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 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. 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 transcriptional 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 AATAAA 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 TnTA 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)GA . . . TTT motif and its derivatives originally proposed by Zaret and Sherman (12). For positioning elements a TTAAGAAC motif, an A8 stretch or the canonical AATAAA element have been discussed (9). Little is known about the exact sequence requirement for the poly(A) site, but Cn or Ta 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-
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 . . . TAGTGA . . . TTG 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 requiring 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 (MATα gal2 SUC2 mal2::URA3 [MATα gal2 SUC2 mal2 CUP1]). X2180–1A (MATα gal2 SUC2 mal2 CUP1). S. cerevisiae strain RH1631 (MATα ara3-2 gen4-101 ura3-52 ar4 hist7::URA3) was used for transformation of all the constructed integrative test gene plasmids. All mutant ARO4/HIS7 alleles were integrated into the yeast strain RH1548 (MATα ara3-2 gen4-101 ura3-52 ar4 hist7::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 Bal31 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 SphI/BamHI fragment of the ARO4/HIS7 locus with a created ClaI site at position +405 relative to the translational start codon of the HIS7 gene. The plasmid was linearized either with ClaI or EcoRV and subsequently treated with Bal31 exonuclease to obtain 5′ and 3′ deletions of the region, respectively. After cloning of a ClaI/HindIII/EcoRV 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 pME966 (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 pME966 (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 (MATα ara3-2 gen4-101 ura3-52 ar4 hist7::URA3). The procedure was described previously for the wild type HIS7-lacZ construct pME966 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 HindIII fragment of URA3 into the XhoI site, by inserting the 1.1-kilobase BamHI fragment of pME729 (24) into the BamHI site of the polylinker and finally eliminating a multiple cloning site (double-stranded OLeCI-OLCE2) into the ClaI site of the 1.1-kilobase BamHI fragment. The different mutated alleles of the ARO4/HIS7 intergenic region were amplified by using OLC826 and OLC827 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 KpnI and BglII.

**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 OLC386 to OLC407. These oligonucleotides were used in a PCR reaction together with OLC827 as second primers and pME727-DNA as template. The final PCR products were cut with KpnI and BglII 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-methylumbelliferone h⁻¹ ml⁻¹ Assay⁻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-phosphate synthase activities were determined as described in Taka-hashi and Chan (26). Routinely, yeast was cultivated in MV minimal medium to an optical density of approximately 2 at A546, 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 (Aminoce, Silver Spring, MD), and the cell debris was removed by centrifugation (15,000 ⨯ g, 10 min). The supernatant was supplemented 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 erthyrose-4-phosphate (8 mM), 40 μl of phosphoenolpyruvate (10 mM), 50 μl of 0.4 mM potassium phosphate buffer, and 60 μl of H₂O. 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 Na₂O₃ in 0.25 % H₃SO₄ and 60 μl of H₂O. This reaction mixture was boiled for 5 min in a boiling water bath. The mixture was then cooled, 200 μl of NaAsO₂ (2% in 0.5 M HCl) was added, and after the solution turned colorless, 800 μl tribarbituric 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 at onefifth 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. Diethylpyrocatecholate (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
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 \( \beta \)-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 \( \beta \)-galactosidase as 100%. Each number represents an average value of at least six measurements with a standard deviation of not more than 15%.

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 \( \beta \)-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/-345 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/Palmer sequence element (12) 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'...
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. The numbers correspond to the assignment of position +1 to the A nucleotide of the ATG start codon of the HIS7 gene.

3’ deletion up to position –321 relative to the A residue of the translational start codon ATG of the HIS7 gene (deletion ∆–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 ∆–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 ∆–440/–405 in Fig. 4). In this construct the TAG part of the tripartite TAG...TAT-GTA...TTT Zaret/Sherman sequence element was deleted. Any further 5’ deletion (deletions ∆–405/–340 to ∆–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 ∆–392/–340 and ∆–337/–309 3’ processing activity was reduced to below 10% (Fig. 4), whereas in the deletion ∆–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(aggCT) and mut(DTATGT) the middle part was either changed to the sequence AGGCCT or deleted, whereas in mutation mut(gTa) the third part was exchanged for the sequence GATA. In mutation mut(aggCT-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 (∆–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
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 H1S7 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 Bal31 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-
adjacent promoter are overlapping or 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 9 bp 3′ end of a gene is indispensable for its expression in the natural chromosomal environment. 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 Bal31 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.
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 between neighboring genes.

In conclusion the 3’9 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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