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Multiple Factors Prevent Transcriptional Interference at the Yeast *ARO4-HIS7* Locus*

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Increased transcriptional activity may cause transcriptional interference in organisms with compact genomes such as the yeast *Saccharomyces cerevisiae*. Replacement of the yeast *ARO4* promoter by the stronger *ACT1* promoter increases *ARO4* transcription and simultaneously reduces the basal transcription of the downstream *HIS7* gene. The open reading frames of *ARO4* and *HIS7* are tandemly transcribed and are separated by 416 bp. In wild-type cells, a nuclease-resistant site suggests that the two genes are separated by a single positioned nucleosome. Transcriptional interference correlates with *Micrococcus* nuclease accessibility of this otherwise nuclease-resistant site. Deletion analyses of the region between the two open reading frames revealed that transcriptional interference increases upon removal of either parts of the *ARO4* 3′ end or *HIS7* promoter sequences. The abolishment of the Abf1p-binding site within the *HIS7* promoter significantly enhances transcriptional interference, resulting in a histidine auxotrophic strain. Our data suggest that the yeast cell prevents transcriptional interference by the combined action of efficient *ARO4* transcription termination, the positioning of a fixed nucleosome, and transcription factor binding to the *HIS7* promoter.

The arrangement of tandemly transcribed RNA polymerase II genes can jeopardize regulated transcription in a cell by a phenomenon called *transcriptional interference*. As consequence of elevated transcription of the upstream gene, transcription of the adjacent downstream gene might be diminished or even abolished. Transcriptional interference is favored by close proximity of genes that are only separated by short intergenic regions between the corresponding open reading frames (ORFs).[1] It was found in HeLa cells that two closely spaced α-globin genes in an artificial gene construct interfere with each other (1). In yeast, the cryptic promoter within the intron of the *ACT1* gene is occluded by transcription from the actual *ACT1* promoter at the 5′ end of the gene (2). We have described previously (3) that *HIS7* transcription is reduced when the upstream-located *ARO4* gene is transcribed from the strong *ACT1* promoter instead of its natural promoter.

Eukaryotic transcriptional interference is understood as the result of RNA polymerase II complexes that initiate transcription at the promoter of the upstream gene and subsequently read through the promoter of the downstream gene. Therefore, the assembly of functional transcription complexes at the downstream promoter is disturbed, resulting in promoter occlusion. The extent to which the reading through of RNA polymerase II complexes occurs critically depends on the efficiency of transcription termination of the upstream gene (3–5). Deletions of *GAL10* poly(A) signals abolished any activity of the downstream *GAL7* gene, even when the *GAL7* promoter was intact, resulting in a bicistronic read-through transcript. Therefore, in the case of *GAL7*, the promoter was completely occluded. Polymerase profiles raised in transcription run-on experiments for these poly(A) mutant strains confirmed the accumulation of nonterminated polymerase II complexes within the *GAL7* promoter (4). As a consequence of transcriptional interference, it was shown that various transcription factors are not able to bind to their promoter sites any more. This was demonstrated for the tandem HIV-1 promoters integrated into the genome of HeLa cells, where promoter occlusion of the downstream promoter correlated with reduced binding of the transcription factor Sp1 (6). The binding of the Gal4p transcriptional activator to the *GAL7* promoter was reduced in a similar fashion by read-through transcription initiated at the upstream *GAL10* promoter. Interestingly, Gal4p overexpression can suppress this effect (7).

Eukaryotic transcription is affected by the DNA accessibility of promoter sequences. Nucleosome structures in intergenic regions might therefore play an important role in the prevention of transcriptional interference. An example for prevention of transcriptional interference by a positioned nucleosome was described in *Drosophila melanogaster*. There, reconstituted chromatin with rDNA templates resulted in a positioned upstream nucleosome that is recruited by termination factor TTF-I. This nucleosome can act as barrier to transcriptional interference of the downstream ribosomal RNA genes that are transcribed by the RNA polymerase I complex (8).

ARO4 and *HIS7* are adjacent genes of *Saccharomyces cerevisiae* that are transcribed into the same direction. The intergenic region between both ORFs consists of 416 bp. Both genes encode amino acid biosynthetic enzymes required for the formation of aromatic amino acids and histidine, respectively. Here, we address the question of which parts of the entire *ARO4-HIS7* intergenic region antagonize transcriptional interference. We show that both *ARO4* mRNA 3′ end formation signals and specific *HIS7* promoter sequences diminish transcriptional interference. Moreover, we present a correlation between transcriptional interference and the intergenic chromatin structure.

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[1] The abbreviations used are: ORF, open reading frame; HIV, human immunodeficiency virus; MNase, *Micrococcus* nuclease.

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TABLE I
Yeast strains used in this study

Strain	Genotype	Reference no.
RH1381	*MATαaro3–2gcn4–101 ura3–52*	23
RH1833	*MATαaro3–2gcn4–101 ura3–52 ΔHIS7(−391/−341)*	3
RH1834	*MATαaro3–2gcn4–101 ura3–52 ΔHIS7(−336/−310)*	3
RH1836	*MATαaro3–2gcn4–101 ura3–52 ΔHIS7(−299/−281)*	3
RH1781	*MATαaro3–2gcn4–101 ura3–52 ΔHIS7(−220/−189)*	3
RH2642	*MATαaro3–2gcn4–101 ura3–52 ARO4::URA3-^{P}ACT-ARO4*	This work
RH2643	*MATαaro3–2gcn4–101 ura3–52 ΔHIS7(−391/−341)ARO4::URA3-^{P}ACT1-ARO4*	This work
RH2644	*MATαaro3–2gcn4–101 ura3–52 ΔHIS7(−336/−310)ARO4::URA3-^{P}ACT1-ARO4*	This work
RH2645	*MATαaro3–2gcn4–101 ura3–52 ΔHIS7(−299/−281)ARO4::URA3-^{P}ACT1-ARO4*	This work
RH2646	*MATαaro3–2gcn4–101 ura3–52 ΔHIS7(−220/−189)ARO4::URA3-^{P}ACT1-ARO4*	This work
RH1616	*MATαaro3–2gcn4–101 ura3–52 HIS7::^{P}his7-lacZ*	15
RH1815	*MATαaro3–2gcn4–101 ura3–52 HIS7::^{P}his7(−391/−341)-lacZ*	3
RH1816	*MATαaro3–2gcn4–101 ura3–52 HIS7::^{P}his7(−336/−310)-lacZ*	3
RH1818	*MATαaro3–2gcn4–101 ura3–52 HIS7::^{P}his7(−299/−281)-lacZ*	3
RH1819	*MATαaro3–2gcn4–101 ura3–52 HIS7::^{P}his7(−285/−245)-lacZ*	3
RH1822	*MATαaro3–2gcn4–101 ura3–52 HIS7::^{P}his7(−241/−212)-lacZ*	3
RH1824	*MATαaro3–2gcn4–101 ura3–52 HIS7::^{P}his7(−220/−189)-lacZ*	3
RH1825	*MATαaro3–2gcn4–101 ura3–52 HIS7::^{P}his7(−190/−171)-lacZ*	3
RH1826	*MATαaro3–2gcn4–101 ura3–52 HIS7::^{P}his7(−171/−139)-lacZ*	3
RH2174	*MATαaro3–2gcn4–101 ura3–52 HIS7::^{P}his7-lacZARO4::URA3-^{P}ACT1-ARO4*	This work
RH2632	*MATαaro3–2gcn4–101 ura3–52 HIS7::^{P}his7(−391/−341)-lacZARO4::URA3-^{P}ACT1-ARO4*	This work
RH2633	*MATαaro3–2gcn4–101 ura3–52 HIS7::^{P}his7(−336/−310)-lacZARO4::URA3-^{P}ACT1-ARO4*	This work
RH2634	*MATαaro3–2gcn4–101 ura3–52 HIS7::^{P}his7(−299/−281)-lacZARO4::URA3-^{P}ACT1-ARO4*	This work
RH2635	*MATαaro3–2gcn4–101 ura3–52 HIS7::^{P}his7(−285/−245)-lacZARO4::URA3-^{P}ACT1-ARO4*	This work
RH2636	*MATαaro3–2gcn4–101 ura3–52 HIS7::^{P}his7(−241/−212)-lacZARO4::URA3-^{P}ACT1-ARO4*	This work
RH2637	*MATαaro3–2gcn4–101 ura3–52 HIS7::^{P}his7(−220/−189)-lacZARO4::URA3-^{P}ACT1-ARO4*	This work
RH2638	*MATαaro3–2gcn4–101 ura3–52 HIS7::^{P}his7(−190/−171)-lacZARO4::URA3-^{P}ACT1-ARO4*	This work
RH2639	*MATαaro3–2gcn4–101 ura3–52 HIS7::^{P}his7(−171/−139)-lacZARO4::URA3-^{P}ACT1-ARO4*	This work
RH1830	*MATαaro3–2gcn4–101 ura3–52 HIS7::^{P}his7(mut-ABS)-lacZ*	17
RH2640	*MATαaro3–2gcn4–101 ura3–52 HIS7::^{P}his7(mut-ABS)-lacZARO4::URA3-^{P}ACT1-ARO4*	This work

EXPERIMENTAL PROCEDURES

Yeast Strains, Growth Conditions, and Plasmids—Yeast strains and genotypes used in this work are listed in Table I. For all experiments described here, strains were cultivated in minimal vitamin medium (9) supplemented with the required amino acids according to Rose *et al.* (10). To generate functional fusions of the *ACT1* promoter with the *ARO4* ORF at the original *ARO4* locus, a *Sal*I/*Bst*EII 3.1-kb fragment from plasmid pME1429 (3) was transformed in the respective strains. The cassette consists of the *ACT1* promoter fused to the first half of the *ARO4* ORF. Upstream, the cassette carries the divergently orientated *URA3* auxotrophic marker gene, which is itself preceded by the *ARO4* 5′-untranslated region for homologous integration. Transformants that had replaced the wild-type *ARO4* locus by this cassette by homologous recombination were selected by uracil prototrophy and confirmed in Northern hybridizations by increased *ARO4* mRNA levels and by PCR.

RNA Analysis—Total RNA from *S. cerevisiae* was isolated according to Cross and Tinkelenberg (11). 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 with the Prime It II DNA Labeling Kit from Stratagene. One-kb PCR fragments generated with the oligonucleotides ARO-OLV19 (5′-taccggatccagacgacagagttcttg-3′) and ARO-OLV11 (5′-cctcaagacgtcttcagtagtttcccaacc-3′), HIS-OL1 (5′-gtggtaacctacagtcactaacc-3′) and HIS-OL2 (5′-ccgatcgatactttatcagcacc-3′), and ACT-OL1 (5′-gctgctttggttattgataacgg-3′) and ACT-OL2 (5′-cacttgtggtgaacgatagatgg-3′) served as probes for the *ARO4*, *HIS7,* and *ACT1* genes, respectively. In all cases, template was genomic DNA of strain RH1381. Band intensities were visualized by autoradiography and quantified using a BAS-1500 phosphorimaging scanner (Fuji).

Genomic Chromatin Preparation and Nuclease Digestions—These methods have been described previously (12). Biodyne B nylon membranes were used for Southern transfer. Probes were labeled by the random primer method (13).

Indirect End Labeling—Chromosomal DNA from the nuclease digestion was digested with *Xba*I and *Mlu*I and fractionated in 1.2% agarose gels. The fractionated DNA was blotted on the nylon membrane by the alkaline blotting method and hybridized with a radioactively labeled 250-bp PCR product generated with oligonucleotides HIS7-CHR1 (5′-gagattaaagaaattgtcaga-3′) and HIS7-CHR2 (5′-caagtattgagga gaaatggta-3′), annealing just downstream of the *Xba*I site. A DNA ladder consisting of multiples of 256 bp was used for size estimation (14).

β-Galactosidase Assay—β-Galactosidase activities were determined by using permeabilized yeast cells and the fluorogenic substrate 4-methylumbelliferyl-β-D-galactoside as described previously (15). Yeast cells were cultivated in minimal vitamin medium overnight, diluted to an absorbance of ~0.5 at 546 nm, and cultivated for another 6 h before assay. One unit of β-galactosidase activity is defined as 1 nmol 4-methylumbelliferone h^{-1} ml^{-1} A_{546}^{-1}. The values presented are the means of at least four independent cultures, each of them measured three times. S.D.s were <20%.

RESULTS

^{P}ACT1-driven ARO4 Transcription Creates a MNase-sensitive Site within Nucleosomal DNA That Separates the ARO4 Terminator from the HIS7 Promoter—The replacement of the natural promoter of the *ARO4* gene by the stronger *ACT1* promoter causes transcriptional interference with the downstream *HIS7* gene, reducing *HIS7* transcription in comparison to wild-type expression (3). Because eukaryotic gene expression and its tight regulation in terms of transcriptional initiation and termination processes must take place in the presence of highly ordered chromatin structure, we wanted to know whether the transcriptional interference between the *ARO4* and *HIS7* gene is manifested in chromatin changes. Therefore, we analyzed the chromatin structure of the *ARO4-HIS7* intergenic region in absence and presence of transcriptional interference.

The chromatin structure was investigated by MNase protection experiments. Crude nuclear extracts from overnight cultures grown in minimal vitamin medium from strains with the wild-type *ARO4* promoter (RH1381) or the *^{P}ACT1-ARO4* fusion (RH2642), respectively, were partially digested with MNase and further treated as described previously (12). In wild-type cells, the *ARO4* 3′ region immediately downstream of the ORF is sensitive to MNase (Fig. 1). This short sensitive region was followed by a strongly protected region, which corresponds in length to a positioned nucleosome. The *HIS7* promoter further downstream was again sensitive to MNase. Although the mutant strain with the *^{P}ACT1-ARO4* fusion gene showed a largely similar chromatin pattern, an additional band within the pro-

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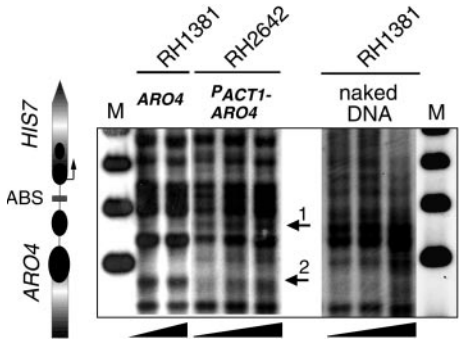

FIG. 1. **MNase protection experiments of the *ARO4-HIS7* intergenic region.** Strain RH1381 possesses the *ARO4* gene with its natural promoter (*ARO4*), whereas strain RH2642 has an *ARO4* allele driven by the *ACT1* promoter (^{P}*ACT1-ARO4*). Chromatin of RH1381 shown in the autoradiography was digested with 9 and 18 units of MNase, and chromatin of RH2642 was digested with 3, 9, and 18 units of MNase (all for 6 min). On the *far left* and *far right lanes*, a size marker of 256 bp is shown (*M*). The *arrows* indicate differences in chromatin structure between both strains. *Black ovals* in the scheme on the *left* reflect protected regions representing positioned nucleosomes. *ABS* represents the binding site for transcription factor Abf1p that was previously shown to bind the *HIS7* promoter, thereby supporting basal *HIS7* expression (3).

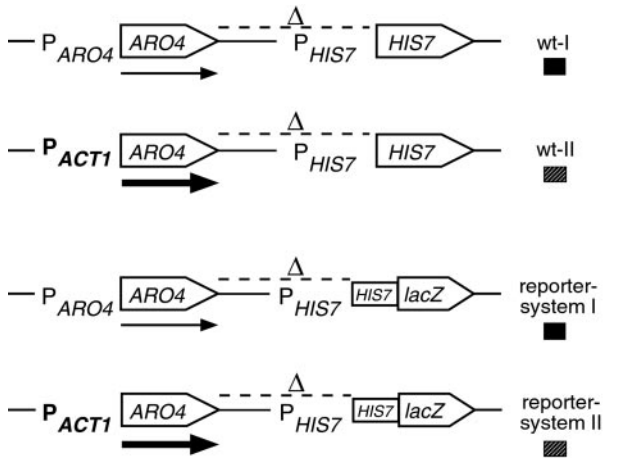

FIG. 2. **Scheme of the reporter systems used to determine DNA regions that antagonize transcriptional interference.** The *first line* shows the wild-type *ARO4-HIS7* locus (*wt-I*). The *second line* represents the *ARO4* allele that is driven from the *ACT1* promoter (*wt-II*). *Lines 3* and *4* represent the alleles corresponding to *lines 1* and *2*, but with *his7-lacZ* reporter fusions instead of wild-type *HIS7* (reporter systems I and II).

tected region between the *ARO4* 3′-end region and the *HIS7* promoter became obvious (Fig. 1, *arrow 1*). This sensitive site already appeared at very low MNase concentrations (chromatin digest with 3 units of MNase for 6 min) and got more pronounced at 9 and 18 units of MNase. In contrast, this MNase-sensitive site is faint in the *ARO4* wild-type strain, even for the digest with 18 units of MNase.

A feasible consequence of enhanced *ARO4* transcription could be that nonterminated, still-transcribing RNA polymerase II complexes weaken the DNA histone interaction at the respective nucleosome, thereby drastically increasing this otherwise very weak MNase-sensitive site. In addition, a sensitive site at the end of the ORF of the wild-type *ARO4* gene appears to be less sensitive in the case of the ^{P}*ACT1-ARO4* fusion gene (Fig. 1, *arrow 2*). Apparently, the chromatin structure of the very 3′ end of the *ARO4* ORF is also subjected to changes upon strong *ARO4* transcription. These changes of chromatin structures may be directly related to the termination efficiency and transcriptional interference.

Specific Deletions within the ARO4 3′-Untranslated Region or the HIS7 Promoter Increase Interference between ARO4 and HIS7 Transcription—The DNA in between the two ORFs of *ARO4* and *HIS7* possesses elements required for efficient 3′-end formation of the *ARO4* mRNA and others that promote efficient *HIS7* transcription (3). Here we intended to define DNA regions between these ORFs whose loss enhances transcriptional interference caused by increased *ARO4* transcription. We established a reporter system with the *HIS7* gene replaced by the quantifiable chimeric ^{P}*his7-lacZ* gene, preceded by the *ARO4* gene driven from either its natural promoter (Fig. 2, *reporter-system I*) or the *ACT1* promoter (Fig. 2, *reporter-system II*). To determine regions in between both ORFs that counteract transcriptional interference, specific β-galactosidase activities for various small intergenic deletions were measured. DNA elements that diminish transcriptional interference were identified by comparison of the read-outs of the two reporter systems for each deletion construct (Fig. 3). To maintain the original chromosomal context, the reporter system was established at the authentic *ARO4-HIS7* locus, with the separating nucleosome positioned approximately from –235 to –381 relative to the *HIS7* ATG start codon. Deletions were chosen to cover several DNA motifs that fulfill different functions, including the Zaret/Sherman element (*Z*/*S*) required for *ARO4* mRNA 3′-end formation, three sites defining the actual poly(A) addition sites, C+T- and A+T-rich regions, the Abf1p-protein binding site (*ABS*), and both Gcn4p recognition elements (*GCRE1* and *GCRE2*) (Fig. 3).

When measured in reporter system I, deletions that cover the Zaret/Sherman element or poly(A) sites as elements of *ARO4* mRNA 3′-end formation (RH1815, RH1816, and RH1818) did not affect the ^{P}*his7-lacZ* expression compared with that of the wild-type intergenic region (RH1616). In reporter system II, however, a 52-bp deletion that eliminated the Zaret/Sherman element (RH2632) reduced the specific β-galactosidase activity to about 28% of reporter system I (RH1815). Moreover, a 28-bp deletion that removed the first poly(A) site strongly reduced ^{P}*his7-lacZ* expression if present in reporter system II (RH2633). Only about 22% activity was left in comparison to reporter system I with this deletion (RH1816). Further deletions of the second and third poly(A) site (reporter system I, RH1818; reporter system II, RH2634), C+T-rich (reporter system I, RH1819; reporter system II, RH2635) and A+T-rich (reporter system I, RH1835; reporter system II, RH2638) stretches, or the binding sites for Gcn4p (Gcn4p recognition elements; reporter system I, RH1822/RH1826; reporter system II, RH2636/RH2639) did not increase transcriptional interference. A 28-bp deletion that covered the Abf1p-binding site in reporter system II (RH2637) displayed a severe loss of specific β-galactosidase activity and almost shut off any *his7-lacZ* expression. In the background of reporter system I, this deletion alone reduced *his7-lacZ* expression to about one-third of the wild-type promoter.

In summary, the data obtained from our reporter system suggest that Abf1p binding to the *HIS7* promoter is an essential element that antagonizes transcriptional interference. Furthermore, the Zaret/Sherman element and the first poly(A) site, which together are responsible for efficient *ARO4* 3′-end formation, obviously counteract transcriptional interference. Deletions within these regions enhance transcriptional interference. No deletion has resulted in higher β-galactosidase activities in reporter system II compared with reporter system I, suggesting that there are no *cis*-elements that support transcriptional interference.

Single Nucleotide Exchanges within the Abf1p-binding Site Increase Transcriptional Interference—The results obtained thus far with the deletion constructs suggested an important

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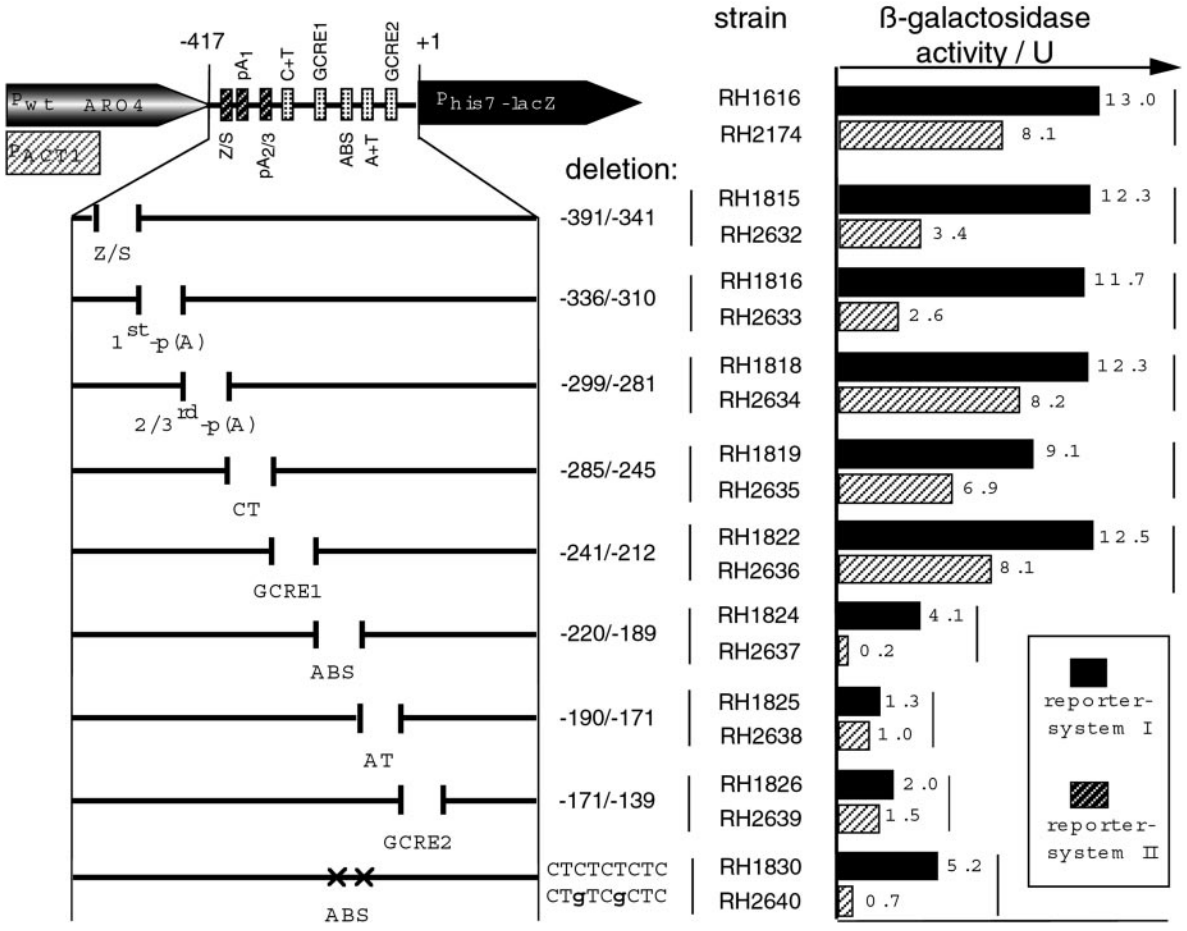

FIG. 3. **Effects of deletions of the *ARO4-HIS7* intergenic region on *his7-lacZ* activity.** The strains carry either the *ARO4* gene possessing its natural wild-type promoter (reporter system I) or the more efficient *ACT1* promoter (reporter system II). β-Galactosidase activities in units (measured as nmol 4-methylumbelliferone h^{-1} ml^{-1} A_{546}^{-1}) are indicated in the chart on the *right side* for strains with various deletions throughout the intergenic region. The scheme on the *left side* visualizes the exact locations of the deletions according to the translational start side (+1) and the DNA motifs at these positions. *Z/S* stands for the Zaret/Sherman motif necessary for correct *ARO4* 3′-end formation, *p(A)* stands for the sites where the pol(A) tail is added to the *ARO4* mRNA 3′ end, and *CT* stands for a C+T-rich element. In the *HIS7* promoter, *GCRE* stands for Gcn4p recognition element, *ABS* stands for Abf1p-binding site, and *AT* stands for an A+T-rich sequence.

contribution of Abf1p binding in the prevention of transcriptional interference at the wild-type *ARO4-HIS7* locus under conditions where *ACT1*-driven transcription also weakens the DNA-protein interaction of the separating nucleosome. We investigated whether it has been the broader context of the deleted 28-bp promoter region or solely the abolished binding of Abf1p itself that antagonized transcriptional interference. Therefore, we investigated the ^{P}*his7-lacZ* expression of a mutant strain with two single nucleotide exchanges within the Abf1p-binding site that were previously shown to abolish binding of Abf1p (16, 17).

In the background of wild-type *ARO4* expression in reporter system I (RH1830), single nucleotide exchanges within the Abf1p-binding site by themselves reduced ^{P}*his7-lacZ* expression to about 35% of that of wild-type. Integrated in reporter system II (RH2640), these nucleotide exchanges caused a further strong reduction in β-galactosidase activity to about 9% of wild-type ^{P}*his7-lacZ* expression. This result demonstrated that it was in fact the binding of Abf1p to its binding site within the *HIS7* promoter, and not a broader promoter context, that antagonized transcriptional interference at the *ARO4-HIS7* locus. It is possible that binding of Abf1p to its *cis*-element competes with the transcription of a nonterminated polymerase II complex and thus blocks transcriptional interference.

Transcriptional Interference Causes Histidine Auxotrophy for a HIS7 Promoter Mutant without Abf1p-binding Site by Prevention of Its Transcription—The data of the reporter systems that derived from a *lacZ* reporter gene have shown that the Abf1p-binding site and elements required for efficient *ARO4* mRNA 3′-end formation are important to prevent transcriptional interference. We then investigated whether the increased transcriptional interference of these deletion mutants gave rise to malfunctions in cells that harbor the wild-type *HIS7* gene. Therefore, the growth rates of these strains were determined in medium without histidine.

When the *ARO4* gene was driven from its own promoter, the wild-type's growth rate of about 0.28 h^{-1} was not changed in strains with deletions in the *ARO4* 3′-end region (strains RH1833, RH1834, and RH1836 in Fig. 4*A*). The growth rate nearly halved with the deletion of the Abf1p-binding site in the *HIS7* promoter (0.18 h^{-1}, RH1781). The combination of a deleted first poly(A) site with an induced *ARO4* expression (RH2644) also significantly reduced the growth rate in comparison to the wild-type (0.20 h^{-1}). Deletion of the Abf1p-binding site in combination with the *ACT1-ARO4* fusion gene (RH2646) was so deleterious for the cell that it resulted in a histidine auxotrophic growth phenotype (Fig. 4, *A* and *B*).

We compared the effects of the transcriptional interference as obtained by the *his7-lacZ* chimeric genes and the growth tests with the quantified *HIS7* mRNA steady-state levels determined by Northern hybridizations (Fig. 5). The fusion of the *ACT1* promoter to the *ARO4* gene increases *ARO4* mRNA levels ~4-

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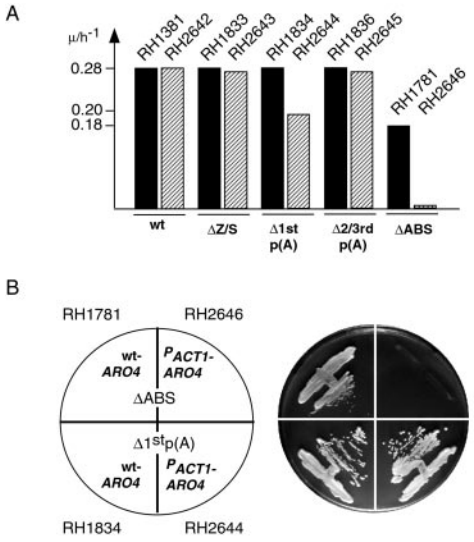

FIG. 4. **Transcriptional interference causes histidine auxotrophy for a *HIS7* promoter deletion that eliminates the Abf1p-binding site.** The growth of yeast strains was tested on minimal vitamin medium lacking histidine. Strains RH1781 and RH1834 possess the *ARO4* gene with its natural promoter and carry deletions in either the *HIS7* promoter (ΔABS) or the *ARO4* 3′-end region (Δ1st p(A)). Strains RH2646 and RH2644 have the natural promoter of the *ARO4* gene replaced by the *ACT1* promoter and carry either the *HIS7* promoter deletion ΔABS or the *ARO4* 3′-end deletion Δ1st p(A).

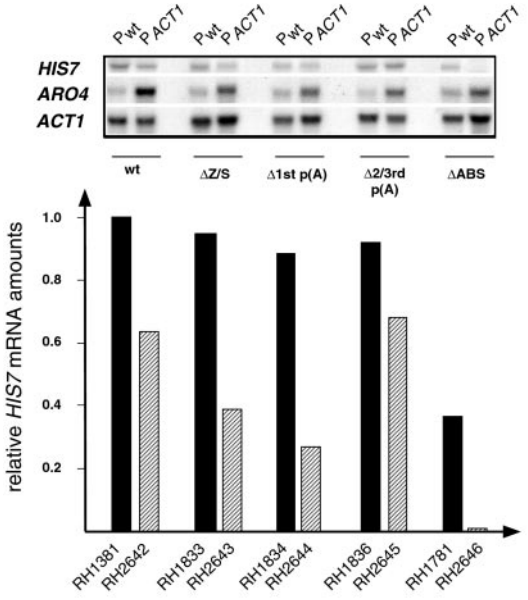

FIG. 5. **Effects of deletions of the *ARO-HIS7* intergenic region on *HIS7* transcript levels.** Northern hybridization experiments of selected yeast strains with deletions in either the *ARO4* 3′-untranslated region (*Z/S, p(A)*) or the *HIS7* promoter covering the Abf1p-binding site (*ABS*) were performed. Quantifications were performed by phosphorimaging analyses and are presented as the averages of at least two hybridizations with total RNAs from three independent cultures. Quantifications of the *ARO4* mRNA amounts revealed a 4-fold increase in average if the gene is transcribed from the *ACT1* promoter.

fold (Fig. 5). Enhanced *ARO4* transcription in the background of the wild-type *HIS7* gene with the wild-type *ARO4-HIS7* intergenic region (RH2642) resulted in *HIS7* mRNA levels of about 60% in comparison to wild-type *ARO4* (RH1381). Deletion of the Zaret/Sherman element as *ARO4* 3′-end formation signal in the background of increased *ARO4* transcription (RH2643) reduced the *HIS7* mRNA levels to ~40% in comparison to the natural *ARO4* promoter (RH1833). When the first *ARO4* poly(A) site was deleted, the reduction of *HIS7* transcript levels as a consequence of enhanced *ARO4* transcription was even more pronounced (RH2644), namely, 30% of the respective strain with wild-type *ARO4* expression (RH1834). In contrast, the deletion covering the second and third *ARO4* poly(A) addition site did not show obvious differences in *HIS7* transcript levels caused by transcriptional interference.

The deletion within the *HIS7* promoter that covers the Abf1p-binding site in the wild-type *ARO4* background (RH1781) already reduced *HIS7* mRNA levels to 40% in comparison to the wild-type *HIS7* promoter. However, in combination with high *ARO4* transcription from the *ACT1* promoter (RH2646), *HIS7* transcripts were no longer detectable. This result confirmed the transcriptional interference as detected before in both the reporter system with the *his7-lacZ* reporter gene and the growth defect on histidine-deficient medium.

We have also investigated whether the single nucleotide exchanges within the Abf1p-binding site alone can change the chromatin structure of the intergenic region without *ACT1*-driven overexpression of *ARO4*. However, no changes in comparison to the wild-type intergenic region have been detected (data not shown). Therefore, we suggest that it is the DNA binding of the Abf1 protein itself that somehow blocked the transcribing polymerase II complex, rather than a reorganization of the nucleosomal structure by Abf1p that subsequently prevents transcriptional interference.

Taken together, these results imply that binding of the ubiquitous transcription factor Abf1p to the *HIS7* promoter counteracts transcriptional interference caused by enhanced *ARO4* expression, which is itself accompanied by nucleosomal changes. *ARO4* 3′-end formation signals also prevented transcriptional interference, although less efficiently than Abf1p binding. By name, these were the *ARO4* mRNA 3′-processing motif, originally described by Zaret and Sherman for *CYC1* (18), and the major site where the *ARO4* mRNA transcript is finally cleaved and processed.

DISCUSSION

Cells have developed mechanisms that enable individually regulated expression of adjacent genes that are located in close proximity without influencing one another. One essential parameter to prevent read-through transcription is the efficient termination of transcription of the upstream gene. In eukaryotic cells, this process is characterized by the combination of events that generate the mRNA 3′ end, followed by its polyadenylation and the actual termination of transcription (that is, the release of the elongation complex from the DNA template). To initiate transcription at the downstream promoter, an efficient recruitment of the transcriptional preinitiation complex at the initiation site is necessary. In addition to this recruitment, regulated gene expression requires efficient binding of gene-specific transcriptional activators to the promoter upstream of the transcriptional initiation site. The efficiency of both the 3′-end formation/termination and the initiation of transcription at the downstream gene must be adjusted to the "strength" of the two adjacent genes for their different levels of expression. Otherwise, transcriptional interference reduces or even abolishes the expression of the downstream gene by promoter occlusion. Because the eukaryotic DNA is closely associated with histone proteins, these processes must take place in the context of a highly ordered chromatin structure. Here we show that a nucleosome is strictly localized in such a position between two tandemly arranged yeast genes that it may guard the more downstream gene from transcriptional interference under normal circumstances. This assumption is corroborated through the finding that increased transcription of the more upstream gene weakens this nucleosome.

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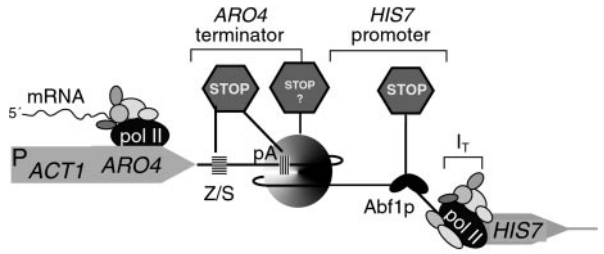

FIG. 6. **Summary of the *ARO4-HIS7* intergenic elements that antagonize transcriptional interference.** The *ARO4* gene is highly transcribed by RNA polymerase II complexes (*pol II*) driven from the *ACT1* promoter. For reinitiation of a functional RNA polymerase II complex at the initiation site of the *HIS7* promoter (I_T), the transcription of upstream *ARO4* has to be efficiently terminated. Elements blocking read-through RNA polymerase II complexes are the Zaret/Sherman element (*Z/S*) and the major poly(A) site (*pA*) within the *ARO4* terminator and the Abf1p binding at the *HIS7* promoter. Moreover, the positioned nucleosome seems to be a barrier to transcriptional interference.

We determined additional elements located in between the ORFs of the two yeast genes that contribute to antagonizing transcriptional interference (Fig. 6). Elements were identified that are necessary to separate transcription of the two adjacent genes. mRNA 3′-end formation signals such as the Zaret/Sherman element as well as the site where the nascent transcript is cleaved and the poly(A) tail is added represent borders that belong to the preceding *ARO4* gene. The removal of these elements significantly increased transcriptional interference at that locus. Termination of transcription by RNA polymerase II and its release from the DNA template were previously shown to be linked to mRNA 3′-end processing (4). Destruction of poly(A) signals probably results in reduced termination events, leading to increased transcription far beyond the poly(A) site of a gene and thereby impairing the activity of downstream promoters (3, 19). However, future transcription run-on experiments for this locus should confirm transcribing RNA polymerase II complexes driven from the upstream promoter into the ORF of the downstream *HIS7* gene.

Another border marked by the downstream *HIS7* gene is the presence of the general DNA-binding factor Abf1p in its promoter. Besides its role as an activator of *HIS7* transcription, it seems to have the additional function of forming a protective barrier against read-through transcription initiated at the upstream *ARO4* gene. This roadblock function of Abf1p is supported by the observation that deletion/mutation of the Abf1p-binding site had different effects in both the wild-type and the *PACT1-ARO4* systems on *his7-lacZ* expression (Fig. 3). Recent investigations focusing on the *GAL10-GAL7* locus in yeast or the tandem HIV-1 promoters integrated in HeLa cells also support such a link between termination and promoter activity (6, 7). By *in vivo* footprinting, it was demonstrated that reduced 3′-end processing activity of the *GAL10* gene directly weakens the binding of the transcription factor Gal4p to the adjacent *GAL7* promoter and thereby reduces its transcription. In the *GAL10-GAL7* system, overexpression of Gal4p seems to counteract some of the transcriptional interference. Because Abf1p is an abundant protein in the yeast cell and it also binds the *HIS7* promoter consistently during inactivated *HIS7* transcription, testing its overexpression in terms of lowering transcriptional interference does not seem promising. The binding of transcription factor Sp1 to the downstream promoter of tandemly localized HIV-1 promoters is significantly increased by insertion of an efficient transcriptional termination element upstream of the occluded promoter. A recent report stated that efficient termination enabled by the murine transcript release factor PTRF augments downstream ribosomal gene transcription by enhancing reinitiation at the ribosomal DNA promoters (20). Although previous reports demonstrated an influence of Abf1p binding on the local chromatin structure of promoters of the *QCR8* and *RPS28A* genes (21, 22), we could not detect any changes in nucleosome distribution at the *ARO4-HIS7* locus upon destruction of the Abf1p-binding site (data not shown). Possibly, in common with other promoters of typical housekeeping genes, the *HIS7* promoter has a pre-set accessible chromatin structure that is not directly dependent on the presence or absence of Abf1p.

The different factors that in concert seem to prevent transcriptional interference are outlined in Fig. 6. Because there is also an alteration of the nucleosomal structure at the 3′ end of the *ARO4* open reading frame upon high *ARO4* transcription, efficient termination of transcription might require a defined chromatin structure at the very end of a gene. A link between the positioning of an upstream nucleosome, transcriptional initiation at downstream promoters, and transcriptional interference was not yet described for an RNA polymerase II-transcribed gene. For genes encoding ribosomal RNA, it was shown that the positioning of a nucleosome at an upstream terminator element is required to allow transcription from the downstream promoter. To position this nucleosome, the DNA-binding termination factor TTF-I, homologous to the yeast Reb1p, was shown to be necessary (8).

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