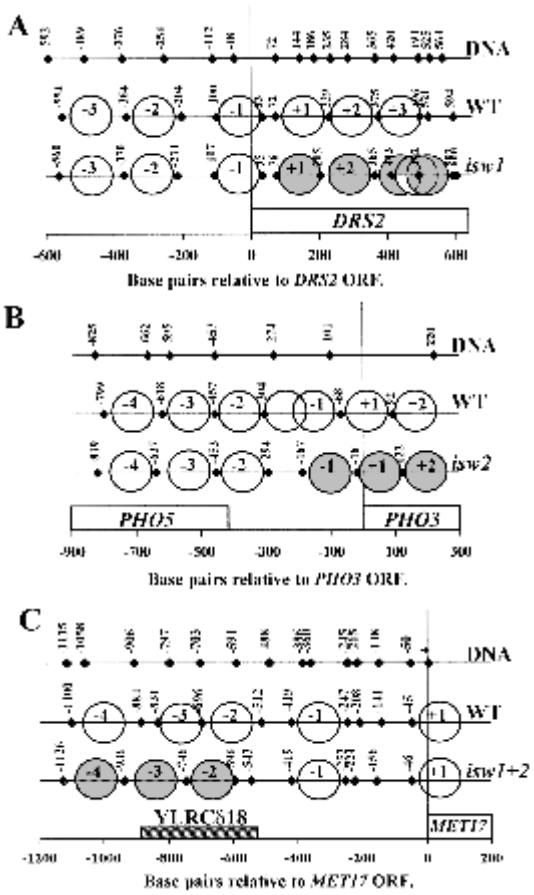

Figure 2. Inferred nucleosome positions at (*A*) *DRS2*, (*B*) *PHO3*, and (*C*) the solo Ty δ element at *MET17*. MNase cleavage sites are plotted relative to the nearest ORF for naked DNA (DNA) and chromatin in wild-type (WT) and *isw* mutant yeast. Nucleosomes are represented by circles 150 bp in diameter and are numbered relative to the ORF. Nucleosomes with altered positions are denoted in grey.

nucleosomes into specific positions. Given that the chromatin structures we have described all occur in potential regulatory DNA, we next asked whether the changes are a cause or consequence of changes in gene expression.

ISWI-dependent gene expression

Under identical growth conditions to those used to analyze chromatin structure, the *isw* mutant chromatin structures at *INO1*, *PHO3*, and Ty1 elements all correlate with a relative increase in expression: Figure 3B shows that basal levels of *INO1* RNA are raised in *isw2* and *isw1 isw2* mutant yeast relative to wild type. Figure 3C shows that acid phosphatase activity in *isw2* yeast is fivefold higher than in the wild-type and *isw1* strains during growth in rich media. The activity of a *PHO5* promoter-*lacZ* fusion reporter plasmid under identical growth conditions shows no increase in activity in *isw2* yeast. Taken together with the repressed chromatin

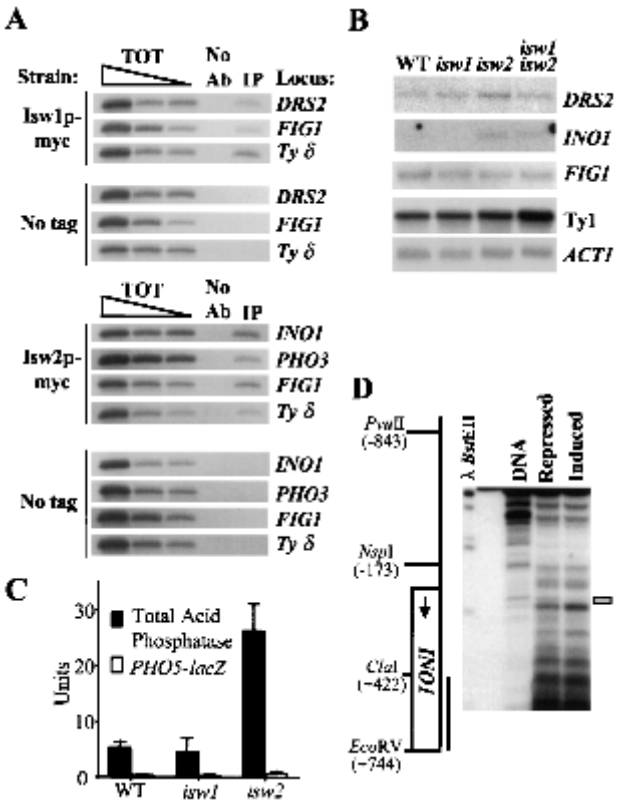

Figure 3. (*A*) ChIP of DNA in regions of Isw1p- and/or Isw2p-dependent chromatin in yeast strains expressing Isw1/2p with C-terminal myc tags and analyzed by PCR. *TOT* lanes contain input chromatin and dilutions at 1:10 and 1:20. *IP* and *NoAb* lanes contain ChIP samples with or without anti-myc monoclonal antibody, respectively. IP and NoAb samples from Isw1p–myc strains are 3.75× more concentrated that those from Isw2p-myc yeast and are accompanied by similarly concentrated untagged controls. (*B*) Northern analysis of *DRS2*, *INO1*, *FIG1*, and Ty1 transcripts in wild-type (WT) and *isw1/2* mutant yeast with *ACT1* as loading control. (*C*) Pho3p activity increases fivefold in *isw2* yeast. Graph showing acid phosphatase activity (Pho5p + Pho3p; Rogers et al. 1982) deriving from expression of chromosomal *PHO5* and *PHO3*, and β-galactosidase activity from a plasmid borne *lacZ* reporter fused to the *PHO5* promoter in CEN.PK2 yeast grown in rich (high phosphate concentration) media. Error bars show standard deviation ($n = 6$). Levels of Pho3p acid phosphatase activity are also reflected in *PHO5*/*PHO3* transcript levels as assayed by Northern blotting (data not shown). (*D*) Chromatin structure of *INO1* during gene induction is different to that occurring in the absence of Isw2p. MNase chromatin cleavage patterns are shown for yeast grown in YPD (Repressed) and after transfer to 1% potassium acetate and growth for 60 min (Induced). Under these conditions, microarray analysis of *INO1* normalized transcript levels are 799 and 18,669 units, respectively (J. Mellor, unpubl.). MNase for chromatin digests was used at 150 U/mL. An MNase cleavage site protected in induced cells is marked with a grey box.

structures observed at *PHO5* in Figure 1A, this result suggests that the increased acid phosphatase activity in *isw2* yeast is likely to derive from de-repression of *PHO3*. Interestingly, the pool of Ty1 transcript is more abundant only in the *isw1 isw2* double mutant, which adds further support to the conclusion that both Isw1p and Isw2p function together at Ty1 δ chromatin. In contrast to *INO1*, *PHO3*, and Ty1 elements, the expression levels of *DRS2* and *FIG1* are not substantially perturbed in any of the *isw* mutants. The fact that *DRS2* and *FIG1* expression remains constant despite changes in chromatin structure suggests that the ISWI-dependent chromatin structures at these loci are a direct product of ISWI function rather than an indirect consequence of gene activation or repression. We also analyzed MNase cleavage patterns at *INO1* during basal expression in rich media and after 23-fold induction on transfer into starvation/sporulation media (Fig. 3D). On induction, a single MNase cleavage site normally present at –180 bp becomes protected, suggesting that transcription from *INO1* is accompanied by a small change in chromatin structure. However, this change in MNase cleavage pattern is completely different from what we observe in the absence of Isw2p (Fig. 1C). Thus, *INO1* transcription per se does not produce the *isw2* mutant chromatin structure.

The Isw1p- and Isw2p-dependent chromatin structures we observe suggest that remodeling activity is localized to short arrays of two or three nucleosomes rather than affecting general nucleosome position or large chromosomal domains. This result argues that Isw1p and Isw2p activity is targeted to particular DNA regions by one or more *trans*-acting factors. *Drosophila* ISWI, as part of the NURF complex, cooperates with GAGA factor to disrupt nucleosomes on the *hsp70* promoter in vitro (Tsukiyama and Wu 1995). However, no GAGA-like factor appears to exist in *Saccharomyces cerevisiae*. We therefore analyzed DNA sequences underlying and flanking the ISWI-dependent regions of yeast chromatin for putative recognition sequences of DNA-binding proteins. Possible candidates included Cbf1p, Fkh1/2p, Pho2p, Ste12p, Thi2p, and Ume6p. The simplest model, in which a DNA-bound factor acts as a tether for either Isw1p or Isw2p, would predict that absence of either DNA-binding protein or remodeling ATPase will produce an identical alteration in chromatin structure. Of the DNA-binding proteins we tested, the absence of Ume6p produced exactly that result at the *INO1* locus. A Ume6p-binding motif at *INO1*, termed URS1, is required for repression of the gene during vegetative growth (Jackson and Lopes 1996), a similar phenotype to *isw2* yeast. Figure 4A shows that MNase cleavage pattern at *INO1* in *ume6* mutant yeast is identical to the *isw2* pattern shown in Figure 1C. A similar result was also obtained very recently at the *REC104* promoter by Goldmark et al. (2000), who implicate Ume6p in the recruitment of Isw2p to early meiotic genes in yeast. However, MNase cleavage at *PHO3*, which is also Isw2p dependent, shows a wild-type pattern in the absence of Ume6p (Fig. 4B). This result is recapitulated in a ChIP analysis (Fig. 4C,D): ChIP of *INO1* promoter DNA with Isw2p is abolished in *ume6* mutant yeast, whereas ChIP of the *PHO3* promoter is not. We conclude that Ume6p acts to recruit Isw2p to DNA at *INO1* but not at *PHO3*. Ume6p is therefore not the only recruitment factor for Isw2p.

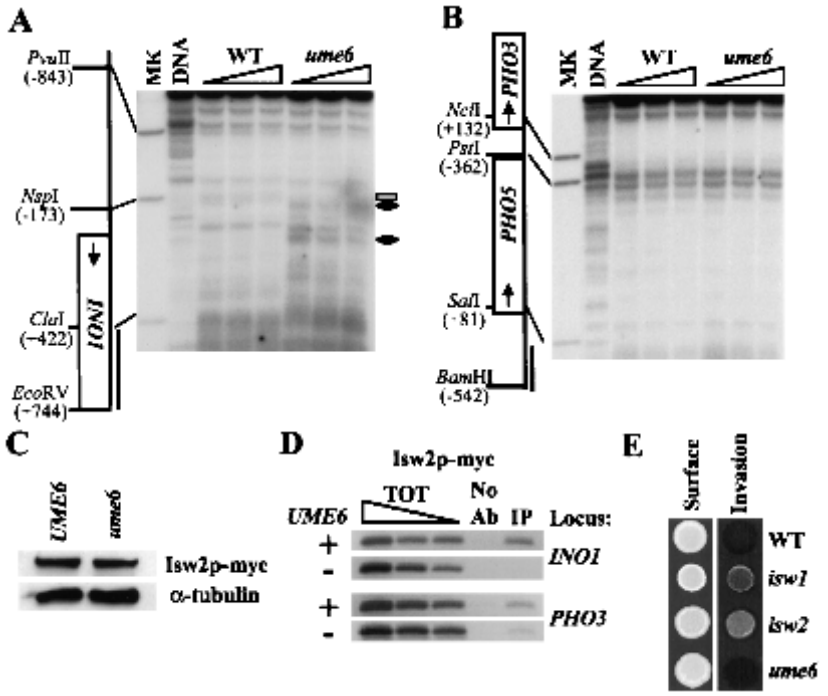

Figure 4. (*A*) Indirect-end-label analysis of MNase chromatin cleavage patterns at *INO1* showing a chromatin structure that is Ume6p dependent. Analysis as described in Figure 1. A Ume6p-binding motif, termed URS1, is present at –193 bp. The chromatin structure in the *ume6* mutant is identical to that observed in *isw2* mutants (Fig. 1C). (*B*) Indirect-end-label analysis of MNase chromatin cleavage patterns at *PHO3* showing chromatin structure is independent of Ume6p. Analysis as described in Figure 1. (*C*) Isw2p-myc expression is not regulated by Ume6p. Western blot of protein extracts from yeast strains expressing myc-tagged Isw2p in the presence and absence of Ume6p probed with anti-myc and anti-α-tubulin monoclonal antibodies. (*D*) ChIP of *INO1* and *PHO3* DNA with Isw2p-myc in wild-type and *ume6* mutant yeast. Analysis as described in Figure 2A. Absence of Ume6p abolishes *INO1* ChIP but not *PHO3* ChIP. (*E*) Haploid invasive growth into solid rich media by *isw1* and *isw2* but not *ume6* mutant yeast. CEN.PK2 background yeast are normally competent to undergo both haploid invasive and diploid pseudohyphal growth under appropriate starvation conditions (Roberts and Fink 1994). Unregulated invasive growth on YPD rich agar was uncovered by removing surface growth with water and a gloved finger.

It has been shown previously that diploid *isw2* yeast are unable to undergo meiosis (Trachtulkova et al. 2000), and recent work has confirmed that Isw2p functions to regulate early meiotic genes (Goldmark et al. 2000). Meiosis in yeast is a response to starvation. Several of the genes we have studied or that show changes in expression in *isw* mutant transcriptome analysis (Hughes et al. 2000) are not solely involved in meiosis but are regulated more widely by starvation. For instance, *INO1* is induced in the absence of inositol and choline, and *PHO3* by starvation for thiamine (Rogers et al 1982). We therefore looked for defects in other aspects of the starvation response in *isw* mutants. Figure 4E shows that both *isw1* and *isw2* mutant yeast exhibit enhanced haploid invasive growth (HIG) on rich media (Roberts and Fink 1994). Invading cells lacking Isw1p show an elongated morphology and polar budding, while the absence of Isw2p results in aberrant cell morphology (J. Mellor, unpubl.). The absence of Ume6p does not result in morphological changes or enhanced HIG (Fig. 4E; data not shown), again suggesting that another recruitment factor exists for Isw2p. We conclude that the ISWI complexes in yeast regulate the developmental responses of yeast to starvation.

Materials and methods

Strains and media

For analysis of Isw1p- and Isw2p-dependent chromatin structures, two sets of isogenic strains were used: YTT166/W1588–4C (*ISW1, ISW2*), YTT186 (*isw1::ADE2*), YTT196 (*isw2::LEU2*), and YTT199 (*isw1::ADE2, isw2::LEU2*) were a gift from Toshio Tsukiyama (Tsukiyama et al. 1999). CEN.PK2-1D (*ISW1, ISW2*) and CEN.KR9-6C (*isw1::KanMX*) are EUROSCARF strains. CEN.RP2 (*isw2::URA3*) and CEN.RP1+2 (*isw1::KanMX isw2::URA3*), which are isogenic to CEN PK2-1D, were a generous gift from Raymond Poot and Patrick Varga-Weiss. For analysis of Ume6p-dependent chromatin, we used EUROSCARF strains YO3566 (*ume6::KanMX*) and its isogenic wild-type parent BY4741. Deletion of *UME6* and C-terminal tagging of Isw1p and Isw2p with c-myc epitopes in the CEN.PK2 background was performed by gene replacement (Longtine et al. 1998). Primers are available from the authors. Rich media (YPD) contained 1% (w/v) Bactopeptone, 1% (w/v) yeast extract, and 2% (w/v) D-glucose, and yeast were grown at 29°C. Liquid cultures were harvested within a range of cell densities determined to represent mid-log/prediauxic shift phase. Haploid invasive growth was assayed as described by Roberts and Fink (1994) after growth of cells on YPD agar plates for 48 h.

In vivo chromatin analysis

Chromatin was digested with MNase in permeabilized yeast cells according to the general method of Kent et al. (1993) and Wu and Winston (1997), using the rapid sphaeroplasting modification described by Kent and Mellor (1995). Chromatin samples, each containing DNA from 2.0×10^8 cells, were digested with 75, 150, and 300 U/mL of MNase for 3 min at 37°C. Equivalent amounts of purified genomic DNA were digested with 10 U/mL MNase at 22°C for 40 sec to provide "naked" DNA controls. Further samples of purified DNA were cleaved with restriction enzymes and pooled in various combinations to provide marker digests. MNase-treated samples and marker mixes were digested to completion with appropriate restriction

enzymes and analyzed by indirect-end-labeling (Wu 1980), as described by Kent et al. (1994). Experiments were repeated at least twice and in both isogenic strain backgrounds.

Probes

Probes were derived from DNA fragments amplified by PCR from yeast genomic DNA. PCR products were cleaved with appropriate restriction enzymes and gel purified before radiolabeling by random priming. Hybridization and washing of Southern and Northern blots was carried out in aqueous buffer as described by Kent et al. (1994).

Interpretation of MNase digests

Molecular weights of MNase cleavage products were assigned using One-Dscan (Scanalytics) based on the mobility of marker bands. Given the specificity of MNase for nucleosomal linker DNA, protection of a MNase cleavage site normally observed in purified DNA and replacement by two flanking chromatin-specific cleavage sites ~150 bp apart is taken to imply the presence of a translationally positioned nucleosome.

Northern analysis

RNA was isolated from 2×10^8 cell aliquots of cultures, grown under identical conditions to those used for chromatin analysis, using the RNeasy midi kit (QIAGEN), and processed according to the manufacturer's protocols. *INO1* and *FIG1* transcript was measured using RNA from YTT strains, and Ty 1 and *DRS2* transcript using CEN.PK2 strains. Indirect-end-labels were used as probes where appropriate. The Ty 1 probe was a gift from Jef Boeke.

Enzymatic assays

Cells were assayed for acid phosphatase activity as described by Svaren et al. (1994). β-galactosidase activity was assayed as described by Hirst et al. (1994). The values for the assays are plotted as averages of three independent experiments each performed in duplicate. The *PHO5-lacZ* plasmid was made by PCR amplification of the region of *PHO5* from the *Pml*I site at –250 bp to +258 bp, at which place a *Hin*dIII site was introduced. This fragment was fused in frame to *lacZ* introduced into pRS426 containing the *PHO5* upstream sequences on an *Eco*RI (–2667 bp) to *Pml*I (–250 bp) fragment.

ChIP assays

ChIP was performed as described by Meluh and Broach (1999). Cultures were fixed in 1% formaldehyde for 2 h at room temperature. Chromatin from 3.0×10^8 cell equivalents was incubated for 15 h with (IP) or without (NoAb) anti-myc monoclonal antibody from clone 9E10 (Sigma) at a 1:200 dilution. NoAb and IP samples were resuspended in 40 µL for Isw1p–-myc analysis and in 150 µL for Isw2p-myc analysis. With primer pairs amplifying 300 bp, 5 µL aliqouts of DNA were analyzed by PCR. DNA from input chromatin (TOT) represents 0.5% of total with 1:10 and 1:20 dilutions. Products were fractionated on 1.5% agarose gels containing ethidium bromide and photographed in negative. Primer pairs amplify the following regions: *INO1*: –200 to +100 bp; *PHO3*: –120 to +180 bp; Ty δ at *MET17*: –1100 to –800; *FIG1*: –200 to +100; *DRS2*: +230 to +530.

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