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 an 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 *CHAP* 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 (MATa, SWI/ADR6, ura3–52, leu2–801, his3–200, ade2–101, trp1–Δ1, his3–Δ200, leu2–Δ1), CY88 (MATa, same as CY26 except *swi1::LEU2*, JSY112 (MATa, same as CY26 except *adr1::LEU2*), and EPY10 (MATa, same as CY26 except *adr1::LEU2*, *swi1::LEU2*).

*The Journal of Biological Chemistry* Vol. 275, No. 11, Issue of March 17, pp. 7612–7618, 2000

Printed in U.S.A.

© 2000 by The American Society for Biochemistry and Molecular Biology, Inc.
Role of Adr1p Domains in Nucleosome Remodeling

Yeasts 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 with lithium acetate (28).

Plasmids—The Adr1p derivatives used were as follows: pADR1 ATP3, consisting of the first 172 amino acids of Adr1p inserted in pRS314 (CEN6, ARS3, and TRP1); pADR1 ATP2-AD1, 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 ade1 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 analysis (30).

Cells exponentially growing (A600 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 mM 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 mM 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‘-GTGGTGGACCTTA-ACGACTTGCGCTAA-C-3‘) specific for the ADH2 gene (from +710 to +864 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 palindrome 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 carrying a deletion 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 ADH22 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 acids; MOPS, 4-morpholinepropanesulfonic acid; UAS, upstream activating sequence.

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: Taq1-HindIII fragment, 102 base pairs. Each group of octals represents a family of multiple overlapping nucleosomes, the borders of which were mapped at high resolution (25).
Role of Adr1p Domains in Nucleosome Remodeling

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, and SWI1-sw1. 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 SWI1/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-swi1 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 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 redressed 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-
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 ADHIII 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 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 nucleosome. 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. 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 Adr1p 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 DNA binding domain fused to the 238-aa ADR1 minigene. The 181-aa construct contains the DNA binding domain; the 238-aa construct contains the DNA binding domain fused to the 43-amino acid activation domain; and the 1323-aa construct contains the DNA binding domain fused to the 238-aa ADR1 minigene containing the DNA binding domain fused to the 43-amino acid activation domain. The 181-aa construct contains the DNA binding domain; the 238-aa construct contains the DNA binding domain fused to the 43-amino acid activation domain; and the 1323-aa construct contains the DNA binding domain fused to the 238-aa ADR1 minigene containing the DNA binding domain fused to the 43-amino acid activation domain.

**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 BamHI and HindIII (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 32P DNA probe. Lane N contains deproteinized pFA DNA reacted in vitro with MN, BamHI, and HindIII. 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.
Role of Adr1p Domains in Nucleosome Remodeling

Fig. 6. Chromatin remodeling driven by full-length Adr1p is characterized by two kinetically distinct MN 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, and 2 units/0.25 ml (U)), deproteinized, and digested with BanHI and HindIII (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 to a 32P-labeled pFA DNA reacted in vitro with MN, BanHI and HindIII. The three relevant nucleosomes, the schematic map of which is shown in Fig. 1, are represented as oval, 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 derepressing conditions in which the transcription is on.

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 oval, 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 ADR1 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.

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 Gα1p 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 cromatin 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 become weakened. 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 overcoming nucleosome-exerted repression. Specifically, 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 (47, 48).

Acknowledgments—We thank E. T. Young for providing strains and comments, F. Co-ferr and J. Velona for helpful discussion, and G. Camilloni and G. Micheli for the invaluable help with computer facilities.

REFERENCES

1. Grunstein, M. (1990) Trends Genet. 6, 395–400
2. van Holde, K. E., and Zlatanova, J. (1996) BioEssays 18, 697–700
3. Wold, P. A., and Kurumizaka, H. (1998) Prog. Nucleic Acids Res. 61, 379–422
4. Grunstein, M. (1997) Nature 389, 349–352
5. Kadonaga, J. T. (1998) Cell 92, 367–371
6. Workman, J. L., and Kingston, R. E. (1998) Annu. Rev. Biochem. 67, 545–579
7. Almer, A., Rudolph, A., Hinnen, A., and Horz, W. (1986) EMBO J. 5, 2689–2696
8. Perez-Ortin, J. E., Estruch, F., Matalanna, E., and Franco, L. (1987) Nucleic Acids Res. 15, 6937–6956
9. Fedor, M. J., and Kornberg, R. D. (1989) Mol. Cell. Biol. 9, 1721–1732
10. Anezrod, J. D., Reagan, M. S., and Majors, J. (1993) Genes Dev. 7, 857–869
11. Cavalli, G., and Thoma, F. (1993) EMBO J. 12, 4603–4613
12. Winston, F., and Carlson, M. (1992) Trends Genet. 8, 387–391
13. Cairns, B. R., Lorch, Y., Li, Y., Lacomis, L., Erdjument-Bromage, H., Tempst, P., Laurent, B., and Kornberg, R. D. (1996) Cell 87, 1249–1250
14. Marcus, G. A., Silverman, N., Berger, S. A., Horuchi, J., and Guarente, L. (1994) EMBO J. 13, 4807–4815
15. Grant, P. A., Duggan, L., Cote, J., Roberts, S. M., Brownell, J. E., Candau, R., Ohba, R., Owen-Hughes, T., Allis, C. D., Winston, F., Berger, S. L., and Workman, J. L. (1997) Genes Dev. 11, 1640–1650
16. Roberts, S. M., and Winston, F. (1997) Genetics 147, 451–465
17. Tsukiyama, T., Palmer, L., Landel, C. C., Shiloach, J., and Wu, C. (1999) Genes Dev. 13, 686–697
18. Pollard, K. J., and Peterson, C. L. (1998) BioEssays 20, 771–780
19. Holstege, F. C. P., Jennings, E. G., Wyrick, J. J., Lee, T. I., Hengartner, C. J., Green, M. R., Golub, T. R., Lander, E. S., and Young, R. A. (1998) Cell 95, 717–728
20. Gaudreau, L., Schmid, A., Blaschke, D., Pfahme, H., and Horz, W. (1997) Cell 89, 55–62
21. Ptashne, M., and Gann, A. (1997) Nature 386, 569–577
22. Stafford, G. A., and Morse, R. H. (1997) J. Biol. Chem. 272, 11526–11534
23. Price, A. T., Hwang, Y.-P., McDonnell, D. P., and O’Malley, B. W. (1991) J. Biol. Chem. 266, 18179–18187
24. Moreira, J. M. A., and Holmgren, S. (1998) EMBO J. 17, 6025–6038
25. Verdone, L., Camilloni, G., Di Mauro, E., and Caserta, M. (1996) Mol. Cell. Biol. 16, 1978–1988
26. Denis, C. L., Ciriaci, M., and Young, E. T. (1981) J. Mol. Biol. 148, 355–368
27. Young, E. T., Saario, J., Kacherovsky, N., Chao, A., Sloan, J. S., and Dombeck, K. M. (1998) J. Biol. Chem. 273, 24080–24083
28. Ito, H., Fukuda, Y., Murata, K., and Kimura, A. (1983) J. Biol. Chem. 258, 854–860
29. Verdone, L., Cesari, F., Denis, C. L., Di Mauro, E., and Caserta, M. (1997) J. Biol. Chem. 272, 30828–30834
30. Wu, C. (1980) Nature 86, 854–860
31. Venditti, S., and Camilloni, G. (1994) Mol. Gen. Genet. 242, 100–104
32. Schmitt, M. E., Brown, T. A., and Trumpower, B. L. (1990) Nucleic Acids Res. 18, 3091–3092
33. Sluseter, J., Yu, J., Cox, D., Chan, R. L. V., Smith, M., and Young, E. T. (1986) Mol. Cell. Biol. 6, 4532–4536
34. Eisen, A., Taylor, W. E., Blumberg, H., and Young, E. T. (1988) Mol. Cell. Biol. 8, 523–530
35. Taguchi, A. K. W., and Young, E. T. (1987a) Genetica 116, 523–530
Role of Adr1p Domains in Nucleosome Remodeling

36. Taguchi, A. K. W., and Young, E. T. (1987b) Genetics 116, 531–540
37. Peterson, C. L., and Herskowitz, I. (1992) Cell 68, 573–583
38. Chiang, Y.-C., Komarnitsky, P., Chase, D., and Denis, C. L. (1996) J. Biol. Chem. 271, 32359–32365
39. Happel, A. M., Swanson, M. S., and Winston, F. (1991) Genetics 128, 69–77
40. Svaren, J., Schmidt, J., and Hersh, W. (1994) EMBO J. 13, 4856–4862
41. McAndrew, P. C., Svaren, J., Martin, S. R., Hersh, W., and Goding, C. R. (1998) Mol. Cell. Biol. 18, 5818–5827
42. Thukral, S. K., Tavianini, M. A., Blumberg, H., and Young, E. T. (1989) Mol. Cell. Biol. 9, 2360–2369
43. Balasubramanian, B., and Morse, R. H. (1999) Mol. Cell. Biol. 19, 2977–2985
44. Morse, R. H. (1993) Science 262, 1563–1566
45. Stargell, L. A., and Struhl, K. (1996) Trends. Genet. 12, 311–315
46. Cosma, M. P., Tanaka, T., and Nasmyth, K. (1999) Cell 97, 299–311
47. Tsukiyama, T., and Wu, C. (1997) Curr. Opin. Genet. Dev. 7, 182–191
48. Travers, A. A. (1999) Cell 96, 311–314
Two Distinct Nucleosome Alterations Characterize Chromatin Remodeling at the
Saccharomyces cerevisiae ADH2 Promoter
Ernesto Di Mauro, Steven G. Kendrew and Micaela Caserta

J. Biol. Chem. 2000, 275:7612-7618.
doi: 10.1074/jbc.275.11.7612

Access the most updated version of this article at http://www.jbc.org/content/275/11/7612

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

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

This article cites 48 references, 22 of which can be accessed free at
http://www.jbc.org/content/275/11/7612.full.html#ref-list-1