MOLECULAR AND CELLULAR BIOLOGY, Feb. 1987, p. 780–786
0270-7306/87/020780-07$02.00/0

Vol. 7, No. 2

GAL4 of *Saccharomyces cerevisiae* Activates the Lactose-Galactose Regulon of *Kluyveromyces lactis* and Creates a New Phenotype: Glucose Repression of the Regulon

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Received 15 August 1986/Accepted 17 November 1986

A *Kluyveromyces lactis* mutant defective in *lac9* cannot induce β-galactosidase or galactokinase activity and is unable to grow on lactose or galactose. When this strain was transformed with the *GAL4* positive regulatory gene of *Saccharomyces cerevisiae* it was able to grow on lactose or galactose as the sole carbon source. Transformants bearing *GAL4* exhibited a 4.5-h generation time on galactose or lactose, versus 24 h for the nontransformed *lac9* strain. A *K. lactis lac9* strain bearing two integrated copies of *GAL4* showed 3.5-fold induction of β-galactosidase activity and 1.8-fold induction of galactokinase activity compared with 15.6-fold and 4.4-fold induction, respectively, for the *LAC9* wild-type strain. In transformants bearing 10 integrated copies of *GAL4*, the induced level of β-galactosidase was nearly as high as in the *LAC9* wild-type strain. In addition to restoring lactose and galactose gene expression, *GAL4* in *K. lactis lac9* mutant cells conferred a new phenotype, severe glucose repression of lactose and galactose-inducible enzymes. Glucose repressed β-galactosidase activity 35- to 74-fold and galactokinase activity 14- to 31-fold in *GAL4* transformants, compared with the 2-fold glucose repression exhibited in the *LAC9* wild-type strain. The *S. cerevisiae MEL1* gene was repressed fourfold by glucose in *LAC9* cells. In contrast, the *MEL1* gene in a *GAL4 lac9* strain was repressed 20-fold by glucose. These results indicate that the GAL4 and LAC9 proteins activate transcription in a similar manner. However, either the *LAC9* or *GAL4* gene or a product of these genes responds differently to glucose in *K. lactis*.

The galactose-melibiose regulon of *Saccharomyces cerevisiae* and the galactose-lactose regulon of *Kluyveromyces lactis* have several common features. Both have a galactose gene cluster with the order *GAL7-GAL10-GAL1* (2, 24). These clusters code for galactose-1-phosphate (gal-1-P) uridyl transferase (*GAL7*), UDP-galactose-4-epimerase (*GAL10*), and galactokinase (*GAL1*) (10, 24). Each regulon also contains an unlinked permease gene, galactose permease in *S. cerevisiae* (*GAL2* [28]) and lactose permease in *K. lactis* (*LAC12* [24]), and a hydrolyase gene, α-galactosidase in *S. cerevisiae* (*MEL1* [17]) and β-galactosidase in *K. lactis* (*LAC4* [27]). Both regulons can be induced with galactose or a dissacharide, melibiose for *S. cerevisiae* (17) and lactose for *K. lactis* (8). Both are positively regulated, by *GAL4* in *S. cerevisiae* (10) and *LAC9* in *K. lactis* (unpublished results), as well as negatively regulated, by *GAL80* in *S. cerevisiae* (11) and *LAC10* in *K. lactis* (9).

These phenomenological and organizational similarities suggest functional and mechanistic similarities between the two regulons. Recently it has been shown that the *K. lactis GAL1-GAL10-GAL7* cluster is inducible by galactose in *S. cerevisiae* (Webster and Dickson, unpublished results), indicating *GAL4* and *GAL80* regulation of the *K. lactis* cluster. These data directly imply that *GAL4* and *LAC9* activate transcription in a similar manner. No direct implication between the similarity of *GAL80* and *LAC10* can be drawn from these data. Since GAL4 protein must bind to upstream activator sequences (UAS_{GAL}) (3, 14) in order to activate transcription, one would expect the *K. lactis GAL* genes to have sequences related to UAS_{GAL}, and they do (4; S. Bhairi, Ph.D. thesis, University of Kentucky, Lexington, 1984; Webster and Dickson, unpublished results).

Despite the phenomenological and organizational similarities the regulons are strikingly different in their response to glucose. The galactose-melibiose regulon is severely repressed by glucose (1), while the galactose-lactose regulon is not (8), even though other *K. lactis* genes are severely repressed (20). Circumstantial evidence suggests that differences in the GAL4 and LAC9 proteins may underlie the difference in the way the regulons respond to glucose. Footprint analysis of the 5′-flanking region of the *GAL1* and *GAL10* genes in *S. cerevisiae* detects *GAL4*-dependent binding to the region in nuclei from cells grown in glycerol plus lactic acid and on glycerol, lactic acid, and galactose medium, but not on glycerol, lactic acid, and glucose medium (14, 19). Overall, the implication is that glucose repression acts via interference with the binding of the *GAL4* protein to UAS and that in *K. lactis* the corresponding binding of the *LAC9* protein to UAS is relatively insensitive to interference by glucose.

These observations and considerations prompted us to determine just how similar in function the GAL4 and LAC9 proteins are. In particular we asked whether the *GAL4* gene can complement a *lac9*-defective strain of *K. lactis* and restore galactose inducibility and whether such complementation exhibits weak or strong glucose repressibility. To assess the expression of an *S. cerevisiae* galactose-melibiose regulon target gene in such cells, we constructed *K. lactis* strains bearing the *S. cerevisiae MEL1* gene. Our results provide clear evidence that *GAL4* can complement the transcriptional activation function of *LAC9*. A remarkable

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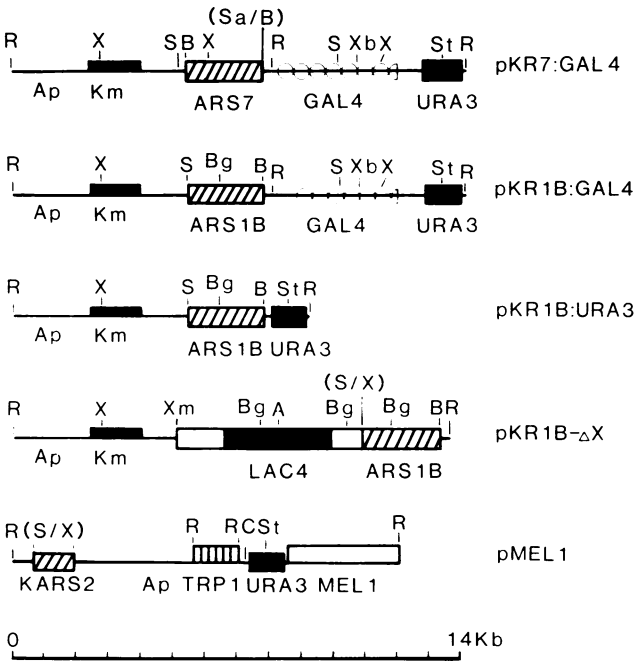

FIG. 1. Structure of yeast plasmids. Plasmids containing the *S. cerevisiae GAL4* gene were constructed by inserting a 6-kb *Eco*RI fragment containing *GAL4* and *URA3*, obtained from plasmid SJ3 (16), into the *Eco*RI site of pKR7 (30) to give pKR7:GAL4 and into the *Eco*RI site of pKR1B (30) to give pKR1B:GAL4. The construction of pKR1B-ΔX has been described (29). Plasmid pMEL1 was constructed by transferring a 4.83-kb *Cla*I-*Bam*HI fragment from pMP550 (22), containing the *S. cerevisiae URA3* and *MEL1* genes, into the corresponding restriction sites in pKARS2 (5). Plasmid sequences: solid thin line, pBR322; cross-hatched box, *K. lactis ARS*; Km, the Tn*903* gene that confers resistance to kanamycin in *E. coli* and the antibiotic G418 in yeast; Ap, ampicillin resistance; *TRP1* and *URA3*, *S. cerevisiae* genes that complement the *trp1* and the *ura3* mutations of *K. lactis*, respectively; *LAC4*, β-galactosidase gene from *K. lactis*. Abbreviations: A, *Asp*718; B, *Bam*HI; Bg, *Bgl*II; C, *Cla*I; H, *Hin*dIII: R, *Eco*RI; Sa, *Sau*3A; S, *Sal*I; St, *Stu*I; X, *Xho*I; Xb, *Xba*I; Xm, *Xma*III. Restriction sites shown in parentheses were lost during construction of plasmids. Not shown is an *Asp*718 site about 100 bp to the right of the *Bgl*II site in *ARS1B*. Not all *Eco*RI or *Cla*I sites present in these plasmids are indicated. Original references should be consulted for their location.

aspect of this complementation is the severe glucose repressibility that is bestowed on the *K. lactis* galactose-lactose regulon.

MATERIALS AND METHODS

Strains and media. Strains 9B383 (*lac9-3 ura3 met2*) and 10B383 (*ura3-1 met2-2*) came from crossing a *ura3* derivative (Dickson, unpublished data) of wild-type strain Y1140 (26) and a *lac9-3 met2-2* strain derived from MS26 (26). The *trp1* marker was introduced by crossing strain 10B383 to a *trp1-1 his2-2* strain to yield strain 7B520 (*ura3-1 trp1-1 his2-2*). The *trp1* marker in strain 10B383 was derived from strain SD11 (*lac4 trp1* [5]) by crossing out the *lac4* marker and introducing a *his2* marker. As described in the text, strain 7B520 was used to introduce the *trp1* markers into strains W and Q. All strains except SD11 are derivatives of Y1140 (26). The conditions for growing cells in minimal medium containing lactose (MinLac) or galactose (MinGal) have been described (24). Carbon sources were added to a final concentration of 2% (wt/vol) as indicated.

Plasmids and yeast transformation procedures. The plasmids used in these studies are presented in Fig. 1. *K. lactis* was transformed with plasmid DNA by the procedure of Sreekrishna et al. (30) except that 60% instead of 40% polyethylene glycol 4000 (BDH Chemicals Ltd., Poole, England) was used to promote cell fusion.

Miscellaneous procedures. Procedures for measuring β-galactosidase and galactokinase specific activities in cell extracts have been described (24). The procedure of Post-Brittenmiller et al. (22) was used to measure α-galactosidase activity, except that cell extracts were used in place of whole-cell homogenates: both procedures gave similar α-galactosidase activity. β-Glucosidase activity was measured in the same way as β-galactosidase activity except that *p*-nitrophenyl-β-D-glucopyranoside (Sigma Chemical, St. Louis, Mo.) was the substrate. A molar extinction coefficient of 18.3×10^3 at 400 nm was used for calculating specific enzyme activities.

Yeast DNA was isolated by the procedure of Davis et al. (6). For restriction endonuclease cutting, 2 μg of DNA was digested in a total volume of 100 μl; digestions in smaller volumes were often incomplete due to unidentified inhibitory compounds. Southern blots were done by the method of Reed and Mann (23). DNA probes for Southern blots were labeled with [α-^{32}P]dCTP (3,000 Ci/mmol; New England Nuclear Corp.) by a published procedure (13).

RESULTS

***GAL4* complements *lac9* for growth on lactose and galactose.** Strains of *K. lactis* that are defective in *lac9* grow very slowly on MinLac and MinGal plates and are not inducible for β-galactosidase, galactokinase, epimerase, or transferase activity (unpublished results). Based on this phenotype, *LAC9* is considered a positive regulator of the lactose-galactose regulon of *K. lactis*. As discussed in the Introduction, circumstantial evidence suggests that *GAL4* and *LAC9* might share some functions. As an initial direct test of this possibility, strain 9B383 (*ura3 lac9*) of *K. lactis* was transformed with pKR7:GAL4 (Fig. 1), and Ura$^+$ transformants were selected. Transformants grew at a moderate rate on MinLac or MinGal plates, suggesting that *GAL4* was complementing *lac9*. Growth rates were determined by growing cells in liquid medium. Transformants of the *lac9* strain carrying pKR7:GAL4 grew on galactose or lactose with a generation time of about 4 to 4.5 h (Fig. 2). On lactose there was a lag in growth that may reflect a low basal rate of intracellular lactose transport, which is induced slowly. For controls, strain 9B383 was transformed with the parent vector pKR7. These transformants had a generation time of about 24 h on lactose or galactose (Fig. 2). For comparison, a *LAC9* strain of *K. lactis* had a generation time of 105 ± 5 min under these growth conditions (data not shown). From these experiments we conclude that *GAL4* was able to complement *lac9* for growth on lactose and galactose.

***GAL4* complements *lac9* for induction of β-galactosidase activity.** From the preceding results we would expect a *lac9* strain carrying *GAL4* to show either induction of the lactose-galactose catabolic enzymes or a high constitutive level of the enzymes. To distinguish between these alternatives, we measured the kinetics of β-galactosidase induction. To facilitate comparison with experiments presented later, we used the *K. lactis* vector pKR1B, which can be integrated into *K. lactis* chromosomes more readily than pKR7. Strain 9B383 was transformed with pKR1B:GAL4 (Fig. 1), and Ura$^+$ colonies were examined for induction of β-galactosidase

activity. A *lac9* strain carrying pKR1B:GAL4 in the autonomous state had a normal uninduced level of β-galactosidase activity which slowly increased during 24 h of growth in the presence of inducer and reached about 30% of the level of enzyme activity found in a *LAC9* strain transformed with the control plasmid pKR1B:URA3 (Fig. 3). Thus, *GAL4* confers an inducible, not a constitutive, phenotype on the lactose-galactose regulon of *K. lactis*. The low level of enzyme induction observed in these populations of transformed cells is most likely due to plasmid instability, since strains carrying stably integrated copies of *GAL4* showed higher enzyme levels (see below).

***GAL4* confers a new phenotype on *K. lactis*.** Wild-type *K. lactis* shows very little (twofold) glucose repression of the lactose-galactose regulon (8), although this yeast does show severe glucose repression of other genes (20). In contrast, *S. cerevisiae* shows severe glucose repression of the melibiose-galactose regulon (1) and many other genes (7, 12, 32). To determine whether severe glucose repression of the lactose-galactose regulon occurs when *GAL4* is controlling the regulon, we measured β-galactosidase induction in a *GAL4* transformant of the *lac9*-defective strain 9B383. β-Galactosidase activity was not induced in this strain when both glucose and galactose were present in the culture medium (Fig. 3). For example, cells contained 20 times less β-galactosidase activity after 24 h of growth in the presence of glucose (0.7 U of enzyme per A_{600} unit) than in the absence of glucose (14.2 U of enzyme per A_{600} unit). In the *LAC9* control strain carrying pKR1B:URA3 grown in the presence of both glucose and galactose, glucose repressed β-galactosidase activity about twofold (46 versus 25 U of enzyme per A_{600} unit). This evidence suggests that *GAL4* activity is more sensitive than *LAC9* activity to glucose repression.

Integration of *GAL4* into a *K. lactis* chromosome. The vectors pKR7, pKR1B, and their derivatives are present in multiple copies, 5 to 10 per cell, and a large fraction of cells (50 to 75%) lack the vector even when cells are grown under conditions that select for a vector-borne gene, either G418 or *URA3* selection pressure (30). To avoid dealing with a heterogeneous population of cells and with a variable number of *GAL4* per cell, we integrated pKR1B:GAL4 into a *K. lactis* chromosome. The plasmid was cleaved at its unique *Bgl*II site located in *ARS1B* (Fig. 1) and then used to obtain Ura$^+$ transformants of strain 9B383. The vector was cleaved with *Bgl*II to direct integration to the chromosomal *ARS1B* locus (25). Four transformants showing stability for the Ura$^+$ and G418^r phenotypes were obtained. All four were also Lac$^+$. They were screened first for resistance to various concentrations of G418 since there is a correspondence between the number of kanamycin resistance (Km^r) genes in a cell and their resistance to G418 (unpublished results). On this basis strains W and A were resistant to 100, strain K to 300, and strain Q to 500 μg of G418 per ml. As shown below, these resistance levels corresponded to the relative number of copies of the integrated vector.

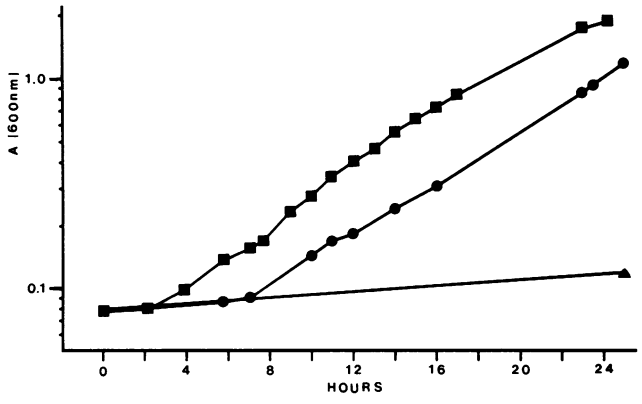

FIG. 2. Growth rate of a *K. lactis lac9* strain transformed with the *GAL4* gene of *S. cerevisiae*. *K. lactis* strain 9B383 (*ura3 lac9*) transformed with pKR7:GAL4 was grown at 30°C in supplemented minimal medium lacking uracil and containing 2% galactose (■) or 2% lactose (●) as the sole carbon source. The control (▲) was strain 9B383 transformed with the parent vector pKR7 grown on medium containing 2% lactose and uracil (10 μg/ml). Plasmids were maintained by selection for the *URA3* gene.

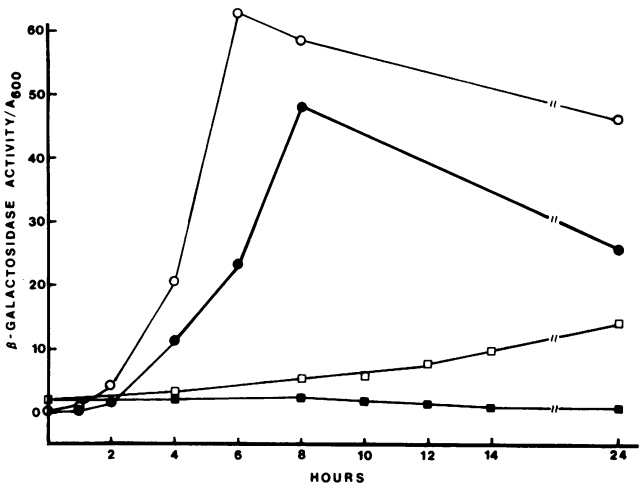

FIG. 3. Induction and glucose repression of β-galactosidase activity in a *lac9*-defective strain carrying *GAL4* on an autonomously replicating plasmid. *K. lactis* strain 9B383 (*ura3 lac9*) transformed with pKR1B:GAL4 was pregrown to saturation at 30°C in supplemented minimal medium lacking uracil and containing 2% sorbitol. At time zero the cells were diluted to an A_{600} of 0.05 and grown in the same medium plus 2% galactose in the absence (□) or presence (■) of 2% glucose. The *LAC9* control strain 10B383 transformed with pKR1B:URA3 was grown in the same way in the absence (○) and presence (●) of 2% glucose. At the end of 14 h of growth the cultures were diluted 50-fold into fresh prewarmed medium to maintain log-phase growth until the 24-h time point.

Although integration of pKR1B:GAL4 was directed to the chromosomal *ARS1* by cleaving the vector within *ARS1B*, the vector could conceivably have integrated at *LAC9* if *GAL4* had some DNA sequence homology to *LAC9*. If *GAL4* had integrated at *LAC9* then tetrads from a cross of the integrated strain to a *LAC9* strain should all have the parental ditype configuration (4 Lac$^+$:0 Lac$^-$). From the cross W × 7B520 there were four parental ditype, seven tetratype (3 Lac$^+$:1 Lac$^-$), and three nonparental ditype (2 Lac$^+$:2 Lac$^-$) tetrads. From the cross Q × 7B520 there were one parental ditype, nine tetratype, and three nonparental ditype tetrads. Thus, the vector was not integrated near *lac9* in strain W or Q. The meiotic behavior of these crosses was verified by scoring progeny spores for the vector-borne phenotypes Ura$^+$ (*URA3*) and G418^r resistance (Km^r). In both crosses we observed cosegregation of the Lac$^+$ (*GAL4*), Ura$^+$ (*URA3*), and G418^r phenotypes, as expected for close linkage of the vector genes. These results indicate that in both strains Q and W the vector integrated at one site, but close linkage of multiple integration sites cannot be

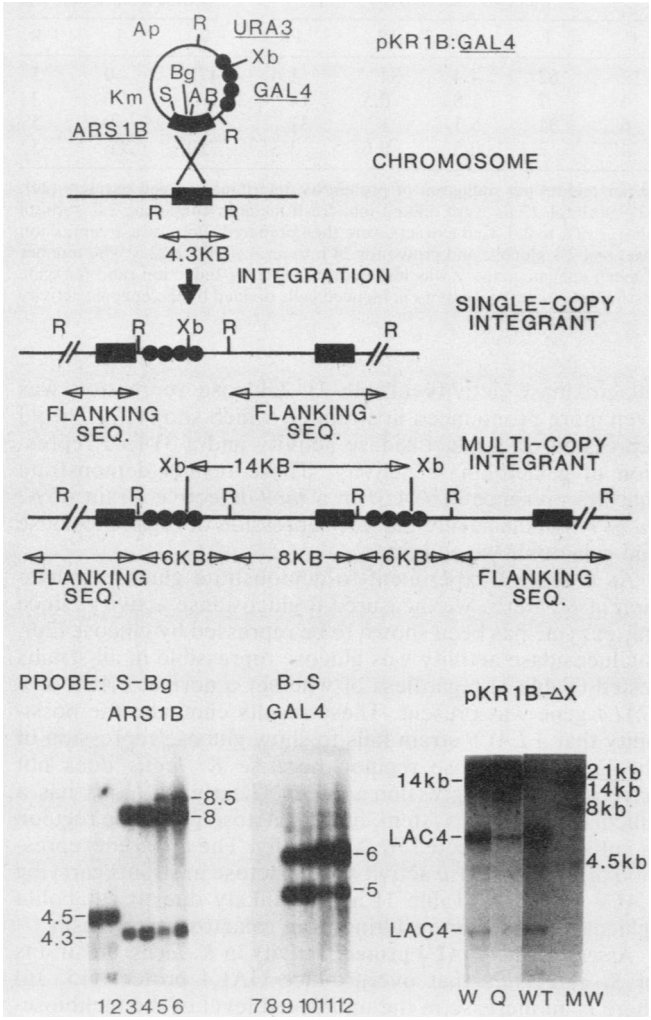

FIG. 4. *K. lactis* strain carrying *GAL4* integrated at the chromosomal *ARS1* locus. Southern blots were used to determine the chromosomal integration site of pKR1B:GAL4 in *K. lactis* strains W, WW, Q, and QQ. The results of a single- or multiple-copy integration event at the chromosomal *ARS1* locus are diagrammed at the top. Total DNA was extracted from yeast, and 2 μg was cleaved with EcoRI (lanes 1 to 12). The cleaved DNA was fractionated on a 0.6% agarose gel, and then the DNA was transferred to a nitrocellulose filter. The filters were hybridized to the indicated probes, which were labeled with ^{32}P. The *GAL4* probe was a DNA fragment with a *Bam*HI linker 4 bases in front of the ATG initiation codon and a *Sal* site about 1.2 kb downstream. The filter representing lanes 1 to 6 was reprobed to give the autoradiogram represented by lanes 7 to 12. DNA is from *K. lactis*: lanes 1 and 7, strain 7B520; lanes 2 and 8, strain 9B520 (*lac9*); lanes 3 and 9, strain W; lanes 4 and 10, strain WW; lanes 5 and 11, strain Q; lanes 6 and 12, strain QQ. The molecular weight (MW) markers were lambda DNA cleaved with *Ava*I. Southern blot analysis (right panel) was used to determine the number of copies of pKR1B:GAL4 present in strains W and Q. Two micrograms of total yeast DNA was cleaved with the restriction endonuclease *Asp*718, an isoschizomer of *Kpn*I, separated on a 0.6% agarose gel, and transferred to a nitrocellulose filter. The filter was probed with ^{32}P-pKR1B-ΔX (Fig. 1). Lanes: W, strain W; Q, strain Q; WT, strain 7B520 (*LAC9*); MW, molecular weight markers (linearized plasmid DNAs of known size): pBR322, 4.3 kb; pKR1B, 8 kb; pKR1B-ΔX, 14 kb; pKR1B-*LAC4*-1 (29), 21 kb.

excluded completely. From each of these crosses we kept a *trp1* progeny spore, termed WW or QQ, for further analysis.

The chromosomal location of the integrated vector was also examined by Southern blots. Total DNA from strain Q or QQ, strain W or WW, and the parent strains 9B383 and 7B520 was digested with the restriction endonuclease *Eco*RI, blotted onto a filter, and probed with the 1-kilobase (kb) ^{32}P-labeled *Bgl*II-*Sal*I fragment of *ARS1B* (Fig. 4). The untransformed parent strains showed a 4.5-kb band of hybridization corresponding to the chromosomal *Eco*RI fragment carrying *ARS1* (lanes 1 and 2, Fig. 4). If the vector integrated by homologous recombination via its *Bgl*II site with the chromosomal *ARS1B*, then the 4.5-kb *Eco*RI fragment should not be present in the transformed strains. If one copy of the vector integrated, then there should be two new bands of hybridization representing sequences flanking the integrated vector. If more than one copy of the vector integrated in tandem, then there should be an 8-kb band representing the *Eco*RI vector fragment carrying *ARS1B* plus two bands representing sequences flanking the integrated vector (Fig. 4). All transformants lacked the 4.5-kb *Eco*RI fragment found in the untransformed parent, indicating that at least one copy of the vector had integrated into the chromosomal *ARS1B* region. Strains W and WW (lanes 3 and 4, respectively, Fig. 4) were identical and showed two new bands of hybridization at 4.3 and ca. 8 kb. Strains Q and QQ (lanes 5 and 6, respectively) showed these two bands plus a third band at about 8.5 kb. It is not clear from these data whether one or more than one copy of the vector was integrated. For example, in strains W and WW the 8-kb band could either represent a flanking *Eco*RI fragment, which would mean that the transformants carried only one copy of the vector, or contain two comigrating bands, one a flanking sequence and one the vector repeat. In this case the strains would contain two or more integrated vectors. To determine whether one or more copies of the vector had integrated tandemly, we did Southern blots (data not shown) with total yeast DNA cleaved with a restriction endonuclease, *Xba*I, that only cleaves the vector once, in *GAL4*. The rationale for using this approach is diagrammed in Fig. 4. The blots revealed that multiple copies of *GAL4* were present in all strains examined. The blots also suggested that one copy of *GAL4* had been partially deleted (the 5-kb band, Fig. 4, lanes 9 to 12).

Further Southern blots were performed with several restriction endonucleases and various probes in an attempt to resolve the structure of the integrated vectors. These results (data not shown) suggest that a region of the vector containing the *Stu*I site in *URA3* and the *Eco*RI site between *URA3* and the pBR322 sequences has been deleted in one of the integrated vector sequences. As we discuss below, even though one copy of *GAL4* was perturbed in strains Q, QQ, W, and WW, this perturbation did not appear to cause GAL4 protein to be overproduced to any measurable extent.

The number of copies of *GAL4* in strains Q and W was determined (Fig. 4) by cleaving total DNA with the restriction endonuclease *Asp*718 and hybridizing the Southern blot to ^{32}P-pKR1B-ΔX (plasmid diagrammed in Fig. 1). This plasmid hybridized to five DNA fragments found in wild-type *K. lactis*. Two of the five *Asp*718 fragments represented a single-copy gene, *LAC4*, which codes for β-galactosidase. The largest *Asp*718 chromosomal fragment, 22 kb, carried *ARS1B*. The DNA fragment of about 4 kb represented a chromosomal sequence adjacent to *ARS1B*. Autoradiograms were quantitated by densitometry, and all bands of hybridization were normalized to the largest *LAC4* band of about 6

TABLE 1. Enzyme induction and repression in *K. lactis* carrying integrated *GAL4*[a]

LAC9 allele (strain)	β-Galactosidase activity					Galactokinase activity					β-Glucosidase activity		
	U	I	I/U	R	I/R	U	I	I/U	R	I/R	U	I	R
LAC9 (10B383)	93 (9)	1,451	15.6	744	2.0	12	62	4.4	34	1.8	471	9	5
lac9/GAL4 (W)	70 (4)	247	3.5	7	35	4	7	1.8	0.5	14	595	70	3
lac9/GAL4 (Q)	216 (9)	1,250	5.8	17	74	6	31	5.1	1	31	719	9	5
lac9 (9B383)	59 (7)	40	0.7	14	2.4	4	2	0.5	0.6	3.3	710	286	5

[a] Specific enzyme activities are expressed as nanomoles of substrate hydrolyzed per minute per milligram of protein as determined in cell extracts (24). Uninduced cells were grown to saturation at 30°C on minimal medium containing 2% sorbitol. Cells were diluted into fresh medium containing 2% sorbitol (uninduced, U) or 2% sorbitol plus 2% galactose (induced, I) and grown for 24 h to an A_{600} of 1 to 2.5. Cell extracts were then prepared. For glucose repression (R), 24-h-induced cells were diluted into medium containing 2% sorbitol, 2% galactose, and 2% glucose and grown for 24 h to an A_{600} of 1 to 2.5. The number of independent experiments is shown in parentheses. The standard deviation of the mean enzyme activity was less than ±15%. I/U, Induction ratio (enzyme activity in induced cells divided by the enzyme activity in uninduced cells); I/R, repression ratio (enzyme activity in induced cells divided by the enzyme activity in repressed cells).

kb (Fig. 4). From four Southern blots we determined that strain W (and strain WW, data not shown) had 2 copies of *GAL4* and strain Q (and strain QQ, data not shown) had 10 copies. These calculations assume that the 14-kb *Asp*718 fragment represents a vector repeat sequence, the 11-kb band represents a deleted vector sequence, and the 21-kb fragment represents a flanking sequence.

Enzyme induction and repression in strains carrying integrated *GAL4*. Before making quantitative comparisons of enzyme levels, we determined that a steady-state level of induced enzyme activity was achieved after 12 h of induction by growth in the presence of galactose and remained unchanged for up to 36 h. For convenience we used a 24-h induction period. A 24-h period was also used for determination of glucose repression.

Under the growth conditions used here a *LAC9* strain of *K. lactis* showed a 15.6-fold induction of β-galactosidase activity (Table 1) and a 4.4-fold induction of galactokinase activity. The *lac9* strain showed a slight decrease in both enzymes following addition of galactose to the culture. Strain W, carrying two copies of *GAL4*, showed a 3.5-fold induction of β-galactosidase activity and a 1.8-fold induction of galactokinase activity. Strain Q, with 10 copies of *GAL4*, showed a 5.8-fold induction of β-galactosidase activity and a 5.1-fold induction of galactokinase activity. In strain Q the induced level of β-galactosidase activity was nearly as high as in the *LAC9* strain, but the fold induction was low because strain Q had a high uninduced level of the enzyme. Analogous results were obtained for galactokinase activity except that the uninduced level of enzyme activity was low in both *GAL4* strains (Table 1). These results demonstrate that *GAL4* can mimic the transcriptional activation function of *LAC9*, but multiple copies of *GAL4* are needed to obtain the same induction level obtained with one copy of *LAC9*.

Since the lactose-galactose regulon of *K. lactis* and the melibiose-galactose regulon of *S. cerevisiae* respond differently to glucose, we were interested in assessing whether the response in *K. lactis* would be perturbed with the regulon under *GAL4* control. The response of cells to glucose was measured by growing induced cells for 24 h in the presence of glucose and inducer (galactose). In the wild-type *LAC9* strain of *K. lactis*, there was a twofold repression of enzyme activity (Table 1). The same was true of the *lac9* strain, suggesting that either the observed drop in enzyme activity is independent of *LAC9* function or that the *lac9* mutation is leaky. These alternatives cannot be distinguished until a *lac9* deletion strain is available. In contrast to these results, strains carrying *GAL4* displayed strong glucose repression. For example, strain W showed a 35-fold repression of β-galactosidase activity and a 14-fold repression of galactokinase activity (Table 1). Glucose repression was even more pronounced in strain Q, which showed a 74-fold repression of β-galactosidase activity and a 31-fold repression of galactokinase activity. These results demonstrate that the presence of *GAL4* in a *lac9*-defective strain of *K. lactis* results in severe glucose repression of β-galactosidase and galactokinase activity.

As a control experiment to demonstrate glucose repression in *K. lactis*, we measured β-glucosidase activity, since this enzyme has been shown to be repressed by glucose (20). β-Glucosidase activity was glucose repressible in all strains tested (Table 1) regardless of whether a normal *LAC9* or a *GAL4* gene was present. These results eliminate the possibility that a *LAC9* strain fails to show glucose repression of the lactose-galactose regulon because *K. lactis* does not have a glucose repression system. Clearly *K. lactis* has a glucose repression system, but the lactose-galactose regulon is not severely affected by it normally. The apparent repression of β-glucosidase activity by galactose in strains carrying *LAC9* or *GAL4* (Table 1) is most likely due to catabolite (glucose) repression resulting from galactose catabolism.

Assessment of GAL4 protein activity in *K. lactis*. In strains of *S. cerevisiae* that overproduce GAL4 protein (15, 16) there is an increase in the uninduced level of the melibiose-galactose regulon enzymes. We reasoned that if GAL4 protein was overproduced in strain W or strain Q of *K. lactis*, then the uninduced level of the lactose-galactose regulon enzymes should be elevated: the protein level could be so high that induction would not produce an increase in enzyme activity. Only strain Q had an elevated uninduced level of β-galactosidase activity: the level of galactokinase was normal. Both strains, Q and W, induced a β-galactosidase and galactokinase activity (Table 1). These data argue that GAL4 protein activity is sufficient in strain Q (10 copies of *GAL4*) but not in strain W (2 copies of *GAL4*) to cause an increase in the uninduced level of β-galactosidase activity.

To obtain another assessment of the level of GAL4 protein in *K. lactis*, we introduced *MEL1* into this yeast on an autonomous vector, *pMEL1* (Fig. 1). This *S. cerevisiae* gene, which codes for α-galactosidase, is regulated by *GAL4* and should thus serve as an independent assessment of the relative GAL4 protein abundancy in *K. lactis*. The *GAL4*-containing strains WW and QQ and the *LAC9* strain ZZ had the same uninduced level of α-galactosidase activity (Table 2). All three strains induced α-galactosidase activity four- to fivefold. We conclude that GAL4 protein activity in *K. lactis* strains QQ and WW is as effective in stimulating *MEL1* expression as LAC9 protein activity in a *LAC9* strain.

Several other results presented in Table 2 deserve comment. Most notably, *MEL1* expression was more severely

TABLE 2. Induction and repression of *MEL1* in *K. lactis*[a]

LAC9 allele (strain)	α-Galactosidase activity					β-Galactosidase activity					Galactokinase activity				
	U	I	I/U	R	I/R	U	I	I/U	R	I/R	U	I	I/U	R	I/R
LAC9 (ZZ)	103	539	5.2	142	3.8	157	5,441	35	2,888	1.9	7.3	121	17	105	1.2
lac9/GAL4 (WW)	129	462	3.6	71	6.5	260	732	2.8	32	23	4.3	31	7.2	1.1	28
lac9/GAL4 (QQ)	132	470	3.6	23	20	1,773	3,131	1.8	62	51	9.1	68	7.5	2.2	31
lac9 (DD)	30	64	2.1	24	2.7	98	28	0.30	20	1.4	5	4	0.8	2	2

[a] All strains were transformed with pMEL1. The *LAC9* host strain was 7B520, while the *lac9* host strain was 9B383. The fraction of cells carrying pMEL1 was determined prior to making extracts by measuring the frequency of Trp^+ cells in the population. The range for each strain was: ZZ, 40 to 70%; WW, 40 to 60%; QQ, 80 to 90%; DD, 70 to 90%. Enzyme values were not corrected for these variations. See Table 1, footnote *a*, for details. Tryptophan was not included in the medium in order to select for cells carrying pMEL1. The data represent the average of two experiments.

repressed by glucose in the presence of *GAL4* than in the presence of *LAC9*. These results directly imply that *GAL4* confers glucose repression on the galactose-melibiose regulon in *S. cerevisiae*. Second, α-galactosidase activity was more inducible in the *LAC9* than in the *lac9* strain, indicating that *MEL1* is responding to *LAC9*. Third, α-galactosidase activity (Table 2), but not β-galactosidase or galactokinase activity (Table 1), was induced in the *lac9*-defective strain. This suggests that the LAC9 protein produced by the *lac9-2* allele may retain some activity for *MEL1*. Finally, in accordance with the data in Table 1, strain QQ, which had 10 copies of *GAL4*, had a higher uninduced level of β-galactosidase activity than strain WW, which had 2 copies of *GAL4*.

For reasons that we do not understand, all of the uninduced values for β-galactosidase activity shown in Table 2 are higher than those shown in Table 1. This does not seem to be a general trend in these strains, since the values for galactokinase were similar in the two tables. We also note that the induced level of β-galactosidase activity in the *LAC9* strain 10B383 (Table 1) was lower than the corresponding values in the *LAC9* strain 7B520 (Table 2). This difference is due to a propensity of cell extracts made from strain 10B383 to loose β-galactosidase activity rapidly. The value in Table 2 for induced β-galactosidase activity is typical of the 3,000 to 5,000 U we normally see (8, 9).

DISCUSSION

Our results demonstrate that the *GAL4* gene of *S. cerevisiae* can complement a strain of *K. lactis* defective in *lac9* and enable the strain to grow on galactose and lactose. For *K. lactis* to grow on galactose, it must induce expression of *GAL1*, *7*, and *10* (24) and a permease, *LAC12* (unpublished results). To grow on lactose it must induce *LAC4* (27) plus *LAC12* (unpublished results). Thus, *GAL4* is able to activate transcription of all the known structural genes in the galactose-lactose regulon. The GAL4 protein activates transcription in *S. cerevisiae* by binding to a 17-base-pair (bp) UAS (3, 14). We would expect GAL4 protein to function the same way in *K. lactis*, but to do so each gene in the *K. lactis* galactose-lactose regulon would have to have a proper UAS. From DNA sequence analyses all genes in the regulon have a UAS (4; Bhairi, Ph.D. thesis; Webster and Chang, unpublished results) that is related to the UAS_{GAL} of *S. cerevisiae*. By implication LAC9 protein must activate transcription by binding to these UASs. Alternatively, mixing of *lac9* mutant protein subunits and GAL4 subunits might produce an active protein in which the UAS binding is provided by either the LAC9 or the GAL4 subunit and transcription activation activity is provided by the other subunit.

A priori the *GAL4* gene in *K. lactis* could give either inducible or constitutive expression of the galactose-lactose regulon. Measurements of β-galactosidase and particularly galactokinase activity demonstrate that strains carrying either 2 or 10 copies of *GAL4* are inducible for these enzyme activities (Tables 1 and 2). Inducibility implies that GAL4 protein activity is somehow blocked in the absence of galactose. A possible candidate for blocking is the LAC10 protein (9), which at the phenomenological level appears to act in *K. lactis* like the GAL80 protein of *S. cerevisiae*.

The severe glucose repression of the galactose-lactose regulon in strains Q and W strongly suggests that glucose repression somehow involves *GAL4*. This possibility is further supported by the data for *MEL1* expression in *K. lactis*, which show that expression of this gene is more severely repressed by glucose in a *GAL4* than in a *LAC9* strain (Table 2). In *S. cerevisiae* the UAS_{GAL} seemed to have GAL4 protein bound in the absence and presence of inducer; only in the presence of glucose is there a lack of binding (14, 19). It is not known how glucose causes this effect on GAL4 protein. Glucose or a metabolite could directly or indirectly reduce the affinity of GAL4 protein for UAS. Whatever the mechanism, it seems reasonable to assume that it is similar in *S. cerevisiae* and *K. lactis*. The implication is that in *K. lactis* GAL4 protein senses the glucose repression signal but the LAC9 protein does not. Clearly, however, other models could explain our data. For example, glucose or a metabolite could reduce the effective concentration of GAL4 protein by inhibiting translation of GAL4 mRNA or by promoting degradation of GAL4 protein. Improved analytical techniques for measuring the activity of the GAL4 protein and its intracellular level will be needed to distinguish between these mechanisms. In this regard, we were unable to detect GAL4 protein in *K. lactis* strains Q, WW, and QQ and in wild-type *S. cerevisiae* by Western blotting (data not shown).

Glucose repression of the galactose-melibiose regulon requires the *GAL82* and *GAL83* functions, which are specific for the regulon, and the *REG1* function, which is not specific to the regulon and serves a more global role in glucose repression (21). Our data suggest that *K. lactis* may have similar genes. Now that the *LAC9* gene has been isolated and its nucleotide sequence determined (L. V. Wray, Jr., M. W. Witte, R. C. Dickson, and M. I. Riley, Mol. Cell. Biol., in press), it may be possible to determine, by using chimeric *LAC9-GAL4* fusion genes, the region(s) in the GAL4 and LAC9 proteins that mediates the differential response to glucose in *K. lactis*.

Strains of *K. lactis* transformed with pMEL1 showed a high uninduced level of α-galactosidase activity regardless of whether *LAC9* or *GAL4* was present (Table 2). These levels were at least 10-fold higher than the uninduced level seen in *S. cerevisiae* (22; Wray et al., submitted). Two nonexclusive mechanisms could explain these observations. First, pMEL1 was present at 4 to 6 copies per cell, which could cause the

uninduced level of enzyme activity to rise. Second, there seems to be a species difference in regulon activity. The *GAL1*, *GAL7*, and *GAL10* gene products are not measurable in uninduced *S. cerevisiae* (1, 16; Wray et al., in press) but are readily measurable in uninduced *K. lactis* (Tables 1 and 2) (24). The mechanism(s) underlying this species difference is not known. The induced level of *MEL1* expression (α-galactosidase activity) in *K. lactis* (Table 2) and *S. cerevisiae* (Wray et al., in press) was similar, within a factor of 2, regardless of whether *LAC9* or *GAL4* was present. Thus, it is only the uninduced level of gene expression that is different between these yeasts.

The similarities and differences between the galactose-melibiose regulon and the galactose-lactose regulon provide a unique opportunity for studying how *trans*-acting regulatory proteins activate transcription, how these proteins connect to other components of the regulons, and how the regulons are interfaced to global control circuits, including carbon catabolite (glucose) repression.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grant GM-22749 to R.C.D. and grant GM-27925 to J.E.H. from the National Institutes of Health and by grant 50004 from the University of Kentucky PSP to M.I.R.

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