

Two Distinct Nucleosome Alterations Characterize Chromatin Remodeling at the *Saccharomyces cerevisiae* *ADH2* Promoter*

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Glucose depletion derepresses the *Saccharomyces cerevisiae* *ADH2* gene; this metabolic change is accompanied by chromatin structural modifications in the promoter region. We show that the *ADR6/SWI1* gene is not necessary for derepression of the wild type chromosomal *ADH2*, whereas the transcription factor Adr1p, which regulates several *S. cerevisiae* functions, plays a major role in driving nucleosome reconfiguration and *ADH2* expression. When we tested the effect of individual domains of the regulatory protein Adr1p on the chromatin structure of *ADH2*, a remodeling consisting of at least two steps was observed. Adr1p derivatives were analyzed in derepressing conditions, showing that the Adr1p DNA binding domain alone causes an alteration in chromatin organization in the absence of transcription. This alteration differs from the remodeling observed in the presence of the Adr1p activation domain when the promoter is transcriptionally active.

It has become increasingly clear in the last 10 years that eukaryotic gene regulation at the level of transcription is strictly connected to the structural organization of the genome (1–3). Genetic loci that are going to become active in certain tissues, following a precise timing during development, require DNA binding transcription factors to implement the regulatory function exerted by nucleosomes. In addition, protein complexes capable of reconfiguring nucleosomes in an ATP-dependent manner or through the addition or removal of acetyl groups from histones or other factors have been genetically and biochemically characterized (4–6). The core of each complex is represented by proteins that have been conserved during evolution from yeast to humans, suggesting the existence of common mechanisms for turning on specific functions in differentiated cells or in a unicellular context.

Saccharomyces cerevisiae has proven to be a useful system for studying chromatin remodeling induced by various environmental stimuli at the level of specific promoters (7–11). For several genes, the dependence of transcription on complexes such as SWI/SNF (12), RSC (13), ADA/GCN5 (14), or SAGA (15, 16) or factors such as ISWI (17) has been documented. Nevertheless, this kind of requirement is not a widespread phenomenon (18), and in some instances, these complexes act as negative regulators (19). Activation of genes involved in galactose

or phosphate metabolism, for example, requires the function of their specific DNA binding transcription factors, the Gal4p and the Pho4p proteins, respectively. In these instances, the dependence on the above mentioned chromatin remodeling complexes stands only in particular cases, in which the activators function gets weakened (20, 21).

One of the unresolved questions concerns the real function of DNA binding transcription factors. Two scenarios were proposed. 1) the most important function of activators is to directly favor the recruitment of the transcription initiation complex, which in turn modifies the nucleosome structure, as in the case of Gal4p (10, 22), Pho4p (20, 21), and estrogen receptor derivatives produced in *S. cerevisiae* (23); and 2) the regulatory role of positive factors implies a first step in which transcription-independent binding of the activator to its target promoter allows the establishment of a specific architecture that favors the subsequent contact between the activation domain and the transcription initiation complex. Evidence in support of this second hypothesis has been presented recently: yeast mutants defective in TATA-binding protein function are able to remodel chromatin at the *CHA1* locus (24).

We have used the *ADH2* promoter coding for the enzyme alcohol dehydrogenase II as a model system to investigate this problem. This gene is tightly repressed by glucose and is derepressed when the glucose content of the medium is lowered. During derepression, the promoter undergoes structural changes at the level of a few nucleosomes, mainly the –1 and +1 nucleosomes, containing the TATA box and the RNA initiation sites, respectively (25). We have demonstrated (25) that this chromatin remodeling requires the transcription factor Adr1p (26), which also activates genes involved in peroxisome biogenesis and glycerol metabolism.

Here, we show that a major component of the SWI/SNF complex is not required for the wild type chromosomal *ADH2* derepression. Furthermore, to understand the role of individual Adr1p domains in the steps leading to *ADH2* derepression, we analyzed the function of a group of constructs coding for portions of the regulatory molecule. In cells containing the Adr1p DNA binding domain alone, in the absence of transcription, a predominant change in the structure of the TATA box containing nucleosome –1 was seen. When a small acidic domain of 43 amino acids recently characterized to possess the strongest activation potential of the entire Adr1p molecule (27) was added to the DNA binding domain, a qualitatively different nucleosome modification was observed, and at the same time, *ADH2* transcription level reached its maximum.

EXPERIMENTAL PROCEDURES

Yeast Strains and Media—*S. cerevisiae* strains used in this study were as follows: CY26 (MAT α , SWI/ADR6, *ura3-52*, *lys2-801*^a, *ade2-101*^b, *trp1- Δ 1*, *his3- Δ 200*, *leu2- Δ 1*), CY58 (MAT α , same as CY26 except *swi1::LEU2*); JSY112 (MAT α , same as CY26 except *adr1::LEU2*), and EPY10 (MAT α , same as CY26 except *adr1::LEU2*, *swi1::LEU2*).

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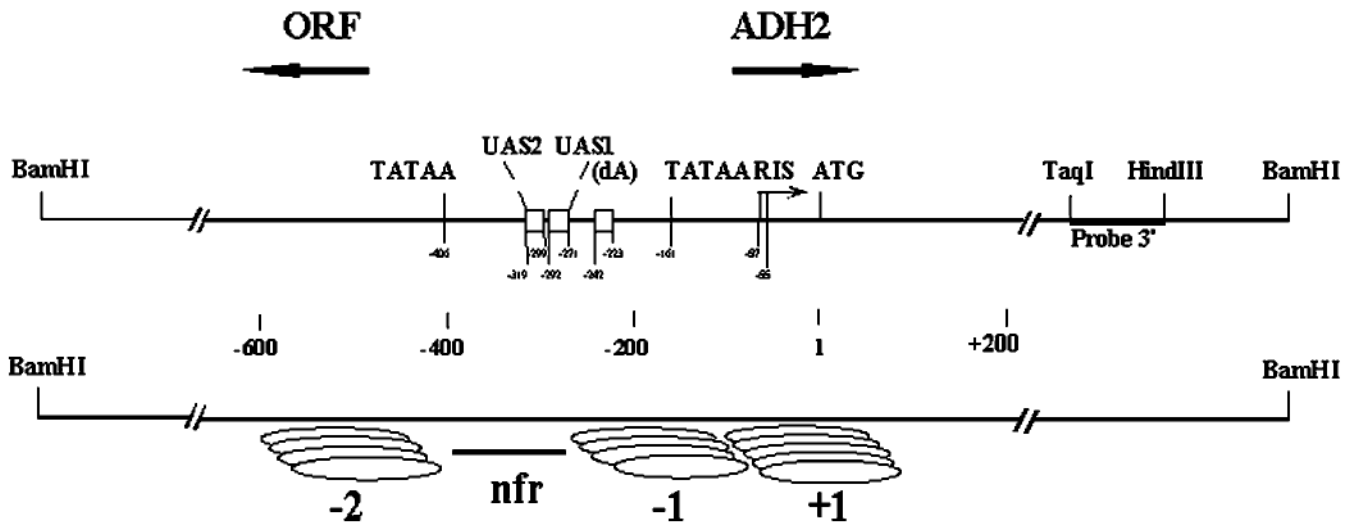


FIG. 1. Schematic map of the *ADH2* gene. The positions of the relevant elements are given relative to the ATG (A is at position +1). *RIS*, RNA initiation site; *ORF*, open reading frame; *nfr*, nucleosome-free region. Probe 3': *TaqI-HindIII* fragment, 102 base pairs. Each group of ovals represents a family of multiple overlapping nucleosomes, the borders of which were mapped at high resolution (25).

Yeast strains carrying no plasmid were grown in YPD medium (1% yeast extract, 2% bacto peptone, 3% glucose). To obtain *ADH2* derepression, the cells were collected by centrifugation, washed once with water, and resuspended in the same volume of fresh YP medium containing 0.05% glucose for the appropriate time.

Cells carrying the plasmids described below were grown in YNB medium (0.68% yeast nitrogen base) supplemented with the required amino acids and 3 or 0.05% glucose.

For yeast transformations, cells were made competent by treatment with lithium acetate (28).

Plasmids—The *Adr1p* derivatives used were as follows: pADR1_{Δ172}, consisting of the first 172 amino acids of *Adr1p* inserted in pRS314 (*CEN6*, *ARSH4*, and *TRP1*); pADR1_{Δ172-AD}, same as above with the addition of a peptide containing amino acids 420–462 of *Adr1p*; and pADR1 s, consisting of the entire *ADR1* gene inserted in pRS314. The construction of these molecules has been described previously (27). These plasmids were used to transform the *adr1* disrupted strain JSY112. As a control, the same strain was also transformed with the vector pKD8 (*CEN3*, *ARS1*, and *TRP1*).

pFA plasmid DNA (29) was used to prepare the probe for the indirect end labeling analysis and as deproteinized material for control reactions with micrococcal nuclease (MN).¹

Chromatin Analysis—The analysis of nucleosome position and/or structure was performed by using MN digestion of spheroplasts coupled with the indirect end labeling procedure (30).

Cells exponentially growing (A_{600} 0.5/ml) in *ADH2* repressing (3% glucose) or derepressing (0.05% glucose) conditions were washed once with water and then resuspended in zymolyase buffer (1 M sorbitol, 50 mM Tris-HCl, pH 7.5, 10 mM 2-mercaptoethanol). Incubation with zymolyase was for 20 min at 30 °C. The resulting spheroplasts were collected by centrifugation and resuspended in nystatin buffer (1 M sorbitol, 20 mM Tris-HCl, pH 8.0, 1.5 mM CaCl₂, 50 mM NaCl, 100 μg/ml nystatin) in order to permeabilize cell membranes for the subsequent treatment with MN. (For more details on the use of nystatin for chromatin analysis, see Ref. 31.) Incubation with MN was for 15 min at 37 °C, and the reaction was stopped with 5 mM EDTA, 1% SDS (final concentrations). The samples were then treated with proteinase K for 2 h at 56 °C and purified by phenol-chloroform extraction and ethanol precipitation.

After secondary digestion with the appropriate restriction endonuclease, the samples were run on 1.5% agarose gels in Tris-borate TBE buffer and transferred to nitrocellulose filters. Southern blot and hybridization were performed by standard procedures.

RNA Analysis—Aliquots containing the same number of cells were collected by centrifugation, and total RNA was prepared as described previously (32). After spectrophotometric determination of the RNA amount present in each aliquot, 10 μg of RNA were loaded onto 1.2%

agarose-MOPS gels containing formaldehyde and ethidium bromide.

Northern blot analysis was performed by standard procedures. For hybridization, a 5'-end-labeled oligonucleotide (5'-GTTGGTAGCCTTA-ACGACTGCGCTAAC-3') specific for the *ADH2* gene (from +710 to +684 of the coding region) was used.

RESULTS

Fig. 1 shows the chromatin structure of the repressed *ADH2* promoter, with the position of the three relevant nucleosomes relative to the regulatory and basal transcription elements. Upstream activating sequence 1 (UAS1) is a 22-base pair palindromic sequence recognized by the DNA binding transcription factor *Adr1p* (33); UAS2 contains the consensus sequence for several proteins including *Adr1p* (34). The high resolution mapping of this region, showing the peculiar organization in families of octamer particles with the same rotational orientation, was described previously (25). When the sequencing of the yeast genome was completed, we became aware of the presence of a divergently located transcription unit, the putative TATA box of which turned out to be protected by the upstream edge of nucleosome -2.

The *ADH2* promoter is derepressed when the glucose content of the medium is lowered. The nucleosomes -1 and +1, protecting the TATA box and the RNA initiation sites, respectively, undergo a structural modification (25) extending to the nucleosomes +2 and -2, which is actually part of the adjacent transcription unit. We have shown that the RNA polymerase II catalytic subunit is not required for this modification, suggesting that the transcription process *per se*, at least at the elongation step, is not the cause of the change (29). Up to now, *Adr1p* is the only protein shown to be required for the chromatin remodeling at the *ADH2* promoter. We therefore looked at additional factors involved: the result of the analysis of a strain containing a disruption of the *SWI1* gene is described below.

The *ADR6/SWI1* Gene Is Not Involved in the Wild Type Chromosomal *ADH2* Derepression—*ADR6* was initially identified in a screen for mutants able to decrease Ty-activated *ADH2* expression, and it was shown to be required also for *ADH2*⁺ expression, when the derepressing carbon source is glycerol (35, 36). The *ADR6* gene was subsequently shown to be identical to *SWI1* (37), a component of the SWI/SNF chromatin remodeling complex, required for the transcription of a subset of *S. cerevisiae* genes (19). More recently, a *snf2* deletion was shown to have no effect on the ability of *LexA-ADR1* fusions to activate a *LexA-lacZ* reporter (38).

¹ The abbreviations used are: MN, micrococcal nuclease; aa, amino acid(s); MOPS, 4-morpholinepropanesulfonic acid; UAS, upstream activating sequence.

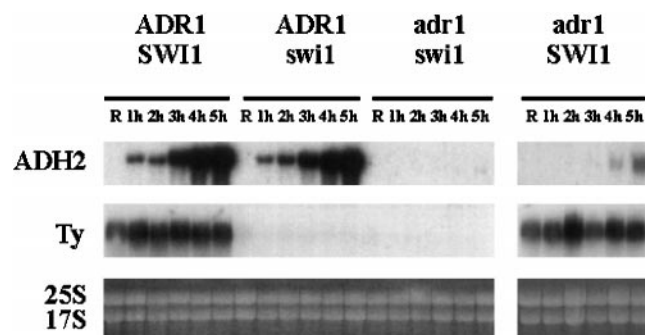


FIG. 2. Disruption of the *SWI1* gene does not affect the derepression of wild type chromosomal *ADH2*. Northern blot analysis of the kinetics of *ADH2* mRNA accumulation during derepression, for four isogenic strains (CY26, CY58, EPY10, and JSY112: first, second, third, and fourth groups of samples from *left*, respectively). *R*, repressing conditions (3% glucose). Times refer to hours of derepression. Total RNA was prepared from an equal number of cells at each time point: the ethidium bromide staining of rRNA (*bottom panel*) is shown as a control for equal loading. Ty transcription was detected by using a Ty1 internal probe.

Thus, we tested directly whether in the normal chromosomal context, the accumulation of mRNA from the *ADH2* promoter was affected by a *SWI1* disruption. Fig. 2 shows the results of a comparison among four isogenic strains: *SWI1-ADR1*, *swi1-ADR1*, *swi1-adr1*, and *SWI1-adr1*. This comparison provides a complete picture of the effect of disrupting *SWI1*, in both the presence and the absence of the major activator of the system. *ADH2* derepression, obtained by lowering the glucose content of the medium to 0.05%, is clearly not influenced by a disruption of *SWI1* (Fig. 2, second group of samples from *left*), whereas the lack of *ADR1* (fourth group) strongly reduces transcription, as expected. Interestingly, when both functions are lost, as in the *swi1-adr1* strain, *ADH2* transcription is almost totally turned off (Fig. 2, third group of samples from *left*), suggesting a role for *SWI1* in the *ADR1*-independent mRNA accumulation. As a control for a defect in *SWI1* function, we have rehybridized the same filter with a probe for a Ty element, transcription of which is known to be reduced in SWI/SNF defective strains (39); consistent with this observation, we found that Ty mRNA is missing in the *swi1* disrupted strains (Fig. 2, second and third groups from *left*).

We next analyzed the chromatin structure of isogenic *ADR1-SWI1* and *ADR1-sw1* strains. The results are shown in Fig. 3: consistent with the lack of influence of *SWI1* on *ADH2* transcription, chromatin remodeling at the *ADH2* promoter occurs also in the *swi1* defective strain (second group of samples) when the cells are incubated in derepressing medium (0.05% glucose). The nucleosomal organization of an isogenic *adr1-SWI1* strain in the same conditions is shown in parallel to underline the relevance of *ADR1* function in the structural modifications that accompany *ADH2* derepression. When *ADR1* is disrupted, the further disruption of *SWI1* does not alter the chromatin pattern which maintains the repressed configuration (data not shown).

We conclude that derepression of the *ADH2* promoter, in its normal chromosomal location, does not require a functional copy of the *SWI1* gene.

Adr1p DNA Binding and Activation Domains Induce Two Distinct Nucleosome Modifications at the Chromosomal *ADH2* Promoter—In a previous study (29), we searched for factors required for chromatin remodeling at the *ADH2* promoter: most of the factors we have analyzed are involved in the control of mRNA accumulation but do not affect nucleosome structure. No factor was found to influence remodeling without affecting transcription, suggesting a dependence of the second event on

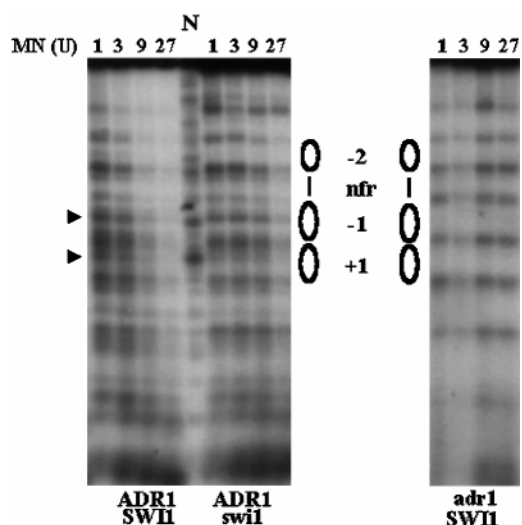


FIG. 3. Disruption of the *SWI1* gene does not prevent chromatin remodeling. MN analysis of 3 isogenic strains (CY26, CY58, and JSY112: first, second, and third groups of samples from *left*, respectively). Nystatin-permeabilized spheroplasts from cells grown for 3 h in YP medium containing 0.05% glucose were treated with the indicated amounts of MN (*U*, units/0.25 ml), deproteinized, and digested with *Bam*HI and *Hind*III (map positions are -1202 and +760, respectively). The samples were electrophoresed through 1.5% agarose-TBE gels and transferred to a nitrocellulose membrane. The filters were hybridized with the 3' DNA probe. Lane *N* contains deproteinized pFA DNA reacted *in vitro* with MN, *Bam*HI, and *Hind*III. The three relevant nucleosomes, spanning the basic and regulated promoter elements, are represented as ovals. *nfr*, nucleosome-free region. Arrowheads indicate the MN sensitivities present in the chromatin (in derepressing conditions) and in the naked samples at the level of the -1 and +1 nucleosomes.

the first.

Because up to now the only factor required for both remodeling and transcription has been the *Adr1p* activating protein, we asked whether the chromatin modifying activity present in *Adr1p* is distinct from its function as transcriptional activator. This question has already been addressed in the case of other two proteins, the Gal4p activator (10, 22) and the Pho4p activator, which drives remodeling and transcription at the *PHO5* promoter (40). In this latter case, the two activities (nucleosome reconfiguration and transcriptional activation) could not be separated, as both were associated with a small region of the Pho4p activation domain (41). No chromatin remodeling was observed when utilizing the DNA binding domain only (40). Thus, we have readdressed this problem in the case of the *Adr1p* activator.

Because of the large size of this factor, we analyzed a reduced version of *Adr1p*, recently characterized by Young *et al.* (27). This molecule consists of the two zinc finger DNA binding domain, which is known to interact with the UAS1 sequence in the *ADH2* promoter (34), fused to a 43-amino acid (amino acids 420–462) acidic domain, which has been shown to be the strongest activation domain of the entire *Adr1p* (27). The natural nuclear targeting signal (42) is included at the N terminus of the molecule. Fig. 4A illustrates the constructs we have used for our analysis: (i) the DNA binding domain (181 aa); (ii) the DNA binding domain fused to the 43-amino acid activation domain (238 aa); and (iii) the full-length protein (1323 aa). The construction of these molecules was described previously (27). Transcription of all of these constructs is driven by the *ADR1* natural promoter on centromeric plasmids that we used to transform an *ADR1* disrupted strain.

Fig. 4B shows the results of a comparative analysis of *ADH2* mRNA accumulation in derepressing conditions for the various *Adr1p* derivatives. When the *ADR1* disrupted strain was trans-

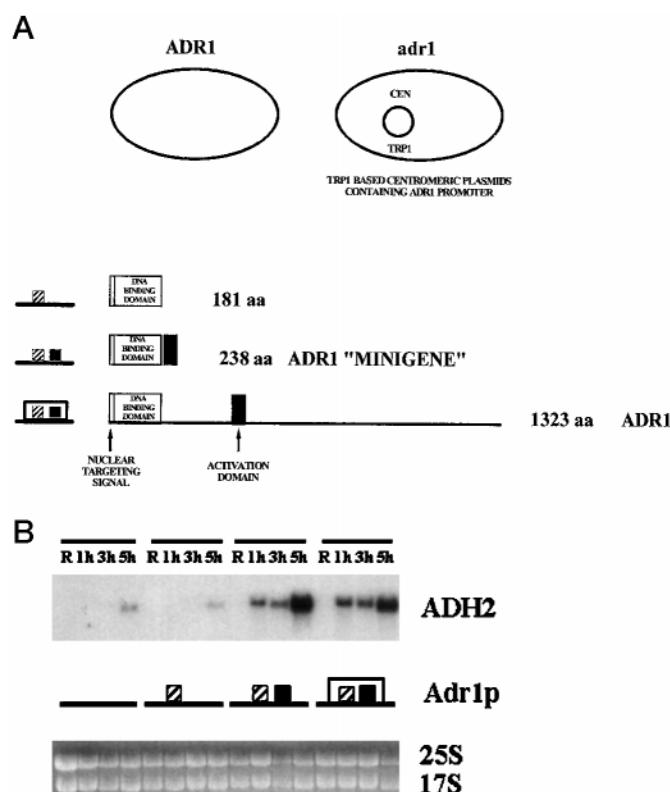


FIG. 4. Analysis of the effect of *Adr1p* derivatives on *ADH2* derepression. A, schematic representation of the constructs used. Different portions of the *ADR1* gene were inserted in centromeric plasmids carrying the *ADR1* promoter, as described previously (27). The natural nuclear targeting signal (42) is included at the N terminus of each molecule. The 181-aa construct contains the DNA binding domain; the 238-aa *ADR1* minigene contains the DNA binding domain fused to the 43-amino acid activation domain; and the 1323-aa construct contains the full-length *Adr1p*. B, Northern analysis of the kinetics of *ADH2* mRNA accumulation during derepression in the presence of different *Adr1p* portions. First group of samples from left: *adr1* disrupted strain (JSY112) transformed with pKD8 (vector alone). Second, third, and fourth groups from left: the same strain transformed with the 181-, 238-, and 1323-aa constructs, respectively. Hatched and black boxes represent the *Adr1p* DNA binding and activation domains, respectively.

formed with the vector alone (Fig. 4B, first group of samples from left) or with the plasmid containing the *Adr1p* DNA binding domain (second group), transcription was very low. When the construct containing both the DNA binding and the activation domains (Fig. 4B, third group from left) was used for transformation, the kinetics of mRNA accumulation was almost indistinguishable from that shown by the plasmid containing the entire protein (fourth group). The transcriptional behavior of these four constructs is in agreement with the *ADH2* enzyme levels described recently (27).

In order to understand whether the nucleosome modifications occurring at the *ADH2* promoter are the direct consequence of the presence of the *Adr1p* activation domain or are induced by the binding of the protein to DNA, we analyzed the chromatin structure of the *ADH2* promoter in the presence of the various *Adr1p* derivatives in derepressing conditions (0.05% glucose, for 3 h). Fig. 5 shows the following results: (i) in the case of the vector alone (first group of samples) chromatin remodeling did not occur, as expected; (ii) when only the DNA binding domain was present (second group of samples from left), a change in the structure of nucleosomes -1 and $+1$ was observed. In particular, this change consists of two bands appearing at the level of the -1 nucleosome and one band at the level of the $+1$. Arrowheads in Fig. 5 indicate the MN cleavage

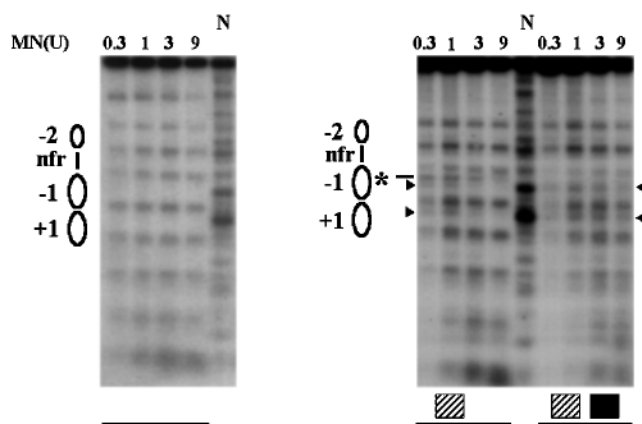


FIG. 5. Two distinct nucleosome modifications are induced by *Adr1p* DNA binding and activation domains. MN analysis of the *adr1* disrupted strain (JSY112) transformed with three different *Adr1p* constructs: vector alone (first group of samples from left), 181-aa construct (second group), and 238-aa construct (third group). Nystatin-permeabilized spheroplasts from cells grown for 3 h in YNB medium containing 0.05% glucose were treated with the indicated amounts of MN (*U*, units/0.25 ml), deproteinized, and digested with *Bam*HI and *Hind*III (map positions are -1202 and $+760$, respectively). The samples were electrophoresed through 1.5% agarose-TBE gels and transferred to a nitrocellulose membrane. The filters were hybridized with the 3' DNA probe. Lane *N* contains deproteinized pFA DNA reacted *in vitro* with MN, *Bam*HI, and *Hind*III. The three relevant nucleosomes, the schematic map of which is shown in Fig. 1, are represented as ovals. *nfr*, nucleosome-free region. The asterisk indicates the structural alteration specifically induced by the *Adr1p* DNA binding domain. Arrowheads indicate the MN sensitivities present in the chromatin (in derepressing conditions) and in the naked samples at the level of the -1 and $+1$ nucleosomes. Hatched and black boxes represent the *Adr1p* DNA binding and activation domains, respectively.

products that are present also in the *in vitro* treated samples (lane *N*), whereas an asterisk indicates the MN cleavage product that is present exclusively in the *in vivo* samples. The band with the asterisk is located halfway between the UAS1 sequence (recognized by *Adr1p*) and the TATA box, close to a poly(dA) tract of 20 adenines. The fact that this band is not visible in the *in vitro* treated sample at the level of the -1 nucleosome suggests that it is not due to loosening of DNA histones contacts but rather to a local DNA deformation on the surface of that nucleosome or alternatively to a redistribution of its borders (see under "Discussion"). The MN sensitivities induced by the *Adr1p* DNA binding domain alone disappeared at the highest enzyme dose used. (iii) When the construct containing the DNA binding domain plus the 43-amino acid activation domain was tested (Fig. 5, third group of samples from left), the same chromatin remodeling occurred as was observed previously with the entire *Adr1p* (see Refs. 25 and 29 and Fig. 3). This remodeling is identified by the appearance of the two bands also visible in the *in vitro* treated sample at the level of the -1 and $+1$ nucleosomes. In this case, the band marked with the asterisk in Fig. 5 was not observed. The MN sensitivities induced by the construct containing both *Adr1p* domains were still visible, although less intense, at the highest enzyme dose used.

The nucleosome modification induced by the *Adr1p* DNA binding domain in the absence of transcription is therefore distinct both qualitatively and quantitatively from that observed when the transcriptional activation domain is also present.

Chromatin Remodeling Driven by Full-length Adr1p Is Characterized by Two Kinetically Distinct Nucleosome Modifications—Because of the difference in nucleosome modifications observed in the absence or in the presence of the *Adr1p* activation domain, we analyzed more carefully the chromatin re-

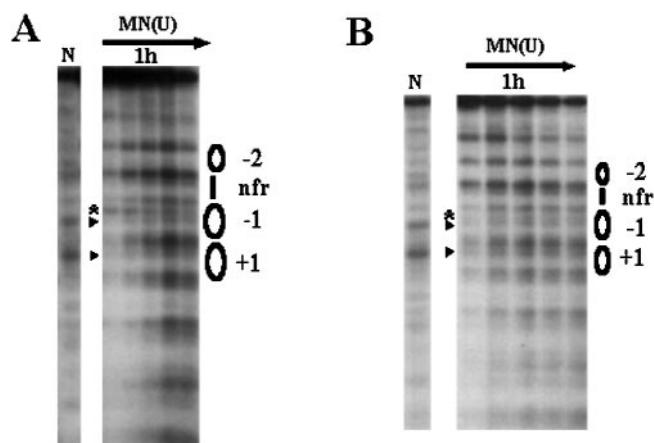


FIG. 6. Chromatin remodeling driven by full-length *Adr1p* is characterized by two kinetically distinct nucleosome modifications. A, MN analysis of the wild type strain (CY26). Nystatin-permeabilized spheroplasts from cells grown for 1 h in YP medium containing 0.05% glucose were treated with increasing amounts of MN (0.125, 0.25, 0.5, 1, and 2 units/0.25 ml (U)), deproteinized, and digested with *Bam*HI and *Hind*III (map positions are -1202 and $+760$, respectively). The samples were electrophoresed through 1.5% agarose-TBE gels and transferred to a nitrocellulose membrane. The filters were hybridized with the 3' DNA probe. Lane N contains deproteinized pFA DNA reacted *in vitro* with MN, *Bam*HI and *Hind*III. The three relevant nucleosomes, the schematic map of which is shown in Fig. 1, are represented as ovals. *nfr*, nucleosome-free region. The asterisk indicates the structural alteration specifically induced by the *Adr1p* DNA binding domain. Arrowheads indicate the MN sensitivities present in the chromatin (in derepressing conditions) and in the naked samples at the level of the -1 and $+1$ nucleosomes. B, same as in A but using *ADR1-swi1* cells from strain CY58.

modeling induced by the full-length transcription factor. Fig. 6A shows the results obtained by using low MN amounts on spheroplasts from wild type cells grown in derepressing conditions for a short length of time (0.05% glucose for 1 h). The very first alteration induced is visible on the -1 nucleosome and is marked with an asterisk; this band is not present in the *in vitro* treated sample and coincides with that observed in the presence of the *Adr1p* DNA binding domain alone (Fig. 5, asterisk). At increasing amounts of MN, the efficiency of cleavage at this site decreased, whereas the two bands marked with arrowheads in Fig. 6A, present in the -1 and $+1$ nucleosomes, became more and more visible.

Therefore, by using low MN amounts and early derepression times (1 h), it is possible to distinguish the very first modification occurring in the TATA box containing nucleosome -1 at the *ADH2* locus *in vivo*. This modification is due to the binding of the *Adr1p* transcription factor to UAS1, in close proximity to the first altered nucleosome; in fact, it can also be observed in the absence of the *Adr1p* activation domain (see Fig. 5). As derepression proceeded, both nucleosomes -1 and $+1$ showed a significant loss of protection of the two MN cleavage sites marked by arrowheads in Figs. 5 and 6, suggesting loosening of DNA histone contacts.

We conclude that chromatin remodeling driven by the full-length *Adr1p* molecule is characterized by two kinetically distinct nucleosome modifications, one due to the binding of the protein to DNA and the other one induced by its activation domain.

Fig. 6B shows the results of a similar analysis performed with the *ADR1-swi1* strain: in agreement with the lack of requirement of the *SWI1* function in the *ADH2* chromatin remodeling (see Fig. 3), the MN cleavage site induced by the *Adr1p* DNA binding domain in the nucleosome -1 (asterisk) is visible also in this mutant strain. Nevertheless, the TATA box containing nucleosome -1 becomes accessible to MN in wild

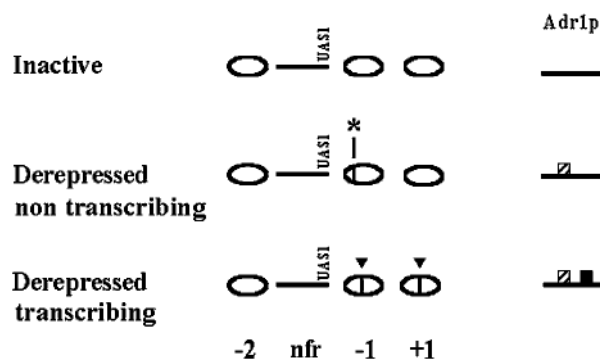


FIG. 7. Schematic representation of the events occurring at the *ADH2* promoter during derepression. The three relevant nucleosomes, the schematic map of which is shown in Fig. 1, are represented as ovals. *nfr*, nucleosome-free region. UAS1 is the binding site for *Adr1p*. The asterisk indicates the structural alteration specifically induced by the *Adr1p* DNA binding domain. Arrowheads indicate the MN sensitivities present in the chromatin and naked samples at the level of the -1 and $+1$ nucleosomes. Three possible states of the promoter are shown depending on the absence or the presence in the cells of specific *Adr1p* portions. *Inactive*: in the absence of *Adr1p*, no chromatin change is observed, and transcription is off. *Derepressed nontranscribing*: in the presence of the *Adr1p* DNA binding domain alone, transcription is off, but the nucleosome -1 is specifically modified in the vicinity of the UAS1. *Derepressed transcribing*: when the *Adr1p* activation domain is also present, both nucleosomes -1 and $+1$ are structurally altered, and transcription is on.

type (Fig. 6A) and *swi1* (Fig. 6B) cells following different kinetics.

DISCUSSION

We have shown that the chromatin remodeling that occurs at the *S. cerevisiae ADH2* promoter upon derepression is characterized by two distinct structural alterations. The first consists of a localized modification induced by the *Adr1p* DNA binding domain at the level of the nucleosome -1 (see the band marked with an asterisk in Figs. 5 and 6); this cleavage site, appearing under derepressing conditions on the particle containing the TATA box, is located halfway between the UAS1, where *Adr1p* binds, and the TATA box. This early remodeling step occurs in the absence of transcription, as shown by the fact that after lowering the glucose content of the medium, the *ADH2* mRNA level accumulated in the *ADR1* disrupted strain transformed with the *Adr1p* DNA binding domain does not differ from the level obtained in the *ADR1* disrupted strain transformed with the control vector. The MN sensitivity induced by the DNA binding domain in derepressing conditions is not present in the naked sample, suggesting a localized deformation on the surface of nucleosome -1 that can be recognized by MN. Alternatively, the new band could be due to a redistribution of the upstream borders of nucleosome -1 as a consequence of *Adr1p* binding to UAS1. A MN cleavage site with very similar characteristic has been recently described in the case of Gal4p binding to episomal DNA (43).

The second type of structural alteration, induced when the activation domain is also present, is more stable and involves both nucleosomes -1 and $+1$; this latter particle contains the RNA initiation sites. In this case, the two MN cleavage sites, showing up in derepressing conditions, coincide with those visible in the naked sample at the level of nucleosomes -1 and $+1$, suggesting a partial unwrapping of these particles in conditions in which the transcription is on.

A simplified scheme of these findings is shown in Fig. 7, in which three possible states of the *ADH2* promoter are presented. In the absence of *Adr1p*, the *ADH2* promoter remains structurally and functionally inactive when the cells are shifted to derepressing conditions. In the presence of the *Adr1p* DNA

binding domain alone, a localized modification on the -1 nucleosome, in the vicinity of the UAS1 sequence, occurs: the promoter is structurally derepressed but functionally inactive. When the *Adr1p* activation domain is also present, a distinct structural alteration occurs at the transcribing *ADH2* locus: the promoter is fully derepressed and functionally active. The three possible states of the promoter, due to the absence or the presence of different *Adr1p* portions, can be considered as an ordered sequence of events occurring at the *ADH2* locus during derepression.

We believe that the observed differences are not due to a reduced binding ability of the small 181-amino acid construct for the following reasons: (i) *in vitro* gel retardation assay with these derivatives has shown that the binding abilities of the 181- and 238-amino acid constructs are equivalent (27); and (ii) the difference between the two types of structural modification is qualitative and not only quantitative.

The observation that the chromatin remodeling induced by two different domains of the same protein, the *Adr1p* transcription factor, are qualitatively different is novel. In fact, in the case of the *Pho4p* activator, it is clear that no chromatin remodeling occurs when utilizing the DNA binding domain only (40). As for the *Gal4p* transcription factor, it appears that derivatives lacking the activation domain are still able to reconfigure nucleosomes on episomal DNA, although to a lesser extent (22, 44). In this latter case, the chromatin alterations observed in the absence or in the presence of the activation domain are equal but quantitatively different. In the case of *Adr1p*, however, we find that the DNA binding and the activation domains are able to induce two qualitatively different types of structural modification at the *ADH2* promoter in its natural chromosomal location.

The existence of two steps in the process of chromatin remodeling during derepression, one of which occurs in the absence of the transcription factor activation domain, suggests that at least two functions can be attributed to *Adr1p*. First, the protein reconfigures nucleosomes in the immediate vicinity of its binding site allowing the basal promoter elements to assume the most appropriate structure for the subsequent activation. Second, the protein recruits the transcription machinery through its activation domain, allowing mRNA accumulation. Therefore, a DNA binding factor plays a role in overcoming nucleosome-exerted repression as a prerequisite for transcriptional activation and not as a trivial consequence of RNA polymerase II recruitment, as suggested (20, 21). A recent study of the *CHAI* promoter in yeast has shown that mutants defective in TBP function are still able to remodel chromatin (24): based on the proposal of a two-step mechanism in the response to acidic activators *in vivo* by TBP (45), the authors suggest that remodeling of the nucleosome covering the TATA sequence would precede the formation of a stable preinitiation complex. Our results strongly favor this model. The two distinct nucleosome modifications, characterizing the *ADH2* promoter, are also in agreement with a recent work demonstrating an ordered recruitment of transcription and chromatin remodeling factors in the case of a cell cycle-regulated promoter (46).

In a speculative model of control of gene expression at the level of transcription initiation, the existence of two separate events could represent an energy saving mechanism. Each event would offer an opportunity for regulation and, in case of unfavorable conditions, the activation of a specific gene could be blocked after the earliest step of derepression.

The separation between the two steps will be useful in the search for factors involved in the two types of structural modification. We have investigated the role of the *ADR6/SWI1*

gene, a member of the SWI/SNF protein complex, in wild type chromosomal *ADH2* derepression and found that it is not required for nucleosome modification and transcription. This result is in contrast with previous work showing *ADR6/SWI1* involvement in *ADH2* expression (35–37). The discrepancy could be due to the different derepressing conditions used: here, we utilized low (0.05%) glucose for a few hours, whereas in the previous works, either glycerol (35, 36) or glycerol, ethanol, and sucrose for at least 20 generations (37) were utilized. The requirement of *SWI1* in the constitutive *ADH2* expression, driven by insertion of Ty elements (35), can be explained as follows: the UAS1 sequence, where *Adr1p* binds, is no longer adjacent to the upstream borders of the nucleosome -1 , because of the inserted elements, and the function of the activator becomes weakened.

It could be argued that the simplest situation is the one in which a DNA binding transactivator works alone to drive nucleosome reconfiguration, whereas in the presence of a more complex promoter architecture, the requirement of additional protein systems is fundamental. An easy prediction would be that in higher eukaryotes, the majority of the genes require one of the numerous chromatin remodeling complexes recently characterized (6, 47, 48).

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**Two Distinct Nucleosome Alterations Characterize Chromatin Remodeling at the
Saccharomyces cerevisiae ADH2 Promoter**

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