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Common Chromatin Architecture, Common Chromatin Remodeling, and Common Transcription Kinetics of Adr1-Dependent Genes in *Saccharomyces cerevisiae*†

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ABSTRACT: The chromatin structure of several *Saccharomyces cerevisiae ADR1*-dependent genes was comparatively analyzed in vivo in order to evaluate the role of promoter architecture in transcriptional control. In repressing conditions (high glucose) a nucleosome particle always obstructs the TATA box, immediately adjacent to an upstream-located nucleosome-free region containing a cluster of Adr1 binding sites. Upon derepression the TATA box-containing nucleosome is destabilized according to a mechanism shared by all of the genes studied. The transcription factor Adr1 is always required for the observed chromatin remodeling. mRNA accumulation of all of the genes analyzed is strongly delayed in the absence of the acetyltransferase Gcn5 and is decreased in the presence of a temperature-sensitive Esa1 mutant. The results suggest that a defined promoter chromatin structure, controlled by DNA conformational features, is relevant for the activation of coregulated genes.

The recent development of genome-wide analyses allows comparative studies aimed at deciphering the function of the DNA sequences determined. The application of the chromatin immunoprecipitation methodology (*1*) to microarray-based analysis (genome-wide location analysis; *2*−*5*) leads to the unraveling of complex regulatory networks (*6*, *7*). In simple organisms such as *Saccharomyces cerevisiae*, the availability of mutants allows the integration of the information derived from genome-wide location analyses with the genetically determined phenotypes (*8*).

Regulation of gene expression generally depends on the presence of protein complexes acting as coactivators or corepressors that assist promoter-specific transcription factors. These protein complexes belong to one of two major groups, depending on the enzymatic activity of their catalytic subunit: ATP-dependent chromatin remodeling complexes (*9*−*12*) and histone covalent modifiers (*13*−*15*). Some promoters are mainly dependent on the first group of protein complexes and others on the second group. Certain promoters, as, for instance, those regulated by the cell cycle, require the cooperation of both types of chromatin modification complexes (*16*, *17*). Therefore, it appears that each promoter has a specific chromatin organization that requires the involvement of defined enzymatic activities capable of regulating its transcriptional state.

While information on factor and cofactor requirements for the control of promoters is accumulating rapidly, a genome-wide analysis of nucleosome positioning on gene regulatory regions is lacking. This information is required for the understanding of the rules governing the basic process leading to the buildup of chromatin architecture and to factor recruitment.

We report the comparative analysis of the chromatin organization of a group of functionally heterogeneous *S. cerevisiae* genes which are normally repressed by glucose and require, to be derepressed under low glucose conditions, the activity of the same transcriptional activator, the Adr1 protein. The results show that the analyzed genes are characterized by a conserved promoter architecture, which drives the repression state and, eventually, chromatin remodeling and transcriptional activation.

EXPERIMENTAL PROCEDURES

Yeast Strains and Media. S. cerevisiae strains used in this study are as follows: CY26 (wild type), *MAT*α, *ura3-52*, *lys2-801*^{a}, *ade2-101*^{o}, *trp1-*Δ*1*, *his3-*Δ*200*, *leu2-*Δ*1*; JSY112 (*adr1*), same as CY26 except *adr1::LEU2*; YDS2 (wild type), *MAT***a**, *trp1-1*, *his3-11,15*, *ade2-1*, *leu2-1*, *can1-100*; WJY139 (*gcn5*), same as YDS2 except *gcn5::URA3*; LPY3431 (wild type), *MAT***a**, *his3*Δ*200*, *leu2-3,112*, *trp1*Δ*1*, *ura3-52*; and LPY3430 (*esa1* temperature sensitive), same as LPY3431 except *esa1::HIS3*, *esa1-L327S::URA3* (kindly provided by L. Pillus).

Yeast strains were grown in YPD medium (1% yeast extract, 2% bactopeptone, 3% glucose). To obtain derepression of the various promoters, 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.

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Enzymes. All nucleases were purchased from Roche. Zymolyase 100T was purchased from Seikagaku Corp.

Chromatin Analysis. The analysis of nucleosome positioning was performed by micrococcal nuclease (MN) digestion of spheroplasts coupled with the indirect end-labeling procedure (*18*). Cells exponentially growing (A_{600} 0.3 OD/mL) in 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 β-mercaptoethanol). Incubation with zymolyase (0.01 mg/OD) was for 20 min at room temperature. 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 $CaCl_2$, 50 mM NaCl, 100 μg/mL nystatin) in order to permeabilize cell membranes for the subsequent treatment with MN (*19*). Incubation with MN was for 15 min at 37 °C, and the reaction was stopped with 5 mM EDTA and 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 TBE buffer and transferred to nitrocellulose filters. Southern blot and hybridization were performed by standard procedures. The DNA fragments to be utilized as probes for the hybridization were obtained by PCR amplification starting from a couple of converging oligonucleotides and purified yeast genomic DNA as template. A 149 bp restriction fragment (*Bam*HI−*Kpn*I) from plasmid ER1-1 (pUC18 containing the catalase A gene) was used for the chromatin analysis of the *CTA1* gene. For all of the other genes the oligonucleotides used were as follows: 5′GCTAGGCTTGCTAGTTATATG3′ and 5′CGAAGCTTTGTCTGGATCATT3′ for the *POX1* gene (starting positions 249 and 336, respectively); 5′CCAACAAGATTAAGGTTGGGC3′ and 5′AGCGAACTCGTCTTGATCCTT3′ for the *POT1* gene (starting positions 406 and 623, respectively); 5′GTACCATGAAATCCACTGTTATG3′ and 5′CTGCATATGCGTTGTACCAA3′ for the *ADY2* gene (starting positions 610 and 755, respectively).

RNA Analysis. Aliquots containing the same number of cells were collected by centrifugation, and total RNA was prepared as previously described (*20*). After spectrophotometric determination of the RNA amount present in each aliquot, 10 μg of RNA was loaded onto 1.2% agarose−MOPS gels containing formaldehyde and ethidium bromide.

Northern blot analysis was performed by standard procedures. The DNA fragments to be utilized as probes for the hybridization were obtained by PCR amplification starting from a couple of converging oligonucleotides and purified yeast genomic DNA as template. A 1207 bp restriction fragment (*Bam*HI−*Eco*RI) from plasmid ER1-1 (pUC18 containing the catalase A gene) was used for the CTA1 gene. For all of the other genes the oligonucleotides used were as follows: 5′GCTAGGCTTGCTAGTTATATG3′ and 5′CGAAGCTTTGTCTGGATCATT3′ for the *POX1* gene (starting positions 249 and 336, respectively); 5′CCAACAAGATTAAGGTTGGGC3′ and 5′AGCGAACTCGTCTTGATCCTT3′ for the *POT1* gene (starting positions 406 and 623, respectively); 5′GTACCATGAAATCCACTGTTATG3′ and 5′CTGCATATGCGTTGTACCAA3′ for the *ADY2* gene (starting positions 610 and 755, respectively).

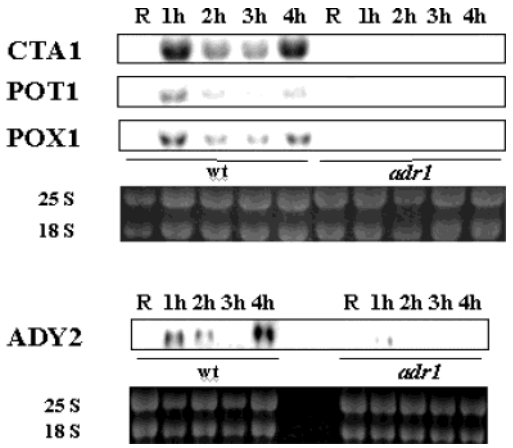

FIGURE 1: Northern analysis of *ADR1* dependence. mRNA accumulation for four genes, among those showing the highest dependence on Adr1, was analyzed by Northern: two independent filters were hybridized with the indicated genes. Cells were collected in repressing conditions (3% glucose, R) or at different times after the culture was shifted to derepressing conditions (0.05% glucose). wt, CY26, and *adr1*, JSY112. 25S and 18S indicate the two major yeast rRNA species.

RESULTS

Northern Analysis of ADR1 Dependence. The transcription factor Adr1 was originally shown to be required for the activation of the *ADH2* gene, coding for the enzyme alcohol dehydrogenase (*21*), and was later found to be involved also in the biogenesis and function of peroxisomes (*22*). A recent genome-wide expression profile analysis has revealed that the transcription of many more genes than previously expected depends on this protein, when the cells are grown in low glucose medium (*23*).

We have performed a Northern analysis in order to confirm the dependence on *ADR1* of a group of genes shown by microarrays to be strongly downregulated. The results of this analysis for four genes belonging to two different functional classes (*CTA1*, *POX1*, and *POT1*, involved in peroxisomes biogenesis and β-oxidation, and *ADY2*, involved in nitrogen utilization) are shown in Figure 1. A wild-type strain and its isogenic *adr1* were analyzed in both repressing (R = 3% glucose) and derepressing conditions (0.05% glucose) at different times. For all of the genes tested, transcription is clearly dependent on the presence of a functional Adr1 protein. Activation occurs during the first hour after the shift to derepressing conditions; then mRNA accumulation drops to increase again at later times. A similar biphasic pattern has been described for the activation of the glucose-repressed gene *SUC2* (*24*).

Chromatin Analysis of ADR1-Dependent Promoters. To compare the chromatin organization of the selected genes, we performed a micrococcal nuclease (MN) treatment of nystatin-permeabilized spheroplasts from wild-type and *adr1* cells in both repressing and derepressing conditions. This analysis was previously used to map the position of relevant nucleosomes on the *ADH2* gene (*25*). Figure 2 shows the results for the *CTA1*, *POX1*, *POT1*, and *ADY2* genes. According to standard definitions, protected regions of 140−200 bp are assumed to represent the footprint of a histone

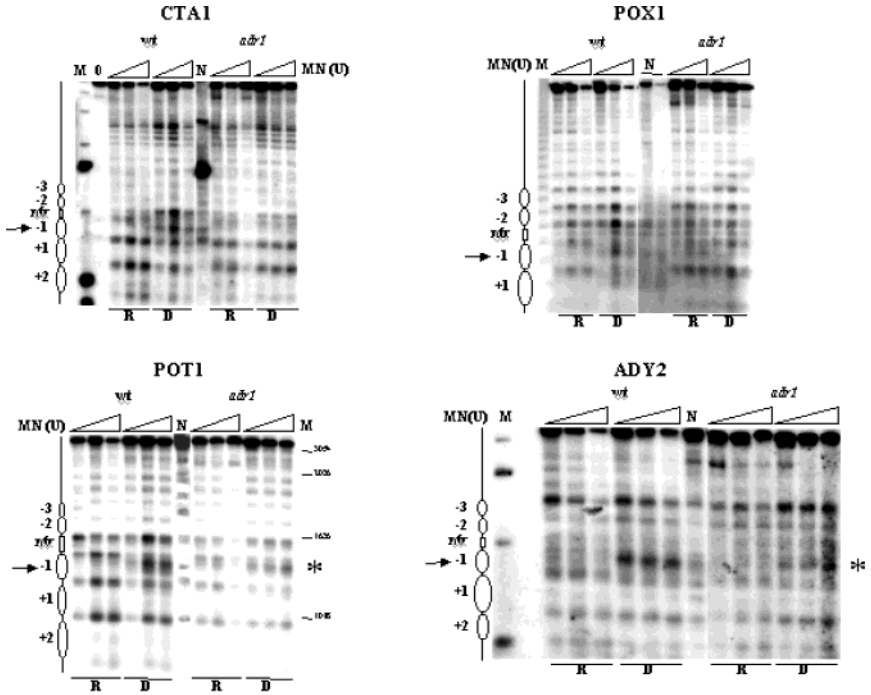

FIGURE 2: Chromatin analysis of *ADR1*-dependent promoters. MN treatment of nystatin permeabilized spheroplasts was performed with the following amounts of MN: 0.2, 0.4, and 0.8 units/0.25 mL for each condition. N, purified yeast genomic DNA treated in vitro with MN. After purification the samples were treated with restriction endonucleases as follows: *Bam*HI for *CTA1*, *Eco*RI for *POT1* and *ADY2*, and *Hin*dIII for *POX1*. The DNA probes used for the hybridization are described in Experimental Procedures. Nucleosomes are represented by ovals. Nfr, nucleosome-free region; R, repressing conditions (3% glucose); D, derepressing conditions (0.05% glucose, 1 h); M, molecular weight marker: 1 kb DNA ladder for *CTA1* and *ADY2* and 123 bp DNA ladder for *POX1* and *POT1*. The black arrow points to the nucleosome where the major chromatin remodeling event occurs. wt, CY26, and *adr1*, JSY112.

Table 1: Length of Nucleosome-Protected Areas and Nuclesome-Free Regions As Determined by the Low-Resolution Analysis Shown in Figure 2[a]

	length (bp)				
	nucl −3	nucl −2	nfr	nucl −1	nucl +1
CTA1	200	140	80	195	170
POX1	165	140	140*	160	175
POT1	195	150	115	180	180
ADY2	150	160	100	150	190

[a] By using semilogarithmic plots the length of the relevant areas of protection, assumed to represent the footprint of a histone octamer and, therefore, interpreted as positioned nucleosomes (*26*), was determined for every gene analyzed. Nucleosome-free regions (nfr) are deduced because of the shorter length. The only exception to the rule is represented by the POX1 promoter (nfr = 140*): in this case, the nucleosome-free region is clearly inferred by the presence of a MN cut in the middle of the area.

octamer and are therefore interpreted as positioned nucleosomes (*26*). Shorter regions are accordingly interpreted (*26*) as nucleosome-free regions (see Table 1). In repressing conditions (3% glucose), in every gene tested a nucleosome-free region upstream of the TATA box-containing nucleosome is observed. This region is characterized by the presence of several putative Adr1 binding sites (see Discussion). In the wild-type strain, chromatin remodeling involving mainly the −1 nucleosome (containing the TATA box), and to a lesser extent the +1, is constantly observed in derepressing conditions (0.05% glucose).

As described in the case of the *ADH2* gene (*25*), the chromatin remodeling is strongly dependent on the presence of a functional Adr1 protein, as shown by the lack of increased accessibility to MN in the *adr1* strain (Figure 2). For two genes, namely, *POT1* and *ADY2*, a residual *ADR1*-independent chromatin reorganization is observed (Figure 2, asterisks in the two bottom panels), suggesting the involvement in the remodeling event of additional proteins, possibly specific for each promoter. In the case of the *POT1* gene, a role for the Isw2 complex in nucleosome positioning has been reported (*27*). We tested whether the Isw2 or the Isw1 complexes are involved in chromatin remodeling at the *POT1* promoter by analyzing a set of isogenic strains (wild type, *isw1*, *isw2*, *isw1-isw2*). We found that in repressing conditions (3% glucose) the position of the −1 and the +1 nucleosomes is shifted downstream in the *isw2* and *isw1-isw2* strains, as expected (*27*). Nevertheless, in derepressing conditions (0.05% glucose) chromatin remodeling of these two nucleosomes can be observed exactly as in the wild-type strain, suggesting that neither of the complexes is required for the activation process (data not shown).

Alignment of the ADR1-Dependent Promoters. The similarity of the chromatin organization observed among the four promoters tested (Figure 2) allows the alignment of the inferred nucleosome maps. The map of the *ADH2* promoter previously determined (*25*) is also included. Figure 3 shows the results of this alignment. It is evident that the nucleosome distributions on the considered promoters, from the nucleosome-free region (nfr) rightward, are perfectly aligned, thus underlying the relevance of nucleosome positioning in the organization of a consensus promoter architecture. Instead, no alignment is possible along the position of the ATG sequence or the TATA box. The fact that these promoters modify such common architecture according to a morphologically and kinetically similar mechanism links this architecture to gene regulation.

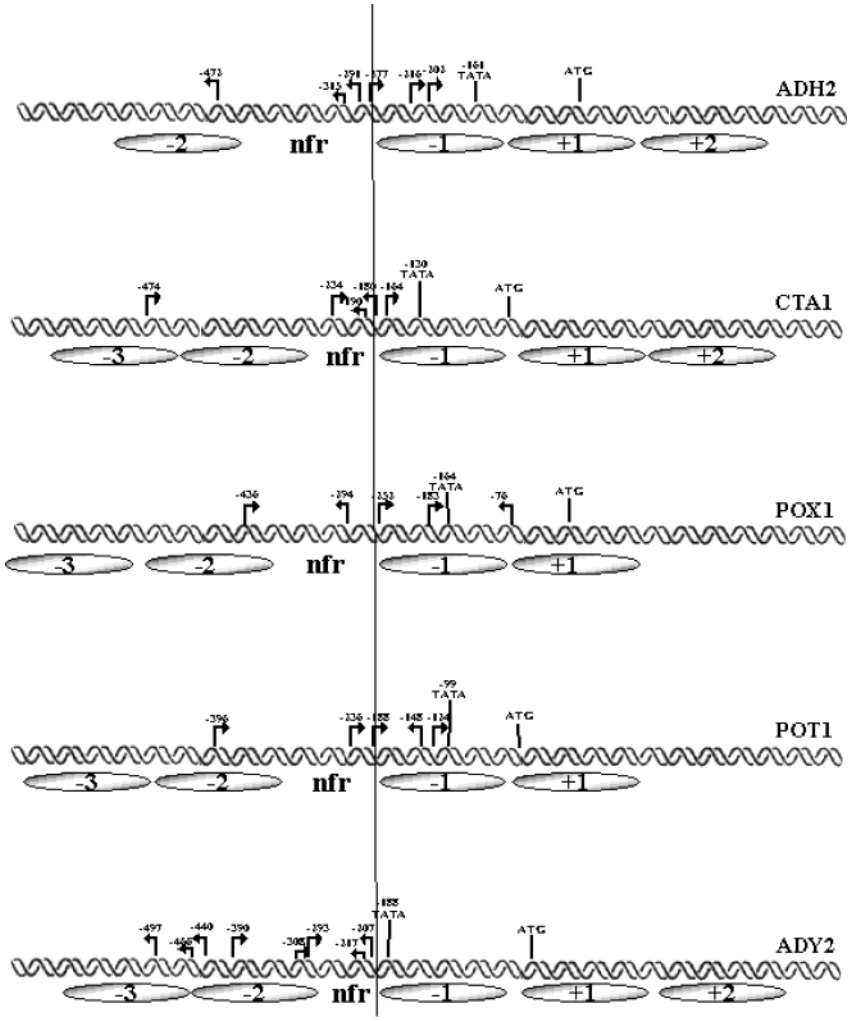

FIGURE 3: Alignment of the *ADR1*-dependent promoters. Nucleosome maps derived from the chromatin analysis shown in Figure 2. The *ADH2* map was adapted from previous data (*25*). The arrows indicate the position and the direction of the putative Adr1 binding sites. The consensus derived from the analysis of 50 genes having the highest degree of *ADR1* dependence is T/GT/CGGRG (*23*). We have also taken into consideration the shorter consensus YGGRG and GGRG, based on Adr1 binding analysis (*48*). The alignment is centered on the position of the upstream borders of the TATA box-containing nucleosome −1.

From close inspection of the position and of the orientation of the putative Adr1 binding sites (indicated by arrows in Figure 3) in the nucleosome-free region, a correlation of these sites with the different types of nucleosome remodeling observed (Figure 2) emerges, as follows: (i) a palindrome consisting of two Adr1 binding sites is present immediately upstream of the −1 nucleosome when the remodeling is exclusively dependent on *ADR1* (*ADH2*, *CTA1*, and *POX1* genes); (ii) multiple sites not forming a palindrome but having the same orientation are present when the remodeling depends only partly on *ADR1* (*POT1* and *ADY2* genes).

The Kinetics of mRNA Accumulation of ADR1-Dependent Genes Are Influenced by the Lack of Gcn5 and Esa1. Because of the similarity in the structural organization of the ADR1-dependent promoters, we tested whether the molecular mechanism underlying the chromatin remodeling observed during their derepression would be conserved. We reason that if the same type of molecules are involved, in the absence of a particular cofactor or in the presence of defined mutants the effect on mRNA accumulation would be the same for all of the genes tested. Previous work on the *ADH2* promoter has shown that the acetyltransferase Gcn5 is required to ensure the correct timing for the appearance of mRNA (*28*). We analyzed by Northern the mRNA accumulation for the *CTA1*, *POX1*, *POT1*, and *ADY2* genes in a couple of isogenic strains, wild type and *gcn5*. The results are shown in Figure 4A. For all of the genes tested, lack of Gcn5 causes a strong delay in transcription: in the wild-type strain mRNA is already abundant 30 min after the shift to derepressing conditions and then decreases with time to reappear later on (according to the biphasic pattern shown in Figure 1), whereas in the *gcn5* deletion mutant the immediate answer to glucose depletion is abolished. We then asked whether the requirement for the acetyltransferase Esa1, observed in the case of the *ADH2* promoter, would stand also in the case of the ADR1-dependent genes under investigation. The results for the *CTA1* and *POT1* genes are shown in Figure 4B: mRNA accumulation is already decreased in the *esa1* mutant at the permissive temperature and is almost abolished at the nonpermissive temperature. The same behavior was observed also for the *ADY2* and *POX1* genes (data not shown).

We hypothesize that transcriptional activation occurs via a common molecular mechanism for all of the genes possessing the same type of promoter chromatin organization.

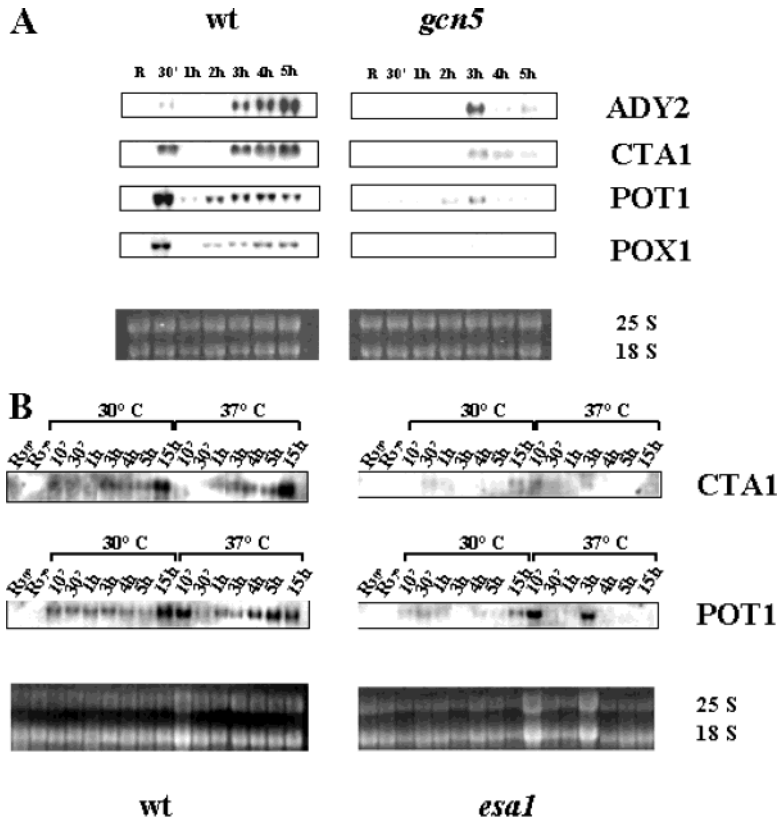

FIGURE 4: Kinetics of mRNA accumulation of ADR1-dependent genes in the absence of Gcn5 and Esa1. (A) A couple of isogenic strains, wild type (YDS2) and *gcn5* (WJY139), were analyzed by Northern to reveal the appearance of mRNA during derepression of the *CTA1*, *POX1*, *POT1*, and *ADY2* genes. Cells were collected in repressing conditions (3% glucose, R) or at different times after the culture was shifted to derepressing conditions (0.05% glucose). 25S and 18S indicate the two major yeast rRNA species. (B) A couple of isogenic strains, wild type (LPY3431) and temperature-sensitive *esa1* (LPY3430), were analyzed by Northern. Cells were collected in repressing conditions (3% glucose, R) at both 30 and 37 °C or at different times after the culture was shifted to derepressing conditions (0.05% glucose) at both 30 and 37 °C.

DISCUSSION

A Common Chromatin Architecture Characterizes ADR1-Dependent Promoters. Regulation of gene expression at the level of transcription is intimately linked to promoter chromatin organization. This has been clearly demonstrated by the involvement of protein complexes such as ATP-dependent remodelers and/or histone tail covalent modifiers in regulating the structure and the accessibility of the relevant sequences to the transcription machinery.

Because of the lack of genome-wide nucleosome assays allowing global phenotypic analysis with respect to mutations in genes coding for chromatin-related proteins, we have undertaken a gene-by-gene study of the structure of several promoters regulated by the transcription factor Adr1. This protein has been implicated in the chromatin remodeling event that accompanies derepression of the *ADH2* gene (*25*, *28*−*31*). Upon binding to its recognition site (UAS1) in the vicinity of the *ADH2* promoter, consequent to the shift of the cells to low glucose conditions, Adr1 is able to induce the sliding of the TATA box containing nucleosome by few nucleotides, by means of its transcription−activation domain (*30*). Since a recent microarray analysis has shown that many functionally heterogeneous *S. cerevisiae* genes are indeed regulated by Adr1 (*23*), we asked whether the chromatin remodeling observed at the *ADH2* promoter is a peculiarity of that gene or whether Adr1 has a more general function in nucleosome repositioning. The results of this gene-by-gene study reveal that in derepressing conditions Adr1-dependent remodeling of the TATA box containing nucleosome occurs (Figure 2) in every promoter. This suggests that the presence of Adr1 binding sites in the vicinity of the basal promoter is necessary and sufficient to drive a chromatin change in different sequence contexts. Interestingly, we found that in repressing conditions all of the promoters possess common chromatin architecture, characterized by the presence of a nucleosome-free region immediately upstream of the TATA box-containing nucleosome bound to be remodeled during activation. By using episomal constructs, it was shown that the presence of a nucleosome-free region upstream of the core promoter is a critical requirement for transcription in vivo (*32*). In the case of the chromosomal *ADR1*-dependent genes, this architectural feature results in the perfect alignment of all of the promoters (Figure 3). A number of yeast promoters have been shown to possess in the inactive state a nucleosome on the TATA box which is altered during the activation process (refs *33*−*35* and references cited therein). Nevertheless, the new finding here is the fact that the molecular mechanism and the cofactor involvement (see the Gcn5 and Esa1 requirement, Figure 4) in the activation of all of the *ADR1*-dependent genes tested are conserved, presumably as a consequence of the common promoter structure. A recent report on the SUC2 gene (*24*), which is glucose repressed just as the Adr1-dependent genes are, shows the same double requirement for Gcn5 and Esa1, suggesting that this is possibly a more general feature of glucose-repressible genes.

What Determines the Common Chromatin Architecture? The result concerning the perfect alignment of all of the promoters is unexpected given that the only sequence that these functionally heterogeneous genes have in common at their 5′-end is a cluster of Adr1 binding sites which, in repressing conditions, are not occupied by the protein (*23*, *36*). One possible explanation for this finding could be provided by the presence on the promoters of all the analyzed *ADR1*-dependent genes of a protein or protein complex that controls the position of the TATA box-containing nucleosome or helps in maintaining the upstream region free of nucleosome particles. Alternatively, it is the DNA sequence information itself that is responsible for the nucleosome positioning. Both explanations have been repeatedly discussed in the past (*26*, *35*, *37*). Evidence for proteins with this role in chromatin organization was reported (*38*−*42*). In the intergenic region of the two divergent promoters *GAL1* and *GAL10* a binding site for the factor Y-Grf2-Reb1 exists which is necessary and sufficient for the maintenance of a nucleosome-free region encompassing sites for the transcription factor Gal4 (*38*, *39*). However, the relevance of Reb1 in determining nucleosome positioning was criticized (*43*, *44*). A role for Abf1 and ORC in the control of nucleosome positioning at the level of a replication origin was shown (*40*, *42*). A similar role for the pleiotropic transcription factor Rap1 has also been described (*41*).

Our sequence analysis of the 5′ region of the five *ADR1*-dependent promoters tested (data not reported) has not revealed a sequence consensus for a common factor that could explain the observed conserved chromatin organization. We have identified several potential factor binding sites which are specific for each promoter or for defined subsets: oleate-responsive elements (ORE) are present in the *CTA1*, *POX1*, and *POT1* promoters, but the heterodimeric Oaf1-Pip2 transcription factor, which binds to it, is active only in derepressing conditions (*45*); a binding site for the Cat8 protein (CSRE = carbon source responsive element) is present only in the *ADH2* and *ADY2* 5′ region (*23*).

When the promoter sequences of 50 genes showing the highest degree of *ADR1* dependence were analyzed in the search for consensus binding sites, the highest scoring motif that emerged was T/GT/CGGRG (*23*), corresponding to the Adr1 binding site. Nevertheless, as demonstrated by chromatin immunoprecipitation experiments, Adr1 does not bind to its DNA sites in repressing conditions (*23*, *36*). An alternative possibility is that architectural proteins, with scarce recognizable sequence specificity, are responsible for the maintenance of the common promoter structure. Not having found evidence of proteins involved in nucleosome positioning in the group of *ADR1*-regulated genes examined, we hypothesize that an influence is exerted by DNA features such as curvature and flexibility. Both properties are related to the repetition of AA tracts in phase with the B DNA periodicity and could be responsible for the common promoter architecture of this functionally heterogeneous group of genes having in common the possibility to be activated by the same transcription factor Adr1. These DNA features were already shown to be relevant in determining in vivo nucleosome positioning (*46*, *47*). A similar conclusion was drawn from the analysis of the *ILV1* promoter (*43*).

In this perspective, DNA conformational information would strongly contribute to the logics of transcriptional regulation.

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