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The Adjacent Yeast Genes *ARO4* and *HIS7* Carry No Intergenic Region*

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The region between the open reading frames of the adjacent yeast genes *ARO4* and *HIS7* consists of 417 base pairs (bp). Termination of *ARO4* transcription and initiation of *HIS7* transcription has to take place within this interval, because both genes are transcribed into the same direction. We show that the *ARO4* terminator and the *HIS7* promoter are spatially separated, nonoverlapping units. The *ARO4* terminator includes 84 bp of the *ARO4* 3′-untranslated region with several redundant *ARO4* 3′ end processing signals. Deletion of the *ARO4* terminator does reduce but not completely shut down its expression. The adjacent region of 40 bp is neither required for correct *ARO4* 3′ end formation nor for *HIS7* initiation but contains the nucleotides corresponding to the wild type mRNA 3′ ends. The following 280 bp are required for the *HIS7* promoter. Replacement of the housekeeping *ARO4* promoter by the stronger *ACT1* promoter leads to reduced *HIS7* expression due to transcriptional interference. This underlines the compactness of the yeast genome carrying virtually no intergenic regions between adjacent genes.

The sequencing of the genome of the budding yeast *Saccharomyces cerevisiae* has revealed the remarkable compactness of its genome. This results from the short size of regions between the open reading frames. Open reading frames of divergent promoters on average are only 618 bp[1] apart. Open reading frames of convergent terminators are separated by 326 bp on average. Arrangements with a terminator-promoter combination are spaced by 517 bp. Assuming nonoverlapping units this leads to a deduced and calculated average size of 309 bp for a promoter and 163 bp for a terminator (1). For the regulated expression of the yeast genome, it is important that transcription of an upstream located gene does not interfere with the initiation of transcription of an immediately downstream located gene.

The goal of this study was to test for a concrete terminator-promoter combination in yeast the size of the terminator and the promoter. In addition, we wanted to know whether both units are overlapping or whether there is an intergenic spacer region between the terminator and the promoter.

In eukaryotes, the process of transcriptional termination is poorly understood. A number of different assays have been developed to measure termination in RNAP II genes, including poly(A) site competition, transcriptional interference (2), and reverse transcription-polymerase chain reaction. Using these methods, termination sequences in mammalians have been identified between two closely spaced genes, human complement genes C2 and factor B. A binding site has been identified in the termination signal that binds the protein MAZ. It seems plausible that the proven ability of MAZ to bend DNA may relate to the RNAP II termination process (3). In *S. cerevisiae*, *in vitro* studies with the *ADH2* and *GAL7* genes lead to the hypothesis that the coupling of a RNAP II pause site to a functional polyadenylation signal results in transcription termination (4).

In yeast, as in all eukaryotes, the 3′ ends of mRNAs are generated by a processing reaction that takes place in the cell nucleus (for review see Refs. 5–8). The mRNA precursors first lose a 3′-terminal noncoding fragment by endonucleolytic cleavage and then receive a poly(A) tail by polymerization of AMP. In higher eukaryotes two sequence elements define a poly(A) site. One is the almost invariant AAUAAA hexanucleotide, about 15 nucleotides upstream of the poly(A) addition site. The second signal, located downstream of the poly(A) site, is either a run of Us or a poorly defined GU-rich sequence (6). In yeast, however, the situation seems to be more complex. A highly conserved consensus sequence as found in higher eukaryotes is lacking. Sequences that have been identified to play an important role in mRNA 3′ end formation of one gene are often absent or nonfunctional in other genes. In general, the yeast mRNA 3′ end formation signals seem to be more degenerate, redundant, and disperse (8).

In yeast, the 3′ processing signal has been proposed to consist of three elements (9). The far upstream element directs the efficiency of the processing site, whereas the near upstream element is required for the positioning of the poly(A) site. The third element is the poly(A) site itself. Two classes of far upstream elements have been discussed (10). An efficient, unidirectional class contains the T_5TA sequence motif proposed by Henikoff and Cohen (11) or derivatives thereof. A less efficient class functions in both orientations and is defined by the tripartite TAG . . . TA(T)GTA . . . TTT motif and its derivatives originally proposed by Zaret and Sherman (12). For positioning elements a TTAAGAAC motif, an A_8 stretch or the canonical AATAAA element have been discussed (9). Little is known about the exact sequence requirement for the poly(A) site, but CA_n or TA_n sequences within the permissive distance appear to be preferred (13).

Numerous studies have been performed in yeast where either individual promoters or individual mRNA 3′ end forma-

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[1] The abbreviations used are: bp, base pair(s); PCR, polymerase chain reaction; DAHP, 3-deoxy-D-arabino-heptulosonate-7-phospate; PIPES, piperazine-*N*,*N*′-bis(2-ethanesulfonic acid); MOPS, morpholinopropanesulfonic acid.

tion signals have been analyzed in various test systems. It is hardly known how different mRNA 3′ end formation signals affect different promoters in a single test system. Therefore, the aim of this study was to investigate effects on a mRNA 3′ end formation signal and a promoter simultaneously.

The *ARO4* gene encodes the tyrosine-regulated 3-deoxy-D-arabino-heptulosonate-7-phosphate synthase catalyzing the first step in the Shikimate pathway (14). Its poly(A) site contains the tripartite TAG . . . TATGTA . . . TTT motif proposed by Zaret and Sherman (12) and belongs to the class of bidirectionally functional poly(A) sites (10). The *HIS7* gene is located just downstream of the *ARO4* gene on yeast chromosome II. It encodes the bifunctional glutamine amidotransferase:cyclase catalyzing the fifth and sixth step in the *de novo* histidine biosynthesis (15). Basal transcription of *HIS7* requires the global factor Abf1p, and it is activated under conditions of amino acid starvation and adenine starvation conditions by Gcn4p and Bas1/2p, respectively (16). The two genes are transcribed in the same direction with a normal spacing of 417 bp between the open reading frames.

We show that the *ARO4* terminator and the *HIS7* promoter are nonoverlapping, spatially separated units. The signals directing proper *ARO4* 3′ end formation are spread over 84 bp of the *ARO4* 3′-untranslated region. Various point mutations have no effect on the ability of *ARO4* 3′ end formation, suggesting the presence of multiple redundant signals. Deletion of the complete *ARO4* 3′ end processing signal reduces but does not completely shut down *ARO4* expression. Replacement of the housekeeping *ARO4* promoter by the efficiently transcribing *ACT1* promoter leads to reduced *HIS7* expression due to transcriptional interference between these two genes. Because 280 bp are required for the *HIS7* promoter, there are only about 40 bp between these two genes where the actual poly(A) addition sites are located.

EXPERIMENTAL PROCEDURES

Strains, Media, and General Methods—All yeast strains were derivatives of standard laboratory strains *S. cerevisiae* X2180–1A (*MAT***a** *gal2 SUC2 mal CUP1*) and X2180–1B (*MAT*α *gal2 SUC2 mal CUP1*). *S. cerevisiae* strain RH1631 (*MAT***a** *ura3-52*) was used for transformation of all the constructed integrative test gene plasmids. All mutant *ARO4*/*HIS7* alleles were integrated into the yeast strain RH1548 (*MAT*α *aro3-2 gcn4-101 ura3-52 aro4 his7::URA3*). *Escherichia coli* strain DH5α (17) was used for the propagation of plasmid DNA. Enzymatic manipulations and cloning of DNA were performed as described in Sambrook *et al.* (18). Yeast strains were cultivated in YEPD complete medium (19) or MV minimal medium (20). Yeast transformation (21), DNA isolation (22), and Southern analysis (23) were previously described. Oligonucleotides were synthesized by MWG-Biotech (Ebersberg, Germany).

Construction of the Internal Deletions of the ARO4/HIS7 Intergenic Region—The various internal deletion mutations of the *ARO4*/*HIS7* intergenic region were constructed by *Bal*31 exonuclease treatment of the linearized plasmid pME947. Plasmid pME947 was constructed based on the pGEM-7Zf (+) plasmid (Promega, Madison, WI) by insertion of the 1.9-kilobase *Sph*I/*Bam*HI fragment of the *ARO4*/*HIS7* locus with a created *Cla*I site at position −405 relative to the translational start codon of the *HIS7* gene. The plasmid was linearized either with *Cla*I or *Eco*RV and subsequently treated with *Bal*31 exonuclease to obtain 5′ and 3′ deletions of the region, respectively. After cloning of a *Cla*I/*Hin*dIII/*Eco*RV adapter, appropriate 5′ and 3′ deletion fragments were combined to obtain the internal deletions of the *HIS7* promoter. This resulted in the plasmids pME951 to pME956 (3′ deletions), pME966 to pME971 (5′ deletions), and pME991 to pME995, pME997, pME999, and pME1001 (internal deletions).

Construction of Translational HIS7-lacZ Fusions—The respective integrative HIS7-*lacZ* fusion constructs based on the plasmids pME951 to pME956 (3′ deletions), pME966 to pME971 (5′ deletions), and pME991 to pME995, pME997, pME999, and pME1001 (internal deletions) were constructed on the basis of the pME947 derivatives, carrying the respective *HIS7* promoter mutations, as described previously for pME696 (15).

Integration of the HIS7-lacZ Fusion Constructs—All *HIS7-lacZ* fusion constructs were integrated as single copy into the yeast genome at the *ARO4*/*HIS7* locus of yeast strain RH1548 (*MAT*α *aro3-2 gcn4-101 ura3-52 aro4 his7::URA3*). The procedure was described previously for the wild type *HIS7-lacZ* construct pME696 resulting in strain RH1616 (15).

Construction of the Test Gene—Plasmid pME800 was constructed on the basis of pSP64 (Promega, Madison, WI) to obtain an integrative vector. Vector pSP64 was modified by cloning the 1.1-kilobase *Hin*dIII fragment of *URA3* into the *Xho*I site, by inserting the 1.1-kilobase *Bam*HI fragment of pME729 (24) into the *Bam*HI site of the polylinker and by introducing a multiple cloning site (double-stranded OLCE1-OLCE2) into the *Cla*I site of the 1.1-kilobase *Bam*HI fragment. The different mutated alleles of the *ARO4*/*HIS7* intergenic region were amplified by using OLCS26 and OLCS27 as primers and the plasmids pME951 to pME956 (3′ deletions), pME966 to pME971 (5′ deletions) and pME991 to pME995, pME997, pME999 and pME1001 (internal deletions) as templates in a PCR reaction and cloned into the multiple cloning site of plasmid pME800 after restriction with *Kpn*I and *Bgl*II.

Site-directed Mutagenesis of the ARO4/HIS7 Intergenic Region—Site-directed mutations in the *ARO4*/*HIS7* intergenic region were introduced using the PCR technique (25). Oligonucleotides carrying specific mutations were OLCS36 to OLCS40. These oligonucleotides were used in a PCR reaction together with OLCS27 as second primers and pME947-DNA as template. The final PCR products were cut with *Kpn*I and *Bgl*II and cloned into plasmid pME800.

β-Galactosidase Activity Assay—β-Galactosidase activities were determined by using permeabilized yeast cells and the fluorogenic substrate 4-methylumbelliferyl-β-D-galactoside as described earlier (15). Routinely, yeast cells were cultivated in MV minimal medium overnight, diluted to an optical density of approximately 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-methyl-umbelliferone h^{-1} ml^{-1} $A_{546}{}^{-1}$. The given values are the means of at least four independent cultures. The standard errors of the means were less than 20%.

DAHP Synthase Activity Assay—3-Deoxy-D-arabino-heptulosonate-7-phospate synthase activities were determined as described in Takahashi and Chan (26). Routinely, yeast was cultivated in MV minimal medium to an optical density of approximately 2 at A_{546}, harvested by centrifugation and washed three times with potassium phosphate buffer (50 mM potassium phosphate, pH 7.6, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM EDTA, 1 mM dithiothreitol). The cells were resuspended in 5 ml of potassium phosphate buffer, disrupted in a French press (Aminco, Silver Spring, MD), and the cell debris was removed by centrifugation. Finally, the supernatant was applied to a PD 10 column (Pharmacia Biotech Inc. Uppsala, Sweden). 50 μl of crude cell extract was incubated for 10 min in 50 μl of erythrose-4-phosphate (8 mM), 40 μl of phosphoenolepyroat (10 mM), 50 μl of 0.4 M potassium phosphate buffer, and 60 μl of H_2O. The enzymatic reaction was stopped by adding 50 μl of trichloroacetic acid (20%). 100 μl of the reaction solution was added to 100 μl of 20 mM $NaIO_4$ in 0.25 M H_2SO_4 and incubated for 30 min at 37 °C. This reaction was stopped by adding 200 μl of $NaAsO_2$ (2% in 0.5 M HCl). After the solution turned colorless, 800 μl thiobarbituric acid (0.3%) was added, and the mixture was boiled for 10 min. The absorption of the product was measured at 550 nm.

Isolation of Total RNA from S. cerevisiae—Yeast cells were grown overnight in a 100-ml culture to an optical density at 546 nm of about 2. The cells were spun at 6000 × *g* for 5 min on one-fifth volume of ice and resuspended in 6 ml of PLE buffer (100 mM PIPES, 100 mM LiCl, 1 mM EDTA, pH 7.4). After centrifugation at 6000 × *g* for 5 min at 4 °C, the cells were resuspended in 300 μl of ice-cold PLE buffer and 100 μl of ice-cold dichloromethane-saturated phenol equilibrated with PLE buffer. Diethylpyrocarbonate (1%, v/v) was added to inactivate RNases. Sterilized glass beads 0.45 mm in diameter were added, and the cells were disrupted by vigorous shaking for six 15 s periods with cooling on ice in between. Nucleic acids were extracted once with 1 volume of dichloromethane-saturated phenol equilibrated with PLE buffer, 0.05 g of bentonite, and 1% (w/v) sodium dodecyl sulfate and twice with 1 volume of dichloromethane-saturated phenol equilibrated with PLE buffer. Total RNA was precipitated by 1.5 volumes of ice-cold isopropanol, and the concentration was determined spectrophotometrically. The precipitated RNA was stored at −20 °C.

RNA Analysis—For Northern (RNA) hybridization experiments, approximately 10 μg of total RNA was precipitated, resuspended, and denatured in 30 μl of sample buffer (50%, v/v, deionized formamide, 6% v/v formaldehyde, 1 × loading buffer, 10% [v/v] 10 mM Tris-1 mM EDTA [TE] buffer) for 15 min at 65 °C and put on ice. The RNA was separated on a denaturing formaldehyde agarose gel. The 1.4% (w/v) agarose gel

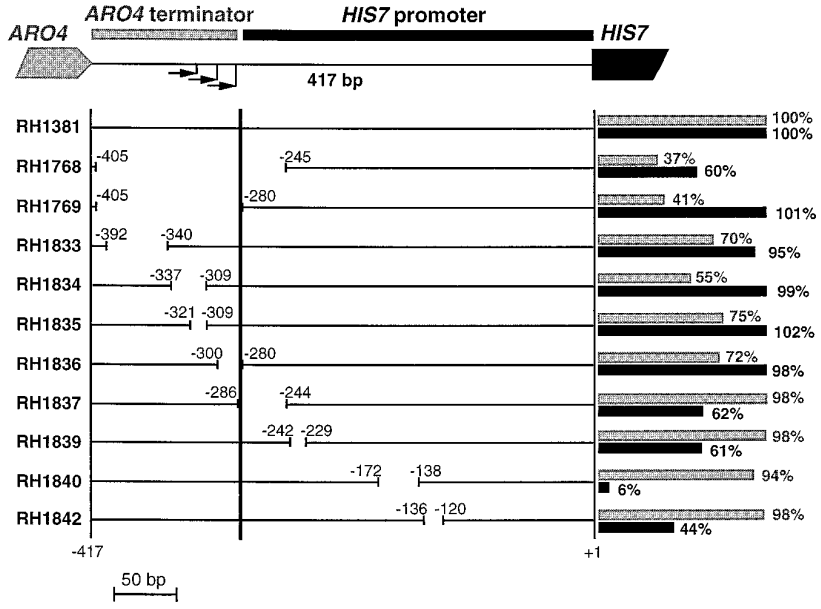

FIG. 1. **The *ARO4* terminator and the *HIS7* promoter are nonoverlapping and spatially separated.** The DNA region between the *ARO4* and the *HIS7* open reading frames is shown. The positions of the three mapped *ARO4* mRNA 3′ ends are indicated by *black arrows*. The tested deletion constructs of the *ARO4*/*HIS7* intergenic region are shown below. End points of the deletions are indicated by numbers representing the positions relative to the A residue of the ATG start codon of the *HIS7* gene. The *ARO4*-derived enzyme activity was measured as DAHP synthase activity and is shown in *shaded boxes*, whereas the *HIS7*-encoded enzyme activity was measured as β-galactosidase activity from corresponding *HIS7-lacZ* fusions and is indicated by *black boxes*. Numbers are relative values, with the specific wild type enzyme activity for the *ARO4*-encoded enzyme DAHP synthase and the wild type activity for the *HIS7-lacZ* fusion-encoded β-galactosidase as 100%. Each number represents an average value of at least six measurements with a standard deviation of not more than 15%.

(3%, v/v, formaldehyde, 20 mM MOPS, 5 mM sodium acetate, 1 mM EDTA) was run for 3 h at 60 V in a buffer containing 20 mM MOPS, 5 mM sodium acetate, and 1 mM EDTA. The gel was soaked twice in 25 mM Na phosphate buffer for 20 min each time, and the RNA was transferred onto a nylon membrane (Amersham, Buckinghamshire, UK) by electroblotting (2 A, 50 V) for 3 h in 25 mM Na phosphate buffer. After washing in 2 × SSC (1 × SSC is 0.15 M NaCl plus 0.15 M sodium citrate), drying on 3MM paper, and cross-linking under UV light (254 nm) for 5 min, the membrane with the bound RNA was hybridized at 42 °C with a labeled fragment for 24 h in 50 ml of a hybridization mixture (50%, v/v, formamide, 50 mM sodium phosphate, pH 6.5), 800 mM NaCl, 1 mM EDTA, 0.5% sodium dodecyl sulfate, 10 × Denhardt's solution, 150 μg of calf thymus DNA per ml, 500 μg of torula yeast RNA/ml). The fragment, representing the 440-bp *Mlu*I/*Xho*I DNA element of the *ACT1* 5′ region, was randomly radiolabeled as described previously (27). The RNA was visualized by autoradiography. Band intensities from autoradiographs were quantified with a PhosphorImager (Molecular Dynamics, Sunnyvale, CA).

RESULTS

The ARO4 Terminator and the HIS7 Promoter Are Nonoverlapping and Spatially Separated Units—The spacing between the open reading frames of the *ARO4* gene and the *HIS7* gene consists of 417 bp. We wanted to know whether deletions within this region result in interference between *ARO4* transcription and the initiation of transcription of the *HIS7* promoter. Therefore a deletion analysis of the *ARO4*/*HIS7* intergenic region was performed. *ARO4* expression was determined by measuring DAHP synthase activity, which is the gene product. *HIS7* transcription was monitored by determining β-galactosidase activities of strains carrying respective translational *HIS7-LacZ* fusions integrated in single copies at the *ARO4*/*HIS7* locus (Fig. 1). All strains had a *gcn4-101* genetic background to avoid interference with the general control of amino acid biosynthesis in yeast.

Deletion of large parts of the *ARO4* 3′-untranslated region in the yeast strains RH1768 (Δ −405/−245 relative to the *HIS7* AUG start codon) and RH1769 (Δ −405/−280) (Fig. 1) including the mapped poly(A) sites (14) and the tripartite Zaret/Sherman sequence element (12, 10) reduced *ARO4* activity to 37 and 41%, respectively, compared with wild type activity. Smaller deletions of 52 bp in RH1833, 28 bp in RH1834, 12 bp in RH1835, or 20 bp in RH1836 moderately reduced *ARO4* expression leading to between 55 and 75% of wild type activity. All these deletions were within the first 140 bp of the *ARO4* 3′-untranslated region and had no effect on *HIS7* expression. The four strains RH1837, RH1839, RH1840, and RH1842 carry various deletions between 13 and 42 bp in length, all located more than 140 bp downstream of the end of the *ARO4* open reading frame within the *HIS7* promoter. None of these four deletions affected *ARO4* expression, but all of them reduced *HIS7* expression.

In summary, any deletion within the first 140 bp of the *ARO4* 3′-untranslated region had a significant effect on *ARO4* expression but did not affect *HIS7* transcription. By contrast, all deletions within the next 280 bp affected *HIS7* transcription, but none of them had any effect on *ARO4* expression. These results strongly suggest that the *ARO4* termination sequences are located within the first 140 bp of the untranslated region between the *ARO4* and the *HIS7* genes and do not overlap with the *HIS7* promoter. Therefore, the *ARO4* termination sequences and the *HIS7* promoter sequences are located within spatially clearly separated units.

A Region of Maximal 40 bp between ARO4 and HIS7 Is Not Necessary for Efficient ARO4 mRNA 3′ End Formation nor for HIS7 Promoter Activity but Contains the ARO4 Wild type mRNA 3′ End Positions—To define whether there is any intergenic spacer region between *ARO4* and *HIS7*, the sequences required for *ARO4* mRNA 3′ end formation were analyzed more precisely. We tested *ARO4* 3′ end modifications in an artificial test system that we had established earlier (28). The *ARO4* polyadenylation element represents the class of yeast 3′ processing sites which function in both orientations in an *in vivo* test system (10). The 3′-untranslated region of the *ARO4* gene contains the tripartite sequence motif TAG . . . TATGTA . . . TTT, which was proposed to represent a processing consensus element in yeast (Fig. 2) (12). Modifications of the *ARO4* 3′-

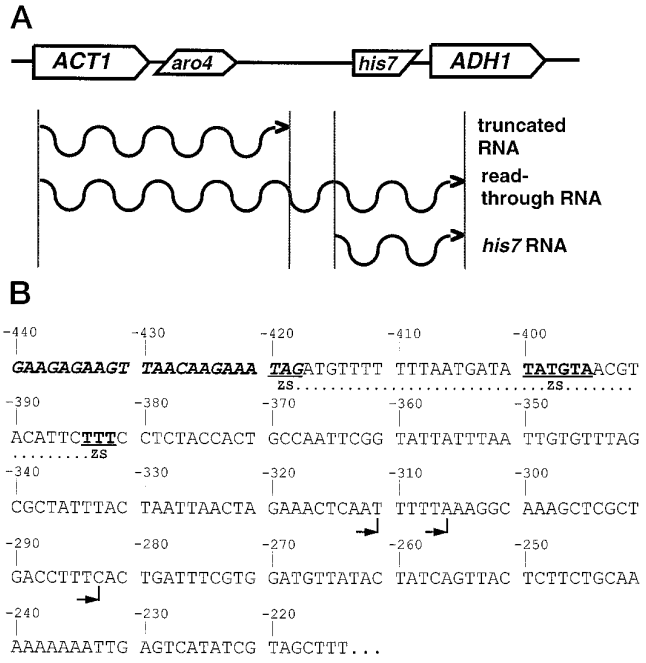

FIG. 2. ***In vivo* test cassette for either wild type or mutant mRNA 3′ processing signals in *S. cerevisiae*.** *A*, the test cassette consists of the *ACT1* promoter fused to the *ADH1* terminator. Functional 3′ processing sites were cloned between the *ACT1* promoter and the *ADH1* terminator and result in short truncated transcripts, whereas nonfunctional sites result in long readthrough transcripts. Because the complete *HIS7* promoter is cloned into the test cassette, a short transcript initiated at this promoter and ending in the *ADH1* terminator is expected. *B*, the primary sequence of the *ARO4* 3′-untranslated region and a part of the open reading frame (in *boldface italic type*) are shown. The tripartite Zaret/Sherman (*ZS*) motif TAG . . . TATGTA . . . TTT is a putative consensus element and is *underlined* and in *boldface type*. The three mapped *ARO4* 3′ ends are indicated by *black arrows*. The numbers correspond to the assignment of position +1 to the A nucleotide of the ATG start codon of the *HIS7* gene.

untranslated region included 3′ and 5′ end, internal deletions, and specific point mutations inserted into the complete element (Fig. 3). The modified *ARO4* 3′ end elements were cloned into the multiple cloning site of the test gene consisting of the *ACT1* promoter and the *ADH1* terminator (Fig. 2) (28). The test gene was integrated into the chromosome at the *URA3* locus, thereby avoiding multicopy effects. The effects of all modifications were analyzed at the transcript level by performing Northern blot analysis. Functional 3′ processing elements resulted in short truncated transcripts, whereas nonfunctional elements resulted in long readthrough transcripts as schematically drawn in Fig. 2.

3′ deletion up to position −321 relative to the A residue of the translational start codon ATG of the *HIS7* gene (deletion Δ −321/−104 in Fig. 4) resulted in a 3′ processing efficiency (86% truncated transcript) similar to that of the complete wild type *ARO4*/*HIS7* intergenic region (83–86% truncated transcripts). Further deletion to position −337 completely abolished 3′ end formation (deletion Δ −337/−104 in Fig. 4). Therefore the downstream boundary for a completely functional *ARO4* 3′ processing element in the test system was located in the −337 to −321 region. The mapped 3′ ends (positions −311, −306, and −283) (14) are located downstream of this boundary suggesting that they are not important for the efficiency of mRNA 3′ end formation in the test gene.

5′ deletion of the part containing the *ARO4* open reading frame including 12 bp of the 3′-untranslated region had no effect on 3′ end processing (deletion Δ −440/−405 in Fig. 4). In this construct the TAG part of the tripartite TAG . . . TATGTA . . . TTT Zaret/Sherman sequence element was deleted. Any further 5′ deletion (deletions Δ −405/−340 to Δ −405/−211 in Fig. 4) resulted in the complete loss of *ARO4* 3′ end formation. We therefore conclude that no parts of the *ARO4* open reading frame are involved in 3′ end formation and the 5′ boundary of the 3′ processing element must be located somewhere downstream of position −405.

This finding was confirmed by analyzing internal deletion constructs of this region. In the deletions Δ −392/−340 and Δ −337/−309 3′ processing activity was reduced to below 10% (Fig. 4), whereas in the deletion Δ −321/−309 the ability to process 3′ ends was restored to almost wild type level (77% truncated transcript), substantiating the 3′ boundary between positions −337 and −321. None of the internal deletions downstream of position −300 affected 3′ end formation. Therefore the *ARO4* 3′ end processing element could be delimited to the 84 bp between positions −405 and −321. Any internal deletion within this part leads to a complete loss of proper 3′ end generation. Interestingly, neither the TAG part of the tripartite Zaret/Sherman sequence element nor the mapped poly(A) sites are within the boundaries of this element.

In a set of point mutations, the involvement of the tripartite TAG . . . TATGTA . . . TTT Zaret/Sherman sequence in *ARO4* 3′ end formation was further analyzed. The first TAG part of the element is identical with the *ARO4* stop codon. In mutations mut(TAa) and mut(Tga) (Fig. 4) this element was replaced by one of the alternative stop codons TAA or TGA, respectively. In the mutations mut(agcGT) and mut(ΔTATGT) the middle part was either changed to the sequence AGCGT or deleted, whereas in mutation mut(gTa) the third part was exchanged for the sequence GTA. In mutation mut(agcGT-gTa) both the middle and the third element were mutated. None of these point mutations or small deletions had any effect on *ARO4* 3′ end formation in the *in vivo* test system. We therefore conclude that several redundant 3′ processing signals must be spread over a maximum of 84 bp between position −405 (which is 12 bp downstream of the *ARO4* stop codon) and position −321 relative to the *HIS7* AUG start codon. Taking into account that the *HIS7* promoter reaches approximately to position −280 relative to the *HIS7* start codon (Fig. 1), the intergenic region between the *ARO4* and the *HIS7* genes consists of 40 bp at most. This region carries all mRNA 3′ ends that were mapped *in vitro* (positions −311, −306, and −283). Thus, virtually no intergenic region exists between *ARO4* and *HIS7* underlining the compactness of the yeast genome.

Deletion of the ARO4 Poly(A) Signal Reduces Its Expression—In the deletion (Δ −405/−280) all the sequences required for *ARO4* 3′ end formation in the artificial test system were removed. Strain RH1769 carrying this deletion in the untranslated region between the *ARO4* and the *HIS7* genes showed a decreased *ARO4* expression level. *ARO4* expression in this strain was about 40% when compared with wild type expression levels (Fig. 1). In contrast, deletion of the *ARO4* 3′ end processing signals did not affect the expression of the *HIS7* gene located downstream (Fig. 1). We therefore concluded that deletion of the 3′ processing signals reduces *ARO4* expression to about 40% compared with its wild type expression level, indicating the existence of cryptic 3′ end forming signals.

Overexpression of the ARO4 Gene Lacking its 3′ Processing Signals Shuts Down Expression of the Downstream Located HIS7 Gene—The *ARO4* terminator and the *HIS7* promoter are separate elements, and deletion of the whole *ARO4* terminator does not influence *HIS7* expression (Fig. 1). This seemed surprising to us, because theoretically we expected that the role of a terminator is not only to correctly process mRNA 3′ ends but also to avoid transcriptional interference between two adjacent

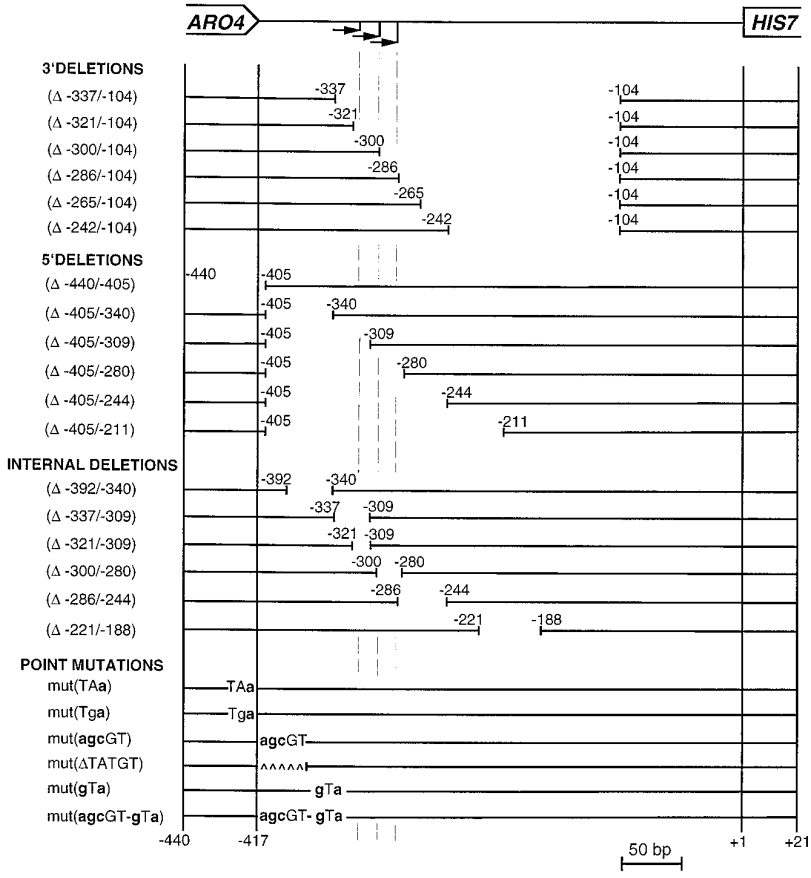

FIG. 3. **Schematic representation of the mutations constructed in the *ARO4/HIS7* intergenic region.** The numbers indicated above the constructs represent the boundaries of the deletions relative to the A nucleotide of the *HIS7* ATG start codon. The nucleotide exchange in the point mutations are indicated with *lowercase boldface letters*, whereas a *caret* indicates deletion of a single nucleotide.

genes. Thus, we further investigated the role of the *ARO4* terminator for its ability to prevent interference between the transcription of the *ARO4* and the *HIS7* genes.

Replacement of the *ARO4* promoter by the *ACT1* promoter increased its expression 4-fold and caused a reduction of *HIS7* expression to 50% of the wild type expression (Fig. 5). This effect was even more pronounced using the yeast strain RH1815 carrying a 52-bp deletion within the *ARO4* 3′ end processing signal reducing *ARO4* expression to 70%. In this strain *HIS7* activity was slightly reduced to 95% compared with wild type activity. Here, replacement of the *ARO4* promoter by the strong *ACT1* promoter leading to the yeast strain RH2172 reduced *HIS7* activity to 30% of wild type activity. These results indicated that expression of the *ARO4* gene under the control of the strong *ACT1* promoter at its original chromosomal locus interfered with the initiation of transcription at the downstream located *HIS7* promoter and therefore caused a reduction of *HIS7* expression. This effect is even more pronounced when simultaneously the *ARO4* terminator is lacking.

In the *ACT1-ARO4* 3′ end formation test gene where the *ACT1* promoter is fused to the *ARO4/HIS7* intergenic region with only 90 bp of the open reading frame in between, no transcript initiated at the *HIS7* promoter could be detected (Fig. 6). Therefore, we tested whether this is due to the strong initiation at the *ACT1* promoter and the incomplete 3′ end formation at the *ARO4* polyadenylation site in the *ACT1-ARO4* hybrid gene. Two constructs served as controls. In the first construct the *ACT1* promoter was destroyed by *Bal*31 digestion. With no transcript initiated at the strong *ACT1* promoter, no interference was expected between the *ACT1-ARO4* hybrid transcript and the initiation at the *HIS7* promoter. Therefore a short transcript initiated at the *HIS7* promoter was expected. In the second construct the strong polyadenylation signal of the *GCN4* gene (28) was cloned between the *ACT1* promoter and the *ARO4/HIS7* intergenic region. In this construct the discrepancy between the strong *ACT1* promoter and the weak *ARO4* terminator should be abolished, and therefore a transcript initiated at the *HIS7* promoter was expected.

In a Northern blot experiment with RNA isolated from the yeast strains RH2169 (with inserted *GCN4* terminator) and RH2171 (with destroyed *ACT1* promoter), a short transcript initiated at the *HIS7* promoter could be detected by hybridization with a radiolabeled, 215-bp *ADH1* probe. No such transcript was detected using RNA isolated from the yeast strain RH2160 with an intact *ACT1* promoter and no inserted *GCN4* terminator (Fig. 6).

Hybridization of RNA isolated from the yeast strain RH2169 (with inserted *GCN4* terminator) with the radiolabeled 524 bp *ACT1* probe led to a great amount of *ACT1-GCN4* hybrid transcript. The strong *ACT1* promoter directed high levels of initiation of transcription and the downstream inserted strong *GCN4* terminator resulted in complete termination of transcription. In the strain RH2171 the *ACT1* promoter was completely destroyed, because no transcript could be visualized by hybridization of RNA from this strain with the *ACT1* probe. In the strain RH2160 (wild type *ARO4/HIS7* intergenic region) both truncated and readthrough transcripts were present, indicating incomplete processing of the *ACT1-ARO4* hybrid mRNA. These results demonstrated that expression of the *ACT1-ARO4* hybrid mRNA abolished initiation of transcription at the *HIS7* promoter located downstream due to transcriptional interference between these two genes.

In summary, deletion of the *ARO4* terminator has no effect on *HIS7* transcription. Overexpression of the *ARO4* gene by the *ACT1* promoter reduces *HIS7* expression by a factor of two. Simultaneous overexpression of *ARO4* and deletion of its ter-

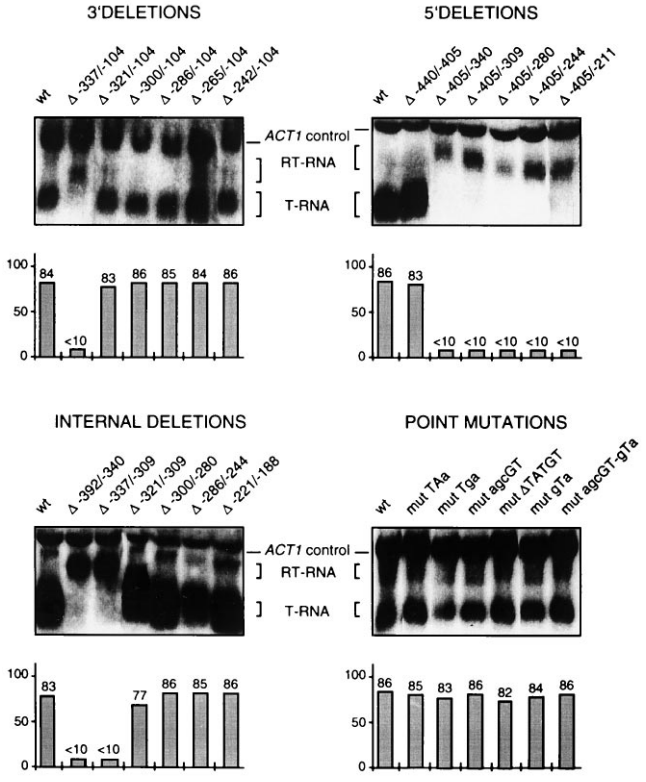

FIG. 4. **Effects of the modifications in the *ARO4/HIS7* intergenic region on mRNA 3′ end processing.** Northern hybridization analysis was performed with total RNA isolated from the wild type and mutated strains. The truncated RNA (*T-RNA*) and the readthrough RNA (*RT-RNA*) were visualized with a radiolabeled probe derived from the *ACT1* promoter. The wild type *ACT1* transcript was visualized with the same probe and was used as control. The 3′ processing efficiencies were determined by using a PhosphorImager. All values represent the ratio between truncated transcripts and the total amount of transcripts, *i.e.* T-RNA/(T-RNA + RT-RNA), and each is the average of evaluations of at least three Northern blots. The standard deviation did not exceed 10%. The 3′ processing efficiency with the wild type *ARO4/HIS7* intergenic region was approximately 84% (82–86%).

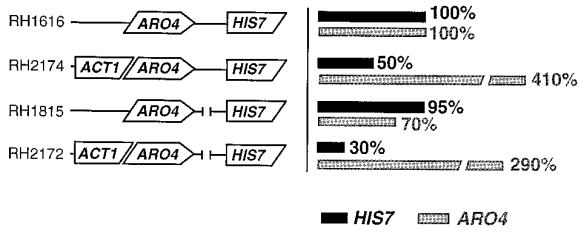

FIG. 5. **Effects of the *ACT1/ARO4* fusion on *HIS7* expression.** β-Galactosidase activities (*black boxes*) and DAHP synthase activities (*shaded boxes*) of the four strains RH1616, RH2174, RH1815, and RH2172 carrying respecive *HIS7/lacZ* fusion constructs are shown. The strain RH1616 represents the wild type *ARO4/HIS7* intergenic region, in the strain RH2174 the *ARO4* promoter was replaced by the *ACT1* promoter, in the strain RH1815 the sequences of the *ARO4/HIS7* intergenic region between positions −392 and −340 relative to the *HIS7* start codon were deleted, and finally the strain RH2172 was constructed by replacement of the *ARO4* promoter by the *ACT1* promoter in the strain RH1815. The wild type activity was set to 100%. The numbers indicated represent the average value obtained by at least six measurements. The standard deviation did not exceed 15%.

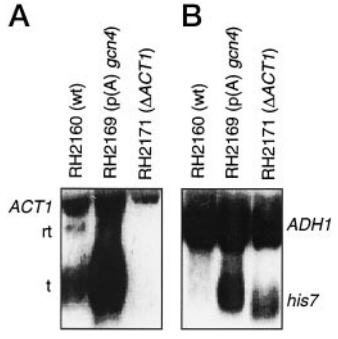

FIG. 6. **Northern experiments with different *ACT1-ARO4* hybrid genes.** The strain RH2160 carries the wild type *ARO4/HIS7* intergenic region inserted in the *in vivo* test cassette. In strain RH2169 the strong 3′ processing signals of the *GCN4* gene were cloned between the *ACT1* promoter and the *ARO4/HIS7* intergenic region. In strain RH2171 the *ACT1* promoter was destroyed by *Bal*31 digestion. In *panel A*, the blot was hybridized with a radiolabeled 542-bp fragment of the *ACT1* promoter to monitor *ACT1-ARO4* hybrid transcripts, whereas in *panel B* a 215-bp fragment of the *ADH1* terminator was used to monitor *HIS7-ADH1* transcripts.

minator reduces *HIS7* expression to 30% of wild type level. Finally, as shown in Fig. 6*B* (*first lane*), a shortened distance between the *ACT1* promoter and the *ARO4* terminator with just a little part of the *ARO4* open reading frame in the *ACT1-ARO4* hybrid gene completely shuts down *HIS7* expression. Therefore strain RH2160, where the *ACT1* promoter is fused to the *ARO4-HIS7* intergenic region with only 90 bp of the *ARO4* open reading frame in between and integrated into the yeast genome, raises no *HIS7* transcript.

DISCUSSION

This study had three major results. (*a*) We wanted to know whether the authentic 3′ end of a gene is indispensable for its expression at its natural chromosomal locus. We found that we can delete the *ARO4* 3′ end signal. Therefore, the *ARO4* 3′ end signal is not essential but required for efficient *ARO4* gene expression. (*b*) We wanted to know whether the *ARO4* 3′ end formation signals can generally block transcriptional interference and guarantee efficient *HIS7* expression. We found that 4-fold increased *ARO4* expression reduces *HIS7* expression by a factor of two. (*c*) We wanted to know whether in yeast a terminator and an adjacent promoter are overlapping or whether there is intergenic space between two adjacent genes. Our results suggest two independent nonoverlapping units and no intergenic region between *ARO4* and *HIS7*.

Part of our analysis concerns the question of how essential the 3′ end of a gene is for its expression in the natural chromosomal environment. The *ARO4* 3′ processing signal includes several redundant elements that are located within 84 bp starting about 12 bp downstream of the *ARO4* stop codon. Any deletion within this region reduced *ARO4* expression to between 35 and 75% when compared with the wild type activity. Interestingly, deletion of the complete *ARO4* 3′ end signal reduces *ARO4* expression to 41% when compared with wild type but does not completely shut down its expression. Thus, the complete 3′ end of *ARO4* is only important for the efficiency of gene expression but is not essential for gene expression per se. The cell seems to be able to cope with the lack of the *ARO4* 3′ end by using cryptic signals within the *HIS7* promoter for *ARO4* mRNA 3′ end formation.

Furthermore, the effect of enhanced *ARO4* transcription on the initiation of the downstream located *HIS7* gene was investigated. Small deletions within the 3′ processing and termination region of the *ARO4* gene reduced its expression but had no effect on *HIS7* transcription. Even a 52-bp deletion only hardly reduced *HIS7* expression compared with the wild type expression level. By contrast, a shortened *ACT1-ARO4* hybrid gene, where the *ACT1* promoter was directly linked to the 3′-untranslated region of the *ARO4* gene, with only 90 bp of the open reading frame in between caused total *HIS7* promoter occlusion with no detectable transcript initiated at the downstream

located *HIS7* promoter. Expression of the complete *ARO4* gene under the control of the *ACT1* promoter resulted in 4-fold increased *ARO4* expression, and simultaneous *HIS7* expression was reduced by a factor of two. This effect was even more pronounced when parts of the *ARO4* poly(A) signal were deleted. In conclusion the 3′ end of a gene is adjusted to its own promoter. Deletion of a poly(A) signal affects the expression of a downstream located gene only if the activity of the upstream promoter is simultaneously increased. The adjustment of the 3′ end formation signal for a mRNA is necessary to prevent transcriptional interference with the adjacent gene. In some further studies, the mechanism should be investigated in more detail, by which transcriptional interference between neighboring genes is prevented.

One remarkable feature of the yeast genome is its compact architecture, resulting from short intergenic regions. Some statistical calculations with the yeast genome revealed an average of 309 bp for a promoter (1). This theoretical value fits well with the observed 280 bp for the *HIS7* promoter. The calculated size of an average yeast terminator consists of 163 bp. In this study we mapped the *ARO4* poly(A) signals within a region of 84 bp starting 12 bp downstream of the *ARO4* stop codon. Adding these 12 bp to the poly(A) signal and taking into account that the actual poly(A) addition sites were mapped within 40 bp downstream of the poly(A) signal, the *ARO4* terminator consists of 136 bp. Therefore, the theoretically calculated sizes for yeast promoters and terminators fit well with the concrete situation between the open reading frames of the *ARO4* and *HIS7* genes. Within the 40 bp between the *ARO4* 3′ end processing signals and the *HIS7* promoter, the actual *ARO4* 3′ ends are located. In conclusion, there is virtually no intergenic region between the *ARO4* and *HIS7* genes underlining the compact architecture of the yeast genome.

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