Nucleosome Position-Dependent and -Independent Activation of *HIS7* Expression in *Saccharomyces cerevisiae* by Different Transcriptional Activators

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ARO4 and *HIS7* are two tandemly orientated genes of *Saccharomyces cerevisiae* that are transcribed into the same direction. The *ARO4* terminator and the *HIS7* promoter regions are sensitive to *Micrococcus* nuclease (Mnase) and separated by a positioned nucleosome. The *HIS7* promoter is target for the transcription factors Gcn4p and Bas1p/Bas2p that activate its transcription upon amino acid starvation and purine limitation, respectively. Activation of the *HIS7* gene by Gcn4p overexpression but not by Bas1p/Bas2p releases an ordered nucleosome distribution to yield increased Mnase sensitivity throughout the intergenic region. This remodeling is *SNF2* dependent but mostly *GCN5* independent. Accordingly, *SNF2* is necessary for the Gcn4p-mediated transcriptional activation of the *HIS7* gene. *GCN5* is required for activation upon adenine limitation by Bas1p/Bas2p. Our data suggest that activation of *HIS7* transcription by Gcn4p and Bas1p/Bas2p is supported by a nucleosome position-dependent and -independent mechanism, respectively. Whereas Gcn4p activation causes Swi/Snf-mediated remodeling of the nucleosomal architecture at the *HIS7* promoter, the Bas1p/Bas2p complex presumably activates in combination with Gcn5p-dependent histone acetylation.

Regulation of transcription involves specific transcription factors and multiple complexes that act directly or indirectly by changing the nucleosomal structure of the chromatin (2). In general, 147 bp of DNA coil around a histone octamer that together form the nucleosome, the smallest unit of chromatin structure. Tight interactions between promoter DNA and/or upstream activation sequences with the histone octamer can repress gene transcription and result in silent or basally transcribed genes (26, 40). The process of destabilizing nucleosomes in order to facilitate access for the general transcription machinery to promoters requires not only sequence specific transcription factors but also cooperation with histones and cofactors that help to remodel or displace nucleosomes (31). Genetic studies and subsequent biochemical analyses have identified a number of factors required for transcriptional regulation in the context of chromatin structure. The multitude of these proteins functions as part of large complexes, such as the Swi/Snf complex; the RSC (for remodeling the structure of chromatin), Ada, and SAGA complexes; and the Srb/mediator/holoenzyme complex (32, 28).

Swi/Snf was purified as a 2-MDa protein complex that is composed of the Swi1, Swi2/Snf2, Swi3, Snf5, and Snf6 proteins plus five additional polypeptides (7). Swi/Snf can bind to nucleosomes and DNA, thereby creating loops in nucleosomal arrays or naked DNA, respectively, bringing distant sites into close proximity (4). In an ATP-dependent fashion it can repo-

sition nucleosomes in *cis* on the same DNA molecule (47), with the *SWI2/SNF2* gene encoding the DNA-dependent ATPase activity (33). Transcriptome analyses with a *swi2/snf2* mutant strain have revealed that Swi/Snf controls transcription of only 6% of all *Saccharomyces cerevisiae* genes and that the control is exerted at the level of individual promoters rather than over chromosomal domains (39). Swi/Snf thereby both activates and represses transcription of different target genes. Recruitment of Swi/Snf to specific promoters by DNA-binding regulatory proteins, as well as targeting of the complex by the general transcription machinery, has been suggested (48).

Two high-molecular-mass Ada-Gcn5 complexes (0.8 and 1.8 MDa) have been biochemically isolated from *S. cerevisiae* and shown to be able to acetylate nucleosomes both in vitro and in vivo at specific lysine residues of histones H3 and H4 (14). Both complexes share Gcn5p, Ada2p, and Ada3p, whereas the larger one additionally contains Spt proteins (Spt20p, Spt3p, Spt8p, and Spt7p) and is called SAGA (for Spt/ADa/Gcn5 acetyltransferase). Gcn5p comprises the histone acetyltransferase (HAT) activity to acetylate histones in promoter regions in a manner that is correlated with Gcn5p-dependent transcriptional activation and HAT activity in vitro (23). SAGA interacts with both TATA-binding protein and acidic transcriptional activators such as the herpesvirus VP16 activation domain and yeast Gcn4p, suggesting that the complex might also have a transcriptional adaptor function for some promoters (15).

HIS7 is a typical housekeeping gene of *S. cerevisiae*, and its gene product is necessary for the biosynthesis of the amino acid histidine as well as purines (21). Its expression has previously been shown to be activated by two major stimuli: the lack of amino acid availability and limitation of external purines

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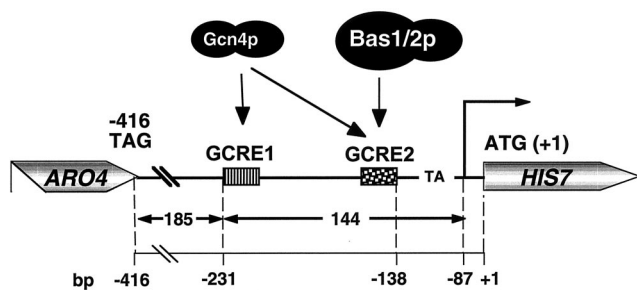
FIG. 1. Diagram of the yeast *HIS7* promoter. Two binding sites for Gcn4p, GCRE1 and GCRE2, are functional parts of the promoter. GCRE2 additionally functions as recognition element for the heterodimeric transcription factor Bas1p/Bas2p. TA at position -120 reflects the putative TATA element. The arrow at -87 indicates the initiator element of the major transcriptional start site. The positions are relative to the translational start codon AUG, which is shown as $+1$.

(37). Starvation for amino acids triggers increased expression of Gcn4 protein that in turn activates transcription of amino acid biosynthetic genes (general control of amino acid biosynthesis) (6, 18). Upon starvation, two Gcn4p-recognition elements (GCREs) within the *HIS7* promoter are targeted by the transcription factor, thereby mediating a sixfold increase in *HIS7* expression (Fig. 1) (37). The absence of purines causes a twofold increase in *HIS7* expression and is mediated by the heterodimeric transcription factor Bas1/Bas2p that shares a common binding site with Gcn4p, the TATA-proximal GCRE2 (Fig. 1). This activation requires both factors Bas1p and Bas2p (37). Nevertheless, both activation pathways act independently of each other and, moreover, are additive upon simultaneous amino acid and purine limitation (37). Located 416 bp upstream of the *HIS7* gene is the *ARO4* gene, encoding an enzyme that is required for the biosynthesis of aromatic amino acids (22). Both transcriptional units are separated by a positioned nucleosome in between them (45).

A recent study on the field of chromatin elucidated how different chromatin-related coactivators act together on the regulation of genes (28). However, up to now there has been little information about how chromatin-modifying complexes such as Swi/Snf or HATs contribute to the regulation of a single gene under different activation systems. Whether different transcriptional activators require different, possibly independently acting, chromatin-modifying activities or whether they compete in these activities is poorly understood. We show here that two different transcription factors, although sharing a common binding site, use different chromatin-modifying activities to achieve their goal of transcriptional gene activation. Transcriptional *HIS7* activation by Gcn4p requires the Swi/Snf-dependent rearrangement of nucleosomes, whereas Bas1/Bas2p-mediated *HIS7* transcription requires the presence of Gcn5p HAT activity.

MATERIALS AND METHODS

Yeast strains and growth conditions. The yeast strains and their genotypes used here are listed in Table 1. For high *GCN4* expression, strains were transformed with plasmid p238 harboring a constitutively highly expressed *GCN4* allele (27). To prepare strains FY1553 and FY1360 starving for histidine by using its analog 3-aminotriazole (3-AT), histidine prototrophy was initially regained by transforming these strains with plasmid pRS303 (*HIS3*) (35), yielding strains

RH2561 and RH2563. Strains RH2569 and RH2570 with deletions of *GCN5* and translational *Phis7-lacZ* fusions were obtained by transforming RH1615 and RH1616 with the deletion cassette of plasmid pME1236. The inserted KAN^R marker gene was then removed by using the Cre-loxP recombination system as described earlier (17).

Strains were cultivated in minimal-vitamin medium (25). Adenine repression was achieved by supplementation with 0.3 mM adenine (37).

Plasmids. The plasmids used in the present study are listed in Table 1. Plasmid pME1236, carrying the *gcn5::KAN^R* deletion cassette, was created by replacing the *GCN5* coding sequence with the kanamycin resistance cassette by a PCR-based three-step cloning strategy, with plasmids pME1234 and pME1235 as intermediates. The other plasmids used here have been described previously.

Genomic chromatin preparation and nuclease digestions. These methods have been described previously (42). Biodyne B nylon membranes (Pall, Dreieich, Germany) were used for Southern transfer. Probes were labeled by the random primer method (11).

Indirect end labeling. Chromosomal DNA from the nuclease digestion was digested with *Xba*I and *Mlu*I and fractionated on 1.2% agarose gels. The fractionated DNA was blotted on a Hybond-N nylon membrane by the alkaline blotting method and then hybridized with a radioactively labeled 250-bp PCR amplicon, generated with oligonucleotides HIS7-CHR1 (5′-GAGATTAAAGA AATTGTCAGA-3′) and HIS7-CHR2 (5′-CAAGTATTGAGGAGAAATGGT A-3′), with annealing just downstream of the *Xba*I site. A DNA ladder consisting of multiples of 256 bp was used for calibration (43).

RNA analysis. Total RNA from *S. cerevisiae* was isolated according to the method of Cross and Tinkelenberg (9). For Northern hybridization analysis, 20 μg of total RNAs were separated on a formaldehyde-agarose gel and transferred to a positively charged nylon membrane (Biodyne B; Pall) by capillary blotting. Hybridization with specific DNA probes was performed after ^{32}P labeling of the respective DNA fragments with the Prime-It II DNA labeling kit from Stratagene. The *HIS7* probe was generated with oligonucleotides HIS-OL1 (5′-GTG GTAACCTACAGTCACTAACC-3′) and HIS-OL2 (5′-CCGATCGATACTTT ATCAGCACC-3′), and the *ACT* probe was generated with oligonucleotides ACT-OL1 (5′-GCTGCTTTGGTTATTGATAACGG-3′) and ACT-OL2 (5′-C ACTTGTGGTGAACGATAGATGG-3′). Signal intensities were visualized by autoradiography and quantified by using a BAS-1500 Phospho-Imaging scanner (Fuji).

β-Galactosidase assay. β-Galactosidase activities were determined by using permeabilized yeast cells and the fluorogenic substrate 4-methylumbelliferyl-β-D-galactoside (MUF) as described previously (21).

RESULTS

Positioned nucleosomes flank an open *HIS7* promoter. The nucleosomes within the yeast *HIS7* promoter were mapped by *Micrococcus* nuclease (Mnase) treatment of crude nuclei extracts. In a first approach, the *HIS7* promoter was investigated for cells grown without amino acid starvation and in the absence of adenine. Under these conditions the cells express only low amounts of Gcn4p, and the lack of adenine results in Bas1/Bas2p-mediated *HIS7* expression. Since this expression level of *HIS7* corresponds to the expression in minimal medium, it is here referred to as "basal" expression. We have used this nomenclature in the discussion below. Crude nuclear extracts from overnight cultures were partially digested with Mnase and treated further as previously described (42). The comparison of the Mnase-treated chromatin with that of naked DNA revealed that under these conditions the *HIS7* promoter with its binding sites for Gcn4p and Bas1p/Bas2p lays in a region sensitive to the nuclease (Fig. 2, region A of lanes 1 to 4). This extended sensitive region A is flanked on both sites by protected DNA stretches, here referred to as positioned nucleosomes N1 and N2. Thus, N1 covers the transcriptional initiation site of the *HIS7* promoter. The *ARO4* gene is located 417 bp upstream of *HIS7* and is transcribed in the same direction (21). The site that defines where the poly(A) tail is added during *ARO4* mRNA 3′-end processing (pA) lies within nu-

TABLE 1. Yeast strains and plasmids used in this study

Strain or plasmid	Genotype or description[a]	Reference or source
Strains		
FY1353	*MATα his3Δ200 ura3-52 leu2Δ1 lys2-173R2*	39
RH2561	Same as FY1353 but with plasmid pRS303 (*HIS3*)	This work
FY1354	*MATα his3Δ200 ura3-52 leu2Δ1 lys2-173R2 gcn5Δ::HIS3*	39
FY1360	*MATa his3Δ200 ura3-52 leu2Δ1 lys2-173R2 snf2Δ::LEU2*	39
RH2563	Same as FY1360 but with plasmid pRS303 (*HIS3*)	This work
FY1352	*MATa his3Δ200 ura3-52 leu2Δ1 lys2-173R2 gcn5Δ::HIS3 snf2Δ::LEU2*	39
RH1381	*MATa ura3-52 aro3-2 gcn4-101*	37
RH1616	*MATa ura3-52 aro3-2 his7-lacZ gcn4-101*	37
RH2029	*MATa ura3-52, gcn4-101 bas1-2 bas2-2*	45
RH2569	*MATa ura3-52 aro3-2 his7-lacZ gcn5Δ::loxP*	This work
RH2570	*MATa ura3-52 aro3-2 his7-lacZ gcn4-101 gcn5Δ::loxP*	This work
RH1619	*MATa ura3-52 aro3-2 his7(mut GCRE1)-lacZ gcn4-101*	37
RH1622	*MATa ura3-52 aro3-2 his7(mut GCRE2)-lacZ gcn4-101*	37
RH1830	*MATa ura3-52 aro3-2 his7(mut ABS)-lacZ gcn4-101*	36
Plasmids		
pBluescript II SK(+)	Commercial cloning vector with polylinker; *lacZ*	Stratagene
pUG6	*loxP-kanMX-loxP* module with Kan[r] marker flanked by *TEF2* promoter and terminator, Amp[r]	17
pME1768	*loxP-kanMX-loxP* module from pUG6 in pBluescript II SK(+)	
pME2034	774-bp *GCN5* 3′ fragment in pBluescript II SK(+)	This work
pME2035	862-bp *GCN5* 5′ fragment in pBluescript II SK(+)	This work
pME2036	1.6-kb *kanMX* fragment from pME1768 in between *GCN5* 5′ and 3′ fragment	This work
pSH47	*cre* recombinase expression vector	17
pRS303	Yeast integrative vector; *lacZ HIS3*	35
pCB159	p*GAL-BAS1* (*Spe*I-*Mlu*I 3.6-kb fragment) in B656 backbone	44
p238	YCp50 carrying a *GCN4* allele with mutated uORFs[b]	27

[a] Kan[r], kanamycin resistance; Amp[r], ampicillin resistance.
[b] uORFs, upstream open reading frames.

cleosome N2 that separates the *ARO4* gene from the *HIS7* promoter. The sensitive region further upstream encodes a Zaret/Sherman element that is required for efficient *ARO4* 3′-end processing (36). A further nucleosome N3 is positioned at the very 3′-end of the *ARO4* open reading frame.

Although Gcn4p expression is rather low during growth with sufficient supply of amino acids, binding to the GCREs in the *HIS7* promoter could be responsible for the accessible chromatin detected. We therefore tested the nucleosome positions of cells lacking any Gcn4p with a *gcn4Δ* strain. However, they display the same Mnase-sensitive promoter region flanked by nucleosomes on both sides, as shown for cells with low Gcn4p levels (Fig. 2, lanes 5 to 10). A positioned nucleosome N4 in the 3′ end of the *ARO4* open reading frame is absent in cells with low Gcn4p levels but present in Gcn4p-lacking cells.

Gcn4p overexpression releases the ordered chromatin structure at the intergenic region. Cells starving for amino acids increase expression of Gcn4p, the master regulator of the general control system of amino acid biosynthesis. For experimental purposes, amino acid starvation is often induced by adding 3-AT or 5-methyltryptophan to the growth culture. 3-AT and 5-methyltryptophan are amino acid analogs acting as false feedback inhibitors of the histidine or tryptophan biosynthetic pathways, respectively. The general control network can also be activated by using a constitutively highly expressed *GCN4* allele (27). For Gcn4p-dependent *HIS7* activation both induction methods were shown to increase its transcription (21, 37).

Basing our studies on the *HIS7* promoter chromatin structure of the basally expressed gene as described above (for low Gcn4p, Bas1/Bas2p activation caused by absence of adenine),

we now addressed the question of whether activation of *HIS7* expression mediated by Gcn4p overexpression affects the *HIS7* promoter chromatin structure.

To answer this question, we investigated the chromatin structure with a strain that harbors a highly expressed *GCN4* allele and therefore constitutively expressing the general control system (27). Cells were cultivated in the presence of adenine so that the Gcn4p activation of *HIS7* is not affected by Bas1/Bas2p. In striking contrast to the ordered *HIS7* promoter chromatin structure described for the basal expression (Fig. 2, lanes 1 and 2), which is also observed for growth with 0.3 mM adenine (Fig. 3, lanes 1 and 2), several Mnase-sensitive bands in close proximity appear throughout the entire *ARO4-HIS7* intergenic region as a result of Gcn4p overexpression (Fig. 3, lanes 5 and 6). Unambiguous localization of positioned nucleosomes that protect the DNA from the nuclease activity in that region is no longer feasible. We conclude that Gcn4p overexpression alters the chromatin structure at the *ARO4-HIS7* intergenic region specifically and at the same time causes increased *HIS7* and *ARO4* transcription. We conclude that the *HIS7* activation by high Gcn4p levels is associated with severe changes of the local *ARO4-HIS7* chromatin structure, whereas the regulation of *HIS7* transcription mediated by Bas1p/Bas2p seems not to be associated with any remodeling of nucleosomes.

Different Bas1/2p levels do not affect the nucleosome positioning at the *HIS7* promoter. We investigated further the impact of Bas1/2p on *HIS7* chromatin by varying its cellular levels. To eliminate conflicting data due to the Gcn4p response, the experiments were performed with yeast of a *gcn4Δ*
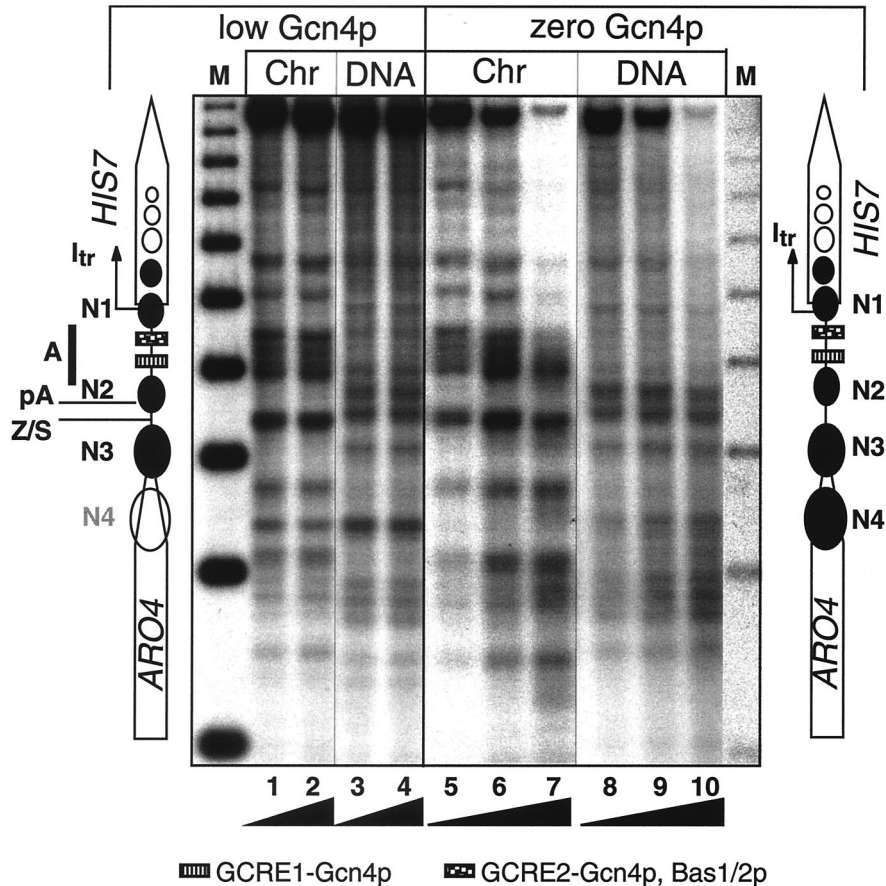
FIG. 2. Chromatin structure at the *ARO4-HIS7* intergenic region. Mnase protection experiments are shown with chromatin from strains FY1353 (wild-type *GCN4*) or RH1381 (*gcn4Δ*). Cells were cultivated in the absence of exogenous adenine. Control Mnase digests of naked DNA are shown in lanes 3, 4, 8, 9, and 10. Lanes M on the far left and right show a 256-bp DNA-ladder (42). Black ramps at the bottom indicate increasing amounts of Mnase and exactly represent 20 and 40 U for lanes 1 and 2; 20, 40, and 60 U for lanes 5, 6, and 7; 4 and 8 U for lanes 3 and 4; and 4, 8, and 12 U for lanes 8, 9, and 10, respectively. The diagrams on the left and right show the location of the open reading frames of *ARO4* and *HIS7*. Protected regions are shown as black ovals N1-N3 (here referred to as positioned nucleosomes). The binding sites for Gcn4p and Bas1p/Bas2p are indicated at the bottom. The site of *HIS7* transcriptional initiation is indicated by I$_{tr}$. pA, poly(A) addition site of the *ARO4* mRNA; Z/S, Zaret/Sherman element required for efficient *ARO4* mRNA 3′-end processing.

genetic background. Cells were cultivated in the absence of adenine in the growth medium to induce Bas1/2p-dependent *HIS7* transcription. The chromatin mappings of strain RH2029 (*bas1Δ* and *bas2Δ*), which lacks any Bas1/Bas2 protein, revealed a distribution of Mnase-sensitive and Mnase-protected sites identical to the *BAS1/2* wild-type strain RH1381 (Fig. 4, lanes 1 to 3 and 4 to 6). The absence of Bas1/2p does not cause changes at the *HIS7* promoter chromatin structure. We conclude that the heterodimeric complex Bas1/2p is not required to keep the *HIS7* promoter and its *cis*-acting elements accessible. Overexpression of *BAS1* is toxic for cell viability. We analyzed *HIS7* transcription and its promoter chromatin structure after a 2-h burst of *BAS1* expression to test whether toxicity correlates with a changed nucleosome structure. High *BAS1* expression was achieved with a high-copy-number plasmid, pCB159 (Table 1), carrying *BAS1* under the control of the *GAL1* promoter, induced by shifting cells to medium with galactose as single carbon source. Surprisingly, *HIS7* transcription is not only not affected by high Bas1p levels but in fact nearly prevented under these circumstances (data not shown).

The *HIS7* chromatin structure during *BAS1* overexpression remains unaffected and is identical to that of zero or low Bas1p levels, shown in lanes 10 to 12 of Fig. 4. Thus, Bas1p, in conjunction with Bas2p, neither requires nor triggers chromatin remodeling to fulfill its function at the *HIS7* promoter.

Remodeling of the *HIS7* promoter chromatin structure upon Gcn4p overexpression is *SNF2* dependent. Chromatin remodeling upon transcriptional repression or activation of a variety of genes requires a functional Swi/Snf complex. As described above, the chromatin structure of the *HIS7* promoter changes upon high Gcn4p expression. An influence of Swi/Snf on *HIS7* chromatin is therefore conceivable for the described Gcn4p-mediated chromatin change. By using Mnase protection experiments we investigated the *HIS7* promoter chromatin of cells with a *snf2Δ* background. We analyzed whether the ordered chromatin structure of the basal expressed *HIS7* promoter or the Gcn4p-dependent change in chromatin structure is Swi/Snf dependent. Experiments with the *snf2Δ* mutant strain grown in the presence of low Gcn4p levels show a *HIS7* promoter chromatin structure similar to the wild-type strain grown under
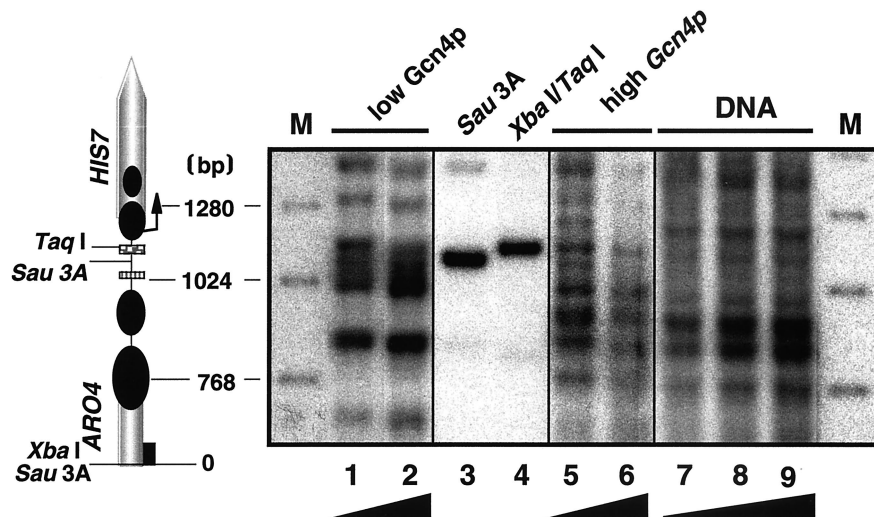
FIG. 3. Chromatin structure of the *ARO4-HIS7* intergenic region at low and high intracellular Gcn4p levels. Yeast cells were grown in the presence of 0.3 mM adenine to prevent Bas1p/Bas2p-mediated *HIS7* transcription. The Mnase protection pattern as described in Fig. 2 for cells with basal *HIS7* expression levels of strain FY1353 can be seen in lanes 1 and 2. Strain FY1353[p238] expresses constitutively high amounts of Gcn4p and yields a protection pattern as shown in lanes 5 and 6. In lanes 3 and 4, restriction digests with *Sau*3A and *Xba*I/*Taq*I show DNA fragments precisely located within the *HIS7* promoter. The diagram on the left and the black ramps at the bottom are as described for Fig. 2.

basal gene expression conditions (Fig. 5 A, compare lanes 1 and 2 with lanes 5 and 6). The positions of sensitive, as well as protected, regions within the intergenic region are similar for the *snf2* mutant and the wild-type *SNF2* strains. This suggests that the Swi/Snf complex is not involved in the establishment and maintenance of the defined *HIS7* promoter chromatin structure at low Gcn4p-levels (low Gcn4p, adenine limitation).

However, the basal transcription of *HIS7* is affected by the Swi/Snf complex, since in comparison to wild-type cells the *snf2*Δ strain showed a twofold increase in *HIS7* mRNA levels (Fig. 6, lanes 1 and 5). The mechanism of Swi/Snf action remains unsolved here. One possibility is that it actually binds as a repressor to the promoter or, alternatively, this might be the result of an indirect effect. However, the transition in
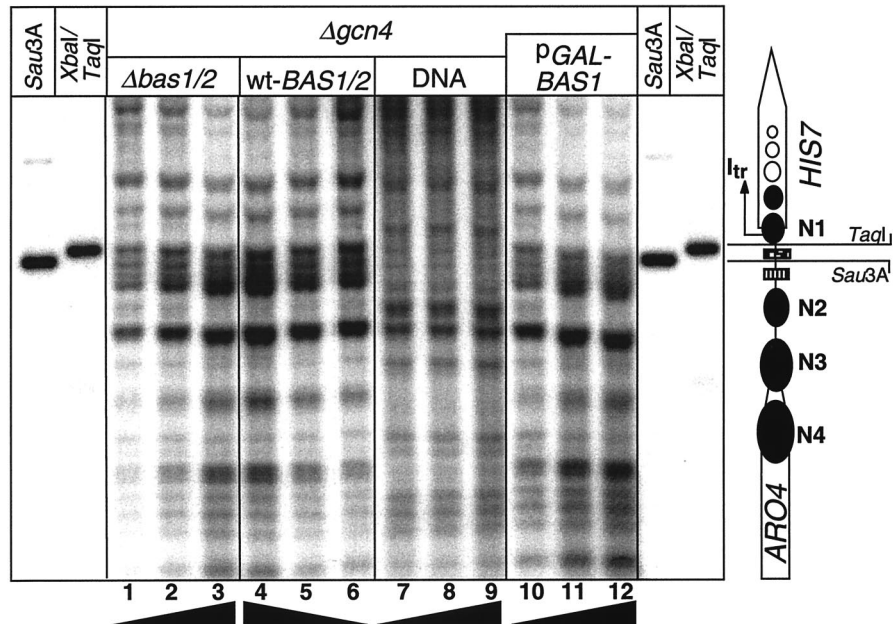

FIG. 4. Chromatin structure of the *ARO4-HIS7* intergenic region at various intracellular Bas protein levels. Yeast cells were grown in the absence of adenine. Lanes: 1 to 3, chromatin of strain RH2029; 4 to 6, chromatin of strain RH1381; 10 to 12, chromatin of strain RH1381 with the *BAS1* overexpression plasmid pCB159 (44). On this high-copy-number plasmid the *BAS1* open reading frame is fused to the *GAL1* promoter. After shifting from glucose to galactose medium, *BAS1* expression is strongly induced (44). The location of the promoter region is labeled by Southern hybridizations of chromosomal DNA digested with either *Xba*I/*Taq*I or *Sau*3A.
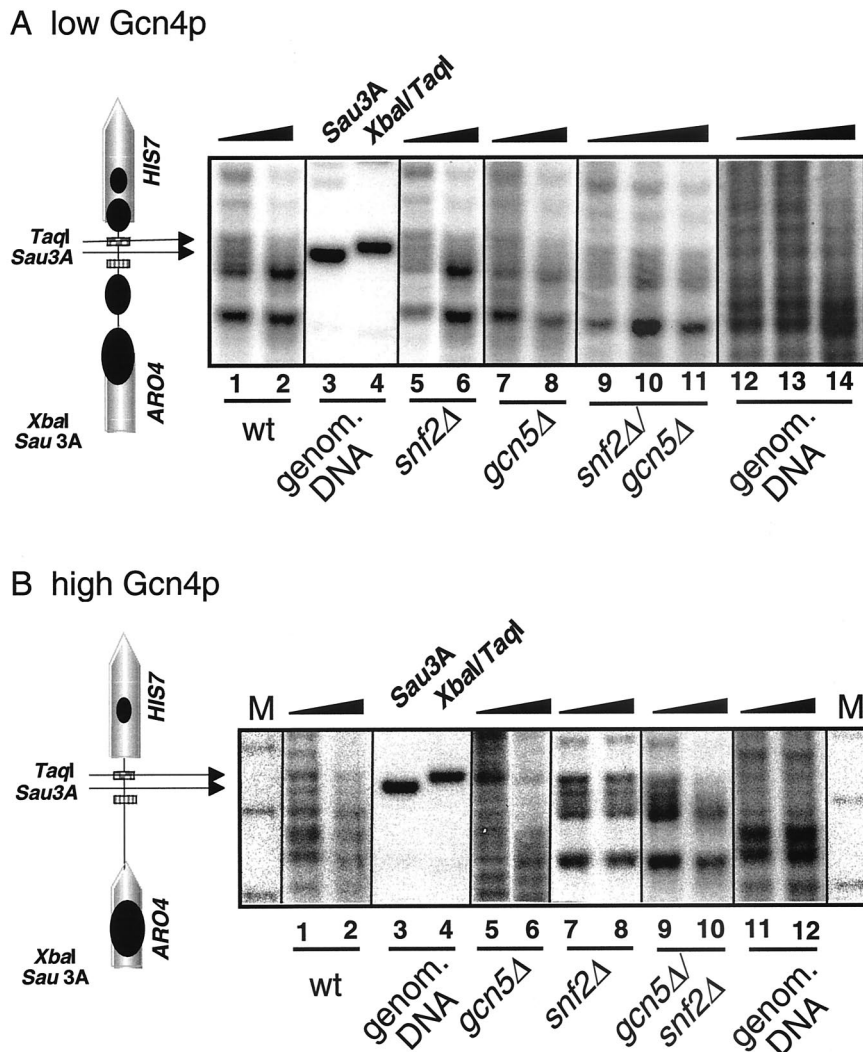
FIG. 5. Chromatin structure of the *ARO4/HIS7* intergenic region of strains deficient in nucleosome modifying activity. Yeast strains FY1360 (*snf2Δ*), FY1354 (*gcn5Δ*), or FY1352 (*snf2Δ/gcn5Δ*) were cultivated without exogenous adenine. Experiments were performed with strains expressing either low (A) or high (B) amounts of Gcn4p from wild-type *GCN4* (wt) or the additional *CGCN4* (constitutively expressed *GCN4*) allele of p238, respectively.

chromatin structure during high Gcn4p expression as described above is prevented in the *snf2Δ* background. In the absence of *SNF2*, the chromatin of the *HIS7* promoter remains in its ordered structure of the basal transcribed promoter (Fig. 5 B, lanes 1, 2, 7, and 8). Therefore, the Swi/Snf complex is essential for the nucleosomal change upon Gcn4p-overexpression. Consistent with this influence on chromatin, Gcn4p-dependent activation of *HIS7* transcription is almost absent in the *snf2Δ* background (Fig. 6, lanes 5 and 6).

GCN5 is only required for the Gcn4p-independent *HIS7* expression. Besides the remodeling of nucleosomes, histones are also targets for various modifications, including acetylation and/or deacetylation. Reversible acetylation of histones H3 and H4 has been shown to be associated with transcriptional gene activation. *GCN5* encodes a respective HAT activity and is essential for the function of the yeast HAT complexes SAGA and Ada.

We then tested whether *GCN5* is involved in the Gcn4p-mediated *HIS7* transcription. Northern hybridization with total

RNA of *gcn5Δ* mutant cells revealed that Gcn4p activation of *HIS7* transcription is similar to that of the *GCN5* wild-type strain (Fig. 6, lanes 3 and 4). Therefore, Gcn4p-dependent *HIS7* activation seems to be independent of Gcn5p-containing HAT complexes. However, since the double mutation *gcn5Δ snf2Δ* led to a much stronger reduction of *HIS7* transcription than *Δsnf2* by itself does, a synergistic contribution of both activities seems obvious. Chromatin mappings Furthermore, showed that the remodeling of the nucleosomes upon Gcn4p overexpression as described above also takes place in *gcn5Δ* mutant cells (Fig. 5B, lanes 5 and 6). Gcn4p-dependent *HIS7* gene expression and its associated nucleosome remodeling do not require functional Gcn5p-containing HAT complex. In contrast, the Bas1p/Bas2p-dependent basal *HIS7* transcription is halved in a *gcn5Δ* background (Fig. 6, lanes 1 and 3). The nucleosome mappings, depicted in Fig. 5A (lanes 7 and 8), show no changes in the absence of Gcn5p.

Gcn5p is required for the Gcn4p-independent activation of the *HIS7* promoter by adenine limitation. Low amounts of
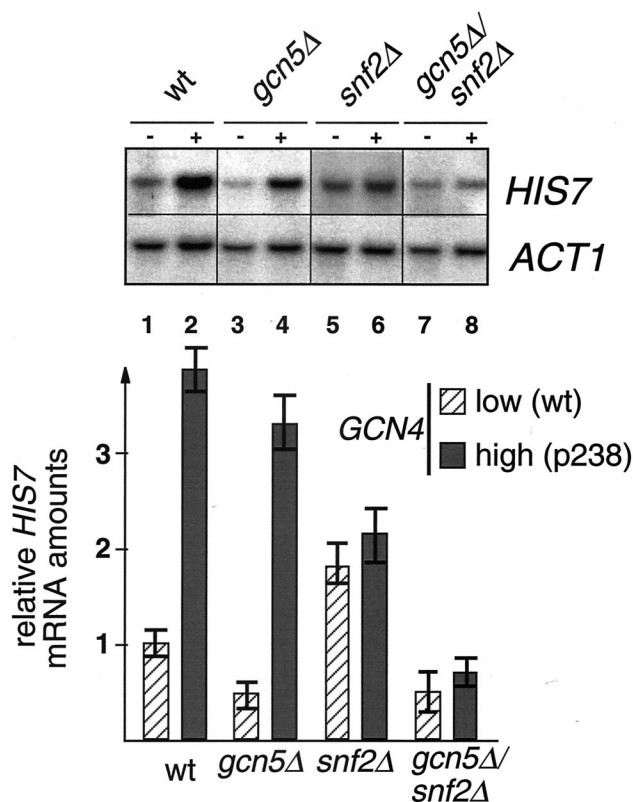
FIG. 6. *HIS7* mRNA levels in yeast cells expressing either a low or a high amount of Gcn4 protein after cultivation without exogenous adenine. Deletions of components required for chromatin modification are as indicated. Low Gcn4p amounts are expressed from the wild-type *GCN4* gene (−), whereas high Gcn4p amounts are expressed from plasmid p238 (+) for strains FY1353 (wt), FY1360 (*snf2D*), FY1354 (*gcn5D*), and FY1352 (*snf2Δ/gcn5D*). Four independent RNA isolations were hybridized twice in Northern experiments and then equalized to *ACT1* mRNA levels, resulting in the mean values given in the graph.
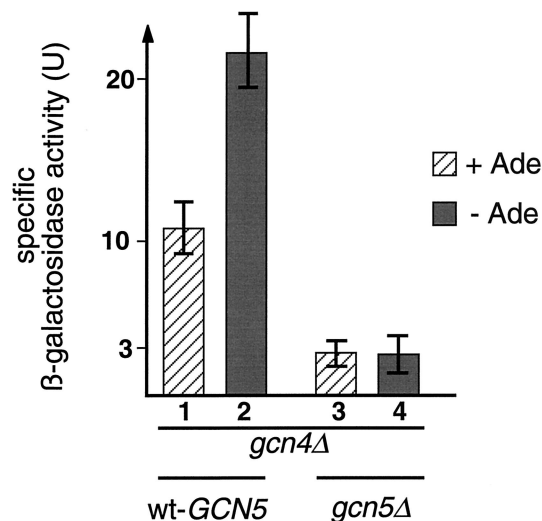

FIG. 7. *HIS7* promoter driving a *lacZ* reporter in cells defective in *GCN4*, as well as with defects in chromatin modification. The effects of external adenine on *his7-lacZ* expression for strains RH1616 (*GCN5*) and RH2570 (*gcn5D*) were measured as specific β-galactosidase activity. The graphs give average values of three individual enzyme assays performed with crude extracts from four independent cultures. Strains were cultivated either in adenine-deficient medium (−Ade) or in medium containing 0.3 mM adenine (+Ade). Specific β-galactosidase units correspond to nanomoles/(hour · milliliter · optical density at 546 nm)$^{-1}$.

Gcn4p are expressed in cells even during the absence of amino acid starvation. It is known that these low amounts are responsible for various promoters to maintain certain expression levels. To confirm that the Gcn5p-dependency of the basal *HIS7* expression is mediated through Bas1p/Bas2p and not through low Gcn4p, we tested the role of *GCN5* in the *gcn4Δ* background. Specific β-galactosidase activities of cells harboring a translational *his7-lacZ* fusion instead of the wild-type *HIS7* gene were measured to determine *HIS7* expression. The activity of the reporter gene is strongly reduced in cells harboring the *gcn5Δ* background. In the presence of adenine when *his7-lacZ* expression is low anyway, deletion of *GCN5* further reduces it to ca. 30% of that value (Fig. 7, lanes 1 and 3). Furthermore, any activation of *his7-lacZ* expression upon adenine limitation is prevented in a *gcn5Δ* background (Fig. 7, lane 4). This means that the Bas1p/Bas2p-dependent activation of *his7-lacZ* requires the HAT activity encoded from *GCN5* to enable increased expression upon adenine limitation.

In summary, the *HIS7* transcription is highly activated upon Gcn4p overexpression by mechanisms that require a functional Swi/Snf complex. This activation is accompanied by changes of

the promoter chromatin structure. This response of disruption or randomizing nucleosomes does not depend on *GCN5* but on *SWI2/SNF2*. In contrast, the Bas1p/Bas2p-mediated *HIS7* expression during purine limitation conditions takes place with the aid of Gcn5p. Nucleosome remodeling as in the case of Gcn4p overexpression was not detected.

DISCUSSION

We have shown here that two eukaryotic transcriptional activators that share a common *cis* element in the promoter of their common target gene *HIS7* require the activities of two different nucleosome-associated protein complexes. One of them, the jun-like transcription factor Gcn4p depends on the multiprotein complex Swi/Snf, leading to the remodeling of the promoter chromatin structure. The second factor, the heterodimeric complex Bas1p/Bas2p, requires Gcn5p/HAT complexes, whereas nucleosome remodeling does not take place (Fig. 8).

Activation by Gcn4p overexpression but not Bas1p/Bas2p induction alters an ordered chromatin structure at the *HIS7* promoter. The *HIS7* gene is an example for a yeast housekeeping gene that is regulated by two different and independent activation pathways. As a result of amino acid starvation, the transcription factor Gcn4p activates the transcription. In the absence of amino acid starvation, when yeast cells are not supplemented with purines and have to synthesize them de novo, the heterodimeric transcription factor Bas1p/Bas2p activates *HIS7* expression to a level that has been termed basal expression (3). Supplementation with adenine represses this
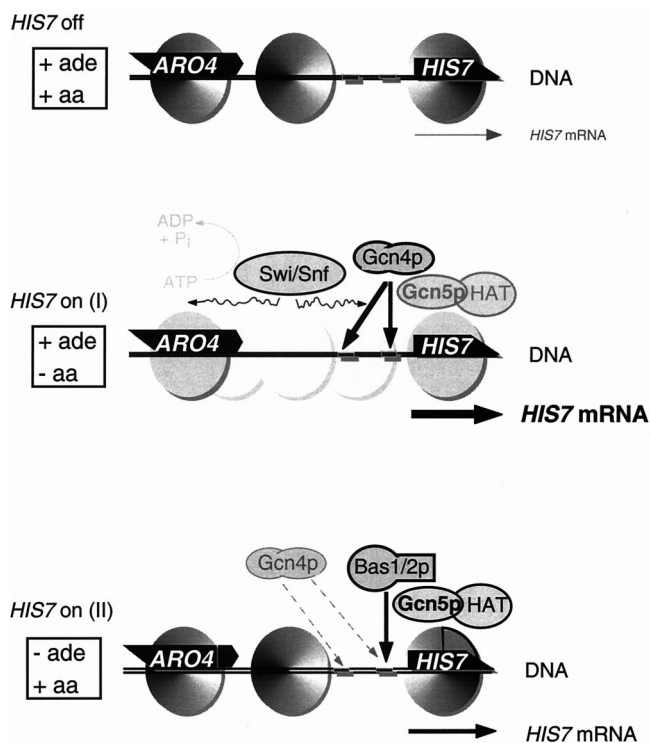
FIG. 8. Model of the alternative mechanisms of nucleosome-dependent transcriptional activation of yeast *HIS7* initiated by two different transcription factors. +ade/+aa, Growth in the presence of adenine and amino acids, that is, without any acid starvation; +ade/−aa, growth in presence of adenine but without amino acids, that is, with amino acid starvation; −ade/+aa, growth without adenine but in the presence of amino acids, that is, with purine starvation. Dark circles represent positioned nucleosomes with strong DNA-histone interactions.

basal expression to a lower level that corresponds to the *HIS7* expression of a *bas1Δ* or *bas2Δ* mutant strain (37).

Changes of chromatin structure at promoters during gene activation are a common phenomenon previously reported for numerous genes as, e.g., *PHO5*, *PHO8*, *SUC2*, *CHA1*, *HIS4*, *GAL10*, and *CUP1* (1, 8, 10, 12, 16, 26, 34). Activators that specifically increase gene expression comprise transcription factors of different DNA-binding motif classes, including the basic-helix-loop-helix activator Pho4p, the acidic Cys$_6$-Zn cluster activators Gal4p or Cha4p, and the basic-leucine-zipper Gcn4p.

Yeast *HIS7* is an example of a gene with a promoter chromatin structure that changes upon the activation by one but not the other physiological operating activator (Fig. 7). High Gcn4p levels change the chromatin structure within the *ARO4-HIS7* intergenic region and at the same time increase *HIS7* transcription. We assume that the simultaneous binding to GCRE1 and GCRE2 causes the observed nucleosome randomization or disruption at the locus. This would support the previously described synergistic nature of *HIS7* transcription by the binding of Gcn4p to two binding sites within the *HIS7* promoter (37). Different levels of Bas1/2p influence *HIS7* transcription (*BAS1* overexpression astonishingly even represses it) but do not affect the chromatin structure.

Gcn4p-dependent *HIS7* chromatin remodeling demands for a functional Swi/Snf-complex. Genome-wide expression analysis revealed that ca. 6% of all yeast genes are affected twofold or more by the inactivation of Swi/Snf. The affected genes are subdivided into two groups: one with reduced amounts of transcript and the other with an increased transcript level (20, 39). Remodeling by the Swi/Snf-complex can in general have two consequences. One is that repressive nucleosomes are removed from the promoter region, thereby enforcing gene activity. The other is that nucleosomes are positioned to promoter elements, leading to the repression of gene activity.

An active role of the Swi/Snf complex in Gcn4p-mediated activation of the yeast *HIS3* gene was previously described (29). However, a preferential accessibility for Mnase to the *HIS3* promoter was shown to be a general property of the DNA sequence and not mediated by the Gcn4p-binding site (24). Moreover, several studies have described direct interactions between transcription factors and Swi/Snf that support the recruitment of the remodeling activity to the promoter by binding the DNA-bound activator (30, 46, 48).

The chromatin of the noninduced *HIS7*-promoter is an accessible open one, sustaining weak *HIS7* expression (Fig. 7). This open structure is compatible with the Bas1p/Bas2p-mediated *HIS7* activation during adenine limitation and does not require further remodeling. However, this open configuration does not seem to be suitable for high *HIS7* expression at high Gcn4p levels. High *HIS7* expression levels caused by Gcn4p overexpression result in the randomization or disruption of nucleosomes within the *ARO4-HIS7* intergenic region. Mechanistically, this remodeling is coupled to the Swi/Snf complex.

The Bas1p/Bas2p-dependent adenine response on *HIS7* expression depends on a functional SAGA/Ada (Gcn5p) complex. The HAT activity of the SAGA or Ada complexes in yeast is encoded by *GCN5* and is necessary for the transcriptional activation of several genes (5), including both Gcn4p-regulated and Gcn4p-independent genes. Previous studies reported Gcn5p dependence for the Gcn4p activation of an artificial *PHO5* promoter that also harbors a binding site for Gcn4p and was therefore inducible by amino acid starvation. Moreover, the same *PHO5* promoter is activated upon phosphate limitation by the transcription factor Pho4p and yet independently of Gcn5p (41). Other genes that require a functional *GCN5* gene for the Gcn4p-dependent transcriptional activation are *HIS3*, *TRP3*, and *ILV1* (13). In contrast, the Gcn4p-dependent activation of the *HIS4* and *ARG4* genes has been shown to be strictly *GCN5* independent (13). In common with these latter genes the Gcn4p response of the *HIS7* promoter does not require *GCN5*. The Gcn4p-binding sites of the *HIS4*, *ARG4*, and *HIS7* promoters nearly perfectly matches the consensus sequence 5′-TGACTC-3′, whereas the *HIS3*, *TRP3*, and *ILV1* promoters possess weak Gcn4p-binding sites (19, 38). Possibly, the requirement for Gcn5p increases with the decreasing strength of the respective Gcn4p recognition element.

However, since *GCN5* is required for basal Bas1p/Bas2p-dependent *HIS7* transcription, the situation is complex for this promoter, enabling individual responses to different stimuli by using different mechanisms. Altogether, we demonstrated that the transcriptional regulation of the *HIS7* gene by two independent activation pathways uses different chromatin-modify-

ing machineries. Gcn4p changes the nucleosomal distribution at the *HIS7* promoter upon its overexpression. This process requires a functional Swi/Snf complex but no functional SAGA/Ada (Gcn5p) complex. Bas1p/Bas2p-dependent *HIS7* activation, in contrast, requires a functional SAGA/Ada (Gcn5p) complex but is not associated with chromatin remodeling. Astonishingly, this is possible even though both activators share a common *cis* element of the *HIS7* promoter. Chromatin immunoprecipitation studies shall further elucidate the recruitment of the cofactors to the *HIS7* promoter chromatin and their relationship to the bound transcription factors at the different stages of *HIS7* expression.

ACKNOWLEDGMENTS

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