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Positive Regulation of the β-Galactosidase Gene from *Kluyveromyces lactis* Is Mediated by an Upstream Activation Site That Shows Homology to the *GAL* Upstream Activation Site of *Saccharomyces cerevisiae*

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In contrast to the *Escherichia coli lac* operon, the yeast β-galactosidase gene is positively regulated. In the 5′-noncoding region of the *Kluyveromyces lactis LAC4* gene, we mapped an upstream activation site (UAS) that is required for induction. This sequence, located between positions −435 and −326 from the start of translation, functions irrespective of its orientation and can confer lactose regulation to the heterologous *CYC1* promoter. It is composed of at least two subsequences that must act in concert. One of these subsequences showed a strong homology to the UAS consensus sequence of the *Saccharomyces cerevisiae GAL* genes (E. Giniger, S. M. Varnum, and M. Ptashne, Cell 40:767–774, 1985). We propose that this region of homology located at about position −426 is a binding site for the product of the regulatory gene *LAC9* which probably induces transcription of the *LAC4* gene in a manner analogous to that of the *GAL4* protein.

The yeast *Kluyveromyces lactis* is a eucaryotic unicellular organism that can utilize lactose as a sole carbon source. It offers an interesting possibility in that the regulation of its *LAC* genes can be compared directly with the analogous *lac* operon of *Escherichia coli*. As is typical for eucaryotes these genes are not organized in an operon but are coordinately regulated (8, 30). Both the *LAC4* gene, encoding β-galactosidase (33), and the *LAC12* gene, encoding lactose permease (34), are inducible by lactose and galactose. Their regulation is thereby closely coupled to that of the *GAL* genes. The lactose permease gene and the β-galactosidase gene have been cloned (9, 34) and have been shown to be tightly linked, mapping within 13 kilobase pairs (kbp) (34). The structural genes of the Leloir pathway enzymes *GAL1*, *GAL10*, and *GAL7* (in analogy to the *Saccharomyces cerevisiae* genes) are also clustered and show the same gene order *GAL7 GAL10 GAL1* as in *S. cerevisiae* (30).

Recessive regulatory *K. lactis* mutants have been isolated (10, 32; M. Ruzzi and A. Walker-Jonah, unpublished data) in which expression of the *LAC* as well as the *GAL* genes (8; Ruzzi and Walker-Jonah, unpublished data) are affected. They fall into two classes: noninducible (*lac9*) and constitutive (*lac10*) mutations (10, 32).

In *S. cerevisiae* both the *GAL* genes and the *MEL1* gene, which encode α-galactosidase, are transcriptionally regulated by the products of the regulatory genes *GAL4* and *GAL80* (29; for a review, see reference 27). Recessive mutations in *GAL4* are noninducible (11), and *gal80* mutants are constitutive (12). It has been shown that the *GAL4* protein binds to the 5′-noncoding regions of the genes regulated by galactose (2, 13), thereby activating transcription in the presence of galactose. Under noninducing conditions the *GAL80* gene product probably inactivates the *GAL4* protein (25, 26, 28). The DNA binding site for *GAL4* has been mapped (2, 13) and coincides with the GAL upstream activation sites (UAS_G) that were previously shown to be required for induction in *cis* (36).

In *K. lactis LAC4* is the best-characterized gene of lactose-galactose metabolism. The gene has been cloned by complementation of an *E. coli lacZ* mutant (9). It is transcriptionally regulated (23). We have mapped and sequenced the promoter region (4) and presented evidence that regulation of transcription by lactose and galactose is mediated by a positive regulatory factor that interacts with sequences in the 5′-noncoding region of the *LAC4* gene (6). In this study we mapped and characterized the sequences involved in the induction of the *LAC4* gene in more detail and have defined an upstream activation site named UAS_L.

The analysis of UAS_L revealed a strong homology with the *GAL4* protein binding site in *S. cerevisiae*, which establishes a direct link to one of the best-studied regulatory systems in yeast cells, the regulation of the *GAL* genes in *S. cerevisiae*. Because the *LAC9* gene complements a *gal4* mutation (31), we propose that the *LAC9* protein binds to UAS_L and activates transcription of the *LAC4* gene as well as the other lactose- and galactose-inducible genes in a way analogous to that of *GAL4* in *S. cerevisiae*.

MATERIALS AND METHODS

Bacterial strains and plasmids. *E. coli* K514 (*hsrK hsmK* derivative of strain C600) and JA300 (*thr leuB6 thi thyA trpC1117 hsrK hsmK* Str^r) (35) were used. Plasmids pK147 and pK7 have been described previously (6). Plasmid pLG669-Z (17) was obtained from L. Guarente.

In plasmid pLL26 a *Sal*I *LAC4* promoter fragment (−675 to −26) (see below) was inserted in front of a slightly modified *E. coli lacZ* structural gene in the *E. coli*-yeast vector pLV1 (see below). The source of the *lacZ* gene was the plasmid piWiT72, which was kindly supplied by B. v.

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TABLE 1. Sequences around the deletion endpoints

Plasmid	Sequence[a]
pKL4	a g c t t a t c a t c g CGTCGACGAATTCT
	−670
pK114	a g c t t a t c a t c g CGTCGACGGCGGAA
	−435
pK147	a g c t t a t c a t c g CGTCGACGGATTTC
	−254
pK147-423	AGCGGAAATTTGTGTCGACGGATTTC
	−423 −254
pK147-381	GTTACCGGAATTCGTCGACGGATTTC
	−381 −254
pK147-326	ACTACTTGAAGTCGTCGACGGATTTC
	−326 −254
pK147-292	ACACCCGGAATTCGTCGACGGATTTC
	−292 −254
pK147-292.2	GTGAACAGAATTCGTCGACGGATTTC
	−670 −254
pK7	a g c t t a t c a t c g CGTCGACGCGGTCG
	−392
pK7-423	AGCGGAAATTTGTGTCGACGCGGTCG
	−423 −392
pK7-423.2	GTGAACAGAATTCGTCGACGCGGTCG
	−670 −392
pK4	a g c t t a t c a t c g CGTCGACGAGGTAT
	−176
pK4-292	ACACCCGGAATTCGTCGACGAGGTAT
	−292 −176
pK4-174	AAAGGCGGAATTCGTCGACGAGGTAT
	−174 −176
pK4-174.2	GTGAACAGAATTCGTCGACGAGGTAT
	−670 −176
pK6-174	AAAGGCGGAATTCGTCGACGTTCTTT
	−174 −139

[a] pBR sequences are shown in lowercase letters; linker sequences are underlined.

Wilken-Bergmann. In this plasmid a synthetic promoter supplying an ATG initiation codon was fused to codon 6 of the *lacZ* gene, thereby substituting the original first five amino acids of β-galactosidase by nine different ones. We used a *Nco*I site overlapping the ATG codon to insert a *Sal*I linker at this position so that the 5′ end of the *lacZ* gene read as follows:

GTCGACGC ATG GCA ATG CCA AGC TTT CCG GGG AAT TCA
Met Ala Met Pro Ser Phe Pro Gly Asn Ser*

where the asterisk is amino acid 6 of *E. coli* β-galactosidase. The *lacZ* gene was inserted as a *Sal*I-*Bam*HI fragment into the *tet* gene of pEK2 (kindly supplied by E. Kellermann) to give pLV1. pEK2 is a derivative of YRp7 (35) that contains an autonomously replicating sequence for *K. lactis* KARS2 (7) inserted as a *Bam*HI-*Bgl*II fragment into the unique *Bgl*II site of YRp7.

Plasmid pKL4 (see legend to Fig. 1) is derived from pKlac2 (6) by eliminating pBR322 sequences 5′ to the *LAC4* gene up to the unique *Sal*I site of pKlac2.

Yeast strains. The *K. lactis* wild-type strain was CBS2360. *K. lactis* SD11 is a *lac4 trp1* derivative of CBS2360 (7).

Transformation of yeast. *K. lactis* SD11 was transformed as described by Klebe et al. (22). SD11 transformants were selected as Trp^+. The restriction pattern of the transforming plasmids was reaffirmed after retransformation into *E. coli*.

Assay of β-galactosidase. β-Galactosidase activity was measured in crude extracts as described previously (6), with *o*-nitrophenyl-β-D-galactopyranoside used as a substrate. One unit of enzyme activity was defined as the change in the A_{420} per minute per milligram of protein. The protein concentration was measured as described by Lowry (24), with bovine gamma globulin (Serva, Heidelberg, Federal Republic of Germany) used as the standard.

Deletion construction. The construction of 5′ deletions of pKlac2 has been described previously (6).

A set of 3′ deletions was obtained from the plasmid pSH4. pSH4 is a subclone of the *LAC4* 5′ end. The 2.1-kb *Eco*RI-*Hin*dIII fragment was cloned into the polylinker region of pUR250 (U. Ruther, Ph.D. thesis, Universität Köln, Cologne, Federal Republic of Germany, 1983). A *Sal*I linker (5′-CGTCGACG-3′; Boehringer GmbH, Mannheim, Federal Republic of Germany) was introduced at the *Eco*RI site after the sticky ends were filled in with DNA polymerase I (Klenow fragment), which resulted in a *Sal*I site flanked by two *Eco*RI sites (5′-GAATTCGTCGACGAATTC-3′).

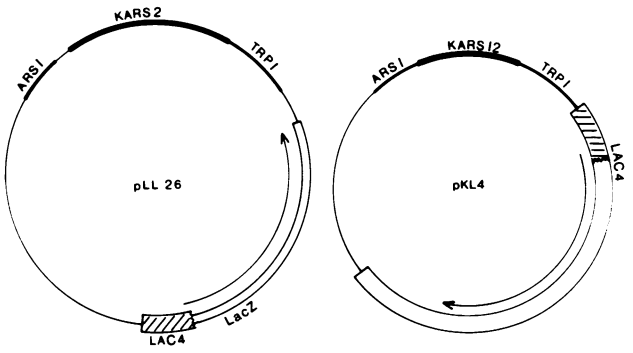
The *LAC4* promoter fragments 381, 292, 174, and 26 were generated by BAL 31 starting from the unique *Cla*I site of pSH4 at position +356 (4) of the *LAC4* coding region. The plasmid was religated in the presence of *Eco*RI linkers (CGGAATTCCG; Boehringer). The deletion endpoints were determined by sequencing. *Sal*I linkers were added to the promoter fragment after the *Eco*RI restriction sites were filled in.

The naming of the fragments indicates the position of the last *LAC4* nucleotide at the 3′ end of the fragments with respect to the start of translation in the intact *LAC4* gene. The 5′ end of all fragments is given by the *Sal*I site at position −675; *LAC4* sequences start with position −670.

Fragments 326 and 423 were obtained by using the conveniently located restriction sites *Taq*I and *Ava*II, respectively. In each case the fragments were blunt-end ligated into the *Sal*I site of pBR322 after the ends were filled in or after *Exo*III treatment (for the *Ava*II site). From the resulting plasmids the *LAC4* sequences could be obtained on *Sal*I fragments that were inserted into the unique *Sal*I site of the 5′ deletions. The sequence of all deletion junctions shown in Fig. 2 is listed in Table 1.

RESULTS

As we have shown previously (6), the *LAC4* gene containing 670 base pairs (bp) of the 5′- and about 1 kb of the 3′-noncoding region is correctly regulated when located on a plasmid. To identify the sequences that are responsible for regulation, we isolated a series of promoter fragments as described above, extending from position −675 to various positions at the 3′ end (e.g., a fragment from −675 to −26 is named fragment 26).



β-Galactosidase activity in transformants:

	pLL26	pKL4
induced	440	11400
non-ind.	10	300
ratio i/ni	44	38

FIG. 1. Fusion of the *LAC4* promoter to the bacterial *lacZ* gene showing that all sequences required for regulation of *LAC4* reside in the 5'-noncoding region. Plasmids pLL26 and pKL4 consist of pBR322 sequences (thin line), the *TRP1 ARS1* fragment from *S. cerevisiae* (thick line), a *K. lactis* fragment confering autonomous replication (*KARS*) (black box), and a β-galactosidase gene (*lacZ* from *E. coli* or *LAC4* from *K. lactis*). In each case the *LAC4* 5'-noncoding region from nucleotides −668 to −26 is indicated by a hatched box. In the case of pLL26 this sequence is linked to the *lacZ* structural gene by a synthetic linker (supplying the ATG initiation codon), whereas the hatched box in pKL4 is part of the intact *LAC4* gene. Details of the constructions are described in the text. β-Galactosidase activities in transformants with plasmids pLL26 and pKL4 are compared. Enzyme levels were measured in crude extracts by cleavage of *o*-nitrophenyl-β-D-galactopyranoside, as described in the text, after cells were grown in 2% glucose (noninducing [non-ind.] conditions) or 2% galactose–0.5% glucose (induced). The ratio of the induced versus noninduced (i/ni) enzyme activity is indicated. The copy number of the two plasmids was comparable, as determined by Southern analysis. Numbers represent milliunits per milligram of protein.

In a first fusion we ensured that the signals conferring transcriptional regulation of the *LAC4* gene resided within the 5' end of the gene. Therefore, fragment 26, which encompassed almost the whole 5' region, was inserted in front of the *E. coli lacZ* structural gene on an *E. coli*-yeast shuttle vector resulting in the plasmid pLL26 (see above for details; Fig. 1). Position −26 of the *LAC4* 5'-noncoding region was fused to the *lacZ* gene by 17 bp of synthetic linker DNA supplying the ATG initiation codon. pLL26 and a control plasmid, pKL4, which carries the intact *LAC4* gene, were introduced into *K. lactis* SD11 (*lac4 trp1*). β-Galactosidase activities measured in crude extracts of transformants grown in yeast nitrogen base plus lactose (induced) and yeast nitrogen base plus glucose (noninduced) were compared (Fig. 1). In pLL26 the *LAC4* promoter fragment 26 conferred a 40-fold induction to *lacZ* expression, which was similar to that of the intact *LAC4* gene in pKL4. Thus, the regulatory sequences reside in the 5'-noncoding region between nucleotides −670 and −26.

We have shown previously (6) that the regulation of the cloned *LAC4* gene is not affected by deleting the 5'-noncoding region up to position −435, whereas a deletion up to position −392 severely reduced induction and a deletion to position −254 totally abolished induction (compare pK114, pK7, and pK147; Fig. 2). To localize the sequences that are essential for induction of the *LAC4* gene, we constructed internal deletions in the 5'-noncoding region.

The 3' border of an essential region is located between position −326 and −381: pK147-292 (fusion of −292 and −254) and pK147-326 (fusion of −326 and −254) show normal induction, whereas pK147-381 (fusion of −381 and −254) totally lost induction. When fragment 292 was inserted in an opposite orientation (pK147-292.2) normal induction was also observed. Thus, fragment 292 has characteristics of UAS_L. It activates gene expression and it acts irrespective of its orientation.

Analysis of the *LAC4* nucleotide sequence had shown a region of dyad symmetry in the upstream region located at about position −330 (4). The wild-type expression of pK147-326 (fusion of −326 and −254) lacking one-half of the inverted repeat shows that disruption of this symmetrical structure has no effect on induction of the *LAC4* promoter by lactose. Because, as mentioned above, sequences upstream from position −435 do not effect the *LAC4* induction (compare pK114; Fig. 2), UAS_L should be located between positions −435 and −326.

The residual activity of pK7 (deletion to −392; Fig. 2) and the normal regulation of the internal deletion pK7-423 (fusion of −423 and −392) show that UAS_L is composed of at least two distinct elements, one that is upstream of −423 (segment A) and another that is downstream of −392 (segment B). Inversion of fragment −423 (pK7-423.2) slightly affects induction by a factor of 2 to 3, which indicates the requirement for a certain spacial relationship between the two elements.

The deletions described to this point affect regions upstream of position −254. To determine whether additional elements downstream of position −254 contribute to UAS_L function, we tested an internal deletion pK4-292 (fusion of −292 and −176). This deletion drastically affected induction, resulting in about 10-fold reduced enzyme levels under inducing conditions (Fig. 2). The insertion of 15 bp (linker and a 3-bp duplication) at position −176 had no effect, as shown by the construct pK4-174. In contrast, when fragment 174 was placed in the opposite orientation, as in pK4-174.2, the level of induction was lowered to the same extent as that in pK4-292. The region between −254 and −176 thus contains sequences the function of which is orientation or position dependent, or both. These properties usually are not observed for UAS elements.

We propose that the reduction in *LAC4* expression in pK4-292 might be caused by the deletion of a TATA-like sequence, TATTTAATA, that is located at about position −230. The deletion of two other TATA boxes at −170 and −140 (4) also results in a lower expression, as shown by pK6-174 (fusion of −174 and −139), but the effect was less drastic than that with pK4-292. Further work is in progress to determine which TATA box(es) influence(s) the transcription initiation sites.

The properties of a UAS element were shown by fragment 292 (see above). To test whether this fragment is sufficient to confer regulation by lactose, we fused it to a heterologous promoter. For this purpose we used the plasmid pLG669-Z (17) which carries a fusion of the *CYC1* promoter of *S. cerevisiae* with the structural *lacZ* gene of *E. coli*. The *CYC1-lacZ* fusion has already been used to show that certain regulatory sequences function as a UAS (18). The expression of the *CYC1* promoter is normally under the control of two UAS sequences which are located between *Xho*I sites (15, 16). Therefore, the two UAS elements can be easily deleted and substituted by any UAS-like sequence to be

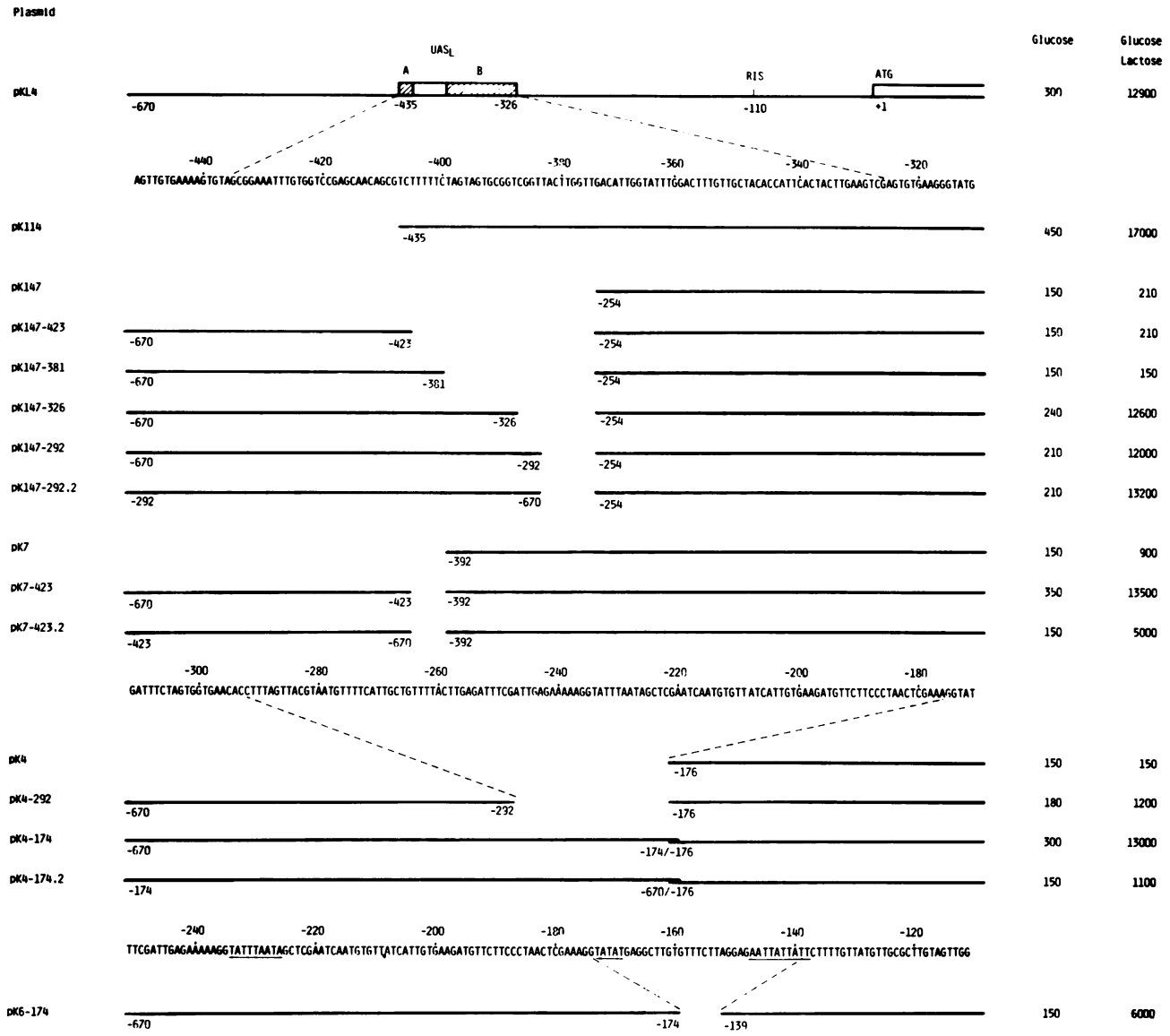


FIG. 2. Effects of internal deletions within the 670-bp upstream region of the cloned *LAC4* gene on β-galactosidase activity in *K. lactis* SD11 transformants. The constructs are derivatives of pKL4 (Fig. 1), as described in the text. The 5′ end of the *LAC4* gene is schematically shown; the RNA initiation site (RIS) and the start of translation (ATG) are indicated. The position of UAS$_L$ is a result from the deletion analysis. The hatched boxes within UAS$_L$ are segments A and B, as defined in the text. Lines represent the *LAC4* sequences that are still present in the various deletions. The numbers refer to the distance from the ATG codon and indicate the nucleotide to which linker sequences were added (compare with Table 1 for the sequence of deletion junctions). The sequence of the *LAC4* upstream region is given in three partially overlapping segments above the relevant deletions. TATA-like sequences between −250 and −110 are underlined. Each deletion plasmid was introduced into strain SD11, and enzyme activity was determined in crude extracts of the transformants. β-Galactosidase activities are expressed in milliunits per milligram of protein as defined in the text. Plasmid loss was tested by measuring retention of the Trp$^+$ phenotype and was comparable under both growth conditions.

tested. The fused promoter then comes under the control of the inserted UAS.

In pLG669-Z (Fig. 3) we deleted the *CYC1* sequence upstream of position −248, which carried the *CYC1* UASs (resulting in plasmid pLC0), and inserted fragment 292 in both orientations at this position (plasmid pLC1 and pLC2). At the unique *Sal*I site of pLG669-Z we inserted an autonomously replicating sequence of *K. lactis*, KARS12, and the *ARS1-TRP1* fragment of *S. cerevisiae* (35). *K. lactis* SD11 was transformed with the three plasmids, and β-galactosidase activity was measured.

The fused UAS$_L$-*CYC1* promoter was induced by lactose, irrespective of the orientation of fragment 292 in the fusion (Fig. 3). This indicates that the information on fragment 292 alone is sufficient to confer the *LAC4*-specific regulation.

Evidence for homology between the regulatory elements of the lactose and galactose genes in *K. lactis* and the galactose and melibiose genes in *S. cerevisiae* exists. As mentioned above, the cloned *LAC9* gene from *K. lactis* on a multicopy plasmid can complement a *gal4* mutation of *S. cerevisiae* (31), and vice versa, a mutation within the *K. lactis LAC9* gene results in a noninducible phenotype of the lactose-galactose regulon, which can be relieved by the *GAL4* gene product (M. I. Riley et al., unpublished data).

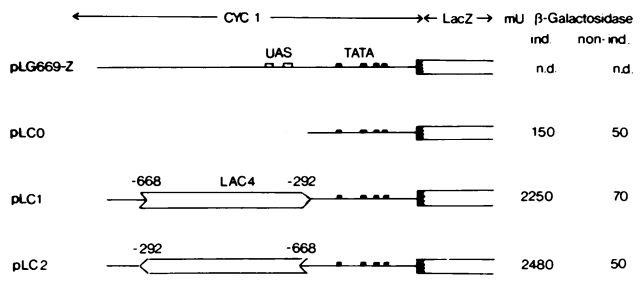


FIG. 3. Lactose regulation of the *CYC1*-promoter in fusions with the *LAC4* UAS. The diagram shows the relevant part of the plasmids used to determine the β-galactosidase activity directed by the *S. cerevisiae CYC1* promoter in a *CYC1-lacZ* gene fusion (17). Details of the constructs are described in the text. The location of the UAS (open boxes) and TATA boxes (closed boxes) in the *CYC1* promoter region are indicated. pLC0 does not contain a UAS; *CYC1* sequences upstream of the *Xho*I site at −247 were removed. In pLC1 and pLC2 the *CYC1* UASs between −247 and −670 were replaced by the *LAC4* regulatory region from −675 and −292 in the original and reverse orientation, respectively. β-Galactosidase levels in *K. lactis* SD11 transformed with the indicated plasmids were measured after growth in galactose (inducing [ind.] conditions) or glucose (noninducing [non-ind.] conditions) and are expressed in milliunits per milligram of protein, as defined in the text.

UAS_G, which is involved in the induction of the *S. cerevisiae* structural genes *GAL1* and *GAL10*, has been shown to be the binding site for the *GAL4* protein; the binding occurs to four related 17-bp sequences (2, 13). A unique near consensus, 17-bp synthetic oligonucleotide has been shown to be sufficientto mediate *GAL4*-dependent activation of transcription when placed in front of the *GAL1* gene (13). A comparison of the upstream sequences of six galactose-inducible genes in *S. cerevisiae* has revealed the presence of this sequence (2) in all of them.

On the basis of these observations we addressed the question as to whether there is any homology between UAS_L and UAS_G. By computer analysis we compared the consensus sequence proposed by Giniger et al. (13) as being a *GAL4* protein recognition sequence, with 700 bp of *LAC4* 5′-flanking sequences. Both strands were scanned with the 9-bp half sequence $CGGA\frac{G}{C}GACA$. The most obvious homology was found in a region between −434 and −418. These sequences are aligned in Fig. 4, which shows that 11 of 17 bp are identical. Seventeen base pairs were identical to the slightly extended 23-bp consensus sequence described by Bram et al. (2) when a 1-bp gap was inserted (data not shown). Because our deletions mapped an essential element of the *LAC4* UAS between positions −435 and −423, we propose that segment A of UAS_L is identical to the region of homology with the *GAL4* UAS and might represent a binding site for the *LAC9* gene product. As shown by the constructs pK147-423 and pK147-381, however, this sequence is not sufficient to mediate regulation by lactose. We could not detect any repeat of the region of homology between −391 and −326, where we mapped the second essential element of UAS_L. So the basis for the importance of these sequences still remains to be elucidated.

DISCUSSION

The fusion of heterologous promoter elements has been applied to a number of other yeast genes (1, 18, 19), as well as control elements of higher eucaryotes (5), and has demonstrated the mosaiclike structure of eucaryotic promoters. In most yeast genes at least two promoter elements have been identified: the sequences surrounding the transcription initiation site(s), including one or several TATA-boxes, and a positive control element, UAS, which activates transcription in response to the inducer (for a review see reference 14). The UAS can function in both orientations and also can function in connection with a heterologous TATA box. In this respect the *LAC4* gene is a typical yeast gene in that it possesses a UAS element that confers regulation by lactose and galactose and a TATA-like element that is close to the transcription initiation sites.

One focus in this study was the identification of the UAS of *LAC4*. We mapped this element, UAS_L, between positions −435 and −326. Analysis of internal deletions led to the conclusion that UAS_L is composed of at least two essential sequences (segments A and B) located between positions −435 and −423 and between positions −392 and −326, respectively. Interestingly, the region between positions −434 and −418 shows a high homology to the *GAL4* protein binding site of *S. cerevisiae* as proposed by Giniger et al. (13). This sequence was detected by computer comparison as the region of highest homology within the 700 bp at the 5′ end of *LAC4*. In fragment 423, which shows wild-type induction when fused to the −392 deletion of pK7, the last five nucleotides of the 17-bp UAS_G consensus sequence were deleted. The sequence of the *Sal*I linker that is fused to the deletion endpoint, however, resembles the wild-type sequence. As shown below, if one allows for a 1-bp gap, two of the last four bases are identical and the last one is still a purine.

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                    -434                -418
Wild-type LAC4      CGGAAATTTGTGGTCCG
pK7-423             CGGAAATTTGTG TCGACGC
                                -423
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We therefore propose that the 17-bp UAS_G consensus sequence is functionally identical to segment A of UAS_L, which encompasses the region between positions −434 and −418.

Segment A represents an example of an evolutionarily conserved regulatory sequence. We suggest that this sequence is a binding site for the *LAC9* gene product. The *LAC9* gene of *K. lactis* was recently cloned by complementation of a *gal4* mutant of *S. cerevisiae* (31). Comparison of the amino acid sequence of the *LAC9* protein, as deduced from the DNA sequence with the *GAL4* protein, showed several regions of homology. One conserved region was located at the N-terminal end, which was shown to represent

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GAL     CGGA(G/C)gacaGTcGTCCG
LAC4    CGGAaatttGTgGTCCG
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FIG. 4. Homology of *LAC4* regulatory sequences with the *GAL4* protein binding site. The upper line shows the consensus sequence deduced by Giniger et al. (13) by comparison of the regulatory regions of *GAL1*, *GAL10*, and *GAL7*. This sequence is aligned with the *LAC4* sequence between −434 and −418. Lowercase letters are used for nonidentical nucleotides. The *LAC4* sequence was found as the region of highest homology within the 700-bp upstream region by scanning with the sequence $CGGA\frac{G}{C}GACA$ with a computer program (Pustell sequence analysis programs; International Biotechnologies Inc.). The previously published *LAC4* sequence (4) contains a mistake in this region. The corrected version is shown here.

a DNA-binding domain of the *GAL4* protein (20, 21), and a second one was found at the C-terminal end, which might be required for transcriptional activation (3). We suggest that the *LAC9* gene acts in a way analogous to that of the *GAL4* protein of *S. cerevisiae* by directly binding to segment A of UAS_L, which mediates activation of *LAC4* transcription.

Our suggestion that segment A is a binding site for a regulatory protein is supported by data presented elsewhere (K. D. Breunig and P. Kuger, submitted for publication). The sequence between −436 and −412 is protected against DNase I digestion when a DNA fragment containing UAS_L is incubated with a *K. lactis* crude extract.

Because our deletion data have shown that a second region of UAS_L is essential to mediate the regulation of *LAC4*, we addressed the question as to whether segment B could represent an additional *LAC9* protein binding site(s). We looked for repeats of segment A in the region between positions −392 and −326, but we found no significant homology. Several sequences slightly resembled the 17-bp sequence, but such homologies were found throughout the 700-bp region that was scanned. Thus, at present we cannot decide whether segment B contains additional *LAC9* protein binding site(s) or whether it is an element of a different nature.

The deletion analysis of the *LAC4* 5′-noncoding region revealed another promoter element between positions −292 and −176. It contained a TATA-like sequence at position −230. The fact that this element did not function when placed upstream of the UAS_L (compare pK4-174.2; Fig. 2) supports the hypothesis that this might be the functional TATA box (or one of several). A further analysis of this promoter element certainly requires analysis of RNA initiation sites and is in progress.

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